US 20220050103A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2022/0050103 A1**

Marvin et al. (43) **Pub. Date: Feb. 17, 2022**

(54) **GENETICALLY ENCODED BIOSENSORS**

(71) Applicant: **Howard Hughes Medical Institute**, Chevy Chase, MD (US)

(72) Inventors: **Jonathan S. Marvin**, Arlington, VA (US); **Loren Looger**, Sterling, VA (US)

(21) Appl. No.: **17/515,289**

(22) Filed: **Oct. 29, 2021**

Related U.S. Application Data

(60) Division of application No. 16/902,160, filed on Jun. 15, 2020, now Pat. No. 11,162,942, which is a continuation of application No. 16/002,697, filed on Jun. 7, 2018, now Pat. No. 10,684,282, which is a continuation-in-part of application No. 15/904,574, filed on Feb. 26, 2018, now Pat. No. 10,060,920, which is a division of application No. 15/664,326, filed on Jul. 31, 2017, now Pat. No. 9,939,437, which is a division of application No. 14/350,199, filed on Nov. 18, 2014, now Pat. No. 9,719,992, filed as application No. PCT/US2012/059219 on Oct. 8, 2012.

(60) Provisional application No. 61/544,867, filed on Oct. 7, 2011.

Publication Classification

(51) **Int. Cl.**

G01N 33/557	(2006.01)
G01N 33/68	(2006.01)
G01N 33/58	(2006.01)
C07K 14/195	(2006.01)
C07K 14/245	(2006.01)
C07K 14/435	(2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/557*** (2013.01); ***G01N 33/68*** (2013.01); ***G01N 33/582*** (2013.01); ***C07K 14/195*** (2013.01); ***C07K 14/245*** (2013.01); *G01N 2400/00* (2013.01); ***C07K 14/43595*** (2013.01); *G01N 2458/00* (2013.01); *C07K 2319/20* (2013.01); *C07K 2319/24* (2013.01); *C07K 2319/60* (2013.01); ***G01N 33/6812*** (2013.01)

(57) **ABSTRACT**

The present disclosure provides, inter alia, genetically encoded recombinant peptide biosensors comprising analyte-binding framework portions and signaling portions, wherein the signaling portions are present within the framework portions at sites or amino acid positions that undergo a conformational change upon interaction of the framework portion with an analyte.

Specification includes a Sequence Listing.

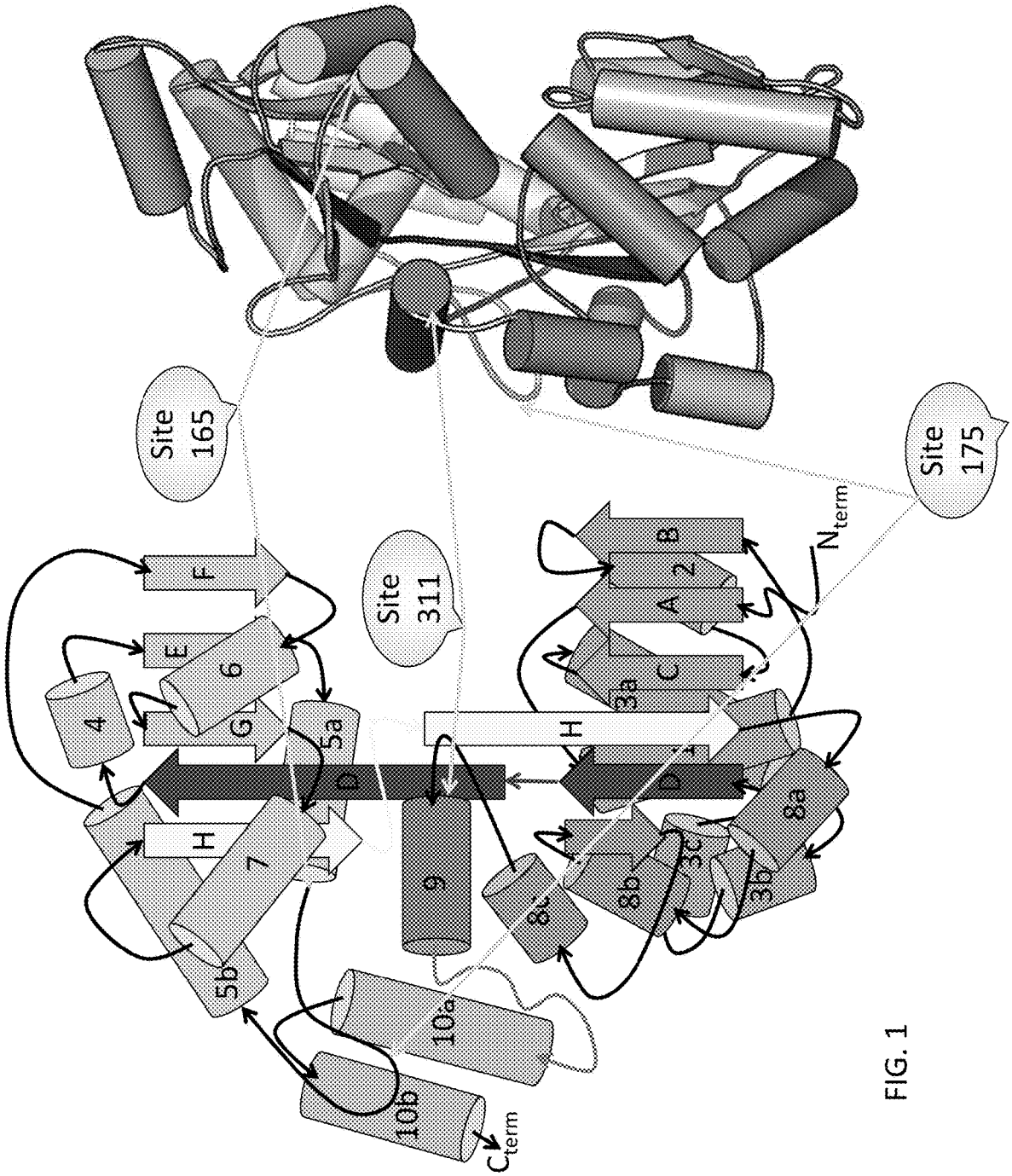

Site 165
Site 311
Site 175
N_{term}
C_{term}
F
E
6
G
4
5a
D
H
7
5b
10a
10b
9
8a
8b
8c
3b
3c
3a
C
A
2
B
H
D'
1

FIG. 1

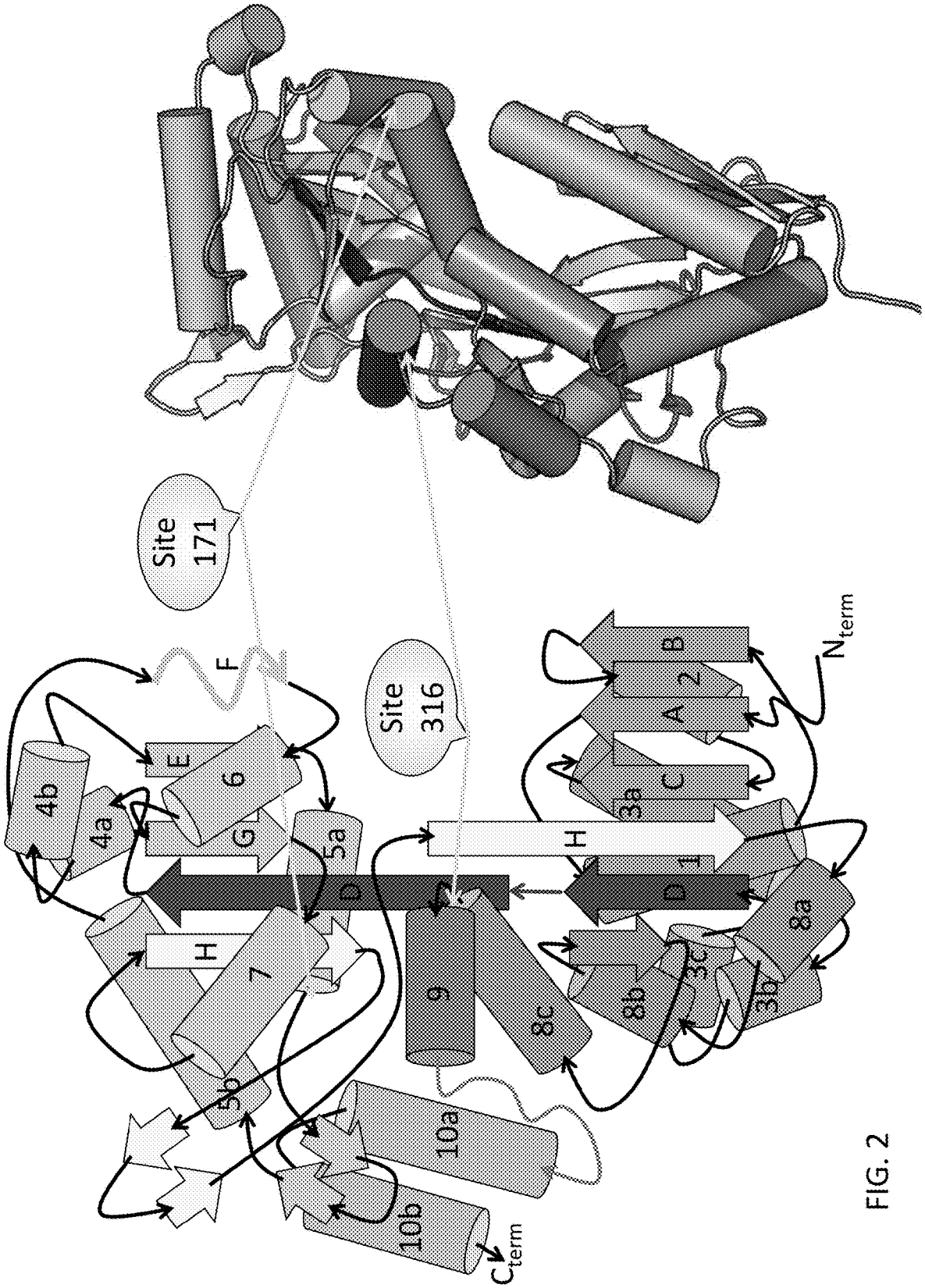

Site 171
Site 316
F
E
6
4b
4a
G
5a
H
D
H
7
5b
9
8c
10a
10b
C_{term}
N_{term}
B
2
A
C
3a
1
D
8b
3c
3b
8a

FIG. 2

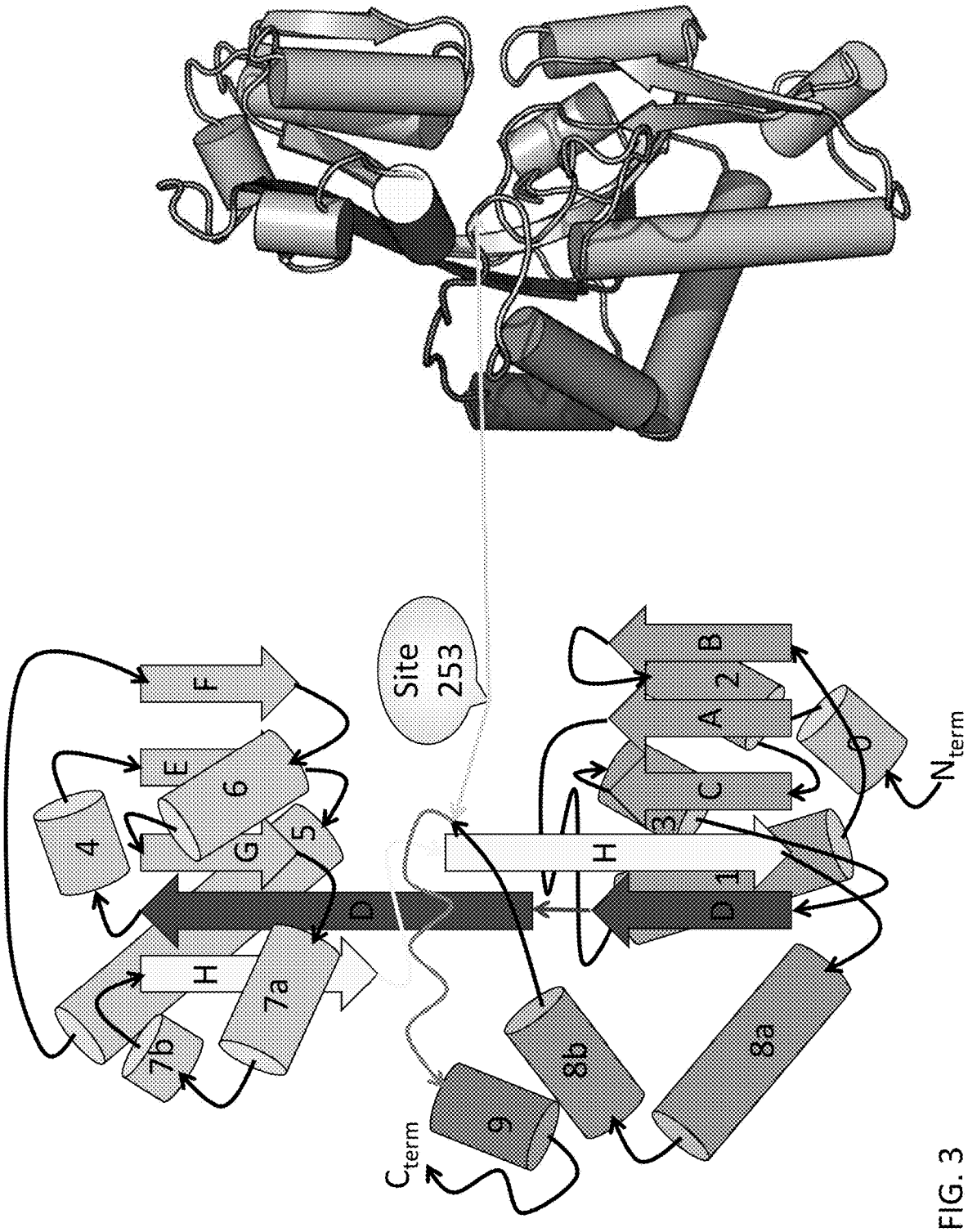


FIG. 3

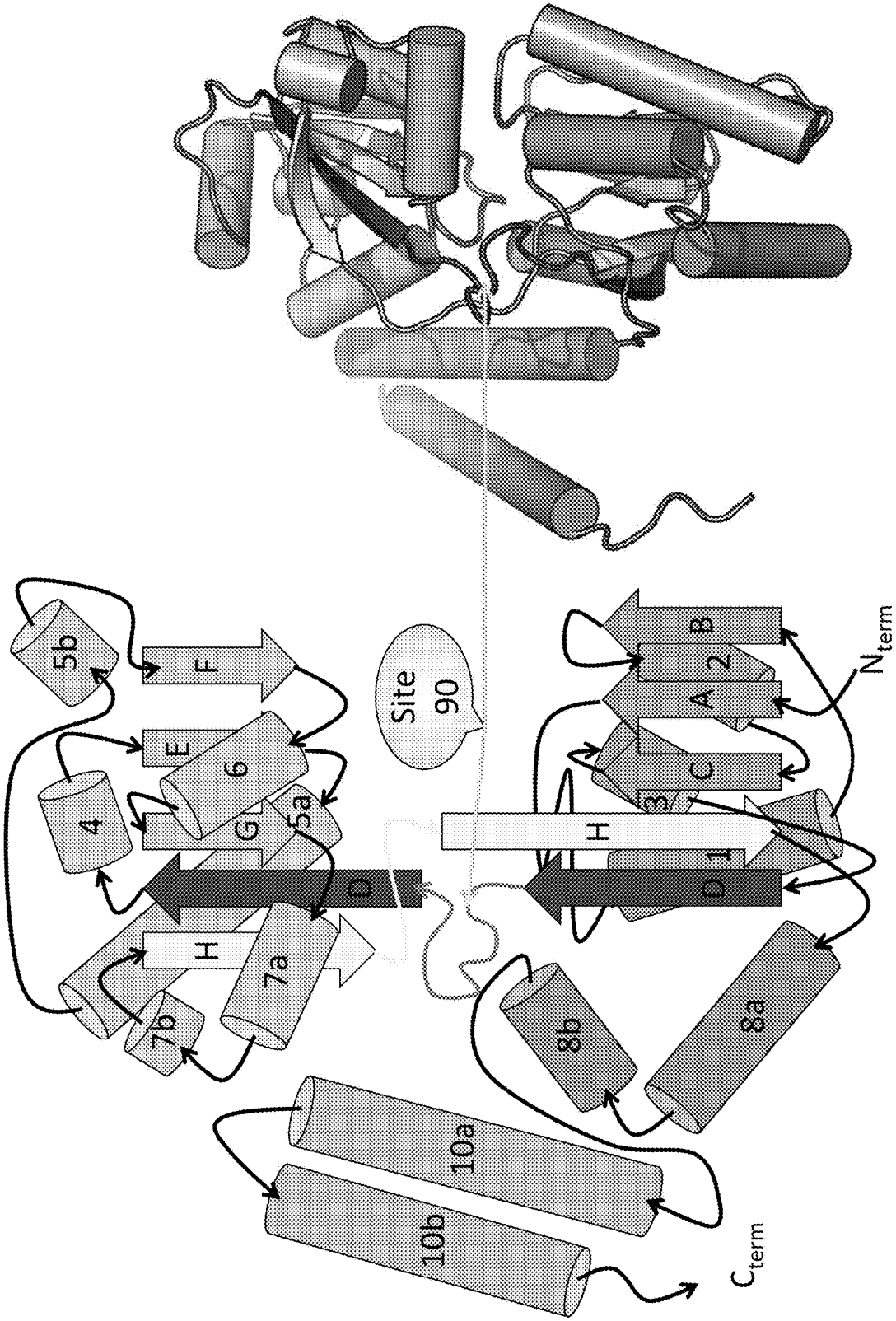


FIG. 4

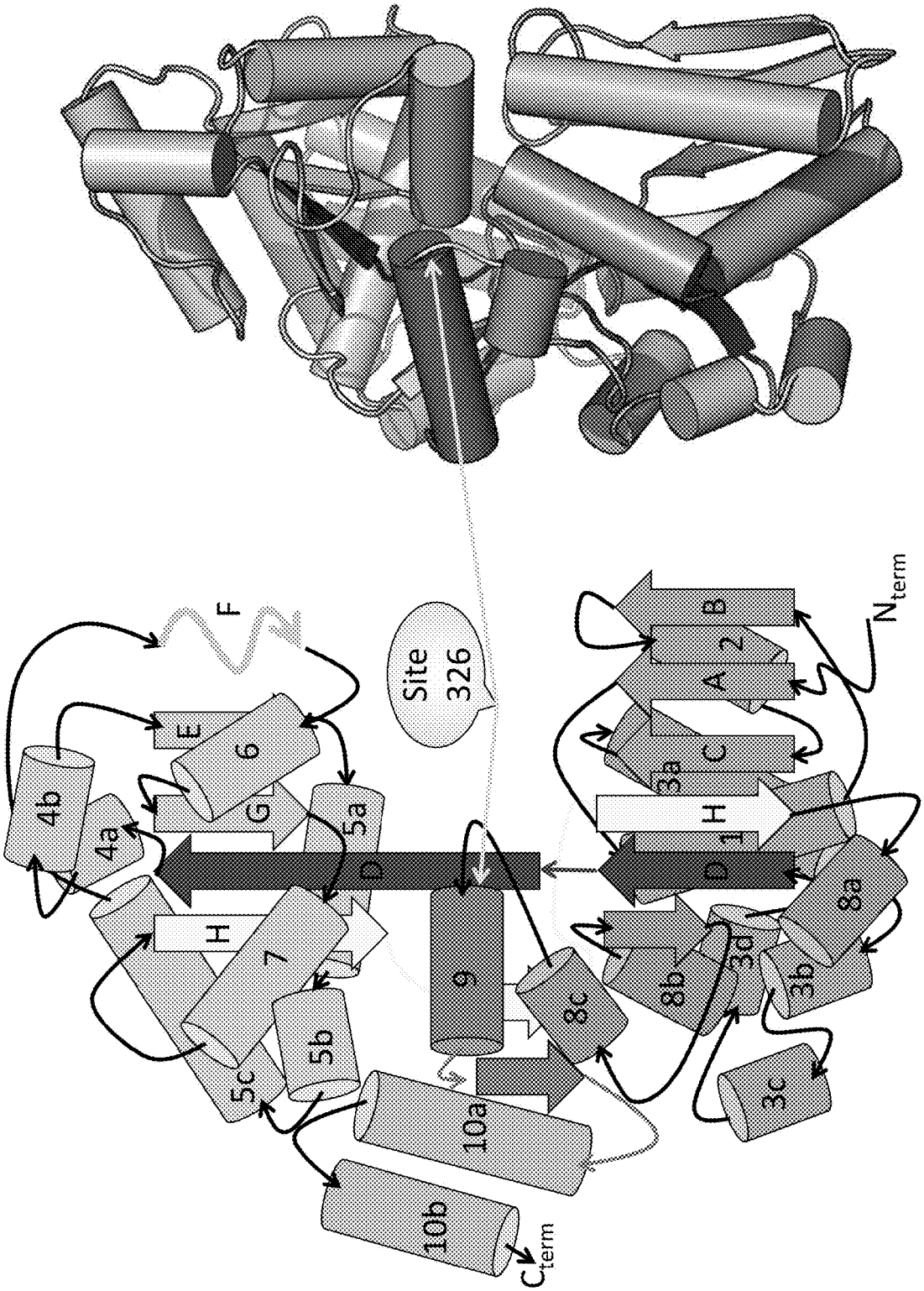

F
Site
326
E
6
4b
4a
G
5a
D
H
7
5c
5b
9
8c
10a
10b
C_{term}
N_{term}
B
2
A
3a
C
H
1
D
8a
8b
3d
3b
3c

FIG. 5

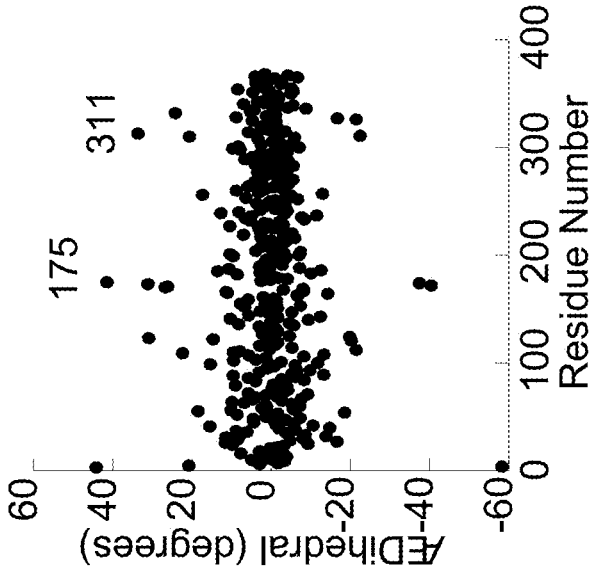

ΔDihedral (degrees)
60
40
20
0
-20
-40
-60
0
100
200
300
400
Residue Number
175
311

FIG. 6B

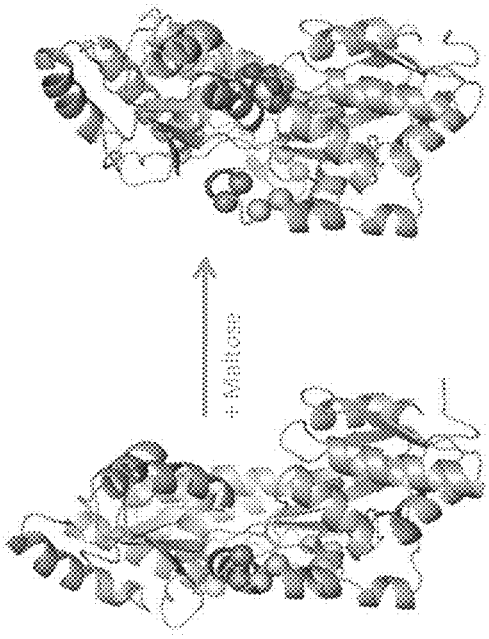

+Maltose

FIG. 6A

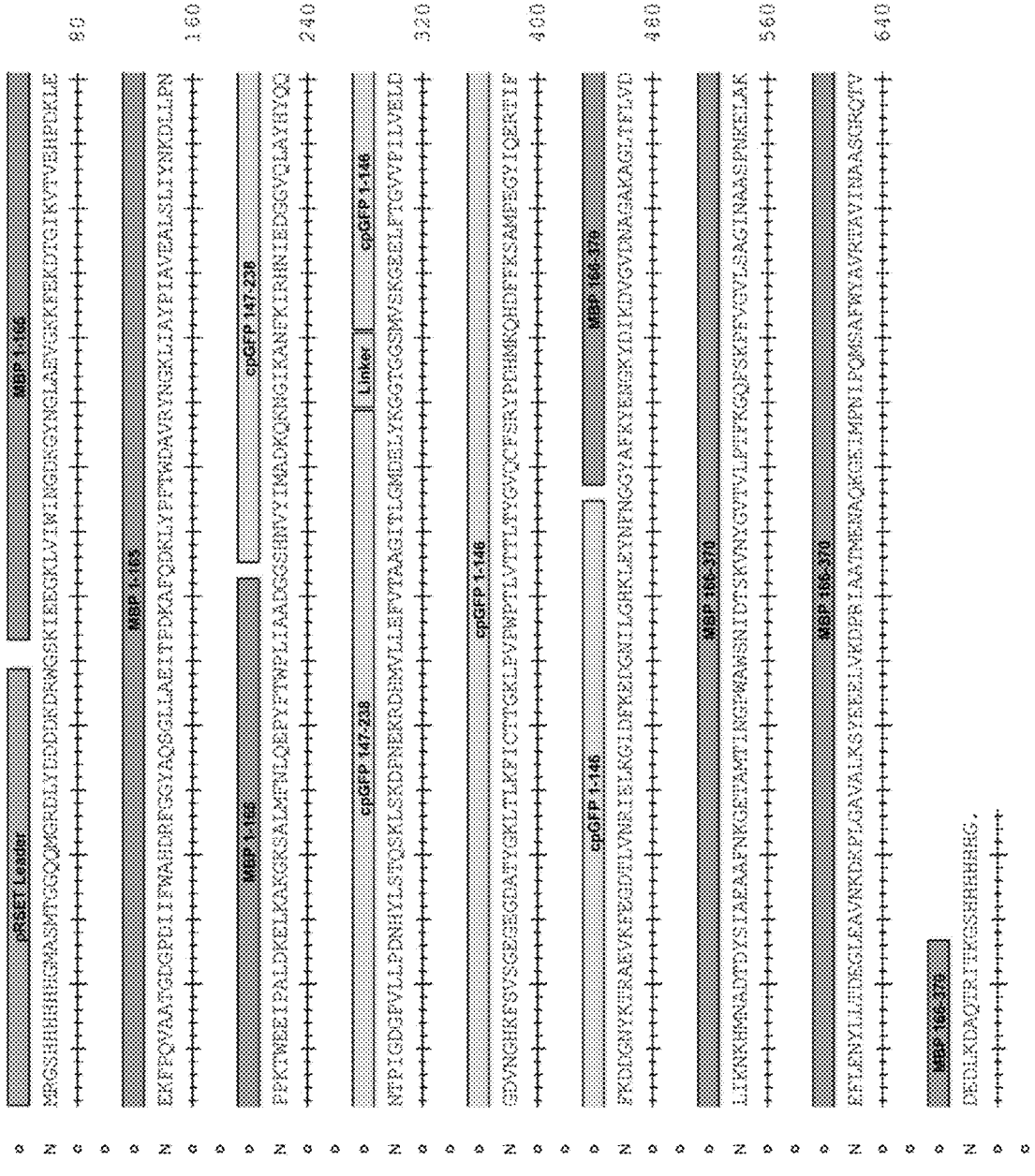

pRSET Leader
MBP 1-166
MBP 1-165
cpGFP 147-238
MBP 166
Linker
cpGFP 1-146
MBP 166-370
MBP 166-372
MBP 166-376

FIG. 7A

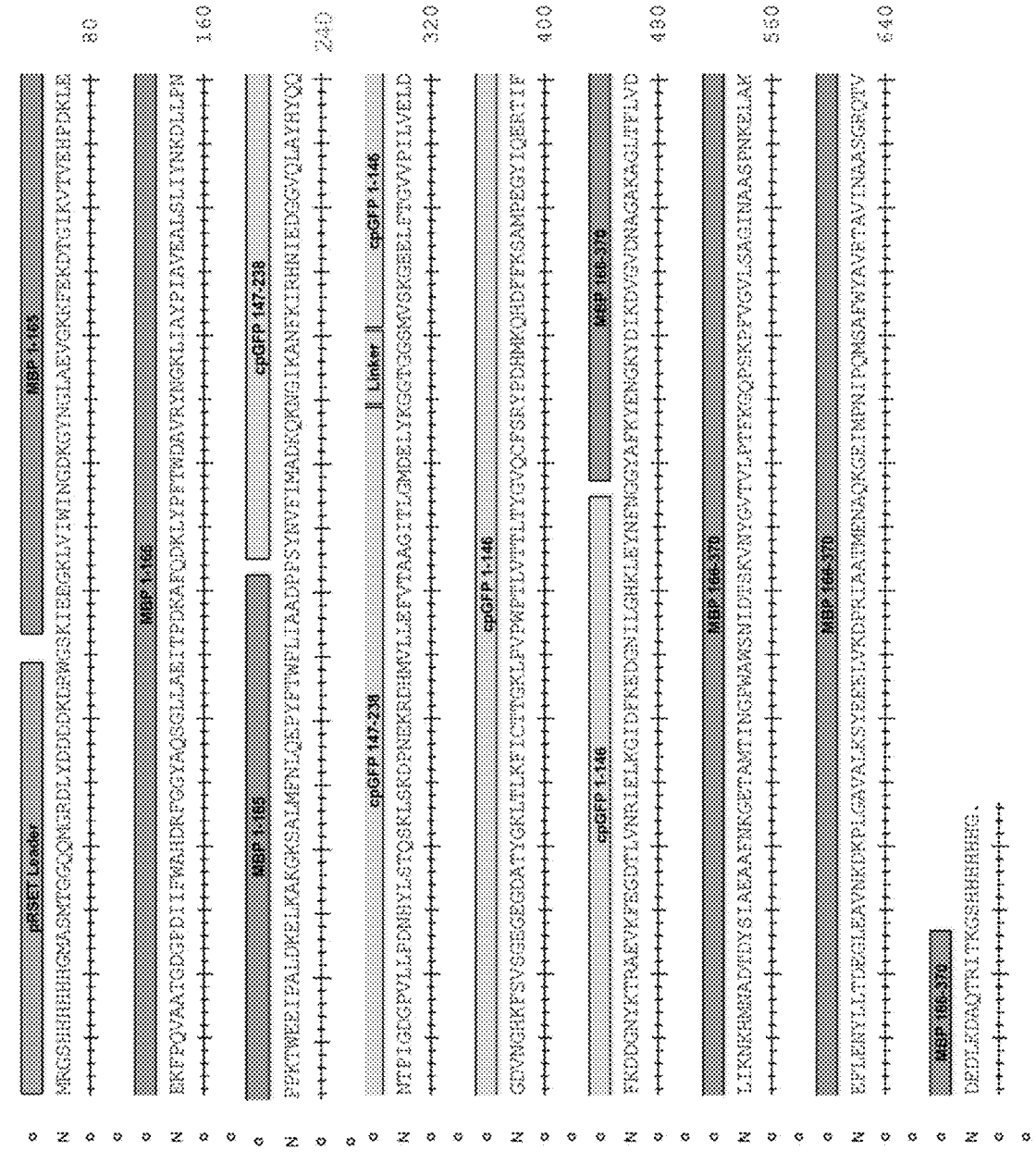

pRSET Leader
MBP 1-165
MBP 1-166
cpGFP 147-238
sMBP 1-165
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
MBP 166-370
MBP 166-370
MBP 166-370
MBP 166-370
80
160
240
320
400
480
560
640

FIG. 7B

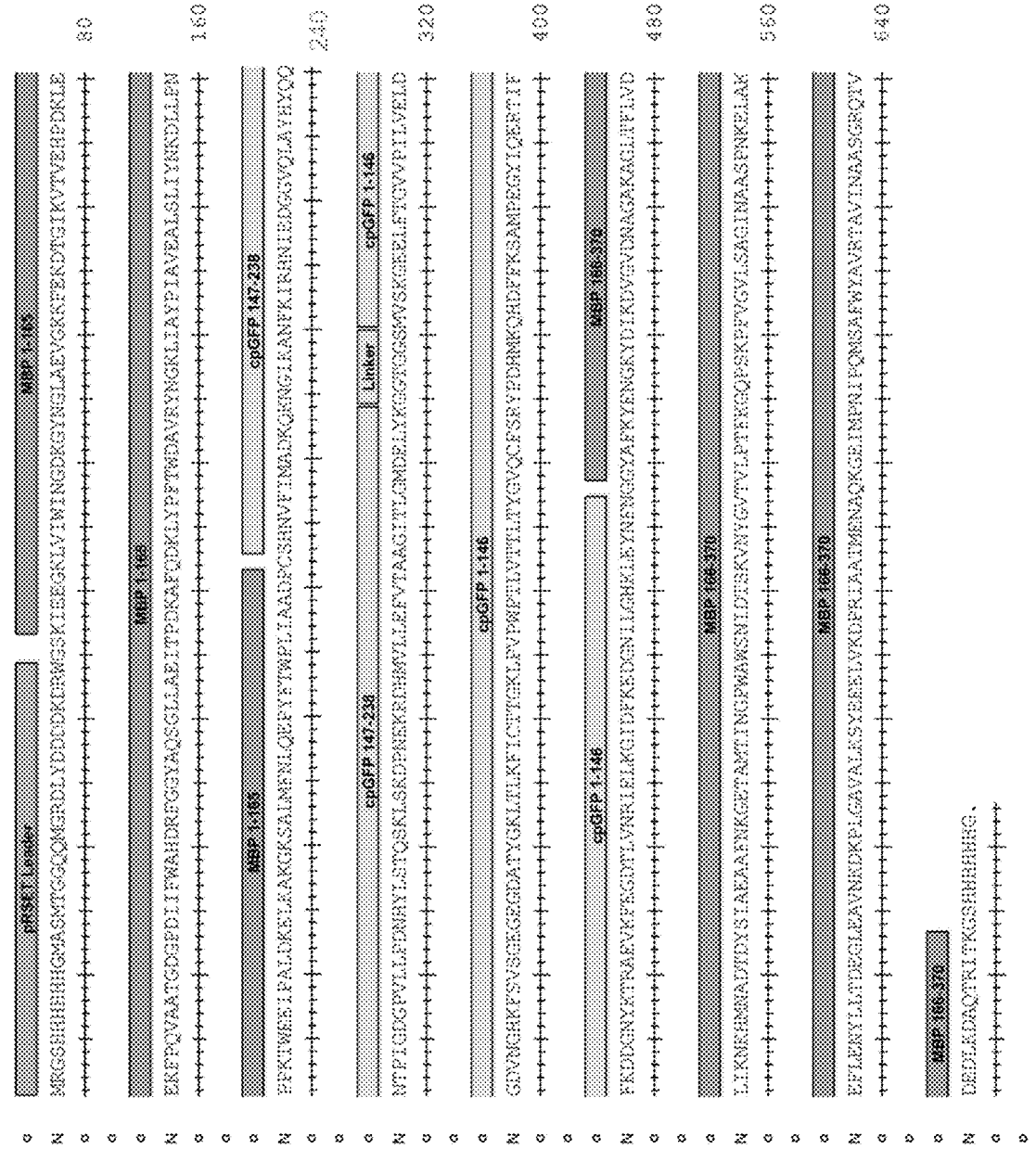

pRSET Leader
MBP 1-165
MBP 1-166
cpGFP 147-238
MBP 1-165
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
MBP 166-370
MBP 166-370
MBP 166-370
MBP 166-370

FIG. 7C

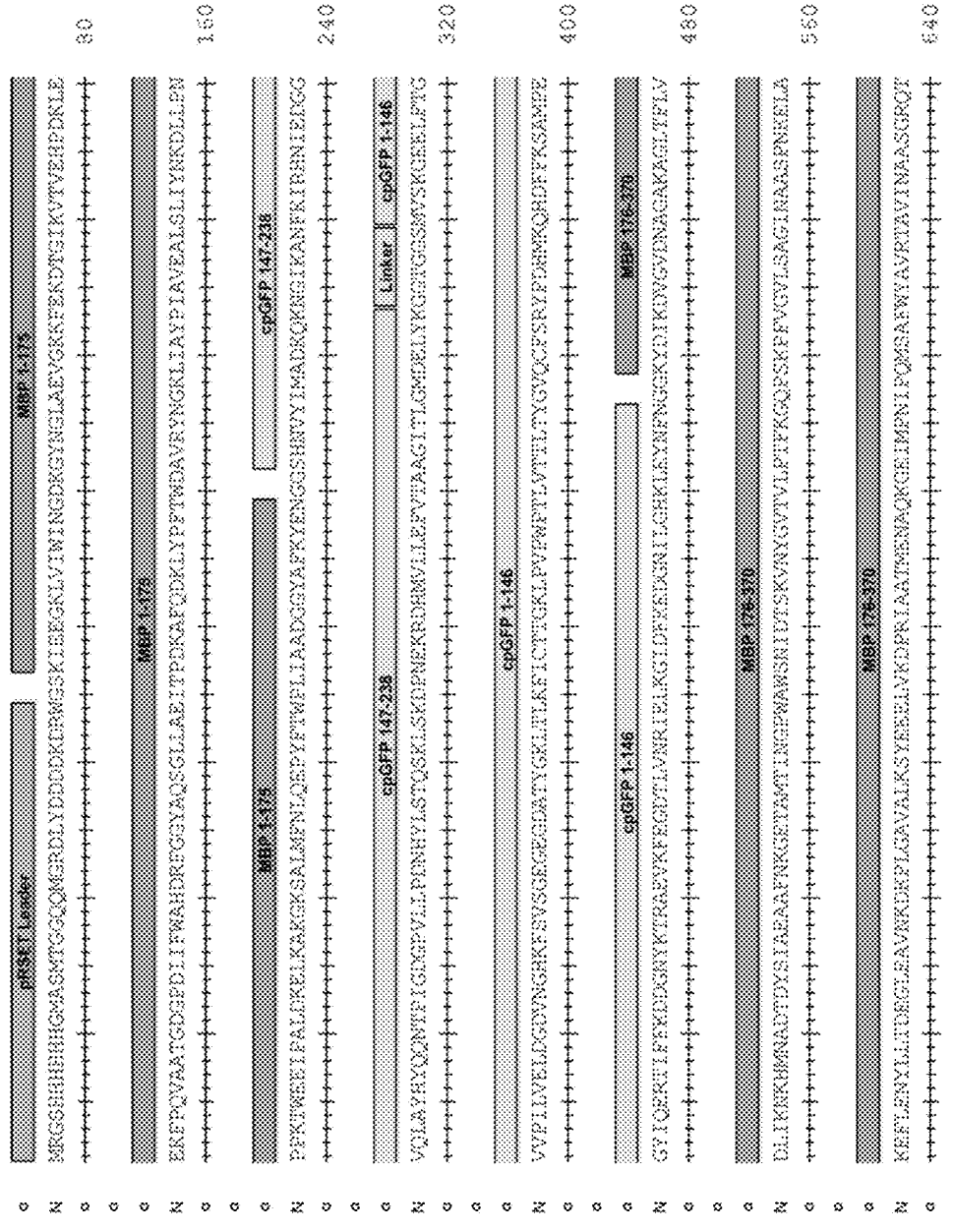

pRSET Leader
MBP 1-175
MBP 1-175
cpGFP 147-238
MBP 1-175
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 147-238
cpGFP 1-146
cpGFP 1-146
MBP 176-370
MBP 176-370
MBP 176-370
MBP 176-370

FIG. 8A

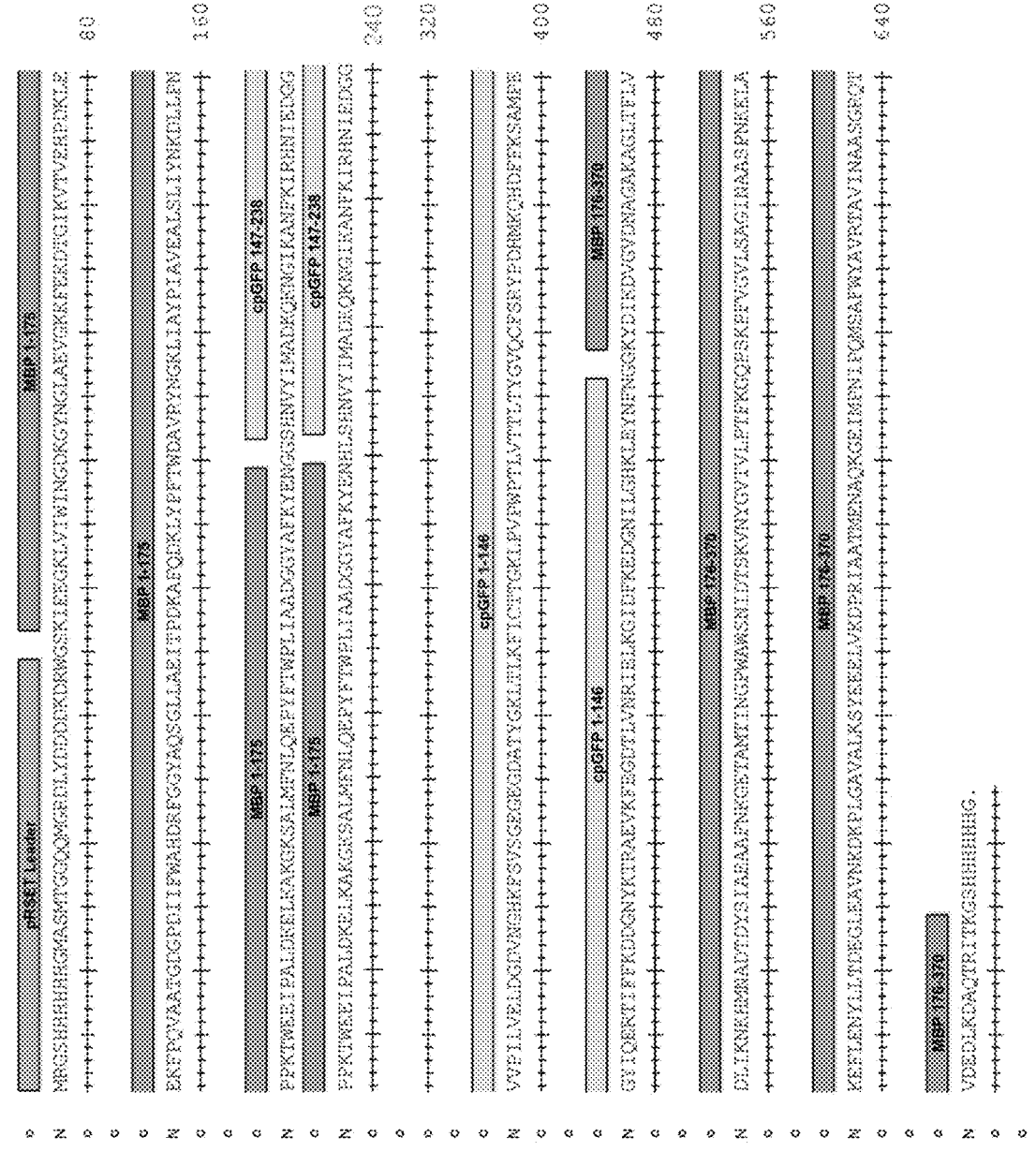

pRSET Leader
MBP 1-370
MBP 1-173
cpGFP 147-238
MBP 1-175
cpGFP 1-146
MBP 176-370

FIG. 8B

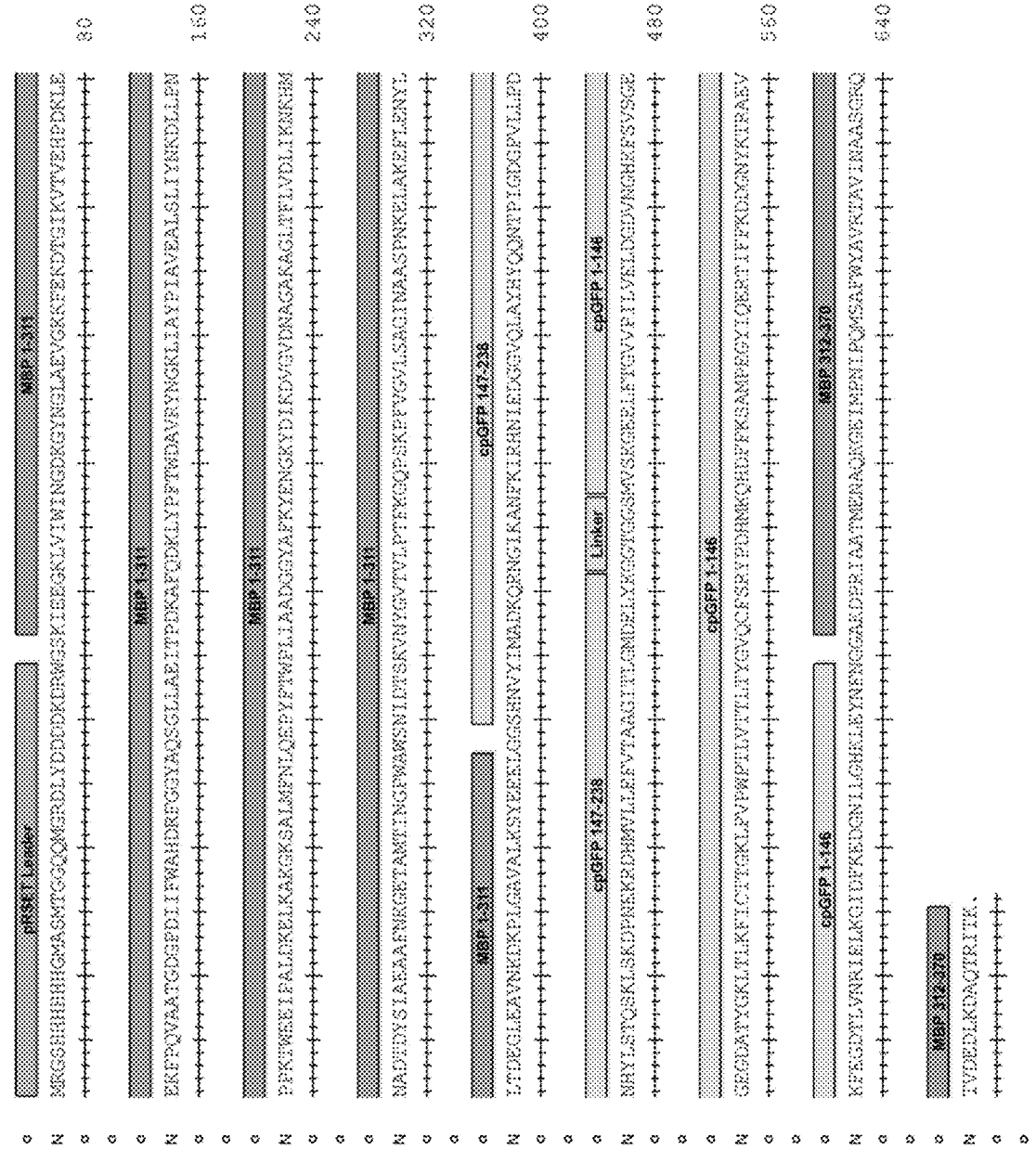

pRSET Leader
MBP 1-311
MBP 1-311
MBP 1-311
MBP 1-311
cpGFP 147-238
MBP 1-311
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
MBP 312-370
MBP 312-370
30
150
240
320
400
480
560
640

FIG. 9A

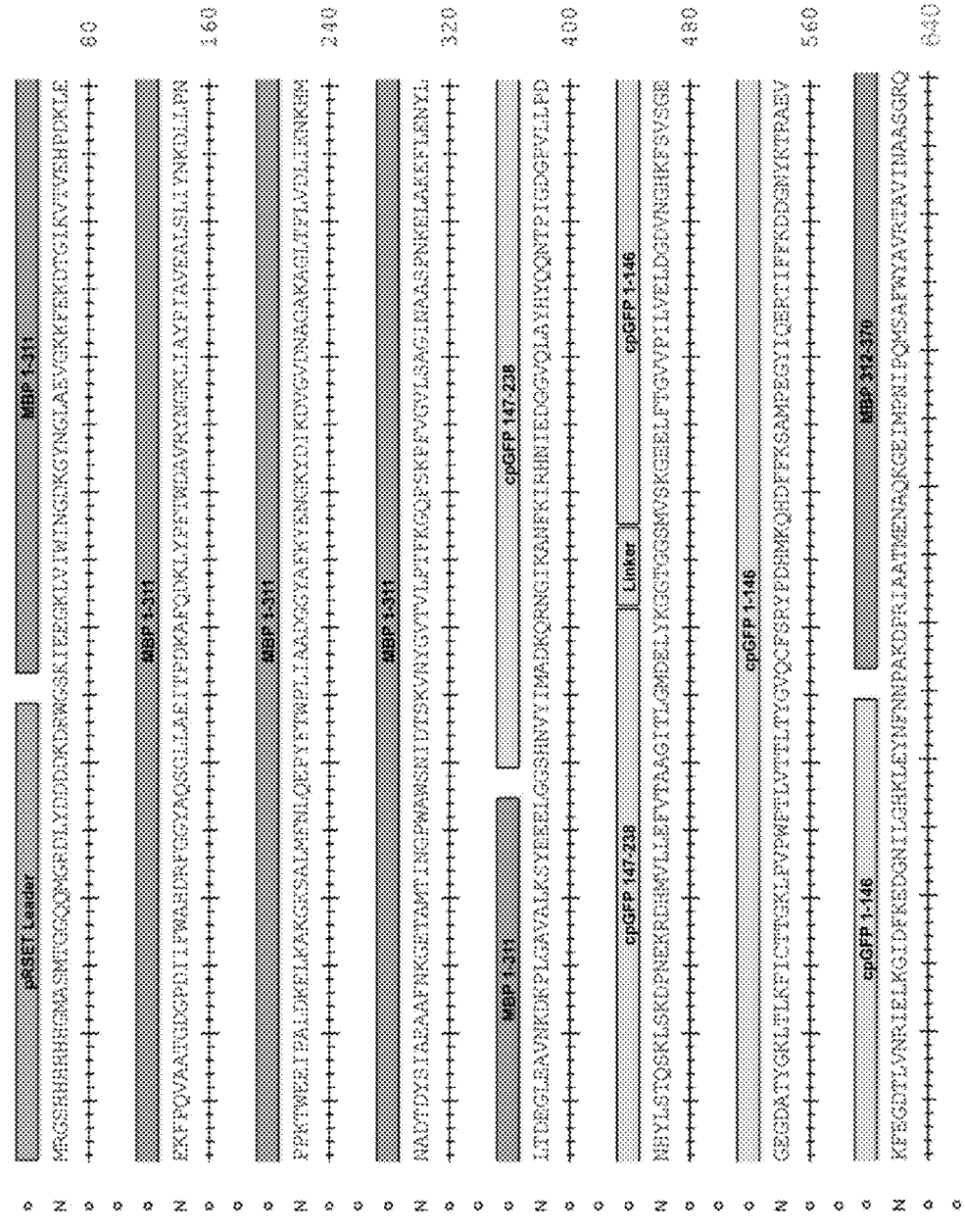

pRSET Leader
MBP 1-311
MBP 1-311
MBP 1-311
MBP 1-311
MBP 1-311
cpGFP 147-238
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
MBP 312-370
MBP 312-370

FIG. 9B

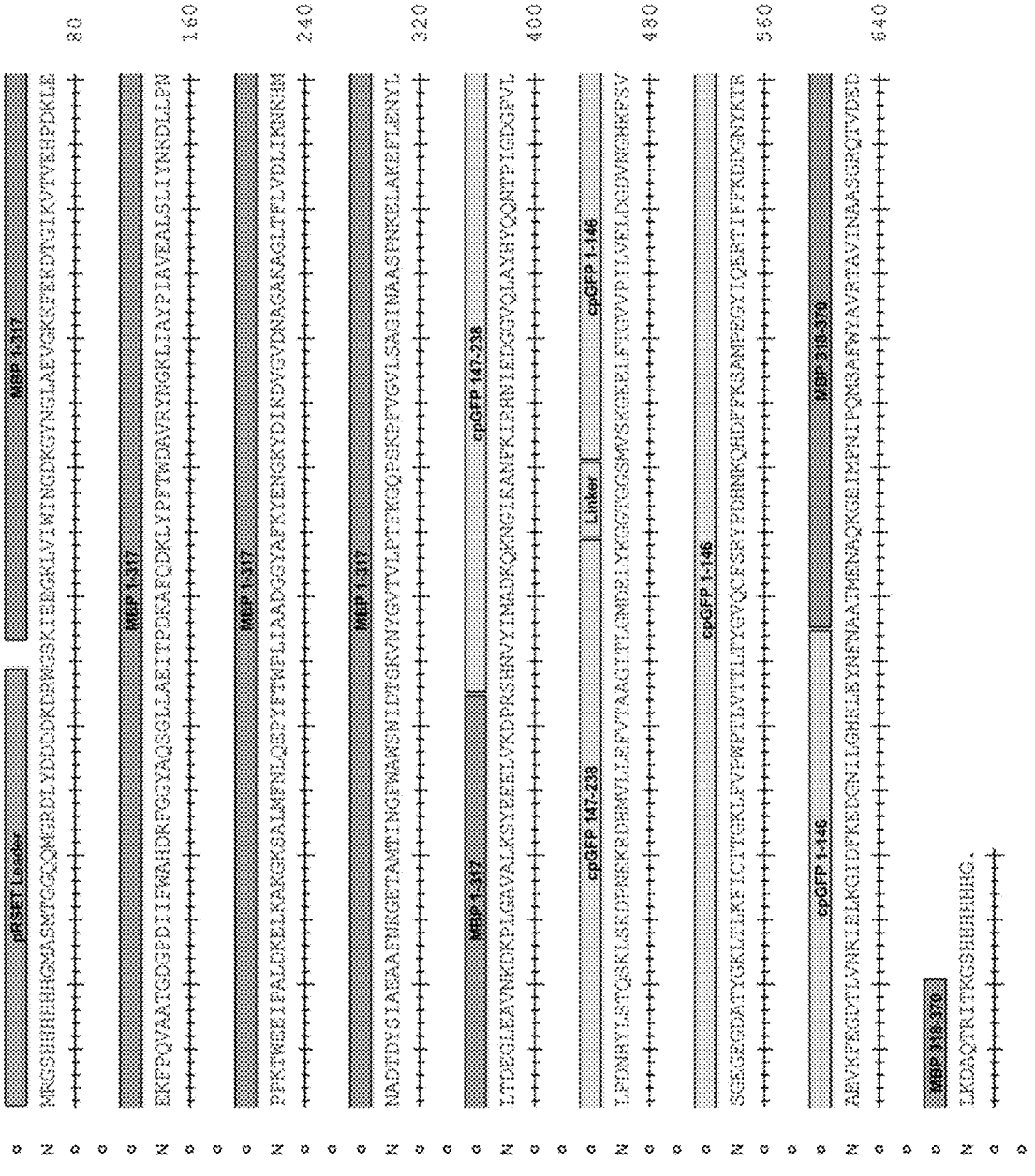

pRSET Leader
MBP 1-317
MBP 1-317
MBP 1-317
MBP 1-317
MBP 1-317
cpGFP 147-238
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
MBP 318-370
MBP 318-370
0
80
160
240
320
400
480
560
640

FIG. 10

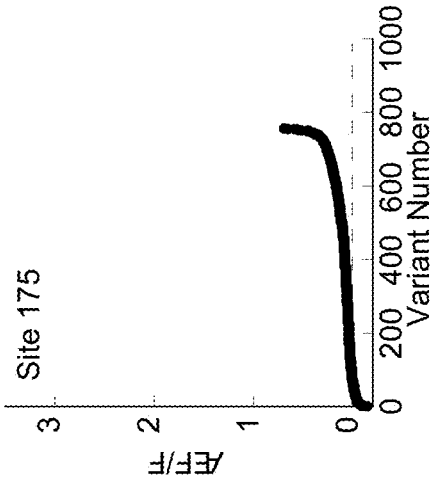


FIG. 11A

FIG. 11B

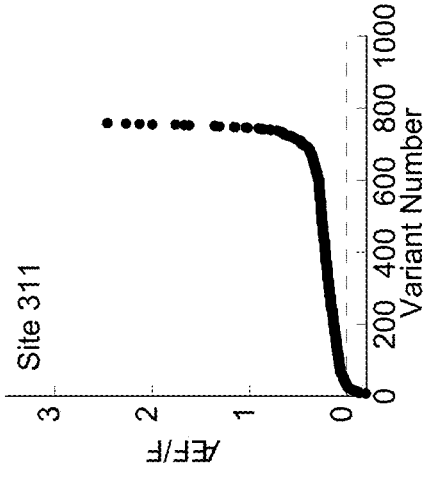


FIG. 11C

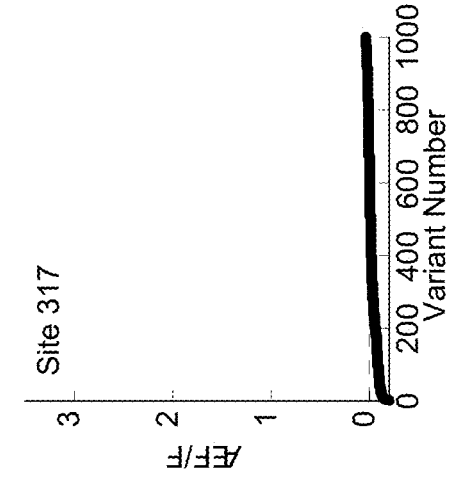


FIG. 11D

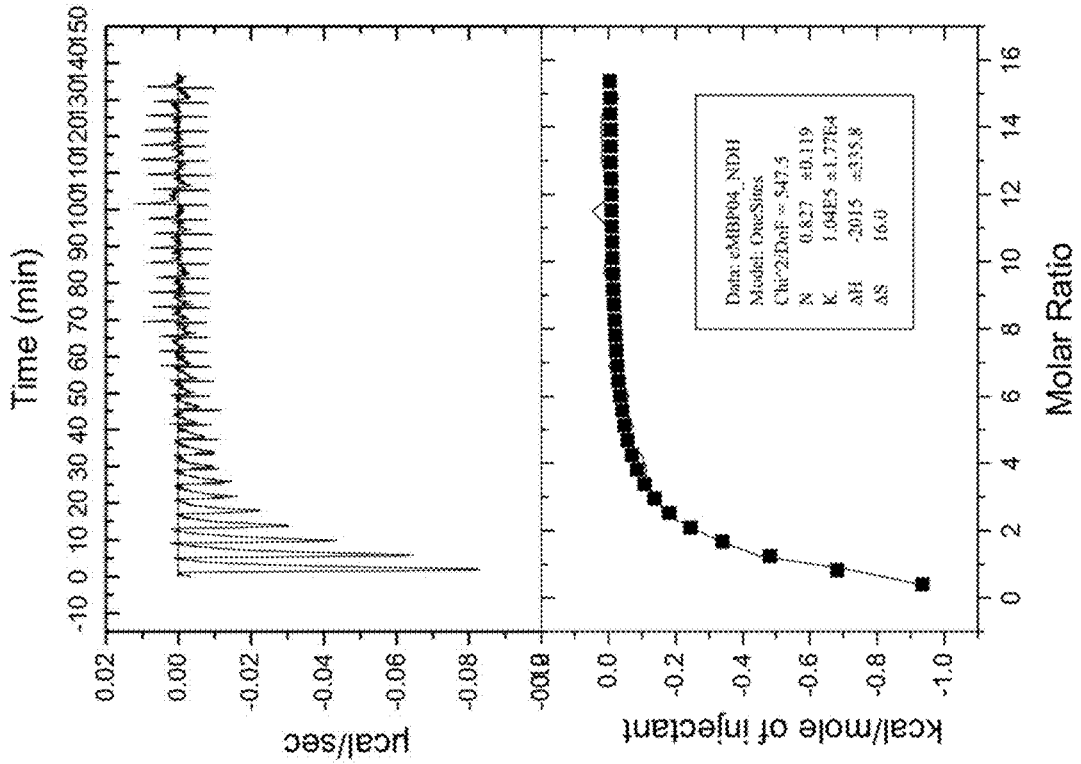

Time (min)
-10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
µcal/sec
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
kcal/mole of injectant
0 2 4 6 8 10 12 14 16
Molar Ratio
Data: eMBP04_NDH
Model: OneSites
Chi^2/DoF = 347.5
N 0.827 ±0.119
K 1.04E5 ±1.77E4
ΔH -2015 ±355.8
ΔS 16.0

FIG. 12

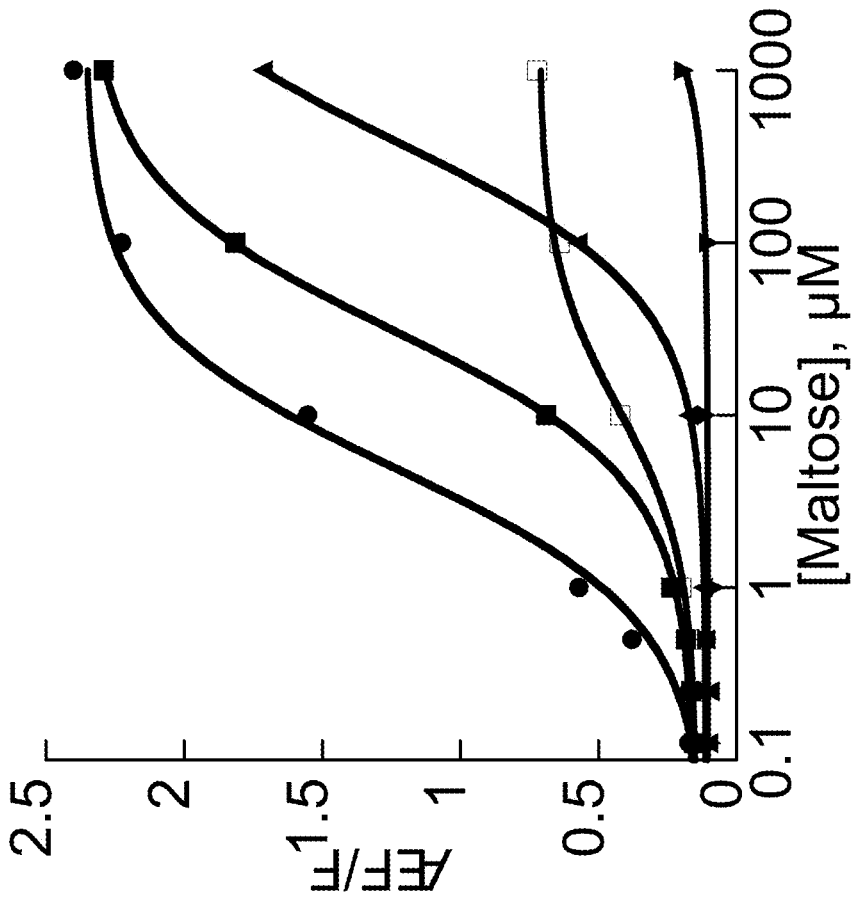


FIG. 13

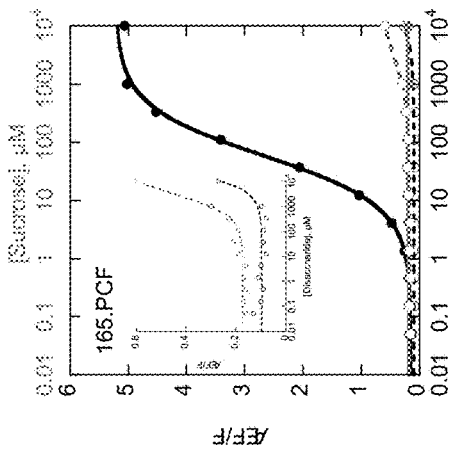


FIG. 14B

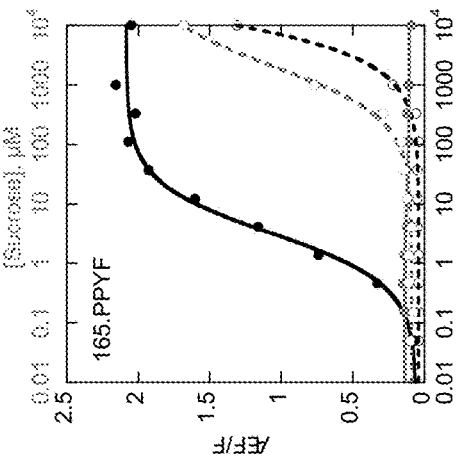


FIG. 14A

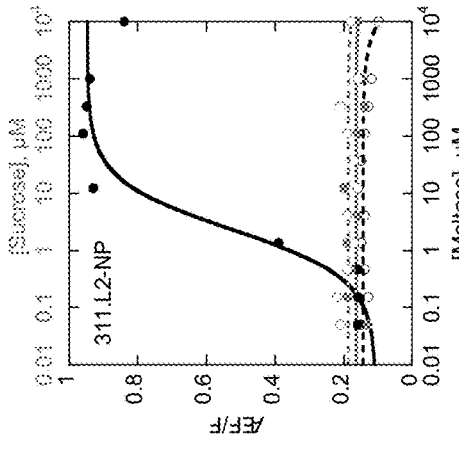


FIG. 14D

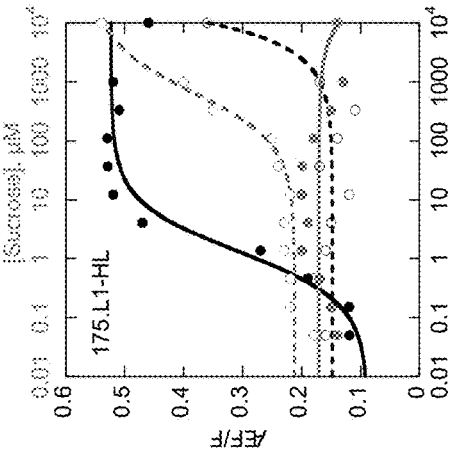


FIG. 14C

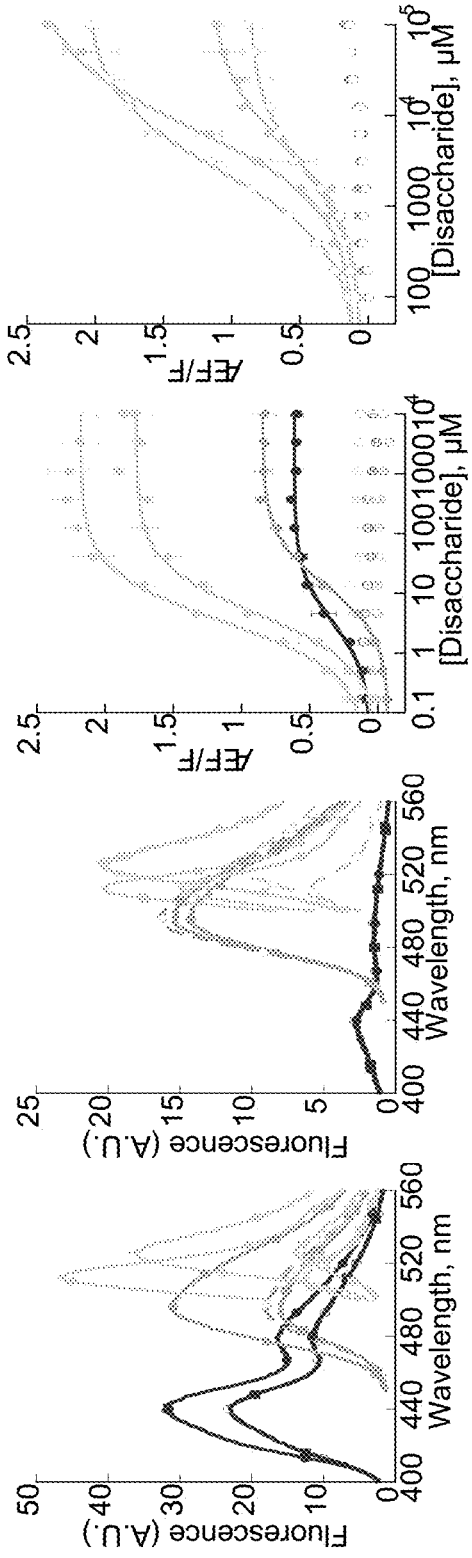


FIG. 15A

FIG. 15B

FIG. 15C

FIG. 15D

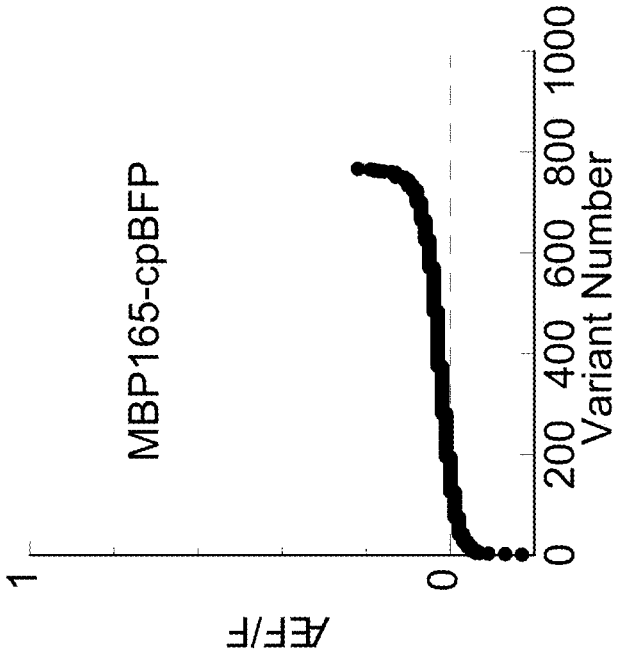


FIG. 16

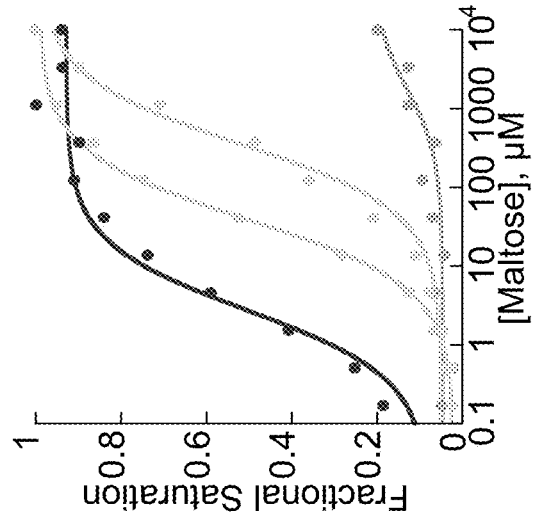


FIG. 17B

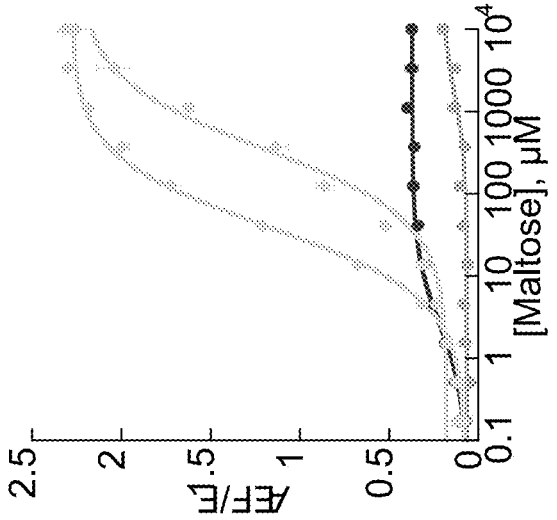


FIG. 17A

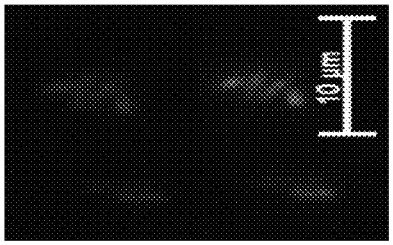


FIG. 18C

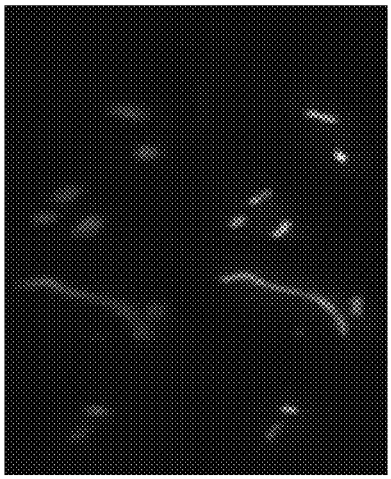


FIG. 18B

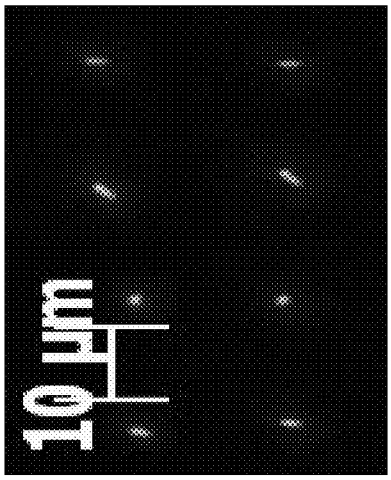


FIG. 18A

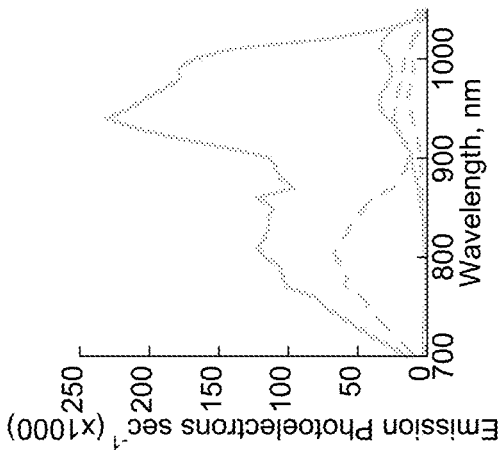


FIG. 19B

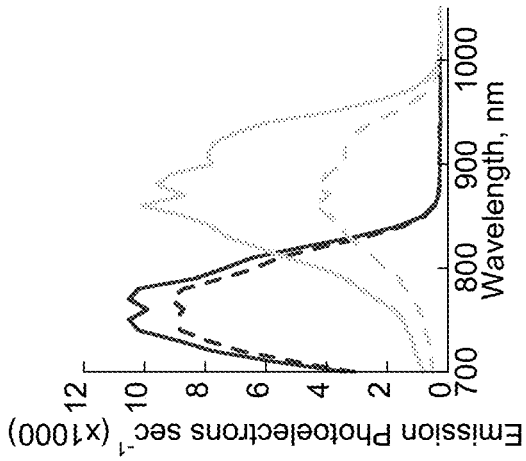


FIG. 19A

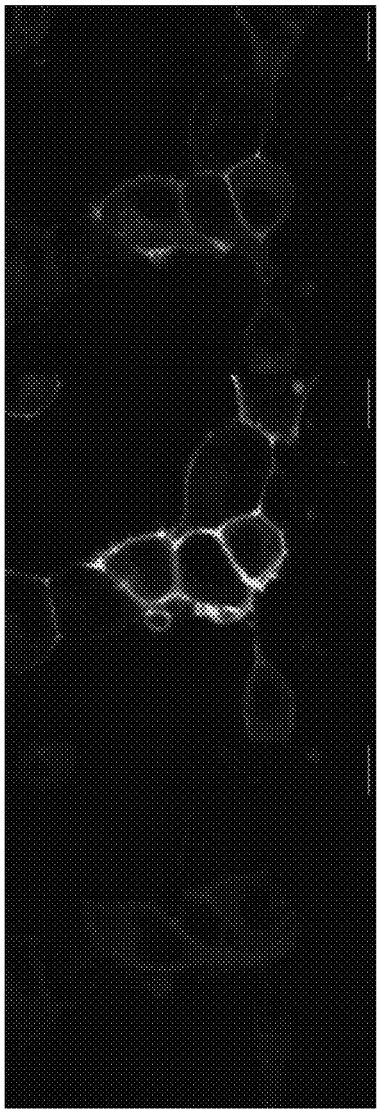


FIG. 20C

FIG. 20B

FIG. 20A

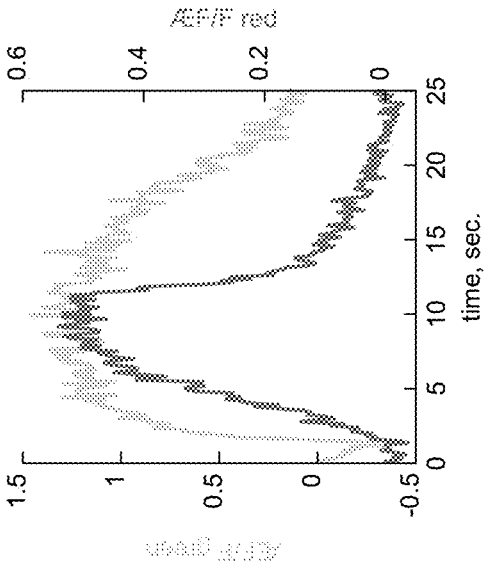


FIG. 21B

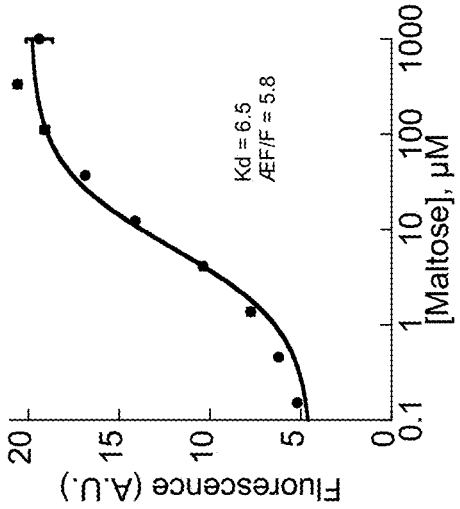


FIG. 21A

FIG. 22D

FIG. 22C

FIG. 22B

FIG. 22A

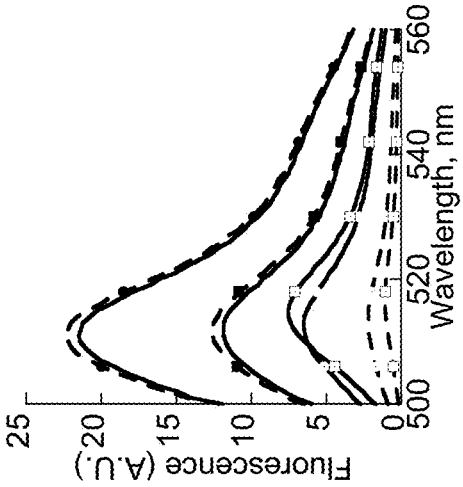


FIG. 23A

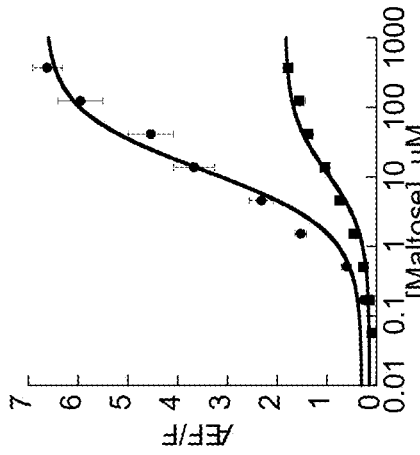


FIG. 23B

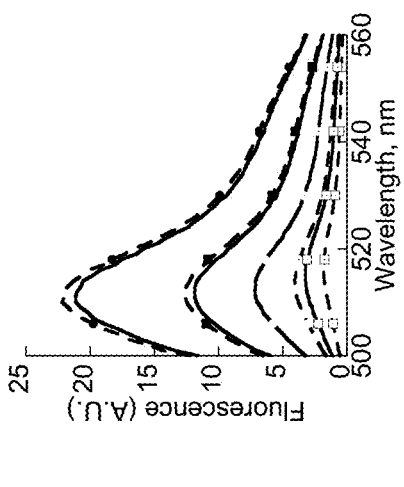


FIG. 23C

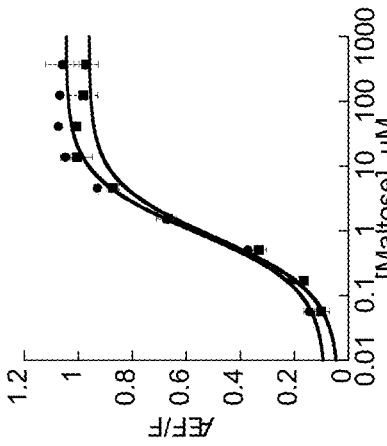


FIG. 23D

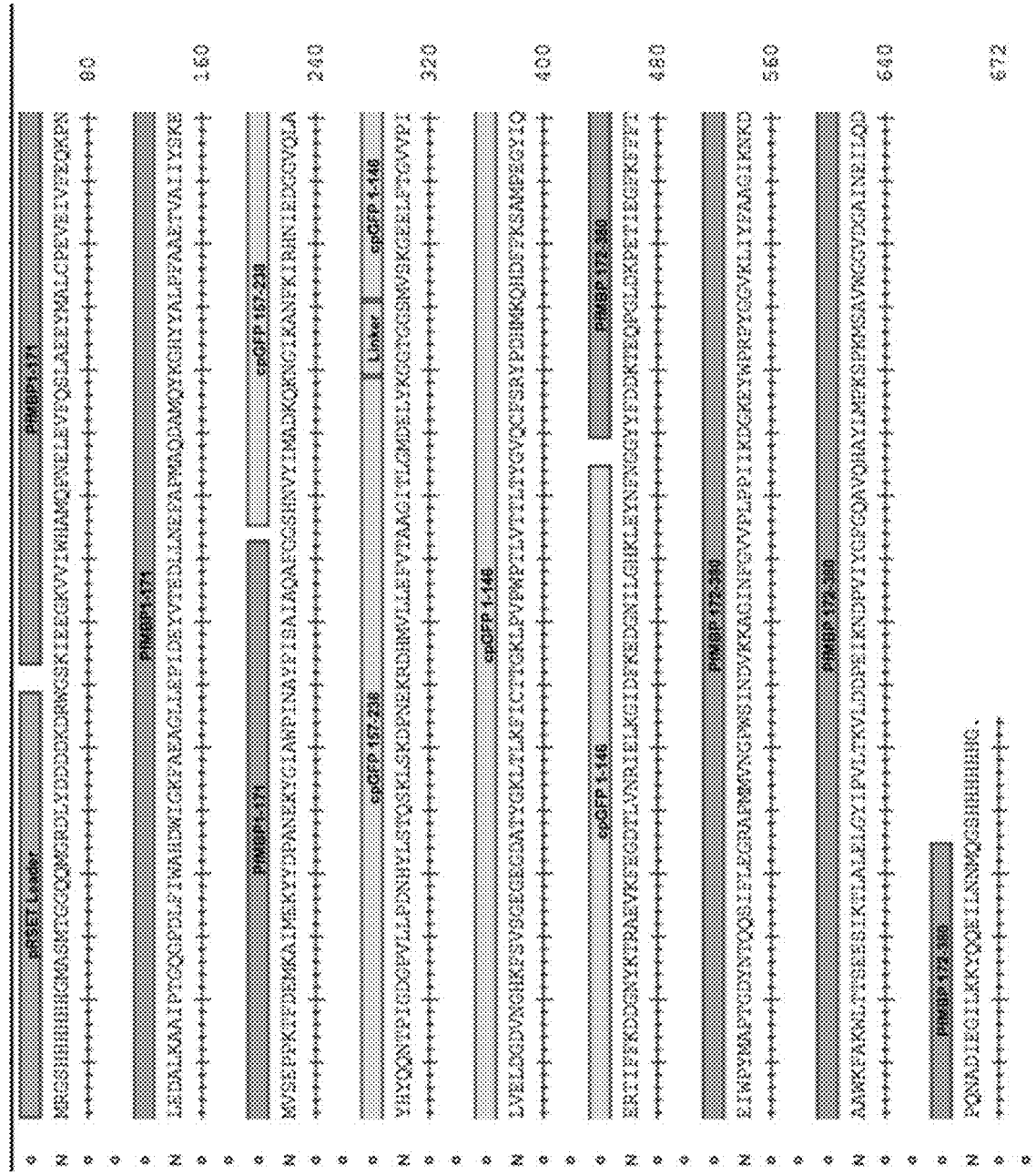


FIG. 24A

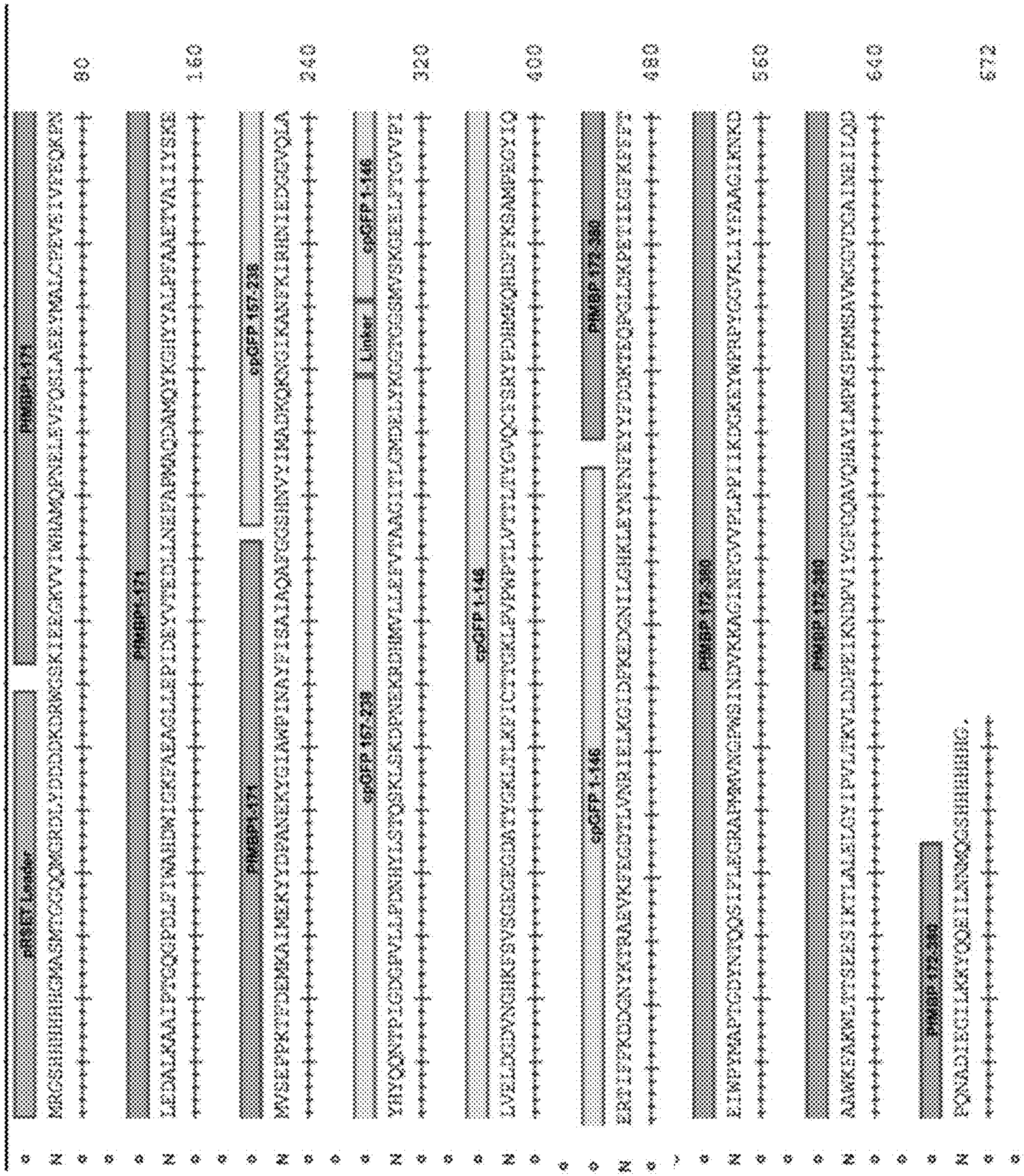

pRSET Leader
PMBP1-171
cpGFP 157-238
Linker
cpGFP 1-146
PMBP 172-380
80
160
240
320
400
480
560
640
672

FIG. 24B

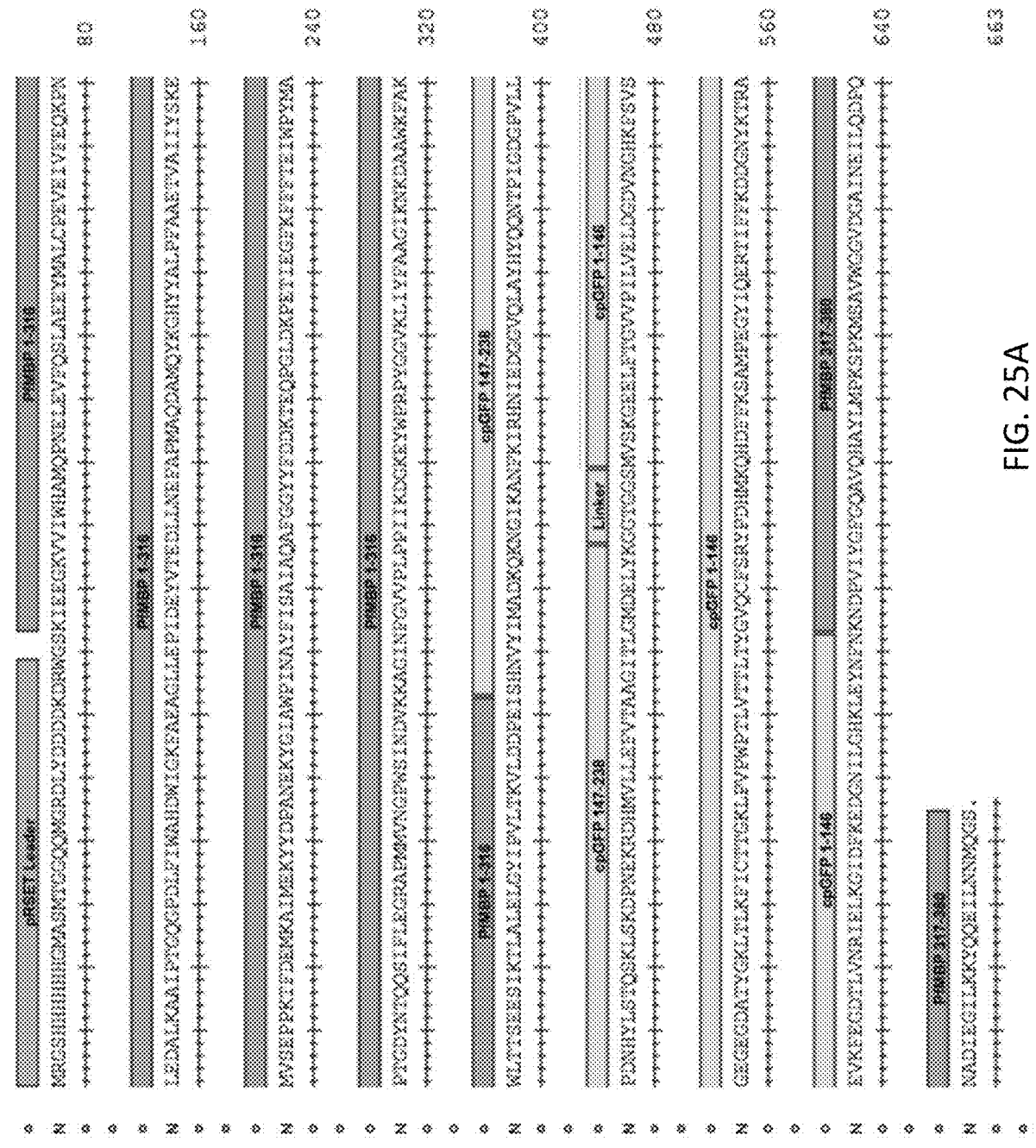

pRSET Leader
PhiBP 1-316
PhiBP 1-316
PhiBP 1-316
PhiBP 1-316
spGFP 147-238
PhiBP 1-316
spGFP 147-238
Linker
spGFP 1-146
spGFP 1-146
spGFP 1-146
PhiBP 317-388
PhiBP 317-388
80
160
240
320
400
480
560
640
663

FIG. 25A

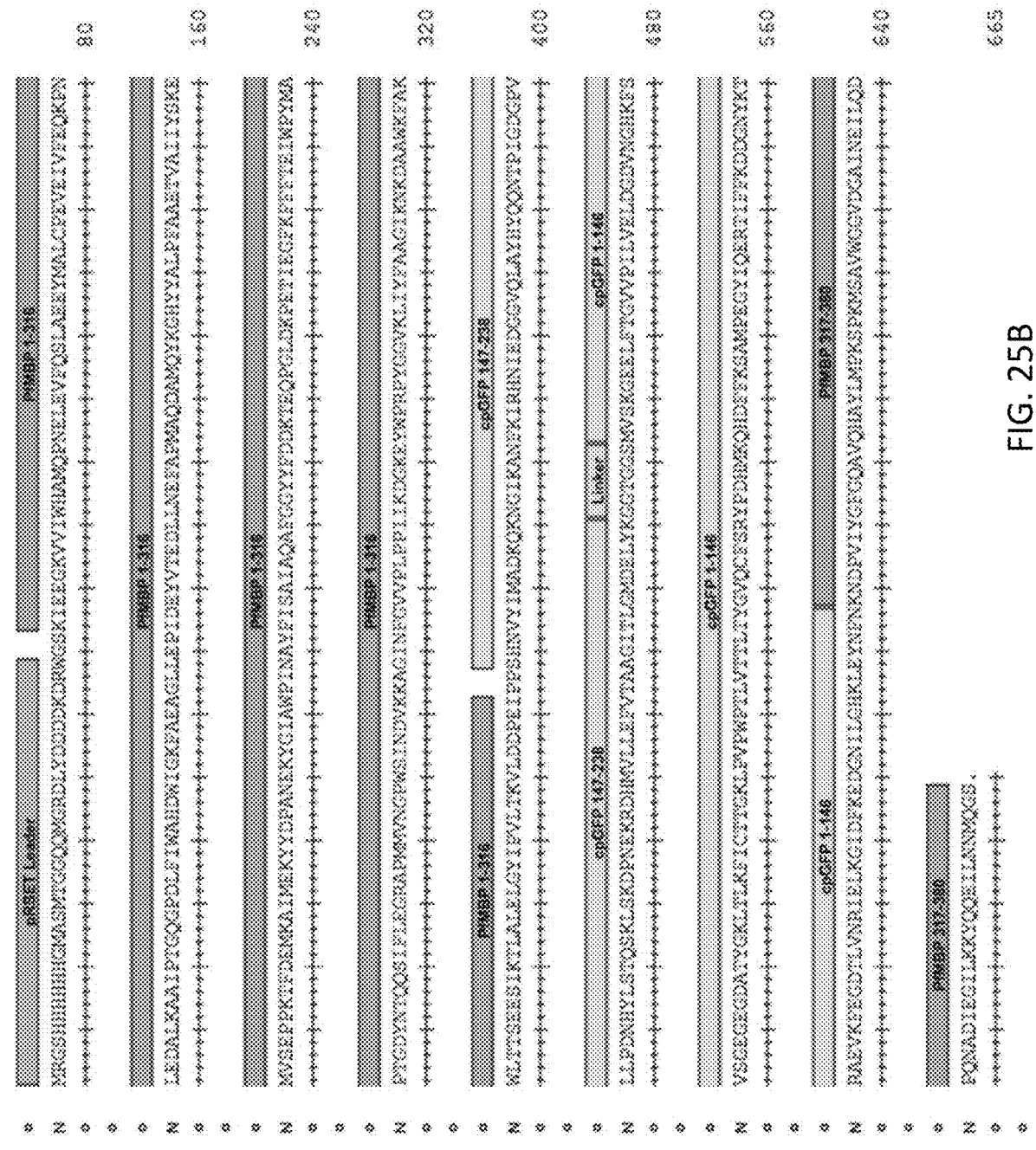

pRSET Leader
PhiBP 1-316
PhiBP 1-316
PhiBP 1-316
PhiBP 1-316
PhiBP 1-316
spGFP 147-238
spGFP 1-146
Linker
spGFP 147-238
spGFP 1-146
spGFP 1-146
PhiBP 317-380
PhiBP 317-380
80
160
240
320
400
480
560
640
665

FIG. 25B

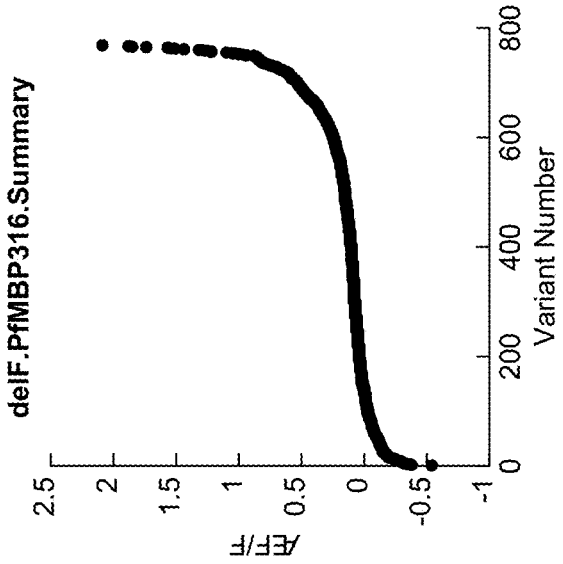


FIG. 26B

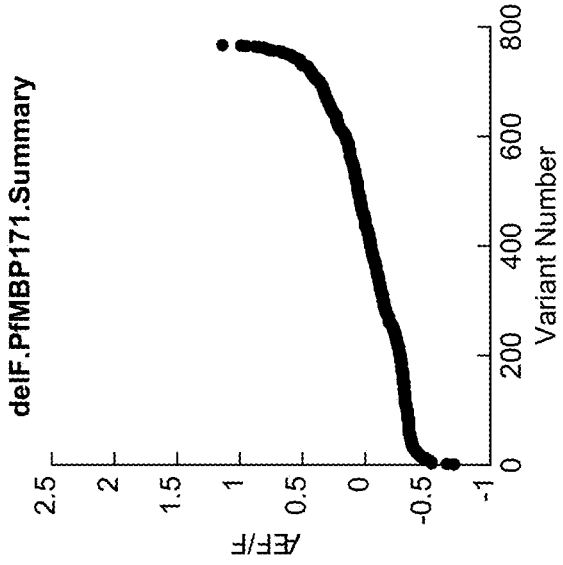


FIG. 26A

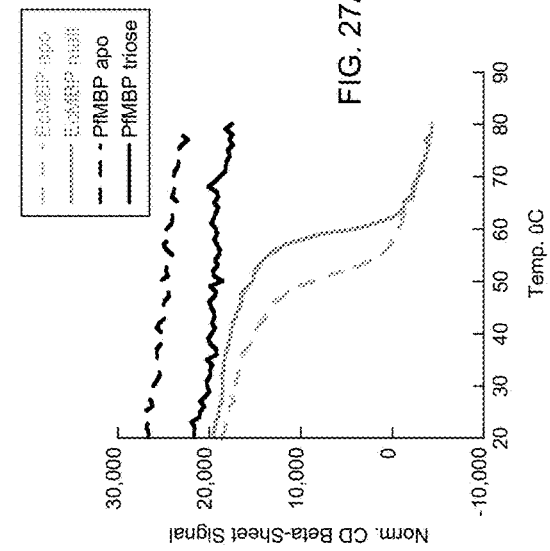


FIG. 27A

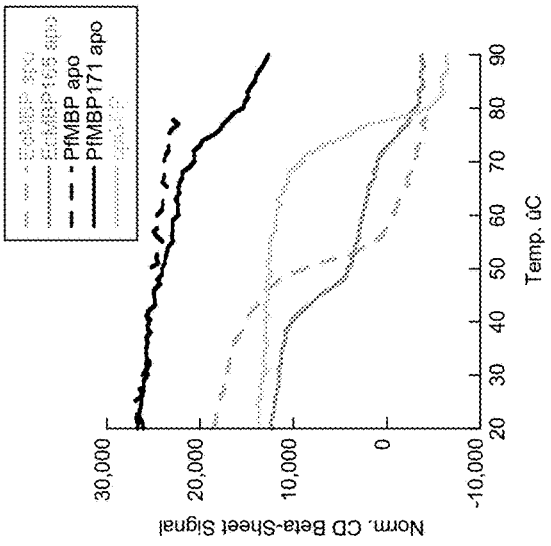


FIG. 27B

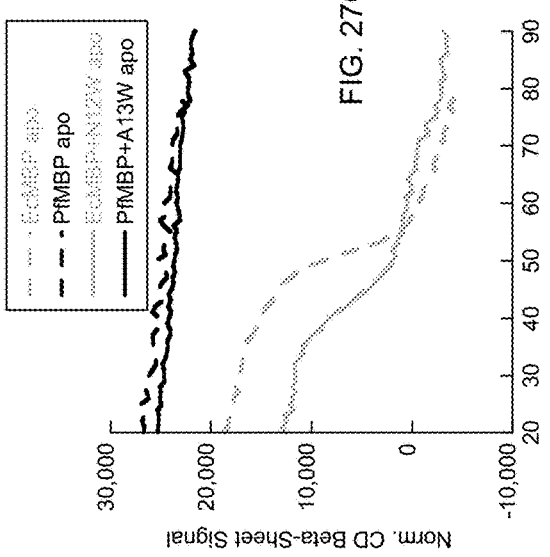


FIG. 27C

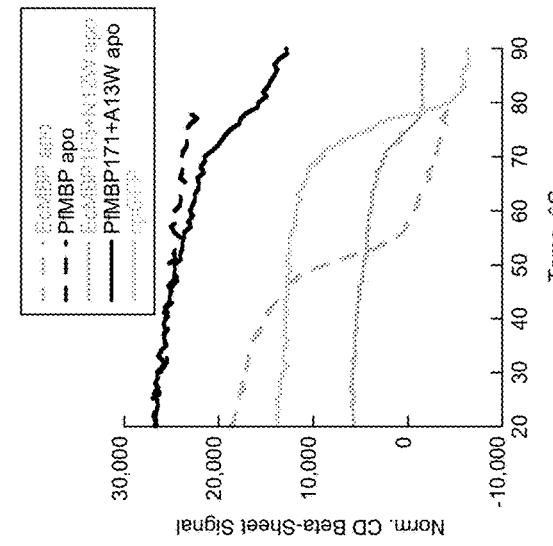


FIG. 27D

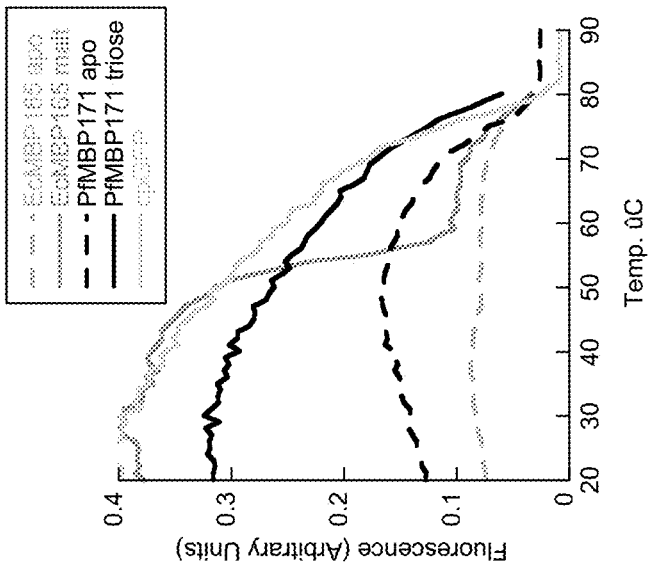


FIG. 28A

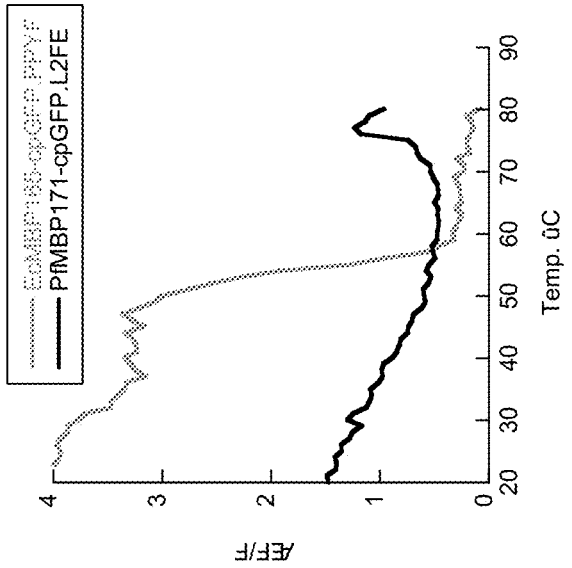


FIG. 28B

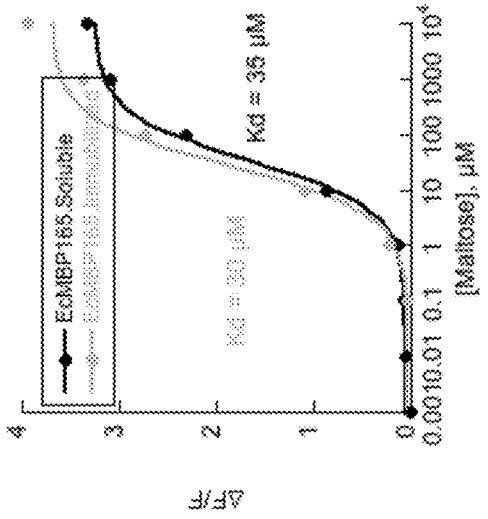


FIG. 28E

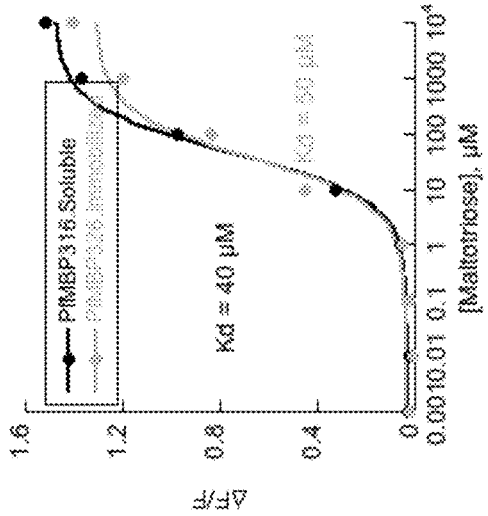


FIG. 28D

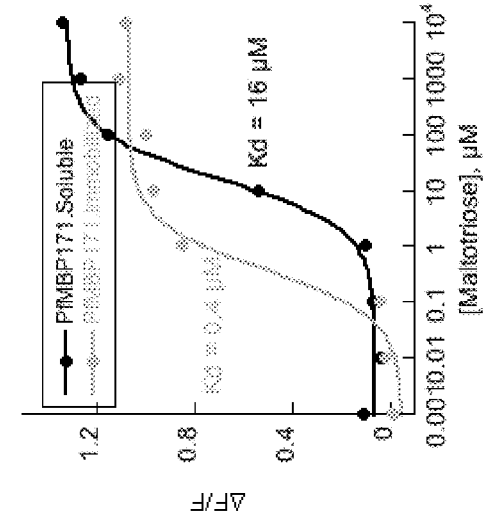


FIG. 28C

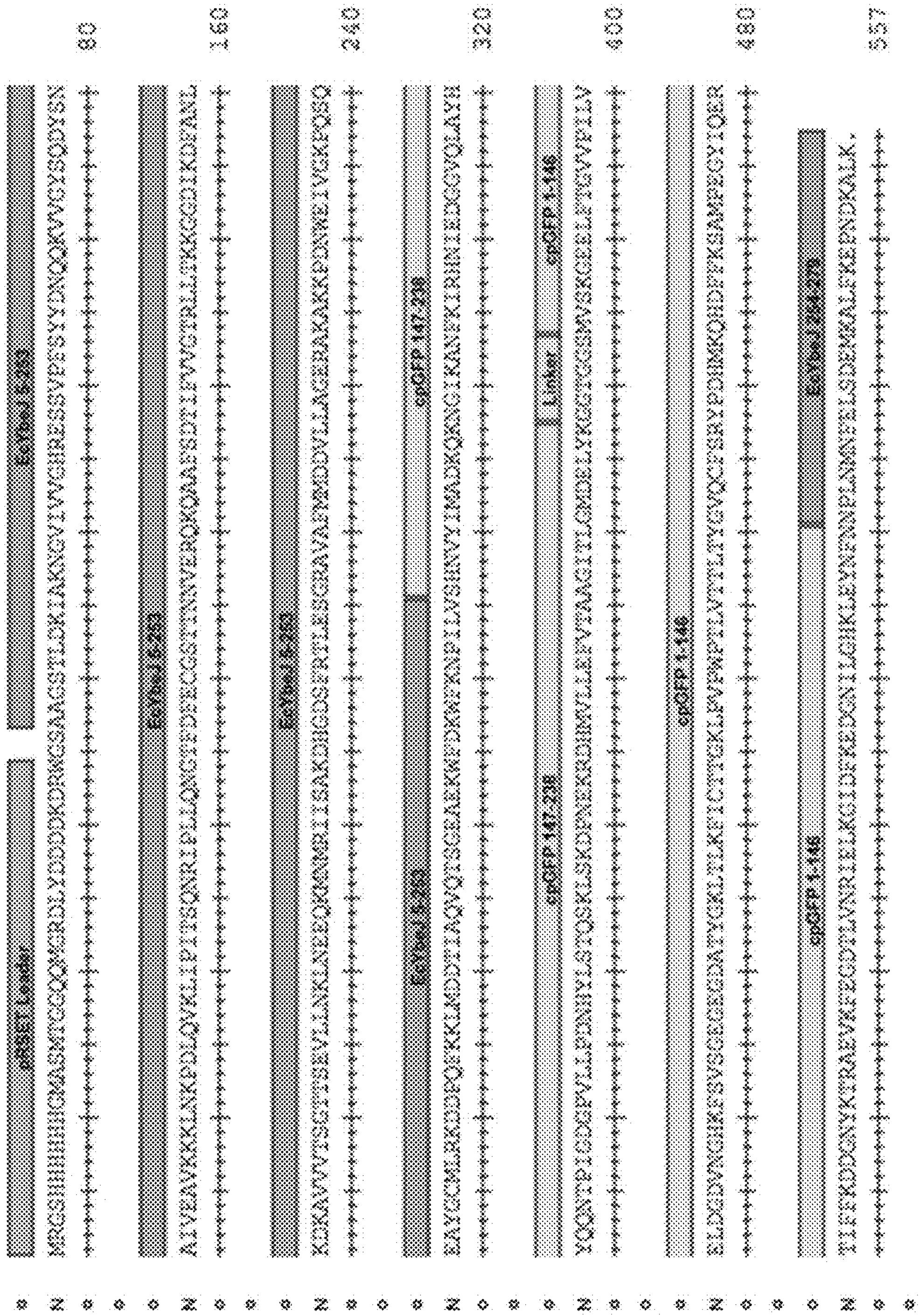

pRSET Leader
EcYbeJ 5-253
cpGFP 147-238
Linker
cpGFP 1-146
EcYbeJ 254-278
80
160
240
320
400
480
557

FIG. 29A

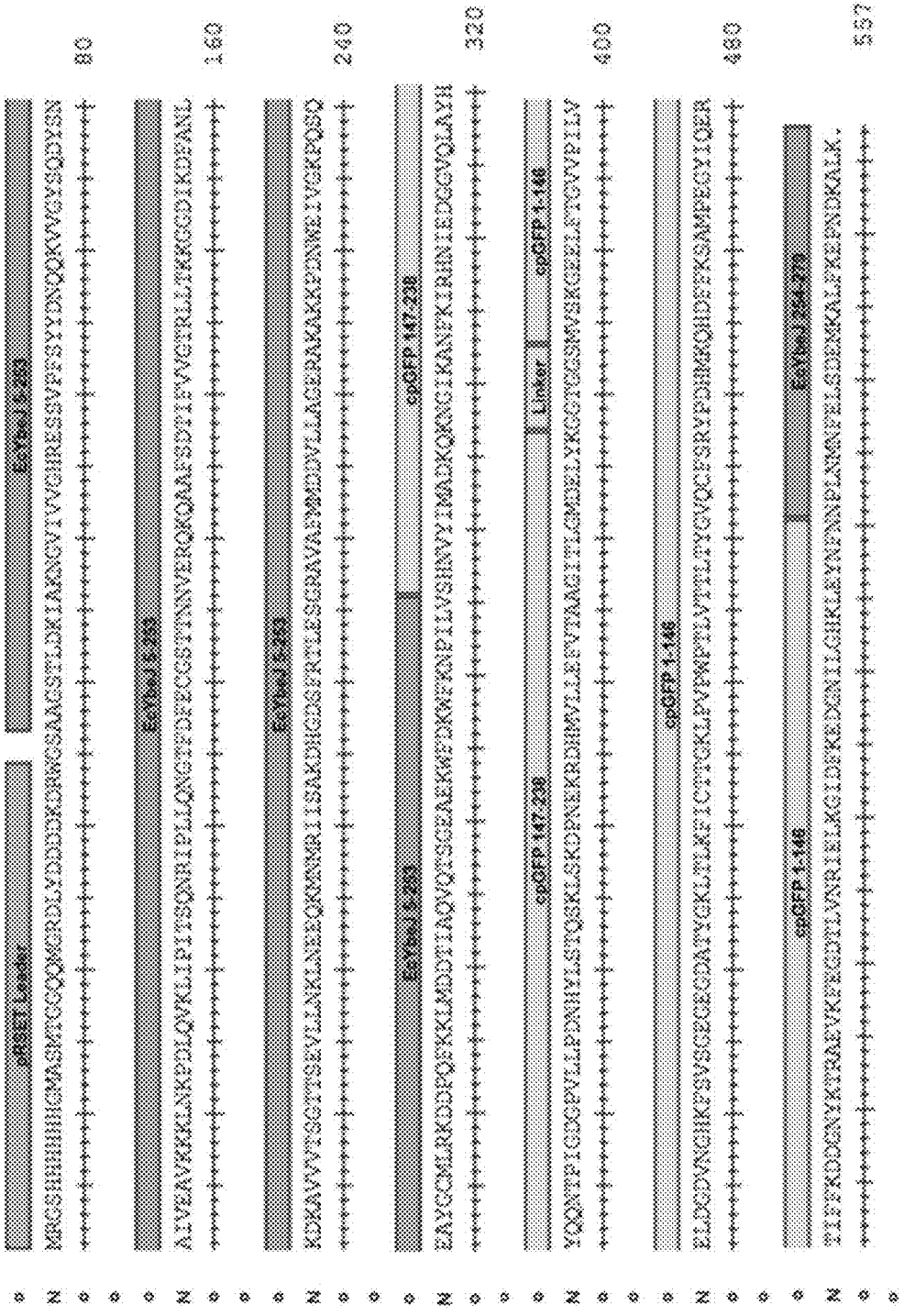

pBBE1 Leader
EcTad 1-283
80
EcTad 1-283
160
EcTad 1-283
240
EcTad 1-283
sfGFP 147-238
320
sfGFP 147-238
Linker
sfGFP 1-146
400
sfGFP 1-146
480
sfGFP 1-146
EcTad 284-479
557

FIG. 29B

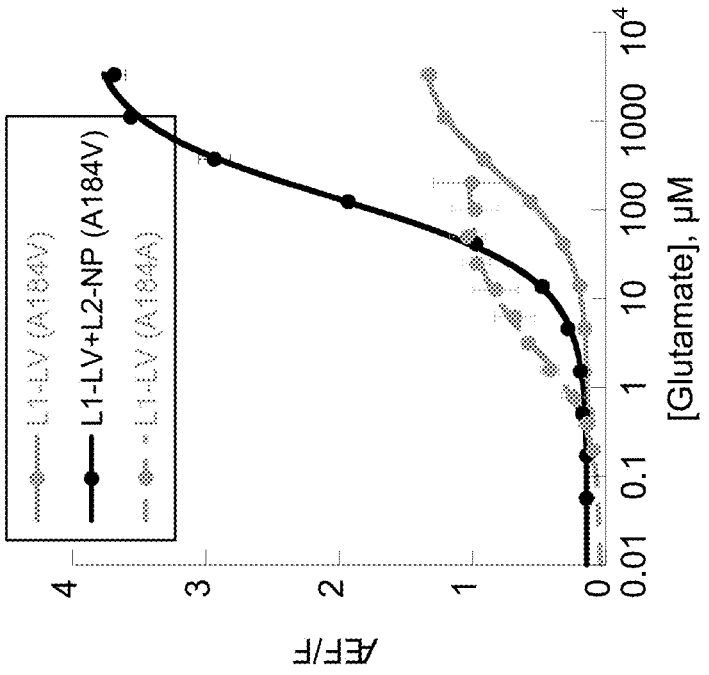

L1-LV (A184V)
L1-LV+L2-NP (A184V)
L1-LV (A184A)
ΔF/F
0
1
2
3
4
0.01
0.1
1
10
100
1000
10^4
[Glutamate], μM

FIG. 30

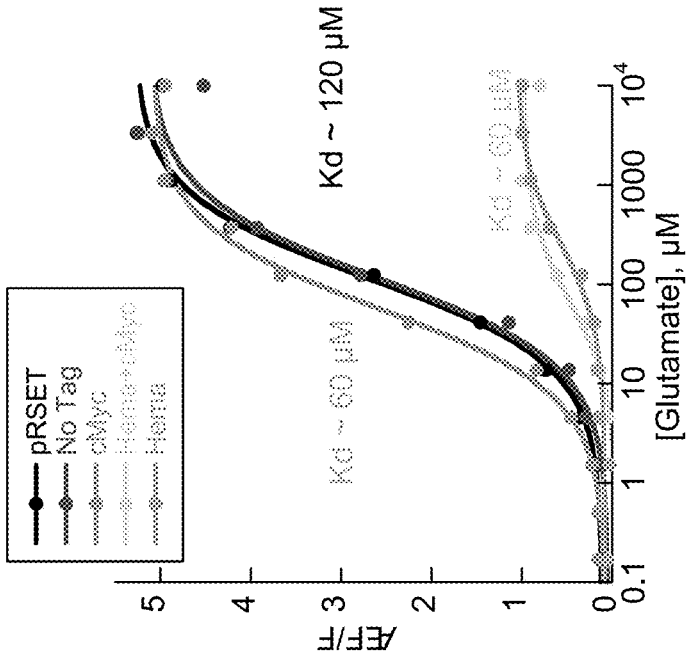

pRSET
No Tag
cMyc
Human-ubiq
Homa
ΔEF/F
0
1
2
3
4
5
0.1
1
10
100
1000
10^4
[Glutamate], µM
Kd ~ 120 µM
Kd ~ 60 µM
Kd ~ 60 µM

FIG. 31

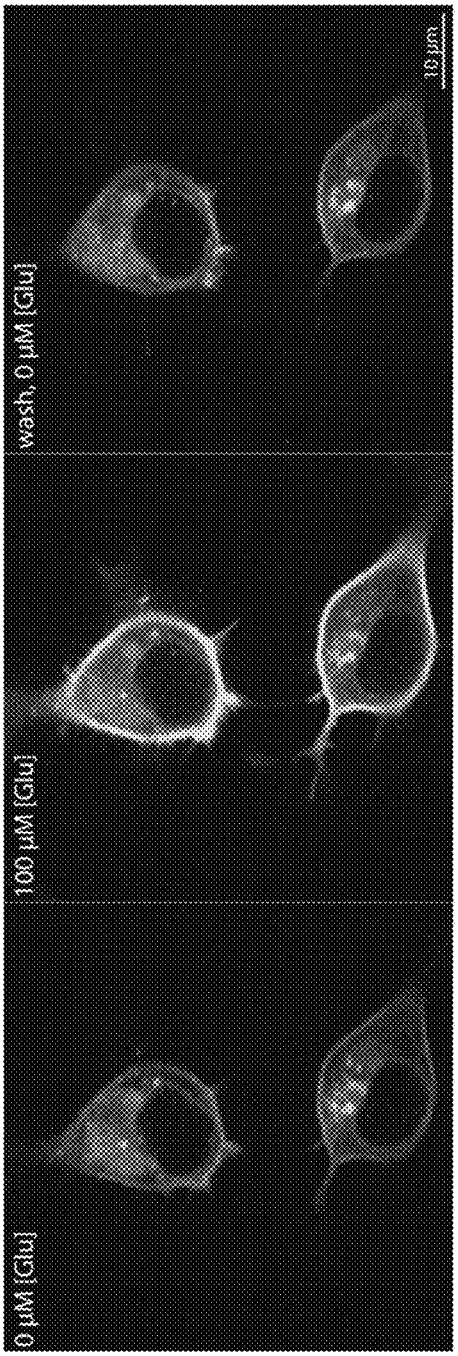


FIG. 32A

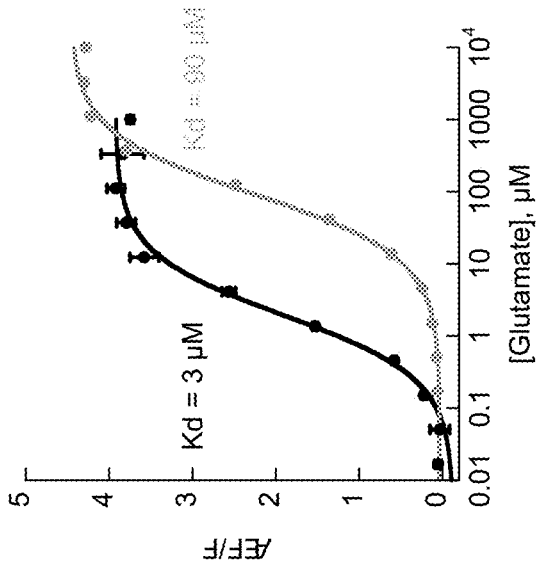


FIG. 32B

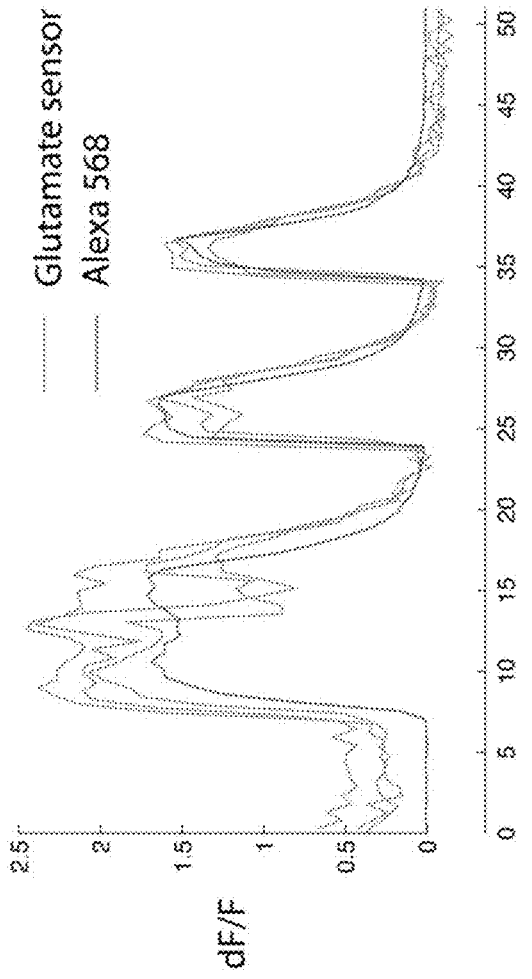

Glutamate sensor
Alexa 568
dF/F
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
40
45
50
Time (s)

FIG. 33

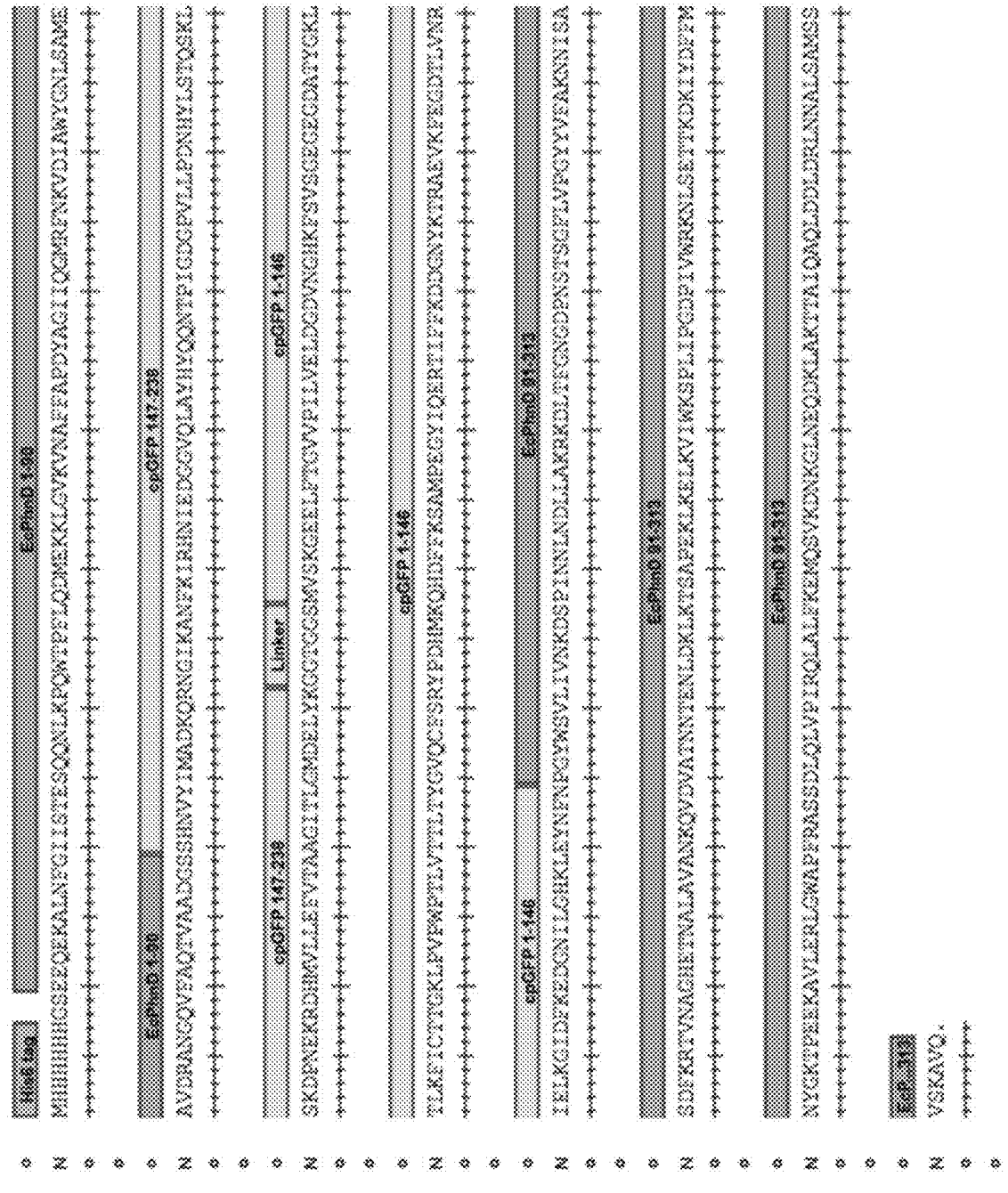

His tag
EcPhoD 1-98
80
EcPhoD 1-98
spGFP 147-238
160
spGFP 147-238
Linker
spGFP 1-146
240
spGFP 1-146
320
spGFP 1-146
EcPhoD 81-313
400
EcPhoD 81-313
480
EcPhoD 81-313
560
EcPhoD 81-313
567

FIG. 34A

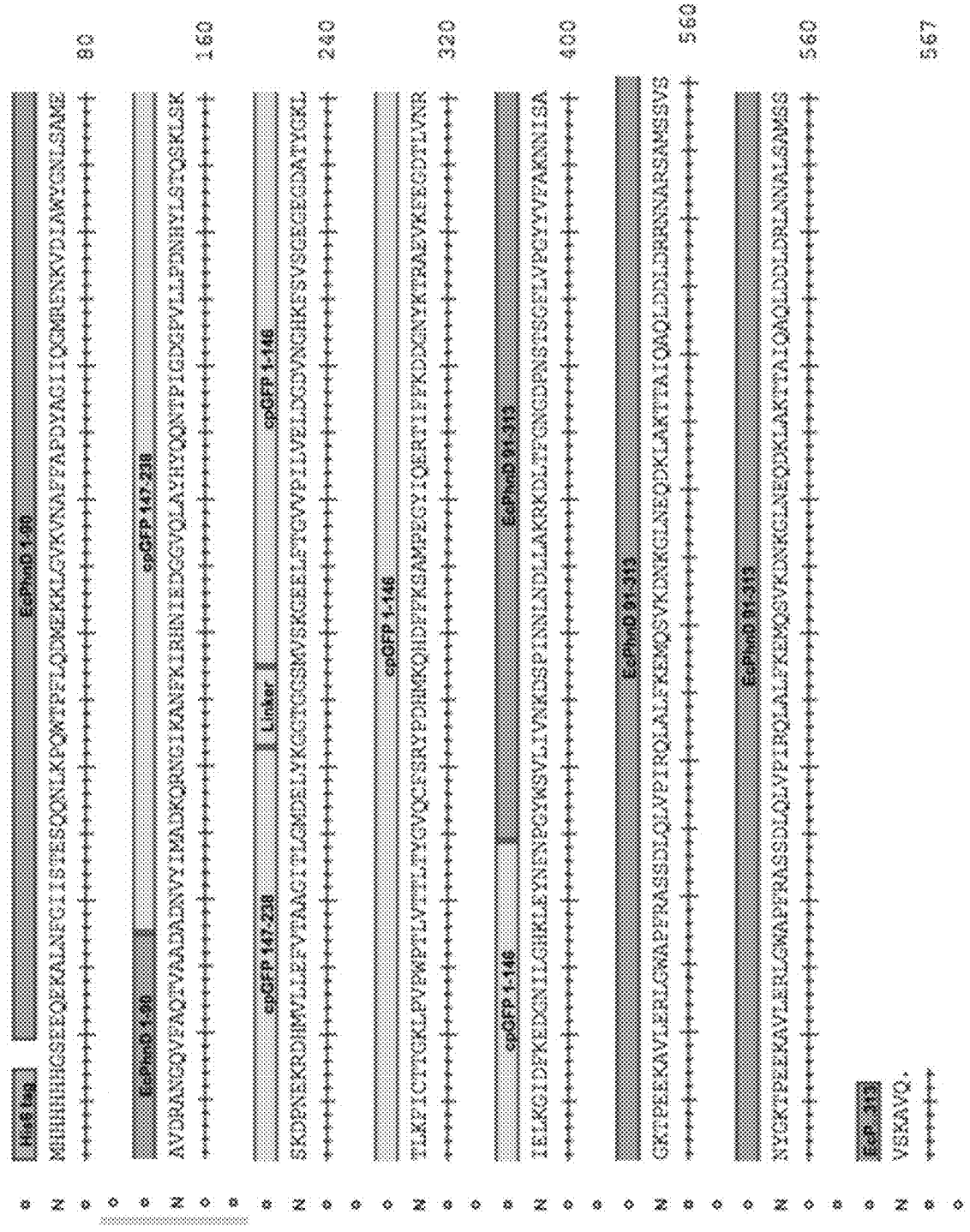

His6 tag
EcPhoD 1-98
80
EcPhoD 1-98
spGFP 147-238
160
spGFP 147-238
Linker
spGFP 1-146
240
spGFP 1-146
320
spGFP 1-146
EcPhoD 81-313
400
EcPhoD 81-313
560
EcPhoD 81-313
560
EcPhoD 81-313
567

FIG. 34B

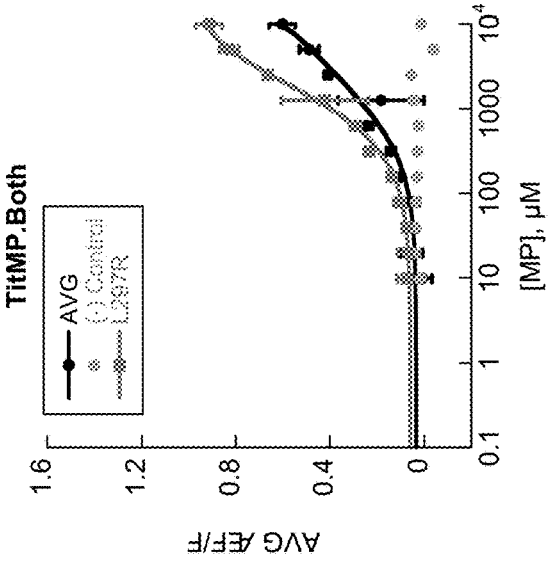


FIG. 35C

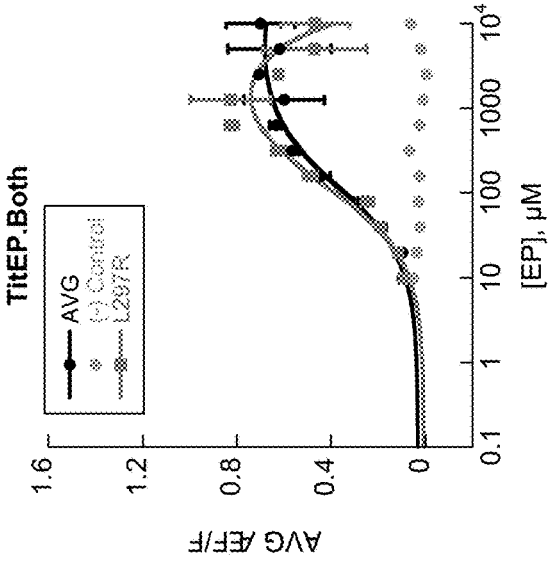


FIG. 35B

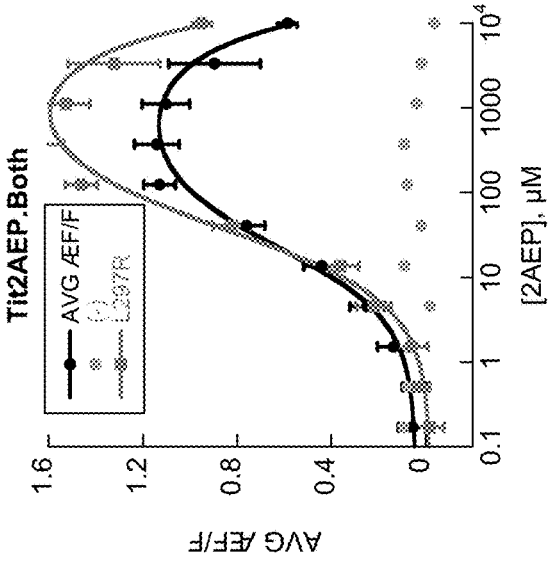


FIG. 35A

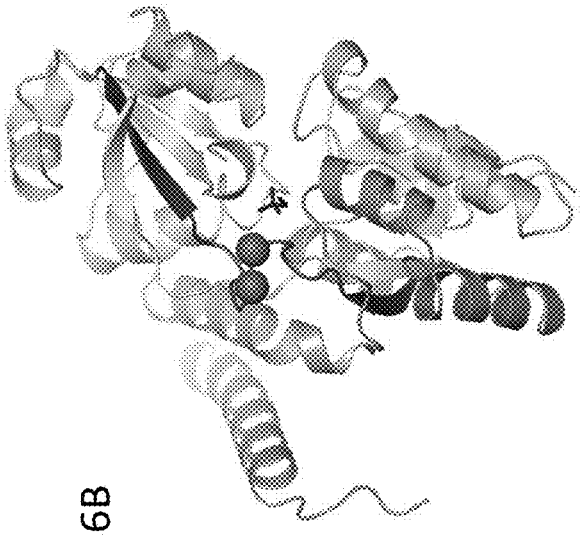


FIG. 36B

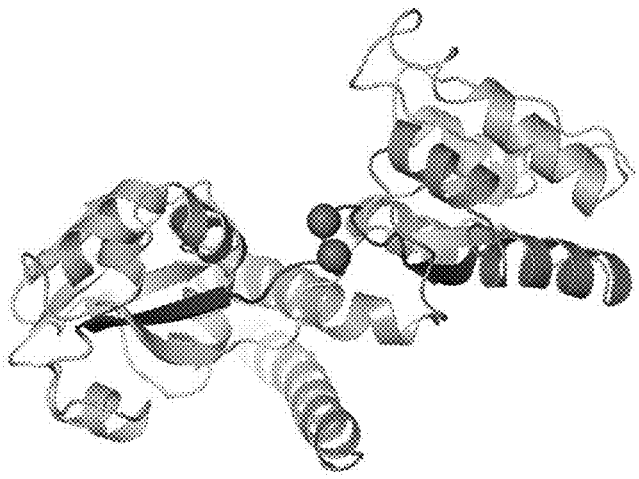


FIG. 36A

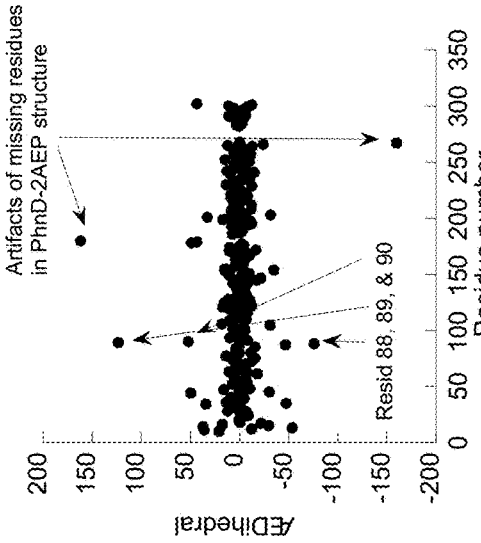


FIG. 36C

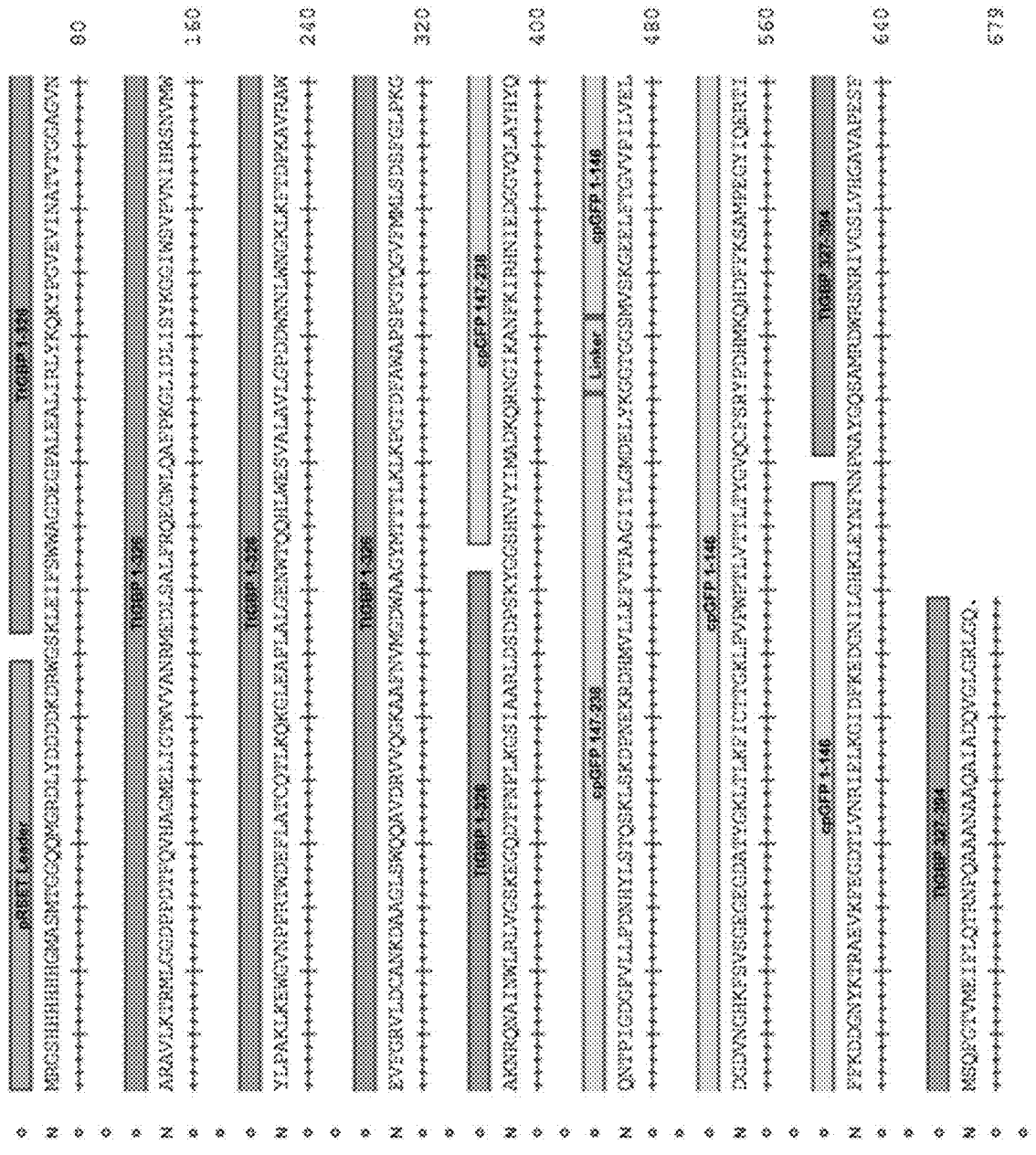

pBAD Leader
TolB 1-328
80
TolB 1-326
160
TolB 1-326
240
TolB 1-326
320
TolB 1-326
400
TolB 1-326
sfGFP 147-238
sfGFP 1-146
Linker
480
sfGFP 1-146
560
sfGFP 1-146
TolB 327-394
640
TolB 327-394
678

FIG. 37A

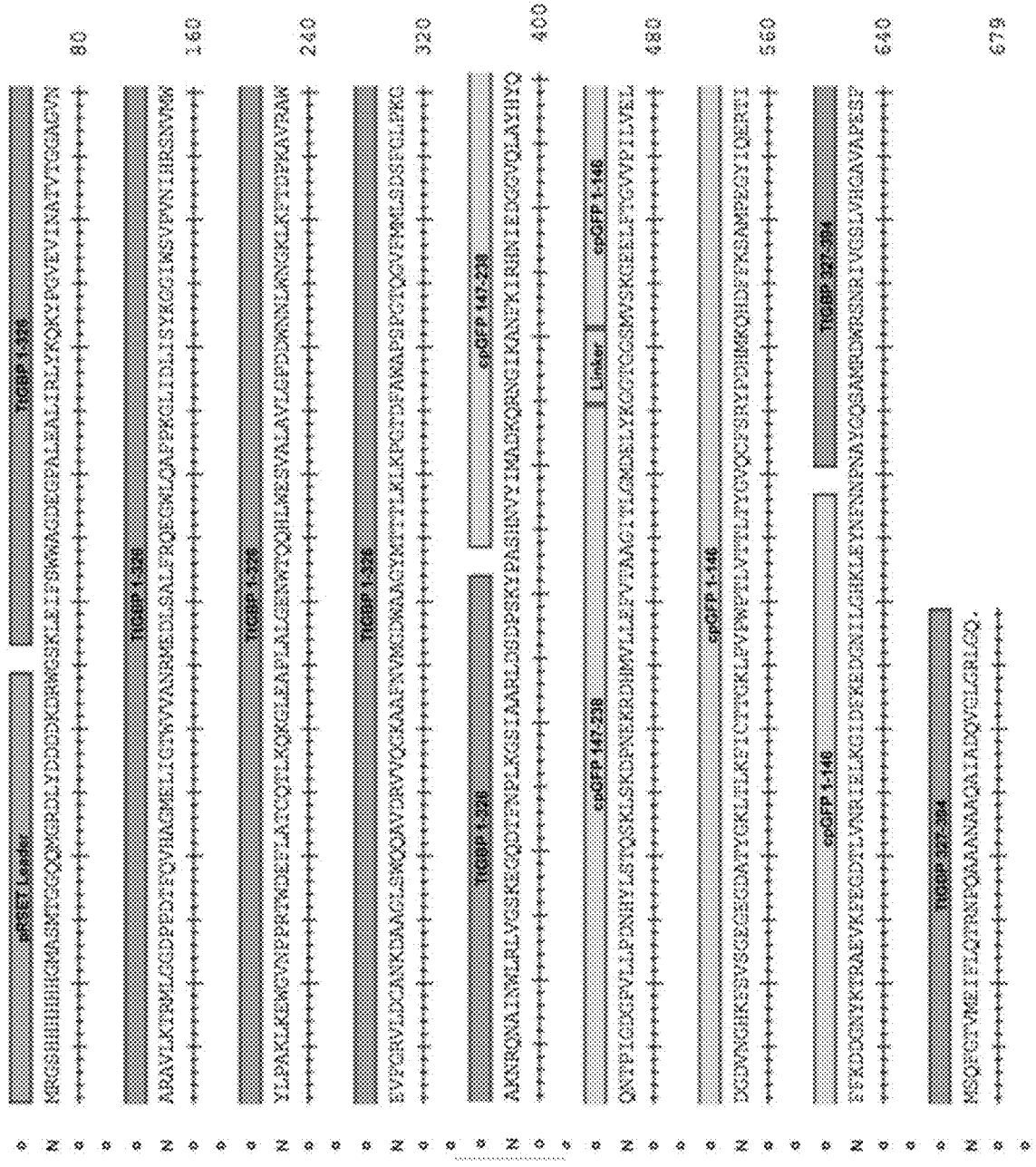

pRSET Leader
TGBP L328
80
TGBP L328
160
TGBP L328
240
TGBP L328
320
TGBP L328
sfGFP 147-238
400
sfGFP 147-238
Linker
sfGFP 1-146
480
sfGFP 1-146
560
sfGFP 1-146
TGBP 227-328
640
TGBP 227-328
675

FIG. 37B

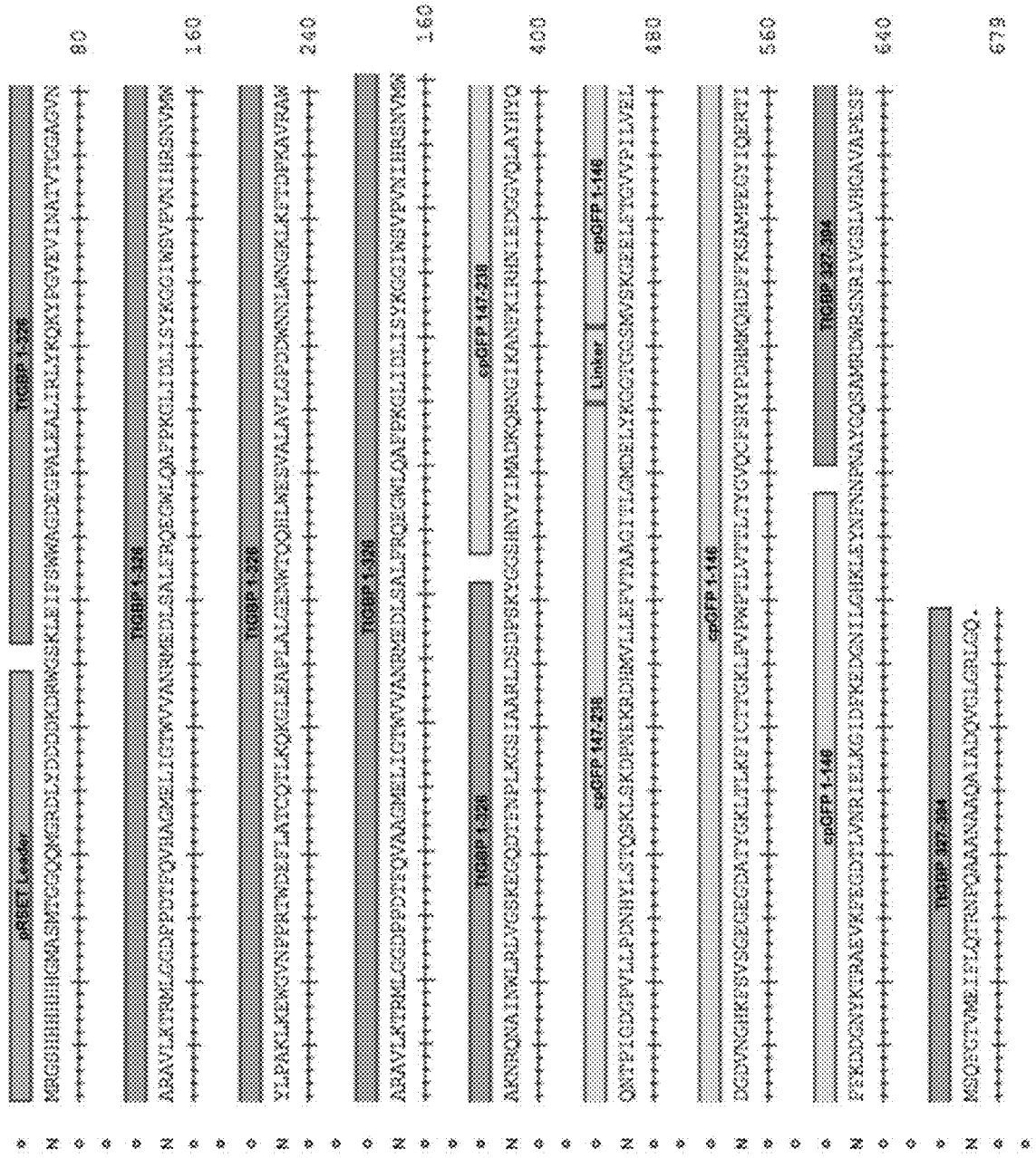

pRSET Leader
TGBP 1-328
TGBP 1-328
TGBP 1-328
TGBP 1-328
spGFP 147-238
spGFP 1-146
Linker
spGFP 147-238
spGFP 1-146
spGFP 1-146
TGBP 147-328
TGBP 147-328
80
160
240
160
400
480
560
640
678

FIG. 37C

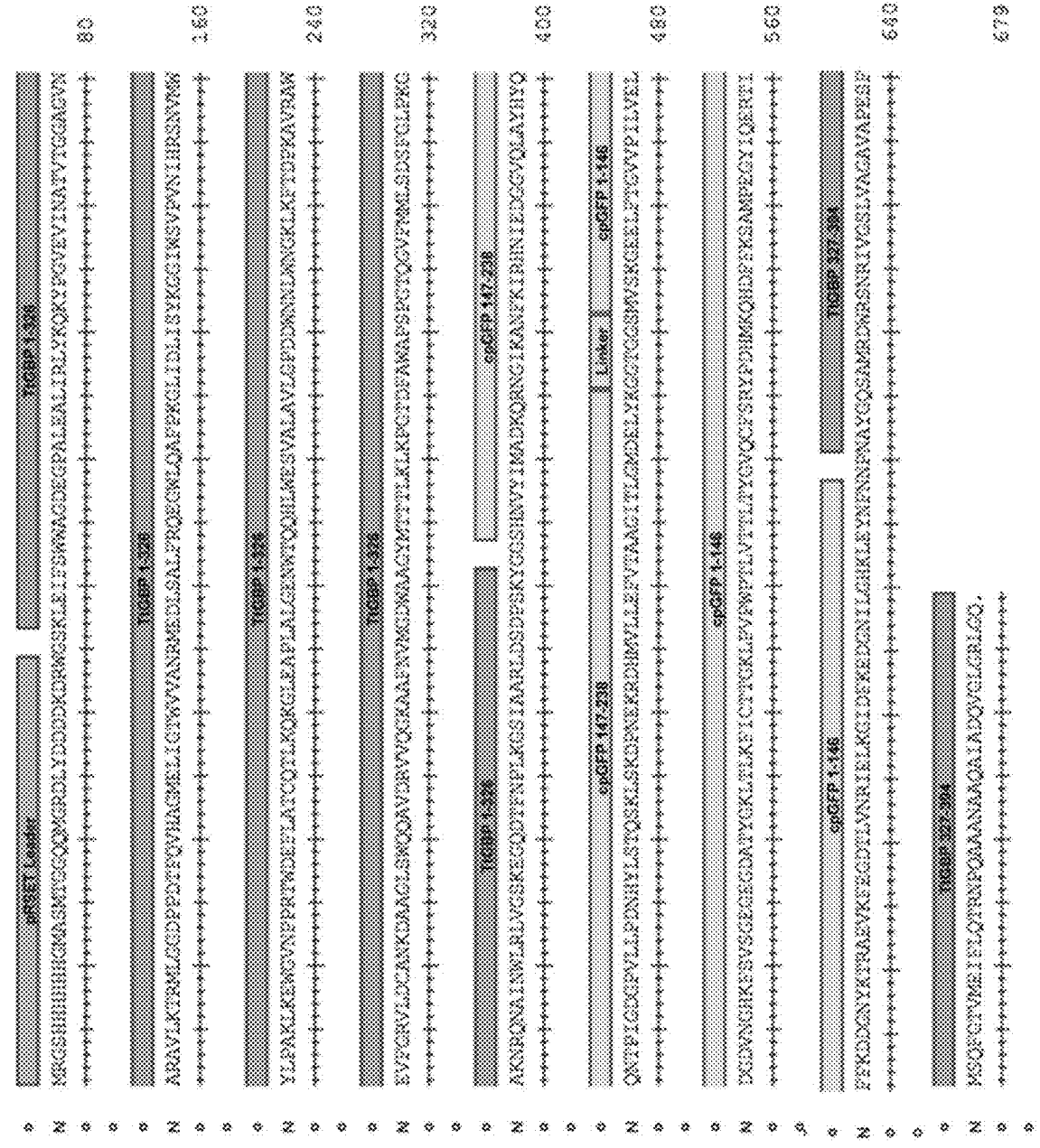

pPSET Leader
TcBIP 1-336
80
TcBIP 1-336
160
TcBIP 1-336
240
TcBIP 1-336
320
TcBIP 1-336
spGFP 167-238
spGFP 1-146
Linker
400
spGFP 167-238
spGFP 1-146
480
spGFP 1-146
560
spGFP 1-146
TcBIP 337-384
640
TcBIP 337-384
679

FIG. 37D

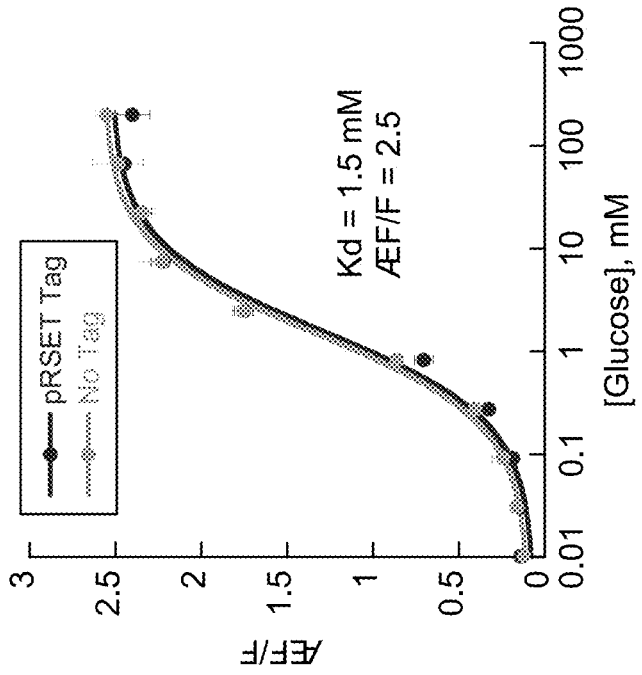

pRSET Tag
No Tag
Kd = 1.5 mM
ÆF/F = 2.5
ÆF/F
[Glucose], mM
0
0.5
1
1.5
2
2.5
3
0.01
0.1
1
10
100
1000

FIG. 38

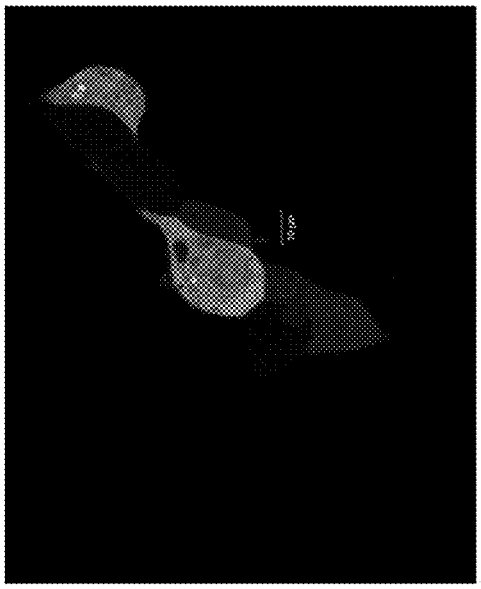


FIG. 39

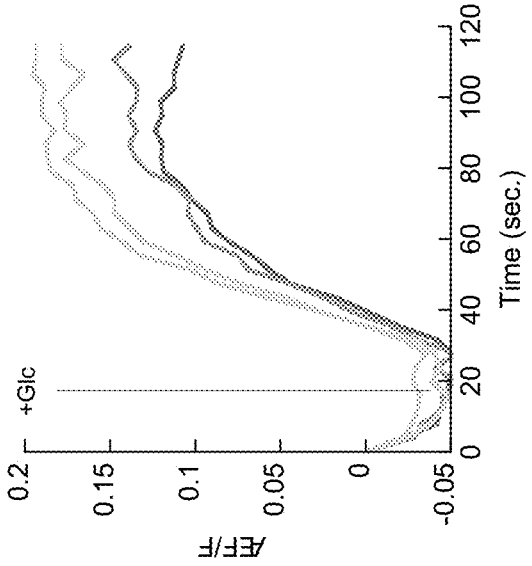


FIG. 40B

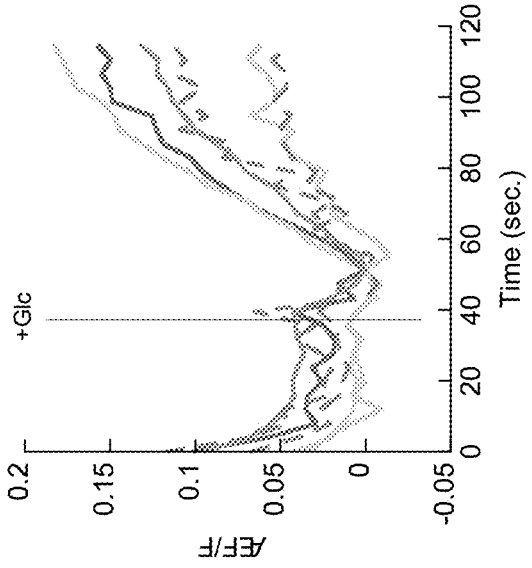


FIG. 40A

MKIKTGARIL ALSALTTMMF SASALAKIEE GKLVIWINGD
KGYNGLAEVG KKFEEKDTGIK VTVEHPDKLE EKFPQVAATG
DGPDIIFWAH DRFGGYAQSG LLAEITPDKA FQDKLYPFTW
DAVRYNGKLI AYPIAVEALS LIYNKDLLPN PPKTWEEIPA
LDKELKAKGK SALMFNLQEP YFTWPLIAAD GGYAFKYENG
KYDIKDVGVD NAGAKAGLTF LVDLIKNKHM NADTDYSIAE
AAFNKGETAM TINGPWAWSN IDTSKVNYGV TVLPTFKGQP
SKPFVGVLSA GINAASPNKE LAKEFLENYL LTDEGLEAVN
KDKPLGAVAL KSYEEELAKD PRIAATMENA QKGEIMPNIP
QMSAFWYAVR TAVINAASGR QTVDEALKDA QTRITK

FIG. 41

MRRATYAFAL LAILVLGVVA SGCIGGGTTT
PTQTSPATQP TTTQTPTQTE TQAVECGSGK
VVIWHAMQPN ELEVFQSLAE EYMALCPEVE
IVFEQKPNLE DALKAAIPTG QGPDLFIWAH
DWIGKFAEAG LLEPIDEYVT EDLLNEFAPM
AQDAMQYKGH YYALPFAAET VAIIYNKEMV
SEPPKTFDEM KAIMEKYYDP ANEKYGIAWP
INAYFISAIA QAFGGYYFDD KTEQPGLDKP
ETIEGFKFFF TEIWPYMAPT GDYNTQQSIF
LEGRAPMMVN GPWSINDVKK AGINFGVVPL
PPIIKDGKEY WPRPYGGVKL IYFAAGIKNK
DAAWKFAKWL TTSEESIKTL ALELGYIPVL
TKVLDDPEIK NDPVIYGFGQ AVQHAYLMPK
SPKMSAVWGG VDGAINEILQ DPQNADIEGI
LKKYQQEILN NMQG

FIG. 42

MNAKIIASLA FTSMFSLSTL LNPAYAEEQE
KALNFGIIST ESQQNLKPQW TPFLQDMEKK
LGVKVNAFFA PDYAGIIQGM RFNKVDIAWY
GNLSAMEAVD RANGQVFAQT VAADGSPGYW
SVLIVNKDSP INNLNDLLAK RKDLTFGNGD
PNSTSGFLVP GYYVFAKNNI SASDFKRTVN
AGHETNALAV ANKQVDVATN NTENLDKLKT
SAPEKLKELK VIWKSPLIPG DPIVWRKNLS
ETTKDKIYDF FMNYGKTPEE KAVLERLGWA
PFRASSDLQL VPIRQLALFK EMQSVKDNKG
LNEQDKLAKT TAIQAQLDDL DRLNNALSAM
SSVSKAVQ

FIG. 43

MQLRKPATAI LALALSAGLA QADDAAPAAG
STLDKIAKNG VIVVGHRESS VPFSYYDNQQ
KVVGYSQDYS NAIVEAVKKK LNKPDLQVKL
IPITSQNRIP LLQNGTFDFE CGSTTNNVER
QKQAAFSDTI FVVGTRLLTK KGGDIKDFAN
LKDKAVVVTS GTTSEVLLNK LNEEQKMNMR
IISAKDHGDS FRTLESGRAV AFMMDDALLA
GERAKAKKPD NWEIVGKPQS QEAYGCMLRK
DDPQFKKLMD DTIAQVQTSG EAEKWFDKWF
KNPIPPKNLN MNFELSDEMK ALFKEPNDKA
LN

FIG. 44

MRKWLLAIGM VLGLSALAQG GKLEIFSWWA
GDEGPALEAL IRLYKQKYPG VEVINATVTG
GAGVNARAVL KTRMLGGDPP DTFQVHAGME
LIGTWVVANR MEDLSALFRQ EGWLQAFPKG
LIDLISYKGG IWSVPVNIHR SNVMWYLPAK
LKEWGVNPPR TWDEFLATCQ TLKQKGLEAP
LALGENWTQQ HLWESVALAV LGPDDWNNLW
NGKLKFTDPK AVRAWEVFGR VLDCANKDAA
GLSWQQAVDR VVQGKAAFNV MGDWAAGYMT
TTLKLKPGTD FAWAPSPGTQ GVFMMLSDSF
GLPKGAKNRQ NAINWLRLVG SKEGQDTFNP
LKGSIAARLD SDPSKYNAYG QSAMRDWRSN
RIVGSLVHGA VAPESFMSQF GTVMEIFLQT
RNPQAAANAA QAIADQVGLG RLGQ

FIG. 45

MIRTLSLKFM LAGAVCMATL TAGSAFAAEP
ESCGTVRFSD VGWTDITATT ATATTILEAL
GYETDVKVLS VPVTYTSLKN KDIDVFLGNW
MPTMEADIAP YREDKSVETV RENLAGAKYT
LATNAKGAEL GIKDFKDIAA HKDELDGKIY
GIEPGNDGNR LIIDMVEKGT FDLKGFEVVE
SSEQGMLAQV ARAEKSGDPI VFLGWEPHPM
NANFKLTYLS GGDDVFGPNY GGATVHTNVR
AGYTTECPNV GKLLQNLSFS LQMENEIMGK
ILNDGEDPEK AAAAWLKDNP QSIEPWLSGV
ATKDGGDGLA AVKAALGL

FIG. 46

MGGGRSTETS SSSGGDGGAT KKKVVVGTDA
AFAPFEYMQK GKIVGFDVDL LDAVMKAAGL
DYELKNIGWD PLFASLQSKE VDMGISGITI
TDERKQSYDF SDPYFEATQV ILVKQGSPVK
NALDLKGKTI GVQNATTGQE AAEKLFGKGP
HIKKFETTVV AIMELLNGGV DAVITDNAVA
NEYVKNNPNK KLQVIEDPKN FASEYYGMIF
PKNSELKAKV DEALKNVINS GKYTEIYKKW
FGKEPKLDRL

FIG. 47

MKKSLLSAVA LTAMVAFGGS AWADVVIAVG
APLTGPNAAF GAQIQKGAEQ AAKDINAAGG
INGEQIKIVL GDDVSDPKQG ISVANKFVAD
GVKFVVGHFN SGVSIPASEV YAENGILEIT
PAATNPVFTE RGLWNTFRTC GRDDQQGGIA
GKYLADHFKD AKVAIIHDKT PYGQGLADET
KKAANAAGVT EVMYEGVNVG DKDFSALISK
MKEAGVSIIY WGGLHTEAGL IIRQAADQGL
KAKLVSGDGI VSNELASIAG DAVEGTLNTF
GPDPTLRPEN KELVEKFKAA GFNPEAYTLY
SYAAMQAIAG AAKAAGSVEP EKVAEALKKG
SFPTALGEIS FDEKGDPKLP GYVMYEWKKG
PDGKFTYIQQ

FIG. 48

MNIKGKALLA GCIALAFSNM ALAEDIKVAV
VGAMSGPVAQ YGDQEFTGAE QAVADINAKG
GIKGNKLQIV KYDDACDPKQ AVAVANKVVN
DGIKYVIGHL CSSSTQPASD IYEDEGILMI
TPAATAPELT ARGYQLILRT TGLDSDQGPT
AAKYILEKVK PQRIAIVHDK QQYGEGLARA
VQDGLKKGNA NVVFFDGITA GEKDFSTLVA
RLKKENIDFV YYGGYHPEMG QILRQARAAG
LKTQFMGPEG VANVSLSNIA GESAEGLLVT
KPKNYDQVPA NKPIVDAIKA KKQDPSGAFV
WTTYAALQSL QAGLNQSDDP AEIAKYLKAN
SVDTVMGPLT WDEKGDLKGF EFGVFDWHAN
GTATDAK

FIG. 49

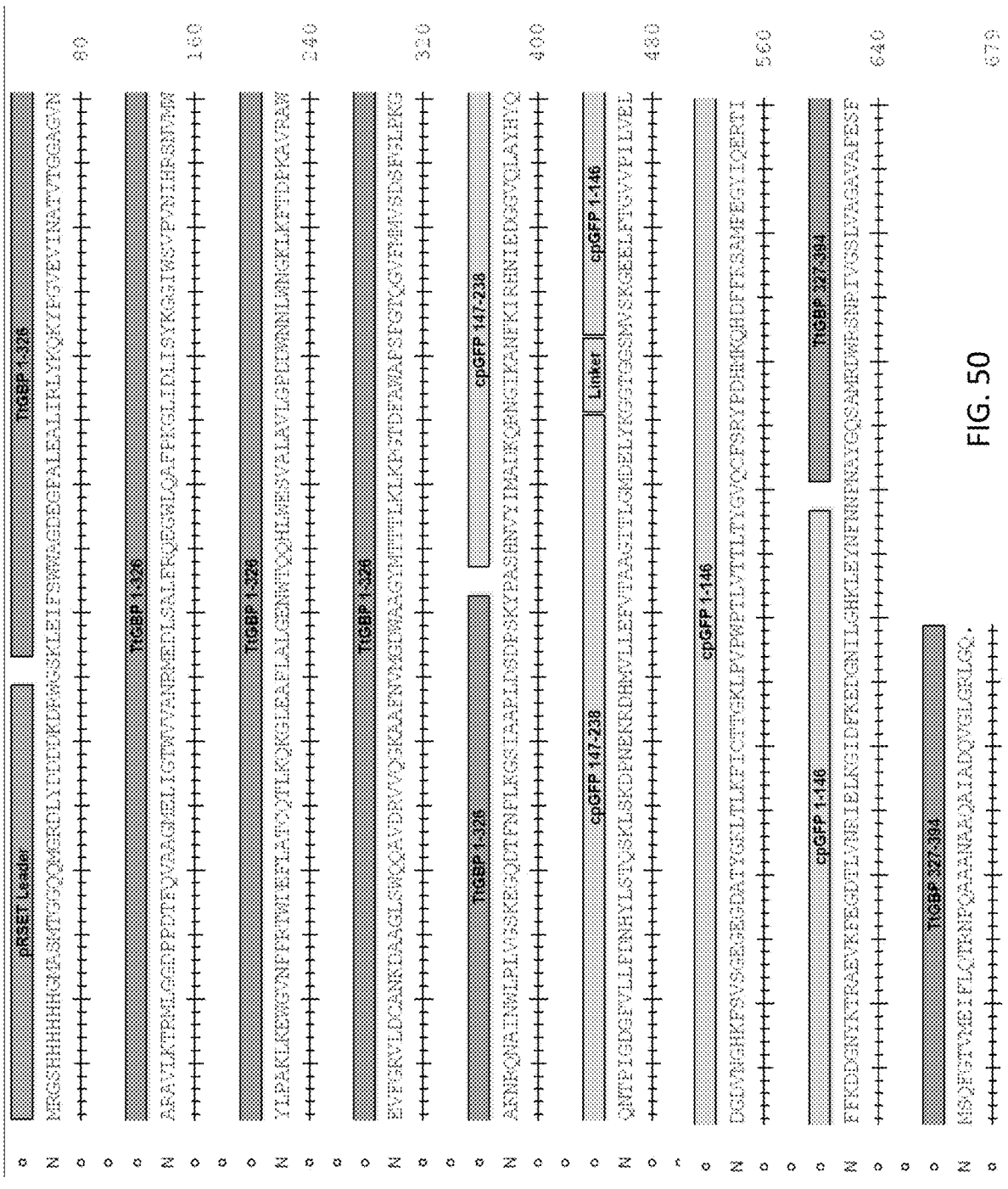

pRSET Leader
TtGBP 1-326
TtGBP 1-326
TtGBP 1-326
TtGBP 1-326
TtGBP 1-326
cpGFP 147-238
cpGFP 147-238
Linker
cpGFP 1-146
cpGFP 1-146
cpGFP 1-146
TtGBP 327-394
TtGBP 327-394

FIG. 50

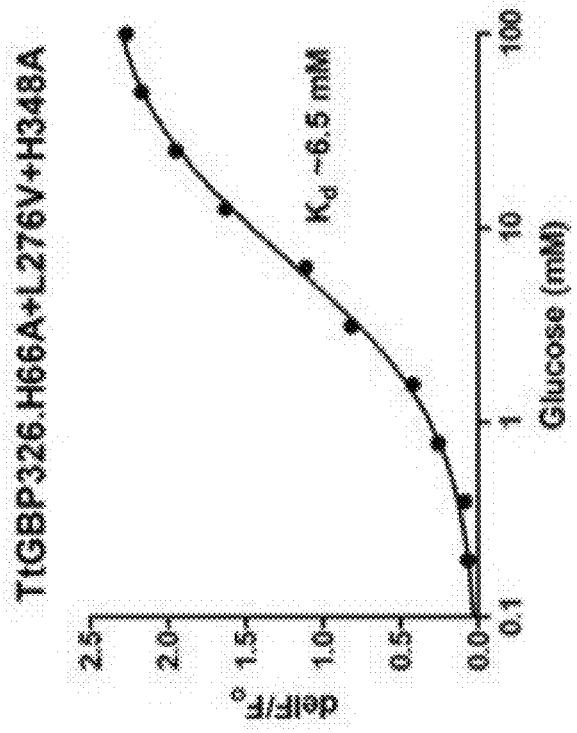

TtGBP326.H66A+L276V+H348A
K_d ~6.5 mM
dolF/F$_o$
2.5
2.0
1.5
1.0
0.5
0.0
0.1
1
10
100
Glucose (mM)

FIG. 51

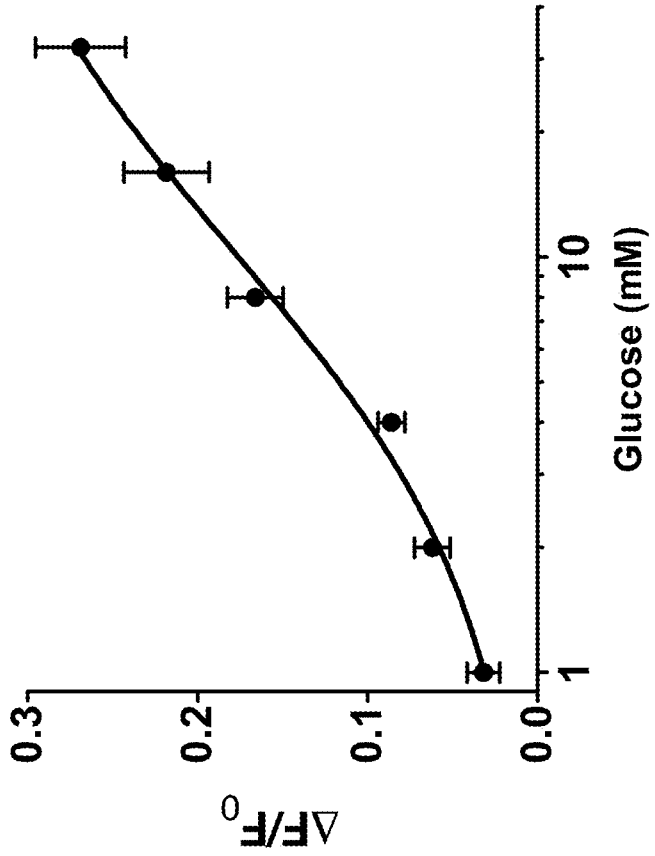


FIG. 52

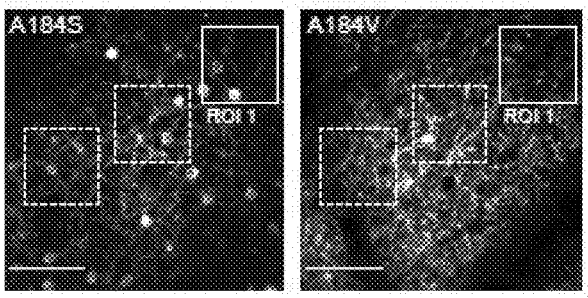


FIG. 53A

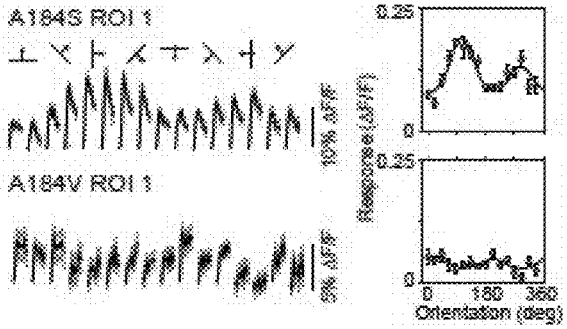


FIG. 53B

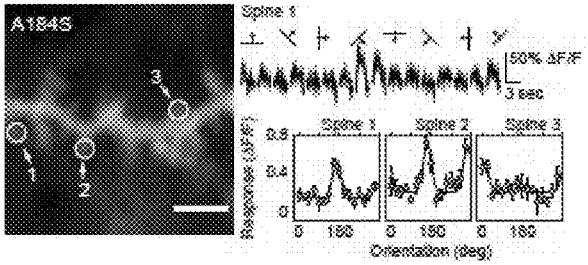


FIG. 53C

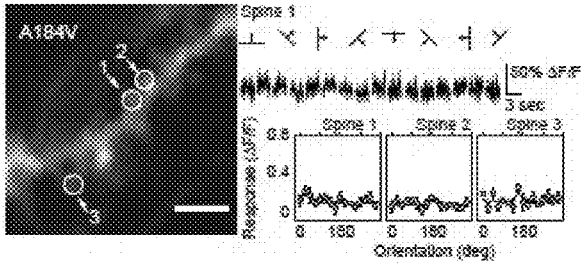


FIG. 53D

FIG. 54A

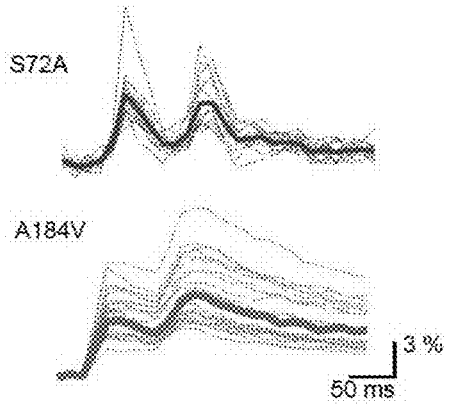


FIG. 54B

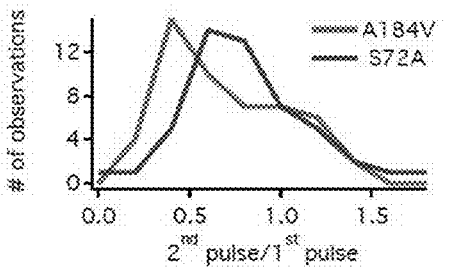


FIG. 54C

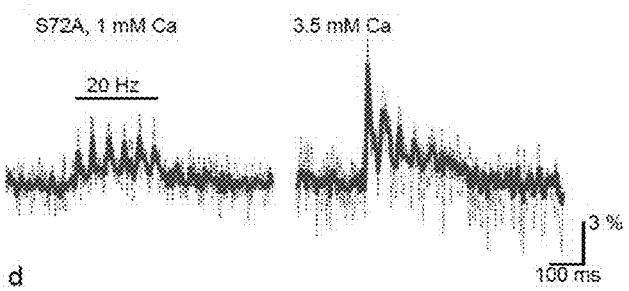
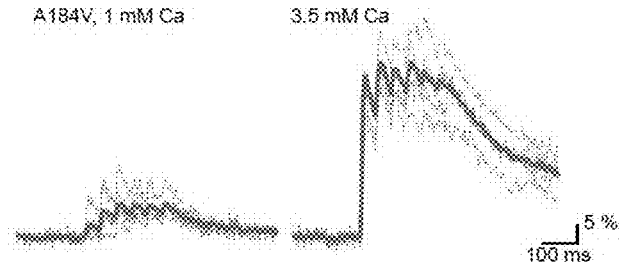


FIG. 54D



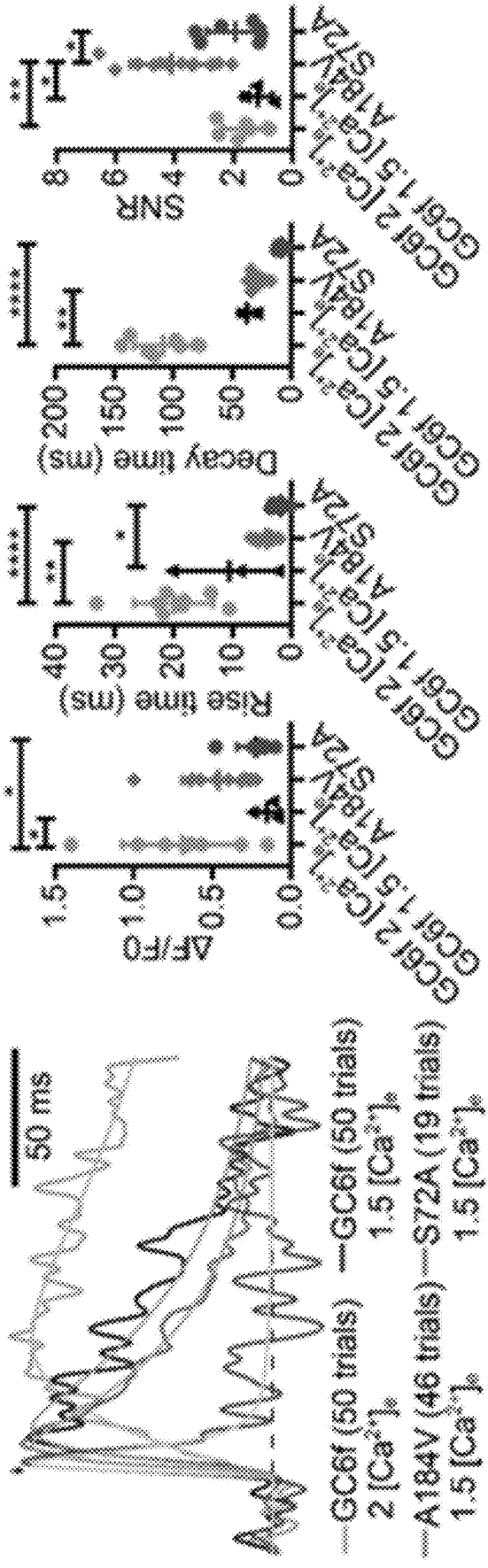

50 ms
—GC6f (50 trials)
2 [Ca2+]h
—GC6f (50 trials)
1.5 [Ca2+]h
—A184V (46 trials)
1.5 [Ca2+]h
—S72A (19 trials)
1.5 [Ca2+]h
ΔF/F0
Rise time (ms)
Decay time (ms)
SNR
GCaMP2
GCaMP1.5
GCaMP1.5 A184V
GCaMP1.5 S72A

FIG. 55A

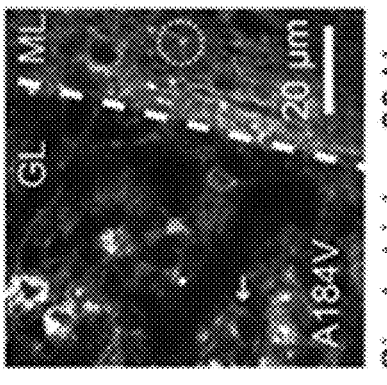
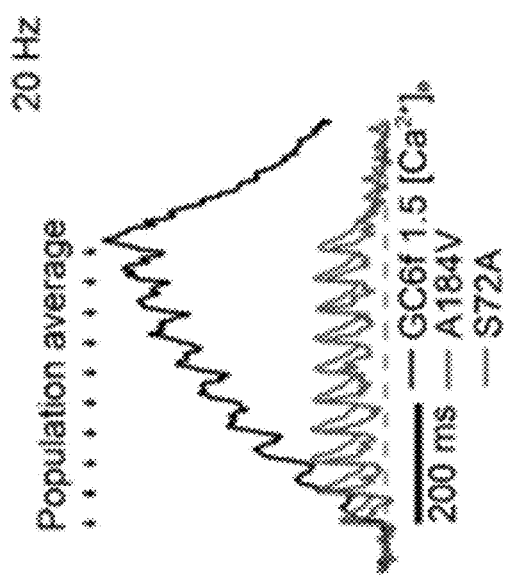


FIG. 55B



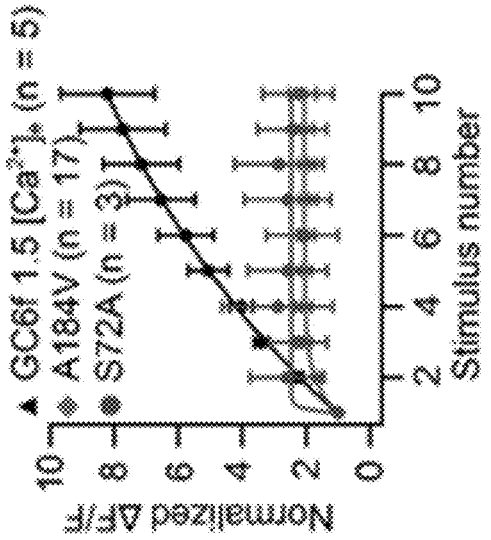
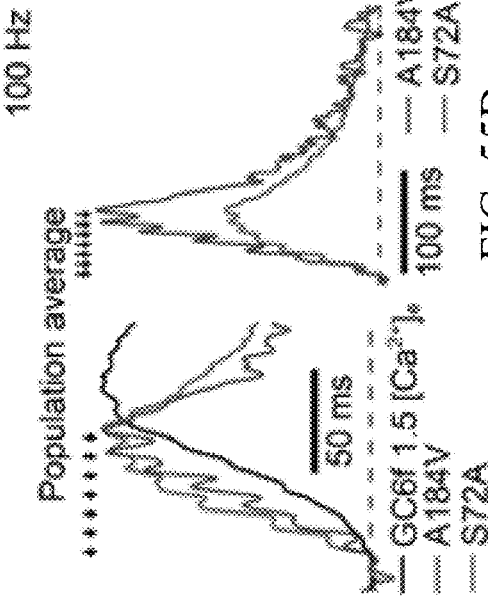


FIG. 55C



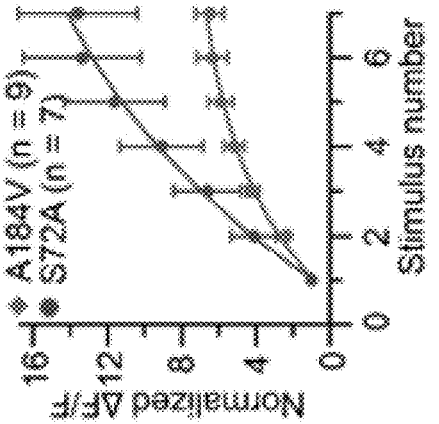


FIG. 55D

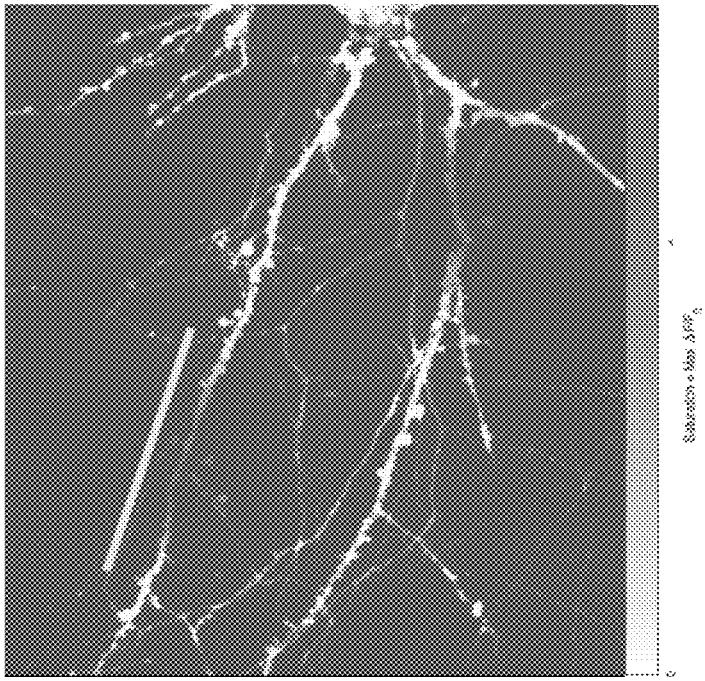


FIG. 56A

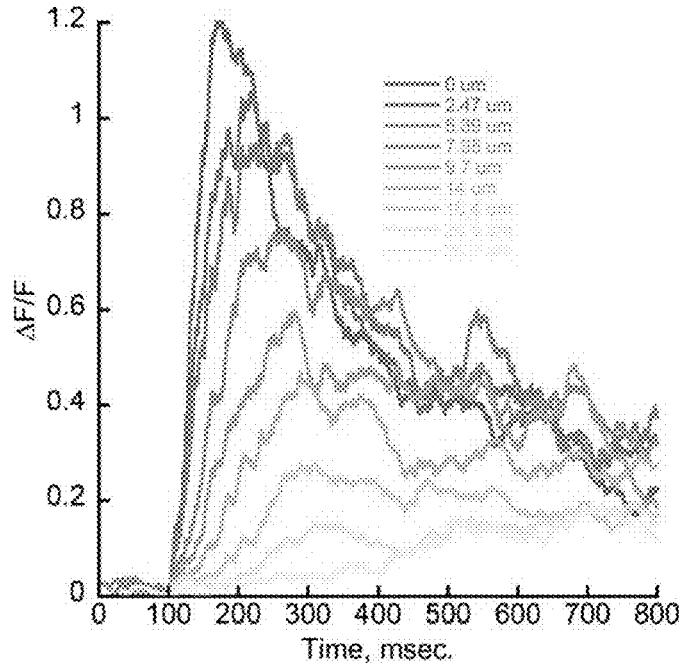


FIG. 56B

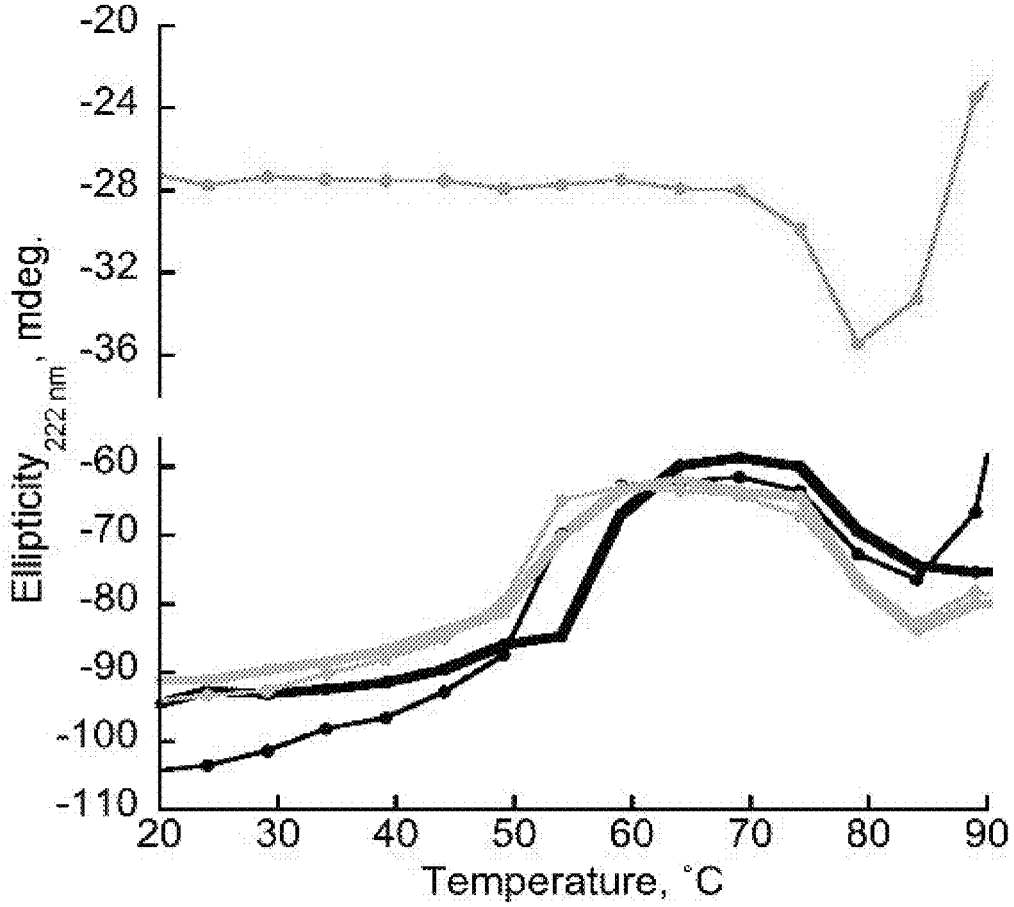

-20
-24
-28
-32
-36
-60
-70
-80
-90
-100
-110
Ellipticity222 nm, mdeg.
20
30
40
50
60
70
80
90
Temperature, ˚C

FIG. 57

FIG. 58A

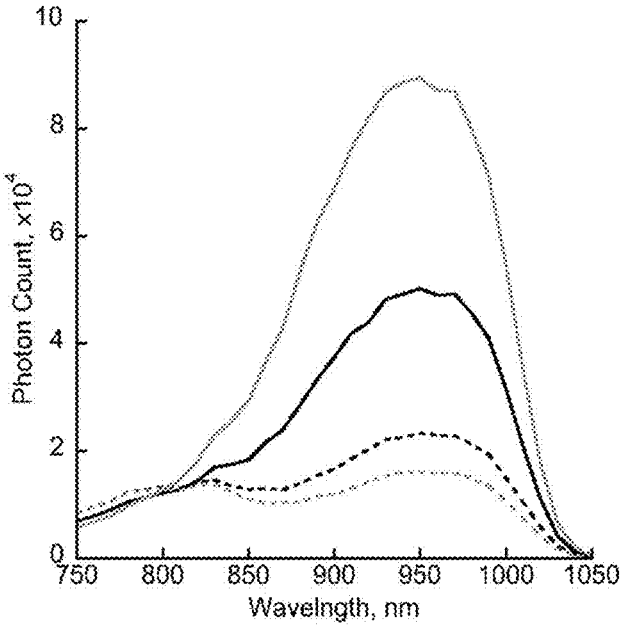


FIG. 58B

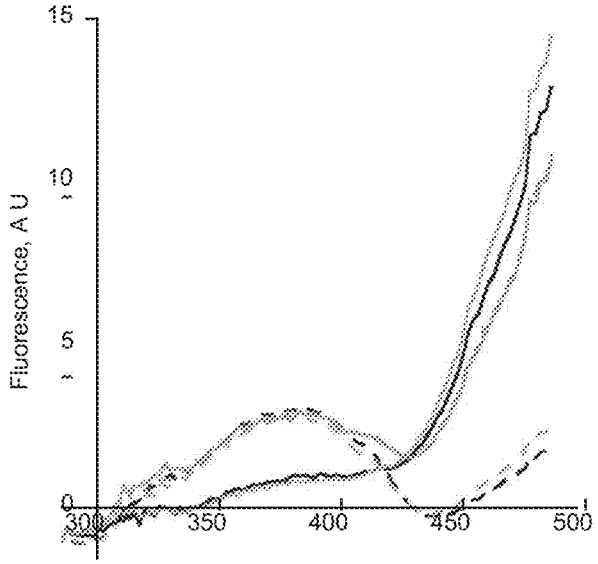


FIG. 58C

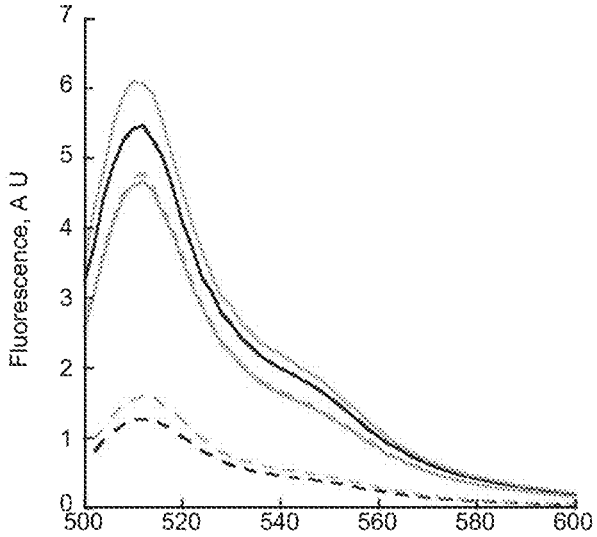
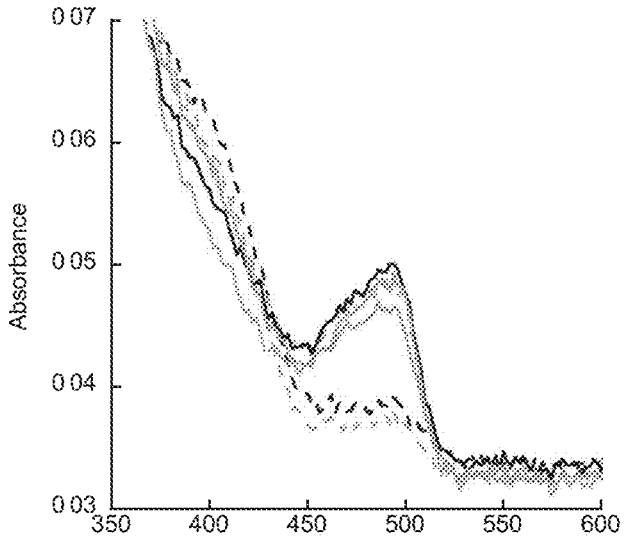


FIG. 58D



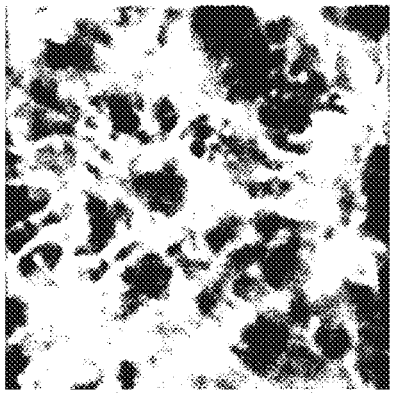


FIG. 59A

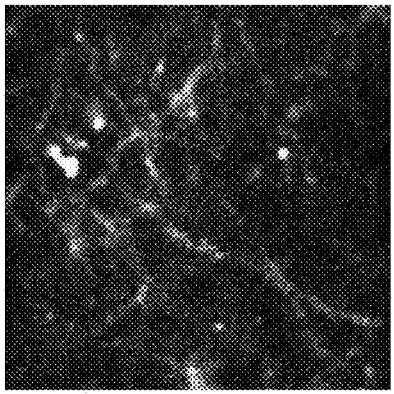


FIG. 59B

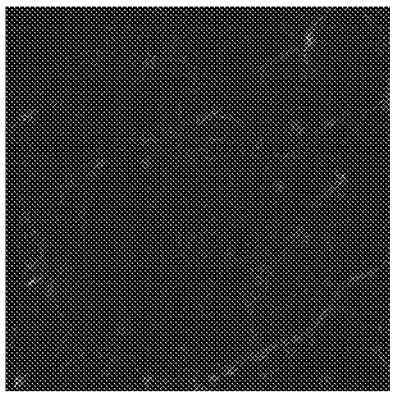


FIG. 59C

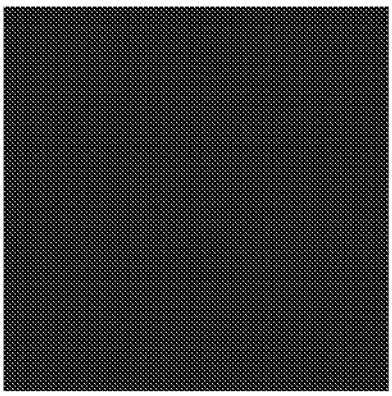


FIG. 59D

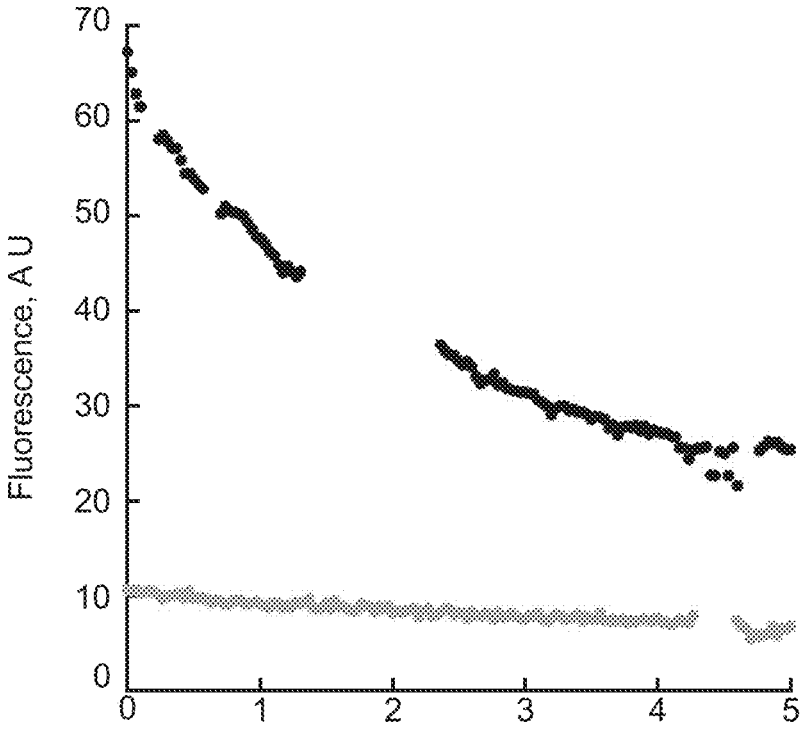
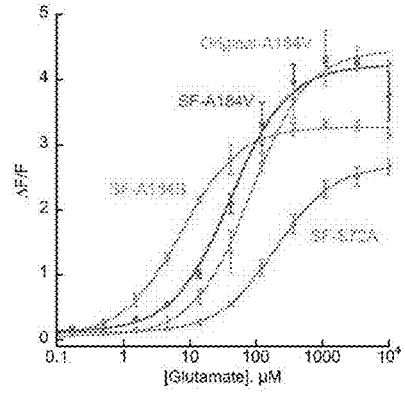


FIG. 59E

FIG. 60A



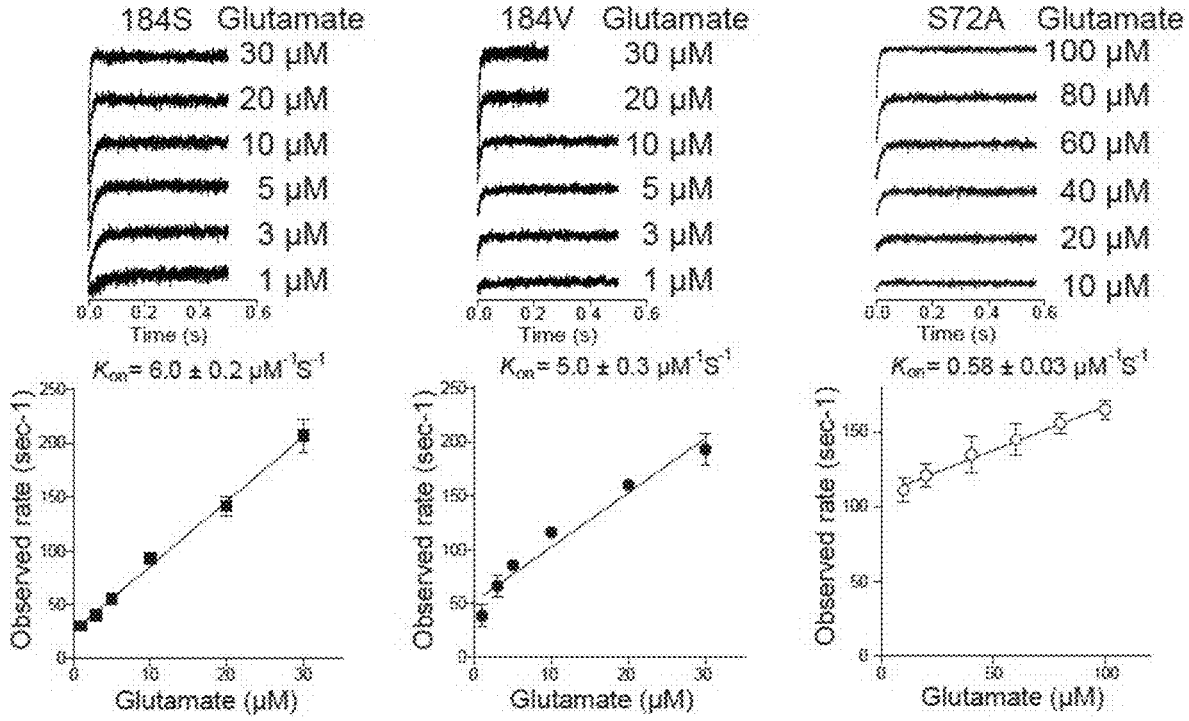


FIG. 60B

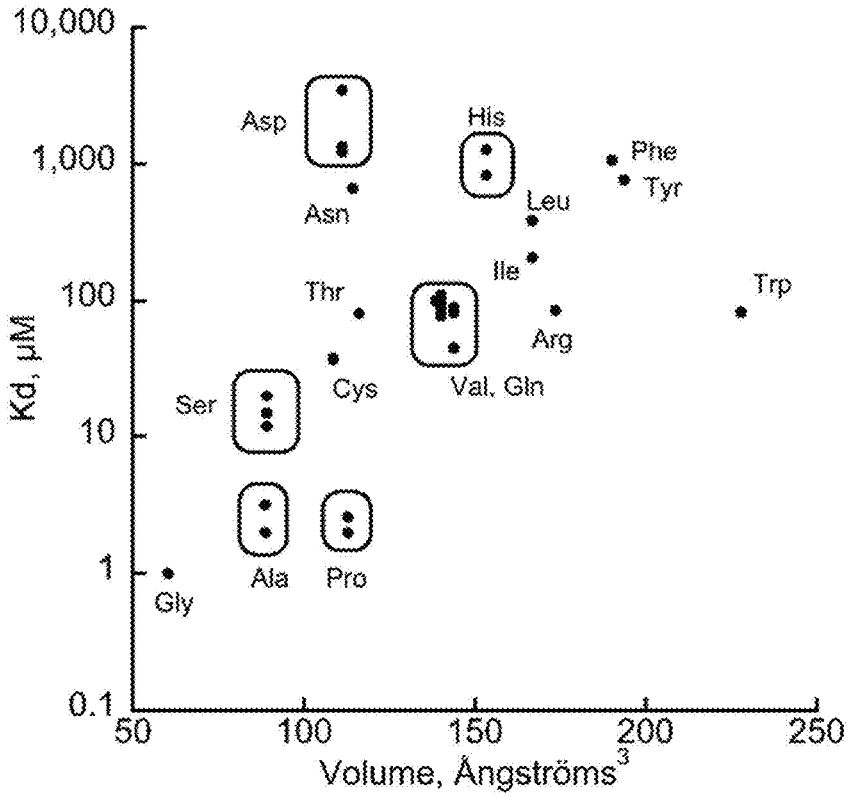

10,000
1,000
100
10
1
0.1
Kd, μM
50
100
150
200
250
Volume, Ångströms3
Asp
Asn
His
Phe
Tyr
Leu
Ile
Thr
Trp
Arg
Cys
Val, Gln
Ser
Ala
Pro
Gly

FIG. 61

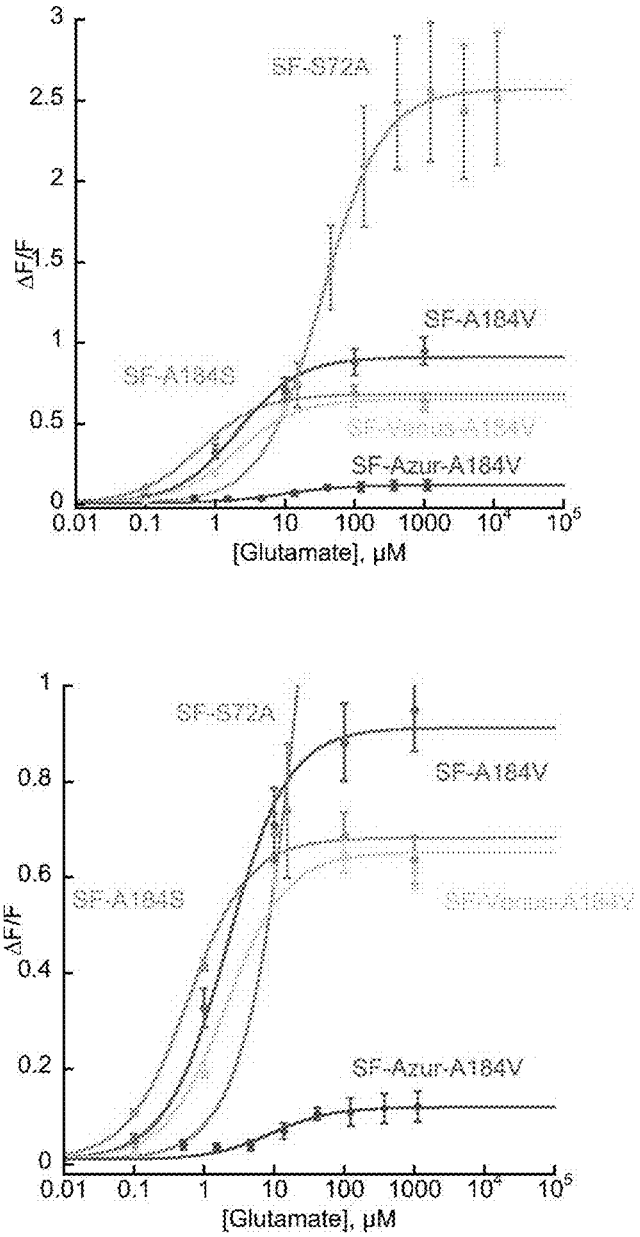
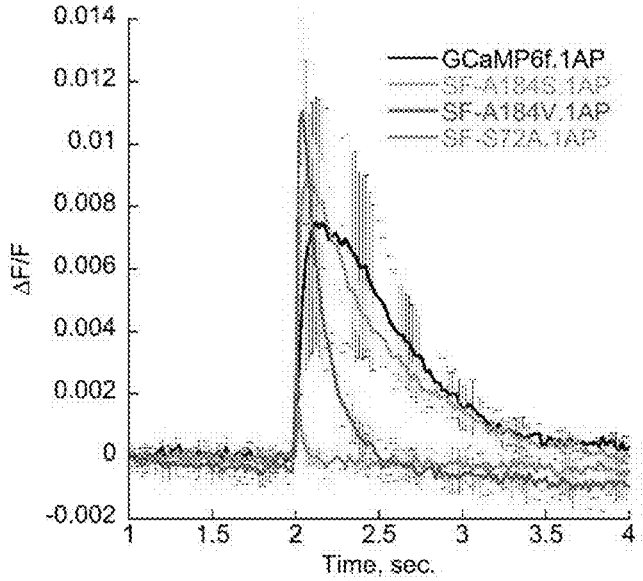

SF-S72A
SF-A184V
SF-A184S
SF-Azur-A184V
ΔF/F
[Glutamate], μM
0.01
0.1
1
10
100
1000
0
0.5
1
1.5
2
2.5
3

FIG. 62


0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
-0.002
ΔF/F
1
1.5
2
2.5
3
3.5
4
Time, sec.
GCaMP6f.1AP
SF-A184S.1AP
SF-A184V.1AP
SF-S72A.1AP

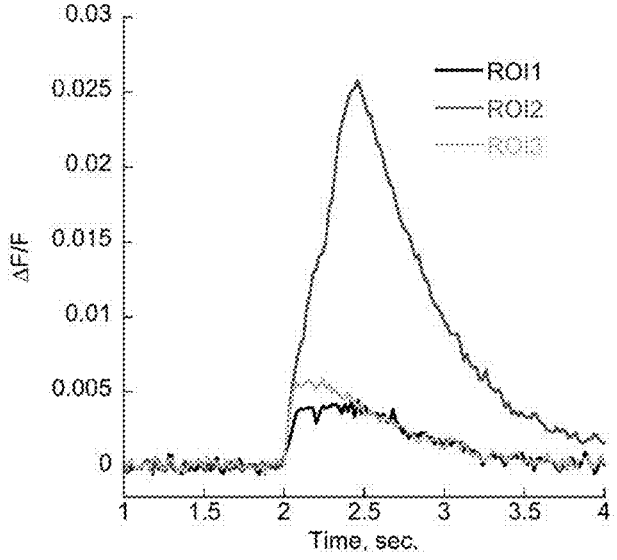

0.03
0.025
0.02
0.015
0.01
0.005
0
ΔF/F
1
1.5
2
2.5
3
3.5
4
Time, sec.
ROI1
ROI2
ROI3

FIG. 63A

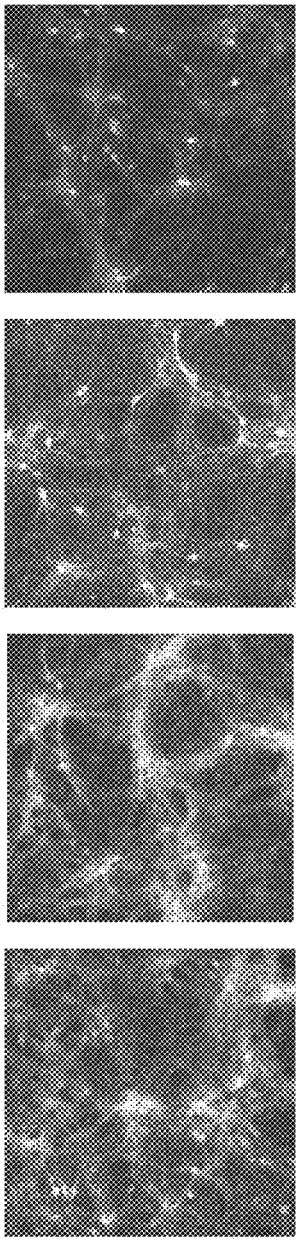


FIG. 63B

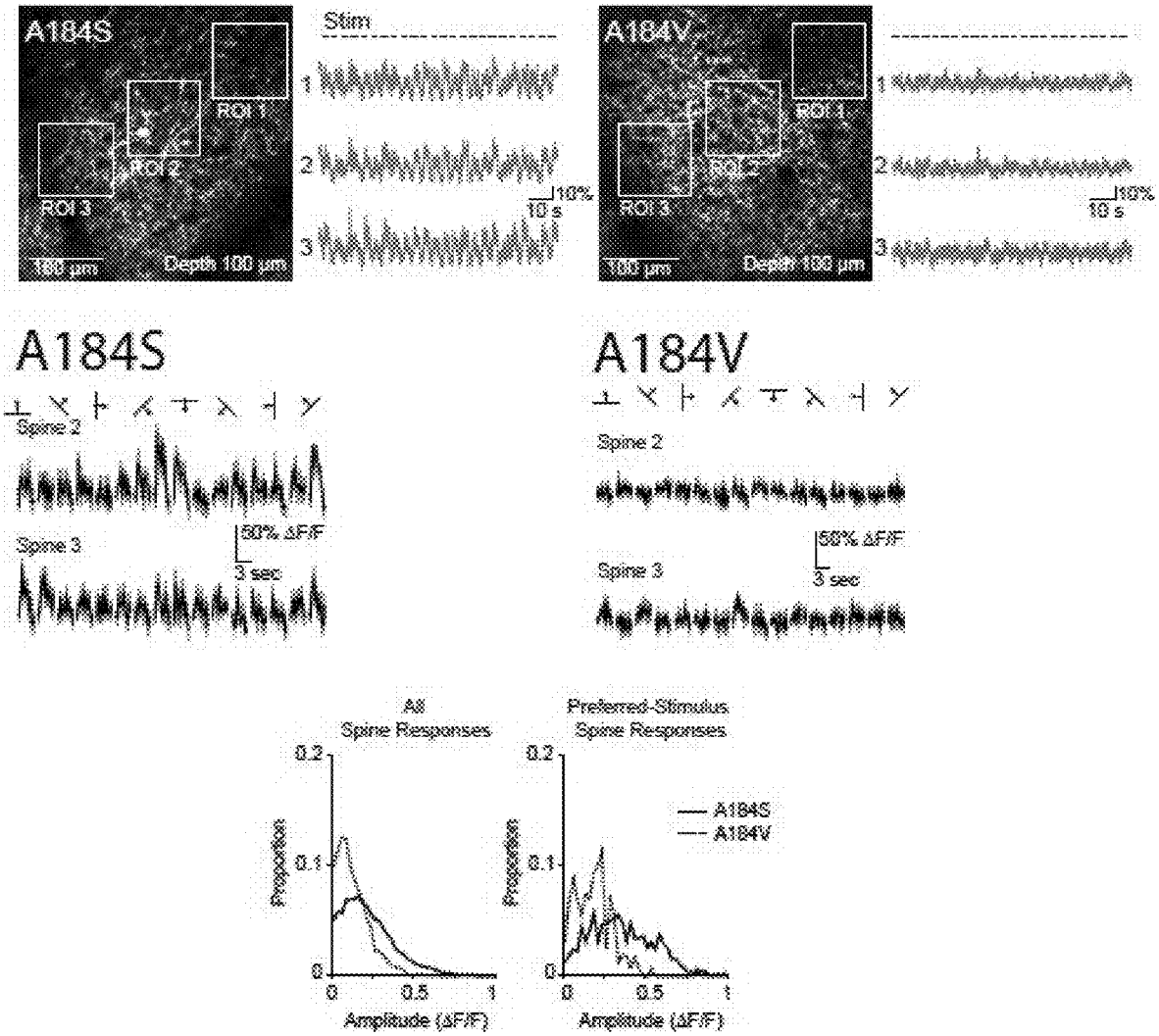

A184S
Stim
ROI 1
ROI 2
ROI 3
100 µm
Depth 100 µm
1
2
3
10%
10 s
A184V
ROI 1
ROI 2
ROI 3
100 µm
Depth 100 µm
1
2
3
10%
10 s
A184S
Spine 2
Spine 3
50% ΔF/F
3 sec
A184V
Spine 2
Spine 3
50% ΔF/F
3 sec
All
Spine Responses
Preferred-Stimulus
Spine Responses
Proportion
0.2
0.1
0
0
0.5
1
Amplitude (ΔF/F)
A184S
A184V

FIG. 64

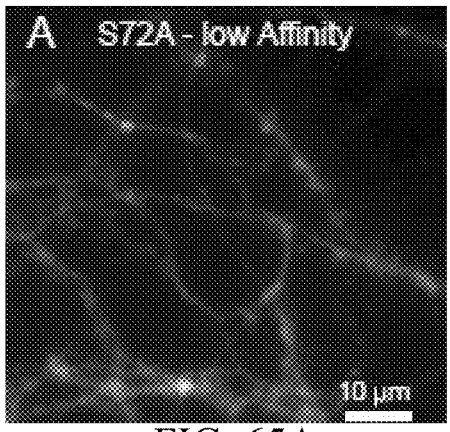


FIG. 65A

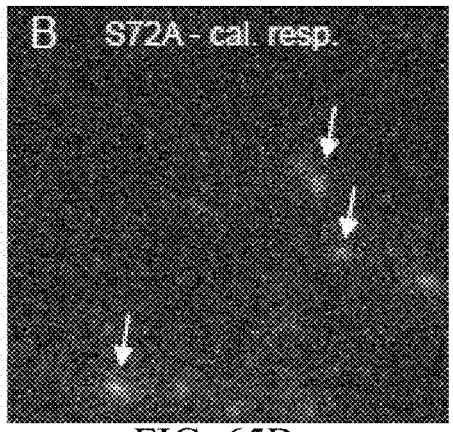


FIG. 65B

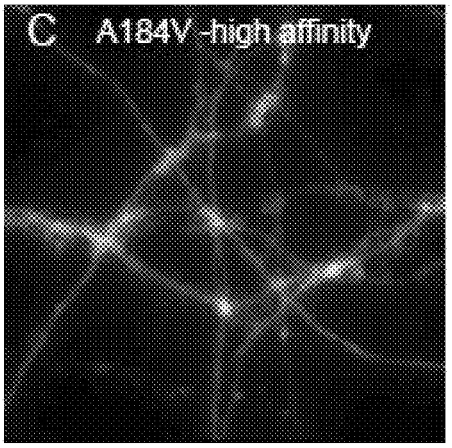


FIG. 65C

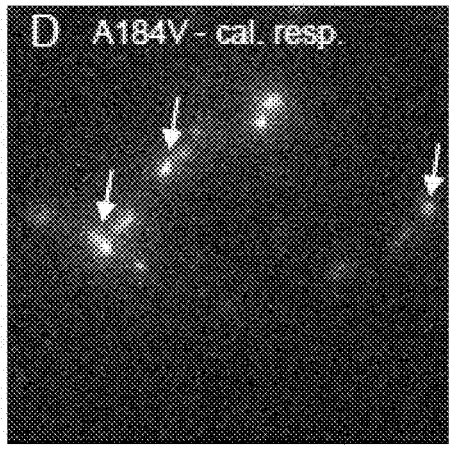


FIG. 65D

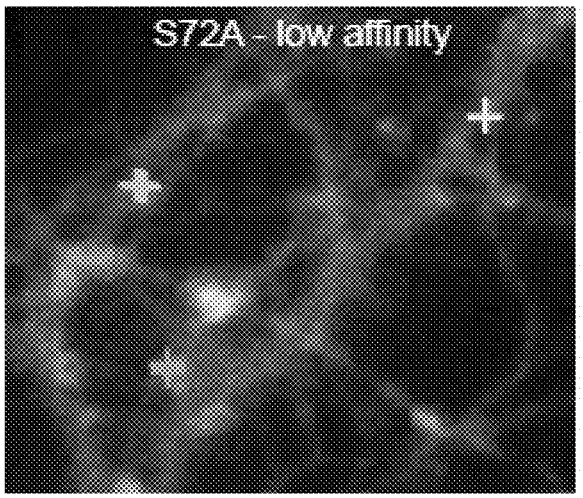


FIG. 66A

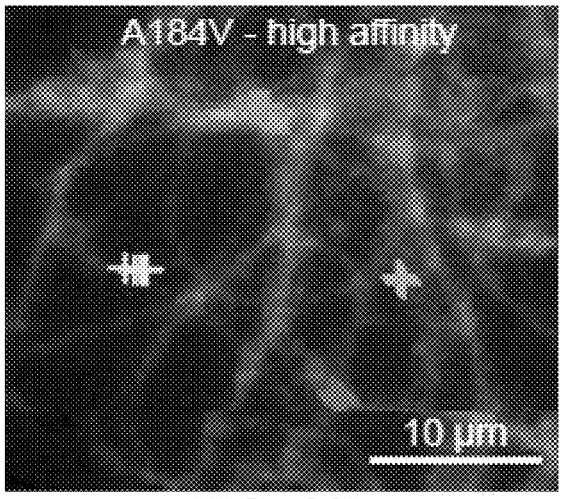


FIG. 66B

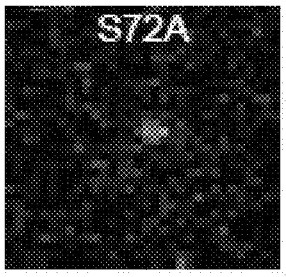


FIG. 66C

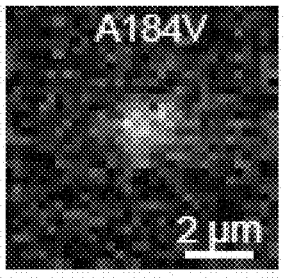


FIG. 66D

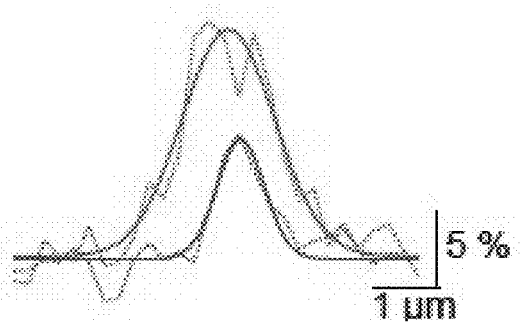


FIG. 66E

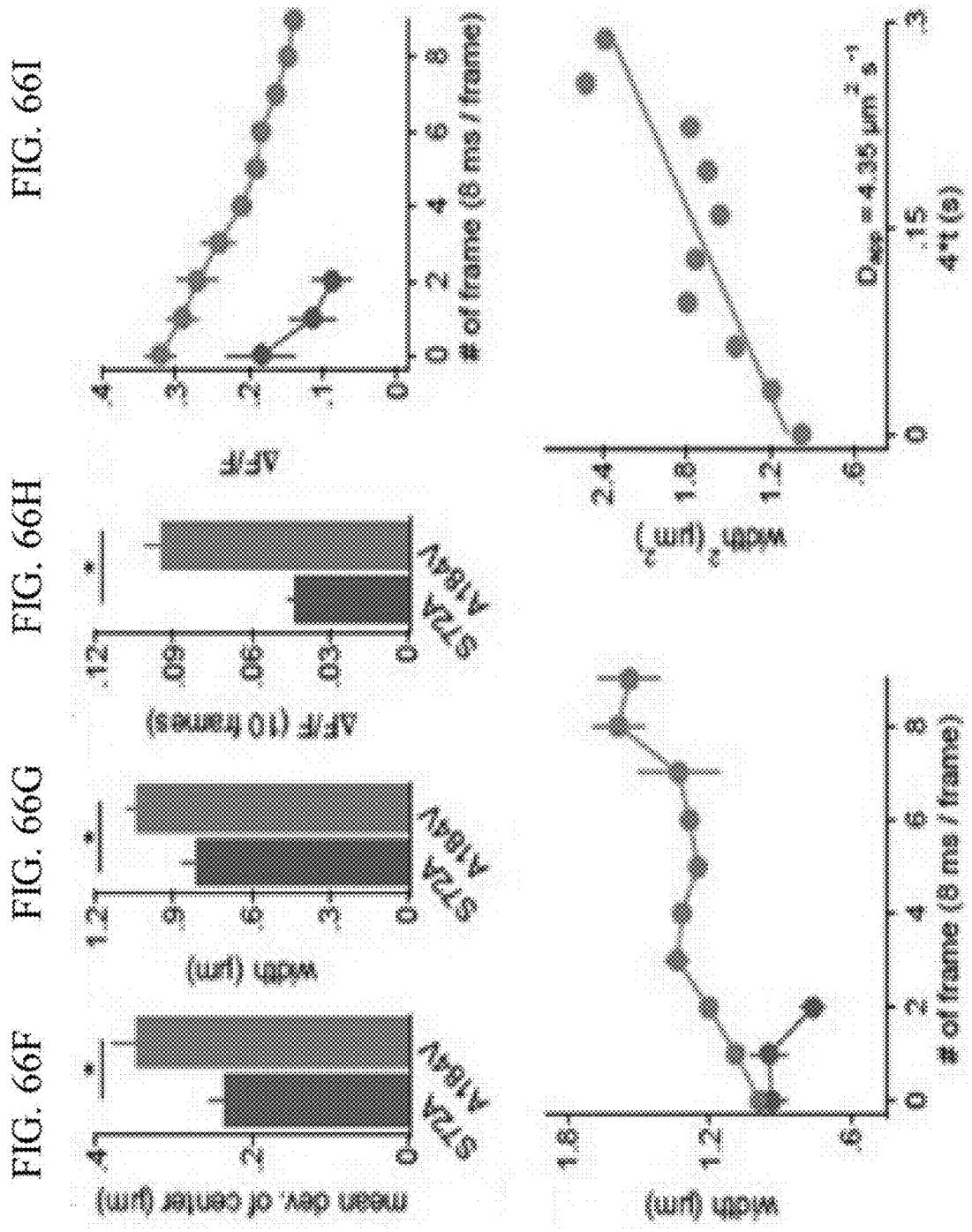


FIG. 66F
mean dev. of center (μm)
STZ1
ATG9
FIG. 66G
width (μm)
STZ1
ATG9
FIG. 66H
ΔF/F (10 frames)
STZ1
ATG9
FIG. 66I
ΔF/F
# of frame (8 ms / frame)
FIG. 66J
width (μm)
# of frame (8 ms / frame)
width² (μm²)
D_app = 4.35 μm² s⁻¹
4*t (s)

IgG secretion signal
[redacted]
[redacted] Linker [redacted]
[redacted]
Myc epitope
PDGFR transmembrane domain 513-561

SF-iGluSnFR

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]LQVDEQK
LISEEDLNAVG

QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 67A

B **SF-Venus-iGluSnFR**
Mutations in GFP: T203Y and Y65G to shift the color. Mutations F46L and S72A to increase chromophore maturation.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]LQVDEQK
LISEEDLNAVG

QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 67B

SF~Azurite~iGluSnFR

Mutations in GFP: T65S and Y66H to shift the color. V150I and V224R to improve maturation and brightness.
Linker1 mutations: GltI-cpSFGFP connection from PILV[redacted]NV to PILG[redacted]NV
Linker2 mutations: cpSFGFP-GltI connection from YNFNN[redacted]N to YNFNE[redacted]N

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]GGTGGS[redacted]

[redacted]

[redacted]LQVDEQK
LISEEDLNAVG

[redacted]

QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 67C

FIG. 68A

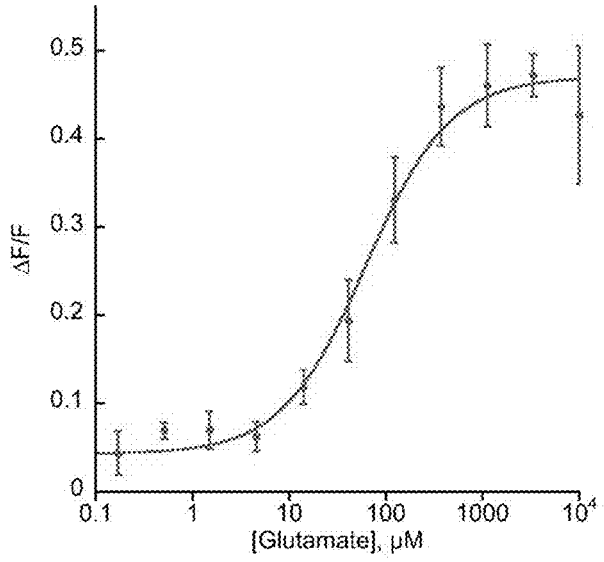


FIG. 68B

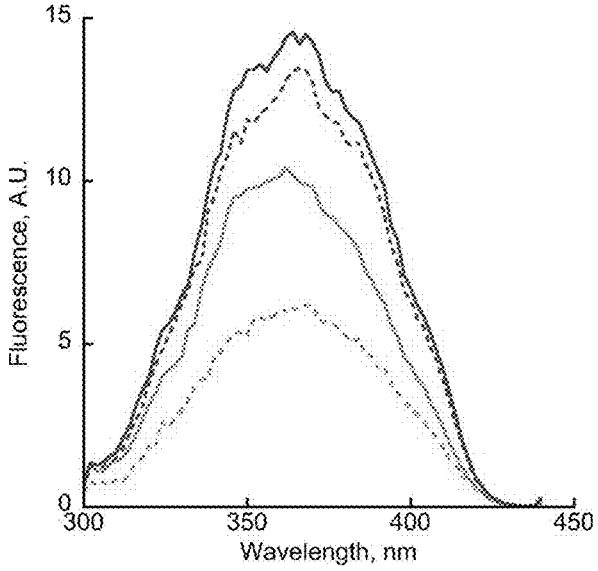


FIG. 68C

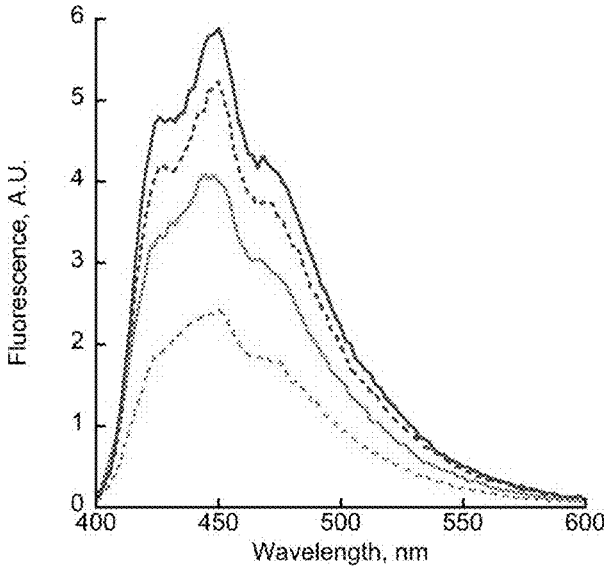


FIG. 68D

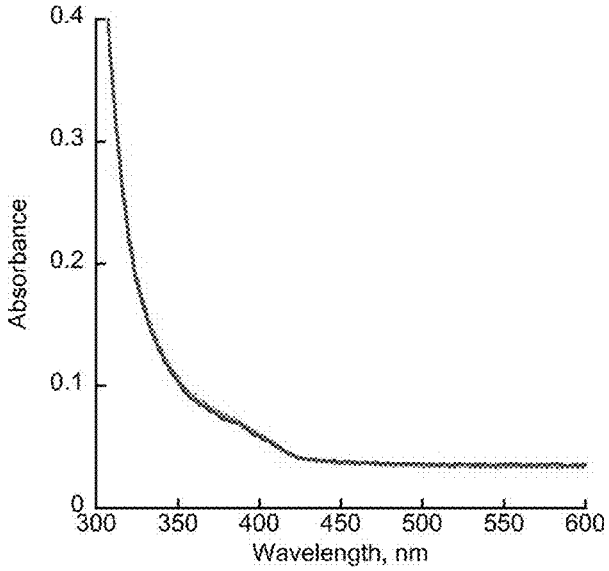


FIG. 69A

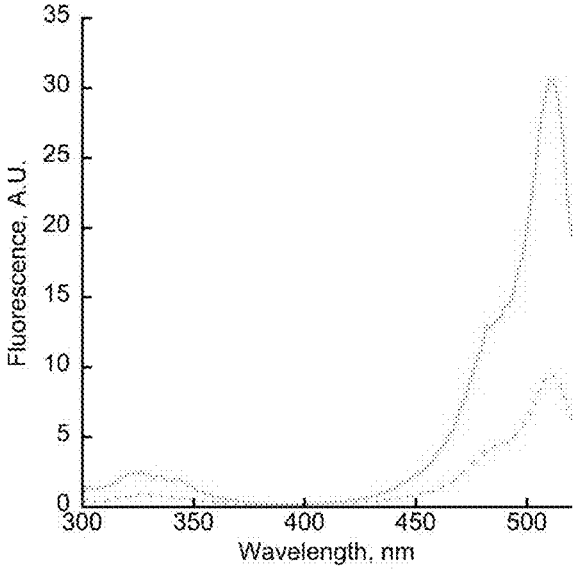


FIG. 69B

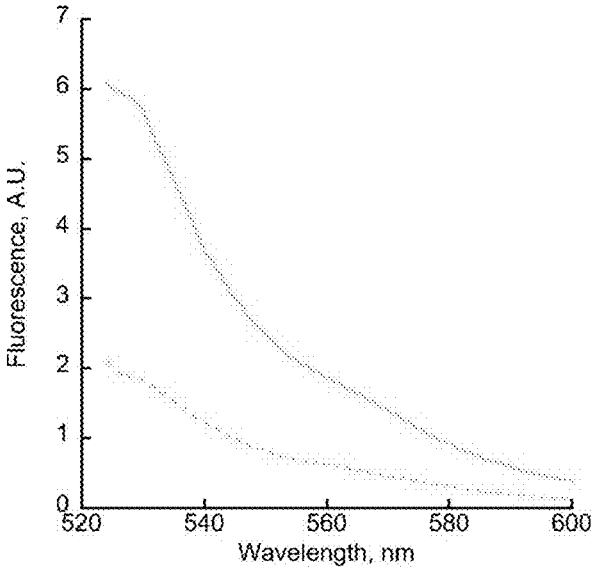


FIG 69C

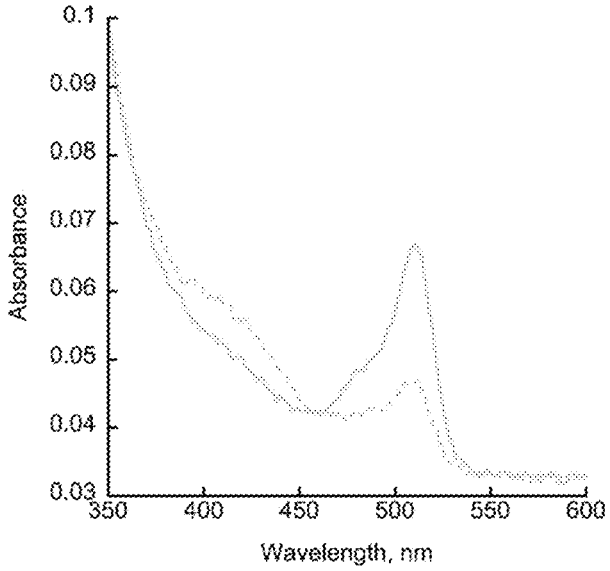
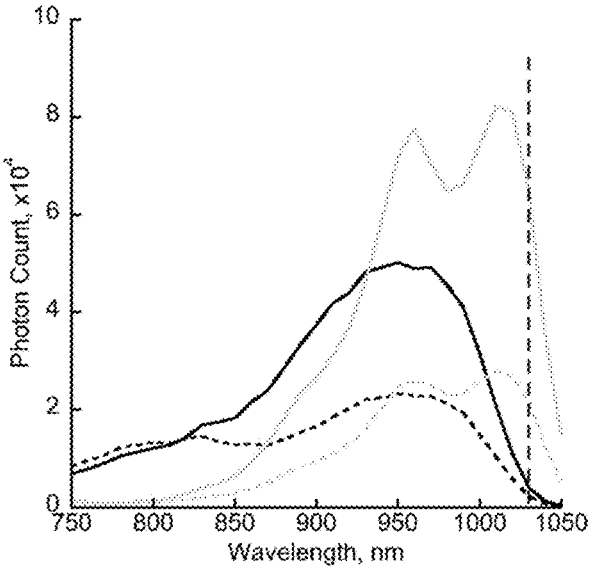


FIG. 69D



SF-iGluSnFR.A184V (SEQ ID NO: 176)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKKKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFMMDDVLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNNPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SF-iGluSnFR.A184S (SEQ ID NO: 177)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKKKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFmMDDSLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNNPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 70A

SF-iGluSnFR.S72A (SEQ ID NO: 178)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKRKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFMMDDVLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNSPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SF-Venus-iGluSnFR.A184V (SEQ ID NO: 179)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKRKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFMMDDVLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNSPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 70B

SF-Venus-iGluSnFR.A184S (SEQ ID NO: 180)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKKKLNKPDLQVKLIPITaQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFmMDDSLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSyQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKLICTT
GKLPVPWPTLVTTLgYGVQCFaRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNNPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SF-Venus-iGluSnFR.S72A (SEQ ID NO: 181)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKKKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFMMDDVLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILVSHNVYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSyQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKLICTT
GKLPVPWPTLVTTLgYGVQCFaRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNNPLNMNFELSDEMKALFKEPNDKALKLQVDEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 70C

SF-Azurite-iGluSnFR (SEQ ID NO: 182)

METDTLLLWVLLLWVPGSTGDRSAAGSTLDKIAKNGVIVVGHRESSVPFSYYDNQQKVVGYSQDYSNAIV
EAVKKKLNKPDLQVKLIPITSQNRIPLLQNGTFDFECGSTTNNVERQKQAAFSDTIFVVGTRLLTKKGGD
IKDFANLKDKAVVVTSGTTSEVLLNKLNEEQKMNMRIISAKDHGDSFRTLESGRAVAFMMDDVLLAGERA
KAKKPDNWEIVGKPQSQEAYGCMLRKDDPQFKKLMDDTIAQVQTSGEAEKWFDKWFKNPILGVHNIYITA
DKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTT
GKLPVPWPTLVTTLSHGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRD
HMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKL
TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEV
KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRD
HMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKL
TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEV
KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNNPNAYGQSAMRDWRSNRIVGSLVAGAVAPESFMSQP
GTVMEIFLQTRNPQAAANAAQAIADQVGLGRLGQLQVDEQKLISEEDLNAVGQDTQEVIVVPHSLPFKVV
VISAILALVVLTIISLIILIMLWQKKPR

iDexSnFR (SF-GlucoseSensor)(SEQ ID NO: 183)

METDTLLLWVLLLWVPGSTGDRSKLEIFSWWAGDEGPALEALIRLYKQKYPGVEVINATVTGGAGVNARA
VLKTRMLGGDPPDTFQVAAGMELIGTWVVANRMEDLSALFRQEGWLQAFPKGLIDLISYKGGIWSVPVNI
HRSNVMWYLPAKLKEWGVNPPRTWDEFLATCQTLKQKGLEAPLALGENWTQQHLWESVALAVLGPDDWNN
LWNGKLKFTDPKAVRAWEVFGRVLDCANKDAAGLSWQQAVDRVVQGKAAFNVMGDWAAGYMTTTLKLKPG
TDFAWAPSPGTQGVFMMLSDSFGLPKGAKNRQNAINWLRLVGSKEGQDTFNPLKGSIAARLDSDPSKYPA
SHNVLITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRD
HMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKL
TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEV
KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNNPNAYGQSAMRDWRSNRIVGSLVAGAVAPESFMSQP
GTVMEIFLQTRNPQAAANAAQAIADQVGLGRLGQLQVDEQKLISEEDLNAVGQDTQEVIVVPHSLPFKVV
VISAILALVVLTIISLIILIMLWQKKPR

FIG. 70D

iGABASnFR (SEQ ID NO:184)

METDTLLLWVLLLWVPGSTGDRSESINFVSWGGSTQDAQKQAWADPFSKASGITVVQDGPTDYGKLKAMV
ESGNVQWDVVDVEADFALRAAAAEGLLEPLDFSVIQRDKIDPRFVSDHGVGSIIFSFVLGYNEGKLCASKP
QDWTALFDTKTYPGKRALYKWPSPGVLELALLADGVPADKLYPLDLDRAFKKLDTIKKDIVWWGGCAQSQ
QLLASGEVSMGQFWNGRIHALQEDGAPVGVSWKQNLVMADILVVPKGTKNKAAAMKFLASASSAKGQDDF
SALTAYAPVNIDSVQRLDIGQVFTADKQKNGTMANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDN
HYLSTQSVLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGVVPILVELDGDVNG
HKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQ
ERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFPPPATTDPEGAYETVKKEYK
RKWNIVWLKPLGFNNTYTLTVKDELAKQYNLKTFSDLAKISDKLILGATMFFLEGPDGYPGLQKLYNFKFK
HTKSMDMGIRYTAIDNNEVQVIDAWATDGLLVSHKLKILEDDKAFFPPYYAAPIIRQDVLDKHPELKDVLN
KLANQISLEEMQKLNYKVDGEGQDPAKVAKEFLKEKGLILQVDEQKLISEEDLN

iAChSnFR

SEQ ID NO: 185

MHHHHHHGYPYDVPDYAGAQPARSANDTVVVGSIIFTEGIIVANMVAEMIEAHTDLKVVRKLNLGGVNVN
FEAIKRGGANNGIDIYVEYTGHGLVDILGFPEPNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQN
TPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGV
VPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQH
DFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFPPPATT
DPEGAYETVKKEYKRKWNIVWLKPLGFNNTYTLTVKDELAKQYNLKTFSDLAKISDKLILGATMFFLEGP
DGYPGLQKLYNFKFKHTKSMDMGIRYTAIDNNEVQVIDAWATDGLLVSHKLKILEDDKAFFPPYYAAPII
RQDVLDKHPELKDVLNKLANQISLEEMQKLNYKVDGEGQDPAKVAKEFLKEKGLILQVDEQKLISEEDLN

FIG. 70E

SEQ ID NO: 186

METDTLLLWVLLLWVPGSTGDRSANDTVVVGSIIFTEGIIVANMVAEMIEAHTDLKVVRKLNLGGVNVNF
EAIKRGGANNGIDIYVEYTGHGLVDILGFPEPNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNT
PIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMSKGEELFTGVV
PILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHD
FFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFPPPATTD
PEGAYETVKKEYKRKWNIVWLKPLGFNNTYTLTVKDELAKQYNLKTFSDLAKISDKLILGATMFFLEGPD
GYPGLQKLYNFKFKHTKSMDMGIRYTAIDNNEVQVIDAWATDGLLVSHKLKILEDDKAFFPPYYAAPIIR
QDVLDKHPELKDVLNKLANQISLEEMQKLNYKVDGEGQDPAKVAKEFLKEKGLILQVDEQKLISEEDLNA
VGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

FIG. 70F

Structure I

Wherein:

" 

" is a framework portion;

" 

" is a first signaling portion;

"—" is an optional linker;

wherein the signaling portion is present at a site within the framework portion that undergoes a conformational change upon interaction of the framework portion with an analyte

FIG. 71

GENETICALLY ENCODED BIOSENSORS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Divisional of, and claims the benefit of priority under 35 U.S.C. § 121 to, U.S. application Ser. No. 16/902,160 filed Jun. 15, 2020, now allowed, which is a Continuation of, and claims priority under 35 U.S.C. § 121 to, U.S. application Ser. No. 16/002,697 filed Jun. 7, 2018, which is a Continuation-In-Part of, and claims priority under 35 U.S.C. § 120 to, U.S. application Ser. No. 15/904,574 filed Feb. 26, 2018, which is a Divisional application of, and claims the benefit of priority under 35 U.S.C. § 121 to, U.S. application Ser. No. 15/664,326 filed Jul. 31, 2017, which is a Divisional application of, and claims the benefit of priority under 35 U.S.C. § 121 to, U.S. application Ser. No. 14/350,199 filed Nov. 18, 2014, which is a U.S. National Phase application of, and claims the benefit of priority under 35 U.S.C. 371 to, International Application No. PCT/US2012/059219 filed Oct. 8, 2012, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Application No. 61/544,867 filed Oct. 7, 2011.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing that has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. The ASCII copy, created on 25 Oct. 2021, is named 30872_0020003_SEQ.txt, and is 254553 bytes in size.

TECHNICAL FIELD

[0003] This disclosure relates to genetically encoded biosensors and methods for the design, production, and use of such biosensors.

BACKGROUND

[0004] Protein-based sensors that transduce microscopic binding events into macroscopically observable signals are available to allow real-time visualization of a variety of biological events and/or molecules (Frommer et al., Chem. Soc. Rev., 38:2833-2841, 2009). Such sensors can be targeted and/or expressed in living cells, tissues, and organisms, and permit imaging with minimally invasive techniques (Okumoto, Curr. Opin. Biotechnol., 21:45-54, 2010). Application of these sensors is limited by the narrow range of analytes that can be detected and/or by their inability to distinguish signal over noise.

SUMMARY

[0005] In one aspect, a recombinant peptide biosensor is provided that includes an analyte-binding framework portion and a signaling portion, wherein the signaling portion is present within the framework portion at a site or amino acid position that undergoes a conformational change upon interaction of the framework portion with a defined, specific, or selected analyte.

[0006] In one embodiment, the signaling portion is allosterically regulated by the framework portion such that signaling from the signaling portion is altered upon interaction of the framework portion with the analyte. In some embodiments, signaling by the signaling portion detectably increases upon interaction of the framework portion with the analyte. In some embodiments, signaling by the signaling portion detectably decreases upon interaction of the framework portion with the analyte. In some embodiments, signaling by the signaling portion is proportional to the level of interaction between the framework portion and the analyte.

[0007] In some embodiments, the signaling portion is a superfolder (SF) fluorescent protein (see, for example, Pedelacq et al., 2006, Nature Biotechnol., 24:79-88), a protein that exhibits robust folding, even when fused to a protein that folds poorly. In some embodiments, the SF protein is circularly permuted. In some embodiments, the SF protein is a green fluorescent protein, a yellow fluorescent protein, a red fluorescent protein, or a blue fluorescent protein.

[0008] In some embodiments, the framework portion has a first structure in the absence of an analyte and a second structure, that is detectably distinct from the first structure, in the presence of the analyte. In some embodiments, the conformational change between the first structure and the second structure allosterically regulates the signaling portion. In some embodiments, the framework portion is a periplasmic binding protein (PBP) or a variant of a PBP.

[0009] In some embodiments, the analyte-binding framework portion binds specifically to an analyte selected from the group consisting of glucose, maltose, phosphonate, glutamate, GABA, and ACh.

[0010] In another aspect, a recombinant peptide biosensor is provided that includes an amino acid sequence having at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NOs: 176-182, wherein the recombinant peptide biosensor binds specifically to glutamate.

[0011] In one embodiment, the recombinant peptide biosensor includes a recombinant peptide biosensor selected from the group consisting of SEQ ID NOs: 176-182 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glutamate. In some embodiments, the recombinant peptide biosensor includes a recombinant peptide biosensor selected from the group consisting of SEQ ID NOs: 176-182.

[0012] In still another aspect, a recombinant peptide biosensor is provided that includes an amino acid sequence having at least 90% identity to a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 183, wherein the recombinant peptide biosensor binds specifically to glucose.

[0013] In some embodiments, the recombinant peptide biosensor includes a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 183 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glucose. In some embodiments, the recombinant peptide biosensor includes a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 183.

[0014] In one aspect, a recombinant peptide biosensor is provided that includes an amino acid sequence having at least 90% identity to a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 184, wherein the recombinant peptide biosensor binds specifically to GABA.

[0015] In one embodiment, the recombinant peptide biosensor includes a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 184 comprising 10 or fewer conservative amino acid substitutions, wherein the recom-

binant peptide biosensor binds specifically to GABA. In one embodiment, the recombinant peptide biosensor includes a recombinant peptide biosensor having the sequence shown in SEQ ID NO: 184.

[0016] In another aspect, a recombinant peptide biosensor is provided that includes an amino acid sequence having at least 90% identity to a recombinant peptide biosensor having a sequence selected from the group consisting of SEQ ID NO: 185 and 186, wherein the recombinant peptide biosensor binds specifically to ACh.

[0017] In one embodiment, the recombinant peptide biosensor includes a recombinant peptide biosensor having a sequence selected from the group consisting of SEQ ID NO: 185 and 186 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to ACh. In one embodiment, the recombinant peptide biosensor includes a recombinant peptide biosensor having a sequence selected from the group consisting of SEQ ID NO: 185 and 186.

[0018] In one aspect, a nucleic acid is provided that encodes a recombinant peptide biosensor as described herein.

[0019] In one aspect, a vector is provided that includes a nucleic acid as described herein.

[0020] In one aspect, a cell is provided that includes a nucleic acid as described herein.

[0021] In one aspect, a cell is provided that includes a vector as described herein.

[0022] In one aspect, a kit is provided that includes a recombinant peptide biosensor as described herein, a nucleic acid as described herein, a vector as described herein, and/or the cell as described herein.

[0023] In still another aspect, a method is provided for detecting glutamate, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor, the peptide biosensor having an amino acid sequence selected from the group consisting of SEQ ID NOs: 176-182, and correlating the level of fluorescence with the presence of glutamate.

[0024] In some embodiments, the recombinant peptide biosensor is expressed from a nucleic acid. In some embodiments, the method includes contacting the recombinant peptide biosensor with a sample comprising glutamate. In some embodiments, the method includes correlating the level of fluorescence with a concentration of glutamate. In some embodiments, the method includes comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of glutamate. In some embodiments, the method is performed in vitro.

[0025] In some aspects, a method for detecting glucose is provided, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor, the peptide biosensor having an amino acid sequence shown in SEQ ID NO: 183, and correlating the level of fluorescence with the presence of glucose.

[0026] In some aspects, a method for detecting GABA is provided, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor, the peptide biosensor having an amino acid sequence shown in SEQ ID NO: 184, and correlating the level of fluorescence with the presence of GABA.

[0027] In some aspects, a method for detecting ACh is provided, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor, the peptide biosensor having an amino acid sequence selected from the group consisting of SEQ ID NOs: 185 and 186, and correlating the level of fluorescence with the presence of ACh.

[0028] In some aspects, a method for detecting a defined, selected, or specific analyte is provided, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor of claim **1**; and correlating the level of fluorescence with the presence of a defined, selected, or specific analyte

[0029] In some embodiments, the recombinant peptide biosensor is expressed from a nucleic acid. In some embodiments, the method includes contacting the recombinant peptide biosensor with a sample comprising the analyte. In some embodiments, the method includes correlating the level of fluorescence with a concentration of the analyte. In some embodiments, the method includes comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte. In some embodiments, the method is performed in vitro. In some embodiments, the analyte is selected from the group consisting of glutamate, glucose, GABA, and ACh.

[0030] The present disclosure provides genetically encoded recombinant peptides containing an analyte-binding framework portion linked (e.g., operably linked) to a signaling portion, wherein the signaling portion is allosterically regulated by the framework portion upon interaction of the framework portion with an analyte (e.g., a defined, selected, and/or specific analyte). These constructs can be used as biosensors, e.g., to transduce microscopic binding events into macroscopically observable signals.

[0031] The present disclosure provides, in part, recombinant peptides for use as biosensors (e.g., recombinant peptide biosensors) that include (e.g., comprise, consist essentially of, or consist of), e.g., include at least, an analyte-binding framework portion and a signaling portion. As described in further detail herein, such signaling portions are present within the framework portion at a site or amino acid position that undergoes a conformational change (e.g., a conformational change sufficient to alter a physical and/or functional characteristic of the signaling portion, e.g., a substantial conformational change) upon interaction of the framework portion with a defined, specific, or selected analyte (e.g. such as an analyte to which the framework portion or a region thereof, and/or the biosensor, specifically binds).

[0032] For example, in some instances, the signaling portion is allosterically regulated by the framework portion such that signaling from the signaling portion is altered (e.g. wherein a first level of signaling is altered or changed to a second level of signaling that can be distinguished using routine methods of detection from the first) upon interaction of the framework portion with the analyte. In some instances, signaling by the signaling portion can detectably increase or decrease upon interaction of the framework portion with the analyte. In some instances, signaling by the signaling portion upon interaction of the biosensor with a defined, specific, or selected analyte (e.g. such as an analyte to which the framework portion or a region thereof, and/or

the biosensor, specifically binds) can be proportional or can correlate with to the level of interaction between the framework portion and the analyte such that the level of interaction can be determined from the signaling or alteration thereof.

[0033] In some instances, framework portions of the biosensors disclosed herein have a first structure in the absence of an analyte and a second structure that is detectably distinct from the first structure in the presence of the analyte. In some instances, the conformational change between the first structure and the second structure allosterically regulates the signaling portion.

[0034] In some instances, framework portions of the biosensors disclosed herein can be, or can include (e.g., comprise, consist essentially of, or consist of), periplasmic binding proteins (PBP) or variants of a PBP. In some instances, exemplary PBPs or variants thereof can include, but are not limited to, peptides with at least 90% identity to a peptide selected from the group consisting of SEQ ID NO:105, SEQ ID NO: 106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO: 110, SEQ ID NO:111, SEQ ID NO:113, and SEQ ID NO:114. In some instances, exemplary PBPs or variants thereof can include, but are not limited to, peptides with at least 95% identity to a peptide selected from the group consisting of SEQ ID NO:105, SEQ ID NO: 106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO: 110, SEQ ID NO:111, SEQ ID NO:113, and SEQ ID NO:114. In some instances, exemplary PBPs or variants thereof can include, but are not limited to, peptides selected from the group consisting of SEQ ID NO:105, SEQ ID NO: 106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO: 110, SEQ ID NO:111, SEQ ID NO:113, and SEQ ID NO:114. In some instances, exemplary PBPs or variants thereof can include, but are not limited to, peptides selected from the group consisting of SEQ ID NO:105, SEQ ID NO: 106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO: 110, SEQ ID NO:111, SEQ ID NO:113, and SEQ ID NO:114 comprising 10 or fewer conservative amino acid substitutions. PBPs or variants thereof disclosed herein can be truncated.

[0035] In some instances, signaling portions of the biosensors disclosed herein can be or can include (e.g., comprise, consist essentially of, or consist of) one or more (e.g., one, two three, four, five, and less than ten) circularly permuted fluorescent proteins (cpFPs). Such cpFPs can be include but are not limited to, for example, green fluorescent proteins, yellow fluorescent proteins, red fluorescent proteins, and/or blue fluorescent proteins.

[0036] In some instances, biosensors disclosed herein, e.g., analyte-binding framework portions of biosensors disclosed herein, can bind (e.g., bind specifically) to glucose. Such sensors can be referred to as glucose binding biosensors or glucose biosensors.

[0037] In some instances, biosensors disclosed herein, e.g., analyte-binding framework portions of biosensors disclosed herein, can bind (e.g., bind specifically) to maltose. Such sensors can be referred to as maltose binding biosensors or maltose biosensors.

[0038] In some instances, biosensors disclosed herein, e.g., analyte-binding framework portions of biosensors disclosed herein, can bind (e.g., bind specifically) to phosphonate.

[0039] Such sensors can be referred to as phosphonate binding biosensors or phosphonate biosensors.

[0040] In some instances, biosensors disclosed herein, e.g., analyte-binding framework portions of biosensors disclosed herein, can bind (e.g., bind specifically) to glutamate. Such sensors can be referred to as glutamate binding biosensors or glutamte biosensors.

[0041] In some instances, biosensors disclosed herein can include (e.g., comprise, consist essentially of, or consist of): an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53, wherein the recombinant peptide biosensor binds specifically to maltose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to maltose; and/or a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53.

[0042] In some instances, biosensors disclosed herein can include (e.g., comprise, consist essentially of, or consist of): an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63, wherein the recombinant peptide biosensor binds specifically to glutamate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glutamate; and/or a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63.

[0043] In some instances, biosensors disclosed herein can include (e.g., comprise, consist essentially of, or consist of): an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78, wherein the recombinant peptide biosensor binds specifically to phosphonate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to phosphonate; and/or a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78.

[0044] In some instances, biosensors disclosed herein can include (e.g., comprise, consist essentially of, or consist of): an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94, wherein the recombinant peptide biosensor binds specifically to glucose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glucose; and/or a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94.

[0045] In some instances, biosensors disclosed herein can include (e.g., comprise, consist essentially of, or consist of): SEQ ID NO:91; SEQ ID NO:92; SEQ ID NO:93; SEQ ID NO:95.

[0046] In some instances, any recombinant biosensor disclosed herein can be isolated and/or purified. The terms "isolated" or "purified," when applied to a biosensor dis-

closed herein includes nucleic acid proteins and peptides that are substantially free or free of other cellular material or culture medium when produced by recombinant techniques, or substantially free or free of precursors or other chemicals when chemically synthesized.

[0047] The disclosure also provides, in part, nucleic acids (e.g., isolated and/or purified nucleic acids) encoding any one or more of the recombinant peptide biosensors disclosed herein. For example, nucleic acids can encode: an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53, wherein the recombinant peptide biosensor binds specifically to maltose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to maltose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and 53; an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63, wherein the recombinant peptide biosensor binds specifically to glutamate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glutamate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 62 and 63; an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78, wherein the recombinant peptide biosensor binds specifically to phosphonate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to phosphonate; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 77 and 78; an amino acid sequence with at least 90% identity to a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94, wherein the recombinant peptide biosensor binds specifically to glucose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94 comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to glucose; a recombinant peptide biosensor selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94; and/or SEQ ID NO:91; SEQ ID NO:92; SEQ ID NO:93; SEQ ID NO:95.

[0048] In some instances, the disclosure includes vectors containing one or a plurality of the nucleic acids disclosed herein and cells containing such vectors. In some instances, the disclosure provides cells containing one or a plurality of nucleic acids disclosed herein.

[0049] In some instances, the disclosure includes kits related to the biosensors and nucleic acids disclosed herein Such kits can include or contain, for example, a biosensor, a nucleic acid encoding a biosensor, vectors, and/or cells, provided herein.

[0050] In some instances, the disclosure provides methods related to the biosensors and nucleic acids disclosed herein. Such methods can include methods of making, using, and/or selling the biosensors and nucleic acids disclosed herein. For example, methods can include methods for producing genetically encoded recombinant peptide biosensors. In such instances, methods can include, for example, selecting a framework portion that binds specifically to a target analyte and that undergoes a conformational change upon interacting binding to the target analyte, identifying a site or amino acid position within the selected framework portion where or around which the conformational change occurs, and inserting a signaling portion into the site or amino acid position. In some instances, framework portions include periplasmic binding proteins (PBPs) disclosed herein. Exemplary PBPs include PBPs that bind (e.g., bind specifically) to glucose.

[0051] In some instances, the present disclosure includes methods for detecting glucose, e.g., in a sample containing a level of glucose. Such methods can include, detecting a level of fluorescence emitted by a recombinant peptide biosensor, the peptide biosensor having an amino acid sequence selected from the group consisting of SEQ ID NO: 91, 92, 93 and 94, and correlating the level of fluorescence with the presence of glucose. In some instances, recombinant peptide biosensors used in the methods herein are expressed from nucleic acids. In some instances, methods include contacting the recombinant peptide biosensor with a test sample (e.g., a sample comprising glucose). In some instances, methods can include the level of fluorescence emitted by a biosensor (e.g., a biosensor bound to glucose) with a concentration glucose in the sample. Such correlation can include, for example, comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of glucose. In some instance, the level of fluorescence emitted by the recombinant peptide biosensor in the presence (e.g., bound or bound specifically to) of a sample comprising a known concentration or range of concentrations of glucose is stored on an electronic database.

[0052] One of skill will appreciate that such methods can be adapted for any defined, specific, or selected analyte. For example, in some instances, the disclosure provides methods for detecting a defined, selected, or specific analyte. These methods can include detecting a level of fluorescence emitted by a recombinant peptide biosensor expressed from a nucleic acid and correlating the level of fluorescence with the presence the defined, selected, or specific analyte. In some instances, methods include contacting the recombinant peptide biosensor with a sample comprising the analyte. In some instances, methods include correlating the level of fluorescence with a concentration of the analyte. In some instances, methods include comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte, wherein the level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte is stored on an electronic database.

[0053] In some instances, the present disclosure provides methods for detecting a defined, selected, or specific analyte, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor of any one of claims **1-36**; and correlating the level of fluorescence with the presence of a defined, selected, or specific analyte. In some instances, recombinant peptide biosensors can be

expressed from a nucleic acid. In some instances, methods can include contacting the recombinant peptide biosensor with a sample comprising the analyte. In some instances, methods can include correlating the level of fluorescence with a concentration of the analyte and, optionally, comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte. In some instances, the level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte is stored on an electronic database.

[0054] Methods herein can be performed in vitro.

[0055] In some instances, the present disclosure provides compositions containing any one or a plurality of the peptide biosensors and/or nucleic acids disclosed herein.

[0056] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

[0057] Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

[0058] FIG. **1** Cartoon representation showing ligand bound *Escherichia coli* malto-dextrin-binding protein (EcMBP) and potential circularly-permuted fluorescent protein (cpFP) insertion sites.

[0059] FIG. **2** Cartoon representation showing ligand bound *Pyrococcus furiosus* maltotriose binding protein (PfMBP) and potential cpFP insertion sites.

[0060] FIG. **3** Cartoon representation showing ligand bound *E. coli* glutamate-binding protein (EcYbeJ) and potential cpFP insertion sites.

[0061] FIG. **4** Cartoon representation showing ligand bound *E. coli* phosphonate-binding protein (EcPhnD) and potential cpFP insertion sites.

[0062] FIG. **5** Cartoon representation showing ligand bound *Thermus thermophilus* glucose binding protein (TtGBP) and potential cpFP insertion sites.

[0063] FIG. **6**A-B Changes in EcMBP upon maltose binding and locations at which circularly-permuted fluorescent protein (cpFP) was inserted are shown as colored spheres at the Cα positions. Yellow: 165-166, Green: 175-176, Cyan: 311-312, Violet: 317-318 (FIG. **6**A). (FIG. **6**B) shows backbone structural changes. The Cα dihedral is calculated from the four atoms: Cαi+2, Cαi+1, Cαi, Cαi−1. ΔDihedral is calculated as the difference in dihedrals between the closed (1ANF) and open (1OMP) states of MBP, and corrected to fall within a range of −180° to 180°. The regions near residues 175 and 311 are labeled. There is a crystallographic artifact at the N-terminus resulting in the appearance of significant structural changes.

[0064] FIG. **7**A Amino acid sequence of MBP-165-cpGFP (SEQ ID NO:1).

[0065] FIG. **7**B Amino acid sequence of MBP-165-cpGFP. PPYF (SEQ ID NO:2).

[0066] FIG. **7**C Amino acid sequence of MBP-165-cpGFP. PCF (SEQ ID NO:3).

[0067] FIG. **8**A Amino acid sequence of MBP-175-cpGFP (SEQ ID NO:4).

[0068] FIG. **8**B Amino acid sequence of MBP-175-cpGFP. L1-HL (SEQ ID NO:5).

[0069] FIG. **9**A Amino acid sequence of MBP-311-cpGFP (SEQ ID NO:6).

[0070] FIG. **9**B Amino acid sequence of MBP-311-cpGFP. L2-NP (SEQ ID NO:7).

[0071] FIG. **10** Amino acid sequence of MBP-317-cpGFP (SEQ ID NO:8).

[0072] FIGS. **11**A-**11**D Line charts showing EcMBP plot of ΔF/F for clarified lysate screen of cpGFP linker-screens at insertion points 165, 175, 311, and 317. The horizontal dashed line at zero indicates no fluorescence change. Standard deviations in ΔF/F are less than 10% of an average ΔF (repetitions for MBP165-cpGFP.PPYF yields ΔF/F values of 2.51, 2.63, and 2.54).

[0073] FIG. **12** Isothermal titration calorimetry (ITC) of MBP317-cpGFP with maltose.

[0074] FIG. **13** Graph showing EcMBP165-cpGFP.PPYF affinity variant binding maltose-binding curves. Binding curves for affinity variants of MBP165-cpGFP.PPYF. Data is fit to a single-binding site isotherm. Curve-fit affinities are: WT binding pocket, 5 μM (●); W230A, 32 μM (■); W62A, 375 μM (▲); W340A, >1 mM (▼); 1329W, 11 μM (□).

[0075] FIGS. **14**A-**14**D Line graphs showing maltose and sucrose binding curves for wild-type and 5-7 variants of the EcMBP-cpGFP sensors. Maltose (black) and sucrose (red) binding curves for wild-type (filled, solid lines) and 5-7 variants (open, dashed lines) of the MBP-cpGFP sensors. MBP165-cpGFP.PPYF (FIG. **14**A); MBP165-cpGFP.PCF (FIG. **14**B); MBP175-cpGFP.L1-HL (FIG. **14**C); MBP311-cpGFP.L2-NP (FIG. **14**D).

[0076] FIGS. **15**A-**15**D Line graphs showing emission spectra for colored variants of EcMBP sensors. Fluorescence emission spectra of the MBP165-Blue, Cyan, Green, and Yellow wild-type sensors (FIG. **15**A) and the 5-7 variants (FIG. **15**B) in the absence of ligand (dashed lines, open circles), with 10 mM maltose (solid lines, filled circles), or 10 mM sucrose (solid lines, filed squares). Sensors were excited at 383, 433, 485, and 485 nm, respectively. Titration of maltose and sucrose in the Blue, Cyan, Green, and Yellow MBP165 wild-type sensors (FIG. **15**C) and for the 5-7 variants (FIG. **15**D). Filled circles are titration of maltose, open circles are titration of sucrose. For the wild-type sensors, Kds for maltose binding are: Blue 3.3 Cyan 13 μM, Green 4.5 μM, Yellow 3.3 μM. No sucrose binding is observed. For the 5-7 variants, Kd of Green is 2.4 mM (sucrose) and 7.1 mM (maltose). Kd of Yellow is 2.5 mM (sucrose) and 4.5 mM (maltose).

[0077] FIG. **16** Plot of ΔF/F for clarified lysate screen of MBP165-cpBFP linker-screen. The horizontal dashed line at zero indicates no fluorescence change.

[0078] FIGS. **17**A-**17**B Line graphs showing maltose binding. Blue (wt binding pocket) has an affinity of 2.7 μM. Green (W230A) has an affinity of 40 μM. Yellow (W62A) has an affinity of 350 μM. Cyan (W340A) has an affinity of

approximately 1.7 mM. Data is plotted at ΔF/F (FIG. **17**A) or normalized to Fractional Saturation (FIG. **17**B).

[0079] FIGS. **18**A-**18**C Images bacterial cells expressing (FIG. **18**A) EGFP, (FIG. **18**B) PPYF, or (FIG. **18**C) PPYF. T203V in the absence (top) and presence (bottom) of maltose.

[0080] FIGS. **19**A-**19**B Line graphs showing EcMBP-cpGFP.PPYF.T203V 2-photon excitation spectra. MBP165-cpAzurite.L2-FE (FIG. **19**A), -cpCFP.PCF (FIG. **19**A), -cpGFP.PPYF (FIG. **19**B), and -cpYFP.PPYF (FIG. **19**B) were excited at the wavelengths indicated and emission measured through appropriate wavelength filters. Two graphs are shown to present different y-axis scales. Optimal ΔF/F values for 2-photon excitation of the spectral variants of MBP165 are: –cpAzurite, 1.1 (ex 760 nm); –cpCFP, 2.3 (ex 830-960 nm); –cpGFP, 10.0 (ex 940 nm); –cpYFP, 2.6 (ex 940 nm).

[0081] FIGS. **20**A-**20**C Images showing EcMBP-cpGFP. PPYF.T203V expressing HEK cells. Images of individual HEK293 cells expressing membrane displayed PPYF. T203V in the absence of maltose (FIG. **20**A), in the presence of 1 mM maltose (FIG. **20**B), and after washout with maltose-free buffer (FIG. **20**C). Scale bars are 10 μm.

[0082] FIGS. **21**A-**21**B Graphs showing quantification of fluorescence of EcMBP-cpGFP.PPYF.T203V when displayed on the surface of HEK cells. (FIG. **21**A) Concentration dependence. (FIG. **21**B) Observed fluorescence after a "puff" of HBSS solution containing 1 mM maltose and 2.5 nM Alexa Fluor® 568 (Invitrogen, Carlsbad, Calif.).

[0083] FIGS. **22**A-**22**D Cartoon representations and close-up views of inter-domain linkers and selected amino acids of the cpGFP chromophore environment of the structure of MBP175-cpGFP.L1-HL (FIG. **22**A and FIG. **22**B) and MBP311-cpGFP.L2-NP (FIG. **22**C and FIG. **22**D) bound to maltose. The MBP domain is colored as in FIG. **1**. The cpGFP domain is green and the inter-domain linkers are colored white. The cpGFP chromophore is displayed as sticks and the bound maltose as red and white spheres. Ordered water molecules are represented as red spheres. Selected hydrogen bonds are displayed as dashed black lines. β-strands 10 and 11 of cpGFP are displayed as semi-transparent for clarity. The 2Fo-Fc electron density map calculated with the displayed residues omitted from the model is shown as blue mesh.

[0084] FIGS. **23**A-**23**D EcMBP-cpGFP: effect of T203V mutation on fluorescence. (FIG. **23**A) Emission spectra of 1 μM purified eGFP (filled circles), cpGFP (filled squares), MBP165-cpGFP.PPYF (open circles), and MBP165-cpGFP. PPYF+T203V (open squares) in the absence (dashed lines) or presence (solid lines) of 1 mM maltose. cpGFP is half as bright as eGFP, and the saturated MBP165-cpGFP.PPYF variants are about half as bright as cpGFP. (FIG. **23**B) Titration of maltose for MBP165-cpGFP.PPYF (filled squares), and MBP165-cpGFP.PPYF+T203V (filled circles). Affinities for each protein are the same, but with different ΔF/F. (FIG. **23**C) Emission spectra of 1 μM purified eGFP (filled circles), cpGFP (filled squares), MBP311-cpGFP.L2-NP (open circles), and MBP311-cpGFP.L2-NP+ T203V (open squares) in the absence (dashed lines) or presence (solid lines) of 1 mM maltose. Note that mutation T203V decreases the fluorescence of both the apo-state and the saturated state of MBP311-cpGFP.L2-NP. (FIG. **23**D) Titration of maltose for MBP311-cpGFP.L2-NP (filled squares), and MBP311-cpGFP.L2-NP+T203V (filled circles). Affinities for each protein are the same, but with ΔF/F slightly increased for the T203V variant.

[0085] FIG. **24**A Amino acid sequence of PfMBP171-cpGFP (SEQ ID NO:50)

[0086] FIG. **24**B Amino acid sequence of PfMBP171cpGFP.L2-FE (SEQ ID NO:51)

[0087] FIG. **25**A Amino acid sequence of PfMBP316-cpGFP (SEQ ID NO:52)

[0088] FIG. **25**B Amino acid sequence of PfMBP316-cpGFP.L1-NP (SEQ ID NO:53)

[0089] FIG. **26**A-**26**B Plot of ΔF/F for clarified lysate screen of cpGFP linker-screens at insertion points 171 (FIG. **26**A) and 316 (FIG. **26**B).

[0090] FIGS. **27**A-**27**D Plot of Beta-sheet circular dichroism (CD) signal as a function of temperature.

[0091] FIGS. **28**A-**28**B PfMBP Fluorescence vs. temperature. (FIG. **28**A) Plot of fluorescence as a function of temperature in the presence (solid) or absence (dashed) of ligand. (FIG. **28**B) Plot of ΔF/F as a function of temperature. Using the data from FIG. **27**A, ΔF/F for each protein (Fbound-Fapo/Fapo) was calculated for each temperature.

[0092] FIGS. **28**C-**28**E Line graphs showing the function of immobilized and soluble proteins.

[0093] FIG. **29**A Amino acid sequence of EcYbeJ253-cpGFP (SEQ ID NO:62).

[0094] FIG. **29**B Amino acid sequence of EcYbeJ253-cpGFP.L1LVL2NP (SEQ ID NO:63).

[0095] FIG. **30** EcYbeJ binding curves. Plot of ΔF/F as a function of [Glutamate], μM. The first generation sensor, EcYbeJ253.L1-LV (with the A184V) mutation (grey, solid) has an affinity for glutamate of about 100 μM and a ΔF/F of 1.2. The reversion of that affinity mutation, V184A, in the L1-LV background increases affinity to 1 (grey dashed). The second generation sensor, with the L2-NP linker optimization and the A184V mutation, has a ΔF/F of at least 4 and an affinity for glutamate of about 100 μM (black solid).

[0096] FIG. **31** EcYbeJ Hema/cMyc analysis. The effect of N- and C-terminal tags on ΔF/F and glutamate affinity were determined by expressing variously tagged versions of the EcYbeJ253.L1LVL2NP protein in bacteria. The presence of the pRSET leader sequence (black) has no effect on ΔF/F (~5) or affinity (~120 when compared to the version without a tag (grey). The addition of the cMyc tag to the C-terminus retains ΔF/F and increases affinity slightly, to 60 μM. The addition of the N-terminal hemagglutinin tag, with (green) or without (orange) the cMyc tag, decreases ΔF/F substantially.

[0097] FIGS. **32**A-**32**B EcYbeJ253-cpGFP.L1LVL2NP. pMinDis expressed in HEK293 cells. (FIG. **32**A) Images of the sensor expressing HEK cells in the absence of glutamate (left), with 100 μM glutamate (center), and re-imaged after wash-out of glutamate with buffer (right). (FIG. **32**B) By measuring the equilibrium ΔF/F with different concentrations of glutamate in the buffer, an in situ binding affinity (black) can be obtained. The surface displayed sensor has a higher affinity (3 μM) for glutamate than the soluble sensor (grey), which is about 90 μM.

[0098] FIG. **33** EcYbeJ253-cpGFP.L1LVL2NP.pMinDis expressed in neuronal culture, and responds rapidly to added glutamate (green). Red shows signal of 2.5 nM Alexa Fluor® 568 (Invitrogen, Carlsbad, Calif.), also in pipette.

[0099] FIG. **34**A Amino acid sequence of EcPhnD90-cpGFP (SEQ ID NO:77).

[0100] FIG. **34**B Amino acid sequence of EcPhnD90-cpGFP.L1AD+L297R+L301R (SEQ ID NO: 78).

[0101] FIGS. **35**A-**35**C EcPhnD90-cpGFP Binding Curves. For both the L1AD and the L1AD+L297R+L301R variants, binding was determined for (FIG. **35**A) 2-aminoethylphosphonate (2AEP), (FIG. **35**B) methylphosphonate (MP), and (FIG. **35**C) ethylphosphonate (EP).

[0102] FIGS. **36**A-**36**C The crystal structures of the ligand-free (FIG. **36**A), open state (with H157A mutation to the binding pocket) and the ligand-bound (FIG. **36**B), closed state of EcPhnD clearly shows a large conformational change. Residues in between which cpGFP is inserted in EcPhnD90-cpGFP are marked by red spheres, in the equatorial strand (red). (FIG. **36**C) Analysis of the change in Cα dihedral (ΔDihedral) clearly shows that residues for which there is the greatest ΔDihedral upon going from the open to the closed state are residues 88 (ΔDihedral=)−75°, 89 (ΔDihedral=123°), and 90 (ΔDihedral=52°).

[0103] FIG. **37**A Amino acid sequence of TtGBP326-cpGFP (SEQ ID NO:91).

[0104] FIG. **37**B Amino acid sequence of TtGBP326.L1-PA (SEQ ID NO:92).

[0105] FIG. **37**C Amino acid sequence of TtGBP326. H66A (SEQ ID NO:93).

[0106] FIG. **37**D Amino acid sequence of TtGBP326. H348A (SEQ ID NO:94).

[0107] FIG. **38** TtGBP326-cpGFP Binding Curves. Plot of ΔF/F as a function of [Glucose], mM.

[0108] FIG. **39** An image showing TtGBP326-cpGFP expressed as a transgenic reporter of intracellular glucose in cultured human cells.

[0109] FIGS. **40**A-**40**B Are line graphs showing that the addition of extracellular glucose increases TtGBP326-cpGFP fluorescence in human cells.

[0110] FIG. **41** Amino acid sequence of *Escherichia coli* maltodextrin-binding protein (EcMBP) (SEQ ID NO: 105).

[0111] FIG. **42** Amino acid sequence of *Pyrococcus furiosus* maltose-binding protein (PfMBP) (SEQ ID NO: 106).

[0112] FIG. **43** Amino acid sequence of *E. coli* glutamate-binding protein (EcYbeJ) (SEQ ID NO:107).

[0113] FIG. **44** Amino acid sequence of *E. coli* phosphonate-binding protein (EcPhnD) (SEQ ID NO:108).

[0114] FIG. **45** Amino acid sequence of *Thermus thermophilus* glucose-binding protein (TtGBP) (SEQ ID NO:109).

[0115] FIG. **46** Amino acid sequence of UniProt accession number Q92N37 (SEQ ID NO: 110).

[0116] FIG. **47** Amino acid sequence of UniProt accession number DOVWX8 (SEQ ID NO:111).

[0117] FIG. **48** Amino acid sequence of UniProt accession number Q7CX36 (SEQ ID NO:112).

[0118] FIG. **49** Amino acid sequence of UniProt accession number POAD96 (SEQ ID NO:113).

[0119] FIG. **50** Amino acid sequence of TtGBP326.L1PA. L2NP.H66A.H348A.L276V (SEQ ID NO:114).

[0120] FIG. **51** A line graph showing binding of TtGBP326.L1PA.L2NP.H66A.H348A.L276V to glucose.

[0121] FIG. **52** A line graph showing fluorescence increase upon addition of glucose to HEK293 cells expressing TtGBP326.LIPA.L2NP.H66A.H348A.L276V on their extracellular surface.

[0122] FIG. **53**A-D SF-iGluSnFR.A184S shows larger responses to visual stimuli than SF-iGluSnFR.A184V. (FIG. **53**A) Two-photon standard-deviation projection of SF-iGluSnFR.A184S and A184V expressed in ferret visual cortex (A184S: 190 μm, A184V: 175 μm, scale bar 100 μm). (FIG. **53**B) Trial-averaged stimulus-evoked responses (shown for ROI 1) reveal robust orientation tuning and peak amplitudes of ~30% ΔF/F for A184S. Peak responses plotted as a function of stimulus orientation show robust selectivity with the A184S variant. For the A184V variant, stimulus-evoked fluctuations are too small (~5% ΔF/F) to generate robust tuning plots. (FIG. **53**C) Two-photon standard-deviation projection of an isolated dendritic segment with active spines revealed with SF-iGluSnFR.A184S. Individual dendritic spines are driven selectively and strongly by drifting gratings. Orientation tuning from peak responses shows large spine responses (30-50% ΔF/F) and, importantly, reveals that spines on a single dendritic branch can receive differently tuned excitatory input. (FIG. **53**D) Same as in (FIG. **53**C) for SF-iGluSnFR.A184V. Dendritic spine responses with A184V are weak and almost unresolvable.

[0123] FIG. **54**A-D SF-iGluSnFR. S72A permits resolution of multiple glutamate release events in cultured mouse embryonic hippocampal neurons. (FIG. **54**A) Single (dashed) and averaged (solid) traces of SF-iGluSnFR.S72A (blue) and SF-iGluSnFR.A184V (red) response to 20 Hz paired electrical stimuli. (FIG. **54**B) Histogram showing intensity second pulse to first pulse response. (FIG. **54**C) The faster off-rate of S72A can be used to observe vesicle release depression. Higher concentrations of extracellular calcium can increase vesicle release, leading to vesicle exhaustion as the train of field pulses progresses. (FIG. **54**D) The slow decay of A184V obscures this depression.

[0124] FIG. **55**A-D S72A variant shows faster bouton fluorescence signals resulting from single or trains of electrical stimulation mouse cerebellar brain slice. (FIG. **55**A) Averaged response from single boutons expressing GCaMP6f (GC6f) at 2 mM $[Ca^{2+}]_{extracellular}$ (green), GC6f at 1.5 mM $[Ca^{2+}]_e$ (black), SF-iGluSnFR.A184V at 1.5 mM $[Ca^{2+}]_e$ (A184V, red) and SF-iGluSnFR.S72A at 1.5 mM $[Ca^{2+}]_e$ (S72A, blue), normalized to peak response. In parenthesis the number of trials used to calculate the average. Right, summary plots of $\Delta F/F_0$, 10-90% rise time, 50% decay time and signal-to-noise-ratio (SNR). Multiple comparisons were performed with the Kruskal-Wallis test and the Dunn's multiple comparisons test. *P<0.05, **P<0.01, ****P<0.0001. (FIG. **55**B) Two-photon fluorescent image of granule cells and parallel fibers expressing A184V in cerebellum slice (GL—granule layer, ML—molecular layer). Yellow arrows indicate labeled soma of granule cells, and circle indicate boutons from parallel fibers. Bottom, example of single trial A184V fluorescence responses to 20 Hz electrical stimulation (red) and the average of 10 trials (purple). (FIG. **55**C) Population average fluorescence responses to 20 Hz stimulation (n boutons=5 GC6f; n=17, A184V; n=3, S72A). Traces are normalized to the peak of the first response. (FIG. **55**D) Population average of response to 100 Hz electrical stimulation (n boutons=9 GC6f; n=9, A184V; n=7, S72A) normalized to the maximum amplitude (left) or to the peak of the first response (middle), and average response of all the boutons. n is number of boutons. Black arrows indicate time of electrical stimulation.

[0125] FIG. **56**A-B High-speed two photon imaging (1016 Hz frame rate) of a neuron expressing SF-Venus-iGluSnFR. (FIG. **56**A) RuBi-glutamate was uncaged for 10 msec. at each of two 5 μm spots (red arrowheads) on the dendrites. Saturation denotes the glutamate transient amplitude. Yel-

low line indicates locations for traces shown in (FIG. **56**B). (FIG. **56**B) Recorded traces at nine pixels at various distances from the uncaging focus, along the yellow line in (FIG. **56**A). The traces are approximate maximum likelihood solutions recovered with the FADE algorithm. (Kazemipour et al., Proceedings of the 2017 Asilomar Conference on Signals, Systems, and Computers, October 29-November 1, Pacific Grove, Calif.), which incorporates dynamics having arbitrarily fast rise but slow decay. This recording is of a single uncaging event, without averaging.

[0126] FIG. **57** Circular dichroism of iGluSnFR and SF-iGluSnFR. 20 μM purified and dialyzed protein in 0.1×PBS was analyzed by circular dichroism (Chirascan, Applied Biophysics). Grey, iGluSnFR; black, SF-iGluSnFR; green, cpSFGFP; thick line, with 1 mM glutamate; thin line, no glutamate. Spectra were collected with a 1 sec. sampling time after equilibration for 2 min at each temperature. The first unfolding transition is shifted from about 50° C. to 55° C. by inclusion of the Superfolder mutations to cpGFP. Interestingly, the second transition, at about 75° C., which parallels the transition of cpSFGFP alone, is unchanged.

[0127] FIG. **58**A-D Spectra of SF-iGluSnFR. (FIG. **58**A) 2-photon cross-section of purified, soluble iGluSnFR (grey) and SF-iGluSnFR (black) in the ligand-free (dashed line) and glutamate-saturated (solid line) state. Excitation (FIG. **58**B), emission (FIG. **58**C), and absorption spectra (FIG. **58**D) of iGluSnFR (grey), SF-iGluSnFR (black), and cpSFGFP (green) with glutamate (solid line) and without (dashed line).

[0128] FIG. **59**A-E Representative images of (FIG. **59**A) SF-iGluSnFR and (FIG. **59**B) iGluSnFR in mouse somatosensory cortex taken at 0.9 μm/pixel, 0.126 nsec dwell time per μm, 80 mW power, prior to bleaching. 20 nl of AAV2/1.hSynapsin1.iGluSnFR or SF-iGluSnFR (identical virus titer, prepared by the same person) was injected three weeks before imaging. Contrast adjusted to 10 grayscales in both images to make original iGluSnFR observable. Mean signal-to-noise ratios (n=2 animals) are 66 vs. 14 (80 mW power) and 2.4 vs. 0.3 (5 mW power). (FIG. **59**C) & (FIG. **59**D) Representative images of SF-iGluSnFR and iGluSnFR taken with 5 mW power, which is more typical in live imaging conditions. (FIG. **59**E) Bleaching of SF-iGluSnFR (black) and original iGluSnFR (grey) at 80 mW power and 10× zoom (0.09 μm/pixel, 1.26 nsec dwell time per μm).

[0129] FIG. **60**A-B In vitro binding affinity. (FIG. **60**A) Titration of bacterially expressed iGluSnFR and SF-iGluSnFR and variants. Affinities (K_d) for original iGluSnFR, SF-iGluSnFR.A184S, SF-iGluSnFR.A184V, and SF-iGluSnFR.S72A are 84±7 μM, 7.5±0.4 μM, 41±7 μM, and 200±5 μM respectively. (FIG. **60**B) Kinetics of glutamate binding by stopped-flow fluorescence spectroscopy. Equal volumes of 1 μM SF-iGluSnFR (A184S, A184V, or S72A) and glutamate (variable concentration) were mixed in an SX.18MV stopped-flow spectrometer (Applied Photophysics, Surrey, UK). Representative traces shown. Pseudo-first order analysis indicates that the on-rate of binding for SF-iGluSnFR.A184S, A184V, S72A are 6, 5, and 0.6 μM^{-1} sec^{-1}, respectively. The off rates, as determined by the y-intercept, are 25 sec^{-1}, 52 sec^{-1}, and 108 sec^{-1} respectively. Error bars are standard deviation of three measurements.

[0130] FIG. **61** Binding affinity screening. Pellets of bacterially expressed A184X variants of iGluSnFR were washed 5 times in PBS to remove bound glutamate. After freezing and thawing, pellets were clarified by centrifugation and titrated with glutamate to screen for their affinity for glutamate. There is a general trend of larger amino acids resulting in weaker affinity.

[0131] FIG. **62** Affinity of SF-iGluSnFR variants displayed on the surface of neurons. AAV2/1.hSynapsin1.SF-iGluSnFR variants (1 μl of 1E13 GC/ml) were used to infect rat hippocampal neuronal culture 3 days after culturing. After 10 days in vitro, fluorescence was monitored under continuous flow of buffer with varying concentrations of glutamate. Affinities (K_d) for SF-iGluSnFR.A184S, SF-iGluSnFR.A184V, and SF-iGluSnFR.S72A are 0.6, 2.1, and 34 μM respectively. Affinities for SF-Venus.A184V and SF-Azurite.A184V are 2.0 and 9 μM respectively. Bottom panel is zoom-in of top panel.

[0132] FIG. **63**A-B Rise and decay of fluorescence signal resulting from a single field stimulation (1 msec., 90 mA) in rat hippocampal culture (10 DIV, 7 DPI) in non-flowing buffer (FIG. **63**B). Traces in FIG. **63**A are the average of three ROIs (bottom) and three trials (top); error bars are standard deviation of those nine measurements. The large error for GCaMP6f results from back propagating action potentials, which can be seen in differences from individual ROIs.

[0133] FIG. **64** Examples of individual responses for ROIs 1, 2, and 3 (top). Responses of individual Spines #2 and #3 (from FIG. **53**) (middle). Histogram showing distribution of spine responses (bottom). Response amplitudes across individual trials were consistently greater for A184S than the A184V when examining all stimulus-evoked responses.

[0134] FIG. **65**A-D Mouse neuronal culture images. The fluorescent labeling pattern and intensity of primary hippocampal neurons transduced with AAV2/1.hSynapsin1.SF-iGluSnFR.S72A or with AAV2/1.hSynapsin1-SF-iGluSnFR.A184V at DIV4 and imaged at DIV13 looked qualitatively similar for both variants and as expected for a membrane targeted protein. To resolve fast stimulus associated changes in fluorescence, a time series of 100 frames at 60 Hz during a paired-pulse stimulation paradigm was acquired. Basal fluorescence before stimulation was clearly stronger for A184V, the high affinity sensor, leading to a higher SNR (FIG. **65**A and FIG. **65**C). However, when dividing each frame by an average of the pre-stimulus images for both variants of SF-iGluSnFR localized spots where fluorescence increases was observed (FIG. **65**B, FIG. **65**D, arrows), likely representing synaptic release sites. ROIs were defined based on these spots, and fluorescence within these ROIs (background subtracted) was averaged for every image in the time series.

[0135] FIG. **66**A-J Vesicle release sites can be localized by identifying the center of stimulus-evoked SF-iGluSnFR fluorescence changes. (FIGS. **66**A and **66**B) Representative images of SF-iGluSnFR.S72A and SF-iGluSnFR.A184V expression in primary neuron cultures. Markers indicate the centers of Gaussians fitted to fluorescence profiles calculated across identified release sites from consecutive stimulation trials (such as shown in (FIG. **66**E)). Note that the scatter of the centers of the localized release sites is substantially larger for SF-iGluSnFR.A184V (16-25 stimulation trials per experiment with inter-stimulus intervals of 20-60 s, 20 frames before and 10 frames after stimulation were recorded). (FIGS. **66**C and **66**D) Spots of increased fluorescence as they occur immediately after electrical stimulation when neurons are expressing SF-iGluSnFR.S72A or

SF-iGluSnFR.A184V. 10 frames after the stimulus were averaged and divided by an average of 5 frames before stimulation. In this way, structures, which do not change fluorescence after simulation (background/inactive dendritic segments) will become 1. The lookup table of these images was adjusted to range from 1 to 1.5. (FIG. **66**E) Line profiles calculated across the response sites shown in (FIG. **66**C) and (FIG. **66**D) (dashed lines) and superimposed Gaussian fits (lines). The width of the fitted Gaussian profiles were 0.57 and 1.11 μm for SF-iGluSnFR.S72A and SF-iGluSnFR.A184V, respectively. (FIG. **66**F) Localization is more precise for SF-iGluSnFR. S72A. For each selected responding site (n=28-53), the mean deviation of the center of the Gaussians across the stimulation trials was calculated. These values were averaged and bar graphed for each SF-iGluSnFR variant. (FIGS. **66**G and **66**H) Width and amplitude of fitted Gaussian functions are significantly larger for the high affinity A184V sensor. (FIG. **661**) Gaussian fits to profiles obtained from individual (not averaged) frames after stimulus reveal the persistence of the SF-iGluSnFR.A184V variant. (FIG. **66**J) Left: Gaussians fitted to the SF-iGluSnFR.A184V-mediated signal progressively broaden over time indicating that also sensor molecules remote to the site of release bind glutamate. Right: Same data as on left, but plotted as width^2 over 4*t. The data points can be approximated by a line consistent with a diffusional spread of glutamate. The slope of the fitted line estimates the apparent (A184V-slowed) diffusion coefficient (D_{app}) of synaptically released glutamate to be 4.3 μm^2/s in vitro. This value is orders of magnitude smaller than the diffusion coefficient of free glutamate in solution (~600-700 μm^2/s) indicating that A184V not only prolongs but also substantially localizes glutamate molecules at the sites of release.

[0136] FIG. **67**A-C Annotated amino acid sequences of SF-iGluSnFR (FIG. **67**A), SF-Azurite-iGluSnFR (FIG. **67**C), and SF-Venus-iGluSnFR (FIG. **67**B). Domains colored as indicated. Affinity modulating mutations S72A and A184V/S are indicated by orange arrow. Mutations from SF-iGluSnFR to SF-Venus-iGluSnFR and SF-Azurite-iGluSnFR indicated in red.

[0137] FIG. **68**A-D Characterization of soluble, purified SF-Azurite-iGluSnFR. (FIG. **68**A) Titration of SF-Azurite-iGluSnFR yields a K_d of 62±11 μM, error bars are standard deviation of three measurements. Excitation (FIG. **67**B), emission (FIG. **67**C), and absorption (FIG. **68**D) spectra of SF-Azurite-iGluSnFR (light blue) and Azurite (dark blue), with glutamate (solid line) and without (dashed line).

[0138] FIG. **69**A-D Spectra of SF-Venus-iGluSnFR. Excitation (FIG. **69**A), emission (FIG. **69**B), and absorbance (FIG. **69**C) spectra of SF-Venus-iGluSnFR (yellow) with (solid line) and without (dashed line) glutamate. (FIG. **69**D) 2-photon spectrum with SF-iGluSnFR (black) and vertical 1030 nm markup included for reference.

[0139] FIG. **70**A-F Annotated amino acid sequences of the SF biosensors disclosed herein. Affinity modulating mutations S72A and A184V/S are indicated with small case letters. For SEQ ID NOs: 176-182, each domain is indicated with underlining as follows: IgG secretion signal ; GltI5-253; SF-GFP147-238; Linker ; SF-GFP1-146; Glt1254-279; Myc epitope ; PDGFR transmembrane domain 513-561 . SF-iGluSnFR.A184V (SEQ ID NO: 179); SF-iGluSnFR.A184S (SEQ ID NO: 177); SF-iGluSnFR.S72A (SEQ ID NO:178); SF-Venus-iGluSnFR.A184V (SEQ ID NO: 179; mutations at residues T203Y and Y65G to shift the color and at residues F46L and S72A to increase chromophore maturation are shown in lower case); SF-Venus-iGluSnFR.A184S (SEQ ID NO: 180; mutations at residues T203Y and Y65G to shift the color and at residues F46L and S72A to increase chromophore maturation are shown in lower case); SF-Venus-iGluSnFR.S72A (SEQ ID NO: 181; mutations at residues T203Y and Y65G to shift the color and at residues F46L and S72A to increase chromophore maturation are shown in lower case); SF-Azurite-iGluSnFR (SEQ ID NO: 182; mutations at residues T65S and Y66H to shift the color and at residues V150I and V224R to improve maturation and brightness are shown in lower case; Linker1 mutations: GltI-cpSFGFP connection from PILVSHNV (SEQ ID NO: 187) to PILGYHNV (SEQ ID NO: 188); Linker2 mutations: cpSFGFP-GltI connection from YNFNNPLN (SEQ ID NO: 189) to YNFNEQLN (SEQ ID NO: 190)); iDexSnFR (or SF-GlucoseSensor) (SEQ ID NO: 183); iGABASnFR (SEQ ID NO: 184; cpSFGFP was inserted after D276 of the Pf622 starting sequence. Insertion of cpSFGFP is after residue D276 of Pf622. Residues RS near the N-terminus encode BglII, and residues LQ at the C-terminus encode PstI. Mutations included in iGABASnFR include: affinity modulating hinge mutation: Pf622: F101L; Pf622-SFGFP interface: Pf622: N260A; Linker 1: SHNVY (SEQ ID NO: 191) of SFGFP to LAQVR (SEQ ID NO: 192) (SFGFP: S147L, H148A, N149Q, Y151R); Linker 2: SFGFP (SEQ ID NO: 193): F145W; Linker 2: SVLAP (SEQ ID NO: 194) of Pf622 to ANLAP (SEQ ID NO: 195) (Pf622: S277A, V278N); Binding site mutation: Pf622: F102G/Y. Underlining indicates the domain as follows: IgG secretion signal Pf6222-276; SF-GFP147-238; Linker; SF-GFP1-146; Pf622277-320; Myc epitope PDGFR transmembrane domain 513-561 Binding site mutation F102 indicated with a small case letter; and iAChSnFR (*E. coli* expression vector shown in SEQ ID NO:185 with the domains indicated as follows: pHHM His tag, leader sequence *Thermoanaerobacter*sp.X513cholinebindingproteinsequence; Linkerregions; Circularlypermutedsuper-folderEGFP; Myc tag C -terminal sequence and mammalian expression vector shown in SEQ ID NO: 186 with the domains indicated as follows: IgG secretion sequence leader *Thermoanaerobacter*sp.X513cholinebindingproteinsequence; Linkerregions; Circularlypermutedsuper-folderEGFP; PDGFR transmembrane sequence) .

[0140] FIG. **71** A schematic of Structure I as described herein.

DETAILED DESCRIPTION

[0141] The present disclosure is based, at least in part, on the discovery of structures and methods related to and useful for genetically encoded biosensors. Specifically, the disclosure provides genetically encoded recombinant or chimeric peptides for use as biosensors and methods for the design, production, and use of such biosensors. As described below, these sensors can be employed (e.g., expressed) in biological systems to detect and/or monitor a wide range of target analytes (e.g., a defined, selected, and/or specific analytes) due, in part, to the signal change generated by the sensors upon binding to their respective analyte(s), which signal change allows bound and unbound sensors to be distinguished.

[0142] While the disclosure encompasses generic biosensors and methods related thereto, examples of particular

binding sensors, including biosensors for detecting maltose, sucrose, maltotriose, glutamate, phosphonate, and glucose are also disclosed.

Compositions

[0143] Provided herein are genetically encoded biosensors, i.e., nucleic acids encoding peptides, and/or the encoded peptides (e.g., isolated peptides), for use as biosensors. Biosensors herein include genetically encoded recombinant peptides containing an analyte-binding framework portion linked (e.g., operably linked) to at least one independent signaling portion, wherein the independent signaling portion is allosterically modulated or regulated by the framework portion upon interaction of the framework portion with an analyte (e.g., a defined, selected, and/or specific analyte), such that signaling from the signaling portions is altered upon interaction of the framework portion with the analyte.

[0144] In some instances, an independent signaling portion is present at a site within the framework portion that undergoes a conformational change upon interaction of the framework portion with an analyte such that the conformational change allosterically modulates or regulates signaling by the signaling portion. For example, biosensors herein can include structure I.

[0145] In some instances, signaling by the signaling portion is detectably altered upon interaction (e.g., binding) of the framework portion with an analyte. For example, signaling by the signaling portion can detectably increase or detectably decrease upon interaction (e.g., binding) of the framework portion with an analyte. In some cases, biosensors have a signal change upon binding (e.g., specific binding) to their respective analyte of at least about, for example, ±0.5, and/or an increase or decrease in signal of at least about, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 250%, 500%, 750%, 1000%, or more than 1000%, e.g., relative to unbound biosensor. In some increases, the level of signal change is linked to background signal. Values represented here can be converted and/or expressed into any conventional units using ordinary skill. For example, units can be expressed as 'signal change' (as used above), ΔF/F and/or as signal-to-noise ratio (e.g., ΔF/F multiplied by the square root of the number of photons collected). In some instances, signaling by a biosensor can be intensity based.

[0146] In some instances, biosensors herein are distinguishable from Forster resonance energy transfer, also known as fluorescence resonance energy transfer (FRET)-based sensors, which require donor and acceptor chromophores, e.g., that function in concert, in that they include independently functioning or detectable signaling portions. For example, in some instances, signaling by a first signaling portion of a biosensor herein is independent of signaling by a second signaling portion within the same or a distinct biosensor. As noted above, signaling portions are allosterically regulated by the framework portion to which they are linked upon interaction of the framework portion with an analyte (e.g., a defined, selected, and/or specific analyte).

Framework Portions

[0147] Framework portions include genetically encoded macromolecules (e.g., proteins or peptides) that undergo conformational alteration (e.g., a structural change) upon interaction (e.g., binding) with, or to, an analyte (e.g., an analyte-binding dependent conformational alteration). For example, genetically encoded framework portions can have a first structure in the absence of an analyte (e.g., in an unbound or open state) and a second structure, that is detectably distinct (e.g., differences in structures before and after a conformational change can be observed using methods known in the art) from the first structure, in the presence of an analyte (e.g., in a bound or closed state), e.g., under physiologic conditions. In some instances, the conformational change that occurs upon interaction with an analyte (e.g., an analyte-binding dependent conformational alteration) is detectably distinct (e.g., can be observed using methods known in the art) from a conformational change that may occur for the same protein or peptide under other physiological conditions (e.g., a change in conformation induced by altered temperature, pH, voltage, ion concentration, phosphorylation).

[0148] Methods for identifying proteins or peptides that exhibit suitable conformational characteristics and/or for observing differences in structure between structures or before and after a conformational change are known in the art and/or are described herein. Such methods can include, for example, one or more of structural analysis, crystallography, NMR, EPR using Spin label techniques, Circular Dichroism (CD), Hydrogen Exchange surface Plasmon resonance, calorimetry, and/or FRET.

[0149] In some instances, framework portions can have a first structure in the absence of an analyte (e.g., in an unbound or open state) and a second structure, that is detectably distinct (e.g., can be observed using methods known in the art) from the first structure, in the presence of an analyte (e.g., in a bound or closed state), e.g., under physiologic conditions, wherein the structural change between the open and closed state can allosterically modulate an independent signaling portion recombinantly (e.g., artificially introduced) present within the framework portion (see, e.g., Structure I).

[0150] Framework portions can also interact (e.g., bind) with at least one analyte (e.g., at least one defined, specific, and/or selected analyte). In some instances, a framework portion can interact specifically with one analyte (e.g., at least one defined, specific, and/or selected analyte). In such cases, affinity of binding between the framework binding peptide and the analyte can be high or can be controlled (e.g., with millimolar, micromolar, nanomolar, or picomolar affinity). Alternatively, the single framework binding protein can bind two or more analytes (e.g., two or more defined, specific, and/or selected analytes). In such cases, affinity of binding to the two or more analytes can be the same or distinct. For example, the affinity of binding can be greater for one analyte than it is for a second or third, etc., analyte. In some instances, binding between a framework portion and an analyte (e.g., at least one defined, specific, and/or selected analyte) have an affinity of for example, 10 mM to 1 pM.

[0151] As used herein, the term "analyte" can include naturally occurring and/or synthetic sugars, amino acids, proteins (e.g., proteins, peptides, and/or antibodies), hormones, ligands, chemicals (e.g., small molecules), pharmaceuticals, nucleic acids, cells, tissues, and combinations thereof.

[0152] In some instances, biosensors can include one, two, or more framework binding portions that bind (e.g., binds

specifically) a single analyte (e.g., a single defined, specific, and/or selected analyte) or distinct analytes (e.g., two or more distinct defined, specific and/or selected analytes). Alternatively or in addition, the framework portion can be chimeric. In such cases, a first part of the framework portion can be a first peptide or can be derived from a first peptide, and a second part of the framework portion can be a second peptide or can be derived from a second peptide, wherein the first a second peptides are combined to result in a single peptide.

[0153] Accordingly, framework portions can include macromolecules that undergo a conformational change upon interaction with an analyte. One non-limiting example of a suitable macromolecule is Calmodulin (CaM). CaM is in an extended shape in the absence of Ca^{2+} and in a condensed conformation in the presence of Ca^{2+} (Kuboniwa et al., Nat. Struc. Biol., 2:768-776, 1996 and Fallon and Quiocho, Structure, 11:1303-1307, 2003).

[0154] In some instances, a framework binding portion can be a bacterial protein or can be derived from a bacterial protein. Suitable bacterial proteins can include, but are not limited to, for example, periplasmic binding proteins (PBPs).

[0155] PBPs from bacteria are generally useful in the biosensors herein at least because they undergo dramatic conformational changes upon ligand binding (Ouiocho et al. Mol. Microbiol., 20:17-225, 1996). X-ray crystal structures of the apo (open) and bound (closed) forms of various PBPs reveal that these proteins have two (typically, although some have more) domains that undergo a large hinge-twist movement relative to each other in a Venus flytrap manner (Dwyer and Hellinga, Curr. Opin. Struc. Biol., 14:495-504, 2004). This conformational change has been exploited to create a number of FRET-based genetically encoded sensors (see, e.g., Deuschle et al., Pro. Sci, 14:2304-2314, 2005; Deuschle et al., Cytometry, 64:3-9, 2005; Okumoto et al., Proc. Natl. Acad. Sci. USA., 102:8740-8745, 2005; Bogner and Ludewig, J. Fluoresc., 17:350-360, 2007; and Gu et al., FEBS Letters, 580:5885-5893, 2006). In addition, the ligand-binding diversity of the PBP superfamily is large (Dwyer and Hellinga, Curr. Opin. Struc. Biol., 14:495-504, 2004).

[0156] In some instances, framework portions can include, for example, one or more of: arabinose-binding protein(s), glucose/galactose-binding protein(s), histidine-binding protein(s), maltose-binding protein(s), glutamine-binding protein(s), maltotriose-binding protein(s), RBP, ribose-binding protein(s), acetylcholine binding protein(s), choline binding protein(s), lysine binding protein(s), arginine binding protein(s), gamma aminobutyric acid (GABA) binding protein (s), ion-binding protein(s), peptide-binding protein(s), lactate-binding protein(s), histamine-binding protein(s), and/or Leucine/Isoleucine/Valine binding protein(s), including full length proteins, fragments, and/or variants thereof.

[0157] In some instances, exemplary framework portions can include: SEQ ID NO:105, which is *Escherichia coli* maltodextrin-binding protein (EcMBP) (UniProt accession number POAEX9); SEQ ID NO: 106, which is *Pyrococcus Furiosus* maltotriose-binding protein (PfMBP) (UniProt accession number P58300); SEQ ID NO:107, which is *E. coli* glutamate-binding protein (EcYbeJ) (UniProt accession number Q1R3F7); SEQ ID NO:108, which is *E. coli* phosphonate-binding protein (EcPhnD) (UniProt accession number P37902); and/or SEQ ID NO:109, which is *Thermus thermophilus* glucose-binding protein (TtGBP) (UniProt accession number Q72KX2, including full length proteins, fragments, and/or variants thereof.

[0158] In some instances, exemplary framework portions can include SEQ ID NO: 110 (UniProt accession number Q92N37); SEQ ID NO:111 (UniProt accession number D0VWx8, SEQ ID NO:112 (UniProt accession number Q7CX36), and/or SEQ ID NO:113 (UniProt accession number POAD96, including full length proteins, fragments, and/or variants thereof.

[0159] In some embodiments, exemplary framework portions can include residues 24-272 and 517-542 of SEQ ID NO: 176 (SF-iGluSnFR.A184V); residues 24-272 and 517-542 of SEQ ID NO: 177 (SF-iGluSnFR.A184S); residues 24-272 and 517-542 of SEQ ID NO: 178 (SF-iGluSnFR.S72A); residues 24-272 and 517-542 of SEQ ID NO: 179 (SF-Venus-iGluSnFR.A184V); residues 24-272 and 517-542 of SEQ ID NO: 180 (SF-Venus-iGluSnFR.A184S); residues 24-272 and 517-542 of SEQ ID NO: 181 (SF-Venus-iGluSnFR.S72A); residues 24-271 and 519-541 of SEQ ID NO: 182 (SF-Azurite-iGluSnFR); residues 24-350 and 595-664 of SEQ ID NO: 183 (iDexSnFR or SF-GlucoseSensor); residues 24-298 and 543-586 of SEQ ID NO: 184 (iGABASnFR); residues 25-99 and 348-545 of SEQ ID NO: 185 (iAChSnFR *E. coli* expression sequence); or residues 24-98 and 347-544 of SEQ ID NO: 186 (iAChSnFR mammalian expression sequence).

[0160] In some instances, framework portions, or biosensors, do not include signal peptides, or portions of signal peptides, that would otherwise be present in the peptide from which the framework portion is derived.

Signaling Portions

[0161] Biosensors herein include one or more genetically encoded signaling portions (e.g., independent signaling portions) within the amino acid sequence of a framework portion at a site(s) within the framework portion that undergo(es) a conformational change upon interaction of the framework portion with an analyte (e.g., a defined, specific, and/or selected analyte).

[0162] Signaling portions (e.g., independent signaling portions) include genetically encoded molecules (e.g., peptides or proteins) that can be allosterically induced to emit a detectable signal (e.g., an analyte-binding dependent signal).

[0163] In some instances, the detectable signal is detectably distinct (e.g., can be distinguished using methods known in the art and/or disclosed herein) from a signal emitted by the molecule prior to allosteric inducement (e.g., signaling portions can emit a detectable signal in two detectably distinct states. For example, first signal can be emitted in unbound state and a second signal can be emitted in bound state). As noted above, in some instances, the detectable signal is proportional to the degree of allosteric inducement. In some instances, if two or more signaling portions are present in a biosensor, then two or more detectably distinct signals can be emitted by the biosensor.

[0164] In some instances, a genetically encoded independent signaling portion is a genetically encoded fluorescent protein (FP), e.g., a macromolecule containing a functional group (e.g., a fluorophore) that absorbs energy of a specific wavelength and re-emits energy at a different (but equally specific) wavelength, including, for example, circularly permuted FP (cpFP). In some instances, a signaling portion is

a "superfolder" FP (e.g., Pedelacq et al., 2006, Nat. Biotech., 24:79-88), e.g., a circularly permuted SF FP.

[0165] As used herein, the term "fluorophore" relates to a functional group in a molecule which will absorb energy of a specific wavelength and re-emit energy at a different (but equally specific) wavelength. In some instances, fluorophore containing molecules include fluorescent proteins. The fluorophore in green fluorescent protein (GFP) includes Ser-Tyr-Gly sequence (i.e., Ser65-dehydroTyr66-Gly67), which is post-translationally modified to a 4-(p-hydroxybenzylidene)-imidazolidin-5. Exemplary genetically encoded fluorescent proteins include, but are not limited to, fluorescent proteins from coelenterate marine organisms, e.g., *Aequorea victoria, Trachyphyllia geoffroyi*, coral of the *Discosoma* genus, *Rennilla mulleri, Anemonia sulcata, Heteractis crispa, Entacmaea quadricolor*, and/or GFP (including the variants S65T and EGFP, *Rennilla mulleri* GFP), cyan fluorescent protein (CFP), including Cerulean, and mCerulean3 (described by Markwardt et al., PLoS ONE, 6(3) e17896.doi:10.1371/journal.pone.0017896), CGFP (CFP with Thr203Tyr: Has an excitation and emission wavelength that is intermediate between CFP and EGFP), yellow fluorescent protein (YFP, e.g., GFP-Ser65Gly/Ser72Ala/Thr203Tyr; YFP (e.g., GFP-Ser65Gly/Ser72Ala/Thr203Tyr) with Va168Leu/Gln69Lys); Citrine (i.e., YFP-Va168Leu/Gln69Met), Venus (i.e., YFP-Phe46Leu/Phe64Leu/Met153Thr/Val163Ala/Ser175Gly), PA-GFP (i.e., GFP-Val/163Ala/Thr203His), Kaede), red fluorescent protein (RFP, e.g., long wavelength fluorescent protein, e.g., DsRed (DsRed1, DsRed2, DsRed-Express, mRFP1, drFP583, dsFP593, asFP595), eqFP611, and/or other fluorescent proteins known in the art (see, e.g., Zhang et al., Nature Reviews, Molecular and Cellular Biology, 3:906-908, 2002).

[0166] As set forth above, in some instances, fluorophore containing molecules include fluorescent proteins that can be or that are circularly permutated. Circular permutation methods are known in the art (see, e.g., Baird et al., Proc. Natl. Acad. Sci., 96:11241-11246, 1999; Topell and Glockshuber, Methods in Molecular Biology, 183:31-48, 2002) as are "superfolder" (SF) proteins (e.g., Pedelacq et al., 2006, Nat. Biotech., 24:79-88) (e.g., circularly permuted SF proteins).

[0167] In some instances, single-FP sensors have a number of advantages: they preserve spectral bandwidth for multi-analyte imaging; their saturated states may be nearly as bright as the parental FP, and their ligand-free states may be arbitrarily dim, providing large theoretical fluorescence increases. This allows for much greater changes in fluorescence and thus increased signal-to-noise ratios and greater resistance to photobleaching artifacts (Tian et al., Nat. Methods, 6:875-881, 2009).

[0168] In some instances, issues arising from long-term effects such as gene regulation and protein expression and degradation can be identified by simply fusing the intensity-based sensor to a another fluorescent protein of different color, to serve as a reference channel.

[0169] In some instances, biosensors can include circularly permuted YFP (cpYFP) as a cpFP. cpYFP has been used as a reporter element in the creation of sensors for H_2O_2 (HyPer) (Belousov et al., Nat. Methods, 3:281-286, 2006), cGMP (FlincG) (Nausch et al., Proc. Natl. Acad. Sci. USA., 105:365-370, 2008), ATP:ADP ratio (Perceval) (Berg et al., Nat. Methods., 105:365-370, 2008), and calcium ions (Nakai et al., Nat. Biotechno., 19:137-141, 2001), including full length, fragments, and/or variants thereof.

[0170] In some embodiments, exemplary sensor portions can include residues 273-516 of SEQ ID NO: 176 (SF-iGluSnFR.A184V); residues 273-516 of SEQ ID NO: 177 (SF-iGluSnFR.A184S); residues 273-516 of SEQ ID NO: 178 (SF-iGluSnFR.S72A); residues 273-516 of SEQ ID NO: 179 (SF-Venus-iGluSnFR.A184V); residues 273-516 of SEQ ID NO: 180 (SF-Venus-iGluSnFR.A184S); residues 273-516 of SEQ ID NO: 181 (SF-Venus-iGluSnFR.S72A); residues 272-518 of SEQ ID NO: 182 (SF-Azurite-iGluSnFR); residues 351-594 of SEQ ID NO: 183 (iDexSnFR or SF-GlucoseSensor); residues 299-544 of SEQ ID NO: 184 (iGABASnFR); residues 104-343 of SEQ ID NO: 185 (iAChSnFR *E. coli* expression sequence); or residues 103-342 of SEQ ID NO: 186 (iAChSnFR mammalian expression sequence).

Linker Portions

[0171] As shown in Structure I, biosensors herein can optionally include one or more genetically encoded linkers positioned between or operably linking the framework portion and the signaling portion. Linker portions can include at least one naturally occurring or synthetic amino acid (discussed below) as exemplified by SEQ ID NOs: 9-49, 54-61, 64-76, 79-90, 95-104. In some instances, linker can include one or more of SEQ ID NOs: 9-49, 54-61, 64-76, 79-90, 95-104, and/or portions of SEQ ID NOs: 9-49, 54-61, 64-76, 79-90, 95-104. For example, linkers can include, but are not limited to, one or more of: PxSHNVY (SEQ ID NO:114), xPSHNVY (SEQ ID NO:115), xxSHNVY (SEQ ID NO:116), xxSHNVF (SEQ ID NO:117), PxSHNVF (SEQ ID NO:118), PxSYNVF (SEQ ID NO:119), xxSYNVF (SEQ ID NO:120), PxSYNVF (SEQ ID NO:121), xxSYNVF (SEQ ID NO:122), PxSxNVY (SEQ ID NO:123), PxSHxVY (SEQ ID NO:124), PxSHNxY (SEQ ID NO:125), PxSHNVx (SEQ ID NO:126), FNxxY (SEQ ID NO:127), FNxY (SEQ ID NO:128), FNY (SEQ ID NO:129), FxY (SEQ ID NO:130), xxY (SEQ ID NO:131), WxY (SEQ ID NO:132), xKY, (SEQ ID NO:133), FNPxY (SEQ ID NO:134), FNxPY (SEQ ID NO:135), HNS (SEQ ID NO:136), GGS (SEQ ID NO:137), xxS (SEQ ID NO:138), xxK (SEQ ID NO:139), GGK (SEQ ID NO:140), PXS (SEQ ID NO:141), xPS (SEQ ID NO:142), Px (SEQ ID NO:143), xP (SEQ ID NO:144), IxxS (SEQ ID NO:145), NxPK (SEQ ID NO:146), NPcK (SEQ ID NO:147), PPxSH (SEQ ID NO:148), PPxxSH (SEQ ID NO:149), PPPxSH (SEQ ID NO:150), PPxPSH (SEQ ID NO:151), xxSH (SEQ ID NO:152), PPxx (SEQ ID NO:153), FNxKN (SEQ ID NO:154), FNxxKN (SEQ ID NO:155), FNxPKN (SEQ ID NO:156), FNPxKN (SEQ ID NO:157), FNxx (SEQ ID NO:158), N, ADGSSH (SEQ ID NO:159), ADxxSH (SEQ ID NO:160), ADxPSH (SEQ ID NO:161), ADPxSH (SEQ ID NO:162), ADxx (SEQ ID NO:163), ADxxSH (SEQ ID NO:164), FNPG (SEQ ID NO:165), FNxxPG (SEQ ID NO:166), xxPG (SEQ ID NO:167), FNxx (SEQ ID NO:168), FNPx (SEQ ID NO:169), KYxxSH (SEQ ID NO:170), KYPxSH (SEQ ID NO:171), KYxPSH (SEQ ID NO:172), FxxP (SEQ ID NO:173), FNxP (SEQ ID NO:174), and/or FNPx (SEQ ID NO:175), where "x" indicates any amino acid.

[0172] In some embodiments, exemplary linker portions can include residues 365-370 of SEQ ID NO: 176 (SF-iGluSnFR.A184V); residues 365-370 of SEQ ID NO: 177

(SF-iGluSnFR.A184S); residues 365-370 of SEQ ID NO: 178 (SF-iGluSnFR.S72A); residues 365-370 of SEQ ID NO: 179 (SF-Venus-iGluSnFR.A184V); residues 365-370 of SEQ ID NO: 180 (SF-Venus-iGluSnFR.A184S); residues 365-370 of SEQ ID NO: 181 (SF-Venus-iGluSnFR.S72A); residues 365-370 of SEQ ID NO: 182 (SF-Azurite-iGluSnFR); residues 443-448 of SEQ ID NO: 183 (iDexSnFR or SF-GlucoseSensor); residues 391-396 of SEQ ID NO: 184 (iGABASnFR); residues 100-103 and 344-347 of SEQ ID NO: 185 (iAChSnFR *E. coli* expression sequence); or residues 99-102 and 343-346 of SEQ ID NO: 186 (iAChSnFR mammalian expression sequence).

Exemplary Biosensor Constructs

[0173] As noted above, biosensors herein include genetically encoded biosensors, i.e., nucleic acids encoding biosensors, and/or the encoded biosensors (e.g., isolated biosensors), for use as biosensors. In some instances, nucleic acids encoding biosensors include isolated nucleic acids. In some instances, the portion of a nucleic acid encoding a biosensor can include a single reading frame encoding the biosensor. For example, a biosensor can be encoded by a portion of a nucleic acid that falls within a start codon and a stop codon. In some instances, biosensors are isolated (e.g., biosensors are substantially free of contaminating and/or non-biosensor components).

[0174] In some instances, biosensors can include, for example, one or more framework portions selected from the group consisting of: arabinose-binding protein(s), glucose/galactose-binding protein(s), histidine-binding protein(s), maltose-binding protein(s), maltotriose-binding protein(s), glutamine-binding protein(s), RBP, ribose-binding protein (s), acetylcholine binding protein(s), choline binding protein (s), lysine binding protein(s), arginine binding protein(s), gamma aminobutyric acid (GABA) binding protein(s), ion-binding protein(s), peptide-binding protein(s), lactate-binding protein(s), histamine-binding protein(s), and/or Leucine/Isoleucine/Valine binding protein(s), including full length proteins, fragments, and/or variants thereof, including full length proteins, fragments and/or variants thereof, and at least one independent signaling portion present at a site within the framework portion that undergoes a conformational change upon interaction of the framework portion with an analyte.

[0175] In some instances, biosensors can include, for example, one or more framework portions selected from the group consisting of: SEQ ID NO:105, which is *Escherichia coli* maltodextrin-binding protein (EcMBP) (UniProt accession number POAEX9); SEQ ID NO: 106, which is *Pyrococcus Furiosus* maltose-binding protein (PfMBP) (UniProt accession number P58300); SEQ ID NO:107, which is *E. coli* glutamate-binding protein (EcYbeJ) (UniProt accession number Q1R3F7); SEQ ID NO:108, which is *E. coli* phosphonate-binding protein (EcPhnD) (UniProt accession number P37902); and/or SEQ ID NO:109, which is *Thermus thermophilus* glucose-binding protein (TtGBP) (UniProt accession number Q72KX2), including full length proteins, fragments and/or variants thereof, and at least one independent signaling portion present at a site within the framework portion that undergoes a conformational change upon interaction of the framework portion with an analyte.

[0176] In some instances, biosensors can include, for example, one or more framework portions selected from the group consisting of: SEQ ID NO: 110 (UniProt accession number Q92N37); SEQ ID NO:111 (UniProt accession number DOVWx8, SEQ ID NO:112 (UniProt accession number Q7CX36), and/or SEQ ID NO:113 (UniProt accession number POAD96), including full length proteins, fragments and/or variants thereof, and at least one independent signaling portion present at a site within the framework portion that undergoes a conformational change upon interaction of the framework portion with an analyte.

[0177] In some instances, biosensors include any one or more:

[0178] Maltose biosensors SEQ ID NOs: 1-8 (e.g., *Escherichia coli* maltodextrin-binding protein (EcMBP)) or SEQ ID NOs: 50-53 (e.g., *Pyrococcus furiosus* maltose-binding protein (PfMBP)), including full length proteins, fragments and/or variants thereof;

[0179] Glutamate biosensors SEQ ID NOs: 62-63 (e.g., *E. coli* glutamate-binding protein (EcYbeJ)) or SEQ ID NOs: 176-182, including full length proteins, fragments and/or variants thereof;

[0180] Phosphonate biosensors SEQ ID NOs: 77-78 (e.g., *E. coli* phosphonate-binding protein (EcPhnD)), including full length proteins, fragments and/or variants thereof;

[0181] Glucose biosensors SEQ ID NOs: 91-94 (e.g., *Thermus thermophilus* glucose-binding protein (TtGBP)) and SEQ ID NO: 183, including full length proteins, fragments and/or variants thereof;

[0182] GABA biosensors SEQ ID NO: 184, including full length proteins, fragments and/or variants thereof; and/or

[0183] ACh biosensors SEQ ID NOs: 185 & 186, including full length proteins, fragments and/or variants thereof.

[0184] In some instances, nucleic acids encoding, and/or amino acid sequences of, any of the framework portions, signaling portions, linker portions, or the entire biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) disclosed herein can be modified to generate fragments (e.g., truncated peptides) and/or variants (e.g., peptides with a defined sequence homology to the peptides disclosed herein). Variants can include framework portions, signaling portions, linker portions, or biosensors with amino acid sequences with homology to the framework portions, signaling portions, linker portions, or biosensors disclosed herein and/or truncated forms of the framework portions, signaling portions, linker portions, or biosensors herein. In some instances, truncated forms of the framework portions, signaling portions, linker portions, or biosensors herein can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 50-100, 101-150, fewer amino acids than the framework portions, signaling portions, linker portions, and/or biosensors herein, e.g., wherein the truncated biosensor variants retain at least at portion of the binding and/or signaling properties of same biosensor without truncation (e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the binding and/or signaling properties of the same biosensor without truncation). In addition, truncations can be made at the amino-terminus, the carboxy-terminus, and/or within the body of the framework portions, signaling portions, linker portions, and/or biosensors herein.

[0185] While variants are generally observed and discussed at the amino acid level, the actual modifications are typically introduced or performed at the nucleic acid level. For example, variants with 95%, 96%, 97%, 98, or 99%

sequence identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 can be generated by modifying the nucleic acids encoding SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 using techniques (e.g., cloning techniques) known in the art and/or that are disclosed herein.

[0186] As with all peptides, polypeptides, and proteins, including fragments thereof, it is understood that modifications to the amino acid sequence can occur that do not alter the nature or function of the peptides, polypeptides, or proteins. Such modifications include conservative amino acids substitutions and are discussed in greater detail below.

[0187] The peptides, polypeptides, and proteins, including fragments thereof, provided herein are biosensors whose activity can be tested or verified, for example, using the in vitro and/or in vivo assays described herein.

[0188] In some instances, any of the framework portions, signaling portions, or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) described herein can be modified and varied so long as their desired function is maintained. For example, the polypeptides can be modified as long as the resulting variant polypeptides have the same or better characteristics as the polypeptide from which they derived. For example, the variants can have the same or better affinity for their respective analyte.

[0189] In some instances, the interacting face of a modified peptide can be the same (e.g., substantially the same) as an unmodified peptide (methods for identifying the interacting face of a peptide are known in the art (Gong et al., BMC: Bioinformatics, 6:1471-2105 (2007); Andrade and Wei et al., Pure and Appl. Chem., 64(11):1777-1781 (1992); Choi et al., Proteins: Structure, Function, and Bioinformatics, 77(1):14-25 (2009); Park et al., BMC: and Bioinformatics, 10:1471-2105 (2009)), e.g., to maintain binding to an analyte. Alternatively, amino acids within the interacting face can be modified, e.g., to decrease binding to an analyte and/or to change analyte specificity.

[0190] The interacting face of a peptide is the region of the peptide that interacts or associates with other molecules (e.g., other proteins). Generally, amino acids within the interacting face are naturally more highly conserved than those amino acids located outside the interacting face or interface regions of a protein. In some instances, an amino acid within the interacting face region of any of the framework portions or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) disclosed herein can be the same as the amino acid shown in any of the framework portions or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) disclosed herein or can be include conservative amino acid substitutions. In some instances, an amino acid within the interacting face region any of the framework portions or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) disclosed herein can be substituted with an amino acid that increases the interaction between the framework portion or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) and an analyte.

[0191] In some instances, genetically encoded biosensors can include peptides that have at least 80, 85, 90, 95, 96, 97, 98, 99 percent identity to the framework portions, signaling portions, or the biosensor sequence (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) (e.g., any amino acid sequence) described herein. Those of skill in the art readily understand how to determine the identity of two polypeptides. For example, the identity can be calculated after aligning the two sequences so that the identity is at its highest level.

[0192] Another way of calculating identity can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, Adv. Appl. Math, 2:482 (1981), by the identity alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection.

[0193] The same types of identity can be obtained for nucleic acids by, for example, the algorithms disclosed in Zuker, Science 244:48-52 (1989); Jaeger et al., Proc. Natl. Acad. Sci. USA 86:7706-10 (1989); Jaeger et al., Methods Enzymol. 183:281-306 (1989), which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity and to be disclosed herein.

[0194] Amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, or deletional modifications. Insertions include amino and/or terminal fusions as well as intra-sequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions can be made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional modifications are those in which at least one residue has been removed and a different residue inserted in its place. In some instances, substitutions can be conservative amino acid substitutions. In some instances, variants herein can include one or more conservative amino acid substitutions. For example, variants can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, or 40-50 conservative amino acid substitutions. Alternatively, variants can include 50 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer conservative amino acid substitutions. Such substitutions generally are made in accordance with the following Table 1 and are referred to as conservative substitutions. Methods for predicting tolerance to protein modification are known in the art (see, e.g., Guo et al., Proc. Natl. Acad. Sci., USA, 101(25):9205-9210 (2004)).

TABLE 1

Conservative Amino Acid Substitutions

Amino Acid	Substitutions (others are known in the art)
Ala	Ser, Gly, Cys
Arg	Lys, Gln, His
Asn	Gln, His, Glu, Asp
. . .	
Asp	Glu, Asn, Gln
Cys	Ser, Met, Thr
Gln	Asn, Lys, Glu, Asp, Arg
Glu	Asp, Asn, Gln
Gly	Pro, Ala, Ser
His	Asn, Gln, Lys
Ile	Leu, Val, Met, Ala
Leu	Ile, Val, Met, Ala
Lys	Arg, Gln, His
Met	Leu, Ile, Val, Ala, Phe
Phe	Met, Leu, Tyr, Trp, His
Ser	Thr, Cys, Ala
Thr	Ser, Val, Ala
Trp	Tyr, Phe
Tyr	Trp, Phe, His
Val	Ile, Leu, Met, Ala, Thr

[0195] In some instances, substitutions are not conservative. For example, an amino acid can be replaced with an amino acid that can alter some property or aspect of the peptide. In some instances, non-conservative amino acid substitutions can be made, e.g., to change the structure of a peptide, to change the binding properties of a peptide (e.g., to increase or decrease the affinity of binding of the peptide to an analyte and/or to alter increase or decrease the binding specificity of the peptide).

[0196] Modifications, including the specific amino acid substitutions, are made by known methods. By way of example, modifications are made by site-specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the modification, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis.

Nucleic Acids

[0197] The disclosure also features nucleic acids encoding the biosensors (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) described herein, including variants and/or fragments of the biosensors (e.g., variants and/or fragments of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186). These sequences include all degenerate sequences related to the specific polypeptide sequence, i.e., all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the polypeptide sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed polypeptide sequences.

[0198] In some instances, nucleic acids can encode biosensors with 95, 96, 97, 98, or 99 identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186.

[0199] In some instances, nucleic acids can encode SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, or 40-50 conservative amino acid substitutions.

[0200] In some instances, nucleic acids can encode SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 containing 50 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer conservative amino acid substitutions

[0201] Also provided herein are vectors comprising the biosensors (e.g, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) described herein, including variants and/or fragments of the biosensors (e.g, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186). For example:

[0202] Vectors can include nucleic acids that encode biosensors with 95, 96, 97, 98, or 99 identity to SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186.

[0203] Vectors can include nucleic acids that encode SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, or 40-50 conservative amino acid substitutions.

[0204] Vectors can include nucleic acids that encode SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186 containing 50 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer conservative amino acid substitutions

[0205] Examples of suitable vectors include, but are not limited to, plasmids, artificial chromosomes, such as BACs, YACs, or PACs, and viral vectors. As used herein, vectors are agents that transport the disclosed nucleic acids into a cell without degradation and, optionally, include a promoter yielding expression of the nucleic acid molecule in the cells into which it is delivered.

[0206] Viral vectors can include, for example, Adenovirus, Adeno-associated virus, herpes virus, Vaccinia virus, Polio virus, Sindbis, and other RNA viruses, including these viruses with the HIV backbone. Any viral families which share the properties of these viruses which make them suitable for use as vectors are suitable. Retroviral vectors, in general are described by Coffin et al., Retroviruses, Cold Spring Harbor Laboratory Press (1997), which is incorporated by reference herein for the vectors and methods of making them. The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-20 (1987); Massie et al., Mol. Cell. Biol. 6:2872-83 (1986); Haj-Ahmad et al., J. Virology 57:267-74 (1986);

Davidson et al., J. Virology 61:1226-39 (1987); Zhang et al., BioTechniques 15:868-72 (1993)). Recombinant adenoviruses have been shown to achieve high efficiency after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma, and a number of other tissue sites. Other useful systems include, for example, replicating and host-restricted non-replicating Vaccinia virus vectors.

[0207] Non-viral based vectors can include expression vectors comprising nucleic acid molecules and nucleic acid sequences encoding polypeptides, wherein the nucleic acids are operably linked to an expression control sequence. Suitable vector backbones include, for example, those routinely used in the art such as plasmids, artificial chromosomes, BACs, YACs, or PACs. Numerous vectors and expression systems are commercially available from such corporations as Novagen (Madison, Wis.), Clontech (Pal Alto, Calif.), Stratagene (La Jolla, Calif.), and Invitrogen/Life Technologies (Carlsbad, Calif.). Vectors typically contain one or more regulatory regions. Regulatory regions include, without limitation, promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, protein binding sequences, 5' and 3' untranslated regions (UTRs), transcriptional start sites, termination sequences, polyadenylation sequences, and introns.

[0208] Promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as polyoma,

[0209] Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis B virus, and most preferably cytomegalovirus (CMV), or from heterologous mammalian promoters, e.g. β-actin promoter or EF1α promoter, or from hybrid or chimeric promoters (e.g., CMV promoter fused to the β-actin promoter). Of course, promoters from the host cell or related species are also useful herein.

[0210] Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence itself. They are usually between 10 and 300 base pairs in length, and they function in cis. Enhancers usually function to increase transcription from nearby promoters. Enhancers can also contain response elements that mediate the regulation of transcription. While many enhancer sequences are known from mammalian genes (globin, elastase, albumin, fetoprotein, and insulin), enhancers derived from a eukaryotic cell viruses can be used. Examples of such can include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0211] The promoter and/or the enhancer can be inducible (e.g. chemically or physically regulated). A chemically regulated promoter and/or enhancer can, for example, be regulated by the presence of alcohol, tetracycline, a steroid, or a metal. A physically regulated promoter and/or enhancer can, for example, be regulated by environmental factors, such as temperature and light. Optionally, the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize the expression of the region of the transcription unit to be transcribed. In certain vectors, the promoter and/or enhancer region can be active in a cell type specific manner. Optionally, in certain vectors, the promoter and/or enhancer region can be active in all eukaryotic cells, independent of cell type. Promoters of this type can include the CMV promoter, the SV40 promoter, the β-actin promoter, the EF1α promoter, and the retroviral long terminal repeat (LTR).

[0212] The provided vectors also can include, for example, origins of replication and/or markers. A marker gene can confer a selectable phenotype, e.g., antibiotic resistance, on a cell. The marker product is used to determine if the vector has been delivered to the cell and once delivered is being expressed. Examples of selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hygromycin, puromycin, and blasticidin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. Examples of other markers include, for example, the *E. coli* lacZ gene, green fluorescent protein (GFP), and luciferase. In addition, an expression vector can include a tag sequence designed to facilitate manipulation or detection (e.g., purification or localization) of the expressed polypeptide. Tag sequences, such as GFP, glutathione S-transferase (GST), polyhistidine, c-myc, hemagglutinin, or FLAG™ tag (Kodak; New Haven, Conn.) sequences typically are expressed as a fusion with the encoded polypeptide. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino terminus.

[0213] The disclosure further provides cells comprising the biosensors (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) described herein, including variants and/or fragments of the biosensors (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186). Cells can include, for example, eukaryotic and/or prokaryotic cells. For example, cells can include, but are not limited to cells of *E. coli, Pseudomonas, Bacillus, Streptomyces*; fungi cells such as yeasts (*Saccharomyces*, and methylotrophic yeast such as *Pichia, Candida, Hansenula*, and *Torulopsis*); and animal cells, such as CHO, R1.1, B-W and LM cells, African Green Monkey kidney cells (for example, COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (for example, Sf9), human cells and plant cells. Suitable human cells can include, for example, HeLa cells or human embryonic kidney (HEK) cells. In general, cells that can be used herein are commercially available from, for example, the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Va. 20108. See also F. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., (1998).

[0214] Optionally, the biosensors (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) described herein, including variants and/or fragments of the biosensors (e.g., SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) can be located in the genome of the cell (e.g., can be stably expressed in the cell) or can be transiently expressed in the cell.

[0215] Methods of making the provided cells are known and the method of transformation and choice of expression vector will depend on the host system selected. Transformation and transfection methods are described, e.g., in F. Ausubel et al., Current Protocols in Molecular Biology, John

Wiley & Sons, New York, N.Y., (1998), and, as described above, expression vectors may be chosen from examples known in the art.

[0216] There are a number of compositions and methods which can be used to deliver the nucleic acid molecules and/or polypeptides to cells, either in vitro or in vivo via, for example, expression vectors. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based deliver systems. Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein.

[0217] By way of example, the provided polypeptides and/or nucleic acid molecules can be delivered via virus like particles. Virus like particles (VLPs) consist of viral protein (s) derived from the structural proteins of a virus. Methods for making and using virus like particles are described in, for example, Garcea and Gissmann, Current Opinion in Biotechnology 15:513-7 (2004). The provided polypeptides can be delivered by subviral dense bodies (DBs). DBs transport proteins into target cells by membrane fusion. Methods for making and using DBs are described in, for example, Pepperl-Klindworth et al., Gene Therapy 10:278-84 (2003). The provided polypeptides can be delivered by tegument aggregates. Methods for making and using tegument aggregates are described in International Publication No. WO 2006/110728.

[0218] Also provided are transgenic animals comprising one or more cells the biosensors (e.g, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186) described herein, including variants and/or fragments of the biosensors (e.g, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186). As used herein, the term animal refers to non-human animals, including, mammals, amphibians and birds. Specifically, examples include sheep, feline, bovines, ovines, pigs, horses, rabbits, guinea pigs, mice, hamsters, rats, non-human primates, and the like. As used herein, transgenic animal refers to any animal, in which one or more of the cells of the animal contain a heterologous nucleic acid. The heterologous nucleic acid can be introduced using known transgenic techniques. The nucleic acid is introduced into the cell, directly or indirectly. For example, the nucleic acid can be introduced into a precursor of the cell or by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The nucleic acid may be integrated within a chromosome, or it may be an extrachromosomally replicating DNA.

[0219] Methods for making transgenic animals using a variety of transgenes have been described in Wagner et al. (1981) Proc. Nat. Acad. Sci. USA, 78:5016-5020; Stewart et al. (1982) Science, 217:1046-1048; Constantini et al. (1981) Nature, 294:92-94; Lacy et al. (1983) Cell, 34:343-358; McKnight et al. (1983) Cell, 34:335-341; Brinstar et al. (1983) Nature, 306:332-336; Palmiter et al. (1982) Nature, 300:611-615; Palmiter et al. (1982) Cell, 29:701-710; and Palmiter et al. (1983) Science, 222:809-814. Such methods are also described in U.S. Pat. Nos. 6,175,057; 6,180,849; and 6,133,502.

[0220] By way of example, the transgenic animal can be created by introducing a nucleic acid into, for example, an embryonic stem cell, an unfertilized egg, a fertilized egg, a spermatozoon or a germinal cell containing a primordial germinal cell thereof, preferably in the embryogenic stage in the development of a non-human mammal (more preferably in the single-cell or fertilized cell stage and generally before the 8-cell phase). The nucleic acid can be introduced by known means, including, for example, the calcium phosphate method, the electric pulse method, the lipofection method, the agglutination method, the microinjection method, the particle gun method, the DEAE-dextran method and other such method. Optionally, the nucleic acid is introduced into a somatic cell, a living organ, a tissue cell or other cell by gene transformation methods. Cells including the nucleic acid may be fused with the above-described germinal cell by a commonly known cell fusion method to create a transgenic animal.

[0221] For embryonic stem (ES) cells, an ES cell line may be employed, or embryonic cells may be obtained freshly from a host, e.g., mouse, rat, guinea pig, and the like. Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of appropriate growth factors, such as leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination or integration of the nucleic acid. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected. The chimeric animals are screened for the presence of the nucleic acid, and males and females having the modification are mated to produce homozygous progeny transgenic animals.

[0222] Kits comprising one or more containers and the nucleic acid sequences, polypeptides, vectors, cells, provided herein, or combinations thereof, are also provided. For example, provided is a kit comprising (i) a nucleic acid sequence encoding a biosensor described herein (e.g, one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186), including variants and/or fragments of the biosensor (e.g, variants or fragments of one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186), (ii) a polypeptide comprising a biosensor described herein (e.g, one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186), including variants and/or fragments of the biosensor (e.g, variants or fragments of one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186), (iii) a vector comprising the nucleic acid of (i), (iv) a cell comprising the nucleic acid or (i) and/or the polypeptide of (ii), (v) a cell comprising the vector of (iii). The kit can comprise any combination of (i)-(v). Optionally, the kit further comprises reagents for using the nucleic acid or peptide biosensors, vectors, and/or cells. For example, if the kit comprises cells, the kit may also comprise cell culture medium. Optionally, the kit further

comprises instructions for use. Optionally, the kit further comprises a GPCR, a GPCR-encoding nucleic acid sequence.

Design and Production/Manufacture Methods

[0223] Using the methods described herein, it is possible to design, produce, and/or adapt genetically encoded biosensors to assays for a variety of classes of analytes. The provided materials and methods facilitate the discovery of new compounds targeting a wide array of protein targets, including but not limited to: endogenous targets responsible for disease state progression, targets on pathogens for treating infectious diseases, and endogenous targets to be avoided (thus screening early for potential drug side effects and toxicity).

[0224] Methods herein provide systematic and generic approaches for the design and production of genetically encoded recombinant peptides containing an analyte-binding framework portion linked (e.g., operably linked) to a signaling portion, wherein the signaling portion is allosterically modulated or regulated by the framework portion upon interaction of the framework portion with an analyte. Generally, methods include: (i) selecting one or more target analytes; (ii) selecting a framework portion (e.g., a PBP) that interacts with (e.g., interacts specifically with) or binds to (e.g., binds specifically to) the target analyte and that undergoes a conformational change upon interacting with or binding to the analyte; (iii) identifying sites or amino acid positions within the framework portion (e.g., the PBP) where the conformational change occurs; and (iv) inserting or cloning a signaling portion into the site or amino acid position identified in (iii). Methods can, optionally, further include: (v) modifying or optimizing linker sequences between the framework portion and the signaling portion, for example, by genetic manipulation (e.g., by point mutation); (vi) modifying or optimizing analyte binding; (vii) modifying the signal generated by the biosensor; and/or (viii) cloning the biosensor into a suitable vector.

[0225] In some instances: (iii) includes identification of insertion sites by analysis of the structure (e.g., crystal structure) of the selected framework portion (e.g., the selected PBP) in one or both of its open and closed states to determine amino acid positions at which analyte-binding dependent structural changes occur. In instances where structures for both open and closed states are not available, analysis can be conducted by analogy to a structurally similar framework portion (e.g., PBP); (iv) includes cloning a signaling portion (e.g., a cpFP) at the site identified in (iii) such that the analyte-binding dependent structural change observed in (iii) will result in a conformational change in the signaling portion (e.g., the cpFP) and allosteric modulation of the signaling portion; (v) includes generating a library of mutants of biosensors with distinct linker sequences (e.g., by point mutation), screening the library of mutants to identify mutants with enhanced properties (e.g., improved signal-to-noise ratio), and selecting mutants with enhanced properties (e.g., improved signal-to-noise ratio); (vi) includes increasing or decreasing binding or affinity of the framework portion to the analyte, e.g., by modifying amino acids in the interacting face of the framework portion or regions within the framework portion that are critical for analyte binding; (vii) includes increasing or decreasing signal emission by the signaling portion and/or changing the color of the signal where the signaling portion is a FP (e.g., a cpFP). Methods including (i)-(viii) are exemplified in the Examples section herein.

Methods of Use

[0226] The disclosure further provides methods for using the biosensors disclosed herein (e.g., one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186), including variants and/or fragments of the biosensor (e.g., variants or fragments of one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, 53, 62, 63, 77, 78, 91, 92, 93, 94, and/or 176-186)) to detect analytes, e.g., in biological systems. Such methods can include, for example:

[0227] Use of a maltose biosensor disclosed herein (e.g., one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and/or 53 including variants and/or fragments of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 50, 51, 52, and/or 53) to detect maltose, e.g., in a biological system;

[0228] Use of a glutamate biosensor disclosed herein (e.g., one or more of SEQ ID NOs: 62, 63, and/or 176-182 including variants and/or fragments of SEQ ID NOs: 62, 63, and/or 176-182) to detect glutamate, e.g., in a biological system;

[0229] Use of a phosphonate biosensor disclosed herein (e.g., one or more of SEQ ID NOs: 77 and/or 78 including variants and/or fragments of SEQ ID NOs: 77 and/or 78) to detect phosphonate, e.g., in a biological system; and/or

[0230] Use of a glucose biosensor disclosed herein (e.g., one or more of SEQ ID NOs: 91, 92, 93, 94, and/or 183 including variants and/or fragments of SEQ ID NOs: 91, 92, 93, 94, and/or 183) to detect glucose, e.g., in a biological system.

[0231] Use of a GABA biosensor disclosed herein (e.g., SEQ ID NO: 184 including variants and/or fragments of SEQ ID NO: 184) to detect GABA, e.g., in a biological system.

[0232] Use of an ACh biosensor disclosed herein (e.g., one or more of SEQ ID NOs: 185 and/or 186 including variants and/or fragments of SEQ ID NOs: 185 and/or 186) to detect ACh, e.g., in a biological system.

[0233] Techniques for performing such methods are known in the art and/or are exemplified herein. For example, methods can include introducing one or more biosensors into a biological system (e.g., a cell); expressing the one or more biosensors in the biological system (e.g., the cell); monitoring the signal emitted by the expressed biosensor in the biological system; and correlating the signal emitted by the expressed biosensor in the biological system with a level of the analyte in the biological system.

EXAMPLES

[0234] The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

[0235] Example 1: Maltose Indicators

[0236] Genetically encoded maltose indicators were generated using *Escherichia coli* maltodextrin-binding protein (EcMBP) as a framework and either circularly permuted β-lactamase (cpBla) or circularly permuted fluorescent protein (cpFP) as a signal. Data describe below suggest that cpBla and cpFP are not interchangeable.

[0237] Allosteric coupling of ligand binding to fluorescence was hypothesized to require:

[0238] i) that the site in into which cpGFP is inserted have the capacity to transduce the global conformational change the scaffold protein (EcMBP in this example) to the local environment of the chromophore in cpGFP; and

[0239] ii) that the local environment of the chromophore (e.g., linkers) be optimized to maximize the difference in emission between unbound (apo) and the bound (in this example maltose-bound) states.

Example 1A: Identification of cpGFP Insertion Sites in EcMBP

[0240] Potential insertion sites were identified using the crystal structures of the maltose-bound, closed form of EcMBP (Ouiocho et al., Structure, 5:997-1015, 1997) and the ligand-free, open form of EcMBP shown in FIG. **1** (Sharff et al., Biochemistry, 31:10657-10663, 1992) to guide rational design of EcMBP-cpGFP fusions that would result in maltose-dependent GFP fluorescence.

[0241] For (i), the change in dihedral angle (defined by the Cα atoms spanning four residues) was analyzed to identify maltose-dependent structural changes in sequentially adjacent residues (FIG. **6**); this analysis showed that the Cα chain is "torqued" around residues 175 (ΔDihedral=)+41° and 311 (ΔDihedral=)−22° upon ligand binding. This sequential conformational change was predicted to be coupled to structural changes of an inserted cpGFP, resulting in maltose-dependent fluorescence for the fusion protein.

[0242] Previous studies using randomly digested and reassembled circularly permuted β-lactamase (cpBla) and EcMBP showed maltose-dependent β-lactamase activity in proteins with insertions of cpBla at EcMBP residues 165 and 317 (Guntas et al., Chem. Biol., 11:1483-1487, 2004; Guntas and Ostermeier, J. Mol. Biol., 336:263-273, 2004).

[0243] Since the ΔDihedral of EcMBP165 is +11° (moderate change) and EcMBP317 is +2° (no real change), four EcMBP-cpGFP templates were constructed by inserting cpGFP into EcMBP at sites 165, 175 (identified herein), 311 (identified herein), and 317 to test our predictive method and the interchangeability of cpBla and cpGFP at sites identified from the EcMBP-cpBla screen. These constructs were named MBP165-cpGFP, MBP175-cpGFP, MBP311-cpGFP, and MBP317-cpGFP (names were modified to encompass variants (e.g., with modified linker sequences). The cpGFP used is cpGFP146 described in Baird et al. (Proc. Natl. Acad. Sci., USA, 96:11241-11246, 1999). PCR assembly was used to construct fusion proteins with GlyGly-linkers between EcMBP and each terminus of cpGFP. The amino acid sequence of each construct is shown in FIGS. **6**-**9**. The sequences of SEQ ID NOs:1-3 shown in FIGS. **7**A-**7**C (i.e., MBP165-cpGFP) differ in the linker sequence between MBP 1-165 and cpGFP 147-238 (linker 1: see the line ending in amino acid 240)). The sequences of SEQ ID NOs: 4-5 shown in FIGS. **8**A-**8**B (i.e., MBP175-cpGFP) differ in the sequence between MBP 1-175 and cpGFP 147-238 (linker 1: see the line ending in amino acid 240)). The sequences of SEQ ID NOs: 6-7 shown in FIGS. **9**A-**9**B (i.e., MBP311-cpGFP) differ in the sequence between cpGFP 1-146 and MBP 312-370 (linker 2: see the line ending in amino acid 640)). Each construct includes 3 linkers: A linker between the C-terminus of the C-terminal portion of MBP and the N-terminus of cpGFP (i.e., linker 2), a linker between the N-terminus of cpGFP and C-terminus of the N-terminal portion of MBP, and a linker in cpGFP (i.e., linker 3).

Example 1B: Linker Optimization

[0244] Libraries of variants of SEQ ID NOs: 1-8 were generated with randomized linkers by single-stranded uracil template mutagenesis (see Kunkel et al., Methods Enzymol., 204:125-139, 1991) using the primers listed below:

165 Linker 1 Primers:

(SEQ ID NO: 9)
PLIAADGxxNVYIM

(SEQ ID NO: 10)
PLIAADxxNVYIM

(SEQ ID NO 11)
PLIAADGGxxNVYIM

(SEQ ID NO: 12)
PLIAADGxPNVYIMG

(SEQ ID NO: 13)
PLIAADGIxNVYIMG

(SEQ ID NO: 14)
PLIAADPxSHNVYIM

(SEQ ID NO: 15)
PLIAADxPSHNVYIM

(SEQ ID NO: 16)
PLIAADxxSHNVYIM

(SEQ ID NO: 17)
PLIAADxxSHNVFIM

(SEQ ID NO: 18)
PLIAADPxSHNVFIM

(SEQ ID NO: 19)
PLIAADPxSYNVFIM

(SEQ ID NO: 20)
PLIAADxxSYNVFIM

(SEQ ID NO: 21)
PLIAADPxSYNVFIM

(SEQ ID NO: 22)
PLIAADxxSYNVFIM

(SEQ ID NO: 23)
PLIAADPxSxNVYIM

(SEQ ID NO: 24)
PLIAADPxSHxVYIM

(SEQ ID NO: 25)
PLIAADPxSHNxYIM

(SEQ ID NO: 26)
PLIAADPxSHNVxIM

165 Linker 2 Primers:

(SEQ ID NO: 27)
KLEYNFNxxYAFKYEN

(SEQ ID NO: 28)
KLEYNFNxYAFKYEN

(SEQ ID NO: 29)
KLEYNFNYAFKYEN

-continued

(SEQ ID NO: 30)
KLEYNFxYAFKYEN

(SEQ ID NO: 31)
KLEYNxxYAFKYEN

(SEQ ID NO: 32)
KLEYNWxYAFKYEN

(SEQ ID NO: 33)
KLEYNxKYAFKYEN

(SEQ ID NO: 34)
KLEYNFNPxYAFKYEN

(SEQ ID NO: 35)
KLEYNFNxPYAFKYEN

175 Linker 1 Primers:

(SEQ ID NO: 36)
AFKYENxxSHNVYIM

175 Linker 2 Primers:

(SEQ ID NO: 37)
KLEYNFNxxKYDIKDV

311 Linker 1 Primers:

(SEQ ID NO: 38)
KSYEELxxSHNVYIM

(SEQ ID NO: 39)
KSYEELPxSHNVYIM

(SEQ ID NO: 40)
KSYEELxPSHNVYIM

311 Linker 2 Primers:

(SEQ ID NO: 41)
KLEYNFNxxAKDPRIA

(SEQ ID NO: 42)
KLEYNFNPxAKDPRIA

(SEQ ID NO: 43)
KLEYNFNxPAKDPRIA

317 Linker 1 Primers:

(SEQ ID NO: 44)
ELAKDPRxSHNVYIM

(SEQ ID NO: 45)
ELAKDPRxxSHNVYIM

(SEQ ID NO: 46)
ELAKDPRxxxSHNVYIM

317 Linker 2 Primers:

(SEQ ID NO: 47)
KLEYNFNxAATMENA

(SEQ ID NO: 48)
KLEYNFNxxAATMENA

(SEQ ID NO: 49)
KLEYNFNxxxAATMENA

[0245] Where "x" indicates that a degenerate primer (with DNA sequence "NNS") was used to encode all 20 possible amino acids.

[0246] About 400 variants were screened in semi-high-throughput fashion, measuring fluorescence intensity of clarified cell lysate in the absence and presence of 10 mM maltose.

[0247] Insertion of cpGFP as MBP317, a site previously reported for cpBla, did not show maltose-dependent fluorescence (FIG. **11**) even though the framework protein still bound maltose, as determined by isothermal titration calorimetry (FIG. **12**). These data demonstrate that identification of insertion sites by a method other than insertion of cpGFP (such as insertion of cpBla) is not sufficient to identify sites that transduce ligand binding to changes in fluorescence intensity

[0248] Insertion of cpGFP at residue 165 of EcMBP (EcMBP165-cpGFP), another position reported in cpBla studies (Guntas and Ostermeier, supra) with -GlyGly-linkers flanking the cpGFP resulted in a protein in which fluorescence increased 20% ($\Delta F/F=0.2$) upon addition of saturating maltose.

[0249] Screening a fully-degenerate, length-two library ("XX") at either the EcMBP-cpGFP linker (linker 1) or the cpGFP-EcMBP linker (linker 2) yielded proteins with maltose-dependent fluorescent increases >300% or decreases >50% (FIG. **11**). Many of the variants with increased $\Delta F/F$ values had linkers containing proline(s). Subsequent libraries constructed from oligonucleotides encoding XP or PX and randomization of the residues in GFP from residue 146 to 150 were screened, yielding a final variant with: a two-proline EcMBP-cpGFP linker, a two-glycine cpGFP-EcMBP linker, GFP-H148Y, and GFP-Y151F. This variant, called "EcMBP165-cpGFP.PPYF" (abbreviated PPYF (SEQ ID NO:2)) has a $\Delta F/F=2.5$, a Kd for maltose of 3 μM. Screens also identified variant EcMBP311-cpGFP.L2-NP (-AsnPro- at linker 2 (SEQ ID NO:7)), which has a $\Delta F/F$ of 1.0 and a Kd for maltose of 2 μM. This variant has an inferior maltose-dependent fluorescence increase than PPYF, but demonstrates generality of the cpFP insertion method.

[0250] EcMBP175-cpGFP was also screened with XX linkers, and a few variants with $\Delta F/F\approx 1$ were identified (FIG. **11**). One mutant, with the first linker encoding HL (EcMBP175-cpGFP.L1-HL (SEQ ID NO:5)), has a $\Delta F/F=0.5$ and a Kd for maltose of 1.3 μM.

[0251] These data support that choice of insertion site by structural analysis is preferable to random insertion.

Example 1C: Modifying Ligand Binding and/or Fluorescent Properties of Sensors

[0252] One objective in the development of generic biosensors is for the framework to permit independent optimization of binding and signaling properties. Analysis of whether biosensors herein permit such optimization was tested using the high-SNR sensor PPYF, by: (i) rationally altering maltose-binding affinity; (ii) changing the ligand-binding specificity from maltose to sucrose, and (iii) creating a family of sensors in multiple colors.

[0253] As a first step, the impacts of mutations of three tryptophan side-chains in the maltose-binding pocket (W230, W62, and W340) were tested. These sites have previously been shown to lower the affinity of EcMBP for maltose by one, two, or three orders of magnitude, respectively, when mutated to alanine (Martineau et al., J. Mol. Biol., 214:337-352, 1990). A mutation to the hinge region, I329W, was also made to PPYF, as this has been shown to increase maltose affinity by about 2-fold in both wild-type EcMBP (Marvin and Hellinga, Nat. Struc. Biol., 8:795-798, 2001) and in the EcMBP-cpBla switches (Guntas et al., Chem. Biol., 11:1483-1487, 2004; Kim and Ostermeier, Arch. Biochem. Biophys., 446:44-51, 2006). As shown in FIG. **13**, for the PPYF sensor, the three tryptophan-to-alanine binding-pocket mutations behaved as expected, low-

ering affinity by between one and three orders of magnitude. In contrast, the I329W mutation did not increase affinity as expected, but rather decreased it. ΔF/F also decreased. This data suggests that the mechanism of fluorescence change in this sensor is dependent on subtle interactions between EcMBP and cpGFP that are linked to the I329W mutation.

[0254] As an alternative test for changing the ligand-binding specificity of the sensor while preserving fluorescence signaling, "5-7" mutations (D14L, K15F, W62Y, E111Y), previously shown to confer EcMBP with an affinity for sucrose (Guntas and Mansell, Proc. Natl. Acad. Sci., 102:11224-11229, 2005), were made to PPYF. As shown in FIG. **14**A, the mutations conferred to the sensor about 2 mM affinity for sucrose and ~3 mM affinity for maltose. To address a discrepancy between expected (micromolar) and observed (millimolar) affinity for disaccharides, the 5-7 mutations were made to sensors with cpGFP inserted at different positions in EcMBP, and with different linker compositions. In the context of EcMBP165-cpGFP.PCF, the 5-7 mutations conferred very low (but observable) binding preference for sucrose over maltose (FIG. **14**B). The trend of higher (but still weak) affinity for sucrose (~0.6 mM) over maltose (~6 mM) continued when the 5-7 mutations are made in the context of EcMBP175-cpGFP.L1-HL (FIG. **14**C). In the context of EcMBP311-cpGFP.L2-NP, the 5-7 mutations appeared to eliminate all binding (FIG. **14**D). The preference for sucrose over maltose of the 5-7 variants of the sensors is consistent with the binding properties of the 5-7 variants of EcMBP alone and EcMBP-cpBla (Guntas and Mansell, Proc. Natl. Acad. Sci., 102:11224-11229, 2005). The lower affinity for both ligands of the 5-7 variants of the sensors may be the consequence of the inserted cpGFP shifting the open and closed equilibrium.

[0255] These data suggest that ligand binding and fluorescent properties of biosensors can be independently modified.

Example 1D: Modifying Sensor Color

[0256] The color of GFP can be altered by changing the amino acids that either comprise or interact with the chromophore (see Shaner et al., J. Cell. Sci. 120:4247-4260, 2007, for a review).

[0257] Using PPYF as a template, mutations Y66W (to yield a cyan variant, "cpCFP"), L64F+T65G+V68L+T203Y (yellow, "cpYFP"), and Y66H (blue, "cpBFP") mutations were made (see Cubitt et al., Trends Biochem., 20:448-455, 1995, for exemplary methods). As shown in FIG. **15**, the variants exhibit fluorescence emission spectra consistent with their respective intended designs.

[0258] The ΔF/F of the color variants in response to maltose is different (in each case inferior) from the ΔF/F of 2.5 observed in PPYF-green. The EcMBP165-cpYFP.PPYF sensor, which has the same covalent chromophore structure as PPYF, has the greatest ΔF/F of the three spectral variants (FIG. **15**A). EcMBP165-cpCFP.PPYF has a lower ΔF/F than the green and yellow variants, but by incorporating previously identified mutations, (L1-PC+GFP-Y151F; the resulting protein is called EcMBP165.cpCFP.PCF), a variant with ΔF/F=0.8 was obtained (FIG. **15**A).

[0259] The EcMBP165-cpBFP.PPYF variant, while dimly fluorescent, is not a sensor, and a screen of 800 linker variants failed to produce any variant with ΔF/F >0.2 (FIG. **16**).

[0260] Since EcMBP165-cpBFP.PPYF was very dim, Azurite mutations T65S+V150I+V224R were included to increase brightness and stability, and make EcMBP165-cpAzurite a good template for linker screening. Using oligonucleotides encoding XX amino acid linkers, a variant was obtained, EcMBP165-cpAzurite.L2-FE, that had ΔF/F=0.8 (FIG. **15**).

Example 1E: Modifying Sensor Color and Ligand Specificity/Affinity

[0261] The four sucrose-binding "5-7" mutations described above that conferred weak sucrose affinity in the green sensor (EcMBP165-cpGFP.PPYF) were converted to blue, cyan, and yellow maltose sensors (EcMBP165-cpAzurite.L2-FE, EcMBP165-cpCFP.PCF, and EcMBP165-cpYFP.PPYF). The green and yellow sensors showed increased fluorescence upon addition of 10 mM sucrose, but the cyan and blue proteins did not (FIG. **15**A). Like the green variant, the yellow variant had no detectable sucrose affinity with the wild type binding pocket (FIG. **15**C) and millimolar affinity for both sugars, with preference for sucrose over maltose (FIG. **15**D).

[0262] As seen in FIG. **17**, as maltose concentration increased, the blue sensor increased in fluorescence first (Kd ~2.7 μM), then the green (Kd ~40 μM), then the yellow (Kd ~350 μM), and at high maltose concentrations, the cyan variant began to increase its fluorescence (Kd ~1.7 mM).

Example 1F: Imaging Bacteria

[0263] The ultimate value of genetically encoded fluorescent sensors is in their utility for observing analyte flux in living cells and organisms. In a simple proof-of-principle experiment, *Escherichia coli* expressing PPYF or PPYF.T203V (see "Second-generation maltose sensors" below) were imaged in the green fluorescence channel in the absence of maltose, and then re-imaged after addition of saturating maltose to the media.

[0264] As shown in FIG. **18**, bacteria expressing the sensors clearly became brighter, while control bacteria expressing EGFP appeared unchanged. Increased fluorescence was quantified by measuring the peak (gray-value) pixel intensity of each bacterium. Those expressing PPYF undergo an approximate doubling of fluorescence (bacterium-averaged $\Delta F/F=1.1\pm0.4$), those expressing PPYF.T203V have slightly increased ΔF/F ($\Delta F/F=1.29\pm0.2$), while those expressing EGFP have no change in fluorescence ($\Delta F/F=-0.01\pm0.05$).

Example 1F: 2-Photon Imaging of Mammalian Cells

[0265] Multi-photon microscopy opened new frontiers for in vivo fluorescence imaging, in particular for neuronal activity visualization through the use of genetically encoded calcium indicators (Tian et al., Nat. Methods, 3:281-286, 2009; Denk et al., Science, 248:73-76, 1990; Denk and Svoboda, Neuron, 18:351-357, 1997).

[0266] To demonstrate that the maltose sensors described herein have the potential to be used for 2-photon imaging, fluorescence excitation spectra were collected. As shown in FIG. **19**, with a 535 nm bandpass emission filter (50 nm bp), EcMBP165-cpGFP.PPYF showed a 10-fold maltose-dependent increase in fluorescence when excited at 940 nm. All

four spectral variants showed a significant maltose-dependent increase in 2-photon fluorescence.

Example 1G: Sub-Cloning Maltose Sensors

[0267] EcMBP165-cpGFP.PPYF.T203V (see "Second-generation maltose sensors" below) were cloned into a modified version of the pDisplay vector (Invitrogen) for extracellular display on the surface of transiently transfected human embryonic kidney (HEK293) cells.

[0268] As shown in FIG. **20**, the sensor localized to the plasma membrane and increased in brightness in a concentration-dependent manner when perfused with buffers of varying maltose concentration. The ΔF/F is 5.8-fold, very close to that of the soluble protein produced in *E. coli*, with the mid-point of the maltose-dependent fluorescence increase being 6.5 μM (FIG. **21**A), very similar to the affinity determined on purified protein (5 μM). Furthermore, the surface displayed sensor responded rapidly to a pulse of 1 mM maltose (FIG. **21**A), indicating that the time course for its action is useful for transient events.

Example 1H: Crystal Structure Analysis of Maltose Sensors

[0269] High-resolution structures of several of the maltose sensors described above were generated. Crystallization trials were performed with EcMBP165-cpGFP.PPYF, EcMBP175-cpGFP.L1-HL, and EcMBP311-cpGFP.L2-NP in the presence and absence of excess maltose, from which both EcMBP175-cpGFP.L1-HL and EcMBP311-cpGFP.L2-NP crystallized in the presence of maltose. X-ray structures were solved to 1.9 and 2.0 Å resolution, respectively, by molecular replacement (FIGS. **22**A-**22**C).

[0270] The structures of the cpGFP and EcMBP domains of the sensors are superimposable with published crystal structures of cpGFP (from GCaMP2; RMSD=0.36 and 0.38 Å, respectively, for comparing 221 common Cα atoms) and EcMBP-maltose (RMSD=0.43 and 0.37 Δ, 370 Cα). The structure of EcMBP is largely unperturbed by insertion of the cpGFP domain; only residues around the 175 and 311 insertion sites showed any significant displacement.

[0271] GFP-H148, which H-bonds the GFP chromophore in the structure of native GFP, also directly H-bonded to the chromophore in the EcMBP175-cpGFP.L1-HL-maltose structure (FIG. **22**B), although a different rotamer was observed. In the EcMBP311-cpGFP.L2-NP-maltose structure, GFP-H148 is pulled away from the chromophore and is largely replaced by the Asn from linker 2, which makes H-bond interactions to both strand 8 of the GFP barrel and the chromophore phenolate oxygen (through a water molecule, FIG. **22**D). GFP-H148, meanwhile, seemed to stabilize the conformation of linker 2 of EcMBP311-cpGFP.L2-NP by H-bonding the backbone carbonyl of the linker 2 Asn. There is some solvent access to the cpGFP chromophore through the hole in the GFP barrel created by circular permutation, although the inter-domain linkers block much of the opening in both structures. Relatively few contacts are made between the cpGFP and EcMBP domains.

[0272] Based on the structures of two maltose-bound sensors, the sensing mechanism likely involves a shift in the relative position of linker 1 and linker 2 induced by the conformational change in the EcMBP domain associated with maltose binding (FIG. **5**). The register shift of interactions between the two linkers could alter the proximity of linker 2 and nearby side-chains to the cpGFP chromophore and change the water structure in the cpGFP opening, leading to a shift in the chromophore protonation equilibrium. This might explain why rigid proline is preferred in either linker, since conformational changes upon ligand binding might be better propagated through the rigid linkers to the cpGFP chromophore environment.

Example 1I: Generation of Second-Generation Maltose Sensors

[0273] In an attempt to increase brightness and ΔF/F of GCaMP, the local environment of the chromophore was altered by randomizing residues within cpGFP, and screening for improved variants (Tian et al., nat. Methods, 6:875-881, 2009).

[0274] As shown in FIG. **23**, in the context of EcMBP165-cpGFP.PPYF, the T203V mutation decreases the fluorescence emission of the apo-state by half (FIG. **23**A), while saturated fluorescence and affinity are unchanged (FIG. **23**B), increasing ΔF/F to 6.5. In the maltose-saturated state, PPYF itself has about a quarter the brightness of EGFP, and half the brightness of cpGFP.

[0275] In the context of EcMBP311-cpGFP.L2-NP, the T203V mutation decreases the brightness of both the apo-state and the saturated-state equally, resulting in no significant change in ΔF/F (FIGS. **23**C and D).

[0276] These results indicate that the benefits of the T203V mutation are not universally transferable, and that cpGFP-based fluorescent sensors need to be optimized individually.

Example 2: Maltotriose Indicators

[0277] Genetically encoded maltotriose indicators were created using *Pyrococcus furiosus* maltotriose binding protein. As described below, only the structure of the ligand-bound state *P. furiosus* maltotriose binding protein (PfMBP) is available. As shown in FIGS. **1** and **2**, PfMBP is homologous to EcMBP (compare FIGS. **1** and **2**). Two sensors were made, PfMBP171 and PfMBP316, the insertion points for which were selected based on homology to EcMBP165 and EcMBP311, respectively. Linkers were optimized. PfMBP sensors have a ΔF/F of ~1.2.

[0278] *Pyrococcus furiosus* is a thermophilic organism. Proteins from thermophiles have been shown to be more amenable to mutation than those from mesophiles (Bloom et al., Proc. Natl. Acad. Sci., 103:5869-5874, 2006). As an alternative to developing new sensors by inserting cpGFP into PBPs, it should also be possible to generate new sensors by changing the ligand-binding specificity of an existing PBP-based sensor.

[0279] It has previously been shown that the binding sites of PBPs can be reengineered to accommodate novel ligands (Looger et al., Nature, 423:185-190, 2003). However, those re-design efforts used framework proteins from mesophiles and suffered from poor stability. We hypothesized that PfMBP, which is intrinsically more stable than EcMBP, is more tolerant of mutations. To test this hypothesis, we characterized and compared the stability of PfMBP to EcMBP, PfMBP-cpGFP sensors to EcMBP-cpGFP sensors, PfMBP binding site mutants to EcMBP binding site mutants, and PfMBP-cpGFP sensor binding site mutants to EcMBP-cpGFP sensor binding site mutants. Conclusively, the PfMBP variants were more stable than the EcMBP variants.

Finally, we demonstrate that the increased thermo-stability of the PfMBP-cpGFP sensors is useful for the measurement of maltotriose at temperatures as high at 60° C., whereas the EcMBP-cpGFP sensors are only useful for the measurement of maltose at temperatures as high as 40° C.

Example 2A: Identification of cpGFP Insertion Sites in PfMBP

[0280] The ligand-bound (closed) structure of PfMBP is available (Evdokimov et al., J. Mol. Biol., 305:891-904, 2001), but the unbound structure is not. Accordingly, insertion sites for the PfMBP-cpGFP sensors were identified by homology to EcMBP.

[0281] Sites were selected based on the structural similarities between PfMBP and EcMBP. Two sites were selected. One of these sites is EcMBP311, which is homologous to PfMBP316. This site is at juncture between the end of the cluster of helices (Helices 8a, 8b, 8c) and the start of the "equatorial" spanning helix (Helix 9). Another site that was made into a sensor in EcMBP was EcMBP165, which is homologous to PfMBP171. cpGFP was inserted into PfMBP at each of these sites. The sequences of the resulting constructs, PfMBP171-cpGFP and PfMBP316-cpGFP, are shown in FIGS. **24** and **25**, respectively.

Example 2B: Linker Optimization

[0282] Libraries of variants of SEQ ID NOs: 50-53 were generated with randomized linkers by single-stranded uracil template mutagenesis using the primers listed below:

175 Linker 1 Primers:

AIAQAFxxSHNVYIMA (SEQ ID NO: 54)

AIAQAFPxSHNVYIMA (SEQ ID NO: 55)

171 Linker 2 Primers:

KLEYNFNxxYYFDDKTE (SEQ ID NO: 56)

316 Linker1 Primers

VLDDPExxHNVYIM (SEQ ID NO: 57)

VLDDPEIxxSHNVYIM (SEQ ID NO: 58)

316 Linker2 Primers

KLEYNFxxNDPVIY (SEQ ID NO: 59)

KLEYNFNxPKNDPVIY (SEQ ID NO: 60)

KLEYNFNPxKNDPVIY (SEQ ID NO: 61)

[0283] Where "x" indicates that a degenerate primer (with DNA sequence "NNS") was used to encode all 20 possible amino acids.

[0284] Several thousand variants were screened in semi-high-throughput fashion, measuring fluorescence intensity of clarified cell lysate in the absence and presence of 1 mM maltotriose.

[0285] Screening a fully-degenerate, length-two library ("XX") at either the PfMBP171-cpGFP linker (linker 1) or the cpGFP-PfMBP linker (linker 2) yielded proteins with maltotriose-dependent fluorescent increases >100% or decreases >20% (FIG. **26**A). A variant from this group with a GlyGly PfMBP-cpGFP linker and a PheGlu cpGFP-PfMBP linker was selected for further characterization. This variant, called "PfMBP171-cpGFP.L2FE" has a ΔF/F=1.2, a Kd for maltotriose of <1 μM.

[0286] Screening a fully-degenerate, length-two library ("XX") at either the PfMBP316-cpGFP linker (linker 1) or the cpGFP-PfMBP linker (linker 2) also yielded proteins with maltotriose-dependent fluorescent increases >100% or decreases >20% (FIG. **26**B). A variant from this group with a GlyGly PfMBP-cpGFP linker and a PheGlu cpGFP-PfMBP linker was selected for further characterization. This variant, called "PfMBP316-cpGFP.L1-NP" has a ΔF/F=1.2, a Kd for maltotriose of 40 μM.

[0287] These data support that structurally homologous frameworks can be compared to identify insertion sites for cpGFP.

Example 2C: Characterization of the Thermostability of the PfMBP and PfMBP-cpGFP Compared to EcMBP and EcMBP-cpGFP

[0288] Thermal stability of PfMBP171-cpGFP.L2FE was measured using circular-dichroism (CD) and compared to the original EcMBP and PfMBP binding proteins, along with cpGFP. Following the changes by means of CD allowed determination of whether different transitions happened in alpha, beta, or both kinds of structures.

[0289] Given that cpGFP is a beta barrel, strong transitions in the beta signal alone were associated with changes in this kind of structure. In the same way, transitions in both kinds of signals were associated with the binding protein structure. As shown in FIG. **27**A, PfMBP is significantly more thermostable than EcMBP. In fact, while EcMBP denatured at about 50° C., PfMBP did not denature at temperatures less than 80° C. Also, the addition of maltose to EcMBP stabilized the protein by about 10° C.

[0290] As shown in FIG. **27**B, the stability of the EcMBP component of the EcMBP165-cpGFP.PPYF sensor decreased from 50° C. to 45° C. with insertion of cpGFP, while the intrinsic stability of cpGFP in the sensor remained unchanged. There was little change in the stability of the PfMBP component of the PfMBP171-cpGFP.L2FE sensor with insertion of cpGFP (FIG. **27**B). Moreover, PfMBP seemed to exert a small stabilizing effect over the inserted cpGFP, as shown by the change in the steepness and melting point of the curve of the soluble form and the PfMBP171-cpGFP.L2FE sensor. All the associations made between transitions and domain unfolding were supported by CD spectra taken at the beginning and the end of each temperature ramp.

[0291] Analysis of whether the PfMBP scaffold was more tolerant of mutation than the EcMBP scaffold was also performed. Proof-of-principle mutations were made to the ligand-binding sites of EcMBP and PfMBP, and their respective sensors. In EcMBP, Asn12 was mutated to Trp to result in steric clashes with the surrounding residues, and backbone, of the binding pocket. The homologous mutation in PfMBP is Ala13Trp, which would be expected to have the same effect.

[0292] As shown in FIG. **27**C, N12W decreased the Tm of EcMBP from 50° C. to 40° C., while the corresponding mutation in PfMBP, A13W, had no noticeable effect. This data confirms that the thermo-philic protein is more tolerant

of mutations to the binding site. Furthermore, in the context of the sensors, the N12W mutation to EcMBP165-cpGFP. PPYF completely destabilized the binding protein component of the sensor (FIG. **27**D), while the A13W mutation in PfMBP171-cpGFP.L2FE had no effect on stability (FIG. **27**D).

Example 2D: Tolerance of PfMBP Sensor to Increased Temperature

[0293] Fluorescence of the protein in the apo and ligand-bound states at was measured at different temperatures.

[0294] As shown in FIG. **28**A, fluorescence of the EcMBP165-cpGFP.PPYF sensor in the bound state was higher than it is in the apo-state at lower temperatures, by about 4-fold. However, at around 55° C. (the unfolding transition of the EcMBP component) the fluorescence of the EcMBP165-cpGFP.PPYF sensor dropped precipitously. As a result, EcMBP165-cpGFP.PPYF is unsuitable for detection of maltose at temperatures greater than 50° C. (FIG. **28**B). In contrast, PfMBP171-cpGFP.L2FE retained its maltotriose binding capabilities at high temperatures (FIGS. **28**A and **28**B), and is limited only by the intrinsic fluorescence of the cpGFP component, which decays at about 80° C. (FIG. **28**A).

Example 2E: Measurement of Maltodextrins in Hot Liquids

[0295] To demonstrate that the soluble and immobilized sensors function similarly, PfMBP171-cpGFP.L2FE, PfMBP316-cpGFPL1XXX, and EcMBP165-cpGFP.PPYF. T203V were immobilized via their N-terminal poly-histidine tags on to the surface of Ni-NTA coated glass. In a fluorescence plate reader, the immobilized proteins performed similarly to their soluble counterparts (see FIGS. **28**C, **28**D, and **28**F).

[0296] Next, a prototype device was constructed, with a light guide providing the excitation light and returning the fluorescent emitted light back to the photodetector, the bio-sensor protein immobilized to Ni-NTA coated coverslips, and the coverslip attached to the end of the light guide. The "wand" of the detector was dipped into different compositions of solutions, each with varying concentrations of maltose or maltotriose. Experiments were performed at different temperatures. PfMBP-cpGFP sensor performed better at higher temperatures (as high as 60° C.) than the EcMBP-cpGFP sensor.

Example 3: Glutamate Indicators

[0297] Glutamate indicators were created from *Escherichia coli* glutamate-binding protein (EcYbeJ). As with PfMBP in Example 2, only the structure of the ligand-bound EcYbeJ is available. EcYbeJ is homologous to EcMBP, but to a lesser degree. The best homology match between a site in EcYbeJ and a site in a binding protein for which an intensity-based sensor has already been created is EcYbeJ253 and EcMBP311 (described herein). As shown in FIG. **3**, both sites are at the junction of "Rising Helix 8" and the "Equatorial Helix/Coil." The amino acid composition of the cpGFP and EcYbeJ junction was made the same as that of the EcMBP311-cpGFP sensor (Linker 2=NP). The amino acid composition of the EcYbeJ junction and cpGFP was optimized to LV (Linker 1=LV). The variant has a ΔF/F of 5.

Example 3A: Identification of cpGFP Insertion Sites

[0298] The ligand-bound (closed) structure of *Shigella flexneri* glutamate binding protein is available (Fan et al., Protein Pept. Lett., 13:513-516, 2006). This protein has only 4 amino acid mutations relative to EcYbeJ, and is thus an appropriate model.

[0299] Insertion sites for the EcYbeJ-cpGFP sensors were identified by homology to EcMBP. Based on the topology map (FIG. **3**), position 311 in EcMBP was identified as an acceptable insertion site for EcYbeJ. EcMBP311 is equivalent to EcYbeJ253. EcYbeJ253 is at juncture between the end of the cluster of helices (Helices 8a, 8b, 8c) and the start of the "equatorial" spanning helix (Helix 9). In YbeJ, the structure that is homologous to the equatorial helix is the equatorial coil (depicted in red, to match the red coloring of Helix 9).

[0300] Intrinsic affinity of wild-type YbeJ for glutamate (~1 μM) was too high to permit high-throughput screening of linker libraries. Endogenous glutamate (from the growth media) saturates the sensor, making measurement of the unbound state technically challenging. A mutation to YbeJ (A184V), in the "hinge" of the protein were made. Mutation of this residue to Trp or Arg have previously been shown to decrease affinity in FRET-based sensors (see Okumoto et al., Proc. Natl. Acad. Sci., 102:8740-8745, 2005). EcYbeJ253 (A184V)-cpGFP has an affinity for glutamate of about 100 μM. All references to EcYbeJ253-cpGFP, unless otherwise noted, refer to the A184V variant. The sequences of the EcYbeJ constructs are shown in FIG. **29**.

Example 3B: Linker Optimization

[0301] Libraries of variants of SEQ ID NOs: 62-63 were generated with randomized linkers by single-stranded uracil template mutagenesis using the primers listed below:

253 Linker 1 Primers:

FKNPIPPxSHNVYIMA (SEQ ID NO: 64)

FKNPIPPxxSHNVYIMA (SEQ ID NO: 65)

FKNPIPPPxSHNVYIMA (SEQ ID NO: 66)

FKNPIPPxPSHNVYIMA (SEQ ID NO: 67)

KWFKNPIxxSHNVYIMA (SEQ ID NO: 68)

FKNPIPPxxNVYIMAD (SEQ ID NO: 69)

KWFKNPIxxNVYIMAD (SEQ ID NO: 70)

253 Linker 2 Primers:

KLEYNFNxKNLNMNF (SEQ ID NO: 71)

KLEYNFNxxKNLNMNF (SEQ ID NO: 72)

KLEYNFNxPKNLNMNF (SEQ ID NO: 73)

-continued

(SEQ ID NO: 74)
KLEYNFNPxKNLNMNF

(SEQ ID NO: 75)
GHKLEYNxxLNMNF

(SEQ ID NO: 76)
KLEYNFNxxLNMNF

[0302] Where "x" indicates that a degenerate primer (with DNA sequence "NNS") was used to encode all 20 possible amino acids.

[0303] Several thousand variants were screened in semi-high-throughput fashion, measuring fluorescence intensity of clarified cell lysate in the absence and presence of 10 mM glutamate.

[0304] Screening a fully-degenerate, length-two library ("XX") at the EcYbeJ253-cpGFP linker (linker 1) identified a sensor with glutamate-dependent fluorescent increases of ~100%. This variant has a LeuVal EcYbeJ-cpGFP linker (L1-LV) and was used as the framework for optimization of the cpGFP-EcYbeJ253 linker (linker 2). The results of that screen yielded a protein with glutamate-dependent fluorescent increase of ~500% and a linker 2 composition of AsnPro. As shown in FIG. **30**, this variant, called "EcYbeJ253-cpGFP.L1LVL2NP" has a ΔF/F=5, a Kd for glutamate of 100 μM. Interestingly, the composition of the second linker, AsnPro, is the same as the linker composition of EcMBP311-cpGFP.L2NP.

Example 3C: Detection of Extracellular Glutamate

[0305] EcYbeJ253-cpGFP.L1LVL2NP was cloned into the pDisplay™ vector to allow targeting and anchoring of the sensor to the plasma membrane. The resulting construct was transfected into cultured mammalian cells (HEK293) to visualize the addition of glutamate to extracellular media. Constructs were also generated in a bacterial expression vector with the epitope tags individually and in combination.

[0306] As shown in FIG. **31**, the hemagglutinin tag interferes with the fluorescence change. EcYbeJ253-cpGFP.L1LVL2NP was re-cloned into a derivative of the pDisplay™ vector, lacking the hemagglutinin tag, called pMinDis (for Minimal Display). This new construct, when expressed in HEK293 cells, shows a change in fluorescence intensity under 2-photon excitation that is approximately the same as the soluble protein (see FIG. **32**) with higher affinity, of about 1 μM (see FIG. **32**).

[0307] To demonstrate that the sensor is functional in neurons, and not just cultured HEK cells, the gene from EcYbeJ253-cpGFP.L1LVL2NP was cloned into an adeno-associated virus vector (AAV) under control of the synapsin promoter. Virus particles were generated and used to infect cultured primary hippocampus neurons from rats 7 days after culturing. 14 days after culturing (and 7 days after infection), the infected neurons were imaged under 2-photon microscopy (FIG. **33**).

Example 4: Phosphonate Indicators

[0308] An indicator for phosphonate compounds was created from *Escherichia coli* phosphonate-binding protein (EcPhnD). In this instance, only the structure of the ligand-bound state was available at the time the sensor was conceived. EcPhnD is homologous to EcMBP to a lesser degree and to EcYbeJ to a greater degree. The best homology match between a site in EcPhnD and a site in a binding protein for which an intensity-based sensor has already been created is EcPhnD90 and EcYbeJ253. There is no "Rising Helix 8" in EcPhnD, but there is an "Equatorial Helix/Coil" (FIG. **4**). cpGFP was inserted at the Equatorial Helix/Coil and linkers were optimized to yield a sensor with ΔF/F of 1.2. EcPhnD is a dimmer, so, a pair of mutations (L297R+L301R) were made to convert it to a monomer. The monomer variant has a ΔF/F of 1.6.

Example 4A: Identification of cpGFP Insertion Sites in EcPhnD

[0309] Insertion sites for the EcPhnD-cpGFP sensors were identified using the ligand-bound (closed) structure of EcPhnD by homology to EcMBP. Based on the topology map (FIG. **4**), position 311 in EcMBP was identified as an acceptable insertion site in EcPhnD. EcMBP311 corresponds to EcPhnD90. This site is at the point where the rising strand (Strand D) of EcPhnD has a small bend in it that runs equatorial to the rest of the sheets in the protein. Even though it is topologically different from the "equatorial" spanning helix (Helix 9) of EcMBP its equatorial alignment is similar, and with just the closed structure at the time, in an environment that was expected to undergo significant dihedral change upon binding ligand. Sequences of EcPhnD constructs are shown in FIG. **34**.

Example 4B: Linker Optimization

[0310] Libraries of variants of SEQ ID NOs: 77-78 were generated with randomized linkers by single-stranded uracil template mutagenesis using the primers listed below:

90 Linker 1 Primers:

(SEQ ID NO: 79)
QTVAADGSSHNVYIMA

(SEQ ID NO: 80)
QTVAADxxSHNVYIMA

(SEQ ID NO: 81)
QTVAADxPSHNVYIMA

(SEQ ID NO: 82)
QTVAADPxSHNVYIMA

(SEQ ID NO: 83)
QTVAADxxNVYIMA

(SEQ ID NO: 84)
QTVAADxxSHNVYIMA

(SEQ ID NO: 85)
VFQTVAxxSHNVYIMA

90 Linker 2 Primers:

(SEQ ID NO: 86)
HKLEYNFNPGYWSVLI

(SEQ ID NO: 87)
HKLEYNFNxxPGYWSVLI

(SEQ ID NO: 88)
HKLEYNxxPGYWSVLI

(SEQ ID NO: 89)
HKLEYNFNxxYWSVLI

(SEQ ID NO: 90)
HKLEYNFNPxYWSVLI

[0311] Where "x" indicates that a degenerate primer (with DNA sequence "NNS") was used to encode all 20 possible amino acids.

[0312] Several thousand variants were screened in semi-high-throughput fashion, measuring fluorescence intensity of clarified cell lysate in the absence and presence of 100 uM 2AEP.

[0313] Screening a number of fully-degenerate, libraries at the EcPhnD90-cpGFP linker (linker 1) yielded a protein with 2AEP-dependent fluorescent increases of >100%. This variant has a AlaAsp EcPhnD-cpGFP linker (L1-AD) and a ΔF/F of 1.2. The variant came from a linker that also deleted two residues, effectively making the insertion point of cpGFP occur after residue D88, and then skipping to residue P91 at the cpGFP-EcPhnD linker.

[0314] It was observed from the crystal structure that EcPhnD forms a dimer. To disrupt the dimer inter-face and potentially simplify the observable binding behavior of the EcPhnD protein, two mutations, L297R and L301R, were introduced into the dimerization helices. These mutations were expected, by charge repulsion, to disrupt the dimer interface. As shown in FIG. **35**, incorporation of L279R and L301R mutations into EcPhnD90-cpGFP.L1AD caused ΔF/F to increases to 1.6 in response to 2AEP.

[0315] Further attempts to crystallize the open, ligand-unbound form of the protein were successful after making a mutation to the binding site, H157A, that substantially decreased affinity for phosphonate compounds. This mutant was crystallized in the absence of ligand, and the open state of the protein solved. The ΔDihedral analysis (FIG. **36**) showed that the region of greatest dihedral change was the group of residues from 88-90, just one amino acid away from the site chosen by homology to the equatorial helix.

[0316] These data further indicate that ΔDihedral metric is sufficient for identifying sites in PBPs into which cpGFP can be inserted and result in intensity-based fluorescent sensors.

Example 5: Glucose Indicators

[0317] Glucose indicators were created from *Thermus thermophilus* glucose binding protein (TtGBP). In this instance, only the structure of the ligand-bound state is available. TtGBP is very homologous to EcMBP and PfMBP (compare FIG. **5** with FIGS. **1** and **2**). The insertion point (TtGBP326) was chosen by homology to EcMBP311 and PfMBP316. The amino acid composition of the cpGFP and TtGBP junction was made the same as that of the EcMBP311-cpGFP and EcYbeJ253 sensors (Linker 2=NP). Linker 1 was optimized (Linker 1=PA) and the TtGBP326 sensor have a ΔF/F of ~2.5. To improve its utility for the measuring glucose concentrations in human blood, the affinity was weakened from its native ~1 μM to 1.5 mM by mutation of two residues in the binding pocket (H66A+ H348A).

Example 5A: Identification of cpGFP Insertion Sites in TtGBP

[0318] The ligand-bound (closed) structure of TtGBP is available (Cuneo et al., J. Mol. Biol., 362:259-270, 2006). Accordingly, insertion sites for the TtGBP-cpGFP sensors were identified by homology to EcMBP and PfMBP. Based on the topology map (FIG. **5**), it is apparent that TtGBP, PfMBP, and EcMBP are structurally similar in the closed, ligand-bound state. Positions in EcMBP determined by the dihedral analysis (see above) were predicted to be acceptable insertion sites in TtGBP. EcMBP311 is homologous to TtGBP326. This site is at juncture between the end of the cluster of helices (Helices 8a, 8b, 8c) and the start of the "equatorial" spanning helix (Helix 9). The amino acid sequence of the TtGBP construct is shown in FIG. **37**.

Example 5B: Linker Optimization

[0319] Libraries of variants of SEQ ID NO:91 were generated with randomized linkers by single-stranded uracil template mutagenesis using the primers listed below:

```
326 Linker 1 Primers:
                                (SEQ ID NO: 95)
DSDPSKYxxSHNVYIM

                                (SEQ ID NO: 96)
DSDPSKYPxSHNVYIM

                                (SEQ ID NO: 97)
DSDPSKYxPSHNVYIM

                                (SEQ ID NO: 98)
RLDSDPSxxSHNVYIM

                                (SEQ ID NO: 99)
DSDPSKYxxNVYIM

326 Linker 2 Primers:
                              (SEQ ID NO: 100)
KLEYNFNxxNAYGQSA

                              (SEQ ID NO: 101)
KLEYNFxxPNAYGQSA

                              (SEQ ID NO: 102)
GHKLEYNxxNAYGQSA

                              (SEQ ID NO: 103)
KLEYNFNxPNAYGQSA

                              (SEQ ID NO: 104)
KLEYNFNPxNAYGQSA
```

[0320] Where "x" indicates that a degenerate primer (with DNA sequence "NNS") was used to encode all 20 possible amino acids.

[0321] Several hundred variants were screen in semi-high-throughput fashion, measuring fluorescence intensity of clarified cell lysate in the absence and presence of 10 mM glucose.

[0322] Linker 1 was optimized (Linker 1=PA) and the TtGBP326-cpGFP.L1PAL2NP sensor has a ΔF/F of ~2.5 (see FIG. **38**). Additionally, the TtGBP sensor was tested with and without the N-terminal pRSET tag and no difference was observed. Specifically, both sensors exhibited an affinity for glucose of about 1.5 mM and a ΔF/F of 2.5.

[0323] Data showing that it was possible to construct a glucose sensor by replacing the EcMBP or PfMBP with TtGBP, retaining the composition of linker 2, and optimizing the composition of linker 1, indicates that the methods for generating sensors disclosed herein can be used to generate sensors using any suitable framework.

Example 5C: Detecting Changes in Glucose Concentration in Vivo

[0324] The TtGBP326-cpGFP.L1PAL2NP sensor was cloned into a variant of the pDisplay™ vector lacking the N-terminal secretion sequence, the N-terminal hemaggluti-

nin tag, the C-terminal cMyc tag, and the C-terminal PDGFR membrane anchoring domain.

[0325] The TtGBP sensor was cloned into a mammalian expression vector (based on the pDisplay™ vector described in Example 3 above) with the secretion, epitope, and transmembrane anchoring peptides removed, thus resulting in cytosolic expression of the TtGBP326-cpGFP.L1PAL2NP+H66A+H348A sensor. The construct was transfected into HEK293 cells. As shown in FIG. **39**, the TtGBP sensor was expressed in the cytosol.

[0326] As shown in FIG. **40**, addition of 10 mM glucose to the media increases fluorescence.

[0327] The TtGBP326-cpGFP.L1PAL2NP+H66A+H348A sensor was further modified by L276V mutation to produce TtGBP326.L1PA.L2NP.H66A.H348A.L276V (see FIG. **50**). As shown in FIG. **51**, this construct has an affinity for glucose of 6.5 mM.

[0328] Additionally, the TtGBP326.L1P1.L2NP.G66A.H348A.L276V was cloned into the pMinDis derivative of the pDisplay vector and expressed on the extracellular surface of HEK293 cells. After exchanging the HEK293 cell media for PBS, addition of glucose to the PBS led to an increase in fluorescence (see FIG. **52**).

[0329] These data indicate, in part, that the pRSET tag is not essential to the function of the sensor and that the TtGBP326-cpGFP.L1PAL2NP sensor is capable of detecting changes in the concentration of glucose inside or on the external surface of human cells.

Example 6: Stability, Affinity and Chromatic Variants of the Glutamate Sensor iGluSnFR

Example 6A: In Vivo Assessment of iGluSnFR Brightness in Apical Dendrites in Mouse Somatosensory Cortex

[0330] Wildtype C57/B6 mice were purchased from the Jackson Laboratory and group housed in the Janelia animal facility. Mice were injected at 8 weeks of age with AAV2/1.hSynapsin1.iGluSnFR.A184S or SF-iGluSnFR.A184S, at identical titers (1×10^{13} genomic copies per milliliter, GC/ml), volumes (20 nl), and locations (3 mm lateral to midline, 1.4 mm caudal to bregma, and 0.3 mm below the cortical surface). After viral injection, a craniotomy (3 mm diameter) was made over the injection site, and the skull was replaced with a #1.5 Schott glass and fixed in place with dental acrylic (Lang Dental Manufacturing), which also secured a titanium head bar to the skull for head-mounting during imaging experiments.

[0331] In vivo two-photon imaging experiments were performed during a state of 'quite wakefulness', after having been habituated to head fixation the prior 2-3 days. Period water rewards were given to keep animals hydrated and passive. For comparisons of intensity and bleaching, a custom two-photon microscope emitting 960 nm light from a Coherent Chameleon ultrafast laser was used. All experiments were performed using a 25×, 1.5 NA Olympus objective immersed in water. Image acquisition was performed with ScanImage (Vidrio) software and analyzed post hoc using ImageJ (NIH). Images were acquired at a variety of speeds/zooms, and powers in order to assess the impact of pulse energy and dwell time on bleaching and intensity. Images at each setting were acquired for 5 seconds. To analyze the data, images were averaged and thresholded to create a signal (above threshold) and background mask. Signals in these masks were then averaged, and SNR was calculated from these as (signal-background)/(standard deviation of background). Bleaching percentage was calculated as the average intensity in the first 25% of the trace, divided by the last 25% of the trace.

Example 6B: Ferret Visual Cortex Assessment of SF-iGluSnFR.A184S and A184V

[0332] All procedures were approved by the Max Planck Florida Institute for Neuroscience Institutional Animal Care and Use Committee and adhered to the standards of the National Institutes of Health. Juvenile female ferrets (*Mustela putorius furo*, Marshall Farms) were used. Animals were housed in a vivarium under 16 hour light/8 hour dark cycle. The full methodological details for functional two-photon imaging of ferret visual cortex is previous described in detail (Wilson et al., 2016, Nat. Neurosci., 19:1003-9).

[0333] Briefly, juvenile female ferrets (*Mustela putorius furo*, Marshall Farms) aged P21-22 (n=2) were anesthetized with ketamine (50 mg/kg, IM) and isoflurane (1-3%) delivered in O_2, then intubated and artificially respirated. Atropine (0.2 mg/kg, SC) and a 1:1 mixture of lidocaine and bupivacaine administered subcutaneously in the scalp. Animals were kept at 37° C. A small craniotomy (0.8 mm) was made over the visual cortex 7-8 mm lateral and 2-3 mm anterior to lambda. AAV2/1.hSynapsin1.Cre (Penn Vector Core) was diluted in phosphate-buffered saline (Sigma) and mixed with AAV2/1.hSynapsin-FLEX.SF-iGluSnFR.A184S or A184V for expression in layer 2/3 cortical neurons. Beveled glass micropipettes were lowered into the brain and 400-500 nl of virus were injected over 5 minutes at multiple depths below the pia. Following, the craniotomy was filled with 1% w/v agarose.

[0334] After four weeks, ferrets were anesthetized with 50 mg/kg ketamine and 1-3% isoflurane. Atropine (0.2 mg/kg, SQ) and bupivacaine were administered. Animals were kept at 37 to 38° C., artificially respirated, and given intravenous fluids. Isoflurane (1-2%) was used throughout the surgical procedure to maintain a surgical plane of anesthesia. ECG, endtidal CO_2, external temperature, and internal temperature were continuously monitored. A custom titanium headplate was implanted on the skull at the viral injection site and the dura retracted to reveal the cortex. A custom insert with a single 4 mm coverglass (0.17 mm thickness) was placed onto the brain to gently compress the underlying cortex and dampen biological motion during imaging. The cranial window was hermetically sealed using a stainless steel retaining ring and Vetbond. Tropicamide Ophthalmic Solution and Phenylephrine Hydrochloride Ophthalmic Solution were applied and contact lenses were inserted into both eyes. Upon completion of the surgical procedure, Isoflurane was gradually reduced and pancuronium (2 mg/kg/hour) was delivered IV to immobilize the animal.

[0335] The animal was placed under the microscope 25 cm from the stimulus monitor, with the monitor subtending 130 degrees in azimuth and 74 degrees in elevation. Imaging was performed using a Bergamo II (Thorlabs) running ScanImage 5 or ScanImage 2015[19] (Vidrio Technologies) with dispersion compensated 950 nm excitation provided by an Insight DS+ (Spectraphysics). Average excitation power after the exit pupil of the objective (16×, CFI75, Nikon Instruments) ranged from 25 to 40 mW. Two-photon frame triggers from ScanImage were synchronized with stimulus information using Spike2 (CED). Visual stimuli were gen-

erated using PsychoPy (Peirce, 2007, J. Neurosci. Methods, 162:8-13). Full-field drifting square-wave gratings (16 directions, 100% contrast, 0.1 cycles/°, 4 cycles/sec., 3 sec. stimulus period followed by 2-3 sec. ISI, plus a blank) were presented to the contralateral eye in a pseudorandom sequence for 8 trials.

[0336] Images were corrected for in-plane motion using a correlation-based approach (MATLAB). ROI drawing was performed in ImageJ (Schindelin et al., 2012, Nat. Methods, 9:676-82). Fluorescence time-courses were computed as the mean of all pixels within the ROI at each time point and were extracted as described in Sage et al. (2012, ImageJ User developer Conference 1:1). Fluorescence time courses were then synchronized with stimulus information, and visually evoked responses were computed as changes in fluorescence relative to the baseline fluorescence. Peak ΔF/F responses for field ROIs and dendritic spines ROIs were computed using the Fourier analysis to calculate mean and modulation amplitudes for each stimulus presentation, which were summed together.

Example 6C: Mouse Neuronal Culture Analysis

[0337] Primary Hippocampal Neuron Cultures

[0338] Primary hippocampal neuron cultures were prepared from embryonic mice (E16) as described previously (Woitecki et al., 2016, J. Neurosci., 36:2561-70). Hippocampi were rinsed 3-5 times in Hank's Balanced Salt Solution (HBSS, Life technologies) and digested with trypsin (25 mg/ml, Life Technologies) for 20 min at 37° C. followed by DNase I (1 mg/ml; Roche). Subsequently, the tissue was dissociated using cannulas (three times 0.9 mm×40 mm; three times 0.45 mm×23 mm) and the solution was passed through a Nylon cell strainer (100 μm; BD Biosciences). The mesh was rinsed with 4-10 ml basal medium eagle (BME, Life technologies) supplemented with 0.5% glucose (Sigma-Aldrich), 10% fetal calf serum (FCS), 2% B-27, and 0.5 mM L-glutamine (all Life Technologies) to collect all cells. After counting, the cells were plated on cover slips in a 24-well cell culture plate at a density of 70,000 cells per 24-well and cultured in a humidified incubator at 37° C. and 5% CO_2.

[0339] Viral Vector Production

[0340] Recombinant AAV2/1 genomes were generated by large scale triple transfection of HEK293 cells as described previously (Marvin et al., 2013, Nat. Methods, 10:162-70). The adeno-associated virus (AAV) plasmid coding for SF-iGluSnFR.S72A or SF-iGluSnFR.A184V, helper plasmids encoding rep and cap genes (pRV1 and pH21), and adenoviral helper pFΔ6 (Stratagene) were transfected using the calcium phosphate transfection method. Cells were harvested ~72 h after transfection. To purify the virus, cell pellets were lysed in the presence of 0.5% sodium deoxycholate (Sigma) and 50 units/ml Benzonase endonuclease (Sigma). rAAV viral particles were purified from the cell lysate by HiTrap heparin HP column purification (GE Healthcare) and then concentrated using Amicon Ultra Centrifugal Filters (Millipore) until a final stock volume of 500 μl was reached.

[0341] Viral Transduction and Image Acquisition

[0342] Primary hippocampal neurons were transduced with AAV2/1.hSynapsin1.SF-iGluSnFR.S72A or with AAV2/1.hSynapsin1.SF-iGluSnFR.A184V on DIV4 and imaged on DIV13. A low amplitude field stimulation (1 msec, 20 mA, platinum bar electrodes) was applied to recruit a small fraction (~20%) of neurons. Images were acquired with an EM-CCD camera (frame time 5-50 msec) and a stabilized LED light source of cultures visualized through a coverslip with high NA objective. All experiments were performed in Tyrode's solution (1 ml/min) at RT. Low and high affinity versions of SF-iGluSnFR were expressed in a comparable manner.

[0343] Glutamate Release Site Localization

[0344] Primary hippocampal neurons were transduced with rAAV-SF-iGluSnFR. S72A or with rAAV-SF-iGluSnFR.A184V on DIV3-5 and used for experiments on DIV13-18. A low amplitude electrical field stimulation (1 msec., 20 mA, platinum bar electrodes) was applied to activate a small fraction (~20%) of neurons only. Per experiment, stimuli were applied 16-25 times at an inter-stimulus interval of 20-60 sec. Images were acquired with an EM-CCD camera (Hamamatsu ImagEM X1, 8 ms exposure, 125 Hz acquisition rate) attached to an inverted microscope (Nikon T1 Eclipse) using a triggered, stabilized LED light source (Cairn OptoLED with 470 nm excitation wavelength, 470/40 emission filter and 525/50 excitation filter). Cells were imaged through a coverslip with a high NA objective (Zeiss, 63×, 1.4 NA, water). All experiments were performed in saline (1 ml/min, as described above) at room temperature.

[0345] In each experiment, 30 images were acquired per stimulation trial (20 before and 10 after stimulation). Each of the 30 images was registered with StackReg Plugin in ImageJ to the first image. The image series was then normalized to the average of 5 frames before stimulation to distinguish responding sites (>1) and non-responding structures (~1). For selection of responding sites to be included in the analysis, 10 normalized images subsequent to the stimulus in the first trial were averaged. All spots of increased fluorescence (FIG. **64***c,d*) that reached at least 50% of the ΔF/F value of the brightest spot in the image were defined as responding sites and used for further analysis. The spatial extent of glutamate release sites was quantified by extracting a brightness profile based on a line (length: 12-30 pixels, width: 3 pixels) drawn along the underlying neurite. These profiles were calculated for each stimulation trial and each responding site in an experiment and fitted by Gaussians with Igor Pro 6.3 (Wavemetrics).

[0346] In each experiment (n=6 and 8 for S72A and A184V, respectively, each consisting of 16-25 trials) the mean deviation of the center (X_0 position), the average width and the average amplitude of the fitted Gaussians were calculated per response site and averaged across all experiments and statistically compared by an unpaired Mann-Whitney test, n=28 and 53 for S72A and A184V, respectively).

Example 6D: Cerebellar Parallel Fiber Analysis

[0347] Stereotaxic Injections.

[0348] To fluorescently label boutons of parallel fibers, stereotaxic injections of viral vectors expressing SF-iGluSnFR or GCaMP6f into cerebellar vermis were performed. The following vectors were used: AAV-DJ.hSynapsin.SF-iGluSnFR (1.9×10^{13} GC/ml), AAV2/1.hSynapsin.SF-iGluSnFR.S72A (2.6×10^{13} GC/ml), AAV-DJ.CAGFLEX.SF-iGluSnFR.S72A (6.3×10^{12} GC/ml) or AAV-DJ.hSynapsin.GCaMP6f (1.2×10^{13} GC/ml). Mice between 30 and 60 days old were deeply anesthetized before surgery with a mixture of hypnotic (ketamine 1.5%, Mérial) and analgesic (xylazine

0.05%, Bayer) anesthetics mixed in NaCl and injected in the peritoneum. A local anesthetic (xylocaine 2% gel, Newpharma) was applied on top of the location of the cranial incision. The anesthetized mouse was then placed on a stereotaxic frame adaptor comprising adjustable ear bars and tooth holder. The skull was then perforated at the injection site with a surgical drill. The vermis was identified using the Paxinos and Franklin mouse brain atlas. The injection of viral constructs in the vermis (100 nl; 6.5 mm caudal to bregma, lateral 0.2 mm, ventral 3.6 mm and 3.4 mm) was performed by slow infusion (100 nl/min) with steel needles (26G×50 mm and 36G×70 mm, Phymep) connected to a pump via a catheter and a Hamilton syringe. Injected mice were then kept 2 to 4 weeks to allow transgene expression.

[0349] Slice Preparation

[0350] All protocols were approved by the ethics committee CEEA-Paris1. Cerebellar acute slices were prepared from adult CB6F1 mice (F1 cross of BalbC and C57Bl/6J) or Gabra6 mice (B6; 129P2-Gabra6$^{tm2(cre)Wwis}$/Mmucd) of postnatal day 41 to 123. The mice were killed by rapid decapitation, after which the brains were quickly removed and placed in an ice-cold solution containing (in mM): 2.5 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 25 glucose, 230 sucrose, and 0.5 ascorbic acid bubbled with 95% O_2 and 5% CO_2. Coronal slices were cut from the dissected cerebellar vermis using a vibratome (Leica VT1200S). After preparation, the slices were incubated at 32° C. for 30 minutes in the following solution (in mM): 85 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 25 glucose, 75 sucrose and 0.5 ascorbic acid. Slices were then transferred to an external recording solution containing (in mM): 125 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose and 0.5 ascorbic acid, and maintained at room temperature for up to 6 hours. All slice recordings were performed at 36-38° C.

[0351] Transmitted Light and Fluorescence Imaging

[0352] Parallel fiber and boutons expressing SF-iGluSnFR or GCaMP6f were identified using an Ultima two-photon scanning scanhead (Bruker Nano Surfaces Division, Middleton, Wis., USA) that was mounted on an Olympus BX61W1 microscope, equipped with a water-immersion objective (60×, 1.1 NA, Olympus Optical, Tokyo, Japan) and infrared Dodt-gradient contrast. Two-photon excitation was performed with a pulsed Ti: Sapphire laser (DeepSee, Spectra-Physics, France) tuned to 920 nm for imaging morphology, glutamate and Ca^{2+} fluorescence detection.

[0353] Boutons from parallel fibers were identified by increase fluorescence as response to 100 or 300 Hz trains. The probe response was evoked with 60 μs voltage pulses 5-15 V above threshold (Digitimer Ltd, Letchworth Garden City, UK) using a patch pipette (typically with a tip resistance of 4-6 MΩ) filled with ACSF and placed in the molecular layer adjacent to labelled parallel fibers. Activation of boutons was routinely confirmed by verifying increase in fluorescence in response to 100 or 300 Hz trains of stimulation. Line-scan imaging through boutons was performed at dwell time of 0.8 μsec per pixel, for 300 to 800 msec. Individual traces were background subtracted and averaged with no smoothing or filtration for single events for SF-iGluSnFR, or background subtracted and averaged with smoothing for GCaMP6f, 20 Hz and 100 Hz trains. SNR was calculated from the peak of the fit to the fluorescent events divided by the average SD of a 20 msec baseline window. Data were analyzed and presented using custom-written macros in Igor Pro.

Example 6E: Fast Imaging of SF-Venus-iGluSnFR

[0354] Primary Rat Hippocampal Neuron Cultures

[0355] A mixed cell culture (neurons and glia) was prepared from Sprague-Dawley rat pups (Charles River Laboratories). Briefly, P0 pups were decapitated, and the brains were dissected into ice-cold neural dissection solution (NDS, 10 mM HEPES (Sigma) in HBSS (Invitrogen), pH 7.4). Hippocampi were dissected and cut into small pieces to facilitate enzyme digestion. Hippocampi pieces were transferred using a large bore pipette into a 15 ml conical tube and incubated with enzyme digest solution (Papain, Worthington Biologicals) at 37° C. for 30 min. After 30 min., the enzyme solution was removed, and Plating Media (MEM media containing 10% FBS) was added and tissue pieces were triturated resulting in mostly single cells. The cell suspension was filtered using a 45 um filter. The filtered cell suspension was centrifuged, and the resulting cell pellet was re-suspended with Plating Media and counted.

[0356] For electroporation, 1 μg of DNA was mixed with 1×10^6 cells using the Amaxa Nucleofector II instrument. Cells were plated onto coverslips coated with Poly-D-Lysine (Sigma) and kept at 37° C., 5% CO_2 in PM for ~24 hours and then in NbActiv4 (BrainBits) was added for the duration with medium exchanges every 4 days.

[0357] Glutamate Uncaging and Imaging

[0358] Rat hippocampal culture was imaged on DIV19 at room temperature in HEPES buffered Tyrode's solution (145 mM NaCl, 2.5 mM KCl, 10 mM glucose, 10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4).

[0359] Excitation was with a 1030 nm, 5 MHz, 190 fsec laser (Menlo Systems, model: Bluecut). Average power was 39 mW at the sample. Fluorescence collected at 560/80 nm with a Hamamatsu MPPC detector. The field of view is a 256 um diameter circle, 1280 pixels across. The bath contained HEPES buffered Tyrode's solution plus 10 μM NBQX and 150 μM RuBi-Glutamate (Tocris). Glutamate uncaging was performed with 420 nm fiber-coupled LEDs (Thorlabs M420F2). The tips of the fibers were imaged onto the sample plane through the same objective used for activity imaging.

Example 6F: Summary of Results

[0360] The intensity-based glutamate-sensing fluorescent reporter (iGluSnFR) (Marvin et al., 2013, Nat. Methods, 10:162-70) has become an invaluable tool for studying glutamate dynamics in diverse systems, including retina (Park et al., 2014, J. Neurosci., 34:3976-81; Borghuis et al., 2013, J. Neurosci., 33:10972-85), mouse olfactory bulb (Brunert et al., 2016, J. Neurosci., 36:6820-35) and cat visual cortex (O'Herron et al., 2016, Nature, 534:378-82). Beyond specific circuits, iGluSnFR also allows mesoscale "functional connectomic" mapping (Xie et al., 2016, J. Neurosci., 36:1261-72) and mechanistic studies of Huntington's disease (Jiang et al., 2016, J. Neurosci., 36:3453-70), synaptic spillover (Rosa et al., 2015, eLife, 4:728), cortical spreading depression (Enger et al., 2015, Cerebral Cortex, 25:4469-76) and exocytotic vesicle fusion (Bao et al., 2016, Nat. Struct. Biol., 23:67-73). However, iGluSnFR is insufficient for some applications due to poor expression (in some brain regions), and kinetics that do not match the time

courses of some observations. Here, we describe variants that are functionally brighter (due to increased expression on cell membrane), have tighter or weaker affinity (resulting from slower or faster off-rates), and fluoresce blue, green, or yellow.

[0361] Replacement of circularly permuted eGFP with circularly permuted "superfolder" GFP (Pedelacq et al., 2006, Nat. Biotech., 24:79-88) (SF-iGluSnFR) yielded 5-fold higher soluble-protein expression levels in bacteria (0.5 μmol/1 L growth vs. 0.1 μmol/1 L). Circular dichroism indicates an increase in melting temperature transition (T_m) of ~5° C. (FIG. **57**). The 2-photon cross-section and excitation, emission, and absorption spectra of SF-iGluSnFR are similar to the original (FIG. **58***a-d*). Head-to-head comparison of SF-iGluSnFR with original iGluSnFR in mouse somatosensory cortex shows substantially more robust expression by the former (FIG. **59***a,b*). Under typical imaging conditions (<20 mW, 130-nanosecond dwell time per pixel), SF-iGluSnFR is bright enough for repeated imaging, while original iGluSnFR is too dim (FIG. **59***c,d*). While a faster 2-photon in vivo photobleaching rate was observed for SF-iGluSnFR in somatosensory cortex (FIG. **59***e*), partially-bleached SF-iGluSnFR was still brighter than iGluSnFR. Thus, SF-iGluSnFR will have superior expression in vivo, where the quantity of deliverable DNA can be limiting.

[0362] While the affinity of membrane-displayed iGluSnFR (4 μM) is adequate for some in vivo applications, tighter variants are needed for circumstances of limiting glutamate concentrations, such as at sparsely-firing synapses. Additionally, measuring glutamate release events with raster scanning microscopes requires variants with slower off-rates so that the decay time from glutamate binding is long enough to be sufficiently sampled at the operating frame rate for most experiments (typically <100 Hz). Replacement of eGFP with superfolder GFP increases the in vitro affinity of soluble SF-iGluSnFR two-fold compared to original iGluSnFR (40 μM vs. 80 FIG. **60***a*). To further modulate affinity, the conformational coupling between the open-closed equilibrium of bacterial periplasmic binding proteins (PBPs, e.g. the glutamate-binding protein in iGluSnFR) and their ligand-binding affinity (Marvin et al., 2001, Nat. Struct. Biol., 8:795-8) was exploited. Briefly, mutation of residues in the "hinge" of PBPs can allosterically alter affinity, without compromising the stereochemical integrity of the ligand-binding site. In a bacterial lysate assay, an A184X library of the iGluSnFR glutamate-binding domain (mutated to valine in the original iGluSnFR) was screened. Reversion to alanine or other small amino acids tightened affinity, while larger side chains weakened affinity (FIG. **61**).

[0363] A184S was introduced into SF-iGluSnFR to generate a tighter variant. (Reversion A184A had a low ΔF/F.) Affinities of purified soluble protein were 7 μM and 40 μM for the A184S and A184V (unmutated from iGluSnFR) SF-iGluSnFR variants, respectively (FIG. **60***a*). The tighter affinity of the A184S variant arises from a slower off-rate (FIG. **60***b*). The affinity variants were re-cloned into an AAV vector containing an IgG secretion signal and a PDGFR transmembrane domain. Viral expression on cultured rat hippocampal neurons (AAV2/1.hSynapsin1.SF-iGluSnFR) yields glutamate affinities about an order of magnitude tighter than the soluble form (0.7 μM and 2 μM for A184S and A184V, respectively; FIG. **62**). A similar increase in affinity upon membrane tethering was seen with the original sensor (Marvin et al., 2013, Nat. Methods, 10:162-70). Whole-field stimulation (50 Hz) of these cultures shows that their relative half-times of fluorescence decay parallel their in vitro kinetics, with all variants having faster decay than GCaMP6f (FIG. **63**).

[0364] In vivo, the tighter/slower SF-iGluSnFR.A184S variant shows improved detection of stimulus-evoked glutamate release in the ferret visual cortex in response to presented drifting gratings (FIG. **53***a,b*). Peak amplitudes reached 30% ΔF/F for SF-iGluSnFR.A184S but only 5% ΔF/F for SF-iGluSnFR.A184V when imaged at 30 Hz. Greater ΔF/F of SF-iGluSnFR.A184S allows extraction of robust orientation tuning curves compared to SF-iGluSnFR.A184V. Enhanced sensitivity of the A184S variant also allowed orientation-selective responses to be resolved in individual dendritic spines (FIG. **53***c,d*). Synaptic glutamate release as measured with SF-iGluSnFR.A184S was not only strongly selective for visual stimuli, but response amplitudes across individual trials were consistently greater than the A184V variant when examining all stimulus-evoked responses (A184S median ΔF/F=16%, n=72 spines; A184V median ΔF/F=9%, n=22 spines; p=2e-115, Wilcoxon rank-sum test) or only preferred stimuli (A184S median ΔF/F=27%, n=72 spines; A184V median ΔF/F=14%, n=22 spines; p=9e-23, Wilcoxon rank-sum test) (FIG. **64**).

[0365] While slow off-rate variants of SF-iGluSnFR are better for detecting individual synaptic events by temporal summation of fluorescence, faster off-rate variants are needed for temporal resolution spiking dynamics and at large synapses where glutamate clearance is limiting. A weaker variant of SF-iGluSnFR (S72A) was made by removing a hydrogen bond between the protein and glutamate. Soluble SF-iGluSnFR.S72A has 200 μM affinity for glutamate (FIG. **60***a*), arising from a combination of both slower on-rate and faster off-rate (FIG. **60***b*). In neuronal culture, S72A has an affinity of 35 μM, an order of magnitude weaker than its parent, A184V (FIG. **62**).

[0366] In rat neuronal culture, without buffer perfusion, fluorescence of the culture (not localized to specific structures) returns to baseline within 100 msec. of a single electrical stimulation for S72A, faster than A184V, A184S, or GCaMP6f (FIG. **63**). In mouse neuronal culture (FIG. **65**), the substantially faster off-rate of S72A provides enhanced temporal resolution of paired (20 Hz) electrical stimuli over the A184V variant (FIG. **54***a,b*), making it useful for assessing short-term synaptic plasticity. A train of 6 electrical pulses (20 Hz) in 1 mM extracellular Ca^{2+} can be resolved as equal, individual release events by observation with S72A, while A184V yields an integrated signal (FIG. **54***c,d*). In 3.5 mM extracellular Ca^{2+}, vesicles are released with higher probability during the initial stimulation (Dodge et al., 1967, J. Physiol., 193:419-32). This can be observed by S72A, as reported by a reduction in fluorescence response as the train of field pulses progresses (FIG. **54***c*), while these differences are obscured by the slower decay of A184V (FIG. **54***d*). Thus, while S72A has a lower ΔF/F in response to the same amount of glutamate being released (due to weaker affinity), its faster kinetics provides enhanced temporal resolution of synaptic activity. Similarly, S72A provides enhanced spatial resolution of glutamate release over A184V (FIG. **66**).

[0367] With fast rise and decay times, it was examined whether SF-iGluSnFR could be used as an alternative to GCaMP6f for monitoring neuronal activity in mouse cer-

ebellar brain slice. Single cerebellar granule cell bouton responses to single action potentials (APs) could indeed be resolved using fast linescan detection (<1 ms per line; FIG. **55***a*), and were much faster than GCaMP6f rise and decay times at both 2 mM and 1.5 mM extracellular calcium. The S72A variant had by far the fastest response (S72 half decay 7.9±1.0 ms, A184V 28.1±1.6 ms, GCaMP6f 1.5 mM $[Ca^{2+}]_e$ 37.9±3.9 ms, GCaMP6f 1.5 mM $[Ca^{2+}]_e$ 108.6±8.8 ms). The signal-to-noise-ratios (SNRs) were best for A184V, but even S72A produced better SNRs than GCaMP6f under physiological extracellular calcium concentrations (1.5 mM). The superior SNR of A184V showed putative single vesicle release events in single trials (FIG. **55***b*). However, if many bouton responses are pooled and averaged for each trial, single spike detection at 20 Hz is feasible (see average trace, FIG. **55***b*). For 20 Hz stimuli, both the A184V and S72A variants produced little accumulation of bouton fluorescence after 10 stimuli as compared to GCaMP6f (FIG. **55***c*), similar to the dendritic responses in culture (FIG. **54**). For 100 Hz train stimuli, discrete release events could be detected, in contrast to GCaMP6f (FIG. **55***d*). Note the poor temporal precision of the train response, in contrast to A184V and S72A. Thus both A184V and S72A enable a larger dynamic range of reported firing frequencies, with S72A providing the largest range due to its low affinity. Moreover, the fast kinetics of SF-iGluSnFR.A184V and SF-iGluSnFR.S72A could be used for a more reliable estimate of spike times (versus GCaMP6f), and are much better suited to high-frequency spike detection (>100 Hz) which is necessary for the high instantaneous firing rates of cerebellar granule cells (van Beugen et al., 2013, Frontiers in Neural Circuits, 7:95).

[0368] Introduction of chromophore mutations from GFP variants Azurite (Mena et al., 2006, Nat. Biotech., 24:1569-71) or Venus (Nagai et al., 2002, Nat. Biotech., 20:87-90) to SF-iGluSnFR led to functional blue and yellow versions, respectively. The former required re-optimization of the residues that link the FP with the glutamate-binding protein. The latter was a straightforward modular replacement. (Annotated amino acid sequences are given in FIG. **67**). SF-Azurite-iGluSnFR has significantly lower ΔF/F (FIG. **68**), perhaps a result of intrinsic differences in chromophore structure. SF-Venus-iGluSnFR has similar affinity and maximum fluorescence response to glutamate as SF-iGluSnFR, but with red-shifted excitation and emission spectra (FIG. **69**). Importantly, its 2-photon excitation spectrum is sufficiently red-shifted to allow strong excitation at 1030 nm (FIG. **69**), compatible with relatively inexpensive, powerful femtosecond fiber lasers (Tang et al., 2009, J. Biomed. Optics, 14:030508). These powerful lasers enable simultaneous excitation of many foci, enabling very fast (1016 Hz) large-area imaging by recording projections of the sample and computationally reconstructing images (Kazemipour, et al., 2018). In neuronal culture, two near-simultaneous pulses of glutamate uncaging can be resolved with both high spatial and temporal resolution by measuring fluorescence changes in a neuron expressing SF-Venus-iGluSnFR.A184V (FIG. **56**).

[0369] The iGluSnFR variants described here increase the power of genetically encoded glutamate imaging. Affinity variants with altered kinetics broaden the range of observable glutamate release events. Chromatic mutants allow fast imaging with cheap lasers, and potential utility in multicolor imaging. Improved membrane targeting and photostability will be valuable in all applications.

OTHER EMBODIMENTS

[0370] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 197

<210> SEQ ID NO 1
<211> LENGTH: 662
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 1

```
Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                  10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
```

-continued

```
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Ser His Asn Val Tyr Ile
        195                 200                 205

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg
    210                 215                 220

His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln Gln
225                 230                 235                 240

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
                245                 250                 255

Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
            260                 265                 270

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
        275                 280                 285

Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser Lys
    290                 295                 300

Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
305                 310                 315                 320

Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
                325                 330                 335

Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
            340                 345                 350

Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly
        355                 360                 365

Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
    370                 375                 380

Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile Phe
385                 390                 395                 400

Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
                405                 410                 415

Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
            420                 425                 430

Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn Gly
        435                 440                 445

Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val
    450                 455                 460

Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp
465                 470                 475                 480

Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala
                485                 490                 495
```

-continued

```
Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro
            500                 505                 510

Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr
        515                 520                 525

Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val
    530                 535                 540

Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys
545                 550                 555                 560

Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val
                565                 570                 575

Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu
            580                 585                 590

Glu Leu Val Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln
        595                 600                 605

Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr
    610                 615                 620

Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val
625                 630                 635                 640

Asp Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser His
                645                 650                 655

His His His His His Gly
            660


<210> SEQ ID NO 2
<211> LENGTH: 662
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 2

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
```

-continued

```
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Pro Pro Ser Tyr Asn Val Phe Ile
        195                 200                 205

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg
    210                 215                 220

His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln Gln
225                 230                 235                 240

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
                245                 250                 255

Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
            260                 265                 270

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
        275                 280                 285

Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser Lys
    290                 295                 300

Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
305                 310                 315                 320

Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
                325                 330                 335

Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
            340                 345                 350

Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly
        355                 360                 365

Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
    370                 375                 380

Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile Phe
385                 390                 395                 400

Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
                405                 410                 415

Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
            420                 425                 430

Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn Gly
        435                 440                 445

Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val
    450                 455                 460

Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp
465                 470                 475                 480

Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala
                485                 490                 495

Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro
            500                 505                 510

Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr
        515                 520                 525

Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val
    530                 535                 540

Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys
545                 550                 555                 560

Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val
                565                 570                 575
```

-continued

```
Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu
            580                 585                 590

Glu Leu Val Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln
        595                 600                 605

Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr
    610                 615                 620

Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val
625                 630                 635                 640

Asp Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser His
                645                 650                 655

His His His His His Gly
            660


<210> SEQ ID NO 3
<211> LENGTH: 659
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 3

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Pro Cys Ser His Asn Val Phe Ile
        195                 200                 205

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg
    210                 215                 220

His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln Gln
225                 230                 235                 240

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
```

-continued

```
               245                 250                 255

Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
            260                 265                 270

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
        275                 280                 285

Met Asp Glu Leu Tyr Lys Gly Gly Ser Met Val Ser Lys Gly Glu Glu
    290                 295                 300

Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val
305                 310                 315                 320

Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr
                325                 330                 335

Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
            340                 345                 350

Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys
        355                 360                 365

Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser
    370                 375                 380

Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp
385                 390                 395                 400

Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
                405                 410                 415

Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly
            420                 425                 430

Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn Gly Gly Tyr Ala
        435                 440                 445

Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp
    450                 455                 460

Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys
465                 470                 475                 480

Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala
                485                 490                 495

Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp
            500                 505                 510

Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro
        515                 520                 525

Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala
    530                 535                 540

Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu
545                 550                 555                 560

Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp
                565                 570                 575

Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Val
            580                 585                 590

Asp Lys Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu
        595                 600                 605

Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg
    610                 615                 620

Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Asp
625                 630                 635                 640

Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser His His His His
                645                 650                 655
```

-continued

His His Gly

<210> SEQ ID NO 4
<211> LENGTH: 661
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 4

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
35 40 45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
50 55 60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65 70 75 80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
85 90 95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
100 105 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
115 120 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
130 135 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145 150 155 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
165 170 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
180 185 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu
195 200 205

Asn Gly Gly Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
210 215 220

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly
225 230 235 240

Val Gln Leu Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
245 250 255

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu
260 265 270

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
275 280 285

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly
290 295 300

Gly Thr Gly Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly
305 310 315 320

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
325 330 335

-continued

```
Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu
            340                 345                 350

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro
        355                 360                 365

Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr
    370                 375                 380

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
385                 390                 395                 400

Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr
                405                 410                 415

Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
            420                 425                 430

Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
        435                 440                 445

His Lys Leu Glu Tyr Asn Phe Asn Gly Gly Lys Tyr Asp Ile Lys Asp
    450                 455                 460

Val Gly Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp
465                 470                 475                 480

Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala
                485                 490                 495

Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro
            500                 505                 510

Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr
        515                 520                 525

Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val
    530                 535                 540

Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys
545                 550                 555                 560

Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val
                565                 570                 575

Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu
            580                 585                 590

Glu Leu Val Asp Lys Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln
        595                 600                 605

Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr
    610                 615                 620

Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Gln Thr Val Asp
625                 630                 635                 640

Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser His His
                645                 650                 655

His His His His Gly
            660
```

<210> SEQ ID NO 5
<211> LENGTH: 663
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 5

```
Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
```

-continued

```
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu
        195                 200                 205

Asn His Leu Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
    210                 215                 220

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly
225                 230                 235                 240

Val Gln Leu Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
                245                 250                 255

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu
            260                 265                 270

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
        275                 280                 285

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly
    290                 295                 300

Gly Thr Gly Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly
305                 310                 315                 320

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
                325                 330                 335

Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu
            340                 345                 350

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro
        355                 360                 365

Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr
    370                 375                 380

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
385                 390                 395                 400

Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr
                405                 410                 415
```

-continued

```
Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
            420                 425                 430

Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
        435                 440                 445

His Lys Leu Glu Tyr Asn Phe Asn Gly Gly Lys Tyr Asp Ile Lys Asp
    450                 455                 460

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
465                 470                 475                 480

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
                485                 490                 495

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
            500                 505                 510

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
        515                 520                 525

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
    530                 535                 540

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
545                 550                 555                 560

Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala
                565                 570                 575

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
            580                 585                 590

Glu Glu Leu Val Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
        595                 600                 605

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
    610                 615                 620

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
625                 630                 635                 640

Val Asp Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser
                645                 650                 655

His His His His His His Gly
            660


<210> SEQ ID NO 6
<211> LENGTH: 655
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 6

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
```

-continued

```
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu
        195                 200                 205

Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala
    210                 215                 220

Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met
225                 230                 235                 240

Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly
                245                 250                 255

Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp
            260                 265                 270

Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly
        275                 280                 285

Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala
    290                 295                 300

Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu
305                 310                 315                 320

Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly
                325                 330                 335

Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Gly Gly Ser His Asn
            340                 345                 350

Val Tyr Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe
        355                 360                 365

Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His
    370                 375                 380

Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
385                 390                 395                 400

Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu
                405                 410                 415

Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
            420                 425                 430

Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met
        435                 440                 445

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
    450                 455                 460

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
465                 470                 475                 480

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
                485                 490                 495
```

-continued

```
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
            500                 505                 510

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
        515                 520                 525

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg
    530                 535                 540

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
545                 550                 555                 560

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
                565                 570                 575

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
            580                 585                 590

Phe Asn Gly Gly Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn
        595                 600                 605

Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe
    610                 615                 620

Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln
625                 630                 635                 640

Thr Val Asp Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys
                645                 650                 655


<210> SEQ ID NO 7
<211> LENGTH: 655
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 7

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
```

-continued

```
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu
        195                 200                 205

Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala
    210                 215                 220

Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met
225                 230                 235                 240

Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly
                245                 250                 255

Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp
            260                 265                 270

Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly
        275                 280                 285

Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala
    290                 295                 300

Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu
305                 310                 315                 320

Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly
                325                 330                 335

Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Gly Gly Ser His Asn
            340                 345                 350

Val Tyr Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe
        355                 360                 365

Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His
    370                 375                 380

Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
385                 390                 395                 400

Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu
                405                 410                 415

Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
            420                 425                 430

Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met
        435                 440                 445

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
    450                 455                 460

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
465                 470                 475                 480

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
                485                 490                 495

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
            500                 505                 510

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
        515                 520                 525

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg
    530                 535                 540

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
545                 550                 555                 560

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
                565                 570                 575

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
            580                 585                 590
```

-continued

```
Phe Asn Asn Pro Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn
        595                 600                 605

Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe
    610                 615                 620

Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln
625                 630                 635                 640

Thr Val Asp Glu Asp Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys
                645                 650                 655


<210> SEQ ID NO 8
<211> LENGTH: 659
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 8

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn
        35                  40                  45

Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu
    50                  55                  60

Lys Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu
65                  70                  75                  80

Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile
                85                  90                  95

Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu
            100                 105                 110

Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe
        115                 120                 125

Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile
    130                 135                 140

Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn
145                 150                 155                 160

Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys
                165                 170                 175

Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe
            180                 185                 190

Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu
        195                 200                 205

Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala
    210                 215                 220

Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met
225                 230                 235                 240

Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly
                245                 250                 255

Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp
            260                 265                 270

Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly
```

-continued

```
        275                 280                 285

Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala
    290                 295                 300

Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu
305                 310                 315                 320

Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly
                325                 330                 335

Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Val Lys Asp Pro Arg
            340                 345                 350

Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys
        355                 360                 365

Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu
    370                 375                 380

Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
385                 390                 395                 400

Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp
                405                 410                 415

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            420                 425                 430

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        435                 440                 445

Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
    450                 455                 460

Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
465                 470                 475                 480

Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
                485                 490                 495

Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
            500                 505                 510

Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
        515                 520                 525

Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile
    530                 535                 540

Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
545                 550                 555                 560

Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
                565                 570                 575

Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
            580                 585                 590

Glu Tyr Asn Phe Asn Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu
        595                 600                 605

Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg
    610                 615                 620

Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Asp
625                 630                 635                 640

Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys Gly Ser His His His His
                645                 650                 655

His His Gly
```

<210> SEQ ID NO 9
<211> LENGTH: 14

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 9

Pro Leu Ile Ala Ala Asp Gly Xaa Xaa Asn Val Tyr Ile Met
1 5 10

<210> SEQ ID NO 10
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 10

Pro Leu Ile Ala Ala Asp Xaa Xaa Asn Val Tyr Ile Met
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 11

Pro Leu Ile Ala Ala Asp Gly Gly Xaa Xaa Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 12
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 12

Pro Leu Ile Ala Ala Asp Gly Xaa Pro Asn Val Tyr Ile Met Gly
1 5 10 15

<210> SEQ ID NO 13
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 13

Pro Leu Ile Ala Ala Asp Gly Ile Xaa Asn Val Tyr Ile Met Gly
1 5 10 15

<210> SEQ ID NO 14
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 14

Pro Leu Ile Ala Ala Asp Pro Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 15
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 15

Pro Leu Ile Ala Ala Asp Xaa Pro Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 16
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 16

Pro Leu Ile Ala Ala Asp Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 17
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)

-continued

<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 17

Pro Leu Ile Ala Ala Asp Xaa Xaa Ser His Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 18
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 18

Pro Leu Ile Ala Ala Asp Pro Xaa Ser His Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 19
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 19

Pro Leu Ile Ala Ala Asp Pro Xaa Ser Tyr Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 20
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 20

Pro Leu Ile Ala Ala Asp Xaa Xaa Ser Tyr Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 21

-continued

Pro Leu Ile Ala Ala Asp Pro Xaa Ser Tyr Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 22
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 22

Pro Leu Ile Ala Ala Asp Xaa Xaa Ser Tyr Asn Val Phe Ile Met
1 5 10 15

<210> SEQ ID NO 23
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 23

Pro Leu Ile Ala Ala Asp Pro Xaa Ser Xaa Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 24
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 24

Pro Leu Ile Ala Ala Asp Pro Xaa Ser His Xaa Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 25
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)

-continued

<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 25

Pro Leu Ile Ala Ala Asp Pro Xaa Ser His Asn Xaa Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 26
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 26

Pro Leu Ile Ala Ala Asp Pro Xaa Ser His Asn Val Xaa Ile Met
1 5 10 15

<210> SEQ ID NO 27
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 27

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10 15

<210> SEQ ID NO 28
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 28

Lys Leu Glu Tyr Asn Phe Asn Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10 15

<210> SEQ ID NO 29
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

oligopeptide

<400> SEQUENCE: 29

Lys Leu Glu Tyr Asn Phe Asn Tyr Ala Phe Lys Tyr Glu Asn
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 30

Lys Leu Glu Tyr Asn Phe Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 31

Lys Leu Glu Tyr Asn Xaa Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 32

Lys Leu Glu Tyr Asn Trp Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 33

-continued

Lys Leu Glu Tyr Asn Xaa Lys Tyr Ala Phe Lys Tyr Glu Asn
1 5 10

<210> SEQ ID NO 34
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 34

Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Tyr Ala Phe Lys Tyr Glu Asn
1 5 10 15

<210> SEQ ID NO 35
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 35

Lys Leu Glu Tyr Asn Phe Asn Xaa Pro Tyr Ala Phe Lys Tyr Glu Asn
1 5 10 15

<210> SEQ ID NO 36
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 36

Ala Phe Lys Tyr Glu Asn Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 37
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 37

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Lys Tyr Asp Ile Lys Asp Val
1 5 10 15

-continued

<210> SEQ ID NO 38
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 38

Lys Ser Tyr Glu Glu Leu Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 39

Lys Ser Tyr Glu Glu Leu Pro Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 40
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 40

Lys Ser Tyr Glu Glu Leu Xaa Pro Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 41
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 41

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Ala Lys Asp Pro Arg Ile Ala
1 5 10 15

<210> SEQ ID NO 42
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 42

Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Ala Lys Asp Pro Arg Ile Ala
1 5 10 15

<210> SEQ ID NO 43
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 43

Lys Leu Glu Tyr Asn Phe Asn Xaa Pro Ala Lys Asp Pro Arg Ile Ala
1 5 10 15

<210> SEQ ID NO 44
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 44

Glu Leu Ala Lys Asp Pro Arg Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 45
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 45

Glu Leu Ala Lys Asp Pro Arg Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 46
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:

-continued

<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 46

Glu Leu Ala Lys Asp Pro Arg Xaa Xaa Xaa Ser His Asn Val Tyr Ile
1 5 10 15

Met

<210> SEQ ID NO 47
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 47

Lys Leu Glu Tyr Asn Phe Asn Xaa Ala Ala Thr Met Glu Asn Ala
1 5 10 15

<210> SEQ ID NO 48
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 48

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Ala Ala Thr Met Glu Asn Ala
1 5 10 15

<210> SEQ ID NO 49
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 49

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Xaa Ala Ala Thr Met Glu Asn
1 5 10 15

Ala

<210> SEQ ID NO 50
<211> LENGTH: 671
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

-continued

<400> SEQUENCE: 50

```
Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                  10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Val Val Ile Trp His Ala
        35                  40                  45

Met Gln Pro Asn Glu Leu Glu Val Phe Gln Ser Leu Ala Glu Glu Tyr
    50                  55                  60

Met Ala Leu Cys Pro Glu Val Glu Ile Val Phe Glu Gln Lys Pro Asn
65                  70                  75                  80

Leu Glu Asp Ala Leu Lys Ala Ala Ile Pro Thr Gly Gln Gly Pro Asp
                85                  90                  95

Leu Phe Ile Trp Ala His Asp Trp Ile Gly Lys Phe Ala Glu Ala Gly
            100                 105                 110

Leu Leu Glu Pro Ile Asp Glu Tyr Val Thr Glu Asp Leu Leu Asn Glu
        115                 120                 125

Phe Ala Pro Met Ala Gln Asp Ala Met Gln Tyr Lys Gly His Tyr Tyr
    130                 135                 140

Ala Leu Pro Phe Ala Ala Glu Thr Val Ala Ile Ile Tyr Ser Lys Glu
145                 150                 155                 160

Met Val Ser Glu Pro Pro Lys Thr Phe Asp Glu Met Lys Ala Ile Met
                165                 170                 175

Glu Lys Tyr Tyr Asp Pro Ala Asn Glu Lys Tyr Gly Ile Ala Trp Pro
            180                 185                 190

Ile Asn Ala Tyr Phe Ile Ser Ala Ile Ala Gln Ala Phe Gly Gly Ser
        195                 200                 205

His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
    210                 215                 220

Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala
225                 230                 235                 240

Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
                245                 250                 255

Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro
            260                 265                 270

Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
        275                 280                 285

Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly
    290                 295                 300

Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
305                 310                 315                 320

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
                325                 330                 335

Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
            340                 345                 350

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
        355                 360                 365

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
    370                 375                 380

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln
385                 390                 395                 400
```

-continued

```
Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
                405                 410                 415

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
            420                 425                 430

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
        435                 440                 445

Tyr Asn Phe Asn Gly Gly Tyr Tyr Phe Asp Asp Lys Thr Glu Gln Pro
    450                 455                 460

Gly Leu Asp Lys Pro Glu Thr Ile Glu Gly Phe Lys Phe Phe Phe Thr
465                 470                 475                 480

Glu Ile Trp Pro Tyr Met Ala Pro Thr Gly Asp Tyr Asn Thr Gln Gln
                485                 490                 495

Ser Ile Phe Leu Glu Gly Arg Ala Pro Met Met Val Asn Gly Pro Trp
            500                 505                 510

Ser Ile Asn Asp Val Lys Lys Ala Gly Ile Asn Phe Gly Val Val Pro
        515                 520                 525

Leu Pro Pro Ile Ile Lys Asp Gly Lys Glu Tyr Trp Pro Arg Pro Tyr
    530                 535                 540

Gly Gly Val Lys Leu Ile Tyr Phe Ala Ala Gly Ile Lys Asn Lys Asp
545                 550                 555                 560

Ala Ala Trp Lys Phe Ala Lys Trp Leu Thr Thr Ser Glu Glu Ser Ile
                565                 570                 575

Lys Thr Leu Ala Leu Glu Leu Gly Tyr Ile Pro Val Leu Thr Lys Val
            580                 585                 590

Leu Asp Asp Pro Glu Ile Lys Asn Asp Pro Val Ile Tyr Gly Phe Gly
        595                 600                 605

Gln Ala Val Gln His Ala Tyr Leu Met Pro Lys Ser Pro Lys Met Ser
    610                 615                 620

Ala Val Trp Gly Gly Val Asp Gly Ala Ile Asn Glu Ile Leu Gln Asp
625                 630                 635                 640

Pro Gln Asn Ala Asp Ile Glu Gly Ile Leu Lys Lys Tyr Gln Gln Glu
                645                 650                 655

Ile Leu Asn Asn Met Gln Gly Ser His His His His His His Gly
            660                 665                 670


<210> SEQ ID NO 51
<211> LENGTH: 671
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 51

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Val Val Ile Trp His Ala
        35                  40                  45

Met Gln Pro Asn Glu Leu Glu Val Phe Gln Ser Leu Ala Glu Glu Tyr
    50                  55                  60

Met Ala Leu Cys Pro Glu Val Glu Ile Val Phe Glu Gln Lys Pro Asn
```

-continued

```
65                  70                  75                  80

Leu Glu Asp Ala Leu Lys Ala Ala Ile Pro Thr Gly Gln Gly Pro Asp
                85                  90                  95

Leu Phe Ile Trp Ala His Asp Trp Ile Gly Lys Phe Ala Glu Ala Gly
            100                 105                 110

Leu Leu Glu Pro Ile Asp Glu Tyr Val Thr Glu Asp Leu Leu Asn Glu
        115                 120                 125

Phe Ala Pro Met Ala Gln Asp Ala Met Gln Tyr Lys Gly His Tyr Tyr
    130                 135                 140

Ala Leu Pro Phe Ala Ala Glu Thr Val Ala Ile Ile Tyr Ser Lys Glu
145                 150                 155                 160

Met Val Ser Glu Pro Pro Lys Thr Phe Asp Glu Met Lys Ala Ile Met
                165                 170                 175

Glu Lys Tyr Tyr Asp Pro Ala Asn Glu Lys Tyr Gly Ile Ala Trp Pro
            180                 185                 190

Ile Asn Ala Tyr Phe Ile Ser Ala Ile Ala Gln Ala Phe Gly Gly Ser
        195                 200                 205

His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
    210                 215                 220

Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala
225                 230                 235                 240

Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
                245                 250                 255

Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro
            260                 265                 270

Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
        275                 280                 285

Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly
    290                 295                 300

Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
305                 310                 315                 320

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
                325                 330                 335

Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
            340                 345                 350

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
        355                 360                 365

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
    370                 375                 380

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln
385                 390                 395                 400

Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
                405                 410                 415

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
            420                 425                 430

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
        435                 440                 445

Tyr Asn Phe Asn Phe Glu Tyr Tyr Phe Asp Asp Lys Thr Glu Gln Pro
    450                 455                 460

Gly Leu Asp Lys Pro Glu Thr Ile Glu Gly Phe Lys Phe Phe Phe Thr
465                 470                 475                 480
```

-continued

```
Glu Ile Trp Pro Tyr Met Ala Pro Thr Gly Asp Tyr Asn Thr Gln Gln
                485                 490                 495

Ser Ile Phe Leu Glu Gly Arg Ala Pro Met Met Val Asn Gly Pro Trp
            500                 505                 510

Ser Ile Asn Asp Val Lys Lys Ala Gly Ile Asn Phe Gly Val Val Pro
        515                 520                 525

Leu Pro Pro Ile Ile Lys Asp Gly Lys Glu Tyr Trp Pro Arg Pro Tyr
    530                 535                 540

Gly Gly Val Lys Leu Ile Tyr Phe Ala Ala Gly Ile Lys Asn Lys Asp
545                 550                 555                 560

Ala Ala Trp Lys Phe Ala Lys Trp Leu Thr Thr Ser Glu Glu Ser Ile
                565                 570                 575

Lys Thr Leu Ala Leu Glu Leu Gly Tyr Ile Pro Val Leu Thr Lys Val
            580                 585                 590

Leu Asp Asp Pro Glu Ile Lys Asn Asp Pro Val Ile Tyr Gly Phe Gly
        595                 600                 605

Gln Ala Val Gln His Ala Tyr Leu Met Pro Lys Ser Pro Lys Met Ser
    610                 615                 620

Ala Val Trp Gly Gly Val Asp Gly Ala Ile Asn Glu Ile Leu Gln Asp
625                 630                 635                 640

Pro Gln Asn Ala Asp Ile Glu Gly Ile Leu Lys Lys Tyr Gln Gln Glu
                645                 650                 655

Ile Leu Asn Asn Met Gln Gly Ser His His His His His His Gly
            660                 665                 670


<210> SEQ ID NO 52
<211> LENGTH: 662
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 52

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Val Val Ile Trp His Ala
        35                  40                  45

Met Gln Pro Asn Glu Leu Glu Val Phe Gln Ser Leu Ala Glu Glu Tyr
    50                  55                  60

Met Ala Leu Cys Pro Glu Val Glu Ile Val Phe Glu Gln Lys Pro Asn
65                  70                  75                  80

Leu Glu Asp Ala Leu Lys Ala Ala Ile Pro Thr Gly Gln Gly Pro Asp
                85                  90                  95

Leu Phe Ile Trp Ala His Asp Trp Ile Gly Lys Phe Ala Glu Ala Gly
            100                 105                 110

Leu Leu Glu Pro Ile Asp Glu Tyr Val Thr Glu Asp Leu Leu Asn Glu
        115                 120                 125

Phe Ala Pro Met Ala Gln Asp Ala Met Gln Tyr Lys Gly His Tyr Tyr
    130                 135                 140

Ala Leu Pro Phe Ala Ala Glu Thr Val Ala Ile Ile Tyr Ser Lys Glu
```

-continued

```
145                 150                 155                 160

Met Val Ser Glu Pro Pro Lys Thr Phe Asp Glu Met Lys Ala Ile Met
                165                 170                 175

Glu Lys Tyr Tyr Asp Pro Ala Asn Glu Lys Tyr Gly Ile Ala Trp Pro
            180                 185                 190

Ile Asn Ala Tyr Phe Ile Ser Ala Ile Ala Gln Ala Phe Gly Gly Tyr
        195                 200                 205

Tyr Phe Asp Asp Lys Thr Glu Gln Pro Gly Leu Asp Lys Pro Glu Thr
    210                 215                 220

Ile Glu Gly Phe Lys Phe Phe Phe Thr Glu Ile Trp Pro Tyr Met Ala
225                 230                 235                 240

Pro Thr Gly Asp Tyr Asn Thr Gln Gln Ser Ile Phe Leu Glu Gly Arg
                245                 250                 255

Ala Pro Met Met Val Asn Gly Pro Trp Ser Ile Asn Asp Val Lys Lys
            260                 265                 270

Ala Gly Ile Asn Phe Gly Val Val Pro Leu Pro Pro Ile Ile Lys Asp
        275                 280                 285

Gly Lys Glu Tyr Trp Pro Arg Pro Tyr Gly Gly Val Lys Leu Ile Tyr
    290                 295                 300

Phe Ala Ala Gly Ile Lys Asn Lys Asp Ala Ala Trp Lys Phe Ala Lys
305                 310                 315                 320

Trp Leu Thr Thr Ser Glu Glu Ser Ile Lys Thr Leu Ala Leu Glu Leu
                325                 330                 335

Gly Tyr Ile Pro Val Leu Thr Lys Val Leu Asp Asp Pro Glu Ile Ser
            340                 345                 350

His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala
        355                 360                 365

Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala
    370                 375                 380

Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu
385                 390                 395                 400

Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro
                405                 410                 415

Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala
            420                 425                 430

Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly
        435                 440                 445

Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    450                 455                 460

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
465                 470                 475                 480

Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
                485                 490                 495

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            500                 505                 510

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
        515                 520                 525

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln
    530                 535                 540

Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
545                 550                 555                 560
```

-continued

```
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                565                 570                 575

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            580                 585                 590

Tyr Asn Phe Asn Lys Asn Asp Pro Val Ile Tyr Gly Phe Gly Gln Ala
        595                 600                 605

Val Gln His Ala Tyr Leu Met Pro Lys Ser Pro Lys Met Ser Ala Val
    610                 615                 620

Trp Gly Gly Val Asp Gly Ala Ile Asn Glu Ile Leu Gln Asp Pro Gln
625                 630                 635                 640

Asn Ala Asp Ile Glu Gly Ile Leu Lys Lys Tyr Gln Gln Glu Ile Leu
                645                 650                 655

Asn Asn Met Gln Gly Ser
            660


<210> SEQ ID NO 53
<211> LENGTH: 664
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 53

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Ile Glu Glu Gly Lys Val Val Ile Trp His Ala
        35                  40                  45

Met Gln Pro Asn Glu Leu Glu Val Phe Gln Ser Leu Ala Glu Glu Tyr
    50                  55                  60

Met Ala Leu Cys Pro Glu Val Glu Ile Val Phe Glu Gln Lys Pro Asn
65                  70                  75                  80

Leu Glu Asp Ala Leu Lys Ala Ala Ile Pro Thr Gly Gln Gly Pro Asp
                85                  90                  95

Leu Phe Ile Trp Ala His Asp Trp Ile Gly Lys Phe Ala Glu Ala Gly
            100                 105                 110

Leu Leu Glu Pro Ile Asp Glu Tyr Val Thr Glu Asp Leu Leu Asn Glu
        115                 120                 125

Phe Ala Pro Met Ala Gln Asp Ala Met Gln Tyr Lys Gly His Tyr Tyr
    130                 135                 140

Ala Leu Pro Phe Ala Ala Glu Thr Val Ala Ile Ile Tyr Ser Lys Glu
145                 150                 155                 160

Met Val Ser Glu Pro Pro Lys Thr Phe Asp Glu Met Lys Ala Ile Met
                165                 170                 175

Glu Lys Tyr Tyr Asp Pro Ala Asn Glu Lys Tyr Gly Ile Ala Trp Pro
            180                 185                 190

Ile Asn Ala Tyr Phe Ile Ser Ala Ile Ala Gln Ala Phe Gly Gly Tyr
        195                 200                 205

Tyr Phe Asp Asp Lys Thr Glu Gln Pro Gly Leu Asp Lys Pro Glu Thr
    210                 215                 220

Ile Glu Gly Phe Lys Phe Phe Phe Thr Glu Ile Trp Pro Tyr Met Ala
```

-continued

```
225                 230                 235                 240

Pro Thr Gly Asp Tyr Asn Thr Gln Gln Ser Ile Phe Leu Glu Gly Arg
                245                 250                 255

Ala Pro Met Met Val Asn Gly Pro Trp Ser Ile Asn Asp Val Lys Lys
            260                 265                 270

Ala Gly Ile Asn Phe Gly Val Val Pro Leu Pro Pro Ile Ile Lys Asp
        275                 280                 285

Gly Lys Glu Tyr Trp Pro Arg Pro Tyr Gly Gly Val Lys Leu Ile Tyr
    290                 295                 300

Phe Ala Ala Gly Ile Lys Asn Lys Asp Ala Ala Trp Lys Phe Ala Lys
305                 310                 315                 320

Trp Leu Thr Thr Ser Glu Glu Ser Ile Lys Thr Leu Ala Leu Glu Leu
                325                 330                 335

Gly Tyr Ile Pro Val Leu Thr Lys Val Leu Asp Asp Pro Glu Ile Pro
            340                 345                 350

Pro Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
        355                 360                 365

Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln
    370                 375                 380

Leu Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
385                 390                 395                 400

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys
                405                 410                 415

Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
            420                 425                 430

Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr
        435                 440                 445

Gly Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val
    450                 455                 460

Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser
465                 470                 475                 480

Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu
                485                 490                 495

Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu
            500                 505                 510

Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp
        515                 520                 525

His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr
    530                 535                 540

Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr
545                 550                 555                 560

Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu
                565                 570                 575

Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys
            580                 585                 590

Leu Glu Tyr Asn Phe Asn Lys Asn Asp Pro Val Ile Tyr Gly Phe Gly
        595                 600                 605

Gln Ala Val Gln His Ala Tyr Leu Met Pro Lys Ser Pro Lys Met Ser
    610                 615                 620

Ala Val Trp Gly Gly Val Asp Gly Ala Ile Asn Glu Ile Leu Gln Asp
625                 630                 635                 640
```

-continued

Pro Gln Asn Ala Asp Ile Glu Gly Ile Leu Lys Lys Tyr Gln Gln Glu
645 650 655

Ile Leu Asn Asn Met Gln Gly Ser
660

<210> SEQ ID NO 54
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 54

Ala Ile Ala Gln Ala Phe Xaa Xaa Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

<210> SEQ ID NO 55
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 55

Ala Ile Ala Gln Ala Phe Pro Xaa Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

<210> SEQ ID NO 56
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 56

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Tyr Tyr Phe Asp Asp Lys Thr
1 5 10 15

Glu

<210> SEQ ID NO 57
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

-continued

<400> SEQUENCE: 57

Val Leu Asp Asp Pro Glu Xaa Xaa His Asn Val Tyr Ile Met
1 5 10

<210> SEQ ID NO 58
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 58

Val Leu Asp Asp Pro Glu Ile Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 59
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 59

Lys Leu Glu Tyr Asn Phe Xaa Xaa Asn Asp Pro Val Ile Tyr
1 5 10

<210> SEQ ID NO 60
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 60

Lys Leu Glu Tyr Asn Phe Asn Xaa Pro Lys Asn Asp Pro Val Ile Tyr
1 5 10 15

<210> SEQ ID NO 61
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 61

Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Lys Asn Asp Pro Val Ile Tyr
1 5 10 15

-continued

<210> SEQ ID NO 62
<211> LENGTH: 556
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 62

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Arg Trp Gly Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile Ala Lys Asn
35 40 45

Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro Phe Ser Tyr
50 55 60

Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp Tyr Ser Asn
65 70 75 80

Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro Asp Leu Gln
85 90 95

Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro Leu Leu Gln
100 105 110

Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn Asn Val Glu
115 120 125

Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val Val Gly Thr
130 135 140

Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe Ala Asn Leu
145 150 155 160

Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser Glu Val Leu
165 170 175

Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg Ile Ile Ser
180 185 190

Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser Gly Arg Ala
195 200 205

Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu Arg Ala Lys
210 215 220

Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro Gln Ser Gln
225 230 235 240

Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln Phe Lys Lys
245 250 255

Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly Glu Ala Glu
260 265 270

Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val Ser His Asn
275 280 285

Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe
290 295 300

Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His
305 310 315 320

Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
325 330 335

Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu

-continued

```
            340                 345                 350

Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
        355                 360                 365

Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met
    370                 375                 380

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
385                 390                 395                 400

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
                405                 410                 415

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
            420                 425                 430

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
        435                 440                 445

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
    450                 455                 460

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg
465                 470                 475                 480

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
                485                 490                 495

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
            500                 505                 510

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
        515                 520                 525

Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu Met Lys
    530                 535                 540

Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys
545                 550                 555


<210> SEQ ID NO 63
<211> LENGTH: 556
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 63

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile Ala Lys Asn
        35                  40                  45

Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro Phe Ser Tyr
    50                  55                  60

Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp Tyr Ser Asn
65                  70                  75                  80

Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro Asp Leu Gln
                85                  90                  95

Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro Leu Leu Gln
            100                 105                 110

Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn Asn Val Glu
        115                 120                 125
```

-continued

```
Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val Val Gly Thr
    130                 135                 140

Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe Ala Asn Leu
145                 150                 155                 160

Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser Glu Val Leu
                165                 170                 175

Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg Ile Ile Ser
            180                 185                 190

Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser Gly Arg Ala
        195                 200                 205

Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu Arg Ala Lys
    210                 215                 220

Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro Gln Ser Gln
225                 230                 235                 240

Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln Phe Lys Lys
                245                 250                 255

Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly Glu Ala Glu
            260                 265                 270

Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val Ser His Asn
        275                 280                 285

Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe
    290                 295                 300

Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His
305                 310                 315                 320

Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp
                325                 330                 335

Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu
            340                 345                 350

Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile
        355                 360                 365

Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met
    370                 375                 380

Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
385                 390                 395                 400

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
                405                 410                 415

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
            420                 425                 430

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
        435                 440                 445

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
    450                 455                 460

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg
465                 470                 475                 480

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
                485                 490                 495

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
            500                 505                 510

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
        515                 520                 525

Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu Met Lys
```

-continued

```
    530                 535                 540

Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys
545                 550                 555


<210> SEQ ID NO 64
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 64

Phe Lys Asn Pro Ile Pro Pro Xaa Ser His Asn Val Tyr Ile Met Ala
1               5                   10                  15


<210> SEQ ID NO 65
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 65

Phe Lys Asn Pro Ile Pro Pro Xaa Xaa Ser His Asn Val Tyr Ile Met
1               5                   10                  15

Ala


<210> SEQ ID NO 66
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 66

Phe Lys Asn Pro Ile Pro Pro Pro Xaa Ser His Asn Val Tyr Ile Met
1               5                   10                  15

Ala


<210> SEQ ID NO 67
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid
```

-continued

<400> SEQUENCE: 67

Phe Lys Asn Pro Ile Pro Pro Xaa Pro Ser His Asn Val Tyr Ile Met
1 5 10 15

Ala

<210> SEQ ID NO 68
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 68

Lys Trp Phe Lys Asn Pro Ile Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

Ala

<210> SEQ ID NO 69
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 69

Phe Lys Asn Pro Ile Pro Pro Xaa Xaa Asn Val Tyr Ile Met Ala Asp
1 5 10 15

<210> SEQ ID NO 70
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 70

Lys Trp Phe Lys Asn Pro Ile Xaa Xaa Asn Val Tyr Ile Met Ala Asp
1 5 10 15

<210> SEQ ID NO 71
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

-continued

<400> SEQUENCE: 71

Lys Leu Glu Tyr Asn Phe Asn Xaa Lys Asn Leu Asn Met Asn Phe
1 5 10 15

<210> SEQ ID NO 72
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 72

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Lys Asn Leu Asn Met Asn Phe
1 5 10 15

<210> SEQ ID NO 73
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 73

Lys Leu Glu Tyr Asn Phe Asn Xaa Pro Lys Asn Leu Asn Met Asn Phe
1 5 10 15

<210> SEQ ID NO 74
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 74

Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Lys Asn Leu Asn Met Asn Phe
1 5 10 15

<210> SEQ ID NO 75
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 75

Gly His Lys Leu Glu Tyr Asn Xaa Xaa Leu Asn Met Asn Phe
1 5 10

-continued

<210> SEQ ID NO 76
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 76

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Leu Asn Met Asn Phe
1 5 10

<210> SEQ ID NO 77
<211> LENGTH: 567
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 77

Met His His His His His His Gly Ser Glu Glu Gln Glu Lys Ala Leu
1 5 10 15

Asn Phe Gly Ile Ile Ser Thr Glu Ser Gln Gln Asn Leu Lys Pro Gln
20 25 30

Trp Thr Pro Phe Leu Gln Asp Met Glu Lys Lys Leu Gly Val Lys Val
35 40 45

Asn Ala Phe Phe Ala Pro Asp Tyr Ala Gly Ile Ile Gln Gly Met Arg
50 55 60

Phe Asn Lys Val Asp Ile Ala Trp Tyr Gly Asn Leu Ser Ala Met Glu
65 70 75 80

Ala Val Asp Arg Ala Asn Gly Gln Val Phe Ala Gln Thr Val Ala Ala
85 90 95

Asp Gly Ser Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Arg Asn
100 105 110

Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly
115 120 125

Val Gln Leu Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
130 135 140

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu
145 150 155 160

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
165 170 175

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly
180 185 190

Gly Thr Gly Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly
195 200 205

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
210 215 220

Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu
225 230 235 240

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro

-continued

```
               245                 250                 255

Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr
            260                 265                 270

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
        275                 280                 285

Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr
    290                 295                 300

Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
305                 310                 315                 320

Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
                325                 330                 335

His Lys Leu Glu Tyr Asn Phe Asn Pro Gly Tyr Trp Ser Val Leu Ile
            340                 345                 350

Val Asn Lys Asp Ser Pro Ile Asn Asn Leu Asn Asp Leu Leu Ala Lys
        355                 360                 365

Arg Lys Asp Leu Thr Phe Gly Asn Gly Asp Pro Asn Ser Thr Ser Gly
    370                 375                 380

Phe Leu Val Pro Gly Tyr Tyr Val Phe Ala Lys Asn Ile Asn Ile Ser
385                 390                 395                 400

Ala Ser Asp Phe Lys Arg Thr Val Asn Ala Gly His Glu Thr Asn Ala
                405                 410                 415

Leu Ala Val Ala Asn Lys Gln Val Asp Val Ala Thr Asn Asn Thr Glu
            420                 425                 430

Asn Leu Asp Lys Leu Lys Thr Ser Ala Pro Glu Lys Leu Lys Glu Leu
        435                 440                 445

Lys Val Ile Trp Lys Ser Pro Leu Ile Pro Gly Asp Pro Ile Val Trp
    450                 455                 460

Arg Lys Asn Leu Ser Glu Thr Thr Lys Asp Lys Ile Tyr Asp Phe Phe
465                 470                 475                 480

Met Asn Tyr Gly Lys Thr Pro Glu Glu Lys Ala Val Leu Glu Arg Leu
                485                 490                 495

Gly Trp Ala Pro Phe Arg Ala Ser Ser Asp Leu Gln Leu Val Pro Ile
            500                 505                 510

Arg Gln Leu Ala Leu Phe Lys Glu Met Gln Ser Val Lys Asp Asn Lys
        515                 520                 525

Gly Leu Asn Glu Gln Asp Lys Leu Ala Lys Thr Thr Ala Ile Gln Ala
    530                 535                 540

Gln Leu Asp Asp Leu Asp Arg Leu Asn Asn Ala Leu Ser Ala Met Ser
545                 550                 555                 560

Ser Val Ser Lys Ala Val Gln
                565


<210> SEQ ID NO 78
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 78

Met His His His His His His Gly Ser Glu Glu Gln Glu Lys Ala Leu
1               5                   10                  15
```

-continued

```
Asn Phe Gly Ile Ile Ser Thr Glu Ser Gln Gln Asn Leu Lys Pro Gln
            20                  25                  30

Trp Thr Pro Phe Leu Gln Asp Met Glu Lys Lys Leu Gly Val Lys Val
        35                  40                  45

Asn Ala Phe Phe Ala Pro Asp Tyr Ala Gly Ile Ile Gln Gly Met Arg
    50                  55                  60

Phe Asn Lys Val Asp Ile Ala Trp Tyr Gly Asn Leu Ser Ala Met Glu
65                  70                  75                  80

Ala Val Asp Arg Ala Asn Gly Gln Val Phe Ala Gln Thr Val Ala Ala
                85                  90                  95

Asp Ala Asp Asn Val Tyr Ile Met Ala Asp Lys Gln Arg Asn Gly Ile
            100                 105                 110

Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val Gln
        115                 120                 125

Leu Ala Tyr His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
    130                 135                 140

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys
145                 150                 155                 160

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
                165                 170                 175

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly
            180                 185                 190

Gly Thr Gly Gly Ser Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly
        195                 200                 205

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
    210                 215                 220

Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu
225                 230                 235                 240

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro
                245                 250                 255

Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr
            260                 265                 270

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
        275                 280                 285

Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr
    290                 295                 300

Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
305                 310                 315                 320

Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
                325                 330                 335

His Lys Leu Glu Tyr Asn Phe Asn Pro Gly Tyr Trp Ser Val Leu Ile
            340                 345                 350

Val Asn Lys Asp Ser Pro Ile Asn Asn Leu Asn Asp Leu Leu Ala Lys
        355                 360                 365

Arg Lys Asp Leu Thr Phe Gly Asn Gly Asp Pro Asn Ser Thr Ser Gly
    370                 375                 380

Phe Leu Val Pro Gly Tyr Tyr Val Phe Ala Lys Asn Asn Ile Ser Ala
385                 390                 395                 400

Gly Lys Thr Pro Glu Glu Lys Ala Val Leu Glu Arg Leu Gly Trp Ala
                405                 410                 415

Pro Phe Arg Ala Ser Ser Asp Leu Gln Leu Val Pro Ile Arg Gln Leu
```

-continued

```
            420                 425                 430

Ala Leu Phe Lys Glu Met Gln Ser Val Lys Asp Asn Lys Gly Leu Asn
        435                 440                 445

Glu Gln Asp Lys Leu Ala Lys Thr Thr Ala Ile Gln Ala Gln Leu Asp
    450                 455                 460

Asp Leu Asp Arg Arg Asn Asn Ala Arg Ser Ala Met Ser Ser Val Ser
465                 470                 475                 480

Asn Tyr Gly Lys Thr Pro Glu Glu Lys Ala Val Leu Glu Arg Leu Gly
                485                 490                 495

Trp Ala Pro Phe Arg Ala Ser Ser Asp Leu Gln Leu Val Pro Ile Arg
            500                 505                 510

Gln Leu Ala Leu Phe Lys Glu Met Gln Ser Val Lys Asp Asn Lys Gly
        515                 520                 525

Leu Asn Glu Gln Asp Lys Leu Ala Lys Thr Thr Ala Ile Gln Ala Gln
    530                 535                 540

Leu Asp Asp Leu Asp Arg Leu Asn Asn Ala Leu Ser Ala Met Ser Ser
545                 550                 555                 560

Val Ser Lys Ala Val Gln
                565


<210> SEQ ID NO 79
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide

<400> SEQUENCE: 79

Gln Thr Val Ala Ala Asp Gly Ser Ser His Asn Val Tyr Ile Met Ala
1               5                   10                  15


<210> SEQ ID NO 80
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 80

Gln Thr Val Ala Ala Asp Xaa Xaa Ser His Asn Val Tyr Ile Met Ala
1               5                   10                  15


<210> SEQ ID NO 81
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 81
```

-continued

Gln Thr Val Ala Ala Asp Xaa Pro Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

<210> SEQ ID NO 82
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 82

Gln Thr Val Ala Ala Asp Pro Xaa Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

<210> SEQ ID NO 83
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 83

Gln Thr Val Ala Ala Asp Xaa Xaa Asn Val Tyr Ile Met Ala
1 5 10

<210> SEQ ID NO 84
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 84

Gln Thr Val Ala Ala Asp Xaa Xaa Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

<210> SEQ ID NO 85
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 85

Val Phe Gln Thr Val Ala Xaa Xaa Ser His Asn Val Tyr Ile Met Ala
1 5 10 15

-continued

<210> SEQ ID NO 86
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 86

His Lys Leu Glu Tyr Asn Phe Asn Pro Gly Tyr Trp Ser Val Leu Ile
1 5 10 15

<210> SEQ ID NO 87
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 87

His Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Pro Gly Tyr Trp Ser Val
1 5 10 15

Leu Ile

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 88

His Lys Leu Glu Tyr Asn Xaa Xaa Pro Gly Tyr Trp Ser Val Leu Ile
1 5 10 15

<210> SEQ ID NO 89
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 89

His Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Tyr Trp Ser Val Leu Ile
1 5 10 15

<210> SEQ ID NO 90
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

-continued

oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 90

```
His Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Tyr Trp Ser Val Leu Ile
1               5                   10                  15
```

<210> SEQ ID NO 91
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 91

```
Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp Glu
        35                  40                  45

Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr Pro
    50                  55                  60

Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val Asn
65                  70                  75                  80

Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
                85                  90                  95

Thr Phe Gln Val His Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
            100                 105                 110

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
        115                 120                 125

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
    130                 135                 140

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
145                 150                 155                 160

Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg Thr
                165                 170                 175

Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly Leu
            180                 185                 190

Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu Trp
        195                 200                 205

Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn Leu
    210                 215                 220

Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala Trp
225                 230                 235                 240

Glu Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp Ala Ala Gly
                245                 250                 255

Leu Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly Lys Ala Ala
            260                 265                 270

Phe Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr Thr Thr Leu
        275                 280                 285
```

-continued

```
Lys Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser Pro Gly Thr
    290                 295                 300

Gln Gly Val Phe Met Met Leu Ser Asp Ser Phe Gly Leu Pro Lys Gly
305                 310                 315                 320

Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly Ser
                325                 330                 335

Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala Ala
            340                 345                 350

Arg Leu Asp Ser Asp Pro Ser Lys Tyr Gly Gly Ser His Asn Val Tyr
        355                 360                 365

Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe Lys Ile
    370                 375                 380

Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln
385                 390                 395                 400

Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
                405                 410                 415

Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg
            420                 425                 430

Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu
        435                 440                 445

Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser
    450                 455                 460

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
465                 470                 475                 480

Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
                485                 490                 495

Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
            500                 505                 510

Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
        515                 520                 525

Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
    530                 535                 540

Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile
545                 550                 555                 560

Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
                565                 570                 575

Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
            580                 585                 590

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn
        595                 600                 605

Asn Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg Ser Asn
    610                 615                 620

Arg Ile Val Gly Ser Leu Val His Gly Ala Val Ala Pro Glu Ser Phe
625                 630                 635                 640

Met Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr Arg Asn
                645                 650                 655

Pro Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln Val Gly
            660                 665                 670

Leu Gly Arg Leu Gly Gln
        675
```

-continued

<210> SEQ ID NO 92
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic recombinant peptide biosensor

<400> SEQUENCE: 92

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20 25 30

Arg Trp Gly Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp Glu
35 40 45

Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr Pro
50 55 60

Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val Asn
65 70 75 80

Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
85 90 95

Thr Phe Gln Val His Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
100 105 110

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
115 120 125

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
130 135 140

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
145 150 155 160

Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg Thr
165 170 175

Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly Leu
180 185 190

Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu Trp
195 200 205

Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn Leu
210 215 220

Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala Trp
225 230 235 240

Glu Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp Ala Ala Gly
245 250 255

Leu Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly Lys Ala Ala
260 265 270

Phe Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr Thr Thr Leu
275 280 285

Lys Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser Pro Gly Thr
290 295 300

Gln Gly Val Phe Met Met Leu Ser Asp Ser Phe Gly Leu Pro Lys Gly
305 310 315 320

Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly Ser
325 330 335

Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala Ala
340 345 350

-continued

```
Arg Leu Asp Ser Asp Pro Ser Lys Tyr Pro Ala Ser His Asn Val Tyr
        355                 360                 365

Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe Lys Ile
    370                 375                 380

Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln
385                 390                 395                 400

Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
                405                 410                 415

Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg
            420                 425                 430

Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu
        435                 440                 445

Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser
    450                 455                 460

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
465                 470                 475                 480

Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
                485                 490                 495

Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
            500                 505                 510

Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
        515                 520                 525

Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
    530                 535                 540

Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile
545                 550                 555                 560

Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
                565                 570                 575

Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
            580                 585                 590

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn
        595                 600                 605

Asn Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg Ser Asn
    610                 615                 620

Arg Ile Val Gly Ser Leu Val His Gly Ala Val Ala Pro Glu Ser Phe
625                 630                 635                 640

Met Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr Arg Asn
                645                 650                 655

Pro Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln Val Gly
            660                 665                 670

Leu Gly Arg Leu Gly Gln
        675


<210> SEQ ID NO 93
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 93

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15
```

-continued

```
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp Glu
        35                  40                  45

Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr Pro
    50                  55                  60

Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val Asn
65                  70                  75                  80

Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
                85                  90                  95

Thr Phe Gln Val His Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
            100                 105                 110

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
        115                 120                 125

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
    130                 135                 140

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
145                 150                 155                 160

Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg Thr
                165                 170                 175

Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly Leu
            180                 185                 190

Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu Trp
        195                 200                 205

Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn Leu
    210                 215                 220

Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala Trp
225                 230                 235                 240

Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
                245                 250                 255

Thr Phe Gln Val Ala Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
            260                 265                 270

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
        275                 280                 285

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
    290                 295                 300

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
305                 310                 315                 320

Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly Ser
                325                 330                 335

Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala Ala
            340                 345                 350

Arg Leu Asp Ser Asp Pro Ser Lys Tyr Gly Gly Ser His Asn Val Tyr
        355                 360                 365

Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe Lys Ile
    370                 375                 380

Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln
385                 390                 395                 400

Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
                405                 410                 415
```

-continued

```
Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg
            420                 425                 430

Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu
        435                 440                 445

Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser
    450                 455                 460

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
465                 470                 475                 480

Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
                485                 490                 495

Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
            500                 505                 510

Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
        515                 520                 525

Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
    530                 535                 540

Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile
545                 550                 555                 560

Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
                565                 570                 575

Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
            580                 585                 590

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn
        595                 600                 605

Asn Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg Ser Asn
    610                 615                 620

Arg Ile Val Gly Ser Leu Val His Gly Ala Val Ala Pro Glu Ser Phe
625                 630                 635                 640

Met Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr Arg Asn
                645                 650                 655

Pro Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln Val Gly
            660                 665                 670

Leu Gly Arg Leu Gly Gln
        675


<210> SEQ ID NO 94
<211> LENGTH: 678
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 94

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp Glu
        35                  40                  45

Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr Pro
    50                  55                  60

Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val Asn
65                  70                  75                  80
```

-continued

```
Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
                85                  90                  95

Thr Phe Gln Val His Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
            100                 105                 110

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
        115                 120                 125

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
    130                 135                 140

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
145                 150                 155                 160

Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg Thr
                165                 170                 175

Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly Leu
            180                 185                 190

Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu Trp
        195                 200                 205

Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn Leu
    210                 215                 220

Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala Trp
225                 230                 235                 240

Glu Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp Ala Ala Gly
                245                 250                 255

Leu Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly Lys Ala Ala
            260                 265                 270

Phe Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr Thr Thr Leu
        275                 280                 285

Lys Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser Pro Gly Thr
    290                 295                 300

Gln Gly Val Phe Met Met Leu Ser Asp Ser Phe Gly Leu Pro Lys Gly
305                 310                 315                 320

Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly Ser
                325                 330                 335

Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala Ala
            340                 345                 350

Arg Leu Asp Ser Asp Pro Ser Lys Tyr Gly Gly Ser His Asn Val Tyr
        355                 360                 365

Ile Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe Lys Ile
    370                 375                 380

Arg His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln
385                 390                 395                 400

Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
                405                 410                 415

Tyr Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg
            420                 425                 430

Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu
        435                 440                 445

Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser
    450                 455                 460

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
465                 470                 475                 480
```

-continued

```
Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
                485                 490                 495

Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
            500                 505                 510

Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
        515                 520                 525

Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
    530                 535                 540

Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile
545                 550                 555                 560

Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
                565                 570                 575

Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
            580                 585                 590

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn
        595                 600                 605

Asn Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg Ser Asn
    610                 615                 620

Arg Ile Val Gly Ser Leu Val Ala Gly Ala Val Ala Pro Glu Ser Phe
625                 630                 635                 640

Met Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr Arg Asn
                645                 650                 655

Pro Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln Val Gly
            660                 665                 670

Leu Gly Arg Leu Gly Gln
        675


<210> SEQ ID NO 95
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 95

Asp Ser Asp Pro Ser Lys Tyr Xaa Xaa Ser His Asn Val Tyr Ile Met
1               5                   10                  15


<210> SEQ ID NO 96
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 96

Asp Ser Asp Pro Ser Lys Tyr Pro Xaa Ser His Asn Val Tyr Ile Met
1               5                   10                  15
```

-continued

<210> SEQ ID NO 97
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 97

Asp Ser Asp Pro Ser Lys Tyr Xaa Pro Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 98
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 98

Arg Leu Asp Ser Asp Pro Ser Xaa Xaa Ser His Asn Val Tyr Ile Met
1 5 10 15

<210> SEQ ID NO 99
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 99

Asp Ser Asp Pro Ser Lys Tyr Xaa Xaa Asn Val Tyr Ile Met
1 5 10

<210> SEQ ID NO 100
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 100

Lys Leu Glu Tyr Asn Phe Asn Xaa Xaa Asn Ala Tyr Gly Gln Ser Ala
1 5 10 15

<210> SEQ ID NO 101
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 101

Lys Leu Glu Tyr Asn Phe Xaa Xaa Pro Asn Ala Tyr Gly Gln Ser Ala
1 5 10 15

<210> SEQ ID NO 102
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 102

Gly His Lys Leu Glu Tyr Asn Xaa Xaa Asn Ala Tyr Gly Gln Ser Ala
1 5 10 15

<210> SEQ ID NO 103
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 103

Lys Leu Glu Tyr Asn Phe Asn Xaa Pro Asn Ala Tyr Gly Gln Ser Ala
1 5 10 15

<210> SEQ ID NO 104
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 104

Lys Leu Glu Tyr Asn Phe Asn Pro Xaa Asn Ala Tyr Gly Gln Ser Ala
1 5 10 15

<210> SEQ ID NO 105
<211> LENGTH: 396
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 105

Met Lys Ile Lys Thr Gly Ala Arg Ile Leu Ala Leu Ser Ala Leu Thr

-continued

```
1               5                   10                  15

Thr Met Met Phe Ser Ala Ser Ala Leu Ala Lys Ile Glu Glu Gly Lys
            20                  25                  30

Leu Val Ile Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu
        35                  40                  45

Val Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu
    50                  55                  60

His Pro Asp Lys Leu Glu Glu Lys Phe Pro Gln Val Ala Ala Thr Gly
65                  70                  75                  80

Asp Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr
                85                  90                  95

Ala Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln
            100                 105                 110

Asp Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys
        115                 120                 125

Leu Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn
    130                 135                 140

Lys Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala
145                 150                 155                 160

Leu Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn
                165                 170                 175

Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly
            180                 185                 190

Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly
        195                 200                 205

Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu
    210                 215                 220

Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu
225                 230                 235                 240

Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp
                245                 250                 255

Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val
            260                 265                 270

Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu
        275                 280                 285

Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu
    290                 295                 300

Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn
305                 310                 315                 320

Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu
                325                 330                 335

Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys
            340                 345                 350

Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala
        355                 360                 365

Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp
    370                 375                 380

Glu Ala Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys
385                 390                 395
```

<210> SEQ ID NO 106

-continued

<211> LENGTH: 434
<212> TYPE: PRT
<213> ORGANISM: Pyrococcus furiosus

<400> SEQUENCE: 106

Met Arg Arg Ala Thr Tyr Ala Phe Ala Leu Leu Ala Ile Leu Val Leu
1 5 10 15

Gly Val Val Ala Ser Gly Cys Ile Gly Gly Gly Thr Thr Thr Pro Thr
20 25 30

Gln Thr Ser Pro Ala Thr Gln Pro Thr Thr Thr Gln Thr Pro Thr Gln
35 40 45

Thr Glu Thr Gln Ala Val Glu Cys Gly Ser Gly Lys Val Val Ile Trp
50 55 60

His Ala Met Gln Pro Asn Glu Leu Glu Val Phe Gln Ser Leu Ala Glu
65 70 75 80

Glu Tyr Met Ala Leu Cys Pro Glu Val Glu Ile Val Phe Glu Gln Lys
85 90 95

Pro Asn Leu Glu Asp Ala Leu Lys Ala Ala Ile Pro Thr Gly Gln Gly
100 105 110

Pro Asp Leu Phe Ile Trp Ala His Asp Trp Ile Gly Lys Phe Ala Glu
115 120 125

Ala Gly Leu Leu Glu Pro Ile Asp Glu Tyr Val Thr Glu Asp Leu Leu
130 135 140

Asn Glu Phe Ala Pro Met Ala Gln Asp Ala Met Gln Tyr Lys Gly His
145 150 155 160

Tyr Tyr Ala Leu Pro Phe Ala Ala Glu Thr Val Ala Ile Ile Tyr Asn
165 170 175

Lys Glu Met Val Ser Glu Pro Pro Lys Thr Phe Asp Glu Met Lys Ala
180 185 190

Ile Met Glu Lys Tyr Tyr Asp Pro Ala Asn Glu Lys Tyr Gly Ile Ala
195 200 205

Trp Pro Ile Asn Ala Tyr Phe Ile Ser Ala Ile Ala Gln Ala Phe Gly
210 215 220

Gly Tyr Tyr Phe Asp Asp Lys Thr Glu Gln Pro Gly Leu Asp Lys Pro
225 230 235 240

Glu Thr Ile Glu Gly Phe Lys Phe Phe Phe Thr Glu Ile Trp Pro Tyr
245 250 255

Met Ala Pro Thr Gly Asp Tyr Asn Thr Gln Gln Ser Ile Phe Leu Glu
260 265 270

Gly Arg Ala Pro Met Met Val Asn Gly Pro Trp Ser Ile Asn Asp Val
275 280 285

Lys Lys Ala Gly Ile Asn Phe Gly Val Val Pro Leu Pro Pro Ile Ile
290 295 300

Lys Asp Gly Lys Glu Tyr Trp Pro Arg Pro Tyr Gly Gly Val Lys Leu
305 310 315 320

Ile Tyr Phe Ala Ala Gly Ile Lys Asn Lys Asp Ala Ala Trp Lys Phe
325 330 335

Ala Lys Trp Leu Thr Thr Ser Glu Glu Ser Ile Lys Thr Leu Ala Leu
340 345 350

Glu Leu Gly Tyr Ile Pro Val Leu Thr Lys Val Leu Asp Asp Pro Glu
355 360 365

Ile Lys Asn Asp Pro Val Ile Tyr Gly Phe Gly Gln Ala Val Gln His

-continued

```
    370                 375                 380

Ala Tyr Leu Met Pro Lys Ser Pro Lys Met Ser Ala Val Trp Gly Gly
385                 390                 395                 400

Val Asp Gly Ala Ile Asn Glu Ile Leu Gln Asp Pro Gln Asn Ala Asp
                405                 410                 415

Ile Glu Gly Ile Leu Lys Lys Tyr Gln Gln Glu Ile Leu Asn Asn Met
            420                 425                 430

Gln Gly


<210> SEQ ID NO 107
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 107

Met Asn Ala Lys Ile Ile Ala Ser Leu Ala Phe Thr Ser Met Phe Ser
1               5                   10                  15

Leu Ser Thr Leu Leu Asn Pro Ala Tyr Ala Glu Glu Gln Glu Lys Ala
            20                  25                  30

Leu Asn Phe Gly Ile Ile Ser Thr Glu Ser Gln Gln Asn Leu Lys Pro
        35                  40                  45

Gln Trp Thr Pro Phe Leu Gln Asp Met Glu Lys Lys Leu Gly Val Lys
    50                  55                  60

Val Asn Ala Phe Phe Ala Pro Asp Tyr Ala Gly Ile Ile Gln Gly Met
65                  70                  75                  80

Arg Phe Asn Lys Val Asp Ile Ala Trp Tyr Gly Asn Leu Ser Ala Met
                85                  90                  95

Glu Ala Val Asp Arg Ala Asn Gly Gln Val Phe Ala Gln Thr Val Ala
            100                 105                 110

Ala Asp Gly Ser Pro Gly Tyr Trp Ser Val Leu Ile Val Asn Lys Asp
        115                 120                 125

Ser Pro Ile Asn Asn Leu Asn Asp Leu Leu Ala Lys Arg Lys Asp Leu
    130                 135                 140

Thr Phe Gly Asn Gly Asp Pro Asn Ser Thr Ser Gly Phe Leu Val Pro
145                 150                 155                 160

Gly Tyr Tyr Val Phe Ala Lys Asn Asn Ile Ser Ala Ser Asp Phe Lys
                165                 170                 175

Arg Thr Val Asn Ala Gly His Glu Thr Asn Ala Leu Ala Val Ala Asn
            180                 185                 190

Lys Gln Val Asp Val Ala Thr Asn Asn Thr Glu Asn Leu Asp Lys Leu
        195                 200                 205

Lys Thr Ser Ala Pro Glu Lys Leu Lys Glu Leu Lys Val Ile Trp Lys
    210                 215                 220

Ser Pro Leu Ile Pro Gly Asp Pro Ile Val Trp Arg Lys Asn Leu Ser
225                 230                 235                 240

Glu Thr Thr Lys Asp Lys Ile Tyr Asp Phe Phe Met Asn Tyr Gly Lys
                245                 250                 255

Thr Pro Glu Glu Lys Ala Val Leu Glu Arg Leu Gly Trp Ala Pro Phe
            260                 265                 270

Arg Ala Ser Ser Asp Leu Gln Leu Val Pro Ile Arg Gln Leu Ala Leu
        275                 280                 285

Phe Lys Glu Met Gln Ser Val Lys Asp Asn Lys Gly Leu Asn Glu Gln
```

-continued

```
    290                 295                 300

Asp Lys Leu Ala Lys Thr Thr Ala Ile Gln Ala Gln Leu Asp Asp Leu
305                 310                 315                 320

Asp Arg Leu Asn Asn Ala Leu Ser Ala Met Ser Ser Val Ser Lys Ala
                325                 330                 335

Val Gln


<210> SEQ ID NO 108
<211> LENGTH: 302
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 108

Met Gln Leu Arg Lys Pro Ala Thr Ala Ile Leu Ala Leu Ala Leu Ser
1               5                   10                  15

Ala Gly Leu Ala Gln Ala Asp Asp Ala Ala Pro Ala Ala Gly Ser Thr
            20                  25                  30

Leu Asp Lys Ile Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu
        35                  40                  45

Ser Ser Val Pro Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly
    50                  55                  60

Tyr Ser Gln Asp Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys
65                  70                  75                  80

Leu Asn Lys Pro Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln
                85                  90                  95

Asn Arg Ile Pro Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly
            100                 105                 110

Ser Thr Thr Asn Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp
        115                 120                 125

Thr Ile Phe Val Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp
    130                 135                 140

Ile Lys Asp Phe Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser
145                 150                 155                 160

Gly Thr Thr Ser Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys
                165                 170                 175

Met Asn Met Arg Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg
            180                 185                 190

Thr Leu Glu Ser Gly Arg Ala Val Ala Phe Met Met Asp Asp Ala Leu
        195                 200                 205

Leu Ala Gly Glu Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile
    210                 215                 220

Val Gly Lys Pro Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys
225                 230                 235                 240

Asp Asp Pro Gln Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val
                245                 250                 255

Gln Thr Ser Gly Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn
            260                 265                 270

Pro Ile Pro Pro Lys Asn Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        275                 280                 285

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Asn
    290                 295                 300
```

-continued

<210> SEQ ID NO 109
<211> LENGTH: 414
<212> TYPE: PRT
<213> ORGANISM: Thermus thermophilus

<400> SEQUENCE: 109

Met Arg Lys Trp Leu Leu Ala Ile Gly Met Val Leu Gly Leu Ser Ala
1 5 10 15

Leu Ala Gln Gly Gly Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp
20 25 30

Glu Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr
35 40 45

Pro Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val
50 55 60

Asn Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro
65 70 75 80

Asp Thr Phe Gln Val His Ala Gly Met Glu Leu Ile Gly Thr Trp Val
85 90 95

Val Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly
100 105 110

Trp Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys
115 120 125

Gly Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met
130 135 140

Trp Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg
145 150 155 160

Thr Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly
165 170 175

Leu Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu
180 185 190

Trp Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn
195 200 205

Leu Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala
210 215 220

Trp Glu Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp Ala Ala
225 230 235 240

Gly Leu Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly Lys Ala
245 250 255

Ala Phe Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr Thr Thr
260 265 270

Leu Lys Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser Pro Gly
275 280 285

Thr Gln Gly Val Phe Met Met Leu Ser Asp Ser Phe Gly Leu Pro Lys
290 295 300

Gly Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly
305 310 315 320

Ser Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala
325 330 335

Ala Arg Leu Asp Ser Asp Pro Ser Lys Tyr Asn Ala Tyr Gly Gln Ser
340 345 350

Ala Met Arg Asp Trp Arg Ser Asn Arg Ile Val Gly Ser Leu Val His
355 360 365

-continued

```
Gly Ala Val Ala Pro Glu Ser Phe Met Ser Gln Phe Gly Thr Val Met
    370                 375                 380

Glu Ile Phe Leu Gln Thr Arg Asn Pro Gln Ala Ala Ala Asn Ala Ala
385                 390                 395                 400

Gln Ala Ile Ala Asp Gln Val Gly Leu Gly Arg Leu Gly Gln
                405                 410


<210> SEQ ID NO 110
<211> LENGTH: 318
<212> TYPE: PRT
<213> ORGANISM: Rhizobium meliloti

<400> SEQUENCE: 110

Met Ile Arg Thr Leu Ser Leu Lys Phe Met Leu Ala Gly Ala Val Cys
1               5                   10                  15

Met Ala Thr Leu Thr Ala Gly Ser Ala Phe Ala Ala Glu Pro Glu Ser
            20                  25                  30

Cys Gly Thr Val Arg Phe Ser Asp Val Gly Trp Thr Asp Ile Thr Ala
        35                  40                  45

Thr Thr Ala Thr Ala Thr Thr Ile Leu Glu Ala Leu Gly Tyr Glu Thr
    50                  55                  60

Asp Val Lys Val Leu Ser Val Pro Val Thr Tyr Thr Ser Leu Lys Asn
65                  70                  75                  80

Lys Asp Ile Asp Val Phe Leu Gly Asn Trp Met Pro Thr Met Glu Ala
                85                  90                  95

Asp Ile Ala Pro Tyr Arg Glu Asp Lys Ser Val Glu Thr Val Arg Glu
            100                 105                 110

Asn Leu Ala Gly Ala Lys Tyr Thr Leu Ala Thr Asn Ala Lys Gly Ala
        115                 120                 125

Glu Leu Gly Ile Lys Asp Phe Lys Asp Ile Ala Ala His Lys Asp Glu
    130                 135                 140

Leu Asp Gly Lys Ile Tyr Gly Ile Glu Pro Gly Asn Asp Gly Asn Arg
145                 150                 155                 160

Leu Ile Ile Asp Met Val Glu Lys Gly Thr Phe Asp Leu Lys Gly Phe
                165                 170                 175

Glu Val Val Glu Ser Ser Glu Gln Gly Met Leu Ala Gln Val Ala Arg
            180                 185                 190

Ala Glu Lys Ser Gly Asp Pro Ile Val Phe Leu Gly Trp Glu Pro His
        195                 200                 205

Pro Met Asn Ala Asn Phe Lys Leu Thr Tyr Leu Ser Gly Gly Asp Asp
    210                 215                 220

Val Phe Gly Pro Asn Tyr Gly Gly Ala Thr Val His Thr Asn Val Arg
225                 230                 235                 240

Ala Gly Tyr Thr Thr Glu Cys Pro Asn Val Gly Lys Leu Leu Gln Asn
                245                 250                 255

Leu Ser Phe Ser Leu Gln Met Glu Asn Glu Ile Met Gly Lys Ile Leu
            260                 265                 270

Asn Asp Gly Glu Asp Pro Glu Lys Ala Ala Ala Ala Trp Leu Lys Asp
        275                 280                 285

Asn Pro Gln Ser Ile Glu Pro Trp Leu Ser Gly Val Ala Thr Lys Asp
    290                 295                 300

Gly Gly Asp Gly Leu Ala Ala Val Lys Ala Ala Leu Gly Leu
305                 310                 315
```

-continued

<210> SEQ ID NO 111
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Geobacillus stearothermophilus

<400> SEQUENCE: 111

Met Gly Gly Gly Arg Ser Thr Glu Thr Ser Ser Ser Ser Gly Gly Asp
1 5 10 15

Gly Gly Ala Thr Lys Lys Lys Val Val Val Gly Thr Asp Ala Ala Phe
20 25 30

Ala Pro Phe Glu Tyr Met Gln Lys Gly Lys Ile Val Gly Phe Asp Val
35 40 45

Asp Leu Leu Asp Ala Val Met Lys Ala Ala Gly Leu Asp Tyr Glu Leu
50 55 60

Lys Asn Ile Gly Trp Asp Pro Leu Phe Ala Ser Leu Gln Ser Lys Glu
65 70 75 80

Val Asp Met Gly Ile Ser Gly Ile Thr Ile Thr Asp Glu Arg Lys Gln
85 90 95

Ser Tyr Asp Phe Ser Asp Pro Tyr Phe Glu Ala Thr Gln Val Ile Leu
100 105 110

Val Lys Gln Gly Ser Pro Val Lys Asn Ala Leu Asp Leu Lys Gly Thr
115 120 125

Ile Gly Val Gln Asn Ala Thr Thr Gly Gln Glu Ala Ala Glu Lys Leu
130 135 140

Phe Gly Lys Gly Pro His Ile Lys Lys Phe Glu Thr Thr Val Val Ala
145 150 155 160

Ile Met Glu Leu Leu Asn Gly Gly Val Asp Ala Val Ile Thr Asp Asn
165 170 175

Ala Val Ala Asn Glu Tyr Val Lys Asn Asn Pro Asn Lys Lys Leu Gln
180 185 190

Val Ile Glu Asp Pro Lys Asn Phe Ala Ser Glu Tyr Tyr Gly Met Ile
195 200 205

Phe Pro Lys Asn Ser Glu Leu Lys Ala Lys Val Asp Glu Ala Leu Lys
210 215 220

Asn Val Ile Asn Ser Gly Lys Tyr Thr Glu Ile Tyr Lys Lys Trp Phe
225 230 235 240

Gly Lys Glu Pro Lys Leu Asp Arg Leu
245

<210> SEQ ID NO 112
<211> LENGTH: 370
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens

<400> SEQUENCE: 112

Met Lys Lys Ser Leu Leu Ser Ala Val Ala Leu Thr Ala Met Val Ala
1 5 10 15

Phe Gly Gly Ser Ala Trp Ala Asp Val Val Ile Ala Val Gly Ala Pro
20 25 30

Leu Thr Gly Pro Asn Ala Ala Phe Gly Ala Gln Ile Gln Lys Gly Ala
35 40 45

Glu Gln Ala Ala Lys Asp Ile Asn Ala Ala Gly Gly Ile Asn Gly Glu
50 55 60

-continued

```
Gln Ile Lys Ile Val Leu Gly Asp Asp Val Ser Asp Pro Lys Gln Gly
65                  70                  75                  80

Ile Ser Val Ala Asn Lys Phe Val Ala Asp Gly Val Lys Phe Val Val
                85                  90                  95

Gly His Phe Asn Ser Gly Val Ser Ile Pro Ala Ser Glu Val Tyr Ala
            100                 105                 110

Glu Asn Gly Ile Leu Glu Ile Thr Pro Ala Ala Thr Asn Pro Val Phe
        115                 120                 125

Thr Glu Arg Gly Leu Trp Asn Thr Phe Arg Thr Cys Gly Arg Asp Asp
    130                 135                 140

Gln Gln Gly Gly Ile Ala Gly Lys Tyr Leu Ala Asp His Phe Lys Asp
145                 150                 155                 160

Ala Lys Val Ala Ile Ile His Asp Lys Thr Pro Tyr Gly Gln Gly Leu
                165                 170                 175

Ala Asp Glu Thr Lys Lys Ala Ala Asn Ala Ala Gly Val Thr Glu Val
            180                 185                 190

Met Tyr Glu Gly Val Asn Val Gly Asp Lys Asp Phe Ser Ala Leu Ile
        195                 200                 205

Ser Lys Met Lys Glu Ala Gly Val Ser Ile Ile Tyr Trp Gly Gly Leu
    210                 215                 220

His Thr Glu Ala Gly Leu Ile Ile Arg Gln Ala Ala Asp Gln Gly Leu
225                 230                 235                 240

Lys Ala Lys Leu Val Ser Gly Asp Gly Ile Val Ser Asn Glu Leu Ala
                245                 250                 255

Ser Ile Ala Gly Asp Ala Val Glu Gly Thr Leu Asn Thr Phe Gly Pro
            260                 265                 270

Asp Pro Thr Leu Arg Pro Glu Asn Lys Glu Leu Val Glu Lys Phe Lys
        275                 280                 285

Ala Ala Gly Phe Asn Pro Glu Ala Tyr Thr Leu Tyr Ser Tyr Ala Ala
    290                 295                 300

Met Gln Ala Ile Ala Gly Ala Ala Lys Ala Ala Gly Ser Val Glu Pro
305                 310                 315                 320

Glu Lys Val Ala Glu Ala Leu Lys Lys Gly Ser Phe Pro Thr Ala Leu
                325                 330                 335

Gly Glu Ile Ser Phe Asp Glu Lys Gly Asp Pro Lys Leu Pro Gly Tyr
            340                 345                 350

Val Met Tyr Glu Trp Lys Lys Gly Pro Asp Gly Lys Phe Thr Tyr Ile
        355                 360                 365

Gln Gln
    370


<210> SEQ ID NO 113
<211> LENGTH: 367
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 113

Met Asn Ile Lys Gly Lys Ala Leu Leu Ala Gly Cys Ile Ala Leu Ala
1               5                   10                  15

Phe Ser Asn Met Ala Leu Ala Glu Asp Ile Lys Val Ala Val Val Gly
            20                  25                  30

Ala Met Ser Gly Pro Val Ala Gln Tyr Gly Asp Gln Glu Phe Thr Gly
```

-continued

```
        35                  40                  45

Ala Glu Gln Ala Val Ala Asp Ile Asn Ala Lys Gly Gly Ile Lys Gly
    50                  55                  60

Asn Lys Leu Gln Ile Val Lys Tyr Asp Asp Ala Cys Asp Pro Lys Gln
65                  70                  75                  80

Ala Val Ala Val Ala Asn Lys Val Val Asn Asp Gly Ile Lys Tyr Val
                85                  90                  95

Ile Gly His Leu Cys Ser Ser Ser Thr Gln Pro Ala Ser Asp Ile Tyr
            100                 105                 110

Glu Asp Glu Gly Ile Leu Met Ile Thr Pro Ala Ala Thr Ala Pro Glu
        115                 120                 125

Leu Thr Ala Arg Gly Tyr Gln Leu Ile Leu Arg Thr Thr Gly Leu Asp
    130                 135                 140

Ser Asp Gln Gly Pro Thr Ala Ala Lys Tyr Ile Leu Glu Lys Val Lys
145                 150                 155                 160

Pro Gln Arg Ile Ala Ile Val His Asp Lys Gln Gln Tyr Gly Glu Gly
                165                 170                 175

Leu Ala Arg Ala Val Gln Asp Gly Leu Lys Lys Gly Asn Ala Asn Val
            180                 185                 190

Val Phe Phe Asp Gly Ile Thr Ala Gly Glu Lys Asp Phe Ser Thr Leu
        195                 200                 205

Val Ala Arg Leu Lys Lys Glu Asn Ile Asp Phe Val Tyr Tyr Gly Gly
    210                 215                 220

Tyr His Pro Glu Met Gly Gln Ile Leu Arg Gln Ala Arg Ala Ala Gly
225                 230                 235                 240

Leu Lys Thr Gln Phe Met Gly Pro Glu Gly Val Ala Asn Val Ser Leu
                245                 250                 255

Ser Asn Ile Ala Gly Glu Ser Ala Glu Gly Leu Leu Val Thr Lys Pro
            260                 265                 270

Lys Asn Tyr Asp Gln Val Pro Ala Asn Lys Pro Ile Val Asp Ala Ile
        275                 280                 285

Lys Ala Lys Lys Gln Asp Pro Ser Gly Ala Phe Val Trp Thr Thr Tyr
    290                 295                 300

Ala Ala Leu Gln Ser Leu Gln Ala Gly Leu Asn Gln Ser Asp Asp Pro
305                 310                 315                 320

Ala Glu Ile Ala Lys Tyr Leu Lys Ala Asn Ser Val Asp Thr Val Met
                325                 330                 335

Gly Pro Leu Thr Trp Asp Glu Lys Gly Asp Leu Lys Gly Phe Glu Phe
            340                 345                 350

Gly Val Phe Asp Trp His Ala Asn Gly Thr Ala Thr Asp Ala Lys
        355                 360                 365


<210> SEQ ID NO 114
<211> LENGTH: 677
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      recombinant peptide biosensor

<400> SEQUENCE: 114

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1               5                   10                  15
```

-continued

```
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
            20                  25                  30

Arg Trp Gly Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala Gly Asp Glu
        35                  40                  45

Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln Lys Tyr Pro
    50                  55                  60

Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala Gly Val Asn
65                  70                  75                  80

Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp Pro Pro Asp
                85                  90                  95

Thr Phe Gln Val Ala Ala Gly Met Glu Leu Ile Gly Thr Trp Val Val
            100                 105                 110

Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln Glu Gly Trp
        115                 120                 125

Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser Tyr Lys Gly
    130                 135                 140

Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn Val Met Trp
145                 150                 155                 160

Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro Pro Arg Thr
                165                 170                 175

Trp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln Lys Gly Leu Glu
            180                 185                 190

Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln His Leu Trp Glu
        195                 200                 205

Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp Asn Asn Leu Trp
    210                 215                 220

Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val Arg Ala Trp Glu
225                 230                 235                 240

Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp Ala Ala Gly Leu
                245                 250                 255

Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly Lys Ala Ala Phe
            260                 265                 270

Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr Thr Thr Leu Lys
        275                 280                 285

Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser Pro Gly Thr Gln
    290                 295                 300

Gly Val Phe Met Met Val Ser Asp Ser Phe Gly Leu Pro Lys Gly Ala
305                 310                 315                 320

Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu Val Gly Ser Lys
                325                 330                 335

Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser Ile Ala Ala Arg
            340                 345                 350

Leu Asp Ser Asp Pro Ser Lys Tyr Pro Ala Ser His Asn Val Tyr Ile
        355                 360                 365

Met Ala Asp Lys Gln Arg Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg
    370                 375                 380

His Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Tyr His Tyr Gln Gln
385                 390                 395                 400

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
                405                 410                 415

Leu Ser Thr Gln Ser Lys Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
```

-continued

```
            420                 425                 430

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
        435                 440                 445

Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser Lys
    450                 455                 460

Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
465                 470                 475                 480

Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly
                485                 490                 495

Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
            500                 505                 510

Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly
        515                 520                 525

Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe
    530                 535                 540

Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile Phe
545                 550                 555                 560

Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu
                565                 570                 575

Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys
            580                 585                 590

Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Phe Asn Asn
        595                 600                 605

Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg Ser Asn Arg
    610                 615                 620

Ile Val Gly Ser Leu Val Ala Gly Ala Val Ala Pro Glu Ser Phe Met
625                 630                 635                 640

Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr Arg Asn Pro
                645                 650                 655

Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln Val Gly Leu
            660                 665                 670

Gly Arg Leu Gly Gln
        675


<210> SEQ ID NO 115
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 115

Xaa Pro Ser His Asn Val Tyr
1               5


<210> SEQ ID NO 116
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      oligopeptide
```

-continued

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 116

Xaa Xaa Ser His Asn Val Tyr
1 5

<210> SEQ ID NO 117
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 117

Xaa Xaa Ser His Asn Val Phe
1 5

<210> SEQ ID NO 118
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 118

Pro Xaa Ser His Asn Val Phe
1 5

<210> SEQ ID NO 119
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 119

Pro Xaa Ser Tyr Asn Val Phe
1 5

<210> SEQ ID NO 120
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

-continued

<400> SEQUENCE: 120

Xaa Xaa Ser Tyr Asn Val Phe
1 5

<210> SEQ ID NO 121
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 121

Pro Xaa Ser Tyr Asn Val Phe
1 5

<210> SEQ ID NO 122
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 122

Xaa Xaa Ser Tyr Asn Val Phe
1 5

<210> SEQ ID NO 123
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 123

Pro Xaa Ser Xaa Asn Val Tyr
1 5

<210> SEQ ID NO 124
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

-continued

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 124

Pro Xaa Ser His Xaa Val Tyr
1 5

<210> SEQ ID NO 125
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 125

Pro Xaa Ser His Asn Xaa Tyr
1 5

<210> SEQ ID NO 126
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 126

Pro Xaa Ser His Asn Val Xaa
1 5

<210> SEQ ID NO 127
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 127

Phe Asn Xaa Xaa Tyr
1 5

<210> SEQ ID NO 128
<211> LENGTH: 4
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 128

Phe Asn Xaa Tyr
1

<210> SEQ ID NO 129
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 129

Phe Asn Tyr
1

<210> SEQ ID NO 130
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 130

Phe Xaa Tyr
1

<210> SEQ ID NO 131
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 131

Xaa Xaa Tyr
1

<210> SEQ ID NO 132
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

-continued

<400> SEQUENCE: 132

Trp Xaa Tyr
1

<210> SEQ ID NO 133
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 133

Xaa Lys Tyr
1

<210> SEQ ID NO 134
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 134

Phe Asn Pro Xaa Tyr
1 5

<210> SEQ ID NO 135
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 135

Phe Asn Xaa Pro Tyr
1 5

<210> SEQ ID NO 136
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 136

His Asn Ser
1

<210> SEQ ID NO 137

-continued

<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 137

Gly Gly Ser
1

<210> SEQ ID NO 138
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 138

Xaa Xaa Ser
1

<210> SEQ ID NO 139
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 139

Xaa Xaa Lys
1

<210> SEQ ID NO 140
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 140

Gly Gly Lys
1

<210> SEQ ID NO 141
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 141

-continued

Pro Xaa Ser
1

<210> SEQ ID NO 142
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 142

Xaa Pro Ser
1

<210> SEQ ID NO 143
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 143

Pro Xaa
1

<210> SEQ ID NO 144
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 144

Xaa Pro
1

<210> SEQ ID NO 145
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 145

Ile Xaa Xaa Ser
1

-continued

<210> SEQ ID NO 146
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 146

Asn Xaa Pro Lys
1

<210> SEQ ID NO 147
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 147

Asn Pro Cys Lys
1

<210> SEQ ID NO 148
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 148

Pro Pro Xaa Ser His
1 5

<210> SEQ ID NO 149
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 149

Pro Pro Xaa Xaa Ser His
1 5

<210> SEQ ID NO 150
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

-continued

<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 150

Pro Pro Pro Xaa Ser His
1 5

<210> SEQ ID NO 151
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 151

Pro Pro Xaa Pro Ser His
1 5

<210> SEQ ID NO 152
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 152

Xaa Xaa Ser His
1

<210> SEQ ID NO 153
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 153

Pro Pro Xaa Xaa
1

<210> SEQ ID NO 154
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

-continued

<400> SEQUENCE: 154

Phe Asn Xaa Lys Asn
1 5

<210> SEQ ID NO 155
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 155

Phe Asn Xaa Xaa Lys Asn
1 5

<210> SEQ ID NO 156
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 156

Phe Asn Xaa Pro Lys Asn
1 5

<210> SEQ ID NO 157
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 157

Phe Asn Pro Xaa Lys Asn
1 5

<210> SEQ ID NO 158
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 158

Phe Asn Xaa Xaa

-continued

1

<210> SEQ ID NO 159
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 159

Asn Ala Asp Gly Ser Ser His
1 5

<210> SEQ ID NO 160
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 160

Ala Asp Xaa Xaa Ser His
1 5

<210> SEQ ID NO 161
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 161

Ala Asp Xaa Pro Ser His
1 5

<210> SEQ ID NO 162
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 162

Ala Asp Pro Xaa Ser His
1 5

<210> SEQ ID NO 163
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 163

Ala Asp Xaa Xaa
1

<210> SEQ ID NO 164
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 164

Ala Asp Xaa Xaa Ser His
1 5

<210> SEQ ID NO 165
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide

<400> SEQUENCE: 165

Phe Asn Pro Gly
1

<210> SEQ ID NO 166
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 166

Phe Asn Xaa Xaa Pro Gly
1 5

<210> SEQ ID NO 167
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (1)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 167

-continued

Xaa Xaa Pro Gly
1

<210> SEQ ID NO 168
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 168

Phe Asn Xaa Xaa
1

<210> SEQ ID NO 169
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 169

Phe Asn Pro Xaa
1

<210> SEQ ID NO 170
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 170

Lys Tyr Xaa Xaa Ser His
1 5

<210> SEQ ID NO 171
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 171

Lys Tyr Pro Xaa Ser His
1 5

-continued

<210> SEQ ID NO 172
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 172

Lys Tyr Xaa Pro Ser His
1 5

<210> SEQ ID NO 173
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 173

Phe Xaa Xaa Pro
1

<210> SEQ ID NO 174
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 174

Phe Asn Xaa Pro
1

<210> SEQ ID NO 175
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 175

Phe Asn Pro Xaa
1

<210> SEQ ID NO 176
<211> LENGTH: 606
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 176

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
20 25 30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
35 40 45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
50 55 60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65 70 75 80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro
85 90 95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
100 105 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
115 120 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
130 135 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145 150 155 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
165 170 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
180 185 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu
195 200 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
210 215 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225 230 235 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
245 250 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
260 265 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
275 280 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
290 295 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305 310 315 320

Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Val Leu Ser Lys Asp
325 330 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
340 345 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
355 360 365

-continued

```
Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            420                 425                 430

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510

Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 177
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 177

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
            20                  25                  30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
        35                  40                  45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
    50                  55                  60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65                  70                  75                  80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro
                85                  90                  95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
            100                 105                 110
```

-continued

```
Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
        115                 120                 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Ser Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
            260                 265                 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            420                 425                 430

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510
```

-continued

```
Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 178
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 178

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
            20                  25                  30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
        35                  40                  45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
    50                  55                  60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65                  70                  75                  80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ala Gln Asn Arg Ile Pro
                85                  90                  95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
            100                 105                 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
        115                 120                 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255
```

-continued

```
Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
            260                 265                 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            420                 425                 430

Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510

Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 179
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide
```

-continued

<400> SEQUENCE: 179

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                  10                  15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
            20                  25                  30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
        35                  40                  45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
    50                  55                  60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65                  70                  75                  80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro
                85                  90                  95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
            100                 105                 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
        115                 120                 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
            260                 265                 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400
```

-continued

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Leu
405 410 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
420 425 430

Thr Leu Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met
435 440 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
450 455 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465 470 475 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
485 490 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
500 505 510

Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
515 520 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
530 535 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545 550 555 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
565 570 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
580 585 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
595 600 605

<210> SEQ ID NO 180
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 180

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
20 25 30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
35 40 45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
50 55 60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65 70 75 80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ala Gln Asn Arg Ile Pro
85 90 95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
100 105 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
115 120 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe

-continued

```
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Ser Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
            260                 265                 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Leu
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            420                 425                 430

Thr Leu Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510

Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540
```

-continued

```
Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 181
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 181

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
            20                  25                  30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
        35                  40                  45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
    50                  55                  60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65                  70                  75                  80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro
                85                  90                  95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
            100                 105                 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
        115                 120                 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Val
            260                 265                 270

Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
```

-continued

```
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Leu
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
            420                 425                 430

Thr Leu Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510

Tyr Asn Phe Asn Asn Pro Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 182
<211> LENGTH: 606
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 182

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15
```

-continued

```
Gly Ser Thr Gly Asp Arg Ser Ala Ala Gly Ser Thr Leu Asp Lys Ile
            20                  25                  30

Ala Lys Asn Gly Val Ile Val Val Gly His Arg Glu Ser Ser Val Pro
        35                  40                  45

Phe Ser Tyr Tyr Asp Asn Gln Gln Lys Val Val Gly Tyr Ser Gln Asp
    50                  55                  60

Tyr Ser Asn Ala Ile Val Glu Ala Val Lys Lys Lys Leu Asn Lys Pro
65                  70                  75                  80

Asp Leu Gln Val Lys Leu Ile Pro Ile Thr Ser Gln Asn Arg Ile Pro
                85                  90                  95

Leu Leu Gln Asn Gly Thr Phe Asp Phe Glu Cys Gly Ser Thr Thr Asn
            100                 105                 110

Asn Val Glu Arg Gln Lys Gln Ala Ala Phe Ser Asp Thr Ile Phe Val
        115                 120                 125

Val Gly Thr Arg Leu Leu Thr Lys Lys Gly Gly Asp Ile Lys Asp Phe
    130                 135                 140

Ala Asn Leu Lys Asp Lys Ala Val Val Val Thr Ser Gly Thr Thr Ser
145                 150                 155                 160

Glu Val Leu Leu Asn Lys Leu Asn Glu Glu Gln Lys Met Asn Met Arg
                165                 170                 175

Ile Ile Ser Ala Lys Asp His Gly Asp Ser Phe Arg Thr Leu Glu Ser
            180                 185                 190

Gly Arg Ala Val Ala Phe Met Met Asp Asp Val Leu Leu Ala Gly Glu
        195                 200                 205

Arg Ala Lys Ala Lys Lys Pro Asp Asn Trp Glu Ile Val Gly Lys Pro
    210                 215                 220

Gln Ser Gln Glu Ala Tyr Gly Cys Met Leu Arg Lys Asp Asp Pro Gln
225                 230                 235                 240

Phe Lys Lys Leu Met Asp Asp Thr Ile Ala Gln Val Gln Thr Ser Gly
                245                 250                 255

Glu Ala Glu Lys Trp Phe Asp Lys Trp Phe Lys Asn Pro Ile Leu Gly
            260                 265                 270

Tyr His Asn Ile Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys
        275                 280                 285

Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu
    290                 295                 300

Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
305                 310                 315                 320

Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Val Leu Ser Lys Asp
                325                 330                 335

Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Arg Thr Ala
            340                 345                 350

Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly
        355                 360                 365

Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
    370                 375                 380

Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg
385                 390                 395                 400

Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe
                405                 410                 415

Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
```

-continued

```
            420                 425                 430

Thr Leu Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
        435                 440                 445

Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
    450                 455                 460

Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala
465                 470                 475                 480

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
                485                 490                 495

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
            500                 505                 510

Tyr Asn Phe Asn Glu Gln Leu Asn Met Asn Phe Glu Leu Ser Asp Glu
        515                 520                 525

Met Lys Ala Leu Phe Lys Glu Pro Asn Asp Lys Ala Leu Lys Leu Gln
    530                 535                 540

Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
545                 550                 555                 560

Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
                565                 570                 575

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
            580                 585                 590

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 183
<211> LENGTH: 728
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 183

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Lys Leu Glu Ile Phe Ser Trp Trp Ala
            20                  25                  30

Gly Asp Glu Gly Pro Ala Leu Glu Ala Leu Ile Arg Leu Tyr Lys Gln
        35                  40                  45

Lys Tyr Pro Gly Val Glu Val Ile Asn Ala Thr Val Thr Gly Gly Ala
    50                  55                  60

Gly Val Asn Ala Arg Ala Val Leu Lys Thr Arg Met Leu Gly Gly Asp
65                  70                  75                  80

Pro Pro Asp Thr Phe Gln Val Ala Ala Gly Met Glu Leu Ile Gly Thr
                85                  90                  95

Trp Val Val Ala Asn Arg Met Glu Asp Leu Ser Ala Leu Phe Arg Gln
            100                 105                 110

Glu Gly Trp Leu Gln Ala Phe Pro Lys Gly Leu Ile Asp Leu Ile Ser
        115                 120                 125

Tyr Lys Gly Gly Ile Trp Ser Val Pro Val Asn Ile His Arg Ser Asn
    130                 135                 140

Val Met Trp Tyr Leu Pro Ala Lys Leu Lys Glu Trp Gly Val Asn Pro
145                 150                 155                 160
```

-continued

```
Pro Arg Thr Trp Asp Glu Phe Leu Ala Thr Cys Gln Thr Leu Lys Gln
                165                 170                 175

Lys Gly Leu Glu Ala Pro Leu Ala Leu Gly Glu Asn Trp Thr Gln Gln
            180                 185                 190

His Leu Trp Glu Ser Val Ala Leu Ala Val Leu Gly Pro Asp Asp Trp
        195                 200                 205

Asn Asn Leu Trp Asn Gly Lys Leu Lys Phe Thr Asp Pro Lys Ala Val
    210                 215                 220

Arg Ala Trp Glu Val Phe Gly Arg Val Leu Asp Cys Ala Asn Lys Asp
225                 230                 235                 240

Ala Ala Gly Leu Ser Trp Gln Gln Ala Val Asp Arg Val Val Gln Gly
                245                 250                 255

Lys Ala Ala Phe Asn Val Met Gly Asp Trp Ala Ala Gly Tyr Met Thr
            260                 265                 270

Thr Thr Leu Lys Leu Lys Pro Gly Thr Asp Phe Ala Trp Ala Pro Ser
        275                 280                 285

Pro Gly Thr Gln Gly Val Phe Met Met Leu Ser Asp Ser Phe Gly Leu
    290                 295                 300

Pro Lys Gly Ala Lys Asn Arg Gln Asn Ala Ile Asn Trp Leu Arg Leu
305                 310                 315                 320

Val Gly Ser Lys Glu Gly Gln Asp Thr Phe Asn Pro Leu Lys Gly Ser
                325                 330                 335

Ile Ala Ala Arg Leu Asp Ser Asp Pro Ser Lys Tyr Pro Ala Ser His
            340                 345                 350

Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn
        355                 360                 365

Phe Lys Ile Arg His Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp
    370                 375                 380

His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
385                 390                 395                 400

Asp Asn His Tyr Leu Ser Thr Gln Ser Val Leu Ser Lys Asp Pro Asn
                405                 410                 415

Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
            420                 425                 430

Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser
        435                 440                 445

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
    450                 455                 460

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Arg Gly Glu
465                 470                 475                 480

Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe Ile Cys
                485                 490                 495

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
            500                 505                 510

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
        515                 520                 525

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
    530                 535                 540

Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala Glu Val
545                 550                 555                 560

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
```

-continued

```
               565                 570                 575

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
            580                 585                 590

Phe Asn Asn Pro Asn Ala Tyr Gly Gln Ser Ala Met Arg Asp Trp Arg
        595                 600                 605

Ser Asn Arg Ile Val Gly Ser Leu Val Ala Gly Ala Val Ala Pro Glu
    610                 615                 620

Ser Phe Met Ser Gln Phe Gly Thr Val Met Glu Ile Phe Leu Gln Thr
625                 630                 635                 640

Arg Asn Pro Gln Ala Ala Ala Asn Ala Ala Gln Ala Ile Ala Asp Gln
                645                 650                 655

Val Gly Leu Gly Arg Leu Gly Gln Leu Gln Val Asp Glu Gln Lys Leu
            660                 665                 670

Ile Ser Glu Glu Asp Leu Asn Ala Val Gly Gln Asp Thr Gln Glu Val
        675                 680                 685

Ile Val Val Pro His Ser Leu Pro Phe Lys Val Val Val Ile Ser Ala
    690                 695                 700

Ile Leu Ala Leu Val Val Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile
705                 710                 715                 720

Met Leu Trp Gln Lys Lys Pro Arg
                725


<210> SEQ ID NO 184
<211> LENGTH: 650
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 184

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Glu Ser Ile Asn Phe Val Ser Trp Gly
            20                  25                  30

Gly Ser Thr Gln Asp Ala Gln Lys Gln Ala Trp Ala Asp Pro Phe Ser
        35                  40                  45

Lys Ala Ser Gly Ile Thr Val Val Gln Asp Gly Pro Thr Asp Tyr Gly
    50                  55                  60

Lys Leu Lys Ala Met Val Glu Ser Gly Asn Val Gln Trp Asp Val Val
65                  70                  75                  80

Asp Val Glu Ala Asp Phe Ala Leu Arg Ala Ala Ala Glu Gly Leu Leu
                85                  90                  95

Glu Pro Leu Asp Phe Ser Val Ile Gln Arg Asp Lys Ile Asp Pro Arg
            100                 105                 110

Phe Val Ser Asp His Gly Val Gly Ser Phe Leu Phe Ser Phe Val Leu
        115                 120                 125

Gly Tyr Asn Glu Gly Lys Leu Gly Ala Ser Lys Pro Gln Asp Trp Thr
    130                 135                 140

Ala Leu Phe Asp Thr Lys Thr Tyr Pro Gly Lys Arg Ala Leu Tyr Lys
145                 150                 155                 160

Trp Pro Ser Pro Gly Val Leu Glu Leu Ala Leu Leu Ala Asp Gly Val
                165                 170                 175
```

-continued

```
Pro Ala Asp Lys Leu Tyr Pro Leu Asp Leu Asp Arg Ala Phe Lys Lys
            180                 185                 190

Leu Asp Thr Ile Lys Lys Asp Ile Val Trp Trp Gly Gly Gly Ala Gln
        195                 200                 205

Ser Gln Gln Leu Leu Ala Ser Gly Glu Val Ser Met Gly Gln Phe Trp
    210                 215                 220

Asn Gly Arg Ile His Ala Leu Gln Glu Asp Gly Ala Pro Val Gly Val
225                 230                 235                 240

Ser Trp Lys Gln Asn Leu Val Met Ala Asp Ile Leu Val Val Pro Lys
                245                 250                 255

Gly Thr Lys Asn Lys Ala Ala Ala Met Lys Phe Leu Ala Ser Ala Ser
            260                 265                 270

Ser Ala Lys Gly Gln Asp Asp Phe Ser Ala Leu Thr Ala Tyr Ala Pro
        275                 280                 285

Val Asn Ile Asp Ser Val Gln Arg Leu Asp Leu Ala Gln Val Arg Ile
    290                 295                 300

Thr Ala Asp Lys Gln Lys Asn Gly Ile Met Ala Asn Phe Lys Ile Arg
305                 310                 315                 320

His Asn Val Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln
                325                 330                 335

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr
            340                 345                 350

Leu Ser Thr Gln Ser Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp
        355                 360                 365

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly
    370                 375                 380

Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Ser Lys Gly
385                 390                 395                 400

Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
                405                 410                 415

Asp Val Asn Gly His Lys Phe Ser Val Arg Gly Glu Gly Glu Gly Asp
            420                 425                 430

Ala Thr Asn Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
        435                 440                 445

Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val
    450                 455                 460

Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
465                 470                 475                 480

Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Ser Phe
                485                 490                 495

Lys Asp Asp Gly Thr Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
            500                 505                 510

Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
        515                 520                 525

Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Trp Asn Ala Asn
    530                 535                 540

Leu Ala Pro Asn Leu Pro Thr Ala Tyr Val Lys Asp Gln Ile Thr Leu
545                 550                 555                 560

Asp Phe Ala Tyr Trp Ala Lys Asn Gly Pro Ala Ile Ala Thr Arg Trp
                565                 570                 575

Asn Glu Trp Leu Val Lys Leu Gln Val Asp Leu Gln Val Asp Glu Gln
```

-continued

```
            580                 585                 590

Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly Gln Asp Thr Gln
        595                 600                 605

Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys Val Val Val Ile
    610                 615                 620

Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile Ser Leu Ile Ile
625                 630                 635                 640

Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
                645                 650


<210> SEQ ID NO 185
<211> LENGTH: 560
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 185

Met His His His His His His Gly Tyr Pro Tyr Asp Val Pro Asp Tyr
1               5                   10                  15

Ala Gly Ala Gln Pro Ala Arg Ser Ala Asn Asp Thr Val Val Val Gly
            20                  25                  30

Ser Ile Ile Phe Thr Glu Gly Ile Ile Val Ala Asn Met Val Ala Glu
        35                  40                  45

Met Ile Glu Ala His Thr Asp Leu Lys Val Val Arg Lys Leu Asn Leu
    50                  55                  60

Gly Gly Val Asn Val Asn Phe Glu Ala Ile Lys Arg Gly Gly Ala Asn
65                  70                  75                  80

Asn Gly Ile Asp Ile Tyr Val Glu Tyr Thr Gly His Gly Leu Val Asp
                85                  90                  95

Ile Leu Gly Phe Pro Glu Pro Asn Val Tyr Ile Thr Ala Asp Lys Gln
            100                 105                 110

Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp
        115                 120                 125

Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
    130                 135                 140

Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
145                 150                 155                 160

Val Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
                165                 170                 175

Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr
            180                 185                 190

Lys Gly Gly Thr Gly Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr
        195                 200                 205

Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His
    210                 215                 220

Lys Phe Ser Val Arg Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys
225                 230                 235                 240

Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp
                245                 250                 255

Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg
            260                 265                 270
```

-continued

```
Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro
        275                 280                 285

Glu Gly Tyr Val Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr
    290                 295                 300

Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn
305                 310                 315                 320

Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu
                325                 330                 335

Gly His Lys Leu Glu Tyr Asn Phe Pro Pro Pro Ala Thr Thr Asp Pro
            340                 345                 350

Glu Gly Ala Tyr Glu Thr Val Lys Lys Glu Tyr Lys Arg Lys Trp Asn
        355                 360                 365

Ile Val Trp Leu Lys Pro Leu Gly Phe Asn Asn Thr Tyr Thr Leu Thr
    370                 375                 380

Val Lys Asp Glu Leu Ala Lys Gln Tyr Asn Leu Lys Thr Phe Ser Asp
385                 390                 395                 400

Leu Ala Lys Ile Ser Asp Lys Leu Ile Leu Gly Ala Thr Met Phe Phe
                405                 410                 415

Leu Glu Gly Pro Asp Gly Tyr Pro Gly Leu Gln Lys Leu Tyr Asn Phe
            420                 425                 430

Lys Phe Lys His Thr Lys Ser Met Asp Met Gly Ile Arg Tyr Thr Ala
        435                 440                 445

Ile Asp Asn Asn Glu Val Gln Val Ile Asp Ala Trp Ala Thr Asp Gly
    450                 455                 460

Leu Leu Val Ser His Lys Leu Lys Ile Leu Glu Asp Asp Lys Ala Phe
465                 470                 475                 480

Phe Pro Pro Tyr Tyr Ala Ala Pro Ile Ile Arg Gln Asp Val Leu Asp
                485                 490                 495

Lys His Pro Glu Leu Lys Asp Val Leu Asn Lys Leu Ala Asn Gln Ile
            500                 505                 510

Ser Leu Glu Glu Met Gln Lys Leu Asn Tyr Lys Val Asp Gly Glu Gly
        515                 520                 525

Gln Asp Pro Ala Lys Val Ala Lys Glu Phe Leu Lys Glu Lys Gly Leu
    530                 535                 540

Ile Leu Gln Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
545                 550                 555                 560


<210> SEQ ID NO 186
<211> LENGTH: 608
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      polypeptide

<400> SEQUENCE: 186

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                   10                  15

Gly Ser Thr Gly Asp Arg Ser Ala Asn Asp Thr Val Val Val Gly Ser
            20                  25                  30

Ile Ile Phe Thr Glu Gly Ile Ile Val Ala Asn Met Val Ala Glu Met
        35                  40                  45

Ile Glu Ala His Thr Asp Leu Lys Val Val Arg Lys Leu Asn Leu Gly
    50                  55                  60
```

-continued

```
Gly Val Asn Val Asn Phe Glu Ala Ile Lys Arg Gly Gly Ala Asn Asn
65                  70                  75                  80

Gly Ile Asp Ile Tyr Val Glu Tyr Thr Gly His Gly Leu Val Asp Ile
                85                  90                  95

Leu Gly Phe Pro Glu Pro Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys
            100                 105                 110

Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Val Glu Asp Gly
        115                 120                 125

Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
    130                 135                 140

Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Val
145                 150                 155                 160

Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu
                165                 170                 175

Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
            180                 185                 190

Gly Gly Thr Gly Gly Ser Met Ser Lys Gly Glu Glu Leu Phe Thr Gly
        195                 200                 205

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
    210                 215                 220

Phe Ser Val Arg Gly Glu Gly Glu Gly Asp Ala Thr Asn Gly Lys Leu
225                 230                 235                 240

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro
                245                 250                 255

Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr
            260                 265                 270

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
        275                 280                 285

Gly Tyr Val Gln Glu Arg Thr Ile Ser Phe Lys Asp Asp Gly Thr Tyr
    290                 295                 300

Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
305                 310                 315                 320

Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
                325                 330                 335

His Lys Leu Glu Tyr Asn Phe Pro Pro Pro Ala Thr Thr Asp Pro Glu
            340                 345                 350

Gly Ala Tyr Glu Thr Val Lys Lys Glu Tyr Lys Arg Lys Trp Asn Ile
        355                 360                 365

Val Trp Leu Lys Pro Leu Gly Phe Asn Asn Thr Tyr Thr Leu Thr Val
    370                 375                 380

Lys Asp Glu Leu Ala Lys Gln Tyr Asn Leu Lys Thr Phe Ser Asp Leu
385                 390                 395                 400

Ala Lys Ile Ser Asp Lys Leu Ile Leu Gly Ala Thr Met Phe Phe Leu
                405                 410                 415

Glu Gly Pro Asp Gly Tyr Pro Gly Leu Gln Lys Leu Tyr Asn Phe Lys
            420                 425                 430

Phe Lys His Thr Lys Ser Met Asp Met Gly Ile Arg Tyr Thr Ala Ile
        435                 440                 445

Asp Asn Asn Glu Val Gln Val Ile Asp Ala Trp Ala Thr Asp Gly Leu
    450                 455                 460
```

-continued

```
Leu Val Ser His Lys Leu Lys Ile Leu Glu Asp Asp Lys Ala Phe Phe
465                 470                 475                 480

Pro Pro Tyr Tyr Ala Ala Pro Ile Ile Arg Gln Asp Val Leu Asp Lys
                485                 490                 495

His Pro Glu Leu Lys Asp Val Leu Asn Lys Leu Ala Asn Gln Ile Ser
            500                 505                 510

Leu Glu Glu Met Gln Lys Leu Asn Tyr Lys Val Asp Gly Glu Gly Gln
        515                 520                 525

Asp Pro Ala Lys Val Ala Lys Glu Phe Leu Lys Glu Lys Gly Leu Ile
    530                 535                 540

Leu Gln Val Asp Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala
545                 550                 555                 560

Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro
                565                 570                 575

Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr
            580                 585                 590

Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
        595                 600                 605


<210> SEQ ID NO 187
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 187

Pro Ile Leu Val Ser His Asn Val
1               5


<210> SEQ ID NO 188
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 188

Pro Ile Leu Gly Tyr His Asn Val
1               5


<210> SEQ ID NO 189
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide

<400> SEQUENCE: 189

Tyr Asn Phe Asn Asn Pro Leu Asn
1               5


<210> SEQ ID NO 190
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
```

-continued

peptide

<400> SEQUENCE: 190

Tyr Asn Phe Asn Glu Gln Leu Asn
1 5

<210> SEQ ID NO 191
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 191

Ser His Asn Val Tyr
1 5

<210> SEQ ID NO 192
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 192

Leu Ala Gln Val Arg
1 5

<210> SEQ ID NO 193
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 193

Ser Phe Gly Phe Pro
1 5

<210> SEQ ID NO 194
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 194

Ser Val Leu Ala Pro
1 5

<210> SEQ ID NO 195
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic peptide

<400> SEQUENCE: 195

Ala Asn Leu Ala Pro
1 5

-continued

<210> SEQ ID NO 196
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligopeptide
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Any amino acid

<400> SEQUENCE: 196

Pro Xaa Ser His Asn Val Tyr
1 5

<210> SEQ ID NO 197
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic 6xHis tag

<400> SEQUENCE: 197

His His His His His His
1 5

What is claimed is:

1. A recombinant peptide biosensor comprising an analyte-binding framework portion and a signaling portion, wherein the signaling portion is present within the framework portion at a site or amino acid position that undergoes a conformational change upon interaction of the framework portion with a defined, specific, or selected analyte, wherein the recombinant peptide biosensor comprises a amino acid sequence having at least 85% sequence identity to SEQ ID NO: 184.

2. The recombinant peptide biosensor of claim **1**, wherein the signaling portion is allosterically regulated by the framework portion such that signaling from the signaling portion is altered upon interaction of the framework portion with the analyte.

3. The recombinant peptide biosensor of claim **1**, wherein signaling by the signaling portion detectably increases upon interaction of the framework portion with the analyte.

4. The recombinant peptide biosensor of claim **1**, wherein signaling by the signaling portion detectably decreases upon interaction of the framework portion with the analyte.

5. The recombinant peptide biosensor of claim **1**, wherein signaling by the signaling portion is proportional to the level of interaction between the framework portion and the analyte.

6. The recombinant peptide biosensor of claim **1**, wherein the framework portion has a first structure in the absence of an analyte and a second structure, that is detectably distinct from the first structure, in the presence of the analyte.

7. The recombinant peptide biosensor of claim **6**, wherein the conformational change between the first structure and the second structure allosterically regulates the signaling portion.

8. The recombinant peptide biosensor of claim **1**, wherein the framework portion is a periplasmic binding protein (PBP) or a variant of a PBP.

9. The recombinant peptide biosensor of claim **1**, wherein the signaling portion is a circularly permuted super fluorescent (SF) protein.

10. The recombinant peptide biosensor of claim **9**, wherein the SF protein is selected from the group consisting of a green fluorescent protein, a yellow fluorescent protein, a red fluorescent protein, and a blue fluorescent protein.

11. The recombinant peptide biosensor of claim **1**, wherein the analyte-binding framework portion binds specifically to gamma aminobutyric acid (GABA).

12. A recombinant peptide biosensor comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:184, wherein the recombinant peptide biosensor binds specifically to GABA.

13. The recombinant peptide biosensor of claim **12** having SEQ ID NO:184 and comprising 10 or fewer conservative amino acid substitutions, wherein the recombinant peptide biosensor binds specifically to GABA.

14. The recombinant peptide biosensor of claim **12**, comprising a recombinant peptide biosensor having SEQ ID NO:184.

15. A nucleic acid encoding the recombinant peptide biosensor of claim **1**.

16. A vector comprising the nucleic acid of claim **15**.

17. A cell comprising the nucleic acid of claim **15**.

18. A cell comprising the vector of claim **16**.

19. A kit comprising the recombinant peptide biosensor of claim **1**, the nucleic acid of claim **15**, the vector of claim **16**, the cell of claim **17**, and/or the cell of claim **18**.

20. A method for detecting GABA, the method comprising detecting a level of fluorescence emitted by a recombi-

nant peptide biosensor, the peptide biosensor having the amino acid sequence shown in SEQ ID NO:184, and correlating the level of fluorescence with the presence of GABA.

21. The method of claim **20**, wherein the recombinant peptide biosensor is expressed from a nucleic acid.

22. The method of claim **20**, comprising contacting the recombinant peptide biosensor with a sample comprising GABA.

23. The method of claim **22**, comprising correlating the level of fluorescence with a concentration of GABA.

24. The method of claim **23**, comprising comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of GABA.

25. The method of claim **24**, wherein the method is performed in vitro.

26. A method for detecting a defined, selected, or specific analyte, the method comprising detecting a level of fluorescence emitted by a recombinant peptide biosensor of claim **1** in the presence of said analyte; and correlating the level of fluorescence with the presence of a defined, selected, or specific analyte.

27. The method of claim **26**, wherein the recombinant peptide biosensor is expressed from a nucleic acid.

28. The method of claim **26**, comprising contacting the recombinant peptide biosensor with a sample comprising the analyte.

29. The method of claim **28**, comprising correlating the level of fluorescence with a concentration of the analyte.

30. The method of claim **29**, comprising comparing the level of fluorescence with a level of fluorescence emitted by the recombinant peptide biosensor in the presence of a sample comprising a known concentration or range of concentrations of the analyte.

31. The method of claim **30**, wherein the method is performed in vitro.

32. The method of claim **30**, wherein the analyte is GABA.

* * * * *