



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : G01N 33/50, C12Q 1/48, 1/25</p>	A3	<p>(11) International Publication Number: WO 98/45704</p> <p>(43) International Publication Date: 15 October 1998 (15.10.98)</p>
<p>(21) International Application Number: PCT/DK98/00145</p> <p>(22) International Filing Date: 7 April 1998 (07.04.98)</p> <p>(30) Priority Data: 0392/97 7 April 1997 (07.04.97) DK</p> <p>(71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): THASTRUP, Ole [DK/DK]; Birkevej 37, DK-3460 Birkerød (DK). PETERSEN BJØRN, Sara [DK/DK]; Klampenborgvej 102, DK-2800 Lyngby (DK). TULLIN, Søren [DK/DK]; Karl Gjellerups Alle 18, DK-2860 Søborg (DK). KASPER, Almholt [DK/DK]; Eigilsgade 32, 4. tv, DK-2300 København S (DK). SCUDDER, Kurt [US/DK]; Lavendelhaven 70, DK-2830 Virum (DK).</p> <p>(74) Common Representative: NOVO NORDISK A/S; attn. Lars Kellberg, Novo Allé, DK-2880 Bagsvaerd (DK).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p>(88) Date of publication of the international search report: 22 April 1999 (22.04.99)</p>	
<p>(54) Title: A METHOD FOR EXTRACTING QUANTITATIVE INFORMATION RELATING TO AN INFLUENCE ON A CELLULAR RESPONSE</p>		
<p>(57) Abstract</p> <p>Cells are genetically modified to express a luminophore, e.g., a modified (F64L, S65T, Y66H) Green Fluorescent Protein (GFP, EGFP) coupled to a component of an intracellular signalling pathway such as a transcription factor, a cGMP- or cAMP-dependent protein kinase, a cyclin-, calmodulin- or phospholipid-dependent or mitogen-activated serine/threonine protein kinase, a tyrosine protein kinase, or a protein phosphatase (e.g. PKA, PKC, Erk, Smad, VASP, actin, p38, Jnk1, PKG, IkappaB, CDK2, Grk5, Zap70, p85, protein-tyrosine phosphatase 1C, Stat5, NFAT, NFkappaB, RhoA, PKB). An influence modulates the intracellular signalling pathway in such a way that the luminophore is being redistributed or translocated with the component in living cells in a manner experimentally determined to be correlated to the degree of the influence. Measurement of redistribution is performed by recording of light intensity, fluorescence lifetime, polarization, wavelength shift, resonance energy transfer, or other properties by an apparatus consisting of e.g. a fluorescence microscope and a CCD camera. Data stored as digital images are processed to numbers representing the degree of redistribution. The method can be used as a screening program for identifying a compound that modulates a component and is capable of treating a disease related to the function of the component.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 98/00145

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 C12Q1/48 C12Q1/25

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 11094 A (NOVONORDISK AS ;THASTRUP OLE (DK); TULLIN SOEREN (DK); POULSEN LAR) 27 March 1997	1-27, 30-40, 44-60, 64-82,88
Y	see the whole document see claims	28,29, 41,61-63
X	WO 91 01305 A (UNIV WALES MEDICINE) 7 February 1991	1-27, 30-40, 42-60, 64-84, 87,88
Y	see page 4, line 15 - line 20 see claims see examples 1-10	28,29, 41,61-63

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"Z" document member of the same patent family

Date of the actual completion of the international search

19 January 1999

Date of mailing of the international search report

25. 02. 1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hoekstra, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 98/00145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07463 A (UNIV COLUMBIA ;WOODS HOLE OCEANOGRAPHIC INST (US); CHALFIE MARTIN) 16 March 1995 cited in the application	1-27, 30-40, 42-60, 64-84, 87,88
	see claim 26	
Y	see the whole document	28,29, 41,61-63

Y	WO 96 23898 A (NOVONORDISK AS ;THASTRUP OLE (DK); TULLIN SOEREN (DK); POULSEN LAR) 8 August 1996 see the whole document	28,29, 41,61-63
X	see page 8-17	42,43, 46,47

X	WO 96 03649 A (UNIV NORTH CAROLINA) 8 February 1996 see page 49; example 6.10	45

P,X	WO 97 20931 A (US HEALTH ;HTUN HAN (US); HAGER GORDON L (US)) 12 June 1997 see claims 41-58	40,44

P,X	WO 97 30074 A (CYTOGEN CORP ;UNIV NORTH CAROLINA (US)) 21 August 1997 see page 57	44

P,X	WO 98 02571 A (TSIEN ROGER Y ;CUBITT ANDREW B (US); UNIV CALIFORNIA (US)) 22 January 1998	1-27, 30-40, 42-50, 52-54, 57-60, 64-82,88
	see claims	

E	WO 98 30715 A (ISACOFF EHUD Y ;SIEGAL MICAH S (US); UNIV CALIFORNIA (US); CALIFOR) 16 July 1998 see the whole document	1-84,87, 88

	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 98/00145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>SAKAI, N. ET AL.: "Direct visualization of the translocation of the gamma subspecies of protein kinase c in living cells using fusion proteins with green fluorescent protein." THE JOURNAL OF CELL BIOLOGY, vol. 139, no. 6, 15 December 1997, pages 1465-1476, XP002078902 see the whole document</p>	1-43,46, 47,49, 53-57, 59-82,88
O,X	<p>& Direct visualization of the translocation of the gamma subspecies of protein kinase c in living cells using fusion proteins with green fluorescent protein.Meeting held at 22-23.03.97 cited in the application see abstract</p>	
X	<p>--- SCHMIDT, D.J. ET AL.: "Dynamic analysis of alpha-PKC-GFP chimera translocation events in smooth muscle with ultra-high speed 3D fluorescence microscopy" FASEB JOURNAL, vol. 11, no. 3, 28 February 1997, page A505 XP002077257 cited in the application see abstract</p>	1-43,46, 47,49, 53-57, 59-82,88
X	<p>--- GERISCH, GUENTHER ET AL: "Chemoattractant-controlled accumulation of coronin at the leading edge of Dictyostelium cells monitored using a green fluorescent protein-coronin fusion protein" CURR. BIOL. (1995), 5(11), 1280-5 CODEN: CUBLE2;ISSN: 0960-9822, XP002089510 see abstract p 1281, right col, second full , last sentence</p>	1,40,43, 45
X	<p>--- SIDOROVA, JULIA M. ET AL: "Cell cycle-regulated phosphorylation of Swi6 controls its nuclear localization" MOL. BIOL. CELL (1995), 6(12), 1641-58 CODEN: MBCEEV;ISSN: 1059-1524, XP002089512 see the whole document</p>	40,43,44
X	<p>--- HAN HTUN ET AL: "VISUALIZATION OF GLUCOCORTICOID RECEPTOR TRANSLOCATION AND INTRANUCLEAR ORGANIZATION IN LIVING CELLS WITH A GREEN FLUORESCENT PROTEIN CHIMERA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 10, May 1996, pages 4845-4850, XP002029560 see the whole document</p>	1-40,44, 64-72

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 98/00145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAREY K L ET AL: "EVIDENCE USING A GREEN FLUORESCENT PROTEIN-GLUCOCORTICOID RECEPTOR CHIMERA THAT THE RAN/TC4 GTPASE MEDIATES AN ESSENTIAL FUNCTION INDEPENDENT OF NUCLEAR PROTEIN IMPORT" THE JOURNAL OF CELL BIOLOGY, vol. 133, no. 5, June 1996, pages 985-996, XP000670316 cited in the application see the whole document ---	1-40, 44, 64-72
X	OGAWA H ET AL: "LOCALIZATION, TRAFFICKING, AND TEMPERATURE-DEPENDENCE OF THE AEQUOREA GREEN FLUORESCENT PROTEIN IN CULTURES VERTEBRATE CELLS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, no. 25, 5 December 1995, pages 11899-11903, XP002029556 see the whole document ---	1-40, 44, 64-72
X	WESTPHAL, MONIKA ET AL: "Microfilament dynamics during cell movement and chemotaxis monitored using a GFP - actin fusion protein" CURR. BIOL. (1997), 7(3), 176-183 CODEN: CUBLE2;ISSN: 0960-9822, XP002090291 see page 181, left-hand column, line 1 ---	1, 40, 43, 45
X	TODA, TAKASHI ET AL: "The fission yeast sts5+ gene is required for maintenance of growth polarity and functionally interacts with protein kinase C and an osmosensing MAP kinase pathway" J. CELL SCI. (1996), 109(9), 2331-2342 CODEN: JNCSAI;ISSN: 0021-9533, XP002090292 see abstract ---	40, 42
A	WEBB, CHRIS D. ET AL: "Use of green fluorescent protein for visualization of cell-specific gene expression and subcellular protein localization during sporulation in Bacillus subtilis" J. BACTERIOL. (1995), 177(20), 5906-11 CODEN: JOBAAY;ISSN: 0021-9193, XP002089513 see the whole document ---	44
A	WO 94 23039 A (CANCER RES INST ROYAL ;MARSHALL CHRISTOPHER JOHN (GB); ASHWORTH AL) 13 October 1994 see the whole document -----	1-84, 87, 88

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 98/00145

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 83-84 and claim 87 relate to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition (Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy).
2. Claims Nos.: 85,86
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 85,86

The subject-matter (compounds per se) is solely characterised in claims 85 and 86 by the result to be achieved, no support of a technical character is derivable from the description for the technical formulation of the subject of the search, accordingly no scope of a search could be defined and a meaningful search is hence not possible.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Partially: 1-43, 46, 59-82 and 88; Entirely: 47, 49, 53-57

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being serine/threonine protein kinases

2. Claims: Partially: 1-41, 43, 59-82 and 88; Entirely: 48

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being tyrosine kinases

3. Claims: Partially: 1-43, 46, 59-82 and 88; Entirely: 50, 51

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being cAMP dependent protein kinases.

4. Claims: Partially: 1-43, 46, 59-82 and 88; Entirely: 52

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being cGMP dependent protein kinases

5. Claims: Partially: 1-43, 59-82 and 88; Entirely: 58

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being protein phosphatases

6. Claims: Partially: 1-41, 43, 59-82 and 88; Entirely: 44

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being transcription factors

7. Claims: Partially: 1-41, 43, 59-82 and 88; Entirely: 45

Methods for extracting information from influences on a living cell involving observing spatial redistribution or modulation of a luminophore linked to a biologically active molecule, in particular to a molecule involved in intracellular signalling pathways, nucleic acids encoding fusion proteins comprising both the luminophore and the biological active molecule, cells containing and expressing these nucleic acids, as well as methods and apparatuses involving above products, in so far as related to the biologically active protein being to proteins associated with the cytoskeletal network

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 98/00145

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9711094	A	27-03-1997	AU 4482996 A	09-04-1997
			CA 2232727 A	27-03-1997
			EP 0851874 A	08-07-1998

WO 9101305	A	07-02-1991	AU 6054590 A	22-02-1991
			CA 2064766 A	23-01-1991
			EP 0484369 A	13-05-1992
			JP 5501862 T	08-04-1993
			US 5683888 A	04-11-1997

WO 9507463	A	16-03-1995	US 5491084 A	13-02-1996
			AU 694745 B	30-07-1998
			AU 7795794 A	27-03-1995
			CA 2169298 A	16-03-1995
			EP 0759170 A	26-02-1997
			JP 9505981 T	17-06-1997

WO 9623898	A	08-08-1996	AU 4483096 A	21-08-1996
			CA 2217700 A	08-08-1996
			EP 0815257 A	07-01-1998

WO 9603649	A	08-02-1996	AU 3146095 A	22-02-1996
			CA 2195629 A	08-02-1996
			EP 0772773 A	14-05-1997
			JP 10503369 T	31-03-1998

WO 9720931	A	12-06-1997	AU 1283497 A	27-06-1997
			CA 2239951 A	12-06-1997

WO 9730074	A	21-08-1997	AU 2272397 A	02-09-1997

WO 9802571	A	22-01-1998	AU 3801997 A	09-02-1998

WO 9830715	A	16-07-1998	AU 5090498 A	03-08-1998

WO 9423039	A	13-10-1994	AU 677834 B	08-05-1997
			AU 6382394 A	24-10-1994
			EP 0703984 A	03-04-1996
			JP 9501302 T	10-02-1997
			AU 696939 B	24-09-1998
			AU 1586195 A	29-08-1995
			CA 2182967 A	17-08-1995
			EP 0742827 A	20-11-1996
			WO 9521923 A	17-08-1995
			JP 9508795 T	09-09-1997
