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(54) Title: MULTI-FUNCTIONAL MOLECULES COMPRISING GLYCANS AND USES THEREOF

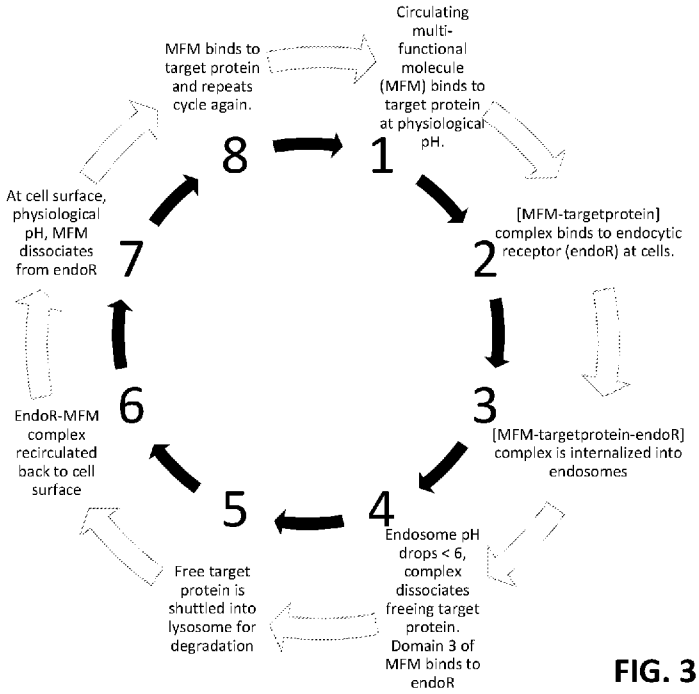

FIG. 3

(57) Abstract: Provided herein are multi-functional molecules comprising: (1) a first moiety that binds to an endocytic receptor; and (2) a second moiety that binds to a target; and optionally, (3) a third moiety that binds to an endocytic receptor. In some embodiments, a first moiety binds to a target under a first set of conditions and/or a second set of conditions. In some embodiments, a second moiety binds to a target under a first set of conditions. In some embodiments, a third moiety binds to an endocytic receptor under a second set of conditions. Further provided herein are compositions comprising multi-functional molecules disclosed herein and/or nucleic acids encoding the same, as well as methods of making and using the same.

[Continued on next page]

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MULTI-FUNCTIONAL MOLECULES COMPRISING GLYCANS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 63/497,567 filed on April 21, 2023, and U.S. Provisional Patent Application No. 63/505,844 filed on June 2, 2023, the entire contents of each of which are hereby incorporated by reference in their entirety.

BACKGROUND

[0002] Increasing the bioavailability and/or efficacy of therapeutic molecules has been a long-standing challenge.

SUMMARY

[0003] The present disclosure identifies certain challenges with the limited bioavailability and/or efficacy of many existing therapeutic molecules. For example, the present disclosure identifies limitations of therapeutic molecules such as antibodies which bind to a target of interest outside a cell (e.g., in circulation) and are degraded along with the bound target via internalization into cells (e.g., via endocytosis and lysosomal degradation). Internalization (and degradation) of a therapeutic molecule (e.g., an antibody) bound to a target is beneficial for degrading the target, but this also prevents said therapeutic molecule from being able to bind to additional instances of the target thus reducing its overall efficacy. Such therapeutic molecules (e.g., antibodies) need to be dosed more frequently and/or at higher doses to obtain a desired therapeutic effect. Thus, there is a need to develop therapeutic molecules that can bind to and cause degradation of a target, while not getting degraded in the process.

[0004] Without wishing to be bound by any particular theory, technologies provided in the present disclosure can address certain limitations identified in existing therapeutic molecules. For example, technologies provided herein can avoid the normal degradation of certain therapeutic molecules by being recycled out of the cell after delivering the target to appropriate cellular machinery (e.g., a lysosome). This in turn can result in degradation of a target but not

degradation of the therapeutic molecule itself thus increasing the overall efficacy of the therapeutic molecule. In some embodiments, this can allow for reduced frequency of dosing and/or dosing at lower doses compared to existing therapeutic molecules.

[0005] For example, technologies provided herein can achieve selective target degradation, but not degradation of a multi-functional molecule which binds to the target, by utilizing, e.g., pH dependent binding of one or more moieties on a multi-functional molecule. In some embodiments, a multi-functional molecule disclosed herein comprises a first moiety that binds to an endocytic receptor under a first set of conditions and/or a second set of conditions (e.g., in the presence of a cation such as Ca2+ and/or within a specified pH range) and a second moiety that binds to a target at a first pH (e.g., a pH of 6.7-8) but not at a second pH (e.g., a pH of 6.5-5.6) or a third pH (e.g., pH of less than 5.5). Such a multi-functional molecule allows for internalization of the target bound to the second moiety into a cell expressing an endocytic receptor bound by the first moiety. Without wishing to be bound by theory, in some embodiments, upon internalization into an endosome having a second, lower pH, the target dissociates from the multi-functional molecule and the target is trafficked into a late endosome and/or lysosome for degradation. In some embodiments, a multi-functional molecule, which is now free from its target, can be transported back to the cell surface (e.g., exocytosed). In some embodiments, exocytosis occurs via binding of a moiety (e.g., a first moiety) of the multi-functional molecule to one or more receptors in an endosome, e.g., one or more receptors involved in exocytosis. Accordingly, said multi-functional molecule is recycled and is able to participate again in one or more binding events, e.g., to promote the degradation of one or more targets.

[0006] As another example, a multi-functional molecule disclosed herein comprises: (1) a first moiety that binds to an endocytic receptor under a first set of conditions (e.g., in the presence of a cation such as Ca2+ and/or within a specified pH); (2) a second moiety that binds to a target at a first pH (e.g., a pH of 6.7-8) but not at a second pH (e.g., a pH of 6.5-5.6) or a third pH (e.g., pH of less than 5.5); and (3) a third moiety that binds to an endocytic receptor under a second set of conditions (e.g., at a second pH but not at a first pH or a third pH). For clarity, a second pH at which a third moiety binds an endocytic receptor is the same second pH at which a second moiety does not bind to a target. Such a multi-functional molecule allows for internalization of the target bound to the second moiety into a cell expressing an endocytic receptor bound by the first moiety. Without wishing to be bound by any particular theory, in

some embodiments, upon internalization into an endosome having a second pH, the target dissociates from the multi-functional molecule and the target is trafficked into a late endosome and/or lysosome for degradation. In some embodiments, a multi-functional molecule which is now free from its target and is in an endosome having a second pH, is able to bind to an endocytic receptor via the third moiety and can be transported back to the extracellular surface of the cell. In some embodiments, the cell surface has the first pH and this promotes dissociation of the third moiety from the endocytic receptor. Accordingly, said multi-functional molecule is recycled and is able to participate in one or more binding events, e.g., to promote the degradation of one or more targets.

[0007] In some embodiments, multi-functional molecules that bind to a target, resulting in degradation of a target while not being degraded themselves, are also referred to as catalytic degraders.

[0008] Accordingly, the present disclosure provides a multi-functional molecule, comprising: (a) a first moiety that specifically binds to an endocytic receptor (e.g., a first endocytic receptor); (b) a second moiety that specifically binds to a target at a first pH; and optionally (c) a third moiety that specifically binds to an endocytic receptor (e.g., a second endocytic receptor) at a second pH.

[0009] In some embodiments, a first endocytic receptor and a second endocytic receptor are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

[0010] In some embodiments, a first endocytic receptor and a second endocytic receptor are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

[0011] Also provided herein is a multi-functional molecule, comprising: (a) a first moiety that specifically binds to an endocytic receptor; and (b) a second moiety that specifically binds to a target at a first pH.

[0012] In some embodiments, a first moiety binds to an endocytic receptor under a first set of conditions and/or a second set of conditions. In some embodiments, a first moiety binds to an endocytic receptor under a first set of conditions. In some embodiments, a first moiety binds to an endocytic receptor under a second set of conditions. In some embodiments, a

first moiety binds to an endocytic receptor under a first set of conditions and a second set of conditions.

[0013] In some embodiments, a first moiety does not bind to or has lower affinity binding to an endocytic receptor under a third set of conditions.

[0014] In some embodiments, an endocytic receptor bound by a first moiety under a first set of conditions and an endocytic receptor bound by a first moiety under a second set of conditions are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

[0015] In some embodiments, an endocytic receptor bound by a first moiety under a first set of conditions and an endocytic receptor bound by a first moiety under a second set of conditions are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

[0016] The disclosure further provides multi-functional molecule, comprising: (a) a first moiety that specifically binds to an endocytic receptor under a first set of conditions; (b) a second moiety that specifically binds to a target at a first pH; and (c) a third moiety that specifically binds to an endocytic receptor under a second set of conditions.

[0017] In some embodiments of any of the multi-functional molecules disclosed herein, a first set of conditions comprises: (i) the presence of a cation (e.g., Ca2+); (ii) a first pH; or (iii) both (1) and (2).

[0018] In some embodiments of any of the multi-functional molecules disclosed herein, a second set of conditions comprises: (i) a second pH (e.g., a pH of about pH 6.5 to about pH 5.6); (ii) presence in an intracellular vesicle which:(a) expresses, or has detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof, (b) has no detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof; (c) has the ability to export a molecule to an extracellular space; (d) expresses one or more receptors that can bind to a molecule and export the molecule to an extracellular space; (e) does not have the ability to degrade a molecule; (f) has no or minimal proteolytic activity (e.g., hydroylase activity); or (g) any combination or all of (a)-(f); (iii) an early endosome or a recycling endosome; or (iv) any combination or all of (i)-(iii).

[0019] In some embodiments, a second set of conditions comprises a second pH.

[0020] In some embodiments, a polypeptide associated with an early endosome and/or a recycling endosome comprises a GTPase, a sorting nexin, or a combination thereof. In some embodiments, a GTPase comprises Arf6, Rab4, Rab5, Rab8, Rab10, Rab11, Rab22a, Rab35 or variants or functional fragments thereof.

[0021] In some embodiments, an early endosome and/or a recycling endosome is a vesicle in which Rab conversion (e.g., a reduction in Rab5 and/or an increase in Rab7) has not occurred, e.g., has not been initiated or has not been completed. In some embodiments, an early endosome and/or a recycling endosome does not express or does not have detectable presence of a polypeptide associated with Rab conversion, e.g.,SAND-1/Mon1.

[0022] In some embodiments, an early endosome and/or a recycling endosome does not express or has no detectable presence of Rab7.

[0023] In some embodiments, an endocytic receptor bound by a first moiety and an endocytic receptor bound by a third moiety are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

[0024] In some embodiments, an endocytic receptor bound by a first moiety and an endocytic receptor bound by a third moiety are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

[0025] In some embodiments of any of the multi-functional molecules disclosed herein, a third set of conditions comprises (i) a third pH (e.g., less than pH 5.5); (ii) presence in an intracellular vesicle which: (a) has no detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof, (b) expresses or has detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof; (c) has the ability to degrade a molecule; (d) has proteolytic activity (e.g., hydroylase activity); (e) any combination or all of (a)-(d); or (iii) a late endosome or a lysosome; or (iv) any combination or all of (i)-(iii).

[0026] In some embodiments, a polypeptide associated with a late endosome and/or a lysosome comprises Rab7, one or more subunits of a homotypic fusion and vacuole protein sorting (HOPS) complex, SAND-1/Mon1, Ccz1, one or more subunits of an ESCRT complex, or variants or fragments thereof. In some embodiments, a subunit of a HOPS complex comprises Vps39 or a variant or a fragment thereof.

[0027] In some embodiments, a late endosome and/or a lysosome is a vesicle in which Rab conversion (e.g., a reduction in Rab5 and/or an increase in Rab7) has occurred, e.g., has been initiated or has been completed. In some embodiments, a late endosome and/or a lysosome expresses or has detectable presence of a polypeptide associated with Rab conversion, e.g., SAND-1/Mon1.

[0028] In some embodiments, a late endosome and/or a lysosome does not express or has no detectable presence of Rab5.

[0029] Also provided herein are polynucleotides encoding a multi-functional molecule disclosed herein, host cells expressing the same, and methods of making the same.

[0030] Further provided herein are compositions comprising a multi-functional molecule disclosed herein. In some embodiments, a composition is a pharmaceutical composition. In some embodiments, a pharmaceutical composition comprises one or more excipients.

[0031] This disclosure also provides methods of using a multi-functional molecule disclosed herein or compositions comprising the same. Provided herein, inter alia, is a method of administering a multi-functional molecule disclosed herein or a composition comprising the same.

[0032] In some embodiments, a subject administered a multi-functional molecule disclosed herein or a composition comprising the same has a disease or disorder, or one or more symptoms of a disease or disorder disclosed herein. In some embodiments, a disease or disorder is chosen from: an autoimmune disease, a metabolic disease, a genetic disease, a fibrotic disease, a rare disease, a neurodegenerative disease, a vascular disease, a cancer, or a disease requiring enzyme replacement therapy. In some embodiments, a cancer is a solid tumor or a hematological cancer.

[0033] Also provided herein is a method of delivering a target to a cell, comprising contacting a cell with a complex comprising a multi-functional molecule disclosed herein and a target, under conditions sufficient to deliver a complex comprising a multi-functional molecule to a cell. In some embodiments, contacting comprises internalization of a complex into a compartment of a cell. In some embodiments, a compartment is an endosome. In some embodiments, a target dissociates from a complex in an endosome. In some embodiments, a target is degraded. In some embodiments, degradation occurs in a lysosome. In some

embodiments, a multi-functional molecule from which a target dissociated remains in an endosome. In some embodiments, a multi-functional molecule is delivered to a surface of a cell via binding of a third moiety to an endocytic receptor in an endosome.

[0034] This disclosure provides, inter alia, a method of degrading a target, comprising contacting a cell with a complex comprising a multi-functional molecule disclosed herein and a target, under conditions sufficient to degrade s target in s cell. In some embodiments, degradation of a target can be modulated by altering a number of glycans of a first moiety. In some embodiments, increasing a number of glycans increases a rate of degradation of a target.

[0035] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a first moiety comprises one or more glycans. In some embodiments, a first moiety is conjugated to a second moiety and/or a third moiety at one or more glycosylation sites. In some embodiments, a first moiety is present on a peptide. In some embodiments, a peptide comprising the first moiety is linked to a second moiety and/or third moiety.

[0036] In some embodiments, a glycan comprises a terminal GlcNac.

[0037] In some embodiments, a glycan comprises a terminal GalNac.

[0038] In some embodiments, a glycan comprises a terminal Gal.

[0039] In some embodiments, a glycan is an N-glycan. In some embodiments, a N-glycan is linked to a second moiety and/or third moiety at 1, 2, 3, 4 or 5 N-glycosylation sites.

[0040] In some embodiments, a glycan structure comprises GlcNAc2-Man3-GlcNAc2, GalNAc2-GlcNAc2-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, GlcNAcl-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, Gal1- GlcNAc2-Man3-GlcNAc2, GalNAc1-GlcNAc2-Man3-GlcNAc2, GlcNAc3-Man3-GlcNAc2, GlcNAc4-Man3-GlcNAc2, Gal3-GlcNAc3-Man3-GlcNAc2, GalNAc3-GlcNAc3-Man3-GlcNAc2, GalNAc4-GlcNAc4-Man3-GlcNAc2, Gal4-GlcNAc4-Man3-GlcNAc2, or Man-6-P -N-glycan.

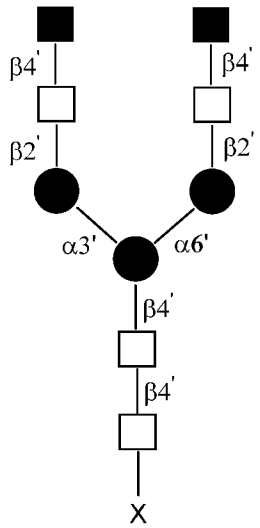
[0041] In some embodiments, increasing a number of glycan structures on the multi-functional molecule increases the rate of lysosomal degradation as compared to an otherwise similar multi-functional molecule with fewer glycan structures.

[0042] In some embodiments, a number of glycan structures comprise 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more or 10 or more.

[0043] In some embodiments, a glycan structure comprises a monoantennary structure, biantennary structure, a triantennary structure, or a tetraantennary structure.

[0044] In some embodiments, a glycan structure comprises a biantennary structure. In some embodiments, a glycan structure comprises a biantennary GalNAc. In some embodiments, a biantennacy GalNac binds to an asialoglycoprotein receptor (ASGPR) or a fragment or variant thereof, or a complex comprising ASGPR.

[0045] In some embodiments, a N-glycan has a structure of:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the second moiety or third moiety.

[0046] In some embodiments, a N-glycosylation site is naturally occurring.

[0047] In some embodiments, a N-glycosylation site is engineered into the amino acid sequence of a second moiety and/or third moiety.

[0048] In some embodiments, an endocytic receptor bound by a first moiety (e.g., a first endocytic receptor), is or comprises ASGPR or a fragment or variant thereof. In some embodiments, when an endocytic receptor bound by a first moiety (e.g., a first endocytic receptor) is ASGPR, a second moiety and/or third moiety are conjugated to, or linked to, a first moiety (e.g., on a peptide) having a glycan structure comprising a terminal GalNac.

[0049] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a first pH is between pH 6.7 to pH 8. In some embodiments, a first pH is or comprises a pH of at least 6.7, at least 6.8, at least 6.9, at least 7.0, at least 7.1, at least 7.2, at least 7.3, at least 7.4, at least 7.5, at least 7.6, at least 7.7, at least 7.8, at least 7.9, or at least 8.0. In some embodiments, a first pH is or comprises a pH of about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, or about 8.0. In some embodiments, a first pH is or comprises a pH of 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.

[0050] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a second pH is between pH 6.5 to pH 5.6. In some embodiments, a second pH is or comprises a pH of about 6.5, a pH of about 6.4, a pH of about 6.3, a pH of about 6.2, a pH of about 6.1, a pH of about 6.0, a pH of about 5.9, a pH of about 5.8, a pH of about 5.7, or a pH of about 5.6. .

[0051] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a third pH is less than pH 5.5.

[0052] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a first moiety binds to an endocytic receptor (e.g., a first endocytic receptor) under a first set of conditions. In some embodiments, a first moiety binds to an endocytic receptor in the presence of one or more cations. In some embodiments, one or more cations comprise Ca 2+. In some embodiments, a first moiety binds to an endocytic receptor (e.g., a first endocytic receptor) in a pH dependent manner. In some embodiments, a first moiety binds to an endocytic receptor (e.g., a first endocytic receptor) at a first pH. In some embodiments, a first moiety has reduced binding or does not bind to an endocytic receptor (e.g., a first endocytic receptor), at a second pH and/or at a third pH. In some embodiments, reduced binding is assessed as compared to binding of a first moiety to an endocytic receptor (e.g., a first endocytic receptor) at a first pH.

[0053] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a first moiety binds to an endocytic receptor under a second set of conditions, e.g., as disclosed herein. In some embodiments, a first moiety binds to an endocytic receptor in a pH dependent manner. In some embodiments, a first moiety binds to an endocytic receptor at a second pH. In some

embodiments, a first moiety has reduced binding or does not bind to an endocytic receptor at a third pH. In some embodiments, reduced binding is assessed as compared to binding of a first moiety to an endocytic receptor at a third pH.

[0054] In some embodiments, an endocytic receptor (e.g., a first endocytic receptor) is or comprises an endocytic lectin receptor.

[0055] In some embodiments, an endocytic receptor (e.g., a first endocytic receptor) is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0056] In some embodiments, an endocytic receptor (e.g., a first endocytic receptor) is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0057] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a second moiety binds to a target at a first pH. In some embodiments, a second moiety has reduced binding, or does not bind to a target at a second pH.

[0058] In some embodiments, reduced binding is assessed as compared to binding of a second moiety to a target at a first pH. In some embodiments, a second moiety undergoes a pH dependent conformation change. In some embodiments, a conformation change prevents binding or reduced binding to a target at a second pH.

[0059] In some embodiments, a second moiety comprises one or more peptides that specifically binds to a target.

[0060] In some embodiments, a target is a secreted protein.

[0061] In some embodiments, a target is an antibody.

[0062] In some embodiments, a target is an auto-antibody.

[0063] In some embodiments, a target is membrane-bound.

[0064] In some embodiments, a second moiety comprises an antibody agent. In some embodiments, an antibody agent comprises an antigen binding fragment having (1) at least one amino acid substituted with a histidine; and/or (2) insertion of at least one histidine. In some embodiments, an antibody agent comprises an Fc domain having one or more mutations to alter binding to a receptor, e.g., FcRn.

[0065] In some embodiments, a binding affinity of an antibody agent of a second moiety to a target at a first pH (KD at first pH) is at least 1.5-fold lower compared to binding of an antibody agent to a target at a second pH (KD at second pH).

[0066] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a third moiety binds to an endocytic receptor (e.g., a second endocytic receptor) at a second pH. In some embodiments, a third moiety has reduced binding, or does not bind to an endocytic receptor (e.g., a second endocytic receptor), at a first pH. In some embodiments, reduced binding is assessed as compared to binding of an endocytic receptor (e.g., a second endocytic receptor) at a second pH.

[0067] In some embodiments, a third moiety undergoes a pH-dependent conformation change. In some embodiments, a conformation change prevents binding or reduces binding of an endocytic receptor (e.g., a second endocytic receptor) at a first pH.

[0068] In some embodiments, a third moiety comprises one or more peptides that specifically binds to an endocytic receptor (e.g., a second endocytic receptor).

[0069] In some embodiments, a third moiety binds to an endocytic receptor (e.g., a second endocytic receptor) with an affinity that is at least an affinity required for transporting a multi-functional molecule to a cell surface.

[0070] In some embodiments, a multi-functional molecule with a third moiety bound to an endocytic receptor (e.g., a second endocytic receptor) is delivered to a cell surface via exocytosis.

[0071] In some embodiments, a third moiety does not bind to an endocytic receptor (e.g., a second endocytic receptor), with an affinity that is at or higher than an affinity required for transporting a multi-functional molecule to a lysosome.

[0072] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is or comprises an endocytic lectin receptor.

[0073] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0074] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0075] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is not a neonatal Fc receptor (FcRn).

[0076] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is or comprises a neonatal Fc receptor (FcRn).

[0077] In some embodiments, an endocytic receptor (e.g., a second endocytic receptor), is or comprises a Siglec, one or more SNARE proteins, or a multi-drug transporter.

[0078] In some embodiments, a third moiety comprises an antibody agent.

[0079] In some embodiments, a binding affinity of an antibody agent of a third moiety to an endocytic receptor (e.g., a second endocytic receptor) at a second pH (KD at second pH) is at least 1.5-fold lower compared to binding of an antibody agent of a third moiety to an endocytic receptor (e.g., a second endocytic receptor) at a second pH (KD at second pH).

[0080] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, binding of a second moiety to a target forms a first complex comprising a multi-functional molecule and a target. In some embodiments, binding of a first moiety to an endocytic receptor (e.g., a first endocytic receptor), forms a second complex. In some embodiments, a second complex comprises a first complex, and an endocytic receptor (e.g., a first endocytic receptor). In some embodiments, binding of a first moiety to an endocytic receptor (e.g., a first endocytic receptor), internalizes a second complex into a cell, e.g., into an endosome.

[0081] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule is characterized in that upon internalization into an endosome, a first moiety dissociates from an endocytic receptor (e.g., a first endocytic receptor). In some embodiments, an endosome has the second pH.

[0082] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule is characterized in that upon internalization into an endosome a target dissociates from a second moiety in the presence of a second pH. In some embodiments, dissociation occurs in an intracellular compartment of a cell. In some embodiments, dissociation of a target from a second moiety results in degradation of a target. In some embodiments, degradation occurs in a lysosome.

[0083] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule is characterized in that upon internalization into an endosome a third moiety binds to an second endocytic receptor (e.g., a second endocytic receptor), at a second pH and forms a third complex.

[0084] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule is characterized in that binding of a third moiety to an endocytic receptor (e.g., a second endocytic receptor), delivers a third complex to a surface of a cell. In some embodiments, a surface of a cell has the first pH. In some embodiments, a third moiety dissociates from an endocytic receptor (e.g., a second endocytic receptor), at a first pH. In some embodiments, a multi-functional molecule which is dissociated from an endocytic receptor (e.g., a second endocytic receptor), at a surface of a cell is not bound to a cell surface.

[0085] In some embodiments, a multi-functional molecule which is dissociated from an endocytic receptor (e.g., a second endocytic receptor) at a surface of a cell and is not bound to a cell surface, is able to bind (e.g., repeated binding) to one or more targets with a second moiety at a first pH. In some embodiments, said multi-functional molecule is able to bind to one or more endocytic receptors, e.g., first endocytic receptor, with a first moiety. In some embodiments, a multi-functional molecule is able to bind to one or more endocytic receptors, e.g., second

endocytic receptor, with a third moiety upon internalization into a cell compartment with a second pH.

[0086] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more first moieties of (a): a first moiety that specifically binds to an endocytic receptor (e.g., a first endocytic receptor).

[0087] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more second moieties of (b): a second moiety that specifically binds to a target at a first pH.

[0088] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more third moieties of (c) a third moiety that specifically binds to an endocytic receptor (e.g., a second endocytic receptor) at a second pH.

[0089] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more linkers. In some embodiments, one or more linkers are situated between a first and second moieties. In some embodiments, one or more linkers are situated between a first and third moieties. In some embodiments, one or more linkers are situated between a second and third moieties.

[0090] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more peptides comprising a first moiety linked to one or more second moieties with one or more linkers.

[0091] In some embodiments of any of the multi-functional molecules disclosed herein, compositions comprising the same or methods of using the same, a multi-functional molecule comprises one or more peptides comprising a first moiety linked to one or more third moieties with one or more linkers.

[0092] In some embodiments, one or more linkers comprise a Gly-Ser linker, or an EAAAK linker.

[0093] Additional features of multi-functional molecules disclosed herein, nucleic acids encoding the same, compositions comprising multi-functional molecules or nucleic acids encoding the same, and methods of making and using the same are provided throughout the present disclosure.

Definitions

[0094] In this application, unless otherwise clear from context, (i) the term "a" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; (iii) the terms "comprising" and "including" may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) the terms "about" and "approximately" may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

[0095] ***Endosome***: As used herein, the term "endosome" refers to an intracellular membrane-enclosed structure which has a pH of about 6.5 to about 5.6. In some embodiments, an endosome has a pH of about 6.5. In some embodiments, an endosome is not a late endosome. In some embodiments, an endosome is not a lysosome. In some embodiments, an endosome does not have proteolytic activity. In some embodiments, an endosome does not degrade a biological molecule, e.g., a multi-functional molecule disclosed herein. In some embodiments, an endosome comprises an early endosome, and/or a recycling endosome. In some embodiments, an endosome comprises a recycling endosome, e.g., one or more compartments and/or tubules of an endocytic recycling compartment.

[0096] ***Glycans:*** As used herein, the term "glycan" refers to one or more saccharides or sugar chains that can be attached to a protein or lipid to form a glycoconjugate. A glycan conjugated to a protein forms a glycoprotein. A glycan conjugated to a nitrogen atom of an amino acid residue is an N-linked glycan and a glycan conjugated to an oxygen atom of an amino acid residue is an O-linked glycan. As will be appreciated by those of ordinary skill in the art, the structure of a glycan indicates if a specific glycan is an N-linked glycan.

[0097] ***Glycoengineered***: As used herein, the term "glycoengineered," or an equivalent thereof means a process of glycosylating a particular protein (e.g., a multi-functional molecule, or specific moiety thereof as disclosed herein), or a modified protein made by such process. In

some embodiments, the process uses a host cell system that has one or more enzymes (e.g., pathways) that provides for glycosylation of a protein; in some other embodiments, the process is performed by chemically attaching one or more glycans to a protein, e.g., using Click chemistry. Such a host cell system can be genetically engineered to introduce a glycosylation pathway to selectively glycosylate a particular protein with a particular glycan structure. A host cell used to generate a glycoengineered protein can include, for example, a recombinant nucleic acid encoding a protein; and a recombinant nucleic acid encoding a heterologous glycosyltransferase. The host cell system used for glycoengineering (e.g., to generate a glycoengineered protein) can introduce, eliminate or modify N-linked glycosylation. The host cell system used for glycoengineering (e.g, to generate a glycoengineered protein) can introduce, eliminate or modify O-linked glycosylation. The host cell used for glycoengineering or to generate a glycoengineered target protein can be a mammalian cell, an insect cell, a yeast cell, a bacterial cell, a plant cell, a microalgae, or a protozoa. The protozoa used for glycoengineering can be a species of Leishmania. In some embodiments, a glycoengineered protein also includes a protein that has been engineered to be selectively glycosylated at one or more specific sites when generated in the host cell system.

[0098] ***Glycosylation site:*** As used herein, the term "glycosylation site" refers to a site of glycosylation in a protein. Such a glycosylation site, also referred to as a glycosite herein, can be naturally present in the amino acid sequence of a protein or recombinantly engineered into the protein by addition or substitution or deletion of amino acids. In some embodiments, a glycosylation site is present in a so-called glycotag that is fused to a moiety of a multi-functional molecule disclosed herein. In certain embodiments, a glycotag is fused to a protein to create a bispecific binding protein. As used herein a glycotag refers to a peptide containing consensus N-glycosylation site sequence fused to N- or a C-terminal or both termini of a protein or polypeptide. In some embodiments, the glycotag is fused to the C-terminus of a moiety of a multi-functional molecule disclosed herein via a peptide linker. In some embodiments, the glycotag is fused to the N-terminus of a moiety of a multi-functional molecule disclosed herein via a peptide linker. In some embodiments, the peptide linker is a consensus peptide sequence. In some embodiments, the consensus peptide sequence is 1, 2, 3, 4, 5, 6, 7 or more amino acid residues in length. In some embodiments, the bifunctional protein provided herein contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more glycotags.

[0099] ***Endocytic receptor:*** As used herein, the term "endocytic receptor" refers to a receptor or a fragment thereof that is capable of (1) binding to a molecule (e.g., a multi-functional molecule) and internalizing the molecule into a cell; and/or (2) binding to a molecule (e.g., a multi-functional molecule) in an endosome and exporting the molecule (e.g., a multi-functional molecule) out of the cell. In some embodiments, an endocytic receptor recognizes and binds to one or more glycans on a multi-functional molecule. In some embodiments, binding of an endocytic receptor to a molecule (e.g., a multi-functional molecule) internalizes the target into a cell, e.g., into an endosome. In some embodiments, an endocytic receptor is or comprises an endocytic lectin receptor. In some embodiments, an endocytic receptor is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof. In some embodiments, an endocytic receptor is or comprises a neonatal Fc receptor (FcRn). In some embodiments, an endocytic receptor is or comprises a Siglec (Sialic acid-binding immunoglobulin-type lectins), one or more SNARE proteins, or a multi-drug transporter. In some embodiments, an endocytic receptor is capable of internalizing a molecule into a cell (e.g., endocytosing). In some embodiments, an endocytic receptor is capable of exporting a molecule out of a cell (e.g., exocytosing). In some embodiments, an endocytic receptor is capable of internalizing a molecule into a cell (e.g., endocytosing) and exporting a molecule out of a cell (e.g., exocytosing).

[0100] ***About:*** The term "about", when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about" in that context. For example, in some embodiments, the term "about" may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0101] ***Administration:*** As used herein, the term "administration" typically refers to the administration of a composition to a subject or system, for example to achieve delivery of an agent that is, or is included in or otherwise delivered by, the composition. Those of ordinary skill

in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example an animal or a human. In some embodiments, an animal is a domestic animal, such as a companion animal, e.g., a dog or a cat; in some embodiments, an animal is an animal used in agriculture (e.g., farming [e.g., a cow, a sheep or a horse]) or for recreation. For example, in some embodiments, administration may be systemic or local. Those skilled in the art will be aware of appropriate administration routes for use with particular therapies described herein, for example which include bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc.), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may be by injection (*e.g.*, intramuscular, intravenous, or subcutaneous injection). In some embodiments, injection may involve bolus injection, drip, perfusion, or infusion. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time. In some embodiments, an antibody agent can be formulated for oral delivery. For example, one with skill in the art will understand that an antibody agent disclosed herein can be formulated for oral delivery using technologies developed by Oramed (https://www.oramed.com/) or Premas (https://www.premasbiotech.com/).

[0102] ***Adult***: As used herein, the term "adult" refers to a human eighteen years of age or older. In some embodiments, a human adult has a weight within the range of about 90 pounds to about 250 pounds.

[0103] ***Affinity***: As is known in the art, "affinity" is a measure of the tightness with which two or more binding partners associate with one another. In some embodiments, a binding affinity of a moiety of a multi-functional molecule to a binding partner is about 0.01 picomolar (pM) to about 900 nanomolar (nM). Those skilled in the art are aware of a variety of assays that can be used to assess affinity, and will furthermore be aware of appropriate controls for such assays. In some embodiments, affinity is assessed in a quantitative assay. In some embodiments,

affinity is assessed over a plurality of concentrations (e.g., of one binding partner at a time). In some embodiments, affinity is assessed in the presence of one or more potential competitor entities (e.g., that might be present in a relevant – e.g., physiological – setting). In some embodiments, affinity is assessed relative to a reference (e.g., that has a known affinity above a particular threshold [a "positive control" reference] or that has a known affinity below a particular threshold [a "negative control" reference"]. In some embodiments, affinity may be assessed relative to a contemporaneous reference; in some embodiments, affinity may be assessed relative to a historical reference. Typically, when affinity is assessed relative to a reference, it is assessed under comparable conditions.

[0104] ***Avidity:*** As is known in the art, "avidity" is a measure of the accumulated strength of multiple non-covalent interactions between two or more binding partners in a complex. Those skilled in the art are aware of a variety of assays that can be used to assess avidity, and will furthermore be aware of appropriate controls for such assays. In some embodiments, avidity can be determined by (1) a binding affinity of two or more binding partners in a complex; (2) valency of each of the binding partners in a complex; and/or (3) structural arrangements of two or more binding partners in a complex. In some embodiments, the avidity of binding between two or more binding partners is more than a sum of each binding affinity between the two or more binding partners. In some embodiments, avidity is also referred to as apparent affinity or functional affinity. In some embodiments of a multi-functional molecule disclosed herein, a first moiety comprising one or more glycans which can bind to a receptor (e.g., an endocytic receptor) contributes to binding avidity of the multi-functional molecule. In some embodiments, an endocytic receptor is ASGPR or a fragment or variant thereof. In some embodiments, avidity is assessed in a quantitative assay. In some embodiments, avidity is assessed over a plurality of concentrations. In some embodiments, avidity is assessed in the presence of one or more potential competitor entities (e.g., that might be present in a relevant – e.g., physiological – setting). In some embodiments, avidity may be assessed relative to a contemporaneous reference; in some embodiments, avidity may be assessed relative to a historical reference. Typically, when avidity is assessed relative to a reference, it is assessed under comparable conditions.

[0105] ***Agent***: As used herein, the term "agent", may refer to a physical entity or phenomenon. In some embodiments, an agent may be characterized by a particular feature and/or effect. In some embodiments, an agent may be a compound, molecule, or entity of any

chemical class including, for example, a small molecule, polypeptide, nucleic acid, saccharide, lipid, metal, or a combination or complex thereof. In some embodiments, the term "agent" may refer to a compound, molecule, or entity that comprises a polymer. In some embodiments, the term may refer to a compound or entity that comprises one or more polymeric moieties. In some embodiments, the term "agent" may refer to a compound, molecule, or entity that is substantially free of a particular polymer or polymeric moiety. In some embodiments, the term may refer to a compound, molecule, or entity that lacks or is substantially free of any polymer or polymeric moiety.

[0106] ***Amino acid:*** in its broadest sense, as used herein, refers to any compound and/or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term "amino acid" may be used to refer to a free amino acid; in some embodiments it may be used to refer to an amino acid residue of a polypeptide.

[0107] ***Animal:*** as used herein refers to a member of the animal kingdom. In some embodiments, "*animal*" refers to humans; unless otherwise specified, in many embodiments, a

human may be of either gender and/or at any stage of development. In some embodiments, "*animal*" refers to non-human animals; unless otherwise specified, in many embodiments, a non-human animal may be of any gender and/or at any stage of development. In certain embodiments, a non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, an animal may be, for example, a mammal, a bird, a reptile, an amphibian, a fish, an insect, a worm, *etc.*. In some embodiments, an animal may be a transgenic animal, genetically engineered animal, and/or a clone.

[0108] ***Antibody agent****:* As used herein, the term "antibody agent" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. In the case of an autoimmune disease, the antigen to which a pathogenic autoantibody binds is also referred to as an "autoantigen." As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a "Y-shaped" structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)– an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y's stem). A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another "switch". Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complementarity determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies

fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present disclosure include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. In some embodiments, antibodies produced and/or utilized in accordance with the present disclosure include one or more modifications on an Fc domain, e.g., an effector null mutation, e.g., a LALA, LAGA, FEGG, AAGG, or AAGA mutation. For purposes of the present disclosure, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of dog, cat, mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are human, humanized, primatized, chimeric, etc, as is known in the art. Moreover, the term "antibody" as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, in some embodiments, an antibody utilized in accordance with the present invention is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., VHH [e.g., a camelid VHH] or NAR) alternative scaffolds or antibody mimetics (e.g., anticalins, FN3 monobodies, DARPins, Affibodies, Affilins, Affimers, Affitins, Alphabodies, Avimers, Fynomers, Im7, VLR, VNAR, Trimab, CrossMab, Trident); nanobodies, binanobodies, F(ab')2, Fab', di-sdFv, trifunctional antibodies, diabodies, and minibodies. etc. In some embodiments, relevant formats may be or include: Adnectins®; Affibodies®; Affilins®;

Anticalins®; Avimers®; BiTE®s; cameloid antibodies; Centyrins®; ankyrin repeat proteins or DARPINs®; dual-affinity re-targeting (DART) agents; Fynomers®; shark single domain antibodies such as IgNAR; immune mobilixing monoclonal T cell receptors against cancer (ImmTACs); KALBITOR®s; MicroProteins; Nanobodies® minibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); TCR-like antibodies;, Trans-bodies®; TrimerX®; VHHs. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody format is or comprises a VHH, e.g., a camelid VHH. In some embodiments, a VHH is a multivalent VHH, e.g., a bivalent VHH. In some embodiments, an antibody comprises a single domain antibody, e.g., comprising one or more additional domains such as an Fc, a half-Fc (e.g., comprising an interchain cysteine mutant), an albumin domain, or combinations thereof. In some embodiments, an antibody comprises a single chain Fv, e.g., comprising one or more additional domains such as an Fc, a half-Fc (e.g., comprising an interchain cysteine mutant), an albumin domain, or combinations thereof. In some embodiments, an antibody comprises a polypeptide- Fc fusion. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc], or other pendant group [e.g., poly-ethylene glycol, etc.]).

[0109] ***Antibody fragment***: As used herein, an "antibody fragment" refers to a portion of an antibody or antibody agent as described herein, and typically refers to a portion that includes an antigen-binding portion or variable region thereof. An antibody fragment may be produced by any means. For example, in some embodiments, an antibody fragment may be enzymatically or chemically produced by fragmentation of an intact antibody or antibody agent. Alternatively, in some embodiments, an antibody fragment may be recombinantly produced (i.e., by expression of an engineered nucleic acid sequence. In some embodiments, an antibody fragment may be wholly or partially synthetically produced. In some embodiments, an antibody fragment (particularly an antigen-binding antibody fragment) may have a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 amino acids or more, in some embodiments at least about 200 amino acids.

[0110] ***Antigen***: The term "antigen", as used herein, refers to an agent that elicits an immune response; and/or (ii) an agent that binds to a T cell receptor (*e.g.,* when presented by an MHC molecule) or to an antibody. In some embodiments, an antigen elicits a humoral response

(e.g., including production of antigen-specific antibodies); in some embodiments, an elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen). In some embodiments, and antigen binds to an antibody and may or may not induce a particular physiological response in an organism. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments other than a biologic polymer [e.g., other than a nucleic acid or amino acid polymer) etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a glycan. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (e.g., together with other materials, for example in an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source). In some embodiments, antigens utilized in accordance with the present invention are provided in a crude form. In some embodiments, an antigen is a recombinant antigen.

[0111] ***Approximately:*** As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0112] ***Binding***: Those skilled in the art will appreciate that the term "binding", as used herein, typically refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

[0113] ***CDR:*** as used herein, refers to a complementarity determining region within an antibody variable region. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. A "*set of CDRs*" or "*CDR set*" refers to a group of three or six CDRs that occur in either

a single variable region capable of binding the antigen or the CDRs of cognate heavy and light chain variable regions capable of binding the antigen. Certain systems have been established in the art for defining CDR boundaries (e.g., Kabat, Chothia, etc.); those skilled in the art appreciate the differences between and among these systems and are capable of understanding CDR boundaries to the extent required to understand and to practice the claimed subject matter.

[0114] ***Composition:*** Those skilled in the art will appreciate that the term "composition" may be used to refer to a discrete physical entity that comprises one or more specified components. In general, unless otherwise specified, a composition may be of any form – e.g., gas, gel, liquid, solid, etc.

[0115] ***Comprising:*** A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as "comprising" or "consisting essentially of" one or more named elements or steps also describes the corresponding, more limited, and closed-ended composition or method "consisting of" (or "consists of") the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.

[0116] ***Moiety:*** The term "moiety" as used herein refers to a section or portion of an entity. In some embodiments, a "moiety" is associated with a particular structural and/or functional feature of the entity so that, when the moiety is physically separated from the rest of its parent entity, it substantially or entirely retains the particular structural and/or functional feature. Alternatively, or additionally, a moiety may be or include a portion of an entity that, when separated from that (parent) entity and linked with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features that characterized it in the parent entity. In some embodiments, a moiety is a

section or portion of a molecule (e.g., a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a moiety is a section of a polypeptide; in some such embodiments, a moiety is characterized by a particular structural element (e.g., a particular amino acid sequence or sequence motif, alpha-helix character, alpha-sheet character, coiled-coil character, random coil character, etc.), and/or by a particular functional feature (e.g., binding activity, enzymatic activity, folding activity, signaling activity, etc.).

[0117] ***Epitope:*** as used herein, includes any moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component. In some embodiments, an epitope is comprised of a plurality of chemical atoms or groups on an antigen. In some embodiments, such chemical atoms or groups are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, such chemical atoms or groups are physically near to each other in space when the antigen adopts such a conformation. In some embodiments, at least some such chemical atoms are groups are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized).

[0118] ***Functional:*** As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[0119] ***Fragment:*** A "fragment" of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a polymer fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (e.g., residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (e.g., residues) found in the whole polymer. The whole material or entity may in some embodiments be referred to as the "parent" of the fragment.

[0120] ***Homology:*** As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, e.g., between polypeptide molecules. In some embodiments, polymeric molecules such as antibodies are considered to be "homologous" to one

another if their sequences are at least 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 80%, 85%, 90%, 95%, or 99% similar.

[0121] ***Human***: In some embodiments, a human is an embryo, a fetus, an infant, a child, a teenager, an adult, or a senior citizen.

[0122] ***Humanized:*** as is known in the art, the term "*humanized*" is commonly used to refer to antibodies (or antibody components) whose amino acid sequence includes V_H and V_L region sequences from a reference antibody raised in a non-human species (e.g., a mouse), but also includes modifications in those sequences relative to the reference antibody intended to render them more "*human-like*", i.e., more similar to human germline variable sequences. In some embodiments, a "*humanized*" antibody (or antibody component) is one that immunospecifically binds to an antigen of interest and that has a framework (FR) region having substantially the amino acid sequence as that of a human antibody, and a complementary determining region (CDR) having substantially the amino acid sequence as that of a non-human antibody. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor immunoglobulin) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In some embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin constant region. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include a C_H1, hinge, C_H2, C_H3, and, optionally, a C_H4 region of a heavy chain constant region. In some embodiments, a humanized antibody only contains a humanized V_L region. In some embodiments, a humanized antibody only contains a humanized V_H region. In some certain embodiments, a humanized antibody contains humanized V_H and V_L regions.

[0123] ***Identity***: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "substantially identical" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences,

for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0124] ***"Improve," "increase", "inhibit"*** or ***"reduce":*** As used herein, the terms "improve", "increase", "inhibit', "reduce", or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a particular system (e.g., in a single individual) under otherwise comparable conditions absent presence of (e.g., prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may be or comprise a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment.

[0125] ***Peptide:*** The term "peptide" as used herein refers to a polypeptide that is typically relatively short, for example having a length of less than about 100 amino acids, less than about 50 amino acids, less than about 40 amino acids less than about 30 amino acids, less

than about 25 amino acids, less than about 20 amino acids, less than about 15 amino acids, or less than 10 amino acids.

[0126] ***Pharmaceutical composition***: As used herein, the term "pharmaceutical composition" refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, a pharmaceutical composition may be specially formulated for administration in a particular form (e.g., in a solid form or a liquid form), and/or may be specifically adapted for, for example: oral administration (for example, as a drenche [aqueous or non-aqueous solutions or suspensions], tablet, capsule, bolus, powder, granule, paste, etc, which may be formulated specifically for example for buccal, sublingual, or systemic absorption); parenteral administration (for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation, etc); topical application (for example, as a cream, ointment, patch or spray applied for example to skin, lungs, or oral cavity); intravaginal or intrarectal administration (for example, as a pessary, suppository, cream, or foam); ocular administration; nasal or pulmonary administration, etc.

[0127] ***Polypeptide:*** As used herein refers to a polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may comprise or consist of only natural amino acids or only non-natural amino acids. In some embodiments, a polypeptide may comprise D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may comprise only D-amino acids. In some embodiments, a polypeptide may comprise only L-amino acids. In some embodiments, a polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains, at the polypeptide's N-terminus, at the polypeptide's C-terminus, or any combination thereof. In some embodiments, such pendant groups or modifications may be selected from the group consisting of acetylation, amidation,

lipidation, methylation, pegylation, etc., including combinations thereof. In some embodiments, a polypeptide may be cyclic, and/or may comprise a cyclic portion. In some embodiments, a polypeptide is not cyclic and/or does not comprise any cyclic portion. In some embodiments, a polypeptide is linear. In some embodiments, a polypeptide may be or comprise a stapled polypeptide. In some embodiments, the term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure; in such instances it is used herein to refer to polypeptides that share the relevant activity or structure and thus can be considered to be members of the same class or family of polypeptides. For each such class, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known; in some embodiments, such exemplary polypeptides are reference polypeptides for the polypeptide class or family. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class; in some embodiments with all polypeptides within the class). For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that may in some embodiments be or comprise a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some embodiments, a relevant polypeptide may comprise or consist of a fragment of a parent polypeptide. In some embodiments, a useful polypeptide as may comprise or consist of a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent), so that the polypeptide of interest is a derivative of its parent polypeptide.

[0128] ***Reference:*** As used herein describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent, animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[0129] ***Specific binding:*** As used herein, the term "specific binding" refers to an ability to discriminate between possible binding partners in the environment in which binding is to occur. A binding agent that interacts with one particular target when other potential targets are present is said to "*bind specifically*" to the target with which it interacts. In some embodiments, specific binding is assessed by detecting or determining degree of association between the binding agent and its partner; in some embodiments, specific binding is assessed by detecting or determining degree of dissociation of a binding agent-partner complex; in some embodiments, specific binding is assessed by detecting or determining ability of the binding agent to compete an alternative interaction between its partner and another entity. In some embodiments, specific binding is assessed by performing such detections or determinations across a range of concentrations.

[0130] ***Specific:*** The term "specific", when used herein with reference to an agent having an activity, is understood by those skilled in the art to mean that the agent discriminates between potential target entities or states. For example, an in some embodiments, an agent is said to bind "specifically" to its target if it binds preferentially with that target in the presence of one or more competing alternative targets. In many embodiments, specific interaction is dependent upon the presence of a particular structural feature of the target entity (e.g., an epitope, a cleft, a binding site). It is to be understood that specificity need not be absolute. In some embodiments, specificity may be evaluated relative to that of the binding agent for one or more other potential target entities (e.g., competitors). In some embodiments, specificity is evaluated relative to that of a reference specific binding agent. In some embodiments specificity is

evaluated relative to that of a reference non-specific binding agent. In some embodiments, the agent or entity does not detectably bind to the competing alternative target under conditions of binding to its target entity. In some embodiments, binding agent binds with higher on-rate, lower off-rate, increased affinity, decreased dissociation, and/or increased stability to its target entity as compared with the competing alternative target(s).

[0131] ***Specificity***: As is known in the art, "specificity" is a measure of the ability of a particular ligand to distinguish its binding partner from other potential binding partners.

[0132] ***Substantially:*** As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0133] ***Substantial identity:*** as used herein refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially identical" if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul et al., Methods in Enzymology; Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis et al., Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al, (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475,

500 or more residues. In the context of a CDR, reference to "*substantial identity*" typically refers to a CDR having not more than a small number (*e.g.*, 3, 2, or 1) an amino acid sequence changes relative to that of a reference CDR. In some embodiments, a CDR that is *substantially identical* to a reference CDR differs from that reference CDR by one or more amino acid changes at the end of the reference CDR; in some such embodiments, the relevant CDR is identical to the reference CDR other than at one or both ends. As is known in the art, CDR elements typically have a length within a range of a few amino acids (*e.g.*, 3, 4, 5, 6, or 7) to about 20 or 30 amino acids (*see,* for example, Collis *et al. J. Mol. Biol.* 325:337, 2003, incorporated herein by reference); thus, in some embodiments, a CDR may be considered to be substantially identical to a reference CDR when it shares at least about 80% (or less for a shorter CDR), at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or at least about 100% identity with the reference CDR.

[0134] ***Substantial sequence homology***: The phrase "substantial homology" is used herein to refer to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "substantially homologous" if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues will appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "hydrophobic" or "hydrophilic" amino acids, and/or as having "polar" or "non-polar" side chains Substitution of one amino acid for another of the same type may often be considered a "homologous" substitution. Typical amino acid categorizations are summarized below:

Amino acid name	3 letter code	1-letter code	Polarity	Charge
Alanine	Ala	A	nonpolar	neutral
Arginine	Arg	R	polar	positive
Asparagine	Asn	N	polar	neutral
Aspartic acid	Asp	D	polar	negative
Cysteine	Cys	C	nonpolar	neutral

Glutamic acid	Glu	E	polar	negative
Glutamine	Gln	Q	polar	neutral
Glycine	Gly	G	nonpolar	neutral
Histidine	His	H	polar	positive
Isoleucine	Ile	I	nonpolar	neutral
Leucine	Leu	L	nonpolar	neutral
Lysine	Lys	K	polar	positive
Methionine	Met	M	nonpolar	neutral
Phenylalanine	Phe	F	nonpolar	neutral
Proline	Pro	P	nonpolar	neutral
Serine	Ser	S	polar	neutral
Threonine	Thr	T	polar	neutral
Tryptophan	Trp	W	nonpolar	neutral
Tyrosine	Tyr	Y	polar	neutral
Valine	Val	V	nonpolar	neutral

Ambiguous Amino Acids	**3-Letter**	**1-Letter**
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment

search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul, et al., Methods in Enzymology; Altschul, et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis, et al., Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al., (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, at least 425, at least 450, at least 475, at least 500 or more residues.

[0135] ***Treat:*** As used herein, the term "treat," "treatment," or "treating" is used to refer to one or more of partial or complete alleviation, amelioration, relief, inhibition, prevention, delay of onset of, reduction in severity of and/or reduction in frequency (e.g., incidence) of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, treatment may be prophylactic; for example may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits early signs of the disease, disorder, and/or condition, and may, for example, decrease risk of developing pathology associated with the disease, disorder, and/or condition and/or delay onset and/or decrease rate of development or worsening of one or more features of a disease, disorder and/or condition.

[0136] ***Treatment***: As used herein, the term "treatment" (also "treat" or "treating") refers to administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder

and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors, e.g., that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition. Thus, in some embodiments, treatment may be prophylactic; in some embodiments, treatment may be therapeutic.

[0137] ***Variant:*** The term "variant", as used herein, refers to a molecule or entity (*e.g.*, that are or comprise a nucleic acid, protein, or small molecule) that shows significant structural identity with a reference molecule or entity but differs structurally from the reference molecule or entity, e.g., in the presence or absence or in the level of one or more chemical moieties as compared to the reference molecule or entity. In some embodiments, a variant also differs functionally from its reference molecule or entity. In many embodiments, whether a particular molecule or entity is properly considered to be a "variant" of a reference is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, a biological or chemical reference molecule in typically characterized by certain characteristic structural elements. A variant, by definition, is a distinct molecule or entity that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule or entity. To give but a few examples, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular structural motif and/or biological function; a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to on another in linear or three-dimensional space. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (*e.g.,* carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (*e.g.,* that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some

embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a "variant" of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. Typically, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. Often, a variant polypeptide or nucleic acid comprises a very small number (*e.g.*, fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues (*i.e.*, residues that participate in a particular biological activity) relative to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference. In some embodiments, a reference polypeptide or nucleic acid is one found in nature. In some embodiments, a reference polypeptide or nucleic acid is a human polypeptide or nucleic acid.

BRIEF DESCRIPTION OF THE DRAWING

[0138] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings(s) will be provided by the Office upon request and payment of the necessary fee.

[0139] The Figures described below, which together make up the Drawing, are for illustration purposes only, not for limitation.

[0140] **FIG. 1** is a schematic of an exemplary multi-functional molecule disclosed herein. Moiety 1 can include one or more glycans and bind to an endocytic receptor. Moiety 2 can be a moiety that specifically binds to a target in a pH dependent manner. Moiety 3 can be a moiety that specifically binds to an endocytic receptor in a pH dependent manner. As an example, the endocytic receptor bound by Moiety 3 can be the same or different as the endocytic receptor bound by Moiety 1.

[0141] **FIGs. 2A-2E** depict an exemplary proposed mechanism of action for an exemplary multi-functional molecule comprising three moieties shown in **FIG. 1**. **FIG. 2A** shows: (Step 1) binding of the second moiety (blue circle) of the multi-functional molecule to a target (red) at a first (higher) pH (e.g., as described herein), and formation of a first complex comprising the multi-functional molecule and the target; and (Step 2) binding of the complex to a first endocytic receptor (shown in yellow as inserted in the lipid bilayer) on a cell via the first moiety (blue box) and formation of a second complex. **FIG. 2B** shows (Step 3) internalization of the second complex into an endosome having a second (lower) pH (e.g., as described herein). **FIG. 2C** shows (Step 4) dissociation of the target from the second moiety, dissociation of the first moiety from the first endocytic receptor, and binding of the third moiety (yellow box) to the second endocytic receptor, all occurring at the second (lower) pH in the endosome. Binding of the third moiety to the second endocytic receptor forms the third complex. Additionally, this figure also shows (Step 5) shuttling of the dissociated target into a lysosome for degradation. **FIG. 2D** shows (Step 6) delivery of the third complex to the cell surface; and (Step 7) dissociation of the third moiety from the second endocytic receptor at the first (higher) pH of the cell surface thus being released from the cell surface. **FIG. 2E** shows (Step 8) that the multi-functional molecule, which is now dissociated from all binders (binders to the first, second and third moieties), is able to participate in one or more binding events and repeat Steps 1-7.

[0142] **FIG. 3** is a schematic of an exemplary proposed mechanism of action for an exemplary multi-functional molecule (MFM) comprises three moieties as disclosed herein. The proposed mechanism in **FIG. 3** is similar to that shown in **FIGs. 2A-2E**.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0143] Disclosed herein, *inter alia*, are multi-functional molecules that utilize pH dependence and/or cation dependence to modulate binding of one or more moieties in different

cellular spaces. The ability to modulate binding and dissociation of one or more moieties of a multi-functional molecule to a cognate binding partner allows for selective degradation of desired molecules, e.g., targets, and not degradation of multi-functional molecules disclosed herein. In some embodiments, a multi-functional molecule that has been internalized and dissociated from its target can be released back to an extracellular surface of a cell, e.g., recycled. This recycling of a multi-functional molecule can increase potency of a multi-functional molecule, for example, by increasing availability of a multi-functional molecule to participate in one or more rounds of binding events. **FIGs. 2A-2E** depict an exemplary set of binding events associated with an exemplary multi-functional molecule disclosed herein.

Multi-functional catalytic degraders

[0144] Disclosed herein are multi-functional molecules comprising (1) a first moiety that binds to an endocytic receptor under a first set of conditions and/or a second set of conditions (e.g., in a cation- or pH-dependent manner); (2) a second moiety that binds to a target at a first pH (e.g., a pH of 6.7-8) but not at a second pH (e.g., a pH of 6.5-5.6) or a third pH (e.g., a pH of less than 5.5), and optionally (3) a third moiety that binds to an endocytic receptor under a second set of conditions (e.g., at a second pH but not at a first pH). In some embodiments, conditions under which a first moiety binds to an endocytic receptor are also referred to as a first set of conditions or a second set of conditions. In some embodiments, conditions under which a third moiety binds to an endocytic receptor are also referred to as a second set of conditions.

[0145] As disclosed herein, an endocytic receptor bound by a first moiety of a multi-functional molecule disclosed herein can be referred to as (1) a first endocytic receptor, or (2) an endocytic receptor bound by a first moiety. Similarly, an endocytic receptor bound by a third moiety of a multi-functional molecule disclosed herein can be referred to as (1) a second endocytic receptor, or (2) an endocytic receptor bound by a third moiety.

[0146] In some embodiments, a multi-functional molecule disclosed herein comprises a first moiety that binds to an endocytic receptor (e.g., in the presence of one or more cations and/or within a specific pH range), and a second moiety that binds to a target at a first pH (e.g., a pH of 6.7-8) but not at a second pH (e.g., a pH of 6.5-5.6) or a third pH. In some embodiments, a multi-functional molecule comprising a first moiety and a second moiety can bind to a target with a second moiety at a first pH, e.g., at the cell surface. In some embodiments, said binding

forms a complex comprising the multi-functional molecule and target, which can further bind to an endocytic receptor on the surface of a cell via the first moiety, e.g., in the presence of one or more cations (forming a further complex comprising a multi-functional molecule, target, and endocytic receptor). Binding of the further complex to the endocytic receptor can result in internalization of the complex into an intracellular compartment such as an endosome (e.g., an early endosome and/or a recycling endosome) which has a second pH but not a third pH. Without wishing to be bound by theory, in some embodiments, upon internalization into an endosome having a second pH (e.g., a pH of 6.5-5.6), the target dissociates from the second moiety of a multi-functional molecule and the target is trafficked into a late endosome and/or a lysosome for degradation. In some embodiments, in an endosome having a second pH and/or in the absence of one or more cations, a first moiety can also dissociate from an endocytic receptor. In some embodiments, a first moiety that has dissociated from an endocytic receptor can bind to one or more (e.g., same or different) endocytic receptors in an endosome. In some embodiments, in an endosome having a second pH and/or in the absence of one or more cations, a first moiety does not dissociate from an endocytic receptor. In some embodiments, a multi-functional molecule which is now free from its target and/or endocytic receptor binding via a first moiety, can be transported back to a plasma membrane on the cell surface (e.g., exocytosed). In some embodiments, exocytosis occurs via binding of a moiety (e.g., a first moiety or a second moiety) of the multi-functional molecule to one or more endocytic receptors in an endosome, e.g., receptors capable of trafficking molecules to the plasma membrane of the cell surface . Accordingly, said multi-functional molecule is recycled and is able to participate in one or more additional binding events.

[0147] In some embodiments, a multi-functional molecule disclosed herein comprises: (1) a first moiety that binds to an endocytic receptor under a first set of conditions (e.g., in the presence of a cation such as Ca2+ and/or within a specific pH range); (2) a second moiety that binds to a target at a first pH (e.g., a pH of 6.7-8) but not at a second pH (e.g., a pH of 6.5-5.6); and (3) a third moiety that binds to an endocytic receptor under a second set of conditions, e.g., at a second pH but not at a first pH. In some embodiments, a multi-functional molecule comprising a first moiety, a second moiety, and a third moiety can bind to a target with a second moiety at a first pH, e.g., at the cell surface. In some embodiments, said binding forms a complex comprising the multi-functional molecule and target, which can further bind to an endocytic receptor on the surface of a cell via the first moiety, e.g., in the presence of one or more cations and/or within a specific pH range (forming a further complex comprising a multi-functional molecule, target, and

endocytic receptor). Binding of the further complex to the endocytic receptor can result in internalization of the complex into an intracellular compartment such as an endosome which has a second pH. Without wishing to be bound by theory, in some embodiments, upon internalization into an endosome having a second pH (e.g., a pH of 6.5-5.6), the target dissociates from the second moiety of a multi-functional molecule and the target is trafficked into a lysosome for degradation. In some embodiments, in an endosome having a second pH and/or in the absence of one or more cations, a first moiety can also dissociate from an endocytic receptor. In some embodiments, in an endosome having a second pH, a multi-functional molecule which is now free from its target and is in an endosome having a second pH, is able to bind to an endocytic receptor via the third moiety and can be transported back to the extracellular surface of the cell. In some embodiments, the cell surface has the first pH and this promotes dissociation of the third moiety from the endocytic receptor. In some embodiments, a multi-functional molecule is recycled and is able to participate in one or more binding events, e.g., to promote the degradation of one or more targets.

[0148] In some embodiments, a first moiety described herein binds to an endocytic receptor described herein, e.g., a first endocytic receptor. In some embodiments, a first moiety binds to an endocytic receptor in the presence of one or more cations, e.g., Ca2+ and/or within a specific pH range, e.g., a first pH range. In some embodiments, a first moiety does not bind to an endocytic receptor in the absence of one or more cations, e.g., Ca2+. In some embodiments, an extracellular surface of a cell has one or more cations, e.g., at a sufficient concentration, to allow binding of a first moiety to an endocytic receptor. In some embodiments, an intracellular compartment of a cell, e.g., an endosome, does not have one or more cations, e.g., does not have a sufficient concentration of one or more cations to allow binding of a first moiety to an endocytic receptor, or the endosome has an insufficient concentration of one or more cations, e.g., only sufficient to allow reduced binding of a first moiety to an endocytic receptor.

[0149] In some embodiments, a first moiety binds to an endocytic receptor under a second set of conditions, e.g., as described herein.

[0150] In some embodiments, a third moiety described herein binds to an endocytic receptor described herein, e.g., a second endocytic receptor, under a second set of conditions. In some embodiments, a third moiety binds to an endocytic receptor at a second pH, e.g., a in an endosome. In some embodiments, a third moiety has reduced or no binding to an endocytic receptor at a first pH, e.g., at an extracellular surface of a cell.

[0151] In some embodiments, an endocytic receptor bound by a first moiety and an endocytic receptor bound by a third moiety are the same endocytic receptor. In some embodiments, a first endocytic receptor (e.g., bound by a first moiety), and a second endocytic receptor (e.g., bound by a third moiety) are the same endocytic receptor. In some embodiments, a same endocytic receptor is an endocytic receptor having the same structure (e.g., sequence and/or secondary structure), and/or function. For example, for a particular endocytic receptor A having two molecules in a cell, A' and A''; a same endocytic receptor refers to A' and/or A''. In this example, a first endocytic and second endocytic receptor bound by the first and third moieties respectively can be the same molecule A' (that is a first endocytic receptor and second endocytic receptor are the same). Following this example, a first endocytic and second endocytic receptor bound by the first and third moieties respectively can also be the same molecule A'' (that is a first endocytic receptor and second endocytic receptor are the same). As another example, a first endocytic and second endocytic receptor bound by the first and third moieties respectively can be two different molecules of receptor A; wherein the first moiety binds to the first endocytic receptor A' and the third moiety binds to the second endocytic receptor A'', or vice versa (that is a first endocytic receptor and second endocytic receptor are the same).

[0152] In some embodiments, an endocytic receptor bound by a first moiety and an endocytic receptor bound by a third moiety are different. In some embodiments, a first endocytic receptor (e.g., bound by a first moiety), and a second endocytic receptor (e.g., bound by a third moiety) are different. In some embodiments, a different endocytic receptor is an endocytic receptor having a different structure (e.g., sequence and/or secondary structure), and/or function.

Binding conditions and endocytic recycling pathway

[0153] A multi-functional molecule described herein can utilize an endocytic recycling system present in a cell to internalize and/or externalize (e.g., recycle) a multi-functional molecule and/or a complex comprising the same.

[0154] Endosomes and endosomal recycling is well-studied and described e.g., in Maxfield FR and McGraw TW, *Nature Reviews Molecular Cell Biology* (2004), Volume 5, pp. 121-132; Grant BD and Donaldson JG, *Nature Reviews Molecular Cell Biology* (2009), Volume 10, pp. 597-608, Poteryaev D. et al., (2010) *Cell* vol. 141, pp. 497-508; and Cabrera M. and Ungermann C. (2010) *Cell* vol. 141, pp. 404-406, the entire contents of each of which are hereby

incorporated by reference in their entireties. As described in Maxfield (2004), the endocytic recycling system is comprised of many organelles and components many of which are not fully characterized, at least in part, due to the heterogeneity that exists in this system. For example, internalization into a cell can occur via clathrin coated-pit formation or non-clathrin coated-pit formation. Additionally, receptor mediated endocytosis typically occurs via clathrin coated-pit formation. Once internalized, molecules can be present in early endosomes or sorting endosomes from which molecules can be trafficked to an endocytic recycling compartment (recycling endosomes) or trafficked to other compartments for degradation (e.g., late endosomes or lysosomes). Molecules that are trafficked to an endocytic recycling compartment (recycling endosomes) can then be returned to the plasma membrane at the cell surface (recycling).

[0155] In some embodiments, an endosome as disclosed herein comprises an early endosome. In some embodiments, an early endosome is also referred to as a sorting endosome. In some embodiments, an early endosome expresses or has detectable presence of one or more GTPase, one or more SNARE proteins. In some embodiments, a GTPase expressed in an early endosome comprises Rab5 or a variant or fragment thereof. In some embodiments, an early endosome expresses EEA1 or a variant or fragment thereof.

[0156] Hu Y. et al., *Translational Neurodegeneration* (2015) 4:18, DOI 10.1186/s40035-015-0041-1 discloses exemplary polypeptides that can be expressed in early endosomes. Additional exemplary polypeptides that can be expressed in an early endosome are disclosed in Tables 2 and 3 of Grant and Donaldson, *Nature Reviews Molecular Cell Biology* (2009) 10, 597-608, DOI 10.1038/nrm2755, which listings are incorporated by reference in their entireties.

[0157] In some embodiments, an endosome as disclosed herein comprises a recycling endosome. In some embodiments, a recycling endosome comprises one or more components of an endocytic recycling compartment such as tubules. In some embodiments, a recycling endosome expresses or has detectable presence of one or more GTPases, one or more sorting nexins, or one or more accessory proteins. In some embodiments, a GTPase expressed in an early endosome comprises Rab11 or a variant or fragment thereof, or Rab 4 or a variant or fragment thereof, or Arf6 or a variant or fragment thereof, or any combination thereof. In some embodiments, a recycling endosome expresses EHD4 or a variant or fragment thereof. In some embodiments, a recycling endosome expresses a sorting nexin or a variant or fragment thereof.

[0158] Hu (2015) also discloses exemplary polypeptides that can be expressed in endocytic recycling. Additional exemplary polypeptides that can be expressed in a recycling endosome are disclosed in Tables 2 and 3 of Grant and Donaldson (2009), which listings are incorporated by reference in their entireties.

[0159] Rab5 is typically expressed in early endosomes and Rab7 is typically expressed in late endosomes. As disclosed in Cabrera and Ungermann (2010), early endosomes can be converted to late endosomes by an exchange of Rab GTPases which involves a reduction in (or loss of) Rab5, and an increase in Rab7. This process is also referred to as Rab conversion. Poteryaev (2010) discloses that SAND-1 / Mon1 can act as a switch and facilitate Rab conversion. For example, Poteryaev (2010) teaches that SAND-1/Mon1 displaces Rabx-5 (a Rab5 guanine exchange factor from early endosomes thus interrupting the positive feedback loop for Rab5 activation, and that SAND-1 is actively involved in the recruitment of Rab7 to endosomes.

[0160] In some embodiments, an early endosome and/or a recycling endosome is a vesicle in which Rab conversion (e.g., a reduction in Rab5 and/or an increase in Rab7) has not occurred, e.g., has not been initiated or has not been completed.

[0161] In some embodiments, an early endosome and/or a recycling endosome does not express or does not have detectable presence of a polypeptide associated with Rab conversion. In some embodiments, a polypeptide associated with Rab conversion comprises SAND-1/Mon1 or a variant or fragment thereof.

[0162] In some embodiments, an early endosome and/or a recycling endosome does not express or has no detectable presence of Rab7.

[0163] In some embodiments, an endosome as disclosed herein does not comprise a late endosome or a lysosome, or any other intracellular compartment in which a molecule can be degraded.

[0164] In some embodiments, a late endosome expresses or has detectable presence of one or more GTPases. In some embodiments, a GTPase expressed in a late endosome is Rab7 or a variant or fragment thereof.

[0165] In some embodiments, a late endosome expresses or has detectable presence of one or more subunits of a homotypic fusion and vacuole protein sorting (HOPS) complex, SAND-1/Mon1, Ccz1, one or more subunits of an ESCRT complex, or variants or fragments of

any of the foregoing. In some embodiments, a subunit of a HOPS complex comprises Vps39 or a variant or a fragment thereof.

[0166] In some embodiments, a late endosome and/or a lysosome is a vesicle in which Rab conversion (e.g., a reduction in Rab5 and/or an increase in Rab7) has occurred, e.g., has been initiated or has been completed. In some embodiments, a late endosome and/or a lysosome expresses or has detectable presence of a polypeptide associated with Rab conversion, e.g., SAND-1/Mon1.

[0167] In some embodiments, a late endosome and/or a lysosome does not express or has no detectable presence of Rab5.

[0168] In some embodiments, a multi-functional molecule utilizes an endocytic recycling system in a cell to internalize a multi-functional molecule or a complex comprising the same via binding of a first moiety to an endocytic receptor. In some embodiments, a multi-functional molecule utilizes an endocytic recycling system in a cell to externalize (e.g., recycle to a plasma membrane on the cell surface) a multi-functional molecule or a complex comprising the same via binding of a first moiety to an endocytic receptor and/or binding of a third moiety to an endocytic receptor. In some embodiments, upon internalization, a target which may be bound to a second moiety of a multi-functional molecule may dissociate from a second moiety and be degraded, e.g., by trafficking to a late endosome and/or lysosome. In some embodiments, a multi-functional molecule is not degraded, e.g., by not being trafficked to a late endosome and/or a lysosome.

[0169] In some embodiments, a multi-functional molecule disclosed herein comprises a plurality of moieties each of which can bind to a receptor and/or target under certain conditions. Binding of a moiety to a receptor and/or target under a specified condition allows for the moiety to be internalized into a cell, trafficked into a particular cellular compartment, and/or returned to the plasma membrane at the cell surface (e.g., recycled).

First set of conditions

[0170] A first set of conditions as disclosed herein comprise conditions which are typically observed on the surface of a cell and not in an internal compartment of a cell.

[0171] In some embodiments, a multi-functional molecule disclosed herein comprises a first moiety that binds under a first set of conditions.

[0172] In some embodiments, a multi-functional molecule disclosed herein comprises a second moiety that binds under a first set of conditions.

[0173] In some embodiments, a first set of conditions comprises a first pH (e.g., a pH of about pH 6.7 to about pH 8). In some embodiments, a first pH is or comprises a pH of at least 6.7, at least 6.8, at least 6.9, at least 7.0, at least 7.1, at least 7.2, at least 7.3, at least 7.4, at least 7.5, at least 7.6, at least 7.7, at least 7.8, at least 7.9, or at least 8.0.

[0174] In some embodiments, a first pH is or comprises a pH of about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, or about 8.0.

[0175] In some embodiments, a first pH is or comprises a pH of 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.

[0176] In some embodiments, a first pH comprises a pH of 6.7 to 8.0, pH of 6.7 to 7.9, pH of 6.7 to 7.8, pH of 6.7 to 7.7, pH of 6.7 to 7.6, pH of 6.7 to 7.5, pH of 6.7 to 7.4, pH of 6.7 to 7.3, pH of 6.7 to 7.2, pH of 6.7 to 7.1, pH of 6.7 to 7.0, pH of 6.7 to 6.9, or pH of 6.7 to 6.8. In some embodiments, a first pH comprises a pH of 6.8 to 8.0, pH of 6.9 to 8.0, pH of 7.0 to 8.0, pH of 7.1 to 8.0, pH of 7.2 to 8.0, pH of 7.3 to 8.0, pH of 7.4 to 8.0, pH of 7.5 to 8.0, pH of 7.6 to 8.0, pH of 7.7 to 8.0, pH of 7.8 to 8.0, pH of 7.9 to 8.0.

[0177] In some embodiments, an extracellular surface of a cell and/or space adjacent thereto has a first pH, e.g., as described herein.

[0178] In some embodiments, a first set of conditions comprises the presence of a cation. In some embodiments, a cation comprises Ca2+.

[0179] In some embodiments, a cation is present at a cell surface. In some embodiments, an extracellular surface of a cell has one or more cations, e.g., at a sufficient concentration, to allow binding of a first moiety to an endocytic receptor.

[0180] In some embodiments, a cation is not present in an intracellular compartment, e.g., an endosome. In some embodiments, an intracellular compartment of a cell, e.g., an endosome, does not have a sufficient concentration of one or more cations to allow binding of a first moiety to an endocytic receptor. In some embodiments, an endosome has an insufficient

concentration of one or more cations, e.g., only sufficient to allow reduced binding of a first moiety to an endocytic receptor.

Second set of conditions

[0181] A second set of conditions as disclosed herein comprise conditions which are typically observed in an intracellular compartment which has minimal or no proteolytic activity. In some embodiments, a multi-functional molecule disclosed herein comprises a first moiety that binds under a second set of conditions. In some embodiments, a multi-functional molecule disclosed herein comprises a third moiety that binds under a second set of conditions. Binding of a first moiety and/or a third moiety of a multi-functional molecule under a second set of conditions allows for trafficking of an internalized multi-functional molecule back to a plasma membrane at the cell surface (e.g., recycling of a multi-functional molecule).

[0182] In some embodiments, a second set of conditions comprises a second pH. In some embodiments, a second pH is or comprises a pH of about 6.5 to about 5.6. In some embodiments, a second pH is or comprises a pH of about 6.5, a pH of about 6.4, a pH of about 6.3, a pH of about 6.2, a pH of about 6.1, a pH of about 6.0, a pH of about 5.9, a pH of about 5.8, a pH of about 5.7, or a pH of about 5.6. In some embodiments, a second pH is or comprises a pH of 6.5, a pH of 6.4, a pH of 6.3, a pH of 6.2, a pH of 6.1, a pH of 6.0, a pH of 5.9, a pH of 5.8, a pH of 5.7, or a pH of 5.6.

[0183] In some embodiments, an intracellular compartment such as an endosome has a second pH, e.g., as described herein. In some embodiments, an endosome has a second pH of about 6.5 to about 5.6.

[0184] In some embodiments, a second set of conditions comprises presence in an intracellular vesicle comprising an endosome, e.g., an early endosome and/or a recycling endosome. In some embodiments, an intracellular vesicle associated with a second set of conditions does not include a late endosome and/or a lysosome.

[0185] In some embodiments, a second set of conditions comprises presence in an early endosome (sorting endosome).

[0186] In some embodiments, a second set of conditions comprises presence in a recycling endosome.

[0187] In some embodiments, a second set of conditions comprises presence in an intracellular vesicle which expresses, or has detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof. In some embodiments, a polypeptide associated with an early endosome and/or a recycling endosome comprises a GTPase, a sorting nexin or a combination thereof. In some embodiments, a GTPase comprises Arf6, Rab4, Rab5, Rab8, Rab10, Rab11, Rab22a, Rab35 or variants or functional fragments thereof.

[0188] In some embodiments, a second set of conditions comprises presence in an intracellular vesicle which has no detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof.

[0189] In some embodiments, a second set of conditions comprises presence in an intracellular vesicle which has the ability to export a molecule to an extracellular space. In some embodiments, an intracellular vesicle expresses one or more receptors that can bind to a molecule and export the molecule to an extracellular space.

[0190] In some embodiments, a second set of conditions comprises presence in an intracellular vesicle which does not have proteolytic activity. In some embodiments, proteolytic activity comprises hydrolyase activity.

Third set of conditions

[0191] A third set of conditions as disclosed herein comprise conditions which are typically observed in an intracellular compartment with proteolytic activity.

[0192] In some embodiments, a multi-functional molecule disclosed herein does not bind to a receptor and/or target under a third set of conditions.

[0193] In some embodiments, a third set of conditions comprises a third pH. In some embodiments, a third pH is a pH of less than 5.5. In some embodiments, a late endosome has a third pH. IN some embodiments, a lysosome has a third pH. In some embodiments, a third pH is a pH at which a target that has dissociated from a second moiety of an internalized multi-functional molecule is degraded.

[0194] In some embodiments, an intracellular compartment such as an endosome does not have a third pH, e.g., a pH less than 5.5.

[0195] In some embodiments, a third set of conditions comprises presence in an intracellular vesicle which has no detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof.

[0196] In some embodiments, a third set of conditions comprises presence in an intracellular vesicle which expresses or has detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof. In some embodiments, a polypeptide associated with a late endosome and/or a lysosome comprises Rab7 or a variant or fragment thereof.

[0197] In some embodiments, a third set of conditions comprises presence in an intracellular vesicle which has the proteolytic activity, e.g., hydrolyase activity.

[0198] In some embodiments, a third set of conditions comprises presence in a late endosome.

[0199] In some embodiments, a third set of conditions comprises presence in a lysosome.

Additional Elements

[0200] In some embodiments, a multi-functional molecule disclosed herein comprises a first moiety that binds to a first endocytic receptor, a second moiety that binds to a target at a first pH, a third moiety that binds to a second endocytic receptor at a second pH, and one or more additional elements. In some embodiments, a multi-functional molecule comprises a linker, a spacer, a signal peptide, a tag, a half-life extender or a combination thereof.

[0201] In some embodiments, a multi-functional molecule disclosed herein comprises one or more first moieties.

[0202] In some embodiments, a multi-functional molecule disclosed herein comprises one or more second moieties.

[0203] In some embodiments, a multi-functional molecule disclosed herein comprises one or more third moieties.

[0204] In some embodiments, a multi-functional molecule disclosed herein comprises one or more linkers. In some embodiments, linkers are situated between a first and a second

moiety. In some embodiments, linkers are situated between a first and a third moiety. In some embodiments, linkers are situated between a second and a third moiety.

[0205] In some embodiments, a multi-functional molecule comprises one or more peptides comprising a first moiety linked to one or more second moieties with a linker.

[0206] In some embodiments, a multi-functional molecule comprises one or more peptides comprising a first moiety linked to one or more third moieties with a linker.

[0207] In some embodiments, a linker comprises a Gly-Ser linker, or an EAAAK linker In some embodiments, a linker comprises a (Gly-Gly-Gly-Gly-Ser)n linker, wherein n is an integer between 0 to 20.

[0208] In some embodiments, a signal peptide is derived from a *Leishmania* species. In certain embodiments, a signal peptide is derived from *Leishmania tarentolae*. In certain embodiments, the signal peptide is an invertase signal peptide from derived from *Leishmania tarentolae*. In certain embodiments, a signal peptide comprises an amino acid sequence of SEQ ID NO: 35, or a portion thereof. In certain embodiments, a signal peptide comprises an amino acid sequence of SEQ ID NO: 36, or a portion thereof. In certain embodiments, a signal peptide is processed and removed from the multi-functional molecule.

[0209] Exemplary signal peptide: SPinv, a modified signal peptide from *Leishmania tarentolae* invertase, SEQ ID NO: 35: MIASSVRHAVILLLVAVAMMAAVIA

[0210] Exemplary signal peptide: SPinv, the native signal peptide from *Leishmania tarentolae* invertase, SEQ ID NO: 36: MIASSVRHAVILLLVAVAMMAAAVIA.

[0211] In some embodiments, a tag comprises a His tag, a Myc tag, or a GST tag, In some embodiments, a tag comprises a cleavable tag.

[0212] In some embodiments, a half-life extender comprises albumin or a fragment or a variant thereof.

[0213] In some embodiments, a half-life extender comprises a Fc domain, e.g., with or without mutations in an Fc domain.

First moiety

First moiety comprising peptide

[0214] A multi-functional molecule disclosed herein may comprises a first moiety that comprises one or more peptides and that binds to an endocytic receptor.

[0215] In some embodiments, a first moiety comprises an antibody agent. In some embodiments, an antibody agent comprises a full antibody, a Fab fragment, an scFv, a nanobody, a duobody, or a single domain antibody (e.g., a VHH).

[0216] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor under a first set of conditions, e.g., as described herein.

[0217] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor under a second set of conditions, e.g., as described herein.

[0218] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor under a first set of conditions, e.g., as described herein, and under a second set of conditions, e.g., as described herein.

[0219] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor at a first pH and/or a second pH. In some embodiments, a first moiety has reduced binding, or does not bind to an endocytic receptor at the third pH. In some embodiments reduced binding is assessed as compared to binding of the first moiety to the endocytic receptor at a first pH or a second pH. In some embodiments, binding affinity of the first moiety at the third pH (KD at third pH) is at least 1.5-fold lower compared to: the binding of the first moiety at a first pH (KD at first pH) or binding of the first moiety at a second pH (KD at second pH).

[0220] In some embodiments of a first moiety comprising one or more peptides, a first moiety undergoes a pH dependent conformation change. In some embodiments, a conformation change prevents binding or reduced binding to the endocytic receptor at the third pH.

[0221] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to the endocytic receptor with an affinity that allows for internalization of the multi-functional molecule into an intracellular compartment in the cell. In some embodiments, a first moiety binds to the endocytic receptor with an affinity that is at least an affinity required for internalizing the multi-functional molecule into an intracellular compartment in the cell (e.g., if the binding affinity required for internalization is "A nM," then the binding affinity of the first moiety to the first endocytic receptor is at least "A nM" or a numerical value that is smaller than "A nM").

[0222] As is well understood in the field and will be appreciated by one with knowledge in the field, a higher binding affinity is reflected by a lower dissociation constant (K_D), and a lower binding affinity is reflected by a higher dissociation constant (K_D). A K_D value can be measured by binding assays known in the field and provided as molar concentrations (e.g., micromolar [μM], nanomolar [nM], picomolar [pM], etc.). As a matter of example, a binding affinity of 10 nM is stronger (higher affinity) than a binding affinity of 100 nM (lower affinity).

[0223] In some embodiments, a first moiety stays bound to the endocytic receptor upon internalization into the intracellular compartment. In some embodiments, a first moiety does not stay bound to the endocytic receptor upon internalization into the intracellular compartment. In some embodiments, a first moiety binds a different endocytic receptor upon internalization into the intracellular compartment.

[0224] In some embodiments, an intracellular compartment is an endosome. In some embodiments, an endosome comprises an early endosome and/or a recycling endosome. In some embodiments, an intracellular compartment has a second pH.

[0225] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to the endocytic receptor with an affinity that allows for transporting the multi-functional molecule to the cell surface. In some embodiments, a first moiety binds to the endocytic receptor with an affinity that is at least an affinity required for transporting the multi-functional molecule to the cell surface (e.g., if the binding affinity required for transporting the multi-functional molecule to the cell surface is "B nM," then the binding affinity of the first moiety to the endocytic receptor is at least "B nM" or a numerical value that is smaller than "B nM").

[0226] In some embodiments of a first moiety comprising one or more peptides, a first moiety does not bind to the endocytic receptor with an affinity that allows for transporting the multi-functional molecule to a late endosome and/or lysosome. In some embodiments, a first moiety does not bind to the endocytic receptor with an affinity that is at or higher than an affinity required for transporting the multi-functional molecule to a late endosome and/or lysosome (e.g., if the binding affinity required for transporting the multi-functional molecule to a late endosome and/or lysosome is "C nM," then the binding affinity of the first moiety to the endocytic receptor is a numerical value that is larger than "C nM").

[0227] In some embodiments of a first moiety comprising one or more peptides, a multi-functional molecule with the first moiety bound to the endocytic receptor is delivered (e.g., recycled) to the plasma membrane at the cell surface.

[0228] In some embodiments of a first moiety comprising one or more peptides, a first moiety is bound to the same endocytic receptor for internalization into the cell and delivery to the plasma membrane at the cell surface.

[0229] In some embodiments of a first moiety comprising one or more peptides, a first moiety is bound to a different endocytic receptor for internalization into the cell and delivery to the plasma membrane at the cell surface.

[0230] In some embodiments of a first moiety comprising one or more peptides, upon delivery of the multi-functional molecule to the plasma membrane at the cell surface, the first moiety dissociates from the endocytic receptor thereby releasing the multi-functional molecule from the plasma membrane at the cell surface.

[0231] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0232] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor which is or comprises ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0233] In some embodiments of a first moiety comprising one or more peptides, a first moiety binds to an endocytic receptor which is or comprises a Siglec, one or more SNARE proteins, or a multi-drug transporter.

[0234] In some embodiments, a first moiety comprises a peptide that is about 5 amino acids in length to about 500 amino acids in length. In some embodiments, a first moiety comprises a peptide that is about 5 amino acids, about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about

40 amino acids, about 45 amino acids, about 50 amino acids, about 55 amino acids, about 60 amino acids, about 65 amino acids, about 70 amino acids, about 75 amino acids, about 80 amino acids, about 85 amino acids, about 90 amino acids, about 95 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids in length.

[0235] In some embodiments, a first moiety comprises a peptide that is at least 5 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 95 amino acids, at least 100 amino acids, at least 200 amino acids, at least 300 amino acids, at least 400 amino acids, at least 500 amino acids in length.

[0236] In some embodiments, a first moiety comprises one or more peptides that are about 50 amino acids in length to about 5000 amino acids in length in total. In some embodiments, a first moiety comprises one or more peptides that are about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids, about 600 amino acids, about 700 amino acids, about 800 amino acids, about 900 amino acids, about 1000 amino acids, about 1500 amino acids, about 2000 amino acids, about 2500 amino acids, about 3000 amino acids, about 3500 amino acids, about 4000 amino acids, about 4500 amino acids, about 5000 amino acids in length in total.

[0237] In some embodiments, a first moiety comprises 1, 2, 3, 4, 5, or more peptides that specifically bind to an endocytic receptor.

First moiety comprising glycans

[0238] A multi-functional molecule disclosed herein may comprise a first moiety comprising one or more glycans which specifically binds to an endocytic receptor, e.g., a first endocytic receptor as disclosed herein.

[0239] In some embodiments, one or more glycans are conjugated to the second and/or third moieties at one or more glycosylation sites.

[0240] In some embodiments, one or more glycans are present on a peptide that is conjugated (e.g., linked) to a second and/or third moiety.

[0241] Without being bound by any particular theory, glycan engagement with endocytic carbohydrate binding proteins and receptors enables different biological pathways. These essential biological pathways are involved in modulating immune responses, mediating protein clearance, protein turnover, and controlling trafficking of soluble glycoproteins, glycolipids and any natural molecule containing a glycan moiety. The glycan-receptor interaction is determined by the glycan structure. Glycan binding receptors are highly diverse and can be exploited by glycoengineering to develop novel therapeutics.

[0242] In some embodiments, a first moiety of a multi-functional molecule disclosed herein comprises one or more glycans and specifically binds to one or more endocytic receptors. Endocytic receptors are ubiquitous in human and can be found on different cells.

[0243] In some embodiments, an endocytic receptor is or comprises a receptor that can bind to and transport a molecule (e.g., a multi-functional molecule) across one or more cell membranes.

[0244] In some embodiments, an endocytic receptor is or comprises an endocytic lectin receptor. In some embodiments, the endocytic receptor is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0245] In some embodiments, an endocytic receptor is or comprises FcRn.

[0246] In some embodiments, an endocytic receptor is or comprises a Siglec, a SNARE protein, or a multidrug transporter.

[0247] In some embodiments, provided herein is a multi-functional molecule comprising a first moiety comprising a glycan comprising terminal GlcNAc.

[0248] In some embodiments, provided herein is a multi-functional molecule comprising a first moiety comprising a glycan comprising terminal GalNAc.

[0249] In some embodiments, provided herein is a multi-functional molecule comprising a first moiety comprising a glycan comprising terminal Gal.

[0250] In some embodiments, a multi-functional molecule provided herein can comprise (i) a binding specificity to one or more target protein(s) and (ii) one or more N-glycan(s) with binding specificities to one or more endocytic receptor(s).

[0251] In some embodiments, a multi-functional molecule comprises one type of N-glycan with binding specificity to one type of endocytic receptor.

[0252] In some embodiments, a multi-functional molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more N-glycosylation sites (or glycosites; such as an N-glycosylation consensus sequence). These N-glycosylation sites can be glycosylated by an N-glycan such that the resulting binding protein can engage with or bind to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more endocytic receptor molecules.

[0253] In some embodiments, a multi-functional molecule comprises two types of N-glycans with binding specificities to two different endocytic receptors. In certain embodiments, a multi-functional molecule provided herein can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more polypeptide chains. Each chain can be produced in a different cell line. In certain embodiments, the multi-functional molecule can be an antibody and one type of N-glycan is on the Fc domain and another type of N-glycan is on the Fab domain (e.g., the variable regions) of the antibody.

[0254] In some embodiments, a multi-functional molecule provided herein has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more glycosites. In some embodiments, in a population of multi-functional molecule, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% of the glycosites in the population at one specific position are glycosylated. In certain embodiments, in a population of multi-functional molecule, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% of the glycosites in the population are glycosylated. N-glycans that can be present at the glycosites of the multi-functional molecule provided herein are described herein.

[0255] In some embodiments, a glycosite is an N-glycosylation consensus sequence. The consensus sequence can be N-X-S/T, or N-X-C, wherein X is any amino acid except proline.

[0256] In some embodiments, an N-glycan is conjugated to the second moiety and/or third moiety at at least one, two, three, or four N-glycosylation sites. In some embodiments, an N-glycan is conjugated to the second moiety and/or third moiety at one, two, three, or four N-glycosylation sites.

[0257] In some embodiments, an N-glycosylation site is engineered into the amino acid sequence of the second moiety and/or third moiety.

[0258] In some embodiments, an N-glycan is present on a peptide of a first moiety. In some embodiments, an N-glycan is conjugated to the peptide at at least one, two, three, or four N-glycosylation sites. In some embodiments, an N-glycan is conjugated to the peptide at one, two, three, or four N-glycosylation sites.

[0259] In some embodiments, an N-glycosylation site is engineered into the amino acid sequence of the first moiety.

[0260] In some embodiments, an N-glycosylation site is naturally occurring.

[0261] In certain embodiments, one or more of the N-glycosylation sites are engineered into the amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety of the multi-functional molecule (*i.e.* one or more of the N-glycosylation sites are not present in a wild-type, or naturally occurring form of the first moiety). In certain embodiments, at least one of the N-glycosylation sites is engineered into the amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, at least two of the N-glycosylation sites are engineered into the amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, at least three of the N-glycosylation sites are engineered into amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, at least four of the N-glycosylation sites are engineered into the amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, one or more of the engineered N-glycosylation sites are glycotags fused to the N- and/or C-terminus of the amino acid sequence of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety via a peptide linker. In certain embodiments, a glycotag is fused to the N-terminus of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, a glycotag is fused to the C-

terminus of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, a glycotag is fused to the N- and the C-terminus of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety. In certain embodiments, one or more of the N-glycosylation sites are natural N-glycosylation sites (*i.e.* one or more of the N-glycosylation sites are present in a wild-type, or naturally occurring form of a peptide of the first moiety, a peptide of the second moiety and/or a peptide of the third moiety). In certain embodiments, at least one of the N-glycosylation sites is a natural N-glycosylation site. In certain embodiments, at least two of the N-glycosylation sites are natural N-glycosylation sites.

[0262] In some embodiments, provided herein is a multi-functional molecule comprising a first moiety comprising an N-glycan selected from the group consisting of GlcNAc2Man3GlcNAc2, GalNAc2GlcNAc2Man3 GlcNAc2, Gal2GlcNAc2Man3GlcNAc2, Man3 GlcNAc, GlcNAc lMan3 GlcNAc2, Gal2GlcNAc2Man3 GlcNAc2, Gal 1 GlcNAc2Man3 GlcNAc2, GalNAc 1 GlcNAc2Man3 GlcNAc2, GlcNAc3Man3 GlcNAc2, GlcNAc4Man3 GlcNAc2, Gal3GlcNAc3Man3 GlcNAc2, GalNAc3 GlcNAc3Man3 GlcNAc2, GalNAc4GlcNAc4Man3GlcNAc2, Gal4GlcNAc4Man3GlcNAc2, or Man-6-P -N-glycan.

[0263] In some embodiments, increasing the number of glycan structures on a multi-functional molecule increases the rate of lysosomal degradation as compared to an otherwise similar multi-functional molecule with fewer glycan structures.

[0264] In some embodiments, the number of glycan structures on a multi-functional molecule disclosed herein is 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more or 10 or more glycan structures.

[0265] In some embodiments, a multi-functional molecule disclosed herein comprises a glycan structure having a monoantennary structure.

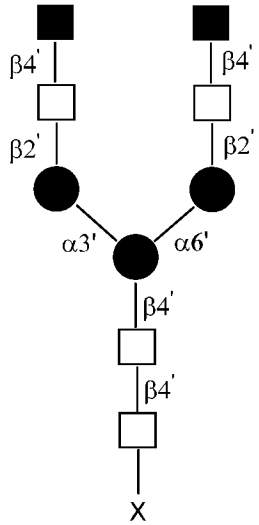
[0266] In some embodiments, a multi-functional molecule disclosed herein comprises a glycan structure having a biantennary structure.

[0267] In some embodiments, a multi-functional molecule disclosed herein comprises a glycan structure having a triantennary structure.

[0268] In some embodiments, a multi-functional molecule disclosed herein comprises a glycan structure having a tetraantennary structure.

[0269] In some embodiments, the glycan structure comprises a biantennary structure. In some embodiments, the glycan structure comprises a biantennary GalNAc. In some embodiments, the biantennary GalNac binds to an asialoglycoprotein receptor (ASGPR) or a fragment or variant thereof, or a complex comprising ASGPR.

[0270] In some embodiments, the N-glycan has a structure of:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the first moiety, second moiety or third moiety.

[0271] In some embodiments, the N-glycan specifically binds to one or more endocytic receptors. In some embodiments, the N-glycan specifically binds to ASGPR.

[0272] In some embodiments, the endocytic receptor is or comprises ASGPR or a fragment or variant thereof, or a complex comprising ASGPR. In some embodiments, when the endocytic receptor is ASGPR, the glycan structure of the second moiety comprises a terminal GalNac.

[0273] ASGPR-mediated degradation, for example, in the hepatocyte, has many applications. ASPGR binding to the N-glycan structure disclosed herein can result in the selective degradation of one or more target proteins. By way of example, ASGPR-mediated degradation can lead to removal of cytokines, chemokines and hormones. Additionally, ASGPR-mediated degradation can be used for the delivery of the target molecules to the endosome.

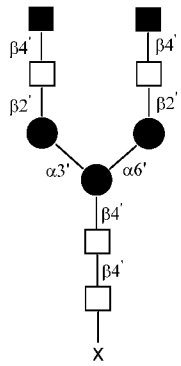
Thus, ASGPR-mediated degradation is applicable for various diseases, while limiting systemic toxicity.

[0274] In certain embodiments, the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more N-glycosylation sites can be glycosylated by the N-glycan such that the resulting multi-functional molecule can engage with or bind to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more endocytic receptor molecules. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 2 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 3 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 4 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 5 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 6 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 7 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 8 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 9 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at at least 10 N-glycosylation sites.

[0275] In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 2 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 3 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 4 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 5 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 6 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 7 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 8 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 9 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated by the N-glycan at 10 N-glycosylation sites. In certain embodiments, the multi-functional molecule is glycosylated at an

Asn amino acid residue of the multi-functional molecule. In certain embodiments, the N-glycosylation site is an N-glycosylation consensus sequence. In certain embodiments, the N-glycosylation site comprises a consensus sequence of N-X-S/T or N-X-C, wherein X is any amino acid except proline.

[0276] In certain embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95, or at least 98% of the N-glycosylation sites are occupied by an N-glycan. In certain embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95, or at least 98% of the N-glycosylation sites are occupied by an N-glycan of the structure:

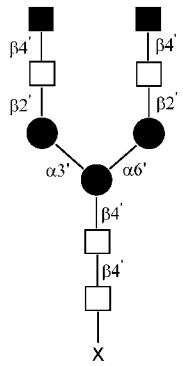


linked to the multi-functional molecule at one or more N-glycosylation sites, wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue the black circle represents a mannose (Man) residue, and X represents an amino acid residue of the multi-functional molecule. In certain embodiments, at least 10% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 20% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 30% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 40% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 50% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 60% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 70% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 80% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 90% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at

least 95% of the N-glycosylation sites are occupied by the N-glycan. In certain embodiments, at least 98% of the N-glycosylation sites are occupied by the N-glycan.

[0277] In certain embodiments, the N-glycan is linked to the multi-functional molecule at at least one N-glycosylation site. In certain embodiments, the N-glycan is linked to the multi-functional molecule at at least two N-glycosylation sites. In certain embodiments, the N-glycan is linked to the multi-functional molecule at one, two, three, or four N-glycosylation sites. In certain embodiments, the N-glycan is linked to the multi-functional molecule at one N-glycosylation site. In certain embodiments, the N-glycan is linked to the multi-functional molecule at two N-glycosylation sites. In certain embodiments, the N-glycan is linked to the multi-functional molecule at three N-glycosylation sites. In certain embodiments, the N-glycan is linked to the multi-functional molecule at four N-glycosylation sites. In certain embodiments, the N-glycan is linked to the multi-functional molecule at an Asn amino acid residue of the multi-functional molecule. In certain embodiments, the N-glycan is linked to the multi-functional molecule at an N-glycosylation consensus sequence. In certain embodiments, the N-glycan is linked to the multi-functional molecule at a consensus sequence of N-X-S/T or N-X-C, wherein X is any amino acid except proline.

[0278] In certain embodiments, the multi-functional molecule is glycosylated at two or more N-glycosylation sites by an N-glycan of the structure:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue the black circle represents a mannose (Man) residue, and X represents an amino acid residue of the multi-functional molecule, and wherein two of the N-glycosylation sites are separated by at least 5 , at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acids. In certain embodiments, the N-glycan is linked to the multi-functional molecule at

two N-glycosylation sites separated by a distance of about 5-10, about 10-20, about 20-30, about 30-40, about 40-50, about 50-60, about 60-70, about 70-80, about 80-90, about 90-100, about 100-150, about 150-200, or about 200-300 amino acids. In certain embodiments, the amino acid separation between the N-glycosylation sites is the number of amino acids between the terminal amino acids of the N-glycosylation consensus sequence. Without being bound by theory, the multi-functional molecule folds in space and, thus, has a three-dimensional geometry in addition to its primary amino acid structure. Also without being bound by theory, this three-dimensional geometry, including the position of the N-glycan is not static but dynamic (see, for example, Re, S., *et al* Biophysical Reviews, 4, 179-187 (2012)). Notwithstanding, in certain embodiments, the distance between N-glycosylation sites and/or N-glycans on a multi-functional molecule may be from an equilibrium geometry of the multi-functional molecule, as determined by any standard means known in the art, including for example computational modelling studies. In certain embodiments, the distance between the N-glycosylation sites and/or N-glycans is chosen to minimize steric hindrance. In certain embodiments, the distance between the N-glycosylation sites and/or N-glycans is chosen based on the separation of ASGPR receptors on a cell surface. In certain embodiments, the distance between the N-glycosylation sites and/or N-glycans is chosen to be similar (e.g. no more than twice, or no less than half) to the separation of ASGPR receptors on a cell surface. In certain embodiments, the N-glycan is linked to the multi-functional molecule at an Asn amino acid residue of the multi-functional molecule (e.g., a moiety of a multi-functional molecule). In certain embodiments, the N-glycan is linked to the multi-functional molecule at an N-glycosylation consensus sequence. In certain embodiments, the N-glycan is linked to the multi-functional molecule at a consensus sequence of N-X-S/T or N-X-C, wherein X is any amino acid except proline.

Second moiety comprising target binding domain

[0279] Also disclosed herein are multi-functional molecules comprising a second moiety that specifically binds to a target in a pH dependent manner. In some embodiments, a second moiety binds to a target at a first pH, e.g., a pH at an extracellular surface of a cell. In some embodiments, a second moiety has reduced binding (e.g., reduced binding affinity), or does not bind to a target at a second pH, e.g., a pH in an intracellular compartment of a cell, e.g., an

endosome. In some embodiments, reduced binding affinity is assessed as compared to binding affinity of a second moiety to a target at a first pH.

[0280] Exemplary modifications to an antigen binding fragment of an antibody agent for pH dependent antigen binding are disclosed, e.g., in Igawa T et al., (2010) *Nature Biotechnology* 28:11, pp. 1203-1208; and U.S. Patent 9,540,449, the entire contents of each of which are hereby incorporated by reference. As taught in these references, modifying one or more amino acids in a complementarity determining region (CDR) of an antibody to a Histidine can confer pH-dependent binding wherein the modified antibody binds to a target at a physiological pH and dissociates from (or binds with a lower affinity to) a target at an acidic pH.

[0281] In some embodiments, a second moiety comprises one or more peptides that specifically binds to a target. In some embodiments, a second moiety comprises an antibody agent that binds to a target. In some embodiments, an antibody agent comprises an antigen binding fragment. In some embodiments, an antibody agent comprises a full antibody, a Fab fragment, an scFv, a nanobody, a duobody, a single domain antibody (e.g., a VHH). In some embodiments, the antibody agent comprises a VHH, e.g., a camelid VHH or a bivalent VHH.

[0282] In some embodiments, an antibody agent comprises an antigen binding fragment having (1) at least one amino acid substituted with a histidine as compared to a wild type sequence; or (2) insertion of at least one histidine as compared to a wild type sequence, or (3) both.

[0283] Furthermore, U.S. Patent 9,540,449 also discloses mutations to an Fc domain which enhance or diminish antibody binding to the FcRn receptor. For example, mutations in the CH2 or CH3 regions of an Fc domain can increase the affinity of the Fc domain to FcRn in an acidic environment, e.g., in an endosome. Such Fc mutations disclosed therein are hereby expressly incorporated by reference.

[0284] In some embodiments, an antibody agent comprises an Fc domain having one or more mutations to alter binding to a receptor, e.g., FcRn. For example, in embodiments of a multi-functional molecule comprising a first moiety (e.g., glycans) that binds to an endocytic receptor and a second moiety that binds to a target in a pH dependent manner, a second moiety comprises an antibody agent having a Fc domain with one or more mutations to improve binding to a receptor (e.g., FcRn) in an acidic environment (e.g., second pH of endosome). In some

embodiments, a mutated Fc domain allows for binding of the second moiety to FcRn in an endosome and allow transport of the multi-functional molecule back to the surface of the cell.

[0285] In some embodiments, a second moiety undergoes a pH dependent conformation change. In some embodiments, a conformation change prevents binding or results in reduced binding to a target at a second pH. In some embodiments, at a second pH a second moiety undergoes a conformation change that blocks one or more epitopes at which a target can bind a second moiety. In some embodiments, at a first pH said one or more epitopes of a second moiety are available for binding to a target.

[0286] In some embodiments, binding affinity of an antibody agent of a second moiety to a target at a first pH (KD at first pH) is at least 1.5-fold lower, at least 2-fold lower, at least 4-fold lower, at least 6-fold lower, at least 8-fold lower, at least 10-fold lower, or at least 20-fold lower compared to a binding of an antibody agent to a target at a second pH (KD at second pH).

[0287] In some embodiments, an antibody agent of a second moiety has a ratio of Kd at a second pH / Kd at a first pH of greater than about 3.0. In some embodiments, an antibody agent of a second moiety has a ratio of Kd at a second pH / Kd at a first pH of less than about 0.8.

[0288] Binding properties of an antibody to a particular antigen can be measured by half-life of the antibody-antigen interaction. In some embodiments, an antibody agent of a second moiety has a ratio of half-life (t½) at a second pH / t½ at a first pH of less than about 1.0. In some embodiments, an antibody agent of a second moiety has a ratio of half-life (t½) at a second pH / t½ at a first pH of greater than about 0.15. In some embodiments, an antibody agent of a second moiety has a lower t½ at a second pH as compared to a t½ at a first pH. In some embodiments, a t½ at a second pH is at least 2-fold, 5-fold, 10-fold, 20-fold, or 50-fold lesser than a t½ at a first pH.

[0289] In some embodiments, a target is a secreted protein. In some embodiments, a target is an antibody. In some embodiments, a target is an auto-antibody.

[0290] In some embodiments, a target is membrane-bound.

[0291] In some embodiments, a second moiety comprises a peptide that is about 5 amino acids in length to about 500 amino acids in length. In some embodiments, a first moiety comprises a peptide that is about 5 amino acids, about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, about 50 amino acids, about 55 amino acids, about 60

amino acids, about 65 amino acids, about 70 amino acids, about 75 amino acids, about 80 amino acids, about 85 amino acids, about 90 amino acids, about 95 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids in length.

[0292] In some embodiments, a second moiety comprises a peptide that is at least 5 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 95 amino acids, at least 100 amino acids, at least 200 amino acids, at least 300 amino acids, at least 400 amino acids, at least 500 amino acids in length.

[0293] In some embodiments, a second moiety comprises one or more peptides that are about 50 amino acids in length to about 5000 amino acids in length in total. In some embodiments, a second moiety comprises one or more peptides that are about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids, about 600 amino acids, about 700 amino acids, about 800 amino acids, about 900 amino acids, about 1000 amino acids, about 1500 amino acids, about 2000 amino acids, about 2500 amino acids, about 3000 amino acids, about 3500 amino acids, about 4000 amino acids, about 4500 amino acids, about 5000 amino acids in length in total.

[0294] In some embodiments, a second moiety comprises 1, 2, 3, 4, 5, or more peptides that specifically bind to a target.

[0295] In some embodiments, a multi-functional molecule comprises one or more second moieties.

Third moiety

[0296] Also disclosed herein are multi-functional molecules comprising a third moiety that specifically binds to an endocytic receptor (e.g., a second endocytic receptor) in a pH dependent manner. In some embodiments, a third moiety binds to an endocytic receptor at a second pH, e.g., a pH found in an intracellular compartment of a cell, e.g., an endosome. In

some embodiments, a third moiety has reduced binding (e.g., reduced binding affinity), or does not bind to an endocytic receptor at a first pH, e.g., a pH found in the extracellular environment of a cell. In some embodiments, reduced binding affinity is assessed as compared to binding affinity of a third moiety to an endocytic receptor at a second pH.

[0297] In some embodiments, a third moiety has reduced binding (e.g., reduced binding affinity), or does not bind to an endocytic receptor at a third pH (e.g., a pH found in a late endosome and/or a lysosome).

[0298] In some embodiments, a third moiety comprises one or more peptides that specifically binds to an endocytic receptor. In some embodiments, a third moiety comprises an antibody agent that binds to an endocytic receptor. In some embodiments, an antibody agent comprises an antigen binding fragment. In some embodiments, an antibody agent comprises a full antibody, a Fab fragment, an scFv, a nanobody, a duobody, a single domain antibody (e.g., a VHH). In some embodiments, the antibody agent comprises a VHH, e.g., a camelid VHH or a bivalent VHH.

[0299] In some embodiments, a third moiety undergoes a pH dependent conformation change. In some embodiments, a conformation change prevents binding or results in reduced binding to an endocytic receptor at a first pH. In some embodiments, at a first pH a third moiety undergoes a conformation change that blocks one or more epitopes at which an endocytic receptor can bind a third moiety. In some embodiments, at a second pH said one or more epitopes of a third moiety are available for binding to a endocytic receptor.

[0300] In some embodiments, a binding affinity of an antibody agent of a third moiety to an endocytic receptor at a second pH (KD at second pH) is at least 1.5-fold lower, at least 2-fold lower, at least 4-fold lower, at least 6-fold lower, at least 8-fold lower, at least 10-fold lower or at least 20-fold lower compared to the binding of an antibody agent of a third moiety to an endocytic receptor at a second pH (KD at second pH).

[0301] In some embodiments, an antibody agent of a second moiety has a ratio of Kd at a first pH / Kd at a second pH of greater than about 3.0. In some embodiments, an antibody agent of a second moiety has a ratio of Kd at a first pH / Kd at a second pH of less than about 0.8.

[0302] Binding properties of an antibody to a particular antigen can be measured by half-life of the antibody-antigen interaction. In some embodiments, an antibody agent of a third moiety has a ratio of half-life (t½) at a first pH / t½ at a second pH of less than about 1.0. In some

embodiments, an antibody agent of a third moiety has a ratio of half-life (t½) at a first pH / t½ at a second pH of greater than about 0.15. In some embodiments, an antibody agent of a third moiety has a lower t½ at a first pH as compared to a t½ at a second pH. In some embodiments, a t½ at a first pH is at least 2-fold, 5-fold, 10-fold, 20-fold, or 50-fold lesser than a t½ at a second pH.

[0303] In some embodiments, a third moiety binds to an endocytic receptor, e.g., a second endocytic receptor, with an affinity that allows for transporting a multi-functional molecule to a cell surface. In some embodiments, a third moiety binds to an endocytic receptor, e.g., a second endocytic receptor, with an affinity that is at least an affinity required for transporting a multi-functional molecule to a cell surface (e.g., if the binding affinity required for transporting the multi-functional molecule to the cell surface is "D nM," then the binding affinity of the third moiety to the endocytic receptor is at least "D nM" or a numerical value that is smaller than "D nM").

[0304] In some embodiments, a multi-functional molecule with a third moiety bound to an endocytic receptor, e.g., a second endocytic receptor, is delivered to the cell surface via exocytosis.

[0305] In some embodiments, a third moiety does not bind to an endocytic receptor, e.g., a second endocytic receptor, with an affinity that is at or higher than an affinity required for transporting a multi-functional molecule to a lysosome.

[0306] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is or comprises an endocytic lectin receptor.

[0307] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0308] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR. In some

embodiments, when an endocytic receptor is ASGPR or a fragment or variant thereof, a third moiety comprises an antibody agent that binds to ASGPR.

[0309] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is not the neonatal Fc receptor (FcRn).

[0310] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is or comprises the neonatal Fc receptor (FcRn).

[0311] In some embodiments, an endocytic receptor, e.g., a second endocytic receptor, is or comprises a Siglec, one or more SNARE proteins, or a multi-drug transporter.

[0312] In some embodiments, a third moiety comprises a peptide that is about 5 amino acids in length to about 500 amino acids in length. In some embodiments, a third moiety comprises a peptide that is about 5 amino acids, about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, about 50 amino acids, about 55 amino acids, about 60 amino acids, about 65 amino acids, about 70 amino acids, about 75 amino acids, about 80 amino acids, about 85 amino acids, about 90 amino acids, about 95 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids in length.

[0313] In some embodiments, a third moiety comprises a peptide that is at least 5 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 55 amino acids, at least 60 amino acids, at least 65 amino acids, at least 70 amino acids, at least 75 amino acids, at least 80 amino acids, at least 85 amino acids, at least 90 amino acids, at least 95 amino acids, at least 100 amino acids, at least 200 amino acids, at least 300 amino acids, at least 400 amino acids, at least 500 amino acids in length.

[0314] In some embodiments, a third moiety comprises one or more peptides that are about 50 amino acids in length to about 5000 amino acids in length in total. In some embodiments, a third moiety comprises one or more peptides that are about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, about 100 amino acids, about 200 amino acids, about 300 amino acids, about 400 amino acids, about 500 amino acids, about 600 amino acids, about 700 amino acids, about 800 amino acids, about

900 amino acids, about 1000 amino acids, about 1500 amino acids, about 2000 amino acids, about 2500 amino acids, about 3000 amino acids, about 3500 amino acids, about 4000 amino acids, about 4500 amino acids, about 5000 amino acids in length in total.

[0315] In some embodiments, a third moiety comprises 1, 2, 3, 4, 5, or more peptides that specifically bind to an endocytic receptor.

[0316] In some embodiments, a multi-functional molecule comprises one or more third moieties.

Nucleic acid sequences encoding multi-functional molecules

[0317] The present disclosure, among other things, provides nucleic acid sequences encoding a multi-functional molecule as described herein.

[0318] In some embodiments, a nucleic acid sequence is or comprises single stranded DNA (e.g., as in certain viral vectors). In some embodiments, a nucleic acid is or comprises double stranded DNA (e.g., as in certain viral vectors and/or certain plasmids). In some embodiments, a nucleic acid is or comprises RNA (e.g., as in certain viral vectors and/or as in mRNA therapeutics), etc.

[0319] Nucleic acids encoding a multi-functional molecule may be modified to include codons that are optimized for expression in a particular cell type (e.g., a Leishmania cell) or organism. Codon optimized sequences are synthetic sequences, and preferably encode an identical polypeptide (or biologically active fragment of a full length polypeptide which has substantially the same activity as the full length polypeptide) encoded by a non-codon optimized parent polynucleotide. In some embodiments, a coding region of a nucleic acids encoding a multi-functional molecule described herein, in whole or in part, may include an altered sequence to optimize codon usage for a particular cell type (e.g., a eukaryotic or prokaryotic cell). For example, a coding sequence for an antibody agent (e.g., antigen binding fragment) as described herein may be optimized for expression in a bacterial cells. Alternatively, the coding sequence may be optimized for expression in a mammalian cell (e.g., a CHO cell). Such a sequence may be described as a codon-optimized sequence.

[0320] Nucleic acid constructs of the present disclosure may be inserted into an expression vector or viral vector by methods known to the art, and nucleic acids may be operably linked to an expression control sequence. A vector comprising any nucleic acids or fragments

thereof described herein is further provided by the present disclosure. Any nucleic acids or fragments thereof described herein can be cloned into any suitable vector and can be used to transform or transfect any suitable host (e.g., Leishmania host cell). Selection of vectors and methods to construct them are commonly known to persons of ordinary skill in the art.

[0321] In some embodiments, nucleic acids and vectors of the present disclosure are isolated and/or purified. The present disclosure also provides a composition comprising an isolated or purified nucleic acid, optionally in the form of a vector. Isolated nucleic acids and vectors may be prepared using standard techniques known in the art including, for example, alkali/SDS treatment, CsCl binding, column chromatography, agarose gel electrophoresis, and/or other techniques well known in the art. The composition can comprise other components as described further herein.

[0322] Any method known to one skilled in the art for the insertion of nucleic acids into a vector may be used to construct expression vectors encoding a multi-functional molecule described herein under control of transcriptional and/or translational control signals. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombination (see, e.g., Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989); and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994), each of which is hereby incorporated by reference in its entirety).

Compositions and pharmaceutical compositions

[0323] A composition disclosed herein may comprise and/or deliver one or more multi-functional molecules disclosed herein or nucleic acids encoding one or more multi-functional molecules disclosed herein.

[0324] In some embodiments, a composition disclosed herein comprises a multi-functional molecule comprising a first moiety and a second moiety. In some embodiments, a composition disclosed herein comprises a multi-functional molecule comprising one or more first moieties, and one or more second moieties.

[0325] In some embodiments, a composition disclosed herein comprises a multi-functional molecule comprising a first moiety, a second moiety, and a third moiety. In some

embodiments, a composition disclosed herein comprises a multi-functional molecule comprising one or more first moieties, one or more second moieties, and one or more third moieties.

[0326] In some embodiments, a composition disclosed herein comprises a plurality of multi-functional molecule comprising a first moiety and a second moiety. In some embodiments, a composition comprising a plurality of multi-functional molecules comprises 1, 2, 3, 4, 5, or more multi-functional molecules.

[0327] In some embodiments, a composition disclosed herein comprises a plurality of multi-functional molecule comprising a first moiety, a second moiety, and a third moiety. In some embodiments, a composition comprising a plurality of multi-functional molecules comprises 1, 2, 3, 4, 5, or more multi-functional molecules.

[0328] In some embodiments, disclosed herein is a composition comprising a population of multi-functional molecules, wherein the population of multi-functional molecules has an N-glycan profile that is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or about 100% homogeneous at one or more of the N-glycosylation site(s).

[0329] In some embodiments, the homogeneity of the N-glycan profile at one or more of the N-glycosylation sites is determined by N-glycan analysis, glycopeptide analysis or intact protein analysis.

[0330] In some embodiments, the N-glycan profile comprises about 30% to 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% of the N-glycan of the structure provided herein.

[0331] In some embodiments, the population of multi-functional molecules has an N-glycan profile comprising about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% of the N-glycan of the structure provided herein among all glycans in the N-glycan profile.

[0332] In some embodiments, multi-functional molecules disclosed herein or a composition comprising the same may be useful to treat and/or prevent a disease described herein, or to ameliorate a symptom associated with a disease, disorder or condition described herein.

[0333] The present disclosure also provides pharmaceutical compositions that, when administered to a subject (e.g., a human subject) suffering from a disease associated with a soluble protein as disclosed herein, deliver a multi-functional molecule as described herein to such subject. Thus, in some embodiments, the present disclosure provides pharmaceutical compositions that comprise or deliver one or more multi-functional molecules or one or more polynucleotides encoding such as described herein.

[0334] In some embodiments, a pharmaceutical composition is or comprises a composition according to the present disclosure.

[0335] Typically, a pharmaceutical composition includes a multi-functional molecule as described herein, or a nucleic acid that encodes it in combination with one or more pharmaceutically acceptable carriers or excipients such as, for example one or more buffers, diluents, fillers, salts, solubilizers, stabilizers, and/or other materials as is known in the art. Those skilled in the art will be aware of a variety of carrier components appropriate to a particular active type (*e.g.,* polypeptide versus nucleic acid, viral vector vs plasmid versus RNA, *etc.*) and/or route of administration (*e.g.,* parenteral, enteral, *etc.*).

[0336] In some embodiments, a pharmaceutical composition, may comprise or deliver two or more different multi-functional molecules, so that such agents may be administered in combination (*e.g.,* substantially simultaneously or sequentially) to subject(s).

[0337] In some embodiments, a pharmaceutical composition may contain one or more agents that, for example, may improve stability of the composition and/or its active agent (*e.g.,* to particular storage conditions and/or period(s) of time), facilitate delivery of the composition and/or its active agent, and/or otherwise enhance effectiveness (and/or reduce one or more undesirable side effects) of the active agent or composition once administered.

[0338] Alternatively or additionally, in some embodiments, a provided pharmaceutical composition may comprise or deliver another active agent in addition to a multi-functional molecule as described herein.

Methods of treatment and/or prevention

[0339] In accordance with various embodiments, multi-functional molecules, compositions, and methods provided herein may be used to treat any disease, disorder or

condition, thereby improving at least one sign or symptom of a disease, disorder or condition. In some embodiments, a disease, disorder or condition disclosed herein is one in which degradation of one or more targets would be useful to treat one or more signs or symptoms of a disease, disorder or condition.

[0340] In some embodiments, provided herein are methods of treating a disease or disorder associated with a target, e.g., a soluble protein. In some embodiments, a target binds to a second moiety of a multi-functional molecule disclosed herein. In some embodiments, a target is an antibody (e.g., an autoantibody) or a fragment thereof. In some embodiments, a target is an antigen recognized by an autoantibody, or a fragment thereof. In some embodiments, a target is a secreted protein. In some embodiments, a target is cleaved off a cell surface. In some embodiments, a target is an enzyme. In some embodiments, a target is a chemokine or cytokine.

[0341] In some embodiments, provided herein are methods of treating a disease or disorder chosen from: an autoimmune disease, a metabolic disease, a genetic disease, a fibrotic disease, a rare disease, a neurodegenerative disease, a vascular disease, a cancer, or a disease requiring enzyme replacement therapy.

[0342] In some embodiments, a disease or disorder is an autoimmune disorder, e.g., as disclosed herein.

[0343] In some embodiments, a disease or disorder is a metabolic disease. In some embodiments, a metabolic disease is chosen from: diabetes, mitochondrial disorders, hemochromatosis, Gaucher's disease, amyloid neuropathy; amyloidosis; cirrhosis; glycogen storage diseases; hepatic lipase deficiency (LIPC); medullary cystic kidney disease; phenylketonuria; or polycystic kidney disease.

[0344] In some embodiments, a disease or disorder is a genetic disease.

[0345] In some embodiments, a disease or disorder is a fibrotic disease.

[0346] In some embodiments, a disease or disorder is a cancer or a metastasis thereof.

[0347] In some embodiments, a cancer is a solid tumor or a metastasis thereof. In some embodiments, a solid tumor is sarcoma or a carcinoma. In some embodiments, a solid tumor is chosen from:fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian

cancer, prostate cancer, hepatocellular carcinoma, squamous eel! carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors. In some embodiments, a CNS tumor is chosen from glioma (e.g., brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pineaioma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases.

[0348] In some embodiments, a cancer is a hematological cancer or a metastasis thereof. In some embodiments, a hematological cancer is chosen from chronic lymphocytic leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, acute myelogenous leukemia and acute myeloblastic leukemia), myeloma (e.g., multiple myeloma) lymphoma (e.g., Hodgkin's lymphoma or non-Hodgkin's lymphoma.

[0349] A subject to be treated with methods described herein can be e.g., a patient having, or at risk of having, or is diagnosed as having a disease, disorder or condition disclosed herein.

[0350] Those skilled in the art, reading the present disclosure, will appreciate that provided compositions, may be useful for treating a disease associated with a target, e.g., a soluble protein, e.g., as disclosed herein.

[0351] In some embodiments, a method of treating and/or preventing a disease, disorder or condition in a subject comprises administering to a subject a composition according to the present disclosure. In some embodiments, administration of a composition reduces a level a target as compared to a subject who has not been administered the composition or as compared to the same subject prior to administration of the composition. In some embodiments, a reduction in the level of a target treats and/or prevents one or more signs and/or symptoms of the disease, disorder or condition.

[0352] In some embodiments, a composition according to the present invention is used to reduce and/or degrade a target in a subject having a disease, disorder or condition. In some

embodiments, a target is a soluble protein, e.g., which can bind to a second moiety of a multi-functional molecule disclosed herein.

[0353] In some embodiments, a method of degrading a target comprises contacting a cell with a complex comprising a multi-functional molecule disclosed herein and a target, under conditions sufficient to degrade a target in a cell. In some embodiments, a multi-functional molecule is delivered to the cell or administered to a subject comprising the cell, such that the target and the multi-functional molecule form a complex. In some embodiments, degradation of the target can be modulated by altering the number of glycans of the first moiety. In some embodiments, increasing the number of glycans increases the rate of degradation of the target.

[0354] In some embodiments, a composition according to the present invention is used to reduce the risk of a disease, disorder or condition associated with expression of a target.

[0355] In some embodiments, methods disclosed herein comprise delivering a target to a cell, the method comprising contacting a cell with a complex comprising a multi-functional molecule and a target, under conditions sufficient to deliver the complex comprising the multi-functional molecule to the cell. In some embodiments, contacting comprises internalization of a complex into a compartment of a cell. In some embodiments, a compartment is an endosome. In some embodiments, a target dissociates from a complex in an endosome. In some embodiments, a target is degraded. In some embodiments, degradation occurs in a lysosome. In some embodiments, a multi-functional molecule from which a target dissociated remains in an endosome. In some embodiments, a multi-functional molecule is delivered to a surface of a cell via binding of a third moiety to an endocytic receptor in an endosome.

Autoimmune diseases

[0356] Compositions disclosed herein may be used to treat and/or prevent autoimmune diseases. In some embodiments, an autoimmune disease is associated with autoantibodies and/or autoantigens. In some embodiments, administration to a subject of a multi-functional molecule or a composition comprising the same in which a second moiety binds to an autoantibody (or a fragment thereof), an autoantigen, and/or an immune complex comprising the same, may be useful in degrading the autoantibody, autoantigen and/or immune complex. In some embodiments, degradation of an autoantibody, autoantigen and/or immune complex comprising the same treats and/or prevents a sign or symptom of an autoimmune disease. Exemplary

autoimmune diseases that may be treated and/or prevented with a multi-functional molecule or a composition comprising the same as provided herein.

[0357] In some embodiments, an autoimmune disease is ANCA vasculitis, e.g., as described herein.

[0358] In some embodiments, an autoimmune disease is membranous nephropathy, e.g., as described herein.

[0359] In some embodiments, an autoimmune disease is iTTP, e.g., as described herein.

[0360] In some embodiments, an autoimmune disease is IgA nephropathy, e.g., as described herein.

ANCA vasculitis

[0361] ANCA vasculitis is a systemic disease that may involve ears, nose and throat, lungs, kidneys, heart, digestive system, nervous system, eyes, skin, musculoskeletal tract and, infrequently, other organs (Hilhorst, M. et al. J Am Soc Neprhol, 2015). ANCA vasculitis may be induced by autoimmunity (e.g., anti-neutrophil autoantibodies) to neutrophil granule proteins (e.g., neutrophil autoantigens), such as myeloperoxidase (MPO) or proteinase 3 (PR3) as described herein. Anti-neutrophil autoantibodies against PR3 or MPO can alone or in combination result in vasculitis (e.g., small to medium-vessel vasculitis). In some embodiments, anti-neutrophil autoantibodies that specifically bind to PR3 and/or MPO cause ANCA vasculitis. Additional anti-neutrophil autoantibodies have been identified in subjects with ANCA vasculitis, which may be causative of or contribute to development or severity of a neutrophil autoantibody associated disease, including LAMP-2 and PTX3.

[0362] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-neutrophil autoantibody, or a fragment or a complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-neutrophil autoantibody, or a fragment or a complex thereof, can be used to treat and/or prevent ANCA vasculitis.

Microscopic Polyangiitis (MPA)/perinuclear ANCA

[0363] Autoimmunity to MPO (e.g., generation of anti-MPO autoantibodies) is strongly associated with Microscopic Polyangiitis (MPA)/perinuclear ANCA. MPA is often characterized by vasculitis limited to the kidneys. Studies have shown that 1 out of 3 patients with MPA progress to dialysis or kidney transplantation (Hilhorst, M. et al. J Am Soc Neprhol, 2015).

[0364] In some embodiments, ANCA vasculitis is Microscopic Polyangiitis (MPA)/perinuclear ANCA.

[0365] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-MPO autoantibody, or a fragment or a complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-MPO autoantibody, or a fragment or a complex thereof, can be used to treat and/or prevent Microscopic Polyangiitis (MPA)/perinuclear ANCA.

Granulomatosis with Polyangiitis (GPA)/cytoplasmic ANCA

[0366] Autoimmunity to PR3 (e.g., generation of PR3 autoantibodies) is strongly associated with Granulomatosis with Polyangiitis (GPA)/cytoplasmic ANCA (Formerly called Wegener's Granulomatosis). GPA if often characterized by granulomatous inflammation of the respiratory tract, necrotizing small-vessel vasculitis, and glomerulonephritis. A hallmark of GPA is granulomatous inflammation. Granuloma formation is thought to be initiated by small aggregates of neutrophils surrounding necrotic areas (microabscess) (Hilhorst, M. et al. J Am Soc Neprhol, 2015).

[0367] In some embodiments, ANCA vasculitis is Granulomatosis with Polyangiitis (GPA)/cytoplasmic ANCA.

[0368] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-PR3 autoantibody or a fragment or a complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-PR3 autoantibody or a fragment or a complex thereof, can be used to treat and/or prevent Granulomatosis with Polyangiitis (GPA)/cytoplasmic ANCA.

Membranous Nephropathy

[0369] Membranous nephropathy (MN) is a glomerular disease that can occur at all ages. In adults, it is the most frequent cause of nephrotic syndrome. Membranous nephropathy is an autoimmune disease which is characterized by a thickening of glomerular capillary walls due to immune complex deposition on the subepithelial side of the glomerular basement membrane (GBM). Immune deposits typically comprise of immunoglobulin G (IgG) (e.g., one or more anti-podocyte autoantibodies), one or more podocyte autoantigens, and/or one or more complement complexes (e.g., a membrane attack complex [MAC]) (Ronco et al., (2021) "Membranous Nephropathy" *Nature Reviews: Disease Primer* 7:69). The complement MAC C5b-9 induces a variety of downstream pathways which can result in podocyte injury. Among the pathways which can be induced by the complement MAC include: protein kinases, lipid metabolism, reactive oxygen species, growth factors, gene transcription, endoplasmic reticulum stress and the ubiquitin-proteasome system (Ke et al., (2022) BMC Nephrology 23:313).

[0370] Depending on the cause, membranous nephropathy can be classified as primary (idiopathic) or secondary membranous nephropathy, which account for 75%–80% and 20%–25% of MN, respectively. Primary or idiopathic membranous nephropathy cases are most common and are often associated with autoantibodies recognizing podocyte autoantigens which can form immune complexes along the glomerular basement membrane (GBM). Secondary membranous nephropathy cases are often associated with autoimmune diseases, malignancies, infections, and/or drugs. A recent report has suggested a role for VEGFA in the pathogenesis of idiopathic membranous nephropathy. According to Ke et al. 2022, VEGFA induced activation of the PI3K-Akt signaling pathway can lead to vascular hyperpermeability resulting in increased filtration of inflammatory factors, complements, and cytokines.

[0371] In some embodiments, administration of a multi-functional molecule can be used to treat and/or prevent Membranous nephropathy.

<u>Anti-podocyte autoantibodies and podocyte autoantigens</u>

[0372] Membranous nephropathy (e.g., idiopathic membranous nephropathy) may be induced by autoimmunity to one or more autoantigens expressed on podocytes (e.g., by the development of anti-podocyte autoantibodies). In some embodiments, anti-podocyte autoantibodies or immune complexes comprising the same form deposits at the glomerular basement membrane (GBM). In some embodiments, anti-podocyte autoantibodies or immune complexes comprising the same may cause podocyte injury. In some embodiments, anti-podocyte autoantibodies or immune complexes comprising the same may cause thickening of the

GBM. In some embodiments, anti-podocyte autoantibodies or immune complexes comprising the same contribute to and/or result in Membranous nephropathy (e.g., idiopathic membranous nephropathy).

[0373] Podocytes are cells in Bowman's capsule in the kidneys that wrap around capillaries of the glomerulus. Podocytes make up the epithelial lining of Bowman's capsule, the third layer through which filtration of blood takes place. Podocytes express a number of polypeptides (e.g., autoantigens) including but not limited to: phospholipase A2 receptor (PLA2R), Thrombospondin Type-1 Domain–Containing 7A (THSD7A), neutral endopeptidase (NEP), Neural Epidermal Growth Factor like 1 Protein (NELL-1), Exostosin 1 (EXT1), Exostosin 2 (EXT2), Semaphorin 3B (SEMA3B; UniProt/Swiss-Prot Accession No. Q13214), Neural Cell Adhesion Molecule 1 (NCAM1; UniProt/Swiss-Prot Accession No.P13591), and Protocadherin 7 (PCDH7; UniProt/Swiss-Prot Accession No. O60245) (Ronco et al. 2021).

[0374] In some embodiments, a podocyte autoantigen is expressed in a podocyte, e.g., in the cytoplasm, nucleus, peri-nucleus, or in a compartment in a cell. In some embodiments, a podocyte autoantigen is expressed on the cell surface of podocytes. In some embodiments, diseases associated with anti-podocyte autoantibodies such as membranous nephropathy (e.g., idiopathic membranous nephropathy) are associated with increased and/or aberrant expression of one or more podocyte autoantigens. In some embodiments, membranous nephropathy (e.g., idiopathic membranous nephropathy) is associated with increased and/or aberrant expression of one or more anti-podocyte autoantibodies or immune complexes comprising the same.

[0375] In some embodiments, a podocyte autoantigen comprises: PLA2R, THSD7A, NEP, NELL1, EXT1, EXT2, SEMA3B, NCAM1, PCDH7, or a combination thereof.

[0376] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-podocyte autoantibody, or a fragment or a complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-podocyte autoantibody, or a fragment or a complex thereof, can be used to treat and/or prevent membranous nephropathy.

Thrombotic thrombocytopenic purpura

[0377] Thrombotic thrombocytopenic purpura (TTP) is a life-threatening rare disorder characterized by a deficiency in the activity of ADAMTS13 (a disintegrin and metalloproteinase

with a thrombospondin type 1 motif, member 13) (Ercig B. et al. J. Biol. Chem., 2021). ADAMTS13 is a mettaloprotease that cleaves the von Willebrand factor (VWF) thus preventing accumulation of ultralarge VWF (ULVWF) multimers. A limited number of cases are congenital, however approximately 95% of cases are of an acquired, autoimmune nature – immune TTP (iTTP) – in which autoantibodies targeting ADAMTS13 cause loss of ADAMTS13 activity and/or promote ADAMTS13 polypeptide clearance resulting in accumulation of highly pro-thrombotic ULVWF multimers (Ercig B et al 2021). iTTP has an annual incidence rate of 1.5 to 6 cases per million adults per year. The acute phase of iTTP often presents itself with purpura, fever, neurological manifestations, renal dysfunctions, hemolytic anemia with schistocytes, and thrombocytopenia.

[0378] When enzymatically active, ADAMTS13 cleaves VWF or ULVWF (e.g., on the surface of endothelial cells). VWF is produced by vascular endothelium and megakaryocytes. VWF circulates in a globular form; however, exposure to shear forces and binding to exposed collagen at the site of a damaged vessel promotes its unfolding. VWF recruits platelets to sites of vascular injury. The ability of VWF to bind circulating platelets is dependent on its multimeric size, with largest multimers being most potent in capturing platelets during primary hemostasis. Cleavage of VWF serves to reduce the size of VWF polymers in circulation. Under normal physical conditions, the multimeric size of VWF is controlled by ADAMTS13. In the absence of a functional ADAMTS13 polypeptide, VWF polymers are not adequately processed, resulting in spontaneous adhesion of blood platelets, which presents as severe, life-threatening microvascular thrombosis (Ercig B. et al. J. Biol. Chem., 2021).

[0379] Under normal conditions, ADAMTS13 polypeptides circulate in a closed conformation. Upon binding to VWF, ADAMTS13 polypeptides adopt a short-lived transient open conformation that allows for interaction with VWF.

[0380] In iTTP patients, ADAMTS13 polypeptides may adopt an open conformation. Without wishing to be bound by any particular theory, anti-ADAMST13 autoantibodies can induce a conformational change in ADAMTS13 resulting in exposure of one or more neoepitopes (Roose E et al., (2020) vol. 136, number 3). It has been reported that in some cases of iTTP, open conformation ADAMTS13 precedes a drop in ADAMTS13 activity thus suggesting that open conformation ADAMTS13 may be useful as a biomarker for iTTP.

[0381] In some embodiments, conformational changes of ADAMTS13 polypeptide may result in exposure of neoepitopes that may trigger the development of autoantibodies. In

some embodiments, an open ADAMTS13 polypeptide conformation can be used as a biomarker for iTTP. In some embodiments, anti-ADAMTS13 autoantibodies can change the conformation of ADAMTS13 from a closed conformation to an open conformation.

[0382] In some embodiments, administration of a multi-functional molecule can be used to treat and/or prevent iTTP.

Anti-ADAMTS13 autoantibodies

[0383] iTTP may be induced by autoimmunity to ADAMTS13 (e.g., by the development of anti-ADAMTS13 autoantibodies) (Roose 2020; Ercig 2021). Anti-ADAMTS13 autoantibodies may inhibit ADAMTS13 activity and/or clear ADAMTS13 from circulation.

[0384] Deficiency in ADAMTS13 may result in accumulation of ULVWF multimers, which spontaneously bind to platelets and may lead to formation of microthrombi that obstruct the microvasculature. In some embodiments, binding of an anti-ADAMTS13 autoantibody to an ADAMTS13 increases accumulation of VWF or ULVWF. In some embodiments, anti-ADAMTS13 autoantibodies or immune complexes comprising the same may reduce and/or inactivate ADAMTS13 thereby causing endothelial cell injury. In some embodiments, anti-ADAMTS13 autoantibodies or immune complexes comprising the same may cause platelet activation. In some embodiments, binding of an anti-ADAMTS13 autoantibody to an ADAMTS13 increases microthrombi or platelet-rich clots.

[0385] In some embodiments, an anti-ADAMTS13 autoantibody specifically binds to an epitope of an ADAMTS13 polypeptide, e.g., as described herein. In some embodiments, an epitope is a linear epitope. In some embodiments, an epitope is a conformational epitope.

[0386] In some embodiments, iTTP is associated with increased and/or aberrant anti-ADAMTS13 autoantibodies.

[0387] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-ADAMTS13 autoantibody, or a fragment or a complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-ADAMTS13 autoantibody, or a fragment or a complex thereof, can be used to treat and/or prevent iTTP.

IgA nephropathy

[0388] IgAN is a disease of the kidney. The disease is considered to be an immune-complex-mediated glomerulonephritis, which is characterized by deposition of IgA either alone or with other immunoglobulins (e.g., IgG and/or IgM) and/or with complement components, in the glomerular mesangium. (Wyatt RJ and Julian BA, (2013) *NEJM* 368:25). Nephropathy results and is defined by proliferative changes in the glomerular mesangial cells. IgAN is one of the most common types of chronic glomerulonephritis and a frequent cause of end-stage renal disease.

[0389] Without wishing to be bound by any particular theory, it is proposed that IgAN may develop by the following four steps. The first step is the appearance in the circulation of increased levels of aberrantly O-galactosylated IgA1 (e.g., galactose deficient IgA1 [gd-IgA1]). This IgA1 may also exhibit reduced O-linked sialylation and a reduction in N-acetygalactosamine (GalNAc) residues at the hinge region of IgA1. The second step is the generation of IgG and/or IgA autoantibodies directed against the aberrantly O-galactosylated hinge region of gd-IgA1, with the third step being the formation of anti-gd-IgA1:gd-IgA1 immune complexes. The fourth step is the variable development of inflammatory and fibrotic processes in the kidney, triggered by the deposition of anti-gd-IgA1:gd-IgA1 immune complexes in the mesangium (Selvskandan et al. Frontiers in Immunology, 2020).

[0390] In some embodiments, IgA deposits occur by accumulation of IgA1 immune complexes (e.g., gd-IgA1 immune complexes and/or IgA1 immune complexes having a normal O-glycosylation). In some embodiments, IgA deposits occur by accumulation of gd-IgA1 immune complexes. In some embodiments, IgA deposits occur by accumulation of IgA1 immune complexes. In some embodiments, IgA deposits occur by accumulation of anti-gd-IgA1 immune complexes, e.g., along with the gd-IgA1 antigen (Selvskandan, H. et al. Frontiers in Immunology, 2020). In some embodiments, an immune complex comprising IgA1, gd-IgA1, and/or anti-gd-IgA1 comprises an antigen recognized by the antibody, one or more components of a complement system, one or more additional immunoglobulins, or combinations thereof.

[0391] In some embodiments, an autoimmune disease is a disease associated with increased and/or aberrant IgA. In some embodiments, an autoimmune disease is IgA nephropathy (IgAN).

[0392] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an IgA1 antibody or a fragment or complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an IgA1 antibody or a fragment or complex thereof, can be used to treat and/or prevent IgA nephropathy.

[0393] In some embodiments, a multi-functional molecule comprises a second moiety which binds to a gd-IgA1 antibody or a fragment or complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to a gd-IgA1 antibody or a fragment or complex thereof, can be used to treat and/or prevent IgA nephropathy.

[0394] In some embodiments, a multi-functional molecule comprises a second moiety which binds to an anti-gd-IgA1 autoantibody or a fragment or complex thereof. In some embodiments, administration of a multi-functional molecule comprising a second moiety which binds to an anti-gd-IgA1 autoantibody or a fragment or complex thereof, can be used to treat and/or prevent IgA nephropathy.

Administration

[0395] In some embodiments, of the disclosure, provided are methods comprising administering to a subject a composition according to the present disclosure. In some embodiments, a method comprises administering to a subject a pharmaceutical composition comprising a multi-functional molecule according to the present disclosure.

[0396] Delivery of a multi-functional molecule can be achieved *e.g.,* by administration of a pharmaceutical composition as described herein, such as a pharmaceutical composition that comprises a multi-functional molecule or a nucleic acid that encodes it, for example via oral ingestion, inhalation, topical application or parenteral administration (*e.g.,* cutaneous, subcutaneous, intraperitoneal, intramuscular or intravenous injection). In some embodiments, administration is by intravenous or intramuscular injection. In some embodiments, local administration may be or comprise topical administration (*e.g.,* to the skin) or parenteral administration (*e.g.,* by injection to a site of deposition such as to the kidney).

[0397] In some embodiments, delivery of a multi-functional molecule can be achieved *e.g.,* by administration of a pharmaceutical composition as described herein, such as a

pharmaceutical composition that comprises a multi-functional molecule or a nucleic acid that encodes it, may be oral, rectal, ophthalmic (including intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal, intracranial, intratracheal, and epidural). Those skilled in the art will be aware of typical guiding principles for formulation of pharmaceutical compositions for administration by such routes. For example, such techniques may include the step of bringing into association a multi-functional molecule or a nucleic acid that encodes it and the pharmaceutical carrier(s) or excipient(s). In some embodiments, compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0398] In some embodiments, the administration step comprises intravenous injection, intraperitoneal injection, subcutaneous injection, transdermal injection, or intramuscular injection.

[0399] In some embodiments, a composition according to the present disclosure are delivered to a subject suffering from or susceptible to a disease associated with a soluble protein, e.g., as described herein.

Dosing Regimens

[0400] In some embodiments, a method comprises administering a composition once. In some embodiments, a method comprises administering a composition repeatedly.

[0401] In some embodiments, administration of a composition is continued to maintain improvement in one or more sign or symptom of a disease, disorder or condition (e.g., remission (e.g., keep anti-neutrophil autoantibodies low and/or undetectable) and/or avoid relapse of a cancer).

[0402] Amounts of multi-functional molecule administered in a single dose may depend on the nature and/or severity of the condition being treated and/or on the nature of prior treatments that the patient has undergone. In some embodiments, the attending physician decides the amount of multi-functional molecule with which to treat each individual patient. In some embodiments, the attending physician initially administers low doses of multi-functional molecules of the present invention and observe the patient's response. In some embodiments,

larger doses are administered until an optimal therapeutic effect is obtained for the patient, after which dosage is not increased further.

Characterization of multi-functional molecules

[0403] The present disclosure provides, among other things, multi-functional molecules that specifically bind to a target and thereby causing degradation of a target. In some embodiments target degradation comprises internalization into a cell for degradation (e.g., by transporting the target to a late endosome and/or a lysosome).

[0404] In some embodiments, multi-functional molecules according to the present disclosure is used to degrade and/or reduce the levels of a target.

[0405] In some embodiments, the present disclosure provides multi-functional molecules characterized in that when administered to a cell, tissue, or subject, the multi-functional molecules which is bound to a target via the second moiety, and to an endocytic receptor via a first moiety results in degradation of the target.

[0406] In some embodiments, degradation comprises internalization into a cell. In some embodiments, degradation comprises lysosomal degradation. In some embodiments, degradation occurs in a liver cell.

[0407] In some embodiments, multi-functional molecules disclosed herein are characterized in that when administered to a cell, tissue, or subject, the binding of a second moiety to a target forms a first complex comprising a multi-functional molecule and a target. In some embodiments, binding of a first moiety to a first endocytic receptor forms a second complex. In some embodiments, a second complex comprises a first complex and a first endocytic receptor bound by a first moiety. In some embodiments, binding of a first moiety to a first endocytic receptor internalizes a second complex into a cell, e.g., into an endosome.

[0408] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome, a first moiety dissociates from a first endocytic receptor. In some embodiments, an endosome has a second pH. In some embodiments, an endosome does not have a third pH. In some embodiments, an endosome comprises an early endosome. In some embodiments, an endosome comprises a recycling endosome.

[0409] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome, the first moiety does not dissociate from the endocytic receptor.

[0410] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome, the first moiety dissociates from the endocytic receptor.

[0411] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome, the first moiety binds a different endocytic receptor. In some embodiments, a different endocytic receptor comprises an endocytic receptor having a different structure and/or function compared to the endocytic receptor which was bound to the first moiety at internalization into the cell.

[0412] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome, the first moiety binds the same endocytic receptor. In some embodiments, a different endocytic receptor comprises an endocytic receptor having the same structure and/or function compared to the endocytic receptor which was bound to the first moiety at internalization into the cell.

[0413] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome a target dissociates from a second moiety in the presence of a second pH. In some embodiments, dissociation occurs in an intracellular compartment of a cell. In some embodiments, dissociation of a target from a second moiety results in degradation of a target. In some embodiments, degradation occurs at a third pH. In some embodiments, degradation occurs in a late endosome and/or a lysosome.

[0414] In some embodiments, a multi-functional molecule from which the target has dissociated is bound to an endocytic receptor with the first moiety.

[0415] In some embodiments, a multi-functional molecule disclosed herein is characterized in that binding of the first moiety to the endocytic receptor delivers (e.g., recycles) the multi-functional molecule to a plasma membrane at the surface of the cell.

[0416] In some embodiments, a first moiety dissociates from the endocytic receptor at a plasma membrane comprising a first pH.

[0417] In some embodiments, a multi-functional molecule which is dissociated from the endocytic receptor is not bound to the cell surface.

[0418] In some embodiments, a multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more endocytic receptors with the first moiety.

[0419] In some embodiments, a multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more targets with the second moiety at the first pH.

[0420] In some embodiments, a multi-functional molecule disclosed herein is characterized in that upon internalization into an endosome the third moiety binds to the second endocytic receptor at the second pH and forms a third complex.

[0421] In some embodiments, a multi-functional molecule disclosed herein is characterized in that binding of the third moiety to the second endocytic receptor delivers the third complex to the surface of the cell. In some embodiments, a surface of the cell has the first pH. In some embodiments, the third moiety dissociates from the second endocytic receptor at the first pH. In some embodiments, the multi-functional molecule which is dissociated from the second endocytic receptor at the surface of the cell is not bound to the cell surface.

[0422] In some embodiments, a multi-functional molecule is able to bind (e.g., repeated binding) to one or more targets with the second moiety at the first pH. In some embodiments, a multi-functional molecule is able to bind (e.g., repeated binding) to one or more first endocytic receptors with the first moiety. In some embodiments, a multi-functional molecule is able (e.g., repeated binding) to bind to one or more second endocytic receptors with the third moiety upon internalization into a cell compartment with the second pH.

[0423] In some embodiments, repeated binding of a multi-functional molecule to one or more targets and/or one or more endocytic receptors allows for increased removal (e.g., degradation) of a target when said multi-functional molecule is administered to a subject, as compared to administration of an otherwise comparable second moiety that is not part of a multi-functional molecule. In some embodiments, repeated binding of a multi-functional molecule to one or more targets allows for administration of a multi-functional molecule or a composition comprising the same at a reduced frequency and/or at a lower dose to degrade a target and/or achieve a therapeutic effect.

Method of making multi-functional molecules

[0424] The present disclosure, among other things, provides methods of making a multi-functional molecule comprising a first moiety that binds to an endocytic receptor (e.g., in the presence of one or more cations), a second moiety that binds to a target in a pH dependent manner and a third moiety that binds to an endocytic receptor in a pH dependent manner..

[0425] A multi-functional molecule disclosed herein can be made using methods disclosed in U.S. Provisional Patent Application No. 63/410,955 filed on September 28, 2022 and U.S. Provisional Patent Application No. 63/410,936, filed on September 28, 2022 the entire contents of each of which are hereby incorporated by reference.

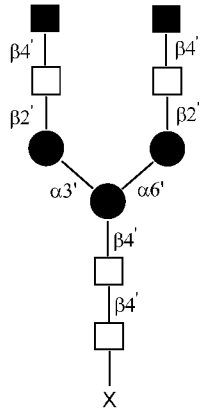
[0426] For example, in US 63/410,955, Section 5.3 Leishmania discloses Leishmania host cells; Section 5.4 discloses exemplary methods of genetically engineering a Leishmania cell for expressing multi-functional molecules, Section 5.5 disclose exemplary methods of culturing Leishmania host cells; and Section 5.6 discloses exemplary uses of Leishmania host cells as an expression system. As would be understood by persons with ordinary skill in the art, such methods and host cells can also be used for making multi-functional molecule disclosed herein.

[0427] In some embodiments, the one or more glycans of the second moiety are conjugated to the first moiety at one or more glycosylation sites with *in vivo* glycosylation, e.g., in a cell. In some embodiments, a cell is a Leishmania host cell. In some embodiments, a cell is a glycoengineered yeast host cell, e.g., glycoengineered Pichia pastoris host cell.

[0428] In some embodiments, the one or more glycans of the second moiety are conjugated to the first moiety at one or more glycosylation sites with chemical conjugation, e.g., using Click chemistry.

[0429] Also disclosed herein are methods for making a multi-functional molecule. In one embodiment, provided herein is a method of producing a multi-functional molecule *in vivo*, using a *Leishmania* host cell described herein. In some embodiments, provided herein is a method for producing a multi-functional molecule, said method comprising (i) culturing a *Leishmania* host cell under conditions suitable for polypeptide production and (ii) isolating said multi-functional molecule. In a specific embodiment, the *Leishmania* host cell comprises: (a) a recombinant nucleic acid encoding a multi-functional molecule; and (b) a recombinant nucleic acid encoding one or more recombinant N-acetylgalactosamine (GalNAc) transferases. In certain embodiments, the *Leishmania* host cell is capable of producing multi-functional molecule

comprising a biantennary, GalNAc-terminated N-glycan. In particular, the *Leishmania* host cells provided herein is capable of producing multi-functional molecule comprising an N-glycan of the following structure:

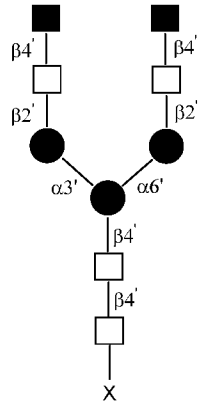


wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the multi-functional molecule.

[0430] In certain embodiments, the multi-functional molecule produced by the *Leishmania* host cell is a therapeutic polypeptide, *i.e.*, a polypeptide used in the treatment of a disease or disorder. For example, the multi-functional molecule produced by the *Leishmania* host cell can be peptide or an antibody.

Leishmania host cells

[0431] Provided herein are *Leishmania* host cells for the production of multi-functional molecule disclosed herein, or a population of multi-functional molecule, wherein the *Leishmania* host cells comprise: (a) a recombinant nucleic acid encoding a multi-functional molecule disclosed herein; and (b) a recombinant nucleic acid encoding one or more recombinant N-acetylgalactosamine (GalNAc) transferases. In certain embodiments, the *Leishmania* host cells provided herein are capable of producing multi-functional molecule comprising a biantennary, GalNAc-terminated N-glycan. In particular, the *Leishmania* host cells provided herein are capable of producing multi-functional molecule comprising an N-glycan of the following structure:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the multi-functional molecule.

[0432] In certain embodiments, the *Leishmania* host cells provided herein comprise a recombinant nucleic acid encoding one or more recombinant N-acetylgalactosamine (GalNAc) transferases disclosed herein. In certain embodiments, the *Leishmania* host cells provided herein comprise a recombinant nucleic acid encoding one or more additional recombinant glycosyltransferases disclosed herein. In certain embodiments, one or more endogenous enzymes disclosed herein from the glycan biosynthesis pathway of the the *Leishmania* host cells provided herein have been deleted, mutated and/or functionally inactivated. In certain embodiments, the *Leishmania* host cells provided herein further comprise a recombinant nucleic acid encoding heterologous UDP-GalNAc biosynthetic pathway proteins capable of generating UDP-GalNAc. In certain embodiments, the *Leishmania* host cells provided herein comprise a recombinant nucleic acid encoding a heterologous UDP-GalNAc transporter protein capable of transporting UDP-GalNAc to the secretory pathway.

[0433] In certain embodiments, the *Leishmania* host cells provided herein have been genetically engineered such that the formation of an O-linked GlcNAc on a polypeptide produced in the *Leishmania* host cell is reduced or eliminated. *Leishmania* host cells that have been genetically engineered to reduce or eliminate the formation of an O-linked GlcNAc are described, for example, in WO 2021/140143, which is incorporated herein by reference in its entirety.x

[0434] In certain embodiments, the *Leishmania* host cells provided herein below are genetically engineered using the methods described herein. In certain embodiments, the

Leishmania host cells provided herein below are cultured according to the methods described herein.

[0435] Other suitable host cells comprise liver cells, myeloid cells, immune cells, endothelial cells, parenchymal cells or epithelial cells. In some embodiments, the immune cell is a dendritic cell, a macrophage, a monocyte, a microglia cell, a granulocyte or a B lymphocyte.

Methods of Culturing *Leishmania* Host Cells

[0436] Provided herein are methods for culturing *Leishmania* host cells. In one embodiment, the *Leishmania* host cells are cultured using any of the standard culturing techniques known in the art. For example, cells are routinely grown in rich media like Brain Heart Infusion, Trypticase Soy Broth or Yeast Extract, all containing 5 µg /ml Hemin. Additionally, incubation is done at 26°C in the dark as static or shaking cultures for 2-3 days. In some embodiments, cultures of recombinant cell lines contain the appropriate selective agents. Non-limiting exemplary selective agents are provided in Table 2.

[0437] Table 2: Selective agents used during transfection (50% concentration for preselection and 100% concentration for main selection) and standard culturing of *L. tarentolae*. Double amounts of the selective agents could be used if higher selection pressure was intended.

Selective agent	**Resistance conferring gene**	**Concentration (100%)**	**Concentration (50%)**
		main selection/ standard culturing	preselection
Nourseothricin	*sat*	50 µg/ml	25 µg/ml
Geneticin	*neo*	50 µg/ml	25 µg/ml
Paromomycin	*neo*	300 µg/ml	150 µg/ml
Zeocin	*ble*	150 µg/ml	75 µg/ml
Hygromycin	*hyg*	50 µg/ml	25 µg/ml
Blasticidin	*bsd*	5 µg/ml	2.5 µg/ml
Puromycin	*pac*	5 µg/ml	2.5 µg/ml

[0438] In certain embodiments, the *Leishmania* host cells are cultured in a growth medium comprising GalNAc. In certain embodiments, the growth medium comprises at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6 mM, at least 7 mM, at least 8 mM, at least 9 mM, at least 10 mM, at least 11 mM, at least 12 mM, at least 13 mM, at least 14 mM, at least 15 mM, at least 16 mM, at least 17 mM, at least 18 mM, at least 19 mM, or

at least 20 mM GalNAc. In certain embodiments, the growth medium comprises about 1 mM to about 5 mM, about 5 mM to about 10 mM, about 10 mM to about 15 mM, or about 15 mM to about 20 mM GalNAc. In certain embodiments, the growth medium comprises about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, or about 20 mM GalNAc. In certain embodiments, the growth medium comprises about about 10 mM GalNAc.

[0439] In certain embodiments, the *Leishmania* host cells are cultured in a growth medium comprising GlcNAc. In certain embodiments, the growth medium comprises at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6 mM, at least 7 mM, at least 8 mM, at least 9 mM, at least 10 mM, at least 11 mM, at least 12 mM, at least 13 mM, at least 14 mM, at least 15 mM, at least 16 mM, at least 17 mM, at least 18 mM, at least 19 mM, or at least 20 mM GlcNAc. In certain embodiments, the growth medium comprises about 1 mM to about 5 mM, about 5 mM to about 10 mM, about 10 mM to about 15 mM, or about 15 mM to about 20 mM GlcNAc. In certain embodiments, the growth medium comprises about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, or about 20 mM GlcNAc.

[0440] In certain embodiments, a *Leishmania* host cell may be used as an expression system for making a multi-functional molecule disclosed herein or a population of multi-functional molecule. In certain embodiments, the multi-functional molecule degrader may be a heterologous, non-*Leishmania* protein, such as a therapeutic protein (e.g., an antibody). Other methods of producing *Leishmania* host cells for use as expression systems are known and may also be used, for example, see WO 2019/002512, WO 2021/140144 and WO 2021/140143, each of which are incorporated herein by reference in their entirety. Use of *Leishmania* host cells to make monoclonal antibodies are also known. Exemplary methods are described in WO 2022/053673, which is incorporated herein by reference in its entirety.

[0441] The compositions comprising the *Leishmania* host cells can comprise additional components suitable for maintenance and survival of the *Leishmania* host cells, and can additionally comprise additional components required or beneficial to the production of glycoengineered bifunctional degraders by the *Leishmania* host cells, e.g., inducers for inducible promoters, such as arabinose, IPTG.

Yeast or filamentous fungal host cells

[0442] Provided herein are yeast or filamentous fungal host cells for the production of multi-functional molecule disclosed herein, or a population of multi-functional molecule. In some embodiments, a yeast or filamentous fungal host cell is a *K. lactis* host cells. In some embodiments, a yeast or filamentous fungal host cell is a *Pichia pastoris* host cell. In some embodiments, a yeast or filamentous fungal host cell is a *Pichia methanolica* host cell. In some embodiments, a yeast or filamentous fungal host cell is a *Hansenula* host cell.

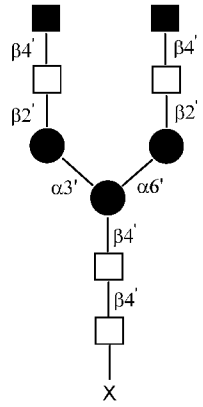
[0443] Exemplary yeast or filamentous fungal host cells that can be used to produce multi-functional molecule disclosed herein are disclosed in U.S. Patent 8,206,949, the entire contents of which are hereby incorporated by reference.

[0444] Exemplary yeast or filamentous fungal host cells that can be used to produce multi-functional molecule disclosed herein are disclosed in U.S. Patent7,981,660, the entire contents of which are hereby incorporated by reference.

[0445] Exemplary yeast or filamentous fungal host cells that can be used to produce multi-functional molecule disclosed herein are disclosed in U.S. Patent 8,883,483, the entire contents of which are hereby incorporated by reference.

[0446] In some embodiments, a yeast or filamentous fungal host cell has been genetically modified to produce glycoproteins with a predominant N-glycan glycoform.

[0447] In certain embodiments, the yeast or filamentous fungal host cells provided herein are capable of producing multi-functional molecule comprising a biantennary, GalNAc-terminated N-glycan. In particular, the yeast or filamentous fungal host cells provided herein are capable of producing multi-functional molecule comprising an N-glycan of the following structure:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of a moiety of the multi-functional molecule.

ENUMERATED EMBODIMENTS

[0448] Embodiment 1. A multi-functional molecule, comprising:

[0449] (a) a first moiety that specifically binds to an endocytic receptor; and

[0450] (b) a second moiety that specifically binds to a target at a first pH.

[0451] Embodiment 2. The multi-functional molecule of embodiment 1, wherein the first moiety binds to an endocytic receptor under a first set of conditions and/or a second set of conditions.

[0452] Embodiment 3. The multi-functional molecule of embodiment 1 or 2, wherein the first moiety does not bind to or has lower affinity binding to an endocytic receptor under a third set of conditions.

[0453] Embodiment 4. The multi-functional molecule of embodiment 2, wherein the endocytic receptor bound by the first moiety under the first set of conditions and the endocytic receptor bound by the first moiety under the second set of conditions are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

[0454] Embodiment 5. The multi-functional molecule of embodiment 2, wherein the endocytic receptor bound by the first moiety under the first set of conditions and the

endocytic receptor bound by the first moiety under the second set of conditions are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

[0455] Embodiment 6. A multi-functional molecule, comprising:

[0456] (a) a first moiety that specifically binds to an endocytic receptor under a first set of conditions;

[0457] (b) a second moiety that specifically binds to a target at a first pH; and

[0458] (c) a third moiety that specifically binds to an endocytic receptor under a second set of conditions.

[0459] Embodiment 7. The multi-functional molecule of embodiment 6, wherein the endocytic receptor bound by the first moiety and the endocytic receptor bound by the third moiety are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

[0460] Embodiment 8. The multi-functional molecule of embodiment 6, wherein the endocytic receptor bound by the first moiety and the endocytic receptor bound by the third moiety are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

[0461] Embodiment 9. The multi-functional molecule of any one of embodiments 2-8, wherein the first set of conditions comprises:

[0462] (i) a first pH (e.g., a pH of about pH 6.7 to about pH 8);

[0463] (ii) presence of a cation (e.g., Ca 2+);

(iii) both (i) and (ii).

[0464] Embodiment 10. The multi-functional molecule of any one of embodiments 2-9, wherein the second set of conditions comprises:

[0465] (i) a second pH (e.g., a pH of about pH 6.5 to about pH 5.6);

[0466] (ii) presence in an intracellular vesicle which: (a) expresses, or has detectable presence of, a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof, (b) has no detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof; (c) has the ability to export a molecule to an extracellular space; (d) expresses one or more

receptors that can bind to a molecule and export the molecule to an extracellular space; (e) does not have the ability to degrade a molecule; (f) has no or minimal proteolytic activity (e.g., hydroylase activity); (g) any combination or all of (a)-(h);

[0467] (iii) an early endosome or a recycling endosome; or

[0468] (iv) any combination or all of (i)-(iii).

[0469] Embodiment 11. The multi-functional molecule of embodiment 10, wherein a polypeptide associated with an early endosome and/or a recycling endosome comprises a GTPase, a sorting nexin, or a combination thereof.

[0470] Embodiment 12. The multi-functional molecule of embodiment 11, wherein a GTPase comprises Arf6, Rab4, Rab5, Rab8, Rab10, Rab11, Rab22a, Rab35 or variants or functional fragments thereof.

[0471] Embodiment 13. The multi-functional molecule of any one embodiments 3-12, wherein the third set of conditions comprises:

[0472] (i) a third pH (e.g., less than pH 5.5);

[0473] (ii) presence in an intracellular vesicle which: (a) has no detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof, (b) expresses, or has detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof; (c) has the ability to degrade a molecule; (d) has proteolytic activity (e.g., hydroylase activity); (e) any combination or all of (a)-(d); or

[0474] (iii) a late endosome or a lysosome; or

[0475] (iv) any combination or all of (i)-(iii).

[0476] Embodiment 14. The multi-functional molecule of embodiment 13, wherein a polypeptide associated with a late endosome and/or a lysosome comprises Rab7 or a variant or fragment thereof.

[0477] Embodiment 15. The multi-functional molecule of embodiment 13 or 14, wherein the first moiety has reduced binding, or does not bind to an endocytic receptor at the third pH.

[0478] Embodiment 16. The multi-functional molecule of embodiment 15, wherein reduced binding is assessed as compared to binding of the first moiety to the endocytic receptor at a first pH or a second pH.

[0479] Embodiment 17. The multi-functional molecule of embodiment 16, wherein the binding affinity of the first moiety at the third pH (KD at third pH) is at least 1.5-fold lower compared to: the binding of the first moiety at a first pH (KD at first pH) or binding of the first moiety at a second pH (KD at second pH).

[0480] Embodiment 18. The multi-functional molecule of any one the preceding embodiments, wherein the first moiety undergoes a pH dependent conformation change.

[0481] Embodiment 19. The multi-functional molecule of embodiment 18, wherein the conformation change prevents binding or reduced binding to the endocytic receptor at the second pH and/or third pH.

[0482] Embodiment 20. The multi-functional molecule of any one the preceding embodiments, wherein the first moiety binds to the endocytic receptor with an affinity that is at least an affinity required for internalizing the multi-functional molecule into an intracellular compartment in the cell.

[0483] Embodiment 21. The multi-functional molecule of embodiment 20, wherein the first moiety stays bound to the endocytic receptor upon internalization into the intracellular compartment.

[0484] Embodiment 22. The multi-functional molecule of embodiment 21, wherein the first moiety does not stay bound to the endocytic receptor upon internalization into the intracellular compartment.

[0485] Embodiment 23. The multi-functional molecule of embodiment 22, wherein the first moiety binds a different endocytic receptor upon internalization into the intracellular compartment.

[0486] Embodiment 24. The multi-functional molecule of any one of embodiments 20-23, wherein the intracellular compartment is an endosome.

[0487] Embodiment 25. The multi-functional molecule of embodiment 24, wherein the endosome comprises an early endosome and/or a recycling endosome.

[0488] Embodiment 26. The multi-functional molecule of any one of embodiments 20-25, wherein the intracellular compartment has a second pH.

[0489] Embodiment 27. The multi-functional molecule of embodiment 26, wherein the intracellular compartment has a pH of about pH 6.5 to about pH 5.6.

[0490] Embodiment 28. The multi-functional molecule of any one of embodiments 20-27, wherein the first moiety binds to the endocytic receptor with an affinity that is at least an affinity required for transporting the multi-functional molecule to the cell surface.

[0491] Embodiment 29. The multi-functional molecule of any one of embodiments 20-27, wherein the first moiety does not bind to the endocytic receptor with an affinity that is at or higher than an affinity required for transporting the multi-functional molecule to a late endosome and/or lysosome.

[0492] Embodiment 30. The multi-functional molecule of any one embodiments 20-29, wherein the multi-functional molecule with the first moiety bound to the endocytic receptor is delivered (e.g., recycled) to the plasma membrane at the cell surface.

[0493] Embodiment 31. The multi-functional molecule of embodiment 30, wherein the first moiety is bound to the same endocytic receptor for internalization into the cell and delivery (e.g., recycling) to the plasma membrane at the cell surface.

[0494] Embodiment 32. The multi-functional molecule of embodiment 30, wherein the first moiety is bound to a different endocytic receptor for internalization into the cell as compared to delivery (e.g., recycling) to the plasma membrane at the cell surface.

[0495] Embodiment 33. The multi-functional molecule of embodiment 32, wherein upon delivery of the multi-functional molecule to the plasma membrane at the cell surface, the first moiety dissociates from the endocytic receptor thereby releasing the multi-functional molecule from the plasma membrane at the cell surface.

[0496] Embodiment 34. The multi-functional molecule of any one embodiments 1-33, wherein the endocytic receptor is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2,

DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0497] Embodiment 35. The multi-functional molecule of any one embodiments 1-34, wherein the endocytic receptor is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0498] Embodiment 36. The multi-functional molecule of any one embodiments 1-33, wherein the endocytic receptor is or comprises a Siglec, one or more SNARE proteins, or a multi-drug transporter.

[0499] Embodiment 37. The multi-functional molecule of any one embodiments the preceding embodiments, wherein the first moiety comprises one or more peptides that specifically binds to an endocytic receptor.

[0500] Embodiment 38. The multi-functional molecule of embodiment 37, wherein the first moiety comprises an antibody agent.

[0501] Embodiment 39. The multi-functional molecule of embodiment 38, wherein the antibody agent comprises a full antibody, a Fab fragment, an scFv, a nanobody, a duobody, or a single domain antibody (e.g., a VHH).

[0502] Embodiment 40. The multi-functional molecule of any one of the preceding embodiments, wherein the first moiety comprises one or more glycans.

[0503] Embodiment 41. The multi-functional molecule of any one of embodiments 6-40, wherein the first moiety is conjugated to the second moiety and/or the third moiety at one or more glycosylation sites.

[0504] Embodiment 42. The multi-functional molecule of any one of embodiments 6-36 or 40-41, wherein the first moiety is present on a peptide.

[0505] Embodiment 43. The multi-functional molecule of embodiment 42, wherein the peptide comprising the first moiety is linked to the second moiety and/or third moiety.

[0506] Embodiment 44. The multi-functional molecule of any one embodiments 9-43, wherein the first pH is about pH 6.7 to about pH 8.

[0507] Embodiment 45. The multi-functional molecule of any one of embodiments 10-44, wherein the second pH is about pH 6.5 to about pH 5.6.

[0508] Embodiment 46. The multi-functional molecule of any one of the preceding embodiments, wherein the first moiety binds to the first endocytic receptor in the presence of one or more cations.

[0509] Embodiment 47. The multi-functional molecule of embodiment 46, wherein the one or more cations comprise Ca 2+.

[0510] Embodiment 48. The multi-functional molecule of any one of the preceding embodiments, wherein the first moiety binds to the endocytic receptor in a pH dependent manner.

[0511] Embodiment 49. The multi-functional molecule of any one of the preceding embodiments, wherein the first moiety binds to the endocytic receptor at the first pH.

[0512] Embodiment 50. The multi-functional molecule of any one of the preceding embodiments, wherein the first moiety binds to the endocytic receptor at the second pH.

[0513] Embodiment 51. The multi-functional molecule of any one of embodiments 1-49, wherein the first moiety has reduced binding or does not bind to the endocytic receptor bound by the first moiety, at the second pH.

[0514] Embodiment 52. The multi-functional molecule of embodiment 51, wherein reduced binding is assessed as compared to binding of the first moiety to the endocytic receptor bound by the first moiety, at the first pH.

[0515] Embodiment 53. The multi-functional molecule of any one of embodiments 40-52, wherein the endocytic receptor bound by the first moiety, is or comprises an endocytic lectin receptor.

[0516] Embodiment 54. The multi-functional molecule of any one embodiments 40-52, wherein the endocytic receptor bound by the first moiety, is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0517] Embodiment 55. The multi-functional molecule of any one of embodiments 40-54, wherein the endocytic receptor bound by the first moiety, is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0518] Embodiment 56. The multi-functional molecule of any one of embodiments 40-55, wherein the glycan comprises a terminal GlcNac.

[0519] Embodiment 57. The multi-functional molecule of any one of embodiments 40-56, wherein the glycan comprises a terminal GalNac.

[0520] Embodiment 58. The multi-functional molecule of any one of embodiments 40-57, wherein the glycan comprises a terminal Gal.

[0521] Embodiment 59. The multi-functional molecule of any one of embodiments 40-58, wherein the glycan is an N-glycan.

[0522] Embodiment 60. The multi-functional molecule of embodiment 59, wherein the N-glycan is linked to the second moiety and/or third moiety at 1, 2, 3, 4 or 5 N-glycosylation sites.

[0523] Embodiment 61. The multi-functional molecule of any one of embodiments 40-60, wherein the glycan structure comprises GlcNAc2-Man3-GlcNAc2, GalNAc2-GlcNAc2-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, GlcNAcl-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, Gal1- GlcNAc2-Man3-GlcNAc2, GalNAc1-GlcNAc2-Man3-GlcNAc2, GlcNAc3-Man3-GlcNAc2, GlcNAc4-Man3-GlcNAc2, Gal3-GlcNAc3-Man3-GlcNAc2, GalNAc3-GlcNAc3-Man3-GlcNAc2, GalNAc4-GlcNAc4-Man3-GlcNAc2, Gal4-GlcNAc4-Man3-GlcNAc2, or Man-6-P -N-glycan.

[0524] Embodiment 62. The multi-functional molecule of any one of embodiments 40-61, wherein increasing a number of glycan structures on the multi-functional molecule increases the rate of lysosomal degradation as compared to an otherwise similar multi-functional molecule with fewer glycan structures.

[0525] Embodiment 63. The multi-functional molecule embodiment 62, wherein a number of glycan structures comprise 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more or 10 or more.

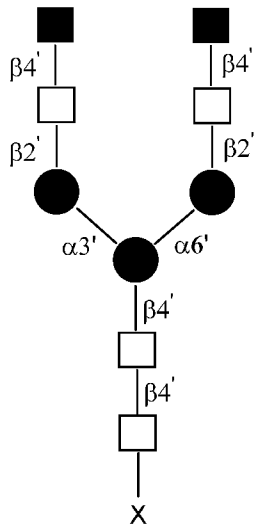
[0526] Embodiment 64. The multi-functional molecule of embodiment 62 or 63, wherein a glycan structure comprises a monoantennary structure, biantennary structure, a triantennary structure, or a tetraantennary structure.

[0527] Embodiment 65. The multi-functional molecule of any one of embodiments 62-64, wherein a glycan structure comprises a biantennary structure.

[0528] Embodiment 66. The multi-functional molecule of embodiment 65, wherein the glycan structure comprises a biantennary GalNAc.

[0529] Embodiment 67. The multi-functional molecule of embodiment 65 or 66, wherein the biantennary GalNac binds to an asialoglycoprotein receptor (ASGPR) or a fragment or variant thereof, or a complex comprising ASGPR.

[0530] Embodiment 68. The multi-functional molecule of any one of embodiments 59-67, wherein the N-glycan has a structure of:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the second moiety or third moiety.

[0531] Embodiment 69. The multi-functional molecule of any one of embodiments 59-68, wherein the N-glycan is conjugated to the second moiety and/or third moiety at at least one, two, three, or four N-glycosylation sites.

[0532] Embodiment 70. The multi-functional molecule of any one of embodiments 59-68, wherein the N-glycan is conjugated to the second moiety and/or third moiety at one, two, three, or four N-glycosylation sites.

[0533] Embodiment 71. The multi-functional molecule of any one of embodiments 59-70, wherein the N-glycosylation site comprises a consensus sequence of N-X-S/T or N-X-C, wherein X is any amino acid except proline.

[0534] Embodiment 72. The multi-functional molecule of any one of embodiments 59-71, wherein the N-glycosylation site is naturally occurring.

[0535] Embodiment 73. The multi-functional molecule of any one of embodiments 59-71, wherein the N-glycosylation site is engineered into the amino acid sequence of the second moiety and/or third moiety.

[0536] Embodiment 74. The multi-functional molecule of any one of the preceding embodiments, wherein the endocytic receptor bound by the first moiety, is or comprises ASGPR or a fragment or variant thereof.

[0537] Embodiment 75. The multi-functional molecule of embodiment 74, wherein when the endocytic receptor bound by the first moiety, is ASGPR, the second moiety and/or third moiety are conjugated to, or linked to, a first moiety (e.g., on a peptide) having a glycan structure comprising a terminal GalNac.

[0538] Embodiment 76. The multi-functional molecule of any one of the preceding embodiments, wherein the second moiety has reduced binding, or does not bind to a target at the second pH and/or the third pH.

[0539] Embodiment 77. The multi-functional molecule of embodiment 76, wherein reduced binding is assessed as compared to binding of the second moiety to the target at the first pH.

[0540] Embodiment 78. The multi-functional molecule of any one of the preceding embodiments, wherein the second moiety undergoes a pH dependent conformation change.

[0541] Embodiment 79. The multi-functional molecule of embodiment 78, wherein the conformation change prevents binding or reduced binding to a target at the second pH and/or third pH.

[0542] Embodiment 80. The multi-functional molecule of any one of the preceding embodiments, wherein the second moiety comprises one or more peptides that specifically binds to a target.

[0543] Embodiment 81. The multi-functional molecule of any one of the preceding embodiments, wherein the target is a secreted protein.

[0544] Embodiment 82. The multi-functional molecule of any one of the preceding embodiments, wherein the target is an antibody.

[0545] Embodiment 83. The multi-functional molecule of any one of the preceding embodiments, wherein the target is an auto-antibody.

[0546] Embodiment 84. The multi-functional molecule of any one of embodiments 1-80, wherein the target is membrane-bound.

[0547] Embodiment 85. The multi-functional molecule of any one of the preceding embodiments, wherein the second moiety comprises an antibody agent.

[0548] Embodiment 86. The multi-functional molecule of embodiment 85, wherein the antibody agent comprises an antigen binding fragment having (1) at least one amino acid substituted with a histidine; or (2) insertion of at least one histidine.

[0549] Embodiment 87. The multi-functional molecule of embodiment 85 or 86, wherein the antibody agent comprises an Fc domain having one or more mutations to alter binding to a receptor, e.g., FcRn.

[0550] Embodiment 88. The multi-functional molecule of any one of embodiments 85-87, wherein the binding affinity of the antibody agent of the second moiety to the target at the first pH (KD at first pH) is at least 1.5-fold lower compared to: the binding of the antibody agent to the target at the second pH (KD at second pH) or the binding of the antibody agent to the target at the third pH (KD at third pH).

[0551] Embodiment 89. The multi-functional molecule of any one of embodiments 6-88, wherein the third moiety has reduced binding, or does not bind to the endocytic receptor at the first pH and/or the third pH.

[0552] Embodiment 90. The multi-functional molecule of embodiment 89, wherein reduced binding is assessed as compared to binding of the third moiety to the endocytic receptor, at the second pH.

[0553] Embodiment 91. The multi-functional molecule of embodiment 89 or 90, wherein the third moiety undergoes a pH-dependent conformation change.

[0554] Embodiment 92. The multi-functional molecule of embodiment 91, wherein the conformation change prevents binding or reduces binding of the third moiety to the endocytic receptor at the first pH and/or the third pH.

[0555] Embodiment 93. The multi-functional molecule of any one of embodiments 6-92, wherein the third moiety comprises one or more peptides that specifically binds to the endocytic receptor.

[0556] Embodiment 94. The multi-functional molecule of embodiment 93, wherein the third moiety binds to the endocytic receptor, with an affinity that is at least an affinity required for transporting the multi-functional molecule to the plasma membrane at the cell surface.

[0557] Embodiment 95. The multi-functional molecule of any one of embodiments 6-94, wherein the multi-functional molecule with the third moiety bound to the endocytic receptor bound, is delivered (e.g., recycled) to the plasma membrane at the cell surface.

[0558] Embodiment 96. The multi-functional molecule of embodiment 95, wherein the third moiety does not bind to the endocytic receptor with an affinity that is at or higher than an affinity required for transporting the multi-functional molecule to a late endosome and/or a lysosome.

[0559] Embodiment 97. The multi-functional molecule of any one of embodiments 6-96, wherein the endocytic receptor bound by the third moiety, is or comprises an endocytic lectin receptor.

[0560] Embodiment 98. The multi-functional molecule of any one of embodiments 6-97, wherein the endocytic receptor bound by the third moiety, is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, and mannose 6 phosphate receptor (M6PR), or a combination thereof.

[0561] Embodiment 99. The multi-functional molecule of embodiment 98, wherein the endocytic receptor bound by the third moiety, is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

[0562] Embodiment 100. The multi-functional molecule of any one of embodiments 6-96, wherein the endocytic receptor bound by the third moiety, is not the neonatal Fc receptor (FcRn).

[0563] Embodiment 101. The multi-functional molecule of any one of embodiments 6-96, wherein the endocytic receptor bound by the third moiety, is or comprises the neonatal Fc receptor (FcRn).

[0564] Embodiment 102. The multi-functional molecule of any one of embodiments 6-96, the endocytic receptor bound by the third moiety, is or comprises a Siglec, one or more SNARE proteins, or a multi-drug transporter.

[0565] Embodiment 103. The multi-functional molecule of any one of embodiments 6-102, wherein the third moiety comprises an antibody agent.

[0566] Embodiment 104. The multi-functional molecule embodiment 103, wherein the binding affinity of the antibody agent of the third moiety to the second endocytic receptor, at the second pH (KD at second pH) is at least 1.5-fold lower compared to the binding of the antibody agent of the third moiety to the second endocytic receptor, at a second pH (KD at second pH).

[0567] Embodiment 105. The multi-functional molecule of any one of the preceding embodiments, wherein binding of the second moiety to a target forms a first complex comprising the multi-functional molecule and the target.

[0568] Embodiment 106. The multi-functional molecule of any one of the preceding embodiments, wherein binding of the first moiety to the endocytic receptor forms a second complex.

[0569] Embodiment 107. The multi-functional molecule of embodiment 106, wherein the second complex comprises the first complex, and the endocytic receptor bound by the first moiety.

[0570] Embodiment 108. The multi-functional molecule of embodiment 106 or 107, wherein binding of the first moiety to the endocytic receptor internalizes the second complex into a cell.

[0571] Embodiment 109. The multi-functional molecule of embodiment 108, wherein the second complex is internalized into an endosome.

[0572] Embodiment 110. The multi-functional molecule of embodiment 109, wherein the endosome comprises an early endosome and/or a recycling endosome.

[0573] Embodiment 111. The multi-functional molecule of embodiment 109 or 110, characterized in that upon internalization into an endosome, the first moiety dissociates from the endocytic receptor.

[0574] Embodiment 112. The multi-functional molecule of embodiment 111, wherein the endosome has the second pH.

[0575] Embodiment 113. The multi-functional molecule of embodiment 111, wherein the endosome does not have the third pH.

[0576] Embodiment 114. The multi-functional molecule of any one of embodiments 109-113, characterized in that upon internalization into an endosome, the first moiety does not dissociate from the endocytic receptor.

[0577] Embodiment 115. The multi-functional molecule of any one of embodiments 109-113, characterized in that upon internalization into an endosome, the first moiety dissociates from the endocytic receptor.

[0578] Embodiment 116. The multi-functional molecule of embodiment 115, characterized in that upon internalization into an endosome, the first moiety binds a different endocytic receptor, e.g., an endocytic receptor having a different structure and/or function compared to the endocytic receptor which was bound to the first moiety at internalization into the cell.

[0579] Embodiment 117. The multi-functional molecule of embodiment 115, characterized in that upon internalization into an endosome, the first moiety binds the same endocytic receptor, e.g., an endocytic receptor having the same structure and/or function compared to the endocytic receptor which was bound to the first moiety at internalization into the cell.

[0580] Embodiment 118. The multi-functional molecule of any one of embodiments 109-117, characterized in that upon internalization into an endosome the target dissociates from the second moiety in the presence of the second pH.

[0581] Embodiment 119. The multi-functional molecule of embodiment 118, wherein dissociation occurs in an intracellular compartment of a cell.

[0582] Embodiment 120. The multi-functional molecule of embodiment 118 or 119, wherein dissociation of the target from the second moiety results in degradation of the target.

[0583]

[0584] Embodiment 121. The multi-functional molecule of embodiment 120, wherein degradation occurs at a third pH.

[0585] Embodiment 122. The multi-functional molecule of embodiment 120 or 121, wherein degradation occurs in a late endosome and/or a lysosome.

[0586] Embodiment 123. The multi-functional molecule of any one of embodiments 118-122, wherein the multi-functional molecule from which the target has dissociated is bound to an endocytic receptor with the first moiety.

[0587] Embodiment 124. The multi-functional molecule of any one of embodiments 118-123, characterized in that binding of the first moiety to the endocytic receptor delivers (e.g., recycles) the multi-functional molecule to a plasma membrane at the surface of the cell.

[0588] Embodiment 125. The multi-functional molecule of any one of embodiments 118-124, wherein the first moiety dissociates from the endocytic receptor at a plasma membrane comprising a first pH.

[0589] Embodiment 126. The multi-functional molecule of embodiment 125, wherein the multi-functional molecule which is dissociated from the endocytic receptor is not bound to the cell surface.

[0590] Embodiment 127. The multi-functional molecule of embodiment 125 or 126, wherein the multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more endocytic receptors with the first moiety.

[0591] Embodiment 128. The multi-functional molecule of any one of embodiments 125-127, wherein the multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more targets with the second moiety at the first pH.

[0592] Embodiment 129. The multi-functional molecule of any one of embodiments 105-122, characterized in that upon internalization into an endosome the third moiety binds to the endocytic receptor at the second pH and forms a third complex.

[0593] Embodiment 130. The multi-functional molecule of embodiment 129, characterized in that binding of the third moiety to the endocytic receptor delivers (e.g., recycles) the third complex to a plasma membrane at the surface of the cell.

[0594] Embodiment 131. The multi-functional molecule of embodiment 130, wherein the surface of the cell has the first pH.

[0595] Embodiment 132. The multi-functional molecule of embodiment 131, wherein the third moiety dissociates from the endocytic receptor bound by the third moiety, at the first pH.

[0596] Embodiment 133. The multi-functional molecule of embodiment 132, wherein the multi-functional molecule which is dissociated from the endocytic receptor bound by the third moiety, at the surface of the cell is not bound to the plasma membrane at the cell surface.

[0597] Embodiment 134. The multi-functional molecule of embodiment 133, wherein the multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more targets with the second moiety at the first pH.

[0598] Embodiment 135. The multi-functional molecule of embodiment 133 or 134, wherein the multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more endocytic receptors with the first moiety.

[0599] Embodiment 136. The multi-functional molecule of any one of embodiments 133-135, wherein the multi-functional molecule is able to bind to (e.g., exhibit repeated binding) one or more endocytic receptors with the third moiety upon internalization into a cell compartment with the second pH.

[0600] Embodiment 137. The multi-functional molecule of any one of the preceding embodiments, comprising one or more first moieties of (a).

[0601] Embodiment 138. The multi-functional molecule of any one of the preceding embodiments, comprising one or more second moieties of (b).

[0602] Embodiment 139. The multi-functional molecule of any one of embodiments 6-138, comprising one or more third moieties of (c).

[0603] Embodiment 140. The multi-functional molecule of any one of the preceding embodiments, wherein the molecule comprises one or more linkers.

[0604] Embodiment 141. The multi-functional molecule of embodiment 140, wherein the one or more linkers are situated between the first and second moieties.

[0605] Embodiment 142. The multi-functional molecule of embodiment 140 or 141, wherein the one or more linkers are situated between the first and third moieties.

[0606] Embodiment 143. The multi-functional molecule of any one of embodiments 140-142, wherein the linkers are situated between the second and third moieties.

[0607] Embodiment 144. The multi-functional molecule of any one of embodiments 40-143, wherein the multi-functional molecule comprises one or more peptides comprising the first moiety linked to one or more second moieties with a linker.

[0608] Embodiment 145. The multi-functional molecule of any one of embodiments 40-144, wherein the multi-functional molecule comprises one or more peptides comprising the first moiety linked to one or more third moieties with one or more linkers.

[0609] Embodiment 146. The multi-functional molecule of any one of embodiments 140-145, wherein the one or more linkers comprise a Gly-Ser linker, or an EAAAK linker.

[0610] Embodiment 147. A polynucleotide encoding the multi-functional molecule of any one of the preceding embodiments.

[0611] Embodiment 148. A composition comprising the multi-functional molecule of any one of embodiments 1-146.

[0612] Embodiment 149. A composition comprising a population of multi-functional molecules of any one of embodiments 1-146, wherein the population of multi-functional molecules has an N-glycan profile that is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or about 100% homogeneous at one or more of the N-glycosylation site(s).

[0613] Embodiment 150. The composition of embodiment 149, wherein the homogeneity of the N-glycan profile at one or more of the N-glycosylation sites is determined by N-glycan analysis, glycopeptide analysis or intact protein analysis.

[0614] Embodiment 151. The composition of embodiment 149, wherein the N-glycan profile comprises about 30% to 40%, about 40% to about 50%. about 50% to about 60%, about

60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% of the N-glycan of the structure provided in embodiment 36.

[0615] Embodiment 152. The composition of embodiment 149, wherein the population of multi-functional molecules has an N-glycan profile comprising about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% of the N-glycan of the structure provided in embodiment 36 among all glycans in the N-glycan profile.

[0616] Embodiment 153. The composition of any one of embodiments 148-152, wherein the composition is a pharmaceutical composition.

[0617] Embodiment 154. The composition of embodiment 153, wherein the pharmaceutical composition comprises one or more excipients.

[0618] Embodiment 155. A Leishmania host cell expressing a multi-functional molecule of any one of embodiments 1-146.

[0619] Embodiment 156. A method comprising administering to a subject a pharmaceutical composition of embodiment 153 or 154.

[0620] Embodiment 157. The method of embodiment 156, wherein the method is a treatment method.

[0621] Embodiment 158. The method of embodiment 156, wherein the method is a prevention method.

[0622] Embodiment 159. The method of any one of embodiments 156-158, wherein the subject has a disease or disorder, or one or more symptoms of a disease or disorder.

[0623] Embodiment 160. The method of embodiment 159, wherein the disease or disorder is associated with a soluble protein.

[0624] Embodiment 161. The method of embodiment 159 or 160, wherein the disease or disorder is chosen from: an autoimmune disease, a metabolic disease, a genetic disease, a fibrotic disease, a rare disease, a neurodegenerative disease, a vascular disease, a cancer, or a disease requiring enzyme replacement therapy.

[0625] Embodiment 162. The method of embodiment 161, wherein the cancer is a solid tumor or a hematological cancer.

[0626] Embodiment 163. A method of delivering a target to a cell, comprising

[0627] contacting a cell with a complex comprising the multi-functional molecule of any one of embodiments 1-146 and a target, under conditions sufficient to deliver the complex comprising the multi-functional molecule to the cell.

[0628] Embodiment 164. The method of embodiment 163, wherein contacting comprises internalization of the complex into a compartment of the cell.

[0629] Embodiment 165. The method of embodiment 164, wherein the compartment is an endosome.

[0630] Embodiment 166. The method of embodiments 165, wherein the endosome comprises an early endosome and/or a recycling endosome.

[0631] Embodiment 167. The method of embodiment 165, wherein the endosome does not comprise a late endosome and/or a lysosome.

[0632] Embodiment 168. The method of embodiment 165 or 166, wherein the target dissociates from the complex in the endosome.

[0633] Embodiment 169. The method of embodiment 168, wherein the dissociated target is transported to a different compartment.

[0634] Embodiment 170. The method of embodiment 169, wherein the different compartment is or comprises a late endosome and/or a lysosome.

[0635] Embodiment 171. The method of any one of embodiments 168-170, wherein the target is degraded.

[0636] Embodiment 172. The method of embodiment 171, wherein degradation occurs in a late endosome and/or a lysosome.

[0637] Embodiment 173. The method any one of embodiments 163-172, wherein the multi-functional molecule from which the target dissociated remains in the endosome.

[0638] Embodiment 174. The method of embodiment 173, wherein the multi-functional molecule is delivered to the surface of the cell via binding of the first moiety to an endocytic receptor in the endosome.

[0639] Embodiment 175. The method of embodiment 173, wherein the multi-functional molecule is delivered to the surface of the cell via binding of the third moiety to an endocytic receptor in the endosome.

[0640] Embodiment 176. A method of degrading a target, comprising

[0641] contacting a cell with a complex comprising the multi-functional molecule of any one of embodiments 1-146 and a target, under conditions sufficient to degrade the target in the cell

[0642] Embodiment 177. The method of embodiment 176, wherein degradation of the target can be modulated by altering the number of glycans of the first moiety.

[0643] Embodiment 178. The method of embodiment 177, wherein increasing the number of glycans increases the rate of degradation of the target.

[0644] Embodiment 179. The method of any one of embodiments 163-178, wherein the cell is *in vitro*.

[0645] Embodiment 180. The method of any one of embodiments 163-178, wherein the cell is *in vivo*, e.g., in a subject.

[0646] Embodiment 181. The method of any one of embodiments 163-178, wherein the cell is *ex vivo*.

[0647] Embodiment 182. The method of any one of embodiments 163-181, wherein contacting comprises administering a multi-functional molecule of any one of embodiments 1-146, or a pharmaceutical composition of embodiment 153 or 154 to a subject.

[0648] Embodiment 183. The method of any one of embodiments 156-178, 180 or 182, wherein the subject is a mammal.

[0649] Embodiment 184. The method of embodiment 183, wherein the subject is a human.

INCORPORATION BY REFERNECE

[0650] Each publication, including scientific references to the scientific literature, patent references, and electronic databases, websites and other references accessible through the

internet, are hereby incorporated by reference herein, in their entirety, for their disclosure relevant to this specification and as cited herein.

EQUIVALENTS

[0651] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

Claims

What is claimed is:

1. A multi-functional molecule, comprising:

(a) a first moiety that specifically binds to an endocytic receptor; and

(b) a second moiety that specifically binds to a target at a first pH.

2. The multi-functional molecule of claim 1, wherein the first moiety:

(i) binds to an endocytic receptor under a first set of conditions;

(ii) binds to an endocytic receptor under a second set of conditions; and/or

(iii) does not bind to or has lower affinity binding to an endocytic receptor under a third set of conditions.

3. A multi-functional molecule, comprising:

(a) a first moiety that specifically binds to an endocytic receptor under a first set of conditions;

(b) a second moiety that specifically binds to a target at a first pH; and

(c) a third moiety that specifically binds to an endocytic receptor under a second set of conditions.

4. The multi-functional molecule of claims 2 or 3, wherein:

(i) the endocytic receptor bound by the first moiety under the first set of conditions and the endocytic receptor bound by the first moiety under the second set of conditions are different endocytic receptors, e.g., endocytic receptors having different structure and/or function; or

(ii) the endocytic receptor bound by the first moiety and the endocytic receptor bound by the third moiety are different endocytic receptors, e.g., endocytic receptors having different structure and/or function.

5. The multi-functional molecule of claims 2 or 3, wherein:

(i) the endocytic receptor bound by the first moiety under the first set of conditions and the endocytic receptor bound by the first moiety under the second set of conditions are

the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function; or

(ii) the endocytic receptor bound by the first moiety and the endocytic receptor bound by the third moiety are the same endocytic receptor, e.g., endocytic receptors having the same structure and/or function.

6. The multi-functional molecule of any one of claims 2-5, wherein the first set of conditions comprises:

(i) a first pH (e.g., a pH of about pH 6.7 to about pH 8);

(ii) presence of a cation (e.g., Ca 2+); or

(iii) both (i) and (ii).

7. The multi-functional molecule of any one of claims 2-6, wherein the second set of conditions comprises:

(i) a second pH (e.g., a pH of about pH 6.5 to about pH 5.6);

(ii) presence in an intracellular vesicle which:

(a) expresses, or has detectable presence of, a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof,

(b) has no detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof;

(c) has the ability to export a molecule to an extracellular space;

(d) expresses one or more receptors that can bind to a molecule and export the molecule to an extracellular space;

(e) does not have the ability to degrade a molecule;

(f) has no or minimal proteolytic activity (e.g., hydroylase activity);

(g) any combination or all of (a)-(h);

(iii) an early endosome or a recycling endosome; or

(iv) any combination or all of (i)-(iii).

8. The multi-functional molecule of any one of claims 2-7, wherein the third set of conditions comprises:

(i) a third pH (e.g., less than pH 5.5);

(ii) presence in an intracellular vesicle which:

(a) has no detectable presence of a polypeptide associated with an early endosome and/or a recycling endosome, or a variant or a functional fragment thereof,

(b) expresses, or has detectable presence of a polypeptide associated with a late endosome and/or a lysosome, or a variant or a functional fragment thereof;

(c) has the ability to degrade a molecule;

(d) has proteolytic activity (e.g., hydroylase activity);

(e) any combination or all of (a)-(d); or

(iii) a late endosome or a lysosome; or

(iv) any combination or all of (i)-(iii).

9. The multi-functional molecule of claim 8, wherein the first moiety has reduced binding, or does not bind to an endocytic receptor at the third pH, optionally wherein reduced binding is assessed as compared to binding of the first moiety to the endocytic receptor at a first pH or a second pH.

10. The multi-functional molecule of any one the preceding claims, wherein the first moiety undergoes a pH dependent conformation change, optionally wherein the conformation change prevents binding or reduced binding to the endocytic receptor at the second pH and/or third pH.

11. The multi-functional molecule of any one the preceding claims, wherein the first moiety binds to the endocytic receptor with an affinity that allows internalization of the multi-functional molecule into an intracellular compartment in the cell, optionally wherein the first moiety does not stay bound to the endocytic receptor upon internalization into the intracellular compartment.

12. The multi-functional molecule of claim 11, wherein the first moiety binds a different endocytic receptor upon internalization into the intracellular compartment, optionally wherein the intracellular compartment is an endosome and/or wherein the intracellular compartment has a second pH.

13. The multi-functional molecule of any one of the preceding claims, wherein

(i) the first moiety binds to the endocytic receptor with an affinity that allows for transporting the multi-functional molecule to the cell surface, and/or

(ii) the first moiety does not bind to the endocytic receptor with an affinity that allows for transporting the multi-functional molecule to a late endosome and/or lysosome.

14. The multi-functional molecule of claim 13, wherein upon delivery of the multi-functional molecule to the plasma membrane at the cell surface, the first moiety dissociates from the endocytic receptor thereby releasing the multi-functional molecule from the plasma membrane at the cell surface.

15. The multi-functional molecule of any one the preceding claims, wherein the first moiety comprises one or more peptides that specifically binds to an endocytic receptor, wherein the endocytic receptor is chosen from: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, mannose 6 phosphate receptor (M6PR), a Siglec, one or more SNARE proteins, a multi-drug transporter, or a combination thereof.

16. The multi-functional molecule of the preceding claims, wherein the endocytic receptor is ASGPR or a fragment or variant thereof, or a complex comprising ASGPR.

17. The multi-functional molecule of any one of the preceding claims, wherein the first moiety comprises an antibody agent, optionally wherein the antibody agent comprises a full antibody, a Fab fragment, an scFv, a nanobody, a duobody, or a single domain antibody (e.g., a VHH).

18. The multi-functional molecule of any one of the preceding claims, wherein the first moiety comprises one or more glycans, optionally wherein the one or more glycans are conjugated to the second moiety and/or the third moiety at one or more glycosylation sites.

19. The multi-functional molecule of any one of claims 6-18, wherein
(i) the first pH is about pH 6.7 to about pH 8; and/or
(ii) the second pH is about pH 6.5 to about pH 5.6.

20. The multi-functional molecule of any one of the preceding claims, wherein the first moiety binds to the endocytic receptor in a pH dependent manner, optionally wherein:
(i) the first moiety binds to the endocytic receptor at the first pH; and/or
(ii) the first moiety binds to the endocytic receptor at the second pH.

21. The multi-functional molecule of any one of claims 18-20, wherein the glycan comprises a terminal GlcNac, a terminal GalNac, or a terminal Gal.

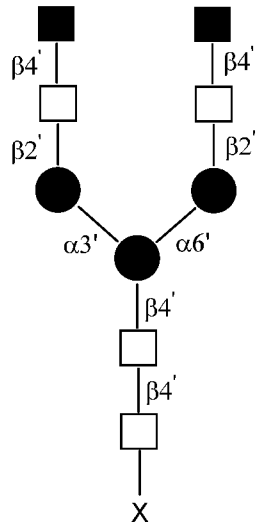
22. The multi-functional molecule of any one of claims 18-21, wherein the one or more glycans is an N-glycan, optionally wherein the N-glycan is linked to the second moiety and/or third moiety at 1, 2, 3, 4 or 5 N-glycosylation sites.

23. The multi-functional molecule of any one of claims 18-22, wherein the one or more glycans comprises a glycan structure comprising GlcNAc2-Man3-GlcNAc2, GalNAc2-GlcNAc2-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, GlcNAcl-Man3-GlcNAc2, Gal2-GlcNAc2-Man3-GlcNAc2, Gal1- GlcNAc2-Man3-GlcNAc2, GalNAc1-GlcNAc2-Man3-GlcNAc2, GlcNAc3-Man3-GlcNAc2, GlcNAc4-Man3-GlcNAc2, Gal3-GlcNAc3-Man3-GlcNAc2, GalNAc3-GlcNAc3-Man3-GlcNAc2, GalNAc4-GlcNAc4-Man3-GlcNAc2, Gal4-GlcNAc4-Man3-GlcNAc2, or Man-6-P -N-glycan.

24. The multi-functional molecule of claim 23, wherein the glycan structure comprises a biantennary structure, optionally wherein the glycan structure comprises a biantennary GalNAc.

25. The multi-functional molecule of claim 24, wherein the biantennary GalNac binds to an asialoglycoprotein receptor (ASGPR) or a fragment or variant thereof, or a complex comprising ASGPR.

26. The multi-functional molecule of any one of claims 22-25, wherein the N-glycan has a structure of:



wherein the black square represents an N-acetyl galactosamine (GalNAc), the white square represents an N-acetylglucosamine (GlcNAc) residue and the black circle represents a mannose (Man) residue, and wherein X represents an amino acid residue of the second moiety or third moiety.

27. The multi-functional molecule of any one of claims 22-26, wherein the N-glycan is conjugated to the second moiety and/or third moiety at at least one, two, three, or four N-glycosylation sites.

28. The multi-functional molecule of any one of claims 22-27, wherein the N-glycosylation site is naturally occurring or engineered into the amino acid sequence of the second moiety and/or third moiety.

29. The multi-functional molecule of any one of the preceding claims, wherein the second moiety has reduced binding, or does not bind to a target at the second pH and/or the third pH, optionally wherein the second moiety undergoes a pH dependent conformation change and the conformation change prevents binding or reduced binding to a target at the second pH and/or third pH.

30. The multi-functional molecule of any one of the preceding claims, wherein the second moiety comprises one or more peptides that specifically binds to a target wherein:

the target comprises a secreted protein, an antibody, or an autoantibody, or

the target is membrane-bound.

31. The multi-functional molecule of any one of the preceding claims, wherein the second moiety comprises an antibody agent, optionally wherein the antibody agent comprises an antigen binding fragment having (1) at least one amino acid substituted with a histidine; or (2) insertion of at least one histidine, or wherein the antibody agent comprises an Fc domain having one or more mutations to alter binding to a receptor, e.g., FcRn.

32. The multi-functional molecule of any one of claims 3-31, wherein the third moiety has reduced binding, or does not bind to the endocytic receptor at the first pH and/or the third pH, optionally wherein the third moiety undergoes a pH-dependent conformation change and wherein the conformation change prevents binding or reduces binding of the third moiety to the endocytic receptor at the first pH and/or the third pH.

33. The multi-functional molecule of any one of claims 3-32, wherein the third moiety comprises one or more peptides that specifically binds to the endocytic receptor, optionally wherein the endocytic receptor comprises: an asialoglycoprotein receptor (ASGPR); a mannose binding receptor, a Cluster of Differentiation 206 (CD206) receptor; a DC-SIGN (Cluster of Differentiation 209 or CD209) receptor; a C-Type Lectin Domain Family 4 Member G (LSECTin) receptor; a macrophage inducible Ca2+-dependent lectin receptor (Mincle); a L-SIGN CD209L receptor; dectin-1; dectin -2, langerin, macrophage mannose 2 receptor, BDCA-2, DCIR, MBL, MDL, MICL, CLEC2, DNGR1, CLEC12B, DEC-205, mannose 6 phosphate receptor (M6PR), a Siglec, one or more SNARE proteins, a multi-drug transporter, or any combination thereof.

34. The multi-functional molecule of any one of claims 3-33, wherein the third moiety binds to the endocytic receptor, with an affinity that allows for transporting the multi-functional molecule to the plasma membrane at the cell surface.

35. The multi-functional molecule of any one of claims 3-34, wherein the endocytic receptor bound by the third moiety, is or comprises the neonatal Fc receptor (FcRn), a Siglec, one or more SNARE proteins, or a multi-drug transporter.

36. The multi-functional molecule of any one of claims 3-35, wherein the third moiety comprises an antibody agent.

37. The multi-functional molecule of any one of the preceding claims, wherein the molecule comprises one or more linkers, wherein the one or more linkers are situated:

(i) between the first and second moieties;

(ii) between the first and third moieties; and/or

(iii) between the second and third moieties.

38. A polynucleotide encoding the multi-functional molecule of any one of the preceding claims.

39. A composition comprising the multi-functional molecule of any one of claims 1-37, or the polynucleotide of claim 38.

40. A composition comprising a population of multi-functional molecules of any one of claims 1-37, wherein the population of multi-functional molecules has an N-glycan profile that is at least 30% homogeneous at one or more of the N-glycosylation site(s).

41. The composition of claim 39 or 40, wherein the composition is a pharmaceutical composition, optionally wherein the pharmaceutical composition comprises one or more excipients.

42. A Leishmania host cell expressing a multi-functional molecule of any one of claims 1-37.

43. A method comprising:

administering to a subject a pharmaceutical composition of claim 41.

44. The method of claim 43, wherein the method is a treatment method or a prevention method.

45. The method of claim 43 or 44, wherein the subject has a disease or disorder, or one or more symptoms of a disease or disorder, optionally wherein the disease or disorder is associated with a soluble protein.

46. The method of claim 45, wherein the disease or disorder is chosen from: an autoimmune disease, a metabolic disease, a genetic disease, a fibrotic disease, a rare disease, a neurodegenerative disease, a vascular disease, a cancer, or a disease requiring enzyme replacement therapy.

47. A method of delivering a target to a cell, comprising

contacting a cell with a complex comprising the multi-functional molecule of any one of claims 1-37 and a target, under conditions sufficient to deliver the complex comprising the multi-functional molecule to the cell,

optionally wherein contacting comprises internalization of the complex into a compartment of the cell.

48. The method of claim 47, wherein the compartment is an endosome, optionally wherein the endosome comprises an early endosome and/or a recycling endosome.

49. The method of claim 47 or 48, wherein the target:

(i) dissociates from the complex in the endosome, optionally wherein the dissociated target is transported to a different compartment; and/or

(ii) is degraded, optionally wherein degradation occurs in a late endosome and/or a lysosome.

50. The method of claim 49, wherein the multi-functional molecule from which the target dissociated:

(i) remains in the endosome;

(ii) is delivered to the surface of the cell via binding of the first moiety to an endocytic receptor in the endosome; and/or

(iii) is delivered to the surface of the cell via binding of the third moiety to an endocytic receptor in the endosome.

51. A method of degrading a target, comprising

contacting a cell with a complex comprising the multi-functional molecule of any one of claims 1-37 and a target, under conditions sufficient to degrade the target in the cell.

52. The method of any one of claims 47-51, wherein the cell is:

(i) *in vitro*;

(ii) *in vivo*, e.g., in a subject; or

(iii) *ex vivo*.

53. The method of any one of claims 47-52, wherein contacting comprises administering a multi-functional molecule of any one of claims 1-37, or a pharmaceutical composition of claim 41 to a subject.

54. The method of any one of claims 43-46 or 52-53, wherein the subject is a mammal.

55. Use of the multi-functional molecule of any one of claims 1-37 in the manufacture of a medicament for treating a disease or disorder in a subject in need thereof, wherein treating comprises administering the multi-functional molecule to the subject.

56. A composition comprising the multi-functional molecule of any one of claims 1-37 for use in treating a disease or disorder in a subject in need thereof, wherein the use comprises administering the multi-functional molecule to the subject.

57. Use of the multi-functional molecule of any one of claims 1-37 in the manufacture of a medicament for delivering a target to a cell, wherein the use comprises:

contacting a cell with a complex comprising the multi-functional molecule and a target, under conditions sufficient to deliver the complex comprising the multi-functional molecule to the cell,

optionally wherein contacting comprises internalization of the complex into a compartment of the cell.

58. A composition comprising the multi-functional molecule of any one of claims 1-37 for use in delivering a target to a cell, wherein the use comprises:

contacting a cell with a complex comprising the multi-functional molecule and a target, under conditions sufficient to deliver the complex comprising the multi-functional molecule to the cell,

optionally wherein contacting comprises internalization of the complex into a compartment of the cell.

59. The use of claim 55 or the composition for use of claim 54, wherein the subject is a mammal.

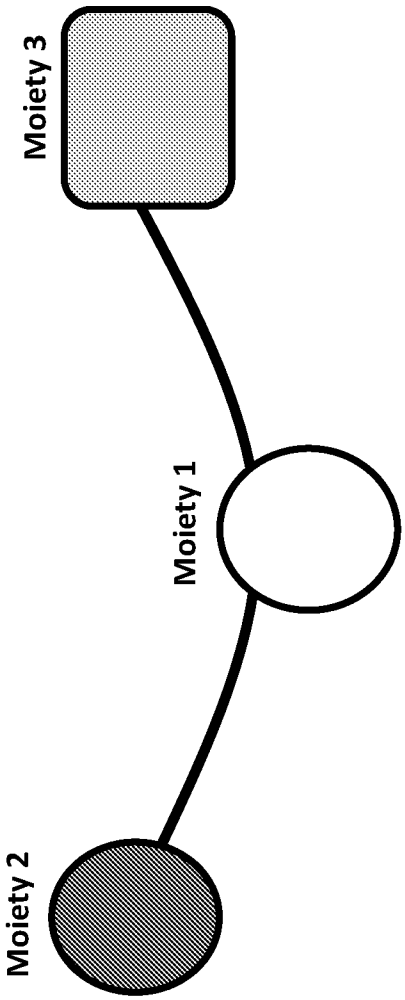


FIG. 1

FIG. 2B

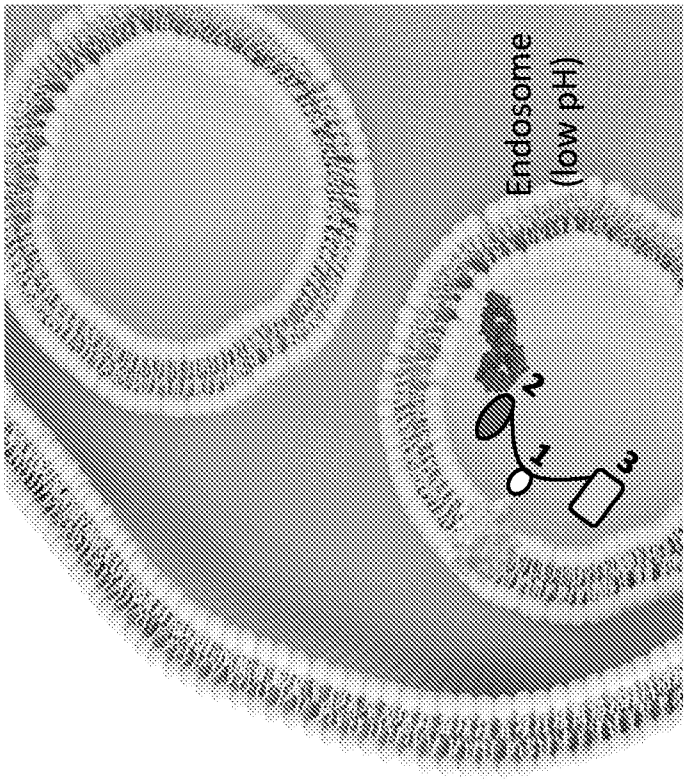


FIG. 2A

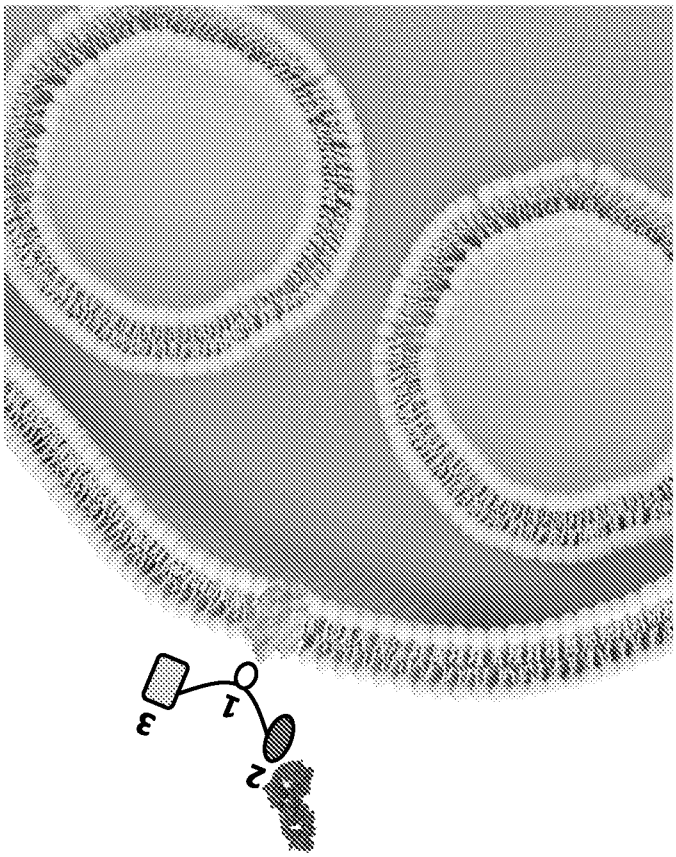


FIG. 2D

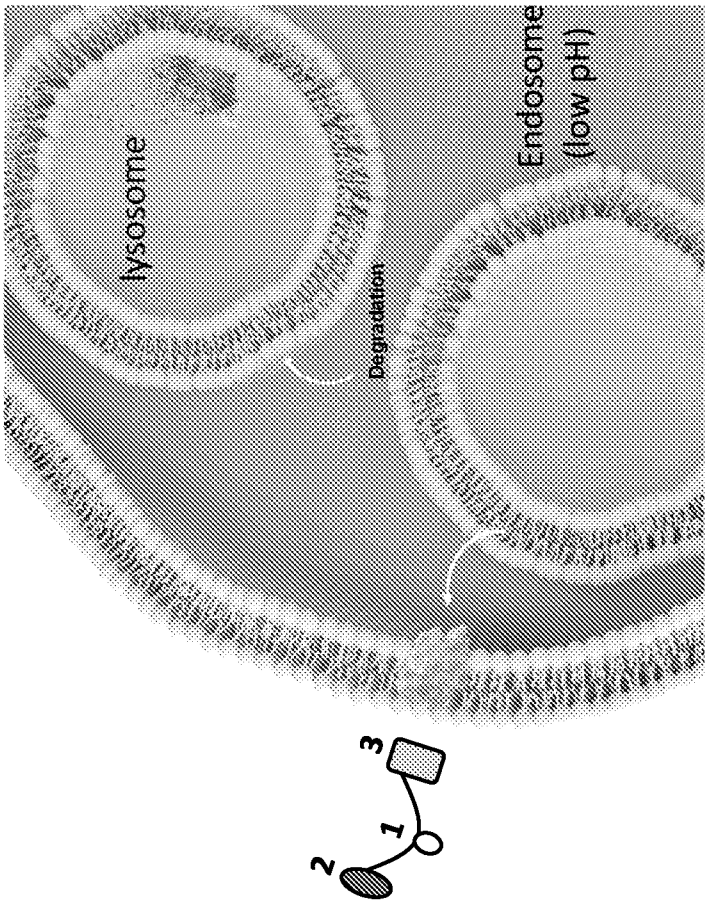

lysosome
Degradation
Endosome
(low pH)
1
2
3

FIG. 2C

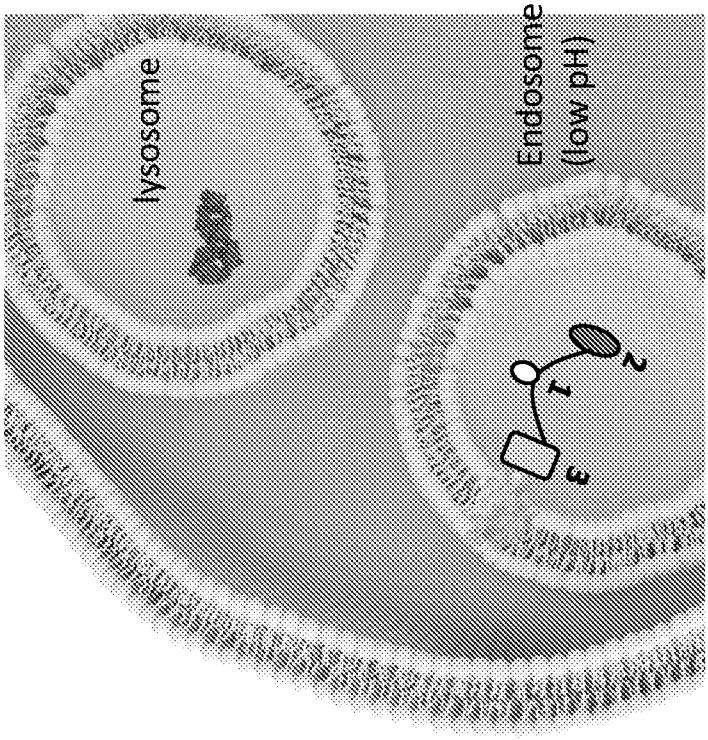
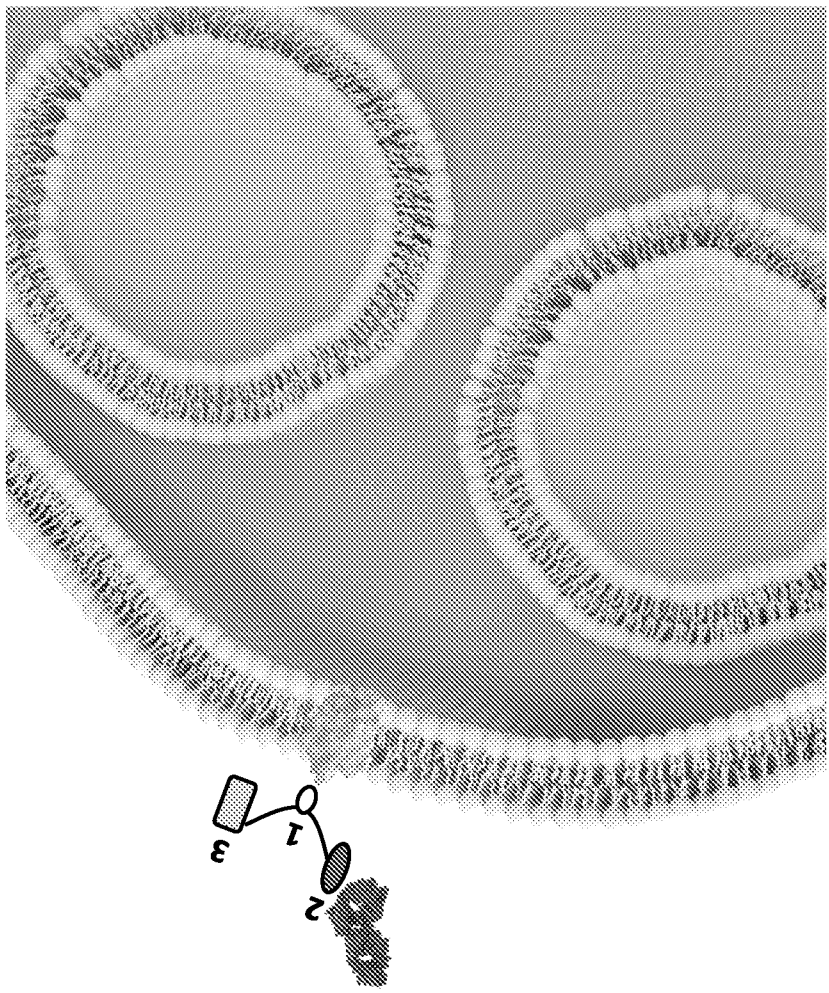

lysosome
Endosome
(low pH)
1
2
3

FIG. 2E



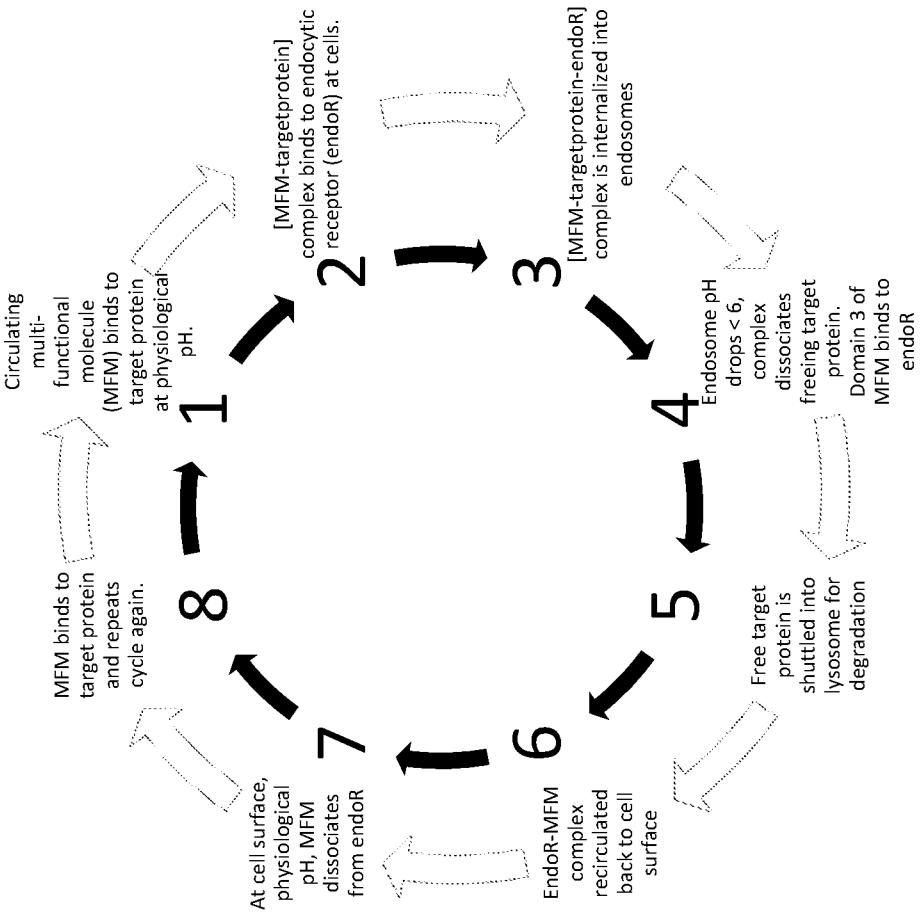

1
Circulating multi-functional molecule (MFM) binds to target protein at physiological pH.
2
[MFM-targetprotein] complex binds to endocytic receptor (endoR) at cells.
3
[MFM-targetprotein-endoR] complex is internalized into endosomes
4
Endosome pH drops < 6, complex dissociates freeing target protein. Domain 3 of MFM binds to endoR
5
Free target protein is shuttled into lysosome for degradation
6
EndoR-MFM complex recirculated back to cell surface
7
At cell surface, physiological pH, MFM dissociates from endoR
8
MFM binds to target protein and repeats cycle again.

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/053854

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/54 A61K47/61 A61P43/00
ADD.

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)
A61K A61P

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 practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/199621 A1 (UNIV YALE [US]) 17 October 2019 (2019-10-17) examples claims	1-41, 43-59
	- - - - -	
X	WO 2019/199634 A1 (UNIV YALE [US]) 17 October 2019 (2019-10-17) examples claims	1-41, 43-59
	- - - - -	
X	WO 2022/053673 A1 (LIMMATECH BIOLOGICS AG [CH]) 17 March 2022 (2022-03-17) cited in the application examples table 7 claims	1-41, 43-59
	- - - - -	
	-/- -	

X Further documents are listed in the continuation of Box C.

X See patent family 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 application or patent 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2024	17/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dullaart, Anwyn

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/053854

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 765 192 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 13 August 2014 (2014-08-13) examples claims	1-41, 43-59
X	WO 2022/200390 A2 (GLYCOERA AG [CH]) 29 September 2022 (2022-09-29) examples claims	1-41, 43-59
X	WO 2022/200388 A1 (GLYCOERA AG [CH]) 29 September 2022 (2022-09-29) examples claims	1-41, 43-59
X	WO 2013/022721 A1 (MERCK SHARP & DOHME [US]; MEEHL MICHAEL [US] ET AL.) 14 February 2013 (2013-02-14) examples claims	1-41, 43-59
X	WO 2014/152137 A2 (GLYCOBIA INC [US]; FISHER ADAM C [US] ET AL.) 25 September 2014 (2014-09-25) examples claims	1-41, 43-59
X	WO 2022/099307 A1 (CHO PHARMA INC; CHO PHARMA USA INC [US]) 12 May 2022 (2022-05-12) examples claims	1-41, 43-59
X	US 2017/362338 A1 (DAVIDSON ROBERT [US] ET AL) 21 December 2017 (2017-12-21) examples claims	1-41, 43-59
X	WO 2020/247642 A1 (ARIZONA BOARD OF REGENTS ON BEHALF OF ARIZONA STATE UNIVERSITY [US] ET) 10 December 2020 (2020-12-10) examples claims	1-41, 43-59

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/053854

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DANIEL J KARST ET AL: "Modulation and modeling of monoclonal antibody N-linked glycosylation in mammalian cell perfusion reactors", BIOTECHNOLOGY AND BIOENGINEERING, vol. 114, no. 9, 23 May 2017 (2017-05-23), pages 1978-1990, XP071115449, ISSN: 0006-3592, DOI: 10.1002/BIT.26315 abstract figures page 1989	1-41, 43-59
X	EP 2 762 166 A1 (CHUGAI PHARMACEUTICAL CO LTD [JP]) 6 August 2014 (2014-08-06) examples claims	1-41, 43-59
X	WO 2019/002512 A2 (LIMMATECH BIOLOGICS AG [CH]) 3 January 2019 (2019-01-03) cited in the application examples claims	42
X	WO 2019/234021 A1 (LIMMATECH BIOLOGICS AG [CH]) 12 December 2019 (2019-12-12) examples claims	42
X	WO 2021/140143 A1 (LIMMATECH BIOLOGICS AG [CH]) 15 July 2021 (2021-07-15) cited in the application examples claims	42
X,P	WO 2023/235522 A1 (BLAZE BIOSCIENCE INC [US]) 7 December 2023 (2023-12-07) examples claims	1-41, 43-59
X,P	WO 2024/068753 A1 (GLYCOERA AG [CH]) 4 April 2024 (2024-04-04) examples claims	1-41, 43-59
X,P	WO 2024/068768 A1 (GLYCOERA AG [CH]) 4 April 2024 (2024-04-04) examples claims	42

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2024/053854

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/053854

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019199621 A1	17-10-2019	CA 3134765 A1	17-10-2019
		EP 3773729 A1	17-02-2021
		US 2021139436 A1	13-05-2021
		US 2024083859 A1	14-03-2024
		WO 2019199621 A1	17-10-2019
WO 2019199634 A1	17-10-2019	CA 3134610 A1	17-10-2019
		CN 112236169 A	15-01-2021
		EP 3773727 A1	17-02-2021
		US 2021145974 A1	20-05-2021
		WO 2019199634 A1	17-10-2019
WO 2022053673 A1	17-03-2022	AU 2021342346 A1	23-03-2023
		CA 3192770 A1	17-03-2022
		EP 4211163 A1	19-07-2023
		JP 2023542104 A	05-10-2023
		US 2024067714 A1	29-02-2024
		WO 2022053673 A1	17-03-2022
EP 2765192 A1	13-08-2014	EP 2765192 A1	13-08-2014
		EP 3617313 A1	04-03-2020
		JP 6271251 B2	31-01-2018
		JP 6672251 B2	25-03-2020
		JP 7250718 B2	03-04-2023
		JP 2018099118 A	28-06-2018
		JP 2020105199 A	09-07-2020
		JP 2023078345 A	06-06-2023
		JP WO2013051294 A1	30-03-2015
		US 2015299313 A1	22-10-2015
		US 2022324966 A1	13-10-2022
		WO 2013051294 A1	11-04-2013
WO 2022200390 A2	29-09-2022	AU 2022246021 A1	05-10-2023
		CA 3210709 A1	29-09-2022
		EP 4314053 A2	07-02-2024
		JP 2024513757 A	27-03-2024
		WO 2022200390 A2	29-09-2022
WO 2022200388 A1	29-09-2022	AU 2022244103 A1	05-10-2023
		CA 3211056 A1	29-09-2022
		EP 4314052 A1	07-02-2024
		JP 2024513755 A	27-03-2024
		WO 2022200388 A1	29-09-2022
WO 2013022721 A1	14-02-2013	AR 087433 A1	26-03-2014
		AU 2012294656 A1	20-02-2014
		CA 2843640 A1	14-02-2013
		CN 103889444 A	25-06-2014
		EP 2744510 A1	25-06-2014
		JP 2014525922 A	02-10-2014
		KR 20140057589 A	13-05-2014
		TW 201311720 A	16-03-2013
		US 2014235537 A1	21-08-2014
		US 2016289290 A1	06-10-2016
		WO 2013022721 A1	14-02-2013
WO 2014152137 A2	25-09-2014	CA 2906671 A1	25-09-2014
		CN 105378096 A	02-03-2016

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/053854

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		EP 2971030 A2	20-01-2016
		JP 2016518114 A	23-06-2016
		KR 20160021075 A	24-02-2016
		SG 10201707565W A	30-10-2017
		SG 11201508347Y A	27-11-2015
		US 2014273163 A1	18-09-2014
		US 2016177355 A1	23-06-2016
		WO 2014152137 A2	25-09-2014
WO 2022099307 A1	12-05-2022	AU 2021376384 A1	08-06-2023
		CA 3196069 A1	12-05-2022
		EP 4240759 A1	13-09-2023
		JP 2023549677 A	29-11-2023
		KR 20230104192 A	07-07-2023
		TW 202233233 A	01-09-2022
		US 2022143172 A1	12-05-2022
		WO 2022099307 A1	12-05-2022
US 2017362338 A1	21-12-2017	US 2017362338 A1	21-12-2017
		US 2022242970 A1	04-08-2022
WO 2020247642 A1	10-12-2020	US 2022298211 A1	22-09-2022
		WO 2020247642 A1	10-12-2020
EP 2762166 A1	06-08-2014	AU 2012317418 A1	01-05-2014
		BR 112014007687 A2	13-06-2017
		CA 2850194 A1	04-04-2013
		CA 3186128 A1	04-04-2013
		CN 103974721 A	06-08-2014
		CN 108144058 A	12-06-2018
		CN 110563838 A	13-12-2019
		CN 110639014 A	03-01-2020
		EP 2762166 A1	06-08-2014
		EP 3680251 A1	15-07-2020
		HK 1198578 A1	30-04-2015
		HK 1252829 A1	06-06-2019
		JP 6093305 B2	08-03-2017
		JP 6261785 B2	17-01-2018
		JP 6998748 B2	18-01-2022
		JP 2017114882 A	29-06-2017
		JP 2018058891 A	12-04-2018
		JP 2020079296 A	28-05-2020
		JP 2023018124 A	07-02-2023
		JP WO2013047752 A1	30-03-2015
		KR 20140076593 A	20-06-2014
		KR 20200051048 A	12-05-2020
		KR 20200096692 A	12-08-2020
		KR 20230066646 A	16-05-2023
		MX 361713 B	14-12-2018
		RU 2014117505 A	10-11-2015
		SG 10201610870X A	27-02-2017
		SG 11201401101X A	28-08-2014
		TW 201321412 A	01-06-2013
		TW 201726745 A	01-08-2017
		TW 202120542 A	01-06-2021
		TW 202309079 A	01-03-2023
		US 2014335089 A1	13-11-2014
		US 2019112393 A1	18-04-2019

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/053854

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		US 2022389118 A1	08-12-2022
		WO 2013047752 A1	04-04-2013
WO 2019002512 A2	03-01-2019	AU 2018294504 A1	23-01-2020
		CN 111108211 A	05-05-2020
		CN 117487873 A	02-02-2024
		EP 3645732 A2	06-05-2020
		JP 7437162 B2	22-02-2024
		JP 2020530273 A	22-10-2020
		JP 2023134519 A	27-09-2023
		KR 20200022486 A	03-03-2020
		US 2021332403 A1	28-10-2021
		WO 2019002512 A2	03-01-2019
WO 2019234021 A1	12-12-2019	NONE	
WO 2021140143 A1	15-07-2021	AU 2021205617 A1	28-07-2022
		CA 3166726 A1	15-07-2021
		EP 4087921 A1	16-11-2022
		JP 2023510260 A	13-03-2023
		US 2023287327 A1	14-09-2023
		WO 2021140143 A1	15-07-2021
WO 2023235522 A1	07-12-2023	NONE	
WO 2024068753 A1	04-04-2024	WO 2024068753 A1	04-04-2024
		WO 2024068768 A1	04-04-2024
WO 2024068768 A1	04-04-2024	WO 2024068753 A1	04-04-2024
		WO 2024068768 A1	04-04-2024