

ENDOCYTIC AND SCAVENGER RECEPTOR-TARGETING CHIMERIC PROTEINS AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/599,308, filed November 15, 2023 and U.S. Provisional Patent Application No. 63/529,231, filed July 27, 2023, the disclosure of each of which is incorporated by reference herein in its entirety.

2. SEQUENCE LISTING

[0002] This application contains an electronic Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled “14789-002-228_SEQ_LISTING.xml”, was created on July 22, 2024, and is 82,106 bytes in size.

3. BACKGROUND

[0003] Targeted protein degradation (TPD) has emerged as a solution for eliminating the activity of difficult-to-target pathogenic proteins. One TPD approach uses proteolysis-targeting chimeras (PROTACs). PROTACs are primarily designed to target intracellular proteins for degradation. Approximately 35% of the human protein-coding genes have predicted secreted transcripts with at least one predicted transmembrane region¹ and are thus located extracellularly. Extracellular proteins, including secreted and membrane-anchored proteins, can be degraded via the endosome-lysosome or proteasome pathway. Lysosomal targeting chimeras (LYTACs) have emerged as a novel strategy for the degradation of extracellular proteins. Extracellular protein degradation is beneficial compared to inhibitory or blocking-based modalities if blocking the pathogenic secreted proteins is not sufficient to provide a clinical benefit or if blocking a receptor is not adequate to halt downstream signaling that drives the disease. Additionally, in cases where a pathogenic protein is difficult to drug via classical occupancy-driven approaches due to multiple functionalities or absence of binding domains, extracellular TPD can be employed as a pharmacological intervention whereby degrader binding triggers an event that reduces the cellular levels of the target protein, “event-driven pharmacology.”²

[0004] Recent studies have shown complex chimeric molecules, composed of a ligand for an internalizing receptor such as C-type lectins or cytokine receptors, can lead to degradation of extracellular targeted proteins. For example, Bertozzi and co-workers have synthesized LYTACs molecules which are composed of asialoglycoprotein receptor (ASGPR) or cation-independent Mannose-6-phosphate receptor (M6PR) ligands by chemical conjugation of a complex synthetic carbohydrate-molecule, ASGPR or M6PR ligand, to a monoclonal antibody for targeting EGFR or HER2 for degradation. These LYTAC molecules were able to degrade targeted cell surface receptors such as EGFR and HER2.³ Wells and co-workers have created cytokine receptor-targeting chimeras (KineTACs) by a complex protein engineering method to create a bifunctional protein. They showed that KineTACs containing certain cytokines, *e.g.*, CXCL12, can be used to degrade a variety of proteins of interest via lysosomal degradation.⁴ The challenges in all recent emerging studies are: 1) Using limited number of receptors that can mediate degradation 2) requires over-complicated chemical synthesis or protein engineering approach that limits applicability, broadness of application, and potential safety issues due to immunogenicity or triggering undesirable immune reactions.

[0005] Further, previously described lysosomal degradation modalities tend to lack cell and tissue specificity, particularly for targeted degradation of pathogenic membrane proteins. For example, the mannose-6-phosphate receptor (M6PR) is often used, however, M6PR is expressed ubiquitously and therefore lacks cell specificity. The ASGPR endocytic receptor is highly cell-specific, however, ASGPR is exclusively expressed in hepatocytes making it undesirable for membrane protein degradation outside of applications targeting hepatocyte membrane pathogenic proteins. Accordingly, identifying receptors that mediate lysosomal degradation and are highly enriched in the target cells that cause a disease will allow development of cell-specific degraders that are effective and efficient in treating the disease.

[0006] Scavenger receptors (ScaRs) are cell-surface receptors with different biological functions. ScaRs can bind to diverse ligands and enhance the elimination of altered-self or non-self-ligands. The functional mechanisms that lead to their clearance of harmful substances involve pathways such as phagocytosis and endocytosis.⁵ Endocytic receptors (EnyRs) are cell-surface receptors, and their main function is binding to their ligands and forming a complex which can be internalized via endocytosis pathway.⁶ The present disclosure provides methods of addressing the challenges mentioned above utilizing ScaRs and EnyRs to bind and degrade pathogenic proteins and peptides and thereby treat disease.

4. SUMMARY

- [0007] In one aspect, the present disclosure provides a chimeric protein comprising:
- a receptor binding moiety capable of binding to a scavenger receptor (ScaR); and
 - a target binding moiety capable of binding to a target protein or peptide.
- [0008] In another aspect, the present disclosure provides a chimeric protein comprising:
- a receptor binding moiety capable of binding to an endocytic receptor (EnyR); and
 - a target binding moiety capable of binding to a target protein or peptide
- [0009] In one embodiment, the receptor binding moiety of the chimeric protein is a peptide.
- [0010] In one embodiment, the receptor binding moiety binds to the EnyR or ScaR at a location different from the pocket to which a natural ligand of the EnyR or ScaR binds.
- [0011] In one embodiment, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is lower than the binding affinity of the target binding moiety to the target protein or peptide. In one embodiment, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is at least an order of magnitude lower than the binding affinity of the target binding moiety to the target protein or peptide.
- [0012] In one embodiment, the chimeric protein comprises two or more receptor binding moieties. In one embodiment, the protein comprises two or more target binding moieties.
- [0013] In one embodiment, the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to a different part of the same ScaR or EnyR. In one embodiment, the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to a different epitope of the same ScaR or EnyR. In one embodiment, the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different part of the same target protein or peptide. In one embodiment, the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different epitope of the same target protein or peptide. In one embodiment, the chimeric protein further comprises a linker that connects the receptor binding moiety and target binding moiety.
- [0014] In one embodiment, the expression of the target protein or peptide is upregulated in a disease or disorder. In one embodiment, the disease or disorder is cancer. In one embodiment, the cancer is a solid tumor or hematological cancer. In one embodiment, the

solid tumor is ovarian cancer, esophageal/gastric cancer, cervical cancer, bladder cancer, breast cancer, central nervous system cancer, colorectal cancer, gastrointestinal cancer, endocrine cancer, eye cancer, female genitourinary cancer, head and neck cancer, liver cancer, lung cancer, skin cancer, soft tissue cancer, bone cancer, squamous cell cancer, pancreatic cancer, kidney cancer, or prostate cancer. In one embodiment, the hematological cancer is leukemia, lymphoma, or multiple myeloma.

[0015] In one embodiment, the disease or disorder is a receptor-mediated autoimmune disease. In one embodiment, the target protein or peptide is an immune checkpoint molecule, immune cell inhibitory signal molecule, immune cell inhibitory receptor, "don't-eat-me" signal molecule, "don't-eat-me" signal receptor, immune co-stimulatory signal molecule, immune co-stimulatory receptor, immune modulation receptor, or cytokine receptor. In one embodiment, the target protein or peptide is an "eat-me" signal receptor, T-cell receptor, B-cell receptor, major histocompatibility complex (MHC) molecule, or CD8/CD4). In one embodiment, the disease or disorder is rheumatoid arthritis, type 1 diabetes, psoriasis, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, or Sjögren's Syndrome. In one embodiment, the disease or disorder is myasthenia gravis, anti-NMDA receptor encephalitis, or idiopathic membranous nephropathy. In one embodiment, the disease or disorder is anti-NMDA receptor encephalitis, and the target protein or peptide is an NMDA receptor. In one embodiment, the disease or disorder is idiopathic membranous nephropathy, and the target protein or peptide is phospholipase A2 receptor.

[0016] In one embodiment, the target protein or peptide is a soluble extracellular protein. In one embodiment, the target protein or peptide is a cell-surface receptor of a cell. In one embodiment, the target protein or peptide is an anchored protein or peptide.

[0017] In one embodiment, the ScaR is a ScaR listed in **Table 1**. In one embodiment, the EnyR is an EnyR listed in **Table 2**. In one embodiment, the receptor binding moiety is a natural ligand of ScaR or a fragment of a natural ligand of ScaR. In one embodiment, the receptor binding moiety comprises a peptide mimicking the natural ligand. In one embodiment, the receptor binding moiety comprises a peptide selected from those listed in **Table 1** or a fragment of a peptide selected from those listed in **Table 1**. In one embodiment, the receptor binding moiety is a natural ligand of EnyR or a fragment of a natural ligand of EnyR. In one embodiment, the receptor binding moiety comprises a peptide mimicking the natural ligand.

[0018] In one embodiment, the receptor binding moiety comprises a peptide selected from those listed in **Table 1** or a fragment of a peptide selected from those listed in **Table 1**.

[0019] In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises an scFv. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a Fab fragment. In one embodiment, the chimeric protein is a chimeric protein as described in **Table 3**. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a cyclic protein or peptide. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a branched protein or peptide. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a designed ankyrin repeat protein (DARPin).

[0020] In one embodiment, the chimeric protein is capable of binding to a ScaR or EnyR and a target protein expressed by the same cell. In one embodiment, the cell is a cancer cell. In one embodiment, the co-expression of the ScaR or EnyR and a target protein is specific to the cancer cell. In one embodiment, the chimeric protein is capable of binding to ScaR or EnyR and the target protein or peptide to form a complex that is internalized into a cell and degraded by a lysosome.

[0021] In another aspect, the present disclosure provides a method of treating a disease or disorder in a subject comprising administering to the subject a chimeric protein according to any embodiment disclosed herein, wherein the expression of the target protein or peptide is upregulated in the disease or disorder. In one embodiment, the disease or disorder is cancer. In one embodiment, the cancer is a solid tumor or hematological cancer. In one embodiment, the solid tumor is bladder cancer, breast cancer, central nervous system cancer, colorectal cancer, gastrointestinal cancer, endocrine cancer, eye cancer, female genitourinary cancer, head and neck cancer, liver cancer, lung cancer, skin cancer, soft tissue cancer, bone cancer, squamous cell cancer, pancreatic cancer, kidney cancer, or prostate cancer. In one embodiment, the solid tumor is non-small cell lung cancer, breast cancer, or gastroesophageal cancer. In one embodiment, the hematological cancer is leukemia, lymphoma, or multiple myeloma.

[0022] In another aspect, the present disclosure provides a method of degrading a target protein or peptide, the method comprising contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR), wherein the chimeric protein comprises: (a) a receptor binding moiety capable of binding to the ScaR or EnyR; and (b) a target binding moiety capable of binding to a target protein or peptide, wherein the contacting the target protein or peptide with the chimeric protein binds the target protein or peptide to the chimeric protein to

generate a target-bound protein or peptide; wherein the method further comprises binding a ScaR or an EnyR to the chimeric protein to generate a bound chimeric complex; and wherein when the chimeric protein is bound to both a ScaR or EnyR and the target protein or peptide, the ScaR or EnyR internalizes the target protein or peptide and directs it to a lysosome.

[0023] In some embodiments, the target protein or peptide is directed to the lysosome at least 50%, at least 60%, at least 70%, or at least 80% of the times that the ScaR or EnyR binds to the target-bound chimeric protein. In one embodiment, degradation of the target protein or peptide is initiated within 1 hour of the contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR). In one embodiment, at least 50% of the target protein or peptide is directed to the lysosome within 4 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR). In one embodiment, at least 50% of the target protein or peptide is degraded within 4 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR). In one embodiment, at least 80% of the target protein or peptide is directed to the lysosome within 24 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR). In one embodiment, at least 80% of the target protein or peptide is degraded within 24 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR). In some embodiments, the bound chimeric complex is directed to the lysosome. In some embodiments, the percent of receptor- and target protein or peptide-bound chimeric protein that is directed to the lysosome is measured by fluorescence-based assay or a biochemical fraction assay. In some embodiments, the cell is in an *in vitro* assay. In some embodiments, the percent of target-bound chimeric protein that is degraded is measured by flow cytometry and/or Western Blot. In some embodiments, the cell is in an *in vitro* assay. In some embodiments, the cell is in a mammalian subject.

[0024] In certain embodiments, the target protein or peptide is a soluble extracellular protein. In certain embodiments, the target protein or peptide is a cell-surface receptor of a cell. In certain embodiments, the target protein or peptide is an anchored protein or peptide. In certain embodiments, the receptor binding moiety is a natural ligand of ScaR, a fragment of a natural ligand of ScaR, or a peptide mimicking a natural ligand of ScaR. In certain embodiments, the receptor binding moiety is a natural ligand of EnyR, a fragment of a natural

ligand of EnyR, or a peptide mimicking a natural ligand of EnyR. In certain embodiments, the receptor binding moiety is capable of binding to HB-EGF, IGF2R, CD63, or SCARA5/SCARA3. In certain embodiments, the receptor binding moiety is an scFV of an antibody of CD63. In certain embodiments, the receptor binding moiety comprises an amino acid sequence of SEQ ID NO:47. In certain embodiments, the receptor binding moiety is capable of binding to SCARA5/SCARA3. In certain embodiments, the target binding moiety is capable of binding to HER2. In certain embodiments, the target binding moiety is trastuzumab, a Fab of trastuzumab, or a scFV of trastuzumab. In certain embodiments, the chimeric protein comprises a target binding moiety capable of binding to EGFR.

[0025] In certain embodiments, the target binding moiety is a first target binding moiety, and the chimeric protein comprises a second target binding moiety that is capable of binding to EGFR. In certain embodiments, the target binding moiety that is capable of binding to EGFR is a scFV of cetuximab. In certain embodiments, the target binding moiety that is capable of binding to EGFR is an anti-EGFR affibody.

[0026] In certain embodiments, the EnyR is not a transferrin receptor. In certain embodiments, at least one of the receptor binding moiety and target binding moiety is not an IgG antibody. In certain embodiments, the chimeric protein is not a bispecific antibody.

5. DRAWINGS

[0027] **FIG. 1** depicts mode of action of EnyrTAC and SCARTAC molecules.

[0028] **FIG. 2** depicts one embodiment of a chimeric protein that comprises a receptor binding moiety linked to a target binding moiety.

[0029] **FIG. 3** depicts a heatmap of ScaR and EnyR expression scores for selected human cell lines.

[0030] **FIG. 4** depicts a chimeric protein comprising multiple target binding moieties binding to a same target protein (an extracellular membrane protein) and receptor binding moiety to a ScaR or EnyR.

[0031] **FIG. 5** depicts a chimeric protein comprising multiple receptor binding moieties capable of binding to different ScaRs and EnyRs expressed on the same cell.

[0032] **FIG. 6** depicts a chimeric protein comprising a scFv target binding moiety (1), a linker (2), and a scFV receptor binding moiety (3).

[0033] **FIG. 7** depicts a chimeric protein comprising a Fab target binding moiety (1), two linkers (2), each bound to natural ligand of ScaR and/or EnyR (3).

[0034] **FIG. 8** depicts a heatmap of HER2 expression levels in different human cancer lines based on transcriptomic and proteomic data.

[0035] **FIG. 9** depicts HER2 quantification in SKOV3 and HeLa cells by flow cytometry and Western blot. Panel A shows surface expression of HER2 measured by flow cytometry, with results presented as median fluorescence intensity (MFI). Panel B depicts Western-blot analysis of total protein extracts from SKOV3 and HeLa cells.

[0036] **FIG. 10** depicts a heatmap of expression levels of ScaR and EnyR candidates which are expressed in SKOV3 and HeLa cell lines.

[0037] **FIGs. 11A and 11B** confirm candidate EnyR expression in SKOV3 and HeLa cell lines using flow cytometry and Western blot. **FIG. 11A** confirms the expression of CD63 and IGF2R by flow cytometry, while **FIG. 11B** confirms the expression of IGF2R and proHB-EGF with Western blot.

[0038] **FIG. 12** provides schematic representations of chimeric proteins as described in **Table 3**.

[0039] **FIG. 13** confirms the production of chimeric proteins as described in **Table 3**.

[0040] **FIG. 14** provides quantification data of HER2 in (A) SKOV3 and (B) HeLa cells treated with Trastuzumab Fab fragment (control), chimeric protein #9a, or #10a. Panel A shows surface expression of HER2 measured by flow cytometry, with results presented as median fluorescence intensity (MFI). Panel B depicts the total HER2 quantification in SKOV3 cells by Western-blot, equal amounts of total protein extract loaded.

[0041] **FIG. 15** provides quantification data of HER2 in SKOV3 and HeLa cells treated with IgG isotype control, Trastuzumab, and EnyRTAC #17a. Panel A shows surface expression of HER2 measured by flow cytometry, with results presented as median fluorescence intensity (MFI). Panel B depicts the total HER2 expression in SKOV3 by Western-blot, equal amounts of total protein extract loaded.

[0042] **FIG. 16** provides quantification data of HER2 in SKOV3 and HeLa cells treated with EnyRTACs based on the scFv derived from Trastuzumab: Trastuzumab scFv (control), EnyRTAC #1g, EnyRTAC #16b, EnyRTAC #16a, and EnyRTAC #18a. The surface expression of HER2 was measured by flow cytometry, and the results are presented as the median fluorescence intensity (MFI) for SKOV3 (Panel A) and for HeLa (Panel B).

[0043] **FIG. 17** provides schematic representations of tri-specific chimeric proteins (Panel A) and confirms the production of chimeric proteins as described in **Table 3** (Panel B).

[0044] FIG. 18 provides a schematic depiction of EnyRTAC #24a (Panel A) and confirms the production thereof (Panel B).

[0045] FIG. 19 provides data quantifying surface HER2 in SKOV3 cells treated with different concentrations of EnyRTAC #24a.

[0046] FIG. 20 provides data quantifying surface HER2 in SKOV3 cells after treatment with EnyRTAC #24a after 6 hours and 24 hours.

[0047] FIG. 21 provides data quantifying tumor growth in a xenograft tumor model (NSG mice injected with SKOV3 cells) after intertumoral injection with EnyRTAC #24a or Trastuzumab Fab.

6. DETAILED DESCRIPTION

[0048] Described herein are chimeric molecules capable of binding to a target protein or peptide of interest and a cell receptor that has the ability to internalize the resulting protein complex followed by transport into the lysosome for degradation and removal of the protein complex. The chimeric molecules comprise a receptor-binding moiety that bind to the cell receptor and a target binding moiety that bind to a protein or peptide of interest, such as a pathogenic protein or peptide, for targeted degradation.

[0049] The terms “peptide,” “polypeptide” and “protein” refer to an oligomer or polymer of amino acid residues and are not limited to a minimum length of the product. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. Furthermore, proteins disclosed herein include proteins having modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity.

[0050] Conventional notation is used herein to portray polypeptide and protein (including enzyme) sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus. Amino acid residues in the proteins or peptides disclosed herein may be either D- or L- configuration.

[0051] As used herein, when a target binding moiety or receptor binding moiety is described as “capable of binding to” a target protein or peptide or receptor, respectively, it is meant that the moiety (target or receptor binding) interacts via non-covalent interactions with the target protein or peptide or receptor with a binding affinity (K_D) of at least 10 μ M.

[0052] As used herein, the term “binding affinity” generally refers to the strength of the sum of noncovalent interactions between a single binding site of a molecule and its binding

partner. Unless indicated otherwise, “binding affinity” refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair. Method for measuring affinity of a binder to a target are well-known in the art. In one embodiment, the “ K_D ” can be measured by competition enzyme-linked immunoabsorbent assay (cELISA), flow cytometry, kinetic exclusion assay (KinExA), or microscale thermophoresis (MST), biolayer interferometry (BLI) using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA) or using surface plasmon resonance (SPR) assays by BIACORE™, using, for example, a BIACORE™-2000 or a BIACORE™-3000 (BIAcore, Inc., Piscataway, NJ).

[0053] Low-affinity binding moieties generally bind its target slowly and tend to dissociate readily, whereas high-affinity binding moieties generally bind target faster and tend to remain bound longer. As used herein, “high binding affinity” refers to binding affinities in the picomolar to low nanomolar range (*e.g.*, 10 nM or less). As used herein, “low binding affinity” refers to binding affinities in the low micromolar range (*e.g.*, 0.1 μ M to 10 μ M).

[0054] In one embodiment, the chimeric molecule is a chimeric protein, wherein the receptor-binding moiety is capable of binding to a scavenger receptor (ScaR). Such a chimeric protein is termed herein a scavenger receptor-targeting chimera (ScaRTAC). In one embodiment, the chimeric molecule is a chimeric protein, wherein the receptor-binding moiety is capable of binding to an endocytic receptor (EnyR). Such a chimeric protein is termed herein an endocytic receptor-targeting chimera (EnyRTAC). As used herein, the term “chimeric protein(s)” collectively refers to both ScaRTACs and EnyRTACs.

[0055] The overall arrangement, or format, of a chimeric protein may be as described in Section 6.1. Various aspects of suitable receptor binding moieties capable of binding to ScaR or EnyR are discussed in Section 6.2. Various aspects of suitable target binding moieties capable of binding to a target protein or peptide are discussed in Section 6.3. The mode of action by which target proteins may be targeted and degraded are discussed in Section 6.4. Design of chimeric proteins is discussed in Section 6.5. Pharmaceutical compositions comprising the chimeric proteins disclosed herein are discussed in Section 6.7. Various therapeutic applications of the chimeric proteins disclosed herein are discussed in Section 6.8.

[0056] The chimeric proteins disclosed herein are capable of binding to a target extracellular protein or peptide to mediate lysosomal or proteasomal degradation of the target extracellular protein or peptide. The target extracellular protein or peptide may be any extracellular protein, *e.g.*, a cell surface membrane protein, such as a receptor or enzyme. In one embodiment, the extracellular target protein is cross-linked with the ScaR or EnyR by

virtue of being bound to the chimeric protein which is bound to a ScaR or EnyR and subsequently undergoes internalization into the cell and protein degradation via downstream lysosomal degradation pathways. In some embodiments, the downstream lysosomal degradation pathways are mediated by the endocytosis of the ScaR or EnyR. An exemplary process of binding and internalization is shown in **FIG. 1**.

[0057] The efficiency by which a chimeric protein disclosed herein is able to bind both the target protein or peptide and the ScaR or EnyR and subsequently be internalized into the cell also will be influenced by the rate of association or “association rate” or “ k_{on} ,” as well as an “off-rate” or “rate of dissociation” or “dissociation rate” or “ k_{off} ,” of each of the binding moieties present in the chimeric proteins. For example, the dissociated rate of the target binding moiety to the target protein or peptide tunes the time bound to the target protein or peptide. Similarly, the association rate (k_{on}) of the receptor binding moiety to the receptor tunes potential sink effects. Association rate dissociation rate can also be determined with the same competition enzyme-linked immunoabsorbent assay (cELISA), flow cytometry, kinetic exclusion assay (KinExA), or microscale thermophoresis (MST), biolayer interferometry (BLI) using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA) or using surface plasmon resonance (SPR) assays by BIACORE™, using, for example, a BIACORE™-2000 or a BIACORE™-3000 (BIAcore, Inc., Piscataway, NJ).

[0058] In one embodiment, the chimeric protein binds first to a target protein or peptide and thereafter binds to the ScaR or EnyR receptor. The binding of the chimeric protein to the target protein or peptide and the receptor forms a complex comprising the chimeric protein, the target protein or peptide, and the ScaR or EnyR. Without being bound by theory, the complex, as a consequence of being bound to the ScaR or the EnyR, may be internalized into the ScaR- or EnyR-expressing cell. In certain embodiments, the chimeric protein may then enter the endosomal pathway for degradation in the lysosome. In this way, the chimeric proteins disclosed herein may be used to target and degrade specific protein or peptides, *e.g.*, proteins or peptides upregulated or involved in pathogenesis of disease or disorder (“pathogenic protein or peptides”). Therefore, the chimeric proteins disclosed herein, which aid in the degradation of pathogenic proteins, may be beneficial for use in the treatment of a disease in which expression and/or activity of the pathogenic target protein is implicated.

[0059] In one aspect, the present disclosure provides a chimeric protein comprising:

(a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a receptor able to undergo endocytosis, phagocytosis, autophagy, and/or transcytosis; and

(b) at least one target binding moiety capable of binding to a target protein or peptide.

[0060] In one aspect, the present disclosure provides a chimeric protein comprising:

(a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and

(b) at least one target binding moiety capable of binding to a target protein or peptide.

[0061] In one embodiment, the receptor binding moiety is capable of binding to a ScaR. Therefore, in one embodiment, the present disclosure provides a ScaRTAC comprising:

(a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR; and

(b) at least one target binding moiety capable of binding to a target protein or peptide.

[0062] In one embodiment, the receptor binding moiety is capable of binding to an EnyR. Therefore, in another embodiment, the present disclosure provides an EnyRTAC comprising:

(a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to an EnyR; and

(b) at least one target binding moiety capable of binding to a target protein or peptide.

[0063] **FIG. 2** depicts one embodiment of a chimeric protein as disclosed herein, comprising:

a) a target binding moiety that is capable of binding to a target extracellular protein, which may be a soluble or anchored protein;

b) a receptor binding moiety that is capable of binding to EnyR or ScaR, and

c) a linker attaching the target binding moiety to the receptor binding moiety.

6.1 Chimeric Protein Format & Linkers

[0064] A chimeric protein provided herein can have any format provide it comprises:

(a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and

(b) at least one target binding moiety capable of binding to a target protein or peptide.

[0065] A chimeric protein can comprise any number of receptor binding moieties and any number of target binding moieties. The one or more receptor binding domains can be linked

to one or more target binding domains in numerous ways to form a chimeric protein. Non-limiting examples of different formats of chimeric proteins are shown in **FIGs. 2, 4, 5, 6, 7, 12, and 17**.

[0066] In any embodiment disclosed herein, “a” or “the” target binding moiety can include one or more, such as two, three, or four target binding moieties. Similarly, in any embodiment disclosed herein, “a” or “the” receptor binding moiety can include one or more, such as two, three, or four receptor binding moieties. For example, in one embodiment, the chimeric protein comprises one receptor binding moiety and one target binding moiety. In one embodiment, the chimeric protein comprises one receptor binding moiety and two target binding moieties. In one embodiment, the chimeric protein comprises one receptor binding moiety and three target binding moieties. In one embodiment, the chimeric protein comprises two receptor binding moieties and one target binding moiety. In one embodiment, the chimeric protein comprises two receptor binding moieties and two target binding moieties. In one embodiment, the chimeric protein comprises two receptor binding moieties and three target binding moieties. In one embodiment, the chimeric protein comprises three receptor binding moieties and one target binding moiety. In one embodiment, the chimeric protein comprises three receptor binding moieties and two target binding moieties.

[0067] In one embodiment, the chimeric protein comprises two or more target binding moieties. In one embodiment, the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different part of the same target protein or peptide, as shown in **FIG. 4**. In one embodiment, the chimeric protein comprises two or more target binding moieties such that multiple target proteins or peptide may be bound. In one embodiment, the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different epitope of the same target protein or peptide, such as shown in **FIG. 4**. In one embodiment, the chimeric protein comprises two or more receptor binding moieties. In one embodiment, the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to two or more different ScaRs or EnyRs, such as shown in **FIG. 5** and **FIG. 17**. In one embodiment, the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to a different epitope of the same ScaR or EnyR.

[0068] Similarly, the affinity of each of the receptor binding moiety and target binding moiety is not limited but should be sufficient to effect stable binding of the chimeric protein to both ScaR or EnyR and the target protein or peptide, respectively. In certain embodiments, at least one of the receptor binding moiety and target binding moiety has high affinity to its

target (*e.g.*, ScaR or EnyR or the target protein or peptide, respectively). In one embodiment, both the receptor binding moiety and the target binding moiety have high binding affinity to ScaR and/or EnyR and the target protein or peptide, respectively. In one embodiment, one of the receptor binding moiety and target binding moiety has high affinity to its target and one of the receptor binding moiety and target binding moiety has low affinity to its target. In one embodiment, the receptor binding moiety has a high affinity to its target ScaR and/or EnyR and the target binding moiety has low affinity to the target protein or peptide. In one embodiment, the receptor binding moiety has a low affinity to its target ScaR and/or EnyR and the target binding moiety has high affinity to the target protein or peptide, particularly in instances where the target ScaR and/or EnyR and the target protein or peptide are expressed on the same cell.

[0069] In yet another embodiment, the binding affinity of the receptor binding moiety to its target ScaR and/or EnyR is lower than the binding affinity of the target binding moiety to the target protein or peptide. Such embodiments may be advantageous in that binding of the chimeric protein occurs first to a target protein or peptide and thereafter to the ScaR or EnyR receptor, such that chimeric protein that is not yet bound to a target protein is not internalized prior to formation of the ternary complex (target protein or peptide, chimeric protein, and target receptor). In one embodiment, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is at least an order of magnitude lower than the binding affinity of the target binding moiety to the target protein or peptide. In one embodiment, the binding of the affinity of the receptor binding moiety to ScaR or EnyR is low and the binding affinity of the target binding moiety to the target protein or peptide is high. In one embodiment, the binding of the affinity of the receptor binding moiety to ScaR or EnyR is low and the binding affinity of the target binding moiety to the target protein or peptide is high and the ScaR or EnyR and target protein or peptide are expressed on the same cell.

[0070] In one embodiment, the binding of the affinity of the receptor binding moiety to ScaR or EnyR is significantly lower than the binding affinity of the target binding moiety to the target protein or peptide is high and the ScaR or EnyR and target protein or peptide are expressed on the same cell.

[0071] In one embodiment, the ratio of the binding affinity of the receptor binding moiety to the target binding moiety (*i.e.*, $K_{D, \text{receptor}}:K_{D, \text{target}}$) is about 1:1 to about 10000:1, such as about 1:1 to 10:1, about 1:1 to 100:1, about 1:1 to about 1000:1, about 5:1 to about 10:1, about 5:1 to about 100:1, about 5:1 to about 1000:1, about 10:1 to about 100:1, about 10:1 to about 1000:1, about 100:1 to about 1000:1, or any value therebetween, including, but not

limited to about 2:1, about 5:1, about 10:1, about 50:1, about 100:1, about 250:1, about 500:1, about 750:1, about 1000:1, about 2500:1, about 5000:1, about 7500:1, or about 10000:1. In some embodiments, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is about 0.1 nM to about 10 nM and the binding affinity of the target binding moiety to the target protein or peptide is about 0.1 pM to about 1 nM.

[0072] Further discussion of binding affinity of suitable receptor binding moieties to ScaR or EnyR is discussed in further detail in Section 6.2. Binding affinity of the suitable target binding moieties to a target protein or peptide is discussed in further detail in Section 6.3.

[0073] The target binding moiety and receptor binding moiety may be any protein or peptide that is capable of binding to a target protein or peptide and receptor (*e.g.*, ScaR or EnyR), respectively. In one embodiment, at least one of the target binding moiety and receptor binding moiety is a peptide, polypeptide, or protein. In one embodiment, the receptor binding moiety is a natural ligand, fragment of a natural ligand, or derivative thereof, such a mimetic of a natural ligand or fragment of a natural ligand of the receptor. In one embodiment, the receptor binding moiety is an antibody, fragment thereof (*e.g.*, Fab), or scFv of the receptor. In one embodiment, at least one of the receptor binding moiety and target binding moiety comprises a protein. In one embodiment, at least one of the receptor binding moiety and target binding moiety is not an antibody. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a single chain variable fragment (scFv) of an antibody, such as shown in **FIG. 6**. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a fragment antigen-binding region (Fab) of an antibody that such as shown in **FIG. 7**. In one embodiment, the receptor binding moiety comprises a natural ligand of a ScaR or EnyR, fragment of a natural ligand of a ScaR or EnyR, or a mimic of a natural ligand of a ScaR or EnyR, and the target binding moiety comprises an antibody, Fab, or scFv. In one embodiment, the receptor binding moiety comprises an antibody, Fab, or scFv of a ScaR or EnyR and the target binding moiety comprises an antibody, Fab, or scFv of a target protein. In one embodiment, the receptor binding moiety comprises an scFv of a ScaR or EnyR and the target binding moiety comprises an scFv of a target protein. In one embodiment, the receptor binding moiety comprises an scFv of a ScaR or EnyR and the target binding moiety comprises an antibody of a target protein. In one embodiment, at least one of the target binding moiety and receptor binding moiety is not an IgG antibody. In one embodiment, neither the target binding moiety nor the receptor binding moiety is an IgG antibody. In one embodiment, the chimeric protein

is not a bispecific antibody. In one embodiment, the chimeric protein is not a bispecific diabody. In one embodiment, the chimeric protein is not a multispecific antibody. In one embodiment, the chimeric protein is not a bispecific Fab2. In one embodiment, the chimeric protein is not a bispecific camelid antibody. In one embodiment, the chimeric protein is not a knob in hole bispecific IgG or knob in hole bispecific Fc-Fab. In one embodiment, the chimeric protein is not a Fc-Fab. In one embodiment, the chimeric protein is not a bispecific peptibody scFv-Fc. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a cyclic peptide. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a branched peptide. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a designed ankyrin repeat protein (DARPin). In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises an affibody. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises an antibody mimetic. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises a single domain antibody. In one embodiment, one or both of the receptor binding moiety and target binding moiety comprises an IgG1 or IgG4. Particular peptides or proteins that are suitable for use as receptor binding moieties and target binding moieties are described in further detail below in Sections 6.2 and 6.3, respectively.

[0074] A chimeric protein, as disclosed herein, may optionally include a linker to couple the target binding moiety to the receptor binding moiety. In certain embodiments, the linker is a bond. Because any chimeric protein disclosed herein may have multiple receptor binding moieties and/or multiple target binding moieties, such as discussed in Section 6.1, a chimeric protein may have more than one linker. Such linkers may link two receptor binding moieties to one another, two target binding moieties to one another, or a target binding moiety to a receptor binding moiety. In one embodiment, a chimeric protein having two or more target binding moieties comprises a linker joining the two or more target binding moieties to each other. In one embodiment, a chimeric protein having two or more receptor binding moieties comprises a linker to join the two or more receptor binding moieties to each other. The number of linkers present within a chimeric protein is not particularly limited and will depend on the number of individual receptor and target binding moieties present. Similarly, the organization of the target and receptor binding moieties with respect to each other is not particularly limited. Any target binding moiety can be joined with any other target binding moiety or a receptor moiety, *e.g.*, with one or more linkers, in any order or arrangement. For example, a chimeric protein may comprise a linear arrangement, a branched arrangement, star

arrangement, cyclic arrangement, any irregularly shaped arrangement, or any combination thereof with regard to the receptor and binding moieties. The arrangement of receptor and target binding moieties within the chimeric protein is not particularly limited. For example, receptor and target binding moieties may alternate or two or more receptor binding moieties may be bound together coupled to two or more target binding moieties bound together.

[0075] The composition of the one or more linkers is not particularly limited and should maintain a stable linkage between the binding moieties which it connects during storage of the chimeric protein and after administration, *e.g.*, in a subject's body (*e.g.*, in blood plasma) through internalization of the complex formed upon binding of the receptor binding moiety to ScaR or EnyR and binding of the target binding moiety to the target protein or peptide. For example, a suitable linker should be absent immunogenic T or B cell epitopes and post-translational modification sites when a production cell line is used to generate the chimeric protein. For example, if CHO, yeast, insect, or HEK1 cells are used to generate the chimeric protein, the linker should devoid serine residues to avoid O-glycosylation. As used herein, the term "subject" refers to human and non-human subjects, especially mammalian subjects. In one embodiment, the subject is a human.

[0076] In one embodiment, the linker is rigid. In one embodiment, the linker is flexible and allows movement of the receptor binding moiety and/or target binding moiety with respect to one another, such that once one of the receptor binding moiety and target binding moiety is bound to the receptor or target protein or peptide, respectively, the non-bound binding moiety is able to freely move to facilitate (or at least not hinder) binding to its target. In another embodiment, the target binding moiety is coupled to the receptor binding moiety via a peptide or by a peptidic bond (*e.g.*, the chimeric protein comprises or is a fusion protein). In a chimeric protein having two or more linkers, the two or more linkers may be the same or different.

[0077] The length of the linker is not particularly limited. In some embodiments, the linker is a peptide comprising 1 to 20 amino acids, such as 1 to 15 amino acids, 1 to 10 amino acids, or 1 to 5 amino acids. In some embodiments, the linker comprises 5 to 20 amino acids. In some embodiments, the linker comprises 5 amino acids. In some embodiments, the linker comprises 15 amino acids. In yet other embodiments, the linker comprises 20 amino acids. In some embodiments, the linker comprises one or more multimer of the amino acid sequence GGGGA (SEQ ID NO:58). In one embodiment, the linker comprises an amino acid sequence of GGGGAGGGGAGGGGAGGGGA (SEQ ID NO:56). In one embodiment, the linker comprises an amino acid sequence of GGGGAGGGGAGGGGA (SEQ ID NO:57).

6.2 Receptor Binding Moieties

[0078] The chimeric proteins disclosed herein comprise at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR. As used herein, the ScaR or EnyR to which the receptor binding moiety is capable of binding may also be called the “target” receptor, such as a “target ScaR” or “target EnyR.” In identifying a target receptor, such as a target ScaR or target EnyR, it is meant that a chimeric protein comprises a receptor binding moiety that is capable of binding said target receptor.

[0079] In one embodiment, the receptor binding moiety comprises a motif capable of binding to the target receptor and a lysosomal sorting sequence (LSS), to facilitate the trafficking of the bound chimeric protein to the lysosome once internalized. One of ordinary skill in the art will recognize suitable LSS sequences that may be used for this purpose. In one embodiment, the LSS is NPGY (SEQ ID NO:50). Other non-limiting examples of suitable LSS sequences include DXXLL (SEQ ID NO:51), DDSDEDLL (SEQ ID NO:52), YXXØ (SEQ ID NO:53), wherein Ø is a bulky hydrophobic residue (*e.g.*, F, I, L, M, or V) and X is any amino acid, NPXY (SEQ ID NO:61), wherein X is any amino acid, EESEERDDHLL (SEQ ID NO:62), [DE]XXXL[LI], wherein X is any amino acid and bracketed amino acids are optional, (SEQ ID NO:63), NPFX (SEQ ID NO:59), wherein X is any amino acid, SFHDDSDEDLLHI (SEQ ID NO:60), and ubiquitin.

[0080] As discussed herein, the binding affinity of the receptor binding moiety of the target receptor is at least 0.1 μ M. In one embodiment, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is about 0.1 nM to about 10 nM, such as about 0.1 nM to about 7.5 nM, about 0.1 nM to about 5 nM, about 0.1 nM to about 2.5 nM, about 0.1 nM to about 1 nM, about 0.5 nM to about 10 nM, about 0.5 nM to about 7.5 nM, about 0.5 nM to about 5 nM, about 0.5 nM to about 2.5 nM, about 1 nM to about 10 nM, about 1 nM to about 7.5 nM, about 1 nM to about 5 nM, or about 1 nM to about 2.5 nM. In another embodiment, the binding affinity of the receptor binding moiety to ScaR and/or EnyR is about 0.1 nM to about 1 nM, such as about 0.1 nM to about 0.75 nM, about 0.1 nM to about 0.5 nM, about 0.1 nM to about 0.25 nM, about 0.25 nM to about 1 nM, about 0.25 nM to about 0.75 nM, about 0.25 nM to about 0.5 nM, about 0.5 nM to about 1 nM, about 0.5 nM to about 0.75 nM, or about 0.75 nM to about 1 nM.

[0081] Specific aspects of example embodiments of receptor binding moieties that can be used to target the ScaR and EnyR are discussed further hereinbelow. These descriptions are not intended to limit the ScaR or EnyR targeted by a chimeric protein, as disclosed herein,

but merely provide representative examples of ScaRs or EnyRs that may be suitable as targets of a chimeric protein that may be used according to the methods disclosed herein to degrade pathogenic proteins or peptides.

6.2.1 Scavenger Receptor Binding Moieties

[0082] In one embodiment, the receptor to which the receptor binding moiety is capable of binding is a ScaR. ScaRs are a large family of cell-surface receptors that are membrane-associated pattern recognition receptors that bind and internalize a wide array of structurally diverse ligands. The ScaR may be any known ScaR, such as a ScaR belonging to class SR-A1, SR-A1.1, SR-A3, SR-A4, SR-A5, SR-A6, SR-B1, SR-B2, SR-D1, SR-E1, SR-E2, SR-E3, SR-E4, SR-F1, SR-F2, SR-G1, SR-H1, SR-H2, SR-I1, SR-I2, SR-I3, SR-J1, SR-K1, SR-L1, or SR-L2.

[0083] In one embodiment, the target ScaR is as listed in the “Exemplary Members” column of **Table 1**. In one embodiment, the target ScaR is SCARA5/SCARA3.

[0084] In one embodiment, the receptor binding moiety comprises a natural ligand, fragment thereof, or mimic thereof of a ScaR. In one embodiment, the receptor binding moiety comprises a natural ligand, fragment thereof, or mimic thereof of a natural binder SCARA5/SCARA3. In one embodiment, the receptor binding moiety binds to the same pocket as a natural ligand of the ScaR, *e.g.*, the same pocket as a natural ligand of SCARA5/SCARA3. In one embodiment, the receptor binding moiety comprises of SEQ ID NO:9. In one embodiment, the receptor binding moiety binds to a different location than the natural binding pocket on the ScaR, *e.g.*, different than the pocket to which SCARA5/SCARA3 binds.

[0085] In one embodiment, the receptor binding moiety comprises a natural ligand of ScaR, fragment thereof, or a derivative thereof, such as a peptide mimicking a natural ligand of a ScaR. In one embodiment, the receptor binding moiety comprises a natural ligand, fragment thereof, or mimic thereof of a natural binder of a ScaR, *e.g.*, of SCARA5/SCARA3 and a lysosomal sorting sequence. In one embodiment, the lysosomal sorting sequence is NPGY (SEQ ID NO:50). In one embodiment, the receptor binding moiety comprises of SEQ ID NO:9 fused to a lysosomal sorting sequence as disclosed herein. In one embodiment, the receptor binding moiety comprises of SEQ ID NO:9 fused to a lysosomal sorting sequence of SEQ ID NO:50.

[0086] In one embodiment, the receptor binding moiety comprises a natural ligand of ScaR, fragment thereof, or a derivative thereof, such as a peptide mimicking a natural ligand of a ScaR.

[0087] In one embodiment, the receptor binding moiety comprises a natural ligand, fragment thereof, or mimic thereof of a natural binder of a ScaR, *e.g.*, of SCARA5/SCARA3 and a lysosomal sorting sequence. In one embodiment, the lysosomal sorting sequence is NPGY (SEQ ID NO:50). In one embodiment, the receptor binding moiety comprises SEQ ID NO:9 fused to a lysosomal sorting sequence as disclosed herein. In one embodiment, the receptor binding moiety comprises of SEQ ID NO:9 fused to a lysosomal sorting sequence of SEQ ID NO:50.

[0088] In one embodiment, the receptor binding moiety comprises an antibody, Fab, or scFv of a ScaR, *e.g.*, of SCARA5/SCARA3. In one embodiment, the receptor binding moiety comprises scFv of ScaR, *e.g.*, of SCARA5/SCARA3.

[0089] In one embodiment, the receptor binding moiety comprises an antibody, Fab, or scFv of a ScaR, *e.g.*, of SCARA5/SCARA3, and a lysosomal sorting sequence. In one embodiment, receptor binding moiety comprises scFv of ScaR, *e.g.*, of SCARA5/SCARA3, and a lysosomal sorting sequence. In one embodiment, the lysosomal sorting sequence is NPGY (SEQ ID NO:50).

[0090] Non-limiting examples of target ScaRs (including consensus nomenclature classes and exemplary members thereof) and exemplary ligands that may be suitable as receptor targeting moieties are described in **Table 1** below. Each listed ligand includes a number corresponding to a reference to a source that disclosed the ligand as a ligand of a ScaR. The list of references, each of which are incorporated by reference herein with respect to their disclosure of the various ligands that bind to ScaRs, may be found in Section 8.

Table 1: Exemplary ScaRs and their Ligands

Consensus Nomenclature “Class”	Exemplary Members (human UniProt No.)	Exemplary Ligands
SR-A1	SCARA1 (CD204, UniProt No. P21757) MSR1 (UniProt Nos. B4DDJ5, E5RFW8, H0YBY2), SR-AI	○ EYQFVANVEEEAWINEKMTLVASEDYGDTLAAIQGLLKKHEAFETDFTVHKDRVNDVCTNGQDLIKNNHHEENISSKMGGLNGKVSDEKAAQQRKAKLDENSAFLQFNWKADV VESWIGEKENSLKTDDYGRDLSSVQTLLTKQETFDAGLQAFQQEGIANITALKDDQLLAAK HVQSKAIEARHASLMKRWSQLLANSAARKKKLLE (SEQ ID NO:1)⁷ ○ HEVQRFHRDADETKEWIEKNQALNTDNYGHDLASVQALQRKHEGFERDLAALGDKVN SLGETAERLIQSHPESAEDLQEKCTELNQAWSSLGKRADQRKAKLG (SEQ ID NO:2)⁷ ○ LSLERFLRCWSDAPA (SEQ ID NO:3)⁸ ○ LSLERFLRCWSDSPR (SEQ ID NO:4)⁸ ○ DWFKAFYDKVAEKFKEAF (SEQ ID NO:5)⁹
		○ AGYLLGLLOOLAAAALOOLL-NH₂ (SEQ ID NO:6)¹⁰ ○ RRRRRRRR (SEQ ID NO:7)¹¹
		○ MAASPARPAVLALTGLALLLLCWGPGGSGNKLKMLQKREAPVPTKTVAVDENKAK EFLGSLKRQKRQLWDRTRPEVQQWYQQFLYMGFDEAKFEDDITYWLNRRDRNGHEY YGDDYYQRHYDEDESAIGPRSPYGFRHGASVNYDDY (SEQ ID NO:8)¹²
		○ AGYLLGLLOOLAAAALOOLL-NH₂ (SEQ ID NO:6)¹⁰ ○ RRRRRRRR (SEQ ID NO:7)¹¹
		○ MKLVTFIPLLVTISLCSYSATAFLINKVPLPVDKLAPLPLDNILPFMDPLKLLKTLGISVEHL VEGLRKCVNELGPEASEAVKKLLEALSHLV (SEQ ID NO:9)¹³
		○ F^DRFK-NH₂ (SEQ ID NO:10)¹⁴
SR-B1	SCARB1 (UniProt No. Q8WTV0, also called CD36L1), SR-BI	
SR-B2	CD36 (UniProt No. P16671, also called SCARB3), PAS4	○ DWLKAFYDKVAEKLKEAFPDWAKAAAYDKAAEKAKEAA (SEQ ID NO:11)¹⁵ ○ CSVTCG (SEQ ID NO:12)^{16, 17}

Consensus Nomenclature “Class”	Exemplary Members (human UniProt No.)	Exemplary Ligands
SR-D1	CD68 (UniProt No. P34810, also called SCARD1, macrophage)	○ DCAIVYAYDPCL (SEQ ID NO:13)¹⁸
SR-E1	LOX-1 (UniProt No. P78380, also called OLR1)	○ MAASPARPAVLALTGLALLLLCWGPGISGNKLKMLQKREAPVPTKTKVAVDENKAK EFLGSLKRQKRQLWDRTRPEVQQWYQQFLYMGFDEAKFEDDITYWLNDRNRNGHEYGGD YYQRHYDEDSAIQPRSPYGFHGSVNYDDY (SEQ ID NO:8) ○ QLWDRTRPEVQQWYQQFLYMGFDEAKFEDDITYWLNDRNRNGHEYGGDYQRHYDEDS AIGPR (SEQ ID NO:14)¹²
SR-E2	Dectin-1 (UniProt No. Q9BXN2, also called CLEC7A)	○ Galectin-9, Annexins, Vimentin, Tropomyosin¹⁹
SR-E3	Mannose receptor 1 (UniProt No. P22897, also called CD206, MRC1)	○ CSPGAK (SEQ ID NO:15)²⁰ ○ CSPGAKVRC (SEQ ID NO:16)²¹ ○ KFRKAFKRFF (SEQ ID NO:17)²¹
SR-H2	FEEL-2 (UniProt No. Q8WWQ8, also called STAB2)	○ CRLTLTVRKC (SEQ ID NO:18)²²
SR-K1	CD44 (UniProt No. P16070)	○ EGEWVPYP (SEQ ID NO:19)²³ ○ VEGEGEEGEEY (SEQ ID NO:20)²⁴ ○ FTEAESNMNDLV (SEQ ID NO:21)²⁴ ○ EEDFGEEAEEEA (SEQ ID NO:22)²⁴

Consensus Nomenclature “Class”	Exemplary Members (human UniProt No.)	Exemplary Ligands
SR-L1, SR-L2		○ YHFGCEDPLCR (SEQ ID NO:23)²⁵ ○ HPWCCGLRLDLR (SEQ ID NO:24)²⁵ ○ CWSYAVHPEC (SEQ ID NO:25)²⁶ ○ CMPSVILPSC (SEQ ID NO:26)²⁶ ○ LRKLRKRLLRDADDL (SEQ ID NO:27)²⁷ ○ LRKLRKRLLRDADDLRLKLRKRLRDADDL (SEQ ID NO:28)²⁷ ○ TEELRVRLASHLRKLRKRL (SEQ ID NO:29)²⁸ ○ PWNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHREIEGRVMV/TFENVPEEDGTR ○ FHRQASKCDSHGTHLAGVVSGRDAGVAKGASMRSLRVLNCQKGTVSGTLGLEFIRKS ○ QLVQPVGPLVLLPLAGGYSRVLNAACQRLARAGVVLVTAAGNFRDDACLYSPASAPEV ○ ITVGATNAQDQPVTLGTLGTFNFGRCVDLFAPEGDIIGASSYCSCTCFVSQSGTAQAAAHVA ○ GIAAMMLSAPELTLAELRQRLIHFSAKDVINEAWFPEDQRVLTPLNVAALPPSTHGAGW ○ QLFCRTVW (SEQ ID NO:30)²⁹ ○ KFTIVFPHNQKGNWKNVPSNYHYCPSSDDLNWHNLDLGTALQVKMPKSHKAIQADGWM ○ CHASKWVTTCDFRWYGPKYITHSIRSFPTSPVEQCKESIEQTKQGTWLNPGFPPQSCGYATV ○ TDAEAAIVQVTPHHVLVDEYTGWVDSQFINGKCSNDICPTVHNSITTWHSYKVKGLCD ○ SNLISDTITFFSEDGELSSLGKEGTGFRSNYFA YETGDK ACKMQYCKHWGVRLPSGVWFE ○ MADKDLFAARFPECPEGSSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPV ○ DLSYLAPKNPGTGPVFTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTTTTERELWDDWAP ○ YEDVEIGPNGVLR TSLGYKFPL YMIGHGMLDSDLHLSSK AQVFEHPHIQDAAS (SEQ ID NO:31)⁸ ○ KNESSTNATNTKQWRDETKGFRDEAKRFK (SEQ ID NO:32)³⁰ ○ MKVLW AALLVTLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRW ○ VQTLSEQVQEEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETARLSKELQA ○ AQARLGADMEDVCGRLVQYRGEVQAMLGQSTEELRVRLASHLRKLRKRLRDADDLQK ○ RLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGOPLQERAQAWGERLRA
	LDLR (UniProt No. P01130), LRP1 (UniProt No. Q07954)	

Consensus Nomenclature “Class”	Exemplary Members (human UniProt No.)	Exemplary Ligands
SR-F1	SCARF1 (UniProt No. Q14162, also called SREC-I)	RMIEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEK VQAAVGTSAAPVPSDNH (SEQ ID NO:33) ³¹
		○ YSREKNQPKPSKRESGEEFRMEKLNQWEKAQRLHLPVRLAELHADLKIQERDELAWKKLLDGLDEDEGEKEARLIRNLNVILAKYGLDGKKDARQVTSNSLSGTQEDGLDDPRLEKLWHKAKTSGKFSGEELDKLWREFLHHKEKVHEYNVLLETLSRTEEIHENVISPSDLSDIKGSVLHSRHTELKEKLRNSINQGLDRLRRVSHQGYSTEAEEFEPRVIDLWDLAQSANLTDKEL EAFREELKHFEAKIEKHNYQKQLEIAHEKLRHAESVGDGERVSRGREKHALLEGR TKELGYTVKKHLQDLSGRISRAR (SEQ ID NO:34) ³²
		○ EPAVYFKEQFLDGDGWTSRWIESKHKSDFGKFVLSGKFYGDEEKDKGLQTSQDARFYA LSASFEPFSNKGQTLVVQFTVKHEQNIDCGGGYVKLPNSLDQTDMHGDSYINIMFGPDI CGPGTKKVHVIFNYKGKNVLINKDIRCKDDEFTHLYTLIVRPDNTYEVKIDNSQVESGSLE DDWDFLPPKKIKDPDASKPEDWDERAKIDDPDTSKPEDWDKPEHIPDPDAKKPEDWDEE MDGEWEPPVIQNPEYKGEWKPRQIDNPDKYGTWHPIDNPESPDPSIYAYDNFGLGLDLWQVKSGTIFDNFLITNDEAYAEFGNETWGVTKAAEKQMKDKQDEEQRL (SEQ ID NO:35) ³³
		○ EPAVYFKEQFLDGDGWTSRWIESKHKSDFGKFVLSGKFYGDEEKDKGLQTSQDARFYA LSASFEPFSNKGQTLVVQFTVKHEQNIDCGGGYVKLPNSLDQTDMHGDSYINIMFGPDI CGPGTKKVHVIFNYKGKNVLINKDIRCKDDEFTHLYTLIVRPDNTYEVKIDNSQVESGSLE (SEQ ID NO:36) ³³

6.2.2 Endocytic Receptor Binding Moieties

[0091] In another embodiment, the target receptor is an endocytic receptor (EnyR). EnyRs are receptors that undergo endocytosis to import macromolecules from outside a cell (“internalization”). In one embodiment, the EnyR undergoes endocytosis upon binding of a receptor binding moiety of a chimeric protein as described herein. In one embodiment, the EnyR subsequently delivers the endocytosed material to a lysosome. In one embodiment, the endocytosis by the EnyR is clathrin-dependent. In another embodiment, the endocytosis by the EnyR is clathrin-independent.

[0092] Non-limiting examples of target EnyRs that may be used as a target receptor (*e.g.*, a chimeric protein comprises a receptor binding moiety that binds to) include G-protein coupled receptors (GPCRs), receptor tyrosine kinase receptors (RTKs), and transmembrane receptors.

[0093] Non-limiting examples of GPCRs that may be used as a target receptor include adrenoreceptors, chemokine receptors, and coagulation factor II receptor.

[0094] Non-limiting examples of transmembrane receptors that may be used as a target receptor include folate receptors (*e.g.*, FOLR1, FOLR2, FOLR3), interleukin 2 receptors (*e.g.*, IL2RA, IL2RB, IL2RG), low density lipoprotein receptors (LDLR), aminopeptidase N, sortilin 1, and transferrin receptor (TFRC).

[0095] Non-limiting examples of RTKs that may be used as a target receptor include colony stimulating factor receptors (*e.g.*, CSF1R), epidermal growth factor receptor (EGFR), Erb-b2 receptor tyrosine kinase receptors (*e.g.*, ERBB2, ERBB3), fibroblast growth factor receptors (*e.g.*, FGFR1, FGFR2, FGFR3, FGFR4), Fms-related tyrosine kinase 1 (FLT1), vascular endothelial growth factor receptors (*e.g.*, VEGFR1), insulin-like growth factor receptors (*e.g.*, IGF1R, IGF2R), MET receptor tyrosine kinase, neurotrophic tyrosine kinase receptor (*e.g.*, NTRK1), platelet-derived growth factor receptors (*e.g.*, PDGFRA), and transforming growth factor beta receptors (*e.g.*, TGFBR1, TGFBR2).

[0096] In one embodiment, the target EnyR is a RTK or a transmembrane receptor. In one embodiment, the target EnyR is not a GPCR. In one embodiment, the target EnyR is not a chemokine receptor. Said differently, in one embodiment, a chimeric protein, as described herein, comprises a receptor binding moiety that does not specifically bind to a GPCR (*e.g.*, has a binding affinity of 1 mM or higher). In one embodiment, a chimeric protein as described herein comprises a receptor binding moiety that does not specifically bind to a cytokine receptor (*e.g.*, has a binding affinity of 1 mM or higher).

[0097] In one embodiment, the target EnyR is not a GPCR. In one embodiment, the target EnyR is not LGR5. In one embodiment, the target EnyR is not an EGFR, *e.g.*, is not HER3. In

one embodiment, the target EnyR is not LY75, MST1R, MSLN, or TNFRSF10B. In one embodiment, the target EnyR is not an E3 ligase, *e.g.*, is not RNF43.

[0098] In one embodiment, the target EnyR is a transmembrane receptor. In one embodiment, the target EnyR is transferrin receptor (TFRC). In one embodiment, the target EnyR is TFRC. In one embodiment, the target EnyR is aminopeptidase N. In one embodiment, the target EnyR is sortilin-1. In one embodiment, the target EnyR is lysosomal-associated membrane protein (CD63).

[0099] In one embodiment, the target EnyR is not a transferrin receptor. In one embodiment, the target EnyR is not transferrin receptor protein-1. In certain embodiments, the EnyR is not a transmembrane glycoprotein. In one embodiment, the EnyR is not M6P. In one embodiment, the target EnyR is not CD71, MUC1, integrin $\alpha\beta 6$ (or any subunit thereof), CEACAM5, EpCAM, B7-H3, or CDH17.

[00100] In one embodiment, the target EnyR is a receptor tyrosine kinase. In one embodiment, the target EnyR is a growth factor receptor. In one embodiment, the target EnyR is an epidermal growth factor receptor. In one embodiment, the target EnyR is membrane-bound heparin-binding epidermal growth factor-like growth factor (proHB-EGF). In one embodiment, the target EnyR is an insulin-like growth factor receptor (IGFR). In one embodiment, the target EnyR is insulin-like growth factor 2 receptor (IGF2R).

[00101] In one embodiment, the target EnyR is as listed in the left column of **Table 2**.

[00102] In one embodiment, receptor binding moiety comprise a natural ligand of an EnyR, a fragment thereof, or a derivative thereof, such a peptide mimicking a natural ligand or a fragment of a natural ligand. In one embodiment, the receptor binding moiety binds to the same pocket as the natural ligand of the EnyR. In one embodiment, the receptor binding moiety binds to the EnyR at a location different from the natural ligand binding pocket of the EnyR. In one embodiment, the receptor binding moiety is an antibody, Fab, or scFv of the EnyR. In one embodiment, the receptor binding moiety is not an antibody, Fab, or scFv of the EnyR.

Non-limiting examples of exemplary ligands of target EnyRs that may be used for designing chimeric proteins, as disclosed herein, appear in the right column of **Table 2**. Each listed ligand includes a number corresponding to a reference to a source that disclosed the ligand as one of EnyR. The list of references, each of which are incorporated by reference herein with respect to their disclosure of the various ligands that bind to EnyRs, may be found in Section 8.

Table 2: Exemplary EnyRs and their Ligands

Receptor Name	Exemplary Ligands
Pro-Heparin-binding epidermal growth factor–like growth factor receptor (proHB-EGF, UniProt No. Q99075)	○ SPGHKTQPFLHDGYAVSWNTVEDSIIRTFQGESGHDIKI TAENTPLPIAGVLLPTIPGKLDVNKSKTHISVNGRKIRMR CRAIDGDVTFRCPKSPVYVGNVHANLHVAFHRSSSEKI HSNEISSDSIGVLGYQKTVDHTKVNSKLSLFFEIKS (SEQ ID NO:37) ³⁴
insulin like growth factor 2 receptor (IGF2R, UniProt No. P11717)	○ LCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEEC CFRSCDLALLETYCATPAKSE (SEQ ID NO:38) ^{35,36,48}
Aminopeptidase N (APN5/CD13, UniProt No. P15144)	○ NGR (SEQ ID NO:39) ³⁷
Transferrin receptor protein 1 (TfR1, UniProt No. P02786)	○ THRPPMWSPVWP (SEQ ID NO:40) ³⁸ ○ HAIYPRH (SEQ ID NO:41) ³⁹ ○ PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVWW ESYGTEWSSYRTTPPVLDSDGSFFLYSKLTVTREEWQQG FVFTCGVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:42) ⁴⁰
Sortilin-1 (UniProt. No. Q92673)	○ SCEKEVVSAQPATFLARSPHVGKDVCEGEGHFCHDNQ TCCRDNRQGWACCPYRQGVCCADRRHCCPAGFRCAAR GTKCLRREAPRWDAPLRDPALRQLL (SEQ ID NO:43) ^{41,42} ○ SDVYCEVCEFLVKEVTKLIDNNKTEKEILDAFDKMCSKL PKSLSEECQEVVDTYGSSILSILLEEVSPELVCSMLHLCSG TRLPALTVHVTPQKDGGFCEVCKKL VGYLDRNLEKNST KQEILAALEKGC SFLPDYPYQKQCDQFVAEYEPVLIEIL VE VMDPSFVCLKIGACPSAHKPLLGTEKCIWGPSYWCQNT E TAAQCNAVEHCKRHVWN (SEQ ID NO:44) ⁴³
CXCR7 (UniProt No. P25106)	○ ARLDVASEFRKKWNKWALSR (SEQ ID NO:45) ⁴⁴ ○ FRKKWNKWALSR (SEQ ID NO:46) ⁴⁴
CD63 (Uniprot No. P08962)	○ scFv with SYYN (CDR-H1, SEQ ID NO:64), YTKRGYTDYNPSLRS (CDR-H2, SEQ ID NO:65), MGWGSHAFDM (CDR-H3, SEQ ID NO:66), RASQSVSSSYLA (CDR-L1, SEQ ID NO:67), GASSRAT (CDR-L2, SEQ ID NO:68), QQYGSSPWT (CDR-L3, SEQ ID NO:69) ⁴⁹ , such as QVQLQESGPKVVKPSETLSLTCTVSGGSISSYYWNWIRQ SPGKGLEWIGYTKRGYTDYNPSLRSRVTISEDTSKNQFSL RISSVTAADTAVYYCAQMGWGSHAFDMWVGQTMVAV SSGGGGAGGGGAGGGGAGGGGAIEVLTQSPGTLSPG ERATLSCRASQSVSSSYLA WYQQKPGQAPRLLIYGASSR ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP WTFGQGTKVEIK (SEQ ID NO:47)

[00103] In one embodiment, target EnyR is proHB-EGF, IGF2R, or CD63. In one embodiment, receptor binding moiety is a natural ligand of proHB-EGF, IGF2R, or CD63, a fragment of a natural ligand of proHB-EGF, IGF2R, or CD63, or a variant thereof, such as a mimic of a natural ligand or fragment of a natural ligand of proHB-EGF, IGF2R, or CD63. In

one embodiment, receptor binding moiety is a natural ligand of proHB-EGF or IGF2R, a fragment of a natural ligand of proHB-EGF or IGF2R, or a variant thereof, such as a mimic of a natural ligand or fragment of a natural ligand of proHB-EGF or IGF2R. In one embodiment, the receptor binding moiety comprises an amino acid sequence of SEQ ID NOs:37, 38 or 53.

[00104] In certain embodiments, the receptor binding moiety is an antibody, Fab, or scFv of an EnyR. In one embodiment, the receptor binding moiety is an antibody, Fab, or scFv of proHB-EGF, IGF2R, or CD63. In one embodiment, the receptor binding moiety is an antibody, Fab, or scFv of CD63. In one embodiment, the receptor binding moiety is not an antibody, Fab, or scFv of proHB-EGF or IGF2R. In one embodiment, the receptor binding moiety is an scFv of CD63, a which comprises an amino acid sequence of SEQ ID NO:47.

6.3 Target Binding Moiety

[00105] Chimeric proteins disclosed herein comprise at least one target binding moiety capable of binding to a target protein or peptide. In one embodiment, the target protein or peptide is a pathogenic protein or peptide. In one embodiment, the pathogenic protein or peptide is a secreted protein. In one embodiment, the pathogenic protein or peptide is a soluble extracellular protein. In one embodiment, the pathogenic protein or peptide is a cell-surface receptor of a cell (such as a cancer cell). In one embodiment, the pathogenic protein or peptide is an anchored protein or peptide. In one embodiment, the expression of the pathogenic peptide or protein is upregulated in cancer cells. In one embodiment, the pathogenic peptide or protein is an immune checkpoint, immune cell inhibitory signal molecule, immune cell inhibitory receptor, "don't-eat-me" signal molecule, "don't-eat-me" signal receptor, immune co-stimulatory signal molecules, immune co-stimulatory receptor, immune modulation receptor, or cytokine receptor. In one embodiment, the target protein or peptide is an "don't-eat-me" signal receptor, or similarly activating receptors, a T-cell receptor, a B-cell receptor, a major histocompatibility complex (MHC) molecule, or CD8/CD4.

[00106] The means by which a target binding moiety binds to a target binding protein or peptide is not particularly limited. For example, a target binding moiety may comprise an antibody or a fragment thereof, such as a Fab. A target binding moiety may comprise a nanobody or a scFv. If the target protein or peptide is a receptor, the target binding moiety may comprise a natural ligand, a peptide mimicking a natural ligand, or fragment of a natural ligand, of the receptor (*e.g.*, receptor binding portion of the natural ligand or mimic thereof).

[00107] As discussed herein, the binding affinity of the target binding moiety is at least about 0.1 μ M. In one embodiment, the binding affinity of the target binding moiety to a target protein or peptide is about 0.1 pM to about 1 nM, such as about 0.1 pM to about 0.75 nM, about 0.1 pM to about 0.5 nM, about 0.1 pM to about 0.25 nM, about 0.1 pM to about 0.1 nM, about 0.5 pM to

about 1 nM, about 0.5 pM to about 0.75 nM, about 0.75 pM to about 1 nM, about 1 pM to about 1 nM, about 1 pM to about 0.75 nM, about 1 pM to about 0.5 nM, about 1 pM to about 0.25 nM, 5 pM to about 1 nM, about 5 pM to about 0.75 nM, about 5 pM to about 0.5 nM, about 5 pM to about 0.25 nM, about 5 pM to about 0.1 nM.

[00108] In one embodiment, the target binding moiety comprises an antibody, antigen-binding fragment (Fab), or single chain variable fragment (scFv) of the target protein or peptide. In one embodiment, the target protein or peptide is a membrane-bound protein. In one embodiment, the membrane-bound protein comprises a member of the human epidermal growth factor receptor family. In one embodiment, the target binding moiety comprises an antibody, Fab, or scFv of human epidermal growth factor receptor 2 (HER2; also referred to as CD340).

[00109] In one embodiment, the target binding moiety comprises Trastuzumab, which comprises a heavy chain having an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT
LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:48) and a light chain
comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFGSGRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTL
SKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:49).

[00110] In one embodiment, the target binding moiety comprises a variable domain of Trastuzumab. In one embodiment, the target binding moiety comprises the light chain variable complementarity-determining regions (*e.g.*, CDR1, CDR2, and CDR3) of Trastuzumab. In one embodiment, the target binding moiety comprises a F(ab')₂, Fab' or rIgG fragment of Trastuzumab.

[00111] In one embodiment, the target binding moiety comprises a Fab of Trastuzumab, which comprises a heavy chain having an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT
LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA

VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:54) and a light chain having an amino acid sequence of SEQ ID NO:49.

[00112] In one embodiment, the target binding moiety comprises a scFv of Trastuzumab, which comprises an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFGSGRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGGAGGGGAGGG
GAGGGGAEVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARI
YPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD
YWGGQTLVTVSS (SEQ ID NO:55), which comprises a light chain having amino acid

sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS
RFGSGRSGTDFTLTISLQPEDFATYYCQQHYTTPPTFGQGTKVEIK (SEQ ID NO:73)

linked via a peptide linker of SEQ ID NO:56 to a heavy chain having a sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQTL
VTVSS (SEQ ID NO:74).

6.4 Internalization and Lysosomal Degradation

[00113] In certain embodiments, a chimeric protein, as disclosed herein, brings a target protein or peptide into proximity (*e.g.*, “induces proximity”) with a ScaR or EnyR to enable lysosomal degradation. In certain embodiments, lysosomal degradation can be described in several steps, including 1) binding of the chimeric protein to a target protein or peptide; 2) inducing of proximity of the target-bound chimeric protein to recruit a ScaR or EnyR; 3) binding of the target-bound chimeric protein to the ScaR or EnyR to form a ternary complex; 4) formation of a vesicle; 5) encapsulation of the ternary complex by an endosome; 6) merging of endosome and lysosome; and 7) lysosomal degradation of the ternary complex. In certain embodiments and depending on the particular type of ScaR or EnyR, the vesicle may be further coated by clathrin or caveolin.

[00114] Without being bound by theory, a chimeric protein, as disclosed herein, once bound to a target protein or peptide (*e.g.*, pathogenic protein or peptide) and a target ScaR or EnyR, may be internalized into the ScaR- or EnyR-expressing cell. Internalization of the target protein or peptide reduces the biological activity of the target protein or peptide in the extracellular space. In certain embodiments, the internalized chimeric protein, which is still bound to the target protein or peptide may enter the endosomal pathway for degradation in a lysosome of the cell. In one embodiment, the internalization is mediated by clathrin. Endocytosis can be measured by a variety of assays available commercially or previously described, such as those described by

Burgstaller et al.⁴⁵ and Tomihari et al.⁴⁶ Each of the cited references by Burgstaller et al. and Tomihari et al. is incorporated herein by reference with respect to their description of assays that may be used to measure endocytosis. In certain embodiments, the chimeric protein, including the target protein to which it is bound, is not recycled back outside of the cell.

[00115] Target-Bound Chimeric Protein Degradation: In methods disclosed herein, an endocytic receptor (EnyR) or scavenger receptor (ScaR) binds to a chimeric protein that has bound to a target protein or peptide (herein “target-bound chimeric protein”), internalizes the target-bound chimeric protein, and directs the target-bound chimeric protein to a lysosome. In certain embodiments, the EnyR or ScaR does not recycle the target-bound chimeric protein back outside of the cell or to the cell surface. In certain embodiments, the EnyR or ScaR is not capable of recycling the target-bound chimeric protein back outside of the cell or to the cell surface.

[00116] In certain embodiments, an EnyR or a ScaR, once bound to the target-bound chimeric protein, will internalize and direct the target-bound chimeric protein to the lysosome at least 30% of the times that the receptor binds to the target-bound chimeric protein. In certain embodiments, an EnyR or a ScaR, once bound to a target-bound chimeric protein, will internalize and direct the target-bound chimeric protein to the lysosome at least 40% of the times that the receptor binds to the target-bound chimeric protein. In certain embodiments, an EnyR or a ScaR, once bound to a target-bound chimeric protein, will internalize and direct the target-bound chimeric protein to the lysosome at least 50% of the times that the receptor binds to the target-bound chimeric protein. In certain embodiments, the EnyR or ScaR is not capable of recycling the target-bound chimeric protein back outside of the cell or to the cell surface.

[00117] In certain embodiments, target-bound chimeric protein is directed to the lysosome 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 99% of the times that the receptor binds to a target-bound chimeric protein. In certain embodiments, a target-bound chimeric protein is directed to the lysosome 30%-99% of the time. In certain embodiments, a target-bound chimeric protein directed to the lysosome 30%-95%, 30%-90%, 30%-80%, 30%-70%, or 30%-60% of the time. In certain embodiments, a target-bound chimeric protein is directed to the lysosome 50%-99% of the time. In certain embodiments, a target-bound chimeric protein directed to the lysosome 50%-95%, 50%-90%, 50%-80%, or 50%-70% of the time. In certain embodiments, a target-bound chimeric protein is directed to the lysosome 60%-99%, 60%-95%, 60%-90%, or 60%-80% of the time. In certain embodiments, a target-bound chimeric protein is directed to the lysosome 70%-99%, 70%-95%, or 70%-90% of the time. In certain embodiments, a target-bound chimeric protein is directed to the lysosome 80%-99%, 80%-95% or 80%-90% of the time. In certain embodiments, the target-bound chimeric protein is directed to the lysosome 90%-99% or 90%-95% of the time. In certain

embodiments, the target-bound chimeric protein is directed to the lysosome 95%-99% of the time.

[00118] The efficiency by which a ScaR or EnyR is able to bind to and successfully direct a target-bound chimeric protein to a lysosome for degradation, as disclosed herein, may be evidenced, *e.g.*, by measuring or detecting accumulation or presence of the target-bound chimeric protein in the lysosome or by measuring lysosomal degradation of that target-bound chimeric protein. Methods for detecting accumulation of or lysosomal degradation of target-bound chimeric protein in the lysosome will be well known to one of ordinary skill in the art. For example, fluorescence microscopy could be used. A target protein or peptide as described herein can be labeled (*e.g.*, tagged with a fluorescent molecule) and introduced together with a chimeric protein as described herein into a cell culture of cells expressing the desired EnyR or ScaR (*e.g.*, SKOV3 cells, HeLa cells). For example, the chimeric protein could also be labeled such that its accumulation in the lysosome could be detected and/or measured separately from that of the target protein or peptide. After a certain incubation time, the presence of the tagged target protein or peptide (and/or chimeric protein) in lysosomes can be detected by imaging methods known in the art (*e.g.*, by fluorescence microscopy). For example, lysosomal localization and degradation of a target protein or peptide can be visualized using a labeling kit, such as one of the LysoLight™ Deep Red Antibody Labeling Kits by ThermoFisher Scientific, which utilize a fluorophore that is only active upon enzymatic cleavage by the lysosomal specific enzyme, Cathepsin B. Outside of the lysosome, LysoLight™ Deep Red has little to no observable background fluorescence, and only upon localization to the protease rich environment of the lysosome, does LysoLight™ become activated and brightly fluoresce. As another example, lysosomal degradation can also be quantified by a protocol using red fluorescent protein (RFP) and green fluorescent protein (GFP) published by Tomihari et al.,⁴⁶ and also used in Ikatura et al.⁵¹, each of which is incorporated herein by reference with respect to the methods disclosed for measuring lysosomal localization and accumulation of a target protein or peptide. Localization of target-bound chimeric protein to the lysosome may also be visualized and/or quantified by tagging a target protein or peptide with a fluorescent probe and using a dye that stains acidic organelles, such as lysosomes, *e.g.*, one of the LysoTracker® dyes by ThermoFisher, such as described in Banik et al.,⁵⁰ which is incorporated herein by reference with respect to the methods disclosed for measuring lysosomal localization and accumulation of a target protein or peptide. In yet another example, one or both of the target protein and chimeric protein could be tagged with a pH-sensitive tag that changes fluorescence intensity based on pH to detect protein localization within the acidic environment of the lysosomes. Alternatively, biochemical fractionation could be utilized, wherein after incubation of a ScaR or EnyR-expressing cell line with the chimeric

protein and target protein or peptide, lysosomes are isolated using density gradient centrifugation or other biochemical methods. Thereafter, the presence of the target protein in lysosomal fractions could be measured using Western blotting or mass spectrometry.

[00119] The efficiency by which a ScaR or EnyR is able to degrade a target protein or peptide, as disclosed herein, will be well known to one of ordinary skill in the art. For example, methods such as flow cytometry and/or Western Blot, as described in the Examples herein, may be used to quantify the amount of degraded protein or peptide. For example, total expression of a particular target protein or peptide may be analyzed by Western Blot while surface expression of a surface-bound target protein or peptide may be assessed by flow cytometry.

[00120] In certain embodiments, lysosomal degradation of a target-bound chimeric protein initiates within 1 hour of introduction of the target protein or peptide and chimeric protein into a cell culture. In certain embodiments, lysosomal degradation of a target-bound chimeric protein initiates within 45 minutes of introduction of the target protein or peptide and chimeric protein into a cell culture. In certain embodiments, lysosomal degradation of a target-bound chimeric protein initiates within 30 minutes of introduction of the target protein or peptide and chimeric protein into a cell culture.

[00121] In certain embodiments, at least 30% of the target protein or peptide initially introduced into a cell culture together with a chimeric protein, as disclosed herein, will be degraded (*e.g.*, by being internalized and directed to the lysosome) within 4 hours. That is, at least 30% of the target protein or peptide will be degraded within 4 hours of exposing the target receptor and the target protein or peptide to the chimeric protein. In certain embodiments, at least 50% of the target protein or peptide will be degraded within 3 hours. In certain embodiments, at least 50% of the target protein or peptide will be degraded within 4 hours. In certain embodiments, at least 50% of the target protein or peptide will be degraded within 5 hours. In certain embodiments, at least 80% of the target protein or peptide will be degraded within 10 hours. In certain embodiments, at least 80% of the target protein or peptide will be degraded within 12 hours. In certain embodiments, at least 80% of the target protein or peptide will be degraded within 15 hours. In certain embodiments, at least 80% of the target protein or peptide will be degraded within 20 hours. In certain embodiments, at least 80% of the target protein or peptide will be degraded within 24 hours. In certain embodiments, at least 85% of the target protein or peptide will be degraded within 24 hours.

[00122] In one embodiment, the target protein or peptide is a soluble protein or peptide. In certain embodiments, an EnyR or a ScaR, once bound to a soluble target-bound chimeric protein, will internalize and direct the soluble target-bound chimeric protein to the lysosome at least 50%

(*e.g.*, 50%-95%), at least 60% (*e.g.*, 60%-95%), or at least 70% (*e.g.*, 70%-95%) of the times that the receptor binds to the soluble target-bound chimeric protein.

[00123] In one embodiment, the target protein or peptide is an extracellular membrane-bound protein. In certain embodiments, an EnyR or a ScaR, once bound to a membrane-bound target-bound chimeric protein, will internalize and direct the membrane-bound target-bound chimeric protein to the lysosome at least 30% (*e.g.*, 30%-95%), at least 40% (*e.g.*, 40%-95%), or at least 50% (*e.g.*, 50%-95%) of the times that the receptor binds to a membrane-bound target-bound chimeric protein.

[00124] Receptor- and Target-Bound Chimeric Protein Degradation: In certain embodiments, an endocytic receptor (EnyR) or scavenger receptor (ScaR) binds to a target-bound chimeric protein, internalizes the resulting complex, including the ScaR or EnyR, chimeric protein, and target protein or peptide (herein "bound chimeric complex"), and directs the bound chimeric complex to a lysosome. That is, in certain embodiments, the EnyR or ScaR is also degraded and is not recycled to the cell surface. One of skill in the art would be familiar with methods for determining and/or quantifying directing of the EnyR or ScaR as part of the bound chimeric complex. For example, recombinant cell lines could be produced such that the EnyR or ScaR contains a detectable (*e.g.*, fluorescent) tag that can be quantified and visualized in a similar manner as described for fluorophores used to tag the target protein or peptide and/or chimeric protein.

[00125] In certain embodiments, a chimeric protein, as described herein, possesses higher potency for degrading a particular target protein or peptide (*e.g.*, a greater percentage of target-bound chimeric protein will be directed to the lysosome for degradation in a certain amount of time) as compared to a monospecific-binding scaffold or protein degrader (*e.g.*, a monoclonal antibody, a Fab fragment, an scFv, a designed ankyrin repeat protein, a VHH, an inhibitor cystine knot, an affitin, an affibody, an affimer, an avimer, a polypeptide, or cyclic peptide). In certain embodiments, a chimeric protein, as described herein, possesses a degradation potency for a particular target protein or peptide that is at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, or at last 100% higher, as compared to a monospecific-binding scaffold or protein degrader.

[00126] Therefore, provided herein is a method of degrading a target protein or peptide, the method comprising contacting the target protein or peptide with a chimeric protein, as disclosed herein, wherein the chimeric protein comprises (a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to the ScaR or an EnyR; and (b) at least one target binding moiety capable of binding to the target protein or peptide. The contacting may be done in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic

receptor (EnyR). In certain embodiments, contacting of the target protein or peptide with the chimeric protein binds each of the target protein or peptide and the EnyR or ScaR to the chimeric protein to generate a bound chimeric complex. In certain embodiments, contacting of the target protein or peptide with the chimeric protein binds the target protein or peptide to the chimeric protein to generate a target-bound chimeric protein, and the method further comprises binding a ScaR or an EnyR to the target-bound chimeric protein to form a bound chimeric complex. In certain embodiments, when the chimeric protein is bound to both a ScaR or EnyR and the target protein or peptide, the ScaR or EnyR internalizes the target protein or peptide and directs it to a lysosome. In certain embodiments, when the chimeric protein is bound to both a ScaR or EnyR and the target protein or peptide, the ScaR or EnyR internalizes the target-bound chimeric protein and directs it to a lysosome. In certain embodiments, when the chimeric protein is bound to both a ScaR or EnyR and the target protein or peptide, the bound chimeric complex is directed to the lysosome.

[00127] In one embodiment, the receptor binding moiety comprises a ligand as described in any embodiment in Section 6.2, such as listed in **Table 1** or in **Table 2**. In one embodiment, the receptor binding moiety is a natural ligand of the receptor, a fragment of a natural ligand of the receptor, or a mimic of any of the foregoing. In one embodiment, the chimeric protein comprises a receptor binding moiety as described in **Table 3** in the Examples. In one embodiment, the receptor binding moiety comprises an LSS. In one embodiment, the receptor binding moiety binds SCARA3/SCARA5. In one embodiment, the receptor binding moiety binds CD63.

[00128] In one embodiment, the target binding moiety is as described in any embodiment of Section 6.3. In one embodiment, the target binding moiety is an antibody or fragment thereof, such as an IgG, Fab, or scFv. In one embodiment, the target binding moiety binds a growth factor receptor. In one embodiment, the target binding moiety binds HER2. In one embodiment, the target binding moiety binds EGFR.

[00129] In one embodiment, the target protein or peptide is endocytosed into a cell (*i.e.*, undergoes endocytosis) once bound to a chimeric protein, as disclosed herein and that target-bound chimeric protein binds to a ScaR or EnyR. In one embodiment, the target protein or peptide is endocytosed into a cell only after that target-bound chimeric protein binds to a ScaR or EnyR. In one embodiment, the target protein or peptide is endocytosed into a cell after that target-bound chimeric protein binds to a ScaR or EnyR as part of regular cellular processes. In one embodiment, the endocytosis is clathrin-mediated endocytosis (CME). In one embodiment, the endocytosis is clathrin-independent endocytosis. In one embodiment, the degrading of the target protein or peptide is via lysosomal degrading. In one embodiment, the target protein is

expressed on the same cell as the target ScaR or EnyR. In one embodiment, the cell is a cancer cell.

6.5 Exemplary Chimeric Proteins

[00130] A chimeric protein, as disclosed herein, comprises at least one receptor binding moiety having one or more of the attributes as described in Section 6.2 and a target binding moiety having one or more of the attributes as described in section 6.3 and exhibits activity as described in Section 6.4. Further examples of chimeric proteins are disclosed in Section 7, the examples.

[00131] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to HB-EGF (*e.g.*, a ligand of HB-EGF such as SEQ ID NO:37 or 61) and a target protein binding moiety that binds to HER2. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to HB-EGF (*e.g.*, a ligand of HB-EGF such as SEQ ID NO:37 or 61) and a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55) as the target protein binding moiety. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to HB-EGF (*e.g.*, a ligand of HB-EGF such as SEQ ID NO:37 or 61) and HER2 Fab (*e.g.*, Trastuzumab Fab) as the target protein binding moiety. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to HB-EGF (*e.g.*, a ligand of HB-EGF such as SEQ ID NO:37 or 61) and HER2 IgG (*e.g.*, Trastuzumab) as the target protein binding moiety.

[00132] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to IGF2R (*e.g.*, a ligand of IGF2R such as SEQ ID NO:38) and a target protein binding moiety that binds to HER2. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to IGF2R (*e.g.*, a ligand of IGF2R such as SEQ ID NO:38) and HER2 Fab (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55) as the target protein binding moiety.

[00133] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47) and a target protein binding moiety that binds to HER2. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47) and a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55) as the target protein binding moiety. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47) and HER2 IgG (*e.g.*, Trastuzumab) as the target protein binding moiety.

[00134] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to SCARA5/SCARA3 (*e.g.*, a SCARA5/SCARA3 ligand) and a target protein binding moiety that binds to HER2. In certain embodiments, a chimeric protein comprises a receptor

binding moiety that binds to SCARA5/SCARA3 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47) and a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55) as the target binding moiety.

[00135] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63, a target protein binding moiety that binds to HER2, and a target protein binding moiety that binds to EGFR.

[00136] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47), a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55) as a first target protein binding moiety, and a target protein binding moiety that binds to EGFR as a second protein binding moiety. In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63, a first target protein binding moiety that binds to HER2, and an EGFR affibody (*e.g.*, SEQ ID NO:70) as a second protein binding moiety. In certain embodiments, the EGFR affibody comprises an amino acid sequence of:

VDNKFNKEMWAAWEEIRNLPNLNGWQMTAFIASLVDDPSQSANLLAEAKKLNDAPK (SEQ ID NO:70). In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63 (*e.g.*, an anti-CD63 scFV such as SEQ ID NO:47), a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55), as a first target protein binding moiety, and an EGFR affibody (*e.g.*, SEQ ID NO:70) as a second protein binding moiety.

[00137] In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63, a first target protein binding moiety that binds to HER2, and an EGFR scFV (*e.g.*, a Cetuximab scFV, such as SEQ ID NO:71) as a second protein binding moiety. In certain embodiments, the Cetuximab scFV comprises an amino acid sequence of:

DILLTQSPVILSVSPGERVVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFS
GSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKGGGGSGGGGSGGGGS
GGGGQVQLKQSGPGLVQPQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQG
TLVTVSA (SEQ ID NO:71). In certain embodiments, a chimeric protein comprises a receptor binding moiety that binds to CD63, a HER2 scFV (*e.g.*, a Trastuzumab scFV such as SEQ ID NO:55), as a first target protein binding moiety, and an EGFR scFV (*e.g.*, a Cetuximab scFV, such as SEQ ID NO:71), as a second protein binding moiety.

6.6 Chimeric Protein Design

[00138] In another aspect, the present disclosure provides a method of designing a chimeric protein, as disclosed herein. In one embodiment, suitable receptor binding moieties of a chimeric protein can be identified by first identifying co-expression of ScaR or EnyR with a target protein

on a cell. Identifying co-expression may be carried out, *e.g.*, by identifying particular ScaR and/or EnyRs that are expressed by cells of a subject to which the chimeric protein will be administered. In one embodiment, the cell is a cancer cell. For example, **FIG. 3** depicts a heatmap based on transcriptomic and proteomic data from Human Protein Atlas (RNA HPA cell line gene data) to identify expression of certain ScaRs and EnyRs in various human cell lines, some of which are cancer cell lines. Higher values/darker regions indicate highest expressed ScaR/EnyR in each cell line.

6.7 Pharmaceutical Compositions

[00139] Chimeric proteins, as disclosed herein, can be included in a pharmaceutical composition for administration, *e.g.*, to a subject for treating a disease, disorder, or condition. As such, the present disclosure provides a pharmaceutical composition comprising:

- i. a chimeric protein, the chimeric protein comprising:
 - (a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and
 - (b) at least one target binding moiety, wherein the target binding moiety is capable of binding to a target protein or peptide; and
- ii. at least one pharmaceutically acceptable excipient.

[00140] In one embodiment, the receptor binding moiety comprises a ligand as described in any embodiment in Section 6.2, such as listed in **Table 1** or in **Table 2**. In one embodiment, the receptor binding moiety is a natural ligand of the receptor, a fragment of a natural ligand of the receptor, or a mimic of any of the foregoing. In one embodiment, the chimeric protein comprises a receptor binding moiety as described in **Table 3** in the Examples. In one embodiment, the receptor binding moiety comprises an LSS. In one embodiment, the receptor binding moiety binds SCARA3/SCARA5. In one embodiment, the receptor binding moiety binds CD63.

[00141] In one embodiment, the target binding moiety is as described in any embodiment of Section 6.3. In one embodiment, the target binding moiety is an antibody or fragment thereof, such as an IgG, Fab, or scFv. In one embodiment, the target binding moiety binds a growth factor receptor. In one embodiment, the target binding moiety binds HER2. In one embodiment, the target binding moiety binds EGFR.

[00142] The pharmaceutically acceptable excipient can be one or more compatible solid or liquid fillers, diluents, other excipients, or encapsulating substances that are suitable for administration into a human or veterinary subject (*e.g.*, a physiologically acceptable and/or pharmacologically acceptable). The pharmaceutically acceptable excipient can be co-mingled with one or more of the active components, *e.g.*, a hybrid molecule, and with each other, when more than one pharmaceutically acceptable excipient is present in the pharmaceutical

composition, in a manner so as not to substantially impair the desired pharmaceutical efficacy. Pharmaceutically acceptable materials typically are capable of administration to a subject without the production of significant undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. It is, for example, desirable for a composition comprising a pharmaceutically acceptable excipient not to be immunogenic when administered to a human subject for therapeutic purposes.

[00143] Pharmaceutical compositions comprising the chimeric protein, as disclosed herein, can additionally contain suitable buffering agents, including, for example, acetic acid in a salt, citric acid in a salt, boric acid in a salt, and phosphoric acid in a salt. Pharmaceutical compositions can also optionally contain suitable preservatives, such as benzalkonium chloride, chlorobutanol, parabens, and thimerosal. Pharmaceutical compositions can be presented in unit dosage form and can be prepared by any suitable method, many of which are well known in the art of pharmacy. Such methods include the step of bringing the chimeric protein, as disclosed herein, into association with one or more pharmaceutically acceptable excipients.

[00144] A pharmaceutical composition suitable for parenteral administration conveniently comprises a sterile aqueous preparation of the composition, which preferably is isotonic with the blood of the recipient. This aqueous preparation can be formulated of known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also can be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed, such as synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables. Excipient formulations suitable for oral, subcutaneous, intravenous, intramuscular, and the like, administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

[00145] Preparation of pharmaceutical compositions of the invention and their various routes of administration can be carried out in accordance with methods well known in the art. The delivery systems useful in the context of the invention include time-released, delayed release, and sustained release systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The pharmaceutical composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the pharmaceutical composition, thereby increasing convenience to

the subject and the physician, and can be particularly suitable for some pharmaceutical compositions disclosed herein.

[00146] Many types of release delivery systems are available and known to those of ordinary skill in the art. Suitable release delivery systems include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids such as sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and triglycerides; hydrogel release systems; systatic systems; peptide-based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patents 4,452,775, 4,667,014, 4,748,034, and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patents 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[00147] Generally, a chimeric protein or pharmaceutical composition comprising a chimeric protein, as disclosed herein, is suitably packaged, *e.g.*, in a vial, pouch, ampoule, and/or any container appropriate for a therapeutic method. Components can be provided as concentrates (including lyophilized compositions), which can be further diluted prior to use, or they can be provided at the concentration of use. For use of a chimeric protein *in vivo*, single dosages can be provided in sterilized containers having the desired amount and concentration of components.

6.8 Therapeutic Uses

[00148] Degradation of a target protein or peptide by binding it to via a chimeric protein to a ScaR or EnyR may be used to treat, prevent, or alleviate symptoms of a disease, disorder, or condition in which the target protein or peptide is implicated.

[00149] As used herein, the term “treating” or “treatment” as used herein means the treating or treatment of a disease or medical condition in a subject, such as a mammal (particularly a human) that includes: (a) preventing the disease or medical condition from occurring, such as, prophylactic treatment of a subject; (b) ameliorating the disease or medical condition, such as, eliminating or causing regression of the disease or medical condition in a subject; (c) suppressing the disease or medical condition, for example by, slowing or arresting the development of the disease or medical condition in a subject; or (d) alleviating a symptom of the disease or medical condition in a subject. In some embodiments, the term “treating,” or “treatment” excludes a prophylactic treatment.

[00150] For example, the target protein or peptide may be a signaling molecule that advances pathogenesis or progression of the disease, disorder, or condition. In another example, the target protein or peptide may be a signaling molecule that affects the pathophysiology of a disease, disorder, or condition. In another example, the target protein or peptide may be a molecule that inhibits degradation or blocking of a signaling molecule that advances pathogenesis or progression of the disease, disorder, or condition. In another example, the target protein or peptide may be a molecule that inhibits a subject's immune system to slow or reverse pathogenesis or progression of the disease, disorder, or condition. In another example, the target protein or peptide may be a molecule that contributes to a symptom of the disease, disorder, or condition. In one embodiment, the expression of the target protein or peptide is upregulated as a result of the pathogenesis or progression of the disease, disorder, or condition.

[00151] Therefore, in one embodiment, provided herein is a method of slowing or reversing pathogenesis or progression of a disease, disorder, or condition in a subject, wherein the pathogenesis is directly or indirectly mediated by a target protein or peptide, the method comprising administering to the subject the chimeric protein comprising (a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and (b) at least one target binding moiety capable of binding to the target protein or peptide. In one embodiment, the method is a treatment of the disease, disorder, or condition. In one embodiment, the treatment also alleviates the symptoms of the disease, disorder, or condition.

[00152] In another embodiment, provided herein is a method of reducing the symptoms of a disease, disorder, or condition in a subject, wherein the symptoms are directly or indirectly mediated by a target protein or peptide, the method comprising administering to the subject the chimeric protein comprising (a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and (b) at least one target binding moiety capable of binding to the target protein or peptide.

[00153] In another embodiment, provided herein is a method of preventing the onset of pathogenesis of a disease, disorder, or condition in a subject, wherein the pathogenesis is directly or indirectly mediated by a target protein or peptide, the method comprising administering to the subject a chimeric protein comprising (a) at least one a receptor binding moiety, wherein the receptor binding moiety is capable of binding to a ScaR or an EnyR; and (b) at least one target binding moiety capable of binding to the target protein or peptide.

[00154] In one embodiment, the receptor binding moiety comprises a ligand as described in any embodiment in Section 6.2, such as listed in **Table 1** or in **Table 2**. In one embodiment, the receptor binding moiety is a natural ligand of the receptor, a fragment of a natural ligand of the

receptor, or a mimic of any of the foregoing. In one embodiment, the chimeric protein comprises a receptor binding moiety as described in **Table 3** in the Examples. In one embodiment, the receptor binding moiety comprises an LSS. In one embodiment, the receptor binding moiety binds SCARA3/SCARA5. In one embodiment, the receptor binding moiety binds CD63.

[00155] In one embodiment, the target binding moiety is as described in any embodiment of Section 6.3. In one embodiment, the target binding moiety is an antibody or fragment thereof, such as an IgG, Fab, or scFv. In one embodiment, the target binding moiety binds a growth factor receptor. In one embodiment, the target binding moiety binds HER2. In one embodiment, the target binding moiety binds EGFR.

[00156] In one embodiment, the disease, disorder, or condition is cancer, such as a solid tumor or hematological cancer. Non-limiting examples of solid tumors that may be treated by administering a chimeric protein as described herein include ovarian cancer, esophageal/gastric cancer, cervical cancer, bladder cancer, breast cancer, central nervous system cancer, colorectal cancer, gastrointestinal cancer, endocrine cancer, eye cancer, female genitourinary cancer, head and neck cancer, liver cancer, lung cancer, skin cancer, soft tissue cancer, bone cancer, squamous cell cancer, pancreatic cancer, kidney cancer, or prostate cancer. Non-limiting examples of hematological cancers that may be treated by administering a chimeric protein as described herein include are leukemia, lymphoma, or multiple myeloma. In one embodiment, the cancer is ovarian cancer. In one embodiment, the cancer is cervical cancer.

[00157] In another embodiment, the disease or disorder is a receptor-mediated autoimmune disease. In one embodiment, the disease or disorder is rheumatoid arthritis, type 1 diabetes, psoriasis, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, or Sjögren's Syndrome. In one embodiment, the disease or disorder is Graves' disease, myasthenia gravis, anti-NMDA receptor encephalitis, or idiopathic membranous nephropathy. In one embodiment, the disease or disorder is anti-NMDA receptor encephalitis, and the target protein or peptide is an NMDA receptor. In another embodiment, the disease or disorder is idiopathic membranous nephropathy, and the target protein or peptide is phospholipase A2 receptor. In another embodiment, the disease or disorder is ovarian cancer or cervical cancer and the target protein or peptide is HER2. In another embodiment, the disease or disorder is non-small cell lung cancer, breast cancer, or gastroesophageal cancer and the target protein or peptide is EGFR. In another embodiment, the disease or disorder is non-small cell lung cancer, breast cancer, or gastroesophageal cancer and the chimeric protein comprises two target binding moieties, one capable of binding HER2 and one capable of binding EGFR.

7. EXAMPLES

[00158] Example 1: Heat Map Analysis to Identify ScaR and EnyR expression

[00159] FIG. 3 depicts a heat map identifying expression of ScaRs and EnyRs in various human cell lines. Transcriptomic read counts for ScaRs/EnyRs from Human Protein atlas (RNA HPA cell line gene data) were normalized for each cell line (1 is highest expressed ScaR/EnyR in each cell line, others are ratios thereof). This allows for each cell line to select the most promising (highest expressed) ScaR/EnyR. For example, according to FIG. 3 the expression of TFRC is high in JURKAT (acute T cell leukemia), K-562 (myelogenous leukemia), and MCF-7 (breast cancer) cell lines. This suggests that a chimeric protein comprising a receptor binding moiety comprising a natural ligand of TFRC may be suitable for treating acute T cell leukemia, myelogenous leukemia, and/or breast cancer. Similarly, the expression of LAMP1 is high in HEK293, HUVEC/TERT2, SK-OV-3, and U2OS cells, suggesting that a chimeric protein comprising a receptor binding moiety comprising a natural ligand of LAMP1 may be suitable for treating various cancers, such as ovarian cancer or osteosarcoma, as well.

[00160] While FIG. 3 depicts a 2-dimensional heat map, a 3-dimensional heat map could be generated in an analogous manner to identify co-expression of ScaR or EnyR and a target protein in each cell line.

[00161] Example 2: Transcriptomic and proteomic analyses of different human cancer cell lines for prediction of expression level of HER2

[00162] To predict the level of expression of a target protein for degradation, *e.g.*, HER2, transcriptomic and proteomic analyses were performed on different human cancer cell lines using datasets available from the Human Protein Atlas and Sanger Cell model passport. Based on a panel of available cell lines, SKOV3 (ovarian adenocarcinoma cell line with epithelial morphology) and HeLa (cervical adenocarcinoma cell line with epithelial morphology) cell lines were selected to represent a HER2 high-expressing and low-expressing cell line, respectively, with a predicted difference of approximately one magnitude in expression (see FIG. 8). These cell lines are herein referred to as targeted cell lines. High levels of HER2 expression in SKOV3 cells and low levels of expression in HeLa cells are predicted.

[00163] Example 3: Validation of expression of HER2 in targeted cell lines

[00164] Based on transcriptomic and proteomic analysis, the SKOV3 cell line was identified as a high-expressing HER2, while the HeLa cell line was identified as a low-expressing HER2. Flow cytometry (FIG. 9, panel A) and Western-blot (FIG. 9, panel B) analyses demonstrated that the expression level of HER2 on SKOV3 is significantly higher than that on HeLa. These targeted cells were used for HER2 degradation. FIG. 9, panel A shows surface expression of HER2 measured by flow cytometry, with results presented as median fluorescence intensity

(MFI). **FIG. 9, panel B** depicts Western-blot analysis of total protein extracts from SKOV3 and HeLa cells. Total protein extracts corresponding to different cell densities loaded. The intensity of the HER2 bands in the upper part of panel B is correlated with the density of cells in both SKOV3 and HeLa cells, similar to β -actin in the lower part of panel B, confirming efficient extraction of total HER2 by the described protocol. Comparison of the ratio of HER2 band intensity to cell density clearly demonstrates that SKOV3 cells express a significantly higher level of HER2 compared to HeLa cells, while this ratio is comparable for β -actin in both SKOV3 and HeLa cells.

[00165] Example 4: Identification of targeted cells specific ScaRs/EnyRs

[00166] Based on reported ScaRs and EnyRs, candidate ScaRs/EnyRs were selected that are highly expressed on SKOV3 and HeLa cells using transcriptomic data. Cell line-specific data was extracted from the RNA HPA cell line gene data from the Human Protein Atlas using the Python data analysis library (available at <http://pandas.pydata.org/>), referenced to the ENS IDs of the ScaRs/EnyRs, and sorted by normalized transcript levels. The selected high-scoring ScaR/EnyR candidates were graphed using Matplotlib (available at <http://matplotlib.org/>), which is shown in **FIG. 10**. Based on the highest score, three (3) EnyR candidates were selected (1) HB-EGF, (2) IGF2R, and (3) CD63. In addition, it was predicted that the level of expressions of these three EnyRs is comparable in SKOV3 and HeLa cell lines.

[00167] Example 5: Confirmation of expression of target endocytic receptors in targeted cells

[00168] Flow cytometry and Western-blot analyses were used to confirm the expression of EnyRs HB-EGF, IGF2R, and CD63 in SKOV3 and HeLa cell lines. **FIG. 11A** reports the expression of CD63 and IGF2R, as analyzed by flow cytometry in SKOV3 and HeLa cell lines. **FIG. 11B** depicts the expression of proHB-EGF, and IGF2R confirmed by Western Blot analysis. These results collectively confirm the transcriptomic analyses prediction of Example 4, indicating that HB-EGF, IGF2R, and CD63 are expressed in SKOV3 and HeLa cells. In addition, it was confirmed that the level of expression of the three EnyRs was comparable between SKOV3 and HeLa cells, also as predicted by transcriptomic analysis.

[00169] Example 6: HER2-degrading chimeric protein design

[00170] As will be described in further detail, a variety of chimeric proteins have been engineered by linking (*e.g.*, fusing or chemically linking) target-binding moieties and a receptor binding moiety (*e.g.*, ScaR or EnyR binding moiety) in a variety of diverse formats (see, *e.g.*, **Table 3**, **FIG. 12**, and **FIG. 17**, where black motif refers to the target-binding moiety and the lighter motif(s) refer(s) to the receptor binding moiety). Chimeric proteins prepared as disclosed herein comprising a HER2 binder based on Trastuzumab as target binding moiety. As one of skill

in the art will know, Trastuzumab is an approved monoclonal antibody that binds to the juxtamembrane domain of HER2. The Trastuzumab-based binder was an IgG, Fab, or scFv of Trastuzumab.

A number of chimeric proteins targeting HER2 via either an IgG, Fab, or scFv of Trastuzumab were tested, as shown in **Table 3**. In chimeric protein #1g, heparin binding epidermal-like growth factor was targeted using a polypeptide consisting of 26 amino acids derived from the HB-EGF binding protein from *Corynebacterium diphtheriae*⁴⁷: SSDSIGVLGYQKTVDHTKVNSKLSLF (SEQ ID NO:72). In chimeric protein #9a, SEQ ID NO:37 was used to target the HB-EGF receptor. In chimeric protein #10a, SEQ ID NO:38, which is a polypeptide consisting of 60 amino acids derived from insulin-like growth factor fused to the c-terminus of the heavy chain of insulin-like growth factor was used to target the IGF2 receptor (IGF2R). In each of chimeric proteins #16a, #16b, and #17a, an anti-CD63 single-chain variable fragment of SEQ ID NO:47 was used to target CD63⁴⁹. In chimeric protein #18a, SEQ ID NO:7 fused to SEQ ID NO:50 was used to target SCARA5. The linker in chimeric protein #1g was GGGGAGGGGAGGGGAGGGGA (SEQ ID NO:56). The linker in chimeric proteins #9a, #10a, and #17a was GGGGAGGGGAGGGGA (SEQ ID NO:57). The linker in chimeric protein #16a and #16b was GGGGA (SEQ ID NO:58). **Table 3: Prepared Chimeric Proteins**

Chimeric Protein	Format	Linker	Target Receptor // Receptor Binding Moiety	Target Protein // Target Binding Moiety
#1g	receptor binding ligand – linker – target binding peptide	SEQ ID NO:56	HB-EGF // SEQ ID NO:72	HER2 // Trastuzumab scFv
#9a	receptor binding ligand – linker – C-terminus of target binding Fab heavy chain	SEQ ID NO:57	HB-EGF // SEQ ID NO:37	HER2 // Trastuzumab Fab
#10a	receptor binding ligand – linker – C-terminus of target binding Fab heavy chain	SEQ ID NO:57	IGF2R // SEQ ID NO:38	HER2 // Trastuzumab Fab
#16a	target binding scFv – linker – receptor binding scFv	SEQ ID NO:58	CD63 // SEQ ID NO:47	HER2 // Trastuzumab scFv
#16b	receptor binding scFv – linker – target binding scFv	SEQ ID NO:58	CD63 // SEQ ID NO:47	HER2 // Trastuzumab scFv
#17a	receptor binding scFv – linker – C-terminus of target binding IgG heavy chain	SEQ ID NO:57	CD63 // SEQ ID NO:47	HER2 // Trastuzumab IgG
#18a	receptor binding ligand + LSS tag – target binding scFv	bond	SCARA5/SCARA3 // SEQ ID NO:7	HER2 // Trastuzumab scFv

Chimeric Protein	Format	Linker	Target Receptor // Receptor Binding Moiety	Target Protein // Target Binding Moiety
#22a	receptor binding scFv – C-terminus of the heavy chain of a target 1 binding IgG AND light chain of the target 1 binding IgG – target 2 binding affibody	bond	CD63 // SEQ ID NO:47	Target 1: HER2 // Trastuzumab scFv AND Target 2: EGFR // Anti-EGFR affibody (SEQ ID NO:70)
#22b	receptor binding scFv – C-terminus of heavy chain of target 1 binding IgG AND light chain of the target 1 binding IgG – target 2 binding scFv	n/a	CD63 // SEQ ID NO:47	Target 1: HER2 // Trastuzumab scFv AND Target 2: EGFR // Cetuximab scFv (SEQ ID NO:71)
#24a	receptor binding scFv – linker – target binding Fab	SEQ ID NO:57	CD63 // SEQ ID NO:47	HER2 // Trastuzumab Fab

[00171] Example 7: Chimeric protein production

[00172] Engineered protein sequences referred to in **Table 3** were transiently expressed using a standard Chinese hamster ovary cell platform. Culture medium was collected and purified by Ni NTA Beads or Protein A, where appropriate. **FIG. 13** and **FIG. 17**, panel B confirms the production of each chimeric protein in **Table 3**. To determine the purity level of chimeric proteins in purified protein samples, both reduced and non-reduced samples were resolved by SDS Page and stained with Coomassie blue.

[00173] Example 8: HER2 degradation in targeted cells

[00174] It has been demonstrated that the different chimeric protein formats consisting of different HER2 binder formats derived from Trastuzumab, such as single-chain variable fragment, Fab fragment, and full-length monoclonal antibody, combined with different motifs that bind to diverse EnyRs, such as IGF2R, HB-EGF, and CD63, results in tunable degradation of HER2.

[00175] SKOV3 and HeLa cells were treated with Trastuzumab Fab (as a control), chimeric protein #9a and chimeric protein #10a. Surface expression of HER2 was analyzed by flow cytometry. Total HER2 expression was analyzed by Western Blot.

[00176] Trastuzumab Fab fragment based chimeric proteins #9a and #10a, each of which was composed of Trastuzumab Fab fused via a peptide of SEQ ID NO:56 to a polypeptide that binds to HB-EGF (chimeric protein #9a) or a polypeptide that is part of the natural ligand of IGF2R (chimeric protein #10a), demonstrated 35-65% degradation of surface HER2 comparing to a

Trastuzumab Fab fragment control, as shown in **FIG. 14**. Panel A shows surface expression of HER2 with results presented as median fluorescence intensity (MFI) measured by flow cytometry. Panel B depicts the total HER2 quantification in SKOV3 cells by Western Blot.

[00177] In line with this finding, total HER2 quantification demonstrated that SKOV3 cells treated with chimeric protein #9a degraded 35% of total HER2 (*e.g.*, intra- and extracellular HER2) and chimeric protein #10a degraded 55% of total HER2, whereas Trastuzumab Fab fragment (control) showed no degradation potency. Similarly, HeLa cells treated with chimeric protein #9a degraded 45% of total HER2 and chimeric protein #10a degraded 65% of total HER2.

[00178] This demonstrates that the Fab fragment fused to different HB-EGF and IGF2R binding motifs can elicit significant degradation compared to Trastuzumab Fab fragment treated cells.

[00179] As shown in **FIG. 15**, panel A, Trastuzumab full length IgG-based chimeric protein #17a, composed of IgG fused to an anti-CD63 scFv, demonstrated 65% and 75% cell surface HER2 degradation in SKOV3 and HeLa cells, respectively, whereas trastuzumab only demonstrated 30% surface HER2 degradation. In line with this finding, total HER2 quantification demonstrated that SKOV3 cells treated with chimeric protein #17a degraded 60% of total HER2 while Trastuzumab degraded only 30% of Trastuzumab treated cells (see **FIG. 15**, panel B).

[00180] **FIG. 16** summarizes the degradation potency of each of chimeric proteins #1g, #16a, #16b, and #18a in comparison to Trastuzumab control, demonstrating that the fusion of the Trastuzumab scFV with a wide variety of moieties capable of binding to a variety of endocytic and scavenger receptors yield potent chimeric proteins for degrading HER2. This is particularly important for the development of smaller-sized chimeric proteins, the use of which may exhibit enhanced tissue penetration properties.

[00181] These results demonstrate that the rapid chimeric protein design process produces targeted protein degraders that possess high degradation potency and tunability. Additionally, thanks to design flexibility, diverse degrader chimeras can be generated based on desirable drug candidate attributes, which are essential for the safe and efficient development of drug candidates. This information collectively demonstrates that the process established here, comprising bioinformatic analysis and methods for designing chimeric proteins, is an innovative and unique approach for developing precision medicine. This approach enables targeting of indications that are difficult to treat due to a lack of efficient treatment options.

[00182] Example 9: Methods Used in Examples 2-8**[00183] Cell Culture**

[00184] Adherent SKOV3 and HeLa cells were cultured in 6-well plates at 37°C in a 5% CO₂ atmosphere. SKOV3 and HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and grown to approximately 70% confluency before treatment. Cells were detached using Verve solution and either pelleted for protein extraction or used for flow cytometry.

[00185] For HER2 degradation, SKOV3 and HeLa cells were cultivated as indicated above. After overnight incubation, the medium was removed, and fresh medium containing 100nM of EnyRTAC or ScaRTAC (**Table 1**) and corresponding control molecules, IgG isotype, Trastuzumab, and Fab fragment, were added.

[00186] Protein Extraction

[00187] Cells were harvested, and the pellet was washed twice with ice-cold PBS. The pellets were then mixed with RIPA lysis buffer (Thermo Fisher Scientific) containing a protease inhibitor cocktail (Thermo Fisher Scientific) and 10mM EDTA. This mixture was incubated for 15 minutes at 4°C with gentle shaking. The suspension was centrifuged at 20,000 x g for 15 minutes at 4°C. The total protein concentration was measured using the BCA protocol (Thermo Fisher Scientific). The supernatant was transferred into a new microcentrifuge tube and frozen at -80°C until it was used.

[00188] Flow Cytometry Analysis

[00189] After 24 hours, cells treated with EnyRTACs, as described in cell culture protocol, cells were washed with PBS, detached using Versene solution (Gibco) and transferred to a 96 V-bottom plate for staining. To avoid unspecific antibody binding, cells were blocked using Fc Receptor Binding Inhibitor Polyclonal Antibody (Invitrogen) and subsequently stained with live/dead cell exclusion dye (Zombie Dyes, BioLegend). Surface staining was performed with fluorophore-conjugated antibodies for 30 minutes at 4°C in FACS buffer (PBS, 2% FCS, 0.5 mM EDTA). Stained samples were fixed using IC fixation buffer (eBioscience) until further analysis. For intracellular staining, cells were stained with fluorophore-conjugated antibodies in 1x Permeabilization buffer (eBioscience) for 30 minutes at room temperature according to the manufacturer's instruction. Samples were acquired on LSR II Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo 10.8 (TreeStar Inc). Doublets, cell debris and dead cells were excluded before performing downstream analysis. Antibodies used listed in **Table 2**.

[00190] Western-blot Analysis

[00191] Aliquoted frozen extracted protein samples were mixed with Lämmli buffer and heated for 5 minutes at 95°C. The protein extracts were loaded onto 1 SurePAGE™ Bis-Tris gel

(10x8, 4-12%) and run in 1x MOPS buffer for 40 minutes at 200 V. A semi-dry transfer protocol was used to transfer proteins from the gels onto PVDF membranes using IBlot2 (Thermo Fisher Scientific). The membranes were blocked for 1-4 hours at room temperature in PBST buffer containing 0.1% Casein. After blocking, the membrane was incubated with the primary antibody in PBST buffer overnight at 4°C. The membrane was washed three times with excess washing buffer (PBST 0.05% Casein) and incubated with the secondary antibody for 1 hour at room temperature. The membrane was washed four times with PBST and developed by adding the developing solution, HRP-substrate Radiance Plus, and the image was acquired with iBright 150 (Thermo Fisher Scientific). Antibodies used listed in **Table 4**.

Table 4

Antibody ID	Target	Application
Pertuzumab	HER2	Flow cytometry; labeled with AF-488
BioLegend 353003	CD63	Flow cytometry
BioLegend 364205	IGF2R	Flow cytometry
Bioconcept 4967S	Beta-Actin	Western Blot
Bioconcept 14363S	IG2R	Western Blot
Bioconcept 2242S	HER2	Western Blot
BioLegend PA5-109805	HB-EGF	Western Blot

[00192] Example 10: HER2 degradation *In Vivo*

[00193] To demonstrate EnyRTAC functionality and activity *in vivo*, a Trastuzumab Fab fragment-based chimeric protein, #24a, composed of Trastuzumab Fab fused via a peptide to an anti-CD63 scFv, was designed and produced in CHO cells (see **FIG. 18**). To exclude bias in HER2 degradation as a result of the HER2 binding moiety, the degradation potency of the Trastuzumab Fab fragment was compared to Trastuzumab and an isotype IgG control. It was demonstrated that the Trastuzumab Fab fragment did not elicit HER2 degradation compared to the isotype control, in contrast to Trastuzumab, which showed 20-25% HER2 degradation compared to the isotype control. Additionally, it was demonstrated that product #24a can degrade 55% of HER2 in SKOV3 cells compared to the Trastuzumab Fab fragment control (see **FIG. 19**). No significant difference in HER2 degradation in SKOV3 cells treated with 100 nM and 500 nM of product #24a after 24 hours of treatment was observed, indicating maximum HER2 degradation potency had been reached at 100 nM. To determine the onset of HER2 degradation in SKOV3 cells treated with 100 nM of product #24a and Trastuzumab, HER2 levels were measured 6- and 24-hours post-treatment. It was observed that HER2 degradation in SKOV3 cells treated with product #24a reached 45% after 6 hours and increased to 55% after 24 hours of treatment, while HER2 degradation was measured at 10% and 30%, respectively, at the same time points for Trastuzumab when compared to the Trastuzumab Fab (see **FIG. 20**).

[00194] To prepare a xenograft tumor model for *in vivo* study, ten (10) NSG mice (NOD-scid-gc) were injected subcutaneously with 1×10^6 SKOV3 cells. Tumor growth was determined by measuring volume, and the injection was initiated when the tumor size reached 50-100 mm³. Two groups, with 5 mice each, were injected intratumorally twice, with a time interval of two days, with 6 mg/kg of product #24a and Trastuzumab Fab fragment. Two days after the last injection, the mice were euthanized, and SKOV3 cells were isolated from the tumor mass by cutting the tumor in small pieces and using collagenase digestion. The level of HER2 in SKOV3 cells was measured by flow cytometry using fluorescently-labelled Pertuzumab. The group of mice treated with product #24a showed a significant reduction in HER2 compared to the Trastuzumab Fab fragment control group (see FIG. 21). These results demonstrated that EnyRTAC, product #24a, is functional *in vivo* and can degrade HER2. In addition, EnyRTAC, product #24a, was well-tolerated in mice.

[00195] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entireties and to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[00196] While exemplary embodiments provided herein have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments described herein can be employed in practicing the subject matter provided herein. All such equivalents are considered to be within the scope of the claimed subject matter and are encompassed by the appended claims.

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CLAIMS

1. A chimeric protein comprising:
 - a. a receptor binding moiety capable of binding to a scavenger receptor (ScaR);
and
 - b. a target binding moiety capable of binding to a target protein or peptide.
2. A chimeric protein comprising:
 - a. a receptor binding moiety capable of binding to an endocytic receptor (EnyR);
and
 - b. a target binding moiety capable of binding to a target protein or peptide.
3. The chimeric protein of claim 1 or claim 2, wherein the receptor binding moiety is a peptide.
4. The chimeric protein of any one of claims 1-3, wherein the receptor binding moiety binds to the EnyR or ScaR at a location different from the pocket to which a natural ligand of the EnyR or ScaR binds.
5. The chimeric protein of any one of claims 1-4, wherein the binding affinity of the receptor binding moiety to the ScaR and/or the EnyR is lower than the binding affinity of the target binding moiety to the target protein or peptide.
6. The chimeric protein of any one of claims 1-4, wherein the binding affinity of the receptor binding moiety to the ScaR and/or the EnyR is at least an order of magnitude lower than the binding affinity of the target binding moiety to the target protein or peptide.
7. The chimeric protein of any one of claims 1-4, wherein the binding affinity of the receptor binding moiety to the ScaR and/or the EnyR is about 0.1 nM to about 10 nM and the binding affinity of the target binding moiety to the target protein or peptide is about 0.1 pM to about 1 nM.
8. The chimeric protein of any one of claims 1-7, wherein the chimeric protein comprises two or more receptor binding moieties.
9. The chimeric protein of claim 8, wherein the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to a different part of the same ScaR or EnyR.

10. The chimeric protein of claim 8, wherein the chimeric protein comprises two or more receptor binding moieties, each independently capable of binding to a different epitope of the same ScaR or EnyR.
11. The chimeric protein of any one of claims 1-10, wherein the chimeric protein comprises two or more target binding moieties.
12. The chimeric protein of claim 10, wherein the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different part of the same target protein or peptide.
13. The chimeric protein of claim 10, wherein the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different epitope of the same target protein or peptide.
14. The chimeric protein of claim 10, wherein the chimeric protein comprises two or more target binding moieties, each independently capable of binding to a different target protein or peptide.
15. The chimeric protein of any one of claims 1-14, wherein the chimeric protein further comprises a linker that connects the receptor binding moiety and target binding moiety.
16. The chimeric protein of any one of claims 1-15, wherein expression of the target protein or peptide is upregulated in a disease or disorder.
17. The chimeric protein of claim 16, wherein the disease or disorder is cancer.
18. The chimeric protein of claim 17, wherein the cancer is a solid tumor or hematological cancer.
19. The chimeric protein of claim 18, wherein the solid tumor is ovarian cancer, esophageal/gastric cancer, cervical cancer, bladder cancer, breast cancer, central nervous system cancer, colorectal cancer, gastrointestinal cancer, endocrine cancer, eye cancer, female genitourinary cancer, head and neck cancer, liver cancer, lung cancer, skin cancer, soft tissue cancer, bone cancer, squamous cell cancer, pancreatic cancer, kidney cancer, or prostate cancer.
20. The chimeric protein of claim 18, wherein the hematological cancer is leukemia, lymphoma, or multiple myeloma.

21. The chimeric protein of claim 16, wherein the disease or disorder is a receptor-mediated autoimmune disease.
22. The chimeric protein of claim 16, wherein the disease or disorder is rheumatoid arthritis, type 1 diabetes, psoriasis, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, or Sjögren's Syndrome.
23. The chimeric protein of claim 16, wherein the disease or disorder is myasthenia gravis, anti-NMDA receptor encephalitis, or idiopathic membranous nephropathy.
24. The chimeric protein of claim 16, wherein the disease or disorder is anti-NMDA receptor encephalitis, and the target protein or peptide is an NMDA receptor.
25. The chimeric protein of claim 16, wherein the disease or disorder is idiopathic membranous nephropathy, and the target protein or peptide is phospholipase A2 receptor.
26. The chimeric protein of any one of claims 1-23, wherein the target protein or peptide is a soluble extracellular protein.
27. The chimeric protein of any one of claims 1-23, wherein the target protein or peptide is a cell-surface receptor of a cell.
28. The chimeric protein of any one of claims 1-23, wherein the target protein or peptide is an anchored protein or peptide.
29. The chimeric protein of claim 1 or any one of claims 3-28, wherein the receptor binding moiety is a natural ligand of ScaR, a fragment of a natural ligand of ScaR, or a peptide mimicking a natural ligand of ScaR.
30. The chimeric protein of claim 29, wherein the ScaR is a ScaR listed in **Table 1**.
31. The chimeric protein of claim 30, wherein the receptor binding moiety comprises a peptide selected from those listed in **Table 1** or a fragment of a peptide selected from those listed in **Table 1**.
32. The chimeric protein of any one of claims 2-28, wherein the receptor binding moiety is a natural ligand of EnyR, a fragment of a natural ligand of EnyR, or a peptide mimicking a natural ligand of EnyR.
33. The chimeric protein of claim 32, wherein the EnyR is an EnyR listed in **Table 2**.

34. The chimeric protein of claim 33, wherein the receptor binding moiety comprises a peptide selected from those listed in **Table 2** or a fragment of a peptide selected from those listed in **Table 2**.
35. The chimeric protein of any one of claims 1-34, wherein the target protein or peptide is an immune checkpoint molecule, immune cell inhibitory signal molecule, immune cell inhibitory receptor, "don't-eat-me" signal molecule, "don't-eat-me" signal receptor, immune co-stimulatory signal molecule, immune co-stimulatory receptor, immune modulation receptor, or cytokine receptor.
36. The chimeric protein of any one of claims 1-34, wherein the target protein or peptide is an "eat-me" signal receptor, T-cell receptor, B-cell receptor, major histocompatibility complex (MHC) molecule, or CD8/CD4).
37. The chimeric protein of any of one of claims 1-36, wherein one or both of the receptor binding moiety and target binding moiety comprises an scFv.
38. The chimeric protein of any of one of claims 1-37, wherein the receptor binding moiety comprises a natural ligand of the receptor, fragment of a natural ligand of the receptor, or derivative of the natural ligand or natural ligand fragment of the receptor and the target binding moiety comprises an scFv.
39. The chimeric protein of any of one of claims 1-38, wherein one or both of the receptor binding moiety and target binding moiety comprises a Fab fragment.
40. The chimeric protein of any of one of claims 1-39, wherein the receptor binding moiety comprises a natural ligand of the receptor, fragment of a natural ligand of the receptor, or derivative of the natural ligand or natural ligand fragment of the receptor and the target binding moiety comprises a Fab.
41. The chimeric protein of any of one of claims 1-40, wherein the chimeric protein is a chimeric protein as described in **Table 3**.
42. The chimeric protein of any of one of claims 1-40, wherein one or both of the receptor binding moiety and target binding moiety comprises a cyclic protein or peptide.
43. The chimeric protein of any of one of claims 1-40, wherein one or both of the receptor binding moiety and target binding moiety comprises a branched protein or peptide.

44. The chimeric protein of any of one of claims 1-40, wherein one or both of the receptor binding moiety and target binding moiety comprises a designed ankyrin repeat protein (DARPin).
45. The chimeric protein of any of one of claims 1-44, wherein the chimeric protein is capable of binding to a ScaR or EnyR and a target protein expressed by the same cell.
46. The chimeric protein of claim 45, wherein the cell is a cancer cell.
47. The chimeric protein of claim 46, wherein the co-expression of the ScaR or EnyR and a target protein is specific to the cancer cell.
48. The chimeric protein of any of one of claims 1-47, wherein the chimeric protein is capable of binding to ScaR or EnyR and the target protein or peptide to form a complex that is internalized into a cell and degraded by a lysosome.
49. The chimeric protein of any of one of claims 1-48, wherein the receptor binding moiety is capable of binding to HB-EGF, IGF2R, CD63, or SCARA5/SCARA3.
50. The chimeric protein of claim 49, wherein the receptor binding moiety is capable of binding to HB-EGF.
51. The chimeric protein of claim 49, wherein the receptor binding moiety is capable of binding to IGF2R.
52. The chimeric protein of claim 49, wherein the receptor binding moiety is capable of binding to CD63.
53. The chimeric protein of claim 52, wherein the receptor binding moiety is an scFV of an antibody of CD63.
54. The chimeric protein of claim 53, wherein the receptor binding moiety comprises an amino acid sequence of SEQ ID NO:47.
55. The chimeric protein of claim 49, wherein the receptor binding moiety is capable of binding to SCARA5/SCARA3.
56. The chimeric protein of any one of claims 1-55, wherein the target binding moiety is capable of binding to HER2.

57. The chimeric protein of claim 56, wherein the target binding moiety is trastuzumab, a Fab of trastuzumab, or a scFV of trastuzumab.
58. The chimeric protein of any one of claims 1-55, wherein the target binding moiety is capable of binding to EGFR.
59. The chimeric protein of any one of claims 1-10 and 15-57, wherein the target binding moiety is a first target binding moiety, and the chimeric protein comprises a second target binding moiety that is capable of binding to EGFR.
60. The chimeric protein of claim 58 or claim 59, wherein the target binding moiety that is capable of binding to EGFR is a scFV of cetuximab.
61. The chimeric protein of claim 58 or claim 59, wherein the target binding moiety that is capable of binding to EGFR is an anti-EGFR affibody.
62. A method of treating a disease or disorder in a subject comprising administering to the subject the chimeric protein of any one of claims 1-61, wherein the expression of the target protein or peptide is upregulated in the disease or disorder.
63. The method of claim 62, wherein the disease or disorder is cancer.
64. The method of claim 63, wherein the cancer is a solid tumor or hematological cancer.
65. The method of claim 64, wherein the solid tumor is bladder cancer, breast cancer, central nervous system cancer, colorectal cancer, gastrointestinal cancer, endocrine cancer, eye cancer, female genitourinary cancer, head and neck cancer, liver cancer, lung cancer, skin cancer, soft tissue cancer, bone cancer, squamous cell cancer, pancreatic cancer, kidney cancer, or prostate cancer.
66. The method of claim 64, wherein the hematological cancer is leukemia, lymphoma, or multiple myeloma.
67. The method of claim 64, wherein the solid tumor is non-small cell lung cancer, breast cancer, or gastroesophageal cancer.
68. A method of degrading a target protein or peptide, the method comprising contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR), wherein the chimeric protein comprises:

- a. at receptor binding moiety capable of binding to the ScaR or EnyR; and
 - b. a target binding moiety capable of binding to a target protein or peptide,
- wherein the contacting the target protein or peptide with the chimeric protein binds the target protein or peptide to the chimeric protein to generate a target-bound protein or peptide;
- wherein the method further comprises binding a ScaR or an EnyR to the chimeric protein to generate a bound chimeric complex; and
- wherein when the chimeric protein is bound to both a ScaR or EnyR and the target protein or peptide, the ScaR or EnyR internalizes the target protein or peptide and directs it to a lysosome.
69. The method of claim 68, wherein the target protein or peptide is directed to the lysosome at least 50%, at least 60%, at least 70%, or at least 80% of the times that the ScaR or EnyR binds to the target-bound chimeric protein.
70. The method of claim 68 or 69, wherein degradation of the target protein or peptide is initiated within 1 hour of the contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR).
71. The method of any one of claims 68-70, wherein at least 50% of the target protein or peptide is degraded within 4 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR).
72. The method of any one of claims 68-71, wherein at least 80% of the target protein or peptide is degraded within 24 hours of contacting the target protein or peptide with a chimeric protein in the presence of a cell expressing a scavenger receptor (ScaR) or an endocytic receptor (EnyR).
73. The method of any one of claims 68-70, wherein the bound chimeric complex is directed to the lysosome.
74. The method of any claim 70 or 73, wherein the percent of target-bound chimeric protein that is degraded is measured by flow cytometry and/or by Western Blot.
75. The method of claim 68, wherein the cell is in an *in vitro* assay.
76. The method of claim 68, wherein the cell is in a mammalian subject.

77. The method of any one of claims 68, wherein the receptor binding moiety binds to the EnyR or ScaR at a location different from the pocket to which a natural ligand of the EnyR or ScaR binds.
78. The method of any one of claims 68-77, wherein the binding affinity of the receptor binding moiety to the ScaR and/or the EnyR is lower than the binding affinity of the target binding moiety to the target protein or peptide.
79. The method of any one of claims 68-78, wherein the chimeric protein comprises two or more target binding moieties.
80. The method of any one of claims 68-79, wherein the target protein or peptide is a soluble extracellular protein.
81. The method of any one of claims 68-79, wherein the target protein or peptide is a cell-surface receptor of a cell.
82. The chimeric protein of any one of claims 68-79, wherein the target protein or peptide is an anchored protein or peptide.
83. The method of any one of claims 68-82, wherein the ScaR is a ScaR listed in **Table 1**.
84. The method of any one of claims 68-82, wherein the EnyR is an EnyR listed in **Table 2**.
85. The method of claim 83, wherein the receptor binding moiety is a natural ligand of ScaR, a fragment of a natural ligand of ScaR, or a peptide mimicking a natural ligand of ScaR.
86. The method of claim 85, wherein the receptor binding moiety comprises a peptide selected from those listed in **Table 1** or a fragment of a peptide selected from those listed in **Table 1**.
87. The method of any one of claims 84, wherein the receptor binding moiety is a natural ligand of EnyR, a fragment of a natural ligand of EnyR, or a peptide mimicking a natural ligand of EnyR.
88. The method of claim 87, wherein the receptor binding moiety comprises a peptide selected from those listed in **Table 2** or a fragment of a peptide selected from those listed in **Table 2**.

89. The method of any one of claim 68-88, wherein one or both of the receptor binding moiety and target binding moiety comprises an scFv.
90. The method of any one of claim 68-88, wherein the receptor binding moiety comprises a natural ligand of the receptor, fragment of a natural ligand of the receptor, or derivative of the natural ligand or natural ligand fragment of the receptor and the target binding moiety comprises an scFv.
91. The method of any one of claim 68-88, wherein one or both of the receptor binding moiety and target binding moiety comprises a Fab fragment.
92. The method of any one of claim 68-88, wherein the receptor binding moiety comprises a natural ligand of the receptor, fragment of a natural ligand of the receptor, or derivative of the natural ligand or natural ligand fragment of the receptor and the target binding moiety comprises a Fab.
93. The method of any one of claim 68-88, wherein the receptor binding moiety is capable of binding to HB-EGF, IGF2R, CD63, or SCARA5/SCARA3.
94. The method of claim 93, wherein the receptor binding moiety is an scFV of an antibody of CD63.
95. The chimeric protein of any of one of claims 68-94, wherein the chimeric protein is a chimeric protein as described in **Table 3**.
96. The method of claim 53 or 94, wherein the receptor binding moiety comprises an amino acid sequence of SEQ ID NO:47.
97. The method of claim 93, wherein the receptor binding moiety is capable of binding to SCARA5/SCARA3.
98. The method of any one of claim 68-97, wherein the target binding moiety is capable of binding to HER2.
99. The method of any one of claim 68-98, wherein the target binding moiety is trastuzumab, a Fab of trastuzumab, or a scFV of trastuzumab.
100. The method of any one of claim 68-99, wherein the target binding moiety is capable of binding to EGFR.

101. The method of any one of claim 68-99, wherein the target binding moiety is a first target binding moiety, and the chimeric protein comprises a second target binding moiety that is capable of binding to EGFR.
102. The method of claim 100 or claim 101, wherein the target binding moiety that is capable of binding to EGFR is a scFV of cetuximab.
103. The method of claim 100 or claim 101, wherein the target binding moiety that is capable of binding to EGFR is an anti-EGFR affibody.
104. The chimeric protein or method of any preceding claim, wherein the EnyR is not a transferrin receptor.
105. The chimeric protein or method of any preceding claim, wherein at least one of the receptor binding moiety and target binding moiety is not an IgG antibody.
106. The chimeric protein or method of any preceding claim, wherein the chimeric protein is not a bispecific antibody.