

[0008a] In an aspect of the present disclosure, there is provided a targeting conjugate comprising a targeting moiety and an effector molecule, wherein the targeting moiety specifically binds to a target molecule, wherein the effector molecule is conjugated to the targeting moiety via a conjugation site, and wherein the effector molecule can be released from the targeting conjugate via cleavage.

[0008b] In another aspect of the present disclosure, there is provided a targeting conjugate comprising a targeting moiety conjugated to a therapeutic agent having a formula selected from the group consisting of compounds of Formulae (1)-(7).

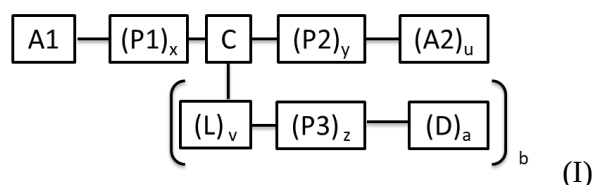
[0008c] In a further aspect of the present disclosure, there is provided a composition comprising a plurality of targeting conjugates disclosed herein.

[0008d] In another aspect of the present disclosure, there is provided a composition disclosed herein, for use in treating a disease in an individual.

[0008e] In a further aspect of the present disclosure, there is provided a composition disclosed herein, for use in diagnosing a disease in an individual, wherein the effector molecule is a detectable label, and wherein detection of the detectable label is indicative of the presence of the disease.

[0008f] In another aspect of the present disclosure, there is provided a method of making a targeting conjugate disclosed herein, wherein the method comprises conjugating the effector molecule to the targeting moiety.

[0009] In some embodimentexamples, there is provided a targeting conjugate comprising a targeting moiety and an effector molecule, wherein the effector molecule is conjugated to the targeting moiety via a conjugation site, wherein the effector molecule can be released from the targeting conjugate via cleavage. In some embodimentexamples, the targeting conjugate comprises the structure of Formula I:



wherein: A1 is a first targeting moiety; A2 is a second targeting moiety; P1 is a first cleavage site; P2 is a second cleavage site; P3 is a third cleavage site; C is the conjugation site; L is a linker; D is the effector molecule; x = 0 or 1; y = 0 or 1; z = 0 or 1; u = 0 or 1; v = 0 or 1; a=1-20; and b=1-20. In some embodimentexamples, z = 0. In some embodimentexamples, z = 1. In some embodimentexamples, y = 0. In some embodimentexamples, y = 1. In some embodimentexamples,

$x = 0$. In some embodimentexamples, $x = 1$. In some embodimentexamples, $u = 0$. In some embodimentexamples, $u = 1$. In some embodimentexamples, a is 1 or more. In some embodimentexamples, b is 1 or more.

[0010] In some embodimentexamples according to any one of the targeting conjugates described above, the targeting conjugate comprises a targeting peptide. In some embodimentexamples, the targeting conjugate comprises an antibody or antigen-binding fragment thereof. In some embodimentexamples, the antibody or antigen-binding fragment thereof is selected from the group consisting of: a human antibody, a humanized antibody, a chimeric antibody, a monospecific antibody, a multispecific antibody, a diabody, a nanobody, an scFv, an scFab, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, and a dsFv. In some embodimentexamples, the targeting moiety comprises an antibody-peptide fusion protein. In some embodimentexamples, the antibody-peptide fusion protein comprises an Fc region. In some embodimentexamples, the peptide is fused to the C-terminus of the Fc region.

[0011] In some embodimentexamples according to any one of the targeting conjugates described above, the targeting conjugate comprises a targeting moiety that specifically binds to a target molecule (*e.g.*, cell surface molecule) at a target site. In some embodimentexamples, the target site is a site of a disease. In some embodimentexamples, the disease is tumor. In some embodimentexamples, the target molecule is selected from the group consisting of: PD1, PD-L1, Trop2, CTLA-4, LAG-3, TIM-3, 4-1BB, CD40, OX40, CD47, SIRP α , HER2, HER3, EGFR, VEGF, VEGFR2, CD19, CD20, CD22, CD30, CD33, CD38, CD79, integrin $\alpha v \beta 3$, $\alpha v \beta 6$, MUC1, PMSA, uPAR, and angiopep-2.

[0012] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the cleavage is triggered by a condition at a target site for the targeting moiety. In some [embodimentexample](#), the condition at a target site is selected from the group consisting of: protease, pH change, redox change, hypoxia, oxidative stress, hyperthermia, and extracellular ATP concentration. In some [embodimentexamples](#), the target site is a site of a disease. In some [embodimentexample](#), the disease is tumor.

[0013] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the cleavage is by a protease. In some [embodimentexamples](#), the protease is selected from the group consisting of: urokinase plasminogen activator (uPA), legumain, plasmin, TMPRSS3, TMPRSS4, TMPRSS6, MMP1, MMP2, MMP-3, MMP-9, MMP-8, MMP- 14, MT1-MMP, CATHEPSIN D, CATHEPSIN K, CATHEPSIN S, ADAM 10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, TACE, human neutrophil elastase, beta-secretase, fibroblast associated protein, matriptase, PSMA and PSA. In some [embodimentexamples](#), the protease is uPA. In some [embodimentexamples](#), the cleavage site comprises an amino acid sequence selected from the amino acid sequences of SEQ ID NOs: 50-55.

[0014] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the effector molecule is selected from the group consisting of a therapeutic agent, an oligonucleotide, and a detectable label. In some [embodimentexamples](#), the effector molecule is a therapeutic agent. In some [embodimentexample](#), the effector molecule is selected from the group consisting of a protein-based drug, a small molecule drug, a cytotoxic agent, a toxin, an immunomodulatory agent, an anti-inflammatory agent, an anti-infective agent, and an epigenetic modulating agent. In some [embodimentexamples](#), the effector molecule is an oligonucleotide. In some [embodimentexamples](#), the oligonucleotide is about 2 to about 100 nucleotides long. In some [embodimentexamples](#), the oligonucleotide is selected from the group consisting of: a double stranded DNA, a single stranded DNA, a double stranded RNA, a single stranded RNA, an antisense RNA, a small interference RNA, a microRNA, a short hairpin RNA (shRNA), and a CpG (Cytosine-phosphodiester-Guanine) oligonucleotide. In some [embodimentexamples](#), the oligonucleotide is a CpG oligonucleotide. In some [embodimentexamples](#), the oligonucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 66-67.

[0015] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the targeting conjugate comprises two or more effector molecules.

[0016] In some [embodimentexample](#) according to any one of the targeting conjugates described above, the effector molecule is a cytotoxic agent selected from the group consisting of an

anthracycline, an auristatin, a camptothecin, a combretastatin, a dolastatin, a duocarmycin, an enediyne, a geldanamycin, an indolino-benzodiazepine dimer, a maytansine, a puromycin, a pyrrolobenzodiazepine dimer, a taxane, a vinca alkaloid, a tubulysin, a hemiasterlin, a spliceostatin, a pladienolide, and stereoisomers, isosteres, analogs, or derivatives thereof. In some [embodimentexamples](#), the effector molecule is SN38 or a derivative thereof. In some [embodimentexamples](#), the effector molecule is MMAE or a derivative thereof. In some [embodimentexamples](#), the effector molecule is selected from compounds of Formulae (1)-(7). In some [embodimentexamples](#), the target conjugate comprises two or more small molecule effector molecules.

[0017] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, wherein the targeting conjugate comprises the structure of Formula I, A1 is a first targeting peptide, or a first antibody or antigen-binding fragment thereof recognizing a first target molecule, and A2 is a second targeting peptide, or a second antibody or antigen-binding fragment thereof recognizing a second target molecule. In some [embodimentexamples](#), the first target molecule and the second target molecule are the same. In some [embodimentexamples](#), the first target molecule and the second target molecule are different. In some [embodimentexamples](#), A1 is connected to the N-terminus of the conjugation site (C), and wherein A2 is connected to the C-terminus of the conjugation site (C).

[0018] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the conjugation site is a covalent conjugation site. In some [embodimentexamples](#), the conjugation site is an endogenous conjugation site. In some [embodimentexamples](#), the conjugation site is an engineered conjugation site introduced into the targeting moiety. In some [embodimentexamples](#), the conjugation site is present in a peptide fused to the targeting moiety. In some [embodimentexamples](#), the conjugation site is a transglutaminase conjugation site, such as a glutamine-containing tag. In some [embodimentexamples](#), the conjugation site comprises a plurality of glutamine-containing tags that are fused to each other in tandem. In some [embodimentexamples](#), the conjugation site comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-12.

[0019] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, the conjugation site is a non-covalent conjugation site. In some [embodimentexamples](#), the conjugation site comprises an oligonucleotide binding polypeptide. In some [embodimentexamples](#), the conjugation site comprises a CpG binding polypeptide. In some [embodimentexamples](#), the conjugation site comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-65.

[0020] In some [embodimentexamples](#) according to any one of the targeting conjugates described above, wherein the targeting conjugate comprises the structure of Formula I, L is represented

by the formula: SH₂-Spacer, MAL-Spacer, NH₂-Spacer, or Osu-Spacer (Osu: oxysuccinimide). In some [embodimentexamples](#), L is represented by the formula: (Gly)_n-(PEG)_m-VC-PAB-(DMAE)_k (Formula II), wherein n≥1, m≥2, and k = 0 or 1. In some [embodimentexamples](#), L is represented by the formula: (Gly)_n-(PEG)_m-Val-Ala-PAB-(DMAE)_k, wherein n≥1, m≥2, and k = 0 or 1. In some [embodimentexamples](#), L is represented by (Gly)_n-(PEG)_m-P-PAB-(DMAE)_k (Formula III), wherein n, m and k are integers, n≥1, m≥2, and k = 0 or 1, P is a cleavage site.

[0021] In some [embodimentexamples](#), there is provided a targeting conjugate comprising a targeting moiety conjugated to a therapeutic agent having a formula selected from the group consisting of compounds of Formulae (1)-(7).

[0022] Also described herein is a composition comprising a plurality of any one of the targeting conjugates described above. In some [embodimentexamples](#), the average ratio of the effector molecule and the targeting moiety in the composition is at least about 1:1. In some [embodimentexamples](#), at least two of the targeting conjugates in the composition comprise different numbers of effector molecules. In some [embodimentexamples](#), wherein the effector molecule is a therapeutic agent or an oligonucleotide, the composition is a pharmaceutical composition. In some [embodimentexamples](#), wherein the effector molecule is a detectable label, the composition is a diagnostic composition.

[0023] In some [embodimentexamples](#), there is provided a method of treating a disease in an individual, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above.

[0024] In some [embodimentexamples](#), there is provided a method of diagnosing a disease in an individual, comprising administering to the individual an effective amount of the diagnostic compositions described above, wherein the detection of the detectable label is indicative of the presence of the disease.

[0025] Also provided herein is a method of making any one of the targeting conjugates describe above, wherein the method comprises conjugating the effector molecule to the targeting moiety.

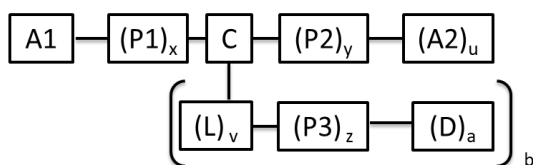
BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIGs. 1A-10C depicts a schematic structure of an exemplary targeting conjugate, comprising A1: a first targeting moiety, such as an antibody or antigen-binding fragment or a targeting peptide (including a multispecific antibody and a fusion protein) with or without protease cleavage sites; A2: a second targeting moiety, such as an antibody or antigen-binding

CLAIMS

What is claimed is:

1. A targeting conjugate comprising a targeting moiety and an effector molecule, wherein the targeting moiety specifically binds to a target molecule, wherein the effector molecule is conjugated to the targeting moiety via a conjugation site, and wherein the effector molecule can be released from the targeting conjugate via cleavage.
2. The targeting conjugate of claim 1, wherein the targeting conjugate comprises the following structure:



wherein:

A1 is a first targeting moiety;

A2 is a second targeting moiety;

P1 is a first cleavage site;

P2 is a second cleavage site;

P3 is a third cleavage site;

C is the conjugation site

L is a linker;

D is the effector molecule;

x = 0 or 1;

y = 0 or 1;

z = 0 or 1;

v = 0 or 1;

u = 0 or 1;

a=1-20; and

b=1-20.

3. The targeting conjugate of claim 1 or 2, wherein the targeting moiety comprises a targeting peptide, or an antibody or antigen-binding fragment thereof.

~~4. The targeting conjugate of claim 3, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of: a human antibody, a humanized antibody, a~~

~~chimeric antibody, a monospecific antibody, a multispecific antibody, a diabody, a nanobody, an scFv, an scFab, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, and a dsFv.~~

~~5. The targeting conjugate of claim 3-4, wherein the targeting moiety comprises an antibody-peptide fusion protein.~~

~~6. The targeting conjugate of any one of claims 1-5, wherein the targeting moiety specifically binds to a target molecule at a target site.~~

~~74. The targeting conjugate of any one of claims 1-63, wherein the cleavage is triggered by a condition at a target site for the targeting moiety.~~

~~58. The targeting conjugate of claim 47, wherein~~

~~i) the condition at the target site is selected from the group consisting of: protease, pH change, redox change, hypoxia, oxidative stress, hyperthermia, and extracellular ATP concentration; and/or~~

~~ii) the target site is a site of a disease.~~

~~9. The targeting conjugate of claim 7 or 8, wherein the target site is a site of a disease.~~

~~10. The targeting conjugate of claim 9, wherein the disease is tumor.~~

~~116. The targeting conjugate of any one of claims 1-510, wherein the cleavage is by a protease.~~

~~7. The targeting conjugate of claim 6, wherein the protease is uPA, and wherein the cleavage site comprises the amino acid sequence of any of SEQ ID NOs: 50-55.~~

~~12. The targeting conjugate of claim 11, wherein the protease is selected from the group consisting of: urokinase plasminogen activator (uPA), legumain, plasmin, TMPRSS3, TMPRSS4, TMPRSS6, MMP1, MMP2, MMP-3, MMP-9, MMP-8, MMP-14, MT1-MMP, CATHEPSIN-D, CATHEPSIN-K, CATHEPSIN-S, ADAM-10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, TACE, human neutrophil elastase, beta-secretase, fibroblast associated protein, matriptase, PSMA and PSA.~~

~~138. The targeting conjugate of any one of claims 1-712, wherein the effector molecule is selected from the group consisting of a therapeutic agent, an oligonucleotide, and a detectable label.~~

~~14. The targeting conjugate of claim 13, wherein the effector molecule is a therapeutic agent.~~

~~15. The targeting conjugate of claim 14, wherein the therapeutic agent is selected from the group consisting of a protein-based drug, a small molecule drug, a cytotoxic agent, a toxin, an immunomodulatory agent, an anti-inflammatory agent, an anti-infective agent, and an epigenetic modulating agent.~~

~~16. The targeting conjugate of claim 13, wherein the effector molecule is an oligonucleotide.~~

~~17. The targeting conjugate of claim 16, wherein the oligonucleotide is about 2 to about 100 nucleotides long.~~

~~18. The targeting conjugate of any one of claims 16 or 17, wherein the oligonucleotide is selected from the group consisting of: a double stranded DNA, a single stranded DNA, a double stranded RNA, a single stranded RNA, an antisense RNA, a small interference RNA, a microRNA, a short hairpin RNA (shRNA), and a CpG oligonucleotide.~~

~~19. The targeting conjugate of claim 18, wherein the oligonucleotide is a CpG oligonucleotide.~~

~~209.~~ The targeting conjugate of any one of claims 1-~~819~~, wherein the targeting conjugate comprises

i) a moiety having the structure of 5' Amino Modifier-Spacer-P_h-CpG ODN, or

ii) a moiety having the structure of 3' Amino Modifier-Spacer-P_h-CpG ODN;

wherein Spacer is (CH₂)_n-(PEG)_m, P is a cleavage site, and wherein h, n and m are integers, h=0 or 1, n≥1, and m≥0.

~~21. The targeting conjugate of any one of claims 1-19, wherein the targeting conjugate comprises a moiety having the structure of 3' Amino Modifier-Spacer-P_h-CpG ODN, wherein Spacer is (CH₂)_n-(PEG)_m, P is a cleavage site, and wherein h, n and m are integers, h=0 or 1, n≥1, and m≥0.~~

~~22. The targeting conjugate of any one of claims 1-21, wherein the targeting conjugate comprises two or more effector molecules.~~

~~23. The targeting conjugate of any one of claims 2-22, wherein z is 0.~~

~~24. The targeting conjugate of any one of claims 2-22, wherein z is 1.~~

- ~~25. The targeting conjugate of any one of claims 2-24, wherein y is 0.~~
- ~~26. The targeting conjugate of any one of claims 2-24, wherein y is 1.~~
- ~~27. The targeting conjugate of any one of claims 2-26, wherein x is 0.~~
- ~~28. The targeting conjugate of any one of claims 2-26, wherein x is 1.~~
- ~~29. The targeting conjugate of any one of claims 2-28, wherein u is 0.~~
- ~~30. The targeting conjugate of any one of claims 2-28, wherein u is 1.~~
- ~~31. The targeting conjugate of any one of claims 2-30, wherein v is 0.~~
- ~~32. The targeting conjugate of any one of claims 2-30, wherein v is 1.~~
- ~~33. The targeting conjugate of any one of claims 2-32, wherein a is 1-20.~~
- ~~34. The targeting conjugate of any one of claims 2-33, wherein b is 1-20.~~
- ~~35. The targeting conjugate of any one of claims 2-34, wherein a is 2-10.~~
- ~~36. The targeting conjugate of any one of claims 2-35, wherein b is 2-10.~~

10. The targeting conjugate of any one of claims 1-9, wherein the target molecule is an immune checkpoint molecule or a tumor antigen.

~~37~~11. The targeting conjugate of any one of claims 2-~~36~~10, wherein A1 is a first targeting peptide, or a first antibody or antigen-binding fragment thereof recognizing a first target molecule, and wherein A2 is a second targeting peptide, or a second antibody or antigen-binding fragment thereof recognizing a second target molecule.

12. The targeting conjugate of claim 11, wherein

- i) the first target molecule is a first immune checkpoint molecule, and the second target molecule is a second immune checkpoint molecule; or
- ii) the first target molecule is a first tumor antigen, and the second target molecule is a second tumor antigen.

13. The targeting conjugate of claim 11, wherein

- i) the first target molecule is an immune checkpoint molecule, and the second target molecule is a tumor antigen; or
- ii) the first target molecule is a tumor antigen, and the second target molecule is an immune checkpoint molecule.

14. The targeting conjugate of claim 13, wherein the immune checkpoint molecule is PD-L1, and/or the tumor antigen is Trop-2.

~~38.~~ The targeting conjugate of claim 37, wherein the first target molecule and the second target molecule are the same.

~~39.~~ The targeting conjugate of claim 37, wherein the first target molecule and the second target molecule are different.

~~40.~~ The targeting conjugate of any one of claims 37-39, wherein A1 is connected to the N-terminus of C, and wherein A2 is connected to the C-terminus of C.

~~41.~~ The targeting conjugate of any one of claims 1-40, wherein the conjugation site is a covalent conjugation site.

4215. The targeting conjugate of ~~claim 41~~any one of claims 1-14, wherein the conjugation site is a transglutaminase conjugation site.

1643. The targeting conjugate of claim 15~~any one of claims 1-42~~, wherein

i) the conjugation site comprises a plurality of glutamine-containing tags that are fused to each other in tandem; and/or

ii) the transglutaminase conjugation site comprises the amino acid sequence of any of SEQ ID NOs: 1-49, and 153-155.

~~44.~~ The targeting conjugate of any one of claims 1-40, wherein the conjugation site is a non-covalent conjugation.

~~45.~~ The targeting conjugate of claim 43, wherein the conjugation site is a binding site for an oligonucleotide binding polypeptide.

~~46.~~ The targeting conjugate of any one of claims 2-45, wherein L is represented by the formula: SH₂-Spacer, MAL-Spacer, NH₂-Spacer, or Osu-Spacer.

4717. The targeting conjugate of any one of claims 2-~~46~~16, wherein L is represented by the formula (Gly)_n-(PEG)_m-P-PAB-(DMAE)_k, (Gly)_n-(PEG)_m-VC-PAB-(DMAE)_k or (Gly)_n-(PEG)_m-Val-Ala-PAB-(DMAE)_k, and wherein n, m and k are integers, n≥1, m≥2, ~~and~~ k is 0 or 1, and P is a cleavage site.

~~48.~~ The targeting conjugate of any one of claims 2-~~46~~, wherein L is represented by the formula: ~~(Gly)_n-(PEG)_m-P-PAB-(DMAE)_k~~, wherein n, m and k are integers, ≥1, m≥2, and k is 0 or 1, ~~P is a cleavage site~~.

18. The targeting conjugate of any one of claims 2-17, wherein

i) u=1, A1 is a full-length anti-PD-L1 antibody, A2 is an anti-Trop-2 scFv, sdAb, or scFab; wherein the optional P1, C, and the optional P2 are fused to the C-terminus of one or both heavy chains of the full-length anti-PD-L1 antibody, and wherein A2 is fused to the C-terminus of the optional P2 at the C-terminus the one or both heavy chains of the full-length anti-PD-L1 antibody; or

ii) u=0, A1 is a full-length anti-PD-L1 antibody, wherein the optional P1, C, and the optional P2 are fused to the C-terminus of one or both heavy chains of the full-length anti-PD-L1 antibody.

4919. The targeting conjugate of claim 814, wherein the therapeutic agent is selected from the group consisting of compounds of Formulae (1)-(7).

20. The targeting conjugate of any one of claims 2-19, wherein:

i) x=1, y=0, z=0, v=1, u=1, a=1-10, and b=2-10; or

ii) x=1, y=0, z=1, v=1, u=1, a=1-10, and b=2-10.

2150. A targeting conjugate comprising a targeting moiety conjugated to a therapeutic agent having a formula selected from the group consisting of compounds of Formulae (1)-(7).

2251. A composition comprising a plurality of targeting conjugates of any one of claims 1-2050.

~~52.—The composition of claim 51, wherein the average ratio of the effector molecule and the targeting moiety in the composition is at least about 1:1.~~

~~53.—The composition of claim 51 or 52, wherein at least two of the targeting conjugates in the composition comprise a different number of effector molecules.~~

~~54.—The composition of any one of claims 51-53, wherein the effector molecule is a therapeutic agent or an oligonucleotide, and wherein the composition is a pharmaceutical composition.~~

5523. A composition of claim 22, for use in ~~method of~~ treating a disease in an individual, comprising ~~administering to the individual an effective amount of the pharmaceutical composition of claim 54.~~

~~56.—The composition of any one of claims 51-53, wherein the effector molecule is a detectable label, and wherein the composition is a diagnostic composition.~~

~~5724.~~ A ~~composition of claim 22, for use in method of~~ diagnosing a disease in an individual, ~~comprising administering to the individual an effective amount of the diagnostic composition of claim 56, wherein the effector molecule is a detectable label, and~~ wherein detection of the detectable label is indicative of the presence of the disease.

~~2558.~~ A method of making a targeting conjugate of any one of claims 1-~~2050~~, wherein the method comprises conjugating the effector molecule to the targeting moiety.