NLPs are nanometer-sized particles that generally consist of amphipathic lipids and apolipoprotein that self-assemble to form a discoidal lipid bilayer, where the hydrophobic periphery of the disc is stabilized by binding to the apolipoprotein. NLPs have been explored for select applications and drug cargo. Nonetheless, no previous studies have evaluated this platform for delivery of fragment antibodies (Fabs).

There thus remains a need in the art for novel NLP delivery platforms, in particular, stable compositions and systems, with good manufacturability, for delivering antigen-binding polypeptides and/or for targeting other therapeutic and diagnostic agents. The present invention meets these and other needs.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure generally relates to nanolipoprotein-polypeptide conjugates and compositions, systems, and methods of making and using the conjugates, where a nanolipoprotein particle is conjugated to polypeptides such as an antigen-binding polypeptide (*e.g.*, a Fab) or a cysteine knot peptide (CKP), to give conjugates, with surprising properties in some ~~embodiment~~examples. Conjugates of the present ~~invention~~disclosure can provide advantages and improvements, such as low toxicity, low immunogenicity, good stability (without cross-linkers), good manufacturability (including feasible production of highly concentrated but low viscosity formulations), minimal to no inter-particle crosslinking, a relatively homogeneous NLP population, and/or enhanced antigen-binding potency.

In one aspect, the disclosure provides a conjugate comprising: a scaffold protein, a membrane-forming lipid, and an antigen-binding polypeptide, wherein said membrane-forming lipids arrange in a lipid bilayer and said scaffold protein encircles said bilayer; and wherein said antigen-binding polypeptide is conjugated to one or more of said membrane-forming lipids on one or both surfaces of said bilayer via a functionalized group on said one or more membrane-forming lipids and a complementary functional group located C-terminally on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide. In one ~~aspect~~example, the ~~invention~~disclosure provides a conjugate comprising a scaffold protein, a membrane-forming lipid, and an antigen-binding polypeptide, where the membrane-forming lipids arrange in a lipid bilayer and the scaffold protein encircles the bilayer; and where the antigen-binding polypeptide is conjugated to one or more of the membrane-forming lipids on one or both surfaces of the bilayer via a functionalized group on one or more membrane-forming lipids and a complementary functional group located C-terminally on the antigen-binding polypeptide. In some ~~embodiment~~examples, the antigen-binding polypeptide is a Fab. In some ~~embodiment~~examples, a spacer connects the functionalized groups to the membrane-forming lipid and/or a spacer connects the complementary functional group to the antigen-binding polypeptide. In some ~~embodiment~~examples, the spacer is GSGS.

In some ~~embodiment~~examples, the functionalized group is selected from the group consisting of a maleimide derivative, a haloacetamide, a pyridyldithio-propionate, a thiosulfate, and a combination of any one or more thereof; and, in some ~~embodiment~~examples, the complementary functional group is a free thiol group, such as a cysteine thiol group, optionally where the cysteine forms a hinge disulfide bond in an antibody from which the antigen-binding polypeptide is derived (*e.g.*, Cys-226 or Cys-227 according to Kabat numbering). In some ~~embodiment~~examples, the scaffold protein is selected from the group consisting of (a) apolipoprotein A, (b) apolipoprotein B, (c) apolipoprotein C, (d) apolipoprotein D, (e) apolipoprotein H, (f) apolipoprotein E, (g) a truncated version of (a)-(f) capable of stabilizing said bilayer, and (h) a combination of any one or more of (a)-(g); and/or the membrane-forming lipid is selected from the group consisting of C_{4-28} fatty-acyl (*e.g.*, a C_{16} fatty-acyl), DMPC, DOPC, DOPS, DOPE, DPPC, and a combination of any one or more thereof. In some ~~embodiment~~examples, one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and the fatty-acyl is conjugated to a short peptide of 20-60 amino acids.

In ~~some embodiments~~another aspect, provided is a conjugate comprising: a scaffold protein, a membrane-forming lipid, and a short peptide of 20-60 amino acids, wherein said membrane-forming lipids arrange in a lipid bilayer and said scaffold protein encircles said bilayer; and wherein said short peptide is conjugated to one or more of said membrane-forming lipids on one or both surfaces of said bilayer. In some ~~embodiment~~examples, the membrane-forming lipids are selected from the group consisting of C_{4-28} fatty-acyl (*e.g.*, a C_{16} fatty-acyl), DMPC, DOPC, DOPS, DOPE, DPPC, and a combination of any one or more thereof. In some ~~embodiment~~examples, the membrane-forming lipid is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to the short peptide of 20-60 amino acids.

In some ~~embodiment~~examples, the fatty-acyl is a C_{16} fatty-acyl. In some ~~embodiment~~examples, the short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, tthe CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some

~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II.

In some ~~embodiment~~examples, the activity of the short peptide in the conjugate (*e.g.*, in an NLP conjugate that comprises a short peptide and an antigen-binding polypeptide) is higher than the activity of the peptide in a conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is between 2-fold and 10-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is about 5-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the short peptide is a CKP or CKP variant having biological activity, and 80-90%, 90-95%, or 95-99% of said activity is retained in said conjugate. In some ~~embodiment~~examples, said conjugate increases an activity or avidity of said short peptide. In some ~~embodiment~~examples, the conjugate (*e.g.*, the peptide-NLP conjugate) further comprises an antigen-binding polypeptide conjugated to one or more of said membrane-forming lipids on one or both surfaces of said bilayer via a functionalized group on said one or more membrane-forming lipids and a complementary functional group located C-terminally on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide In some ~~embodiment~~examples, the conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab). In some ~~embodiment~~examples, the conjugate comprises at least two different peptide-C_{4-28} fatty-acyl conjugates, wherein a first peptide-C_{4-28} fatty-acyl conjugate comprises a first short peptide, and wherein a second peptide-C_{4-28} fatty-acyl comprises a second short peptide that is different from the first short peptide. In some ~~embodiment~~examples, the at least two peptides (*e.g.*, cystine-knot peptides) bind different targets, optionally two, three, four, or eight different targets. In some ~~embodiment~~examples, the two or more short peptides bind two different targets. In some ~~embodiment~~examples, the targets are a pair selected from the group

consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa.

In some ~~embodiment~~examples, the scaffold protein and membrane-forming lipids are in a molar ratio of from 1:60 to 1:100, such as 1:80. In some ~~embodiment~~examples, 20-35% of membrane-forming lipids carry the functionalized groups, such as 20%. The antigen-binding polypeptide may be selected from the group consisting of a Fab, a Fab', a Fab'-SH, a F(ab')2, a single chain Fab (scFab), a single chain Fv (scFv), a VH-VH dimer, a VL-VL dimer, a VH-VL dimer, a single domain, a diabody, a linear antibody, and a combination of any one or more thereof. In particular ~~embodiment~~examples, the antigen-binding polypeptide is a Fab or a Fab-like molecule, such as a human or humanized Fab. The antigen bound by the antigen-binding polypeptide (*e.g.*, a Fab) may be OX40, DR4, GITR, Tie2, factor D, VEGF, MerTK, CD3, and/or Lymphotoxin beta receptor.

The conjugates can provide formats for multivalent and/or multi-specific constructs. For example, a conjugate may comprise two or more antigen-binding polypeptide molecules (*e.g.*, two or more Fab molecules), such as 2-60, 2-32, 10-30, or 20 molecules. In some ~~embodiment~~examples, the spacer comprises PEG or the amino acid sequence of GSGS. In some ~~embodiment~~examples, the conjugate has 1-160, 10-120, 20-100, 40-80, or 60 molecules of said antigen-binding polypeptide; wherein said antigen-binding polypeptide is a Fab and said Fab is connected by a PEG spacer to said membrane-forming lipid. In some ~~embodiment~~examples, the PEG spacer connects said functionalized group to said membrane-forming lipid and has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000.

Such formats can increase activity and/or avidity of the antigen-binding polypeptide, its serum stability and/or its serum half-life (compared to the antigen-binding polypeptide not conjugated to the nanolipoprotein particle (NLP)). In some ~~embodiment~~examples, the serum half-life of the antigen-binding polypeptide is increased by 2-20 fold, 5-15, or 10-fold. In some ~~embodiment~~examples, said conjugate has 5-60, 6-40, or 7-32 molecules of said antigen-binding polypeptide.

In some ~~embodiment~~examples, the conjugate comprises two or more antigen-binding polypeptides (*e.g.* two or more Fabs) binding different targets or epitopes, such as two, three, four, or eight different targets or epitopes. Such formats can provide bispecific constructs, *e.g.*, where the targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor

IXa. In some ~~embodiment~~examples, the antigen-binding polypeptide is a Fab or a Fab-like molecule, optionally a human or humanized Fab.

Conjugates of the ~~invention~~disclosure may be provided in pharmaceutical compositions comprising the conjugate and a pharmaceutically acceptable vehicle. In one aspect, the disclosure provides a pharmaceutical composition comprising the conjugate as disclosed herein and a pharmaceutically acceptable vehicle.

In one aspect, the disclosure provides a liquid formulation comprising 100-300 mg of the conjugate as disclosed herein and having a viscosity of 10-50 cP, optionally wherein said formulation is for administration subcutaneously or via ocular delivery. Another ~~aspect~~ example of the ~~invention~~disclosure provides high concentration, low viscosity liquid formulations, such as a liquid formulation comprising 100-300 mg of conjugate and having a viscosity of 10-50 cP. In some ~~embodiment~~examples, the pharmaceutical composition, or liquid formulation, is administered subcutaneously or via ocular delivery. In some ~~embodiment~~examples, the conjugate, composition, or formulation is lyophilized, such as in the presence of trehalose; and optionally reconstituted. In particular ~~embodiment~~examples, lyophilization occurs in the presence of 80 mM trehalose; and/or at a concentration of 5 mg conjugate/mL. In some ~~embodiment~~examples, the conjugate comprises 18-20 Fab molecules per conjugate. Advantageously, in some ~~embodiment~~examples, 80-90%, 90-95%, or 95-99% of antigen-binding activity of the antigen-binding polypeptide (*e.g.*, the Fab) is retained following lyophilization and reconstitution.

In one aspect, the disclosure provides a method of preparing a conjugate of a nanolipoprotein particle and an antigen-binding polypeptide, comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide. Another ~~aspect~~ example of the ~~invention~~disclosure provides methods of preparing a conjugate of the ~~invention~~disclosure. In some ~~embodiment~~examples, the method comprises a) providing a scaffold protein and a membrane-forming lipid under conditions allowing assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, where one or more membrane-forming lipids present a functionalized group on one or both surfaces of the particle; b) allowing the particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to the functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of the particle and the antigen-binding polypeptide. In some ~~embodiment~~examples, step b) follows step a) without an intervening step to remove some or all unassembled membrane-forming lipids. In particular ~~embodiment~~examples, the functionalized group is a maleimide derivative and the functional group is a cysteine thiol group, such as where the cysteine forms a hinge disulfide bond in an antibody from which the antigen-binding polypeptide is derived (*e.g.*, Cys-226 or Cys-227 according to Kabat numbering).

In some ~~embodiment~~examples, the functionalized group does not conjugate to the scaffold protein at the low pH, *e.g.*, at pHs of 5.5 to 6.5, 5-6, or a pH of 6.

In some ~~embodiment~~examples of the methods, a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide. In some ~~embodiment~~examples, the spacer is a PEG spacer that connects said functionalized group to said membrane-forming lipid. In some ~~embodiment~~examples, the PEG spacer has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000.

In some ~~embodiment~~examples, one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to a short peptide of 20-60 amino acids. In some ~~embodiment~~examples, the fatty-acyl is a C_{16} fatty-acyl. In some ~~embodiment~~examples, the short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II. In some ~~embodiment~~examples, the membrane-forming lipids comprise a peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide. In some ~~embodiment~~examples, the activity of the short peptide in the conjugate is higher than the activity of the peptide in a conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is between 2-fold and 10-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is about 5-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the short peptide is a CKP or CKP variant having biological activity, and 80-90%, 90-95%, or 95-99% of said activity is retained in said conjugate. . In some ~~embodiment~~examples, the conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen

binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab). In some ~~embodiment~~examples, the conjugate comprises at least two different peptide-C_{4-28} fatty-acyl conjugates, wherein a first peptide-C_{4-28} fatty-acyl conjugate comprises a first short peptide, and wherein a second peptide-C_{4-28} fatty-acyl comprises a second short peptide that is different from the first short peptide. In some ~~embodiment~~examples, the at least two peptides (*e.g.*, cystine-knot peptides) bind different targets, optionally two, three, four, or eight different targets. In some ~~embodiment~~examples, the two or more short peptides bind two different targets. In some ~~embodiment~~examples, the targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa.

In ~~some embodiments~~one aspect, provided is a method of preparing a conjugate of a nanolipoprotein particle and a short peptide of 20-60 amino acids (*e.g.*, a cystine-knot peptide), comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein; wherein one or more of said membrane-forming lipids comprises a C_{4-28} fatty-acyl (*e.g.*, a C_{16} fatty-acyl) and fatty-acyl is conjugated to said short peptide; and optionally purifying the peptide conjugate. In some ~~embodiment~~examples, the short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II. In some ~~embodiment~~examples, said membrane-forming lipids comprise a peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide.

In some ~~embodiment~~examples, the conjugate comprises at least two different peptide-C_{4-28} fatty-acyl conjugates, wherein a first peptide-C_{4-28} fatty-acyl conjugate comprises a first short peptide, and wherein a second peptide-C_{4-28} fatty-acyl comprises a second short peptide that is different from the first short peptide. In some ~~embodiment~~examples, theleast two peptides (*e.g.*, cystine-knot peptides) bind different targets, optionally two, three, four, or eight different targets. In some ~~embodiment~~examples, the two or more short peptides bind two different targets. In some ~~embodiment~~examples, said targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa. In some ~~embodiment~~examples, short peptide is a CKP or CKP variant having biological activity, and 80-90%, 90-95%, or 95-99% of said activity is retained in said conjugate. In some ~~embodiment~~examples, one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; and further comprising the step of: b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; wherein said step b) follows said step a) without an intervening step to remove some or all unassembled membrane-forming lipids; and/or without an intervening step to enrich the peptide conjugate of step a). In some ~~embodiment~~examples, said functionalized group is a maleimide derivative and said functional group is a cysteine thiol group, optionally wherein said cysteine amino acid residue forms a hinge disulfide bond in the antibody from which the antigen-binding polypeptide is derived (such as Cys-226 or Cys-227). In some ~~embodiment~~examples, said functionalized group does not conjugate to said scaffold protein at said low pH. In some ~~embodiment~~examples, said low pH is a pH of 5.5 to 6.5, a pH of 5-6, or a pH of 6. In some ~~embodiment~~examples, a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide. In some ~~embodiment~~examples, said spacer is a PEG spacer that connects said functionalized group to said membrane-forming lipid. In some ~~embodiment~~examples, said PEG spacer has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000. In some ~~embodiment~~examples, said conjugate increases an activity or avidity of said short peptide. In some ~~embodiment~~examples, said conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab).In some ~~embodiment~~examples, the activity of

the short peptide in the conjugate is higher than the activity of the peptide in a conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is between 2-fold and 10-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is about 5-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide.

Also provided is a conjugate produce by any of the methods herein. In one aspect, the disclosure provides a conjugate produced by the method disclosed herein.

In one aspect, the disclosure provides a method of reducing lipid-scaffold protein conjugation in preparing a conjugate of a nanolipoprotein particle and an antigen-binding polypeptide, comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group at a low pH that favors conjugation of said functionalized group to said functional group rather than to said scaffold protein; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide. Accordingly, another ~~aspect~~ example of the ~~invention~~disclosure provides a method of reducing lipid-scaffold protein conjugation in preparing a conjugate of a nanolipoprotein particle and an antigen-binding polypeptide. The method may comprise a) providing a scaffold protein and a membrane-forming lipid under conditions allowing assembly of a nanolipoprotein particle comprising a lipid bilayer of membrane-forming lipids encircled by the scaffold protein, where one or more membrane-forming lipids presents a functionalized group on one or both surfaces of the particle; b) allowing the particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group at a low pH that favors conjugation of the functionalized group to the functional group rather than to the scaffold protein (*e.g.*, a pH of about 5.55 to about 6.5, about 5 to about 6, or about 6). In some ~~embodiment~~examples, step b) follows step a) without an intervening step to remove some or all unassembled membrane-forming lipids. In particular ~~embodiment~~examples, the functionalized group is a maleimide derivative and the functional group is a cysteine thiol group, such as where the cysteine forms a hinge disulfide bond in an antibody from which the antigen-binding polypeptide is derived (*e.g.*, Cys-226 or Cys-227 according to Kabat numbering). In some ~~embodiment~~examples, said membrane-forming lipids is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to a short peptide of 20-60 amino acids. In some ~~embodiment~~examples, said fatty-acyl is a C_{16} fatty-acyl. In some ~~embodiment~~examples, said short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, said membrane-forming lipids comprise a

peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide.

In one aspect, the disclosure provides a method of increasing avidity, activity, and/or potency of an antigen-binding polypeptide, said method comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide, thereby increasing avidity, activity, and/or potency of said antigen-binding polypeptide. Another ~~aspect~~ example of the ~~invention~~disclosure provides methods of increasing avidity, activity, and/or potency of an antigen-binding polypeptide (*e.g.*, a Fab) (compared to the polypeptide's avidity, activity, and/or potency when not conjugated to the NLP). In some ~~embodiment~~examples, the method comprises a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide, thereby increasing avidity, activity, and/or potency of the antigen-binding polypeptide (compared to the polypeptide's avidity, activity, and/or potency when not conjugated to the NLP). In particular ~~embodiment~~examples, the antigen-binding polypeptide is a Fab or a Fab-like molecule; in particular ~~embodiment~~examples, the conjugate comprises at least 6 molecules, *e.g.*, 10-60, 15-30, or 20 molecules, of antigen-binding polypeptide. In some ~~embodiment~~examples, said antigen-binding polypeptide is a Fab or a Fab-like molecule connected by a PEG spacer to said membrane-forming lipid; and said conjugate comprises 1-160, 10-120, 20-100, 40-80, or 60 molecules of said antigen-binding polypeptides. In some ~~embodiment~~examples, the PEG spacer connects said functionalized group to said membrane-forming lipid and has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000. In some ~~embodiment~~examples, said conjugate is stabilized. In some ~~embodiment~~examples, the conjugate is stabilized, such as conjugates comprising 7-60, 7-32, 40-60, or 50 molecules of said antigen-binding polypeptides, compared to conjugates comprising fewer or no antigen-binding polypeptides (*e.g.*, fewer than 7 Fabs). In some ~~embodiment~~examples, the conjugate further comprises one or more molecules of a second antigen-binding polypeptide that binds a different target; and/or the conjugate is provided in a liquid formulation comprising 100-300 mg of said conjugate and having a viscosity of 10-50 cP.

In some ~~embodiment~~examples, one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to a short peptide of 20-60 amino acids, and the method increases the avidity, activity, and/or potency of the short peptide. In some ~~embodiment~~examples, said fatty-acyl

is a C_{16} fatty-acyl. In some ~~embodiment~~examples, said short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II. In some ~~embodiment~~examples, said membrane-forming lipids comprise a peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide. In some ~~embodiment~~examples, the conjugate comprises at least two different peptide-C_{4-28} fatty-acyl conjugates, wherein a first peptide-C_{4-28} fatty-acyl conjugate comprises a first short peptide, and wherein a second peptide-C_{4-28} fatty-acyl comprises a second short peptide that is different from the first short peptide. In some ~~embodiment~~examples, said at least two peptides (*e.g.*, cystine-knot peptides) bind different targets, optionally two, three, four, or eight different targets. In some ~~embodiment~~examples, said two or more short peptides bind two different targets. In some ~~embodiment~~examples, said targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa. In some ~~embodiment~~examples, said conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab). In some ~~embodiment~~examples, the activity of the short peptide in the conjugate is higher than the activity of the peptide in a conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is between 2-fold and 10-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples,

the activity of the peptide in the conjugate is about 5-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide.

Another ~~aspect~~ example of the ~~invention~~disclosure provides methods of increasing stability, shelf-life, and/or half-life of an antigen-binding polypeptide (*e.g.*, a Fab) (compared to the polypeptide's avidity, activity, and/or potency when not conjugated to the NLP). In one aspect, the disclosure provides a method of increasing stability, shelf-life, and/or half-life of an antigen-binding polypeptide, said method comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide, thereby increasing stability, shelf-life, and/or half-life of said antigen-binding polypeptide. Also provided are methods of increasing stability, shelf-life, and/or half-life of an NLP (compared to the NLP's increasing stability, shelf-life, and/or half-life when not conjugated to the antigen-binding polypeptide, *e.g.*, a Fab or Fab-like molecule). In one aspect, the disclosure provides a method of increasing stability, shelf-life, and/or half-life of a nanolipoprotein particle, said method comprising a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide, thereby increasing stability, shelf-life, and/or half-life of said nanolipoprotein particle. In some ~~embodiment~~examples, the method comprises a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein, wherein one or more of said membrane-forming lipids presents a functionalized group on one or both surfaces of said particle; b) allowing said particle to contact an antigen –binding- polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; and c) optionally purifying the conjugate of said particle and said antigen-binding polypeptide, thereby increasing stability, shelf-life, and/or half-life of the antigen-binding polypeptide (compared to the polypeptide's stability, shelf-life, and/or half-life when not conjugated to the NLP or conjugated to an NLP having fewer antigen-binding polypeptides,(*e.g.*, fewer than 7 Fabs). In some ~~embodiment~~examples, the conjugate comprises 5-60 molecules of said antigen-binding polypeptide. In particular ~~embodiment~~examples, the antigen-binding polypeptide is a Fab or a Fab-like molecule; in particular ~~embodiment~~examples, the conjugate comprises 7-32 molecules of antigen-binding polypeptide. In some ~~embodiment~~examples, one or more of the membrane-forming lipids is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to a short peptide of 20-60 amino acids. In some ~~embodiment~~examples, said fatty-acyl is a C_{16} fatty-acyl.

In one aspect, the disclosure provides a method of increasing the stability, shelf-life, and/or half-life of a short peptide that is 20-60 amino acids in length, said method comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein; wherein one or more of said membrane-forming lipids comprises a C_{4-28} fatty-acyl (*e.g.*, a C_{16} fatty-acyl) and fatty-acyl is conjugated to said short peptide; and optionally purifying the peptide conjugate; b) modifying one or more of said membrane-forming lipids to a present functionalized group on one or both surfaces of said particle, and c) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; wherein said step c) follows said step b) without an intervening step to remove some or all unassembled membrane-forming lipids; and/or without an intervening step to enrich the peptide conjugate of step b) optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby increasing stability, shelf-life, and/or half-life of said short peptide. Also provided are methods of increasing the stability, shelf-life, and/or half-life of a short peptide that is 20-60 amino acids in length, said method comprising: a) providing a scaffold protein and a membrane-forming lipid under conditions allowing (self-)assembly of a nanolipoprotein particle comprising a lipid bilayer of said membrane-forming lipids encircled by said scaffold protein; one or more of said membrane-forming lipids comprises a C_{4-28} fatty-acyl (*e.g.*, a C_{16} fatty-acyl) and fatty-acyl is conjugated to said short peptide; and optionally purifying the peptide conjugate; b) modifying one or more of said membrane-forming lipids to

a present functionalized group on one or both surfaces of said particle, and c) allowing said particle to contact an antigen-binding polypeptide (*e.g.*, a Fab) having a C-terminally located complementary functional group that conjugates to said functionalized group at low pH in the range of 4.5 to 6.5 pH; wherein said step c) follows said step b) without an intervening step to remove some or all unassembled membrane-forming lipids; and/or without an intervening step to enrich the peptide conjugate of step b). In some ~~embodiment~~examples, a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby increasing stability, shelf-life, and/or half-life of said short peptide. In some ~~embodiment~~examples, the fatty-acyl is a C_{16} fatty-acyl.

In some ~~embodiment~~examples, said short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II.

In some ~~embodiment~~examples, said membrane-forming lipids comprise a peptide-$C_{4\text{-}28}$ fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide.

In some ~~embodiment~~examples, said antigen-binding polypeptide is a Fab or a Fab-like molecule and/or said conjugate comprises 5-60, 6-40, 7-32, 40-60, or 50 molecules of said antigen-binding polypeptides (*e.g.*, said Fab). In some ~~embodiment~~examples, conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen-binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said

conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab).

In some ~~embodiment~~examples of the methods for increasing avidity, activity, potency, stability, shelf-life, and/or half-life, the conjugate further comprises a biologically active agent or a detectable agent. In particular ~~embodiment~~examples, the biologically active agent is a cytotoxic agent selected from the group consisting of daunomycin, doxorubicin, methotrexate, vindesine, a radionuclide, diphtheria toxin, ricin, geldanamycin, a maytansinoid, calicheamicin, or a combination of any one or more thereof. In some ~~embodiment~~examples, the detectable agent is a label selected from the group consisting a radioactive isotope, a fluorophore, a chemiluminescent dye, a chromophore, an enzyme, a metal ion, a nanoparticle, and a combination of any one or more thereof. In some ~~embodiment~~examples, the conjugate is provided in a liquid formulation, said formulation comprising 100-300 mg of said conjugate and having a viscosity of 10-50 cP.

In one aspect, the disclosure provides the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, for use as a medicament. In another aspect, the disclosure provides use of the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, in the manufacture of a medicament for treatment of cancer or ocular disorders. In yet another aspect, the disclosure provides use of the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, in the manufacture of a medicament for inhibiting angiogenesis and/or tumor growth. Other ~~aspects~~ examples of the ~~invention~~disclosure provide a conjugate, pharmaceutical composition, or formulation as provided herein, for use as a medicament; for use in treating cancer or ocular disorders; in the manufacture of a medicament for treatment of cancer or ocular disorders; and/or in the manufacture of a medicament for inhibiting angiogenesis and/or tumor growth.

In one aspect, the disclosure provides the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, for use in treating an individual with an antigen-binding polypeptide (*e.g.*, a Fab), optionally wherein said conjugate, pharmaceutical composition or formulation is for administration with an additional therapeutic agent. Another ~~aspect~~ example of the ~~invention~~disclosure provides a method of treating an individual with an antigen-binding polypeptide (*e.g.*, a Fab) and/or a short peptide, comprising administering to the individual an effective amount of a conjugate, pharmaceutical composition, or formulation provided herein. In some ~~embodiment~~examples, the method further comprises administering an additional therapeutic agent to the individual.

In one aspect, the disclosure provides the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, for use in delivering an antigen-binding polypeptide (*e.g.*, a Fab) to an individual in need thereof, wherein said conjugate, composition, or formulation is to be administered to said individual, thereby delivering said antigen-binding polypeptide to said individual. In another aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, said Fab) and a nanolipoprotein particle, for use in delivering an antigen-binding polypeptide (*e.g.*, a Fab) to an individual in need thereof, in a potent, stable liquid formulation, wherein said liquid formulation is to be administered to said individual; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein said membrane-forming lipids are conjugated to 5-100 molecules of said antigen-binding polypeptide via a functionalized group on said membrane-forming lipid and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby delivering said antigen-binding polypeptide to said individual in a stable liquid formulation. In another aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, said Fab) and a nanolipoprotein particle, for use in delivering an antigen-binding polypeptide (*e.g.*, a Fab) to an individual in need thereof, in a potent, low viscosity formulation, wherein said liquid formulation is to be administered to said individual; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is conjugated to said antigen-binding polypeptide via a functionalized group on said one or more membrane-forming lipids and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide; and wherein said liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg of said conjugate/mL, thereby delivering said antigen-binding polypeptide to said individual in a low viscosity formulation. Still another ~~aspect~~ example provides a method of delivering an antigen-binding polypeptide (*e.g.*, a Fab) to an individual in need thereof, comprising providing a conjugate, pharmaceutical composition, or formulation of the ~~invention~~disclosure, for administration to the individual. Advantageously, the antigen-binding polypeptide (*e.g.*, a Fab) can be delivered in a potent, stable liquid formulation, such as where a conjugate comprises membrane-forming lipids conjugated to 5-60 molecules of antigen-binding polypeptide (*e.g.*, 5-60 Fab molecules) via functionalized groups on the membrane-forming lipids and C-terminally located complementary functional groups on the antigen-binding polypeptide. In some ~~embodiment~~examples, a spacer connects the functionalized group to the membrane-forming lipid and/or a spacer

connects the complementary functional group to the antigen-binding polypeptide. Advantageously, the antigen-binding polypeptide (*e.g.*, a Fab) can be delivered in a potent, low viscosity formulation, such as in a liquid formulation having a viscosity of 10-50 cP and a concentration of 100-300 mg of conjugate/mL.

In some ~~embodiment~~examples of the methods for delivering antigen-binding polypeptides, the antigen-binding polypeptide is a Fab that binds a target selected from the group consisting of factor D, VEGF, Tie2, DR4, and a combination of any one or more thereof; and the formulation is administered via ocular delivery. In some ~~embodiment~~examples of the methods for delivering antigen-binding polypeptides, the antigen-binding polypeptide is a Fab that binds a target selected from the group consisting of OX40, DR4, GITR, Tie2, factor D, VEGF, MerTK, CD3, Lymphotoxin beta receptor, and a combination of any one or more thereof. In particular ~~embodiment~~examples, the antigen-binding polypeptides (*e.g.*, the Fabs) bind at least two different targets, *e.g.*, where the two different targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa.

In one aspect, the disclsoure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, said Fab) and a nanolipoprotein particle, for use in delivering a short peptide 20-60 amino acids in length to an individual in need thereof, in a potent, stable liquid formulation, wherein said liquid formulation is to be administered to said individual; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and wherein said fatty-acyl is conjugated to a short peptide of 20-60 amino acids; wherein said membrane-forming lipids are conjugated to 5-100 molecules of said antigen-binding polypeptide via a functionalized group on said membrane-forming lipid and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby delivering said short peptide to said individual in a stable liquid formulation. Also provided herein is a method of delivering a short peptide 20-60 amino acids in length to an individual in need thereof, in a potent, stable liquid formulation, said method comprising:

administering to an individual a liquid formulation comprising a conjugate of said antigen-binding polypeptide (*e.g.*, said Fab), and a nanolipoprotein particle; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and wherein said fatty-acyl is conjugated to a short peptide of 20-60 amino acids; wherein said membrane-forming lipids are conjugated to 5-100 molecules of said antigen-binding polypeptide via a functionalized group on said membrane-forming lipid and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby delivering said antigen-binding polypeptide to an individual in a stable liquid formulation.

In one aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, said Fab) and a nanolipoprotein particle, for use in delivering a short peptide 20-60 amino acids in length to an individual in need thereof, in a potent, low viscosity formulation, wherein said liquid formulation is to be administered to said individual; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and wherein said fatty-acyl is conjugated to a short peptide of 20-60 amino acids; wherein one or more of said membrane-forming lipids is conjugated to said antigen-binding polypeptide via a functionalized group on said one or more membrane-forming lipids and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide; and wherein said liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg of said conjugate/mL, thereby delivering said short peptide to said individual in a low viscosity formulation. Also provided is a method of delivering a short peptide 20-60 amino acids in length to an individual in need thereof, in a potent, low viscosity formulation, said method comprising:

administering to an individual a liquid formulation comprising a conjugate of said antigen-binding polypeptide (*e.g.*, said Fab) and a nanolipoprotein particle; wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and wherein said fatty-acyl is conjugated to a short peptide of 20-60 amino acids; wherein one or more of said membrane-forming lipids is conjugated to said antigen-binding polypeptide via a functionalized group on said one or more membrane-forming lipids and a C-terminally located complementary functional group on said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide; and wherein said liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg of said conjugate/mL, thereby delivering said antigen-binding polypeptide to an individual in a low viscosity formulation.

In some ~~embodiment~~examples of the methods of delivering, said fatty-acyl is a C_{16} fatty-acyl. In some ~~embodiment~~examples, said short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II. In some ~~embodiment~~examples, the short peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II. In some ~~embodiment~~examples, said membrane-forming lipids comprise a peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide. In some ~~embodiment~~examples, the activity of the short peptide in the conjugate is higher than the activity of the peptide in a conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is between 2-fold and 10-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, the activity of the peptide in the conjugate is about

5-fold higher than the activity of the peptide in the conjugate that does not comprise the antigen-binding polypeptide. In some ~~embodiment~~examples, said conjugate comprise 1-100 molecules of said Fab; optionally said conjugate comprises 5-30, 10-25, 15-20, or 18 molecules of said peptide, and 5-40, 10-35, 15-30, 20-25, or 23 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 3-30, 5-20, 10-15, or 13 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab); or optionally said conjugate comprise 20-80, 25-70, 30-60, 35-50, or 40 molecules of said peptide and 10-60 molecules of said antigen binding polypeptide (*e.g.*, Fab). In some ~~embodiment~~examples, said spacer is a PEG spacer that connects said functionalized group to said membrane-forming lipid. In some ~~embodiment~~examples, the PEG spacer has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000. In some ~~embodiment~~examples, said antigen-binding polypeptide (*e.g.*, a Fab) and said short peptide each bind the same target. In some ~~embodiment~~examples, said antigen-binding polypeptide (*e.g.*, a Fab) and said cystine-knot peptide each bind a different target. In some ~~embodiment~~examples, said antigen-binding polypeptide is a Fab that binds a target selected from the group consisting of factor D, VEGF, Tie2, DR4; and a combination of any one or more thereof; and said formulation is administered via ocular delivery. In some ~~embodiment~~examples, said short peptide binds a target selected from the group consisting of factor D, VEGF, Tie2, DR4, and a combination of any one or more thereof. In some ~~embodiment~~examples, said antigen-binding polypeptide is a Fab that binds a target selected from the group consisting of OX40, DR4, GITR, Tie2, factor D, VEGF, MerTK, CD3, Lymphotoxin beta receptor, and a combination of any one or more thereof. In some ~~embodiment~~examples, said short peptide binds a target selected from the group consisting of OX40, DR4, GITR, Tie2, factor D, VEGF, MerTK, CD3, Lymphotoxin beta receptor, and a combination of any one or more thereof. In some ~~embodiment~~examples, said antigen-binding polypeptides bind at least two different targets. In some ~~embodiment~~examples, said two different targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa. In some ~~embodiment~~examples, said short peptide and said antigen-binding polypeptide bind at least two different targets. In some ~~embodiment~~examples, said two different targets are a pair selected from the group consisting of CD3 and CD19; CD3 and EpCAM; CD3 and CEA; CD16 and CD30; CD16 and CD33; Ang-2 and VEGF-A; and Factor X and Factor IXa.

In one aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, a Fab), a nanolipoprotein particle, and a biologically active agent or a detectable agent, for use in delivering a biologically active agent or a detectable agent to an individual in need thereof, in a stable liquid formulation, wherein said liquid formulation is to be administered to said individual, wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein said membrane-forming lipids are conjugated to 5-100 molecules of said antigen-binding polypeptide via a functionalized group on said membrane-forming lipid and a C-terminally located complementary functional group on said antigen-binding polypeptide; wherein said biologically active agent or said detectable agent is conjugated to at least one of said scaffold protein, said membrane-forming lipid, or said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide, thereby delivering said biologically active agent or said detectable agent to said individual in need thereof in a stable liquid formulation. Yet still another ~~aspect~~ example provides a method of delivering a biologically active agent or a detectable agent to an individual in need thereof, in a stable liquid formulation. the method comprising administering to an individual a liquid formulation comprising a conjugate of the

~~invention~~disclosure and a biologically active agent or a detectable agent, where the conjugate comprises membrane-forming lipids conjugated to 5-60 molecules of antigen-binding polypeptide (*e.g.*, 5-60 Fab molecules) via functionalized groups on the membrane-forming lipids and C-terminally located complementary functional groups on the antigen-binding polypeptide; and where the biologically active agent or detectable agent is conjugated to the scaffold protein, membrane-forming lipid and/or antigen-binding polypeptide of the conjugate. In some ~~embodiment~~examples, a spacer connects the functionalized group to the membrane-forming lipid and/or a spacer connects the complementary functional group to the antigen-binding polypeptide.

In one aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, a Fab), a nanolipoprotein particle, and a biologically active agent or a detectable agent, for use in delivering a biologically active agent or a detectable agent to an individual in need thereof, in a low viscosity formulation, wherein said liquid formulation is to be administered to said individual, wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is conjugated to said antigen-binding polypeptide via a functionalized group on said one or more membrane-forming lipids and a C-terminally located complementary functional group on said antigen-binding polypeptide; wherein said biologically active agent or said detectable agent is conjugated to at least one of said scaffold protein, said membrane-forming lipid, or said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide; and wherein said liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg of said conjugate/mL, thereby delivering said biologically active agent or said detectable agent to said individual in need thereof in a low viscosity formulation. Yet still another ~~aspect~~ example provides a method of delivering a biologically active agent or a detectable agent to an individual in need thereof, in a low viscosity formulation, the method comprising administering to an individual a liquid formulation comprising a conjugate of the ~~invention~~disclosure and a biologically active agent or a detectable agent, where the biologically active agent or detectable agent is conjugated to the scaffold protein, membrane-forming lipid, and/or antigen-binding polypeptide of the conjugate; and where the liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg conjugate/mL. In some ~~embodiment~~examples, a spacer connects the functionalized group to the membrane-forming lipid and/or a spacer connects the complementary functional group to the antigen-binding polypeptide.

In one aspect, the disclosure provides a liquid formulation comprising a conjugate of an antigen-binding polypeptide (*e.g.*, a Fab), a nanolipoprotein particle, and a biologically active agent or a detectable agent, for use in delivering a biologically active agent or a detectable agent to the brain or central nervous system of an individual in need thereof, wherein said liquid formulation is for administration systemically, wherein said nanolipoprotein particle comprises a scaffold protein encircling a bilipid layer of membrane-forming lipids; wherein one or more of said membrane-forming lipids is conjugated to said antigen-binding polypeptide via a functionalized group on said one or more membrane-forming lipids and a C-terminally located complementary functional group on said antigen-binding polypeptide; wherein said biologically active agent or said detectable agent is conjugated to at least one of said scaffold protein, said membrane-forming lipid, or said antigen-binding polypeptide; optionally wherein a spacer connects said functionalized group to said membrane-forming lipid and/or a spacer connects said complementary functional group to said antigen-binding polypeptide; and wherein said conjugate translocates across the blood-brain barrier, thereby delivering said biologically active agent or said detectable agent to the brain or central nervous system of an individual in need thereof. Yet still another ~~aspect~~ example provides a method of delivering a biologically active agent or a detectable agent to the brain or central nervous system of an individual in need thereof, the method comprising systemically administering to an individual a liquid formulation comprising a conjugate of the ~~invention~~disclosure and a biologically active agent or a detectable agent, where the biologically active agent or detectable agent is conjugated to the scaffold protein, membrane-forming lipid, and/or antigen-binding polypeptide; and where the conjugate translocates across the blood-brain barrier. In some ~~embodiment~~examples, a spacer connects the functionalized group to the membrane-forming lipid and/or a spacer connects the complementary functional group to the antigen-binding polypeptide. In particular ~~embodiment~~examples, the scaffold protein is apoE2 and/or apoE4. In particular ~~embodiment~~examples, the liquid formulation is administered intravenously.

In some ~~embodiment~~examples, one or more of said membrane-forming lipids is a C_{4-28} fatty-acyl and said fatty-acyl is conjugated to a short peptide of 20-60 amino acids. In some ~~embodiment~~examples, said fatty-acyl is a C_{16} fatty-acyl. In some ~~embodiment~~examples, said short peptide is a cystine-knot peptide (CKP). In some ~~embodiment~~examples, the CKP is EETI-II In some ~~embodiment~~examples, the short

peptide is CKP variant that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the CKP variant comprises an additional lysine residue at its N-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the wild type CKP is EETI-II. In some ~~embodiment~~examples, said membrane-forming lipids comprise a peptide-C_{4-28} fatty-acyl conjugate and at least one more of DMPC, DOPC, DOPS, DOPE, DPPC (*e.g.*, a peptide-C_{16} fatty-acyl conjugate and DOPC) in the molar ratio of 1:3 to 1:15, or 1:6 to 1:12, or 1:9; and/or said conjugate comprises 1-100, 10-90, 20-80, 30-70, 40-60, or 60 molecules of said short peptide. In some ~~embodiment~~examples, said spacer is a PEG spacer that connects said functionalized group to said membrane-forming lipid. In some ~~embodiment~~examples, said PEG spacer has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000.

In some ~~embodiment~~examples of the methods for delivering a biologically active agent or a detectable agent, the antigen-binding polypeptide is a Fab or a Fab-like molecule. In particular ~~embodiment~~examples, the biologically active agent is a cytotoxic agent selected from the group consisting of daunomycin, doxorubicin, methotrexate, vindesine, a radionuclide, diphtheria toxin, ricin, geldanamycin, a maytansinoid, calicheamicin, or a combination of any one or more thereof. In some ~~embodiment~~examples, the detectable agent is a label selected from the group consisting a radioactive isotope, a fluorophore, a chemiluminescent dye, a chromophore, an enzyme, a metal ion, a nanoparticle, and a combination of any one or more thereof. In particular ~~embodiment~~examples, the membrane-forming lipids are conjugated to 5-60 molecules of antigen-binding polypeptide. Advantageously, the liquid formulation may have a viscosity of 10-50 cP and a concentration of 100-300 mg of conjugate/mL.

In some ~~embodiment~~examples, the liquid formulation is administered intravenously. In some ~~embodiment~~examples, said membrane-forming lipids are conjugated to 5-100 molecules of said antigen-binding polypeptide via a functionalized group on said membrane-forming lipid and a C-terminally located complementary functional group on said antigen-binding polypeptide. In some ~~embodiment~~examples, said antigen-binding polypeptide is a Fab and said Fab is connected via a

PEG spacer to said membrane-forming lipid. In some ~~embodiment~~examples, said PEG spacer connects said functionalized group to said membrane-forming lipid and has a MW of 1000-3000, 1500-2500, 1900-2200, or 2000. In some ~~embodiment~~examples, said liquid formulation has a viscosity of 10-50 cP and a concentration of 100-300 mg of said conjugate/mL.

In one aspect, the disclosure provides a system or kit for delivering an antigen-binding polypeptide to a target cell, the system or kit comprising: one or more components for preparing the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, wherein said components are provided in one or more compartments of said kit or system, and optionally instructions for preparing said conjugate, composition, or formulation and/or instructions for delivering said antigen-binding polypeptide. Another ~~aspect~~ example provides a system or kit for carrying out the methods described herein, *e.g.*, for delivering an antigen-binding polypeptide (*e.g.*, a Fab) to a target cell. In some ~~embodiment~~examples, the system or kit comprises one or more components for preparing a NLP-polypeptide conjugate or peptide-NLP-polypeptide conjugate herein, a pharmaceutical composition herein, or a formulation herein , in one or more compartments of the system or kit. In some ~~embodiment~~examples, instructions are provided for preparing the conjugate, composition, or formulation, or for its use in delivering the antigen-binding polypeptide in accordance with methods of the ~~invention~~disclosure. In particular ~~embodiment~~examples, the antigen-binding protein is a Fab or a Fab-like molecule.

In one aspect, the disclosure provides a system or kit for delivering a short peptide of 20-60 amino acids (*e.g.* a cystine-knot peptide) to a target cell, the system or kit comprising: one or more components for preparing the conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, or the formulation as disclosed herein, wherein said components are provided in one or more compartments of said kit or system, and optionally instructions for preparing said conjugate or composition and/or instructions for delivering said short peptide (*e.g.*, said cystine-knot peptide). Also provided is a system or kit for delivering a short peptide of 20-60 amino acids (*e.g.* a cystine-knot peptide) to a target cell, the system or kit comprising: one or more components for preparing a peptide-NLP conjugate herein, a pharmaceutical composition herein, or a formulation herein , wherein said components are provided in one or more compartments of said kit or system, and optionally instructions for preparing said conjugate or composition and/or instructions for delivering said short peptide (*e.g.*, said cystine-knot peptide).

In ~~a related~~one aspect, provided is a method of increasing the biological activity of a short peptide attached to the surface of a lipid-based nanoparticle, comprising (a) providing a lipid-based nanoparticle comprising a short peptide attached its surface, wherein one or more lipids of the lipid-based nanoparticle presents a functionalized group, and (b) allowing the nanoparticle to contact a polypeptide having a functional group under conditions that favor conjugation of said functionalized group to said functional group. In some ~~embodiment~~examples, the method further comprises purifying the conjugate comprising the lipid based nanoparticle, the short peptide, and the polypeptide. In some ~~embodiment~~examples, the lipid-based nanoparticle comprises a lipid bilayer. In some ~~embodiment~~examples, the lipid-based nanoparticle is a liposome, a solid lipid nanoparticle (SLN), or a nanostructured lipid carrier (NLC). In some ~~embodiment~~examples, a spacer connects said functionalized group to a lipid on the surface of the lipid-based nanoparticle and/or a spacer connects said complementary functional group to said polypeptide. In some ~~embodiment~~examples, the polypeptide is an antigen-binding polypeptide. In some ~~embodiment~~examples, the

antigen-binding polypeptide is selected from the group consisting of a Fab, a Fab', a Fab'-SH, a $F(ab')_2$, a single chain Fab (scFab), a single chain Fv (scFv), a VH-VH dimer, a VL-VL dimer, a VH-VL dimer, a single domain, a diabody, a linear antibody, and a combination of any one or more thereof. In some ~~embodiment~~examples, the short peptide is a CKP or a variant of a CKP that comprises: (a) one or more amino acid insertions, deletions, and/or substitutions in one or more loop sequences relative to the corresponding one or more loop sequences of a wild type CKP; (b) one or more amino acid insertions, deletions, and/or substitutions at the N terminus relative to a wild type CKP; (c) one or more amino acid insertions, deletions, and/or substitutions at the C-terminus relative to a wild type CKP; (d) a chemical modification at the N-terminus relative to a wild type CKP; and/or (e) a chemical modification at the C-terminus relative to a wild type CKP. In some ~~embodiment~~examples, the activity of the short peptide following conjugation of the polypeptide is between 2-fold and 100-fold higher following conjugation of the polypeptide to the lipid-based nanoparticle as compared to the activity of the short peptide before conjugation of the polypeptide to the lipid-based nanoparticle.

BRIEF DESCRIPTION OF THE FIGURES

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

Figures 1A-1D depict the effect of DOPE-MCC on NLP assembly. **Figure 1A** depicts a schematic of DOPE-MCC NLP assembly and conjugation of a Fab. **Figure 1B** depicts MALS/QELS analysis of MW and R_h across the NLP SEC peaks as a function of mol% DOPE-MCC in the NLP. **Figure 1C** depicts TIC chromatograms of apoE422k alone (circles), NLP assembled at pH 7.4 (plain line) and NLP assembled at pH 6.0 (triangles). **Figure 1D** depicts deconvoluted masses from the peak TIC spectra shown in 1D for apoE422k alone (circles), NLP assembled at pH 7.4 (plain line) and NLP assembled at pH 6.0 (triangles).

Figures 2A-2C depict Fab-NLP conjugation, purification and characterization. **Figure 2A** depicts SEC purification of the Fab-NLP after conjugation at a Fab to NLP ratio of 20. The first peak corresponds to the Fab-NLP conjugate, the second to Fab dimer and the third to free unconjugated Fab. The inset shows a zoomed-in version of the Fab-NLP peak with R_h from QELS overlaid across the peak. The left y-axis is absorbance and the right y-axis is the R_h of different fractions of the Fab-NLP conjugate across the peak. **Figure 2B** depicts TIC