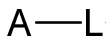
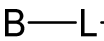
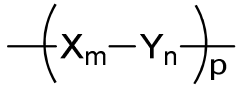
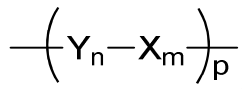
[0012] The present ~~invention~~ disclosure provides novel non-naturally occurring peptides having a cell penetrating function and being capable of forming transfection complexes with a cargo molecule and one or more cationic lipids.

[0013] Disclosed herein are compositions and methods that provide improved efficiency for introducing macromolecules, such as nucleic acids, into cells in culture or in a tissue in vivo. Accordingly, certain ~~embodiments~~ examples provide herein a complex containing, in non-covalent association, a cargo molecule, such as a nucleic acid molecule, a transfection agent and a non-naturally occurring peptide, where the non-naturally occurring peptide contains a membrane-penetrating peptide sequence. In certain ~~aspects~~examples, the complexes contain a macromolecule to be introduced into the cell, such as a peptide, a protein, or a nucleic acid.

[0013a] In one aspect of the disclosure, disclosed herein is a polypeptide having the structure:

, or ; wherein; A is membrane penetrating peptide sequence selected from any one of SEQ ID NO:1-68, or a variant thereof having at least 75% sequence similarity to any one of SEQ ID NO: 1-68; L is a linker peptide sequence having the structure:  or , where each X is independently a neutral amino acid, where each Y is independently a neutral polar amino acid, and where m is an integer from 2 to 50, where n is an integer from 1 to 40, and where p is an integer from 1 to 20; and B is a cationic moiety or a cationic peptide sequence having a net positive charge at physiologic pH.

[0013b] In one aspect of the disclosure, disclosed herein is a complex comprising the polypeptide according to the disclosure and a nucleic acid molecule.

[0013c] In one aspect of the disclosure, disclosed herein is an *in vitro* use of a polypeptide according to the disclosure, or a complex according to the disclosure, for the delivery of at least one nucleic acid molecule into a cell.

[0013d] In one aspect of the disclosure, disclosed herein is a kit containing, in at least a first suitable container, at least one polypeptide according to the disclosure.

[0013e] In one aspect of the disclosure, disclosed herein is a polypeptide according to the disclosure, or a complex according to the disclosure, for use in therapy.

[0014] In one ~~aspect~~ example of the ~~invention~~disclosure, the non-naturally occurring peptides of the present ~~invention~~disclosure have the general structure:

[0015] A—L—B, or

[0016] B—L—A;

[0017] Where A is membrane penetrating peptide, L is either a bond or a linker peptide, and B is a cationic moiety or a cationic polypeptide. In some preferred though non-limiting ~~embodiment~~examples, A is a peptide sequence selected from those set forth in Table 1, or a variant thereof retaining at least a portion of its ability to enhance transfection efficiency. In some ~~embodiment~~examples, the peptide sequence of A is between 5 to about 50 amino acids, and A is characterized in that it improves delivery of a molecule into a cell by at least 50% or more.

[0018] In some examples ~~embodiments~~, the peptide sequence of A is between 5 to about 50 amino acids, and A is characterized in that it improves delivery of a molecule into a cell by at 75% or more.

[0019] In some ~~embodiment~~examples, A is at least 75% identical to any one of the peptides set forth in Table 1, or a variant thereof retaining at least a portion of its ability to enhance transfection efficiency, where A is characterized in that it improves delivery of a molecule into a cell by at least 10% or more. In some ~~embodiment~~examples, A is selected from the list consisting of any one of SEQ ID NO. 1 through SEQ ID NO. 68, or a variant thereof.

[0020] In one ~~aspect~~example of the ~~invention~~disclosure, the non-naturally occurring peptide is selected from any one of the peptides set forth in Table 4.

[0021] Further ~~embodiment~~examples of the present ~~invention~~disclosure are directed to transfection complexes containing the non-naturally occurring peptides described above in combination with one or more transfection reagents, which transfection reagents may include one or more cationic lipids, and optionally one or more helper and/or neutral lipids.

[0022] In some ~~embodiment~~examples, a transfection complex may include a cargo to be delivered to the interior of a cell, or optionally may be administered to an animal or to a human patient who would benefit from the administration thereof. In some exemplary though non-limiting ~~embodiment~~examples, preferred cargo molecules suitable for use with the present ~~invention~~disclosure include nucleic acid molecules such as DNA molecules or RNA molecules. Suitable DNA molecules may include a DNA molecule having an expressible nucleic acid sequence, such as an expression vector or a cDNA molecule comprising an open reading frame encoding a protein. Other suitable molecules that may function as suitable cargo in the practice of the present ~~invention~~disclosure include RNA molecules, such as an mRNA molecule or an RNAi molecule.

[0023] Further ~~embodiment~~examples of the present ~~invention~~disclosure are directed to methods for preparing transfection complexes and to methods for the use thereof to deliver a cargo molecule to the interior of a cell. Methods for preparing a transfection complex can include contacting a cargo molecule with at least one cationic lipid or transfection reagent and the non-naturally occurring peptides of the present ~~invention~~disclosure, optionally in the presence of one or more helper lipids and/or one or more neutral lipids, under conditions that promote the formation of a transfection complex capable of conveying the cargo to the interior of a cell.

[0024] Further ~~embodiment~~examples of the present ~~invention~~disclosure are directed to methods for transfecting cells that include forming transfection complexes comprising at least one cargo molecule, at least one cationic lipid or transfection reagent, and a non-naturally occurring peptides in accordance with the present ~~invention~~disclosure, optionally having one or more helper lipids and/or one or more neutral lipids, and contacting the transfection complex with a cell under conditions that promote the transfection of the cell. Yet further ~~embodiment~~examples of the present ~~invention~~disclosure are directed to pharmaceutical preparations comprising a cargo or a drug to be delivered to an animal or a human subject, at least cationic lipid or transfection reagent and a non-naturally occurring peptide of the present ~~invention~~disclosure, optionally in the presence of one or

more helper lipids and/or one or more neutral lipids, to form a pharmaceutically active complex suitable for the delivery of a drug or biologically active compound to an animal or to a human subject having need thereof for the treatment of a physiological condition or disorder.

[0025] Other ~~embodiment~~examples of the present ~~invention~~disclosure will be apparent to one of ordinary skill in light of the following drawings and description of the ~~invention~~disclosure, and of the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings in which:

[0027] FIG. 1A shows a panel of 10 different cancer cell lines expressing Green Fluorescent Protein (GFP) that were transfected with an expression vector encoding GFP using LIPOFECTAMINE® 3000 in combination with a peptide according to one embodiment;

[0028] FIG. 1B shows 2 different neuronal cell lines expressing GFP that were transfected with an expression vector encoding GFP using LIPOFECTAMINE® 3000 in combination with a peptide according to one embodiment;

[0029] FIG. 1C shows 2 different myoblast cell lines expressing GFP that were transfected with an expression vector encoding GFP using LIPOFECTAMINE® 3000 in combination with a peptide according to one embodiment;

[0030] FIG. 1D shows a kidney fibroblast cell line expressing GFP that were transfected with an expression vector encoding GFP using LIPOFECTAMINE® 3000 in combination with a peptide according to one embodiment;

[0031] FIG. 2 shows a panel of six different cell lines expressing GFP that were transfected with an expression vector encoding GFP using a the indicated commercially available transfection reagent; FUGENE® HD (first column), LIPOFECTAMINE® 2000 (center

CLAIMS

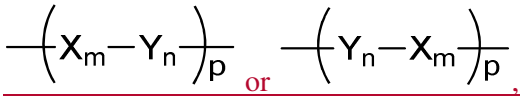
WHAT IS CLAIMED IS:

1. A polypeptide having the structure:

wherein;

A is membrane penetrating peptide sequence selected from any one of SEQ ID NO:1-68, or a variant thereof having at least 75% sequence similarity to any one of SEQ ID NO: 1-68;

L is ~~either a bond or~~ a linker peptide sequence having the structure:



where each X is independently a neutral amino acid,

where each Y is independently a neutral polar amino acid, and

where m is an integer from 2 to 50,

where n is an integer from 1 to 40, and

where p is an integer from 1 to 20; and

B is a cationic moiety or a cationic ~~poly~~peptide sequence having a net positive charge at physiologic pH.

2. The polypeptide according to claim 1, wherein A ~~comprises a~~ is a membrane penetrating peptide sequence selected from any one of SEQ ID NO: 1-68, or a variant thereof having at least 90% sequence similarity to any one of SEQ ID NO: 1-68~~Table 1~~.

~~3. The peptide according to claim 1, wherein A is a peptide comprising 5 to about 50 amino acids, wherein A is characterized in that it improves delivery of a molecule into a cell by at least 10%.~~

~~4. The peptide according to claim 1, wherein A is a peptide comprising 5 to about 50 amino acids, wherein A is characterized in that it improves delivery of a molecule into a cell by between about 50% to about 100%.~~

~~5. The peptide according to claim 1, wherein A is a peptide comprising 5 to about 50 amino acids, wherein A is characterized in that it improves delivery of a molecule into a cell by between about 75% to about 500%~~

~~6. The peptide according to claim 1, wherein A is at least 50% similar to any one of SEQ ID NO. 1 – 68, and A is characterized in that it improves delivery of a molecule into a cell by at least 20%.~~

~~7. The peptide according to claim 1, wherein A is at least 75% similar to any one of SEQ ID NO. 1 – 68, and A is characterized in that it improves delivery of a molecule into a cell by at least 50%.~~

~~8. The peptide according to claim 1, wherein A is at least 90% similar to any one of SEQ ID NO. 1 – 68, and A is characterized in that it improves delivery of a molecule into a cell by at least 100%.~~

~~9.~~3. The polypeptide according to claim 1, wherein A is ~~at least 90% similar to~~selected from any one of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 53, SEQ ID NO. 58, SEQ ID NO. 67 and SEQ ID NO. 68.

~~10.~~4. The polypeptide according to any one of claims 1 to 3, wherein L comprises a 3 to 50 amino acid sequence ~~characterized in that~~and wherein at least 50% of the amino acids in the sequence are neutral.

~~11. The peptide according to claim 1, wherein L comprises a 3 to 50 amino acid sequence characterized in that at least 60% of the amino acids in the sequence are neutral.~~

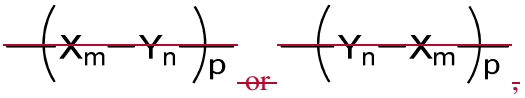
~~12. The peptide according to claim 1, wherein L comprises a 3 to 50 amino acid sequence characterized in that at least 75% of the amino acids in the sequence are neutral.~~

~~13. The peptide according to claim 1, wherein L comprises a 3 to 50 amino acid sequence characterized in that at least 30% of the amino acids in the sequence are neutral and polar.~~

~~14.~~5. The polypeptide according to any one of claims 1 to 3~~claim 1~~, wherein L comprises a 6 to 60 amino acid sequence ~~characterized in that~~and wherein at least 30% of the amino acids in the sequence are neutral and polar.

~~15.~~6. The polypeptide according to any one of claims 1 to 3~~claim 1~~, wherein L comprises a 10 to 25 amino acid sequence ~~characterized in that~~and wherein at least 30% of the amino acids in the sequence are neutral and polar.

~~16. The peptide according to claim 1, wherein L has the structure:~~



~~where each X is independently an neutral amino acid,~~

~~where each Y is independently an neutral polar amino acid, and~~

~~where m is an integer from 3 to 50,~~

~~where n is an integer from 1 to 40, and~~

~~where when L is not a bond, p is an integer from 1 to 20.~~

~~17.~~7. The polypeptide according to any one of claims 1 to 6~~claim 16~~, wherein where m > n.

~~18.~~8. The polypeptide according to any one of claims 1 to 7~~claim 16~~, wherein each X is independently glycine, alanine, valine, leucine or isoleucine.

~~19.~~9. The polypeptide according to any one of claims 1 to 8~~claim 16~~, wherein X is Gly and Y is Ser.

~~20.~~10. The polypeptide according to claim ~~19~~9, where m is 3, n is 1, and p is 3.

~~21. The peptide according to claim 1, wherein B is an amino acid sequence having a net positive charge at physiologic pH.~~

~~22.~~11. The polypeptide according to any one of claims 1 to 10~~claim 1~~, wherein B is a peptide sequence comprising 5-20 positively charged amino acids.

~~23.~~12. The polypeptide according to any one of claims 1 to 11~~claim 1~~, wherein B is a positively charged amino acid sequence comprising 5-20 Arg residues.

~~24. The peptide according to claim 1, wherein B is Arg_5 to Arg_{20}.~~

~~25. A peptide comprising a peptide sequence that is at least 75% similar to a peptide in Table 4 and characterized in that it improves delivery of a molecule into a cell by between about 50% to about 100%.~~

~~26. A transfection complex comprising the peptide of claim 1.~~

~~27. The transfection complex of claim 26, further comprising a cationic lipid.~~

~~28. The transfection complex of claim 27, further comprising a neutral lipid.~~

~~29. A transfection complex comprising a peptide that is at least 75% similar to a peptide sequence selected from the list consisting of SEQ ID NO. 89 through SEQ ID NO. 107, at least one cationic lipid, and at least one helper lipid.~~

~~30. The transfection complex according to claim 29, further comprising at least one cargo molecule.~~

~~31. The transfection complex according to claim 30, wherein said cargo molecule is a nucleic acid.~~

~~32. The transfection complex according to claim 31, wherein said cargo molecule is a DNA or an RNA molecule.~~

13. The polypeptide according to any one of claims 1 to 12, wherein the polypeptide has at least 90% sequence similarity to a peptide sequence selected from any one of SEQ ID NO. 89 through SEQ ID NO. 100 and SEQ ID NO. 102 through SEQ ID NO. 107.

14. The polypeptide according to any one of claims 1 to 13, wherein the polypeptide comprises a sequence selected from any one of SEQ ID NO. 89 through SEQ ID NO. 100, SEQ ID NO. 102 through SEQ ID NO. 105 and SEQ ID NO. 107.

15. A complex comprising the polypeptide according to any one of claims 1-14 and a nucleic acid molecule.

16. An *in vitro* use of a polypeptide according to any one of claims 1-14, or a complex according to claim 15, for the delivery of at least one nucleic acid molecule into a cell.

17. A kit containing, in at least a first suitable container, at least one polypeptide according to any one of claims 1-14.

18. The kit according to claim 17, further comprising one or more containers comprising at least one cationic lipid, optionally further comprising a neutral lipid.

19. The kit according to claim 18, wherein the cationic lipid is selected from the group consisting of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylamonium chloride (DOTMA), dioleoylphosphatidylcholine (DOPE),1,2-Bis(oleoyloxy)-3-(4'-trimethylammonio) propane (DOTAP), 1,2-dioleoyl-3-(4'-trimethylammonio) butanoyl-sn-glycerol (DOTB), 1,2-dioleoyl-3-succinyl-sn-glycerol choline ester (DOSC), dihydroxyl-dimyristylspermine tetrahydrochloride (DHDMS), hydroxyl-dimyristylspermine tetrahydrochloride (HDMS), cholesteryl (4'-trimethylammonio)butanoate (ChoTB), cetyltrimethylammonium bromide (CTAB), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), O,O'-didodecyl-N-[p(2-trimethylammonioethyloxy)benzoyl]-N,N,N-trimethylammonium chloride, spermine conjugated to one or more lipids, spermine conjugated to one or more of 5-carboxyspermylglycine dioctadecylamide (DOGS), N,N^{I},N^{II},N^{III}-tetramethyl-N,N^{I},N^{II},N^{III}-tetrapalmitylspermine (TM-TPS) and dipalmitoylphasphatidylethanolamine 5-carboxyspermylaminde (DPPES), lipopolylysine (polylysine conjugated to DOPE), TRIS (Tris(hydroxymethyl)aminomethane, tromethamine) conjugated fatty acids (TFAs) and/or peptides and/or peptides selected from the group consisting of trilysyl-alanyl-TRIS mono-, di-, and tri-palmitate, (3β-[N--(N',N'-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), N-(α -trimethylammonioacetyl)-didodecyl-D-glutamate chloride (TMAG), dimethyl dioctadecylammonium bromide (DDAB), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanamininiumtrifluoroacetate (DOSPA) and combinations thereof.

20. The kit according to claim 18, wherein the neutral lipid is selected from the group consisting of dioleoylphosphatidylethanol-amine (DOPE), cholesterol or dioleoylphosphidylcholine (DOPC).

21. A polypeptide according to any one of claims 1-14, or a complex according to claim 15, for use in therapy.