[0012] The present 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 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 examples, the complexes contain a macromolecule to be introduced into the cell, such as a peptide, a protein, or a nucleic acid.

In one aspect, there is provided a transfection complex comprising: a cationic lipid, a polyanion cargo molecule; and a polypeptide having the structure:

A—L—B, or

B—L—A;

wherein;

A is a peptide sequence comprising a membrane penetrating peptide sequence that is at least 75% similar to any one of SEQ ID NO. 1 - 68;

L is a linker peptide sequence having the structure:

$-\left(X_m-Y_n\right)_p-$ or $-\left(Y_n-X_m\right)_p-$,

wherein each X is a neutral amino acid, wherein each Y is a neutral polar amino acid, and

wherein m is an integer from 3 to 50, wherein n is an integer from 1 to 40, and wherein p is an integer from 1 to 20; and B is a cationic peptide comprising 5-20 Arg residues.

In another aspect, there is provided a lipid aggregate comprising: a polypeptide that is at least 75% similar to a peptide sequence selected from the group consisting of SEQ ID NO. 89 through SEQ ID NO. 107, at least one cargo molecule; and at least one cationic lipid.

[0014] In one example, the non-naturally occurring peptides of the present 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 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 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, 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 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 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 example, there is provided 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%. In one example, the non-naturally occurring peptide is selected from any one of the peptides set forth in Table 4. In one example, there is provided a transfection complex comprising the peptide of the present invention. In another example, there is provided 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.

[0021] Further examples of the present 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 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 examples, preferred cargo molecules suitable for use with the present invention 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 include RNA molecules, such as an mRNA molecule or an RNAi molecule.

[0023] Further examples of the present 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 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 examples of the present 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 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 examples of the present

CLAIMS

WHAT IS CLAIMED IS:

1. A transfection complex comprising:

a cationic lipid,

a polyanion cargo molecule; and

a polypeptide having the structure:

A—L—B, or

B—L—A;

wherein;

A is a peptide sequence comprising a membrane penetrating peptide sequence that is at least 75% similar to any one of SEQ ID NO. 1 - 68;

L is a linker peptide sequence having the structure:

$-\left(X_m-Y_n\right)_p-$ or $-\left(Y_n-X_m\right)_p-$,

wherein each X is a neutral amino acid,

wherein each Y is a neutral polar amino acid, and

wherein m is an integer from 3 to 50,

wherein n is an integer from 1 to 40, and

wherein p is an integer from 1 to 20; and

B is a cationic peptide comprising 5-20 Arg residues.

2. The transfection complex according to claim 1, wherein the polyanion cargo molecule is a nucleic acid molecule.

3. The transfection complex according to claim 0 or 2, wherein A is a peptide sequence comprising a membrane penetrating peptide sequence that is at least 85% similar to any one of SEQ ID NO. 1 – 68.

4. The transfection complex according to any one of claims 0 to 3, wherein A is a peptide sequence comprising a membrane penetrating peptide sequence that is at least 90% similar to any one of SEQ ID NO. 1 – 68.

5. The transfection complex according to any one of claims 0 to 4, wherein A is a peptide sequence that is at least 85% similar to 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.

6. The transfection complex according to any one of claims 0 to 5, wherein L comprises a 3 to 50 amino acid linker sequence and at least 50% of the amino acids in the linker sequence are neutral.

7. The transfection complex according to any one of claims 0 to 6, wherein L comprises a 3 to 50 amino acid linker sequence and at least 60% of the amino acids in the linker sequence are neutral.

8. The transfection complex according to any one of claims 0 to 7, wherein L comprises a 3 to 50 amino acid linker sequence and at least 75% of the amino acids in the linker sequence are neutral.

9. The transfection complex according to any one of claims 0 to 8, wherein L comprises a 3 to 50 amino acid linker sequence and at least 30% of the amino acids in the linker sequence are neutral and polar.

10. The transfection complex according to any one of claims 0 to 9, wherein L comprises a 6 to 60 amino acid linker sequence and at least 30% of the amino acids in the linker sequence are neutral and polar.

11. The transfection complex according to any one of claims 0 to 10, wherein L comprises a 10 to 25 amino acid linker sequence and at least 30% of the amino acids in the linker sequence are neutral and polar.

12. The transfection complex according to any one of claims 1 to 11, wherein $m > n$.

13. The transfection complex according to any one of claims 1 to 12, wherein each X is selected from the group consisting of glycine, alanine, valine, leucine and isoleucine.

14. The transfection complex according to any one of claims 1 to 13, wherein each X is Gly and each Y is Ser.

15. The transfection complex according to any one of claims 1 to 14, where m is 3, n is 1, and p is 3.

16. The transfection complex according to any one of claims 0 to 15, wherein B is Arg_5 to Arg_{20}.

17. The transfection complex according to any one of claims 1 to 16, wherein the polyanion cargo molecule is a nucleic acid molecule selected from DNA or RNA.

18. The transfection complex of any one of claims 1 to 17, wherein the cationic lipid is selected from the group consisting of N-[I-(2,3-dioleyloxy)propyl]-N,N,N-trimethylamonium chloride (DOTMA), dioleoylphosphatidylcholine (DOPE),l ,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-hydroxyethylammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammoniumbromide (DORIE), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide(DMRIE), O,O'-didodecyl-N-[p(2-trimethylammonioethyloxy)benzoyl]-N,N,N-trimethylammoniumchloride, spermine conjugated to one or more lipids (for example, 5-carboxyspermylglycine dioctadecylamide (DOGS), N,NI,NII,NIII-tetramethyl-N,NI,NII,NIIItet-rapalmitylspermine (TM -TPS) and dipalmitoylphasphatidylethanolamine 5-carboxyspermylaminde (DPPES)), lipopolylysine (polylysine conjugated to DOPE), TRIS(Tris(hydroxymethyl)aminomethane, tromethamine) conjugated fatty acids (TF As), trilysyl-alanyl-TRIS mono-, di-, and tri-palmitate, (3ß-[N--(N',N'dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), N-(a -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.

19. The transfection complex of any one of claims 1 to 18, further comprising a neutral lipid.

20. The transfection complex according to any one of claims 1 to 19, wherein L is at least 85% similar to SEQ ID NO. 73-81.

21. A lipid aggregate comprising:

a polypeptide that is at least 75% similar to a peptide sequence selected from the group consisting of SEQ ID NO. 89 through SEQ ID NO. 107,

at least one cargo molecule; and

at least one cationic lipid.

22. The lipid aggregate according to claim 21, wherein said at least one cargo molecule is a polyanion.

23. The lipid aggregate according to claim 21 or 22, wherein said polyanion is a nucleic acid molecule.

24. The lipid aggregate according to claim 23, wherein said nucleic acid molecule is a DNA or an RNA molecule.

25. The lipid aggregate according to any one of claims 21 to 24, wherein said cargo molecule is contacted with said polypeptide prior to the addition of said cationic lipid.

26. The lipid aggregate according to any one of claims 21 to 25, further comprising at least one neutral lipid.

27. The lipid aggregate according to any one of claims 21 to 26, wherein said cationic lipid is selected from the group consisting of N-[I-(2,3-dioleyloxy)propyl]-N,N,N-trimethylamonium chloride (DOTMA), dioleoylphosphatidylcholine (DOPE),l ,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-hydroxyethylammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammoniumbromide (DORIE), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide(DMRIE), O,O'-didodecyl-N-[p(2-trimethylammonioethyloxy)benzoyl]-N,N,N-trimethylammoniumchloride, spermine conjugated to one or more lipids (for example, 5-carboxyspermylglycine dioctadecylamide (DOGS), N,NI,NII,NIII-tetramethyl-N,NI,NII,NIIItet-rapalmitylspermine (TM -TPS) and dipalmitoylphasphatidylethanolamine 5-carboxyspermylaminde (DPPES)), lipopolylysine (polylysine conjugated to DOPE), TRIS(Tris(hydroxymethyl)aminomethane, tromethamine) conjugated fatty acids (TF As), trilysyl-alanyl-TRIS mono-, di-, and tri-palmitate, (3ß-[N--(N',N'dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), N-(a -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.