[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.

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