MODIFIED MINI-NUCLEOSOME CORE PROTEINS AND USE IN NUCLEIC ACID DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/009,124, filed on April 13, 2020, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] AAV vectors are considered the current gold standard of gene therapy and have shown promise in diverse clinical trials, including clinical trials for, e.g., retinal gene therapy and systemic gene therapy in liver, CNS, and/or other tissues. With the regulatory approval of at least three different gene therapies, the field is poised for many more, so patients can access these life-changing treatments. However, despite being the industry's gold standard, AAV vectors have certain limitations. Improved and/or alternative nucleic acid delivery technologies are needed.

SUMMARY

[0003] The present disclosure provides compositions and methods relating to, among other things, polypeptides that are capable of associating with nucleic acid molecules, wherein the polypeptides include at least one modified amino acid. In various ~~embodiment~~examples, the polypeptides can be used for, e.g., delivering the nucleic acid molecules to a subject in need of gene therapy. Accordingly, the present disclosure includes, among other things, modified polypeptides capable of associating with nucleic acid molecules, as well as compositions including modified polypeptides disclosed herein together with associated nucleic acid molecules. The present disclosure contemplates, without wishing to be bound by any particular scientific theory, that association of a nucleic acid molecule with a modified polypeptide disclosed herein can facilitate delivery of the nucleic acid to a target cell, subject, or other system.

[0004] In particular, the present disclosure includes, among other things, "modified mini-nucleosome core proteins" for delivery of nucleic acids. In various ~~embodiment~~examples, a mini-

nucleosome core protein of the present disclosure can include (a) a nucleic acid binding domain ("NABD"); (b) a nucleic acid release domain; and, optionally, (c) further domains including, e.g., one or more of a targeting domain, a stability domain, an oligomerization domain, and/or a linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can include (a) a nucleic acid binding domain ("NABD"); (b) a nucleic acid release domain; (c) a targeting domain; and, optionally, (d) further domains including, e.g., one or more of a stability domain, an oligomerization domain, and/or a linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can be a modified mini-nucleosome core protein that includes (a) a nucleic acid binding domain ("NABD"); (b) a nucleic acid release domain; (c) a modified amino acid residue; and, optionally, (d) further domains including, e.g., one or more of a targeting domain, a stability domain, an oligomerization domain, and/or a linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can be a modified mini-nucleosome core protein that includes (a) a nucleic acid binding domain ("NABD"); (b) a nucleic acid release domain; (c) a targeting domain; (d) a modified amino acid residue; and, optionally, (e) further domains including, e.g., one or more of a stability domain, an oligomerization domain, and/or a linker domain. As disclosed in herein, a modified a mini-nucleosome core protein can be referred to and/or described as a mini-nucleosome core protein and/or as a protein that is or includes a mini-nucleosome core protein having at least one modified amino acid residue. Accordingly, for clarity, all references, express or implied, to "mini-nucleosome core proteins" in the present disclosure include and encompass modified mini-nucleosome core proteins of the present disclosure. One or more mini-nucleosome core proteins associated with a nucleic acid cargo can be referred to as a "loaded mini-nucleosome." Because a loaded mini-nucleosome that is for delivery of a nucleic acid to a target is non-viral, a mini-nucleosome is an example of a non-viral vehicle for nucleic acid delivery.

[0005] In particular, the present disclosure includes, among other things, "modified mini-nucleosome core proteins" for delivery of nucleic acids. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can include (a) a nucleic acid binding domain ("NABD"); (b) a targeting domain; and, optionally, (c) further domains including, e.g., one or more of a nucleic acid release domain, a stability domain, an oligomerization domain, and/or a

linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can include (a) a nucleic acid binding domain ("NABD"); (b) a targeting domain; (c) a nucleic acid release domain; and, optionally, (d) further domains including, e.g., one or more of a stability domain, an oligomerization domain, and/or a linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can be a modified mini-nucleosome core protein that includes (a) a nucleic acid binding domain ("NABD"); (b) a targeting domain; (c) a modified amino acid residue; and, optionally, (d) further domains including, e.g., one or more of a nucleic acid release domain, a stability domain, an oligomerization domain, and/or a linker domain. In various ~~embodiment~~examples, a mini-nucleosome core protein of the present disclosure can be a modified mini-nucleosome core protein that includes (a) a nucleic acid binding domain ("NABD"); (b) a targeting domain; (c) a nucleic acid release domain; (d) a modified amino acid residue; and, optionally, (e) further domains including, e.g., one or more of a stability domain, an oligomerization domain, and/or a linker domain. As disclosed in herein, a modified a mini-nucleosome core protein can be referred to and/or described as a mini-nucleosome core protein and/or as a protein that is or includes a mini-nucleosome core protein having at least one modified amino acid residue. Accordingly, for clarity, all references, express or implied, to "mini-nucleosome core proteins" in the present disclosure include and encompass modified mini-nucleosome core proteins of the present disclosure. One or more mini-nucleosome core proteins associated with a nucleic acid cargo can be referred to as a "loaded mini-nucleosome." Because a loaded mini-nucleosome that is for delivery of a nucleic acid to a target is non-viral, a mini-nucleosome is an example of a non-viral vehicle for nucleic acid delivery.

[0006] The present disclosure includes the recognition that at least some modified mini-nucleosome core proteins of the present disclosure have advantageous properties including, without limitation, increased bioavailability; increased half-life; increased stability; decreased degradation; increased binding affinity, e.g., for target cells; increased uptake, e.g., by target cells; improved blood-brain barrier penetration; reduced accumulation, e.g., in target cells or tissues; reduced aggregation; reduced precipitation; reduced thermal denaturation; and/or reduced kinetic denaturation, e.g., as set forth herein.

[0007] The present disclosure includes the recognition that at least certain compositions and methods described herein remedy one or more deficiencies associated with AAV vectors, including that:

[0008] 1) AAV is associated with a payload limitation of 4.5 kb DNA length, which limitation prevents use of AAV in treatment of diseases caused at least in part by deficiency in expression of a gene product typically encoded by a nucleic acid larger than 4kb (for example genes like CFTR, HTT, F8, DMD, ABCA4 etc. cannot fit into AAV vectors) (Lai Y. et al, 2010).

[0009] 2) AAV has been known to integrate at low percentage and/or in a site-non-specific manner (Smith R.H., 2008). Random or site-non-specific integration may be deleterious if integration can or does disrupt a tumor suppressor gene or gene important for cellular functions.

[0010] 3) Depending on the serotype of AAV, 25-70 % of humans have preexisting neutralizing antibodies to AAV which means, they would be less likely to benefit for AAV therapy (Fitzpatrick Z., et al 2018).

[0011] 4) Multiple treatments with AAV are highly unlikely to be effective because once a patient is injected, the patient produces a high number of antibodies against the virus. For some diseases where cellular turnover is high (e.g., in the turnover of liver cells or airway epithelial cells) multiple treatments maybe needed. Thus, due to increased antibodies against AAVs following a first treatment, the same vector may not be useful in follow-up treatments or doses.

[0012] 5) Effective treatment of some diseases may require delivery of an enormous payload of particles administered by intravenous injection in order to transduce cells in vivo. A high dose of AAV comes with its own toxicities, which are well documented (Hinderer C. et al, 2018).

[0013] 6) Most diseases are also associated with multi-organ defects and AAV may not be applied to various organs in the same body. One application at one site will raise antibodies and thus may block transduction at other locations in the body when injected in a subsequent treatment or dose.

[0014] Due at least in part to the deficiencies of AAV discussed above, there is a dire need for alternatives to AAV. In at least certain ~~embodiment~~example**s**, non-viral vectors disclosed herein overcome one or more of the deficiencies of AAV discussed above.

[0015] Moreover, prior non-viral vectors are also associated with several barriers to therapeutic efficacy including: i) low transfection/transduction efficiency (Guerra-Crespo M et al, 2003) ii) low particle stability in blood, body fluids and other tissues (Barua and Mitragotri, 2014) ; iii) low cell entry via receptor-mediated endocytosis or cell fusion; iv) low stability in, and low escape from, endosomal and lysosomal compartments; v) low diffusion rate in the cytoplasm; vi) low nuclear pore transit; and vii) low release of DNA to permit biological function in the nucleus (Zabner J. et al, 1995). Several publications have documented inability or low efficiency of prior non-viral vectors to transfect post-mitotic cells (Wilke M. et al, 1996). Certain prior non-viral vectors lack longevity of expression and/or produce low amount of proteins that are not therapeutic enough and cannot be targeted to specific cell types in an efficient manner.

[0016] Thus, despite state-of-the-art research in the field of non-viral vectors, many prior non-viral vectors are not optimal for clinical use. Certain characteristics of at least certain ~~embodiment~~examples discussed herein that contribute to, among other things, clinical utility, can include, without limitation:

[0017] Size and molecular weight: Many prior non-viral vectors that carry DNA molecule have a size of 10- 200nm in diameter (Konstan M.W. et. al, 2004). Their molecular weights can be greater than 300 kDa or greater than 500kDa. The present disclosure provides, among other things, non-viral proteinaceous vehicles, and/or loaded mini-nucleosomes, that are <20nm in diameter and have a molecular weight of <500kDa. In particular ~~embodiment~~examples, a non-viral proteinaceous vehicles, and/or loaded mini-nucleosomes, disclosed herein can pass into the nucleus more efficiently, perhaps, by passive diffusion, at least in part because a typical nuclear pore is only 20nm in diameter, such that <20nm size may allow passage.

[0018] Stability in body fluids: Many prior non-viral vectors are degraded in body fluids like blood or CSF before they can be delivered to target cells (Barua and Mitragotri, 2014). The present disclosure, provides, among other things, non-viral proteinaceous vehicles, and/or loaded mini-nucleosomes, that are physiologically stable and/or have properties that allow them to be stable in blood and/or other body fluids until and after entry into a target cell. At least one goal for these particles to safely reach the nucleus of desired cells.

[0019] Release of particles in nucleus: Many prior non-viral vectors have a very short life time because most release associated nucleic acids before entering target cells, and the remainder release associated nucleic acids in the cytoplasm, where delivered DNA encounters nucleases that destroys DNA (Zabner, J. et al, 1995). Certain prior vectors that make it into the cell nucleus and provide expression levels are very low, if they express at all. The present disclosure also recognizes, among other things, that it can be beneficial to release associated nucleic acids at a slow rate, instead of all at once, which may allow for longevity of expression.

[0020] Cell type specificity: Prior non-viral vectors are not targeted to specific cell types are associated with reduced levels of transduction and thus, reduced expression. The present disclosure provides, among other things, non-viral vectors optimized for cell-type specificity. Certain means of engineering cell-type specificity are described, e.g., in Templeton and Senzer, 2011.

[0021] Taken together, there is a tremendous need for nucleic acid delivery technologies that provide effective levels of expression for a desired duration, are non-immunogenic and non-toxic, and have less limited payload capacity. Moreover, the need for millions of patients of Huntington, Stargardt, Duchenne muscular dystrophy, Cystic Fibrosis, and other conditions treatable by gene therapy clearly presents a need for technology that can help treat these patients.

[0022] The present disclosure provides safe and efficacious non-viral proteinaceous vehicles ("mini-nucleosome core proteins"), and loaded mini-nucleosomes, for delivery of nucleic acids. The present disclosure further recognizes that in at least some instances modified mini-nucleosome core proteins of the present disclosure can have advantageous properties including, without limitation, increased bioavailability; increased half-life; increased stability; decreased degradation; increased binding affinity, e.g., for target cells; increased uptake, e.g., by target cells; improved blood-brain barrier penetration; reduced accumulation, e.g., in target cells or tissues; reduced aggregation; reduced precipitation; reduced thermal denaturation; and/or reduced kinetic denaturation, e.g., as set forth herein.

[0023] In various ~~embodiment~~example~~s~~, a mini-nucleosome core protein is associated with one or more nucleic acids. As disclosed herein a mini-nucleosome core protein associated with one or more nucleic acids can be referred to as a "loaded mini-nucleosome."

[0024] In various ~~embodiment~~examples, a mini-nucleosome core protein includes a nucleic acid release domain that targets a loaded mini-nucleosome to one or more specific cell types for delivery and/or targeted expression of a nucleic acid, such as a gene, in or to one or more specific cell types.

[0025] In various ~~embodiment~~examples, a mini-nucleosome core protein composition (e.g., a composition including one or more loaded mini-nucleosomes) can be titered and/or administered either once or repeatedly based on need. Furthermore, in various ~~embodiment~~examples, a mini-nucleosome core protein or mini-nucleosome composition (e.g., a composition including one or more loaded mini-nucleosomes) is non-immunogenic and non-toxic.

[0026] Mini-nucleosome core proteins disclosed herein can, in certain ~~embodiment~~examples, utilize principles applicable to macromolecule uptake, viral entry into cells, nucleosome formation in eukaryotic cells, cleavage of certain proteins at certain location in the cells, etc.

[0027] Various ~~embodiment~~examples of the compositions and methods provided herein include domains that facilitate one or more of enhanced stability, targeting to specific cell types, and enhanced longevity of expression by slow nucleic acid release.

[0028] In various ~~embodiment~~examples, a mini-nucleosome core protein and/or a mini-nucleosome is stable in body fluids and/or include domains that allow and/or target release in or to the nucleus. In at least a first aspect, the present disclosure provides an engineered polypeptide comprising: (a) a nucleic acid binding domain, and (b) a nucleic acid release domain or a targeting domain, wherein one or more amino acids of the engineered polypeptide is a modified amino acid that comprises: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation; (ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; or (xiv) pegylation. In one example, the present disclosure provides an engineered polypeptide including a nucleic acid binding domain and a nucleic acid release domain, where one or more amino acids of the engineered polypeptide is a modified amino acid, optionally where the modification includes at least one of: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation; (ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; (xiv) pegylation; and/or (xv) any combination thereof. In certain ~~embodiment~~examples, the engineered polypeptide includes a targeting domain.

[0029] In at least a further ~~aspect~~example, the present disclosure provides an engineered polypeptide including a nucleic acid binding domain and a targeting domain, where one or more amino acids of the engineered polypeptide is a modified amino acid, optionally where the modification includes at least one of: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation;

(ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; (xiv) pegylation; and/or (xv) any combination thereof; where engineered polypeptide optionally further includes a nucleic acid release domain.

[0030] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, each of two or more amino acids of an engineered polypeptide is a modified amino acid. In some ~~embodiment~~examples, at least one of the modified amino acids includes a modification chain including two or more modifications selected from: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation; (ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; (xiv) pegylation; and (xv) any combination thereof. In some ~~embodiment~~examples, the modification increases the stability, half-life, and/or bioavailability of the engineered polypeptide. In some ~~embodiment~~examples, the modification increases the affinity and/or avidity of the engineered polypeptide with a binding partner, optionally where the binding partner is a receptor, cell, or cell membrane. In some ~~embodiment~~examples, the modification increases the affinity or avidity of the engineered polypeptide with a nucleic acid. In some ~~embodiment~~examples, the modification decreases precipitation and/or aggregation of the engineered polypeptide.

[0031] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a nucleic acid binding domain is derived from a histone polypeptide sequence. In some ~~embodiment~~examples, the nucleic acid binding domain is or includes the amino acid sequence KRHRK. In some ~~embodiment~~examples, the nucleic acid binding domain is or includes an amino acid sequence that includes KRHRK, RRRRR, RRLARR, KKAKAAAKPKK, KKDGKKRKR, KKKLK, KKRIRK, RKKSK, KKPKK, or a combination thereof. In some ~~embodiment~~examples, the nucleic acid binding domain is a modified nucleic acid binding domain in that the nucleic acid binding domain includes one or more modified amino acids.

[0032] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 397-422, where the targeting domain is phosphorylated. In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 423-428, where the targeting domain is sulfated. In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting

domain having the sequence of any one of SEQ ID NOs: 429-434, where the targeting domain is glycosylated.

[0033] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 435-440, where the targeting domain is prenylated. In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 441-446, where the targeting domain is methylated. In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 447-459, where the targeting domain is sialylated. In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a cell attachment targeting domain, a beta galactose binding domain, a fucose binding domain, a heparin binding domain, a sialic acid binding domain, a glycoprotein binding domain, a carbohydrate binding domain, a lysophosphatidic acid binding domain, a cAMP binding domain, a hyaluronan binding domain, a chondroitin sulfate binding domain, an integrin binding domain, a nucleolin binding domain, a collagen binding domain, a clathrin binding domain, a Fc receptor binding domain, an actin binding domain, an endocytosis motif, a nuclear localization signal, or a combination thereof.

[0034] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a cell attachment targeting domain. In some ~~embodiment~~examples, the cell attachment targeting domain is or includes an amino acid sequence that includes WGREERQ, NTQIH, WNNKTPH, TPH, VNRWS, XBBBXXBX, ARKKAAKA, QRR, SRR, WEPSRPFPVD, HRRTRKAPKRIRLPHIR, KRTGQYKLGSKTGPGQK, KKTK, KLRSQLVKK, RRRCGQKKK, BX(7)B, RIQNLLKITNLRIKFVK, KKEKDIMKKTI, KGE, RGD, RGDS, TTVVNPKYEGK, ERMSQIKRLLS, WRHRARS, GFOGER, LFDLM, WGREERQ, QSTEKRG, LPNTG, or a combination thereof.

[0035] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is an internalization domain. In some ~~embodiment~~examples, the internalization domain is or includes an amino acid sequence that includes FXDXF, PPSY, FEDNFVP, YIRV, YADW, YTQV, KKRPKP, SSDDE, RRASS, (YXXL)2, LPLTG, LAFTG, or a combination thereof.

[0036] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a cell-type specific targeting domain. In some ~~embodiment~~examples, the cell-type specific targeting domain is or includes an amino acid sequence that includes ASSLNIA, KKEEEKKEEEKKEEE, LIFHKEQ, KFNKPFVFLI, QPEHSST, EYHHYNK, NGR, GEKGEP, KTKKK, KALKKK, KGKKK, CSVTCG, LRE, YKYNLNGRES, YRSL, $KGGK_7$, KKKQYTSIHHG, KDEL, LADQDYTKTA, or a combination thereof.

[0037] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a targeting domain is a modified targeting domain in that the targeting domain includes one or more modified amino acids. In some ~~embodiment~~examples, the nucleic acid release domain is or includes an amino acid sequence that includes GRKKRRQRRRPQ, KRH, KSVKKRSVSEIQ, NRRKKRAL, KFERQ, VRGP, NKDS, NRDN, ANNR, or a combination thereof.

[0038] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, a the nucleic acid release domain is a modified nucleic acid release domain in that the nucleic acid release domain includes one or more modified amino acids.

[0039] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, an engineered polypeptide further includes a poly-arginine domain. In some ~~embodiment~~examples, the poly-arginine domain is a modified poly-arginine domain in that the poly-arginine domain includes one or more modified amino acids.

[0040] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, an engineered polypeptide further includes a nuclear internalization signal or a nuclear import machinery binding domain. In some ~~embodiment~~examples, the nuclear internalization signal or nuclear import machinery binding domain is or includes an amino acid sequence that includes KKKYKLK, KKRKLE, TRSK, HRKRKR, NKRKRK, AEKSKKK, RKSK, KRVK, KRK, LQQTPLHLAVI, RRPR, PRPR, RPPP, RKKRKGK, PAAKRVKLD, KLKIKRPVK, PKKKRKV, QRKRQK, DSPE, FQVT, QSTEKRG, RQGLID, Cyclic RKKH, or a combination thereof. In some ~~embodiment~~examples, the nuclear internalization signal or a nuclear import machinery binding domain is a modified nuclear internalization signal or a nuclear import machinery binding domain in that the nuclear internalization signal or a nuclear import machinery binding domain includes one or more modified amino acids.

[0041] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, an engineered polypeptide further includes a stability domain. In some ~~embodiment~~examples, the stability domain is or includes an amino acid sequence that includes YTRF, GDAY, LLEE, RKKRRQRRR, YKSL, YENF, FQDL, YIGSR, IKVAV, or a combination thereof. In some ~~embodiment~~examples, the stability domain is a modified stability domain in that the stability domain includes one or more modified amino acids.

[0042] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, an engineered polypeptide further includes an oligomerization domain. In some ~~embodiment~~examples, the oligomerization domain is selected from the oligomerization domains of Table 11, optionally where the oligomerization domain is positioned at the C-terminus of the engineered polypeptide. In some ~~embodiment~~examples, the oligomerization domain is a modified oligomerization domain in that the oligomerization domain includes one or more modified amino acids.

[0043] In at least certain ~~embodiment~~examples of various aspects of the present disclosure, an engineered polypeptide further includes a linker. In some ~~embodiment~~examples, the linker is a linker according to any one of SEQ ID NOs: 154-250. In some ~~embodiment~~examples, the linker is a modified linker in that the linker includes one or more modified amino acids.

[0044] In some ~~embodiment~~examples, one or more amino acids of an engineered polypeptide is a phosphorylated amino acid. In some ~~embodiment~~examples, the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid. In some ~~embodiment~~examples, the phosphorylated amino acid is present in a linker domain or targeting domain.

[0045] In some ~~embodiment~~examples, one or more amino acids of an engineered polypeptide is a sulfated amino acid. In some ~~embodiment~~examples, the sulfated amino acid is a serine, threonine, or tyrosine amino acid. In some ~~embodiment~~examples, the sulfated amino acid is present in a linker domain or targeting domain.

[0046] In some ~~embodiment~~examples, one or more amino acids of an engineered polypeptide is an acetylated amino acid. In some ~~embodiment~~examples, the acetylated amino acid is a lysine amino acid. In some ~~embodiment~~examples, the acetylated amino acid is present in a linker domain or targeting domain.

[0047] In some ~~embodiment~~examples, one or more amino acids of an engineered polypeptide is a mannosylated amino acid. In some ~~embodiment~~examples, the mannosylated amino acid is a serine amino

acid. In some ~~embodiment~~examples, the mannosylated amino acid is present in a linker domain or targeting domain.

[0048] In at least one further aspect, the present disclosure provides a polynucleotide that encodes the amino acid sequence of an engineered polypeptide as disclosed herein. In one example, the present disclosure provides a polynucleotide that encodes the amino acid sequence of an engineered polypeptide of the present disclosure, such as a DNA or RNA polynucleotide. In at least one further ~~aspect~~example, the present disclosure provides a vector including polynucleotide of the present disclosure. In at least one further aspect, the present disclosure provides a cell comprising an engineered polypeptide as disclosed herein, or a polynucleotide as disclosed herein. In one example, the present disclosure provides a cell including an engineered polypeptide of the present disclosure or a vector of the present disclosure.

[0049] In at least one further ~~aspect~~example, the present disclosure provides method of making an engineered polypeptide of the present disclosure, the method including expressing a polynucleotide of the present disclosure in a cell. In some ~~embodiment~~examples, the method further includes isolating the engineered polypeptide from the cell.

[0050] In at least one further aspect, the present disclosure provides a composition including: (i) at least one polynucleotide, and (ii) at least one engineered polypeptide of the present disclosure. In some ~~embodiment~~examples, the at least one polynucleotide is or includes DNA or RNA. In some ~~embodiment~~examples, the at least one polynucleotide includes a nucleotide sequence encoding a polypeptide. In some ~~embodiment~~examples, the at least one polynucleotide is or includes mRNA. In some ~~embodiment~~examples, the at least one polynucleotide includes an inhibitory RNA. In some ~~embodiment~~examples, the inhibitory RNA is a gRNA, siRNA, miRNA, or shRNA. In some ~~embodiment~~example, the composition includes at least two engineered polypeptides of the present disclosure, where a first engineered polypeptide of present disclosure is able to oligomerize with a second engineered polypeptide of the present disclosure. In some ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:2,000. In some ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:1,000, between 1:1 and 1:500, between 1:1 and 1:200, between 1:1 and 1: 100, between 1:1 and 1:50, between 1:3 and 1:1,000, between 1:3 and 1:500, between 1:3 and 1:200, between 1:3 and 1: 100, or between 1:3 and 1:50. In some ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:200 and 1:2,000, between 1:200 and 1:1000, or between 1:200 and 1:500. In some ~~embodiment~~examples, the composition includes a pharmaceutical carrier.

[0051] In at least one further aspect, the present disclosure provides the composition as disclosed herein for use in therapy. In at least one further aspect, the present disclosure provides an *ex vivo* or *in vitro* method comprising adding a composition as disclosed herein to a system, wherein the system is a cell or a tissue. In at least one further ~~aspect~~example, the present disclosure provides a method that includes administering a composition of the present disclosure to a system, where the system is a cell, tissue, or subject. In some ~~embodiment~~examples, after administration, the modification increases the stability, half-life, and/or bioavailability of the composition in the system. In some ~~embodiment~~examples, after administration, the modification increases the affinity or avidity of the composition with a binding partner in the system, optionally where the binding partner is a receptor, cell, or cell membrane. In some ~~embodiment~~examples, after administration, the modification decreases precipitation and/or aggregation of the composition in the system. In some ~~embodiment~~examples, after administration, the modification increases the rate at which the composition enters one or more cells in the system. In some ~~embodiment~~examples, after administration, the modification increases delivery of the composition to one or more cells in the system. In some ~~embodiment~~examples, after administration, the modification increases delivery of the nucleic acid of the composition to one or more cells in the system. In some ~~embodiment~~examples, the system is a mammalian subject and, after administration, the modification decreases accumulation of the composition in liver. In some ~~embodiment~~examples, the system is a mammalian subject and, after administration, the modification increases the amount of composition that crosses the blood-brain barrier.

[0052] In some ~~embodiment~~examples, the present disclosure provides a method that includes administering a composition of the present disclosure including at least one polynucleotide and at least one engineered polypeptide to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a phosphorylated amino acid. In some ~~embodiment~~examples, the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid. In some ~~embodiment~~examples, the phosphorylated amino acid is present in a linker domain or targeting domain. In some ~~embodiment~~examples, the composition is delivered to cells of the central nervous system (CNS).

[0053] In some ~~embodiment~~examples, the composition is delivered to CNS neurons. In some ~~embodiment~~examples, the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia. In some ~~embodiment~~examples, the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells. In some ~~embodiment~~examples, the polynucleotide encodes an expression product that is expressed in cells to which the composition

is delivered. In some ~~embodiment~~examples, the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.

[0054] In some ~~embodiment~~examples, the present disclosure provides a method that includes administering a composition of the present disclosure including at least one polynucleotide and at least one engineered polypeptide to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a sulfated amino acid. In some ~~embodiment~~examples, the sulfated amino acid is a serine, threonine, or tyrosine amino acid. In some ~~embodiment~~examples, the sulfated amino acid is present in a linker domain or targeting domain. In some ~~embodiment~~examples, the composition is delivered to cells of the central nervous system (CNS). In some ~~embodiment~~examples, the composition is delivered to CNS neurons. In some ~~embodiment~~examples, the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia. In some ~~embodiment~~examples, the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells. In some ~~embodiment~~examples, the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered. In some ~~embodiment~~examples, the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.

[0055] In some ~~embodiment~~examples, the present disclosure provides a method that includes administering a composition of the present disclosure including at least one polynucleotide and at least one engineered polypeptide o a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is an acetylated amino acid. In some ~~embodiment~~examples, the acetylated amino acid is a lysine amino acid. In some ~~embodiment~~examples, the acetylated amino acid is present in a linker domain or targeting domain. In some ~~embodiment~~examples, the composition is delivered to CNS neurons. In some ~~embodiment~~examples, the composition is delivered to retinal cells. In some ~~embodiment~~examples, the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells. In some ~~embodiment~~examples, the composition is delivered to photoreceptor cells, optionally wherein the photoreceptor cells include one or both of rods and cones. In some ~~embodiment~~examples, the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered. In some ~~embodiment~~examples, the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.

[0056] In some ~~embodiment~~examples, the present disclosure provides a method that includes administering a composition of the present disclosure including at least one polynucleotide and at least one engineered polypeptide to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a mannosylated amino acid. In some ~~embodiment~~examples, the mannosylated amino acid is a serine amino acid. In some ~~embodiment~~examples, the mannosylated amino acid is present in a linker domain or targeting domain. In some ~~embodiment~~examples, the composition is delivered to CNS neurons. In some ~~embodiment~~examples, the composition is delivered to retinal cells. In some ~~embodiment~~examples, the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells. In some ~~embodiment~~examples, the composition is delivered to photoreceptor cells include one or both of rods and cones. In some ~~embodiment~~examples, the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered. In some ~~embodiment~~examples, the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.

[0057] In at least one further aspect, the present disclosure provides a method of condensing a polynucleotide, including contacting the polynucleotide with a polypeptide of the present disclosure.

[0058] In at least one further aspect, the present disclosure provides a method of neutralizing the charge of a polynucleotide, including contacting the polynucleotide with a polypeptide of the present disclosure.

[0059] In at least one further ~~aspect~~example, the present disclosure provides a composition including an engineered polypeptide and at least one nucleic acid, the engineered polypeptide including a nucleic acid binding domain, a targeting domain, and a nucleic acid release domain, where one or more amino acids of the engineered polypeptide is a modified amino acid, optionally where the modification includes at least one of: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation; (ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; (xiv) pegylation; and/or (xv) any combination thereof; optionally where the composition is for use in delivering a nucleic acid to a subject or system.

[0060] In at least one further ~~aspect~~example, the present disclosure provides a composition including an engineered polypeptide and at least one nucleic acid, the engineered polypeptide including a nucleic acid binding domain and a targeting domain, where one or more amino acids of the engineered polypeptide is a modified amino acid, optionally where the modification includes at least one of: (i) phosphorylation; (ii) sulfation; (iii) glycosylation; (iv) prenylation; (v) methylation; (vi) sialylation; (vii) lipidation and/or lipoylation; (viii) acetylation; (ix) hydroxylation; (x) palmitoylation; (xi) mannosylation; (xii) myristoylation; (xiii) fucosylation; (xiv) pegylation; and/or (xv) any combination thereof; optionally where the composition is for use in delivering a nucleic acid to a subject or system. In some ~~embodiment~~examples, the engineered polypeptide comprises a targeting domain.

[0061] In at least one ~~aspect~~example, the present disclosure provides an engineered polypeptide that includes a nucleic acid binding domain and a targeting domain, which engineered polypeptide can be a mini-nucleosome core protein. A loaded mini-nucleosome can be or provide a non-viral vector that includes an engineered polypeptide (e.g., a mini-nucleosome core protein) as described herein and at least one nucleic acid molecule as provided herein or otherwise known in the art.

[0062] In some ~~embodiment~~examples, an engineered polypeptide (e.g., a mini-nucleosome core protein) that is or includes a nucleic acid binding domain is derived from a histone polypeptide sequence and/or a nucleic acid binding domain that is or includes the amino acid sequence KRHRK. In certain ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a nucleic acid binding domain that is or includes an amino acid sequence that includes KRHRK, RRRRR, RRLARR, KKAKAAAKPKK, KKDGKKRKR, KKKLK, KKRIRK, RKKSK, KKPKK, or a combination thereof, but not limited to it.

[0063] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a nucleic acid binding domain derived from any histone protein sequence or those described in Table 3 or a combination of the sequences described herein but not limited to it. These nucleic acid binding domains may be derived from various human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the “NABD” with changes to the amino acid sequence. One skilled in the art may also contemplate placing the

"NABD" in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0064] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a targeting domain that is a cell attachment targeting domain, a beta galactose binding domain, a fucose binding domain, a heparin binding domain, a sialic acid binding domain, a glycoprotein binding domain, a carbohydrate binding domain, a lysophosphatidic acid binding domain, a cAMP binding domain, a hyaluronan binding domain, a chondroitin sulfate binding domain, an integrin binding domain, a nucleolin binding domain, a collagen binding domain, a clathrin binding domain, a Fc receptor binding domain, an actin binding domain, an endocytosis motif, a nuclear localization signal, or a combination thereof but not limited to it. Some examples of those domain are described in Table 5 but is not limited to these. These domains may be derived from any human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the targeting domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the targeting domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0065] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a targeting domain that is an internalization domain wherein the internalization domain is or includes an amino acid sequence that includes FXDXF, PPSY, FEDNFVP, YIRV, YADW, YTQV, KKRPKP, SSDDE, RRASS, (YXXL)2, LPLTG, LAFTG, or a combination thereof but not limited to it. These domains may be derived from human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the internalization domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the internalization domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0066] Those of skill in the art will appreciate that, as used in protein sequences throughout the present specification, an "X" can refer to any amino acid unless otherwise specified. Thus, unless otherwise specified, an "X" is a placeholder for a single amino acid, which position could be filled by any single amino acid known to those of skill in the art.

[0067] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a cell attachment targeting domain that is or includes an amino acid sequence selected from WGREERQ, NTQIH, WNNKTPH, TPH, VNRWS, XBBBXXBX, ARKKAAKA, QRR, SRR, WEPSRPFPVD, HRRTRKAPKRIRLPHIR, KRTGQYKLGSKTGPGQK, KKTK, KLRSQLVKK, RRRCGQKKK, BX(7)B, RIQNLLKITNLRIKFVK, KKEKDIMKKTI, KGE, RGD, RGDS, TTVVNPKYEGK, ERMSQIKRLLS, WRHRARS, GFOGER, LFDLM, WGREERQ, QSTEKRG, LPNTG, and a combination thereof, where X can be any amino acid, but not limited to it.

[0068] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a targeting domain that is an internalization domain cell-type specific targeting domain wherein the cell-type specific targeting domain is or includes an amino acid sequence that includes ASSLNIA, KKEEEKKEEEKKEEE, LIFHKEQ, KFNKPFVFLI, QPEHSST, EYHHYNK, NGR, GEKGEP, KTKKK, KALKKK, KGKKK, CSVTCG, LRE, YKYNLNGRES, YRSL, $KGGK_7$, KKKQYTSIHHG, KDEL, LADQDYTKTA, or a combination thereof but not limited to it. These domains may be derived from human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the targeting domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the targeting domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0069] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a poly-arginine domain with varying length or multiple poly-arginine domains throughout the polypeptide sequence.

[0070] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a nuclear internalization signal or a nuclear import machinery binding domain. The engineered polypeptide, the nuclear internalization signal or a nuclear import machinery binding domain can be or include an amino acid sequence that includes KKKYKLK, KKRKLE, TRSK, HRKRKR, NKRKRK, AEKSKKK, RKSK, KRVK, KRK, LQQTPLHLAVI, RRPR, PRPR, RPPP, RKKRKGK, PAAKRVKLD, KLKIKRPVK, PKKKRKV, QRKRQK, DSPE, FQVT, QSTEKRG, RQGLID, Cyclic RKKH, or a combination thereof but not limited to it. These domains may be derived from human proteins or other organisms. One skilled in the art may

contemplate modifying or engineering the nuclear internalization signal with changes to the amino acid sequence. One skilled in the art may also contemplate placing the nuclear internalization signal in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0071] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a nucleic acid release domain. The nucleic acid release domain is or includes an amino acid sequence that includes GRKKRRQRRRPQ, KRH, KSVKKRSVSEIQ, NRRKKRAL, KFERQ, VRGP, NKDS, NRDN, ANNR, or a combination thereof but not limited to it. These domains may be derived from various proteins that are substrates of peptidases, enzymes or other proteins found in humans or other organisms. Some nucleic acid release domains may also be derived from autolysis sites of various proteins. One skilled in the art may contemplate modifying or engineering the nucleic acid release domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the nucleic acid release signal in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0072] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure further including a stability domain. In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure can include a stability domain that is or includes an amino acid sequence that includes YTRF, GDAY, LLEE, RKKRRQRRR, YKSL, YENF, FQDL, YIGSR, IKVAV, or a combination thereof but not limited to it. These domains may be derived from human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the stability domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the stability domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0073] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes an oligomerization domain. In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure can include an oligomerization domain is selected from the oligomerization domains of Table 11 but not limited to it. The position of oligomerization domain is positioned at the C-terminus of an engineered polypeptide of the present disclosure or at any other locations. These domains may be derived from human proteins or other organisms. One skilled in the art

may contemplate modifying or engineering the oligomerization domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the oligomerization domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0074] In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure includes a Linker. In some ~~embodiment~~examples, an engineered polypeptide of the present disclosure can include a Linker selected, without limitation, from the exemplary domains of Table 12. The position of linker in an engineered polypeptide of the present disclosure may be in between other domains and any other locations. These Linkers may be derived from human proteins or other organisms. One skilled in the art may contemplate modifying or engineering the linker domain with changes to the amino acid sequence. One skilled in the art may also contemplate placing the linker domain in reverse sequence or by switching amino acid positions within the domain or adding posttranslational modifications to amino acids.

[0075] In various ~~embodiment~~examples, two or more engineered polypeptides of the present disclosure can oligomerize.

[0076] In some ~~embodiment~~examples, the present disclosure includes a composition that includes an engineered polypeptide of the present disclosure (e.g., a mini-nucleosome core protein) together with at least one polynucleotide. In some ~~embodiment~~examples, the polypeptide is a DNA or RNA polynucleotide. In some ~~embodiment~~examples, the polypeptide is a or includes an inhibitory RNA, wherein the inhibitory RNA is a gRNA, siRNA, miRNA, or shRNA. In various ~~embodiment~~examples, the polypeptide(s) and polynucleotide(s) are not associated but are together in a composition, e.g., a kit or solution. In various ~~embodiment~~examples, the polypeptide(s) and polynucleotide(s) are associated, e.g., condensed, e.g., to form a loaded mini-nucleosome. In certain ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:2,000 or between 1:3 and 1:2,000. In certain ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:2,000. In certain ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:1,000, between 1:1 and 1:500, between 1:1 and 1:200, between 1:1 and 1: 100, between 1:1 and 1:50. In certain ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:3 and 1: 1,000, between 1:3 and 1:500, between 1:3 and 1:200, between 1:3 and 1: 100, or between 1:3 and 1:50. In certain

~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:200 and 1:2,000, between 1:200 and 1:1000, or between 1:200 and 1:500. In certain ~~embodiment~~examples, the ratio of polynucleotides to engineered polypeptides is between 1:1 and 1:50, 1:1 and 1:40, 1:1 and 1:30, 1:1 and 1:20, 1:1 and 1:10, 1:1 and 1:5, 1:1 and 1:4, 1:1 and 1:3, or 1:1 and 1:2. One skilled in the art may also contemplate chemical modifications to the DNA or RNA molecules.

[0077] In some ~~embodiment~~examples, a composition provided herein that includes a mini-nucleosome core protein and/or a loaded mini-nucleosome) can be administered to or contacted with a cell, tissue, or subject. The conditions of application may be in in vitro, ex vivo or in vivo. Such engineered cell may include a pharmaceutical carrier, e.g., that may be used in, or is compatible with, delivery of therapeutic materials (e.g., a composition provided herein that includes a mini-nucleosome core protein and/or a loaded mini-nucleosome) to various parts of human body for example brain, retina, gut, pancreas, lung etc. without any limitations.

[0078] In some ~~embodiment~~examples, a method of condensing a polynucleotide may include contacting a polynucleotide with a mini-nucleosome core protein as described herein. The method may include process of neutralizing the charge of a polynucleotide or condensation of the polynucleotide into nano-sized particles, including contacting the polynucleotide with a mini-nucleosome core protein described herein.

[0079] In some ~~embodiment~~examples, the mini-nucleosome core protein may be a branched peptide or a cyclic peptide but not limited to these characteristics. One skilled in the art may contemplate changing the characteristics of mini-nucleosome core protein to obtain enhanced tropism to various cell types.

[0080] The present disclosure further provides a polynucleotide encoding an engineered polypeptide (e.g., a mini-nucleosome core protein) as provided herein. The polynucleotide encoding the engineered polypeptide can be a DNA polynucleotide or an RNA polynucleotide. In some instances, the present disclosure provides a vector including a polynucleotide that encodes an engineered polypeptide of the present disclosure. In some ~~embodiment~~examples, the present disclosure provides a cell that includes a polynucleotide encoding an engineered polypeptide (e.g., a mini-nucleosome core protein) as provided herein, a vector including such polynucleotide, or includes the sequence of such polynucleotide. In certain ~~embodiment~~examples, an engineered polypeptide of the present disclosure can be isolated from one or more such cells.

[0081] In various ~~embodiment~~examples, one or more amino acids of an engineered polypeptide of the present disclosure has at least 80% sequence identity with an amino acid sequence selected from SEQ ID NOs: 336-388 (e.g., at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with an amino acid sequence selected from SEQ ID NOs: 336-388).

[0082] In various ~~embodiment~~examples, one or more amino acids of an engineered polypeptide of the present disclosure (e.g., a mini-nucleosome core protein) is pegylated, acetylated, methylated, glycosylated, phosphorylated, sumoylated, amidated, lipidated, prenylated, lipoylated, alkylated, acylated, glycated, nitrosylated, sulfated, carbamylated, carbonylated, neddylated, biotinylated, or ribosylated.

DEFINITIONS

[0083] *About:* The term "about," when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about" in that context. For example, in some embodiments, the term "about" may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referenced value.

[0084] *Administration:* As used herein, the term "administration" typically refers to administration of a composition to a subject or system to achieve delivery of an agent that is, or is included in, the composition. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc. In some particular embodiments, administration may be bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or include, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc.), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some

CLAIMS

1. An engineered polypeptide comprising:

(a) a nucleic acid binding domain, and

(b) a nucleic acid release domain or a targeting domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid that comprises, optionally wherein the modification comprises at least one of:

(i) phosphorylation;

(ii) sulfation;

(iii) glycosylation;

(iv) prenylation;

(v) methylation;

(vi) sialylation;

(vii) lipidation and/or lipoylation;

(viii) acetylation;

(ix) hydroxylation;

(x) palmitoylation;

(xi) mannosylation;

(xii) myristoylation;

(xiii) fucosylation; or

(xiv) pegylation; and/or

(xv) any combination thereof.

2. The engineered polypeptide of claim 1, wherein the engineered polypeptide comprises a targeting domain.

3. An engineered polypeptide comprising a nucleic acid binding domain and a targeting domain,

wherein one or more amino acids of the engineered polypeptide is a modified amino acid, optionally wherein the modification comprises at least one of:

(i) phosphorylation;

(ii) sulfation;

(iii) glycosylation;

(iv) prenylation;

(v) methylation;

~~(vi) sialylation;~~

~~(vii) lipidation and/or lipoylation;~~

~~(viii) acetylation;~~

~~(ix) hydroxylation;~~

~~(x) palmitoylation;~~

~~(xi) mannosylation;~~

~~(xii) myristoylation;~~

~~(xiii) fucosylation;~~

~~(xiv) pegylation; and/or~~

~~(xv) any combination thereof;~~

~~wherein engineered polypeptide optionally further comprises a nucleic acid release domain.~~

~~4. The engineered polypeptide of any one of claims 1-3, wherein each of two or more amino acids of the engineered polypeptide is a modified amino acid.~~

~~5. The engineered polypeptide of any one of claims 1-4, wherein at least one of the modified amino acids comprises a modification chain comprising two or more modifications selected from:~~

~~(i) phosphorylation;~~

~~(ii) sulfation;~~

~~(iii) glycosylation;~~

~~(iv) prenylation;~~

~~(v) methylation;~~

~~(vi) sialylation;~~

~~(vii) lipidation and/or lipoylation;~~

~~(viii) acetylation;~~

~~(ix) hydroxylation;~~

~~(x) palmitoylation;~~

~~(xi) mannosylation;~~

~~(xii) myristoylation;~~

~~(xiii) fucosylation;~~

~~(xiv) pegylation; and~~

~~(xv) any combination thereof.~~

~~6~~2. The engineered polypeptide of ~~any one of claims 1-5~~claim 1, wherein the modification increases:

(i) the stability of the engineered polypeptide,

(ii) the half-life of the engineered polypeptide,

(iii) the bioavailability of the engineered polypeptide,

(iv) the affinity of the engineered polypeptide with a binding partner,

(v) the avidity of the engineered polypeptide with a binding partner, or

(vi) a combination thereof.

~~the stability, half-life, and/or bioavailability of the engineered polypeptide.~~

~~7. The engineered polypeptide of any one of claims 1-6, wherein the modification increases the affinity and/or avidity of the engineered polypeptide with a binding partner, optionally wherein the binding partner is a receptor, cell, or cell membrane.~~

~~8. The engineered polypeptide of any one of claims 1-7, wherein the modification increases the affinity or avidity of the engineered polypeptide with a nucleic acid.~~

~~9. The engineered polypeptide of any one of claims 1-8, wherein the modification decreases precipitation and/or aggregation of the engineered polypeptide.~~

~~10. The engineered polypeptide of any one of claims 1-9, wherein the nucleic acid binding domain is derived from a histone polypeptide sequence.~~

~~11. The engineered polypeptide of any one of claims 1-10, wherein the nucleic acid binding domain is or comprises the amino acid sequence KRHRK.~~

~~12~~3. The engineered polypeptide of ~~any one of claims 1-11~~claim 1 or 2, wherein the nucleic acid binding domain is or comprises an amino acid sequence ~~that comprises~~of KRHRK, RRRRR, RRLARR, KKAKAAAKPKK, KKDGKKRKR, KKKLK, KKRIRK, RKKSK, KKPKK, or a combination thereof.

~~13. The engineered polypeptide of any one of claims 1-12, wherein the nucleic acid binding domain is a modified nucleic acid binding domain in that the nucleic acid binding domain comprises one or more modified amino acids.~~

~~14~~4. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 397-422, and wherein the targeting domain is phosphorylated.

~~15~~5. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 423-428, and wherein the targeting domain is sulfated.

~~16~~6. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 429-434, and wherein the targeting domain is glycosylated.

~~17. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 435-440, wherein the targeting domain is prenylated.~~

~~18. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 441-446, wherein the targeting domain is methylated.~~

~~19. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a targeting domain having the sequence of any one of SEQ ID NOs: 447-459, wherein the targeting domain is sialylated.~~

~~20. The engineered polypeptide of any one of claims 2-13, wherein the targeting domain is a cell attachment targeting domain, a beta galactose binding domain, a fucose binding domain, a heparin binding domain, a sialic acid binding domain, a glycoprotein binding domain, a carbohydrate binding domain, a lysophosphatidic acid binding domain, a cAMP binding domain, a hyaluronan binding domain, a chondroitin sulfate binding domain, an integrin binding domain, a nucleolin binding domain, a collagen binding domain, a clathrin binding domain, a Fc receptor binding domain, an actin binding domain, an endocytosis motif, a nuclear localization signal, or a combination thereof.~~

~~21~~7. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is a cell attachment targeting domain.

~~22~~8. The engineered polypeptide of claim ~~21~~7, wherein the cell attachment targeting domain is or comprises an amino acid sequence ~~that comprises~~of WGREERQ, NTQIH, WNNKTPH, TPH, VNRWS, XBBBXXBX, ARKKAAKA, QRR, SRR, WEPSRPFPVD, HRRTRKAPKRIRLPHIR, KRTGQYKLGSKTGPGQK, KKTK, KLRSQLVKK, RRRCGQKKK, BX(7)B, RIQNLLKITNLRIKFVK, KKEKDIMKKTI, KGE, RGD, RGDS, TTVVNPKYEGK, ERMSQIKRLLS, WRHRARS, GFOGER, LFDLM, WGREERQ, QSTEKRG, LPNTG, or a combination thereof.

~~23~~9. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is an internalization domain.

~~24~~10. The engineered polypeptide of claim ~~23~~9, wherein the internalization domain is or comprises an amino acid sequence ~~that comprises~~of FXDXF, PPSY, FEDNFVP, YIRV, YADW, YTQV, KKRPKP, SSDDE, RRASS, (YXXL)2, LPLTG, LAFTG, or a combination thereof.

~~25~~11. The engineered polypeptide of any one of claims ~~2-13~~1-3, wherein the targeting domain is a cell-type specific targeting domain.

~~26~~12. The engineered polypeptide of claims ~~25~~11, wherein the cell-type specific targeting domain is or comprises an amino acid sequence ~~that comprises~~of ASSLNIA, KKEEEKKEEEKKEEE, LIFHKEQ, KFNKPFVFLI, QPEHSST, EYHHYNK, NGR, GEKGEP, KTKKK, KALKKK, KGKKK, CSVTCG, LRE, YKYNLNGRES, YRSL, $KGGK_7$, KKKQYTSIHHG, KDEL, LADQDYTKTA, or a combination thereof.

~~27~~13. The engineered polypeptide of any one of claims 1-3~~2-26~~, wherein the targeting domain is a modified targeting domain in that the targeting domain comprises one or more modified amino acids.

~~28~~14. The engineered polypeptide of any one of claims 1-~~27~~13, wherein the nucleic acid release domain is or comprises an amino acid sequence ~~that comprises~~of GRKKRRQRRRPQ, KRH, KSVKKRSVSEIQ, NRRKKRAL, KFERQ, VRGP, NKDS, NRDN, ANNR, or a combination thereof.

~~29~~15. The engineered polypeptide of any one of claims 1-~~28~~14, wherein the nucleic acid release domain is a modified nucleic acid release domain ~~in that the nucleic acid release domain~~ comprising~~es~~ one or more modified amino acids.

~~30~~16. The engineered polypeptide of any one of claims 1-~~29~~15, further comprising a poly-arginine domain.

~~31. The engineered polypeptide of claim 30, wherein the poly-arginine domain is a modified poly-arginine domain in that the poly-arginine domain comprises one or more modified amino acids.~~

~~32~~17. The engineered polypeptide of any one of claims 1-~~31~~16, further comprising a nuclear internalization signal or a nuclear import machinery binding domain.

~~33. The engineered polypeptide of claim 32, wherein the nuclear internalization signal or nuclear import machinery binding domain is or comprises an amino acid sequence that comprises KKKYKLK, KKRKLE, TRSK, HRKRKR, NKRKRK, AEKSKKK, RKSK, KRVK, KRK, LQQTPLHLAVI, RRPR, PRPR, RPPP, RKKRKGK, PAAKRVKLD, KLKIKRPVK, PKKKRKV, QRKRQK, DSPE, FQVT, QSTEKRG, RQGLID, Cyclic RKKH, or a combination thereof.~~

~~34~~18. The engineered polypeptide of ~~claim 32 or claim 33~~claim 17, wherein the nuclear internalization signal or a nuclear import machinery binding domain ~~is a modified nuclear internalization signal or a nuclear import machinery binding domain in that the nuclear internalization signal or a nuclear import machinery binding domain~~ comprises one or more modified amino acids.

~~35~~19. The engineered polypeptide of any one of claims 1-~~34~~18, further comprising a stability domain.

~~36. The engineered polypeptide of claim 35, wherein the stability domain is or comprises an amino acid sequence that comprises YTRF, GDAY, LLEE, RKKRRQRRR, YKSL, YENF, FQDL, YIGSR, IKVAV, or a combination thereof.~~

~~37. The engineered polypeptide of claim 35 or 36, wherein the stability domain is a modified stability domain in that the stability domain comprises one or more modified amino acids.~~

~~38~~20. The engineered polypeptide of any one of claims 1-~~37~~19, further comprising an oligomerization domain.

~~39. The engineered polypeptide of claim 38, wherein the oligomerization domain is selected from the oligomerization domains of Table 11, optionally wherein the oligomerization domain is positioned at the C-terminus of the engineered polypeptide.~~

~~40. The engineered polypeptide of claim 38 or claim 39, wherein the oligomerization domain is a modified oligomerization domain in that the oligomerization domain comprises one or more modified amino acids.~~

~~41~~21. The engineered polypeptide of any one of claims 1-~~40~~20, wherein the polypeptide comprises a linker.

~~42. The engineered polypeptide of claim 41, wherein the linker is a linker according to any one of SEQ ID NOs: 154-250.~~

~~43. The engineered polypeptide of claim 41 or 42, wherein the linker is a modified linker in that the linker comprises one or more modified amino acids.~~

~~44. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a phosphorylated amino acid.~~

~~45. The engineered polypeptide of claim 44, wherein the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid.~~

~~46. The engineered polypeptide of claim 44 or claim 45, wherein the phosphorylated amino acid is present in a linker domain or targeting domain.~~

~~47. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a sulfated amino acid.~~

~~48. The engineered polypeptide of claim 47, wherein the sulfated amino acid is a serine, threonine, or tyrosine amino acid.~~

~~49. The engineered polypeptide of claim 47 or claim 48, wherein the sulfated amino acid is present in a linker domain or targeting domain.~~

~~50. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is an acetylated amino acid.~~

~~51. The engineered polypeptide of claim 50, wherein the acetylated amino acid is a lysine amino acid.~~

~~52. The engineered polypeptide of claim 50 or claim 51, wherein the acetylated amino acid is present in a linker domain or targeting domain.~~

~~53. The engineered polypeptide of any one of claims 1-43, wherein one or more amino acids of the engineered polypeptide is a mannosylated amino acid.~~

~~54. The engineered polypeptide of claim 53, wherein the mannosylated amino acid is a serine amino acid.~~

~~55. The engineered polypeptide of claim 53 or 54, wherein the mannosylated amino acid is present in a linker domain or targeting domain.~~

~~56~~22. A polynucleotide that encodes the amino acid sequence of an engineered polypeptide of any one of claims 1-~~55~~21.

~~57. The polynucleotide of claim 56, wherein the polynucleotide is DNA or RNA.~~

~~58. A vector comprising the polynucleotide of claim 56 or 57.~~

~~59~~23. A cell comprising an engineered polypeptide of any one of claims 1-~~55~~21 or~~,~~ a polynucleotide of claim ~~56 or 57, or a vector of claim 58~~22.

~~60. A method of making an engineered polypeptide of any one of claims 1-55, the method comprising expressing a polynucleotide of any one of claims 56-58 in a cell.~~

~~61. The method of claim 60, further comprising isolating the engineered polypeptide from the cell.~~

~~62~~24. A composition comprising:

(i) at least one polynucleotide, and

(ii) at least one engineered polypeptide of any one of claims 1-~~55~~21.

~~63. The composition of claim 62, wherein the at least one polynucleotide is or comprises DNA or RNA.~~

~~64. The composition of claim 62 or 63, wherein the at least one polynucleotide comprises a nucleotide sequence encoding a polypeptide.~~

~~65. The composition of any one of claims 62-64, wherein the at least one polynucleotide is or comprises mRNA.~~

~~66. The composition of claim 62 or 63, wherein the at least one polynucleotide comprises an inhibitory RNA.~~

~~67. The composition of claim 66, wherein the inhibitory RNA is a gRNA, siRNA, miRNA, or shRNA.~~

~~68. The composition of any one of claims 62-67, comprising at least two engineered polypeptides of any one of claims 1-55, wherein a first engineered polypeptide of any one of claims 1-55 is able to oligomerize with a second engineered polypeptide of any one of claims 1-55.~~

~~69. The composition of any one of claims 62-68, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:1 and 1:2,000.~~

~~70. The composition of claim 69, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:1 and 1:1,000, between 1:1 and 1:500, between 1:1 and 1:200, between 1:1 and 1: 100, between 1:1 and 1:50, between 1:3 and 1:1,000, between 1:3 and 1:500, between 1:3 and 1:200, between 1:3 and 1: 100, or between 1:3 and 1:50.~~

~~71. The composition of any one of claims 62-70, wherein the ratio of polynucleotides to engineered polypeptides of any one of claims 1-55 is between 1:200 and 1:2,000, between 1:200 and 1:1000, or between 1:200 and 1:500.~~

~~72. The composition of any one of claims 62-71, comprising a pharmaceutical carrier.~~

2573. ~~A method comprising administering a~~The composition of ~~any one of claims 62-72~~claim 24 for use in therapy ~~to a system, wherein the system is a cell, tissue, or subject~~.

26. An *ex vivo* or *in vitro* method comprising adding a composition of claim 24 to a system, wherein the system is a cell or a tissue.

~~74. The method of claim 73, wherein, after administration, the modification increases the stability, half-life, and/or bioavailability of the composition in the system.~~

~~75. The method of claim 73 or 74, wherein, after administration, the modification increases the affinity or avidity of the composition with a binding partner in the system, optionally wherein the binding partner is a receptor, cell, or cell membrane.~~

~~76. The method of any one of claims 73-75, wherein, after administration, the modification decreases precipitation and/or aggregation of the composition in the system.~~

~~77. The method of any one of claims 73-76, wherein, after administration, the modification increases the rate at which the composition enters one or more cells in the system.~~

~~78. The method of any one of claims 73-77, wherein, after administration, the modification increases delivery of the composition to one or more cells in the system.~~

~~79. The method of any one of claims 73-78, wherein, after administration, the modification increases delivery of the nucleic acid of the composition to one or more cells in the system.~~

~~80. The method of any one of claims 73-79, wherein the system is a mammalian subject and, after administration, the modification decreases accumulation of the composition in liver.~~

~~81. The method of any one of claims 73-80, wherein the system is a mammalian subject and, after administration, the modification increases the amount of composition that crosses the blood-brain barrier.~~

~~82. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a phosphorylated amino acid.~~

~~83. The method of claim 82, wherein the phosphorylated amino acid is a serine, threonine, or tyrosine amino acid.~~

~~84. The method of claim 82 or claim 83, wherein the phosphorylated amino acid is present in a linker domain or targeting domain.~~

~~85. The method of any one of claims 82-84, wherein the composition is delivered to cells of the central nervous system (CNS).~~

~~86. The method of any one of claims 82-84, wherein the composition is delivered to CNS neurons.~~

~~87. The method of any one of claims 82-84, wherein the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia.~~

~~88. The method of any one of claims 82-84, wherein the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells.~~

~~89. The method of any one of claims 82-88, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.~~

~~90. The method of any one of claims 82-89, wherein the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.~~

~~91. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a sulfated amino acid.~~

~~92. The method of claim 91, wherein the sulfated amino acid is a serine, threonine, or tyrosine amino acid.~~

~~93. The method of claim 91 or claim 92, wherein the sulfated amino acid is present in a linker domain or targeting domain.~~

~~94. The method of any one of claims 91-93, wherein the composition is delivered to cells of the central nervous system (CNS).~~

95. The method of any one of claims 91-93, wherein the composition is delivered to CNS neurons.

96. The method of any one of claims 91-93, wherein the composition is delivered to CNS astrocytes, microglia, oligodendrocytes, or glia.

97. The method of any one of claims 91-93, wherein the composition is delivered to spinal cord cells, optionally wherein the spina cord cells are spinal cord neurons or spinal cord glial cells.

98. The method of any one of claims 91-97, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.

99. The method of any one of claims 91-98, wherein the subject is a mammalian subject and the administration is intrathecal, intracranial, or intra-cisterna magna.

100. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is an acetylated amino acid.

101. The method of claim 100, wherein the acetylated amino acid is a lysine amino acid.

102. The method of claim 100 or claim 101, wherein the acetylated amino acid is present in a linker domain or targeting domain.

103. The method of any one of claims 100-102, wherein the composition is delivered to CNS neurons.

104. The method of any one of claims 100-102, wherein the composition is delivered to retinal cells.

105. The method of any one of claims 100-102, wherein the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells.

106. The method of any one of claims 100-102, wherein the composition is delivered to photoreceptor cells, optionally wherein the photoreceptor cells include one or both of rods and cones.

~~107. The method of any one of claims 100-106, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.~~

~~108. The method of any one of claims 100-107, wherein the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.~~

~~109. A method comprising administering a composition of any one of claims 62-72 to a cell, tissue, or subject, wherein one or more amino acids of the engineered polypeptide is a mannosylated amino acid.~~

~~110. The method of claim 109, wherein the mannosylated amino acid is a serine amino acid.~~

~~111. The method of claim 109 or claim 110, wherein the mannosylated amino acid is present in a linker domain or targeting domain.~~

~~112. The method of any one of claims 109-111, wherein the composition is delivered to CNS neurons.~~

~~113. The method of any one of claims 109-111, wherein the composition is delivered to retinal cells.~~

~~114. The method of any one of claims 109-111, wherein the composition is delivered to retinal neurons, optionally wherein the retinal neurons include one or more of photoreceptors, bipolar cells, retinal ganglion cells, horizontal cells, and amacrine cells.~~

~~115. The method of any one of claims 109-111, wherein the composition is delivered to photoreceptor cells include one or both of rods and cones.~~

~~116. The method of any one of claims 109-115, wherein the polynucleotide encodes an expression product that is expressed in cells to which the composition is delivered.~~

~~117. The method of any one of claims 109-116, wherein the subject is a mammalian subject and the administration is intravitreal, suprachoroidal, or subretinal.~~

~~118~~27. A method of condensing a polynucleotide, comprising contacting the polynucleotide with a polypeptide of any one of claims 1-~~55~~21.

~~119~~28. A method of neutralizing the charge of a polynucleotide, comprising contacting the polynucleotide with a polypeptide of any one of claims 1-~~55~~21.

~~120. A composition comprising an engineered polypeptide and at least one nucleic acid,~~

~~the engineered polypeptide comprising a nucleic acid binding domain and a nucleic acid release domain,~~

~~wherein one or more amino acids of the engineered polypeptide is a modified amino acid,~~ ~~optionally wherein the modification comprises at least one of:~~

~~(i) phosphorylation;~~

~~(ii) sulfation;~~

~~(iii) glycosylation;~~

~~(iv) prenylation;~~

~~(v) methylation;~~

~~(vi) sialylation;~~

~~(vii) lipidation and/or lipoylation;~~

~~(viii) acetylation;~~

~~(ix) hydroxylation;~~

~~(x) palmitoylation;~~

~~(xi) mannosylation;~~

~~(xii) myristoylation;~~

~~(xiii) fucosylation;~~

~~(xiv) pegylation; and/or~~

~~(xv) any combination thereof;~~

~~optionally wherein the composition is for use in delivering a nucleic acid to a subject or system.~~

~~121. The composition of claim 120, wherein the engineered polypeptide comprises a targeting domain.~~

~~122. A composition comprising an engineered polypeptide and at least one nucleic acid,~~

~~the engineered polypeptide comprising a nucleic acid binding domain and a targeting domain,~~

~~wherein one or more amino acids of the engineered polypeptide is a modified amino acid,~~ ~~optionally wherein the modification comprises at least one of:~~

~~(i) phosphorylation;~~

~~(ii) sulfation;~~

~~(iii) glycosylation;~~

(iv) prenylation;

(v) methylation;

(vi) sialylation;

(vii) lipidation and/or lipoylation;

(viii) acetylation;

(ix) hydroxylation;

(x) palmitoylation;

(xi) mannosylation;

(xii) myristoylation;

(xiii) fucosylation;

(xiv) pegylation; and/or

(xv) any combination thereof;

optionally wherein the composition is for use in delivering a nucleic acid to a subject or system.

123. The engineered polypeptide of any one of claims 1-55, the polynucleotide of any one of claims 56-57, the vector of claim 58, the cell of claim 59, the method of any one of claims 60-61, the composition of any one of claims 62-72, 120, 121, or 122, or the method of any one of claims 73-119, wherein the engineered polypeptide has at least 80% sequence identity with an amino acid sequence selected from SEQ ID NOs: 336-388.