CLAIMS

1. A composition comprising:
   (a) a membrane impermeable cargo that binds or is to be delivered to an intracellular biological target; and
   (b) a bioconjugate for mediating cytosolic/nuclear or intracellular delivery of said cargo, the bioconjugate comprising a synthetic peptide shuttle agent conjugated to a biocompatible non-anionic hydrophilic polymer.

2. The composition of claim 1, wherein the synthetic peptide shuttle agent comprises a core amphipathic alpha-helical motif at least 12 amino acids long having a solvent-exposed surface comprising a discrete positively-charged hydrophilic face and a discrete hydrophobic face (shuttle agent core motif).

3. The composition of claim 2, wherein the biocompatible non-anionic polymer is conjugated to the synthetic peptide shuttle agent N- and/or C-terminal with respect to said shuttle agent core motif (e.g., at the N or C terminus of the shuttle agent).

4. The composition of any one of claims 1 to 3, wherein conjugation of the biocompatible non-anionic hydrophilic polymer to the shuttle agent:
   (i) raises the minimum effective concentration of the shuttle agent as compared to a corresponding unconjugated shuttle agent (e.g., as determined *in vitro* in cultured HeLa cells);
   (ii) reduces the cytotoxicity of the shuttle agent as compared to a corresponding unconjugated shuttle agent (e.g., as determined *in vitro* in cultured HeLa cells);
   (iii) broadens the effective concentration window of the shuttle agent as compared to a corresponding unconjugated shuttle agent (e.g., as determined *in vitro* in cultured HeLa cells);
   (iv) alters the *in vivo* biodistribution of the shuttle agent and/or cargo as compared to a corresponding unconjugated shuttle agent; or
   (v) any combination of (i) to (iv).

5. The composition of any one of claims 1 to 4, wherein the concentration of the bioconjugate in the composition is sufficient to achieve increased delivery of the cargo to the intracellular biological target, as compared to a corresponding composition comprising an unconjugated synthetic peptide shuttle agent.

6. The composition of any one of claims 1 to 5, wherein the concentration of the bioconjugate in the composition is at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 μM.

7. The composition of any one of claims 1 to 6, wherein the biocompatible non-anionic hydrophilic polymer has a linear, branched, hyper-branched, or dendritic structure.

8. The composition of any one of claims 1 to 7, wherein the biocompatible non-anionic hydrophilic polymer is a polyether moiety, a polyester moiety, a polyoxazoline moiety, a polyvinylpyrrolidone moiety, a polyglycerol moiety, a polysaccharide moiety, any non-anionic derivative thereof, hydrophilic peptide or polypeptide linker moiety, a polysiloxane moiety, a polylysine moiety, a non-anionic polynucleotide analog moiety (e.g., a charge-neutral polynucleotide analog moiety having a phosphorodiamidate backbone, an amide (e.g., peptide) backbone, a methylphosphonate backbone, a neutral phosphotriester backbone, a sulfone backbone, or a triazole backbone; or a cationic polynucleotide analog moiety having an aminoalkylated phosphoramidate backbone, a guanidinium backbone, an S-methylthiourea backbone, or a nucleosyl amino acid (NAA) backbone), or any combination thereof.

9. The composition of any one of claims 1 to 8, wherein the biocompatible non-anionic hydrophilic polymer comprises a polyethylene glycol (PEG) moiety and/or a polyester moiety.

10. The composition of any one of claims 1 to 9, wherein biocompatible non-anionic polymer:

(a) has a mass of at least 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-, 21-, 22-, 23-, 24-, 25-, 26-, 27-, 28-, 29-, 30-, 31-, 32-, 33-, 34-, 35-, 36-, 37-, 38-, 39-, or 40-fold of the mass of the synthetic peptide shuttle agent;

(b) has a mass of between 1-, 2-, 3-, 4-, 5-fold to 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-, 21-, 22-, 23-, 24-, 25-, 26-, 27-, 28-, 29-, 30-, 31-, 32-, 33-, 34-, 35-, 36-, 37-, 38-, 39-, or 40-fold of the mass of the synthetic peptide shuttle agent;

(c) has a mass of between about 1 to 80 kDa, 1 to 70 kDa, 1 to 60 kDa, 1 to 50 kDa, 1 to 40 kDa, 2 to 80 kDa, 2 to 70 kDa, 2 to 60 kDa, 2 to 50 kDa, 2 to 40 kDa, 3 to 80 kDa, 3 to 70 kDa, 3 to 60 kDa, 3 to 50 kDa, 3 to 40 kDa, 4 to 80 kDa, 4 to 70 kDa, 4 to 60 kDa, 4 to 50 kDa, 4 to

40 kDa, 5 to 80 kDa, 5 to 70 kDa, 5 to 60 kDa, 5 to 50 kDa, 5 to 40 kDa, 5 to 35 kDa, 10 to 35 kDa, 10 to 30 kDa, 10 to 25 kDa, or 10 to 20 kDa; or

(d) any combination of (a) to (c).

11. The composition of any one of claims 1 to 10, wherein the biocompatible non-anionic hydrophilic polymer is conjugated to the synthetic peptide shuttle agent via a cleavable or non-cleavable linkage.

12. The composition of any one of claims 1 to 11, wherein the biocompatible non-anionic hydrophilic polymer is further conjugated to said cargo via a cleavable or non-cleavable linkage.

13. The composition of any one of claims 1 to 12, wherein the bioconjugate is a multimer comprising at least two synthetic peptide shuttle agents tethered together via said biocompatible non-anionic hydrophilic polymer.

14. The composition of claim 13, wherein:

(a) the multimer tethers together at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 synthetic peptide shuttle agents; tethers together up to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, or 256 synthetic peptide shuttle agents; and/or tethers together up to 2^n synthetic peptide shuttle agents, wherein n is any integer from 2 to 8; and/or

(b) the synthetic peptide shuttle agent monomer concentration in the composition is at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410,

420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1500, 2000, 2500, or 3000 μM.

15. The composition of claim 13 or 14, wherein the multimer comprises a branched, hyper-branched, or dendritic PEG or polyester core.

16. The composition of any one of claims 13 to 15, wherein the biocompatible non-anionic hydrophilic polymer comprises cleavable linkages enabling untethering of synthetic peptide shuttle agents following administration (e.g., when exposed to the reducing cellular environment).

17. The composition of any one of claims 1 to 16, wherein the cargo is not covalently linked to the synthetic peptide shuttle agent(s).

18. The composition of any one of claims 1 to 16, wherein the cargo is:

(a) covalently linked to the synthetic peptide shuttle agent(s) and/or to the biocompatible non-anionic hydrophilic polymer via a cleavable bond such that the cargo detaches therefrom through cleavage of said bond following administration (e.g., when exposed to the reducing cellular environment, and/or but prior to, simultaneously with, or shortly after being delivered intracellularly); or

(b) covalently linked to the synthetic peptide shuttle agent(s) and/or to the biocompatible non-anionic hydrophilic polymer via a non-cleavable bond.

19. The composition of any one of claims 1 to 18, wherein the cargo lacks a cell penetrating domain.

20. The composition of any one of claims 1 to 19, wherein the cargo is or comprises a therapeutic cargo and/or a diagnostic cargo.

21. The composition of any one of claims 1 to 20, wherein the cargo is or comprises a peptide, recombinant protein, nucleoprotein, polysaccharide, small molecule, non-anionic polynucleotide analog moiety (e.g., a charge-neutral polynucleotide analog moiety having a phosphorodiamidate backbone, an amide (e.g., peptide) backbone, a methylphosphonate backbone, a neutral phosphotriester backbone, a sulfone backbone, or a triazole backbone; or a cationic polynucleotide analog moiety having an

aminoalkylated phosphoramidate backbone, a guanidinium backbone, an S-methylthiourea backbone, or a nucleosyl amino acid (NAA) backbone), or any combination thereof.

22. The composition of any one of claims 1 to 21, wherein:

(a) the cargo is or comprises: a recombinant protein which is an enzyme, an antibody or antibody conjugate or antigen-binding fragment thereof, a transcription factor, a hormone, a growth factor; a nucleoprotein cargo which is a deoxyribonucleoprotein (DNP) or ribonucleoprotein (RNP) cargo (e.g., an RNA-guided nuclease, a Cas nuclease, such as a Cas type I, II, III, IV, V, or VI nuclease, or a variant thereof that lacking nuclease activity, a base editor, or a prime editor, a CRISPR-associated transposase, or a Cas-recombinase (e.g., recCas9), Cpf1-RNP, Cas9-RNP); or

(b) the biocompatible non-anionic hydrophilic polymer is or comprises: a phosphorodiamidate morpholino oligomer (PMO), a peptide nucleic acid (PNA), a methylphosphonate oligomer, or a short interfering ribonucleic neutral oligonucleotide (siRNN), and the cargo is an antisense synthetic oligonucleotide (ASO) comprised in the biocompatible non-anionic hydrophilic polymer.

23. The composition of any one of claims 1 to 22, wherein said shuttle agent core motif is sufficient to increase cytosolic/nuclear intracellular transduction of said cargo (e.g., *in vitro* in cultured cells such as HeLa cells) and comprises: a discrete positively-charged hydrophilic face harboring a cluster of positively charged residues on one side of the helix defining a positively charged angle of 40° to 160°, 40° to 140°, 60° to 140°, or 60° to 120° in Schiffer-Edmundson helical wheel representation; and/or a discrete hydrophobic face harboring a cluster of hydrophobic amino acid residues on an opposing side of the helix defining a hydrophobic angle of 140° to 280°, 160° to 260°, or 180° to 240° in Schiffer-Edmundson helical wheel representation.

24. The composition of claim 23, wherein:

(a) at least 20%, 30%, 40%, or 50% of the residues in the hydrophobic cluster are hydrophobic residues (e.g., hydrophobic residues selected from phenylalanine, isoleucine, tryptophan, leucine, valine, methionine, tyrosine, cysteine, glycine, and alanine; or selected from phenylalanine, isoleucine, tryptophan, and/or leucine);

(b) at least 20%, 30%, 40%, or 50% of the residues in the positively charged cluster are positively charged residues (e.g., positively charged residues selected from lysine and arginine)

(c) the shuttle agent core motif has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, or 5.5;

(d) the shuttle agent core motif has a maximal length of 14, 15, 16, 17, 18, 19, or 20 residues; or

(e) any combination of (a) to (d).

25. The composition of any one of claims 1 to 24, wherein the synthetic peptide shuttle agent is a peptide of between 15, 16, 17, 18, 19 or 20 to 150 amino acids in length, wherein any combination of at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or all of following parameters are respected:

- the peptide is soluble in aqueous solution (e.g., having a grand average of hydropathicity (GRAVY) index of less than -0.35, -0.40, -0.45, -0.50, -0.55, or -0.60);
- the hydrophobic face comprises a hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing 12 to 50% of the amino acids of the peptide, based on an open cylindrical representation of the alpha-helix having 3.6 residues per turn;
- the peptide has a hydrophobic moment (μH) of 3.5 to 11;
- the peptide has a predicted net charge of +3, +4, +5, +6, +7, +8, +9 to +10, +11, +12, +13, +14, or +15 at physiological pH;
- the peptide has an isoelectric point (pI) of 8 to 13 or 10 to 13;
- the peptide is composed of 35% to 65% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;
- the peptide is composed of 0% to 30% of any combination of the amino acids: N, Q, S, and T;
- the peptide is composed of 35% to 85% of any combination of the amino acids: A, L, K, or R;
- the peptide is composed of 15% to 45% of any combination of the amino acids: A and L, provided there being at least 5% of L in the peptide;
- the peptide is composed of 20% to 45% of any combination of the amino acids: K and R;
- the peptide is composed of 0% to 10% of any combination of the amino acids: D and E;
- the difference between the percentage of A and L residues in the peptide (% A+ L), and the percentage of K and R residues in the peptide (K + R), is less than or equal to 10%; and

– the peptide is composed of 10% to 45% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T and H, and preferably less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of D and/or E.

26. The composition of any one of claims 1 to 25, wherein synthetic peptide shuttle agent comprises a histidine-rich domain, optionally wherein the histidine-rich domain is: (i) positioned towards the N terminus and/or towards the C terminus of the shuttle agent; (ii) is a stretch of at least 3, at least 4, at least 5, or at least 6 amino acids comprising at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% histidine residues; and/or comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 consecutive histidine residues; or (iii) both (i) and (ii).

27. The composition of any one of claims 1 to 26, wherein said synthetic peptide shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues (e.g., separating N-terminal and a C-terminal segments of the shuttle agent; or positioned N- and/or C-terminal of said shuttle agent core motif).

28. The composition of any one of claims 1 to 27, wherein the synthetic peptide shuttle agent: does not comprise a cell penetrating domain (CPD), a cell-penetrating peptide (CPP), or a protein transduction domain (PTD); or does not comprise a CPD fused to an endosome leakage domain (ELD).

29. The composition of any one of claims 1 to 28, wherein the shuttle agent comprises an endosome leakage domain (ELD) and/or a cell penetrating domain (CPD).

30. The composition of any one of claims 1 to 29 for use in therapy, wherein the composition is formulated to be administered *in vivo*.

31. The composition as defined in any one of claims 1 to 29 for use in therapy, wherein the composition is formulated to be administered

(a) intravenously or parenterally (e.g., intrathecal), or intranasally; or
(b) to target organs or tissues (e.g., lung, liver, pancreas, spleen, heart, brain, bladder, and/or kidney) contacting or proximal to bodily fluids and/or secretions (e.g., mucus membranes, such as those lining the respiratory tract); or
(c) both (a) and (b).

32. The composition as defined in any one of claims 1 to 29 for use in therapy, wherein the cargo is a therapeutic cargo (e.g., that binds or is to be delivered to an intracellular therapeutic target), or for the manufacture of a medicament for treating a disease or disorder ameliorated by cytosolic/nuclear and/or intracellular delivery of the cargo in a subject.

33. A bioconjugate as defined in any one of claims 1 to 29.

34. The bioconjugate of claim 33, wherein the synthetic peptide shuttle agent is conjugated via a non-cleavable bond to a cargo for intracellular delivery; or wherein the synthetic peptide shuttle agent is conjugated via a cleavable bond to a cargo for intracellular delivery, preferably such that the cargo detaches therefrom through cleavage of said bond, thereby enabling the cargo to be delivered to the cytosol/nucleus.

35. The bioconjugate of claim 34, wherein the synthetic peptide shuttle agent comprises a core amphipathic alpha-helical motif at least 12 amino acids long having a solvent-exposed surface comprising a discrete positively-charged hydrophilic face and a discrete hydrophobic face (shuttle agent core motif), and wherein the cargo is conjugated to the synthetic peptide shuttle agent N- and/or C-terminal with respect to said shuttle agent core motif, preferably such that the cargo detaches therefrom through cleavage of said bond or degradation of the shuttle agent, thereby enabling the cargo to be delivered to the cytosol/nucleus.

36. The bioconjugate of any one of claims 33 to 35, wherein:

(a) the shuttle agent is conjugated to the cargo via the biocompatible non-anionic hydrophilic polymer;
(b) the shuttle agent is conjugated to the cargo is as defined in any one of claim 17 to 22;
(c) the shuttle agent is as defined in any one of claims 23 to 27; or
(d) any combination of (a) to (c).

37. The bioconjugate of any one of claims 33 to 36 for use in cargo transduction to the cytosol/nucleus of target eukaryotic cells.

38. A cargo comprising a D-retro-inverso nuclear localization signal peptide conjugated to a detectable label (e.g., a fluorophore).

39. The cargo of claim 38 for use in intracellular delivery.

40. A composition comprising a synthetic peptide shuttle agent covalently conjugated in a cleavable or non-cleavable fashion to a membrane impermeable cargo that binds or is to be delivered to an intracellular biological target, wherein:

(a) the shuttle agent is as defined in any one of claims 1 or 23 to 29;

(b) the membrane impermeable cargo is as defined in any one of claims 19 to 22;

(c) the shuttle agent is conjugated to the cargo in a manner as defined in claim 18;

(d) the shuttle agent is at concentration of at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 μM;

(e) the composition is for the use as defined in any one of claims 30 to 32; or

(f) any combination of (a) to (e).

41. The composition as defined in any one of claims 1 to 32 or 40, wherein the composition is formulated to be intranasally administered, wherein the cargo is a therapeutic cargo for treating or preventing a lung or respiratory disease or disorder (e.g., cystic fibrosis, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), or lung cancer).

42. The composition of claim 41, further comprising a mucolytic agent, an anti-inflammatory agent (e.g., steroid), a bronchodilator (e.g., albuterol), an antibiotic (e.g., aminoglycoside), or any combination thereof.

43. The composition of claim 41 or 42, formulated for inhalation such as in a nebulizer or an inhaler (e.g., metered dose inhaler or dry powder inhaler).

44. The composition as defined in any one of claims 1 to 32 or 34 to 43, or the bioconjugate as defined in any one of claims 33 to 39:

(a) for use in delivering the membrane impermeable cargo to an intracellular biological target, wherein the composition is formulated to be intravenously administered; or

(b) for use in delivering the membrane impermeable cargo to an intracellular biological target in the lungs, wherein the composition is formulated to be intranasally administered.