WHAT IS CLAIMED IS:

1. A method of loading a payload to an extracellular vesicle (EV) comprising mixing the payload with the EV in a loading buffer comprising a salt at a concentration lower than about 200 mM, about 190 mM, about 180 mM, about 170 mM, about 160 mM, about 150 mM, about 140 mM, about 130 mM, about 120 mM, or about 110 mM.

2. The method of claim 1, wherein the salt comprises NaCl (sodium chloride), KCl (potassium chloride), PO_4 (phosphate salt), $CaCl_2$ (calcium chloride), $MgCl_2$ (magnesium chloride), Mg_2SO_4 (magnesium sulfate), $ZnCl_2$ (zinc chloride), $MnCl_2$ (manganese chloride), $MnSO_4$ (manganese sulfate), NaSCN (sodium thiocyanate), KSCN (potassium thiocyanate), LiCl (lithium chloride), K_2HPO_4 (dipotassium phosphate), Na_2SO_4 (sodium sulfate), $NaPO_4$ (sodium phosphate), K_2SO_4 (potassium phosphate), sodium acetate, sodium bromide, sodium iodide, potassium bromide, lithium bromide, sodium fluoride, potassium fluoride, lithium fluoride, lithium iodide, potassium acetate, lithium acetate, potassium iodide, calcium sulfate, sodium sulfate, chromium trichloride, chromium sulfate, sodium citrate, iron (III) chloride, yttrium (III) chloride, potassium sulfate, ferrous chloride, calcium citrate, magnesium phosphate, ferric chloride, arginine-HCl, or any combination thereof, wherein the loading buffer comprising the salt increases the loading of the payload to the EV by at least about 2 fold compared to a corresponding loading buffer without the salt or with a lower concentration of the salt.

3. The method of claim 1 or 2, further comprising mixing the payload with the EV at a loading temperature higher than 4 ºC, wherein the loading temperature increases the loading of the payload to the EV by at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold or more compared to the loading at a reference temperature, which is lower than the loading temperature.

4. The method of any one claims 1 to 3, further comprising mixing the payload with the EV for a loading duration longer than about one hour, wherein the loading duration increases the loading of the payload to the EV by at least about 2 fold, at least about 3 fold, at least about 4 fold,

at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold or more compared to the loading for a reference duration, which is shorter than the loading duration.

5. A method of loading a payload to an extracellular vesicle (EV) comprising mixing the payload with the extracellular vesicle in a loading buffer comprising a salt at a concentration of between about 1 mM and about 450 mM, at a loading temperature of between about 5 ºC to about 70 ºC, and for a loading duration between about 1 hour and about seven days.

6. The method of any one of claims 1 to 5, further comprising, after the mixing, measuring the amount of payload loaded to the EVs and determining the load density of the EVs, wherein one or more loading parameters are adjusted to further increase the load density of the EVs, and wherein the one or more loading parameters are selected from: a salt concentration of the loading buffer, loading temperature, loading duration, payload feed concentration, EV feed concentration, or a combination thereof.

7. A method of decreasing the dissociation of a payload from an extracellular vesicle (EV) after EV loading comprising loading the payload to the EV according to the method of any one of claims 1 to 6, wherein the dissociation of the payload from the EV is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% compared to the corresponding dissociation observed when the payload is not loaded to the EV.

8. A method of increasing the stability of an extracellular vesicle (EV) loaded with a payload comprising loading the payload to the EV according to the method of any one of claims 1 to 6, wherein the stability of the EV loaded with the payload is increased by at least about at least about 1 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 12 fold, at least about 14 fold, at least about 16 fold, at least about 18 fold, at least about 20 fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about

40-fold, at least about 45-fold, or at least about 50-fold or more compared to the corresponding stability observed when the payload is not loaded to the EV.

9. A method of improving one or more properties of an extracellular vesicle after loading with a payload comprising loading the payload to the EV according to the method of any one of claims 1 to 6, wherein the one or more properties comprise: inter-particle interaction, particle rigidity, particle size, or a combination thereof, and wherein the improvement in the one or more properties allow for increased filterability of the EV.

10. The method of any one of claims 1 to 9, wherein the loading buffer further comprises one or more of the following components: tris, sucrose, glucose, trehalose, mannose, sorbitol, mannitol, glycerol, histidine, arginine, methionine, tryptophan, tyrosine, sodium phosphate, potassium phosphate, polymers, surfactants (*e.g.*, polysorbates 80 and 20), chelating agents (*e.g.*, EDTA, citrate), inorganic acids, or any combination thereof.

11. The method of any one of claims 1 to 10, wherein the payload comprises a peptide, a small molecule, an oligonucleotide, an antisense oligonucleotide (ASO), a PMO, an mRNA, an miRNA, an lcRNA, an antagomir, a tRNA, a siRNA, peptide nucleic acid, a cell penetrating peptide, an adjuvant, a protein, a carbohydrate, a sugar, an amino acid, or any combination thereof, and wherein the payload in the loading buffer is at a concentration between about 100 and about 2500 µM.

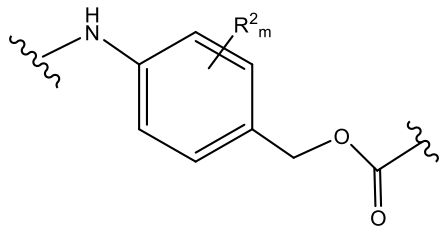
12. The method of any one of claims 1 to 11, wherein the payload is conjugated to an anchoring moiety, wherein the anchoring moiety comprises a sterol, GM1, a lipid, a phospholipid, a vitamin, a small molecule, a peptide, or a combination thereof.

13. The method of claim 12, wherein the anchoring moiety further comprises a linker between the payload and the anchoring moiety, wherein the linker has the formula:

$-A_a-Y_y-$

wherein each –A- is independently an amino acid unit, a is independently an integer from 1 to 12; -Y- is a spacer unit, and y is 0, 1, or 2,

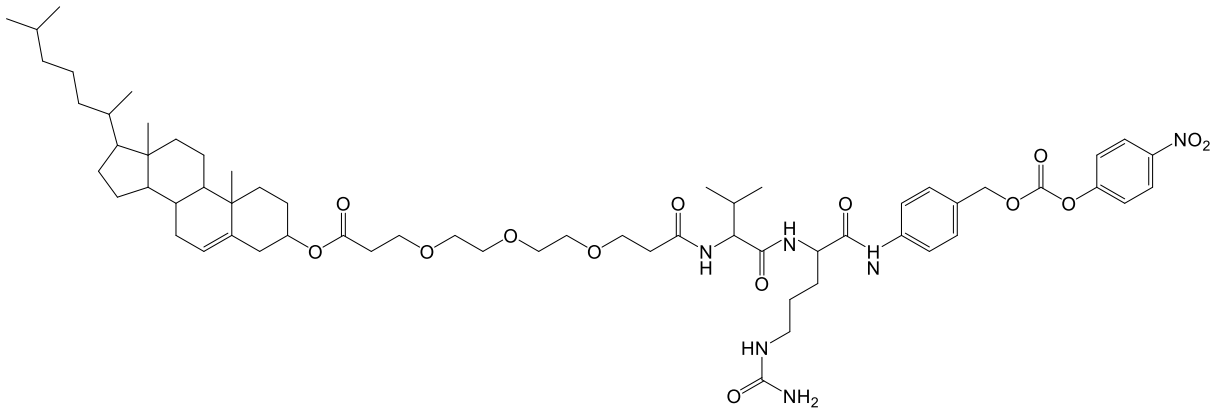
wherein $–Y_y-$ has the formula (V):



(V),

wherein each R^2 is independently C_{1-8} alkyl, -O-(C_{1-8} alkyl), halogen, nitro, or cyano; and m is an integer from 0 to 4.

14. The method of claim 12, wherein the anchoring moiety comprises:



15. The method of claim 13, which comprises (i) a payload comprising the nucleotide sequence set forth in any one of SEQ ID NOs: 41 to 112, and (ii) a linker selected from the linker combinations of TABLE 1 or TABLE 2, wherein the payload is conjugated to a cholesterol.

16. The method of claim 15, wherein the payload comprises 5'GbsCbsAbsdAsdGsdAsdTs(5MdC)s(5MdC)s(5MdC)sdGsdGsdAsdTsdTs(5MdC)sdGsGbsTbs Cb3', wherein Nb is an LNA, dN is a DNA, (5MdC) is 5-Methyl-dC, s is phosphorothioate, TEG is triethylene glycol, and HEG is hexaethylene glycol.

17. The method of any one of claims 1 to 16, wherein the EV further comprises a protein in the membrane of the EV, wherein the protein comprises prostaglandin F2 receptor negative regulator (the PTGFRN protein); basigin (the BSG protein); immunoglobulin superfamily member 2 (the IGSF2 protein); immunoglobulin superfamily member 3 (the IGSF3 protein); immunoglobulin superfamily member 8 (the IGSF8 protein); integrin beta-1 (the ITGB1 protein); integrin alpha-4 (the ITGA4 protein); 4F2 cell-surface antigen heavy chain (the SLC3A2 protein);

a class of ATP transporter proteins (the ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4 proteins); a functional fragment thereof; and any combination thereof.

18. An extracellular vesicle (EV) prepared by the method of any one of claims 1 to 17.

19. A pharmaceutical composition comprising the EV of claim 18.

20. Use of the EV of claim 18 or pharmaceutical composition of claim 19 in the manufacture of a medicament for the treatment of a disease or condition in a subject in need thereof.