CLAIMS

1. Isolated extracellular vesicles comprising an exogenous cargo molecule, wherein the exogenous cargo molecule comprises an active agent chemically bound to at least one carbohydrate or derivative thereof, wherein the active agent is selected from a small molecule, protein, peptide, polypeptide, lipid, and a nucleic acid.

2. The isolated EVs according to claim 1, wherein the carbohydrate is selected from a monosaccharide, disaccharide, trisaccharide, tetrasaccharide and oligosaccharide or wherein the carbohydrate derivative is selected from a saccharide linked to an amino acid, polyphenol, or lipid.

3. The isolated EVs according to claim 2, wherein the monosaccharide is selected from glucose, ribose, arabinose, galactose, mannose, and xylose; the disaccharide is selected from sucrose, lactose and maltose; the trisaccharide is selected from maltotriose and raffinose; a saccharide linked to an amino acid is D-ribose-L-cysteine; a saccharide linked with a polyphenol is selected from (–)-epigallocatechin gallate 3′-O-α-D-glucoside, isoquercitrin, baicalin and puerarin; and a saccharide linked with a lipid is a cerebroside, such as glucocerebroside, optionally wherein the active agent is bound to the carbohydrate via a linker.

4. The isolated EVs according to any one of claims 1 to 3, wherein the active agent is a nucleic acid.

5. The isolated EVs according to claim 4, wherein the nucleic acid is an oligonucleotide, optionally wherein the oligonucleotide is selected from RNA, RNAi, siRNA, shRNA, miRNA, and or short activating RNA (saRNA).

6. The isolated EVs cargo according to any one of claims 1 to 5, wherein the active agent is covalently bound to a carbohydrate or derivative thereof via a cleavable linkage.

7. A method of loading isolated extracellular vesicles (EVs) with cargo molecules, comprising incubating a population of EVs with the carbohydrate molecules as an active agent or with cargo molecules comprising an active agent chemically bound to at least one carbohydrate or derivative thereof, wherein the active agent is selected from a small molecule, protein, peptide, lipid, polypeptide, and a nucleic acid.

8. The method according to claim 7, wherein the carbohydrate is selected from monosaccharide, disaccharide, trisaccharide, tetrasaccharide and oligosaccharide and wherein the carbohydrate derivative is selected from an amino acid, polyphenol, and lipid linked to a carbohydrate, optionally wherein the active agent is bound to said carbohydrate via a linker.

9. The method according to claim 8, wherein:

(i) the monosaccharide is selected from glucose, ribose, arabinose, galactose, mannose, and xylose;

(ii) the disaccharide is selected from sucrose, lactose and maltose;

(iii) the trisaccharide is maltotriose; or

(iv) the derivative comprising a saccharide linked with an amino acid is from D-ribose-L-cysteine;

(v) derivative comprising a saccharide linked with a polyphenol is selected from (–)-epigallocatechin gallate 3′-O-α-D-glucoside, isoquercitrin, baicalin, puerarin, flavonoids, and isoflavonoids;

(vi) the derivative comprising a saccharide linked with a lipid is cerebroside, optionally wherein the cerebroside is glucocerebroside.

10. The method according to claim 7, characterized by at least one of:

(i) the linker is DBCO-C6-acid;

(ii) the active agent molecule is a nucleic acid;

(iii) the active agent molecule is a nucleic acid is an oligonucleotide, optionally wherein the oligonucleotide is selected from RNA, RNAi, siRNA, shRNA, saRNA, and miRNA inhibitors;

(iv) wherein the method is performed in the presence of insulin, optionally wherein the amount of the loaded exogenous cargo molecule in the resulting EVs is at least 20% higher than in EVs loaded in the absence of insulin;

(v) the EVs are exosomes; or

(vi) wherein the extracellular vesicles are derived from adherent cells expressing mesenchymal markers, optionally wherein the adherent cells expressing mesenchymal markers are mesenchymal stem cells (MSC).

11. The method according to claim 7, wherein the active agent molecule is a nucleic acid bound to a carbohydrate selected from glucose and sucrose.

12. The method according to any one of claims 7 to 11, wherein the method comprising electroporation or the use of a transfection reagent such as a lipid transfection reagent or wherein the method takes place in the absence of electroporation and in the absence of a transfection reagent.

13. Isolated EVs obtainable or obtained by the method of any one of claims 7 to 12.

14. A pharmaceutical composition comprising a population of the isolated EVs comprising an exogenous cargo molecule comprising an active agent according to any one of claims 1 to 6 or claim 13, and a pharmaceutically acceptable carrier and/or excipient.

15. An exogenous conjugate molecule comprising a nucleic acid covalently bound to at least one carbohydrate or derivative thereof.

16. The conjugate molecule according to claim 15, wherein the carbohydrate is selected from a monosaccharide, disaccharide, trisaccharide, tetrasaccharide and oligosaccharide and wherein the carbohydrate derivative is selected from a saccharide linked to an amino acid, polyphenol, or lipid, optionally wherein the monosaccharide is selected from glucose, ribose, arabinose, galactose, mannose, and xylose; the disaccharide is selected from sucrose lactose and maltose; the trisaccharide is selected from maltotriose and lactose; a saccharide linked to an amino acid is D-ribose-L-cysteine; a saccharide linked with a polyphenol is selected from (–)-epigallocatechin gallate 3′-O-α-D-glucoside, isoquercitrin, baicalin and puerarin; and a saccharide linked with a lipid is a cerebroside, such as glucocerebroside.

17. The conjugate molecule according to any one of claims 15 to 16, characterized by at least one of:

(i) the nucleic acid is bound to a carbohydrate via a linker, optionally via a cleavable linker; or

(ii) the nucleic acid is an oligonucleotide.

18. The conjugate molecule according to claim 17, wherein the oligonucleotide is selected from RNA, RNAi, siRNA, shRNA, miRNA, and saRNA.

19. The conjugate molecule according to claim 18, wherein the oligonucleotide is siRNA.

20. The conjugate molecule according to claim 19, wherein the conjugate comprises siRNA bound to a carbohydrate selected from glucose and sucrose.