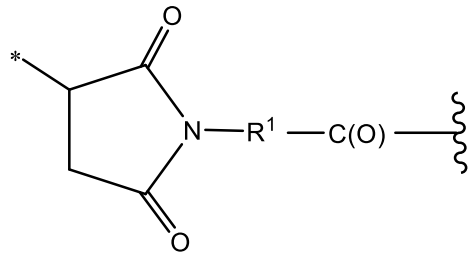
WHAT IS CLAIMED IS:

1. An extracellular vesicle (EV) comprising a biologically active molecule covalently linked to the EV via a maleimide moiety.

2. The extracellular vesicle of claim 1, wherein the maleimide moiety has the formula (I):



(I),

wherein

R^1 is selected from the group consisting of -C_{1-10} alkylene-, -C_{3-8} carbocyclo-, -O-(C_{1-8} alkylene)-, -arylene-, -C_{1-10} alkylene-arylene-, -arylene-C_{1-10} alkylene-, -C_{1-10} alkylene-(C_{3-8} carbocyclo)-, -(C_{3-8} carbocyclo)-C_{1-10} alkylene-, -C_{3-8} heterocyclo-, -C_{1-10} alkylene-(C_{3-8} heterocyclo)-, -(C_{3-8} heterocyclo)-C_{1-10} alkylene-, -$(CH_2CH_2O)_r$-, and -$(CH_2CH_2O)_r$-CH_2-;

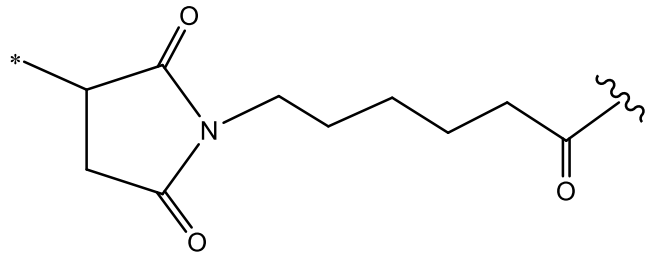
r is an integer from 1 to 10;

* indicates the covalent attachment site of the maleimide moiety to the EV; and,

the wavy line indicates the attachment site of the maleimide moiety to the biologically active molecule.

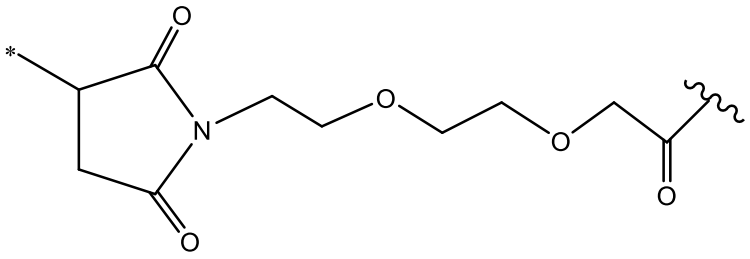
3. The extracellular vesicle of claim 2, wherein R^1 is –$(CH_2)_s$-, wherein s is 4, 5, or 6.

4. The extracellular vesicle of claim 2 or 3, wherein the maleimide moiety has the formula (II), where R^1 is –$(CH_2)_5$-:



(II).

5. The extracellular vesicle of claim 2, wherein the maleimide moiety has the formula (III), where R^1 is -$(CH_2CH_2O)_r$-CH_2-, where r is 2:



(III).

6. The extracellular vesicle of any one of claims 1-5, wherein the maleimide moiety is covalently linked to a functional group present on the EV, wherein the functional group is a sulfhydryl group.

7. The extracellular vesicle of claim 6, wherein the sulfhydryl group is on a protein on the surface of the EV.

8. The extracellular vesicle of any one of claims 1-7, wherein the maleimide moiety is linked to the biologically active molecule by a linker.

9. The extracellular vesicle of claim 8, wherein the linker comprises a cleavable linker.

10. The extracellular vesicle of claim 9, wherein the cleavable linker is cleaved by a protease.

11. The extracellular vesicle of claim 10, wherein the protease is a cathepsin.

12. The extracellular vesicle of claim 8, wherein the linker is a reduction-sensitive linker, or an acid labile linker.

13. The extracellular vesicle of any one of claims 8-12, wherein the linker has the formula (IV):

$$-A_a-Y_y- \qquad \text{(IV)},$$

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.

14. The extracellular vesicle of claim 13, wherein $-A_a-$ is a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, or a hexapeptide.

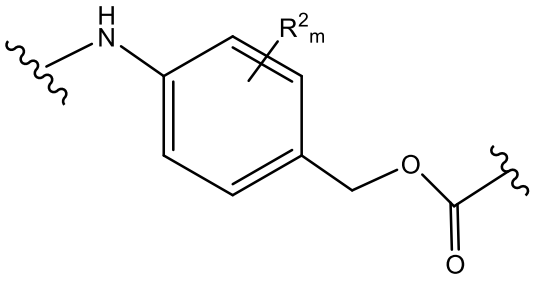
15. The extracellular vesicle of claim 14, wherein a is 2 and $–A_a-$ is selected from the group consisting of valine-alanine, valine-citrulline, phenylalanine-lysine, N-methylvaline-citrulline, cyclohexylalanine-lysine, and beta-alanine-lysine.

16. The extracellular vesicle of claim 15, wherein said $–A_a-$ is valine-alanine or valine-citrulline.

17. The extracellular vesicle of any one of claims 13-16, wherein y is 1.

18. The extracellular vesicle of any one of claims 13-17, wherein –Y- is a self-immolative spacer.

19. The extracellular vesicle of claim 18, 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.

20. The extracellular vesicle of claim 19, wherein m is 0, 1, or 2.

21. The extracellular vesicle of claim 20, wherein m is 0.

22. The extracellular vesicle of any one of claims 8-21, wherein the cleavable linker is valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.

23. The extracellular vesicle of any one of claims 13-17, wherein –Y- is a non self-immolative spacer.

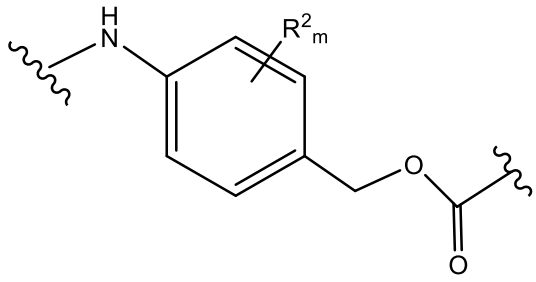
24. The extracellular vesicle of claim 23, wherein the non self-immolative spacer is –Gly- or –Gly-Gly-.

25. The extracellular vesicle of claim 8, wherein the linker is an acid labile linker.

26. The extracellular vesicle of claim 25, wherein the acid labile linker comprises a cis-aconitic linker, a hydrazide linker, a thiocarbamoyl linker, or any combination thereof.

27. The extracellular vesicle of claim 25 or 26, wherein the acid labile linker comprises a spacer unit to link the biologically active molecule to the acid labile linker.

28. The extracellular vesicle of claim 27, wherein the spacer unit has the formula (V):



(V),

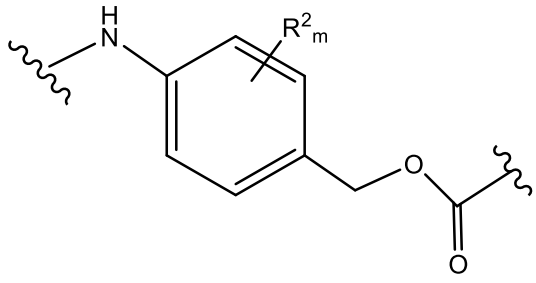
wherein each R^2 is independently C_{1-8} alkyl, -O-(C_{1-8} alkyl), halogen, nitro, or cyano; and m is an integer from 0 to 4.

29. The extracellular vesicle of claim 8, wherein the linker is a non-cleavable linker.

30. The extracellular vesicle of claim 29, wherein the non-cleavable linker comprises tetraethylene glycol (TEG), polyethylene glycol (PEG), succinimide, or any combination thereof.

31. The extracellular vesicle of claim 29 or 30, wherein the non-cleavable linker comprises a spacer unit to link the biologically active molecule to the non-cleavable linker.

32. The extracellular vesicle of claim 31, wherein the spacer unit has the formula (V):



(V),

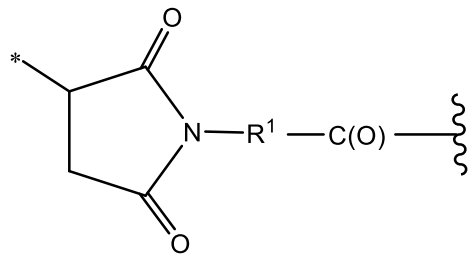
wherein each R^2 is independently C_{1-8} alkyl, -O-(C_{1-8} alkyl), halogen, nitro, or cyano; and m is an integer from 0 to 4.

33. An extracellular vesicle comprising a biologically active molecule and a cleavable linker, wherein the cleavable linker connects the EV to the biologically active molecule.

34. The extracellular vesicle of claim 33, wherein the cleavable linker comprises valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.

35. The extracellular vesicle of claim 33 or 34, further comprising a maleimide moiety, which links the EV to the cleavable linker via a functional group present on the EV.

36. The extracellular vesicle of claim 35, wherein the maleimide moiety has the formula (I):



(I), wherein

R^1 is selected from the group consisting of -C_{1-10} alkylene-, -C_{3-8} carbocyclo-, -O-(C_{1-8} alkylene)-, -arylene-, -C_{1-10} alkylene-arylene-, -arylene-C_{1-10} alkylene-, -C_{1-10} alkylene-(C_{3-8} carbocyclo)-, -(C_{3-8} carbocyclo)-C_{1-10} alkylene-, -C_{3-8} heterocyclo-, -C_{1-10} alkylene-(C_{3-8} heterocyclo)-, -(C_{3-8} heterocyclo)-C_{1-10} alkylene-, -$(CH_2CH_2O)_r$-, and -$(CH_2CH_2O)_r$-CH_2-;

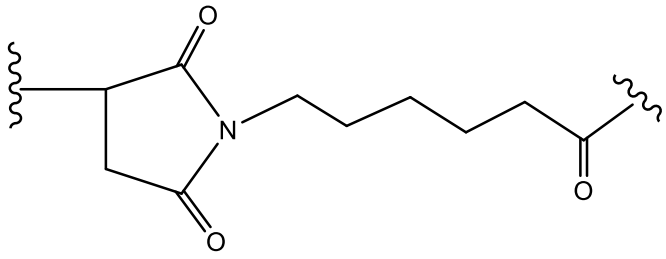
r is an integer from 1 to 10; and

* indicates the covalent attachment site of the maleimide moiety to the EV; and,

the wavy line indicates the attachment site of the maleimide moiety to the biologically active molecule.

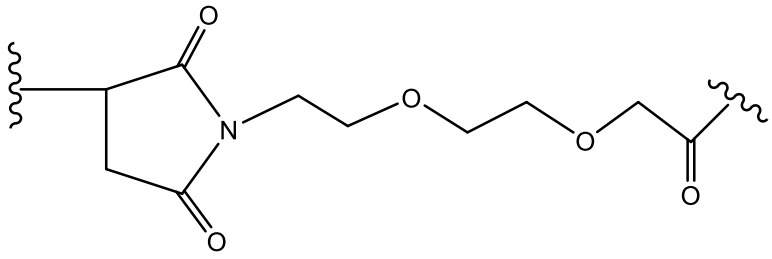
37. The extracellular vesicle of claim 36, wherein R^1 is –$(CH_2)_s$-, wherein s is 4, 5, or 6.

38. The extracellular vesicle of claim 36 or 37, wherein the maleimide moiety has the formula (II), where R^1 is $–(CH_2)_5$-:



(II).

39. The extracellular vesicle of claim 36, wherein the maleimide moiety has the formula (III), where R^1 is $-(CH_2CH_2O)_r-CH_2-$, where r is 2:



(III).

40. The extracellular vesicle of any one of claims 34-39, wherein the maleimide moiety is covalently linked to a functional group present on the EV.

41. The extracellular vesicle of claim 40, wherein the functional group is on a glycan on the EV.

42. The extracellular vesicle of claim 40, wherein the functional group is sulfhydryl.

43. The extracellular vesicle of claim 41 or 42, wherein the functional group is on a protein on the surface of the EV.

44. The extracellular vesicle of claim 43, wherein the protein is a scaffold moiety.

45. The extracellular vesicle of claim 44, wherein the protein is a PTGFRN polypeptide, a BSG polypeptide, a IGSF2 polypeptide, a IGSF3 polypeptide, a IGSF8 polypeptide, a ITGB1 polypeptide, a ITGA4 polypeptide, a SLC3A2 polypeptide, a ATP transporter polypeptide, or a fragment thereof.

46. An extracellular vesicle comprising a maleimide moiety, a cleavable linker, and a biologically active molecule, wherein the maleimide moiety links the EV to the cleavable linker, and the cleavable linker connects the maleimide moiety to the biologically active molecule.

47. The extracellular vesicle of any one of claims 1-46, wherein the biologically active molecule is a polypeptide, a peptide, a polynucleotide (DNA and/or RNA), a chemical compound, or any combination thereof.

48. The extracellular vesicle of claim 47, wherein the biologically active molecule is a chemical compound.

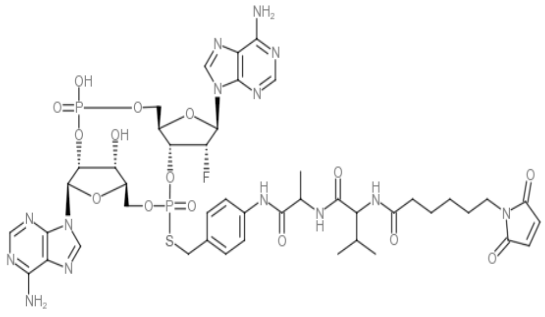
49. The extracellular vesicle of claim 48, wherein the chemical compound is a small molecule.

50. The extracellular vesicle of claim 49, wherein the small molecule is a proteolysis-targeting chimera (PROTAC).

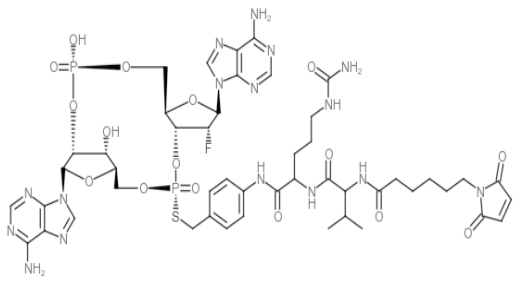
51. The extracellular vesicle of claim 47, wherein the biologically active molecule is nucleotide, wherein the nucleotide is a stimulator of interferon genes protein (STING) agonist.

52. The extracellular vesicle of claim 51, wherein the STING agonist comprises a cyclic dinucleotide STING agonist or a non-cyclic dinucleotide STING agonist.

53. The extracellular vesicle of claim 52, comprising a (maleimide moiety)-(cleavable linker)-(biologically active molecule) having the formula (VI) or (VII):

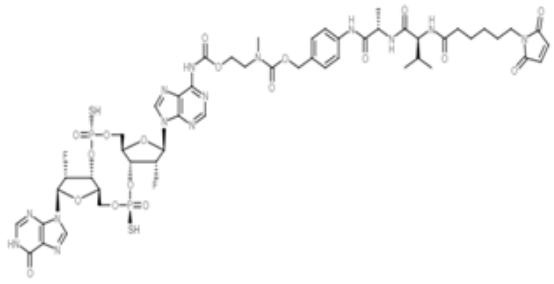


(VI),



(VII), or a pharmaceutically acceptable salt thereof.

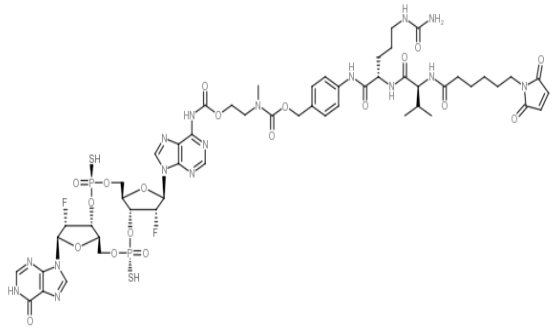
54. The extracellular vesicle of claim 52, comprising a (maleimide moiety)-(cleavable linker)-(biologically active molecule) having the formula (VIII), (IX), (X), or (XI):



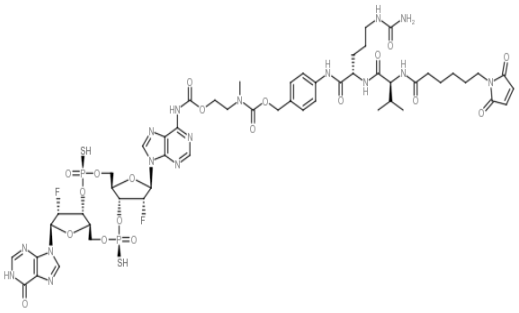
(VIII),



(IX),



(X),



(XI),

or a pharmaceutically acceptable salt thereof.

55. The extracellular vesicle of any one of claims 1-5, 8-32, and 35-45, wherein the EV is modified to expose a functional group on the surface to covalently link the maleimide moiety.

56. The extracellular vesicle of claim 55, wherein the functional group is a sulfhydryl group.

57. The extracellular vesicle of claim 56, wherein the functional group is exposed by treating the EV with a reducing agent.

58. The extracellular vesicle of claim 57, wherein the reducing agent comprises TCEP (Tris(2-carboxyethyl)phosphine), DTT (dithiothreitol), BME (2-mercaptoethanol), a thiolating agent, or any combination thereof.

59. The extracellular vesicle of claim 58, wherein the thiolating agent comprises Traut's reagent (2-iminothiolane).

60. The extracellular vesicle of any one of claims 1 to 59, wherein the EV is an exosome.

61. A pharmaceutical composition comprising the extracellular vesicle of any one of claims 1 to 60 and a pharmaceutically acceptable carrier.

62. A method of conjugating a biologically active molecule to an EV, comprising linking a maleimide moiety to the EV.

63. The method of claim 62, wherein the linking comprises treating the EV with a reducing agent.

64. The method of claim 63, wherein the reducing agent is comprises TCEP (Tris(2-carboxyethyl)phosphine), DTT (dithiothreitol), BME (2-mercaptoethanol), a thiolating agent, or any combination thereof.

65. The method of claim 64, wherein the thiolating agent comprises Traut's reagent (2-iminothiolane).

66. The method of any one of claims 62 to 65, wherein the linking further comprises bringing the reduced EV in contact with the maleimide moiety.

67. The method of claim 66, wherein the maleimide moiety is linked to a biologically active molecule prior to the linking to the EV.

68. The method of claim 67, wherein the maleimide moiety is further attached to a linker to connect the maleimide moiety to the biologically active molecule.

69. A kit comprising the EV of any one of claim 1 to 60 and instructions for use.

70. A kit comprising reagents to conjugate a biologically active molecule to an EV, and instructions to conduct the conjugation, thereby making the EV of any one of claims 1 to 60.

72. 71. Use of the EV of any one of claims 1 to 60 in the manufacture of a medicament for treating or preventing a disease or disorder in a subject in need thereofwherein the disease or disorder

is a cancer, an inflammatory disorder, a neurodegenerative disorder, a central nervous diseases, or a metabolic disease.

73. The use of claim 71 or 72, wherein the EV is to be administered intravenously, intraperitoneally, nasally, orally, intramuscularly, subcutaneously, parenterally, intratumorally, intrathecally, or intraocularly.

74. The EV of any one of claims 1 to 60 for use in treating or preventing a disease or disorder in a subject in need thereof.

75. An extracellular vesicle (EV) comprising at least one biologically active molecule covalently linked to a scaffold moiety via a maleimide moiety.

76. The extracellular vesicle of claim 75, wherein the maleimide moiety is a bifunctional molecule.

77. The extracellular vesicle of claims 75 or 76, wherein the maleimide moiety comprises at least one linker or spacer.

78. The extracellular vesicle of claim 77, wherein the linker is a cleavable linker.

79. The extracellular vesicle of any one of claims 75 to 78, wherein the scaffold moiety is a scaffold protein or a scaffold lipid.

80. The extracellular vesicle of claim 79, wherein the scaffold protein is a Scaffold X protein.

81. The extracellular vesicle of claim 80, wherein the Scaffold X protein is a PTGFRN polypeptide, a BSG polypeptide, a IGSF2 polypeptide, a IGSF3 polypeptide, a IGSF8 polypeptide, a ITGB1 polypeptide, a ITGA4 polypeptide, a SLC3A2 polypeptide, a ATP transporter polypeptide, or a fragment thereof.

82. The extracellular vesicle of any one of claims 75 to 81, wherein the biologically active molecule comprises a vaccine antigen, a vaccine adjuvant, or any combination thereof.

83. The extracellular vesicle of any one of claims 75 to 81, wherein the biological active molecule comprises a STING, an ASO, a synthetic antineoplastic agent (e.g., MMAE), a cytokine

release inhibitor (e.g., MCC950), an mTOR inhibitor (e.g,. Rapamycin), an autotaxin inhibitor (e.g., PAT409), an LPA1 antagonist (e.g., AM152), or any combination thereof.

84. The extracellular vesicle of any one of claims 75 to 83, wherein the extracellular vesicle further comprises a targeting moiety, a tropism moiety, an anti-phagocytic signal, or any combination thereof.

85. The extracellular vesicle of claim 84, wherein the targeting moiety, tropism moiety, anti-phagocytic signal, or combination thereof is linked to the extracellular vesicle via a maleimide moiety.