WHAT IS CLAIMED IS:

1. A composition comprising a plurality of intact bacterially derived minicells, comprising a first minicell composition, wherein:

(a) each minicell comprises and encloses at least 400,000 molecules of a compound that displays autofluorescence, wherein the compound is PNU-159682;

(b) the minicell is attached to a bispecific antibody comprising (i) a first arm with specificity for a minicell surface structure and (ii) a second arm with specificity for epidermal growth factor receptor (EGFR);

(c) the composition contains less than about 5 EU free endotoxin; and

(d) the composition contains minimal or no PNU-159682 and/or nucleic acid trapped on the surface of the minicells.

2. The composition of claim 1, wherein the composition further comprises a second minicell composition, wherein:

(a) each minicell in the second composition comprises and encloses a therapeutic nucleic acid molecule;

(b) each minicell in the second composition is attached to a bispecific antibody comprising (i) a first arm with specificity for a minicell surface structure and (ii) a second arm with specificity for epidermal growth factor receptor (EGFR);

(c) the composition contains less than about 5 EU free endotoxin; and

(d) the composition contains minimal or no nucleic acid trapped on the surface of the minicells.

3. The composition of claim 1 or claim 2, wherein the epidermal growth factor receptor (EGFR) is present in a brain tumor.

4. The composition of any one of claims 1 to 3, wherein the first minicell composition comprises minicells, each of which encloses at least about 200 ng of PNU-159682.

5. The composition of any one of claims 2-4, wherein the therapeutic nucleic acid molecule comprises an RNA sequence.

6. The composition of any one of claims 2-5, wherein the therapeutic nucleic acid molecule comprises or corresponds to a gene that is associated with tumor suppression selected from the p53 gene, the retinoblastoma gene, and the gene encoding tumor necrosis factor.

7. A method of making the composition of any one of claims 1-6, comprising loading a plurality of minicells with a desired compound without resort to centrifugation, comprising

(a) incubating the plurality in a volume of an incubation solution of the compound in a buffered liquid, wherein the volume is on the order of about 100 mls; and then

(b) subjecting the plurality to multiple washing steps, each comprising cross-flow filtration of the minicells with a volume of buffered liquid that is about 20 liters, wherein none of the washing steps employs centrifugation of the minicells.

8. The method of claim 7, wherein a binary ionic compound, which is different from the desired compound, is dissolved in the incubation solution to a concentration that is on the order of about 200 mM or more.

9. The method of claim 7 or 8, wherein:

(a) step (b) comprises three to five washing steps; and/or

(b) the desired compound is fluorescent; and/or

(c) the incubating is for a period of about4 hours or more; and/or

(d) the desired compound is biologically active; and/or

(e) the desired compound is a small molecule drug of about 1500 Daltons or less; and/or

(f) the small molecule drug is cytotoxic; and/or

(g) the small molecule drug is activated *in vivo*.

10. The method of any one of claims 7-9, wherein the desired compound is PNU-159682.

11. Use of an effective amount of a composition according to any of claims 1-6, in the manufacture of a medicament for treating cancer in a patient in need thereof.

~~1. A composition comprising an intact and nonliving bacterial vesicle that encloses a fluorescent small molecule drug, which is not doxorubicin, irinotecan, bisantrene, topotecan, epirubicin, daunorubicin, mitoxantrone, Oregon Green® 488-conjugated paclitaxel, or BODIPY® FL-conjugated vinblastine.~~

~~2. The composition of claim 1, wherein the vesicle is an intact, bacterially derived minicell.~~

~~3. The composition of claim 1 or 2, wherein the minicell encloses at least about 500,000 molecules of the small molecule drug.~~

~~4. The composition of any one of claims 1-3, wherein the vesicle is a killed bacterial cell.~~

~~5. The composition of any one of claims 1-4, wherein the small molecule drug:~~

~~(a) is biologically active; and/or~~

~~(b) is cytotoxic; and/or~~

~~(c) is activated *in vivo*.~~

~~6. The composition of any one of claims 1-5, wherein the small molecule drug is a morpholinyl anthracycline derivative.~~

7. The compostion of claim 6, wherein the small molecule drug is PNU-159682.

8. A composition comprising an intact and nonliving bacterial vesicle that encloses a compound of formula D-L-F or a salt thereof, wherein D is the residue of a small molecule drug, L is a linker, and F is a fluorescent moiety.

9. The composition of claim 8, wherein the linker has a half-life of between about 6 hours and about 24 hours.

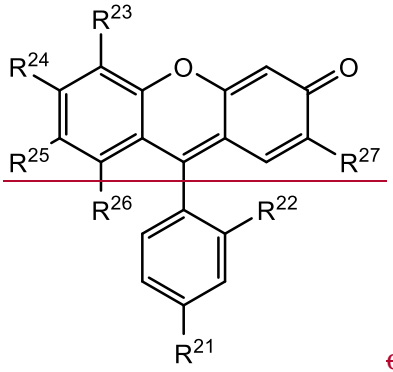
10. The composition of claim 8 or 9, wherein the linker is degraded in the endosome of a mammalian cell.

11. The composition of any one of claims 8-10, wherein:

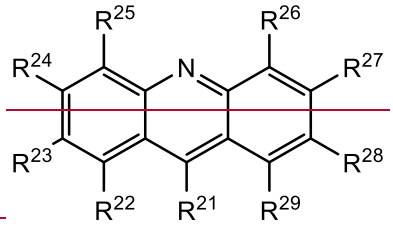
(a) the linker is selected from the group consisting of a bond, -OC(O)-$(CHR^{40})_qNR^{41}C(O)$- and -OC(O)-$(CHR^{40})_qC(O)NR^{41}$-$(CHR^{42})_u$-NH-C(S)-NH-, wherein R^{40}, R^{41} and R^{42} are independently -H or C_{1-4} alkyl, and q and u are independently 1, 2, 3, 4, 5, 6 or 7; or

(b) the linker is selected from the group consisting of -O-C(O)-(CH_2)NHC(O)- and -O-C(O)-$(CH_2)_3C(O)NH-(CH_2)_6$-NH-C(S)-NH-.

12. The composition of any one of claims 8-11, wherein the fluorescent moiety is:



or



wherein

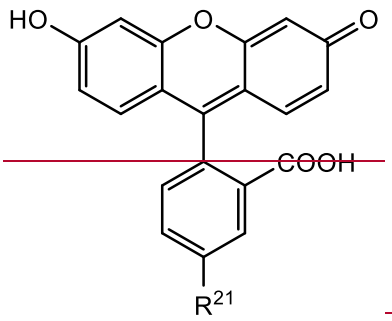
R^{21} is -H, -OH, -COOH, -O-C(O)-(C_{1-4}-alkyl), -C(O)-O-(C_{1-4}-alkyl), C_{1-4}-alkoxy, halo, or -R^d;

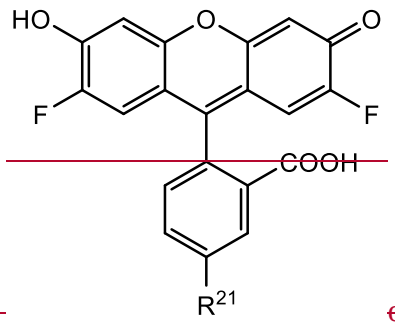
R^{22} is -H, -OH, -COOH, C_{1-4}alkyl, substituted C_{1-4}-alkyl, C_{1-4} alkoxy, substituted C_{1-4}-alkoxy, -O-C(O)-(C_{1-4}-alkyl), -O-C(O)-(substituted C_{1-4}-alkyl), -C(O)-O-(C_{1-4} alkyl), -C(O)-O-(substituted C_{1-4}-alkyl), -O-CH_2-O-(C_{1-4}-alkyl), -S-CH_2-O-(C_{1-4}-alkyl), or -R^d;

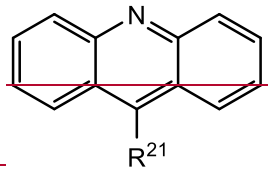
each R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28} and R^{29} are independently -H, halo, -OH, -CH_3 or -R^d;

provided at least one of R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28} and R^{29} is -R^d; wherein R^d is a point of connection to the linker.

13. The composition of any one of claims 8-11, wherein the fluorescent moiety is:

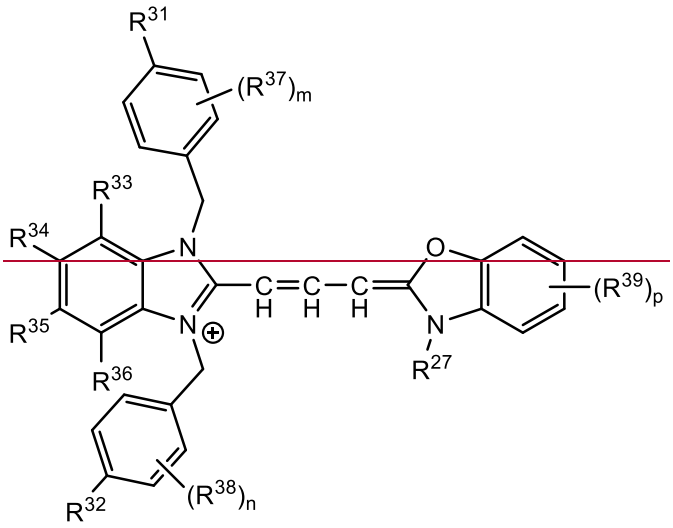


,


or


wherein R^{21} is the point of connection to the linker.

14. The composition of any one of claims 8-11, wherein the fluorescent moiety is:



wherein

R^{31} and R^{32} are independently -H, -OH, C_{1-4} alkyl, C_{1-4} haloalkyl, -O-C(O)-(C_{1-4} alkyl), -C(O)-O-(C_{1-4} alkyl), C_{1-4} alkoxy, halo, or R^d;

R^{34} and R^{35} are independently -H, halo, -OH, -COOH, C_{1-4}alkyl, substituted C_{1-4} alkyl, C_{1-4} alkoxy, -O-C(O)-(C_{1-4} alkyl), -C(O)-O-(C_{1-4} alkyl), -O-CH_2-O-(C_{1-4} alkyl), -S-CH_2-O-(C_{1-4} alkyl), or R^d;

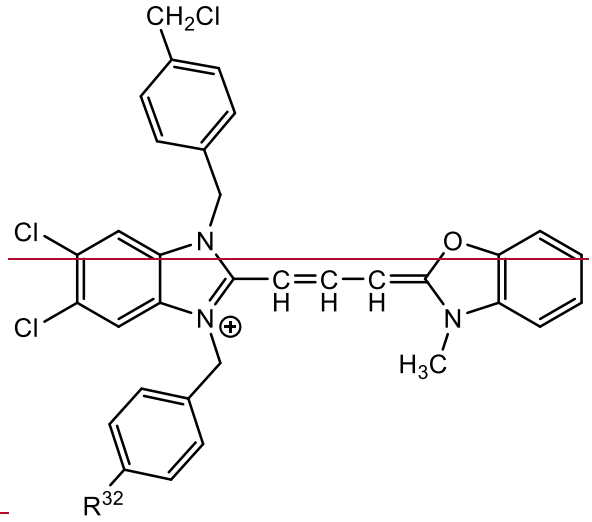
each R^{33} and R^{36} is independently -H, halo, -OH, $-CH_3$ or R^d;

each R^{37}, R^{38} and R^{39} is independently halo, -OH, $-CH_3$ or R^d;

each m, n, and p is independently 0, 1, 2, 3 or 4;

provided at least one of R^{31}, R^{32}, R^{34}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38} and R^{39} is R^d; wherein R^d is a point of connection to the linker.

15. The composition of claim 14, wherein the fluorescent moiety is:



wherein R^{32} is the point of connection to the linker.

16. A composition comprising an intact, bacterially derived bacterial vesicle enclosing a compound that comprises an active agent bound through a linker to an energy transfer moiety, wherein the active agent is other than Oregon Green® 488-conjugated paclitaxel and BODIPY® FL-conjugated vinblastine.

17. The composition of claim 16, wherein the energy transfer moiety:

(a) is a light emitting moiety;

(b) comprises a conjugated pi system; and/or

(c) comprises an acridinyl moiety, a xanthenyl moiety, or a benzimidazolyl moiety.

18. A method of loading a plurality of minicells with a desired compound without resort to centrifugation, comprising

(a) incubating the plurality in a volume of an incubation solution of the compound in a buffered liquid, wherein the volume is on the order of about 100 mls or more; and then

(b) subjecting the plurality to multiple washing steps, each comprising cross-flow

~~filtration of the minicells with a volume of buffered liquid that is on the order of liters, wherein none of the washing steps employs centrifugation of the minicells.~~

~~19. The method of claim 18, wherein a binary ionic compound, which is different from the desired compound, is dissolved in the incubation solution to a concentration that is on the order of about 200 mM or more.~~

~~20. The method of claim 18 or 19, wherein:~~

~~(a) step (b) comprises three to five washing steps; and/or~~

~~(b) the desired compound is fluorescent; and/or~~

~~(c) the incubating is for a period of about4 hours or more; and/or~~

~~(d) the desired compound is biologically active; and/or~~

~~(e) the desired compound is a small molecule drug of about 1500 Daltons or less; and/or~~

~~(f) the small molecule drug is cytotoxic; and/or~~

~~(g) the small molecule drug is activated *in vivo*.~~

~~21. The method of any one of claims 18-20, wherein the desired compound is of formula D-L-F or a salt thereof, wherein D is the residue of a small molecule drug, L is a linker, and F is a fluorescent moiety.~~

~~22. The method of claim 21, wherein:~~

~~(a) the linker has a half-life of between about 6 hours and about 24 hours; and/or~~

~~(b) the linker is degraded in the endosome of a mammalian cell.~~

~~23. Use of an effective amount of a composition according to any one of claims 1-17, wherein the compound is cytotoxic, in a medicament for treating cancer in a patient in need thereof.~~