WHAT IS CLAIMED IS:

1. A composition comprising:

(a) a complex comprising a chitosan-derivative nanoparticle comprising amino-functionalized chitosan and at least one nucleic acid molecule, wherein the at least one nucleic acid molecule is non-covalently bound to the chitosan-derivative nanoparticle at an amino to phosphorous (N:P) molar ratio of greater than 3:1, thereby forming a derivatized chitosan nucleic acid complex having a positive charge; and

(b) a plurality of linear block copolymers non-covalently bound to the chitosan-derivative nanoparticle, wherein said linear block copolymers comprise at least one polyanionic (PA) anchor region and at least one polyethylene glycol (PEG) tail region, wherein the PEG-PA molecules are non-covalently bound to the chitosan-derivative nanoparticle, and

wherein the composition comprises an amino to anion (N:A) molar ratio that is greater than ~~about~~ 1:100 and less than ~~about~~ 10:1.

2. The composition according to claim 1, wherein said linear block copolymer is a diblock copolymer comprising a PA anchor region and a PEG tail region.

3. The composition according to claim 1, wherein said linear block copolymer is a triblock copolymer comprising a central PA anchor region flanked by two PEG tail regions, or alternatively a central PEG tail region flanked by two PA anchor regions.

4. The composition according to claim 1, wherein the PA ~~tail~~ anchor region comprises a polypeptide, wherein the polypeptide is negatively charged.

5. The composition according to claim 1, wherein the PA ~~tail~~ anchor region comprises a carbohydrate, wherein the carbohydrate is negatively charged.

6. The composition according to claim 5, wherein the carbohydrate comprises a plurality of carboxylate, phosphate, and/or sulfate moieties.

7. The composition according to claim 6, wherein the carbohydrate is a glycosaminoglycan.

8. The composition according to claim 1, wherein PEG-PA molecules comprise:

(a) PEG-polyglutamic acid (PEG-PGA) molecules;

(b) PEG-polyaspartic acid (PEG-PAA) molecules; or

(c) PEG-hyaluronic acid (PEG-HA) molecules,

or a combination of 1, or 2, or all of (a)-(c).

9. The composition according to any one of claims 1 to 6, wherein the PEG portion of the PEG-PA molecules comprise a weight average molecular weight (Mw) of from ~~about~~ 500 Da to ~~about~~ 50,000 Da, preferably from ~~about~~ 1,000 Da to ~~about~~ 10,000 Da, more preferably from ~~about~~ 1,500 Da to ~~about~~ 7,500 Da, yet more preferably from ~~about~~ 3,000 Da to ~~about~~ 5,000 Da, most preferably about 5,000 Da.

10. The composition according to any one of claims 1 to 9, wherein the PA portion of the PEG-PA molecules comprise a weight average molecular weight (Mw) of from ~~about~~ 500 Da to ~~about~~ 3,000 Da, more preferably from ~~about~~ 1,000 Da to ~~about~~ 2,500 Da, more preferably about 1,500 Da.

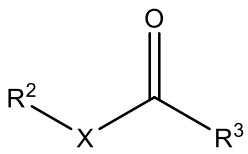
11. The composition according to any one of claims 1 to 9, wherein the PA portion of the PEG-PA molecules comprises a~~, e.g., linear,~~ polypeptide comprising from about 5 to about 25 acidic amino acids.

12. The composition according to any one of claims 1 to 11, wherein the N:P molar ratio is greater than ~~about~~ 3:1 and less than ~~about~~ 100:1, more preferably greater than ~~about~~ 5:1 and less than ~~about~~ 50:1, yet more preferably greater than ~~about~~ 5:1 and less than ~~about~~ 30:1, yet more preferably greater than ~~about~~ 5:1 and less than ~~about~~ 20:1, yet more preferably greater than ~~about~~ 5:1 and less than ~~about~~ 10:1, most preferably about 7:1.

13. The composition according to any one of claims 1 to 12, wherein the N:A molar ratio is greater than ~~about~~ 1:75 and less than ~~about~~ 8:1, more preferably greater than ~~about~~ 1:50 and less than ~~about~~ 6:1, yet more preferably greater than ~~about~~ 1:25 and less than ~~about~~ 6:1, yet more preferably greater than ~~about~~ 1:10 and less than ~~about~~ 6:1, yet more preferably greater than ~~about~~ 1:5 and less than ~~about~~ 6:1. -

14. The composition according to any one of claims 1 to 11, wherein the N:P molar ratio is from ~~about~~ 1:8 to ~~about~~ 30:1 ~~(e.g., to about 20:1, 15:1, 10:1, 8:1, or 7:1)~~, and wherein the P:A molar ratio is from ~~about~~ 1:50 to ~~about~~ 1:5, more preferably wherein the N:A molar ratio is from ~~about~~ 1:10 to ~~about~~ 5, more preferably from ~~about~~ 1:5 to ~~about~~ 2, more preferably from ~~about~~ 1:3 to ~~about~~ 1.5, more preferably from ~~about~~ 1:2.5 to ~~about~~ 1, yet more preferably wherein the N:P:A ratio is about 7:1:7; about 7:1:12; or about 7:1:17.

15. The composition according to any one of claims 1 to 14, wherein the chitosan-derivative nanoparticle comprises a polyol of Formula II or is functionalized with a polyol of Formula II:



II

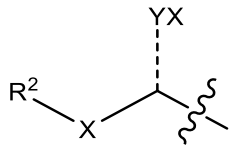
wherein

(a) R^2 is selected from: H and hydroxyl;

(b) R^3 is selected from: H and hydroxyl; and

(c) X is selected from C_2-C_6 alkylene optionally substituted with one or more hydroxyl substituents.

16. The composition according to any one of claims 1 to 14, wherein the chitosan-derivative nanoparticle comprises a polyol of Formula III:



III

wherein:

-----Y is =O or $–H_2$;

R^2 is selected from: H and hydroxyl;

R^3 is selected from: H and hydroxyl;

X is selected from: C_2-C_6 alkylene optionally substituted with one or more hydroxyl substituents; and

 denotes the bond between the polyol and the derivatized chitosan.

17. The composition according to any one of claims 1 to 16, wherein amino-functionalized chitosan is arginine, lysine, or ornithine functionalized, preferably arginine functionalized.

18. The composition according to any one of claims 1 to 17, wherein the composition is stable:

(a) for at least 24 hours in fasted state simulated intestinal fluid; or

(b) for at least 1 h dispersed in mammalian urine at 37 ° C.

19. The composition according to any one of claims 1 to 18, wherein the composition further comprises a surfactant, excipient, and/or a storage stability agent.

20. The composition according to claim 19, wherein the composition comprises the storage stability agent, preferably wherein the storage stability agent is a monosaccharide, a disaccharide, a polysaccharide, or a reduced alcohol thereof, yet more preferably wherein the storage stability agent is selected from trehalose and mannitol.

21. The composition according to claim 19 or 20, wherein the composition comprises the surfactant, preferably wherein the surfactant comprises a poloxamer, more preferably wherein the poloxamer is poloxamer 407.

22. The composition according to any one of claims 1 to 21, wherein the at least one nucleic acid comprises RNA.

23. The composition according to any one of claims 1 to 21, wherein the at least one nucleic acid comprises DNA.

24. The composition according to any one of claims 1 to 23, wherein the composition is stable for, or for at least, 48 h, or 1 week at 4 °C in an aqueous dispersion comprising the composition dispersed in purified water.

25. The composition according to claim 24, wherein the composition is stable in the aqueous dispersion after freeze/thaw and/or drying/rehydration, preferably wherein the drying comprises spray drying, lyopholization, spray freeze drying, evaporation,or supercritical drying, more preferably wherein the drying comprises lyopholization or spray drying.

26. The composition according to claims 24 or 25, wherein the composition exhibits a polydispersity index of less than 0.2 after~~, e.g.,~~ at least~~,~~ 48 h~~,~~ or 1 week at 4 °C in the aqueous dispersion.

27. A method for making any one of the compositions of claims 1 to 26, the method comprising:

(a) providing a complex comprising the chitosan-derivative nanoparticle comprising amino-functionalized chitosan and the at least one nucleic acid; and

(b) mixing the complex with a solution comprising PEG-PA molecules, thereby forming a reaction mixture comprising the composition.

28. The method of claim 27, wherein the complex of (a) is provided at a nucleotide concentration of from 0.01 mg/mL to 25 mg/mL, more preferably from 0.05 to 10 mg/mL, more preferably from 0.10 to 5 mg/mL, more preferably from 0.10 to 2 mg/mL.

29. The method of claim 27 or 28, wherein (a) and (b) are mixed at a (v/v) ratio of from 1:10 to 10:1, preferably from 1:5 to 5:1, more preferably from 1:2 to 2:1, yet more preferably at a ratio of about 1:1.

30. The method of claim 27, 28, or 29, wherein the method further comprises concentrating the reaction mixture, preferably the concentrating comprises ultrafiltration, solvent sublimation, and/or solvent evaporation, more preferably the ultrafiltration comprises tangential flow filtration.

31. The method of claim 27, wherein the amino-functionalized chitosan comprises or is functionalized with a hydrophilic polyol.

32. A method for making any one of the compositions of claims 1 to 26, the method comprising simultaneously or sequentially admixing amino-functionalized chitosan, at least one nucleic acid, and PEG-PA molecules, thereby forming a reaction mixture comprising the composition.

33. The method of claim 32, wherein the method comprises simultaneously combining the amino-functionalized chitosan, at least one nucleic acid, and the PEG-PA molecules, thereby forming a reaction mixture comprising the composition.

34. Use of a composition according to any one of claims 1 to 26, or a composition produced by any of the methods of claims 27 to 33 for the manufacture of a formulation for~~A method of~~ transfecting a cell with a nucleic acid~~ comprising contacting the cell with a composition according to any one of claims 1 to 22, or a composition produced by any of the methods of claims 23 to 33~~.

35. The use~~method~~ of claim 34, wherein the cell is a cell comprising or derived from a mucosal tissue.

36. The use~~method~~ of claim 35, wherein the mucosal tissue is lung tissue, nasal tissue, ocular tissue, vaginal tissue, bladder tissue, or gastrointestinal tract tissue.

37. The use~~method~~ of claim 35, wherein the cell is an intestinal cell of a subject ~~and the contacting comprises orally or rectally administering the composition to the subject~~.

38. The use~~method~~ of claim 35, wherein the cell is a cell of the bladder ~~and the contacting comprises intravesical administration of the composition to the subject~~.

39. The method of any one of claims 27 to 33, wherein the method provides decreased muco-adhesion as compared to particles that do not comprise the ~~polymer component comprising PEG-PA~~ linear block copolymers.