What is claimed is:

Claims

1. A soluble stimulus responsive polymer comprising a polyvinylaminepolyelectrolyte backbone comprising one or more hydrophobic groups attached to the backbone, wherein the polymer is capable of binding and precipitating a biomolecule of interest in a sample upon addition of a stimulus.

2. The soluble stimulus responsive polymer of claim 1, wherein the polyelectrolyte backbone comprises at least two monomeric units.

3. The soluble stimulus responsive polymer of claim 2, wherein the polyelectrolyte backbone comprises at least three monomeric units.

4. The soluble stimulus responsive polymer of claim 2, wherein each monomeric unit in the polyelectrolyte backbone comprises a charge.

5. The soluble stimulus responsive polymer of claim 2, wherein at least 50% of the monomeric units comprise a charge.

6. The soluble stimulus responsive polymer of claim 1, wherein the biomolecule of interest is a therapeutic polypeptide.

7. The soluble stimulus responsive polymer of claim 1, wherein the biomolecule of interest is an impurity present in a sample along with a therapeutic polypeptide.

8. The soluble stimulus responsive polymer of claim 7, wherein the impurity is selected from the group consisting of host cell protein, endotoxin, DNA, RNA, viruses, lipids, whole cells and cellular debris.

9. The soluble stimulus responsive polymer of claim 1, wherein the stimulus is a multivalent ion.

10. The soluble stimulus responsive polymer of claim 9, wherein the multivalent ion is phosphate or citrate.

11. The soluble stimulus responsive polymer of claim 7, wherein the therapeutic polypeptide is an antibody.

12. The soluble stimulus responsive polymer of claim 11, wherein the antibody is a monoclonal antibody.

~~13. A stimulus responsive polymer selected from the group consisting of polyallylamine, polyallylamine modified with a benzyl group, polyvinylamine and polyvinylamine modified with a benzyl group, wherein the stimulus is phosphate or citrate.~~

13.~~14.~~ A method of separating a target molecule from one or more impurities in a sample, wherein the method comprises the steps of:

(a) providing a sample comprising a target molecule and one or more impurities;

(b) contacting the sample with a soluble stimulus responsive polymer comprising a polyelectrolyte backbone comprising one or more hydrophobic groups attached to the backbone ~~according to claim 1~~ , thereby to form a complex of polymer and the one or more impurities; and

(c) adding a stimulus to the sample, thereby to precipitate the complex out of solution,

thereby to separate the target molecule from one or more impurities.

14.~~15.~~ The method of claim 13~~14~~, wherein the method further comprises one or more filtration steps.

15.~~16.~~ The method of claim 13~~14~~, wherein the method further comprises one or more chromatography step.

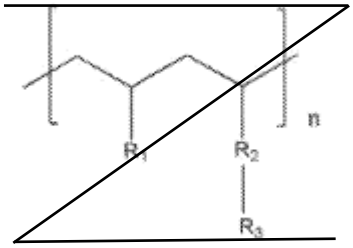
16.~~17.~~ The method of claim 13~~14~~, wherein the stimulus is a multivalent ion.

17.~~18.~~ The method of claim 16~~17~~, wherein the multivalent ion is phosphate or citrate.

18.~~19.~~ The method of claim 13~~14~~, wherein the polyelectrolyte backbone comprises at least two monomeric units, at least 50% of the units comprising a charge.

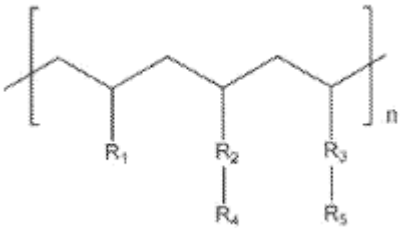
19.~~20.~~ The method of claim 13~~14~~, wherein the polyelectrolyte backbone comprises at least three monomeric units, at least 50% of the units comprising a charge.

~~21. The stimulus responsive polymer comprising the following structure:~~



~~wherein the polymer comprises a carbon containing polyelectrolyte backbone; R_1 and R_2 are charged groups which form a part of the backbone; R3 is a hydrophobic group attached to a charged group in the backbone; and n is the number of monomeric units in the polymer, wherein n is equal to or greater than 2.~~

20.~~22.~~ ~~The~~A soluble stimulus responsive polymer comprising the following structure:



~~W~~wherein the polymer comprises a carbon containing polyelectrolyte backbone; R_1, R_2 and R_3 are charged groups which form a part of the backbone; R_4 is a hydrophobic group attached to a charged group in the backbone; R_5 is a group possessing a charge opposite of the charge found in R_1, R_2 or R_3; and n is the number of monomeric units in the polymer, wherein n is equal to or greater than 2.

21.~~23.~~ A method of separating an antibody from one or more impurities in a sample ~~using a stimulus responsive polymer according to claim 1,~~ while minimizing residual amounts of polymer, the method comprising the steps of:

(a) providing a sample comprising an antibody and one or more impurities;

(b) contacting the sample with a soluble stimulus responsive polymer comprising a polyelectrolyte backbone comprising one or more hydrophobic groups attached to the backbone ~~according to claim 1~~ under a first set of conditions suitable for the polymer to bind one or more impurities, thereby to form a first complex of polymer and one or more impurities, wherein the first set of conditions comprise adjusting pH or salt concentration of the sample ~~before,~~ during or after the addition of the polymer;

(c) precipitating the first complex from the sample under a second set of conditions;

(d) contacting the sample with a stimulus comprising a multivalent ion, thereby to form a second complex of residual polymer and multivalent ion; and

(e) precipitating the second complex;

thereby to separate the antibody from one or more impurities in the sample while reducing the amount of residual polymer in the sample.

22.~~24.~~ The method of claim 21~~23~~, wherein the antibody is a monoclonal antibody.

23.~~25.~~ The method of claim 21~~23~~, wherein the one or more impurities is selected from the group consisting of host cell protein, DNA, RNA, viruses, antibody aggregates and cell culture additives.

24.~~26.~~ The method of claim 21~~23~~, wherein the sample comprises a feedstock.

25.~~27.~~ The method of claim 21~~23~~, wherein the sample comprises cell culture media into which an antibody is secreted.

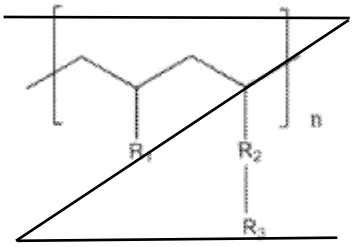
26.~~28.~~ The method of claim 21~~23~~, further comprising one or more chromatography steps.

27.~~29.~~ The method of claim 21~~23~~, further comprising one or more filtration steps.

~~30. The polymer of claim 1, wherein the polyelectrolyte backbone comprises a polyamine backbone.~~

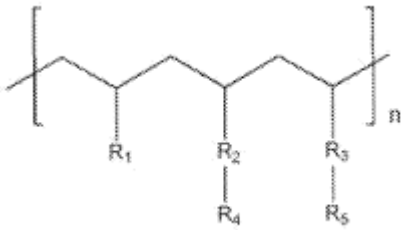
28.~~31.~~ The polymer of claim 1, wherein the hydrophobic group is a phenyl group.

~~32. A stimulus responsive polymer useful for purifying a biomolecule of interest from a sample, the polymer comprising the following structure:~~



~~wherein the polymer comprises a carbon containing polyelectrolyte backbone; R_1, R_2 are amines form a part of the backbone; R_3 is a hydrophobic modifying group present on 20% to 75% of the amine groups and consists of a phenyl group or derivative thereof; and n is the number of monomeric units in the polymer, wherein n is equal to or greater than 2.~~

29.~~33.~~ A soluble stimulus responsive polymer for separating a target molecule from one or more impurities comprising the following formula:



wherein the polymer comprises a carbon containing polyelectrolyte backbone; R_1, R_2 and R_3 are amine groups which form a part of the backbone; R4 is a phenyl group which is attached to 5% to 75% of the R_1, R_2 or R_3 groups; R_5 is a cation exchange group

modifying 5% to 50% of the R_1, R_2 or R_3 groups; and n is the number of monomeric units in the polymer, wherein n is equal to or greater than 2.

30.~~34.~~ A method of separating an antibody from one or more impurities in a sample using a stimulus responsive polymer, while minimizing residual amounts of polymer, the method comprising the steps of:

(a) providing a sample comprising an antibody and one or more impurities;

(b) contacting the sample with a soluble stimulus responsive polymer comprising a polyelectrolyte backbone comprising one or more hydrophobic groups attached to the backbone under a first set of conditions suitable for the polymer to bind one or more impurities, thereby to form a first complex of polymer and one or more impurities, wherein the first set of conditions comprise adjusting pH or salt concentration of the sample ~~before,~~ during or after the addition of the polymer;

(c) flocculating the first complex from the sample under a first set of conditions;

(d) removing the flocculated solids;

(e) contacting the sample with a multivalent ion, thereby to form a second complex of residual polymer and multivalent ion; and

(f) precipitating the second complex

thereby to separate the antibody from one or more impurities in the sample while reducing the amount of residual polymer in the sample.

31.~~35.~~ A method of separating a target molecule from one or more impurities in a sample, wherein the method comprises the steps of:

(a) providing a sample comprising a target molecule and one or more impurities;

(b) contacting the sample with a soluble multivalent ion responsive polymer comprising a polyelectrolyte backbone comprising one or more hydrophobic groups attached to the backbone, wherein a multivalent ion is added ~~before,~~ during or after addition of the polymer, thereby to form a complex of polymer and the one or more impurities;

(c) precipitating the complex out of solution,

thereby to separate the target molecule from one or more impurities.

32.~~36.~~ The method of claim 31~~35~~, wherein the multivalent ion stimulus is phosphate or citrate.

33.~~37.~~ The method of claim 31~~35~~, wherein the stimulus responsive polymer is a copolymer.

34.~~38.~~ The method of claim 31~~35~~, further comprising the step of removing a residual amount of polymer after step (c), wherein the step comprises the use of a membrane or resin modified with a multivalent ion.

35.~~39.~~ The method of claim 34~~38~~, wherein the multivalent ion is phosphate.

36. The method of claim 13, wherein the polymer is selected from polyallylamine modified with a hydrophobic group and polyvinylamine modified with a hydrophobic group.

37. The method of claim 36, wherein the hydrophobic group is a benzyl group.

38. The method of claim 30, wherein the polymer is selected from polyallylamine modified with a hydrophobic group and polyvinylamine modified with a hydrophobic group.

39. The method of claim 38, wherein the hydrophobic group is a benzyl group.

40. The method of claim 31, wherein the polymer is selected from polyallylamine modified with a hydrophobic group and polyvinylamine modified with a hydrophobic group.

41. The method of claim 40, wherein the hydrophobic group is a benzyl group.