

## SUMMARY OF THE INVENTION

**[0007]** In one aspect, the present invention refers to method method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample, wherein the biological target associated with the solid is separated from the sample by filtration or sedimentation, and washed by re-suspending it in a clean buffer then recovering the solid by sedimentation or filtration~~with the proviso that sample does not include polyethylene glycol 6000 (PEG 6000) at an amount of 15% or greater.~~ Examples disclosed herein provide methods for selectively separating biological targets from a sample, including the steps of (i) providing a solid having one or more ureide moieties at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of biological targets in the sample binds to the ureides on the surface of the solid; and (iii) separating the solid from the sample. In some examples, the biological targets are considered “large.” The term “large,” as used herein in reference to biological targets, will be understood to include individual biological targets or assemblages of biological targets with an aggregate size from about 20 microns to millions of Daltons. Exemplary biological targets include cells and cellular substructures such as organelles, inclusion bodies, endotoxins, aggregates and viruses. Solids having one or more ureides at their surfaces particularly include ureide crystals or ureides immobilized on a surface. In certain examples the ureide particularly includes allantoin.

**[0008]** In certain examples, the sample is the local environment and the method provides means for selectively removing biological targets from that environment. In some such examples the sample is air, contacted with a solid material having one or more ureides on its surface. In one particular examples the airborne biological target is a virus or other pathogenic agent, and the ureide is allantoin.

**[0009]** In certain examples, the sample is a liquid, contacted with a solid material having one or more ureides on its surface. In one such example, the sample is water, the biological targets include pathogenic microbes or derivative substructures thereof such as endotoxins, and the ureide is allantoin at a supersaturating concentration. In another such example, the sample is a protein preparation containing a desired protein such as containing a recombinant protein, and the ureide is allantoin at a supersaturating concentration. In certain such examples, the protein preparation may contain cells, cell debris, microbes and substructures thereof including endotoxins to be removed from the liquid. In certain such examples, organic multivalent ions may be combined with the ureide to enhance the removal of unwanted materials. In certain such examples, the sample treated with the ureide and/or organic multivalent ions may be combined with other solids comprising organic multivalent ions to further enhance the removal of unwanted materials.

**[0010]** In certain examples the biological targets may be recovered from the solid having one or more ureides at its surface by dissolving the solid, or by disrupting the interaction of the target with the ureide. In one such example, the ureide is allantoin. In one such

example, the method provides the additional step of recovering a virus from the solid wherein the sample contains a desired virus to be purified. In another such example, the method provides the additional step of recovering a cellular organelle from the solid wherein the sample contains a desired organelle to be purified. In a further aspect, the present invention refers to a method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby at least 90% of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample. In another aspect, the present invention refers to a method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample, wherein the biological target to be bound to the allantoin is a virus being subjected to treatment to inactivate the virus.

### BRIEF DESCRIPTION OF THE DRAWINGS

- [0011]** Figure 1 shows a time course plot indicating a reduction in high molecular weight aggregates and reduction of host cell protein contaminants in accordance with methods disclosed herein.
- [0012]** Figures 2A and 2B show size exclusion chromatography profiles before and after treatment of IgM-84 antibody in accordance with methods disclosed herein.
- [0013]** Figure 3 shows size exclusion chromatography profiles before and after the treatment of an anti-HER2 monoclonal IgG antibody in accordance with methods disclosed herein.

### DETAILED DESCRIPTION

**[0014]** It has been surprisingly discovered that a solid having one or more ureides at its surface mediates a sufficiently strong affinity for biological targets, including large biological targets, that they can be selectively extracted from a sample simply by removing the solid to which they are bound. The extracted biological targets can be subsequently discarded or recovered. Large biological targets are understood to include individual targets or assemblages of targets with an aggregate size from 20 microns to millions of Daltons (D), and particularly include cells and cellular substructures such as organelles, microbes and substructures of microbes such as endotoxins, and aggregates. Smaller biological targets particularly including proteins may exhibit less affinity for ureides on a solid surface, which facilitates their separation from the larger targets bound to the ureide.

**[0015]** In some embodiments, the biological target has a size in a range selected from the group consisting of (1) from about 10 nm to about 200 nm, (2) from about 200 nm to about 1 micron, (3) from about 1 micron to about 20 microns, and (4) about 20 microns or greater. One skilled in the art will appreciate that the exact size of the biological target will be a function of the nature of the target itself. Where smaller biological targets are to be purified, methods disclosed herein may be combined with other purification techniques to provide the desired degree of purification.

**[0016]** Without being bound by any particular theory, experimental data indicate that the affinity of allantoin may be mediated through van der Waals interactions, particularly including hydrogen bonds. Such bonds are affected by pH, conductivity, and the presence of organic

## Claims

1. A method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample, wherein the biological target associated with the solid is separated from the sample by filtration or sedimentation, and washed by re-suspending it in a clean buffer then recovering the solid by sedimentation or filtration.
2. The method of claim 1, wherein the biological target has a size in a range selected from the group consisting of (1) from about 10 nm to about 200 nm, (2) from about 200 nm to about 1 micron, (3) from about 1 micron to about 20 microns, and (4) about 20 microns or greater.
3. The method of claim 1, wherein the biological target is a large biological target having a molecular weight in a range from the group consisting of (1) From about 100 kiloDaltons to about 500 kiloDaltons, (2) from about 500 kiloDaltons to about 1 megaDalton, (3) from about 1 megaDalton to about 10 megaDaltons, and (4) about 10 megaDaltons or greater.
4. The method of claim 1 wherein the biological target is a cell.
5. The method of claim 4, wherein the cell comprises one selected from the group consisting of (i) a mammalian cell, (ii) an insect cell, (iii) a yeast cell, (iv) a bacterial cell, and (v) another biological cell.
6. The method of claim 1, wherein the biological target comprises a substructure of a cell.
7. The method of claim 6, wherein the cellular substructure comprises one from the group consisting of (i) an organelle, (ii) an inclusion body, (iii) an endotoxin, or (iv) another cellular substructure.

8. The method of claim 7, wherein the cellular substructure comprises an organelle selected from the group consisting of (i) an exosome, (ii) a ribosome, (iii) a mitochondrion, (iv) a chloroplast, or (v) another cellular substructure.
9. The method of claims 1-8, wherein at least 50% of the biological target binds to the allantoin.
10. The method of claim 9, wherein at least 75% of the biological target binds to the allantoin.
11. The method of claim 9, wherein at least 90% of the biological target binds to the allantoin.
12. The method of claim 9, wherein at least 95% of the biological target binds to the allantoin.
13. The method of claim 9, wherein at least 99% of the biological target binds to the allantoin.
14. The method of any one of claims 1-13, wherein substantially all of the biological target binds to the allantoin.
15. The method of any one of claims 1-14, wherein the solid comprises allantoin crystals.
16. The method of any one of claims 1-15, wherein the allantoin is crystalline and the solid is in the form of a powder.
17. The method of claim 15, wherein the allantoin is in an aqueous liquid and is solid by virtue of being present at a supersaturating concentration.
18. The method of claim 15, wherein the allantoin is in an aqueous liquid and the allantoin is at a concentration consisting of one from the group (i) greater than 0.56%, (ii) 0.56 to 20%, (iii) 5-10%, (iv) 1 to 2%, (v) 2 to 5%, (vi) 20-50%.
19. The method of claim 1, wherein the allantoin is supersaturated allantoin and the clean buffer comprises allantoin at or close to saturation.

20. The method of claim 1 or 19, wherein the washing step may be repeated one or more times.
21. The method of any one of claims 1, 19 and 20, wherein the biological target is separated from at least some of the allantoin by solubilizing the at least some of the allantoin.
22. The method of claim 21, wherein the biological target is subsequently concentrated by diafiltration.
23. The method of claim 22, wherein resolubilizing the biological target is simultaneous with diafiltration.
24. The method of claim 22 or 23, wherein the biological target is subsequently purified by an additional fractionation method.
25. The method of claim 1, wherein the sample is a liquid.
26. The method of claim 1 or 25, wherein the sample is a cell culture harvest containing cells or lysed cells.
27. The method of claim 26, wherein the cells may have been previously removed.
28. The method of claim 25, wherein the sample is partially purified.
29. The method of claim 25, wherein the sample is highly purified.
30. The method of claim 1 or 25, wherein the sample is aqueous.
31. The method of claim 1 or 25, wherein the sample is air.
32. The method of claim 1, wherein the biological target is a desired biological target to be purified.

33. The method of claim 1, wherein the biological target is an undesired biological target to be removed.
34. The method of claim 1, wherein the biological target may comprise more than one species of biological target to be removed.
35. The method of claim 33 or 34, wherein the sample is treated with one or more species of soluble organic multivalent ions with the same net charge at an aggregate concentration ranging from 0.001 to 0.1%.
36. The method of claim 35, wherein the soluble organic multivalent ion comprises one or more species more from the group of ethacridine, chlorhexidine, or octanoic acid at a concentration ranging from 0.001 to 0.1%.
37. The method of claim 33 or 34, wherein the sample is treated with one or more species of immobilized organic multivalent ions of the same or different net charge, on a solid surface under conditions where a desired protein is substantially unbound to the immobilized organic multivalent ions.
38. The method of claim 33 or 34, wherein the sample is treated with both soluble and immobilized organic multivalent ions, and wherein at least one species of immobilized organic multivalent ions has a net charge opposite to the soluble organic multivalent ions.
39. The method of claim 38, wherein the immobilized organic multivalent ions include species of opposite net charge.
40. The method of any one of claims 36-39, wherein at least one species of organic multivalent cation comprises a metal affinity functionality.
41. The method of any one of claim 36-40, wherein treatment with soluble organic multivalent ions occurs first, or exposure to soluble and immobilized organic multivalent ions occurs essentially at the same time.
42. The method of any one of claims 1-41, wherein in the method is practiced at a pH value selected from the group consisting of (i) 7, (ii) 6.5 to 7.5, (iii) 6 to 8, (iv) 5 to 9, (v) 4-10, (vi) less than 4, and (vii) an intermediate pH value.

43. The method of any one of claims 1-42, wherein in the method is practiced at a conductivity selected from the group consisting of (i) 12-15 mS/cm, (ii) 5-20 mS/cm, (iii) 1-30 mS/cm (iv) 1-50 mS/cm (v) 1-100 mS cm (vi) less than 1 mS/cm, (vii) more than 100 mS/cm, and (viii) an intermediate conductivity value.

44. The method of any one of claims 1-43, wherein the biological target to be bound to the allantoin is incubated together with the allantoin for a period of time selected from the group consisting of (i) 1 minute, (ii) 5 minutes, (iii) 10 minutes, (iv) 15 minutes, (v) 30 minutes, (vi) 60 minutes, (vii) more than 60 minutes, or (viii) an intermediate period of time.

45. The method of claim 1, wherein the biological target to be bound to the allantoin is a virus being subjected to treatment to inactivate the virus.

46. The method of claim 45, wherein the virus inactivating treatment is one from the group consisting of exposure to (i) a pH of 3 to 5, (ii) a pH of 9-11, (iii) ethacridine, (iv) chlorhexidine, (v) benzalkonium chloride, (vi) a surfactant, (vii) tri(n-butyl)phosphate, (viii) methylene blue, (ix) polyethyleneimine, (x) urea, (xi) guanidine, (xii) caprylic acid, (xiii) sodium chloride, or (xiv) arginine.

47. A method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby at least 90% of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample.

48. A method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample, wherein the biological target to be bound to the allantoin is a virus being subjected to treatment to inactivate the virus.

49. The method of claim 48, wherein the biological target associated with the solid is separated from the sample by filtration or sedimentation, and washed by re-suspending it in a clean buffer then recovering the solid by sedimentation or filtration.

## Claims

1. A method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby a substantial fraction of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample, with the proviso that sample does not include polyethylene glycol 6000 (PEG-6000) at an amount of 15% or greater.
2. The method of claim 1, wherein the biological target has a size in a range selected from the group consisting of (1) from about 10 nm to about 200 nm, (2) from about 200 nm to about 1 micron, (3) from about 1 micron to about 20 microns, and (4) about 20 microns or greater.
3. The method of claim 1, wherein the biological target is a large biological target having a molecular weight in a range from the group consisting of (1) From about 100 kiloDaltons to about 500 kiloDaltons, (2) from about 500 kiloDaltons to about 1 megaDalton, (3) from about 1 megaDalton to about 10 megaDaltons, and (4) about 10 megaDaltons or greater.
4. The method of claim 1 wherein the biological target is a cell.
5. The method of claim 4, wherein the cell comprises one selected from the group consisting of (i) a mammalian cell, (ii) an insect cell, (iii) a yeast cell, (iv) a bacterial cell, and (v) another biological cell.
6. The method of claim 1, wherein the biological target comprises a substructure of a cell.
7. The method of claim 6, wherein the cellular substructure comprises one from the group consisting of (i) an organelle, (ii) an inclusion body, (iii) an endotoxin, or (iv) another cellular substructure.
8. The method of claim 7, wherein the cellular substructure comprises an organelle selected from the group consisting of (i) an exosome, (ii) a ribosome, (iii) a mitochondrion, (iv) a chloroplast, or (v) another cellular substructure.

9. The method of claims 1-8, wherein at least 50% of the biological target binds to the allantoin.
10. The method of claim 9, wherein at least 75% of the biological target binds to the allantoin.
11. The method of claim 9, wherein at least 90% of the biological target binds to the allantoin.
12. The method of claim 9, wherein at least 95% of the biological target binds to the allantoin.
13. The method of claim 9, wherein at least 99% of the biological target binds to the allantoin.
14. The method of any one of claims 1-13, wherein substantially all of the biological target binds to the allantoin.
15. The method of any one of claims 1-14, wherein the solid comprises allantoin crystals.
16. The method of any one of claims 1-15, wherein the allantoin is crystalline and the solid is in the form of a powder.
17. The method of claim 15, wherein the allantoin is in an aqueous liquid and is solid by virtue of being present at a supersaturating concentration.
18. The method of claim 15, wherein the allantoin is in an aqueous liquid and the allantoin is at a concentration consisting of one from the group (i) greater than 0.56%, (ii) 0.56 to 20%, (iii) 5-10%, (iv) 1 to 2%, (v) 2 to 5%, (vi) 20-50%.
19. The method of claim 1, wherein the biological target associated with the solid is separated from the sample by filtration or sedimentation.
20. The method of claim 19, wherein the biological target is washed by re-suspending it in a clean buffer then recovering the solid by sedimentation or filtration.

21. The method of claim 20, wherein the allantoin is supersaturated allantoin and the clean buffer comprises allantoin at or close to saturation.
22. The method of claim 20 or 21, wherein the washing step may be repeated one or more times.
23. The method of any one of claims 19-22, wherein the biological target is separated from at least some of the allantoin by solubilizing the at least some of the allantoin.
24. The method of claim 23, wherein the biological target is subsequently concentrated by diafiltration.
25. The method of claim 24, wherein resolubilizing the biological target is simultaneous with diafiltration.
26. The method of claim 24 or 25, wherein the biological target is subsequently purified by an additional fractionation method.
27. The method of claim 1, wherein the sample is a liquid.
28. The method of claim 1 or 27, wherein the sample is a cell culture harvest containing cells or lysed cells.
29. The method of claim 28, wherein the cells may have been previously removed.
30. The method of claim 27, wherein the sample is partially purified.
31. The method of claim 27, wherein the sample is highly purified.
32. The method of claim 1 or 27, wherein the sample is aqueous.
33. The method of claim 1 or 27, wherein the sample is air.
34. The method of claim 1, wherein the biological target is a desired biological target to be purified.

35. The method of claim 1, wherein the biological target is an undesired biological target to be removed.

36. The method of claim 1, wherein the biological target may comprise more than one species of biological target to be removed.

37. The method of claim 35 or 36, wherein the sample is treated with one or more species of soluble organic multivalent ions with the same net charge at an aggregate concentration ranging from 0.001 to 0.1%.

38. The method of claim 37, wherein the soluble organic multivalent ion comprises one or more species more from the group of ethacridine, chlorhexidine, or octanoic acid at a concentration ranging from 0.001 to 0.1%.

39. The method of claim 35 or 36, wherein the sample is treated with one or more species of immobilized organic multivalent ions of the same or different net charge, on a solid surface under conditions where a desired protein is substantially unbound to the immobilized organic multivalent ions.

40. The method of claim 35 or 36, wherein the sample is treated with both soluble and immobilized organic multivalent ions, and wherein at least one species of immobilized organic multivalent ions has a net charge opposite to the soluble organic multivalent ions.

41. The method of claim 40, wherein the immobilized organic multivalent ions include species of opposite net charge.

42. The method of any one of claims 38-41, wherein at least one species of organic multivalent cation comprises a metal affinity functionality.

43. The method of any one of claims 38-42, wherein treatment with soluble organic multivalent ions occurs first, or exposure to soluble and immobilized organic multivalent ions occurs essentially at the same time.

44. The method of any one of claims 1-43, wherein in the method is practiced at a pH value selected from the group consisting of (i) 7, (ii) 6.5 to 7.5, (iii) 6 to 8, (iv) 5 to 9, (v) 4-10, (vi) less than 4, and (vii) an intermediate pH value.

45. The method of any one of claims 1-44, wherein in the method is practiced at a conductivity selected from the group consisting of (i) 12-15 mS/cm, (ii) 5-20 mS/cm, (iii) 1-30 mS/cm (iv) 1-50 mS/cm (v) 1-100 mS cm (vi) less than 1 mS/cm, (vii) more than 100 mS/cm, and (viii) an intermediate conductivity value.

46. The method of any one of claims 1-45, wherein the biological target to be bound to the allantoin is incubated together with the allantoin for a period of time selected from the group consisting of (i) 1 minute, (ii) 5 minutes, (iii) 10 minutes, (iv) 15 minutes, (v) 30 minutes, (vi) 60 minutes, (vii) more than 60 minutes, or (viii) an intermediate period of time.

47. The method of claim 1, wherein the biological target to be bound to the allantoin is a virus being subjected to treatment to inactivate the virus.

48. The method of claim 47, wherein the virus inactivating treatment is one from the group consisting of exposure to (i) a pH of 3 to 5, (ii) a pH of 9-11, (iii) ethacridine, (iv) chlorhexidine, (v) benzalkonium chloride, (vi) a surfactant, (vii) tri(n-butyl)phosphate, (viii) methylene blue, (ix) polyethyleneimine, (x) urea, (xi) guanidine, (xii) caprylic acid, (xiii) sodium chloride, or (xiv) arginine.

49. The method of any one of claims 1-48, with the proviso that sample does not include polyethylene glycol 6000 (PEG-6000) at an amount of 5% or greater.

50. The method of claim 49, with the proviso that sample does not include polyethylene glycol 6000 (PEG-6000) at an amount of 2% or greater.

51. The method of claim 49, with the proviso that sample does not include polyethylene glycol 6000 (PEG-6000).

52. The method of claim 49, with the proviso that sample does not include polyethylene glycol at an amount of 2% or greater.

53. The method of claim 49, with the proviso that sample does not include polyethylene glycol.

54. A method of selectively separating a biological target from a sample comprising the biological target material or suspected of comprising the biological target comprising the steps of (i) providing a solid comprising allantoin at its surface; (ii) contacting the sample with the solid, whereby at least 90% of the biological target in the sample binds to the allantoin; and (iii) separating the solid from the sample.