~~What is claimed is:~~

CLAIMS

1.~~——~~ A method of manufacturing a recombinant therapeutic protein, the method comprising:

providing a system comprising a first vessel and a second vessel, where the first vessel comprises a liquid comprising ~~the~~a recombinant therapeutic protein and the second vessel contains a volume of a sterilizing gas; and

flowing a first volume of the liquid out of the first vessel ~~and~~into the second vessel through ~~a~~the volume of the sterilizing gas ~~and into a second vessel~~contained within a headspace of the second vessel, wherein the method results in the manufacturing of the recombinant therapeutic protein.

2. The method of claim 1, wherein the ~~volume of sterilizing gas is disposed within a head space of the second vessel.~~

~~3. The method of claim 2, wherein the~~ second vessel further comprises:

(i) a fluid inlet configured such that the first volume of the liquid entering the second vessel passes through the ~~head space~~headspace;

(ii) a fluid outlet configured such that liquid exiting the second vessel is flowed from below the ~~sterilizing gas-filled head space;~~headspace;

(iii) at least one gas inlet; and

(iv) at least one gas outlet,

wherein the fluid inlet is in fluid communication with the first vessel.

~~4~~3. The method of claim ~~3~~2, wherein the first vessel comprises a fluid outlet in fluid communication with the fluid inlet of the second vessel.

~~5~~4. The method of claim 1, wherein during the ~~process~~method the second vessel is at least partially filled with a liquid.

~~6~~5. The method of claim ~~2~~1, wherein the sterilizing gas is sparged into the second vessel.

~~7~~6. The method of claim ~~2~~1, wherein the sterilizing gas is introduced directly into the ~~head space~~headspace of the second vessel.

~~8~~

7. The method of claim 1, wherein the first vessel is a sterilized vessel.

~~9~~8. The method of claim 1, wherein the system is a biological manufacturing system.

~~10~~9. The method of claim 1, wherein the method further ~~comprising~~comprises:

(i) flowing a second volume of fluid from the second vessel into a first multi-column chromatography system (MCCS1);

(ii) capturing the recombinant therapeutic protein in the second volume of fluid using the MCCS1, wherein the eluate from the MCCS1 comprising the recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2); and

(iii) purifying and polishing the recombinant therapeutic protein using the MCCS2, wherein the eluate from the MCCS2 comprises the recombinant therapeutic protein.

~~11~~10. The method of claim ~~10~~9, wherein the method is integrated and runs continuously from the first vessel to the eluate from the MCCS2.

~~12~~11. The method of claim ~~10~~9, wherein the MCCS1 is a first periodic countercurrent chromatography system (PCCS1).

~~13~~12. The method of claim ~~10~~9, wherein the MCCS2 is a second periodic countercurrent chromatography system (PCCS2).

~~14~~13. The method of claim ~~10~~9, wherein the MCCS1 is a first periodic countercurrent chromatography system (PCCS1) and the MCCS2 is a second periodic countercurrent chromatography system (PCCS2).

~~15~~14. The method of claim 1, wherein the recombinant therapeutic protein is an antibody or antibody fragment.

~~16~~15. The method of claim 1, wherein the recombinant therapeutic protein is an enzyme.

~~17~~16. The method of claim ~~1~~15, wherein the enzyme is α-galactosidase-A.

~~18~~17. The method of claim ~~1~~15, wherein the enzyme is ~~ϑ~~ beta-glucosidase.

18. The method of claim 15, wherein the enzyme is alglucosidase.

19. The method of claim 1, wherein the ~~enzyme is alglucosidase~~sterilizing gas comprises ozone.

20. The method of claim 1, wherein the sterilizing gas comprises ~~ozone~~ethylene oxide.

21. The method of claim 1, wherein the sterilizing gas comprises ~~ethylene oxide~~nitrogen dioxide.

22. The method of claim 1, wherein the sterilizing gas comprises ~~nitrogen dioxide.~~

~~23. The method of claim 1, wherein the sterilizing gas comprises~~ vaporized hydrogen peroxide.

~~24~~23. The method of any one of claims 1-~~23~~22, wherein the first vessel is a bioreactor.

~~25~~24. The method of claim ~~24~~23, wherein the bioreactor is a perfusion bioreactor.

~~26~~25. The method of claim ~~24~~23, wherein the bioreactor is a fed-batch bioreactor.

~~27~~26. The method of claim ~~24~~23, wherein the bioreactor is a production bioreactor.

~~28~~ 27. The method of any one of claims 1-~~23~~22, wherein the first vessel is a chromatography system.

~~29~~ 28. The method of any one of claims 1-~~23~~22, wherein the first vessel is an ultrafiltration/diafiltration system.

~~30. The method of any one of claims 1-23, wherein the first volume of the liquid is a liquid culture medium that is substantially free of mammalian cells.~~