

We claim:

1. ~~—A process for manufacturing a purified protein of interest, the process comprising the step of:~~

~~(a) —culturing mammalian cells in one or more single-use perfusion bioreactors comprising a liquid culture medium under conditions that allow the cells to secrete the protein into the liquid culture medium for a production cultivation period of at least 10 days, wherein, periodically or continuously, during the production cultivation period, fresh sterile liquid culture medium is added into the one or more perfusion bioreactors, being mixed contemporaneously from a plurality of different concentrated medium component solutions and an aqueous diluent, to maintain a constant culture volume in each of the perfusion bioreactor(s), in direct relation to volumes of the culture that are continuously or periodically removed from each of the perfusion bioreactor(s) as volumes of permeate or cell bleed, and wherein the removed volumes of permeate are automatically and fluidly fed from the one or more single-use perfusion bioreactor(s) into a single-use surge vessel and thence into a first chromatography system, whereby the protein is collected in a protein isolate fraction.~~

2. ~~The process of Claim 1, further comprising the step of:~~

~~(b) switching the protein isolate fraction into a low pH or detergent viral inactivation system and, if needed, a neutralization system, to obtain a virally inactivated product pool comprising the protein.~~

3. ~~The process of Claim 2, further comprising the steps of:~~

~~(c) introducing the virally inactivated product pool into a second chromatography system to obtain a purified product pool comprising the protein;~~

~~(d) switching the purified product pool comprising the protein into an optional third chromatography system and/or a viral filtration system to obtain a virus-free filtrate comprising the protein; and~~

~~(e) switching the virus-free filtrate into an ultrafiltration/diafiltration system to obtain a composition comprising the purified protein of interest.~~

4. ~~—The process of Claim 1, wherein the protein of interest is a recombinant protein.~~

5. ~~—The process of Claim 1, wherein the protein of interest is a therapeutic protein.~~

6.——~~The process of Claim 1, Claim 2, or Claim 3, wherein one or more of the first chromatography system, the second chromatography system, the third chromatography system, the low pH or detergent viral inactivation system, the neutralization system, the viral filtration system, or the ultrafiltration/diafiltration system, comprise a single use component(s).~~

7.——~~The process of Claim 1, wherein the mammalian cells are cultured in two, three, four, five, or six single use perfusion bioreactors.~~

8.——~~The process of Claim 1, wherein the one or more single use bioreactor(s) can contain a volume of liquid culture medium about 50 L to about 4000 L.~~

9.——~~The process of Claim 1, wherein the fresh sterile liquid culture medium is added to the one or more perfusion bioreactors, by injecting the plurality of different concentrated medium component solutions at fixed ratios relative to one another, directly into the perfusion bioreactor(s), while an aqueous diluent is also added at varied ratio(s) relative to the plurality of different concentrated component solutions, to maintain a constant culture volume in each perfusion bioreactor(s).~~

10.——~~The process of Claim 1, wherein the fresh sterile liquid culture medium is added to the one or more perfusion bioreactors, by injecting the plurality of different concentrated medium component solutions and the aqueous diluent at fixed ratios relative to one another, directly into the perfusion bioreactor(s), to maintain a constant culture volume in each perfusion bioreactor(s).~~

11.——~~The process of Claim 1, wherein the fresh sterile liquid culture medium is added to the one or more perfusion bioreactors, by injecting the plurality of different concentrated medium component solutions and the aqueous diluent, at fixed ratios relative to one another, into a mixing chamber wherein fresh sterile liquid culture medium is mixed contemporaneously before being added to each perfusion bioreactor(s) to maintain a constant culture volume.~~

12.——~~The process of Claim 1, wherein an automated controller comprising a detector is used to measure the fluid volume in the single use surge vessel, and a processor varies the pump speeds of the first chromatography system to maintain a pre-set volume range in the single use surge vessel.~~

~~13.—The process of Claim 2 or Claim 3, wherein one or more of steps (b), (c), (d), or (e) is performed automatically and fluidly in an uninterrupted flow from the previous step, and wherein a surge vessel is employed between one or more steps, and a processor varies the pump speed in a subsequent step to regulate the pre-set volume range of the surge vessel preceding the subsequent step.~~

~~14.—The process of Claim 13, wherein in-line or in-vessel conditioning of pH and/or conductivity load is performed between the one or more of steps (b), (c), (d), or (e).~~

~~15.—The process of Claim 1, wherein:~~

~~(i) a process automation system is in electronic communication with at least the one or more single-use perfusion bioreactors, the single-use surge vessel, and the first chromatography system;~~

~~(ii) the process automation system stores a first set of control modules to control operation of at least one single-use perfusion bioreactor of the one or more single-use perfusion bioreactors;~~

~~(iii) the process automation system stores a second set of control modules to control operation of feed tanks;~~

~~(iv) the process automation system stores a third set of control modules to control operation of collection tanks; and~~

~~(v) the at least one single-use perfusion bioreactor is logically configured to be coupled to one or more feed tanks, one or more collection tanks, or a filter bank.~~

~~16.—The process of Claim 15, wherein the at least one single-use perfusion bioreactor is disposed on a skid and the skid includes a plurality of communication interfaces to electronically couple the at least one single-use perfusion bioreactor to a plurality of pieces of portable equipment.~~

~~17.—The process of Claim 16, further comprising:~~

~~determining, by the process automation system, that the single-use surge vessel has been coupled to a communication interface of the plurality of communication interfaces based on data received via the communication interface, the data indicating an identifier of the single-use surge vessel and a function of the single-use surge vessel; and~~

~~determining, based at least partly on the identifier and the function of the single-use surge vessel, that the single-use surge vessel is a collection tank and that the third set of control modules is to control operation of the single-use surge vessel.~~

~~18. — The process of Claim 17, further comprising:~~

~~determining, by the process automation system, that a mixing vessel has been coupled to an additional communication interface of the plurality of communication interfaces based on additional data received via the additional communication interface, the additional data indicating an additional identifier of the mixing vessel and an additional function of the mixing vessel; and~~

~~determining, based at least partly on the additional identifier and the additional function of the mixing vessel, that the mixing vessel is a feed tank and that the second set of control modules is to control operation of the mixing vessel.~~

~~19. — The process of Claim 1, wherein the production cultivation period is at least 20 days.~~

201. An automated facility for manufacturing a purified protein of interest, the facility comprising:

(a) one or more single-use perfusion bioreactors capable of containing a liquid culture medium under conditions that allow cultured cells to secrete the protein into the liquid culture medium for a production cultivation period of at least 10 days; wherein the single-use perfusion bioreactor(s) are adapted to receive fresh sterile liquid culture medium fluidly through an inlet into each of the perfusion bioreactor(s) in direct relation to volumes of conditioned culture medium that are continuously or periodically removed from each of the perfusion bioreactor(s) as volumes of permeate or cell bleed during the production cultivation period;

(b) a plurality of reservoirs, each adapted for containing a concentrated medium component solution or aqueous diluent, the plurality of reservoirs being fluidly connected to the inlet into each of the perfusion bioreactor(s) directly, or indirectly via an optional mixing vessel adapted for receiving from the plurality of reservoirs the concentrated culture medium component solutions and aqueous diluent at predetermined ratios and contemporaneously mixing them, the optional mixing vessel being fluidly connected directly to the inlet into each of the perfusion bioreactor(s);

- (c) a first single-use surge vessel (SUSV1) into which said removed volumes of permeate are automatically and fluidly fed from the one or more single-use perfusion bioreactor(s); and
 - (d) a first chromatography system, adapted to automatically and fluidly receive cell-free permeate from the SUSV1, whereby the protein is captured in a protein isolate fraction; and
- wherein the automated facility is controlled by a process automation system (PAS) optionally

~~21.~~ ~~The automated facility of Claim 20,~~ further comprising:

- (e) a low pH or detergent viral inactivation system and, if needed, a neutralization system, adapted to automatically and fluidly receive the protein isolate fraction from the first chromatography system, whereby a virally inactivated product pool comprising the protein is obtained; and
- (f) a holding vessel or a second single-use surge vessel, adapted for receiving the virally inactivated product pool.

~~22.~~ The automated facility of Claim ~~120~~, further comprising:

- (g) a second chromatography system adapted to fluidly receive from the holding vessel or the second single-use surge vessel the virally inactivated product pool, whereby a purified product pool comprising the protein is obtained;
- (h) an optional third chromatography system and/or a viral filtration system adapted to fluidly receive the purified product pool comprising the protein from the second chromatography system, whereby a virus-free filtrate comprising the protein is obtained; and
- (i) an ultrafiltration/diafiltration system adapted to fluidly receive the virus-free filtrate from the second chromatography system or from the third chromatography system and/or the viral filtration system, whereby the purified protein of interest is obtained.

~~23.~~ The automated facility of Claim ~~201 or 2~~, wherein one or more single-use perfusion bioreactors can contain a volume of liquid culture medium of about 50 L to about 4000 L.

~~24.~~ The automated facility of Claim ~~201~~, further comprising an automated controller comprising a detector to measure the fluid volume in SUSV1, and a processor to vary the pump speeds of the first chromatography system to maintain a pre-set volume range in SUSV1.

~~255.~~ The automated facility of Claim [201](#), wherein one or more of the first chromatography system, the second chromatography system, the third chromatography system, the low pH or detergent viral inactivation system, the neutralization system, the viral filtration system, or the ultrafiltration/diafiltration system, comprise a single-use component(s).

~~266.~~ The automated facility of Claim [201](#), further comprising, and fluidly connected directly downstream from the first chromatography system:

- (i) a second single-use surge vessel; or
- (ii) at least two automatically switchable alternate single-use collection vessels (SUCV1 and SUCV2) adapted for receiving the protein isolate fraction;

wherein (i) and (ii) are adapted to receive the protein isolate fraction from the first chromatography system and to fluidly feed the protein isolate fraction to the low pH or detergent viral inactivation system.

~~27.~~ ~~The automated facility of Claim 21,~~ wherein [preferably](#) the low pH or detergent viral inactivation system and, if needed, the neutralization system, comprises:

- (i) a second single-use surge vessel adapted for receiving the protein isolate fraction; or
- (ii) at least two automatically switchable alternate single-use collection vessels (SUCV1 and SUCV2) adapted for receiving the protein isolate fraction;

wherein viral inactivation, and if needed neutralization, is conducted within the second single-use surge vessel, or within SUCV1 and SUCV2.

~~287.~~ The automated facility of Claim [201](#), further comprising a hollow fiber membrane, a series of depth filters, or a filtration cart, before the permeate is automatically and fluidly fed to the SUSV1.

~~298.~~ The automated facility of Claim [241](#), comprising in (f) a single-use surge vessel adapted for receiving the virally inactivated product pool.

~~309.~~ The automated facility of Claim [201](#), further comprising a heat exchanger upstream of the SUSV1 [and/or](#).

~~31.~~ ~~The automated facility of Claim 20, further comprising~~ a filtration system upstream of the SUSV1.

[3210](#). The automated facility of Claim [221](#), wherein one or more of:

- (i) the second chromatography system;
- (ii) the optional third chromatography system;
- (iii) the viral filtration system; and
- (iv) the ultrafiltration/diafiltration system,

is automatically and fluidly connected to the previous system, and wherein a surge vessel is optionally employed to regulate the uninterrupted flow of material between the connected systems.

[3311](#). The automated facility of any of Claims [20-221](#), wherein:

at least the one or more single-use perfusion bioreactors, SUSV1, the first chromatography system, the low pH or detergent viral inactivation system, the holding vessel or single-use surge vessel, the second chromatography system, the optional third chromatography system and/or the viral filtration system, and the ultrafiltration/diafiltration system comprise first pieces of equipment that are arranged in a first configuration of a production line for the purified protein of interest; and

a first plurality of control modules are implemented to control operation of the first pieces of equipment.

[3412](#). The automated facility of Claim [3311](#), wherein:

second pieces of equipment are arranged in a second configuration of a production line for an additional purified protein of interest, the second configuration of the production line including at least the one or more single-use perfusion bioreactors, the first chromatography system, the low pH or detergent viral inactivation system, the second chromatography system, the ultrafiltration/diafiltration system, and a plurality of mixing vessels;

the second configuration of the production line being different from the first configuration of the production line;

a second plurality of control modules are implemented to control operation of the second pieces of equipment;

at least one mixing vessel of the plurality of mixing vessels is included in both the first configuration and the second configuration; and

the at least one mixing vessel has a first function in the first configuration and a second function in the second configuration, the second function being different from the first function.

[3513](#). The automated facility of any of Claims [20-221](#), further comprising a portable filter bank, the portable filter bank including a plurality of filter assemblies, wherein:

a first filter assembly of the plurality of filter assemblies includes a first filter and a second filter assembly of the plurality of filter assemblies includes a second filter; and

a production facility control system:

monitors a pressure within the first filter assembly as material flows through the first filter assembly;

determines that the pressure within the first filter assembly is at least a threshold value; and

sends a signal to cause a diverter valve coupled to the first filter assembly and the second filter assembly to operate to cause the material to flow into second filter assembly.

[3614](#). The automated facility of Claim [201](#), wherein the one or more single-use perfusion bioreactors is capable of containing a liquid culture medium under conditions that allow the cultured cells to secrete the protein into the medium for a production cultivation period of at least 20 days.

[3715](#). The automated facility of Claim [201](#), wherein the protein of interest is a recombinant protein.

[3816](#). The automated facility of Claim [201](#), wherein the protein of interest is a therapeutic protein.

[17](#). The automated facility of Claim 1, wherein:

(i) the process automation system is in electronic communication with at least one or more single use-perfusion bioreactors, the single-use surge vessel and the first chromatography system;

(ii) the process automation system stores a first set of control modules to control operation of at least one single-use perfusion bioreactor of the one or more single use perfusion bioreactors;

(iii) the process automation system stores a second set of control modules to control operation of feed tanks;

(iv) the process automation stores a third set of control modules to control operation of collection tanks; and

(v) the at least one single use perfusion bioreactor is logically configured to be coupled to one or more feed tanks, one or more collection tanks, or a filter bank,

wherein preferably the at least one single use perfusion bioreactor is disposed on a skid and the skid includes a plurality of communication interfaces to electronically couple the at least one single use perfusion bioreactor to a plurality of pieces of portable equipment.

~~39.—A process for manufacturing a purified protein drug substance comprising a protein of interest, the process comprising the steps of:~~

~~(a) —culturing mammalian cells in one or more single-use perfusion bioreactors comprising a liquid culture medium under conditions that allow the cells to secrete the protein into the medium for a production cultivation period of at least 10 days, wherein, periodically or continuously, during the production cultivation period, fresh sterile liquid culture medium is added into the one or more perfusion bioreactors, to maintain a constant culture volume in each of the perfusion bioreactor(s), in direct relation to volumes of the culture that are continuously or periodically removed from each of the perfusion bioreactor(s) as volumes of permeate or cell bleed, and wherein the removed volumes of permeate are automatically and fluidly fed from the one or more single-use perfusion bioreactor(s) into a single-use surge vessel and thence into a first chromatography system, whereby the protein is collected in a protein isolate fraction;~~

~~(b) switching the protein isolate fraction into a low pH or detergent viral inactivation system and, if needed, a neutralization system, to obtain a virally inactivated product pool comprising the protein;~~

~~(c) introducing the virally inactivated product pool into a second chromatography system to obtain a purified product pool comprising the protein;~~

~~(d) switching the purified product pool comprising the protein into an optional third chromatography system and/or a viral filtration system to obtain a virus-free filtrate comprising the protein; and~~

~~(e) switching the virus-free filtrate into an ultrafiltration/diafiltration system to obtain the purified protein drug substance comprising the protein of interest.~~

~~40. The process of Claim 39, wherein the fresh sterile liquid culture medium is mixed contemporaneously from a plurality of different concentrated medium component solutions and an aqueous diluent, before being added into the one or more perfusion bioreactors to maintain a constant culture volume in each of the perfusion bioreactor(s).~~

~~41. The process of Claim 39, wherein the protein of interest is a recombinant protein.~~

~~42. The process of Claim 39, wherein the protein of interest is a therapeutic protein.~~

~~43. An automated facility for manufacturing a purified protein drug substance, the facility comprising:~~

~~(a) one or more single-use perfusion bioreactors capable of containing a liquid culture medium under conditions that allow cultured mammalian cells to secrete a protein of interest into the medium for a production cultivation period of at least 10 days; wherein the single-use perfusion bioreactor(s) are adapted to receive fresh sterile liquid culture medium fluidly into each of the perfusion bioreactor(s) in direct relation to volumes of conditioned culture medium that are continuously or periodically removed from each of the perfusion bioreactor(s) as volumes of permeate or cell bleed during the production cultivation period;~~

~~(b) a first single-use surge vessel (SUSV1) into which said removed volumes of permeate are automatically and fluidly fed from the one or more single-use perfusion bioreactor(s);~~

~~(c) a first chromatography system, adapted to automatically and fluidly receive permeate from the SUSV1, whereby the protein is captured in a protein isolate fraction;~~

~~(d) a low pH or detergent viral inactivation system and, if needed, a neutralization system, adapted to automatically and fluidly receive the protein isolate fraction from the first chromatography system, whereby a virally inactivated product pool comprising the protein is obtained;~~

~~(e) a holding vessel or a single-use surge vessel, adapted for receiving the virally inactivated product pool;~~

~~(f) — a second chromatography system adapted to fluidly receive from the holding vessel or single use surge vessel the virally inactivated product pool, whereby a purified product pool comprising the protein is obtained;~~

~~(g) — an optional third chromatography system and/or a viral filtration system adapted to fluidly receive the purified product pool comprising the protein from the second chromatography system, whereby a virus free filtrate comprising the protein is obtained; and~~

~~(h) — an ultrafiltration/diafiltration system adapted to fluidly receive the virus free filtrate from the second chromatography system or from the third chromatography system and/or the viral filtration system, whereby the purified protein drug substance is obtained; and~~

~~wherein the automated facility is controlled by a process automation system (PAS).~~

~~44. — The automated facility of Claim 43, wherein a plurality of reservoirs, each adapted for containing a concentrated medium component solution or aqueous diluent, are fluidly connected to the perfusion bioreactor(s) directly, or indirectly via an optional mixing vessel adapted for receiving from the plurality of reservoirs the concentrated culture medium component solutions and aqueous diluent at predetermined ratios and contemporaneously mixing them, the optional mixing vessel being fluidly connected directly to the perfusion bioreactor(s).~~

~~45. — The automated facility of Claim 43, wherein the protein of interest is a recombinant protein.~~

~~46. — The automated facility of Claim 43, wherein the protein of interest is a therapeutic protein.~~

~~47. — The automated facility of any of Claims 20-22, or Claim 43, wherein the facility is configured for operation in a continuous format.~~

~~48. — The process of any of Claims 1-3, Claim 6, or Claim 39, wherein the process is conducted in a continuous format.~~

~~49. — The process of any of Claims 1-3, Claim 6, or Claim 39, wherein the first chromatography system is sanitized with a chemical sanitant solution comprising peracetic acid before use.~~

~~50. The process of Claim 3 or Claim 39, wherein the ultrafiltration/diafiltration system comprises a single pass tangential flow filtration (SPTFF), and the operating pressure of the SPTFF is controlled in a range of about 0.25 psi to about 60 psi.~~

~~51. The process of Claim 3 or Claim 39, wherein the ultrafiltration/diafiltration system comprises inline depth filtration (ILDF), and the operating pressure of the ILDF is controlled in a range of about 0.25 psi to about 60 psi.~~