US Patent Application No. 20160333046 also identified a number of detergents that show robust viral clearance, as well as ecological safety.

As such, there is a need for detergents that are effective for inactivation of envelope viruses, particularly detergents that are considered eco-friendly, for use within viral clearance process steps in biomanufacturing. The invention described herein meets this need by identifying effective, viral inactivating detergents that could be incorporated into the biomanufacturing processes for multiple therapeutic modalities.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

The ~~invention~~disclosure provides a method for inactivating envelope virus in a fluid known or suspected to contain at least one enveloped virus, comprising obtaining a fluid known or suspected to contain at least one enveloped virus; exposing the fluid to a detergent from Table 1; at a concentration and for a time sufficient to cause viral inactivation. In one aspect, the present disclosure provides a method for inactivating enveloped virus in a fluid known or suspected to contain at least one enveloped virus, comprising obtaining a fluid known or suspected to contain at least one enveloped virus; and exposing the fluid to a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a concentration and for a time sufficient to cause viral inactivation, wherein the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In another aspect, the present disclosure provides a method for inactivating enveloped viruses during purification of a recombinant protein of interest comprising obtaining a fluid comprising the recombinant protein of interest which is known or suspected to contain at least one virus; subjecting the fluid to at least one detergent, wherein the detergent has a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7 at a concentration and for a time sufficient to cause inactivation of enveloped viruses in the fluid; and subjecting the viral inactivated fluid to at least one unit operation which includes at least a filtration step or a chromatography step, wherein the concentration is 5X of its CMC and the time is at least 10 minutes. In another aspect, the present disclosure provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; subjecting the harvested fluid containing the recombinant protein of interest to a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-

12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; processing the viral inactivated fluid containing the recombinant protein of interest through at least two additional unit operations; and obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes. In another aspect, the present disclosure provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; processing the fluid containing the recombinant protein of interest through at least two unit operations, wherein during at least one unit operation there is a step where the fluid containing the recombinant protein of interest is combined with a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; and obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

In one ~~embodiment~~example, the detergent has a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7. In one ~~embodiment~~example the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, or CAS 85618-20-8. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 10 minutes. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 30 minutes. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 60 minutes or longer. In one ~~embodiment~~example the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In one ~~embodiment~~example the concentration of the detergent is at least 5X of its CMC. In a related ~~embodiment~~example the concentration of the detergent is at least 7.5X of its CMC. In another related ~~embodiment~~example the concentration of the detergent is at least 10X of its CMC. In one ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5ºC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 15ºC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 20ºC. In another ~~embodiment~~example inactivation is determined using a $TCID_{50}$ assay. In one ~~embodiment~~example the fluid comprises a recombinant protein of interest. In one ~~embodiment~~example the fluid is harvested host cell culture fluid. In one ~~embodiment~~example the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step or a chromatography step. In a related ~~embodiment~~example the fluid is eluate collected from depth filtration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography. In one related ~~embodiment~~example the fluid is a pool containing harvested cell culture fluid, eluate from depth filtration,

eluate from affinity chromatography, eluate from ion exchange chromatography, eluate from multimodal chromatography, eluate from hydrophobic interaction chromatography or eluate from hydroxyapatite chromatography. In another related ~~embodiment~~example the affinity chromatography is Protein A, Protein G, Protein A/G, or Protein L chromatography. In one ~~embodiment~~example, the detergent concentration is 5X of its CMC and the time is at least 10 minutes.

The ~~invention~~disclosure provides a method for inactivating enveloped viruses during purification of a recombinant protein of interest comprising obtaining a fluid comprising the recombinant protein of interest which is known or suspected to contain at least one virus; subjecting the fluid to at least one detergent, wherein the detergent has a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7 at a concentration and for a time sufficient to cause inactivation of enveloped viruses in the fluid; and subjecting the viral inactivated fluid to at least one unit operation which includes at least a filtration step or a chromatography step. In one ~~embodiment~~example the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 10 minutes. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 30 minutes. In another related ~~embodiment~~example the fluid is exposed to the detergent for at least 60 minutes or longer. In another ~~embodiment~~example the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In a related ~~embodiment~~example the concentration of the detergent is at least 5X of its CMC. In a related ~~embodiment~~example the concentration of the detergent is at least 7.5X of its CMC. In another related ~~embodiment~~example the concentration of the detergent is at least 10X of its CMC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5ºC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 15ºC. In another related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 20ºC. In another ~~embodiment~~example inactivation is determined using a $TCID_{50}$ assay. In another ~~embodiment~~example the fluid comprises a recombinant protein of interest. In another ~~embodiment~~example the fluid is harvested host cell culture fluid. In another ~~embodiment~~example the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step or a chromatography step. In another ~~embodiment~~example the fluid is eluate collected from depth filtration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography. In another ~~embodiment~~example the fluid is a pool containing harvested cell culture fluid, eluate from depth filtration, eluate from affinity chromatography, eluate from ion exchange chromatography, eluate from multimodal chromatography, eluate from hydrophobic interaction chromatography or eluate from hydroxyapatite chromatography. In a related ~~embodiment~~example the affinity chromatography is Protein A, Protein G, Protein

A/G, or Protein L chromatography. In another ~~embodiment~~example the chromatography is selected from affinity chromatography, Protein A chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography; hydrophobic interaction chromatography; mixed modal or multimodal chromatography, or hydroxyapatite chromatography. In another ~~embodiment~~example the fluid is harvested host cell culture fluid and the unit operation includes depth filtration. In another ~~embodiment~~example the fluid is harvested host cell culture fluid and the unit operation includes Protein A affinity chromatography. In another ~~embodiment~~example the fluid is Protein A eluant and the unit operation includes depth filtration. In another ~~embodiment~~example the unit operation includes depth filtration. In one ~~embodiment~~example the unit operation includes microfiltration. In one ~~embodiment~~example, the detergent concentration is 5X of its CMC and the time is at least 10 minutes.

The ~~invention~~disclosure also provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; processing the fluid containing the recombinant protein of interest through at least two unit operations, wherein during at least one unit operation there is a step where the fluid containing the recombinant protein of interest is combined with a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; and obtaining an isolated, purified, recombinant protein of interest. In one ~~embodiment~~example the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 10 minutes. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 30 minutes. In another related ~~embodiment~~example the fluid is exposed to the detergent for at least 60 minutes or longer. In another ~~embodiment~~example the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In a related ~~embodiment~~example the concentration of the detergent is at least 5X of its CMC. In a related ~~embodiment~~example the concentration of the detergent is at least 7.5X of its CMC. In another related ~~embodiment~~example the concentration of the detergent is at least 10X of its CMC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5ºC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 15ºC. In another related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 20ºC. In another ~~embodiment~~example inactivation is determined using a $TCID_{50}$ assay. In another ~~embodiment~~example at least one unit operation includes a capture chromatography step selected from affinity chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In another ~~embodiment~~example at least one unit operation includes a polish chromatography step selected from ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal

chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In another ~~embodiment~~example at least one unit operation includes a step selected from virus filtration, depth filtration, and UF/DF. In another ~~embodiment~~example the unit operation including the viral inactivation step occurs prior to a unit operation which includes affinity chromatography. In another ~~embodiment~~example a unit operation including affinity chromatography occurs before the unit operation including the viral inactivation step. In one ~~embodiment~~example the unit operation including the viral inactivation step occurs prior to a unit operation which includes depth filtration. In one ~~embodiment~~example is provided an isolated, purified, recombinant protein of interest according the method described above. In one ~~embodiment~~example is provided a pharmaceutical composition comprising the isolated protein of interest according to the method described above. In one ~~embodiment~~example, the detergent concentration is 5X of its CMC and the time is at least 10 minutes.

The ~~invention~~disclosure also provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest; harvesting cell culture fluid containing the recombinant protein of interest; subjecting the harvested fluid containing the recombinant protein of interest to a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; processing the viral inactivated fluid containing the recombinant protein of interest through at least two additional unit operations; and obtaining an isolated, purified, recombinant protein of interest In one ~~embodiment~~example the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8. In one ~~embodiment~~example the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 10 minutes. In a related ~~embodiment~~example the fluid is exposed to the detergent for at least 30 minutes. In another related ~~embodiment~~example the fluid is exposed to the detergent for at least 60 minutes or longer. In another ~~embodiment~~example the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC). In a related ~~embodiment~~example the concentration of the detergent is at least 5X of its CMC. In a related ~~embodiment~~example the concentration of the detergent is at least 7.5X of its CMC. In another related ~~embodiment~~example the concentration of the detergent is at least 10X of its CMC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5° C to 22° C. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 5ºC. In a related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 15ºC. In another related ~~embodiment~~example exposure of the fluid to the detergent occurs at a temperature of at least 20ºC. In another ~~embodiment~~example inactivation is determined

using a $TCID_{50}$ assay. In another ~~embodiment~~example at least one unit operation includes a capture chromatography step selected from affinity chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In another ~~embodiment~~example at least one unit operation includes a polish chromatography step selected from ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In another ~~embodiment~~example at least one unit operation includes a step selected from virus filtration, depth filtration, and UF/DF. In another ~~embodiment~~example the unit operation including the viral inactivation step occurs prior to a unit operation which includes affinity chromatography. In another ~~embodiment~~example a unit operation including affinity chromatography occurs before the unit operation including the viral inactivation step. In one ~~embodiment~~example the unit operation including the viral inactivation step occurs prior to a unit operation which includes depth filtration. In one ~~embodiment~~example is provided an isolated, purified, recombinant protein of interest according the method described above. In one ~~embodiment~~example is provided a pharmaceutical composition comprising the isolated protein of interest according to the method described above. In one ~~embodiment~~example, the detergent concentration is 5X of its CMC and the time is at least 10 minutes.

The ~~invention~~disclosure also provides an isolated, purified, recombinant protein of interest according to the methods described herein. The ~~invention~~disclosure also provides a pharmaceutical composition comprising the isolated protein of interest according to the methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Structurally different detergent classes identified to have viral inactivating properties.

Figure 2: Mass Spec Elution peaks for: A: mAb Elution after ProA purification. B: Blank run after detergent injection to verify clearance of detergent from detector. C: Spiked C12E9 detergent (0.03%) into water.

Figure 3: Turbidity data for a mixture of detergents and HCCF at room temperature (25°C) and at 5°C. Triton X, triangle; Anapoe C12E8 (APO128), diamond; Anapoe C12E8 (APO129), square; no detergent control, circle.

Figure 4: %HMW and %LMW for detergent spiked samples before and after affinity chromatography. Triton X, triangle; Anapoe C12E8 (APO128), diamond; Anapoe C12E8 (APO129), square; no detergent control, circle; TDA-9 dashed line and star; second no detergent control, dashed line, open circle.

Figure 5: %HMW and %LMW for detergent spiked BiTE® #1 samples before and after affinity chromatography.

CLAIMS

What is claimed is

~~1. A method for inactivating envelope virus in a fluid known or suspected to contain at least one enveloped virus, comprising~~

~~obtaining a fluid known or suspected to contain at least one enveloped virus;~~

~~exposing the fluid to a detergent from Table 1;~~

~~at a concentration and for a time sufficient to cause viral inactivation.~~

~~2~~1. ~~The method according to claim 1,~~ A method for inactivating enveloped virus in a fluid known or suspected to contain at least one enveloped virus, comprising

obtaining a fluid known or suspected to contain at least one enveloped virus; and

exposing the fluid to a detergent ~~wherein the detergent has~~having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a concentration and for a time sufficient to cause viral inactivation, wherein the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC).

~~3~~2. The method according to claim ~~2~~1, wherein the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, ~~or~~ and CAS 85618-20-8.

~~4~~3. The method according to claim 1 wherein the fluid is exposed to the detergent for at least 30 seconds to at least 60 minutes or longer, preferably for at least 10 minutes.

~~5. The method according to claim 4 wherein the fluid is exposed to the detergent for at least 10 minutes.~~

~~6. The method according to claim 4 wherein the fluid is exposed to the detergent for at least 30 minutes.~~

~~7. The method according to claim 4, wherein the fluid is exposed to the detergent for at least 60 minutes or longer.~~

~~8. The method according to claim 1, wherein the concentration of the detergent is at least 2.5X to at least 10X or higher of its Critical Micelle Concentration (CMC).~~

~~9~~4. The method according to claim ~~8~~1, wherein the concentration of the detergent is at least 5X of its CMC.

~~10. The method according to claim 8, wherein the concentration of the detergent is at least 7.5X of its CMC.~~

~~11. The method according to claim 8, wherein the concentration of the detergent is at least 10X of its CMC.~~

~~12~~5. The method according to claim 1, wherein exposure of the fluid to the detergent occurs at a temperature of at least 5º C to 22º C, preferably at least 15ºC.

~~13. The method according to claim 12, wherein exposure of the fluid to the detergent occurs at a temperature of at least 5ºC.~~

~~14. The method according to claim 12, wherein exposure of the fluid to the detergent occurs at a temperature of at least 15ºC.~~

~~15. The method according to claim 12, wherein exposure of the fluid to the detergent occurs at a temperature of at least 20ºC.~~

~~16~~6. The method according to claim 1, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

~~17. The method according to claim 1, wherein inactivation is determined using a $TCID_{50}$ assay.~~

~~18~~7. The method according to claim 1, wherein the fluid comprises a recombinant protein of interest.

~~19~~8. The method according to claim 1, wherein the fluid is harvested host cell culture fluid.

~~20~~9. The method according to claim 1, wherein the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step or a chromatography step, preferably wherein the fluid is

(i) eluate collected from depth filtration, microfiltration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography, or

(ii) a pool containing harvested cell culture fluid, eluate from depth filtration, eluate from microfiltration, eluate from affinity chromatography, eluate from ion exchange chromatography, eluate from multimodal chromatography, eluate from hydrophobic interaction chromatography, or eluate from hydroxyapatite chromatography, optionally wherein the affinity chromatography is Protein A, Protein G, Protein A/G, or Protein L chromatography.

~~21. The method according to claim 20, wherein the fluid is eluate collected from depth filtration, microfiltration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography.~~

~~22. The method according to claim 20, wherein the fluid is a pool containing harvested cell culture fluid, eluate from depth filtration, eluate from microfiltration, eluate from affinity chromatography, eluate from ion exchange chromatography, eluate from multimodal chromatography, eluate from hydrophobic interaction chromatography, or eluate from hydroxyapatite chromatography.~~

~~23. The method according to claim 22, wherein the affinity chromatography is Protein A, Protein G, Protein A/G, or Protein L chromatography.~~

~~24~~10. A method for inactivating enveloped viruses during purification of a recombinant protein of interest comprising

obtaining a fluid comprising the recombinant protein of interest which is known or suspected to contain at least one virus;

subjecting the fluid to at least one detergent, wherein the detergent has a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7 at a concentration and for a time sufficient to cause inactivation of enveloped viruses in the fluid; and

subjecting the viral inactivated fluid to at least one unit operation which includes at least a filtration step or a chromatography step, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

~~25. The method according to claim 24, wherein the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8.~~

~~26. The method according to claim 24, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.~~

~~27. The method according to claim 24, wherein the fluid comprises a recombinant protein of interest.~~

~~28. The method according to claim 24, wherein the fluid is harvested host cell culture fluid.~~

~~29. The method according to claim 24, wherein the fluid is from an effluent stream, eluate, pool, storage or hold from a unit operation comprising a harvest step, a filtration step, or a chromatography step.~~

~~30. The method according to claim 24, wherein the fluid is eluate collected from depth filtration, microfiltration, affinity chromatography, ion exchange chromatography, multimodal chromatography, hydrophobic interaction chromatography or hydroxyapatite chromatography.~~

~~31. The method according to claim 24, wherein the fluid is a pool containing harvested cell culture fluid, eluate from depth filtration, eluate from microfiltration, eluate from affinity chromatography, eluate from ion exchange chromatography, eluate from multimodal chromatography, eluate from hydrophobic interaction chromatography or eluate from hydroxyapatite chromatography.~~

~~32. The method according to claim 31, wherein the affinity chromatography is Protein A, Protein G, Protein A/G, or Protein L chromatography.~~

~~33~~11. The method according to claim ~~24~~10, wherein the chromatography is selected from affinity chromatography, Protein A chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography; hydrophobic interaction chromatography; mixed modal or multimodal chromatography, or hydroxyapatite chromatography.

~~34~~12. The method according to claim ~~24~~10, wherein:

(i) the fluid is harvested host cell culture fluid and the unit operation includes depth filtration;

(ii) the fluid is harvested host cell culture fluid and the unit operation includes microfiltration;

(iii) the fluid is harvested host cell culture fluid and the unit operation includes Protein A affinity chromatography; or

(iv) the fluid is Protein A eluant and the unit operation includes depth filtration.

~~35. The method according to claim 24, wherein the fluid is harvested host cell culture fluid and the unit operation includes microfiltration.~~

~~36. The method according to claim 24, wherein the fluid is harvested host cell culture fluid and the unit operation includes Protein A affinity chromatography.~~

~~37 The method according to claim 24, wherein the fluid is Protein A eluant and the unit operation includes depth filtration.~~

~~38~~13. The method according to claim ~~24~~10, wherein the unit operation includes depth filtration or microfiltration.

~~39. The method according to claim 24, wherein the unit operation includes microfiltration.~~

~~40~~14. A method for producing an isolated, purified, recombinant protein of interest comprising

establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest;

harvesting cell culture fluid containing the recombinant protein of interest;

subjecting the harvested fluid containing the recombinant protein of interest to a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS 250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus;

processing the viral inactivated fluid containing the recombinant protein of interest through at least two additional unit operations; and

obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

~~41. The method according to claim 40, wherein the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8.~~

~~42. The method according to claim 40, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.~~

~~43. An isolated, purified, recombinant protein of interest according to claim 40.~~

~~44. A pharmaceutical composition comprising the isolated protein of interest according to claim 40.~~

~~45~~15. A method for producing an isolated, purified, recombinant protein of interest comprising

establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein and culturing the cells to express the recombinant protein of interest;

harvesting cell culture fluid containing the recombinant protein of interest;

processing the fluid containing the recombinant protein of interest through at least two unit operations, wherein during at least one unit operation there is a step where the fluid containing the recombinant protein of interest is combined with a detergent having a CAS registry number of CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, CAS 85618-20-8, CAS 181135-58-0, CAS 181135-57-9, CAS

250692-65-0, CAS 228579-27-9, CAS 349477-49-2, CAS 70504-28-8, CAS 59080-45-4, CAS 69984-73-2, CAS 148616-91-5, CAS 148565-55-3, CAS 69227-93-6, CAS 82494-09-5, CAS 253678-67-0, CAS 106402-05-5, or CAS 93911-12-7, at a detergent concentration and for a time sufficient to cause inactivation of enveloped virus; and

obtaining an isolated, purified, recombinant protein of interest, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.

~~46. The method according to claim 45, wherein the detergent is selected from CAS 3055-99-0, CAS 3055-98-9, CAS 9043-30-5, and CAS 85618-20-8.~~

~~47. The method according to claim 45, wherein the concentration is 5X of its CMC and the time is at least 10 minutes.~~

~~48. The method according to claim 45, wherein at least one unit operation includes a capture chromatography step selected from affinity chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography.~~

~~49. The method according to claim 45, wherein at least one unit operation includes a polish chromatography step selected from ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography.~~

~~50. The method according to claim 45, wherein at least one unit operation includes a step selected from virus filtration, depth filtration, and UF/DF.~~

~~51. The method according to claim 45, wherein the unit operation including the viral inactivation step occurs prior to a unit operation which includes affinity chromatography.~~

~~52. The method according to claim 45, wherein a unit operation including affinity chromatography occurs before the unit operation including the viral inactivation step.~~

~~53. The method according to claim 45, wherein the unit operation including the viral inactivation step occurs prior to a unit operation which includes depth filtration.~~

~~54. An isolated, purified, recombinant protein of interest according to claim 45.~~

~~55. A pharmaceutical composition comprising the isolated protein of interest according to claim 45.~~