

CLAIMS

1. A process of purifying a human immunodeficiency virus (HIV) envelope (Env) protein, comprising:
 - a. obtaining a cell sample, such as a cell supernatant, comprising the HIV Env protein;
 - b. adjusting the pH of the cell sample to about 5.0 to thereby precipitate host cell proteins (HCPs) in the cell sample;
 - c. removing the precipitated HCPs from the cell sample by depth filtration to obtain a filtrate comprising the HIV Env protein; and
 - d. purifying the HIV Env protein in the filtrate by chromatography.
2. The process of claim 1, wherein the sample is incubated at about pH 5, preferably for about 1-3 hours, preferably about 3 hours, to precipitate the HCPs.
3. The process of claim 1 or 2, wherein the chromatography comprises
 - a. applying the filtrate comprising the protein to ultrafiltration (UF) and diafiltration (DF) to obtain a retentate comprising the HIV Env protein; and
 - b. applying the retentate to a first column to purify the HIV Env protein.
4. The process of claim 3, wherein the HIV Env protein is HIV-1 gp140 protein, and the first column comprises a multimode resin comprising hydrophobic interaction and cation exchange properties.
5. A process for purifying HIV-1 gp140 protein, comprising capturing the protein on a multimodal resin comprising hydrophobic interaction and cation exchange properties, and eluting a purified fraction from the multimodal resin, wherein the purity of the HIV-1 gp140 protein is substantially increased as compared to the protein in the mixture when it is loaded on the multimodal resin.
6. A process for purifying HIV-1 gp140 protein, comprising the steps of:
 - i) providing a composition comprising HIV-1 gp140 protein and other, non-desired proteins, such as host cell proteins derived from the host cell in which HIV-1 gp140 protein is expressed;
 - ii) capturing the HIV-1 gp140 protein on a multimodal resin comprising hydrophobic interaction and cation exchange properties, and eluting a purified fraction comprising the HIV-1 gp140 protein from the multimodal resin;
 - iii) applying the purified fraction of step ii) to an anion exchange resin to bind the

- HIV-1 gp140 protein, and eluting a further purified fraction comprising the HIV-1 gp140 protein from the anion exchange resin; and
- iv) subjecting the further purified fraction of step iii) to a multimodal resin that has anion-exchange and hydrophobic functionalities, and eluting a further purified HIV-1 gp140 protein.
7. The process of claim 6, wherein the HIV-1 gp140 protein is clade C gp140 protein, preferably comprising SEQ ID NO: 1.
 8. The process of claim 6 or 7, wherein the HIV-1 gp140 protein binds to the multimodal resin in step ii) and is eluted later.
 9. The process of any one of claims 6-8, wherein the multimodal resin in step iv) is used in flow-through mode.
 10. The process of any one of claims 6-9, further comprising the step of applying the further purified fraction of HIV-1 gp140 protein of step iii) to an affinity medium resin, and eluting a further purified fraction comprising the HIV-1 gp140 protein from said resin, preferably before subjecting this fraction to step iv).
 11. The process of claim 10, wherein the HIV-1 gp140 protein is mosaic gp140 protein, preferably comprising SEQ ID NO: 2.
 12. The process of claim 10 or 11, wherein the affinity medium resin comprises ligand sulfate or dextran sulfate.
 13. The process of any one of claims 10-12, wherein the HIV-1 gp140 protein binds to the affinity medium resin and is eluted later.
 14. The process of any one of claims 6 to 13, wherein the composition in step i) is produced by host cells in a bioreactor.
 15. The process of claim 14, the bioreactor has a volume of between about 1L to about 20000L, preferably about 10 L to about 16500 L.
 16. The process of any one of claims 6 to 15, wherein the process further comprising adjusting the pH of the composition to about 5.0 to thereby precipitate host cell proteins (HCPs) in the composition before step i).
 17. The process of any one of claims 6 to 16, wherein the purified HIV-1 gp140 protein is subject to a viral retentive filtration step.
 18. The process of any one of claims 6 to 17, wherein the purified HIV-1 gp140 protein is subject to ultrafiltration (UF) and diafiltration (DF).
 19. The process of any one of claims 6 to 18, wherein the purified HIV-1 gp140 protein is subject to a final formulation step.

20. A vaccine comprising the protein isolated by the method of any one of claims 1 to 19, and a pharmaceutically acceptable carrier.