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Poster Presentation

Purification of a chimeric virus-like particle from a complex culture medium

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Background

Virus-like particles are promising delivery vectors for molecular therapy since they combine the major advantages of viral vectors with minimal, or even with complete depletion of the viral vectors disadvantages. A chimeric Simian – Human Immunodeficiency virus-like particle (VLP) have been constructed by fusion of Simian matrix protein (p17) and HIV-1 p6 protein which assemble as viral-like particles and when produced in HEK 293T cells are released to the culture medium [1]. The purification of these VLPs from a complex culture medium involves an ultrafiltration/dialysis step, using a membrane which excludes proteins above 300kDa, and an anionic chromatography in which the particle is eluted at high salt concentrations.

Results

293 cells were transfected with the vectors encoding the chimeric protein SIV p17-HIV p6. The chimeric protein has a molecular weight of about 24kDa that assemble in spherical structures of about 80 nm. Preliminary studies with analytical ultracentrifugation indicated that this spherical structure has a molecular weight above 1000 kDa. Therefore we expect particle retention in 300 kDa cut-off membranes, which were thus used as primary purification/concentration step.

Figure 1 shows a diagram of the concentration and dialysis of 45 mL supernatant of transfected 293T cells using a 300 kDa cut-off ultrafiltration membrane, and Figure 2 shows the correspondent denaturing 12% poliacrilamide gel and western blot analysis.

As shown our VLP was successfully retained and concentrated with the 300 kDA cut off membrane. To further remove residual impurity compounds we optimize an anion exchange (AEX) chromatography, which yielded a pure VLPs solution containing no proteinaceous compounds (Figure 3).

Conclusion

Purified virus-like particles were obtained from a complex mammalian culture medium using a simple two-step

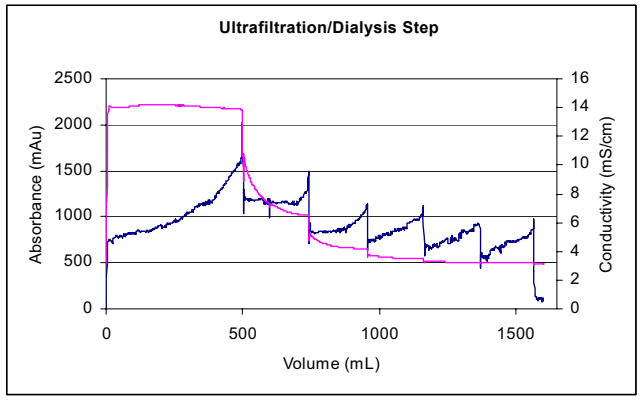

Figure 1
Variation of the absorbance during supernatant concentration and dialysis. Firstly all supernatant were concentrated to 5 mL and then diluted with 15 mL of 20 mM phosphate buffer at pH 8 and then concentrated again to 5 mL (this as been repeated five times). After each dilution it is visible protein concentration.

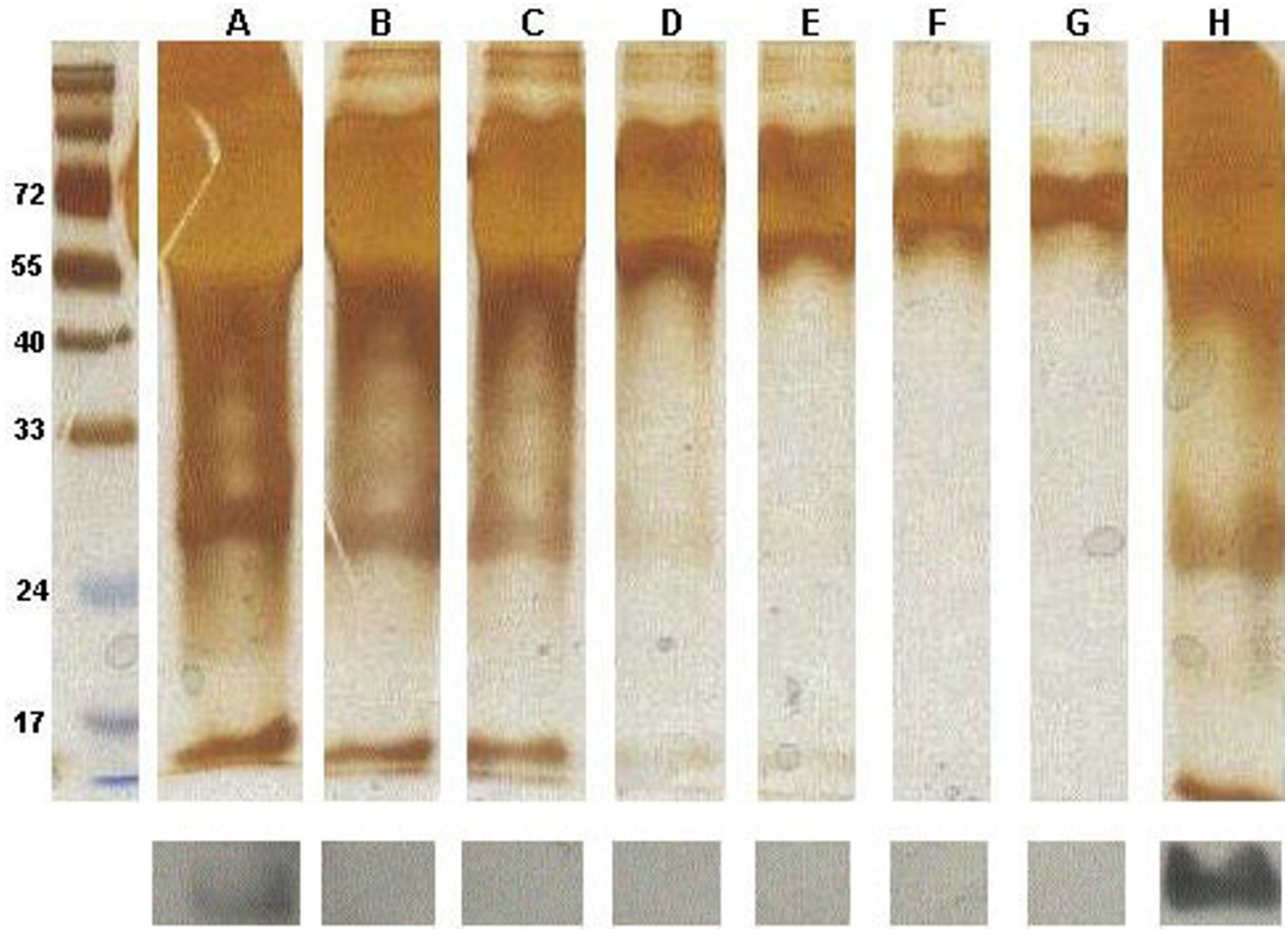

Figure 2
Silver Staining of a denaturing 12% poliacrilamide gel presenting the protein pattern during centration/dialysis in a 300 kDa ultrafiltration membrane. Western blot were performed using a conjugated antibody, anti-HA HRP Roche). Molecular Weight markers were marked in kDa. Lane **A**. Supernatant of transfected 293T prior to concentration **B.** First permeate **C**. Second permeate **D**. Third permeate **E**. Fourth permeate **F**. Fifth permeate **G**. Sixth permeate **H**. Concentrated supernatant.

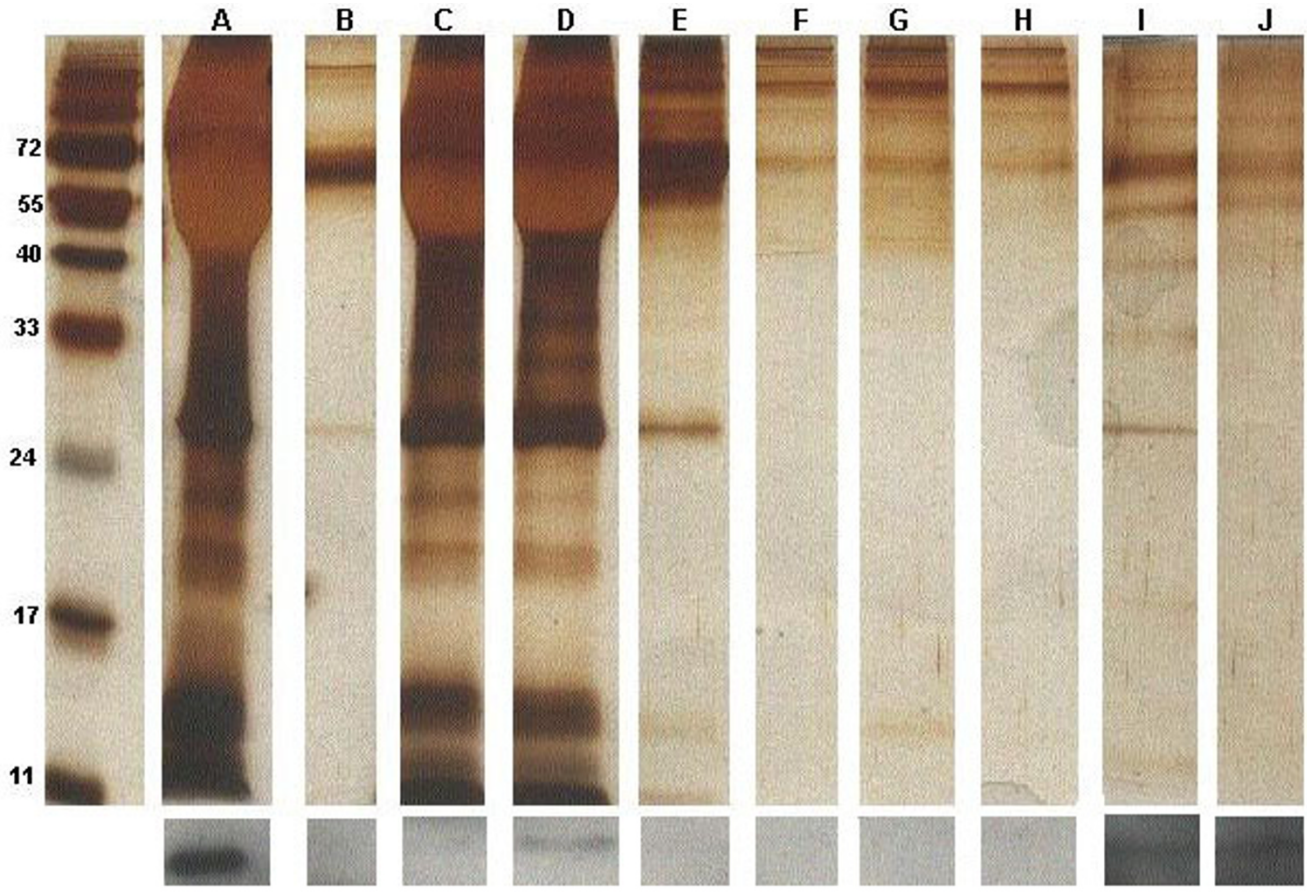

Figure 3
Silver Staining of a denaturing 12% poliacrilamide gel presenting the protein pattern during the q-sepharose chromatography. Western blot were performed using a conjugated antibody, anti-HA HRP (Roche). Molecular Weight markers were marked in kDa. Lane **A** – Concentrated supernatant; lanes **B to H** – Unretained and washed material. Lanes **I and J** – Eluted fractions from the AEX column.

purification process: a concentration/dialysis step, using an ultrafiltration membrane, and an anionic chromatography.

Acknowledgements

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References

1. Costa MJL, Pedro L, Matos APA, Aires-Barros MR, Belo JA, Gonçalves J, Ferreira GNM: **Construction of chimeric Simian – Human Immunodeficiency virus-like particles.** in press.