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INFLUENCE OF DNA-PROTEIN INTERACTIONS ON PURIFICATION AND ASSEMBLY OF VIRUS-LIKE PARTICLES

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Modular virus-like-particles (VLPs), presenting foreign antigens on their surface, are promising candidates for a wide range of future vaccines. A viable production pathway is the expression and purification of viral structural proteins and their subsequent *in vitro* assembly into VLPs, in a bioprocess environment. One promising approach is the use of murine polyomavirus major capsid protein VP1 as a carrier of modular epitopes from vaccine targets. This platform technology uses *E. coli* as an expression system and showed promising results in creating VLP vaccines candidates directed at influenza, Group A Streptococcus and other infectious pathogens. However, like other viral capsomeres and viral structures, purification using chromatography techniques remains a challenge as conventional high capacity ion exchange matrices suffer from low binding capacities.

In this work the role of DNA-protein interaction during the purification of VLP precursor capsomeres is investigated. It is found that modular VP1 capsomeres coat DNA molecules forming large DNA-protein complexes that are unable to access the pores of chromatographic resins resulting in inefficient column binding. By increasing the salt concentration of the buffer above 0.3M NaCl, the DNA-protein complexes dissociate. At intermediate salt concentrations salt-tolerant ion-exchange resins can be used to efficiently capture and purify VP1 capsomeres, as the salt breaks the aggregates but is insufficient to interfere with binding to the salt-tolerant matrix. This approach increases the binding capacity of VLP precursor proteins by at least a magnitude over published laboratory-based methods.

Minimizing the interaction of VP1 capsomeres and host cell DNA not only led to the development of efficient and scalable purification strategies, it also has a significant influence on VLP assembly. Assembly of DNA-protein complexes at appropriate ionic strength leads to the formation of worm like structures instead of VLPs. On the other hand, DNA had no influence on the assembly if high salt conditions are used, as has been common practice in the literature to date.

This work demonstrates conclusively that control of VP1-DNA aggregation and dissociation is essential for the efficient bioprocessing of precursor viral structural proteins. Such control leads to enhanced binding on chromatographic resins and efficient and scalable purification strategies that remove both DNA and host cell protein.

As DNA binding is an inherent property of many precursor viral structural proteins and viral capsids, similar approaches using salt tolerant ion exchange resins, at elevated salt concentrations, are also likely applicable for purification of other modular and non-modular VLP vaccine candidates.

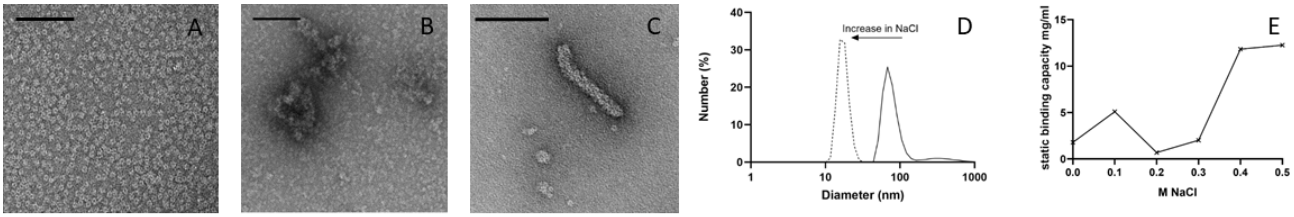

Figure 1 – (A) Purified VP1 capsomeres in 0.1 M NaCl. (B) Addition of E. coli DNA leads to formation of DNA-protein aggregates. (C) Dialysis of DNA-protein aggregates against assembly buffer leads to formation of worm like structures. (D) Dynamic light scattering showed a dissociation of DNA-VP1 complexes by increasing NaCl. (E) Static binding capacity of VP1 from clarified supernatant on CaptoMMC at pH 7.5. Scale bar represents 100 nm.