

A CLICK CHEMISTRY STRATEGY TO SPECIFICALLY MONITOR AND IMPROVE PURIFICATION OF INFLUENZA VIRUS-LIKE PARTICLES

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Virus-like particles (VLPs) constitute a promising platform in vaccine development and targeted drug-delivery. However, most applications use simple, non-enveloped VLPs that present less technical challenges, not only to produce and purify, but also in terms of characterization, compared to enveloped VLPs. Recent advances in upstream processing, new product quality requirements and other regulatory issues, as well as the search for more cost-effective processes, led to the need to develop more efficient downstream processes for biopharmaceuticals.^[1] In that sense, new monitoring and product characterization methods, which can be applied at all stages of downstream processing, are needed.

Here is reported a valuable platform for the downstream processing and monitoring of the *in vivo* production of site-specifically functionalized enveloped Influenza VLPs. This strategy involves a two-step procedure that consists of residue-specific replacement of methionine by an analog (azidohomolanine) that enabled for post-expression functionalization with a fluorophore.^[2]

Importantly, this platform does not impact VLP production or purification processes, and allows functionalization without deleterious effect on hemagglutinin biological function. As a proof of concept a complete downstream processing was performed, including clarification, capture and polishing steps. A flow cytometry analysis (FACS) step was added to achieve a refined discrimination and separation between VLPs and baculovirus - the major impurity of the process.^[3] This was further confirmed using atomic force microscopy (AFM). This tool allowed to accurately monitor our product, achieve higher product recovery yields and higher impurity removal levels. The versatile system presented here is broadly applicable to the production of functionalized enveloped VLPs, for vaccine design, targeted drug delivery and molecular imaging.

[1] CRAMER et al., *Curr Opin Chem Eng*, 1, 27-37, 2011.

[2] BANERJEE et al., *J. Virol.*, 85, 7546-7554, 2011.

[3] VAN DER VLIST et al., *Nature Protoc*, 7.7, 1311-1326, 2012.