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Simplifying the difficult task of vesicle purification

Throughout their life-cycle, bacterial and mammalian cells have been shown to shed portions of their outermost lipid membranes that often contain carbohydrates, proteins, and nucleic acids on their surfaces as well as within their lumen. Referred to as extracellular vesicles (EV), outer membrane vesicles (OMV) or exosomes these biological particles carry out various *in vivo* cellular functions that include: cell-cell communication, host defense mechanisms, gene transfer and delivery of virulence factors. These vesicles, or proteoliposomes, are increasingly being found to play significant roles in many disease processes as both diagnostic markers of disease and indications of disease progression in people. Use of EV/OMV for therapeutic applications is also being actively explored with primary indications in the area of vaccine based pharmaceuticals. Advanced scientific tools for engineering biological processes have allowed researchers better control over the composition of EV/OMV, however, as cells tend to shed EV/OMV throughout their life-cycle, additional tools are necessary to enrich target EV/OMV that contain the desired payload from the bulk population of vesicles (Fig. 1A).

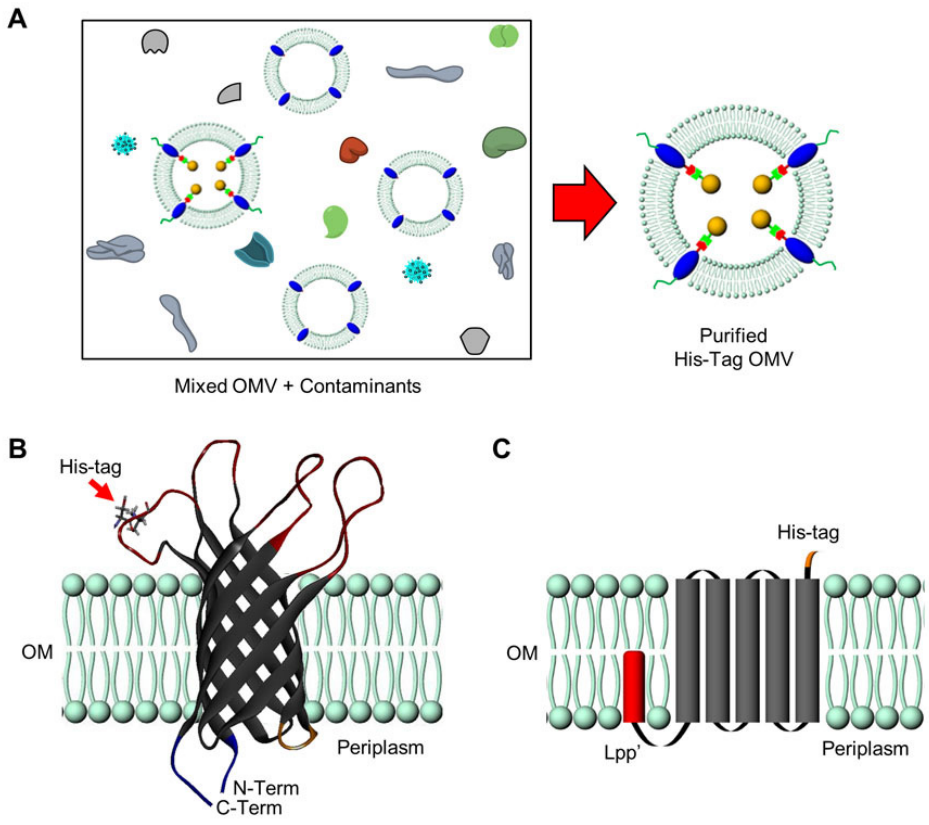

Fig. 1. A) Schematic representation of selective affinity purification of His-tag protein-loaded OMV from native non-loaded OMV and other culture media contaminants. B & C) Recombinant membrane proteins were used to present the histidine sequence on the exterior of the OMV.

Purification of EV/OMV can be costly requiring specialized equipment for filtration and prolonged cycles of ultracentrifugation. We sought to simplify the preparation of EV/OMV while simultaneously enriching for those EV/OMV containing a specific cargo. In a series of proof-of-concept experiments, we utilized a recombinant bacterial membrane protein and a synthetic membrane anchor protein encoding a histidine amino acid repeat that is presented on the exterior of the bacterial OMV (Fig. 1B,C). Using a common purification technique, immobilized metal affinity chromatography (IMAC), we showed that we could selectively enrich from bulk media the OMV containing our engineered proteins. This method significantly reduced the overall complexity and cost associated with OMV purification.

In our manuscript, we outline a simplified protocol in which an excess of IMAC resin is incubated with bacterial culture media. The high affinity of binding due to multiple copies of the histidine epitope tag on each OMV allows for a batch washing protocol where resin is gently pelleted via low speed centrifugation. Captured EV readily elute from the IMAC resin with the addition of a competing small molecule (imidazole). We confirmed resin capture of the engineered OMV using fluorescence microscopy and scanning electron microscopy (Fig. 2A). Purified OMV were quantitated using particle tracking instrumentation to confirm there were no significant changes to morphology in addition to western blotting to assess capture efficiency and purity (Fig. 2B). As observed in the western blot, minimal target OMV were lost in the flow-through or wash fractions demonstrating a high recovery of engineered OMV.

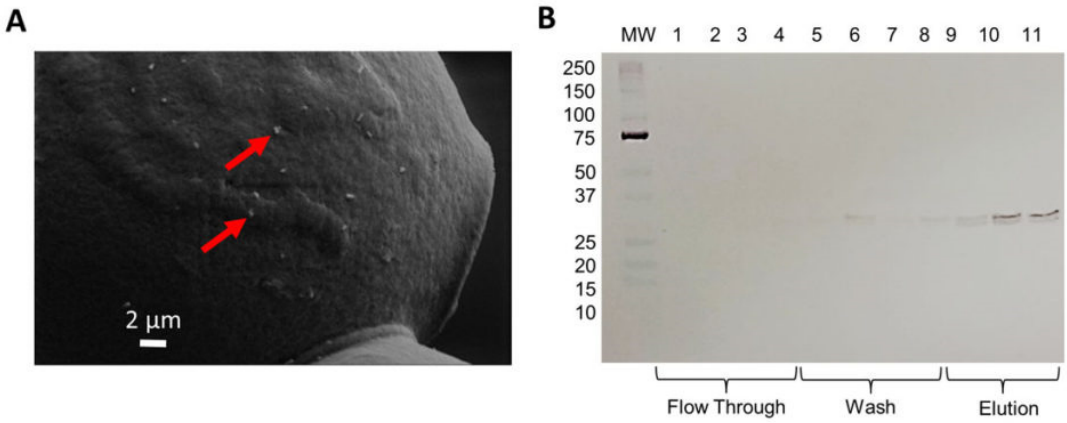

Fig. 2. A) SEM image of OMV bound to IMAC capture resin. B) Western blot of the elution profile of OMV from IMAC resin utilizing an anti-His-tag antibody.

While the proof-of-concept studies were carried out utilizing the bacterium *Escherichia coli* the described EV/OMV isolation technique can be applied to any bacterial or mammalian system being studied. This powerful purification strategy allows for the separation of cargo-loaded EV from non-loaded EV by linking the external affinity epitope to internal modification sites (Fig. 1B), which is unlike any other EV/OMV purification strategy available. This advanced purification capability will allow for the production and purification of diverse pharmaceutical and industrial biological products that were previously thought to not be possible as a result of purity limitations. While new discoveries are continually being made in the area of

EV/OMV research this purification tool will help shape how EV/OMV production and utilization for pharmaceutical and industrial applications is carried out in the future.

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