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Multi-approach Characterization of Bacterial Extracellular Vesicles (bevs) to Unravel Their Structural and Molecular Architecture

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Bacterial extracellular vesicles (BEVs) are nanoscale lipid bilayer structures involved in cell-to-cell communication, biofilm formation, and host-pathogen interactions. Despite their biological relevance, the biophysical properties governing their formation, stability, and molecular cargo remain underexplored. In this study, we employ a multi-approach characterization strategy to investigate the biochemistry and biophysics behind BEVs comprehensively. Escherichia coli DH5α-derived BEVs are isolated and purified by ultracentrifugation and size exclusion chromatography (SEC). Nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and flow cytometry are employed to elucidate the physicochemical properties of BEVs. SDS-PAGE and Western blotting further confirm the presence of potential vesicular markers, providing insights into their molecular composition. Transmission electron microscopy (TEM) is then used for the ultrastructural analysis. This study aims to establish a systematic characterization framework for BEVs, highlighting the necessity of a multidisciplinary approach to study them. This study represents a step toward addressing key gaps in the field, including the absence of universal biomarkers for bacterial vesicles and the need for improved methods to characterize their lipid structure and biophysical properties.