From flat to curved: bilayer asymmetry encodes membrane remodelling directionality

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The Endosomal Sorting Complex Required for Transport (ESCRT) machinery plays a central role in membrane remodeling and scission across diverse cellular processes, including multivesicular body (MVB) biogenesis, autophagosome closure, and cytokinetic abscission (1). During MVB formation, ESCRTs not only sort transmembrane cargoes on endosomal membranes but also deform membranes through cycles of polymerization and depolymerization (2). A key unresolved question is what determines the directionality of membrane budding. Here, we propose that budding direction emerges from molecular asymmetry—either through crowding of transmembrane cargoes confined to a single leaflet or through asymmetric lipid distributions.

Using in vitro reconstitution with purified ESCRT and cargo components, we show that saturating giant unilamellar vesicles (GUVs) with model cargoes on a single leaflet induces membrane curvature in a concentration-dependent manner, consistent with a crowding-driven mechanism. Cargo-induced crowding further promotes early ESCRT-III buckling, revealing a cooperative interplay between protein asymmetry and ESCRT-mediated remodeling. In budding yeast, we show that stalling cargo internalization at the endosome leads to internal membrane deformation and sorting defects.

Beyond protein-driven asymmetry, we find that lipid asymmetry also contributes to directional budding. In an in vitro GUV system, enzymatic conversion of sphingomyelin into ceramide alters lipid distribution and biases membranes toward inward budding. These results support a model in which sphingolipid metabolism regulates membrane asymmetry to promote curvature formation. To resolve the spatial organization of cargo molecules, lipid asymmetry, and ESCRT assemblies, we employ ultrastructural approaches including cryo-electron microscopy and Tokuyasu cryosectioning. By integrating in vitro reconstitution, yeast cell biology, and structural biology, we provide evidence that ESCRT-driven membrane remodeling is governed by molecular asymmetry, with both protein crowding and lipid distribution shaping membrane curvature and directing budding.

References:

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