Ezrin enrichment on curved cell membranes requires phosphorylation or interaction with a curvature-sensitive partner

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Proper spatial localisation of proteins that connect cortical actin and the plasma membrane is essential for cell shaping and function. *In vivo*, the ERM protein ezrin is associated on membranes that are flat, or with positive or negative curvature. To assess the curvature-sensing of ezrin, we use cell biology and *in vitro* approaches that associate cryo-electron microscopy (cryo-EM) and mechanical measurements on model membranes containing PIP_2 and purified ezrin. We observe that ezrin (ezrinWT) and its constitutively phosphorylated mutant (ezrinTD) self-assemble in an anti-parallel manner, zipping adjacent membranes. Phosphorylation reduces ezrin intermolecular interactions, induces a conformational change, and facilitates ezrin binding to actin filaments, as shown by cryo-EM, and promotes ezrin binding to positively curved membrane. While neither ezrinTD nor ezrinWT senses negative membrane curvature alone, we demonstrate that their enrichment in negatively curved cellular membranes such as protrusions requires them to interact with curvature sensors, e.g. I-BAR-domain proteins. Overall, our work corroborates a role for ezrin, not as a curvature sensor but rather in the mechanical cohesion of membranes with actin.

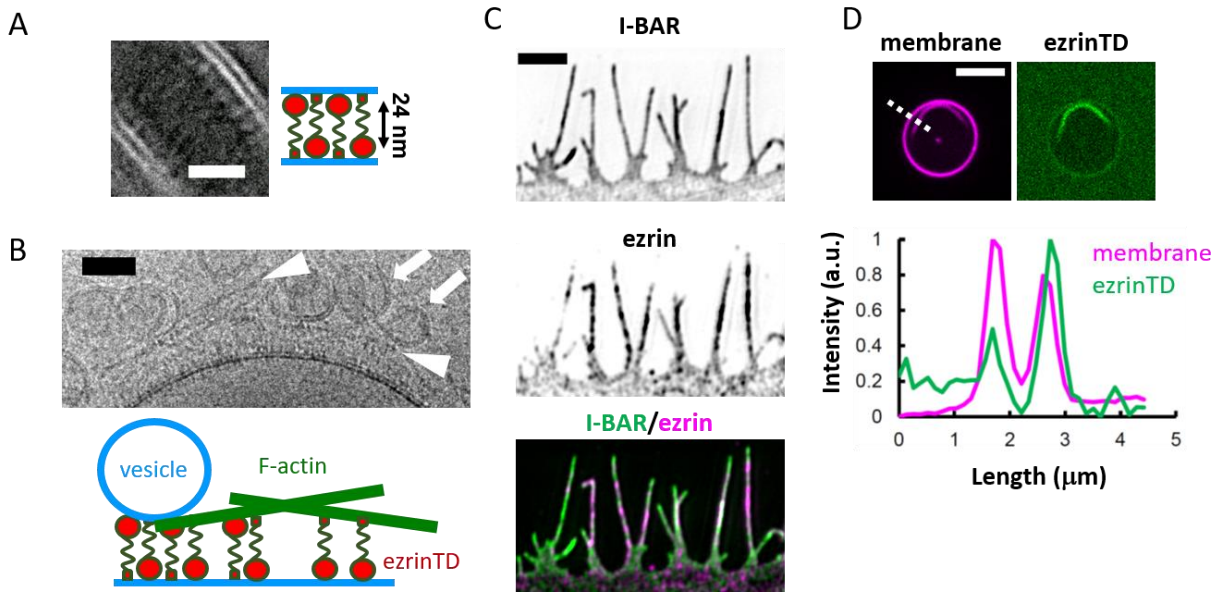

Figure 1. (A) Representative cryo-electron micrograph of PIP_2-membranes tethered by ezrinTD. Scale bar, 20 nm. (B) Representative cryo-electron micrograph of PIP_2-containing LUVs incubated with muscle F-actin in the presence of ezrinTD. Arrowheads indicate F-actin and arrows indicate tethered vesicles. (C) Representative structured illumination microscopy images of cellular protrusions of LLC-PK1 cells transfected with GFP-I-BAR domain and immunolabeled for endogenous ezrin. Scale bar, 2 μm (D) (Top) Representative confocal images of ezrinTD in IRSp53 I-BAR domain induced tubules. Scale bar, 5 μm (Bottom) Normalized fluorescence intensity profiles along the line drawn from outside the GUV towards the interior of the GUV, as indicated in the top image.