Ezrin enrichment on curved membranes requires a specific conformation or interaction with a curvature-sensitive partner.

Résumé

One challenge in cell biology is to decipher the biophysical mechanisms governing protein enrichment on curved membranes and the resulting membrane deformation. The ERM protein ezrin is abundant and associated with cellular membranes that are flat, positively or negatively curved. Using in vitro and cell biology approaches, we assess mechanisms of ezrin’s enrichment on curved membranes. We evidence that wild-type ezrin (ezrinWT) and its phosphomimetic mutant T567D (ezrinTD) do not deform membranes but self-assemble anti-parallelly, zipping adjacent membranes. EzrinTD’s specific conformation reduces intermolecular interactions, allows binding to actin filaments, which reduces membrane tethering, and promotes ezrin binding to positively-curved membranes. While neither ezrinTD nor ezrinWT senses negative curvature alone, we demonstrate that interacting with curvature-sensing I-BAR-domain proteins facilitates ezrin enrichment in negatively-curved membrane protrusions. Overall, our work demonstrates that ezrin can tether membranes, or be targeted to curved membranes, depending on conformations and interactions with actin and curvature-sensing binding partners.

ProLIF - quantitative integrin protein-protein interactions and synergistic membrane effects on proteoliposomes.

Résumé

Integrin transmembrane receptors control a wide range of biological interactions by triggering the assembly of large multiprotein complexes at their cytoplasmic interface. Diverse methods have been used to investigate interactions between integrins and intracellular proteins, and predominantly include peptide-based pulldowns and biochemical immuno-isolations from detergent-solubilised cell lysates. However, quantitative methods to probe integrin-protein interactions in a more biologically relevant context where the integrin is embedded within a lipid bilayer have been lacking. Here, we describe ‘protein-liposome interactions by flow cytometry’ (denoted ProLIF), a technique to reconstitute recombinant integrin transmembrane domains (TMDs) and cytoplasmic tail (CT) fragments in liposomes as individual subunits or as αβ heterodimers and, via flow cytometry, allow rapid and
quantitative measurement of protein interactions with these membrane-embedded integrins. Importantly, the assay can analyse binding of fluorescent proteins directly from cell lysates without further purification steps. Moreover, the effect of membrane composition, such as PI(4,5)P₂ incorporation, on protein recruitment to the integrin CTs can be analysed. ProLIF requires no specific instrumentation and can be applied to measure a broad range of membrane-dependent protein-protein interactions with the potential for high-throughput/multiplex analyses.


**Adhesion to nanofibers drives cell membrane remodeling through 1D wetting**

*Nature Communications*: [DOI: org/10.1101/393744](https://doi.org/10.1101/393744)

**Résumé**

The shape of cellular membranes is highly regulated by a set of conserved mechanisms. These mechanisms can be manipulated by bacterial pathogens to infect cells. Human endothelial cell plasma membrane remodeling by the bacterium *Neisseria meningitidis* is thought to be essential during the blood phase of meningococcal infection, but the underlying mechanisms are unknown. Here we show that plasma membrane remodeling occurs independently of Factin, along meningococcal type IV pili fibers, by a novel physical mechanism we term « one dimensional » membrane wetting. We provide a theoretical model that gives the physical basis of 1D wetting and show that this mechanism occurs in model membranes interacting with model nanofibers, and in human cells interacting with model extracellular matrices. It is thus a new general principle driving the interaction of cells with their environment at the nanoscale that is diverted by meningococcus during infection.


**Septin-based readout of PI(4,5)P₂ incorporation into membranes of giant unilamellar vesicles**

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**Résumé**

Septins constitute a novel class of cytoskeletal proteins. Budding yeast septins self-assemble into non-polar filaments bound to the inner plasma membrane through specific interactions with L- α-phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂). Biomimetic in vitro assays using Giant Unilamellar Vesicles (GUVs) are relevant tools to dissect and reveal insights in proteins-lipids interactions, membrane mechanics and curvature sensitivity. GUVs doped with PI(4,5)P₂ are challenging to prepare. This report is dedicated to optimize the
incorporation of PI(4,5)P2 lipids into GUVs by probing the proteins-PI(4,5)P2 GUVs interactions. We show that the interaction between budding yeast septins and PI(4,5)P2 is more specific than using usual reporters (phospholipase Cd1). Septins have thus been chosen as reporters to probe the proper incorporation of PI(4,5)P2 into giant vesicles. We have shown that electro-formation on platinum wires is the most appropriate method to achieve an optimal septin-lipid interaction resulting from an optimal PI(4,5)P2 incorporation for which, we have optimized the growth conditions. Finally, we have shown that PI(4,5)P2 GUVs have to be used within a few hours after their preparation. Indeed, over time, PI(4,5)P2 is expelled from the GUV membrane and the PI(4,5)P2 concentration in the bilayer decreases.