O-05.3 Short talk

Structural and functional implications of in vivo phase separation of membrane protein in in Escherichia coli

Dmitrii Linnik [1], Sumajra Sultanji [1], Gea Schuurman-Wolters [1], Rinse De Boer [1], Christiaan Punter [1], Ivan Maslov [1], Bert Poolman [1]

[1] Rijksuniversiteit Groningen, Groningen, Netherlands

In bacteria liquid-liquid phase-separation (LLPS) controls protein activity and dynamically organizes (macro)molecules without the need for membrane-bound compartments. LLPS of cytoplasmic proteins has extensively been studied, but phase-separation of membrane proteins is unchartered territory. In this work we induce in vivo condensation of lactose permease (LacY), a widely-studied model monomeric inner membrane protein in E. coli, and evaluated how it affected LacY function. Addition of PopTag to LacY results in a predominantly polar localization of the fusion protein. We show the condensate-like behavior of LacY-Pop using fluorescence recovery after photobleaching (FRAP), photoactivated localization microscopy (PALM), and single-molecule displacement mapping (SMdM). In a series of perturbation experiments we show that nucleoid exclusion is not critical for LacY-Pop polar localization, while local membrane curvature plays a role. Radiolabeled lactose transport experiments show that LacY in condensates is active and outcompetes native LacY even upon hyperosmotic stress. Finally, we designed and characterized heterocondensates of LacY with its downstream lactose-metabolizing enzyme, β-galactosidase LacZ. Our findings contribute to the field of biomolecular condensates and the engineering of spatially-controlled metabolic networks and their coupling to membrane processes.