Distortion, Dynamics and Destruction: Real-Time Probing of Vesicle Solubilisation using Single-Molecule FRET Spectroscopy

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The solubilisation of lipid membranes by detergents is commonly used for the purification and isolation of membrane proteins. Biophysical studies of detergent-induced solubilisation pathways using ensemble-averaging methods have provided an integrated picture of the solubilisation mechanism that involves three states: detergent monomers are taken up in stage I without disruption; detergent-saturated membranes lead to the generation of mixed detergent-lipid micelles in stage II; and complete membrane solubilisation results in the formation of mixed micelles in stage III. Although the three-state hypothesis is a didactic and simple thermodynamic model, the transient nature of the process induces much more complex and diverse transitions between regimes. These changes include transmembrane lipid motion (flip-flop), swelling, breakdown of the membrane permeability barrier and fusion. To date, whether these processes are independent of each other and take place sequentially, or if they are interconnected, is an open question. Here we present a biophysical toolbox for quantifying the solubilisation dynamics of single vesicles induced by the non-ionic detergent Triton-X 100, and explore their kinetic details above and below the critical micellar concentration. By leveraging recent advances in model-membrane biochemistry with single-molecule fluorescence spectroscopy techniques, we have unambiguously separated, within a single FRET trajectory the swelling, permeabilisation and lysis steps of the TX-100 induced process in real-time.