TAMING MEMBRANES: FUNCTIONAL IMMOBILIZATION OF BIOLOGICAL MEMBRANES IN HYDROGELS

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ABSTRACT

Single molecule studies on membrane proteins embedded in their native environment are hampered by the intrinsic difficulty of immobilizing elastic and sensitive biological membranes without interfering with protein activity. Here, we present hydrogels composed of nano-scaled fibers as a generally applicable tool to immobilize biological membrane vesicles of various size and lipid composition. Importantly, membrane proteins immobilized in the hydrogel as well as soluble proteins are fully active. The triggered opening of the mechanosensitive channel of large conductance (MscL) reconstituted in giant unilamellar vesicles (GUVs) was followed in time on single GUVs. Thus, kinetic studies of vectorial transport processes across biological membranes can be assessed on single, hydrogel immobilized, GUVs. Furthermore, protein translocation activity by the membrane embedded protein conducting channel of bacteria, SecYEG, in association with the soluble motor protein SecA was quantitatively assessed in bulk and at the single vesicle level in the hydrogel. This technique provides a new way to investigate membrane proteins in their native environment at the single molecule level by means of fluorescence microscopy.

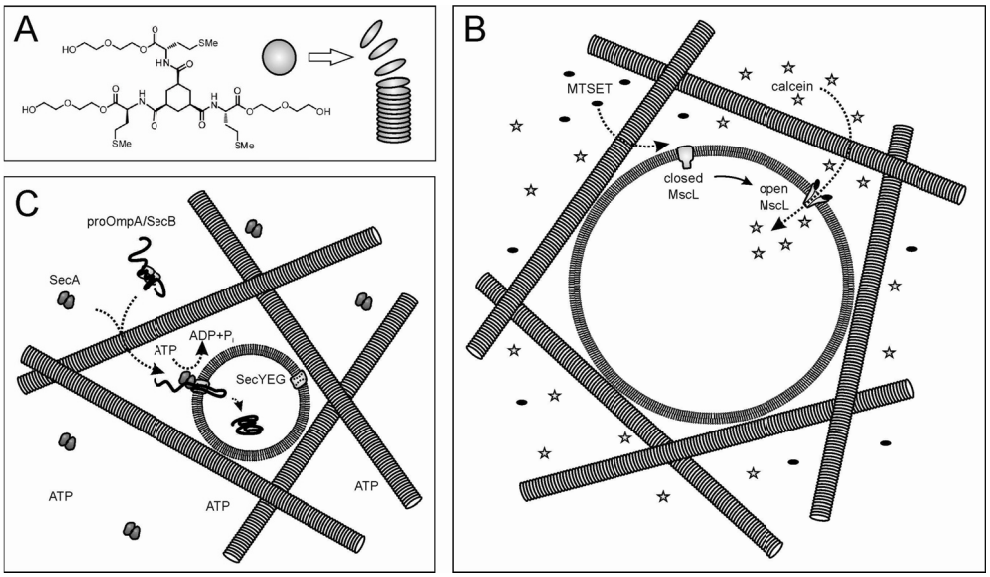

Figure 1: Hydrogels composed of self-assembling organic gelators immobilize membrane vesicles. (A) Gelator molecule 1 is based on 1,3,5-cyclohexyltricarboxamide and self-assembles into fibers of 20-100 nm. (B) Schematic representation of a hydrogel immobilized GUV with embedded MscL channel. The lipid bilayer is impermeable for the soluble fluorophore calcein when MscL occupies the closed conformation. Addition of MTSET triggers the opening of genetically engineered MscL and allows influx of calcein into the lumen of the GUV. (C) *In vitro* protein translocation into hydrogel immobilized PLs. SecA translocates the fluorescently labeled preprotein proOmpA through the SecYEG channel by multiple cycles of ATP hydrolysis. Inside the PL, proOmpA is protected against an externally added protease. Molecules in (B) and (C) are not drawn in scale.