40e Fabrication and Characterization of Solid-Supported Membranes on Silica Beads Using Bacteriorhodopsin Conjugates as Integrated Anchors

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Supported lipid bilayer membranes have gained a lot of importance over the past decade due to their potential applicability in various areas of bioengineering. They serve as models of biological cell membranes and are a very attractive candidate for use in the design of biomimetic interfaces. Untethered bilayers are formed onto solid supports via spontaneous spreading of lipid vesicles on hydrophilic surfaces and they float on the surface on a thin, lubricating water layer (1-3nm). This thin water layer is sufficient to provide the membrane lipids with the fluidity but presents issues when other biomolecules are embedded into these membranes, as these molecules tend to interact with the supporting surface and tend to loose their mobility and structure. In addition, these structures are presumably unstable under shear flow or processing conditions. Several attempts have been made to address this issue with a general approach to separate the membrane from the solid support with a polymer cushion, although most of the work on polymer supported membranes have been limited to flat geometries.

We have developed a new method to form tether-supported membranes on spherical silica microparticles or other surfaces of arbitrary topology. Fabrication of supported membranes on spherical particles has various advantages compared with flat geometries. This can be argued on the basis of greater surface to volume ratio on spherical geometries when compared with flat surfaces. We have synthesized bacteriorhodopsin conjugates with polyethylene-glycol polymer tethering chains that are used to anchor the bilayers onto the surface. The silica bead surfaces employed in theses studies have been extensively modified to provide them with highly specific anchoring biofunctionality and also to keep non-specific interactions to minimal level.

The assembly of supported membranes on silica particles presents challenges in terms of verifying the nature of the structures that are obtained. Evidence for high lateral mobility of the lipids within the bilayer is the ultimate check to establish the formation of a fluidic lipid bilayer. Fluorescence Recovery after Photobleaching (FRAP) is a widely used technique to study the lateral mobility in lipid bilayers. Confocal FRAP measurements, in tandem with co-localization studies, were used to analyze the supported membrane complexes. We have observed that the approach that is generally taken to form supported bilayer on flat surfaces does not work as in the non-tethered case, i.e. spreading lipid vesicles on a hydrophilic support. This technique led to immobilization of intact liposomes on the bead surface, which did not fuse to give rise to continuous bilayer structure. The use of fusogenic PEG to promote the fusion of surface-immobilized vesicles was also unsuccessful. We succeeded in fabricating the tethered membrane with essential fluidity using the detergent based self-assembly approach. Silica beads displaying tethered bacteriorhodopsin on the surface were mixed with detergent solubilized lipids and the removal of detergent using dialysis resulted in self-assembly of lipid bilayer around the particles. These constructs can potentially be used to reconstitute various membrane proteins in the tether-supported lipid bilayer for enhanced stability and functionality under a wider range of processing conditions required for various applications, e.g. biosensing, high throughput screening, biocatalysis etc.