A fusion assay based on pore-spanning membranes.

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Fusion of biological membranes is a central requirement for many cellular processes. In recent years a great variety of fusion assays based on artificial membrane systems has been developed. Such reductionist approaches mainly account for our current knowledge on fusion processes. However, there are still a number of drawbacks associated with these assays. Thus, we aim to establish a new vesicle-planar membrane fusion assay to be able to gain insight into protein-mediated fusion processes starting from docking, via hemifusion to full fusion. To achieve this goal, membranes suspending the pores of a highly ordered porous material were established, which have the advantage that they are very robust, mechanically stable and the membranes are accessible from both sides.

Our results demonstrate the successful fusion (lipid mixing) of large unilamellar vesicles with pore-spanning membranes. Micro-BLMs were prepared by the painting-technique resulting in a lipid bilayer doped with Oregon Green DHPE. The addition of large unilamellar vesicles (600 nm in diameter), doped with Texas Red DHPE, to the pore-suspending membrane allow for the observation of single fusion events by means of fluorescence microscopy. Lipid mixing was followed by the distribution of the Texas Red fluorescence in the plane of the pore-suspending membrane while simultaneously, quenching of the Oregon Green fluorescence due to Förster resonance energy transfer (FRET) between the Oregon Green and Texas Red dye was monitored. Simultaneously, the release of a water soluble dye entrapped in the vesicle lumen is observed.