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Supported Lipid Membranes: A Neutron Probe!

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Biological membranes play an extremely critical role in both intra and extracellular cellular functions. They form an intricate and highly complex network of lipids and proteins held together by hydrophobic interactions, with dimensions of the order of a few nanometers. These complex systems are studied by using both integrated and reconstituted model systems that mimic natural membranes closely. Model supported bilayer systems (SLBs) allow us to probe various cellular processes such as ligand-receptor interactions [1], and cellular signalling events [2].

The process of SLB formation follows two important mechanisms. First, there is rupture and adhesion of vesicles on the solid surface, often explained by a simple continuum approach, wherein information regarding adsorption, deformation and rupture of vesicles is obtained by treating the bilayer as a thin two-dimensional sheet embedded into a three dimensional space [3,4]. Second, vesicles form patchy blobs that evolve into uniform bilayers [4]. The role of the underlying solid support is relatively unexplored compared to other parameters. In my opinion, it is one of the most pertinent features in designing stable SLBs. It is known that hydrophilicity is an important feature in solid supports, and work on the role of surfaces such as Gold, mica and TiO_2 have been explored to determine their effect of vesicle rupture and subsequent bilayer formation. However, a thorough analysis on its role in retaining critical membrane lateral mobility and stability and translocation of membrane proteins remain largely unexplored.

While SLBS are great for investigating various cellular processes, they do not provide a suitable environment for mimicking trans-membrane proteins having large peripheral domains. The underlying 10-20 Å hydration layer is thick enough to maintain mobility of lipid molecules, however it is not sufficiently thick to maintain the mobility or prevent denaturation of proteins resulting from contact with the solid support. The addition of a suitable polymer cushion layer decouples the membrane from the substrate and maintains enough fluidity and hydration that counters protein denaturation. Another potential advantage is that the polymer layer in effect prevents any nonspecific adsorption of trans-membrane proteins and proteins from aqueous solutions. Care has to be taken that the polymer chosen is both thermodynamically and mechanically stable which can be tuned by tweaking the wetting behaviour of the hydrated polymer interface. Stable films can only be obtained when complete wetting is ensured. Also of importance is the repulsive nature between the membrane and the surface which ensures the stability of the bilayer [5,6].

One of the most challenging aspects of SLBs has been characterizing their structural properties and deriving information on the stability of the films. There have been several efforts utilizing surface probes that have good sensitivity in aqueous mediums in recent years. For instance, reflection interference contrast microscopy (RICM) has been used to investigate self assembly and lateral organization of SLBs. Micro-ellipsometry has been used for high resolution measurements for accurately measuring thickness of polymer/lipid interfaces on Si/SiO_2 and metallic solid supports while SPR can be effectively employed to study SLBs on Gold surfaces [7].

Reflectivity techniques have been of particular interest of late for their ability to measure mass-density profiles. Neutron reflectivity in particular offers several unique advantages over conventional techniques. Neutrons are sensitive to carbon hydrogen oxygen and nitrogen, important biological constituents. Moreover, neutrons are extremely sensitive to isotopic variations of deuterium for hydrogen. This variation helps in obtaining substantially different scattering length density (SLD) for biological systems. For instance, it is possible to selectively tag an adsorbed protein sub-layer of a stratified film with deuterium while the rest of the film is saturated with protons. It is therefore possible to study various events such as protein penetration into lipid layers and protein-protein interactions while measuring partial volume fractions calculated from the SLD profiles. Researchers have demonstrated that dipalmitoylphosphatidylcholine (DPPC) bilayers were adsorbed onto planar substrates from vesicles in solution by using neutron reflectivity to monitor vesicle adsorption at fixed values of Q using chain deuterated and chain-protonated lipids [8,9]. In another study neutron reflectivity was successfully used study membrane fluctuation and to model the elastic behavior of cell membranes using a thermoresponsive tethered polymer cushion with tunable thickness composed of poly (N-isopropylacrylamide-co-methacroylbenzophenone (3%)) [9-11].

It is clear that neutron reflectivity is a versatile tool for the characterization of biological membranes and surfaces. Recent improvements have enabled to determine compositional depth profiles with Angstrom level accuracy. Combining neutron reflectivity with other conventional techniques opens up newer arenas to accurately study interaction between proteins with model lipid membranes and interfaces. The experimental approaches outlined above along with our present understanding of SLB formation on solid supports should be extended to more complex systems incorporating a combination polymer and protein tagged liposomes and by incorporating various trans-membrane proteins and lipid compositions. The final frontier should be to extend these lab scale experiments to transcend into viable, technologically relevant applications.

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