NIRT: Nanoscale engineering of bilaterally accessible biomembrane mimics

NSF NIRT Grant 0304062

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Biomembranes are one of the most common structures encountered in living organisms and are indispensible for the functioning of life. On the one hand, they provide a structural basis for compartmentalization for complex biosynthesis and signal processing and amplification, and on the other, a dynamic environment for embedded membrane proteins. Such proteins depend on the lipid matrix to function in biochemical reaction cascades as signal transformers, molecular shuttles, or recognition sites. Exploitation of the unique properties of biomembranes may consequently open exciting new opportunities for a variety of technical application ranging from biosensorics to nanoscale responsive reactors enabling spatiotemporal control of complex chemistry, or high-speed DNA sequencing. However, serious challenges to the current capabilities of nanotechnology exist: Lipid bilayers are formidably dynamic and at the same time fragile structures, in that they consist of a leaflet that is merely 5 nm thick and has the lateral fluidity of an oil phase. Attempts to reinforce synthetic lipid membrane mimics by preparing bilayers close to solid interfaces have in the past invariably led to a severe reduction, or even elimination, of the system dynamics, rendering the membrane defunct. Moreover, since native transmembrane proteins can protrude from the membrane interior bilaterally into the adjacent aqueous media, the close association of a synthetic bilayer with a solid substrate will in most cases prevent the incorporation of such proteins in the first place.

Approach. This project is devoted to the nanoscale engineering of biomembrane mimics that overcome these limitations by adopting biomimetic, multiscale routes to membrane stabilization on porous substrates. We pursue novel concepts for the molecular-level engineering of membrane scaffolds that involve ultrathin nanoporous gold leaf (NPGL), biomimetic polymer brushes and two-dimensional (2D) protein crystals to synthesize stabilized functional membranes. A unique biomolecular toolkit on the basis of bacterial surface-layer proteins (S-layers) and their biospecific interaction with secondary cell wall polymers (SCWP) provides a flexible framework for the biocompatibilization of inorganic surfaces. Selected transmembrane proteins, such as α-hemolysin from *Staphylococcus aureus* and the *Bacillus anthracis* PA_{63} and its cofactors provide challenging test cases for the functionalization of stabilized membrane mimics.

Bacterial S-layers and SCWP. S-layers are the most commonly observed cell surface structures in prokaryotic organisms – bacteria and archaea (*1*). They consist of a single protein or glycoprotein, that is specific for a particular bacterial or archaeal species, with MW's from 40 to 200 kDa, depending on the molecular species it derives from. Similarly, structural properties of S-layers formed by different organisms, such as their degree of glycosilation or lattice symmetry, vary greatly. The latter may be oblique, square or hexagonal with unit cell dimensions in the range of 3 to 30 nm. On the other hand, general features are similar for many of the S-layers studied so far. The crystalline sheets are generally 5 to 15 nm thick. They consist of highly porous meshworks (30% to 70% porosity) with pores of uniform size and morphology in the 2 to 8 nm range. Since the S-layer comprises a porous crystal lattice, they control molecular traffic to the membrane surface: Based on the exclusion limit of the isoporous network in relation to the size of a diffusing species, that species will be admitted or rejected. Studies on the structure-

function relationship of different S-layers from *Bacillaceae* have hinted at specific (lectin-type) binding domains on the N-terminal part of the proteins for SCWP, that, in turn, covalently link to the peptidoglycan matrix of the cell wall (*2*). SCWPs are believed to control the crystallization of the protein monomers and to couple the protein assembly to the peptidoglycan matrix.

Using an S-layer based molecular construction set, hybrid protein–lipid heterostructures will be prepared on NPGL carrier substrates. The vast complexity of the design options (Figure 1) illustrates well the broad potential impact of this approach. Within the NIRT collaboration, which includes the Center for NanoBiotechnology (Prof. Sleytr) at the University of Natural Resources and Applied Life Sciences (Vienna, Austria) and the Materials Research group (Prof. Knoll) at the MPI for Polymer Research (Mainz, Germany), we concentrate on two aspects: (A) Design of laterally continuous biomembrane architectures that incorporate SCWP-derived polymer cushions that take advantage of biospecific interactions with the conjugated S-layer protein. This polymer cushion decouples the membrane from a supporting solid-state substrate whose surface bears a monomolecular protein sheet crystal, the S-layer. (B) The use of NPGL as porous ultrafiltration structures, as a basis for macroscopic support.

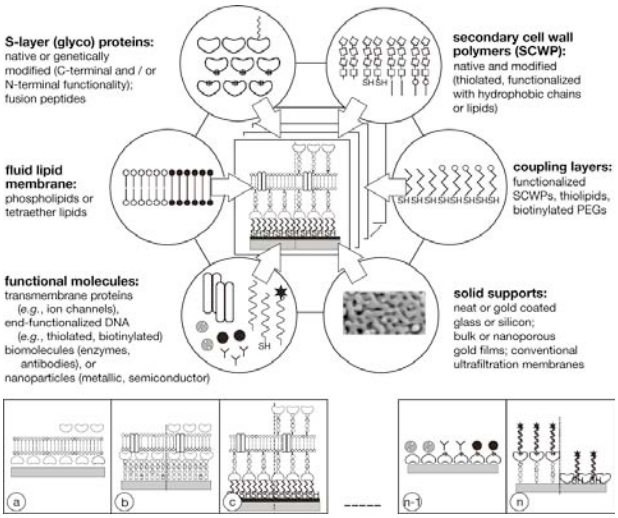

Figure 1: Schematic representation of a biomolecular construction kit based on S-layers and their conjugated SCWP employed for the nanoscale engineering of bilaterally accessible, yet macroscopically rugged biomembrane mimics.

Nanoporous gold leaf. NPGL is formed by selective dissolution of silver from freestanding silver/gold films, ~100 nm thick, to form ~10 nm diameter pores. Current successes integrating NPGL into the molecular construction kit include fabrication of freestanding NPGL floating on aqueous solution and a simple post-processing method for adjusting the pore size by "electrochemical annealing" (*3*). We have shown that NPGL films chemically tethered to glass slides exhibit surface plasmon resonance (SPR). The high surface area of NPGL coupled with the intrinsic sensitivity of SPR suggests exciting possibilities for applying NPGL in biosensor applications. Furthermore, in collaboration with the Knoll group, we have tethered the transmembrane protein cytochrome-c oxidase (Cyt-cOx) to NPGL and built a tethered bilayer around these proteins. Evidence of membrane formation was derived from electrochemical impedance spectroscopy (EIS), which was observed to correlate with SPR resonance shifts. Cyt-cOx activity as a proton pump was maintained after tethering to NPGL and the construction of the lipid bilayer.

MD simulations of charged polyglycane brushes. Molecular simulations of SCWP brushes and their interaction with conjugated S-layer proteins form a basis for the molecular engineering of polymer cushions for biomembrane mimics. Our approach is to map SCWPs onto a coarse-grained (bead-spring) polymer representation that involves polymer brush theory, experimental data on specific SCWPs available from our European collaborators, and detailed atomistic MD simulations of short-chain segments in water and salt ions. Using a combination of *ab initio* quantum mechanical calculations and MD simulations, we determined a unique set of partial charges, minimum energy conformations, and torsion angle potentials for short-chain oli-

gomers in water (*4*). We found that polymer conformational preferences in aqueous solution are sensitive to the partial charge assignments, as well as the hydration of the charged chemical groups comprising the SCWP monomers. Consequently, extensive MD simulations were carried out to examine the effect of hydration on SCWP conformations for both continuum and explicit water models. Our results indicate that the SCWP brush properties can be accurately described using a coarse-grained (bead-spring) representation of the polymer in continuum water (*4*).

Protein ion channels. It is well established that protein ion cannels can be used to detect and characterize ions, polynucleotides and specific proteins. These and other nanopore-based applications would benefit if channels could be functionally reconstituted and immobilized in unsupported and robust thin films. Toward that goal, we demonstrated that two different protein ion channels (*B. anthracis* PA_{63} and *S. aureus* α-hemolysin) were fully functional in two different polymerizable lipid membranes in the liquid crystalline state. Moreover, the α-hemolysin channel remained completely functional after the membrane was polymerized. Specifically, the channel, which was shown earlier to permit the translocation of individual DNA molecules in non-polymerizable membranes, still does so in membranes formed from polymerizable lipids. In addition, the characteristic pattern of current blockades, caused by the polynucleotide threading through the channel, is essentially unchanged upon UV-induced membrane polymerization (*5*).

Three toxins secreted by *B. anthracis* – protective antigen (PA), lethal factor (LF) and edema factor (EF) – form the molecular basis of anthrax-induced cell death. To gain insight into the mechanism by which anthrax kills host cells, we studied the interaction between either LF or EF and activated PA (PA_{63}) in planar bilayer membranes using electrophysiology techniques. We observed that LF and EF convert the current-voltage relationship of PA_{63} from slightly nonlinear to highly rectifying (*6*), see Fig. 2, LF forms a very tight complex with the PA_{63} ion channel (*7*) – apparent binding constant, K_d < 50 pM – and a known antibody against PA_{63} prevents blocking of the channel by LF. This latter result demonstrates the efficacy of a method to screen potential therapeutics against any of the three deadly anthrax proteins. In addition, a theoretical model for the PA_{63} channel was used to better explain these findings. Consistent with several independent sources of biophysical data, this model illustrates the formidable barrier that the channel pore presents to LF (or EF) translocation, a result already stimulating new research into the cellular process by which LF and EF enter, and thereby kill, cells.

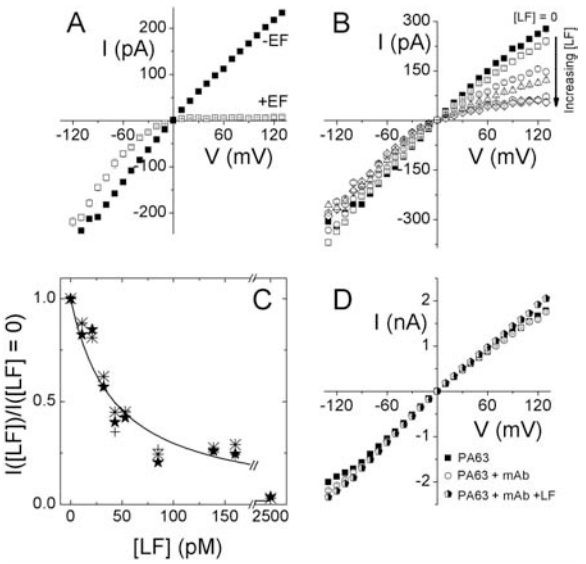

Figure 2: PA_{63} in bilayer lipid membranes. A, B: dependence on EF and LF. C: binding isotherm for LF to PA_{63}. D: interference of a PA-specific antibody with LF binding to PA_{63}.

References

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