Functional Toxin Pores in Tethered Bilayers: Membrane Association of α-hemolysin

Several toxins form pores on the surface of susceptible cells that penetrate the membrane barrier. The pores allow unregulated ion diffusion across the membrane, leading to cell death (lysis). The protein α-hemolysin (αHL) is a toxin produced by the bacteria *Staphylococcus aureus* which is the subject of recent study, in particular because it shows promise for development as a stochastic biosensor for small ions [1], proteins [2], and large molecules, including DNA [3, 4]. Using a system that we previously designed and optimized, we have incorporated the protein into a robust biomimetic solid-tethered bilayer membrane (tBLM). Monitoring the incorporated pore's ion transport properties with electrical impedance spectroscopy, we verified that the toxin retains its natural functionality. This enabled the characterization of the interaction between the membrane and protein using neutron reflectometry (NR) at the Cold Neutrons for Biology and Technology's Advanced Neutron Reflectometer/Diffractometer (AND/R) [5].

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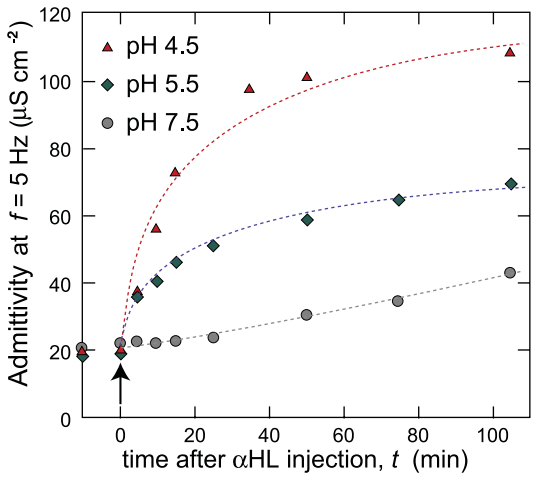

FIGURE 1:
Change in tBLM-covered electrode admittivity at f = 5 Hz, indicative of αHL incorporation, as a function of buffer pH.

The development of practical biosensors based on natural membrane proteins, such as αHL, is a subject of considerable effort. However, many problems must be overcome in the design of a system suitable for technical manipulation and measurement while preserving the biological activity of the protein. Natural cell membranes are intrinsically complex and disordered, a specific molecular architecture of lipids, sterols, proteins, glycolipids, etc., which drives the development of simpler model systems that are more amenable to preparation and characterization. Biosensors must also be robust, preferably reusable, and allow the behavior of the membrane-associated protein to be readily probed.

Using a common approach [6] we have addressed these issues in developing a solid-supported, tethered bilayer membrane (tBLM), which can be formed in a simple fashion on gold-covered surfaces, and which is surface-decoupled through a controllable hydrated polymer spacer [7]. The tBLMs were optimized for αHL incorporation using electroimpedance spectroscopy (EIS), a technique that is capable of rapidly probing the ion transport properties of the membrane pore. The increase in the tBLM admittivity is a function of the number of active pores incorporated (Fig. 1).

The electrical parameters of the toxin in the tBLM can compared with αHL in unsupported black lipid membranes, where they have been comprehensively studied [8]. Unfortunately, unsupported lipid membranes are limited for structural studies of the protein because of their limited size (≈ 10^2 mm^2), and unsuitable for biosensor development as they are fragile, being sensitive to shock and vibration. However, the comparison of the effects of polymers on pore conductivity [8] and response to pH [9], confirms that the toxin has retained its functionality in the tBLM, with the implication that the protein has also retained its native structure.

Using the optimized conditions, incorporation of αHL into the biomimetic tBLM occurs at a surface coverage large enough that its effect is readily observable in the raw neutron reflectivity (Fig. 2). These effects can be fitted using a simple layer model, which accounts for the most prominent feature of the protein, its large cap domain exterior to the membrane. In order to obtain more information about the interactions between the protein and the tBLM it was assumed that the in the tBLM the αHL channel x-ray crystal structure is valid.

The modeling of the NR shows that the protein spans the tBLM, with the stem penetrating ≈ 5 Å into the headgroup region on the inner leaflet (Fig. 3). The protein rim appears to interact strongly with the lipid headgroups, and even perturbs the hydrophobic tails, of the outer leaflet. This confirms the predictions of Song *et al.,* from analysis of the crystal structure that the cap region would be "probably in close proximity, if not in direct contact, with the membrane bilayer" [10]. The overhang between the membrane-penetrating stem domain and the rim domains is lined with basic and acidic residues, forming a suitable enclosure for the zwitterionic phospholipid headgroups, while the very edge of the rim contains a number of aromatic residues that can interact with the tail region of the lipid bilayer. The extension of the stem into the polar headgroup region on the far side of the membrane also stabilizes the band of charged residues that rings the bottom of the stem domain.

The NR modeling shows that the protein is incorporated into the bilayer at ≈ 36 % of the estimated maximum possible packing density of the protein. This packing density, ≈ 1 channel per 310 nm^2, is qualitatively similar to that observed in lysed cells [11]. The incorporation of such a large amount of the protein in the bilayer, without bilayer collapse, emphasizes the robustness of the solid-supported system.

The authors acknowledge useful discussions with Dr Hirsh Nanda. This work was supported by the NSF (grant nos. 0555201 and 0457148). ML and AND/R are supported, through the CNBT, by the NIH under grant no. 1 R01 RR14812 and by The Regents of the University of California. JJK is supported in part by the NIST Office of Law Enforcement Standards and a NIST Single Molecule Manipulation and Measurement Competence Award.

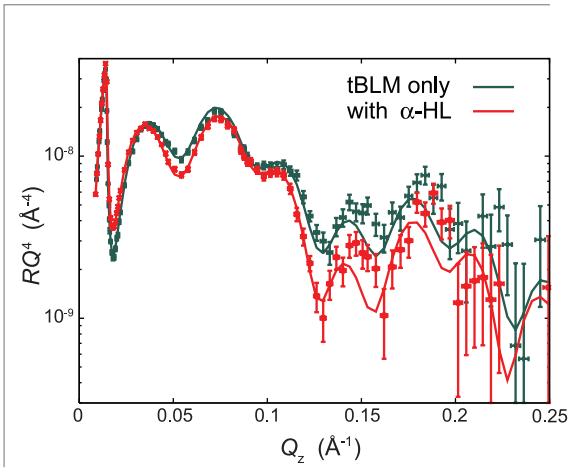

FIGURE 2:
NR and fitted models of the tBLM before and after αHL exposure, measured in D_2O. Fits were also constrained to other water contrasts (not shown).

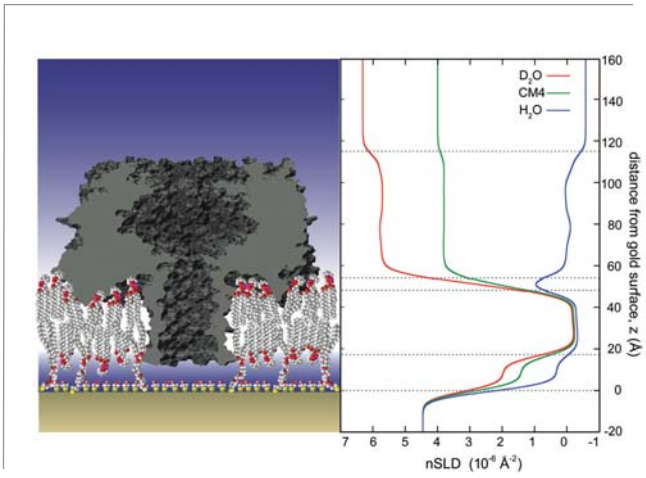

FIGURE 3:
(Left) A scaled cartoon of the functional αHL pore incorporated into a tBLM on gold, derived from the neutron scattering length density (nSLD) profile depicted on the right. (Right) The nSLD profile derived from simultaneous fitting of data measured in D_2O, H_2O and a D_2O:H_2O mixture (CM4) using the x-ray crystal structure of the protein. The combination of EIS and neutron reflectometry on a robust tethered membrane system has enabled the combined study of the structure and the functionality of a toxin with potential biotechnology applications. The functionality of the membrane is preserved in the biomimetic environment, and the neutron reflectometry reveals that the x-ray crystal structure is well-preserved in the membrane-bound form of the protein. The scattering also reveals details of the intimate binding between the protein and the membrane, confirming predictions based on the toxin structure.

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