quantify STxB interactions with the membrane under various reaction conditions and to study membrane-bound structures of STxB with neutron scattering. The influence of Gb3 and pH conditions (matching endosomes where binding takes place or the Golgi where release is thought to occur) on binding will be discussed.

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Comparative Analysis of Block by Poly-Ethyleneglycol of Canonical and Low-Conductance Oligomeric Assemblies of Alpha-Hemolysin: Mechanistic Implications

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Recent studies using pore forming membrane proteins in single-molecule detection have concentrated exclusively on analyte interaction with the so-called ''canonic pore'', displaying a certain conductance under given ionic conditions and presumably corresponding to a pore structure assembled from a particu-lar number of monomers. For instance, for the pore-forming toxin alpha-hemolysin, a conductance of approximately 1 nS in 1 M KCl at pH 7.5 is thought to correspond to a heptameric assembly, while a smaller conductance level is likely to be due to pores formed by a smaller number of monomers [1-4] Using a recently developed microarray device for parallel and high-resolution recording from suspended micro-bilayres (Microelectrode cavity array, MECA [5-7] we have begun to study the properties of the lower con-ductance form of aHL-mediated pores. The adavntage of the parallel recording system for these studies is that single canonical pores can be recorded simultaneously with low conductance forms under identical conditions. We found that low-conductance variants tend to appear after prolonged incubation of mono-mers in recording solution (3 M KCl). Interestingly, these smaller pores are blocked by poly-ethyleneglycol (PEG) oligomers in a fashion very similar to the larger canonical pores [8], showing a simi-lar resolution of PEG mass [6,9-11]. However, surprisingly, the relative position of the maxima in the histo-gram of relative residual conductances are shifted to larger values for the smaller pores. This finding has potential implications for the mechanism of the block by PEG, in that it suggests that PEG entry into the pore adds a resistance in series with the resistance of the internal constriction site.

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Aerolysin Block by Single Polyethylenegycol Oligomers: Mass Sensitivity and Voltage Dependence

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Electrophysiological studies of the interaction of polymers with pores formed by bacterial toxins (1) provide a window on single molecule interaction with proteins in real time, (2) report on the behavior of macromolecules in confinement and (3) enable label-free single molecule sensing technologies. Using pores formed by the staphylococcal toxin alpha-hemolysin (aHL), a particulary pertinent observation was that under high salt conditions (3-4 M KCl) the current through the pore is blocked for periods of hundreds of microseconds to milliseconds by polyethyleneglycol (PEG) oligomers (degree of polymerization approx. 10-60). Notably, this block showed monomeric sensitivity on PEG mass, allowing the construction of mass spectra from the residual current values.

Here, we show that the current through aerolysin (AeL) from Aeromonas hydrophila is also blocked by PEG but with important differences in the voltage-dependence of the interaction kinetics. While PEG blocking events of aHL show maximal dwell times at a transmembrane voltage of approximately +40 mV (stemside) and are very short at stemside-(-) voltages, blocks of AeL are so short as to be not resolvable (bandwidth 20 kHz) at stemside-(+) voltages but increase in duration with increasing values of stemside-(-) voltage up to −200 mV. Importantly, the interaction also shows monomer sensitivity to PEG mass, and at >100 mV stemside-(-) voltage long dwell times durations and high driving force combine to provide particularly precise determination of residual current, resulting in high peak-to-floor ratio mass spectra. These findings may potentially be understood as a consequence of the relatively high content of negative charges of the AeL pore compared to aHL and suggest that comparative studies of polymer interactions with different pore proteins are important in elucidating the underlying physicochemical mechanisms.

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Polyamidoamine Dendrimers as Universal Pore-Blocking Binary Toxin Inhibitors

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Attaching multiple functional groups onto an inert scaffold is very beneficial for drug design objectives. These multi-ligand compounds often possess an additive or cooperative affinity towards multiple binding sites which is significantly higher than that of a single functional group interacting with a single binding site. Here we explore a new group of potential multivalent pore-blocking antitoxins - dendrimers, which are the repeatedly branched polymers with all bonds emanating from a central core. Dendrimers are unique highly branched macromolecules with numerous groundbreaking biomedical applications under development. In this study, we identified polyamidoamine (PAMAM) dendrimers as novel blockers for the pore-forming B components of the binary anthrax toxin (PA_{63}) and Clostridium botulinum C2 toxin (C2IIa). These pores are essential for delivery of the enzymatic A components of the internalized toxins from endosomes into the cytosol of target cells. We demonstrate that at low μM concentrations, cationic PAMAM dendrimers block PA_{63} and C2IIa to inhibit channel mediated transport of the A components, thereby protecting HeLa and Vero cells from intoxication. By channel reconstitution and high-resolution current recording, we show that the PAMAM dendrimers obstruct transmembrane PA_{63} and C2IIa pores in planar lipid bilayers at nM concentrations. These findings suggest a new potential role for the PAMAM dendrimers as effective polyvalent channel-blocking inhibitors, which can protect human target cells from intoxication with binary toxins from pathogenic bacteria.

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Imaging the Assembly of Perfringolysin O

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Cholesterol-dependent cytolysins such as Perfringolysin O (PFO) lyse cells by forming large pores in the target cell membrane; they contribute to infections ranging from food poisoning to pneumonia and listeriosis. PFO monomers bind the target membrane and then oligomerize via a pre-pore intermediate to form pores of 20-50 subunits. Here we study the assembly of these pores using single-molecule fluorescence imaging in Droplet Interface Bilayers. We track the increase in brightness as monomers assemble to form individual pores. We observe significant fluctuations in the number of subunits that occur during the assembly of an individual pore.

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Key Residues in Vibrio Cholerae Cytolysin Involved in Membrane Binding

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Vibrio cholerae cytolysin (VCC) is a pore-forming toxin (PFT) secreted by the human pathogen Vibrio cholerae that has a predominant role in lethality in ΔCT (classical cholera toxin null) strains. VCC is a potent toxin with the ability to lyse cells in vitro at picomolar concentrations. In order to form heptameric lytic pores, VCC makes high-affinity interactions with cell membranes utilizing a combination of glycan receptors and lipid/cholesterol interactions. While previous research in our lab has illuminated aspects of glycan interactions made by one of the two lectin domains attached to VCC, knowledge regarding non-glycan mediated interactions between the host cell membrane and VCC's membrane-contacting rim domain is still lacking. To better understand direct membrane interactions, we performed systematic alanine scanning mutagenesis to over 30 amino acid residues predicted to interact with the membrane based on the crystal structure of the heptameric pore. We found several residues that when mutated to alanine, display drastically decreased protein activity; in some cases more than a 1000-fold loss. To ensure that the observed loss in activity is not related to a destabilization of the protein, we confirmed that our most drastic mutants are well behaved in solution and are not prone to aggregation. We also showed that the decrease in activity is not due to any