invasive types [Choi et al 2013, Human Pathology], during normal development of a tissue, or wound-healing.
We show how change of membrane cytoskeletal features can be quantified in single cells, by measuring the diffusion of fluorescently tagged transmembrane (TM) proteins level using camera-based FCS (bimFCS) technique. Data is analyzed in two populations of diffusion states: fast, in between cytoskeleton fences, and slow, due to hopping effect over them. Our technique makes quantification of average fence spacing and the protein-fence interaction strength possible for the ROI observed.
Using bimFCS, we analyzed the diffusion behavior of TM proteins in TM12 (epithelial) and TM12T (mesenchymal) breast cancer cell membranes. Cytoskeletal features (average fence spacing, protein-fence interaction strength) of invasive type TM12T cells show clear differences from the ones of in situ type TM12 cells.
We also observed changes during the EMT in single cell level, after wound simulating scratches by pipette-tip. Over a period of 24 hours wound-healing experiment on stage, at physiological temperature, we observed critical changes of cytoskeletal features by comparing the slow and fast diffusion coefficients for differentiating TM12 cells during the process.

405-Pos Board B185
'In vivo in Silico': Effects of Membrane Complexity on Protein-Lipid Interactions, Lipid Nano-Domains and Curvature
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Advances in lipidomics reveal the compositional complexity of cell membranes. These compositional complexities alter how the membrane interacts with proteins and hence have the potential to control the functional behaviour of proteins. It has become clear that the cell membrane does not only act as a barrier but plays an important role in controlling the function of proteins including the regulation of the epidermal growth factor receptor by the glycolipid GM3 and cholesterol modulation of G-protein coupled receptors (GPCRs).
Previous computational studies of membrane proteins have generally been limited to simple single component symmetric membrane models. We have developed an approach to create highly complex asymmetric biologically relevant membrane models allowing us to obtain a better understanding of membrane properties and enabling us to explore functionally important protein-lipid interactions.
We explore properties of biological relevant membranes, such as nano-domain formation by certain lipid types, and also more macroscopic properties such as membrane undulation and curvature in relation to lipid species and lipid clustering. Reaching system dimensions of > 100 nm allow us to explore membrane dynamics on length scales comparable with experimental ones. Our simulations indicate stabilization of curved areas by lipid nano-domains and a correlation between composition and curvature.
We can start to explore the influence of the lipid bilayer environment on protein properties such as oligomerisation of GPCRs in an ''in vivo in silico'' approach in contrast to previous studies being limited to ''in vitro in silico'' models. We can explore the binding of drugs entering the protein binding site from the membrane environment. Additionally we have moved even further towards in vivo conditions by introducing a cytoskeleton mimic into our large systems allowing us to explore the confinement this underlying network provides.

406-Pos Board B186
Morphological Modifications of the Early Secretory Pathway in Differentiating Skeletal Muscle Cells
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Skeletal muscle (SKM) cells present a regular and striking arrangement of organelles and membrane systems that is essential for their function. During SKM differentiation, membrane systems as transverse (T) tubules, sarcoplasmic reticulum (SR), and Calcium Release Units (CRUs) follow a highly coordinated plan. In addition to remodeling processes, SKM cell differentiation involves synthesis and transport of proteins addressed to build up the contractile apparatus, the SR and the sarcolemma. This process most probably requires an important involvement of early secretory pathway components as endoplasmic reticulum (ER), ER exit sites (ERES), Golgi Complex (GC), and vesicular traffic in general.
In terminally differentiated SKM cells, early secretory pathway components are regularly arranged; the GC is organized fiber-type dependent and, formed by small elements localized around the nucleus and in throughout all the fiber. It has been shown that early secretory pathway components undergo a dramatic reorganization during SKM cell differentiation resulting in the formation of smaller GC elements that are strongly associated to ERES. Whether GC reorganization consists in fragmentation or ex-novo biogenesis is not known, and whether its co-localization with ERES is the result of structural hindrance by the myofibrils and membrane systems, or depends on interaction mechanisms has not been explained.
The present work aims at better understanding how GC and ERES achieve their mature structure, what is their role during SKM differentiation, and whether morphological modifications are followed by alterations of membrane traffic efficiency. Therefore we investigated the processes underlying the morphological modifications of the secretory pathway structures as CG and ERES during SKM differentiation by applying live imaging techniques.
We report preliminary data on the morphological modifications occurring to GC and ERES in differentiated C2C12 cells.

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Phase Transition and Formation of Transmembrane Pore in Stretched Phospholipid Bilayer Including Cholesterol: Molecular Dynamics Simulation
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We perform molecular dynamics (MD) simulations of stretched phospholipid/cholesterol bilayers to understand effects of cholesterol on the dynamics of pore formation in the bilayer under mechanical stresses. The stretched DPPC/cholesterol bilayers including 40 mol% cholesterol from the liquid-ordered (L_O) phase are simulated with constant *NPzAT* MD simulations by applying various areal strains ε_A, defined by the increase ratio of the area of the stretched bilayer to the un-stretched bilayer, until a pore forms ($\varepsilon_A \sim 1.4$). From the visual inspection of snapshots obtained by MD simulations, we found the dynamics of the pore formation can be divided into four stages. In the first stage ($0.0 \leq \varepsilon_A \leq 0.3$), DPPC molecules become disordered monotonically. In the second stage ($0.3 < \varepsilon_A \leq 1.0$), DPPC molecules of the two monolayers start to interdigitate in a part of the bilayer and the order of DPPC molecules are recovered slightly. This indicates the L_O phase transforms to the coexisting phase of L_O and an interdigitated gel (L_βI) like phases. The region of the L_βI-like phase grows with the increase of ε_A and dominates near the upper limit of this stage. In the third stage ($1.0 < \varepsilon_A \leq 1.2$), DPPC molecules in the L_βI-like phase bilayer become disordered again, and in the fourth stage ($1.2 < \varepsilon_A \leq 1.4$), a transmembrane pore forms. The critical areal strain (~1.4), where the pore forms, for the DPPC/cholesterol bilayer is about double of that reported for the pure DPPC bilayer previously, in qualitative agreement with corresponding experimental data. We suspect the phase transition from L_O to L_βI-like phase is involved into the toughness of the DPPC/cholesterol bilayer under mechanical stresses.

Membrane Active Peptides and Toxins I

408-Pos Board B188
Characterizing the Shiga Toxin-Receptor Interaction
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The Shiga toxin (Stx) secreted by *Shigella dysenteria* and the shigatoxigenic group of *Escherichia coli* (STEC) causes over a million deaths annually and poses a global health problem. Stx invades its host by binding to globotriaosylceramide (Gb3) at the cell surface. Once internalized, it is trafficked to the Golgi via early endosomes, bypassing the late endosomes and lysosomes, hence avoiding degradation. This sorting is mediated by its B subunit (StxB) binding to the coiled-coil domain of GPP130, a host cell transmembrane protein that cycles constitutively between the endosomes and the Golgi. However, this interaction is only poorly characterized, in particular in context of membranes. Detailed characterization of this interaction is expected to offer new targets for therapeutic intervention, aiding rational drug design. Here we report the development of a physiologically relevant *in vitro* assay that uses sparsely tethered bilayer lipid membranes (stBLMs) to enable studies of the toxin-receptor interaction. To mimic the transmembrane receptor, we anchored hexa-histidine tagged $GPP130_{36\text{-}247}$, comprising the coiled-coil domain of full-length GPP130, on stBLM surfaces via conjugation to Ni^{2+}-presenting NTA lipids. We are thus able to establish a significant membrane surface density of His-$GPP130_{36\text{-}247}$ that can be used to

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.

409-Pos Board B189

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.

410-Pos Board B190

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