calcein leakage assay and UV resonance Raman spectroscopy. Effect of small changes in the primary structure of the peptide on the membrane rupturing activity is discussed.

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457-Pos

Simultaneous Single-Channel Recording and Fluorescence Imaging of Calcium Flux Reveals the Behaviour of Individual Antimicrobial Peptide Pores

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We have used simultaneous single-channel recording and total internal reflection fluorescence (TIRF) microscopy to investigate the behaviour of antimicrobial peptides in artificial droplet-on-hydrogel lipid bilayers (DHB).

These pore-forming peptides play an important role in many organisms by providing resistance to infection. An improved understanding of their mechanism of action is essential in the development of new antibiotics.

Our study focuses on two peptides thought to follow different pore-formation mechanisms. Alamethicin is produced by the fungus *Trichoderma viride* and is understood to form barrel-stave pores. Magainin II is found in the skin of the African clawed frog *Xenopus laevis* and is thought to follow a toroidal-pore model.

Using a fluorescent calcium indicator we are able to detect the ion flux through individual alamethicin and magainin II pores and can monitor multiple pores at once. We observe multiple conductance states from single alamethicin pores and see that magainin II forms stable pores.

458-Pos

Peptide-Induced Domain Formation in Supported Lipid Bilayers: Direct Evidence By Combined Atomic Force and Polarized Total Internal Reflection Fluorescence Microscopy

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Direct visualization of the mechanism(s) by which peptides induce localized changes to the structure of membranes has tremendous potential for understanding the structure-function relationship in antimicrobial and cell-penetrating peptides. We have applied a combined imaging strategy to track the interaction of a model amidated antimicrobial peptide, PFWRIRIRR-amide, with bacterial membrane-mimetic supported phospholipid bilayers comprised of POPE:TOCL. Our in situ studies revealed rapid reorganization of the POPE:TOCL membrane into localized TOCL-rich domains with a concomitant change in the organization of the membranes themselves, as reflected by changes in fluorescent membrane probe order parameter, upon introduction of the peptide.

459-Pos

Time-Resolved, Single-Cell Study of the Attack of the Antimicrobial Peptide Ll-37 on Live E. Coli Cells

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Human LL-37 is an antimicrobial peptide whose amphiphilic helices selectively degrade bacterial membranes by a mechanism that is poorly understood. We are using single-cell, two-color fluorescence microscopy to directly observe the attack of rhodamine-LL-37 on live E. coli cells in real time. The cells express either periplasmic or cytoplasmic GFP. This enables quantitative correlation of the extent of LL-37 adsorption with leakage or lysing of GFP from the two different compartments, while simultaneously monitoring cell growth. At 15 uM, LL-37 lyses the periplasm to GFP and halts growth in 2-4 min, long before the cytoplasm lyses to GFP at 20-30 min. At 6 uM, rhodamine-LL-37 binding occurs in three distinct waves, with Wave 2 correlating in time with the halting of cell growth (t = 7-10 min). Wave 1 coats the cell periphery uniformly, but Wave 2 preferentially attacks the septal region and slowly spreads outward towards the poles. This suggests that the cell division machinery may be a target of LL-37-induced cell death. We will use FRET to discern the penetration depth of LL-37 during the different waves of attack and a variety of mutant strains to correlate the LL-37 attack with formation of the Z-ring and additional parts of the divisome. These methods will enable quantitative comparison of antimicrobial attack on real bacterial membranes with studies of lysing of synthetic lipid bilayers. They will be applicable to a wide variety of antimicrobial agents and bacterial species.

Interfacial Protein-Lipid Interactions I

460-Pos

Membrane Diffusion of PH Domain-PIP3 Complexes: the Effects of Target Lipid Stoichiometry on Diffusion Constant Probed Using Single-Molecule Fluorescence Microscopy

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Pleckstrin homology (PH) domains are recruited to specific membrane surfaces during a variety of cell signaling events, including those occurring at the leading edge of chemotaxing cells. This recruitment is often driven by molecular recognition of specific phosphatidylinositol phosphate lipids, such as phosphatidylinositol-(3,4,5)-trisphosphate (PIP3). Using single-molecule fluorescence microscopy, we have recently shown that when bound to PIP3 on the surface of a supported lipid bilayer, the PH domain of GRP1 diffuses at the same rate as an individual phospholipid molecule diffusing in the same type of bilayer. More generally, we hypothesize that protein lateral diffusion constant will decrease as the number of lipid molecules tightly bound to the protein increases. Here, we probe the effects of PIP3 stoichiometry on the diffusion constants of constructs containing one, two, or three GRP1 PH domains coupled by flexible linkers. To a first approximation, we find that the lateral diffusion constants of these engineered PH domain constructs are inversely proportional to the number of bound PIP3 molecules. This observation suggests that the frictional contributions of multiple, tightly bound lipids are additive, at least when the binding sites are located on separate domains. At the meeting we will present our latest diffusion constant measurements, which will shed light on the mechanisms of peripheral membrane protein diffusion, and will provide useful calibration points in molecular dynamics simulations of proteins docked to membranes. Overall, single molecule diffusion methods provide a new, much needed window into the lipid interactions of membrane targeting proteins.

461-Pos

The Autism-Related H93R PTEN Mutant Shows Enhanced Plasma Membrane Binding But Reduced Activity

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The tumor suppressor, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), is a phosphoinositide (PI) phosphatase specific for the 3-position of the inositol ring. PTEN has been implicated in autism for a subset of patients with macrocephaly. Various studies identified patients in this subclass with one normal and one mutated PTEN gene. We characterize the binding, structural properties, activity and subcellular localization of one of these autism-related mutants, H93R PTEN, using fluorescence quenching, SPR and ITC. The membrane association of the mutant protein with solid-supported membranes (tBLMs) is investigated with neutron reflection. We observe that H93R PTEN shows enhanced binding to phosphatidylserine (PS)-containing membranes, in contrast to *wt* PTEN. On the other hand, binding to membranes that contain $PI(4,5)P_2$, a requirement for allosteric activation of PTEN, was strongly reduced for the H93R mutant. H93R and *wt* PTEN share the same secondary structure. However, while α-helical content increases in *wt* PTEN upon binding to $PI(4,5)P_2$, this is not observed for the H93R mutant. Consistent with the increased affinity of H93R PTEN to PS, we find in cell-based studies that the association of the mutant with the plasma membrane is strongly enhanced in comparison to *wt* PTEN. Unexpectedly, this does not enhance $PI(3,4,5)P_3$ turnover, but instead reduces enzyme activity significantly. We hypothesize that the tight binding of H93R PTEN to PS prevents $PI(4,5)P_2$ from interacting with the protein, thereby inhibiting allosteric activation, which is a requirement for binding to and turnover of the substrate, $PI(3,4,5)P_3$.

462-Pos

Unusual Thermal Stability of Human Secreted Phospholipase A2 Enzymes

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Analysis of the thermal stability of proteins in general and enzymes in particular is important for understanding their molecular mechanisms and for their analytical or industrial exploitations. While enzymes with extreme