cally photo-convert mitochondria inside axons and neuromuscular junctions from green to red fluorescence *in vivo*. Importantly, the amount of UV light required for photo-conversion did not damage the tissue, the nerve or the mitochondria. Moreover, photo-converted mitochondria maintained their red fluorescence for remarkably long periods. These mitochondria remained in the junctions 48 hours following the initial photo-conversion. It was possible to photo-convert mitochondria in a small sub-region of a single neuromuscular junction from green to red. Time lapse imaging showed the turnover of mitochondria as some of the red mitochondria were replaced by new incoming green mitochondria two days post photo-conversion.

These experiments provide the first *in vivo* observations of the axonal transport and dynamics of a critical neuronal organelle and will be useful for studying how axonal transport may be distorted in mouse models of neurodegenerative diseases.

Platform BE: Peptide & Toxin Ion Channels

2668-Plat Electron Density Image of Alamethicin Pore: Constructed by X-Ray Anomalous Diffraction

Shuo Qian[1], Wangchen Wang[1], Lin Yang[2], Huey W. Huang[1]

[1] *Rice University, Houston, TX, USA,*

[2] *National Synchrotron Light Source,Brookhaven National Laboratory, Upton, NY, USA.*

We report the first attempt to reconstruct the electron density of a peptide-induced transmebrane pore by x-ray diffraction. Lipidic structures can be long-ranged correlated into periodically ordered lattices that are amenable to diffraction analysis. We used a brominated lipid, di18:0(9,10Br)PC and the multiwavelength anomalous diffraction (MAD) method (BJ *91*, 736, 2006) in order to solve the phase problem. The same diffraction patterns were obtained from samples prepared in different peptide-lipid ratios. This implies that the pore size is determined by the energy of pore formation, not by the peptide-lipid ratios in the samples. We first extracted the diffraction intensities belonging to bromines alone. The Patterson function strongly indicates a barrel-stave model and rules out the toroidal model. Accordingly we modeled the distribution of bromines to obtain the phases, which were then used to construct the experimental electron density. Unlike protein crystals, lipid structures are described by the molecular distribution, rather than by atomic positions. This intrinsic feature corresponds to low resolution diffraction. Only the high values of the electron density are clearly revealed by such low resolution diffraction. The clearly visible regions include the distribution of phosphate groups of the lipids and the distribution of heavy atoms bromines that are bound to carbon 9 and 10 of each hydrocarbon chain. The headgroup and the chain distributions completely defined the lipid assembly. Unfortunately the peptide alamethicin does not possess high electron density; therefore their positions are not visible. Nevertheless the lipid assembly for the alamethicin pore is unmistakably that of a barrel-stave construction. The radius of the lumen is 17.8 A, which is consistent with a barrel-stave pore formed by 8 alamethicin peptides.

2669-Plat Characterizing Ion Channels with Membrane-Immobilized Polymers

Brian Nablo, Joseph Robertson, Vincent Stanford, John Kasianowicz

National Institute of Standards and Technology, Gaithersburg, MD, USA.

Nonelectrolyte polymers are commonly used to estimate the limiting aperture of ion channels. The pore diameter is deduced from the hydrodynamic radius of the critical polymer mass capable of inhibiting ion flux. The excessive polymer concentrations required for these measurements produce potentially detrimental solution and osmotic conditions which could perturb the ion channel structure. Here, we demonstrate how membrane immobilized polymers can be used as "molecular rulers" to probe channel features without requiring high concentrations of aqueous polymer.

2670-Plat Probing Single-molecule Ion Channel Dynamics By Combined Patch-clamp Single-molecule Imaging Microscopy

Somes Das, H. Peter Lu**hplu@bgsu.edu

Bowling Green State University, Bowling Green, OH, USA.

The kinetic behavior of ion channel proteins is regulated by subtle conformational changes that are often difficult to characterize by conventional ensemble-averaged static structure analysis and by interpretations of ion-channel electrophysiological measurements. By combining real-time single-molecule fluorescence imaging measurements with real-time single-channel electric current measurements in artificail lipid bilayers and in living cell membranes, we were able to probe single ion-channel-protein conformational changes simultaneously, and thus providing an understanding the dynamics and mechanism of ion-channel proteins at the molecular level. Subtle structural dynamics of ion channels play an important role in regulating channel function and selectivity. This technical innovation has been used to gain an understanding of how ion-channel activities are regulated by the conformational change dynamics of the dye-labeled colicin channels. We were able to probe fluctuating polypeptide-domain diffusional motions of single-molecule colicin channels across a lipid bilayer. A new solvation-desolvation model for colicin ion-channel dynamics has been postulated based on our experimental results.

2671-Plat Imaging the mechanisms of Aβ42 peptide toxicity

Angelo Demuro, Ian Parker

UCI, Irvine, CA, USA.

Converging evidence points to the oligomeric form of Aβ42-peptide as the primary toxic species in Alzheimer's disease via an uncontrolled elevation of intracellular free-Ca^{2+} [1]. However, many questions remain about the molecular mechanisms and sites of action by which Aβ42 exerts this toxicity. Here we use *Xenopus* oocytes as a model cell system together with advanced fluorescence

microscopy techniques to investigate the effects of intracellular versus extracellular application of Aβ42-oligomer to voltage clamped *Xenopus* oocytes. Extracellular application of 1μg/ml of Aβ42 in the oligomeric form triggered the appearance of multiple localized fluorescence Ca^{2+} signals throughout the image field, similar to those previously seen for ion channels [2]. We interpret these punctuate fluorescent transients to result from Ca^{2+} influx from the extracellular medium because they were absent when:

(a) the oocyte membrane potential was reduced from −120 to −20 mV;
(b) calcium was removed from the extracellular media and
(c) zinc (10 μM) was added to the media.

On the other hand, intracellular injections of 20 nl of 1 μg/ml Aβ42 oligomers triggered sparse Ca^{2+} transients with temporal evolution very different from the punctate Ca^{2+} transients seen by extracellular application. These signals resembled the Ca^{2+} 'puffs' that arise from Ca^{2+} liberation from intracellular stores triggered by the second messenger IP3. Our results reiforce the hypothesis that Aβ42 forms calcium permeable channels, and suggest a very different mechanism of toxicity when applied extracellularly or intracellularly.

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2672-Plat High-throughput Strategies for Discovering Non-toxic Antimicrobial Peptides: Which Aspects of Biological Activity are Correlated to Pore-forming Activity in Lipid Vesicles?

Ramesh Rathinakumar, Jessica R. Marks, Joshua M. Rausch, William C. Wimley

Tulane University Health Sciences Center, New Orleans, LA, USA.

An enigmatic image emerges from studying structure-function relationships of pore-forming, antimicrobial peptides. The mechanism of pore formation in vesicles is best described by the "carpet model" or "sinking raft model" in which amphipathic peptides bind to the surface of a membrane and self-assemble on that surface to create an unfavorable mass, charge or surface tension asymmetry which is relieved by transient pore formation. Peptide-lipid "pores" in vesicles do not require a specific, hydrogen bonded structure but instead require a balance of physicochemical interactions with each other and the membrane lipids. These results suggest that pore-forming antimicrobial peptides follow a non-structural paradigm in biomolecule design. We have been screening rational combinatorial libraries of peptides with alternating dyad repeat motifs to find beta-sheet sequences that assemble into pores in lipid bilayer vesicles. We have discovered dozens of potent pore-formers. Selected peptides form transient, non-specific pores in lipid vesicles which do not make a water-filled pore. All selected peptides thus function via a carpet model mechanism in vesicles. Without exception, the selected pore-forming peptides also have potent and broad spectrum antimicrobial activity at low μM concentrations. Selected peptides kill Gram positive and Gram negative bacteria as well as fungi and they do so by rapidly permeabilizing the microbial membranes. Most of the pore formers have little hemolytic or cytotoxicity activity against human cells. Interestingly, among the dozens of pore-forming peptides we have discovered, there is little sequence similarity, but rather a similarity in the physicochemical potential for membrane interactions and self-assembly. We show here that simple high-throughput screens for pore formation in synthetic phospholipid vesicles select for peptides that have all the desired properties of a therapeutically useful, non-toxic, broad spectrum antimicrobial peptide.

2673-Plat Plasma Membrane Porating Domain In Poliovirus 2B Protein. A Short Peptide Mimics Viroporin Activity

Jose L. Nieva[1], Vanesa Madan[2], Silvia Sanchez-Martinez[1], Natascia Vedovato[3], Giorgio Rispoli[3], Luis Carrasco[2]

[1] *University of Basque Country, Bilbao, Spain,*
[2] *Universidad AutÃ³noma, Madrid, Spain,*
[3] *UniversitÃ di Ferrara, Ferrara, Italy.*

Picornavirus 2B, a non-structural protein required for effective viral replication, has been implicated in cell membrane permeabilization during the late phases of infection. Here, we have approached the molecular mechanism of this process by assessing the pore-forming activity of an overlapping peptide library that spanned the complete 2B sequence. At non-cytopathic concentrations, only the P3 peptide, spanning 2B residues 35–55, effectively assembled hydrophilic pores that allowed diffusion of low-molecular weight solutes across the cell plasma membrane (IC50 approx. 4x10-7 M) and boundary liposome bilayers (starting at peptide-to-lipid mole ratios $>$ 1:10000). Circular dichroism data were consistent with its capacity to fold as a helix in a membrane-like environment. Furthermore, addition of this peptide to a sealed plasma-membrane model, consisting of retinal rod outer segments patch-clamped in a whole-cell configuration, induced within seconds ion channel activity at concentrations as low as 10-8 M. Thus, we have established a "one-helix" 2B version that possesses the intrinsic pore-forming activity required to directly and effectively permeabilize the cell plasma membrane. We conclude that 2B viroporin can be classified as a genuine pore-forming toxin of viral origin, which is intracellularly produced at certain times post-infection.

2674-Plat Inhibition of Anthrax Channels in Planar Lipid Bilayers by Cyclodextrin Derivatives: A Single Channel Study

Ekaterina M. Nestorovich[1], Vladimir A. Karginov[2], Anna Rossovsky[1], Sergey M. Bezrukov[1]

[1] *NICHD, Bethesda, MD, USA,*
[2] *Innovative Biologics, Inc., Manassas, VA, USA.*

Single heptameric channels of the *Bacillus anthracis* protective antigen, PA, were reconstituted into planar lipid membranes, and their conductance, complex kinetic behavior, open channel noise, and inhibition by cationic aminopropylthio-β-cyclodextrin, AmPrβCD, were analyzed. AmPrβCD interacts strongly with the anthrax channel lumen blocking PA-induced transport at subnanomolar concentrations (in 0.1 M KCl).

The characteristic properties of this process strongly depend on the applied transmembrane voltage, membrane lipid charge, and bathing electrolyte concentration. The residence time of AmPrβCD in the pore changes linearly with the voltage. This type of behavior is typical for the charged pore blocker and suggests that AmPrβCD effectively blocks the pore from the physiologically relevant cis-side instead of translocating through the channel. The efficacy of the blocker changed drastically when neutral lipid was substituted by negatively charged one. It is remarkable that by introducing the negative charge to the membrane we significantly decreased the ability of the highly positively charged compound to block the channel. As a possible explanation of this effect we discuss the "charge inversion model".

The dissociation constant of AmPrβCD binding to the channel demonstrates almost 3 orders of magnitude increase when KCl concentration changes from 0.1M to 1M. This unusually high sensitivity to salt concentration is, however, something that could be expected for the binding of a (7+) charged compound.

We also show that even though the binding parameters are mostly determined by electrostatics, some specific blocker-pore interactions are involved.

2675-Plat Applications of Nanosensors based on Derivatives of Gramicidin A

Steven Blake[1], Ricardo F. Capone[2], Sebastian Munoz Correa[2], Jerry Yang[1], Michael Mayer[2]

[1] *University of California, San Diego, La Jolla, CA, USA,*
[2] *University of Michigan, Ann Arbor, MI, USA.*

Detection of chemical processes on a single molecule scale is the ultimate goal of sensitive analytical assays. We recently reported the possibility to detect chemical modifications on individual molecules by monitoring a change in the single ion channel conductance of derivatives of gramicidin A (gA) upon reaction with analytes in solution (Capone *et al.*, *J. Am. Chem. Soc.*, **2007**, *129*, 9737–9745). These peptide-based nanosensors detect reaction-induced changes in the charge of gA derivatives that were engineered to carry specific functional groups near their C-terminus. Here, we introduce several novel applications of gA-based sensors for monitoring chemical and biochemical reactions. Based on the results, we proposed that charge-based ion channel sensors offer tremendous potential for ultrasensitive functional detection since a single chemical modification of each individual sensing element can lead to readily detectable changes in channel conductance.

Platform BF: Membrane Receptors & Signal Transduction

2676-Plat Microsecond Time Scale Molecular Dynamics Simulations : Endocannabinoid Entry Into The Cannabinoid CB2 Receptor Via The Lipid Bilayer

Patricia H. Reggio[1], Alan Grossfield[2], Dow Hurst[1], Klaus Gawrisch[3], Scott Feller[4], Mike Pitman[5]

[1] *UNC Greensboro, Greensboro, NC, USA,*
[2] *University of Rochester Medical Center, Rochester, NY, USA,*
[3] *NIAAA, NIH, Rockville, MD, USA,*
[4] *Wabash College, Crawfordsville, IN, USA,*
[5] *IBM Thomas J. Watson Research Center, Yorktown Heights, NY, USA.*

It is commonly assumed that Class A GPCR ligands enter and exit the receptor via extracellular space. While this assumption makes sense for charged, hydrophilic ligands such as the cationic neurotransmitters, a similar entrance/exit point is difficult to rationalize for hydrophobic ligands such as 2-arachidonoylglycerol (2-AG), the endogenous ligand of the Class A cannabinoid CB2 receptor. In work reported here, we tested the hypothesis that 2-AG may enter CB2 via the lipid bilayer. Microsecond time scale molecular dynamics simulations of 2-AG (NVT ensemble, T=310K, with velocity resampling occuring every nanosecond) were conducted in a system composed of the CB2 receptor in a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer. The system contained 124 POPC and 38 2-AG molecules. Simulations revealed that 2-AG can enter CB2 from the POPC bilayer by inserting between transmembrane helix 6 (TMH6) and TMH7 extracellular to the highly conserved W6.48(258). The initial interaction site for the 2-AG head group is S7.39(285), however, the ligand quickly establishes a long-standing interaction with D275 in the EC-3 loop of CB2. The entry of 2-AG into the CB2 binding pocket produces rearrangements in the intracellular domains of CB2 including a