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

transient (150 ns) break in a key salt bridge between D6.30(240) and R(136) and movement of the IC-3 loop away from the TMH bundle towards lipid.

[Support: NIH RO1 DA03934 and KO5 DA021358 (PHR)]

2677-Plat Bimolecular Fluorescence Complementation And Resonance Transfer Used To Demonstrate Secretin Receptor Dimerization Without Higher Order Oligomerization

Kaleeckal G. Harikumar, Renee M. Happs, Laurence J. Miller

Mayo Clinic, Scottsdale, AZ, USA.

Oligomeric complexes of GPCRs have been proposed to exist and to affect receptor function. However, the number of receptor molecules in such complexes is unknown. We previously utilized bioluminescence resonance energy transfer (BRET) to demonstrate that the Family B G protein-coupled secretin receptor exists in cells as an oligomeric complex. Now, we utilize bimolecular fluorescence complementation and resonance transfer to explore the number of receptor molecules in this complex. Bimolecular fluorescence complementation was achieved by co-expressing secretin receptors fused at their carboxyl terminus with the non-fluorescent carboxyl-terminal and amino-terminal portions of yellow fluorescent protein (YFP). While neither construct was fluorescent when expressed alone, when both were expressed in the same cell, bright yellow fluorescence was observed at the cell surface. These receptor constructs were capable of binding secretin specifically and saturably, to yield a full cAMP response. Of particular interest, no significant BRET signal could be elicited from expression of the Rlu-tagged secretin receptor (donor) with these two receptor constructs (acceptor). This suggested that two, but not three copies of the secretin receptor were present within the oligomeric complex. This was confirmed using a broad range of concentrations of donor and acceptor. Analogous observations were also made using FRET methodology with the site of donor varied to each of the intracellular loops and tail domain. The interpretation that secretin receptors achieve dimers and not higher order oligomers within the plasma membrane is consistent with our recent mapping of the relevant interface as representing only TM segment four, providing only a single surface for dimerization. It is noteworthy that this dimerization has functional importance for signaling, although not for secretin binding.

2678-Plat Role of HER2 and HER3 Overexpression on EGF Receptor Signaling

Yi Zhang, Harish Shankaran, Lee Opresko, Haluk Resat

Pacific Northwest National Laboratory, Richland, WA, USA.

Epidermal growth factor receptor (EGFR/HER1) is a member of the HER/ErbB family of receptors. Overexpression of HER2 and HER3 has been associated with high rate of tumor formation and development of resistance to treatment. Particularly HER2 plays a key role in aberrant physiological responses. Overexpression of HER2 and HER3 not only shifts EGF receptor distribution between cellular compartments but also alters its signaling properties. HER2 alters EGFR signaling patterns through forming heterodimers, and HER2 coupled EGFR-HER3 interactions lead to prolonged and enhanced downstream signal transduction processes that regulate cell growth, differentiation and migration.

We have quantitatively examined the effect of HER2 and HER3 overexpression on EGFR signaling using a combination of experimental and computational methods. Human mammary epithelial cell (HMEC) line 184A1-1 was transduced with HER2 or HER3 genes and then subcloned to establish a set of genetically related cell lines expressing different levels of HER2 or HER3. The receptor phosphorylation levels in different cellular compartments were monitored in ELISA experiments. Collected data was analyzed in the context of a multi-receptor network model for HER family of receptors that include and unify the receptor trafficking and signaling properties. Parameters of our kinetic model were optimized by fitting them to the experimental data. Our model-based analysis reveals the trends in how the overexpression of HER2 and HER3 alters the signaling properties of HER1-3 receptors.

Our results show that:

1. Internalization rate of EGFR slows down as the HER2/3 expression levels increase.
2. EGFR-HER2 heterodimers are less stable than the EGFR homodimers implying that their association may be transient in character.
3. Number of phosphorylated tyrosine sites depends on the formed receptor dimer types, which indicate the potential high potency of the HER2 receptor in leading to aberrant behavior.

2679-Plat No Abstract

2680-Plat Ligand Stabilized Conformations in G-protein Coupled Receptors: Insight into Activation and Ligand Efficacy

Nagarajan Vaidehi

Beckman Research Institute of the City of Hope, Duarte, CA, USA.

The superfamily of membrane bound proteins called G-protein coupled receptors (GPCRs) play critical physiological role in cell communication and are targets for many diseases. There are ligands that exhibit varied efficacies at the same GPCR, thus stabilizing different receptor conformations. 3D structural information of these ensemble of conformations would be very critical to understand the molecular basis of ligand efficacy. Great strides are being made in obtaining high resolution structures for GPCRs. Biophysical methods including computations are useful to probe the structure function relationships in GPCRs. We have developed a computational method, Ligand Induced Transmembrane Conformational changes (LITCon), based on systematic simultaneous rotational orientation optimization of the seven transmembrane helices, followed by MD simulations in explicit lipid and water for predicting the conformational changes that occur on ligand binding. This method maps the

conformational changes that occur on ligand binding with systematic energy based rotational methods and maps the allosteric conformational changes with MD simulations. Receptor conformational states for rhodopsin bound to trans-retinal, β2-adrenergic receptor bound to five different ligands with varied efficacies (agonist, partial agonists of two different chemical structure, weak partial agonist, inverse agonist). The inter-residue distances obtained from these predictions will be correlated to experimental results and discussed. The role of allosteric antagonists in inducing conformational changes in chemokine receptors CCR2, CCR5 and CCR3 will be discussed. I will also discuss on how the results from this study can be applied in designing mutation/fluorescence experiments to study activation mechanism and designing drugs that fit a particular receptor conformation.

2681-Plat Genetics, Nanotechnology And A New Microscope (PAM) Reveal Molecular Details Of ErbB Tyrosine Kinase Receptors

Donna J. Arndt-Jovin[1], Cornelia Fritsch[2], Guy M. Hagen[1], Wouter Caarls[1], Diane S. Lidke[3], Thomas M. Jovin[1]

[1] *Max Planck Institute for Biophysical Chemistry, Goettingen, Germany,*
[2] *University of Sussex, Brighton, United Kingdom,*
[3] *University of New Mexico, Albuquerque, NM, USA.*

/>Activation of the erbB receptors by the extracellular binding of peptide ligands triggers signaling cascades for cellular motility, cell division, and differentiation. We genetically tagged ErbB proteins with fluorescent proteins or acyl carrier protein (ACP) sequences. Photo- and chemically-stable semiconductor "quantum dots" (QDs), were targeted to receptors on the external cell surface. By combining these ligands and new, high-resolution microscopy techniques, we gain insights into molecular interactions and downstream signaling pathways. The results were acquired with a new generation, commercial, Programmable Array optically sectioning fluorescence Microscope (PAM) for rapid (20 Hz), light-efficient 3D imaging. The stand-alone module, including light source(s) and detector(s), features a ferroelectric liquid-crystal-on-silicon (LCoS) spatial light modulator.

(1) *Activated ErbB1 mobility and retrograde transport*. QD-coupled EGF allows visualization in living cells of individual EGFR receptors, the diffusion of which has been determined on different cell types with the PAM. Utilizing these probes we discovered a systematic retrograde transport on filopodia of EGFR following EGF binding and activation. The process is linked to treadmilling of actin filaments. This phenomenon acts as a biosensor, in that receptors are transferred from remote sites of activation to the transduction mechanisms in the cell body. We have mutated specific tyrosine residues in the cytoplasmic tail of the EGFR to identify the adaptor molecules mediating the transport as well as used peptides that cause a dominant negative phenotype in live cell PAM imaging.

(2) *Partition of activated receptor complexes*. The fate of activated receptors determines the extent and magnitude of signaling, and may provide insights as to how to inhibit oncogenic growth. Pulse chase experiments with the PAM shed new light on the effect of various ligands in cells expressing different combinations of the RTKs.

2682-Plat Imaging Avidity: T Cell Receptor Aggregation Measured in Live Cells using Quantum Dots and Image Correlation Spectroscopy

David L. Kolin[1], Sarah Boyle[2], Jonathan P. Schneck[2], Paul W. Wiseman[1], Michael Edidin[2]

[1] *McGill University, Montreal, QC, Canada,*
[2] *Johns Hopkins University, Baltimore, MD, USA.*

Changes in receptor organization are used by both prokaryotic and eukaryotic cells as a means of modulating their dynamic ranges of responsiveness to environmental cues. Specifically within the immune system this has been studied when naïve T cells become activated after antigenic stimulation (Fahmy, et al., Immunity 2001; *14*:135–143). We present a novel method of measuring T cell receptor (TCR) aggregation in live cells using quantum dot labeling and image correlation spectroscopy. 2C TCR transgenic cells in culture were observed from the naïve state to 12 days after activation. Cells were first labeled with biotin-MHC/peptide known to bind the TCR, and then streptavidin-quantum dots before imaging on an emCCD-equipped microscope, giving single-dot imaging sensitivity. The image series acquired were analyzed using k-space image correlation spectroscopy (Kolin, et al., Biophys. J. 2006; *91*:3061–3075), which allowed the aggregation of TCR to be quantified by two different approaches. The first uses spatial intensity fluctuations in an image to measure the clustering of receptors, while the second detects activation via changes in the intensity correlation function of the blinking QDs. In contrast to fluorescence activated cell sorting, which requires ~10^5 cells, our new approach can detect the activation state on individual cells and only requires ~40 cells. T cells exhibited maximum activation 3–4 days after initial activation, when their TCR degree of aggregation was an order of magnitude greater than the naïve state. This new technology has powerful applications as it can be applied to just a few cells and could be used to detect microheterogenity in receptor organization of cells in vivo.

Platform BG: Self-Assembled Session: Calsequestrin and the Cellular Ca^{+} Store of Skeletal and Cardiac Muscle

2683-Plat Lumenal Calcium Regulation of Single Cardiac Ryanodine Receptor Channels

Jia Qin[1], Giorgia Valle[2], Alma Nani[1], Alessandra Nori[2], Silvia G. Priori[3], Pompeo Volpe[2], Michael Fill[1]

[1] *Rush University Medical Center, Chicago, IL, USA,*
[2] *University of Padova, Padova, Italy,*
[3] *University of Pavia, Pavia, Italy.*

The lumenal Ca^{2+} regulation of cardiac ryanodine receptor (RyR2) was explored at the single channel level. Heavy SR microsomes isolated from rat cardiac muscle were fused into planar bilayers. Two lumenal Ca^{2+} regulatory mechanism(s) were identified and distinguished by their Ca^{2+} vs. Mg^{2+} sensitivity. One is a RyR2-resident mechanism which is likely mediated by a lumenal Ca^{2+} site on the channel protein itself. This mechanism operates in the