molecules to the extracellular ligand-binding domains (LBDs) of these receptors drives the opening of cation-permeable transmembrane pores. Ligand-binding alters the conformational free energy landscape of LBD closure, which provides useful reversible work for opening the gate of the transmembrane ion channel. Using all-atom molecular dynamics simulations, we computed absolute LBD-ligand binding free energies for a set of different ligands to AMPA and NMDA receptor LBDs using a methodology formulated on the basis of potentials of mean force. The free energy of the full ligand-binding process is the sum of the free energy contributions from ligand-docking into an open LBD and LBD closure. Alterations in the free energy landscape of LBD closure are correlated with whether the bound ligand is a full agonist, partial agonist, or antagonist.

3166-Plat
Pore Architecture and ion Sites of Acid Sensing ion Channels and P2X Receptors
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Acid-sensing ion channels are proton-activated, sodium-selective channels composed of three subunits, and members of the degenerin/epithelial sodium channel (DEG/ENaC) superfamily. These eukaryotic channels have essential roles in sodium homeostasis, taste, and pain. Despite their roles in biology, there is little knowledge of the structural and chemical principles underlying their ion channel architecture and ion-binding sites. Here we present the crystal structure of a functional acid-sensing ion channel in a desensitized state at 3 angstrom resolution, the location of the desensitization gate, and the trigonal antiprism coordination of cesium ions bound in the extracellular vestibule. Comparison of the acid-sensing ion channel structure with the P2X receptor reveals unanticipated similarities and mechanical principles in different ligand-gated ion channels.

3167-Plat
Energetics of Allosteric ion Binding to a Ligand-Gated ion Channel
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Allosteric regulation of ligand-gated ion channels (LGICs) is ubiquitous, involving discrete transitions between resting, conducting, and desensitized states, driven by agonist binding. Small molecules that bind at specific sites have also been reported to act as allosteric modulators in many LGICs, such as glutamate, P2X, and Cys-loop receptors, but an understanding of the underlying molecular mechanisms is sparse. Of these, perhaps the best studied are kainate subtype glutamate receptors which require both extracellular Na+ and Cl- for receptor function. Studies on glutamate receptors (iGluRs) have defined the ligand binding domain (LBD) dimer assembly as the key functional unit that controls channel activation and desensitization. Using crystallographic and electrophysiological approaches, we have previously shown that for kainate, but not AMPA iGluRs, the binding of Na+ and Cl- ions to discrete, electrostatically coupled sites in the extracellular LBD dimer regulates the rate of entry into the desensitized state, which occurs when the dimer interface ruptures and the channel closes. We have now dissected the energetic effects of allosteric ions on kainate receptor dimer stability in solution using analytical ultracentrifugation. Our results show that Na+ and Cl- ions modulate dimer affinity as much as 50-fold, and that removal of either ion disrupts the dimer. We further tested the generality of this model of ion action for Ca2+ modulation of the orphan iGluR delta2 that crystallizes as a dimer which binds Ca2+. Our results indicate that ions can contribute substantial free energy to active state stabilization in both these receptors, and we postulate that in contrast to AMPARs, the dimer interface in these receptors may be intrinsically weak to serve a functional role: Allowing ion modulation. Our results provide quantitative measurements of the energetic effects of allosteric ion binding on a LGIC.

3168-Plat
Structure and Dynamics of Nicotinic Acetylcholine Receptor at the Cell Membrane
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A combination of ensemble averaging methods (confocal FRAP and FCS) and single molecule experimental techniques (single-particle tracking, high-resolution fluorescence microscopy, patch-clamp) was used to study the supramolecular organization of the acetylcholine receptor (AChR), receptor dynamics at the cell surface, and the kinetics of receptor internalization. Chol depletion produced gain-of-function of single-channel dwell time. Submicron-sized particles could be resolved into AChR ''nano-clusters'' with a peak size distribution of ~55 nm by superresolution STED and GSDIM microscopies. Chol depletion reduced the number of nanoclusters, increasing their size, and changed their supramolecular organization on larger scales (0.5-3.5 microns). FRAP, FCS and SPT experiments provided information on the dynamics of AChR nanoclusters, disclosing the dependence of their mobility on Chol content and cortical cytoskeleton. Chol content at the plasmalemma may thus modulate cell-surface organization and dynamics of receptor nanoclusters, and fine-tune receptor channel function to temporarily compensate for acute AChR loss from the cell surface.

3169-Plat
Structure Rearrangement of the Pore in P2X Receptors During Gating
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P2X receptors are cation-selective channels that open upon binding extracellular ATP. In mammals, seven P2X receptor subunits have been cloned ($P2X_{1-7}$), which can form functional homomeric as well as heteromeric channels. The recently published X-ray crystal structure of a P2X receptor confirmed that these channels are trimers and that each subunit has two transmembrane (TM) segments, a large extracellular segment containing the ligand binding site, and intracellular N and C termini. The crystallized channel appears to be in a closed state, with an extended plug of hydrophobic residues in the pore-lining TM2 helix forming a gate to prevent ion permeation. Although the structure reveals the overall molecular design of the protein, how the pore opens upon ATP binding is still unknown. Here we study the interaction of metal ions as well as MTS reagents of variable sizes with Cys residues introduced into the TM2 helices of P2X receptors. Our results suggest that the crystal structure is representative of closed P2X receptor channels in native membranes, and that the TM2 helices straighten in a translational motion that produces a modest opening of the outer pore and an accompanying constriction of the inner pore. Consideration of these constraints in light of the distinct arrangement of subunit interfaces in the ligand-binding and pore domains supports a model wherein the ligand-binding domains rotate relative to one another to straighten the TM2 helices and open the pore.

3170-Plat
Analysis of Structural Rearrangements during P2X1 Receptor Activation by Voltage Clamp Fluorometry
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P2X receptors (P2XRs) are non-selective cation channels which are activated upon binding of extracellular ATP. They are assembled as homo- or heteromers from three subunits with two transmembrane domains each and show no sequence homology to any other known ion channel or ATP-binding protein. Most recently, the first crystal structure of a P2X receptor has been resolved confirming many predictions that were based on the interpretation of mutagenesis studies. However, the conformational changes governing channel opening, desensitization and recovery remain unknown, as does the exact mode of ATP binding. The P2X1 receptor subtype is characterized by nanomolar affinity for ATP and a rapid desensitization, followed by a prolonged recovery period before reactivation is possible. Here we used voltage clamp fluorometry to identify domains undergoing conformational changes during ligand binding, activation, desensitization and recovery from desensitization of the P2X1 receptor. We have identified six residues in the extracellular domain of the P2X1 receptor that, upon substitution with cysteine, are accessible for TMRM labeling. Upon activation by ATP, five of these mutants showed significant changes in fluorescence. The kinetics of the fluorescence changes could be correlated with receptor activation or desensitization indicating that the changes were reporting discrete conformational changes. In addition, binding of the competitive antagonist NF449 produced a fluorescence change in three of these mutants. In conclusion, our observations provide insight into the conformational changes occurring during the P2X opening and desensitization and suggest that movements in a cysteine rich domain that projects over the supposed ATP binding site are involved in these processes.

Platform AU: Biotechnology & Bioengineering

3171-Plat
Parallel, Non-Contact Trapping and Translation of Yersenia Pestis Bacteria with Optoelectronic Tweezers
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Studies of host-pathogen (HP) interactions at the single cell level are critical for understanding the often elaborate, dynamical processes involved in pathogen

invasion of hosts and disease initiation and progression. In order to monitor the pathogen invasion process, most often one mixes a solution containing pathogens into a culture of host cells, and then hopes the host cell being monitored is invaded by a pathogen. These chance encounters have been monitored using fluorescence microscopy, allowing for example fluorescent effector proteins to be monitored as they are injected into a host cell (Enninga, Mounier et al. 2005; Schlumberger, Muller et al. 2005). Such ''mix and hope'' strategies are simple, direct, and necessary for initial studies. However, they do not provide a path to obtain large sample sizes, control of timing of pathogen invasion, or a way to determine how many bacteria are required to defeat host defenses. We present recent results from using optoelectronic tweezers (OET) (Chiou, et al. 2005) as a tool for manipulating single pathogenic bacteria, opening a promising route for controlled initiation of HP interactions. Optoelectronic trapping uses laser-excited carriers inside a thin film of amorphous or crystalline Si to create a non-uniform electric field. These carriers, coupled with an electric field modulated in the MHz range produces strong field gradients. The object being trapped is polarized by the electric field, and gradients in the electric field create a potential well which traps the object. This trapping is obtained at extremely low optical intensities ($<1 W/cm^{\wedge 2}$) and does not require functionalization of the pathogen in order to facilitate delivery to the host, opening up novel possibilities for massively parallel studies of HP interactions.

3172-Plat

A Suitably Compliant Microenvironment Commits Mesenchymal Stem Cells to Differentiate into Muscle Like Cells Which Restore Muscular Defects in Dystrophic Models

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Human mesenchymal stem cells (hMSCs) express markers of different lineages when grown on matrices of an elasticity that mimics various tissues of mesenchymal origin. Of particular interest to our group is the possibility of using this approach to drive hMSCs into skeletal muscle lineage for repair of damaged skeletal muscle. In order to identify and determine the necessary *in vitro* culture conditions that will lead to optimal commitment of cells to the muscle lineage *in vivo*, we have been developing biophysical techniques to allow stem cell precommitment to a specific lineage prior to implantation in a diseased tissue by controlling matrix stiffness under *in vitro* culture conditions. This technology has led to a highly reproducible approach to optimizing stem cell fate for human transplantation. The physical nature of a cell's microenvironment - including elasticity of the surrounding tissue - appears to exert a significant influence on cell morphology, cytoskeleton and gene expression. Numerous gel systems - particularly polyacrylamide gels - have tunable elasticity that can be adjusted over several orders of magnitude from extremely soft to stiff, mimicking the elasticity of a wide range of tissues by controlling the extent of polymer cross-linking. Here, we use cross-linked polyacrylamide hydrogels that mimic the true *in-vivo* muscle-like elasticity (10-15 kPa) to induce hMSCs to differentiate into myoblasts expressing key early markers of muscle differentiation program (Pax7, myoD). They also fuse to form myotube like structures expressing late skeletal muscle markers like troponin I in vitro. We have recently used these committed cells on our *in vivo* animal models of muscular dystrophy and successfully demonstrated that they have a very high potential for integrating into skeletal muscle and rescuing the muscular defects.

3173-Plat

Single-Molecule DNA Biosensors for Quantitative Transcription Factor Detection

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We have developed two single-molecule fluorescence biosensors that can quantitatively detect single or multiple transcription factors (TFs) with high sensitivity and specificity. The first sensor is based on TF-based coincidence of two DNA fragments, each containing half of a particular TF binding sequence. In the absence of the particular transcription factor, the DNA fragments diffuse independently. The coincidence is detectable using 2-colour alternating laser excitation (ALEX) spectroscopy using either solution or surface-based approaches.

We have detected a single transcription factor (lactose repressor - lacR) at a concentration of 100pM without the need for amplification steps. Further we can detect TFs in a quantitative manner using a simple kinetic model, without the need for a calibration curve. Using an inducible plasmid for catabolite activator protein (CAP), we show quantitative detection of changes in gene expression in bacterial cell lysates over time.

We extend this first sensor to simultaneously detect two transcription factors (lacR and CAP) in the same solution. Two assays were designed to implement two basic Boolean logic operators (AND and OR). We demonstrate correct functioning of these operators using solution and surface-based approaches with nM TF concentrations. These operators can be cascaded to form arbitrarily complex intelligent sensing assays for true multiplexed detection of several TFs in one experiment.

The second sensor is based on the common phenomenon of TF-induced DNA bending. The design is uni-molecular with a donor-acceptor pair in close proximity and a binding site for the TF of interest. On binding of the TF (CAP), the DNA is bent, increasing the donor-acceptor distance. This can be detected via a change in FRET (Förster Resonance Energy Transfer) in a quantitative manner. Current investigations are focused on using this sensor for TF detection in cell lysates and in-vivo.

3174-Plat

A Microfluidic Device to Maintain Islet-Associated Endothelial Cells During Long-Term Tissue Culture

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Endothelial cells (EC) are integral to the characteristically dense vasculature of pancreatic islets. This vasculature enables accurate blood-glucose sensing and rapid secretion of insulin into the blood stream. It also provides pro-survival hemodynamic signals to EC. How EC and beta cells interact to affect glucose stimulated insulin response is an actively debated topic. However, long-term studies in the *ex vivo* tissue are limited by the loss of EC over a period of days in traditional culture. We postulate that the EC die in part from an absence of the shear and media exchange provided by hemodynamic fluid flow. To test the role of hemodynamic forces on EC, we created a microfluidic device capable of supplying a range of fluid flow to *ex vivo* islets. Our protocol controls temperature, pH and bubble formation using two hot plates and a syringe pump for long-term desk top experimentation. Using this microfluidic device with immunofluorescence microscopy, we examined the morphological response of islet-EC to a variety of flow rates for 24 and 48 hours. Our results show more than twice the average percent area and connectivity of EC in islets treated in the device as compared to no-flow controls stored in traditional cell culture. Using this device with varying media viscosity, we determined that the differences in morphology are due to media exchange and not shear-activated survival. As well, we are currently evaluating the effect of fluid flow on beta cell survival by ensuring normal glucose stimulated calcium and insulin response. Overall, our data indicates that flow in a microfluidic device provides a reliable *co-culture* environment enabling the long-term study of cell biology in the pancreatic islet.

3175-Plat

Engineering Lipid Bilayer Platforms for High Throughput Cell-Free Electrophysiology

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Ion channels are crucial physiologically, involved in nearly all biological processes. Their many roles make them important drug targets as well as targets to avoid, as for drug safety screening (e.g. the hERG channel). Patch clamp provides the highest quality measurements of ion channel function and is used by the pharmaceutical industry to test drug interactions with ion channels, but has poor cost and throughput performance. As an alternative ion channel measurement platform, artificial lipid bilayers are well established to provide a highly controllable environment capable of measurement at the single molecule level, although they also suffer from their own technological shortcomings. Previously, we developed an artificial lipid bilayer platform which is capable of shipping and indefinite storage. Here we present the continued technological development of this system which has resulted in faster bilayer formation times and the ability to package, store, and ship ion channels with the bilayer chip. We have also begun to integrate array chip designs with automated and high throughput solution manipulation and ion channel measurement hardware, advancing this platform closer to operator-free involvement and low cost high throughput cell-free electrophysiology.

3176-Plat

Towards Dark Quencher Based Real Time DNA Sequencing

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Third-generation DNA sequencing technologies are expected to transform biomedical research and health care. Although powerful single-molecule DNA