a highly specific pathogen-derived peptide binds to the T cell receptor (TCR). However, the concomitant signaling events are not specific to the TCR raising the question how T cells recognize specific signals for activation. The organization of signaling proteins in time and space may establish hierarchies and, ultimately, control signaling outcomes that determine cell function in health and disease.
By manipulating lipid packing densities (Rentero et al PLoS One, 2008) and quantify membrane order microscopically (Gaus et al, J Cell Biol, 2005), our previous data revealed that membrane lipids and proteins co-operate to form stable membrane domains and protein clusters that are necessary for full T cell activation. To understand the underlying principles of the organization of signaling proteins in the T cell membrane, we established super-resolution microscopy approaches based on photo-activation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). These are a single molecule imaging technique that allows us to quantify the number of proteins participating in signaling clusters, the number of clusters and the ratio of proteins within clusters (Owen et al. J Biophotonics, 2010). In other words, we are able to quantify signaling efficiency and thus determine how lipids influence the organization and regulation of signaling process.

979-Plat
Competing Negative and Positive Feedback Generate Specific T Cell Responses by Tuning Duration and Amplitude of Itk Activation
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T cells, key orchestrators of adaptive immunity, sense pathogen-derived antigen peptides through T cell receptors (TCRs). Developing T cells express TCRs of random antigen specificity that interact with self-peptides with a wide range of affinity. A strict selection process warrants generation of a functional, protective but self-tolerant T cell repertoire by removing T cell precursors failing to interact or stimulated strongly by self-peptides, and inducing survival and maturation for low-affinity/mild TCR signals. How different TCR signals can have such vastly different outcomes is ill understood. Among crucial TCR effectors, the oligomeric enzyme Interleukin-2 inducible T cell kinase (Itk) controls early (min scale) TCR signaling. Transient Itk activation is controlled by a positive feedback feeding into a negative feedback. Both are mediated by the soluble small messenger molecule inositol(1,3,4,5)tetrakisphosphate (IP4) generated via signal-dependent metabolism of membrane lipids (Huang et al, Science 2007). We combine computational modeling and biochemical experiments to elucidate the role of antigen affinity and Itk oligomerization in regulating duration and amplitude of Itk and T cell activation. Our results suggest that high affinity peptides cause strong but short-lived Itk activation necessary to induce downstream Ras and MAPK activation. Low affinity antigens cause prolonged Itk activation with smaller amplitudes. This is sufficient to activate Erk, an essential mediator for survival in developing T cells. Our findings also suggest that certain modes of Itk oligomerization can inhibit signaling by low-affinity peptides. Regulation of transient Itk activation by IP4 may point to a novel mechanism used by different cell signaling networks to generate specific functional decisions. In developing T cells, it may contribute to an enigmatic TCR signal splitter that determines whether TCR engagement causes death or survival and maturation.

980-Plat
High-Throughput Measurement of GPCR Stability At Femtomole Scale
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The inherent instability of heptahelical G protein-coupled receptors (GPCRs) during purification and reconstitution is a primary impediment to biophysical studies and to obtaining high-resolution crystal structures. New approaches to stabilize receptors during purification and to screen reconstitution procedures are needed. Here we report the development of a novel homogeneous time-resolved fluorescence assay (HTRF) to quantify properly folded CC-chemokine receptor 5 (CCR5). The assay permits high-throughput thermal stability measurements of femtomole quantities of CCR5 in detergent and in engineered nanoscale apolipoprotein bound bilayer (NABB) particles. We show that recombinant expressed CCR5 can be incorporated into NABB particles in high yield, resulting in greater thermal stability compared with CCR5 in detergent solution. We also demonstrate that CCR5 binding to the HIV-1 cellular entry inhibitors maraviroc, AD101, CMPD 167, and vicriviroc dramatically increases receptor stability. The HTRF assay technology reported here is applicable to other membrane proteins and could greatly facilitate structural studies of GPCRs.

981-Plat
Caveolae Regulate Bone Morphogenetic Protein 2 Signaling as Identified with Imaging and a Systems Biology Approach
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The goal of this study was to identify crucial signaling components at the plasma membrane and calculate their dynamic involvement of Bone Morphogenetic Protein (BMP) induced Smad signaling. This was achieved by integrating Family of Image Correlation Spectroscopy (FICS) data and molecular biology results with an approach of formulating a mathematical model followed by experimental verification.
The lipid bilayer of cellular membranes is highly dynamic and heterogeneous consisting of domains enriched in distinct lipids such as sphingolipids and cholesterol. These domains are involved with the regulation of signal transduction pathways through membrane and receptor dynamics. One subset of these domains are flasked-shaped invaginations enriched in Caveolin-1, named caveolae. These domains regulate signaling.
During development, BMP2 regulates the polarity of the embryo and determines cell fate during neurogenesis, osteogenesis and adipogenesis. BMP receptors (BMPRs) localize to distinct domains on the plasma membrane and shuttle between these domains in order to initiate signaling. However the dynamics underlying this initiation are poorly understood and it is challenging to integrate and analyze complex data from multiple experimental sources. Therefore we incorporated and connected the distribution and dynamics of BMPRs on the plasma membrane as measured by FICS and the effect of localization on signaling by molecular biological methods into a mathematical model. The model identified caveolae to be two times more involved in regulating BMP2 induced Smad signaling compared to other domains (65% of the total Smad signal). We verified the model experimentally by determining regions of Smad phosphorylation. This result derived from an integrated modeling-experimental approach revises current dogmas in cell biology and BMP signaling.

982-Plat
Converting Signalling Pathway of Olfactory Receptor Proteins Into Electronic Read Out
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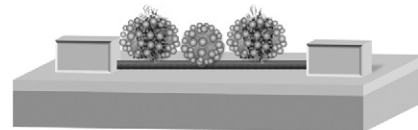
Integration of modern nanoelectronic technology with the potent molecular machines of living organisms offers a pathway to advanced chemical sensing and high throughput screening of ligand binding. Integration of amphiphilic membrane proteins remains a challenging problem despite their vital and varied functionality in living organisms. We have created a nanoelectronic interface to G-protein coupled receptors (GPCRs), a large family of membrane proteins whose roles in the detection of molecules outside eukaryotic cells and initiation of cascades of intracellular responses make them important pharmaceutical targets. Olfactory receptor proteins (ORs) are the most numerous class of GPCRs, representing transcription products of ~ 3% of the mammalian genome. We report a method to integrate ORs with carbon nanotube (NT) transistors. The resulting devices transduce signals associated with odorant binding to ORs in the gas phase under ambient conditions and show responses that are in excellent agreement with results from established assays for OR-ligand binding. The work represents significant progress towards an electronic nose that can be directly compared to biological olfactory systems as well as a general method for the study of GPCR function in multiple domains using electronic readout.



983-Plat
Molecular Modeling of Neurokinin B and Neurokinin-3 Receptor Complex
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The Neurokinin-3 (NK-3) receptor is a member of the Rhodopsin family of G-protein coupled receptors. NK-3 receptor has been regarded as an important drug target due to diverse physiological functions and its possible role in the pathophysiology of psychiatric disorders, including schizophrenia. NK-3

receptor is primarily activated by the tachykinin peptide hormone Neurokinin B (NKB), the most potent natural agonist for the NK-3 receptor. NKB has been reported to play a vital role in the normal human reproduction pathway, potentially life threatening disease such as pre eclampsia and as a neuroprotective agent in case of neurodegenerative diseases. Agonist binding to the receptor is a crucial event in initiating signaling and therefore characterization of the structural features of the agonists can reveal the molecular basis of receptor activation and help in rational design of novel therapeutics. In this study a molecular model for the interaction of the primary ligand NKB with its G-protein coupled receptor NK-3 has been developed. A three-dimensional model for the NK-3 receptor has been generated by homology modeling using rhodopsin as a template. A knowledge based docking of the NMR derived bioactive conformation of NKB to the receptor has been performed utilizing limited ligand binding data obtained from the photo-affinity labeling and site- directed mutagenesis studies. A molecular model for the NKB-NK-3 receptor complex thus obtained sheds light on the topographical features of the binding pocket of the receptor and provides insight into the biochemical data currently available for the receptor. The results of the receptor modeling studies have been used to discuss the molecular determinants for NK-3 receptor selectivity.

PLATFORM V: Excitation-Contraction Coupling

984-Plat

Junctophilin 1 and 2 Interact with the Dihydropyridine Receptor: Knockdown of JP1 and JP2 in Skeletal Muscle Affects the L-Type Calcium Channel Function of the DHPR

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Junctophilins (JPs) play a central role in anchoring the sarcoplasmic reticulum (SR) to the sarcolemma, but also interact with several proteins of the SR and the plasma membrane. We have observed an interaction between JP1 and JP2 and the dihydropyridine receptor (DHPR) in both co-immunoprecipitation and pull-down experiments. This was further supported by immunofluorescence experiments, where Knockdown of JP1 and JP2 appeared to affect the assembly of junctional proteins. Functional experiments performed under voltage-clamp conditions revealed that Knockdown of JPs in cultured myotubes heavily depressed the Ca2+ channel function of the DHPR: JPs Knockdown-positive cells yielded a reduction of the L-type Ca2+ current density that corresponded to an average 50 % reduction of the peak conductance and was accompanied by an ~10 mV right-shift of the voltage-dependence of channel activation. Interestingly, measurements of intracellular Ca2+ transients with the dye rhod-2 showed that there was no substantial alteration of the peak SR Ca2+ release following Knock-down of JPs. Knockdown of the JP proteins in adult fibers also appeared to alter the Ca2+ channel function of the DHPR, though to a lesser extent than in myotubes. The decrease in adult fibers corresponded to an average ~25 % reduction of the peak conductance. As in the myotubes, voltage-activated Ca2+ transients appeared unaffected. Altogether these results suggest that JPs are involved in the organization of a structural and functional platform necessary for the assembly of the e-c coupling components, where their interaction with RyR1 and DHPR may be relevant to the functional cross-talk between plasma membrane channels and intracellular channels in ensuring proper function of the e-c coupling machinery.

985-Plat

$Ca_V\beta_{1a}$ Nuclear Translocation: Potential Mechanisms and Functions

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$Ca_V\beta_{1a}$ Nuclear Translocation: Potential Mechanisms and Functions

$Ca_V\beta$ subunits are traditionally considered constituents of Ca_V complexes (Ca_V1or2, $Ca_V\beta$, Ca_V $\alpha2/\delta$), where they localize at the plasma membrane and serve to regulate channel expression and gating properties. Several recent publications also show $Ca_V\beta$ subunit localization in the nucleus. This phenomenon has been observed under a variety of conditions (different cell types, β subunit isoforms, co-expressed proteins, etc). However, the exact mechanisms responsible for $Ca_V\beta$ subunit nuclear shuttling, as well as a physiological role for this nuclear localization, remain major questions. In the present work, we use primary mouse myoblasts as a model to study $Ca_V\beta$ subunit nuclear localization. These mitotic cells express $Ca_V\beta_{1a}$ protein, despite the absence of any Ca_V1 subunits, and thus provide an ideal model to study non-Ca_V- related function (i.e. nuclear) of $Ca_V\beta$ subunits. Using both immunofluorescence and subcellular fractionation, we show that endogenous and exogenous $Ca_V\beta_{1a}$ proteins are rapidly translocated into the nucleus of myoblasts. We also use shRNA knockdown and transgenic $Ca_V\beta_1$-null myoblasts to examine the importance of $Ca_V\beta_1$ in gene expression and cell proliferation. A mouse model of partial $Ca_V\beta_1$ knockdown is used to determine the role of $Ca_V\beta_{1a}$ in skeletal muscle regeneration following injury *in vivo*. Finally, we attempt to identify nuclear binding partners of $Ca_V\beta_{1a}$-YFP using affinity purification from nuclear fractions coupled with mass spectrometry. Our results support the idea of $Ca_V\beta$ subunits having alternate functions separate from Ca_V's, within the nucleus, and suggest these functions may be specific to progenitor cells.

986-Plat

Cav1.1 Acts as a Voltage Sensor for Two Separate Processes in Skeletal Muscle with Different Voltage Dependence

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In adult muscle fibers, tetanic electrical stimulation induces two separate calcium signals, a fast one related to contraction and dependent on Cav1.1 (DHPR) and RyRs and a slow signal, dependent on DHPR and IP_3Rs. We have recently shown that the amplitude of this slow signal is dependent on frequency of stimulation, having a maximum at 20 Hz. Importantly, this signal participates in the activation of genes related to slow muscle fiber type phenotype. This signal is inhibited by nifedipine, supporting a role for the DHPR in its onset. We have stimulated adult muscle fibers with different concentrations of extracellular K^+, and found calcium signals at more negative membrane potential than those described for triggering calcium release associated with RyR. Moreover, in the same conditions, we observe an increase in mRNA of the slow isoform of TroponinI, as seen in fibers stimulated with a 20Hz train of pulses. These signals have an intracellular origin, because they are still present when working in solutions with no extracellular calcium and 0.1 mM EGTA. Interestingly, they are inhibited by incubation of fibers with 20 uM XestosponginB, a specific inhibitor of IP_3R. These results strongly suggest that the slow signal fires at membrane potential values more negative (Nernst potential estimated -50 mV) than the fast signal (E-C coupling related), where the fast signal threshold (-30 to -20 mV) has not yet been reached. This opens the possibility for a different part of the DHPR molecule being involved in the signal transmission of depolarization to IP_3-production machinery, independently of the process of E-C coupling.

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987-Plat

Gradual Formation and Accumulation of Tubular Aggregates in Fast Twitch Muscle Fibers: SERCA and Calsequestrin Involvement

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Tubular aggregates (TAs), ordered arrays of elongated sarcoplasmic reticulum (SR) membranes, are present in skeletal muscle fibers from patients with various myopathies. TAs have been also described in ageing wild type (WT) mice, where they display a dependence on sex (male), and fiber type (fast twitch). The mechanism(s) leading to TAs formation are, though, not yet clear. Here, we investigated the sequential stages leading to maturation of TAs in extensor digitorum longus (EDL) from male WT and calsequestrin knockout (CASQ-null) mice. A crucial step in the formation of TAs, seems to be the swelling of free SR cisternae at the sarcomere I band. This dilated SR, which is abundant at 1 year of age contains electron-dense material, which likely indicates abnormal accumulation of CASQ. Lately, the enlarged SR sacs mature into multiple and longitudinally oriented tubules containing CASQ, which first elongates into the A band, and then gradually acquire cylindrical shape and uniform size. Apparently the latter changes occur in concert with partial crystallization of sarco(endo)plasmic reticulum Ca2+ ATPases (SERCA) on its surface, as suggested by freeze-fracture (FF) evidence indicative of an inactive ATPase. Finally, multiple small TAs associate to form fewer mature aggregates of very large size. Interestingly, in fibers from CASQ1-knockout mice the initial swelling of the SR does not occur, possibly due to lack of CASQ accumulation, and aggregates of SR tubules remain small/wavy and never develop into ordered aggregates of straight cylinders. Based on these results, we propose the hypothesis that TAs do not participate actively in the fibers' Ca2+ homeostasis, but may simply act as deposit sites for accumulated proteins.