Membrane Topology Analysis of the ON Bipolar Cell Transduction Channel Subunit TRPM1

Agosto MA1, Wensel TG1
1. Department of Biochemistry and Molecular Biology, Baylor College of Medicine

Corresponding author: Theodore G. Wensel, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston TX, E-mail: twensel@bcm.edu

Purpose: Neurotransmission between photoreceptors and downstream ON bipolar cells is mediated by a GPCR signaling cascade, for which the TRPM1 channel subunit is required for function. A high-resolution structure of TRPM1 has not yet been determined. Limited homology between the transmembrane (TM) domain of TRPM1 and those of other TRP family channels suggests that TRPM1 has six TM helices. However, no experimental data regarding the topology of the protein has been reported, and different TM prediction algorithms yield varying results. The purpose of this study is to experimentally determine the topology and orientation of TRPM1 in the membrane.

Methods: A fluorescence protease protection assay, in conjunction with live cell imaging, was employed to assess the membrane topology of TRPM1 and nyctalopin. Human embryonic kidney (HEK293) cells expressing GFP fusion proteins were treated with digitonin to selectively permeabilize the plasma membrane, followed by proteinase K to digest regions accessible to the cytoplasm.

Results: Mouse nyctalopin (NYX), which is known to have an extracellular or ER-luminal N-terminus and a cytoplasmic C-terminus, was used as a control. As expected, the fluorescence of cells expressing NYX-GFP decreased rapidly upon addition of protease. The fluorescence after 4 min of treatment was greatly reduced compared to control cells treated with digitonin but not protease (p<0.001, Mann-Whitney test). In contrast, GFP-NYX was resistant; cells with and without protease treatment were not significantly different. Both GFP-TRPM1 and TRPM1-GFP were localized to intracellular membranes in HEK293 cells. Unlike nyctalopin, the fluorescence of cells expressing TRPM1 with GFP at either the N- or the C-terminus was sensitive to protease treatment (p<0.001 after 4 min).

Conclusions: The fluorescence protease protection assay revealed that both the N- and C-termini of TRPM1 are cytoplasmic, consistent with the presence of six TM helices. Future studies will further dissect the topology of TRPM1 and the location of the TM helices by testing the accessibility of epitope tags and protease cleavage sites engineered into predicted luminal and cytoplasmic loops.
Membrane Topology Analysis of the ON Bipolar Cell Transduction Channel Subunit TRPM1

Araya MK1, Brownell WE2
1. Department of Molecular physiology and Biophysics, Baylor College of Medicine
2. Department of Otolaryngology, Baylor College of Medicine

Corresponding author: Mussie Araya, Department of Molecular physiology and Biophysics, Baylor College of Medicine, Baylor One Plaza, Houstun, TX, E-mail: araya@bcm.edu

Fast neuronal computations, such as coincidence detection, grouping by synchrony and spike-timing-dependent plasticity require neuronal populations to encode rapidly varying stimuli and respond quickly. Theoretical analyses have linked these abilities to the fast-onset dynamics of action potentials (APs). While Hodgkin Huxley theory fails to explain the speed of AP onset, a computational analysis invoking cooperative activation of Na+ ion channels at the axon initial segment (AIS) does. The near simultaneous gating of ion channels results in a hyperpolarized shift in the population activation curve producing a rapid AP initiation. The biophysical basis for intra-channel coupling is unknown and Ca++ or GTP based signaling is too slow. Membrane tethers can generate electromechanical force at frequencies up to 10 kHz. Voltage gated Ion channels are known to be sensitive to various kinds of stimuli including membrane mechanics. Here, we analyze the influence of passive and electrically evoked membrane tension on the dynamics of voltage-gated ion channels of excitable membranes. We propose an electromechanical mechanism for cooperative gating of sodium channels. Specifically, the rapid modulation of membrane tension by membrane potential can provide a fast and direct mechanism for inter-channel coupling. We combined optical tweezers and voltage clamp apparatus to pull membrane tethers from the cells in order to make precise measurement of membrane electromechanical force generation. We find stronger strength of membrane-cytoskeleton adhesion in the AIS suggesting ion channels are clustered by firmly anchoring to the actin based cytoskeleton at AIS. Since cooperative activation is highly dependent on the density of Na+ channels, the clustering of Na+ channels at the AIS can set the stage for cooperative gating between ion channels. Recent experiments also show disruption of cytoskeletal scaffold protein causes deregulation of sodium channel function which impairs network excitability. Concomitant variation between electromechanical force and channel function to determine the membrane’s role in ion channel gating will be discussed.
Determining Membrane Protein-Lipid Binding Thermodynamics Using Native Mass Spectrometry

Cong X\(^1\), Liu Y\(^1\), Liu W\(^1\), Liang X\(^1\), Russell DH\(^2\), Laganowsky A\(^{1,2,3}\)

1. Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Health Science Center
2. Department of Chemistry, Texas A&M University
3. Department of Microbial Pathogenesis & Immunology, College of Medicine, Texas A&M Health Science Center

Corresponding author: Arthur Laganowsky, Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, Texas, E-mail: alaganowsky@ibt.tamhsc.edu

Membrane proteins represent one of the most important targets for drug discovery. They are embedded in diverse biological membrane environments where lipids can modulate their structure and function. The molecular recognition of membrane proteins and the interactions between these proteins and lipids are governed by binding thermodynamics – the free energy, enthalpy and entropy that drive a binding reaction. However, experimental limitations have left the thermodynamics of protein-lipid interactions in biological membranes poorly understood.

Here, we report a method using native mass spectrometry (MS), to determine thermodynamics of individual lipid binding events to integral membrane proteins. Unlike conventional biophysical methods, native MS can resolve and interrogate individual ligand binding events. Coupled with an apparatus to alter and control the temperature, this technique can be further applied in determining binding thermodynamic parameters. We first validated our approach using several soluble protein-ligand systems and similar results were obtained compared with those using isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR). Then we determined for the first time the thermodynamics of individual lipid binding to the ammonia channel (AmtB), an integral membrane protein from Escherichia coli. Remarkably, we observed distinct thermodynamic signatures for the binding of different lipids and entropy-enthalpy compensation for binding lipids of varying chain length. Additionally, using a mutant form of AmtB that abolishes a specific phosphatidylglycerol (PG) binding site, distinct changes were observed in the thermodynamic signatures for binding PG, offering the possibility of mapping key residues involved in specific lipid binding.

Taken together, our native MS-based approach provides invaluable insight into individual lipid binding events to membrane proteins that would otherwise remain intractable by means of other biophysical approaches.

This work was supported by new faculty startup funds from the Texas A&M Health Science Center.
Control of TRPV Upper Gate by Intra-subunit and Inter-subunit Pore-loop-helix Interactions

Dosey T1,2, Wang Z1, Fan G3, Serysheva I1, Chiu W2, Wensel T1
1. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston Texas
2. Integrative Molecular and Biomedical Sciences Program, Baylor College of Medicine, Houston, Texas
3. Department of Biochemistry and Molecular Biology, University of Texas Health Science Center, Houston, Texas

Corresponding Author: Theodore Wensel, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston Texas, twensel@bcm.edu

Transient Receptor Potential (TRP) channels are a family of non-selective cation channels conserved throughout eukaryotes and they are involved in diverse physiological processes including vision, nociception, and insulin signaling. However, our understanding of how TRP channels function has been impeded by the lack of structural information. Therefore, we have focused our research on determining the structure of TRPV2 in distinct conformational states by cryoEM and we have currently achieved a sub-nanometer resolution density map for the open-state. The analysis of this structure as well as the TRPV structures produced by other groups, has suggested that the pore-turret and pore-loop domains control the upper gate. This control appears to be exerted through the simultaneous disruption of intra-subunit interactions by the pore-turret and the stabilization of inter-subunit interactions between the pore-loop and pore-helix domains. Furthermore, mutagenesis and functional analysis is consistent with this model for TRPV upper-gating.

This research was funded by the Welch Foundation and a training fellowship from the Keck Center of the Gulf Coast Consortia, of the Houston Area Molecular Biophysics Program, National Institute of General Medical Sciences (NIGMS), T32GM008280 and by the National Center of Macromolecular Imaging, P41-RR103832.
Guillaume Duret

Engineering the Mechanosensitive Protein Piezo1 for Magnetic Stimulation

Duret G\textsuperscript{1}, Anderson E\textsuperscript{2}, Polali S\textsuperscript{1,3}, Sebesta C\textsuperscript{2}, Robinson J\textsuperscript{1,2,4}
1. Department of Electrical and Computer Engineering, Rice University, Houston, TX
2. Department of Bioengineering, Rice University, Houston, TX
3. Department of Applied Physics, Rice University, Houston, TX
4. Department of Neuroscience, Baylor College of Medicine, Houston, TX

Corresponding author: Guillaume Duret, Electrical and Computer Engineering, 6100 Main Street, Houston, TX 77005-1827, USA - gduret@rice.edu

The magnetic activation of a membrane protein would allow for remote control of neurons and ultimately the stimulation of deep regions of the brain. The implications of such technology span from to behavioral studies on freely moving animals to non-invasive forms of treatment for neurological disorders. Using the advent of small paramagnetic nanoparticles (diameters <10nm) and recent advances in small peptide-sequence recognition proteins (SpyCatcher, nanobodies), we propose to develop a novel gating mechanism responsive to magnetic fields.

We decided to use the mechanosensitive channel Piezo1 as the foundation. This 880kDa trimer spans the membrane 26 to 40 times and seems to only respond to mechanical stress. Our primary goal is to modify Piezo1 to permit binding of artificial super-paramagnetic iron oxide nanoparticles (SPIONs) on relevant sites. Because the domains susceptible to respond to localized mechanical stimuli are unknown, our secondary goal is to understand the roles played by the different regions of Piezo1 on mechano-sensation but also oligomerization and channel formation. We are engineering Piezo1 constructs bearing binding sites in predicted extracellular domains, and are testing the impact of these mutations on Piezo1 activity, oligomerization, localization as well as their ability to bind functionalized magnetic nanoparticles.

In order to quicken the screening process, we have developed a microfluidic device that monitors the Piezo-dependent calcium influx upon mechanical stress on isolated cells in suspension. This stress can be modulated with alterations to pressure, viscosity, and shape of microfluidic channels. Upon selection, the promising candidates are further investigated. Their oligomerization is assessed by Western blot, the binding of SPIONs and the localization are monitored by confocal microscopy and flow cytometry. Finally, the functioning modified Piezo1 that are accessible to nanoparticle are tested for response to magnetic stimulation, using different size SPIONs functionalized with anti-Myc antibodies or streptavidin. A linear magnetic field up to 230 mTesla is generated by an electromagnet integrated to our patch-clamp setup.

We were able to successfully attach magnetic nanoparticle to extracellular domains of Piezo1 and are now testing the impact of the magnetic stimulation on various domains. We have also identified mutations that impact the gating mechanism without impairing trafficking. These findings contribute to a more complete comprehension of the molecular mechanism at play in Piezo1, and bring us closer to the successful construction of a magnetosensitive nanoparticle-protein chimera.
Structure and Function of the Orsay Virus δ Protein

Fan Y1, Guo Y1, Yuan W1, Zhong W1, Tao YJ1
1. Department of BioSciences, Rice University, Houston, Texas, 77005

Corresponding author: Yizhi Jane Tao, Department of BioSciences, Rice University, 6100 Main Street, Houston, Texas, Email: ytao@rice.edu

Caenorhabditis elegans (C. elegans) is an important model organism for addressing fundamental questions in multiple biological fields, including immunology, neurobiology, genetics, developmental biology, and physiology. About four years ago, the first virus known to naturally infect C. elegans was discovered in a wild isolate called JU1580 [1]. Orsay virus (OV), which mainly infects the worm intestine [2], is a positive-sense RNA virus with a bipartite genome. The RNA1 segment encodes the RNA-dependent RNA polymerase (RdRP), while the RNA2 segment encodes the capsid protein (CP) and another protein called δ of unknown function [1]. Additionally, OV can utilize a ribosomal frame-shifting strategy to express a novel capsid-δ fusion protein [3]. To determine the biological function of δ, we carried out a series of structural studies. Our electron microscopy images show that recombinant δ is a filamentous molecule. Furthermore, we have determined the crystal structure of δ(1~66), a δ protein construct containing the first 66 amino acids, to 2.2 Å resolution. δ(1~66) assembles into pentamers with five subunits forming a five-helical bundle. Each δ(1~66) molecule is composed of two α-helices that are linked by a 9-aa loop. Surface representation shows a ~3-5 Å wide channel running through the molecule. In the electron density map, blobs of densities that can be modeled as waters or ions occupy this channel. The symmetry and the shape of the channel bear resemblance to a class of pentameric viroporins. Potential membrane related functions of δ during Orsay infection will also be discussed in light of our structural data.

This work was supported by the Welch Foundation (C-1565 to Y.J.T.) and the National Institutes of Health (NIH).

References:
Coevolution of Residues in the Htr2a and Htr2c Serotonin Receptors Suggests That They Act as a Functional Heterodimer that may be Relevant in Forming Substance Addictions

Fongang B, Zhu M, Anastasio NC, McAllister C, Kudlicki A
Biochemistry and Molecular Biology, University of Texas Medical branch, Galveston, Texas

Serotonin (5-HT) is a monoamine neurotransmitter which regulates activities such as sleep, appetite, substance addiction, and mood. The actions of 5-HT in neurons are transduced by at least 14 subtypes of 5-HT transmembrane receptors grouped into seven distinct classes largely on the basis of their structural and operational characteristics. Evidence and models suggest that some of the receptors may act as heterodimers. Specifically, it has been shown that selective blockade of the 5-HT2A or the activation of the 5-HT2C consistently reduces impulsivity and suppress both the cue- and cocaine-evoked reinstatement. Moreover, recent studies in rats have shown that these receptors always act synergistically to achieve these functions, suggesting a possible contact between their residues in 3D structure. Direct experimental verification of this hypothesis by means of co-crystallization is difficult as they are both transmembrane proteins and their 3D structures have not yet been determined. Here, we used the Direct Coupling Analysis (DCA) method to reveal the native contacts between the heterodimer Htr2a and Htr2c proteins. By studying the co-evolution of residues in the two proteins over more than a hundred species, we identified putative residue contacts and showed that direct contacts between the two proteins are likely limited to extracellular domains. Our results improve the knowledge about serotonin receptors in general and may help understand how these receptors act synergistically to reduce cocaine dependence. Experimentally, the contact sites identified by DCA will be the targets of mutation studies that will confirm the significance of the HTR2a/HTR2C interactions.
Bipolar Cell Degeneration in Pik3c3/Vps34 Conditional Knockout Mice

He F1, R. Agosto MA1, Nichols2, Wensel TG1,2

1. Department of Biochemistry, Baylor College of Medicine, Houston, Texas
2. Department of Ophthalmology, Baylor College of Medicine, Houston, Texas

Corresponding author: Theodore G. Wensel, Department of Biochemistry, Baylor College of Medicine, Houston, Texas. twensel@bcm.edu

Purpose: The type III phosphoinositide 3-kinase (Pik3c3/Vps34) participates in various cellular functions, including intracellular trafficking and cell survival. Our previous studies showed aggressive rod degeneration in rod-specific Vps34 conditional knockout mice due to impairment of autophagy and endosomal pathways. In this study, we investigated Vps34 function in bipolar cells, which are the downstream neurons of the visual phototransduction pathway, using bipolar-specific Vps34 conditional knockout mice.

Methods: Electroporation of plasmid DNA directing expression of DsRed fused to a phosphoinositide binding domain (DsRed-2xHrs) was used to localize PI(3)P in bipolar cells. A mouse line with a conditional functional deletion of Vps34 in bipolar cells was generated by crossing Vps34 floxed mice with a transgenic mouse line that expresses Cre recombinase in bipolar cells using the Purkinje cell protein-2 (Pcp2) promoter. Structural changes in the retina were determined by immunofluorescence and electron microscopy.

Results: PI(3)P was localized to discrete puncta of various sizes in bipolar cells. Loss of Vps34 function in bipolar cells caused significant degeneration of the inner retina. The number of rod ON-bipolar cells and cone type 2-OFF and 6-ON bipolar cells, determined by immunostaining with PCP2 and PKCa antibodies, was significantly reduced in Vps34 knockout mice at 3-4 months, while there were no apparent changes in cone type 3-OFF or 5-ON bipolar cells, horizontal cells, or amacrine cells. The thickness of the inner nuclear and inner plexiform layers was remarkably reduced. No significant degeneration was observed in the photoreceptor cell or ganglion cell layers. Transmission electron microscopy showed an accumulation of vesicles in bipolar cell bodies and a lack of invaginating rod bipolar dendrites in synaptic triads of the outer plexiform layer. Autophagy markers LC3 and p62, as well as ubiquitininated proteins, accumulated and co-localized in rod ON-bipolar cells in Vps34 KO mice. LAMP1 also accumulated, but did not co-localize with the p62/LC3 puncta, indicating abnormal autophagy in bipolar cells in the absence of Vps34.

Conclusions: Vps34 is essential for rod ON bipolar cell survival. The mechanisms of degeneration in the absence of Vps34 may involve disruption of autophagy and/or related pathways.

Acknowledgements: This work was supported by NIH grants R01-EY07981, R01-EY025218, P30-EY002520, the Knights Templar Eye Foundation, and the Welch Foundation.
Near-infrared Photoactivatable Control of Ca2+ Signaling and Optogenetic Immunomodulation

He L1, Zhang Y2, Ma G1, Tan P1, Zang S1, Jing J1, Fang S1, Zhou L3, Wang Y3, Huang Y1, Hogan P4, Gang Han2, #, Zhou Y1, 5, #

1. Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, TX 77030
2. Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA, 01605
3. Beijing Key Laboratory of Gene Resource and Molecular Development, College of Life Sciences, Beijing Normal University, Beijing 100875
4. Division of Signaling and Gene Expression, La Jolla Institute for Allergy and Immunology, La Jolla, CA, 92037
5. Department of Medical Physiology, College of Medicine, Texas A&M University Health Science Center, Temple, Texas 76504

Corresponding authors:
Gang Han, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA, 01605, USA; Email: gang.han@umassmed.edu
Yubin Zhou, Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University Health Science Center, 2121 W Holcombe Blvd, Houston, TX 77030, USA; E-mail: yzhou@ibt.tamhsc.edu

Abstract:
The application of current channelrhodopsin-based optogenetic tools is limited by the lack of strict ion selectivity and the inability to extend the spectra sensitivity into the near-infrared (NIR) tissue transmissible range. Here we present an NIR-stimulable optogenetic platform (termed “Opto-CRAC”) that selectively and remotely controls Ca2+ oscillations and Ca2+-responsive gene expression to regulate the function of non-excitable cells, including T lymphocytes, macrophages and dendritic cells. When coupled to upconversion nanoparticles, the optogenetic operation window is shifted from the visible range to NIR wavelengths to enable wireless photoactivation of Ca2+-dependent signaling and optogenetic modulation of immunoinflammatory responses. In a mouse model of melanoma by using ovalbumin as surrogate tumor antigen, Opto-CRAC has been shown to act as a genetically-encoded “photoactivatable adjuvant” to improve antigen-specific immune responses to specifically destruct tumor cells. Our study represents a solid step forward towards the goal of achieving remote control of Ca2+-modulated activities with tailored function.
Cortical spreading depression (CSD) is a pathological phenomenon associated with traumatic brain injury (TBI), stroke, migraines, and seizures. Typically, following TBIs and other insults, neuronal excitability in and around the area of the injury is affected, with reported increases in local glutamate signaling. Astrocytic glutamate transporters are critical for precise regulation of the extracellular glutamate availability. However, it remains unclear how impaired astrocytic glutamate transport or an acute TBI affect characteristics of the CSD. We quantified the properties of CSD using whole-cell and extracellular electrophysiological recordings, and voltage-sensitive dye imaging (VSDI) in rat visual cortex in vitro. To model impaired astrocytic glutamate transport, we used astrocytic glutamate transporter blocker \((2S, 3S)-3-[3-[4-(trifluoromethyl) benzoylamino] benzyloxy] aspartate\) (TFB/TBOA). In addition, effects of the acute cut through the superficial cortical layers were used to determine effects of acute traumatic brain injury (TBI) on CSD characteristics. Both manipulations; impaired glutamate cycling and acute injury profoundly affected physiological properties of cell firing, latency to CSD formation, and its frequency of occurrence. VSDI revealed significant changes in spatiotemporal dynamics and propagation of the CSD, suggesting that that the cut itself may not initiate CSD depolarizing waves, but rather attract them. Blockade of GLT1 caused significant changes in CSD wave as well, slowing it or even ‘trapping’ its propagation. Our results reveal new information about CSD properties in these pathological conditions and demonstrate an important role of GLT1 in regulation of CSD.
Cannabidiolic Acid Controls Seizure-Like Activity And Neuronal Excitability

Hosseini Zare M1, Abdulla A1, Akulla K1, Ziburkus J1

1. Department of Biology and Biochemistry, University of Houston

Corresponding Author: Jokubas Ziburkus, Department of Biology and Biochemistry, University of Houston, 4800 Calhoun RD, Houston, Texas, jziurkus@uh.edu

Intoxicating tetrahydrocannabinol (THC) and non-intoxicating cannabidiol (CBD) are the two most prevalent phytocannabinoids found in cannabis sativa plants. CBD is well studied in multiple disease models, especially as anti-convulsant in treatment of intractable forms of epilepsy. However, in many strains of cannabis, over 90% of phytocannabinoids are synthetized as non-intoxicating acidic cannabinoids. ‘Raw hemp oil’ extracts prepared using cold temperatures are often dominated by acidic cannabinoids, such as cannabidiolic acid (CBDA), which is the precursor of CBD. Recent evidence suggests that CBDA is an effective anti-emetic (acting through serotonin receptors), anti-inflammatory (Cox-2 inhibition), and it abrogates cancer cell growth. However, it is unknown what role CBDA plays in the central nervous system (CNS) and there is an urgent need to understand the functions of the acidic cannabinoids. Using single cell and network electrophysiological recordings, we compared effects of synthetic CBD (30μM) and CBDA (25μM) on seizure-like activity and neuronal excitability in the hippocampal slices. Both, CBD and CBDA reduced zero magnesium-induced seizure-like activity by reducing action potential firing in the excitatory pyramidal cells. CBDA actions were typically as effective, but delayed by 10-15 minutes, compared to CBD. Only CBDA significantly increased afterhyperpolarizations following the action potentials. In summary, our studies reveal new knowledge about the mechanisms of actions of CBDA in the brain. Further studies of CBDA and other acidic cannabinoids are needed to determine their effectiveness and therapeutic potential in vivo.

Acknowledgment: We thank Professor Raphael Mechoulam for the communications, encouragement, and suggestions about the acidic cannabinoids.
Brain ischemia causes damage to neurons due to reduced oxygen and glucose supply, i.e. oxygen and glucose deprivation (OGD). Cell death after cerebral ischemia may result from elevation in intracellular calcium ($Ca^{2+}$), loss of cell ion homeostasis and glutamate-induced excitotoxicity, which can all result from activation of non-selective cation channels, such as those formed by the Transient Receptor Potential (TRP) proteins. During ischemia/reperfusion to the brain, $Ca^{2+}$ influx to neurons may be induced by the elevation of extracellular glutamate concentration, which elicits several neurotoxic events through activation of glutamate receptors. Among them, the metabotropic glutamate receptors are known to couple to TRPC channel activation, leading to membrane depolarization and sustained $Ca^{2+}$ influx. To determine the contribution of TRPC channels in glutamate-induced $Ca^{2+}$ overload, we measured intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) changes in response to glutamate stimulation in cultured mouse cortical neurons. Glutamate induced a robust increase in $[Ca^{2+}]_i$, which was inhibited by ~50% with the TRPC4/C5 blocker, ML204. In neurons prepared from TRPC4 knockout mice, the glutamate-induced $[Ca^{2+}]_i$ increase was also significantly less than that from the wild type (WT) controls. These results indicate the likely involvement of TRPC4 channels in glutamate-induced $[Ca^{2+}]_i$ rise, a condition commonly associated with OGD. To test if the TRPC channel activity is linked to ischemic neuronal cell death, we used both an in vivo stroke model and an in vitro OGD model. Transient middle cerebral artery (MCA)/common carotid artery (CCA) occlusion resulted in large infarct areas in WT mice. The infarct areas were significantly smaller in the brains of TRPC4−/− mice. In cortical neuron cultures prepared from TRPC1−/−, TRPC4−/− and TRPC1/C4/C5/C6−/− mice, OGD-induced neuronal cell death was significantly less than that from WT mice. In addition, the treatment with ML204 reduced OGD-induced death of WT neurons, showing protection to ischemic damage. These data suggest that TRPC channels play a significant role in neuronal death induced by OGD. Our study reveals that TRPC channels significantly contribute to ischemic cell death through calcium overload and that inhibition of TRPCs may be neuroprotective against brain damage following ischemic stroke.

This work was supported by the NIH [R01 NS092377 and R01 GM092759], the American Heart Association Southwest Affiliate Postdoctoral Fellowship [15POST22630008].
ATM Functions at the Peroxisome to Induce Pexophagy in Response to ROS

Zhang J1,8, Tripathi DN1,8, Jing J1,8, Alexander A2, Kim J3, Powell RT1, Dere R1, Tait-Mulder J4, Lee J.-H5, Paull T5, Pandita RK6, Charaka VK6, Pandita TK6, Kastan MB4,7, Walker CL1

1. Center for Translational Cancer Research, Institute for Biosciences and Technology, Texas A&M University Health Science Center, Houston, TX 77030
2. Department of Experimental Radiation Oncology, UT MD Anderson Cancer Center, Houston, TX 77030
3. Korea Institute of Oriental Medicine, Dajeon, 305-811, South Korea
4. Departments of Oncology, St. Jude Children’s Research Hospital, Memphis, TN 38105
5. Department of Molecular Genetics and Microbiology, University of Texas, Austin, Tx 78712
6. Department of Radiation Oncology, Houston Methodist Hospital, Houston, TX 77030
7. Pharmacology and Cancer Biology, Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710

These authors contributed equally to this work.

Correspondence should be addressed to C.L.W. (cwalker@ibt.tamhsc.edu)

Peroxisomes are highly metabolic, autonomously replicating organelles that generate ROS as a by-product of fatty acid β-oxidation. Consequently, cells must maintain peroxisome homeostasis, or risk pathologies associated with too few peroxisomes, such as peroxisome biogenesis disorders, or too many peroxisomes, inducing oxidative damage and promoting diseases such as cancer. We report that the PEX5 peroxisome import receptor binds ataxia-telangiectasia mutant (ATM) and localizes this kinase to the peroxisome. In response to reactive oxygen species (ROS), ATM signaling activates ULK1 and inhibits mTORC1 to induce autophagy. Specificity for autophagy of peroxisomes (pexophagy) is provided by ATM phosphorylation of PEX5 at Ser141, which promotes PEX5 mono-ubiquitination at Lys 209, and recognition of ubiquitylated PEX5 by the autophagy adapter protein p62, directing the autophagosome to peroxisomes to induce pexophagy. These data reveal an important new role for ATM in metabolism as a sensor of ROS that regulates pexophagy.
Structural Determinants of Raft Partitioning and Plasma Membrane Localization for Single-Pass Transmembrane Proteins

Lorent JH, Diaz-Rohrer BB, Spring K, Levental KR, Levental I

Department of Integrative Biology and Pharmacology, UT Health Science Center at Houston
Corresponding author: Joseph H. Lorent, Department of Integrative Biology and Pharmacology, UT Health Science Center at Houston, 6431 Fannin Street, 77030 Houston, Texas
joseph.h.lorent@uth.tmc.edu

Transmembrane proteins (TMPs) comprise ~30% of the mammalian proteome and mediate nearly all functions of the cell. The subcellular localization, trafficking, signalling, and enzymatic activity of many TMPs is regulated by their association with lipid-driven lateral membrane domains known as lipid rafts. However, the structural determinants of TMP partitioning to raft domains remain almost completely unknown. We hypothesized that structural features of the transmembrane domain (TMD) of single-pass TMPs would guide raft partitioning and raft-dependent sub-cellular trafficking. To explore TMD-dependent raft partitioning, we isolated plasma membranes as Giant Plasma Membrane Vesicles (GPMVs), a model system that allows direct observation of protein partitioning between raft/non-raft phases by fluorescence microscopy. We generated 106 mutants of a model TMP and quantified their partitioning as a function of TMD features, namely hydrophobic length, palmitoylation and accessible surface area. First, raft partitioning was positively related to TMD length and the number of palmitoylations, confirming previous predictions. Investigation of 74 mutants with constant TMD length and palmitoylation status revealed that raft partitioning was also inversely related to TMD accessible surface area, with smaller TMD surfaces preferring raft domains. These results are consistent with a simple physical model wherein raft partitioning is driven by differences in interfacial energy between the TMD and its surrounding lipid matrix in raft and non-raft phases. This model quantitatively predicts raft partitioning solely from amino acid sequence for all tested proteins and yields the first estimate of protein-lipid surface tension in the membrane. Applying the model to the human proteome, we successfully predicted trends in subcellular localization of single-pass TMPs, with plasma membrane proteins generally having higher raft affinity. These results define the general rules for raft partitioning of TMPs and underline the central role of membrane domains in cellular traffic.

We acknowledge the Cancer Prevention and Research Institute of Texas (grant no. R1215) and NIH / NIGMS (grant no. 1R01GM114282) for funding to support this project.
Inside-out Ca2+ Signaling Prompted by the Reorganization of STIM1 Transmembrane Domain

Ma G1, He L1, Wei M2, Liu C3, Jing J1, Tan P1, Wang J3, Wang Y2, Zhou Y1

1. Institute of Biosciences and Technology, Texas A&M University Health Science Center
2. Beijing Key Laboratory of Gene Resource and Molecular Development, Beijing Normal University
3. High Magnetic Field Laboratory, Hefei Institutes of Physical Science, Chinese Academy of Sciences

Corresponding author: Dr. Yubin Zhou, Institute of Biosciences and Technology, Texas A&M University Health Science Center. 2121 W Holcombe Blvd Suite 928, Houston, TX 77030. E-mail: yzhou@ibt.tamhsc.edu

Store-operated Ca2+ entry mediated by STIM1 and ORAI1 constitutes a major Ca2+ entry route in cells of the immune and hematopoietic systems. Aberrant STIM1-ORAI1 signaling is intimately involved in the pathogenesis of immunodeficiency, cardiac hypertrophy and cancer metastasis. The molecular choreography of STIM1-ORAI1 coupling is initiated by endoplasmic reticulum (ER) Ca2+ store depletion with subsequent dimerization/oligomerization of the STIM1 ER-luminal domain, followed by its redistribution toward the plasma membrane (PM) to gate ORAI1 Ca2+ channels. The mechanistic underpinnings of this inside-out Ca2+ signaling, particularly how ER-luminal signals are transmitted toward the cytoplasmic domain (STIM1-CT) and how STIM1-CT escapes inhibitory intramolecular interactions, were largely undefined. By taking advantage of a unique gain-of-function mutation within the STIM1 transmembrane domain (STIM1-TM), here we show that local rearrangement, rather than alteration in the oligomeric state of STIM1-TM, is required to prompt conformational changes in the cytosolic juxtamembrane coiled coil (CC1) region. Importantly, we further unveiled the long-sought contact interface between two critical regions within STIM1-CT that entail autoinhibition. Our findings provide the most compelling evidence to support a conformational switch model in which STIM1-TM reorganization switches STIM1-CT into an extended conformation, thereby unleashing and projecting the ORAI-activating domain toward the ORAI1 Ca2+ channels.
Surface Enhanced Spectroscopy for Lipid Membrane Structure

Matthews JR\textsuperscript{1}, Payne CM\textsuperscript{2}, Hafner JH\textsuperscript{1,2}

\textsuperscript{1} Department of Physics and Astronomy, Rice University, Houston, TX
\textsuperscript{2} Department of Chemistry, Rice University, Houston, TX

Matthews JR, Dept. of Physics and Astronomy, Rice University, 6100 Main Ms-61, Houston Texas, 77005, E-mail: jrm18@rice.edu

Membrane structure is inherently complex, and membrane protein dysfunction is the primary cause of numerous diseases. Understanding the structure of peptide chains inserted in membranes is therefore vitally important for the development of effective diagnosis and treatments. We present progress toward applying Surface Enhanced Raman Spectroscopy (SERS) as a tool for studying lipid membrane structure.

Optical excitation of gold nanoparticles at their size and shape-dependent plasmon resonant frequency induces strong oscillations of the nanoparticle’s free electron gas, leading to bright scattering and large surface fields. These effects lead to SERS – an enhancement of Raman scattering signals in a distance-dependent manner at the molecular scale. This project utilizes gold nanorods, tuned to an excitation laser wavelength of 785 nm, as a substrate for lipid membranes so that their structure can be analyzed by SERS. The immediate scientific objective is to displace the surfactant cetyltrimethylammonium bromide (CTAB) that stabilizes nanorods with the biologically relevant lipid dioleoylphosphatidylcholine (DOPC), and to confirm the displacement by SERS. Gold nanorods were incubated in solutions of DOPC much higher than CTAB to cause direct molecular substitution. Light sonication was used to speed the process. SERS spectra clearly indicate a complete substitution based on the 760 cm\textsuperscript{-1} peak of the CTAB headgroup and the 719 cm\textsuperscript{-1} peak of the DOPC headgroup. The continued presence of a peak at 180cm\textsuperscript{-1} indicates that the bromide ions remain bound to the nanorod surface, serving as the intermediary between the chemically similar DOPC choline group and the CTAB trimethylammonium group. By using SERS, we can conclude that a lipid layer has formed on the nanorod surface with an undetectable amount of remaining surfactant. The nanorod supported lipid layer was shown to have a bilayer configuration through detection of the lipid phase transition at the appropriate transition temperature. Additionally, kinetic exchange of lipid molecules with deuteration lipid variants in solution show that these membranes are loosely bound. Finally, at low lipid concentrations the membranes were shown to collapse from the bilayer state similar to what is seen for surfactants.

Tryptophan was introduced to the nanorod supported lipid membranes and the resulting SERS spectra presents Raman modes specific to the amino acid indicating Tryptophan is interacting with the membrane. DFT simulations are currently underway to replicate the spectral signature from SERS and will provide the necessary information to deduce orientations of molecules in the lipid membranes.

The authors acknowledge support from the Robert A. Welch Foundation under Grant C-1761.
Selective Lipid Sorting Regulates Rac1 Function

Maxwell K\textsuperscript{1,2}, Zhou Y\textsuperscript{1}, Hancock JF\textsuperscript{1,2}

1. Department of Integrative Biology and Pharmacology, McGovern Medical School
2. Graduate School of Biomedical Sciences, Houston, TX

Corresponding author: Kelsey Maxwell, Department of Integrative Biology and Pharmacology, McGovern Medical School, 6431 Fannin St. Houston, TX 77030

Email: kelsey.maxwell@uth.tmc.edu

The small GTPases Ras and Rac1 have been implicated in cancer development and metastasis, and many questions remain regarding their signaling on the plasma membrane (PM). Lipid-anchored Ras GTPases localize to the PM and form spatially distinct nanoclusters that are necessary for downstream signaling. These nanoclusters are segregated not only by isoform-type but also by GDP/GTP loading. Ras nanoclusters have distinct lipid compositions. For example, phosphatidylserine is an essential structural and signaling component of K-Ras but not H-Ras nanoclusters. Recruitment of specific lipids to form compositionally distinct nanoclusters may allow nanoclusters to deliver isoform-specific signal output. Spatial segregation and differential lipid composition of Ras nanoclusters on the PM are mainly determined by differences in the Ras hypervariable regions (HVR) and membrane anchors. The minimal Ras membrane anchors comprise a C-terminal farnesyl-cysteine-methyl-ester, plus dual palmitoyl lipid chains in H-Ras, a single palmitoyl in N-Ras and a polybasic domain in K-Ras. Likewise, Rac1 also functions on the PM and is prenylated, with geranylgeranyl at the C-terminal cysteine residue of the CAAX motif and contains both polybasic sequence and a single palmitoyl in its HVR. I hypothesize that like Ras, Rac1 forms nanoclusters that comprise a distinct lipid composition and act as signaling sites for effector recruitment. The lipid composition of these clusters may contribute to Rac1’s involvement in cell migration and cancer metastasis. Using high-resolution spatial mapping, we show that Rac1 forms nanoclusters on the PM which have distinct lipid compositions from previously studied Ras isoforms. Rac1 nanoclusters incorporate phosphatidic acid (PA), and can be disrupted by inhibiting kinases responsible for PA synthesis. Furthermore, we show that mutations of individual residues in the polybasic or palmitoyl regions of Rac1 affect PM targeting and clustering. The data may ultimately lead to novel and innovative strategies for targeting Rac-related cancers.

This work is funded by M.D. Anderson’s Rosalie B. Hite Graduate Fellowship in Cancer Research.
Novel Adenosine Therapy in Dravet Syndrome

Montier L1, Gu F1, Riffe C1, Megjhani M2, Kulkarni P2, Leasure JL1,3, Roysam B2, Žiburkus J1,4

1. Department of Biology and Biochemistry, University of Houston
2. Department of Electrical and Chemical Engineering, University of Houston
3. Department of Psychology, University of Houston
4. Texas Institute for Measurement, Evaluation, and Statistics (TIMES), University of Houston

Corresponding author: Laura Montier, Department of Biology and Biochemistry, University of Houston, 4800 Calhoun Rd, Houston, TX 77004, LLMontier@uh.edu

Dravet syndrome (DS) is among the most severe and pharmacoresistant forms of childhood epilepsies. The first symptoms of the disease usually manifest at about 6 months of age with the onset of febrile seizures. Seizure incidence often increases in both frequency and severity, and progress into non-febrile seizures by early childhood. In addition to having a high mortality rate of about 20%, Dravet patients suffer from behavioral comorbidities. Social and cognitive dysfunctions have been identified in up to 95% of cases. Using a transgenic DS mouse model with a knock-in mutation in the SCN1A gene that encodes the voltage-gated sodium channel Na\textsubscript{v}1.1, we tested a novel drug strategy. We performed sub-chronic adenosine A1 receptor agonist treatment during early postnatal development and tested effects of this treatment on the long-term survival rate, cognitive and behavioral comorbidities, and astroglial network structure in the hippocampus. Previous work in the laboratory showed that this treatment was effective at reducing hyperthermia-induced seizures in vivo. In our current studies, we discovered that SCN1A mutation increases astrogliosis and alters astrocyte morphology (Farsight 3D image analysis, UH). The prolonged treatment with A1R agonist CPA increased viability rates from 33% to 82, improved sociability, improved hippocampal-based memory in the DS mice. Preliminary data analysis also indicates that the A1R treatment prevents astrogliosis and glial morphological dysfunctions in the SCN1A mouse model of DS. Taken together, our studies show that early postnatal treatment with A1R agonist CPA is beneficial for survival, comorbidity outcomes, and has the ability to restructure neuronal networks in the hippocampus. Further translational studies will determine if A1R agonist could become a viable, adjunct therapy option for patients with DS.

This research is supported by Dravet Syndrome Foundation (J.Ž.), University of Houston SURF and PURS (C.R.), Houston Livestock Show and Rodeo (L.M.), and University of Houston Biology of Behavior Institute (L.M. and F.G.).
Structural Effects Of Phosphorylation On C-terminal Segment Of AMPA Receptor

Caitlin E Nurik, Sudeshna Chatterjee, Suma Priya Sudareshna Devi, David R Cooper, Christy Landes, James Howe, and Vasanthi Jayaraman.

1. Center for Membrane Biology, Department of Biochemistry and Molecular Biology, Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, Texas 77030
2. Department of Chemistry, Rice University, Houston, Texas 77251
3. Department of Pharmacology, Yale University, New Haven, CT 06520

Corresponding author: Dept. of Biochemistry and Molecular Biology, University of Texas Health Science Center, MSB 6.174, 6431 Fannin St., Houston, TX 77030. Tel.: 713-500-6236; Fax: 713-500-7444; E-mail: vasanthi.jayaraman@uth.tmc.edu

The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is one of the two non-NMDA-type ionotropic glutamate receptors. It is the primary contributor to fast excitatory transmission in neurons, which is key to learning and building memory. The AMPA receptor can be divided into four domains. Extracellularly, there is the amino terminal domain, and the more membrane-proximal ligand-binding domain, to which activators like glutamate bind and induce channel opening. The transmembrane domain serves as the actual ion-channel pore and also, of course, links the extracellular domains to the cytoplasmic domain. Of these four domains, the structure of the outermost three has been shown in detailed crystal structures. However, very little is known about the structure of the cytoplasmic domain. Although it is widely thought that this segment is highly disordered, it is unknown whether local order (levels of secondary and/or tertiary structure) may exist in the cytoplasmic terminus, or whether structure changes may occur as conformational shifts due to functional modifications. Previous studies have established protein kinase C (PKC) phosphorylation sites at residues S818, S831, and T840 in the GluA1 subtype receptor, and confirmed their effect to enhance the function of the receptor. These studies examined a representative membrane-proximal section of the GluA1 cytoplasmic terminus comprising residues 809-841 in order to consider local structural changes brought about by the phosphorylation events. The peptide was examined using Fourier transform infrared technology (FTIR) investigation, which showed a conversion to lesser helix content, more extension, in the phosphomimetic sample. Studies of the peptides in a solution of small unilamellar vesicles were conducted and showed that the change in secondary structure is enhanced in the context of close proximity to a charged lipid membrane. To confirm, single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated and phosphomimetic states, in order to gauge the distance between two sets of flor-labeled cysteines in the peptide. Phosphorylation yielded a narrower breadth of FRET efficiencies between labeled cysteines incorporating all three PKC-phosphorylated sites, indicative of more limited dynamics. Again this effect was changed with the charge of the lipid environment. Single-channel electrophysiological studies in progress reveal that the lipid charge environment is a regulator of the functional effects yielded by these phosphorylation events.

Funding provided by NIH RO1 GM094245-01A1 and Houston Area Molecular Biophysics Training Program NIH- 2T32 GM008280-26
Insights Into The Mechanism Of Plug Displacement Of The PapC Usher

Pham T¹, Farabella I², Henderson NS¹, Phan G², Werneburg G³, Geibel S², Topf M², Waksman G², Thanassi DG³, Delcour AH¹

¹. Department of Biology and Biochemistry, University of Houston, Houston, TX
². Institute of Structural and Molecular Biology, Birkbeck College, University College London, London
³. Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, NY

Corresponding author: Anne H. Delcour, Department of Biology and Biochemistry, University of Houston, 3455 Cullen Blvd. Suite #342, Houston, TX 77204, adelcour@uh.edu

Background: The PapC usher is a twin beta barrel pore of the outer membrane of uropathogenic E. coli used for the assembly of the P pilus, a key virulence factor in bacterial colonization of human kidney cells. The usher catalyzes the translocation and ordered addition of folded pilus subunits delivered by the chaperone to the growing pilus. The distal subunit, an adhesin, is the first one delivered to the usher, and confers adhesive properties to the pilus. Each PapC monomer is a 24-stranded β-barrel, flanked by N- and C-terminal globular domains and occluded by a large plug domain (PD). An α-helix and the β5-6 hairpin are additional structural components that may play a role in controlling channel dynamics. The assembly of the pilus proceeds through one of the usher β-barrels and thus requires displacement of the PD. Structural studies revealed that the PD is released to the periplasmic side during pilus biogenesis, but the exact mechanism for PD displacement has not yet been elucidated. Several key residues that may be critical for plug stabilization have been proposed. They cluster in regions at the interface of the plug, the barrel, the α-helix and the hairpin, and appear to belong to either electrostatic or allosteric networks.

Objectives: The goal of the study is to understand the molecular mechanism of release of the PD during pilus assembly through PapC. Two approaches have been used: 1) the study of mutant channels with either single or multiple alanine substitutions at key residues in the allosteric and electrostatic networks; and 2) the investigation of channel dynamics in the presence of a chaperone-adhesin complex, which is believed to trigger the release of the PD.

Methods: We used planar lipid bilayer and patch-clamp electrophysiology to compare the activity of the wildtype channel with that of allosteric and electrostatic network mutants, and to monitor the modulation of channel activity in the presence of the chaperone-adhesin complex. These electrophysiological techniques permit the investigation of the dynamics of a single protein in real-time. Plug displacement events are identified by the sudden opening of the dimeric channel and a rapid transition to the dimeric level of conductance.

Results: Many mutants showed an increased propensity at plug displacement, while others displayed quieter than WT activity. PD displacement is particularly sensitive to the disruption of repulsive interactions between two arginines located on the linker regions connecting the PD to the β-barrel wall. In addition, we have found that the presence on chaperone-adhesin complex facilitates the opening of the PapC pore and appears to engage within the PapC lumen.

Conclusions: We have identified several critical regions involved in the allosteric mechanism that leads to channel opening via plug release upon binding of the first chaperone-subunit complex to the PapC usher.

Funding sources: This work was supported by NIH grants R01GM062987 (to DGT) and F30AI112252 (to GTW), a PhD training grant from the Wellcome Trust (to IF) and funds from the Medical Research Council and the Biotechnology and Biological Sciences Research Council (to MT).
Levosimendan Enhances Memory via Antioxidant Effect

Rababa’h A ¹, Alzoubi K¹, McConnell BK²

¹. Department of Clinical Pharmacy; College of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
². Department of Pharmacological & Pharmaceutical Sciences; College of Pharmacy, University of Houston, Texas Medical Center, Houston TX 77204

Address for Correspondence:
Abeer M. Rababa’h Ph.D., Department of Clinical Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan, E-mail: amrababah@just.edu.jo, Phone: + 962797501326, Fax: + 96265815728.

Introduction: Learning and memory impairment has been associated with elevated the level of reactive oxygen species in the body. It has also been validated through the literature that antioxidants enhance learning and memory. Additionally, it has been shown that inhibition of phosphodiesterase enzyme activity will improve memory deficit. Levosimendan is a cardiac inotropic and vasodilator agent that had been introduced to have a pleotropic effect such as antioxidant, anti-inflammatory and smooth muscle vasodilatory effects. Furthermore, Levosimendan inhibits phosphodiesterase-III (PDE-III) enzyme. In this study, the effect of Levosimendan on learning and memory in rats was investigated.

Methods: The adult male Wistar rats were separated into two groups; control and Levosimendan groups. Levosimendan (12µg/kg) was administrated intraperitoneally once a week for 8 weeks. Radial arm water maze (RAWM) was used to assess spatial learning and memory. Additionally, brain derived neurotrophic factor (BDNF) level, thiobarbituric acid reactive substance (TBARS) level and levels of antioxidant defense biomarkers such as reduced glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ratio, glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) were assessed in the hippocampus. Results: Levosimendan significantly enhances short-term memory and long-term (5 hours) memory. Furthermore, we showed that Levosimendan treated group significantly increases GPx and GSH levels (p<0.05), and significantly decreases TBARS level (p<0.05). There were no significant changes observed between the two study groups in the level of other antioxidants and stress biomarkers such as: SOD, GSSG, GSH/GSSG ratio, catalase and BDNF (p>0.05). Conclusion: Levosimendan as antioxidant agent enhances short term memory and long term (5 hours) memory through potentiating antioxidant defense mechanism in hippocampus.

This study was supported by the deanship of research at Jordan University of Science and Technology, Jordan (grant number 20150056).
A Force Assay for Cell Motility

Rajasekharan V1, 2, Sreenivasan VKA1, Yuan T1, Pereira FA2, Farrell B1

1. Department of Otolaryngology – Head and Neck Surgery, Baylor College of Medicine, Houston, TX 77030
2. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030

Corresponding author: Vivek Rajasekharan, Department of Otolaryngology – Head and Neck Surgery, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX, E-mail: rajasekh@bcm.edu

At the leading dynamic edges of a cancer cell (e.g., filopodia, lamellipodia), pushing (protrusive) and pulling (retractive) forces are produced on the membrane by chemical motors (e.g., actin, myosin and tubulin) acting at or near the plasma membrane as the cytoskeleton undergoes remodeling. The forces generated at an edge of a cell are in the picoNewton range and are detectable with optical tweezers (OT) and atomic force microscopy. We use an optical tweezers (OT) based force measuring device to measure the force at the edge of a living cancer cell. The mechanism entails the use of an optically trapped bead capable of acting as a force sensor, as a handle to form a membrane tube from the cell. This method was initially developed to estimate the non-local bending modulus of plasma membranes and later extended to study membrane-cytoskeleton rupture and unbinding. Most studies monitoring the membrane force at the tip of the formed tube were conducted at slow rates (<100 Hz). In recent reports, by monitoring the membrane force at a faster sampling rate (≥ 1 kHz) transients (e.g., saw tooths, inverse saw tooths) riding atop the plasma membrane force were observed in mast cells, HeLa cells and neurons and it was reasoned that chemical motors, actin or actin in association with myosins were responsible for generating these transients. In other reports, chemical motors actin and β-tubulin have been shown to polymerize and fill the lumen of the formed membrane tube supporting this hypothesis. To detect these transients, we ensure that the temporal response of our instrument is routinely measurable. We describe the design and calibration of this force-measuring device with particular emphasis on the temporal resolution of the instrument and present an example of transients detected from a cancer cell.

Research supported by NIH grants S10 RR027549-01, R21CA152779 and RO1DC00354 and by the Alliance for Nanohealth 1W81XWH-10-2-0125.
**Electrical Coupling between Photoreceptors in the Mammalian Retina: The Rod Connexin is Connexin36**

Ribelayga CP1, Jin NG1, Postma F2, Zhang Z1, Young S3, Silveyra E1, Paul DL2, Massey SC1

1. Ruiz Department of Ophthalmology and Visual Science, The University of Texas Health Science Center at Houston
2. Department of Neurobiology, Harvard University
3. Undergraduate Program, Rice University

Corresponding author: Christophe P. Ribelayga, Ruiz Department of Ophthalmology and Visual Science, The University of Texas Health Science Center at Houston, McGovern Medical School, 6431 Fannin Street, MSB 7.024, Houston, TX 77030, E-mail: Christophe.P.Ribelayga@uth.tmc.edu

**Objectives:** Photoreceptors are electrically coupled via gap junctions. Rod/rod and cone/cone coupling are both present while rod/cone coupling provides an alternative pathway for rod signaling. In mammals, the gap junction expressed in cones has been identified as connexin36 (Cx36). However, the identity of the rod connexin is unknown. To assess the role of Cx36, we compared wild type mice with a pan-Cx36 knockout and both rod and cone specific Cx36 knockouts.

**Methods:** The junctional conductance between pairs of adjacent rods was estimated using a perforated-patch clamp technique in the dark. The distribution of Cx36 in the outer plexiform layer (OPL) was assessed by confocal microscopy.

**Results:** The junctional conductance between pairs of adjacent rods was ~ 150 pS in wild type mice (appropriate littermates) and close to 0 pS in pan-Cx36−/− or rod-Cx36−/− mice. In addition, the rod junctional conductance was ~ 6 pS in cone-Cx36−/− retinas. In the wild-type retina, most of the Cx36 in the OPL was found around the periphery of cone pedicles. A large fraction of this labeling was observed at points of contact between telodendritic processes and rod spherules. Cx36 labeling in the OPL was reduced by more than 90% in both the rod-Cx36−/− and the cone-Cx36−/− lines, except in small clusters underneath the cone pedicles where Cx36 is known to be associated with bipolar cell dendrites. In the rod-Cx36−/− line, a few remaining Cx36 plaques were found at contacts between cone telodendria.

**Conclusions:** Direct measurements of the rod junctional conductance demonstrate that Cx36 is required for rod electrical coupling. Intervening cones apparently account for ~ 90% of rod coupling. The distribution of Cx36 expression in the conditional lines indicates that rod-cone gap junctions require Cx36 on both sides (rod and cone) as the absence of Cx36 on either side prevents the formation of a plaque. This implies that 1) most of the Cx36 plaques observed in the OPL are rod-cone gap junctions. Thus, the rod/cone gap junction is the predominant gap junction in the photoreceptor network and rod/cone coupling provides an important pathway. 2) Cx36 is expressed in rods and required to form rod-cone gap junctions. 3) Altogether, the data strongly indicate that Cx36 is the rod connexin.

**Commercial Relationships:** None

**Support:** This work is supported by the National Institutes of Health (grants EY018640, EY06515, EY010608, OD010768), the Hermann Eye Fund and an Unrestricted Challenge Grant from Research to Prevent Blindness.
Single Molecule Spectroscopic Analysis Of K-Ras Oligomers

Sarkar-Banerjee S, Gorfe AA

1. Department of Integrated Biology and Pharmacology, University of Texas Medical School at Houston.

Corresponding author: Alemayehu A. Gorfe, Department of Integrated Biology and Pharmacology, University of Texas Medical School at Houston, 6431 Fannin, Houston, Texas 77030, E-mail: Alemayehu.G.Abebe@uth.tmc.edu

Ras proteins are small GTPases that control cell growth, differentiation and proliferation. They primarily reside on the inner leaflet of the plasma membrane and are crucial anticancer drug targets. The structural organization of Ras proteins on the plasma membrane has attracted major interest in recent years. Here we examined the distribution of dimers and higher-order oligomers of oncogenic K-Ras G12V and two of its variants, K101E and K101C/E107C, ectopically expressed on the plasma membrane of BHK cells. K101 and E107 are hypothesized to form an inter-monomer ion pair and stabilize K-Ras oligomers. Our analysis was based on fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and image correlation spectroscopy (ICS). Photon count histogram (PCH), raster image correlation spectroscopy (RICS) and number and brightness (N&B) analyses show that K-Ras oligomerization is reduced when the interaction between K101 and E107 is eliminated with a K101E mutation and increases with a double cysteine mutation K101C/E107C. Our data also show that the distribution of monomers, dimers and higher-order oligomers is such that the K101E mutant is mostly monomeric, the WT (K-Ras G12V) has both dimers and higher-order oligomers whereas there is greater percentage of dimers along with other oligomers in K101C/ E107C. These results confirm that K101 and E107 play a key role in K-Ras oligomerization and establish the co-existence of diverse K-Ras oligomeric states.

We thank National Institutes of Health General Medical Sciences (grant No. R01GM100078) for supporting our work.
Defining The Protein:Protein Interaction Interface Of FGF14:NAV1.6 Complex

Singh A1, Ali S1,2, Laezza F1,3,4,5

1. Pharmacology and Toxicology Graduate Program
2. Department of Pharmacology & Toxicology
3. Mitchell Center for Neurodegenerative Diseases
4. Center for Addiction Research
5. Center for Environmental Toxicology, The University of Texas Medical Branch, Galveston, TX 77555

Corresponding Author: Dr. Fernanda Laezza, Department of Pharmacology & Toxicology, 301 University Blvd, Galveston, TX 77555, Email: felaezza@utmb.edu

The voltage-gated Na⁺ (Nav) channel is composed of transmembrane spanning domains and of a cytosolic C-terminal tail which regulates channel function through protein:protein interactions (PPI) with auxiliary proteins, including fibroblast growth factor 14 (FGF14), a member of the intracellular FGF (iFGF) family. In addition to binding to the Nav C-tail, FGF14 forms homodimers and previous structural studies have proposed a conserved surface common for the Nav channel and the iFGF homodimer formation. Seeking for potential differences between the FGF14:Nav1.6-C-tail complex and the FGF14:FGF14 dimer interface, we have engineered model-based amino acid residue mutations at predicted FGF14 hot-spots and begun to screen for their impact on the protein complex stability. Using the in-cell split-luciferase complementation assay to reconstitute the FGF14:Nav1.6-C-tail and the FGF14:FGF14 complex, we identified a point of divergence at the FGF14V160 residue whose mutation led to opposite effects on the relative binding affinity to the FGF14 monomer versus the Nav1.6-C-tail. Functional studies using whole-cell patch-clamp electrophysiology indicated that V160 is a critical residue for FGF14 modulation of Nav1.6-mediated currents that can be abolished by Ala mutation. Intrinsic fluorescence and surface plasmon resonance (SPR) measurements further confirmed V160 in FGF14 as critical amino acid at the interface of the FGF14:Nav1.6 complex. With its unique role in the regulating the FGF14 binding and function, the V160 residue is well-positioned as target site for PPI-based drug development against Nav channels.

Supported by: R01MH095995 (FL) and the Gulf Coast Consortia NIGMS Grant No.1 T32 GM089657-04 (SRA).
pMD-membrane: A Tool to Determine Allosteric Binding Pockets and its Application to Oncogenic K-Ras

Prakash P1, Sayyed-Ahmad A1 and Gorfe AA1

1. Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Texas

Corresponding author: Alemayehu A Gorfe, Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, 6431 Fannin St., MSB 4.108, Houston, TX 77030, alemayehu.g.abebe.uth.tmc.edu

Probe-based molecular dynamics (pMD) simulation is a useful approach to determine druggable binding sites. The premise of pMD is similar to multi-solvent crystallography or NMR fragment-based screening. Applying pMD in the presence of membrane poses a challenge because the probe molecules can partition into the bilayer affecting its structure. We developed an approach, pMD-membrane, where we modify the force field parameters to alleviate this effect. This involves reducing the pairwise non-bonded interaction between selected probe and lipid atoms. We applied pMD-membrane to identify allosteric binding sites in a well-known cancer target, K-Ras. Ras is a lipid-modified GTPase and is involved in a variety of cell signaling pathways. It attaches itself to the inner leaflet of the plasma membrane to perform its biological function. Mutant Ras is associated with a variety of cancers including pancreatic and colorectal cancer. Of the three major human Ras isoforms H-, N- and K-Ras, cancers associated with mutant K-Ras are the most lethal. Although there is strong evidence for the existence of different orientations of H-Ras with respect to membrane plane, the data on K-Ras was not conclusive. Therefore, first, we performed ~8μs all-atom MD simulations of full-length oncogenic (G12D) K-Ras bound to a heterogeneous membrane. MD-derived populations revealed that the K-Ras catalytic domain interacts directly with the membrane with two predominant and distinct modes of interaction. Of the two modes only one was found capable of effector binding. Next, we applied pMD-membrane on the two orientations of membrane-bound K-Ras and different K-Ras mutants, G13D and G12D. The results suggest differential dynamics of the allosteric pockets significantly affect accessibility to probes. Using these and other examples, we will discuss how pMD-membrane can be used to track isoform-dependent or mutation-induced differences in the ligand binding potential of pockets on the surface of Ras proteins.

This research was funded by a two year (2013-2015) training fellowship awarded to PP from the Keck Center of the Gulf Coast Consortia on the Computational Cancer Biology Training Program, Cancer Prevention and Research Institute of Texas (CPRIT) RP140113, PI – Rathindra Bose. We are grateful to the Texas Advanced Computing Center (TACC) for their computational resources.
Allosteric Regulation Of Nuclease Specificity In Human Mitochondrial DNA Repair

Szymanski MR1,3, Yu W1, Gmyrek AM2, White MA2,3, Lee JC2,3, Whitney Yin Y1,3

1. Department of Pharmacology and Toxicology, University of Texas Medical Branch at Galveston, Galveston, TX, 77555
2. Department of Biochemistry and Molecular Biology, University of Texas Medical Branch at Galveston, Galveston, TX, 77555
3. Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch at Galveston, Galveston, TX, 77555

Corresponding author: Y. Whitney Yin, Department of Pharmacology and Toxicology, University of Texas Medical Branch at Galveston, Galveston, TX, 77555. E-mail: ywyin@utmb.edu

Oxidative stress leads to genomic instability and human diseases, thus Base Excision Repair (BER) pathway efficiently corrects DNA damage in mammalian nucleus and mitochondria. However, functions as well as interactions among human mitochondrial BER enzymes remain to be elucidated. Human mitochondrial EXOG (hEXOG), a 5'-exo/endonuclease, is crucial for mitochondrial DNA repair. hEXOG resides in mitochondrial inner membrane and phylogenetically belongs to a nonspecific ββα-Me nuclease family raising a question: how hEXOG performs its functions in BER? Here we present X-ray crystal structures of hEXOG in apo and DNA-bound forms. Combining structural snapshots with solution kinetic and thermodynamic analysis reveals the functional substrate specificity of hEXOG and highlights its role in mitochondrial BER. hEXOG homodimer binds DNA in the cleft between the protein core and the Wing domain that is absent in other ββα-Me family members. The Wing domain forms a docking site for 5'-DNA to acquire structure specificity and an intrinsic tape-measure to precisely hydrolyze two nucleotides from the 5'-terminus. Deletion of the Wing domain abolishes the specific exonuclease activity. hEXOG displays half-site reactivity and its function is allosterically regulated by DNA binding. After cleavage, hEXOG stalls without dissociation from DNA and repair intermediate is stabilized by specific interactions with residues of the Wing domain. This mechanism allows hEXOG to process any DNA damage at the 5'-end and hand-off repair intermediate to other BER enzymes. Our findings provide a novel mechanism of mitochondrial BER and set up a stage for assembly of multi-enzyme BER complex.

The work is supported by grants from NIH (GM 083703, GM110591) to YWY. AMG and JCL are supported by the R. A Welch Foundation (Grant H-0013). MRS was a recipient of J. B. Kempner Postdoctoral Fellowship.
Plasma triglyceride levels are a risk factor for coronary heart disease. Triglyceride metabolism is well characterized, but challenges remain to identify novel paths to lower levels. A metabolomics analysis may help identify such novel pathways and, therefore, provide hints about new drug targets.

In an observational study, causal relationships in the metabolomics level of granularity are taken into account to distinguish metabolites and pathways having a direct effect on plasma triglyceride levels from those which are only associated with or have indirect effect on triglyceride.

The analysis began by leveraging near-complete information from the genome level of granularity using the GDAG algorithm to identify a robust causal network over 122 metabolites in an upper level of granularity. Knowing the metabolomics causal relationships, we enter the triglyceride variable in the model to identify metabolites with direct effect on plasma triglyceride levels. We carried out the same analysis on triglycerides measured over 5 different visits spanning 24 years.

Nine metabolites out of 122 metabolites under consideration influenced directly plasma triglyceride levels. Given these nine metabolites, the rest of metabolites in the study do not have a significant effect on triglyceride levels. Therefore, for the further analysis and interpretations about triglyceride levels, the focus should be on these 9 metabolites out of 122 metabolites in the study.
Zhixian Zhang

Three-Dimensional Structures Associated with Photoreceptor Cilia by Cryo-electron Tomography

Zhang Z\(^1\), He F\(^1\), Schmid MF\(^1\), Wensel TG\(^1\)

\(^1\) Department of Biochemistry and Molecular Biology, Baylor College of Medicine

Corresponding author: Theodore G. Wensel, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, E-mail: twensel@bcm.edu

The outer segment of rod and cone photoreceptor cells is a modified primary cilium. Specialized machinery is needed to assemble and maintain its structure, and to carry out the transport and sorting of molecular components between the inner segment, where most biosynthesis occurs, and the outer segment, where those components are utilized for phototransduction. The narrow connection between the inner and outer segment, known as the connecting cilium, is the location of proteins encoded by genes implicated in inherited diseases known as ciliopathies. Its structural core is a nine + zero microtubule-based axoneme that emerges from a centriole termed the basal body complex. Conserved features of this organelle include the transition fibers and transition zone with a ciliary necklace studding the ciliary membrane. We used cryo-electron tomography to determine the structures of the second basal body (daughter centriole), the connecting cilium and associated machinery in wildtype mice. Recently we used computational sub-tomogram averaging to determine the three-dimensional structure of the basal bodies. The 3D density map reveals a tapered cylindrical structure composed of nine MT bundles from the plus (triplet) end to the minus (doublet) end of the centriole. Additional densities that represent non-tubulin proteins attached to the triplet were revealed, which presumably stabilize the entire basal body. In addition, we have observed transition fibers, located at the ciliary base, connecting to microtubule triplets. The transition zone connects the microtubule doublets of the axoneme to the membrane near the basal body and extends into the connecting cilium toward the outer segment. These structural features in wildtype cilia will help us to understand the normal mechanisms and functions of the many proteins essential for cilium function and photoreceptor survival, and shed light on the ciliopathies that arise from defects in those proteins.

Funded by NIH grants R01-EY07981, P30-EY002520, and P30-GM103832.