dependent activation that rapidly reverses in the dark. Thus, the new red-shifted MAG further validates that the two-component system tolerates variation of the PTL and provides tunable control of channel photoactivation properties.

1922-Pos Board B652
Site-Specific Tagging of Channelrhodopsins with Genetically-Encoded Azido Groups
Benjamin S. Krause[1], Peter Hegemann[1], Thomas Huber[2], Thomas P. Sakmar[2].
[1]Institute of Biology, Experimental Biophysics, Humboldt-University, Berlin, Germany, [2]Laboratory of Chemical Biology & Signal Transduction, www.sakmarlab.org, Rockefeller University, New York, NY, USA.
Channelrhodopsins (ChRs) are light-gated cation channels widely used in optogenetics because they can trigger depolarization of membrane potential upon illumination. In order to investigate the mechanism of channel opening, we used amber stop codon suppression to introduce the unnatural amino acid (uaa) *p*-azido-phenylalanine (azF) into expressed ChRs with high efficiency. Based on the recent crystal structure of a ChR-hybrid, amino acid residues in vicinity of regions that might be involved in the channel gating process were chosen as targets for replacement with azF. AzF-containing mutants were purified from mammalian cells in satisfactory yields with expression levels of up to ~35% compared with wild-type receptor, which matches earlier experience with CCR5 and bovine rhodopsin. We also developed a simple procedure to reconstitute ChR azF mutants into POPC-bilayer-membranes for future spectroscopy studies. The site-specific azF tag provides a useful FT-IR (Fourier Transform Infrared) spectroscopy probe because of its small size and its unique vibrational signature, which is well separated from intrinsic protein backbone signals. FT-IR difference spectroscopy in combination with uaa-mutagenesis can be used to track changes in the electrostatic environment of the azido probe and reveal local structural movements without impairing significantly the native protein architecture. In addition to direct interrogation of azF tags, exploiting the chemical property of the azido group as bio-orthogonal coupling site could allow specific functionalization of ChR. ChR azF mutants were reacted with fluorophore adducts using strain-promoted azide-alkyne cycloaddition chemistry. Coupling efficiency at multiple sites was determined by in-gel fluorescence scanning and UV-Vis spectroscopy. The current work describes for the first time the successful introduction of uaas into ChR variants, demonstrating a robust and powerful technology to investigate function and mechanism of this important class of photoreceptors.

1923-Pos Board B653
Encoding the Light-Sensitivity of Channelrhodopsin-2
Christian Bamann, Thomas Sattig, Ernst Bamberg.
Biophysical Chemistry, Max Planck Institute of Biophysics, Frankfurt, Germany.
Microbial rhodopsins are routinely used as light-controlled switches in neurobiology. Their versatile applicability relies on the simplicity of the optogenetic approach. Light-sensitivity is promoted to the host cell encoded in genetic information. However, the signal output is limited by the expression level and the molecular properties of the rhodopsins. The light-sensitivity of the system can set a limit for its usage. Different strategies might be envisaged how to tune and increase the responsiveness on a molecular level in the case of channelrhodopsin-2 (ChR2), a light-gated cation channel from Chlamydomonas reinhardtii: 1) increased absorption cross-section and quantum efficiency, 2) increased single channel conductance, 3) increased lifetime of the open state and 4) an amplification system. Here, we follow up the different strategies in a combined biophysical and neurobiological approach. As a first step we have developed the tools to study the different properties to have experimental access to the molecular properties from a spectroscopic and electrophysiological side. Especially, the development of fusing different rhodopsins into a single entity allows the discrimination between effects on the expression level and an increased single channel current by using one of the rhodopsins as a molecular ruler. In a next step we looked into the light-induced dynamical changes that accompany the photocycle of ChR2. This strategy allows us to map the conformational changes connected to the open state of the channel. We further generated a more calcium permeable mutant (CatCh, L132C) whose action on tuning the light sensitivity is different. Here, we propose a model that a local calcium concentration increase at the cytoplasmic membrane screens negative surface charges leading to a higher probability of opening the voltage-dependent sodium channels responsible for the onset of action potentials in neurons.

1924-Pos Board B654
Resonance Raman and Low Temperature FTIR Characterization of the Red Shifted Channelrhodopsin 1 from Chlamydomonas Augustae
John I. Ogren[1], Daniel Russano[1], Sergey Mamaev[1], Hai Li[2], Jihong Wang[2], John L. Spudich[2], Kenneth J. Rothschild[1].
[1]Boston University, Boston, MA, USA, [2]University of Texas Health Science Center at Houston, Houston, TX, USA.
Channelrhodopsins (ChRs) control phototaxis in green algae and function as light-gated cation channels when expressed in animal cells. Because ChRs can be functionally expressed in neuronal membranes, this distinct family of microbial rhodopsins have rapidly become an important tool in neuroscience. While the light-activated molecular changes occurring in channelrhodopsin-2 from *Chlamydomonas reinhardtii* (CrChR2) have been extensively studied, little is known about such changes in the diverse groups of other ChRs including the major class of channelrhodopsin-1 (ChR1). Here, we have characterized the structure and molecular changes in ChR1 from *Chlamydomonas augustae* (CaChR1). This ChR has properties advantageous for light modulated neuronal control including a red-shifted λ_{max} and slow light inactivation compared to CrChR2 (Hou, S. et al. (2012) Photochem Photobiol 88, 119-128). Near-infrared confocal resonance Raman spectroscopy (RRS) reveals that in contrast to CrChR2, which contains a mixture of retinal isomers, the retinal chromophore structure of CaChR1 is almost completely all-trans in the light-adapted state similar to many microbial rhodopsins including bacteriorhodopsin. In addition, unlike other ChR1s, such as CrChR1 (ChR1 from *Chlamydomonas reinhardtii*), which exhibit significant shifts in λ_{max} at different pHs, the RRS of CaChR1 including the ethylenic frequency which reflects λ_{max} remains largely unaltered over a wide pH range. This insensitivity to pH is despite the presence of the residue Glu87 (CrChR1 sequence numbering) which has been previously associated with pH sensitivity. Low-temperature FITR difference-spectroscopy reveals that the primary phototransition of CaChR1 to the K photointermediate involves an all-trans to 13-cis retinal isomerization and significant changes in the protein structure including structural changes in the peptide backbone, Asp/Glu residues, Cys residues and internal water molecules. These results are discussed in terms of possible differences in the molecular mechanism of various ChRs.

1925-Pos Board B655
Pado, a Novel Fluorescent Voltage-Sensing Protein, Identified by a Highly Conserved Motif in the S2 Trans-Membrane Segment
Bok Eum Kang, Bradley Baker, Hong Hua Piao, Arong Jung.
CFC, KIST, Seoul, Republic of Korea.
A highly conserved sequence motif in the S2 trans-membrane segment of the voltage-sensing domain was used to search and identify novel voltage-sensing proteins. This motif pulled down in silico proteins with homology to voltage-gated calcium, potassium, sodium and proton channels, as well as voltage-sensing phosphatases in addition to several proteins of unknown function. To test for voltage-sensitive optical responses we fused the putative voltage-sensing domain from 8 novel proteins to the fluorescent protein super ecliptic pHlorin A227D. The cytosolic amino terminus of the Ciona voltage sensing phosphatase was used to improve plasma membrane expression. The voltage-gated proton channel from liver fluke, which we call Pado (파도), gave a large optical signal (>10%) in response to a 200mV depolarization in HEK293. Furthermore, a cation current was detected above a threshold of about 180mV which is associated with an increase in the baseline fluorescence. Inhibition with $Zn2^{+}$ inhibits the movement of S4 and caused a substantial decrease in the optical signal upon membrane depolarization. Decreasing the extracellular pH resulted in a reduced current and also reduced the optical signal upon membrane depolarization. These results suggest that the voltage-gated current is due to the voltage-gated proton channel and that the corresponding change in internal pH affects the fluorescent intensity of Pado (파도). This search criterion is capable of identifying novel voltage-gated proteins that can be used to generate voltage-sensing probes.

1926-Pos Board B656
Light-Regulated $GABA_A$ Receptors: An Optogenetic Toolset for Studying Neural Inhibition
Wan-Chen Lin, Ming-Chi Tsai, Christopher M. Davenport, Caleb M. Smith, Richard H. Kramer.
Molecular and Cell Biology, University of California Berkeley, Berkeley, CA, USA.
$GABA_A$ receptors ($GABA_A$Rs) are the major mediators of inhibitory neurotransmission in the brain. The diverse composition of the $GABA_A$R pentamers, which determines receptor localization and biophysical properties, leads to the heterogeneity and complexity of GABAergic transmission. To

elucidate the distinct roles of different $GABA_AR$ isoforms within a neural circuit, tools allowing precise and specific control over the target receptors are required. Here we present a series of light-regulated $GABA_A$ receptors (LiGABARs) for studying different $GABA_AR$ α-isoforms in the nervous system. Each α-subunit of the LiGABAR is installed a photo-switchable antagonist near the GABA-binding pocket. The installed antagonist, which contains an azobenzene core, reversibly isomerizes to *trans* and *cis* configurations in response to 500-nm and 380-nm lights, respectively. This photoisomerization allows lights to turn off and restore the receptor activity by advancing and withdrawing the antagonist. LiGABARs can be employed in neurons to enable optical control of neural inhibition. For instance, in cortical neurons containing α1-LiGABAR, light switching can cause a ~60% change in the amplitude of the evoked inhibitory postsynaptic current. The target specificity and the spatiotemporal precision offered by optical control make LiGABARs a promising toolset for in-depth investigations of GABAergic transmission.

1927-Pos Board B657

Versatile Optical Control of Voltage-Gated Sodium Channel Function in Engineered HEK293 Cells

Thomas Lila, Andrew Blatz.
Photoswitch Bioscience, Menlo Park, CA, USA.

Optical control of voltage-gated ion channels offers a potentially flexible and inexpensive alternative to patch clamp systems in large-scale compound library characterization. Toward this end, we have engineered HEK293 cells to express a multi-component system conferring precise iterative control over heterologously expressed sodium channels including Nav1.5 and Nav1.7. In combination with sodium channels, balanced expression of an inwardly-rectifying potassium channel plus the Light-gated Glutamate Receptor (LiGluR) has yielded cell lines that maintain resting potentials around −90 mV and can be rapidly depolarized and repolarized via exposure to millisecond 390 nm and 490 nm light pulses, respectively. Prolonged incremental depolarizations in response to varying 390 nm light pulse duration are also possible due to the ''latching'' property of LiGluR in the presence of concanavlin A, enabling a range of sodium channel assay modes. Here, we demonstrate light-controlled protocols capable of discriminating among closed-state, inactivated-state, and use-dependent sodium channel inhibitors, and illustrate the use of voltage-step control in measuring channel voltage gating. We also present progress toward pairing these cell lines with voltage-sensitive dyes and extracellular field recording methods compatible with prospective or modified multiwell instrumentation systems.

Supported by NIH grant R44 GM087755.

1928-Pos Board B658

In Vivo Imaging of Odor-Evoked Responses in the Mouse Olfactory Bulb Using the FP Voltage Sensor ArcLight and the Calcium Sensor GCaMP3

Douglas A. Storace[1,2], Oliver R. Braubach[2,3], Lawrence B. Cohen[1,3], Uhna Sung[3].
[1]Cellular and Molecular Physiology, Yale University, New Haven, CT, USA, [2]NeuroImaging Cluster, Marine Biological Laboratory, Woods Hole, MA, USA, [3]Center for Functional Connectomics, Korea Institute of Science and Technology, Seoul, Korea, Republic of.

Genetically encoded voltage indicators can be used to optically monitor electrical activity from genetically distinct populations of neurons. Recent reports have demonstrated that the FP voltage sensor ArcLight can be used to study intact neural circuits in Drosophila and C. elegans. The first goal of the present study was to evaluate ArcLight as a reporter of electrical activity in the in vivo mouse olfactory bulb using wide-field and 2-photon imaging. The second goal was to selectively target ArcLight to mitral/tufted cells to record the output response of the olfactory bulb. In one set of experiments, an AAV1 vector was used to express ArcLight in the mouse olfactory bulb. In another set of experiments, a floxed version of the AAV1 vector was used in Pcdh21-Cre transgenic mice to selectively target mitral/tufted cells. Odors were presented at different durations and concentrations and the resulting patterns of activation were imaged. ArcLight had sufficient signal size to detect odor evoked responses in single trials using wide-field or 2-photon imaging. Wide-field recordings revealed that ArcLight but not GCaMP3 had sufficient temporal kinetics to clearly distinguish activity elicited by individual breaths of an odor. ArcLight was successfully targeted to mitral/tufted cells using Cre-Lox recombination, and odor-evoked signals were measured. The results indicate that ArcLight can be used to monitor electrical activity of genetically distinct cell types in an in vivo mammalian preparation.

Supported by: US NIH Grants DC005259, Grant WCI 2009-003 from the National Research Foundation of Korea, a James Hudson Brown - Alexander Brown Coxe Fellowship from Yale University, and a Ruth L. Kirschstein National Research Service Award F32DC012981-01A1.

1929-Pos Board B659

Improving Optical Control of Protein Activity by Light-Induced Fluorescent Protein Dissociation

Linlin Fan[1], Xin X. Zhou[1], Mariya Chavarha[1], Michael Z. Lin[1,2].
[1]Department of Bioengineering, Stanford University, Stanford, CA, USA, [2]Department of Pediatrics, Stanford University, Stanford, CA, USA.

Fluorescent proteins have been widely used for imaging while other light-absorbing domains have been used for optical control of protein localization or activity. We recently discovered that a tetrameric variant of the photochromic green fluorescent protein Dronpa exhibits light-mediated dissociation and association, and demonstrated that fluorescent proteins can also be used for reversible optical control of protein activity. We also developed a general design for fluorescent light-inducible proteins (FLiPs) in which a protein domain of interest is fused to two copies of Dronpa (one on each end), so that formation of the Dronpa tetramer occludes the domain of interest at baseline. To simplify the design, we now aim to develop a dimeric form of Dronpa that also exhibits light-mediated dissociation and association. Through structure-guided rational mutagenesis, we have created two dimeric mutants that show robust light-mediated dissociation and reassociation. We compare dimeric and tetrameric forms of Dronpa in photoswitching kinetics and in performance in optical control of protein activity. Using biochemical assays, we also confirm that protein domains of interest within FLiP constructs are occluded from their interaction partners in the pre-illuminated state and this occlusion is relieved by illumination. Our light-regulated Dronpa dimer confirms the utility of fluorescent proteins as optical control elements and the mechanism of action of the FLiP design.

1930-Pos Board B660

Local Optical Temperature Measurements around Magnetosomes within Single Bacteria to Study Size and Geometry Effects on Heating

Heng Huang[1], Rahul Munshi[1], Frank Mueller[2], Dirk Schueler[2], **Arnd Pralle**[1].
[1]Physics, University at Buffalo, Buffalo, NY, USA, [2]Ludwig-Maximilians-University, Munich, Germany.

Using the temperature sensitive of the fluorescence of GFP, we record the local temperature inside magnetotactic bacteria. These bacteria use magnetosomes - membrane and protein enclosed magnetite crystals - for navigating along the earth magnetic field lines. Upon purification, suspensions of magnetosomes are found to be highly effective heaters while subject to radio frequency magnetic field, yielding larger Specific Absorption Rates (SAR) than most of the common ferrofluids, making magnetosomes ideal candidate for application in magnetic field induced cell stimulation or hyperthermia cancer treatment. In the wild-type bacteria the magnetosomes are aligned in well-ordered chains, but mutants are available with different spatial arrangement or crystal size. We demonstrate that GFP fused to a magnetosome protein MamC may function as a molecular-scale temperature probe, and show that wild type magnetosomes raise the local temperature around the magnetosome membrane significantly, upon application of radio frequency magnetic field. The heating is confined to the vicinity of the magnetosomes, as the mutant strain producing the same magnetosomes but expressing a cytosolic GFP does not show heating of the entire cytosol. This approach enables us to study the effect of different spatial arrangement and nano-crystal size on the RF magnetic field induced heating directly in the individual bacteria, and to screen for more efficiently heating mutations. Finite element simulation is performed to understand the relation between spatial arrangement of magnetosomes and their heating efficiency.

1931-Pos Board B661

Interrogation of Downstream Calcium Responses via Optogenetic and Mechanical Stimulation in C. elegans

Gabriel Salzman[1,2], Ramya Parameswaran[1,2], Joshua Riback[1], H.E. Acaron Ledesma[1], John Barrett[1], Corey Seacrist[1], Adam T. Hammond[3].
[1]Biophysical Sciences, University of Chicago, Chicago, IL, USA, [2]Medical Scientist Training Program, University of Chicago, Chicago, IL, USA, [3]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL, USA.

Optogenetic methods have contributed substantially to the experimental analysis of neural function and behavior. In the nematode *Caenorhabditis elegans*, a commonly used model organism for studying neural circuits, optogenetic stimulation of several neuromuscular networks has induced identical behavioral responses to those resulting from mechanical stimulation. This has lead to the acceptance of optogenetic methods as physiologically relevant stimuli. Despite the similarities in macroscopic phenomenology,