tion and site-specific photocrosslinking studies have revealed that AQP4 TMs insert cotranslationally into and accumulate within the Sec61 translocon as the nascent polypeptide exits the ribosome. In contrast, two polar residues in AQP1 (Asn49 and Lys51) prevent TM2 from cotranslationally terminating translocation, and as a result, AQP1 acquires its proper transmembrane topology only after synthesis of TMs 5&6 is complete. We now show that Asn49 and Lys51 functionally interact with Asp185 at the C-terminus TM5 to form a nonlinear quaternary structural motif that influences multiple steps of the AQP folding pathway. Asn49 forms a critical intramolecular hydrogen bond with Asp185 that is required for proper helical packing, monomer formation and water channel function. In contrast, Lys51 forms an intermolecular ionic bond with Asp185 on an adjacent monomer that stabilizes the AQP1 tetramer. Although residues in this motif are highly divergent among AQP family members, they share a conserved quaternary architecture whose functional properties can be transferred en block to other family members. These findings suggest a general mechanism by which evolutionary divergence of closely related membrane proteins can confer new functional properties through alternate folding pathways that give rise to a common final structure. We propose that this sequence diversity contributes to the stability and selectivity of AQP homotetramerization in native tissues.

866-Symp Observing Protein Translocation in SecY and a SecY-Ribosome Complex Through Molecular Dynamics Simulations

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The protein-conducting channel, known as the SecY or Sec61 complex, is a unique membrane-bound protein, assisting both newly formed soluble proteins to cross the endoplasmatic reticulum (ER) membrane and membrane proteins to insert into the ER bilayer. These functions are often performed in tandem with the ribosome, which binds to the channel and feeds the protein in co-translationally. The resolution of a crystal structure of the core monomeric SecY complex from Methanococcus jannaschii four years ago has resulted in almost as many questions as it initially answered. For example, different structural elements such as a 'plug' and a 'pore ring' were both discovered, but their independent roles were still unclear. Additionally, the functional state of the channel, including its oligomeric arrangement, have become contentious issues. We have attempted to address many of these issues with molecular dynamics simulations of the channel, both alone and during translocation of small polypeptides. The flexibility and dynamic nature of both the plug and pore ring were observed, and recent results have clarified their individual roles in blocking the channel. Simulated opening of the lateral gate and subsequent relaxation indicated that the accessory protein SecE may not be very important in holding the gate closed. Simulations of a ribosome-channel complex have also been performed, illustrating in atomic detail how ribosome and channel work together. So far, all results have supported the idea that a SecY monomer forms the active channel.

Symposium 8: Imaging and Controlling Cellular Dynamics in vivo Using Light

867-Symp New Optical Tools For Controlling Neuronal Activity

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Correlating the activity of neurons with behaviour is of fundamental interest in neuroscience. Thus, acutely activating or inhibiting neurons in live and behaving animals is a key approach. With our collaborators G.Nagel, E.Bamberg (MPI Biophysics) and K.Deisseroth (Stanford), we established microbial-type rhodopsins as optical activators or inhibitors of neural function, both in rodent brains and live *Caenorhabditis elegans* nematodes. Channelrhodopsin-2 (ChR2) is a blue-light gated, algal cation-channel, while archaeal Halorhodopsin (NpHR) is a yellow-light driven Cl^--pump.

Depending on the promoters used, ChR2 facilitates rapid photodepolarization and thus activation of different types of *C. elegans* neurons. This allows triggering release of the neurotransmitters GABA or Acetylcholine at neuromuscular junctions, which is accompanied by complete relaxation of muscles (blocking locomotion), or muscle-hypercontraction, respectively. Combined with patch-clamp recording post-synaptic muscles, we now characterize mutations affecting neurotransmission.

Photoactivation of NpHR in cholinergic neurons caused an immediate arrest of locomotion. Since the action spectrum of NpHR is red-shifted relative to ChR2, combining both proteins in the same cell allows for bidirectional optical modulation of neural activity. Indeed, when co-expressed in cholinergic neurons, ChR2 and NpHR could be activated independently and concurrently by blue and yellow light, and NpHR could rapidly and reversibly counteract contractions observed during activation of ChR2 alone.

Towards a functional analysis of the 302 neurons in the *C. elegans* nervous system, we placed ChR2 in mechanosensory and proprioceptive neurons, command neurons that trigger backward locomotion, and dopaminergic cells that normally signal the presence of food. In all of these cells, photoactivation evoked characteristic behaviours.

In sum, ChR2 and NpHR enable repeated and reversible stimulation or inhibition of neurons in live animals with high temporal precision. These optogenetic tools will be instrumental to dissect the neural circuits controlling the numerous behaviours of *C. elegans*.

868-Symp Control of GPCR Pathways and Neuronal Circuits by Light

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A major challenge in understanding the relationship between neural activity and development, and ultimately behavior, is to control simultaneously the activity and intracellular signaling of either many neurons or various subcellular regions within individual neurons. Furthermore, changes of intracellular signaling pathways and changes of ion fluxes over cell membranes of any cell type are

correlated with alterations in cell homeostasis and physiological output. These changes often result in disease. Therefore controlling these signaling pathways and ion fluxes are of major importance for developing strategies, which can re-adjust and reset the altered signals. It is also important to control precisely the intracellular signaling pathways for understanding signaling events and signaling crosstalk of G protein coupled receptor pathways. Rhodopsins and green algae channelrhodopsin are light activated proteins which are currently used to control ion fluxes and GPCR pathways in cells. Here, we will present out data related to the use of vertebrate rhodopsin and channelrhodopsin for ion channel modulation in heterologous systems, control of neuronal excitability in single neurons and in spinal cord.

869-Symp Interplay between Single-Cell and Multi-Cellular Signaling during Glucose-Stimulated Insulin Secretion

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The islet of Langerhans is the functional unit responsible for glucose-stimulated insulin secretion (GSIS), and thus plays a key role in blood glucose homeostasis. The importance of the islet is demonstrated by the proven ability of islet transplants to reverse Type I diabetes pathologies in human patients. Over the last 10 years, we have been interested in understanding the multicellular mechanisms of islet function, and their role in the regulation of blood glucose under normal and pathological conditions. In many ways, the islet appears to function as a syncytium, which exhibits synchronous behavior of membrane action potentials, Ca^{2+} oscillations, and pulsatile insulin secretion across all β-cells in the islet. In other ways, the islet works as individual cells, especially in the regulation of gene transcription. Using our unique quantitative optical imaging methods and novel microfluidic devices, the *dynamics* of these molecular mechanisms can be followed quantitatively in living cells within intact islets. These investigations utilize transgenic and tissue-specific knock-out mouse models with demonstrated phenotypes, as well as traditional biochemical and molecular biological approaches.

870-Symp High-Speed Imaging of Cellular Dynamics in Freely Moving Mice Using Portable Fluorescence Microscopy

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A longstanding research goal has been to develop flexible fiber-optic fluorescence microscopes that provide micron-scale resolution, for the purpose of studying animal behavior and underlying cellular properties concurrently. To date, in vivo fluorescence imaging studies in mammalian subjects have typically been limited to anesthetized animals. To overcome this limitation, we have created high-speed, portable fluorescence microscopes based on micro-optics, fiber-optics, and miniaturized focusing actuators. We concentrated our instrumentation design efforts on the use of mice as our subjects, because of the wide availability of transgenic mouse lines with genetically targeted alterations to cellular properties and resulting behavioral deficits. Our portable microscopes are sufficiently small - about 1 cm in lateral extent and 2.5 g in mass - to be borne on the head of an adult mouse that is freely behaving but tethered via flexible fiber optics and electronic control lines. Using these microscopes we have performed high-speed imaging (up to 100 Hz frame rates) of cerebral microcirculatory dynamics, cerebellar neuronal dynamics, and Bergmann glial calcium transients in freely behaving animals. We expect that fluorescence imaging in freely moving mice will become increasingly prevalent, allowing detailed comparisons of animal behavior, physiological dynamics, and cellular properties between normal and transgenic mouse subjects.

Platform P: Protein Dynamics

871-Plat The Physics of Protein Fluctuations and Conformational Changes

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The different aspects of protein dynamics are investigated by X-ray structure analysis, Mössbauer effect with synchrotron radiation, incoherent neutron scattering and Mössbauer absorption spectroscopy. Experiments were performed in a wide temperature range. Although several different proteins have been investigated yielding similar results, only experiments on myoglobin are discussed. For this protein the most complete data set is available. X-ray structure analysis reveals structural distributions even at 0K. The slightly different structures are called Conformational Substates (CS). With the help of synchrotron radiation the density of phonons coupling to the heme iron of myoglobin was determined in a time regime between ps and fs. The corresponding mean square displacements, msd, increase linearly with temperature indicating a harmonic behavior, which was also seen in the msd obtained from X-ray structure analysis. Incoherent neutron scattering as well as Mössbauer absorption spectroscopy show a non harmonic behavior of the msd above a characteristic temperature T_c (∼180K). An analysis of the spectral shape of the Mössbauer absorption proves that molecules fluctuate between CSs if they can reach the so-called flexible state where slow Brownian oscillations of molecule segments occur. Molecules which do not reach the flexible state can not perform conformational changes. This was proved by CO and H_2O flash photolysis. The kinetics of conformational changes was investigated by the relaxation of a metastable state of myoglobin, created by reducing at low temperatures the Fe^{3+} of met-myoglobin by X-rays. The obtained Fe^{2+} low spin state with H_2O as ligand relaxes with time and temperature to deoxy-myoglobin. Molecules in the flexible state can change the conformation by surmounting the barrier between the states. This process is analyzed by Kramers theory.