enthalpy changes between closed and open. To quantify the energetics of gating, we applied optically generated submillisecond temperature jumps to directly measure the temperature-dependent activation kinetics. The data show that the opening of TRPV1, for example, is accompanied with an enthalpy change of ~100 kcal/mol, five times the energetic changes in voltage- or ligand-dependent gating. To gain insights on the energetic source, we analyzed single-channel and macroscopic kinetics of temperature-dependent gating, both showing that temperature has localized effects on specific gating components. Furthermore, the perturbation of membrane compositions, which altered the physical properties of lipids such as temperature-dependent phase transitions, did not abolish temperature activation of the channel. Thus the thermal sensitivity of the channel appears to be intrinsic to the channel itself, most likely arising from a specific protein domain rather than integration of global thermal effects. Using systematic chimeric analysis, we uncovered a proximal N-terminal region to be crucial for temperature sensing in heat-activated TRPVs. Changing this region both successfully transferred thermal sensitivity to temperature-insensitive isoforms and profoundly altered thermal sensing in temperature-sensitive wild-type channels. Swapping other domains including the whole transmembrane core, the C terminus, and the rest of the N terminus had little effect on the large enthalpy of gating. These results support that thermal TRP channels contain modular thermal sensors for their activation by temperature.

149-Symp
Heat Activation of TRPV Channels
Jie Zheng.
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Activation of TRPV1-4 channels is exquisitely heat-sensitive due to large and balanced changes in entropy and enthalpy. Identification of channel structures that underlie these changes is thus the prerequisite for understanding the molecular mechanism of heat activation. We approach this goal through a combination of methods. We use improved heating methods to accurately control temperature-dependent activation of wildtype channel and mutants which exhibit altered gating behavior, use structural analysis to guide our search for critical domains, and use site-directed fluorescence recordings to monitor conformational changes. For capsaicin receptor TRPV1, heat activation appears to be carried out by the central pore domain, while capsaicin is known to activate the channel by binding to the peripheral S2-S4 region. Structurally and mechanistically separated heat and agonist activation pathways suggest the possibility to selectively manipulate each process in this pain sensor for pharmaceutical purposes. The heat activation process appears to be preserved among TRPV1-3 channels.

150-Symp
Searching for Voltage Sensors in Thermosensitive TRP Channels
Viktorie Vlachova.
Department of Cellular Neurophysiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.
Temperature-activated transient receptor potential (TRP) channels are transmembrane proteins that play important roles in the transduction of thermal and chemical stimuli. In addition to the thermal and chemical cues, these channels can be activated by depolarizing the cell membrane, but the molecular basis of this regulation is unclear. The transmembrane part of the tetrameric TRP channels is structurally related to the voltage-gated K^+ channels in which the conserved charged residues within the fourth transmembrane region (S4) constitute part of a voltage sensor. Compared to these channels, the voltage-dependence of TRP channels is very weak, exhibiting the apparent number of gating charges of less than 1 versus ~12 in K^+ channels, and their putative voltage-sensing domains most likely lie outside the S4 because some of the TRPs completely lack positively charged residues in this region. In the present study, we will attempt to explore the functional roles of selected conserved charged residues which mutations specifically alter the voltage sensitivity of the vanilloid (TRPV) and ankyrin (TRPA1) channels. In particular, we will show that potential voltage-sensing residues in S4 and the S4-S5 linker, when specifically mutated, alter the functionality of these channels with respect to voltage, but also to temperature, agonist, and/or their interactions. Surprisingly, also a single amino acid substitution in the C-terminus led to substantial alterations in the voltage-dependent gating of TRPA1. In summary, the charged residues in S4, the S4-S5 linker, and in the C-terminus contribute to voltage sensing in some thermosensitive TRP channels and, despite their highly conserved nature, regulate the voltage and chemical gating in various related TRP channels in different ways. (Supported by GACR 305/09/0081)

Symposium: Spatial Organization in Prokaryotic Cells: Quantitative Measurements to Quantitative Models

151-Symp
Designing Intracellular Organization for Optimization of Sustainability
Pamela Silver.
Systems Biology, Harvard Medical School, Boston, MA, USA.
Biology presents us with an array of design principles. From studies of both simple and more complex systems, we understand some of the fundamentals of how Nature works. We are interested in using the foundations of biology to engineer cells in a logical and predictable way to perform certain functions. By necessity, the predictable engineering of biology requires knowledge of quantitative behavior of individual cells and communities and the ability to construct reliable models. By building and analyzing synthetic systems, we learn more about the fundamentals of biological design as well as engineer useful living devices with myriad applications. For example, we are interested in building cells that can perform specific tasks, such as remembering past events and thus acting as a biological computer. Moreover, we design cells with predictable biological properties that serve as cell-based sensors, factories for generating useful commodities and improved centers for carbon fixation. We have recently constructed synthetic intracellular protein/RNA structures to increase the efficiency of biological reactions. In doing so, we have made new findings about how cells interact with and impact their environment.

152-Symp
Modeling the Shape and Growth of Bacterial Cells
Ned Wingreen[1], Leon Furchtgott[2], K.C. Huang[3].
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In bacterial cells, the peptidoglycan cell wall is the stress-bearing structure that dictates cell shape. Although many molecular details of the composition and assembly of cell-wall components are known, how the network of peptidoglycan subunits is organized to give the cell shape during normal growth has been relatively unexplored. We have introduced a computational physical model of the bacterial cell wall to complement experimental studies of cell-shape determination, with a particular focus on rod-shaped cells like *Escherichia coli*. First, the model predicts the mechanical response of cell shape to peptidoglycan damage and perturbation. We observe a surprising robustness of cell shape to peptidoglycan defects, helping explain the observed porosity of the cell wall and the ability of cells to grow and maintain their shape even under conditions that limit peptide crosslinking. Interestingly, according to the model, many common bacterial cell shapes can be realized via modest local patterning of peptidoglycan density. Second, we introduced growth processes into the model via insertion of new glycan strands, formation of new peptide crosslinks, and cleavage of old crosslinks (1). The growth model suggests that maintaining a rod shape requires glycan insertion to be insensitive to fluctuations in cell-wall density and stress. Suggestively, in light of the role of MreB in maintaining rod-shaped growth, we find that a simple helical pattern of insertion is sufficient for many-fold elongation without significant loss in rod shape. Finally, we demonstrate that both the length and prestretching of newly inserted strands can regulate cell width. In sum, we show that simple physical rules can allow bacteria to achieve robust, shape-preserving cell-wall growth.
1. Furchtgott L, Wingreen NS, Huang KC. Mechanisms for maintaining cell shape in rod-shaped Gram-negative bacteria. Mol Microbiol. 2011; 81(2):340-53.

153-Symp
A Journey to the Pole: Polar Localization of Proteins in E. coli
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Far from being well-mixed, almost all biological systems exhibit precise spatial and temporal control of protein, mRNA, and DNA localization, demonstrating that cells measure distance and detect proximity with a molecular-scale tool kit. Despite its relatively simple cellular structure and diminutive size, Escherichia coli exhibits a high degree of spatial organization. We examine and quantitatively characterize the phenomena of polar localization at a systems scale by capturing the cell-cycle localization dynamics of all proteins with polar

localization in E. coli. In particular we study the temporal ordering of the arrival of factors at mid-cell, preceding cytokinesis, and at the new pole, after cell division as well as analyzing the asymmetry of protein partitioning on cell division. The great degree of heterogeneity between protein species suggest that a number of different mechanisms are responsible for the phenomena of polar localization.

154-Symp
Spatial Patterns of Protein Secretion on Bacterial Surfaces
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Proteins expressed on bacterial cell surfaces must initially cross the cytoplasmic membrane and then either traverse the thick cell wall in Gram-positive organisms or the thinner cell wall plus outer membrane in Gram-negative organisms. We have used protein tagging techniques to explore the spatial and temporal patterns of protein secretion in both types of bacteria. Overall, despite the profound structural differences between Gram-positive and Gram-negative cell surfaces, we find that both are largely immobile environments where protein behavior is dictated by fundamental physical constraints. For the Gram-negative bacterium E. coli, we used exogenous enzyme-based sequence-specific labeling of LamB to detect the location of newly synthesized exported protein. LamB initially appears in discrete puncta distributed over the cylindrical sides of the cell, rather than uniformly. These results are most consistent with a model where all outer membrane material (including transmembrane proteins and lipopolysaccharides) is added in irregular bursts along the cylindrical sides of the cell, and proteins are essentially immobile with respect to their neighbors. For the Gram-positive bacterium Listeria monocytogenes, we have also observed punctate initial secretion of the surface protein ActA along the cylindrical sides of the cell, and very slow accumulation at cell poles. ActA is a transmembrane protein with a large unstructured ectodomain exposed on the cell surface. We have developed a physical model proposing that the entropic constraint imposed by a small periplasmic space could drive the translocation of a large, unstructured protein across a barrier with a thickness and porosity similar to that of the Listeria cell wall. Consistent with this purely physical model for unstructured protein translocation and polarization in Gram-positive bacteria, we have found that eukaryotic nuclear pore proteins expressed in these bacteria will also translocate and polarize.

Platform: Membrane Receptors & Signal Transduction I

155-Plat
Drug Screening on Signal Transduction Proteins Via μ-Patterned Surfaces
Peter Lanzerstorfer[1], Stefan Sunzenauer[2], Mario Brameshuber[2], Gerhard J. Schütz[2], Shin-Ichiro Takahashi[3], Moritz Bünemann[4], Otmar Höglinger[1], **Julian Weghuber**[1].
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The plasma-membrane of living cells is the major organelle for cellular signaling cascades. To warrant the diverse functions of a cell, communication between the cytoplasm and its organelles and the extracellular space is crucial. Thus, membrane-localized protein receptors activated by various messengers are key to transmit signals into the cell. Defective regulation of these signaling cascades may lead to cell death or uncontrolled proliferation. An important point is the interaction of these receptors with cytosolic proteins. To analyze such interactions we use micro-structured surfaces in combination with fluorescent microscopy (''micro-patterning assay''). This technique was developed to detect protein-protein interactions (Schwarzenbacher et al., 2008; Weghuber et al., 2010) and offers the possibility to measure and quantify also weak or short-lived interactions in-vivo. We visualized the interaction of the following membrane-receptors with their respective intracellular binding partners: EGF-receptor (Grb2), Insulin-receptor (IRS1-4), and ß1- and ß2 adrenergic receptors (Arrestin, G-proteins). Additionally, we analyzed the insulin-dependent transfer of Glucose-transporter 4 (Glut4) to the plasma-membrane. In a next step we started to determine the effects of various messengers (EGF, Insulin, Epinephrine,…) on the described interactions. By doing so we characterized variations in the interaction-properties of these interaction pairs upon application of messenger molecules. Since the micro-patterning assay is a robust technique to analyze a large number of cells within a short time, it is our endeavor to investigate the effects of further, medically relevant messenger molecules (secondary plant metabolites) on the interaction of the aforementioned signaling proteins.
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156-Plat
Homo-FRET Imaging of Membrane Proteins in Living Cells
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The structure-function-activity relationships of transmembrane intracellular adhesion molecules are often mediated by ligand-induced signaling elicited in response to homotypic binding interactions. Understanding the molecular basis for these interactions is therefore critical for elucidating receptor function. A model cellular adhesion protein, carcinoembryonic antigen-related cell-adhesion molecule 1 (CEACAM1), exists as a mixture of monomers and oligomers at the cell surface. Depending on its oligomeric state, CEACAM1 has been previously shown to differentially bind to several protein tyrosine phosphatases, suggesting that CEACAM1's oligomeric state may affect various intracellular signaling pathways (Müller et al., 2009). We are using live cell total internal reflection fluorescence polarization microscopy (TIRFPM) to examine the distribution, association, and ligand accessibility of CEACAM1 at the cell surface. Although we observe heterogeneous spatial and oligomeric distribution of CEACAM1 at the cell surface of unperturbed cells, actin-rich regions with higher concentrations of CEACAM1 seem to comprise largely monomeric CEACAM1. As the functional consequences of these findings are still unclear, our current efforts are focused on identifying the linkages between CEACAM1's spatial and oligomeric distribution and its corresponding downstream signaling processes.

157-Plat
Lateral Dynamics of TNF Receptor I in Living Cells Studied with Single-Particle Tracking and Photoactivatable Fluorescent Probes
Meike Heidbreder[1], Christin Zander[2], Sebastian Malkusch[1], Darius Widera[2], Deepak Nair[3], Barbara Kaltschmidt[2], Christian Kaltschmidt[2], Daniel Choquet[3], Jean-Baptiste Sibarita[3], **Mike Heilemann**[1].
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Tumor necrosis factor receptor 1 (TNF-R1) is an important mediator of inflammation, binding tumor necrosis factor alpha (TNF-α) with high affinity. The functionality of TNF-R1 is closely related to its assembly into tri- and oligomers [1]. Here, we present our studies on the dynamics of TNF-R1 using high-density single-particle tracking in combination with photoactivated localization microscopy (sptPALM) [2]. We quantified single receptor dynamics and generated diffusion coefficient maps of whole cells. We found a heterogeneous distribution of diffusion coefficients indicating receptor species with different mobility, suggesting different molecular organizations. We further investigated receptor dynamics under cholesterol depletion and stimulation with TNF-α. Based on our observation, we designed a refined model on the structural arrangement and activation of TNF-R1 in the plasma membrane.
References
[1] Chan, F. K., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L. and Lenardo, M. J. (2000). A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science 288, 2351-2354.
[2] Manley, S., Gilette, J. M., Lippincott-Schwartz, J. (2010) Single-particle tracking photoactivated-localization microscopy for mapping single-molecule dynamics. Methods in Enzymology, 475, 109-120.

158-Plat
Molecular Determinants of GPCR-G Protein Complex Formation
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The G protein coupled receptor (GPCR) family is comprised of ubiquitous, membrane-bound proteins that are highly conserved in structure, yet varied in their cognate ligand. This allows for regulation of a variety of physiological processes ranging from cardiac contractility and neurotransmission to olfaction and vision. Signaling events downstream of activated GPCRs are initiated through a conserved mechanism of receptor-mediated nucleotide exchange of GDP for GTP in their intracellular binding partner, the heterotrimeric G protein ($G\alpha\beta\gamma$). While much is known about GPCR and $G\alpha\beta\gamma$ function, few