109-Plat
Localization of β-Adrenergic Receptors in Rat Ventricular Myocytes: Sub-Cellular Aspects
Caroline Cros, **Fabien Brette**.
University of Manchester, Manchester, United Kingdom.
In cardiac myocytes, β-adrenergic stimulation is mainly due to β1- and β2-receptors (ARs). In ventricular cardiac myocytes, the sub-cellular distribution of specific β-ARs is unclear: immunocytochemistry data and cAMP recording showed different results (between t-tubules (TT) and surface sarcolemma (SS)). Therefore, the functional distribution of β-ARs in ventricular cardiac myocytes (TT versus SS) is still unclear. This study addresses this point. Rat ventricular cells were enzymatically isolated. Detubulation was achieved using osmotic shock as previously described. Intracellular calcium concentration was recorded using fluorescent dye (fura-2 AM) and cell contraction was induced by field stimulation. Selective β1-adrenergic stimulation was achieved by perfusion of isoprenaline (0.1 μM) and ICI 118,551 (0.1 μM). Selective β2-adrenergic stimulation was achieved by perfusion of salbutamol (10 μM) and atenolol (1 μM). In control cells, β1-adrenergic and β2-adrenergic stimulation caused a significant increase in peak calcium transient (peak CaTr; 236.8 ± 42.9%, n=29 and 24.6 ± 4.2%, n=41, respectively), evaluating full β-adrenergic stimulation (i.e. SS + TT). In detubulated cells, β-adrenergic stimulation had a greater effect on peak CaTr than in control cells (288.1 ± 80.6% increase for β1, n=17 and 83.5 ± 9.0% for β2 n=20; evaluating β-adrenergic stimulation only from SS). From these values, we calculated that the % of increase of peak CaTr from the TT was ~128.3% during β1-adrenergic, and ~0.88% during β2-adrenergic. These data indicates that β1- pathway is functional in SS and TT in ventricular cardiac cells. In contrast, β2-adrenegic stimulation have a physiological effect on CaTr via the SS only. These results are in contradiction with the latest report about the localization of β-adrenergic receptors. However, our study focuses on the functional β-ARs response (i.e. CaTr) instead of the response to the increase of cAMP, which may account for the different conclusion.
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110-Plat
Super Resolution Microscopy Reveals that Caveolin-1 is Required for Antiviral Immune Response
Kristin Gabor[1], Chad Stevens[1], Matthew Pietraszewski[1], Travis Gould[1], Siew Hong Lam[2], Zhiyuan Gong[2], Sam Hess[1], Carol Kim[1].
[1]University of Maine, Orono, ME, USA, [2]National University of Singapore, Singapore, Singapore.
Understanding the organization of lipid rafts enhances our knowledge of the dynamic interactions of the receptors that reside in these membrane nanodomains. Caveolin-1 incorporates into lipid rafts and oligomerizes to induce the formation of caveolae, specialized lipid raft domains characterized by their flask-like morphology. Caveolae are integral for numerous signaling events, including immune responses, but their function in antiviral signaling is largely unexplored. Conventional studies of lipid raft association involve fractionation of detergent resistant membranes, which does not provide spatial and quantitative information. Fluorescence Photoactivation Localization Microscopy (FPALM), a super-resolution microscopy method, can be used to examine the organization and dynamics of single molecules underlying biological processes at the nanoscale. Interferon (IFN) plays a pivotal role in the antiviral response and this study shows that the IFN-receptor (IFN-R) co-localizes with Cav-1 and disperses upon Cav-1 knockdown. Expression levels of an IFN-stimulated gene are preserved upon covalent crosslinking of the IFN-R, despite Cav-1 knockdown, suggesting that caveolae corral or cluster the receptor for downstream signaling. To our best knowledge this is the first report of microscopic visualization of an immune receptor colocalizing with a membrane nanodomain, which is necessary for corralling antiviral receptors for the subsequent downstream signal.

111-Plat
Cytoskeletal Control of Receptor Diffusion in Membrane Promotes CD36 Function and Signaling
Khuloud Jaqaman[1], Hirotaka Kuwata[2], Nicolas Touret[3], Richard Collins[2], William S. Trimble[2], Gaudenz Danuser[1], Sergio Grinstein[2].
[1]Harvard Medical School, Boston, MA, USA, [2]Hospital for Sick Children, Toronto, ON, Canada, [3]University of Alberta, Edmonton, AB, Canada.
Receptor clustering and organization into membrane microdomains is an essential feature of transmembrane signal transduction. The processes that govern the coalescence of receptors into functional aggregates, however, are poorly understood.
CD36 is a clustering-responsive class B scavenger receptor in macrophages, where it binds to multivalent ligands such as oxidized low-density lipoprotein (oxLDL), apoptotic cells and malaria-infected erythrocytes. It is implicated in a wide range of processes, from lipid metabolism to innate immunity to tissue. Biochemical studies suggest that CD36 clustering at the cell surface upon engagement of multivalent ligands triggers signal transduction and receptor-ligand complex internalization. However, it is not known whether CD36 receptors at rest exist as monomers or as oligomers that facilitate the cellular response to ligand exposure, and what factors contribute to CD36 clustering.
To address these questions, we combined quantitative live-cell single-molecule imaging and biochemical approaches to study the dynamics, oligomerization and signaling of CD36 in primary human macrophages. We found that unliganded CD36 receptors exist in the membrane as metastable oligomers that prime the cells to respond to ligand exposure. Temporal multi-scale analysis of single receptor trajectories combined with pharmacological perturbation of the cytoskeleton showed that the movement of CD36 in the membrane was controlled by the submembranous actomyosin meshwork and by microtubules. Specifically, a subset of receptors diffused within cytoskeleton-dependent linear channels which promoted receptor oligomerization by allowing free diffusion in one direction while imposing confinement along the perpendicular direction. Perturbation of this organization markedly decreased CD36-mediated signal transduction. These data demonstrate a critical role for the cytoskeleton in controlling CD36 signaling by organizing the diffusion of receptors within regions of the membrane that increase receptor collision and oligomerization frequency.

112-Plat
Multiscale Simulation of Cadherin-Mediated Cell Adhesion
Yinghao Wu[1], Lawrence Shapiro[1], Avinoam Ben-Shaul[2], Barry Honig[1].
[1]Columbia University, New York, NY, USA, [2]The Hebrew University, Jerusalem, Israel.
Inter-cellular junctions play a pivotal role in the assembly of cells into specific three-dimensional tissues. Cadherins constitute a large family of Ca2+-dependent adhesion molecules that contain an N-terminal ectodomain, a transmembrane anchor and a C-terminal intracelluar region that contains highly conserved binding sites for catenin proteins which provide indirect links to the cytoskeleton. Although the molecular mechanisms underlying cadherin-mediated cell adhesion are still not fully understood, it seems likely that both cis dimers that are formed by binding of extracellular domains of two cadherins on the same cell surface, and trans-dimers formed between cadherins on opposing cell surfaces, play a role in junction formation.
One difficulty that arises in studying any membrane-constrained process is that binding affinities are generally determined for molecules that are free in solution whereas the constraint of a membrane imposes a 2D environment on interacting molecules, for example for membrane-anchored receptors such as cadherins. Here we present a computational strategy to model the process of junction formation based on a knowledge of 3D binding affinities. The cell interfacial region is defined by a simplified system where each of two interacting membrane surfaces is represented as a two-dimensional lattice with each cadherin molecule treated as a randomly diffusing unit. The binding energy for a pair of interacting cadherins in this two-dimensional discrete system is obtained from 3D binding affinities through a renormalization process derived from statistical thermodynamics. The properties of individual cadherins used in the lattice model are based on molecular level simulations. Our results show that within the range of experimentally-measured binding affinities, cadherins condense into junctions driven by the coupling of cis and trans interactions. The key factor appears to be a decrease in the conformational freedom of trans dimers that increases the magnitude of lateral cis interactions.

113-Plat
CXCR4-SDF1 Mediated Chemotaxis - from Tissue to the Single-Molecule Level
Susanne F. Fenz[1], Cassandra Verheul[1], Ewa Snaar-Jagalska[2], Thomas Schmidt[1].
[1]Leiden Institute of Physics: Physics of Life Processes, Leiden, Netherlands, [2]Leiden Institute of Biology: Molecular Cell Biology, Leiden, Netherlands.
Directed cell movement in a chemical gradient, chemotaxis, is not only a prerequisite for many vital processes like e.g. the immune response, but also the basis for cancer spreading in metastasis. Chemotaxis is governed by extracellular gradients of small molecules, the chemokines. The G protein-coupled receptor CXCR4 and its chemokine SDF1α play a crucial role in directing

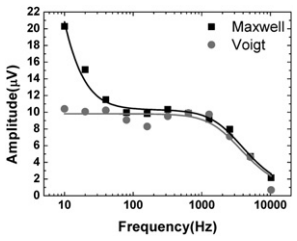
migration of tumor cells to neighbouring tissue as well as in metastasis to distant sites in the body via newly formed blood vessels (angiogenesis).
We investigated CXCR4- SDF1α mediated chemotaxis in mouse fibroblasts in an integrated approach from the tissue to the single-molecule level. First, we characterized cellular migratory potential upon stimulation with SDF1α in wound healing assays applying phase contrast microscopy. We find that transiently transfected cells expressing CXCR4 double their migration speed in comparison to wild type 3T3 cells. Second, we applied single-molecule fluorescence microscopy to study the mobility of the G protein-coupled receptor CXCR4-eYFP in resting cells and upon stimulation with SDF1α. Two fractions of receptors prior to stimulation were identified: half of the receptors were immobile while the other half exhibited free diffusion with D ~ 0.3 $\mu m^2/s$ on short timescales (up to 100 ms). At longer timescales receptors showed confined diffusion within micrometer domains. Global stimulation with SDF1α switched a subset of the receptors from the immobile to the mobile fraction. We predict that the impact of a SDF1α gradient might lead to asymmetric receptor diffusion and subsequently polarized cell behaviour as seen in the wound healing assays.

114-Plat
Quantitative Description of Signaling Downstream of Gq-Coupled Receptors: Similarities and Differences in the Responses of IP3, Calcium, DAG, PKC, and PIP2
Eamonn J. Dickson, Björn H. Falkenburger, Bertil Hille.
University of Washington, Seattle, WA, USA.
Gq-coupled plasma membrane receptors modulate cellular functions by activating phospholipase C (PLC), which hydrolyses the membrane lipid phosphatidylinositol (4,5)-bisphosphate (PIP_2) into the second messengers inositoltrisphosphate (IP_3) and diacylgycerol (DAG). To better understand the mechanisms that govern these partially independent signals we monitored in single, living tsA-201 cells levels of PIP_2, IP_3, calcium, DAG, and PKC by optical probes and current. We compared (i) activation of (low-abundance) endogenous purinergic receptors and overexpressed M_1 muscarinic receptors, and (ii) different concentrations of the muscarinic agonist oxotremorine-M (oxo-M). Whereas the peak responses from reporters of IP_3 (LIBRAvIII) and DAG (C1 domains of PKCγ) scale with abundance of receptor or agonist, downstream production of calcium (Fura4F) and PKC activation (CKAR) do not. Amplitude and duration of calcium signals elicited by 100 μM UTP, 10 nM oxo-M, or 10 μM oxo-M are almost identical. The only difference is a shorter latency with 10 μM oxo-M. These data suggest that a relatively low amount of IP_3 is required for calcium release. This interpretation is supported by the finding that a full-size calcium response can still be elicited after PIP_2 is depleted by recruiting a PI 5-phosphatase to the plasma membrane (by rapamycin-induced dimerization). Duration and late recovery time courses are different between IP_3 (duration=68 s; τ_{off} =55 s) and calcium (duration=110 s; τ_{off}=34 s), suggesting that once a threshold of IP_3 is reached, the calcium signal unfolds. Therefore we conclude that the IP_3 requirement for calcium release must be low. The time point and IP_3 level (from LIBRAvIII) at which the calcium response starts can provide an estimate of this IP_3 threshold. Supported by NIH grants NS08174 & GM83913 and the HFSP.

115-Plat
Vitamin A as an Activator and Sensitizing Chromophore for Rhodopsin
Sadaharu Miyazono, Tomoki Isayama, Clint L. Makino.
Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA, USA.
Absorption of light by rhodopsin isomerizes its 11-cis retinal chromophore to the all-trans conformation. The rhodopsin then activates a biochemical cascade that produces an electrical response by the photoreceptor. Eventually, all-trans retinal dissociates from the opsin and is reduced to vitamin A. The truncated retinal analog, beta-ionone, can pharmacologically activate some types of visual pigment, mimicking the effects of light. Beta-ionone is not normally found in the retina, however, vitamin A is present within the photoreceptor and can reach millimolar concentrations after exposure to bright light. Can vitamin A activate rhodopsin? In suction electrode recordings from isolated green-sensitive rods of salamander, exogenous vitamin A decreased circulating current and flash sensitivity, and accelerated flash response kinetics, changes that can also be seen during exposure to background light. Microspectrophotometric measurements showed that vitamin A accumulated in the outer segments, and an in vitro binding assay confirmed the binding of vitamin A to rhodopsin. These results suggested that vitamin A activated rhodopsin. In addition, suction electrode recordings showed that vitamin A improved the relative sensitivity of rods to UV light and in an in vitro bleaching assay, vitamin A enhanced the bleaching of rhodopsin by UV light. Presumably, both effects involved Forster resonance energy transfer (FRET) from vitamin A to the 11-cis chromophore of rhodopsin. Next, we tested whether FRET could be induced by endogenous vitamin A. After bleaching a large fraction of the rhodopsin in green-sensitive rods to generate vitamin A, relative sensitivity to UV wavelengths did indeed increase. Therefore, vitamin A can bind rhodopsin, activate it and also serve as a sensitizing chromophore.

PLATFORM I: Molecular Mechanics & Force Spectroscopy I

116-Plat
Viscoelasticity of Globular Proteins Measured from the AC Susceptibility
Yong Wang, Giovanni Zocchi.
UCLA, Los Angeles, CA, USA.
We introduce a new method to measure the elasticity and internal viscosity of nanometer size biological molecules such as globular proteins. Gold nanoparticles, tethered to a gold surface by the protein, are driven by an AC electric field while their displacement is synchronously detected by evanescent wave scattering, yielding the mechanical response function of the macromolecular sample in the frequency domain. We apply the method to measure the both the elastic constant and internal viscosity of proteins.



117-Plat
Designed Biomaterials to Mimic the Passive Elastic Properties of Muscles
Shanshan Lv, Yi Cao, Daniel Dudek, John Gosline, **Hongbin Li**.
University of British Columbia, Vancouver, BC, Canada.
The passive elasticity of muscle is largely governed by the I-band part of the giant muscle protein titin, a complex molecular spring composed of a series of individually folded immunoglobulin-like domains as well as largely unstructured unique sequences. These mechanical elements have distinct mechanical properties, and when combined, they provide the desired passive elastic properties of muscle, which are a unique combination of strength, extensibility and resilience. Single-molecule atomic force microscopy (AFM) studies demonstrated that the macroscopic behaviour of titin in intact myofibrils can be reconstituted by combining the mechanical properties of these mechanical elements measured at the single-molecule level. Here we report artificial elastomeric proteins that mimic the molecular architecture of titin through the combination of well-characterized protein domains GB1 and resilin. We show that these artificial elastomeric proteins can be photochemically crosslinked and cast into solid biomaterials. These biomaterials behave as rubber-like materials showing high resilience at low strain and as shock-absorber-like materials at high strain by effectively dissipating energy. These properties are comparable to the passive elastic properties of muscles within the physiological range of sarcomere length and so these materials represent a new muscle-mimetic biomaterial. The mechanical properties of these biomaterials can be fine-tuned by adjusting the composition of the elastomeric proteins, providing the opportunity to develop biomaterials that are mimetic of different types of muscles. We anticipate that these biomaterials will find applications in tissue engineering as scaffold and matrix for artificial muscles.

118-Plat
Influenza Virus Adhesion to Living Cells Measured by Single Virus Force Spectroscopy (SVFS) and Force Probe MD Simulation
Christian Sieben[1], Christian Kappel[2], Anna Wozniak[3], Rong Zhu[4], Christian Rankl[5], Peter Hinterdorfer[4], Helmut Grubmüller[2], Andreas Herrmann[1].
[1]Humboldt-University Berlin, Berlin, Germany, [2]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [3]JPK Instruments AG, Berlin, Germany, [4]Johannes Kepler University Linz, Linz, Austria, [5]Agilent Technologies-Austria, Linz, Austria.
Influenza virus belongs to a wide range of viruses that are enclosed in a lipid envelope. The major spike protein of the viral envelope hemagglutinin (HA) binds sialic acid (SA) residues of glycoproteins on the plasma membrane of