

109-Plat**Localization of β -Adrenergic Receptors in Rat Ventricular****Myocytes: Sub-Cellular Aspects**

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In cardiac myocytes, β -adrenergic stimulation is mainly due to β 1- and β 2-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 β 1-adrenergic stimulation was achieved by perfusion of isoprenaline (0.1 μ M) and ICI 118,551 (0.1 μ M). Selective β 2-adrenergic stimulation was achieved by perfusion of salbutamol (10 μ M) and atenolol (1 μ M). In control cells, β 1-adrenergic and β 2-adrenergic stimulation caused a significant increase in peak calcium transient (peak CaTr; $236.8 \pm 42.9\%$, $n=29$ and $24.6 \pm 4.2\%$, $n=41$, 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 ($288.1 \pm 80.6\%$ increase for β 1, $n=17$ and $83.5 \pm 9.0\%$ for β 2 $n=20$; evaluating β -adrenergic stimulation only from SS). From these values, we calculated that the % of increase of peak CaTr from the TT was $\sim 128.3\%$ during β 1-adrenergic, and $\sim 0.88\%$ during β 2-adrenergic. These data indicates that β 1- pathway is functional in SS and TT in ventricular cardiac cells. In contrast, β 2-adrenergic 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 Gabor¹, Chad Stevens¹, Matthew Pietraszewski¹, Travis Gould¹,Siew Hong Lam², Zhiyuan Gong², Sam Hess¹, Carol Kim¹.¹University of Maine, Orono, ME, USA, ²National 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 Jaqaman¹, Hiroataka Kuwata², Nicolas Touret³, Richard Collins², William S. Trimble², Gaudenz Danuser¹, Sergio Grinstein².¹Harvard Medical School, Boston, MA, USA, ²Hospital for Sick Children, Toronto, ON, Canada, ³University 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 Wu¹, Lawrence Shapiro¹, Avinoam Ben-Shaul², Barry Honig¹.¹Columbia University, New York, NY, USA, ²The 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 intracellular 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. Fenz¹, Cassandra Verheul¹, Ewa Snaar-Jagalska²,Thomas Schmidt¹.¹Leiden Institute of Physics: Physics of Life Processes, Leiden, Netherlands,²Leiden 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 SDF1 α play a crucial role in directing