# Membrane Receptors & Signal Transduction I

**3472-Pos Board B519**
**Protein Flexibility Is One Type Of Biosignal**
**Qinyi Zhao**.
Medical institute, Beijing, China.
According to protein model of biosignal, the protein flexibility is one type of biosignal. In view of protein thermodynamics structure theory, the protein flexibility can influence signal transduction activity by several ways. Firstly, it can modulate the rate of protein conformational change and then influence signal transduction activity (signal activity). Second, it can regulate protein conformational coupling and then regulate activity of other effectors (or signal transduction pathway). Third, it can influence the equilibrium between different protein conformational states and influence the initiation of signal transduction activity. Experimental test indicates that the urea shows anesthetics potency and thus the protein flexibility is related to action mechanism of general anesthetics. The protein flexibility has profound impact on all pathways of signal transduction.

**3473-Pos Board B520**
**Membrane Proteins-bilayer Interplay: Insights From Coarse-grained Self-assembly And Potential Of Mean Force Simulations Of Rhodopsin In Model Bilayers**
**Xavier Periole**[1], Thomas Huber[2], Siewert-Jan Marrink[1], Thomas P. Sakmar[2].
[1]University of Groningen, Groningen, Netherlands, [2]Rockefeller University, New York, NY, USA.
Due to the transient nature of typical protein-protein interactions in biological membranes and other technical limitations, it is extremely difficult to devise robust experimental approaches to elucidate atomic details of complex membrane systems. By offering alternative and complementary information, theoretical and computational approaches have become extremely useful and valuable tools. Here we will present our recent development of new molecular models, which, while conserving the physicochemical properties of the different components in a system, reduce the complexity of the description of a system down to chemical entities (3 to 6 heavy atoms). These coarse-grained models allow simulating systems of size and for time scales relevant to their biological function. The results obtained from self-assembly simulations of visual pigment rhodopsin in model membranes [1] and potentials of mean force (PMF) of rhodopsins association will be presented. The data show that the membrane bilayer responds to the presence of a protein by inhomogeneous and localized deformations at the surface of the protein and that, whereas the amount of deformation (hydrophobic mismatch) strongly affects the propensity of the proteins to aggregate, the location of the deformation correlates with protein-protein interfaces. The PMFs further demonstrate that protein binding energies are strongly correlated to the interfaces involved and that the membrane bilayer has a significant contribution to the energy barriers encountered during association. This work brings new insights to our understanding of the forces driving protein self-organization in membrane bilayers. [1] X. Periole, T. Huber, S.-J. Marrink, and T.P. Sakmar. 2007. G Protein-Coupled Receptors Self-Assemble in Dynamics Simulations of Model Bilayers. J. Am. Chem. Soc. 129, 10126-10132.

**3474-Pos Board B521**
**Docking of Insulin to its Receptor**
Harish Vashisth, **Cameron Abrams**.
Drexel University, Philadelphia, PA, USA.
The human insulin receptor (IR) is a homodimeric membrane-spanning ligand-activated tyrosine kinase. Although a detailed theory describing how binding of the hormone insulin triggers kinase activity of IR remains elusive, the recently published crystal structure of the IR ectodomain offers the unique opportunity to use physical modeling to begin to construct this theory. We present here the results of ~100 ns of explicit-solvent all-atom molecular dynamics (MD) simulations of IR in both the apo and putative T and R-state insulin docked states. Our simulations confirm the large interdomain flexibility of IR and the stability of its dimeric interfaces. More importantly, however, our simulations demonstrate the evolution of large-scale asymmetry in IR relative to the crystal structure, a result that reflects the structural requirements of a ''see-saw'' mechanism that guarantees negative homeotropic allostery in insulin binding. This asymmetry also manifests itself in the opening of one of the two equivalent insulin binding pockets and closing of its partner. This result is significant because it allows for the first time computational docking of an intact molecule of insulin into its binding pocket on an intact IR ectodomain. We use a Monte-Carlo docking algorithm followed by MD equilibration to predict bound states of insulin on IR. These simulations allow us to identify unambiguously the residues on IR that form the ''site-2'' binding epitope which recognizes residues on insulin responsible for its hexamerization.

**3475-Pos Board B522**
**Coupling Atomistic Simulation to a Continuum Based Model to Compute the Mechanical Properties of Focal Adhesions**
**Robert W. Metzke**[1], Mohammad R. K. Mofrad[2], Wolfgang A. Wall[1].
[1]Technische Universität München, Garching, Germany, [2]University of California, Berkeley, Berkeley, CA, USA.
Mechanical Ventilation is causing inflammation in the lung either by rupture of tissue or by overstretching cells and starting signalling cascades. To prevent starting these, it is of great interest to connect ventilation parameters to stresses and strains in the lung on a cellular level.
In order to quantify forces transferred from the tissue to the cell, the mechanical properties of integrins play an important role. Large time and length scale differences between the cell and the integrin molecules make such computations difficult. Given the utility of continuum models based on Finite Element Method, it would be useful to couple the continuum methods to molecular dynamics techniques in order to compute forces between cells and tissue.
For coupling molecular information to the continuum level, we present a technique based on energy transition. Hereby the mechanical properties of on integrin bonds are computed with help of molecular dynamics. In order to model the complete focal adhesion, a spring recruitment model is included on the continuum side, representing more than one molecular bond. The approach includes dynamic effects from both scales. The focal adhesion is represented by a layer of finite elements with the intergin molecule bound to collagen. The deformation gradient of the element is scaled to the size of the protein. The actual gradient and the one from one time step earlier are transferred to the molecular scale. In the molecular dynamics simulations, the energy difference between both deformations is simulated first by slowly deforming and secondly equilibrating the protein. This information is transferred back to the continuum level and, under the assumption that focal adhesions remain connected, the number of bonds are calculated and modelled as multiple non-linear neo-Hookean material, representing parallel springs.

**3476-Pos Board B523**
**In VSMCs, Beta-1 Integrin but not Syndecan-4 Gene Expression is Dependent on Matrix Stiffness**
**Olga V. Sazonova**, Celeste B. Rich, Joyce Y. Wong, Matthew A. Nugent.
Boston University, Boston, MA, USA.
Mechanotransduction is the process by which cells sense and convert mechanical stimuli into biochemical signals, and has been implicated in the development of certain pathologies. During atherosclerosis, the vessel wall experiences progressive stiffening, particularly at the site of developing plaques, and vascular smooth muscle cells are intimately involved in the development and maintenance of these plaques. In vitro, VSMCs have also been shown to be mechanosensing: cell spreading area, migration speed, and expression of characteristic cytoskeletal components is mediated by substrate stiffness. It is likely, then, that changes in arterial mechanics during atherosclerosis contribute to the pathological behavior of VSMCs, but our understanding of this process remains inadequate.
The beta-1 integrin subunit has been implicated in mechanosensing, but its uniqueness as a mechanotransducer has not been established. Syndecan-4 acts in synergy with the alpha5beta1 integrin, though its role in mechanotransduction is unknown.
We hypothesized that beta-1, but not syndecan-4, gene expression is regulated by substrate stiffness. We used polyacrylamide gels functionalized with fibronectin which mimic the mechanical properties of healthy (~30kPa) and diseased (~80kPa) arterial walls. Primary rat VSMCs were grown to 60% confluence on 18kPa, 35kPa, and 80kPa gels with uniform FN surface content, and collected for quantitative PCR analysis. Gene expression of beta-1 increased 1.5x with each increment in stiffness, while syndecan-4 levels were not affected. These results suggest that beta-1 integrin gene expression is sensitive to substrate mechanics, while syndecan-4 expression is not.
Because cell-cell contacts are also capable of mechanotransduction, we also looked at the effects of cell density on beta-1 integrin expression. Preliminary results suggest that increasing cell density eliminates the substrate-mediated increase in beta-1 expression, which suggests that VSMCs integrate mechanical stimuli from various sources to achieve physical homeostasis.

**3477-Pos Board B524**
**Investigation of Signal Transduction through the HAMP Domain from Molecular Dynamics Simulations**
**Taehoon Kim**, Wonpil Im.
The University of Kansas, Lawrence, KS, USA.
The HAMP domain connects the extracellular sensory with intracellular signaling domains in over 7,500 proteins including histidine kinases, adenylyl cyclases, chemotaxis receptors, and phosphatases, and thus plays an essential

role as a link in transmembrane signal transduction. Recently, Hulko et al. determined the solution NMR structure of the HAMP domain (PDB ID:2ASW) of an archaeal protein AF1503, a putative transmembrane receptor [Hulko, M., et al. (2006) *Cell 126*, 929-940]. Based on the NMR structure, we have modeled the relative orientation of transmembrane domains TM1 and TM2 using replica exchange molecular dynamics simulations (REXMD). We then performed MD simulations of the HAMP and TM complex in explicit lipid bilayers to investigate possible thermal motions as well as signal transduction mechanisms. In addition, as Hulko et al. suggested a signal transduction mechanism related to the canonical packing by rotation of the helices, we have investigated the helix rotation propensity through MD simulations. In this work, we will present the simulation results of the HAMP and TM complex as well as the HAMP domain alone.

**3478-Pos Board B525**

**Redistribution Of The Human Mu-opioid Receptor (hMor) In HEK 293 Cell Membranes In Response To Agonists**

**Tanya E. S. Dahms**[1], Dominique Massotte[2], Tianming Sun[1].
[1]University of Regina, Regina, SK, Canada, [2]CNRS/INSERM/ULP, Illkirch cedex, France.

Opioid receptors play a role in a whole battery of physiological processes, including affective behaviour, neuroendocrine physiology and pain perception. The cell membrane, which is the physical environment of the receptor, is known to consist of domains with distinct lipid/protein composition and physical characteristics. Previous work (Moffet *et al.* (2000) *J. Biol. Chem.* 275: 2191-2198.) has shown that the G-protein signalling partners of hMOR localize to the ''so called'' detergent resistant membrane (DRM). Our previous studies using ligand-affinity atomic force microscopy (AFM) of hMOR-expressing Sf9 cell membranes indicated that hMOR localizes to mixed lipid domains, presumably corresponding to detergent soluble membranes (DSM). These results would indicate a physical separation of the receptor and its signalling partner prior to activation. However, the relationship between microdomains characterized using biochemical techniques (DSM/DRM) and membrane rafts defined by biophysical techniques remains unclear. Here we track active hMOR localization and lipid composition in detergent and detergent-free separated membranes. For each fraction, hMOR activity was assessed using a modified binding assay, and Western blot analysis was used to determine the relative amount of lipid raft marker, flotillin-1, and G-proteins. We show a redistribution of the receptor in response to its agonists, implying that hMOR distribution at the cell membrane helps to regulate it function.

**3479-Pos Board B526**

**Evidence for precoupling of inactive M3 muscarinic receptors and $G_q$ Protein**

**Kou Qin**, Kuravi K. Sudha, Nevin A. Lambert.
Medical College of Georgia, 30912, GA, USA.

Two models have been proposed to explain how G protein-coupled receptors (GPCRs) interact with heterotrimeric G proteins to transduce physiological signals. One model suggests that GPCRs and G proteins collide with each other randomly after receptor activation and that binding is transient. An alternative model suggests that GPCRs and G proteins are bound to each other (precoupled) before receptor activation. We have studied interactions between GPCRs and G proteins using fluorescence recovery after photobleaching (FRAP) and avidin-mediated crosslinking in HEK 293 cells. We have previously shown that immobile CFP-labeled $\alpha_{2A}$-adrenoreceptors (C-$\alpha$2ARs) do not decrease the mobility of the G proteins that they activate, consistent with a collision-coupling model. Here we show that immobile CFP-labeled M3 muscarinic receptors (C-M3Rs) decrease the lateral mobility of citrine-labeled $G\alpha_q$. C-M3Rs failed to decrease the mobility of venus-labeled $G\alpha_{oA}$. Conversely, the C-M4Rs (which activate $G_{i/o}$ proteins) failed to decrease the mobility of $G_q$-citrine. Slowing of $G\alpha_q$-citrine by immobile C-M3R was unaffected by an agonist (carbachol) or an inverse agonist (atropine), and thus did not depend on activation of the receptor. Slowing of $G\alpha_q$-citrine by immobile C-M3Rs was enhanced by carbachol when nucleotides were depleted, as predicted by the ternary complex model of G protein coupling. These results suggest that inactive M3Rs precouple with $G_q$ proteins, and that different coupling models apply to different GPCR-G protein pairs.
*Supported by grants GM078319 from the NIH and MCB0620024 from the NSF.*

**3480-Pos Board B527**

**Active $\alpha$2A-receptors induce GTP$\gamma$S release from activated Go proteins and subsequently sequestrate G proteins**

**Leif G. Hommers**, Christian Dees, Moritz Bünemann.
University of Wuerzburg, Wuerzburg, Germany.

Recently, analysis of agonist-induced G protein activation and receptor/G protein interaction has become possible in single living cells by means of FRET. Using these methods, we aimed to investigate whether GTP$\gamma$S-bound Go proteins interact with active $\alpha$2A-receptors and whether bound-GTP$\gamma$S can be released from Go proteins upon this interaction. In order to conduct these experiments, we had to gain control of the intracellular nucleotide composition by permeabilizing the cell membrane of transiently transfected HEK293 cells via short application of saponine.
Agonist-induced receptor/G protein interaction, monitored by means of FRET between tagged $\alpha$2A-receptors and tagged G$\gamma$, was significantly stronger in the presence of low concentration of GDP, GTP and GTP$\gamma$S compared to corresponding 1000-fold higher concentrations of nucleotide. Superfusion of cells with GTP$\gamma$S in the presence of agonist resulted in attenuation of FRET between G protein subunits corresponding to maximal activation of G proteins. When omitting GTP$\gamma$S, the FRET signal recovered with kinetics accelerating with increasing concentration of agonist. This result suggested deactivation of G proteins due to receptor-induced dissociation of GTP$\gamma$S from preactivated G proteins. As a second experiment, non-labeled GTP$\gamma$S could displace radioactively labeled GTP$\gamma$35S in an agonist dependent manner. In the presence of saturating concentration of agonist, increased FRET between G protein subunits suggested a reduced activation of G proteins compared to non-saturating concentration of agonist. This effect was found for both intact cells and permeabilized cells in the presence of low concentration, but not in the presence of high concentration of GTP$\gamma$S. These results suggested (I) interaction of active G proteins with active receptors (II) receptor-induced dissociation of GTP/ GTP$\gamma$S from G proteins and (III) sequestration of inactive nucleotide-free G proteins at the active receptors.

**3481-Pos Board B528**

**Rapid Binding of G$\beta\gamma$ Dimers to the c-terminus of GRK3**

Bettye Hollins, Sudhakiranmayi Kuravi, Gregory J. Digby, **Nevin A. Lambert**.
Medical College of Georgia, Augusta, GA, USA.

One proposed function of macromolecular signaling complexes is acceleration of signal onset, as complexes would obviate the need for collision of randomly-diffusing molecules. Here we study the kinetics of heterotrimeric G protein signaling between molecules that are unlikely to be part of a complex. Binding of the GRK3 c-terminus (GRK3ct) to G$\beta\gamma$ dimers was detected using confocal microscopy and fluorescence or bioluminescence resonance energy transfer (FRET or BRET). Activation of pertussis toxin-insensitive $G_{oA}$ heterotrimers led to translocation of GRK3ct-venus from the cytosol to the plasma membrane with a monoexponential time constant of 431 $\pm$ 35 ms ($n$=9) at room temperature. FRET between a membrane-associated GRK3ct-cerulean (masGRK3ct-C) and G$\beta\gamma$-venus (G$\beta\gamma$-V) increased with a time constant of 213 $\pm$ 32 ms ($n$=10) at 26.5 °C, and 67 $\pm$ 13 ms ($n$=13) at 37 °C. Fluorescence recovery after photobleaching suggested that masGRK3ct-V and heterotrimers were not part of a complex prior to activation. Agonist-induced BRET between masGRK3ct-Rluc8 and G$\beta\gamma$-V decreased as the relative expression of G$\alpha$ increased. This is consistent with buffering of free G$\beta\gamma$ by excess inactive G$\alpha$ subunits, suggesting that masGRK3ct-Rluc8 bound to free G$\beta\gamma$-V dimers rather than to rearranged heterotrimers. These results suggest that G protein signals that occur on a timescale of ~100 ms are not necessarily mediated by preassembled signaling complexes. *Supported by grants GM078319 from the NIH and MCB0620024 from the NSF.*

**3482-Pos Board B529**

**Functional Evidence for Gi-Gq crosstalk through G protein-coupled Receptor Heterocomplexes**

**Miguel Fribourg-Casajuana**[1], Javier González-Maeso[1], Stuart C. Sealfon[1], Diomedes E. Logothetis[2].
[1]Mount Sinai School of Medicine, New York City, NY, USA, [2]Virginia Commonwealth University, Richmond, VA, USA.

We have studied signaling through a complex of two distinct G protein-coupled receptor (GPCR) types: a serotonin (Gq-coupled) and a metabotropic glutamate (Gi-coupled). We used two-electrode voltage clamp in Xenopus oocytes expressing the receptors and used endogenous calcium-activated chloride or heterologously expressed Kir channels to monitor the effects of GPCR signaling. Previous biochemical studies had shown Gi signaling by stimulating the serotonin receptor in the serotonin-glutamate receptor complex (González-Maeso et al., Nature 2008 452:93-97). Our work here focused on studying whether Gq signaling could also take place when stimulating the glutamate receptor.
Upon glutamate receptor activation, oocytes expressing both receptors elicited calcium-activated chloride currents, not observed in oocytes injected with the glutamate receptor alone. Furthermore, these currents could be blocked by a specific regulator of Gq-protein signaling (RGS2) and were accompanied by phosphatidylinositol (3,4)-bisphosphate (PIP2) hydrolysis in the membrane, results consistent with Gq-signaling. Finally, receptor chimeric studies further confirmed that the glutamate-serotonin complex needs to be formed in order to allow Gi-Gq cross activation.