lengths (more than 10 residues). The reasons for this deterioration include:

(a) the difficulty in carrying out sufficiently complete searches of the very high dimensionality of the conformation spaces,
(b) the inherent flexibility of loop regions and,
(c) insufficiently accurate force fields.

As a result, the lowest free energy ensemble represents the native ensemble less well as indicated by larger root mean square deviations from the native loop structure. To improve structural characterization of GPCR loops, we compared predictions of rhodopsin loops derived from application of fairly reliable and fast loop-prediction algorithms for globular proteins (e.g., PLOP [Jacobson, MP et al., *Proteins* 2004; *55*:351–367], MODLOOP [Fiser, A & Sali, A. Bioinformatics 2003;*19*:2500–1], etc.) with those obtained by the MC-SCV method. Results from the combined application of specific loop-prediction algorithms with the MC-SCV method are presented in an attempt to reveal new more effective ways to identify conformations of long GPCR loops that belong to the native energy funnel.

1975-Pos Detecting Key Residues Involving Conformational Change Of Supra-molecules By Elastic Network Normal Mode Analysis

ATSUSHI MATSUMOTO[1], Tetsuji Kamata[2], Junichi Takagi[3], Kenji Iwasaki[3], Kei Yura[1]

[1] *JAPAN ATOMIC ENERGY AGENCY, Kizugawa, Kyoto, Japan,*
[2] *Keio University School of Medicine, Tokyo, Japan,*
[3] *Osaka University, Suita, Osaka, Japan.*

Board B90

Normal mode analysis of elastic network model is widely used these days for studying conformational fluctuations and changes of supra-molecules. The major advantage of this method is that it requires much less computational resources compared to the conventional methods so that it is applicable to huge macromolecular structures such as ribosome and that the results of the calculations agree well with those of the conventional methods and experimental results.

We made slight modification to this method, and applied it to the extracellular domain of integrin. Integrin is a membrane protein with a huge extracellular domain, and participates in cell-cell and cell-extracellular matrix interactions for metazoan. The extracellular domains of a group of integrins are known to perform a large-scale structural change when the protein is activated, but the activation mechanism and generality of the conformational change remain to be elucidated.

We performed normal mode analysis of the elastic network model of the extracellular domain of integrin alphaV beta3 in the bent form and identified key residues dominating the molecular motions. Iterative normal mode calculations demonstrated that the specific non-bonded interactions involving the key residues work as a snap to keep integrin in the bent form. The importance of the key residues for the conformational change was further verified by mutation experiments.

G Proteins

1977-Pos G Protein Coupled Receptor Kinase 2/3 Separate Galphaq And Gbetagamma Subunits During G Protein Activation

Johanna Schleifenbaum, Anne K. Kreile, Martin J. Lohse, Moritz Bunemann

University of Wuerzburg, Wuerzburg, Germany.

Board B92

Ubiquitously expressed G protein coupled receptor kinases 2 and 3 are primarily known to phosphorylate active G protein coupled receptors (GPCR) and therefore to initiate receptor desensitization. GRK2 and 3 have also been demonstrated to bind to Gαq subunits and therefore attenuate Gq mediated signalling. GRK2 actually can bind simultaneously to Gαq and Gβγ subunits to its N- and C-terminus, respectively, as recently illustrated by a crystal structure complex (Tesmer et al., Science 300,1256 *2003*). A striking feature of this structure is a large gap of about 8 nm between αand β γ subunits. We developed a FRET based assay to measure activation of Gq subunits by means of YFP-tagged Gαq and CFP-tagged Gβ γ subunits and determine effects of GRK2 expression on the distance between Gαq and Gβ γ. In nonstimulated cells we couldn't observe an effect of GRK2 on FRET between the G protein subunits. Stimulation of P_2Y_1 receptors induced a fast decrease in FRET, reflecting Gq activation. This activation induced decrease in FRET was potentiated about 3 fold upon coexpression of GRK2 or GRK3, suggesting a GRK dependent separation of αand β γ subunits. We further proved that both Gβ γ and Gαq binding sites were required for this effect by using GRK2-constructs lacking either Gαq or Gβ γ binding sites. Strikingly, even coexpression of both GRK2-truncation constructs did not alter the activation induced FRET signal compared to cells not exogenously expressing GRK2 constructs. These results suggest that in the absence of GRK2 or GRK3 Gαq and Gβ γ subunits do not efficiently dissociate but rather may undergo subunit rearrangement. In the presence of GRK2 activated Gαq and Gβ γ simultaneously and efficiently bind to their respective GRK binding sites as predicted by the crystal structure.

1978-Pos Development of a FRET-based Reconstitution Assay to Probe the Interaction Between the Rho Family GTPases and Defined Synthetic Lipid Membranes

Jared L. Johnson[1], Jon W. Erickson[2], Richard A. Cerione[3]

[1] *Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA,*
[2] *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA,*
[3] *Department of Chemistry and Chemical Biology, Department of Molecular Medicine, Cornell University, Ithaca, NY, USA.*

Board B93

Rho family GTPases play important roles in a variety of fundamental cellular processes, including cytoskeleton reorganization, migration, polarity, vesicle trafficking, and cellular transformation. These outcomes require the correct localization of a given GTPase to the targeted membrane surface. Rho Guanine nucleotide Dissociation Inhibitor (Rho-GDI) is a ubiquitously expressed 22kDa protein that stimulates the removal of Rho family GTPases from membranes, maintaining them in the cytosol as stable heterodimeric complexes.

This study illustrates the utility of a novel FRET assay to characterize GDI facilitated removal of Rho family GTPases, specifically Cdc42, Rac1, and RhoA, from membranes. The GTPase is labeled by binding with fluorescently labeled guanine nucleotides (Mant-nucleotides) and the GTPase-Mant-nucleotide complex is bound to fluorescein labeled membranes that partially quench the GTPase-Mant-nucleotide fluorescence. GDI's removal of GTPase-bound Mant-nucleotide from the proximity of acceptor labeled lipids is indicated by the full restoration of Mant fluorescence upon GDI addition. Titration of GTPase-bound vesicles with increasing amounts of GDI demonstrates that the membrane GTPase extraction and complexing by GDI can be critically dependent on the nucleotide-bound state of the GTPase. Using this real-time FRET read-out, GDI can be used to probe the nature of the interaction between the GTPase and the membrane surface when liposomes of variable composition are used. To that end, we demonstrate here a direct role for isomers of PIP2 on Cdc42, Rac1, and RhoA's affinity for artificial membranes that may provide a more detailed understanding of their critical role at the polarized membrane surface in living cells.

1979-Pos Differential Dissociation Of Gβ γ Dimers From Active $G\alpha_{oA}$ And $G\alpha_s$ Subunits In Living Cells

Gregory J. Digby, Pooja R. Sethi, Nevin A. Lambert

Medical College of Georgia, Augusta, GA, USA.

Board B94

Differential dissociation of Gβ γ dimers from active $G\alpha_{oA}$ and $G\alpha_s$ subunits in living cells

G protein-coupled inwardly rectifying potassium (GIRK) channels are activated by pertussis toxin (PTX)-sensitive G proteins such as G_{oA}. In contrast, PTX-insensitive G proteins such as G_s activate GIRK to a lesser extent, and often must be overexpressed in order to do so. Since GIRK channels are activated by Gβ γ subunits and both families of heterotrimers are thought to liberate free Gβ γ after activation, the molecular mechanisms that account for differential activation of GIRK channels by G_{oA} and G_s heterotrimers are unknown. Here we test the hypothesis that differential activation of GIRK channels reflects differential dissociation of active G_{oA} and G_s heterotrimers. Physical dissociation of heterotrimers containing farnesylated $G\beta_1\gamma_{2/11}$-venus allows these dimers to leave the plasma membrane and translocate to the cell interior, providing a sensitive live-cell assay for heterotrimer dissociation. Activation of heterotrimers containing CFP-TM-$G\alpha_{oA}$ resulted in significantly greater $G\beta_1\gamma_{2/11}$-venus translocation than did activation of heterotrimers containing CFP-TM-$G\alpha_s$. Fluorescence intensity measurements made by imaging and flow cytometry indicated that the abundance of these CFP-TM-Gα subunits at the plasma membrane was comparable. In permeabilized cells loaded with GTPγS immobile CFP-TM-$G\alpha_s$ subunits constrained the lateral mobility of $G\beta_1\gamma_2$-venus dimers to a greater extent than immobile CFP-TM-$G\alpha_{oA}$ subunits, suggesting that the former retained a higher affinity for $G\beta_1\gamma_2$-venus after activation. Finally, we found that both G_s and G_{oA} heterotrimers could activate GIRK channels, although G_s heterotrimers were much less efficacous. These results show that Gβ γ subunits dissociate more readily from active $G\alpha_{oA}$ subunits than active $G\alpha_s$ subunits, and suggest that this difference may contribute to preferential activation of GIRK channels by PTX-sensitive heterotrimers.

Work supported by NSF (MCB 060024), NIH (NS36455/GM 078319), AHA (0715111B)

1980-Pos The Self-Scaffold Model Study of $G\alpha_{i1}$-$G\beta_1\gamma_2$-$PLC\beta_2$ Complex and Oligomerization Study

Jingting Wang, Suzanne Scarlata

Stony Brook University, Stony Brook, NY, USA.

Board B95

Heterotrimeric G protein is activated via its specific seven transmembrane receptor, which catalyzes the exchange of GTP for GDP on the Gα subunit. GTP-bound Gα subunits have a reduced affinity for Gβ γ subunits, however, there are several recent *in vivo* evidence indicate that GTP-bound Gα subunits can still bind to Gβ subunits without dissociation. In this report, we present our study of a model in which G proteins and $PLC\beta_2$ remain complexed through the G protein activation and deactivation cycle *in vitro,* using a combination of biochemical, computational and fluorescence methods. Also, we present evidence that Gα has a second, lower affinity site for Gβ γ. We found that deactivated Gα is capable of binding a second Gβ γ at a reduced affinity. This second site is the main binding site available for activated Gα and leading to a model in which Gα rotates to the second site upon activation.

1981-Pos Both Gi And Go Heterotrimeric G Proteins Are Required To Exert The Full Effect Of Norepinephrine On The B-cell Katp Channel

Ying Zhao[1], Qinghua Fang[2], Susanne G. Straub[1], Geoffrey W.G. Sharp[1]

[1] *Vets School, Cornell Uinversity, Ithaca, NY, USA,*
[2] *Applied and Engineering Physics Cornell Uinversity, Ithaca, NY, USA.*

Board B96

The effects of norepinephrine (NE), an inhibitor of insulin secretion, were examined on membrane potential and the ATP-sensitive K+

channel (KATP) in INS 832/13 cells. Membrane potential was monitored under the whole-cell current clamp mode. NE hyperpolarized the cell membrane, an effect that was abolished by tolbutamide. The effect of NE on KATP channels was investigated in parallel using outside-out single channel recording. This revealed that NE enhanced the open activities of the KATP channels ~2 fold without changing the single channel conductance, demonstrating that NE-induced hyperpolarization was mediated by activation of the KATP channels. The NE effect was abolished cells in cells preincubated with pertussis toxin, indicating coupling to heterotrimeric Gi/Go proteins. To identify the G proteins involved, antisera raised against a and b subunits (anti-Ga-common, anti-Gb, anti-Gai-1/2/3 and anti-Gao) were used. Anti-Ga-common totally blocked the effects of NE on membrane potential and KATP channels. Individually, anti-Gai-1/2/3 and anti-Gao only partially inhibited the action of NE on KATP channels. However, the combination of both completely eliminated the action. Antibodies against Gb had no effects. To confirm these results and to further identify the G-protein subunits involved the blocking effects of peptides containing the eleven amino acids at the C-termini of the a-subunits were used. The data obtained were similar to those derived from the antibody work with the additional information that Gai-3, Gao-1 were not involved. In conclusion, both Gi and Go proteins are required for the full effect of norepinephrine to activate the KATP channel.

Protein-Nucleic Acid Interactions - I

1982-Pos Kinetic Cooperativity In 30S Ribosome Assembly Detected With 2-Photon Fluorescence Fluctuation Spectroscopy In Microfluidics

William K. Ridgeway[1], Effrosyni Seitaridou[2], Alexey V. Karnaukhov[3], Rob Phillips[2], David P. Millar[1], James R. Williamson[1]

[1] *The Scripps Research Institute, La Jolla, CA, USA,*
[2] *California Institute of Technology, Pasadena, CA, USA,*
[3] *Institute of Cell Biophysics Russian Academy of Sciences, Pushchino, Russian Federation.*

Board B97

The bacterial 30S ribosomal subunit is able to self-assemble in vitro into a functional macromolecular machine. The assembly process is thought to be dominated by folding of the core 16S rRNA, while binding of the 20 small-subunit proteins facilitates folding via a tertiary-structure capture mechanism. As monitored by protein binding, the assembly process exhibits significant thermodynamic cooperativity. However, bulk kinetic experiments have elucidated relatively few ordered binding events and so to fully examine kinetic cooperativity we are observing assembly on the level of individual assembly intermediates.

To gain single-intermediate resolution, we have built a 2-photon microscope with single-molecule sensitivity. Three spectrally-distinct fluorophores are simultaneously excited at ~880nm, and photons are recorded and stored for offline analysis. Adaptations of FCS/Coincidence Analysis allow us to extract kinetic traces with ~1second resolution over the duration of the hour-long reaction. 30S Assembly reactions are initiated by mixing three fluorescently-labeled ribosomal proteins with varying amounts of 16S rRNA and unlabeled recombinant ribosomal proteins. From three auto-correlations, three cross-correlations and a triple-correlation, we can monitor eight assembly intermediates and thus detect kinetic cooperativity between any given trio of proteins. Since the technique does not require FRET it can probe interactions between distant domains of the 30S.

Early simulations of reactions highlighted the need for a large dataset of kinetic traces prepared from a combinatorial array of initial reagent concentrations, as well as the need for multiple-start reactions. To facilitate this task, we have designed and built an automated microfluidic reactor which can precisely prepare and initiate the requisite large number of reactions. We are presently investigating late stages of 30S assembly in the 3′Major domain which are thought to involve resolution of misfolded 16S rRNA.

1983-Pos Helicase Superfamily 1 and 2 ATPase Mechanisms

Christopher P. Toseland, Andrew F. Slatter, Maria M. Martinez-Senac, Jackie L. Hunter, Martin R. Webb

MRC-National Institute for Medical Research, London, United Kingdom.

Board B98

Helicases catalyze the unwinding of double-stranded DNA or RNA for a variety of functions through various mechanisms. Helicases are classified into six superfamilies (SF) through conserved sequences. The ATPase mechanisms of two contrasting helicases, one from the highly characterised SF1, and the other from the largest and most diverse family, SF2, will be compared. The SF1 bacterial helicase PcrA is a monomeric enzyme with a role in plasmid replication. This is one of the most characterised helicases and it is known to translocate single-stranded DNA (ssDNA) and move with discrete steps of one base per ATP. The SF2 RecG is a monomeric bacterial helicase which brings about replication fork reversal through the atypical translocation of double stranded DNA. Fork reversal allows repair of an ssDNA lesion which caused the replication fork arrest. A translocation step size of 2–4 bp per ATP has been observed for RecG by determination of the translocation kinetics with oligonucleotide junctions. A variety of biophysical techniques have been applied to determine the ATPase kinetic mechanism and the individual rate constants. This includes utilising fluorescent analogues of ATP, 2′ (3′)-mantATP/ADP (2′ (3′)-*O*-*N*-methylanthraniloyl-ATP/ADP), in rapid-reaction experiments, allowing the nucleotide binding and release kinetics to be explored. These analogues are further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) measures the phosphate release kinetics. Oxygen exchange experiments, using ^{18}O-substituted ATP or phosphate, allow the kinetics of reversal of the hydrolytic cleavage step and phosphate binding to be probed.