the distributions are broad and asymmetric, in excellent agreement with available molecular dynamics simulations and with important implications for the correct interpretation of experiments. Such distributions allow a close prediction of the experimental observables for a number of model and natural membrane peptides, whether amphipathic and bond parallel at the membrane interface or inserted across the membrane in a tilted configuration. Moreover, we show that the predicted distributions result from optimizing the position of peptide residues with respect to the hydrophobic, the interface and the bulk-water regions through a complex interplay of displacement along the membrane normal, tilt and rotation. This leads to a redefinition of the hydrophobic matching peptide adaptation, stressing its dynamic character and so far unconsidered roles of the membrane interface and peptide rotation.

Membrane Proteins - III

1958-Pos Structure-function Relationship Of Helical Membrane Proteins Revealed By Packing Features And Hydrogen-bonding

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Board B73.01

The recent structural elucidation of about one dozen "channels" (in which we include transporters) has provided further evidence that these membrane proteins typically undergo large movements during function. However, it is still not well understood how these proteins achieve the necessary trade-off between stability and mobility. In order to identify specific structural properties of channels, we compared the helix-packing and hydrogen-bonding patterns of channels with those of membrane-coils; the latter is a class of membrane proteins whose structures are expected to be more rigid. We describe in detail how in channels, helix pairs are usually arranged in packing motifs with large crossing angles ($|\tau|\sim40^\circ$), where the (small) side-chains point away from the packing core and the backbones of the two helices are in close contact. We found that this contributes to a significant enrichment of Cα-H—O bonds and to a packing geometry where right-handed parallel ($\tau = -40^\circ \pm 10$) and anti-parallel ($\tau = +140^\circ \pm 25$) arrangements are equally preferred. By sharp contrast, the interdigitation and hydrogen bonding of side-chains in helix pairs of membrane-coils results in narrowly-distributed left-handed anti-parallel arrangements with crossing angles $\tau = -160^\circ \pm 10$ ($|\tau|\sim20^\circ$). In addition, we show that these different helix-packing modes of the two types of membrane proteins correspond to specific hydrogen bonding patterns. In particular, in channels, three times more of the hydrogen-bonded helix pairs are found in parallel right-handed motifs than are non-hydrogen bonded helix pairs. Finally, we discuss how the presence of weak hydrogen-bonds, water-containing cavities and right-handed crossing angles may facilitate the required conformational flexibility between helix pairs of channels, while maintaining sufficient structural stability.

1959-Pos Substitution Rates Of Amino Acid Residues In Transmembrane - Extracellular And Periplasmic- Regions Of Beta-Barrel Membrane Proteins

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Board B74

Beta-barrel membrane proteins are found in Gram-negative bacteria, mitochondria and chloroplasts. They play important roles in metabolism of bacteria, where they are involved in transport of solutes in and out of the cell. Beta-barrel proteins may also act as proteases, lipases and may be important for cell-cell adhesion. Currently, there are about 30 structures of beta-barrels solved. Although the number of beta-barrel folds is fairly small, it is possible to expand the amount of available structural information by homology modeling using existing structures as templates. The scope of structure prediction may be widened by finding remote homologues of the existing structures. To improve the sensitivity of the database searches and the quality of sequence alignments, we study evolutionary history of transmembrane segments of beta-barrel membrane proteins by estimating substitution rates with a Bayesian Monte Carlo approach, which can be used to detect remote homologues. Transmembrane, extracellular and periplasmic regions of beta-barrel proteins experience different evolutionary pressure, which results in different substitution rates. Results on amino acid substitution rates, scoring matrices and database searches for remote homologues will be discussed.

Support from NIH GM079804 is gratefully acknowledged.

1960-Pos Genome Scale Bioinformatics Identification of Peripheral Proteins and Their Membrane-Binding Mechanism

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Board B75

Membrane-binding peripheral proteins bind membranes mostly reversibly and play important roles in many biological processes, such as cell signaling and membrane trafficking. Due to the absence of canonical transmembrane segments, it is difficult to identify them through traditional sequence homology searches. Toward genome-scale identification of peripheral proteins and elucidation of biological rules adopted by these proteins to bind membranes, we employ machine learning protocols in three modules. First, using their structural properties such as surface amino-acid composition, electrostatic characteristics and overall charge, we identify these proteins with more than 90% success rate. Second, the prediction is then extended to using only the sequence-based features which makes the prediction protocol applicable to genome-scale identification. To address the problem of unavailability of a large well-defined negative set, we employ Positive-Unlabeled (PU) learning to identify a

reliable negative set and build a protocol that shows sensitivity larger than 90% for sequence-based prediction. Finally, as a step toward knowledge-mining, we suggest easily-interpretable rules that separate membrane-binding domains from the non-binding ones using alternative decision trees. For example, through pure data-mining we find that features like charge, hydrophobicity, and composition of Trp and Cys are important determinants for membrane-binding. We further investigate such discriminating rules specific to four families of these domains that had statistically-significant number of positive and negative cases: C1, C2, PH and PX. In example, for the C1 domain, we show that in addition to the above mentioned features important for all peripheral protein families, structural features like flexibility, buriability, and hydrophilicity are also crucial. Since our protocol does not rely on sequence-homology or structural-similarity, it can be efficiently used in the identification of novel peripheral domains.

1963-Pos Effect of Mutations on the Stability of a Regular Polyleucine Based Helical Transmembrane Dimer

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Board B78

Membrane proteins are important drug targets as they are known to play a central role in various processes in cells. A large number of membrane proteins consist of helices, a common type of basic structure in proteins, in their transmembrane domains. It is important to study the interactions between these helices to understand the structure and function of membrane proteins. Polyleucine-based peptides form the simplest possible transmembrane helices and have been widely studied experimentally. In this study, we investigated the effect of specific mutations on the helix-helix interactions of polyleucine dimers in DOPC. We mutated leucine to amino acids of different sizes and hydrophilicity to investigate the effect of perturbing the helix-helix interactions on the relative thermodynamic stabilities of the polyleucine helices. We used molecular dynamics simulations and thermodynamic integration method to calculate the free energies for various mutations.

1964-Pos A Free Energy Profile for the Association of WALP23 Transmembrane Helices Calculated with the MARTINI Coarse-Grained Forcefield

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Board B79

Interactions between transmembrane helices play a key role in almost all cellular processes involving membrane proteins. Thermodynamic data on the association of helices is scarce, and a full picture of the forces responsible for specific association of helices remains elusive. We use the new MARTINI coarse-grained membrane protein force field(1) to probe the interactions between WALP23 (leucine-alanine repeat) alpha-helices in a phospholipid bilayer. We calculate the total free energy of association of these helices to be approximately −15 kJ/mol for the parallel case, and −25 kJ/mol for the antiparallel case. This agrees with previously published qualitative data on the preferential antiparallel association of these helices(2), and with quantitative thermodynamic data on a similar leucine-alanine alpha-helix(3). We estimate the favorable enthalpy for association to be greater than the free energy (−30 to −40 kJ/mol), indicating that association is driven by enthalpy with a slightly unfavorable entropy change.

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1965-Pos Self-Assembly and dynamics of the Influenza A M2 channel via Coarse Grain MD simulations

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Board B80

Self-Assembly and dynamics of the Influenza A M2 channel via Coarse Grain MD simulations Membrane protein folding occurs by peptides inserting into the bilayer and forming independently stable transmembrane helices, these then pack into the tertiary structure of the protein. The folding and assembly of small membrane peptides occurs on the μs timescale. This is a problem for conventional atomistic MD, as it is tenfold longer than the upper time limit. However, with CG MD the length of the simulation can be extended to tens of microseconds - long enough to observe the folding of the protein. As the Influenza A proton channel M2 is a relatively simple protein (a homo-tetramer of 97 amino acids), it will provide a good test case for the use of CG MD for protein folding/assembly. CG simulations have been used to produce a model of the M2 monomer inserted into a forming bilayer. Furthermore, with extended CG simulations, four copies of this inserted monomer are observed to tetramerise. The CG-produced tetramer has then been evaluated in terms of both its most occupied conformation and also its dynamics. This shows that the M2 tetramer appears to have dimer-of-dimers-like bundle packing, rather than a tetrameric 4-fold symmetry. Finally, the M2 structure is converted back into a fully atomistic model. Thus CG simulations can be used as a tool to construct simplified models for protein structures that can then converted to an atomistic representation which can be used to resolve the finer detail of the conformation.

1966-Pos Multi Level Insights Into The Ligand Binding Mode Of Membrane Bound Fatty Acid Amide Hydrolase

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Board B81

Fatty acid amide hydrolase (FAAH) is the primary enzyme responsible for endocannabinoid metabolism. This enzyme is biologically active as a homodimer. We have performed multi-level molecular dynamics (MD) simulations at three different levels of granularity (coarse grained, atomistic (united atom/all atom)) levels of the membrane bound FAAH. The atomistic levels included input from Quantum Mechanical/Molecular Mechanical (QM/MM) simulations. The input from QM/MM was in the form of individual snapshots of the FAAH substrate oleamide in complex with part of a hydrated FAAH monomer. These snapshots were superimposed on the atomistic protein structure and subjected to 30ns of simulation. In addition, oleamide was docked into the active site of membrane bound FAAH and subjected to 30 ns of simulation. Our studies investigated the effect of ligand binding on active site geometry and protein pore/cavity size on membrane bound FAAH. We observed more stable behaviour (2.1 Å final frame Root Mean Square Deviation from starting structure) of oleamide structures from QM/MM than those from our docking studies (3.3 final frame Root Mean Square Deviation Å from starting structure). The structures fed in from the QM/MM studies also display a greater number of hydrogen bonds formed between residues in the active site and the ligand. We observe a larger pore size (27.1 Å) in the cavity present between the two monomers, when two ligands are present. The size of the cavity decreases (16.2 Å) when only one ligand is present and further decreases (14.1 Å) in the *apo* state of membrane bound FAAH.

To conclude, our multi level studies have provided insights into the modulation of active site geometry and internal protein pore/cavity size in membrane bound FAAH, and demonstrated the usefulness of multilevel approaches to biomolecular simulation.

1967-Pos Bridging Micro- and Mesoscale Descriptions of Membrane Remodeling by NBAR Domains Through Systematic Coarse-graining

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Board B82

The BAR domain is a homodimer which is known to induce curvature in lipid bilayers during remodeling processes. In experiments, distinct phenomena are observed on long length and timescales (tubulation or vesiculation) depending on BAR domain concentration. However, a detailed understanding of how the average behavior of several hundred interacting BAR domains produces the observed phenomena is lacking. Here we present a coarse-grained model of the membrane-NBAR domain system, which bridges the length and timescale gap between previous studies at atomistic(Blood and Voth, Proc. Nat. Acad. Sc. 2006 *103*:15068) and mesoscopic scales(Ayton et. al., Biophys. J. 2007 *92*:3595). Our model is parameterized from atomistic simulation data—therefore capturing essential interactions in a systematic, rigorous way.

1968-Pos Role of Intracellular and Extracellular Carbonic Anhydrase Isoforms in pH Regulation, Illustrated with Experimental Data and Mathematical Modeling

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Board B83

Carbonic anhydrase (CA) catalyzes the reversible hydration of CO_2 to H^++HCO_3^-. Recently, the role of CA isoforms has been studied in tumors, tissues that are characterized by high metabolic acid-production and poor perfusion. Since most of their acid-output is in the form of CO_2, it has been hypothesized that CAs facilitate acid-removal. To understand the importance of CAs in removing cell-generated acid, we have performed experiments on multi-cellular, cancer-derived 'spheroids' expressing CA isoforms and simulated the data using a three-dimensional, two-compartment diffusion-reaction model. Spheroids (diameter ~0.4mm) were generated from RT112 cells expressing intracellular CAII or extracellular-facing CAIX. Spheroids were AM-loaded with the pH-dye, carboxy-SNARF-1 for confocal intracellular pH (pH_i) imaging, and superfused in 5%CO_2/22mM HCO_3^- medium. At steady-state, spheroids expressing CAII developed intracellular acidosis of 0.3pH units in core-regions relative to the periphery. This pH_i-gradient was greatly collapsed in the presence of CAIX. To study the rate of CO_2 removal, the superfusion was switched rapidly to 0%CO_2 (Hepes-buffered) medium and the ensuing rise in pH_i was measured in core and peripheral regions (0.06mm-wide). In spheroids expressing only intracellular CA, pH_i-changes in the core lagged by ~10s relative to the periphery. In spheroids expressing CAIX, this delay was greatly reduced. In the absence of CA activity (+0.1mM acetazolamide), pH_i-changes in both the periphery and center were considerably slower. These experimental data were best-fitted with the model. Fast CO_2 removal from cells requires

(i) sufficient intracellular CA to generate CO_2 from intracellular titration of acid against HCO_3^-, and
(ii) extracellular CA to facilitate diffusion of CO_2 across the extracellular space, away from cells. Insufficient levels of extracellular CA will lead to spatially uncoordinated pH_i-recovery. Inadequate levels of intracellular CA will produce slower pH_i-changes.

1969-Pos All-atom Model of Rhodobacter sphaeroides LH1-RC-PufX Dimer and its Structural Consequences

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Board B84

In photosynthetic purple bacteria, photosynthesis is carried out by only a handful of proteins that aggregate in the bacterial plasma membrane, forming indentations known as chromatophores. Crystal structures of nearly all key protein complexes in purple bacteria chromatophores have been made available, the exception being the light harvesting complex 1 (LH1). In all species of purple bacteria, LH1 directly surrounds the reaction center (RC) to form the so-called LH1-RC core complex, and currently there exists no high resolution structure for LH1. Additionally, in the Rhodobacter (Rb.) species a polypeptide known as PufX is found within the LH1-RC complex, but its precise location is still under debate. Here we present an all-atom model of the Rb. sphaeroides LH1-RC dimer based on a combination of NMR, X-ray crystallography, cryo-electron microscopy (cryo-EM) and homology modeling. Molecular dynamics simulations of a single LH1-RC dimer in a solvated lipid bilayer (∼700,000 atoms) shows spontaneous bending at the LH1 dimerizing junction, induced mainly by the shape of the RC moieties. The PufX polypeptide was also included in the LH1-RC model and placed near the LH1 gaps, a placement suggested by cryo-EM data. Simulations of the LH1-RC-PufX dimer and lipid bilayer system show that it remains stable after 20 ns of equilibration. Furthermore, simulations of a multiple-dimer system indicate that an array of dimers induces membrane curvature, in agreement with mutant studies that observed formation of tubular chromatophores when only LH1-RC-PufX dimers are present.

1970-Pos Where is the Photosynthetic BC1-Complex Located in the Chromatophore

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Board B85

In purple bacteria, chromatophores are photosynthetic organelles that exist as spherical or lameller invaginations of the inner cellular membrane. The spatial arrangement of the transmembrane proteins in the chromatophore has been shown to influence its photosynthetic efficiency. These same proteins are suspected to play a key role in generating the membrane curvatures necessary for the chromatophore's overall shape. Recent atomic force microscopy (AFM) images of vesicular chromatophores from Rhodobacter sphaeroides have renewed interest in how the self-organization of these key membrane proteins contribute to organelle shape. Interestingly, the cytochrome bc1-complex, which is necessary for photosynthetic function, is absent from the AFM images. This raises the question: where is the bc1-complex located in the chromatophore?

Three potential locations with three unique curvatures exist for the bc1-complex in the chromatophore. These include the spherical bulb of the vesicle with a positive curvature, the membrane surrounding the vesicle with no curvature, and the neck connecting the vesicle to the surrounding membrane with either a negative or saddle-shaped curvature.

Molecular dynamics simulations of single and multiple bc1-complexes in membrane patches were completed to determine the membrane curvature surrounding bc1. Simulations of individual bc1-complexes led to the spontaneous formation of negative membrane curvature, suggesting that the bc1-complex is located in the neck of the chromatophore. The observed curvature was directed along the axis of the cytochrome-C1 subunits in the bc1-dimer, indicating the probable orientation of the bc1-complex within the neck region of the chromatophore. Finally, atomic-level interactions, that may contribute to this curvature were identified and include the asymmetric burial of two amphiphilic helices in the periplasmic leaflet of the lipid bilayer.

1971-Pos Structure, Dynamics, and Position-Resolved Free Energy of Amino Acid Solvation in Membranes From Molecular Simulation

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Board B86

Membrane proteins play key roles in a wide range of processes in thecell, including transport and signaling. The fundamental structural units of this class of proteins are transmembrane helices, where the classical view has long been that exposed residues have to be almost exclusively hydrophobic. Still, several studies have shown that it is possible to insert polar residues, they can be important to drive helix aggregation, and voltage-sensing domains in ion channels might even require charged residues inside the hydrophobic membrane core.

Here, we report on an extensive set of computer simulations that have been used to examine the solvation properties of each amino acid side-chain type in all possible helix positions. Interestingly, polar and charged residues retain significant amounts of water, even to the extent where many of them exist in a microscopic water environment without major distortion of the membrane. Basic sidechains are found to frequently form hydrogen bonds with lipid carbonyl groups, a pattern we also confirm by a narrower dip in their relative occurrence inside bilayers compared to acidic residues. We have further been able to calculate the position-dependent free energy of solvation as a function of the membrane z-coordinate for all amino acid side-chain analogs.

The relative correlation between computational and in vivo scales is quite high, but the former appears to be "compressed" such that

the magnitude of all values are much lower. One possible explanation for this could be the existence of a hydrophobic barrier inside the translocon responsible for the selection of helices to insert into the membrane.

1972-Pos The Roles Of The Pore Ring And The Plug In The SecY Protein-conducting Channel

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Board B87

The translocon, present in all domains of life, is a membrane-bound channel that allows nascent proteins to cross the membrane or to integrate into the membrane. The crystal structure of an archaeal translocon, the SecY complex, revealed a small "plug" domain blocking the center of the channel as well as a pore ring composed of six residues acting as a constriction point. Recent experiments have indicated that deletion of the plug domain neither inactivates the channel nor kills the cell but does increase the channel's resting permeability to ions. New crystal structures of two such plug deletion mutants revealed new plugs had formed, still blocking the channel, leaving open the question of how the plug serves to close the channel. Using molecular dynamics simulations, we have investigated these two mutants to understand the origin of the measured permeability and the roles of the plug and the pore ring. We found the new plugs to be unstable, giving rise to a concomitant increase in flexibility of the pore ring. The rate of permeation of water molecules as well as the force required for simulated translocation of a small deca-alanine helix were used to distinguish between the mutants and the native channel, showing qualitative agreement with experiments. The role of the plug in stabilizing the pore ring was found to be significantly more important than its role as a purely steric barrier in keeping the resting channel closed.

1973-Pos A Continuum Method For Determining Membrane Protein Insertion Energies

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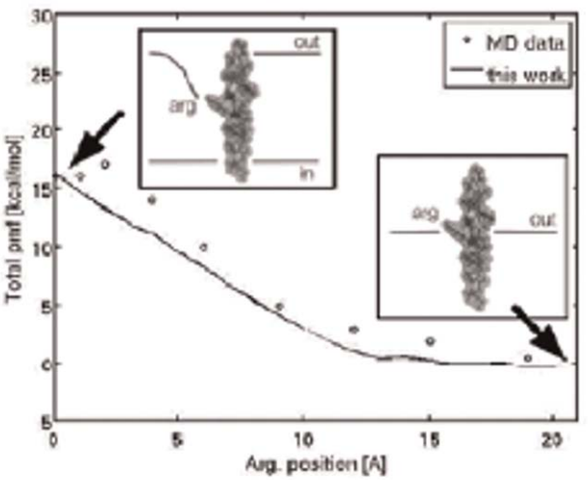
Board B88

Continuum electrostatic approaches have been extremely successful at describing the charged nature of soluble proteins[1]. These calculations also quantitatively reproduce the energetics of partitioning small molecules between bulk phases[2]. However, it is unclear whether continuum methods can be used to understand the electrostatic component of the energy for charged membrane proteins. Recent molecular dynamics (MD) simulations show that the energy required to insert charged molecules into lipid bilayers[3,4] is much smaller than would be expected based on continuum calculations[5]. The primary reason for this failure is the dielectric inhomogeneity of the bilayer and the formation of water defects.

Here, we show that first using elasticity theory to determine the shape of the deformed membrane and subsequently using this shape in a continuum electrostatics calculation circumvents these shortcomings. Our method reproduces the quantitative features of detailed MD simulations at a tiny fraction of the computational cost (See figure).



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1974-Pos Improving Prediction of G-Protein Coupled Receptor Loops

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Board B89

Loop regions in G-protein coupled receptors (GPCRs) exhibit low sequence identity and variable length due to insertions and deletions. Thus homology modeling with the loops of rhodopsin, the only GPCR of known structure, is not feasible. Because of the importance of GPCR loops in signal transduction, *de novo/ab initio* strategies are being developed. Notably, Monte Carlo (MC) simulations in a temperature annealing protocol combined with a scaled collective variables (SCV) technique [Mehler, E.L. et al., *Proteins* 2006; *64*:673–90] and coarse-grained backbone dihedral sampling [Nikiforovich, G.V. & Marshall, G.R. *Biophys. J.* 2005; *89*:3780–89], were recently shown to accurately predict short loop regions of GPCRs. Like other *ab initio* loop prediction methods, the performance of these methods clearly deteriorates at increasing loop

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

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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

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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

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