Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Board B46

Autonomous reconstitution of the seven transmembrane (TM) domains of the human serotonin receptor subtype 6 (5-HT_6) in micelle and lipid bilayer environments is described. Peptides representing the seven α-helical TM domains were synthesized and reconstituted in both detergent micelles and liposomes. Assembly of the seven TM (7TM) peptides was monitored by fluorescence resonance energy transfer (FRET) between donor and acceptor probes labeled at the amino termini of the second and fourth TM-peptides, respectively. FRET analysis suggests that the 7TM assemblies exist as ordered assemblies, and binding studies showed that reconstituted 7TM-peptides in liposomes selectively bind to free serotonin and serotonin-conjugated magnetic beads, yielding a dissociation constant of 0.83 ± 0.14 μM. These results show that the seven individual TM domains of a rhodopsin-type G protein coupled receptor (GPCR) can spontaneously assemble in liposomes into a conformation that mimics a native structure, and further demonstrate that specific interactions between TM helices play a critical role in the folding and stabilizing of GPCRs. Furthermore, the autonomous assembly of 7TM-peptides can be applied to the screening of agonists for GPCRs that are difficult to manipulate.

1931-Pos The Single Channel Water Permeability Coefficient Of Aquaporin-1 Revisited

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Board B47

It is generally accepted that water passes aquaporin-1 (AQP1) at a rate that is close to the diffusion limit. Permeability coefficients of 6 and 11 x 10^{-14} cm^3/subunit/s were derived from swelling experiments carried out with overexpressing oocytes or with reconstituted liposomes. The actual AQP1 transport rate might have been several-fold higher, due to known limitations in accuracy of membrane protein concentration assessment by biochemical or histochemical means. In addition, the unstirred layers in the oocyte system have to be taken into account, and there are variations in the reconstitution efficiency into liposomes. Since such an observation would change the current molecular picture of how water is transported trough single file channels, we now have combined fluorescence correlation spectroscopy (FCS) and scanning electrochemical microscopy to determine the single channel permeability coefficient. FCS was used to count the AQP1 copies reconstituted into planar lipid bilayers and ion sensitive microelectrodes were used to detect water flux induced changes in solute concentration adjacent to these bilayers. The derived value of (4 ± 0.5) x 10^{-14} cm^3/subunit/s is in reasonable agreement with previously published data.

1932-Pos Dependence Of Single Water File Transport Rate On The Solvation Environment At The Channel Mouth

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Board B48

According to atomistic molecular dynamics simulations, the loss of solvation forms the main energetic barrier for water permeation in gramicidin like channels . However, the subsequent conclusion about the invariance of transport rate on channel length is at odds with the experimentally found exponential increase in single channel water permeability as the channel shortens . The observed length dependence may be caused by

(i) water binding to specific accommodation sites in a fully occupied pore or
(ii) liquid vapour oscillations in the channel, i.e. reduced water density in the pore.

Whereas hypothesis

(i) predicts that the re- and dehydration of water in the entry/exit regions negligibly contribute to the total energy barrier, hypothesis
(ii) envisions that the transition step at the channel mouth and the channel permeation step contribute in a comparable fashion to the total permeation resistance.

We now have measured water permeability of gramicidin-A molecules bearing a hydrophobic group (tert-Butyl-diphenylsilyl) at the C-terminus by imposing an osmotic gradient across the hosting lipid bilayer and detected tiny concentration shifts of an impermeable solute in the immediate membrane vicinity. The lower transport rate of the derivative suggested a significant increase in water solvation energy. Exactly the same conclusion was drawn from single channel water permeability measurements carried out after the removal of charged lipid headgroups or the phosphate moieties of the lipids. Thus, both the solvation environment at the channel mouth and channel length are important determinants of single channel water permeability suggesting that liquid vapour oscillations reduce the density of water molecules in the channel.

Membrane Proteins - II

1933-Pos Gating of the Protein Translocation Channel SecY

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Board B49

Department of Cell Biology Harvard Medical School, Boston, MA, USA. Many proteins are translocated across the endoplasmic reticulum (ER) membrane or the bacterial plasma membrane through a conserved channel, formed by a heterotrimeric protein complex (called the Sec61p complex in eukaryotes and the SecY complex in bacteria and archaea). The resting the channel is impermeable to ions and water (Saparov et al., 2007). Channel opening requires the insertion of the translocating polypeptide chain, with the signal sequence intercalated into the lateral gate. Structural rearrangements of the channel must occur before the translocation substrate can insert. Here we show that the energy required for these conformational changes may be provided by the binding of non-translating ribosomes or of the ATPase SecA. In their presence purified SecY complexes, reconstituted into planar membranes, exhibit massive ion channel activity, suggesting that channel partner binding prepares the channel for the subsequent insertion of a translocating chain. The ion flow is likely reduced when the translocating protein occludes the channel.

References

Saparov, S.M., Erlandson, K., Cannon, K., Schaletzky, J., Schulman, S., Rapoport, T.A., and Pohl, P. (2007). Determining the Conductance of the SecY Protein Translocation Channel for Small Molecules. Mol. Cell *26*, 501–509.

1934-Pos The Interprotein Electron Transfer between Sulfite Oxidase and Cytochrome c

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Board B50

Sulfite oxidase is an essential enzyme responsible for the oxidation of sulfite as the terminal step in the metabolism of sulfur containing amino acids. The dimeric enzyme functions via a two domain sequential intramolecular electron transfer from a molybdoterin cofactor to a heme b5 type redox center followed by the subsequent intermolecular reduction of two equivalents of cytochrome c. We have previously shown the utility of electron transfer initiation via the photooxidation of Ru(bpz) labeled cytochrome c in reaction with cytochrome b5, and here extend this method to the electron transfer between sulfite oxidase and cytochrome c. Laser excitation of a complex between reduced sulfite oxidase and Ru-39-cyt c leads to rapid photooxidation of heme c, followed by intracomplex electron transfer from heme b of sulfite oxidase to heme c with a rate constant of 1.0 x 104 s-1. As the ionic strength is increased, the complex dissociates and a slower bimolecular reaction is observed.

1935-Pos Electrochemically-controlled Fluorescence Spectroscopy of Membrane Proteins in a Biomimetic Surface Architecture

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Board B51

Cytochrome c oxidase from *P. denitrificans* was immobilized via the histidine-tag technology on an ITO surface. The immobilization was followed by *in-situ* dialysis of solubilized lipids to form a protein-tethered bilayer lipid membrane (ptBLM) developed previously on metal surfaces (gold, silver)[1]. The ITO surface was used to avoid quenching of the fluorescence and the absorption of light.

The functional preservation and the structural integrity of the membrane protein embedded in the ptBLM system on ITO was verified by electrochemical impedance spectroscopy and cyclic voltammetry. Characteristic changes of the impedance spectra were detected such as the decrease of the resistance due to proton translocation when the enzyme was activated by cytochrome c.

The transmembrane potential self-generated by the CcO during turnover was measured by fluorescence measurements using a confocal microscope. For this purpose The CcO is mixed on the surface with the light harvesting complex LHCII from higher plants as a detector molecule. The fluorescence spectrum of the LHCII undergoes a potential dependent shift of fluorescence wavelength (Stark shift) which can be used to measure the transmembrane potential self-generated by the CcO. The Stark shift was calibrated by applying defined electric fields between the ITO surface and the Ag/AgCl reference electrode.

Moreover, the transmembrane potential was measured using the potential dependent FRET efficiency between the donor di-8-ANEPPS and the acceptor dipicrylamine located within the membrane. The FRET efficiency depends on the distance between donor and acceptor as a function of the potential applied and/or generated.

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1936-Pos In vitro Synthesis of Membrane Proteins into artificial membrane Systems shown by a significant example: vectorial insertion of G-Protein Coupled Receptors

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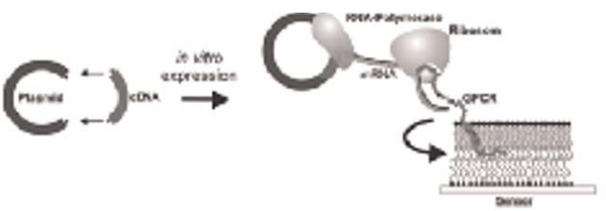
Board B52

The field of GPCR (G-protein coupled receptor) research is currently populated by intense worldwide activities concerning their structural resolution as prerequisite for structural-based drug design. Expression (synthesis), purification and cystallization still pose bottle-necks. There is large interest in functional aspects of GPCR receptors, such as olfactoric receptors, the molecular basis for the sense of smell. The development of an 'artificial nose' for sensing applications is an ongoing issue in basic research, industry and environmental affairs. The molecular concept of olfaction, provides unsurpassed recognition properties, but is not yet addressed in sensor developments.

The reason behind exists in the unavailability of odorant receptor material. Our solution to the problem is the generation of protein not by isolation from cells, but by combination of coding DNA with the protein synthesis machinery of a cell-extract (in vitro transcription and translation). We leave it to the protein synthesis machinery of a cell extract to 'understand' the genetic information of a membrane protein and to generate a protein functionalized membrane for biophysical analysis, such as protein insertion, ligand binding and membrane interactions.



1937-Pos Splice Variant Dependence in Polarized Targeting of Slo Channel in Epithelial Cells

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Board B53

Splice variation can modify Slo traffic by intracellular retention but splicing role in polarized targeting is unknown. In *Madin Darby canine kidney* (MDCK) cells, expressed Slo is targeted to the apical surface, whereas in *human bronchial epithelial* (16HBE14o$^-$) cells channel activity was reported at the basolateral membrane. Here, we tested the hypothesis that splice variation can explain this difference. Using high-resolution confocal microscopy and specific antibodies, we confirmed that in MDCK cells endogenous Slo is mainly expressed at the apical surface; while 16HBE14o$^-$ cells showed expression at both apical and basolateral surfaces. RT-PCR demonstrated that 16HBE14o$^-$ cells have robust expression of a 29 amino acid spliced exon (SV29) but not MDCK cells where its expression was minimal. The role of this spliced exon was tested using viral constructs of Slo attached to EGFP with and without SV29 (Slo-EGFP and Slo-SV29-EGFP). After viral infection of MDCK cells under non-saturating conditions, Slo-EGFP showed apical expression, meanwhile, Slo-SV29-EGFP was also expressed in the basolateral surface suggesting an intrinsic basolateral signal in SV29. Furthermore, fusion of SV29 to a classical apically targeted protein -the non-related neurotrophin receptor p75- caused its targeting to both apical and basolateral surfaces of MDCK cells. Interestingly, expression of Slo-EGFP lacking SV29 in 16HBE14o$^-$ cells resulted in expression at both surfaces indicating that the particular cell machinery also plays a role in directing polarized targeting. Consistent with this view, deletion of the reported Slo apical signal analyzed in cortical collecting duct kidney cells did not result in loss of apical expression in MDCK cells. Thus, our data indicate that SV29 contains an intrinsic signal for Slo basolateral targeting in MDCK cells and that specific cell machinery also plays a role in targeting.

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1938-Pos The Birefringent Myelin Bodies in Renal Development Amplifies the Comprehension of Anderson-Fabry Disease

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Board B54

Maltese-cross birefringence of myelin bodies has become a typical diagnostic characteristic used to evaluate the kidney complications of Anderson-Fabry Disease, an X-linked recessive disorder on chromosome Xq22.1. The myelin bodies seriously damage the glomerular filtration barrier and are responsible for the mechanism of proteinuria. However, our recent work has demonstrated that the birefringent myelin bodies also signify the physiological formation of the kidney during the mesonephros and metanephros stages of development.

1. Under polarization microscopy, these Maltese-crosses are initially observed in E8 in mesonephros. The number of these Maltese-crosses increases during development of the embryo and the size of the birefringent myelin bodies continuously enlarge until E13.

2. In the metanephros phase, the Maltese-crosses are first observed at E14. Both the number and size of the Maltese crosses increase with the development of the embryo. The birefringent Maltese crosses can still be observed until postnatal day, P2 or P3. After this stage, the Maltese crosses with liquid-crystal characteristics can rarely be detected in the matured kidney by polarization microscopy.
3. Our data demonstrated that the Maltese cross configuration is typical of lamellar bodies.

These cytoplasmic inclusion bodies display the concentric lamellation with characteristic "zebra" or "onion skin" appearance. In both mesonephros and metanephros tissues, this lamellar bodies are mainly found in the cytoplasm of proximal tubular epithelial cells. They can also be detected in the lumen of proximal tubules. Thus, we hypothesize that there is an embryonic program for the activation of these myelin bodies. During development of the embryo, this program facilitates kidney organogenesis, but reactivation of the program during adolecence may lead to manifestations of the Disease. Finally, the accumulation of glycophospholipids/glycosphingolipids results in the severe pathological progression ranging from renal insufficiency to end-stage renal failure(ESRF).

1939-Pos Vrfrap: An Effective Approach To Analyse Membrane Compartmentalization. Application To The Problem Of The Hiv Entry

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Board B55

The entry of human immunodeficiency virus into target cells requires successive interactions of the viral envelope glycoprotein gp120 with CD4 and the chemokine receptors CCR5 or CXCR4. We previously demonstrated, by FRET experiments, the constitutive association of CD4 and CCR5 at the surface of living cells *(1)*. We therefore speculated that this interaction may correlate with compartmentalisation of CD4 and CCR5 within the plasma membrane. Here, by means of fluorescence recovery after photobleaching at variable radii (vrFRAP) experiments, we characterise the lateral distribution, the dynamics and stoichiometry of these receptors in living cells stably expressing CD4 and/or CCR5 *(2)*. We found that

(i) these receptors expressed alone are confined into micrometer-sized domains
(ii) these receptors co-expressed revealed a CD4-CCR5 association which occurs outside and inside smaller domains
(iii) the complexes involve multiple CCR5 molecules per CD4.

References

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(2). "CD4 interacts constitutively with multiple CCR5 at the plasma membrane of living cells: a vrFRAP approach"; Baker AM, Saulière A, Gaibelet G., Lagane B., Mazères S., Fourage M, Bachelerie F., Salomé L., Lopez A. and Dumas F., (2007), J. Biol. Chem. (in press)

1940-Pos Expression, Refolding and Characterization of Predicted Beta-Barrel Proteins from Salmonella typhimurium LT2

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Board B56

Approximately 3% of a bacterial genome encodes outer membrane-spanning beta-barrel proteins, yet the number of beta-barrels that have been structurally characterized totals less than 30. Identifying more beta-barrels is of great interest because their myriad functions and accessibility make them tempting targets for novel vaccine and therapy development. We have predicted several genes that encode beta-barrel proteins in *Salmonella typhimurium* LT2 using a prediction algorithm developed in this lab. We are currently characterizing the gene product of one candidate, *ompL*, which was expressed in *Escherichia coli* BL21(DE3). OmpL was found to be associated with the outer membrane fraction using differential solubilization to selectively isolate the proteins of the outer and inner membranes. OmpL was overexpressed into inclusion bodies, which were subsequently denatured with 8 M urea, and then purified by reversed-phase high performance liquid chromatography (RP-HPLC). OmpL was refolded in a mixed detergent system of 3.5 mM SDS (sodium dodecyl-sulfate) and 30 mM DDM (n-dodecyl-β-D-maltopyranoside). Changes in OmpL's mobility through SDS-PAGE suggest that the protein refolded into a native-like conformation as has been observed for other outer membrane proteins. We have determined, via spectropolarimetry, that this protein is beta-sheet rich when refolded. Refolding OmpL in SDS/DDM also caused the tryptophan fluorescence emission to blue-shift slightly and increased its intensity more than two-fold compared to OmpL in SDS alone. OmpL will be inserted into liposomes for further characterization which includes assessing binding to the bilayer via tryptophan fluorescence quenching and characterizing pore formation via liposome swelling assays; several other candidates will be cloned and similarly characterized. The results from this prediction and screening will then be used to generate a public database of outer membrane proteins and their properties.

1941-Pos Stabilization And Characterization Of Membrane Proteins In Solution

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Board B57

Membrane proteins assemblies in solution are often not stable and their characterization is often controversial, because of the association of detergent and lipids sometimes in large amounts, the coexistence of detergent micelles of similar size, and the poor understanding of the effect of changing the solvent composition (see e.g. . Cohen et al. J. Biol. Chem. 2005; Winstone et al. BBRC 2005; Ravaud et al. Biochem. J. 2006). Following our past works on the stabilization of soluble proteins by salt (Ebel & Zaccai, J Mol Recognit 2004), we adapt protocols, mainly based on analytical ultracentrifugation sedimentation velocity, to characterize samples of solubilized membrane proteins (Ebel et al., under press, In: Biophysical approaches of structure and functions of membrane proteins, Pebay-Peyroula Eds, Wiley). We use a thermodynamic approach to rationalize the effect of detergent on protein stability (see e.g. Josse et al., J. Mol. Biol. 2002). Finally, in collaboration with Tribet (ESPCI), Pucci (University of Avignon), Popot and Breyton (IBPC), we characterize complexes of membrane proteins, bacteriorhodopsin and cytochrome b6f, with new compounds, amphipatic polymers or hemifluorinated detergents, which were designed to substitute to detergents for the stabilization of membrane protein assemblies in solution (Gohon et al. Anal. Biochem, 2004; Langmuir 2006 and submitted; Lebaupain et al. Langmuir 2006).

1942-Pos AFM Characterization of Nanolipoprotein Particles and Their Association with Membrane Proteins

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Board B58

Membrane proteins account for more than 30% of cellular proteins. Although these complexes are essential in cellular processes they have proven quite challenging to study due to their hydrophobicity and propensity to aggregate in solution. Recently it has been demonstrated that nanolipoprotein particles (NLPs) can act as a versatile method for stabilizing and solubilizing membrane proteins. NLPs form through self-assembly and consist of amphipathic lipoproteins encircling a lipid bilayer resulting in nanometer scale lipoprotein particles that self-assemble from the apolipoprotein and lipid components. In addition, when membrane proteins are present during NLP self-assembly the membrane protein can be encapsulated within the NLP.

Here we present high resolution AFM imaging of both empty and bacteriorhodopsin (bR) containing NLPs formed through three different techniques; cell-free expression of lipoprotien, and bR, in the presence of 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) liposomes (referred to as co-expression); cell free expression of bR in the presence of pre-formed empty NLPs (referred to as a co-translation); and assembly in solution of purified lipoproteins, bR and DMPC liposomes (referred to as traditional assembly)

Both empty NLPs and bR associated NLPs displayed discrete heterogeneous size distributions. In addition, due to the high Z-resolution of AFM, empty vs. bR containing NLPs could be distinguished due to a 1.5 nm increase in height for NLPs containing bR. Using height differences as a distinguishing feature, the percentage of NLPs containing bR was compared to empty NLPs as a function of NLP size. From this analysis there was an observed increase in percentage of bR associated NLPs for larger NLPs. This was shown to be the case regardless of the method used to produce the NLP. This data suggests a possible mechanism for membrane protein inclusion in NLP particles.

1943-Pos Correlated Single Molecule Imaging Approaches to the Determination of Structure-Function Relationships in Membrane Proteins

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Board B59

Recent efforts by our group and others have demonstrated that in situ atomic force microscopy (AFM) is an exceptionally powerful means of characterizing biomolecular self-assembly, particularly at model membrane interfaces. We describe here our use of this approach, in conjunction with other correlated spectroscopic and optical imaging techniques, including TIRF and FT-IR spectroscopy, to examine conformational changes in model membrane-reconstituted systems, including the insulin-insulin receptor complex, the CorA ion transporter, and the CEACAM family of proteins. This in situ imaging approach provides an excellent means of characterizing domain preference, as well as ligand-binding induced conformational changes and membrane restructuring.

1944-Pos Examination of Reconstitution Condition for AFM Observation of an Ionotropic Glutamate Receptor

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Board B60

Ionotropic glutamate receptors (iGluRs) are important membrane proteins for extracellular signaling in the neuronal networks of the central nervous system. Atomic force microscopy (AFM) enables the nano-scale observation of proteins in a liquid environment, offering a unique opportunity to observe functional biological molecule such as single protein molecules under physiological conditions.

In our studies, we have succeeded in observing structure of purified and reconstituted iGluR in solution using the AFM. The

iGluRs were purified from over-expressed insect cells and then reconstituted into an artificial lipid bilayer by dialysis. We then imaged the reconstituted protein on a substrate under HEPES buffer using AFM. We could observe that its structure consisted of four subunits of a size consistent with the previous electron microscopy reports. In our structural observations, one of the key issues is the sample preparation; single reconstituted proteins needed to be situated in the middle of the lipids domains to ensure that the protein was not influenced by the interfacial regions of the domains.

In this study, we examined the reconstitution condition of proteins for AFM observation of single membrane proteins. We changed the composition of the solution; lipid, detergent and protein, and continued the reconstitution process by dialysis against HEPES buffer. Reconstituted samples were then observed by AFM under the buffer solution. From this study, we determined that the ratio of lipid, detergent and protein were critical to the reconstitution result. At the certain ratio, protein molecules were dispersed into single proteins and reconstituted into the middle of the lipid domains. This result has realized the structural examination of a single receptor protein. Our studies thus suggest the possibility of determining a real-time conformational change of a functioning membrane protein using AFM.

1945-Pos Quantitative Measurements of Protein Dimerization and Activation in Cellular Membranes

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Board B61

Processes in the plasma membrane mediate the communication between a cell and its environment and are thus vital to cell life. Yet, they are difficult to study because they occur in the context of the heterogeneous bilayer mileu. Furthermore, the traditional tools of cell biology used in many studies lack quantitative power and currently provide only a qualitative understanding of these membrane processes. Here we provide the first thermodynamic description of the process of signal transduction across the plasma membrane, as mediated by the membrane receptor Neu (ErbB2), and we calculate the free energy associated with Neu activation. We further find that pathogenic hydrogen bond in the transmembrane domain of RTKs stabilize the active dimeric state by about -1 kcal/mole. These measurements demonstrate that the thermodynamics behind receptor activation in cellular membranes can be carried out in a quantitative manner, based on measurements of phosphorylated (activated) and total number of receptors using Western blots. The method presented here provides a straightforward way to compare the dimerization propensities of membrane receptors that play crucial biochemical roles and are implicated in human diseases.

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1946-Pos Spectroscopic Kinetic and Thermodynamic Studies of Beta-Lactoglobulin with Model Membrane Vesicles

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Board B62

Bovine beta-lactoglobulin (β-LG) is a lipocalin protein found in mammalian milk. In the native state, its secondary structure is dominated by β-sheet, though it has the propensity to form α-helices based on secondary structure predictions. Helical formation has been observed during an early stage of β-LG folding from random coil to native state. Our recent studies showed β-LG can adopt a significant fraction of α-helical conformation upon mixing with synthetic phospholipid vesicles. Here we studied the kinetics of the binding process for β-LG and various vesicles using simultaneous stopped-flow CD and fluorescence. Their comparison showed at least two different phases in the kinetic mechanism. Electrostatic, hydrophobic interactions and lipid packing affect the rate constants. Combining the results of these studies, we have developed a model in which β-LG binds and unfolds on the membrane surface and then forms helical components, which insert into the membrane bilayer. Furthermore, the function of β-LG was studied by calcein leakage experiment showing whether the protein could induce membrane leakage after binding.

1947-Pos Membrane Lipids Modulate Prestin-Associated Charge Movement

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Board B63

A membrane-based motor in the cochlear outer hair cell is required for mammalian hearing. Prestin is an important component of this motor and is a member of the SLC26A anion transporter family. Prestin greatly increases anion (mainly chloride) movement into and out of, as opposed to through, the membrane. The prestin-associated charge movement (q) can be monitored with voltage-clamp and is affected by membrane lipids. Increasing membrane cholesterol shifts the q/V function ~ 50 mV in the hyperpolarizing direction, possibly related to increases in membrane stiffness. The voltage shift shows a saturating concentration dependence and, over time, there is a decrease in total charge moved (presumably from cholesterol induced increase in prestin endocytosis). Decreasing membrane cholesterol shifts the q/V function ~ 50 mV in the depolarizing direction with no effect on the amount of charge moved. Increasing membrane docosahexaenoic acid (DHA) shifts the q/V function in the hyperpolarizing direction < 15 mV. Intriguingly, DHA increases total charge moved (as opposed to decreasing it with cholesterol), raising the possibility that DHA promotes larger conformational changes allowing more chloride to move in and out of the membrane. The results demonstrate lipid-protein interactions are impor-

tant for the functioning of the membrane-based motor. Similar lipid-protein interactions involving cholesterol and DHA have been observed for rhodopsin function.

Supported by research grant DC 00354 from NIH/NIDCD.

1948-Pos Mapping Proton Gradient and Local Dielectric Constant along a Transmembrane Helix

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Board B64

Local electrostatic interactions and hydrogen bonding belong to the major molecular forces that govern membrane protein structure and function but remain to be the most elusive parameters that could be accessed experimentally. In the past penetration of water, metal ions, and protons into model unperturbed lipid bilayers have been accessed by X-ray, EPR, and NMR as well as by molecular dynamics simulations. However, these parameters are likely to be different in the vicinity of membrane proteins. Here we describe mapping of proton gradient and local dielectric properties along the single transmebrane α-helix formed by WALP peptide inserted into anionic DOPG (1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]) bilayers. A series of cysteine WALP mutants were modified with pH-sensitive thiol-specific spin label IMTSL that occupies the volume comparable to that of phenylalanine. This unnatural side chain exhibits pH-dependent EPR spectra allowing us to determine the probe pK_a and corresponding Gibbs free energies from a series of EPR titration experiments. The surface potential of DOPG was determined from titration of spin-labeled pH-sensitive phospholipids we have recently synthesized. Effects of solvent dielectric constant on Gibbs free energy of the label protonation were calibrated in a series of titration experiments in water/ *iso*-propanol mixtures. The combination of these data allowed for separating electrostatic and dielectric contributions to protonation of WALP side chains. Additional EPR oxygen accessibility measurements demonstrated that the position of the probe within the lipid bilayer is only slightly affected by the changes in its ionization state and is within 10% of the depth of the nonprotonated WALP side chain.

Partially Supported by NIH R01GM072897 to AIS.

1949-Pos Board B65 Characterization Of The Putative Fusion Peptide Of The SARS Coronavirus S2 Glycoprotein

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Board B64

SARS is an atypical pneumonia caused by SARS coronavirus. The spike glycoprotein S from SARS-CoV is a surface glycoprotein that mediates viral entry by binding to the cellular receptors and induces membrane fusion, and has been classified as a Class I transmembrane glycoprotein. Class I viral proteins contain a hydrophobic region denominated as the fusion peptide and other hydrophobic region immediately adjacent to the membrane-spanning domain denominated as the pre-transmembrane domain. Although the exact mechanism by which SARS-CoV enters the host cell has not been elucidated, it is most likely similar to other coronaviruses. The fusion peptides interact with and insert into target membranes since this region represents a surface patch with high bilayer-to-water transfer free-energy values. Fusion peptides also participate in inducing lipid rearrangements giving place to hemifusion and pore formation and are also involved in pore enlargement. While much progress has been made in understanding the implication of fusion peptides in the membrane fusion process, available data concerning the fusion peptide of coronaviruses, particularly in the case of SARS-CoV, are scarce. In the present work we identify and report on the determination of the secondary structure and the interaction with model membranes of a peptide pertaining to the fusion domain of SARS-CoV, the structural changes which take place in both, the peptide and phospholipid molecules, induced by membrane binding and interaction with the lipid bilayer through a series of complementary experiments such as leakage, fusion and aggregation. Moreover, we show that SARS-CoV FP strongly partitions into phospholipids membranes and can be localized at different depths depending on the phospholipid composition of the vesicles.

1950-Pos Effects of Residue-Sequence Mutations in the Peptides Mimicking the Transmembrane Domain of Sindbis Virus: Spin-Labeling EPR Studies of Peptide Membrane Insertion

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Board B66

In Sindbis enveloped virus a host-derived membrane bilayer is "sandwiched" between the concentric protein shells and is penetrated by the transmembrane domain anchors of three glycoproteins. Those protein domains are capable of assembling in two strikingly different membranes: mammalian membranes that contain up to 40% of cholesterol and insects membranes that contain shorter unsaturated lipids and no cholesterol. Recently, it was shown that mutations in the transmembrane domain of the Sindbis virus E2 protein produce deferential alterations in the protein association with the lipid bilayer: some mutants were able to grow in insect cells, but not in mammalian cells [1,2]. The Sindbis virus with STM-16 deletion mutation of the E2 transmembrane domain shows the most pronounced differential growth in mammal and insect sells. In the attempt to understand constraints placed upon membrane spanning domains for correct integration into the bilayer we have investigated the interaction of synthetic peptides mimicking wild type transmembrane domain of the E2 glycoprotein and its mutants with lipid bilayers. The phospholipid composition was chosen to represent mammalian and insects' membranes. Results of EPR spin-labeling experiments show that STM-16 peptide adopts a trans-

membrane configuration in bilayers with lipid composition mimicking that of insects. In mammalian cell mimicking membranes that contain cholesterol the STM-16 peptide aggregates at the surface of the bilayer.

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1951-Pos The N-terminus of the ENTH Domain Forms a Boomerang-Shaped Anti-parallel Dimer on the Surface of the Membrane

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Board B67

The epsin NH2-terminal homology (ENTH) domain plays a crucial role in clathrin-mediated endocytosis that is necessary for a diverse number of cellular processes. It has been suggested that N-terminus of ENTH domain can bind to specific lipid PIP2 that has two phosphate groups at position 4 and 5. The crystal structure of the ENTH domain showed that PIP2 helped the formation of helix 0 at the N terminus that was not present in the absence of PIP2. We used site-directed spin labeling EPR to determine the membrane-bound structure of the N terminus of the ENTH domain. Our data confirmed that the N terminus of the ENTH domain inserted into the membrane as an α-helix with an angle of degree 23. Very interestingly, the EPR results showed that helix 0 could oligomerize in the membrane in an anti-parallel fashion. Such a boomerang-shaped membrane binding mode could certainly assist formation of the membrane curvature as other regulator proteins such as the bar domains do.

1952-Pos Defining The Properties Of Cell-impermable Cargo Molecules That Can Be Translocated By The pHLIP-bionanosyringe Across Cell Membrane

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Board B68

Major goals in cancer therapy include the development of approaches to specifically target tumors and deliver drugs across cell membranes. We have discovered a novel approach to target acidic tumors in vivo by employing the pH-selective insertion of a membrane peptide, pHLIP (pH (Low) Insertion Peptide). This 36 amino acid peptide is soluble at physiological pH, while at acidic pH, the transmembrane helix predominates, making the translocation of cell-impermeable molecules linked to its C-terminus through the membrane possible. Our goal is to define the properties of cell-impermeable cargo molecules linked to pHLIP that set the limits on the use of this novel delivery system. To accomplish our objective, we use the wide range of properties that can be obtained using peptides linked to or continuous with the C-terminus of pHLIP. These peptides follow a host-guest model where single amino acids are modified one at a time, allowing us to efficiently vary polarity, charge, shape and size. We have already successfully translocated such a polar cyclic peptide (LogP of ~3). The unique properties of pHLIP made it attractive for the development of a novel class of delivery peptides for the transport of therapeutic and diagnostic agents to acidic tissue sites associated with various pathological processes in vivo.

1953-Pos Antimicrobial Peptide Interactions with Planar Supported Bilayers - Kinetics and Selectivity Study by Electrical Impedance Spectroscopy

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Board B69

Activity of antimicrobial peptides (AMPs) is commonly studied by vesicle interaction and leakage assays, by secondary structure characterization, or by bacterial sterilization. These methods, however, provide an incomplete image of the mechanism of AMP structure and activity.

We explore the utility of impedance spectroscopy as a complementary tool to characterize changes in membrane properties in the presence of AMPs. We show that the recorded changes in membrane resistance and capacitance with time can provide new insights into the mode of AMP action.

1954-Pos Studies of Peptide-Membrane Interactions by Correlated Atomic Force Microscopy and Polarized Attenuated Total Reflection Fourier Transform Infrared Spectroscopy

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Board B70

We have developed an integrated platform that combines atomic force microscopy with polarized ATR-FTIR spectroscopy to study protein-protein and protein-membrane interactions. Such a platform would be ideal for correlating topographical changes as resolved by in situ AFM with specific structural alterations, in either the protein, or the membrane, as detected by polarized ATR-FTIR spectroscopy. Possible applications include investigating membrane-induced protein folding (or refolding) and aggregation behaviour. We report here on the results of preliminary in situ studies of peptide self-assembly in model supported lipid bilayers, including the effect of antimicrobial peptides on lipid order, and peptide fibrillogenesis. Some of the challenges that this approach presents will be discussed.

1955-Pos Polar Residues in Transmembrane Helices can Dramatically Decrease Electrophoretic Mobility in Polyacrylamide Gels Without Helix Dimerization

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Board B71

Polar residues in membrane-spanning helices are known to drive oligomerization in biological and synthetic membranes, as well as in membrane mimetic systems such as detergents. Here we report a study of a hydrophobic peptide that contains either an asparagine (N) or a leucine (L) residue in the 12th position of an otherwise hydrophobic segment of 20 amino acids. These peptides are fully α-helical in detergents. In SDS polyacrylamide gels, several variants of the L12 peptide migrated as monomers of about 3–4,000 Da. However, the N12 peptides always migrated at apparent molecular weights of 7–12,000, suggesting dimers or trimers. In sharp contrast, Forster resonance energy transfer (FRET) experiments in detergent solution showed little evidence of dimerization of N12 under any conditions. Experiments were performed with several variants of N12 and L12 labeled on their amino termini with the dyes fluorescein, TAMRA, Cy3 or Cy5. Experiments were done at concentrations ranging from 25 nM to 50 μM and at acceptor to donor ratios from 1:1 to 10:1. SDS concentrations ranged from 3 to 150 mM and experiments in a dozen other detergents were also done. We conclude that N12 is monomeric in all detergents, despite the fact that it appears to migrate as a dimer in SDS gels. Finally, we performed *in situ* FRET experiments on the bands in polyacrylamide gels. In the gel, there was no excess FRET observed for the slower N12 bands relative to L12 bands. The polar asparagine residue *mimics* dimerization by decreasing electrophoretic mobility. We hypothesize that the polar residue in the otherwise hydrophobic helix alters the interactions with detergent. Physical differences in the peptide-detergent micelles, such as shape and stoichiometry, are responsible for the altered migration of the N12 peptides.

1956-Pos Comparison of Transmembrane helix dimerization in bilayers and detergents using Hill coefficient analysis

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Board B72

Detergent is a common membrane mimetic to study membrane proteins and its small micelle size is particularly attractive for high resolution MNR. However, there are doubts and uncertainties of whether the micelle topology is altering the structure and interactions of membrane proteins, especially when these interactions are weak and can be modulated by different lipid or detergent environments. We used the TM domain of wild type and A391E mutant FGFR3 as an example of weak dimerizing peptides to study how their behavior would change in detergent (SDS). These peptides had been thoroughly characterized in liposomes and in surface supported bilayers. Hill coefficient analysis shows that the dimerization of WT and A391E TM domain in phospolipid bilayers follow the theoretical curve described by a single association constant, though different for WT and A391E. In detergent, both peptides form only dimers and no higher order oligomers, as probed by FRET analysis. However, Hill coefficient analysis indicates anti-cooperativity in WT dimer interactions, suggesting multiple dimer states with different binding constants. At the same time, the A391E mutant behavior is closer to the theoretical trend described by a single binding constant. The observed heterogeneity of the dimeric state can explain the broad peaks in the NMR spectra of those peptides.

The presented analysis can be used to validate the utility of different membrane mimetics used for studying TM helix interactions.

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1957-Pos A Dynamic View Of Hydrophobic Matching From A Free Energy-based Positional And Orientational Landscape Of Peptides In Membranes

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Board B73

The anisotropic environment of the lipid bilayer constraints the molecular position and orientation, allowing the use of minimalistic thermodynamic models for the structural characterization of peptide/membrane complexes. Based on the Wimley-White double scale of partition free energies, a landscape of positional and orientational states for monomeric peptides in membranes is be calculated, from which we select stability-based probability distributions. For the orientational angles (helix tilt and self-rotation)

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.

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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