Board B148

Thermodynamics studies of the binding of a cognate DNA sequence to IHF, a prokaryotic DNA-bending protein, have revealed that, despite the severe distortion of the DNA, which is bent in a U-turn in the complex, the binding is enthalpically driven and entropically unfavored (Holbrook et al., *J. Mol. Biol.* 2001, *310*, 379). These results are in contrast to what is expected if the protein-DNA interactions are driven by the entropic release of ions associated with the free DNA and protein. To gain insight into the nature of the transition state ensemble separating the nonspecific and the specific complex, we have started a detailed investigation of the DNA bending/unbending kinetics, using time-resolved FRET on end-labeled DNA, in response to a ~10 ns laser T-jump perturbation of the IHF-DNA complex. We find that the activation enthalpy for the DNA bending rate (k_{bend}) is ~14 kcal/mol, in accord with a significant enthalpic penalty for kinking DNA. Furthermore, kinetics measurements at different [KCl] indicate that, for [KCl] < 300 mM, k_{bend} is independent of salt, while for [KCl] > 300 mM, k_{bend} decreases with increasing salt. To explain the positive activation enthalpy and the nonlinear salt-dependence of k_{bend}, we propose that in the uphill climb to the transition state ensemble, spontaneous bending/kinking of DNA is rate-limiting at low salt, whereas further bending of the DNA, accompanied by release of ions, becomes rate-limiting at high salt. Thus, the nature of the transition state ensemble depends on the ionic environment. Subsequent conformational changes in the protein that facilitate favorable interactions in the protein-DNA complex, and that contribute to the negative enthalpy change, must occur as the system leaves the transition state, downhill to the final complex.

2034-Pos Thermodynamic Characterization of E. coli Manganese Superoxide Dismutase Binding to Single- and Double-Stranded Polynucleic Acids

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Board B149

Bacterial manganese superoxide dismutase (MnSOD) has been shown to localize to the chromosomal portion of the cell and impart protection from ionizing radiation to DNA. The binding affinity of bacterial MnSOD to non-sequence specific double stranded oligomeric DNA has been quantitated previously by nitrocellulose filter binding and gel shift assays. We have examined the equilibrium binding of E. coli MnSOD containing tryptophan to poly(U), poly (A), poly(C), poly(dU) and double-stranded (ds) DNA. Equilibrium association constants, Kobs, measured by monitoring tryptophan fluorescence quenching, were examined as functions of monovalent salt (MX) concentration and type, as well as temperature, from which ΔG°obs and ΔH°obs were determined. The polynucleotides bind to MnSOD in the following affinity hierarchy, poly(dU)>poly (U)>dsDNA>poly(A)>poly(C). For each polynucleotide, Kobs decreases with increasing [K+]. For polyU, polyA and polyC the values of ΔHobs become less favorable with increasing [K+]; therefore, the salt concentration dependence of ΔG°obs has contributions from entropic and enthalpic origins such that δlogKobs/δ log[K+] is less negative than if it were a simple electrostatic binding event.

2035-Pos Interaction of the Adenoviral IVa2 Protein with the Viral DNA Packaging Sequence

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Board B150

Human adenovirus (Ad) is a non-enveloped dsDNA virus. Ad DNA packaging is the process whereby the Ad genome becomes encapsidated by the viral capsid. Specific packaging is dependent upon the packaging sequence (PS), which is composed of seven repeated elements, called A repeats, and is located near the left end of the viral genome. Two viral proteins, that have been shown to bind to synthetic DNA probes containing A repeats I and II (A-I-II), are also required for DNA packaging. These proteins are called IVa2 and L4-22kDa. Furthermore, in the absence of IVa2, L4-22kDa binding to the A-I-II probe is not detected by a gel-shift assay. These data suggest that both IVa2 and L4-22kDa proteins cooperatively interact with the PS to ensure specific recognition and packaging of the viral genome. In order to begin to define the molecular events that are responsible for initiating DNA packaging, the PS DNA binding properties of the IVa2 and L4-22kDa proteins must be studied quantitatively, in a well defined biochemical system. To this end, we have overexpressed the IVa2 protein in *E. coli*, purified the protein to homogeneity, and have begun to study its interaction with the PS DNA.

Interfacial Protein-Lipid Interactions

2036-Pos Membrane Binding Studies of the GM2 Activator Protein with Phosphatidylcholine Bilayers using EPR and Tryptophan Fluorescence

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Board B151

The GM2 Activator Protein (GM2AP) is a non-enzymatic accessory protein involved in the catabolism of ganglioside GM2. GM2AP is thought to bind GM2 in intralysosomal vesicles at acidic pH, thereby presenting the oligosaccharide head group for hydrolytic cleavage by β-Hexosamidase A (Hex A). A goal of our research is to determine the membrane bound orientation of GM2AP on phosphatidylcholine bilayer surfaces. We currently utilize both site-directed spin labeling (SDSL) electron paramagnetic resonance spectroscopy (EPR) and intrinsic tryptophan fluorescence to ac-

complish this task. Results demonstrating feasibility of the site-directed spin labeling approach, proper protein folding, activity of cysteine mutants compared to wild type, and surface association are presented. Specifically, power saturation EPR experiments utilizing paramagnetic colliders of different solubilities in the aqueous and hydrocarbon phases indicate that two spin labeled cysteine mutants of GM2AP (A60R1 and N136R1), which are located in putative membrane binding loops, do not penetrate into the bilayer. A novel surface-bound paramagnetic collider lipid DOGS-Ni-NTA, which contains a chelated nickel atom, is used to verify the surface location of GM2AP; thus valuable distance information is gained off the surface of the bilayer. Additionally, GM2AP contains three tryptophan residues, two of which (W63 and W131) are located in the putative membrane binding loops. Upon membrane binding at acidic pH, a 1nm blue shift in the tryptophan emission is detected. Modest protection from quenching agents such as acrylamide and idodide is also observed in the presence of POPC vesicles. Taken together, these data are consistent with SDSL EPR results and indicate a surface associated orientation of GM2AP on POPC bilayers.

2037-Pos Effects of Amyloid Oligomers on the Conductance of Lipid Bilayers of Different Lipid Composition

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Board B152

We have found considerable differences in the effects of amyloid oligomers on membranes of different lipid composition. Changing the fatty acid chains of the membrane-forming lipids has a marked influence on the effectiveness of amyloid oligomers in increasing the membrane conductance. The longer the lipid fatty acid chain, the smaller the effect of oligomers on the membrane conductance. Longer fatty acid chains tend to increase the area elastic modulus of the membrane, raising the energy cost to increase or decrease the area per molecule of the lipid. This would have the effect of making it more difficult to thin the membrane or to insert molecules into it. We have proposed that the area elastic modulus is an important determinant of sensitivity to amyloid oligomers, and these experiments support this hypothesis. We have shown that membranes formed from sphingomyelin, a lipid with a very large area elastic modulus, were much less sensitive to Aβ oligomers than membranes formed from dioleolyl phosphatidyl choline (DOPC), and this result was confirmed by our collaborators in Mathias Loeshe's group at Carnegie Mellon and NIST using an entirely different experimental approach. They used neutron reflectometry to show that while Aβ oligomers caused dramatic thinning of membranes formed from DOPC, they had almost no effect on membranes formed from sphingomyelin. They had also used diphytanoyl phosphatidyl choline (DPhyPC) membranes, which also have a high area elastic modulus, in their system. So acting on their suggestion, we tested the effects of Aβ oligomers on membranes containing different percentages of DPhyPC and DOPC.

This work was supported by Alzheimer's Association grant IIRG-06-26167.

2038-Pos KDB: A Potassium Channel Model and Simulation Database

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Board B153

Molecular modelling and simulation play an important role in understanding the relationship between structure and function of ion channels. Many of the questions being addressed require interactions between researchers from many disciplines. For example, both experimental and computational approaches may be used to examine the roles played by lipids in the structure and function of ion channels. We present here a database that contains the summarised results from a large number of classical molecular dynamics simulations of potassium channels and related homology models. The database is high-level and the data contained is intended to help researchers quickly answer a few specific questions. For example, it holds predictions based on our simulations for each ion channel as to which amino acid residues interact with the headgroups and tails of the surrounding phospholipid bilayer. The database is free to access and our hope is that it will be useful to experimentalists who may not have routine access to dynamical models of ion channels. This work is an extension of our existing outer membrane protein (http://sbcb.bioch.ox.ac.uk/ompdb/) and coarse-grain membrane protein insertion (http://sbcb.bioch.ox.ac.uk/cgdb/) databases.

2039-Pos GM2 Activator Protein Interaction with Bilayer Membranes and Effect of Lipid Composition

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Board B154

The GM2 activator protein (GM2AP) is a non-enzymatic accessory protein required for the catabolism of a specific ganglioside, GM2, via hydrolytic cleavage by β-Hexosaminidase A. We have characterized the interaction of GM2AP with lipid vesicles by two different assays:

1. FRET from intrinsic tryptophan to Dansyl-DHPE in vesicles, and
2. sucrose-loaded vesicle pull-down assay with fluorescently labeled GM2AP.

Real time and static FRET measurements are utilized to investigate the effects that pH and lipid composition have on both the membrane partitioning and binding kinetics of GM2AP with lipid vesicles. We find that negatively charged lipids do not alter the

membrane binding of GM2AP but GM2 increase binding affinity as anticipated. Strong binding is observed for pH < 5.2, and this finding is consistent with the biological location of GM2AP, which functions in the acidic lysosome compartments of cells. Surprisingly, our results on His-tagged protein reveal that the His-tag alters both membrane partition and binding kinetics.

2040-Pos Interaction of Neuropeptide Y with charged and uncharged lipid membranes

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Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family with a chain length of 36 amino acids. It belongs to the best conserved peptides in nature, i.e. the amino acid sequences of even evolutionary widely separated species are very similar to each other.

We studied the interaction of human NPY with pure phospholipid membranes. The used liposomes were composed of zwitterionic and negatively charged phospholipids in order to get information about the influence of liposomes surface charge on peptide binding. The interaction of NPY with the liposomes was investigated by means of fluorescence spectroscopy, Electron Paramagnetic Resonance Spectroscopy (EPR) and Nuclear Magnetic Resonance (NMR).

The fluorescence measurements were done with native human NPY using the intrinsic fluorescence of the peptide. The fluorescence intensity of NPY at different lipid-peptide-ratios were analysed using a model, which includes the hydrophobic and the electrostatic interaction between the peptide and the phospholipid membrane.

NMR- and EPR-measurement were done with TOAC labelled NPY. The NMR-relaxation rate of the different lipid segments is increased by the paramagnetic TOAC-label, which is integrated in the backbone of NPY. The distribution of the NPY in the membrane can be determined using this relaxation values. The mobility of the different parts of NPY were determined from EPR-data.

All results indicate, that the binding of NPY to phospholipid membranes depends strongly on the surface charge of the liposomes. The helical part of NPY (AS:14–36) is arranged parallel to the membrane surface and is localized in membrane-water-interface of the liposomes. The unstructured part of NPY sticks out from the membrane and is still high mobile. The fluorescence and NMR-data show, that the penetration depth of the helical part of NPY is reduced in charged membranes in comparison to zwitterionic membranes

2041-Pos Site-directed Mutagenesis Of The Bt Toxin Cyt1A For Intramolecular Distance Determination By FRET

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Board B156

The cytolytic toxin Cyt1A from the spore-forming bacterium *Bacillus thuringiensis* var. *israelensis* is used in the production of environmentally safe insecticides, but its conformation in the lipid membrane and mode of action are not known. Previously, we fluorescently labeled the toxin with 1,5-IAEDANS via the single cysteine residue, and used fluorescence resonance energy transfer (FRET) to determine the average distance between the cysteine and the two neighboring tryptophans in the molecule. Even though the value favorably compared with that determined in the molecular dynamics model of Cyt1A, it is fraught with uncertainty due to the presence of multiple fluorescence energy donors. Herein we produced a single-tryptophan mutant (W161F) of the toxin, which allowed for a less ambiguous and more precise determination of the cysteine-tryptophan distance in the molecule in solution. Importantly, lipid-induced changes in the distance can be used to gain insight into the toxin's conformation upon binding to the membrane. In addition, a cysteine-free single-tryptophan mutant was produced that will serve as a template for additional mutations with cysteine placed in selected locations throughout the toxin molecule. In conjunction with FRET measurements, such a set of mutants will be used for intramolecular distance mapping, which will help us outline the toxin conformation and its changes upon binding to the lipid membrane.

2042-Pos Incomplete Dehydration of the Hydrophobic Face of the Mechanosensitive Channel Inhibitor GsMTx4 during Membrane Partitioning

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Board B157

Recently we have demonstrated that, despite structural similarities, partitioning into the lipid bilayer is not a universal property of the ion-channel blockers [Biophysical J. 2007, 93:L20]. The mechanosensitive channel inhibitor GsMTx4 is the only one that interacts with both anionic and zwitterionic lipids with nearly equal strength.

To gain insight into the determinants of its bilayer interactions we have examined several of its mutants and found to our surprise that none of the mutations had significant effects on membrane partitioning. Another surprising feature of GsMTx4 is the almost complete absence of changes in intrinsic fluorescence during membrane insertion. This anomalous behavior can't be explained by the close proximity of the two indole rings of W6 and W7, as it is also evident in the W6A mutant. The depth of the tryptophan penetration in this mutant and in the wild type, determined from Distribution Analysis of the depth-dependent quenching, is rather significant, but the overall quenching efficiency is poor. To reconcile these observations we suggest the possibility that the hydrophobic face of the GsMTx4 is not completely dehydrated during membrane partitioning. It is likely that the combination of the rigid structure of the highly crosslinked GsMTx4 and the central position of the tryptophans on the hydrophobic interface is responsible for this effect (the "usual" spectral shift is observed for example with SGTx1, in which W30 is located at the side of the hydrophobic patch). We have used the position of the depth-dependent quenching profile as an initial constraint in setting up the MD simulation of GsMTx4 in a POPC bilayer. After equilibration and removal of the constraint, the peptide's position is not changed, but a number of water molecules penetrate deeply into the lipid-peptide interface. GM069783(-04S1)

2043-Pos Characterization of Apolipophorin-III/LPS Complexes

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Board B158

Apolipophorin III (apoLp-III) is an 18 kDa insect apolipoprotein with five amphipathic α-helices, resembling the structure of human apolipoproteins. ApoLp-III has been well documented for its role in lipid transporting processes. Recent studies suggest that apoLp-III plays a role in innate immunity by binding and detoxifying bacterial cell membrane components. Therefore, we have studied the interaction of apoLp-III with LPS from different species to evaluate the role as a pattern recognition protein. Binding of apoLp-III to LPS from *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella enterica* was studied by tyrosine fluorescence spectroscopy, dynamic light scattering and native gel electrophoresis. LPS from *E. coli* (serotype O55:B5) and *Klebsiella pneumoniae* were found to exert the highest increase in apoLp-III tyrosine fluorescence. The LPS/apoLp-III complexes were then analyzed by dynamic light scattering. Addition of apoLp-III to LPS, present in an aggregated form, resulted in a size reduction from 70 to 24 nm for *E. coli* LPS, while the size was reduced from 51 to 10 nm in *K. pneumoniae* LPS. The complex was purified with FPLC using a Superdex-200 size exclusion column. In agreement with the dynamic light scattering data, the complex eluted at a higher volume compared to LPS aggregates. The purified LPS/apoLp-III complex was analyzed for LPS content by the 2-keto-3-deoxyoctanate assay, and apoLp-III content was determined using the bicinchoninic acid protein assay. The molar ratio of the LPS and apoLp-III in the complexes was found to be 15:1 and 8:1 for *E. coli* and *K. pneumoniae* LPS, respectively. Our findings indicate that apoLp-III forms small complexes with LPS from different bacteria which supports its putative role as a pattern recognition protein.

2044-Pos Structural Studies of the Transmembrane and Cytoplasmic Domains of Anthrax Toxin Receptor

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Board B159

The human receptor for anthrax toxin is a single span membrane protein of 368 amino acids that binds to the antigens of *Bacillus anthraces*, the bacterium that causes anthrax. The transmembrane (TM) domain of the receptor spans residues 319–343 and has the sequence G**SI**LAIALLILFLLLALALLWWFWA. Through the use of solid phase peptide synthesis, we have synthesized the native domain (but with Gly instead of Ser) and a related "double-anchored" domain with Trp anchors on either end of the peptide, substituting WW for SI. The cytoplasmic domain of the protein spans residues 344–368 (PLCCTVIIKEVPPPPAEESEENKIK), with a stretch of four prolines in the center of the sequence. Based upon solution NMR of the cytoplasmic domain, it is evident that the structure is not well defined. While the solution NMR spectra of the TM domains (both the single and double anchored) in micelles of deuterated SDS are better defined, there are nevertheless multiple conformations at both 30 °C and 55 °C. This can be seen in the Trp indole ring region of the spectra, where additional peaks are evident. We have incorporated deuterated alanines into both the single- and double-anchored TM domains by solid-phase synthesis and have used the 'GALA' method (van der Wel, et al., *Biophys. J.* **2002**, *83*, 1479) to analyze the ^{2}H-NMR spectra. A key goal is to determine the average orientation of each TM domain in actual hydrated lipid bilayer membranes. It now becomes important to examine the structures of the fused TM and cytoplasmic domains together. For this purpose, the domains are being expressed together in *E. coli*, to allow comparison of the linked domain and separated domain structures.

2045-Pos SUV Solubilization and Nanodisc Formation by Apolipoproteins

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Apolipophorin III (apoLp-III) from *Locusta migratoria* is a model exchangeable apolipoprotein to study apolipoprotein-lipid binding interactions. Promoted by its low stability, the 5 α-helix bundle protein opens upon lipid binding to allow formation of a stable protein-lipid complex. ApoLp-III is able to solubilize phospholipid vesicles, resulting in the formation of 20 nm sized particles termed nanodiscs. They resemble human nascent HDL, an essential lipoprotein for reversed cholesterol transport. Nanodiscs are used to

study the apolipoprotein structure in the lipid bound conformation, and are an excellent model for lipid binding analysis. In the present study we explored the factors that affect nanodisc assembly by investigating phospholipid acyl chain length and cholesterol incorporation. Using small unilamellar phospholipid vesicles (SUVs) nanodisc formation was monitored by measuring the decrease in sample turbidity at 325 nm at the gel-to-crystalline phase transition temperature following apoLp-III addition. To verify nanodisc formation, the complexes were purified by superdex-200 FPLC and analyzed by electron microscopy and native PAGE. ApoLp-III was able to induce a spontaneous transformation of dimyristoylphosphatidylcholine (DMPC) vesicles into nanodiscs, but not dipalmitoylphosphatidylcholine (DPPC) vesicles which required the use of Na-cholate. Decreasing the acyl chain length by one carbon (dipentadecanoylphosphatidylcholine) did not result in nanodisc formation.

However, SUVs made of ditridecanoylphosphatidylcholine were rapidly solubilized. Addition of 5 – 10 % cholesterol (molar) to DMPC SUVs increased the rate of nanodisc formation an order of magnitude but had no effect on DPPC SUVs. These results indicate that acyl chain length is a major factor for apolipoprotein-induced vesicle solubilization and formation of nanodiscs.

2046-Pos Contributions of Membrane Insertion and Angular Orientation in the Interfacial Activation of Phospholipase A_2

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Board B161

Phospholipase A_2 (PLA_2) enzymes undergo activation upon binding to cellular membranes. Interfacial activation is regulated by diverse factors, including the membrane charge, fluidity, mode of membrane binding (insertion, orientation), as well as allosteric conformational effects. However, relative contributions of these factors into the complex kinetics of PLA_2 activation are not well understood. In order to determine the roles of membrane fluidity, charge, membrane insertion, and orientation in PLA_2 activation, we have analyzed relationships between the thermal phase transitions of membrane lipids, membrane surface charge, membrane insertion of PLA_2, angular orientation, and activity of human pancreatic PLA_2. The temperature dependence of the initial catalytic rate of PLA_2 peaks around the lipid phase transition temperature (T_m). High PLA_2 activity can be induced by thermal perturbations of the membrane, such as shifts from higher or lower temperatures to those close to T_m. While PLA_2 activity is several- fold higher against anionic as compared to zwitterionic membranes, the enzyme shows little activity even for anionic membranes at temperatures well below or above T_m. Membrane depth-dependent fluorescence quenching experiments at various temperatures show that despite dramatic effects of the lipid phase transition on PLA_2 activity, membrane insertion of PLA_2 increases only modestly above T_m. Polarized infrared experiments identify a significant change in the angular orientation of membrane-bound PLA_2 during the onset of activity, which corresponds to a transition from a random orientation to a nearly parallel alignment of helices of PLA_2 to the membrane surface. The data show that PLA_2 activity is promoted by membrane structural disorder rather than membrane fluidity or the depth of membrane insertion of the enzyme, and that a transition from a random to a defined, "productive mode" orientation plays an important role in the enzyme activity.

2047-Pos The Obesity-Regulating Peptide BBC1 Interacts with Lipid Membranes in a Surface Charge-Dependent Manner

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BBC1 is a synthetic backbone-cyclic peptide (*c*(Phe(C2)-D-Phe-Arg- (N2)Trp-Gly-NH_2)) designed as a compound mimicking the naturally occurring neuro-peptide alpha-melanocyte stimulating hormone that features an Arg-Trp motif. The natural peptide acts on the human melanocortine-4 receptor involved in obesity and energy regulation. BBC1 was found to penetrate the intestine by a trans-cellular mechanism without causing significant membrane disruption, and is thus considered a member of the unique Intestinal Penetrating Peptide (IPP) family. We investigate the interaction of BBC1 with model lipid bilayers. We find that at bath solution concentrations higher than 60 μM, BBC1 produces membrane instabilities and eventual rupture. The membrane-disrupting activity of BBC1 correlates well with the membrane content of the charged lipid, DOPS. Addition of peptide to membranes that also contain the channel-forming peptide gramicidin A or its covalently linked analogue allowed us to observe the membrane adsorption of BBC1 in real time. The cyclic peptide interacts with the channel and produces transient current interruptions. The on-rate of these events depends linearly on the peptide concentration and scales with the mole percentage of the charged lipid in the membrane. The measured off-rate is surface-charge independent. Thus, our results suggest that the membrane lipid composition plays an important regulatory role in the physiological activity of the BBC1.

2048-Pos Pi-binding Domains And Their Interactions With Phosphoinositides And Other Lipids

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The mechanism of interaction of PH domains with PIP_2-containing lipid bilayers remains uncertain. Whilst crystallographic studies have emphasised PH-IP_3 interactions, biophysical studies indicate a

degree of less specific protein/bilayer interactions. We have used molecular dynamics simulations to characterise the interactions of the PH domain from phospholipase C-δ1 with IP_3 and with PIP_2, the latter in lipid bilayers and in detergent micelles. Simulations of the PH domain in water suggest a reduction in protein flexibility when IP_3 is bound. Simulations of the PH domain bound to PIP_2 in lipid bilayers indicate a tightening of ligand/protein interactions relative to the PH/IP_3 complex, alongside formation of H-bonds between PH sidechains and lipid (PC) headgroups, and some penetration of hydrophobic sidechains into the core of the bilayer. Thus, comparative molecular dynamics simulations reveal how a PI-binding domain undergoes small but significant changes in conformational dynamics on binding to a PIP_2-containing membrane. Related methodologies will be used to explore the behaviour of other PI-binding domains, e.g. FYVE, PX, with PI-containing lipid bilayers.

2049-Pos Interaction Of LL-37 With Model Membrane Systems Of Different Complexity -Influence Of The Lipid

Matrix Eva Sevcsik[1], Georg Pabst[1], Sabine Danner[1], Walter Richter[2], Karl Lohner[1]

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Board B164

Membrane lipid composition influences membrane properties such as e.g. curvature strain, surface charge and fluidity, which are important for membrane function. It also modulates the effect of antimicrobial peptides (AMPs). The paradigm of AMP research is that the cationic peptides interact preferentially with the negatively charged bacterial membranes and not with charge neutral host cell membranes. Therefore, in many studies of AMP/membrane interaction, the focal point of consideration is still lipid headgroup charge. Although model systems must be sufficiently simple to be able to draw conclusions upon the interaction of lipids and peptides on a molecular level, the question must be addressed as to how simple they can be made without losing significance. We studied the interaction of the human multifunctional peptide LL-37 with single phospholipid monolayers, bilayers and bilayers composed of binary lipid mixtures of the four lipid species predominantly used in model membrane experiments (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylglycerol). We found (i) that the effects on the single lipid monolayers are not comparable to the effects on the corresponding bilayers, (ii) four different modes of interaction for LL-37 with bilayers of the four lipids and (iii) that in the binary lipid mixtures, one lipid generally governs the mode of lipid/peptide interaction and this is not necessarily the charged one. Our results suggest that the role of charge in lipid/peptide interaction has perhaps been overestimated. Although electrostatic attraction is undoubtedly important for initial binding of the peptide to a membrane, other lipid properties (such as packing density, the ability to form intermolecular H-bonds, molecular shape and hydrocarbon chain length) play a role at least equal to that of charge in determining the mode of lipid/peptide interaction.

2050-Pos The Preferential Reconstitution Of AMPA Receptor Proteins Into Model Lipid Domains Studied By Atomic Force Microscopy

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Board B165

We report atomic force microscopy (AFM) measurements of the neuroreceptor a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), one of the most common membrane receptor proteins found in the central nervous system (CNS). AMPA receptors are implicated in long term potentiation, a process thought to underlie learning and memory, with up-regulation of AMPAR numbers in the post-synaptic membrane possibly being a key component of this process. In this work we have investigated the dependence of protein reconstitution in model membranes on lipid composition.

We reconstituted purified AMPA receptors into a model lipid system consisting of a mixture that makes up approximately 60% of the synaptic membrane. The mixture forms two distinct domains when immobilised on mica: a high domain (HD) at 7nm and a low domain (LD) at 5nm. We find that the receptors preferentially insert into the HD, while protein that inserts into the LD has a significantly larger protrusion from the membrane. These observations are consistent with hydrophobic matching.

The lateral extent of the lipid domains is typically ~100nm, so they have structural similarities with the lipid rafts observed to occur in synaptic membranes, albeit with much simpler composition. These rafts are loci for insertion of receptor proteins and are believed to act as signalling platforms that organize and compartmentalize neurotransmitter receptors to either enhance or inhibit signalling in CNS synapses. Dynamic AFM measurements reveal information about the mobility of receptors within and between domains which may shed light on this process.

2051-Pos Membrane Tubulation by BAR Domains

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Board B166

Living cells are characterized by intricately curved internal membranes forming organelles that facilitate cellular processes. During the cell life cycle the membrane curvature is sculpted by proteins that act on a nanometer scale, but through concerted action produce cell scale, i.e., micrometer, shapes. We investigate the molecular mechanisms underlying the sculpting process, focusing on a ubiquitous class of membrane-bending proteins, BAR domains, which shape cellular membranes by acting on membrane surfaces. Employing descriptions on four scales (all-atom, residue-based coarse-graining, shape-based coarse-graining, and continuum mechanics),

we demonstrate how BAR domains transform an initially flat membrane into a tubular structure. All-atom molecular dynamics simulations of an individual BAR domain reveal local sculpting; from the results we derive coarse-graining descriptions at two different resolutions, leading to a 200 nanometer-20 microsecond simulation of tube formation. The latter simulation is captured into a mathematical model that is based on continuum mechanics. The results of our study offer a detailed picture of the development of cellular structures through the concerted action of BAR domains.

2052-Pos The Maturation of Human High-Denisty Lipoprotein Particles: Conversion of Disks to Spheres

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High-density lipoproteins (HDL) are protein-lipid particles that circulate in the blood, collecting cholesterol from peripheral tissues and transporting them either to the liver for excretion or to steroidal tissues for use in hormone biogenesis. Apolipoprotein A-I, the major protein component of HDL, is synthesized in the liver and intestines and excreted into the blood, initially forming a particle depleted in lipid. The efflux of lipids and cholesterol from peripheral tissues results in the formation of nascent discoidal particles. The continued loading of lipids and cholesterol and the conversion of cholesterol to esterified cholesterol results in the formation of spherical particles. These spherical particles are the circulating form of HDL and continue to grow in size by accumulating additional lipid and cholesterol molecules. The process by which cholesterol and cholesterol esters are removed from the vasculature is critical for understanding the origin of health benefits attributed to higher circulation of HDL, the "good cholesterol", and in the development of drugs to alter lipoprotein populations. Previous, all-atom and coarse-grained molecular dynamics simulations as well as experimental findings have focused on the structure and assembly of model discoidal HDL particles. For the first time, the assembly of discoidal HDL particles from an initially disordered assembly of proteins and lipids was observed and found to proceed in two broad steps; aggregation of proteins and lipids driven by the hydrophobic effect followed by the optimization of the protein arrangement driven by specific protein-protein interactions. Molecular dynamics is now being used to investigate the structural transformations resulting from the addition of cholesterol and cholesterol esters to discoidal HDL, leading to the formation of spherical particles.

Research supported by NIH RO1-GM33775 (SGS), and RO1-GM067887 and P41-RR05969 (KS).

2053-Pos α-Synuclein Selectively Binds To Anionic Phospholipids In Liquid-disordered Domains

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Board B168

The development of Parkinson's disease (PD) is accompanied with the loss of dopaminergic neurons. Although the mechanisms leading to the death of these cells are still unclear, several genes have been identified which are associated with the development of inheritable monogenic forms of the disease. For the gene α-synuclein three disease related missense mutations have been discovered. In addition amyloid fibrils formed from α-synuclein can be found in protein deposits typical for the PD.

Previous studies indicate that binding of α-synuclein to membranes is critical for its physiological function and the development of PD. We have investigated the association of fluorescence-labeled α-synuclein variants with different types of giant unilamellar vesicles (GUVs) using confocal microscopy. We found that α-synuclein binds with high affinity to anionic phospholipids, when they are embedded in a liquid-disordered as opposed to a liquid-ordered environment. This indicates that not only electrostatic forces but also lipid packing and hydrophobic interactions are critical for the association of α-synuclein with membranes *in vitro*. When compared to wild-type α-synuclein, the disease-causing α-synuclein variant A30P bound less efficiently to anionic phospholipids, while the variant E46K showed enhanced binding. This suggests that the natural association of α-synuclein with membranes is altered in the inherited forms of PD.

2054-Pos Surface Association of the GM2 Activator Protein with Bilayers at Acidic pH: Investigations with SDSL EPR, Fluorescence Spectroscopy and ITC

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Board B169

Ganglioside catabolism is a vital cellular process which occurs in the acidic lysosomal compartments of cells. Accessory proteins, like the GM2 Activator Protein (GM2AP), are required for exohydrolases to degrade gangliosides containing short oligosaccharide head groups. GM2AP is believed to bind and possibly extract GM2 from intralysosomal vesicles, given the appropriate lipid composition. However, very little is known about the binding interactions or the membrane-bound orientation GM2AP. Our research combines a variety of biophysical techniques to probe how GM2AP interacts with lipid bilayers as a function of pH, lipid composition and vesicle size. Results from SDSL EPR and intrinsic TRP fluorescence indicate that GM2AP interacts with POPC under acidic conditions with a surface associated orientation where the putative membrane binding loops do NOT penetrate into the surface. Surprisingly, results for POPC-GM2 vesicles indicate less penetration of the hydrophobic putative membrane binding loops. Results from real time and static FRET, SDSL EPR power saturation and ITC will be presented and discussed in terms of the effects variables such as vesicle size and composition have upon bilayer partitioning, kinet-

ics of binding and orientation of binding. These findings are then related to the biological function of GM2AP.

2055-Pos PI-Specific Phospholipase C Binding to Mixed Component Vesicles Analyzed by FCS

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Board B170

Phosphatidylinositol-specific phospholipase C (PI-PLC) is a secreted bacterial protein that often aids in infectivity of the organism. Characterizing interfacial binding of *Bacillus thuringiensis* PI-PLC (highly homologous to the *B. anthracis* PI-PLC) to membranes is critical for understanding PI-PLC activity. Protein binding experiments also aid in the interpretation of high resolution NMR field cycling studies, used to monitor changes in phospholipid dynamics, arising from PI-PLC binding to small unilamellar vesicles (SUVs). Fluorescence correlation spectroscopy (FCS) was used to measure the affinity of PI-PLC for SUVs composed of anionic phospholipids and increasing mole fractions of the activating, zwitterionic phospholipid phosphatidylcholine (PC). The anionic phospholipids (phosphatidylmethanol (PMe), phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidic acid (PA)) act as competitive inhibitors of PI-PLC. FCS experiments indicate that PC binding is separate from anionic lipid binding, and that PC and anionic phospholipids synergistically enhance the affinity of PI-PLC for vesicles. PI-PLC binds poorly to anionic surfaces at physiological NaCl concentrations while addition of PC, up to 80% mole fraction, increases PI-PLC affinity for SUVs. At PC concentrations above 80%, PI-PLC binding weakens. Interestingly, PI-PLC enzymatic activity does not directly correlate with vesicle binding. The optimum activity is achieved at low mole fractions of PC (10%) and starts to drop when PC is over 60% where vesicle binding is still tight. At very high PC content PI-PLC binding is weaker, consistent with a synergistic effect of both lipids in anchoring the enzyme to bilayers for processive catalysis.

2056-Pos Binding of Anionic Lipids to at Least Three Non-annular Sites on the Potassium Channel KcsA is Required for Channel Opening

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Board B171

As well as the annular or boundary lipids that surround the transmembrane surface of the potassium channel KcsA from *Streptomyces lividans*, X-ray crystallographic studies have detected one anionic lipid molecule bound at each protein-protein interface in the homotetrameric structure, at sites referred to as non-annular sites. The binding constant for phosphatidylglycerol at the non-annular sites has been determined using fluorescence quenching methods with a mutant of KcsA lacking the normal three lipid-exposed Trp residues. Binding is weak, with a binding constant of 0.42 ± 0.06 in units of mol fraction, implying that the non-annular sites will only be ~ 70 % occupied in bilayers of 100 % phosphatidylglycerol. However, the non-annular sites show high selectivity for anionic lipids over zwitterionic lipids, and it is suggested that a change in packing at the protein-protein interface leads to a closing of the non-annular binding site in the unbound state. Increasing the anionic lipid content of the membrane leads to a large increase in open channel probability, from ~ 2.5 % in the presence of 25 mol % phosphatidylglycerol to ~ 62 % in 100 mol % phosphatidylglycerol. The relationship between open channel probability and phosphatidylglycerol content shows cooperativity. The data are consistent with a model in which three or four of the four non-annular sites in the KcsA homotetramer have to be occupied by anionic lipid for the channel to open. The conductance of the open channel increases with increasing concentration of anionic lipid, an effect possibly due to effects of anionic lipid on the concentration of K^+ close to the membrane surface.

2057-Pos Complex Formation and Activation of Coagulation Factors at the Surface of Negatively Charged Membranes

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Board B172

Binding of coagulation factors to negative areas of cellular membrane is a key step in blood clotting cascade. In particular, association of factor VIIa (FVIIa) and tissue factor (TF) at the surface of the membrane results in an increase of the catalytic activity of FVIIa by seven orders of magnitude. While the structure of the FVIIa/TF complex is available, the mechanism of such a significant acceleration is unknown. Here we employ computational methodologies to investigate:

1. the binding characteristics of the membrane-binding domain (the GLA domain) of FVIIa and prothrombin (PT), and the molecular basis of their different membrane binding affinities;
2. the mediatory role of anionic phospholipids in association and complex formation of FVIIa and TF; and
3. the mechanism for the enhanced catalytic rate.

Our recent model of the membrane-bound GLA domain of FVIIa was adopted to construct membrane-bound models of PT, FVIIa, TF, and the FVIIa/TF complex. All systems were equilibrated for at least several tens of nanoseconds in their natural environment. Specific structural features, including increased flexibility of the -loop of the GLA domain, which results in an increased exposure of structurally bound Ca^{2+} ions to negative lipids, and key sequence variations in PT

were identified as the main mechanisms by which PT exhibits significantly stronger membrane binding than FVIIa. The membrane-bound model of the FVIIa/TF complex reveals previously unidentified direct contacts between soluble TF and the membrane (other than those mediated by the known membrane inserting domain). Such contacts have direct implications in the activation mechanism. The differences in fluctuation modes of the free and FVIIa-complexed forms of TF in solution were used to explain the mechanism of the enhanced catalytic activity of FVII upon binding to TF.

2058-Pos Effects Of Membrane-binding Peptides In The Local And Global Curvature Of Lipid Membranes

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Board B173

ASAP1 is part of the protein machinery that alters membranes and the actin cytoskeleton in cellular structures, called invadopodia, thought to mediate invasion of mammary cell carcinoma and uveal melanoma. The molecular mechanisms by which ASAP1 contributes to these structures are not well identified. ASAP1 has two known activities. One is to induce the hydrolysis of GTP that is bound to the protein Arf. This activity depends on the Arf GTPase-activating (GAP) and PH domains, which the protein contains. The second activity is to deform lipid bilayers into tubules. Large Unilamelar Vesicles form tubular structures protruding toward the outside upon addition of ASAP1 to the medium.

First, we developed a consistent model for the GAP activity. We have defined several reaction schemes, some including intermediate steps sensitive to changes in enzyme activation. Using computational tools, we solve the system of ODEs that describe these schemes and compare the model predictions to data obtained from kinetics experiments, done in the presence of lipid bilayers. We find that, although hysteretic reaction schemes are better than linear ones, equations that do not take into account the interaction of protein with the lipid bilayer are not an adequate fit for the data.

As a next step, we test the hypothesis that GAP activity is related to the mechanical activity, developing assays using laser tweezers on a confocal microscope to directly correlate the binding of curvature sensing proteins with local membrane curvature and membrane tension.

2059-Pos Cholera Toxin Penetration and Induced Phase Transformations in Lipid Membranes

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Board B174

Cholera toxin is highly efficient in taking over host organisms. To reap its destructive effects on the cell, these toxins must bind to and infiltrate the cellular membrane, a specialized and controlled barrier. The mechanism by which cholera toxin crosses the membrane remains unresolved. Using x-ray reflectivity and grazing incidence diffraction, we were able to monitor the binding of each toxin, observe perturbations to the packing of the lipid molecules, and gain clues as to how they penetrate into the lipid membranes. Grazing incidence x-ray diffraction revealed the coexistence of two monolayer phases after toxin binding. The first was identical to the monolayer before toxin binding. In regions where toxin was bound, a second membrane phase exhibited a decrease in order as evidenced by a larger area per molecule and tilt angle with concomitant thinning of the monolayer. These results demonstrate that cholera toxin binding induces the formation of structurally distinct, less ordered domains in gel-phases. Furthermore, the largest decrease in lateral order to the monolayer occurred at low pH, supporting a low endosomal pH in the infection pathway.

2060-Pos Interactions Between Profilin And Polyphosphatidylinositol Lipids

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Profilin is a small (12–15 kDa) actin binding protein. Profilin is also involved in the signaling pathway linking receptors in the cell membrane to the microfilament system within the cell. Profilin is thought to play critical roles in this signaling pathway through its interaction with polyphosphoinositides (PPI). To date, profilin's interaction with PPI has only been studied in micelles or small vesicles. Profilin binds with high affinity to small clusters of phosphatidylinositol (4,5) bis-phosphate [$PI(4,5)P_2$] molecules. In this work, we investigated the interactions of profilin with sub-micellar concentrations of $PI(4,5)P_2$ and $PI(3,4,5)P_3$. Fluorescence anisotropy was used to determine the relevant dissociation constants for binding of sub-micellar concentrations of fluorescently labeled PPI lipids to profilin and we show that these are significantly different from those determined for profilin interaction with micelles or small vesicles. Despite the low affinity for submicellar concentration of $PI(4,5)P_2$, profilin was shown to bind to Giant unilamellar vesicles in presence of 1% mole fraction of $PI(4,5)P_2$ Förster Resonance Energy Transfer experiments indicate that profilin is able to recruit adjacent $PI(4,5)P_2$ molecules after initial interaction with the GUV membrane. The implications of these findings are discussed.

2061-Pos Single Molecule Kinetics of ENTH Binding to Lipid Membranes

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Board B176

Transient recruitment of proteins to membranes is a fundamental mechanism by which the cell exerts spatial and temporal control over protein localization and interactions. Thus the specificity and kinetics of peripheral proteins' membrane-residence are an attribute of their function and reactivity. While bulk measurements can quantify average properties of these important protein-membrane associations, they are unable to delineate the details of their underlying mechanism. In here, we report direct visualization of protein-membrane interactions of a representative membrane targeting protein module - the Epsin N terminal homology (ENTH) domain. The use of simple model membranes (i.e. a fluid lipid bilayer deposited on a silica) allows precise control over the membrane physio-chemical properties as well as unprecedented details of membrane integrity during the measurements. Single binding events of the ENTH protein to lipid bilayers was imaged by total internal reflection fluorescence microscopy to discriminate bound and unbound protein. Subsequent single fluorophore tracking permits us to build up distributions of residence times. We report ENTH dissociation rate for various membrane compositions.

2062-Pos Investigating And Modeling Possible Mechanisms By Which Healthy Cell Membranes Become Resistant To Hydrolysis By Secretory Phospholipase A2

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Board B177

Secretory phospholipase A2 (sPLA2) behaves differently toward the membranes of healthy cells compared to those of damaged or dying cells. The enzyme catalyzes rapid and sustained hydrolysis of compromised cells consistent with a simple catalytic mechanism. In contrast, when healthy cells are incubated with sPLA2, they become resistant to hydrolytic attack as manifest by three unusual observations: First, hydrolysis is transient and represents only a small fraction of the total membrane phospholipid content. Second, subsequent addition of sPLA2 fails to generate additional product. Third, the apparent potency of the enzyme to cause the membrane to be refractory is much greater than the potency for catalyzing hydrolysis. The mechanism responsible for this resistance has not yet been identified. Using Monte Carlo and direct analytical methods, we have developed a model capable of explaining all three of these observations. The model requires two salient elements: only a small pool of phospholipids in the healthy cell membrane is available for catalysis by sPLA2, and hydrolyzed phospholipids are re-acylated and restored very slowly to the accessible pool. The requirement for initial hydrolysis (as opposed to the simple physical presence of the enzyme as previously thought) was confirmed experimentally. The model also predicts that total substrate, reacylation rate, and the return rate of phospholipids to the membrane should all be constant as enzyme concentration is varied. This prediction was tested by quantitative analysis of hydrolysis time courses at varied enzyme concentrations. Lastly, initial experiments with a fluorescent probe, merocyanine 540 suggest that resistance may also involve physical changes to the membrane beyond the kinetic mechanisms hypothesized in the model.

Interfacial Protein-Lipid Interactions, Peptides

2063-Pos Interaction of Bactenecin with 1,2-dipalmitoyl-sn-glycero-3-Phosphocholine Monolayers at the Air-Water Interface

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We show results of the interaction of the antimicrobial peptide bactenecin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine monolayers (DPPC) at the air/water interface. We investigated the role of different salt solutions in the subphase on the structure and elastic properties of Bactenecin/DPPC monolayers. Bactenecin interacting with DPPC monolayers change the equilibrium phase LC-LE of DPPC monolayers, depending on the ionic strength and salt type in the subphase. BAM images were obtained in the expansion compression cycles to visualize the Bactenecin-DPPC interaction. AFM images were performed for Bactenecin/DPPC monolayers, where the Bactenecin domains are shown, surrounded by DPPC molecules. We also performed circular dichroism measurements to obtain some information about the peptide conformation in solution and on Langmuir-Blodgett films. Ellipsometry experiments gave us some information of he peptide penetration into DPPC monolayer by measuring the growing of the monolayer size.

2064-Pos The Role of Lipids for the Functional Integrity of Porin

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