immobilized the protein without significantly altering its structure, kinetics, or dynamics, and represent a major step forward toward the goal of ''watching'' individual molecules fold.

2005-Plat
Time Resolved Thermodynamics of Fast Protein Folding in Cytochrome c
Randy W. Larsen.
University of South Florida, Tampa, FL, USA.
One of the earliest studies of fast protein folding involved the photolysis of CO bound Cytochorme c in the presence of 4.5 M Gdn-HCl HCl, pH 7.0. These studies revealed fast coordination of non-native His and Met residues resulting in a 'frustrated' folding pathway resulting from non-native coordination. These early events occur on timescales of less than 10 μs. Similar fast folding can be initiated at lower Gdn-HCl at pHs above 9.5. Under these conditions the non-native coordination occurs with time constants of ~ 300 ns and ~ 3 μs at 22 dg C. Previous time resolved CD spectra suggest that 8% of the native like secondary structure forms in $<$ 1 μs (Goldbeck et al., PNAS (1999), 96, 2782). Here, photoacoustic calorimetry (PAC) has been utilized to probe the thermodynamics associated with fast folding and non-native ligand coordination in CO-Cytochrome c ta pH 12.7 and 350 mM Gdn-HCl. At temperatures below 18 ° C the PAC signals indicate multi-phasic kinetics that can not be fit to simple exponential sums suggesting a distribution of conformations in the presence of CO. The integrated thermodynamics (integrated thermodynamics for processes occurring in $<$ ~ 20 μs) give an enthalpy change of 5 ± 2 kcal/mol and a molar volume change of -0.5 ± 0.4 mL/mol. Taking into account the enthalpy and volume changes associated with CO photo-release from the heme, an enthalpy change of - 12 kcal/mol and volume change of -6 mL/mol is obtained for the 8% folding as well as non-native His and Met coordination. These results will be discussed within the context of the protein funnel mechanism for fast folding in cytochorme c.

2006-Plat
Time-resolved Fret Study Shows Sub-populations of A Globular Protein Molecules at The Refolding Transition Zone
Elisha Haas, Dan Amir, Eitan Lerner.
Bar Ilan University, Ramat Gan, Israel.
Cooperative protein folding invokes discrete folded and unfolded ensembles separated by a free-energy barrier. In contrast, downhill folding involves just one ensemble of protein molecules within a single free-energy well. Common method of monitoring the folding transition which yield mean values cannot resolve the two mechanisms. Time-resolved dynamic resonance nonradiative excitation energy transfer (trFRET), which can yield distributions of conformers in ensembles of partially folded protein molecules was applied. *E. Coli* adenylate kinase (AK) was used as a model in a study of the unfolding/refolding transition. Several mutants were prepared which enabled monitoring the folding transition at different parts of the molecule.
The analysis of trFRET monitored chemically induced unfolding/refolding transition yielded a clear evidence for the presence of two distinct sub-populations at the transition zone. One sub-population was native like and the other was unfolded. The proportion of the size of the two sub-populations was varied as function of the concentration of the denaturant.
These experiments yielded solid evidence in support the model of cooperative, barrier crossing, mechanism of folding this protein. At least for this case, the model of downhill mechanism of folding is not applicable.

Platform AP: Membrane Active Peptides

2007-Plat
Effects of Oxidative Stress on Aggregation and Membrane Interaction of alpha-Synuclein Characterized by Single Molecule Fluorescence
Eva Sevcsik, Elizabeth Rhoades.
Yale University, New Haven, CT, USA.
Oxidative stress has been implicated as a major contributing factor to Parkinson's Disease (PD), a neurodegenerative disorder characterized by the deposition of fibrillar amyloid inclusions in the substantia nigra. The primary protein component of these inclusions is alpha-synuclein (aS), an abundant presynaptic protein, whose natural functions have not yet been resolved but presumably involve synaptic vesicle trafficking. Aggregation of amyloid proteins involves sampling of heterogeneous conformational and oligomeric intermediates, and it is actually these species that have been implicated to be responsible for neuronal cell death, possibly by compromising cell membrane integrity. Here, we use single molecule fluorescence techniques (fluorescence correlation spectroscopy and single molecule Förster energy transfer) to investigate the influence of oxidative modifications to both the protein and the lipid matrix on the molecular mechanisms of aS aggregation and membrane interaction. We find that oxidative modification to either protein or lipid leads to a decrease of aS vesicle binding, with the extent of decrease being dependent on the lipid matrix. As aS natural functions most likely involve synaptic vesicle binding, these results might indicate a loss of aS function due to oxidative stress. Further, oxidized aS shows a different aggregation behavior and does not form amyloid fibrils. The systematic characterization of the effects of oxidation on aS aggregation and membrane interaction will help to refine our understanding of the toxic form(s) of aS in order to identify cellular targets for the design of therapeutics to treat or prevent PD.

2008-Plat
Amyloid-β Ion Channels in Artificial Lipid Bilayers and Neuronal Cells. Resolving a Controversy
Ricardo F. Capone[1,2], Felipe Garcia Quiroz[1], Panchika Prangkio[1], Inderjeet Saluja[1], Anna M. Sauer[1], M.R. Bautista[3], R. Scott Turner[1], Jerry Yang[3], Michael Mayer[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]current address: University of Chicago, Dept. of Medicine, Chicago, IL, USA, [3]University of California, San Diego, CA, USA.
One of the current hypotheses for the pathology of Alzheimer's disease (AD) proposes that amyloid-beta (Aβ) peptides induce uncontrolled, neurotoxic ion flux across cellular membranes. The resulting inability of neurons to regulate their intracellular concentration of ions, in particular calcium ions, has been associated with cell death and may thus contribute to cognitive impairment typical for AD. The exact biophysical mechanism of this ion flux is subject of an ongoing and unresolved controversy. Two mechanisms are currently debated. One proposed mechanism suggests that Aβ assembles into pore-like structures in lipid membranes, leading to stepwise fluctuations of transmembrane current that is typical for ion channels (ion channel hypothesis). The other proposed mechanism postulates a generalized and gradually increasing ion flux as a result of Aβ-induced thinning of membranes.
Here, we resolve this controversy by examining, in detail, the two pivotal protocols for preparing and measuring Aβ induced conductance through planar lipid bilayers and cell membranes. The results clarify that Aβ induces stepwise ion flux across planar lipid bilayers as opposed to a gradual increase in transmembrane current; they show that the previously reported gradual increase in transmembrane current arises from residues of the solvent hexafluoroisopropanol, which is commonly used for the preparation of amyloid samples.
We also examined the effect of Aβ samples on cell membranes. We exposed SH-SY5Y neuroblastoma cells and mouse cortical primary neurons to Aβ at resting potential in the presence and absence of typical ion channel blockers. The results provide additional evidence suggesting that Aβ peptides can form ion channels in cellular membranes that are independent from the postulated ability of Aβ to modulate intrinsic cellular ion channels or transporter proteins.

2009-Plat
Lipid Membrane Penetration Forces from AFM Force Spectroscopy
Elizabeth A. Hager-Barnard, Benjamin D. Almquist, Nicholas A. Melosh.
Stanford University, Stanford, CA, USA.
Understanding how short peptide sequences are able to penetrate cell membranes is important in disease studies and engineering new peptides for drug delivery. While the energetics of membrane penetration has been well studied, the mechanical landscape during contact, translocation, and exit is largely unknown. We used atomic force spectroscopy (AFM) studies on lipid membrane stacks to map the force-distance profile during penetration of short peptides. These force curves reveal the spatial location and magnitudes of penetration barriers that can be related to peptide molecular structure and orientation. We studied the widely used cell penetrating peptide HIV-TAT, a positively charged 9-mer with six arginine groups. The peptides were attached in a single layer at the end of a flat AFM tip giving nanometer spatial resolution relative to the lipid bilayer. Using stacks of lipid membranes rather than individual supported membranes improves data quality by removing substrate effects and providing better statistics.

2010-Plat
Membrane insertion of peptides mimicking E2 domain of Sindbis virus is modulated by cholesterol
Thomas G. Chadwick, Ghada A. Rabah, Brian R. Davies,
Tatyana I. Smirnova.
North Carolina State Univeristy, Raleigh, NC, USA.
In the process of assembly Sindbis enveloped virus uses a host-derived membrane bilayer that is ''sandwiched'' between the concentric protein shells. The transmembrane domains of three glycoproteins penetrate the bilayer and

are capable of assembling in two strikingly different membranes: mammalian membranes that contain up to 40% of cholesterol and insects membranes that contain larger fraction of 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 cells while STM-18 shows almost wild-type behaviour. We have investigated the interaction of synthetic peptides mimicking E2 domain mutants with lipid bilayers with the goal to understand constraints placed upon membrane spanning domains for correct integration into the bilayer. The phospholipid composition was chosen to represent mammalian and insects' membranes. Results of EPR spin-labeling experiments show that both STM-16 and STM-18 peptides adopt a transmembrane configuration in bilayers with lipid composition mimicking that of insects. In mammalian cell mimicking membranes and containing cholesterol the STM-16 peptide aggregates at the surface of the bilayer. Both peptides exhibit transmembrane orientation in bilayers consisting of ''mammalian'' lipid mixture but without cholesterol. Thus, we show that cholesterol content of the lipid mixture modulates insertion of the peptides into bilayer mimicking mammalian cell membrane. Supported by NSF grant MCB-0451510 to TIS.

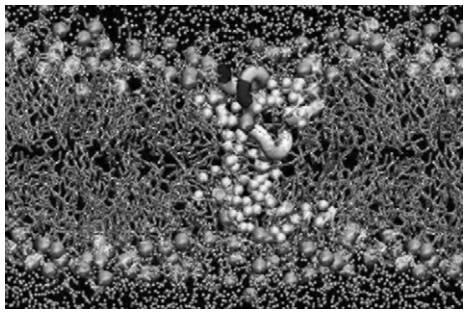
[1] Hernandez, R., *et. al. J Virol* **2003** 77(23), 12710-9.
[2] West, J., *et. al. J. Virol*., **2006** 80:4458-4468.

2011-Plat

Disordered Pore Formation At Rigid/Fluid Boundary Zones As A New Mechanism For Peptide-Membrane Interaction With The Beta-sheeted Antimicrobial Peptide Cateslytin

Frantz Jean-Francois[1], Juan Elezgaray[1], Marie-Héléine Metz-Boutigue[2], **Erick J. Dufourc[1]**.
[1]CNRS, University Bordeaux 1, ENITAB, Pessac, France, [2]INSERM U575, Strasbourg, France.

The peptide cateslytin (RSMRLSFRARGYGFR) produced by enzymatic degradation of stress proteins is remarkably active against a large number of microorganisms including Plasmodium Falciparum responsible for Malaria. Its mode of action on membranes mimicking the external negatively charged membrane of these microorganisms has been studied by circular dichroism, infra-red (attenuated total reflection), high-resolution magic angle sample spinning and wide line solid state NMR, patch clamp and molecular dynamics. The peptide, which is unstructured in solution, adopts an aggregated beta sheet structure upon interacting with negatively charged membranes. Rigid membrane domains are formed upon interaction and separate from more fluid zwitterionic membrane zones. No macroscopic membrane lysis is observed, suggesting that crossing through membranes occurs at phase boundary defects. Patch clamp and all atom molecular dynamics of these zones indicate that disordered pores of ca. 10 Angstrom are formed by a mechanism that is analogous to molecular electroporation (Jean-François et al., Biochemistry & Biophysical J., 2008).



2012-Plat

High-Resolution Structure of Piscidin in Aligned Lipid Bilayers: Implications for Antimicrobial Mode of Action

Myriam Cotten[1], Riqiang Fu[2], Eric D. Gordon[3], Elaina L. Daza[3], Anna S. Kozlova[3], Daniel J. Hibbard[3], Mallorie M. Taylor[3], Jeffrey J. Ditto[3], Milton Truong[2].
[1]Hamilton College, Clinton, NY, USA, [2]National High Magnetic Field Laboratory, Tallahassee, FL, USA, [3]Pacific Lutheran University, Tacoma, WA, USA.

Piscidin is an amphipathic cationic antimicrobial peptide that belongs to a large family of versatile host-defense peptides, which interact, at least initially, with cell membranes in order to perform their function. In the research presented here, we characterize the secondary structure and dynamics of piscidin in order to identify factors optimizing specific molecular interactions that are directly related to its function and mode of action. Our long term goal is to identify common principles that will facilitate the design of pharmaceuticals with broad-spectrum antibacterial activity, minimum induction of bacterial resistance, and low toxicity for mammalian cells.

Previously, we demonstrated that membrane-bound piscidin 1 (p1) adopts an alpha-helical structure, which lies in the plane of the bilayer and experiences fast motions. Here, high-resolution solid-state NMR spectra have been obtained from multiply ^{15}N-backbone-labeled p1 aligned in hydrated lipid bilayers. Analysis of data from twenty sites of this 22-mer reveals two helical segments separated by a kink at Gly_{13}. This kink may help one portion of the peptide insert more deeply in the hydrophobic lipid bilayer, which may be related to the mechanism of membrane disruption. To characterize water exposure, hydrogen-deuterium exchange experiments have been performed on ^{15}N-backbone labeled samples. In addition, solid-state NMR was applied to ^{15}N-side-chain-labeled His-17 p1 to titrate this side chain, which resides at the interface between the hydrophilic and hydrophobic domains of p1. Overall, our atomic-level investigation of the structure, dynamics, and water exposure of membrane-bound piscidin provides new insights into its mode of action and thus help understand how antimicrobial peptides recognize membranes and initiate their activities on microbial cells.

2013-Plat

Membrane Thinning Is Not A Unique Signal Of Pore Formation By Antimicrobial Peptides

Georg Pabst[1], Stephan Grage[2], Sabine Danner-Pongratz[1], Weiguo Jing[1,3], Anne S. Ulrich[2], Anthony Watts[4], Karl Lohner[1], Andrea Hickel[1].
[1]Austrian Academy of Sciences, Graz, Austria, [2]Karlsruhe Institute of Technology, Karlsruhe, Germany, [3]Rockefeller University, New York, NY, USA, [4]Oxford University, Oxford, United Kingdom.

We have observed a hydrocarbon chain length dependent perturbation of saturated acyl chain phosphatidylglycerol bilayers by the antimicrobial peptide peptidyl-glycylleucine-carboxyamide (PGLa) using X-ray diffraction, solid-state ^{2}H-NMR, differential scanning calorimetry and dilatometry. In the gel phase, PGLa assumes a surface alignment and induces a quasi-interdigitated phase, previously reported also for other peptides. This effect is most pronounced for C18 phosphatidylglycerol. Above the lipid chain melting transition, in the fluid phase, the PGLa helix inserts into the membrane above a certain threshold concentration. In this case we found an increase of the membrane thickness and NMR order parameter for C14 and C16 phosphatidylglycerol bilayers, though not for C18. The data is best understood in terms of a close hydrophobic match between the C18 bilayer core and the peptide length when PGLa is inserted with its helical axis normal to the bilayer surface. The C16 acyl chains appear to stretch in order to accommodate PGLa, whereas tilting within the bilayer seems to be energetically favorable for the peptide when inserted into bilayers of C14 phosphatidylglycerol. In contrast to the commonly accepted membrane thinning effect of antimicrobial peptides, the data demonstrate that pore formation does not necessarily relate to changes in the overall bilayer structure.

2014-Plat

Basis For The Broad-spectrum Antimicrobial Activity Of Interfacially-active Peptides

Ramesh Rathinakumar, William C. Wimley.
Tulane University, New Orleans, LA, USA.

Recently, we rationally designed and screened a library of small, membrane pore-forming beta-strand peptides based on interfacial activity: ''the ability of a molecule to partition into the membrane-water interface and to alter the packing and organization of lipids''. From this library we selected ten soluble and highly potent pore-forming peptides using a function based high-throughput screen. Many natural antimicrobial peptides (AMP) act directly on microbes and permeabilize their membranes. Our library peptides were rationally designed to disrupt membranes which were similar to those of microbial membranes. Accordingly, *in vitro* experiments showed that the selected peptides permeabilize live microbial cell membranes and all ten have potent, broad-spectrum activity against many different species bacteria and fungi. These potent, biologically-active peptides apparently undergo nonspecific interactions with membranes. Here we explore the basis for broad-spectrum antimicrobial which is a very poorly understood phenomenon. We find that many members of the library have potent activity against some microbes, however broad-spectrum against multiple classes of microbes is rare overall. Similarly, subtle changes in peptide structure propensity cause the loss of activity against some microbes, but not others. Comparison of structure-function relation of these peptides in lipid vesicles to their activity in biological membrane suggests that ''interfacial activity'' is the basis for biological activity. A very specific and narrow range of interfacial activity gives rise to broad-spectrum antimicrobial activity.