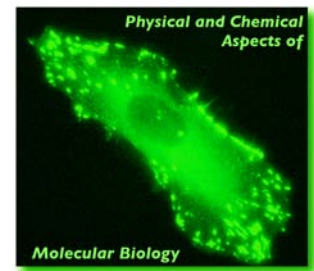


Physical and Chemical Aspects of Molecular Biology

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Abstracts

Talks

A-01 Jonathan Widom, Northwestern University

A genomic code for nucleosome positioning

Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. Nucleosomes are remarkable from a physical perspective because in each nucleosome one persistence length of DNA – a lengthscale of DNA inflexibility – is wrapped in nearly two complete superhelical turns around a protein core. As a consequence of this extreme DNA bending, nucleosomes have higher affinity for particular DNA sequences which are best-able to sharply bend as required by the nucleosome. We have discovered that genomes care where their nucleosomes are located on average, and that genomes manifest this care by encoding an additional layer of genetic information, superimposed on top of other kinds of regulatory and coding information that were previously recognized. We have developed a partial ability to read this nucleosome positioning code and predict the in vivo locations of nucleosomes. We now have two entirely independent approaches to understanding and predicting nucleosome positioning: one is based on a statistical profile of natural nucleosome DNAs, the other, on a dinucleotide mechanics model which itself derives from X-ray crystallographic studies of non-nucleosomal protein-DNA complexes. Our results suggest that genomes utilize the nucleosome positioning code to facilitate specific chromosome functions including transcription factor binding, transcription initiation, and even remodelling of the nucleosomes themselves.

A-02 Jaime Ruiz-Garcia, Universidad Autónoma de San Luis Potosi

Trapping and condensing DNA at the air/water interface

DNA is a highly charged polyelectrolyte and as such it is considered to be completely soluble in pure water. Surprisingly, we found that DNA can be trapped at the air/water interface and does not go back into a pure water subphase. We show that DNA is trapped in a minimum energy at the interface, much bigger than kT , that does not permit its return to the bulk. Once at the interface, DNA molecules condense to form different two-dimensional mesostructures such as foams, giant rings, disks and rods at low density. This condensation occurs without the presence of multivalent cationic ions, as it is required in bulk, for example in condensing DNA toroids. However, we show that monovalent and divalent cations do not change this behavior, but trivalent cations do. At high density, the molecules form a remarkable monomolecular network. At the interface, DNA is only partially immersed in water, which originates that the chains get only partially charged, but the charges are of the same sign. Therefore, this can be considered another case of like-charge attraction, similar to those found in colloids trapped at the air/water interface. However, the origin of the attractive part of the interaction potential is unknown. In addition, we found that DNA at the air/water interface can form 2D smectic-like domains tens of microns in size. Both, the DNA monomolecular networks and the smectic-like domains are interesting from theoretical and application standpoints.

A-03 Joel Stavans, Weizmann Institute of Science

Sex and sensitivity: The fidelity of RecA-assisted homologous recombination

Homologous recombination plays a key role in generating genetic diversity, while maintaining protein functionality. The mechanisms by which RecA enables a single-stranded segment of DNA to recognize a homologous tract within a whole genome are poorly understood. The scale by which homology recognition takes place is of a few tens of base pairs, after which the quest for homology is over. To study the mechanism of homology recognition, RecA-promoted homologous recombination between short DNA oligomers with different degrees of heterology was studied *in vitro*, using fluorescence resonant energy transfer (FRET). RecA can detect single mismatches at the initial stages of recombination, and the efficiency of recombination is strongly dependent on the location and distribution of mismatches. Mismatches near the 5' end of the incoming strand have a minute effect, whereas mismatches near the 3' end hinder strand exchange dramatically. There is a characteristic DNA length above which the sensitivity to heterology decreases sharply. Experiments with competitor sequences with varying degrees of homology yield information about the process of homology search and synapse lifetime. The exquisite sensitivity to mismatches and the directionality in the exchange process cannot be accounted for by equilibrium Statistical Mechanics. Instead we propose a mechanism for homology recognition that can be modeled as a kinetic proofreading cascade.

B-01 Ka-Yee C. Lee, University of Chicago

Cholesterol/phospholipid interactions: Evidence of ordering and displacement of cholesterol by alcohol

The phase diagrams of some cholesterol-lipid mixtures exhibit two immiscibility regions along with a sharp cusp, pointing to a particular stoichiometry for possible lipid/cholesterol complex formation. It has been hypothesized that reactive cholesterol monomers are present in mixtures with cholesterol content greater than that at the cusp. To test this hypothesis, we have examined how the presence of alcohol alters the lipid/cholesterol phase diagram. Lipid/cholesterol/alcohol systems in which various mole fractions of cholesterol are replaced by alcohol reproduce the identical phase diagram as the lipid/cholesterol system, with the cusp position unaltered. Cholesterol uptake by beta-cyclodextrin is large in the ternary system as long as the combined mole fraction of cholesterol and alcohol exceed that at the cusp. X-ray diffraction on these mixtures shows the presence of a broad Bragg peak, indicative of the existence of crystalline order, with coherence length of several molecular dimensions, in mixed lipid/cholesterol systems.

**B-02 José Campos-Terán, UAM Cuajimalpa
with Ramos, Mas-Oliva, Castillo**

Interactions of adsorbed α -helices on hydrophilic substrates

Interaction between amphiphilic α -helical proteins (human apolipoprotein AII) adsorbed on a hydrophilic surface from a buffer solution at pH 4 was studied using an interferometric surface force apparatus. The interaction forces between the adsorbed layers of α -helices are mainly composed of electrostatic double layer forces at large surface distances and of steric repulsive

forces at small distances. Amphiphilicity of the α -helix structure facilitates the formation of protein multilayers next to the surfaces. We also found that the interaction mica-protein is stronger than protein-protein interaction. The results of protein adsorption and force interaction suggest that they are consequences of the protein secondary structure, the amphiphilic α -helix motif.

B-03 Kris N. Dahl, Carnegie Mellon University***Nuclear structure changes in Hutchinson-Gilford progeria syndrome***

Lamin proteins polymerize to form a network of structural filaments at the inner nuclear membrane called the nuclear lamina. Both mechanical stability and mechano-sensitive gene expression are defective in cells lacking certain lamin proteins, but the underlying nature of the mechanical properties of the lamina are poorly understood. We use micromanipulation to quantify the viscoelastic properties of isolated nuclei from human fibroblasts, including those from Hutchinson-Gilford Progeria Syndrome (HGPS) patients. HGPS, a premature aging disease, is caused by mutations in the gene encoding A-type lamins. Nuclear defects similar to HGPS disease are also seen in normal age progression. Nuclei from HGPS patients show significant quantitative reduction in the ability to rearrange, and the HGPS nuclei collapse along major axes, suggesting catastrophic failure to distribute applied forces across the entire lamina. The HGPS lamin network appears to have locally-ordered microdomains, which can explain most of the mechanical differences seen here between HGPS and normal cells and may have functional consequences in disease.

B-04 Volker Kiessling, University of Virginia***Transbilayer organization of complex fluid lipid membranes***

Cell membranes have complex lipid compositions, including an asymmetric distribution of phospholipids between the opposing leaflets of the bilayer. While it has been demonstrated that the lipid composition of the outer leaflet of the plasma membrane is sufficient for the formation of raft-like liquid-ordered (l_o) phase domains, the influence that such domains may have on the lipids and proteins of the inner leaflet remains unknown.

Using tethered polymer supports and a combined Langmuir-Blodgett/vesicle fusion technique allowed us to form asymmetric planar bilayers that mimic plasma membrane asymmetry. While directly supported Langmuir-Blodgett monolayers containing cholesterol-rich liquid ordered phases are inherently unstable when exposed to vesicle suspensions, tethering the monolayers with the lipid-anchored polymer 1,2-dimyristoyl phosphatidylethanolamine-N-[poly(ethylene glycol)-triethoxysilane] increased the stability sufficiently to retain >90% asymmetry for one to two hours. We could show that l_o phase domains in one monolayer of an asymmetric bilayer do not induce the formation of domains in the opposite leaflet when this leaflet is composed of the synthetic lipid palmitoyl-oleoyl phosphatidylcholine (POPC), but do induce domains when this leaflet is composed of natural lipid extracts from porcine brain and cholesterol. Single fluorescent molecule tracking was used to study the lipid diffusion in asymmetric bilayers with coexisting liquid phases. This allowed us to study in detail the diffusion of individual lipids inside, outside, or directly opposed to l_o phase domains. The diffusion of lipids is similar in l_o and l_d phase domains and is not affected by transbilayer coupling indicating that lateral and transverse lipid interactions that give rise to the domain structure are weak in the biological lipid mixtures that were employed in this work. We show how supported asymmetric planar bilayers can be utilized to study aspects of lipid protein interactions.

B-05 Leonardo Dagdug, UAM Iztapalapa

Diffusion and active sites

not available

**B-06 Duncan McGillivray, Australian National University
with Heinrich, Valincius, Ignatiev, Vanderah, Kasianowicz, Lösche**

Toxin interactions with tethered bilayers

*The toxin α -hemolysin (α HL) from *Staphylococcus aureus* is the paradigm of a pore-former in biomembranes. The protein has been extensively investigated in single-molecule electrophysiological studies of free-standing membranes, its atomic-scale structure has been solved by X-ray crystallography, and the suggestion to use the molecular pores in DNA-sequencing devices has received considerable attention. Aiming at both structural studies of its membrane incorporation, and the reconstitution of the protein into a sensor format with long-term stability, we demonstrate the reconstitution of these toxin pores in a biomimetic tethered bilayer membrane (tBLM) on a solid substrate. The electrical characteristics of the pore, determined from electrical impedance spectroscopy, are consistent with measurements of functional proteins in freestanding lipid membranes while being stable over long periods of time (days). Neutron reflectometry shows the protein's cap domain outside the bilayer membrane, and significant impact of the protein on the head group region of the outer membrane leaflet. A more sophisticated analysis of the neutron data using the known X-ray structure of the protein reveals structural details about the interaction of the protein with the membrane. Supported by the NSF under grant no. CBET-0555201.*

C-01 Markus Deserno, Max-Planck-Institute for Polymer Research

Curvature-mediated interactions between membrane proteins lead to aggregation and vesiculation

Cellular tasks such as endocytosis, vesiculation, and protein sorting, or the biogenesis of organelles such as the endoplasmic reticulum or the Golgi apparatus rely on significant protein-assisted membrane remodeling. Special curvature-sensitive proteins may both experience geometry-driven forces and, conversely, induce major changes in membrane shape and topology. Yet, owing to the lipid bilayer's bending stiffness, the latter requires the cooperative action of many individual proteins. Recently generic membrane-based mechanisms have been proposed by biologists as a way to effect the necessary aggregation of proteins which complements the usual specific interactions, but the physical basis for such forces is not as obvious as the recent literature sometimes makes one believe. In this talk I demonstrate, using an efficient generic simulation model for membranes recently developed by us, that generic membrane curvature mediated forces can indeed lead to protein aggregation and trigger subsequent vesiculation, thus providing a firmer basis for existing speculations.

C-02 Arturo Rojo-Domínguez, UAM Cuajimalpa

Molecular modeling of ligand docking in proteins

Proteins are the biological macromolecules with the most versatile functions. They are directly responsible of catalysis, regulation, transport, defense, etc., and their functions depend on the precise orientation of specific chemical groups on their surface. The orientation of such groups depends in turn on the three-dimensional folding of their main chain, directed by the sequence of the amino acids which constitute this polypeptide. Most of the knowledge currently known on protein structure comes from experimental data obtained by X-ray Crystallography and Nuclear Magnetic Resonance. Nevertheless, some proteins are reluctant to be studied with these techniques, and in addition proteins are sequenced at a much higher rate than that of experimental methods for protein structure determination. Hence there is a huge gap between the number of known protein sequences and 3D structures, making computational molecular modeling as the accessible procedure for protein structure determination. Among the different algorithms for this prediction, the most precise is comparative or homology modeling, where a structure with amino-acid sequence similarity to the query protein is required as template. Threading and ab initio procedures are alternative, but less precise forms of protein structure prediction. We will discuss some applications and limitations of protein molecular modeling and computational analysis of protein conformation; the complications derived from the characteristic flexibility and complexity of their main chain and 3D structure; the energetic, kinetic and intermolecular recognition consequences of structural modifications in atomic coordinates; and the protein-ligand prediction challenge, which is the base of both rational drug-design and protein-protein interaction determination for interactome construction. Furthermore, due to the size of protein molecules (typically several thousand atoms) practically all the energy determinations have employed classical methods, but we will discuss two cases where quantum-mechanics studies reveal that classical methods may not be able to adequately describe phenomena.

involved with biological function.

C-03 Maria G. Kurnikova, Carnegie Mellon University

Theoretical/computational analysis of interactions in biological nanopores

An ion channel protein alpha-hemolysin (α HL) forms supramolecular complexes with a polysaccharide beta-cyclodextrin (β CD). The system has potential uses in nanoscale device engineering. Recently, life-times of some of the mutant complexes were studied [1], however, variations in the observed complex life-times were not completely understood in part due to the lack of knowledge of structures of these metastable complexes. We have performed an extensive molecular modeling studies of the β CD- α HL and selected mutant complexes to gain insights into the β CD- α HL interaction mechanisms and to predict possible structures and energetics of the complexes. Thermodynamic integration (TI) and umbrella sampling (US) techniques were used to calculate relative binding affinities of the complexes formed with the wild type α HL, and several mutants. In these studies TI alone was insufficient to accurately calculate corresponding free energy differences. By utilizing a TI/US combination in a novel manner we were able to accurately predict free energy changes in these flexible systems.

[1] Gu et al., J. Gen. Physiol. 118:481 (2001)

**C-04 Michael E. Paulaitis, Ohio State University
with Asthagiri, Pratt**

Role of fluctuations in a snug-fit mechanism of the KcsA K⁺ channel selectivity

Biological ion channels are exquisite nano-scale devices that can sort ions with a high fidelity while also conducting them at a high rate. Understanding their mechanism can provide insights into designing, for example, novel separation processes or sensor devices. The KcsA K⁺ channel is selective for K⁺ over Na⁺ at rates of ion transport approaching the diffusion limit. This selectivity is explained thermodynamically in terms of the favorable partitioning of K⁺ relative to Na⁺ in a narrow selectivity filter in the channel. We examine the molecular basis for this selectivity by calculating the distribution of binding energies for Na⁺ and K⁺ in a simplified model of the filter of the KcsA channel. We find that Na⁺ binds to the filter with a mean binding energy substantially lower than that for K⁺. The difference is comparable to the difference in hydration free energies of Na⁺ and K⁺ in bulk aqueous solution. Thus, the average filter binding energies do not discriminate Na⁺ from K⁺ when measured from a baseline of the difference in bulk hydration free energies. Instead, discrimination can be attributed to the scarcity of favorable binding configurations for Na⁺ compared to K⁺. That relative scarcity is quantified as enhanced binding energy fluctuations and is consistent with constriction of the filter induced by Na⁺ binding.

**C-05 Douglas Tobias, University of California, Irvine
with Freitas, Robinson, Schow, Cheng, White**

Buried charges in membranes: Structural and functional implications for voltage-sensitive transmembrane ion transport

Recently determined crystal structures of membrane proteins show that charged amino acid residues on transmembrane (TM) helices have high exposure to the nonpolar membrane interior, and translocon-mediated TM helix insertion experiments have revealed a low free energy cost for inserting charged residues deeply in a membrane. Both of these observations are surprising in light of expectations based on a continuum electrostatics description of a membrane as a slab of low dielectric constant material sandwiched between regions of high dielectric constant, which predicts a large free energy penalty for inserting a charge in a membrane. In this talk I will report atomistic molecular dynamics simulation studies aimed at understanding how positively charged amino acid sidechains are accommodated by lipid bilayers, and will discuss the implications of our findings for the structure and function of voltage-gated ion channels.

D-01 Armando Gómez-Poyou, Universidad Nacional Autónoma de México

Cross-talk between the subunits in the homodimeric enzyme, triosephosphate isomerase

*In all cells the majority of the enzymes are oligomers. Numerous studies have provided important information on the size and shape and physico-chemical characteristics of the interfaces. However, the dynamic interplay between the subunits of oligomeric enzymes during catalysis are not completely understood. For example in homodimeric triosephosphate isomerase (TIM) only the dimer is catalytically competent, although each monomer possesses the residues required for catalysis. To probe into the functional connection between TIM monomers, we studied triosephosphate isomerase from *Trypanosoma cruzi* (TcTIM) and *T. brucei* (TbTIM). These enzymes are markedly similar in amino acid sequence and three-dimensional structure. In their dimer interface, each monomer has a Cys15 that is surrounded by loop3 of the adjoining*

subunit. Perturbation of the interfacial Cys by methylmethane thiosulfonate (MMTS) causes abolition of catalysis and structural changes. In the two TIMs, the structural arrangements of their Cys15 are almost identical. Nonetheless, the susceptibility of TcTIM to MMTS is nearly 100-fold higher than in TbTIM. Thus, the enzymes provide a unique opportunity to ascertain if the properties of the interface Cys depend on the dynamics of its own monomer or on those of the adjacent subunit. Experiments with mutants of TcTIM that have the interfacial residues of TbTIM, and in TcTIM-TbTIM hybrids that have only one interfacial Cys (C15ATcTIM and wild type TbTIM) showed that the susceptibility of the interfacial Cys depends predominantly on the dynamics of the adjoining monomer, and that the perturbation of one interfacial Cys diminishes the activity by about 60%. We also studied hybrids formed with a C15ATcTIM monomer and a catalytically inert TbTIM monomer (E168D TbTIM); the activity of this hybrid is reduced by ~ 50% when the only interfacial Cys is perturbed. Collectively the data indicate that about half of the activity of each of the two monomers depends on the integrity of each of the two equivalent regions of the interface. This may be part of the explanation of why TIM is an obligatory dimer.

**D-02 Sarah Veatch, Cornell University
with Soubias, Leung, Keller, Thewalt, Gawrisch**

Complex phase behavior in simple membranes: Phase separation and critical phenomena in membranes of DOPC, DPPC, and cholesterol

Coexisting liquid phases in DOPC/DPPC/Cholesterol bilayer membranes are completely described by a thermodynamically consistent ternary phase diagram. Deuterium (^2H) NMR spectra clearly delimit miscibility phase boundaries and are used to evaluate tie-lines connecting liquid-ordered (L_o) and liquid-disordered (L_d) phases. At low temperatures and low cholesterol compositions, the L_o - L_d region intersects a L_o - L_d - S_o (solid-ordered) three-phase triangle which is clearly resolved by ^2H NMR. At high temperature and cholesterol composition, the L_o - L_d region terminates in a line of critical points. Significant spectral broadening is observed by ^2H NMR in the vicinity of known critical points, and is due to a decreased spin-spin relaxation time (T_2) of lipids in these samples. Faster spin-spin relaxation is a result of increased lipid motions with correlation times between 0.1 and 10 microseconds. The observed spectral broadening is consistent with composition fluctuations associated with critical phenomena, and may provide the link between small-scale organization and large-scale phase separation observed in model systems. The effect of impurities is also explored by NMR, and it is found that the miscibility transition is exquisitely sensitive to the addition of fluorescent probes. This finding explains previous discrepancies between NMR and fluorescence microscopy results and provides a possible mechanism by which domain size and stability could be controlled in biomembranes.

**D-03 Arne Gericke, Kent State University
with R. Redfern, D. Redfern, Ross**

Effect of phosphoinositide identity and lateral organization on PTEN binding and structure

Recent reports have shown that PTEN is activated by $\text{PI}(4,5)\text{P}_2$ and that this activation requires a polybasic region located at the N-terminus of the protein. It was hypothesized that $\text{PI}(4,5)\text{P}_2$ aids membrane recruiting and induces a conformational change in PTEN. This study characterizes PTENs binding preferences, investigates its contact points with the lipid bilayer and highlights structural changes upon membrane interaction. Fluorescence quenching experiments

involving PC/phosphoinositide vesicles yielded results consistent with an enhanced PTEN binding to PI(4,5)P₂ or PI(5)P containing vesicles, while the interaction with all other phosphoinositide derivatives was only minor. Addition of PS to these vesicles increased the overall binding but did not alter the binding preferences. Experiments with a truncated PTEN₁₆₋₄₀₃ showed a strongly reduced phosphoinositide affinity and a loss of specificity, while experiments using a peptide representing PTENs N-terminus (PTEN₁₋₂₁) showed preferential PI(4,5)P₂ binding. Infrared spectroscopic measurements furnished results consistent with an increased α -helical secondary structure content in the presence of PI(4,5)P₂/PC vesicles, while other phosphoinositides like PI(3,5)P₂ or PI(3,4,5)P₃ did not cause a structural change. Although PS induced a structural change towards more β -sheet, it did not alter the structural effect of PI(4,5)P₂. In contrast, PTEN₁₆₋₄₀₃ did not show a structural change in the presence of PI(4,5)P₂, while PS induced an enhanced β -sheet content. PTENs structural changes were also associated with a marked change of Trp fluorescence. Supported by the NIH (AR-038910 and NS-21716).

**D-04 Jaime Mas-Oliva, Universidad Nacional Autónoma de México
with Moreno, Castillo**

Disorder-to-order peptide conformational switches for receptor recognition and lipid transfer

One of the major obstacles to a better understanding of the interactions between apolipoprotein-AI, and the scavenger receptor type B class 1 and the ATP binding cassette protein as the key mediators of HDL function, has been the paucity of detailed structural information for apoAI and the modulator apoCI, that would explain receptor recognition and lipid transfer. Based upon state of the art biophysical experimentation using Langmuir monolayers, we have previously shown that surface lateral pressure desorbs apolipoprotein segments from the interface, and proposed to be the switch that triggers both phenomena. Recent experiments in our laboratory have shown that these segments might not keep their predicted α -helix structure, since synthesized peptides derive from complete apolipoprotein sequences show to be non-structured in solution. Therefore, in this study we aim to define that disorder-to-order transitions in these peptides, might be considered as specific switches that could explain conformational-plasticity in apoAI and apoCI.

**D-05 Barbara Baird, Cornell University
with Holowka**

Dynamic membrane interactions during IgE receptor signaling

RBL mast cells have proven to be a useful model for understanding hematopoietic cell signaling and the role of the plasma membrane in this process. This talk will describe our collaborative studies to investigate the basic mechanisms with a combination of experimental approaches. Crosslinking of IgE-Fc ϵ R1 causes their stable association with ordered membrane domains ("lipid rafts") and their consequent phosphorylation by Lyn kinase. Moreover, Lyn in the ordered lipid environment of these rafts is protected from inactivation caused by transmembrane tyrosine phosphatases. To explore the role of transmembrane segments in immunoreceptor signaling, a series of single chain chimeric receptors for human IgE were stably expressed in RBL cells, and their widely different signaling capacities correlate strongly with crosslink-dependent lipid raft association. Defined clustering of IgE-receptors on the micron scale with patterned lipid bilayers enables visualization of interacting components with spatial and temporal resolution. With total internal reflection fluorescence microscopy we find that stimulated exocytosis of

secretory granules and recycling endosomes are targeted differently with respect to the clustered receptors. With the goal of measuring distributions and dynamics of membrane proteins and lipids on the molecular level, we are developing new biophysical tools with fluorescence resonance energy transfer, electron microscopy, and nanofabricated Zero-mode waveguides.

E-01 Igor V. Kurnikov, University of Pittsburgh

Modeling of hemi-gramicidine S-nitroxide conjugates in lipid membranes: Correlation between chemical structure, peptide conformations and biological activity

Recent experiments showed that conjugation of TEMPO nitroxide group with hemigramicidin increased its antiapoptotic activity 1000-fold, apparently by targeting of the antioxidant group to the mitochondrial membrane [1]. However, chemical conjugates of TEMPO with hydrophobic peptides lacking the characteristic β -turn secondary structure of gramicidine S did not exhibit antiapoptotic activity. We performed Molecular Dynamics and Monte-Carlo simulations of nitroxide-peptide conjugates in lipid membranes using explicit and implicit membrane models in order to elucidate relationships between carrier peptide chemical composition, preferred positioning and conformational flexibility inside the membrane, and correlated the simulation results with the reported biological activity of nitroxide-conjugates. We conclude that positioning of the nitroxide group at the interface between polar and non-polar regions of the lipid membrane is critical for its antioxidant and antiapoptotic function.

[1] P. Wipf et al, J. Am. Chem. Soc.127:12460 (2005)

E-02 Alicia Ortega, Universidad Nacional Autónoma de México

Structural energetics of membrane proteins isolated from cardiac and skeletal muscle

not available

E-03 Daniel Zuckerman, University of Pittsburgh

Secret lives of proteins: High and low (dimensionality)

Proteins don't know biology. Rather, they are machines that work, in part, by exploiting thermal fluctuations. These fluctuations have never been quantified in an ensemble picture. On the computational side, this gap is due to under-sampling of the thousands of degrees of freedom present. We have made efforts to improve sampling based on "resolution exchange" methods, which exploit coarse-grained models to enhance atomistic sampling. Along the way, we have found that the configuration spaces of folded proteins are quantifiably low-dimensional in an aggregate sense.

**E-04 Miguel Costas, Universidad Nacional Autónoma de México
with Gómez-Puyou, Pérez-Monfort**

ITC as an aide for drug design: Searching for an effective inhibitor of triosephosphate isomerase

We characterized by calorimetric, crystallographic and biochemical methods the action of a low molecular weight compound that binds to the dimer interface of triosephosphate isomerase from *Trypanosoma cruzi* (TcTIM) and thereby abolishes its function with a high level of selectiv-

ity. The kinetics of TcTIM inactivation by 3-(2-benzothiazolylthio)-1-propanesulfonic acid and isothermal titration calorimetry experiments showed that the binding of two molecules per enzyme are needed for inactivation. The binding of the first molecule is endothermic, and that of the second exothermic. Crystals of a TcTIM-inhibitor complex that diffracted to a resolution of 2 Å showed that the inhibitor is placed at the dimer interface, at less than 4 Å from residues of the two subunits. A relevant feature revealed by the crystal structure of the complex is that the aromatic rings of inhibitor compound are almost perpendicular to the ring of a phenylalanine residue; therefore, it is possible that these two entities interact through attractive edge to face quadrupole interactions stabilizing the TcTIM-inhibitor complex. The inhibitor is more effective at low than at high protein concentrations indicating that it perturbs the association between the two TcTIM monomers. Calorimetric and kinetic data of experiments in which TcTIM was added to a solution of the inhibitor showed that at low concentrations of the inhibitor, inactivation is limited by binding, whereas at high concentrations the events that follow binding become rate-limiting. The portion of the interface of TcTIM that binds the inhibitor and its equivalent region in human TIM differ in amino acid composition and hydrophobic packing. Thus, we show that by focusing on protein-protein interfaces, it is possible to find low molecular weight compounds that are selective for enzymes from parasites. In this context, recent results for a multitypanosomatid inhibitor will be presented.

F-01 Mohammad F. Islam, Carnegie Mellon University**Viscoelasticity of reversible gels of associating rigid rods and their time-dependent gelation**

I will describe recent experiments on the viscoelastic properties of reversible gels of attractive rigid rods: aqueous suspensions of surfactant stabilized single wall carbon nanotubes (SWNTs). The SWNTs are dispersed in water using an anionic surfactant, sodium dodecylbenzene sulfonate. The nanotubes have an average diameter of 1.1 ± 0.3 nm, and an average length of 165 ± 80 nm. Because the persistence length of SWNTs ($l_p \approx 22$ μm) is much larger than the diameter, the nanotubes behave like rigid rods. The SWNT suspensions exhibit a rigidity percolation transition with an onset of solidlike elasticity at a volume fraction of 0.0026; the percolation exponent is 2.3 ± 0.1 . At large strain, the solidlike samples show volume fraction dependent yielding. I will present a simple model to understand these rheological responses and show that the shear dependent stresses can be scaled onto a single master curve to obtain an inter-nanotube interaction energy per bond $\approx 40kBT$. Our experimental observations suggest SWNTs in suspension form interconnected networks with bonds that freely rotate and resist stretching. Suspension elasticity originates from bonds between SWNTs rather than from the stiffness or stretching of individual SWNTs. Finally, I will present a microrheology study of the time dependence of this reversible gelation. We embed fluorescent tracer particles in SWNT suspensions and use optical microscopy tracking techniques to measure the mean squared displacement during gelation. We then apply a time cure superposition to obtain a master curve for the viscoelasticity that extends several decades in frequency. We compare the high frequency dynamics of the SWNTs solutions to those expected for semiflexible and rigid rod polymer systems.

F-02 Mark Chan, Stanford University**DNA-mediated interactions of tethered vesicles**

Reactions between membranes play an important role in many biological processes. A method for tethering lipid vesicles to a supported lipid bilayer via DNA hybridization was developed in

our lab as a scaffold for studying membrane interactions. We have successfully used this system for probing DNA-mediated vesicle-vesicle docking. Spatially separated populations of vesicles presenting complementary DNA are deposited with a microfluidic channel onto a supported bilayer. Vesicles then mix via two-dimensional diffusion, and the kinetics of vesicle-vesicle docking can be monitored by epifluorescence microscopy. Experimental data are compared with lattice-diffusion simulations to extract reaction efficiencies for different vesicle compositions. This efficiency shows a dependence on the average copy number of DNA per vesicle as well as the DNA sequence.

**F-03 Péter Galajda, Princeton University
with Keymer, Muldoon, Park, Austin**

Adaptation on a chip

Using micro- and nanofabrication techniques we constructed adaptive landscapes in microfluidic chips. We studied how the population dynamics of E. coli bacteria depends on the 'geography' of the adaptive landscape. Based on our results we propose a wide variety of use of such devices, from directed evolution to realization of cellular automata.

F-04 David Grainger, University of Utah

Interfacial characterization of nucleic acid diagnostic assays and microarrays

We study how DNA and proteins interact with surfaces in popular microarray diagnostic capture assays, and have used many methods to understand how to optimize diagnostic assay performance using interface design principles in biological environments. Data compared from multiple analysis methods include that from XPS, SPR, radiolabeling, and fluorescence capture assays on surfaces of different hydrophilic chemistries. Surfaces must be able to interact effectively with trace nucleic acids diffusing into the surface zone, while repelling the abundant non-analytes also present in the bulk milieu. Surface selectivity is one parameter. A second important consideration is the DNA polyelectrolyte surface immobilization density resulting brush-like configurations that sterically and electrostatically affect DNA target capture. The microarray uses small spots of immobilized probe DNA from robotic spotting procedures, producing poorly controlled micron spots of rapidly dried DNA deposits, poorly understood in terms of surface properties. This non-equilibrium scenario of DNA-surface immobilization is not well-characterized and critical to assay performance. Despite forecasts, none of these DNA assays are clinically approved for patient use due to their problems. Assay performance depends on understanding these effects in microspot assays.

G-01 Helim Aranda-Espinoza, University of Maryland

Leukocyte adhesion and motility

Leukocyte locomotion over endothelial cells is a critical step that follows firm adhesion. Once firmly adherent, the cell will spread and transmigrate through the endothelial wall to the site of injury. Reflection Interference Contrast Microscopy was used to study neutrophil spreading. The images were analyzed to identify simultaneously the changes in the overall cell adhesion and the zones of close contact with the substrate. We show that for neutrophils, cell spreading is

anisotropic and directional from the very initial stage. The curve describing the spreading area of the cell as a function of time can be fitted piecewise as power laws. All cells follow a slow spreading, fast spreading and finally area saturation. Close contacts occur at opposite sides of the cell where the uropod and lamellipodium develop. The apparition of close contacts seems to indicate a change in adhesion regime. Finally, we explore the effect of flow and protein presentation on the motility of neutrophils. Our findings illustrate that the endothelium regulates neutrophil migration through the presentation of various adhesion ligands at sites of inflammation.

G-02 Ajay Gopinathan, University of California, Merced***Actin polymerization, force production and membrane dynamics during cell motion***

Branched actin networks at the leading edge of a crawling cell evolve via protein-regulated processes such as polymerization, depolymerization, capping, branching and severing. I shall discuss how the cell achieves fine-tuned control of both the morphology and dynamics of the cytoskeletal actin network by employing all the above processes in tandem. I shall also discuss how the polymerization activity leads to protrusive forces and how the coupling between motion of the receptors on the cell surface, actin polymerization activity and the dynamics of the membrane leads to distinct dynamical structures that form at the leading edge during motion. In particular, we find that membrane motion can be wave-like, corresponding to membrane ruffling, or unstable, corresponding to the tendency to form finger-like extensions called filopodia.

G-03 Dolores Bozovic, University of California, Los Angeles***Amplification and spontaneous oscillations in inner ear hair cells***

The first step in auditory processing occurs in the inner ear, and is mediated by hair cells - specialized cells that detect mechanical deflections and perform spectral analysis of incoming sound. The hair cells transduce mechanical stimuli into electrical signals via opening of mechanically gated ion channels. In this talk, we shall present single-cell mechanical recordings acquired in vitro from hair bundles of the bullfrog's sacculus. Despite being immersed in a viscous environment, these cells can nevertheless sustain oscillations, amplify incoming signals, and even support spontaneous oscillations. These and other phenomena indicate the existence of an active process that must pump energy into the cell's motion on a cycle-by-cycle basis. One of the signatures of the active process in these hair bundles is the presence of spontaneous oscillations, which significantly exceed thermal fluctuations. These naturally noisy oscillations can be entrained by a small sinusoidal stimulus, leading to amplification of the applied signal. In the experiments presented here, we have entrained the spontaneous oscillations of an individual hair bundle and measured the force that it exerts as a function of time. By analyzing these results, we are able to quantify the delivery of power by the active process at various phases of the oscillation cycle. Our experimental findings will also be discussed in the context of recent theoretical work in which a hair cell was modeled as a dynamical system that can undergo a Hopf bifurcation.

H-01 Charles M. Knobler, University of California, Los Angeles

The extraordinary effect of pH on the elasticity of viral capsids

Viral capsids provide an ideal test bed for the study of the mechanical properties of assemblies of proteins. The capsid of cowpea chlorotic mosaic virus (CCMV) is 28 nm in diameter and consists of 180 copies of a single 190-residue long protein. Atomic Force Microscopy nanoindentation studies [1] of CCMV capsids at pH 5 show that the capsid can be deformed reversibly and elastically by about 25%. There is a sharp drop in the force vs. indentation curve for deformations that exceed this limit and the reversibility is lost. When the experiments are carried out at pH 6, however, the capsid is softened and found to be remarkably elastic; the force-indentation curve is linear up to indentations of 50% and there is no hysteresis [2]. Finite-element analysis modeling of the response to deformation shows that this behavior can be understood in terms of pH-induced changes in the ratio of the 2-D Young's Modulus to the bending modulus. The response to the pH is associated with the onset of a swelling the capsid.

[1] J. P. Michel, M. M. Gibbons, W. S. Klug, C. M. Knobler, G. J. L. Wuite, C. F. Schmidt, *Proc. Nat. Acad. Sci. USA* 103:6184 (2006)

[2] W. S. Klug, R. F. Bruinsma, J. P. Michel, C. M. Knobler, I. L. Ivanovska, C. F. Schmidt, G. J. L. Wuite, *Phys. Rev. Lett.* 97:228101 (2006)

**H-02 Tommy Nylander, Lund University
with Cardenas, Örberg, Schillén, Lindman**

DNA compaction at interfaces and control of DNA compaction

The behavior of DNA at different types of interfaces and how the formed structures DNA at an interface could be affected by the type of interfaces as well as other compounds such as cationic surfactant will be discussed. The results presented includes DNA interaction with macroscopic flat surfaces, nano-particles, dendrimers and lipid vesicles studied by ellipsometry, light scattering, neutron scattering and reflectometry. Adsorption of DNA-cationic surfactant complexes is determined by a delicate balance of DNA-surface, cationic surfactant-surface, and cationic surfactant-DNA interactions. Particular attention will be paid to how the type of surfaces and DNA (single or double stranded and length) affects the compaction/decompaction of DNA. The aim with our work is to be able to control the DNA compaction at an interface and hereby be able to control the transcription/translation.

H-03 Robijn Bruinsma, University of California, Los Angeles

Physics and the structure of retroviruses

Many viruses can assemble spontaneously in the laboratory from solutions containing capsid proteins and viral genome molecules. The capsid shells of smaller, sphere-like viruses are highly regular with an elegant icosahedral symmetry. The talk will discuss how basic physical and mathematical considerations can provide insight into viral self-assembly and how these methods can be applied to the particular case of HIV-1 and other retroviruses that form irregular capsids.

Posters

P-01 Eduardo Jardón-Valadez, Universidad Nacional Autónoma de México with Ulloa-Aguirre, Piñeiro

Hybrid *ab initio*/homology model and molecular dynamics simulation of the human gonadotropin-releasing hormone receptor

*G-protein coupled receptors (GPCRs) are a large superfamily of membrane receptors involved in the cell transduction of signals. GPCRs are currently considered as important drug targets due to their accessibility from outside of the cells and their functional diversity. However, the lack of knowledge on their three-dimensional structure difficults such an application. Commonly, the alternative is to develop conformational models for the receptor under study. In particular, receptors from the rhodopsine-like GPCRs superfamily are candidates for homology modelling due to its recently resolved high resolution structure. Although the reliability of the obtained conformations in this way is never assured, molecular dynamics simulations in microenvironments that mimic their *in vivo* location in the cell may lead to optimization of the structures, and additionally provide useful information on the dynamic behaviour of the receptor. In this study a conformational model for the human gonadotropin-releasing hormone receptor, based on a hybrid *ab initio*/homology method, is proposed; the obtained structure was simulated by molecular dynamics in a dipalmitoyl-phosphatidylcholine (DPPC) bilayer. A methodology for the modeling and simulation of this type of receptors is proposed and the dynamic behaviour of the protein, the lipids, the water, and the ions necessary to compensate the protein charge at biological pH are analyzed.*

Supported by grants 45991 and J49811-Q from CONACyT. We are grateful to DGSCA (UNAM) for computer time.

P-02 Frank Heinrich, Carnegie Mellon University with McGillivray, Sokolov, Hall, Valincius, Lösche

β -Amyloid oligomers: Mechanisms of toxicity in Alzheimer's disease?

Amyloid plaques associated with dead or damaged neurons are a characteristic property of neuronal cells suffering from Alzheimer's disease. A major component of these plaques is the β -amyloid peptide (1-42), which plays a key role in the progress of Alzheimer's disease. It is widely accepted that soluble oligomers are the primary toxic species of amyloid, although the actual mechanism of cell toxicity remains unclear [1]. Generally, two contrary hypotheses exist: either β A forms ion channels in the membrane [2] or β A leads to membrane thinning and disruption [3]. Neutron reflectivity is able to test both hypotheses by measuring bilayer thickness and completeness in presence of β A oligomers. The neutron experiments presented here reveal interactions of β A with artificial cell membranes composed of phosphocholines using a well-characterized tethered lipid bilayer system. The experiments show a thinning of the membrane in presence of β A. Although the interaction of the peptide with the headgroup region of the membrane is evident, no incorporation of the peptide into the membrane takes place.

[1] R. Kaye et al., Science 300:486 (2003)

[2] S. Micelli et al., Biophys. J. 86:2231 (2004)

[3] R. Kaye et al., J. Biol. Chem. 279:46363 (2004)

Supported by the NSF under grant no. CBET-0555201.

**P-03 Juan Pablo Reyes-Grajeda, Universidad Nacional Autónoma de México
with Mendoza-Espinoza, Moreno, Castillo, Mas-Oliva**

Amyloid-like fibril structures developed by peptides derived from apolipoprotein AI

Protein conformational diseases, including Alzheimer's and Huntington's, result from protein misfolding that results in a distinct fibrillar feature termed amyloid [1]. However, proteins that are disordered in the native state play important roles in molecular recognition and the assembly of functional macromolecular complexes [2]. For instance, unfolding or misfolding is considered a prerequisite for the assembly of amyloid-like fibrils from proteins that are structured in the native state [3]. In this work we have analyzed the structural and self-assembly properties of three short peptides derived from apolipoprotein AI using circular dichroism spectroscopy (CD) and atomic force microscopy (AFM). Every one of these peptides and in special peptide DRV (N-terminal), showed conformational changes mainly from random coil to β -strand-containing structures, in function of the time they were maintained in solution. The propensity of these peptides to form fibrils has been studied and will be discussed in terms of a possible role in the pathophysiology of atherosclerosis.

[1] A. Quist et al., Proc. Natl. Acad. Sci. USA 102:10427 (2005)

[2] A. K. Dunker, Z. Obradovic, Nat. Biotechnol. 19:805 (2001)

[3] B. Bothner, Y. Aubin, R. W. Kriwacki, J. Am. Chem. Soc. 125:3200 (2003)

**P-04 Paola Mendoza-Espinoza, Universidad Nacional Autónoma de México
with Reyes-Grajeda, Moreno, Castillo, Mas-Oliva**

Lipid dependent disorder-to-order conformational switches in apolipoproteins AI and CI derived peptides

not available

**P-05 David Holowka, Cornell University
with Hammond, McLaughlin, Baird**

A novel intracellular structure containing polyphosphatidylinositol and proteins relevant to receptor-mediated Ca²⁺ mobilization

Immunofluorescence microscopy reveals a novel pool of polyphosphatidylinositol -containing membranes in RBL mast cells that is proximal to the plasma membrane and is associated with key proteins that control Ca²⁺ mobilization, including phospholipase C γ and endoplasmic reticulum resident proteins inositol trisphosphate receptors, SERCA2 ATPase, calreticulin, and a subunit of voltage operated Ca²⁺ channels. These membranes are typically localized in one or more micron-sized plaques per cell that are sensitive to disruption in fixed cells by detergents and organic solvents. These structures can also be detected in fixed cells with fluorescent derivatives of the polybasic MARCKS effector domain. Evidence for their role in IgE receptor-mediated Ca²⁺ mobilization includes the inhibition of Ca²⁺ responses in parallel with dispersion of these plaques due to treatment of cells at 4°C. Similar structures are detected in a wide variety of cultured mammalian cells and are commonly localized at growth tips of cell protrusions. These plaques appear to insert into the plasma membrane in response to appropriate stimuli, such as IgE receptor activation. We hypothesize that these membranes serve to connect the endoplasmic reticulum to the plasma membrane.

**P-06 Duane Redfern, Kent State University
with R. Redfern, Jiang, Gericke**

Cholesterol effect on phosphoinositide/phosphatidylcholine mixing behavior

Phosphatidylinositol phosphates have been shown to control membrane trafficking events by attracting proteins to specific cellular sites which requires a tight regulation of phosphoinositide generation and turnover, as well as a high degree of compartmentalization. In part, this compartmentalization is governed by mutual as well as interaction with other lipids in the membrane. We have investigated the lipid mixing behavior of all phosphoinositide derivatives using transmission FTIR and DSC. Domain formation (demixing) of the binary DPPC and DPPI(x,y,z)P_n (n=1,2 or 3) mixtures was found for all investigated mixtures. FRET was used to examine the pH dependent domain formation in the fluid phase for these binary lipid mixtures by using lipids with saturated or unsaturated acyl chain compositions at temperatures above their respective gel/liquid-crystalline phase transition temperature. For all investigated phosphoinositides we found pH dependent domain formation, however, details of the pH dependency was dependent on the position of the phosphomonoester group(s) at the inositol ring as well as the acyl chain composition.

To investigate the effects of cholesterol on phosphoinositide/PC mixing, domain formation in SOPC, PI(4,5)P₂, cholesterol GUV's was examined. We found that the presence of cholesterol enhances phosphoinositide domain formation. To probe the properties of these lipid domains, we used a novel fluorophore with a viscosity dependent emission profile that can be very helpful to highlight different phases in GUV's. For example, the emission wavelength of the fluorophore Di-4-ANEPPDHQ is red shifted from an ordered to a disordered lipid phase and as a result, domains in lipid raft mixtures like DOPC, DPPC and cholesterol can be readily visualized. Using this fluorophore, we found that PI(4,5)P₂ enriched domains in the presence of cholesterol are similar to a fluid lipid phase in contrast to the liquid ordered properties that raft domains possess. In addition, FRET and GUV imaging experiments using fluorescently labeled cholesterol did not furnish results consistent with cholesterol/PI(4,5)P₂ co-localization. Finally, the effect of temperature on phosphoinositide domain formation in GUV's was investigated.

P-07 Edgar Kooijman, Kent State University

An electrostatic hydrogen bond switch as basis for the specific interaction of phosphatidic acid with proteins: What makes phosphatidic acid special among the anionic phospholipids?

Phosphatidic acid (PA) is a minor but important anionic bioactive lipid which, through specific interactions with proteins, plays a central role in several key cellular processes. Yet, PA has a simple phosphate headgroup. To find out what, if anything, is special about the interaction of this lipid with proteins, we first determined the ionization behavior of low concentrations of PA and the related lipid lysophosphatidic acid (LPA) in extended (flat) mixed lipid bilayers using magic angle spinning (MAS) ³¹P NMR. Next, we investigated the interaction of PA with basic amino acid residues in membrane interacting peptides again by MAS ³¹P NMR and by MD simulation, as a model for the interaction of PA with proteins.

Surprisingly, we find that despite identical headgroups, LPA carries more negative charge than PA when present in a phosphatidylcholine (PC) bilayer. Second, deprotonation of PA and LPA was found to be strongly stimulated by the inclusion of phosphatidylethanolamine (PE) in the bilayer, indicating that lipid headgroup charge depends on local lipid composition and will vary

between the different subcellular locations of (L)PA. We present a unifying model to explain these striking results.

The interaction between PA and membrane interacting peptides containing the positively charged amino acids lysine and arginine was investigated next. Surprisingly, our data show that these positively charged amino acids are able to increase the negative charge of PA, and not merely screen this charge. I will discuss these results in the light of the special ionization behavior of PA and will introduce a new model for the interaction of PA with proteins, a model we coined the electrostatic/hydrogen bond switch. We hope to convince you that the electrostatic properties of PA (and LPA) make this simple yet crucial phospholipid so special.

**P-08 Norma Diaz-Vergara, Universidad Nacional Autónoma de México
with Piñero**

A molecular dynamics study of triosephosphate isomerase from Trypanosoma cruzi in water/decane mixtures

Water plays an important role in the structural stability and function of proteins. The local order and dynamics of the water molecules in the vicinity of proteins is remarkably different from those in the solvent bulk. Amazing behaviours of enzymes like catalytic superactivity or strong changes in the thermal stability have been ascribed to the use of different solvents with low water concentrations. To study this matter much experimental work has been performed with enzymes confined in reverse micelles or dispersed in organic solvents. A relationship between the flexibility, the thermal stability and the catalytic activity of proteins has been proposed on the base of such experiments. In spite of the efforts devoted to this concern, no dynamic information at atomic and molecular level exists for this kind of systems. In this work molecular dynamics simulations of the triosephosphate isomerase of Trypanosome cruzi, the parasite causing the Chagas' disease, in several water/decane mixtures are presented. The results show that the dynamic behaviour of the protein does not change regardless the composition of the solvent if a thin water layer is maintained around the protein. An analysis on the local protein structure, the redistribution and mobility of the solvent molecules, the intra and intermolecular hydrogen bonds (protein-protein and protein-solvent), the fluctuation of the protein residues through the trajectory, among other structural and dynamic properties are also presented. Additionally, the methodology employed in this work could be useful for other macromolecules saving much computing time since simulations with non polar explicit solvents are less expensive than simulations with explicit water.

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**P-09 J. Alfredo Freites, University of California, Irvine
with Schow, Tobias, White**

Burying arginines in membranes: The organization of a voltage sensor domain in a lipid bilayer and insights into the stability of membrane proteins

Recent crystallographic structures of two voltage-dependent potassium (Kv) channels suggest that charged amino acid residues on transmembrane (TM) helices have high exposures to the nonpolar membrane interior. We have modeled the following systems in a POPC bilayer in excess water using molecular dynamics (MD) simulations: the TM helix responsible for voltage sensing, the so-called S4 helix; and the whole voltage sensor domain (VSD) of KvAP, a Kv channel from an archaebacterium. Our results reveal that the fluid nature of the lipid bilayer pro-

vides the necessary hydration to these charged systems allowing their stable integration into the membrane. Based on this principle, it is possible to reconcile a large collection of biochemical and biophysical studies of Kv channels that, according to their classical interpretation, were at odds with the direct structural evidence. The unexpected architecture of the VSD of Kv channels has been placed in a broader context by recent in-vitro translation experiments showing that translocon-mediated integration of TM helices into the endoplasmic reticulum membrane is highly sensitive to the presence and position of polar residues within the TM sequence. Accurate measurements of an apparent free energy of insertion as a function of position of an arginine residue in an otherwise hydrophobic helix show a monotonic increase from the termini toward the central position. We recovered a similar free-energy profile in a potential of mean force calculation for moving a single guanidinium ion in a POPC bilayer in excess water along the TM direction. We performed three separate MD simulations of a TM Leu₁₉Arg helix in a POPC bilayer in excess water with the arginine at three different positions. In each case, we found that the peptide remained in a TM configuration. In both the single-ion and peptide systems, we observed the development of a solvation structure around the guanidinium group similar to that observed in the Kv channel systems.

**P-10 Nikolay A. Simakov, Carnegie Mellon University
with Kurnikova**

Modeling of the current-voltage characteristics of the channel formed by anthrax protective antigen using Potential of Mean Force Poisson-Nernst-Planck (PMFNP) theory

Anthrax toxin is the main venom secreted by *Bacillus anthracis*. The channel formed by its protective antigen (PA) is significant for cell intoxication. Structure of the channel has not been resolved experimentally yet. However, a three-dimensional model of the pore was proposed by Nguyen [1]. To verify this model, calculations of I-V characteristics were made and were compared with experimental data [2].

For calculating I-V properties of the PA63 pore, Poisson-Nernst-Planck (PNP) theory of electrodiffusion was used [3,4], where a set of partial differential equations (the Poisson and the Nernst-Planck equations) are solved self-consistently. The model can be significantly improved using the potential of mean force approach. In this approach [5] the position-dependent free energy of ions is calculated first and then used in the PNP calculation. This energy estimates the effect of finite size of ions, dielectric barrier which is created by membrane and protein, and charge distribution in the protein.

[1] T. L. Nguyen, T. L., *J. Biomol. Struct. Dyn.* 22:253 (2004)

[2] K. M. Halverson et al., *J. Biol. Chem.* 280:34056 (2005)

[3] S. Y. Noskov, W. Im, B. Roux, *Biophys. J.* 87:2299 (2004)

[4] A. E. Cardenas, R. D. Coalson, M. G. Kurnikova, *Biophys. J.* 79:80 (2000)

[1] A. B. Mamonov, R. D. Coalson, A. Nitzan, M. G. Kurnikova, *Biophys. J.* 84:3646 (2003)

**P-11 Sol Maria, Universidad Autónoma de San Luis Potosi
with Hernandez-Hernandez, dela Cruz-Medina, Ojeda-Lopez**

MT-Bundling by mixtures of condensing agents

not available

**P-12 Gregory M. Grason, University of California, Los Angeles
with Bruinsma**

Wigner Crystallization of chiral polyelectrolyte bundles

Strong electrostatic correlations are known to play a crucial role in the condensation of highly-charged, stiff biopolymers (such as DNA or filamentous actin) in presence of polyvalent cations. In the limit of very strong electrostatic coupling, the physics governing the effective attraction between the macroions is governed by the Wigner-crystal ground state of the counterion-polymer aggregate. We construct an effective model to describe the thermodynamics of the Wigner-crystalline state of polyvalent counterions absorbed within hexagonal bundles of chiral, rod-like macroions. We argue that the ground state counterion configuration as well as the associated long-wavelength fluctuations about this state are characterized by a frustrated, antiferromagnetic XY **spin** Hamiltonian defined on the Kagomé lattice. The unusual statistical mechanics associated with this model can be treated within a generalized, dual description of interacting vortices (or screw defects), revealing that generically Wigner-crystalline ground states are constructed of arrays of screw-like configurations of counterions which wind helically around the constitute macroions. This description ultimately reveals find that the molecular chirality of the macroions plays an important role in suppressing these domain-like fluctuations of alternating chirality, and hence chirality itself becomes a critical parameter in melting behavior of the Wigner-crystalline state.

**P-13 Ajaykumar Gopal, University of California, Los Angeles
with Knobler**

Are viral genomes special? SAXS studies of viral RNAs

Viral RNAs often self-assemble into thermodynamically stable capsids in the presence of the right capsid protein and pH buffer. Due to the relative simplicity of assembly, it is thought that the RNA molecule is not packaged under mechanical stress unlike in DNA viruses. The current work addresses the basic question "How large are viral RNAs in solution?". By comparing the form factors obtained by small-angle x-ray scattering, we demonstrate that unconstrained viral RNAs (under in-vitro assembly conditions) have dimensions of the order of, but slightly larger than those of the final virus particles. Comparison of the radii of gyration (R_g) and scattering pair distribution functions $[p(r)]$ of several viral mutants and non-viral RNAs of identical nucleotide length demonstrates that wild-type viral RNA consistently folds to a more compact structure. Dummy-atom real-space reconstructions of several viral RNAs reveal that they may share a common coarse-grained anisotropy, suggesting that the nucleotide sequence codes not only for a specific secondary structure, but also for the propensity for a certain 3D shape and size.

**P-14 Paul Grayson, California Institute of Technology
with Han, Winther, Phillips**

Real time visualization of genome ejection from bacteriophage lambda: How internal pressure drives the translocation of DNA

The physical, chemical, and structural features of genome packaging and release in viruses have been the subject of much recent attention. An exciting development was the measurement of DNA ejection from single bacteriophage T5 particles by fluorescence microscopy, which was shown to proceed via stochastic steps between nicks on the DNA, a feature unique

to T5. In this paper we report an application of this assay to bacteriophage lambda. Except for a pause at the end of ejection, before the DNA separates from the capsid, the entire 48.5 kbp lambda genome is translocated in 1-2 s without interruption, consistently reaching speeds of 60 kbp/s. This simple process allows us to quantify the effect of two critical parameters for bacteriophages, finding that a shorter genome length results in lower pressures but a shorter translocation time, while the presence of divalent magnesium ions reduces the pressure and increases friction, increasing the ejection time to 11 s. We believe that the details of the ejection mechanism revealed in this study are generic features of DNA translocation in bacteriophages and have implications for the dynamics of DNA in other biological systems. Additionally, the results presented here clarify a fluorescence experiment [1], which put the lambda ejection time at 30 s. Our interpretation is that the earlier experiment measured the time required to trigger ejection rather than the actual translocation process.

[1] S. L. Novick, J. D. Baldeschwieler, *Biochemistry* 27:7919 (1988)

**P-15 J. R. Vega-Acosta, Universidad Autónoma de San Luis Potosí
with Pinedo-Torres, Cadena-Nava, Ruiz-Garcia, Lavelle, Gelbart, Knobler**

Self-assembly of capsid proteins of the cowpea chlorotic mottle virus (CCMV)

In this work, we present the self-assembly of capsid proteins of the CCMV virus under different pH and ionic strength conditions. The self-assembly is done without the presence of the viral genome. We explore a wider zone respect to the assembly conditions of previous works. We found the same structures that were reported by previous studies like spherical assemblies, bilayers, multilayers and nanotubes. In addition, we found similar structures with a wider range of characteristics, like wider nanotubes, but we also found new structures such as dumbbells and flowers. We also include some preliminary results on the thermodynamic stability of the assemblies.

**P-16 M. Comas-Garcia, Universidad Autónoma de San Luis Potosí
with Ruiz-Garcia**

Physicochemical behavior of the CCMV virus as a nanoparticle at the air/water interface

The CCMV capsid is normally soluble and stable in a buffer solution at pH = 4.8. However, it diffuses in solution and if it gets near the air/water interface, it gets trapped there. The diffusion of the virus is affected by the charge of its capsid. For example, above the isoelectric point (i.p., pH = 3.7) the capsids are negatively charged, and its diffusion towards the air/water interface is slower than at the isoelectric point. In addition, the capsids form stable structures at the interface one layer thick at pH = 4.8 due to the repulsive electrostatic interaction. The interfacial structures formed at the i.p. are thicker, larger and more complex than at higher pH because of electrostatic attraction, since it carries both positive and negative charges.

**P-17 Hiram Beltrán, UAM Cuajimalpa
with Damian-Zea, Andrés-González, Maya, Hernández-Ortega, Toscano, Nieto-Camacho, Ramírez-Apan**

Synthesis, characterization and biologic activities of iminic diphenyl-tin compounds

One-pot reactions with salicylaldehyde (**1**) or 2-hydroxy-naphthaldehyde (**2**), o-aminophenols (**3a-3g**), and diphenyl-tin(IV) oxide (**4**) led to fourteen diphenyl-tin(IV) compounds (**5a-5g**) and

(**6a-6g**) in good yields. All compounds were analyzed by IR, ^1H -, ^{13}C -, and ^{119}Sn -NMR spectroscopy, mass spectrometry and elemental analyses; furthermore in the case of compounds **5b**, **5c**, **5e**, **5g**, **6a**, **6a**·EtOH, **6c**·0.25 CH_2Cl_2 ·0.25 EtOH, **6e** and **6g** by X-ray diffraction. Compounds **5a-5g** and **6a-6g** were tested against six human tumor cell lines, U-251, PC-3, K-562, HCT-15, MCF-7 and SKLU-1, to assess their antitumor activity *in vitro*. The results suggest biological specificity towards U-251, MCF-7 and SKLU cells at doses below 2.5 μM , which are lower than *cis*-platin IC_{50} 's in the three cell lines. Since the inhibitory concentration values for the **5a-5g** series were alike to Ph_2SnCl_2 , but lower for the **6a-6g** derivatives, the results indicated a modest modulation of the activity through the ligand structure, and therefore can be enhanced through further molecular design. **5a-5g** and **6a-6g** were also tested for their antioxidant efficiency in rat brain homogenate showing that **5g** is more active ($\text{IC}_{50}=3.01 \mu\text{M}$) on inhibition of thiobarbituric acid reactive substances (TBARS) than the flavone quercetin (natural antioxidant, $\text{IC}_{50}=4.11 \mu\text{M}$). The TBARS activity (IC_{50}) correlates with the *o*-aminophenol substitutions and a linear combination among sigma Hammett, one bond tin coupling constants and tin chemical shifts against the measured $\text{IC}_{50\text{-TBARS}}$ was found. This correlation indicates that the implied molecular variables can become trackers for the calculation of TBARS inhibitory concentrations in similar systems.

**P-18 Jorge Delgado, Universidad Nacional Autónoma de México
with Lettinga, Castillo**

Phases obtained by shear in a solution of flexible cylindrical micelles

Semidilute and dilute regimes of viscoelastic CTAB/NaSal systems were studied under stationary and non-stationary shear flow conditions. In the diluted regime, we could observe and explain the formation of shear-induced structures (SIS) from fast strain thixotropic loops that decouple the stress coming from non-deforming material (the solvent) and the stress coming from SIS formation. Furthermore, the maximum quantity of SIS at every shear rate can be obtained from ramping-down curves. These curves are independent of the deformation history and can be related with a typical time of SIS formation. In contrast with the dilute regime, where SIS span the fluid; in the semidilute regime the formation of bands in the fluid is common. Using different rheometers and from flow velocity profiles, we obtained the flow properties of different bands in the fluid. We can describe a real interface between bands and an initial hydrodynamic nature of stress oscillations under stationary flow conditions. We found that stress oscillations, typical from a coupling between concentration and flow, are associated with a single band in the fluid. Finally we discuss the idea of a phase transition under shear in the semidilute regime.

**P-19 C. Garza, Universidad Nacional Autónoma de México
with Thieghi, Castillo**

Atomic force microscopy of lyotropic lamellar phases

For the first time, atomic force microscope images of lamellar phases were observed with a freeze fracture technique that does not involve the use of replicas. Samples are rapidly frozen, fractured, and scanned directly with atomic force microscopy at liquid nitrogen temperature in high vacuum. This procedure is used to investigate micro-structured liquids. Lamellar phases in AOT/water and in C_{12}E_5 /water were investigated with this new technique and results compared with X-ray diffraction measurements and other freeze fracture methods reported in the literature. Our results show that the technique is useful to image lyotropic lamellar phases. Estimated repeat distances for lamellar periodicity are consistent with those obtained by X-ray diffraction.