Poking and Sealing Holes: Interactions of Antimicrobial Peptides and Poloxamers with Lipid Membranes

Ka Yee Lee
University of Chicago

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.

Multiscale Motility of Molecular Motors

Reinhard Lipowsky
Max Planck Institut

All directed movements of living organisms are based on molecular machines which perform mechanical work on the nanometer scale. Two different mechanisms for force generation have been identified: pulling forces generated by single motor proteins and pushing forces generated by the growth of single filaments. This talk reviews recent work related to the pulling forces of molecular motors which give rise to a variety of active transport phenomena and structure formation processes that cover many length and time scales. From small to large scales, these phenomena and processes include: Chemomechanical coupling and motor cycles of single molecules; cooperative transport of cargo particles by several motors; active diffusion of cargo particles in slab-like compartments; molecular motor traffic in tube-like compartment; and active structure formation of gliding filaments in contact with immobilized motors. For recent reviews covering these and additional processes, see [1] and [2]

[1] R. Lipowsky, Y. Chai, S. Klumpp, S. Liepelt, and M. Müller, Physica A 372, 34 (2006)
[2] J. Kierfeld, P. Gutjahr, T. Kühne, P. Kraikivski, and R. Lipowsky, J. Comp. Theo. Nanosci. 3, 898 (2006)

How Directional Translocation is Regulated by a DNA Helicase Motor

Klaus Schulten
University of Illinois at Urbana-Champaign

PcrA helicase is one of the smallest motor proteins structurally known in full atomic detail. It translocates from the 3' end to the 5' end of single stranded DNA utilizing the free energy from ATP hydrolysis. The similarities of structure and reaction pathway between PcrA helicase and F1-ATPase suggest a similar mechanochemical mechanism at work in both systems and many other motor proteins.

We report experimental and computational studies of PcrA translocation that demonstrate a domain stepping mechanism in which, during one ATP hydrolysis cycle, the pulling together and pushing apart of two translocation domains is synchronized with alternating mobilities of the individual domains such that PcrA moves unidirectionally along ssDNA. Computational modeling involving combinations of quantum chemical, classical mechanical, and stochastic calculations shows in particular how ATP binding and subsequent hydrolysis influences in a highly delocalized fashion the ability of the individual PcrA domains to move along single stranded DNA. The mechanism is also clearly evident from correlation analysis of molecular dynamics simulations, from low pass filtered elastic network dynamics, and from a so-called coevolution analysis of pairwise mutated side groups. The stated numerical and analytical calculations offer an atomic level view of PcrA's ratchet type motion along DNA. The delocalized nature of the directional translocation could be recognized only by a multi-level analysis, not by any individual methodology.

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Emerging Nanoscale Technology for Single-Cell and Single-Molecule Bioscience

Michael Roukes
California Institute of Technology

Advances in nanoscale devices now allow us to envisage entirely new types of measurements at the level of single cells and molecules. Among these opportunities are mapping forces generated by living cells in real-time, ultimately with piconewton resolution; resolving, calorimetrically, the metabolism of individual cells in real-time; and extending the sensitivity and bandwidth of force spectroscopy to enable following single-molecule stochastic biochemistry in real-time. I'll describe the state-of-the-art, future challenges, and some of the possibilities in these areas -- in hope of stimulating additional imaginative possibilities with the new classes of tools that are emerging.

Understanding the Collective Dynamics of Motile Bacteria

Doug Weibel
University of Wisconsin

We are interested in bacterial motility. An interesting developmental mechanism occurs in many motile strains of bacteria when cells make the phase transition from fluids to surfaces. These cells sense the change in their microenvironment—the chemical and physical interface between cells and their surroundings—and respond by undergoing at least two rapid morphological events: i) upregulating the assembly of flagella; and ii) undergoing multiple rounds of replication without septation. The resulting cells are referred to as 'swarmers' and are capable of moving across surfaces in a coordinated behavior that has been compared to swarming colonies of bees. Swarming is a cooperative mechanism that makes it possible for cells to adapt to changes in their environmental and collectively colonize new niches in search of resources that support their growth. Our understanding of swarming is still its infancy, in part because the tools we need to dissect this behavior quantitatively are still not developed.

In this talk I will describe recent work in our group that is focused on understanding the genetic regulation of swarming and the role of physical/mechanical interactions between swarming cells and their surroundings. We believe that this work will play a role in understanding and preventing infections by pathogenic strains of bacteria. It may also provide an interesting system for studying out-of-equilibrium dynamics.

The Complexity of Proteins

Hans Frauenfelder
Los Alamos National Laboratory

Proteins are most likely the best systems for studies of complexity. They are small enough to be explored in detail and complex enough to yield insight in the concepts and laws that govern complex systems. Four crucial concept that have emerged from the exploration of a "simple" protein, myoglobin, are the existence of a hierarchically organized landscape, of motions linked to the landscape, of the effect of the environment on the landscape and the motions, and of the role of the motions in function. In a simpler form, some of these concepts can also be found in supercooled liquids and glasses. In-depth studies of these concepts are still in progress and the talk will only give a glimpse at the richness of phenomena in proteins.

Rupture, Spreading, and Healing of 2D Fluid Lipid Bilayers at Structured Surfaces

Atul Parikh
University of California, Davis

Interfacial organization of lipids and amphiphiles into a discrete number of molecular layers provides, arguably, one of the most pristine experimental realizations of self-organized, two-dimensional systems. It provides an experimental test-bed for the study of a rich variety of interface-dominated processes, including surface melting, low-dimensional phase transitions, surface dynamics, and phase coexistence and separation. This talk will present recent experimental evidence from our laboratories which highlight the importance of substrate structure (e.g., topography, charge, and surface energies) in influencing the dynamics of formation of interfacial single lipid bilayers and their equilibrium morphologies.

Continuum Models of Large-scale Coherence in Dense Assemblies of Self-propelled Bioparticles: From Shaken Granular Rods to Molecular Motors and Swimming Bacteria

Igor Aronson
Argonne National Laboratory

Dense assemblies of active self-propelled particles, such as vigorously shaken anisotropic grains, microtubules interacting with molecular motor, and hydrodynamically entrained swimming bacteria, often exhibit large-scale spatio-temporal patterns of collective motion whose correlation length greatly exceeds the size of individual particle. Despite a vast difference in the physical mechanisms controlling onset of large-scale coherence, continuum description of such systems can be derived from an analogy with a gas of inelastically colliding granular rods. Thus, starting from a generic stochastic microscopic model of inelastic polar rod-like particles with an anisotropic interaction kernel, we derive set of equations for the local rods concentration and orientation. Above certain critical density of rods the model exhibits spontaneous orientational instability and onset of large-scale coherence. For the system of microtubules interacting with molecular motors we demonstrate that the orientational instability leads to the formation of vortices and asters seen in recent experiments. Similar approach is applied to colonies of swimming bacteria Bacillus subtilis confined in a thin fluid film. The model is formulated in term of two-dimensional equations for local density and orientation of bacteria coupled to the low Reynolds number Navier-Stokes equation for the fluid flow velocity. The collective swimming of bacteria is represented by additional source term in the Navier-Stokes equation. We demonstrate that this system exhibits formation of dynamic large-scale patterns with the typical scale determined by the density of bacteria.

Size Regulation of ss RNA Viruses

Roya Zandi
University of California, Riverside

Under the right circumstances, single-stranded RNA viruses self assemble spontaneously from aqueous solutions containing subunit proteins and genome molecules. While a monodisperse size distribution is common for most icosahedral viruses, the size of the spherical viral shells can vary from one type of virus to another. We study the physical mechanism underlying the size selection of shells among spherical viruses. In particular, we address the effect of genome length and the concentrations of protein and genome on the size of spherical viral capsids. We show that based on genome size, it could be advantageous to have relatively small spherical shells with higher curvature rather than bigger and thus flatter shells. Furthermore, we find that the small ratio of genome to protein concentration could, quite interestingly, result in larger spherical shells. Experimental data on the encapsidation of model genomes supports these findings.

Curvature and Spatial Organization in Biological Membranes

Raghuveer Parthasarathy
University of Oregon

Cellular membranes bend and curve into diverse, controlled shapes as they perform various functions. These deformations make use of the remarkable material properties of biological membranes inherent in their nature as two-dimensional fluids. Membrane curvature is controlled by constituent proteins and lipids, but conversely curvature itself can organize mobile membrane molecules. I'll survey recent experiments that have uncovered intriguing connections between mechanics and biochemistry at membranes, focusing on links between phase separation and curvature and membrane topography at inter-cellular contacts. I'll describe the concepts that emerge from these studies, especially curvature-mediated long-range mechanisms for spatial organization in membranes, and highlight open areas for future research.

Liquid Crystals and the Origin of Life: Living Polymerization and Condensation of Complementary Nanoscale DNA

Noel Clark
University of Colorado

NanoDNA (nDNA), in the form of self-complementary B-DNA oligomers, 6 base pairs to 20 base pairs (2-7 nm) in length, is found to exhibit chiral nematic and columnar liquid crystal (LC) phases, even though such duplexes lack the shape anisotropy required for LC ordering. Structural study shows that these phases are produced by the end-to-end adhesion and consequent stacking of the duplex oligomers into polydisperse anisotropic rod-shaped aggregates, which are then able to orientationally order. Frustration of the complementarity by terminating the oligomers with unmatched single strands reduces the stability the LC phases. Upon cooling solutions in which only a small fraction of the nDNA present is complementary, the duplex-forming oligomers separate into the minority LC phase, condensing into LC droplets and thereby maximally concentrating their oligomer terminal chain ends. Thus, complementarity promotes concentration via the intermediary of LC formation, a link that would provide positive feedback for the selective growth of extended complementary oligomers in a chemical environment where concentration enhances oligomer growth. Scenarios for the appearance of life on earth divide time into pre-biotic and metabolic eras, the latter commencing with the active harvesting of energy. The ubiquity of RNA in the key processes of life leads to the prevailing opinion that RNA or something like it preceded metabolism, appearing in the prebiotic era in the form familiar from DNA and RNA: pairs of linear polymers consisting of water soluble chains stitched together as a pair by the complementary hydrogen bonding of aromatic hydrocarbon side groups (base pairs). The "RNA world", transitioning between the pre-biotic and metabolic eras, required such polymers complex enough to be selectively replicated and to exhibit catalytic activity, something like at least 35 base pairs in length. One of the prevailing mysteries is how, with only energy input and dynamic environmental conditions, such complex molecules could appear out of the "gemisch", the complex mixture of aromatic hydrocarbons, sugars, phosphates, and other inorganics believed to be available in aqueous environments of the prebiotic earth. Clearly, their organizing principle had nothing to do with biology. We will present a new scenario for this development, based on our observations of the liquid crystal phases in nanoscale DNA. The wisdom garnered over the past 50 years or so is that life's information carriers, DNA and RNA, form liquid crystal phases because they are rod-shaped semiflexible polymers. We will argue that it is actually the other way around - life's information carriers are rod shaped polymers because such molecules form liquid crystal phases.

This work was partly supported by NSF Grants DMR 0213918 and DMR 0606528.

Physics of the Retrovirus

Robijn Bruinsma
University of California, Los Angeles

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

Theoretical Studies of Pressure Effects on Folding/unfolding of Proteins and Nuclei Acids

Angel Garcia
Rensselaer Polytechnic Institute

Proteins denature at high hydrostatic pressures, implying that the unfolded proteins in aqueous solution have lower volume than the folded state. A model that explains pressure unfolding requires water to penetrate the protein interior and disrupt the protein hydrophobic core. Previous calculations have shown that alpha helices do not unfold at high pressures, but become more stable. Beta hairpins unfold in a way similar to globular proteins. It is not known what nucleic acids will do at high pressures. Some have argued that nucleic acids will behave like alpha helices and be stabilized by high pressures.

We study the folding and unfolding transitions of a mini protein (trp cage) and of an RNA tetraloop as a function of pressure and temperature. From these simulations we can construct an ellipsoidal P-T stability diagram that describes the fraction folded of these biomolecules over a broad range of pressures and temperatures. These diagrams show that the biomolecules (trp cage and the RNA tetraloop) can cold denature (i.e., unfold upon cooling) at high pressures, as well as heat denature (unfold upon heating) at low pressures.

We will also describe a minimalist model than can capture features of the pressure driven unfolding of proteins and apply it to describe the pressure unfolding of ubiquitin.

This work is supported by the National Science Foundation, MCB-0543769.

Movement of Transfer RNA Through the Ribosome

Kevin Sanbonmatsu
Los Alamos National Laboratory

The rate-limiting step of cognate tRNA selection is the movement inside the ribosome of the aminoacyl-tRNA from the A/T state to the A/A state. We show explicitly that the decoding center interactions observed in the A/A state can be preserved throughout tRNA accommodation using large-scale molecular dynamics simulations. The simulations demonstrate the stereochemical feasibility for accommodation of the tRNA acceptor stem and are consistent with recent structural data.

Nonequilibrium Thermodynamics at the Microscale

Chris Jarzynski
University of Maryland

What do the laws of thermodynamics look like, when applied to microscopic systems such as optically trapped colloids, single molecules manipulated with laser tweezers, and biomolecular machines? Over the past decade or so there has been considerable interest and progress in addressing this question. I will give an overview of some of these developments, with a focus on results pertaining to fluctuations far from thermal equilibrium, and I will argue that these developments have refined our understanding of the second law of thermodynamics.

Assisted Stochastic Sensing of Analytes by a Synthetic Nanopore with Adaptor

Zuzanna Siwy
University of California, Irvine

We have developed a synthetic stochastic sensor (SSS) made of a single conically shaped nanopore in a thick (12 micrometers) polymer membrane with an embedded adaptor. Our SSS uses a single conical nanopore with tip opening as small as 2 nm and sensing length around 50 nm. The nanopore is placed in a conductivity cell with seal resistance >100 GigaOhm and cobalt ions are placed in the surrounding solutions. Cobalt ions convert an insensitive nanopore into a sensitive stochastic sensor. We imagine that cobalt ions transiently bind to the pore walls, bringing the system out of equilibrium and making it very sensitive to any analyte that perturbs the cobalt-nanopore interactions. Our SSS detects micromolar concentrations of an antibiotic, neomycin, and a biomolecule, spermine, and gives different signals when each is present. Experimental experience, and our view of the mechanism of detection, suggest that the sensor will respond to a variety of organic molecules. Possibilities of constructing a chemical oscillator with tens of Hz operating frequency will be discussed as well.

Mechanics of Active Biopolymer Networks

David Weitz
Harvard University

Addition of biomolecular motors to networks of biopolymers can dramatically modify their mechanical properties, providing a route to synthesis of networks whose mechanics are controlled by enzymatic activity. This can lead to stiffening of the network by several orders of magnitude. These materials also offer insight into the mechanics of the cell.

Synchronized Cycles: An Allosteric Model of the Cyanobacterial Circadian Oscillator

David Lubensky
University of Michigan

Like many higher organisms, photosynthetic cyanobacteria exhibit circadian rhythms driven by an autonomous oscillator with a roughly 24-hour period. In a remarkable experiment, Nakajima et al. [Science, 2005] recently showed that this oscillator consists only of the 3 core cyanobacterial clock proteins KaiA, KaiB, and KaiC: These 3 proteins, together with ATP, are sufficient to generate circadian phosphorylation of KaiC in vitro. This system is thus a rare example of a functioning biochemical circuit that can be reconstituted in the test tube. Theoretically, it presents the further challenges that the only reactions driven out of equilibrium are those associated with KaiC phosphorylation and dephosphorylation and that, unlike in most known biochemical oscillators, none of the 3 proteins are created or destroyed. Here, we present a model of the Kai system. At its heart is the assumption, motivated by classical models of allostery, that each individual KaiC hexamer has an intrinsic tendency to be phosphorylated in a cyclic manner. For this property to be translated into a macroscopic oscillation, however, the cycles of the different hexamers must be synchronized. We propose a novel synchronization mechanism that allows us to reproduce a wide range of published data, including temperature compensation of the oscillation period, and to make nontrivial predictions about the effects of varying the concentrations of the Kai proteins.

Simulations of Peptide Inhibitors of Amyloid-b Aggregation

Joan-Emma Shea
University of California, Santa Barbara

Alzheimer's disease is associated with the abnormal self-assembly of the Alzheimer Amyloid-β (Aβ) peptide into aggregate structures. Both the end-product amyloid fibrils as well as smaller soluble oligomers formed in the initial stages of aggregation appear to be toxic to the cell. An attractive therapeutic approach to combat amyloid diseases lies in the development of strategies to inhibit or reverse aggregation. We consider here the 16-22 fragment of the (Aβ) peptide, the shortest sequence of Alzheimer Aβ peptides capable of forming fibrils. An N-methylated version of this peptide has recently been shown to inhibit fibrillogenesis and disassemble Aβ fibrils. We present molecular dynamics simulations of the interaction of this inhibitor peptide with small oligomers of Aβ peptides, as well as with a model fibril. Our simulations suggest that the inhibitor peptide can act on both prefibrillar and fibrillar forms of Aβ, and that the specific mechanism of inhibition depends on the structural nature of the Aβ aggregate.

Mechanics of Growth Control in Animal Development

Boris Shraiman
University of California, Santa Barbara

This talk will focus on the mechanisms that regulate growth of animal limbs and organs and determine their final size and shape. Specifically, it will address the possible role of the mechanical stress generated by non-uniform growth in determining the size of fly wings. Mechanical feedback on growth may be a ubiquitous phenomenon and some new experimental evidence, based on cultured epithelial cells, will be presented.

Protein Dynamics: From Nanoseconds to Microseconds and Beyond

Gerhard Hummer
National Institute of Diabetes and Digestive and Kidney Diseases

Molecular simulations provide a detailed description of the structure, motions, and energetics of biomolecules that has greatly contributed to the understanding of their function. In the first part of my talk, I will focus on the picosecond to nanosecond regime of protein motions, into which time-resolved X-ray crystallography has recently opened up a spectacular new window. On one hand, these experiments permit a quantitative assessment of molecular dynamics simulations, which are shown to capture the directions, amplitudes, and time scales of the motions of protein, ligand, and solvent. On the other hand, this comparison also highlights a unique strength of the simulation approach: the single-molecule simulation trajectories, properly validated against experiments, can provide insights into molecular mechanisms inaccessible even to highly detailed bulk measurements. In the second part of my talk, I will study biomolecular processes at longer time scales of microseconds to milliseconds, such as large-scale protein conformational changes. By extending the concept of structure-based potentials, we perform simulations on coarse-grained energy surfaces that capture the experimentally determined conformers as distinct local minima, much like the Stillinger-Weber inherent structures of disordered media. This procedure allows us to explore large-scale conformation changes, including transitions that involve partial, local unfolding of proteins. In my talk, I will discuss applications to conformational transitions in the calcium sensor calmodulin, as well as the motor proteins kinesin and myosin.

Predicting 3D Structures: Membrane Proteins with Experimental Validation Against Predicted Effects of Mutations Binding Sites of Agonists and Antagonists

William Goddard
California Institute of Technology

A general problem with predicting the structures of biopolymers and membranes is that the experimental tools for structure generally do not provide full atomistic information about structure, which is often averaged over many molecules. However many proteins have very selective active sites for very strong bonding (often pM to nM) of specific ligands. In addition, it is straightforward to probe the details of the binding experimentally and computationally by making point mutations and determining the effect on binding. We have used this strategy to validate our general approach to predicting the 3D Structure of G Protein Coupled Receptors (GPCRs), a very important class of proteins that play a critical role in cell communications (dopamine, histamine, epinephrine, serotonin) and in sensing the outside world (vision, smell, taste, and pain). There are no experimental 3D structures available for human GPCRs despite their importance to pharma. Indeed, considering every form of life there an experimental structure for only a single GPCR: bovine rhodopsin. Consequently we validate our predicted structures by using them to predict the binding site and binding energies for endogenous ligands and for other agonists and antagonists, with the emphasis on how binding changes upon mutations of residues in the predicted binding sites We find that these results are in excellent agreement with available binding and mutation experiments. We will discuss results for some of the following systems: Adrenergic receptors, Dopamine receptors Prostaglandin receptors, Chemokine receptors

Floppy Modes and Nonaffine Deformations in Biopolymer Networks

Erwin Frey
Loyola Marymount University

Fibrous materials are ubiquitous in nature. They form the cytoskeleton of cells and are essential components of the extracellular matrix. Its building blocks are stiff protein filaments and a myriad of associated crosslinking proteins. The interplay between the elasticity of the biopolymers and the binding and elastic properties of the crosslinkers lead to a variety of network architectures [1]. We review recent advances in understanding the elastic properties of these networks in terms of “floppy modes” [2], which are the relevant low-energy excitations characterizing non-affine deformations. This approach might very well serve as a novel paradigm to understand the elasticity of microstructured materials. The theoretical concepts are applied to recent experimental studies of F-actin networks crosslinked with fascin.

Three dimensional tracking of individual quantum dots

Jim Werner
Los Alamos National Laboratory’s Center for Integrated Nanotechnologies

The detection of single molecules by laser-induced fluorescence has emerged as a powerful tool for the characterization and measurement of biological processes. For example, single molecule measurements have been used to investigate enzymatic turnovers, to study the exact step size of molecular motors, and to observe the diffusion and transport of lipids and receptors on cell membranes. Note that in these examples (and *all* single molecule studies by laser-induced fluorescence thus far), the motion of the molecule under investigation was limited to two dimensions: the XY plane of the microscope. We point out perhaps the obvious: most aspects of life, including intracellular signaling and trafficking, are inherently 3-dimensional. Our lab has recently designed and developed fluorescence microscopic instrumentation to follow the 3-D spatial trajectory of an individual fluorescently labeled molecule. With this instrument, we have recently tracked individual semiconductor quantum dots undergoing Brownian motion in three dimensions at rates comparable to many intracellular transport processes (D = 10^-8 cm^2/s or 1 um^2/s). Future applications of this instrumentation include following the trajectory of select proteins inside of a cell and monitoring protein conformation at the single molecule level for long time periods without resorting to surface immobilization.