the mechanism underlying AMPs' ability to disrupt cell membrane defense are not completely understood. We present computational and experimental evidence showing that the β-hairpin PG-1 aggregates and forms ion channels in target cell membranes. We used complementary approaches, including Molecular Dynamics (MD) simulations, Atomic Force Microscopy (AFM) imaging, Planar Lipid Bilayer (PLB) reconstitution and cellular toxicity measurements. MD simulations indicate that PG-1 does not form fibrillar structures on the surface of DOPS/POPE bilayers. However, PG-1 aggregates into channel-like structures with loosely attached subunits when inserted into anionic lipid bilayers. AFM images show no PG-1 fibril formations on the lipid bilayers. However, on a negative non permeable surface, PG-1 formed fibrils that bear some resemblance to amyloids fibers. On the other hand, AFM images show channel-like structures formed by PG-1 when reconstituted in DOPS/POPE bilayers. In PLB electrical conductance measurements, we observed multiple single channel conductances consistent with the heterogeneous oligomeric channel structures seen in AFM images. In addition, PG-1 channel formation seems to be lipid-dependent: PG-1 does not form channels in PC membranes, but forms channels in membranes rich in PE, PG or PS. Unlike amyloid channels, Zn^{2+} does not inhibit PG-1 channel conductance. Microbial cells treated with PG-1 showed antimicrobial activity consistent with ion leakage. The combined results support a model where the β-hairpin PG-1 antibiotic permeates membranes by forming ion conductive channel-like structures and cause cell injury.
Supported by NIH (NIA) and NCI Contract HHSN261200800001E.

52-Plat
S-Layer Self-Assembly on Supported Lipid-Bilayers: The Importance of Amorphous Precursors and Folding Transitions
Sungwook Chung, Seong-Ho Shin, Stephen Whitelam, Carolyn Bertozzi, Jim De Yoreo.
Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
The outermost membranes of many archaea and bacteria are comprised of highly-ordered 2D arrays of surface layer (S-layer) proteins. Their functions include selective transport, structural scaffolding, mineral templating and propagation of or protection from pathogenesis. Although the primary and secondary structures of the isolated proteins determine their governing interactions, their functions emerge from the tertiary and quaternary architecture that stems from S-layer self-assembly, a process that is poorly understood. Here we report results using *in situ* AFM to follow 2D self-assembly of monomeric SbpA of *Lysinibacillus sphaericus* on supported lipid bi-layers (SLBs) at the molecular-scale. We show that the assembly process begins with adsorption of unstructured monomers, which form a mobile phase on the SLBs. These then condense into amorphous clusters, which undergo a phase transition to ordered 2D clusters of 2 to 15 folded tetramers. The ordered clusters then enter a growth phase in which new tetramers form from unstructured monomers exclusively at unoccupied lattice sites along the cluster edges, implying that new tetramer formation is auto-catalytic. We show that the analysis of growth dynamics leads to a quantitative model in which the main rate limiting parameter is the probability of tetramer creation. The estimated energy barrier of 51 kJ/mole for this process is much less than expected form scaling laws for folding of isolated proteins. Finally we present preliminary results from dynamic Monte Carlo simulations that show how the combination of non-specific interactions and directional bonds characteristic of many proteins lead to non-classical assembly pathways, such as the one observed here involving formation of amorphous clusters followed by relaxation to the ordered state.

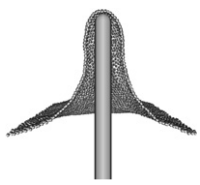
53-Plat
A Predictive Theoretical Model For Clathrin Self-Assembly
Shafigh Mehraeen, Nick Cordella, Andrew J. Spakowitz.
Stanford University, Stanford, CA, USA.
Clathrin is a protein that plays a major role in the creation of membrane-bound transport vesicles in cells. Clathrin forms soccer-ball-shaped lattices that coat a new vesicle as it forms. The clathrin molecule is known to take the shape of a triskelion, a figure with three bent legs. In vitro assembly of clathrin within a solution results in closed, nanoscale assemblies with various shapes and sizes. To understand how clathrin functions, particularly how it forms the lattice, we develop a theoretical model for the thermodynamics and kinetics of clathrin assembly in order to guide experiments toward the design of targeted nanoscale structures. Our model addresses the behavior in 2 and 3 dimensions, relevant to membrane/surface and bulk assembly, respectively. The clathrin triskelions are modeled as effective flexible pinwheels that form leg-leg associations and resist elastic bending and stretching deformations. Thus, the pinwheels are capable of forming a range of ring structures including 5-, 6-, and 7-member rings that are observed experimentally. Our theoretical model employs Brownian dynamics to track the motion of clathrin pinwheels at sufficiently long time scales to achieve complete assembly. With this theoretical model, we predict the phase diagram for clathrin assembly incorporating binding interactions, elastic deformation, and phonon modes. To verify the phase diagram, we perform dynamic simulations for a range of quenches into the phase diagram and compare phase separation across the binodal curve. We show that resulting Brownian dynamics simulations exhibit the hallmark behavior of spinodal decomposition with subsequent coarsening of ordered domains. These simulations demonstrate the effect of quench rate and leg elasticity on the final configurations of the lattice network and cluster-size distribution. We then proceed to discuss the assembly of specific nanoscale structures.

Platform E: Computational Methods

54-Plat
Molecular Dynamics Simulation of Phospholipid Bilayers and Monolayers Using a Polarizable Force Field
Edward Harder[1], Benoit Roux[1], Alex D. MacKerell Jr.[2].
[1]University of Chicago, Chicago, IL, USA, [2]University of Maryland, Baltimore, MD, USA.
The assumptions that underlie empirical force field models based on fixed molecular charge densities become questionable in the strongly heterogeneous electrostatic environment of bilayer membranes. Membranes contain regions that are polar (bulk water) highly charged (zwitterionic lipid head groups) and decidedly non-polar (hydrocarbon core). Using a recently developed polarizable Drude oscillator force field for lipids and water we present a study that illustrates the significant role played by electronic polarization effects in the electrostatic modeling of a phospholipid membrane. Specifically, we show that the inclusion of such many-body polarization effects can bring macroscopic electrostatic properties into quantitative precision with experimental observation.

55-Plat
The Small Angle Scattering Toolbox
Haiguang Liu, Peter H. Zwart.
Lawrence Berkeley National Lab, Berkeley, CA, USA.
Small Angle Scattering (SAS) is a technique used to investigate structure and dynamics of macromolecules in solution. Proteins in buffer conditions close to their physiological environment, are subject to Xray or Neutron scattering experiments. The resulting one-dimensional scattering curves are directly related to their three-dimensional structure. The SAS technique is routinely used to determining the low resolution shape of protein and map specific large scale conformation changes in protein structures.
We present a recently developed computational platform for SAS data analysis and model construction/refinement.The Small Angle Scattering Toolbox (SASTBX) has tools four major modules: (1) Raw data reduction; (2) theoretical scattering profile calculation based on PDB structures; (3) Pair distance distribution function (PDDF) estimation; and (4) 3D model construction and structure refinement.
The toolbox can be utilized to read raw scattering images obtained from the detector to generate an intensity profile. The basic analyses, such as Guiner and Kratky plots can be carried out in real time to assess the sample and data quality while collecting data. The PDDF estimation is a fully automated procedure, linked with a database a known PDDF's allowing for a rough initial classification of the shape of the protein. Model data can be calculated on the basis of a spherical harmonics expansions. Initial structures can be further refined with normal mode movements or rigid-body motions.
The sastbx is built on the open source Computational Crystallography Toolbox (CCTBX). The toolbox is implemented by using Python/C++ hybrid approach: the computing intensive jobs are handled in C++, and the python allows easy integration between other components. The source code will be distributed as open source project.

56-Plat
Large-Scale Simulations of Fluctuating Biological Membranes
Lutz Maibaum[1,2], Andrea Pasqua[1], George Oster[1], Daniel A. Fletcher[1,2], Phillip L. Geissler[1,2].
[1]University of California, Berkeley, CA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
We present a new computational model for lipid bilayers that allows the simulation of membrane systems on the micrometer scale. In our model, each ~25 nm^2 patch of bilayer is represented by a spherical particle. Mimicking the forces of hydrophobic association, many-body interactions suppress the exposure of each sphere's equator to the implicit solvent. This driving force towards high equatorial density stabilizes two-dimensional aggregates without necessitating crystalline order. This allows us to match both the surface



fluidity and bending elasticity of natural lipid membranes. We illustrate the usefulness of our model by computing the response of a bilayer to mechanical perturbations, such as deformations caused by an extending filopod.

57-Plat

Application of Large-Scale First Principles Quantum Mechanical Calculations With the ONETEP Program To Biophysical Problems

Chris-Kriton Skylaris[1], Daniel J. Cole[2], Stephen J. Fox[1], Eeson Rajendra[2], Mike C. Payne[2], Ashok R. Venkitaraman[2].
[1]University of Southampton, Southampton, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom.

Properties and processes at the molecular level are determined by the interactions between electrons and atomic nuclei whose motions are governed by the theory of quantum mechanics. Therefore, an unbiased and highly accurate approach for molecular simulations can be provided by quantum mechanical calculations from ''first principles'', which do not rely on empirical parameters. Density Functional Theory (DFT) is such an approach and is widely used because of its computational efficiency but the computational effort of conventional DFT increases with the third power of the number of atoms. As a result it is practically not feasible even on supercomputers to perform DFT calculations with more than a few hundred atoms. Novel reformulations of DFT based on the one-particle density matrix can lead to computational effort which increases linearly with the number of atoms and hence overcome this length-scale problem. I will briefly describe how this is achieved in our ONETEP linear-scaling DFT program which is designed to achieve the same high level of accuracy as conventional cubic-scaling approaches. Then I will give an overview of current applications we are performing with ONETEP, including quantum mechanical simulations of entire proteins with thousands of atoms participating in protein-protein and protein-ligand complexes.

[1] C.-K. Skylaris, P. D. Haynes, A. A. Mostofi and M. C. Payne. J. Chem. Phys. 122 (2005) 084119.
[2] L. Heady, M. Fernandez-Serra, R. L. Mancera, S. Joyce, A. R. Venkitaraman, E. Artacho, C.-K. Skylaris, L. Colombi Ciacchi and M. C. Payne. J. Med. Chem. 49 (2006) 5141.
[3] C.-K. Skylaris, P. D. Haynes, A. A. Mostofi and M. C. Payne. J. Phys. Condens. Matter 20 (2008) 064209.

58-Plat

Recent Developments of the Molecular Dynamics Flexible Fitting Method

Kwok Yan Chan, Leonardo G. Trabuco, James Gumbart, Klaus Schulten.
University of Illinois at Urbana-Champaign, Champaign, IL, USA.

Molecular Dynamics Flexible Fitting (MDFF) is a computational method that combines structural information from X-ray crystallography and cryo-electron microscopy (cryo-EM). Cryo-EM provides structural data on biomolecules in their functional states but not at atomic resolution, while X-ray crystallography yields atomic-resolution data but for molecules in crystalline form that renders them often non-functional. MDFF employs molecular dynamics (MD) simulations to bridge the two sets of data, by adding an attractive potential derived from cryo-EM maps to the MD force field, driving atoms toward high-density regions while maintaining a stereochemically correct conformation of the molecules. MDFF has already been successfully applied to study several macromolecular complexes such as the ribosome.

To further advance MDFF, we have created a test set consisting of different types of biomolecules in different conformational states. Using this test set, MDFF has been optimized for different types of molecules and conformational changes, providing a useful guideline for users applying MDFF to different biological systems. Moreover, helical symmetry restraints have been incorporated into MDFF as many biological systems studied by MDFF have helical symmetry, such as the family of microbial nitrilases. Information about helical symmetry can now be included in MDFF, improving the quality of the fit on these kinds of systems.

59-Plat

On Bootstrap Techniques For Classifying Projections in Single Particle Electron Microscopy

Hstau Y. Liao[1], Joachim Frank[1,2].
[1]Columbia University, New York, NY, USA, [2]Howard Hughes Medical Institute, New York, NY, USA.

In single-particle reconstruction methods [1], projections of macromolecules at randomly unknown orientations are collected by an electron microscope. Often, several classes of conformations or binding states coexist in a sample. To obtain structures with high accuracy, it is required to separate the classes before reconstruction of the structures takes place. In this work, we take a close look at bootstrap techniques for classifying the projection data.

In the bootstrap techniques for variance estimation [2], the projection images or particles are randomly sampled with replacement from the dataset and a bootstrap volume is reconstructed from each sample, assuming known orientations. In a recent extension of the bootstrap technique to classification [3], each particle is assigned to a volume in the space spanned by the bootstrap volumes, such that the projection of the assigned volume (in the same orientation as the particle) best matches the particle. Finally, a clustering algorithm applied to the assigned volumes determines the class to which the particle belongs.

In this work we explain the rational of these techniques by discussing the nature of the bootstrap volumes: i.e., how they relate to the underlying structural classes. Furthermore, several statistical analyses become easy to study in our framework. Finally, the way the particles are assigned to volumes in the space spanned by the bootstrap volumes is closely examined.

[1] J. Frank, Three Dimensional Electron Microscopy of Macromolecular Assemblies, Oxford, 2006.
[2] P.A. Penczek et al., Estimation of variance in single-particle reconstruction using the bootstrap technique., 154(2):168-83, JSB, 2006.
[3] P.A. Penczek, Personal Communication.

60-Plat

Mypal, a Multi-Resolution Approach For Interactively Locating Functionally Linked Ion Binding Sites

Olivier Delalande[1], Nicolas Férey[1], Benoist Laurent[1], Marc Guéroult[1], Brigitte Hartmann[2], **Marc Baaden**[1].
[1]IBPC, CNRS UPR9080, Paris, France, [2]DSIMB, INTS, Paris, France.

Metal ions drive important parts of biology, yet it remains experimentally challenging to locate their binding sites. Here we present an innovative computational approach. We use interactive steering of charged ions or small molecules in an electrostatic potential map in order to identify potential binding sites. The user interacts with a haptic device and experiences tactile feedback related to the strength of binding at a given site. The potential field is the first level of resolution used in this model. Any type of potential field can be used, implicitly taking into account conditions such as ionic strength, dielectric constants or the presence of a membrane. Furthermore, we represent the accessibility of all binding sites by modelling the shape of the target macromolecule via non-bonded van der Waals interactions between its static atomic or coarse-grained structure and the probe molecule(s). The third level concerns the representation of the molecular probe itself. Ion selectivity can be assessed by using multiple interacting ions as probes. This method was successfully applied to the DNase I enzyme, where we recently identified two new cation binding sites by computationally expensive extended molecular dynamics simulations.

61-Plat

GNEIMO: Constrained Molecular Dynamics Methods For Long Time Scale Simulation of Macromolecules

Gouthaman S. Balaraman[1], Jeff Wagner[2], Rudranarayan Mukherjee[2], Abhinandan Jain[2], Nagarajan Vaidehi[1].
[1]City of Hope, Duarte, CA, USA, [2]Jet Propulsion Laboratory, Pasadena, CA, USA.

The two leading causes for the limitation of the all-atom molecular dynamics (MD) simulation timescales are: 1. calculation of forces that scale as the square of the number of atoms and, 2. the integration time step is limited to 1fs due to the high frequency modes in the protein. High performance technologies and better force calculation algorithms have addressed the former, and we address the latter issue in this work.

Here we report a constrained MD method, GNEIMO (Generalized Newton-Euler inverse mass operator method), that is capable of achieving stable dynamics with integration time steps as large as 10 to 20fs. The GNEIMO method provides a platform to perform long time scale hierarchical simulations ranging from all-atom simulations, coarse-grained dynamics of clusters of few atoms, to dynamics with larger motifs constrained, at lesser computational expense compared to all-atom MD. GNEIMO method uses spatial operator algebra to solve for the internal coordinate dynamics with computational cost scaling linearly as the number of degrees of freedom.

The current implementation of GNEIMO is capable of performing constant temperature Nose-Hoover dynamics, with continuum Generalized Born solvation. We use adaptive step size integration to provide stable dynamics with larger time steps. We have carried out tens of nano-seconds of stable dynamics for proteins with time steps as large as 10 to 20fs for different integrators. We report results from conformational changes of domains in proteins from long time scale dynamics. This implementation integrates the force-field module from the MD program LAMMPS, with constrained dynamics module from NASA-Jet Propulsion laboratory.