Mechanical forces generated by cells mediate shape changes that occur during essential life processes including polarization, division and spreading. While the cell cytoskeleton is recognized for its myriad contributions to force generation, the mechanisms by which the cell membrane may also generate forces are often overlooked. Therefore, we explore the potential that membrane generates mechanical tension on cellular length scales by measuring the traction stresses generated during liposome adhesion and spreading on compliant substrates. We find that giant liposomes devoid of a cytoskeleton generate contractile traction stresses on par with those exerted by living cells. These stresses result from the equilibration of internal, hydrostatic pressures elevated by the membrane tension built by strong adhesion to the substrate. These results highlight the active role of membranes in the generation of mechanical stresses on cellular length scales. Furthermore, it uncovers that the modulation of hydrostatic pressure via membrane tension and adhesion can be channeled to perform mechanical work on the environment, providing a more comprehensive description of cell contractility and force generation.

915-Plat

Determining Material Elastic Properties of Arbitrarily-Shaped Membranes using Molecular Dynamics Simulations with Application to the Inverted Hexagonal Phase

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Accumulating evidence indicates that diverse physiological processes are influenced by the lipid composition of the membrane and by its material properties. This has notably been shown for the function of diverse proteins and their oligomerization, and processes on larger scales such as membrane reshaping and fusion. Determination of the elastic properties of lipidic membranes is therefore of great importance to our understanding of these processes. Experimental approaches to determine the material properties of lipids remain challenging and usually rely on their study in a relaxed environment or in flat bilayers, although it is widely accepted that cell membranes can be under considerable stress and frustration as well as high local curvature. Whether this impacts the measured properties is a matter of debate so that studying membranes under more realistic conditions is key for our understanding how these material properties impact different physiological processes. In this context, we propose a computational method to determine the elastic properties of lipid assemblies of arbitrarily shaped interfaces and use it to study the impact of the curvature of a membrane on its elastic properties. Specifically, we apply the methodology to mixtures of DOPE (dioleoylphosphatidylethanolamine) lipids and cholesterol in the inverted-hexagonal and lamellar phases and find that the bending rigidity for a particular lipid composition critically depends on the geometry of the lipidic system. This dependence correlates on the molecular level to the changes in lipid chain order imposed by the membrane curvature, implying that these results should pertain to other situations where the membrane is deformed, stressed or frustrated that notably emerge around integral membrane proteins or during membrane remodeling processes such as budding.

916-Plat

Mobility of Single-File Water Molecules in Aquaporins

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Confined water is an important element in protein structure and function, yet its physical properties are notoriously difficult to assess. Here we show that the mobility of single-file waters inside aquaporins reaches bulk water mobility. Our assessment is based on measurements of the unitary water channel permeability, p_f [1]. We used stopped-flow experiments to determine the per channel increment in proteoliposome water permeability as a function of protein abundance. Therefore, we substituted (i) semi empirical relationships between vesicle volume and scattered light intensity for an adaptation of the Rayleigh-Gans-Debye equation and (ii) analytically solved the differential equation for the time dependence of vesicle volume on water efflux. Both fluorescence correlation spectroscopy and high speed atomic force microscopy served to determine the exact number of water channels per vesicle. p_f increased in this order: aquaporin-Z [2], aquaporin-1 [3], and GlpF (*E.coli* glycerol facilitator) [4]. The maximal turnover number was equal to $5x10^{10}$ s^{-1}, it thus exceeded previous estimates by as much as 50-fold. The high mobility is consistent with previous reports on the low number of hydrogen bonds formed by the single-file waters in the channel and the distorted geometry of those bonds.

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Platform: Computational and Simulation Methods

917-Plat

Adaptive Boundaries in Multi-Resolution Simulations

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Biomolecular simulations generally require a compromise between forcefield resolution and computational efficiency. Methods that combine multiple levels of resolution promise to extend the ability of simulations to handle bigger systems and more complex processes.
We have developed an explicit/continuum solvent model that is able to reproduce the effects of explicit biomolecular solvation while only including a fraction of the molecules that would otherwise be required. We are extending this approach as a general multi-resolution model where both solvent and other, more complex, molecules change representation as they move across the boundaries of the explicit and continuum domains.
This model includes: (1) new boundary methods that accurately reproduce thermodynamic and kinetic properties in the explicit region exactly, within statistical error, as if they were taken from large, fully explicit simulations; (2) adaptive boundaries that spatially alter the level of detail in response to an evolving molecular environment; and (3) a grand canonical control of molecular components, relaxing the density of chemical species as the simulation progresses (important for studies in crystallization, assembly, etc.).
Our current work is aimed at surmounting a considerable challenge facing all multi-resolution models: transferability to new systems and arbitrary geometries. Overcoming this challenge will be essential in making these multi-resolution models ready to be used 'out of the box' for a range of problems in biophysics.

918-Plat

To Bayes, or Not to Bayes, Information is the Answer

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Biological systems are inherently noisy on a molecular scale. The problem of determining the true state of a system and characterizing the transitions between states whose observables are corrupted by noise is a canonical problem in statistics with a rich history. There has recently been a significant renaissance in the application of Bayesian Statistics (for instance variational Bayes and the use of the Bayesian Information Criterion) to analyze single-molecule biophysics and cell-biology problems. Although these Bayesian techniques regularize the model selection problem to prevent over-fitting through the introduction of a prior probability distribution, Bayesian techniques typically significantly underestimate the complexity that can be resolved by information-based and frequentist analyses and often require the introduction of ad hoc prior distributions. We demonstrate the application of information-based statistics (both canonical and novel) in the analyses of several distinct biological measurements from our laboratory ranging from single-molecule techniques including stoichiometry by bleaching and tether-particle motion to cell biology problems including characterizing cell motility. In each case we compare the resolution of the Bayesian and information-based approaches to demonstrate the increased resolving power of information-based techniques.

919-Plat

Benchmarking and Optimizing Atomistic Forcefields with Density Measurements

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Predictive biophysical models of drug binding and selectivity could accelerate drug discovery. However, a limited understanding of molecular-scale phenomena currently prevents biophysical models from impacting drug design. To assess the quality of the GAFF small molecule forcefield, we have performed

nearly 1000 molecular dynamics simulations of liquids containing drug-like atom types. We compare our simulations to an extensive database of physical properties from NIST's Thermodynamics Research Center. We also evaluate GAFF's performance using density measurements of neat liquids and binary mixtures performed on an in-house automation system.

920-Plat
Implementing Solution X-Ray Scattering Data as Active Constraints in MD Simulations
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X-ray and neutron solution scattering are powerful techniques that provide a measure of biomolecular structure and its diversity. The techniques are highly sensitive to conformational change - however, the measured intensities represent a single global average over all conformations and obscures the extent of structural variations as well as their relative frequency. Thus, the utility of scattering data in molecular simulations depend on the ability to generate the underlying conformational ensemble, guided by only the average values. To resolve this, we recently implemented an explicit-solvent approach to predict the ensemble SWAXS pattern of a biomolecule using molecular dynamics [Chen and Hub, Biophys. J. 107:435-447 (2014)]. By relying upon MD to explicitly sample fast degrees of freedoms in both solvent and solute, accurate predictions were computed for relatively stable proteins. The approach adopted also enables the use of SWAXS data as constraints that provide forces to guide the simulation trajectory, which permits the interpretation of experimental data by directing simulated proteins into compatible conformations. The application of SWAXS-driven MD to biological examples is given in an accompanying talk.
Here, we will address the derivation of such constraints, taking into account the sampling of different degrees of freedom and sources of uncertainty. We find that conformation-dependant SWAXS patterns can be derived over ~1 ns timescales, permitting fast equilibration in constrained-simulations. Uncertainty due to buffer subtraction dominates at low angles q, while experimental and statistical errors dominate at large q. Inclusion of these errors in the constraints do not affect the qualitative behaviour of resulting trajectories. The application of SWAXS-based constraints in the context of both single-state and heterogenous ensembles will also be discussed.

921-Plat
Constant pH Molecular Dynamics in Explicit Solvent with Enveloping Distribution Sampling and Hamiltonian Exchange
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We present a new computational approach for constant pH simulations in explicit solvent based on the combination of the enveloping distribution sampling (EDS) and Hamiltonian replica exchange (HREX) methods. Unlike constant pH methods based on variable and continuous charge models, our method is based on discrete protonation states. EDS generates a hybrid Hamiltonian of different protonation states. A smoothness parameter s is used to control the heights of energy barriers of the hybrid-state energy landscape. A small s value facilitates state transitions by lowering energy barriers. Replica exchange between EDS potentials with different s values allows us to readily obtain a thermodynamically accurate ensemble of multiple protonation states with frequent state transitions. The analysis is performed with an ensemble obtained from an EDS Hamiltonian without smoothing, which strictly follows the minimum energy surface of the end states. The accuracy and efficiency of this method is tested on aspartic acid, lysine, and glutamic acid, which have two protonation states, a histidine with three states, a four-residue peptide with four states, and snake cardiotoxin with eight states. The pKa values estimated with the EDS-HREX method agree well with the experimental pKa values. The mean absolute errors of small benchmark systems range from 0.03 to 0.17 pKa units, and those of three titratable groups of snake cardiotoxin range from 0.2 to 1.6 pKa units. This study demonstrates that EDS-HREX is a potent theoretical framework, which gives the correct description of multiple protonation states and good calculated pKa values.

922-Plat
Mesoscale Modelling of Biomolecules using Continuum Mechanics
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Biophysical techniques that provide structural information at the mesoscale, such as cryo-electron microscopy and 3D tomography, are now sufficiently mature that they merit their own online repository called the EMDataBank (EMDB). We have developed a continuum mechanics description of proteins which uses this new experimental data as input to the simulations. The model is a Finite Element algorithm which we have generalised to include thermal fluctuations, and which is therefore known as Fluctuating Finite Element Analysis (FFEA). While conventional molecular dynamics simulations provide a trajectory in which each individual atomic position fluctuates, an FFEA trajectory shows how the overall shape of the protein changes due to thermal agitation. We have used FFEA to show that the crowded environment of the axoneme impedes the thermal fluctuations of the largest cytoskeletal motor dynein, and have used our model to calculate the reach of the motor in situ. Our modelling highlights the importance of understanding the 3D architecture of biological structures at the mesoscale.

923-Plat
Multilevel Summation Method for Electrostatic Force Evaluation
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The multilevel summation method (MSM) offers an efficient algorithm for evaluating long-range forces in molecular dynamics simulations. MSM is competitive to the ubiquitous particle-mesh Ewald (PME) method, but handles periodic as well as semi-periodic and non-periodic systems. MSM, unlike PME, can calculate dispersion forces without cutoff. The version of MSM available in the simulation program NAMD is described, and its performance and accuracy compared with the PME method. The comparison involves water property calculations such as density, diffusion constant, dielectric constant, surface tension, radial distribution function, and the distance-dependent Kirkwood factor. Excellent agreement between MSM and PME is found also for interface potentials of air-water and membrane-water interfaces, where long-range Coulombic interactions are crucial. Through the use of nested interpolation of softened pair potentials in real space, MSM can be applied to simulations having semi-periodic or non-periodic boundaries. Simulations were performed with periodic boundaries along directions parallel to a membrane surface but not along the direction of the surface normal, to describe membrane pore formation induced by an imbalance of charge across a membrane. With a similar semi-periodic boundary condition, ion conduction through a graphene nanopore was simulated in the presence of an ion gradient. Proteins were also simulated inside a spherical water droplet without any periodic boundary. Finally, MSM is demonstrated to provide better parallel scaling than PME, making MSM more suitable for the simulation of large systems.

924-Plat
Using Long-Timescale Molecular Dynamics Simulations to Benchmark Enhanced Sampling Methods
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All-atom molecular dynamics (MD) simulation is a valuable technique for providing detailed information about the dynamics of biomolecules, but its computational expense can often be prohibitive. This limitation has motivated the development of ''enhanced sampling'' simulation methods - purely algorithmic changes to conventional MD that aim to accelerate the sampling of configurational states. Although many such methods are available, relatively few systematic studies of their performance have been done. Quantitative claims about the performance of enhanced sampling simulations are typically limited to (i) comparisons with conventional MD simulations of small, model systems, which may present qualitatively different sampling challenges than complex biological systems, or (ii) comparisons with experimental data, which may be complicated by discrepancies between the actual experimental conditions and the modeling of those experimental conditions in the enhanced sampling simulation, including errors in the physical model, or force field, used in simulation. An effective alternative approach to quantifying performance of enhanced sampling methods would be to compare the results they obtain on complex biological systems directly to those obtained by conventional MD simulations using the same force field. Here, we use long-timescale conventional MD simulations to assess the performance of certain commonly used enhanced sampling methods in accelerating the sampling of protein conformational changes, protein folding, and ligand binding.