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2023-Pos
BIBEE: a Rigorous and Computationally Efficient Approximation to Continuum Electrostatics
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The computational costs associated with modeling biomolecular electrostatics using continuum theory have motivated numerous approximations, such as Generalized-Born (GB) models, that can be computed in much less time. Unfortunately, most of these approximate models abandon physics in favor of computational efficiency. On the other hand, a new approximation method for molecular electrostatics, called BIBEE (boundary-integral-based electrostatics estimation), retains the underlying physics of continuum theory, but is nearly as efficient as Generalized-Born models. The BIBEE approach derives from well-known results in potential theory and the theory of boundary-integral equations. Three main results demonstrate the value BIBEE may hold for biomolecular analysis and design. First, the integral-equation theory clarifies the origin of accuracy of the Coulomb-field approximation (CFA). Second, BIBEE models offer significantly better accuracy for individual pairwise interactions, relative to GB methods. Third, BIBEE readily provides provable upper and lower bounds to the electrostatic solvation free energy of the original (exact) continuum-theory problem.

2024-Pos
Evaluating Empirical Force Fields Through Combined QM/MM Computations of the Vibrational Stark Effect
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The proper description of the electric environment of the interior of macromolecular structures is a critical challenge for force field methods. To test and validate the CHARMM force field's ability to describe this electric environment, combined QM/MM calculations have been used to calculate the vibrational Stark effect (VSE). The Stark effect refers to the characteristic shift of a specific vibrational frequency upon the introduction of an electric field. In this work, we calculate the Stark shift of several experimentally characterized Stark effect probes (5-cyanoindole, methyl thiocyanate, and fluorobenzene) in several solvents. The solvent environment around the probe is sampled through 20 ns molecular dynamics simulations of each molecule surrounded by several hundred explicit solvent molecules. From these simulations, two hundred snapshots of the solvent environment are collected for the QM/MM analysis. The QM/MM computation uses correlated electronic structure methods to calculate the vibrational spectrum of the VSE probe in the field created by the solvent molecules, which are treated as MM atoms with the CHARMM force field. From these computations, an average Stark shift is determined for each probe molecule and compared to experimental measurements. This information can be directly related to the electric field surrounding the probe molecule, and therefore may be used as a direct test of the ability of a force field to reproduce the electric field around those functional groups. Information from these calculations will act as the basis for additional optimization of the force field to more accurately represent the electric fields in macromolecules.

2025-Pos
Weighted Ensemble Path Sampling for Efficient Calculation of Steady State Properties
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Steady states are common in biological processes, most famously in enzymatic catalysis. We present a rigorous path sampling procedure, generalizing the ''weighted ensemble'' (WE) method, to attain a steady state (SS) efficiently. We apply this procedure to several different systems, from toy models to the folding of the atomistic Trp cage mini-protein. For systems without significant intermediates, we find that the SS-WE procedure is able to attain steady state fairly efficiently. However, for systems with significant intermediates, we develop an enhanced version of SS-WE that shifts probability to speed-up the establishment of a steady state, without perturbing the system's natural dynamics. The enhanced SS-WE approach is able to attain a steady state in significantly less time for systems with significant intermediates, and gives correct results for the steady state rates and probability distribution. First-passage rates are also obtained simultaneously.

2026-Pos
Simulations of Binding Free Energy of Targeted Nanocarriers to Cell Surfaces: the Effects of Antigen Flexural Rigidity, Glycocalyx Resistance, and Shear Flow
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We develop an equilibrium mesoscale model to study the binding free energy of functionalized nanocarriers (NC) to cell surfaces, which plays a central role in targeted drug delivery. Our model is parametrized to mimic interactions between intercellular adhesion molecule 1 (ICAM-1) on cell surface and anti-ICAM (antibody) on NCs and accounts for ICAM-1 diffusion and flexure, bond stiffness, effect of glycocalyx, and shear flow; parameters are chosen from several independent literature experiments. Using umbrella sampling in conjunction with Monte Carlo simulations, we compute the potential of mean force (PMF) as a function of distance between the NC and the cell surface. Our results show that the PMF landscape is rugged along the distance of the NC from the cell surface with multiple equilibrium points separated by free energy barriers of comparable magnitudes. Calculations reveal: (1) a significant effect of the antigen flexural rigidity, namely with decreasing flexural rigidity, even though the multivalency of binding increases, we record decrease in the binding free energy due to increasing entropic penalty; (2) The presence of glycocalyx does not alter multivalency, but significantly reduces the binding free energy; (3) Hydrodynamic shear stress plays a central role in mediating the binding conformations and alters the PMF landscape. Our results provide quantitative assessments of the effects of tunable/controllable properties on the binding of NCs to cell surfaces. Our model provides a rational and unique approach to bridge single molecule and biophysical measurements at the molecular scale with microscopy and flow experiments at the micro and macroscales. This integrative step will enhance optimization of delivery vehicles for use in targeted therapeutics.
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2027-Pos
Multi-Body Knowledge-Based Potentials for Protein Structure Prediction Evaluation
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Knowledge-based potentials have been widely used in the last 20 years for fold recognition, evaluation of protein structure predictions from amino acid sequences, ligand binding, protein design, and many other purposes. The most commonly known are two-body residue-level contact potentials, especially those first introduced by Miyazawa and Jernigan in 1985, and then rederived using an updated, larger protein dataset in 1996. Dense packing of residues in globular proteins is one of their characteristic features. Because of such dense packing cooperative multi-body interactions, especially in protein cores are important. The four-body contact potentials and short-range interaction potentials have been derived by considering different aspects of protein structures than those used to derive pair-contact potentials. The four-body contact potentials are appropriate for representing the cooperative parts of the protein folding process, and we have shown that they are quite successful for recognizing the native structures among hundreds or even thousands of decoys from the Decoys'R'Us database. Short-range interaction energies allow us to estimate free energies from the statistical distribution of local conformational descriptors. We developed two types of four-body potentials: sequential and non-sequential ones. We have found that combining the former ones with short-range interactions yields excellent results for threadings, that significantly outperforms all other methods for coarse-grained models of proteins. We have developed also our knowledge-based potential server http://gor.bb.iastate.edu/potential for coarse-grained protein energy estimations that uses two types of four-body potentials, short-range potentials, and 23 different two-body potentials.

2028-Pos
Prediction of Calcium Binding Site in the RCK1 Domain of BK_{Ca} Channel Using Multisite Cation Model
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Calcium plays a major role in controlling the opening and closing of the large conductance BK_{Ca} channels. Two high affinity binding sites have been identified in the channel structure and one of these sites is the DRDD loop in the N-terminus of the RCK1 domain. Mutation of the first aspartate in this conserved DRDD motif significantly reduces Ca^{2+} sensitivity and hence this residue has been implicated as a coordinating group in the binding site. Here we present results on the prediction of the Ca^{2+} binding site based on a series of detailed computational studies. We use a novel multisite cation model for calcium ion to accurately simulate the ion-coordination. The basic protocol involves multiple

iterations of random ion placement, implicit solvent molecular dynamics simulations and statistical analysis. Our resulting model matches very well with existing mutagenesis data, and subsequent explicit solvent molecular dynamics simulations have been performed using this Ca^{2+} bound structure. Comparison of the dynamics and conformations of the Ca^{2+} bound and unbound simulations reveal a concerted conformational change in the structure and suggest a potential mechanism for calcium dependent activation of these channels.

Imaging & Optical Microscopy II

2029-Pos

Characterization of the Use of Far-Red Dyes for Localization Microscopy of Biological Samples

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Recently it has been shown that conventional fluorescent dyes can be used to achieve super-resolution by single-molecule localization. The use of conventional fluorochromes for this new approach, termed 'localization microscopy', depends critically on the observation of rapid on-off blinking of these dyes in certain chemical environments. Here we characterize the photophysical behavior of several commercially available far-red dyes that have similar properties to the cyanine dye Cy5 and use them to visualize the distribution of proteins in fixed cardiac cells.

To obtain super-resolution images several thousand individual 'blinking'-events, which arise from dye molecules that briefly enter a bright (fluorescent) state, were analyzed and the molecular positions determined by a fitting algorithm. We tested far-red Alexa dyes (647,680,700,750) linked to secondary antibodies for use in immuno-labeling super-resolution microscopy. The dyes were observed in mounting media of various refractive indices containing oxygen scavengers and triplet quenching compounds that favors fluorochrome 'blinking'. Under these conditions typically over 1000 photons per 'blinking'-event were detected and a localization accuracy of ~30 nm (full width at half maximum) was readily achieved with Alexa 680. These accuracies are in good agreement with theoretical calculations when the background present in labeled cellular preparations (largely arising from out-of-focus fluorescence) is taken into account.

For practical super-resolution imaging of immuno-stained preparations, several advantages are provided by these dyes, for example, as compared to blue/green excited fluorochromes less autofluorescence is generally caused by organelles and fixation. We investigate the possibility to use a combination of these dyes for simultaneous localization imaging of multi-labeled preparations excited with a single laser line. This is illustrated using fixed ventricular myocytes that were labeled for β-tubulin, caveolin-3 and ryanodine receptors.

2030-Pos

Synthetic Chromophore Maturation by Split Green Fluorescent Protein (GFP)

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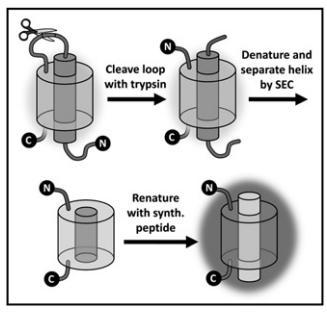
Green fluorescent protein (GFP) undergoes a self-catalyzed cyclization, dehydration, oxidation reaction sequence to form a fluorescent chromophore in the protein's interior. We have developed a system for studying chromophore maturation and the photophysical properties of novel chromophores *in vitro*. Intact GFPs, circularly permutated to locate the interior α-helix at the N-terminus, were expressed with a flexible proteolytic loop inserted on the C-terminal side of the chromophore-containing helix. With trypsin, the helix was severed and subsequently removed by size exclusion chromatography after denaturation. The split GFP was then renatured in the presence of a synthetic peptide which underwent chromophore maturation to acquire fluorescence. Since the complementary helix peptide is fully synthetic we have unprecedented control through specific incorporation of multiple unnatural amino acids. We are studying these effects with a range of spectroscopic techniques including steady state fluorescence, time-correlated and upconversion time-resolved fluorescence in order to better understand the process of chromophore maturation, the photochemistry of the protein, and the kinetics and efficiency of fluorescence reconstitution to inform the strategies for producing more robust *in vivo* probes.



2031-Pos

Dynamics of Individual BK_{Ca} Channels in Live Cells Monitored by Site-Specific Labeling Using Quantum Dots

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In order to monitor the movement of individual large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca} channels) in live cells at real-time, we co-expressed the BK_{Ca} channel tagged at its extracellular N-terminus with the 'acceptor peptide (AP)' for biotin and a genetically modified *E. coli* biotin ligase in various mammalian cells. Using the quantum dots (QDs) coated with streptavidin, we were able to visualize individual BK_{Ca} channels that had been biotinylated intracellularly and then expressed on to the surface of the cells. The channels were determined to be labeled by two QDs in average, based on the levels of quantized fluctuations of fluorescence intensity, known as 'photo-blinkings'. We monitored the movements of BK_{Ca} channels in both live mammalian cell-lines and primary hippocampal neurons using time-lapse imaging. Depending on the type of cells and the location where the channels were expressed within a cell, BK_{Ca} channels showed different patterns and speeds in their movements. We are currently quantifying the movement of individual channels and investigating those protein motifs affecting the channel dynamics. We wish to understand the molecular mechanism of BK_{Ca} channel trafficking and the cellular players involved in.

2032-Pos

Counting Pictures of Cell that Count: A Biotiff-Based Strategy for Indexing Cell Images and Associated Metadata in Large Series of Digital Microscopy-Based Biophysical Result

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Last year we described how BioTIFF code (www.biotiff.org) enables all metadata needed to interpret a given digital image region to be embedded within the same file structure used to store that image (Biophys J. 96: 30a). Here we show how that metadata can then be indexed so that Google-like queries can be performed on any set of BioTIFF files. Indexing can be done either on a single local set of BioTIFF images or a distributed set by using a distributed file system. This then creates a distributed index that has many interesting applications for exploring image relationships across different subsets of images within large sets. For example, single cell (or single molecule) responses measured using digital microscopy-based biophysical methods that relate cellular mechanism to light-based surrogate measures can then be linked to a specific cell in a specific field-of-view of a specific sample. The replication of such results over time in the same lab or in a distributed manner by multiple labs can allow for collaborative replicable science to anchor the evolution of shared understanding of cell physiology mechanisms. We will demonstrate how the approach can be implemented using a plurality of commodity gear meeting certain minimum standards and open source imaging, indexing and search software. This level of transparency and annotation of experimental detail allows for differences in experimental conditions between experiments and labs to be accommodated in open collaborative interpretation of biophysical data.

2033-Pos

Site-Specific, Orthogonal Two Color Labeling of Different Proteins with Flash and Reash in Living Cells

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Multiparameter imaging of independent cellular functions is limitied by the lack of distinct molecular probes that would specifically label two different proteins. Here we report a strategy to simultaneously label two different proteins in living cells with two different fluorophores, FlAsH and ReAsH.

Recently, tetracysteine binding motifs have been improved to selectively bind to FlAsH or ReAsH respectively. We compared the six amino acid motif CCPGCC and the twelve amino acid motif FLNCCPGCCMEP with respect to their affinities for FlAsH and ReAsH. Both fluorophores showed higher affinity for the FLNCCPGCCMEP motif. For both target sequences, FlAsH showed more stable interactions than ReAsH. Using a new labelling protocol we selectively labeled different proteins in the same cell. Our target proteins were localized in different cellular compartments, a plasmamembrane localized G protein-coupled receptor for PTH (PTH-receptor) and the cytosolic β-arrestin2- protein. Our protocol allowed selective labelling of PTH-receptor with ReAsH at a C-terminal FLNCCPGCCMEP motif, while the cytosolic β-arrestin-2 protein was C-terminally modified with the CCPGCC motif and specificly labeled with FlAsH. Both proteins were simultaneously visualized in intact cells, and their interaction was determined by colocalization and fluorescence resonance energy transfer (FRET).

Taken together, our data demonstrate that FlAsH and ReAsH can be used for orthogonal labeling of different target proteins in living cells.