the global minimum in a discrete sampling space. DOMINO decomposes the set of optimized variables into relatively uncoupled but potentially overlapping subsets that can be sampled independently form each other, followed by efficiently gathering the subset solutions into the global minimum.
We have further extended MultiFit for modeling the architecture of macromolecular assemblies by aligning proteomics data into electron-microscopy density maps. The method facilitated the structural modeling of the AAA-ATPase/20S core particle sub-complex of the 26S proteasome [2].
[1] K. Lasker, M. Topf, A. Sali, H. Wolfson. Inferential optimization for simultaneous fitting of multiple components into a cryoEM map of their assembly. Journal of Molecular Biology 388, 180-194, 2009.
[2] F. Forster, K. Lasker, F. Beck, S. Nickell, A. Sali, W. Baumeister. An Atomic Model AAA-ATPase/20S core particle sub-complex of the 26S proteasome. Biochem Biophys Res Commun 388, 228-233, 2009

324-Pos
Multifunctional Gfp Tag: A Useful Tool For Isolation of Protein Complexes
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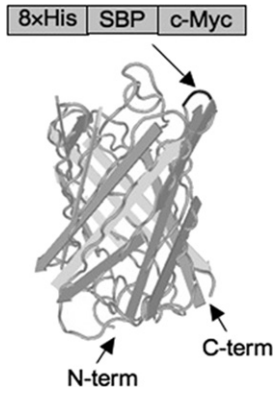
Protein complexes are functional units essential for virtually all cellular processes. To understand molecular mechanisms of the functions, it is necessary to identify and characterize the protein complexes involved. Protein tags are genetically encoded tags and useful tools for detection and isolation of protein complexes. So far, many kinds of protein tags have been developed. We have recently reported a novel multifunctional green fluorescent protein (mfGFP) tag which can be used for cellular localization, composition, and structure of the protein of interest (Kobayashi et al. PLoS ONE, 3, e3822, 2008). mfGFP was engineered by inserting several peptide tags (8×His, SBP, and c-Myc) in tandem into a loop of GFP. In the present study, we developed several variations of mfGFP having different tag systems, which are optimized for isolating various levels of protein complexes from small proteins to large organelles. The mfGFP will be a useful tool for isolation of protein complexes.



325-Pos
Effect of Kinetics on Sedimentation Velocity Profiles and the Role of Intermediates
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We have previously presented a tutorial on direct boundary fitting of sedimentation velocity data for kinetically mediated monomer-dimer systems (Correia & Stafford, 2009). We emphasized the ability of Sedanal to fit for the k_{off} values and measure their uncertainty at the 95% confidence interval. We concluded for a monomer-dimer system the range of well determined k_{off} values is limited to 0.005 to 10^{-5} sec^{-1} corresponding to relaxation times of ~70 to ~33000 sec. More complicated reaction schemes introduce the potential complexity of low concentrations of an intermediate that may also influence the kinetic behavior during sedimentation. This can be seen in a cooperative ABCD system (A+B->C; B+C->D) where C, the 1:1 complex, is sparsely populated ($K_1 = 10^4 M^{-1}$, $K_2 = 10^8 M^{-1}$). Under these conditions a $k_{1,off} < 0.01$ sec^{-1} produces slow kinetic features. The low concentration of species C contributes to this effect while still allowing the accurate estimation of $k_{1,off}$ (although $k_{2,off}$ can readily compensate and contribute to the kinetics). More complex reactions involving concerted assembly or cooperative ring formation with low concentrations of intermediate species also display kinetic effects due to a slow flux of material through the sparsely populated intermediate states. This produces a kinetically limited reaction boundary with partial resolution of individual species during sedimentation. Cooperativity of ring formation drives the reaction and thus separation of kinetics and energetics can be challenging. This situation is experimentally exhibited by systems that form large oligomers or rings, formation of micelles and various protein aggregation diseases including formation of β-amyloid and tau aggregates. Simulations, quantitative parameter estimation by direct boundary fitting and diagnostic features for these systems are presented with an emphasis on the features available in Sedanal to simulate and analyze kinetically mediated systems.

326-Pos
Determining Thermodynamic Parameters of Protein Interactions By Global Analysis of Data From Multiple Techniques
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When studying macromolecular interactions, the thermodynamics and stoichiometry of binding are of considerable interest because they indicate the physical-chemical nature of the biological mechanism. Since a single biophysical technique is limited in the number of observable properties and may provide only insufficient information for more complex systems, one promising approach is the simultaneous consideration of data from multiple biophysical methods. In the past, we have developed a robust computational framework (SEDPHAT) for this purpose which has been widely used in the biophysical community. However, the best strategy for assembling individual data sets into a global analysis has not been explored. It requires understanding of the limitations and consideration of possible systematic errors for each method. In this work, we have performed experiments on a model system (α-chymotrypsin binding to soybean trypsin inhibitor) to study the detailed compatibility of data from calorimetry (ITC), surface binding (SPR), sedimentation (SV) and fluorescence anisotropy. The significance of each data set from the different techniques has been explored through both individual and global analysis with detailed error surface projection using the program, SEDPHAT. We propose a rational strategy for global analysis that deviates from the purely statistical point of view, by rescaling the weights of each data set such that all techniques can make significant contributions. This allows a more detailed picture of the interaction to emerge.

327-Pos
Information Extraction From Simulations-Based Data Fitting of Distributions of Fret Efficiencies from Donors and Acceptors in the Cytoplasm of Living Cells
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Fluorescence Resonance Energy Transfer (FRET) has evolved to the point where the efficiency of energy transfer at each pixel in an image may be obtained after only one scan of the sample and without recourse to photobleaching or external calibration of acceptor excitation. With this method it is now possible to obtain entire distributions of FRET efficiencies in populations of proteins self-associating into oligomeric complexes. To exploit this opportunity, it is necessary to develop tools for analysis of such data. Here we present comparative results from Monte-Carlo simulations for FRET in homogeneous and inhomogeneous spatial distributions of molecules. The FRET efficiencies were interpreted in terms of both average value (as it would be obtained from wide-field microscopy) and statistical distributions of values (as if obtained from scanning optical microscopy). The advantage of an analysis based on the distribution of FRET efficiencies is that it enables one to discriminate between constitutive oligomers and random collisions between diffusing donors and acceptors. We next evaluated the approach based on the distribution of FRET efficiencies with regard to its potential to provide stoichiometric information from whole distributions of FRET efficiencies by using simulation-based data fitting. The experimental FRET data were obtained from a system of donors and acceptors that reside in the cytoplasm of yeast cells (*S. cerevisiae*) and which appear to interact transiently.

DNA Replication, Recombination, & Repair

328-Pos
Molecular Traffic Jams on DNA Highways: Single Molecule Observation of Collisions Between RecBCD Helicase and DNA Binding Proteins
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DNA helicases, polymerases, and other translocases must proceed along a substrate crowded with other DNA-binding proteins. The outcomes of these molecular collisions play a crucial role in shaping multiple metabolic pathways, such as DNA replication and repair. To address the question of how a translocase proceeds along a congested DNA substrate, we have established a high-throughput single molecule assay to observe the motion of RecBCD on individual DNA molecules. RecBCD is a heterotrimeric helicase and exonuclease that initiates homologous DNA recombination at the free dsDNA ends in E. coli. RecBCD is a processive motor enzyme that uses the energy of ATP hydrolysis to digest both strands of dsDNA until the protein encounters the regulatory

sequence chi (5′-GCTGGTGG-3′). The chi sequence occurs roughly once every five kb in the E. coli genome, suggesting that RecBCD must travel for long distances along genomic DNA.
In our assay, we observe the enzymatic activity of RecBCD on individual DNA molecules. Fluorescently labeled RNA polymerase and hydrolytically inactive EcoRI(E111Q) were selected as model roadblock proteins. By preparing a DNA substrate with these fluorescently labeled proteins, we directly observed the outcome of collisions with RecBCD. Our results indicate that RecBCD is able to push and eventually displace multiple proteins without reducing its rate of translocation. These results offer the first direct observation of collisions between a helicase and other proteins along the same DNA helix. We propose that the highly processive, dual motor structure of RecBCD is necessary for stimulating recombination many thousands of bp away from the initial dsDNA break. Our results provide additional evidence that an essential, if underappreciated, aspect of helicase function is the ability to clear dsDNA for further processing by other enzymes.

329-Pos

The DNA-Gate of Gyrase Is Predominantly in the Closed Conformation During DNA Supercoiling

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DNA topoisomerases catalyze the inter-conversion of DNA topoisomers and impact key cellular events such as replication, recombination, and transcription. Gyrase catalyzes the introduction of negative supercoils into DNA *via* a strand-passage mechanism. In the first step, a DNA-segment, the gate-DNA, binds to gyrase. The gate-DNA is cleaved, and a covalent DNA-gyrase complex is formed. A second DNA segment, the transfer-DNA, is passed through the gap, and the gate-DNA is re-ligated. Strand passage requires opening of a transient protein interface at the cleavage site, the so-called DNA-gate, by ~2 nm. The intermediate cleavage complex presents an inherent danger of double strand DNA breaks and thus genome instability, and cleavage complexes have consistently been detected in very low amounts. In contrast, a recent study predicted frequent opening of the topoisomerase II DNA-gate. Here, we present a single molecule FRET study that monitors both the conformation of DNA bound to the DNA-gate of gyrase, and the conformation of the DNA-gate itself. DNA bound to gyrase adopts two different conformations, one slightly, one severely distorted from B-DNA geometry. Distortion requires cleavage, but neither ATP nor a transfer-DNA. The DNA-gate of gyrase is predominantly in the closed conformation, in agreement with <5% of cleavage complexes in equilibrium. Importantly, gyrase with an open DNA-gate is also not significantly populated during the relaxation and supercoiling reactions. Presumably, distortion of the gate-DNA unlatches the DNA-gate, and prepares it for transient release by the transfer-DNA, thus providing a strict coupling of gate-opening to strand passage.

330-Pos

Investigating the Nucleation and Extension Rates of E.coli and Deinococcus RecA Along Duplex DNA

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RecA is a protein which promotes the exchange between two homologous DNA molecules in homologous recombination process. When individual RecA molecules assemble on DNA, the DNA is stretched and underwound to form a nucleoprotein filament with its rigidity and end-to-end length increased. We have developed single-molecule tethered particle motion (TPM) experiments to study the assembly dynamics of RecA proteins on individual duplex DNA molecules. The TPM method is capable of measuring the changes in DNA length by observing the bead's Brownian motion, thus allowing us to monitor RecA nucleation and extension in real-time. Using much shorter DNA (a few hundreds basepairs), TPM experiments offer improved sensitivity, since the DNA length change can be readily detected as soon as a few RecA bounded to duplex DNA molecules. Our experiments indicated a faster nucleation rate compared to the previous reports (Galletto et al., 2006). Moreover, we have compared the nucleation and extension rates of E. coli RecA with the RecA from Deinococcus radiodurans, UV-resistant bacteria, under different nucleotide states, ATP and ATPγS. Deinococcus radiodurans RecA are found to nucleate faster (~1.6×10-2 bp-1min-1) but extend slower (~0.3-1.5 RecA/sec) under ATP.This difference reflects the physiological role of Dr. RecA when extensive UV-damaged DNA molecules are present.

331-Pos

Effect of DNA Supercoiling on DNA Decatenation and Unknotting Followed By Brownian Dynamics Simulations

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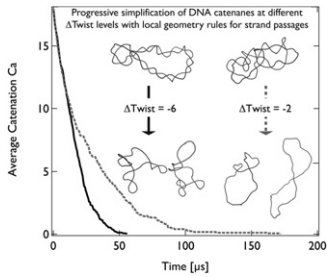

Replication of circular DNA proceeds through a stage of multiply interlinked catenanes that have to be rapidly spatially separated. In addition, knotting of circular DNA has also to be avoided. In bacteria, topology simplification requires participation of two type II topoisomerases: gyrase and topo IV. Several simulation approaches were applied to explain the very efficient topology simplification in that system. Mainly two strategies were explored: in the first one, the system follows its free energy gradient influenced by supercoiling, and in the second one, specific geometrical rules are defined for the selection of strand passages (hooking, chirality). The Monte-Carlo methods usually used to estimate the efficiency of these strategies do not allow to follow DNA topology simplification dynamically, to evaluate its speed, for example. To overcome this limitation, we simulated DNA unknotting and decatenation by Brownian dynamics, which allows for a natural integration of the strategies mentioned above. By following the topological state of the simulated DNA chains (see figure), we show that the combination of supercoiling and local geometrical selection rules provides an important drive for unknotting and decatenation, especially at low topological complexity.

332-Pos

Does T7 DNA Polymerase Backtrack During Proofreading?

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DNA replication is an essential cell process in which the genetic information is copied by replicative DNA polymerases (DNAp). The molecular basis of DNA replication is the addition of nucleotides by DNAp to a growing primer, using single-stranded DNA as a template. High fidelity of the processive T7 DNA polymerase comes from nucleotide selection at the polymerase active site, but is increased several orders of magnitude by an additional intrinsic proofreading ability. In this kinetic process, a partly melted primer shuttles to the exonuclease active site where incorporated mismatches are excised. After excision of erroneous nucleotides, the trimmed primer can shuttle back to the polymerase active site to resume replication. Elucidating the mechanism of the shuttling between these two activities of DNAp is essential for understanding the proofreading mechanism of DNA polymerases.
Transfer of the primer to the exonuclease active site is induced by disruption of the primer-template structure upon the incorporation of a mismatch. Application of tension to the DNA also destabilizes the primer-template structure and can therefore be used to shift the fine-tuned balance between polymerization and proofreading (Wuite *et al*, 2001; Ibarra *et al*, 2009).
Using optical tweezers, we study the kinetic coordination between exonuclease and polymerase activities, while applying different tensions. In these experiments we observe an additional waiting state between proofreading activities, during which the DNAp remains bound to the DNA. The force-dependent rate out of this state suggests that DNAp enters a state comparable to RNA polymerase backtracked state, which was shown to play a role in tuning the fidelity (Shaevitz *et al*, 2003). We speculate that our observed waiting state might play a similar role in the fidelity of DNA polymerase.

333-Pos

A Single Molecule View of the Rad51-ssDNA Interaction

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Homologous recombination (HR) represents an essential DNA repair mechanism in living cells. The central molecular complex of HR is the nucleoprotein filament, a DNA-protein complex in which recombinase protein Rad51 is bounded onto single-stranded DNA (ssDNA) in a helical form. Earlier studies have shown that efficient filament formation is critical for correct DNA repair, therefore a detailed characterization of the interaction between Rad51 and ssDNA is essential to understanding homologous recombination.
We use a combination of single-molecule fluorescence microscopy, optical tweezers and microfluidics to study the interaction of Rad51 with ssDNA. With this approach, we are able to directly visualize Rad51 filament assembly and disassembly on ssDNA at the single-molecule level.