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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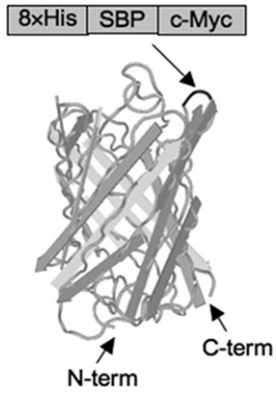
Protein complexes are functional units essential for virtually all cellular processes. To understand molecular mechanisms of the functions, it is necessary to identify and characterize the protein complexes involved. Protein tags are genetically encoded tags and useful tools for detection and isolation 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