X-ray Diffraction

2114-Pos Board B84
Integrative Multi-Resolution Modeling of Pleiomorphic Systems
Mirabela Rusu, Stefan Birmanns.
University of Texas Health Science Center at Houston, Houston, TX, USA.
Biomolecular processes such as transcription, protein folding or cellular motility, often involve organized interactions between multiple biomolecules. Solving the structure of those dynamic assemblies is challenging due to their flexibility, size and complexity. Yet, data collected from various biophysical sources can be integrated to assemble atomic models of such complexes. In particular, high-resolution atomic data, usually available only for component fragments, can be brought into registration with the low-resolution data of the entire system. Multi-resolution modeling techniques are nowadays routinely employed to interpret volumetric data from cryo-electron microscopy or tomography. If the experimental techniques capture dynamic systems in different conformations, flexible registration methods can be applied to refine the atomic models.
We have recently developed a flexible hybrid modeling technique based on feature-extraction methods from signal processing. The deviation of feature vectors is used to guide the molecular structure into the new conformation, whereby efficient spatial interpolation techniques are employed to derive the structure of the entire model.
The feature-point strategy delivers the robustness of the method under the influence of noise and artifacts, but also its efficiency and ease of use. Fast execution and results comparable to simulation techniques enable us now to utilize the spatial interpolation method also in iterative refinement procedures. This makes it feasible to adapt the method also to low-resolution data from tomography or even small angle x-ray scattering, and to integrate those data sources into the hybrid modeling process.
Acknowledgments
This work was supported by startup funds of the University of Texas Health Science Center at Houston, by NIH grant R01GM62968, by a grant from the Gillson Longenbaugh Foundation and by a training fellowship from the W.M. Keck Foundation to the Gulf Coast Consortia through the Keck Center for Interdisciplinary Bioscience Training.

2115-Pos Board B85
Solution Wide-angle X-ray Scattering (waxs) and its Application to Envelope Based Phasing
Xinguo Hong, Quan Hao.
MacCHESS, 200L Wilson Synchrotron Lab, Cornell University, Ithaca, NY, USA.
Solution X-ray scattering from biological macromolecules is an increasingly important technique that yields low-resolution structural information on the molecules, and can be used to monitor their shape and conformational changes (1) ,(2), (3). In contrast to numerous successful applications of small angle X-ray scattering (SAXS), e.g. ref. (4-6), practical use of wide-angle X-ray scattering (WAXS) data has been limited due to the weak scattering despite of the higher-resolution structural information. Recently, we have succeeded in measuring accurate WAXS data on protein solutions at a standard macromolecular diffraction station (7). SAXS data were collected at a dedicated SAXS station, and combined with WAXS data to give a full scattering curve out to 2.5 Å resolution. Both indirect and direct Fourier transforms of the full scattering pattern exhibit that some high resolution aspects of the structural hierarchy and function of a protein can be investigated in solution.
To elucidate the importance of solution WAXS data, we have employed the high resolution scattering curve to solve the phase problem, which remains central to crystallographic structure determination. A 6-dimensional search method of molecular replacement (FSEARCH) was used to locate a low-resolution molecular envelope within the crystallographic unit cell. The most probable model selected from a dozen bead models constructed at 5 Å resolution was employed to phase the single-crystal diffraction data. We find that inclusion of WAXS data is essential for correctly locating the molecular envelope in the crystal unit cell, as well as for locating heavy atom sites (8). The initial phases can be used as a starting point for a variety of phase-extension techniques; successful application of which will result in complete phasing of a crystallographic data set and determination of a macromolecule's internal structure to atomic resolution.

2116-Pos Board B86
X-ray Solution Scattering Combined with Computation Characterizing Protein Folds and Multiple Conformational States: Computation and Application
Sichun Yang[1], Lee Makowski[2], Benoit Roux[1,2].
[1]The University of Chicago, Chicago, IL, USA, [2]Argonne National Laboratory, Chicago, IL, USA.
Small angle X-ray scattering (SAXS) is an increasingly powerful technique to characterize the structure of biomolecules in solution. We present a computational method for accurately and efficiently computing the solution scattering curve from a protein with dynamical fluctuations. The method is built upon a coarse-grained (CG) representation of the protein. This CG approach takes advantage of the low-resolution character of solution scattering. It allows rapid determination of the scattering pattern from conformations extracted from CG simulations to obtain scattering characterization of the protein conformational landscapes. Important elements incorporated in the method include an effective residue-based structure factor for each amino acid, an explicit treatment of the hydration layer at the surface of the protein, and an ensemble average of scattering from all accessible conformations to account for macromolecular flexibility. The CG model is calibrated and illustrated to accurately reproduce the experimental scattering curve of Hen egg white lysozyme. We then illustrate the computational method by calculating the solution scattering pattern of several representative protein folds and multiple conformational states. The results suggest that solution scattering data, when combined with a reliable computational method, have great potential for a better structural description of multi-domain complexes in different functional states, and for recognizing structural folds when sequence similarity to a protein of known structure is low.
Possible applications of the method are discussed.

2117-Pos Board B87
Average Structure Of Cryo-cooled Profilin:actin From An Incommensurately Modulated Crystal
Jason Porta, Jeffrey Lovelace, Gloria Borgstahl.
University of Nebraska Medical Center, Omaha, NE, USA.
Knowledge of the structure of actin in its various conformations is essential for understanding the dynamics of cell motility. A key regulator of actin polymerization is profilin, an abundant protein that associates with actin in a 1:1 complex that serves as a precursor for actin filament formation. Several structures of actin, in the monomeric state and in complexes with actin binding proteins have been solved, but to date, a high-resolution structure of the actin filament has not been solved.
Slightly acidic pH is known to dissociate profilin from actin and to stabilize actin filaments. When profilin:actin crystals are transferred to an acidic pH, they exhibit a modulated diffraction pattern. It has been speculated that these modulated crystals contain actin filaments, or an intermediate state of actin filament formation.
This poster details our successes as we have pursued solving the structure of modulated profilin:actin crystals. This modulation was first observed in the early 1990's but was shelved as there was no way of processing the data at that time. With recent advances in the field of modulated crystallography for small molecules and vast improvements in computing power, we thought it was time to revisit this problem. We have worked through the problems associated with reproducing the original data and can now purify, crystallize and modulate the complex at will. We were able to get the unit cell and q-vector from room temperature modulated images. The q-vector suggests the modulation is incommensurate. Most recently we have been able to cryo-trap the modulated state and have a low-resolution average structure from that data.

Cryo Electron Microscopy & Reconstruction

2118-Pos Board B88
Microfluidic Devices for Time-Resolved Cryo-Electron Microscopy
Zonghuan Lu[1], David Barnard[2], Tanvir R. Shaikh[2], Hisham Mohamed[2], Xing Meng[2], Aymen Yassin[2], Rajendra Agrawal[2], Toh-Ming Lu[1], **Terence Wagenknecht**[2].
[1]Rensselaer Polytechnic Institute, Troy, NY, USA, [2]New York State Dept Health, Albany, NY, USA.
The goal of time-resolved cryo-EM (TRCEM) is to determine structural models for transient functional states of large macromolecules such as ribosomes and ion channels. A challenge of TRCEM is to rapidly mix reactants and then deposit them as a thin aqueous film (<200 nm) on an electron microscope grid in a short time (milliseconds) before quenching of the reaction by plunging into cryogen (e.g., liquid ethane). Previously, we demonstrated that spraying the mixture with an air atomizer produces a sufficiently thin aqueous film when deposited onto a suitably hydrophilic grid. To achieve efficient mixing, low sample consumption (several microliters/sec), and flow rates compatible with air atomization, we designed and fabricated planar passive mixers consisting of