

atomic and ultrafast time resolution combined are lacking, yet will become available. Femtosecond electron diffraction (FED) is a promising tabletop technique with complementary features to XFEL measurements [Miller, R.J.D, Science, 2014, 343, 1108-1116]. It combines the high spatial resolution of diffraction methods with the high temporal resolution of femtosecond optical spectroscopy. The current electron brightness and time-resolution enabled first studies of structural dynamics with atomic resolution on a time-scale of few hundred femtoseconds [Gao, M. et al., Nature, 2013, 496, 343-346] and even gave insight into the structural dynamics during a chemical reaction [Jean-Ruel, H. et al., J. Phys. Chem. A, 2011, 115, 13158-13168]. The main challenge for first applications of FED on proteins is sufficient sample preparation and development. Currently samples need to be single-crystalline, reversible and match a size of about 100-150 nm thickness combined with a lateral size on the order of 100 μm x 100 μm . The presented work will outline and discuss current approaches for sample development on model systems Bacteriorhodopsin and give an overview on the femtosecond electron diffraction method including the limitations for samples. Latest results on a crystalline organic spin-crossover system [Jiang, Y., Ultrafast Phenomena XIX, 2014, in press] as well as on the model system Bacteriorhodopsin are reported to demonstrate the intrinsic capabilities of FED.

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Development of Cryo-Electron Microscopy Sample Preparation for the Examination of Nanobubble

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Cryo-electron microscopy (cryo-EM) has been widely used to characterize bio-macromolecules, such as DNA molecules, proteoliposomes, and protein complexes. The targets of interests were effectively remained the original structure and morphology in amorphous ice. However, in cryo-EM, target of interests in gas phase has not been studied yet. In this study, we utilized cryo-EM to examine the nanobubbles in frozen aqueous solution with a "sandwich" TEM grids assembly. With significantly increased surface area and lower buoyancy, nanobubble system greatly enhances the efficiency of oxygenation and the time of retention in medical application. The substrate of "sandwich" assembly provides sufficient nucleation site for the formation of bubbles. Additionally, elimination of water-air interface increases the possibility to capture bubbles in nano scale. This method demonstrates a promising way to evaluate bubble system using cryo-EM and provides an insight on the study of nanobubble in biomedical application.

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Time-Resolved cryo-EM Study of Ribosome Subunit Association by Mixing-Spraying

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New York, NY, USA. Time-resolved cryogenic electron microscopy (cryo-EM) is a technique for visualizing transient structures in a biological specimen in a pre-equilibrium system. Capturing reactions in the sub-second range has been a practical challenge, due to the requirement of depositing the specimen rapidly on the grid without blotting. To capture faster reactions in the sub-second range, Lu and coworkers [1] developed a mixing-spraying method, which allows a reaction involving two macromolecular components to proceed for tens to hundreds of milliseconds. The first study of ribosome subunit association using time-resolved cryo-EM, by Shaikh et al. [2], were performed at 9.4 ms and 43 ms. In present work, we improved the mixing-spraying method, by designing an environmental chamber and optimizing the EM data yield, and applied the method to the study of ribosome subunit association. We captured the subunit association reaction in a pre-equilibrium state, by mixing the subunits and reacting for 60 ms and 140 ms. Our results showed that at 60 ms and 140 ms time points, 33% and 42% of the large subunits have associated into 70S ribosomes, respectively, compared with 85% in a 15-min incubation control experiment. Three distinct conformations of the 70S ribosome were found: non-rotated, non-rotated with 30S head swiveled and rotated. Our results demonstrate the capability of the mixing-spraying method of time-resolve cryo-EM to visualize multiple states of macromolecules in a reaction within a sub-second time frame. In the future, the mixing-spraying method will be applied to study translation initiation, to gain insights on the role of mRNA, initiator tRNA and initiation factors.

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[1] Lu, Z., et al. (2009). J. Struct. Biol. 168, 388-395.

[2] Shaikh, T.R., et al. (2014). Proc. Natl. Acad. Sci. USA 111, 9822-9827.

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A Computational Modeling of Macromolecular Ensemble Conformation and Blurring in Cryo EM

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It is well known that Cryo EM results in 3D construction in which subunits of a macromolecular complex may appear to be blurred and bloated. This issue affects the accuracy of positional and orientational information about the subunits extracted from such reconstructions. The blurring can be due to classification or averaging steps in the analysis or due to mobility of the quaternary macromolecular structure. Such effects cannot be captured by traditional models of EM. In this paper we propose a mathematical framework and algorithm to model this phenomenon. We use clustering methods such as the K-means algorithm to roughly resolve the different rigid subunits in the 3D density obtained from a macromolecular complex. Then we model the blurring effect in each (resolved) subunit as an ensemble of rigid body motions (i.e., orientations and translations), specifically we use the Gaussian probability density (with unknown covariance and mean) on the group of rigid body motions in 3D. We relate the shape of each resolved (blurred) subunit to the unknown parameters of the Gaussian using the known structure of the subunit (from PDB). This gives a system of equations with unknowns being the parameters of the Gaussian. The system, in general, is underdetermined, however, we impose physically meaningful regularization constraints to obtain unique solutions. Thereby we are able to obtain more accurate orientation and positional information. We show the performance of the algorithm on simulated data. We also discuss prospect of this method in combination (or fusion) with SAXS data in order to reinforce information from both modalities.

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A Computational Modeling of Macromolecular Assemblies in SAXS

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The determination of the structure of large macromolecular complexes is essential to reveal the function of the complexes in biological setting. For that purpose, several experimental methods for the structural determination have been developed and applied such as Electron Microscopy (EM) and Small-Angle X-ray Scattering (SAXS). In this study, we focus on SAXS. In particular, we are interested in determining the spatial relationship between rigid subunits in a given macromolecular complex, which is especially important because conformational dynamics of rigid components in the given complex is directly related to its function. The present work is to develop a more efficient and effective computational framework to reveal spatial relationship between complex subunits. One of important quantities in SAXS includes the so-called distance distribution function (or pair distribution function), which is the distribution of distance between every pair of points in the complex. Given rigid sub-components of the complex, this important quantity can be determined more efficiently when we apply the mathematical concept of the Fourier transform for rigid-body motion group. To this end, we develop a mathematical model to calculate the pair distribution function for a complex structure consisting of several rigid sub-components. This new model is verified with several examples. In the end, our modeling efforts will combine the current methodology with similar ones for other experimental methods (e.g. EM) to reveal more refined biological macromolecular structures.

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Sub-Surface Serial Block Face Scanning Electron Microscopy

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Serial block face scanning electron microscopy (SBF-SEM) provides nanoscale 3D ultrastructure of tissue samples up to several hundred micrometers in size. In SBF-SEM, an ultramicrotome built into the SEM specimen stage successively removes thin sections from a plastic-embedded, heavy metal-stained specimen. After each cut, the freshly exposed block face is imaged at a low incident electron energy using the backscattered electron signal, which is sensitive to heavy atoms in the sample. Although the x-y resolution in the plane of