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Electron Cryomicroscopy of Cellular Machines at Subnanometer Resolution

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Electron cryomicroscopy has become a standard structural tool for molecular and cellular structure research. It has produced 3-dimensional structure models of cellular machines at subnanometer resolutions (3-9 Å) [1]. A cellular machine is made up of multiple molecular components and can undergo conformational changes in the course of different physiological stages. At this range of resolution, long alpha helices and large beta sheets of protein components of cellular machines can be recognized. The experimental and computational process of this technique includes a series of steps as outlined in figure 1.

The first step of this process is to purify the samples to structural homogeneity. In general, this is challenging, because most of the biological assays are based on measurements of function. A functionally pure machine is not necessarily structurally pure. However, this is achievable for some cellular machines which have been studied structurally to this resolution. Examples are acetylcholine receptor, GroEL, acrosomal bundle, microtubule, bacterial flagella, ion channels and icosahedral viruses. The concentration of the samples needed for electron cryomicroscopy is between 0.1-1 mg/ml and each cryo-grid preparation requires 3-5 μl of samples. Holey grids without any support film are generally used to prepare such specimens. Commercially available Quantifoil grids with 1-2 μm hole sizes are commonly used. Glow discharge of the grid is done prior to its usage for rapid freezing. There are commercially available rapid freezing devices such as the Vitrobot, which has robotic control for reproducibly preparing a thin layer of ice to embed the cellular machines of interest. Slightly different freezing parameters may be necessary for each specimen to prepare them optimally in terms of ice thickness and sample distribution on each area of imaging.

The frozen, hydrated specimen is transferred to the cryo-specimen holder of the electron microscope. During the course of electron cryomicroscopy examination, the specimen is kept at or below liquid nitrogen temperature. All ice-embedded specimens are radiation-sensitive. Low dose (<20 electrons/$Å^2$) is used to record images from each specimen area. The data can be recorded on either photographic films or a CCD camera. Due to the recent improvement of performance of the 4k CCD camera, it has become feasible to record images at medium magnification (e.g. 60,000x) with information content better than 9 Å resolution [2]. The choice of the magnification depends on the anticipated resolution of the structure and also the pixel resolution of the detector.

After the image is recorded, there is a pre-screening step to assess the quality of the data. This is done visually and subsequently by computing the Fourier transform intensity of the image frame. The power spectrum of the averaged particle images reveals the contrast transfer function, from which one can derive the potential information content in that particular image frame. The particles are subjected to a series of data processing steps

which includes particle boxing, contrast transfer function correction, particle orientation estimate and refinement and 3-D reconstruction. A number of software packages are available to perform this task, for instance EMAN, which is freely available (http://ncmi.bcm.tmc.edu/~sludtke). This software has been applied in structural determination of cellular machines. The GroEL is an example which structure has been determined to 6 Å resolution without requiring a crystal [3].

Due to the complexity of the multiple components in a cellular machine, dissection of the structural features in the 3-D map of a cellular complex can be quite challenging. Both visualization and structure mining tools are critical to comprehend the vast amount of volume data. *Helixhunter* and *foldhunter* are feature extraction programs [4] that allow one to identify long alpha helices automatically with a scoring function and also the locations of domain folds based on the separately determined crystal structure of a component or a homolog of such a component.

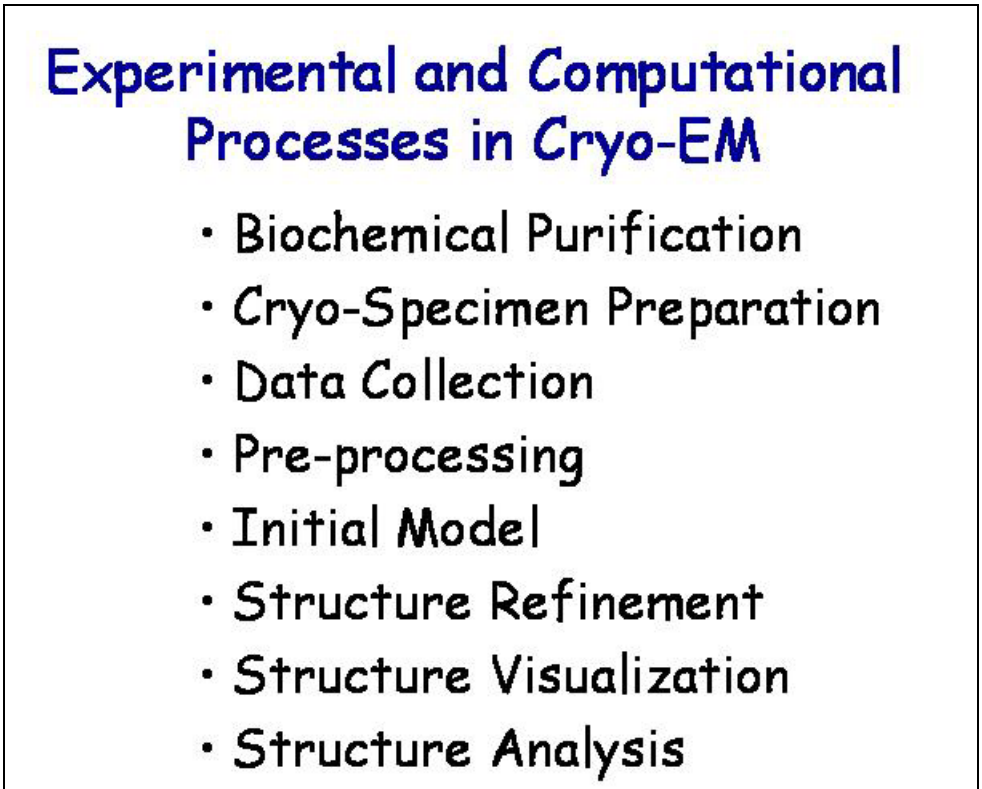

Figure 1. Flow diagram of the steps involved in solving 3-D structure of a biological machine.

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