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Nanometer-scale Electron Microscopy of proteins in liquid

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While cryo-electron microscopy is predominantly used to study structure of proteins and other biological materials it lacks the ability to capture phenomenon under physiologically relevant conditions. Light microscopy, on the other hand is commonly used tool for live imaging but it is diffraction limited with resolution of about 50 nm. Ability to image proteins with nanometer resolution in aqueous state and at room temperature is an overarching goal of biological imaging. Towards this goal we have developed a technique that enabled us to image thin solution layer of actin bundles by encapsulating them between electron transparent windows with 2 nm. The solution of acrosomal bundle is loaded into a liquid chamber with a 300 nm gap between two identical 10 nm thick electron transparent Si_3N_4 windows and inserted inside the column of 120 keV TEM as shown in Figure 1 [1].

The resolution limit both for 100 keV and for 400 keV cryo-electron microscopy for acrosmal bundles have been previously established to be around 2.7 and 0.7 nm [2, 3]. In similar manner from numerous electron images we estimated our resolution to be ~2.7 nm for aqueous protein sandwiched between two 10 nm thick Si_3N_4 windows. Reducing the window thickness will further improve the resolution and contrast of the images. Radiation damage caused by high energy electrons is a drawback of any electron microscopy technique and has been a concern for many years. Radiation damage to a structure of a sample exhibits itself by alteration or destruction of protein structure. We have previously reported that tolerable dose, $D_{1/e}$, the dose at which amplitudes of Fourier peak in periodic structure drops by a factor of e, for acrosomal bundle to be around 25 eV/A^2 for 400keV at cryogenic temperatures[4]. We find the tolerable dose to be $D_{1/e}\ (293K) = 35\ e/A^2$ for the case of proteins in aqueous environment at room temperature. This seemingly enhanced resistance to radiation damage contradicts previous reports on the effect of temperature on radiation damage being at least 10 times worse at room temperature compared to cryogenic conditions[5]. But nevertheless our results on their own merit suggest that aqueous imaging of proteins is possible without any adverse effect of temperature. In conclusion our study shows that that aqueous TEM can be used to study proteins with nanometer resolution in situ. We also find that the radiation damage is not any more severe at room temperature that at cryogenic temperatures.

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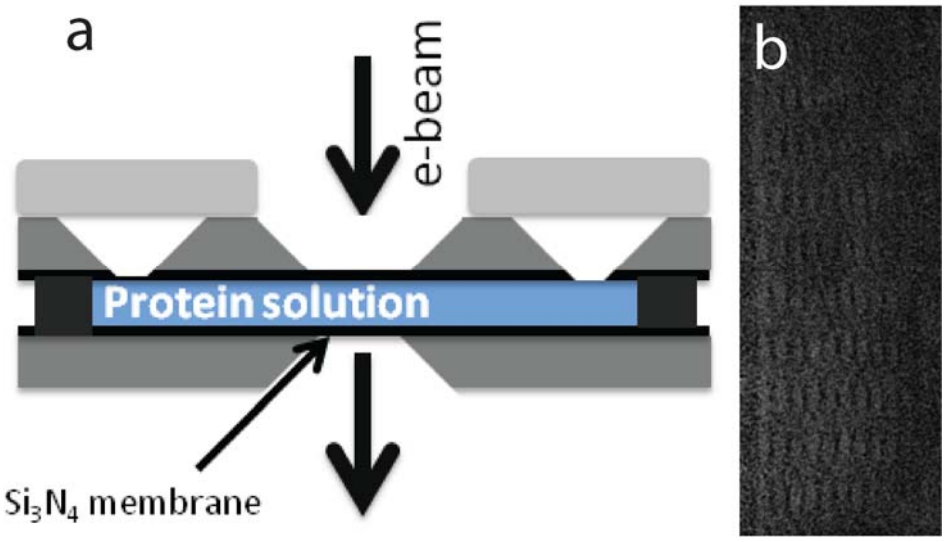

FIG. 1. Imaging proteins in liquid cell. a) Protein solution is loaded into a liquid cell through two large reservoirs and then sealed off by gasket. (b) Image of acrosomal bundle structure in liquid at room temperature obtained at 30000X magnification.

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