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Electron Diffraction of Graphene-covered Catalase Crystals

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The state-of-the-art method to investigate protein structures in a near-native state with electrons is to freeze them rapidly in a layer of amorphous ice and conduct imaging/diffraction at cryogenic temperatures, which is termed cryo-electron microscopy (cryo-EM). There are two major limitations to the cryo-EM. 1) High energy electrons (200-300 keV) cause radiation damage in proteins that limits the maximal dose that can be used for a single protein or protein crystal. 2) A frozen medium at cryogenic temperatures is far from ideal for dynamical observations [1-3].

In our earlier study, we used the so-called graphene liquid cell (GLC) method to encapsulate microtubule proteins between graphene sheets and investigated the radiation damage caused by the electron beam in the protein structure [4]. We benchmarked the GLC sample with a cryo-frozen sample of microtubules and found nearly one order of magnitude higher maximal electron dose before significant damage occurs (D_{max}) with graphene at room temperature compared to a cryo-frozen sample at a resolution of 5 nm, which corresponds to the spacing between the protofilaments of the native microtubules. Besides the damage mitigation effect of graphene on proteins, this method enables studying protein structure at biologically relevant temperatures.

Here, we used a similar GLC approach for encapsulating catalase crystals. The sample was deposited on an ultra-thin amorphous carbon-coated grid, and covered with a graphene-coated grid (Figure 1a). Both grids contained a lacey carbon film supporting either a thin carbon film or a graphene sheet. Electron diffraction was used to investigate radiation damage at different spatial frequencies. Figure 1b shows a transmission electron microscopy (TEM) image of a catalase crystal between graphene and carbon film. Figure 1c-f show a series of electron diffraction images obtained from the same region of one of the crystals. Figure 1g shows the normalized intensities (I/I_0) of the diffraction spot observed at 0.34 nm^{-1} (3 nm, pointed by an arrow in Figure 1c) as a function of cumulative electron dose with an exponential decay fit. We determined the D_{max} as the cumulative dose at which the intensity of the peak in the first image (I_0) dropped by a factor of e = 2.718 [5], and found D_{max} = 137 ± 14 $e^{-}Å^{-2}$ with 10% estimated error. The intensities of the spots at 0.2 nm^{-1} or lower resolution did not exhibit a significant intensity change even after 450 $e^{-}Å^{-2}$ (Figure 1f). Radiation damage is thus a process that strongly depends on the involved spatial distances. Investigating the D_{max} at different resolutions and electron fluxes, and benchmarking the results with cryo-EM is in progress.

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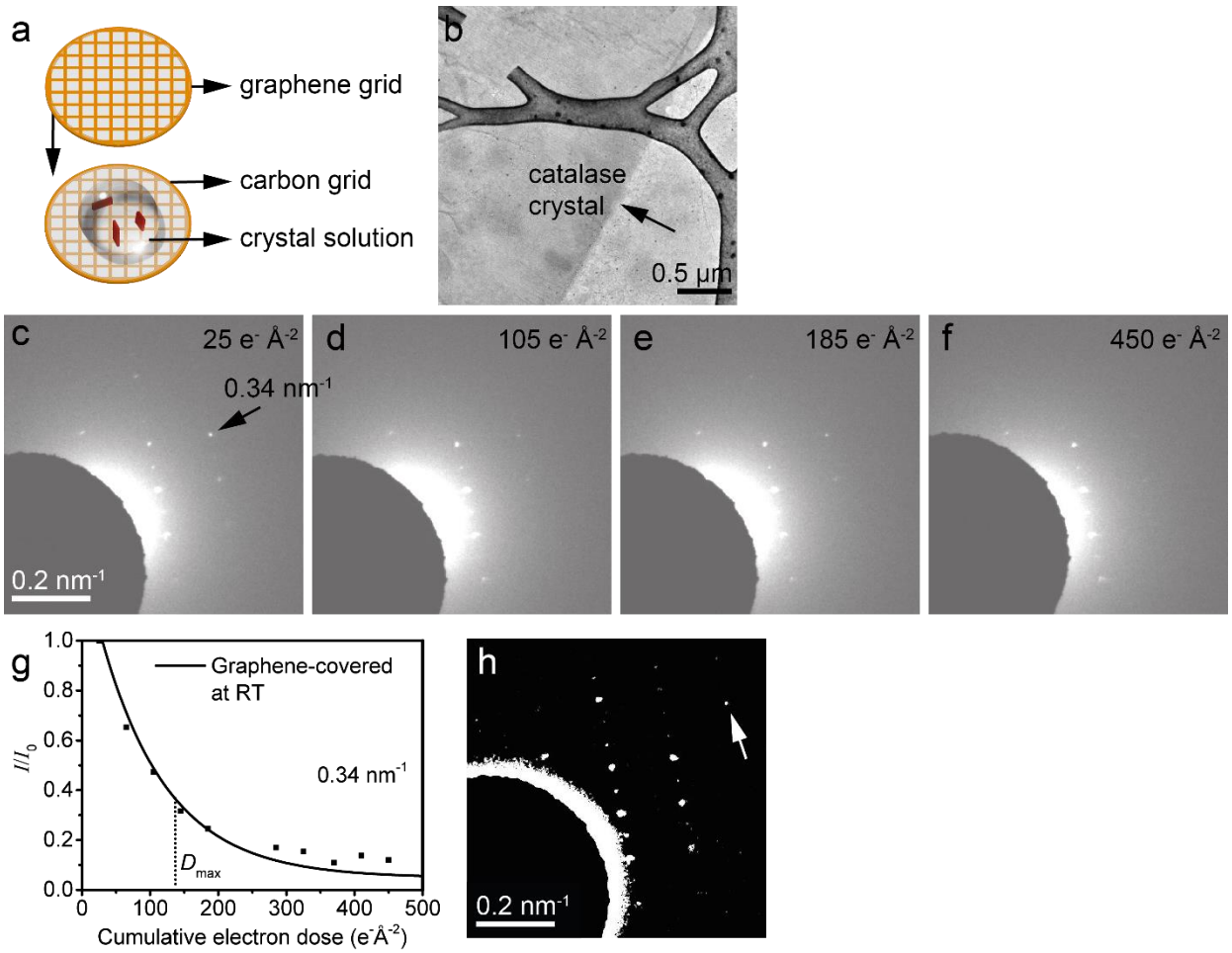

Figure 1. Electron diffraction of graphene-covered catalase crystals at room temperature. a) Schematic representation of the sample preparation method. b) Transmission electron microscopy (TEM) image of a catalase crystal (pointed by arrow) on a carbon grid and covered by graphene. c-f) Electron diffraction images, successively obtained from the same crystal region at increasing cumulative electron doses. The cumulative electron dose is indicated on each image. The scale bar in (c) is 0.2 nm^{-1} and the same for (c-f). Electron flux = 5 $e^{-}Å^{-2}s^{-1}$, exposure time = 5 s per image and accelerating voltage = 200 kV. g) Normalized intensities (I/I_0) of the diffraction spot at 0.34 nm^{-1} as a function of cumulative electron dose with an exponential decay fit. I_0 is the intensity of the peak in the first image of the series. Maximal electron dose before significant damage occurs, D_{max} = 137 ± 14 $e^{-}Å^{-2}$. The diffraction image in (c) after background subtraction to increase the visibility of the peaks. The arrow is pointing to the highest resolution peak obtained at 0.56 nm^{-1} (1.8 nm).

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