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# Molecular Structure Determination Extrapolated to Zero Dose with an Electron Cryomicroscope

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Most high-resolution information loss in cryomicrographs stems from (1) radiation damage, and (2) particle movement in the vitrified specimen during imaging [1]. Recently, we elucidated the physical origins of cryoEM specimen movement and demonstrated a simple strategy for eliminating it [2]. Movement-free imaging not only increases the information content of cryoEM data, but also allows for a new approach to 3D reconstruction from averaged 2D images: zero-dose extrapolation, similar to a previously proposed approach in X-ray crystallography [3]. CryoEM data is typically acquired as a frame series (movies) using fast and efficient direct electron detectors. Currently, cryoEM data processing algorithms calculate a damage-weighted reconstruction by summing the information in each movie frame, down-weighted to account for the amount of movement and damage in that frame [4]. This inevitably results in the incorporation of radiation damage artefacts into the resulting atomic structure. Given the availability of movement-free data, we can now take a different approach. We propose a physical model for how the molecular structure factor S at a Fourier frequency k changes with fluence f due to radiation damage alone during imaging with electrons (Fig. 1). Using this model, we have created a program, zeroDose, that fits the observed structure factors $S_k(f)$ from the time / dose dependent reconstructions. The fits are then used to extrapolate back to the 3D structure of the molecule at zero dose ($S_k(f = 0)$ at every Fourier voxel $k$), before the onset of radiation damage (Fig. 2). In addition, the site-specific radiation sensitivity of the reconstructed molecule can be quantified using the calculated rate of change of every structure factor with fluence. Zero dose extrapolation has been used to determine the structures of several protein complexes: (1) the DPS protein from *E. coli* [2], (2) the light-harvesting 2 antenna complex of the purple bacterium *Mch. purpuratum* [5], and (3) the double-ring photosystem of *G. phototrophica* [6]. Each example, as well as the progressive effects of radiation damage to the molecules, will be shown. The concept of zero-dose extrapolation can be extended to any setting where multiple observations of a structure or measurements of dose dependent phenomena where the dose dependence can be measured with sufficient accuracy to make extrapolations with predictive power.

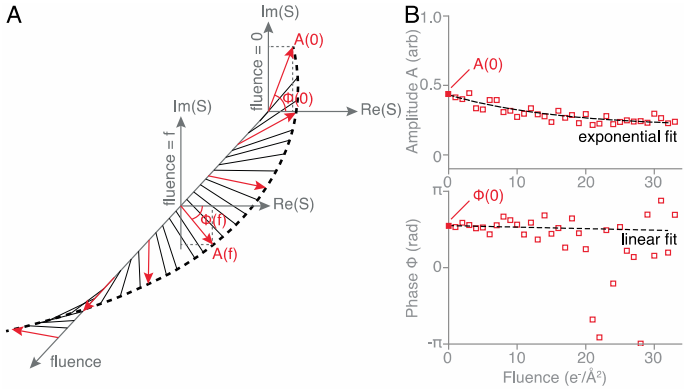


**Figure 1.** (A) Each structure factor (amplitude and phase) of a radiation-sensitive biological molecule changes during irradiation as a function of the total fluence *f*. With a sufficient number of observations, a

physical model describing this radiation damage phenomenon can be fit to the observed values. (B) Example dependence of the amplitude and phase of one structure factor (taken from the 2.3 Å resolution shell) of the DPS molecule. The observed structure factors are marked with empty red squares, and the theoretical fits are indicated by the black dashed lines. The filled red squares show the extrapolated values at zero dose.

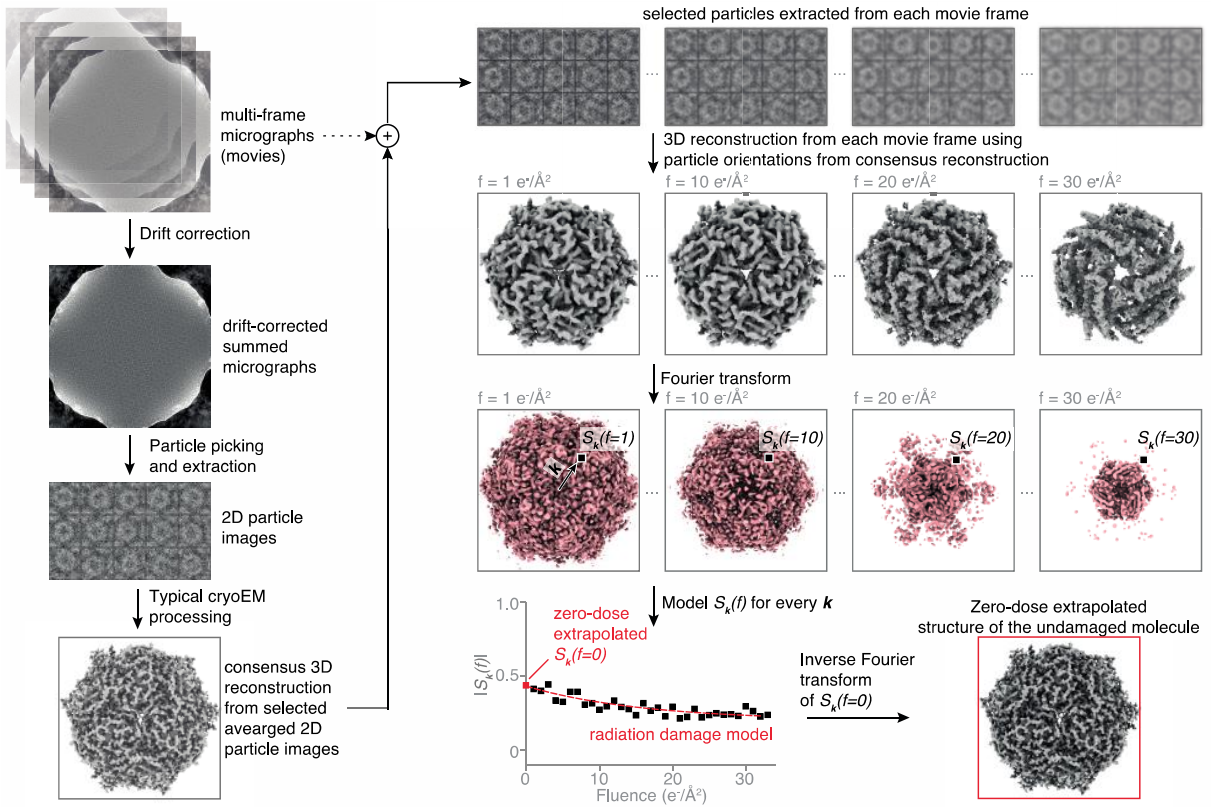


**Figure 2.** Overview of cryoEM data processing with zero dose extrapolation.

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