Paths to Attenuate Radiolysis-Induced Secondary Damage in Biological CryoEM

Matthew Mecklenburg, Shervin S Nia, Ambarneil Saha, Z Hong Zhou

Microscopy and Microanalysis, **30** (Suppl 1), 2024, 1772–1774
https://doi.org/10.1093/mam/ozae044.877

Microscopy AND Microanalysis

Meeting-report

Paths to Attenuate Radiolysis-Induced Secondary Damage in Biological CryoEM

Matthew Mecklenburg[1,*], Shervin S. Nia[1,2,3], Ambarneil Saha[4], and Z. Hong Zhou[1,2,3]

[1]*California NanoSystems Institute (CNSI), University of California, Los Angeles, California, United States*
[2]*Department of Chemistry & Biochemistry, University of California, Los Angeles, Los Angeles, California, United States*
[3]*Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA, United States*
[4]*National Center for Electron Microscopy, Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California, United States*

*Corresponding author: mmecklenburg@cnsi.ucla.edu

The imaging electrons in a transmission electron microscope are a beam of bond-breaking beta-radiation. Imaging soft materials is a challenge because of beam damage and poor contrast between the substrate and specimen. Typically, only 10-100 electrons can strike an atomically sized area before disintegration [1]. Peptide, hydrogen, disulfide bonds etc. are all irreparably broken by radiolysis while being simultaneously imaged. The total dose for loss of useful information is roughly the Henderson limit (often accumulated in a few seconds, see Figure 1), about 20 MGy [2], which is roughly 9 orders of magnitude larger than an average person receives per year [3]. This primary damage cannot be abated in small molecules, protein, or virus samples. In addition, unlike the case for x-rays, the inelastic scattering is more likely than elastic scattering for elements in atomic number less than about iron [4]. This leads to poor contrast using parallel beam imaging modalities.

However, not all received damage is from the primary interaction. The incident electron's schism of binding electrons creates both secondary electrons and heavy radicals that diffuse away from the point of impact [5]. This charged particle propagation further damages these soft structures. The palliative treatment is cryogenic cooling to near liquid nitrogen temperatures. This inhibits secondary damage structure deterioration by factors of 3-100 [6]. Though, thermal diffusion kinetics should be slowed by many orders of magnitude more than is generally observed. Thus, damage cannot only be a thermal diffusion effect; some other influence is at work. Experimental evidence shows there to be little additional benefit at liquid helium temperatures [7, 8].

Our goals are to improve contrast and to acquire as much information as possible before the structure is destroyed. What other methods can be used to decrease the secondary damage [9]? Chemical composition has been shown to play a role in the propagation of radicals [10]. Radical scavengers can be used as sacrificial vanguards to halt their approach to the sample. This has been shown to be effective in x-ray crystallography by extending the intensity of diffraction peaks by 2-9 times depending on the scavenger and crystal [10]. The mass of radicals can also be changed. Hydrogen radicals are hypothesized to be the most damaging [11]. We can alter the mass by substituting hydrogen for deuterium in two places: in the protein, and in the vitreous ice locking the protein in a hydrated state. If we can inhibit the propagation of hydrogen radicals, how much does this improve the fluence we can expose our biological structures to before reaching a critical dose (see Figure 2)?

We will discuss the results of experiments imaging apoferritin in both hydrogen and deuterium radical cases (Figure 3). We will also discuss what additional experiments can be done to inhibit beam damage by combining isotopic changes with radical scavengers, along with a discussion of operating at different temperatures and accelerating voltages. The results of ongoing EELS and 4DSTEM experiments in radiolysis damage will also be presented. In addition, the role of conduction will be discussed, as will new metrics for resolution that are independent of particle number, and resolution metrics that take into account the fluence required for each particular imaging modality [12].

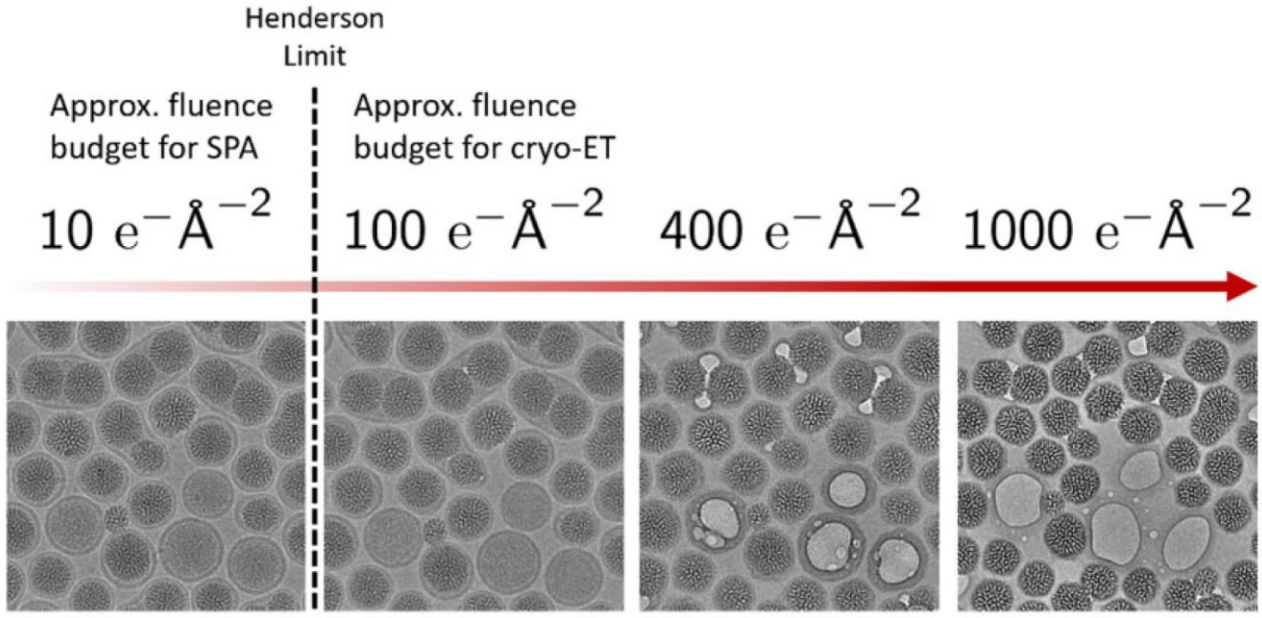

Fig. 1. A limited sampling of a dose series. The atomic-level structural information is lost at roughly 10 $e^-/Å^2$.

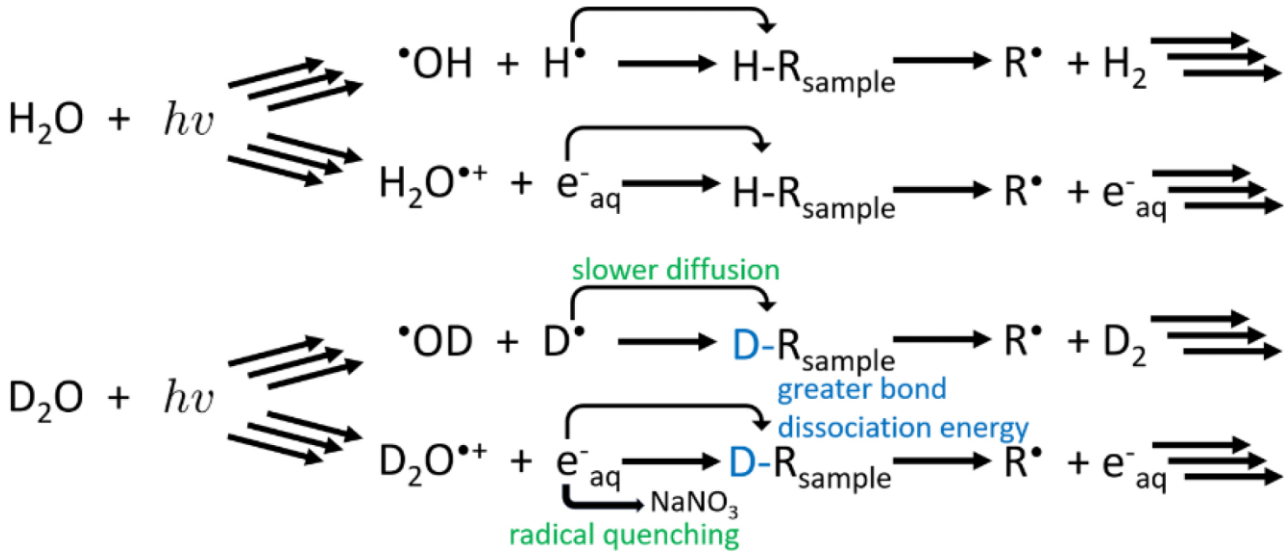

Fig. 2. Hypothesized reaction pathways of radicals and secondary electrons (top) and proposed pathways with solutions implemented (bottom).

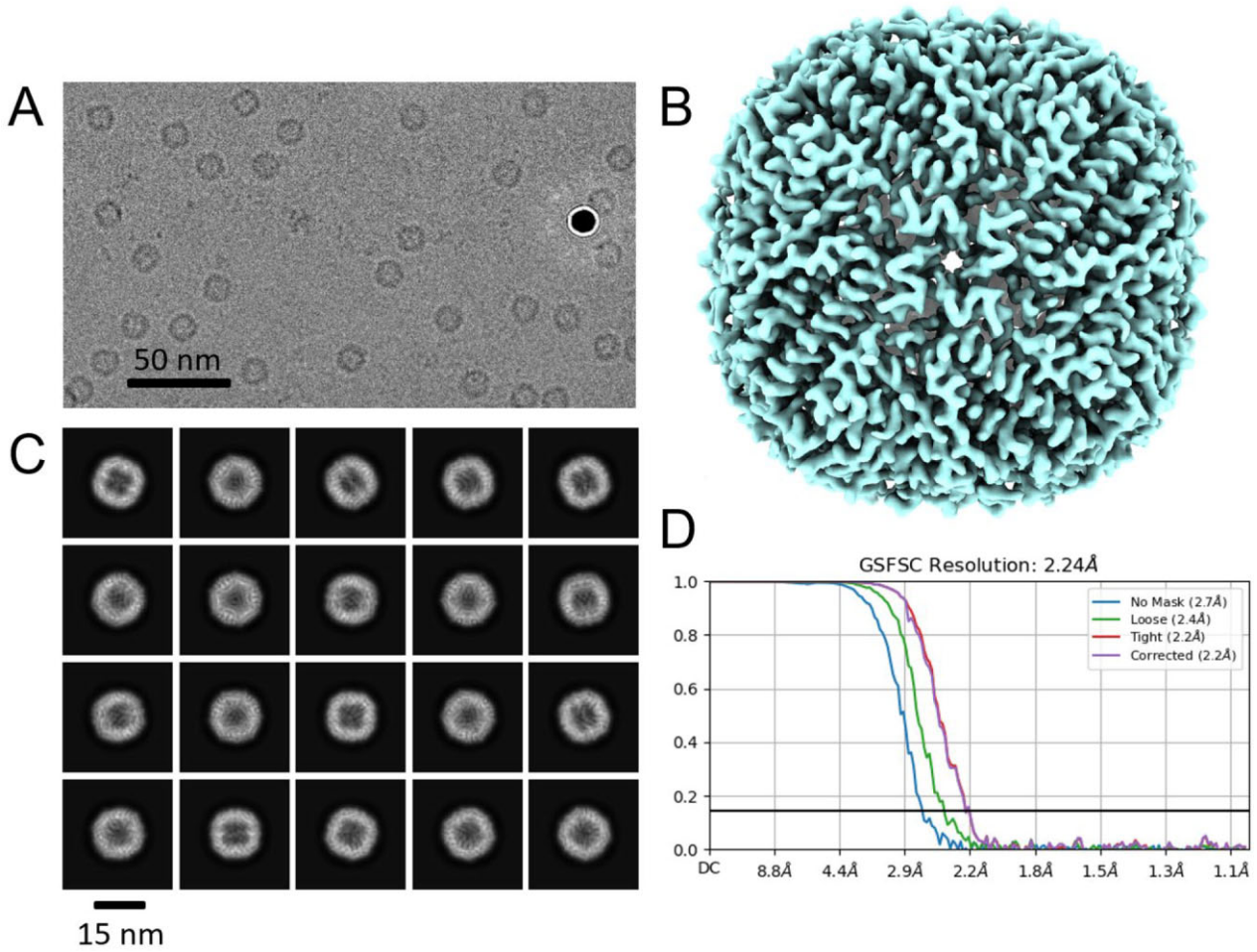

Fig. 3. (A) CTF-corrected and dose-weighted cryoEM micrograph of apoferritin in D_2O. (B) Image acquired on a TEM with a counting camera and energy filter at 300 kV. cryoEM structure of apoferritin dialyzed into D_2O. (C) Selected 2D class averages of ~150,000 particles of apoferritin. (D) Gold-standard Fourier shell correlation of apoferritin maps performed in cryoSPARC.

References

1. R F Egerton *et al.*, *Micron* **35** (2004), p. 399.
2. R Henderson, *Proceedings of the Royal Society B* **241** (1990), p. 6.
3. W Rühm *et al.*, *J Radiat Res.* **59** (2018), p. ii1.
4. L Reimer and H Kohl in "Transmission Electron Microscopy", 5 ed., (Springer, Berlin, Germany) p. 163.
5. R M Glaeser, *Method Enzymol.* **579** (2016), p. 19.
6. R F Egerton, *Micron* **119** (2019), p. 72.
7. K Naydenova *et al.*, *Ultramicroscopy* **237** (2022), p. 113512
8. C V Iancu *et al.*, *J Struct Biol.* **153** (2006) p. 231.
9. J R Fryer, F Holland, *Ultramicroscopy* **11** (1983), p. 67.
10. A I Barker *et al.*, *J Synchrotron Radiat.* **16** (2009), p. 205.
11. A Meents *et al.*, *P Natl Acad Sci* **107** (2009), 1094.
12. The authors acknowledge support from the Electron Imaging Center for Nanomachines (EICN) at the University of California, Los Angeles's California for NanoSystems Institute (CNSI) (RRID:SCR_022900). This work was supported by the BioPACIFIC Materials Innovation Platform of the National Science Foundation under Award No. DMR-1933487. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM145388. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.