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# Comparing Structural and Functional Changes of Biomolecules under Electron Irradiation with Liquid Cell Transmission Electron Microscopy

Trevor Moser[1], James Evans[1,2*]

[1.] Environmental Molecular Sciences Laboratory, Richland, WA, USA.
[2.] School of Biological Sciences, Washington State University, Pullman, WA, USA.
[*] Corresponding author contact: James.Evans@PNNL.Gov

Characterization of interactions between the electron probe and a liquid sample is a critical component in realizing liquid cell transmission electron microscopy (LC-TEM) as a technique for probing biological dynamics and structures at high resolutions in native hydrated environments. Damage resulting from radiolysis of the sample, or from free radicals produced from radiolysis of the aqueous solvent can both result in an alteration of structure and/or functional activity of a biological system. Previous studies on whole organisms within a liquid cell have shown morphological changes and cell shrinking during electron irradiation which may be driven by damage from the electron probe [1]. Further characterization of functional and structural changes in biomolecules as a function of electron dose is needed in order to separate out beam driven artifacts from real biological phenomena

We have previously reported on the electron dose dependent functional activity threshold for fluorescently tagged phospholipid bilayers within an LC-TEM system [2]. By combining fluorescent optical microscopy with LC-TEM of fluorescently tagged lipid bilayers on silicon nitride membranes we have demonstrated that functional activity is altered with electron fluxes as low as 0.0001 e/ $Å^2$, or a dose of ~4 X $10^2$ Gy. These electron fluxes are considerably lower than those currently used standardly in the cryo-electron microscopy (cryo-EM) field for solving high resolution structures of proteins.

While LC-TEM shows significantly lower tolerance to dose for functional stability, there are still open questions as to the impact of electron exposure on protein structure in a liquid environment. Here we investigate dose effects on protein structure in LC-TEM by creating liquid cells which are amenable to both LC-TEM and cryo-EM, so that structures can be solved for equivalent samples at cryogenic temperatures and room temperature under the same imaging conditions on the same instrument. This combination finally allows direct comparison of the approaches where the only variable is physical media state (liquid versus vitrified ice). We offer possible explanations for the variations in structural resolution achieved including temporal as well as damage-based artifacts [3].

References:

[1] TH Moser, T Shokuhfar and JE Evans, Micron **117** (2019), p. 8.
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