MS A031. Advances in Electron Crystallography Methods for Solving Crystal Structures

Investigation of the milling characteristics of different focussed ion beam sources assessed by three-dimensional electron diffraction from crystal lamellae

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Three-dimensional electron diffraction (3D ED) from nanocrystals of biological macromolecules has become an established technique in recent years (Nannenga *et al.*, 2014; Beale *et al.*, 2020). The method requires the use of very small crystals, typically less than 300 nm thick in the direction of the electron beam, due to the strong interaction between electrons and matter (Henderson, 1995). In recent years, focussed ion beam (FIB) milling has been used in the preparation of thin samples for 3D ED. These instruments typically use a gallium liquid metal ion source (Duyvesteyen *et al.*, 2018; Beale *et al.*, 2020). Inductively coupled plasma (ICP) sources in principle offer faster milling rates and lower damage (Burnett *et al.*, 2016). Little work has been done to quantify the damage these sources do to delicate biological samples at cryogenic temperatures. Here, we present an analysis of the effect that milling with plasma FIB (pFIB) instrument has upon lysozyme crystals. We evaluated both argon and xenon plasmas and compared them with crystals milled with a gallium source.

In our analysis, we used 3D ED data quality from lamellae of crystalline lysozyme as a proxy. The introduction of lattice disorder due to damage will result in a loss of information. Diffraction from non-milled wedge-shaped lysozyme crystals of thickness between 100 – 600 nm has previously been reported (Nannenga *et al.*, 2014) and a study of gallium milled lamellae of various thicknesses showed no decrease in diffraction quality down to a targeted thickness of 95 nm at an electron energy of 200 keV (Martynowycz *et al.*, 2021). We fabricated wedge lamellae with a shallow thickness gradient along their length which tapered towards zero at the thin end of each lamella. This allowed data to be collected from very thin crystalline samples. We used an ICP source using argon or xenon and a gallium liquid metal ion source. We acquired 3D ED data and used standard data processing statistics to assess the quality of the diffraction data. We collected a large number of ED datasets from samples prepared using each milling source and include measurements of the thicknesses of the lamellae which are critical in systematically comparing the quality of the diffraction data. We use this information to infer the limits of the depth of the milling damage layer that results from pFIB. We report an upper bound to the depth of the pFIB milling damage layer of between 42.5 nm and 50 nm, corresponding to half the thickness of the thinnest lamellae that resulted in usable diffraction data. We also report a lower bound of between 32.5 nm and 40 nm, based on a literature survey of the minimum amount of diffracting material required for 3D ED.

[1] Nannenga, B. L., Shi, D., Leslie, A. G. W., & Gonen, T. (2014). High-resolution structure determination by continuous-rotation data collection in MicroED. Nature Methods, 11(9), 927–930.
[3] Beale, E. V, Waterman, D. G., Hecksel, C., van Rooyen, J., Gilchrist, J. B., Parkhurst, J. M., de Haas, F., Buijsse, B., Evans, G., & Zhang, P. (2020). A Workflow for Protein Structure Determination from Thin Crystal Lamella by Micro-Electron Diffraction. Frontiers in Molecular Biosciences, 7(August), 1–9.
[4] Henderson, R. (1995). The potential and limitations of neutrons, electrons, and X-rays for atomic resolution microscopy of unstained biological molecules. Quarterly Reviews of Biophysics, 28(2), 171–193.
[5] Duyvesteyn, H. M. E. E., Kotecha, A., Ginn, H. M., Hecksel, C. W., Beale, E. V., De Haas, F., Evans, G., Zhang, P., Chiu, W., & Stuart, D. I. (2018). Machining protein microcrystals for structure determination by electron diffraction. Proceedings of the National Academy of Sciences of the United States of America, 115(38), 9569–9573.
[6] Burnett, T. L., Kelley, R., Winiarski, B., Contreras, L., Daly, M., Gholinia, A., Burke, M. G., & Withers, P. J. (2016). Large volume serial section tomography by Xe Plasma FIB dual beam microscopy. Ultramicroscopy, 161, 119–129.
[7] Martynowycz, M. W., Clabbers, M. T. B., Unge, J., Hattne, J., & Gonen, T. (2021). Benchmarking the ideal sample thickness in cryo-EM. Proceedings of the National Academy of Sciences of the United States of America, 118(49), 1–7.