Hinting at Hydrogens in JEOL CRYO ARM™ Data

High resolution structure determination by electron cryo-microscopy (cryoEM) and Single Particle Analysis (SPA) has progressed to the point where structures can routinely be determined to b e better than 2Å resolution using either a 200 or a 300 kV microscope. At 1.8Å resolution, details like amino acid isoforms can be distinguished[1]. This application note highlights improved results that were obtained on apoferritin at 1.34Å resolution that hint at new features.

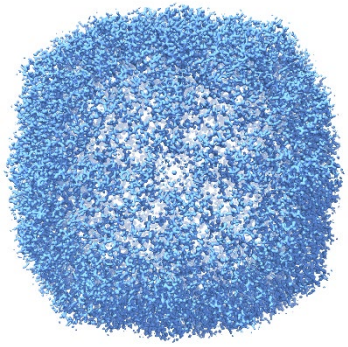


Fig. 1: 1.34Å resolution 3D map of apo-ferritin[3].

Images from frozen-hydrated apoferritin were originally obtained by Kato *et al*. on the JEOL CRYO ARM™ 300 installed at SPring8 (Riken, Japan) and yielded a map at 1.54Å resolution[2]. This data is available under accession code EMPIAR-10204. The images were re-processed using *M* and yielded a map at 1.34Å resolution (Fig. 1)[3]. Figure 2 shows portion of that map along the 4-fold symmetry axis. This map clearly reflects the high quality of the reconstruction. Holes are clearly visible in all of the aromatic residues, e.g. Tyr^{40} or Phe^{51}, but also in the pyrrolidine ring of prolines, e.g. Pro^{127} (Fig. 3). The aromatic residues all show bumps tantalizingly suggesting hydrogens.

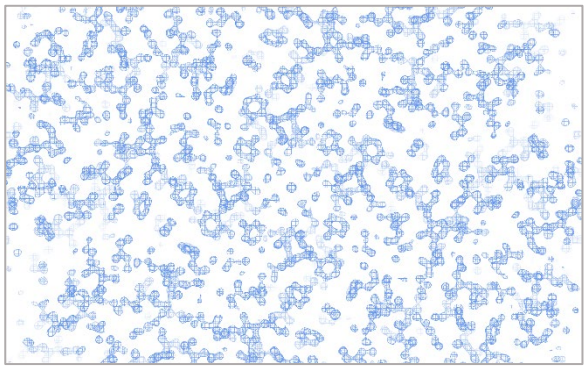
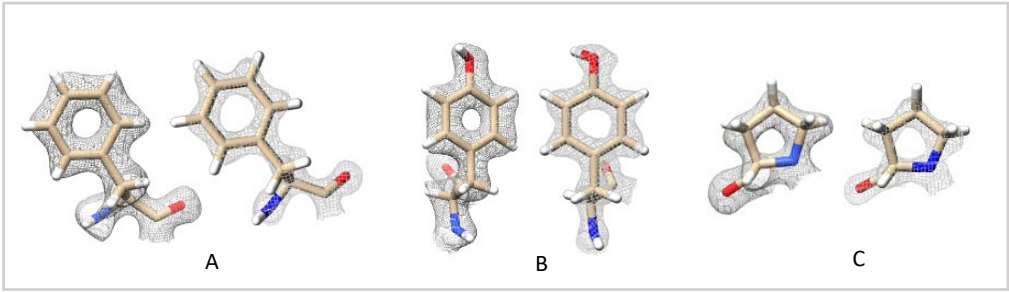


Fig. 2: Portion of the 1.34Å map at the 4-fold symmetry axis.

The JEOL CRYO ARM™ 300 equipped with a cold field emission gun and a direct detector enables the determination of biological macromolecular structures to well below 1.5Å resolution, where details like isoforms are clearly established but also with hints at the presence of hydrogens.

Fig. 3: Selected residues from both maps to demonstrate the improvement in map quality for Phe^{51} (A), Tyr^{40} (B) and Pro^{127} (C) at 1.54 Å (left) and 1.34Å resolution (right).



References:

1. Merk, A., Fukumura, T., Zhu, X., Darling, J.E., Grisshamer, R., Ognejenović and Subramaniam, S. (2020), IUCrJ 7, 639-643.
2. Kato, T., Makino, F., Nakane, T., Terahara, N., Kaneko, T., Shimizu, Y., Motoki, S., Ishikawa, I., Yonekura, K. & Namba, K. (2019). Microsc. Microanal. 25, 998–999. https://doi.org/10.1017/S1431927619005725.
3. Tegunov, D., Xue, L., Dienemann, C., Cramer, P. and Mahamid, J., (2021) Nature Methods 18, 186-193.