

# Atomic resolution structure results from the JEOL 300 kV CRYO ARM™ TEM

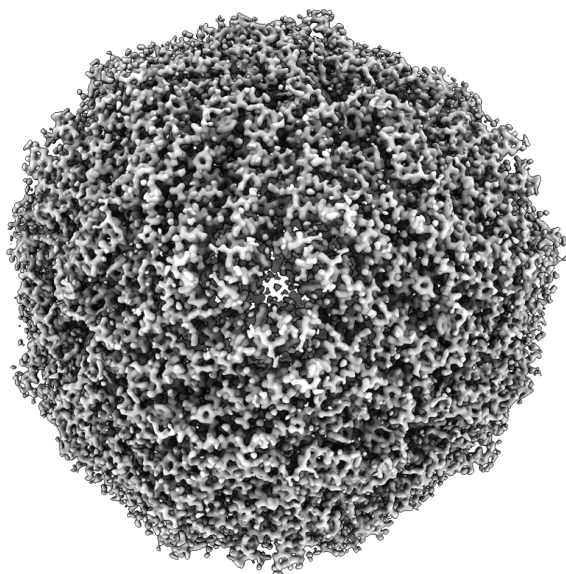


Figure 1.

High resolution structure determination by electron cryo-microscopy (cryoEM) and Single Particle Analysis (SPA) has progressed to the point where structures can be determined routinely to better than 2Å on a 300 kV microscope. Here, we show results from Kato et al. at<sup>1</sup> Osaka University from the JEOL CRYO ARM™ 300 installed at SPring8 (Riken, Japan), that was obtained on mouse heavy chain apo-ferritin at 1.5Å resolution. The 3D map shows surprising details in the map reflecting the high resolution quality of the data.

Images of mouse heavy-chain apo-ferritin<sup>2</sup> were obtained in a multi-day session on the CRYO ARM™ 300 equipped with a Gatan K2 direct electron detector. Control of the camera and microscope was accomplished using JADAS. The Cold Field Emission Gun (CFEG) was flashed automatically every 4 hours and the in-column Omega energy filter was used in zero-loss imaging mode with a 20 eV energy selecting slit width. A total of ~974 movies were collected and processed using Relion-3.1. The final map (Fig. 1) was obtained using only 840 movies with ~120k particles of apo-ferritin at a resolution of 1.54Å using the 0.143 FSC criterion (Fig. 2).

The 3D map shows very well resolved densities for all of the amino acid residues (Fig. 3). As expected, holes are clearly present in the side chain of various aromatic amino acid residues, such as Phe<sup>51</sup>, Trp<sup>93</sup> and Tyr<sup>40</sup>, but also in the pyrrolidine ring of Pro<sup>127</sup>. The map shows clearly defined densities for the carboxyl groups for the acidic residues like Asp<sup>15</sup> and to a somewhat lesser extent for Glu<sup>101</sup>, confirming the high quality of the 3D map that in general come of the CRYO ARMs. Rotamer assignment appears to be possible inspecting Asn<sup>21</sup> similarly to the study of Merk et al. with 1.8-Å data obtained from beta-galactosidase on the JEOL CRYO ARM™ 200<sup>3</sup>.

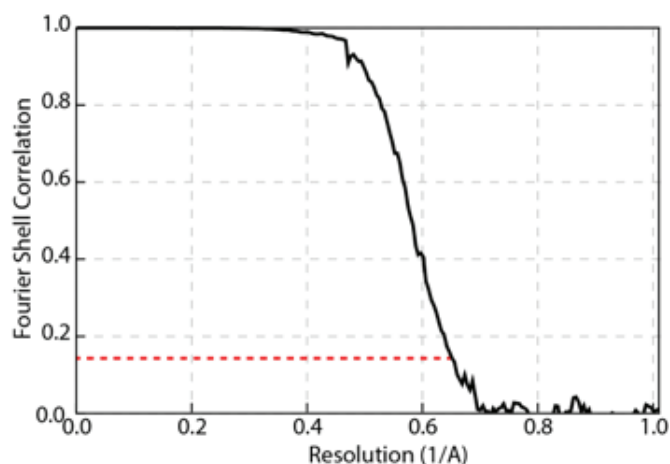


Figure 2.

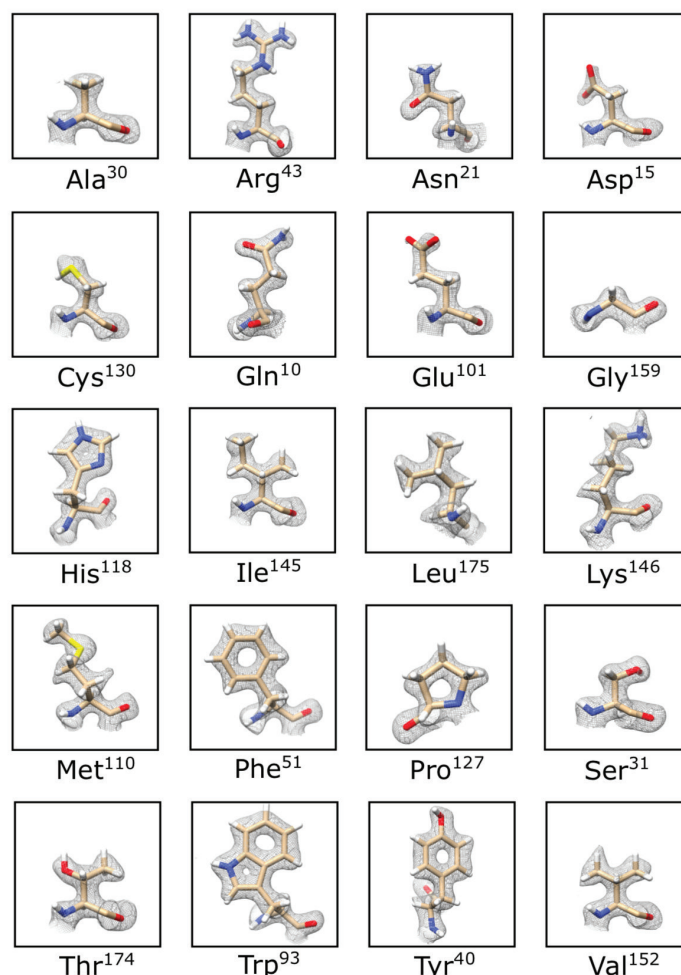


Figure 3.

Fig. 4 showcases the high quality of the map, notably the Met<sup>100</sup> with clear density for the sulfhydryl moiety<sup>4</sup>.

Conclusion: The JEOL CRYO ARM™ 300 equipped with a direct detector allows for the rapid and easy collection of high quality images enabling the determination of biological macromolecular structures below 2Å resolution.

Table 1: Experimental conditions	
Grid type	Quantifoil 200 mesh Cu, R1.2/1.3
Plunger	Vitrobot Mark IV
Microscope	JEOL CRYO ARM™ 300
Camera	Gatan K2
Slit width	20 eV
Magnification	100,000x
Physical pixel	0.495Å
Dose rate	2.3 e <sup>-</sup> /Å <sup>2</sup> /sec
Total dose	100 e <sup>-</sup> /Å <sup>2</sup>
Micrographs	840
Particles	120,295
Symmetry	O
Resolution	1.53Å

#### References:

1. 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>
2. Courtesy of M. Kikkawa, U. Tokyo.
3. Merk, A., Fukumura, T., Zhu, X., Darling, J.E., Grishammer, R., Ognjenović, J. and Subramaniam, S. (2020) *IUCrJ*, 7, 1-5, [doi.org/10.1107/S2052252520006855](https://doi.org/10.1107/S2052252520006855).
4. Images obtained using Chimera courtesy of T. Fukumura.

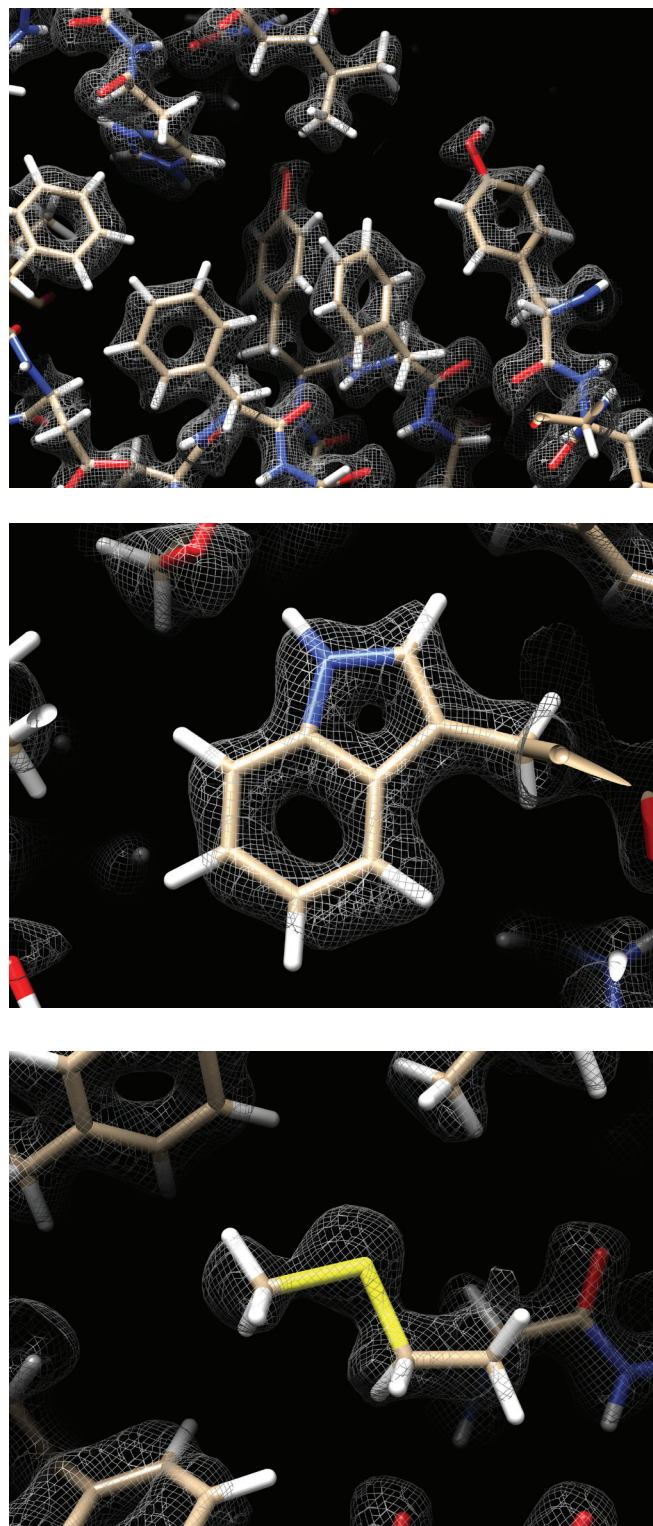


Figure 4.