Atomic resolution structure results from the JEOL 200 kV CRYO ARM™ TEM

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 3Å on a 300 kV microscope. Pioneering efforts have shown that similar results can also be achieved on 200 kV platforms. Similarly, efforts are underway to allow for a structure determination within a single day or even less. Here, we show results from Merk et al. at NIH from the JEOL CRYO ARM™ 200 obtained on beta-galactosidase at 1.8Å resolution[1]. The 3D map shows surprising details in the map reflecting the high resolution quality of the data.

Images were obtained in a single 24-hour imaging session on the CRYO ARM™ 200 equipped with a Gatan K3 direct electron detector. Control of the camera and microscope was accomplished using SerialEM utilizing hole targeting by stage navigation and a 7x7 hole pattern for beam shift and image shift with active beam tilt compensation. 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 30eV energy selecting slit width. A total of ~5000 movies were collected and processed using Relion-3.1 (Tables 1-3). The final map (Fig. 1) was obtained at 1.8Å resolution using ~250k particles of beta-galactosidase (Fig. 2).

The 3D map shows very well resolved densities for all of the amino acid residues (Fig. 3A-E). As expected, holes are clearly present in the side chain of various aromatic amino acid residues, such as Phe^{566}, Trp^{456} and Tyr^{392}, but also in the pyrrolidine ring of Pro^{393} (Fig. 3A). The map shows clearly defined densities for the carboxyl groups for the acidic residues, such as Asp^{447} and Glu^{650}, that were missing in similar work at 200 kV by Wu et al.[2] (Fig. 3D). Resolved densities from hydrogens emanating from asparagines and glutamines allow for the unambiguous identification of the proper rotamer conformation (Fig. 3E).

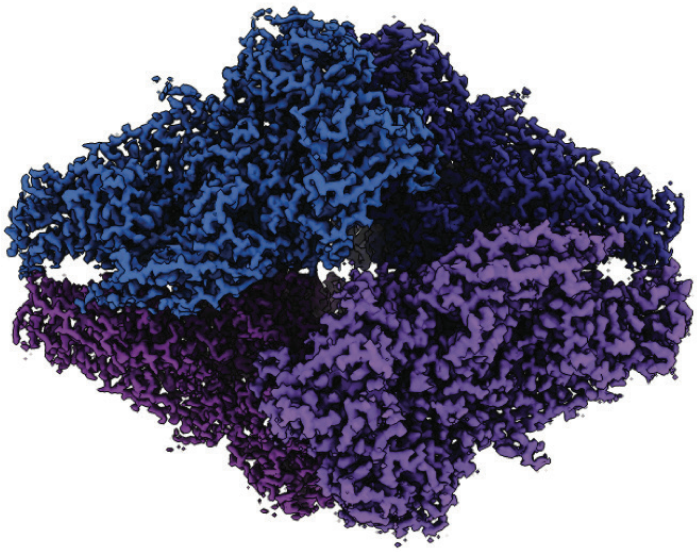

Figure 1.

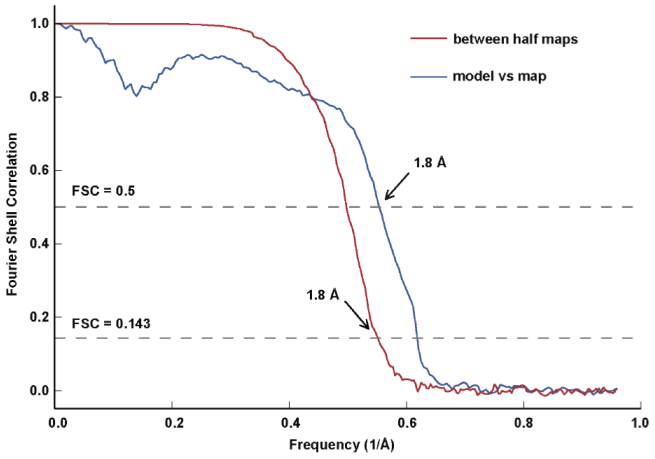

Figure 2.

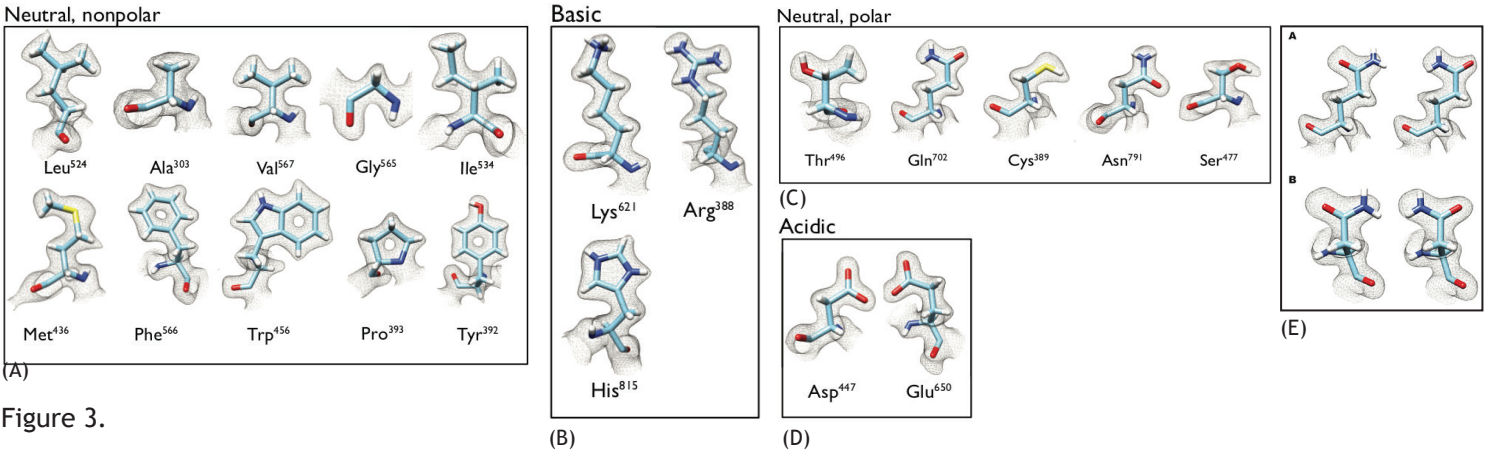

Figure 3.

The B-factor plot of the 1.8Å CRYO ARM™ 200 data and 1.9Å data[3] from a competitive 300 kV electron microscope suggests that fewer particles are needed to reach 1.8Å on the CRYOARM. Fig. 5 showcases the high quality of the map.

Conclusion: The JEOL CRYO ARM™ 200 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.

References:

1. Merk, A., Fukumura, T., Zhu, X., Darling, J.E., Grisshamer, R., Ognejenović and Subramaniam, S. (2020), IUCrJ 7, 1-5 doi. org/10.1107/S2052252520006855.
2. Wu, M., Lander, G.C. and Herzik Jr., M.A. (2019) November 26, bioRxrv @ https://doi.org/10.1101/855643
3. Bartesaghi, A., Aguerrebere, C., Falconieri, V., Banerjee, S., Earl, L.A., Zhu, X., Grigorieff, N., Milne, J.L.S., Sapiro, G., Wu, X. and Subramaniam, S. (2018) Structure 26, 848-856 e843.

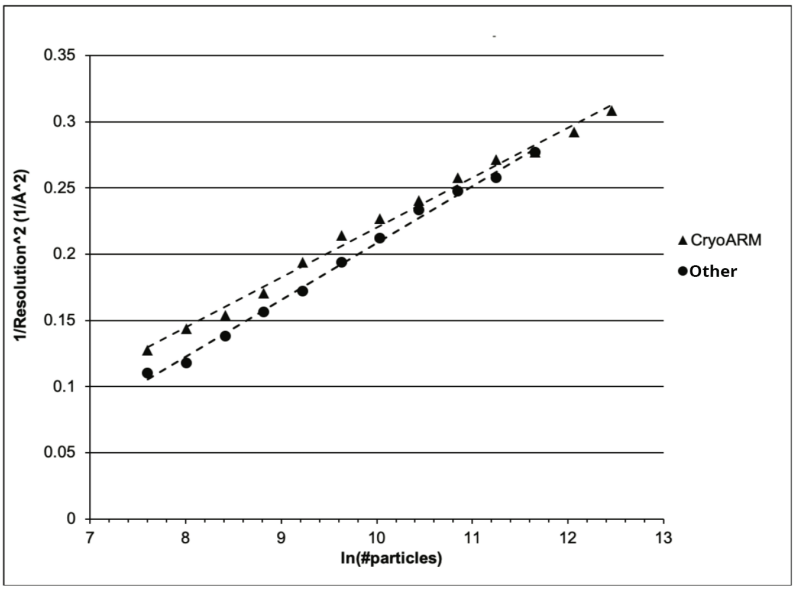

Figure 4.

Table 1: Experimental conditions	
Grid type	Quantifoil 200 mesh Cu, R1.2/1.3
Plunger	Leica EM GP
Microscope	JEOL CRYO ARM™ 200
Camera	Gatan K3
Slit width	30 eV
Magnification	100,000x
Physical pixel	0.52Å
Dose rate	11 e^-/Å^2/sec
Total dose	40 e^-/Å^2
Micrographs	4949
Particles	257,202
Symmetry	D2
Resolution	1.82Å
Map B-factor	-26Å^2

Table 2 Refinement statistics:	
Starting PDB model	5A1A
Atoms	66,138
Hydrogens	30,520
A.a. residues	4056
Waters	3618

Table 3: Validation	
Poor rotamers	0.36%
Ramachandran plot	
Favored	98.12%
Allowed	1.78%
Disallowed	0.1%

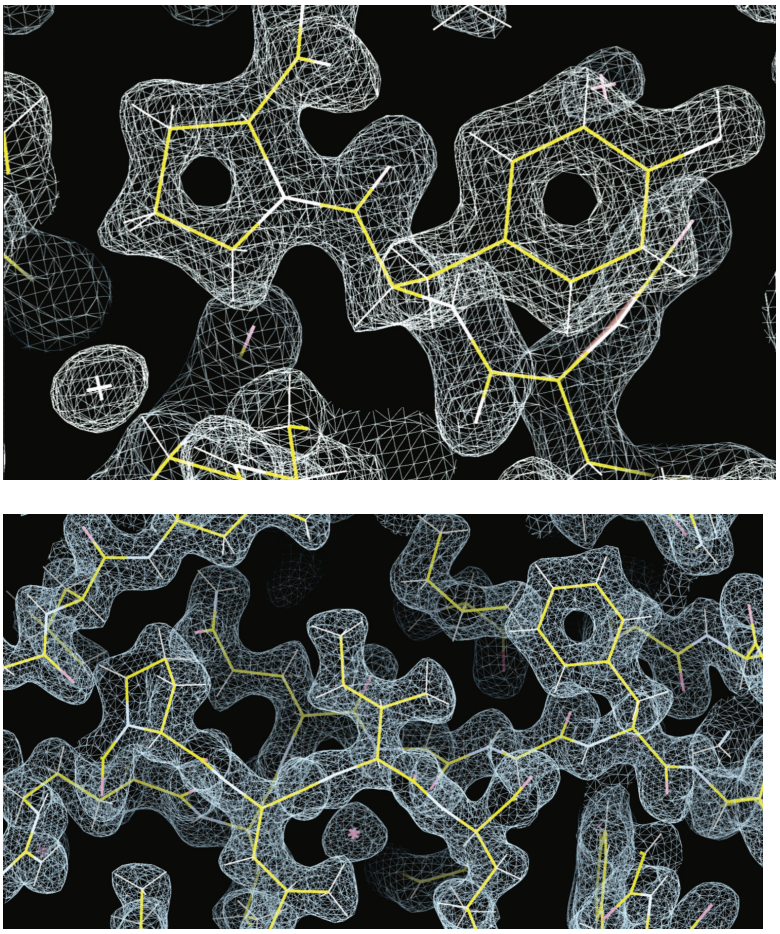

Figure 5.