

## Sub 3Å cryoEM structure using a standard LaB6 120kV TEM upgraded with direct electron detector

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### Background incl. aims

Cryo electron microscopy (Cryo-EM) single particle analysis (SPA) has become a major structural biology technique in recent years. High-resolution cryo-EM structures typically require higher voltage cryo-TEMs with coherent FEG sources, stable columns, autoloader systems and direct electron detectors. These setups are specialised for Cryo-EM work and expensive to establish and maintain. More recently the concept of using 100keV cryo-TEMs has been introduced as a way to make cryoEM more affordable and hence accessible to a larger group of researchers. However, so far, the implementation of these 100keV have relied on specialised cryo-TEMs with FEG sources and better and more stable optics than usually present in common 120keV TEMs.

### Methods

We here explored whether a standard 120keV TEM, commonly available at many places worldwide, can be upgraded with a direct electron detector and used for higher resolution cryo-EM. Our setup consisted of a TFS Tecnai Spirit G2 with lanthanum hexaboride (LaB6) thermionic electron emitter and with a Gatan 626 cryo-transfer holder. The microscope was upgraded with a Gatan Alpine direct detection camera optimised for 100 to 200keV. Automated data collection was set up using Serial EM.

### Results

Using this imaging configuration, we were successful in achieving a 2.7Å reconstruction for the cryo-EM standard apoferritin. We were also able to resolve a more challenging small 64kDa protein haemoglobin to 4.5Å. Furthermore, we solved an asymmetric membrane protein, 160kDa GPCR (M4 muscarinic acid receptor) to a resolution of 4.5Å. Importantly, all the results were achieved using limited datasets ranging from 700-1500 movies, making it feasible to collect these datasets with a side entry cryo-holder.

### Conclusion

These results showcase a potentially widely accessible microscope configuration to obtaining interpretable cryo-EM structures. However, we suggest that the true value of using standard 120keV TEMs is in the potential for many EM facilities and laboratories to setup high quality cryo-EM SPA sample screening without the need to procure specialised Cryo-TEMs. This could hence help to considerably lower the entry barrier for cryo-EM SPA and contribute to the “democratisation” of Cryo-EM.

### **Keywords:**

democratisation, structure, GPCR, screening, optimisation