

Deriving atomic models from cryoEM images recorded on a DE12 DDD camera

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Direct Electron D-12 DDD camera

Campbell MG, Cheng A, Brilot AF, Moeller A,
Lyumkis D, Veesler D, Pan J, Harrison SC, Potter CS,
Carragher B, Grigorieff N, *Structure*, Nov, 2012

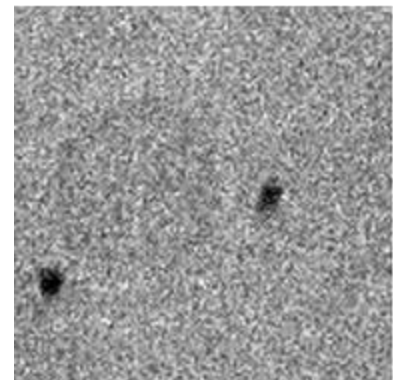


Best resolution of final
3D reconstruction is the
4.4Å structure by movie.

Journal of Structural Biology, 176 (3): 404-8.
December 2011.

Milazzo AC, Moldovan G, Lanman J, Jin L, Bower
JC, Klienfelder S, Peltier ST, Ellisman MH, Kirkland
AI, Xuong NH

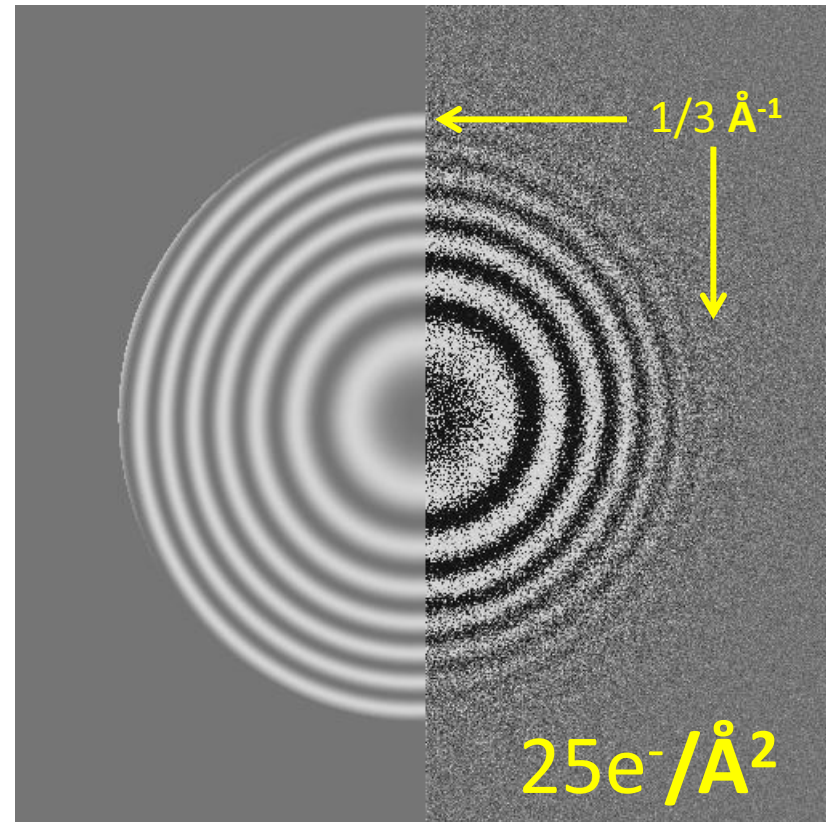
Ultramicroscopy, 110 (7): 744-7. June 2010.



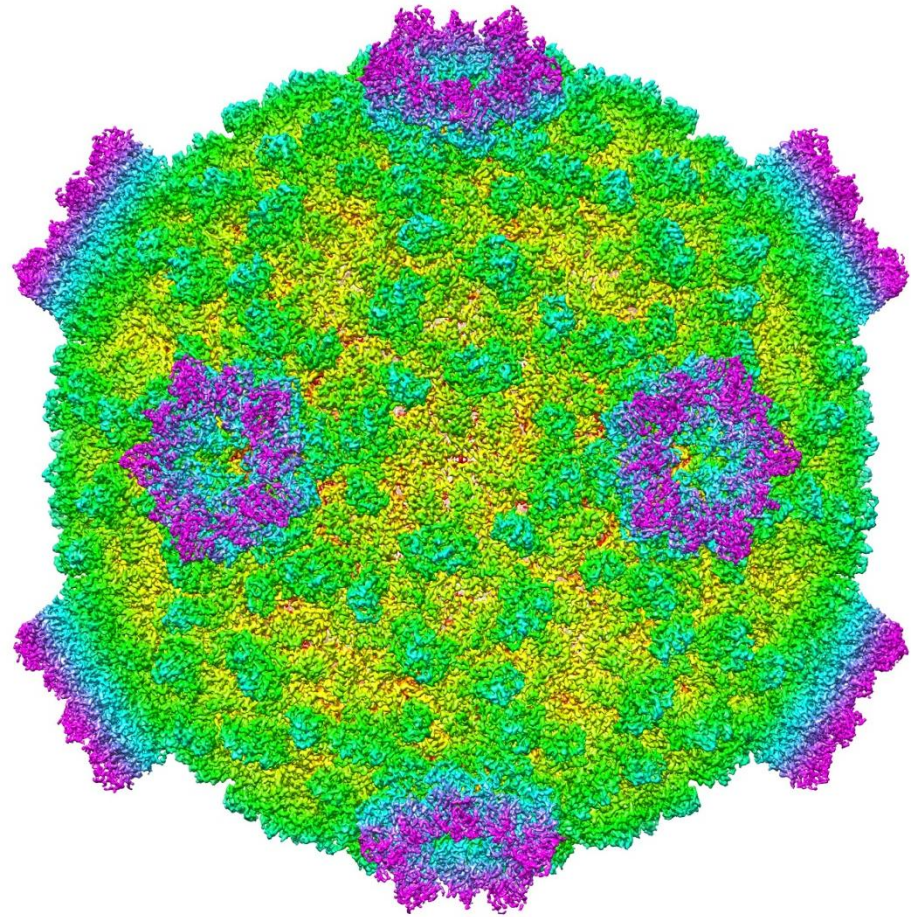
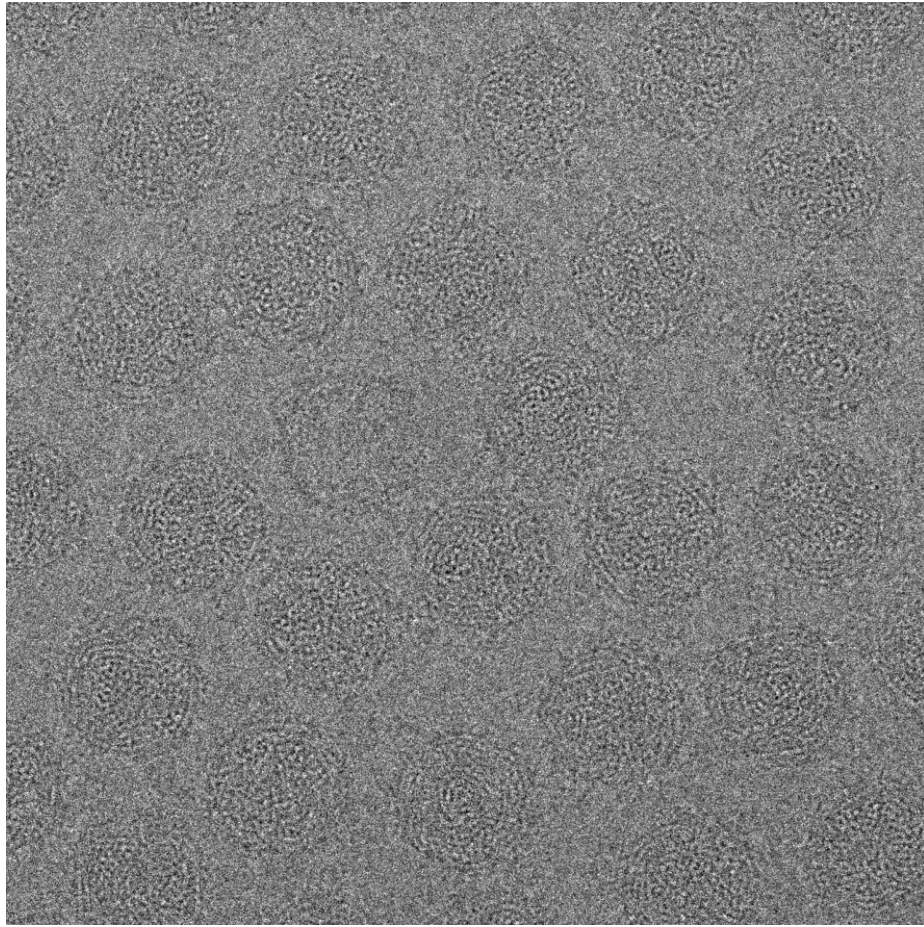
Confirming Thon Ring of Carbon at $\frac{3}{4}$ Nyquist at cryoEM condition

Earlier work at low mag/low resolution: contrast at $\frac{3}{4}$ Nyquist

Bammes BE, Rochat RH, Jakana J, Chen DH, Chiu W
Journal of Structural Biology, 177 (3): 589-601.
March 2012.



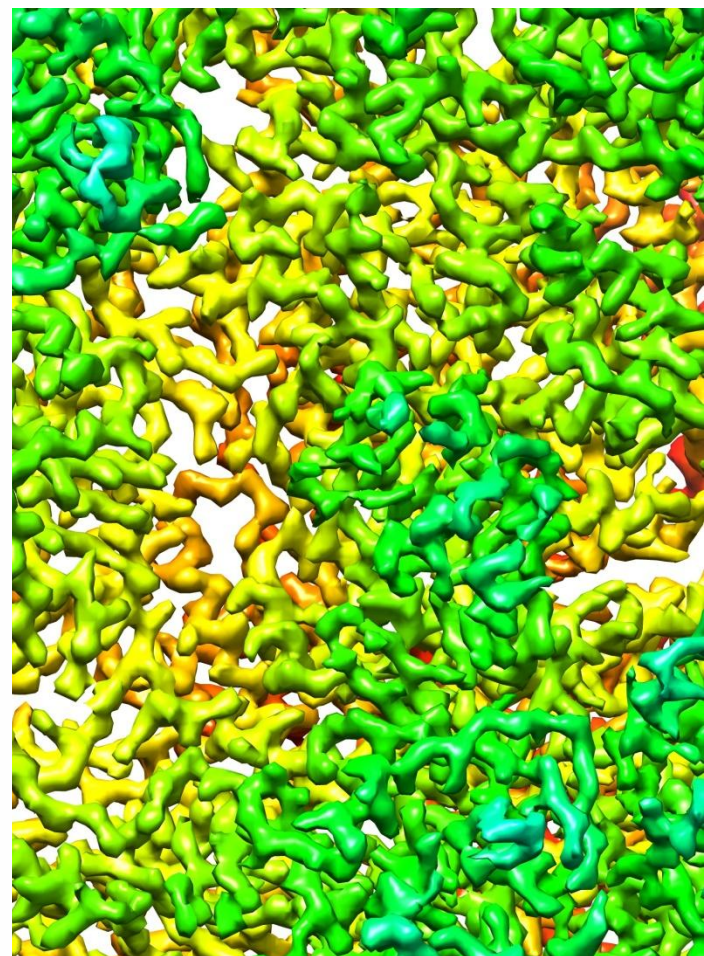
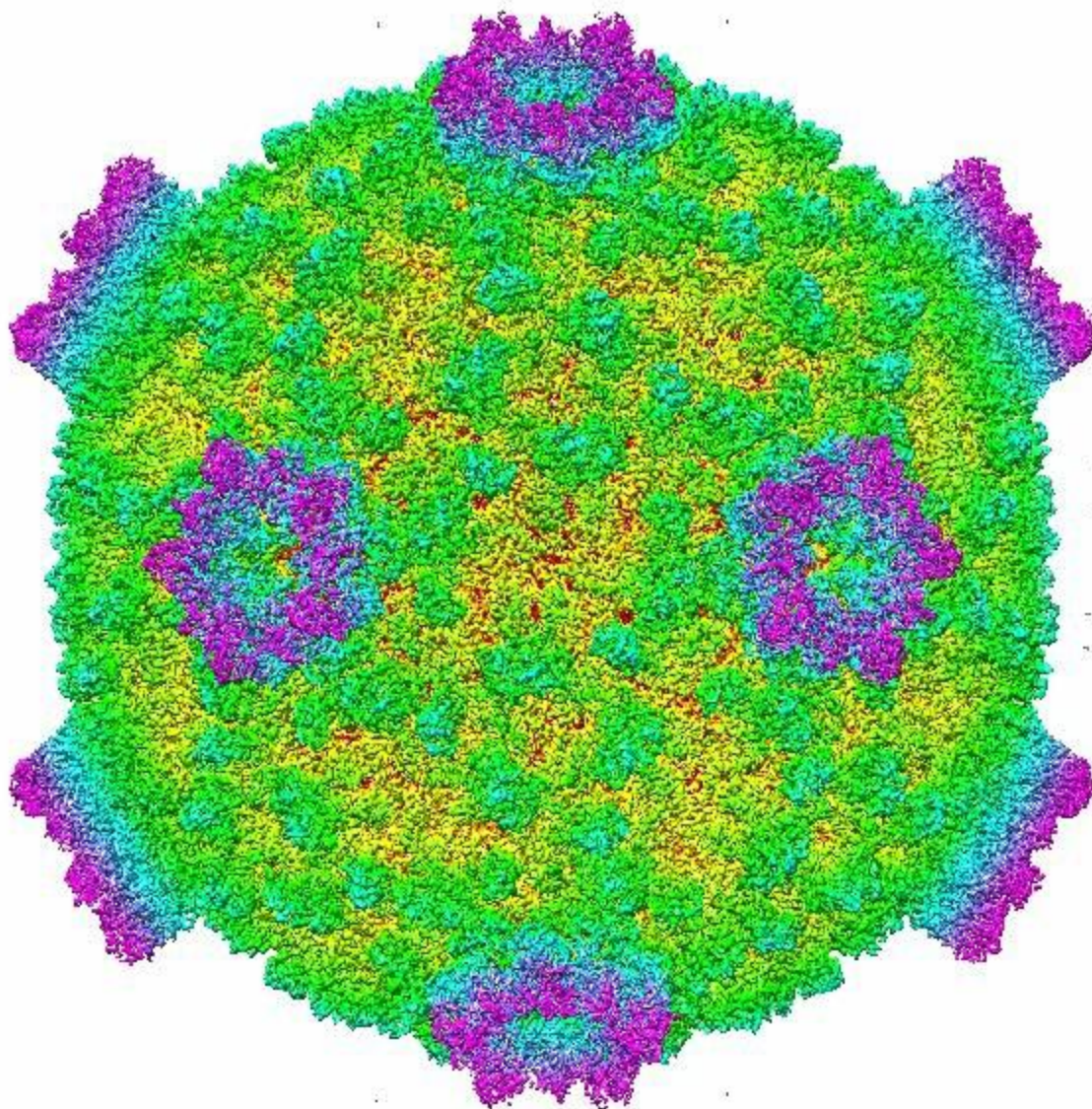
Imaging Cytoplasmic Polyhedrosis Virus (CPV) on DDD camera at 3.5-Å



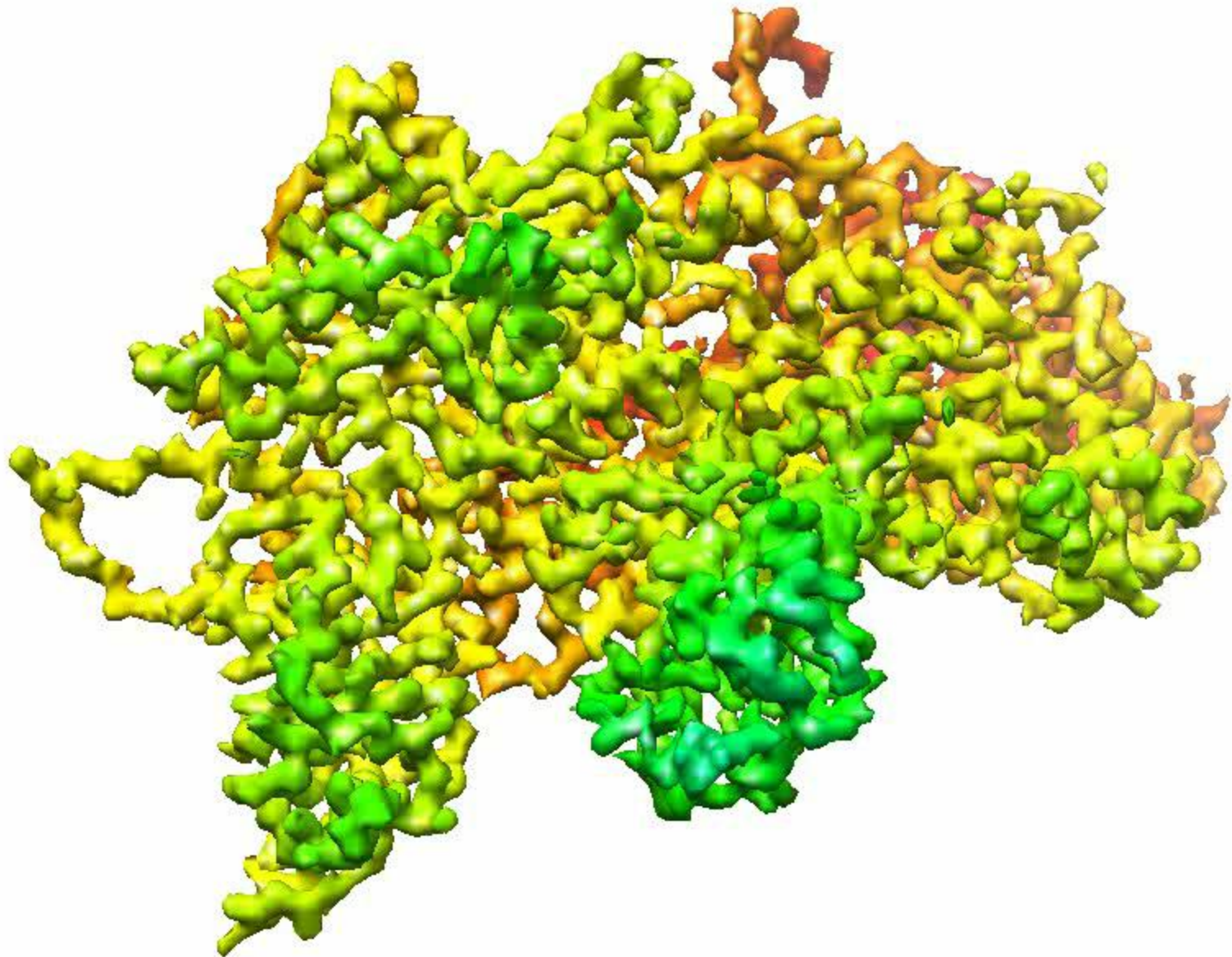
Data Statistics

- Holey Quantifoil grids “baked” by 100kV electrons
- Imaged with Legikon – For all images, including the carbon images, mag=53,600, and pixelsize=1.12Å/pix, dose=25e/Å²
- 2653 DDD pictures with defocus from -0.5 to -3μm were used for processing
- 42082 particles were automatically selected and 33660 (80%) were used for the final reconstruction
- Same program (IMIRS with G3D) was used for alignment and reconstruction of DDD and film data
- The resolution of the capsid is estimated as 3.5Å based on density feature and FSC (Rosenthal and Henderson, *i.e.*, 0.143) after 7 cycles of alignment/refinement

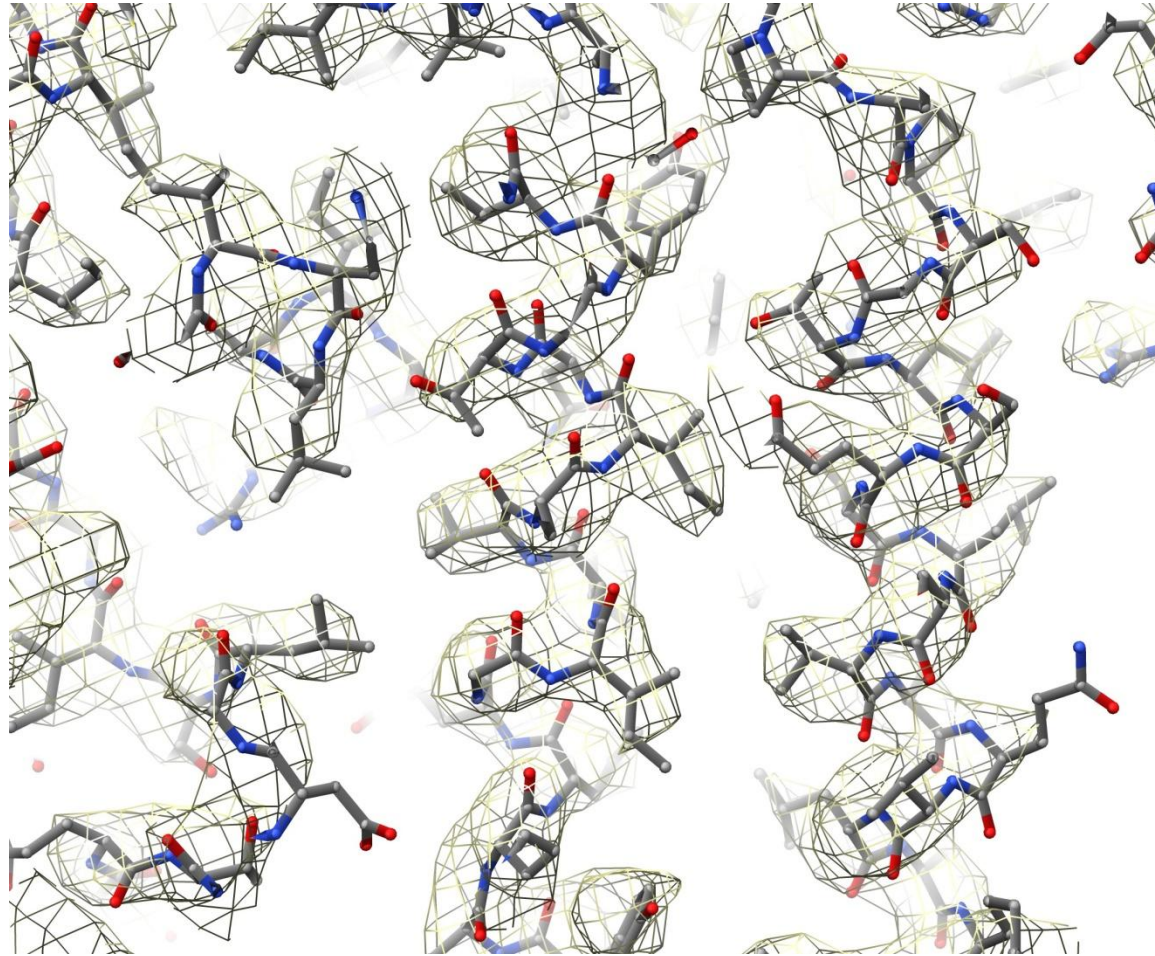
Close-up view of a small region



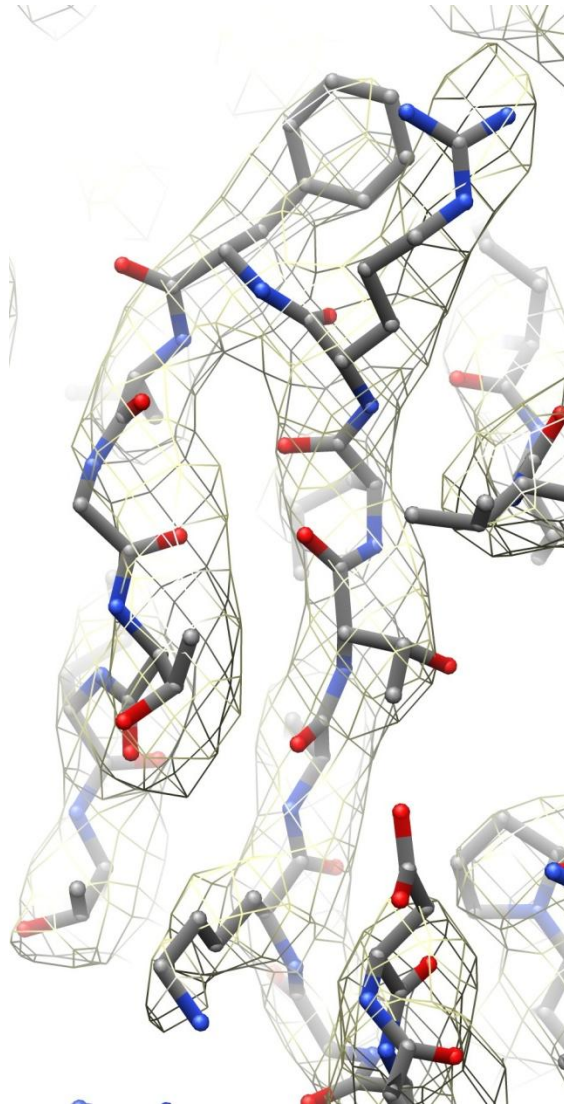
Segmented CSP-A monomer



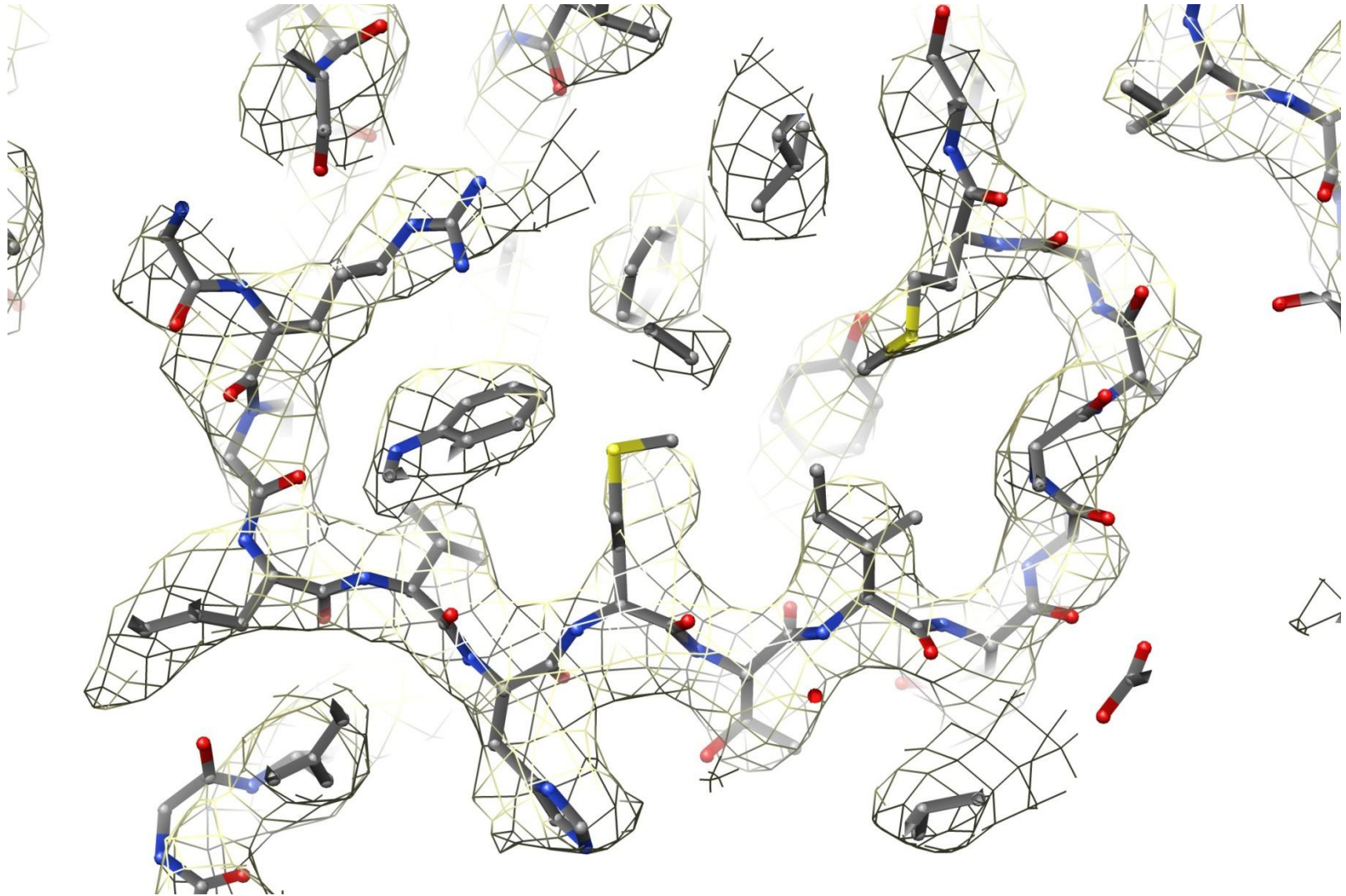
Close-up view of helices



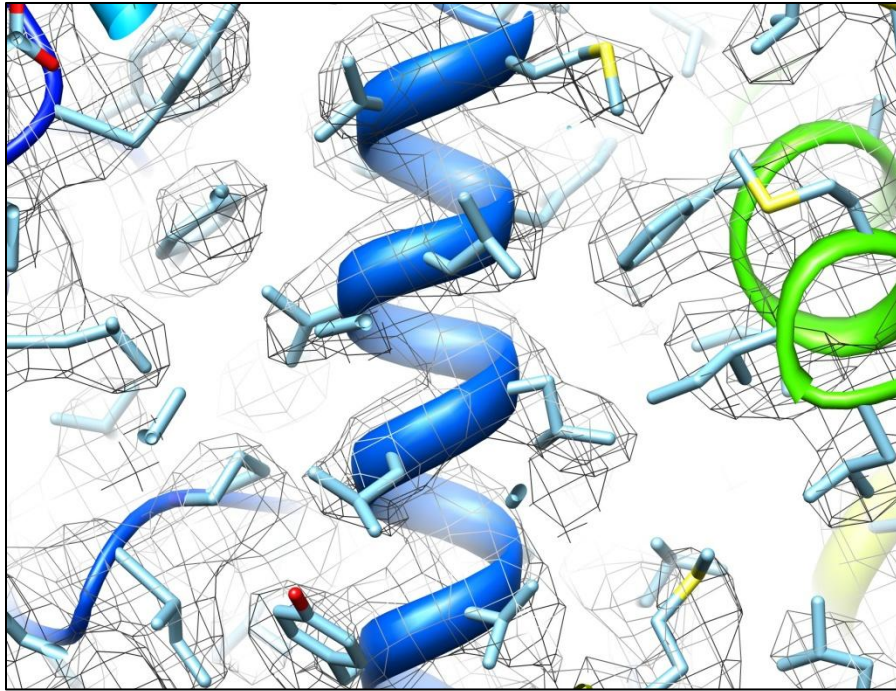
Close-up view of a β -turn



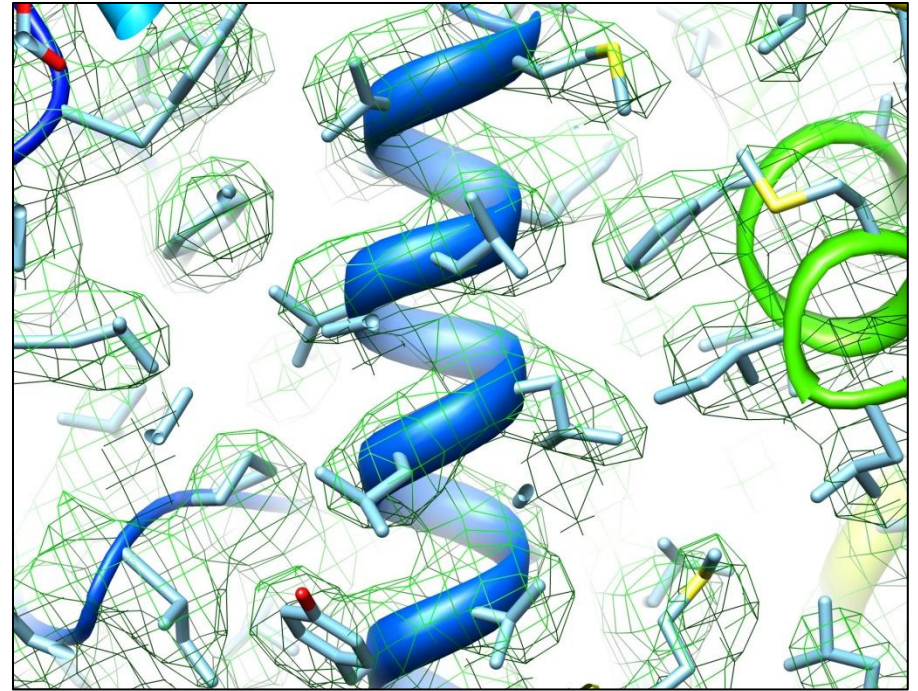
Close-up view of a loop



Comparison between DED and film structures at 3.5 Å

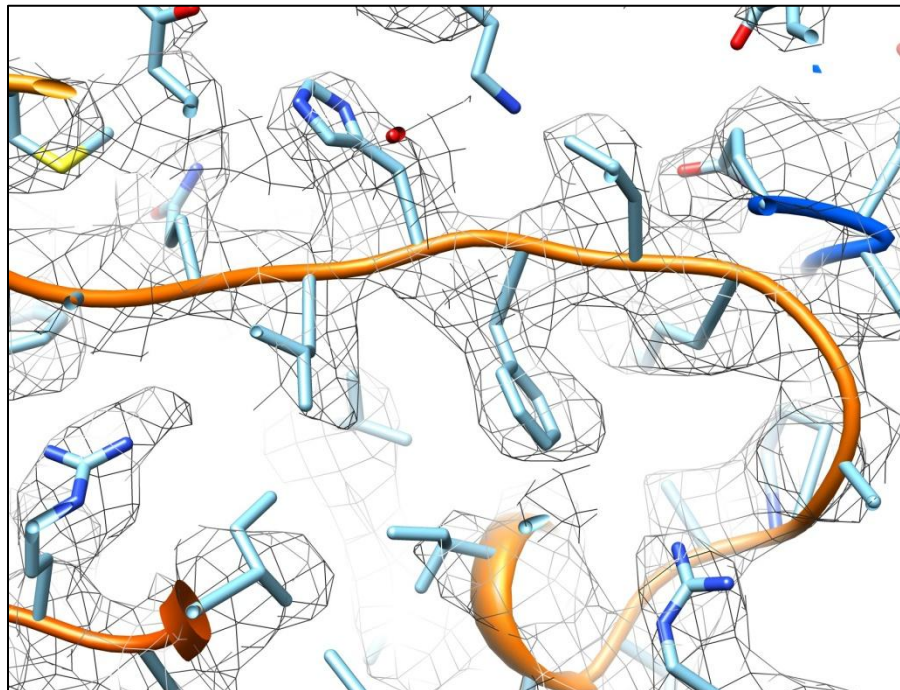


DED

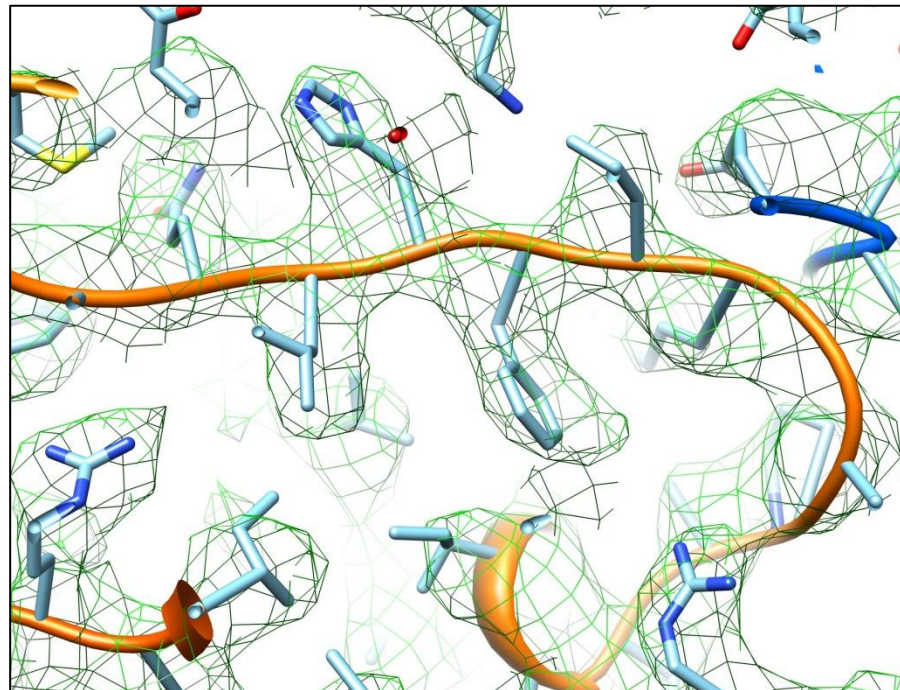


film

Comparison between DED and film structures at 3.5 Å

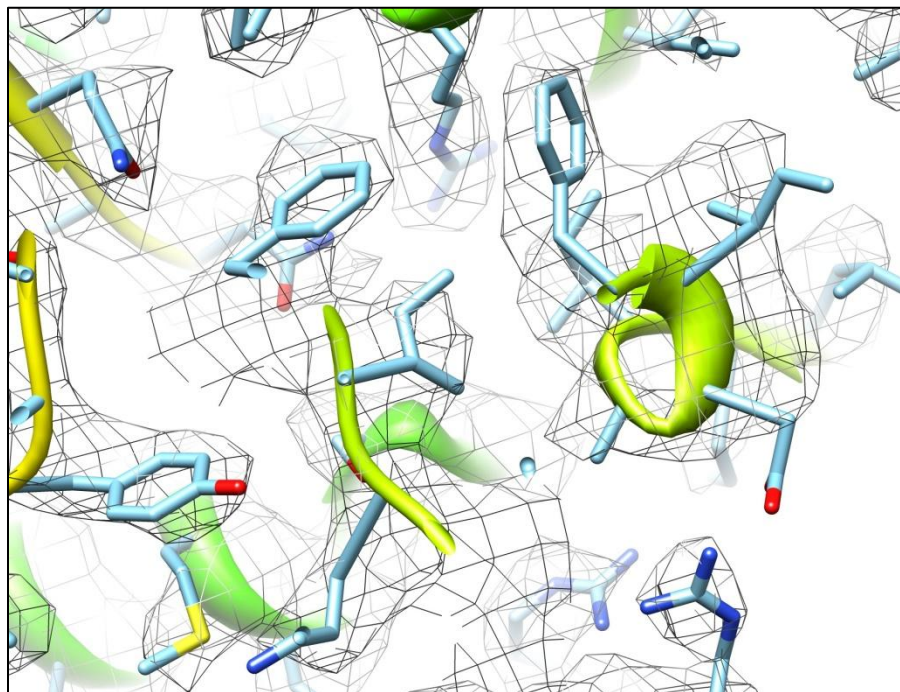


DED

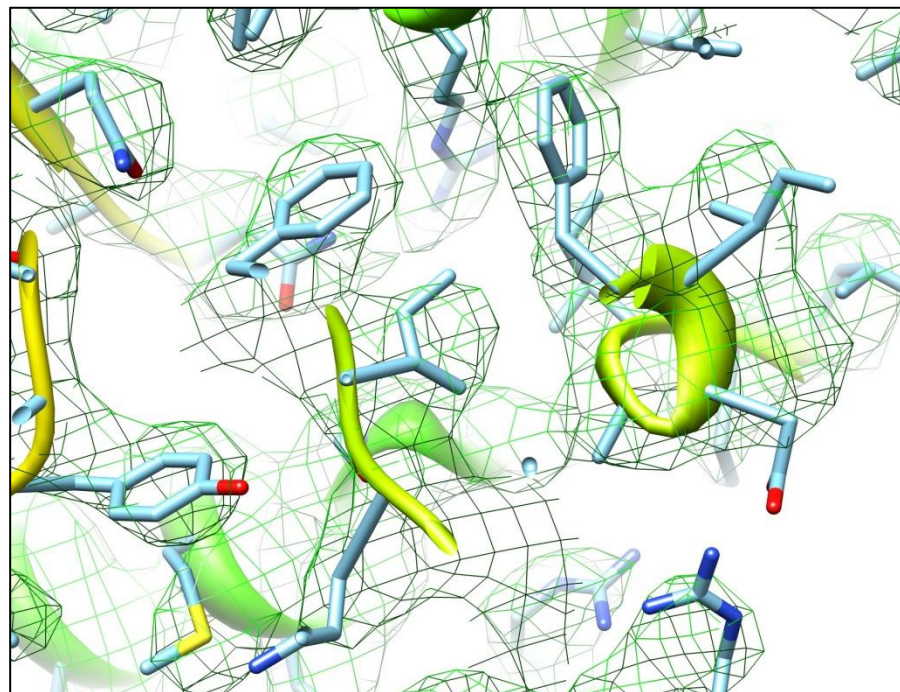


film

Comparison between DED and film structures at 3.5 Å



DED



film

Summary

- DDD (D-12) cryoEM images of CPV and 3D reconstruction at 3.5Å resolution
- Single-frame images of DDD sufficiently good quality – no drift-correction necessary for the Titan Krios
- Direct electron recording has come of age for atomic modeling

Acknowledgement

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FEI company

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