

Type of presentation: Invited

**ID-8-IN-1682 Atomic model of F<sub>420</sub>-dependent [NiFe] hydrogenase by electron cryo-microscopy.**

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It has long been recognized that electron cryo-microscopy has the potential to solve protein structures at near-atomic resolution, but until recently this was a prohibiting task, taking a huge investment in time and effort<sup>1</sup>. With the recent introduction of direct electron detection cameras with much better detective quantum efficiency and high frame rates, images with a higher signal-to-noise ratio can be obtained and alignment of video frames allows to partially correct for the effects of beam-induced particle movement and specimen drift<sup>2,3</sup>. The resulting improvement in image quality makes it possible to obtain better reconstructions with much less data. 3D reconstruction depends on the accurate determination of the relative projection angles of the images of macromolecular complexes, randomly oriented in a vitreous water layer, which is done in an iterative process. New image processing procedures make this process more robust and prevent fitting of high-frequency noise and creating spurious detail<sup>4</sup>. With these new tools we reconstructed a map of the 1.2 MDa, tetrahedrally symmetric complex of the F<sub>420</sub>-reducing [NiFe] hydrogenase (Frh). Frh is an abundant enzyme in methanogenic archaea, regenerating the reduced form of the flavin coenzyme F<sub>420</sub> that is used by several enzymes in the methanogenesis pathway from CO<sub>2</sub> and H<sub>2</sub><sup>5</sup>. It consists of three different subunits with several cofactors, a [NiFe] center, four [4Fe4S] clusters and an FAD forming an electron transfer chain from H<sub>2</sub> to F<sub>420</sub>. 26,000 particle images were selected from 235 electron micrographs, collected on the Falcon II direct electron detector in video mode in a two-day microscope session. The map at 3.4 Å resolution shows all secondary structure as well as clear side chain densities for most residues and the cofactors in the electron transfer chain along with a well-defined substrate access channel. An atomic model for all but a few terminal residues could be built in the density map. From the rigidity of the complex we conclude that catalysis is diffusion-limited and does not depend on protein flexibility or conformational changes<sup>6</sup>.

References:

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<sup>6</sup> Allegretti, M., Mills, D. J., McMullan, G., Kühlbrandt, W. & Vonck, J. *eLife* **3**, e01963, 2014.

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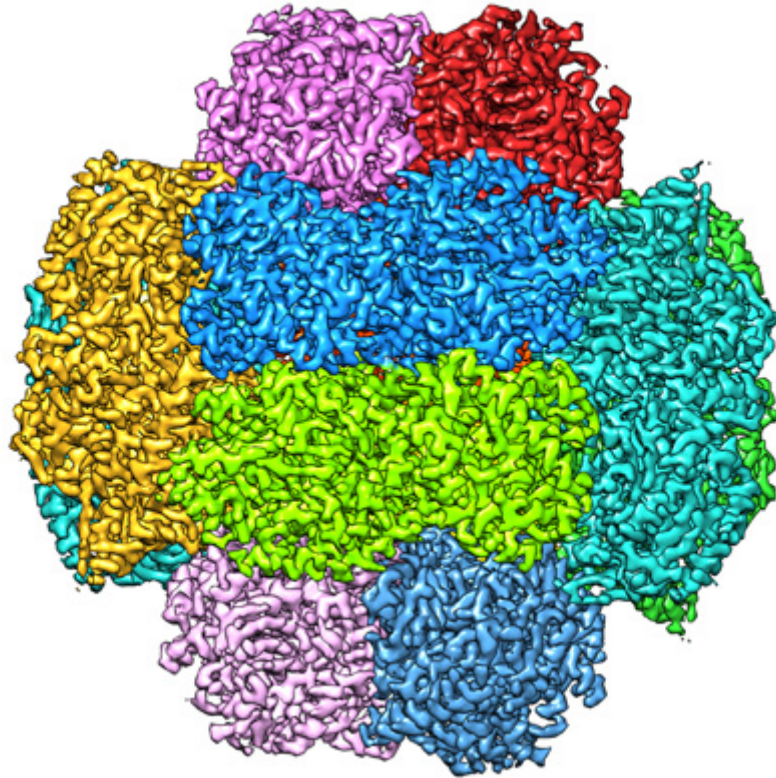


Fig. 1: Density map of the tetrahedral Frh complex at 3.4 Å resolution. Each of the twelve heterotrimers of FrhA, FrhG and FrhB is shown in a different colour.

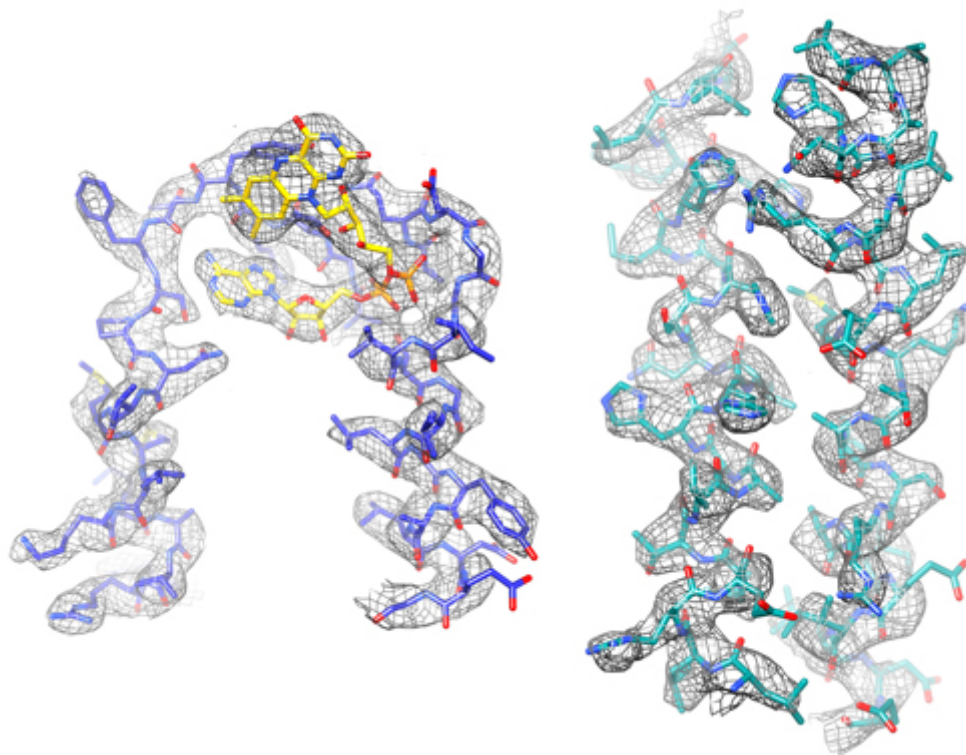


Fig. 2: Details of the density map with fitted atomic model. Left: the FAD cofactor in its binding pocket. Right: two long alpha-helices.