ESRF	Experiment title: *Klebsiella pneumoniae* nitrogenase component 1 (Kp1)	**Experiment number:** LS-1319
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Report:

Nitrogenase catalyses the reduction of dinitrogen to ammonia. It is comprised of two component proteins: component 2 is the electron donor to component 1, and it is the latter which contains the substrate binding and reduction site. Component 1 is a 230kDa $\alpha_2\beta_2$ tetramer. It contains two unique metal-sulfur clusters, the iron-molybdenum cofactor, FeMoco, and the iron-sulfur P-clusters. During our beamtime allocation we collected X-ray data on the NifV$^-$ mutant of Kp1 and also tried to identify gas access channels in the protein.

The *nifv* gene of *K. pneumoniae* encodes a homocitrate synthase. The NifV$^-$ mutant is thus unable to synthesize homocitrate and is therefore incapable of forming the normal Fe-Mo cofactor (FeMoco). Instead it incorporates citrate into the cofactor and as a result displays both altered substrate specificity and poor nitrogen fixing ability. Crystals of this mutant were obtained under similar conditions to the wild-type protein although they did not grow as large. However, X-ray data collection was possible at 1.9 Å

resolution yielding a data set that was 94.2% complete with an overall R_{merge} of 9.4% and I/σ<I> of 15.5. Refinement of the structure is almost complete with an R_{cryst} = 17.1% and R_{free} = 24.3%. A superposition of the mutant onto the wild-type structure reveals that the citrate takes up a very similar configuration to the homocitrate, retaining the bidentate interaction with the Mo of the cofactor. Since one of the "arms" is one CH_2 group shorter in citrate with respect to homocitrate, it froms different hydrogen bonds and perturbs the water structure slightly. This structure is still being analysed and we are not yet in a position to provide a structural explanation for the altered catalytic activity of the mutant.

The gases nitrogen and hydrogen are respectively substrates and products of nitrogenase. Through the analysis of xenon binding sites, we hope to define hydrophobic channels in the protein, which may be routes for gas access to and from the active site. In collaboration with Dr. J. Fontecilla-Camps (Grenoble), we repeated our data collection on a xe-derivatized crystal (we previously collected a poor data set at 3.1Å resolution). The new data set was 95.4% complete with an overall R_{merge} of 3.8% and I/σ<I> of 27.0. From these data we located two clear Xe binding sites per αβ dimer. One of these was located at the protein surface, whilst the other lay adjacent to the FeMoco. We have yet to analyse these data further, but through the identification of low occupancy binding Xe sites, it may be possible to define putative gas access channels within the protein, perhaps connecting the FeMoco to the surface.

The binding of nucleotides to component 1 was also investigated by preincubating crystals with $MgADP^-$ prior to data collection. Unfortunately, none of the crystals diffracted. This may indicate large structural changes are occurring which disrupt the crystal lattice.