ESRF	**Experiment title:** X-ray crystallographic investigation of *Klebsiella pneumoniae* nitrogenase component 1 (Kp1) in different oxidation states.	**Experiment number**: LS-935
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Shifts: 6	**Local contact(s)**: Dr. Wim Burmeister	*Received at ESRF:*

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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 investigated the structural consequences of altering the oxidation state and of adding a substrate to crystals. We also tried to identify gas access channels.

All crystals were grown under reducing conditions. The redox dye thionin will oxidize crystals to a potential of +60mV taking the FeMoco to the +1 state and the P-cluster to the +2 state. Crystals were incubated overnight in saturated solutions of thionin made up in cryoprotectant, and then flash-cooled in liquid nitrogen. A 1.95Å resolution set (96% complete, Rmerge = 7.3%) was collected from a single crystal and compared to a previously collected data set at 1.6Å resolution from a phenosafranin-

soaked crystal giving a mean fractional isomorphous change of 12.3%. The latter oxidizes the protein to a potential of -270mV where the FeMoco remains fully reduced but the P-cluster is oxidized to the +2 state (as with thionin). Therefore we were primarily looking for differences in the FeMoco and its environment, which may only manifest themselves as a slight contraction of the cluster (observable with EXAFS). Unfortunately none were apparent at 1.95Å resolution. It is our intention to extend the resolution of this data set so that we may be able to see subtle structural changes.

In addition to nitrogen, acetylene is a substrate of nitrogenase. The protonation state of an as yet unidentified residue in Kp1 affects the EPR spectrum of the cofactor, suggesting that it may be close to the FeMoco itself. Acetylene reduces this pKa from 8.7 to 8.0. In order to investigate this structurally, crystals were preincubated with acetylene-saturated cryoprotectant prior to flash-cooling. At the start of data collection, diffraction to 1.7Å resolution was observed. However, the X-ray data quality deteriorated during the experiment due to radiation damage (which has not previously been apparent with Kp1 crystals at cryogenic temperatures on less intense sources), such that at the end of the experiment, diffraction to little more than 2.0Å resolution was observable. Consequently the data were processed at this resolution, giving a completeness of 96% and an Rmerge of 8.3%. This data set is still being analysed and thus we are not able to report any structural details at this stage.

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 collected 3.1Å resolution data (98% complete, Rmerge = 6.2%) on a xe-derivatized crystal. Many of the images had several very strong spots, possibly resulting from the magnesium chloride used in the crystallization, suggesting some drying out of the crystal during derivatization. Consequently the data were not very isomorphous with previously collected data, making difference maps very noisy. However one clear xenon site was observed at the protein surface. In the future, we hope to repeat this experiment in order to identify internal xenon sites and thus define putative gas access channels within the protein.