Applications invited immediately for a 4-year BBSRC/DIAMOND funded Ph.D. Studentship

Long wavelength X-ray diffraction experiments for structural studies from biological redox systems

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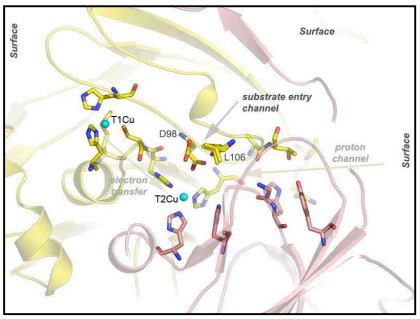
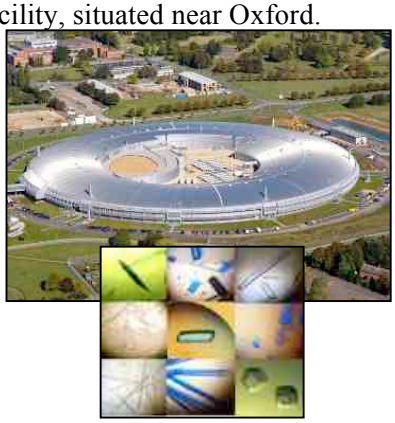
X-ray crystallography is the most powerful method of obtaining three dimensional structures of protein molecules at atomic resolution. This is illustrated by the first Nobel prize for structural biology (1962) and by the recent (2009) Nobel prize in Chemistry. Solving the 'phase problem' is central to protein crystallography. New concerted efforts are being made to use X-ray energies ('long wavelengths') that exploit the intrinsic sulphur containing amino acids for solving *de novo* crystal structures. To further advance the application of long-wavelength x-ray crystallography in *redox biology* (i.e. oxidation-reduction reactions in metalloproteins), appropriate experimental tools, strategies and software for tackling the problems associated with x-ray absorption effects are needed. As absorption effects increase with longer wavelengths, X-ray experiments get very challenging. To address these critical questions we have established a collaboration with DIAMOND to help develop solutions to these outstanding problems, with a focus on the enzymes of the denitrification cycle that contain either single or multiple redox active metal (Cu and/or Fe) centres. The oxidation-reduction reactions essential for their metabolic function depends on the environment of the redox centre and associated (proton-coupled) electron transfer pathways. We have used x-ray crystallography to reveal these pathways and structures in copper containing nitrite reductase (NiR) (2) and have shown that x-ray induced changes to redox states in metalloprotein structure determination is a significant general problem when relating the true redox state of the enzyme to its structure (3). Work will be undertaken by the successful PhD candidate to use our existing 'model' denitrification redox-enzymes to study the effects of x-ray absorption and x-ray photolysis of the metal centres in protein crystals and to work with scientists at DIAMOND to study ways of dealing with x-ray absorption effects, leading to improvements in long x-ray wavelength data collection (4).



The Ph.D. student will be provided a comprehensive training in all aspects of the enzyme structure-function studies and x-ray work including:

1. Expression and purification of denitrification enzymes for structural and functional studies
2. Protein crystallisation
3. X-ray crystallography (data collection, processing, structure refinement and model building)

The student will spend 50% of his/her time at the DIAMOND synchrotron facility, situated near Oxford. Specific work to be undertaken at DIAMOND includes:
(1) Training in use and operation of crystallography beamlines
(2) Testing and benchmarking analytical absorption correction software
(3) Sulphur-SAD phasing on redox proteins at long-wavelengths. A He beam path to be placed at one of the existing beamlines will be designed.



References

(1) Ellis, M., et. al. (2007) JBIC 12, 1119-1127
(2) Antonyuk, S. V. et al. (2005) PNAS 102, 12041-12046
(3) Hough, M. A. et al. J. (2009) Mol. Biol. 378, 353-361
(4) Wagner, A., et. al. (2006). *Acta Cryst. D*62, 1430-1434

For informal enquiries please contact Dr. R.W. Strange and Dr. A. Wagner
Web: www.biophysics.liv.ac.uk and www.diamond.co.uk

Applications are invited from students who are strongly motivated for research and discovery, are ordinarily resident in the UK and either hold or expect to obtain, a First or Upper Second class honours degree in the following subject areas: Physics, Chemistry, Biology or Engineering. Application by email together with a c.v. including the names and contact details of two academic referees, should be sent as soon as possible to: Mrs. Linda J. Marsh, Research Support Office, School of Biological Sciences, The Life Sciences Building, Crown Street, Liverpool L69 7ZB (email: biolres@liverpool.ac.uk, Fax: 0151 795 5122). Review of applications begins from mid February 2010. Please indicate where you first saw the project(s) advertised.