Amman-SESAME-JSPS - October 2002

Structural Biology & Genomics Research at Daresbury

Samar Hasnain

Molecular Biophysics Group

&

College Of Biology & Medicine

Daresbury Laboratory

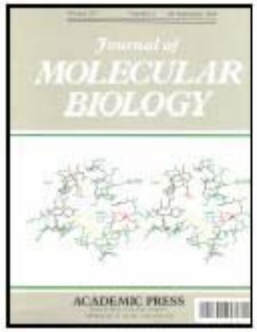
http://www.srs.dl.ac.uk/mbg/

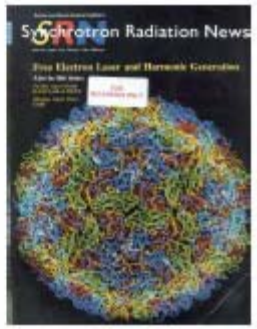
http://www.nwsgc.ac.uk/

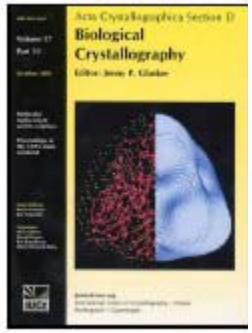
Molecular Biophysics Group

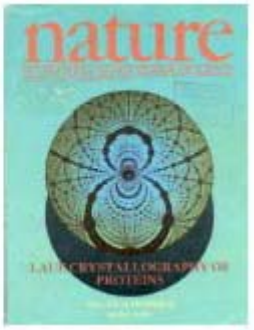
Impact of SRS, Daresbury, on UK structural biology

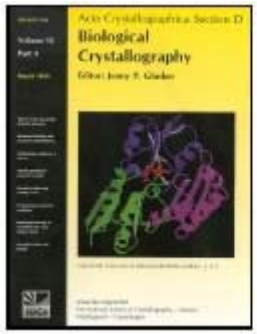
NWSGC

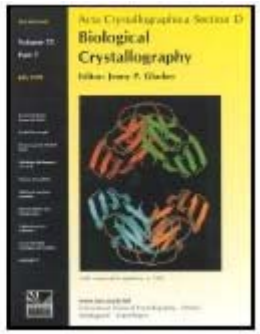


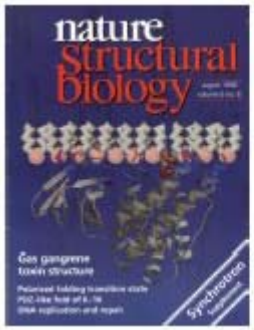


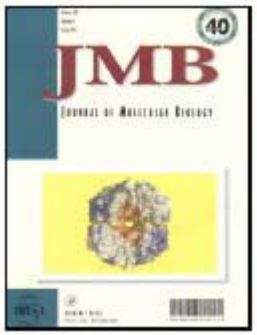




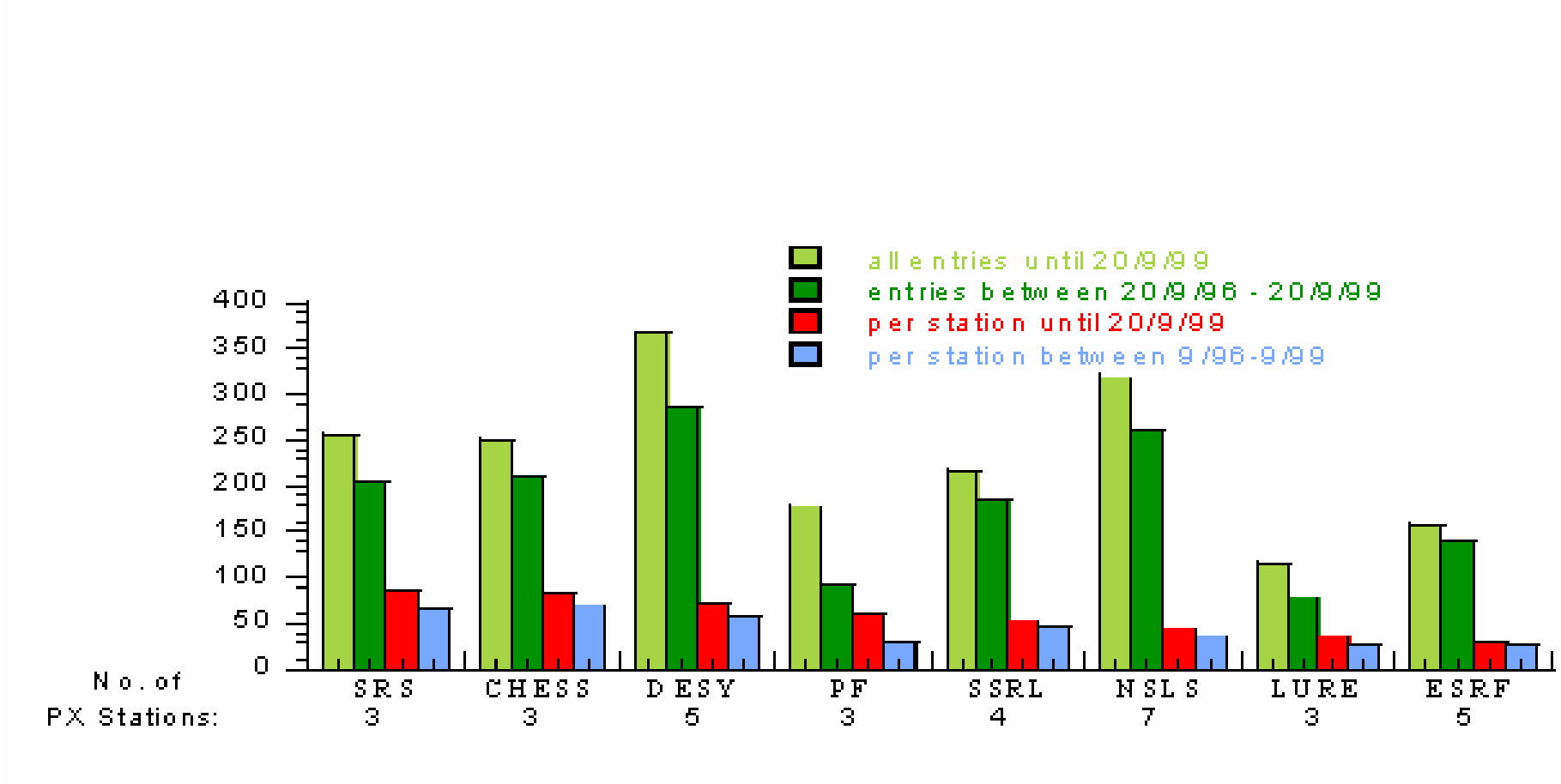




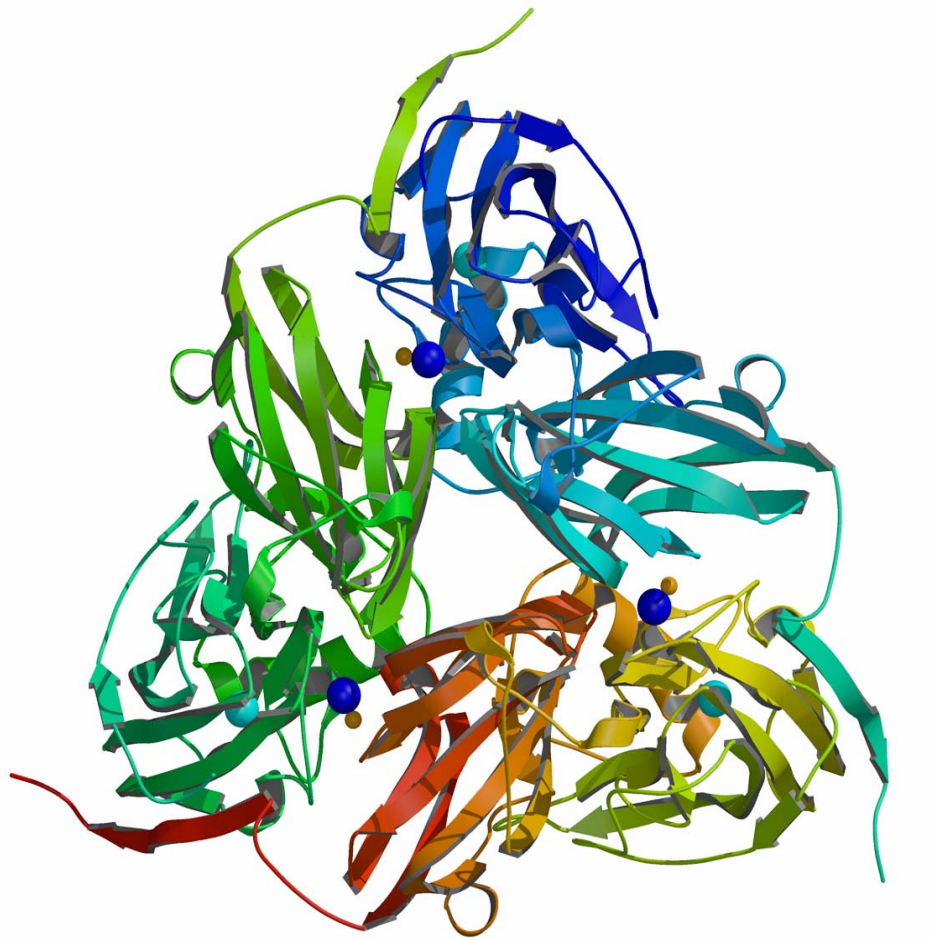




SRS performance in comparison with other major SR facilities 1996-99



Nitrite Reductase



A blue Cu protein from *Alcaligenes xylosoxidans* containing type 1 and type 2 Cu centres.

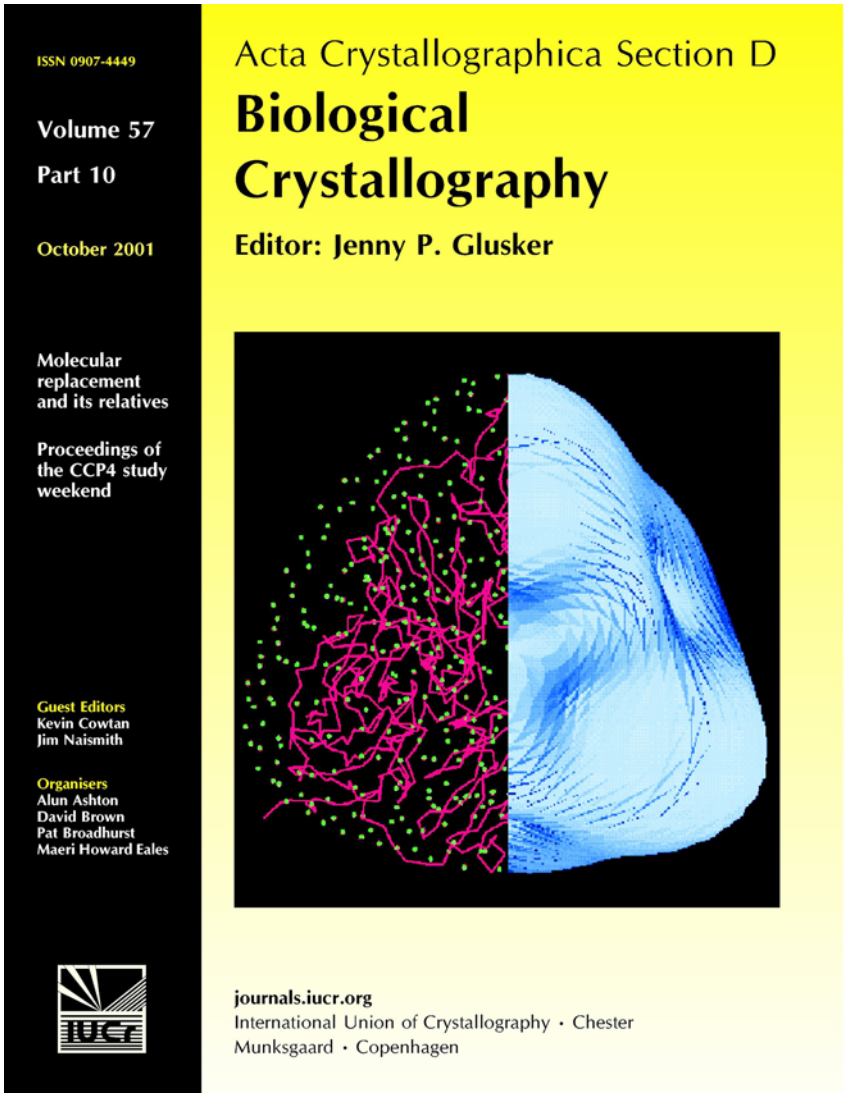
Biologically important as part of the denitrification pathway

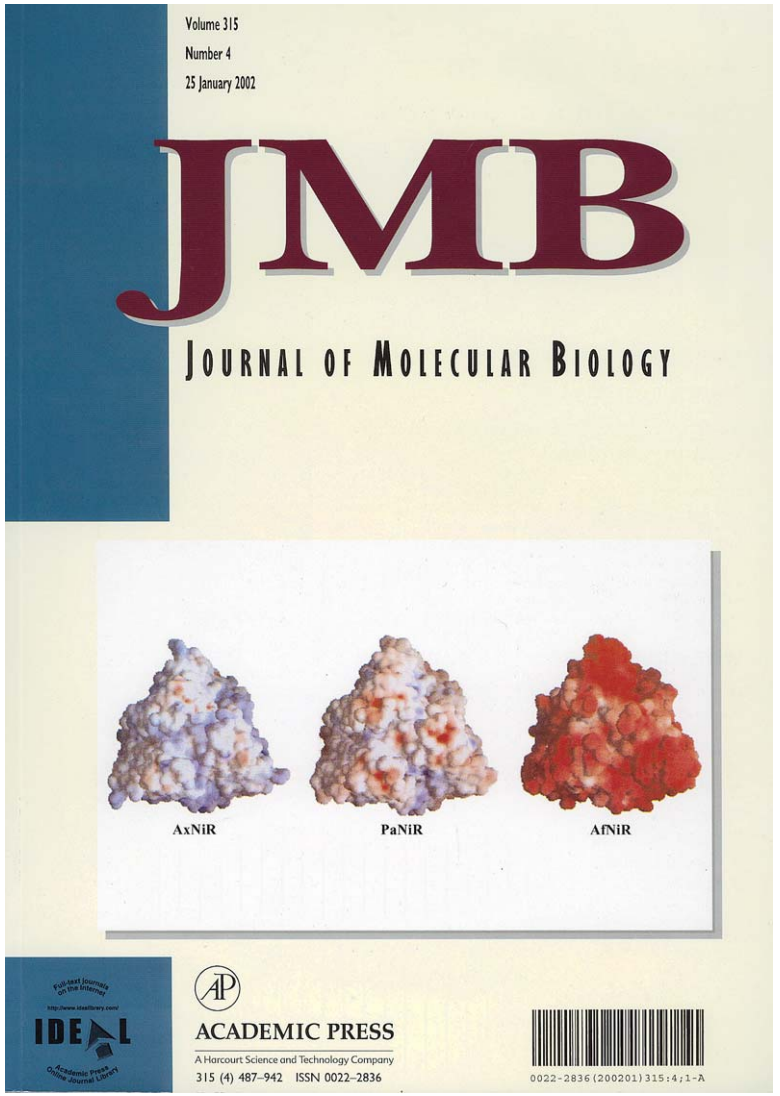
36.5kDa protein containing 336 residues

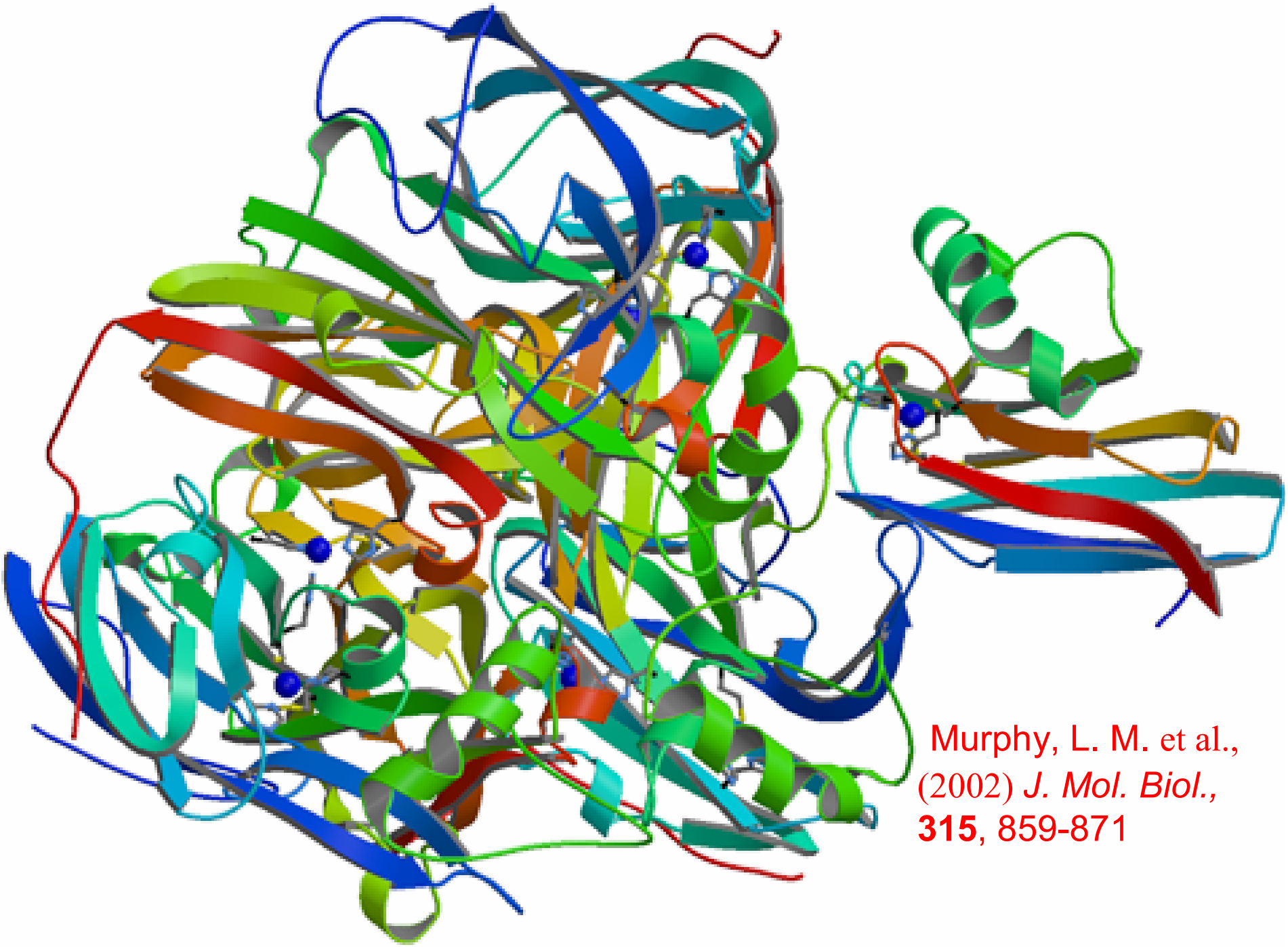
Type 1 Cu is electron donor
Type 2 Cu is catalytic site

$$NO_2^- + e^- + 2H^+ \rightleftharpoons NO + H_2O$$

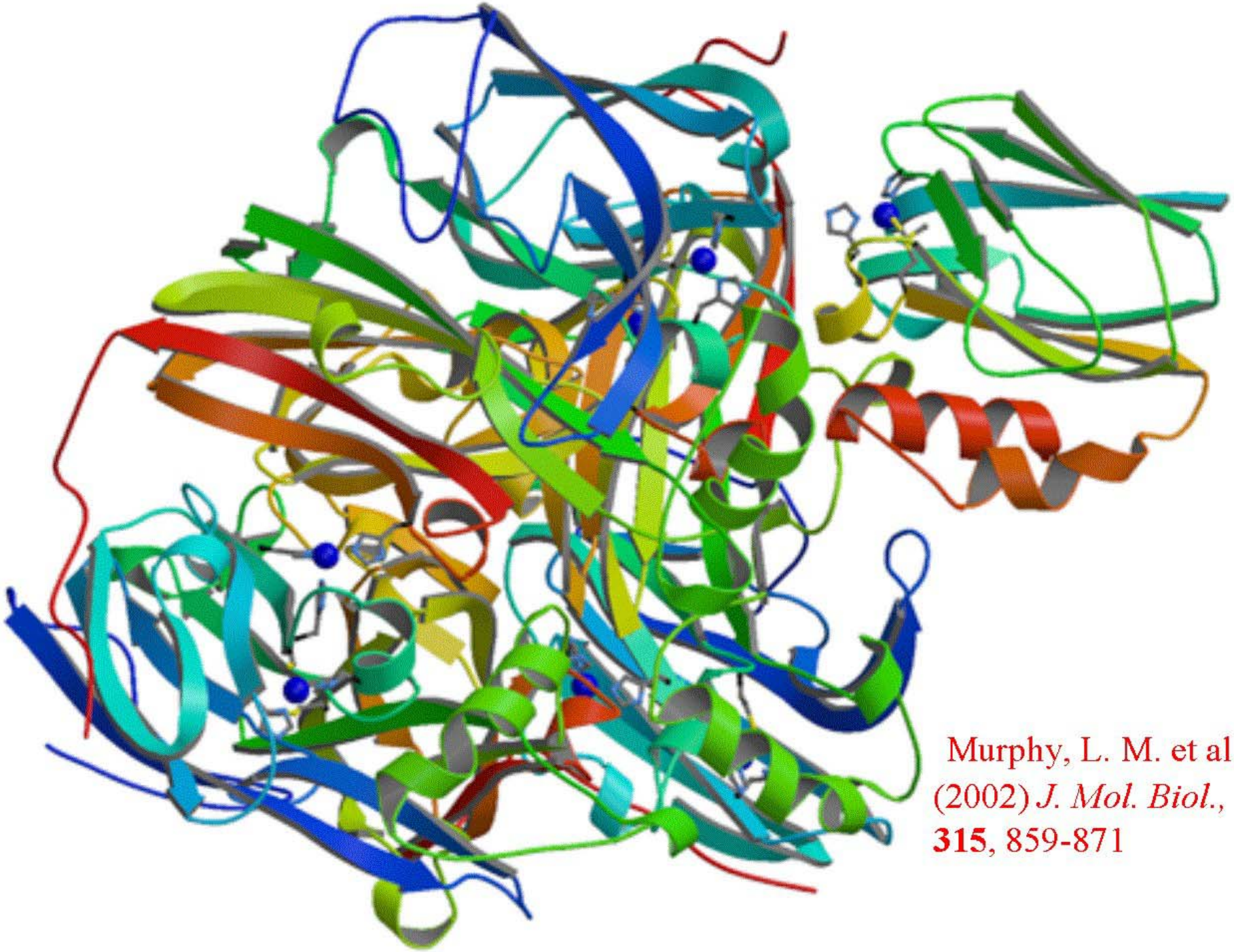
NiR Captures Covers of Recent Journals





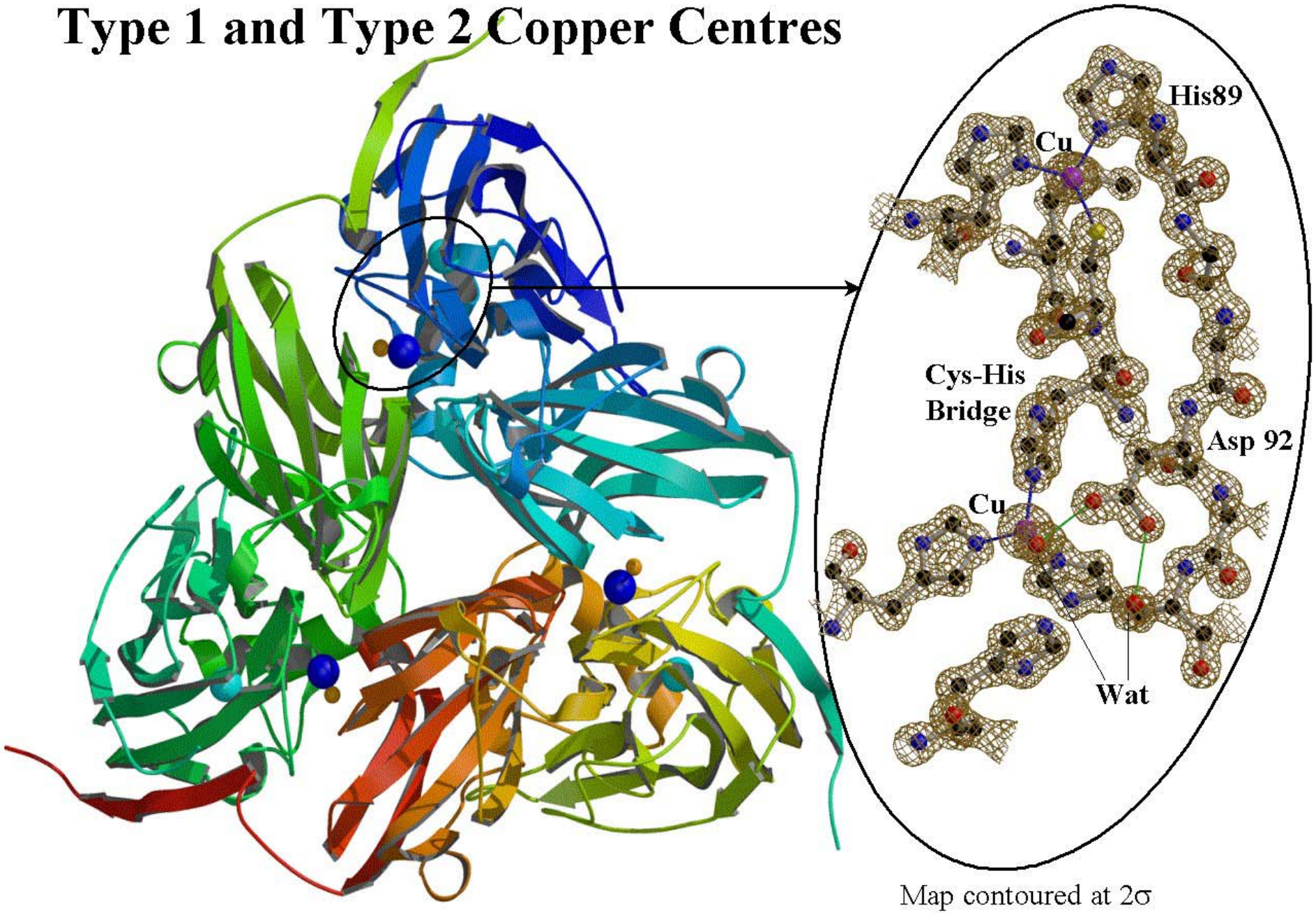


Murphy, L. M. et al.,
(2002) *J. Mol. Biol.*,
315, 859-871



Murphy, L. M. et al.,
(2002) *J. Mol. Biol.*,
315, 859-871

Type 1 and Type 2 Copper Centres

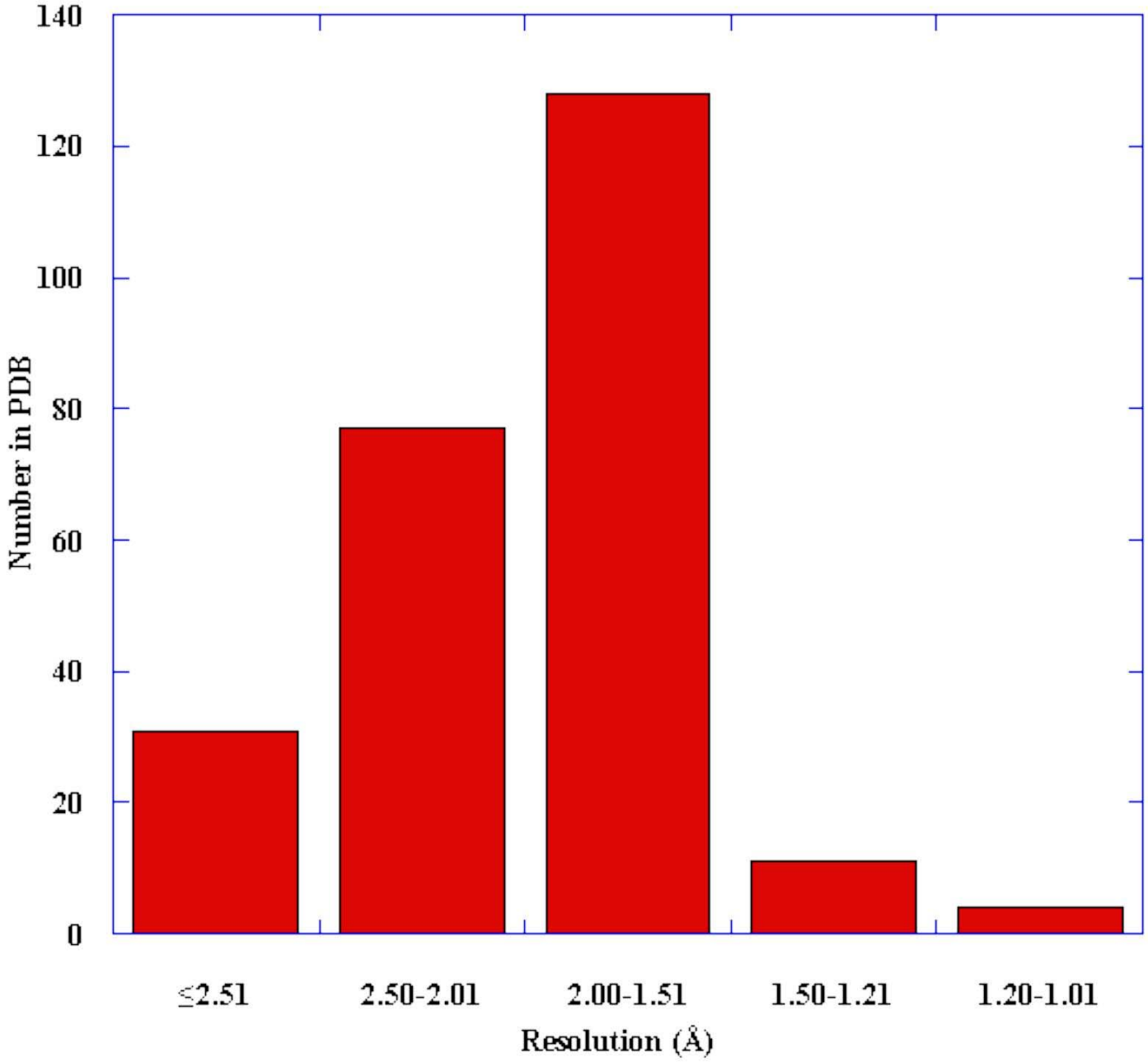


Map contoured at 2σ

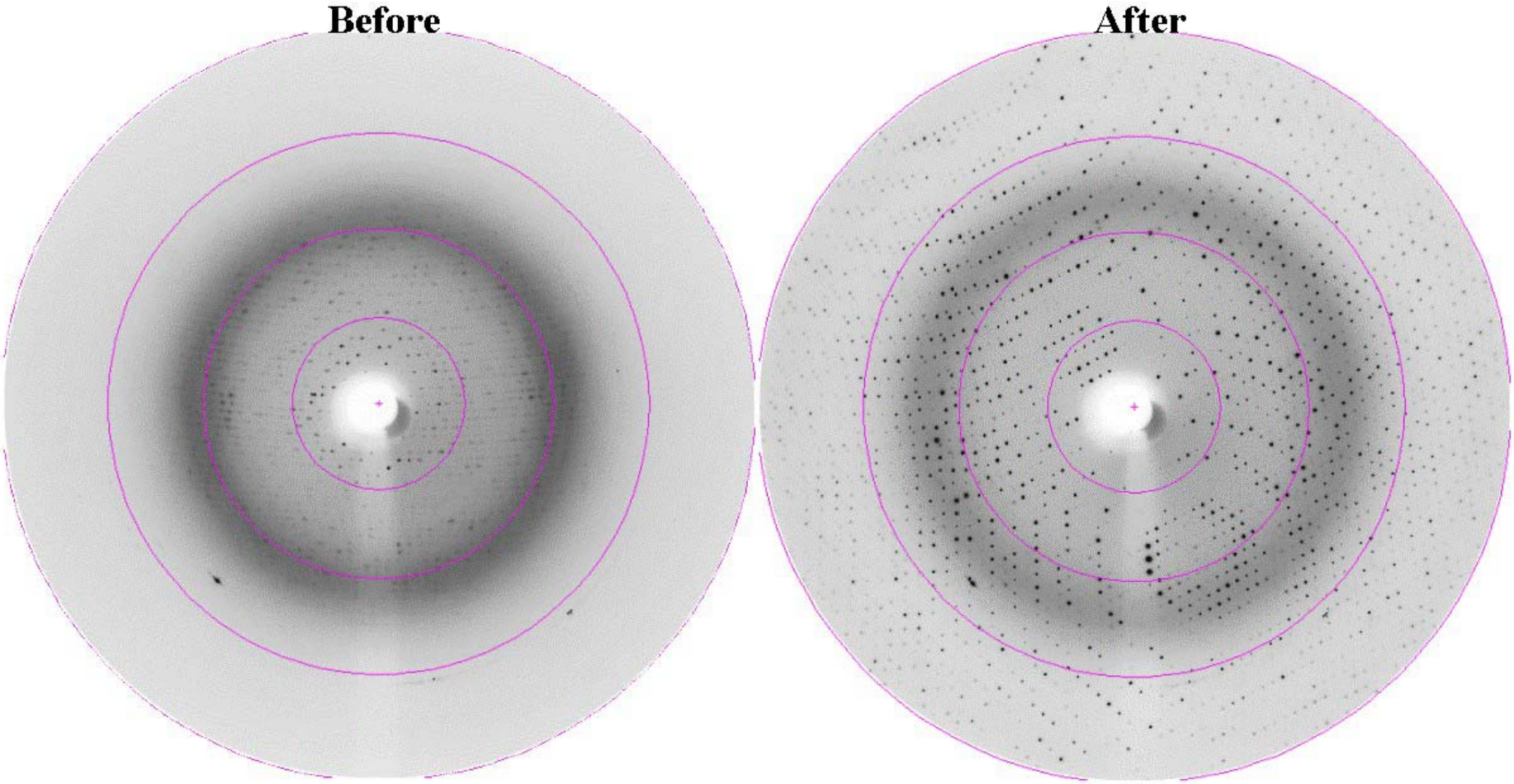
Why Metalloproteins & Atomic Resolution?

- **Metalloproteins make up some 30% of known genomes.**
- **Redox & ligand chemistry of biological metals is exploited to perform a wide variety of chemical reactions.**
- **To understand how the chemistry of metals is utilised to perform a particular function, very high resolution structural data are imperative.**
- **Small changes at the metal centre and its ligands can be amplified by the protein to perform complex biological processes. This is most beautifully illustrated in the case of Haem proteins where small changes at the Fe results in large changes elsewhere which control the allosteric mechanism.**
- **Despite tremendous efforts, only a few crystallographic structures are available at atomic resolution i.e. <1.2Å.**

Resolution of Cu-protein Structures in the PDB



In situ Annealing of NiR Crystals



Resolution ~2Å
Mosaicity 1.5°
Slightly twinned

Resolution 1.04Å (refined)
Mosaicity 0.33°
Twinning lost

Ellis MJ, et al., (2002) *Acta Cryst.* **D58**, 456-458

Structures Determined

Native Protein	**1.04Å resolution**	**SRS Stn. 14.2**
Highest resolution structure of any multicopper oxidase		
Recombinant Protein	***1.15Å resolution***	**SRS Stn. 9.6**
Apo-Recombinant	**1.9Å resolution**	**PF BL6A**

Regulation of T1 Cu Redox Potential

Met144Ala	**2.2Å resolution**	**SRS Stn. 9.6**
Met144Gln	**1.9Å resolution**	**SRS Stn. 9.6**
Met144Leu	**1.9Å resolution**	**SRS Stn. 9.6**

Electron Gating from T1 Cu to T2 Cu

Cys130Ala	**1.35Å resolution**	**SRS Stn. 9.6**
His129Val	**1.9Å resolution**	**SRS Stn. 9.6**

Coupling of Redox Centres - Proton abstraction

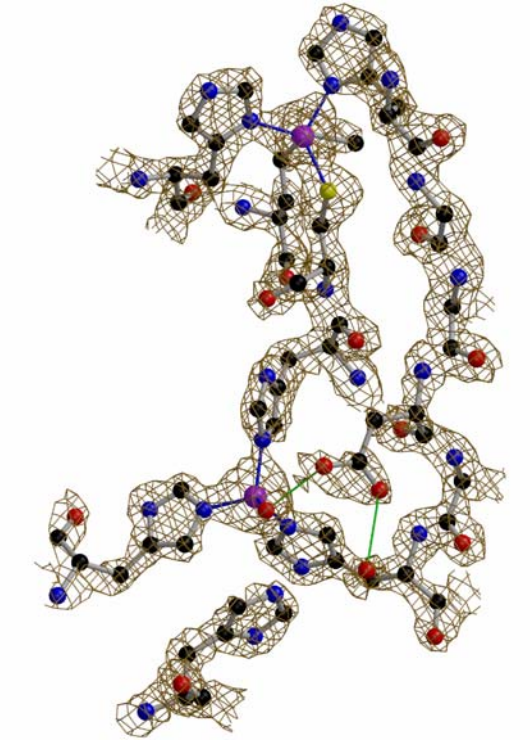
Asp92Asn	**1.9Å resolution**	**SRS Stn. 9.6**
Asp92Glu	***1.12Å resolution***	**SRS Stn. 9.5**

Proton Pumping

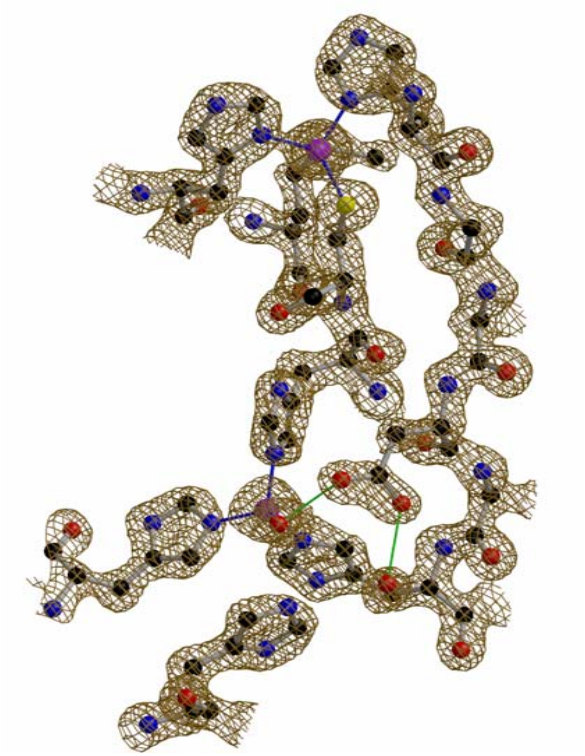
His254Phe	**1.85Å resolution**	**SRS Stn. 9.5**

inactive **reduced activity compared to native** **increased activity compared to native**

Benefits of Atomic Resolution : Map Quality



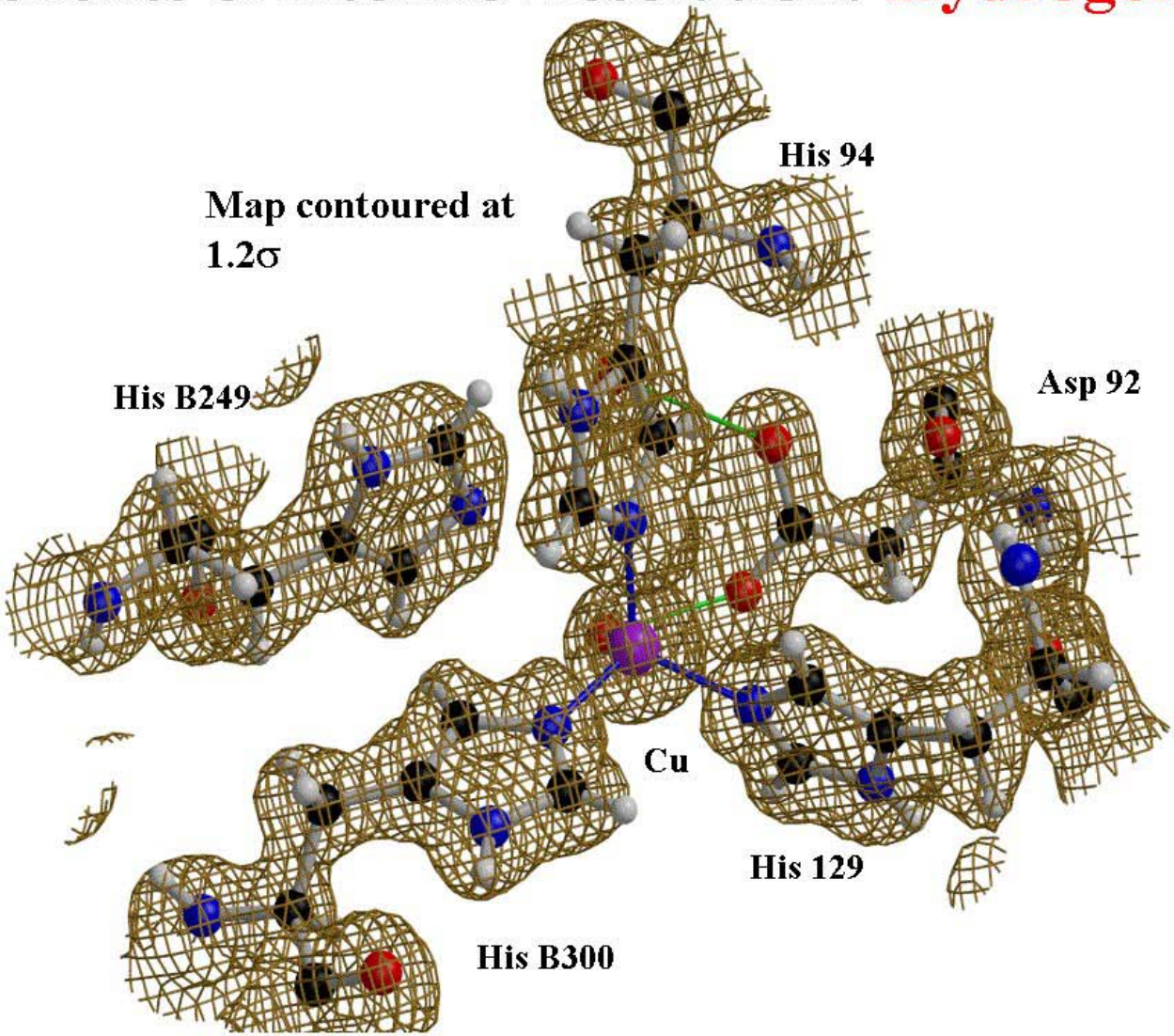
2Å resolution
25023 reflections



1.5Å resolution
58428 reflections

1.04Å resolution
161347 reflections

Benefits of Atomic Resolution: Hydrogen Atom Positions



(Nitrite reductase at 1.04Å)

Benefits of Atomic Resolution: Accuracy of Metrical Information

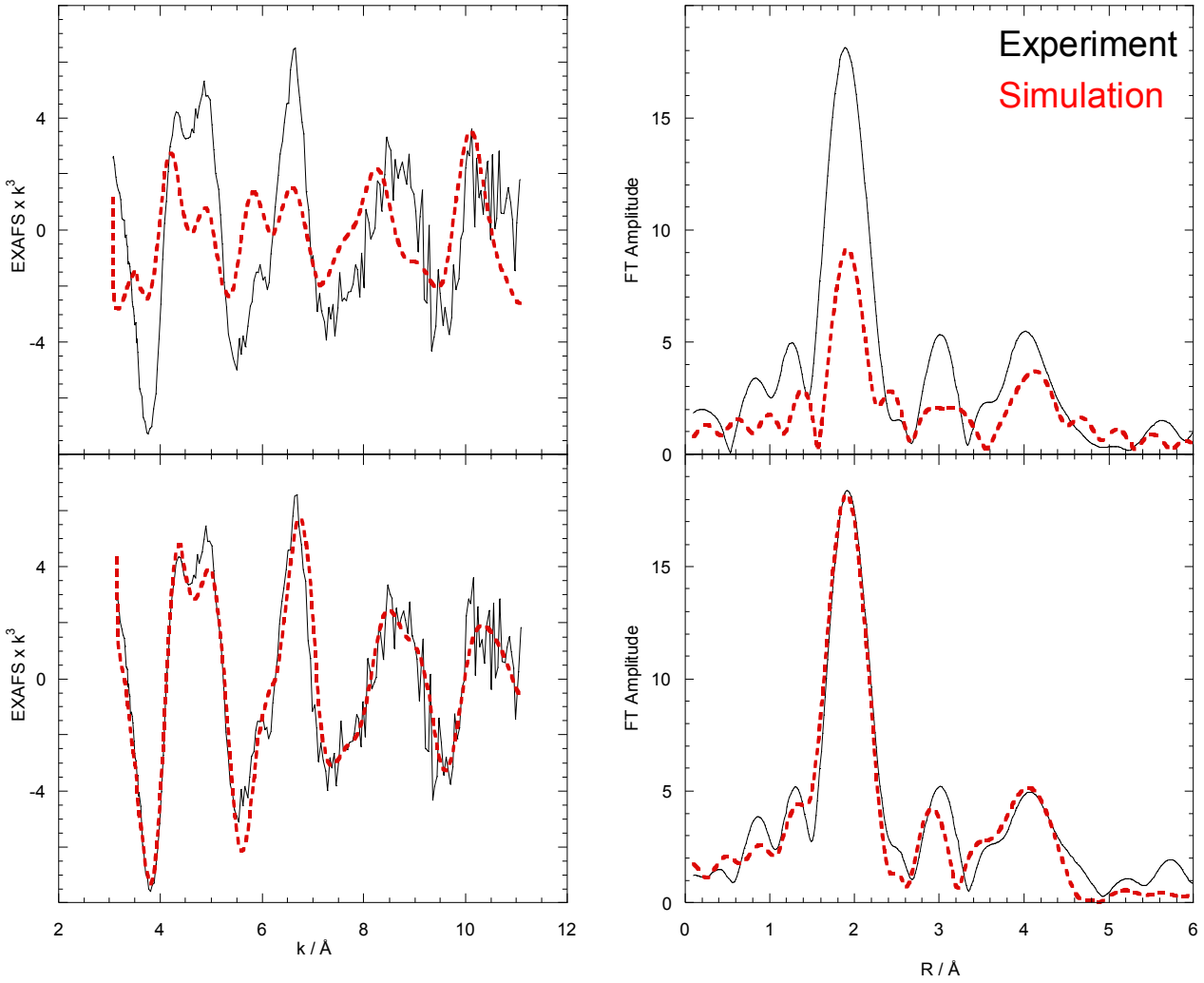
NiR type 2 Cu site EXAFS: simulations using crystallographic coordinates

1.90 Å resolution (1hau.pdb)

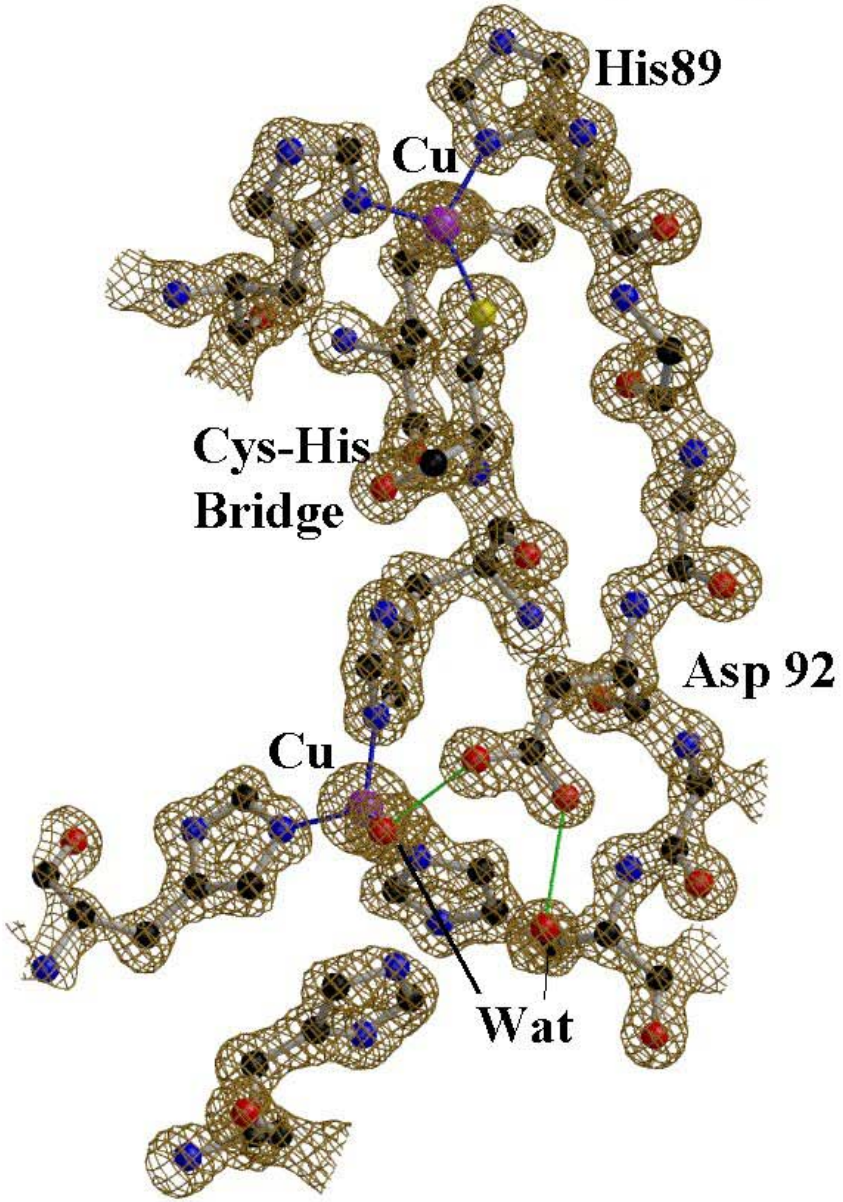
Fit index = 5.4

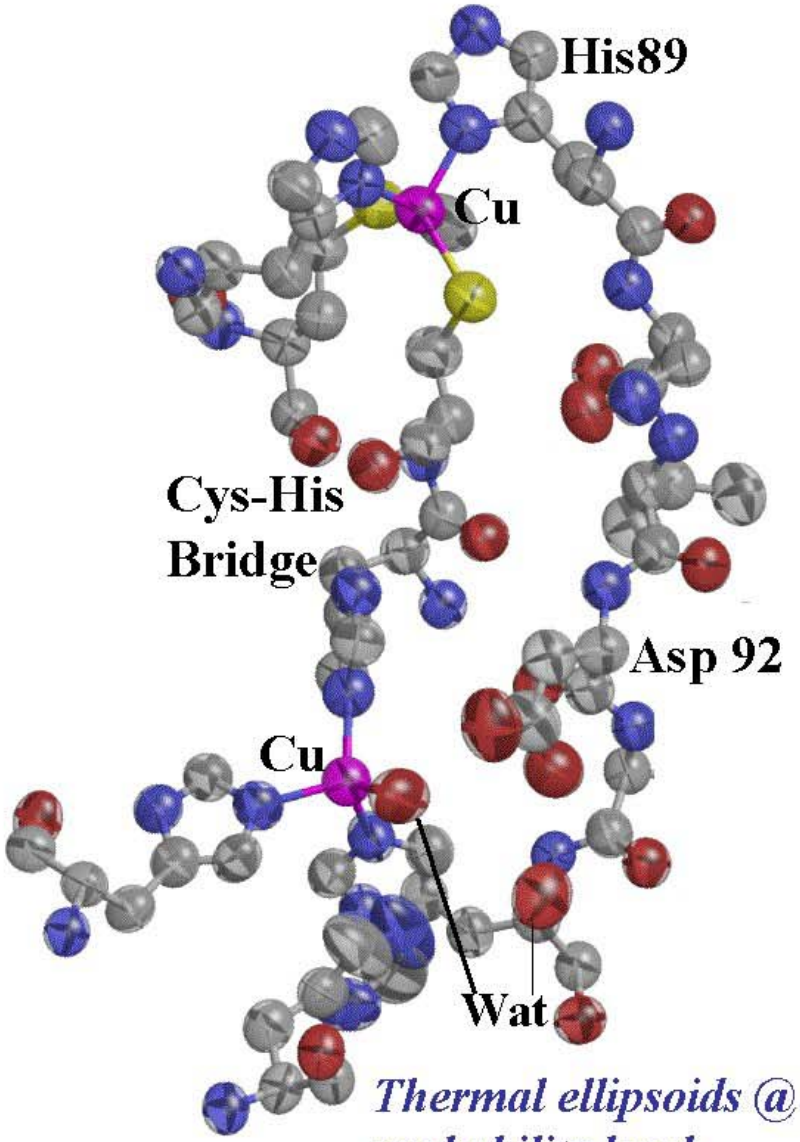
1.04 Å resolution

Fit index = 0.6



Coupling of Redox Centres

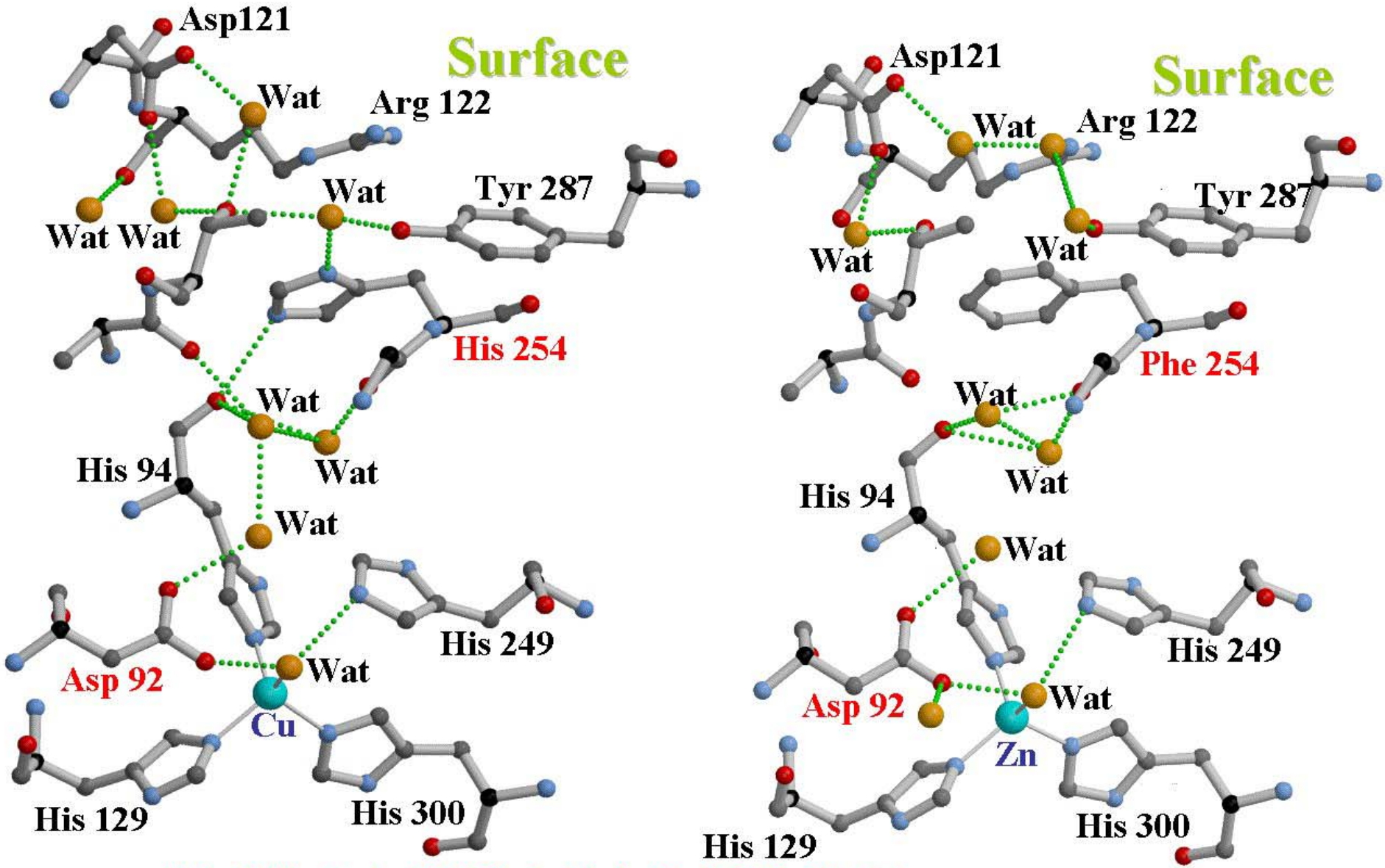




Thermal ellipsoids @ 50% probability level

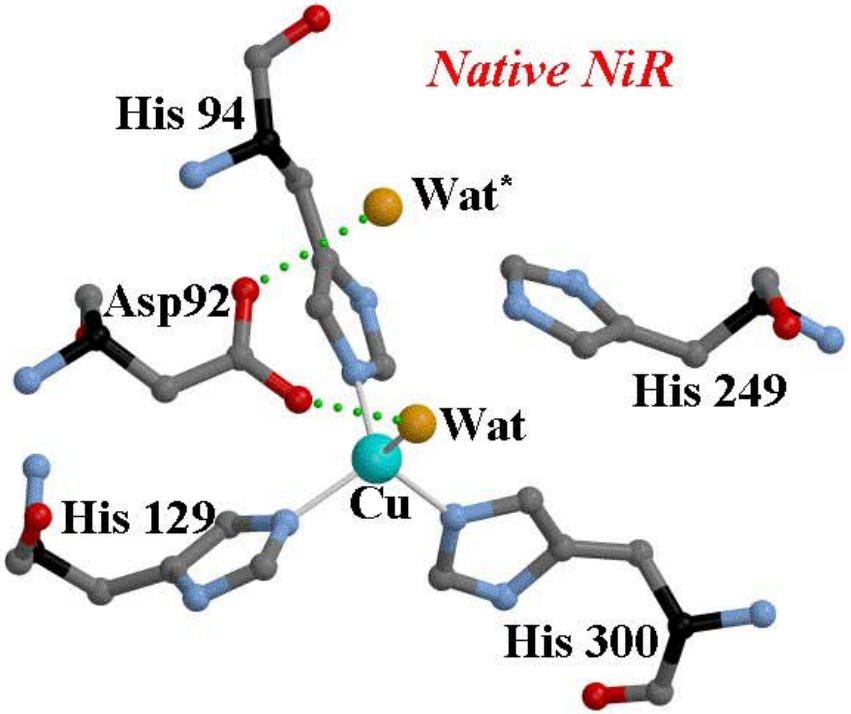
Strange RW, et al., (1999) *J. Mol. Biol.* **287**, 1001-1009

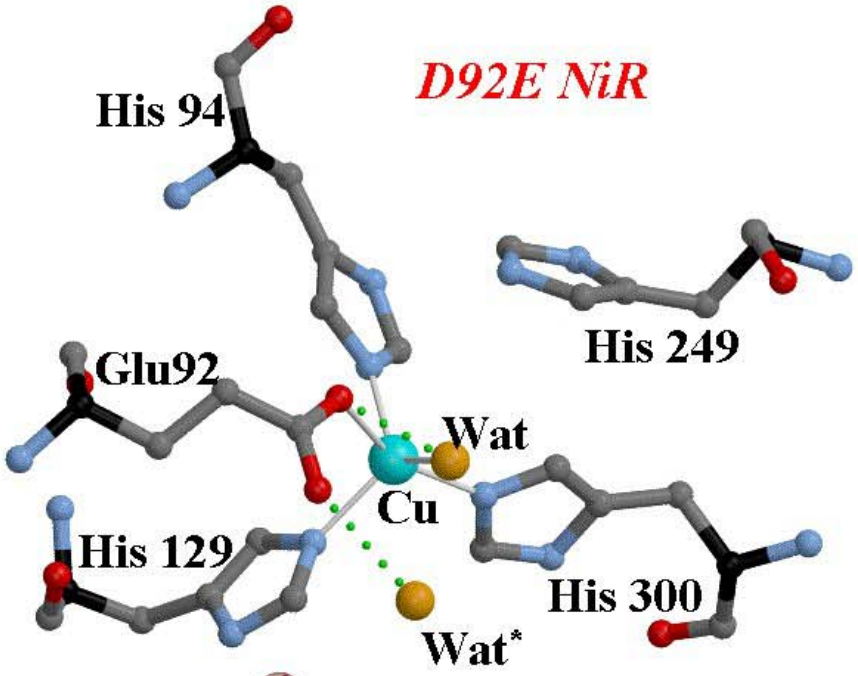
Proton Pump



Ellis MJ, et al., (2002) *J. Mol. Biol.* **316**, 51-64

Proton abstraction - D92E Mutation



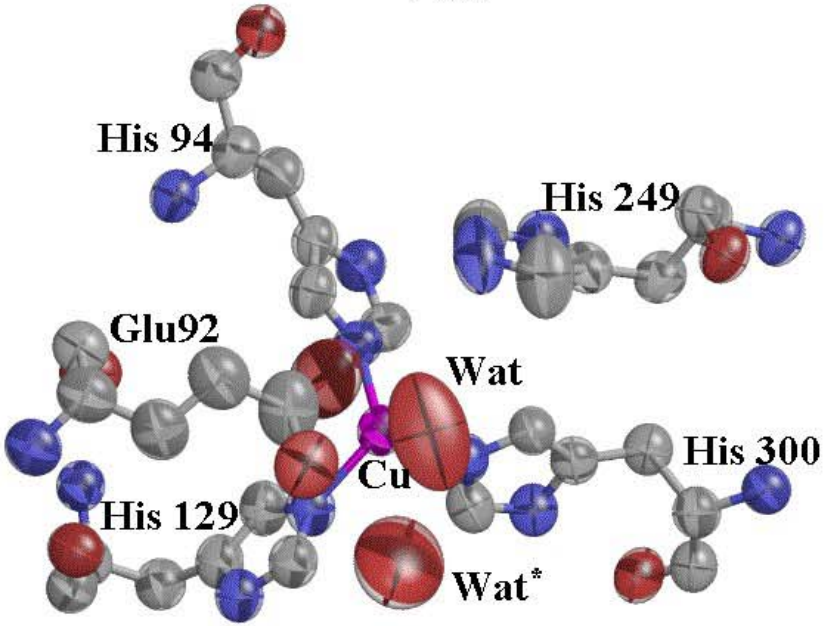


Mutation Asp92Glu has no activity.

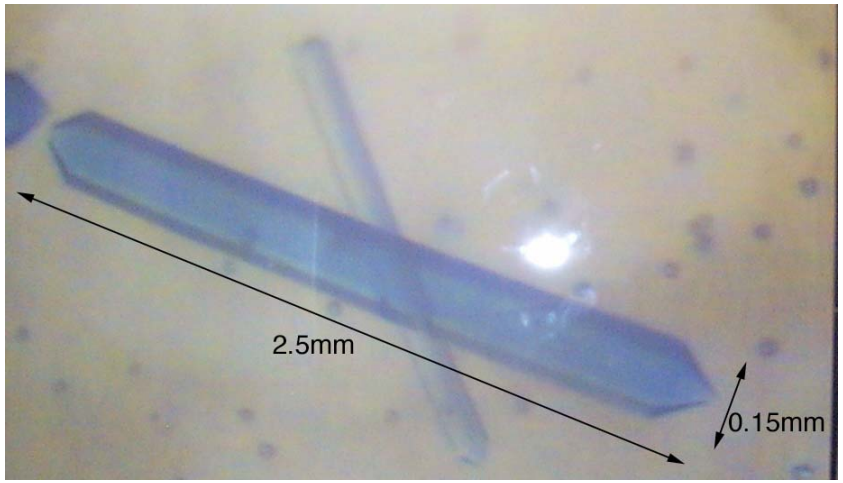
Glu92 directly ligates T2Cu - preventing NO_2^- from co-ordinating to T2Cu.

Wat* moves 6Å from position in native enzyme breaking H-bonded water network.

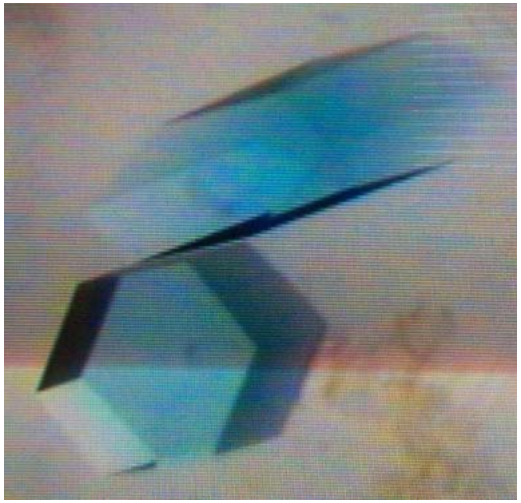
Wat at T2Cu is highly disordered due to Glu92 co-ordination



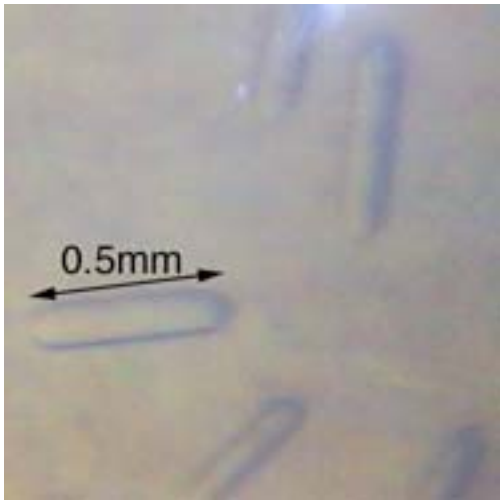
Crystals of NiR



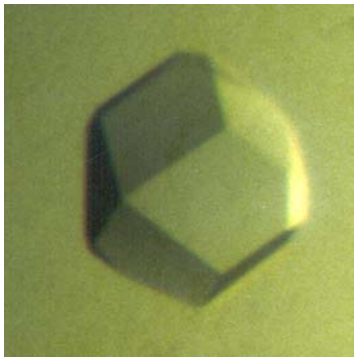
D92E 1.1Å resolution



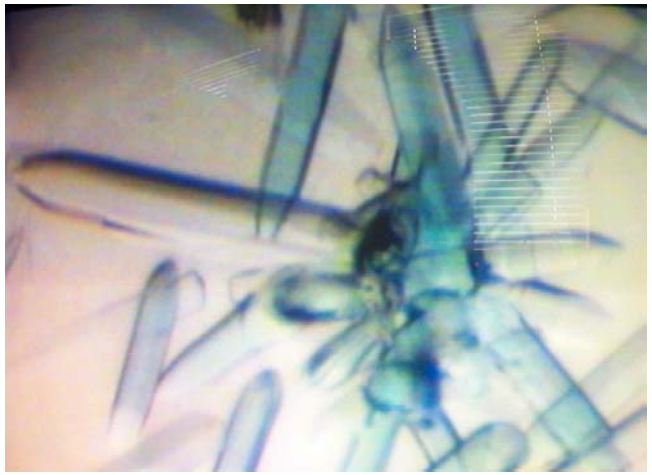
Native 3Å resolution



C130A 1.35Å resolution



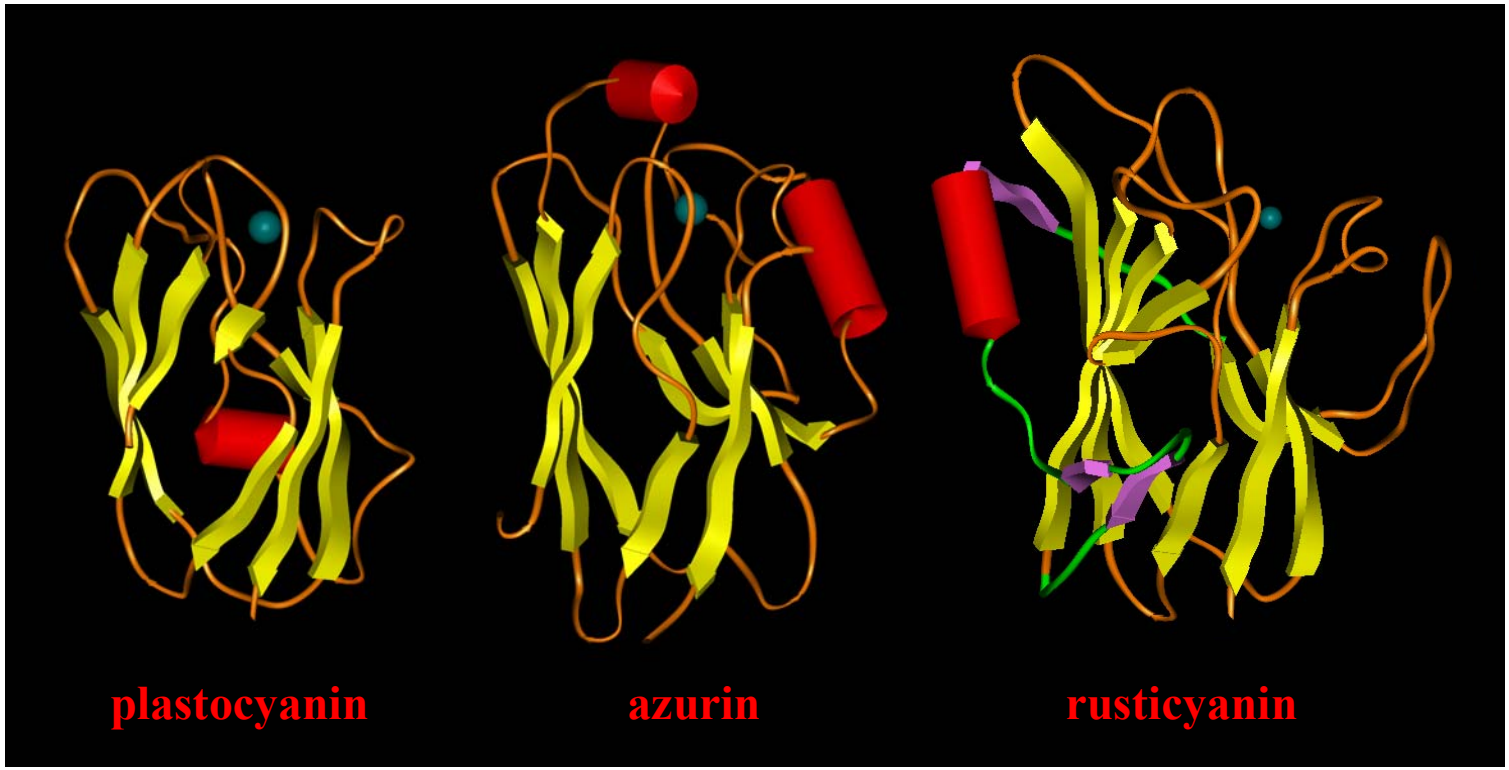
Native 5Å resolution



Native 1.04Å resolution

Combined CD/X-ray Scattering study of

Rusticyanin - a type I “blue” Cu protein

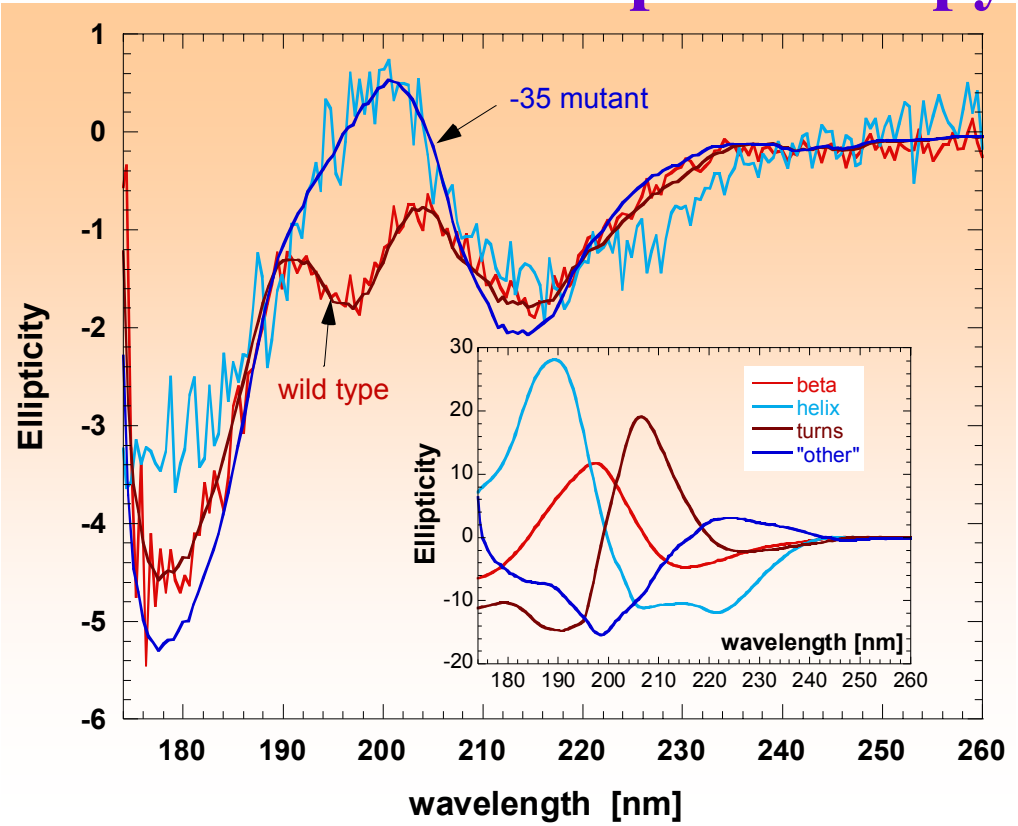


extreme properties of rusticyanin: *high redox potential* (680 mV) and *tolerance of a wide pH range* (ca 1.5-10)

Does the N-terminal “belt” make the protein acid-stable?

Circular-Dichroism Spectroscopy

–35 mutant does not bind copper and is soluble only at pH5 or lower

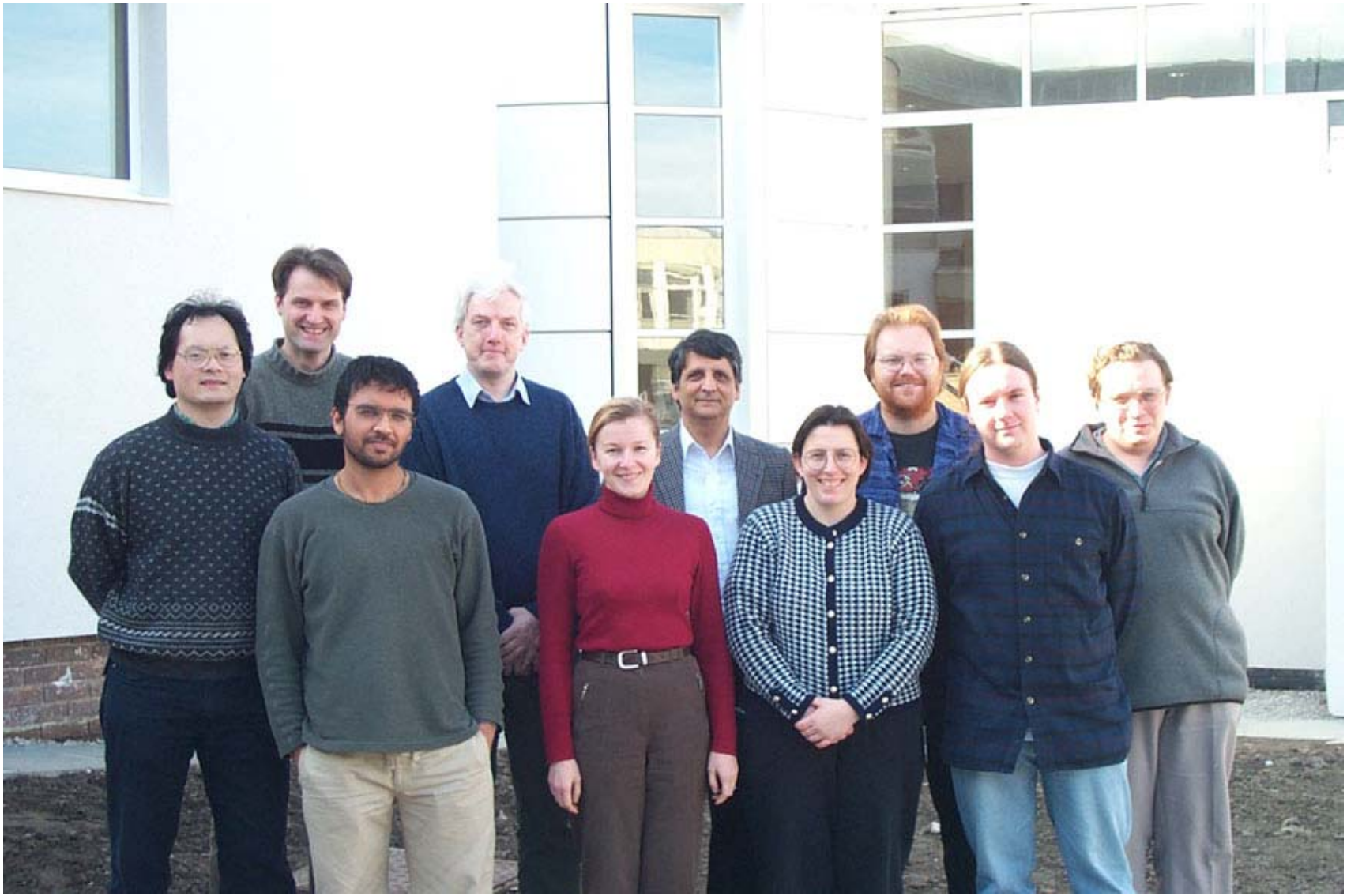


Fit Results

	wild type	-35 mutant
helix	6%	0%
strand	39%	53%
turn	15%	11%
"other"	40%	36%

folded into β-barrel

The Molecular Biophysics Group



North West Structural Genomics Centre

Daresbury Laboratory

THE UNIVERSITY of MANCHESTER

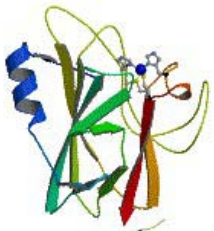
THE UNIVERSITY of LIVERPOOL

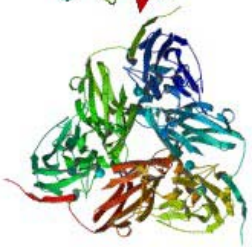
AstraZeneca

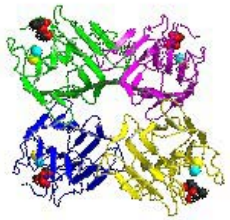
JMU
Liverpool John Moores University

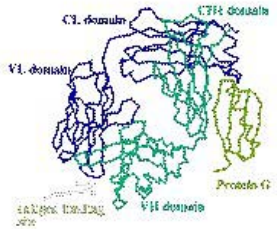
UNIVERSITY OF LEEDS

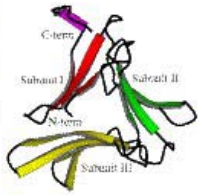
UMIST

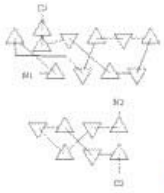


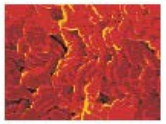


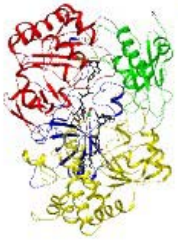












Samar Hasnain
June 2002

bbsrc
EPSRC
Engineering and Physical Sciences Research Council

Medical Research Council MRC
CLRC

• Structural Genomics is a new and rapidly growing interdisciplinary research aimed at extending the vast array of genomic sequence data with a comparable, systematic database of protein structures
• Synchrotron Radiation based X-ray Crystallography is unique in providing very accurate high resolution structures of proteins and their complexes
• Worldwide, structural genomics has been made possible by recent rapid progress in several related key technologies. These include synchrotron based MAD (Multiple wavelength Anomalous Dispersion) phase determination, cloning and recombinant expression, genome sequencing projects and bioinformatic methods of fold assignment and function prediction
• In early 2000, a consortium of several groups in the North West of England proposed the establishment of a structure genomics centre (NWSGC) to exploit the unique resources offered by their close proximity to the UK's current synchrotron radiation source (SRS)

• NWSGC members have brought together expertise in X-ray protein crystallography, pathogens biology, membrane proteins, metalloproteins and thus initiated the first structural genomics effort in the UK.

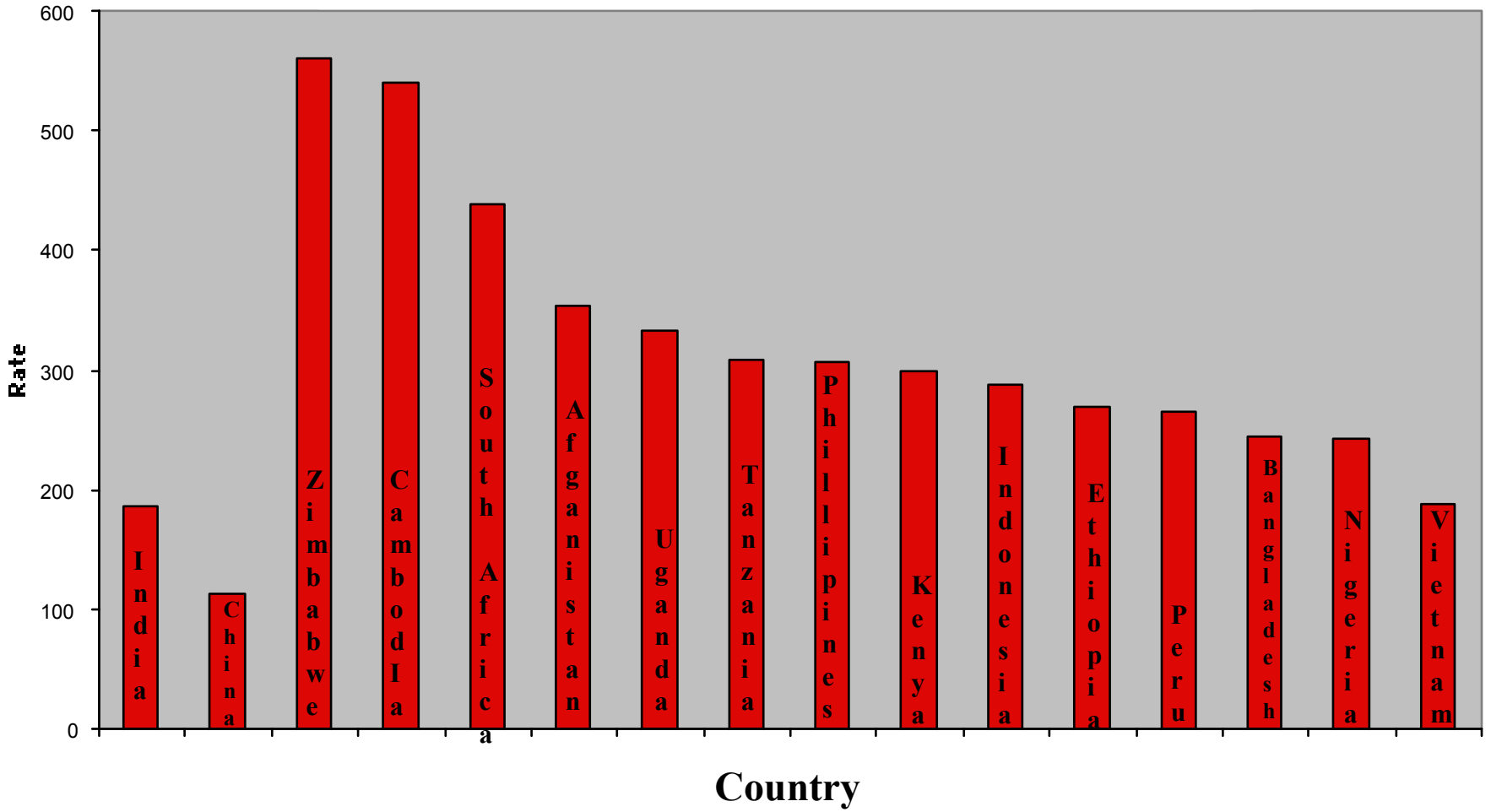
• In summer 2001, the Leeds Bioinformatics group joined.

• In July 2001, UK's research councils (BBSRC, EPSRC & MRC) funded a 5 year grant "NW STRUCTURE GENOMICS CENTRE'S HIGH THROUGHPUT MAD BEAMLINE FOR *PATHOGENS GENOMES*" with NWSGC having 67% share in years 4 &5.

• NWSGC has selected to join the International TB Structural Genomics effort and has established close links with the RIKEN structural genomics programme.

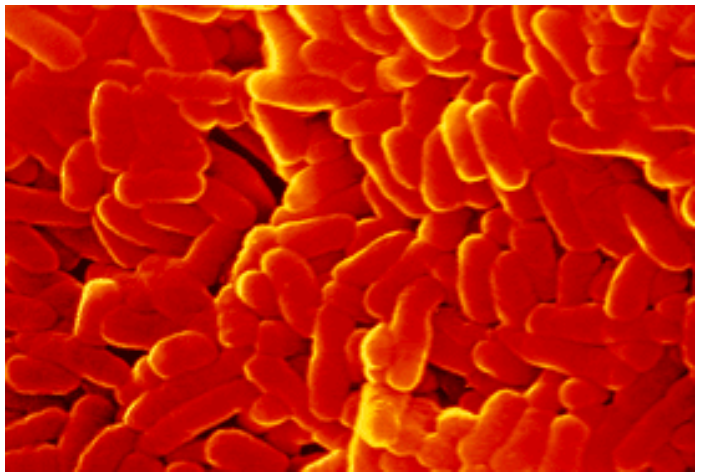
• June 2002, we are participating in two EOI's for EU framework VI proposal; one entitled 'Tuberculosis Drug Development' and other entitled 'Structural Genomics of Metalloproteins : Function and Mis-function'.

TB incidence per 100,000 people



Mycobacterium tuberculosis

- 1/3 of world population currently infected
- 5-10% infective people develop symptoms
- TB kills ~3 million people each year. 8 million annually develop symptoms
- India has the highest incidence of TB (about 1.83 million cases in 1998) and accounts for 23% of the world's cases.
- China is a close second with about 1.41 million cases or 17% of the world's cases.



- Globally, 8% of TB cases are due to HIV
- in sub-Saharan Africa the figure has risen to 80%.

- TB is resistant to a range of antibiotics
- Cell membrane acts as permeability Barrier
- Contains drug modifying enzymes

- Vaccine (BCG): important for control
- Improve efficacy by genetic manipulation
- Attenuated strains devoid of immuno-suppressing genes

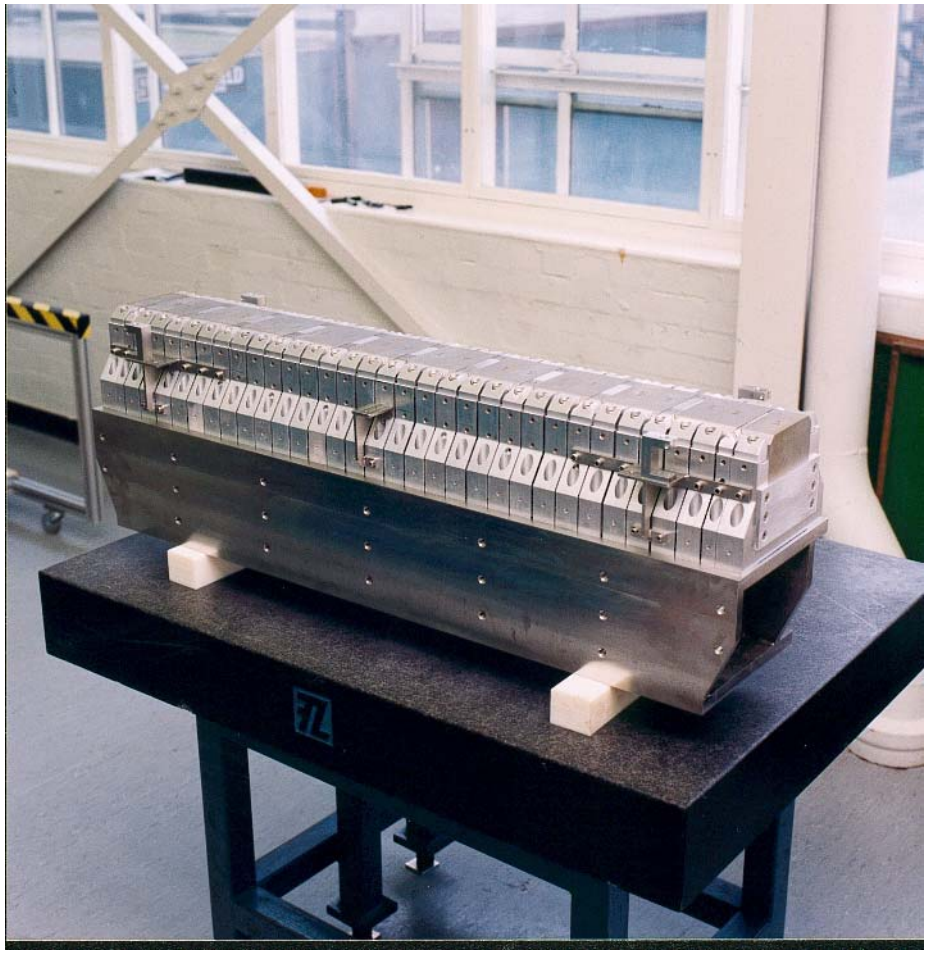
Researcher	Target	Protein	Status
Samar Hasnain	Rv0185	Hypothetical metalloprotein	Crystallising
	Rv2547	Hypothetical metalloprotein	Crystallising
	Rv2865	Hypothetical metalloprotein	Cloned (II)
	Rv0359	Hypothetical metalloprotein	Ligation
	Rv2776c	Probable oxidoreductase	Cloned (II)
	Rv0247c	Probable Iron-sulphur protein	Ligation
	Rv2718c	Probable metalloprotein	Cloned (II)
John Helliwell	Rv0510	hemC, porphobilinogen deaminase	Targeted
	Rv3307	deoD, purine nucleoside phosphorylase	Targeted
Jordi Bella	Rv0171	Part of mce1 operon	Ligation
	Rv1693	Hypothetical protein	Ligation
	Rv1942c	Conserved hypothetical protein	Ligation
	Rv2305	Hypothetical protein	Ligation
	Rv3070	Unknown membrane protein	Ligation
Colin Reynolds	Rv3852	Histone like protein	Targeted
	Rv2986c	Histone like protein	Ligation
	Rv1388	Integration host factor	Targeted
	Rv1407	Similar to other Fmu proteins	Targeted

Researcher	Target	Protein	Status
Lydia Tabernero	Rv0153c	Putative tyrosine-phosphatase	Ligation
	Rv0505c	serB, probable phosphoserine phosphatase	Ligation
	Rv196	part of mce3 operon	Ligation
	Rv2234	ptpA, tyrosine-phosphatase	Ligation
	Rv3042c	serB2, phospherine phosphatase	Ligation
	Rv3628	ppa,inorganic phosphatase	Ligation
	Rv3867	conserved hypothetical protein	Cloned (II)
Mark Ellis	Rv2060	Conserved hypothetical protein	Ligation
	Rv2229c	Putative zinc metalloprotein	Ligation
	Rv2711	ideR, iron dependent repressor	Cloned (II)
	Rv3207c	Putative zinc metallopeptidase	Expressed
	Rv3836	Putative zinc metallopeptidase	Expressed
Michele Cianci	Rv3717	Involved in cell biosynthesis	Targeted
	Rv3919	Involved in cell biosynthesis	Targeted
	Rv2981c	Involved in cell biosynthesis	Targeted
	Rv3712	Involved in cell biosynthesis	Targeted

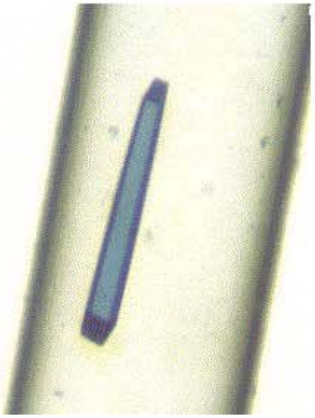
Beamline 10 Statistics

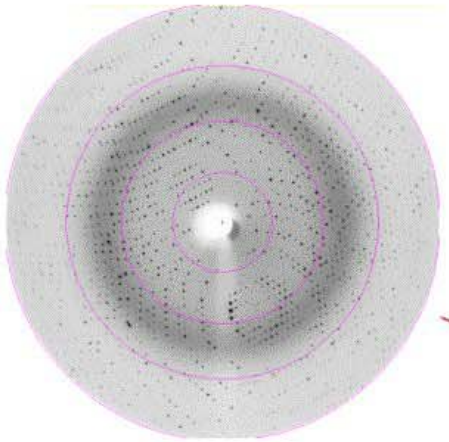
Peak Field	2.4 T
Period	220 mm
Number of Full Strength Poles	9
End Pole Field	1.9 T
Number of End Poles	2
Minimum Magnet Gap	20 mm
Flux at 12.7 keV	4×10^{13}
Flux Advantage over SRS BM	x ~60

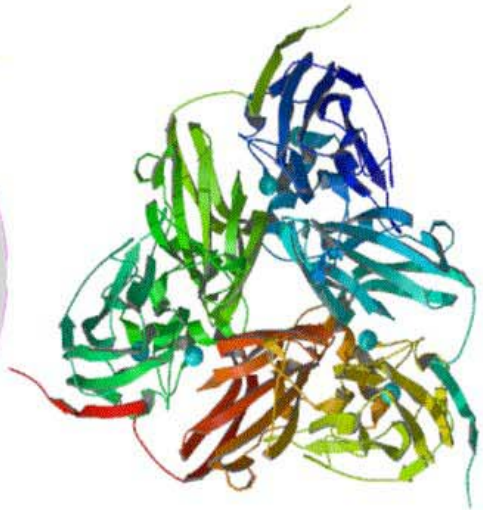
Magnet array ready for mounting on MPW drive mechanism

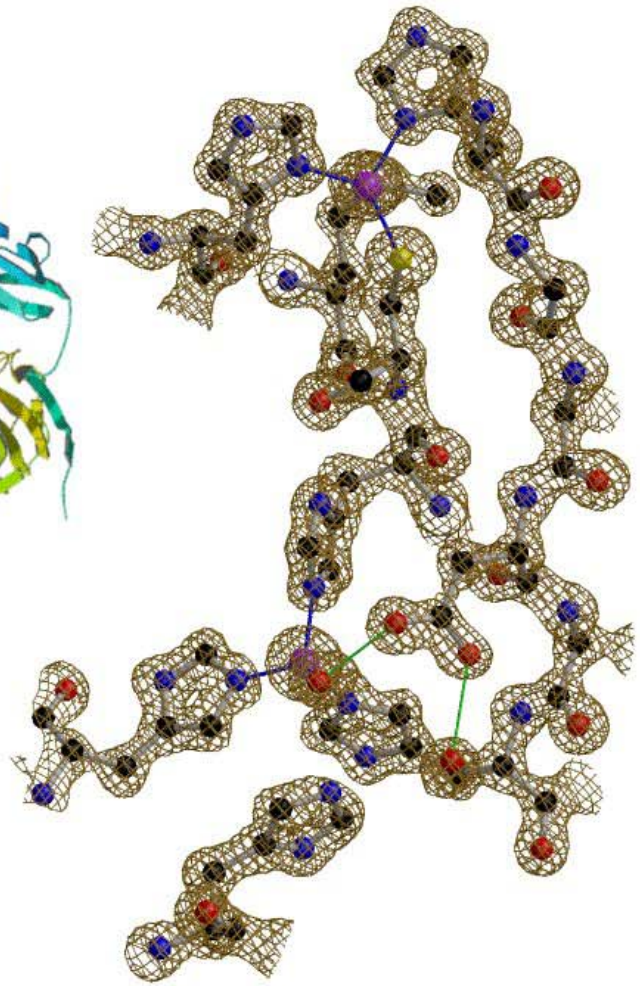


Structure Determination Centre









Characterize all crystals by X-ray diffraction

Collect full X-ray diffraction data sets from the best crystals

Perform crystallographic analysis from data reduction through to final structure refinement

Mycobacterium tuberculosis Structural Genomics Consortium

NESBiC
North of England Structural Biology Centre

Co-operative development with NWSGC

Daresbury Laboratory

SSRL

RIKEN 理研

Structurome Research Group

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Joint Coordinators

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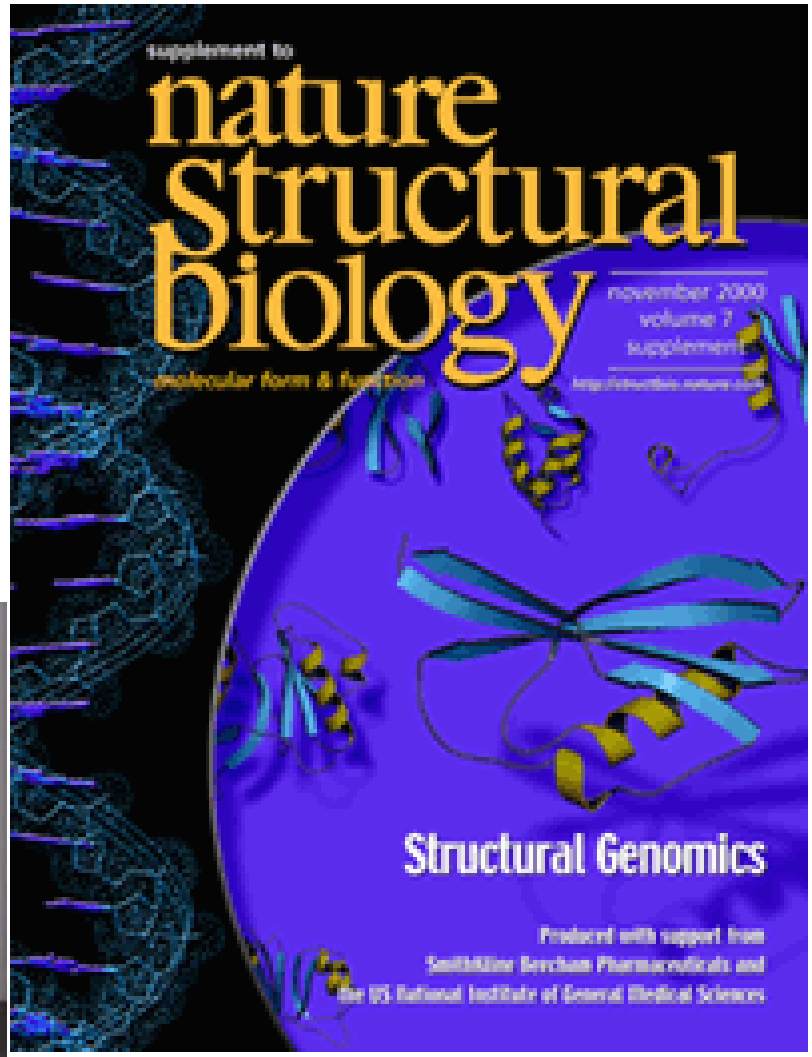

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