Using synchrotron radiation to describe protein catalytic mechanisms

Tuesday 23 Nov 2010 at 17:30 (00h20')

Protein crystallography has been the most successful technique for determining 3D structures from protein molecules. However, the technique has several disadvantages including the need of a crystalline sample, the unavoidable radiation damage and a static, averaged on time and space, resulting model. This is particularly relevant because it has been demonstrated that proteins are dynamic entities whose catalytic properties rely remarkably in their dynamic behavior. Several strategies (for example chemical and physical trap) have been developed in order to extract this dynamical information from protein crystals. We take advantage of the X-ray induced radiation damage to characterize intermediaries of the catalytic cycle from redox enzymes of the Multicopper Oxidase family.

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Presenter : Mr. DE LA MORA, Eugenio (Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico)

Using synchrotron radiation to describe enzyme catalysis

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Instituto de Biotecnología, UNAM

The plan

- Introduction
    - Why do we care about biomolecules?
    - What is biomolecular crystallography?
    - Relevance of X-ray crystallography
- Disadvantages
    - Radiation Damage
- Taking advantage of radiation damage (redox proteins)

Why do we care about biomolecules?

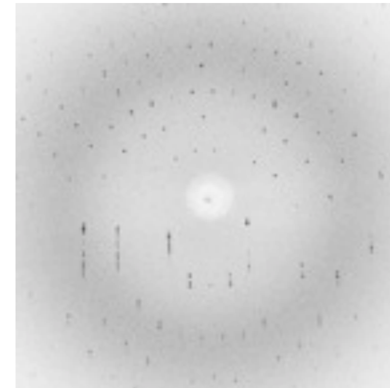
Medical interest (degenerative diseases, virus, etc.)

Complex and efficient machines subjected to evolution
(Ribosome, ATPase)

Nanomaterials (DNA wires, drug delivery, etc.)

*Three dimensional information is necessary because the function is determined by structure

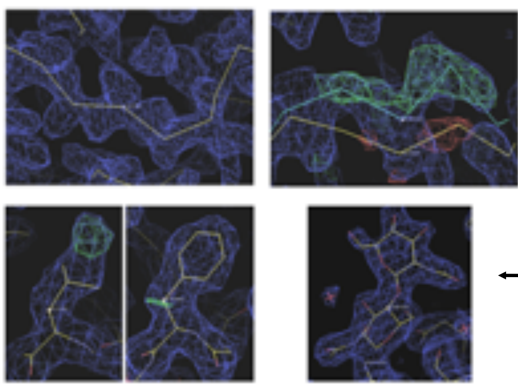
X-ray protein crystallography



Protein Purification

Crystallization

Diffraction



on

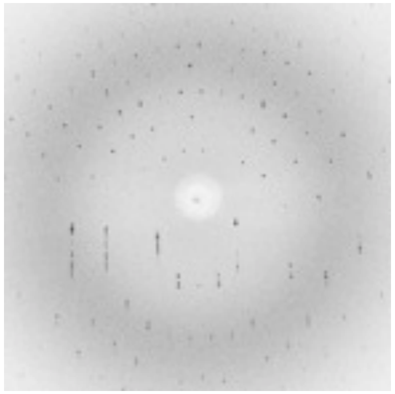
Molecular replacement

Isomorphous replacement

Anomalous dispersion

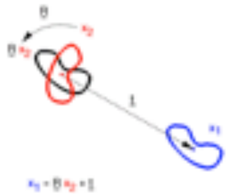
Refinement

X-ray protein crystallography

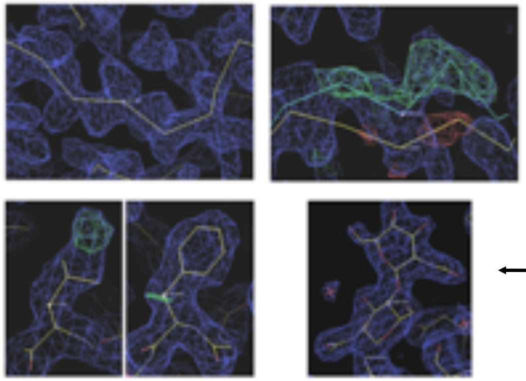


Protein Purification
Diffraction

Crystallization



Molecular replacement



Isomorphous replacement

on

Refinement

Anomalous dispersion

‘Noble’ technique

http://nobelprize.org/

Noble prizes relevant to crystallography:

1901 Phys Wilhelm Conrad Röntgen ‘for the discovery of the remarkable rays’

1914 Phys Max von Laue ‘for his discovery of the diffraction of X-rays by crystals’

1915 Phys Sir W. H. Bragg and W. L. Bragg ‘for their analysis of crystal structure by means of X-rays’

1946 Chem J. B. Sumner (½) ‘for his discovery that enzymes can be crystallized’

1958 Chem Frederick Sanger ‘for his work on the structure of proteins (insulin)’

1962 Chem M. Perutz and J. Kendrew ‘for their studies of the structures of globular proteins’

Med F. Crick, J. Watson and M. Wilkins ‘for their discoveries concerning the molecular

nucleic acids’

‘Noble’ technique

http://nobelprize.org/

1988 Chem J. Deisenhofer, R. Huber and H. Michel ‘for the determination of the three-dimensional structure of a photosynthetic reaction centre’

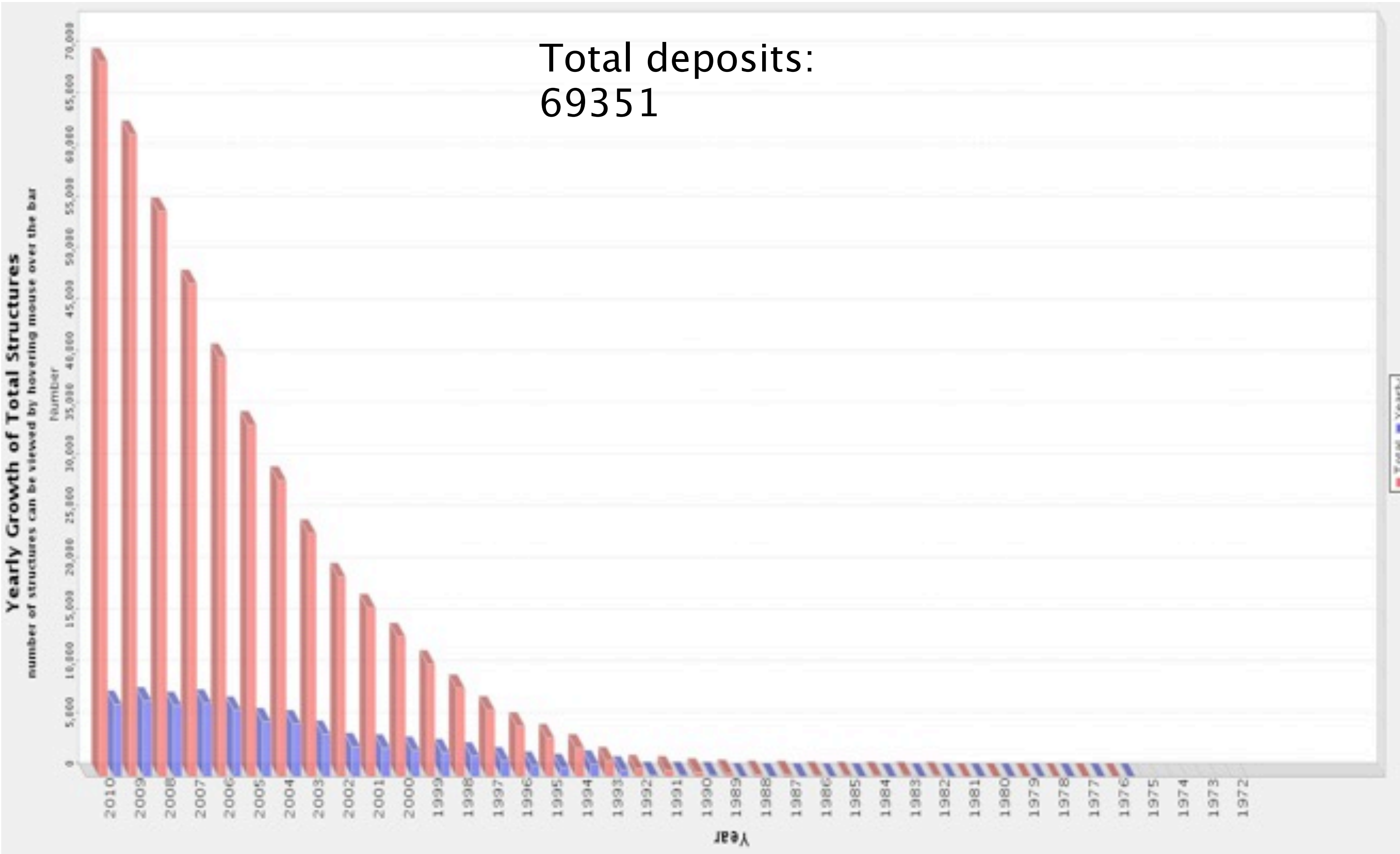
1992 Phys G. Charpak ‘for his invention and development of particle detectors (multiwire proportional chamber) ‘

1997 Chem P. Boyer (¼) and J. Walker (¼) ‘elucidation of the enzymatic mechanism underlying the synthesis of ATP’

2003 Chem R. MacKinnon (½) ‘structural and mechanistic studies of ion channels’

2006 Chem R. D. Kornberg ‘molecular basi transcription’

Protein Data Bank

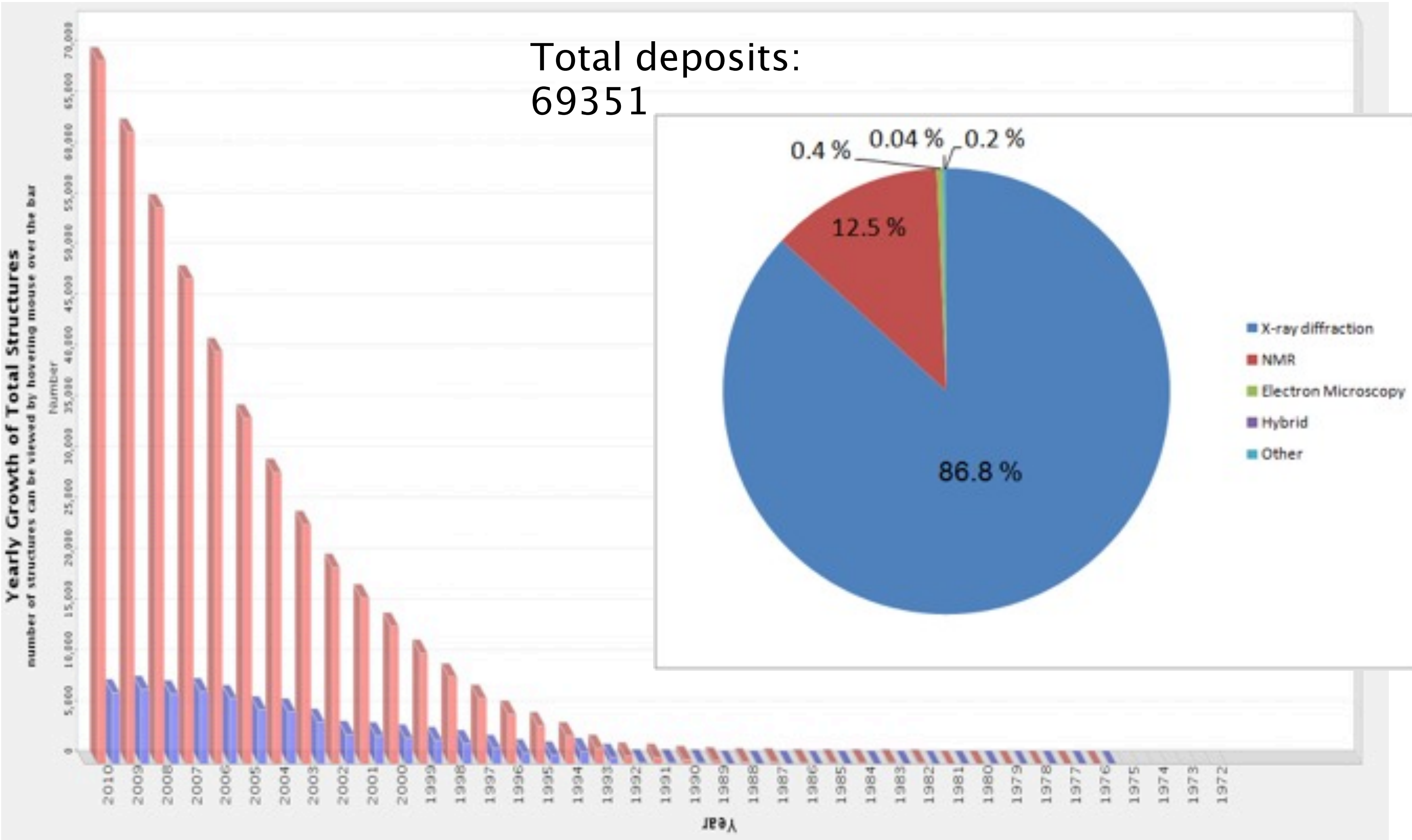


Source: www.pdb.org

UNAM
UNIVERSIDAD NACIONAL DE MEXICO
1910 - 2010

Protein Data Bank

Total deposits:
69351



Source: www.pdb.org

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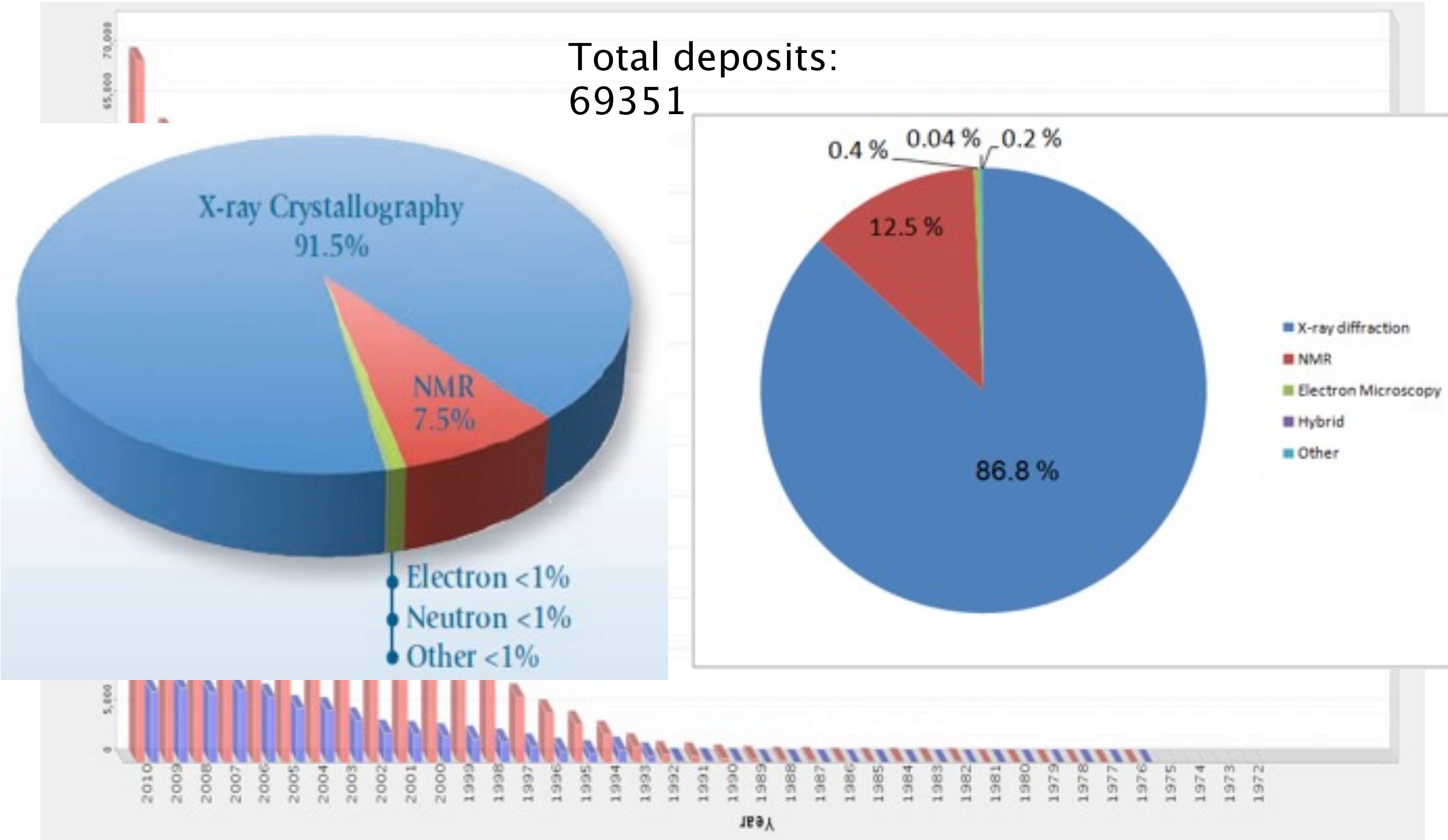
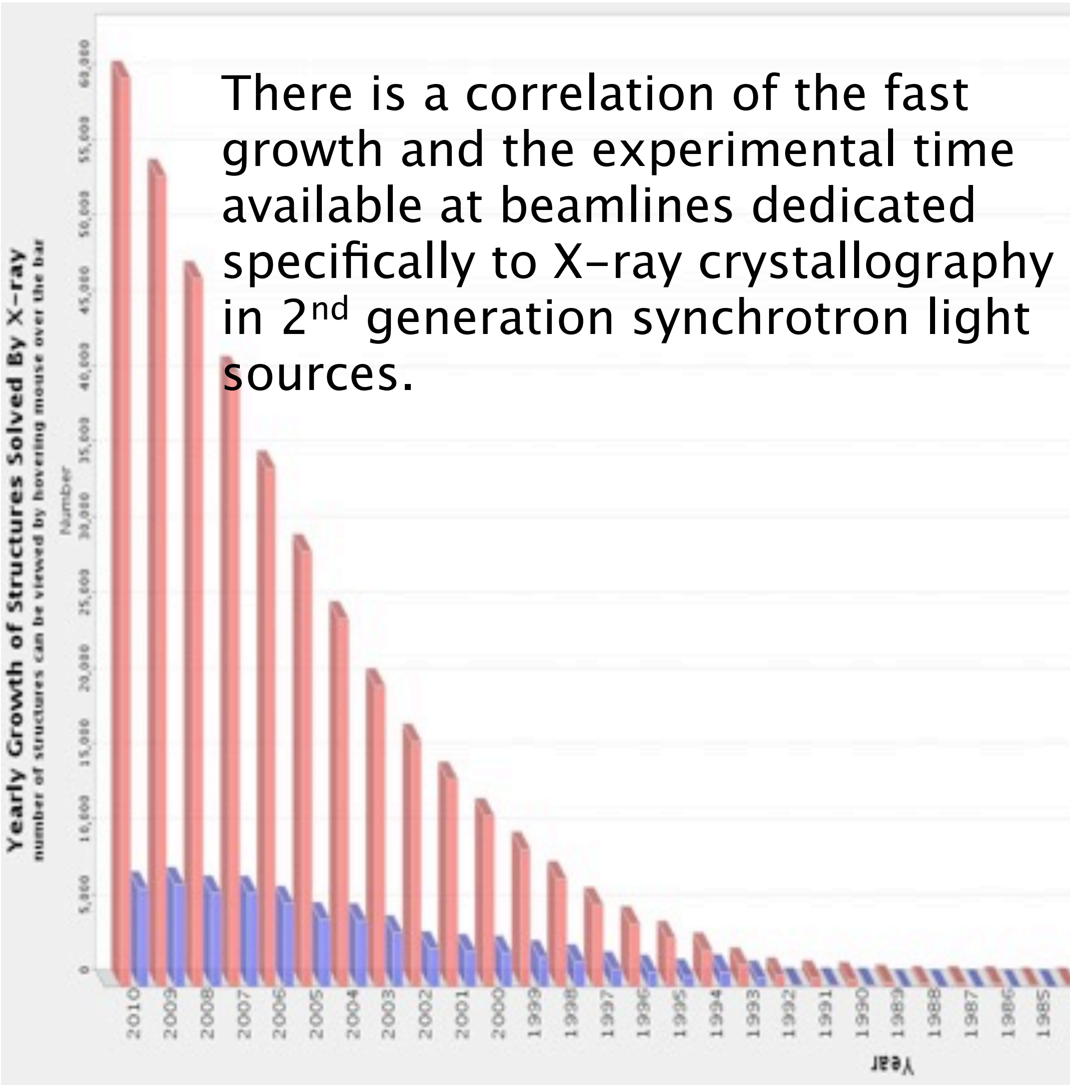
Protein Data Bank



Source: www.pdb.org

2nd Generation Synchrotron Sources



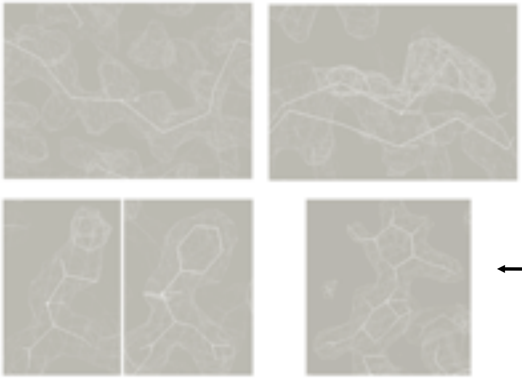
There is a correlation of the fast growth and the experimental time available at beamlines dedicated specifically to X-ray crystallography in 2nd generation synchrotron light sources.

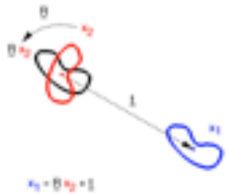
NSLS

Synchrotrons make life easier

Protein Purification
Diffraction

Crystallization





on

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Anomalous dispersion

Refinement

From ‘parasitic’ to a principal role

3rd Generation synchrotron sources

7/49 beamlines dedicated to structural biology

6/50 beamlines dedicated to structural biology

8/34 beamlines dedicated to structural biology

ESRF

Spring 8

APS

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The plan

Introduction

What is biomolecular crystallography?

Why do we care about biomolecules?

Relevance of X-ray crystallography

Disadvantages

Radiation Damage

Taking advantage of radiation damage (redox proteins)

Disadvantages

Need of a crystalline sample (p53)

Phase problem

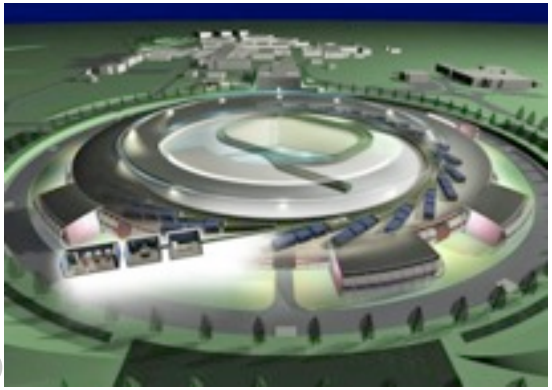
'Static' model averaged in time and space

Radiation damage

Disadvantages

Need of a crystalline sample (p53)

Phase problem



'Static' model averaged in time and sp

Easily solved by anomalous methods

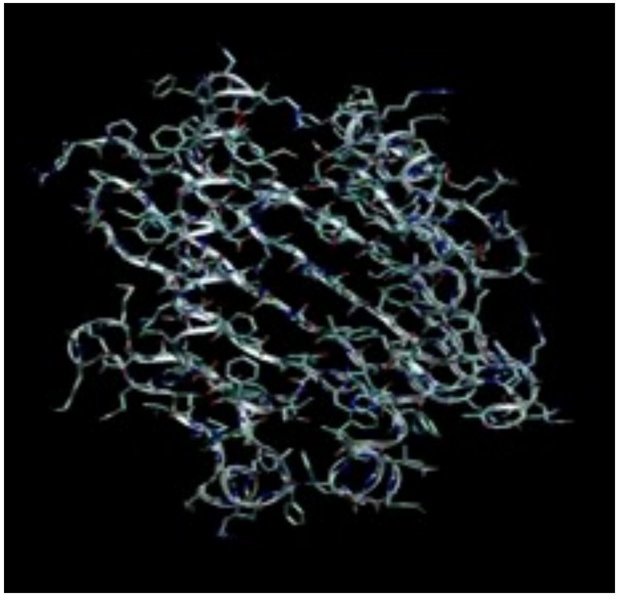
Radiation damage

Disadvantages

Need of a crystalline sample (p53)

Phase problem

‘Static’ model averaged in time and space



Radiation damage

Disadvantages

Need of a crystalline sample (p53)

Phase problem

'Static' model averaged in time and space

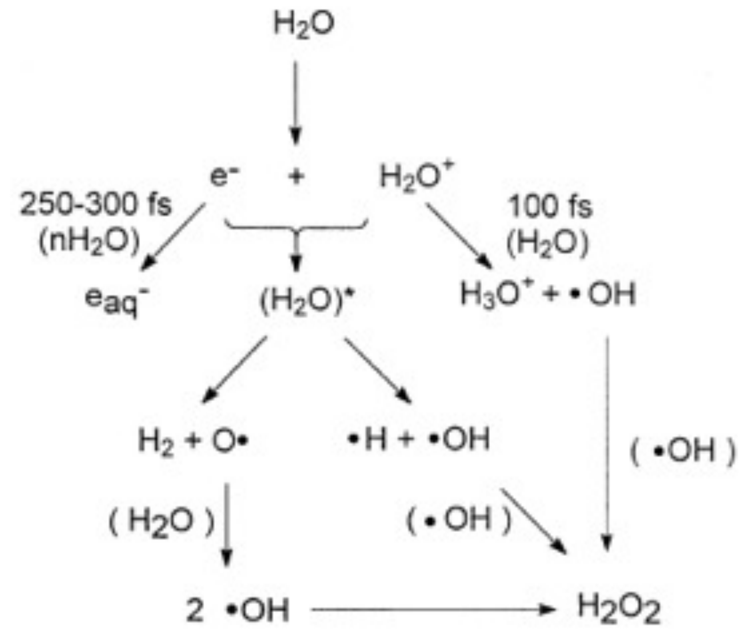
Radiation damage

Radiation damage

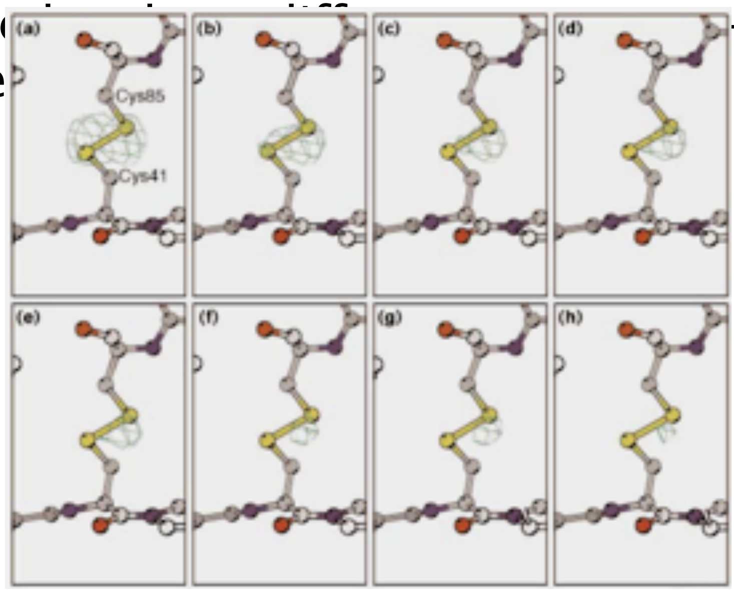
•Rayleigh, Compton and photoelectric effect
(less than 2% of the beam produce diffraction)

•Primary local (unavoidable).- ionization of an atom caused by photoelectron

•Secondary.- formation and migration of several low energy electrons, that are solvat… –
tempe…



Hiroki, A. Pimblott, S. M. and LaVerne, J. A. (2002) J Phys Chem A **106**, 9352-9358



Ravelli,R.B.G. and McSweeney, S.M. (2000). Structure 8.315-328.

Loss of diffraction power- decrease in intensities

Specific damage to metal sites, disulphide bonds, aspartic/glutamic acid, tyrosines, metionines, compromising biological information.

The plan

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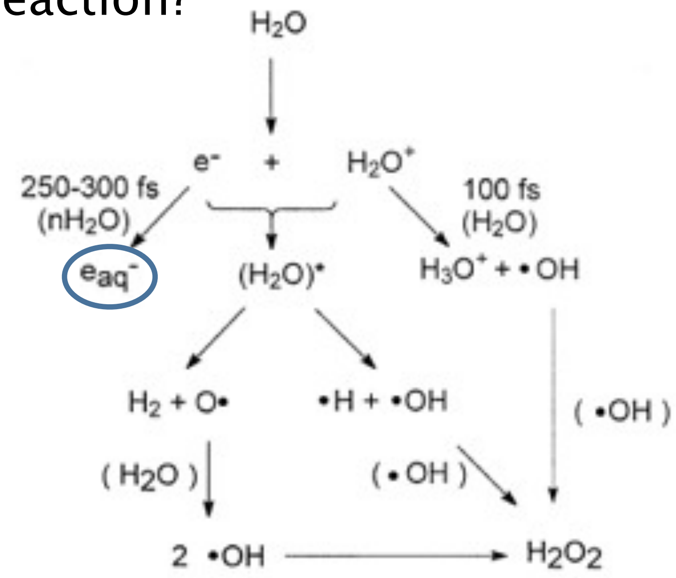
Disadvantages

- Radiation Damage

Taking advantage of radiation damage (redox proteins)

Taking advantage of radiation damage

Can we use electrons produced during X-ray irradiation to trigger a reaction?



Modified from Solomon, E. I., et al. (1999). *Chemical Reviews* **96**, 2563-2605.

Taking advantage of radiation damage

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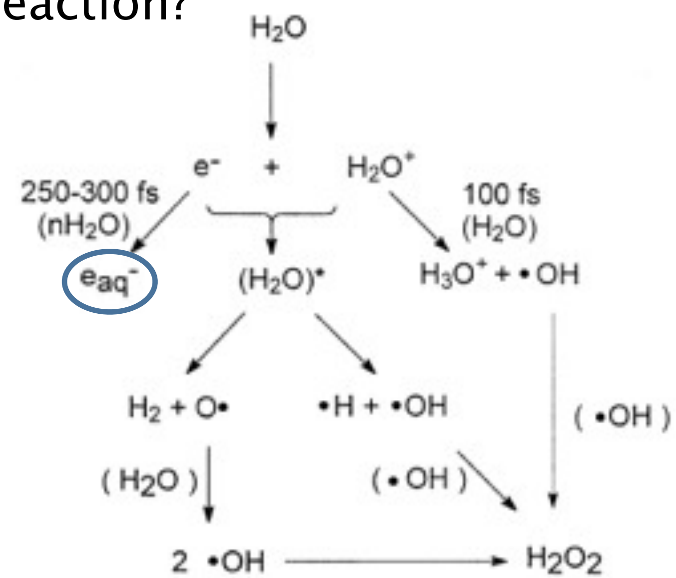
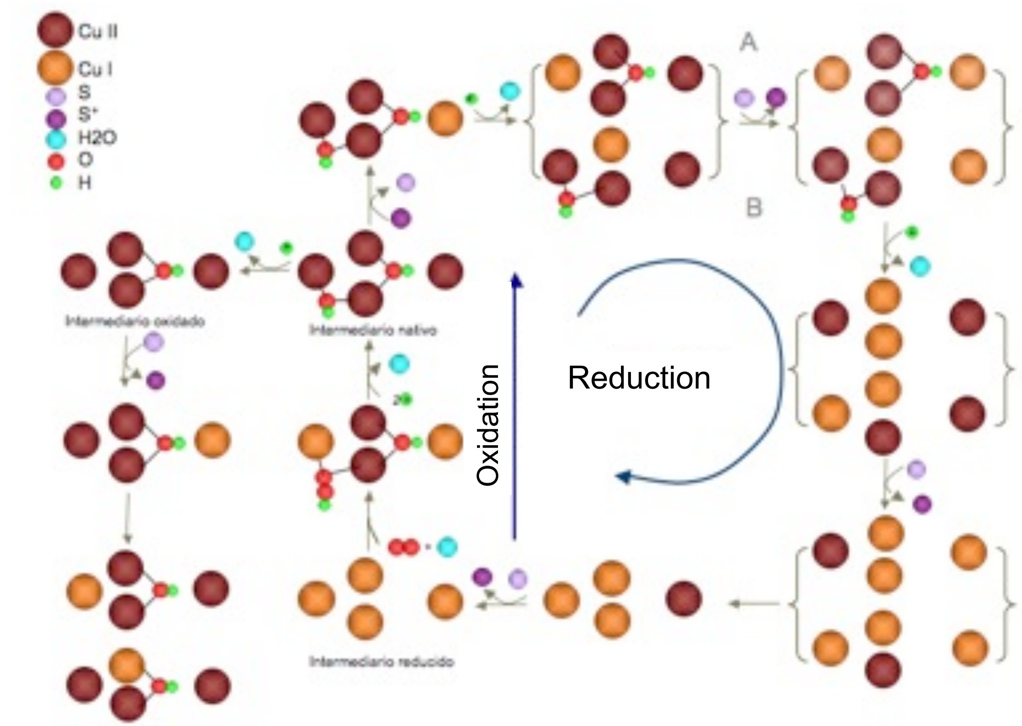


Redox Systems $O_2 + 4e^- + 4H^+ \longrightarrow 2\ H_20$



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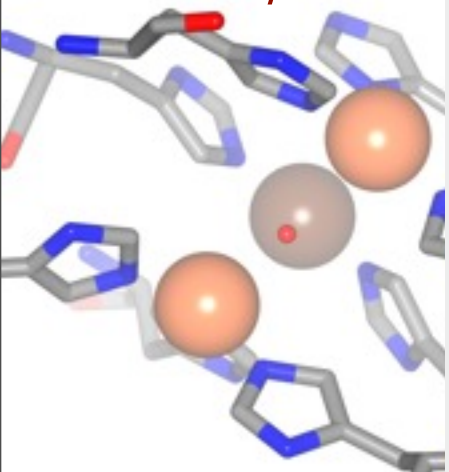
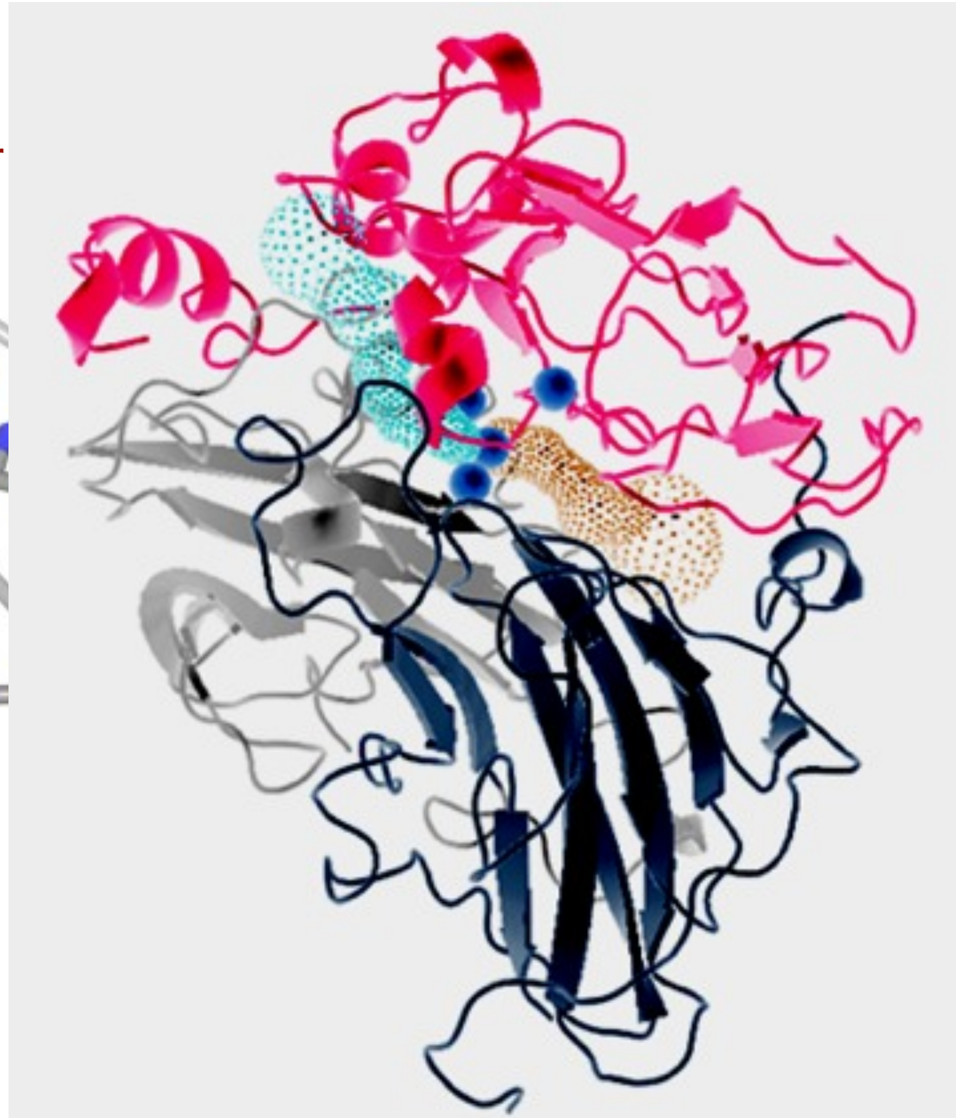
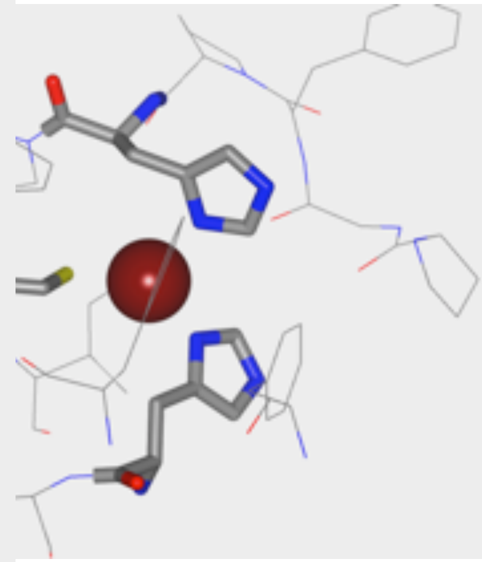
Laccase

Trinuclear center
CuT2/T3

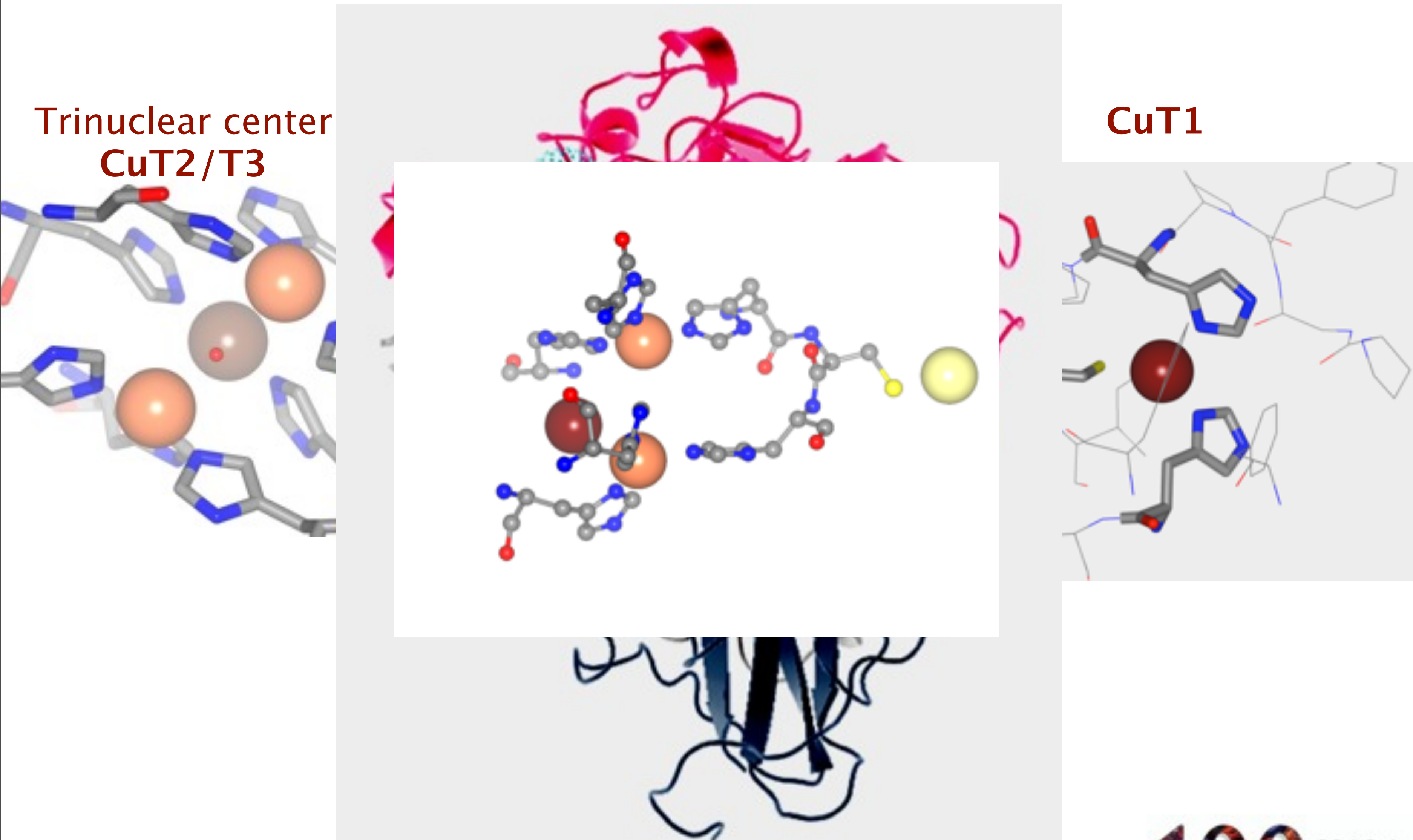




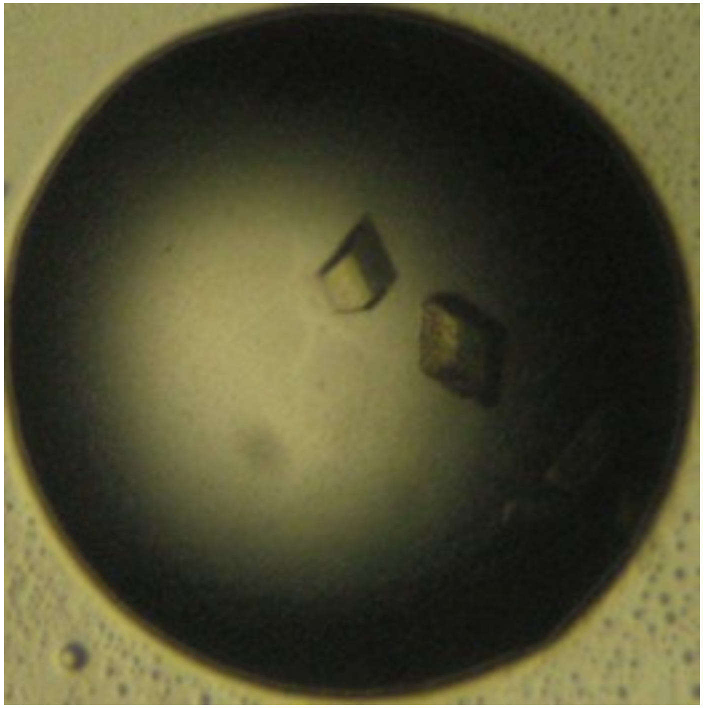
CuT1

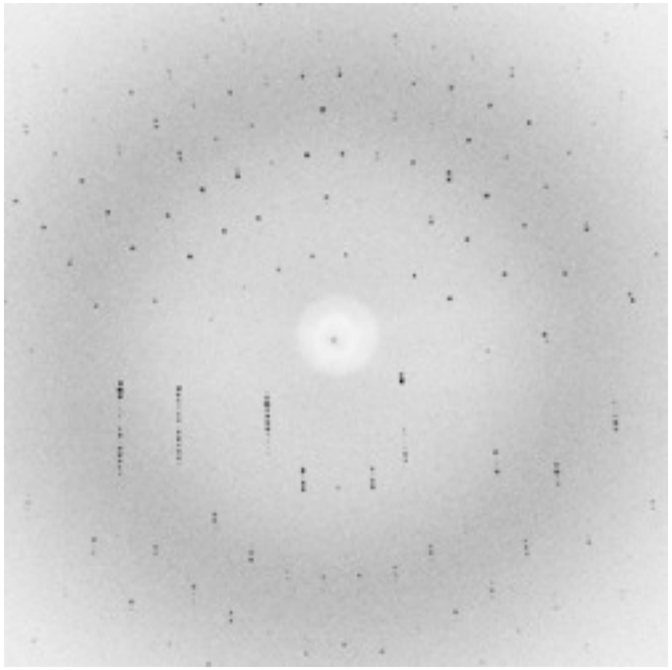


Laccase



Radiolysys of copper sites





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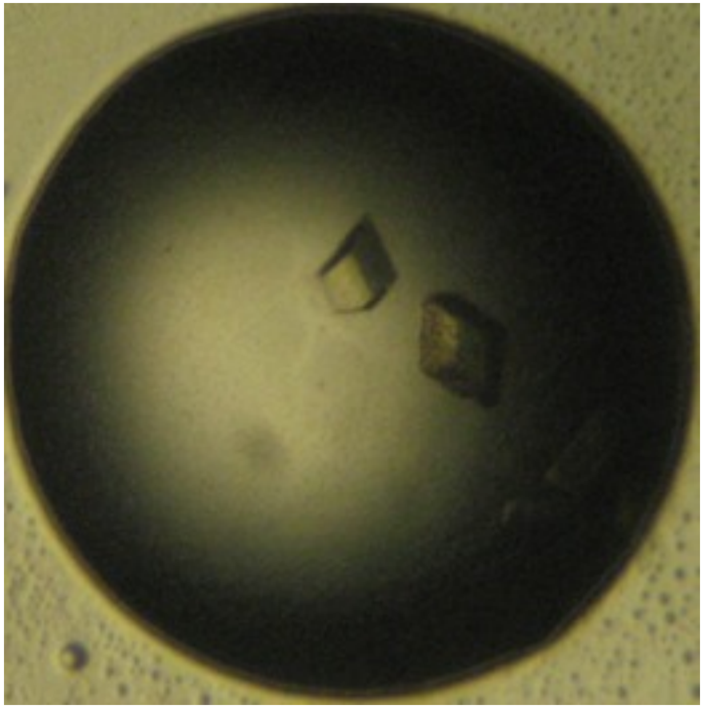
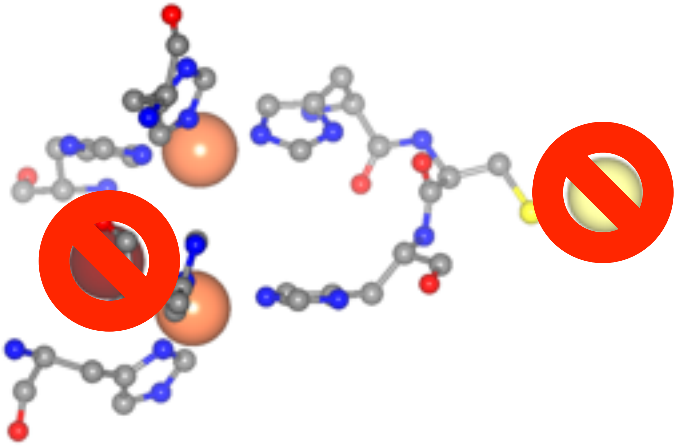
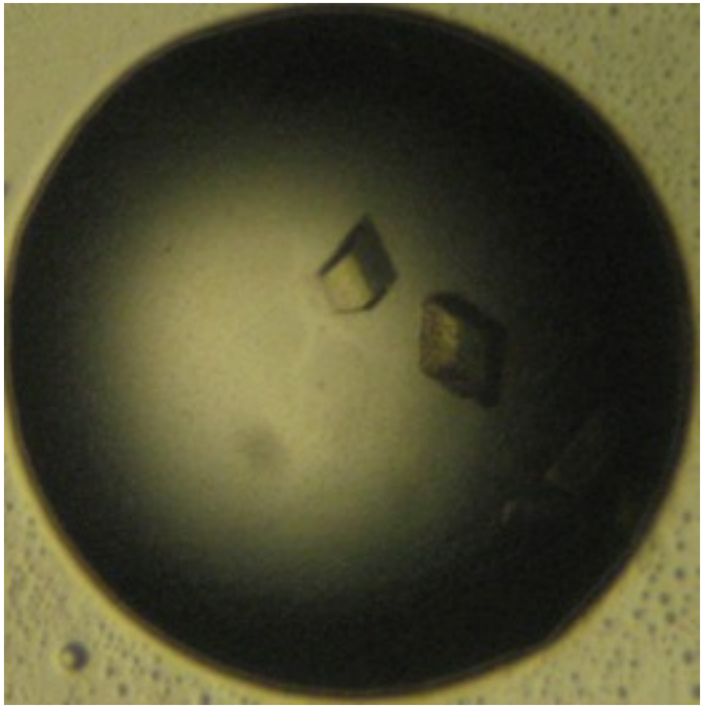
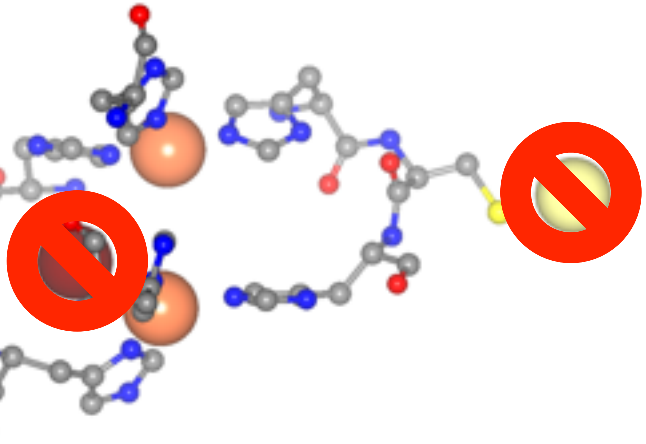




Two of the four copper sites were absent

Radiolysys of copper sites





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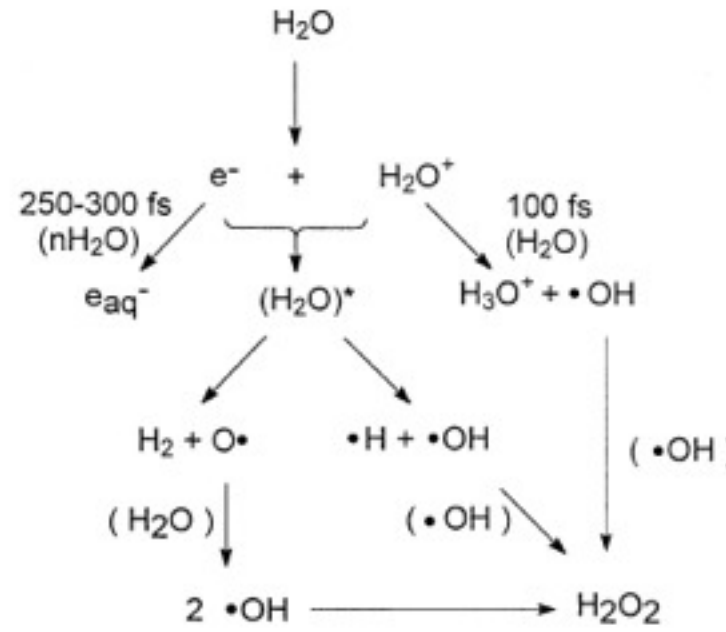
Radiation damage



Hiroki, A. Pimblott, S. M. and LaVerne, J. A. (2002) J Phys Chem A **106**, 9352-9358

Loss of diffraction power– decrease in intensities

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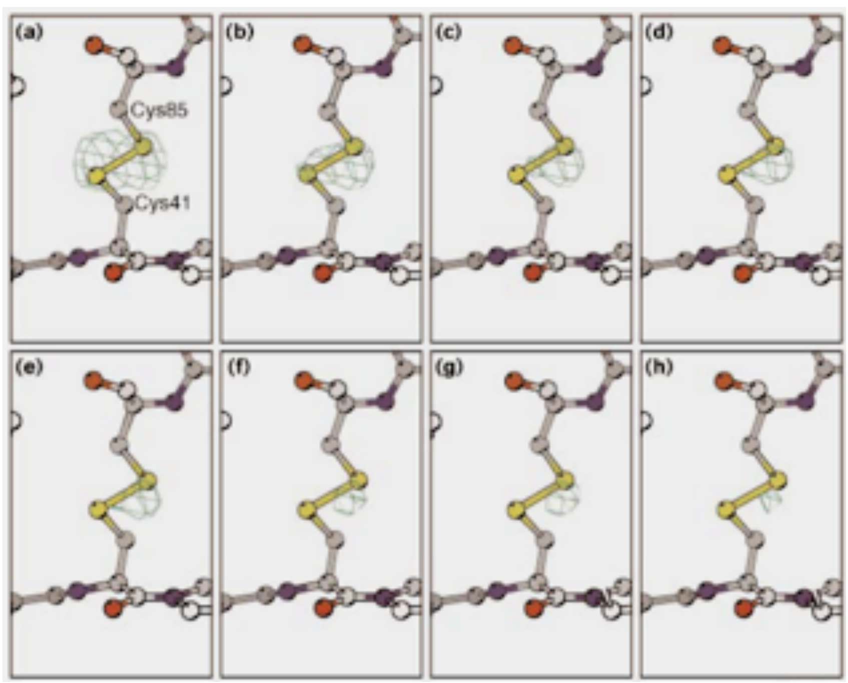
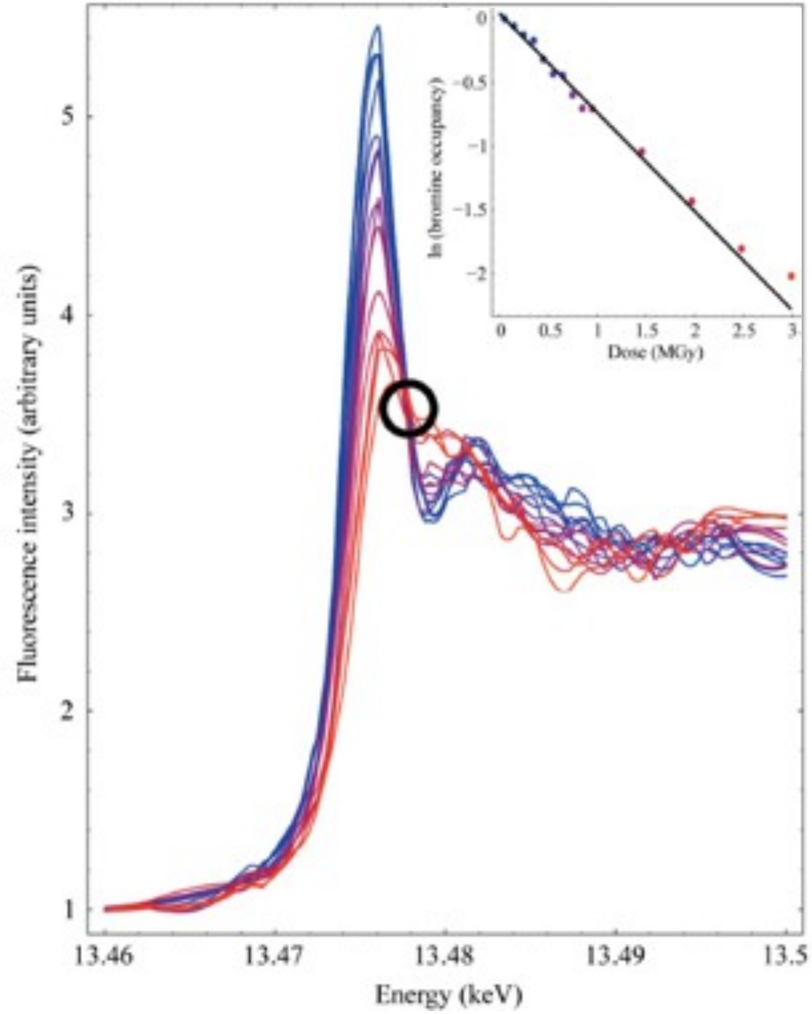


Ravelli,R.B.G. and McSweeney, S.M. (2000). Structure **8**,315-328.

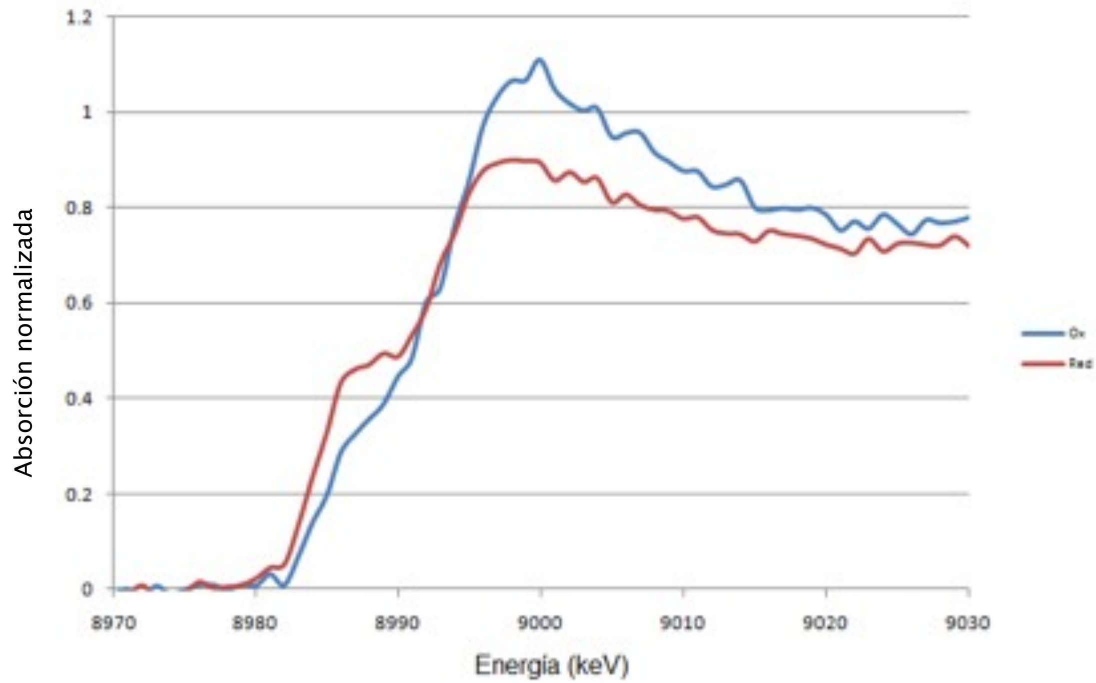
Radiolysis

C-Br bond on RNA molecule



Oliéric, V. *et al*. (2007). *Acta Crystallographica D* **63**, 759-768.

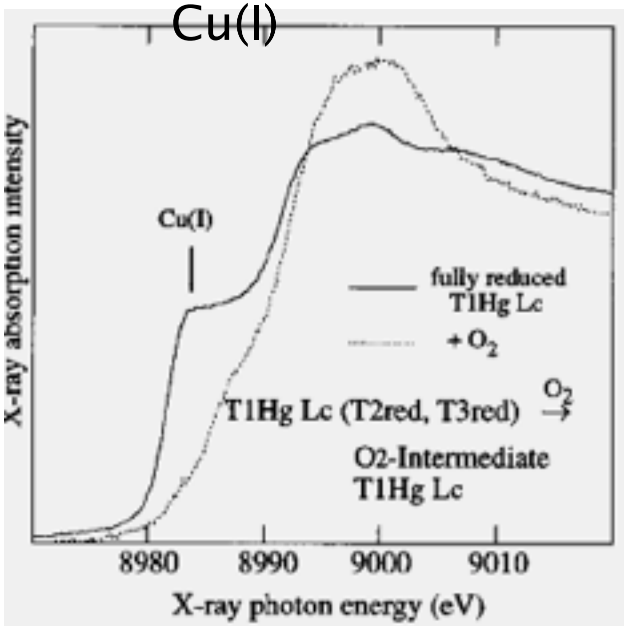
X-ray Absorption Spectroscopy

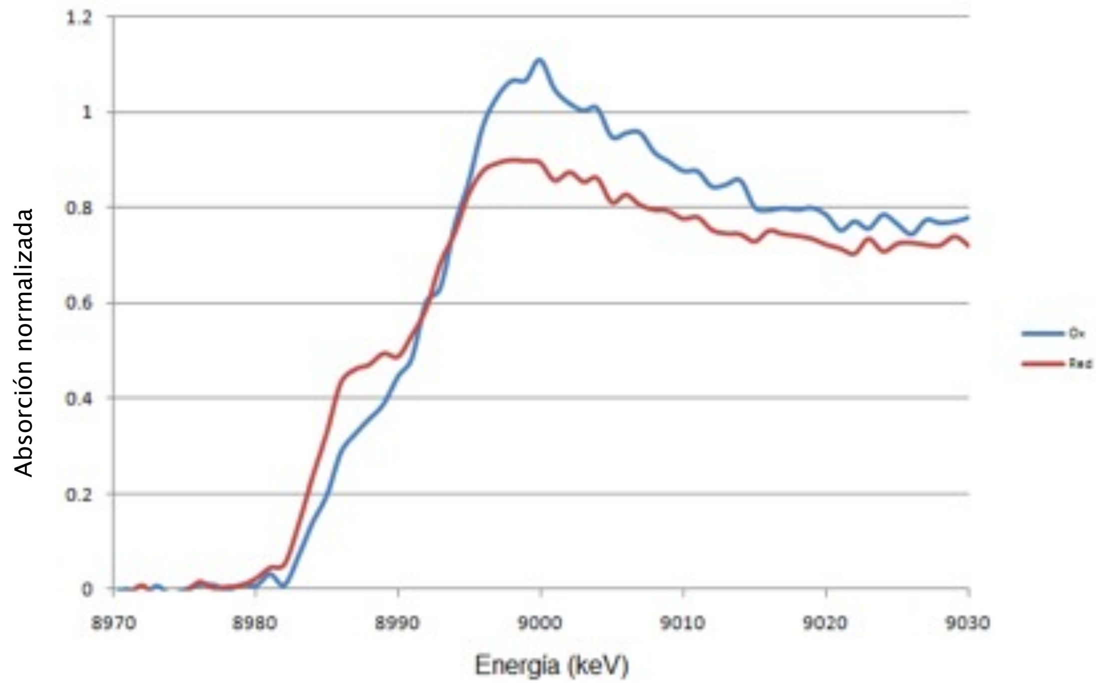


(Collected at Beamline X6A from NSLS)

X-ray Absorption Spectroscopy

Cu(II)⟶
Cu(I)





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Solomon, E. I. et al. 1996. Chemical Reviews 96

X-ray Absorption Spectroscopy

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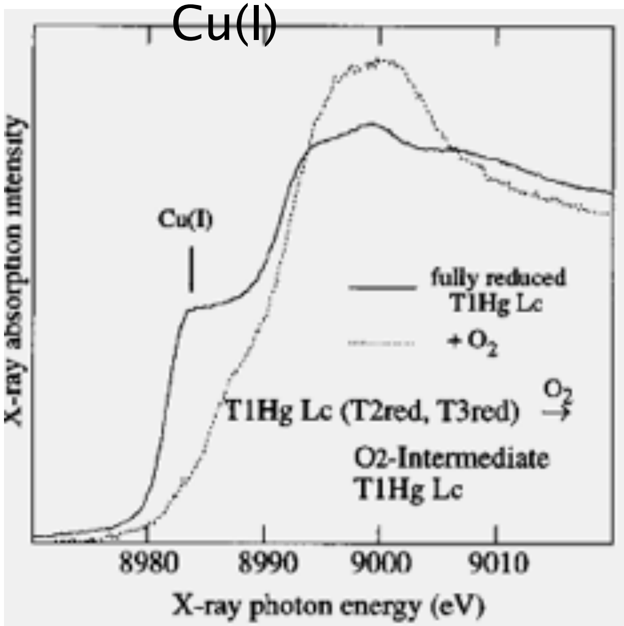
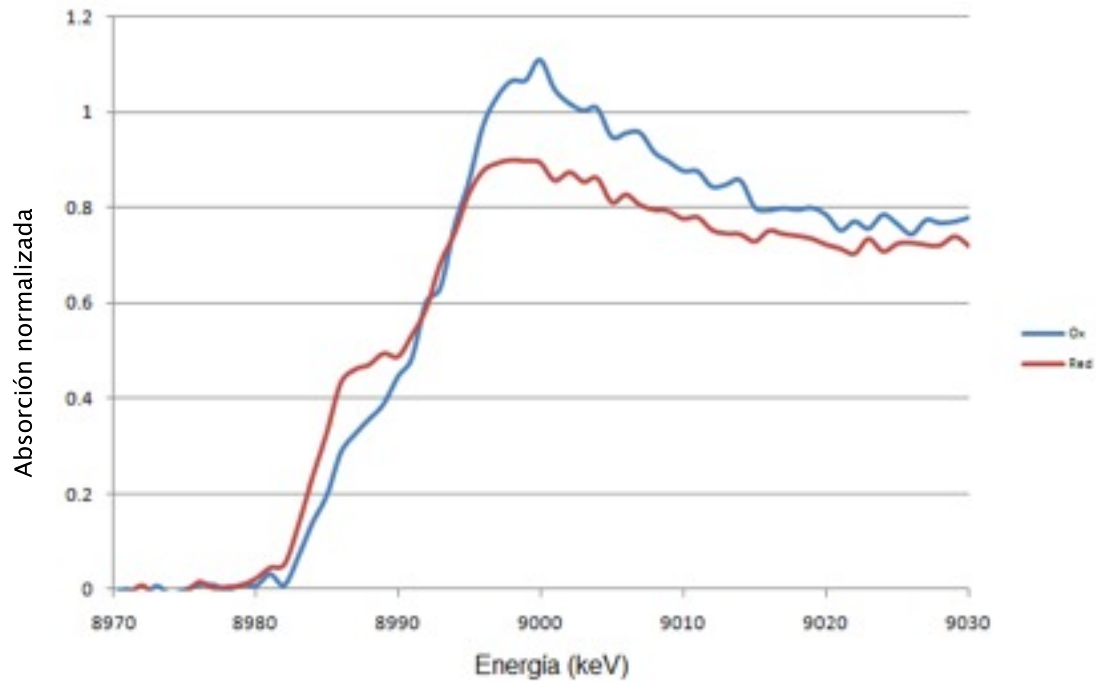


Copper sites are reduced during irradiation



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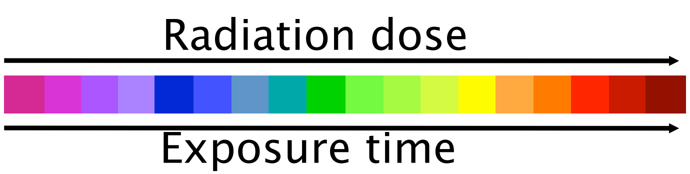
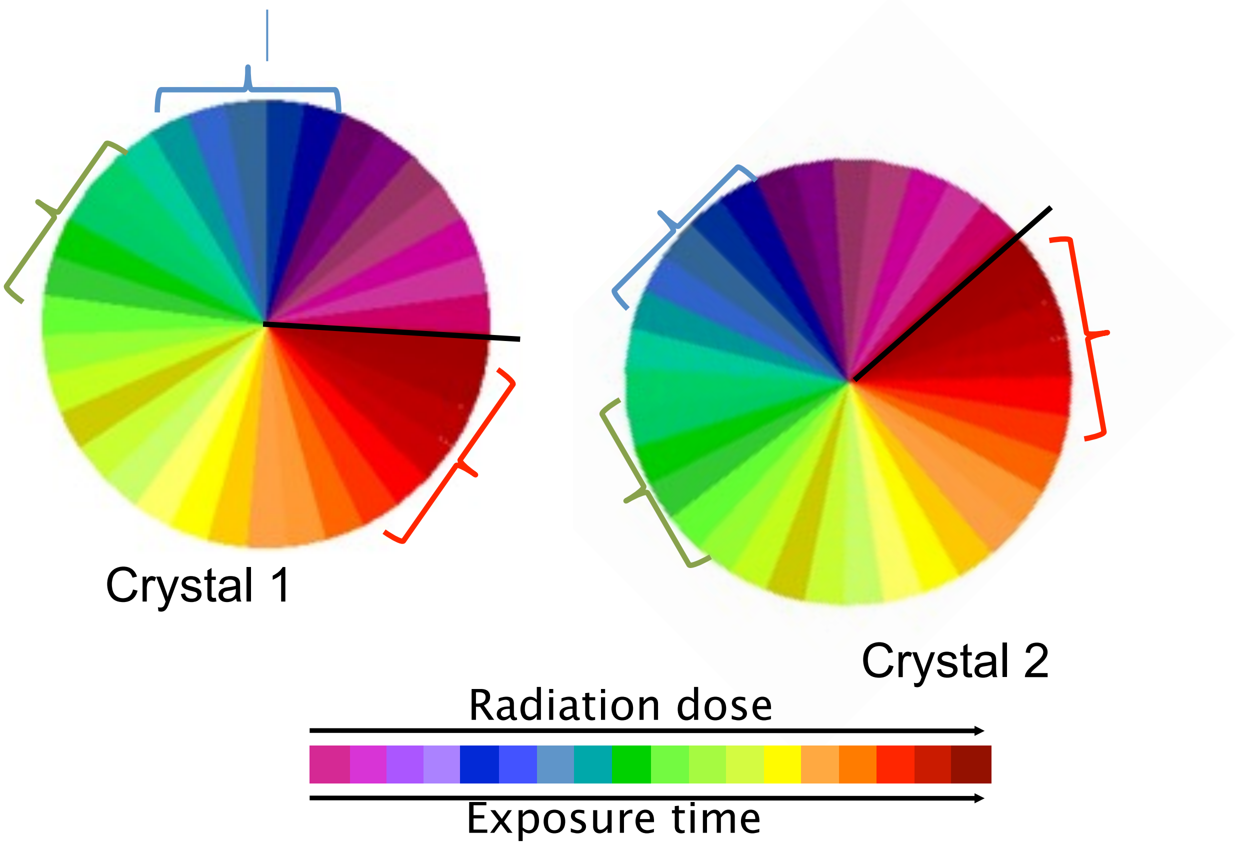
Composite data-sets



Modified from (Berglund, G.I. (2002). *Nature* **417**, 463-468.

Composite data-sets



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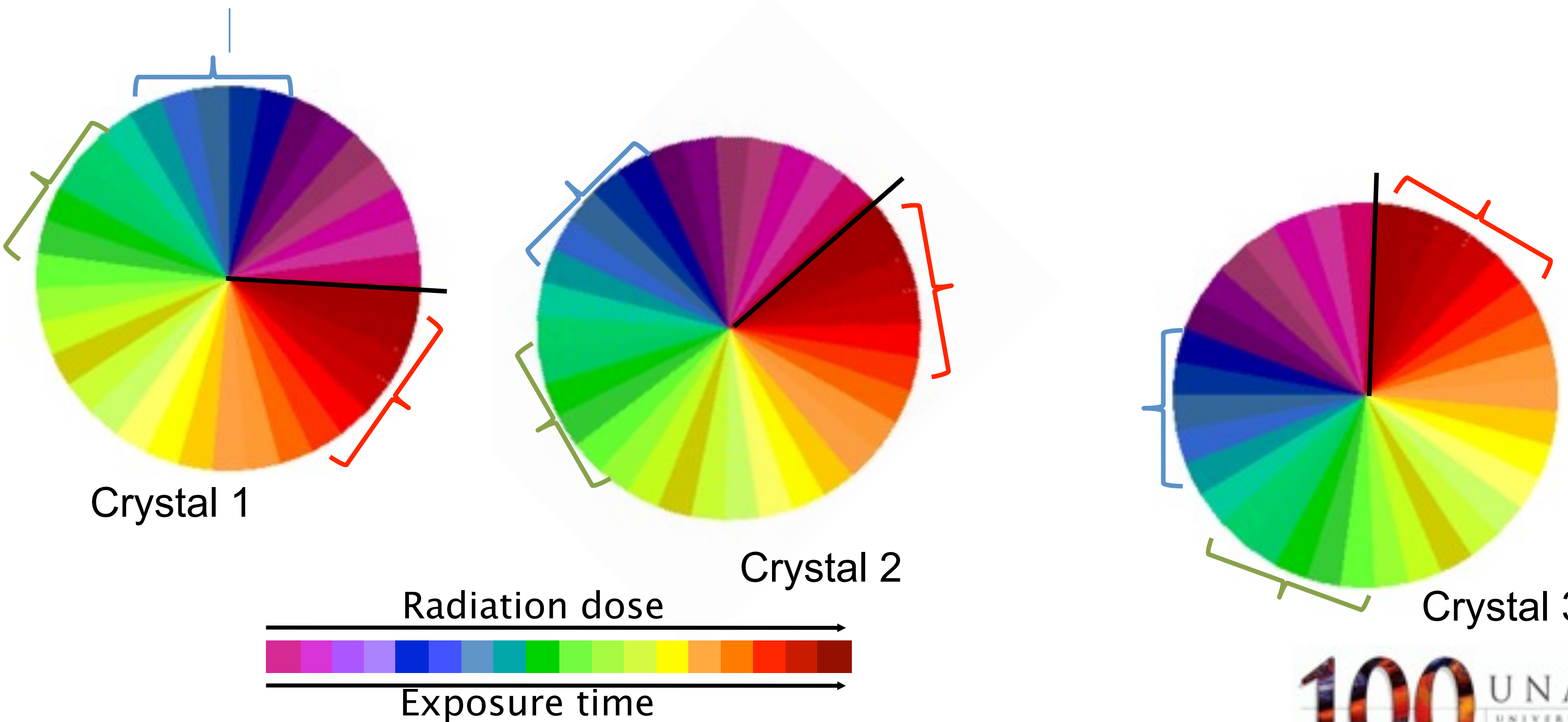

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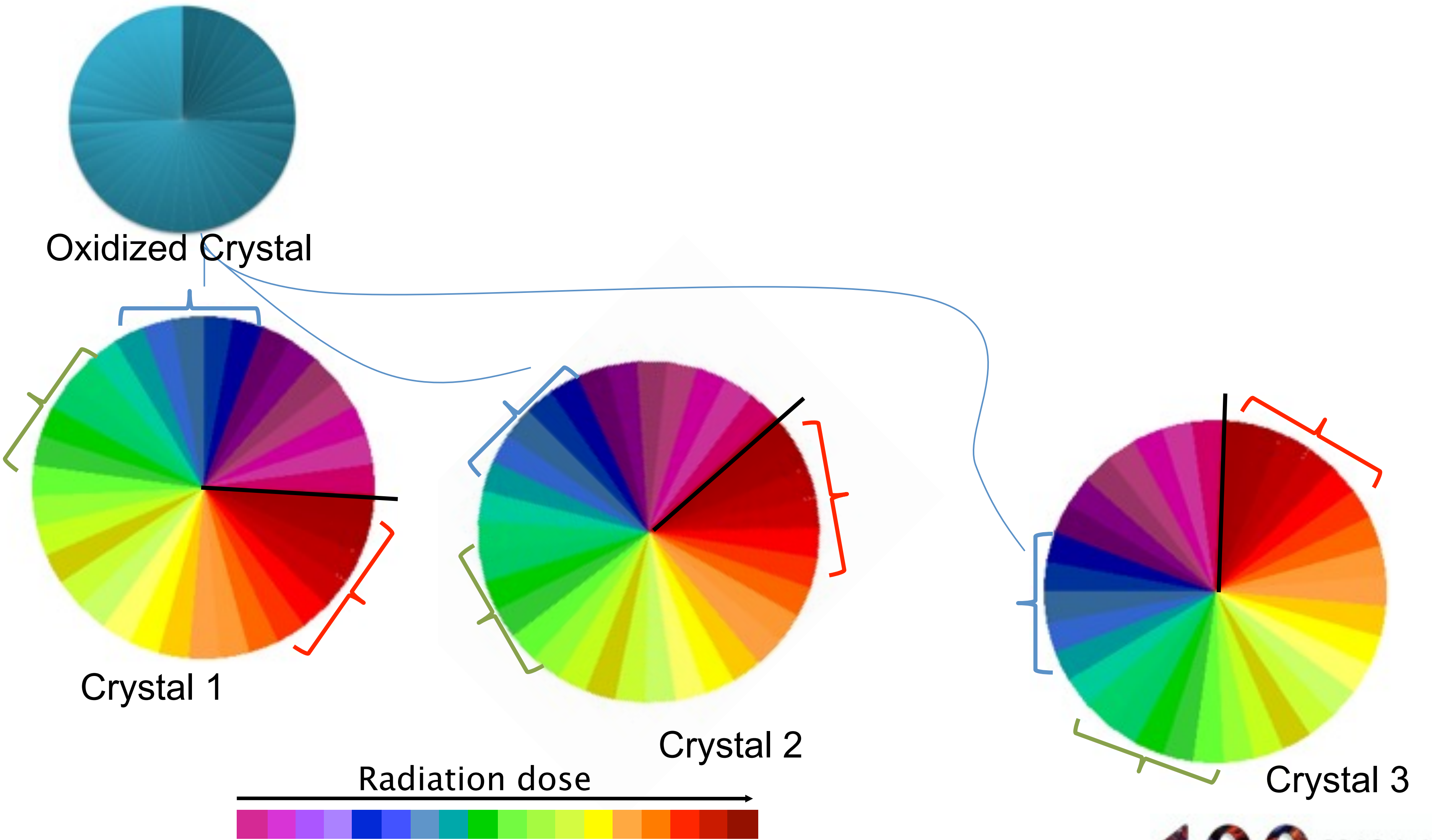

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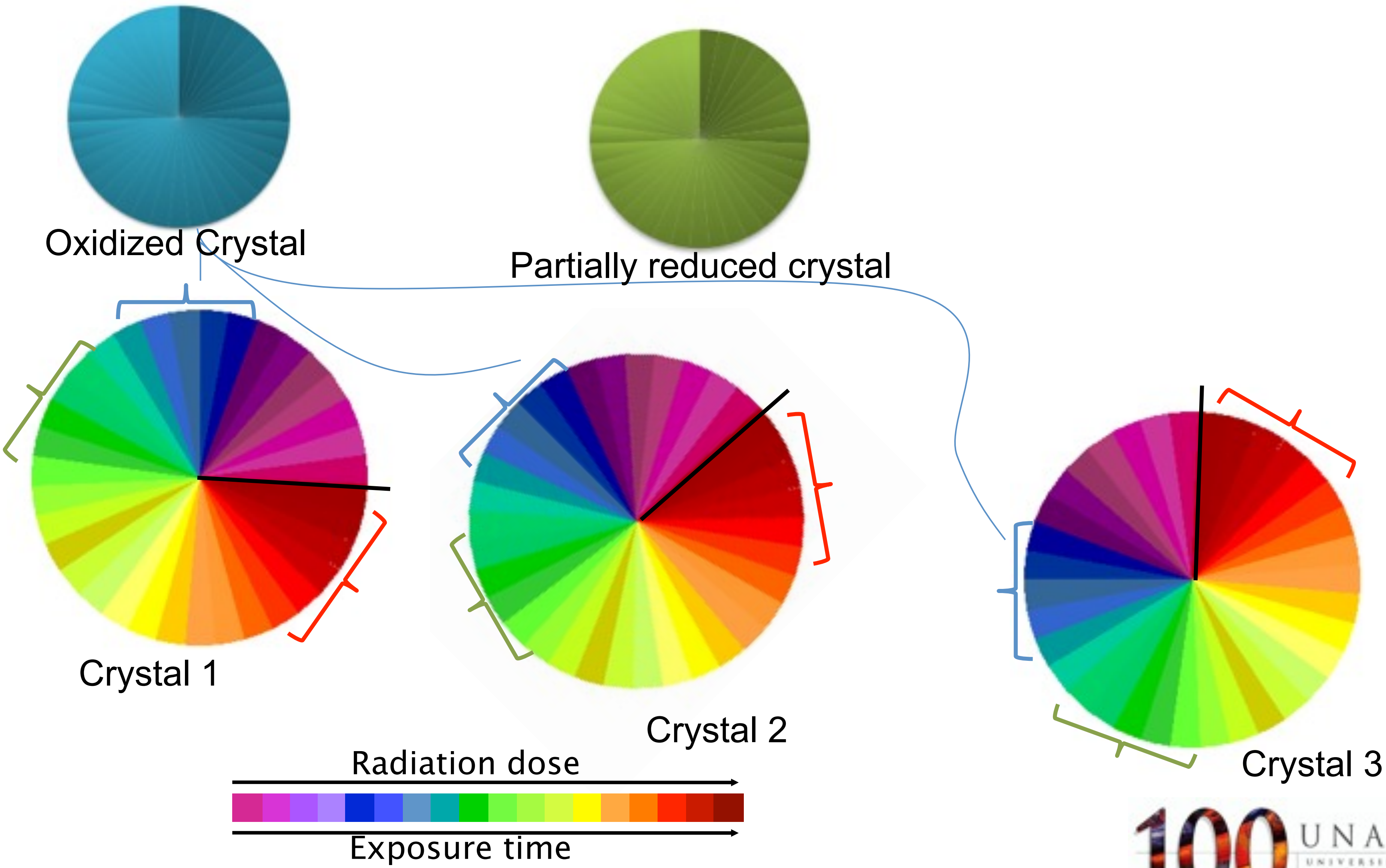

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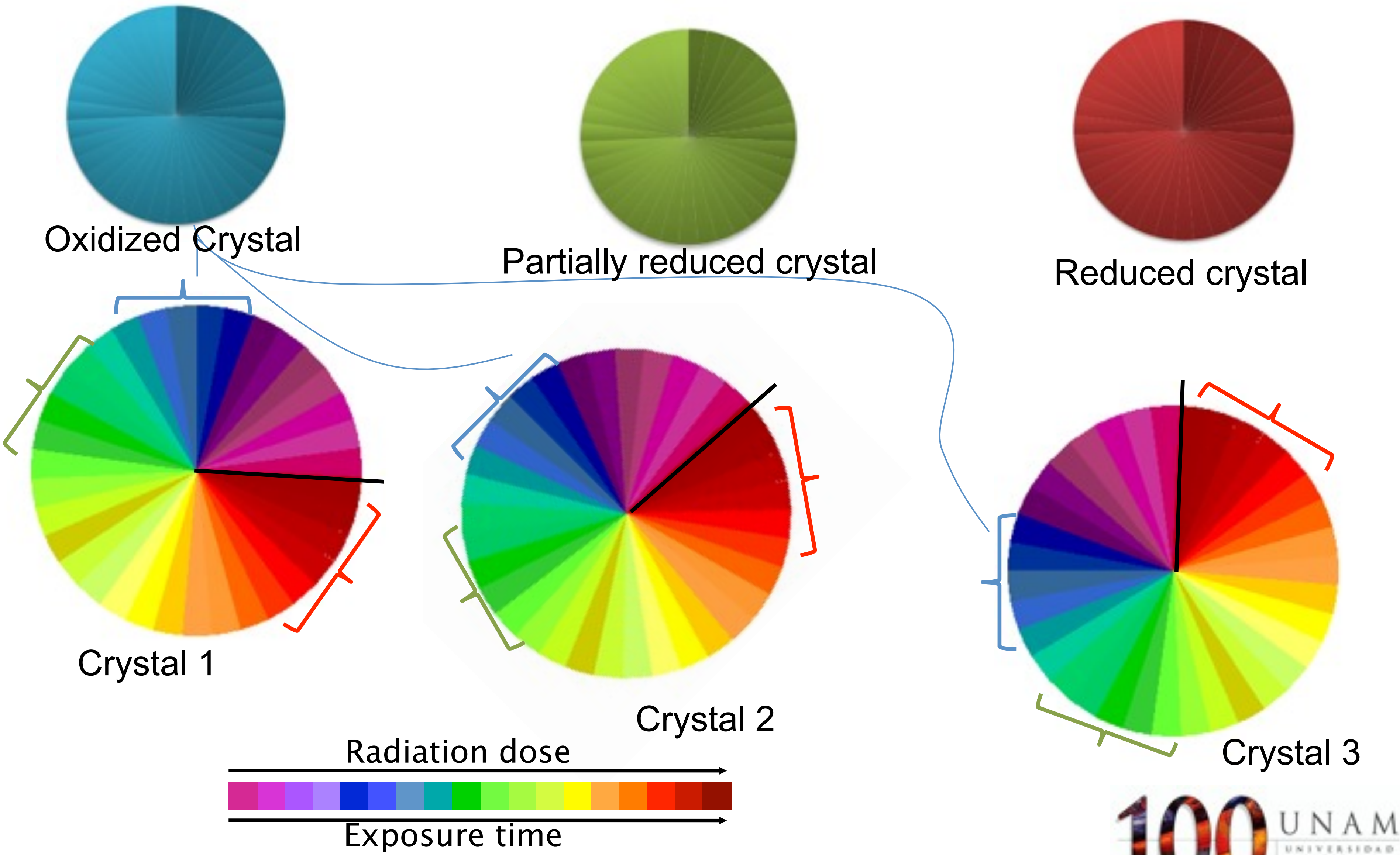

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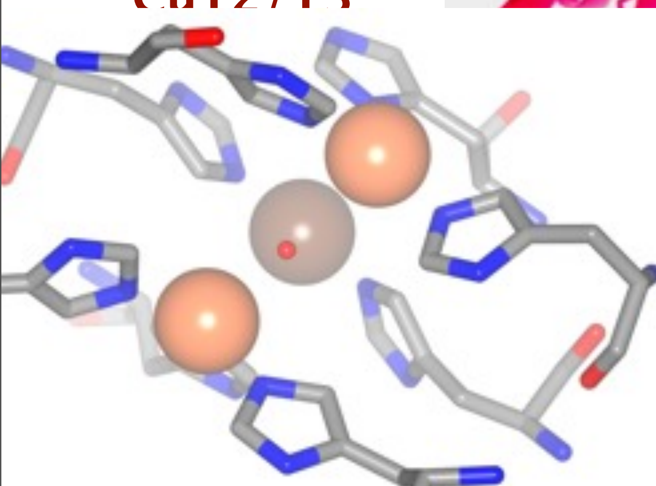
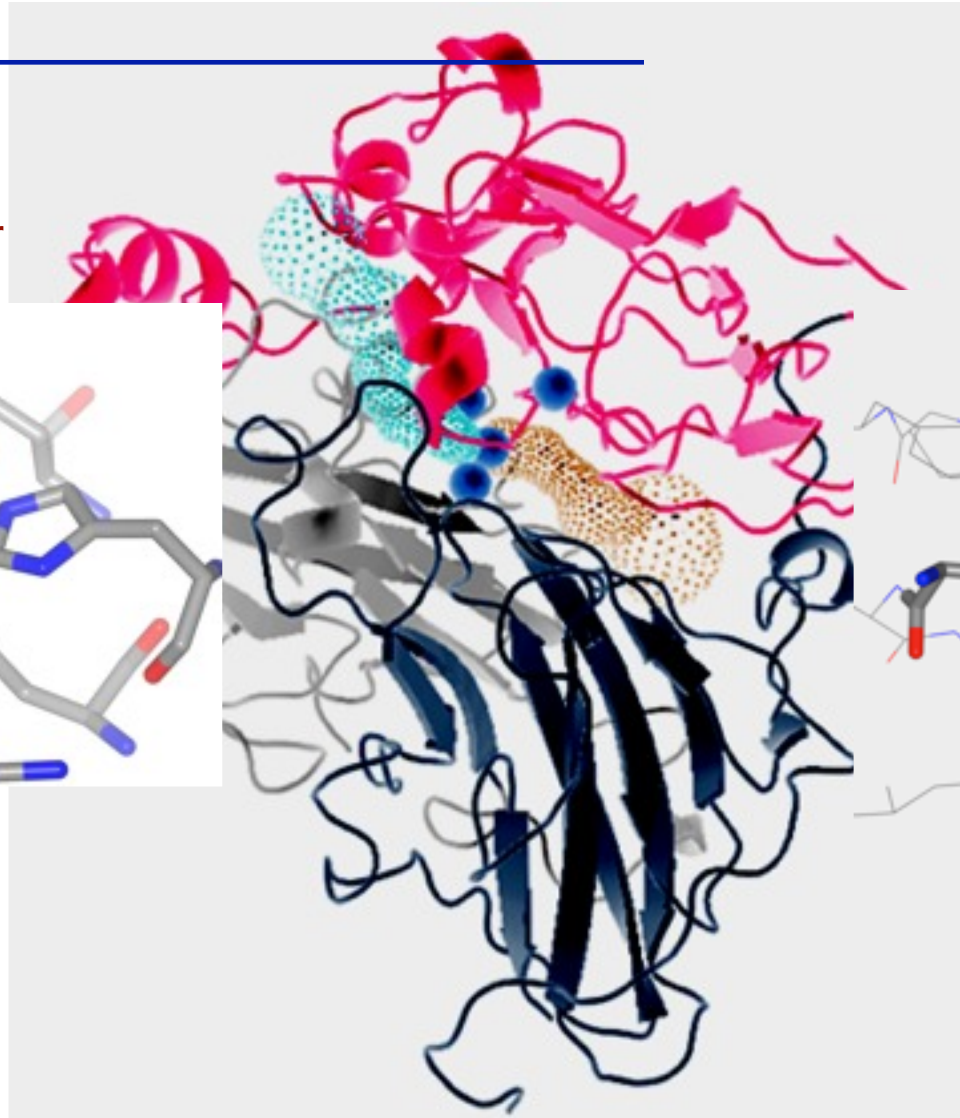
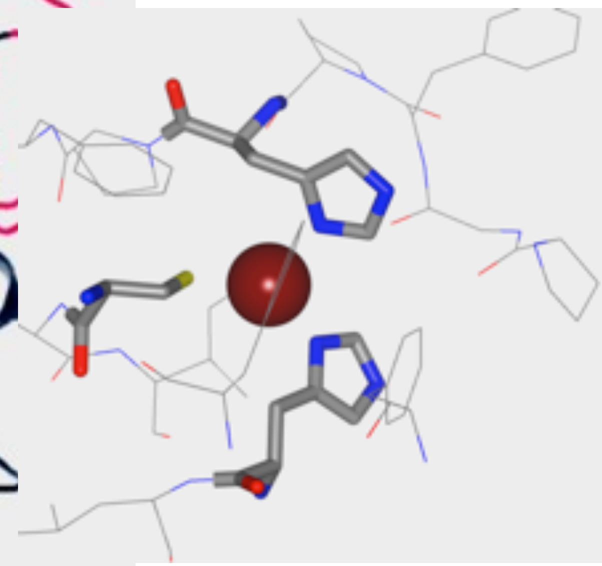
Laccase

Trinuclear center
CuT2/T3



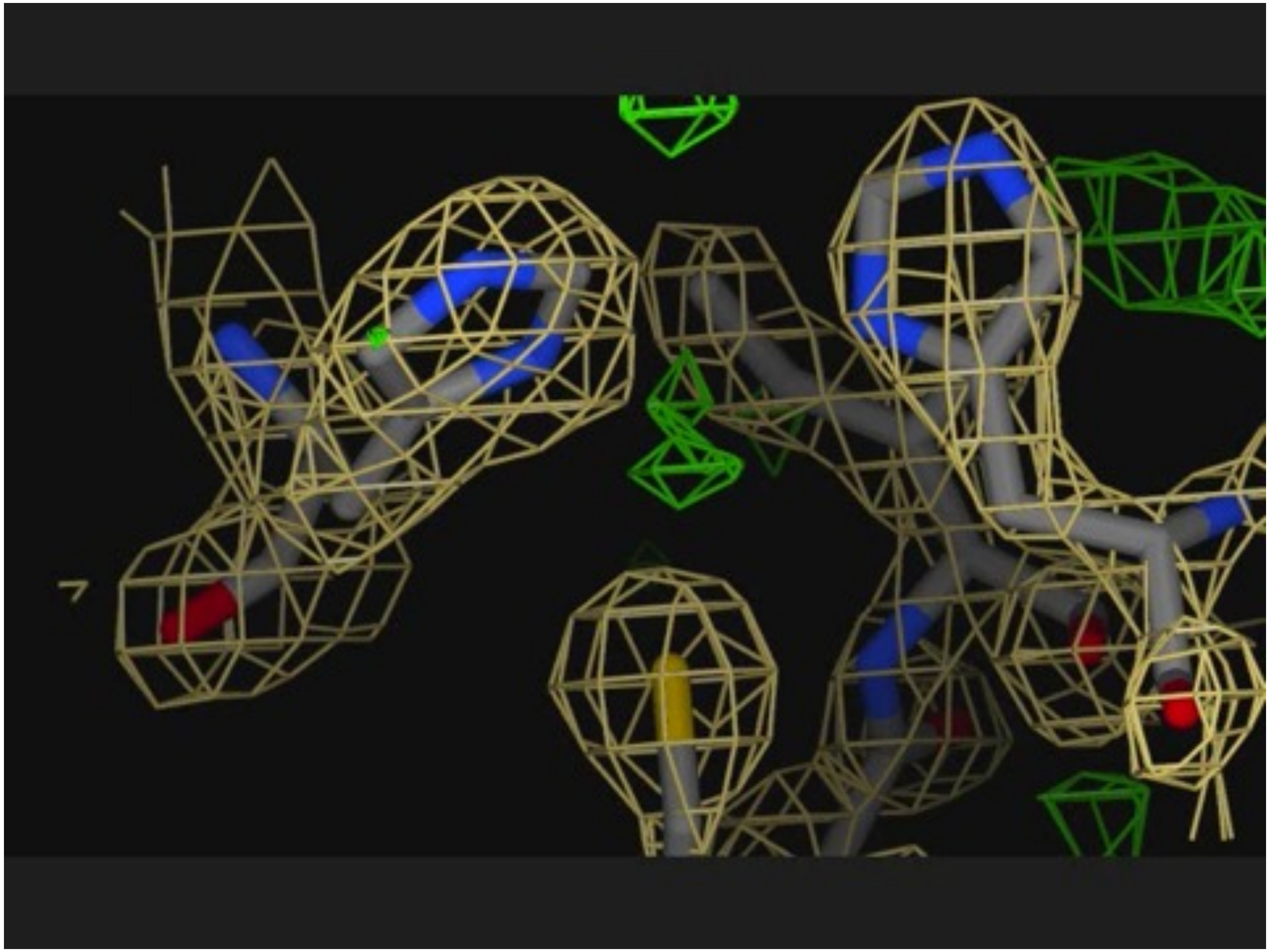


CuT1



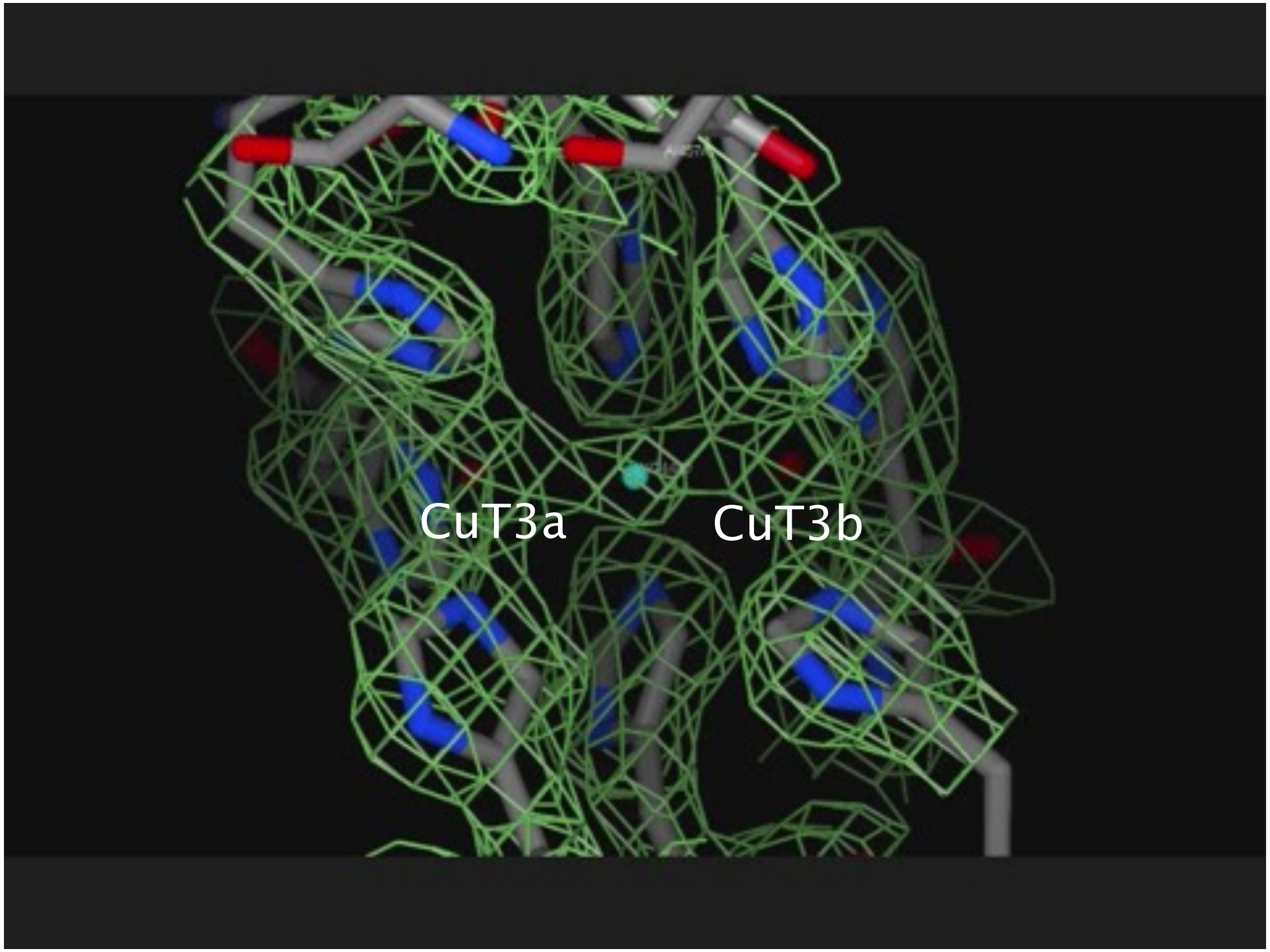
$$O_2 + 4e^- + 4H^+ \longrightarrow 2\ H_20$$

Cu radiolysis



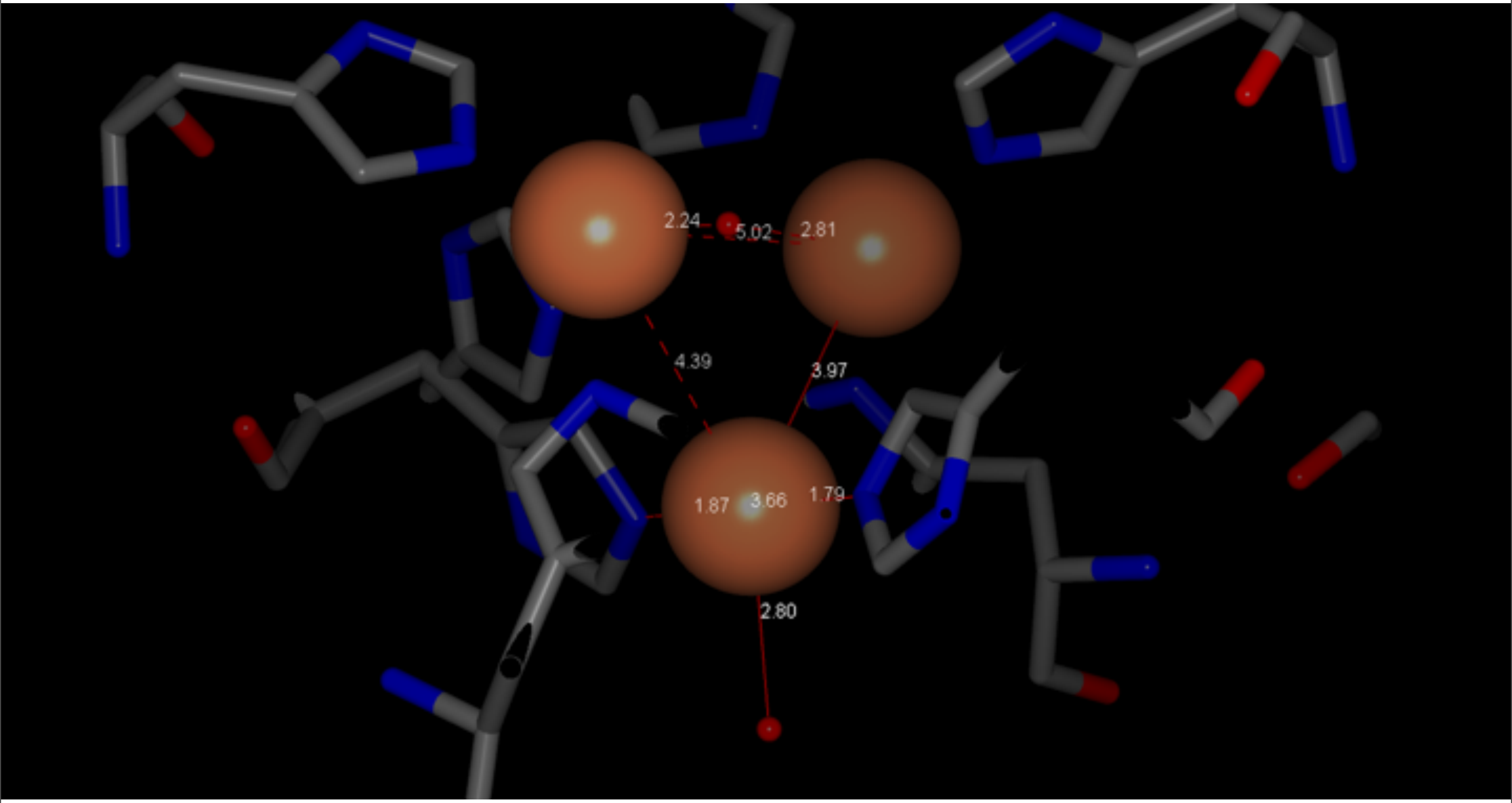
Density corresponding to the CuT1 site appears in the more oxidized structure (partial occupancy)

O_2 reduction



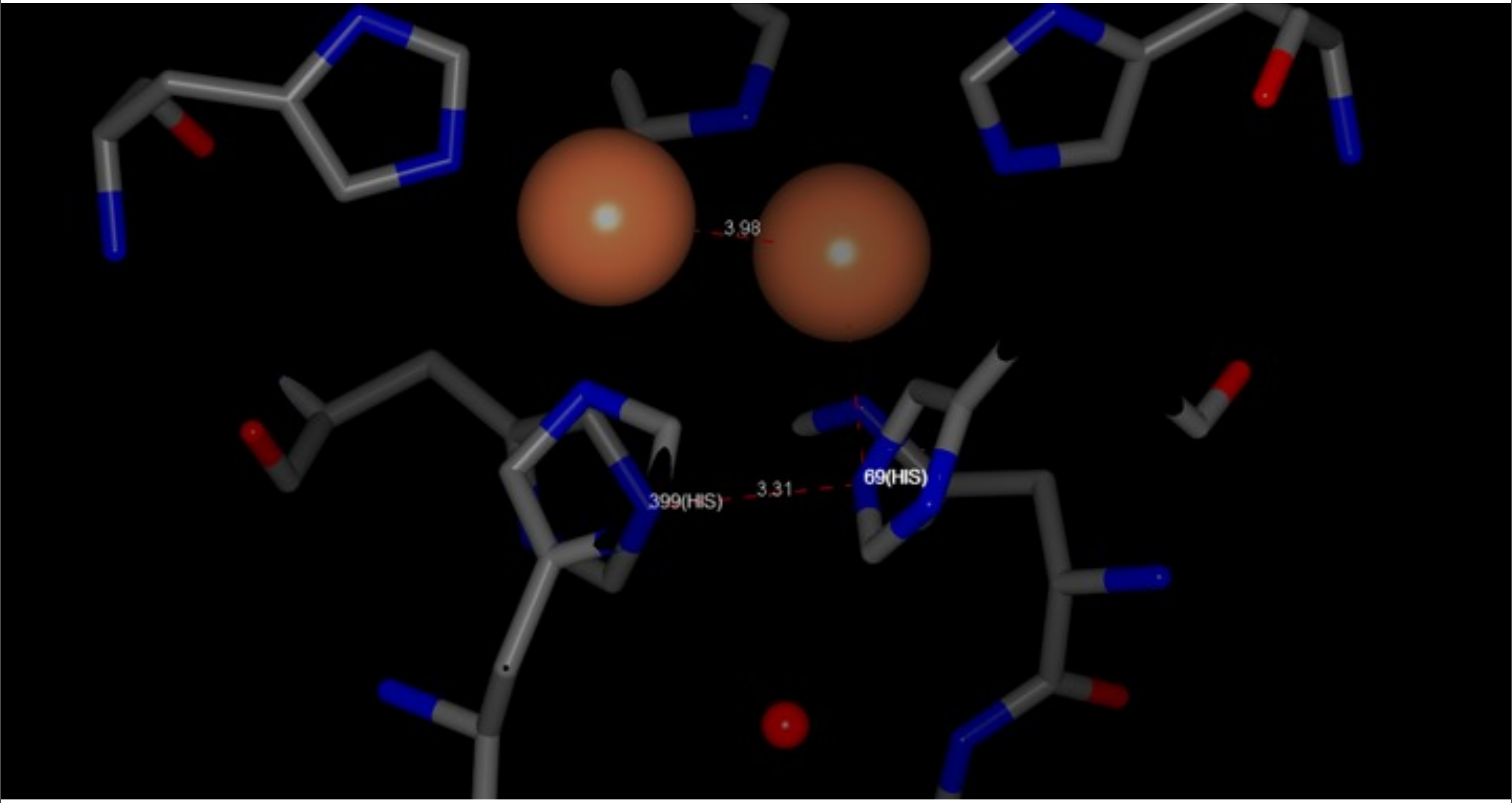
Oxygen intermediates are reduced by X-ray generated electrons

Changes on the coordination distances



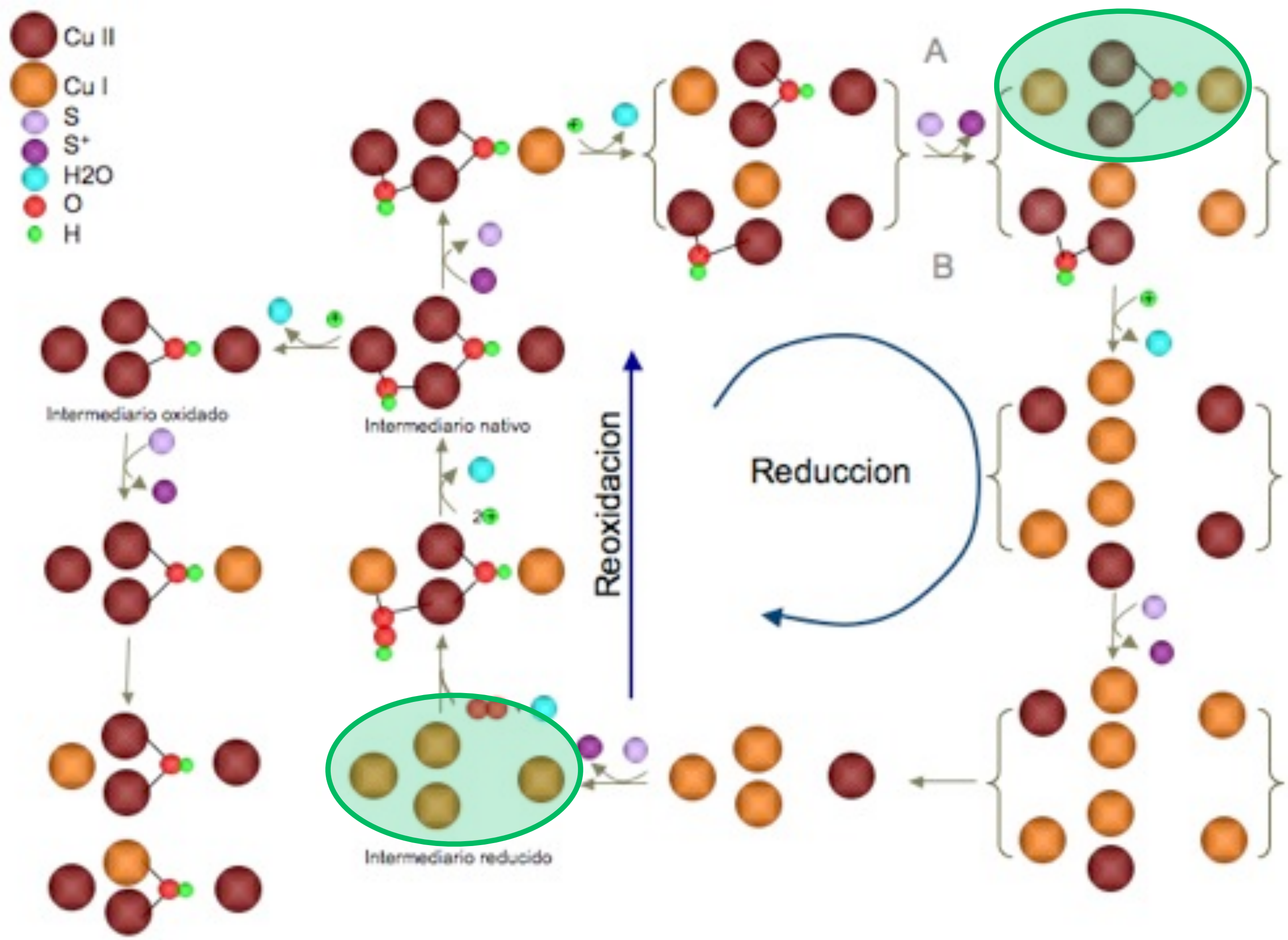
Metal sites are more flexible than we thought

Changes on the coordination distances



Metal sites are more flexible than we thought

Intermediates trapped



Modified from Solomon, E. I., et al. (1999). *Chemical Reviews* **96**, 2563-2605.

Conclusions

It is possible to use X-rays to trigger redox reactions in protein systems

The composite data-set method (Berglund, G.I. *et al*. 2002) allowed us to trap two intermediaries of O_2 reduction

Reduction and radiolysis of metal occurs during the exposure of metalloproteins to X-rays

Acknowledgements

Dr. Enrique Rudiño Piñera

Dr. Brenda Valderrama Blanco

Biol. Sonia Patricia Rojas Trejo

CONACyT y PAPIIT

Thank you

Parenthesis

Scientific production correlates with…

http://www.pdb.org/pdb/general_information/news_publications/annual_reports/annual_report_year_2009.pdf

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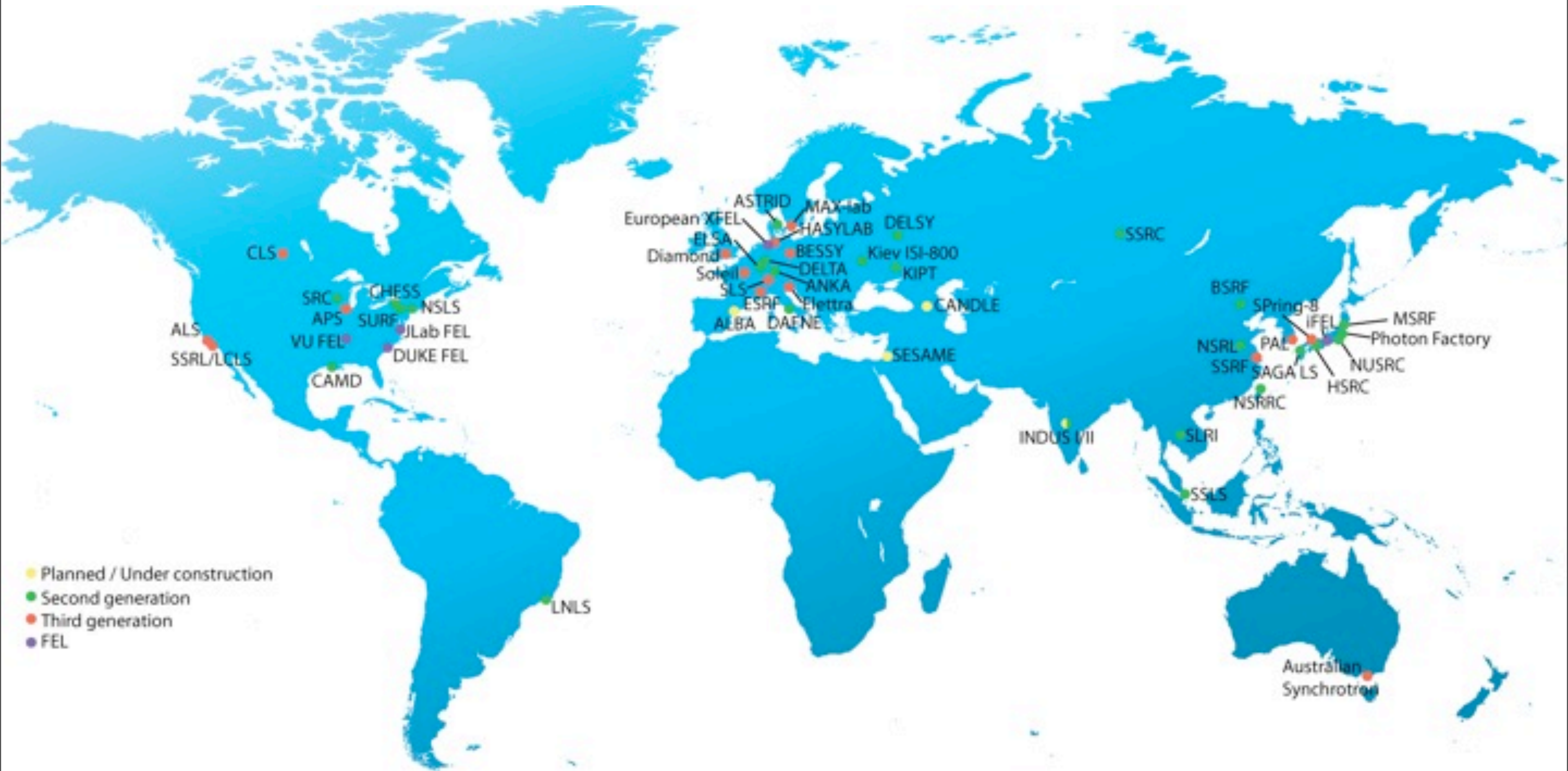
Synchrotron Facilities



http://www.diamond.ac.uk/Home/About/Synchrotrons/World/largemap.html