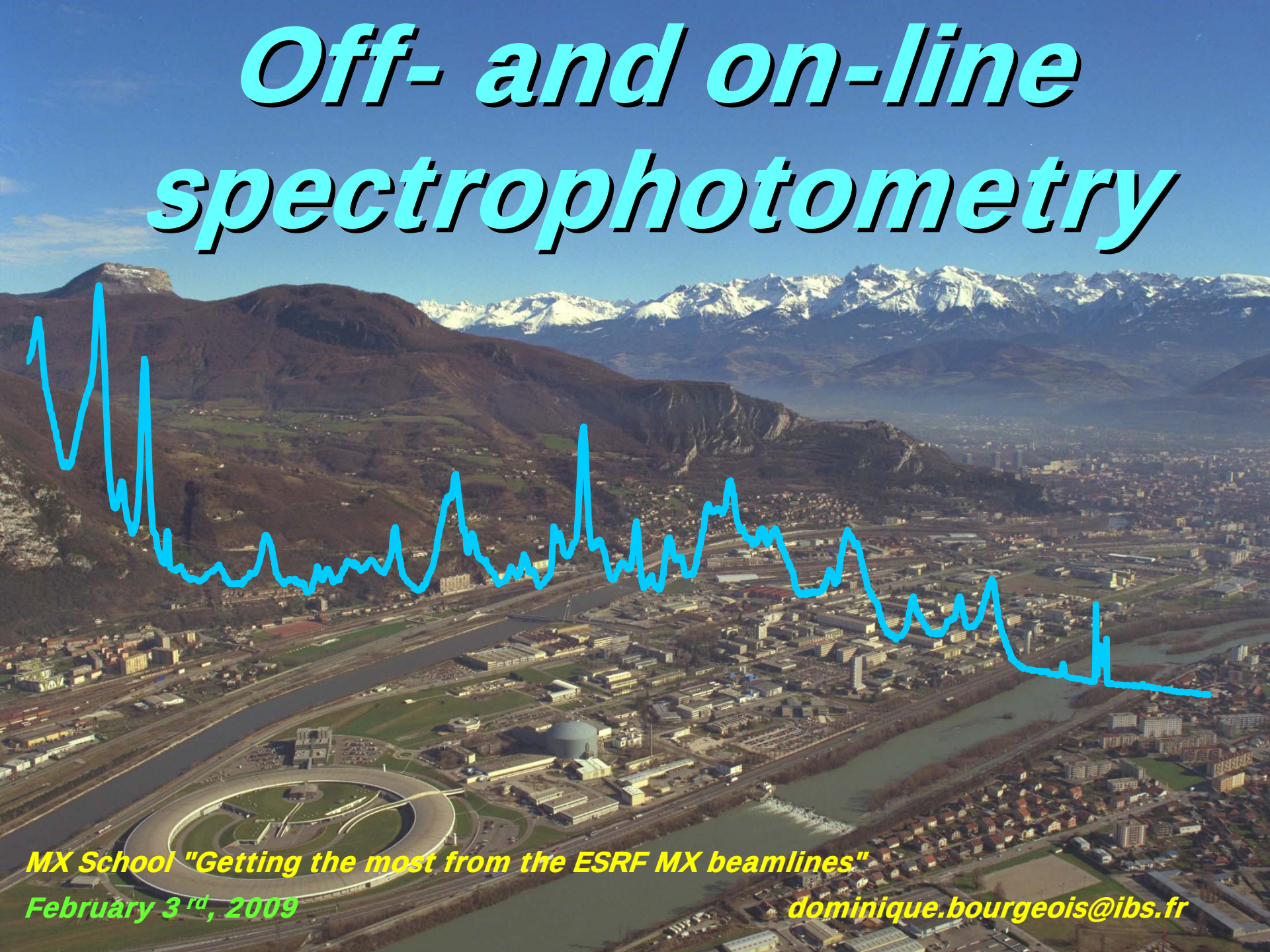
Off- and on-line spectrophotometry



MX School "Getting the most from the ESRF MX beamlines"

February 3rd, 2009

dominique.bourgeois@ibs.fr

Basics

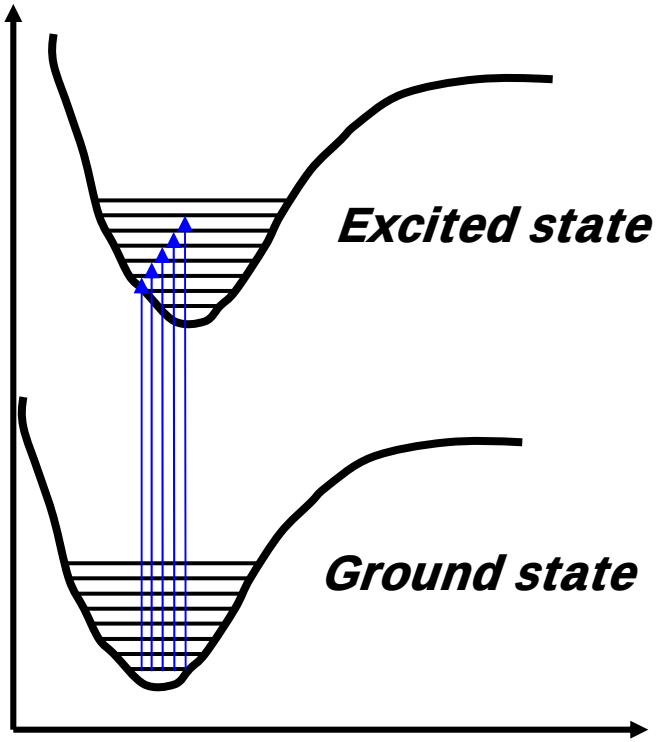
Practical realization

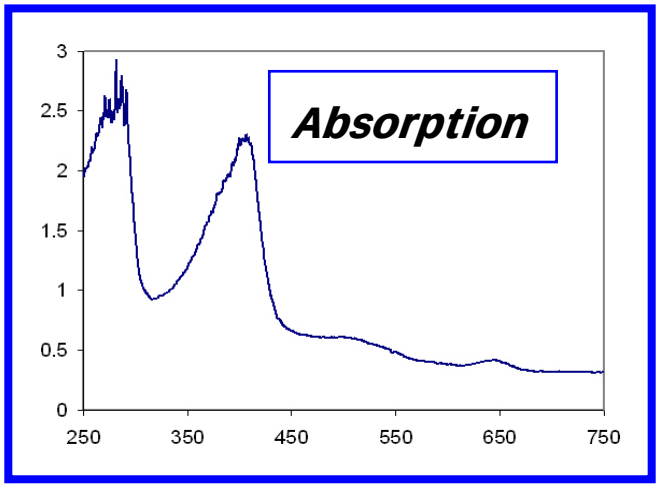
Applications

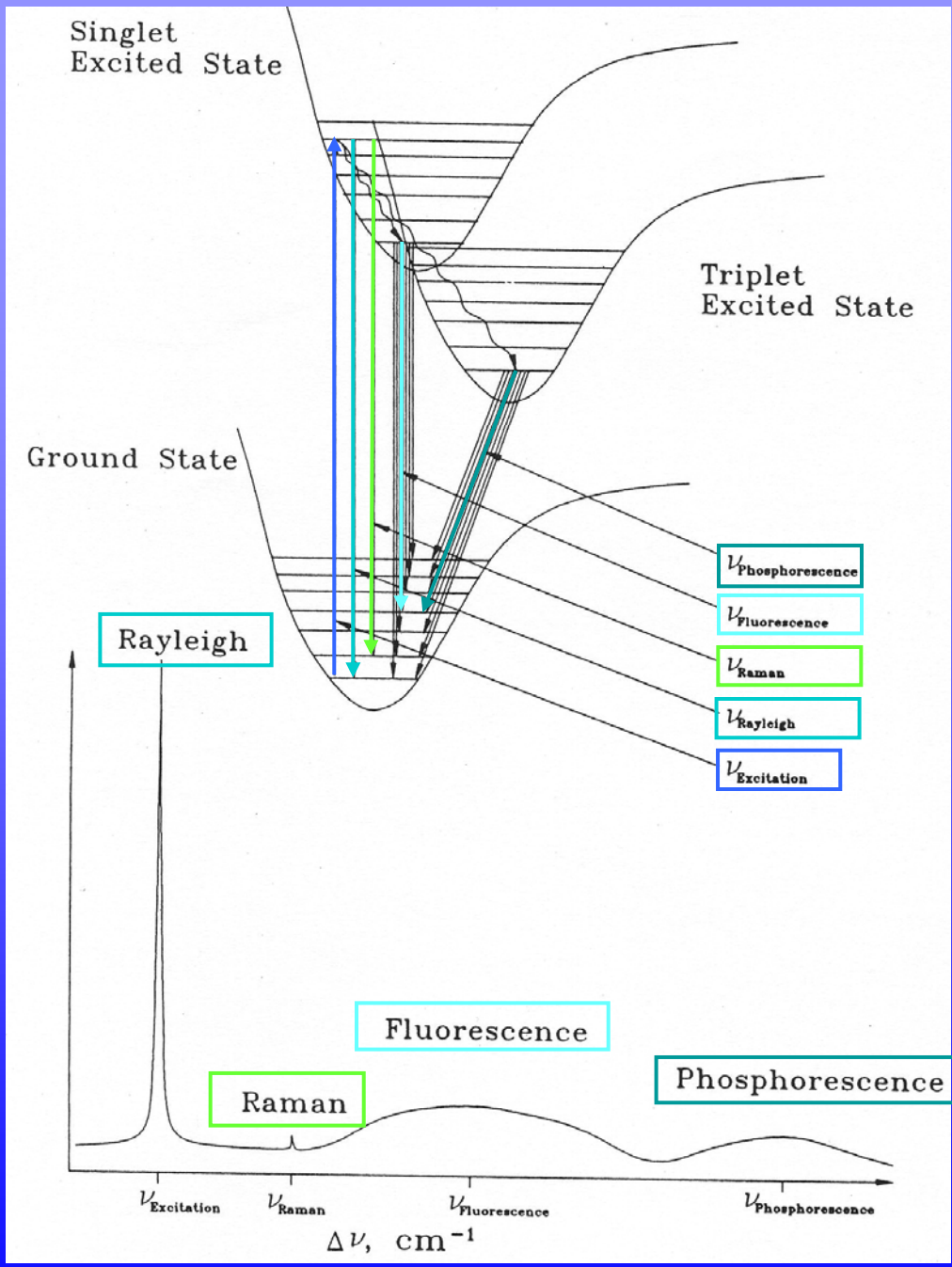
The ESRF Cryobench lab

Superoxide reductase

Basics


Excited state
Ground state

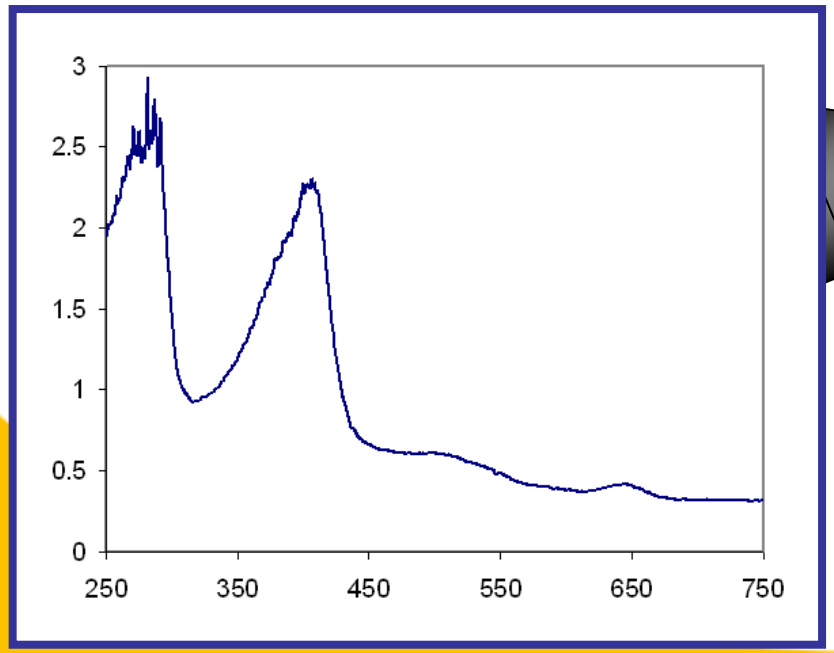

Absorption
3
2.5
2
1.5
1
0.5
0
250
350
450
550
650
750

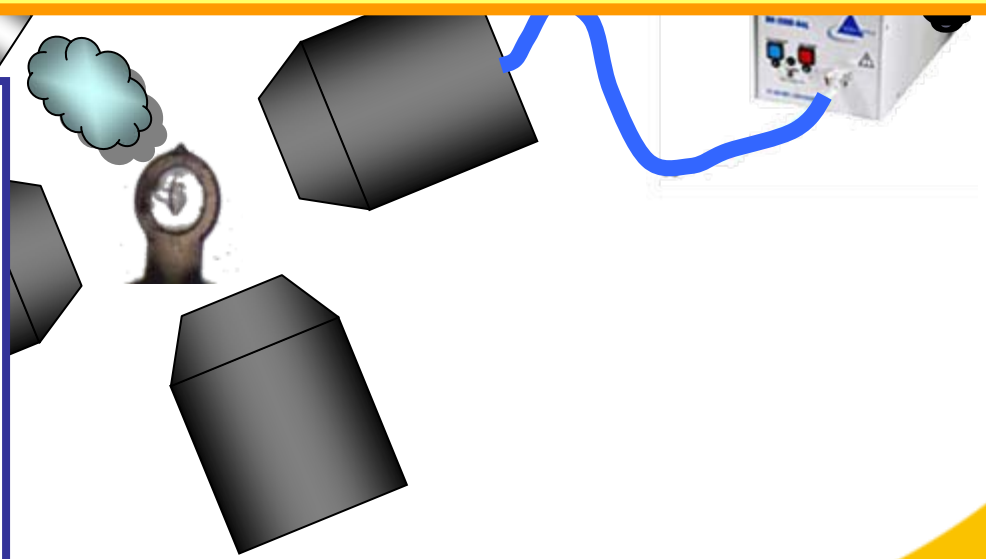

Singlet
Excited State
Triplet
Excited State
Ground State
$\nu_{Phosphorescence}$
$\nu_{Fluorescence}$
ν_{Raman}
$\nu_{Rayleigh}$
$\nu_{Excitation}$
Rayleigh
Fluorescence
Raman
Phosphorescence
$\nu_{Excitation}$
ν_{Raman}
$\nu_{Fluorescence}$
$\nu_{Phosphorescence}$
$\Delta\nu$, cm^{-1}

Practical realization

Absorption spectrum measurement

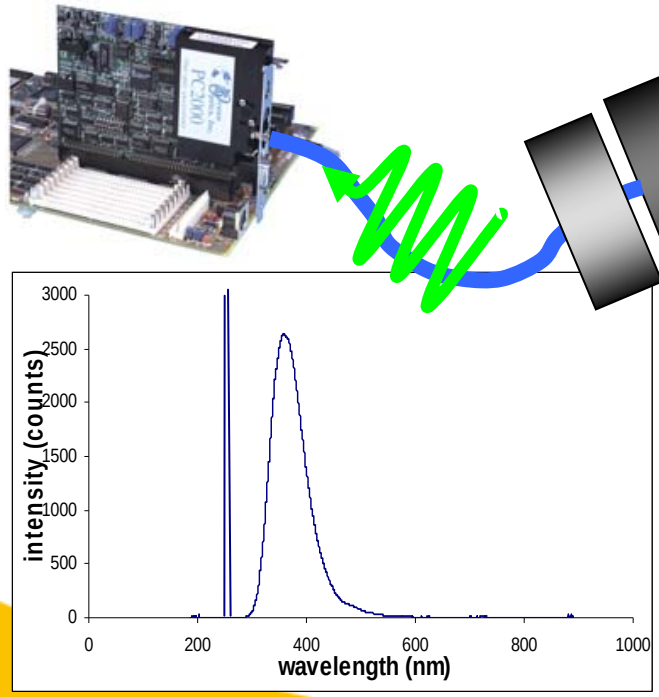
- Measurement time ~ 50 ms
- Pb: optically thick crystals, light scattering effects, orientation dependance, polarization effects





Fluorescence spectrum measurement

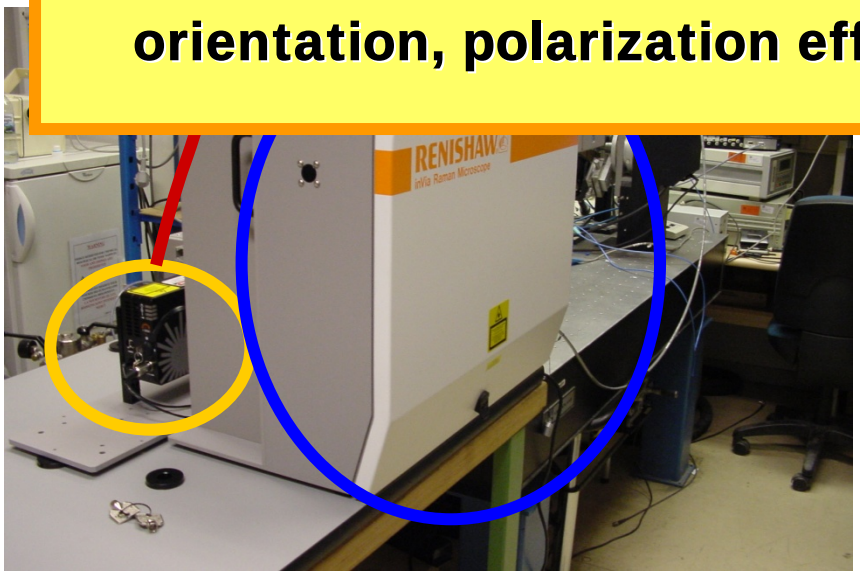
- Measurement time ~ 1 ms (with laser)
- Pb: inner filtering, polarization effects

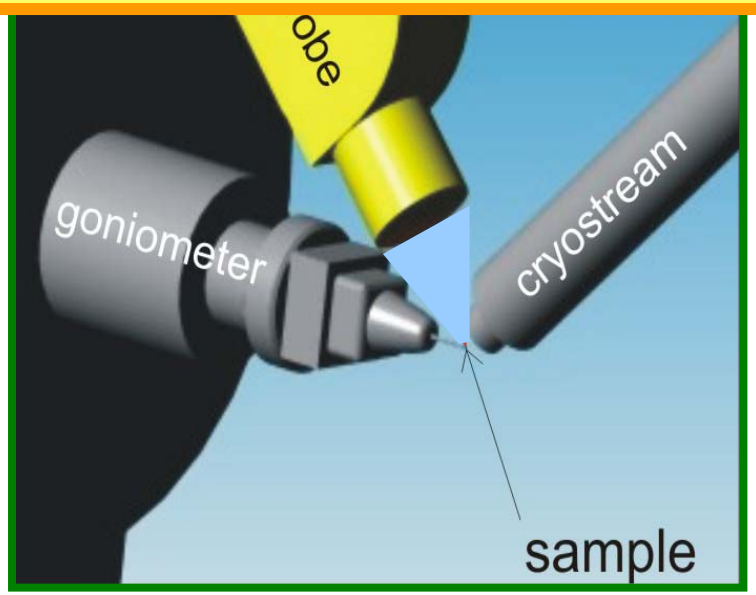


Raman spectrum measurement

785 nm

- Wealth of information
- Measurement time ~ **15 min**, solvent contamination, orientation, polarization effects





In crystallo Raman spectroscopy

Cys vibrations
510, 530 cm^{-1} S-S stretching
725 cm^{-1} Cys C-S stretching

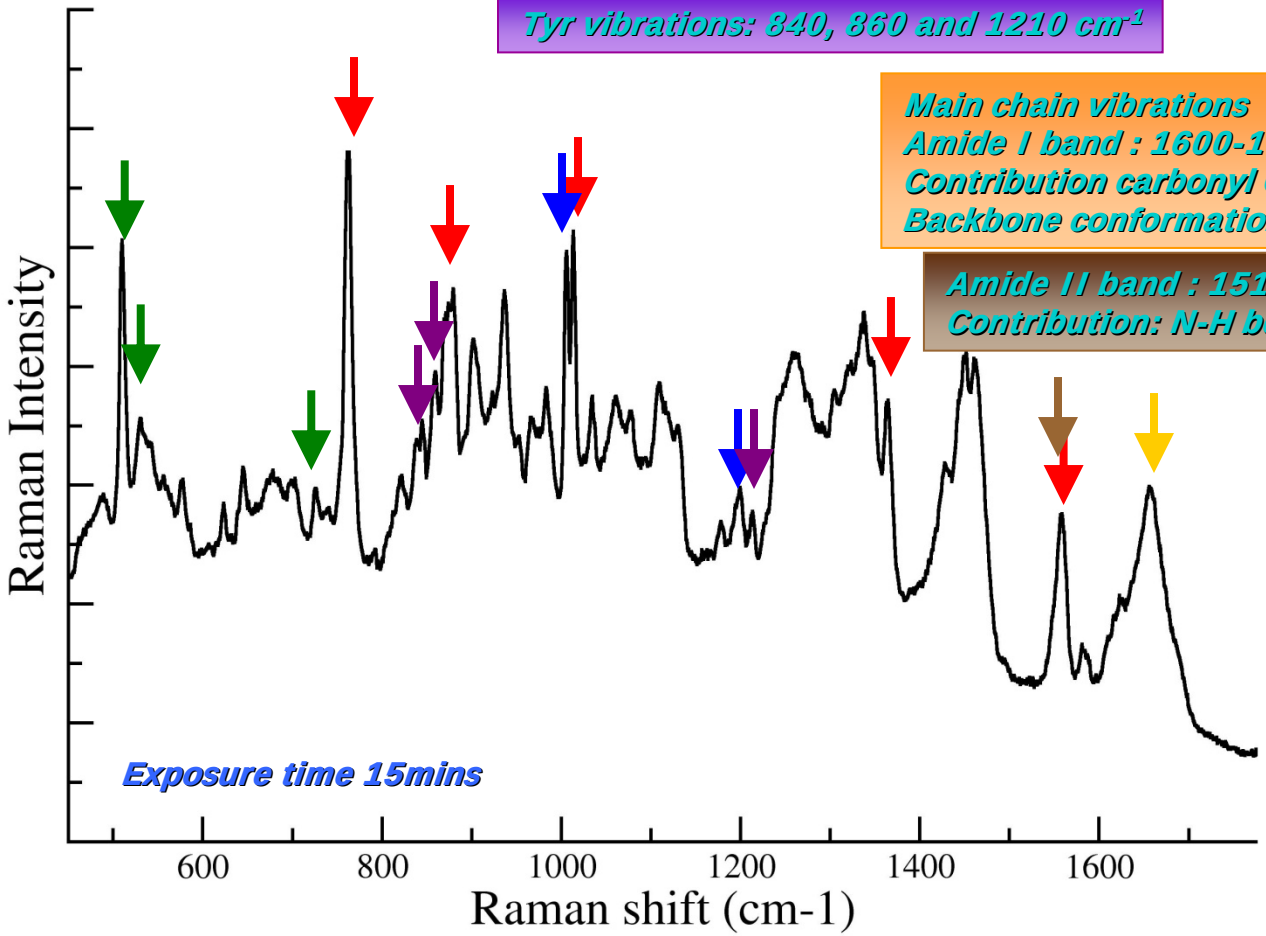
Trp vibrations : 760, 880, 1010, 1350 and 1558 cm^{-1}

Phe vibrations: 1005 and 1200 cm^{-1}

Tyr vibrations: 840, 860 and 1210 cm^{-1}

Main chain vibrations
Amide I band : 1600-1700cm^{-1}
Contribution carbonyl C=0 stretching
Backbone conformation and H-bonding pattern

Amide II band : 1510-1580cm^{-1}
Contribution: N-H bending and C-N stretching

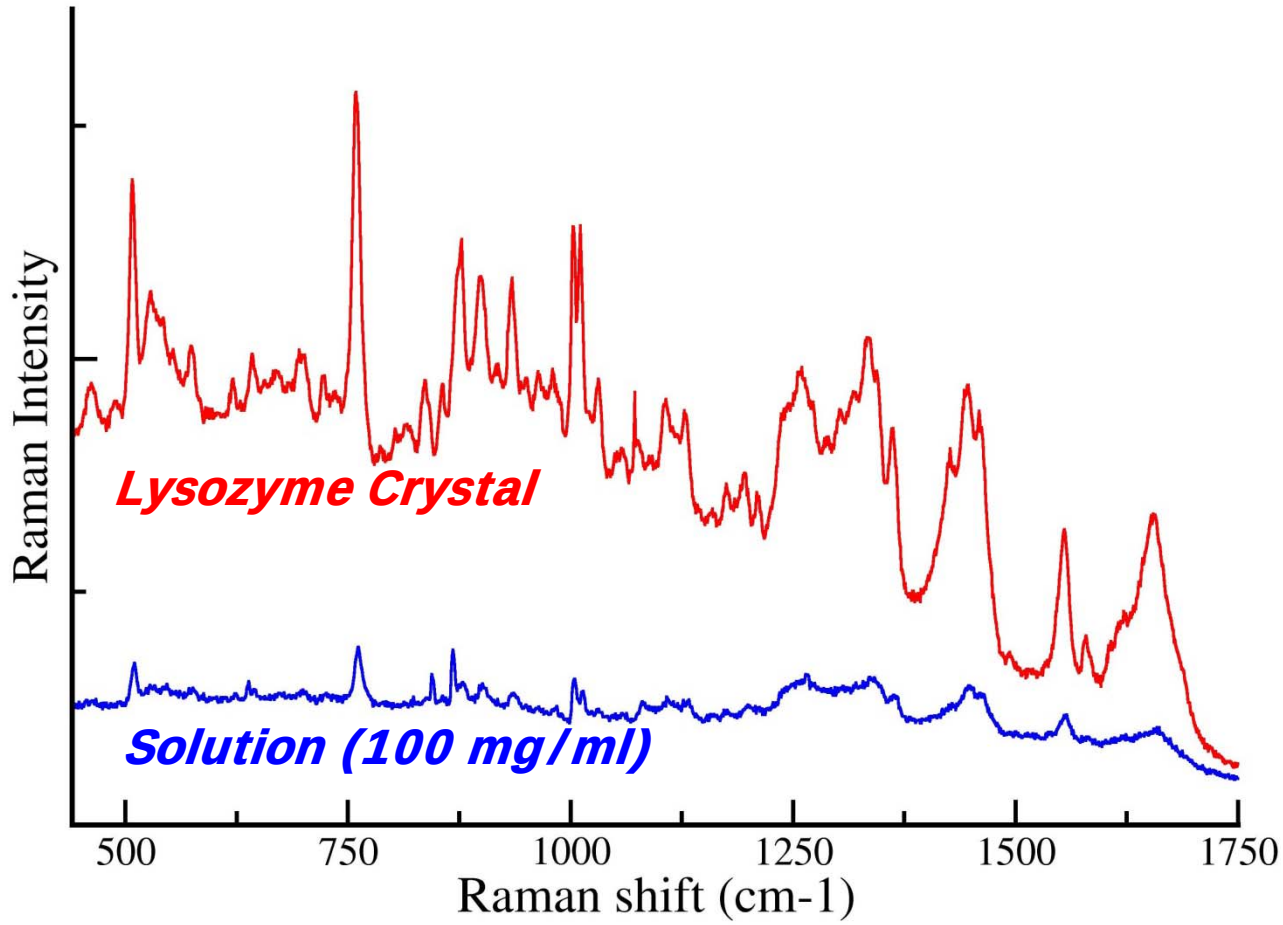


Lysozyme crystal

Raman spectroscopy: advantages of crystals

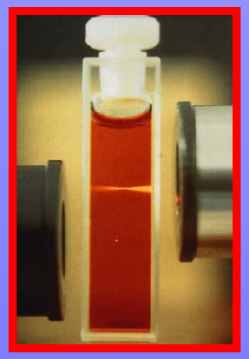
- *High concentration of proteins in crystals 10-50mM (10-100 time more than solution): Higher Raman intensity*
- *Less contribution from solvent in crystals (50%): Lower background in Raman spectra*
- *Selection of conformations in crystals: narrower Raman bands in crystals => better Raman resolution*

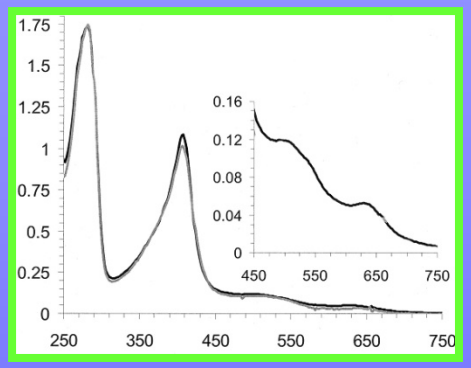
Non-resonant in crystallo Raman data



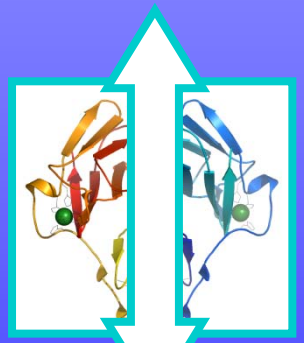
Applications

“In crystallo” spectroscopy ?

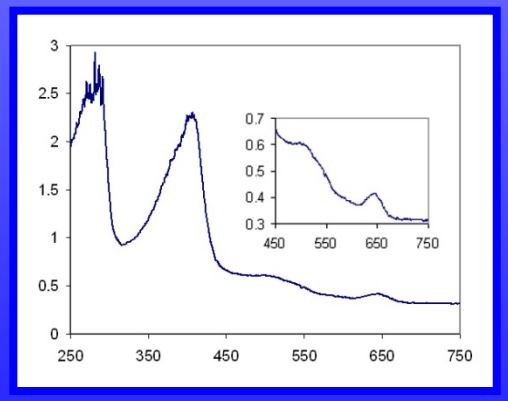




Then



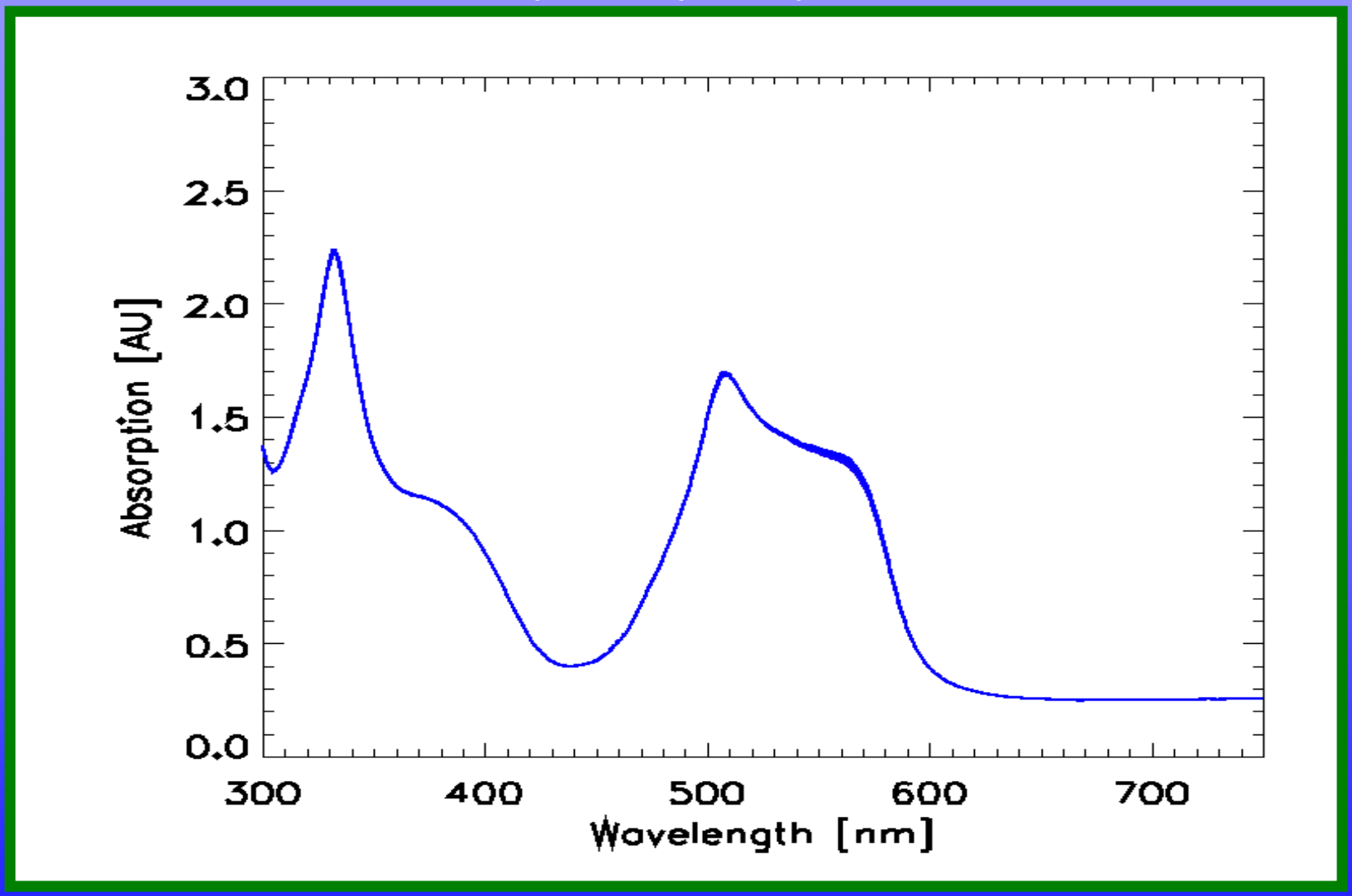
If



a tool to validate the crystal structure.

J. Mc Geehan, EMBL & Dr Xavi Carpena, Parc Cientific de Barcelona

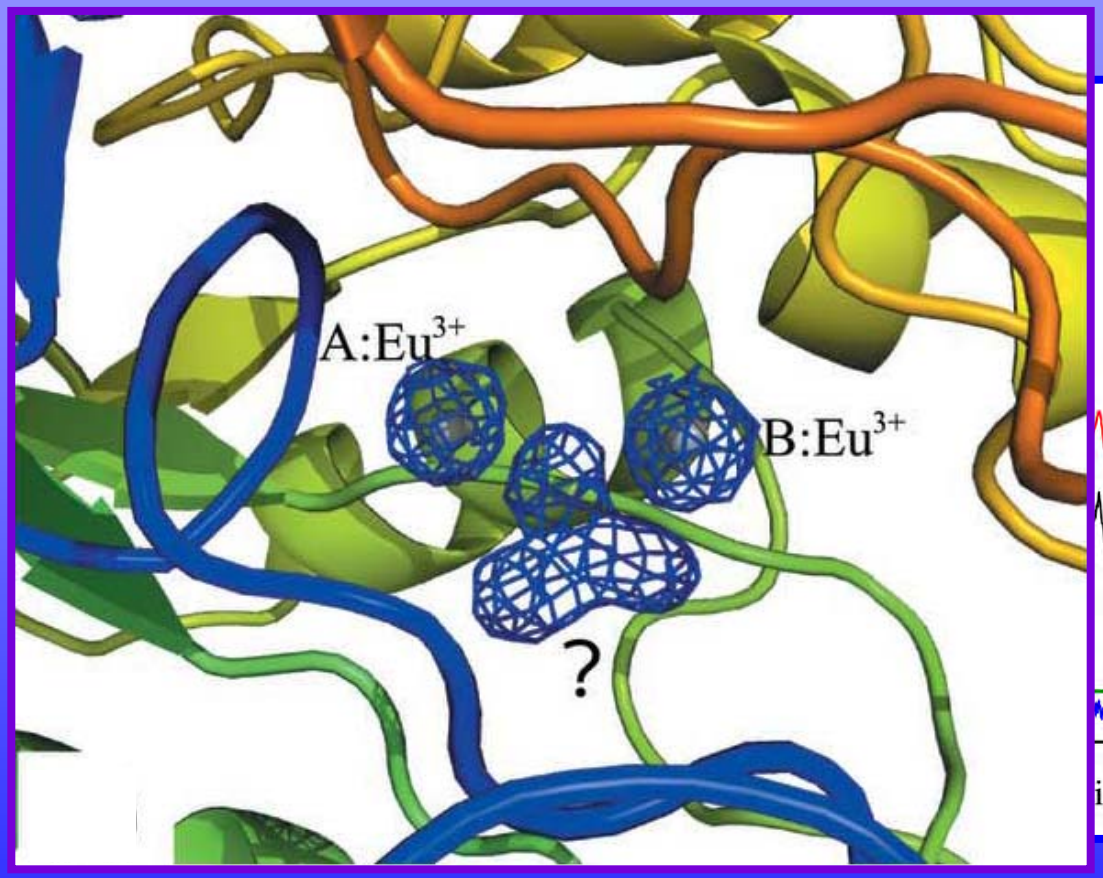
Photoconversion of Alpha-phycoerythrocyanin crystals (absorption)

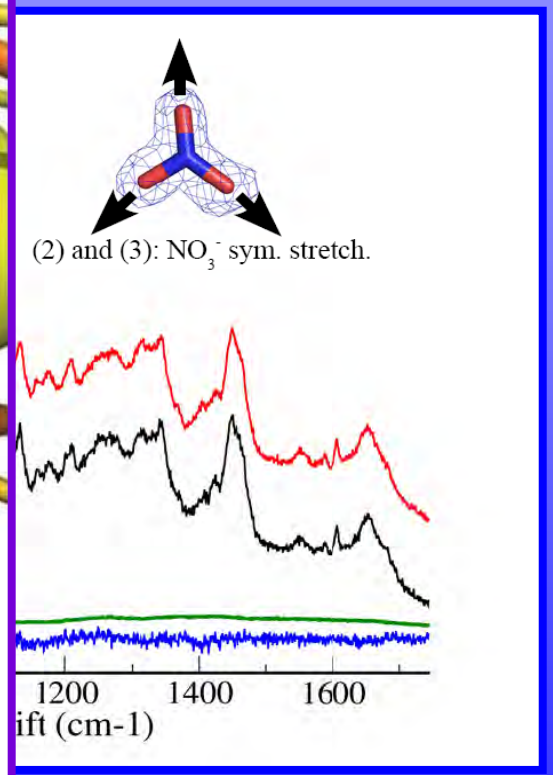


a tool to check activity in the crystalline state.

Marius Schmidt, University of Munich, Germany, Biochemistry 46, 416, 2007

Binding of nitrate to xylose isomerase (Raman)

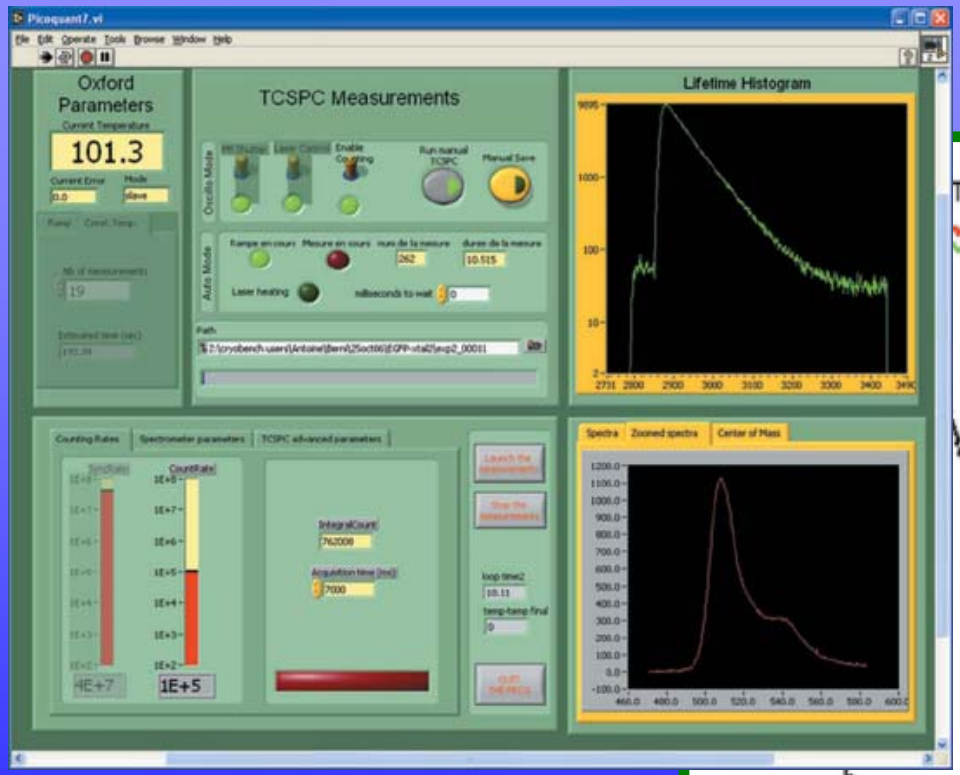


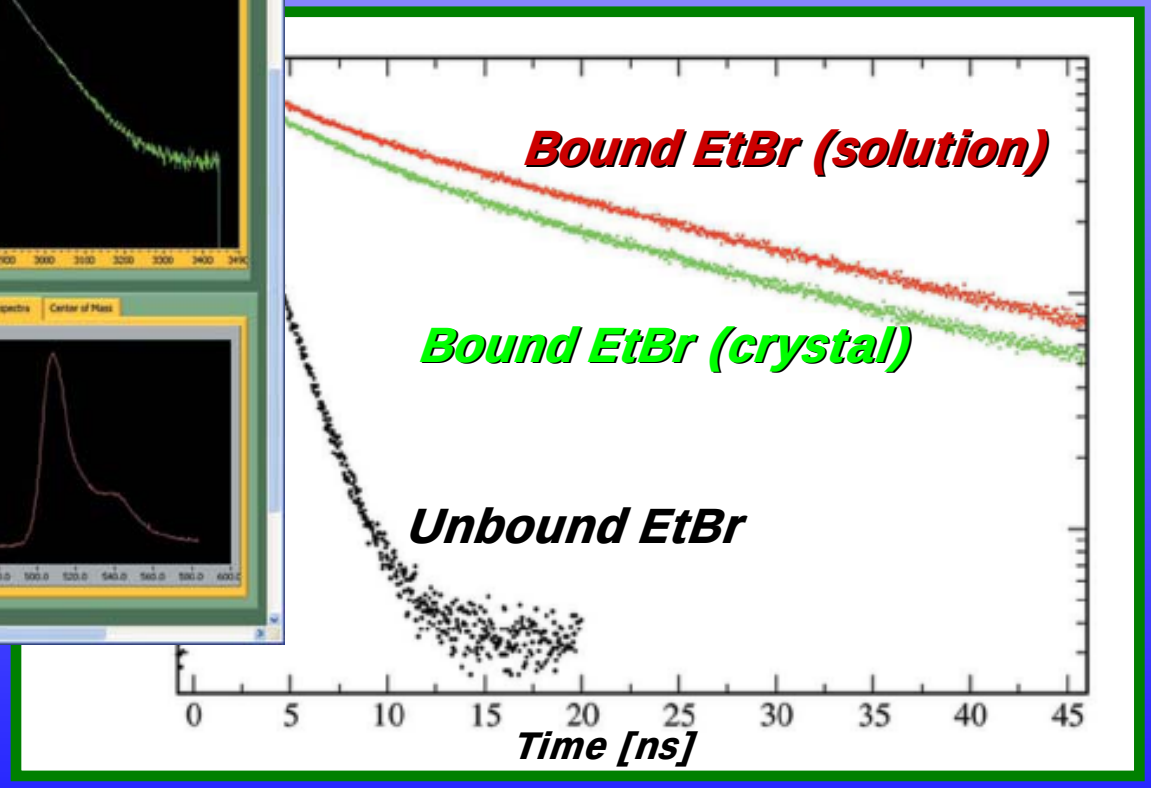


a tool identify bound ligands.

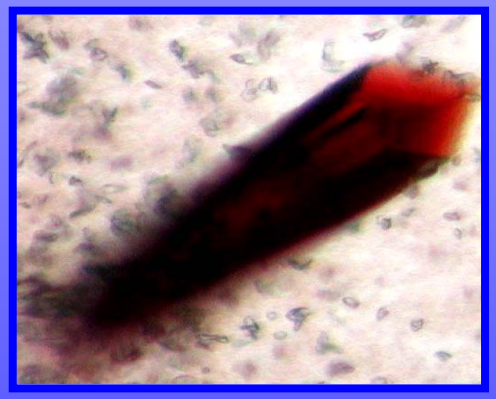
Carpentier et al, J. Appl. Cryst. (2007), 40, 1113–1122.

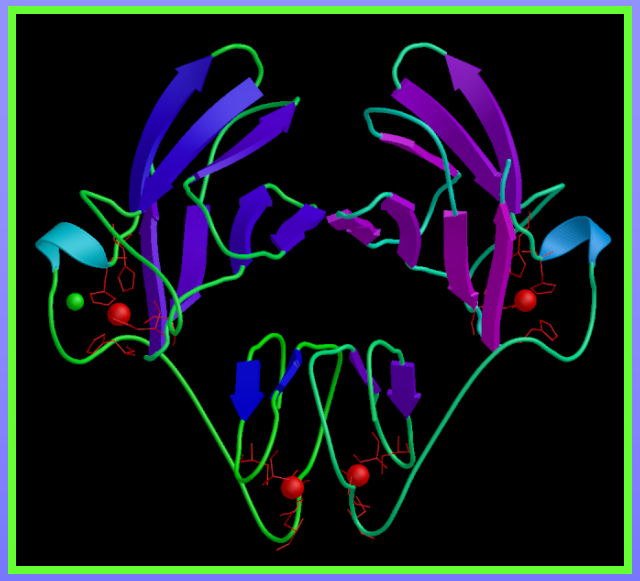
Binding of EtBr to DNA (fluorescence lifetime)

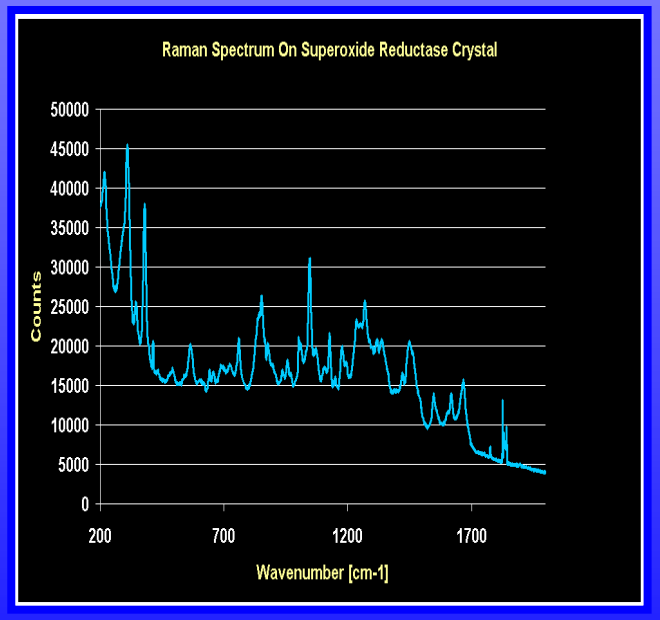




Royant et al, J. Appl. Cryst. (2007), 40, 1105-1112.





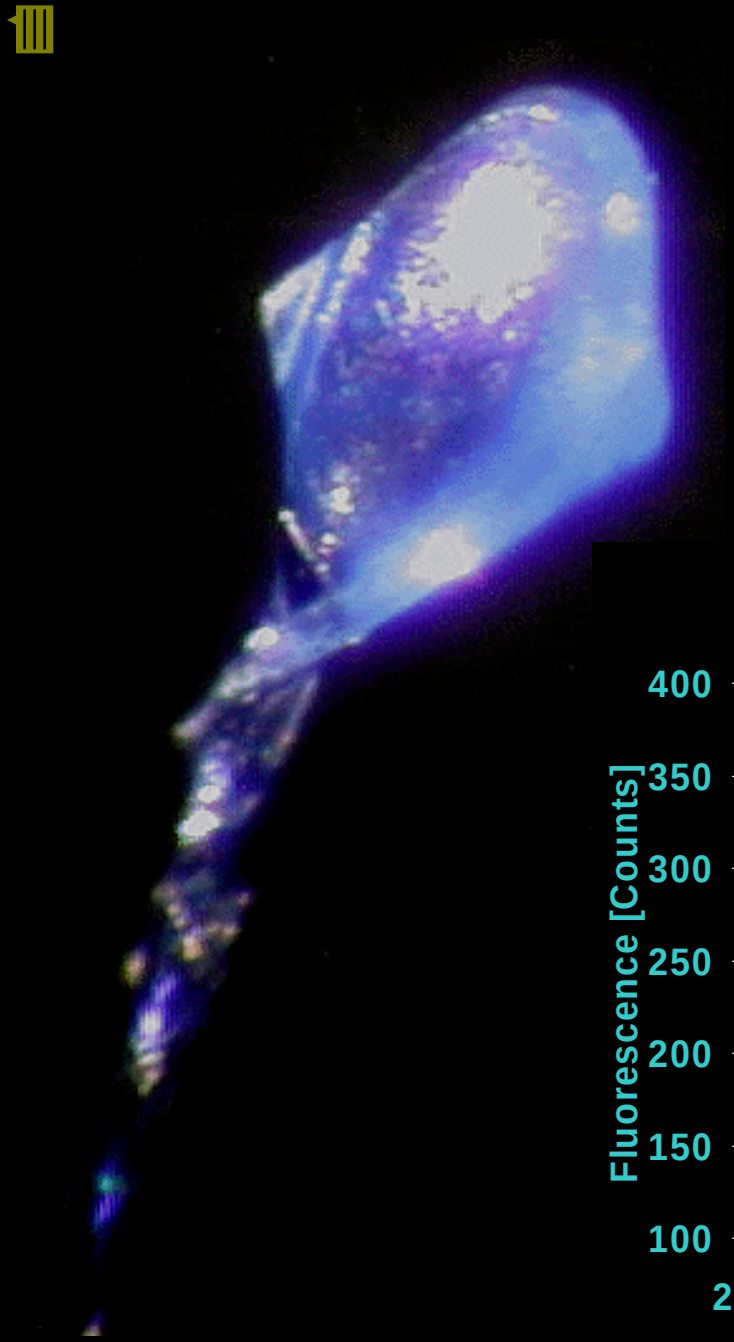


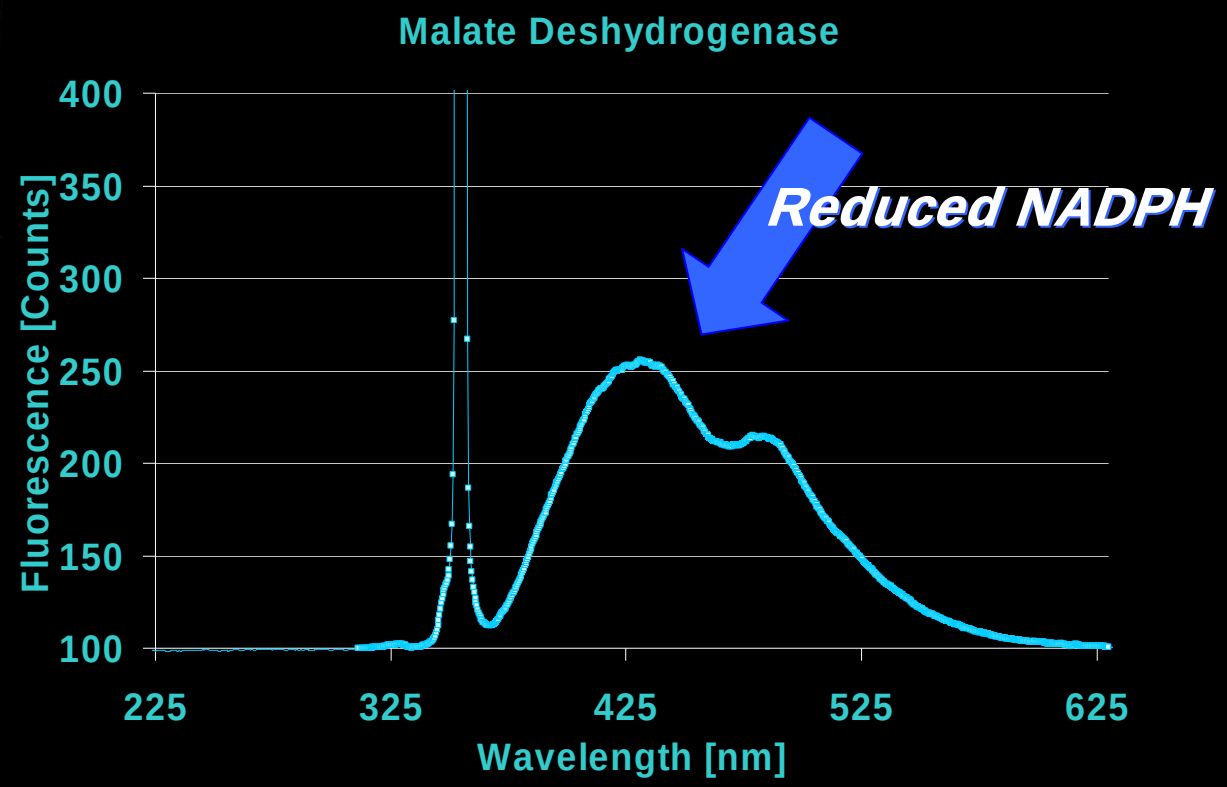
Near-atomic global view

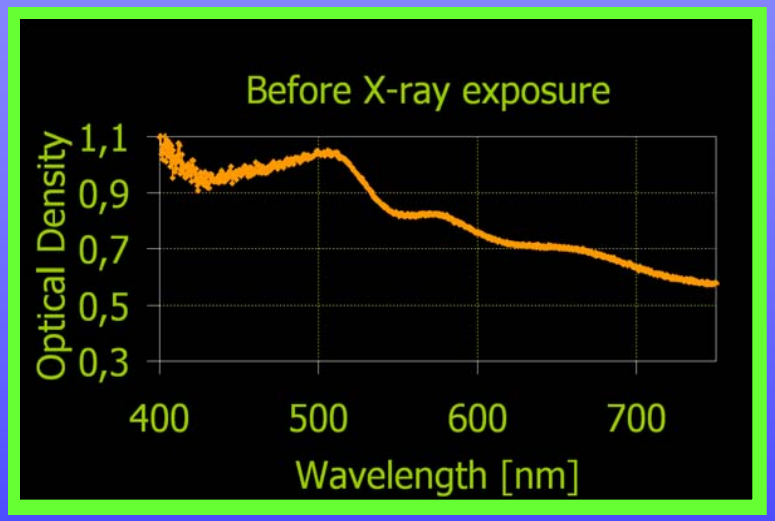
Sub-atomic local view

a tool to complement crystal structure.

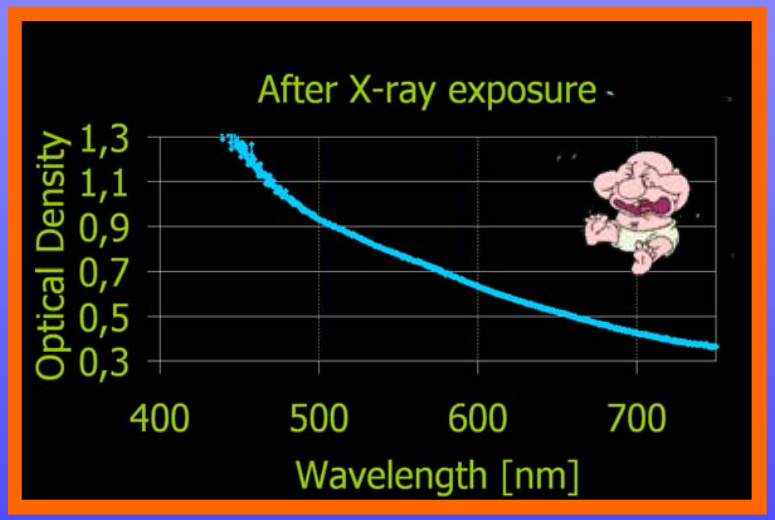
Redox state of NADP(H) (fluorescence)



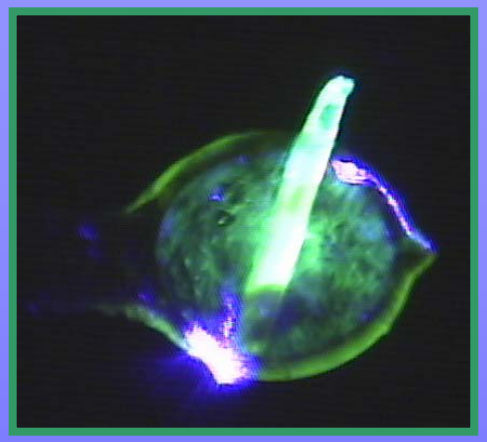




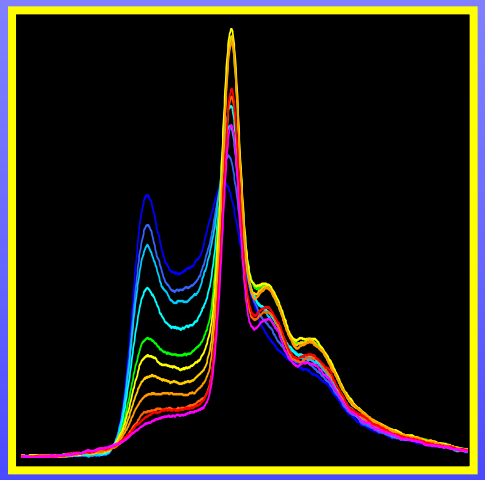
X-rays



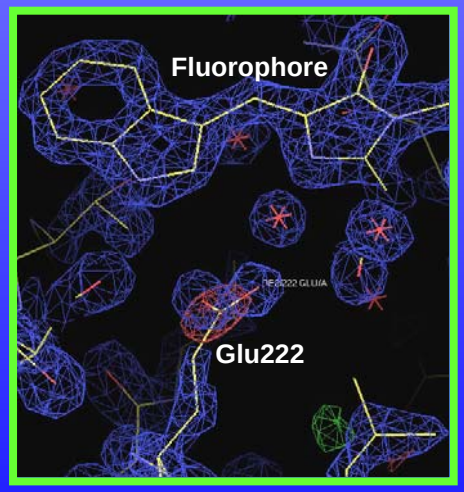
a tool to monitor chemistry induced by X-rays.



In crystallo reaction triggering



Identification of intermediate state



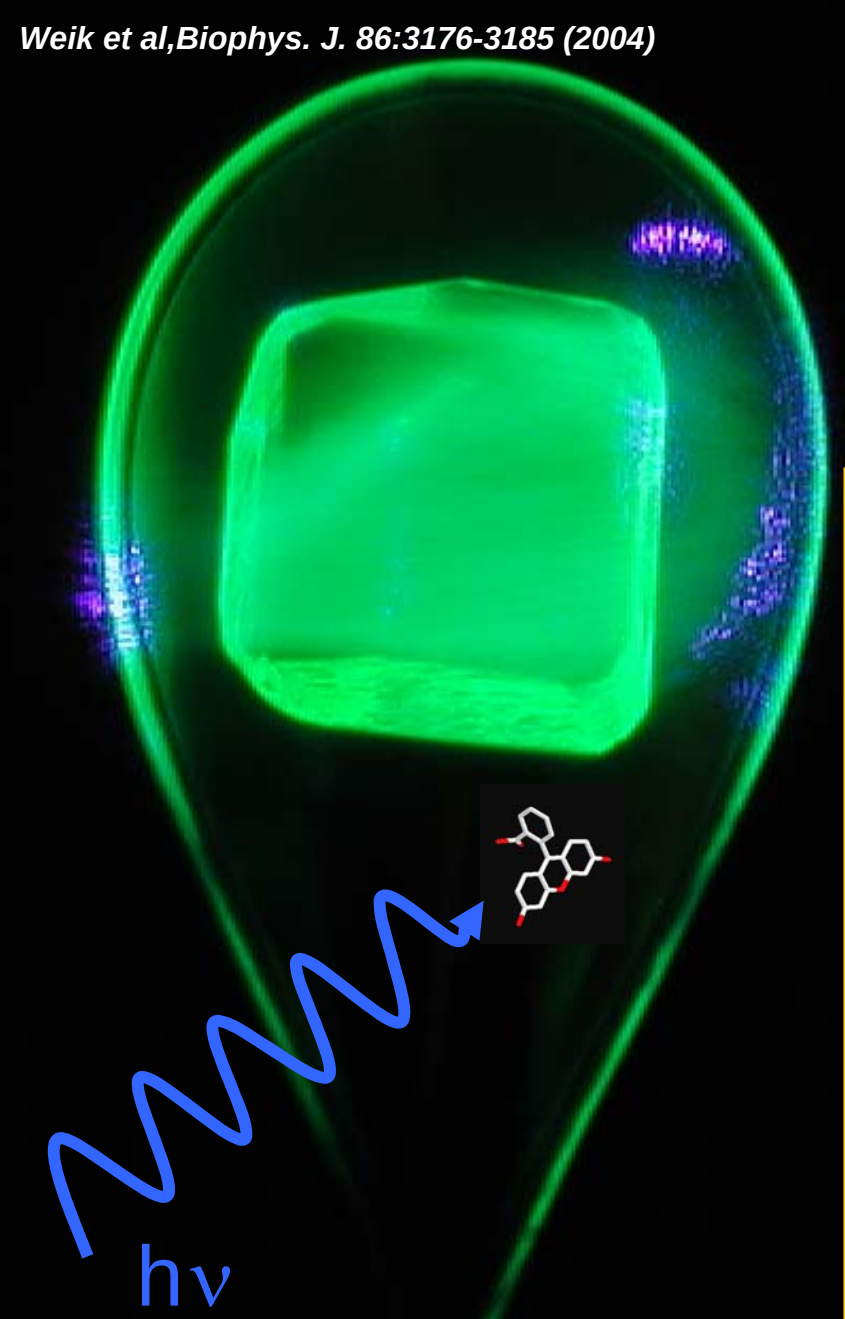
Structure of the intermediate

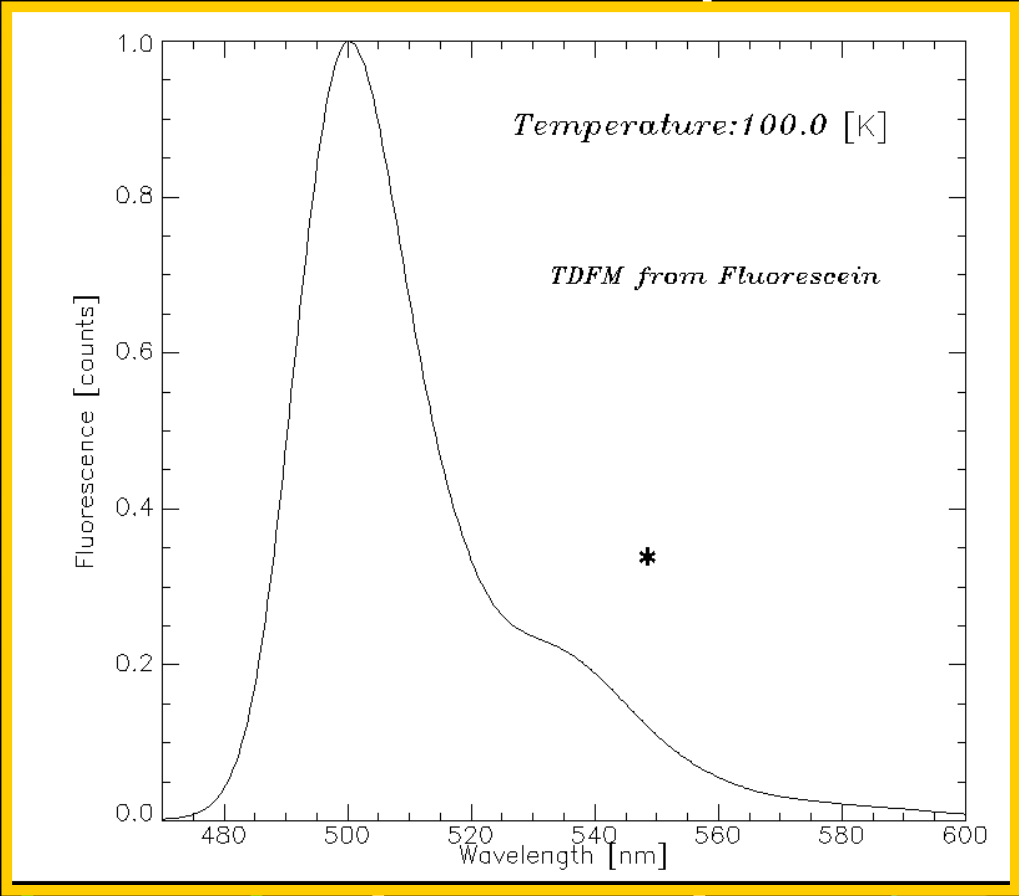
a tool to follow reactions in crystals

Weik et al,Biophys. J. 86:3176-3185 (2004)

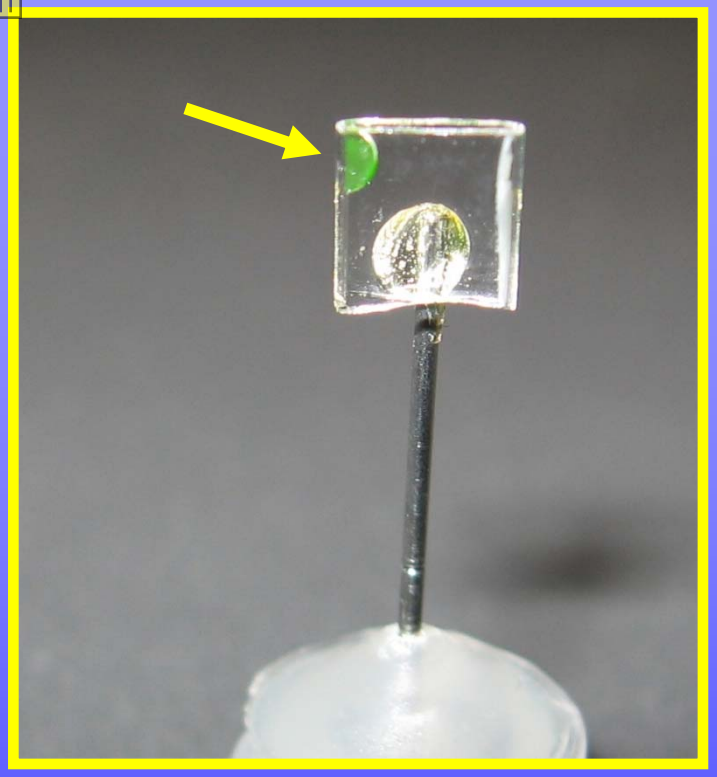
Temperature dependent fluorescence microspectrophotometry (TDFM):

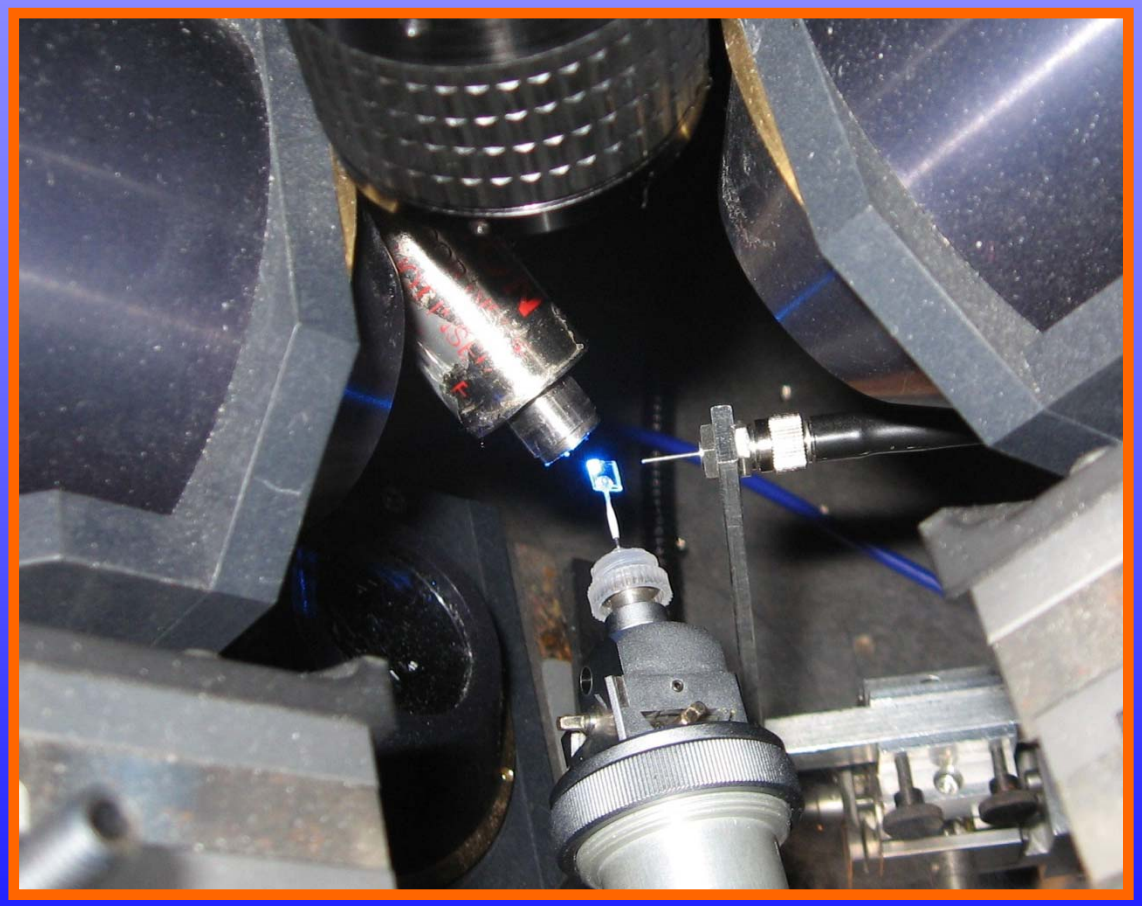
Observing glass transition in nano-volume samples ...





a tool to study dynamics in crystals



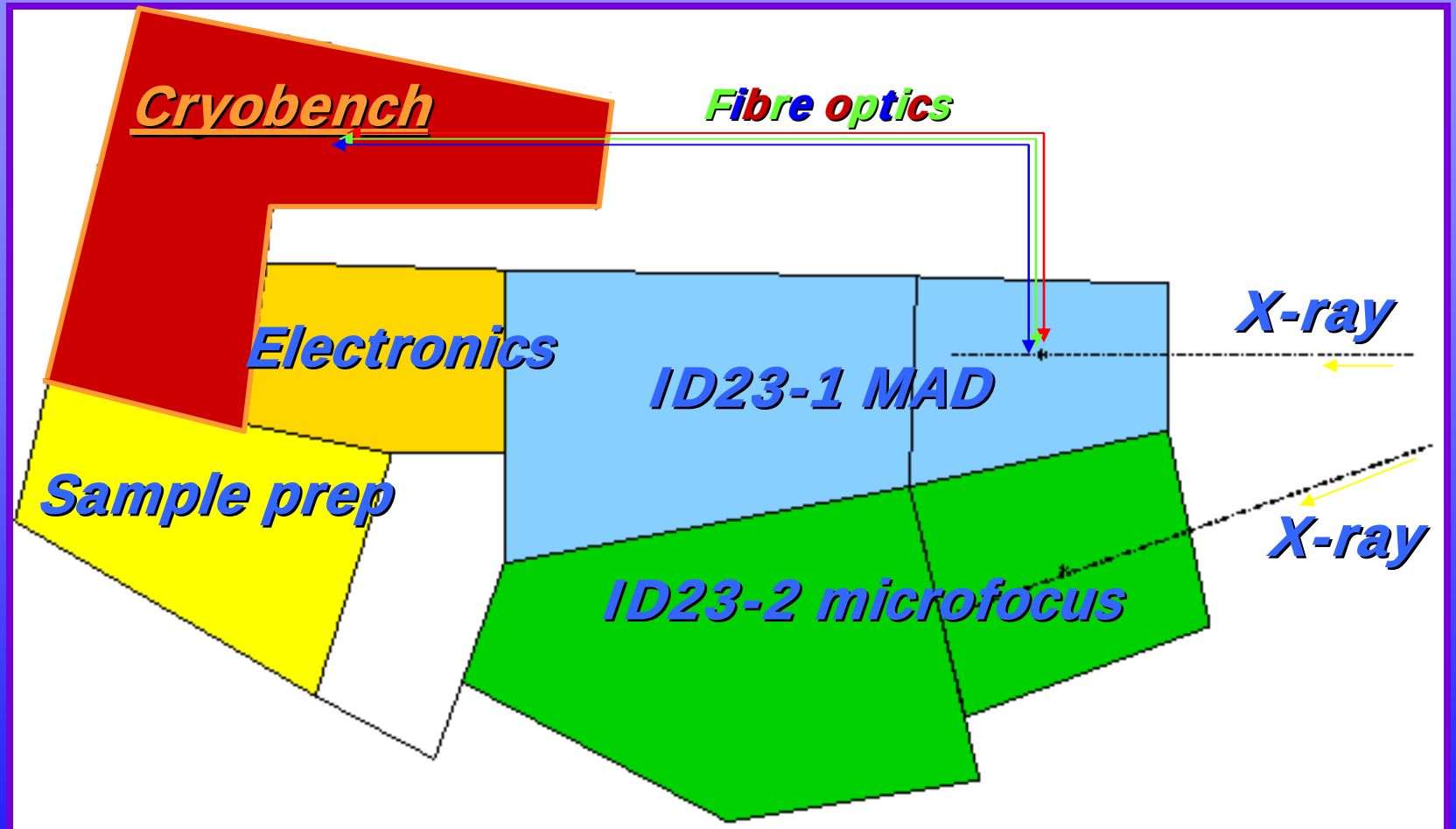


a tool for cryo-enzymology studies in solution

The ESRF Cryobench lab


CRYOBENCH

CRYOBENCH

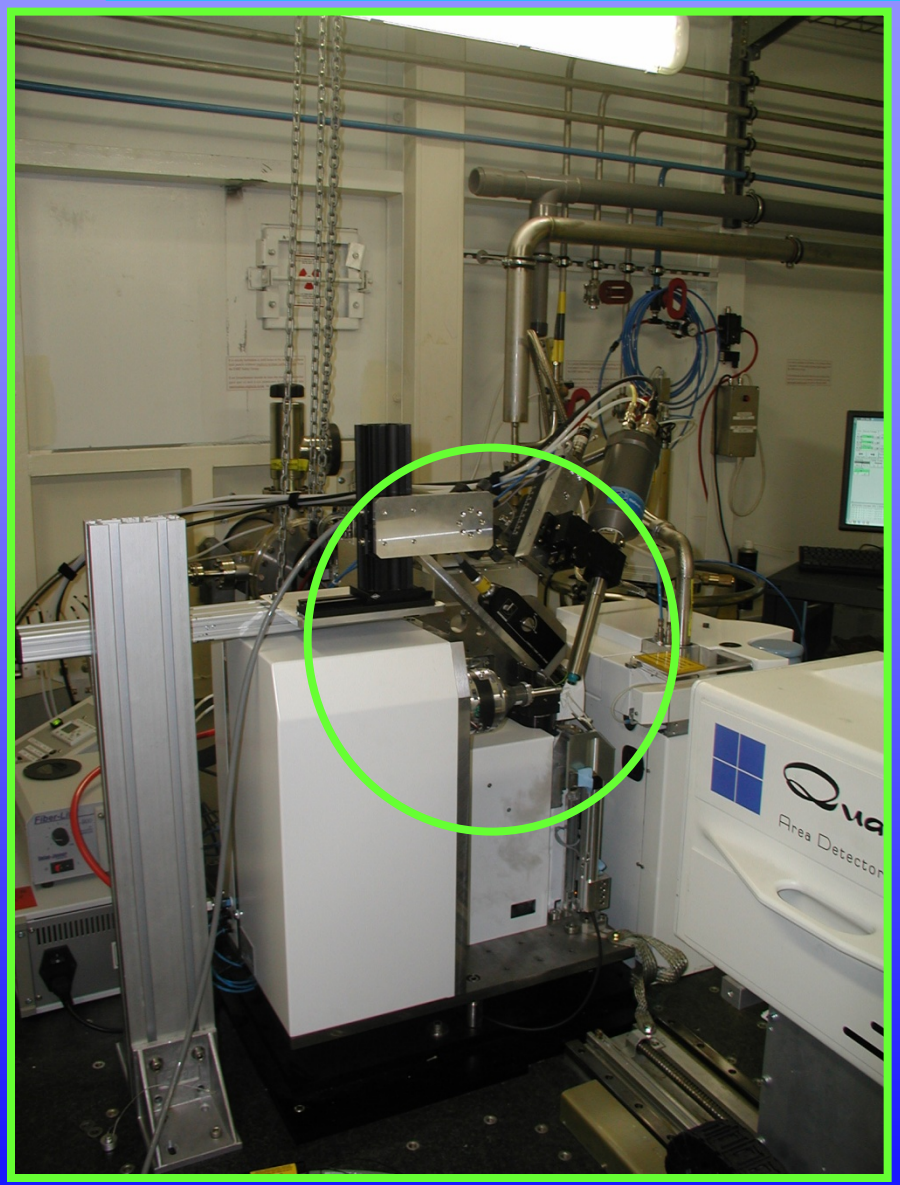


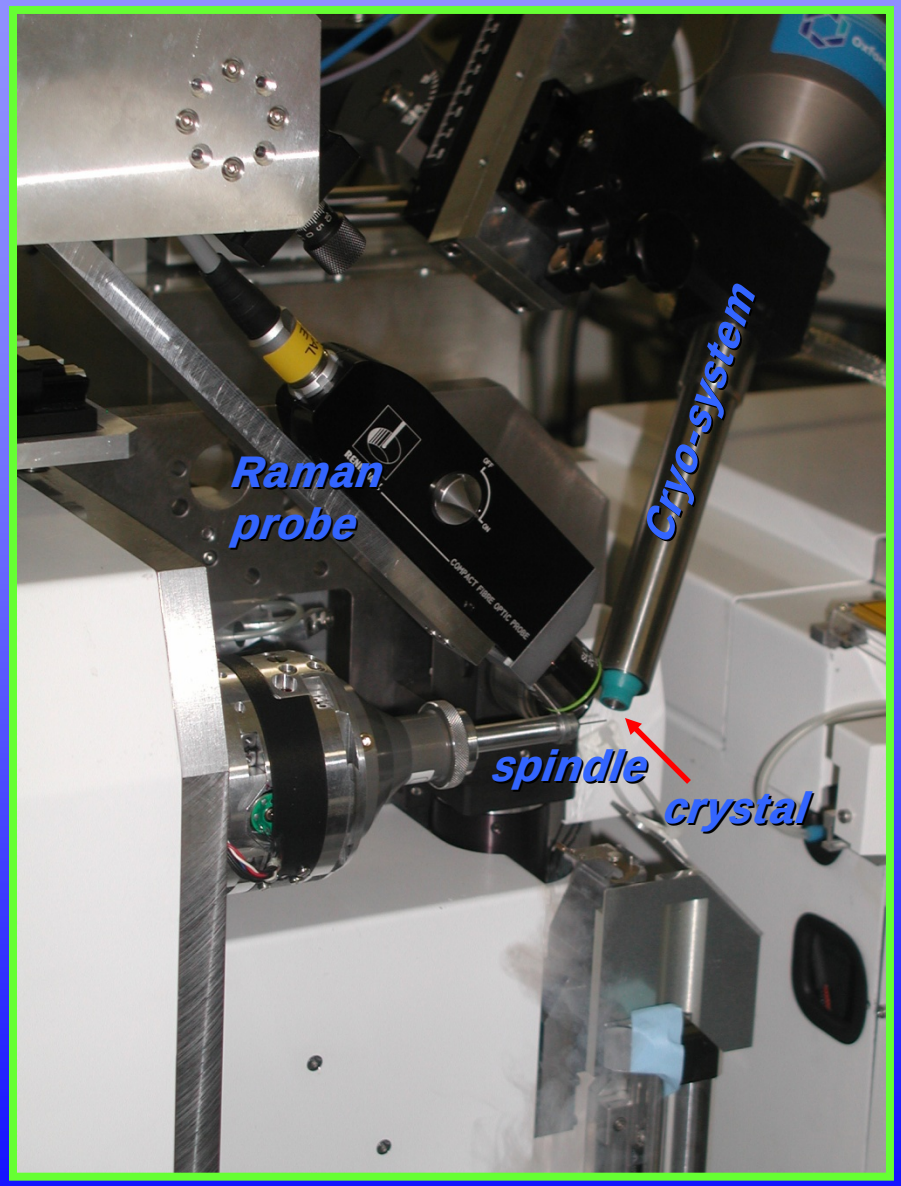
www.esrf.fr/UsersAndScience/Experiments/MX/Cryobench/


Fiber from lasers (fluorescence)
Video-microscope
12X
Fiber from light source (absorption)
Objective 3
Objective 1
Cryostream
Objective 2
Offline
Fiber to spectrometer
Crystal on goniometer head

John McGeehan, Florent Cipriani and Raimond Ravelli, EMBL

On-Line Raman experiment

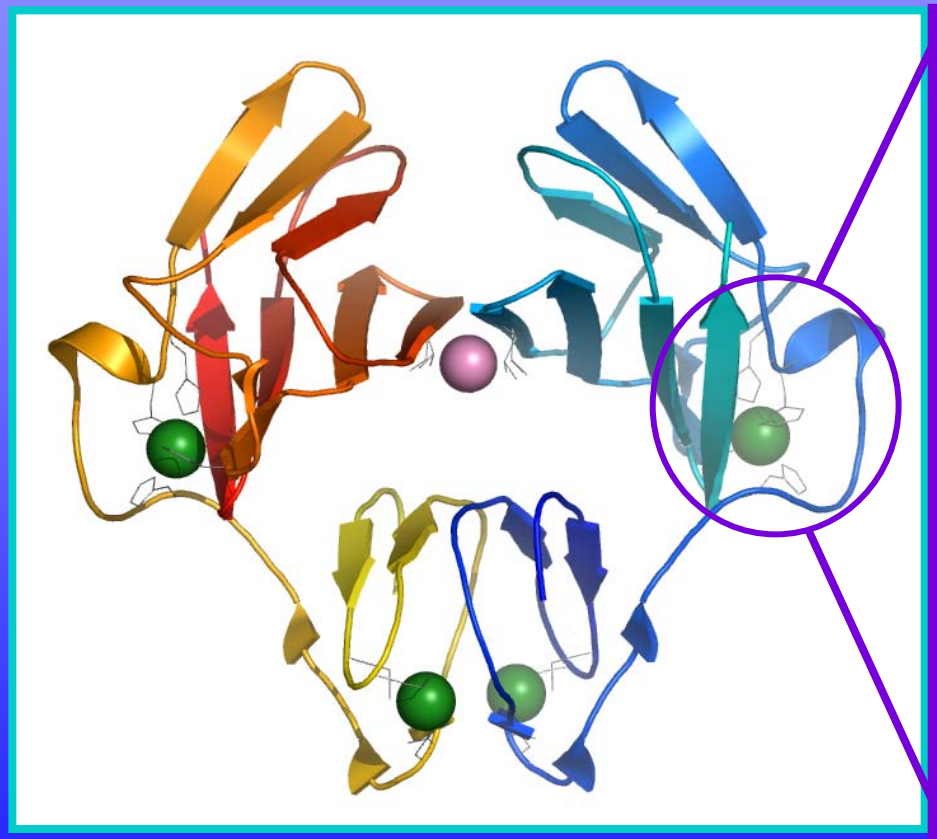


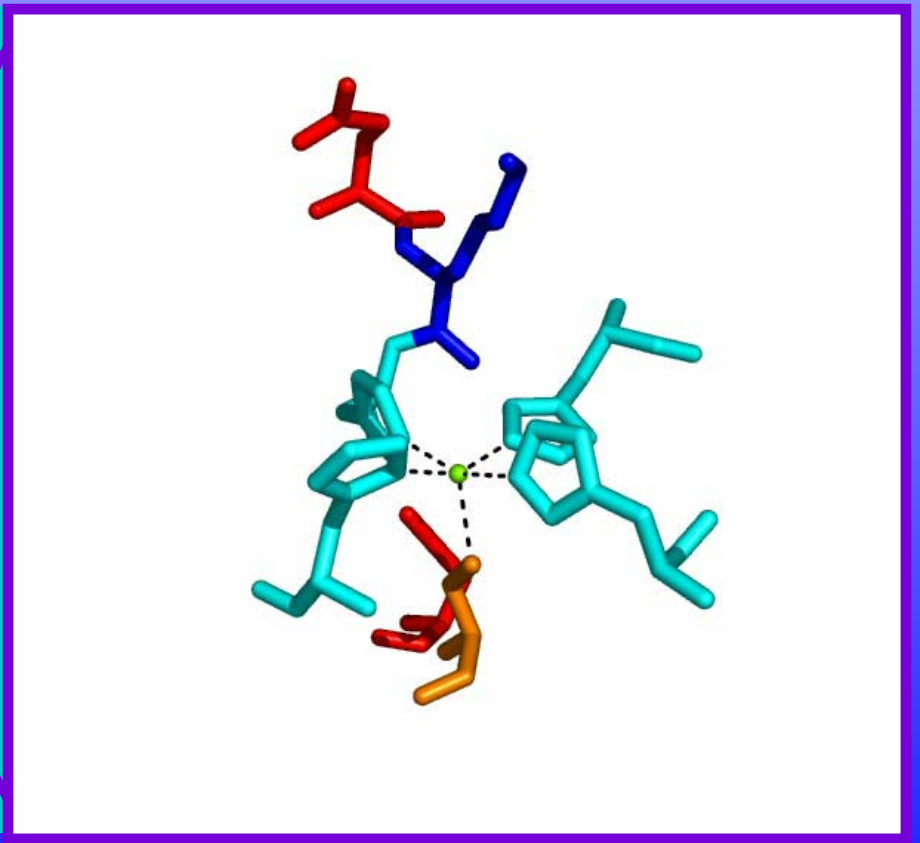


Case example: superoxide reductase

Superoxide reductase:

$O_2^{-\bullet} + 2H^+ + SOR(Fe^{2+}) \rightarrow H_2O_2 + SOR(Fe^{3+})$

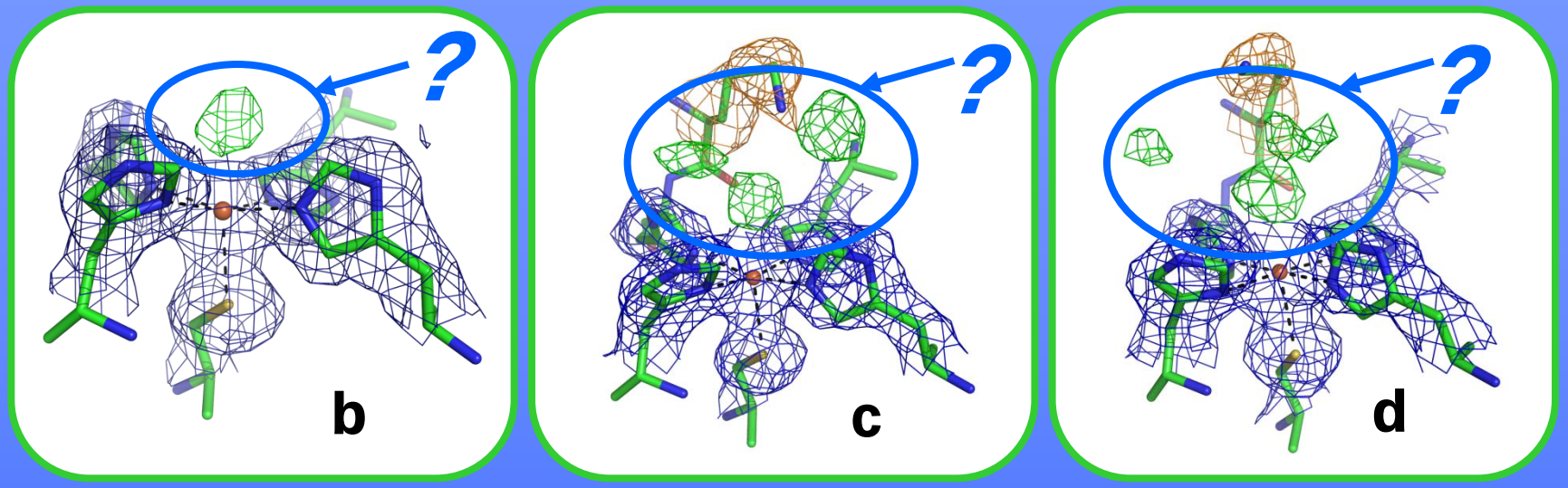




$O_2^{-\bullet} + H^+ + Fe^{2+} \rightarrow Fe^{3+}\text{-}OOH^- \xrightarrow{+ H^+} Fe^{3+} + H_2O_2$

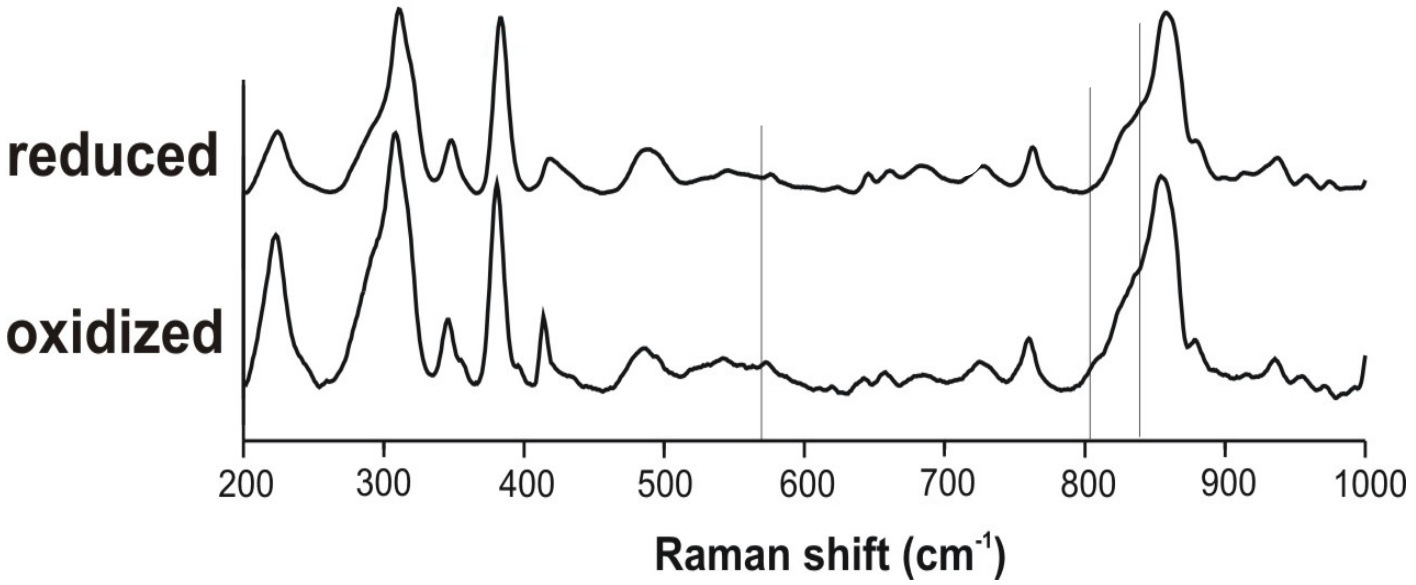
Katona *et al*, *Science* (2007), *316*, 449.

Puzzling crystallographic data

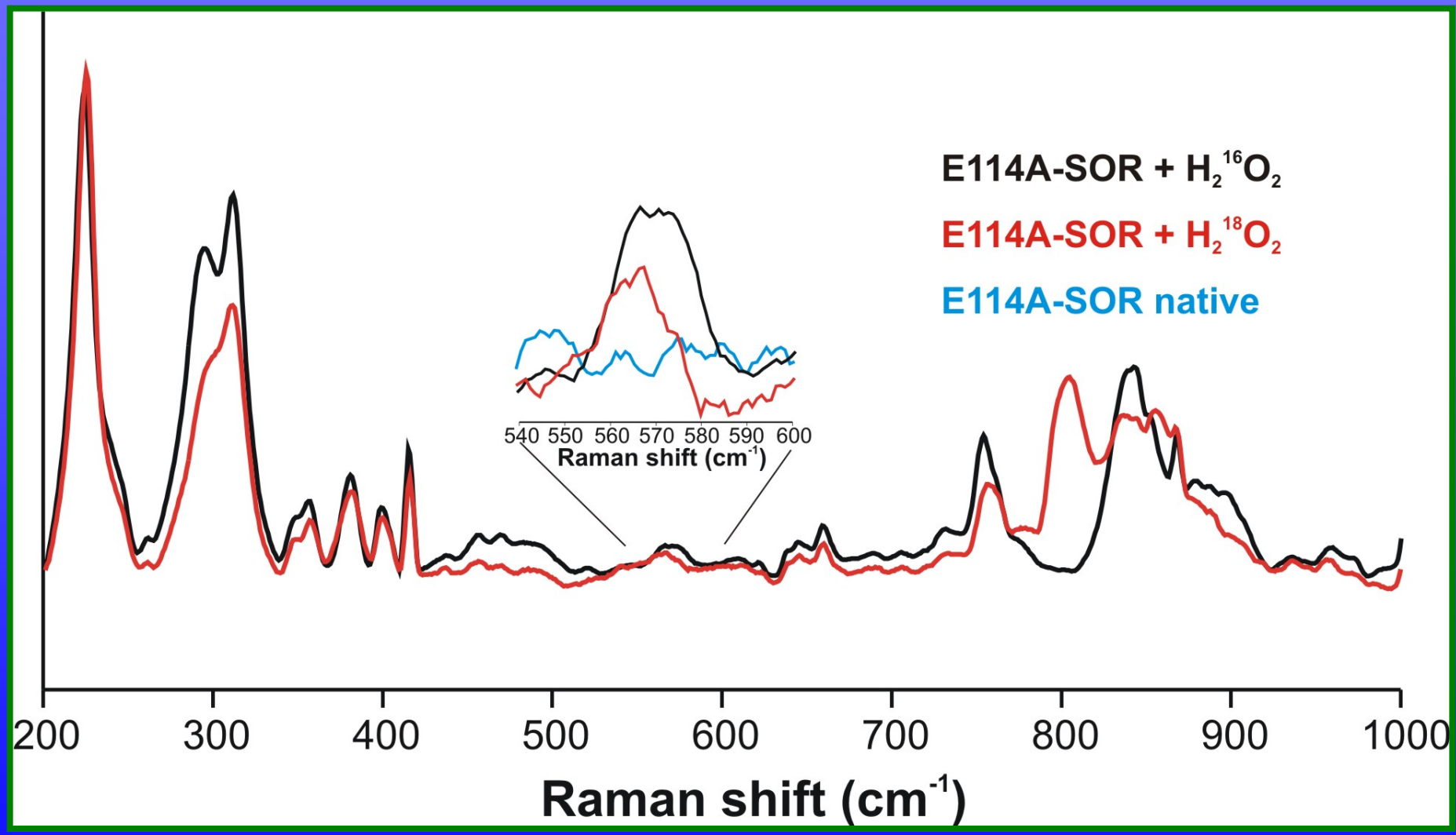


- *Limited crystallographic resolution (1.95 Å)*
- *Multiple observations in the asymmetric unit*
- *Can these be relevant intermediate species?*

In crystallo Raman Spectroscopy of SOR

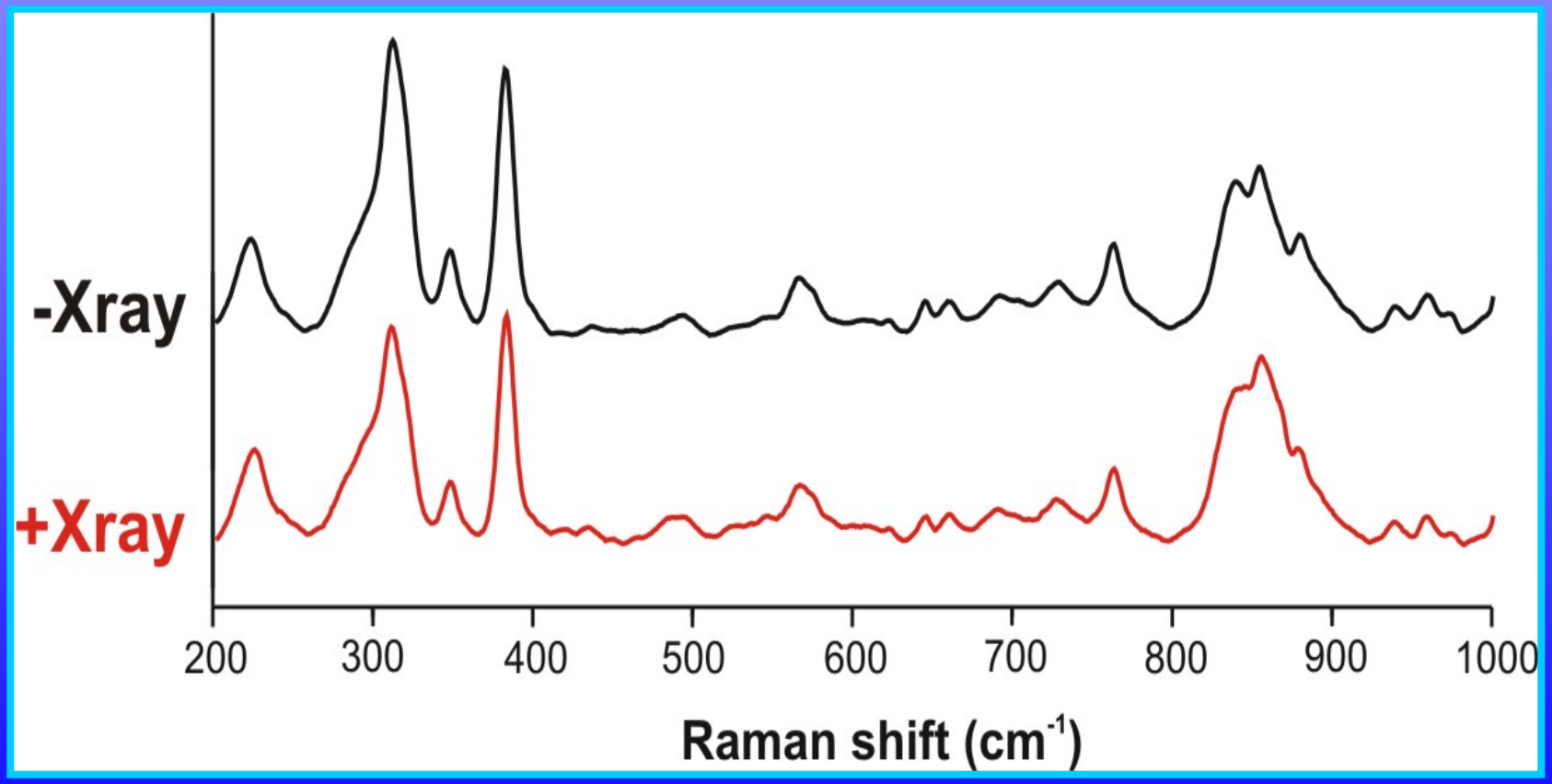


Comparison with solution state

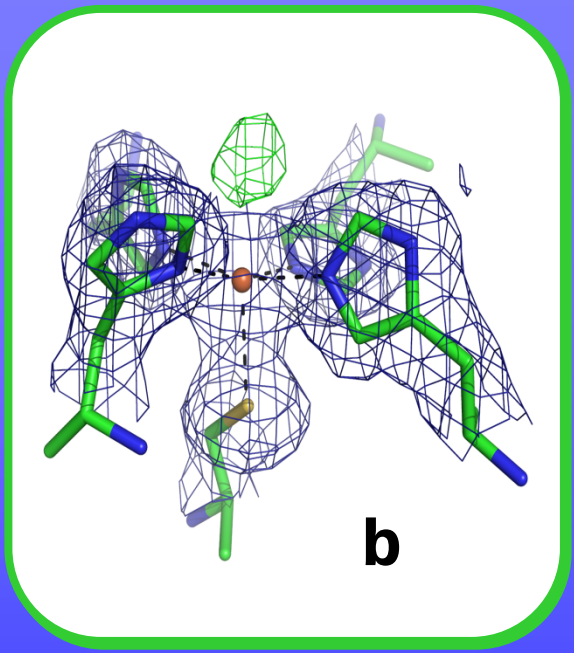


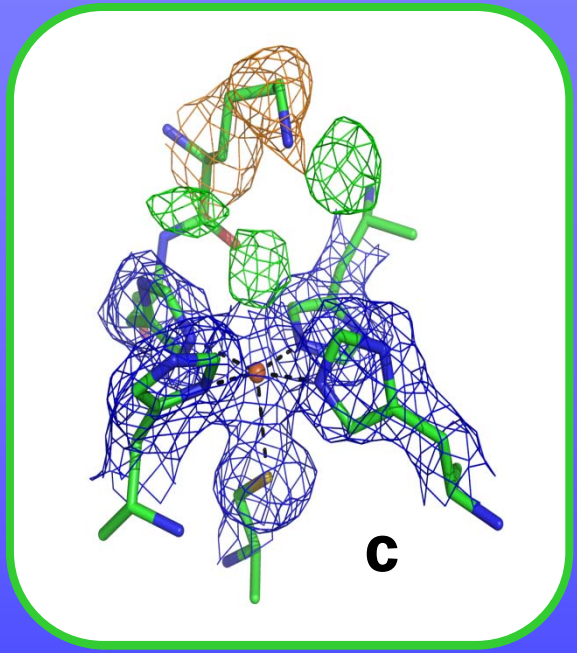
Radiation damage

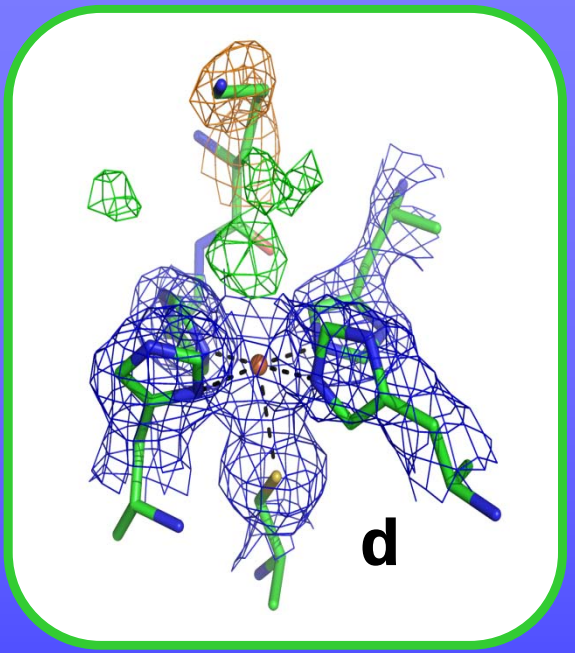
- 3×10^5 Gy absorbed dose does not affect Raman peaks

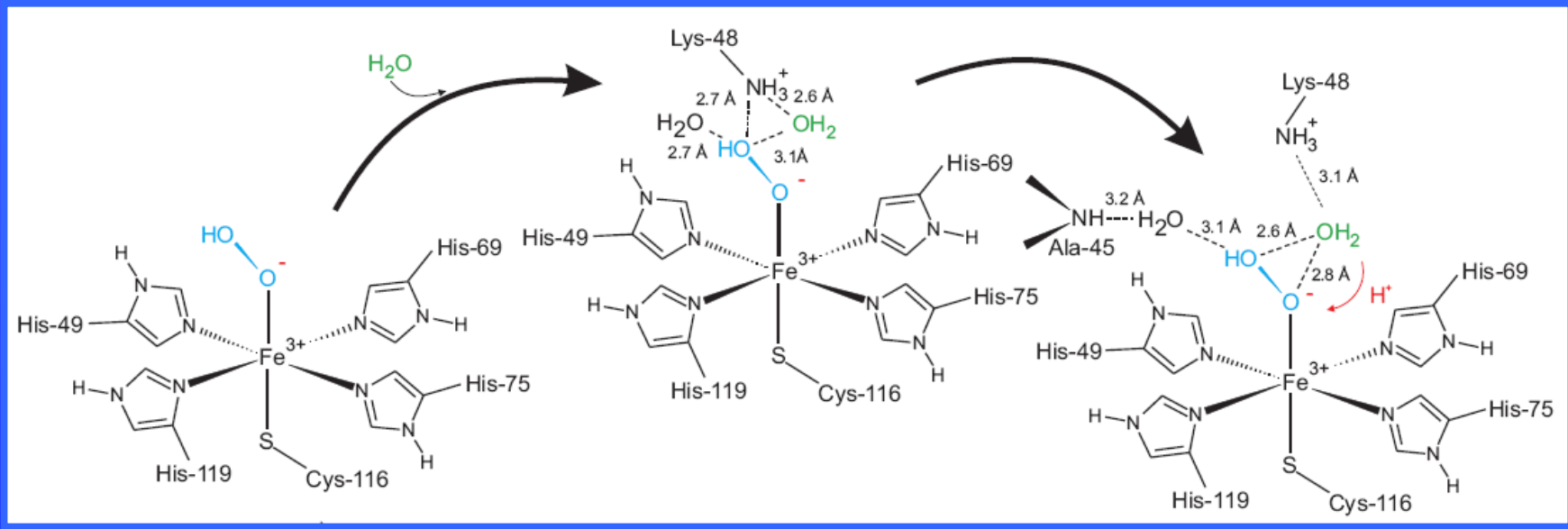


Proposed mechanism ...





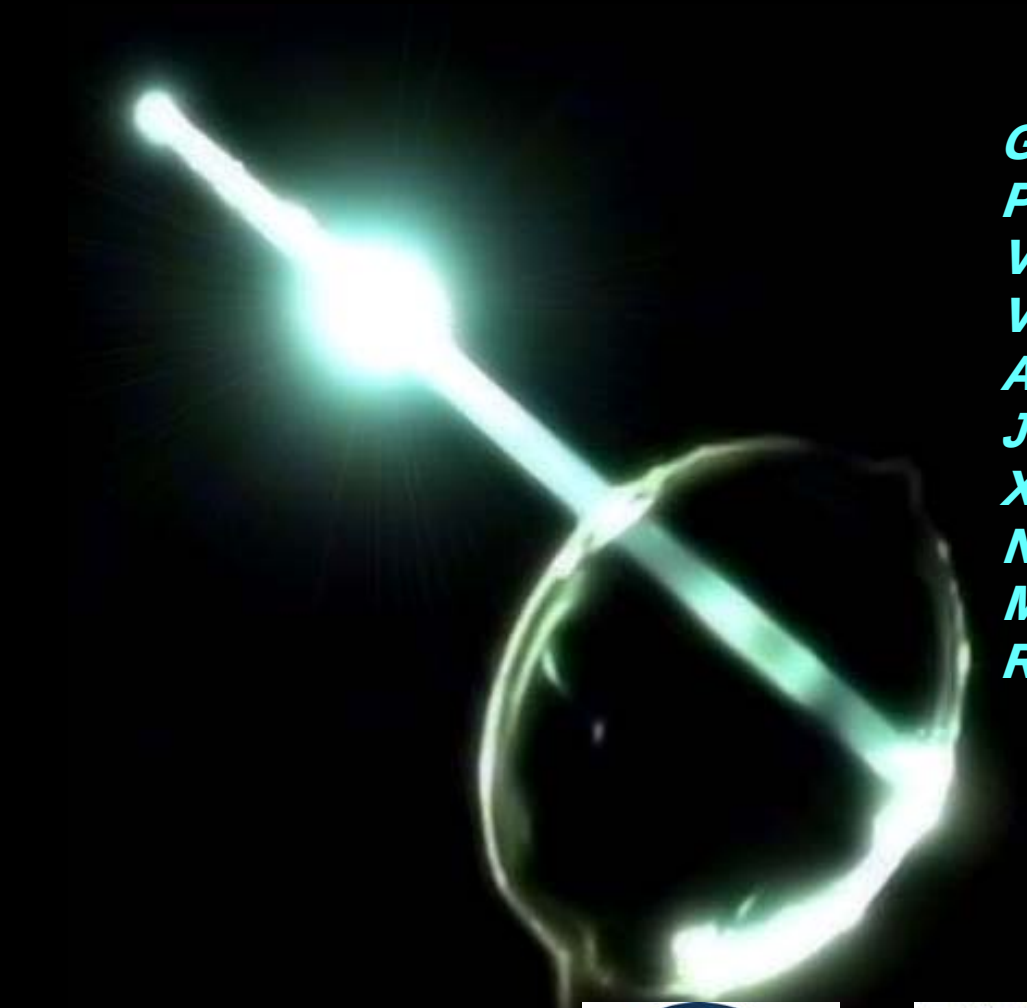




Take home message

- *Growing importance of complementary techniques*
- *In crystallo spectroscopy of particular interest for studies of protein mechanisms and dynamics*
- *Small "niche" in the MX field*
- *Projects are generally difficult and require dedicated efforts over long term.*

Acknowledgments



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Antoine Royant (IBS, Grenoble)
Jérémy Ohana (IBS, Grenoble)
Xavier Vernède (IBS, Grenoble)
Nikolay Tsanov (IBS, Grenoble)
Martin Weik (IBS, Grenoble)
Raimond Ravelli (EMBL Grenoble)