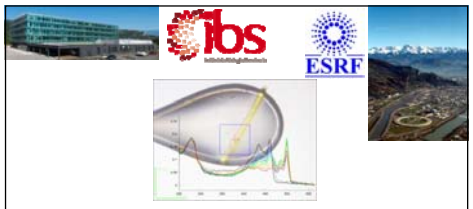


ID29S: optical spectroscopy at the Cryobench

Antoine Royant (IBS/ESRF)

ESRF Users' Meeting 2015: Meeting of MX BAG Representatives and Beamline Staff
Monday Feb 9th, 2015

Vocabulary

- **Optical spectroscopy** = spectroscopy based on UV-visible-IR photons

  * **Electronic spectroscopy**
    - UV-visible absorption
    - fluorescence emission

  * **Vibrational spectroscopy**
    - Raman
    - Infrared

- **Optically visible**: coloured, fluorescent or with a distinct bond vibration mode
- **Online spectroscopy:** spectroscopic characterization happens during diffraction experiment (microspec directly mounted on beamline diffractometer)
- **Offline spectroscopy:** characterization before or after diffraction experiment (microspec used on Cryobench)

Why performing optical spectroscopy in the context of X-ray crystallography?

- To check whether the **crystalline** protein is in a comparable situation as in **solution** (**redox** state, **ligand** /**chromophore** state)

  IDENTIFICATION OF FUNCTIONAL STATE

- To check that the functional state has not been altered by X-rays (**sensitive covalent bonds**, **oxidised metal centers**)

  RADIATION DAMAGE

Optical spectroscopy: for which proteins?

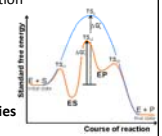
- Naturally coloured/fluorescent proteins
  - redox proteins
  - photoactive proteins
  - photosynthetic proteins
  - fluorescent proteins

- Artificially coloured/fluorescent proteins
  - soaking with exogenous fluorophores
  - caged compounds (protected substrate)

- (Non-)coloured proteins
  - protein/DNA/substrate with specific bonds (S-S, O-Fe, C-Br)

KINETIC PROTEIN CRYSTALLOGRAPHY (KX)

- Protein crystallography -> structure determination of proteins **in a resting state**



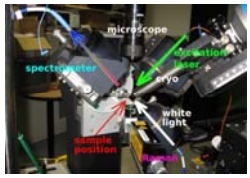
- Proteins often **active** in the crystalline state (reaction rate potentially affected)
- **KX** = structure determination of **unstable species**
  - o Reaction intermediate states (unstable in **time**)
  - o X-ray sensitive states (unstable in **X-ray dose**)
- **MONITORING PROGRESS** by **a complementary spectroscopic technique** whenever possible

The Cryobench (ID29S): offline experimental setup

- Located next, and connected to **beamline ID29** of the ESRF

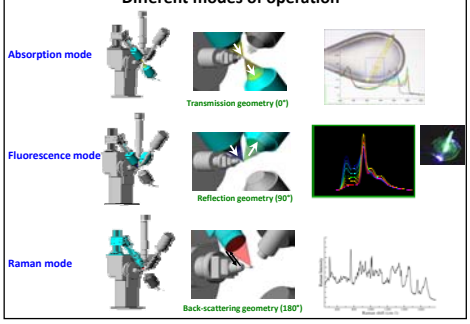
Microspectrophotometer:
- a goniometer
- 4 objectives
- a video microscope
- a cryostream / dehumidifier

= **mimic of the structural biology beamline setup**

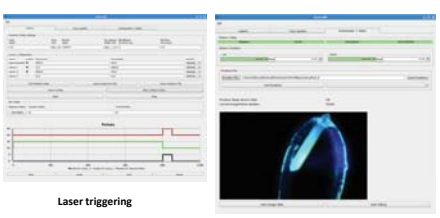


-> For crystals but also nanolitres of solution

Different modes of operation



SpeCuBE: Cryobench control software



Laser triggering

Sample visualisation and goniometer control

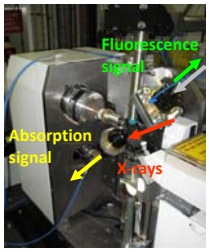
On-line **UV-vis and fluorescence** setup (off-axis)

UV-vis absorption: 0° geometry

Fluorescence: 180° geometry

Only compatible with MD2M (minidiff)

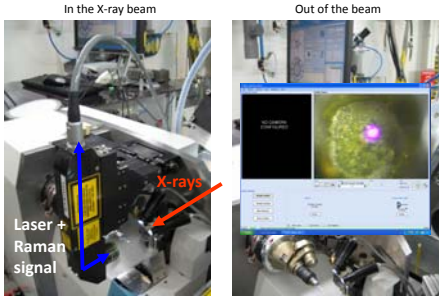
- ID14 (now closed)
- BM30A (FIP)
- Massif3 (to be tested)



McGeehan *et al.* (2009) *J. Synchrotron Rad.* **16,** 163-172

On-line **Raman** setup (on-axis) on ID29

In the X-ray beam | Out of the beam

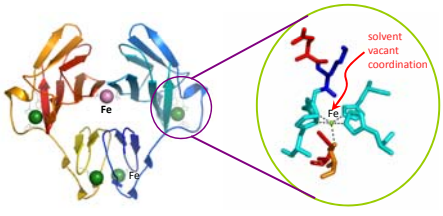


Example of on-line UV-visible abs microspec use (+ off-line Raman)

FUNCTIONAL STATE IDENTIFICATION
+
(OBVIOUS) RADIATION DAMAGE
+
KINETIC CRYSTALLOGRAPHY

Superoxide Reductase (SOR) is an alternative to superoxide dismutase (SOD) in microaerophilic bacteria

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



$O_2^{\cdot -} + H^+ + Fe^{2+} \rightarrow Fe^{3+}\text{-}OOH \rightarrow Fe^{3+} + H_2O_2$ (+ H^+)

Trap the proposed "hydroperoxo" Intermediate *in crystallo ?*

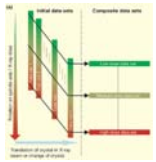
Online UV-vis absorption spectroscopy and composite data sets to solve the structure of oxidised SOR

Adam *et al.* (2004) *Structure* **12**, 1729-1740

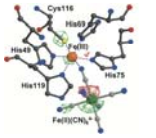
Rapid reduction in the X-ray beam

FUNCTIONAL STATE IDENTIFICATION

RADIATION DAMAGE

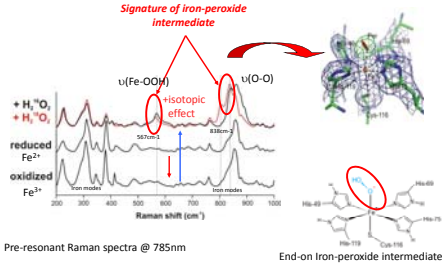


Elaboration of composite data sets



Active site volume expansion upon reduction

In crystallo Raman Spectroscopy of SOR



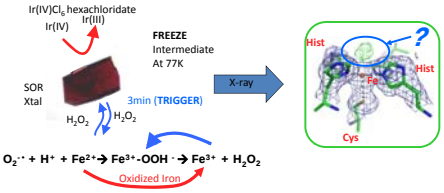
Pre-resonant Raman spectra @ 785nm

End-on Iron-peroxide intermediate

Katona *et al.* (2007) *Science* **316**, 449-453

Puzzling crystallographic data in a KINETIC CRYSTALLOGRAPHY experiment

- Use of law of mass action
- Point-mutant slowing down the reaction



- Limited crystallographic resolution (1.95 Å) (owing to H_2O_2 soaking)
- Unexplained electron density peaks, relevant intermediate species?

Example of on-line Raman use

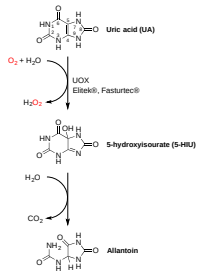
(SUBTLE) RADIATION DAMAGE
+
KINETIC CRYSTALLOGRAPHY

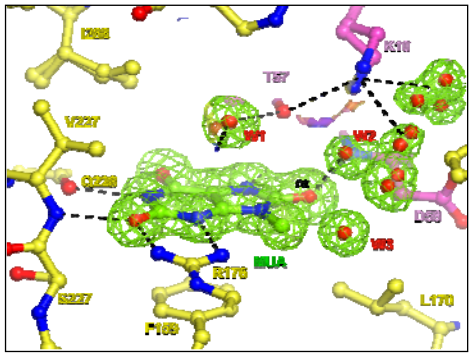
Collaboration with Roberto Steiner

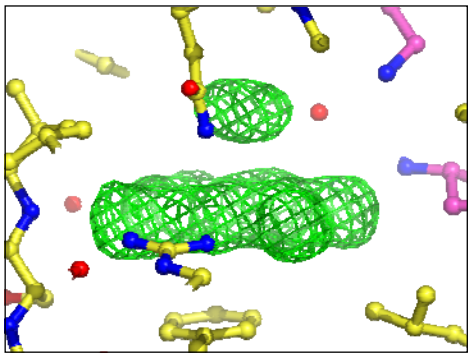
KING'S College LONDON

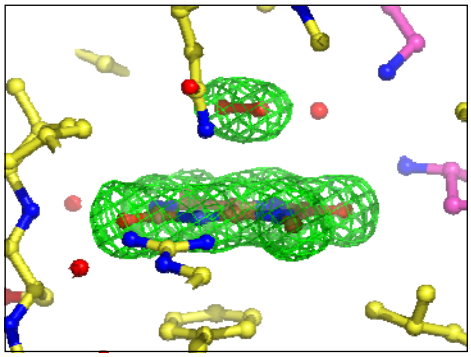
Urate oxidase (UOX) catalysis

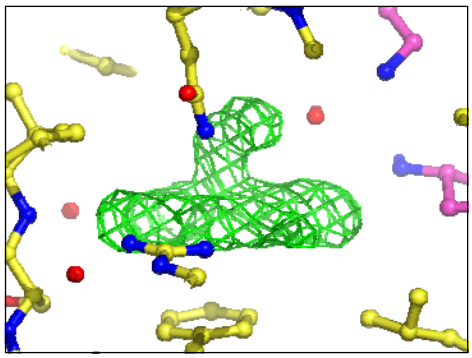
UOX = Cofactor-free oxidase

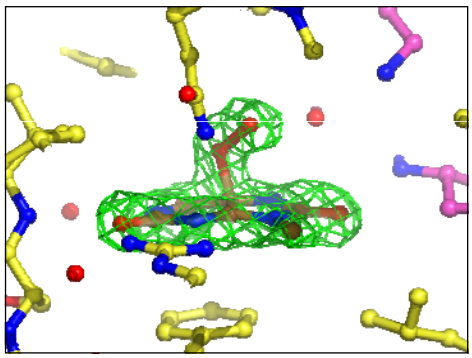




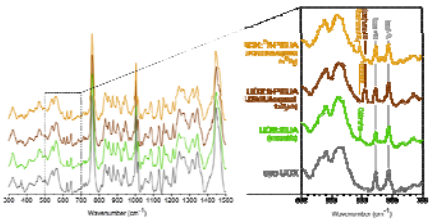




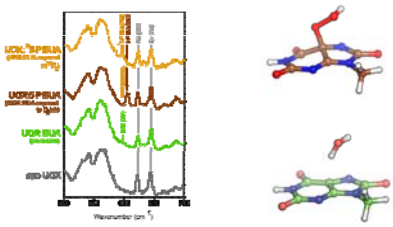




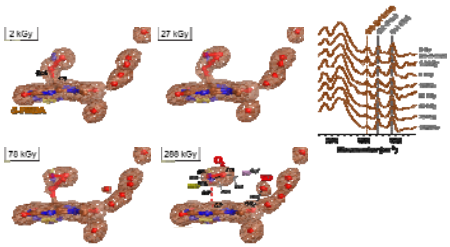
In crystallo Raman



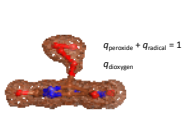
Raman stretching modes

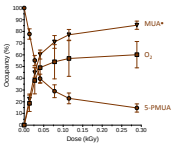


UOX:5-PMUA radiolysis



Conclusions





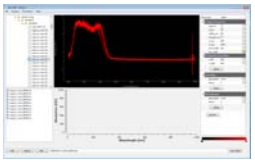
- There is a peroxo intermediate which is very X-ray sensitive (k_1 = 37.5 MGy^{-1})
- X-ray radiolysis offers a view of the E•S•O_2 reactive configuration leading to the C5-(hydro)peroxo intermediate
- Peroxide decay suggests a mechanism of regeneration

In summary

- If your protein is coloured (and crystallized), there are good chances *in crystallo* spectroscopy can bring you some insights – if not, there are still chances
- Beware of X-ray induced **electrons**
- Beware of the **high optical density** of crystals
- **Offline** UV-vis abs., fluorescence, Raman: at the Cryobench (ID29S)
- **Online** UV-vis absorption, fluorescence: BM30A (>100 um beam), Massif3 (15 um)
- **Online Raman**: ID29
- There are 60 days of experiments possible every year at the Cryobench off-, and online [antoine.royant@esrf.fr]
- For more information, see:
  http://www.esrf.eu/UsersAndScience/Experiments/MX/Cryobench/

Perspectives

- At the Cryobench: move to the automation era to increase the ease (and number) of data collections (SC3 + MD2M)
- On the beamlines: a new UV-vis abs and fluorescence microspec is to be designed and built for Massif1 and/or Massif2 (2015-2016)
- Data analysis package currently being developed



Guillaume Gotthard

Acknowledgements

ESRF
Philippe Carpentier
Daniele de Sanctis
Fabien Dobias
David Flot
Thierry Giraud
Guillaume Gotthard
Gordon Leonard
Christop Mueller-Dieckmann
Franc Sever
David von Stetten
The ESRF Structural Biology group

IBS
Virgile Adam
Dominique Bourgeois
Jean-Luc Ferrer
Gergely Katona (now at U. of Gothenburg)
Martin Weik
The Synchrotron group

King's College London
Soi Bui
Roberto Steiner

All Cryobench users
(80+ papers since 2000)