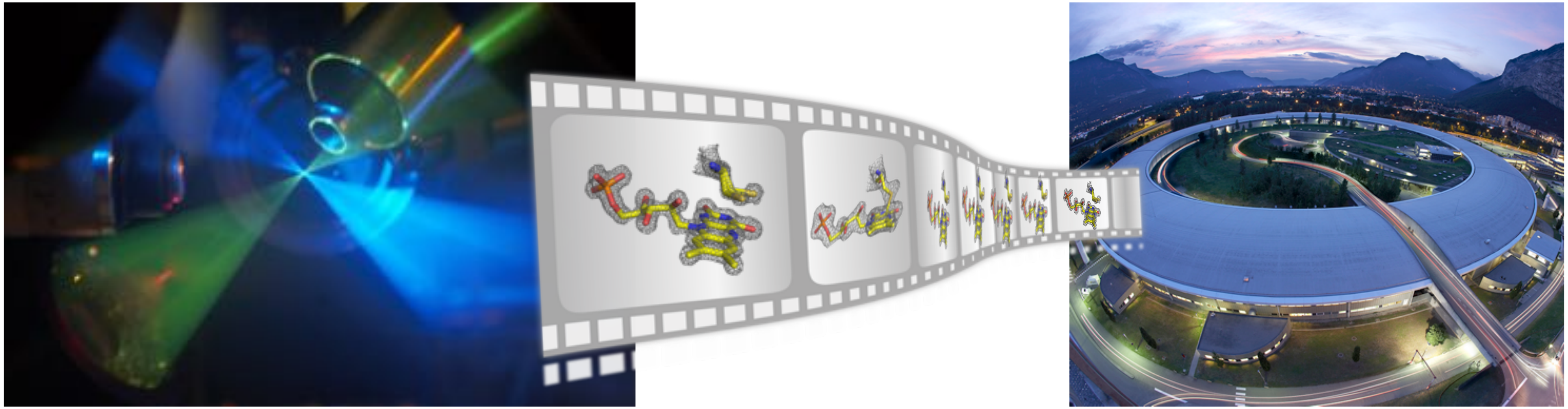


The *in crystallo* optical spectroscopy (icOS) Lab

-

Towards time-resolved icOS (TR-icOS)

Antoine Royant

Institut de Biologie Structurale

& European Synchrotron Radiation Facility

(Grenoble)

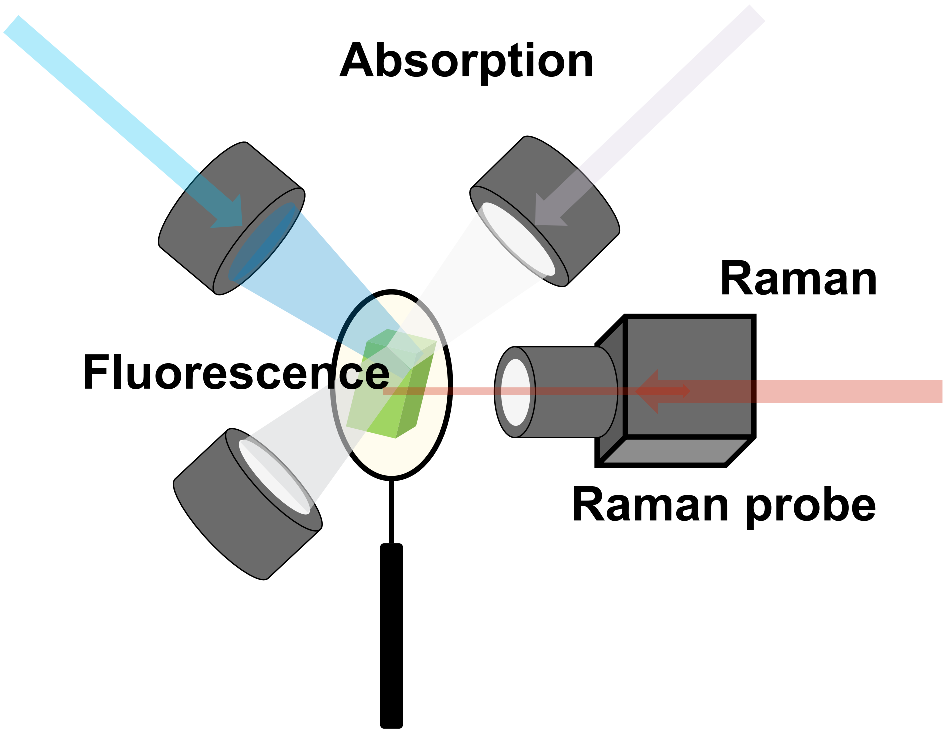
Institut de Biologie Structurale

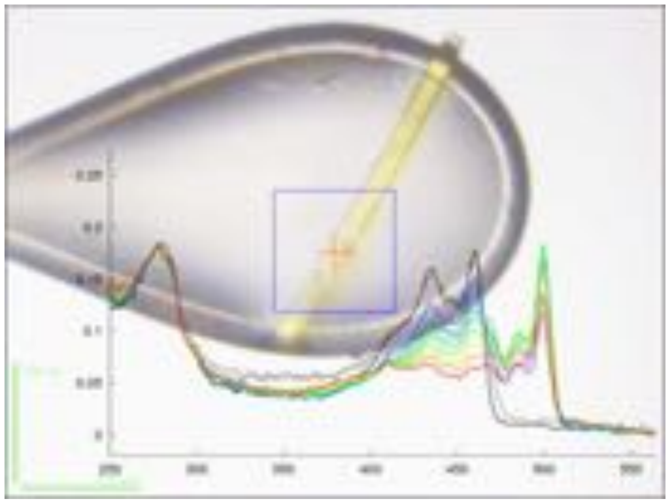
ESRF

Complementary spectroscopy techniques

The *ic*OS Lab, an IBS/ESRF platform, located at the ESRF (formerly known as the Cryobench)

- UV-visible abs/fluorescence/Raman spectra directly measured on crystals at **cryogenic** or **room** temperature





Why performing optical spectroscopy on crystals?

Optical spectroscopy used in complement to MX:

(1) To determine the **functional state** of the crystalline protein

(2) To evaluate the extent of specific **radiation damage** effects

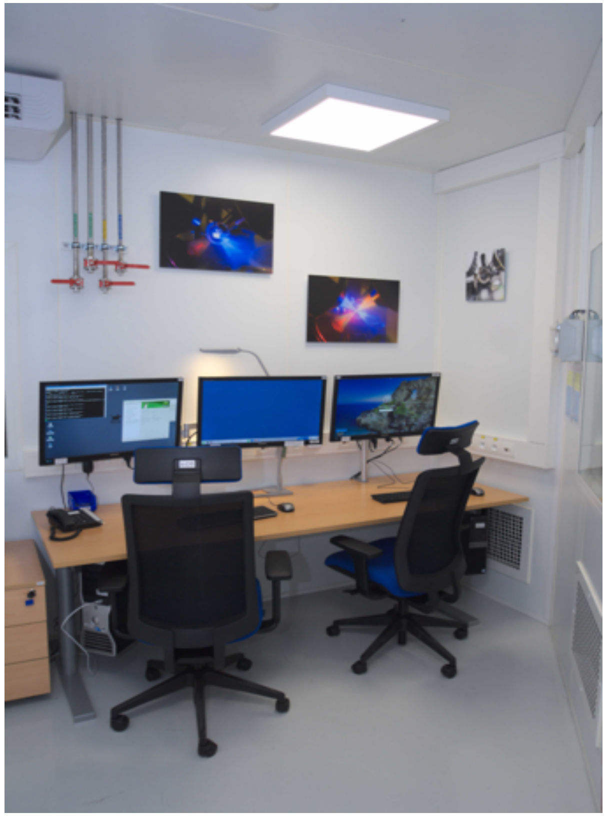
(3) To perform **kinetic crystallography** experiments (structure determination of **unstable species**, in time or dose)

When and where?

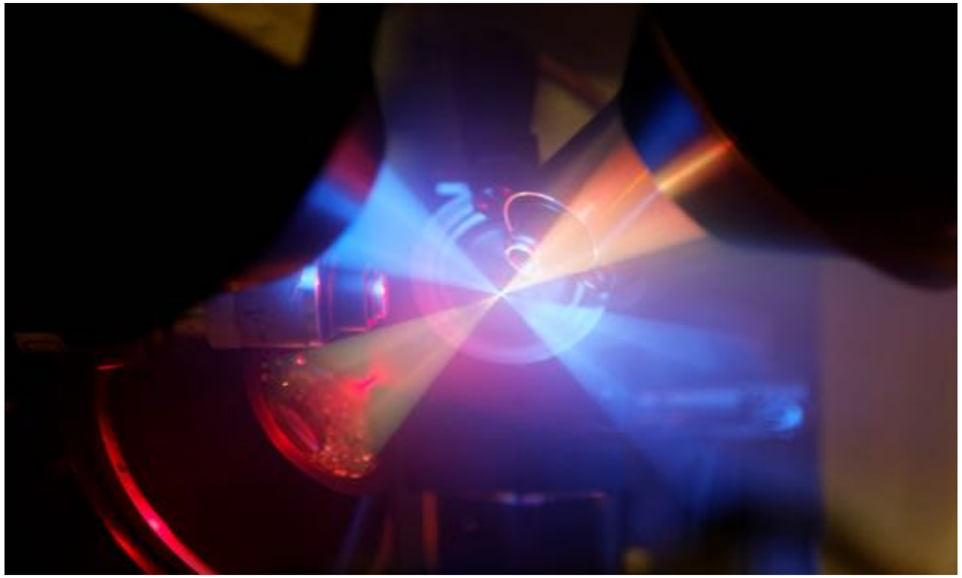
- Before or after the diffraction experiment: **Offline setup**
- During the diffraction experiment: **Online setup**

Offline setup: the icOS Lab – Control Cabin and Experimental Hutch

Now in Chartreuse Hall between new ID29 and ID30B



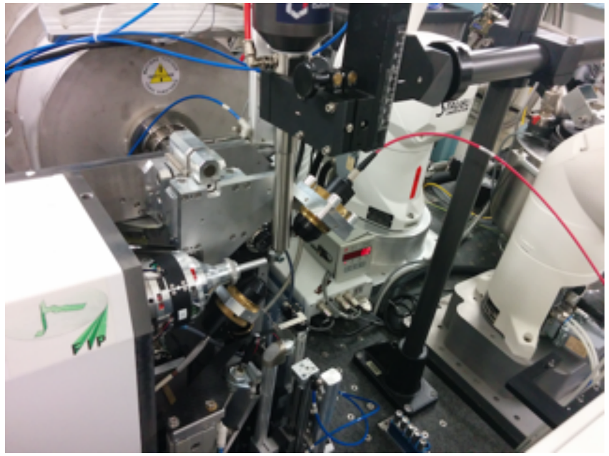
New automated spectroscopy setup

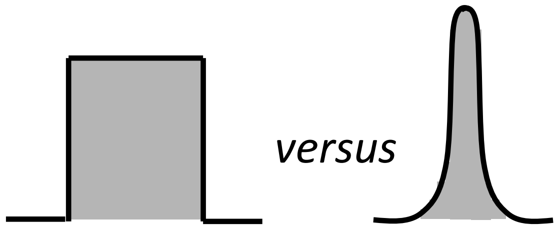


- Standard MX beamline equipment (minidiffractometer MD2M with 3-click sample centring, back/ front lights, on-axis viewer)
- Motorized optical objectives

on-line UV-vis absorption spectroscopy on BM07-FIP2 (large crystals, > 50-100 um)

- FIP2 = reconstruction of BM30A-FIP on BM07
- FIP2: 3x to 10x higher flux + Pilatus detector -> faster data collection -> faster time resolution
- Cryogenic (still very) low dose spectroscopic characterization -> complementary to other MX beamlines
- Importance of keeping a TopHat beam *vs.* Gaussian beam for radiation damage studies

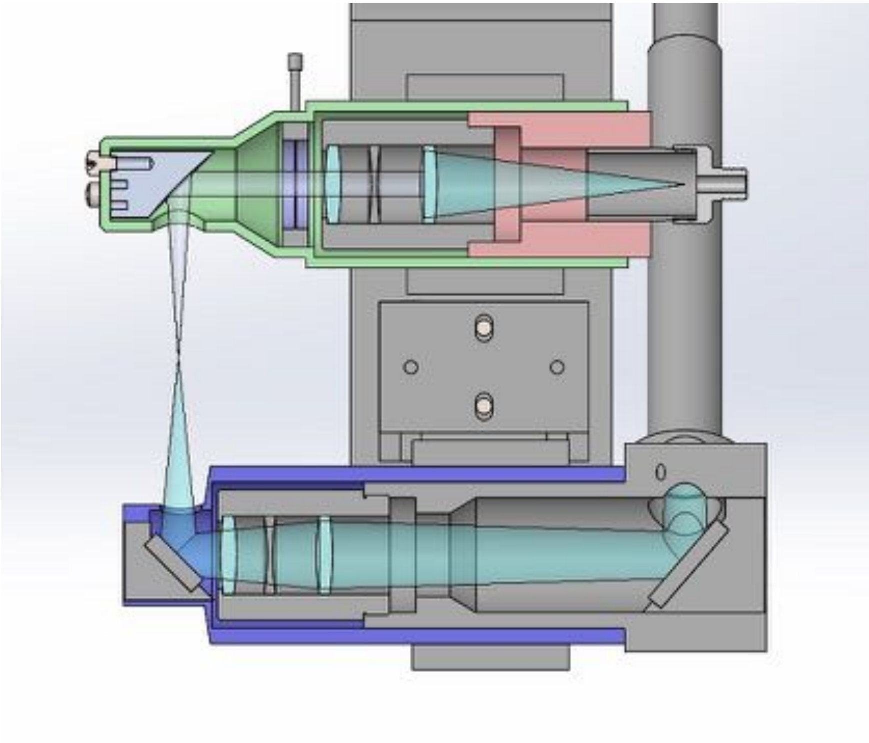


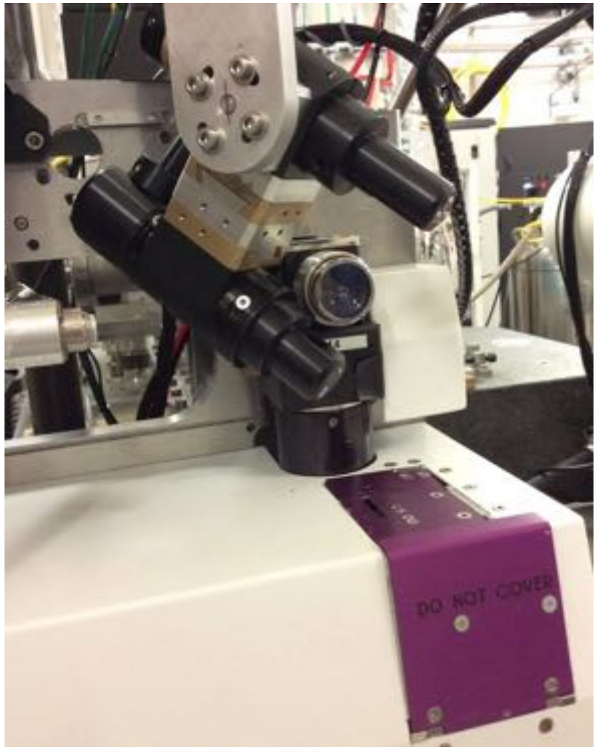


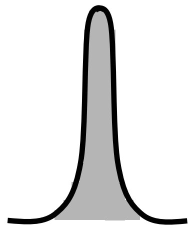
McGeehan *et al.*, *J. Synchrotron Rad.* (2009)

Future: on-line UV-vis absorption spectroscopy on ID30A-3 (smaller crystals, 15-20 um)

- Brand new design (O. Hignette/P. Theveneau, ESRF) - use of parabolic mirrors
- Developed by David von Stetten and now Igor Melnikov

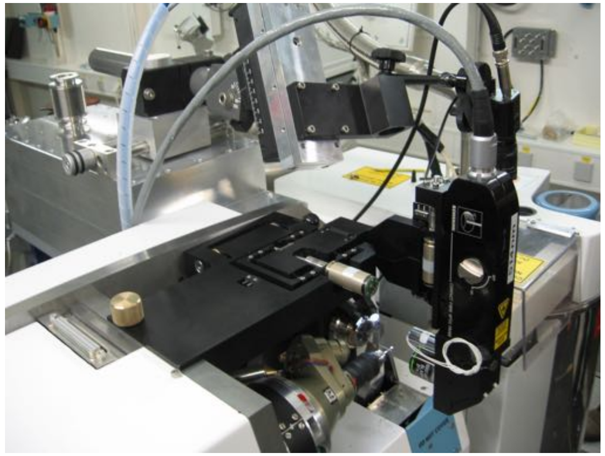


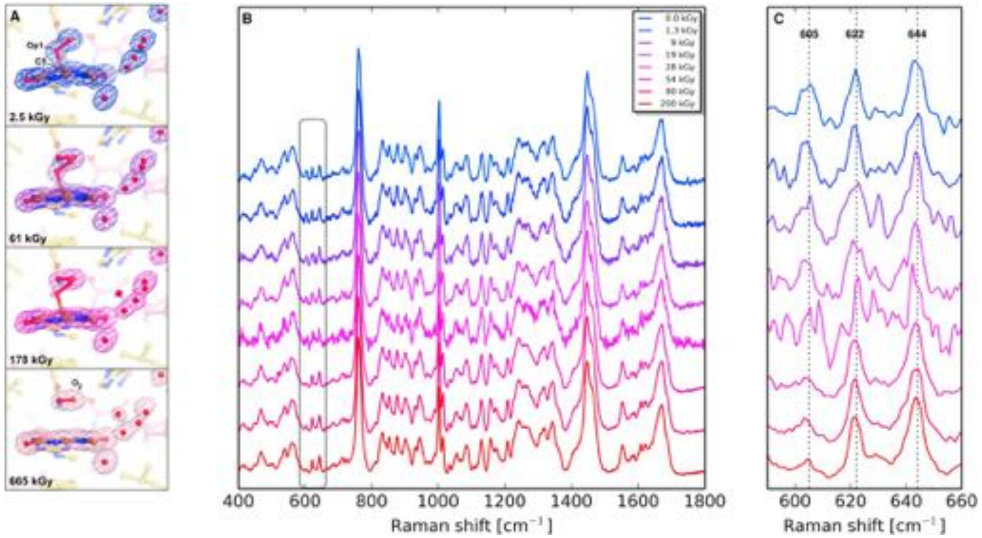


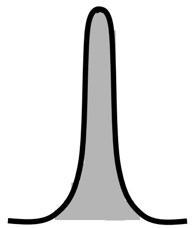


Future: on-line Raman spectroscopy on ID30B

- Online Raman not suitable for ID29 any more (beam size)
- Similar setup as ID29 (6tec/ESRF design), but re-designed to accommodate the MD2S (P. Jacquet, IBS)
- Radiation damage studies − monitoring specific bond breakage or deformation of groups (or of secondary structures)







von Stetten *et al.*, *J. Struct. Biol.* (2017)

Recent *ic*OS highlights

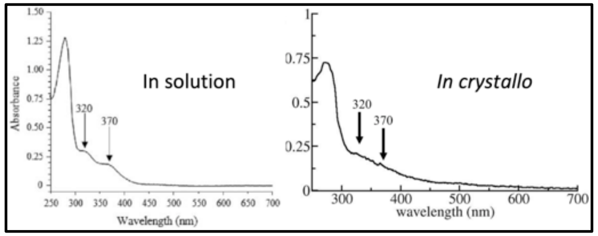
- Carpentier et *al.*, *Nucleic Acids Res.* (2020) Structural, biochemical and functional analyses of the tRNA-monooxygenase enzyme MiaE

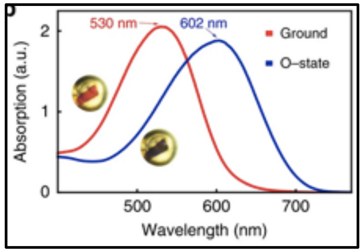
- Kovalev et *al.*, *Nat. Commun.* (2020) − Molecular mechanism of light-driven sodium pumping

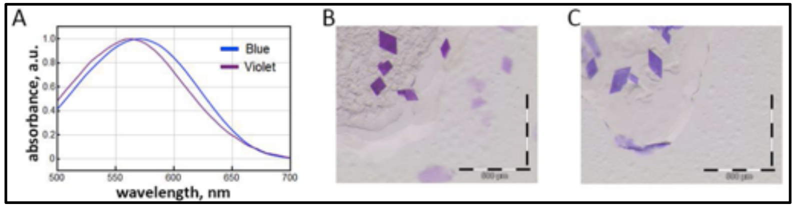
- Kovalev et *al.*, *PNAS* (2020) − High-resolution structural insights into the heliorhodopsin family

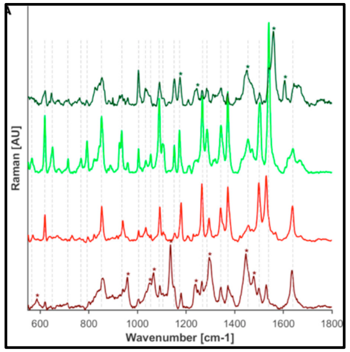
- De Zitter et *al.*, *JACS* (2020) − Mechanistic Investigations of Green mEos4b Reveal a Dynamic Long-Lived Dark State

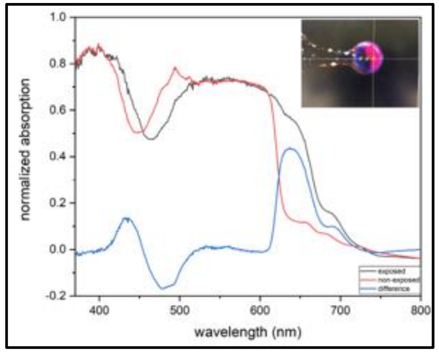
- Xu *et al.*, *PNAS* (2020) − Structural elements regulating the photochromicity in a cyanobacteriochrome





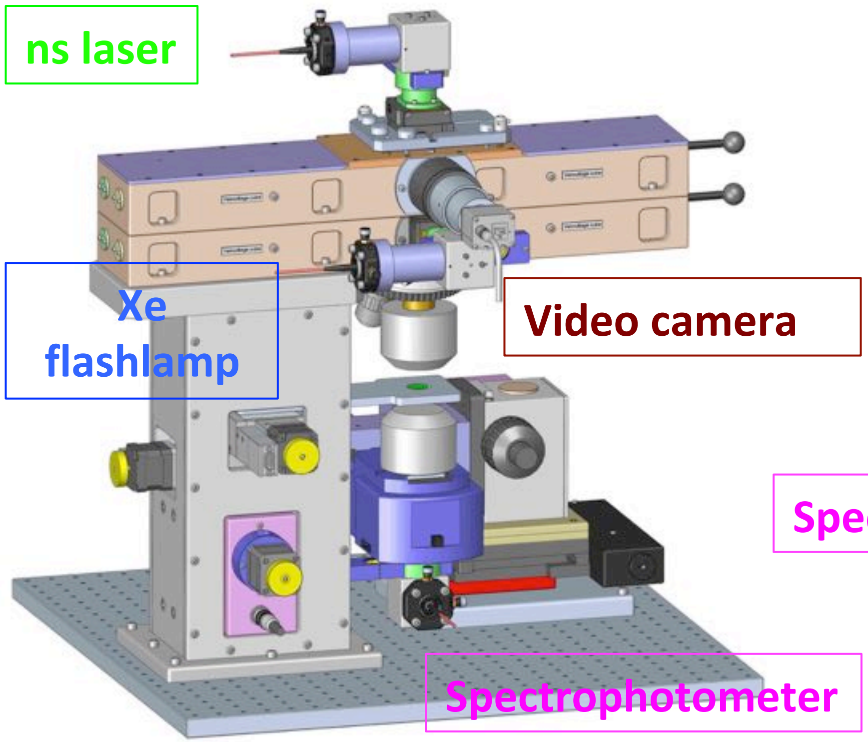


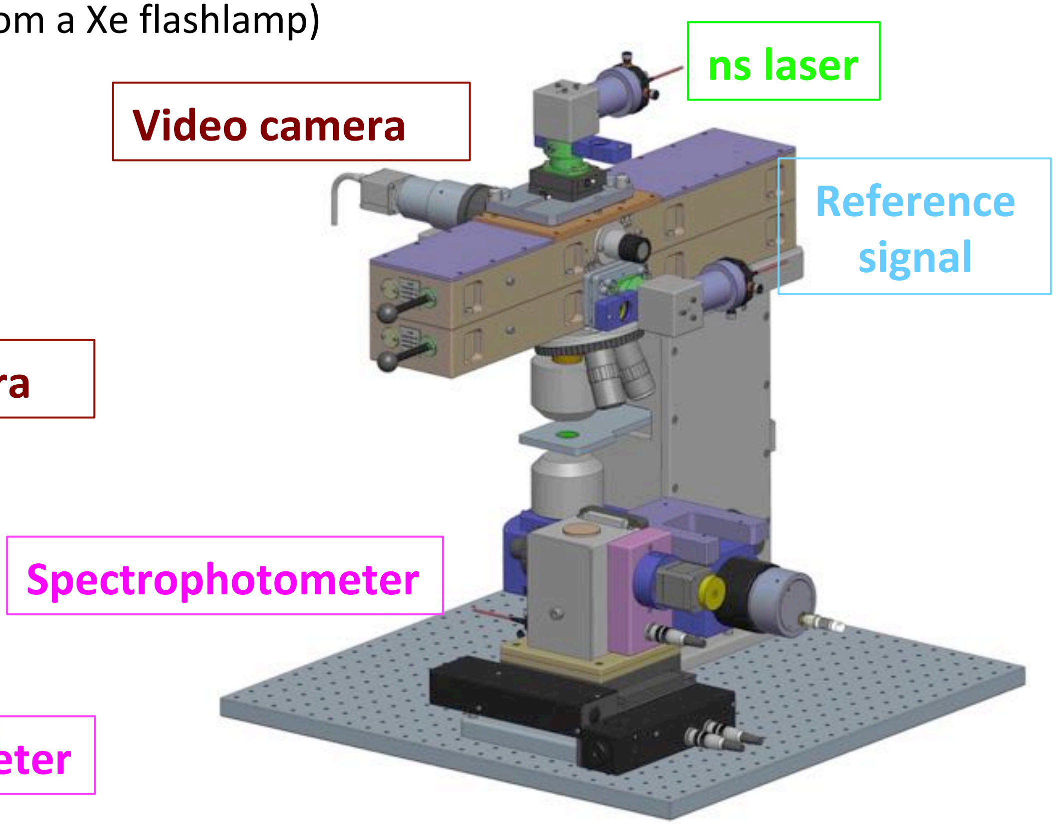




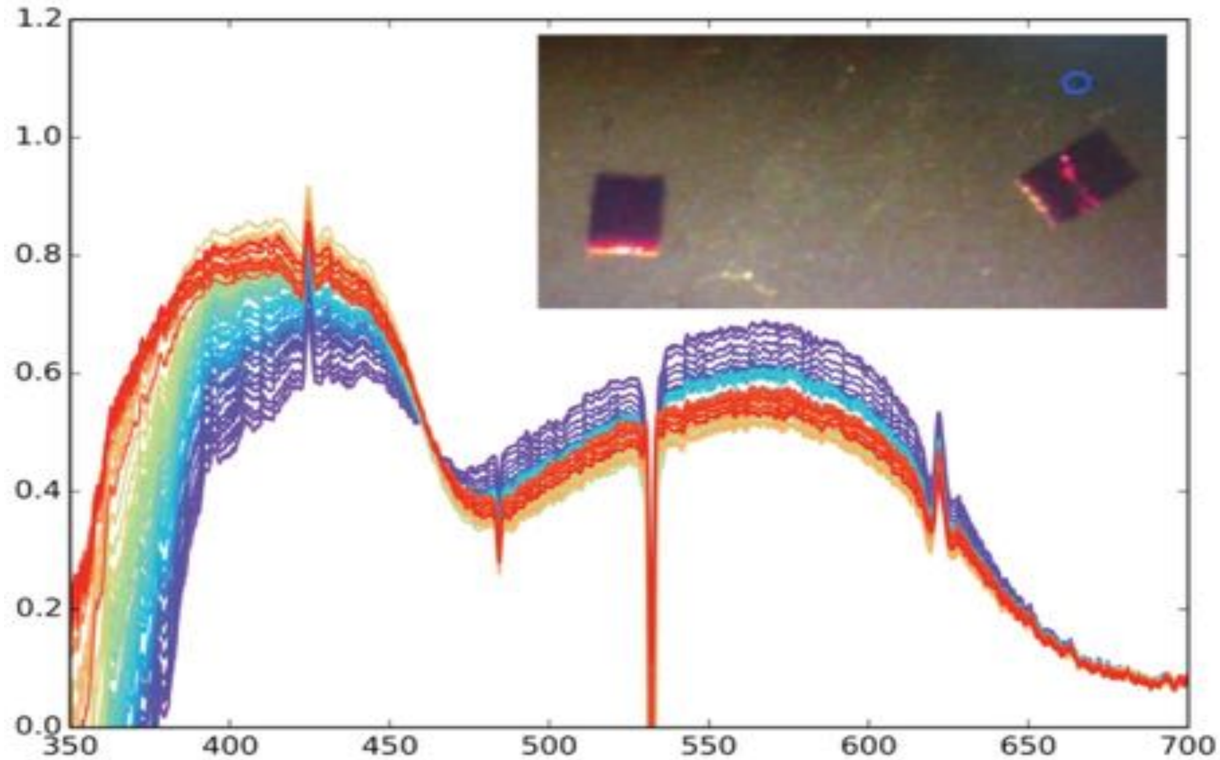
Microsecond time-resolved UV-vis absorption spectroscopy on *ic*OS

- Use with 10-15 um crystals
- Actinic pulse: nanoseconds (from a tunable ns laser)
- Time resolution of probe: microseconds (from a Xe flashlamp)



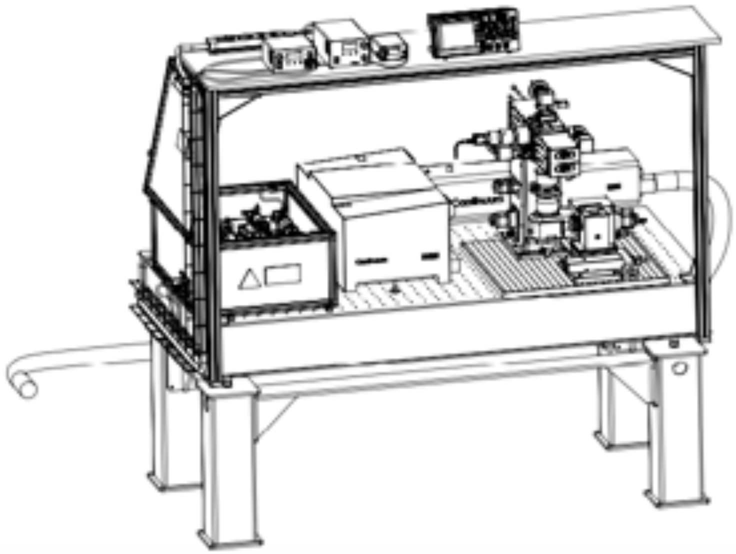


Feasibility experiment on bacteriorhodopsin crystals



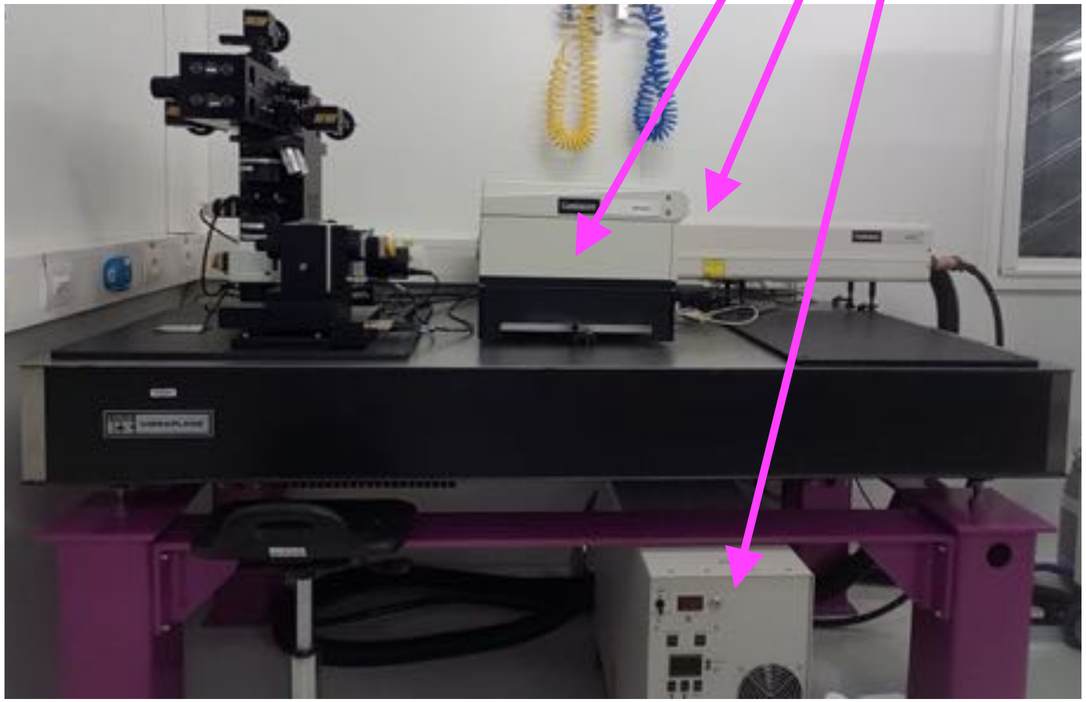
Building the TR-icOS setup – hopefully working end of 2021

Schematic of final setup

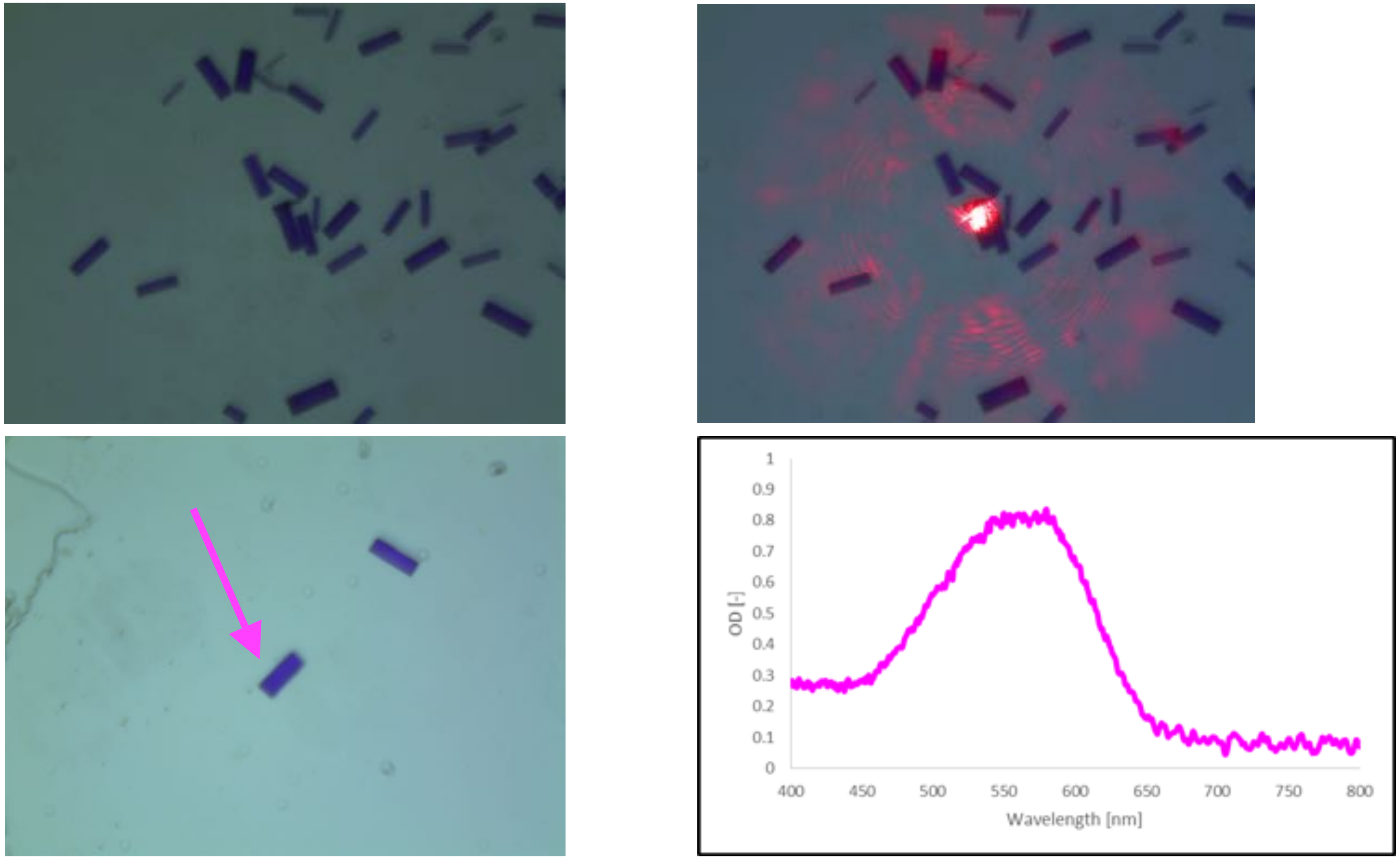


Opto-mechanical setup

Tuneable nanosecond laser



Preliminary results on bacteriorhodopsin crystals



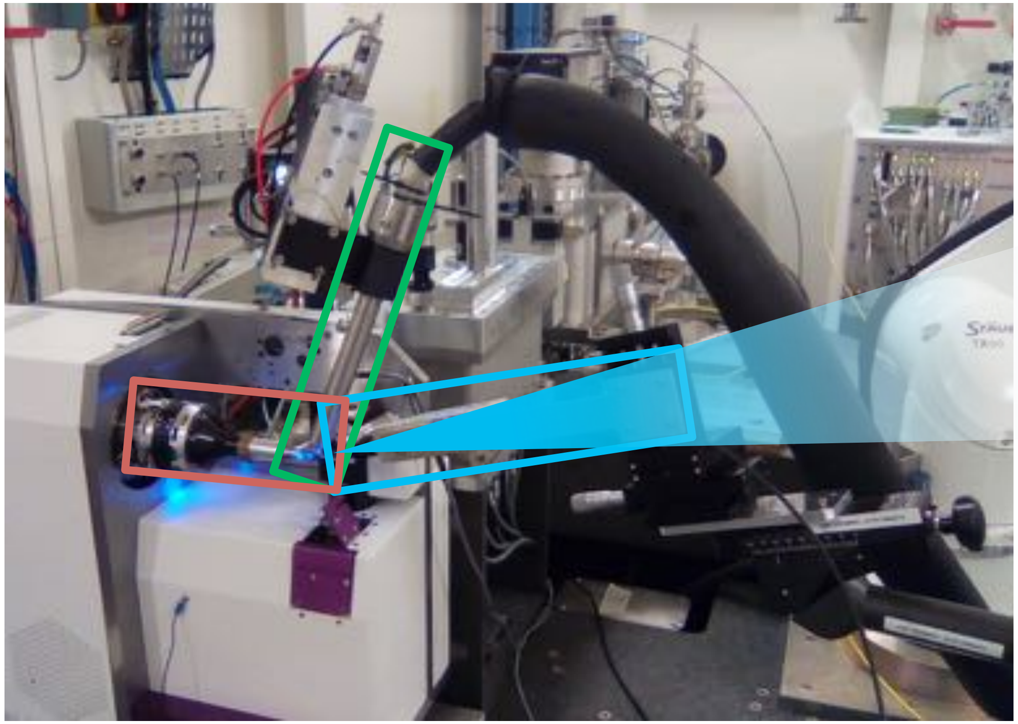
Summary: various available spectroscopies, or soon-to-be

Spectroscopy	Off-line	On-line
UV-vis absorption	***ic*OS**	**BM07-FIP2 (large beam > 50-100 um) ID30A-3 (MASSIF-3) (small beam ~15-20 um)**
Fluorescence	***ic*OS**	
Raman	***ic*OS**	**ID30B**
Time-resolved UV-vis absorption (microsecond)	***ic*OS**	-

Contact: antoine.royant@esrf.fr (+33)47688-1746

Example of a time-resolved crystallography experiment on ID30A-3 aided by off-line time-resolved optical spectroscopy

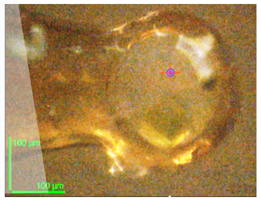
Time-resolved crystallography setup on beamline ID30A-3

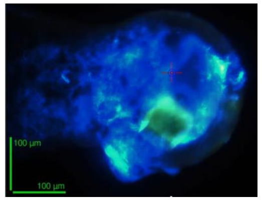


Crystals preservation at RT

HC1 humidity controller

Populate the crystal in light state





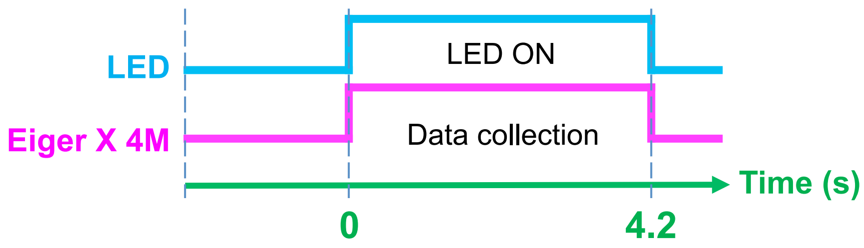
470 nm LED

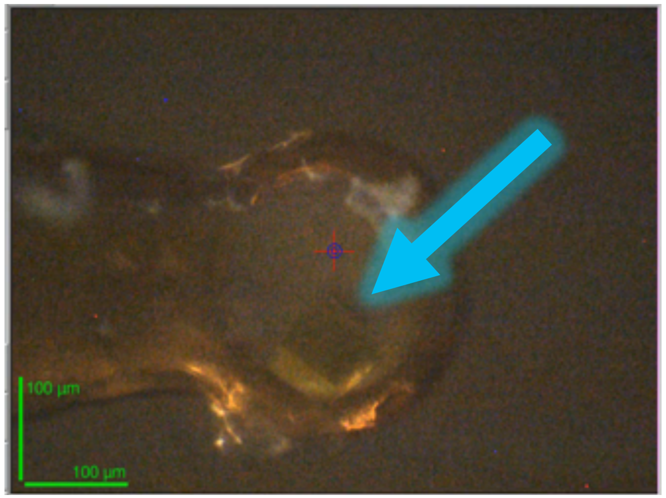
Record oscillation data sets as fast as possible

MD2 Microdiffractometer

Eiger X 4M pixel detector

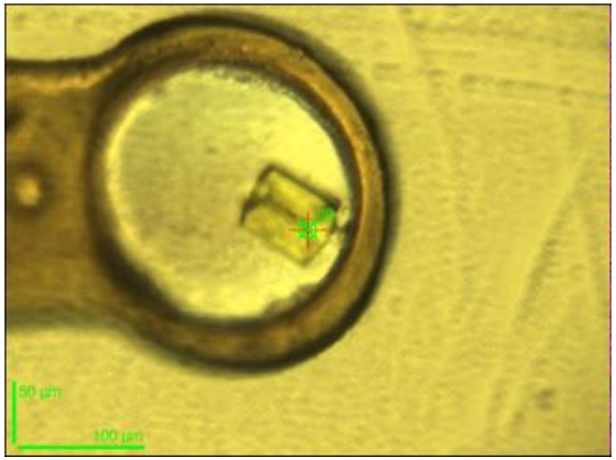
Photoadduct build-up

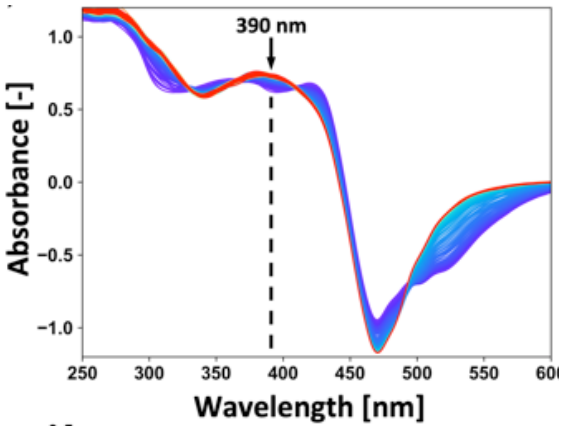


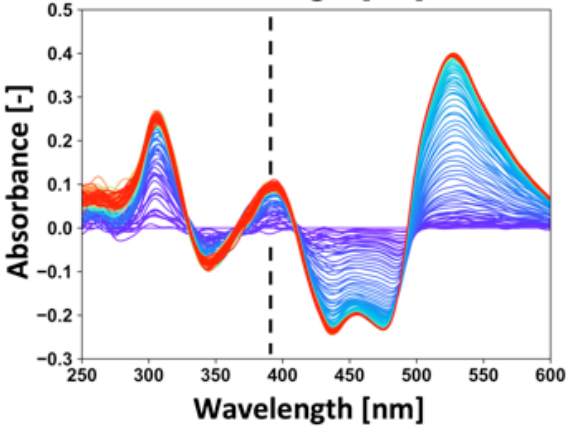


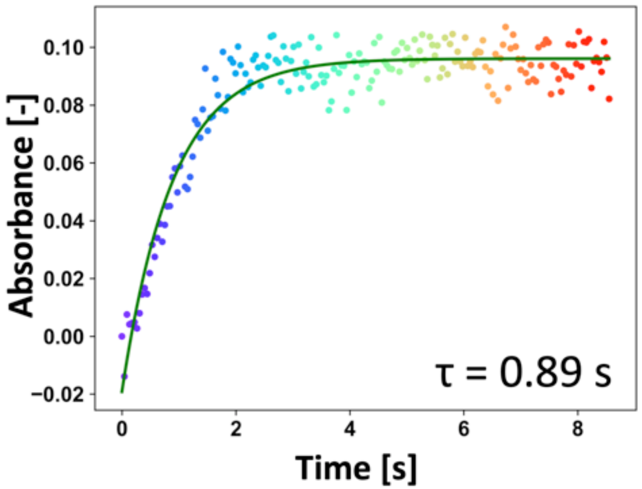
Spectroscopic characterization of photoadduct build-up

Time-resolved UV-vis absorption spectroscopy at the *ic*OS Lab (offline mode), with **41 ms time resolution**

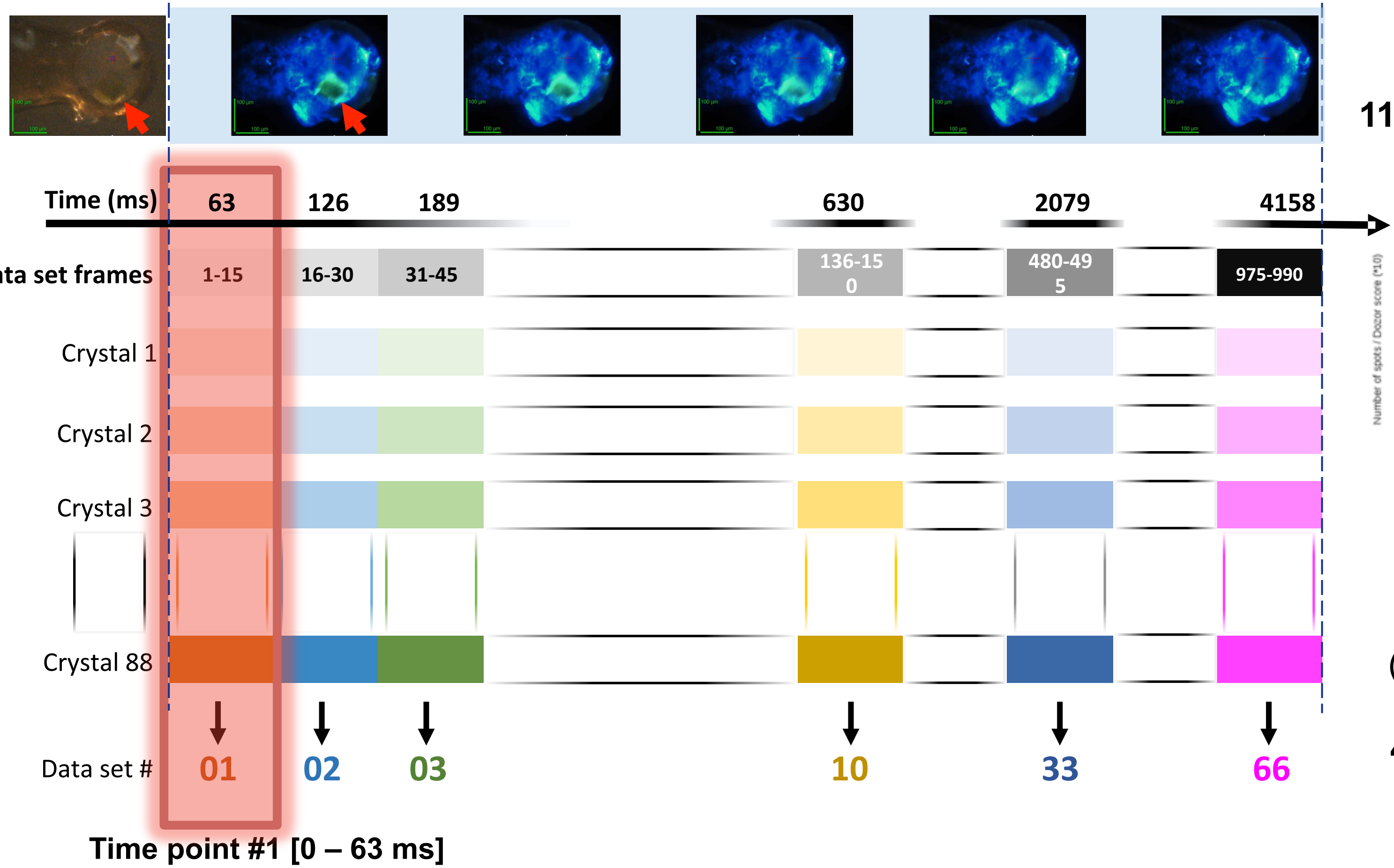




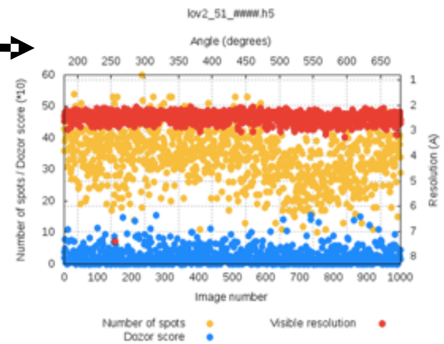




X-ray data collection principle



0.5% transmission
4.5E+10 ph/s flux
115 – 162 kGy / data set

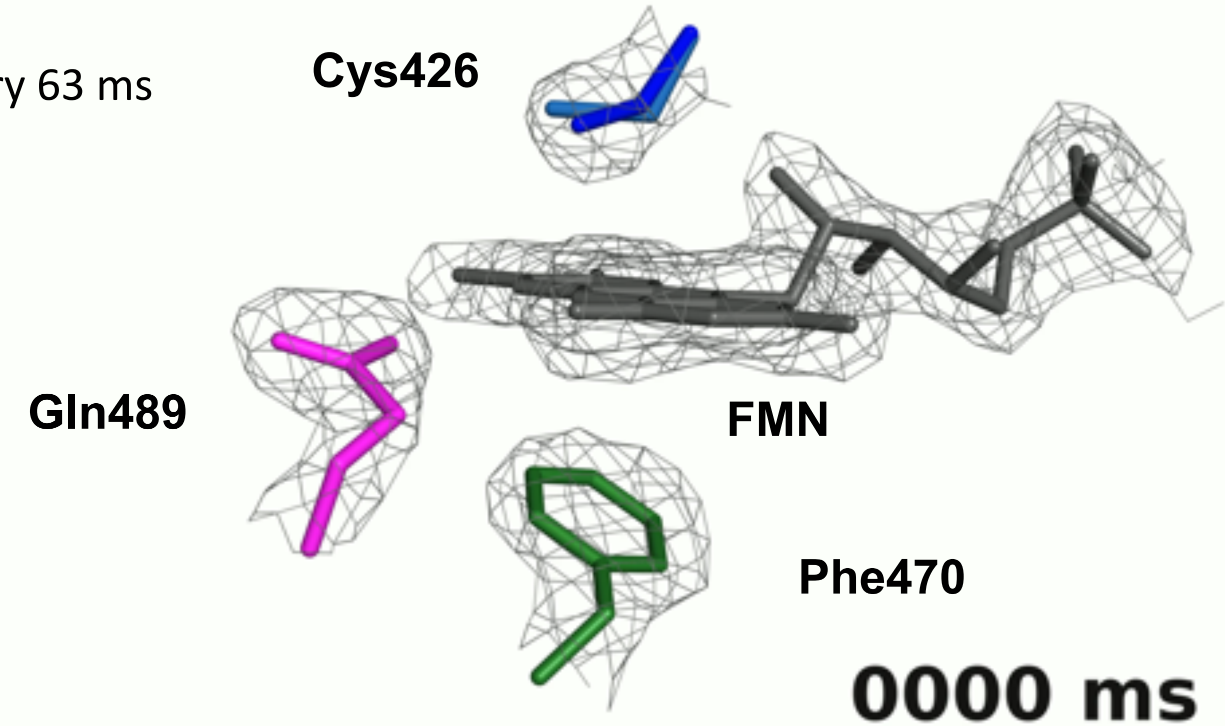


66 complete datasets (resolution **2.3-2.8 Å**) composed by HCA (Santoni *et al.*, *J. Appl. Cryst.* (2017))

4.2 s of build-up probed with a **63 ms time resolution**

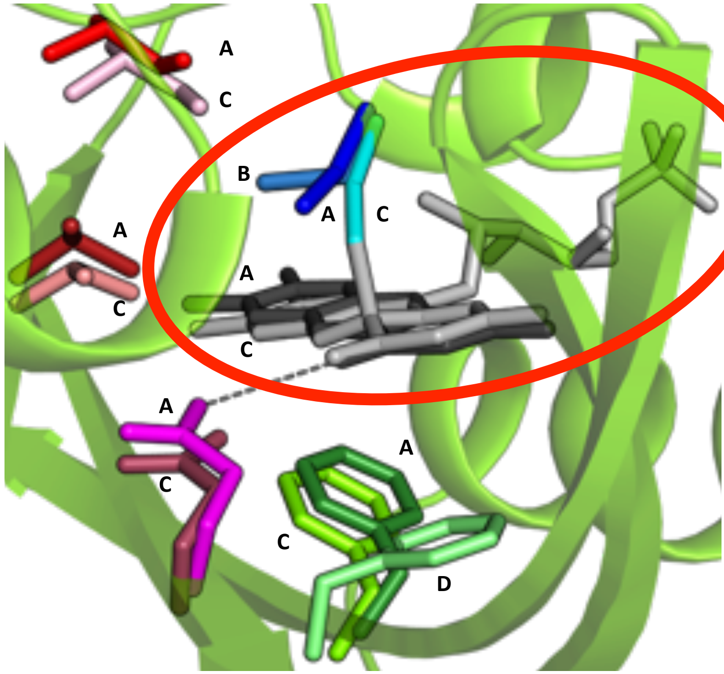
Molecular movie of the photoadduct population build-up in a LOV2 crystal

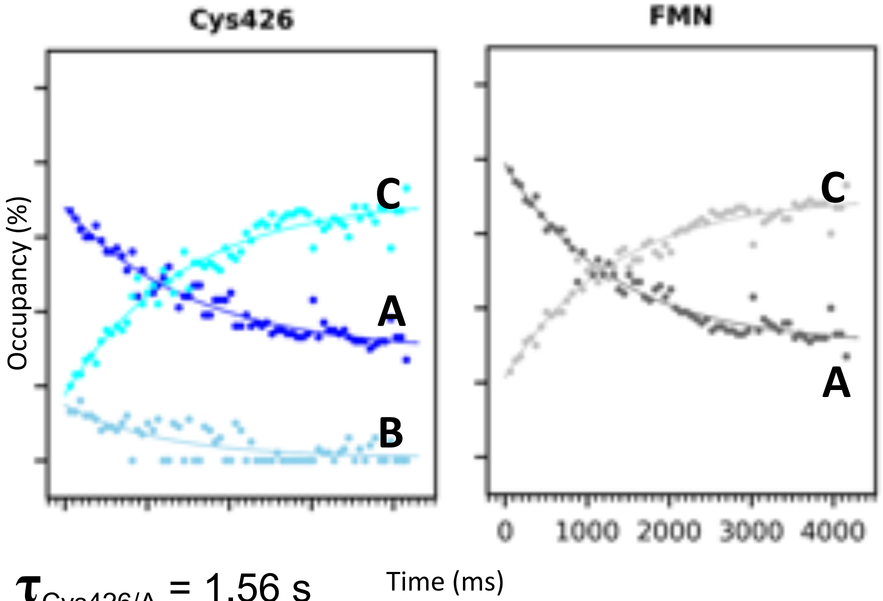
- 66 time points
- One frame every 63 ms
- Slowed x2



Aumonier *et al.*, *IUCrJ* (2020)

Diffraction-based calculation of the build-up time constant





$\tau_{Cys426/A}$ = 1.56 s

$\tau_{Cys426/B}$ = 1.19 s

$\tau_{Cys426/C}$ = 1.43 s

$\boldsymbol{\tau_{average}}$ **= 1.39 s**

$\boldsymbol{\tau_{FMN/AC}}$ **= 1.33 s**

Aumonier *et al.*, *IUCrJ* (2020)

Acknowledgements

Structural Biology Group & Associates

Sylvain Aumonier
Guillaume Gotthard
Hadrien Depernet
Nicolas Caramello
Sylvain Engilberge
David von Stetten
Philippe Carpentier
Thierry Giraud
Alexis van der Kleij
Nathan Spiegel
Pascal Théveneau
Olivier Hignette
Peter van der Linden

David Flot
Deborah Davison
Fabien Dobias
Hugo Caserotto
Jonathan Gigmes
John Surr
Montserrat Soler-Lopez
Igor Melnikov
Gianluca Santoni
Daniele de Sanctis
Andrew McCarthy
Christoph Mueller-Dieckmann
Gordon Leonard

GSY – Synchrotron Group

Christophe Berzin
Yohann Sallaz-Damaz
Eric Mathieu
Philippe Jacquet
Pascale Israel-Gouy
Franck Borel
David Cobessi
Martin Byrdin
Jérôme Dupuy