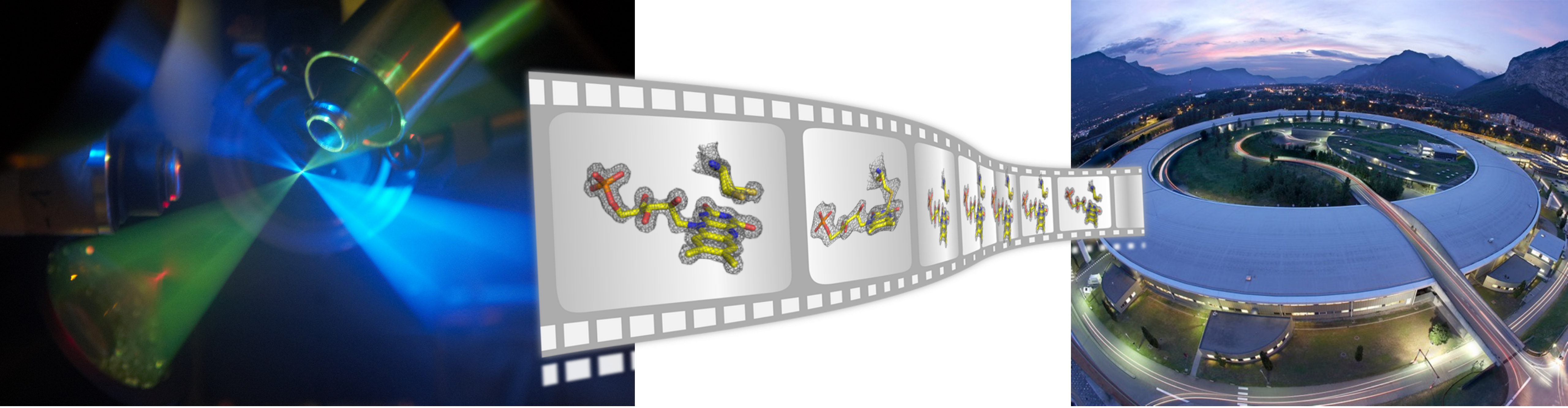


The *in crystallo* optical spectroscopy (*ic*OS) Lab

Antoine Royant

Institut de Biologie Structurale & European Synchrotron Radiation Facility

Institut de Biologie Structurale

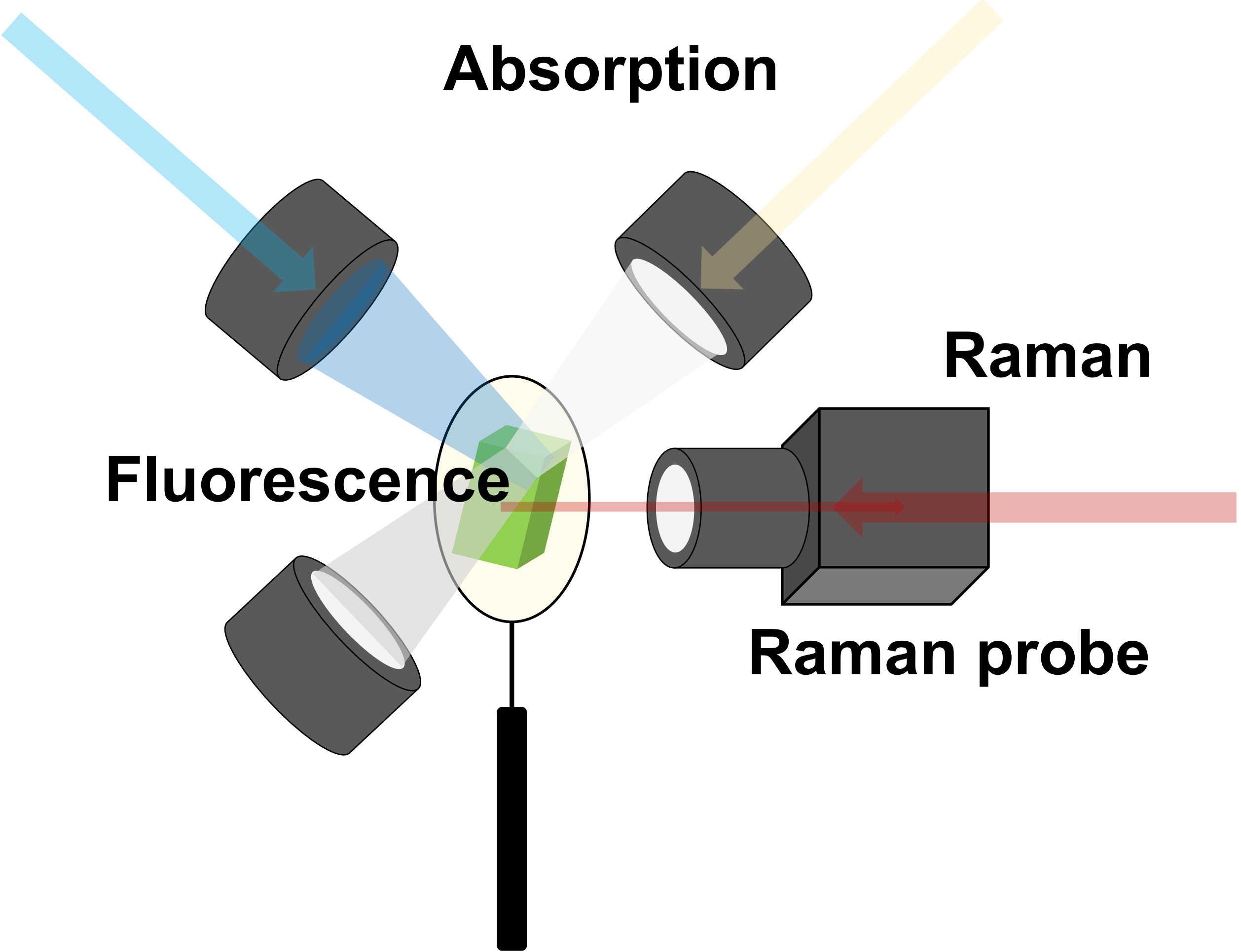
SB BAG meeting
7 February 2022

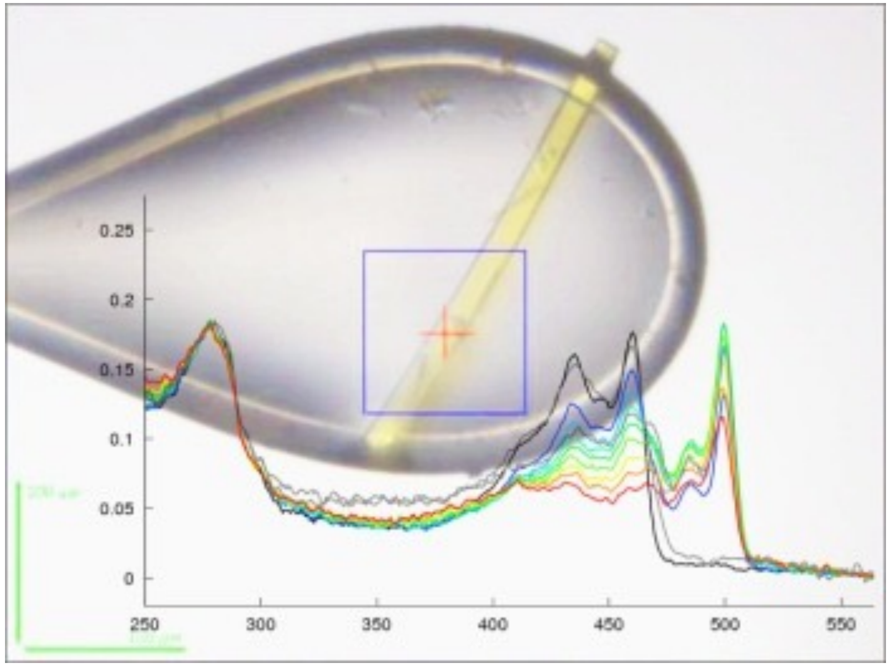
ESRF

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



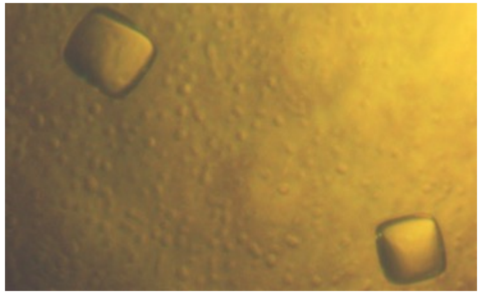
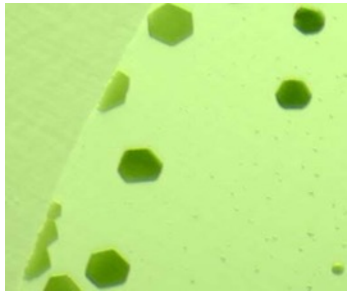


Many proteins are coloured

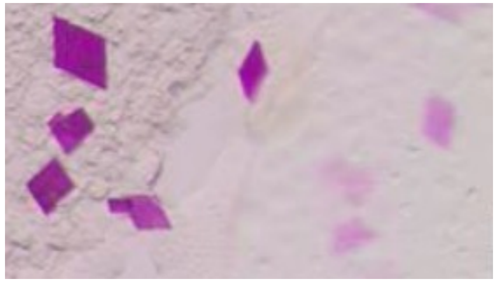
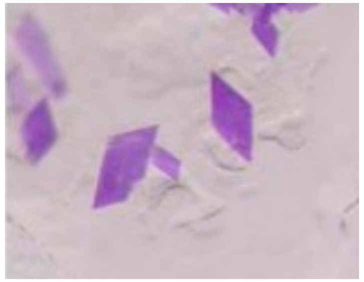
- Metal centres: Fe, Cu, **Co**



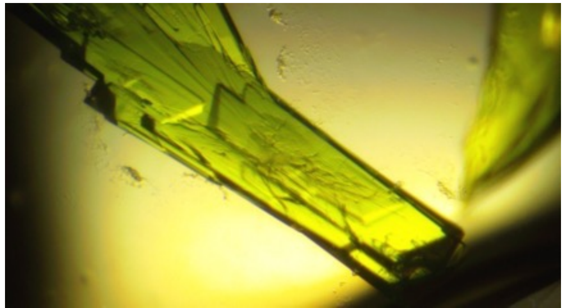
- Cofactor: **Flavins**, NADPH, **chlorophylls**

- Photoactive proteins (**retinal**, biliverdin)

- Fluorescent proteins (**cyan**, **red**, **near-infrared**)



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

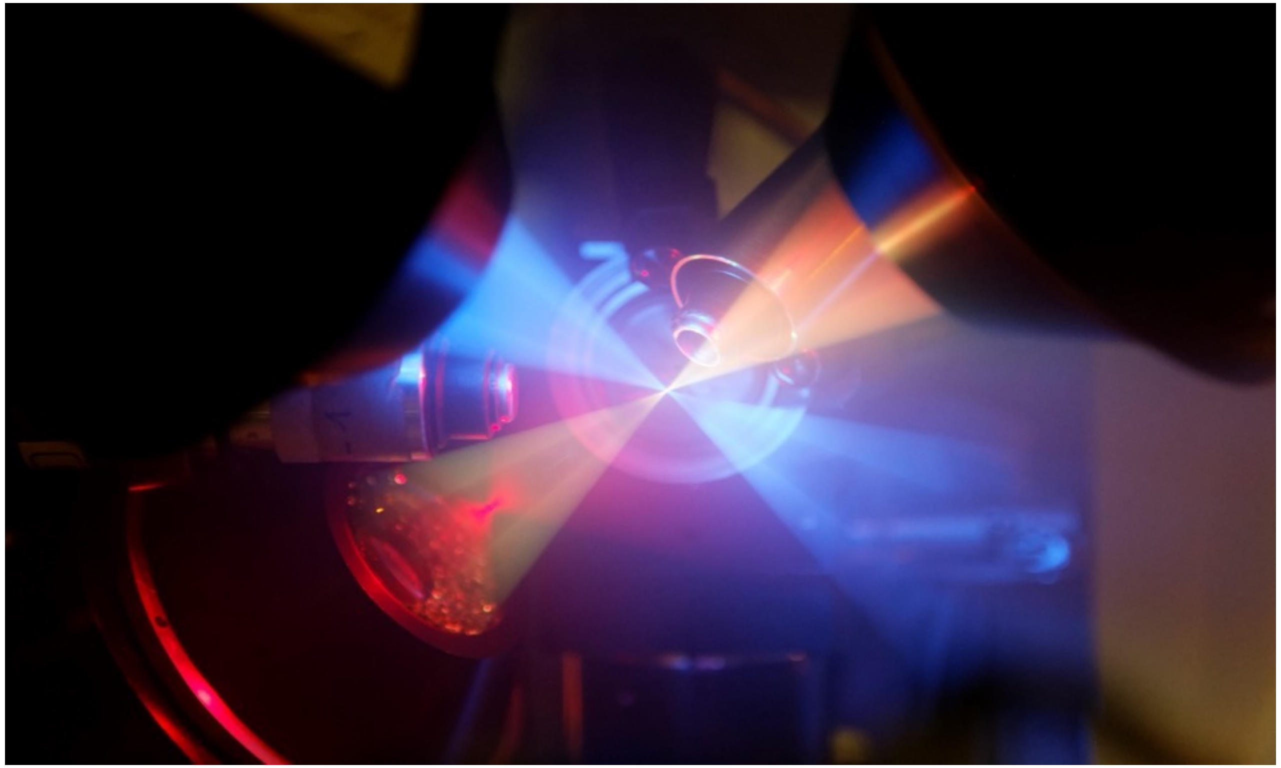
When and where?

- 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 (2018)

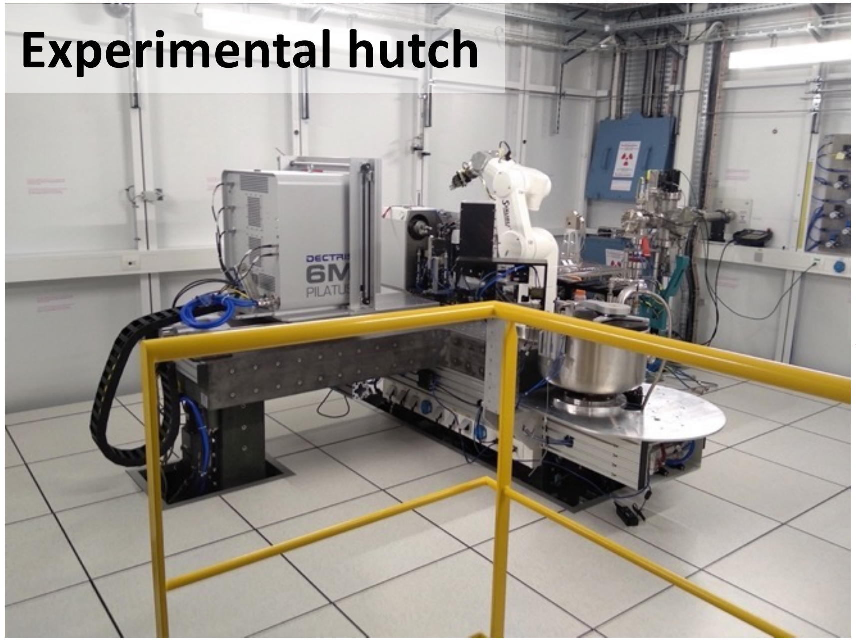


- Standard MX beamline equipment (minidiffractometer MD2M with 3-click sample centring, back/front lights, on-axis viewer)

- Motorized optical objectives

BM07-FIP2: the new French CRG MX beamline (BM30A-FIP replacement)

Experimental hutch



Official opening: 1st Oct. 2021

Detector: Pilatus 2 6M

Sample Changer: G-ROB, taking only **Spine pucks - Crystallisation plates** and **remote mode**: end of 2022

Tophat beam: 50 x 50 µm^2 to 250 x 250 µm^2

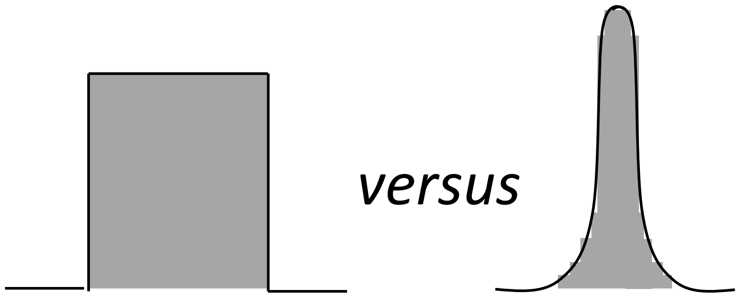
Tunable energy between **7** and **17 keV**

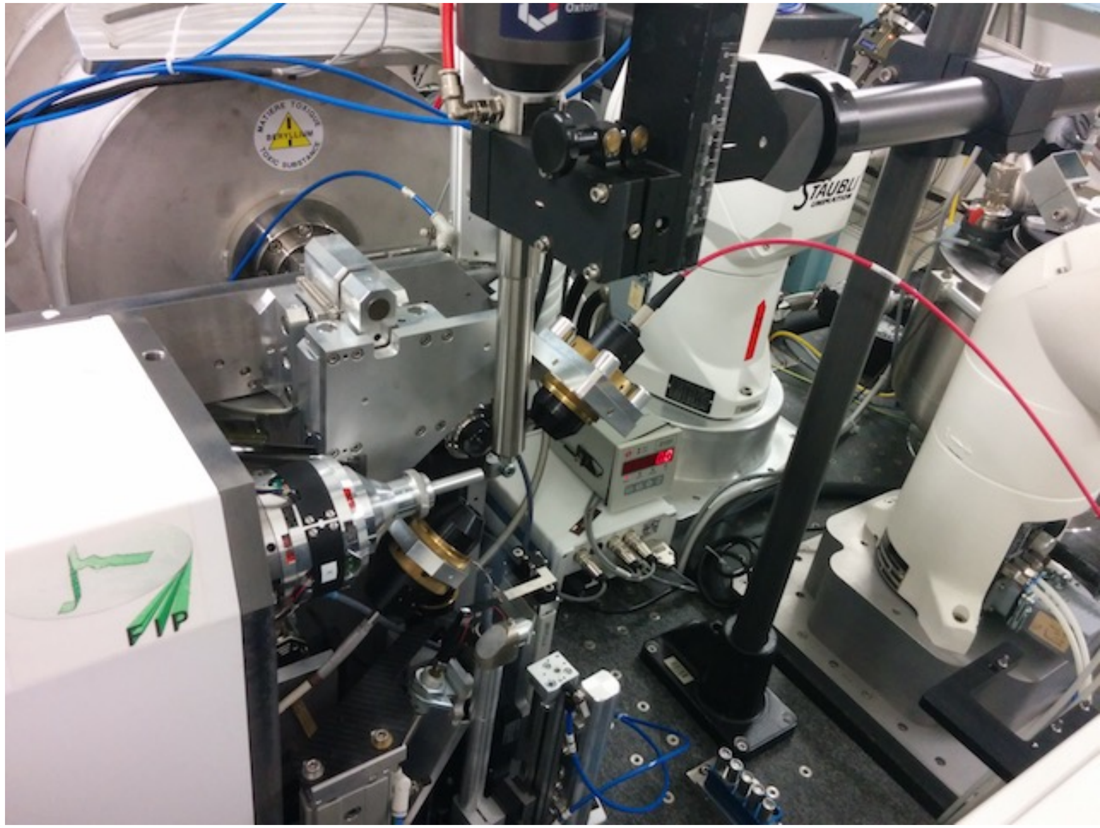
x10 increase in flux after EBS vs. BM30A: **~ 1E+12 ph/s** at 12.7 keV

Typical data collection time: **3 min** (vs. 15-30 min on BM30A)

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

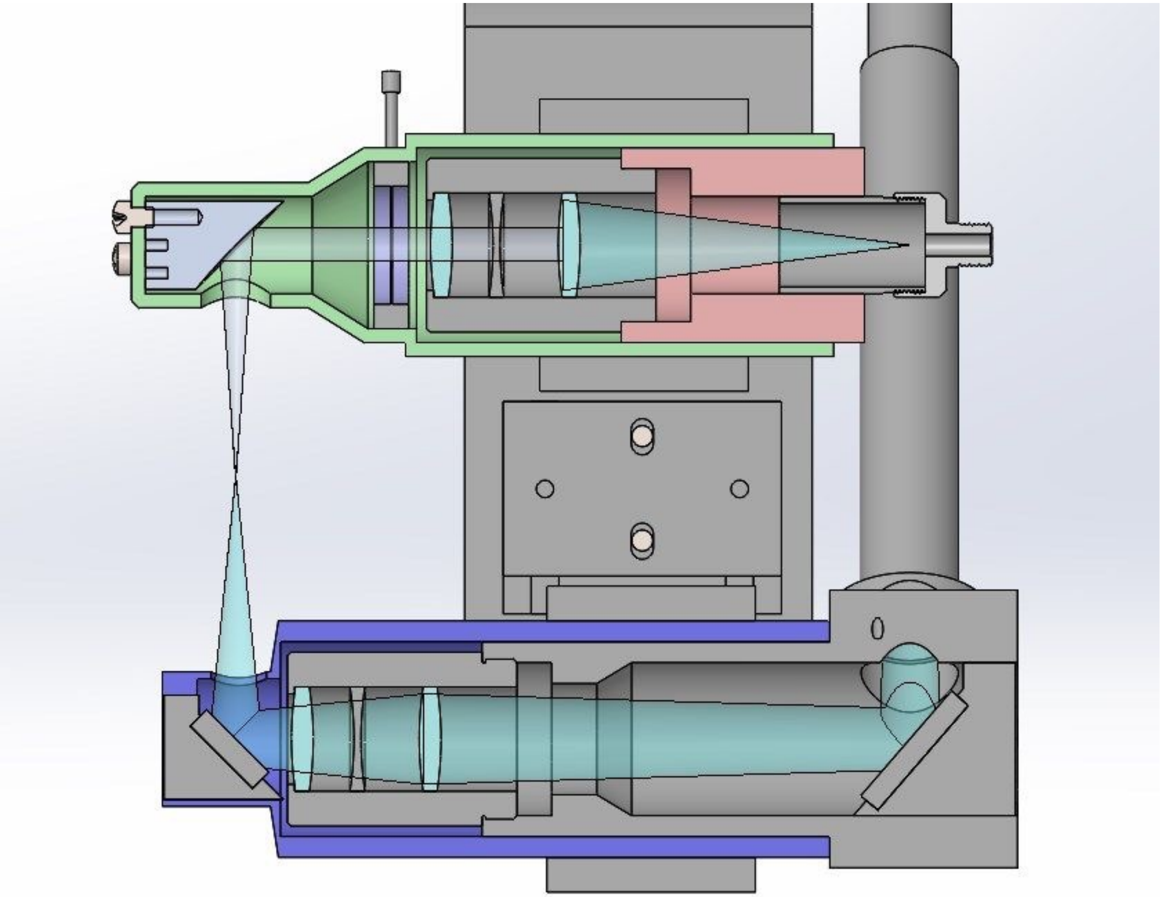
- Use of EMBL/ESRF microspec (McGeehan *et al.* (2009))
- Cryogenic (still very) low dose spectroscopic characterization -> complementary to other MX beamlines
- Importance of keeping a TopHat beam *vs.* Gaussian beam for radiation damage studies

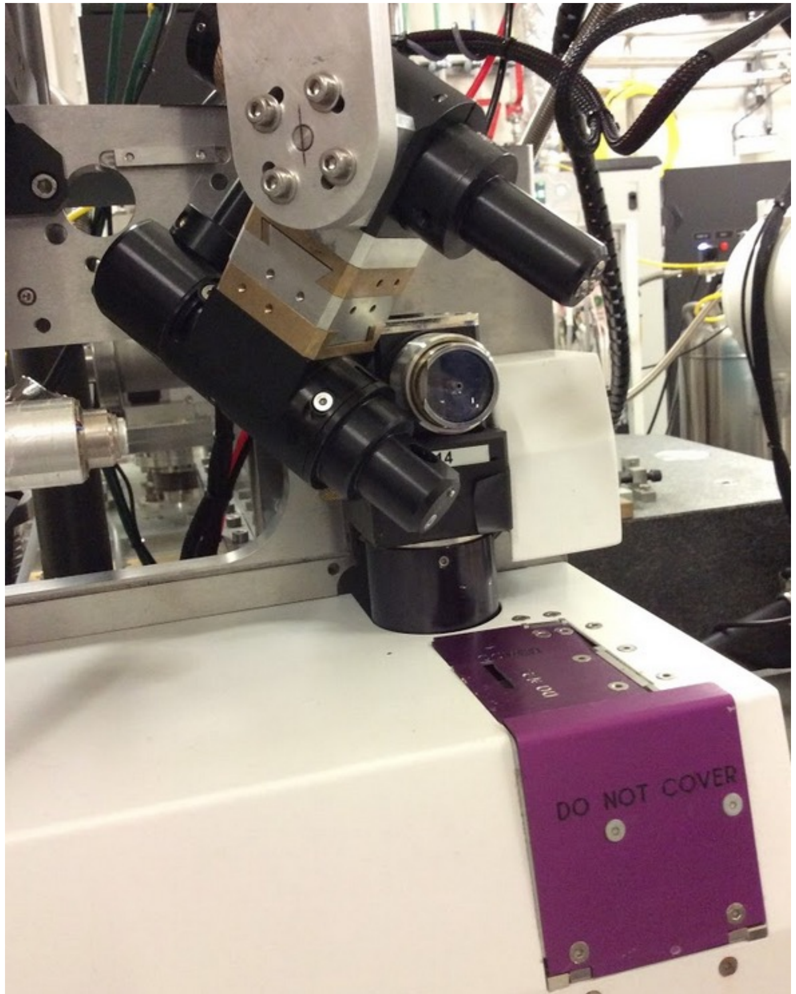


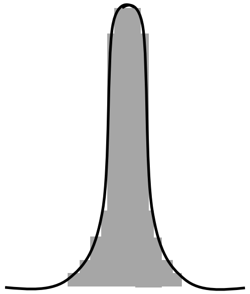


On-line UV-vis absorption spectroscopy on ID30A-3 (smaller crystals, 15-20 µm)

- Brand new design (O. Hignette/P. Theveneau, ESRF) - use of parabolic mirrors
- Developed by D. von Stetten then I. Melnikov

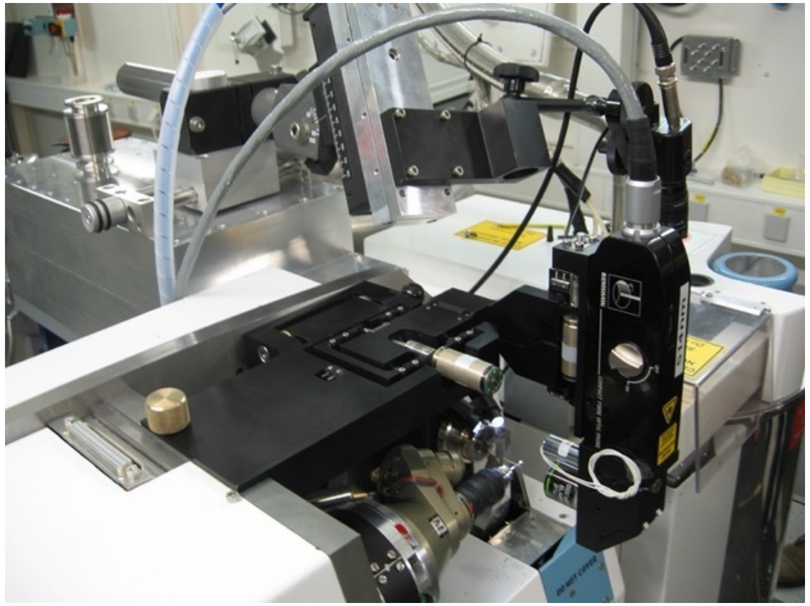


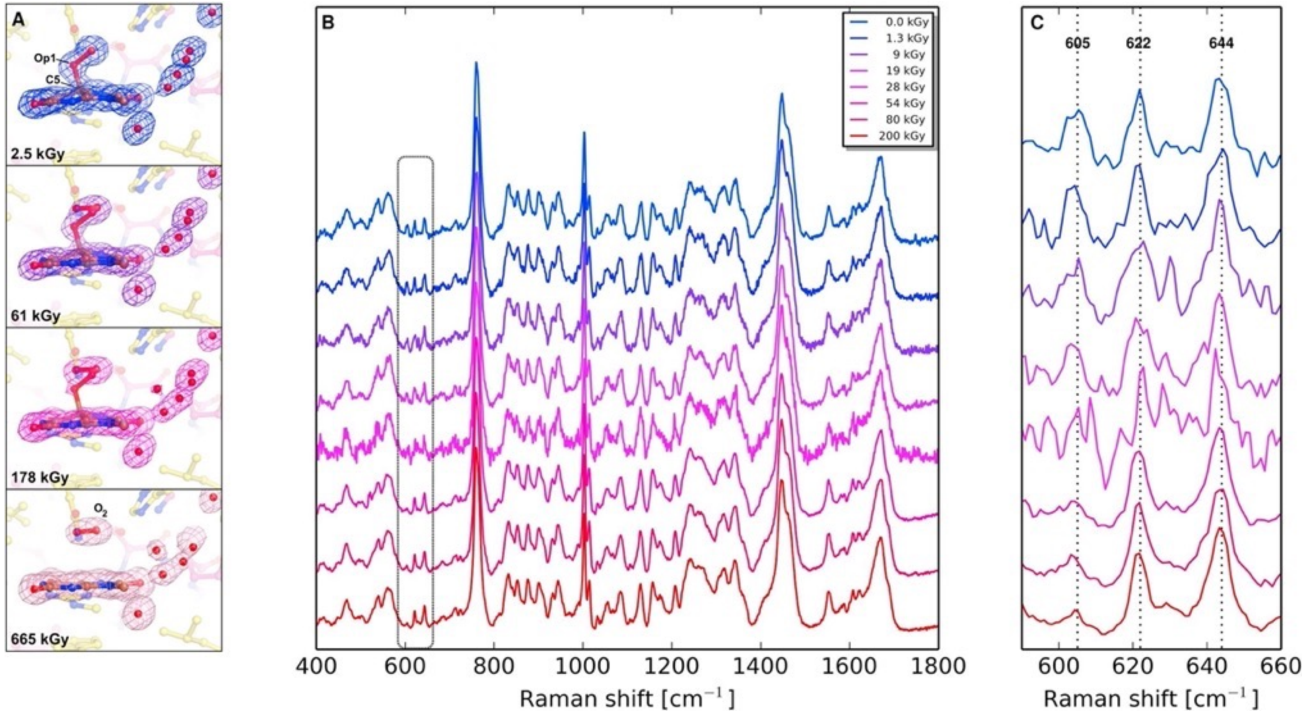




On-line Raman spectroscopy, from ID29 to ID30B

- Originally developed on ID29 (synergy with ID29S-Cryobench) von Stetten *et al.*, *J. Struct. Biol.* (2017)
- Online Raman not suitable for ID29 any more (beam size)
- Will be suitable to the adjustable beam size of ID30B
- Radiation damage studies – monitoring specific bond breakage or deformation of groups (or of secondary structures)





TR-*ic*OS: A setup for Time-Resolved *in crystallo* Optical Spectroscopy

Pump-probe experiments:

- **Pump** = nanosecond pulse from laser
- **Probe** = microsecond pulse from flash-lamp

-> Series of transient UV-vis absorption spectra

See Daniele's presentation

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

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

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Recent *ic*OS highlights

(1) Rodrigues *et al.*, *Nat. Chem. Biol.* (2017) – Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis

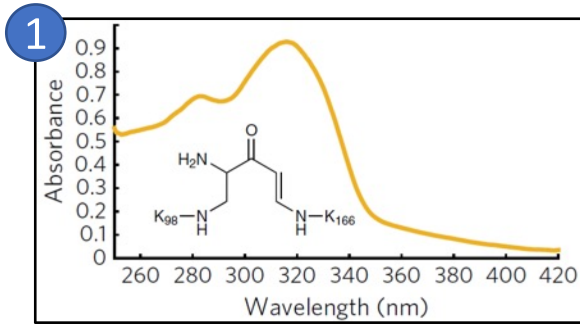
(2) Torra *et al.*, *Sci. Rep.* (2019) – Tailing miniSOG: structural bases of the complex photophysics of a flavin-binding singlet oxygen photosensitizing protein

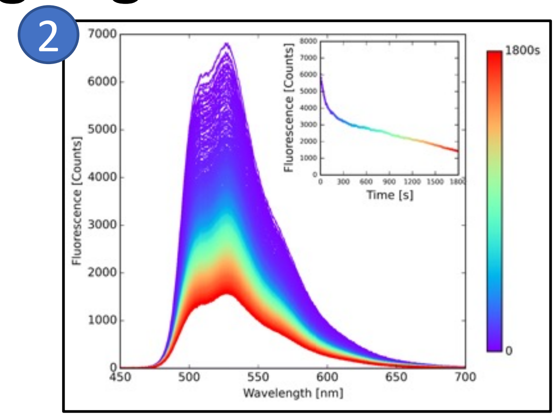
(3) Kovalev *et al.*, *Nat. Commun.* (2020) – Molecular mechanism of light-driven sodium pumping

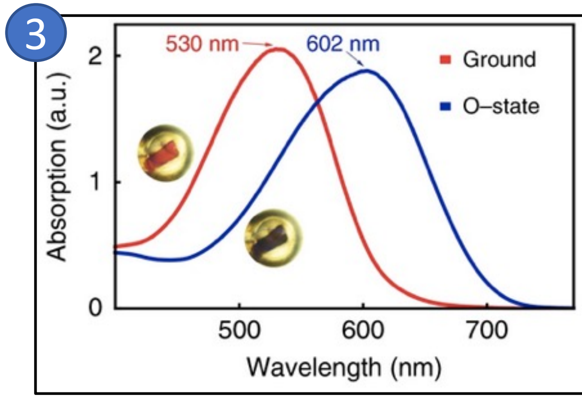
(4) de Zitter *et al.*, *JACS* (2020) – Mechanistic Investigations of Green mEos4b Reveal a Dynamic Long-Lived Dark State

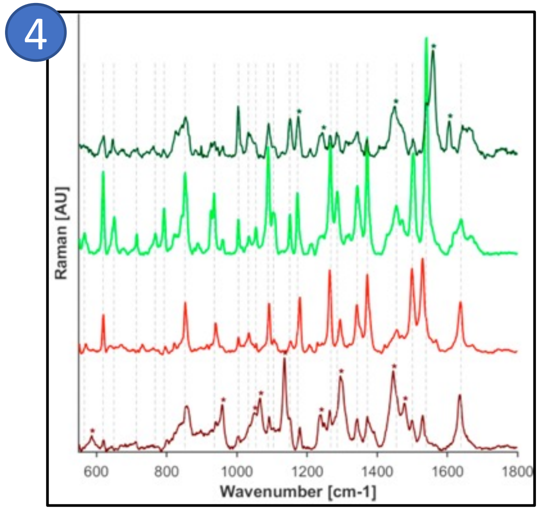
(5) Sorigué *et al.*, *Science* (2021) – Mechanism and dynamics of fatty acid photodecarboxylase

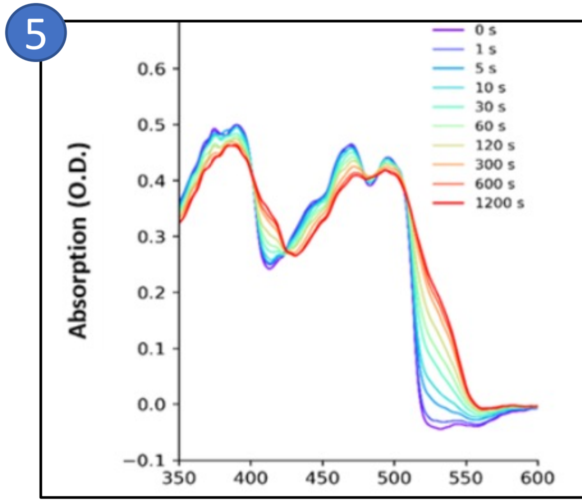
(6) Maestre-Reyna *et al.*, *Nat. Chem.* (accepted) – Serial crystallography captures dynamic control of sequential electron and proton transfer events in a flavoenzyme

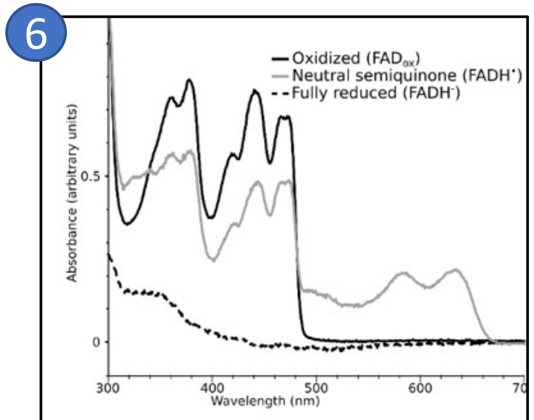












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