Drop-on-Demand Sample Delivery for Studying Biocatalysts in Action at XFELs

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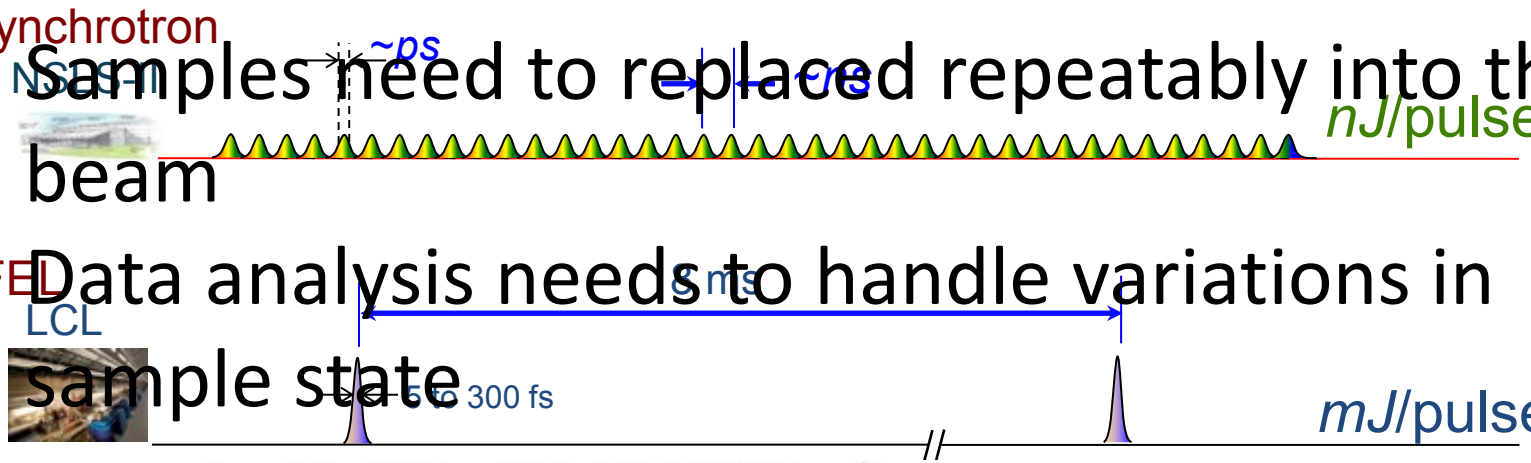
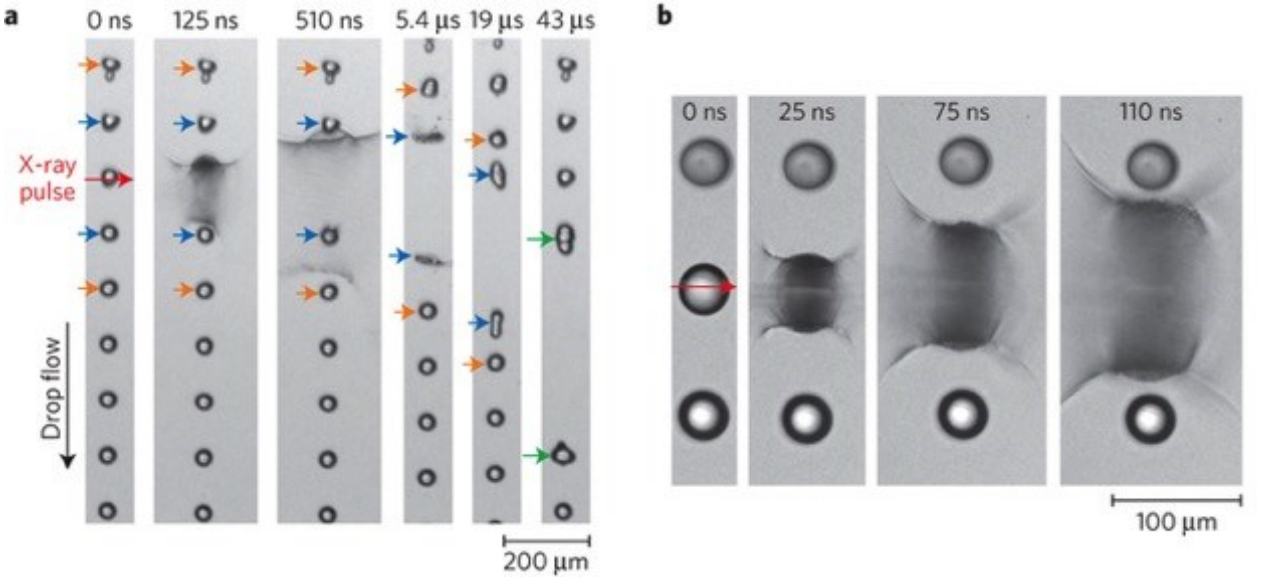
Main Messages

- Atomic resolution, time resolved, room temperature studies of biomolecules are possible with XFELs and highly valuable to advance our understanding
- Multi-modal measurements (e.g. combining diffraction/scattering and spectroscopy) offer more information and make time resolved measurements *better.*

sample delivery challenges at XFELs

- Samples need to replaced repeatably into the beam
- Data analysis needs to handle variations in sample state



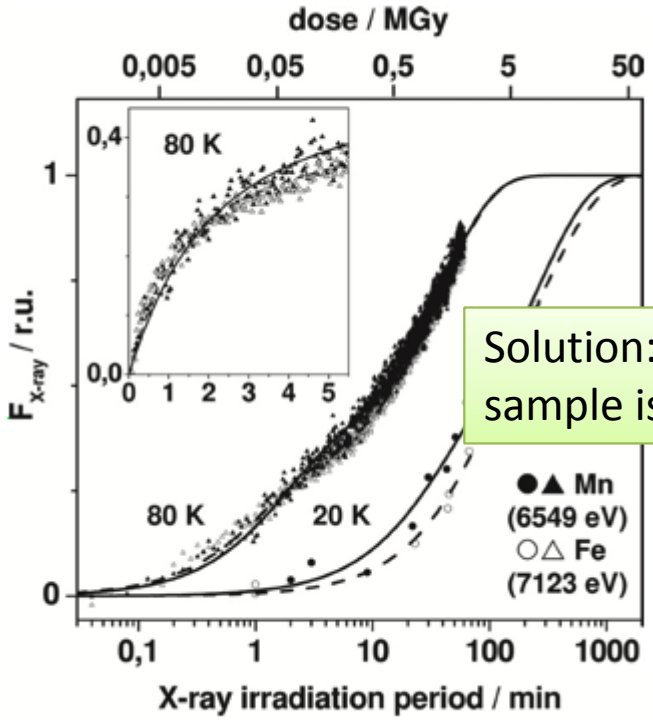


Claudiu Stan, et al. Nature Physics. 2016

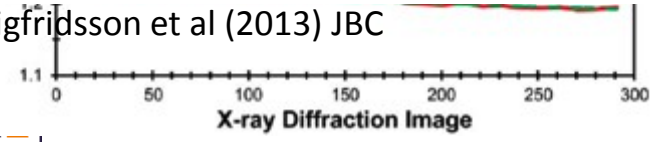
Avoiding Radiation Damage at RT

Reduction of Rieske cluster in Stachedryn demethylase

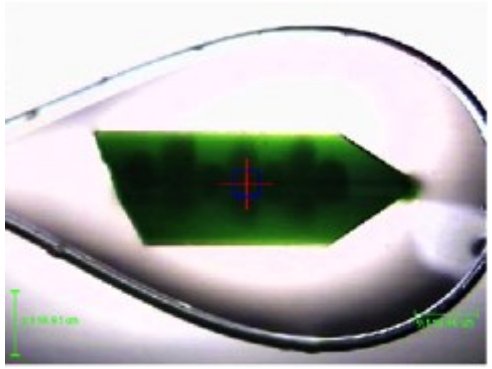
Reduction of RNR active site



Sigfridsson et al (2013) JBC

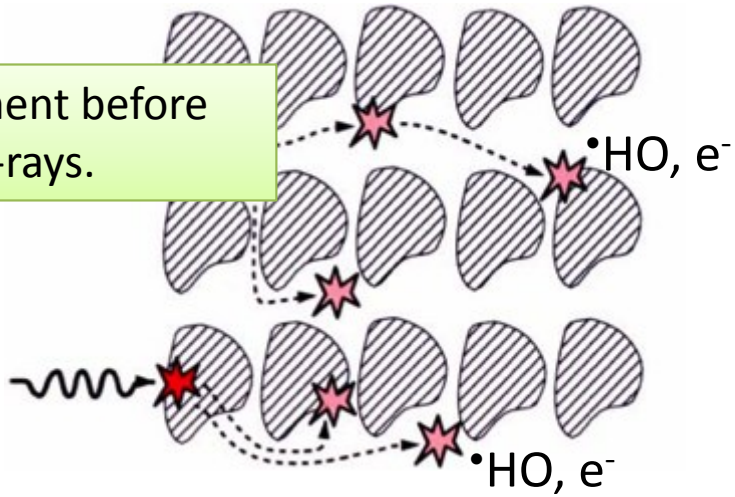


Daughtry et al (2012) JACS



Photosystem II crystal after X-ray diffraction data collection

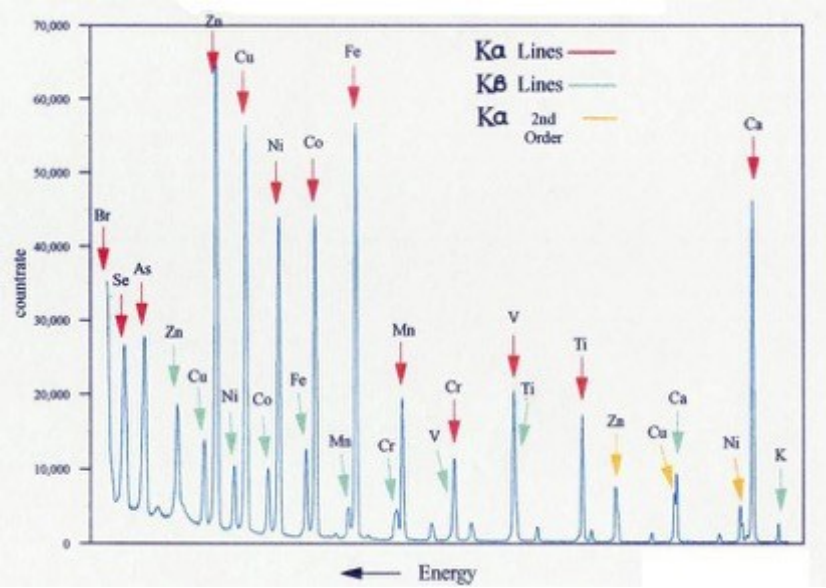
Solution: fast measurement before sample is modified by X-rays.



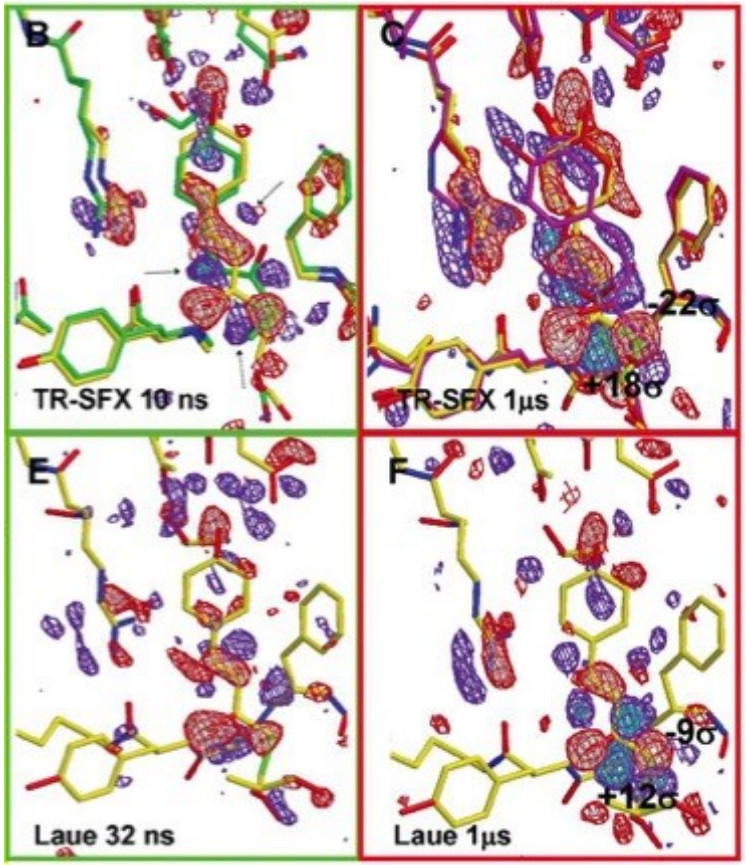
Formation of radicals: 10's fs, cascade leading to ~200 radicals/photon

Diffusion, subsequent damage: ps and longer

Time resolved X-rays methods give a direct atomic, element-specific view



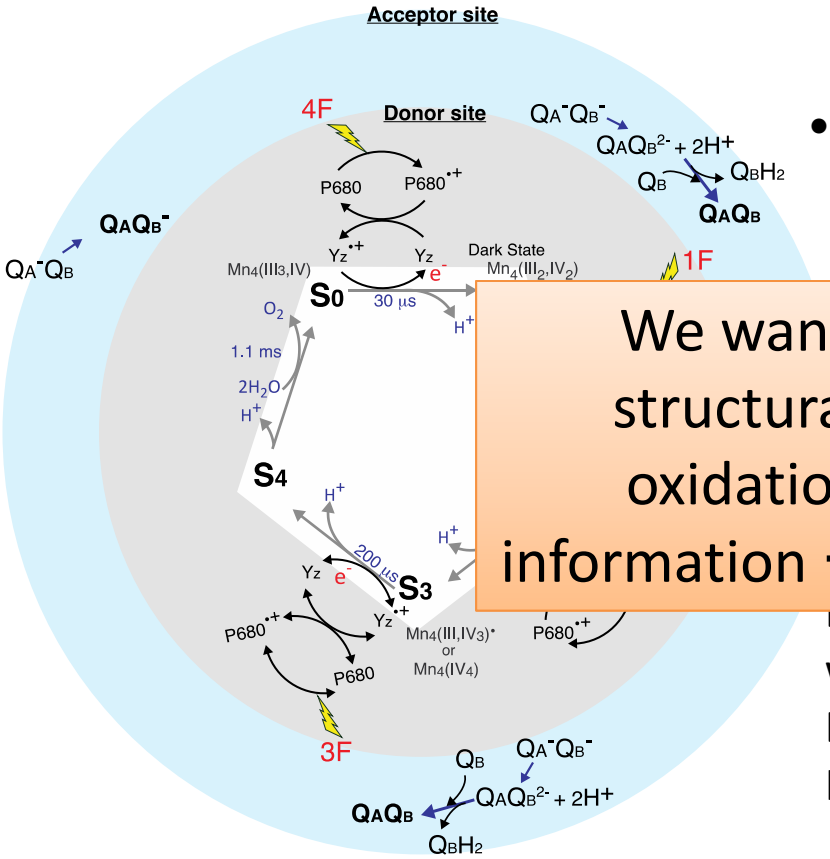
Wikipedia



Tenboer, et al. Science, 2014

Two problems we want to study

Photosystem II: OEC



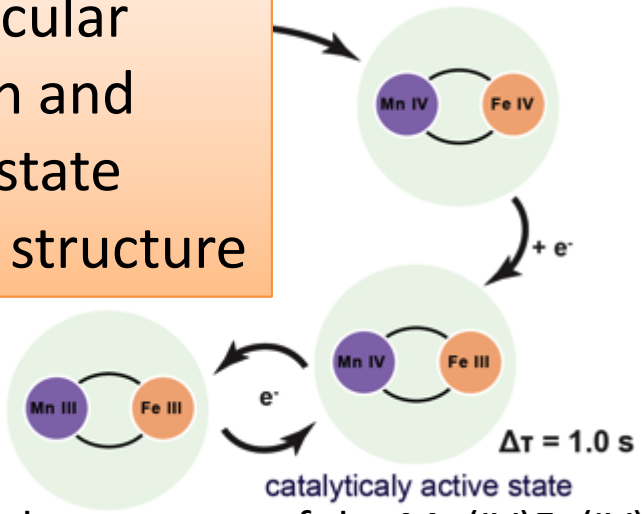
Alonso-Mori, *et al*. Faraday Disc. (2016)

- Structure of S3
  - What waters are bound where
  - Where do inhibitors/other substrates bind
    - *e.g*. Ammonia
- Structure of S4
  - O-O bond formation: lets see it

Ribonucleotide Reductase (R2c)

We want **BOTH** molecular structural information and oxidation state/spin state information → electronic structure

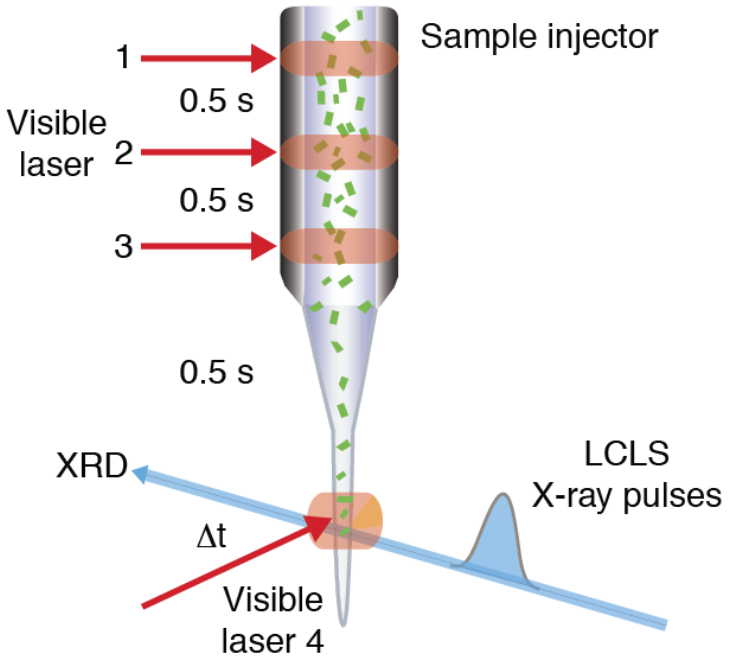
Based on work from Bollinger & Krebs group



- What's the structure of the Mn(IV)Fe(IV) intermediate
- Can we follow the long range PCET in this system?

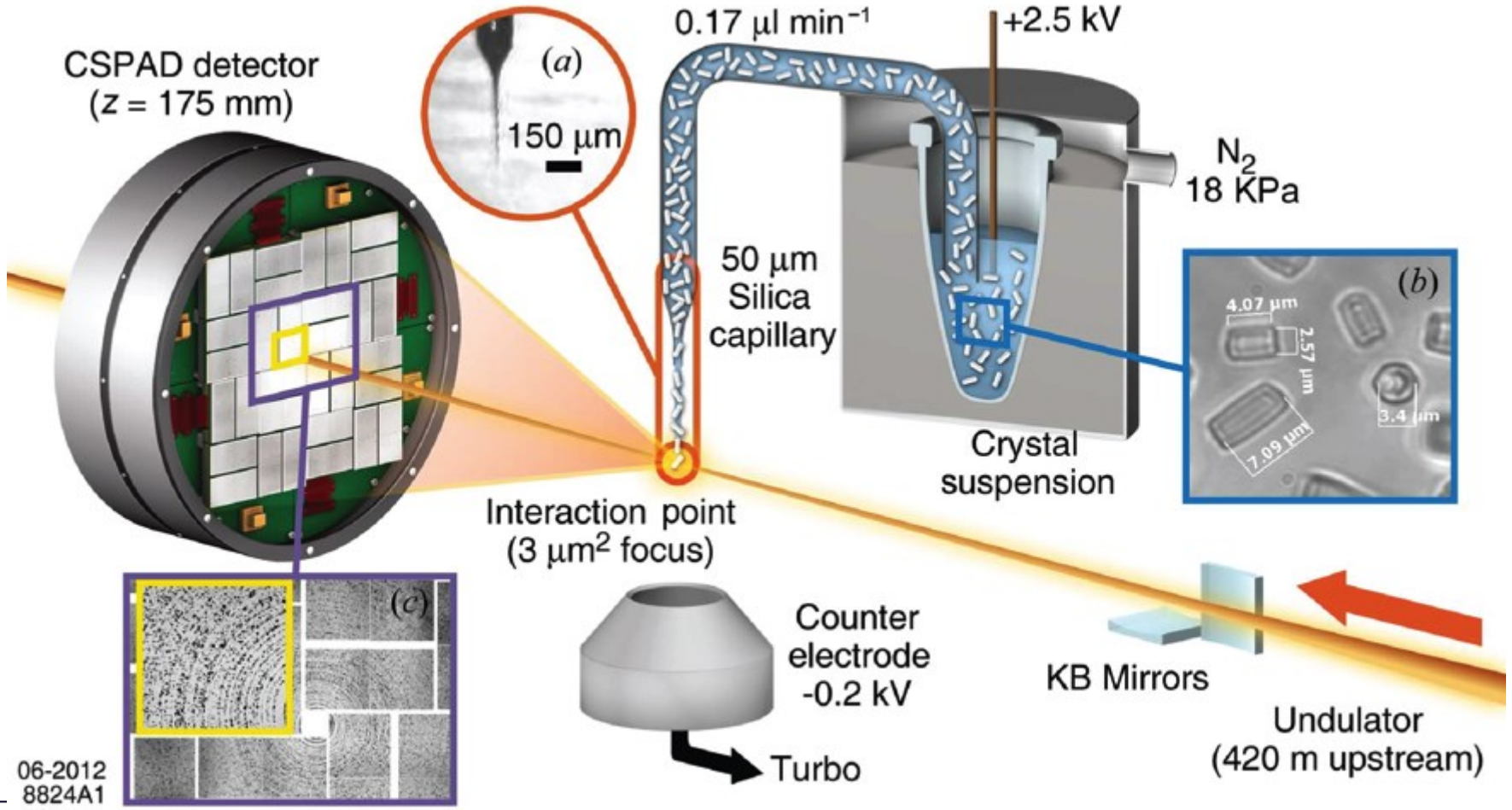
Time scales of interest to us

- Flashing interval for optimal S-state advancement is hundreds of milliseconds
- Formation of S_4 is sub millisecond
- Formation of Fe(IV)Mn(IV) in RNR is <100 ms
- Formation of Fe(III)Mn(IV) in RNR is second scale



Kern et al. Nature. Comm. (2014)

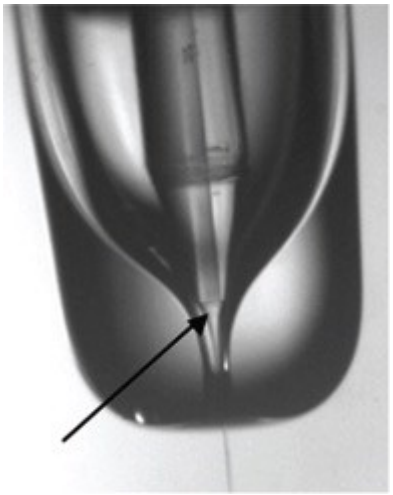
A typical serial x-ray experiment



Sierra, Raymond, et al. *Acta Cryst D*, 2012

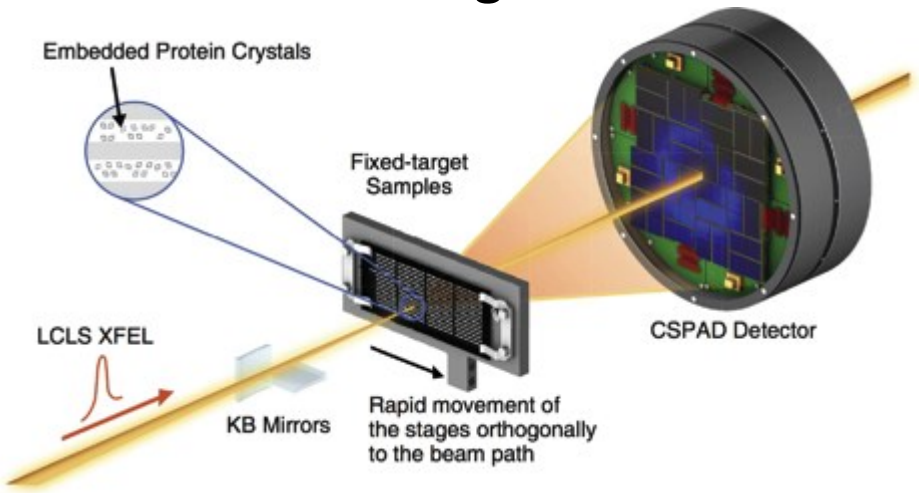
More ways to replace sample

Fast Jets



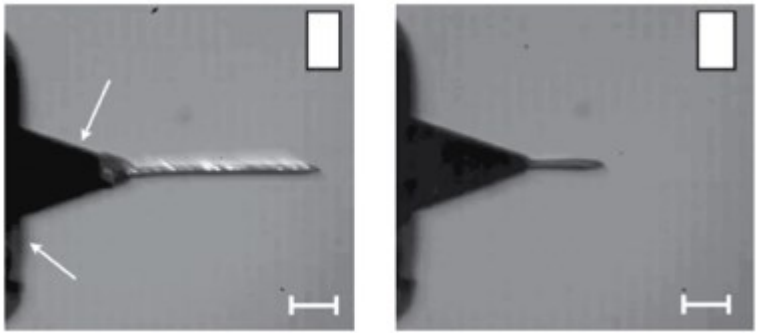
DePonte, D. P., et al. Journal of Physics D (2008)

Rastered Targets



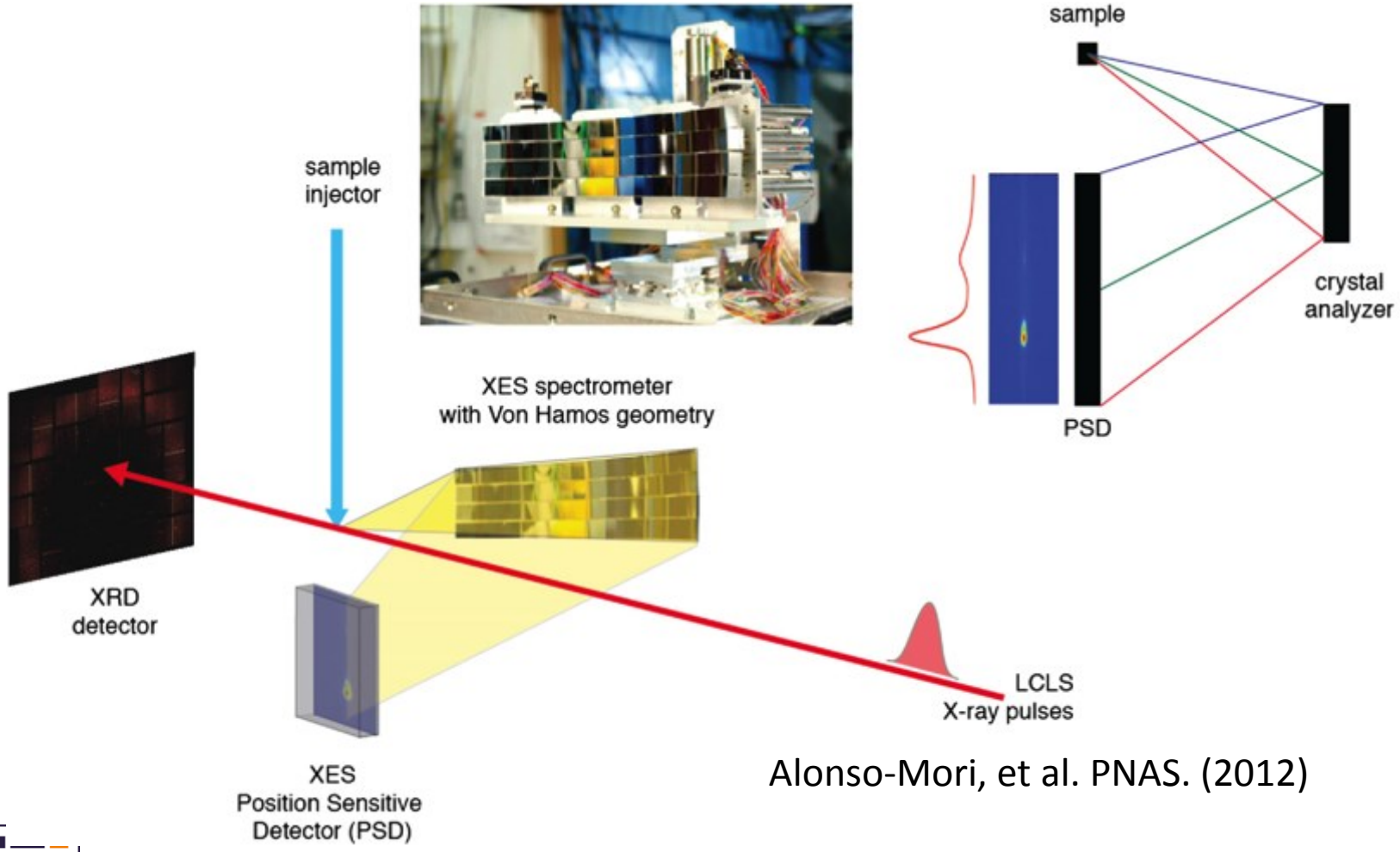
Hunter, Mark, et al. Scientific Reports, (2014)

Slow Jets



Uwe Weierstall, et al. Nature Comm. (2014)

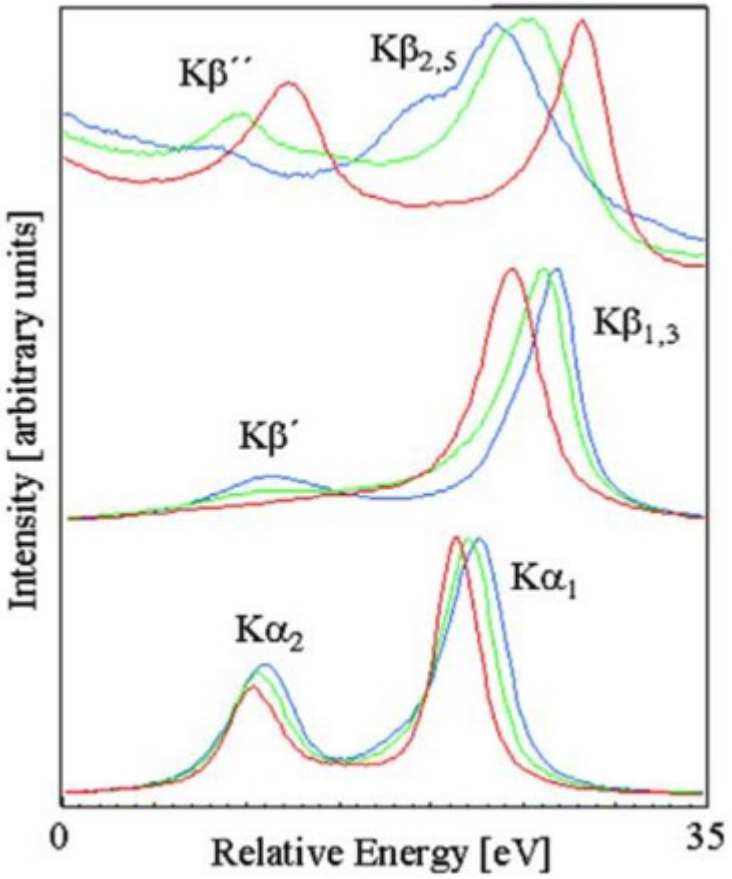
Multi-modal serial x-ray experiment



Alonso-Mori, et al. PNAS. (2012)

Information content of XES

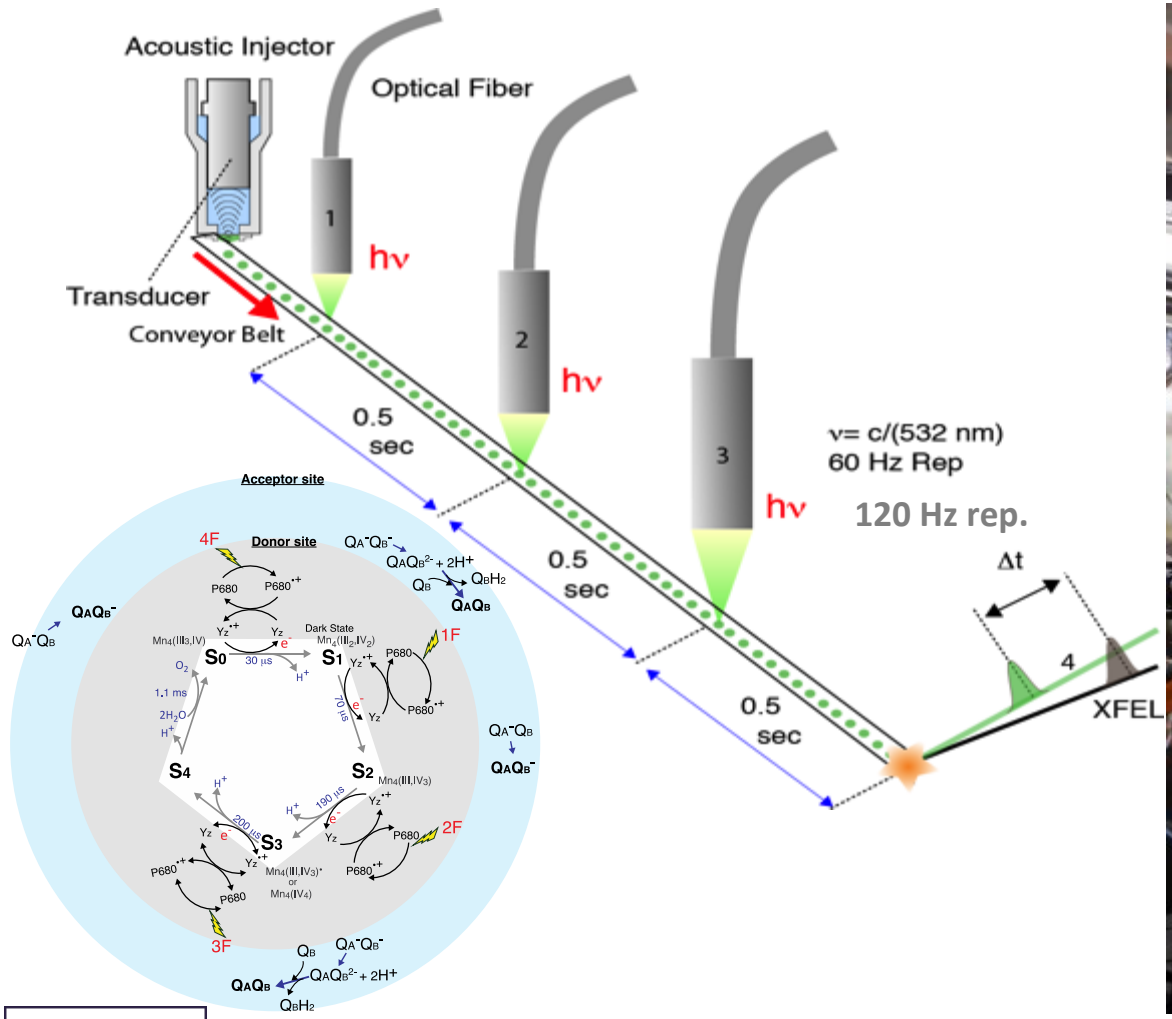
- In situ verification of:
  - No photo-reduction
  - States are being prepared as you think they should
- Probes electronic state:
  - Useful in its own right to study oxidation/reduction kinetics
  - Detect changes from high spin to low spin

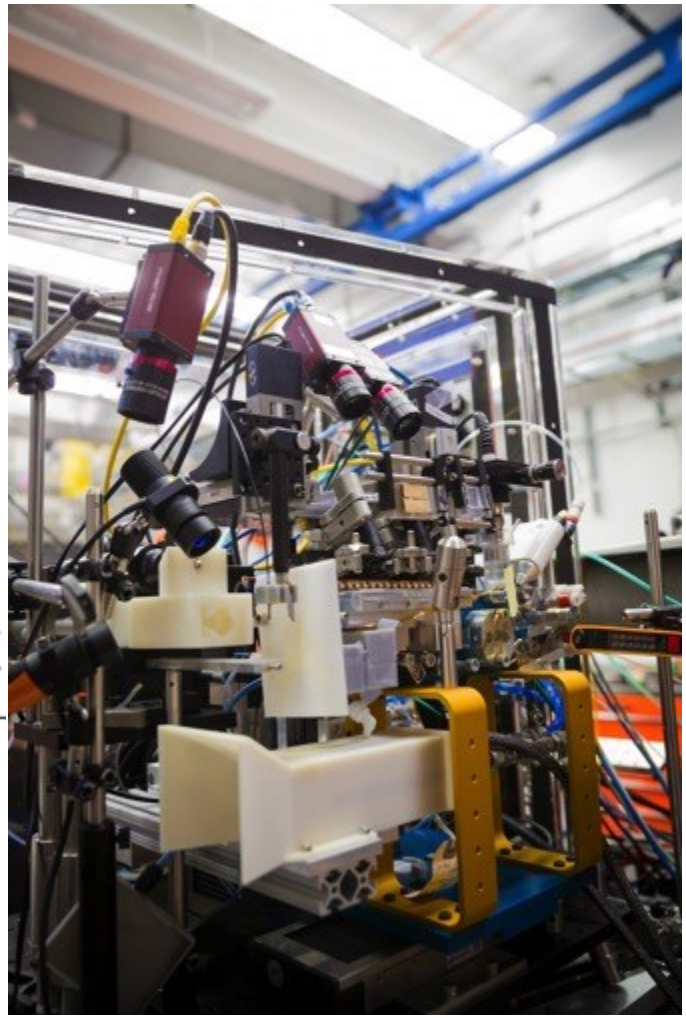


Red = Mn(VII), Green = Mn(IV), Blue = Mn(II)

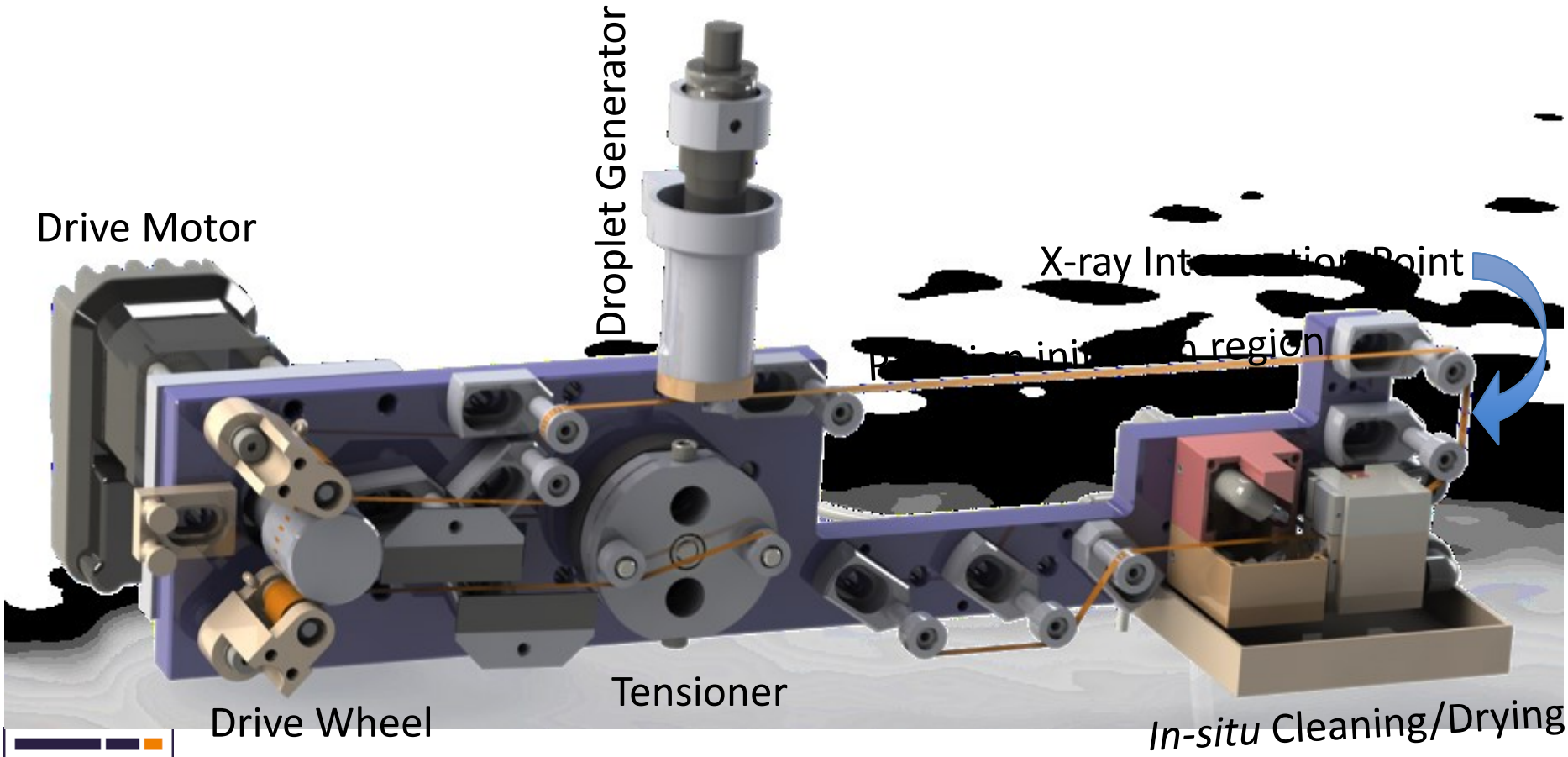
Bergmann, U. Glatzel, P. *Photosynth. Res*. (2009)

Our sample delivery solution

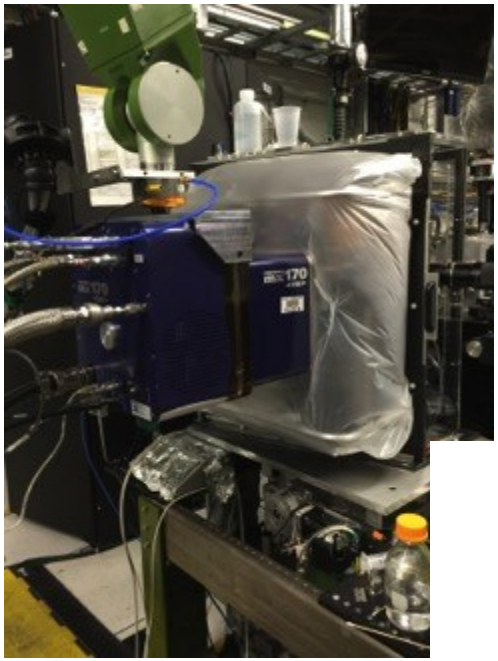


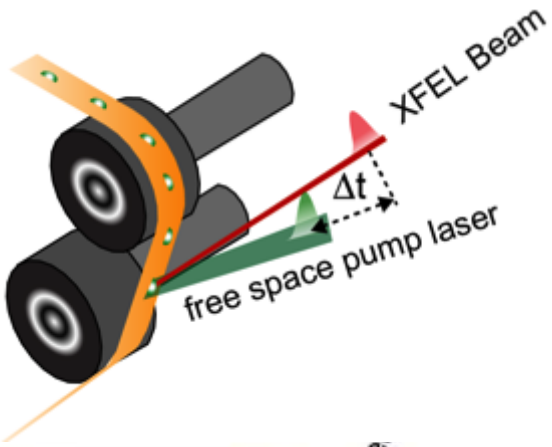


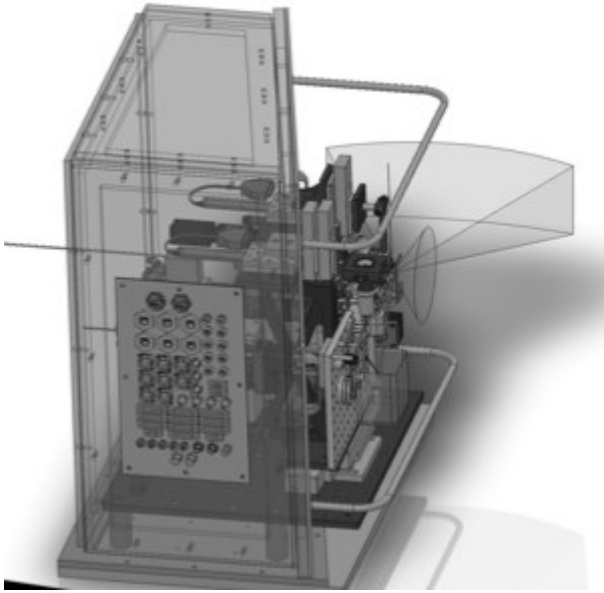
A miniature conveyor system

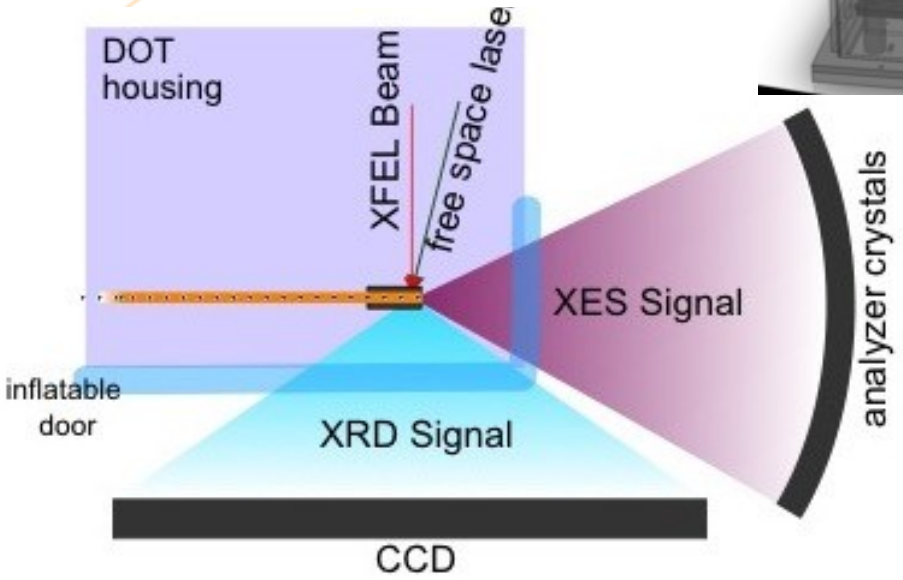


Simultaneous Emission & XRD with the "tape drive"



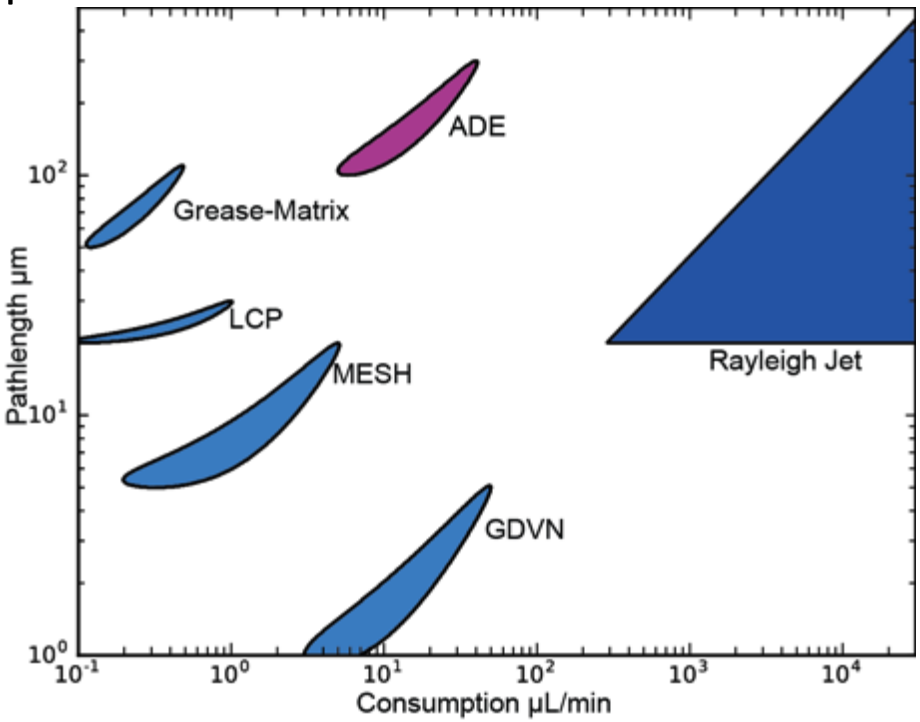




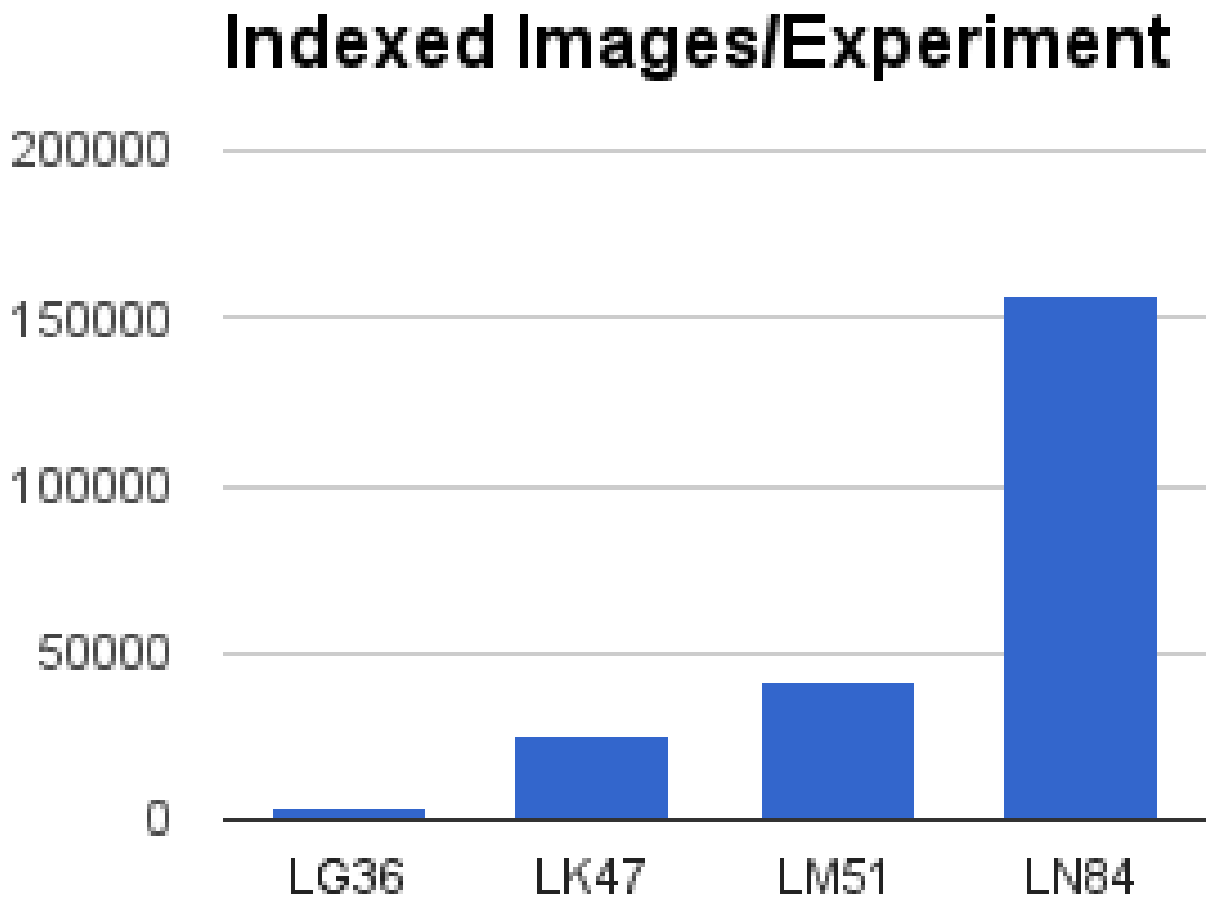


Rationale

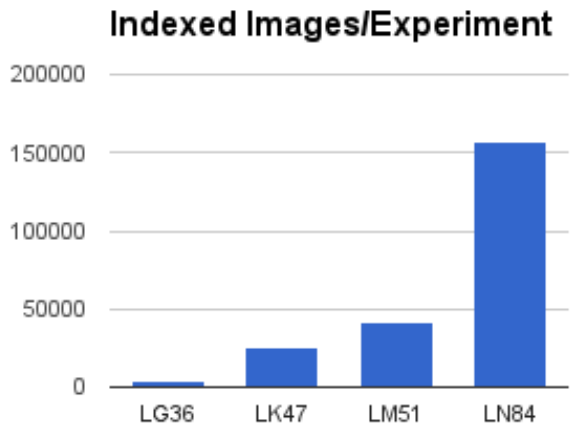
- Flow is discretized into droplets to match XFEL rep-rate
  - No wasted sample between shots
  - Produces defined volumes of a given state for a long time (no accidental mixing)
- Sample is supported on a moving substrate
  - Great control of the sample velocity affords longer time scales more easily
  - Solid support is more robust than *some* jet technologies in terms of positional stability
- Droplet generation method is “friendly” to large crystals and long pathlengths



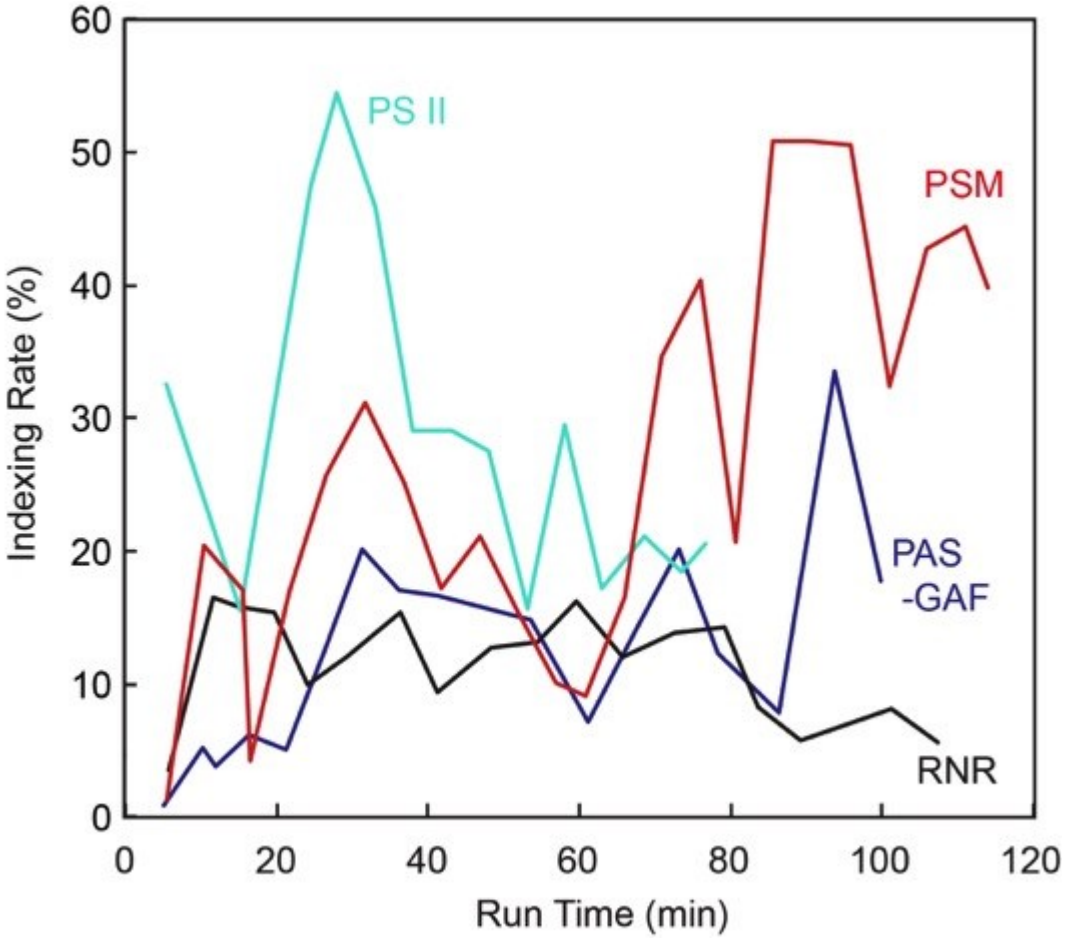
It works well for us



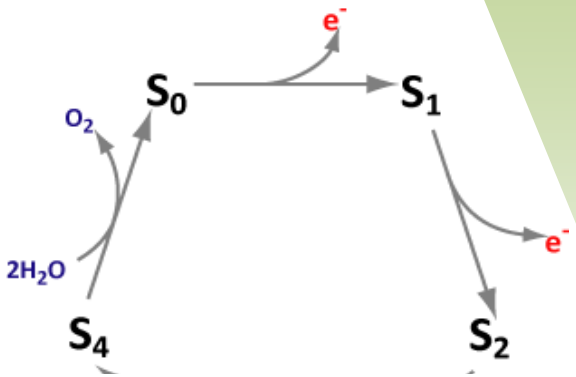
Good Indexing Rate

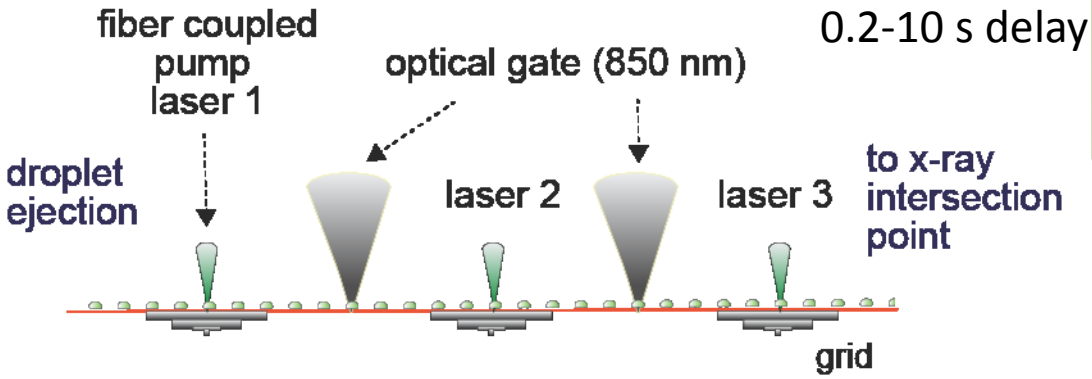


Hitting more often and better hits

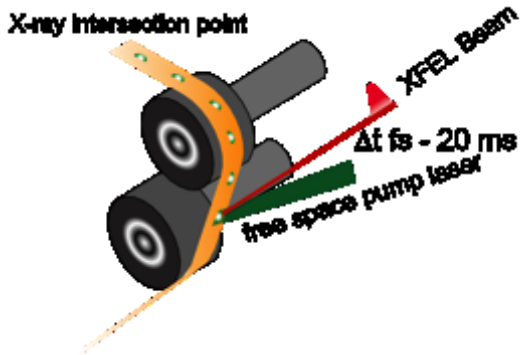


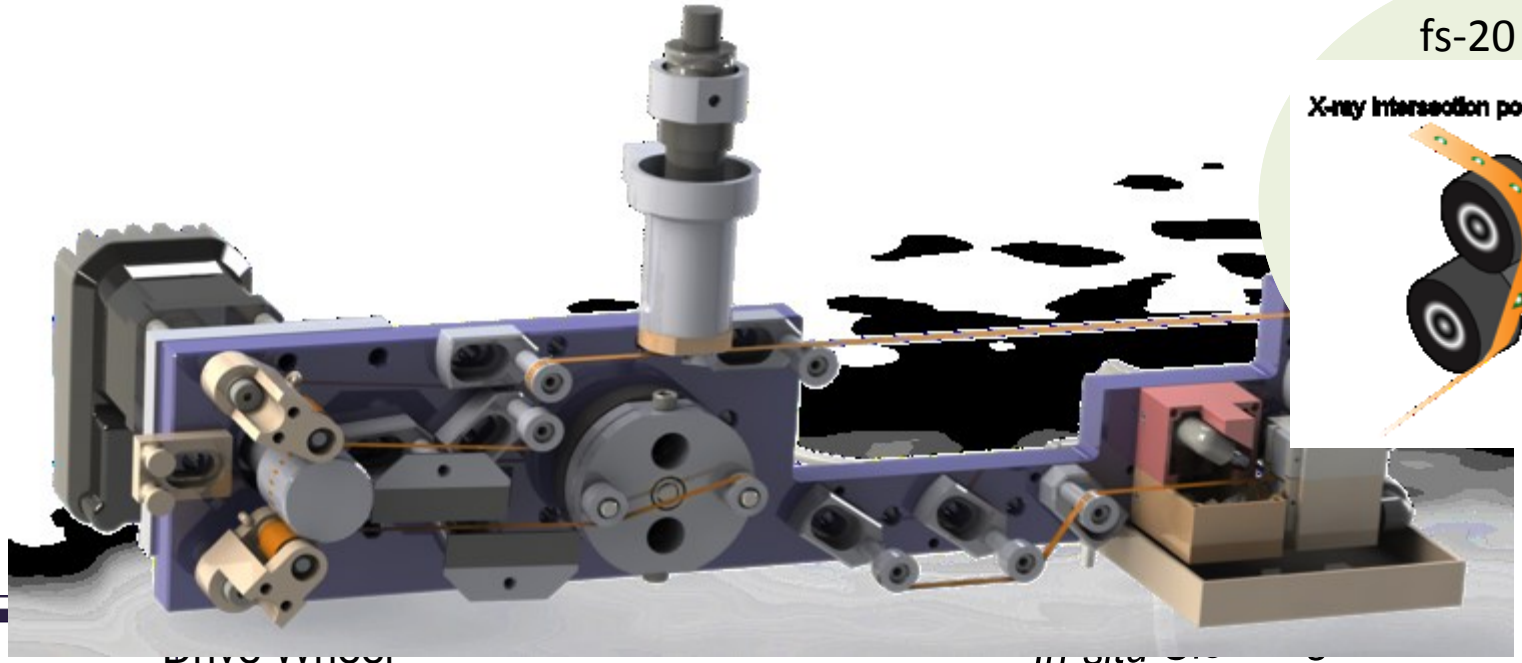
Transient Photosystem II studies





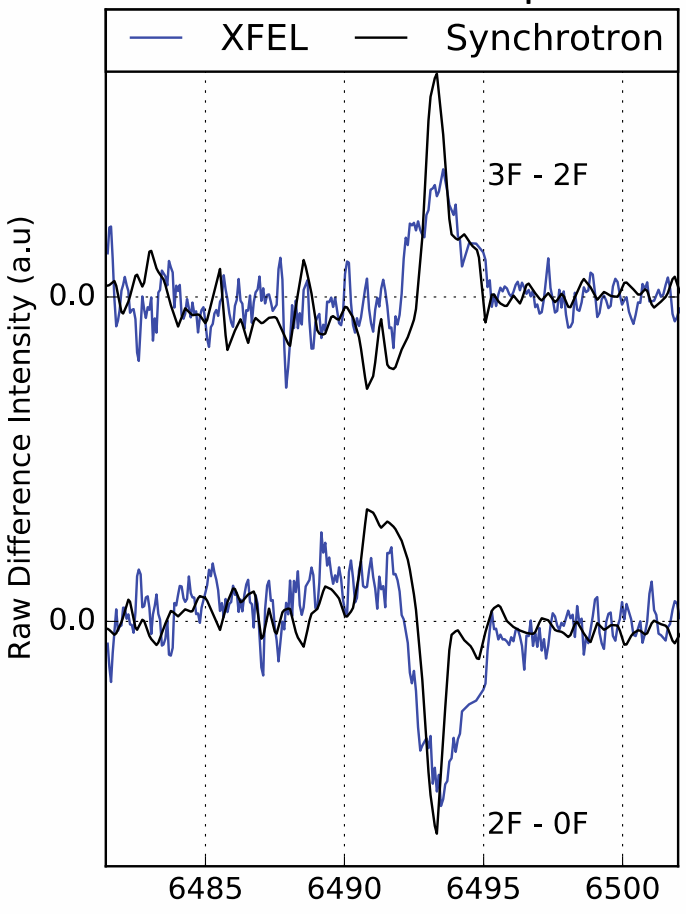
fs-20 ms delay



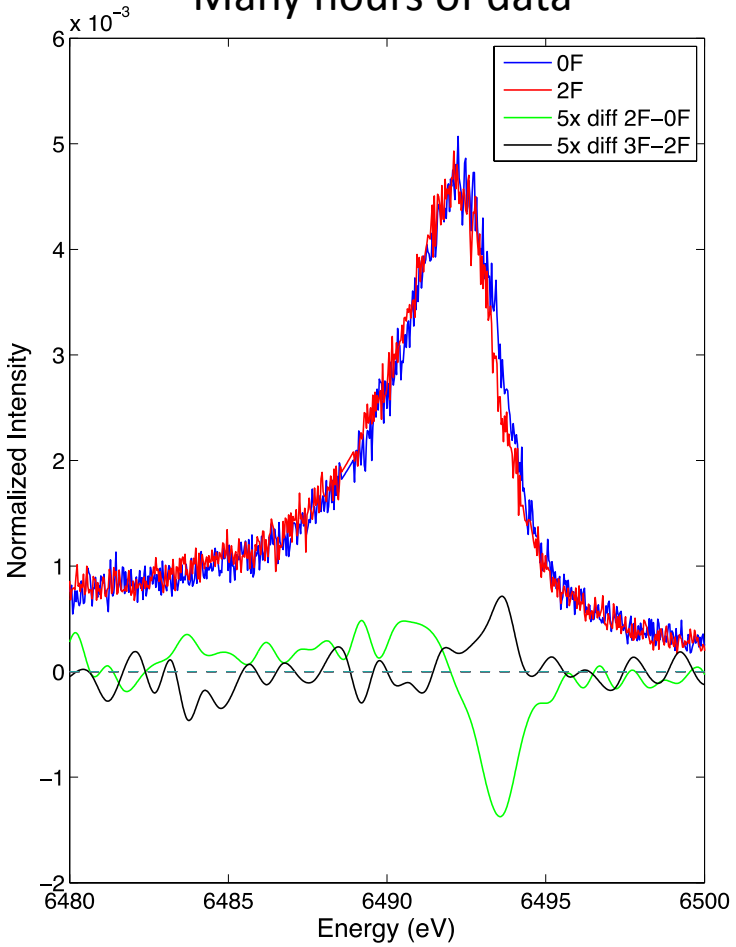


Results: $K\beta_{1,3}$ emission of PS II

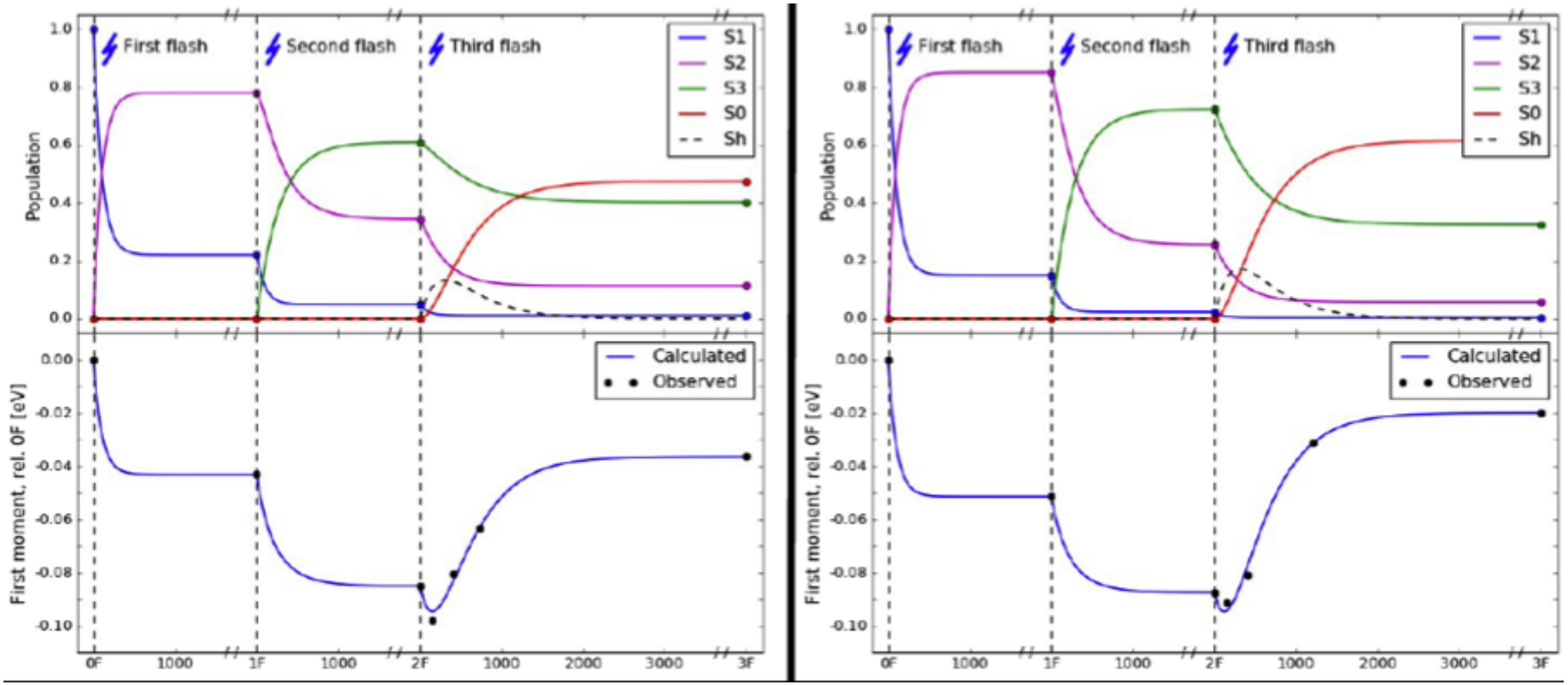
PS II Solution:
~15 minutes of data per diff



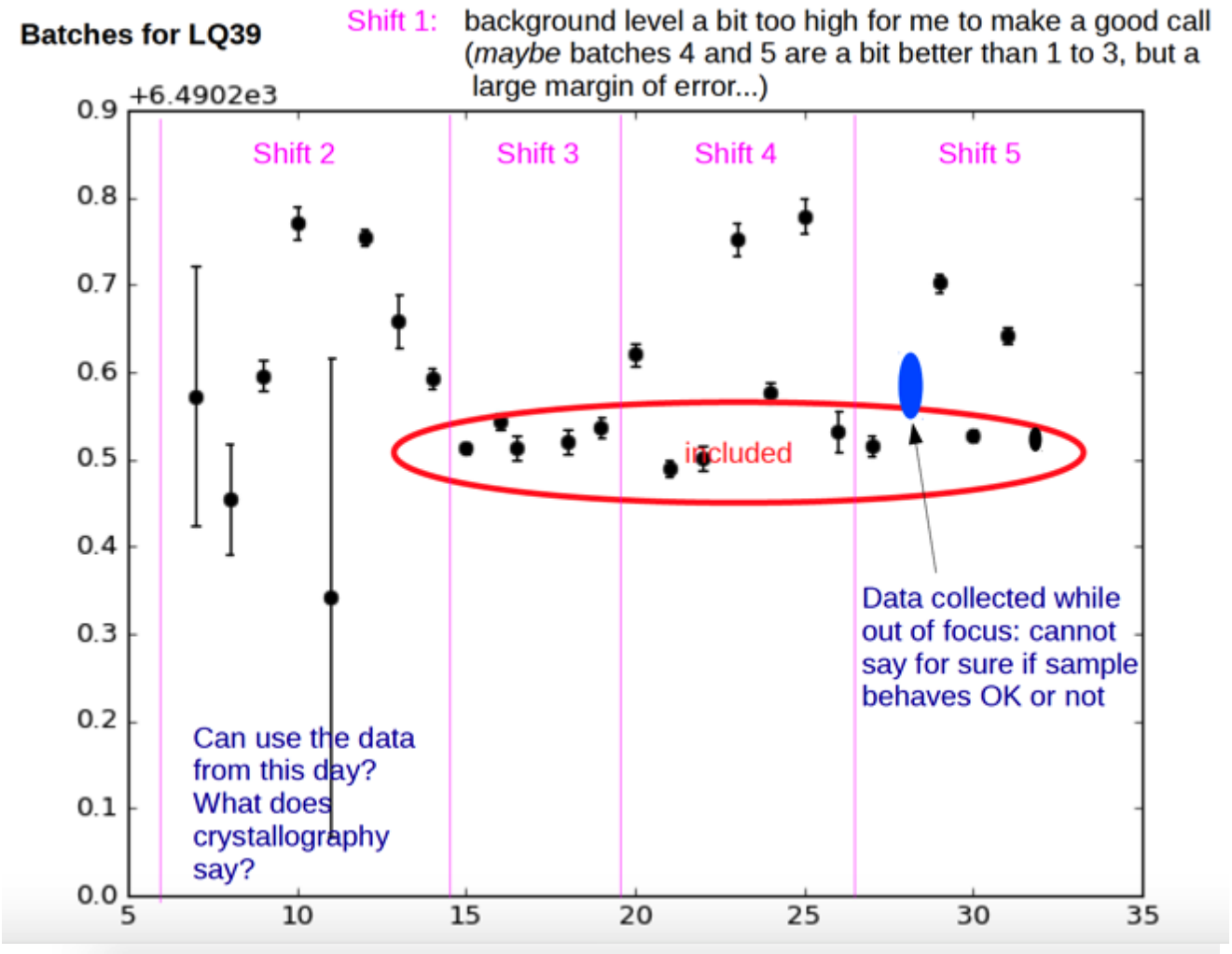
PS II Crystal Slurry:
Many hours of data



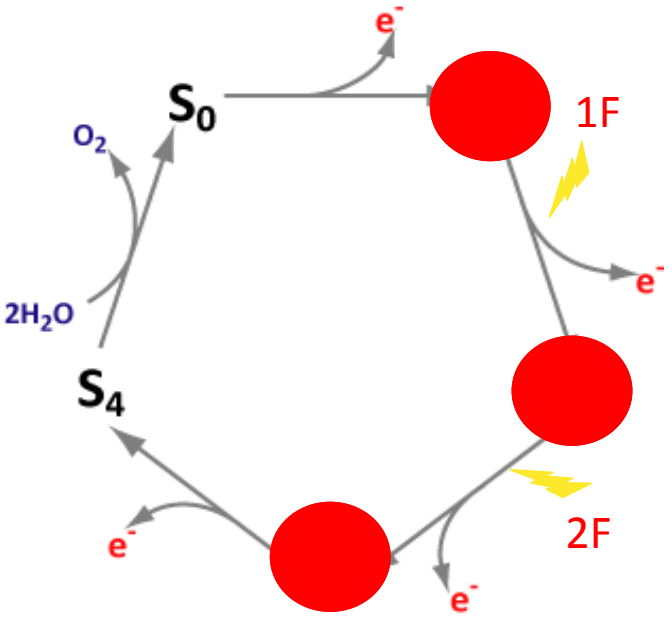
Transient XES of PS II Crystals



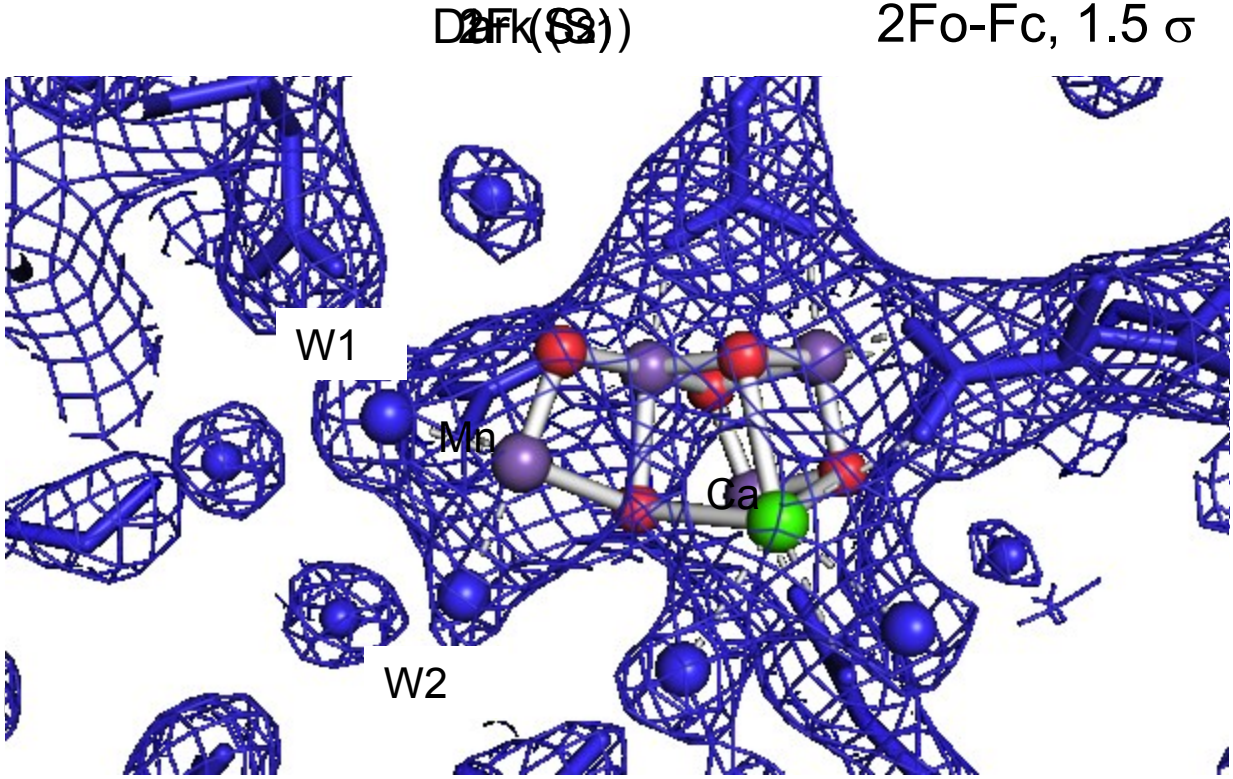
XES can be used to filter XRD



XRD data of 0F, 1F (S_2), and 2F (S_3) at improved Resolution



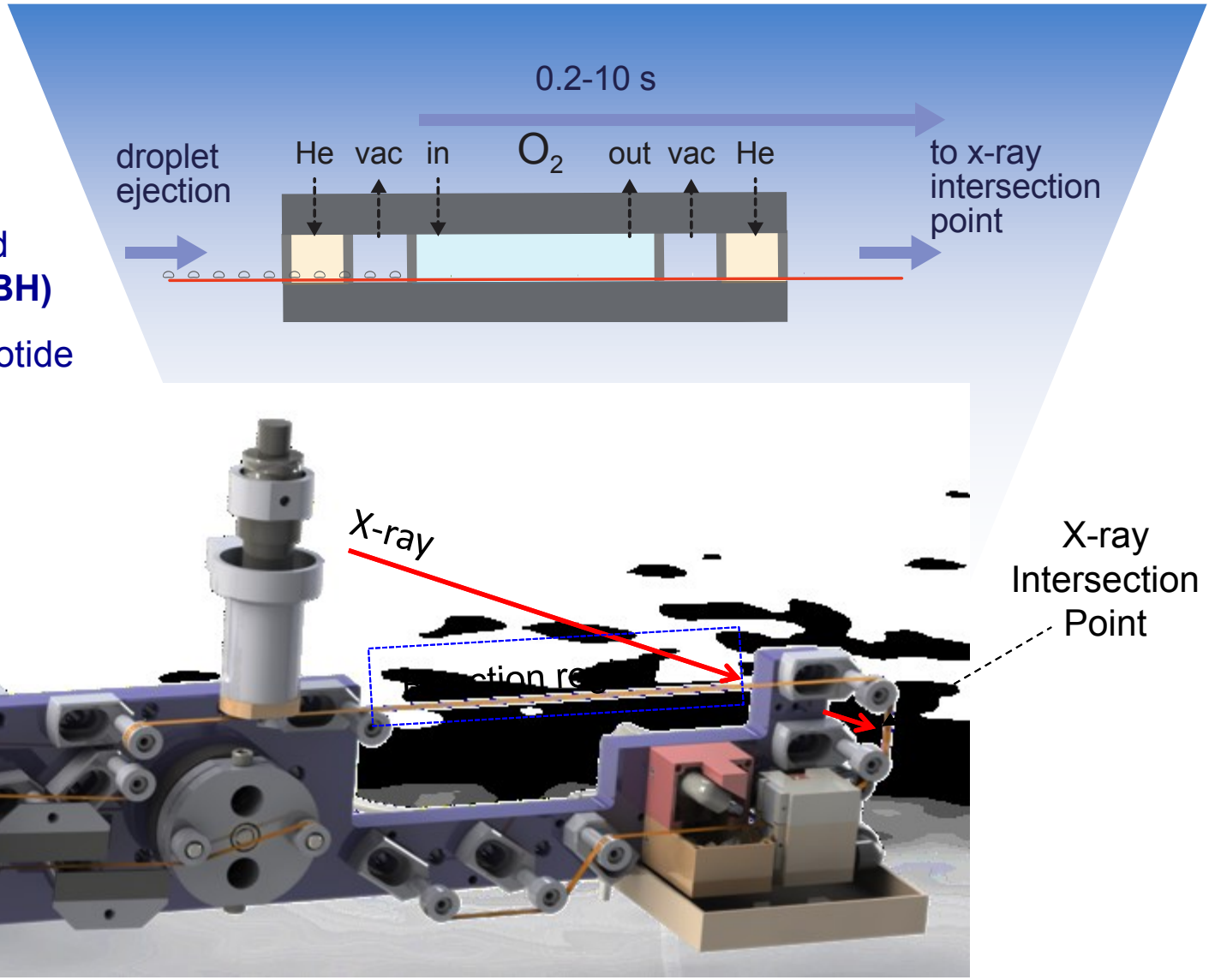
- **High-resolution room temperature structure** of the stable intermediate states **(0F(S_1), 1F(S_2), and 2F (S_3))**, with supporting XES data collected simultaneously to confirm S-state turnover.
- Observation of **changes in atomic positions**.



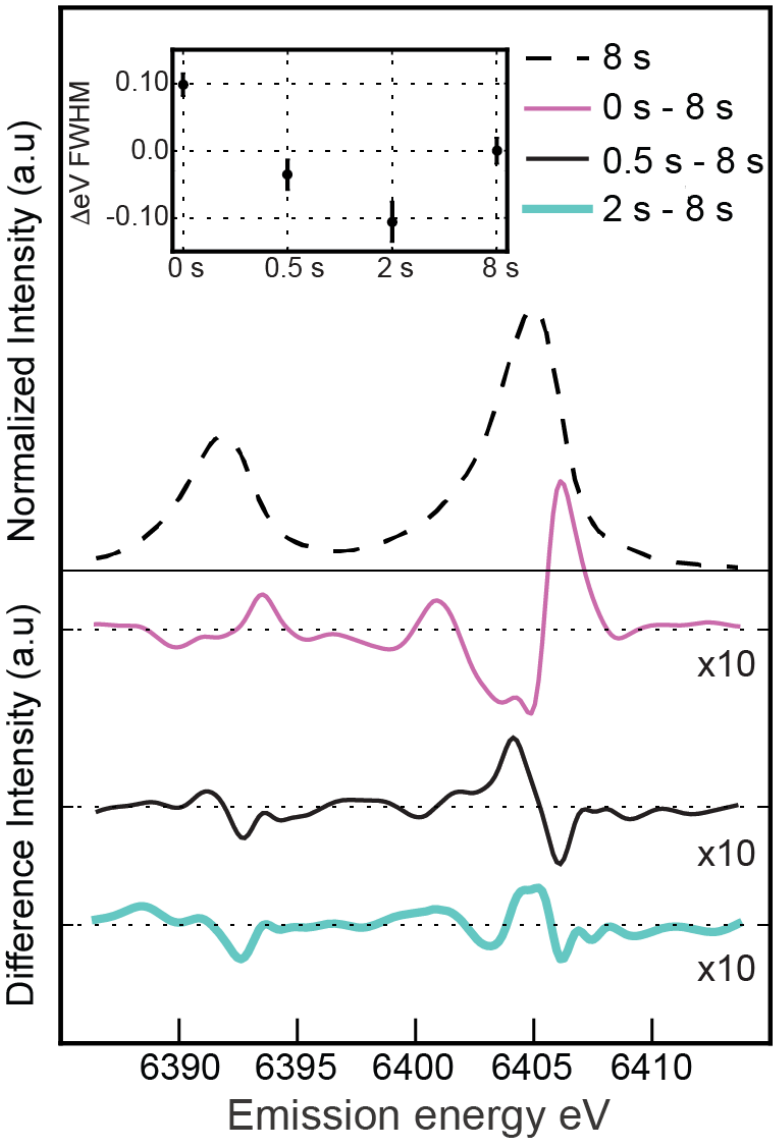
Results: RNR Studies

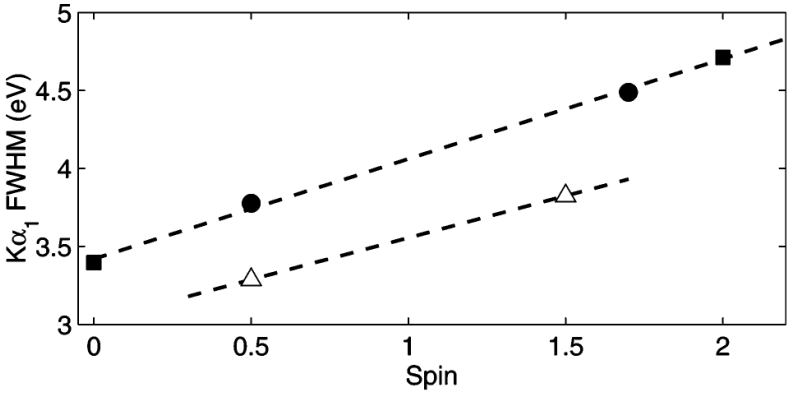
O_2 activation of

- Membrane Bound Hydrogenase **(MBH)**
- Mn/Fe Ribonucleotide reductase **(RNR)**

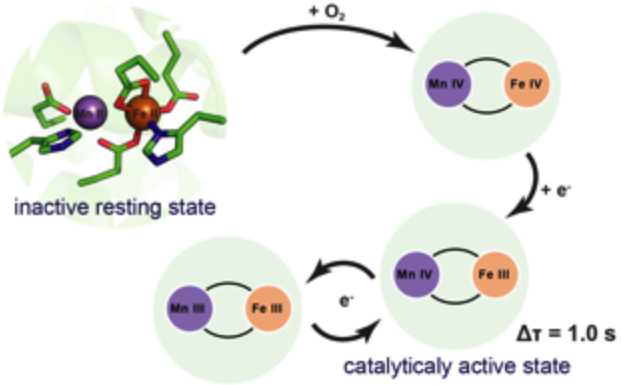


Results: Kα emission of RNR

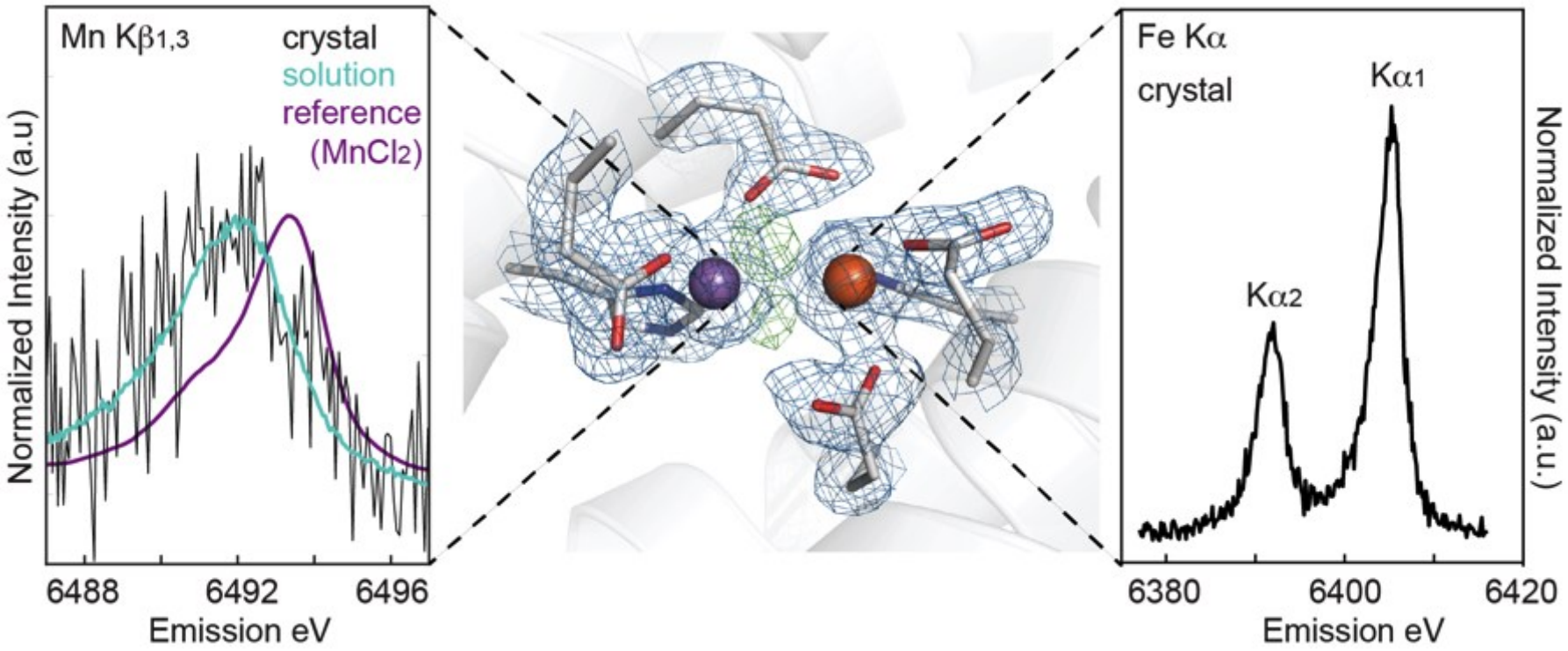




Circle = Fe(III)
Square = Fe(II)
Triangle = Co(II)
ΔeV up → more reduced
ΔeV down → more oxidized
Vanko et al. J. Phys Chem B. (2006)



Results: RNR XRD/Emission



"interim" structure: unit cell variation in crystal prep is causing troubles

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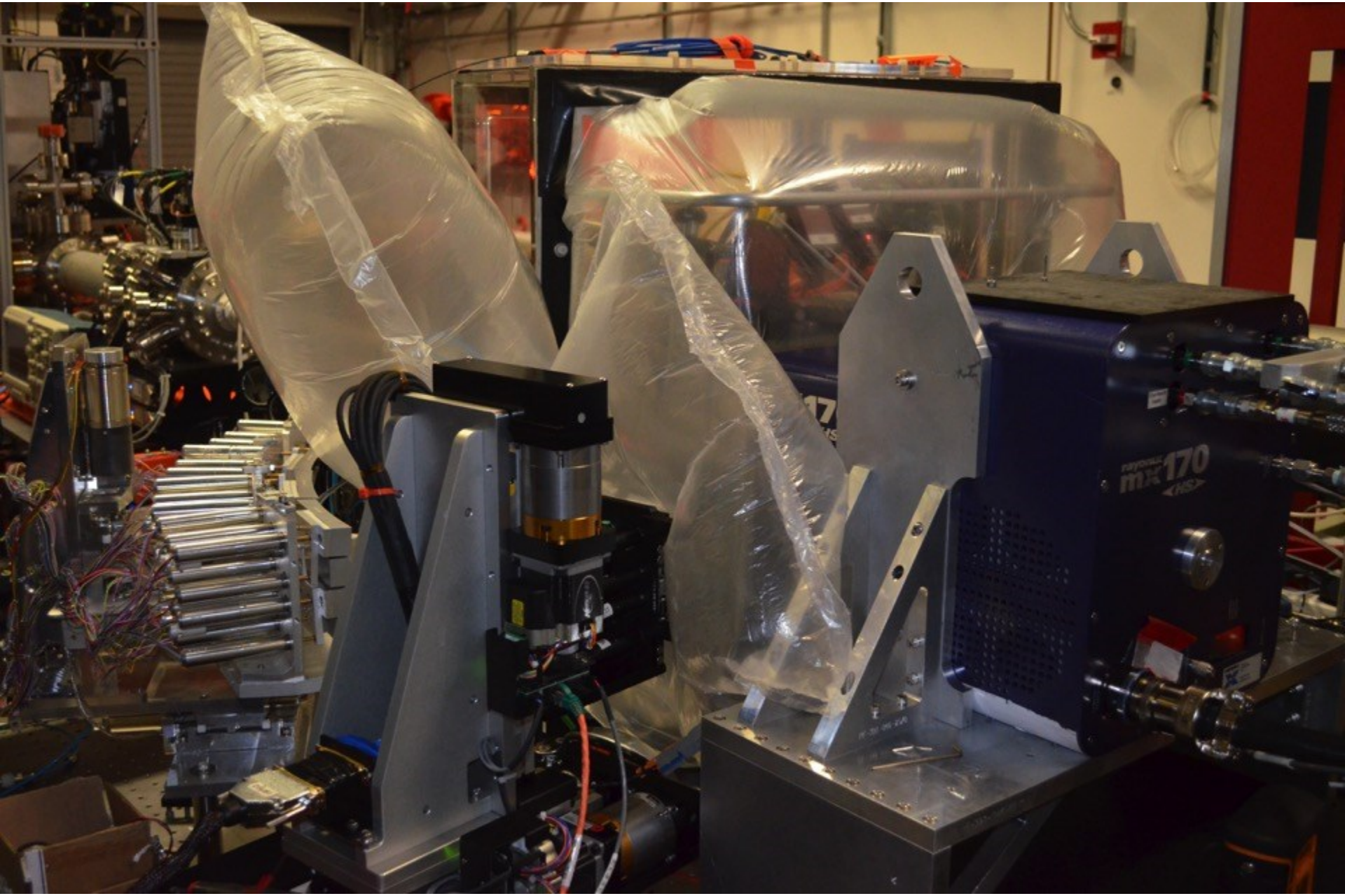
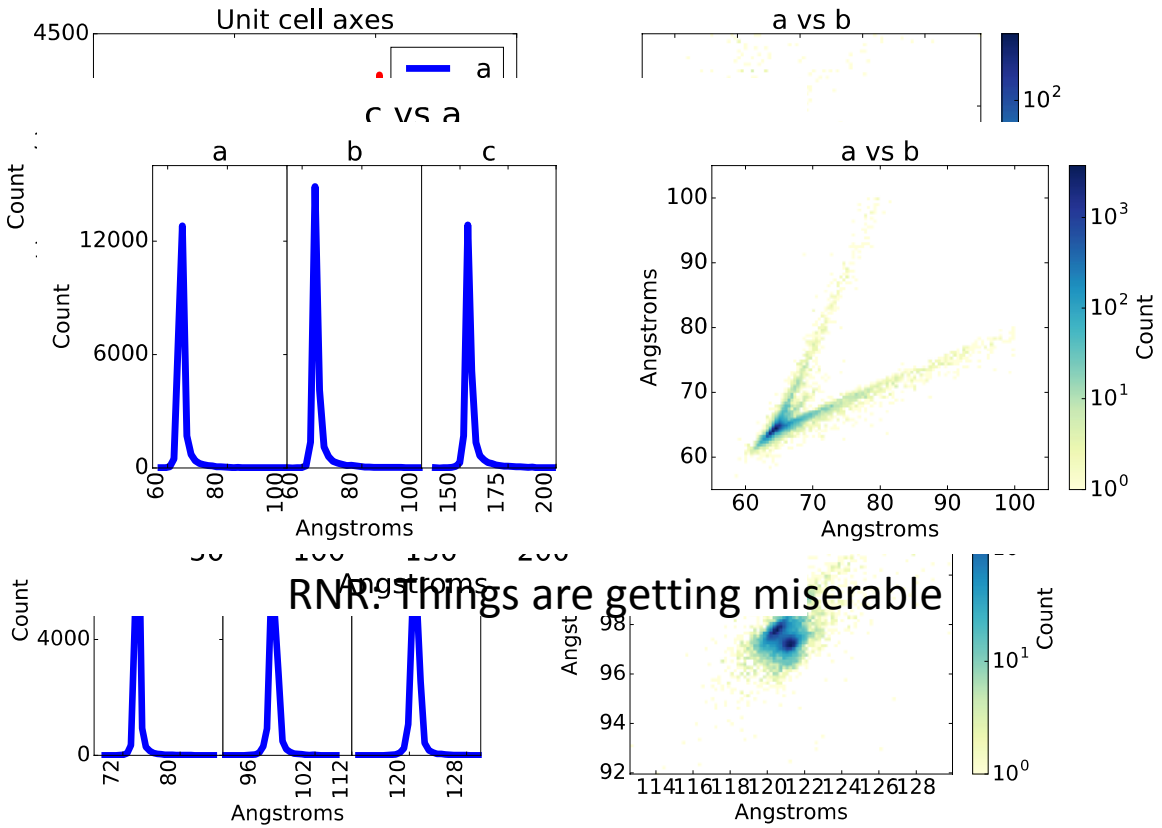
LCLS CXI, XPP, MFX Stanford


mx170

Outlook: Sample inhomogeneity



PAS-GAF (phytochrome)
= Well behaved

; are getting complicated

RNR: Things are getting miserable

MBH = Okay