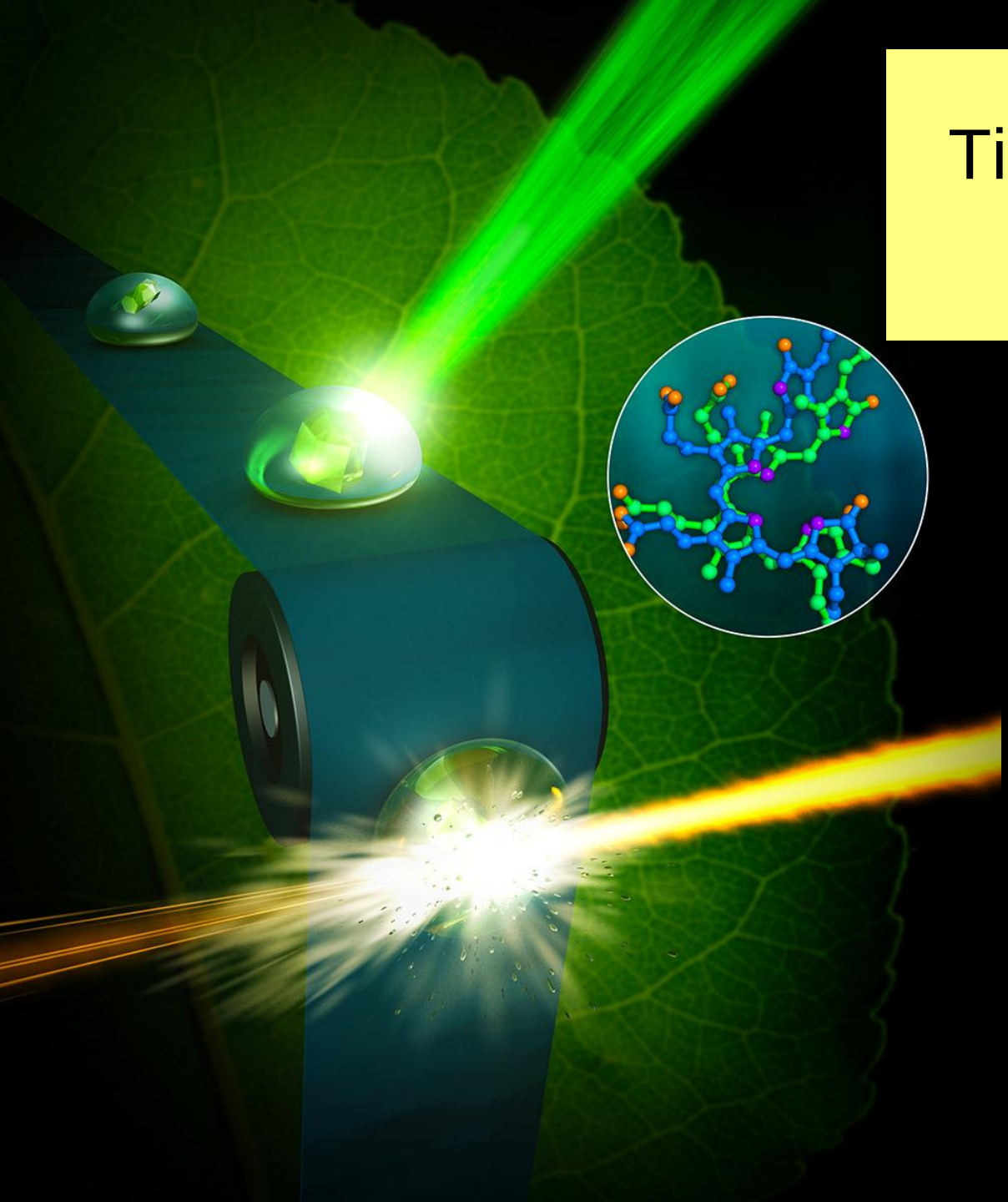

Time-resolved serial MX correlated with emission spectroscopy

Allen M. Orville, Ph.D.

XFEL Hub at Diamond
Diamond Light Source
Harwell Science and Innovation Campus
Didcot, Oxfordshire, OX11 0DE
United Kingdom

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27 – 28 November 2018
XLS | CompactLight User Meeting
CERN, Geneva Switzerland

Some of our collaboration

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Thomas Leissing Ian Clifton
Jos Kamps Anka Lucic
Philipp Bräuer Helen Ginn

Stockholm University

Martin Högbom Hugo Lebrette

Rice University

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Washington University St.

Richard Vierstra **E. Sethe Burgi**

Imperial College London

Jasper van Thor Christopher D

Charité-University Medicine B

Patrick Scheerer Michal Szczepek Andrea Schmidt

PS-II: LBNL, SLAC, BNL, Uppsala Univ., Stanford Univ., UC Berkeley, Helmholtz Zentrum, Humboldt Univ., Umea Univ., Diamond Light Source

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- **U.S. Department of Energy**
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  Chemical Sciences, Geosciences and Biosciences Division
- **Brookhaven National Laboratory, DOE**
  LDRD: 11-008 (Soares)
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Christian G. Roessler Matt Cowan Annie Héroux
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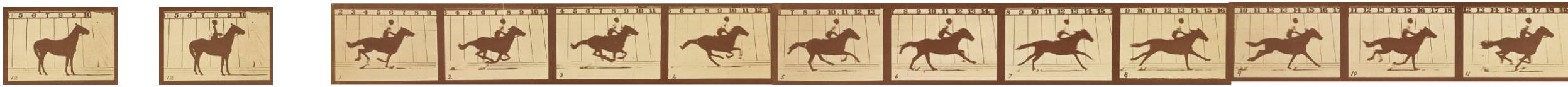
Sergio Carbajo Mark Hunter

Silke Nelson
Mengning Liang

LCLS SACLA European XFEL CFEL SCIENCE BioXFEL

Dynamic Structural Biology

$$E + S_1 \rightleftarrows E{\bullet}S_1 + S_2 \rightleftarrows E{\bullet}S_1{\bullet}S_2 \rightarrow [\,I_1 \rightarrow I_2 \rightarrow I_3 \rightarrow \ldots] \rightarrow E{\bullet}P \rightleftarrows E + P$$



Traditional MX: synchrotrons, macro-crystals, 100 K, **E, E•S$_1$, E•P**; lacks function & dynamics: >90% structures PDB/year

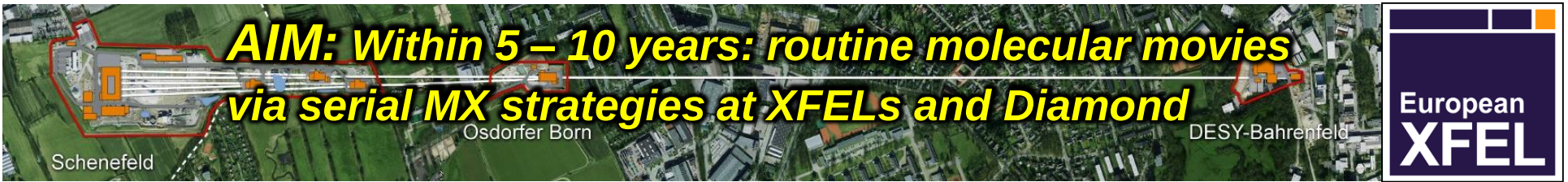
Cryo-EM: important, complements / benefits from MX, low Temp, class averages, limited dynamics, no spectroscopy

Serial MX at XFELs & Diamond to study entire reaction cycles at room temp & pressure (SFX & SMX)

- XFEL fs pulse ≈ bond vibrations
- DLS/VMXi ≈ µs time resolution
- µ-crystal slurries ≈ atomic & electronic data
- E•S equil. µs – ms; Enz. turnover ~60 ms

Entering an era of *dynamic* structural biology… a concept, a set of tools, to collect as much data as possible from every sample and X-ray pulse, enables atomic resolution "movies" of macromolecules engaged in catalysis

Allen M. Orville
BMC Biology (2018) 16:55

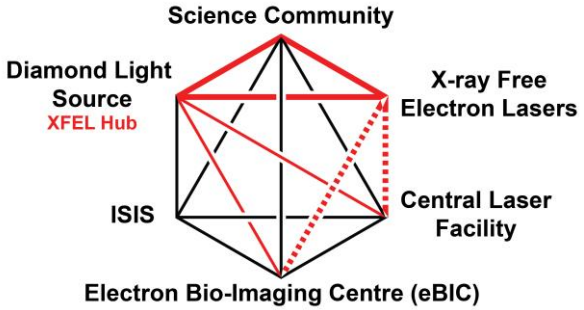


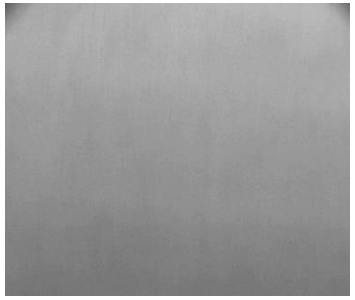
European XFEL

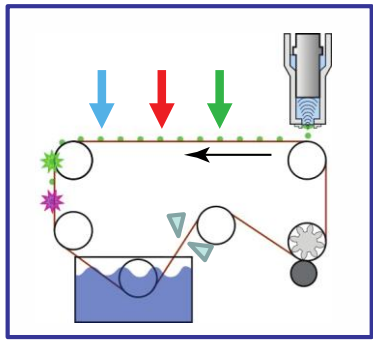
Post-doc positions available

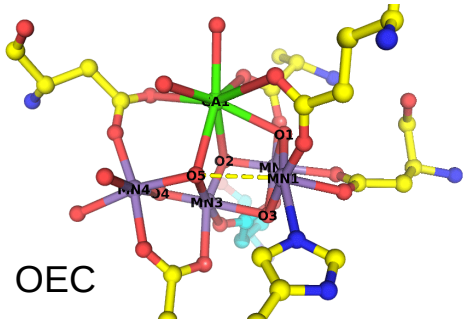
Outline & discussion points

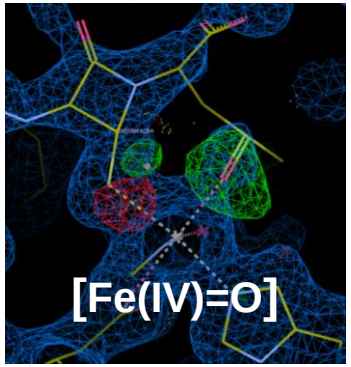
1	**What is the XFEL Hub at Diamond?**
2	**XFEL / SFX / sample delivery, on-demand acoustic injectors**
3	**Science drivers of time-resolved structural biology:** **PS II, RNR, IPNS, and/or Phytochromes, etc**



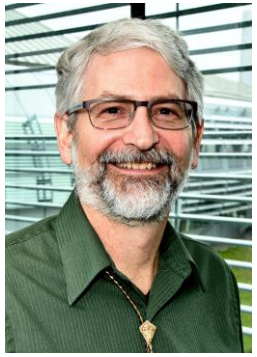








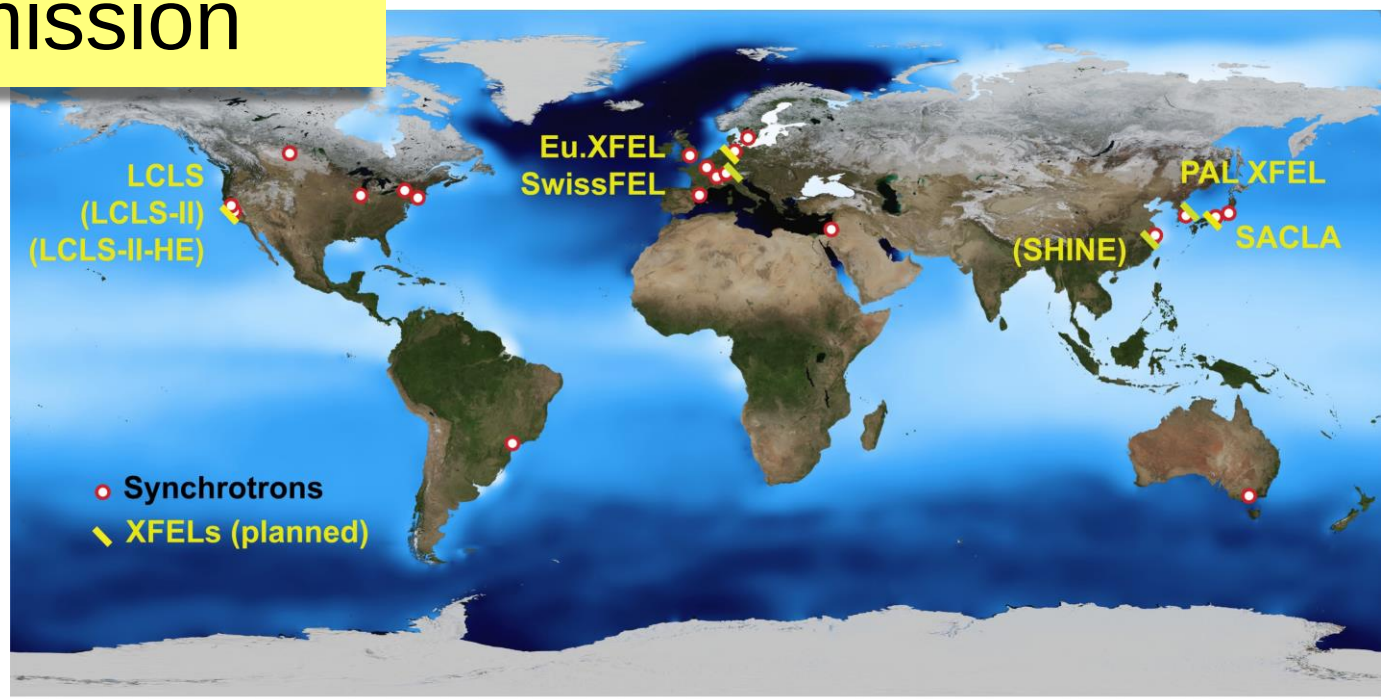
The XFEL Hub team & mission



Allen M. Orville

Pierre Aller

Agata Butryn



XFEL Hub helps facilitate

- (time-resolved) serial structural biology experiments via sample preparation, delivery, data collection, and processing
- the transfer of methods between XFEL, synchrotron, and/or cryo-EM sources
- access to, and data collection from complementary facilities (DLS, CLF, eBIC)

New BAG at Diamond

Dynamic Structural Biology at Diamond & XFELs

- I24 & VMXi with fixed targets, LCP / viscous media injector, on-demand acoustic injectors
- Pump-probe and/or mixing strategies for time resolved studies

Relationships between the Hub, sponsors & community

Wellcome, BBSRC & MRC (Naismith et al)

Diamond Light Source

XFEL Hub

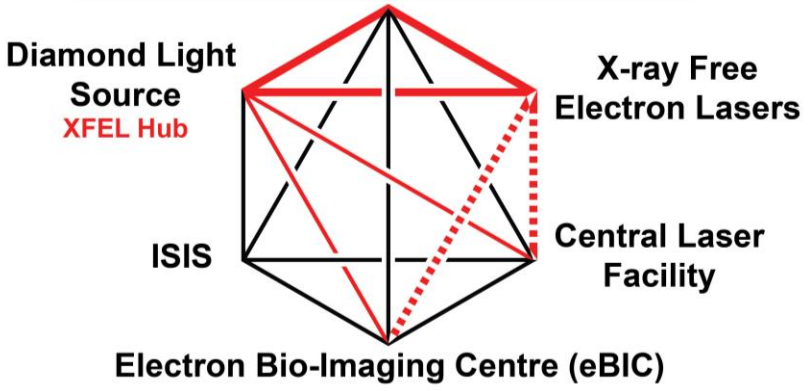
Wellcome Investigator (Orville)

Diamond Light Source

Science Community

Research & Development

- RFI, RCaH, Diamond, XFELs
- Time-resolved serial MX
- IPNS & β-lactamases
- Reactivity in microcrystals
- X-ray emission spectroscopy
- Microfluidics for microcrystals
- On-demand acoustic injector(s)



Service & Training

- Travel assistance UK scientists
- Diamond BAG(s)
- Serial MX processing pipelines

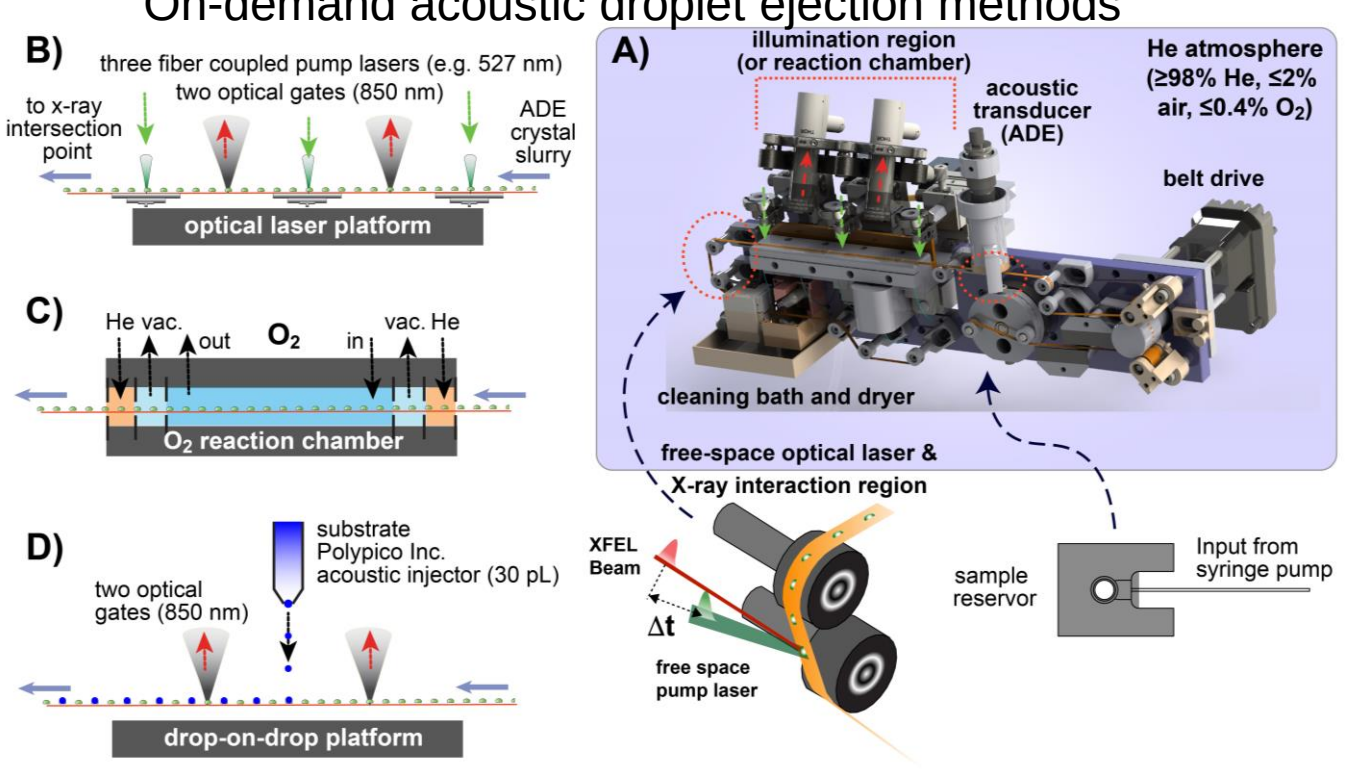
Research & Development

- (tr-)cryoEM grid prep methods
- Serial MX at VMXi ± tr-XES
- Data processing pipeline(s)
- Diamond/CLF PORTO project
- Micro-spectroscopy ± MX
- Novel sample delivery methods

Summary of XFEL beamtime awarded

- AMO moved to Diamond Oct 2015
- 36 beamtime awards w/AMO
- Serial femtosecond crystallography (SFX) data at LCLS, SACLA, and European XFEL
- Most experiments used acoustic tape drive at LCLS/MFX instrument
- Many time-resolved SFX ± time-resolved X-ray emission spectroscopy
- Pump-probe and mixing strategies for time-resolved work
- Currently preparing for five XFEL experiments (two at LCLS and three at SACLA)

On-demand acoustic droplet ejection methods



- Proposals pending at SACLA, Eu.XFEL, SwissFEL and PAL-XFEL
- Many metal-dependent enzymes including: photosystem II, Fe-dependent enzymes that cleave O_2 to create Fe(IV)=O intermediates
- Light sensor systems that modulate activity based upon illumination status
- Enzymes that degrade β-lactam antibiotics

XFEL Hub impact on the UK user community

	UK scientists on expt.	UK scientists XFEL site visits	Hub scientists site visit
2015	3	3	3
2016	25	21	19
2017	70	49	22
2018 *	95	59	34
Total 2015 - 2018	193	132	78

LCLS, SACLA & Eu.XFEL experiments, XFEL Hub involved

Collaboration group	PhDs in group	Publications per year				
		2014	2015	2016	2017	2018 *
BioXFEL, USA	**> 50**	**115**	**148**	**110**	**86**	**43**
CFEL, Germany	**> 50**	**85**	**101**	**48**	**210**	**90**
XFEL Hub at Diamond	**~ 3 – 10**	**4**	**8**	**12**	**14**	**10**

nature International journal of science

Kern et al.,
"Structures of the intermediates of Kok's photosynthetic water oxidation clock"
Nature (**2018**) 563: 421-435

nature COMMUNICATIONS

Wiedorn *et al.*,
"Megahertz serial crystallography"
Nature Communications (**2018**) 9, 4025

nature methods

Fuller et al.,
"Drop-on-demand sample delivery for studying biocatalysts in action at X-ray free-electron lasers"
Nature Methods (**2017**) 14, 443-449

nature COMMUNICATIONS

Roedig et al.,
"High-speed fixed-target serial virus crystallography"
Nature Methods (2017) 14, 805-810

nature International journal of science

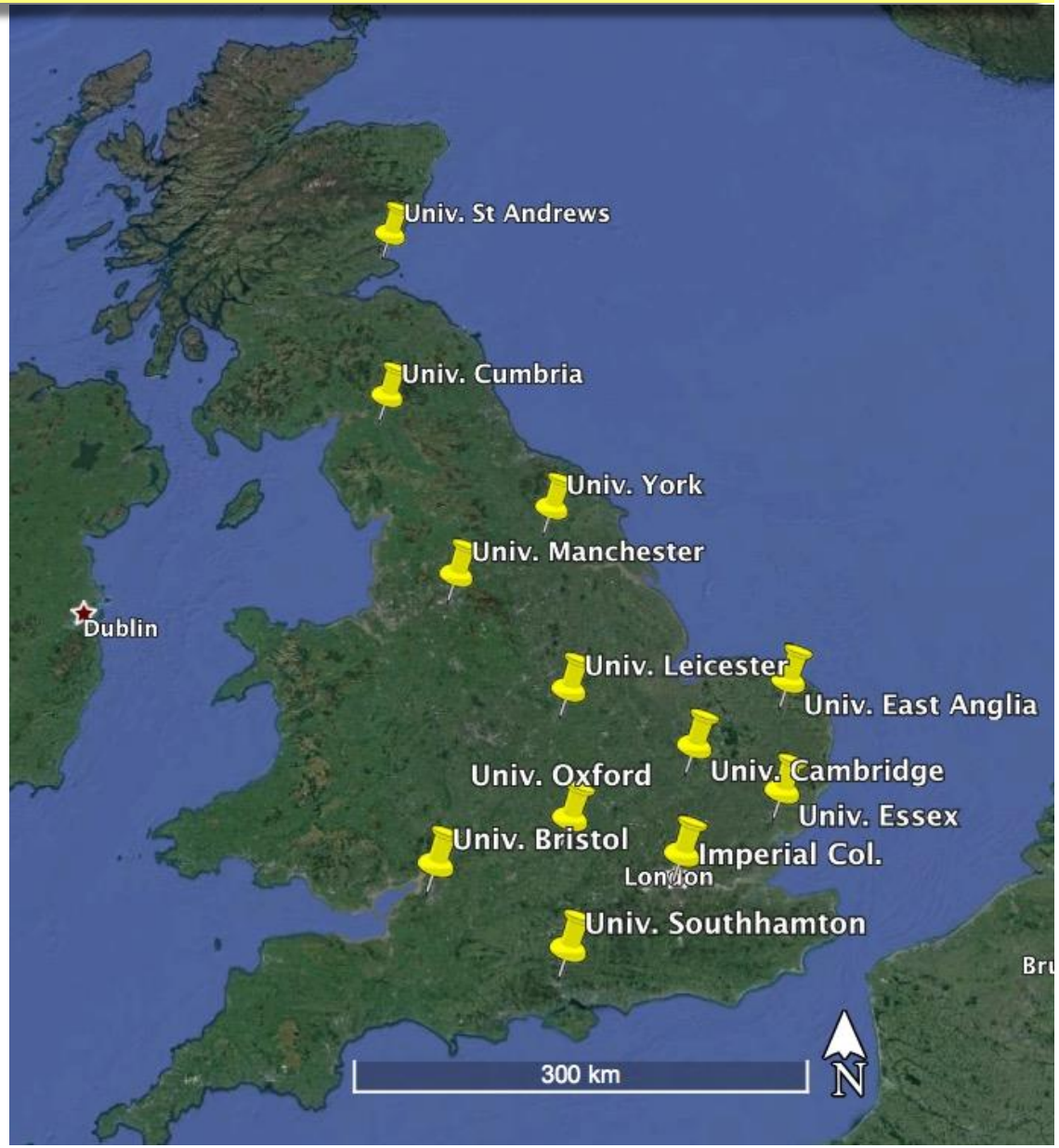
Young et al.,
"Structure of photosystem II and substrate binding at room temperature"
Nature (**2016**) *540*, 453-457

Structure

Roessler et al.,
"Acoustic Injectors for Drop-On-Demand Serial Femtosecond Crystallography"
Structure (**2016**) *24*, 631-640

Some XFEL Hub interactions with UK scientists

- Travel assistance, UK scientists w/ XFEL beamtime
- Non-proprietary R&D
- Apply online; light-touch review by Prof. Jim Naismith
- Typically ~ 4 flight tickets covered, *etc.*
- To date: > 60 UK scientist site-visits (Diamond (I24 and I23), Oxford University (3 different groups), RCaH, Essex University, Imperial College London, University of East Anglia, University of Bristol and University of Leicester)
- To date: > 40 site-visits by XFEL Hub staff
- Average 30 travel awards / year for 2-3 facilities
- Travel to LCLS, SACLA, and European XFEL
- Anticipate increase in demand w/ more facilities



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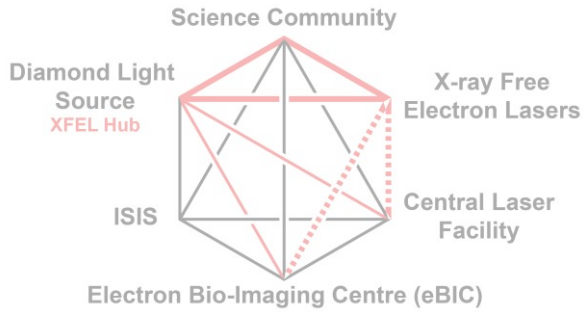
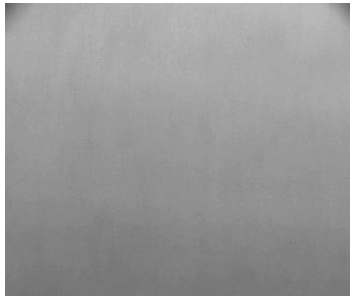
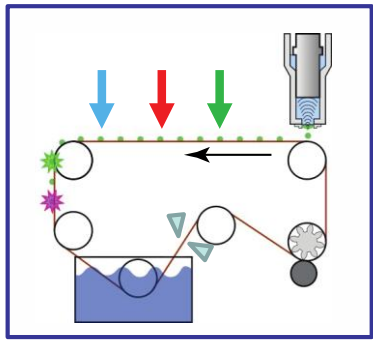
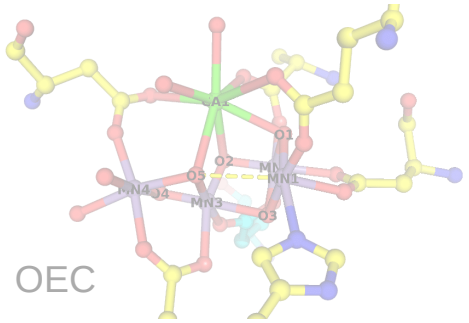
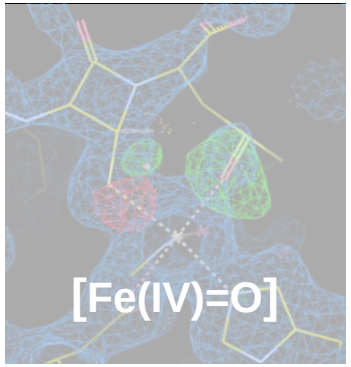


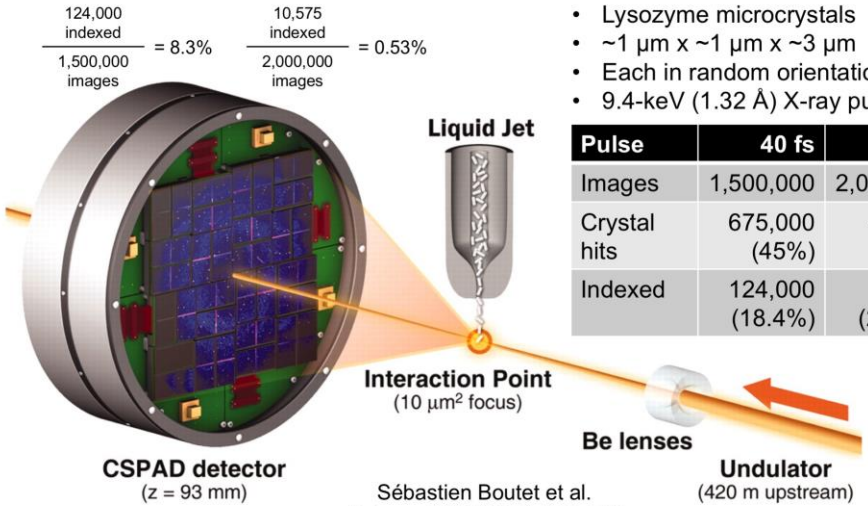








The first high-resolution protein structure determination by SFX methods

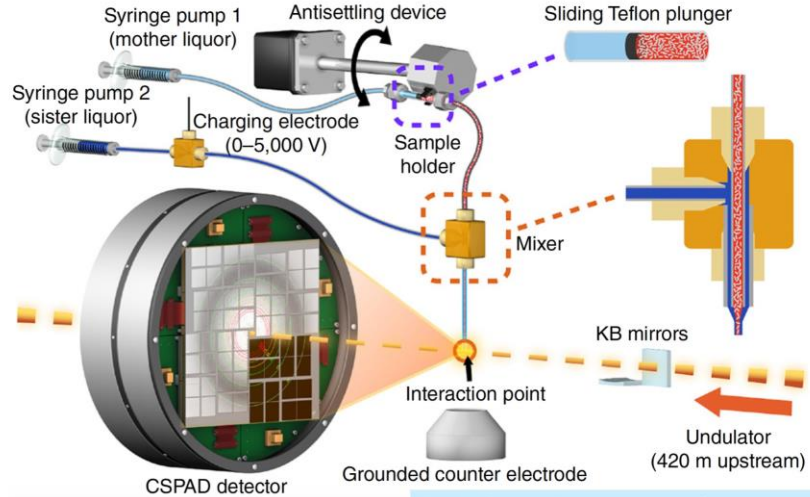


- Lysozyme microcrystals
- ~1 μm x ~1 μm x ~3 μm
- Each in random orientation
- 9.4-keV (1.32 Å) X-ray pulses

Pulse	40 fs	5 fs
Images	1,500,000	2,000,000
Crystal hits	675,000 (45%)	40,000 (2%)
Indexed	124,000 (18.4%)	10,575 (26.3%)

Sébastien Boutet et al.
Science (2012) **337**: 362-364

Concentric-flow MESH injector



Sierra *et al* (2016) *Nat Methods* **13**, 59-62

Microfluidic **E**lectrokinetic **S**ample **H**older (MESH) operates at low flow rates by applying voltage rather than pressure

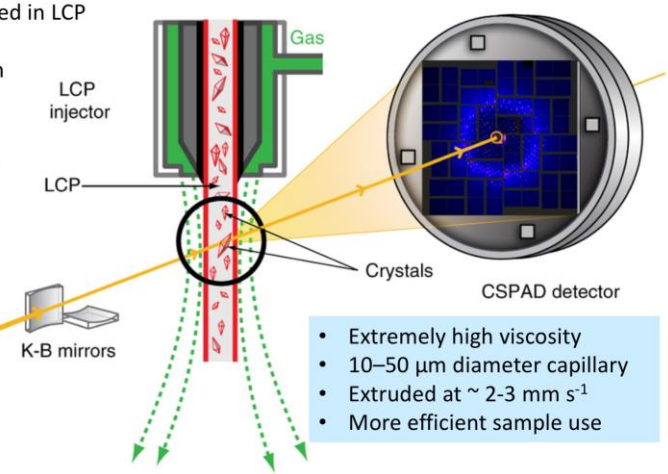
Lipidic cubic phase injectors/extruders: membrane protein serial femtosecond MX

Liu et al (2013) *Science* 342, 1521-1524; Liu et al (2014) *Nature Protocols* 9, 2123

- 5-HT_{2B} receptor microcrystals
- 300 μg protein crystallized in LCP
- ~5 μm x ~5 μm x ~5 μm
- ~1.5-μm-diameter beam
- 9.4-keV x-ray pulses

Pulse	50 fs
Images	4,217,508
Crystal hits	152,651 (3.6%)
Indexed	32,819 (21.5%)

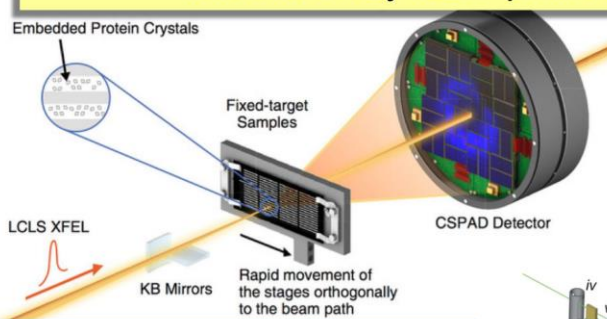
32,819 indexed / 4,217,508 images = 0.78%



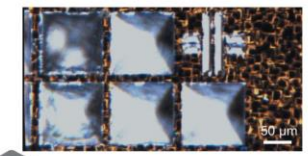
- Extremely high viscosity
- 10–50 μm diameter capillary
- Extruded at ~ 2-3 mm s^{-1}
- More efficient sample use

Weierstall (*Phil. Trans. R. Soc. B* 2014 **369**, 20130337)

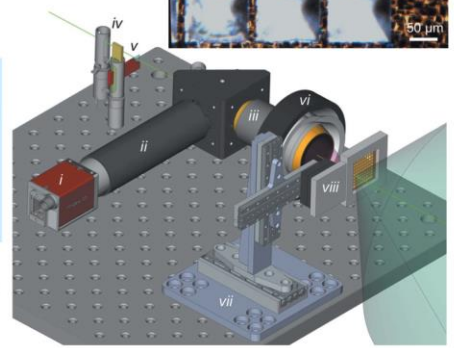
Fixed-target approaches physically move samples into the X-ray laser pulse stream



Hunter et al (2014) *Scientific Reports* | 4 : 6026
Sherrell et al (2015) *J Synch Rad* 22, 1372–1378
Roedig et al (2015) *Scientific Reports* | 5:10451
Roedig et al (2016) *J Appl Crytst* 49, 968–975
Oghbaey et al (2016) *Acta Cryst* D72, 944–955
Owen *et al* (2017) *Acta Cryst* D73, 373–378

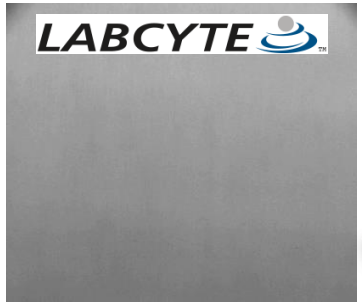


- Data acquisition rates are detector driven at LCLS, SACLA, Diamond…
- Hit rate of ~100%
- Scarce samples, “easy” to load
- Room temp (& cryo too)
- Pump-probe, SFX/SMX ± spectroscopy

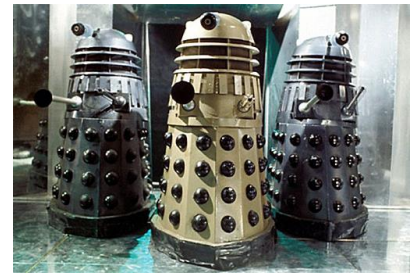


several chip & well sizes
fast controller & software

high-precision stages
high-quality viewing



Potential of drop on demand, acoustic injector methods at XFELs



LCLS total X-rays / s

120 Hz x 50×10^{-15} s

= 6 ps with X-rays

(167×10^9 more dark time)

Billion-fold potential efficiency gain

European XFEL total X-rays / s

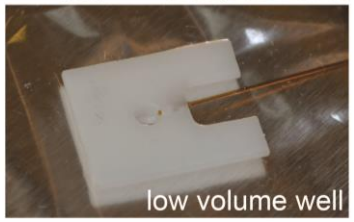
27,000 Hz x 50×10^{-15} s

= 1.4 ns with X-rays

(740×10^6 more dark time)

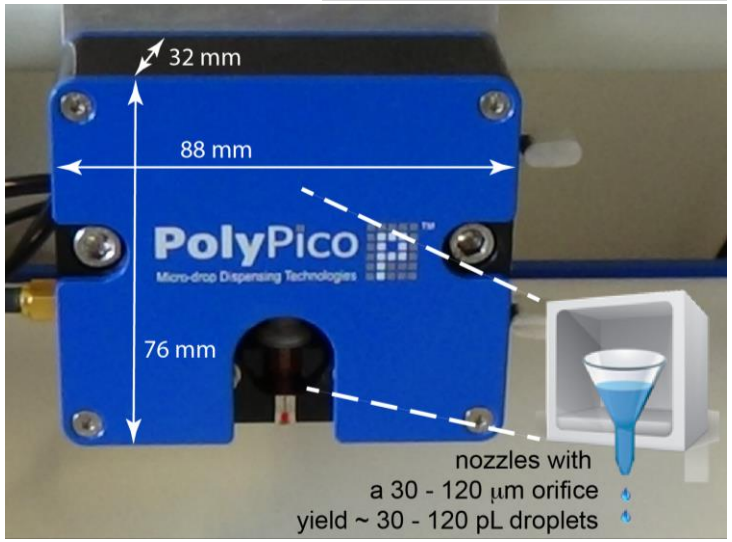
Million-fold potential efficiency gain

ADE system



Some Characteristics of Acoustic Droplet Ejection (ADE)

Transducer frequency (MHz)	~ Droplet volume (pL)	~ Droplet diameter (µm)	~ Crystal size within drop (µm³)
11.5	2500	170	10 – 100
15	1000	125	10 – 75
25	100	60	10 – 40
50	10	25	up to15
75	5	15	up to 5



B)

three fiber coupled pump lasers (e.g. 527 nm)

two optical gates (850 nm)

to x-ray intersection point

ADE crystal slurry

optical laser platform

2016 - 2018

C)

He vac. out

O_2 in

vac. He

O_2 reaction chamber

D)

2019

substrate Polypico Inc. acoustic injector (30 pL)

two optical gates (850 nm)

drop-on-drop platform

A)

illumination region (or reaction chamber)

acoustic transducer (ADE)

He atmosphere (≥98% He, ≤2% air, ≤0.4% O_2)

belt drive

cleaning bath and dryer

free-space optical laser & X-ray interaction region

XFEL Beam

Δt

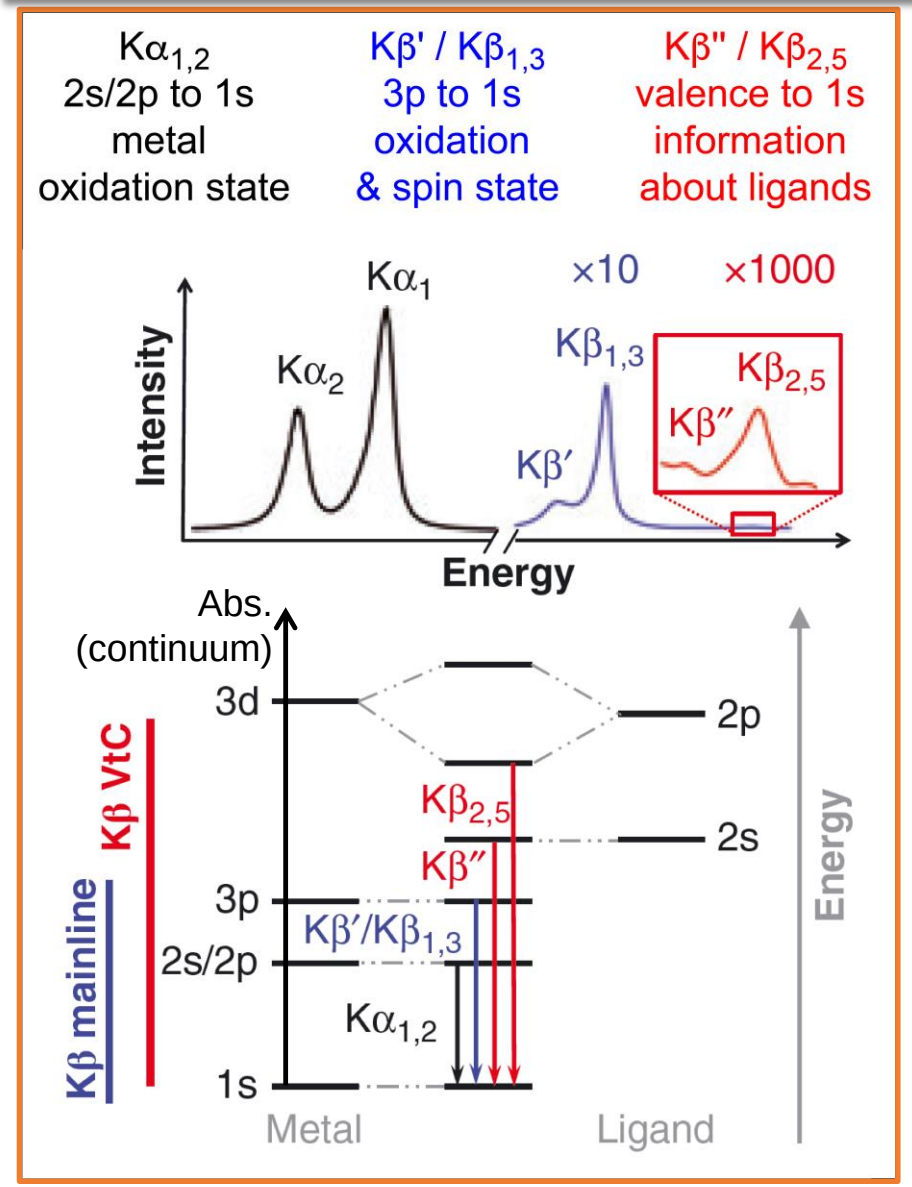
free space pump laser

sample reservor

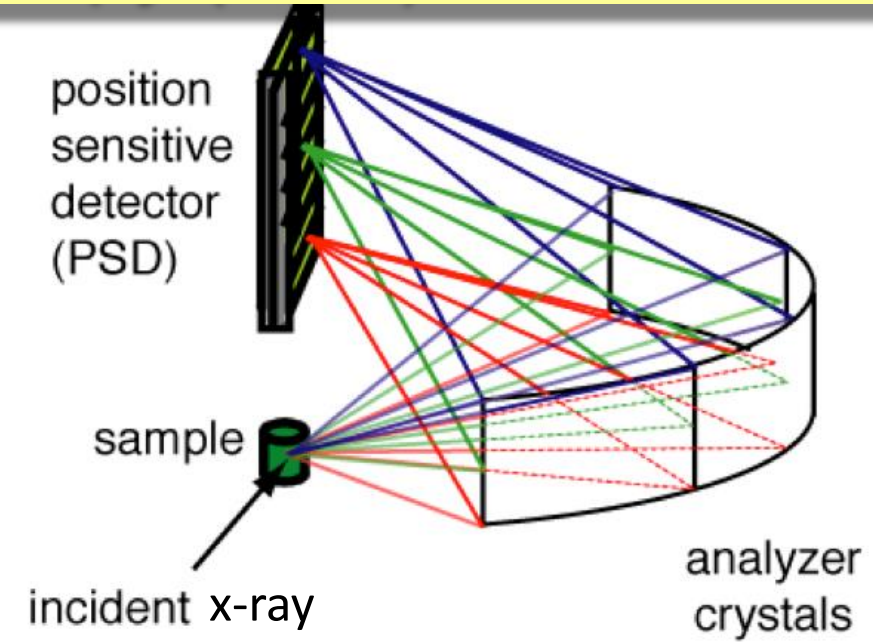
Input from syringe pump

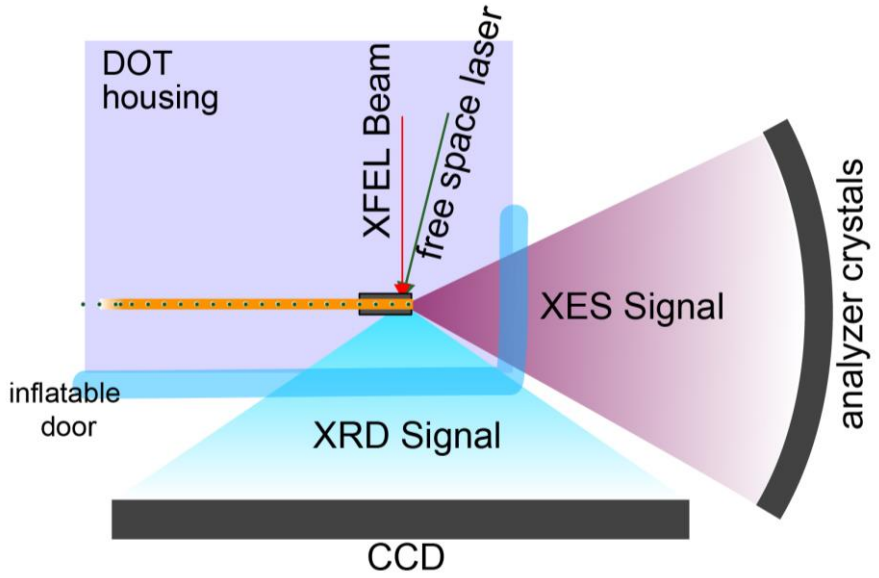
Kern et al (2018) *Nature* 563: 421-435
Fuller and Gul et al (2017) *Nature Methods* 14: 443-449
Young, Ibrahim, Chatterjee et al (2016) *Nature* 540, 453–457

X-ray emission spectroscopy (XES) ± tr-SFX



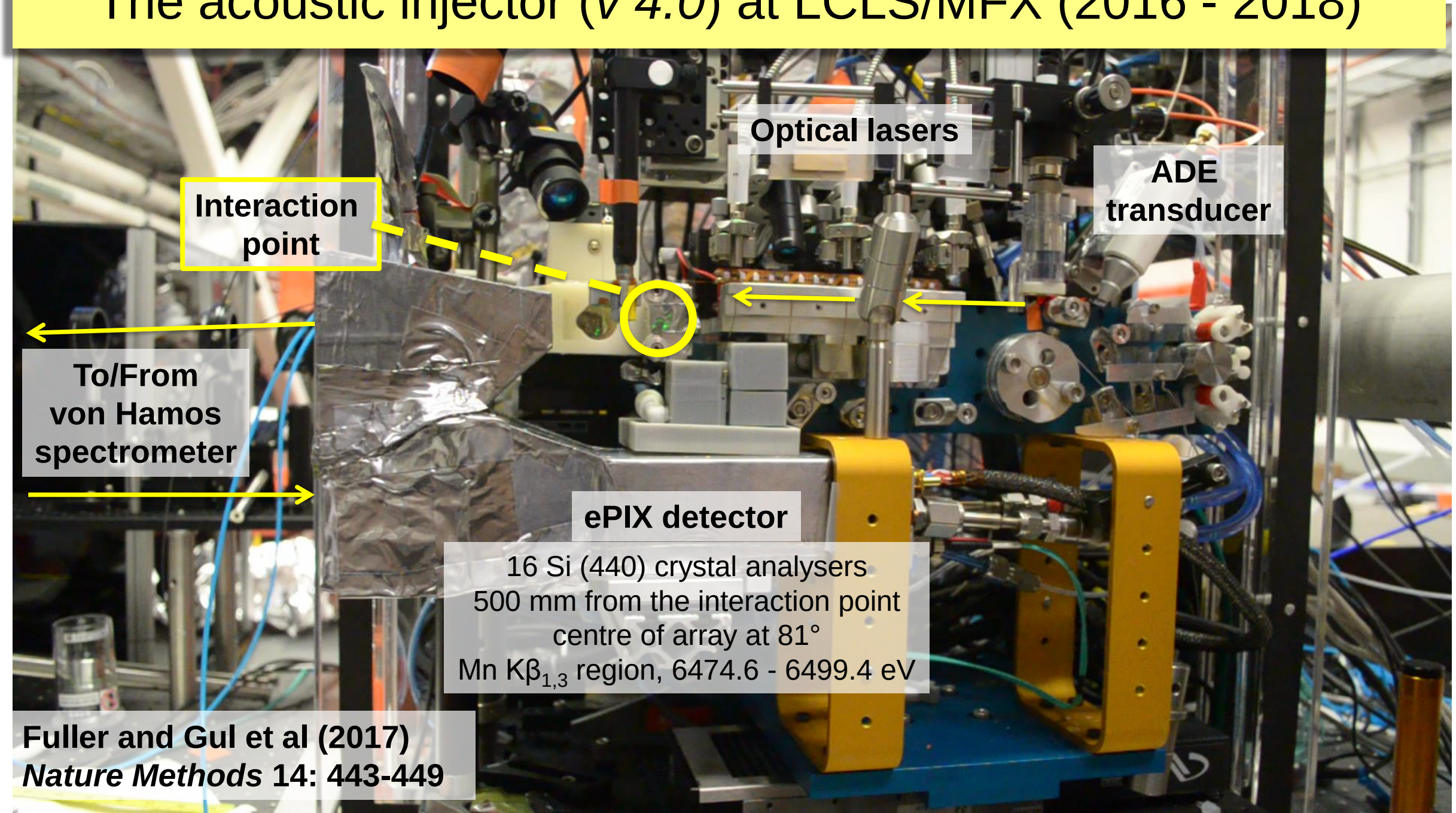
DeBeer & Bergmann (2016)
Encyclopedia of Inorganic and Bioinorganic Chemistry





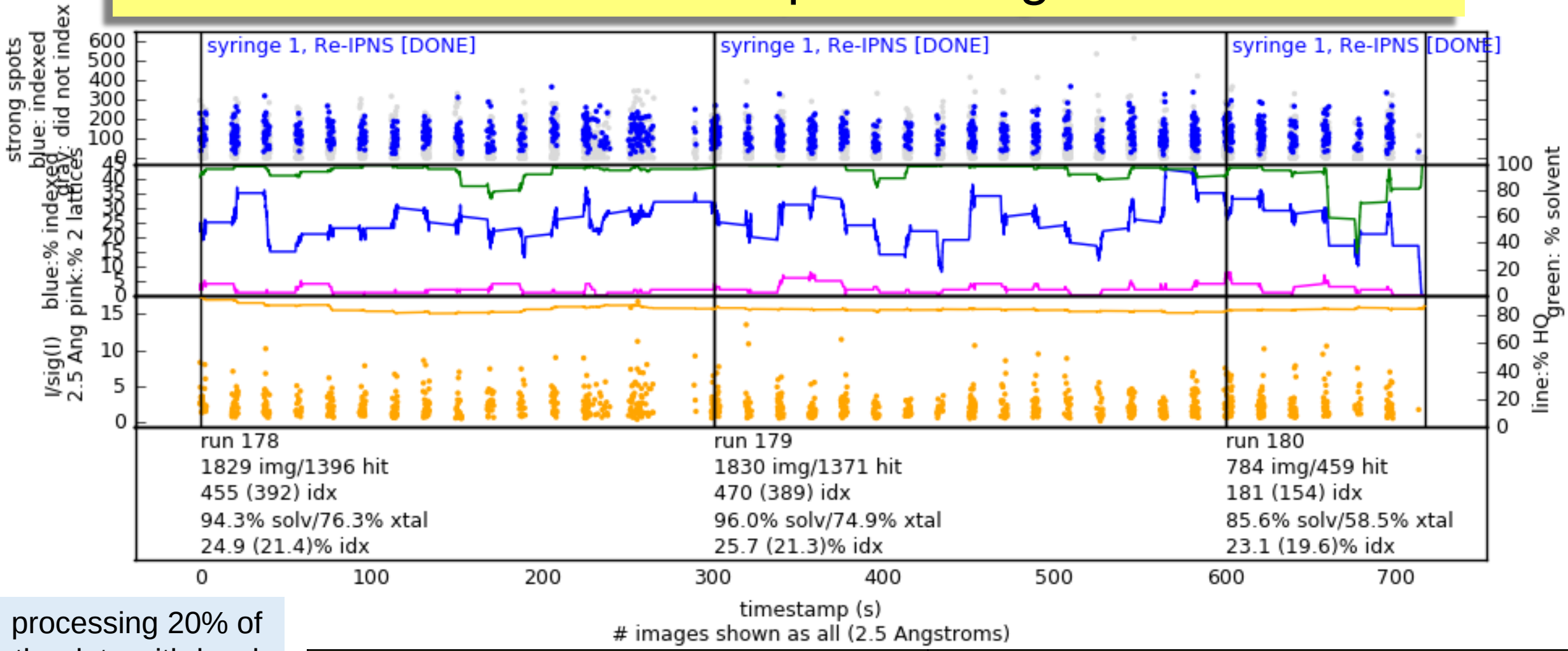
Fuller and Gul et al (2017) *Nature Methods* 14: 443-449

The acoustic injector (*v 4.0*) at LCLS/MFX (2016 - 2018)



Fuller and Gul et al (2017)
***Nature Methods* 14: 443-449**

30 Hz ≈ real time data processing ≈ decisions



processing 20% of the data with local LCLS compute cluster; full processing at NERSC

Are X-rays hitting drops?	✓ green line	Do the crystals index well?	✓ blue & gray dots ✓ blue line
Do the drops contain crystals? Two or more lattices / drop?	✓ blue dots ✓ blue line ✓ pink line	Are crystals of high quality?	✓ red & yellow dots ✓ yellow line

indexed images	25,000					
hit ratio = 5%		image rate	time to collect full SFX dataset			
facility	detector	Hz	seconds	minutes	hours	days
LCLS	CSPAD	120	4167	69.4	1.2	0.0
LCLS	Rayonix	10	50000	833.3	13.9	0.6
LCLS	Rayonix	30	16667	277.8	4.6	0.2
SACLA / PAL-XFEL	MPCCD	60	8333	138.9	2.3	0.1
SwissFEL	Jungfrau	100	5000	83.3	1.4	0.1
Eu.XFEL	Jungfrau *	160	3125	52.1	0.9	0.0
Diamond VMXi	Eiger2	500	1000	16.7	0.3	0.0
Eu.XFEL	AGPID	3,250	154	2.6	0.0	0.0
LCLS-II-HE	ePIX *	10,000	50	0.8	0.0	0.0
SHINE	tbd *	17,000	29	0.5	0.0	0.0

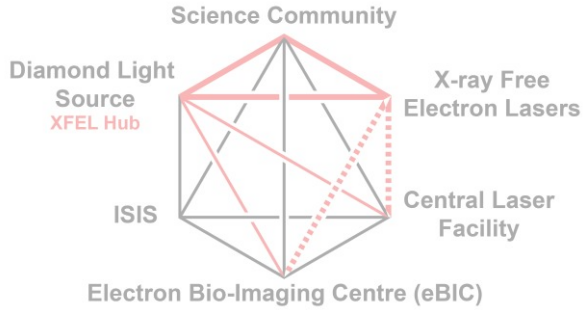
indexed images	25,000				
hit ratio = 80%		image rate	time to collect full SFX dataset		
facility	detector	Hz	seconds	mintues	hours
LCLS	CSPAD	120	260	4.3	0.1
LCLS	Rayonix	10	3125	52.1	0.9
LCLS	Rayonix	30	1042	17.4	0.3
SACLA / PAL-XFEL	MPCCD	60	521	8.7	0.1
SwissFEL	Jungfrau	100	313	5.2	0.1
Eu.XFEL	Jungfrau *	160	195	3.3	0.1
Diamond VMXi	Elger2	500	63	1.0	0.0
Eu.XFEL	AGPID	3,250	10	0.2	0.0
LCLS-II-HE	ePIX *	10,000	3	0.1	0.0
SHINE	tbd *	17,000	2	0.0	0.0

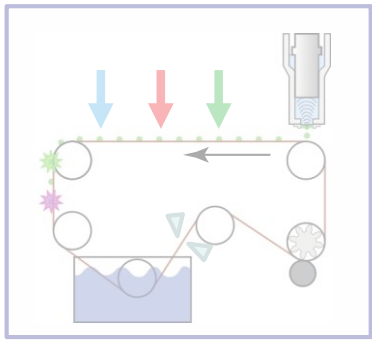
Serial MX data collection rates are driven by hit ratio & detector speed

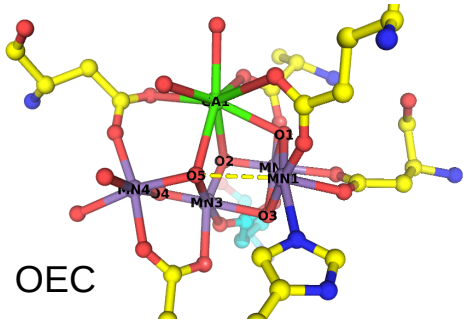
Detector or source speed	Serial MX snap shot
100 Hz (Pilatus)	10 ms
500 Hz (Eiger2)	2 ms
500 Hz + e^- gated	100 µs
10,000 Hz	100 µs
XFEL pulse duration	10 – 50 fs
Eu.XFEL train length	600 µs

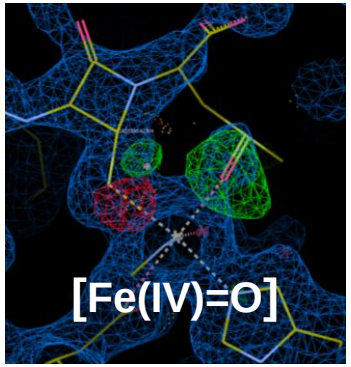
Outline & discussion points

1	What is the XFEL Hub at Diamond?
2	XFEL / SFX / sample delivery, on-demand acoustic injectors
3	**Science drivers of time-resolved structural biology:** **PS II, RNR, IPNS, and/or Phytochromes, etc**

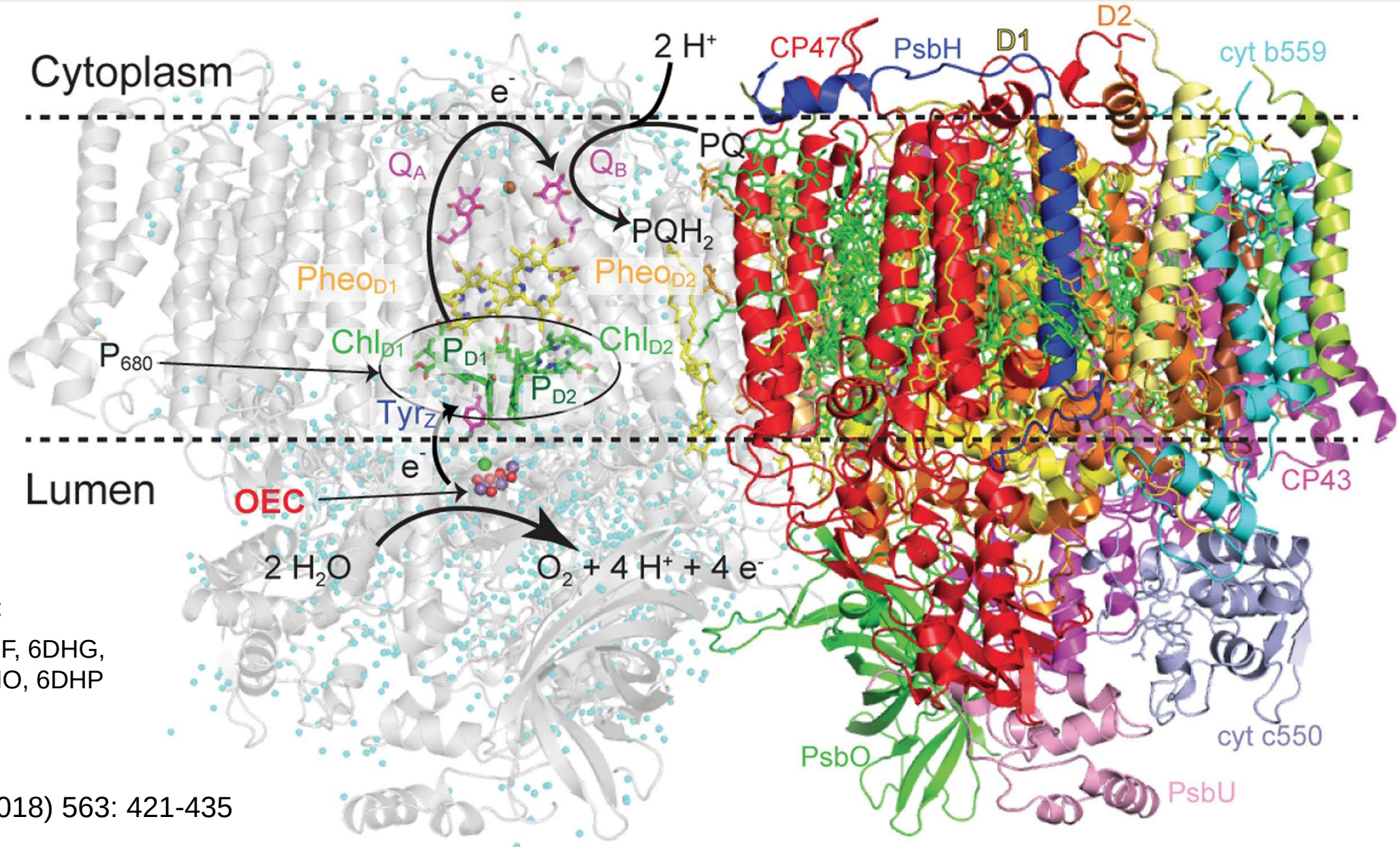








Thermosynechococcus elongatus BP-1 **Photosystem II**



PDB codes:

6DHE, 6DHF, 6DHG,
6DHH, 6DHO, 6DHP

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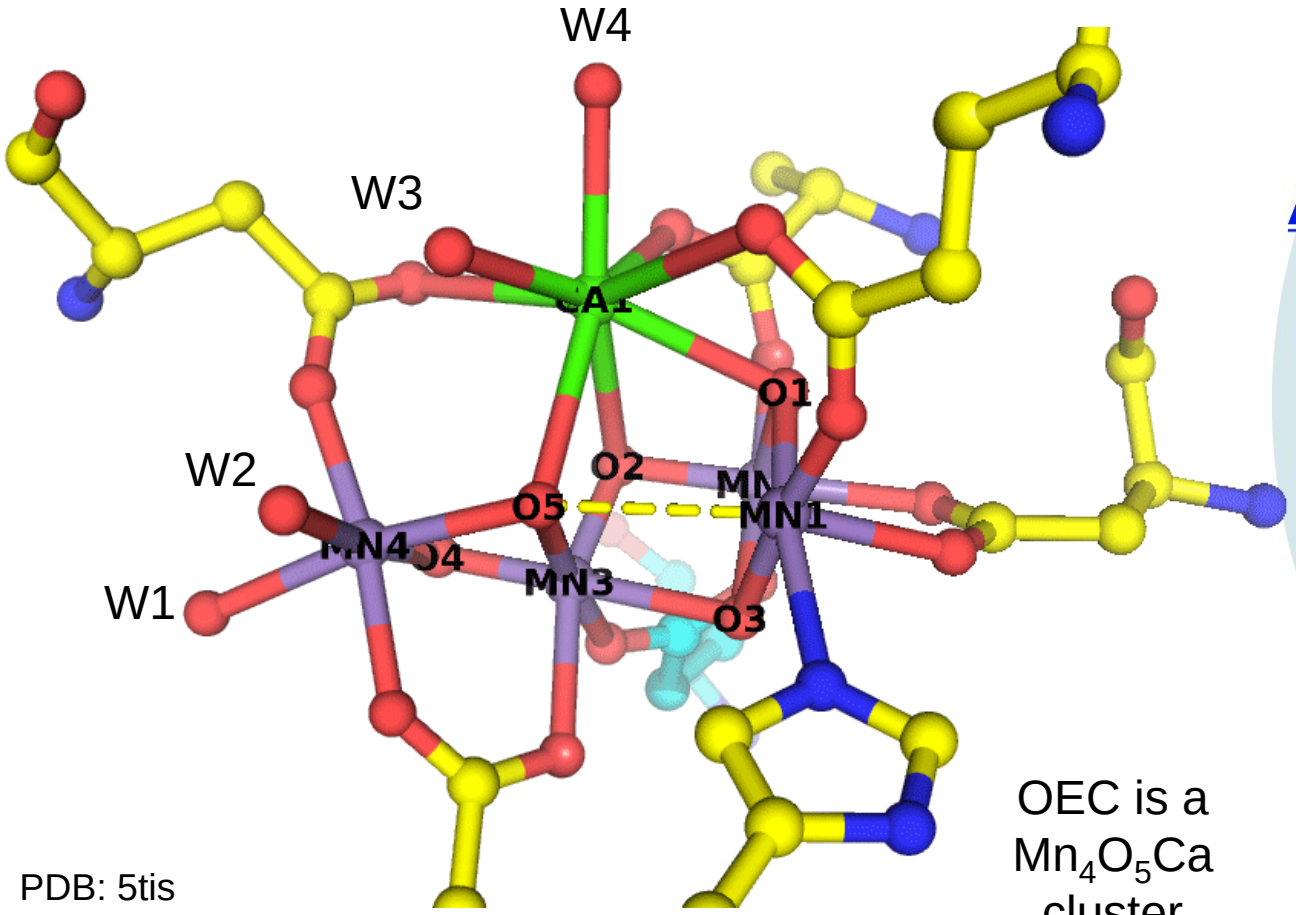
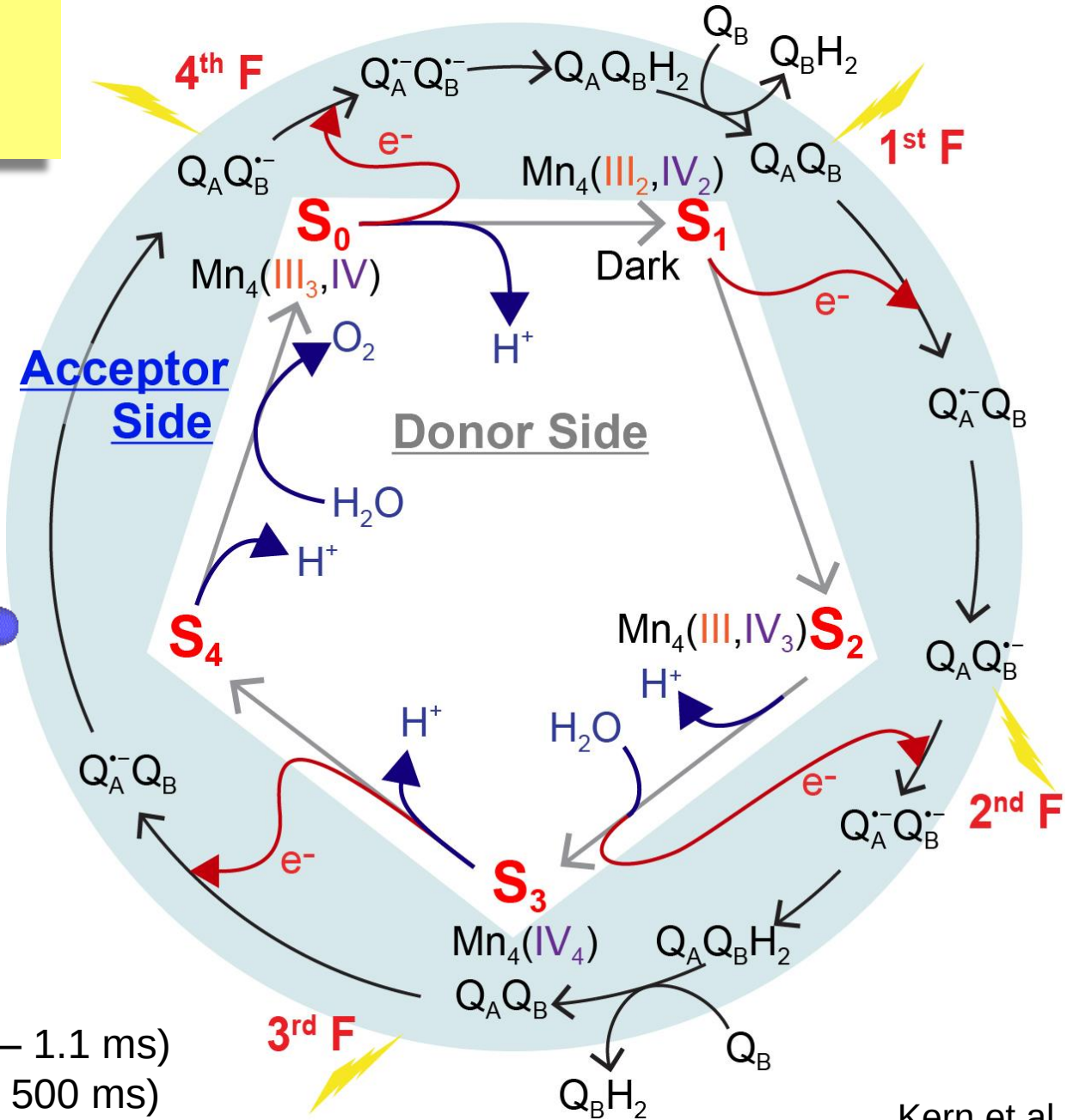
The Kok cycle & the OEC



PDB: 5tis

OEC is a Mn_4O_5Ca cluster



- Reaction rates w/in $S_1 \rightarrow S_0$ Kok cycle are “fast” (30 µs – 1.1 ms)
- e^- acceptor/quinone exchange reactions slower (~ 100 - 500 ms)
- Therefore, we allow equilibration time between illumination flashes.

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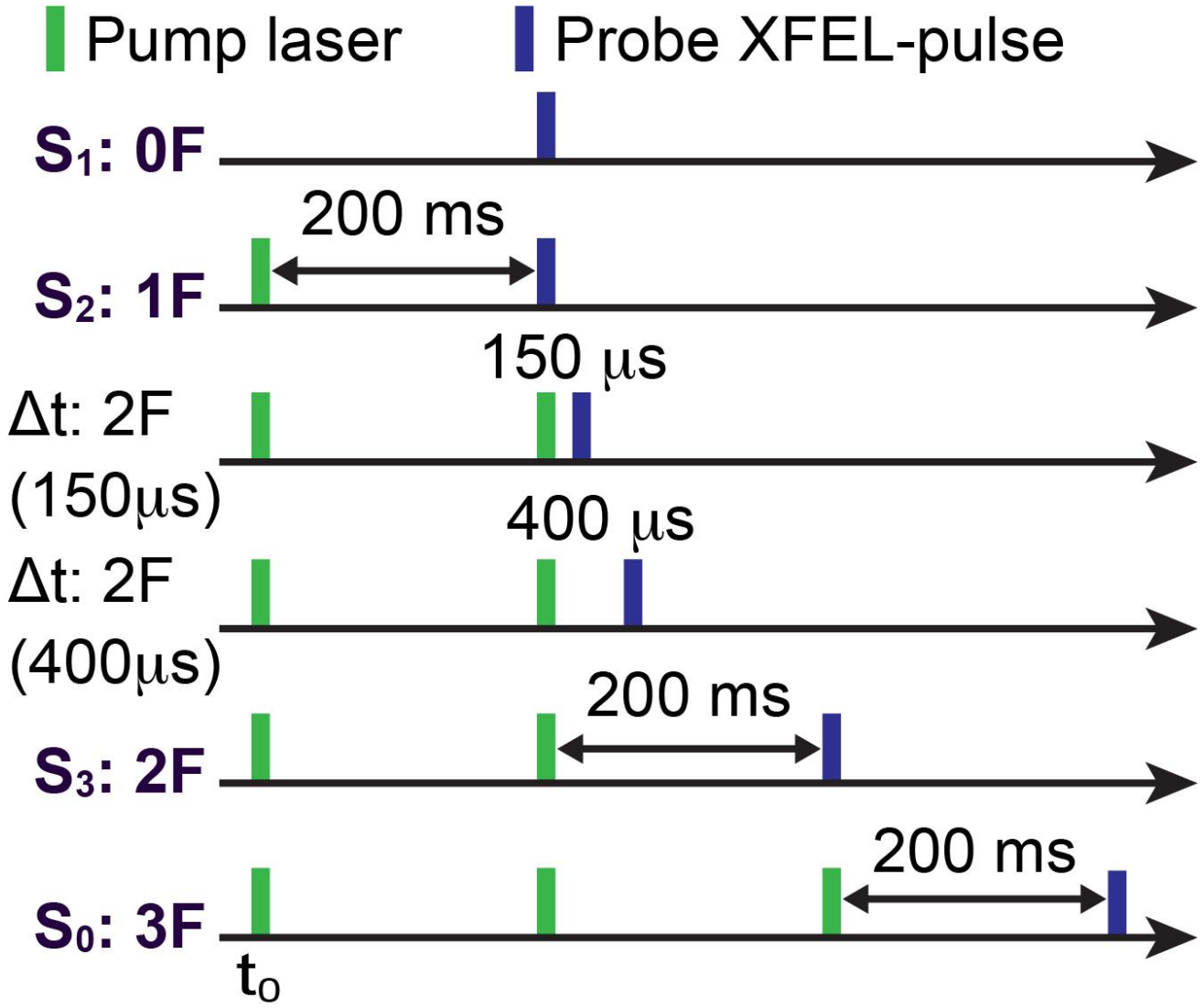
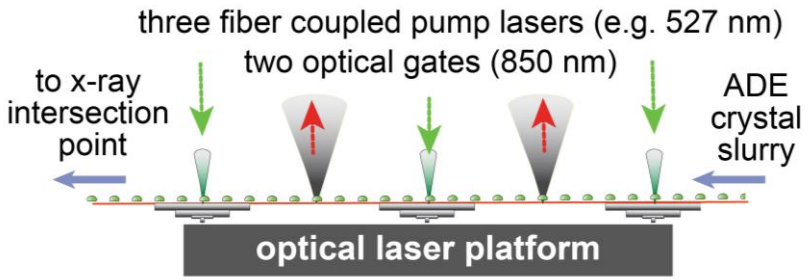
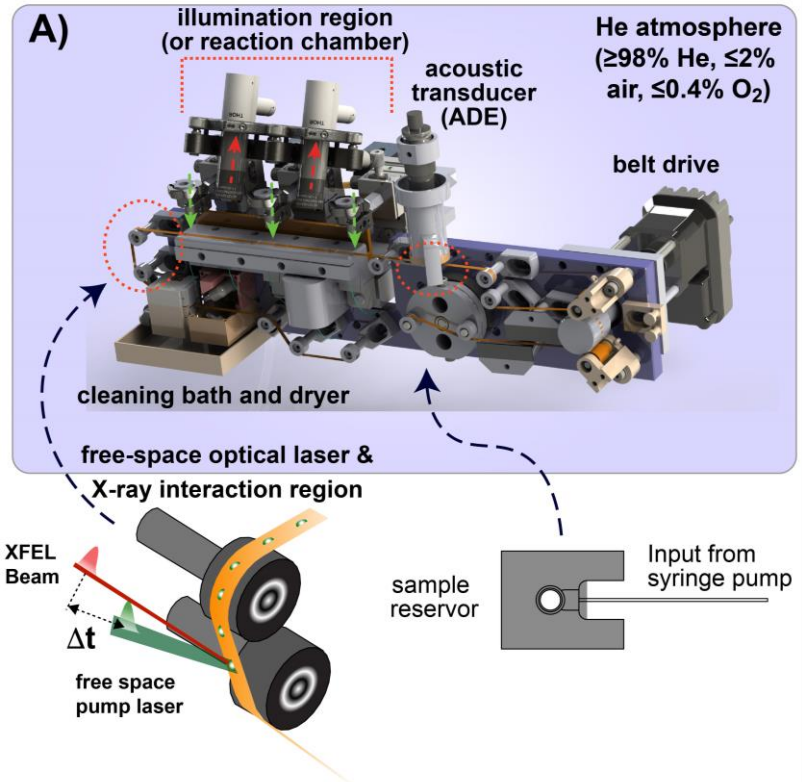
A flexible pump-probe illumination scheme







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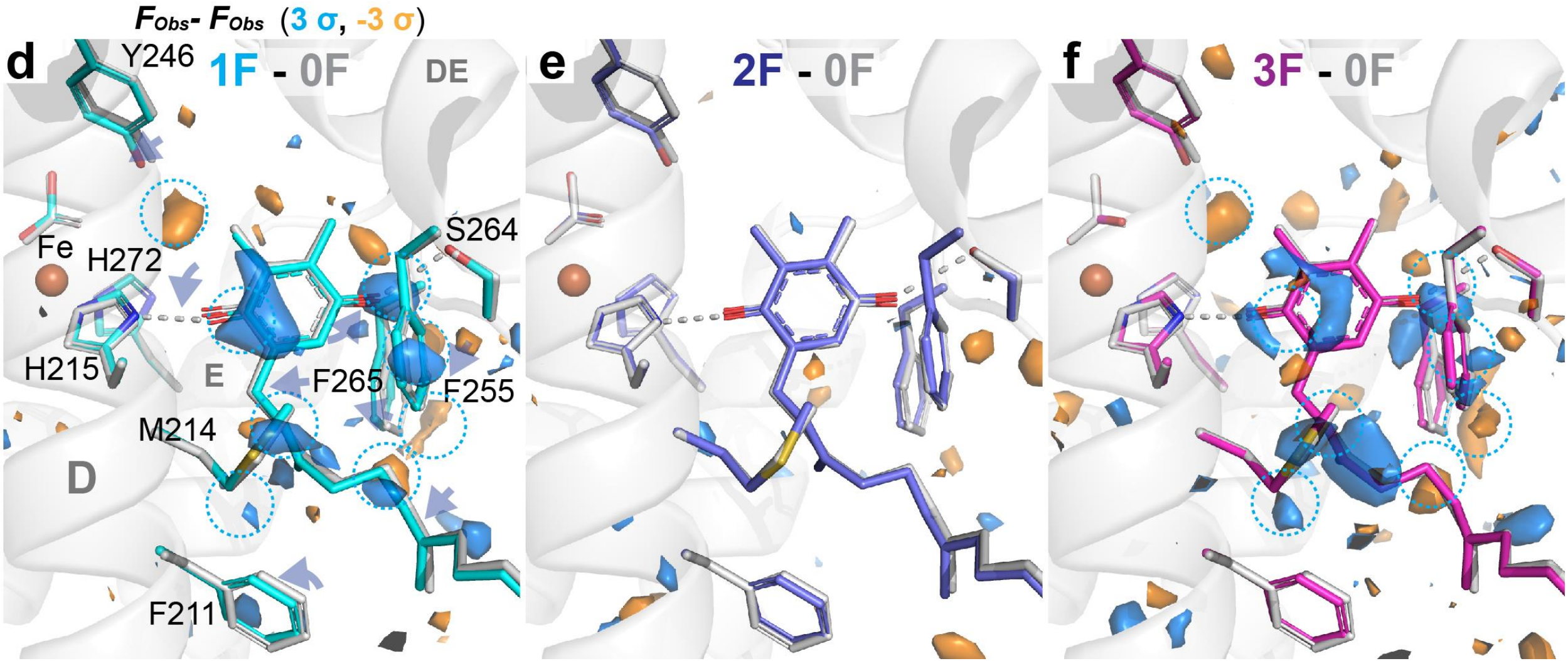
Fuller and Gul et al (2017) *Nature Methods* 14: 443-449

Isomorphous difference maps around plastoquinone QB



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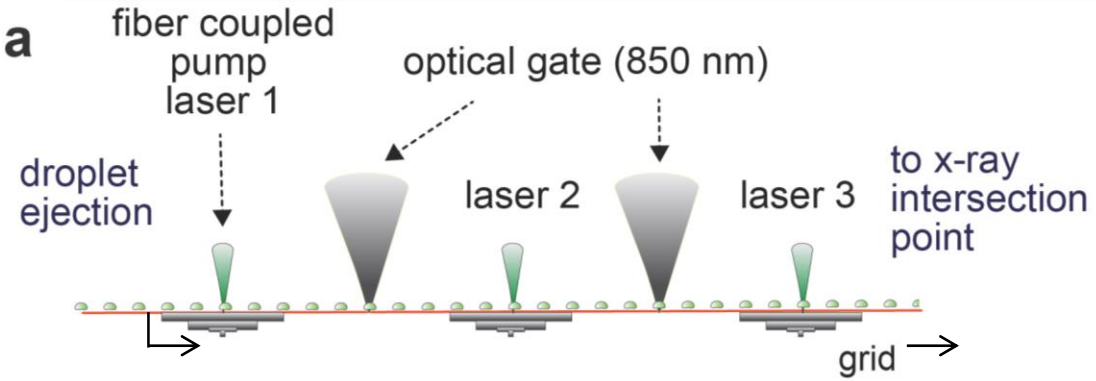
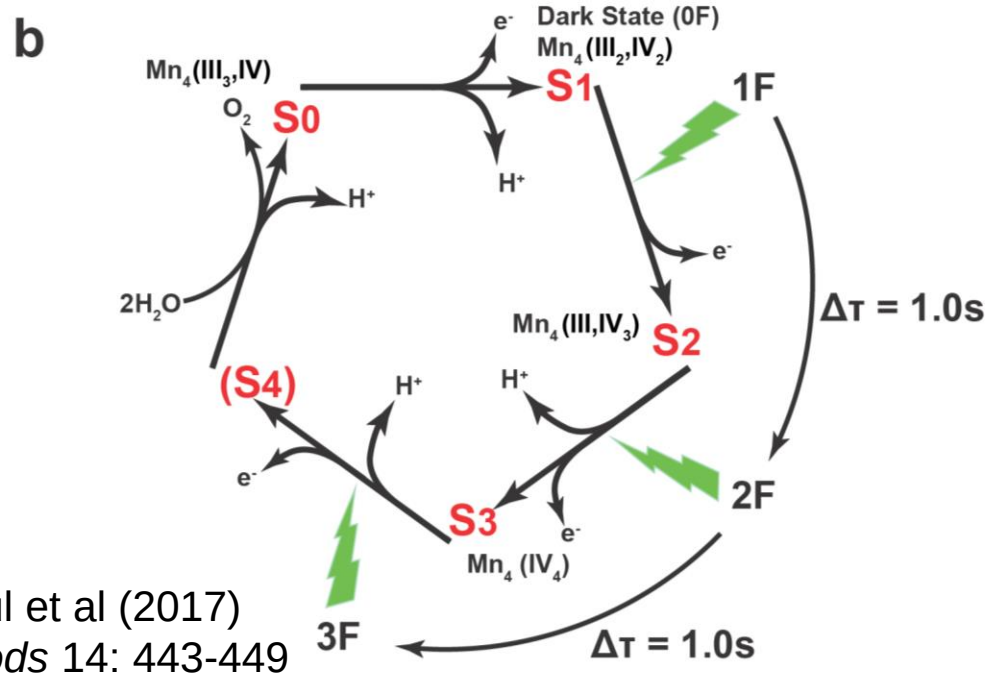
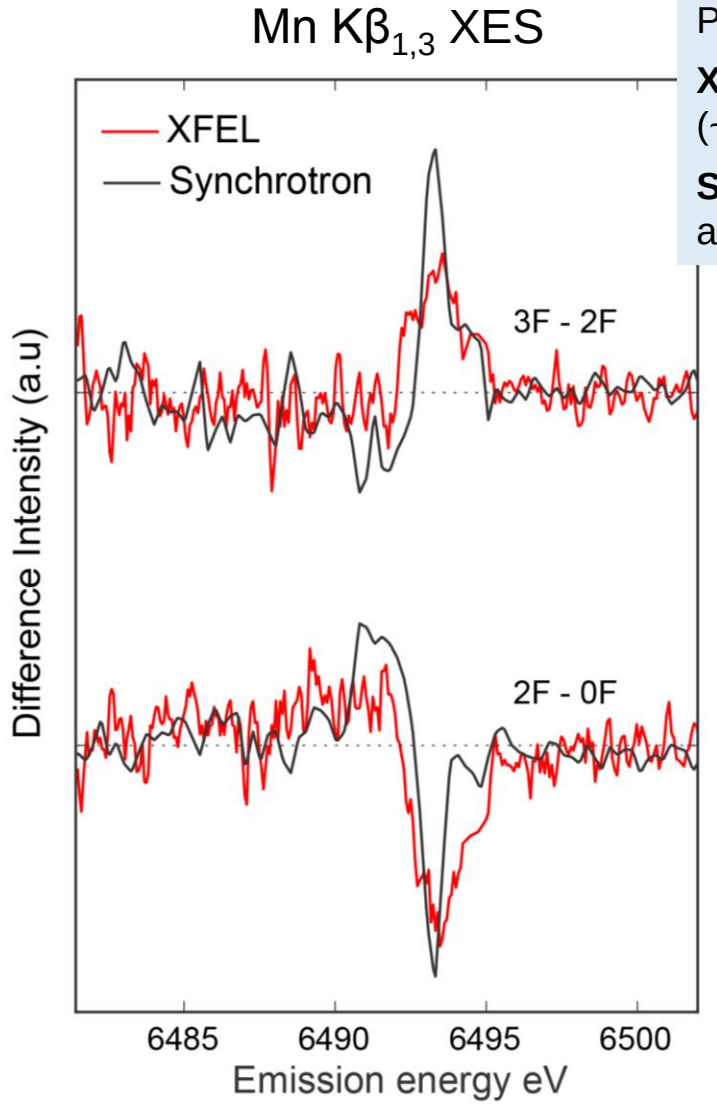
Photosystem II (PS-II): time-resolved, pump-probe SFX ± XES





Fuller and Gul et al (2017)
Nature Methods 14: 443-449



PS-II in solution

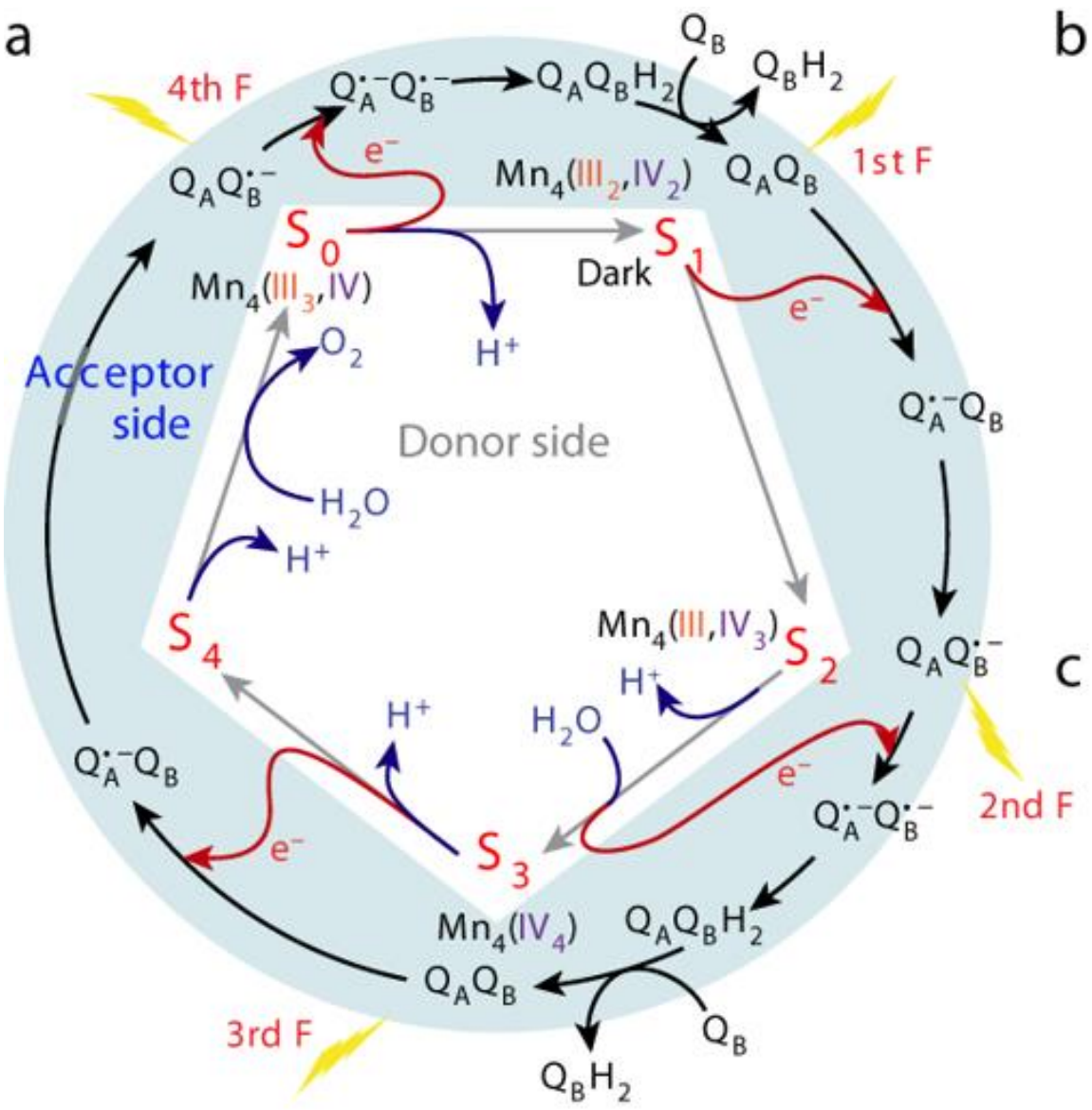
XFEL: at RT (~120,000 shots)

Synch: freeze-quench at 8 K

S_0 Mn(III_3, IV) - **S_3** Mn(IV_4)

S_3 Mn(IV_4) - **S_1** Mn(III_2, IV_2)

Flash-induced oxidation of Mn atoms in OCE



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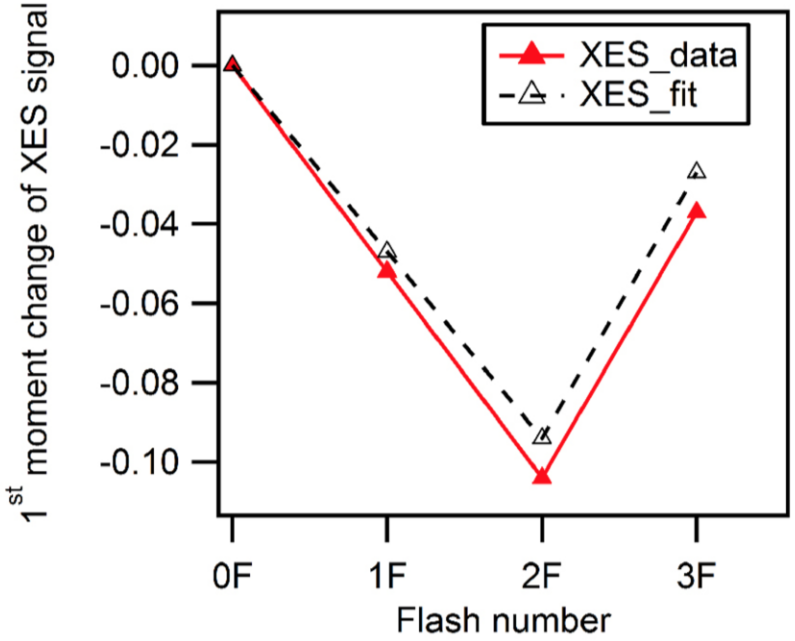
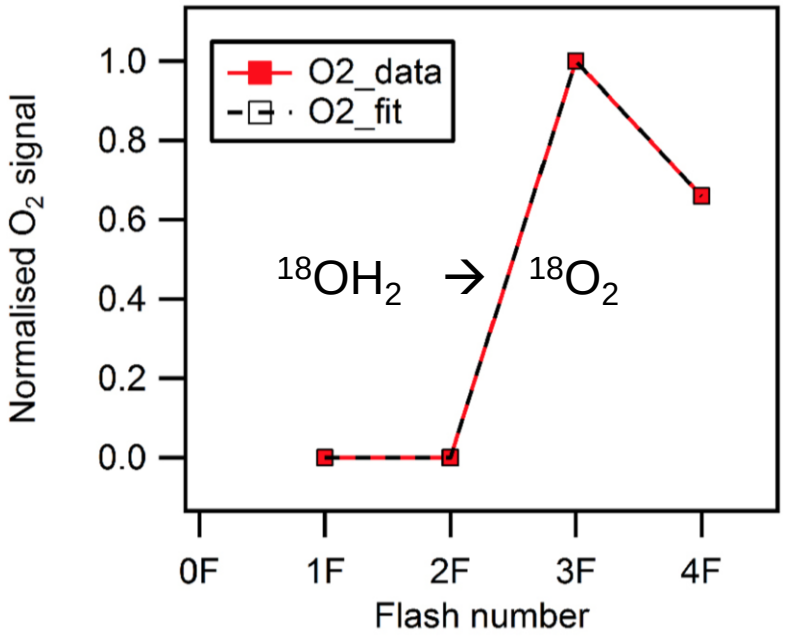
X-ray emission spectroscopy

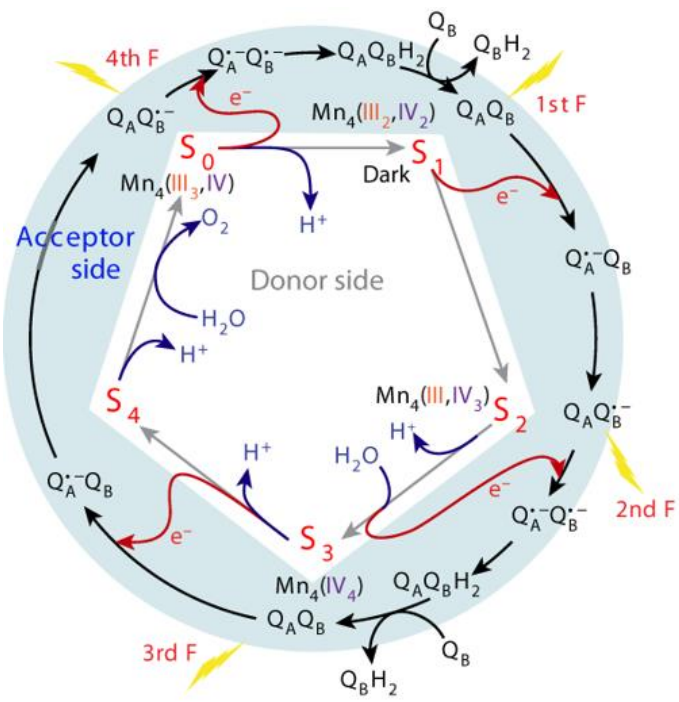


Membrane inlet mass spectroscopy



Flash-induced S-state turnover of PS-II micro-crystals

Sample	S_0		S_1		S_2		S_3	
	XES	O_2	XES	O_2	XES	O_2	XES	O_2
0F	0	0	100	100	0	0	0	0
1F	0	0	8	22	92	78	0	0
2F	0	0	2	5	30	34	68	61
3F	50	48	2	1	5	11	43	40



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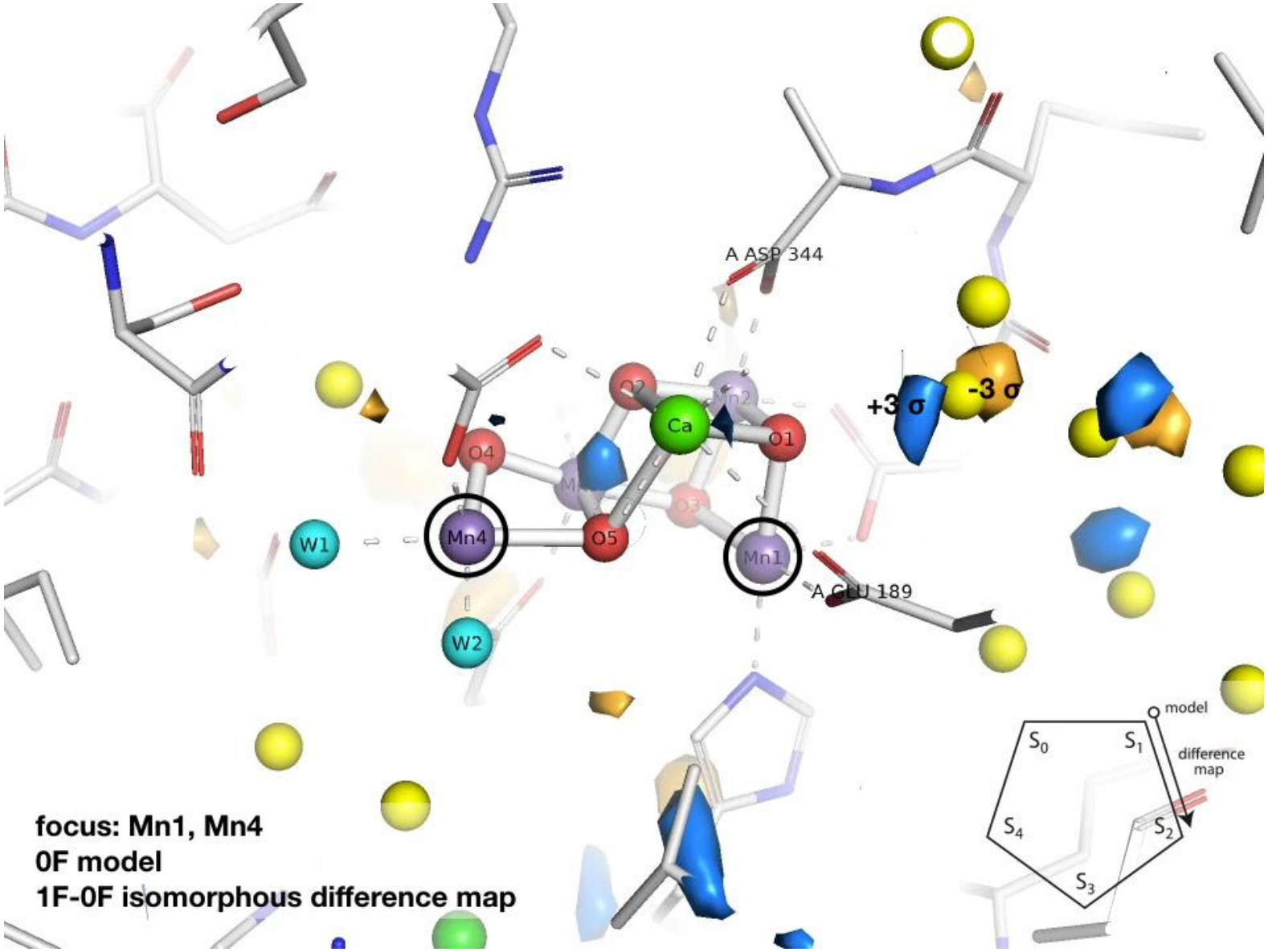
The $S_2 \rightarrow S_3$ transition in photosystem II



- 150 and 400 µs time points after 2nd flash, during $S_2 \rightarrow S_3$ transition
- tr-XES data show oxidation of Mn in the $S_2 \rightarrow S_3$ transition
- tr-SFX data show Mn4 and Mn1 move away from each other by ~ 0.2 Å
- 2.5 and 2.2 Å resolution
- 400 µs data clearly show that Ox is bound to Mn1 and Ca

O_2 formation in the $S_3 \rightarrow S_0$ transition

- Ox and O5 may form O–O bond

  - or -

- Ox may replace O5 after O_2 formed

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Thank you & discussion points

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