Macromolecular Structure & Function via X-Ray Dose- and/or Femtosecond Pulse-Dependent Methods

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Funded in part by:

- **National Institutes of Health**
  National Center for Research Resources (NCRR)
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  Biological and Environmental Research (BER)
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  Chemical Sciences, Geosciences and Biosciences Division

NSLS / NSLS-II & CFN Joint Users' Meeting

May 21, 2014

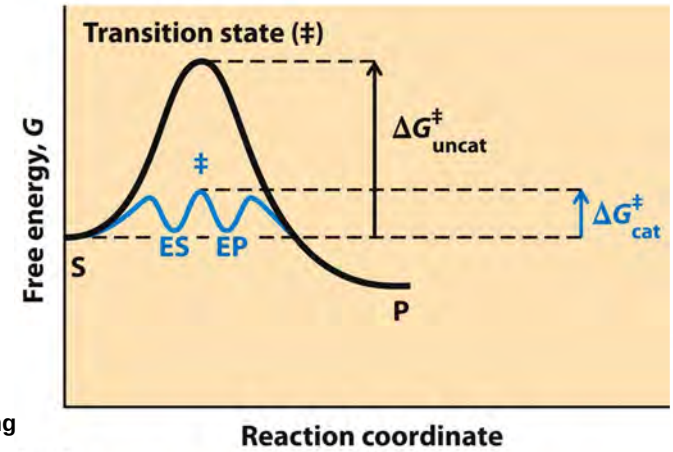

Figure 6-3
Lehninger Principles of Biochemistry, Fifth Edition
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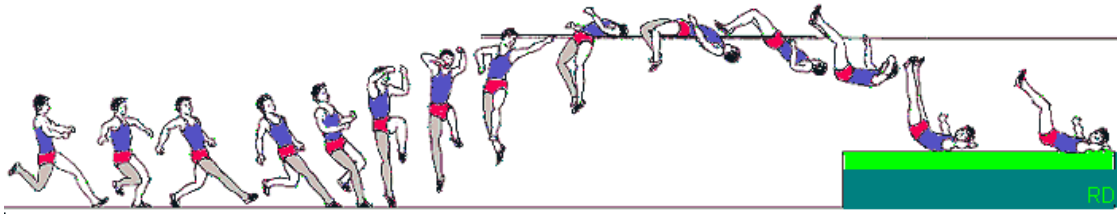
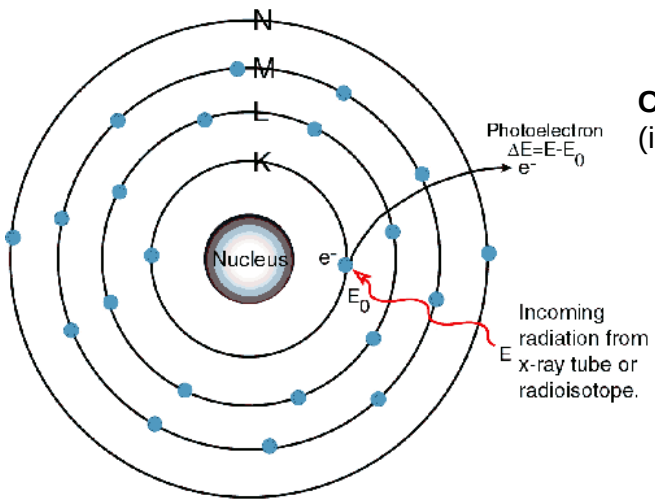
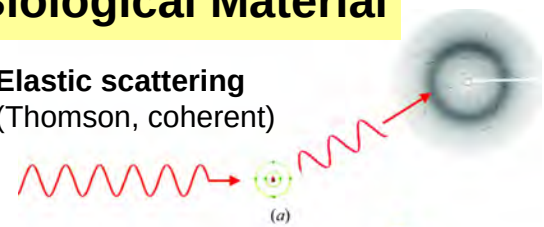
Function: The Sum of Many Parts



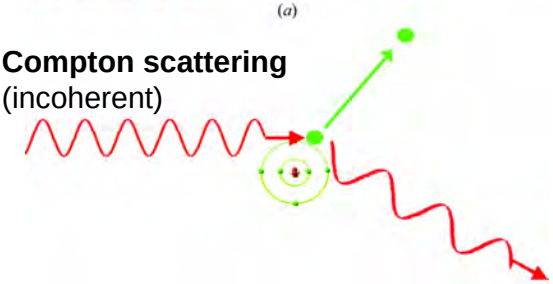
Interaction of X-rays with Biological Material

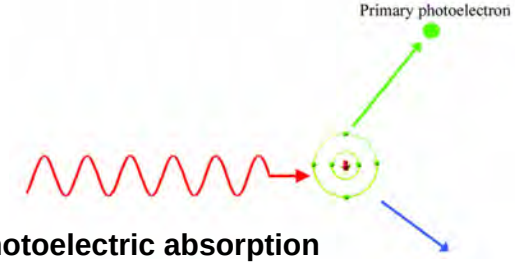


Elastic scattering
(Thomson, coherent)



Compton scattering
(incoherent)





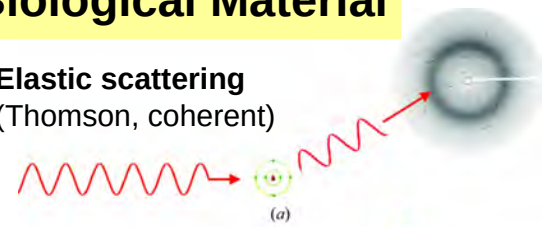
Photoelectric absorption
(ionization of up to 500 other atoms)

Garman, *Acta Cryst*. (2010). D66, 339–351

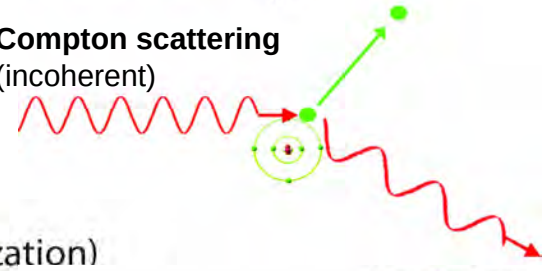
Interaction of X-rays with Biological Material

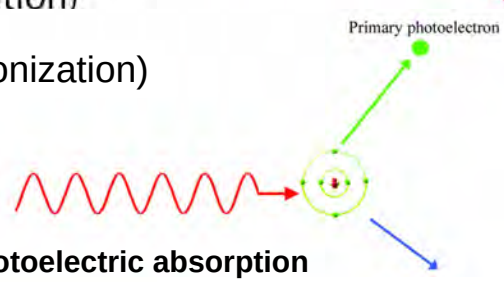
Given 10,000 x-ray photons
98% pass through the sample
2% interact with the sample
Of that 2%:
- **8% (0.15%) diffract = 16 photons**
- **92% deposit energy into sample (184 photons)**
- **Most of them also create ~500 more e- (84,000 electrons)**

Elastic scattering
(Thomson, coherent)



Compton scattering
(incoherent)





$$H_2O \xrightarrow{\text{ionizing radiation}} H_2O^{+\bullet} + e^- \quad \text{(ionization)}$$

$$H_2O \xrightarrow{\text{ionizing radiation}} H_2O^* \quad \text{(electronic ionization)}$$

$$H_2O^{+\bullet} + H_2O \longrightarrow H_3O^+ + {}^\bullet OH$$

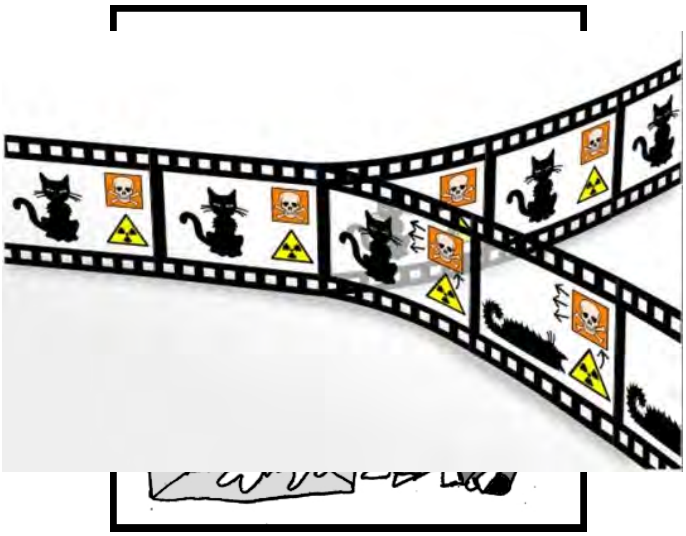
$$e^- + nH_2O \longrightarrow e^-_{aq}$$

$$H_2O^* \longrightarrow H^\bullet + {}^\bullet OH$$

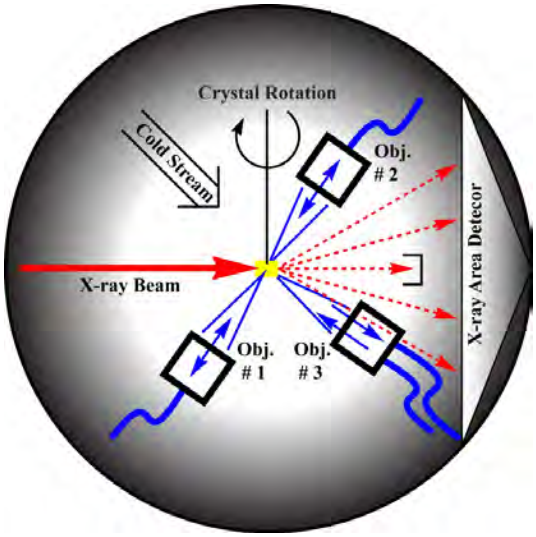
$$e^-_{aq} + H^+ \longrightarrow H^\bullet$$

Photoelectric absorption
(ionization of up to 500 other atoms)

Garman, *Acta Cryst*. (2010). D66, 339–351



Schrodinger's cat
the principle in quantum theory of **superposition …** when does quantum superposition end and reality collapse into one possibility or the other?



Available NOW

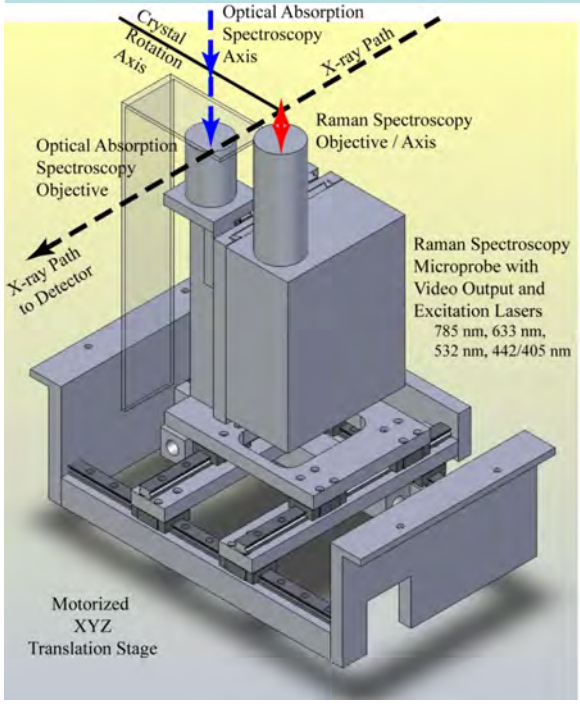
- ***MX at Beamline X26-C***
- ***Optical absorption spectroscopy w/ MX***
- ***Raman spectroscopy w/ MX 785 nm, 633 nm, 532 nm, or 473 nm excitation***

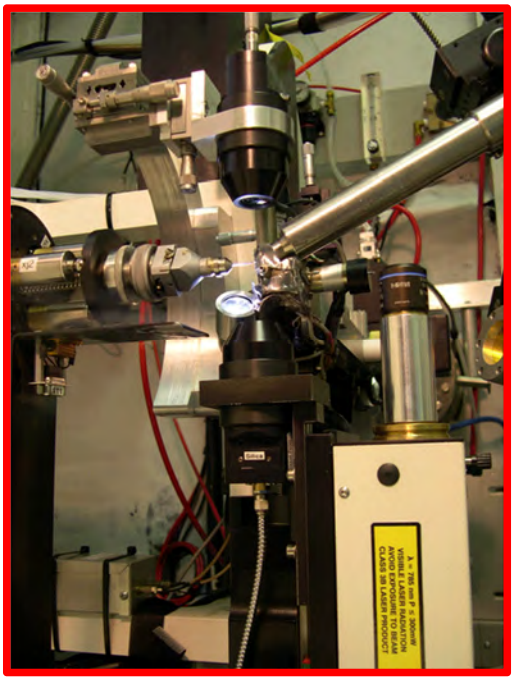
Available Soon

- NSLS-II (SM3)
- Spectroscopies w/ MX
- Acoustic Droplet Ejection

Final Status of Beamline X26-C

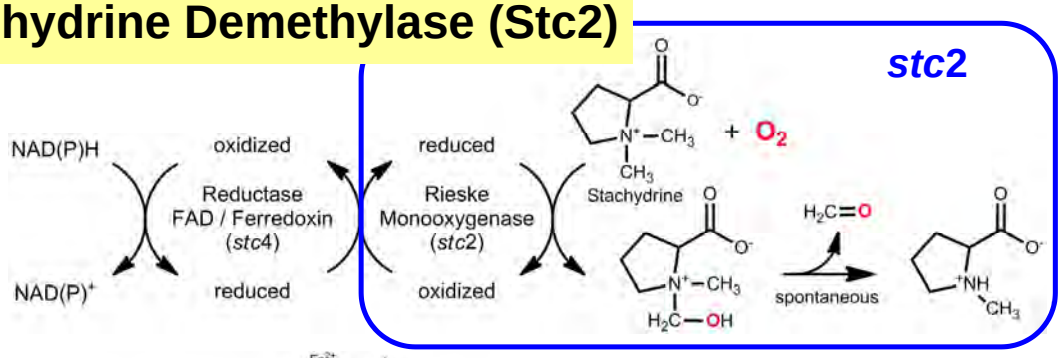
Raman Spec. (785, 633, 532 or 473 nm excitation) + Abs. Spec. + X-ray Diff.

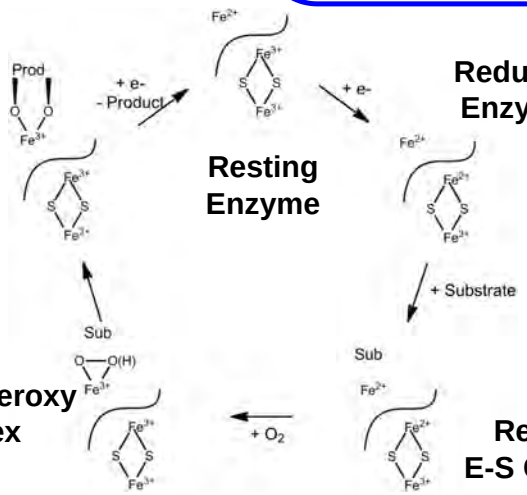




Stoner-Ma et al, (2011) *J. Synchrotron Rad.* **18**, 37–40; Orville et al., (2011) *J. Synchrotron Rad.* **18**, 358–366

Stachydrine Demethylase (Stc2)





Oxidized E-Product Complex

Resting Enzyme

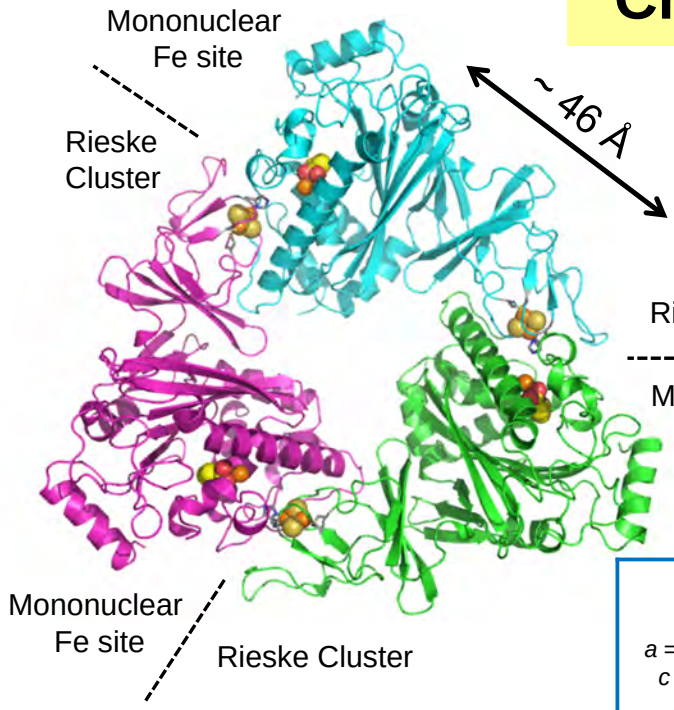
Reduced Enzyme

E-S-Hydroperoxy Complex

Reduced E-S Complex

Resting enzyme (E^{ox}) co-crystalized with substrate should not react, good for crystallography

Stc2 Crystal Structure



α_3 Homotrimer (414 residues)
Rieske Domain
(45 - 153)
Catalytic Domain
(1- 44 & 154 - 412)

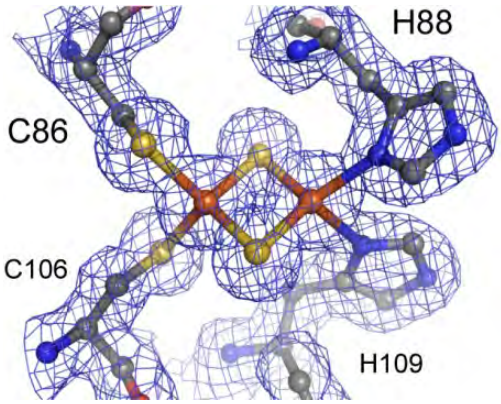
Rieske Cluster

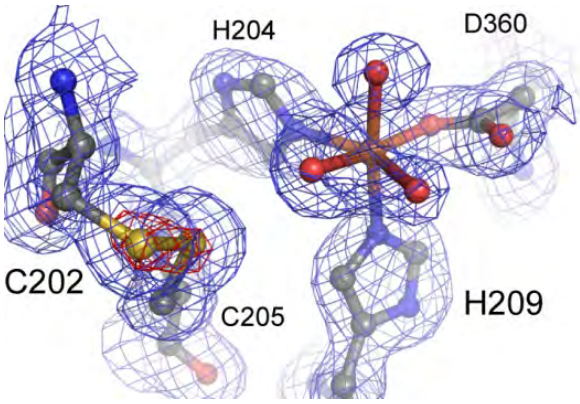
Mononuclear
Fe site
~ 12 Å

$P6_322$	1 Subunit per ASU	28 - 1.6 Å resolution
$a = b = 98$ Å $c = 179$ Å	68,467 unique reflections	R_{merge} 8.0%
99.9 % complete	16-fold redundancy	R / R_{free} 17.8 / 20.9

Daughtry et al. *J. Am. Chem. Soc.* (2012) 134, 2823−2834

Resting *Stc2* Electron Density Maps



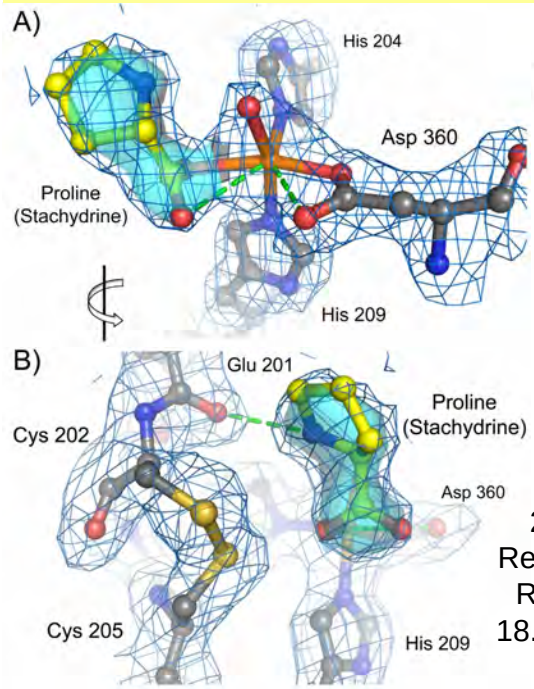


Rieske Center
Electron Transfer
- CXH … CXXH sequence motif
- ~ 46 Å from mononuclear iron within each subunit
- ~ 12 Å across subunit interface

Mononuclear Fe site
Active Site, O_2- Activation
- Distorted octahedral
- His_2 - Asp facial triad w/ 3 solvents
- Disulfide bond ~4.6 Å from iron
- CYHC, conserved sequence

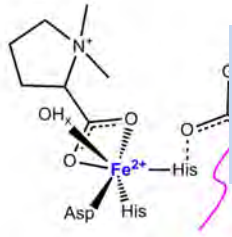
2Fo-Fc (1 σ blue); Fo-Fc (-3 σ red); 1.6 Å resolution; R / R_{free} 17.8 / 20.9 %

Aerobic Enzyme Co-Crystallized with Stachydrine!! ??

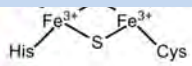


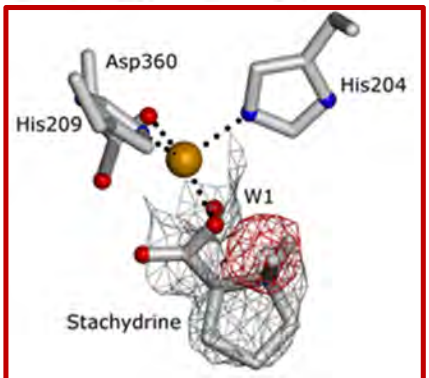
2.2 Å
Resolution
R (R_{free})
18.7 (24.2)

$2F_o$ - F_c 1.0 σ (mesh) & 1.5 σ (surface)



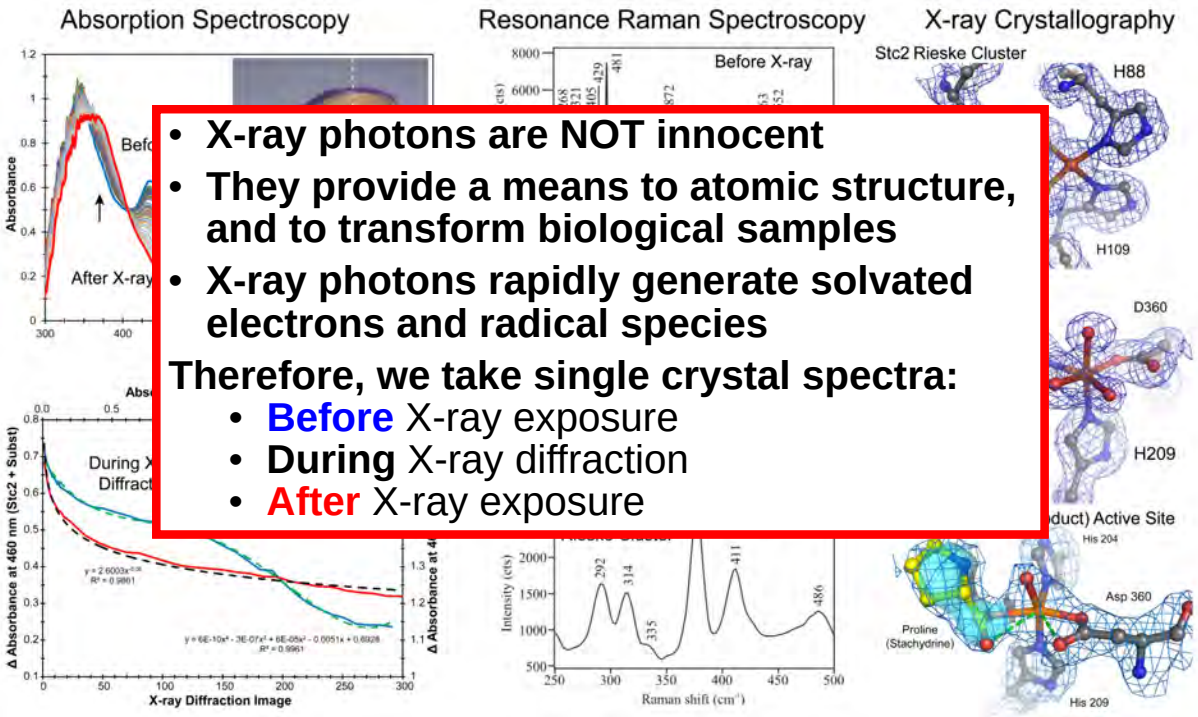
Control:
Stachydrine in solution is **NOT** altered by exposure to high intensity X-rays





$2F_o$ - F_c (grey mesh) 1.0 σ
F_o - F_c (red mesh) -3 σ

Spec. + MX Summary with *Stc2*



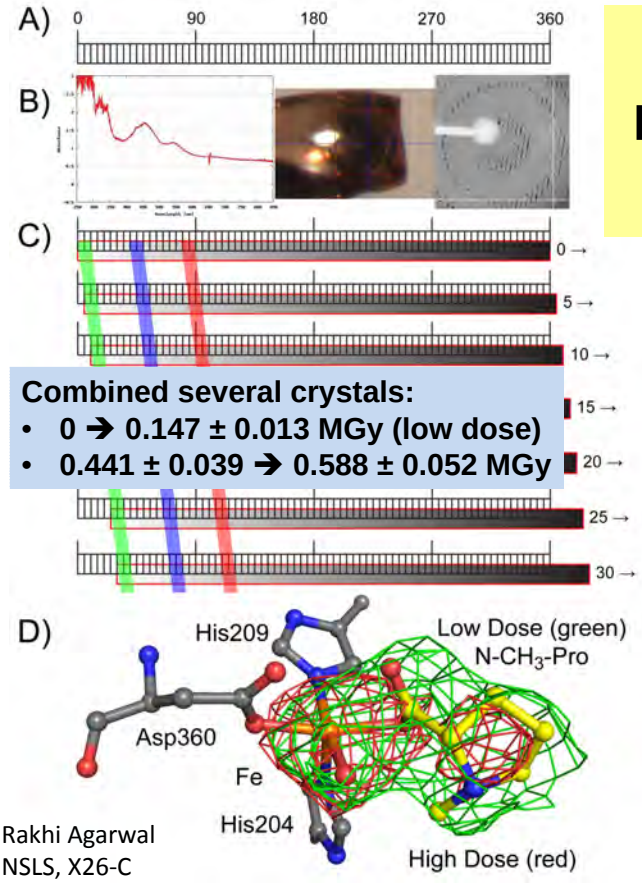
- **X-ray photons are NOT innocent**
- **They provide a means to atomic structure, and to transform biological samples**
- **X-ray photons rapidly generate solvated electrons and radical species**

Therefore, we take single crystal spectra:

- **Before** X-ray exposure
- **During** X-ray diffraction
- **After** X-ray exposure

Daughtry et al. *J. Am. Chem. Soc.* (2012) 134, 2823−2834

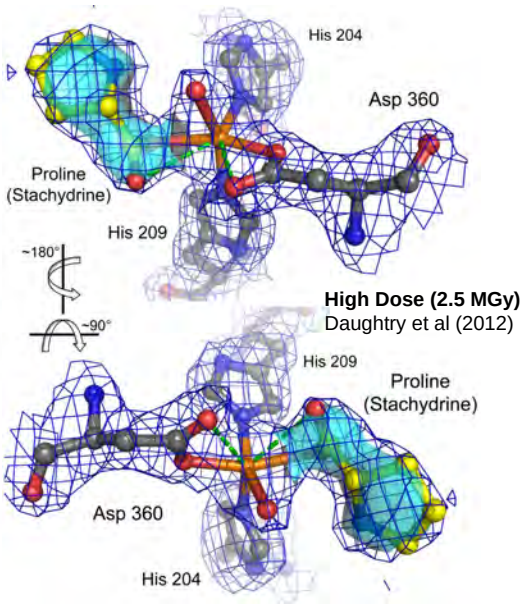
The Photoelectric Effect Promotes Stc2 Turnover at 100K



Combined several crystals:

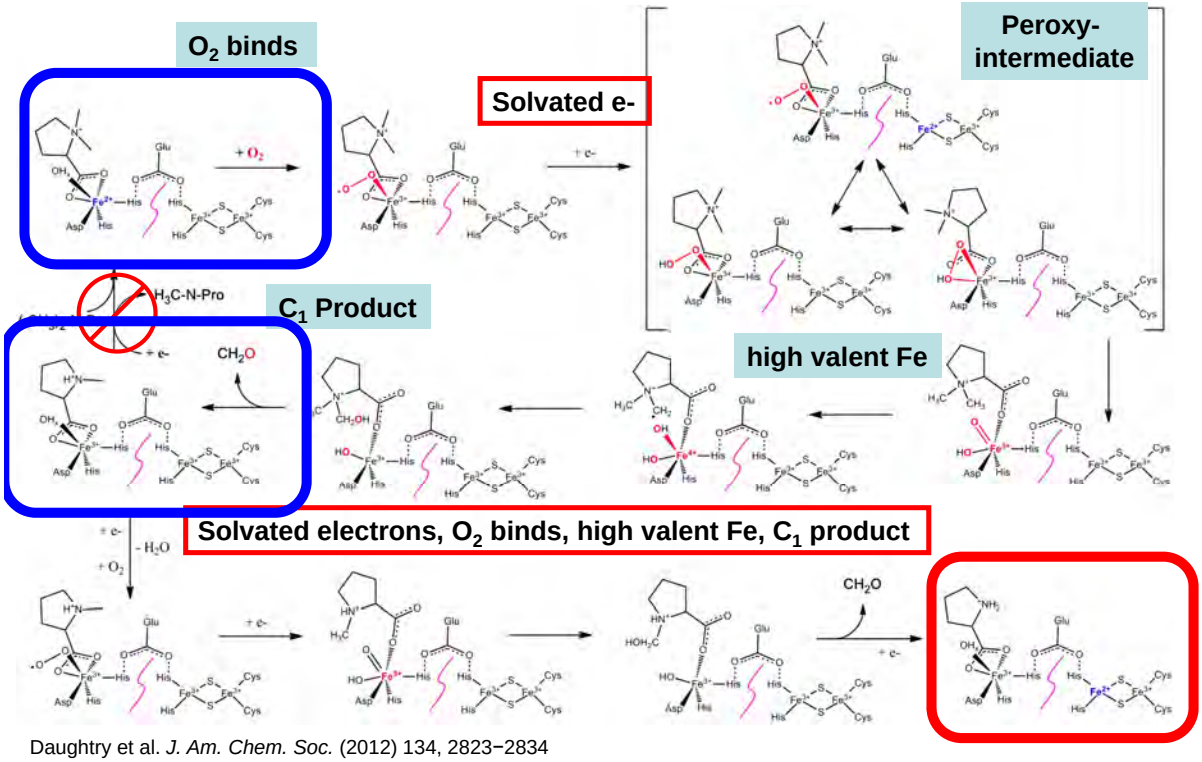
- **0 ➔ 0.147 ± 0.013 MGy (low dose)**
- **0.441 ± 0.039 ➔ 0.588 ± 0.052 MGy**

Rakhi Agarwal
NSLS, X26-C



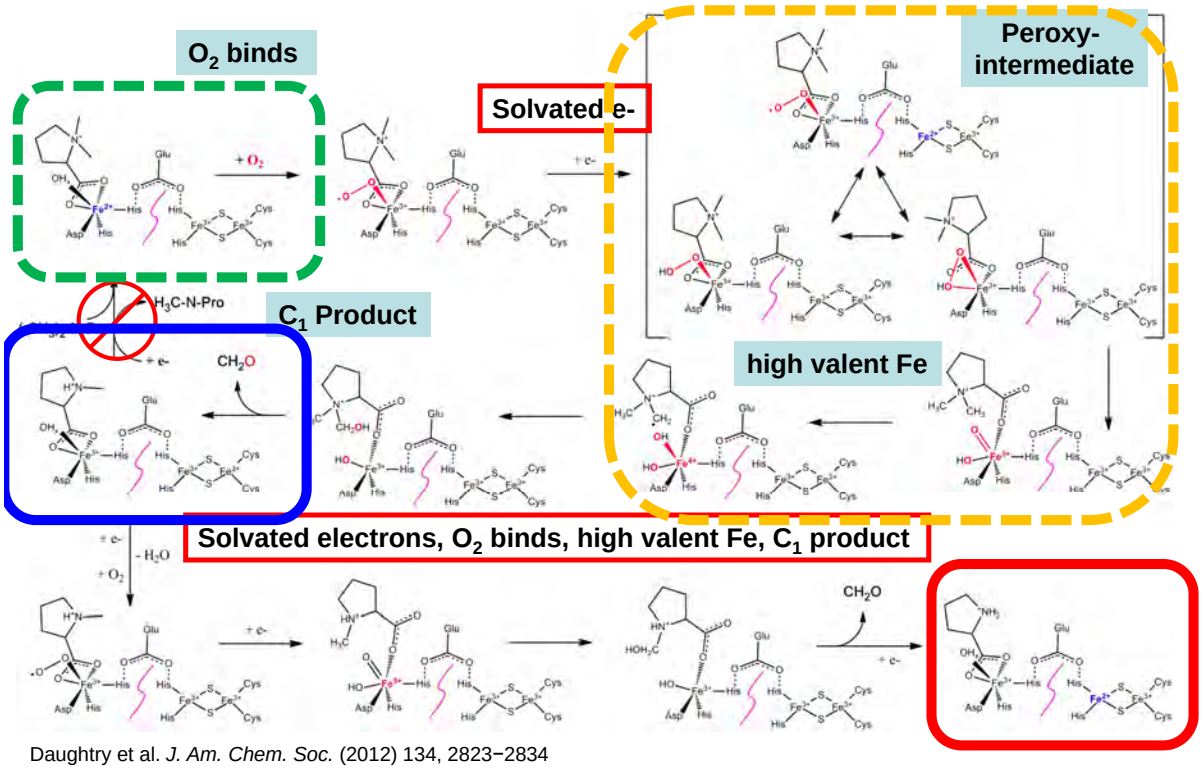
High Dose (2.5 MGy)
Daughtry et al (2012)

in-Crystallo Chemistry w/ X-ray Photons?



Daughtry et al. *J. Am. Chem. Soc.* (2012) 134, 2823−2834

in-Crystallo Chemistry w/ X-ray Photons?



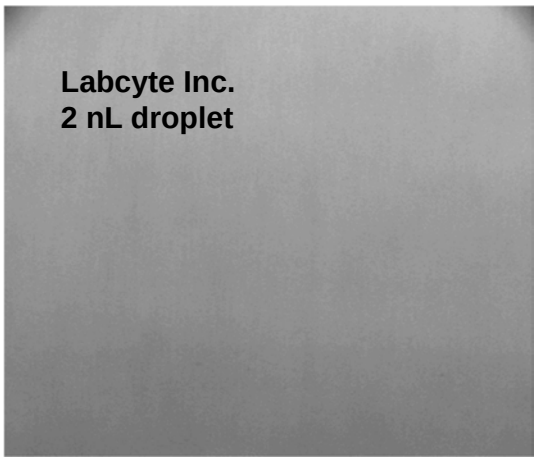
Daughtry et al. *J. Am. Chem. Soc.* (2012) 134, 2823−2834

New X-ray Sources Allow Us to Do More with Less Sample

#39: The Levitating Mouse
The 50 Best Inventions of 2009, *Time.*
"Researchers at NASA's Jet Propulsion Laboratory, led by Yuanming Liu, have figured out how to make the tiny critters float in midair using magnets."

If NASA can levitate a mouse, then we should be able to do something with microcrystals ...

... using **Acoustic Droplet Ejection** (ADE) methods, and **Raster Scan** strategies



We have use ADE methods to launch, catch, and solve structures from 2.5, 1.0 & 0.1 nL droplets containing a slurry of microcrystals!

Biochemistry (2011) **50**:4399–4401 Soares, Engel, Stearns, Datwani, Olechno, Ellson, Skinner, Allaire, and Orville, "Acoustically Mounted Microcrystals Yield High-Resolution X-ray Structures"

J. Synch. Rad. (2013) **20**:805 – 808 Roessler, C. R., Kuczewski, A., Orville, A. M., Allaire, M., Soares, A. S., Héroux, A., "Acoustic Methods for High-Throughput Protein Crystal Mounting"
http://www.youtube.com/watch?v=2xls3eba8RI

"The Big Guns. Powerful X-ray lasers are getting to the heart of matter."

Nature **505**, 604–606 (30 January 2014)

- Mitchell Waldrop highlighted both the tremendous **excitement** and the **difficult challenges** faced by serial femtosecond crystallography (SFX) methods at X-ray free electron laser (XFEL) sources.
- *"Global investments in XFELs over the next few years will total billions of dollars. But to reach their full potential, these machines will have to surmount many more technical hurdles."*
- For example, the current SFX injectors continuously shoot nano- or microcrystal slurries through the X-ray interaction zone, but waste *"more than 99% of the expensive, hard to make nanocrystals."*

In our tests the acoustic injectors were timed to deliver droplets into 50 fs X-ray pulses from the LCLS. We achieved a 50 - 80% droplet hit ratio at frequencies up to the current limit of the laser (120 Hz). Delivering crystals in discrete droplets only when X-rays are present potentiates over a 150 billion-fold reduction in sample consumption. By synchronizing crystal delivery with each X-ray pulse, it is possible to use nearly every crystal and every X-ray pulse.

Timing is Everything!
But, Gravity is Everything Too?

Total X-rays per second
120 Hz x 50×10^{-15} s pulses = 6 ps
(167 x 10^9 more dark time)

BNL injects downward

Labcyte injects upward

Some Characteristics of ADE

Transd. (MHz)	~Vol. (pL)	~Diameter (µm)	~Max. Crystal (µm^3)
11.5	2500	170	100
15	1000	125	77
25	100	60	40
50	10	25	15
75	5	15	5

Drop-on-Demand Acoustic Injectors Deliver Sample *Only* When and Where Needed

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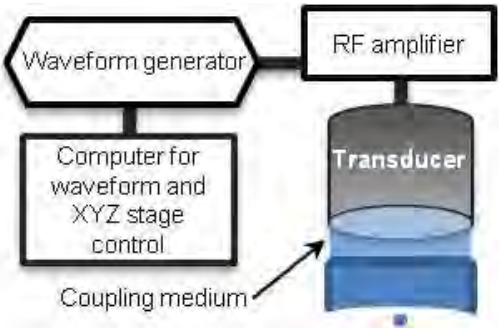
- **National Institutes of Health** NIGMS: Biomedical Technology Research Center (BTRC)
- **U.S. Department of Energy** Biological and Environmental Research (BER)
- **LDRD Brookhaven National Lab**

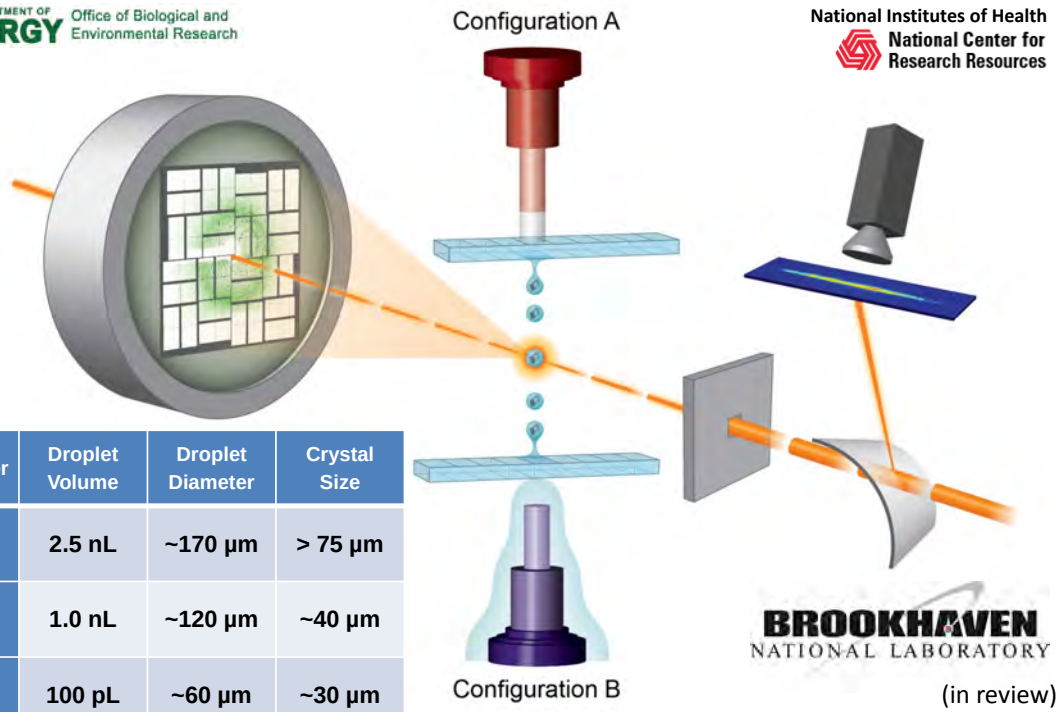


February 19 – 22, 2014
1st Ringberg Workshop on Structural Biology with FELs – from first results to future perspectives

Acoustic Injectors for Drop-on-Demand Serial Femtosecond Crystallography

U.S. DEPARTMENT OF ENERGY Office of Biological and Environmental Research

National Institutes of Health National Center for Research Resources

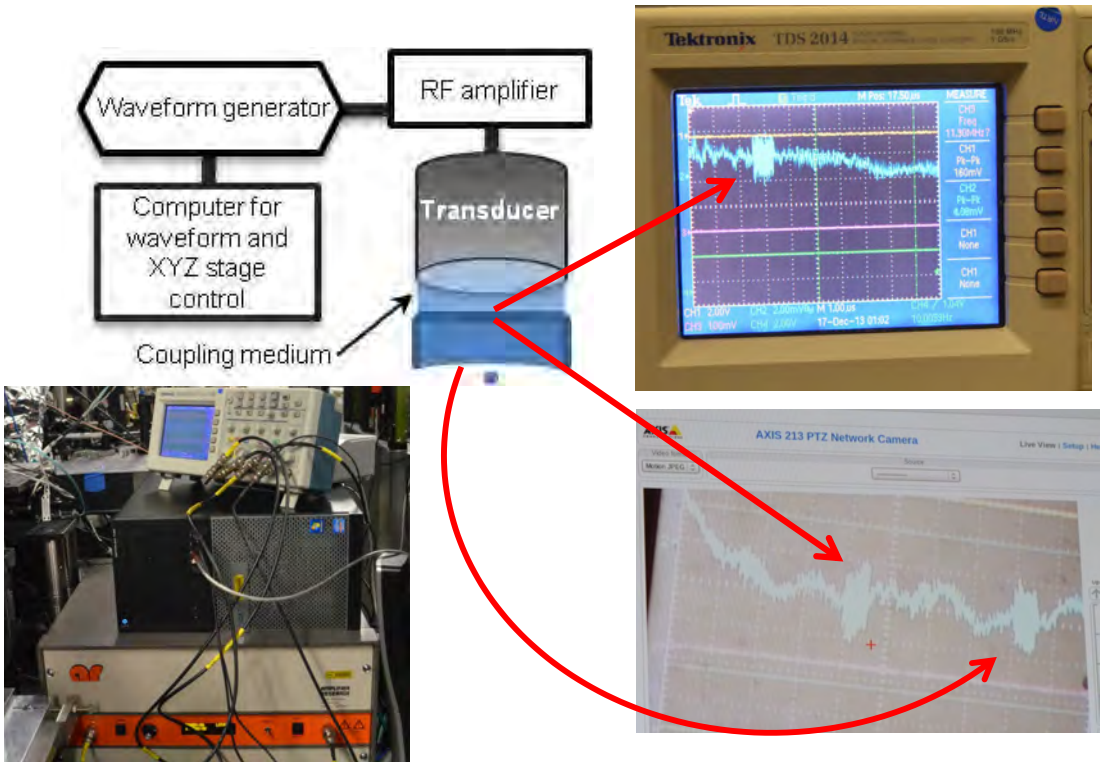


Transducer	Droplet Volume	Droplet Diameter	Crystal Size
11.5 MHz (Conf. B)	2.5 nL	~170 µm	> 75 µm
15 MHz (Conf. A)	1.0 nL	~120 µm	~40 µm
25 MHz (Conf. A)	100 pL	~60 µm	~30 µm

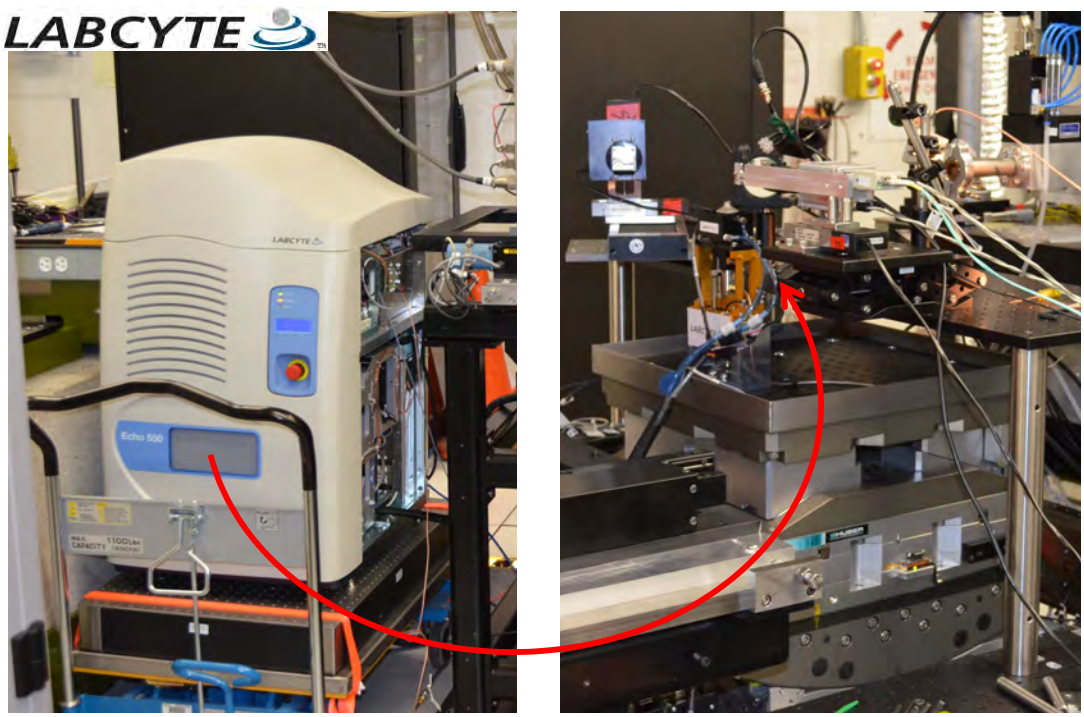
BROOKHAVEN NATIONAL LABORATORY

(in review)
Roessler, Allaire, Soares & Orville *et al*

Return Echoes Used to Align and Tune



Labcyte Modified Echo Injector ↑



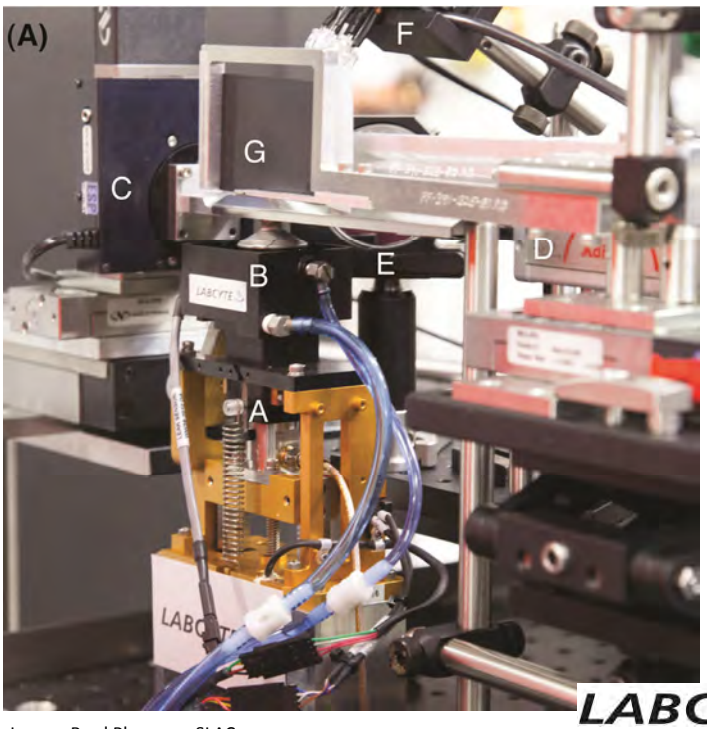
Labcyte Modified Echo Injector ↑

C) sample tray Goniometer 384-well format microplates

G) X-ray transparent stroboscopic reflection film

B) flowing water coupling system

A) transducer assembly



F) stroboscopic LED array

X-ray beam comes in from the right

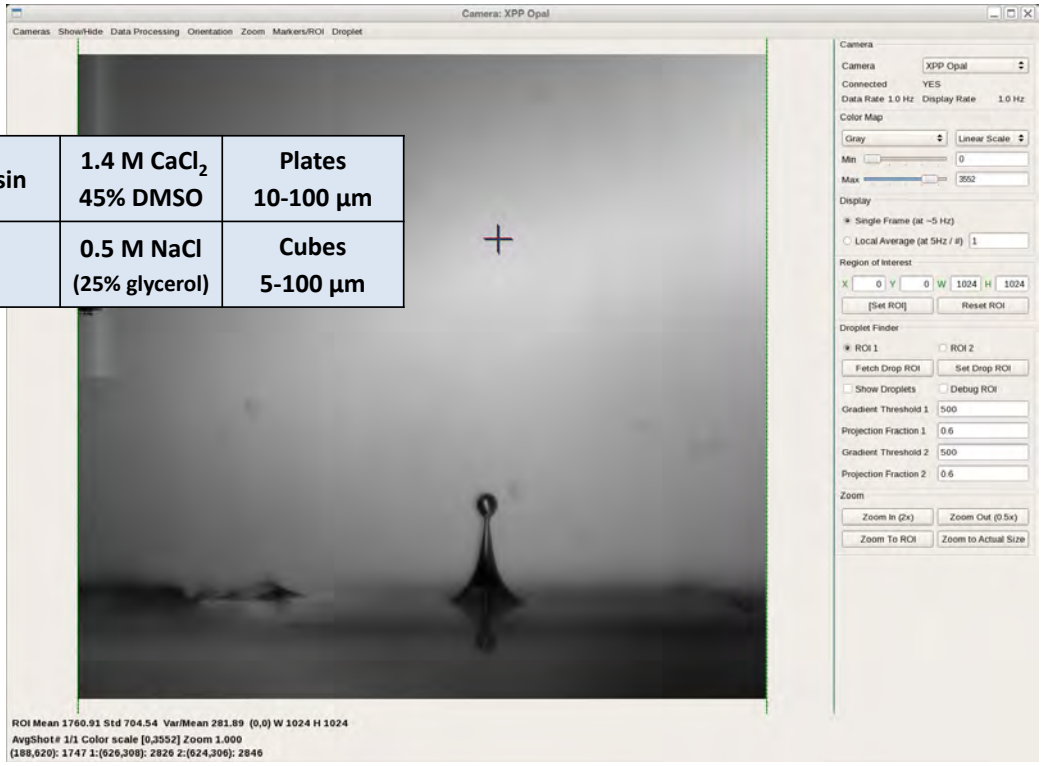
D) off-axis infrared camera

E) 45° mirror w/ center hole for on-axis visualization

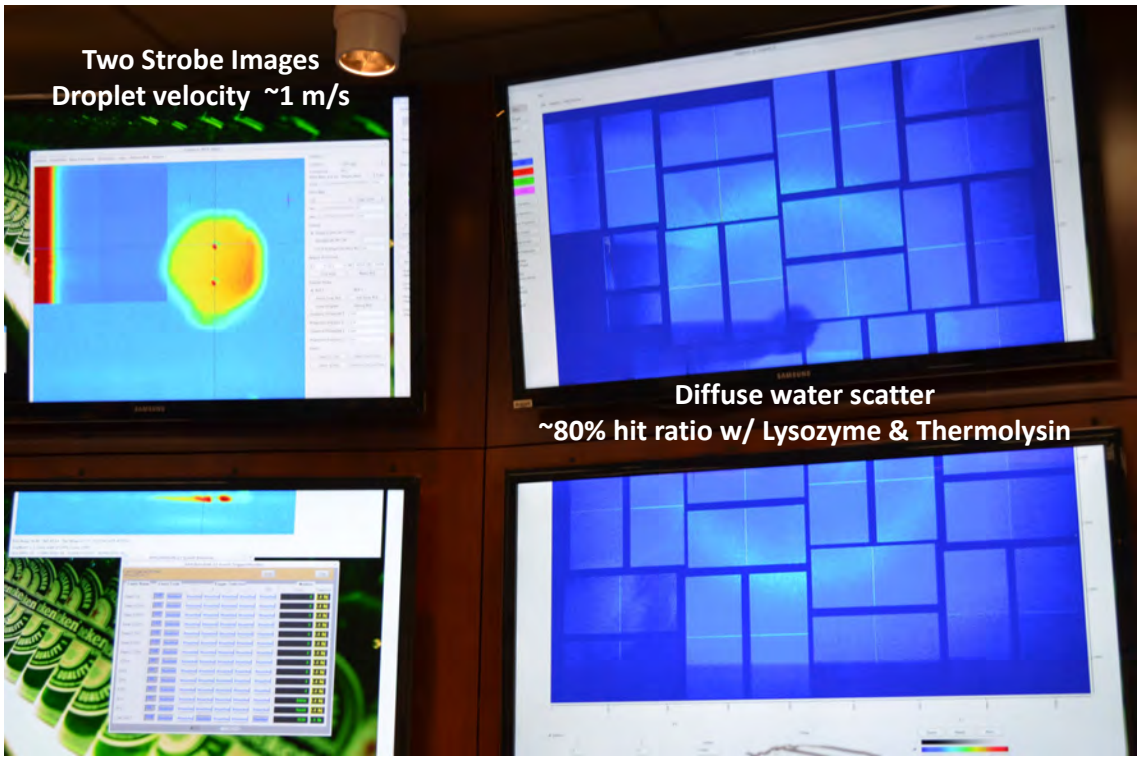
Image: Brad Plummer, SLAC

Injecting ↑ at XPP

Thermolysin	**1.4 M $CaCl_2$ 45% DMSO**	**Plates 10-100 µm**
Lysozyme	**0.5 M NaCl (25% glycerol)**	**Cubes 5-100 µm**



XPP Control Room w/ Labcyte Injector ↑



But

Most (nearly all) of the droplets
did **NOT** yield any diffraction patterns …
→ chemical additives, mixing tests, etc.

In all our test samples …
the crystals sink to the bottom of the well (mother liquor)
We were ejecting from the top of the meniscus

So, during the break we
a) took out the Labcyte instrument and replaced it with
b) the BNL injector turned upside down to inject downward

Inverted Acoustic Injector System ↓

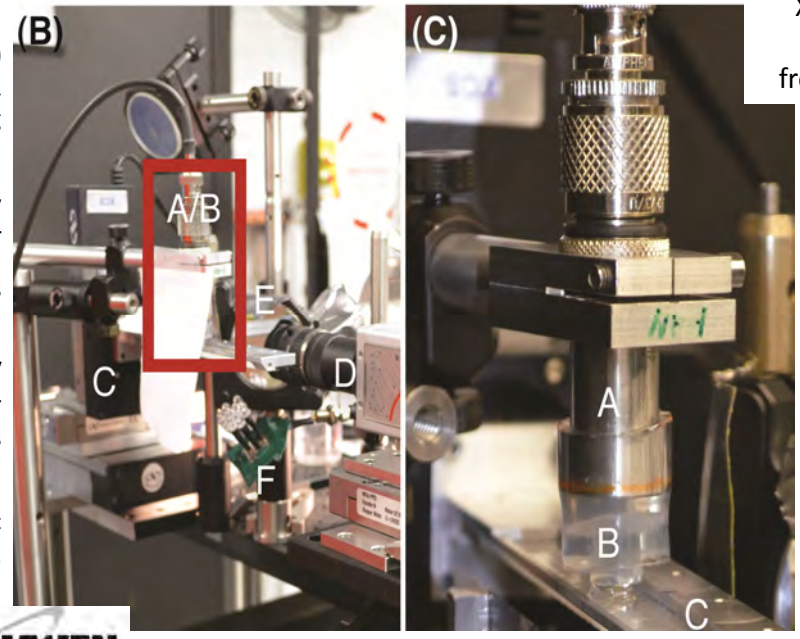
A & B) Transducer & agarose coupling column

C) Sample tray goniometer

D) off-axis infrared camera

E) 45° mirror w/ center hole for on-axis visualization

F) stroboscopic LED array,



X-ray beam comes in from the right

A) Transducer

B) agarose coupling column w/in plastic tubing

C) sample tray goinometer

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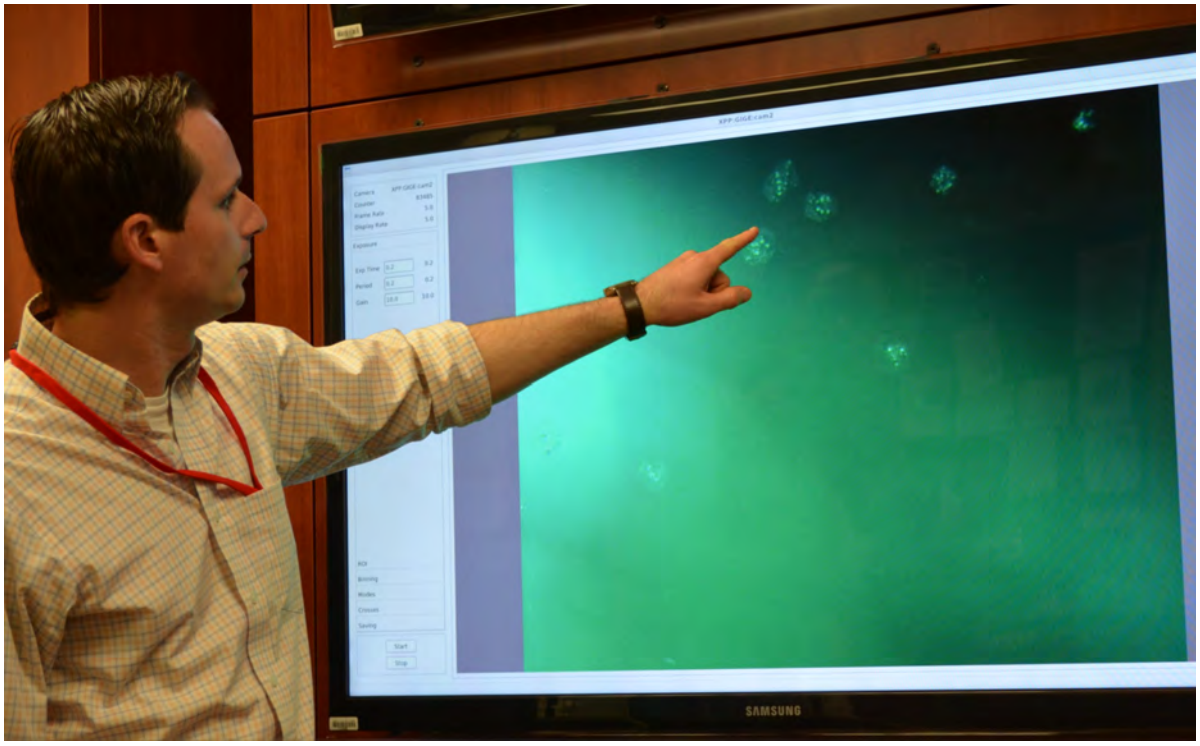
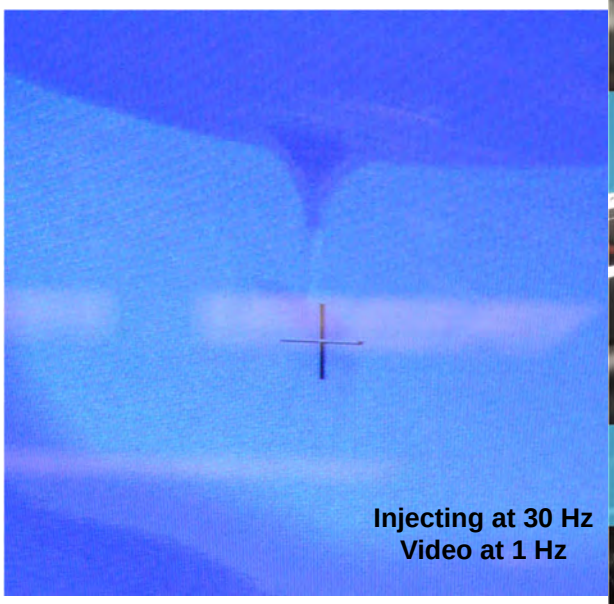
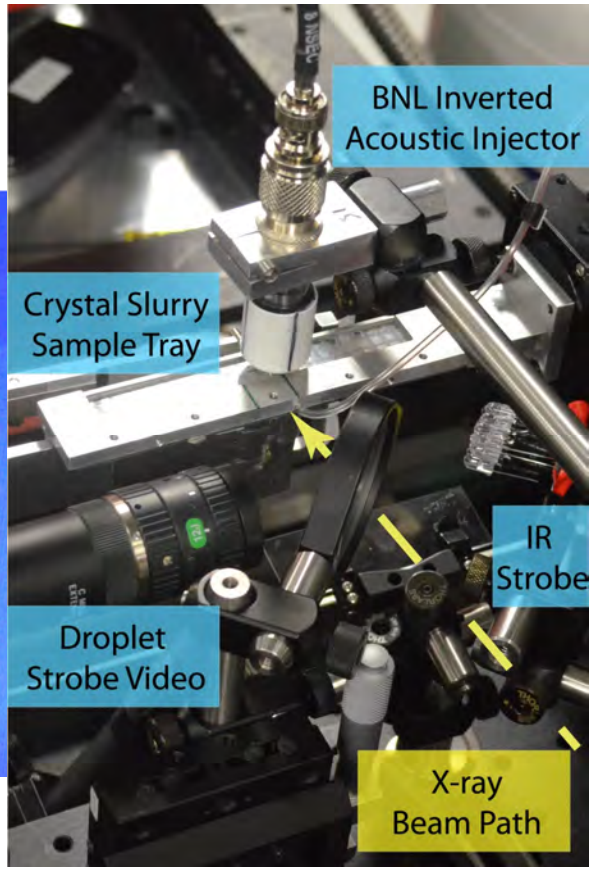
Crystals Ejected with the Inverted Injector Crystals:Droplet Ratio



Acoustic Injection at LCLS / XPP





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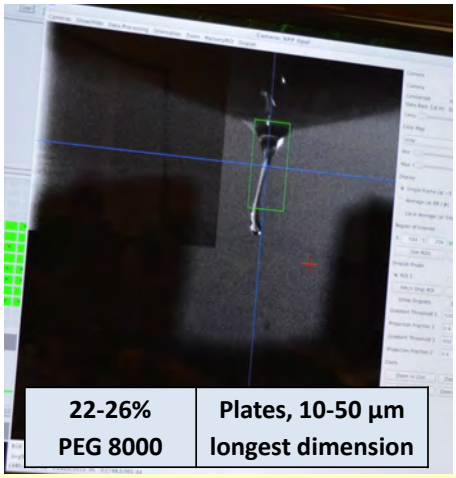
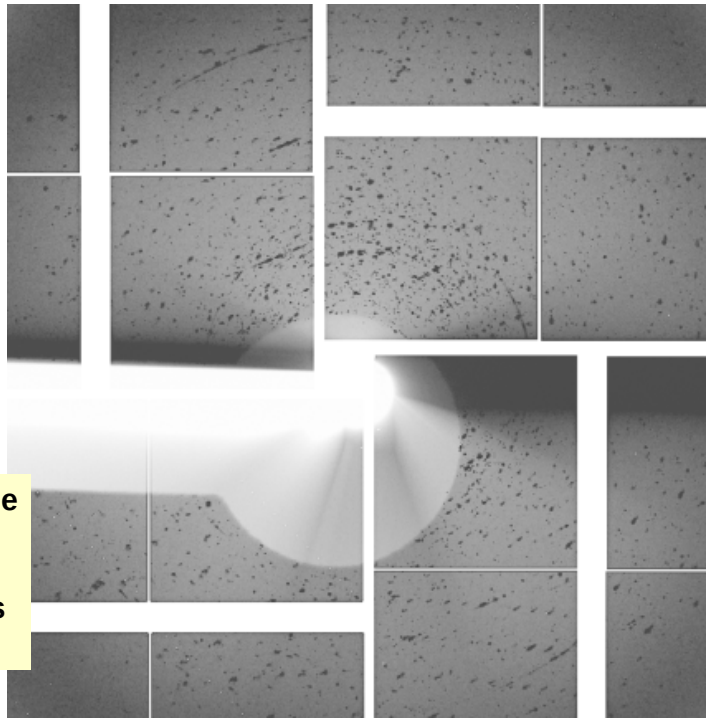
Examples of Crystals (L748 exp.)

Sample	Space group	Unit Cell *a, b, c* (Å) angles (°)	Solvent (%)	Precipitant	Crystal sizes, morphologies
MauG•MADH *bis* Fe(IV)	C2	346 × 55 × 112 90, 107, 90	53	22-26% PEG 8000	Plates, 10-50 µm longest dimension
Stachydrine demethylase E*, ES, E*S	$P6_322$	97 × 97 × 178 90, 90, 120	62	2.5-5% PEG 3350 10% glycerol	Plates & rods, 25-300 µm
Thermolysin	$P6_122$	93 × 93 × 130 90, 90, 120	47	1.4 M $CaCl_2$ 45% DMSO	Plates 10-100 µm
Lysozyme	$P4_32_12$	78 × 78 × 37 90, 90, 90	38	0.5 M NaCl (25% glycerol)	Cubes 5-100 µm
Extracellular hemoglobin (3.6 MDalton)	I222	273 × 320 × 333 90, 90, 90	71	1.6 M K_2HPO_4	Plates, 50-250 µm in the longest dimension
Photosystem II	$P2_12_12_1$	135 × 226 × 303 90, 90, 90	67	8-10% PEG 2000	Rods, 50-400 µm, 50-100 µm or > 150 µm

Composite Maximum Intensity Diffraction for MauG:MADH *bis* Fe(IV)



22-26% PEG 8000	Plates, 10-50 µm longest dimension

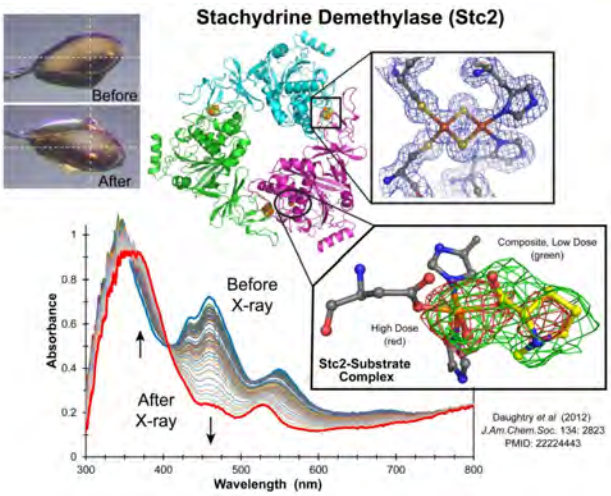


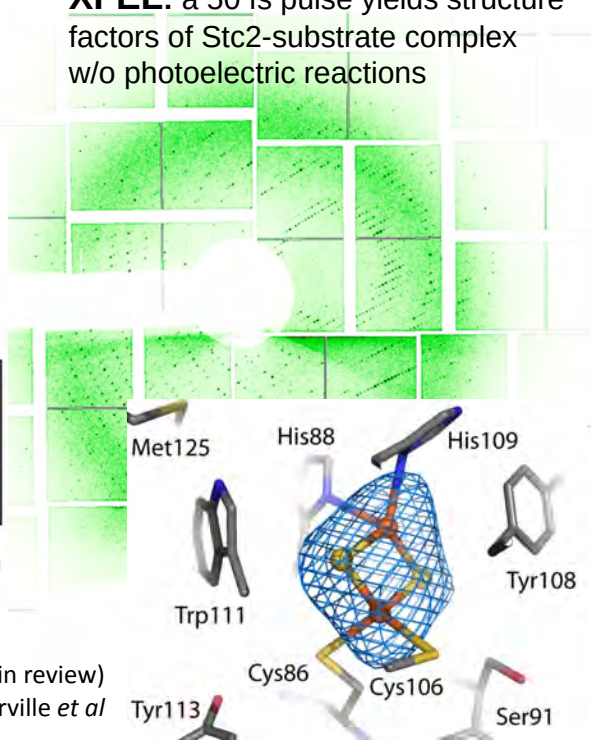
- **Only ~20-30 crystals available**
- **They settle in mother liquor**
- **~1 min. after initiating ↓ injection, diffraction patterns extending to 2.6 Å recorded**

Femtosecond Pulses at the LCLS Can Probe Semi-Stable States & Intermediates

Synchrotron: NSLS Beamline X26-C exploits the photoelectric effect to promote the Stc2 turnover reaction

XFEL: a 50 fs pulse yields structure factors of Stc2-substrate complex w/o photoelectric reactions





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Some of the Participants in Expt. L748

Thank You

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