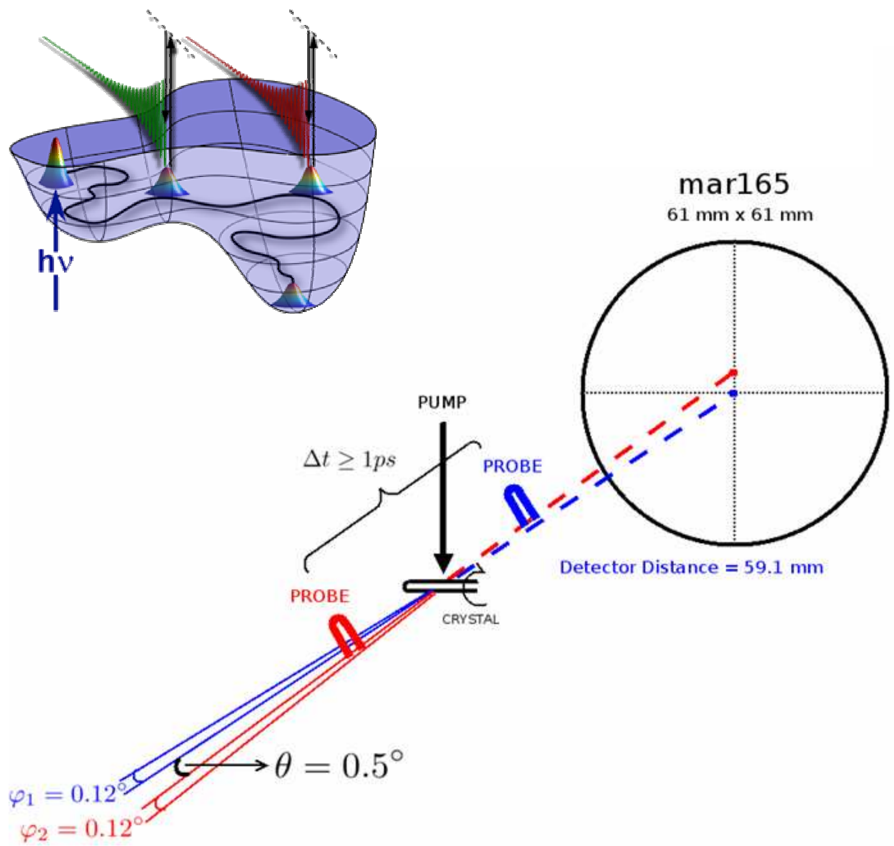
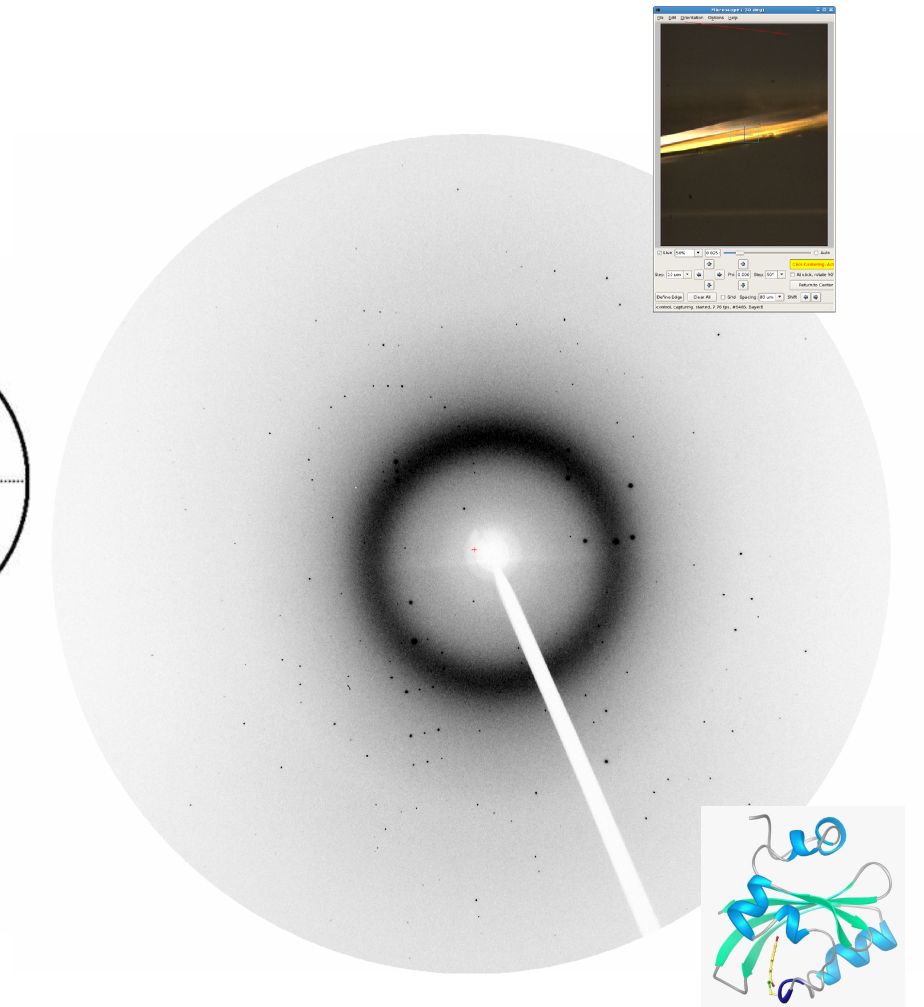
Femtosecond Protein Dynamics using Split-Delay Line Crystallography

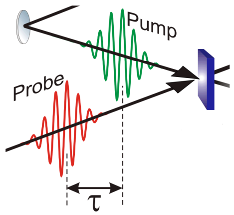
Imperial College London

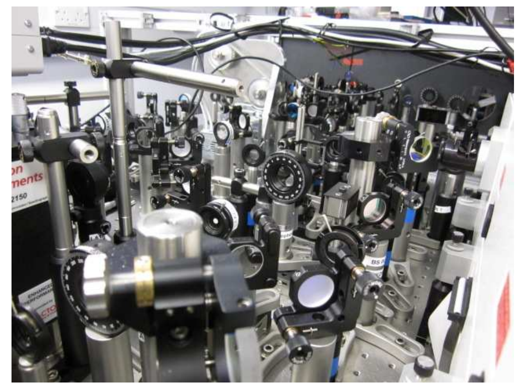
Jasper van Thor



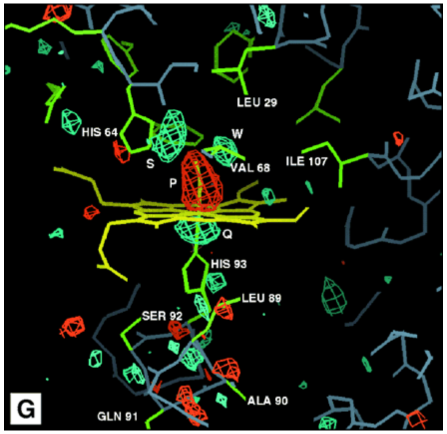


Pump-Probe instruments



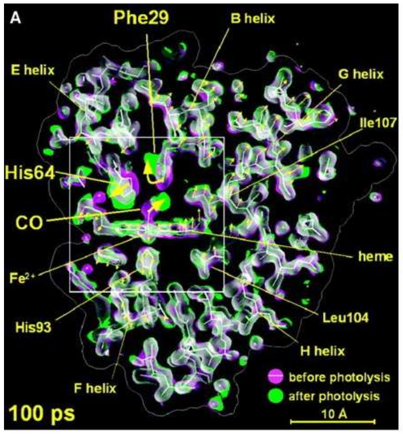
Synchrotron	*XFEL*	*Your lab*
		
50 ps	*fs - ps*	*fs*

Myoglobin-CO



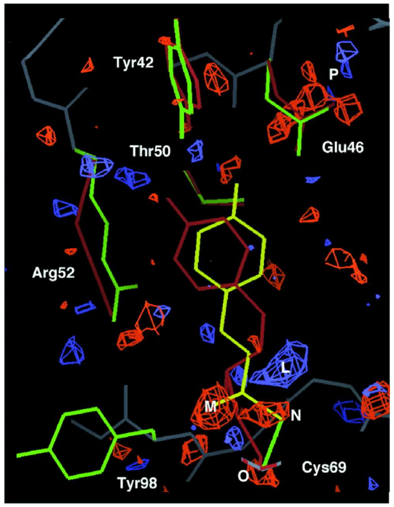
4 ns
Science ***1996***:
274, 1726-1729

Myoglobin-CO



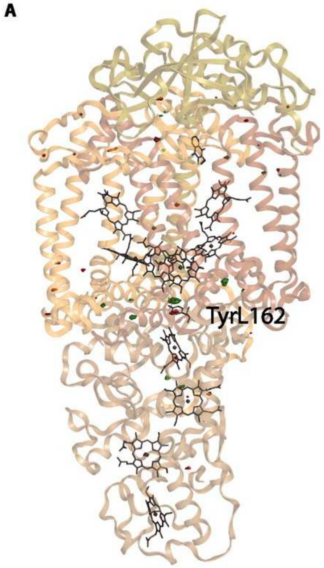
100ps
Science ***2003***:
300, 1944-1947

Photoactive Yellow Protein (PYP)



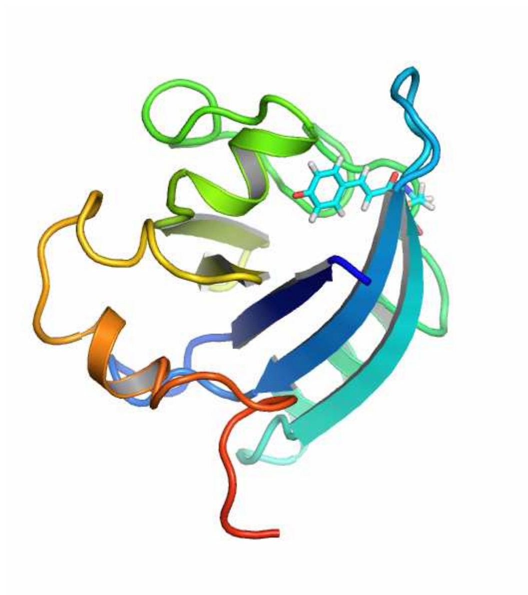
1 ns
Science ***1998***:
279, 1946-1950

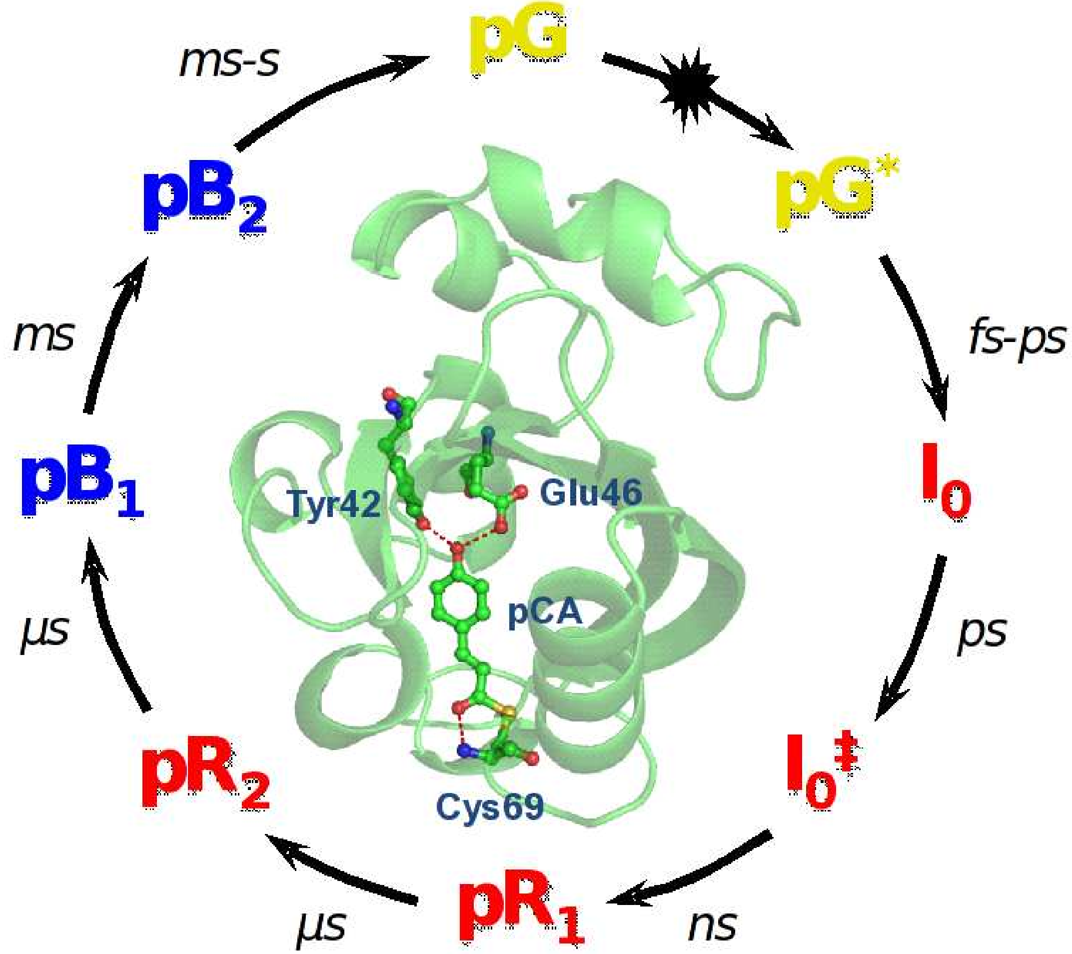
Photosynthetic Reaction Center



3 ms
Science ***2010***:
328, 630-633

Transient structural changes in the PYP photoreceptor



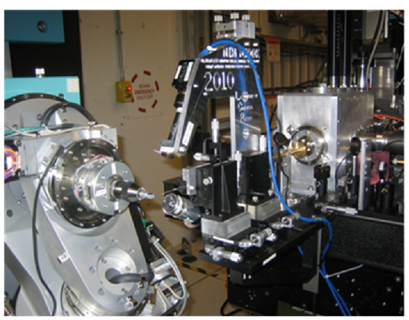


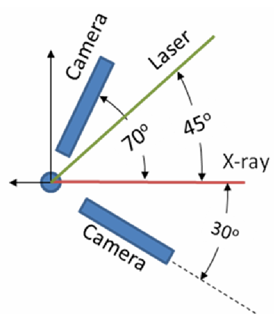
'Photocycle' of the 'Photoactive Yellow Protein'

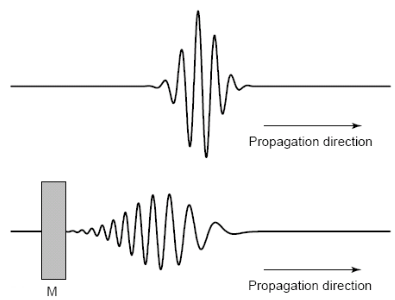
pump-probe fs X-ray diffraction

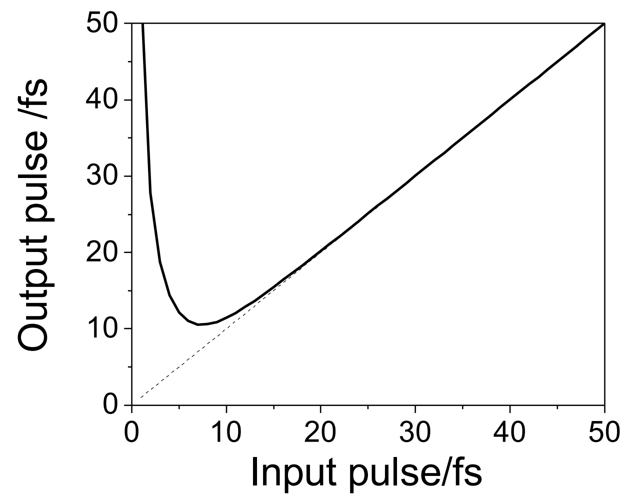
-Time resolution intrinsically limited to ~ 100fs as a result of (1) Multi-photon absorption and (2) group velocity mismatch VIS, X-ray ~ 133 fs/100 μm (group index VIS ~ 1.4) (3) very short intense pulses induce Kerr-effect, self-focussing

- So far, successful pump-probe experiments have relied on *stretching* of optical pulses to avoid non-linear absorption (myoglobin-CO) and circumvents low quantum yield (Photoactive Yellow Protein): No femtosecond synchronisation of sufficient populations has been achieved. Fs time-resolution for X-ray diffraction is dictated by photochemical dynamics





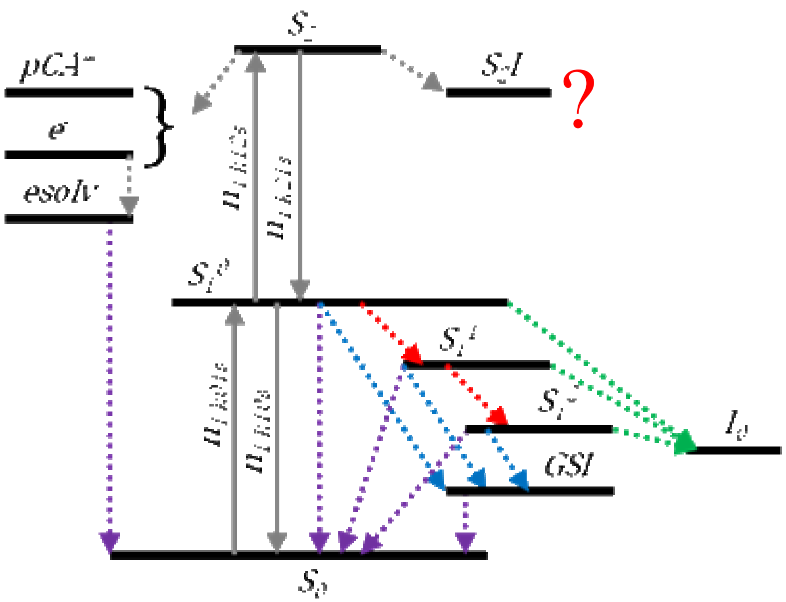


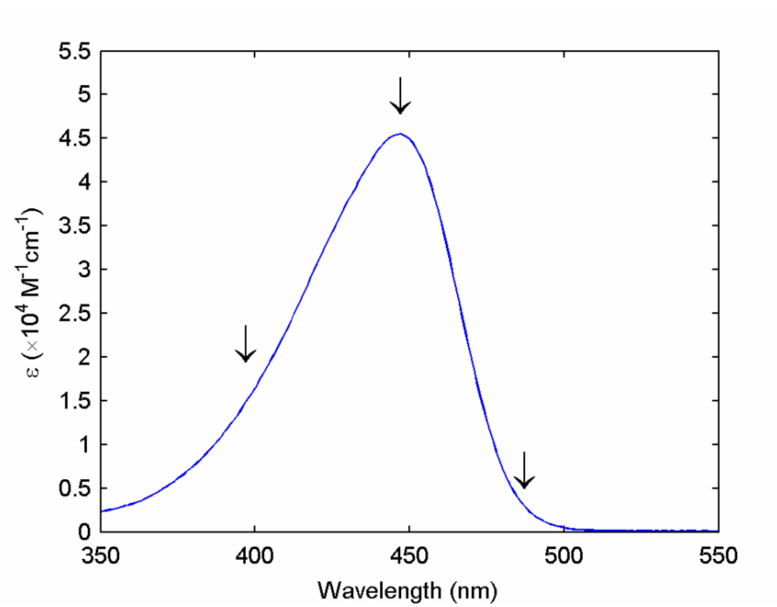


Calculation using GVD 20 fs^2 /100μm of protein crystal ; only expected for < 20 fs pulse duration

PYP

primary photochemical reactions

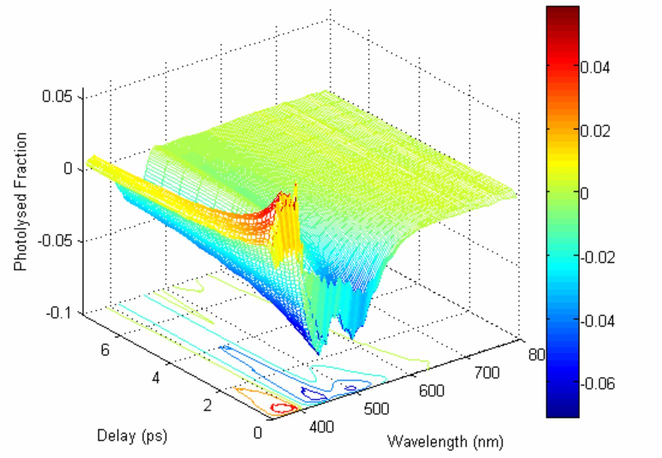


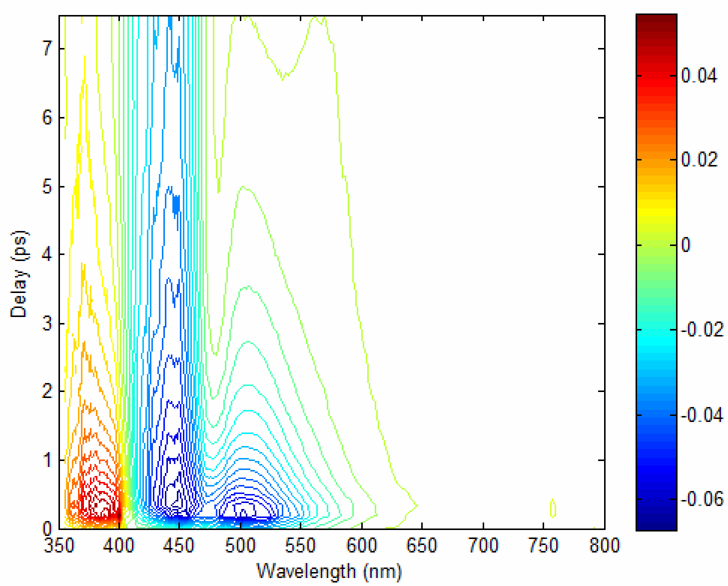


Lincoln et al. 2012. PCCP 14, 15752

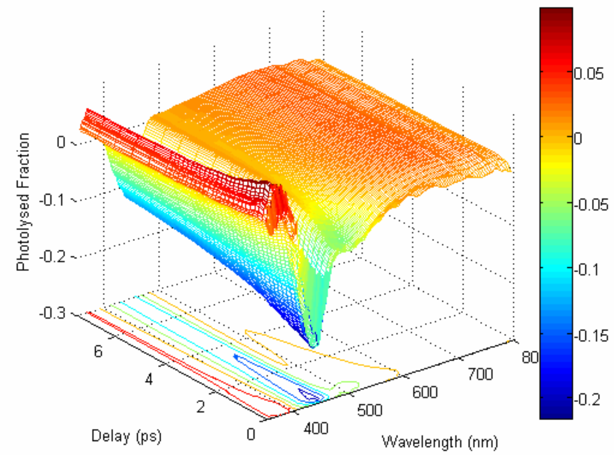
TA – 400 nm

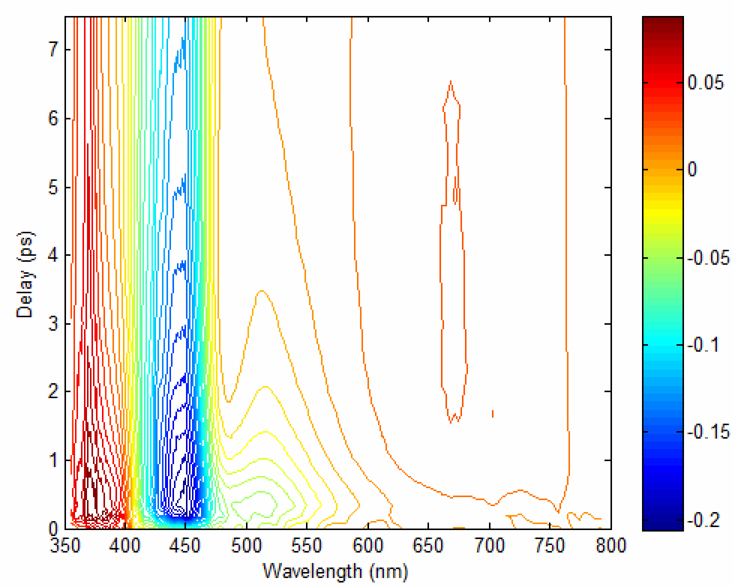
Low Power





High Power





Modelling cross sections from population transfer

$$\frac{d[S_0]}{dt} = -\boldsymbol{k}_{01s}(t)[S_0] + \boldsymbol{k}_{10s}(t)[S_1^0] + \varphi_{S_1^0\to S_0}\boldsymbol{k}_{s_1^0}[S_1^0] + \varphi_{S_1^1\to S_0}\boldsymbol{k}_{s_1^1}[S_1^1] + \varphi_{S_1^2\to S_0}\boldsymbol{k}_{s_1^2}[S_1^2] + \boldsymbol{k}_{s_{GSI}}[S_{GSI}] + \varphi_{S_2\to S_1}\boldsymbol{k}_{S_2}[S_2] \quad (1a)$$

$$\frac{d[S_1^0]}{dt} = \boldsymbol{k}_{01s}(t)[S_0] - \boldsymbol{k}_{10s}(t)[S_1^0] - \boldsymbol{k}_{12s}(t)[S_1^0] + \boldsymbol{k}_{21s}(t)[S_2] - \varphi_{S_1^0\to S_1^1}\boldsymbol{k}_{s_1^0}[S_1^0] - \varphi_{S_1^0\to I_0}\boldsymbol{k}_{s_1^0}[S_1^0] - \varphi_{S_1^0\to S_{GSI}}\boldsymbol{k}_{s_1^0}[S_1^0] - \varphi_{S_1^0\to S_0}\boldsymbol{k}_{s_1^0}[S_1^0] \quad (1b)$$

$$\frac{d[S_1^1]}{dt} = \varphi_{S_1^0\to S_1^1}\boldsymbol{k}_{s_1^0}[S_1^0] - \varphi_{S_1^1\to S_1^2}\boldsymbol{k}_{s_1^1}[S_1^1] - \varphi_{S_1^1\to I_0}\boldsymbol{k}_{s_1^1}[S_1^1] - \varphi_{S_1^1\to S_{GSI}}\boldsymbol{k}_{s_1^1}[S_1^1] - \varphi_{S_1^1\to S_0}\boldsymbol{k}_{s_1^1}[S_1^1] \quad (1c)$$

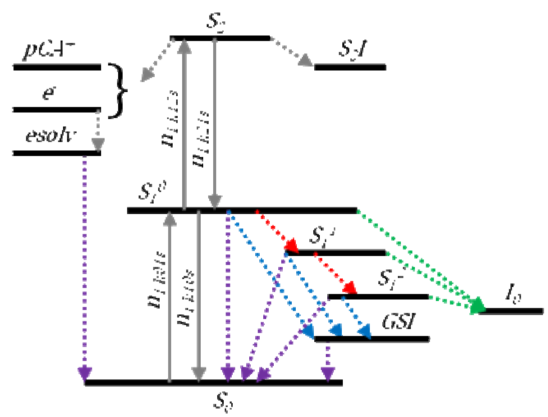
$$\frac{d[S_1^2]}{dt} = \varphi_{S_1^1\to S_1^2}\boldsymbol{k}_{s_1^1}[S_1^1] - \varphi_{S_1^2\to I_0}\boldsymbol{k}_{s_1^2}[S_1^2] - \varphi_{S_1^2\to S_{GSI}}\boldsymbol{k}_{s_1^2}[S_1^2] - \varphi_{S_1^2\to S_0}\boldsymbol{k}_{s_1^2}[S_1^2] \quad (1d)$$

$$\frac{d[I_0]}{dt} = \varphi_{S_1^0\to I_0}\boldsymbol{k}_{s_1^0}[S_1^0] + \varphi_{S_1^1\to I_0}\boldsymbol{k}_{s_1^1}[S_1^1] + \varphi_{S_1^2\to I_0}\boldsymbol{k}_{s_1^2}[S_1^2] - \boldsymbol{k}_{I_0}[I_0] \quad (1e)$$

$$\frac{d[S_{GSI}]}{dt} = \varphi_{S_1^0\to S_{GSI}}\boldsymbol{k}_{s_1^0}[S_1^0] + \varphi_{S_1^1\to S_{GSI}}\boldsymbol{k}_{s_1^1}[S_1^1] + \varphi_{S_1^2\to S_{GSI}}\boldsymbol{k}_{s_1^2}[S_1^2] - \boldsymbol{k}_{S_{GSI}}[S_{GSI}] \quad (1f)$$

$$\frac{d[S_2]}{dt} = \boldsymbol{k}_{12s}(t)[S_1] - \boldsymbol{k}_{21s}(t)[S_2] - \varphi_{S_2\to e^-}\boldsymbol{k}_{S_2}[S_2] - \varphi_{S_2\to S_0}\boldsymbol{k}_{S_2}[S_2] \quad (1g)$$

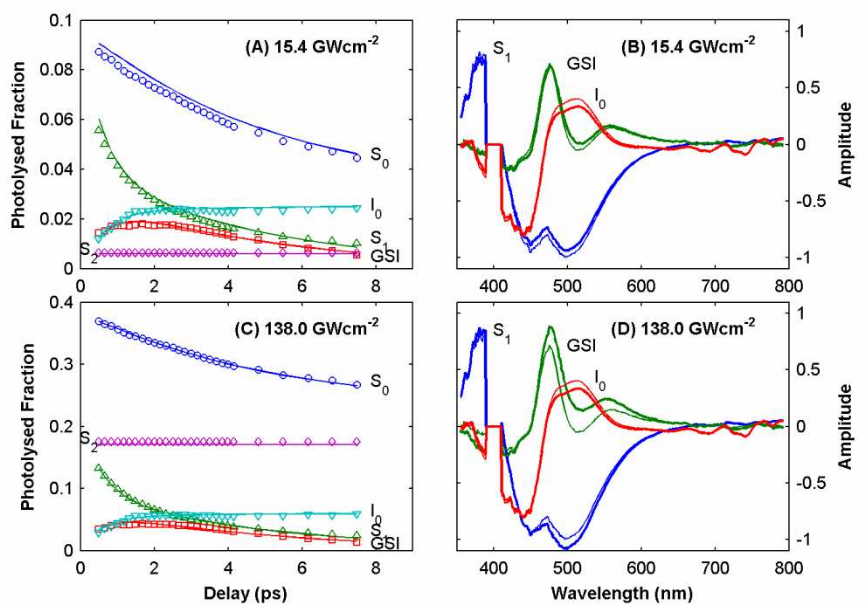
$$\frac{d[e^-]}{dt} = \varphi_{S_2\to e^-}\boldsymbol{k}_{S_2}[S_2] \quad (1h)$$

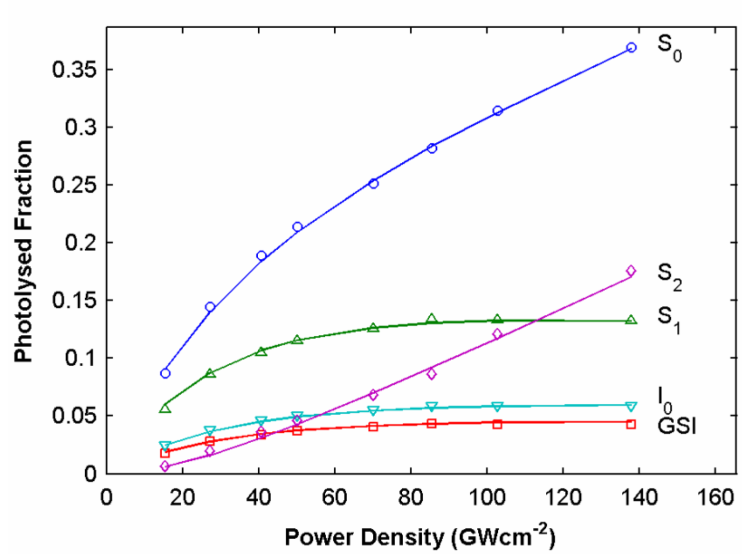


$$\boldsymbol{k}_{ijs}(t) = 3J(t)\,\sigma_{ij}\,\Phi_{ij}\cos^2\theta$$

$$\sigma_{ij} = \frac{1000\times\ln(10)\times\varepsilon_{ijs}}{N_A}$$

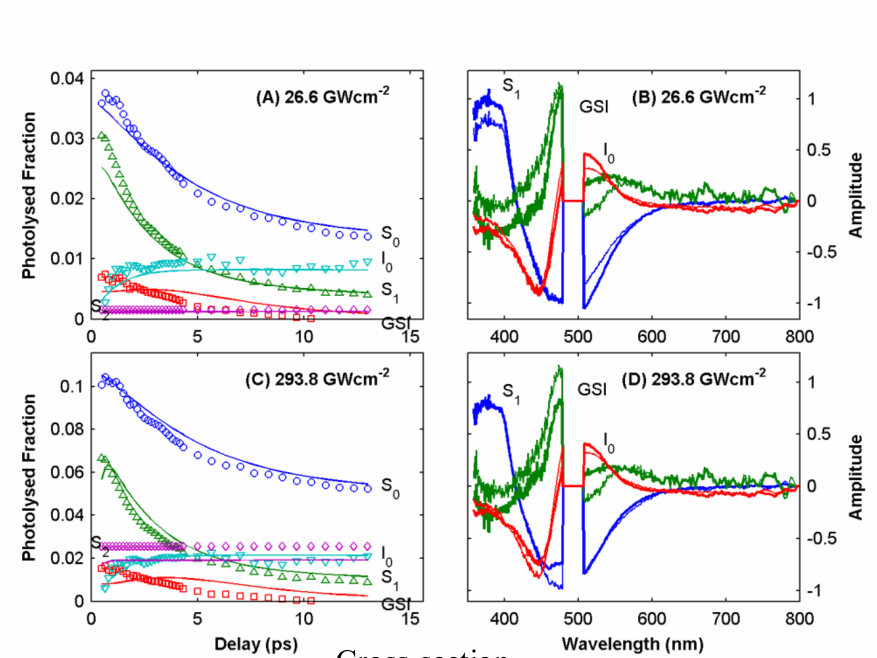
Target Analysis - 400 nm

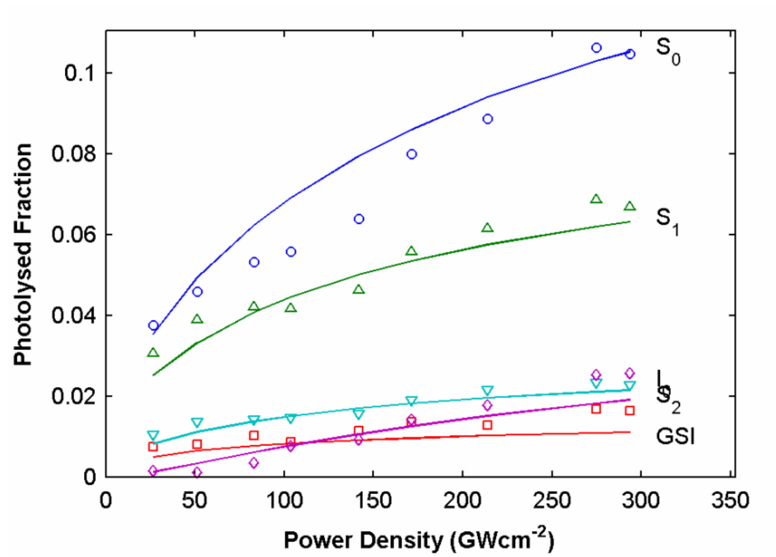




Cross-section (cm²/molecule)			
σ_{01s}	σ_{10s}	σ_{12s}	σ_{21s}
6.31E-17	7.48E-17	3.11E-17	0.00
Branching Yields			
States	$S_1{}^0$	$S_1{}^1$	$S_1{}^2$
S_0	0.05	0.30	0.99
S_{GSI}	0.28	0.61	0.00
I_0	0.26	0.06	0.00
$S_1{}^{i+1}$	0.41	0.02	-
lifetime	0.6	4.6	40.0

Target Analysis - 490 nm





Cross-section

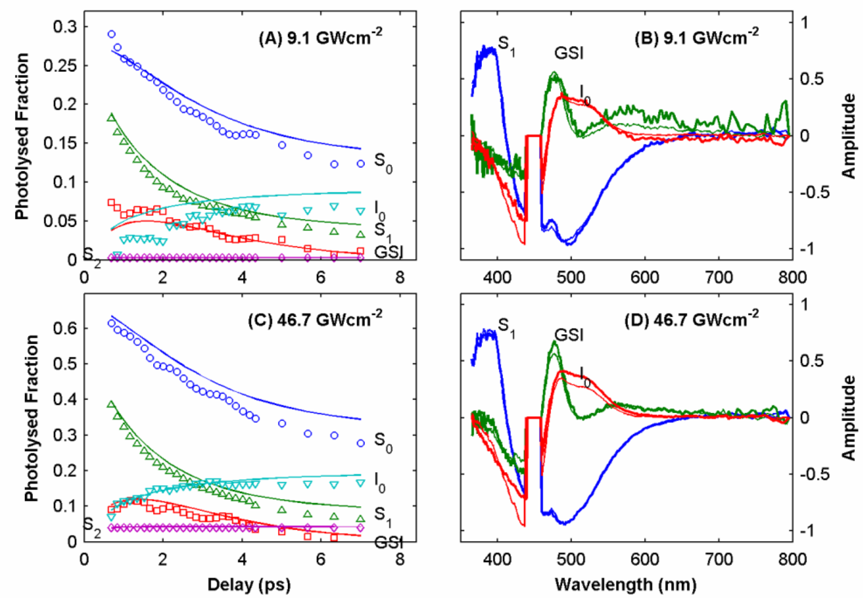
(cm^2/molecule)

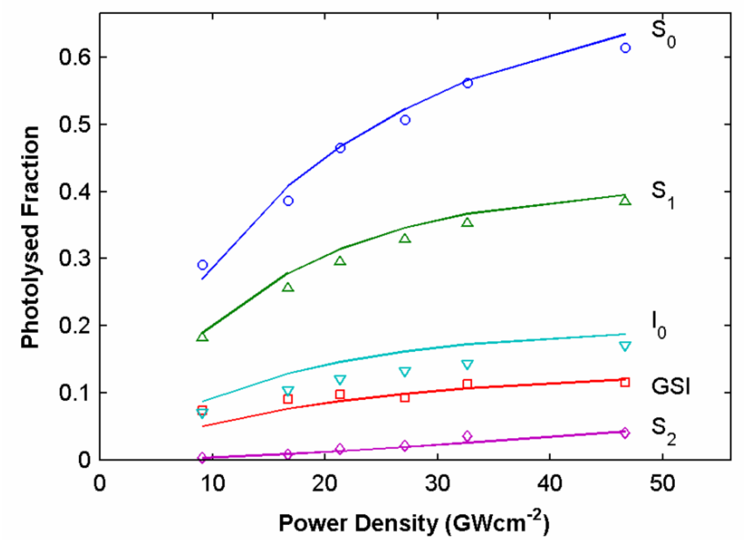
σ_{01s}	σ_{10s}	σ_{12s}	σ_{21s}	σ_{1gsi}	σ_{gsi2}
1.26E-17	2.09E-16	8.41E-18	0.00	6.80E-17	2.48E-16

Branching Yields

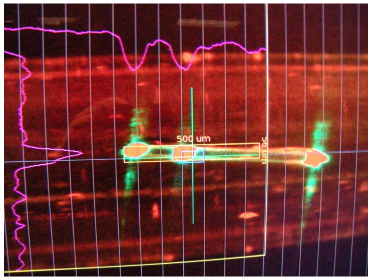
States	$S_1^{\,0}$	$S_1^{\,1}$	$S_1^{\,2}$
S_0	0.06	0.33	0.00
S_{GSI}	0.00	0.41	1.00
I_0	0.27	0.00	0.00
S_1^{i+1}	0.67	0.26	-
lifetime	1.1	2.5	40.0

Target Analysis - 450 nm



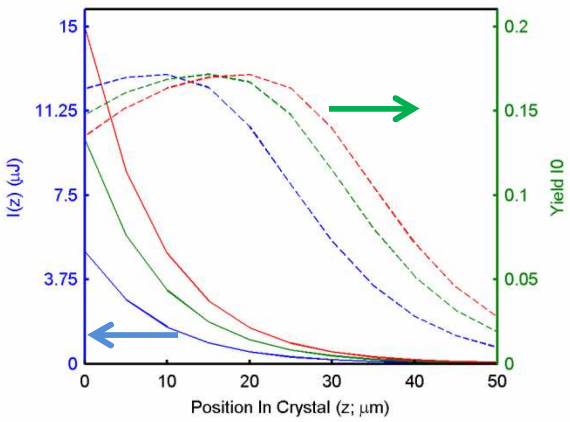


Cross-section (cm²/molecule)			
σ_{01s}	σ_{10s}	σ_{12s}	σ_{21s}
1.68E-16	7.04E-17	1.33E-17	0.00
Branching Yields			
States	S_1^0	S_1^1	S_1^2
S_0	0.00	0.02	0.18
S_{GSI}	0.13	0.52	0.08
I_0	0.15	0.22	0.74
S_1^{i+1}	0.72	0.24	-
lifetime	0.4	1.7	30.0

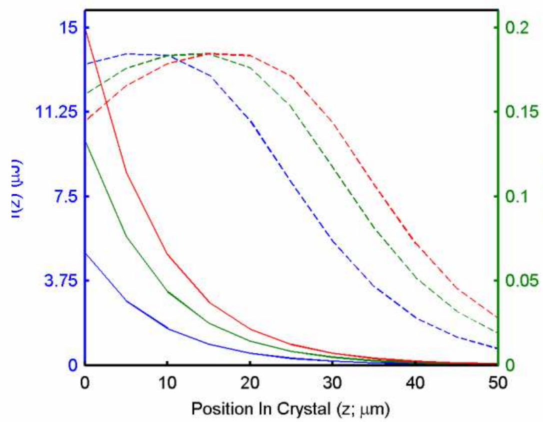


I0 Yield

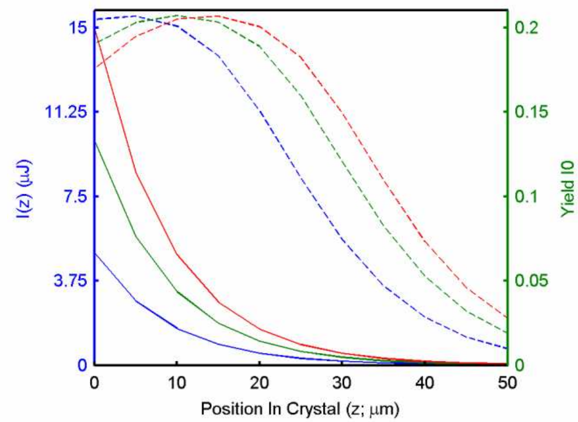
100 fs

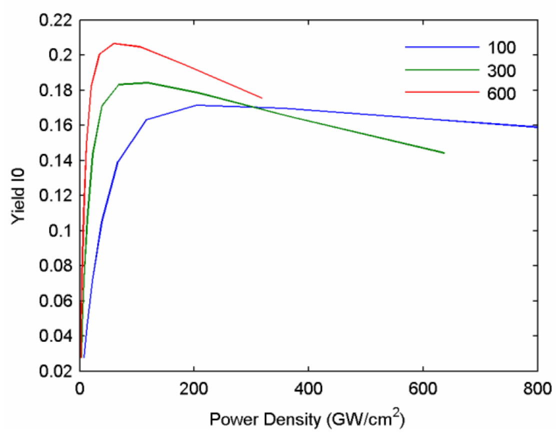


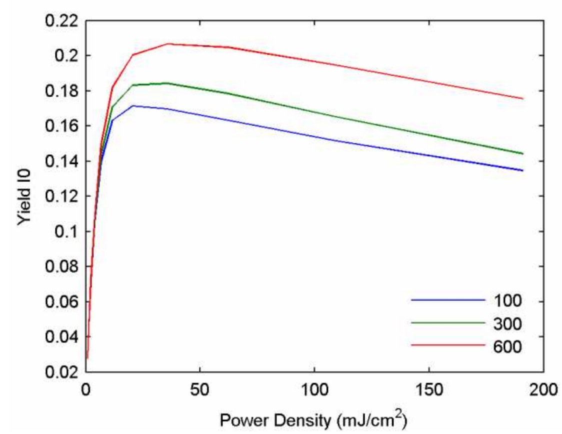
300 fs



600 fs







December 2010:xpp23410

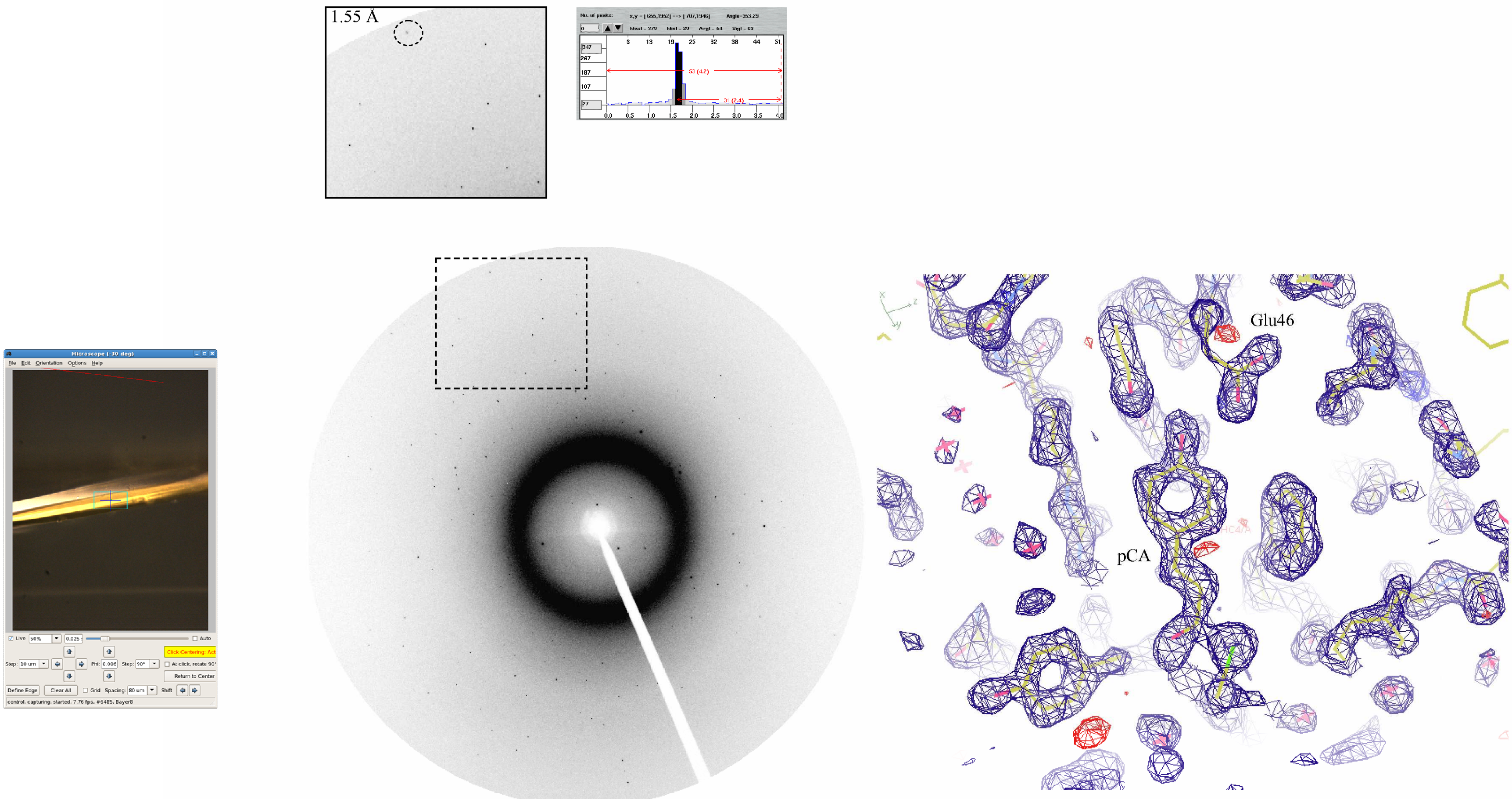


Figure 4. Diffraction of P63 PYP crystals at the XPP beamline, at 8.989 keV, 75 fs and a 2eV bandpass using the 111 monochromator at a detector distance of 68.8mm (1.55 A resolution). Data was scaled, merged (40 degree range at 0.2 degree interval, 85% completeness, 15026 unique reflections, 1.55 A resolution) and phased by rigid body refinement (R = 0.38, R_free = 0.38). Preliminary maps are shown for the ground state (2Fo-Fc; blue) and Fourier difference density at 5 ps delay (F_5ps-F_off; red), with signals visible at the chromophore (pCA) and Glu46. Poor statistics of the preliminary data are caused by collecting with rotations larger than the mosaic spread (which is 0.1 degree)

April 2012: xpp44112

Signal to noise considerations for single crystal femtosecond time resolved crystallography of the Photoactive Yellow Protein

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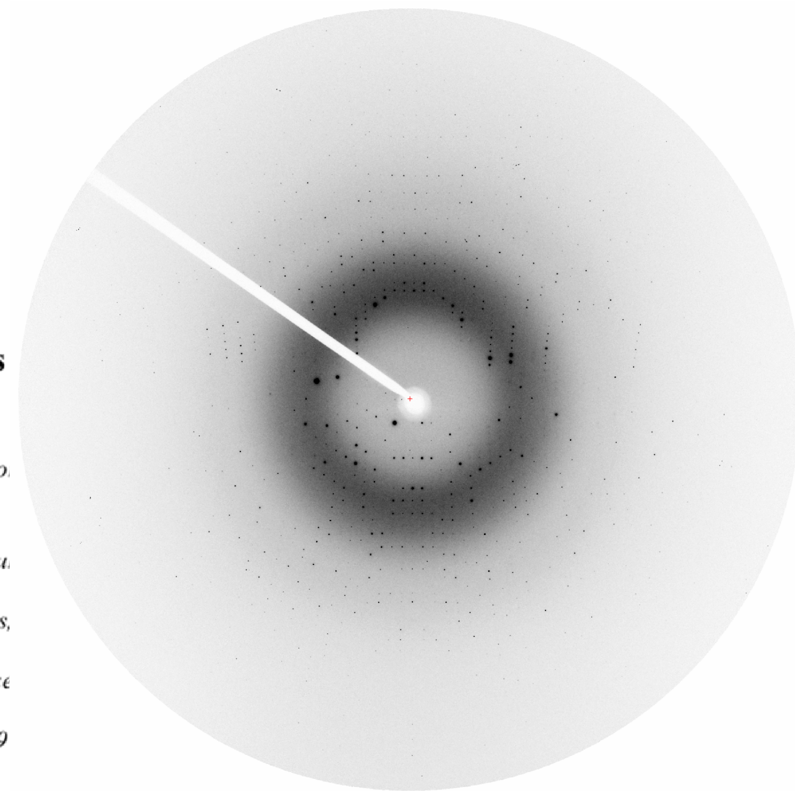
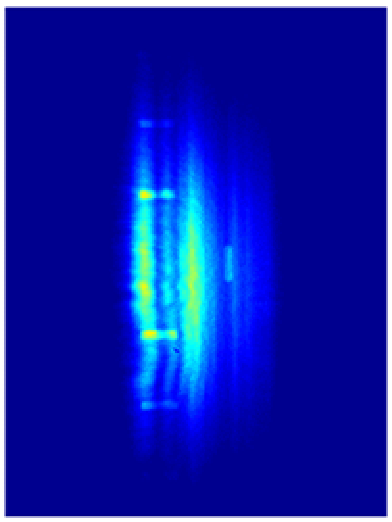


Faraday Disc., 2014, DOI: 10.1039/C4FD00011K

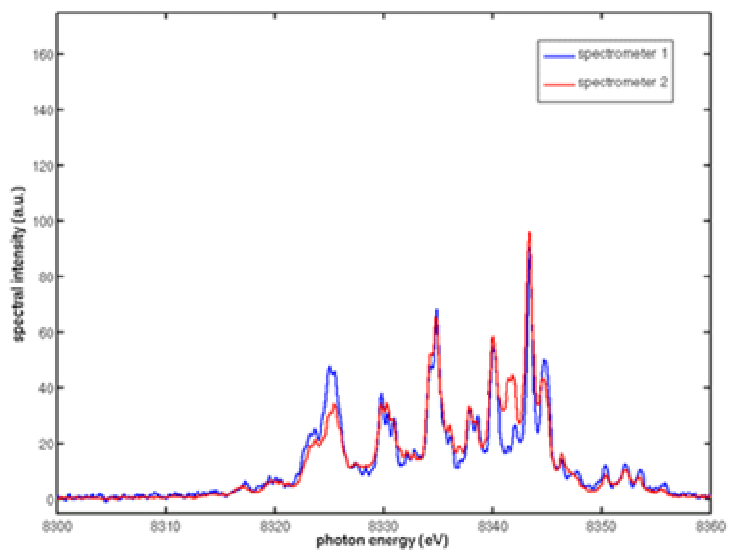
X-rays: 1.3009 Å, ~70 fs, 1.6 eV bandpass,40x40 μm
Pump-probe cross-correlation: ~ 280fs
Pump-probe delays: 600 fs & 900 ns

Crystallographic data quality and reflection amplitude noise

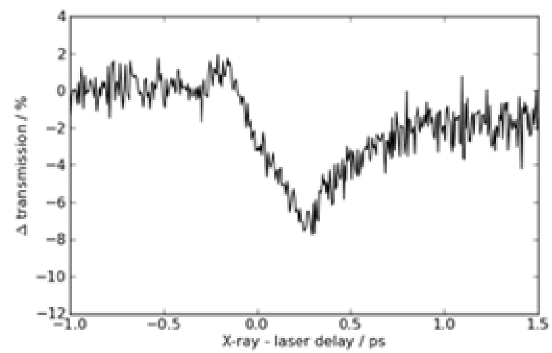
Spatial



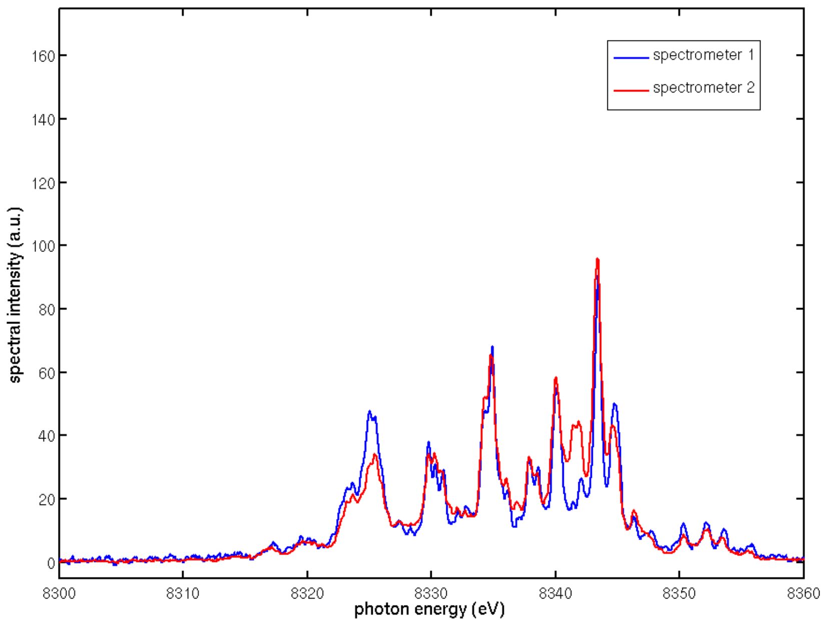
Spectral



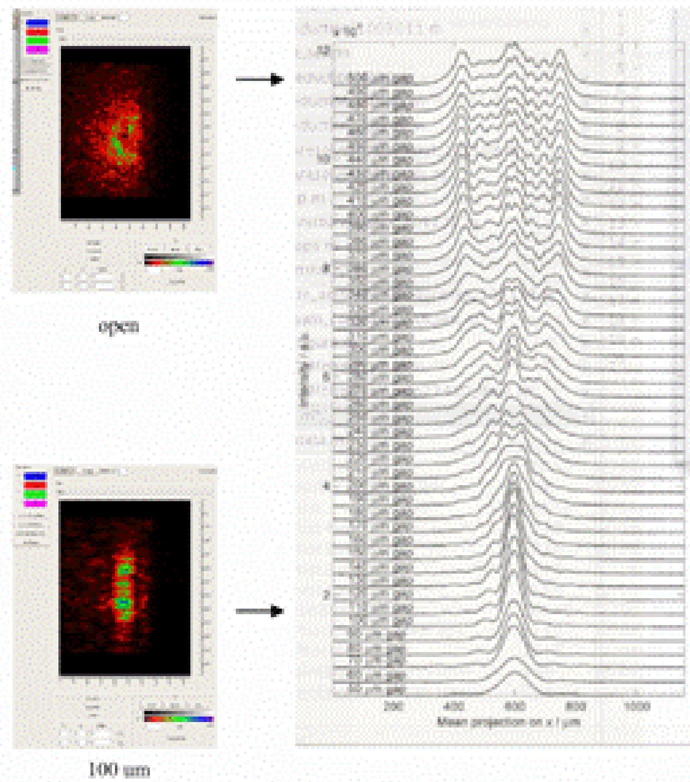
Temporal



Crystallographic data quality and reflection amplitude noise:
Energy selection and spatial selection

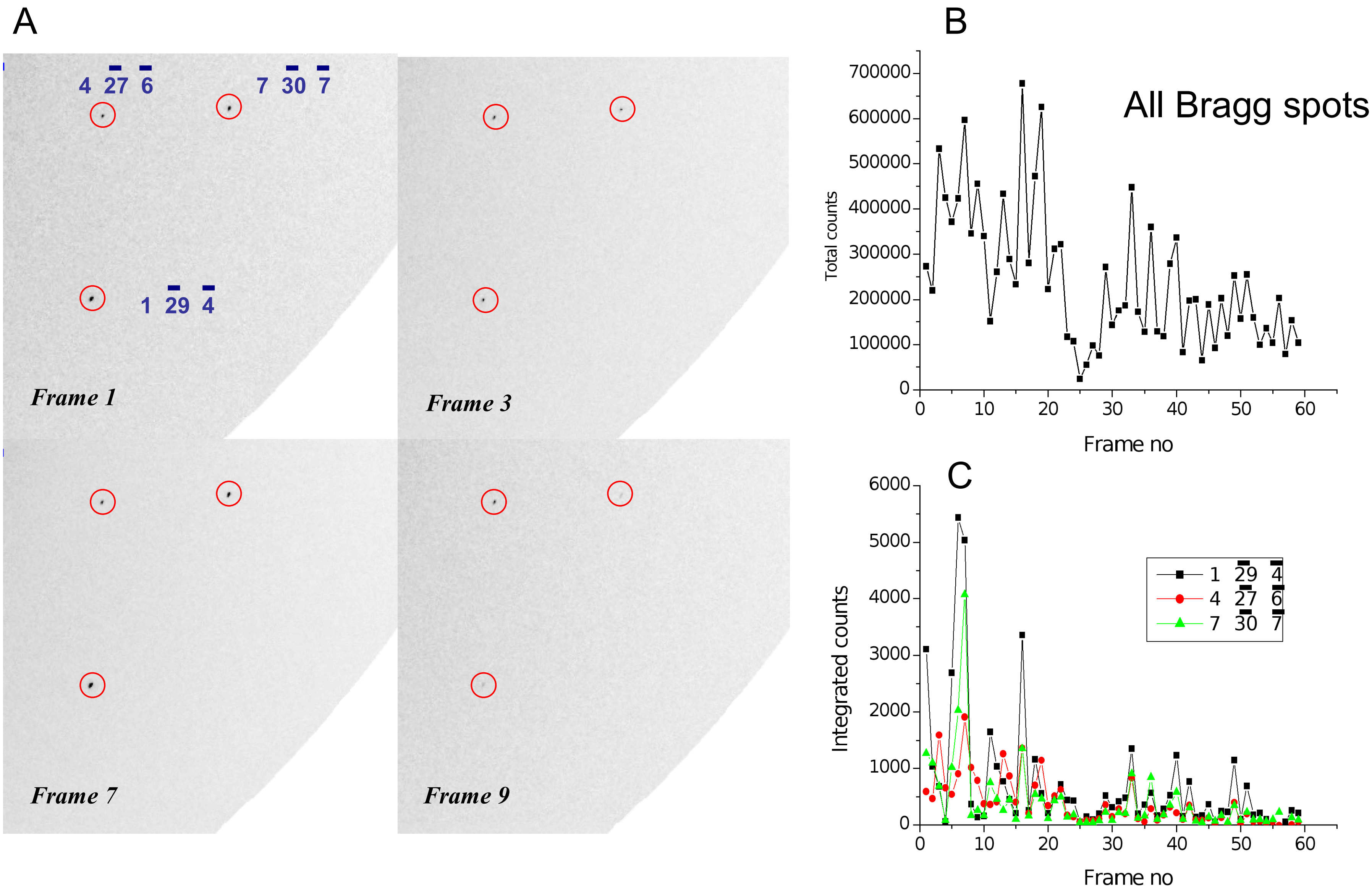


Spectral Jitter

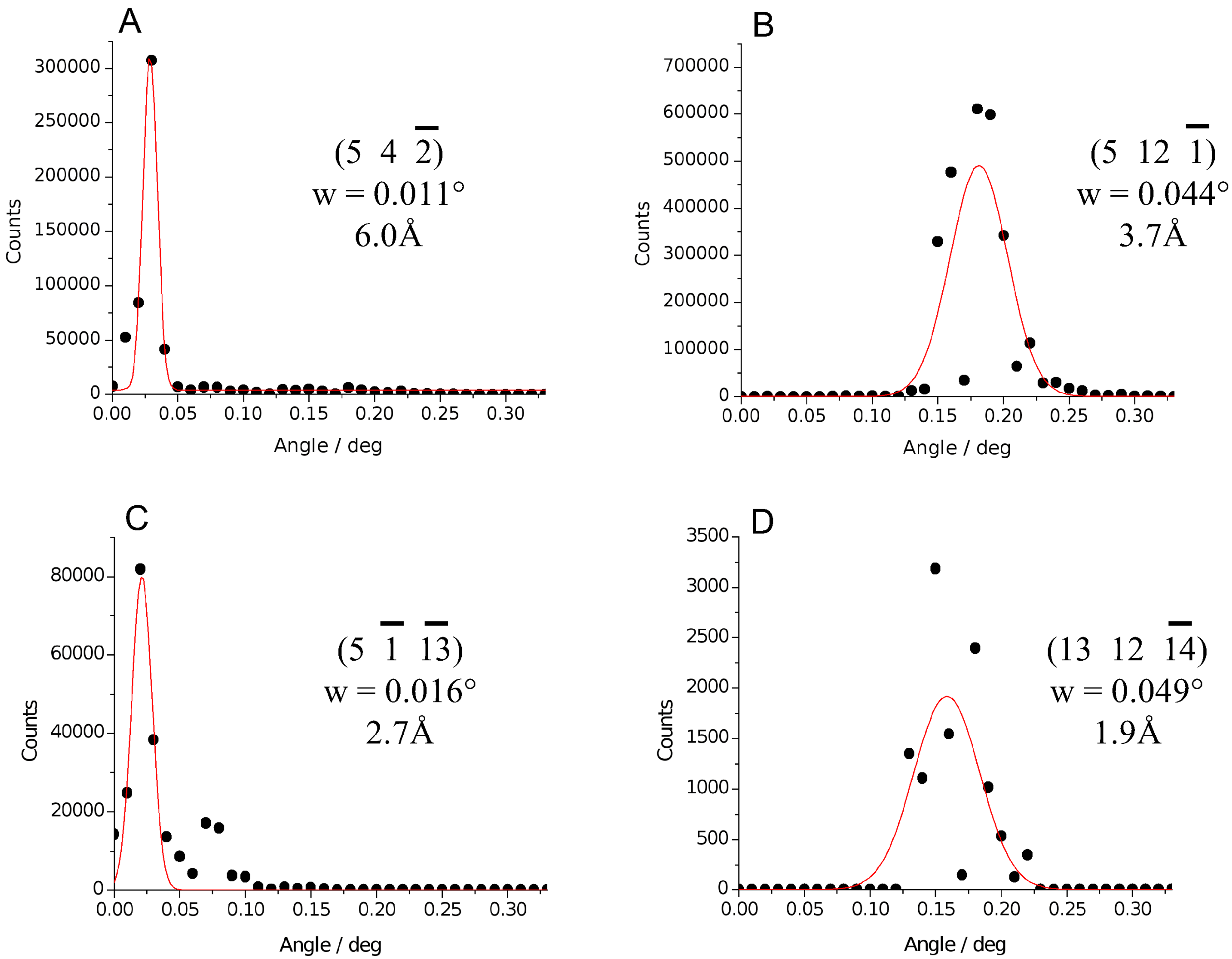


Mode distortions

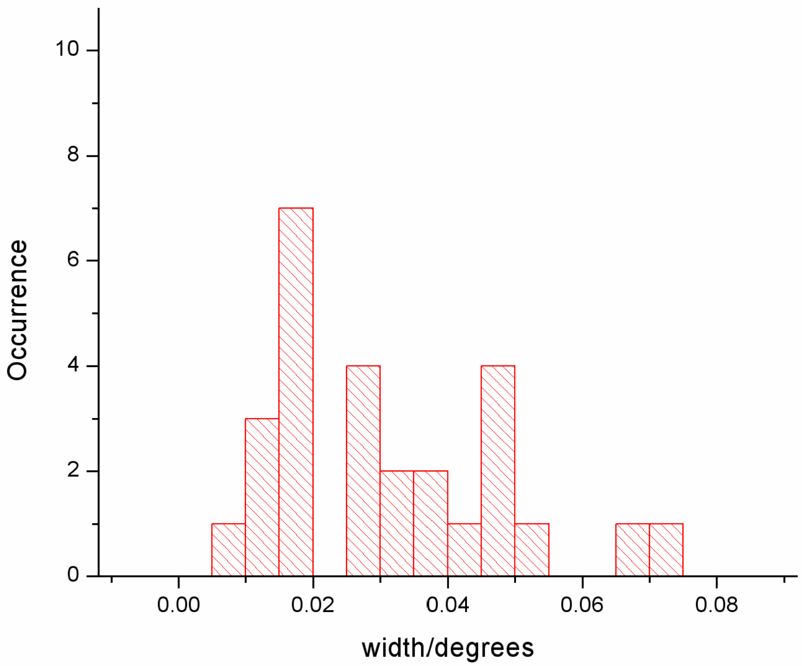
Static crystal orientation, monochromator energy selection 1.6 eV bandpass

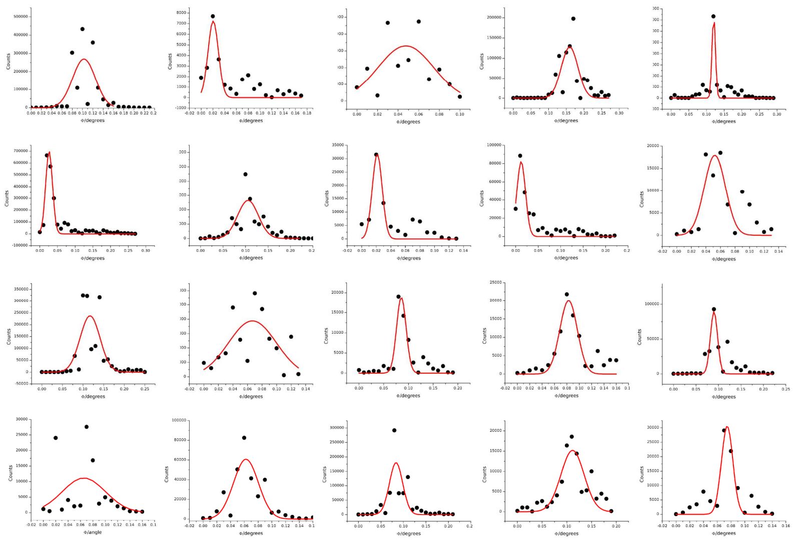


A convergence of 0.007° allows rocking curve measurements

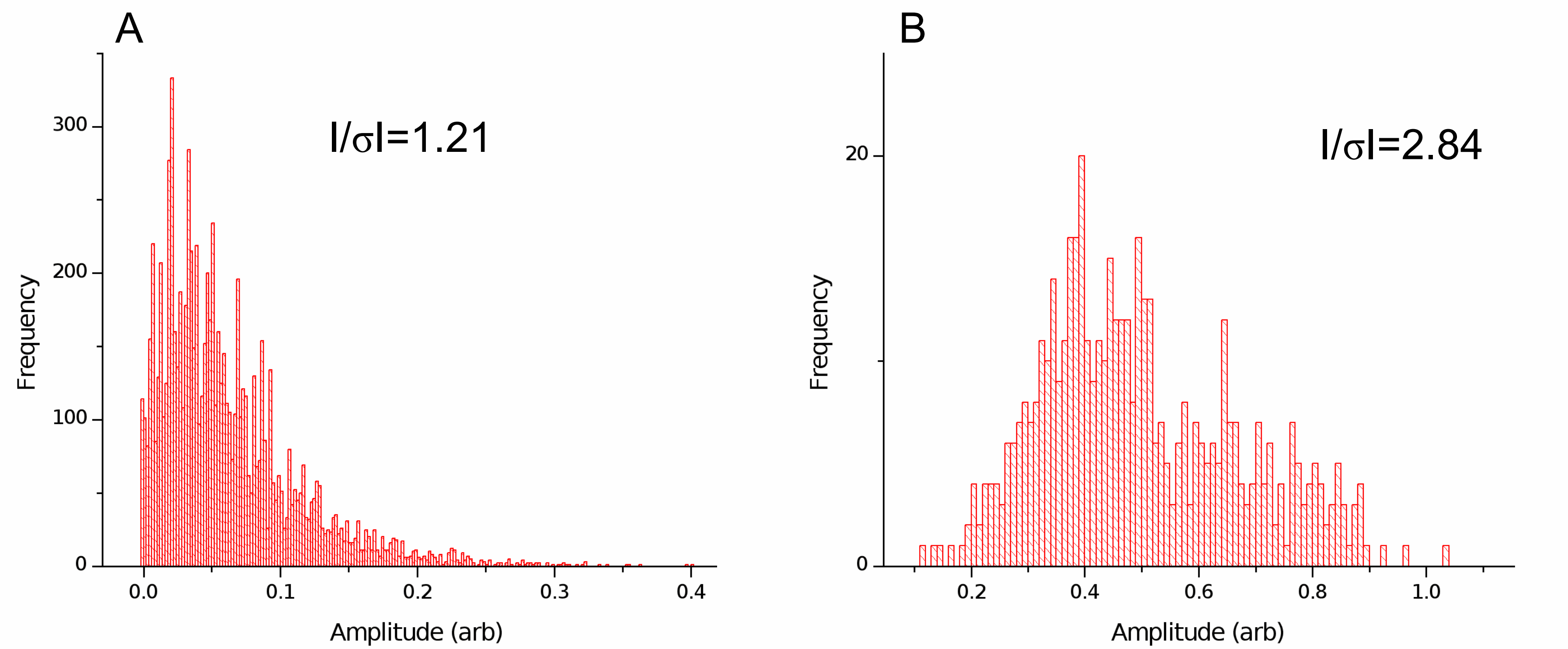


Spread and line defects of rocking curve widths





X-ray intensity noise



xpp44112
8,475 pulse intensities from SASE mode at 9.47 keV with a monochromator.

500 pulses from **self-seeded** mode at 8.4 keV using the entire length of the undulator
Courtesy of Paul Emma and Alberto Lutman, LCLS

MODEL:
Integrated intensity
0.01° rotation/shot

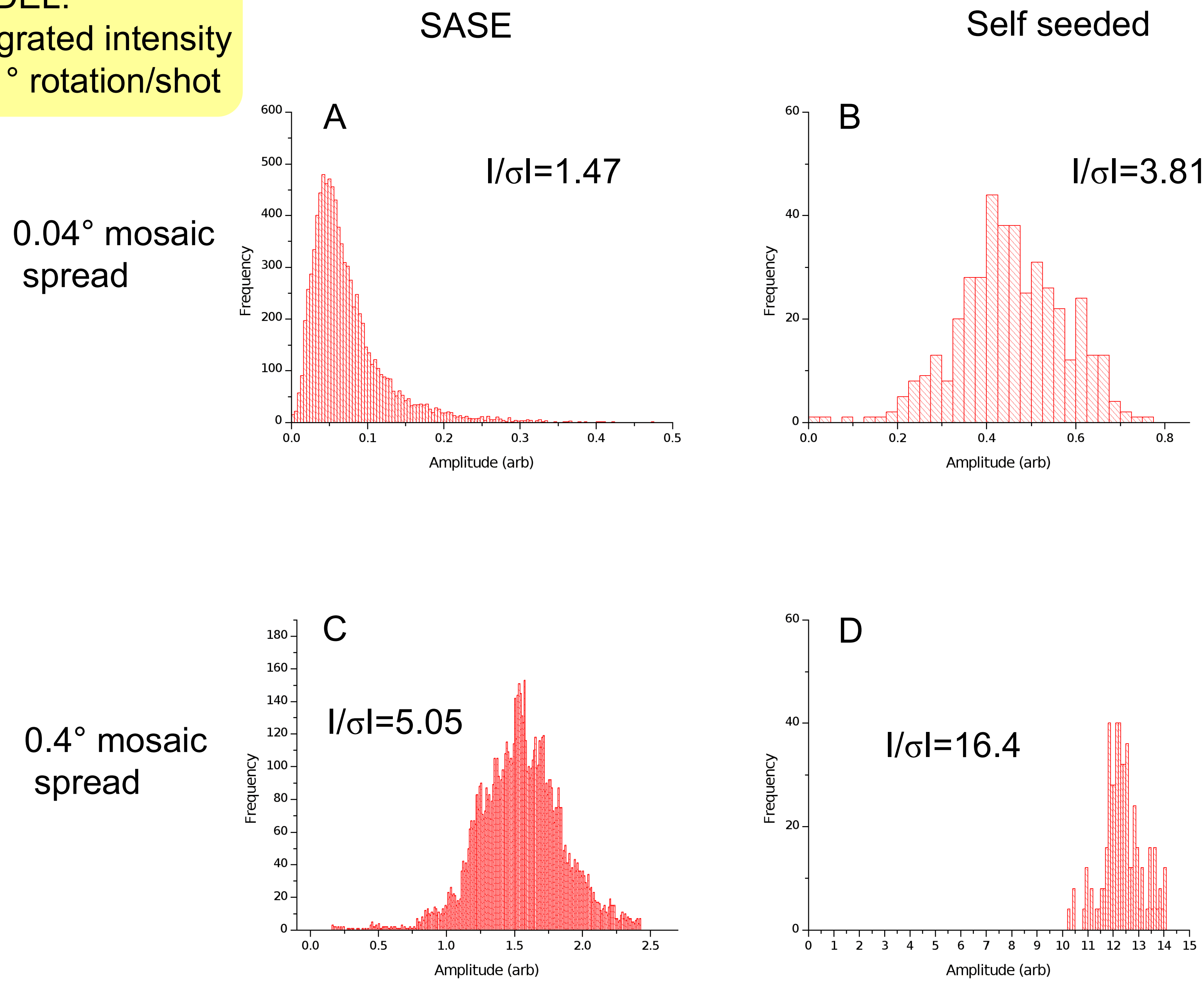


Table 1 Data reduction statistics

	Multi-crystal dataset	Single-crystal dataset
# of frames / # of crystals	96/6	23/1
Space group	$P6_3$	$P6_3$
Unit cell (a,b,c(Å);α,β,γ(°))	66.934, 66.934, 40.986 90.000 90.000, 120.000	66.867, 66.867, 40.985 90.000 90.000, 120.000
Resolution range (Å)	57.97-1.60	100-1.50
Reflections (Total/Unique)	137,459/13,877	20,145/16,947
Rejected outlier (#/%)	9,986/7.3	1,286/6.4
Completeness[a]	84.3(63.6)	51.4(21.6)
I/σI	3.1(1.4)	7.1(1.5)
Average Multiplicity	4.8	1.2
R_{merge}[b]	0.400 (0.534)	0.239 (0.470)
R_{meas}[c]	0.445 (0.614)	N.D.
$R_{p.i.m.}$[d]	0.190 (0.289)	N.D.
PCV[e]	0.635 (0.822)	N.D.
R_{crys}/R_{free} (%)	0.26 (0.23)	N.D.

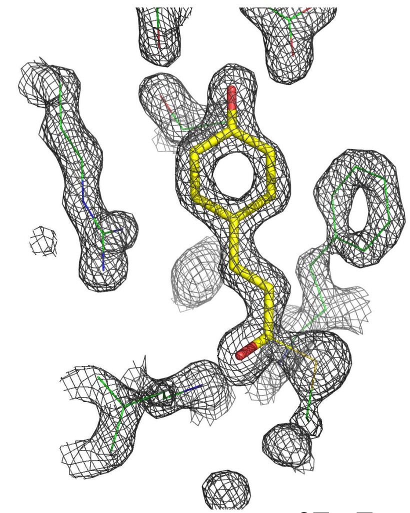
[a] Numbers appearing in parenthesis are for the high resolution shell.

[b] $$R_{merge}(I) = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$$

[c] $$R_{meas}(I) = \sum_{hkl}\sum_i^n \left(\frac{n}{n-1}\right)^{1/2} |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$$

[d] $$R_{p.i.m.}(I) = \sum_{hkl}\sum_i^n \left(\frac{1}{n-1}\right)^{1/2} |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$$

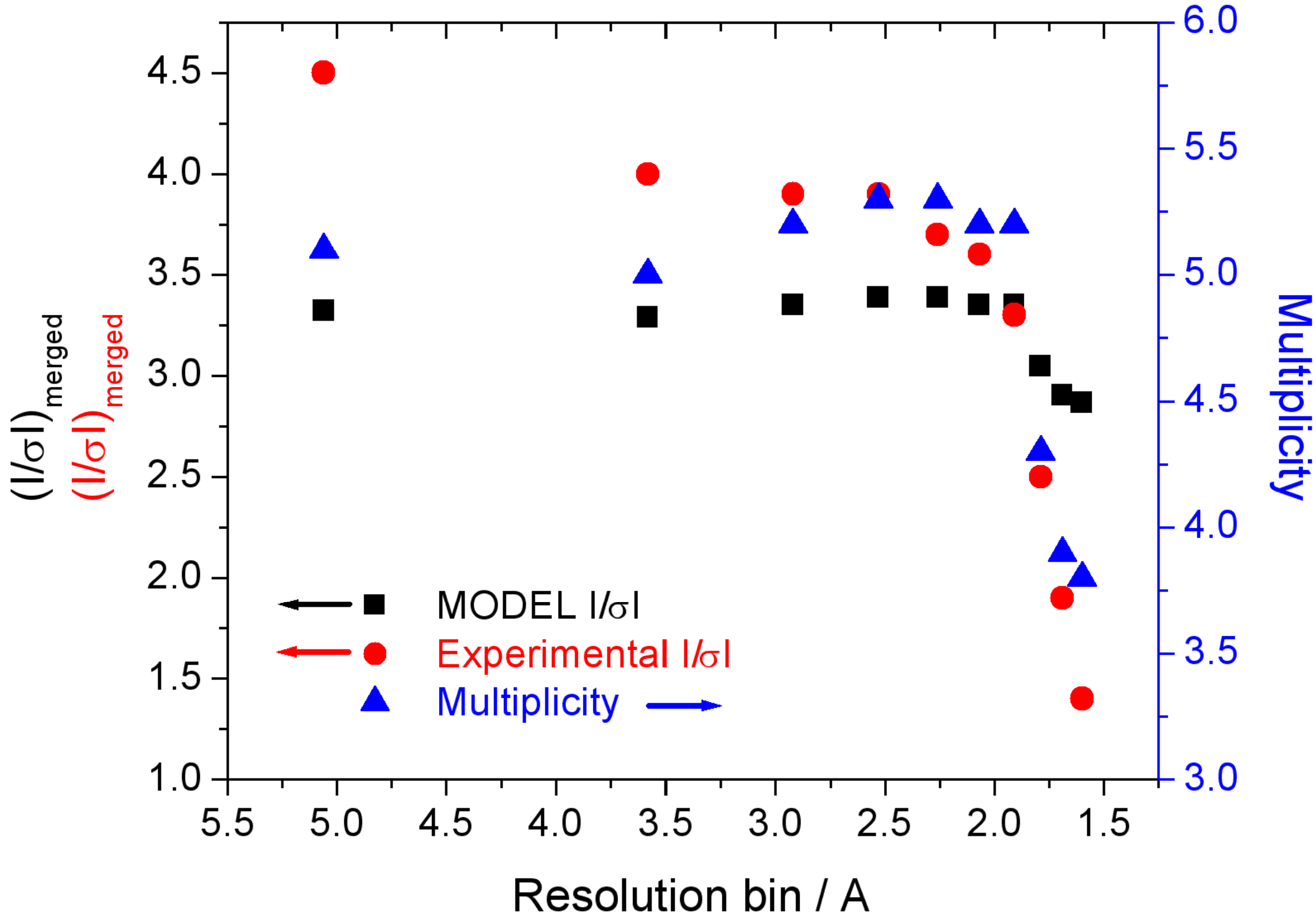
[e] $$PCV(I) = \sum_{hkl}\left(\frac{1}{n-1}\sum_i^n (I_i(hkl) - \langle I(hkl)\rangle)^2\right)^{1/2} / \sum_{hkl}\langle I(hkl)\rangle$$



$2F_O$-F_c

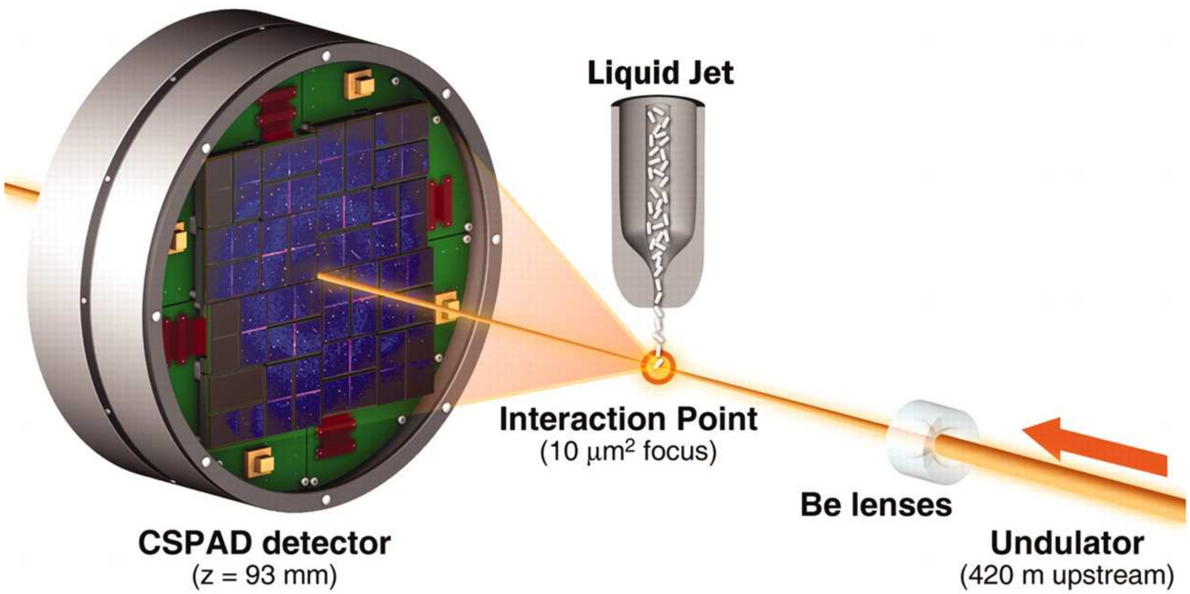
$$\left(\frac{\langle I_o\rangle}{\langle\sigma_I\rangle}\right)_{merged} = n^{1/2}\left(\frac{\langle I_o\rangle}{\langle\sigma_I\rangle}\right)_{unmerged}$$

e.g. $$\sqrt{4}.8\left(\frac{I}{\sigma I}\right) = 3.22$$



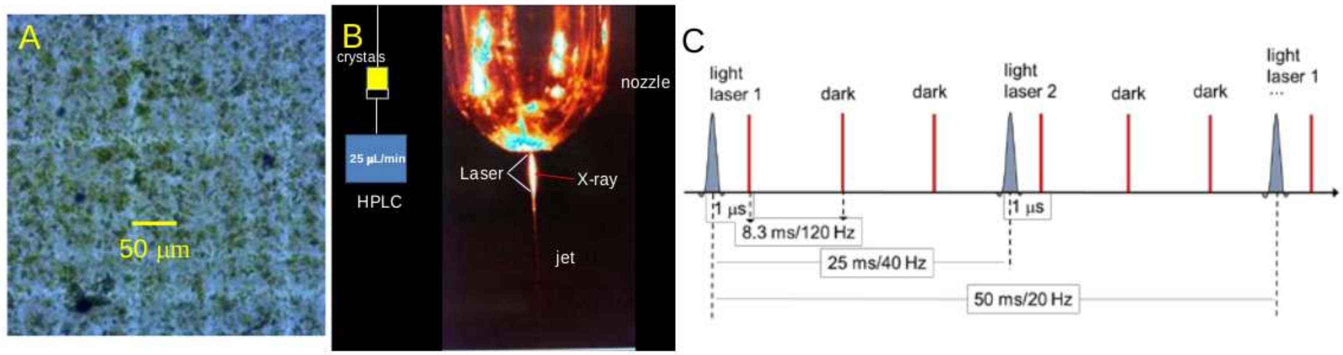
$$\left(\frac{\langle I_o\rangle}{\langle \sigma_I\rangle}\right)_{merged} = n^{1/2}\left(\frac{\langle I_o\rangle}{\langle \sigma_I\rangle}\right)_{unmerged}$$

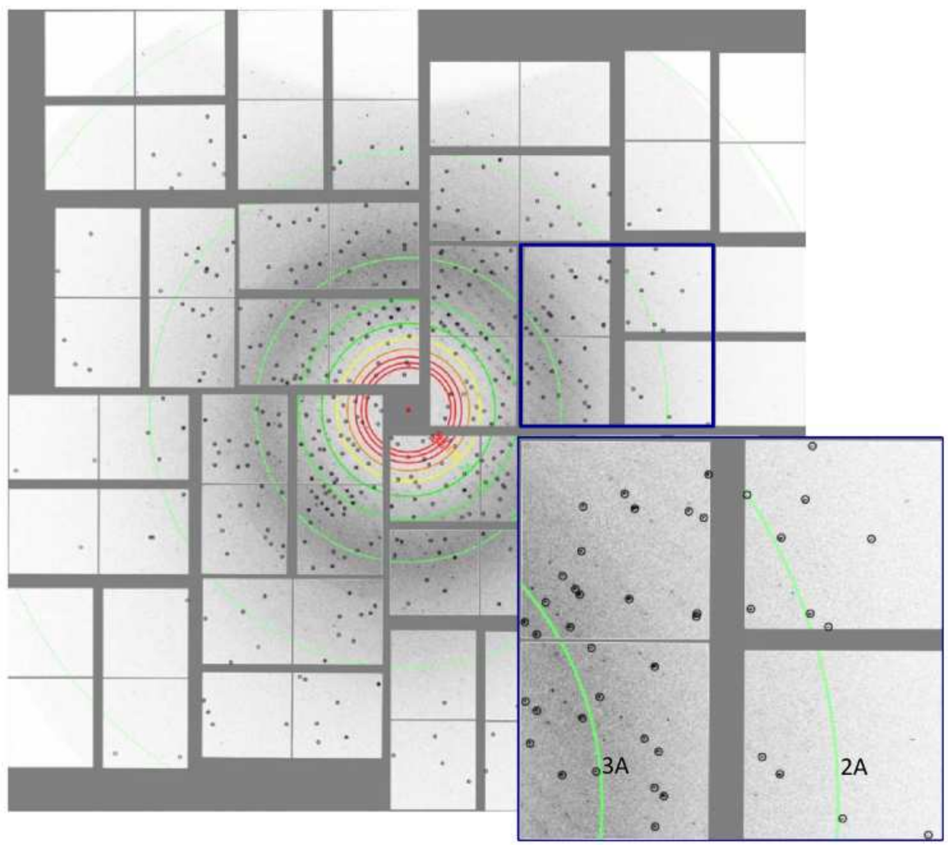
Serial Femtosecond Crystallography:
Monte-Carlo approach to time resolved measurements



Barends et al. Nature **505**(7482), 244-247 (2013).
-SAD Phasing of gadolinium complex of lysozyme resolves ΔI/I ~ 20%
-2,402,199 diffraction patterns.
-59,667 of which were indexed and integrated

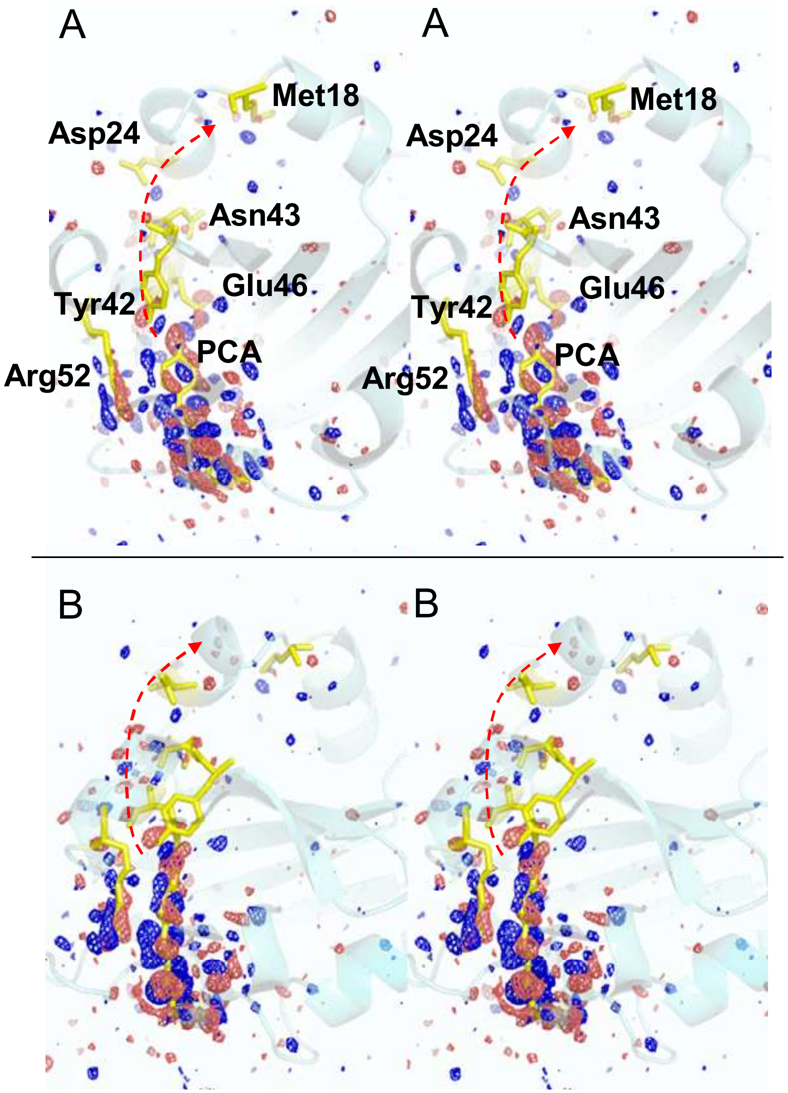
Micro-crystals Jet Timing

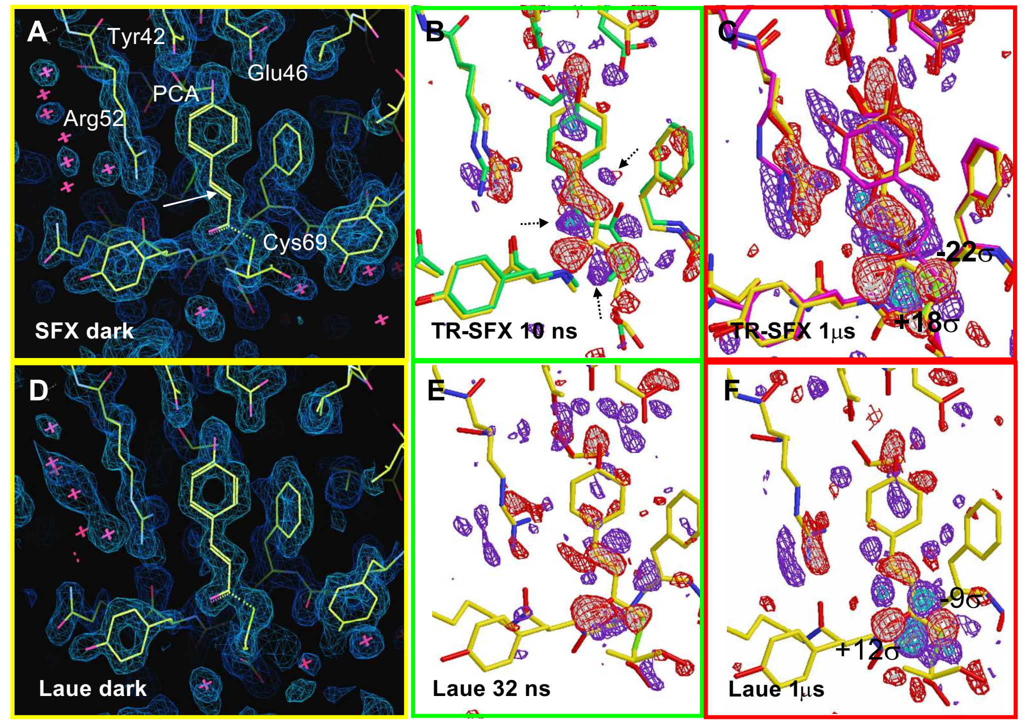




CSPAD image
of PYP diffraction

Microsecond intermediates in PYP at 2Å resolution from SFX

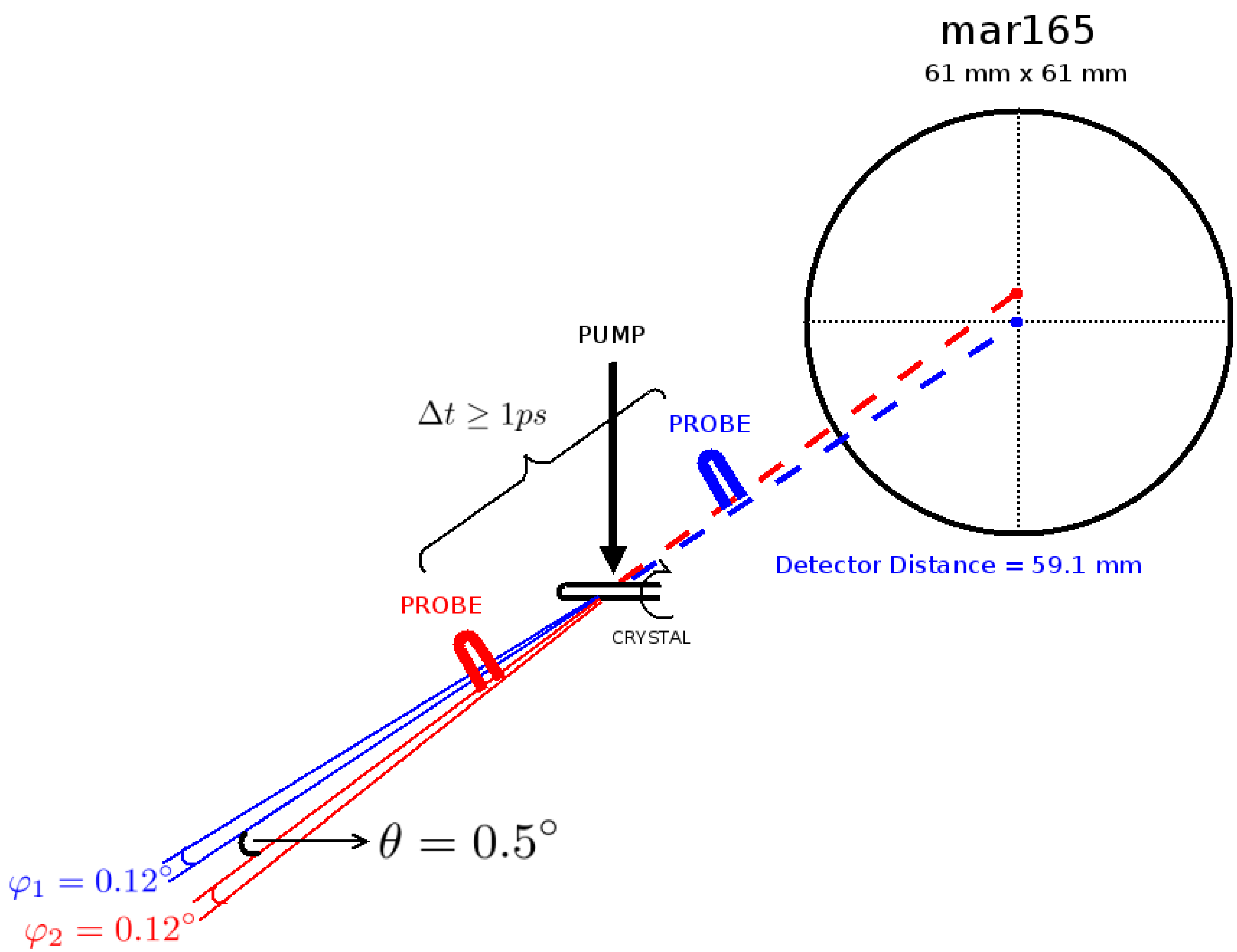




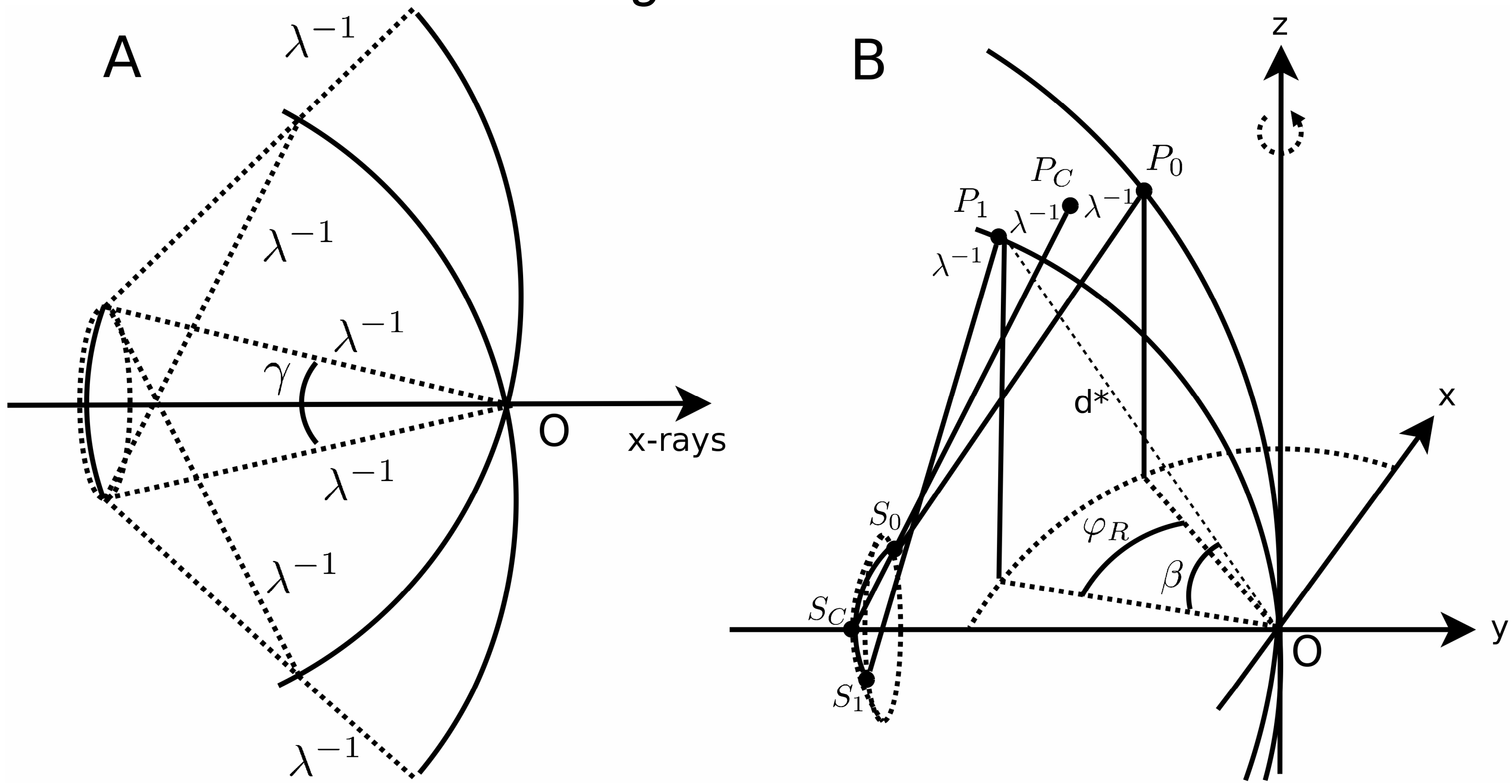
Tenboer et al., 2014. Science. 346; 1242-1246

Proposal for a Probe-Pump-Probe scheme:

Ratiometric detection of ΔF



Convergent Beam Method



$$cos\left(\beta+\frac{\gamma}{2}\right)\leq\frac{\lambda d^{*}}{2}\leq cos\left(\beta-\frac{\gamma}{2}\right)$$

Bragg conditions

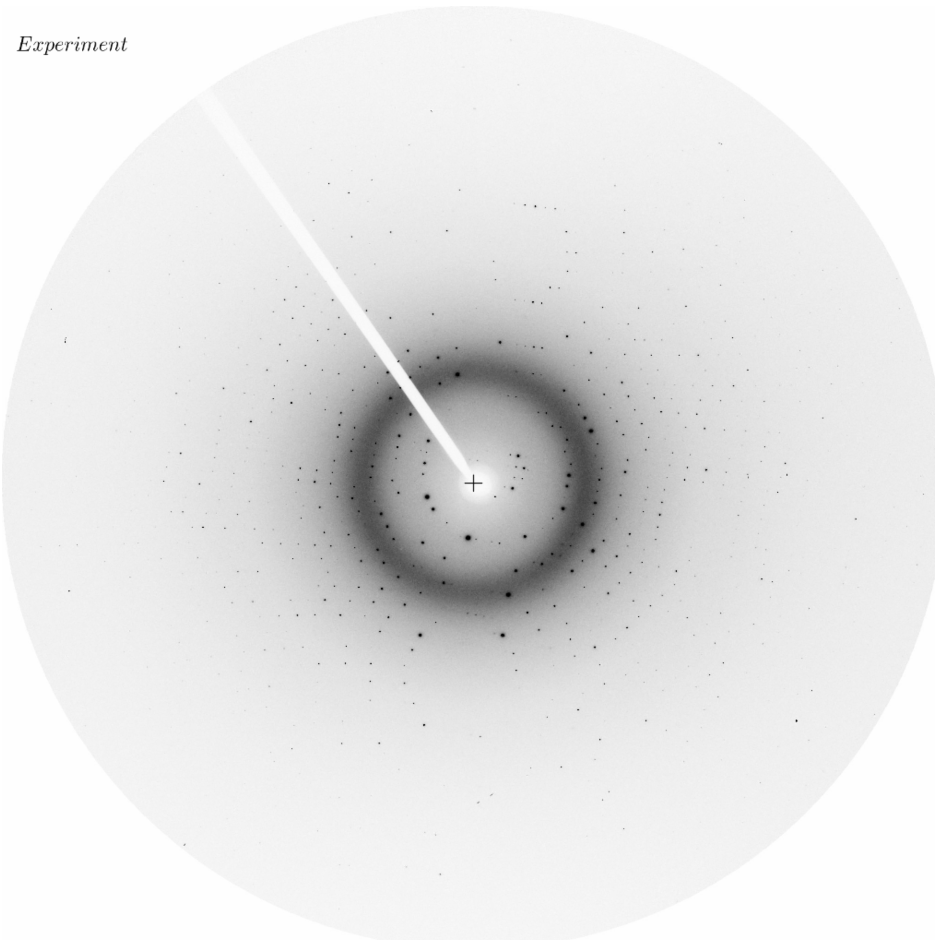
$$\varphi_R \simeq L(\Delta d^{*} cos\theta + \frac{\delta\lambda}{\lambda} d^{*} sin\theta)$$

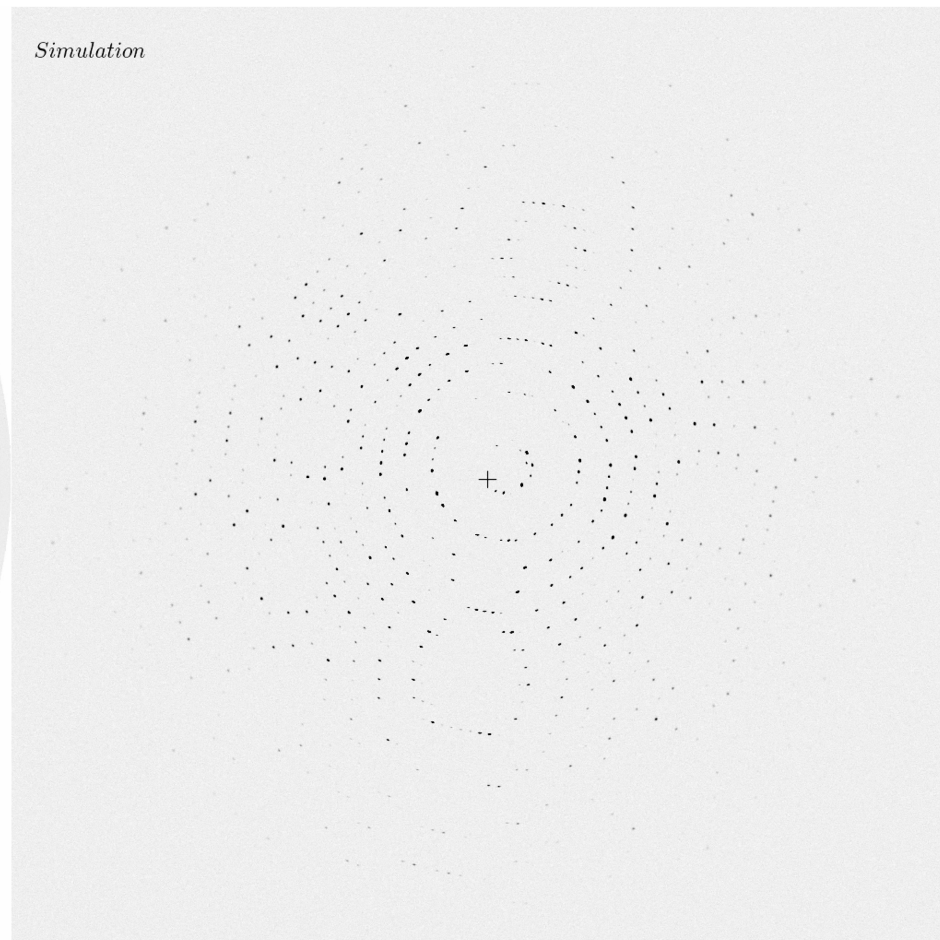
Reflecting Range

$$\Delta = \gamma + \eta$$

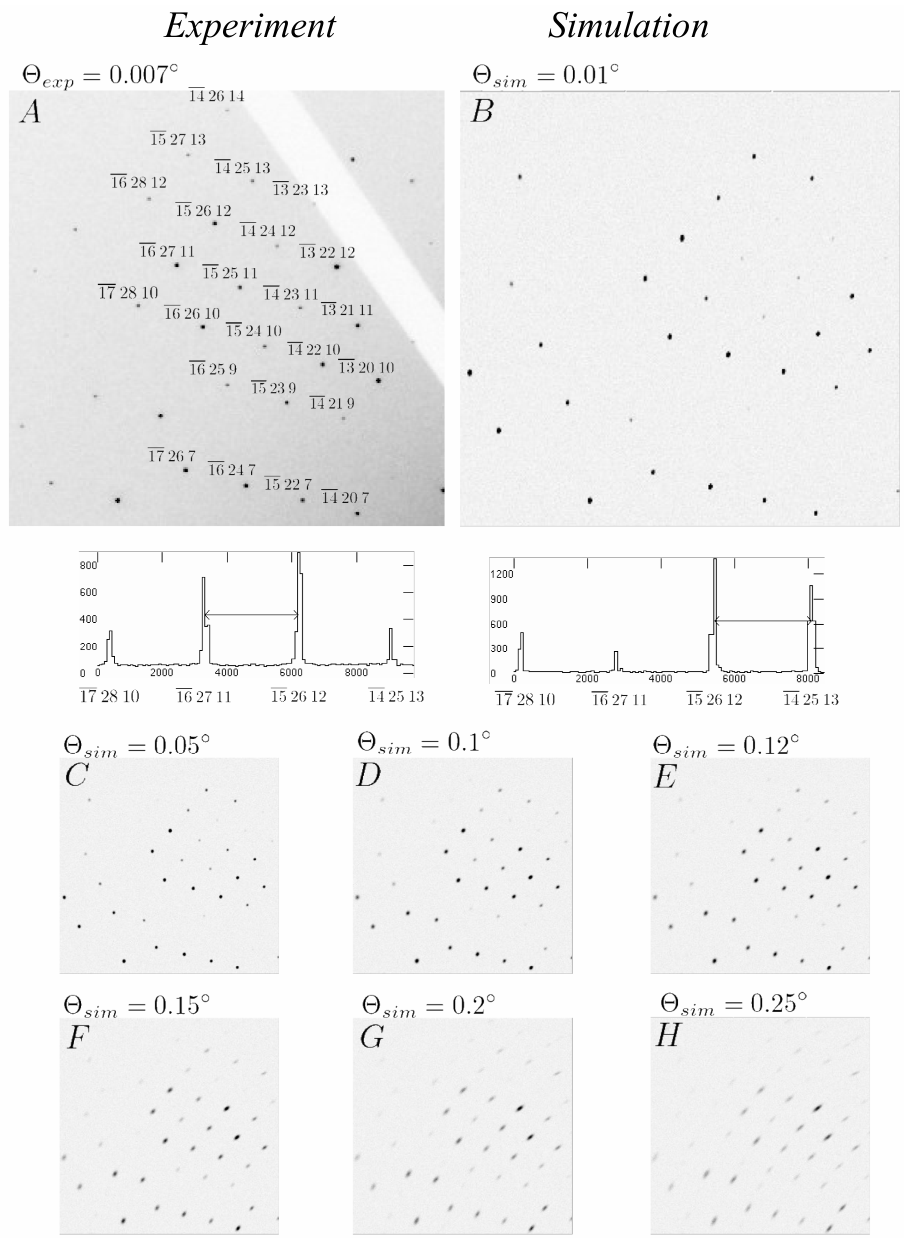
Cross-fire ***and*** mosaic spread

Ray-tracing simulations of LCLS/XPP diffraction data
Monochromatic and Rotation Method

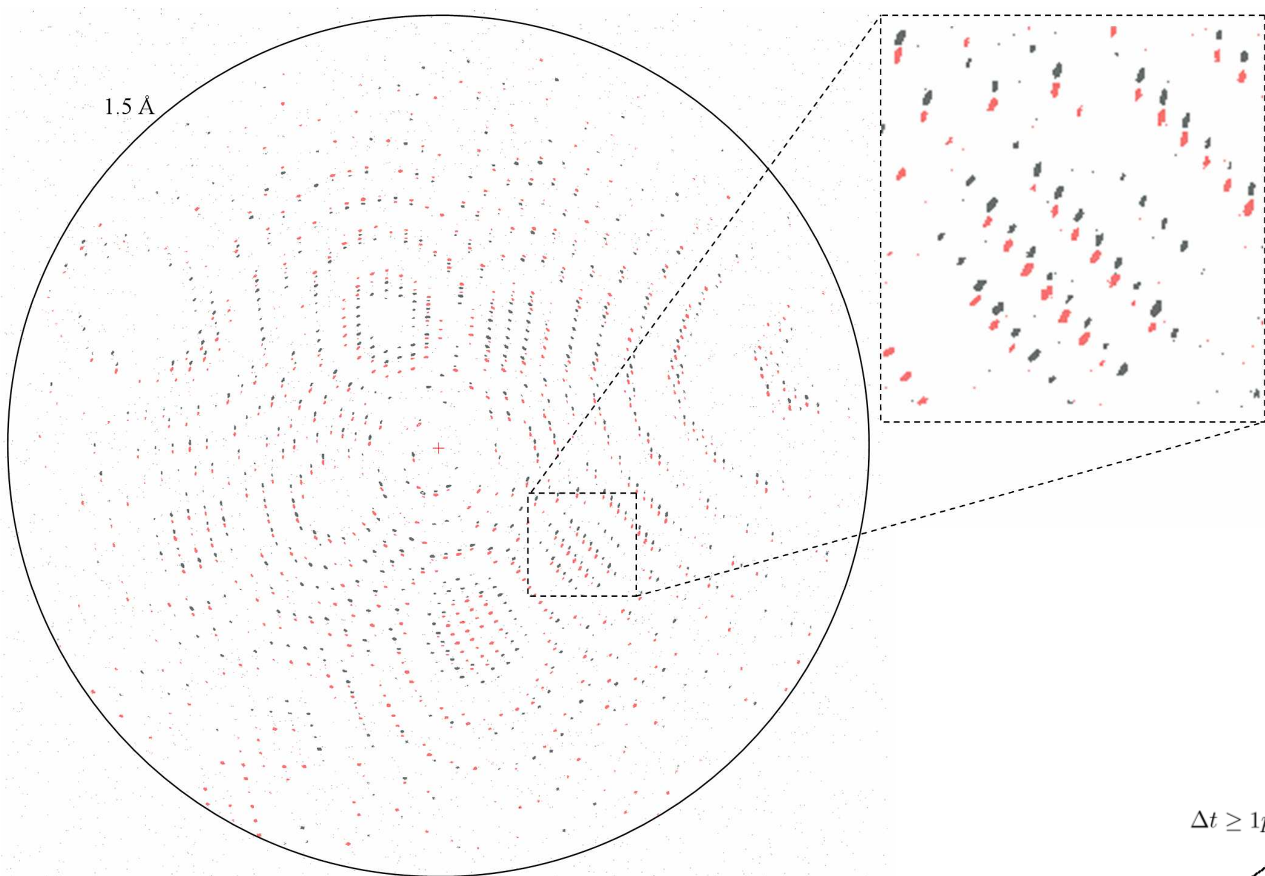


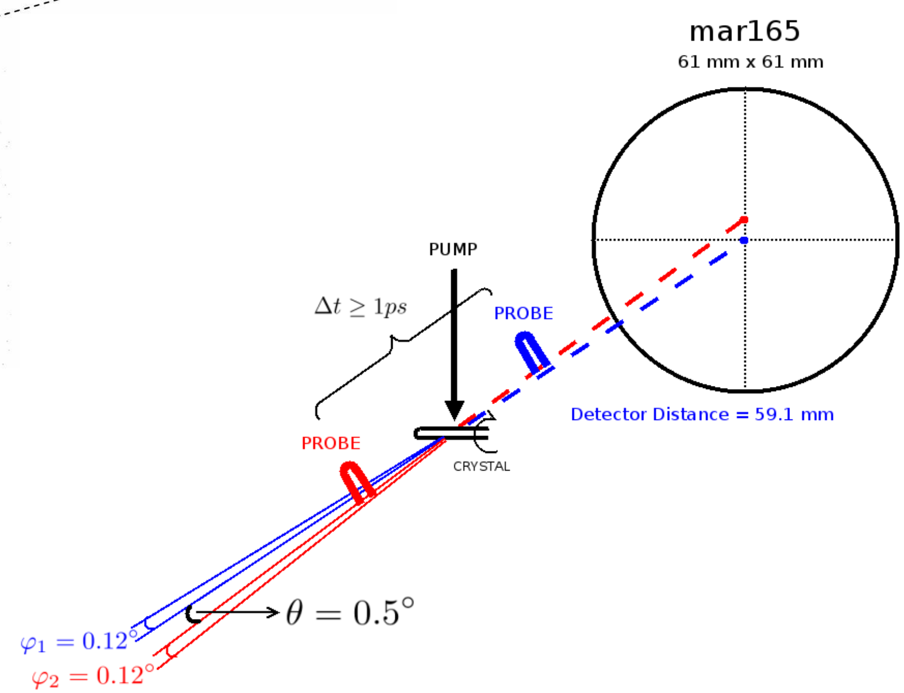


Ray-tracing simulations of LCLS/XPP diffraction data: Convergence angle



Probe-Pump-Probe (Simulation to 1.5 A resolution, 9.4 keV)

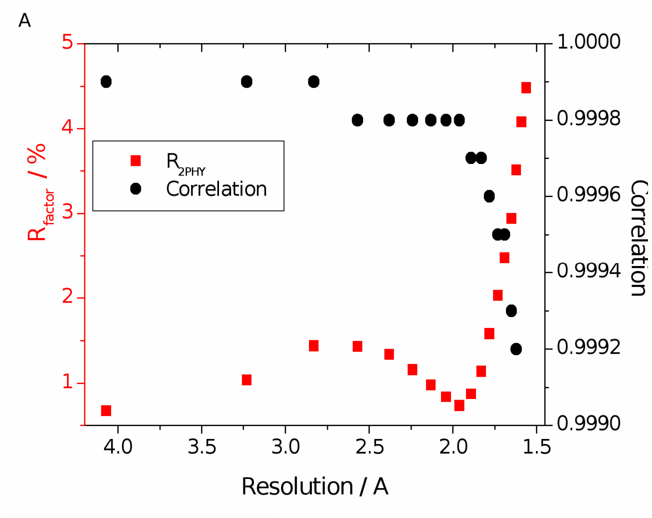


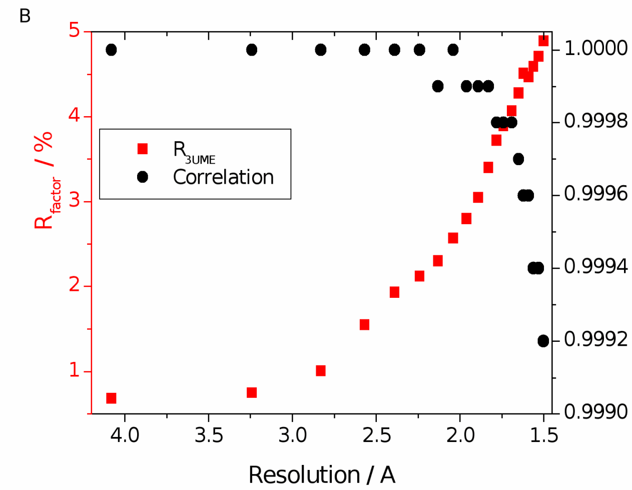


-UNPUMPED
-PUMPED

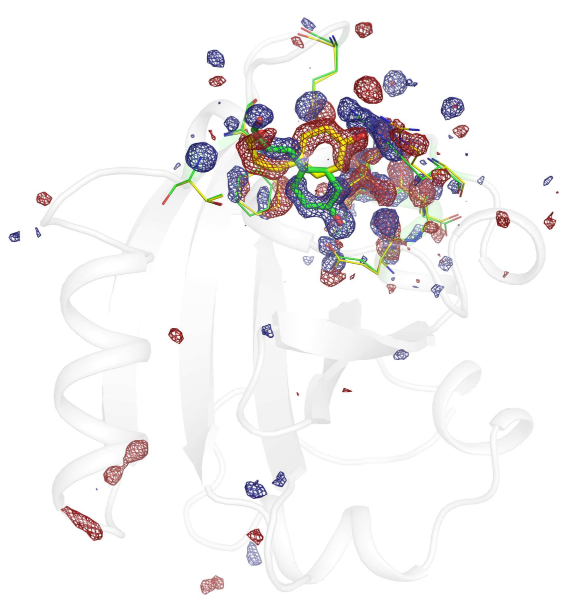
Unpumped

Pumped





Data correlation



Retrieved synthetic
$F_{PUMPED} - F_{UNPUMPED}$

STRUCTURAL DYNAMICS **2**, 000000 (2015)

A split-beam probe-pump-probe scheme for femtosecond time resolved protein X-ray crystallography

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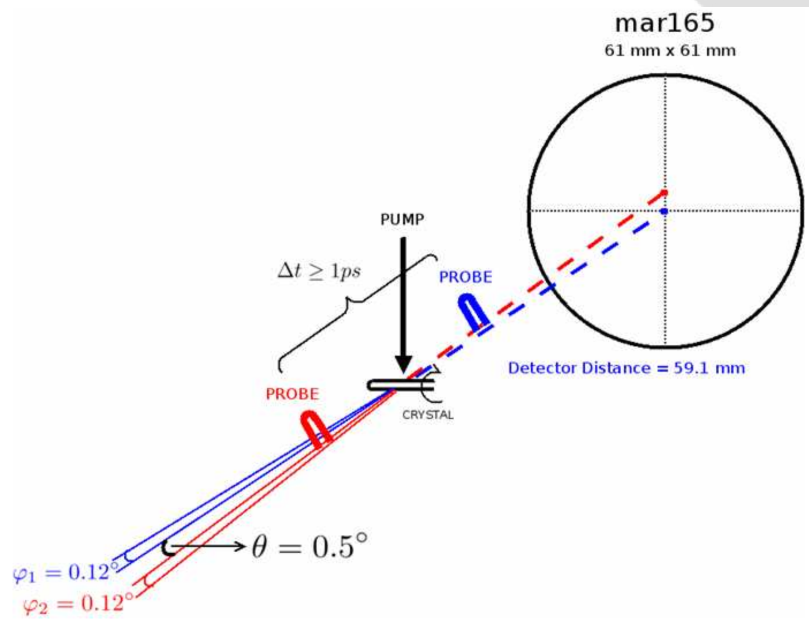
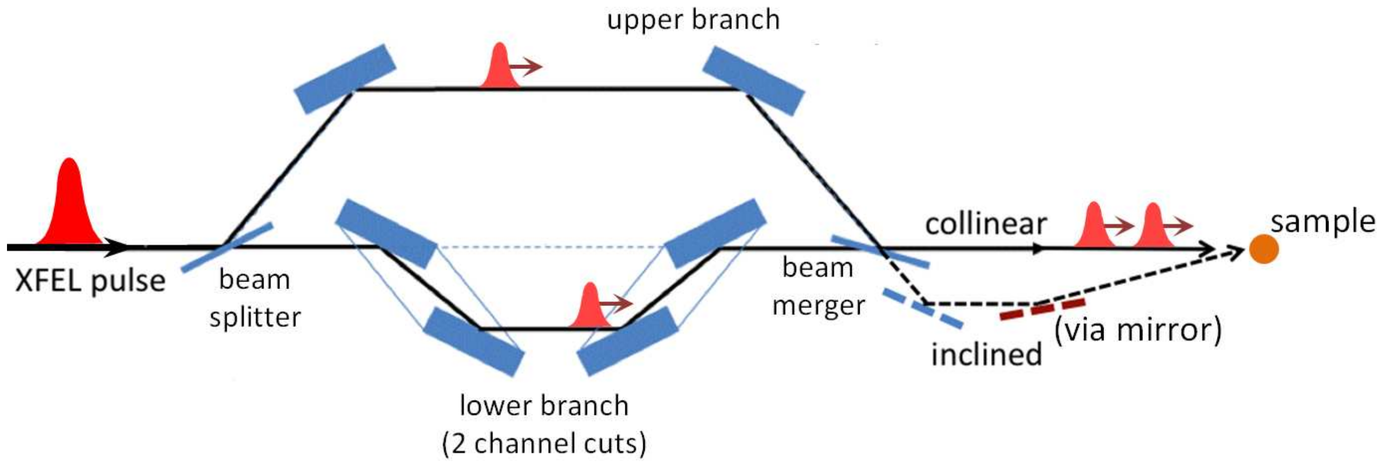


MID station XFEL : SDL design

Technical Design Report: Scientific Instrument MID
Madsen, A.; Hallmann, J. ; Roth, T. ; Ansaldi, G.
XFEL.EU Technical Report 1-191 (2013) [10.3204/XFEL.EU/TR-2013-005]



MID station XFEL : SDL design

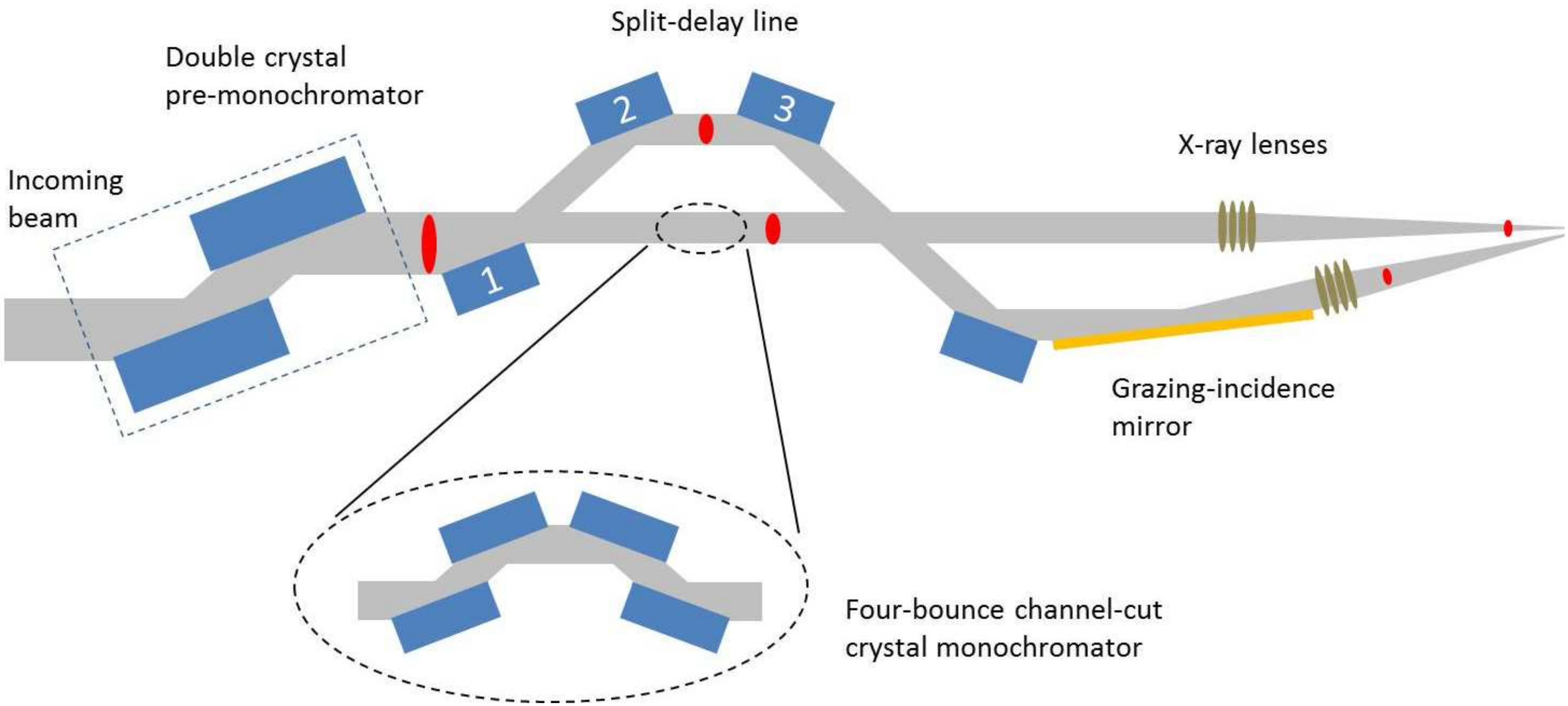
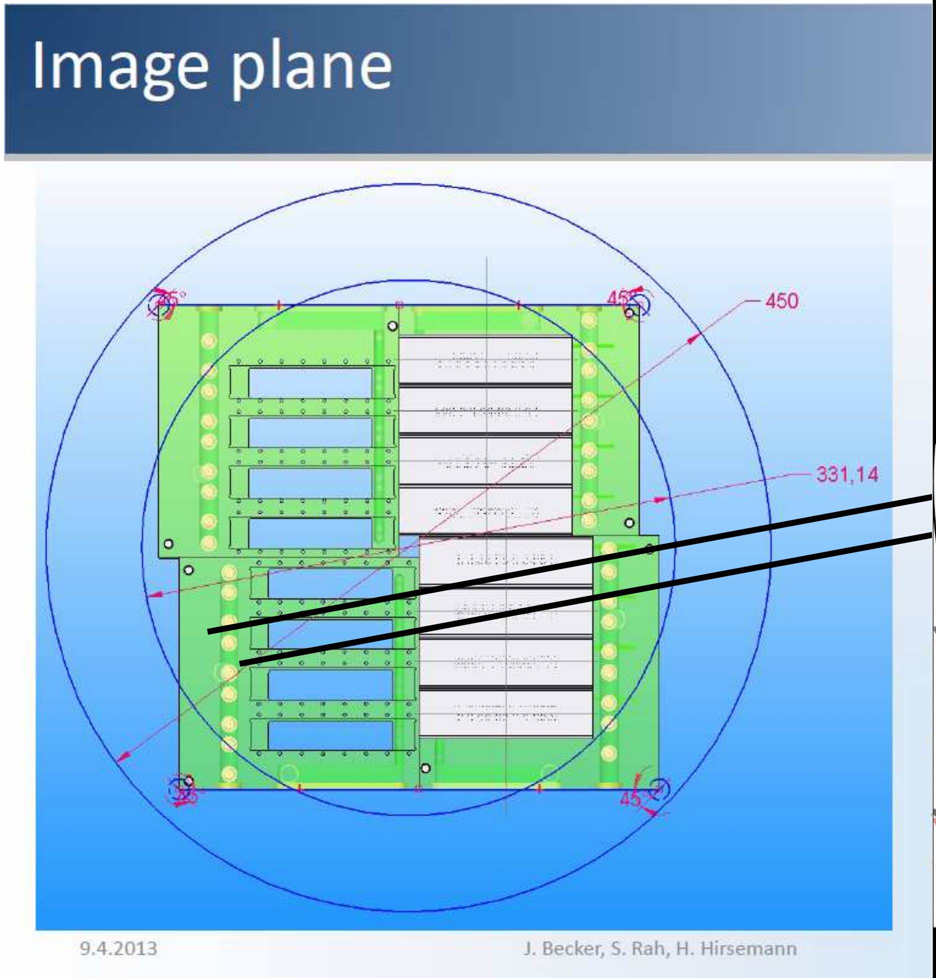
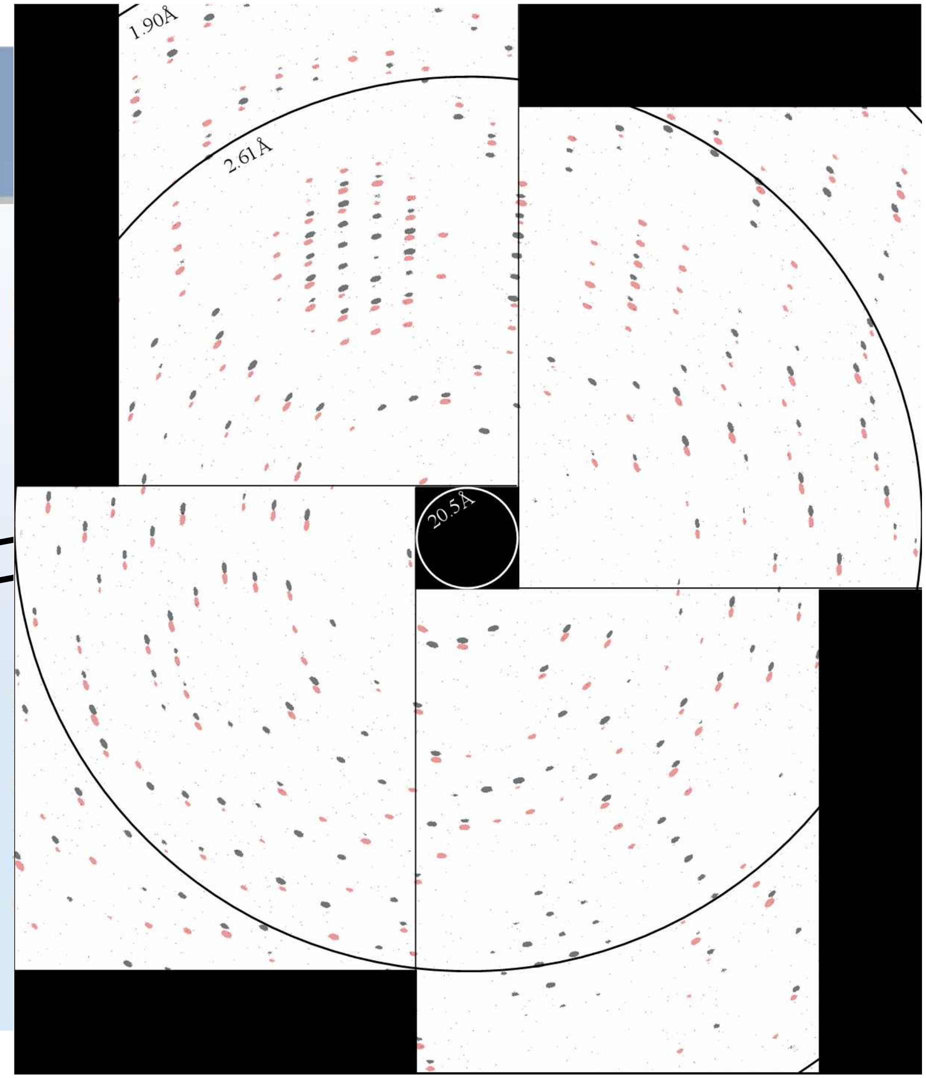


Table 2. Proposed parameters for a split and delay unit

	Single crystal application	SFC application	SDL/MID (XFEL.EU)	
Convergence angle (both beams)	0.12° (optimized value; 0.15° max, no convergence potentially tolerable)	0 - 0.12° (Non-convergent beam acceptable for high-throughput SFC measurement)	Up to ~0.12°, depending on the focusing optics	✓
Angle between beams	0.5° (based on MAR165 detector, and experimental parameters used during XPP44112). Control of the value would allow big and small unit cells (0.1 – 0.8 degrees range)	0.5°	Up to ~0.6°, depending on the mirror material chosen (Si/Pt)	✓
Time delay between pulses	>1 ps. Ideally controlled 1-10 ps	>1 ps.	Design specs: 0-800 ps +/- 3 fs	✓
Bandwidth	1.6 eV	1.6 eV	~6 x 10^{-5} (0.3-0.6 eV) (Si 220 reflection)	✓
Photon energy	9-9.5 keV	9-9.5 keV	5-10 keV (adjustable)	✓
Photons / pulse	> 10^{10} – 10^{11} each beam	< 1.5 x 10^{9} each beam (Non-destructive)	10^{8} - 10^{11} per pulse, tunable, depending on SASE/seeding	✓
Source stability	Not intrinsically required	Not intrinsically required	Desirable	
Repetition rate	Up to full LCLS rate. For PYP: 2 Hz	Detector limited	3.5 kHz, detector limited	✓
Spot size on target	40 µm, round or up to 150 x 40 µm aperture	~1 µm	<1-100 µm, variable	✓
Overlap between beams on target	Better than 5 µm precision	Better than 100nm precision	Design specs: better than 80% overlap area	✓

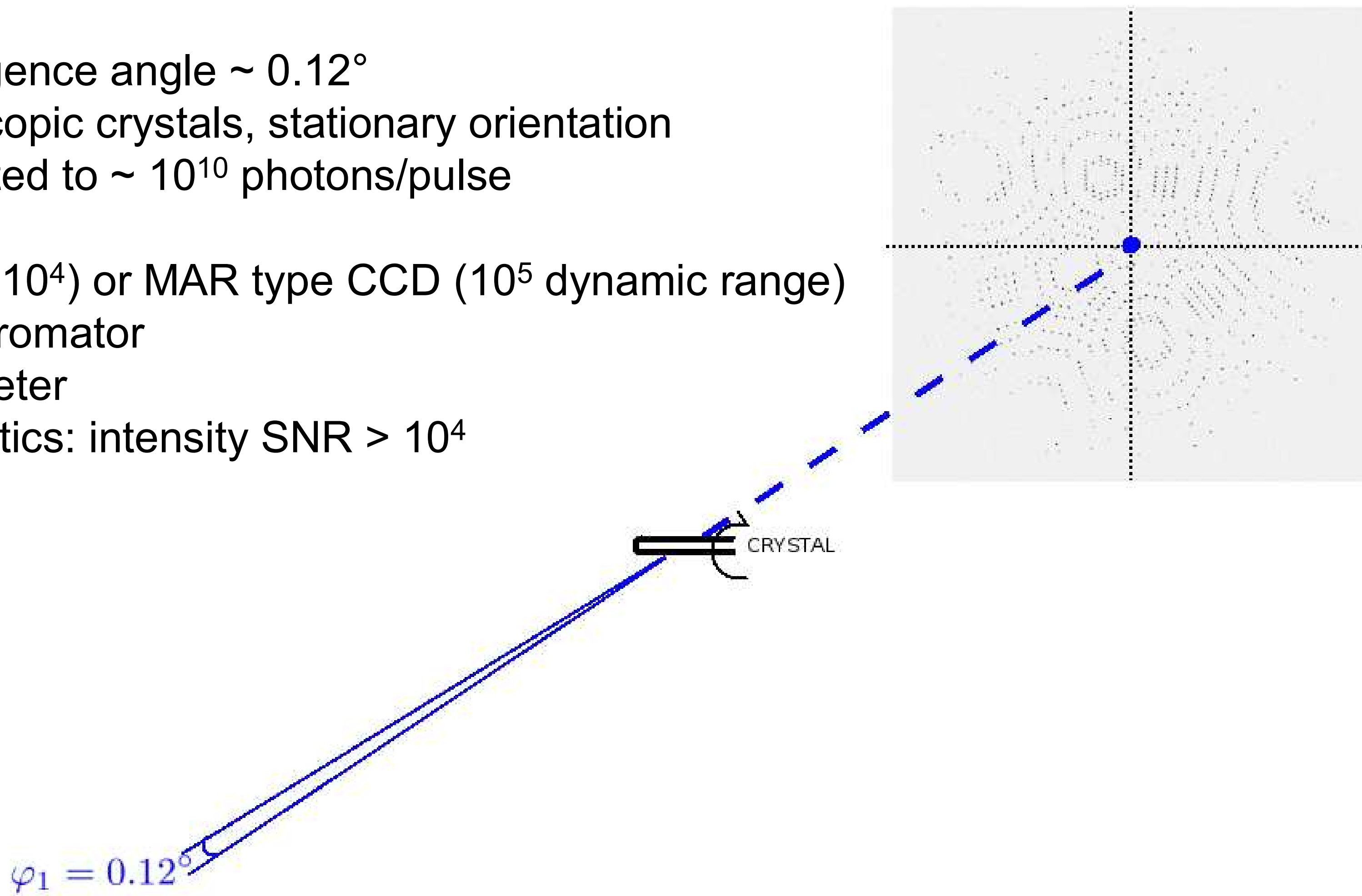
AGIPD detector: 2.5 A resolution limit





Day-1 proposed experiment at MID: Convergent Beam method

- Convergence angle ~ 0.12°
- Macroscopic crystals, stationary orientation
- Attenuated to ~ 10^{10} photons/pulse
- 9 keV
- AGIPD (10^4) or MAR type CCD (10^5 dynamic range)
- Monochromator
- Goniometer
- Diagnostics: intensity SNR > 10^4



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