



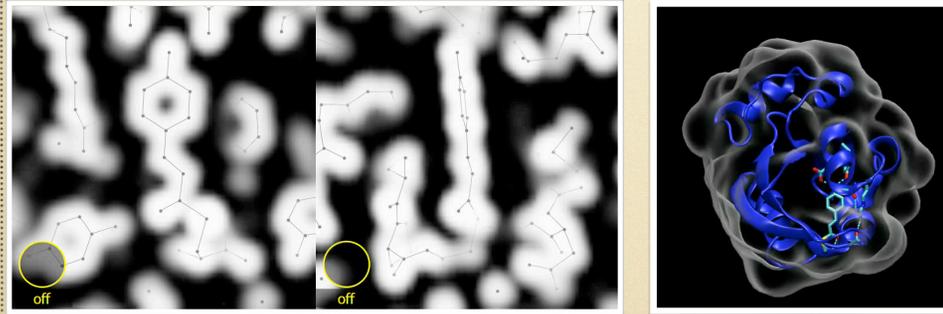
Picosecond Photobiology: What can we do today, and what would we like do do tomorrow?

¹Philip A. Anfinrud, ¹Friedrich Schotte, ¹Hyun Sun Cho, ²Hironari Kamikubo and ²Mikio Kataoka

¹Laboratory of Chemical Physics, NIDDK, NIH, Bethesda, MD, USA

²Graduate School of Materials Science, NAIST, Nara, JAPAN

Picosecond Laue Crystallography Study of Photoactive Yellow Protein



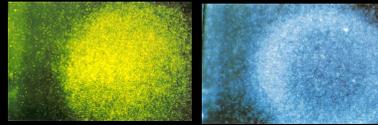
APS Oct 21, 2013

Before coming to the NIH in 1998, my research group at Harvard University was engaged in ultrafast biophysical studies of proteins using spectroscopic techniques, including femtosecond IR spectroscopy. Since coming here, we have continued to develop new methods for studying proteins including picosecond Laue crystallography, and more recently, picosecond SAXS/WAXS. Before I get into what we are doing and how we do it, I thought it prudent to give some background regarding why I think these studies are important. Due in part to my training as a physical chemist, I'm passionate about molecular level details. Nevertheless, it is worthwhile to occasionally retreat from that focus and ponder life at the organismic level, as shown in my next slide.

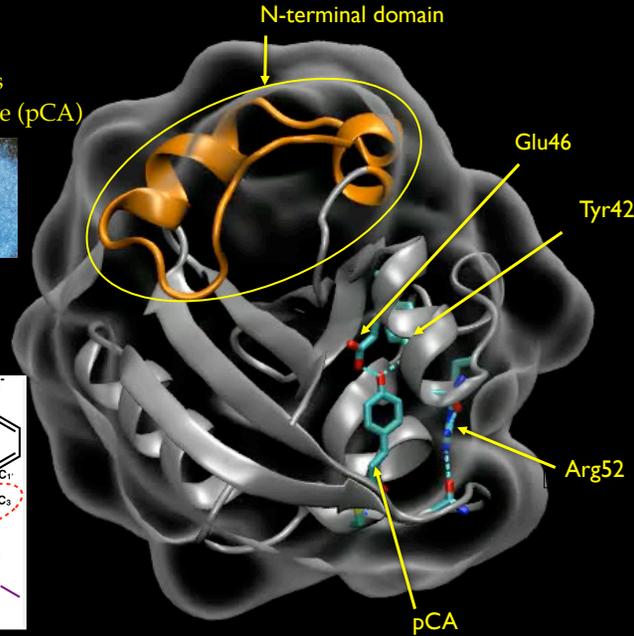
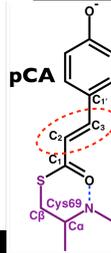
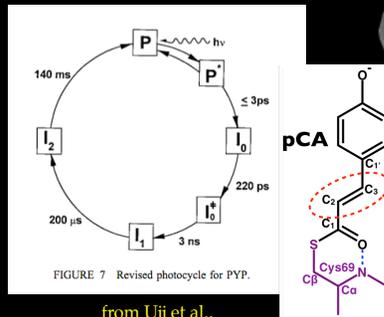


Photoactive Yellow Protein (PYP)

- Halorhodospira halophila
- negative phototaxis
- PYP: 14 kDa, 125 amino acids
- p-coumaric acid chromophore (pCA)



from Sprenger et al.,
J. Bacteriology 175, 3096 (1993)

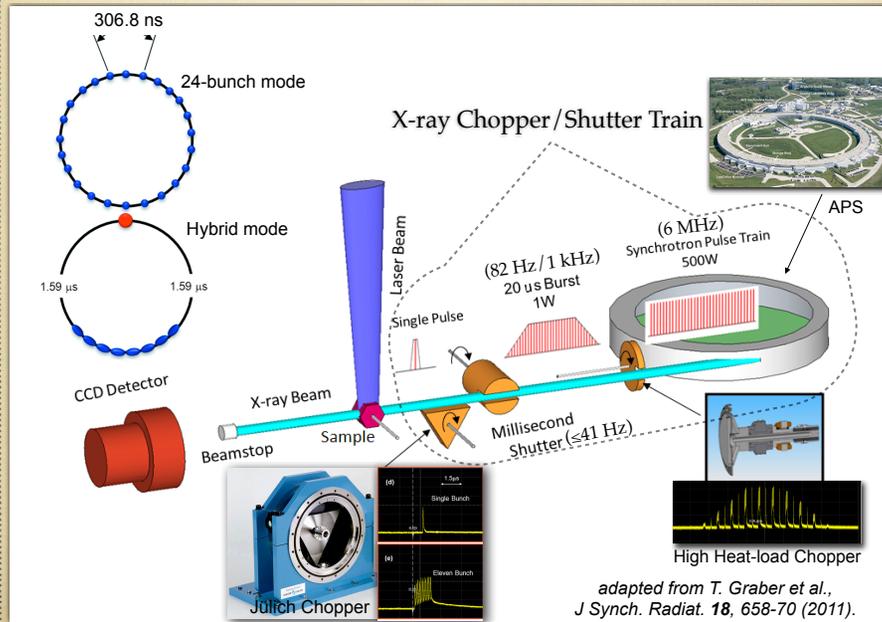


How do signaling proteins function from a molecular point of view? We have chosen PYP as a model system for this investigation. It is a photoreceptor that undergoes structural change upon absorbing a single photon of light. By exciting this protein with a short duration laser pulse, we can control when the structural change begins and study it with near-atomic resolution. In several respects, PYP is an ideal model system. Very large crystals of PYP can be grown, it is fully reversible, and it undergoes a large amplitude signaling-state structural transition. Convert a femtosecond event, and generate a signaling state that is prolonged enough to elicit a response. Negative phototaxis. pCA; trans-cis isomerization; strong H-bonds; water penetration gating by Arg52. PYP photocycle provides a framework for understanding signal transduction in proteins, and for assessing and validating theoretical/computational approaches in protein biophysics



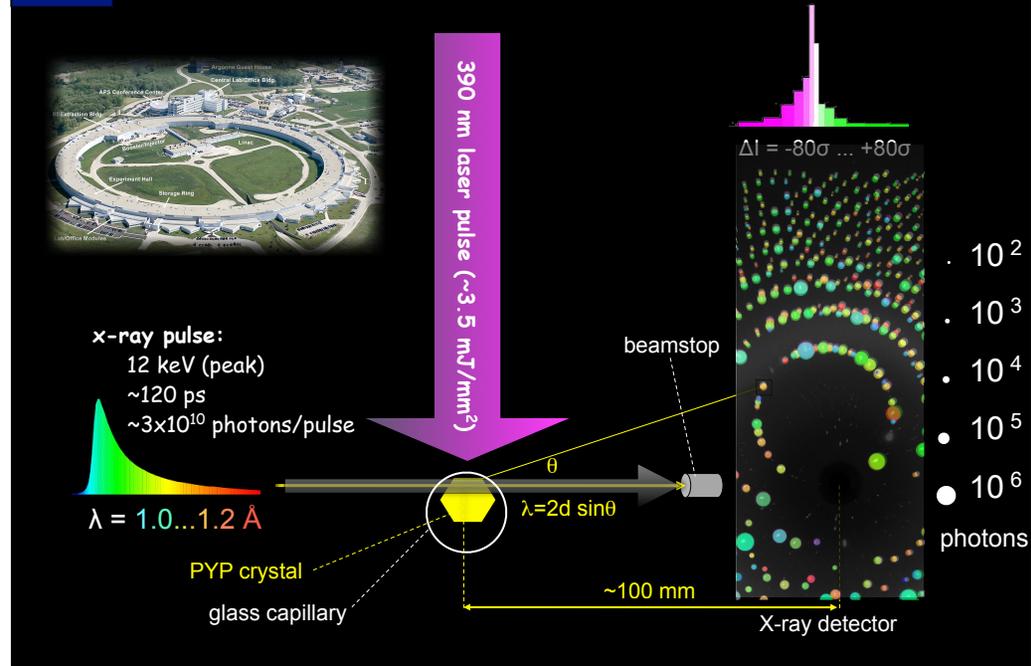
BioCARS High-Flux 14-IDB Beamline

• BioCARS/APS/NIDDK upgrade (2005-2007)

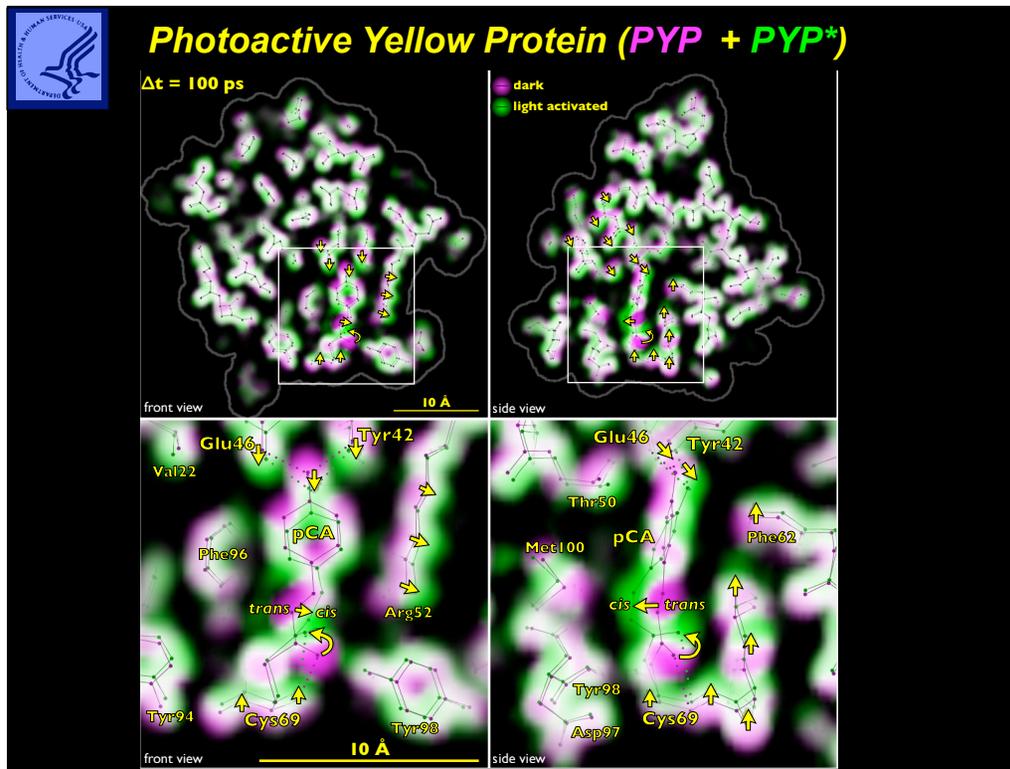




150-ps Time-Resolved Laue Crystallography of PYP via the Pump-Probe Method



We first developed the technique of picosecond time-resolved Laue crystallography at the ESRF.

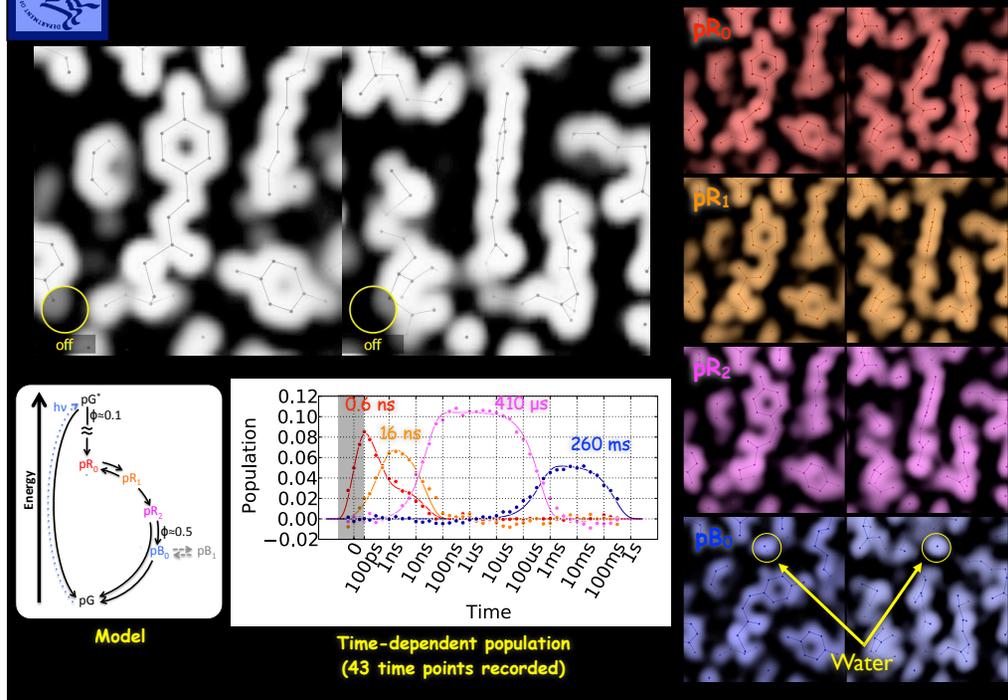


Here is a 100 ps snapshot of PYP structural changes.

- dramatic structural change of the pCA
- trans–cis isomerization shortens length of pCA
- like a winch, pCA pulls the helices inward
- structural differences concentrated near pCA, but also see displacement in helices above the pCA



Global Analysis of Time-resolved Electron Density Maps



10 decades of time with 100 ps time resolution.

4 intermediates, 3 red-shifted, 1 blue shifted

Equilibrium between pR_0 and pR_1

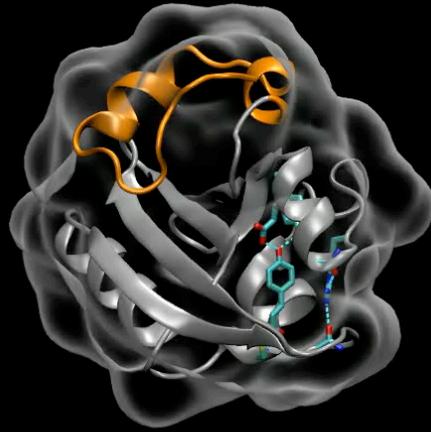
50% of pR_2 takes a short-cut to pG

water penetration

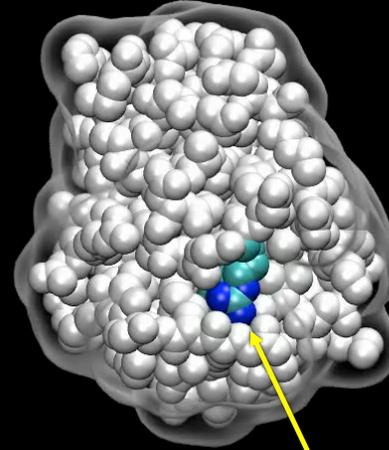


The Arginine 'Switch'

- facilitates pCA protonation in pB₀ state
- facilitates water penetration into the pCA cavity

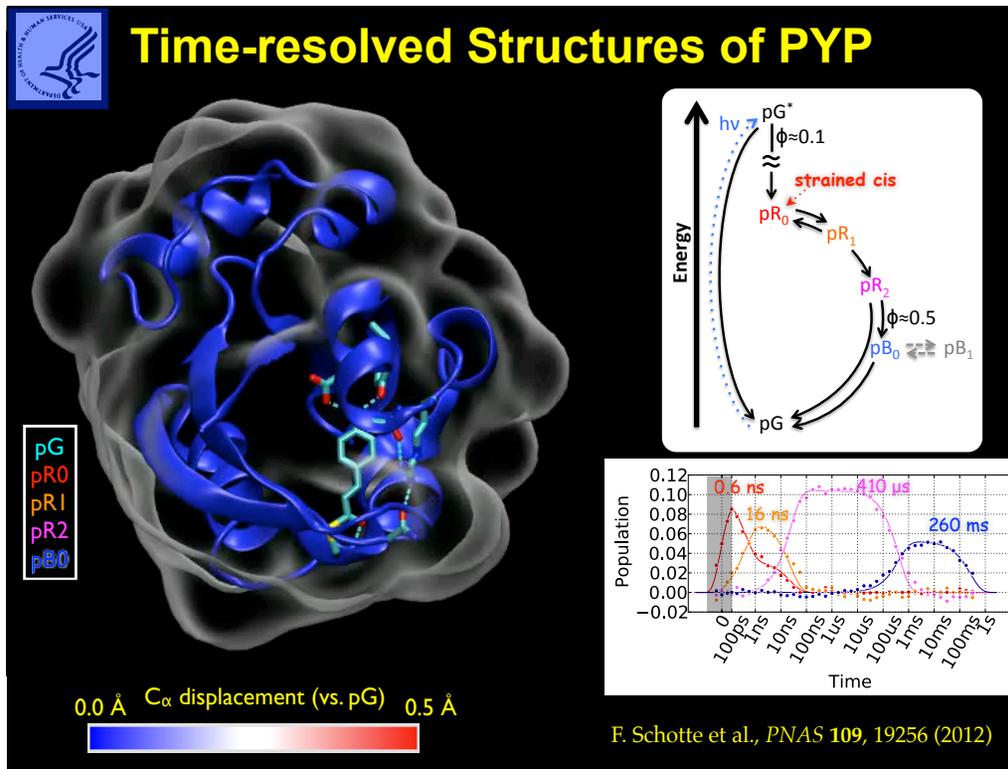


pG



pR2
pB0

Arg52 switches between
'closed' and 'open'
conformations

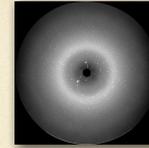


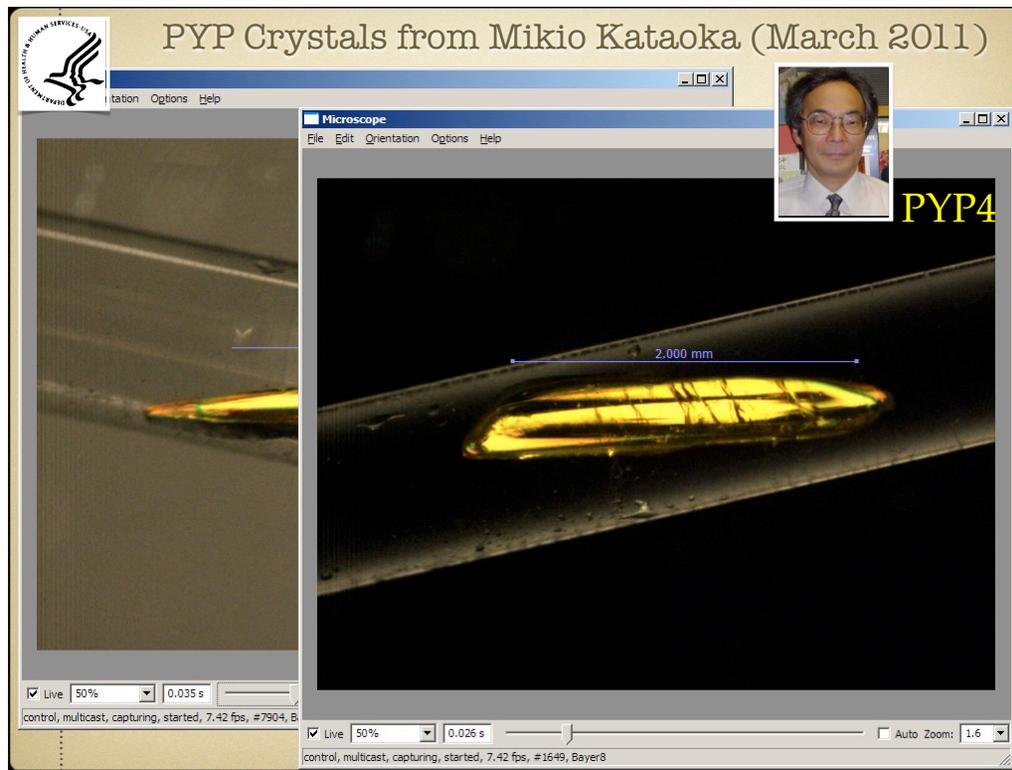
Because the protein crystal prevents large amplitude structural changes, we can't characterize structurally the signaling state, only the structural changes that drive the transition to the signaling state. What we need is a structural technique that works in solution. For this, we developed the method of time-resolved SAXS/WAXS.



What made this study of PYP possible?

- Large protein crystals
- BioCARS High-flux 14-IDB Beamline
- APS Hybrid Mode (~59 nC bunch)
- Jülich High-speed chopper



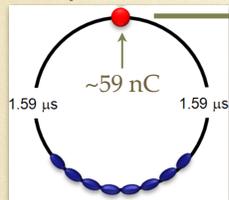


Crystal cracking is evident after acquiring numerous orientations per crystal.

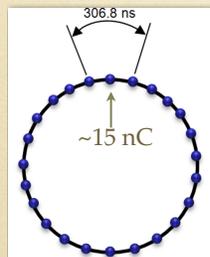


BioCARS High-flux 14-IDB Beamline

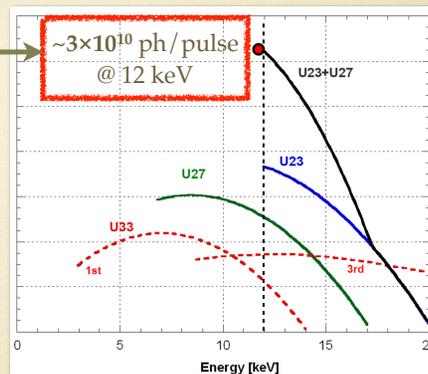
Hybrid mode



24-bunch mode



Two in-line short-period undulators
(U23 + U27) →





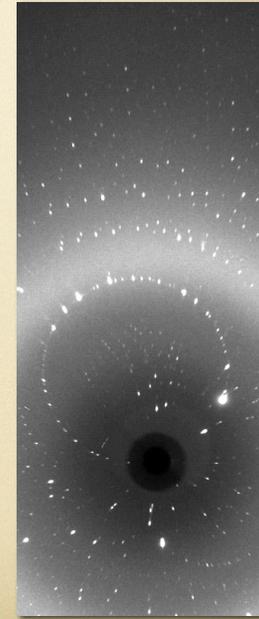
Time-resolved Photobiological Studies are “Photon Starved”

- Uncertainty in the measured intensity of spot hkl is limited by photon-counting statistics, i.e.,

$$(\sigma_I)_{hkl} = (\sqrt{I + B})_{hkl}$$

where I is the integrated number of photons assigned to that hkl , and B is the number of background photons within the footprint of that spot.

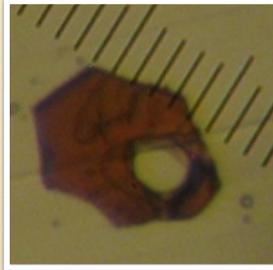
- The average number of photons detected per hkl dictates the S/N of the data set, $\langle I/\sigma_I \rangle$, and the resolution of the structure determination.
- How to boost the S/N :
 - Higher flux source (more photons per pulse)
 - Higher repetition frequency for data acquisition
 - Merge hkl from multiple observations





X-ray Flux: How Much is Too Much? (depends on how tightly it is focused)

- 3×10^{10} photons at 12 keV focused to $\sim 30 \mu\text{m}$ will produce $\sim 6^\circ\text{C}$ temperature jump in the x-ray illuminated volume of the protein crystal.
- $\sim 10^{12}$ photons at 9 keV focused to $\sim 10 \mu\text{m}$ produces an adiabatic temperature jump more than sufficient to generate a 'steam' explosion in the crystal.



MbCO (Michael Soltis, LCLS 2010)

The ensuing shock wave created disorder in the crystal and rendered it non diffracting.



Repetition Rate: How Fast is Too Fast?

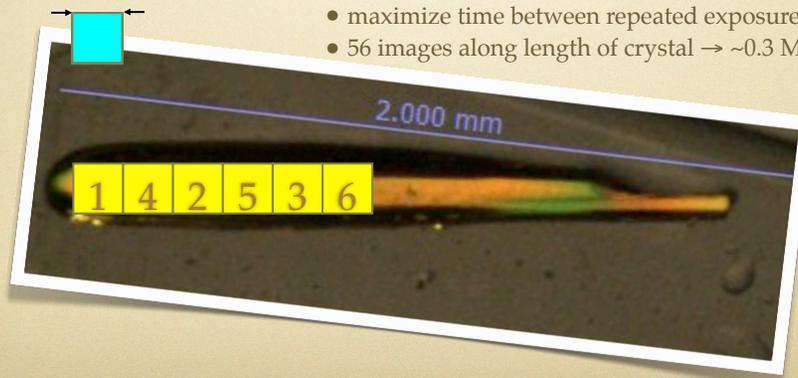
The protein crystal requires time to recover from laser excitation.

- Thermal time constant: few tens of ms ($\sim 100 \mu\text{m}$; proportional to d^2)
- Ground state recovery time: ~ 1 s (can be longer)
- Diffractometer translation rate: ~ 41 Hz (move $600 \mu\text{m}$ with $\sim 2 \mu\text{m}$ precision)
- Detector readout rate: now 0.4 Hz; will be 10 Hz (Rayonix MX340-HS)

6 shots per image

- translate crystal after every shot
- maximize time between repeated exposure
- 56 images along length of crystal $\rightarrow \sim 0.3$ MGy dose

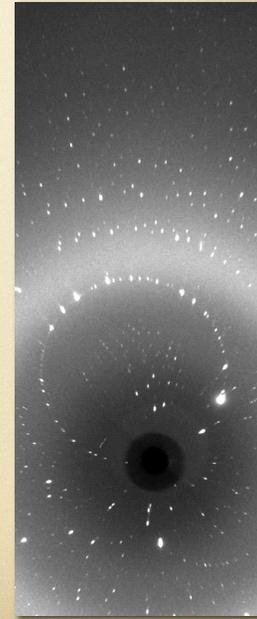
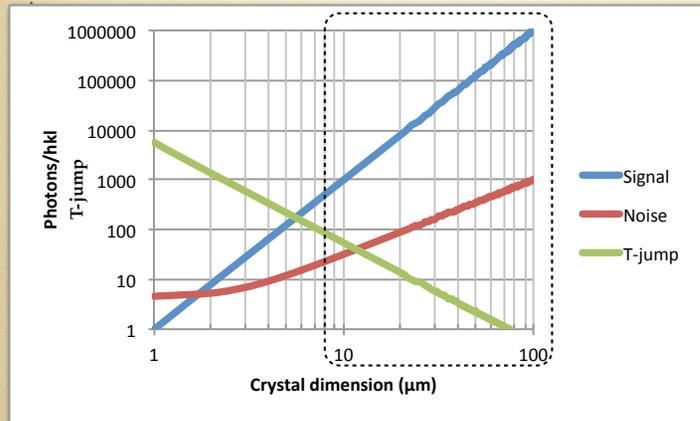
$120 \mu\text{m}$





Signal-to-Noise, Crystal Size, and X-ray Flux Limitations

With 3×10^{10} photons @ 12 keV focused to dimension of the crystal





Q: What would we like to do tomorrow?

A: Study non-reversible enzymatic processes in small crystals

Desired Source Properties	LCLS	APS
• High flux ($\sim 10^{11}$ ph/pulse @ $\sim 1\text{\AA}$)	X	
• Short pulses (≤ 1 ps)	X	
• Small, round focused spot ($\sim 30\ \mu\text{m}$)	X	X
• $\sim 5\%$ bandwidth (Laue)		X
• High pulse-to-pulse stability		X

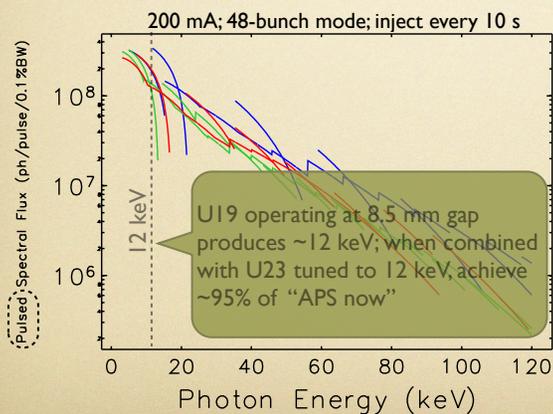


Multibend Achromat Lattice: Flux @ 6 GeV

Quantity	Symbol	Range	Units
Beam energy	E	6	GeV
Natural emittance	ϵ_0	60 - 80	pm
Rms energy spread	σ_δ	0.09 - 0.12	%
Emittance ratio	$\kappa = \epsilon_y/\epsilon_x$	0.1 - 1.0	
Emittance increase due to IBS	-	< 25	%
Horizontal emittance	ϵ_x	30 - 91	pm
Vertical emittance	ϵ_y	40 - 5	pm

Table 2: Basic electron beam properties

- APS currently operates at 7 GeV; lowering to 6 GeV will require shorter period undulators operating at closer gap to achieve comparable energy and flux.



MBA/SCU Super Conducting Undulator; 8.5mm gap; U20.5; U15.5
 MBA/HPM Hybrid permanent magnets; 8.5 mm gap; U28; U25
 APS now 10.75 mm gap; U33; U27

BioCARS Undulators		
Now: 7 GeV 10.5 mm gap	U23	U27
MBA: 6 GeV 8.5 mm gap	U19	U23

[Convert existing U27 to U19]

from MBA_prelim_expected_perf v2.pdf



Multibend Achromat Lattice: Time Resolution

- 2-3 fold electron bunch compression via a superconducting harmonic cavity.

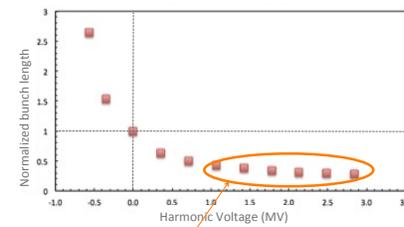
Timing at the APS with Multibend Achromat Lattice

Superconducting harmonic cavities

A. Nassiri

A higher harmonic RF cavity can be used to modify the potential in the RF bucket to either lengthen the electron bunch for improved lifetime or to compress the electron bunch.

Preliminary estimate of bunch compression



A 2-cell SRF harmonic cavity for Super-3HC Project at ELETTRA for bunch lengthening¹

Potential factors of 2-3:

88 MHz mode: 42 → 14 ps

117 MHz mode: 28 → 9.3 ps

Does not include beam dynamics and non-linear effects

¹ G. Penco, Elettra

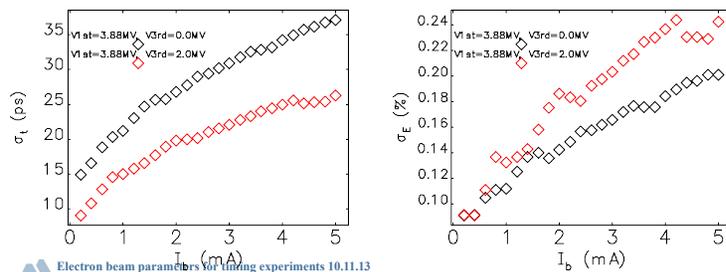


Multibend Achromat Lattice: Time Resolution

- 2-3 fold electron bunch compression via a superconducting harmonic cavity.

Harmonic Cavity

- Under normal operations a harmonic cavity will lengthen the bunch to achieve a lifetime target
- Harmonic cavity can be phased in reverse to shorten the bunch, but will reduce lifetime
- Possible operation of high-charge short bunch may be limited by impedance effects



Electron beam parameters for timing experiments 10.11.13

Vadim Sajaev, Timing Pre-workshop meeting Oct 11th, 2013



Multibend Achromat Lattice: Time Resolution vs. Lifetime

- Bunch compression shortens its lifetime, requiring more frequent injection.
- Might periodic (e.g. ~10 Hz) switch between pulse compression and expansion mitigate lifetime issues?

Table 7: Parameters for ID source points for H7BA-TwoSector-60pm-nux105-nuy34

κ	σ_t	ϵ_x	ϵ_y	β_x	β_y	σ_x	σ'_x	σ_y	σ'_y	σ_δ	$\tau_{10^{14}}$	ΔT_{inj}
	ps	pm	pm	m	m	μm	μrad	μm	μrad	10^{-4}	hour	s
						$N_b = 48$		$f_b = 13.0\text{MHz}$				
1.00	70	41.7	41.7	1.3	2.9	7.4	5.7	10.9	3.8	9.95	1.38	10.4
						$N_b = 81$		$f_b = 22.0\text{MHz}$				
1.00	35	42.5	42.5	1.3	2.9	7.4	5.7	11.0	3.9	1.00×10^1	1.19	5.3
1.00	69	39.6	39.6	1.3	2.9	7.2	5.5	10.6	3.7	9.77	2.23	9.9
						$N_b = 162$		$f_b = 44.0\text{MHz}$				
1.00	21	41.6	41.6	1.3	2.9	7.3	5.7	10.9	3.8	9.95	1.41	3.1
1.00	35	39.6	39.6	1.3	2.9	7.2	5.5	10.6	3.7	9.77	2.23	5.0
0.24	68	56.1	13.3	1.3	2.9	8.5	6.6	6.2	2.2	9.74	3.00	6.7
						$N_b = 216$		$f_b = 58.7\text{MHz}$				
1.00	20	40.7	40.7	1.3	2.9	7.3	5.6	10.8	3.8	9.86	1.72	2.9
1.00	34	38.8	38.8	1.3	2.9	7.1	5.5	10.5	3.7	9.70	2.91	4.9
0.11	68	61.4	6.5	1.3	2.9	8.9	6.9	4.3	1.5	9.79	3.00	5.0
						$N_b = 324$		$f_b = 88.0\text{MHz}$				
1.00	18	39.5	39.5	1.3	2.9	7.2	5.5	10.6	3.7	9.76	2.32	2.6
0.22	35	56.6	12.6	1.3	2.9	8.6	6.6	6.0	2.1	9.74	3.00	3.3
0.10	68	59.8	5.9	1.3	2.9	8.8	6.8	4.1	1.4	9.69	4.31	4.8

round beam

Bunch injection period

Would 3X bunch compression shorten lifetime by ~3X?

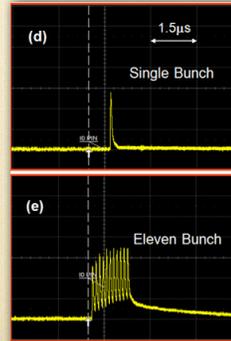
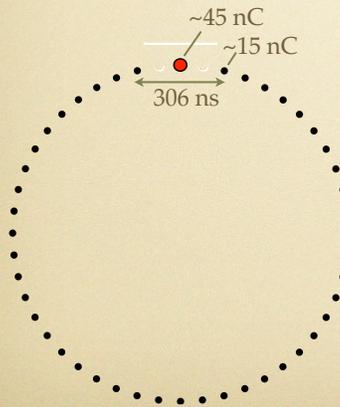
with 2X compression

from MBA_prelim_expected_perf v2.pdf



Multibend Achromat Lattice: “45+1” Mode

- ~45 nC (~12 mA) superbunch with a gap on either side would facilitate isolation of a high-flux pulse for picosecond time resolved studies. Could this become the new Normal Operating Mode?
- The high-speed chopper could also isolate cleanly 45 pulses (~675 nC) to speed the acquisition of data at pump-probe time delays longer than 1 revolution (3.68 μ s).



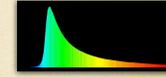
High-speed Chopper



Multibend Achromat Lattice: Bottom Line

Reduced horizontal emittance:

- Focus to smaller, round spot with single K-B mirror pair.
- Round beam means white beam slits can tailor the pink beam spectral bandwidth (beneficial for SAXS/WAXS and Laue).
- Smaller spot requires less laser energy per pulse, and allows more spots across the length of the crystal.

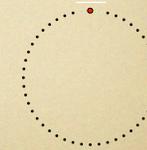


Pulse Duration:

- Superconducting harmonic cavity would allow generation of 2-3 times shorter pulses, which would be welcome.

Fill Pattern:

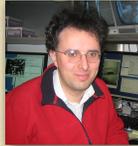
- Desire Normal Operating Mode e.g., 45+1, that allows isolation of a single pulse with a mechanical chopper (e.g., Jülich high-speed chopper).





Acknowledgements

LCP



Dr. Friedrich Schotte



Dr. Hyun Sun Cho



Dr. "Nara" Dashtorajev



Dr. Eric Henry



Bernie Howder



Outside Collaborators



Prof. John Olson
Rice U.



Dr. Michael Wulff
ESRF



Prof. Mikio Kataoka
NAIST

Dr. John Kyndt
Arizona State

NIH/NIDDK/LCP

- Gerhard Hummer
- Ville Kaila

BioCARS (Sector 14 at APS)

- Keith Moffat (PI), Yu-Shen Chen
- Tim Graber, Rob Henning
- Zhong Ren, Vukica Šrajer

Rice University

- John Olson
- Jayashree Soman (Mb, Hb crystals)

LCLS

- Marco Cammarata
- David Fritz

Germany

- Dwayne Miller
- Ilme Schlichting

NAIST

- Hironari Kamikubo

Time-resolved Structures of PYP

