Challenges and Opportunities for Time-Resolved Crystallography at the Next Generation X-ray Sources

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Consortium for Advanced Radiation Sources

The University of Chicago

Time-resolved crystallography

Despite of available static structures of biological macromolecules, the detailed mechanism by which they function often remains elusive.

Challenge:

- detect structural changes
- determine reaction mechanism

Need to capture molecules in action -

From snapshots to movies...

"*Stopping Time*" Gus Kayafas (Ed.)
Photographs by Harold Edgerton

Time-resolved crystallography

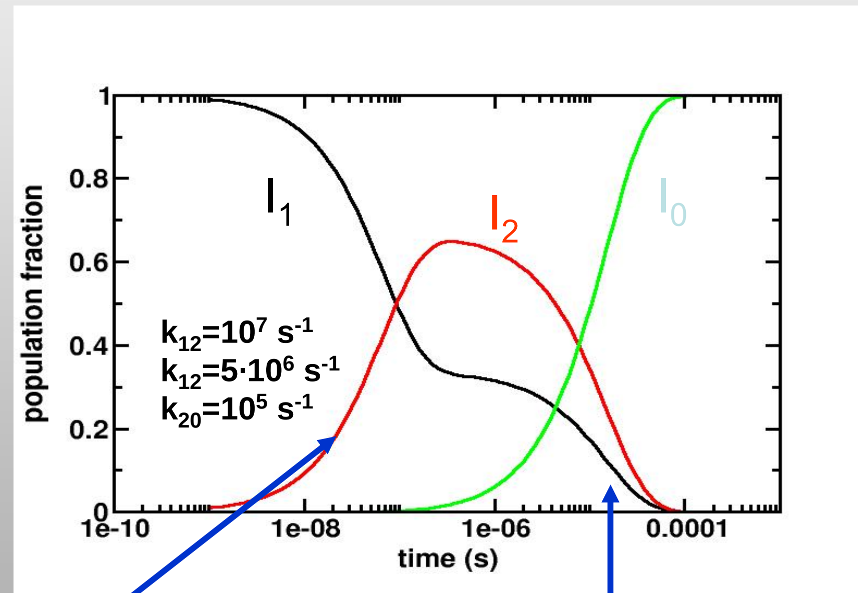
Ultimate goal:

Determine (time-independent) structures of intermediates and reaction mechanism

$$I_0 \xrightarrow{h\nu} I_1 \underset{k_{12}}{\overset{k_{21}}{\rightleftarrows}} I_2 \xrightarrow[k_{20}]{} I_0$$

Concentrations of intermediates:

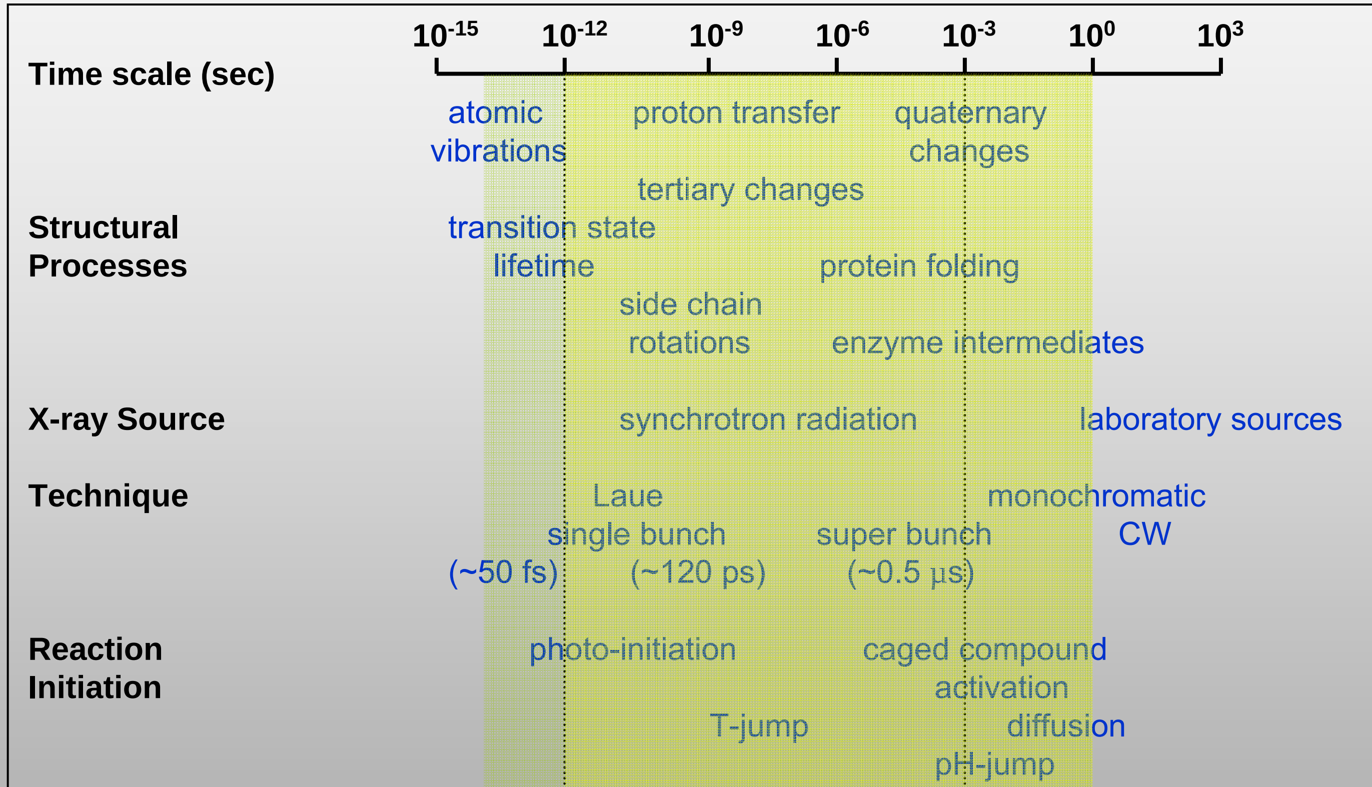
$$C_i(t) = C_{0i} + C_{1i}\exp(-K_1 t) + C_{2i}\exp(-K_2 t)$$



Accumulation detectable?

Mixture of states at most time points

Biological activity involves structural changes



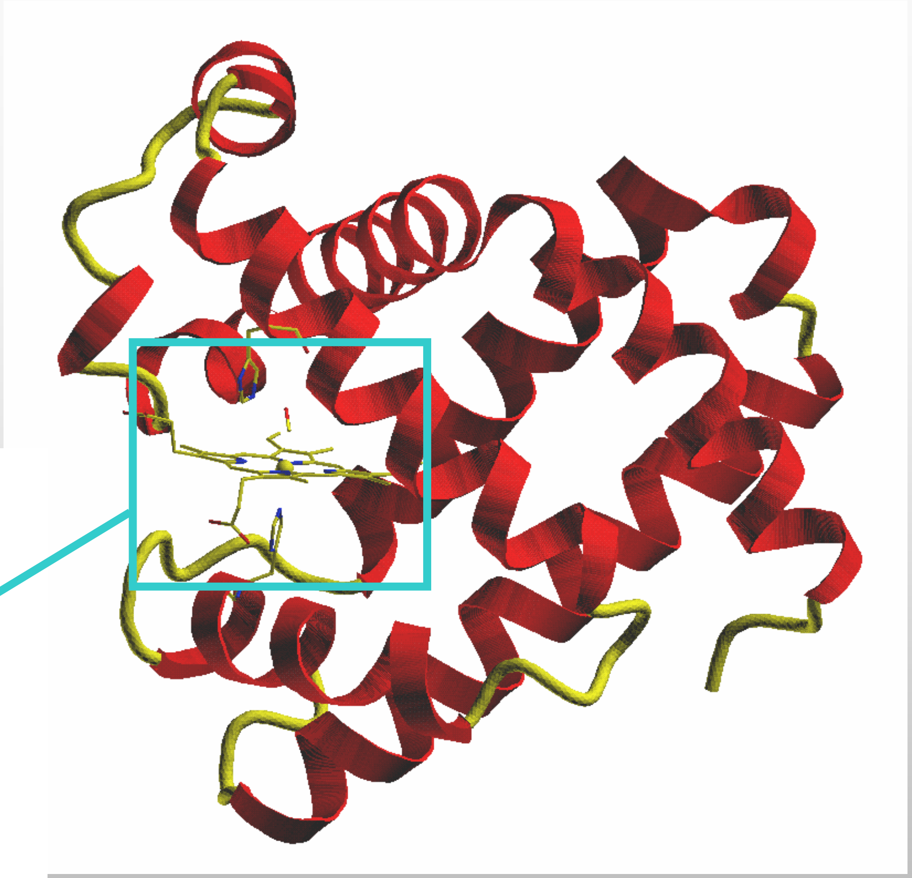
Capture fast processes at ambient temperature without physical or chemical trapping of intermediates

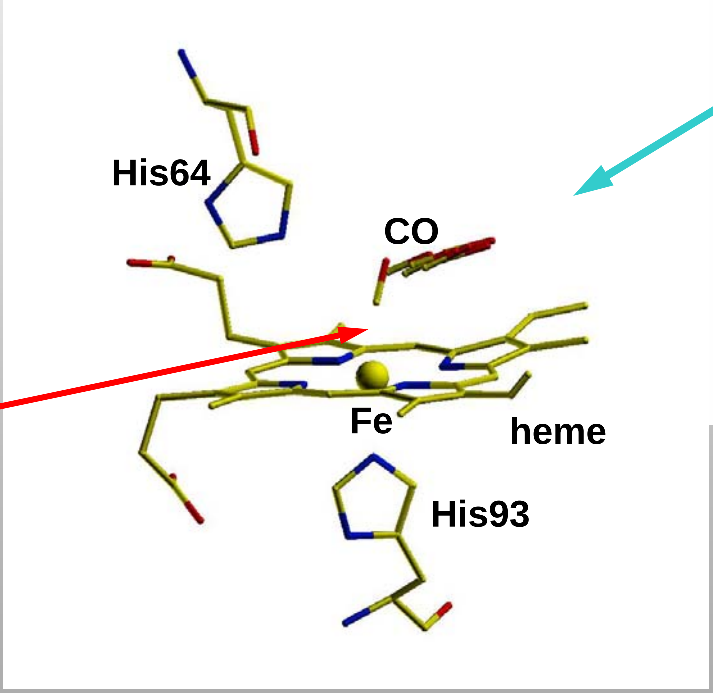
⇒ Reaction initiation & Laue crystallography

Myoglobin (Mb)

Small structural changes (0.2-0.3Å) following ligand photo-dissociation

Time scale: ps – ms





laser pulse breaks Fe-CO bond

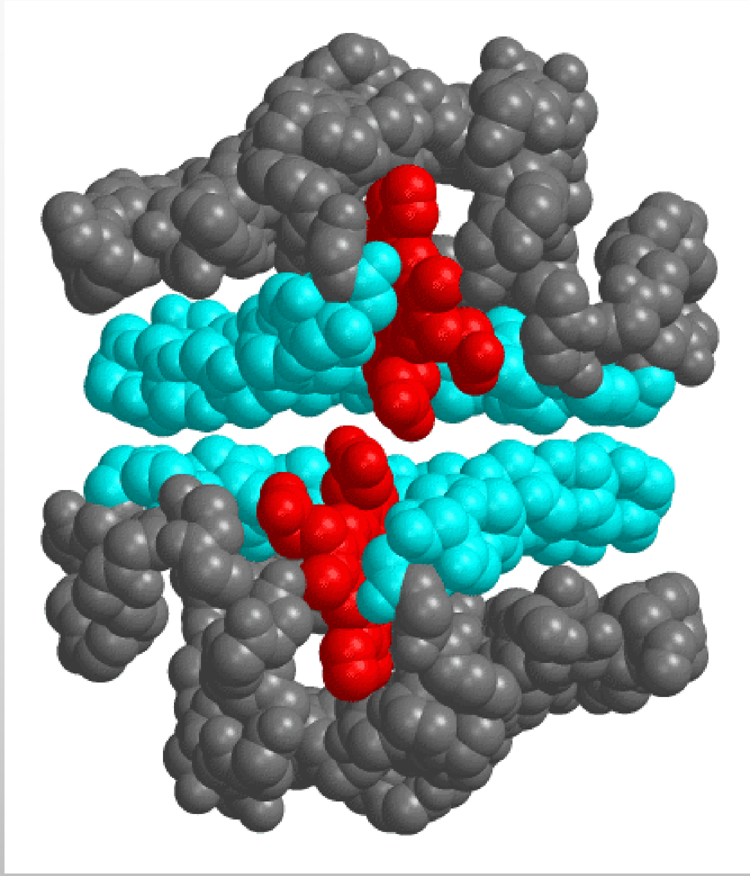
Experiments:

Beamline ID09, ESRF
Beamline 14-ID, APS

Srajer *et al*., Science 274 (1996) 1726-29
Srajer *et al*., Biochemistry 40 (2001) 13802-15
Schotte *et al*., Science 300 (2003) 1944-7

Dimeric Hemoglobin HbI

(from clam *Scapharca Inaequivalvis*)



Model for studies of cooperative protein behavior by time-resolved crystallography

- Cooperative ligand binding demonstrated in crystals
- Structural transitions involved in ligand binding and dissociation localized and not too large: crystals survive quaternary change
- Successful HbI-CO ➞ deoxy HbI ➞ HbI-CO transformation in the crystals
- Crystals diffract to atomic resolution (~1Å)

Knapp *et al.*, Biochem. 42 (2003) 4640-47
Knapp *et* al., PNAS 103 (2006) 7649-54

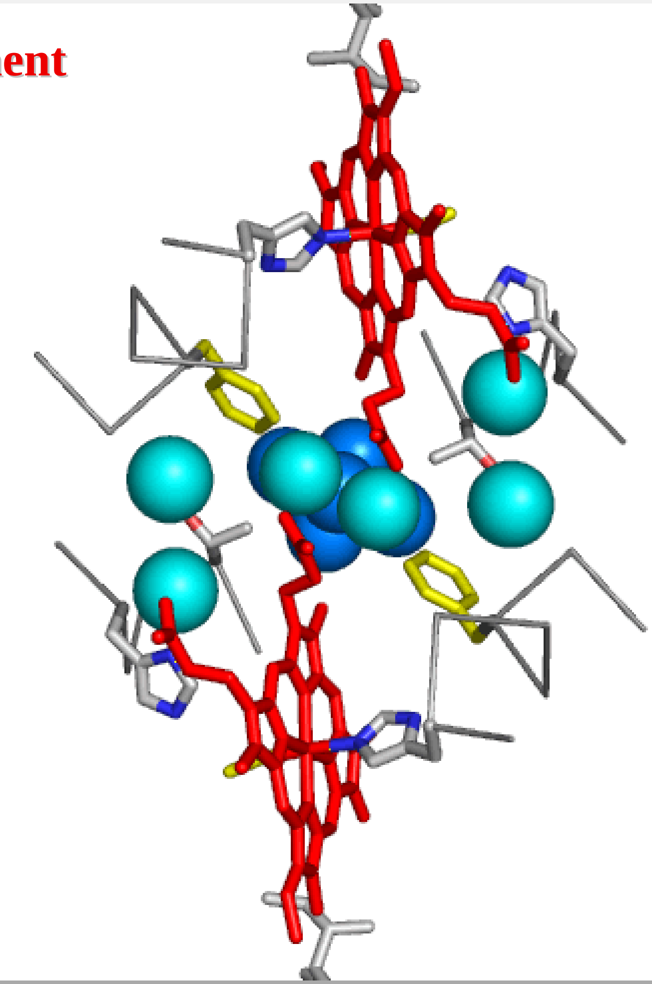
Experiments:
Beamline 14-ID, BioCARS, APS

James Knapp and William Royer
U of Mass Medical School, Worcester, MA

Vukica Srajer, Reinhard Pahl, BioCARS

Hemoglobin (HbI)

Key structural transitions with functional ramifications



Heme movement

F4 Phe flipping

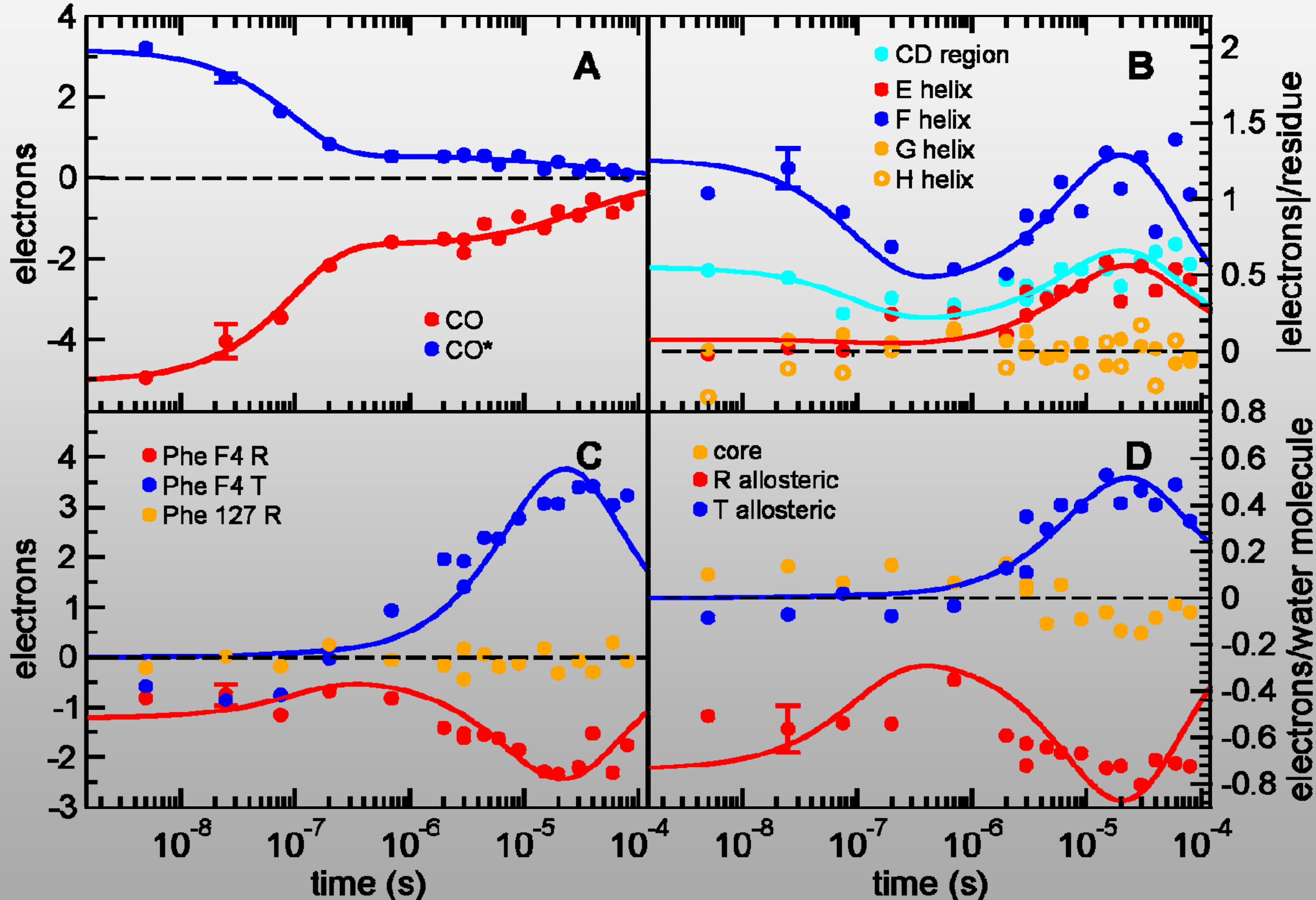
Interface water rearrangement

What is the cascade of structural events?

Do structural intermediates facilitate R to T transition?

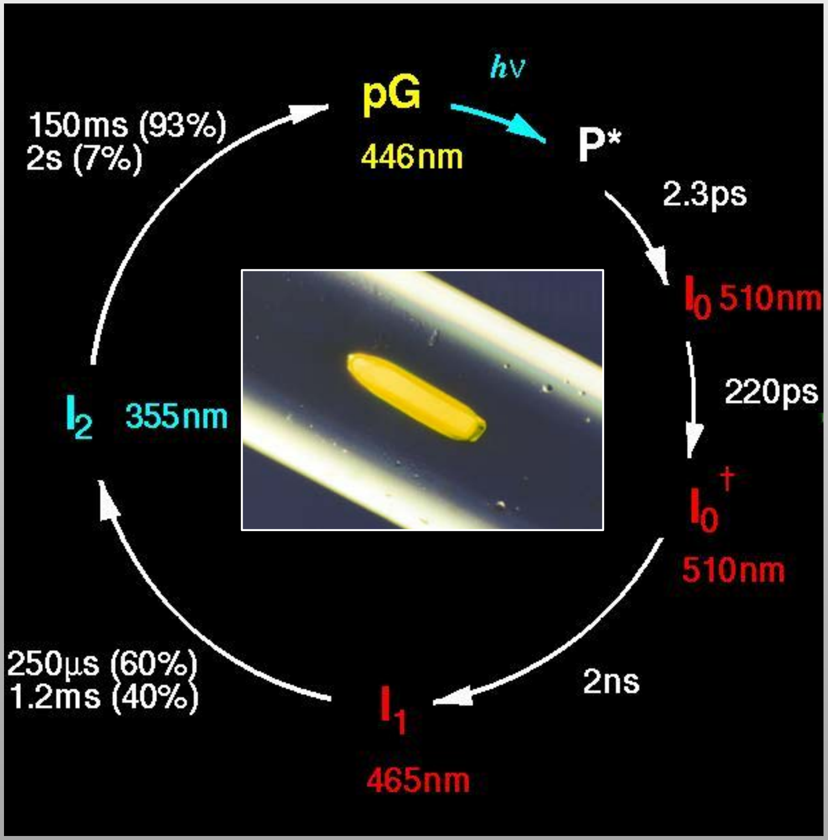
Are these transitions concerted or sequential?

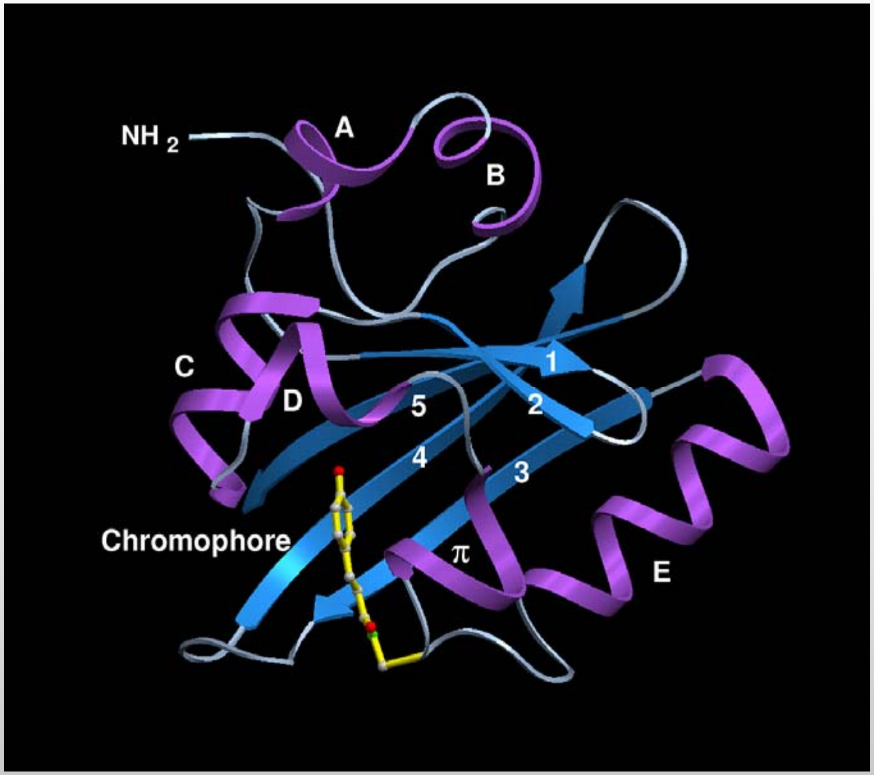
Integrated difference electron density values [F_o(light)-F_o(dark)]



Photoactive Yellow Protein (PYP)

Bacterial blue-light photo-receptor



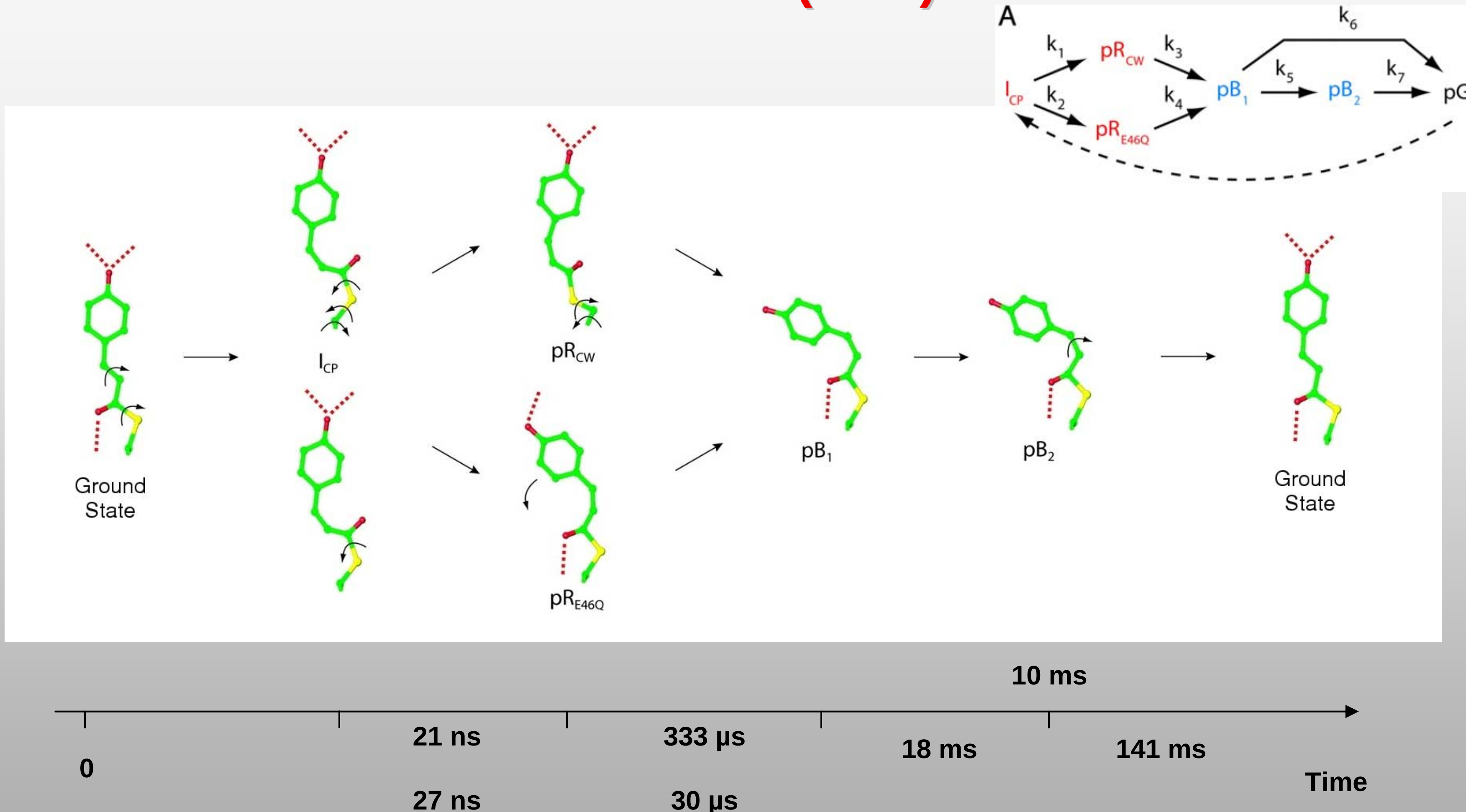


Experiments:

Beamline ID09, ESRF
Beamline 14-ID, APS

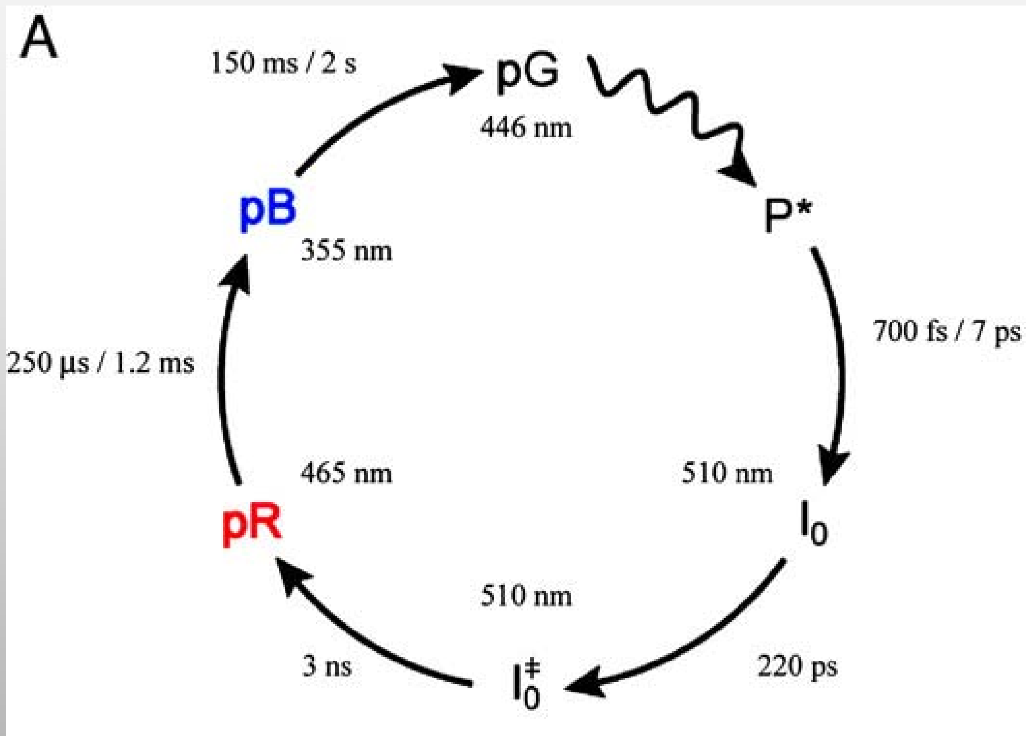
Borgstahl *et al.*, Biochem. 34 (1995) 6278-87
Genick *et al.*, Science 275 (1997) 1471-75
Perman *et al.*, Science 279 (1998) 1946-50
Ren *et al.*, Biochemistry 40 (2001) 13788-801

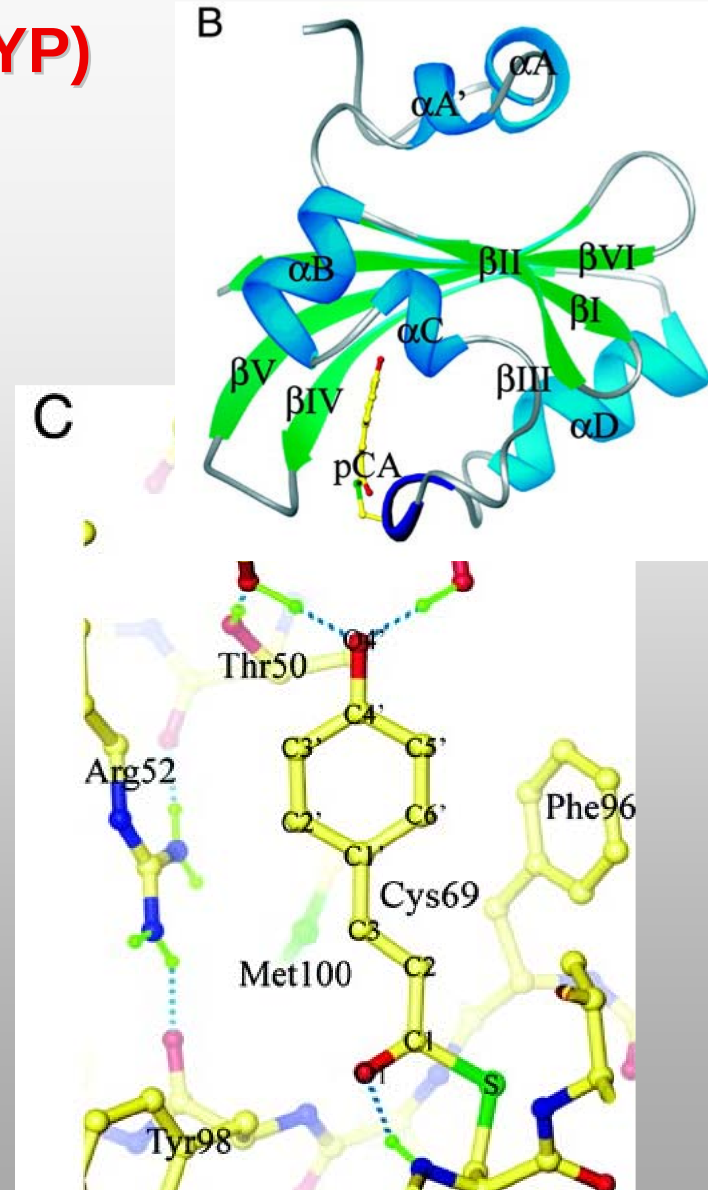
Photoactive Yellow Protein (PYP)



Anderson *et al.*, Structure 12 (2004) 1039-45
Ihee *et al.*, PNAS 102 (2005) 7145-50

Photoactive Yellow Protein (PYP)



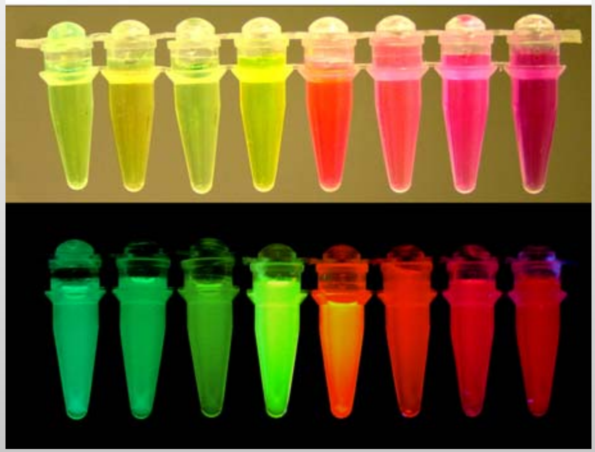


Anderson *et al.*, Structure 12 (2004) 1039-45
Ihee *et al.*, PNAS 102 (2005) 7145-50

Green Fluorescent Protein (GFP)

A Switchable Fluorescent Reporter

- protein-protein interactions
- protein localization
- developmental biology
- gene regulation
- drug screening (HTS, HCS)
- pH sensing
- Ca^{2+} sensing
- single-molecule assays
-
-



Wiedenmann *et al.*, PNAS 101 (2004) 15905-10
Nienhaus *et al.*, PNAS 102 (2005) 9156-59

Enzyme Reactions

Key enzyme in cholesterol biosynthesis in mammals.

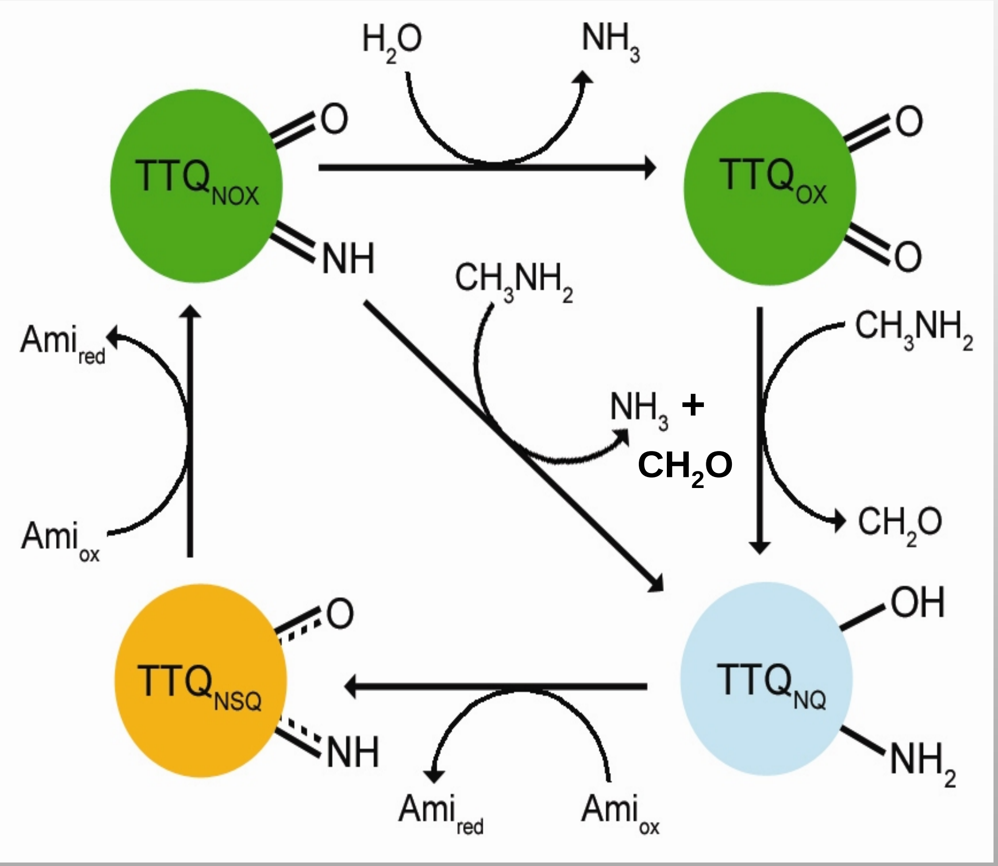
Enzyme is obligate dimer with the active site at the dimer interface.
On cofactor binding, the small domain closes over the NADH adenine ring and a 50 residue flap, disordered in the structure, closes over the active site.

Problem:
Reaction mechanism in current studies is not reversible.
What is different from nature?

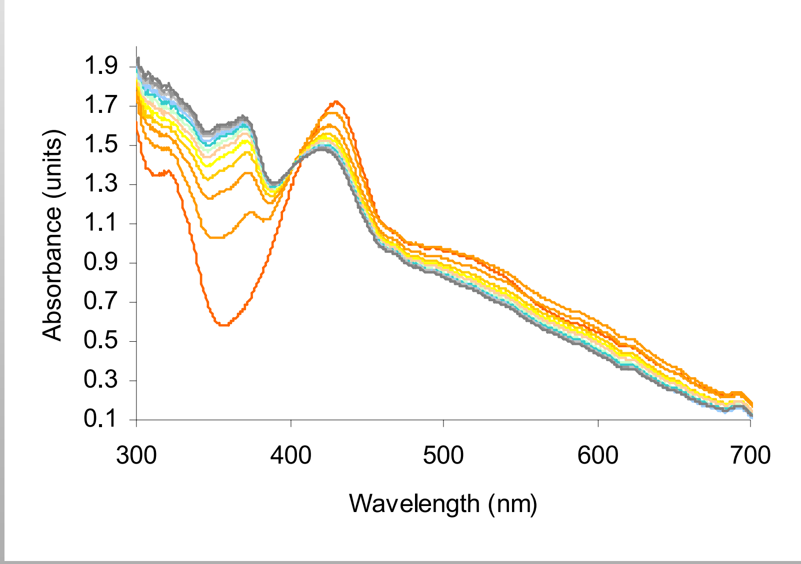
Only limited understanding about the reaction mechanism and function of domains.

Enzyme Reactions

Methylamine dehydrogenase



O-quinone, TTQ_{OX} (resting state)
N-quinol, TTQ_{NQ} ($2e^-$ reduced)
N-semiquinone, TTQ_{NSQ} (1e- reduced)
N-quinone, TTQ_{NOX} (oxidized)



C. Wilmot *et al.* (2006)

Improved X-ray Sources: BioCARS 14-ID Beamline 2006

Insertion device

Tandem undulators U-23 & U-27 are replacing Undulator-A

$E_{min,\ U23}$ = 11.96 keV

$E_{min,\ U27}$ = 6.83 keV

Optics

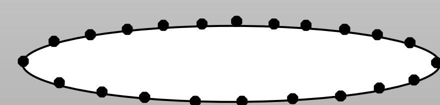
large KB-system (M_{vert}~ 5.2:1, M_{horz}~ 8.3:1)

focal spot ~ 70 x 43 μm^2

10^8 - 10^{10} photons/pulse at sample

$\Rightarrow$ ~ 1 x-ray pulse/image

APS Standard Operating Mode



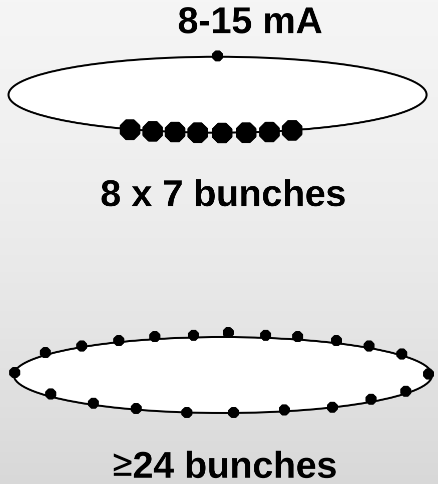
24 bunches

Mechanics

Fast-Shutter : 153 ns spacing between bunches

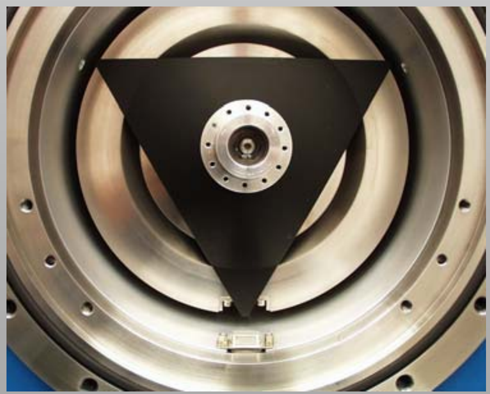
New Experiment – Faster X-ray Shutter

3rd Generation – Synchronous Shutter

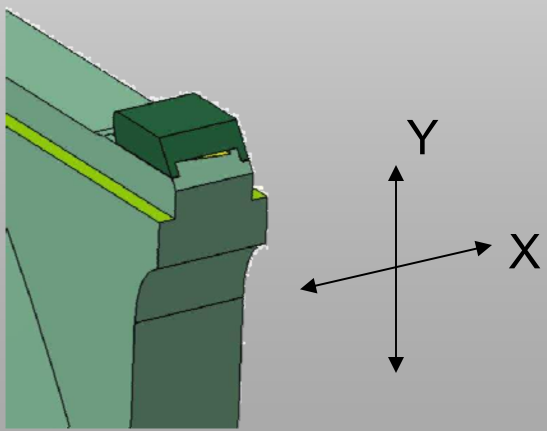


Geometry	TIMETAL (Ti6Al4V) triangle, 166 mm side multiple aperture size vacuum (< 10^{-3} Torr)
Rep. Rate	60,320 rpm (994.7 Hz) T = 1.005 ms
Pulse Width	t_{open}= 190 – 420 ns, 0.46 – 11.5 μs, >ms

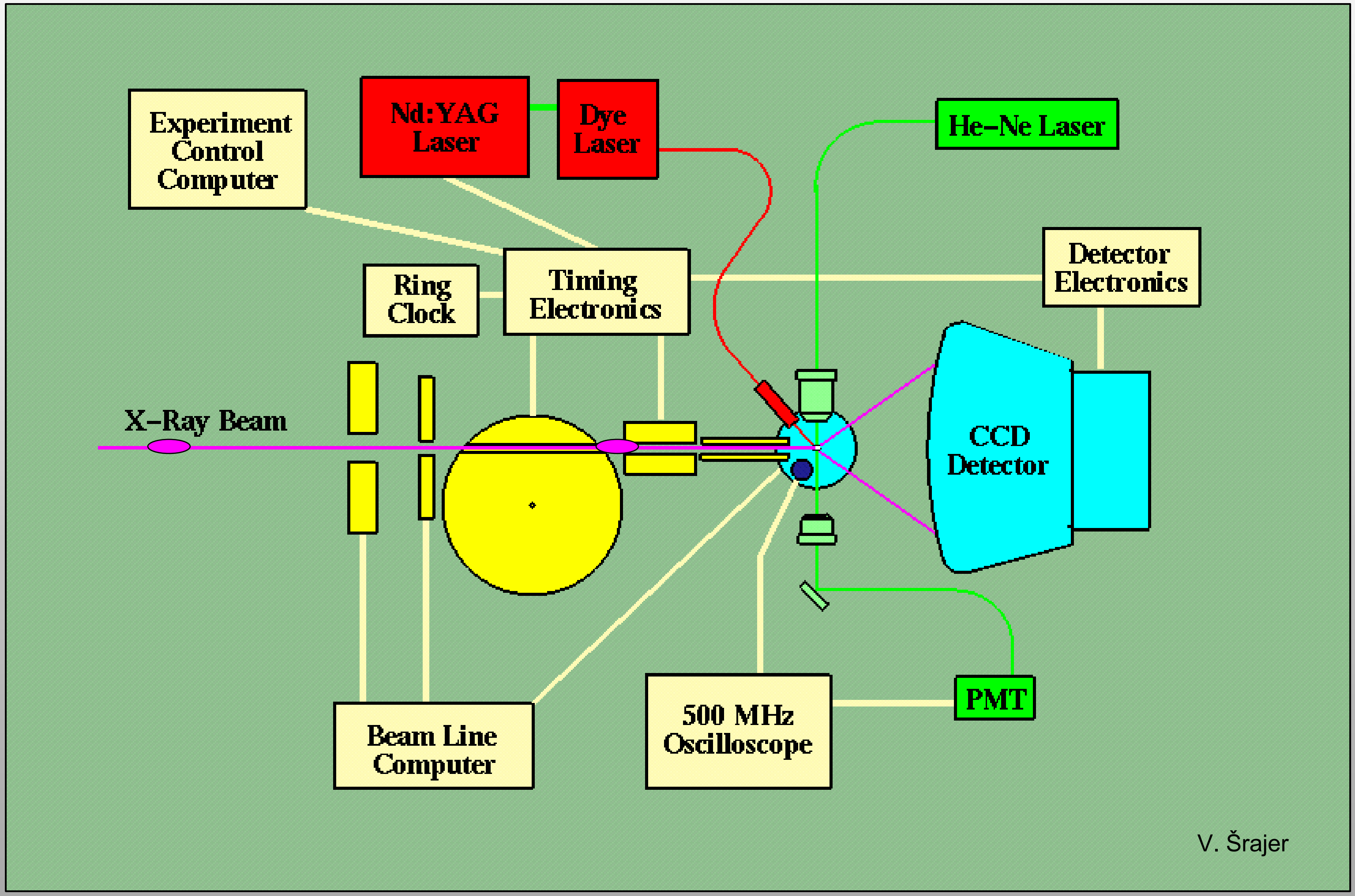
W. Schildkamp,
R. Pahl,
M. Wulff,
B. Lindenau, …



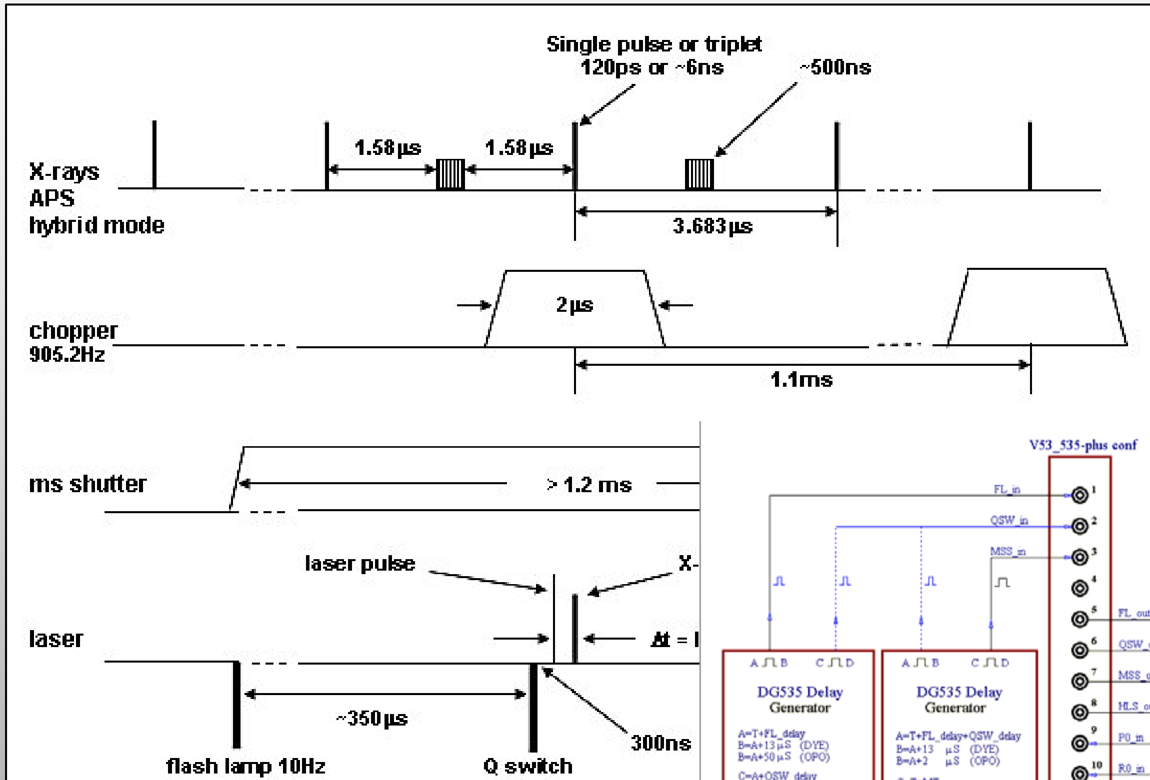
P. Anfinrud,
F. Schotte,
M. Wulff,
B. Lindenau …

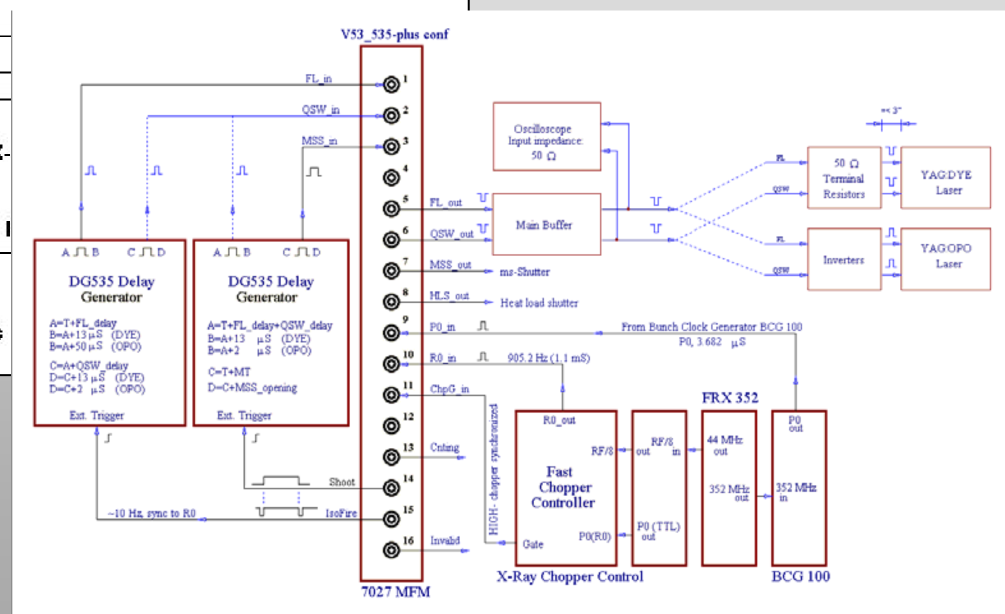


The Experiment



The Experiment

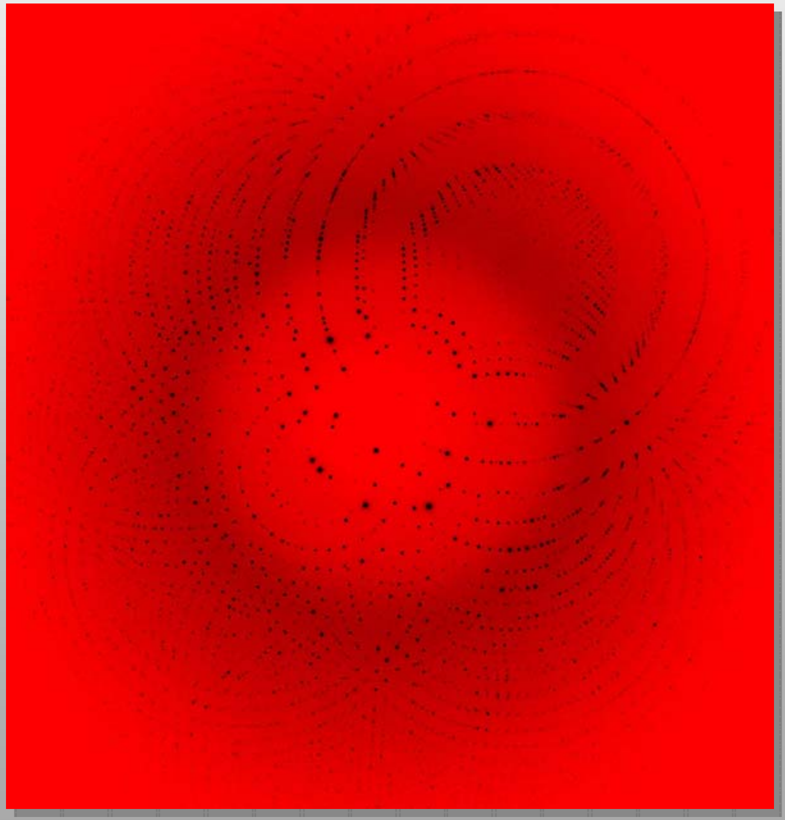




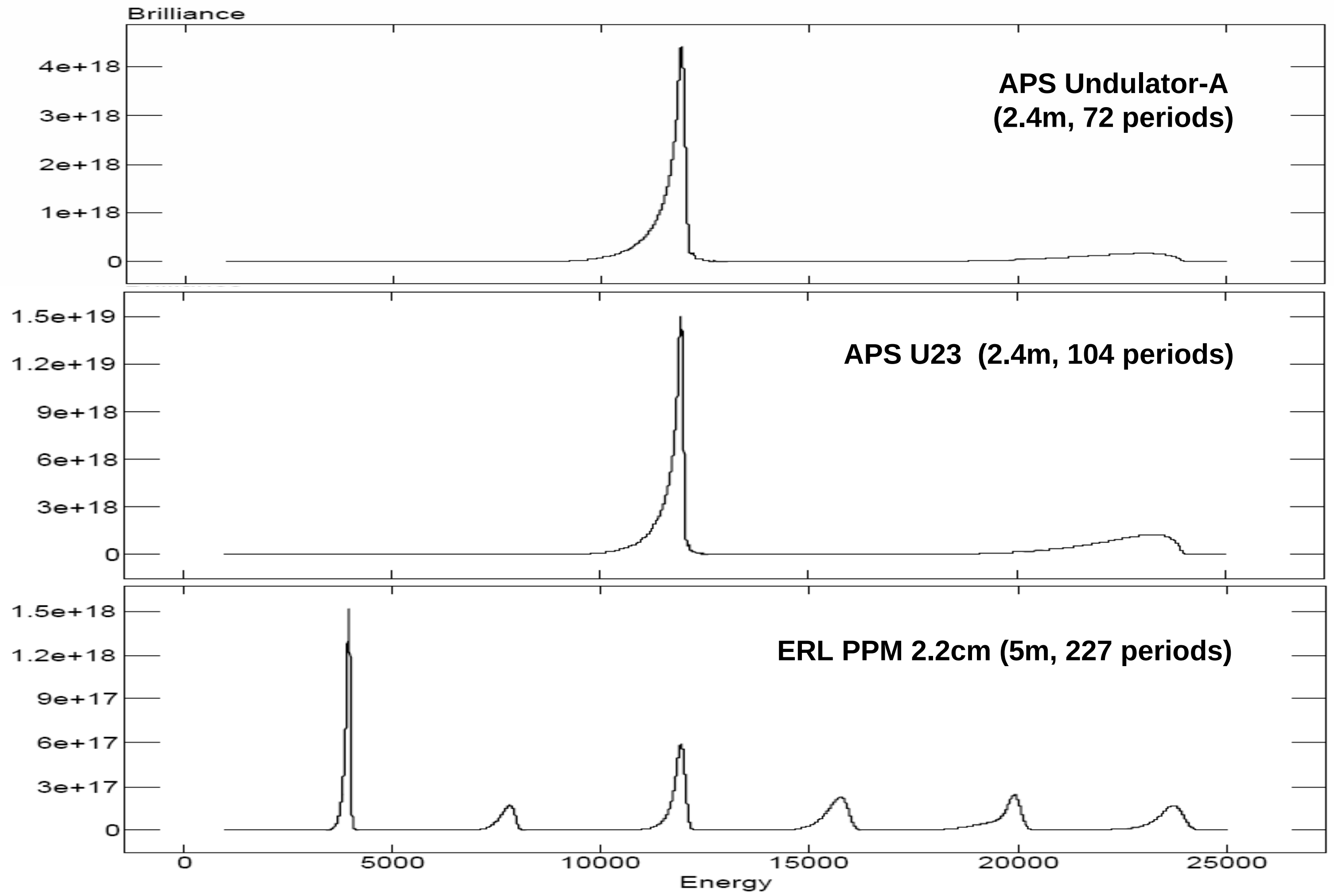
Timing of laser and X-ray pulses

Data Collection Strategy

- **Pump:** *femtosecond* to *nanosecond* laser pulse at appropriate wavelength
- **Probe:** 120 ps or longer x-ray pulses
- **Laser – x-ray delay times:**
  100 ps to seconds
- 1 – 10 laser / x-ray pulses
  per image
- ~1 – 5 sec between laser pulses
- 40 – 60 images per data set
  2-3 deg angular increment
- **Time to collect dataset:**
  5 min – 1 hour elapsed time per data set
  (using conventional CCD detector)



New X-ray Sources



New X-ray Sources

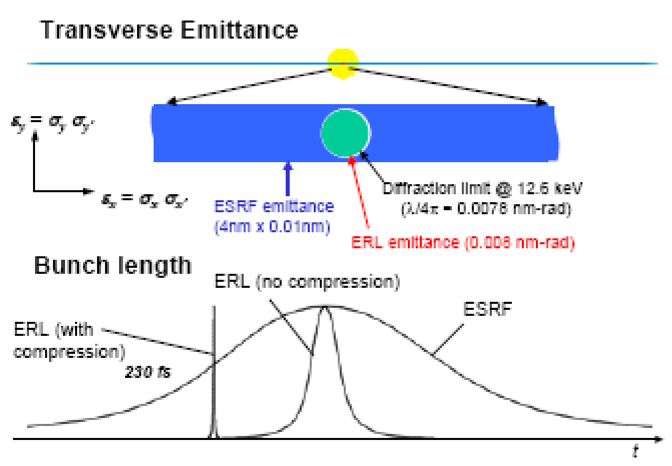


Fig.1 provides a schematic comparison of electron bunch size, in space and time, for storage ring and ERL sources. In the lower figure bunch intensity profiles are normalized to unity. 230fs refers to FWHM (100fs RMS).

APS :

ε_x = 8.135 nm.rad

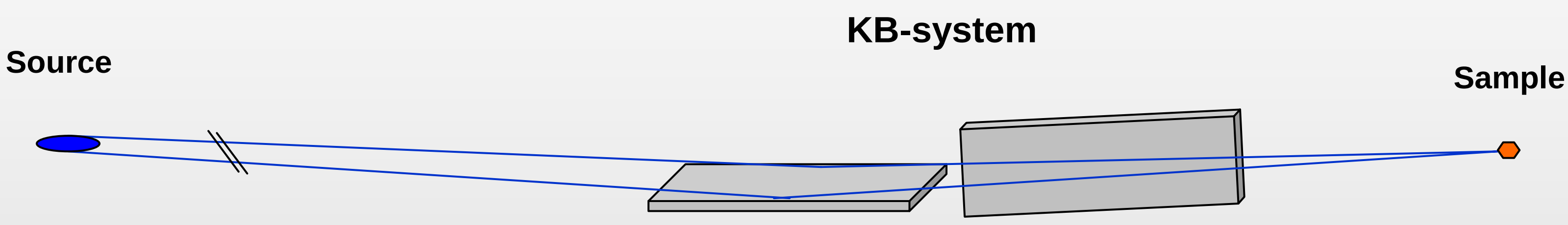
ε_y = 0.065 nm.rad

ERL :

$\varepsilon_{x,y}$ = 0.051 nm.rad

Finkelstein *et al., J.Phys.Chem.Sol. (2006)*

New X-ray Sources



APS source (U23, 24-bunch)

1.031×10^{19} ph/s/mr²/0.1%/mm²

$\sigma_{x,y}$ = 1465, 33 μm, $\sigma'_{x,y}$ = 49.1, 15.6 μrad

bandwidth at 12 keV

~235 eV, $^{\Delta E}/_{E} \geq 1.96\%$

beamline & optics

ø1.4mm mask at 25.6m

sample at 56m

KB-system at 47-50m (M_v=5.2:1, M_H=8.3:1)

focus (ideal)

176 x 6.3 μm²

photons at sample …

8.3×10^{12} ph/s/1.96%/μm²

⇒ 1.2×10^{8} ph/pulse within 10x10 μm²

ERL source (5m, 22mmPPM, mode E)

5.898×10^{17} ph/s/mr²/0.1%/mm²

σ = 65.9 μm, σ' = 45.3 μrad

bandwidth at 12 keV

~270 eV, $^{\Delta E}/_{E}$ ~2.25%

beamline & optics

sample at 50m

KB-system at 47.5m (M~19:1)

focus (ideal)

3.5 x 3.5 μm²

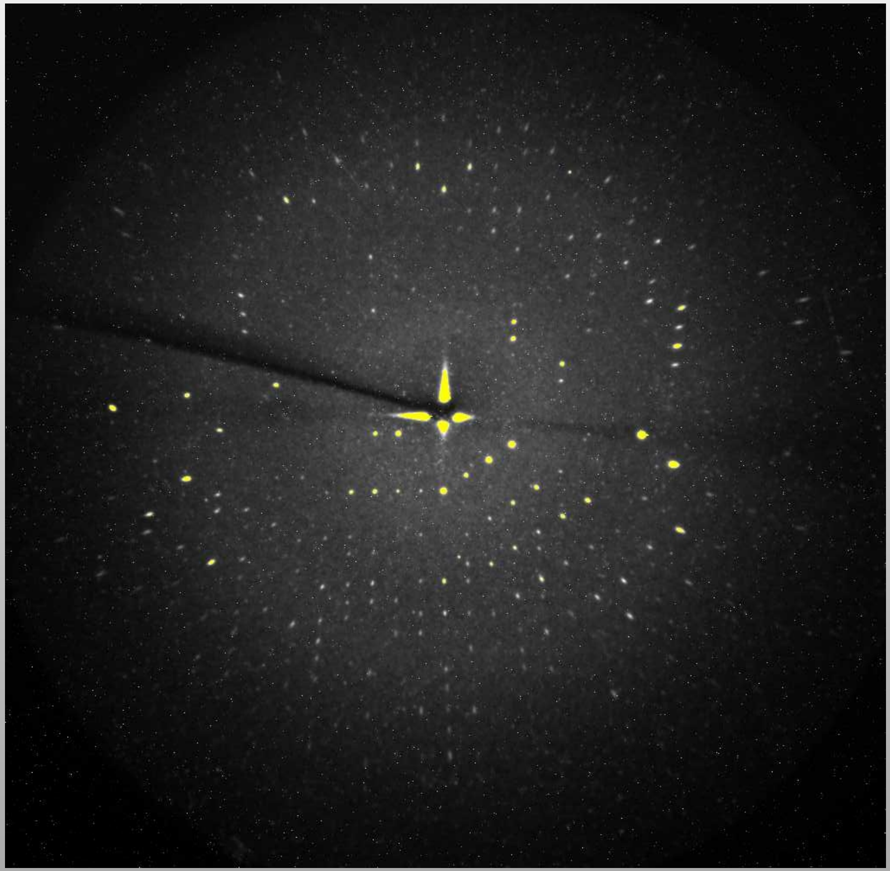
photons in focal spot …

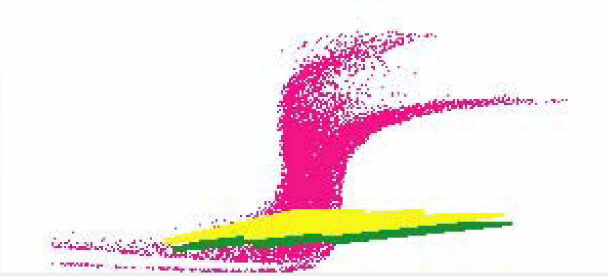
9.8×10^{12} ph/s/2.25%/μm²

⇒ 1.2×10^{8} ph/pulse

SPPS:

First Experiments





Sub-Picosecond Pulse Source

Photoactive Yellow Protein (PYP)

crystal size	200 x 650 μm^2
exposure	3000 pulses
oscillation	3 deg
max. resolution	~1.25 Å

X-rays

9.365 keV, $^{\Delta E}/_{E}$~1.5%
~80fs @ 10 Hz
2 x 10^7 ph/pulse/2x2mm^2
$\Rightarrow$ 2 x 10^9 ph/image

R. Pahl et al. (2004)

Time-resolved Crystallography

The challenges ahead…

Source

specialized insertion devices

Optics

beamline design and quality of optical elements

Timing

flexible rep. rates 10Hz .. 1kHz .. 1 MHz
variable bunch charge, pulse length 50 fs .. 2 ps
trigger accuracy: bunch monitor (EO)

Detectors

large area detectors
fast, and high DQE

Samples

heating, radiation damage (primary and secondary) a problem

Data Analysis

new data collection strategies, analysis and interpretation

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  Shengyang Ruan, Keith Brister, Robert Henning

- **CARS**

  Jim Viccaro, Mati Meron, Timothy Graber, Robert Henning, Peter Eng …

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  ASD, AOD, AES, XFD, XSD

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  Philip Anfinrud, Friedrich Schotte

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SPPS Collaboration

Sub-Picosecond Pulse Source

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Magnus Bergh
Gosta Huldt

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Jochen Schneider
Thomas Tschentscher
Horst Schulte-Schrepping

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Holger Schlarb
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Kelly Gaffney

Copenhagen University
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Richard Neutze

Into a brighter future -

on to exciting science -

Thank You !