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

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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

\[ I_0 \xrightarrow{\text{hv}} I_1 \xrightarrow{k_{21}} I_2 \xrightarrow{k_{12}} I_0 \]

Concentrations of intermediates:

\[ C_i(t) = C_{0i} + C_{1i}\exp(-K_1t) + C_{2i}\exp(-K_2t) \]

Accumulation detectable? Mixture of states at most time points.
**Biological activity involves structural changes**

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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

Experiments:
Beamline ID09, ESRF
Beamline 14-ID, APS

Srajer et al., Science 274 (1996) 1726-29
Srajer et al., Biochemistry 40 (2001) 13802-15
**Dimeric Hemoglobin Hbl**
(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 Hbl-CO $\rightarrow$ deoxy Hbl $\rightarrow$ Hbl-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_0(light)-F_0(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
Ren et al., Biochemistry 40 (2001) 13788-801
Photoactive Yellow Protein (PYP)

Ihee et al., PNAS 102 (2005) 7145-50
Photoactive Yellow Protein (PYP)

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)

\[ CH_2O + NH_3 \rightarrow H_2O + NH_3 \]

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

- $E_{\text{min, }U23} = 11.96$ keV
- $E_{\text{min, }U27} = 6.83$ keV

**Optics**

- large KB-system ($M_{\text{vert}} \approx 5.2:1$, $M_{\text{horz}} \approx 8.3:1$)
- focal spot $\approx 70 \times 43 \mu m^2$

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

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

**Mechanics**

- Fast-Shutter: 153 ns spacing between bunches
New Experiment – Faster X-ray Shutter

3\textsuperscript{rd} Generation – Synchronous Shutter

Geometry

- TIMETAL (Ti6Al4V)
- triangle, 166 mm side
- multiple aperture size
- vacuum (< 10\textsuperscript{-3} Torr)

Rep. Rate

- 60,320 rpm (994.7 Hz)
- \( T = 1.005 \text{ ms} \)

Pulse Width

- \( t_{\text{open}} = 190 – 420 \text{ ns, 0.46 – 11.5 \mu s, >ms} \)

- 8-15 mA

- 8 x 7 bunches

- \( \geq 24 \text{ bunches} \)

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

[Diagram of the experiment setup including labeled components such as Nd:YAG Laser, Dye Laser, He-Ne Laser, Detector Electronics, CCD Detector, PMT, 500 MHz Oscilloscope, Experiment Control Computer, Ring Clock, Timing Electronics, Beam Line Computer, X-Ray Beam, etc.]

V. Šrajer
The Experiment

Timing of laser and X-ray pulses

The Experiment

Timing of laser and X-ray pulses

BioCARS
The University of Chicago

Reinhard Pahl
ERL X-ray Science Workshop, Cornell 2006
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

- APS Undulator-A (2.4m, 72 periods)
- APS U23 (2.4m, 104 periods)
- ERL PPM 2.2cm (5m, 227 periods)
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. 230 fs refers to FWHM (100 fs RMS).


**APS:**

\[ \varepsilon_x = 8.135 \text{ nm.rad} \]
\[ \varepsilon_y = 0.065 \text{ nm.rad} \]

**ERL:**

\[ \varepsilon_{x,y} = 0.051 \text{ nm.rad} \]
New X-ray Sources

APS source (U23, 24-bunch)
1.031 x 10¹⁹ ph/s/mr²/0.1%/mm²
σₓ,y = 1465, 33 µm, σ’ₓ,y = 49.1, 15.6 µrad

bandwidth at 12 keV
~235 eV, ΔE/E ≥ 1.96%

beamline & optics
Ø1.4mm mask at 25.6m
sample at 56m
KB-system at 47-50m (Mᵥ=5.2:1, Mᵥ=8.3:1)
focus (ideal)
176 x 6.3 µm²

photons at sample …
8.3 x 10¹² ph/s/1.96%/µm²

⇒ 1.2 x 10⁸ ph/pulse within 10x10 µm²

ERL source (5m, 22mmPPM, mode E)
5.898 x 10¹⁷ ph/s/mr²/0.1%/mm²
σ = 65.9 µm, σ’ = 45.3 µrad

bandwidth at 12 keV
~270 eV, ΔE/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 x 10¹² ph/s/2.25%/µm²

⇒ 1.2 x 10⁸ ph/pulse
**SPPS:**

First Experiments

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**Photoactive Yellow Protein (PYP)**

- Crystal size: 200 x 650 µm²
- Exposure: 3000 pulses
- Oscillation: 3 deg
- Max. resolution: ~1.25 Å

**X-rays**

- Energy: 9.365 keV, \(\frac{\Delta E}{E} \approx 1.5\%\)
- Duration: ~80 fs @ 10 Hz
- Intensity: \(2 \times 10^7\) ph/pulse/2x2mm²
  \(\Rightarrow 2 \times 10^9\) ph/image

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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
Acknowledgements

- **Keith Moffat Group (University of Chicago)**
  Vukica Srajer, Tsu-yi Teng, Ben Perman
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  Zhong Ren, Marius Schmidt

- **BioCARS**
  Wilfried Schildkamp, Claude Pradervand, Gary Navrotsky
  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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  NIH: NCRR, NIDDK
Into a brighter future -
on to exciting science -

Thank You!