Femtosecond Time-resolved Laue crystallography: Using an ERL to Watch Proteins Function on the Chemical Time Scale

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Time-resolved Laue Crystallography: Probing ligand migration and correlated protein motion in photolyzed carbon monooxy myoglobin
European Synchrotron and Radiation Facility, Grenoble, FRANCE

Time-Resolved X-ray (TReX) Studies
X-ray Generation at ID09B (ESRF)

- 4-bunch mode (~30-nC/pulse; 704-ns spacing)
- Low-beta straight section (H source size: 130-μm FWHM)
- In-vacuum undulator (6-mm gap; 15-keV fundamental)
- Toroidal mirror (maximizes flux via single reflection)
- High-speed chopper (164-ns opening time with 100-μm vertical aperture)

~10^{10} photons focused to 
~100 μm spot (4-bunch mode)
Packing of P6 Myoglobin Crystal (D122N)

\[ a = b = 91.20 \text{ Å}, c = 45.87 \text{ Å}, \alpha = \beta = 90^\circ, \gamma = 120^\circ; \text{ heme plane to } a,b \text{ plane } \angle = 55^\circ \]
"Pink" beam peaked around
~ 15 keV (0.8 Å)
~$10^{10}$ photons per shot;
~ 8 shots per image

$\lambda = 2d \sin \theta$

Spot sizes:
~125-μm FWHM

$\lambda = 0.75...1.0$ Å

~$10^{11}$ photons
Laue diffraction image of MbCO

ca. 4000 usable reflections
Pump-Probe Geometry

~$6 \times 10^{13}$ photons
($\sim 20 \, \mu J; \, 580 \, nm; \, \sim 0.8 \, mJ/mm^2$)

~$3 \times 10^{13}$ hemes in probed volume

X-rays:
~$10^{10}$ photons/pulse

$\Delta T$ in pumped volume $\sim 2 \, ^{\circ}C$

120 microns

60 microns
Pump-Probe Timing (integrated X-ray exposure)
Pump-induced Intensity Changes at 100 ps

\[ \Delta I = -80\sigma \ldots +80\sigma \]
Color-coded maps superimposed: MbCO at 100 ps

- Photolyzed
- Unphotolyzed

- CO
- Fe
- heme
- His64
- Leu29
- Ile107
- His93
- Leu104
Global Tertiary structure changes of photolyzed MbCO are unresolvably fast ($t = 100$ ps)
Extending time-resolved Laue crystallography to the femtosecond time domain: What are the issues?

- Sample reversibility
  - Nonlinear absorption damages chromophore and compromises sample reversibility
  - Can we record “single-shot” Laue diffraction images?

- Flux requirements
  - High-dynamic range diffraction image requires ~16 shots at ESRF
  - Can the Cornell ERL generate suitable X-ray pulse energy for “single-shot” Laue diffraction?

- Repetition frequency limits (for non-exchangeable, crystalline samples)
  - Limited by laser pulse energy deposited in the crystal
    - 3.3 Hz at ESRF with 100 micron spot size
  - To what extent can tighter focusing boost the pump-probe repetition frequency?

- Group velocity mismatch between laser and X-ray pulses
  - Which sample excitation geometries preserves maximum time resolution?
Intense femtosecond excitation converts MbCO (a) to met-Mb (b); (see darkening at the site of exposure).

- Photo-oxidation is triggered by multi-photon absorption via a strongly-absorbing shot-lived (<100 fs) excited state

- Stretching the optical pulse shuts down this channel, but broadens the time resolution

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X-ray flux needed for “single-shot” Laue diffraction

\# X-ray Photons \propto (\text{bunch charge}) \times (\text{undulator length})

- **ESRF Flux (~}10^{10} \text{ photons/shot}**
  - 30 nC at 6 GeV
  - 2 m U17 undulator
  - 16 shots
  - ~10^{11} incident photons

- **ERL**
  - 10 nC at 5 GeV
  - 100 m U17 undulator
  - 1 shot

“FAT” bunch
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Optimum X-ray Focus (~20 micron spot?)

- Volume intercepted by 20 micron X-ray beam:
  - Contains ~ $1.4 \times 10^{12}$ hemes (37 mM for P6 MbCO)
- X-ray induced T-jump when focusing ~$1 \times 10^{11}$ photons at 8 keV down to 20 microns:
  - ~ 50 K
- Laser pulse energy required to photoexcite twice this volume (~2 photons/chromophore):
  - ~2 $\mu$J @ 525 nm
  - T-jump of ~ 4 K
  - ~30 Hz acquisition should be possible (requires fast readout detector)
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Group Velocity Mismatch limits time resolution

\[ n_{\text{vis}} = 1.33 \]
\[ n_{\text{x-ray}} = 1.00 \]

\(~0.7\text{ ps}\)

\(~80\text{ fs}\)
4th Generation X-ray source: Free Electron Laser

~$10^{12}$ photons/shot

~100 fs pulse duration

LCLS at Stanford in 2009?

XFEL in Germany in 2012?
# X-ray Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ESRF</th>
<th>XFEL</th>
<th>LCLS</th>
<th>ERL</th>
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<tbody>
<tr>
<td><strong>Electron energy:</strong></td>
<td>6 GeV</td>
<td>10 GeV</td>
<td>14.35 GeV</td>
<td>5.3 GeV</td>
</tr>
<tr>
<td><strong>X-ray pulse duration:</strong></td>
<td>~150 ps 😞</td>
<td>~100 fs 😃</td>
<td>~100 fs 😃</td>
<td>~200 + fs</td>
</tr>
<tr>
<td><strong>single bunch charge:</strong></td>
<td>~28 nC 😃</td>
<td>~1 nC</td>
<td>~1 nC</td>
<td>1 or 10 nC</td>
</tr>
<tr>
<td><strong>undulator length</strong></td>
<td>2 m</td>
<td>50 m</td>
<td>100 m</td>
<td>100 m</td>
</tr>
<tr>
<td><strong>Spontaneous:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray energy (fundamental):</td>
<td>15 keV (U17)</td>
<td>15 keV (U20.9)</td>
<td>8.2 keV (U30) 😃</td>
<td>8.27 keV (U17) 😃</td>
</tr>
<tr>
<td>X-ray bandwidth (fund.):</td>
<td>~3%</td>
<td>~5%</td>
<td>~5%</td>
<td></td>
</tr>
<tr>
<td>X-ray photons/pulse</td>
<td>~1.4×10¹⁰</td>
<td>~0.9×10¹⁰</td>
<td>~2×10¹⁰</td>
<td></td>
</tr>
<tr>
<td><strong>SASE1:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray energy:</td>
<td>-</td>
<td>12.4 keV</td>
<td>8.2 keV</td>
<td>-</td>
</tr>
<tr>
<td>X-ray bandwidth:</td>
<td>-</td>
<td>0.09%</td>
<td>0.1%</td>
<td>-</td>
</tr>
<tr>
<td>X-ray photons/pulse</td>
<td>-</td>
<td>~1.2×10¹² 😃</td>
<td>~1.1×10¹² 😃</td>
<td>-</td>
</tr>
<tr>
<td><strong>Beam size at crystal/detector (VxH):</strong></td>
<td>~60×100 μm</td>
<td>110 μm</td>
<td>82 μm</td>
<td>20 μm</td>
</tr>
<tr>
<td>Repetition frequency</td>
<td>1 kHz</td>
<td>10 Hz</td>
<td>120 Hz</td>
<td>1 MHz</td>
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Outlook:

- Dual-mode operation of the Cornell ERL would allow no-compromise optimization of time-resolved capabilities (bunch charge, pulse compression, etc.)
- “Fat” bunch operation with a long undulator would enable single-shot Laue diffraction with spontaneous radiation
- Structural studies of proteins on the chemical time scale with near-atomic resolution would unveil mechanisms of protein function at an unprecedented level of detail. Such information is desperately needed to establish a solid foundation for rational drug design.
Acknowledgements

Post-Doctoral fellows

Dr. Friedrich Schotte
NIH
Time-resolved x-ray crystallography

Dr. HyunSun Cho

Dr. Nara Dashdorj

Collaborators

Dr. Michael Wulff
ESRF
X-ray Instrumentation

Dr. Gerhard Hummer
LCP, NIH
MD simulations

Harry Ihee
KAIST
PYP, bR

Marco Cammarata
ESRF
Time-resolved SAXS

Prof. John Olson
Rice University
Myoglobin mutants
(Dr. Jayashree Soman)

Dr. Eric Henry
LCP, NIH
Laue Data Analysis

Prof. George Phillips
Univ. Wisconsin
Refinement
(Roman Aranda and Elena Levin)