BioCARS

Consortium for Advanced Radiation Sources
The University of Chicago
Sector 14, APS

- National user facility for synchrotron-based structural biology
- Supported by NCRR-NIH since its founding in 1992
- Additional recent funding: State of Illinois; APS (BSL-2/3 upgrade); ANSTO, Philip Anfinrud NIDDK/NIH (enhancement of time-resolved capabilities)
- All BioCARS users have been, and remain, general users
- Two experimental stations presently used (14-ID-B and 14-BM-C) for macromolecular crystallography and (recently) fiber diffraction

Sector 14 Layout

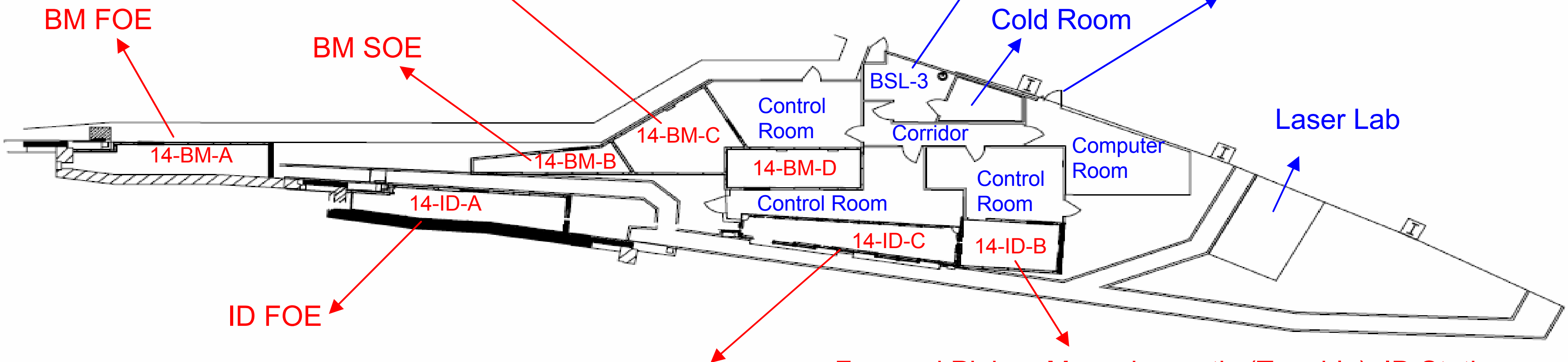
Focused Monochromatic BM Station

Bent conical Si-mirror (Rh coated)
Horizontally deflecting Ge (111) monochromator

Protein and Virus Crystallography
Fiber Diffraction

BSL-2/3 Sample Preparation Room
with Biosafety Cabinet

Facility Main Entrance



Focused Pink or Monochromatic (Tunable) ID Station
(white beam compatible)

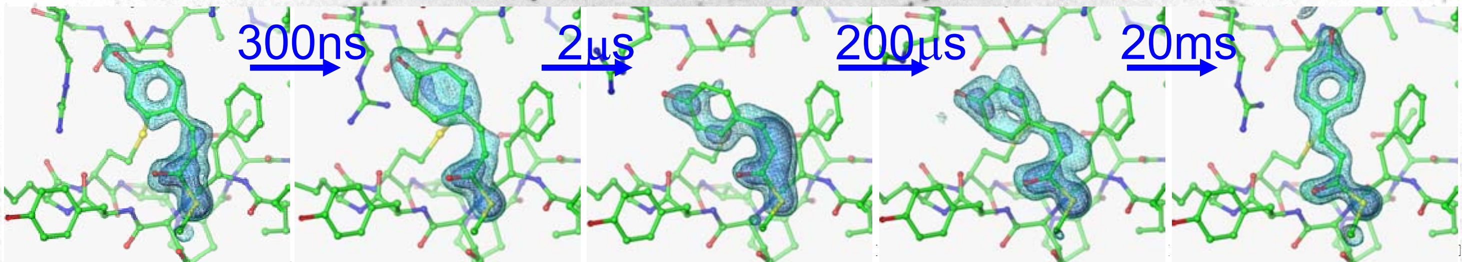
Two In-line Undulators: U23 & U27
KB mirror pair
Kohzu Si (111) monochromator

Time-resolved and Laue crystallography
Protein and Virus Crystallography
Micro-crystallography

BioCARS Primary Focus:
Present and Future

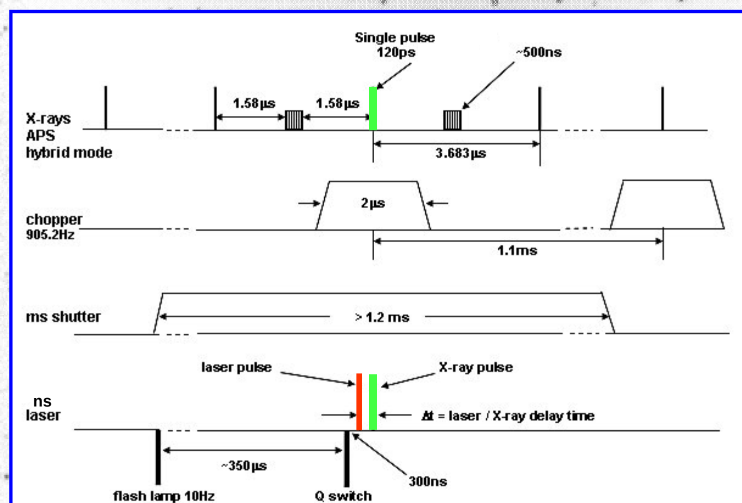
- time-resolved and Laue macromolecular crystallography
  (unique at the APS; one of 3 in the world: ESRF ID09, PF-AR NW14)
- biohazards at the BSL-2 & 3 level
  (unique in the US; one of 2 in the world: Diamond I03)
- Laue and monochromatic micro-crystallography (5-10 μm samples)
- non-standard/customized experiments (e.g. on-line micro-spectrophotometer, topography of protein crystals, fiber diffraction in collaboration with BioCAT)
- standard macromolecular crystallography techniques also supported: SAD/MAD, ultra-high resolution, large unit cells

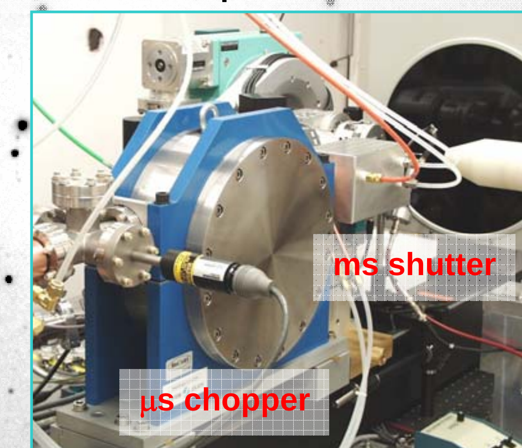
Science at BioCARS: Time-resolved Crystallography
Capturing Macromolecules in Action

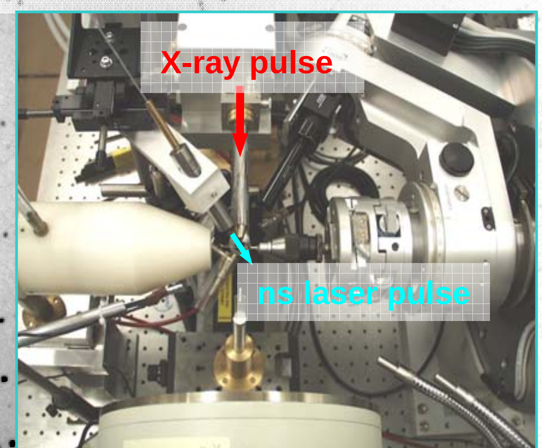


Probe fast structural changes at ambient temperature

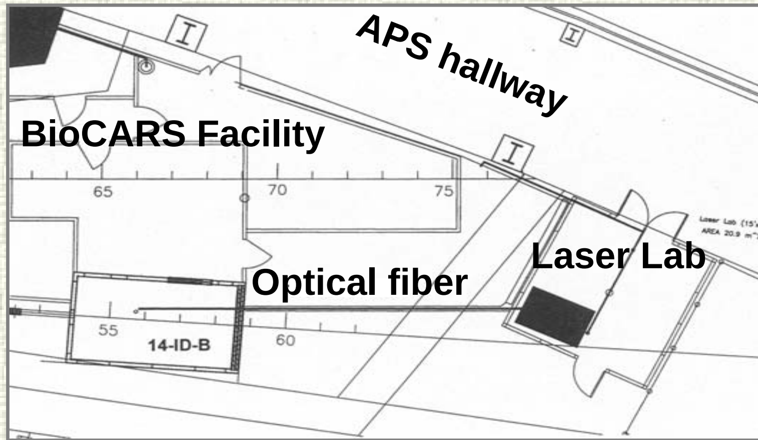
- rapid reaction initiation (pump: short laser pulses, 100fs-10ns)
- rapid data collection (probe: short X-ray pulses, 100ps, or longer pulse trains)
    - stationary crystals / Laue diffraction technique
    - fast X-ray shutter train to isolate a 100ps pulse
    - $>10^9$-10^{10} photons/100ps X-ray pulse
    - focused pink beam, 1-3% bw





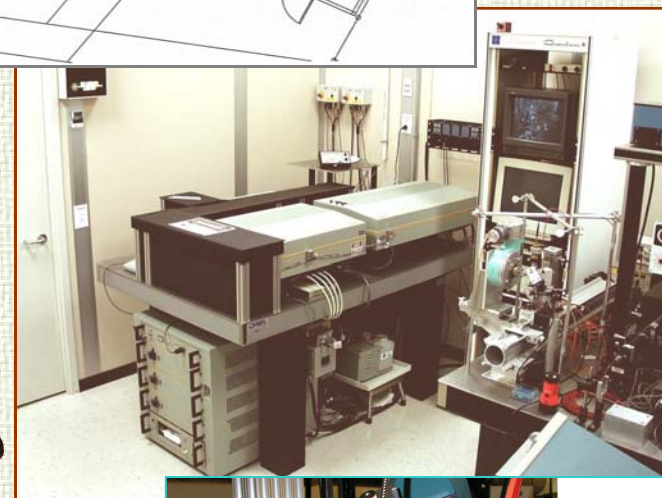


Laser Laboratory



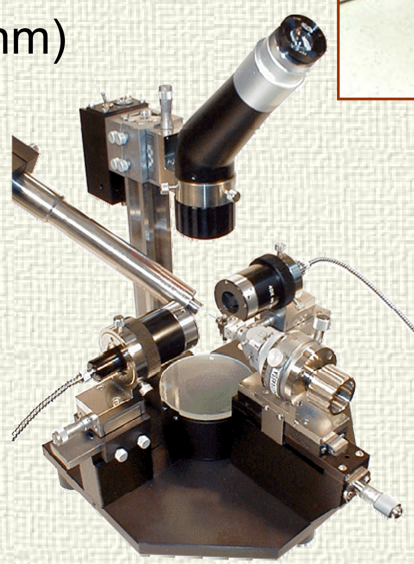
Two ns laser systems:

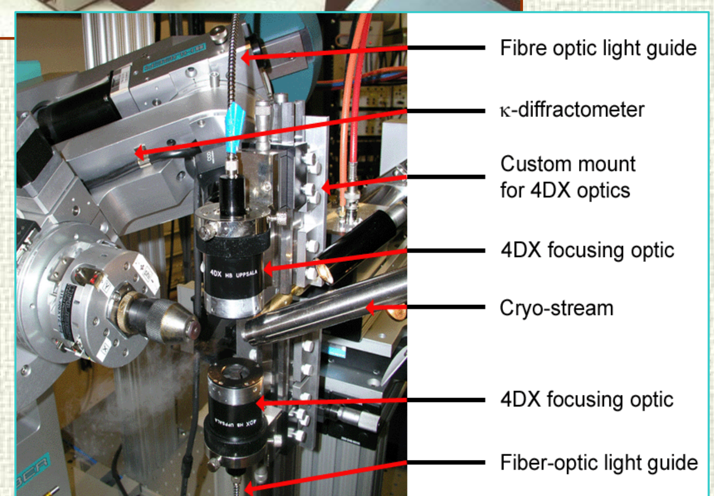
- Nd:YAG pumped dye laser (Continuum)
  - 7ns pulse duration; 10Hz
  - visible (400-650nm)
  - high pulse energy
- OPO laser (Opotek):
  - 4ns pulse duration; 10Hz
  - visible and UV (240-640nm)
  - easy tunability



New ps laser just installed!

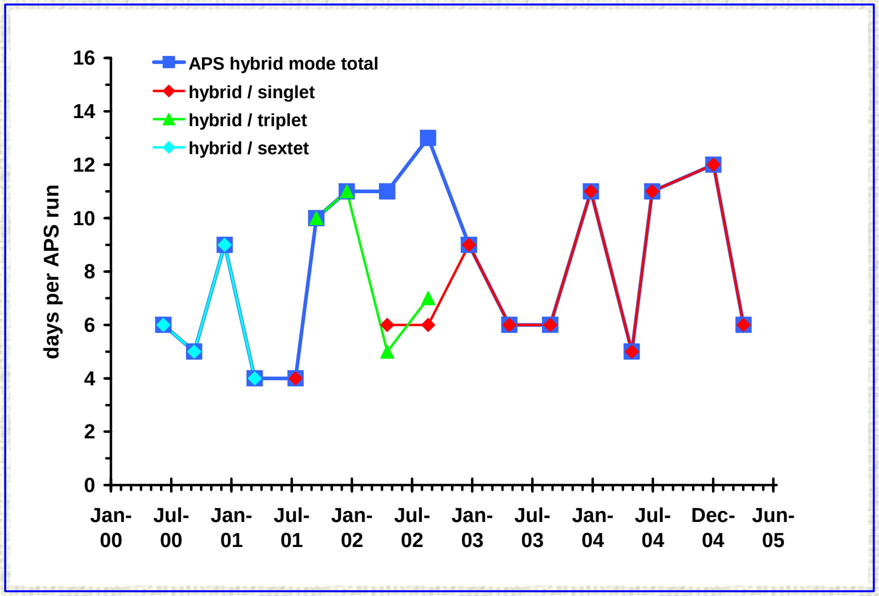
Two micro-spectrophotometers for on-line and off-line crystal absorption measurements.

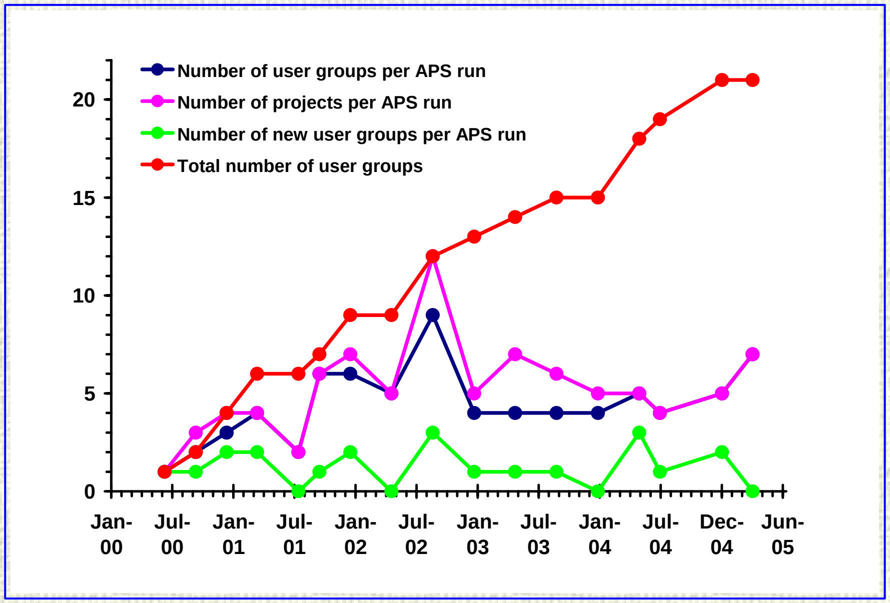




BioCARS Time-resolved Crystallography Users

- User TR operation since Sept 2000
- Laue/TR beamtime:
  - hybrid mode but also 24-bunch mode
  - 2-3 weeks per APS run
  - 1–5 days per user group

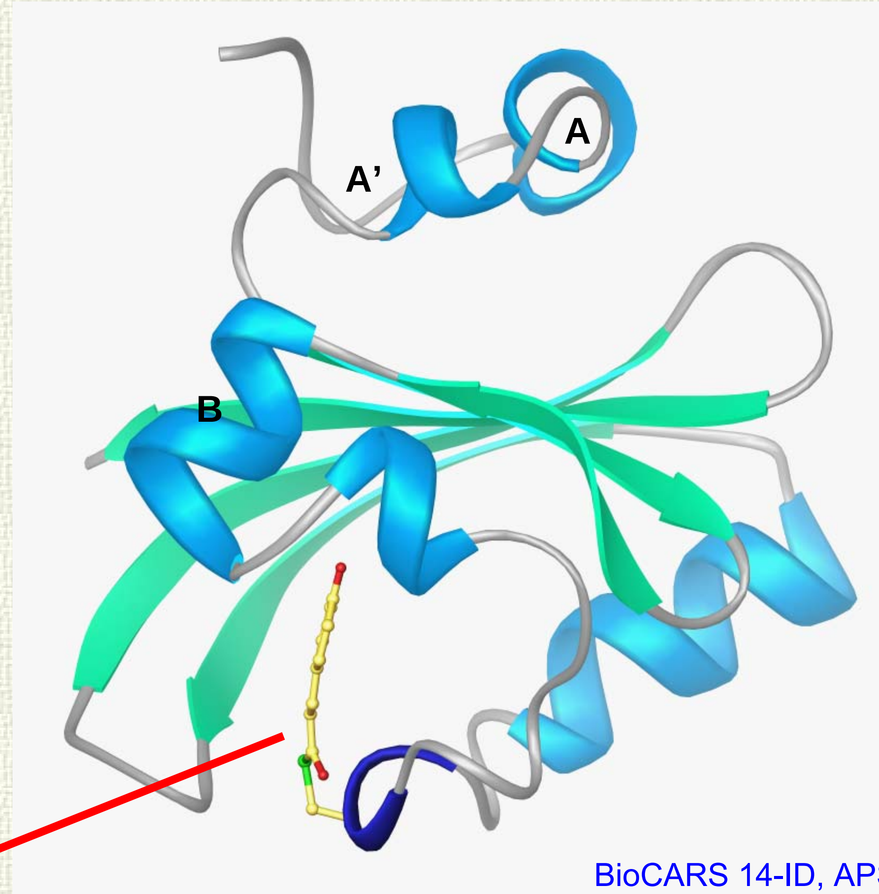




- Time-resolved Crystallography Workshop
  March 10-13, 2001, APS
  86 participants, 27 talks
- Laue Data Processing Workshop
  October 9-11, 2002, BioCARS
- Time-resolved Crystallography Workshop
  May 6-8, 2006, APS
  70 participants, 25 talks

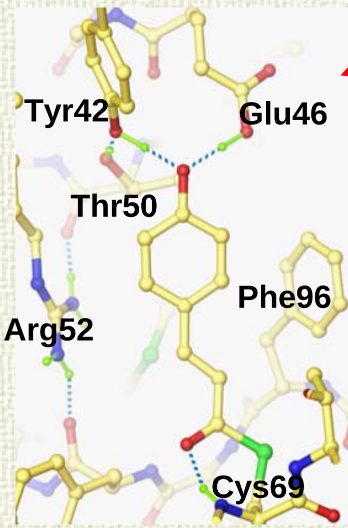
Photoactive Yellow Protein: advanced TR experiment and complete TR data analysis

- Blue light photoreceptor from the purple eubacterium *Ectothiorhodospira halophila*
- Involved in negative phototactic response of *E. halophila* to blue light
- PYP exhibits a photocycle: several intermediates spanning time-scales from <ps to seconds



BioCARS 14-ID, APS

Coumaric Acid Chromophore



light-induced *Trans* to *Cis* isomerization

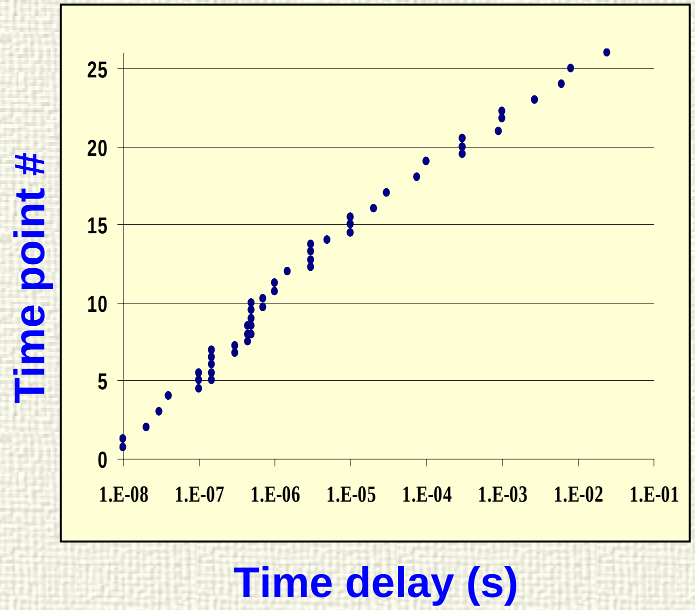
Spencer Anderson, Sudarshan Rajagopal, Harry Ihee, Marius Schmidt, Keith Moffat
University of Chicago

Vukica Srajer, Reinhard Pahl, BioCARS

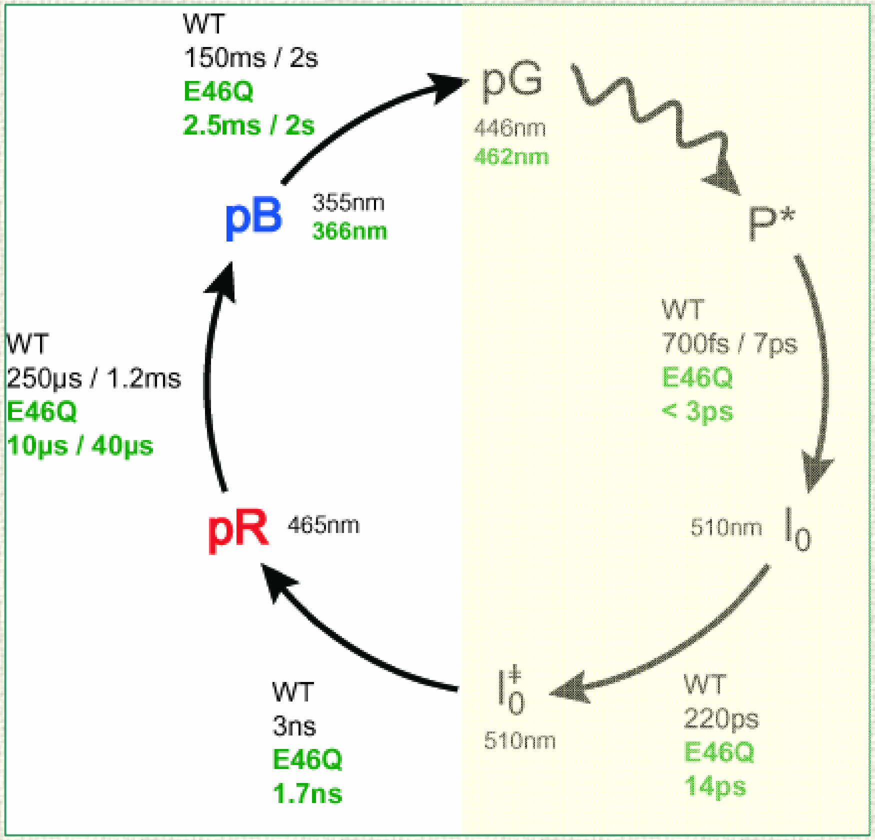
Ihee et al. PNAS 102, 7145 (2005)
Rajagopal et al., Structure 13, 55 (2005)
Anderson et al., Structure 12,1039 (2004)
Rajagopal et al., Acta Cryst. D60, 860 (2004)

Studies of intermediates: PYP E46Q mutant

- 54 Laue data sets collected using 25 crystals, at 30 time delays, from 10ns to 100ms
- Pump: 495-505nm, ~3-5mJ/mm^2, 7ns laser pulses
- Photo-initiation: 15-20%

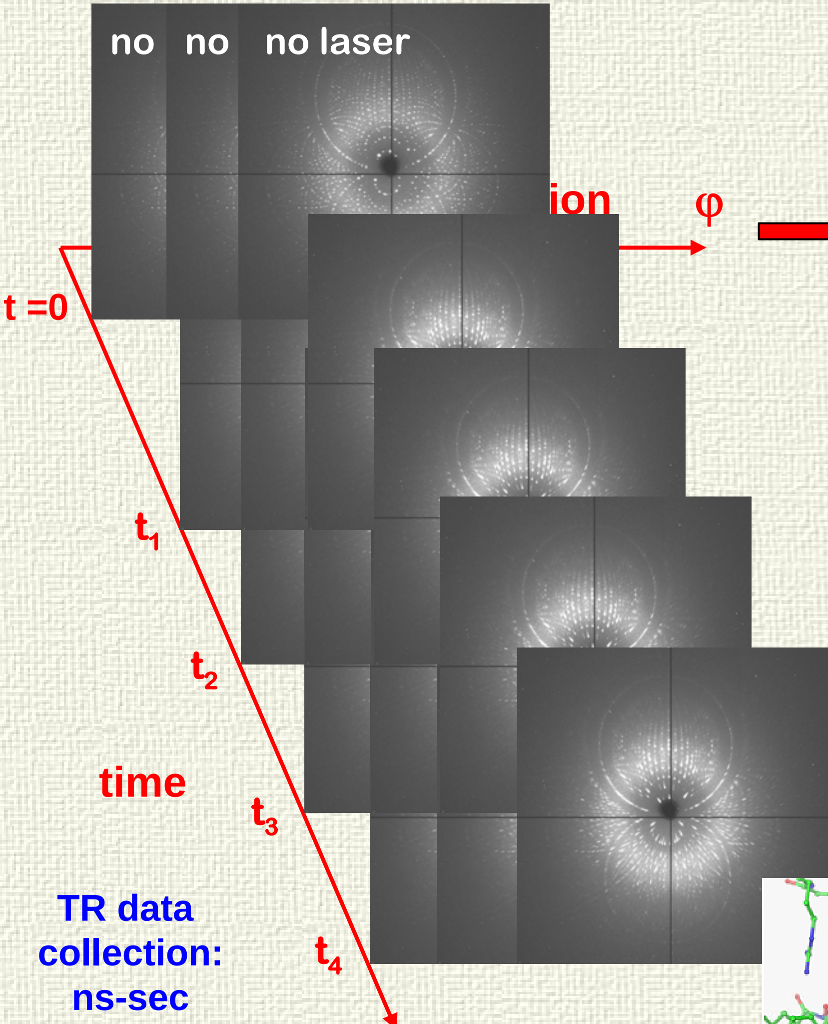


Sub-100ps



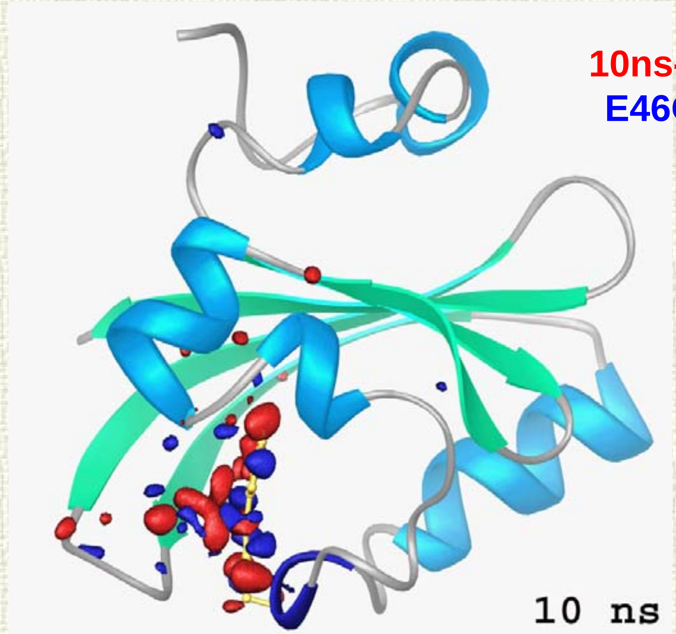
Rajagopal et al., Structure 13, 55 (2005)
Anderson et al., Structure 12,1039 (2004)

TR crystallography at 14-ID: present



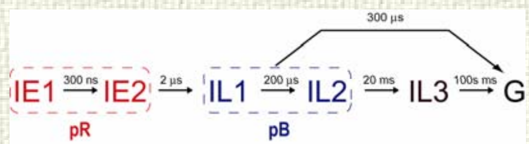
TR data collection: ns-sec

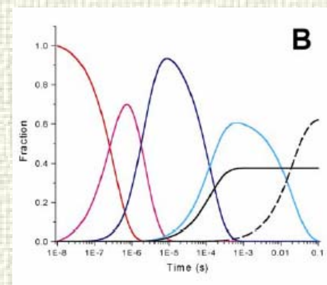
Time-dependent difference electron density map movie, Δρ (t)

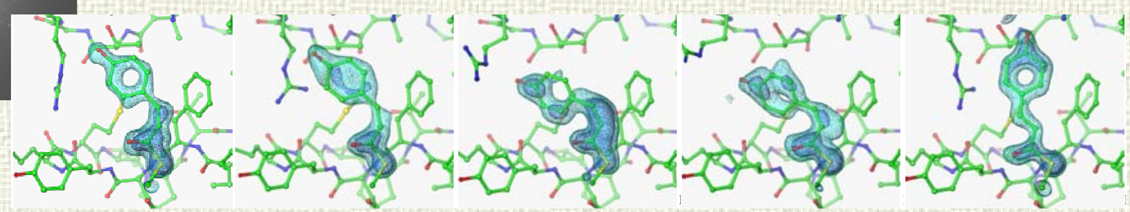


10ns-100ms
E46Q PYP

SVD/post-SVD analysis: mechanism & structures of intermediates







300ns **2µs** **200-300µs** **20ms** **>100ms** **t**

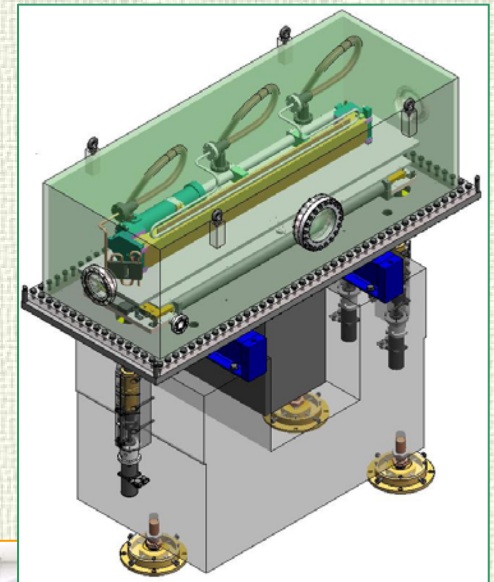
Time-resolved crystallography:
Present status and future challenges

- Mature phase of the technique: demonstrated ability to detect small structural changes even at relatively low levels of reaction initiation (15-40%)
- Development of essential methods for global time-resolved data analysis, such as SVD, is well under way

Challenges for BioCARS:

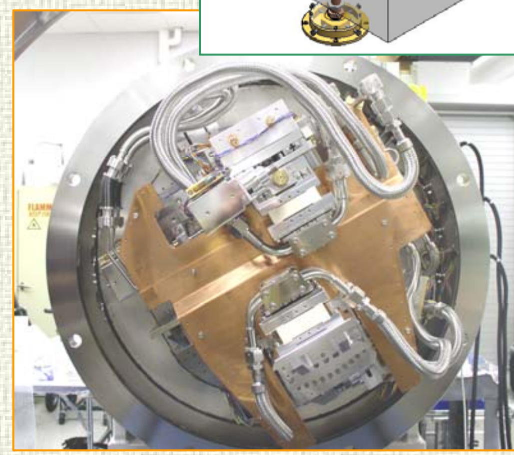
- Expanding time resolution to 100ps and aiming at single 100ps X-ray pulse data collection:
  - two collinear undulators replace Undulator A on 14-ID
  - 14-ID optics upgrade
  - upgrade of the BioCARS fast X-ray chopper
  - ps laser system

14-ID Upgrade

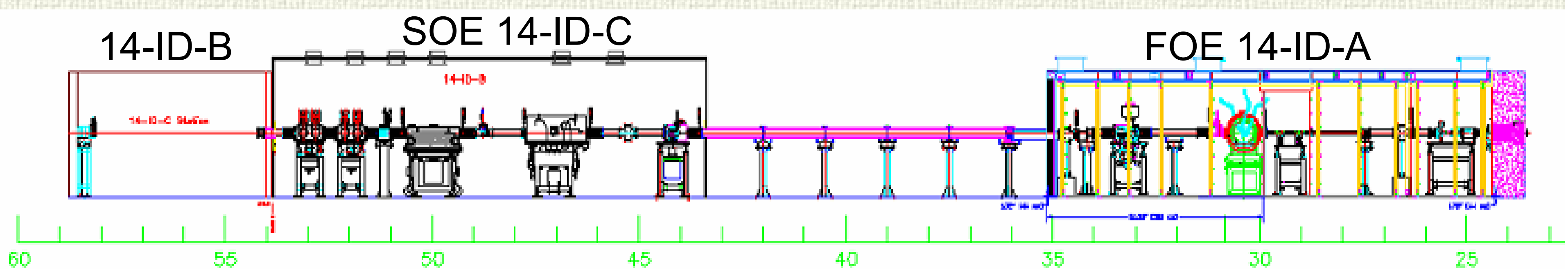


Optics Upgrade:

- New Optics Enclosure (SOE) to house a KB mirror pair:
  - changing from original ~1:1 focusing to ~8.3:1(h)/5.2:1(v) demagnification
  - focused beamsize 75 (h) X 35 (v) μm²
  - both mirrors installed – May 2006 & Jan 2007



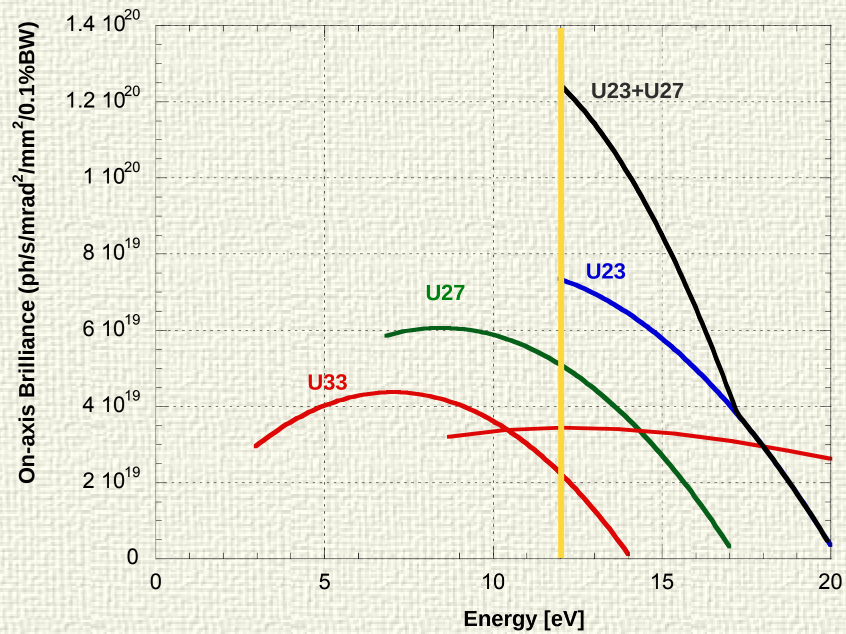
- New Kohzu monochromator (Si 111)
  - installed May 2006



Implementation of 100ps capability

(in collaboration with Philip Anfinrud, supported by funds from NIDDK / NIH):

- Undulator A (U33) replaced by two new collinear undulators U23 and U27
  - U27 installed in Sept 2006, U23 in Jan 2007
  - ≥10^{10} photons/pulse in pink mode, APS 24-bunch mode (4mA/bunch)
- X-ray chopper upgraded (Dec 2006)
  - permits isolation of a single 100ps X-ray pulse in the 24-bunch mode (250ns open time)
  - enhances the beamtime availability for time-resolved research
- New ps laser system (Jan 2007)
  - Ti:Sapphire Spitfire Pro 5: (Spectra Physics) 780nm, 2ps, 5mJ/pulse, 1kHz
  - TOPAS OPA:
    75µJ @ 290-400nm
    >300µJ @ 475-600nm
    >250µJ @ 600-800nm



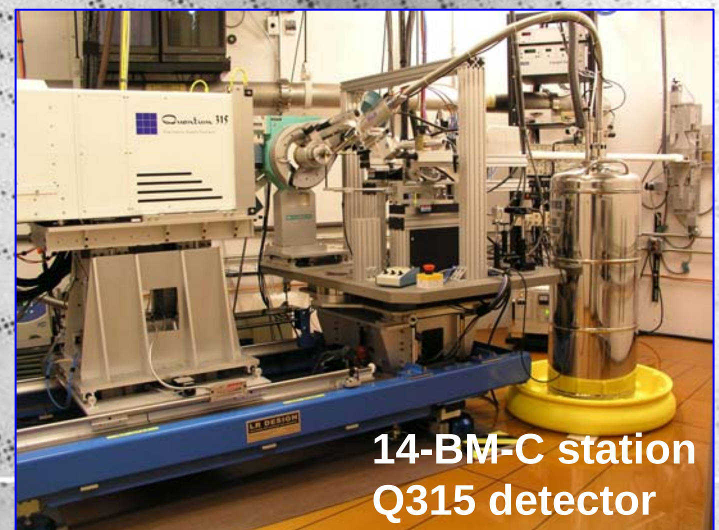
Tuning curves for U33 (Undulator A), U27, and U23. The sum of U23 and U27 is shown in black

Time-resolved crystallography:
Other challenges for BioCARS and user community

- Application of the technique to wider systems of biological interest, photosensitive and beyond ➡ expanding the TR user community
- Reaction initiation: system-specific efforts to determine a suitable reaction initiation method
- Irreversible processes and smaller crystals: need more intense X-ray source (dual undulators and beyond) and faster read-out detectors
- Continuing development and application of essential methods for global time-resolved data analysis, such as SVD and cluster analysis
- Combining experimental results from time-resolved crystallography with computational and theoretical approaches to describe reaction pathways completely, including the transition states

BioCARS: Biosafety Level 2 and 3 Facility

- BioCARS is the only synchrotron-based BSL-2/3 facility in the US
- All stations can be used for BSL-2 and BSL-3 experiments, with all necessary engineering controls and standard operating procedures in place for safe conduct of BSL-2 and BSL-3 experiments.



- Facility upgraded and approved for BSL-3 operation by ANL IBC in November 2004
- 3-6 BSL-2 experiments/year at BioCARS
- 4 BSL-3 experiments since the approval

Background image: HRV-14, courtesy of Michael Rossmann, Purdue University

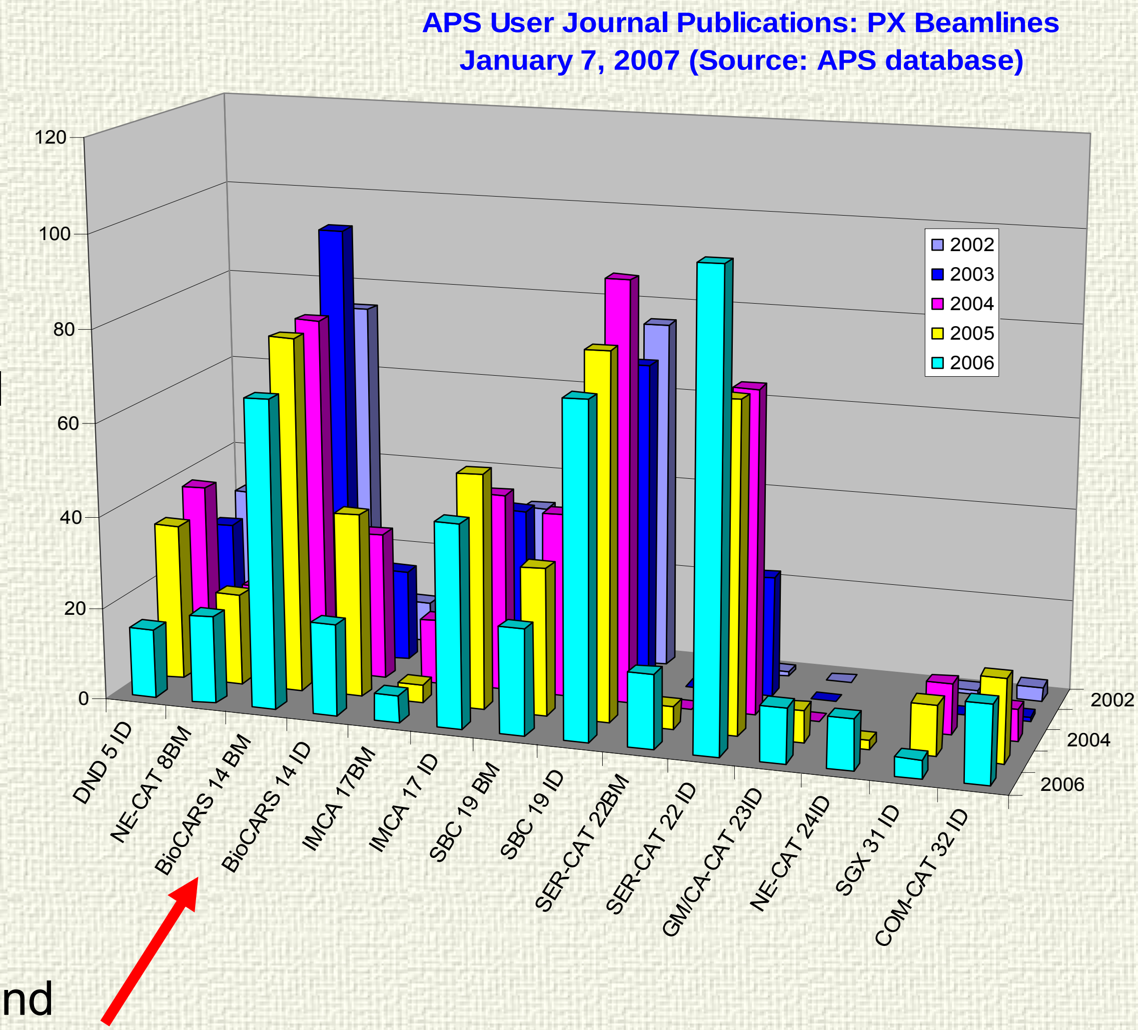
BioCARS User Community and Productivity

- Sept 2002-Sept 2006:
  135 research groups
  337 unique proposals
  772 unique users
  1773 user trips

- BioCARS journal articles account for:
  18%, 14%, 13%, 12% of total APS user journal articles in 2003, 2004, 2005 and 2006

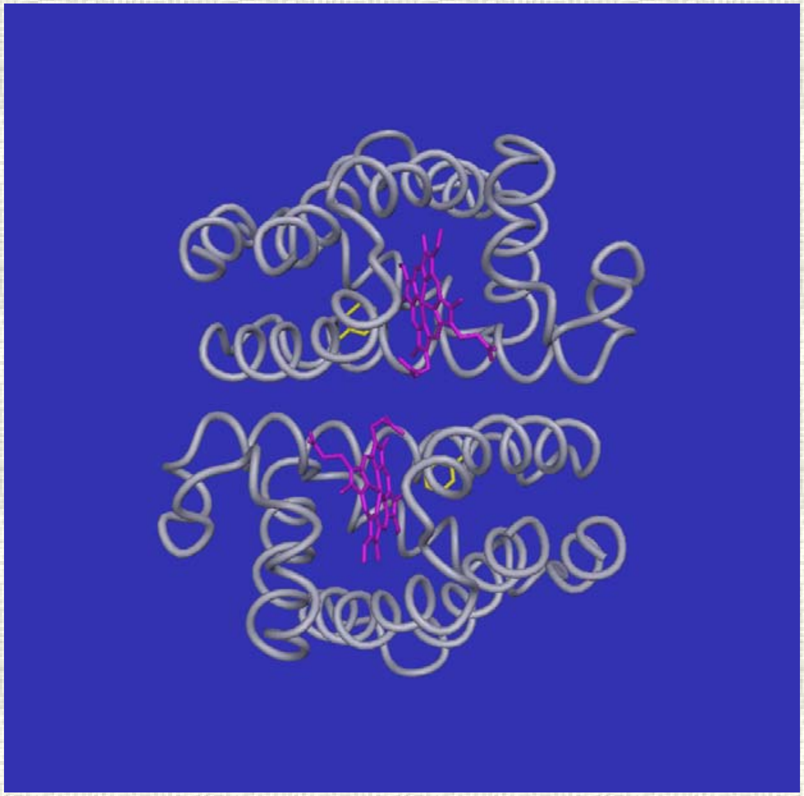
- 1st in 2003, 2nd in 2004, 1st in 2005, 3rd in 2006 among all APS sectors by the number of user journal articles

- ~25% of BioCARS journal articles published in Nature and Cell journals, Science & PNAS



Time-resolved Studies of 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 are localized and not too large: crystals survive quaternary change
- Successful HbI-CO ➡ deoxy HbI ➡ HbI-CO transformation in the crystals
  (Knapp J. and Royer W., Biochemistry 42, 4640, 2003)
- Crystals diffract to atomic resolution (~1Å)

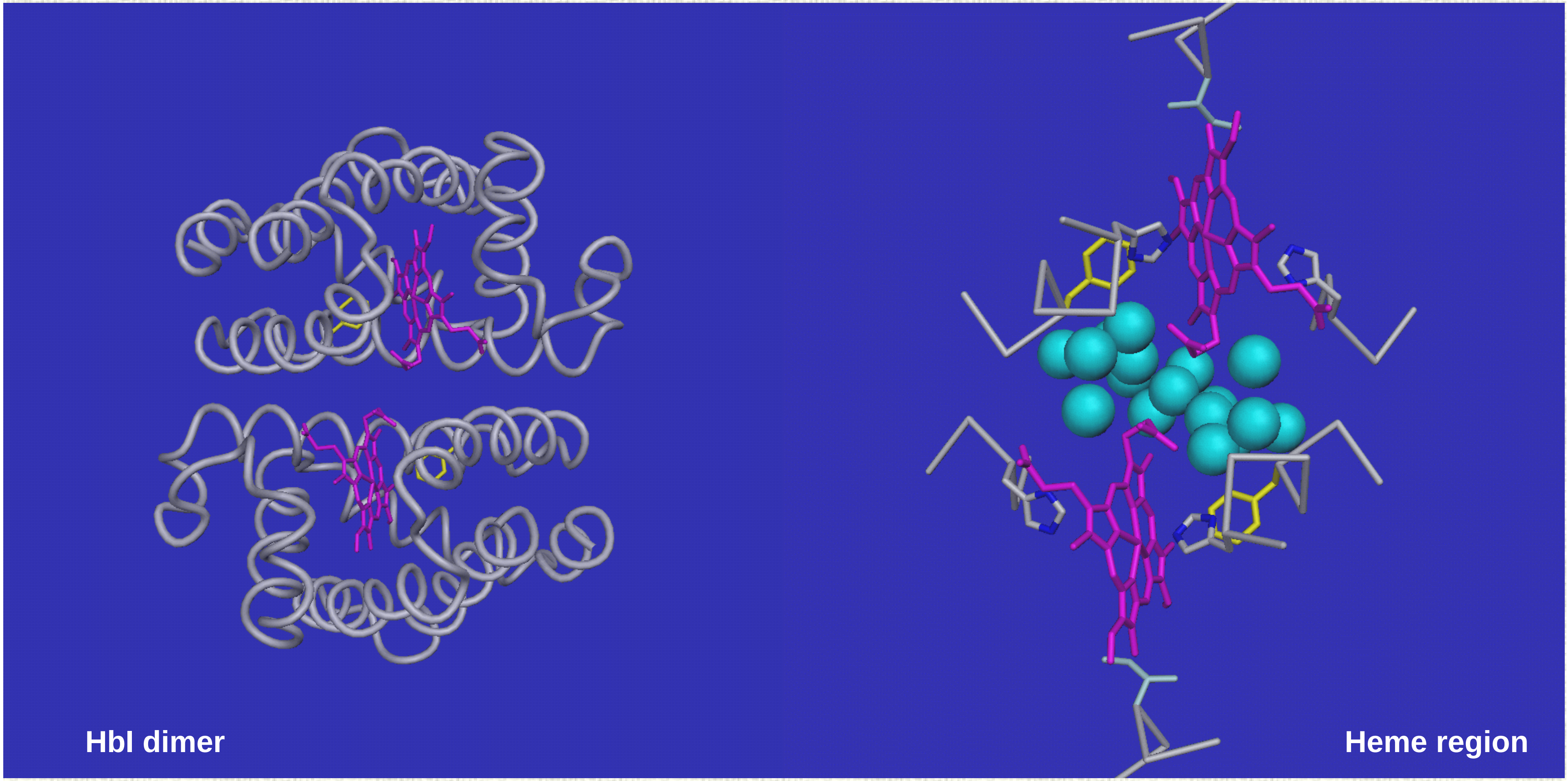
BioCARS 14-ID, APS

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

Vukica Srajer, Reinhard Pahl, BioCARS

Knapp et al., PNAS 103, 7649 (2006)

R (HbI-CO) to T (deoxy) transition: end points from static crystallography data



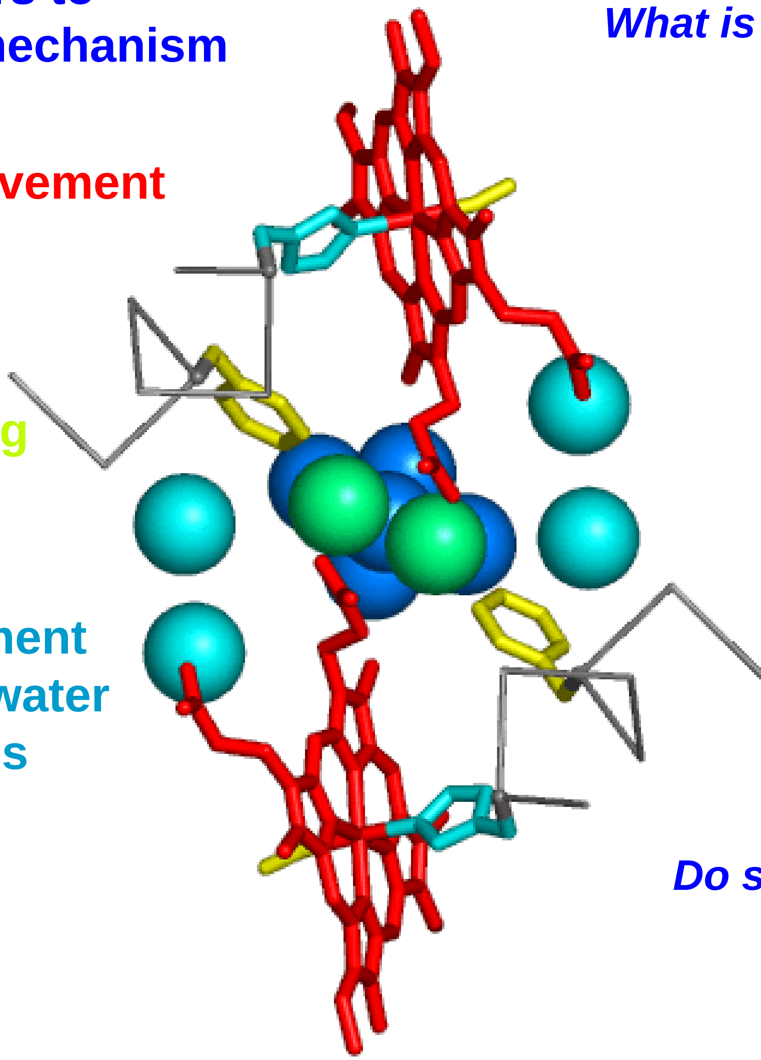
Colors:
- HbI-CO heme (R)
- deoxy HbI heme (T)
- Phe 97
- interface water molecules

Key contributors to cooperativity mechanism

Heme movement

F4 Phe flipping

Rearrangement of interface water molecules



What is the cascade of structural events?

Are these transitions concerted or sequential?

What is the triggering event?

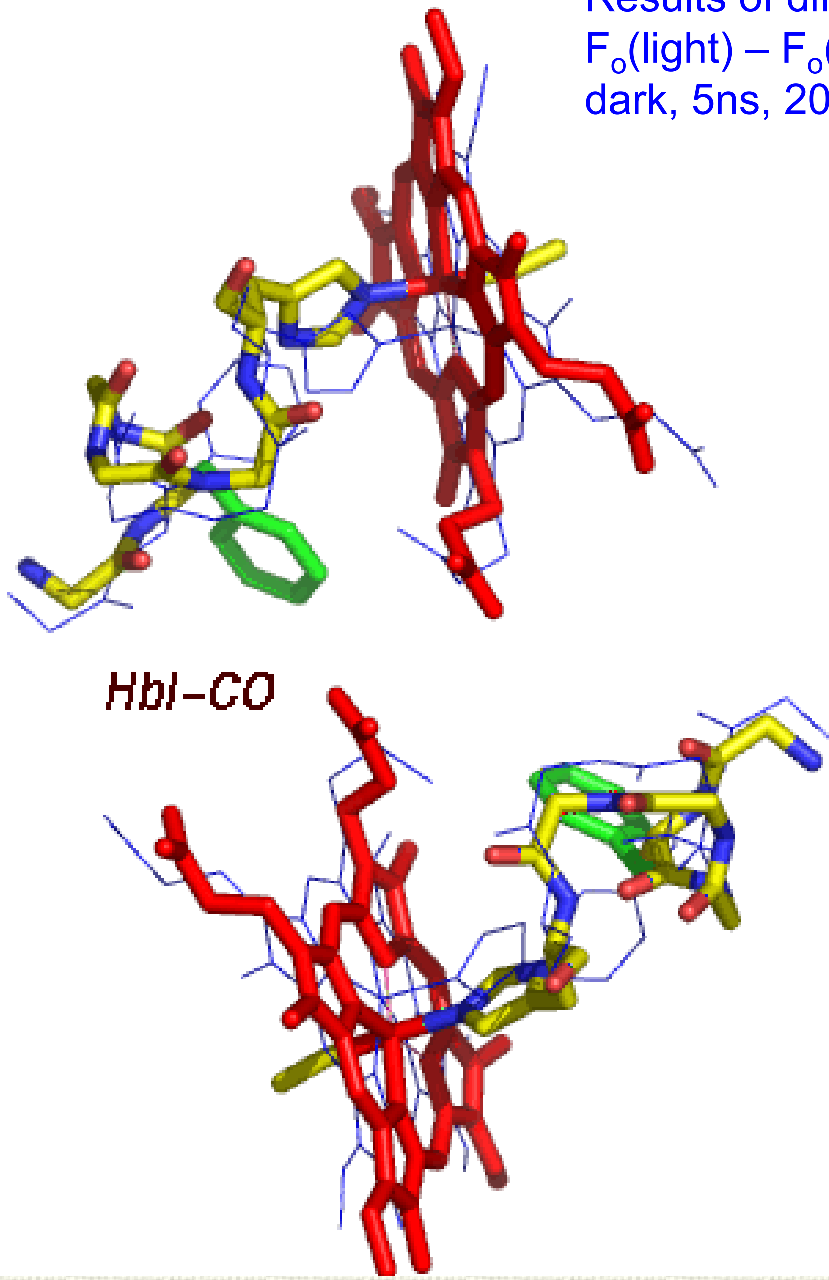
Do structural intermediates facilitate R to T transition?

Results of difference Fourier refinement

F_o(light) – F_o(dark) coefficients:

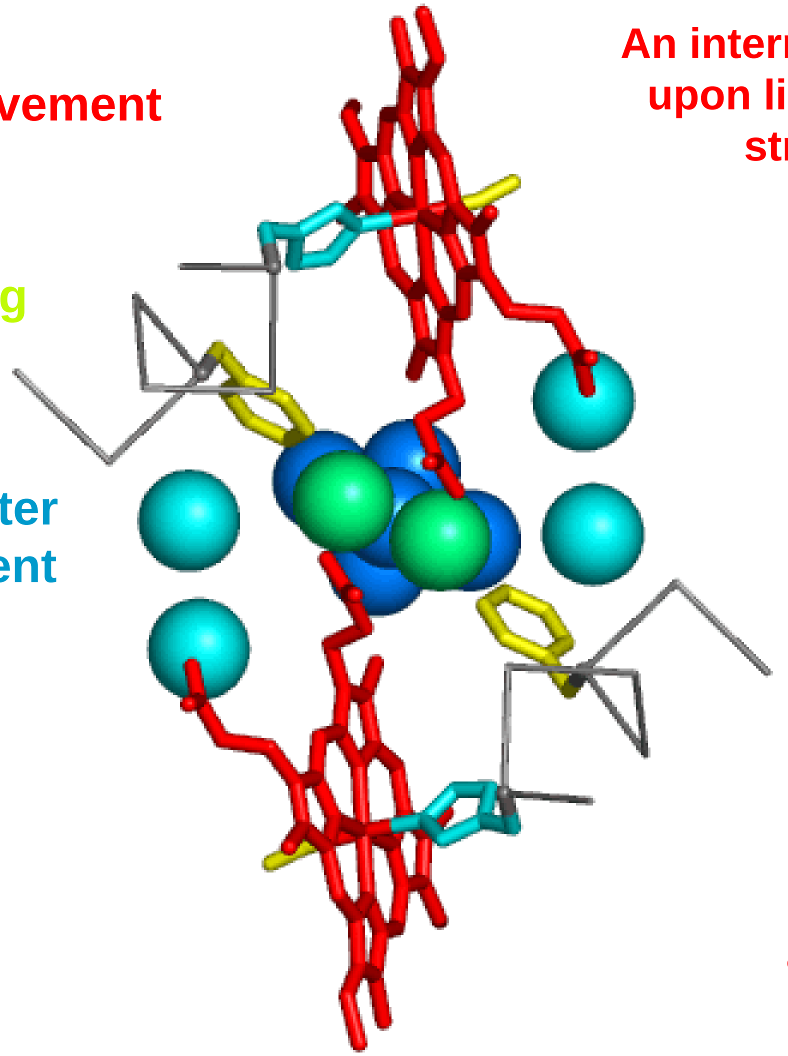
dark, 5ns, 200ns, 700ns, 2μs, 9μs, 80μs

heme and Phe97 transition



Trigger event: rearrangement of water molecules at the dimer interface

Key structural transitions with functional ramifications



What is the cascade of structural events?

An intermediate is formed rapidly (<5ns) upon ligand release, relaxing to T-like structure in μs time domain.

Are these transitions concerted or sequential?

Key allosteric changes appear to be tightly coupled.

What is the triggering event?

Rapid disordering of water molecules H-bonded to heme propionates

Do structural intermediates facilitate R to T transition?

Disordering of water molecules appears to lay the foundation for subsequent heme movement.

Acknowledgements

BioCARS

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