

New Opportunities in Ultrafast Science Using X-Rays

Structural Biology

Keith Moffat
University of Chicago
Department of Biochemistry & Molecular Biology
Institute for Biophysical Dynamics
BioCARS, APS

Frontiers of X-ray Based Structural Biology

- Static : structure determination of molecules, assemblies, organelles, cells...
- Length scales from 0.001nm to 1000nm
- Dynamic : determination of changes in structure
- Time scales from femtoseconds to hundreds of kiloseconds; 20 decades in time

Structural Genomics : The First Frontier of Crystallography

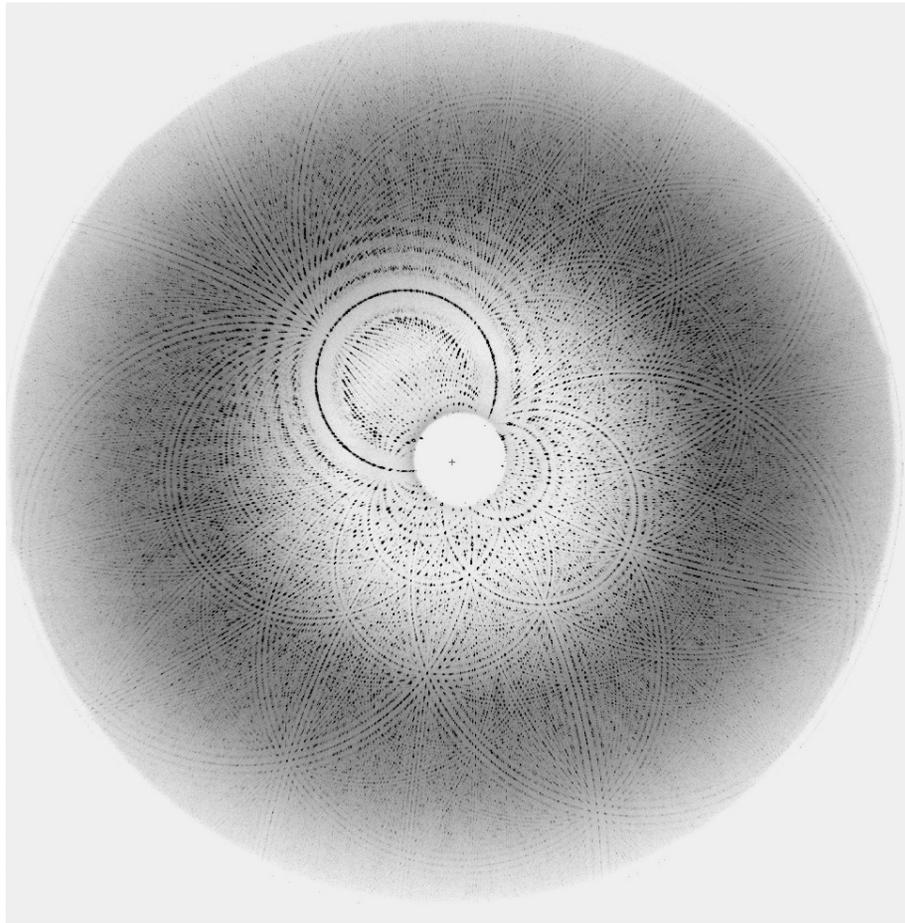
- Goal : high throughput of accurate, static structures
- How many different protein folds? How to identify more drug targets?
- Target identification...cloning/expression/purification... crystallization...X-ray data collection...structure determination...refinement...understanding...comparison with other structures
- X-ray data collection by no means rate-limiting, or even rate-contributing, to overall throughput
- Source brilliance plays a minor role; temporal brilliance plays none

“Molecular machines” : The Second Frontier

- Molecular machines and other very large non-covalent complexes e.g. the ribosome, the proteasome, icosahedral viruses...
- If they crystallize at all, they do so with very large unit cell dimensions of 30 – 200nm; weakly scattering
- Source brilliance plays a major role; temporal brilliance plays none

Laue Diffraction from Cowpea Mosaic Virus

Space group $I23$, $a=317\text{\AA}$



- Data collected on BioCARS 14ID undulator at APS; wavelength range 1.05-1.5 \AA , maximum resolution 2.8 \AA
- Exposure time for pattern ~600 microseconds
- 2507 reflections found for initial alignment
- Rfactor for data scaling 6.2% based on 1820 symmetry equivalent reflections
- 8987 unique reflections measured with average $I/s(I)=15$
- X-ray spectrum computed from processing matches experimental between 1.05 and 1.4 \AA
- Data about 18% complete to 3 \AA resolution, sufficient to solve structure due to high non-crystallographic symmetry

Scattering from Less-Ordered Systems : The Third Frontier

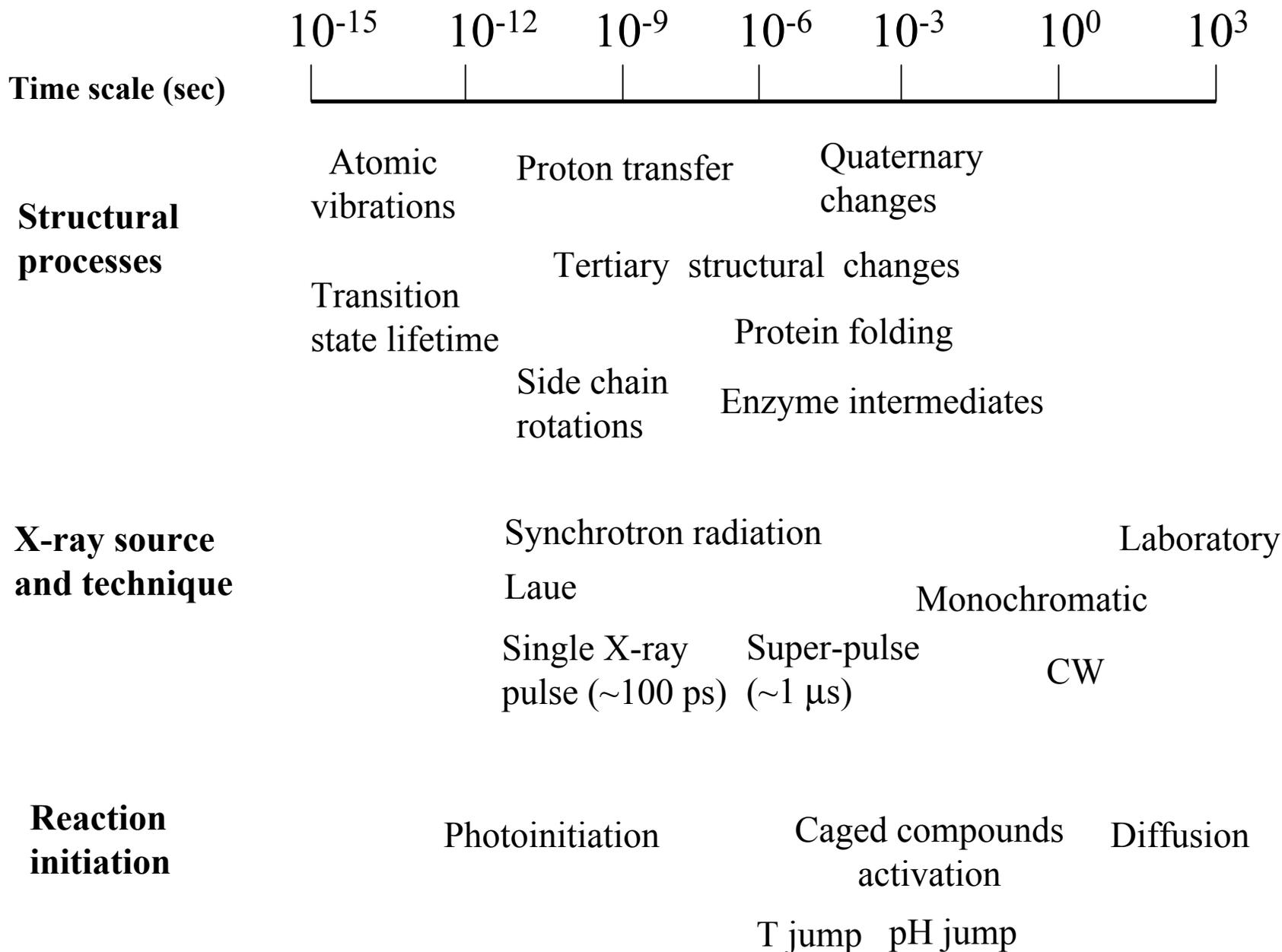
- Two-dimensionally ordered arrays of e.g. integral membrane proteins
- One-dimensionally ordered arrays of e.g. fibers
- Solution
- Imaging of larger structures as single objects : X-ray microscopy
- Source brilliance may play a role; temporal brilliance may play a role in imaging; spatial coherence plays a major role in imaging
- X-ray spectroscopies : limited chemical and structural information, powerful in certain systems

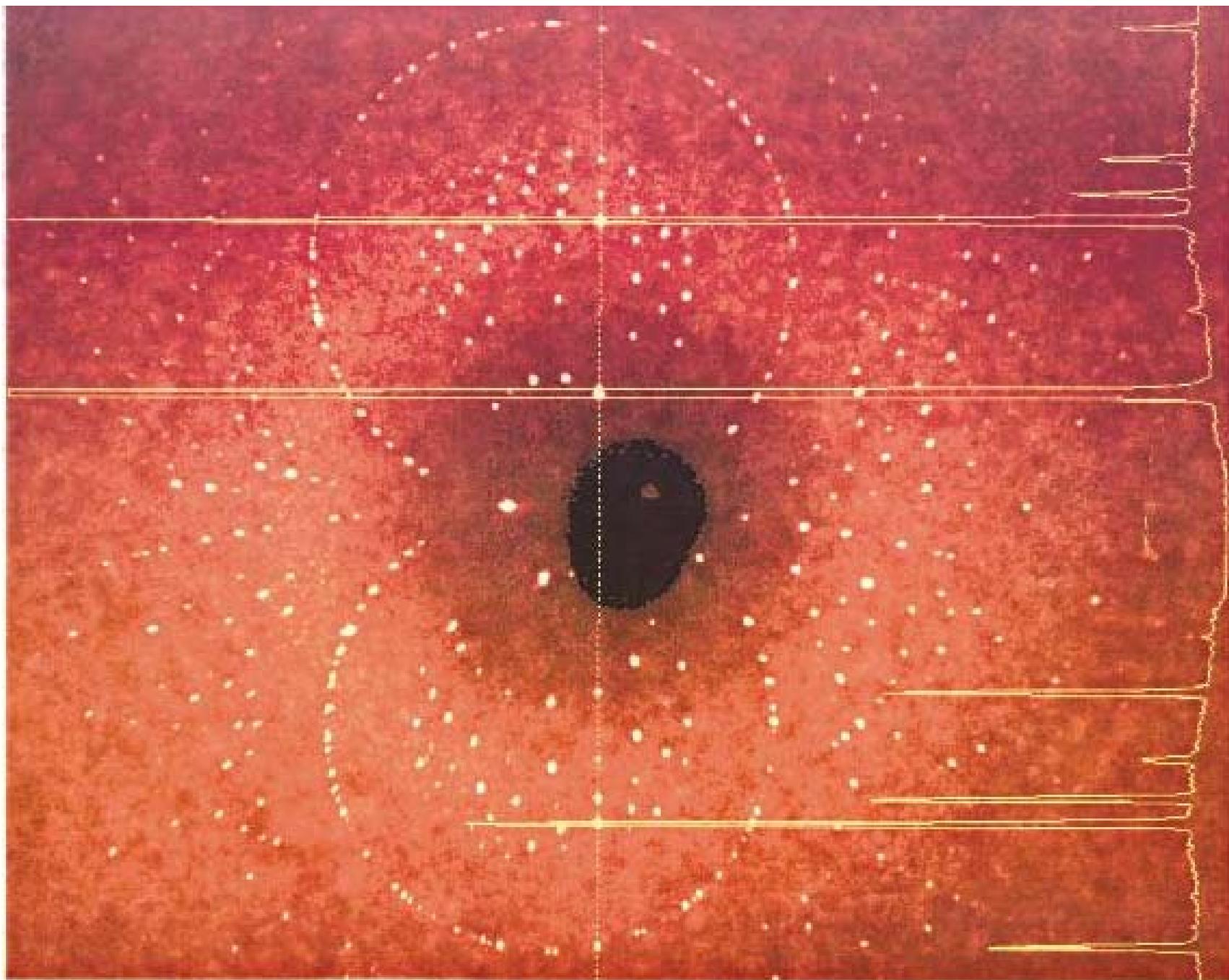
General Considerations

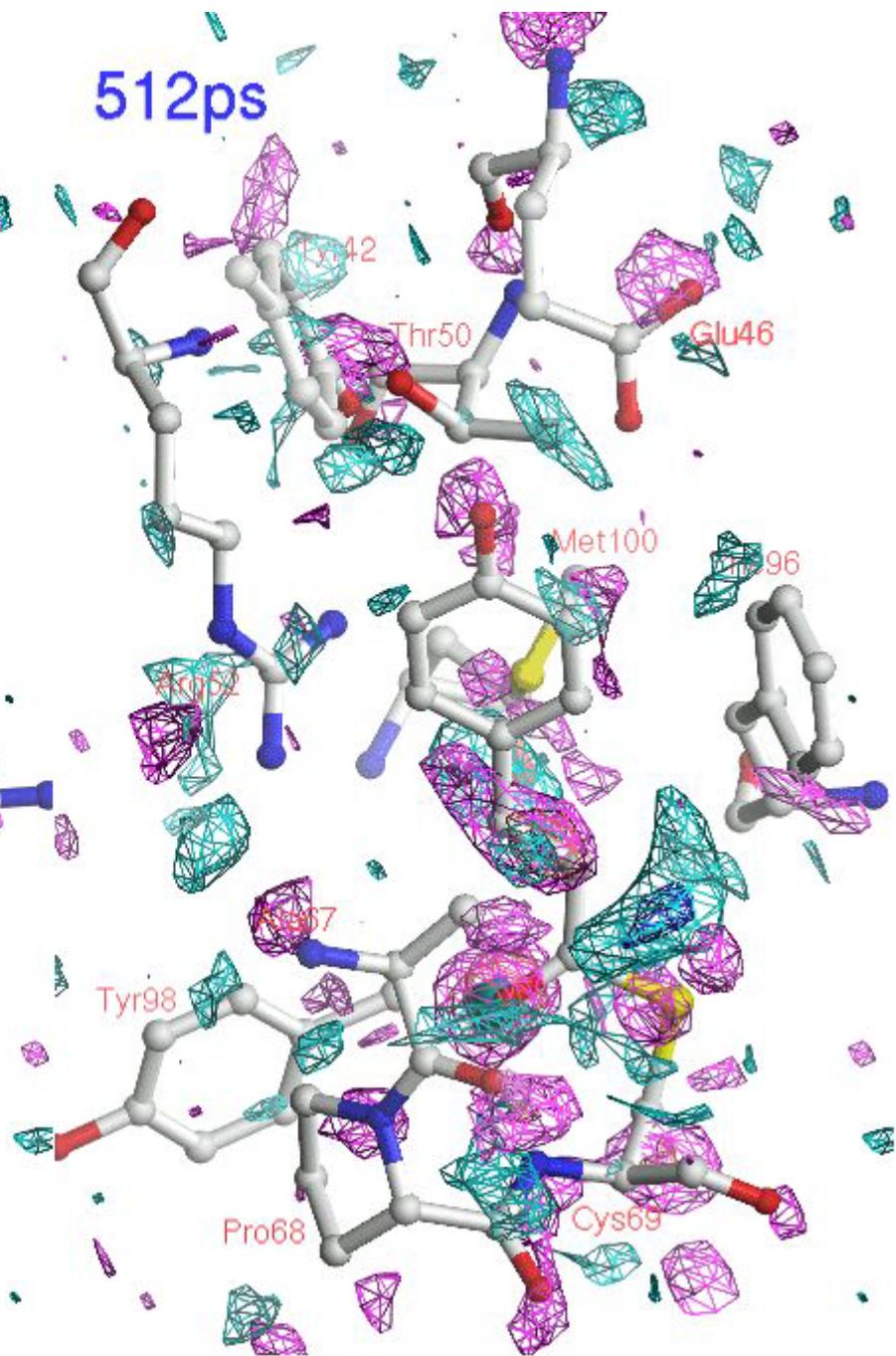
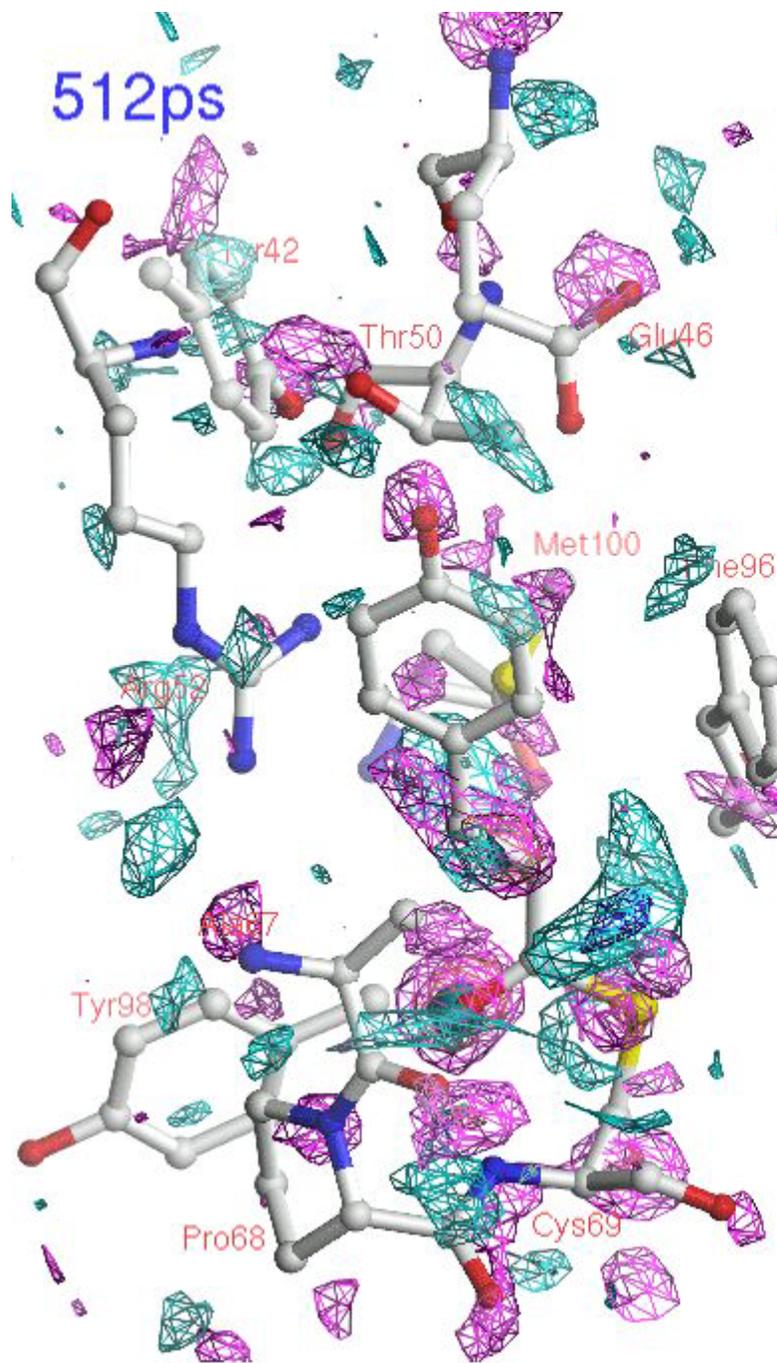
- Each scattering experiment yields a two-dimensional slice through three-dimensional scattering space; four-dimensional if time is an additional variable. Multiple experiments are necessary to accumulate complete data sets
- Radiation damage is critical to all experiments
- Depends ultimately on the ratio of elastic scattering cross-sections that generate signal to inelastic scattering cross-sections that generate noise/damage; inescapable
- Time is also a critical variable in damage processes; may provide room to maneuver!

Time-Resolved Experiments : The Fourth Frontier

- All of chemistry, biochemistry and biology involves motion
- Nevertheless, only a minority of structural biologists are interested in time-resolved phenomena e.g. muscle contraction, cell motility
- Most processes in biochemistry are slow, say greater than one microsecond : large groups of atoms/molecules move large distances, and must traverse large activation energy barriers
- Even fewer structural biologists are interested in ultrafast processes occurring in less than, say, one nanosecond
- Critical overlap with time-resolved optical spectroscopies







Ultrafast Structural Processes

- What processes are intrinsically fast?
- All elementary chemical steps involving bond making, bond breaking, isomerization, solvent rearrangement...
- Steps driven by absorbing a photon
- Fast processes are masked if they follow slower steps; they may be visible if they precede slower steps
- In biology, ultrafast structural processes of interest are largely confined to naturally light-sensitive systems
- Fast photochemical steps are followed by slower – even very slow – structural changes

Experimental considerations

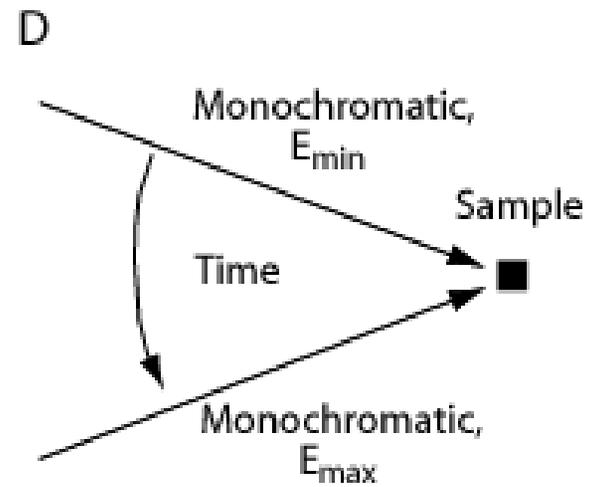
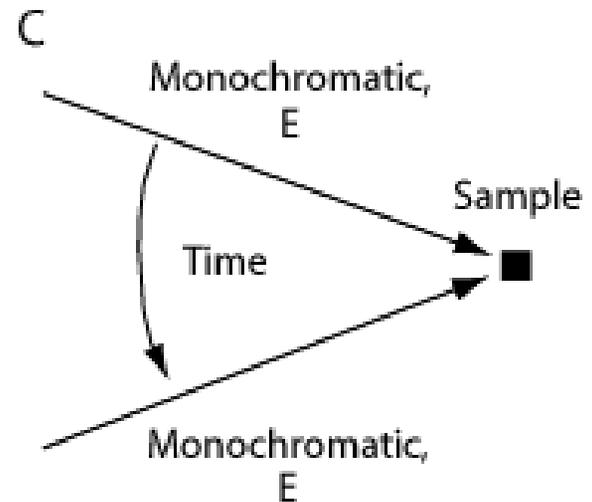
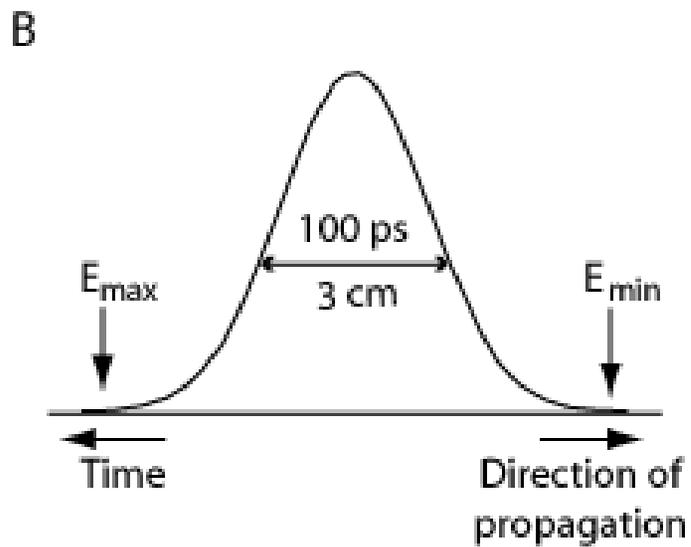
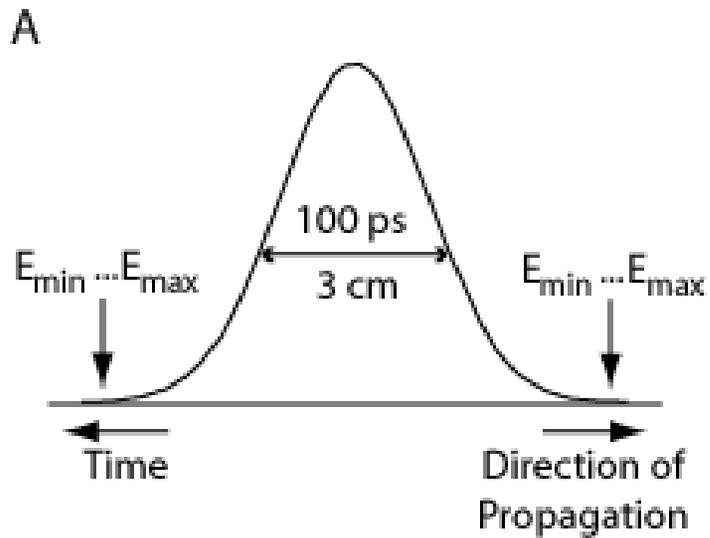
- Pump-probe experiments work well with an integrating detector, but no ultrafast time-slicing, two-dimensional, hard X-ray detector exists
- The essence of an accurate time-resolved experiment is the ability to make repeated measurements on the same sample : non-destructive
- Radiation damage is therefore a major limitation

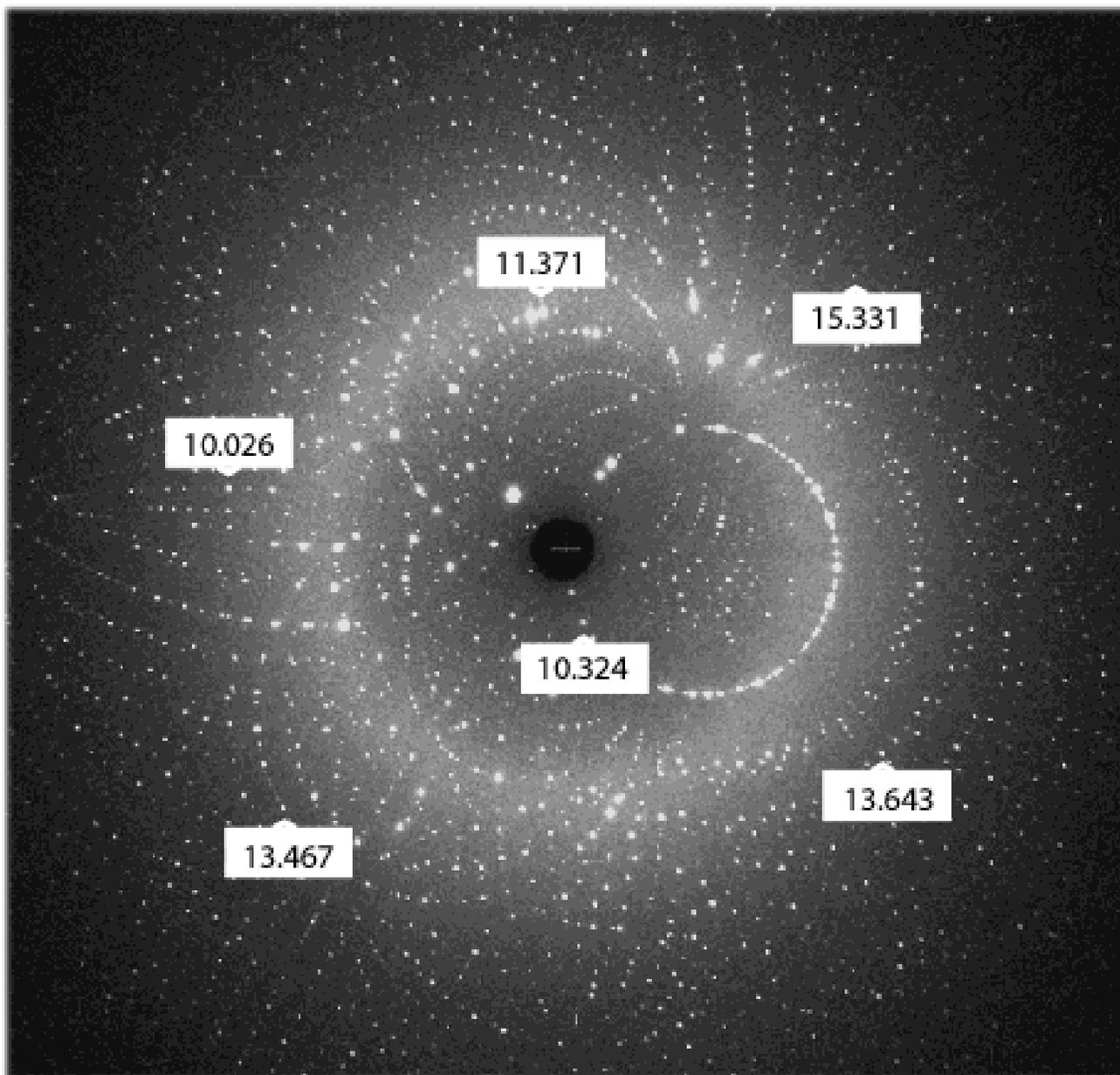
Sources

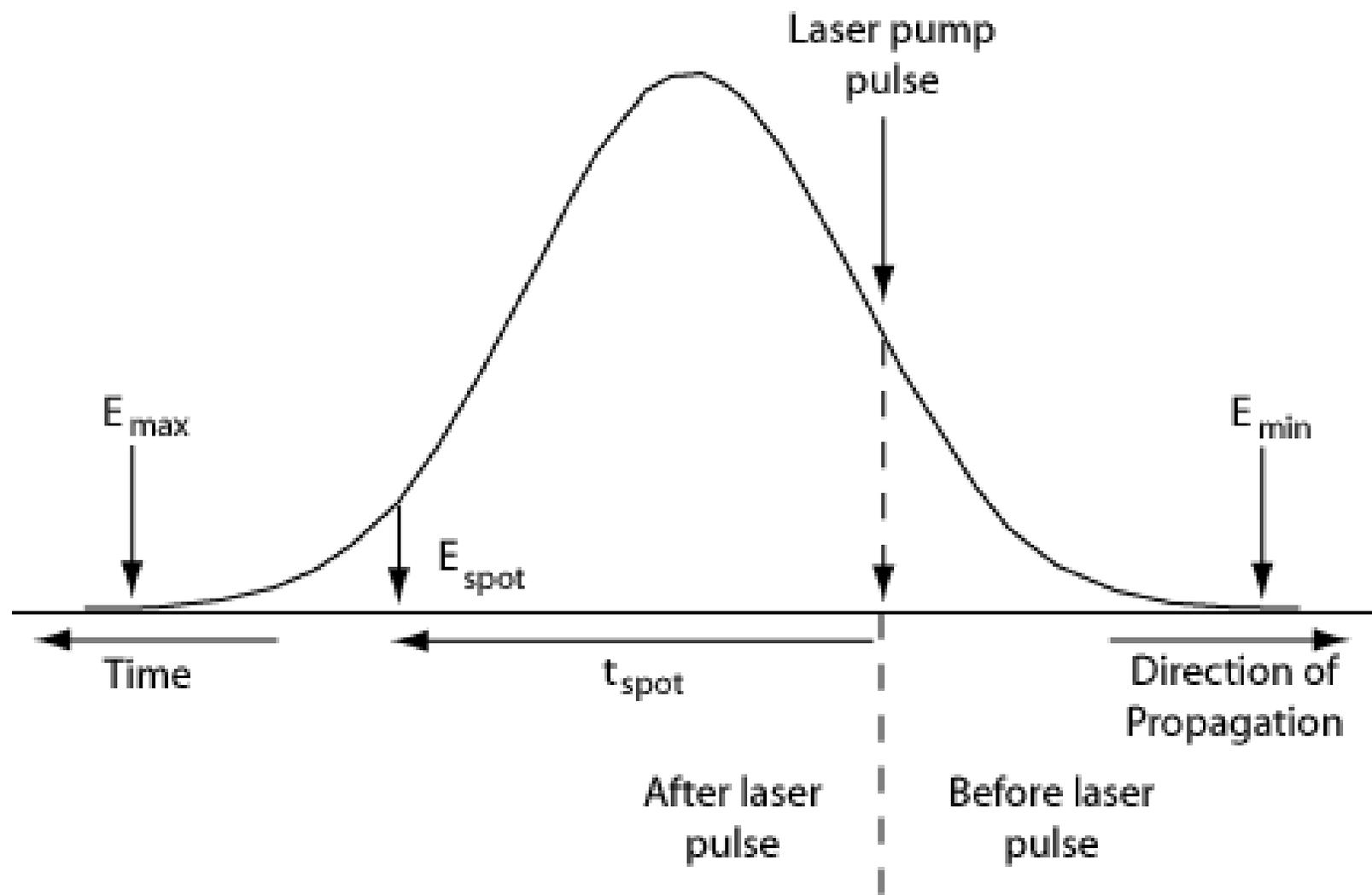
- Storage rings yield pulses ~ 100 ps; this limits the time resolution to that value.
- Get $\sim 10E8$ photons per pulse per 0.1% bandpass at 12 KeV through a 200 micron aperture, at ESRF and APS; would like 100 – 1000 times more, for time-resolved crystallography

How To Enhance The Time Resolution?

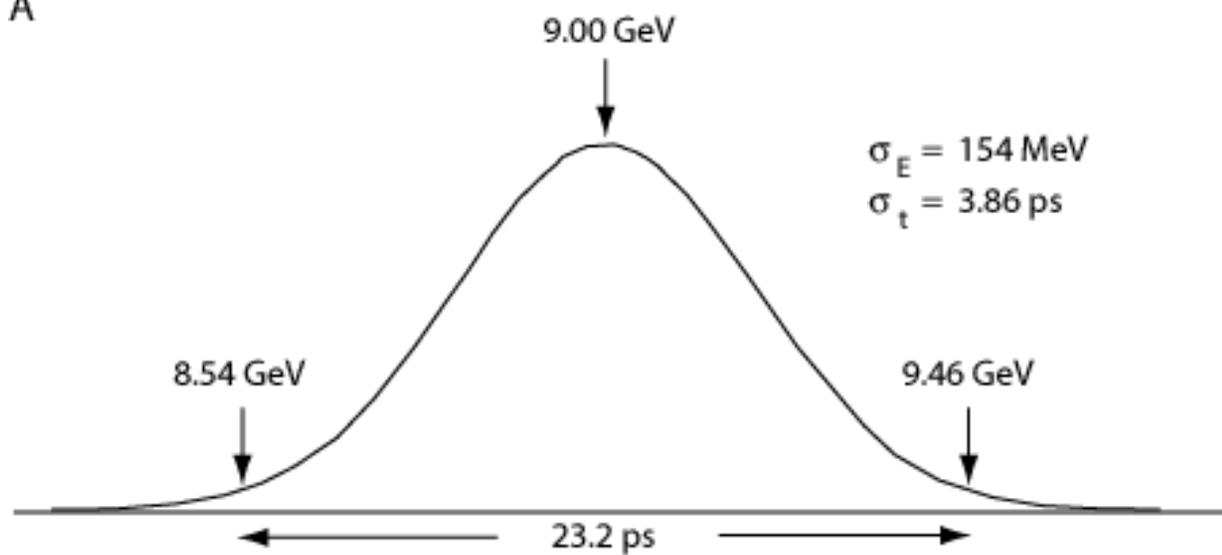
- Need time resolution better than 100 picoseconds
- Shorter pulses e.g. 100 femtoseconds expected from the Sub-Picosecond Photon Source, SPPS; or later from the LCLS
- Radiation damage from a single pulse may be extreme
- Stretch such pulses to much longer, chirped pulses that still afford excellent time resolution (Moffat, J. Chem. Soc. Faraday Trans., in press)
- May surmount part of the radiation damage problem and yield better-quality data







A



B

