Collecting Macromolecular Crystallographic Data at Synchrotrons

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Synchrotrons are useful, not just fashionable

- You can do almost any experiment better and faster at a storage ring than in a conventional lab; and there are experiments that you can *only* do at a storage ring.

What we need to think about

- Why synchrotrons help: Factors, parameters
- How they make things harder
- How synchrotron data collection is different from domestic data collection
- How macromolecular crystallography is different from other storage-ring apps

How synchrotrons help

- Fluence
- Brilliance
- Tunability
- Collimation
- Resources

- 10^{13} Xph/s/mm^2
- 10^{17} Xph/s/mm^2/mrad^2
- E = 12398.0 ± 0.4eV
- FWHM(v) < 100 μm
- Lasers, experts, labs …

Some definitions and units

Quantity	Definition	Units	Value
Flux	# photons / unit time	Xph/ sec	10^{12}
Fluence	flux / unit area	(Xph/sec)/ mm^2	10^{13}
Brilliance	fluence/ solid angle*	Xph/sec/ (mm^2-$mrad^2$)	10^{17}
Brightness	flux/solid angle*	Xph/sec/ $mrad^2$	10^{16}

* Sometimes defined in terms of bandwidth, e.g.
brilliance = (fluence/solid angle)/bandwidth

Which parameters really matter?

- For most macromolecular crystallographic experiments *fluence* is the relevant parameter: we want lots of photons entrained upon a small area
- Brilliance matters with very large unit cells where a high divergence is bad

What does high fluence do?

- Allows us to get good signal-to-noise from small samples
- Allows us to irradiate *segments* of larger samples to counteract decay
- Many experiments per day
- Allows us to contemplate experiments we would never consider with lower fluence

What does high brilliance do?

- How do we separate spots if the unit cell length > 500 Å?
  - Back up the detector
  - Use tiny beams
- Large beam divergence will prevent either of those tools from working

Tunability

- Monochromatic experiments:
  We're allowed to choose the energy that works best for our experiment
- Optimized-anomalous experiments:
  We can collect *F(h,k,l)* and *F(-h,-k,-l)* at the energy where they're most different
- Multiwavelength:
  pick 3-4 energies based on XAS scan and collect diffraction data at all of them

What energies are available?

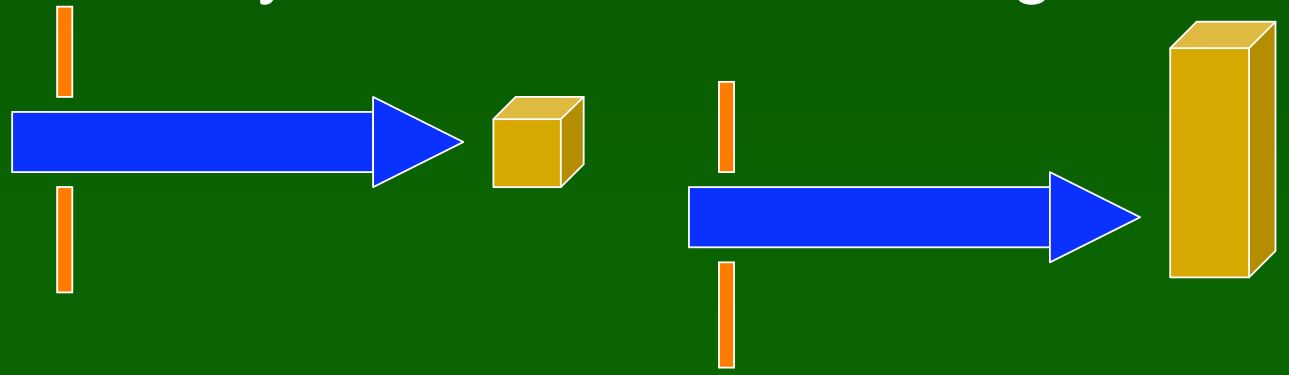
- Depends on the storage ring
- Undulators at big 3rd-generation sources:3-80 KeV
- Protein experiments mostly 5-25 KeV
  - Below 5: absorption by sample & medium
  - Above 25: Edges are ugly, pattern too crowded
- Some beamlines still monochromatic

Energy resolution & spectral width

- Energy resolution: how selective we can reproducibly produce a given energy
  - Typically ~ 0.4 eV at 3rd-Gen sources
  - Need: $\delta E < [E_{peak} - E_{edge}\ (Se)] \sim 1.4$ eV
- Spectral width: how wide the energy output is with the monochromator set to a particular value

Collimation

- Everyone collimates. What's special?
  - Beam inherently undivergent
  - Facility set up to spend serious money making collimation work right
- Result: we can match the beam size to the crystal or to a desired segment of it



Resources

Storage rings are large facilities with a number of resources in the vicinity

- Specialized scientific equipment (lasers)
- Smart, innovative people
- Sometimes: well-equipped local labs where you can do specialized sample preparations

Why *wouldn't* we do this?

- Beamtime is still scarce
- You're away from your home resources
- Disruption of human schedules
  - Travel
  - 24-hour to 48-hour nonstop efforts
  - Bad food
- Extra paperwork:
  Safety, facility security, statistics

How does synchrotron crystallography differ from lab crystallography?

- Time scale very foreshortened
- Multiwavelength means new experimental regimes
- Distinct need for planning and prioritizing experiments
- Robotics: taking hold faster @ beamlines

How does macromolecular crystallography differ from other beamline activities?

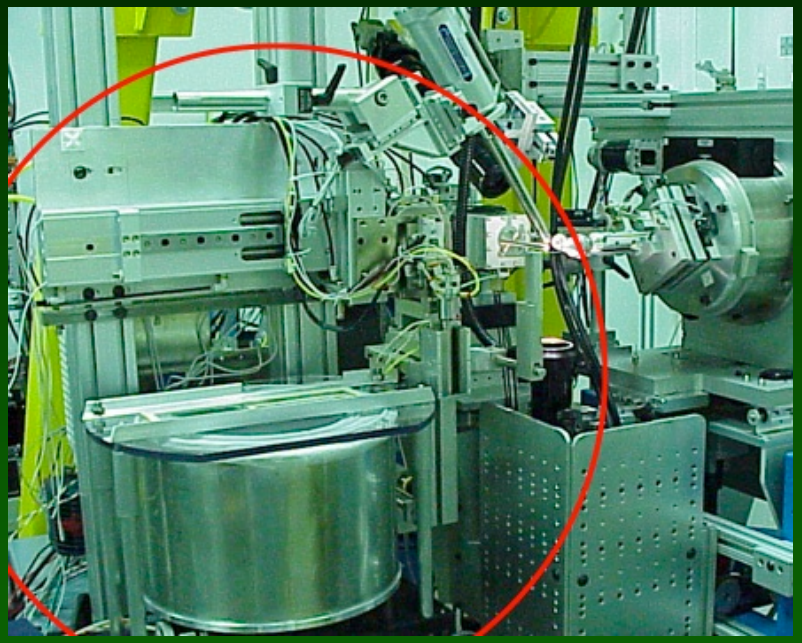
- “Physics and chemistry groups at the beamline do *experiments*;
  crystallographers do *data collection*”
- Expectation: zero or minimal down-time between users
- Often: well-integrated process from sample mounting through structure determination

Where will we collect data?

- SER-CAT: 22-ID
- SBC-CAT: 19-BM
- GM/CA-CAT: 23-ID
- NE-CAT: 8-BM (perhaps)
- DND-CAT: 5-ID
- BioCARS: 14-BM-C

Southeast Regional CAT (22)

- Established ~2002
- Run as an academic consortium of about 25 universities, mostly in the southeast, with some legislative or provost-level support
- 30% of my salary from there!

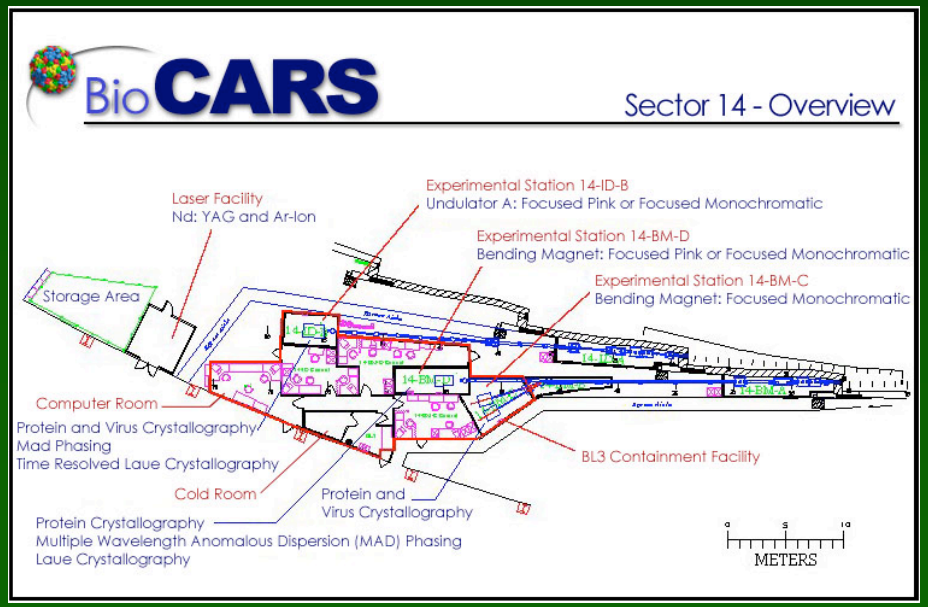


GM/CA-CAT (23)

- Established around 2004 as a site for NIH GM and Cancer grantees, particularly those working on structural genomics and cancer therapeutics
- First APS facility to build out multiple endstations on an insertion device line that are capable of simultaneous use

BioCARS

- Established around 1997 to do cutting-edge crystallographic projects, particularly involving time-resolved techniques and BSL-2 or BSL-3 samples



SBC-CAT

- Oldest macromolecular crystallography facility at the APS
- 19-ID: more structures solved than any other beamline in the world
- Good for all sizes and resolutions