Macromolecular CryoCrystallography @ Synchrotrons

Protocols and Techniques

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Overview

- Biological Macromolecules
  - Proteins, Nucleic Acids, Lipids, Sugars, & Complexes
- Single Crystal X-Ray Diffraction
  - Not powder or fiber or solution scattering
- Synchrotron Data Collection
  - Techniques
- CryoCrystallography
  - Protocols & Advantages
- Examples of Data

Biological Macromolecules

- Proteins
  - 20 Naturally Occurring Amino Acids
  - Anywhere from 20 to ~1500 Amino Acids
- Nucleic Acids
  - 4 Bases
  - DNA/RNA
- Lipids & Sugars
- Protein + NA Complexes
- Membrane Proteins

Single Crystal X-Ray Diffraction

- Provides a Complete Structural Information
- Needs a Crystal (A Challenge)
  - Still takes 6-9 months to get a crystallization condition
- Unit Cell Dimensions:
  - 10 Å (Proteins) | Small Molecules (1 Å)
  - 100 Å (Virus & Complexes)
- Crystal Dimensions: 0.05-1.0mm
- Use 1-2 Å X-Ray Radiation (Cu: 1.54 Å; 8 keV)
- Bond Lengths: ~1.0 – 2.0 Å
- Structural Repository: www.rcsb.org/pdb
  - 34,700 (X-Ray), 6000 (NMR), 225 (EM+)

X-Ray Source 1

- Home Source
  - Rotating Anode
  - Multi-layer Mirror
  - Wide Usage
  - Easy Access
  - Fixed Wavelength
    - Cu (1.54 Å)
    - Cr (2.29 Å)
    - Mo (0.71 Å)

X-Ray Source 2

- Synchrotron
  - Broad Wavelength Selection
  - Bending Magnet & Insertion Devices
  - ~1000 Times Intense
  - Low Divergence (mrad)
  - Small Beam Size (~0.1 x 0.1 mm)
  - Access
  - Travel/Planning

2007 FLAVS & FMS
UCF, Orlando, FL
March 12, 2007
Brilliance* of X-Ray Sources

- Photons/s/mm²/mrad/0.1% bandpass

Sealed Tube Rot Anode Bend Mag Wiggler Benders

- Brilliance

1.0E+07 1.0E+09 1.0E+11 1.0E+13 1.0E+15 1.0E+17 1.0E+19

Why CryoCrystallography?

- A Specialized Field is Now Routine
- 5% in 1995 and >90% in 2006
- But Macromolecular Crystals are
  - Radiation Sensitive
  - Contain 40-70% Solvent
  - Contain Flexible Regions
  - Low Scattering Cross-section

CryoCrystallography

- Breakthrough in 1990
  - T.-Y. Teng (Cornell)
  - Wire Loop
  - Viscous Hydrophilic Solvent
  - Free Standing Crystal
  - Flash Cooling

CryoCrystallography

- Advantages
  - For Macromolecular Crystals
  - Reduces Free Radical Diffusion
  - Reduces Stress on Crystals
  - Reduces Thermal Motion
  - Reduces Extra Scattering

CryoCrystallography

- Cryoprotectants
  - Alcohols
  - Glycerol (20%)
  - Poly Ethylene Glycols (20-30%)
  - 2-Methyl-2,4-pentanediol (30%)
  - Salts
  - Sodium Formate (3M)
  - Lithium Sulfate (2M)

*Photons/s/mm²/mrad/0.1% bandpass
CryoCrystallography

- Problems
  - Crystal Damage
  - Crystal Cracks
  - Chemical Reaction
  - Disorder (Mosaicity)
  - Other
    - Snow Ice
    - Embedded Ice

- Remedies
  - Annealing
  - Screen Solvents
  - Controlled Humidity
  - Protein ↓
  - Water ↑

Problems
- Embedded Ice
  - Hard to Remove
  - Anneal & Flash Cool
  - Problem w/ Lattice
- Snow Ice
  - Nuisance
  - Doesn’t Affect Lattice
  - Problem w/ Processing

Example: Enzyme
- Arginine Kinase 293°K | 12 min
  - 0 h (Home)
  - 12 h (Home), More needed
- Arginine Kinase 100°K | 15 min
  - 0 h (Home)
  - 12 h (Home)
  - 15 h (No LN2)
- Arginine Kinase 100°K | 30 sec
  - 0 h (NSLS – BM-12C)
  - 1.5 h (NSLS – BM-13C)

Example: Virus
- AAV2 293°K | Ambient
  - 24 h (Home | Capillary)
- AAV2 277°K | 4°C
  - 30 s (CHESS | F1 | Capillary)
  - Survived 3 exposures
- AAV2 100°K | Cryo
  - 70 s (CHESS | F1 | CryoLoop)
  - Survived >180 exposures

Example: Protein
- Fibroblast Growth Factor
  - 100°K
  - 40 min (Home)
- Fibroblast Growth Factor
  - 1.1 -1.2Å Diffraction
  - Offset Detector
  - 1.1 -1.2Å Diffraction

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CryoTools
- CryoCap
- CryoLoop
- CryoVial
- CryoTong
- CryoShipper
- CryoPuck

References:
- Thorne et al, MIDS 439 (2005)
SER-CAT Beamline @ APS

Automounter | BCSB

Remote Access | SSRL

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