Crystallographic Phasing

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Goals:
- **Improve** existing phasing capabilities
- Develop **new** phasing techniques
- Phasing with **minimum** biochemical preparation for proteins

Expression → Crystallization → Data collection → Phasing → Structure

SeMet substitute
heavy-atom buffer
heavy-atom soak

Δf' & Δf" Typical Steps in Today’s Protein Crystallography
New Phasing Methods

Protein Crystallography w/o Heavy Atoms?

- Expression
- Crystallization
- Data collection
- $|F(H)|$ & $\alpha(H)$
- Structure

Reference-Beam Diffraction

SAD with native elements, e.g. S

Molecular envelope from SAXS
Reference Beam Diffraction

(a) Conventional oscillation method

(b) Reference-beam diffraction

Phase difference: \( \delta = \alpha_{H-G} + \alpha_G - \alpha_H \) → Triplet Phase

Shen, PRL (1998)
Science (1998)
Reference Beam Diffraction Data Collection

RBD data set: \( I(hkl) \) vs. \( \theta - \theta_G \)

Analogy to MAD data set: \( I(hkl) \) vs. \( \lambda \)
**Dedicated 5-Circle K-Diffractometer**

- **x-rays**
- **θ**
- **2θ**
- **φ**
- **ψ**
- **kappa routine**
- **CCD**

**RBD Theory:** 

\[ I_H(\eta_G) = 1 - \tau \sin \delta \begin{pmatrix} \sin^2 \left( \frac{A \eta_G}{2} \right) \\ \frac{\tau}{\eta_G} \left( \cos \delta + \frac{\tau}{2\eta_G} \right) \left( 1 - \frac{\sin(2A \eta_G)}{2A \eta_G} \right) \end{pmatrix} \]

- **base \( I_0 \)**
- **amp. \( p \)**
- **center \( \theta_0 \)**

**Shen (1999) PRL; Shen (2000) PRB.**

- **initial oscillation image**
- **MOSFLM indexing**
- **initial orientation matrix**
- **refinement of alignment**
- **ADSC CCD control**
- **data collection**
- **triplet-phase \( \delta \)**
- **curve-fitting with interference \( I(\phi) \)**
- **\( \kappa, \phi \) angles for \( G \)**
- **\( G \) along oscillation axis \( \psi \)**
- **integrated intensity vs. \( \theta - \theta_G \)**
- **RBD oscillation images**
- **MOSFLM integration**
- **multiple images at different \( \theta - \theta_G \)**
Experiment Examples

Tetragonal lysozyme P4₃2₁₂

- 14914 RBD profiles in ~ 12 hrs
- 7360 triplet phases with δₘ = 45°

AlPdMn quasicrystal alloy

G = (012012)
Strategies Using Measured Phases

**RBD experiments**

- **Direct methods**: replace math guesses with measured triplet-phases (Weeks, 2000)
- **Recursive method**: using the unique triplet pattern in RBD (Shen, 2002)

\[ \delta = \alpha_{HG} + \alpha_G - \alpha_H \]

\[ \alpha_H = ?? \]

**Future Investigations**

- What is the minimum number of phases needed to be measured in order to solve a structure?
- How do various factors, e.g. number of G data sets, data completeness, space group, measurement errors, crystal quality, etc., affect the likelihood of solutions?
- Are new phasing algorithms possible given the unique pattern of individual phases in a RBD triplet data set?

**NIH grant award (Shen, 2001-06) in collaboration with Hauptman.**

New postdoc: Andy Stewart.
Phasing Programs

supported through MacCHESS

- **Phasing from molecular envelope**
  Hao (MacCHESS)
  - Molecular envelope from SAXS
  - **FSEARCH** to place molecule in unit cell
  - Phase extension to high resolution

- **SAD with direct-methods**
  Hao (MacCHESS); Hauptman, Weeks (HWI)
  - Direct methods routines **SAPI** or **SnB** for heavy-atom positions
  - **OASIS** for SAD phase determination

- **Other phasing programs**
  - **SOLVE** and **RESOLVE**
  - **MLPHARE** (CCP4)
  - **CNS** for MIR, molecular replacement, etc.
  - **Phases** combined with **SnB** $\Rightarrow$ **BnP**
Phasing with Future Coherent Source

⇒ **Advanced phasing algorithms in materials science:**
   *in collaboration with Robinson & Vartanyants (UIUC), Elser (Cornell)*

strains profile $u(r)$ in a nanocrystallite
diffraction imaging of nanocrystals

*Robinson et al. PRL (2001)*

⇒ **From reference-beam diffraction to holography:**

external reference wave
imaging non-crystalline materials

*Leitenberger & Snigerev, JAP (2001)*
Summary: Phasing Initiative

- Integral part of crystallographic facility at CHESS
- Builds on existing collaborations and interests among staff and users
- Unique team of experts in related fields
- New phasing methods would benefit not only CHESS users but all crystallographers