Macromolecular Powder Diffraction

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www.hecra.gr
Can we extract any structural information from a protein polycrystalline sample?
The first protein structure refinement using powder diffraction data:
Whale Metmyoglobin

R. B. Von Dreele
• Sample Preparation
  • Data Collection
  • Data Analysis
  • Case Studies
• Future & Concluding Remarks
POWDER CRYSTALLINE SAMPLES

hundreds of microcrystals

Glass capillary tube containing white protein crystalline precipitate in brass sample holder
Good vs Bad Precipitates

Tutorial 2.

Precipitates

One of the most difficult things for beginners is to recognize promising precipitates and distinguish them from precipitates that are not worth pursuing. Except for drops where the protein is denatured, precipitate can be regarded as a positive drop result. This is because precipitate occurs in a state of supersaturation. Crystals can only grow from supersaturated states, and thus they can grow from precipitates.

How do I tell a "good" precipitate from a "bad" precipitate?

"Good" Precipitate
- Non-amorphous precipitate
- Precipitate shows \textit{birefrigence}
- Precipitate redissolves if given the right conditions

"Bad" Precipitate
- Characteristic brownish tinge to the precipitate
- Skins on the drop
- Precipitate do not redissolve upon dilution

Courtesy: Prof. Terese Bergfors, http://xray.bmc.uu.se/~terese/tutorial2.html
GOOD VS BAD PRECIPITATES

crystals
• Data Collection
1999 - present
ESRF - Grenoble, France

New collaborations with other synchrotrons

FRANCE

http://www.synchrotron-soleil.fr/

JAPAN

http://www.spring8.or.jp/en/

SWITZERLAND

http://www.psi.ch/sls/
Low-angle region of powder diffraction patterns from lysozyme samples. The lower and upper patterns correspond to analyser crystal (ID31) and area detector (BM01A) data respectively. In these experiments, the intensities of certain low angle reflections are modulated by varying the mean electron density of the solvent surrounding the protein molecules in the crystal lattice via variations in the solvent composition. The red and blue spectra correspond respectively to samples soaked in low and high electron-dense solvents.
ID31 data - Pawley in TOPAS

HEWL, $\lambda = 1.251209(6)$ Å

$P_{4_3}2_12$: $a = 79.2688(3)$ Å, $c = 37.9573(2)$ Å

2956 Extracted Intensities up to 3Å resolution.

X’Pert PRO system handles a wide range of applications. It is especially suitable for:

- thin film analysis applications such as rocking curve analysis and reciprocal space mapping,
  - reflectometry
- thin film phase analysis
- residual stress and
- texture analysis

From nanomaterials to bulk samples and proteins
This detector is a member of the Medipix photon counting pixel detector family.

PIXcel is the result of a collaboration with CERN, one of the world’s foremost particle physics laboratories and other research institutes as part of the Medipix2 project.
LEBAIL REFINEMENT OF LAB POWDER DATA USING X’PERT HIGH SCORE PLUS

T3R3: sp. Group: H3
a = 82.480 Å, c = 37.690 Å
4054 extracted intensities

Courtesy: Thomas Degen
thomas.degen@panalytical.com
• Data Analysis
SOFTWARE USED

**Powder Diffraction & CCP14**
- Fit2D
- DASH
- TOPAS
- FULLPROF
- GSAS

**Single Crystal & CCP4**
- CCP4 software package
- MOLREP
- PHASER
- CNS
- REFMAC
- PHOENIX
- WINCOOT
- PYMOL
- CHIMERA

**Home made**
- PRODD
- SFCHECK (modified version)
- Short routines in PYTHON
- Pycluster
- ID31sum

**Other useful software**
- El nemo server
  - [http://www.igs.cnrs-mrs.fr/elnemo/](http://www.igs.cnrs-mrs.fr/elnemo/)
• Case Studies
AN UNKNOWN PROTEIN STRUCTURE SOLVED FROM POWDERS

The Second SH3 domain of Ponsin
The Second SH3 domain of Ponsin

THE CASE OF HUMAN INSULIN
Several polymorphs exist depending on pH of crystallization and concentration of additives.

Great interest in finding new forms of potentially therapeutic applications.

Study microcrystalline insulin crystallized as a function of pH (4 – 8.9) and with phenol – based additives.

3 forms already known with structures (from single crystals) in space groups $C222_1$, $C2$ and $P2_1$. 

MICROCRYSTALLINE INSULIN
HUMAN INSULIN – LIGAND COMPLEXES
CRYSTALLINE PRECIPITATES

PHENOL

RESORCINOL
pH < 5.6

C2

NEW FORM

C 2221

P 21
NEW POLYMORPH
pH 5.18 + resorcinol

\[ a = 114.85 \, \text{Å} \]
\[ b = 334.89 \, \text{Å} \]
\[ c = 49.36 \, \text{Å} \]
\[ \beta = 103.36^\circ \]
\[ v = 1.85 \text{ million } \text{Å}^3 \]
phenol

resorcinol

Unit Cell Volume (Å³)

new $P_2_1$

pH

Karavassili et al.
ENHANCED STRUCTURE REFINEMENTS

Rigid body description of amino acids

Margiolaki et al.
AFMB - Marseille

Research Collaborators
Bruno Canard, Nicolas Papageorgiou & Bruno Coutard
The First Case of a Virus Protein:

nsP3 macro domain of the Mayaro virus (MAYV)
“SEA URCHIN” CRYSTALS OF MAYV
ID14-2, area detector
λ=0.9934 Å
Matthew Bowler, Yves Watier, Nicolas Papageorgiou
PRELIMINARY MODEL AFTER MR SOLUTION AND RB REFINEMENT
Future & Concluding Remarks
CRYSTAL SCREENING

- Polymorph Identification & Ligand Binding
- Crystal size and morphology (XFEL)
- Phase Mapping

• COMBINED USE WITH

- XFEL measurements on nano-crystalline precipitates
- Electron Diffraction on single nano-crystals

  Barty, Küpper, Chapman, Annual Review of Physical Chemistry 01/2013

• Three-dimensional electron crystallography of protein microcrystals
  Shi et al., 2013, eLIFE
CONCLUDING REMARKS

- Protein samples are often easily obtained as microcrystalline precipitates
- Ideal method for Crystal Screening – Phase Identification
- Structure solution and refinement are possible but time consuming
- Good quality data are routinely collected using synchrotrons or modern lab sources
- Combined use with XFEL and ED measurements
ACKNOWLEDGMENTS

X-RAY DIFFRACTION

ESRF, Grenoble

Andy Fitch
Jon Wright
Yves Watier
The ID31 team

Former members:
Lucy Saunders
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<td>PaNalytical, Netherlands</td>
<td>Detlef Beckers, Thomas Degen, Celeste Reiss, Stjepan Prugovecki, Martijn Fransen</td>
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International Workshop 1: Fundamentals of Crystallography
1-7 April 2013 (Tutor: Carmelo Giacovazzo)

International Workshop 2: Powder & Electron Crystallography
8-12 July 2013
Patras Greece

Lectures available from:
http://crystallographypatras.wordpress.com/
• Current Trends in Structural Biology & 7th meeting of the Hellenic Crystallographic Association, September 19th-21st, 2014, FORTH/IMBB, Heraklion, Crete, Greece

• Workshop on “Strategic pipeline planning: from sample preparation to 3D structure determination with bio SAXS and other biophysical techniques” co-organised with National Hellenic Research Foundation. April 5 - 10 - 2014 National Hellenic Research Foundation in Athens, Greece.

• Lectures/presentations on crystallography and its impact in science and applications for students of secondary education. Agricultural University of Athens, NCSRD “Demokritos, University of Patras and others.

• Competitions for secondary education children, (i) a crystal-growing competition; (ii) competition on output inspired by Crystallography, e.g. photographic, video etc. Details will be given in the website of HeCrA (www.hecra.gr ).
IAEA
Coordinated Research Project (CRP) on “Utilisation of accelerator-based real-time and in-situ methods in investigation of materials for energy applications”
2012-2015
CRP code: F12024

UNESCO & L’OREAL Foundations
International Fellowship for Women in Science
2010-2012

Nanomegas
Stavros Nicolopoulos
2012-Present

EU & University of Patras
FP7: SEE-DRUG
PI: George Spyroulias
http://www.seedrug.upatras.gr/
2012-2015

Karatheodoris Foundation & ΕΛΚΕ (UPATRAS)
2010-2013