Results from the SNI nanoArgovia project A3EDPI:

Structure Determination with Electron Crystallography

iNEXT workshop, CEITEC, 29th May 2019

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Electron Diffraction in the News

The CryoEM Method MicroED as a Powerful Tool for Small Molecule Structure Determination

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Electron Diffraction is for Small Crystals
Electrons interact more strongly than X-rays

Powder Crystals are Single Crystals

organic compound

sucrose (ETH coffee bar)

Silicalite–1 / ZSM–5 (Teng Li)

Gruene et al., Chem. EurJ (2018), 24, 2384–2388
Structure Determination by Single Crystal Diffraction

- Diffraction spots: interaction between **wave** and **crystal**
- Experimental result: **Position** and **Intensity** for each spot
Crystal Structure of a Chemical Compound: Determination of 3D atom coordinates

Crystal packing with hydrogen network
CCDC: IRELOH

Intramolecular hydrogen bonding
Deffieux et al., Acta Cryst (1977), B33, 1474
CCDC: EPICZA

samples courtesy Novartis (cf. Clabbers et al., Acta Cryst. (2019), A75, 82–93)
samples courtesy Novartis

Chemistry: Starting point for improvements at atomic level (catalyst efficiency, drug uptake, lacquer brilliance . . .)
How to Turn a TEM into an Electron Diffractometer

EIGER X 1M

- Eiger X 1M designed for X-ray Synchrotron radiation
- $1030 \times 1065$ pixel, $75 \times 75 \, \mu m^2$
- up to $v = 3 kHz$ frame rate: data collection at synchrotron speed
- $3 \, \mu s$ dead time: shutterless data collection
- $\leq 200 keV$: no radiation damage, no beam stop
- 16 or 32 bit image depth & $2.8 \cdot 10^6 \, \text{cts s}^{-1} \text{pixel}^{-1}$: high dynamic range

Diffraction pattern and structure for SAPO–34
Tinti et al., IUCrJ (2018). 5, 190–199

Installation of the EIGER X 1M in 1/2 day (C. Zaubitzer, ScopeM, ETH)

- Removal of the previous camera
- Mounting of the EIGER X 1M with adapter flange
- Shielding and radiation monitoring
- Final shielding after 1/2 day
- Vacuum OK: next morning
- Return to original state: 1 day
- Gatan camera back with auto-justage

Determination of Experimental Parameters

Detector separated from Instrument: no automated read-out (yet)

- Detector distance (alias Camera length)
- Rotation axis
- Direct beam position
- Oscillation width (Rotation per frame)

A3EDPI: values can be calibrated, \textit{e.g.} once/day

Hybrid pixel detectors are radiation hard and require no beamstop. This facilitates determination of detector distance, rotation axis, direct beam position.
Determination of the Rotation Axis

- Rotation axis runs through direct beam and minimum of powder ring
- Rotation axis: region of no spots
- line through 2 points on rotation axis
- \( P_1 = \frac{529}{621} \) and \( P_2 = \frac{458}{702} \)
- \( \tan(\alpha) = \frac{\Delta Y}{\Delta X} = \frac{702-621}{458-529} \)
- \( \text{ROTATION_AXIS} = \cos(\alpha); \sin(\alpha); 0 \)
- Large radius of convergence with XDS (\( \approx \pm 10^\circ \))

Direction of rotation: from minimal error of spindle axis

\[
\begin{array}{cccc}
\text{ROTATION_AXIS} & (XDS.INP) & -0.6979 & -0.7161 & -0.0102 \\
\text{DEVN OF SPINDLE POSN} & (IDXREF.LP) & 0.37^\circ & & +0.6979 & +0.7161 & +0.0102 \\
\end{array}
\]

\begin{array}{ccc}
\text{1.01}^\circ & & \\
\end{array}

Oscillation Width

- Used to be most time consuming parameter to be determined
- Step forward at C–CINA: movie during measurements

\[ \frac{d\phi}{dt} = \frac{\Delta\alpha}{\Delta t} = \frac{58.15^\circ}{25.185 \text{s}} = 2.310^\circ/\text{s} \]

\[ \nu(\text{EIGER}) = 100\text{Hz} \]

\[ \Rightarrow \Delta\phi = 0.0231^\circ/\text{frame} \]
Oscillation Width

- Probe $\alpha$ angle per 0.5s during experiment
- Fit line to measurements
- fast, reproducible
- Oscillation width $\Delta \phi [\degree/\text{frame}] = \frac{d\phi}{dt}/\nu(\text{EIGER})$

Acknowledged: Luca Piazza. Dectris Ltd. for initial Digital Micrograph script

The Electron Diffractometer

- All parts for a dedicated diffractometer are available
- Pieces need to be assembled
- Electron Microscopes (2-10Mio €): many unnecessary features
- Electron Diffractometer: < 500,000 € including detector
Single Crystal Structure from a Pharmacy Powder
Grippostad®, STADA

<table>
<thead>
<tr>
<th>active compounds</th>
<th>non-active compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>paracetamol</td>
<td>gelatine</td>
</tr>
<tr>
<td>ascorbic acid</td>
<td>glycerol tristearate</td>
</tr>
<tr>
<td>caffeine</td>
<td>lactose monohydrate</td>
</tr>
<tr>
<td>chlorphenamine maleate</td>
<td>quinoline yellow (E104)</td>
</tr>
<tr>
<td></td>
<td>erythrosine (E127)</td>
</tr>
<tr>
<td></td>
<td>titanium dioxide (E171)</td>
</tr>
</tbody>
</table>

Single Crystal Structure from a Pharmacy Powder

1. Exp: $a = 6.9$, $b = 9.4$, $c = 11.6$, $\alpha = 90.6$, $\beta = 98.4$, $\gamma = 89.8$

2. CSD: $a = 7.1b = 9.3c = 11.7\alpha = 90.0\beta = 97.7\gamma = 90.0$; CSD search: HXACAN04, $P2_1/n$, Paracetamol,

3. Structure solved with $\leq 40\%$ completeness

4. Difference map reveals hydrogen atoms: data sensitivity

“The existence of multiple crystal forms (polymorphs, solvates, hydrates, etc.) is playing an increasingly important role in establishing and protecting intellectual property rights in the pharmaceutical industry”

(Prof. J. Bernstein, ECM-30 (Aug. 2016), MS50-O2)
Consequence of Grippostad: Screening for Polymorphs

- No lower size limit for electron crystallography
- per sample holder: hundreds – thousands of crystals
Drug Design: Structure of a New Methylene Blue Derivative MBBF$_4$

Dr. J. Holstein & Prof. G. Clever, TU Dortmund
MBBF₄-nanoCrystal (Holstein/Clever, TU Dortmund)

MBBF$_4$ — EIGER and a TEM make a Synchrotron
MBBF$_4$ — Data Accuracy (J. Holstein, TU Dortmund)

Structure of MBBF$_4$
- $R_1 = 22.7\% (2941F_o > 4\sigma_F)$
- $R_1 = 27.2\% (4832F_o)$
- GooF = 1.5
- 564 parameters, 1054 restraints

After addition of hydrogen atoms and restraints: Dual conformation of $BF_4$ becomes visible.

Structure refinement by J. Holstein

Consequence of MBBF$_4$: ED complements XRD

- A dedicated electron diffractometer extends the X-ray diffractometer in every X-ray facility
- Speed of structure determination comparable to X-ray diffractometer
- Reliable Structures from electron diffraction
Preferred Crystal Orientation & the Missing Wedge Problem

(J. Wennmacher et al., “3D-structured supports create complete data sets for electron crystallography”, under revision)
Missing Wedge in Electron Diffraction

- Crystals very often have a **flat shape**: always the same orientation
- Sample support stabilised by Cu-grid
- Copper grid too thick: intransparent for electrons
- Limited rotation range

Wennmacher *et al.*, “3D-structured supports create complete data sets for electron crystallography” under revision
Effect of Missing Data on Map and Structure

Shearing of experimental map results in unreliable coordinates for structure

Wennmacher et al., “3D-structured supports create complete data sets for electron crystallography” under revision
Complete Data from 3D Structured Grids - Coiled carbon grids

Brush Stroke causes carbon layer to coil

• Visual selection of orientation from carbon curvature
• Complete data from 5'ish crystals

Wennmacher et al., “3D-structured supports create complete data sets for electron crystallography” under revision
Complete Data from 3D Structured Grids - Nylon Fibres

Nylon fibres ($\approx 100\text{nm}$ diameter) disturb preferred orientation

- Orientation less obvious from visual inspection
- Possibly more screening required
- Complete data from 5'ish crystals

Wennmacher et al., “3D-structured supports create complete data sets for electron crystallography” under revision
Electron Crystallography of Macromolecules
Protein Crystals in the TEM

Lysozyme, $\approx 2.1\text{Å}$ resolution (Clabbers et al. (2017))

Thermolysin:
$\approx 2 \times 1 \times$ very thin $\mu m^3$
Solvent reduces contrast (sample courtesy I. Schlichting)

Thermolysin:
about 3Å resolution (sample courtesy I. Schlichting)
Comparison of resolution between Electron Diffraction and X-ray diffraction

Some structures from the PDB solved with ED

<table>
<thead>
<tr>
<th>Protein</th>
<th>$d_{\text{min}}$ (Å)</th>
<th>PDB-ID</th>
<th>$d_{\text{min}}$ (Å)</th>
<th>PDB-ID</th>
<th>$d(e^-)/d(\text{X-rays})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>1.80</td>
<td>5K7O</td>
<td>0.94</td>
<td>1IEE</td>
<td>1.9</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>2.80</td>
<td>6HU5</td>
<td>0.94</td>
<td>1IEE</td>
<td>1.9</td>
</tr>
<tr>
<td>Catalase</td>
<td>3.20</td>
<td>5GKN</td>
<td>1.50</td>
<td>1DGF</td>
<td>2.1</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>1.75</td>
<td>5I9S</td>
<td>0.83</td>
<td>2PWA</td>
<td>2.1</td>
</tr>
<tr>
<td>Xylanase</td>
<td>2.30</td>
<td>5K7P</td>
<td>0.97</td>
<td>3AKQ</td>
<td>2.3</td>
</tr>
<tr>
<td>Thaumatin</td>
<td>2.11</td>
<td>5K7Q</td>
<td>0.90</td>
<td>5X9L</td>
<td>2.3</td>
</tr>
<tr>
<td>Trypsin</td>
<td>1.70</td>
<td>5K7R</td>
<td>0.75</td>
<td>4I8H</td>
<td>2.2</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>2.50</td>
<td>5K7T</td>
<td>1.12</td>
<td>5JVI</td>
<td>2.2</td>
</tr>
</tbody>
</table>

ED of proteins only reaches half the resolution of X-rays — in contrast to organic and inorganic compounds.
High resolution data collection for MX-ED

- X-ray: Test crystals (Thaumatin, Lysozyme, ...) easily diffract to 1.2–1Å
- Electron: about 2x worse so far
  1. Rotate sample at high dose with short lifetime but maximum resolution, *e.g.* 5° per crystal
  2. Combine data from many crystals for data completeness
- Outcome determines whether 3D ED will be useful for Structural Biology
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