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**Turning Powder into Single Crystals: Structure Determination of Chemical Compounds with Electrons**

Results from the SNI nanoArgoiva Project *A3EDPI*

presented at the Dpt. of Materials and Environmental Chemistry

University of Stockholm

11th April 2019
1 - Electron Diffraction (ED) in the News
ED in the News
2 - Electron Diffraction is for Small Crystals
Electrons interact more strongly than X-rays

Powder Crystals are Single Crystals

organic compound

Silicalite–1 / ZSM–5 (Teng Li)
Gruene et al., Chem. EurJ (2018), 24, 2384–2388

sucrose (ETH coffee bar)
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

Crystal Structure: 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, see also 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 …)
3 - How to Turn a TEM into an Electron Diffractometer

J. Heidler et al., “Design Guidelines for an Electron Diffractometer for Structural Chemistry and Structural Biology”, under review
EIGER X 1M

- Eiger X 1M designed for X-ray Synchrotron radiation
- 1030x1065 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 & \(2.8 \cdot 10^6 \, \frac{cts}{s \cdot pixel}\): high dynamic range

Diffraction pattern and structure for SAPO–34
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, *e.g.* once/day

Hybrid pixel detectors are radiation hard and require no beam-stop. 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
- $P_1 = 529/621$ and $P_2 = 458/702$
- $\tan(\alpha) = \frac{\Delta Y}{\Delta X} = \frac{702-621}{458-529}$
- $\text{ROTATION\_AXIS}= \cos(\alpha); \sin(\alpha); 0$
- Large radius of convergence with XDS ($\approx \pm 10^\circ$)

Direction of rotation: from minimal error of spindle axis

<table>
<thead>
<tr>
<th>ROTATION_AXIS (XDS.INP)</th>
<th>DEV^N OF SPINDLE POS^N (IDXREF.LP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.6979 -0.7161 -0.0102</td>
<td>-0.6979 -0.7161 -0.0102</td>
</tr>
<tr>
<td>0.37°</td>
<td>+0.0102</td>
</tr>
<tr>
<td>1.01°</td>
<td></td>
</tr>
</tbody>
</table>
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

\[
\frac{d\phi}{dt} \text{[°/s]} = 2.9520 \pm 0.0079, \quad \alpha_0 \text{[°]} = -62.31 \pm 0.19
\]

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

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
4 - Single Crystal Structure from a Pharmacy Powder
Grippostad®, STADA

**active compounds**
- paracetamol
- ascorbic acid
- caffeine
- chlorphenamine maleate

**non-active compounds**
- gelatine
- glycerol tristearate
- lactose monohydrate
- quinoline yellow (E104)
- erythrosine (E127)
- titanium dioxide (E171)
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: \(a = 7.1 \ b = 9.3 \ c = 11.7 \ \alpha = 90.0 \ \beta = 97.7 \ \gamma = 90.0; \) CSD search: HXACAN04, \(P 2_1/n\), 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

5 - Drug Design: Structure of a New Methylene Blue Derivative MBBF$_4$

Dr. J. Holstein & Prof. G. Clever, TU Dortmund
MBBF$_4$-nanoCrystal (Holstein/Clever, TU Dortmund)

$C_{120}H_{120}B_8F_{32}N_{28}S_4$, $M_W = 2,990$ Da
MBBF$_4$ — EIGER and a TEM make a Synchrotron
MBBF$_4$ — Data Accuracy (J. Holstein, TU Dortmund)

Structure of MBBF$_4$
- $R_1 = 22.7\%(2941F_o > 4\sigma_F)$
- $R_1 = 27.2\%(4832F_o)$
- $\text{GooF} = 1.5$
- 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 MBBF₄ Structure

- 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
6 - Preferred Crystal Orientation & the Missing Wedge Problem

(Patent EP 18 202 868)

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

(a)  
(b)  
(c)  
(d)  
(e)  
(f)  
(g)  
(h)
Complete Data from 3D Structured Grids - Coiled carbon grids

Brush Stroke causes carbon layer to coil

(a)  
(b)  
(d)  

4 μm
Nylon fibres (≈ 100nm diameter) disturb preferred orientation

3D structured grids break the preferred orientation of the crystals.
Complete data from < 5 crystals.
7 - 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 $\mu\text{m}^3$
Solvent reduces contrast
(sample courtesy I. Schlichting)

Thermolysin:
about $3\,\text{Å}$ resolution
(sample courtesy I. Schlichting)
Comparison of resolution between Electron Diffraction and X-ray diffraction

Some structures from the PDB

<table>
<thead>
<tr>
<th></th>
<th>$e^-$</th>
<th>X-ray</th>
<th>resol. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d_{\text{min}}$</td>
<td>PDB-ID</td>
<td>$d_{\text{min}}$</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>1.80</td>
<td>5K7O</td>
<td>0.94</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>2.80</td>
<td>6HU5</td>
<td>0.94</td>
</tr>
<tr>
<td>Catalase</td>
<td>3.20</td>
<td>5GKN</td>
<td>1.50</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>1.75</td>
<td>5I9S</td>
<td>0.83</td>
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<tr>
<td>Xylanase</td>
<td>2.30</td>
<td>5K7P</td>
<td>0.97</td>
</tr>
<tr>
<td>Thaumatin</td>
<td>2.11</td>
<td>5K7Q</td>
<td>0.90</td>
</tr>
<tr>
<td>Trypsin</td>
<td>1.70</td>
<td>5K7R</td>
<td>0.75</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>2.50</td>
<td>5K7T</td>
<td>1.12</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
8 - Conclusions

“[…] the present work opens the route to detailed structure analysis […]”

Palatinus et al., Science (2017)

“…the whole field of electron crystallography would benefit from a transmission electron microscope specifically designed as an electron diffractometer.”


Similar statements are found across the decades (Voigt-Martin et al. (1997), Klechkovskaya & Imamov (2001), Dorset (2010), Zou & Hovmöller (2008))

A3EDPI took the final step and created an electron diffractometer for chemical crystallography.
- latest detector technology
- reliable read-out of experimental parameters

DECTRIS QUADRO:
specifically designed for TEM applications
9 - Acknowledgements

- R. Pantelic (PSI/DECTRIS), S. De Carlo (DECTRIS), C. Zaubitzer (ScopeM), J. Wennmacher (PSI), J. Holstein (TU Dortmund), J. Heidler (PSI), A. Fecteau–LeFebvre (C–CINA), K. Goldie (C–CINA), E. Müller (PSI), S. Handschin (ScopeM), H. Stahlberg (C–CINA), N. Blanc (ScopeM), C. Schulze–Briese (DECTRIS)

- B. Luethi (DECTRIS), L. Wagner (DECTRIS), L. Piazza (DECTRIS), D. Mayani (DECTRIS)

- Y. K. Bahk (ETHZ), I. Regeni (TU Dortmund), T. Li (ETHZ), L. Muskalla (Uni Konstanz), A. Pinar (PSI), J.A. van Bokhoven (PSI/ETHZ), G. Clever (TU Dortmund)

- G. Santiso–Quinones (Crystallise!), G. Steinfeld (Crystallise!), R. Mezzenga (ETHZ), U. Shimanovich (Weizmann Inst.), I. Adriansens Martiel (PSI), I. Schlichting (MPI Heidelberg), K. Diederichs (Uni Konstanz), J. Lübben (now Bruker AXS), M. Clabbers (Uni Stockholm)

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SNF Project 169258