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Macromolecular Crystallography with Nanocrystals based on Electron Diffraction

CCP4 Study Weekend
10th January 2017
1 - Electron Diffraction
Structure Determination by Single Crystal Diffraction

- Diffraction spots: interaction between wave and crystal
- Experimental result: **Position** and **Intensity** for each spot

Laue equations:

\[
\begin{align*}
(\vec{S}_o - \vec{S}_i) \cdot \vec{a}^* &= h \\
(\vec{S}_o - \vec{S}_i) \cdot \vec{b}^* &= k \\
(\vec{S}_o - \vec{S}_i) \cdot \vec{c}^* &= l
\end{align*}
\]

Independent of radiation type: \(X, e^-, n\)
Electrons as Radiation Source

- wave–particle dualism (cf de Broglie wavelength)

- typical electron energy: 100–300keV (200keV = 0.02508Å)

- suitable for, but also require small samples: 100keV: < 100nm, 200keV: < 300 – 500nm

- electrons interact with charge: map = electrostatic potential inside crystal

- strong interaction: multiple scattering events do occur

- require high vacuum throughout (10⁻⁸mbar)
The Lens System

- Lenses C1–C3 shape beam
- Crystallography: Parallel beam
- Objective lens: sets effective detector distance to back-focal plane = diffraction mode
- C3 not present in all microscopes

Lenses cause distortions.
2 - Motivation
“Failed” Crystallisation Drops viewed through TEM


“Our work […] shows that [nanocrystals] are commonly observed in crystallization drops […]”
Crystalline Disorder — a Matter of Size?

K Dalle, T Gruene, S Dechert, S Demeshko, and F Meyer, A weakly coupled biologically relevant \( \text{Cu}^{II}_{2} (\mu - \eta^1 : \eta^1 - \text{O}_2) \) cis-peroxo adduct that binds side-on to additional metal ions JACS (2014), 136, 462–46
Nanocrystals

Novartis I:
Ø = 1,700nm = 1.7µm

Novartis II:
Ø = 500nm = 0.5µm

Thermolysin:
≈ 2 × 1 × very thin µm³
Solvent reduces contrast
3 - Electron Diffraction Instrumentation
Medipix / Timepix Detector Family

- first hybrid pixel detector for electrons (cf. Pilatus / Eiger)
- no read–out noise
- high dynamic range
- fast read–out: non–stop sample rotation ("shutterless data collection")
- 512x512 and 1024x1024 pixel cameras installed in Basel (and Pisa (Prof. Mauro Gemmi) and Stockholm (Prof. Sven Hovmöller))

Diffraction image from a MFI type zeolite:
black = 0 counts
red ≥ 1 (carbon scatter + crystal signal) count
Eiger Chip

- Developed at PSI
- 256x256 pixel test chip with 200keV instrument
- pilot for improving phosphor to higher energies $\geq 300$ keV
- higher read–out (up to 8kHz), much lower dead time
- Next: Jungfrau and Mönch with Si, GaAs, or CdTe

Electron diffraction (from an inorganic compound) on a 256x256 Eiger chip
The Rotation Method

- Material Science: diffraction from oriented crystals
- Rotation Method: random orientation
- Standard ("Universal") data collection mode for organic and macromolecular crystallography
- First (?) applications in electron crystallography:
  - Prof. Ute Kolb, Mainz — AD3DT, step motion (≈ 2011)
  - Dr. Wei Wan, Stockholm — RED, beam precession + sample rotation (≈ 2013)
  - Prof. Jan Pieter Abrahams — first diffraction pattern from 3D protein crystals (2011)
  - Dr. Tamir Gonen — first 3D crystal structure in PDB (3J4G, 2013)
- Currently: No connection between goniometer and detector: "manual" rotation leads to very inaccurate oscillation width
- Benefit from well advance integration/ scaling programs (XDS, DIALS, SAINT, evalCCD)
4 - Example Structures
Pharmaceutical I: Visualisation of Hydrogen Atoms

H–atom positions can be refined against electron diffraction data

- Field of view: $3\mu m$
- Crystal: $1.6\mu m \times 400nm$
- $d_{\text{min}} < 0.8\AA$
- $I/\sigma_I(0.91 - 0.81\AA) : 1.8$
- $P2_12_12_1$: 85% completeness
  with 3 crystals
- a=8.06Å b=10.00Å c=17.73Å
- Refinement of hydrogen atom positions
  with mild restraints (SADI)
- 1334 reflections, 195 parameters, 156 restraints (RIGU)
- $R1 = 15.5\%, R_{\text{complete}} = 18.5\%$
Pharmaceutical II: Differentiation of Atom Types

Data quality: recognition of atom types, C vs. O vs. N etc. (CCDC: EPICZA)

- Field of view: 3µm
- Crystal: 400nm diameter
- $d_{\text{min}} = 0.80\text{Å}$
- $I/\sigma_I(0.90 - 0.80\text{Å}) : 2.5$
- $P2_12_12_1$: 92% completeness with 6 crystals ($d_{\text{min}} > 0.84\text{Å}$ : 96%)
- Direct methods: only 1 wrong atom type
- Visualisation of hydrogen atoms
- 1806 refl., 258 param., 267 restraints
- $R_1 = 18.5\%, R_{\text{complete}} = 21.9\%$
Thermolysin (sample courtesy Ilme Schlichting)

- Spacegroup $P6_122$
- Unit Cell 94.3 94.3 130.4 $90^\circ 90^\circ 120^\circ$
- $d_{\text{min}} = 3.5\text{Å}$
- 72.4% completeness
- MR with 3DNZ poly Alanine: TFZ=26.4, LLG=433
- Buccaneer: side chain extension 315/316
- Refmac5: $R_1/\text{Rfree} = 28.0\% / 29.9\%$ (4N5P w/o water)
Lysozyme

1. MR (Phaser) from poly Ala **monomer** determines space group $P2_12_12$ (TFZ=19.8, LLG=335.3)
2. Side chain completion with Buccaneer all except 27 atoms
3. Refinement with refmac5
5 - Electron Crystallography in CCP4

1. Data processing: DIALS (with D. Waterman)

2. Scaling: Aimless

3. MR: Phaser / Molrep

4. Autobuilding: Buccaneer

5. Refinement: Refmac5
   - SOURCE ELECTRON MB
   - MAPC FREE EXLCUDE

6. Model Building: Coot
## 6 - Summary: Electron Crystallography for non–Material Scientists

<table>
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<tr>
<th>Sample Prep</th>
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<th>Processing</th>
<th>Analysis</th>
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<td>++ Detector*</td>
<td>+ Integration</td>
<td>++ Direct Methods</td>
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<td>- Rot^n Axis*</td>
<td>- Param. Stability</td>
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<tr>
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<td>- Lenses</td>
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<tr>
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<td>- Crystal Orient^{n*}</td>
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<td>- Potential Repr.</td>
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* Current project at LBR / PSI
7 - Acknowledgements

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