Macromolecular Crystallography with Electron Diffraction

PSI MX Journal Club — 12th September 2016
1 - Nanocrystals
The (seemingly) Empty Drop

Drops viewed through TEM

How small is “nano”?

Typical protein crystal size for X-rays: 0.2 mm

Typical protein crystal size for electrons: 200 nm

Volumes compare like 6 bath tubs of water vs. 10 μl.
2 - How to react to Radiation Damage
Radiation Damage limits Diffraction

- Henderson / Garman limit: 20–50 MGy = 20–50 MJ/kg before half intensity is lost

- $m \propto V$: nanocrystal can take $10^{-9}$ photons compared to macrocrystal

- Same resolution requires same dose (number of counts on detector)
Means to overcome Radiation Damage

1. More sensitive detectors: hybrid pixel detectors like Pilatus close to ideal (single count reflections)

2. “Measure before destroy”: merge data from few to many individual crystals
   - manually since 1980’s: room temperature data, virus data
   - automated: high intensity free electron lasers, also minimises noise

3. Use electrons instead of X–rays and gain a factor of 1,000 in signal vs. damage
X–ray Interaction with Matter

Interaction of X–rays at 12keV with 100\(\mu m\) soft tissue

- A Transmission 96.6%
- B Photo absorption 3.0%
- C Elastic Scattering 0.2%
- D Compton Scattering 0.2%

Red: Radiation damage Green: Diffraction

Every diffracting photon is accompanied by 16 damaging photons

X–rays Scattering and Electron Scattering

- Probability of inelastic scattering: $10^{-4}$
- Deposited energy: 10keV
- Probability of elastic scattering: $10^{-5} = 10^{-4}/10$
- Damaged per diffracted photon: 100keV

X–rays Scattering and Electron Scattering

- Probability of inelastic scattering: $10^{-4}$
- Deposited energy: 10keV
- Probability of elastic scattering: $10^{-5} = 10^{-4}/10$
- Damage per diffracted photon: 100keV

- Probability of inelastic scattering: 30%
- Deposited energy: 20eV
- Probability of elastic scattering: 10%
- Damage per diffracted electron: 60eV = 0.06keV

2,000 times more damage with X–rays

Other Features of Electrons Scattering

- Small crystals may show less defects
- Hydrogen much better visible
- Map corresponds to electrostatic potential
- Powder consist of single nanocrystals

Sample courtesy Novartis, CSD code IRELOH
Electrons — Cure for Radiation Damage?

1. Limits in sample thickness (200keV electrons: 100% absorption for samples $>1\mu m$, i.e. no signal)

2. Nanocrystals difficult to detect

3. 500nm = 50*100Å: “countable” number of unit cells

4. Cumbersome sample handling

5. No anomalous signal: phasing; chirality

6. Dynamic and multiple scattering obstruct refinement

7. Instruments not made for diffraction
3 - Electron Diffraction
N.B.: 2D and 3D Crystals

The 2012 edition of International Tables F consider “Electron diffraction of protein crystals” (Chapter 19.2) only of 2D crystals.

Here, electron crystallography is the same as X–ray or neutron crystallography:

We measure the diffraction of a 3D crystal, i.e. a solid with periodic repeats in three dimensions, as result of an incoming planar wave.
Structure Determination by Single Crystal Diffraction

- Radiation: X-ray, neutrons, or electrons
- Diffraction spots: interaction between wave and crystal
- Position governed by Laue conditions \((\vec{S}_o - \vec{S}_i) \cdot \vec{a} = h \land (\vec{S}_o - \vec{S}_i) \cdot \vec{b} = k \land (\vec{S}_o - \vec{S}_i) \cdot \vec{c} = l\)
- Experimental result: Position and Intensity for each spot
Electrons: Multiple Scattering

- electron interact strongly with matter = high scattering probability

- nice, as it enables small crystal volumes

- effects that multiple scattering must be taken into account \( I \propto |F|^2 \)
Multiple (Dual) Scattering

- Outgoing ray $\vec{S}_1^o$ acts as incoming ray for reflection $\vec{S}_2^o$.
- Re-reflection with 10% probability at 50 nm path length.
Multiple (Dual) Scattering

Laue Conditions (accordingly $\bar{b}$ and $\bar{c}$):

\[
\begin{align*}
(\vec{S}_o^1 - \vec{S}_i) \cdot \vec{a} &= h_1 \\
(\vec{S}_o^2 - \vec{S}_o^1) \cdot \vec{a} &= h'
\end{align*}
\]

\[
(\vec{S}_o^2 - \vec{S}_i) \cdot \vec{a} = h_1 + h'
\]

Simplest approximation:

\[
I_{\text{exp}}(h_2k_2l_2) \propto |F_{\text{ideal}}(h_2k_2l_2) + \alpha F_{\text{ideal}}(h_1k_1l_1)|^2
\]

- $\alpha$: re-scattering of $S_o^1$
- $F(h_1k_1l_1)$ must be strong
- $F(h',k',l')$ must be strong
- $F(h_2k_2l_2)$ must be weak
- affects high resolution data

Protein crystallographers are used to enormous errors: multiple scattering may just add a bit.
4 - Electron Diffraction Structure of Hen Egg Lysozyme
The Crystal and Diffraction Experiments

Lysozyme nanocrystals measured at NeCEN (Netherlands), FEI Titan Krios microscope with Timepix camera

- 0.1615°/frame fast scan
- 0.048° / frame slow scan
- 11 $e^{-}/\text{Å}^2$ dose
- 10Hz read–out
- $\lambda = 0.02508\text{Å}$
- 2.078m (!) detector distance
- 40° largest wedge, mostly 20°
- few spots to 2.2Å (inset)
Data Processing with XDS

- “spots are spots”: XDS integrates electron diffraction data
- XDS feature per-pixel spatial corrections: covers a few lens distortions
- *caveat*: unset “REFLECTING_RANGE” refines to 20–40°
- default corrections model most likely inappropriate
- weak spots? determine background “along” pixel
Incomplete Data: Merging Data from six Crystals

<table>
<thead>
<tr>
<th>Data integration</th>
<th>Single crystal</th>
<th>Merged data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P2₁2₁2</td>
<td></td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, b, c (Å)</td>
<td>104.56, 68.05, 32.05</td>
<td>66.49Å</td>
</tr>
<tr>
<td>α, β, γ (°)</td>
<td>90.0, 90.0, 90.0</td>
<td></td>
</tr>
<tr>
<td>Number of crystals</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Resolution (Å)¹</td>
<td>32.05-2.50 (2.57-2.50)</td>
<td>57.04-2.50 (2.57-2.50)</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>31.7 (107.3)</td>
<td>35.7 (113.2)</td>
</tr>
<tr>
<td>I/σI</td>
<td>2.92 (1.10)</td>
<td>2.87 (1.10)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>0</td>
<td>69.0 (51.3)</td>
</tr>
<tr>
<td>Reflections</td>
<td>9518 (817)</td>
<td>25148 (1373)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>3445 (236)</td>
<td>5808 (299)</td>
</tr>
<tr>
<td>Redundancy</td>
<td>2.76 (3.46)</td>
<td>4.33 (4.59)</td>
</tr>
</tbody>
</table>

Refinement

| R1 (%)           | 25.90 | 23.54 |
| Rmerge (%)       | 32.49 | 27.21 |
| <B> (Å²)         | 33.08 | 36.49 |
| RmsZ bonds       | 0.779 | 0.765 |
| RmsZ angles      | 0.974 | 0.911 |

PDB–ID 4R0F: P2₁2₁2 with 104.63Å 66.49Å 31.65Å

¹ Resolution for merged dataset.
Structure Solution

1. Molecular Replacement from poly Ala monomer with Phaser uniquely determines space group $P2_12_12_1$ (TFZ=19.8, LLG=335.3)

2. Side chain completion with Buccaneer all except 27 atoms

3. Refinement with refmac5
   - "source electron MB" scattering factors for electrons
   - "mapc free exclude" do not estimate missing reflections: avoid model bias at low completeness

MR solution shows difference density for bulky side chains

Refined map guides model completion
5 - Instruments for Electron Diffraction
Electron Microscopes

- Field emission gun: 100–300keV electrons
- 200keV: $\lambda = 0.02508\text{Å}$
- 2–3 lenses above sample: parallel, narrow beam
- $\geq 1$ lens below sample: effective detector distance
- New: hybrid pixel detector (Timepix, Eiger, Jungfrau...)

(Wikipedia)
Electron Microscope: Imaging Mode

Plane Wave  Object  Diffraction Lens  Image Plane (Detector)
Rays of equal origin focus on detector
Electron Microscope: Imaging Mode

Detector noise and radiation sensitivity require low contrast images.


Plane Wave  Object  Diffraction Lens  Image Plane (Detector)
Electron Microscope: Diffraction Mode

Plane Wave  Object  Diffraction Lens
Electron Microscope: Diffraction Mode

- Plane Wave
- Object
- Diffraction Lens

Image at *Back focal Plane* = ∥Fourier transform of object∥

**For crystals:**

diffraction spots according to Laue conditions

Rays of *equal direction* focus on detector
Summary

- Electron diffraction suitable for structural biology

- Ideal for 100–500nm crystal thickness

- Integration, Solution, Refinement: Methods and programs fit for electrons

- Our results direct for hardware improvements:
  - Goniometer precision and accuracy
  - Lens system optimisation for diffraction
  - Hybrid pixel detectors for 300–1,000 keV electrons
6 - Acknowledgements

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