CCP4 Diamond 2014

Twinning

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Twin: Two or more crystals of the same species are joined together in different orientation

$\alpha$ is the twin fraction:

$I_{1+2} = \alpha I_1 + (1-\alpha)I_2$

The twin law (twin operator) is the operator between the cojoined crystals – an additional symmetry operation.
Identifying the twinning type:

| Non-merohedral twinning | Merohedral twinning | Pseudo-merohedral twinning |

Finding the twin law and the twin fraction $\alpha$

Treat data accordingly
Twin law: Symmetry operator of the crystal system, but not the crystal’s point group
Merohedral twinning

Twin law: Symmetry operator of the crystal system, but not the crystal’s point group
Depending on the twin fraction $\alpha$: The **intensity distribution** has been changed by the twinning. Also, symmetry looks higher!
Merohedral twinning

How to recognize?

- Lower symmetry point group of the trigonal, hexagonal, tetragonal or cubic system
- **Symmetry looks possibly higher than it really is**
- Changed intensity distribution
- $R_{\text{merge}}$ for the higher symmetry
- Typical space group
- No structure solution
Merohedral twinning

Space groups
• Only trigonal, hexagonal and tetragonal space groups
• There is only a limited number of potential twin laws.
• Typical examples:

<table>
<thead>
<tr>
<th>Is:</th>
<th>Looks like:</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4₁</td>
<td>P4₁22 or P4₁₂₂</td>
</tr>
<tr>
<td>P3₁</td>
<td>P₃₁₁₂ or P₃₁₂₁ or P₆₄ or P₆₂</td>
</tr>
</tbody>
</table>

• Merohedral twinning occurs more frequently than commonly recognized!
Is it really a merohedral twin?
How big is the twin fraction \( \alpha \)?

Two intensities \( I_1 \) and \( I_2 \) are related by twin law:

\[
H = \frac{|I_1 - I_2|}{I_1 + I_2}
\]

(For acentric reflections:)

Cumulative probability distribution

\[
N(H) = \begin{cases} 
0 & \text{H<0} \\
\frac{H}{1-2\alpha} & 0 \leq H \leq 1-2\alpha \\
1 & \text{H>1-2\alpha}
\end{cases}
\]

Note: When there is rotation parallel to twinning axis then the distribution will be different.
Merohedral twinning

\[ \alpha = 0.4 \]
\[ \alpha = 0.3 \]
\[ \alpha = 0.2 \]
\[ \alpha = 0.1 \]
\[ \alpha = 0.0 \]

\[ N(H) \]

H
Merohedral twinning

Drawbacks

Perfect twins are not detectable with this method.

\[
H = \frac{|I_1 - I_2|}{I_1 + I_2}
\]

\[I_1 = I_2 \Rightarrow H = 0\]

We need another test!
Merohedral twinning

Yeates-Padilla Test

The reflections with the intensities $I_A$ and $I_B$ are close to each other in reciprocal space:

$$L = \frac{|I_A - I_B|}{I_A + I_B}$$

$$N(L) = \begin{cases} 
0 & \text{L<0} \\
\frac{1}{(1-2\alpha)^2} (\alpha^2 + (1-\alpha)^2 - \frac{8\alpha^2 (1-\alpha)^2}{1-(1-2\alpha)^2 L^2})L & \text{0 \leq L \leq 1} \\
1 & \text{L>1}
\end{cases}$$

When $\alpha=0.5$:

$$N(L) = \begin{cases} 
0 & \text{L<0} \\
\frac{3}{2} (L - \frac{L^3}{3}) & \text{0 \leq L \leq 1} \\
1 & \text{L>1}
\end{cases}$$
Merohedral twinning

Yeates-Padilla Test

This test shows the expected cumulative distributions for perfect twins and untwinned data. Partial twins will be between the curves.

- **Red line**: acentric reflections, perfect twin
- **Yellow line**: acentric reflections, untwinned
- **Blue line**: Centric, untwinned
• Using the Yeates-Padilla test for the PDB shows that twinning occurs more frequently than commonly recognized!

• Both the H test as well as the Yeates-Padilla plot can be generated using POINTLESS, CTRUNCATE or DETWIN via CCP4i.

• The $<|E^2-1|>$ could be too low for twins (below 0.736).
Merohedral twinning
Merohedral twinning
Merohedral twinning

$R_{\text{merge}}$ in Mersacidin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>1.06</td>
</tr>
<tr>
<td>$\langle</td>
<td>E^2 - 1</td>
</tr>
<tr>
<td>$R_{\text{merge}}$ (%) P3$_2$</td>
<td>4.9</td>
</tr>
<tr>
<td>$R_{\text{merge}}$ (%) P3$_2$21</td>
<td>19.5</td>
</tr>
<tr>
<td>$R_{\text{merge}}$ (%) P3$_2$12</td>
<td>44.3</td>
</tr>
</tbody>
</table>

An additional hint could be different $R_{\text{merge}}$ for higher symmetry space group for different crystals of comparable quality.

**Merohedral twinning**

**How to integrate?**

In lower symmetry group with any integration program!

**Structure solution**

<table>
<thead>
<tr>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>O good</td>
</tr>
<tr>
<td>MAD, SAD</td>
<td>O ok</td>
</tr>
<tr>
<td>S-SAD</td>
<td>X too sensitive to noise</td>
</tr>
<tr>
<td>SIR, MIR</td>
<td>X several crystals needed</td>
</tr>
</tbody>
</table>
Merohedral twinning

Refinement

- Several protein refinement programs offer option for the refinement of merohedral twins, for example REFMAC, CNS, phenix.refine and SHELXL. In REFMAC, the twin law is determined automatically.

- Do not use a merohedral twin refinement on data which is not twinned. It will lower the R value possibly, but it is not a valid treatment!
Merohedral twinning

Problematic: R factors

• $R_{\text{free}}$ set should include all twin-related reflections.

• R factors may be lower than in single crystals. (Random R value goes down from 58.5% to 50%!)

• Difference density might have fewer features, as the twinned reflections add noise.
**Merohedral twinning**

<table>
<thead>
<tr>
<th>Job title</th>
<th>restrained refinement</th>
<th>using</th>
<th>no prior phase information</th>
<th>input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do</td>
<td>intensity based</td>
<td>twin refinement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>intensity based</td>
<td>Unassigned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amplitude based</td>
<td>intensity based</td>
<td>Unassigned</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDB in</td>
<td>baker</td>
<td>15lt.pdb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDB out</td>
<td>baker</td>
<td>15lt_refmac1.pdb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIB in</td>
<td>baker</td>
<td>Merge LIBINs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Output lib</td>
<td>baker</td>
<td>15lt.cif</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Twin law: Symmetry operator of the crystal system, but not the crystal’s point group

- Only in tetragonal, trigonal, hexagonal and cubic space groups possible.
- Exact overlap of reciprocal lattices, but different intensity distribution.
- Usually good results.
Reticular merohedry

Most common case: Obverse/reverse twinning in a rhombohedral crystal
Reticular merohedry

The typical case

<table>
<thead>
<tr>
<th>Is:</th>
<th>Looks like:</th>
</tr>
</thead>
<tbody>
<tr>
<td>R32</td>
<td>P3$_1$21</td>
</tr>
<tr>
<td>R3</td>
<td>P3$_1$</td>
</tr>
</tbody>
</table>

1/3 of all reflections are missing.
The missing reflections form a funny pattern, which is inconsistent with any systematic absence.
Pseudo-merohedral twinning

Twin law: Belongs to a higher crystal system than the structure.
Twin law: Belongs to a higher crystal system than the structure.
Pseudo-merohedral twinning is only possible if the real unit cell can be transformed into one of a higher crystal system.

Example:

- Monoclinic with \( a \approx c, \beta \approx 90^\circ \)

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>P2(_1)</td>
<td>P222(_1) or C222(_1)</td>
</tr>
</tbody>
</table>
Pseudo‐merohedral twinning

How to recognize?

• Like a merohedral twin. The real space group belongs to another crystal system than the observed one.

• The overlap of the lattices might not be perfect for all reflections.

• All hints for merohedral twinning might also work for pseudo-merohedral ones.

• $R_{\text{merge}}$ behaves like in merohedral twins.
Pseudo-merohedral twinning

How to treat?

• Treatment is very similar to the one for merohedral twins.

• Most programs that can process merohedral data will also process pseudo-merohedral one.

• Be careful to choose the right (lower symmetry) crystal system.
Twin law: Belongs to a higher crystal system than the structure.

• Recognition from frames or as for merohedral twins.
• Usually good results.
Non-merohedral twinning

Twin law is relatively arbitrary.
How to recognize?

• From detector frames
• By reciprocal lattice viewers: RLATT (*proprietary*) or RLAT4XDS (*available at* [http://www.cb-huebschle.de](http://www.cb-huebschle.de))
• No suitable cell for all reflections (many outliers)
• Cell refinement difficult
• An unusual long cell axis
• Some reflections sharp, others split

**Courtsey of**

Madhumati Sevvana
Example #1: Glucose Isomerase

- tripllett
- three separate centers
- diffraction pattern consists of
  - single reflections
  - overlapping spots
Example #1: Glucose Isomerase

Images courtesy of Madhumati Sevvana
Reflection types

- Non-overlapping reflections
- Partial overlap
- Complete overlap
Cell determination

• For indexing, leave out partial overlaps at first.
• Find a cell that fits a reasonable fraction of spots.
• Use the not-yet-indexed reflections to find an alternative orientation of the same cell; repeat as necessary. (FECKLESS will search for any new cell.)
• Something like this can be done with
  – XDS (use omitted reflections)
  – MOSFLM & FECKLESS (multi keyword in auto-indexing)
  – CELL_NOW (proprietary)
Example #2: Bovine insulin

- Cubic (I2\textsubscript{1}3)
- 51 amino acids
- resolution to 1.60 Å
- interpenetrant twins with approximately the same center

Images courtesy of Madhumati Sevvana
Non-merohedral twinning

**CELL_NOW: Determining the twin law and cell**

Cell for domain 1: 78.040 77.986 78.024 89.99 89.94 90.01
Figure of merit: 0.560  %(0.1): 51.6  %(0.2): 55.2  %(0.3): 62.6

4072 reflections within 0.250 of an integer index assigned to domain 1

Cell for domain 2: 78.040 77.986 78.024 89.99 89.94 90.01
Figure of merit: 0.910  %(0.1): 91.4  %(0.2): 93.6  %(0.3): 94.5

Rotated from first domain by 89.2 degrees about reciprocal axis
0.928 0.207 1.000 and real axis 0.927 0.208 1.000
Twin law to convert hkl from first to this domain (SHELXL TWIN matrix):
0.459 -0.625 0.631
0.824 0.036 -0.565
0.330 0.780 0.532

3564 reflections within 0.250 of an integer index assigned to domain 2,
2751 of them exclusively; 184 reflections not yet assigned to a domain
Non-merohedral twinning

How to integrate?

- Few programs can handle non-merohedral twins:
  - SAINT (Bruker)
  - EVALCCD (free)
  - MOSFLM (free)

- Special file format to hold two domains needed (e.g. HKLF5) or intensities have to be merged.
Non-merohedral twinning

How to integrate?

- Ignoring the twinning: Poor results, if any.
- Omission of all overlaps: Low completeness.
- Omission of all partial overlaps
- Integrating overlaps without deconvolution
- Integrating both domains by profile fitting the overlapping reflections: Good, but complicated.
How to integrate?

- non-overlapping reflections
- partial overlap
- complete overlap
Partially overlapping reflections

- The total intensity of a group of overlapping reflections can be determined precisely!
- Partitioning by 3D profile fit possible.
- Special file format to hold two domains, multiple files, or intensities have to be merged.
Scaling and absorption correction

In TWINABS:

The smallest crystal (red) was furthest from the center.
Non-merohedral twinning

- Twin law relatively arbitrary, often a two-fold rotation
- Recognition from frames or reciprocal lattice
- No exact overlap of reciprocal lattices.
- Cell determination, integration, scaling and refinement can be difficult.
- Detwinning is possible, and this data can be used for structure solution
- If feasible: Try irradiating only a part of the crystal, or get another crystal.
Split crystal

This is NOT a twin!
Split crystal

This is NOT a twin!

- Twin law near to unity
- Indexing gets better with box being bigger
- Bad data quality
- Split crystals give only reasonable data if they have a limited number of domains
Summary

There are several types of twins: Non-merohedral, merohedral and pseudo-merohedral twins.

Twins cannot be detected without prior suspicion.

(Not every data that cannot be solved or properly refined is twinned.)

There are warning signals for twinning in the frames and in the intensity distribution, but not all have to occur.

If the data quality is sufficient, the structure can be elucidated. Don‘t throw your only crystal away just because it‘s twinned!
• Bernhard Rupp, *Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology*, 2004

• **Yeates Server:**
  http://nihserver.mbi.ucla.edu/Twinning


  **Slides:** shelx.uni-ac.gwdg.de/~athorn/
Macroscopic twins

Can you identify the types of twinning?
I am grateful to George Sheldrick and Regine Herbst-Irmer, who freely shared with me their knowledge, ideas and material. The non-merohedral protein data and pictures were taken by Madhumati Sevvana. Additional info from Harry Powell. I also want to thank the Murshudov lab for all the support!