OVERVIEW

Introduction:
- Definitions, origins of twinning

Merohedral twins:
- Recognition, statistical analysis: H plot, Yeates-Padilla plot
- Example mersacidin
- Refinement and R values
- Reticular merohedry

Pseudo-merohedral twins:
- Recognition and treatment

Non-merohedral twins:
- Recognition
- Cell search and integration
- Example bovine insulin

Not a twin!

Summary and literature
Is this a twin?
Definition of twinning

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

\[ \alpha \text{ is the twin fraction: } \]
\[ I_{1+2} = \alpha I_1 + (1-\alpha)I_2 \]

The **twin law** (twin operator) is the operator between the cojoined crystals.
Definition of twinning

Twins result from lattice defects or nuclei intergrowth
Definition of twinning

Macroscopic twin
Desecting a part might give a single crystal!

Microscopic twin
Crystal looks fine, but is twinned.

Images property of Andrea Thorn and Claudia Egerer-Sieber
## Definition of twinning

### Identifying the twinning type:

<table>
<thead>
<tr>
<th>Non-merohedral twinning</th>
<th>Merohedral twinning</th>
<th>Pseudo-merohedral twinning</th>
</tr>
</thead>
</table>

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

### Treat data accordingly
Merohedral twinning

Twin law: Symmetry operator of the crystal system, but not the crystal’s point group

Reciprocal lattice

Crystal lattice
Merohedral twinning

Twin law: Symmetry operator of the crystal system, but not the crystal’s point group
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
- $R_{\text{int}}$ for the higher Laue groups
- Typical space group
- No structure solution
Depending on the twin fraction $\alpha$: The **intensity distribution** has been changed by the twinning. Also, symmetry looks higher!
Merohedral twinning

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

$$N(H) = \frac{1}{2} \left[ 1 + \frac{H}{1 - 2\alpha} \right]$$
Merohedral twinning

Drawbacks:

• **Twin law has to be known**, so the correct related intensities can be compared!

• **Perfect twins are not detectable** with this method.

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

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

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:

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

$$N(L) = \frac{1}{2} \left[ 1 + \frac{L}{1 - 2\alpha} \right]$$
Yeates-Padilla Test

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

- Red: acentric reflections, perfect twin
- Yellow: acentric reflections, untwinned
- Blue: Centric, untwinned
Merohedral twinning

• 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 CTRUNCATE or DETWIN via CCP4i.

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

$R_{\text{merge}}$

**Example Mersacidin**

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

Different $R_{\text{merge}}$ for higher Laue for different crystals

Merohedral twinning

Typical space groups

- Trigonal, hexagonal and tetragonal space groups, e.g.

<table>
<thead>
<tr>
<th>Is:</th>
<th>Looks like:</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4₁</td>
<td>P4₁22 or P4₁2₁2</td>
</tr>
<tr>
<td>P3₁</td>
<td>P3₁12 or P3₁2₁ or P6₄ or P6₂</td>
</tr>
</tbody>
</table>
Merohedral twinning

How to integrate?
In lower Laue group with any integration program!

Structure solution

<table>
<thead>
<tr>
<th>Method</th>
<th>Status</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>O good</td>
<td>Sometimes, detwinning the data helps here!</td>
</tr>
<tr>
<td>MAD, SAD</td>
<td>O ok</td>
<td>SHELXD can use twin laws</td>
</tr>
<tr>
<td>S-SAD</td>
<td>X too sensitive to noise</td>
<td></td>
</tr>
<tr>
<td>SIR, MIR</td>
<td>X several crystals needed</td>
<td></td>
</tr>
</tbody>
</table>
Merohedral twinning

How to refine?

Several protein refinement programs offer option for the refinement of merohedral twins, for example REFMAC (decisions automatic, see tutorial), CNS, phenix.refine and SHELXL.

In REFMAC, even the twin law is determined automatically!

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

![Image of software interface with focused options]

#### Run Refmac5

- **Job title**:
  - restrained refinement
  - using no prior phase information
  - input

- **Input fixed TLS parameters**: no

- **intensity based twin refinement**: baker_8.1.mtz

- **amplitude based twin refinement**: baker_8.1_refmac1.mtz

- **PDB in**: baker 15lt.pdb

- **PDB out**: baker 15lt_refmac1.pdb

- **LIB in**: baker

- **Output lib**: baker 15lt.cif

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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 40%!)

Beware

• 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!
• Do not use detwinned data for refinement!
Merohedral twinning

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
• Correct space group determination and phasing more difficult
Most common case: Obverse/reverse twinning in a rhombohedral crystal
Most common case: Obverse/reverse twinning in a rhombohedral crystal
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.
Pseudo-merohedral twinning

Crystal lattice
Pseudo-merohedral twinning

How to recognize?

• Like a merohedral twin, but 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

Typical space groups

• Monoclinic space group $\text{P2}_1$

<table>
<thead>
<tr>
<th>Is:</th>
<th>Looks like:</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{P2}_1$</td>
<td>$\text{P222}_1$ or $\text{C222}_1$</td>
</tr>
</tbody>
</table>
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) crystal system.
Non-merohedral twinning

Twin law is arbitrary.

Reciprocal lattice

Crystal lattice
Non-merohedral twinning

Twin law is arbitrary.
Non-merohedral twinning
Non-merohedral twinning

How to recognize?

- From detector frames and reciprocal lattice viewer!
- No suitable cell for all reflections (many outliers!)
- Cell refinement difficult
- Unusual long cell axis
- Some reflections sharp, others split
Cell determination

- Search for one cell and then search the not-yet-indexed reflections for the same cell in another orientation.
- This can be done with cell_now, XDS (use omitted reflections)
- Good for viewing of the reciprocal lattice: RLATT (proprietary) or RLAT4XDS (available at http://www.cb-huebschle.de)
Non-merohedral twinning

- Split reflections
- Complete overlap
Non-merohedral twinning

- Split reflections
- Partial overlap
- Complete overlap
Non-merohedral twinning

How to integrate?

• Ignoring the twinning: *No special software needed, but poor results (if any).*

• Omission of all overlapped reflections: *Many integration programs possible, but low completeness of the data.*

• Omission of all partially overlapped reflections

• Use of special software to integrate both domains: *Good, but complicated...*
Non-merohedral twinning

How to integrate?

- Few programs can handle non-merohedral twins:
  - SAINT (Bruker)
  - EVALCCD (free)
- Special file format to hold two domains needed (e.g. HKLF5) or intensities have to be merged.
Non-merohedral twinning

- Twin law arbitrary
- Recognition from frames or reciprocal lattice
- No exact overlap of reciprocal lattices possible.
- Problematic: Cell determination and refinement
- Integration, scaling and merging difficult
- Detwinning is possible, and this data can be used for refinement
- Slightly better results with original data, but only possible in SHELXL
Non-merohedral twinning

Example: Bovine insulin

• 51 amino acids
• Resolution to 1.60 Å
• Cubic (I2₁3)
Non-merohedral twinning

RLATT

Image property of Madhumati Sevvana
Non-merohedral twinning

RLATT
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
Split crystal

This is NOT a twin!

Reciprocal lattice

Crystal lattice
Split crystal

This is NOT a twin!

- Twin law near to unity
- Indexing gets better with box being bigger
- Bad data quality
Summary

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

Twins cannot be detected without prior suspicion.
Not every data set that cannot be solved or properly refined is twinned.

There are warning signals for twinning in the frames and in the intensity distribution. Software tools can help identify the twin law. Not all warning signals 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!**
Literature

• Bernhard Rupp, *Biomolecular Crystallography*: Principles, Practice, and Application to Structural Biology, 2004

• Yeates Server: [http://nihserver.mbi.ucla.edu/Twinning](http://nihserver.mbi.ucla.edu/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.

I also want to thank the Sheldrick group for all the support!