# A Narrow Escape from Merohedral Twinning

**Mark L. Mayer**

**National Institute of Child Health and Human Development**

Merohedral twinning is a special form of disorder that most crystallographers will be forced to deal with at some point in their career. It is different from twinning which arises when crystals fuse during growth, which is easily recognized either in the light microscope, or from diffraction images which reveal the presence of more than one lattice. In the case of merohedral twinning, the crystal contains microdomains in which the same lattice is present but in different orientations related by a twinning operator. As a result, the observed intensities are not accurate, in the sense that they arise from the sum of the unrelated intensities of the twin components. Because the intensities do not correspond to those generated by a single lattice, refinement stalls at unreasonably high R values, and in some cases the structure cannot be solved at all.

Obviously it is important to recognize when merohedral twinning has occurred before wasting time struggling with a stalled refinement or worse, and as a routine practice diffraction data sets should be checked for merohedral twinning before proceeding with structure determination and refinement. The output from the CCP4 program TRUNCATE contains plots of the cumulative intensity distribution, and the moments of the intensities, and gives values expected for untwinned and perfectly twinned (twin fraction 0.5) extremes. If your data passes these tests, which is the most common situation, all is well. The situation becomes more complex when twinning is indicated, since its extent can vary over the fractional range from $> 0$ to $\leq 0.5$.

A very useful program, which my lab now brings to APS on a laptop, is phenix.xtriage, part of the Phenix CCI Apps bundle available from http://www.phenix-online.org/download/cci_apps/. This program will read a .sca or .mtz file, perform a series of data quality checks, and can be run from the command line with just the data file name as input. The output is well organized, easy to read, and tells you almost everything you need to know about your data set, including strength of anomalous signal, checks for translational symmetry, an estimate of number of molecules in the asymmetric unit, and multiple tests for twinning, including checks for higher metric symmetry to exclude the possibility that the data was incorrectly scaled in the wrong space group. When twinning is indicated, the program gives the twin operator, and estimates the twin fraction using both classical methods, and with a maximum likelihood algorithm.

In a recent case in my lab, we recorded a data set for a crystal complex with a new ligand using a home source, and found that it was twinned in the space group H3. Never having dealt with twinned data before we were a bit anxious and unsure how to proceed. We took two approaches, the 1$^{st}$ was to try and work with data we had by (1) detwinning the data using the CCP4 program DETWIN, an approach that was not likely to give much benefit in this case because the twin fraction was close to 0.5, which interferes with separating diffraction by the twinned lattices; and by (2) refining against the twinned data

set, which is possible with phenix.refine or SHELX. Perhaps not surprisingly due to the high twin fraction and the relatively low diffraction limit of the data, refining against the twinned data set with phenix.refine failed to improve the maps to the extent where we were able to observe the bound ligand, a decahydroisoquinoline inhibitor of GluR5 subtype kainate receptors,

Because we had time at APS our 2nd approach was to collect data from additional crystals, with the hope that with a higher resolution data set, our maps would improve to the extent where we could build the small molecule inhibitor, and refine the twinned data set. We collected data sets from multiple crystals, and as soon as scaling was complete ran phenix.xtriage. To our surprise we found that the twin fraction varied from crystal to crystal. The 4th crystal tested was untwinned, diffracted to 1.58 Å, and has been refined to values for Rwork and Rfree of 0.158 and 0. 199 with excellent density for the ligand, including alternative conformations due to ring puckering.

Portions of the logfiles for phenix.xtriage for crystals 1 and 4 follow and illustrate how nicely the results of key tests are summarized:

```
############################################################
##                      phenix.xtriage                    ##
##                                                        ##
##     P.H. Zwart, R.W. Grosse-Kunstleve & P.D. Adams     ##
##                                                        ##
############################################################

Symmetry, cell and reflection file content summary

Miller array info: R3.sca:i_obs,sigma
Observation type: xray.amplitude
Unit cell: (89.221, 89.221, 330.046, 90, 90, 120)
Space group: R 3 :H (No. 146)
Resolution range: 38.3718 2.26093
Completeness in resolution range: 0.981245
Completeness with d_max=infinity: 0.981031

Statistics depending on twin laws
-------------------------------------------------------------------
| Operator  | type | R obs. | Britton alpha | H alpha | ML alpha |
-------------------------------------------------------------------
| -h-k,k,-l |   M  | 0.038  | 0.452         | 0.468   | 0.478    |
-------------------------------------------------------------------

############################################################
##                      phenix.xtriage                    ##
##                                                        ##
##     P.H. Zwart, R.W. Grosse-Kunstleve & P.D. Adams     ##
##                                                        ##
############################################################

Symmetry, cell and reflection file content summary

Miller array info: 42C4a.sca:i_obs,sigma
Observation type: xray.amplitude
Unit cell: (89.241, 89.241, 330.365, 90, 90, 120)
Space group: R 3 :H (No. 146)
Resolution range: 38.3808 1.58067
```

```
Completeness in resolution range: 0.969985
Completeness with d_max=infinity: 0.969912


Statistics depending on twin laws
-------------------------------------------------------------------
| Operator  | type | R obs. | Britton alpha | H alpha | ML alpha |
-------------------------------------------------------------------
| h,-h-k,-l |   M  | 0.476  | 0.015         | 0.037   | 0.022    |
-------------------------------------------------------------------
```

After returning from APS subsequent posts to the CCP4BB elicited a discussion the summary of which was that (1) it is not uncommon for the extent of twinning to vary from crystal to crystal within the same drop, and even within a single crystal, exposure of different areas to X-rays can reveal different twin fractions. (2) It is not necessary to collect complete data sets before running tests for twinning, thus speeding the triage process during synchrotron trips. (3) Except in the case of perfect merohedral twinning, refinement against a twinned data set using either phenix.refine or SHELX is quite easy to perform, and it is probably better to do this than to refine against a detwinned data set.

Of course, for the experienced crystallographer there is nothing new here. Servers to test for twinning have been available for years, but the extreme ease of use of phenix.xtriage, and the range of data checks it performs automatically depending on whether anomalous pairs have been recorded or not, makes it a useful tool to have around during data collection, especially when Zbigniew Dauter is not close by! The lessons I learned from this episode are that dealing with merohedral twinning is not as intimidating as it 1$^{st}$ appeared, and that screening crystals can sometimes avoid it altogether.