Supplementary Notes

Diagnosing merohedral twinning in RNA or DNA crystals and dealing with it.

The standard methods of detecting twinning can fail in cases of structures with pseudo-translations because the effect of a translation, close to the fraction of the unit cell vector on the diffraction pattern is opposite to the effect of twinning. Pseudo-translations increase the fraction of extremely weak and extremely strong intensities, whereas merohedral twinning causes a decrease in intensity variation. In such cases, the statistical analysis of intensities may be insufficient to rule out the possibility of twinning. The only applicable method then is the Padilla & Yeates L-test (1), comparing pairs of intensities within parity sub–classes of reflections (provided that the twin fraction is significantly different from 0.5). Therefore, if the electron density cannot be interpreted unambiguously it is worth considering if a twinning operator exists that might possibly explain the apparent disorder. The program PHENIX (2) can be used to indicate, based on the X-ray diffraction data, the possible twin operators and the twin fraction, the correct space group and NCS symmetry operators.

If the twin fraction is significantly different from 0.5, the X-ray data can be "detwinned" routinely and refinement can proceed normally against the detwinned data. When the twin fraction is 0.5, it is impossible to separate the intensities algebraically but it is still possible to refine an unambiguous model against such data. Programs SHELXL (3), PHENIX (2) or Refmac5 (4) can be used. The main limitation of refining against twinned data with twin fraction 0.5 is that meaningful omit maps cannot be calculated.

Even if twinning cannot be detected statistically, due to pseudo-translational effects, the advantage of detwinning should be evident in the electron density maps, which can be interpreted in terms of an unambiguous model and include details, such as solvent molecules.

Detwinning the [(CUG)$_6$]$_2$ diffraction data deposited (pdb code 1zev) by Mooers et al. (5)

The routine analysis based on the distribution of intensities of the diffraction pattern did not indicate merohedral twinning. However, an off-origin peak (68% of the origin peak height) was detected in the Patterson map, indicating the presence of pseudo-translational symmetry resulting from the periodic character of the helices (see Fig. 1 below). This is likely to mask the statistical effect of twinning on the intensity distribution. We therefore decided to test the hypothesis that the "static

disorder" or "merging of two lattices" reported by Mooers *et al.* (5) is in fact the result of twinning characterised by a 180º rotation about an axis perpendicular to the *c*-axis. The analysis of the structure factors indicated a twin fraction of 0.42 for the assumed twin operator (k h -l). In the course of subsequent refinement, the twin fraction turned out to be indistinguishable from 0.5, in which case it was impossible to detwin the data algebraically. Nevertheless, a unique model could still be refined against such data (see Fig. 2 below). The clarity of the map implies that despite the inherent uncertainties in structure factor amplitudes the phases can be relatively well determined during the refinement.
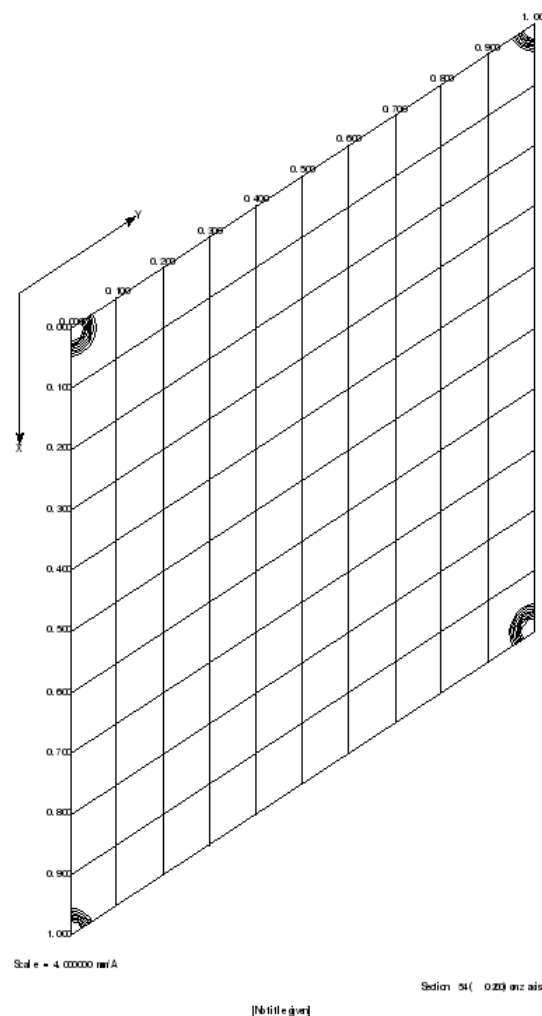

Figure 1. A section through a native Patterson map showing the strong (68% of the origin) peak (0.00, 0.00, 0.20). The peak is 28 Å from the origin along the z-direction and corresponds to the length of one helical twist (10.8 base pairs).
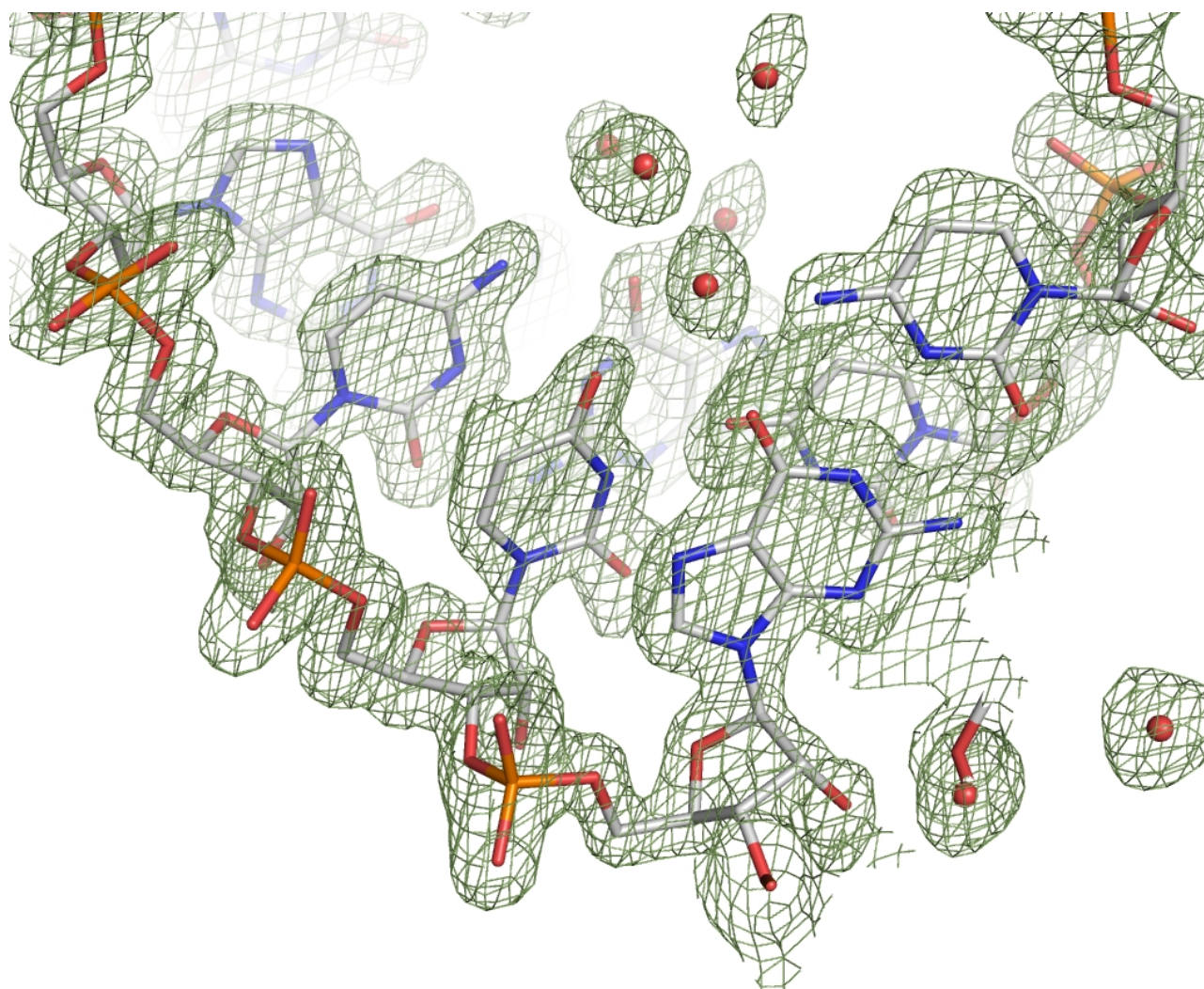
Figure 2. A portion of the $[(CUG)_6]_2$ model and the $2F_o$-F_c map, contoured at the 1σ level, after detwinning the X-ray diffraction data.

References:

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(5) Mooers,B.H., Logue,J.S. and Berglund,J.A. (2005) The structural basis of myotonic dystrophy from the crystal structure of CUG repeats. *Proc. Natl. Acad. Sci. U. S. A*, **102**, 16626-16631.