Pseudo-merohedral twinning. In addition to the cases of true merohedry described above, specific axis ratios can allow twinning, for example in addition in orthorhombic crystals with $a = b$ (appearing tetragonal) or in monoclinic crystals with $\beta \sim 90.0°$ (appearing orthorhombic). In such cases of pseudo-merohedral twinning the apparent higher symmetry implies that the unit cells will appear too small (too many symmetry related copies) to harbor a monomeric motif. When multiple molecules make up the motif, the distinction based on the Matthews probabilities (Chapter 11) is not so clear cut, because it might alternatively be possible that an oligomer axis coincides with a crystallographic axis.

Pseudo-symmetry. As a general rule, twinning analysis becomes more complicated when twinning coincides with translational NCS[40] or pseudo-symmetry. In the case of pseudo-translational symmetry, the intensity distribution tends toward bimodal because one subset of reflections becomes systematically enhanced and the other systematically weakened. The trend toward bimodal shape in turn makes the whole intensity distribution broader. This is essentially the opposite effect of merohedral twinning and manifests itself in the twinning analysis (Table 8-3) as *negative twinning*. The presence of a combination of compensating twinning and translational pseudo-symmetry can lead to near-normal intensity distribution statistics and obscure the detection of twinning.

M·Enz 1997

Intensity statistics for twinned crystals

We can define a statistic H that is a function of the twinning fraction α and which relates the measured diffraction intensities (or the square of the structure amplitudes) as follows:

$$H = \frac{|I_{obs}(\mathbf{h}_1) - I_{obs}(\mathbf{h}_2)|}{I_{obs}(\mathbf{h}_1) + I_{obs}(\mathbf{h}_2)} \qquad (8\text{-}2)$$

where $I_{obs}(\mathbf{h}_1)$ and $I_{obs}(\mathbf{h}_2)$ are the observed intensities resulting from a mixture of the true twin related reflections $I(\mathbf{h}_1)$ and $I(\mathbf{h}_2)$ in a hemihedral twinning case. With the corresponding fractions written as

$$I_{obs}(\mathbf{h}_1) = (1-\alpha)I(\mathbf{h}_1) + \alpha I(\mathbf{h}_2) \text{ and } I_{obs}(\mathbf{h}_2) = (\alpha)I(\mathbf{h}_1) + (1-\alpha)I(\mathbf{h}_2) \qquad (8\text{-}3)$$

we can solve for the true intensities $I(\mathbf{h}_1)$ and $I(\mathbf{h}_2)$ the following linear system:

$$I(\mathbf{h}_1) = \frac{(1-\alpha)I_{obs}(\mathbf{h}_1) - \alpha I_{obs}(\mathbf{h}_2)}{1-2\alpha} \text{ and } I(\mathbf{h}_2) = \frac{-\alpha I_{obs}(\mathbf{h}_1) + (1+\alpha)I_{obs}(\mathbf{h}_2)}{1-2\alpha} \qquad (8\text{-}4)$$

Equations 8-4 allow the recovery of the true intensities from observed twin related pairs provided: (i) we know the twinning operator (otherwise we cannot form the pairs of related reflections); (ii) we know the twinning fraction α; and (iii) that the twinning fraction is not exactly 0.5 (where Equations 8-4 become singular) which is the case for perfect merohedral twinning.

Acta 1988

Enz 97

The twinning fraction can be recovered from analysis of the cumulative probability distribution $N(H)$ for the statistic H from the pairs of twin related intensities.[42] $N(H)$ takes the following algebraic forms:

$N(H) = S(u)$

$$N(H) = \cos^{-1}\left[H/(2\alpha-1)\right]/\pi \quad \text{(centric)} \qquad (8\text{-}5)$$

$2\cos^{-1}[4/(2\alpha-1)]/\pi - 1$

$$N(H) = \left[1 + H/(1-2\alpha)\right]/2 \quad \text{(acentric)} \qquad (8\text{-}6)$$

$N(H) = 4/(1-2\alpha)$

2

The centric function has the sigmoid shape of the arccosine and the acentric function is linear in H. Both functions can be computed and plotted for a number of discrete values for the twinning fraction α for centric and acentric reflections and compared with the actually observed data. In general there are not many centric reflections, so the acentric plots are more meaningful (Figure 8-19). The theoretical experimental distribution that is followed most closely by the experimental data (normalized in resolution shells) provides an estimate of the twinning fraction. This was the first useful statistical procedure for analyzing

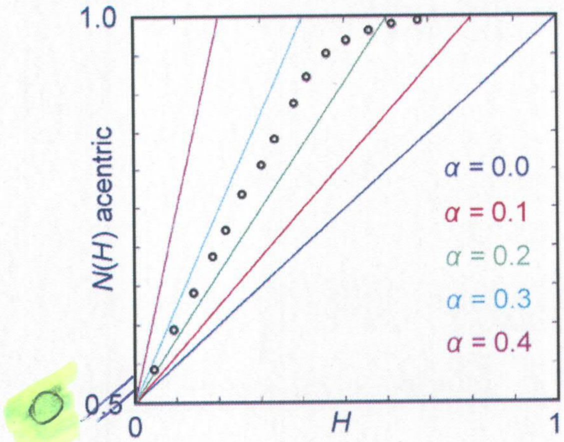

Figure 8-19 Cumulative probability distribution *N(H)* for acentric reflections. The plot shows the theoretical curves for acentric data and the experimental data points. A twinning ratio α of about 0.26 can be interpolated from the graph.

merohedral twinning in macromolecular data, derived by Todd Yeates[43] extending earlier work. Once α is known, the data can be de-twinned (the fractions computed and combined) and treated as normal.

For perfect merohedral twinning, where the expressions (8-5 and 8-6) derived above for H become singular, a number of additional tests based on the moments of E (normalized structure factors) or Z (normalized intensities, $Z = I/\langle \mathrm{I} \rangle$) can be inspected. The CCP4 program *TRUNCATE*, for example, provides such plots, where the moments are plotted in resolution shells. Their expectation values are given in Table 8-3.

The drawback of the above method is that to determine which pairs of reflections are related, one still needs to know the twin operator, that is, try the possible ones, given the apparent space group. The expressions for the cumulative probability distributions of H again become singular for $\alpha \to 0.5$, that is, for perfect hemihedral twinning.

Padilla and Yeates[44] have thus modified the above statistic by considering pairs of locally related reflections instead of twin related reflections. The statistic L is more robust in the presence of anisotropic diffraction and pseudo-centering than the cumulative $N(Z)$ plots ($Z = I/\langle \mathrm{I} \rangle$) derived in Chapter 7. Importantly, L can be evaluated without prior knowledge of the twin law:

$$L = \frac{I_{obs}(\mathbf{h}_1) - I_{obs}(\mathbf{h}_2)}{I_{obs}(\mathbf{h}_1) + I_{obs}(\mathbf{h}_2)} \qquad (8\text{-}7)$$

One can again plot a cumulative distribution function $N(|L|)$ and check for deviations from the expected values for untwinned data. The practical benefit is that Function 8-7 also delivers a defined curve for perfectly twinned data,

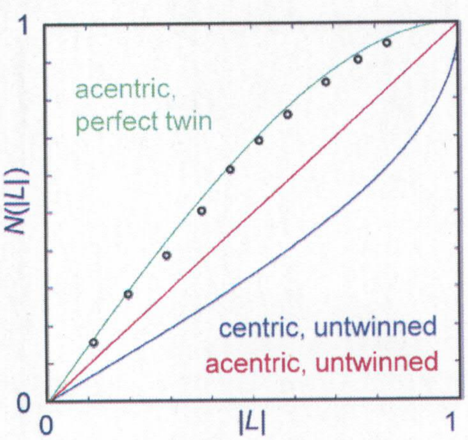

Figure 8-20 Yeates–Padilla plot for the cumulative probability distribution *N*(|*L*|). The graph shows the expected cumulative distribution curves for acentric and centric untwinned data and acentric experimental data (open circles) for a perfect twin. For partial twins, deviations from untwinned data will result in experimental data points located between the calculated curves.