free R value than NCS constraints, then use constraints. If NCS restraints are to be employed, then use the experimental map to design a suitable NCS-restraint scheme (Kleywegt, 1999). Avoid the temptation to model alternative conformations in low-resolution maps or to place putative solvent molecules in every local maximum of the ($F_o - F_c$, α_c) difference map. In other words, be conservative and remember that the maxim 'where freedom is given, liberties are taken' is highly applicable to refinement programs (Hendrickson & Konnert, 1980; Kleywegt & Jones, 1995*b*).

(5) Adopt methodological advances as soon as they become available. Several innovations have only been slowly accepted by the mainstream (*e.g.* the use of databases in building and rebuilding, the use of the free R value, the use of electron-density averaging in molecular-replacement cases, bulk-solvent modelling). The most prominent recent development is the use of likelihood-based refinement programs (Bricogne & Irwin, 1996; Pannu & Read, 1996; Murshudov *et al.*, 1997; Adams *et al.*, 1997; Pannu *et al.*, 1998). These programs produce better models and maps and considerably reduce over-fitting (as assessed by the difference between the free and conventional R values).

(6) Most importantly, the crystallographer should be hyper-critical towards the fruits of his or her own labour. Every intermediate model is a hypothesis to be shot down (Jones & Kjeldgaard, 1994). The crystallographer should be more critical than the supervisor, the supervisor more critical than the referee and the referee more critical than the casual reader. It goes without saying that the reader, casual or not, should have access to model coordinates, experimental data and electron-density maps!

21.1.6. Final model

Once the refinement is finished [*i.e.* once the ($F_o - F_c$, α_c) difference map is featureless (Cruickshank, 1950) and parameter shifts in further refinement cycles are negligibly small], three tasks remain: validation of the final model, description and analysis of the structure, and deposition of the model coordinates and the crystallographic data with the Protein Data Bank (Bernstein *et al.*, 1977).

Until a few years ago, validation of the final model typically entailed calculating the conventional R value, r.m.s. deviations from ideal values of bond lengths and angles, average temperature factors, and a Luzzati-type estimate of coordinate error. Kleywegt & Jones (1995*b*) showed that these statistics are not necessarily even remotely related to the actual quality of a model. Based on these criteria, a backwards-traced protein model was of higher apparent quality than a carefully refined correct model. After this, the realisation sunk in that the best validation criteria are those that assess aspects of the model that are 'orthogonal' to the information used during model refinement and rebuilding. For instance, the main-chain φ and ψ torsion angles are usually not restrained during refinement; this makes the Ramachandran plot such a powerful validation tool (Kleywegt & Jones, 1996*b*, 1998). Other examples of useful independent tests include the profile method of Eisenberg and co-workers (Lüthy *et al.*, 1992), the directional atomic contact analysis method of Vriend & Sander (1993) and the threading-potential method of Sippl (1993).

In general, all quality checks provide necessary, but in themselves insufficient, indications as to whether or not a model is essentially correct. A truly good model should make sense with respect to what is currently known about physics, chemistry, crystallography, protein structures, statistics and (last, but not least) biology and biochemistry (Kleywegt & Jones, 1995*a*). A good model will typically score well on most if not all validation criteria, whereas a poor one will score poorly on many criteria. The same is true at the level of residues: a poor or erroneous region in a model will be characterized by violations of many residue-level quality criteria (Kleywegt & Jones, 1997).

21.1.7. A compendium of quality criteria

In this section, some of the quality and validation criteria that have been used by macromolecular crystallographers are summarized (for more detailed information, the reader is referred to the primary literature). When judging how useful or powerful these criteria are in a certain case, one should keep in mind that any criterion that has been used explicitly or implicitly during model refinement (*e.g.* geometric restraints) or rebuilding (*e.g.* rotamer libraries) does *not* provide a truly independent check on the quality of the model.

Many, but not all, of the criteria discussed below pertain specifically to protein models. Comparatively little work has been performed on the validation of nucleic acid models, although there are indications that there is a need for such procedures (Schultze & Feigon, 1997). The situation would appear to be even worse for hetero-entities (*e.g.* ligands, inhibitors, cofactors, covalent attachments, saccharides, metals, ions; van Aalten *et al.*, 1996; Kleywegt & Jones, 1998).

21.1.7.1. *Data quality*

Although many quality and validation criteria have been developed for assessing coordinate sets of protein models, comparatively few criteria are available for assessing the quality of the crystallographic data.

21.1.7.1.1. *Merging R values*

Possibly the most common mistake in papers describing protein crystal structures is an incorrectly quoted formula for the merging R value (calculated during data reduction),

$$R_{\rm merge} = \sum_h \sum_i |I_{h,\,i} - \langle I_h \rangle| / \sum_h \sum_i I_{h,\,i},$$

where the outer sum (h) is over the unique reflections (in most implementations, only those reflections that have been measured more than once are included in the summations) and the inner sum (i) is over the set of independent observations of each unique reflection (Drenth, 1994). This statistic is supposed to reflect the spread of multiple observations of the intensity of the unique reflections (where the multiple observations may derive from symmetry-related reflections, different images or different crystals). Unfortunately, $R_{\rm merge}$ is a very poor statistic, since its value increases with increasing redundancy (Weiss & Hilgenfeld, 1997; Diederichs & Karplus, 1997), even though the signal-to-noise ratio of the average intensities will be higher as more observations are included (in theory, an N-fold increase of the number of independent observations should improve the signal-to-noise ratio by a factor of $N^{1/2}$). At high redundancy, the value of $R_{\rm merge}$ is directly related to the average signal-to-noise ratio (Weiss & Hilgenfeld, 1997): $R_{\rm merge} \simeq 0.8/\langle I/\sigma(I)\rangle$.

Diederichs & Karplus (1997) have suggested a number of alternative measures that lack most of the drawbacks of $R_{\rm merge}$. Their statistic $R_{\rm meas}$ is similar to $R_{\rm merge}$, but includes a correction for redundancy (m),

$$R_{\rm meas} = \sum_h [m/(m-1)]^{1/2} \sum_i |I_{h,\,i} - \langle I_h \rangle| / \sum_h \sum_i I_{h,\,i}.$$

Another statistic, the pooled coefficient of variation (PCV), is defined as

$$\mathrm{PCV} = \sum_h \{[1/(m-1)] \sum_i (I_{h,\,i} - \langle I_h \rangle)^2\}^{1/2} / \sum_h \langle I_h \rangle.$$

references