To assess the 'geometric strain' in a model on a per-residue basis, the refinement program *X-PLOR* (Brünger, 1992*b*) can produce geometric pseudo-energy plots. In such a plot, the ratio of $E_{\rm geom}(i)$/r.m.s.($E_{\rm geom}$) is calculated as a function of the residue number i. The pseudo-energy term $E_{\rm geom}$ consists of the sums of the geometric and stereochemical pseudo-energy terms of the force field ($E_{\rm geom} = E_{\rm bonds} + E_{\rm angles} + E_{\rm dihedrals} + E_{\rm impropers}$), involving only the atoms of each residue.

It has been observed that the more high-resolution protein structures become available, the more 'well behaved' proteins turn out to be, *i.e.* the distributions of conformational torsion angles and torsion-angle combinations become even tighter than observed previously and the numerical averages tend to shift somewhat (Ponder & Richards, 1987; Kleywegt & Jones, 1998; EU 3-D Validation Network, 1998; MacArthur & Thornton, 1999; Walther & Cohen, 1999).

21.1.7.2.3. *C^α-only models*

Validation of C^α-only models may be necessary if such a model is retrieved from the PDB to be used in molecular replacement or homology modelling exercises; however, not many validation tools can handle such models (Kleywegt, 1997). The C^α backbone can be characterized by C^α—C^α distances (~2.9 Å for a *cis*-peptide and ~3.8 Å for a *trans*-peptide), C^α—C^α—C^α pseudo-angles and C^α—C^α—C^α—C^α pseudo-torsion angles (Kleywegt, 1997). The pseudo-angles and torsion angles turn out to assume certain preferred value combinations (Oldfield & Hubbard, 1994), much like the backbone φ and ψ torsions, and this can be employed for the validation of C^α-only models (Kleywegt, 1997). In addition to these straightforward methods, the mean-field approach of Sippl (1993) is also applicable to C^α-only models.

21.1.7.2.4. *Contacts and environments*

Hydrophobic, electrostatic and hydrogen-bonding interactions are the main stabilizing forces of protein structure. This leads to packing arrangements where hydrophobic residues tend to interact with each other, where charged residues tend to be involved in salt links and where hydrophilic residues prefer to interact with each other or to point out into the bulk solvent. Serious model errors will often lead to violations of such simple rules of thumb and introduce non-physical interactions (*e.g.* a charged arginine residue located inside a hydrophobic pocket; Kleywegt *et al.*, 1996) that serve as good indicators of model errors. Directional atomic contact analysis (Vriend & Sander, 1993) is a method in which these empirical notions have been formalized through database analysis. For every group of atoms in a protein, it yields a score which in essence expresses how 'comfortable' that group is in its environment in the model under scrutiny (compared with the expectations derived from the database). If a region in a model (or the entire model) has consistently low scores, this is a very strong indication of model errors. The *ERRAT* program is based on the same principle, but it is less specific in that it assesses only six types of non-bonded interactions (CC, CN, CO, NN, NO and OO; Colovos & Yeates, 1993).

Hydrogen-bonding analysis can often be used to determine the correct orientation of asparagine, glutamine and histidine residues (McDonald & Thornton, 1995). Similarly, an investigation of unsatisfied hydrogen-bonding potential can be used for validation purposes (Hooft *et al.*, 1996*b*), as can calculation of hydrogen-bonding energies (Morris *et al.*, 1992; Laskowski, MacArthur *et al.*, 1993).

Finally, a model should not contain unusually short non-bonded contacts. Although most refinement programs will restrain atoms from approaching one another too closely, if any serious violations remain they are worth investigating, since they may signal an underlying problem (*e.g.* erroneous omission of a disulfide restraint or incorrect side-chain assignment).

21.1.7.2.5. *Noncrystallographic symmetry*

Molecules that are related by noncrystallographic symmetry exist in very similar, but not identical, physical environments. This implies that their structures are expected to be quite similar, although different relative domain orientations and local variations may occur (*e.g.* owing to different crystal-packing interactions; Kleywegt, 1996). Many criteria have been developed to quantify the differences between (NCS) related models. Some, such as the r.m.s. distance (*e.g.* on all atoms, backbone atoms or C^α atoms) are based on distances between equivalent atoms, measured after a (to some extent arbitrary; Kleywegt, 1996) structural superpositioning operation has been performed. Others are based on a comparison of torsion angles, be it of main-chain φ, ψ angles [*e.g.* $\Delta\varphi$, $\Delta\psi$ plot (Korn & Rose, 1994); multiple-model Ramachandran plot (Kleywegt, 1996); $\sigma(\varphi)$, $\sigma(\psi)$ plot (Kleywegt, 1996); circular variance (Allen & Johnson, 1991) plots of φ and ψ (G. J. Kleywegt, unpublished results); Euclidian φ, ψ distances (Carson *et al.*, 1994) or pseudo-energy values (Carson *et al.*, 1994)] or side-chain χ_1, χ_2 angles [*e.g.* multiple-model χ_1, χ_2 plot (Kleywegt, 1996); $\sigma(\chi_1)$, $\sigma(\chi_2)$ plots (Kleywegt, 1996); circular variance (Allen & Johnson, 1991) plots of χ_1 and χ_2 (G. J. Kleywegt, unpublished results); Euclidian χ_1, χ_2 distances (Carson *et al.*, 1994) or pseudo-energy values (Carson *et al.*, 1994)]. Still other methods are based on analysing differences in contact-surface areas (Abagyan & Totrov, 1997), temperature factors (Kleywegt, 1996) or the geometry of the C^α backbone alone (Flocco & Mowbray, 1995; Kleywegt, 1996). Many of these methods can also be used to compare the structures of related molecules in different crystals or crystal forms (*e.g.* complexes, mutants).

21.1.7.2.6. *Solvent molecules*

Solvent molecules provide an excellent means of 'absorbing' problems in both the experimental data and the atomic model. Neither their position nor their temperature factor are usually restrained (other than by the data and restraints that prevent close contacts) and sometimes even their occupancy is refined. At a resolution of ~2 Å, crystallographers tend to model roughly one water molecule for every amino-acid residue and at 1.0 Å resolution this number increases to ~1.6 (Carugo & Bordo, 1999). When waters are placed, it should be ascertained that they can actually form hydrogen bonds, be it to protein atoms or to other water molecules. Considering that several ions that are isoelectronic with water (Na^+, NH_4^+) are often used in crystallization solutions, one should keep in mind the possibility that some entities that have been modelled as water molecules could be something else (Kleywegt & Jones, 1997). A method to check if water molecules could actually be sodium ions, based on the surrounding atoms, has been published (Nayal & Di Cera, 1996).

21.1.7.2.7. *Miscellaneous*

Many other coordinate-based methods for assessing the validity or correctness of protein models have been developed. These include the profile method of Eisenberg and co-workers (Bowie *et al.*, 1991; Lüthy *et al.*, 1992), the inspection of atomic volumes (Pontius *et al.*, 1996), and the use of threading and other potentials (Sippl, 1993; Melo & Feytmans, 1998; Maiorov & Abagyan, 1998). Some of these methods are described in more detail elsewhere in this volume. The program *WHAT IF* (Vriend, 1990) contains a large array of quality checks, many of which are not available in other programs, that span the spectrum from administrative checks to global quality indicators (Hooft *et al.*, 1996). During the refinement

references