

19. OTHER EXPERIMENTAL TECHNIQUES

the region of the unit cell occupied by protein atoms should be featureless in solvent maps. It can also be assumed that, as an approximation, solvent regions further than 4 Å from the protein surface have bulk solvent characteristics and can be treated as a constant density region. Combining these two regions gives about 50–60% of the total volume of the unit cell.

Knowledge of the density content of such a large percentage of the unit cell places a strong constraint on the overall character of the Fourier transform, a fact that can be used to improve the quality of the experimentally determined phases

19.1.8. Applications of D₂O – H₂O solvent difference maps

19.1.8.1. Orientation of water molecules

In the case of a highly ordered water in a D₂O – H₂O difference map, the oxygen scattering components will cancel (having

identical locations and scattering potential), but because H and D have very different scattering properties (H = –3.8 f, D = +6.7 f), large peaks will be found in the map at the D – H positions. Consequently, these ordered waters can usually be oriented with reasonable accuracy in maps better than 2.0 Å resolution (see Fig. 19.1.3.1b).

19.1.8.2. H/D exchange

Fig. 19.1.8.1 shows examples of density around exchanged and unexchanged amide peptide sites. The density in Fig. 19.1.8.1(a) is characteristic of $2F_o - F_c$ Fourier maps; the D is in positive density, extending off the peptide nitrogen, and the H is represented by negative density, separated and translated off the nitrogen. Fig. 19.1.8.1(b) shows a D₂O – H₂O density map at the same resolution (2.0 Å). Assignment of H/D character is unambiguous, and it is possible to evaluate partial exchange properties more reliably.