19.1. NEUTRON CRYSTALLOGRAPHY
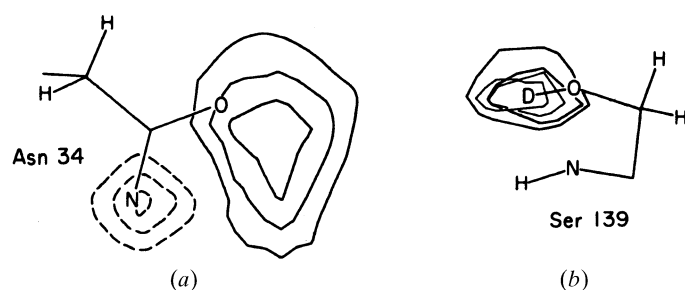

(*a*) (*b*)

Fig. 19.1.5.1. Difference map of Asn34 in the trypsin structure. (*a*) In a
protein X-ray analysis, the difference in scattering intensity between O
and NH$_2$ is much too small to be detected. In contrast, the neutron-
scattering magnitudes of oxygen and nitrogen (5.8 f versus 9.4 f) are
quite dissimilar, and there is additional scattering at the nitrogen site
from the two bound deuterium atoms. The resulting differential is over
350%, quite large enough to be detected for well ordered side chains.
The nitrogen and oxygen positions shown are from the X-ray model.
The difference density indicates that the orientation of the nitrogen and
oxygen atoms is incorrect and should be rotated by 180° around the
C$_\beta$–C$_\gamma$ bond. (*b*) Difference map for Ser139. On well ordered hydroxyl
side chains, the orientation of deuterium atoms can sometimes be
assigned.

in the Fourier map from the true positions and, coupled with their
short bond lengths, complicates the interpretation of the results.
Additionally, it has been shown that small errors in positional and
thermal parameters of the parent atoms can further complicate the
identification of hydrogen-atom positions (Kossiakoff & Spencer,
1981).

19.1.6. Refinement

The methodologies employed to refine neutron data are essentially
the same as those used in most X-ray studies. These include real-
space (Hanson & Schoenborn, 1981; Norvell & Schoenborn, 1976;
Schoenborn & Diamond, 1976), reciprocal-space (Bentley &
Mason, 1980; Phillips, 1984; Wlodawer & Hendrickson, 1982;
Wlodawer & Sjolin, 1981) and restrained difference-map refine-
ment (Kossiakoff & Spencer, 1980; 1981). A joint refinement
technique in which the neutron and X-ray data are refined
simultaneously has been developed (Wlodawer & Hendrickson,
1982). In addition to the normal difficulties encountered in the
refinement of any protein structure, there are several that are
peculiar to the neutron-diffraction technique. These special
problems arise from the close proximity of hydrogen atoms to
their parent atoms, coupled with the effects of the negative
scattering length of the hydrogen atoms. Potential problems exist
when the difference density generated from positional errors of one
atom overlaps an adjacent atom site. The situation is further
complicated by the fact that, because of its negative scattering
length, an error in a hydrogen-atom position is minimized by
moving the atom down the gradient, that is, in the opposite direction
to that required for correcting parent-atom positions. To evaluate
the extent of this problem in refinement, a test was devised using a
2.2 Å data set (Kossiakoff & Spencer, 1981). The coordinates of the
protein trypsin were perturbed by a varying, but known, amount
from their ideal positions. It was determined that, in general,
convergence towards the true coordinate could be obtained when
the coordinate errors were less than 0.3 Å; however, if the parent
atom (an atom with one or more hydrogens attached to it) was
displaced by more than 0.6 Å from its correct position, the effect of
neighbouring hydrogens rendered the calculated shifts inaccurate.
The results of this study support the observations of other
investigators that it is absolutely crucial that the starting phasing

model be of high quality, because the range of convergence of a
neutron analysis is relatively small.

19.1.7. D$_2$O − H$_2$O solvent difference maps

D$_2$O − H$_2$O solvent difference maps provide an unbiased method
for identifying water molecules and exchangeable hydrogens
(Kossiakoff *et al.*, 1992). For several years, the large difference in
the scattering characteristics of neutrons by H$_2$O compared to D$_2$O
has been effectively exploited by using density matching and
exchange labelling in small-angle neutron-scattering experiments.
This difference can likewise be exploited in neutron protein
crystallography to determine the detailed structural characteristics
of protein hydration through the calculation of solvent difference
maps (Shpungin & Kossiakoff, 1986; Kossiakoff *et al.*, 1992). In
practice, such maps are obtained by comparing the changes in
diffracted intensities between two sets of data – one obtained from a
crystal having H$_2$O as the major solvent constituent, and a second
where D$_2$O is the solvent medium. To a good approximation, the
protein-atom contributions to the scattering intensities in both data
sets are equal and cancel, but since H$_2$O and D$_2$O have very
different scattering properties, their differences are accentuated to
reveal an accurate and nearly unbiased representation of the solvent
structure.

The features of a solvent difference map of this type are not as
affected by errors in the phasing model as conventional difference
Fourier maps. In addition, there are refinement procedures that can
be applied to them that lead to significant enhancement in signal/
noise discrimination. The basic feature of the method is a set of
density-modification steps based on the fact that a considerable
amount of information about the density distribution of the
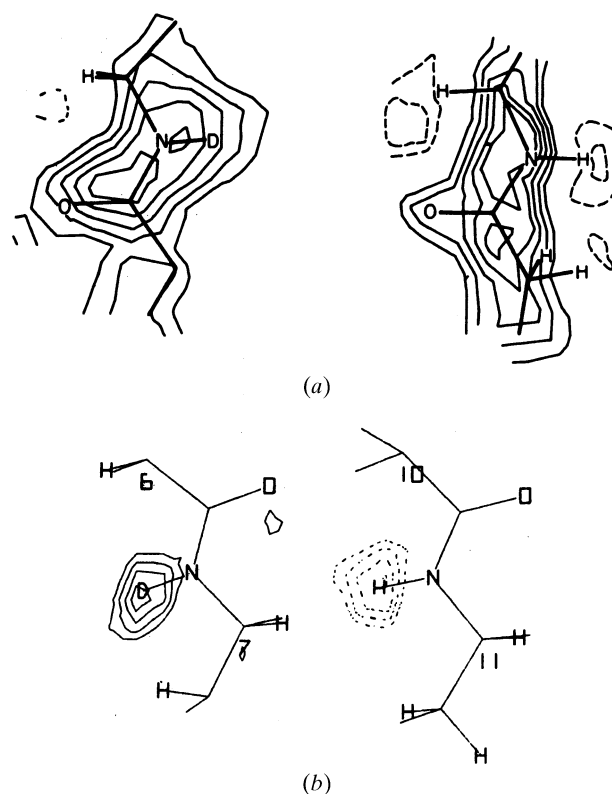crystallographic unit cell is known. For instance, it is known that

(*a*)

(*b*)

Fig. 19.1.8.1. Sections of neutron density maps taken in the plane of the
peptide group. (*a*) $2F_o − F_c$ maps showing an example of an exchanged
and unexchanged amide peptide group. (*b*) D$_2$O − H$_2$O difference
density map showing the same.

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 $D_2O - H_2O$ solvent difference maps

19.1.8.1. *Orientation of water molecules*

In the case of a highly ordered water in a $D_2O - H_2O$ 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. 19.1.3.1*b*).

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 $D_2O - H_2O$ 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.

references