Table 2.2. Refinement statistics for a typical protein structure determination. (Data taken from the structure determination of glucosidase at pH 4.5; PDB code 2NT0)[3]

Resolution (Å)	20–2.2
Reflections	131814
R_{work} / R_{free}	22.0/27.6
No. of protein atoms	1988
Sulfate ions	28
Water	1181
B factors:	
Protein	24.6
rms deviations	
Bond lengths (Å)	0.016
Bond angles (°)	1.7

electron density has a spherical shape and is observed near the surface of the protein. When is such an electron density a water molecule and when is it something else? Unless the electron density feature has a nonspherical shape to indicate that it might be a compound or ion with more than one nonhydrogen atom, such as the commonly observed sulfate or phosphate ions, or glycerol used for cryo-protection, it is assigned to a water molecule. This assignment may not be correct because other individual ions can interact with the protein surface. The resolution range usually found for good protein structure determinations is from approximately 2 to 1.5Å. In this range, electron density for hydrogens is not visible. In fact, resolution of better than 1Å is required to see electron density for hydrogens, and even that is not always sufficient. Consequently, identification of one ion from another is usually not possible, and, in the absence of hydrogen positions, distinguishing water from a cation or anion is usually not possible either.

This leaves an important question: When can the electron density feature be assigned to a water molecule or when is it part of the noise inherent in an electron density map? A number of criteria are applied to make this determination. The two most important ones are the height of the electron density peak (given in terms of the sigma level of the peak relative to the overall average electron density level of the map) and the interactions that a putative water molecule would make with nearby protein atoms if placed into that electron density. Different researchers apply different criteria. Because the height of an electron density peak depends on the occupancy and *B* factor (mobility) of an atom, assignment of a water molecule must take these two factors into account. In general, if an electron density is no longer visible at a σ level of 3, indicating poor occupancy, high mobility, or simply noise, it should not be assigned. Second, if the water molecule at any given position does not interact with the protein in terms of at least some putative hydrogen bonds, of which water is capable of a potential four, it is unlikely to be one. This still does not identify a water molecule unambiguously, but it is the best we can do (Figure 2.14).

It has been shown that small molecules, as small as two or more nonhydrogen atoms, can also interact with the surface of a protein.[21,22] Determining the orientation in which such a molecule binds depends on the shape of the electron

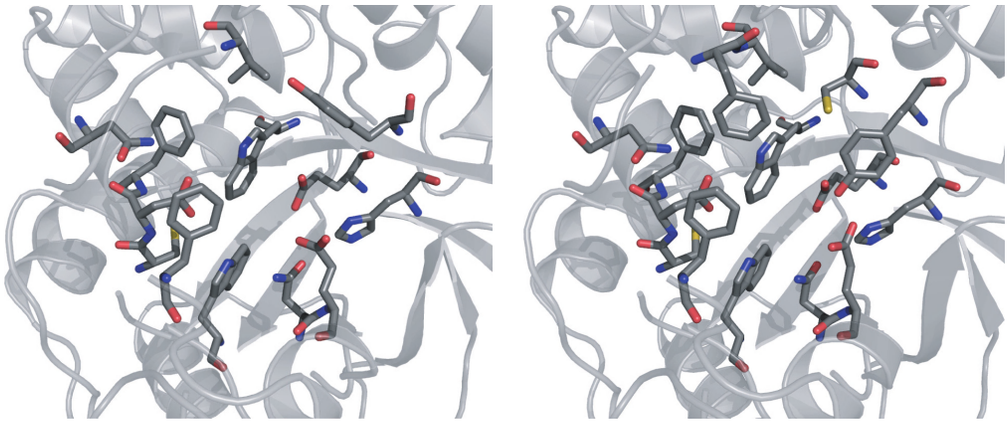

Figure 2.13. The structure of a protein may not be relevant to its catalytic form. In this case, the structure of glucocerebrosidase is subject to changes in configuration as a response to environment, such as the binding of an inhibitor or pH. For instance, the active site of glucocerebrosidase has a different conformation at the pH at which it is synthesized in the endoplasmic reticulum from that at the pH of the lysozome. The conformational changes observed reflect the inactive and active forms of the enzyme in the different compartments of the cell. Data were taken from PDB codes 2NT1 (pH 7.5) and 2NT0 (pH 4.5).[3]