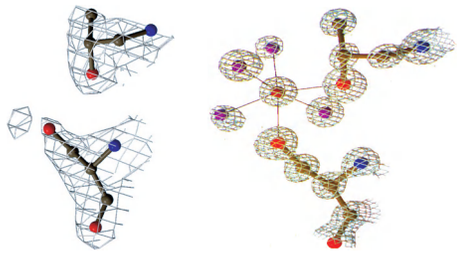


Interpretation of electron density at different resolutions:
2.0Å resolution vs 0.95Å resolution

Figure 2.14. Water molecules are placed in electron density features based on a number of criteria. In the lower resolution structure, the water molecule is assigned based on the height of the electron density feature of a magnitude realistic for an oxygen atom and the location of that feature near hydrogen bonding partners (a carbonyl oxygen atom in this case) if it were a water molecule. At ultrahigh resolution, where the feature is more clearly defined, that "water" molecule turns out to be a cation, probably a sodium ion, identified on the basis of the dominant ion in the mother liquor from which the crystal was obtained. Data were taken from PDB codes 1AMP (1.8Å resolution)[9] and 1RTQ (0.95Å resolution).[10]

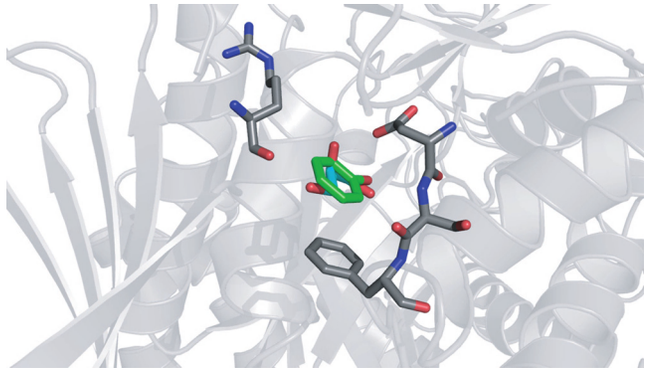
density feature, and, if the shape is not definitive, the chemical interactions that atoms of the molecule can make with atoms of the protein. The obvious interaction is a hydrogen bonding one, but hydrophobic or polar interactions are equally useful to define orientation.

The sites at which small molecules bind can be random. However, some spots on the protein are more prone to interaction with such compounds than others,[23–25] for instance, the active site of an enzyme or an allosteric site on a protein. These are sites that are designed to interact with molecules – substrates or inhibitors or activators – in such a way as to confer specificity to the interaction. Often more than one part of such a site interacts with the recognized molecule. Any one part may therefore be prone to a different type of interaction, such as electrostatic or hydrophobic, depending on the characteristics of the overall compound recognized. Small molecules may make such interactions individually and therefore be attracted to a particular subsite within a larger interaction site.

Any organic ligand can be decomposed into substructures (fragments) that are reminiscent of smaller molecules. Such a small molecule may therefore occupy the same site on a protein as the substructure that it represents. The position of such a small molecule may therefore be used to characterize a particular site on the protein that has a specific affinity for the chemical group represented by the small molecule. If a set of such fragments is found bound to an overall site on the protein, they should map out the binding surface of a region of the protein. The basic assumption is that a specific chemical group will interact with a specific site on a protein in the absence of the rest of a larger ligand molecule. If that is true, the small molecules can be linked together to make a large molecule that fits all of the regions defined by such a mapping procedure. This method was first proposed by Fitzpatrick et al.[21]:

> Thus, acetonitrile may act as a probe to map the amphiphilic regions of the enzyme surface, which would suggest an experimental approach to mapping the complete binding surface of any crystalline protein. By the methods described here, crystals would be transferred to a series of organic solvents, each designed to mimic a particular functional group (e.g., benzene can be used to map binding sites for aromatic groups). Such experiments are directly analogous to computational methods that map the interaction energies of small probe molecules to protein surfaces, thus providing a direct experimental test of such theoretical methods. Once the interaction surface has been mapped by a series of solvent experiments, the various functional groups can be connected to provide specific lead compounds for drug design. Unlike conventional substrate analogues, which interact only with the active center, compounds designed by solvent mapping can exploit additional regions of the protein surface to provide greater specificity and affinity.

Once a set of small molecules are found that associate with a region on the surface of the protein, in principle they can be linked to make a larger compound that contains the combined affinities of the smaller parts as well as the synergistic affinity of the combination (Figure 2.15). Alternatively, the method can be used to build onto an existing framework to optimize the affinity of a starting compound that already binds to the protein. One of the earliest uses of this strategy at the experimental level is the structure/activity relationship by nuclear magnetic resonance (SAR by NMR)



Binding of solvent molecules to the surface of GCase

Figure 2.15. Results of solvent mapping of glucocerebrosidase. A molecule of glycerol (blue) and one of phenol (green) bind to a site on the surface of the protein. Solvent molecules are placed into electron density the same way as inhibitor or water molecules are placed, based on the shape and size of the electron density feature. If several solvent molecules bind in or near the same site on the protein, such a cluster can be used to identify hot spots such as active sites, allosteric sites, or simply binding sites. Data were taken from unpublished structure determinations by Raquel Lieberman.