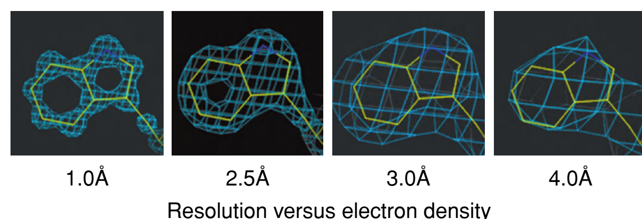

Figure 2.6. The ability to build a model into electron density depends on the quality of that electron density and the resolution at which it is calculated. Here the effect of resolution on the quality of the electron density is illustrated. At high resolution, such as 1.0Å resolution, individual atoms are visible to the extent that carbon and nitrogen atoms are distinguishable. At medium resolution, such as 2.5–3.0Å resolution, the shape of the residue side chain is clearly interpretable even though the individual atoms are not. At low resolution, such as 4Å resolution, the position of the side chain is clear, but the configuration is not and the fit of the model to the electron density is unclear.

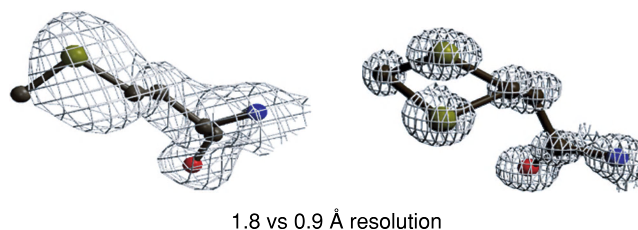

Figure 2.7. Because the structure determined for a protein represents an average over all of the molecules in a unit cell, parts of the protein can have different conformations. The ability to deconvolute these different conformations depends partly on the resolution of the structure determination. Although the electron density at 1.8Å resolution clearly shows the direction in which the side chain points, it does not show the exact location of the end sulfur atom and methyl group. At 0.9Å resolution, this problem is resolved, showing that the sulfur atom can be in two possible conformations, in this case of approximately equal probability. It can also happen that a side chain may point in completely different directions or may have different conformations. Data were taken from PDB codes 1AMP (1.8Å resolution)[9] and 1RTQ (0.95Å resolution).[10]

perfect alignment of all the molecules, and all of the parts of molecules. If the electron density is unequivocal, there is only one molecule or residue, and the shape is unique, the model of the peptide or ligand may be "dropped" into the electron density without a problem. However, if the electron density is not so clear, if it is spread out or not quite connected everywhere, or there may be more than one molecule present in more than one orientation, interpretation becomes complicated.

For instance, a side chain or stretch of peptide may have more than one conformation. If the number is small (i.e., 2), both will usually be apparent if their occupancies are roughly equal. If the number becomes large (i.e., >3 or 4), the electron density may no longer be distinguishable from the noise of the map. A similar phenomenon applies if a ligand is bound to only some of the protein molecules in the crystal and not all. Such partial occupancy produces weak electron density for the ligand, and that may be hard to interpret. At low resolutions the spread of the electron density as a result of multiple conformations is accounted for by a term called the *B* factor or temperature factor: the greater the spread, the larger the *B* factor. Average *B* factors for protein atoms are in the range of 20–30A^2 depending on the resolution of the data. Occupancy is given in terms of a fraction between 0 and 1. These two are related to each other and at the resolutions generally observed for protein/ligand complexes they cannot be distinguished from each other. The *B* factor, which is given for each atom in the coordinate file, consequently takes both into account. It is not uncommon for the *B* factors for a ligand to be significantly higher than those for the protein, accounting at least in part for partial occupancy of the ligand (Figure 2.7).

Electron density maps can be displayed in a number of different ways: the most common for interpretation of protein/ligand complexes are those with coefficients Fo-Fc and 2Fo-Fc. The former displays the difference between the observed electron density and that calculated from a model. Such a difference map highlights missing parts of the model (positive difference electron density) and parts added by the model that are not supported by the data (negative difference electron density). The latter displays the electron density emphasizing the difference between observed and calculated (Figure 2.8).

REFINEMENT

The key structures needed for the purposes of drug design are those of the protein, usually an enzyme, by itself and in the presence of a ligand; and those models should be as accurate as possible. The crystallographic experiment, however, does not provide an "accurate" model; it provides a precise model. The difference refers to the closeness with which the model can be fitted to the observed electron density and how well the two can be made to match, and the "real" structure. What the experimentalist can do is to align the model and the electron density as closely as possibly by a protocol of refinement in which the model is iteratively matched to the electron density, and the measure of fit is recalculated to determine success by the procedure. Iteration is continued until convergence occurs. Refinement is generally carried out with stereochemical restraints that

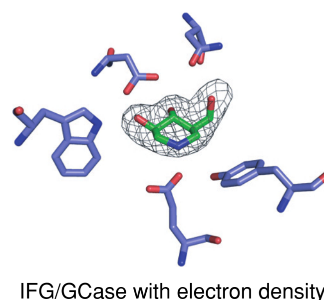

Figure 2.8. The electron density for the inhibitor isofagomine bound to glucocerebrosidase appears in an electron density map that has been calculated from the data for the enzyme-inhibitor complex and the phases from the protein alone. The electron density for the inhibitor has a shape that is consistent with only one orientation of the inhibitor in the active site. Data were taken from PDB code 2NSX.[3]