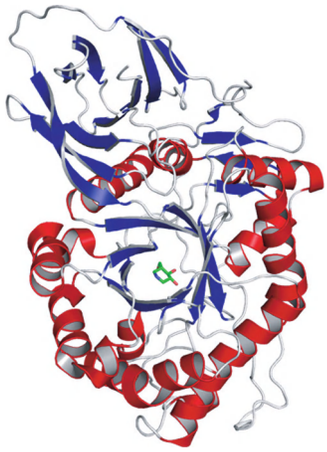


Glucocerebrocidase with IFG bound

Figure 2.4. Common representation of a protein molecule, in this case, acid β-glucosidase with the inhibitor isofagomine (IFG) bound.[3] The ribbon represents the polypeptide, whose path is shown in three dimensions. Secondary structural elements are shown by arrows (beta sheet), helices, and coils (no secondary structure). The position of the inhibitor indicates the location of the active site. Data were taken from PDB code 1OSG.

the structure amplitudes for a protein/ligand complex are combined with the phases for the protein alone, giving an electron density map of the protein with somewhat weaker electron density for the ligand but enough to interpret the structure of the added molecule (Figure 2.4).

How good does the model have to be for success in this process? The answer is not definitive but can be estimated from the figure. Clearly a protein/ligand complex falls into the acceptable range as long as the ligand is a small molecule. However, if the ligand produces major conformational changes to the protein, all bets are off. Our experience is that if the model structure has at least a 50% sequence identity to that of the new, unknown protein, molecular replacement often works (Figure 2.5). Below that rough dividing line, sometimes it works, but many times it does not.[8]

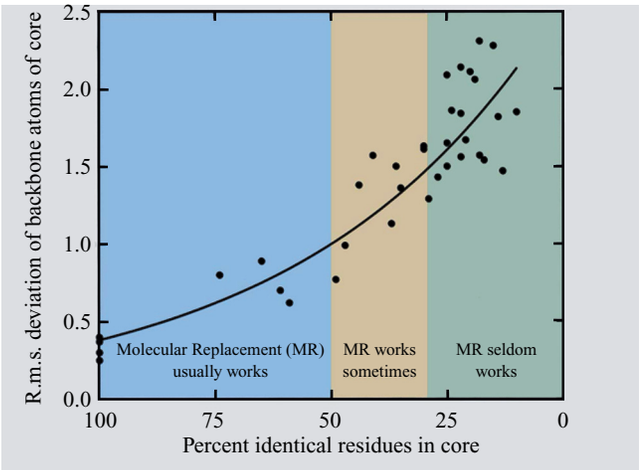
There are many other methods of phase determination that are available if molecular replacement fails or if no related structure exists, but these are outside the scope of this chapter. Suffice it to say that, in modern protein crystallography, phase determination is rarely the bottleneck. We personally have never failed to solve a structure once well-diffracting crystals have been obtained.

ELECTRON DENSITY INTERPRETATION

The electron density map is the end product of an experiment in x-ray diffraction followed by mathematical analysis of the data. It results from a Fourier synthesis with the measured diffraction amplitudes and experimentally determined or calculated phases of each reflection to the highest possible resolution. A number of smoothing operations can enhance the quality of the map but cannot make a silk purse out of a sow's ear. For instance, solvent flattening can sharpen the boundaries between solvent and molecule and thereby improve the observed electron density. If more than one molecule is contained in the asymmetric unit, averaging of the electron densities of these molecules can increase the signal-to-noise ratio of the map. Once the best possible electron density map has been calculated, it must now be interpreted to extract a model of the molecule that produced it.

The electron density map is the objective result of a diffraction study. Now comes the subjective part, the part that is no longer experimental and requires some skill in shape fitting. Building a model into electron density requires interpretation on the part of the operator because more than one fit may be possible. The ability to interpret electron density therefore will depend on a number of the factors already mentioned. Probably the most important is the resolution. Resolution is a measure of the level of detail with which a protein is viewed, and different levels provide different kinds of information. For instance, at 5Å resolution, the limits of the protein (i.e., the protein/solvent boundary), the overall shape of the molecule, and elements of secondary structure are apparent; at 3Å resolution, the general course of the polypeptide chain and the shapes of side chains are interpretable; at 1Å resolution, individual atoms are recognizable not only as individual entities of electron density but also as identifiable atom types. The average structure determination does not achieve that level of resolution, but 1.5–2Å resolution is common, especially for ligand complexes. The electron density in such maps should be easily interpretable (Figure 2.6).

A number of factors contribute to the ease with which interpretation is possible. Because the electron density is an average of any position over all of the unit cells that make up the crystal, a sharp electron density depends on



How Good Must the Model Be?
Irving, Whisstock and Lesk. Proteins **42**, 378-82 (2001).

Figure 2.5. The structural similarity between the model and the unknown determines the probability of success in a molecular replacement experiment. Some guidelines are available but are not absolute. Note that the comparison is made in terms of the identity of core residues, that is, those that are expected to be most similar in terms of sequence and structure between two related structures.[8]