strength of the method is the ability to visually understand the binding of ligands and the conformational changes in both the protein and the ligand that are associated with such interactions. The interpretation of interactions in terms of geometry and distances between interacting parts is essential to the design of new interacting species that can take advantage of these and potentially new interactions, that is, the basis of structure-based drug design.

However, there are also obvious weaknesses in the method. Unless there is obvious disorder in the model of the protein, manifested as poor or absent electron density for parts of it, any one model is a rigid one that may not be an accurate representation of the plasticity of the protein. Thus, any one structure may represent only one form of the protein, of which there may be many. The conditions used for crystallization are rarely representative of the conditions under which the protein functions in the cell. Consequently, interpretation of function from the structure has to consider that the pH, ionic strength, and presence or absence of other molecular species in the model are most likely very different from the native conditions. This usually does not lead to misinterpretation but must be kept in mind nevertheless, because conformational changes may result from such conditions as well as from binding of ligands.

A number of strategies are available to attempt to address these problems. One is to find more than one set of crystallization conditions that vary the pH and constituents of the crystallization solution. Conditions that vary dramatically from high salt to low salt, or at extremes of pH, may provide some insight into the conformational changes that can be associated with such differences in solution conditions. Another is to determine the structure of the protein/ligand complex by two methods: one is to soak the ligand into an existing crystal, and the other is the form the protein/ligand complex in solution and then to crystallize it. If the binding site for the ligand happens to be at a protein/protein interaction site, binding may disrupt this interaction and the integrity of the crystal. Alternatively, the packing of the crystal may prevent the protein from undergoing conformational changes that would occur on ligand interactions in solution. Cocrystallization after binding in solution gets around both of these problems – if the complex crystallizes.

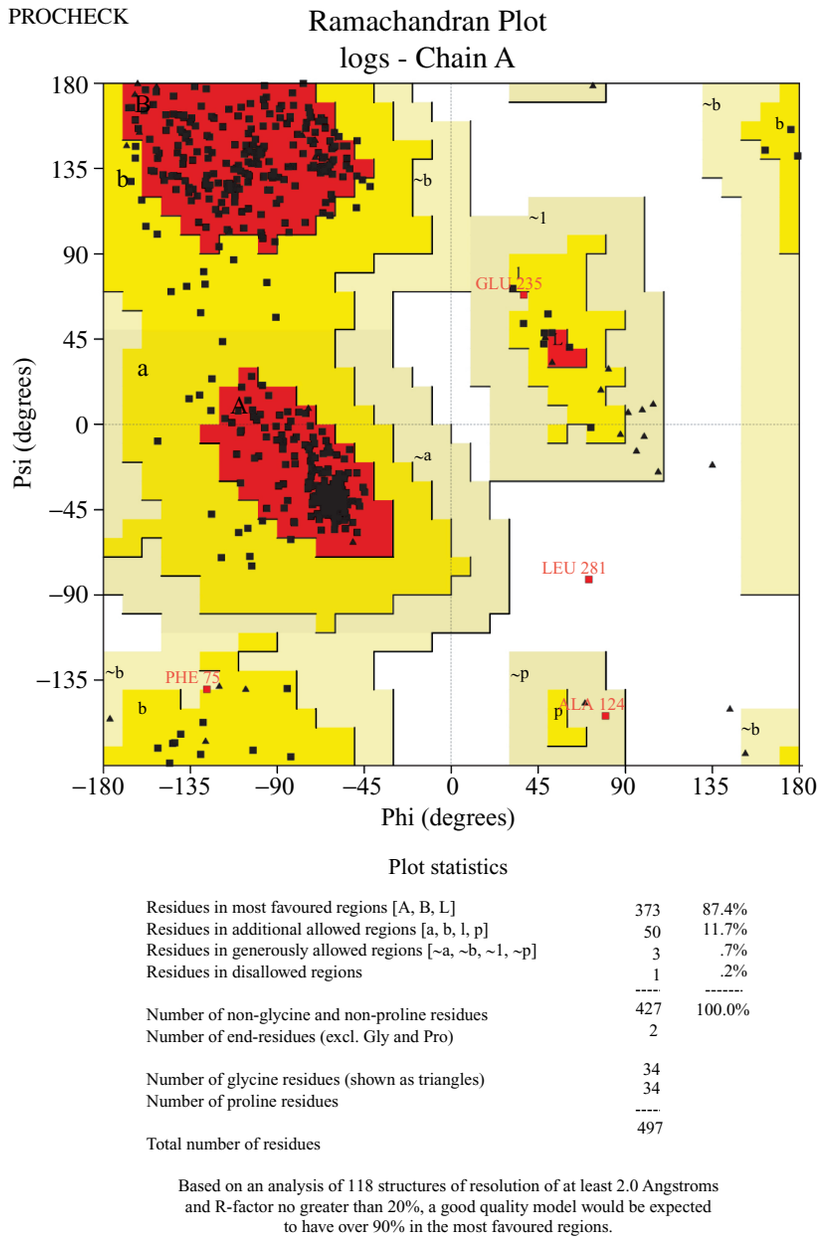

Figure 2.12. Ramachandran plot for glucosidase showing that four residues have angles that do not conform to the ones expected for amino residues in secondary structures. Three of these residues are close enough to be accepted as possible conformations. One of them, Leu281, is considered in an unusual conformation. Inspection of the structural model shows that this residue is in a hydrophobic pocket stabilizing several structural elements. Data were taken from PDB code 1OGS.[2]

FRAGMENT-BASED APPROACHES TO SURFACE MAPPING

Despite the limitations of the crystallographic method, it is clear that the association of small chemical entities can be visualized, just as large ones can, when bound to a protein. The most frequently assigned small molecule on the surface of a protein is water. Once all of the protein is assigned to electron density, and all of the side chains are accounted for, a significant amount of electron density scattered over the surface of the protein is left unaccounted for. Much of that