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X-ray crystallography in the service of structure-based drug design

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Protein crystallography traditionally has been at the base of structure-based drug discovery (SBDD) by providing the structures of protein/ligand complexes that are often the starting point for the design and improvement of specific ligands. Consequently, an awareness of the strengths and weaknesses of this method is important for the success of ligand design. For instance, questions are often raised about the validity of a particular protein structure and whether that structure is relevant to the biological activity of the protein or about the conformation of a bound ligand and whether it represents a productive form. Some of these questions can be answered or at least addressed, whereas others cannot. There is an attempt to address those that can be addressed and to make some suggestions about those that cannot. Therefore, this chapter will focus more on the criteria that can be used to assess the quality of a structure determined by x-ray crystallography and less on the detailed methods used to achieve it.

BASIC CONCEPTS: CRYSTALLIZATION

The basic requirement for a crystal structure is a crystal. Although crystallization of proteins is still more of an art than a science, methods for routine searches of crystallization conditions are indeed available. Historically, the crystallization of proteins was a normal procedure used when working with an enzyme. Because of the known salting out effects of ammonium sulfate, this salt was used to induce selective crystallization, thereby purifying the protein. However, numerous other conditions exist that also promote or prevent crystallization, including pH, presence of counterions or organic compounds, additives with no known rationale, and temperature. A combination matrix of such conditions must be tested to find the best set of conditions that produce not only crystals but crystals of the size and quality required for x-ray diffraction.[1]

How much protein is needed for such a crystallization search? It could be anywhere from micrograms to buckets, depending on how readily conditions are found. What should these crystals look like for a crystallographic experiment? With today's x-ray sources, crystals as small as micrometers on a side are sufficient to obtain measurable diffraction. The most important criterion for successful crystals is the ability to diffract x-rays, and that criterion depends on well-ordered crystals. How to obtain such crystals in a predictable fashion is still not known precisely because each protein seems to have its own characteristics. However, in general, the purity and concentration of the protein, the stability of the protein, and the rate at which crystals form are the dominant features that lead to success (Figure 2.1).

It is a basic fact that if the protein does not crystallize the project cannot proceed. To obtain some structural information despite this roadblock, a number of avenues are available. The most obvious is to obtain a homologous protein from an organism that is different than the target organism and that behaves better with regard to the above criteria. Proteins from thermophiles are especially useful in this context, because they seem to be more thermally stable than their mesophilic counterparts. A number of other approaches are useful in individual cases, such as truncation of a protein to a core domain, selective mutations, and selective modifications. Finally, modification of the protein in a noncovalent fashion, specifically the binding of an inhibitor to an enzyme, often leads to successful crystallization when the apo enzyme is resistant.

For instance, to obtain a crystal structure for the human enzyme glucocerebrosidase, the protein could be purified from tissue directly, or, more commonly when possible, it can be cloned, expressed, and purified from another vector. In the case of glucocerebrocidase, both purified protein from tissue and, later, cloned material from COS cells has been used.[2,3] Mammalian proteins are often posttranslationally modified in their physiological environment, and these modifications, being heterogeneous as a rule, often interfere with crystallizations. Consequently, the proteins may have to be, for instance, deglycosylated to obtain crystals.[3] A protein obtained from a cloned source may lack these modifications or have different ones unique to the cloning cells, and such differences may spell success or failure for crystallization and a structure determination.