

Protein Structure Solution Via Molecular Replacement

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The solution of macromolecular 3-D structures by single-crystal diffraction has become a common and mostly routine process—provided suitable single crystals can be produced. This caveat has lead to difficulties when the proteins form only crystal clusters, highly mosaic single crystals, or micro-crystals. Diffraction of proteins in powder form should broaden the spectrum of candidate proteins, which have historically been overlooked due to unsatisfactory crystal formation. Early work by Von Dreele [1-4] and Margiolaki [5] have demonstrated that the powder diffraction technique is applicable for macromolecules.

In order for this technique to become widespread, the challenges faced by solving the structure of a protein with a non-ideal search model needs to be addressed. In essence, we need to mimic the techniques of single-crystal diffraction, namely molecular replacement. Often, the structure of a related material is known, but it occurs in a different space group. Molecular replacement provides an algorithm of rotation and translation functions to map a related model onto the observed structure factors of the target molecule.

Initial powder studies yielded solutions for molecular replacement of insulin [6] and turkey lysozyme [5], themselves, into alternate space groups. To demonstrate that cross-species molecular replacement is also viable technique for powder diffraction, we present molecular replacement of HEWL using the 60% identical human lysozyme (PDB code: 1LZ1) as the search model. Due to the high incidence of overlaps in powder patterns, we have used four data sets taken at different salt concentrations in a multi-pattern Rietveld

refinement. A superposition of the C α trace of the previously characterized HEWL (PDB code: 194L) and our preliminary molecular replacement solution of the human enzyme reveals the potential of this method (see Figure 1).



Figure 1: Superposition of HEWL (grey) and our human model (Jones Rainbow), shown as Ca traces.

References

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