# Present State of Experimental Phase Determination by Three-beam Diffraction from Macromolecular Crystals

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The phase of the structure factors is lost during a normal x-ray diffraction experiment. Therefore, a direct calculation of electron density from the measured intensities is not possible. Experimental methods to obtain phases require, in most cases, an insertion of heavy atoms into the structure. Then isomorphous replace techniques (MIR) or the MAD technique can be applied. It has been shown that phase information in form of triplet phases can also be obtained by means of three-beam interference experiments. During these experiments, two strong reflections will be excited simultaneously. The wavefield of these two reflections will interfere with eachother via a coupling provided by a third reciprocal lattice vector. Due to this interference, the intensity of the reflections will be changed in a characteristic way that depends on the phase difference (triplet phase) of the structure factors of the three reciprocal lattice vectors involved. A convenient method for generating these three-beam interferences are psi-scan experiments. The prerequisite for these experiments is crystals of low mosaic spread, since otherwise the interference effects of different mosaic blocks overlap and an interpretation is not possible. However, the crystals do not have to be perfect. Three-beam interference experiments have been observed for a number of crystals of small- and medium-size macromolecular structures (e.g., lysozyme, trypsin, proteinase K).[1,2] However, there are some restrictions on the structure factor moduli of those reflections for which triplet phases can be measured. In general, only reflections with large structure factor moduli that give rise to significant interference effects are accessible. Compared to intensity data collection, the speed of an interference experiment is slow (e.g., about 4-6 triplet phases can be determined in one hour from tetr. lysozyme at an ESRF bending magnet beamline). Undulator radiation might speed up the experiment but then radiation decay will be even more severe. It has recently been shown that enough (>800) triplet phases can be measured to calculate a first electron-density map that can be interpreted. The mean-phase error of these measurements compared with the known model is about 20 degrees. Therefore, in principle it is possible to solve the structure of a small protein using the phase information from three-beam interferences. Present investigation is aimed at speeding up the experiment. The first three-beam interference experiments with crystals from an unknown protein structure have already started.

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[1]Huemmer, K., Schwegle, W., Weckert, E., Acta Cryst. A42, 60-62.
[2]Weckert, E., Huemmer, K., Acta Cryst. A53, 108-143.