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Phasing of small proteins by multibeam diffraction using synchrotron radiation

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The phases of structure factors are in general not available in an ordinary two-beam diffraction experiment. By means of a three-beam diffraction experiment, however, in which, in addition to the primary beam, two diffracted beams are excited, thedifference of the structure-factor phases, which is the triplet phase of the involved reflections, can be obtained. The simultaneous excitation of two reflections can be achieved by an azimuthal scan (Ψ-scan) about the reciprocal lattice vector (*rlp*) **h** of a primary reflection that is in diffraction position until the *rlp* of a secondary reflection **g** comes also in diffraction position. The two reflections interfere with each other via *rlp* $\pm$(**h-g**). The information on the triplet phase is contained in the intensity change of the primary reflection due to the excitation of the secondary one.

After this method has been applied successfully to the determination of the absolute structure of small light atom structures and to nonperiodic crystal structures, a gradual development has taken place towards larger structures like small proteins. For multibeam interference profiles with protein crystals in which the interference effects of neighboring three- or multibeam cases do overlap and the overall scattering power is weak and highly collimated, a stable and tuneable synchrotron beam is mandatory, if a large number of phases is required.

Experiments with protein test samples have been carried out at HASYLAB in Hamburg, Germany, and mainly at the ESRF in Grenoble, France. The mean phase error of the measured triplet phases was about 20°. In the case of tetragonal lysozyme, more than 600 triplet phases up to a resolution of 2.5Å have been measured. From these triplet phases, about 550 single phases could be deduced. A 'maximum entropy' extrapolated electron-density map using these reflections already shows the main features of the molecule.