The direct determination of triplet phases $\phi(x) = \phi(h) + \phi(g) - \phi(h-g)$ can be achieved by three-beam interference experiments where two reflections with reciprocal lattice vector $h$ and $g$ are simultaneously excited. The intensity change of the $h$ reflection during a $\gamma$-scan depends on $\phi(h-g)$. This method has been successfully applied to determine the absolute structure of non-centrosymmetric light atom compounds where anomalous dispersion effects can hardly be exploited. Using highly collimated synchrotron radiation (SR) of good stability experimental phase determination is feasible even for small protein structures. Three-beam interference effects have been observed for a number of protein crystals. Tetragonal lysozyme has been chosen as a test candidate for further analyses. About 630 triplet phases have been determined by the use of SR from a bending magnet (Swiss-Norwegian Beamline) at the ESRF in Grenoble, France. These triplets contain reflections up to 2.5 Å resolution. The mean triplet-phase error compared to the known structure is about 20°. Among the measured triplets are 15 $\Sigma_1$-relations which directly give semivariant phases. From these single phase and two origin fixing reflections further single phases can be obtained by measured triplets. Phases that could not be assigned were permuted using magic integers. For each permutation a maximum entropy map was calculated. The entropy of each map as well as a likelihood function proved to be suitable for the indication of maps with small mean phase errors. The extrapolating features of the maximum entropy maps can be used to determine further phases. With this phases a first electron density map has been calculated.

A Yb$^{3+}$-substituted fragment of rat mannose-binding protein A was used in a multiwavelength anomalous dispersion (MAD) experiment to generate a highly accurate and complete set of experimental phases to 1.8 Å resolution (Burling, Weiss, Flaherty and Brunger (1996) Science 271: 72-77). Data were collected at four wavelengths on the X4A beamline at NSLS from a single frozen crystal, using inverse-beam geometry to collect Bijvoet mates. Phases for 99% of the possible reflections to 1.8 Å Bragg spacings were obtained using the least-squares method (MADLSQ) of Hendrickson (1991) Science 254: 51-58), applied to the scaled but unmerged data. An MIR-type probabilistic approach, employing a maximum-likelihood refinement of the heavy atom parameters to provide more realistic figures of merit, was also used to phase the data. The combination of the large anomalous scattering effects at the Yb LIII edge and a large partial structure contribution from 4 Yb$^{3+}$ in the 230-residue asymmetric unit produces extremely large anomalous differences that cause a breakdown of the assumptions commonly used when treating MAD data as an MIR problem. A comparison of phasing by MADLSQ, standard MIR programs, and our modified probabilistic method (see accompanying abstract by A.T.B. F.T.B., K.M.F. W.L.W., session 02.04) will be presented. This set of experimental phases will be used for testing improved refinement methods, including bulk solvent and multi-conformer models.

Protein complexes with xenon and krypton are highly isomorphous with the native structure and can be used as heavy atom derivatives for phase determination. A general method for the preparation of such derivatives is presented. A device has been designed which allows diffraction studies on protein crystals under gas pressures up to 60 bar. Crystal mounting and X-ray data collection do not significantly differ from standard techniques (Schiltz et al. (1994) J. Appl. Cryst. 27, 950-960).

As a test case, X-ray diffraction data at the high-energy side of the krypton K-edge (0.86 Å) were collected on a crystal of elastase (molecular weight of 25.9 kDa) and put under a gas pressure of 56 bar. Although the overall occupancy of the single krypton atom is approximately 0.5 (giving isomorphous and anomalous scattering strengths of respectively 18 and 1.9 electrons), this derivative could be used successfully for phase determination with the SIRAS method (Single Isomorphous Replacement with Anomalous Scattering). After phase improvement by solvent flattening, the resulting electron density map is of exceptionally high quality and displays a correlation coefficient of 0.85 with a map calculated from the refined native structure. Careful data collection and processing, as well as a proper statistical treatment of isomorphous and anomalous signals have proven to be crucial in the determination of this electron-density map.