MS01.03.02 RECENT ADVANCES IN EXPERIMENTAL PHASE DETERMINATION USING THREE-BEAM DIFFRACTION. E. Weckert, Institut für Kristallographie, Universität Karlsruhe (TH), Kaiserstr. 12, D-76128 Karlsruhe, Germany

The direct determination of triplet phases ($\Phi_T=\phi(-h)+\phi(g)+\phi(h-g)$) 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 Ψ-scan depends on Φ_T. 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 16 Σ_1-relations which directly give seminvariant 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 maps as well as a likelihood function proved to be suitable 'figures of merits' 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.

MS01.03.03 ACCURATE HIGH-RESOLUTION PHASES FROM MAD ANALYSIS: EXPERIMENT AND COMPARISON OF PHASING METHODS. William I. Weis+, Kevin M. Flaherty+, F. Temple Burling*, Axel T. Brunger*, +Dept. of Structural Biology, Stanford University School of Medicine, Stanford, CA, USA, *Howard Hughes Medical Institute and Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA

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, Weis, 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 probabalistic approach, employing a maximumlikelihood refinement of the heavy atom parameters to provide more realistic figuresof-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 probabalistic method (see accompanying abstract by A.T.B, F.T.B., K.M.F. W.I.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.

MS01.03.04 XENON AND KRYPTON AS HEAVY ATOMS AND ANOMALOUS SCATTERERS Marc Schiltz & Thierry Prangé LURE, Université Paris-Sud, Bât. 209d, 91405 - Orsay Cedex, France

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.

MS01.03.05 DIRECT METHODS PHASING OF A 450 ATOM STRUCTURE. Gil Privé[1] and David Eisenberg[2]. [1]Ontario Cancer Institute, 610 University Ave., Toronto, Canada M5G 2M9. [2]Molecular Biology Institute, UCLA, Los Angeles, California 90024-1570, USA

The ***Shake-and-Bake*** method can generate automatic solutions for large structures provided that atomic resolution data are available. We have solved the structure of a triclinic crystal containing four independent copies of a twelve residue a-helical peptide using this method. The final structure contains 408 peptide atoms, 2 ethanolamine molecules, 1 MPD, 1 chloride ion and 25 water molecules, for a total of 450 non-hydrogen atoms. The structure has been refined with SHELXL-93 to R=.112 and R_{free}=0.132 for all data to 0.92 Å (23,681 reflections). The success depended critically on the accurate collection of high resolution data, and special care was taken to measure a complete dataset for the P1 crystals. A single crystal was flash frozen at beamline X12C at the NSLS, and data were collected on the 30 cm MAR image plate detector. Different detector 2Q offsets were used to collect the high angle data, and several crystal orientations were required to get complete coverage of the unique hemisphere of reciprocal space. This posed special problems for the data collection strategy because of the very short crystal-to-detector distance used. The structure of alpha-1 reveals an unusual arrangement of helices packed head-to-tail into quasi-infinite columns, which in turn associate laterally into extended sheets of tightly packed α-helices. The sheets then form layers with alternating hydrophobic and hydrophilic interfaces. Certain elements of the intended 4-helix bundle design are found in the structure, as well as many novel features.