s8b.m2.o1 **An Automated Structure Determination System Incorporating SHARP, ARP/wARP and BUSTER/TNT.** E. Blanc[a], C. Vonrhein[a], P. Roversi[b] and G. Bricogne[b,c]. [a]*Global Phasing Ltd, Sheraton House, Castle Park, Cambridge CB3 0AX, United Kingdom.* [b]*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom.* [c]*LURE, Batiment 209D, 91405 Orsay, France.*
Keywords: phase determination, refinement, maximum likelyhood.

An automated software system for macromolecular structure determination has been assembled and tested. It comprises a heavy-atom detector (autoSHARP) used in combination with the heavy-atom refinement and phasing program SHARP, followed by a choice of density modification procedure (DM or SOLOMON) and the automatic map interpretation and model-building program ARP/wARP. The latter has so far used REFMAC as its refinement engine, but it can also call the BUSTER/TNT system for maximum-likelihood structure refinement and take advantage of its powerful maximum-entropy completion feature.

Examples will be presented, which show how this system can not only determine structures with a minimum of user intervention, but also allow weaker phasing signals to be exploited than had so far been possible.

s8b.m2.o2 **Phasing by Three-Beam Diffraction. Present State and Future Prospects.** E. Weckert, R. Müller, J. Zellner, *Institut für Kristallographie, Universität Karlsruhe (TH), 76128 Karlsruhe, Germany*
Keywords: multi-beam diffraction, experimental phasing.

During the last years it has been shown that triplet phases can be determined by three-beam interferences not only from crystals of small molecule compounds but also from crystals of small and medium size proteins[1]. During a three-beam interference experiment the phase information is retrieved from the intensity change due to the interference of two simultaneously excited wave fields inside a crystal. Hereby, a primary reciprocal lattice vector (rlv) **h** is aligned to its diffraction position. This will give rise to a first wavefield. By so-called ψ-scan about **h** the tips of other rlvs **g** are brought to the Ewald sphere generating additional secondary wave fields. These do interfere with the primary one by their respective coupling rlv **h-g** and give rise to the characteristic ψ-scan interference profiles containing the information on the invariant triplet phase $\Phi 3 = \phi(g) + \phi(h - g) - \phi(h)$

In the small molecule regime experimentally determined triplet phases are very helpful for the determination of the absolute structure of light atom compounds (e.g. pure CHN compounds) which can hardly be achieved by other methods.

Three-beam interference effects could be measured from protein crystals with unit cells from 26000Å to 1.2×10^6 Å^3. The main prerequisites for this kind of experiments are crystals of very good quality and a tunable and parallel radiation source. Using the tetragonal form of hen-egg white lysozyme it could be shown that it is possible to measure a number of triplet phases (850, mean phase difference to PDB entry 193R: 17.5º) that would be sufficient for the determination of this protein crystal structure[2,3]. This methodological study revealed that depending on the maximum resolution of the intensity data set available by using a maximum entropy based algorithm to derive single from triplet phases and the application of an automatic refinement procedure[4] about 6.5 to 2.5 triplet phases (for 2.0 to 1.4Å resolution, respectively) per residue are necessary for an automatic solution. Meanwhile about half the number of triplet phases necessary for a successful structure determination of an unknown protein crystal structure have been measured. Efforts in completing this phase set are under way.

The future prospects of this method depend mainly on the availability of crystals of low mosaicity.

[1] E. Weckert, K. Hümmer (1997), *Acta Cryst.* A**53**, 108-143.
[2] Weckert et al. (1999) *Acta Cryst.* D**55**, 1320-1328.
[3] Hölzer et al. (2000) *Acta Cryst.* D**56**, 322-327.
[4] A. Perrakis et al. (1997) *Acta Cryst.* D**53**, 448-455.
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