s8b.m2.o3 **Density constraints and direct phasing** A. Urzhumtsev, V.Y. Lunin, N. Lunina, *LCM³B,UPRESA 7036, Université Nancy I, 54506 Vandoeuvre, France; IMPB RAS, Pushchino 142290 Russia.*
Keywords: direct phasing, density constraints, macromolecules.

Last years, there is an increasing interest to the structure analysis at non atomic resolution which can be explained by a number of reasons : an understanding of the importance of low resolution reflections for refinement and for density map calculation; new technical developments, specially synchrotrons, which made the collection of these data possible; a number of practical cases where the work with conventional methods is not possible or is not successful. Phasing methods based on these low resolution data work as complementary tools for structure determination which in number of situations become the only possible in order to get a crystallographic image.

A number of previous attempts have shown a possibility to solve the phase problem *ab initio*, using some information of a general type in addition to low resolution diffraction magnitudes. However, due to strong and not specific contribution of the bulk solvent to structure factors at the resolution 10-20 Å, the phase search at such low resolution should be either based on the complete models of the unit cell, including that for the bulk solvent, or should not use the models at all.

In the latter case of a search in the phase space, for every phase set taken together with the set of magnitudes measured experimentally, a Fourier synthesis can be calculated, and its features can serve for the search of the solution of the phase problem. Various types of information can be used : restraints on the density values (maximum entropy, histograms, etc.) or on the position of points with a given density value. Such information is usually weak, it does not allow to select unambiguously the solution, however, it allows to increase the percentage of "relatively good variants" in the selected "population of variants", in comparison with the percentage for the initial population of randomly generated ones. Naturally, this enrichment depends on the information and on the criteria used.

At electron density maps, macromolecules in the crystal are usually represented by globular connected regions of the same shape (and, as a consequence, of the same volume). This information on connectivity of macromolecular images was formalised through some numerical criteria and used for the direct phasing. Corresponding enrichment of the "population" of the phase sets by "relatively good variants" was shown to be strong enough in order to provide an image of a reasonably high quality, showing the molecular packing, its shape and important structural features.

Successful applications of this technique to a number of low resolution test cases including some at a 5Å resolution, and to the structure determination of the LDL complex at 27-Å resolution model explaining the known electron microscopy images of this particle, have been reported.

s8b.m2.o4 **Determining the Structure of a Metalloproteinase at Atomic Resolution**. K.E. McAuley, J.-X., Yao, , E.J. Dodson, K.S. Wilson *YSBL, Dept. of Chemistry* and M. M. Woolfson *University of York, Dept. of Physics, York YO10 5DD, U.K.*
Keywords: methods crystallography, phasing, structure solution.

NPII is a zinc-containing neutral metalloproteinase from *Aspergillus oryzae*. There is no apparent sequence homology between this protein and any other protein in the protein data bank.

NPII was crystallised from 1.8 M Na, K phosphate, 0.1M HEPES pH 7.5. The space group is $P2_1$ with unit cell dimensions a = 38.43, b = 34.76, c = 60.28, β = 106.03. Data to 1.005Å were collected at beamline ID14-2 at the ESRF synchrotron facility. The data are 99.7% complete between 30 - 1 Å, and the overall R-merge is 5.2% (24.0% in the outer shell).

The position of the Zn ion was located from the anomalous difference Patterson map. Although the wavelength was not optimised for anomalous scatering by Zn, there was a strong peak in the Patterson map. The coordinates of the Zn were input into the program Acorn[1] and the phase problem was solved by a combination of direct methods and dynamic density modification.

Arp-Warp 5.1 was used to build the main-chain atoms (mode warpNtrace) starting from the phases calculated by Acorn. After 2 building cycles and 12 refinement cycles, 173 residues (out of 177) had been built into 2 protein chains. At this point the R-factor was 19.2% and the Free-R was 22.7%. The side-chains were added using the side-dock option in arp-warp. The missing residues were the N-terminal residue, the C-terminal residue and 2 residues including a cis-proline. These were built into the model using Quanta. Refinement was continued with alternating cycles of maximum likelihood refinement in REFMAC and solvent-building using arp-warp. After 5 cycles, 276 waters had been added and the R-factor was 12.1%, Free-R = 14.5%.

[1] Foadi J., Woolfson, M.M., Dodson, E.J., Wilson, K.S., Jia-xing, Y. & Chao-de, Z. "A Flexible and Efficient Procedure for the Solution and Phase Refinement of Protein Structures", Acta Crystallogr. D, manuscript submitted.