PS02.06.13 DIRECT METHODS PHASE IMPROVEMENT FOR MACROMOLECULAR STRUCTURES USING EITHER SIR OR SAS DATA. D. A. Langs, R. H. Blessing, G. D. Smith, D. Y. Guo. Hauptman-Woodward Medical Research Institute, 73 High St., Buffalo, NY 14203, and Roswell Park Cancer Institute, Buffalo, NY 14263, U.S.A.

Successful efforts at SIR phasing[1] using real data measured for three separate derivatives of cytochrome c_{550} at 2.5Å resolution[2] (mean phase errors 40-47°) were first reported at the 1994 Atlanta ACA meeting. However, phasing methods employing 2.5Å error-free SAS data for the $PtCl_4$ derivative of the same structure, although effective, proved more problematic in adjusting the optimal conditions for successful refinement.[3]

We subsequently showed to our surprise that a modified tangent formula refinement based on Hauptman's SAS triples invariant estimates[4] could produce acceptable solutions which were readily identified by their phase invariant consistency in about 15% of all randomly seeded trials. An easily interpretable map was obtained using 2.5 Å SAS data measured for the $K_2Pt(NO_2)_4$ derivative of Macromomycin[5] (mean phase error 47°).

Structural applications to larger proteins, however, may require improved SIR/SAS triples estimates in order to achieve sufficient phasing power to produce similar results. Two new methods will be described which have the capability of reducing the phase error in Hauptman's initial SIR and SAS triples estimates. These analyses should allow us to apply existing SIR and SAS phasing methodologies to more complex structures.

1. Langs, Guo & Hauptman (1995). Acta Cryst. **A51**. 535-542.
2. Timkovich & Dickerson (1976). J. Biol. Chem. **251**, 4033-4046.
3. Langs & Han (1995). Acta Cryst. **A51**, 542-547.
4. Hauptman (1982). Acta Cryst. **A38**, 289-294, 632-641.
5. VanRoey & Beerman (1989). Proc. Natl. Acad. Sci. **86**, 6587-6591.

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PS02.06.14 UNIQUENESS AND ALGORITHMS FOR MACROMOLECULAR AB INITIO PHASING. R. P. Millane and W.J. Stroud, Whistler Center for Carbohydrate Research and Comutational Science and Engineering Program Purdue University, West Lafayette, Indiana 47907-1160, U.S.A

Using results on uniqueness properties for multidimensional phase retrieval from continuous Fourier data, and arguments from sampling theory for multidimensional band-limited functions, we show that macromolecular ab initio phasing may be feasible in the presence of modest a priori information such as noncrystallographic symmetry and solvent boundaries. In view of this, we develop a modified density modification algorithm which attempts to reconstruct macromolecular phases without any initial phase information. We apply this algorithm to synthetic data from cowpea mosaic virus (CPMV) with 5-fold non-crystallographic symmetry. The algorithm produced good phases at 40 Å resolution where conventional density modification fails, and also shows superior performance at 20 Å resolution.

PS02.06.15 AB-INITIO PHASE DETERMINATION OF PROTEINS WITH HIGH RESOLUTION DATA BY DIRECT METHODS. Monika Mukherjee, Department of Solid State Physics, Indian Association for the Cultivation of Science, Calcutta-700032, India

The direct method program SAYTAN is applied to a known protein structure Rubredoxin (space group $P2_1$, a=15.97, b=41.45, c=24.41Å, β =108.3°, data upto 1Å resolution) containing 393 protein atomic sites, one Fe atom, a sulfate ion and 102 water molecules. By making 1000 trials at different resolutions with sets of initially random phases SAYTAN yielded ab-initio phases for about 800 reflections with mean phase errors (MPE) of 41, 41, 47, 47, 60° for 1, 1.25, 1.5, 1.75 & 2Å resolutions respectively. The phases were extended to 2000 reflections with MPE ~ 62°. Conventional Multan figures of merit were not useful for macromolecular structures, but modified figures of merit proposed by Mukherjee & Woolfson (Acta Cryst.,1993, D49, 9 -12) and successfully applied in the cases of Avian pancreatic polypeptide (space group C2, data upto 0.89Å,302 protein sites +Zn +80 water sites) and 2-Zn insulin (space group R3, data upto 1.5Å, 806 non-hydrogen atoms + 2Zn + solvent atoms; Mukherjee & Woolfson, Acta Cryst., 1995, D51, 626-628) seem capable of selecting better phase sets in Rubredoxin. This modified FOM's were still heavily based on statistical principles i.e. $\int \rho^3 \cdot dv$ should be maximum, a key property of the map for small structures which is not often true for macromolecular structures. Thus from the high resolution data (upto 2.5Å) of the structures comprising of upto 1000 non-H and some heavy atoms in the asymmetric unit useful sets of phases can be obtained using SAYTAN strategy. The best maps obtained from the direct-methods having map correlation coefficient coefficient 0.48 should benefit from further phase refinement before model fitting is attempted.

The procedure was found to be effective even in the case of known protein without any heavy atom RNAP1(1.17Å, 808 non-H atoms + 83 water molecules). It may be hoped that this approach will allow routine application of direct method to unknown protein structures yielding high resolution data.

PS02.06.16 COMPARATIVE QUANTITATIVE ANALYSIS OF β-Si_3N_4 / β-SiC MIXTURES. Jeffrey P. Nicolich, Zoltan Lences, Wolfgang Dressler and Ralf Riedel. Dept. of Materials Science, Dispersive Solids Group, Technical Universtity, Hilpertstraße 31/D, 60295 Darmstadt, Germany

Mean-normalized-intensity (MNI), reference-intensity-ratio (RIR) and full-pattern-fitting (Rietveld) methods of phase quantification are presented on mixtures of β-Si_3N_4 and β-SiC. Various experimental conditions are compared: Measurements were performed on reflection (Siemens D5000) as well as transmission (STOE) geometry diffractometers. Furthermore, runs were made with routine (fast, no sample rotation) and high-quality (slow, sample rotation) data collection.

Silicon carbide - silicon nitride composite materials are investigated by materials scientists for their improved mechanical properties over the single phases. Exceptionally good creep resistance due to pinning of the β-Si_3N_4 grain boundries by β-SiC particles is expected. Two types of additive free β-Si_3N_4 materials were used: "SHS" is produced by self-propagation high-temperature synthesis, "Denka" is recrystallized from α-Si_3N_4 which in turn is produced by direct nitridation of silicon. The β-SiC is commercial Superior Graphite type.

Both β-SiC and "Denka" silicon nitride are equiaxed, with mean grain sizes of approximately 1 micron. "SHS" silicon nitride crystals are elongated up to 10 - 20 microns in length.

PS02.06.17 *GENPAT* SOLUTION OF THE STRUCTURE OF PROTOCATECHUATE-3,4-DIOXYGENASE FROM *PSEUDOMONAS CEPACIA*. Christer E. Nordman, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA

The newly developed molecular-replacement procedure *GENPAT* (Nordman, *Acta Cryst.* 1994, A50, 68-72) has been used to solve the structure of tetrameric (m.wt. 198,000) protocatechuate-3,4-dioxygenase (PCD) from *P. cepacia* (Ludwig, Weber & Ballou, *J. Biol. Chem.* 1984, 259, 14840-14842) using as a model one subunit (m.wt. 48,900) of dodecameric PCD from *P. aeruginosa* (Ohlendorf, Lipscomb

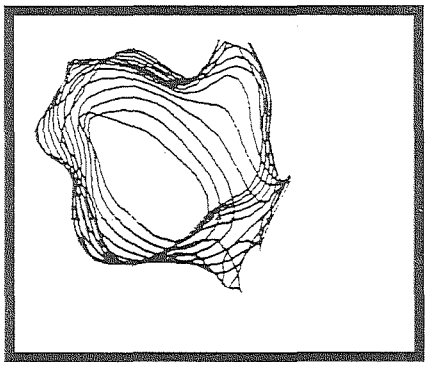
& Weber, *Nature*, 1988, 366, 403-405). The subunit of PCD from *P. aeruginosa* contains 438 residues, and that of *P. cepacia* 431. Both contain one nonheme iron atom. The identity between the two proteins is 48 percent, and there are many deletions and insertions. A conservative search model was used, consisting of 382 residues (1868 atoms) of a polyalanine (glycine) chain, from *P. aeruginosa*, with many gaps, and representing 1.6 percent of the unit cell contents of the *P. cepacia* PCD.

P. cepacia PCD crystallizes in $P2_12_12_1$ with four tetramers per cell. Ludwig *et al.* observed absences of odd orders of ℓ in the $hO\ell$ diffraction pattern, indicating the presence of a non-crystallographic local 2-fold axis at $x \approx 0.25$, and approximately parallel to *c* and suggesting that the local tetramer symmetry is 222. With a tetramer as the asymmetric unit, the rotational search with one monomer would normally give four symmetrically independent solutions. If a noncrystallographic 2-fold axis of the tetramer is parallel to a 2-fold axis in the Patterson, the rotation search solutions reduce to two correspondingly enhanced maxima. Thus the rotation search is facilitated by the orientation of the tetramer 2-fold axis. There is no corresponding enhancement of the maxima in the translation search.

With data to 2.9 Å resolution, the two maxima in the rotation search, mapped as the correlation coefficient, were 0.0809 and 0.0744, or 9.9 and 9.1 σ, respectively, in terms of standard deviations above the background. Spurious peaks were found at up to 3σ; among these were peaks resulting from an approximate 2-fold symmetry within part of the subunit (Ohlendorf *et al.*). The four symmetrically independent solutions to the translation search were found at 3.2 to 4.1σ; the known location of the non-crystallographic 2-fold axis made a fully exhaustive translation search unnecessary.

PS02.06.18 VERY LOW RESOLUTION PHASING ATTEMPTS OF THE RIBOSOMAL 50S PARTICLE FROM T. THERMOPHILUS BY THE FEW ATOMS MODEL METHOD. A.D. Podjarny, A.G. Urzhumtsev and E.A. Vernoslova, UPR de Biologie Structurale, IGBMC, B.P. 163, 67404 Illkirch Cedex, C.U de Strasbourg, France

A suggestion for the phases for the 80 Å resolution X-ray diffraction data from the 50S ribosomal particle of Thermus thermophilus (Volkmann et al., J. Mol. Biol., 216, 239, 1990) has been made using the Few Atoms Model ab initio technique (Lunin et al., Acta Cryst., D51, 896, 1995), in collaboration with A. Yonath and coworkers. This technique generated randomly one million models consisting of 5 pseudo atoms each and selected the 560 solutions which fitted best the observed amplitudes to 60 Å resolution. The selected models were grouped with a clusterisation procedure in a small number of possible solutions. The most adequate one was chosen by imposing the additional constraint that there should be no strong densities on symmetry axes. To refine this result, a second model generation was done imposing stronger amplitude constraints between 120 and 60 Å and density constraints based on the result of the first generation. The map resulting from the second model generation (phased to 80 Å) is shown in the figure. The position and features of the observed envelope agree with those obtained with other ab-initio solution methods and with molecular replacement using models from electron microscopy reconstructions (Volkmann et al., CCP4 Newsletter, 31, 23, 1995).



PS02.06.19 MOLECULAR REPLACEMENT METHOD USING A PARALLEL PROCESSING MACHINE. V.S.Yadava and K.K.Kannan Solid State Physics Division,Bhabha Atomic Research Centre,Bombay-400 085,INDIA.

The molecular replacement method involves six parameters - three rotational and three translational and the correct orientation and position is identified by calculating R-factor at each grid point.

Time requirement: The six-dimensional search requires very large amount of computer time. For a moderate size protien like Carbonic Anhydrase with about 2000 atoms in the molecule and 2000 reflections to 5A requires 20 minutes of cpu time on a Landmark 860 machine for structure amplitude calculations at 1.5Å resolution along the axes for each orientation. For a coarse search with steps of 5 degree in Eulerian angles there are 46656 orientations which require 648 days of computer time. However, with a 64-node parallel-processing system the time required is10 days of the machine time and can be further reduced by using more nodes and faster machines. Program implementation: As the calculations for each orientation are independent of that for others, the different orientations are equally distributed between different nodes. Each node has all the information for calculating structure amplitudes and Rvalue.

Results: The method has been tested first with Human Carbonic Anhydrase (HCA) I data and the same protein as model structure. Next HCA II was used as the model structure for obtaining structure of HCA I. Lowest R-value corresponded to correct orientation and position in both the cases.

PS02.06.20 AUTOINDEXING OF MULTIPHASE POLYCRYSTALS. V.B.Zlokazov FLNP JINR, 141980 Dubna, Moscow region, Russia. E-mail: Zlokazov@main1.jinr.dubna.su

Let a set of interplanar spacings (d_j), $j = 1,2,..,m$ be given, which are diffraction reflections from a *n*-phase polycrystal. The autoindexing problem is solved by minimizing the following functional

$$\sum_{i=1}^{n} \rho\left(\vec{d}, \vec{f}\left(\vec{P}_i, \vec{h}_i\right)\right) + \alpha V\left(\vec{P}_i\right) + \beta N\left(\vec{P}_i, \vec{h}_i, \delta\right) \quad (1)$$

The first member is

$$\rho = \sum_{j=1}^{m} \delta_1\left[d_j, f\left(\vec{P}_i, \vec{h}_j *\right)\right] \quad (2)$$

where $\vec{h}_j *$ = index values, minimizing expressions

$$R_j = \delta_2\left[d_j, f\left(\vec{P}_i, \vec{h}_j\right)\right] \quad (3)$$