

**PS02.06.13 DIRECT METHODS PHASE IMPROVEMENT FOR MACROMOLECULAR STRUCTURES USING EITHER SIR OR SAS DATA.** D. A. Langa, 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<sup>1</sup> using real data measured for three separate derivatives of cytochrome *c*<sub>550</sub> at 2.5 Å resolution<sup>2</sup> (mean phase errors 40-47°) were first reported at the 1994 Atlanta ACA meeting. However, phasing methods employing 2.5 Å error-free SAS data for the PtCl<sub>4</sub> derivative of the same structure, although effective, proved more problematic in adjusting the optimal conditions for successful refinement.<sup>3</sup>

We subsequently showed to our surprise that a modified tangent formula refinement based on Hauptman's SAS triples invariant estimates<sup>4</sup> 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<sub>2</sub>Pt(NO<sub>2</sub>)<sub>4</sub> derivative of Macromomycin<sup>5</sup> (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. Langa, Guo & Hauptman (1995). *Acta Cryst.* **A51**, 535-542.
2. Timkovich & Dickerson (1976). *J. Biol. Chem.* **251**, 4033-4046.
3. Langa & 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.

Support from NIH grant GM-46733 is gratefully acknowledged.

**PS02.06.14 UNIQUENESS AND ALGORITHMS FOR MACROMOLECULAR AB INITIO PHASING.** R. P. Millane and W.J. Stroud, Whistler Center for Carbohydrate Research and Computational 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<sub>1</sub>, a=15.97, b=41.45, c=24.41 Å, β=108.3°, data upto 1 Å 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 & 2 Å 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 0.89 Å, 302 protein sites + Zn + 80 water sites) and 2-Zn insulin (space group R3, data upto 1.5 Å, 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 2.5 Å) 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 (1.17 Å, 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<sub>3</sub>N<sub>4</sub> / β-SiC MIXTURES.** Jeffrey P. Nicolich, Zoltan Lences, Wolfgang Dressler and Ralf Riedel. Dept. of Materials Science, Dispersive Solids Group, Technical University, 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<sub>3</sub>N<sub>4</sub> 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<sub>3</sub>N<sub>4</sub> grain boundaries by β-SiC particles is expected. Two types of additive free β-Si<sub>3</sub>N<sub>4</sub> materials were used: "SHS" is produced by self-propagation high-temperature synthesis, "Denka" is recrystallized from α-Si<sub>3</sub>N<sub>4</sub> 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) protococatechuate-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