01.2-01 PROTEIN STRUCTURE DETERMINATION BY THE SINGLE ISOMORPHOUS REPLACEMENT METHOD WITH A PHASE SELECTION AND REFINEMENT PROCESS. By Bi-Cheng Wang, Biocrystallography Laboratory, Box 12055, V A Medical Center, Pittsburgh, Pa 15240, U.S.A. and Department of Crystallography, University of Pittsburgh, Pittsburgh, Pa 15260, U.S.A.

A novel approach in applying the single isomorphous replacement(SIR) method in protein structure determination is being developed. The goal is first to break the phase ambiguity situation inherent in the conventional SIR technique and then to carry out a phase refinement. A procedure which has given good results involves the modification of the original SIR probability function with the calculated phase information obtained from Fourier inversion. The process is carried out in repeated cycles until the phase angles and other statistical criterea converge. Although anomalous scattering data could easily be incorporated into the method, it is not required. This procedure has been tested on Bence Jones Protein Rhe (Wang et al. J. Mol. Biol. 129, 657-674, 1979) using native and single derivative data only. The "MODIFIED SIR" map at 3 Å resolution not only has reproduced accurately the electron density for the Rhe polypeptide backbone and its side chains but also had a low background. Therefore it is reasonable to assume that the structure of Rhe could have been determined by this procedure with only one isomorphous heavy atom derivative. This technique is being tested on another protein of known structure and several whose structures are unknown. The method and the testing results will be discussed.

01.2-02 PRACTICAL ASSESSMENT OF THE FEASIBILITY OF PHASING PROTEIN STRUCTURE AMPLITUDES USING ANOMALOUS SCATTERING AND PARTIAL STRUCTURE INFORMATION. By Lennart Sjölin*+, Janet L. Smith#, Alexander Wlodawer*+, and Wayne A. Hendrickson#, *LMB, NIADDKD, National Institutes of Health, Bethesda, MD 20205, +National Measurement Laboratory, National Bureau of Standards, Washington, DC 20234, and #Laboratory for the Structured Matter, Naval Research Laboratory, Washington, DC 20375.

We have investigated the practical feasibility of phasing diffraction data from a small protein on the basis of anomalous scattering of both soaked-in heavy atoms and intrinsic sulfurs. As a test case, we have chosen ribonuclease A (MW = 13250, space group $P2_1$). A derivative was prepared by soaking crystals in a solution of K_2PtCl_6. Friedel pairs to 2.5 Å resolution were collected on a diffractometer with care taken to reduce the errors in Bijvoet differences. Scaled data showed rms (ΔF) of 2.2 for acentric data, as compared with 1.3 for the centric, and the anomalous signal was estimated to be 2.8 percent of the rms (F).

The Patterson maps computed with the coefficients $(\Delta F)^2$ were in good agreement with calculations based on the known structure of anomalous scatterers. The positions of three platinum (occupancies of 0.14, 0.13, and 0.04) and 12 sulfur atoms could be refined to R = 0.36 against the strongest 25 percent of the Bijvoet differences to 3.0 A.

We plan to collect data from crystals more heavily soaked in K_2PtCl_6 and will report on resolved-anomalous phasing attempts with these data.

01.2-03 IRON-RESOLVED ANOMALOUS PHASING AND LOCAL SYMMETRY AVERAGING IN THE STRUCTURE SOLUTION OF TRIMERIC HEMERYTHRIN. By Janet L. Smith and Wayne A. Hendrickson, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D. C. 20375, U.S.A.

The structure of trimeric hemerythrin, the respiratory oxygen transport protein of Siphonosoma funafuti, has been solved at 5.5Å resolution from the anomalous scattering of native iron atoms using CuKα X-radiation. The asymmetric unit of the crystal ($P2_1$, a=80.31Å, b=45.11, c=62.58, β=104.8°) consists of a trimer of identical 14,000 Dalton subunits, each containing a dimeric iron active center. Positions of the three iron-centers were determined in an anomalous-difference Patterson map. Electron density maps were calculated for both hands of the iron structure by the resolved-anomalous phasing procedure and the correct hand was selected by comparing threefold-averaged maps. Local symmetry averaging and solvent leveling were used to refine the original phases through successive cycles of averaging/leveling of electron density, Fourier inversion, probabilistic phase combination, Fourier synethesis and least-squares optimization of the triad axis. The molecular envelopes used in this refinement were generated by an automatic procedure from electron density maps threefold averaged without knowledge of the molecular boundary.

The resulting subunit structure is very similar to that of other hemerythrin structures, and once its orientation in the trimer and monoclinic cell were known, it was possible to construct a trimer map from a myohemerythrin electron density function. The structure amplitudes obtained by Fourier inversion of this map agree with those observed for the trimer at an R-index of 0.370 for all data to 5.5Å resolution. The phases from this molecular repositioning are being refined by a procedure similar to that described above. Comparison of the results of the parallel refinements and details of the resolved anomalous phasing procedure will be presented.

We have recently obtained crystals of Siphonosoma cumanense trimeric hemerythrin from A. W. Addison, Drexel University. There are also three hemerythrin subunits per asymmetric unit of this crystal ($C222_1$, a=88.22Å, b=58.26, c=132.88) and we plan an attack similar to that used in the S. funafuti problem. We are interested in this protein as part of our study of quaternary variability in hemerythrins, because the protein has been observed by Addison to form higher aggregates in solution. The presence of twofold axes in the space group $C222_1$ does not preclude the occurrence of hexamers in this crystal.