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 backbone and its side chains but also had a low conventional refinement. Therefore it is reasonable to assume that phase angles and other statistical criteria converge. The process is carried out in repeated cycles until the first to 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 procedure has been tested on Bence Jones protein. Incorporation of the Bijvoet differences to reduce the errors in Bijvoet differences. The electron density maps were in good agreement with calculations based on Patterson maps computed with the coefficients $4F - F$ and $2F - F$. The first to resolution not only has reproduced accurately the electron density for the native and single derivative data only. The "modified SIR" map at 3 Å resolution not only has reproduced accurately the electron density for the Rho polymer雉 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.

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 myohemerythin 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 5.5 Å 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, 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 hemerythins, because the protein has been observed by Addison to form higher aggregates in solution. The presence of twofold axes in the space group C222, does not preclude the occurrence of hexamer in this crystal.