Names and affiliations of applicants (* indicates experimentalists):

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Report:

Crystals of CC3 were obtained in space group $P2_1$ with cell dimensions $a=60.5$ Å, $b=106.3$ Å, $c=80.8$ Å and $\beta=103.5°$. Diffraction data were collected using a 345 mm MAR scanner in 300 mm mode from a small $(0.4 \times 0.2 \times 0.2$ mm$^3)$ frozen crystal of CC3 at three suitable wavelengths near the Fe absorption edge, suitably chosen from an X-ray fluorescence scan. A fourth data set was measured at λ near 0.9 Å. The diffraction images were processed with DENZO and the resulting intensities scaled with SCALEPACK in such a way as to preserve the multiple observations of all the measured Bijvoet mates. The CCP4 program suite was then used to merge the scaled data (ROTAPREP / AGROVATA / TRUNCATE) and to scale together the different wavelength data (SCALEIT).

The anomalous difference Patterson map calculated from data with maximal anomalous differences (λ_2 1.7377 Å) showed a large number of peaks which failed to be interpreted by inspection. The MADSYS suite was then used to extract the $f°_A$ component from the 4-wavelength data set.

The independent 18 Fe sites were then located with SHELXS-97 using a combination of Patterson vector superposition search and partial structure expansion using direct methods. These positions were used to derive the non-crystallographic symmetry operations between the two independent molecules (LSQKAB), each with 9 heme groups instead of 12 as expected. Phase refinement with MLPHARE in the resolution range $10 \geq d \geq 2.9$ Å converged to an overall f.o.m. of 0.78. Density modification, averaging, solvent flattening and phase extension ($20 \geq d \geq 2.4$ Å) procedures (DM) produced a much improved electron density map. Also, phase refinement and density modification calculations ($20 \geq d \geq 2.4$ Å) were carried out with SHARP/SOLOMON to produce an electron density map which was superior in quality to that obtained with MLPHAREYDM and allowed some ambiguities in the electron density to be clearly resolved. Both maps allowed a nearly complete trace of the polypeptide chain, and although only the sequence of the 39 N-terminal residues is known, the quality of these maps allowed the side-chain identification of more than 90% of the residues in the protein. Since in this experiment high quality diffraction data were collected to 1.8 Å resolution it is expected that an electron density map of excellent quality will be obtained in the course of refinement, allowing the identification of most of the remaining side chains. The current model has 291 residues and 9 heme groups in each of the two independent molecules in the unit cell.
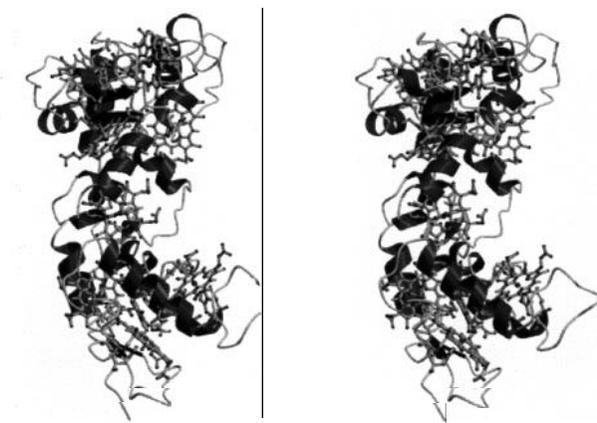
Ribbon diagram stereoview of the CC3 molecule