02-Methods for Structure Determination and Analysis, Computing and Graphics

02.01 – Direct Methods of Phasing Macromolecules

MS-02.01.01 PHASE DETERMINATION, EXTENSION AND REFINEMENT BY MAXIMUM ENTROPY METHODS. E. Prince*[1], L. Sjölin[1,2] and D. M. Collins[3].
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An electron density distribution of the form

$$\rho_k(\mathbf{x}) = \exp\left[\sum_{j=1}^{m} x_j \cos(2\pi \mathbf{h}_j \cdot \mathbf{r}_k - \phi_j)\right]$$

maximizes the entropy, $S = -\sum_{k=1}^{n} \rho_k \ln \rho_k$, under the constraint that structure amplitudes are equal to their experimentally observed values. The values of the logarithmic-space Fourier coefficients, x_j, that give exact agreement with observed amplitudes may be determined by a numerical procedure that is equivalent to finding the unconstrained minimum of a dual function whose Hessian matrix is everywhere positive definite, so that the problem has a unique solution for any given set of trial phases, ϕ_j. $\rho_k(\mathbf{x})$ must be positive, and, as Karle & Hauptman (*Acta Cryst.*, 1950, **3**, 181-187) have shown, if a large enough starting set of phases is known, this condition puts tight restrictions on the amplitudes and phases of other structure factors. Using this procedure, we have developed several tools for the determination, extension and refinement of phases.

The first tool uses an overall constraint on a low-resolution density map to extend phases to higher resolution. Entropy maximization gives a map that is similar to the original map, but with sharper peaks and flatter valleys, which results in non-vanishing structure amplitudes at higher resolution. The phases of these reflections are used with the observed amplitudes to produce a higher resolution map. This tool has been used to complete the solution of several structures, including fragment TR_2C of bull testis calmodulin (Sjölin, L., Svensson, L. A., Prince, E. & Sundell, S., *Acta Cryst.*, 1990, B**46**, 209-215), fragment TR_1C of calmodulin and azurin mutant F114A from pseudomonas aeruginosa.

A second tool allows the most discrepant reflections from a partially determined structure to be fitted, which results in the refinement of all phases. This tool was used to locate a calcium atom in fragment 1 of bovine prothrombin.

If the starting set of phases is not large enough, any set of phases for additional reflections will produce a positive density map. Another tool enables the comparison of the maps produced by various trial phases. Using the entropy of the map as a figure of merit, this tool was used to determine, *ab initio*, a set of phases for the known structure of recombinant bovine chymosin that was in remarkably good agreement with the phases calculated from the refined structure (Sjölin, L., Prince, E., Svensson, L. A. & Gilliland, G. L., *Acta Cryst.*, 1991, A**47**, 216-223). An attempt was then made to determine phases for the incomplete structure of Mojave toxin and the unknown structure of bovine heart creatine kinase. The creatine kinase map clearly shows the position of the molecule and contains features found in protein structures, such as α helices, β sheets and hairpin loops, but its connectivity is not clear enough to enable satisfactory interpretation. Research on this aspect of the problem is continuing.

MS-02.01.02 EXPERIMENTAL DETERMINATION OF TRIPLET PHASES OF MACROMOLECULAR STRUCTURES by K.Hümmer* and E.Weckert, Institut für Kristallographie, Universität (TH), Kaiserstr.12, D-7500 Karlsruhe, Germany

It has been shown that triplet-phase invariants

$$\phi = -\varphi(\mathbf{h}) + \varphi(\mathbf{g}) + \varphi(\mathbf{h}\text{-}\mathbf{g})$$

can be determined experimentally by means of three-beam X-ray diffraction with an accuracy of 45° (Hümmer K., Weckert E. & Bondza H., Acta Cryst. (1990) **A46**, 393-402), where the φ's are the phases of the structure factors involved. The phase information is contained in ψ-scan profiles rotating the crystal around the scattering vector **h** of the primary reflection bringing a secondary reflection **g** into its reflection position. The coherent dynamical interaction of both reflections via the difference vector **h**-**g** results in typical changes of the intensities of the individual reflections due to interference effects which depend on ϕ. In case of small molecule structures with unit cells up to 5 nm^3 using synchrotron radiation it is possible to select a wavelength that adjacent three-beam profiles do not overlap. This is impossible for macromolecular structures because of the dense reciprocal lattice. However, the problem of overlapping multiple beam cases can be reduced to a pseudo three-beam case. Taking into account appropriate structure factor moduli the overlapping multiple-beam cases **h**/**g**'/**h**-**g**' do not significantly affect the ψ-scan profile of a selected three-beam case **h**/**g**/**h**-**g**. It has been found semi-empirically that this condition is met if the ratio

$$Q(\mathbf{hg}) = |F(\mathbf{g})|\ |F(\mathbf{h}\text{-}\mathbf{g})|\ /\ |F(\mathbf{h})|^2$$

is in the range $2 < Q < 6$ and

$$Q(\mathbf{hg}') < 0.2\ Q(\mathbf{hg})$$

though the number of overlaps may be several hundreds.

As to macromolecules the crystal dimensions are usually smaller than the Pendellösung length. It is shown that inspite of this fact the interference contrast is strong enough that it can be exploited for phase determination.
Experimental results are shown for myoglobin, lysozyme and catalase. In case of hen-egg lysozyme (unit cell volume about 250 mm^3) is was possible to measure 80 triplet phases where reflections of the low and medium resolution range up to 4 Å are involved. The mean phase error is 17 degrees compared to the triplet phase calculated from the known structure.
The studies with catalase show that even for structures of that size experimental phase determination may become feasible.
The experimental phase information might be used for solving macromolecular structures in combination with conventional methods.

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