



Fig. 1 SAXS is first used to define the outer envelope of the AxNiR structure (shown as a solid surface in the right hand portion of the figure and as dots on the left hand side). The chains show how the refined `crystal` structure fits within the envelope.

Solution of the phase problem is central to crystallographic structure determination. Small angle X-ray scattering (SAXS) data have proven to be very useful in providing low resolution structural details of proteins and other macromolecules in solution. The spatial parameters of a structure's molecular envelope can be determined in a model-independent manner [1,2]. We have recently proposed and tested a method [3,4,5] to locate such an envelope in a crystallographic unit cell by performing a simultaneous 6 dimensional search on orientation and translation to find the best match between experimental structure factors, F_{obs} and calculated ones, F_c . By using this method (implemented in the program FSEARCH) together with other phase extension techniques, macromolecular structures could potentially be solved without the requirement of incorporating heavy atoms into the protein. FSEARCH has been successful in locating a SAXS molecular shape within the crystallographic unit cell for the cases of the trimeric nitrite reductase (AxNiR, 105kDa; shown in Figure 1) and the dimeric superoxide dismutase (SOD, 32kDa). This method is particularly useful when only low-resolution diffraction data ($>8\text{\AA}$) are available; in this case the conventional Patterson-search based methods may not be appropriate, as the density inside the envelope is uniform, so that there is no discrimination between intra-envelope Patterson

vectors. The program can also be used in general six-dimensional cases for a molecular replacement solution given a pre-determined envelope from any source, such as electron microscopic images (EM), solution scattering (SAXS) or coordinates of a homologous structure, provided that the envelope can be converted to the standard PDB format or expressed in terms of spherical harmonics.

FSEARCH has recently been implemented on the MacCHESS Linux cluster, SIRIUS. A parallel-aware version, MPI_FSEARCH, has been used successfully to perform an exhaustive 6-dimensional search to phase very low resolution x-ray data using a molecular envelope (Liu et al., manuscript in preparation).

It is anticipated that the low resolution phases calculated from the correctly positioned molecular envelope can be used as a good starting point for phase extension through the use of genetic algorithms whereby the mask would be used as a container within the macromolecule's internal structure would be determined. Some preliminary tests are promising and full results will be reported in due course. Once the resolution of the structure has been improved to $\sim 5\text{\AA}$ using this method, phase extension to higher resolutions may be achieved by maximum entropy and density modification methods (e.g. solvent flattening, histogram matching, non-crystallographic symmetry averaging). Thus, it is hoped that this method will greatly facilitate the ab initio structure determination of proteins and provide a good foundation for further structure refinement. The method is still in its infancy but rapid progress could be made as CHESS provides an ideal environment in which SAXS scientists and crystallographers collaborate closely.

The FSEARCH program is now supported by the CCP4 [6].

References

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