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Low-Resolution Crystallography Is Coming of Age

The threshold of what is considered "acceptable" resolution for obtaining mechanistic insights is being pushed by recent structures at 3.8 to 4.7 Å resolution. One of these structures, that of a fully glycosylated SIV gp120 envelope glycoprotein in an unliganded conformation at 4.0 Å resolution, is described in this issue (Chen et al., 2005).

While numerous structures have been reported over the past decade at low resolution, these studies were typically restricted to simple display of electron density maps, to approximate modeling of such maps, rigid body refinement, or model refinement in the presence of high noncrystallographic symmetry. Low-resolution structures are difficult to solve since electron density maps without heavy atom or anomalous scatterers have few or no pronounced markers and few well-defined side chains that could aide in the assignment of molecular fragments. In addition, amplitude-based model refinement progressively diverges as the resolution cutoff is artificially lowered (Brunger and Rice, 1997).

The technique of multiwavelength anomalous dispersion (MAD) (Hendrickson, 1991) can be used to overcome some of the shortcomings of low-resolution crystal structures. First, the identification of heavy atom or selenium positions leads to markers that guide tracing of the macromolecule(s). Second, the high accuracy of the experimental MAD phase probability distributions allows them to be used directly in the refinement process, improving electron density map quality and making refinement more robust, even below 4 Å resolution. For example, the structure of the complete p97/valosin containing protein (VCP) hexamer in complex with the ATP hydrolysis analog ADP ·AlF_x was solved at 4.7 Å resolution with three independent protomers in the asymmetric unit (DeLaBarre and Brunger, 2003). The structure solution process involved MAD phasingand positional (torsion angle simulated annealing and minimization) and group B-factor refinement with a target function that incorporates phase information (Pannu et al., 1998). B-factor sharpening (Bass et al., 2002) of phase-combined electron density maps allowed for the identification of many side chains in addition to the main chain.

The refinement of p97/VCP · ADP ·AlF_x. made use of the standard Crystallography & NMR System protocols (Brunger et al., 1998) except that the bulk solvent model had to be modified and secondary structure restraints added to stabilize the α helices during simulated annealing refinement. Interestingly, B-factor sharpening only performed well when phase-combined maps were used; B-factor sharpened maps obtained by molecular replacement with a partial model were not interpretable. After the p97/VCP · ADP ·AlF_x. structure was solved crystals of p97/VCP complexed with ADP and AMP-PNP were obtained that diffracted to 4.2 and 3.5 Å, respectively (DeLaBarre and Brunger, 2005). Structures were solved and refined with a similar protocol as that for the ADP ·AlF_x complex. These higher resolution structures revealed more side chain positions and essentially confirmed the chain trace of the model solved at 4.7 Å. However, a large portion, the D2 domain, was largely disordered in the "high-resolution" p97/VCP · AMP-PNP complex. In contrast, the electron density maps for the ADP and ADP ·AlF_x complexes that diffracted to lower resolution showed well-defined electron density for the D2 domain. Thus, overall resolution is not necessarily correlated with quality of electron density maps throughout the model.

The work by Chen et al. (2005) described in this issue uses a strategy similar to that used for p97/VCP, but it employs an additional tool: multicrystal averaging. The structure solution is a remarkable achievement due to the limited resolution and radiation sensitivity of the crystals, failure of molecular replacement, and the absence of noncrystallographic symmetry. A combination of phase combination using heavy atom phases, B-factor sharpening, density modification, multicrystal averaging, model building, and secondary structure restrained refinement was used to solve the structure. This paper demonstrates that multicrystal averaging of omit maps can be a powerful tool for electron density map improvement and model improvement. One method that might have helped this structure, had it been available, would have been simultaneous refinement directly against the three or four nonisomorphous data sets. Bulk solvent parameters were modified as described for the structure solution of p97 (DeLaBarre and Brunger, 2003) and B-factor sharpening was successfully employed.

Two other recent examples of crystal structures refined at low resolution illustrate the usefulness of such structures. First, the structure of the tetrahymena ribozyme was solved at 3.8 Å resolution (Guo et al., 2004). Four independent monomers were present, but they showed significant conformational differences, so no averaging was possible. Combinations of MAD and single anomalous dispersion (SAD) datasets were used to solve and refine the structure. B-factor sharpening was used, although bulk solvent refinement failed for unknown reasons. Second, the inactive state of the *E. coli* DNA polymerase clamp loader complex was solved in complex with ATPγS (at 3.5 Å resolution) and with ADP (at 4.1 Å resolution) (Kazmirski et al., 2004). MAD phasing was used for the ATPγS complex, while the ADP complex was solved by molecular replacement with the ATPγS form. B-factor sharpening was unsuccessful for the ADP crystal structure, presumably due to unavailability of experimental phase information.

As mentioned above, incorporation of experimental phase information into maximum likelihood refinement

(Pannu et al., 1998) is essential in order to make the refinement robust at low resolution. It is important to iteratively improve the heavy atom (or selenium) model and experimental phase probability distribution by using the refined model phases as a prior phase probability distribution in order to identify additional scatterers or to improve the parameters of the scatterers (DeLaBarre and Brunger, 2005). During this iterative process, the resulting experimental phase probability distributions improve as assessed by map quality, figures of merit, and free *R* value of the refined model. A recently published method might offer an alternative by incorporating the heavy atom data through a multivariate likelihood function into refinement (Skubak et al., 2004).

As crystallographers will increasingly study larger and larger macromolecular complexes, the intrinsic flexibility of some of these complexes will preclude structure solution at high resolution. Yet, to understand biological function it is important to study assemblies that are as close as possible to their physiological counterparts. Thus, the techniques used by Chen et al. (2005) discussed in this preview, will play an increasingly important role in biological crystallography. In addition, new methods need to be developed—to name a few: aides to interpret noisy low-resolution maps—with current technology essentially no automation can be used, estimators of individual atomic coordinate errors, and statistically correct combination of structural information from a variety of sources. The deposition of low-resolution structures will also require special attention; perhaps electron density maps should be deposited in addition to coordinates and structure factors, so one could judge the accuracy of the model by direct inspection of the maps. These techniques should also be useful for cryo-electron microscopy studies that move closer to the low resolution limits that were discussed here (Fotin et al., 2004; Ludtke et al., 2004).

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