



Figure 1. Ribbon Diagrams Illustrate the Interactions between the IAD of IRF-3 and the IBD Region of CBP

Left: Intramolecular interactions between the IAD of IRF-3 (in green) and the flanking autoinhibitory structures (in red). Phosphorylation sites are in yellow. Right: Intermolecular interactions between the IAD of IRF-3 (in green) and the IBD region of CBP (in blue). The figure was graciously provided by Kai Lin.

cause the interacting residues of IRF-3 involved in IBD association are only partially conserved in other IRF family members, alternate structural motifs and distinct conformations of CBP/p300 may be a necessity to achieve these functional interactions.

While this study provides interesting new perspectives on IRF-3 association with CBP/p300, many important questions remain that will likely have to await structural verification. For example, the structure of full-length IRF-3 with both the DNA binding domain and the IAD has yet to be resolved. Models derived from domain structures may not fully describe the functional interactions of the full-length proteins. In particular, it will be essential to correlate structural models with functional phosphorylation: Is it possible to identify experimentally intermediate IRF-3 structures representing different states of phosphorylation and/or activation? How does the structure of the functionally related IRF-7

correlate with the three-dimensional information available for IRF-3? The answers to these and other important questions will provide insights to the mechanisms of transcriptional regulation in general and to the triggering of antiviral immunity in particular.

**John Hiscott and Rongtuan Lin**  
Lady Davis Institute for Medical Research  
Jewish General Hospital and  
Department of Microbiology & Immunology and  
Department of Medicine  
McGill University  
Montreal H3T 1E2  
Canada

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## Liberating Crystallographers

In this issue of *Structure*, DePristo et al. describe a new program, *RAPPER*, that should automate some of the most time-consuming tasks associated with rebuilding, refining, and completing protein crystal structures at moderate resolutions

When I was a PhD student, the popular perception (which we liked to encourage) was that protein crystallography required great sophistication in mathematics and computer science. Indeed, determining a new crystal structure could take years of challenging work.

But after fixing bugs in programs, struggling with fuzzy loops, and lovingly placing each water molecule into electron density, you would be rewarded with exciting new insights that could often be published in the pages of the highest-profile journals. Unfortunately, the insight you gain from examining the ten-thousandth water molecule is not as great as that from the first, and the process had to become faster and easier if protein crystallography were to achieve its full potential, where many new insights only come from large collections of structures.

The process *has* become faster and easier, with much of the required theoretical sophistication having moved into the computer programs we use. Alwyn

Jones realized that the same structural patterns recur in protein after protein and developed ways to exploit structural databases for manual (and gradually more automated) electron density fitting in the program *O* (Jones, 2004). He also devised criteria, such as real-space R factors and correlation coefficients, to focus attention on the most poorly modeled regions (Jones et al., 1991). Axel Brünger and colleagues introduced simulated annealing into *X-PLOR* and then *CNS* (Brünger et al., 1998), to allow automated refinement to step out of local minima and thereby take over some of the manual work from trained crystallographers. Victor Lamzin and Tassos Perrakis developed the *ARP/wARP* procedure (Perrakis et al., 1999), which iteratively builds a progressively better atomic model for most of the structure by placing atoms into unexplained electron density, removing atoms from low density, refining the modified structure with Refmac5 (Murshudov et al., 1997), and then recognizing structural patterns within the remaining atoms. Automated building can also be carried out with *Resolve*, which assembles overlapping tripeptide fragments (Terwilliger, 2003), or *TEXTAL*, which applies pattern recognition techniques from machine learning (Ioerger and Sacchetini, 2003).

But even with these powerful tools, it is commonly stated that 90% of the effort goes into establishing the last 10% of the structure. Why is the last part so labor intensive? The electron density for poorly ordered solvent molecules, side chains, and surface loops is close to the noise level of the density maps used for rebuilding. A skilled crystallographer can recognize patterns in the noise and interpret some of these poorly ordered parts of the structure correctly, especially if aided by a tool like *O* that helps to build models consistent with prior knowledge. Improving the model improves the calculated phase values and reduces the noise level of the resulting maps, so the last details can slowly be established by an iterative process. Phil Evans likes to say that one refines protein structures ad tedium, i.e., until it has become too tedious to carry on (P.R. Evans, <http://www-structmed.cimr.cam.ac.uk/Course/Fitting/fittingtalk.html>).

Anything that further frees scientists from this repetitive process will be welcomed by protein crystallographers. The program *RAPPER*, described by DePristo et al. (2005) in this issue, promises to provide another significant step along this path. *RAPPER* automates the rebuilding process by identifying regions that fit poorly in the current model, then finding plausible new conformations that fit the electron density map. The heart of *RAPPER* is a conformational sampling engine that generates a population of conformers satisfying knowledge-based constraints. The main chain of each trial conformer is extended by adding a residue with a random backbone conformation, selected from a finely sampled Ramachandran plot and weighted by its propensity. Side chains are added from a finely sampled table of conformers. A population of possibilities is built up from conformers that in addition satisfy packing criteria and place all of their atoms in positive electron density. When a sufficiently large population has been generated, covering the region to be rebuilt, the one

with the best fit (judged by correlation with the electron density map) is saved for the new model. *RAPPER* is then coupled with a refinement program such as *CNS* (Brünger et al., 1998), which optimizes the details of the model and creates maps for a new round of rebuilding.

The individual ingredients—fitting scores, database preferences, iterative rebuilding, and refinement—are found in the other programs, but the whole of the *RAPPER* procedure gives striking results in cases where the others would be expected to fall short. The authors show that a poorly placed model of a lysozyme mutant can be refined automatically using data to only 2.8 Å resolution, giving a final model that is nearly as good as the one obtained by laborious manual refitting. The performance of *RAPPER* may be helped by using finely sampled conformers, instead of minimal sets, and by maintaining a population of possibilities throughout the conformational search. It may also get further in structure completion because it focuses attention on only a few suspect regions; the other programs tend to strike a different balance, rebuilding the entire structure each time to avoid being trapped in an incorrect interpretation.

There is still work to be done. In its current form, *RAPPER* will not yet detect errors in the registration of the sequence with the fold. Another problem is knowing when to stop, by recognizing loops that correlate badly with their electron density because they are completely disordered.

As reported by DePristo et al. (2005), this new program brings us a step closer to the goal of proceeding automatically from medium-resolution diffraction data to final model. I am looking forward, as I am sure many others will, to using *RAPPER* and tools like it in determining our new structures.

#### Randy J. Read

Department of Haematology  
University of Cambridge  
Cambridge Institute for Medical Research  
Wellcome Trust/MRC Building  
Hills Road  
Cambridge CB2 2XY  
United Kingdom

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