PROTEIN BIOCHEMISTRY

X-ray vision reveals conformational changes

A new study demonstrates that X-ray scattering data can reveal even minor conformational changes induced in a protein after ligand binding, offering a potentially valuable tool to expedite biochemical studies and drug design.

High-throughput screening technology is booming these days, and drug discovery research is poised to be one of the biggest beneficiaries of this boom. Numerous techniques enable investigators to scan potential compounds of interest for their ability to get into certain cells or interact with a given molecule. A trickier prospect, however, is the identification of functionally relevant compounds capable of triggering an appropriate biological response. This typically involves the design of highly specialized functional assays, an often complex and laborious process.

Lee Makowski and his colleagues at the Argonne National Laboratory (Argonne, Illinois) envisioned a different approach. "If you look at all the X-ray studies," says Makowski, "they indicate that the substrate binding sites of most enzymes exhibit anywhere from a little bit to a great deal of structural change on binding to substrate... so the question that came to our mind was, can we, in a 30-second experiment, actually detect the structural change induced by a small-molecule ligand?"

Techniques exist for the detection of structural changes, but most lack the sensitivity to detect the more subtle shifts that often accompany ligand binding. One popular technique, small-angle X-ray scattering (SAXS), involves bombarding a sample with a focused X-ray beam and then interpreting the resulting diffraction patterns. The diffraction intensities analyzed in SAXS reveal significant rotational movements by a protein domain but miss smaller changes, such as side-chain reorientations. Makowski's group instead relied on wide-angle X-ray scattering (WAXS); in this method, the data is collected from substantially greater angles, revealing even slight structural alterations at the quaternary, tertiary and secondary level.

Samples are analyzed under physiological conditions, in solution. The protein is first analyzed by itself, then in the presence of ligand and the calculated curves derived from the resulting diffraction data reveal the type and extent of structural rearrangement. For their initial studies, Makowski's team analyzed a variety of protein-ligand pairs, comparing the WAXS data they obtained against predictions from crystallographic studies. Some of these interactions produce major changes, such as the transferrin-iron interaction, which results in a 'venus flytrap' closing movement by the two hinged domains (**Fig. 1**). Others were subtler, such as the

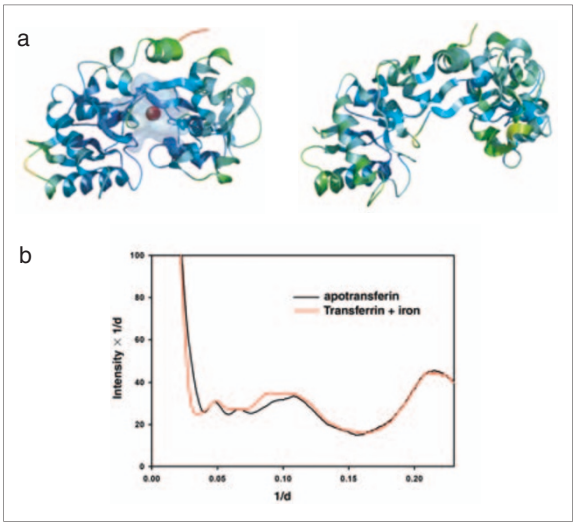

Figure 1 | (**a**) Conformation of transferrin before and after binding ligand. (**b**) Data plot from WAXS analysis of transferrin before (apotransferrin) and after (transferrin + iron) binding iron. The chart plots intensity times scattering vector against the scattering vector, with the differences between the plots revealing the increased lateral dimension of the apo enzyme.

binding of alcohol dehydrogenase to NAD^+, which triggers only a slight alteration at the substrate-binding cleft.

Not only was WAXS capable of revealing the conformational differences between the apo and ligand-bound forms of enzyme, but the data also closely resembled the changes predicted by modeling and crystallographic studies. Based on their experimental data, Makowski's team predicts that WAXS should even be capable of detecting such slight changes as the side-chain reorientation induced in ricin toxin after binding of the inhibitor neopterin. Crystallographic data exist for all of the proteins studied in this article, providing proof of principle for the approach, but Makowski's team believes that the sensitivity and accuracy of this technique will make it possible to reliably detect binding-induced conformational changes for previously uncharacterized enzyme-substrate pairs.

One significant limitation of the system arises from its reliance on access to the latest generation of synchrotron facilities, as the scattering intensity obtained with WAXS is several orders of magnitude lower than with SAXS—and Makowski acknowledges that his team benefited from their access to Argonne's renowned Advanced Photon Source. Nonetheless, WAXS promises considerable benefits for users, including relatively high throughput—each reading takes only three to five seconds—and surprising sensitivity to even small changes in protein conformation.

The system also provides the chance to study protein-ligand interactions in a natural setting. "The disadvantage of screening and looking for a lead drug right now is you almost always have to chemically modify or tag one of the components, either the target protein or the putative lead drug," says coauthor Diane Rodi. "What's beautiful about this technique... is that you don't have to modify either the lead drug or the protein. They're both in solution; they're both in their natural state."

At present, the group is in the process of examining a broad variety of previously characterized protein structures via WAXS in order to better understand the strengths and weaknesses of their approach. From what they've seen so far, however, the Argonne team sees promise for WAXS in the drug discovery process. Says Makowski, "It would be very exciting, from our point of view, to work with an industrial partner to incorporate this kind of a functional or structural screen into a ligand or drug screen pipeline."

Michael Eisenstein

RESEARCH PAPERS

Fischetti, R.F. *et al*. Wide-angle X-ray solution scattering as a probe of ligand-induced conformational changes in proteins. *Chem. Biol.* **11**, 1431–1443 (2004).