

FINDING ACTIVE PROTEINS

When combinatorial chemistry produces new varieties of reagents, the tricky next step is figuring out whether those molecules will be biochemically active. While there are several methods for finding active molecules, they all have limitations. Researchers from Argonne National Laboratory, using the Bio-CAT beamline 18-ID, employed wide-angle x-ray scattering (WAXS) to develop a method for identifying drug candidates.

One way to identify active molecules is to design an assay for the desired function of a protein target and then test each small molecule variant. This approach is labor intensive and time consuming because it means that a different assay must be developed for each drug target of interest. Another approach is based on deriving a more general probe that would identify whether any protein structural change had occurred. A large number of samples could be screened in this way; those that showed promise could then be examined more closely for mechanistic details. Just such a general method, which uses x-ray scattering to detect functional drug candidates, is now available.

The APS served as a unique resource to aid the investigators in their study of WAXS of proteins in solution. The researchers are developing WAXS as a probe for detecting the structural changes that usually occur when a protein binds to a

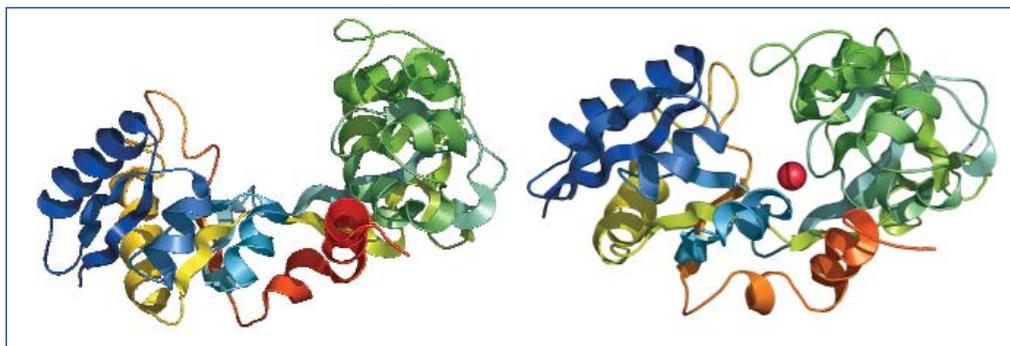


Fig. 1. Computer renditions (PyMOL) of the apo form of the protein transferrin (left) and the iron bound form (right) based upon crystallographic coordinates. From this angle, one can see the great extent to which the protein domains rotate relative to one another upon ligand binding.

functional ligand. Such activity encompasses a broad range of changes in secondary, tertiary, and quaternary structure of the protein. Although nuclear magnetic resonance can be used to characterize proteins in solution, this method requires extensive data collection and analyses. Small-angle x-ray scattering (SAXS) allows researchers to observe changes in the radius of gyration, but not all domain rotations and none of the small

side-chain interactions. Using the WAXS technique, data can be collected quickly and accurately using label-free ligands and targets, making the method well suited to moderate-throughput detection and analysis of protein-ligand interactions. The data collected permits direct estimate of the magnitude of the structural change, without reference to crystallographic coordinates. Although the exact form of the structural change at atomic resolution cannot be inferred from WAXS, both the magnitude and the class of

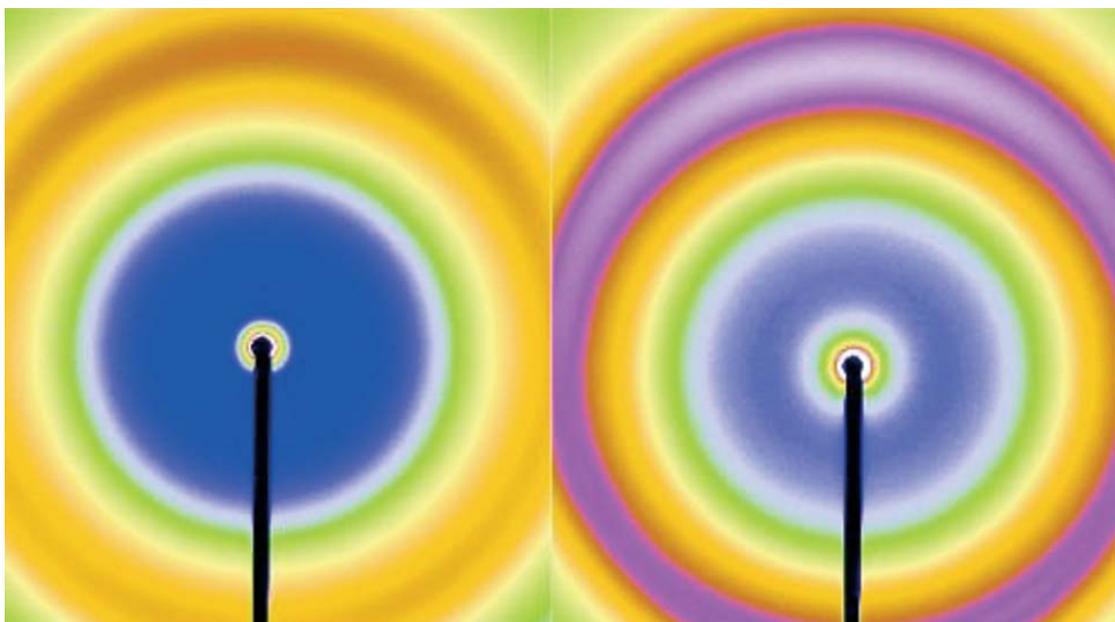


Fig. 2. False color images of the solution scattering patterns from the apo and ligand-bound forms of transferrin, highlighting the differences in diffraction between the structures.

the change can be elucidated. Even without crystallographic data, a combination of SAXS and WAXS has the potential to characterize what happens during ligand binding. As proof of concept, the data demonstrate that WAXS is a sensitive probe for identifying functional ligands through ligand-induced structural changes.

Using proteins in solution, five classes of structural change were studied: transferrin for ligand-induced domain rotation, maltose binding protein for hinge-bending motion, alcohol dehydrogenase for change of the shape of the binding cleft, calmodulin for ligand-induced refolding, and adipocyte lipid binding protein and ricin for side chain reorientations. To minimize damage to the proteins, thin aluminum foils were used as x-ray beam attenuators to control the incident beam flux. Protein concentrations ranged between 12.6 and 48 mg/mL. As a validation, the program CRY SOL was used to predict WAXS patterns from the proteins, using the atomic coordinates from the crystallographic structures.

The data showed that ligand-induced structural changes involving domain movements, as well as smaller ones (such as side chain rearrangements) are detected by WAXS, an *in vitro* biophysical probe. For the most part, measured scattering was in agreement with that predicted by applying the CRY SOL software to the atomic coordinates (Fig. 1). Ligand-induced domain movements, however, were larger than predicted on the basis

of x-ray crystallography alone, consistent with a previous finding that crystal-lattice domain positions may differ from those observed in solution (Fig. 2).

When binding a ligand, most proteins undergo some change in conformation, either local or global. Collecting the necessary WAXS data to characterize these changes takes about 30 s at a third-generation synchrotron source and can be carried out with a wide range of solution conditions for protein-ligand pairs, which do not have to be chemically modified. As such, WAXS can greatly improve the speed and accuracy with which drug candidates can be assessed for their therapeutic potential, enhancing the drug development process. — *Mona Mort*

See: R.F. Fischetti, D.J. Rodi, D.B. Gore, and L. Makowski, "Wide-Angle X-ray Solution Scattering as a Probe of Ligand-Induced Conformational Changes in Proteins," *Chem. Biol.* **11**, 1431 (October 2004).

Author Affiliation: Argonne National Laboratory
Correspondence: lmakowski@anl.gov

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