WATCHING MYOGLOBIN CHANGE IN REAL TIME

X-ray crystallography provides valuable information about proteins and their molecular interactions. But this technique produces static structures of molecules, while proteins in their native state are actually active, moving in solution, changing conformation as they catalyze reactions and bind to different molecules. Researchers from the National Institutes of Health (NIH) and The University of Chicago, working at the BioCARS facility at the APS, have met this limitation head-on by developing a time-resolved x-ray scattering diffractometer that can detect changes in protein scattering patterns in solution, providing researchers with a new, sensitive method for studies of protein structure, function, and dynamics.

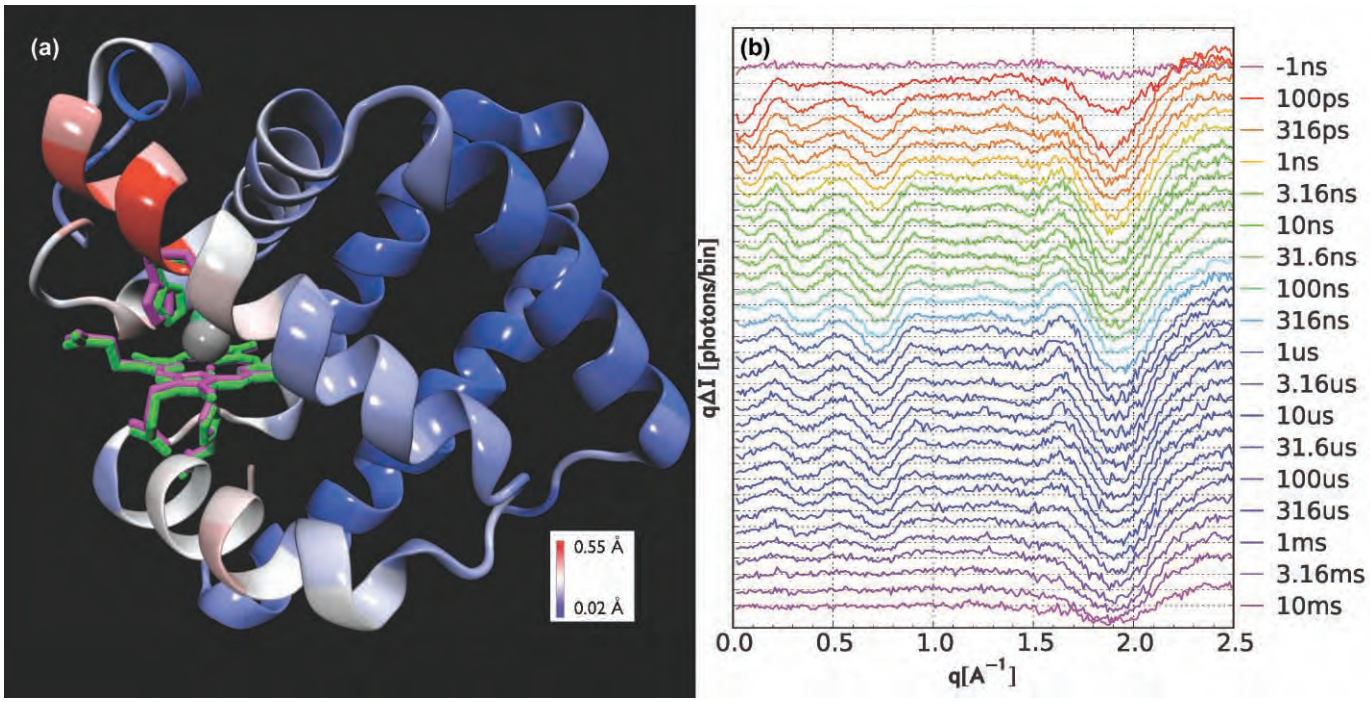

Fig. 1. (a) Structural differences between MbCO and Mb. The heme and nearby histidines are rendered as licorice for both MbCO (magenta) and Mb (green). The back bone is rendered as ribbon and color coded according to the rmsd between these two structures, the global mean of which is less than 0.2 Å. (b) Time-resolved SAXS/WAXS differences. For clarity, the curves are offset from one another and color-coded according to the five states found along the reaction pathway.

X-ray crystallography reveals details about protein structure down to the atomic level. The relative position of each amino acid can be identified and its role in secondary structures such as α-helices and β-sheets can be determined. This technique has provided science with invaluable information about proteins and their molecular interactions, but it has one drawback: It produces static structures of molecules constrained by crystal packing forces. Proteins in their native state are active, moving in solution, changing conformation as they catalyze reactions and bind to different molecules. Spectroscopic techniques can measure changes of proteins in solution but they rely on indirect information from a chromophore and do not detect global structural changes.

In an effort to overcome these limitations, the researchers from the NIH and The University of Chicago have developed a timeresolved x-ray scattering diffractometer that uses small-angle x-ray scattering (SAXS) and wide-angle x-ray scattering (WAXS) to detect changes in protein scattering patterns in solution. This diffractometer, developed on the BioCARS 14-ID beamline at the APS, can probe protein structural changes in solution on short timescales of 100 ps and provides researchers with a new, sensitive method for studies of proteins.

The first step was to develop a high-dynamic-range diffractometer capable of simultaneous coverage of the SAXS and WAXS scattering regions. This was done by positioning the x-ray detector close enough to the sample to access the WAXS region up to q = 2.5 $Å^{-1}$, and making the beamstop diameter small enough to access the SAXS region down to q = 0.02 $Å^{-1}$. For testing the system, the research team chose the muscle protein myoglobin, about which much is already known. Myoglobin binds reversibly to oxygen and carbon monoxide (CO) and the crystal structures of myoglobin with CO bound and unbound have been solved. However, the changes they reveal are subtle, with only very small differences between them. The team set out to test their new diffractometer on myoglobin because they could generate a conformational change with a flash of laser light. This does not require any mixing procedures and thus allows for measurements to be made very quickly upon activation of the conformational change. In the case of myoglobin, a flash of light on myoglobin bound to CO will cause the CO molecule to release. To achieve time resolution as short as 100 ps, these experiments required the ability to isolate a single bunch of x-rays from the APS 24-bunch mode with a mechanical chopper, a feat made possible thanks to a recently completed major upgrade of the BioCARS 14-ID beamline. The unprecedented time-resolved capabilities achieved by this upgrade resulted from a partnership involving BioCARS, NIH/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the APS (see "100-ps Time-Resolved Crystallography at BioCARS," *APS Science 2008*, p. 89 (ANL-08/24 Argonne National Laboratory, May 2009).

After accounting for factors such as the temperature change generated by the absorbed laser light, solvent and helium chamber contributions, protein scattering patterns can be determined. Measurements made on myoglobin using this system showed x-ray scattering differences recorded over times of 100 ps to 10 ms to be rich in structural information. The SAXS data, which are sensitive to the size and shape of the protein, showed a sudden >22 $Å^3$ expansion in the volume of the protein followed by a relaxation 10 ns later to a volume 2 $Å^3$ greater than myoglobin bound to CO before the flash of light. The WAXS data provide higher resolution information, and the combination of SAXS and WAXS data show the subsequent escape of the CO molecule as the conformation of myoglobin changes.

This striking example of the use of a diffractometer that can capture SAXS and WAXS scattering information at the same time on a very small time scale will provide researchers with a valuable new tool for studies of protein structure, function, and dynamics.

— Sandy Field

See: Hyun Sun Cho[1], Naranbaatar Dashdorj[1], Friedrich Schotte[1], Timothy Graber[2], Robert Henning[2], and Philip Anfinrud[1], "Protein structural dynamics in solution unveiled via 100-ps time-resolved x-ray scattering," Proc. Natl. Acad. Sci. USA **107**(16), 7281 (April 20, 2010).
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