

A CLOSER LOOK AT PROTEIN BREATHING

To take a static view of proteins and regard them as simple strings of amino acids that do grunt work in cells would be a mistake. Decades of biomedical research have proven that proteins are often large, complex in structure, and, as is becoming increasingly apparent, undergo sophisticated changes in space and time in order to keep cells functioning properly. Some proteins, when in solution, exhibit dramatic fluctuations in their three-dimensional structures, movement that looks like breathing. Because this movement has usually been studied in relatively dilute solutions, and not in the crowded interior of a cell, it has been difficult to know how much of the motion would actually occur in living systems. Recognizing the need for a new approach to the problem, researchers used the APS to study the breathing motions of a diverse group of five animal proteins. Their results provide badly needed modeling of protein movement in solution and data that can be used widely in biomedical applications, such as therapeutic drug design.

The researchers from Argonne National Laboratory and the Illinois Institute of Technology used computational modeling and wide-angle x-ray scattering (WAXS) experiments performed on the Bio-CAT beamline 18-ID at the APS. They studied the breathing motions of a diverse group of five animal proteins (solutions of bovine hemoglobin, hen egg white lysozyme, hen egg white avidin, bovine serum albumen, and equine myoglobin) that represent a spectrum of size and structural differences.

By observing changes at varying protein concentrations and temperatures, the group was able to quantify spatial changes in this diverse array of molecules and, for the first time, present a set of techniques by which this quantification can be standardized.

The studies revealed motion in the three-dimensional protein structures that increased with decreasing protein concentration and increasing temperature and—perhaps most important—varied widely among the proteins. These findings point to the need for recognizing both the chemical and spatial structure of a protein (as well as its biochemical surroundings) when making estimates of protein movement, which was greatly inhibited at high protein concentration.

The proteins varied widely in their kinetic behavior, which

depended on protein concentration and temperature. Because it exhibited breathing—in the form of rigid body motions—that lends itself well to observation with WAXS, hemoglobin at high concentration was chosen as the reference structure. Compared to hemoglobin, lysozyme at low concentration exhibited much less structural fluctuation. Bovine serum albumin exhibited about the same amplitude of breathing as lysozyme at very low concentration, while myoglobin and avidin exhibited movement comparable to hemoglobin (Fig. 1).

As temperature increased, the breathing in hemoglobin increased slightly; this increase was suppressed as the protein concentration, and accompanying molecular crowding, increased. Thus, the effect of temperature appears to be amplified at lower concentrations. And it seems that when proteins have more room to move (when protein concentration is low and there is more empty space nearby), more breathing occurs.

The chemical composition of a protein, called its primary structure, was also found to be important. Unlike the other proteins studied, the lysozyme and bovine serum albumin, which are stiffened by multiple disulfide bonds, showed relatively little increase in breathing in dilute solutions.

The research team used tech-

niques that allowed an estimate of the size of rigid bodies that move during protein breathing. Concluding that secondary structures, such as alpha-helices, move like rigid bodies during breathing, the authors hypothesized that breathing involves slow collective movements. Another striking result of the study is that a protein's attributes such as size, structure class, subcellular location, or presence of multiple subunits do not seem to correlate with the measured amount of breathing. Thus, based on the usual suspects, there is no way to generalize about the expected amount of structural fluctuation. Each protein must be studied individually to learn its breathing habits under various cellular conditions, underscoring the danger of generalizing protein behavior from a limited sample.

This study provides badly needed modeling of protein movement in solution and data that can be used widely in biomedical applications, such as therapeutic drug design. — *Mona Mort*

See: Lee Makowski^{1*}, Diane J. Rodi¹, Suneeta Mandava¹, David D.L. Minh¹, David B. Gore², and Robert F. Fischetti¹, "Molecular Crowding Inhibits Intramolecular Breathing Motions in Proteins," *J. Mol. Biol.* **375**, 529 (2008). DOI: 10.1016/j.jmb.2007.07.075

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This work and use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract DE-AC02-06CH11357. Bio-CAT is a National Institutes of Health-supported Research Center RR-08630.

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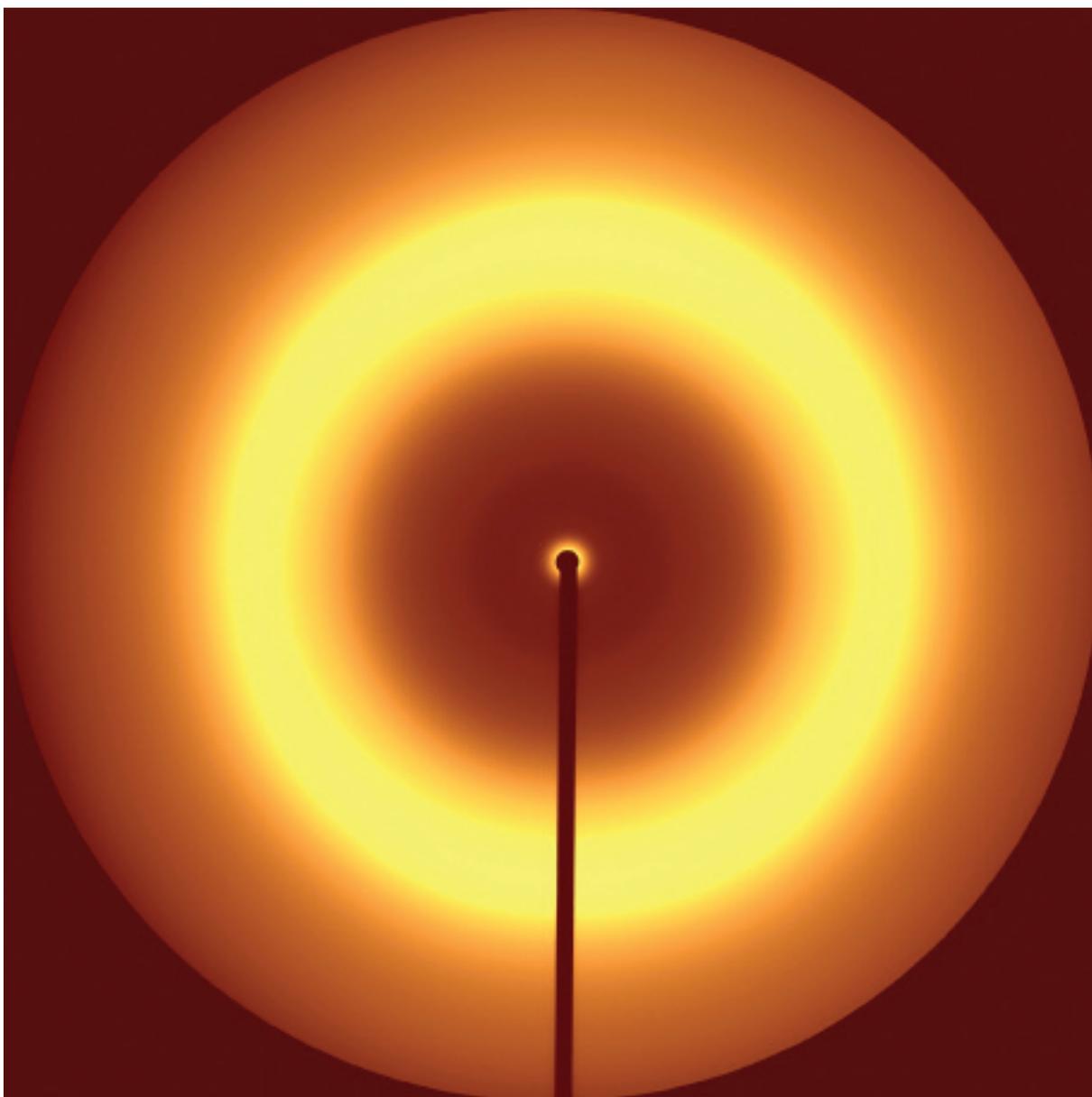


Fig. 1. A false-color scattering pattern from the protein myoglobin.