PROTEINS CAUGHT IN THE ACT

STRUCTURE ANALYSIS: X-ray scattering technique captures conformational changes on nanosecond time scale

IF YOU WANT to watch a movie of a protein in action, a new synchrotron-based structural analysis technique could deliver the goods.

The technique, time-resolved wide-angle X-ray scattering (TR-WAXS), is an enhanced version of WAXS, which has become increasingly popular in the past few years for investigating protein structures. WAXS's time resolution has generally been limited to milliseconds. TR-WAXS improves its time window for observing the kinetics of protein conformational changes by about a millionfold, down to the nanosecond range.

TR-WAXS could provide exactly "what you would want to see—movies of proteins actually functioning," comments Lee Makowski, a macromolecular structure specialist at Argonne National Laboratory. "It's a great new window into trying to understand how proteins work."

A collaborative team devised TR-WAXS and demonstrated its capabilities by using it to visualize nanosecond conformational changes in hemoglobin caused by photodissociation of carbon monoxide. They also used it to watch cytochrome *c* fold and to observe nanosecond conformational changes in myoglobin.

The technique was developed by Marco Cammarata of the European Synchrotron Radiation Facility, in Grenoble, France; Philip Anfinrud of the National Institutes of Health; Antonio Cupane of the University of Palermo, in Italy; Hyotcherl Ihee of the Korea Advanced Institute of Science & Technology, in Daejeon, South Korea; and coworkers (*Nat. Methods*, DOI: 10.1038/nmeth.1255). The researchers hope to eventually further improve the time resolution of TR-WAXS to the picosecond range.

"Virtually everything a protein does, such as binding or releasing a ligand, requires structure changes," Makowski says. "It's hard to get really good data on those changes, but TR-WAXS makes it possible to do so. It opens the door for a lot of future possibilities."

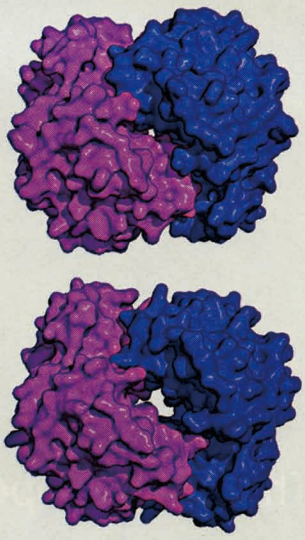
William A. Eaton, a protein physical chemist at NIH, comments that the new technique "is a major breakthrough in investigating kinetics of protein conformational changes. The difference between the deoxy and CO-bound structures of myoglobin is only an angstrom motion of part of the molecule, yet they're picking up the signal. It's remarkable that you can get structural information in solution at such detail with nanosecond time resolution." The technique could make it possible "to watch enzymes work and to see how enzyme conformational changes play a role in enzyme function," Eaton adds.

Existing spectroscopic techniques with equivalent or better time resolution help study protein conformational changes, but they do not provide the detailed structural data that TR-WAXS does. And NMR spectroscopy and X-ray crystallography can calculate protein structures directly, whereas TR-WAXS can only confirm them. But NMR isn't a fast technique, and the crystals used in time-resolved X-ray crystallography tend to restrict those kinds of protein motions that TR-WAXS can analyze under near-physiological conditions.

"We've been talking about doing something like this for a while," Makowski says, but the new work has "put all the pieces together."—STU BORMAN

SUBTLE SHIFT

TR-WAXS-based images of hemoglobin before (top) and after the dissociation of its carbon monoxide ligand. Colors represent the dimeric protein's monomers.



TAKEOVER Bristol-Myers goes straight to ImClone shareholders with higher bid

Bristol-Myers Squibb Chairman James M. Cornelius sidestepped ImClone Systems' management last week by presenting a sweetened buyout offer directly to ImClone shareholders. Bristol-Myers is now willing to pay $62.00 per share, or $4.7 billion, to buy the 83.4% of its drug development partner that it doesn't already own.

In a written response, ImClone Chairman Carl C. Icahn called the hostile offer "absurd" and said Cornelius is being misleading. Although Cornelius alleges that ImClone has resisted meaningful discussions, Icahn insisted that the company has been happy to discuss better offers.

The public haggling has gone on for nearly two months, since Bristol-Myers first made an unsolicited $60.00-per-share bid that ImClone's directors called inadequate. Soon after, Icahn, who owns about 13.5% of ImClone's stock, announced that an unnamed large pharmaceutical firm had made a conditional $70.00-per-share offer (C&EN, Sept. 15, page 20).

Apparently fed up with waiting, Cornelius wrote in a Sept. 22 letter that "bringing our offer directly to the company's stockholders allows them to evaluate the merits of our proposal." He argued that the delays and lack of transparency have created uncertainties that "could hurt the intrinsic value of ImClone's assets."

Among these assets are long-term rights to the anticancer drug Erbitux and related compounds, rights that are claimed by Bristol-Myers. Icahn, however, has stated before that he and Cornelius "disagree that Bristol's rights are clear." Erbitux sales totaled $840 million in the first half of 2008.

Meanwhile, ImClone is waiting for the higher bidder to decide whether to make a firm offer. Icahn said the unnamed company's review will be complete by Sept. 28.—ANN THAYER