A method to study how proteins behave in solution has gotten a major upgrade. Researchers can now perform amide hydrogen-deuterium exchange mass spectrometry (HDXMS) without jury-rigging and can analyze reams of data rapidly, thanks to hardware and software the instrumentation company Waters developed for the technique.

HDXMS involves exchanging certain hydrogen atoms of a protein with deuterium atoms and then locating where the exchanges take place by mass spectrometry. The results indicate the water accessibility and hydrogen-bonding environment of various areas of the protein. When exchanges are measured over time, scientists can infer from the data what the protein’s various parts are doing in solution.

“It’s hard to measure protein motions,” says Natalie G. Ahn, a chemistry professor at the University of Colorado, Boulder, and a Howard Hughes Medical Institute investigator. An early adopter of HDXMS to study protein dynamics, she uses the technique to understand the regulation of protein kinases, enzymes that control cell proliferation. HDXMS is an indirect way to examine how proteins move, she says, but it is “one of the most accessible methods for finding evidence of protein motion that may be relevant to function.”

Researchers use HDXMS to study protein-protein and protein-ligand interactions and conformational changes related to protein activity, among other phenomena. The detailed understanding of protein behavior that HDXMS affords has direct application in drug discovery and development.

For example, the technique can help unravel the mechanism of proteins whose activity depends on communication between different parts of the molecule, says Ganesh S. Anand, an assistant professor of biological science at the National University of Singapore. He is using HDXMS experiments to map the molecular circuits that govern the activity of signaling proteins called response regulators. In bacteria and other organisms, these proteins enable cells to communicate and respond to environmental stimuli. When they are turned on by phosphorylation, which occurs in one domain, they produce an effect at a distant output domain.

How the signal travels through the molecule has been a big question in the field of signaling proteins, Anand says. Well-established models predict linear relays, like electronic cables, connecting the site of phosphorylation to the site of action, he says. A recent HDXMS study in Anand’s lab comparing various forms of the regulatory protein RegA indicates, however, that transmission is not linear (J. Mol. Biol., DOI: 10.1016/j.jmb.2012.01.052). After converging on a parallel circuits to yield an output response, the finding, Anand says, “offers important new insights into inhibitor design.”

Even in drug formulation, HDXMS offers useful insights, Anand says. In work that’s in press at EMBO Journal, his group finds that changes in the osmolality of the protein’s environment affect the protein’s conformation. “Some people think that salts and sugars are inert additives in protein drug formulations,” he says, “but we find that they affect conformation and, therefore, shelf life and bioavailability.”

Developed in the early 1990s, HDXMS has been gaining popularity. “A big part has to do with protein-based pharmaceuticals,” says Elizabeth A. Komives, a professor of chemistry and biochemistry at the University of California, San Diego. She uses HDXMS to study changes in the folding of proteins that occur as they bind to their targets. HDXMS is “the best way to determine whether a protein is a consistent product,” she says. “The fact that drug companies are adopting the method has been a big part of the explosion.”

An HDXMS experiment involves incubating a protein in heavy water for a specific period. During this time, the protein’s most labile hydrogen atoms—those on the amide backbone— will exchange with deuterium from D2O. After predetermined periods, the exchange is quenched by lowering the pH and temperature of the mixture. Next, the deuterated protein is digested with pepsin. Liquid chromatography separates the peptides, which then go to the mass spectrometer for mass analysis.

From quenching to chromatographic separation, operations must take place fairly rapidly and at 0 °C to prevent loss of deuterium from the deuterated protein through further exchange with hydrogen. And therein lies one of the hurdles of HDXMS. Researchers had to adapt a proteomics-capable mass spectrometer to the HDX experiment by using jury-rigged ice buckets, Anand says. “All the tubing used to go through ice; everything was manual and labor-intensive.”

An even bigger headache is data analysis. Michael Eggertson, a senior research chemist at Waters, explains just how much data HDXMS experiments can spew: When digested, a typical 100-kilodalton protein will form up to 200 peptides, each of which must be analyzed. Any experiment must be done at least twice to ensure the data are reproducible. And because HDX is really about measuring changes in protein state,
experiments usually compare proteins under different conditions—for example, proteins from diseased versus normal cells. All the variables—protein states, peptides, replicates, and exposure times—“multiply into a giant multidimensional matrix of data you need to analyze,” Eggertson says.

Waters’ nanoACQUITY ultraperformance liquid chromatography (UPLC) system with HDX technology addresses both the setup and data analysis problems, academic researchers who spoke with C&EN say. Specifically, the UPLC system includes a refrigerated box that allows chromatography to take place in a well-controlled 0 °C environment. Depending on the type of injection system and high-resolution mass spectrometer, the price of a complete system can vary from $400,000 to $750,000, according to Waters. Everyone C&EN asked says the Waters system is one of a kind. Instrument makers Thermo Fisher Scientific and Agilent say that they do not have comparable offerings.

What’s really exciting, researchers say, are the data collection system and the data analysis software. The data collection system, dubbed MS², captures more of the peptides after protein digestion than other data collection systems can. As Ahn explains, the more peptides the experiments recapture, the better one can pinpoint where deuteration occurred. “You can get resolution to one amide if you have many overlapping peptides corresponding to a given sequence,” she says.

Meanwhile, the software system, called DynamX, unites in one package most tasks needed for hydrogen exchange data sets, Ahn explains. “What took days and months is done in half a day,” Anand says. “We can really focus on interpretation.”

Other software to analyze HDXMS data is available, notes John R. Engen, professor of bioanalytical chemistry at Northeastern University. “But DynamX is integrated right into the mass spectrometer’s data acquisition, processing, and control software.”

Engen instigated Waters’ development of HDX technology. “I first contacted Waters in early summer 2005 about the possibility of using UPLC for an HDXMS experiment,” he says, adding that he had been running experiments the old way for 10 years.

UPLC was critical because low-temperature chromatography is otherwise inefficient, limiting the protein sizes that can be analyzed, explains Keith Fadgen, a principal research scientist at Waters. The higher pressures and smaller column particles used in UPLC improve separation, allowing study of larger proteins, such as antibodies, he says.

Fadgen, Eggertson, and proteomics specialist Martha D. Stapels formed the Waters core group that worked with Engen to develop a system dedicated to HDXMS. Engen received the first prototype in the summer of 2007, Fadgen says. After further refinements, Waters expanded access to its HDXMS prototype to several sites worldwide, including Anand’s lab in Singapore. The company commercialized the hardware in 2010 and the complete system in 2011. “Waters did everything right on the instrument development,” Komives says. “They collaborated with leaders in the field.”

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