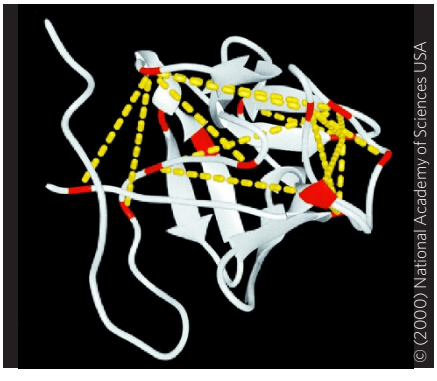
MILESTONE 19

Dynamic protein structures

The native state of most proteins in a cell is a well-defined three-dimensional structure, which is maintained by intramolecular interactions. Detailed knowledge of protein structure allows researchers to determine the molecules' functions and to design inhibitors for medical or research purposes. The traditional methods that are used to elucidate structure, such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, require a lot of time and a large quantity of protein, and, in many cases, the experiments are not successful. In the early 1990s, researchers began to explore how mass spectrometry—a rapid technique that requires only small sample amounts—could be used to provide detailed, dynamic structural information about proteins.

In 1991, several groups began to apply electrospray ionization mass spectrometry (ESI-MS; **Milestone 15**) to analyze the intact, native structures of protein systems. This approach, now known as native mass spectrometry, allowed the groups to examine the conformational changes that occur within proteins during the unfolding process triggered by harsh conditions.

Joseph Loo and colleagues monitored the small globular regulatory protein ubiquitin as it changed from its native conformation under physiological pH and solvent conditions to an extended conformation when exposed to denaturing conditions. In a more complex system, Bruce Ganem, Yu Tsyr Li and Jack Henion used ESI-MS to visualize the interaction of drugs with the cytoplasmic receptor protein FKBP,



Chemical cross-links detected by mass spectrometry provide structural information. Image reproduced with permission from Young *et al.*, *Proc. Natl. Acad. Sci. USA* **97**, 5802–5806 (2000).

demonstrating how mass spectrometry could be used to detect physiologically relevant noncovalent interactions. Yet another group studied noncovalent interactions in myoglobin, a globular protein for which structural information was already available. Viswanatham Katta and Brian Chait built on their work from 1990, which included the refinement of ESI-MS without using organic additives, to examine the impact of protein unfolding on the noncovalent heme-globin domain interaction. This paved the way in 1992 for the first work to report the observation of a functional noncovalent protein-protein interaction, done by Manuel Baca and Stephen Kent. These early papers marked the beginning of an explosion of interest in the use of ESI-MS to examine conformational changes of native proteins in the gas phase, as well as in characterizing noncovalent macromolecular assemblies, an application for which separation by ion mobility (see **Milestone 8**) has been particularly useful.

Katta and Chait had a busy 1991—they were also among the groups developing ESI-MS as a means for examining conformational changes in proteins, using a method called hydrogen-deuterium exchange (HDX). For this work, they used ubiquitin as a model and took advantage of the fact that labile hydrogen atoms in a protein can be replaced by deuterium only when they are exposed to deuterium-containing solvent. In a folded protein, the hydrogens on the surface can be exchanged with deuterium and, because the mass of deuterium is greater than that of hydrogen, this change can be detected by mass spectrometry. As the protein unfolds in harsh conditions, formerly protected hydrogens become exposed and are exchanged for the heavier element, which leads to a change in the mass of the resulting peptides. The hydrogen-to-deuterium exchange rate can thus be used to monitor the timeline of the conformational changes in the protein. In 1993, Zhongqi Zhang and David Smith used HDX to examine conformational changes in the mitochondrial protein cytochrome C upon heating, using high-performance liquid chromatography (see **Milestone 8**) combined with fast-atom bombardment mass spectrometry (see **Milestone 2**). As with native mass spectrometry, these first HDX papers paved the way for assessing protein dynamics in increasingly complex systems.

Nearly ten years after the first structural biology applications of mass spectrometry were published, another development greatly extended the information that could be obtained about protein structure using the technique. Malin Young and colleagues used chemical cross-linkers to covalently bind lysines together in basic fibroblast growth factor 2 (FGF-2) and analyzed the peptides resulting from the protein using time-of-flight mass spectrometry (**Milestone 4**). The cross-links provided information on the comparative positions of the modified lysines, and the researchers combined this data with the output from structure-prediction servers to build a model of the protein's structure. The structure for FGF-2 obtained through this method was similar to that determined by NMR spectroscopy and X-ray crystallography—but it had taken a fraction of the time to achieve.

Taken together, these advances in mass spectrometry research demonstrated that this technique could provide extensive information on the structure and dynamic motion of proteins; this can be further extended in combination with other structural-biology and molecular-modeling methods. These methods will continue to advance as technology and structural information improve, thanks to the trailblazers who started the process.

Rebecca Kirk, Senior Editor,
Nature Communications

ORIGINAL RESEARCH PAPERS Ganem, B., Li, Y. & Henion, J.D. Detection of noncovalent receptor-ligand complexes by mass spectrometry. *J. Am. Chem. Soc.* **113**, 6294–6296 (1991) | Katta, V. & Chait, B.T. Observation of the Heme-Globin complex in native myoglobin by electrospray-ionization mass spectrometry. *J. Am. Chem. Soc.* **113**, 8534–8535 (1991) | Katta, V. & Chait, B.T. Conformational changes in proteins probed by hydrogen-exchange electrospray-ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **5**, 214–217 (1991) | Loo, J.A. *et al.* Solvent-induced conformational changes of polypeptides probed by electrospray-ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **5**, 101–105 (1991) | Zhang, Z. & Smith, D.L. Determination of amide hydrogen exchange by mass spectrometry: a new tool for protein structure elucidation. *Prot. Sci.* **2**, 522–531 (1993) | Young, M.M. *et al.* High-throughout protein fold identification by using experimental constraints derived from intramolecular cross-links and mass spectrometry. *Proc. Natl. Acad. Sci. USA* **97**, 5802–5806 (2000)

FURTHER READING Chowdhury, S.K., Katta, V. & Chait, B.T. Probing conformational changes in proteins by mass spectrometry. *J. Am. Chem. Soc.* **112**, 9012–9013 (1990) | Baca, M. & Kent, S.B.H. Direct observation of a ternary complex between the dimeric enzyme HIV-1 protease and a substrate-based inhibitor. *J. Am. Chem. Soc.* **114**, 3992–3993 (1992) | Robinson, C.V. *et al.* Probing the nature of noncovalent interactions by mass spectrometry. A study of protein–CoA ligand binding and assembly. *J. Am. Chem. Soc.* **118**, 8646–8653 (1996) | Ruotolo, B.T. *et al.* Evidence for macromolecular protein rings in the absence of bulk water. *Science* **310**, 1658–1661 (2005)