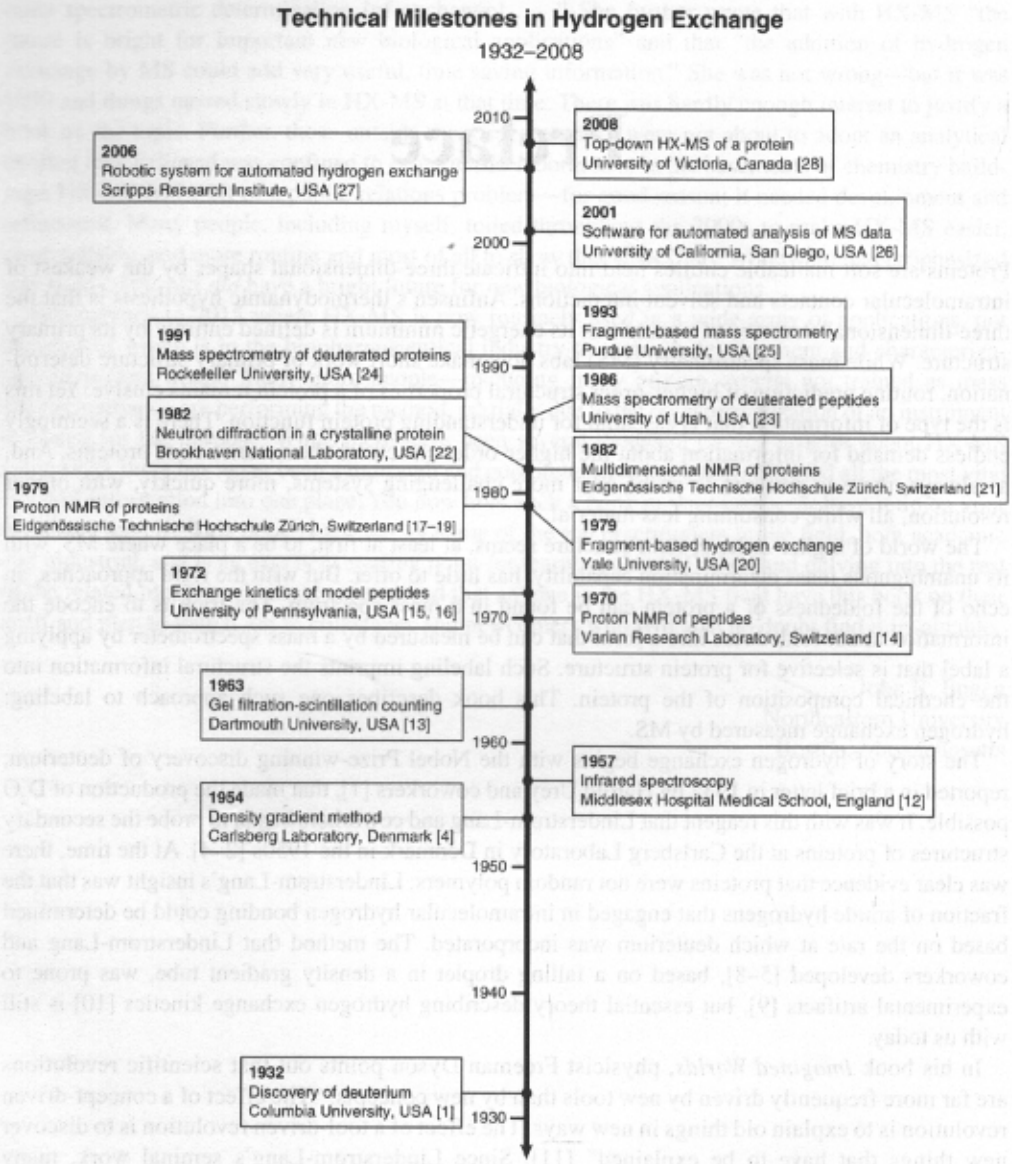
Preface

Proteins are soft malleable entities held into intricate three-dimensional shapes by the weakest of intramolecular contacts and solvent interactions. Anfinsen's thermodynamic hypothesis is that the three-dimensional structure of a protein at its energetic minimum is defined entirely by its primary structure. While mass spectrometry (MS) labs now make short work of primary structure determination, routine prediction of higher-order structural properties of a protein remains elusive. Yet this is the type of information that is essential for understanding protein function. There is a seemingly endless demand for information about the higher-order structural characteristics of proteins. And, as always, there is pressure to probe ever more challenging systems, more quickly, with higher resolution, all while consuming less material.

The world of higher-order protein structure seems, at least at first, to be a place where MS, with its unambiguous mass determination capability, has little to offer. But with the right approaches, an echo of the foldedness of a protein can be found in a mass spectrum. The trick is to encode the information about foldedness into a form that can be measured by a mass spectrometer by applying a label that is selective for protein structure. Such labeling imprints the structural information into the chemical composition of the protein. This book describes one such approach to labeling: hydrogen exchange measured by MS.

The story of hydrogen exchange begins with the Nobel Prize-winning discovery of deuterium, reported in a brief letter in 1932 by Harold Urey and coworkers [1], that made the production of D_2O possible. It was with this reagent that Linderstrøm-Lang and coworkers began to probe the secondary structures of proteins at the Carlsberg Laboratory in Denmark in the 1950s [2–4]. At the time, there was clear evidence that proteins were not random polymers; Linderstrøm-Lang's insight was that the fraction of amide hydrogens that engaged in intramolecular hydrogen bonding could be determined based on the rate at which deuterium was incorporated. The method that Linderstrøm-Lang and coworkers developed [5–8], based on a falling droplet in a density gradient tube, was prone to experimental artifacts [9], but essential theory describing hydrogen exchange kinetics [10] is still with us today.

In his book *Imagined Worlds*, physicist Freeman Dyson points out that scientific revolutions are far more frequently driven by new tools than by new concepts: "The effect of a concept-driven revolution is to explain old things in new ways. The effect of a tool-driven revolution is to discover new things that have to be explained" [11]. Since Linderstrøm-Lang's seminal work, many different tools have been brought to bear on these questions: "How many amide hydrogens exchange?" "How quickly do they exchange?" "Where are they located within the primary structure?" Measurement of amide hydrogen exchange had a long and celebrated history long before Fenn, Tanaka, Karas, and Hillenkamp threw open the doors of protein analysis to mass spectrometrists. Over the last 60 years, as new tools became available, hydrogen exchange evolved from a well-intentioned but unreliable technique into one that is used in perhaps hundreds of labs and now features at least one fully commercialized platform.



Major advances in hydrogen exchange

The timeline recounts some of the major technical advances. By the 1980s, it was possible to obtain site-resolved hydrogen exchange kinetics using multidimensional NMR, at least for small, well-behaved proteins that could be ^{15}N-labeled [21]. An alternative to NMR for measuring hydrogen exchange was developed in Fred Richards' lab in 1979 [20, 29]. The approach, based on proteolysis of the labeled protein under slow exchange conditions followed by chromatographic separation of the labeled peptides, is the forerunner of what is now generally referred to as the bottom-up hydrogen exchange mass spectrometry (HX-MS) experiment. Coupling the chromatography technique with MS was first accomplished by Zhongqi Zhang and David Smith at Purdue in 1993 [25].

(As noted in the timeline, hydrogen exchange in folded polypeptides had been recorded by MS before the work of Zhang and Smith.)

Since 1993, technology to support the MS-based approach has continued to advance. Major milestones along the way include the introduction of software to automate data analysis [26], robotics to automate sample preparation [27], and the achievement of single-residue resolution by MS [28]. MS has essentially removed the protein size limit, enables analysis of complex samples and multiprotein systems, requires much smaller quantities of proteins, and does not require installation of NMR-active nuclei (e.g., ^{13}C and ^{15}N). All of these advantages have led to dramatic growth in the field: a survey of the literature between January 2012 and June 2014 cataloged a total of 234 publications in which HX-MS was used [30]. The field is now composed of some approximately 10^2 labs, roughly an order-of-magnitude increase since I was introduced to the technique at the University of New Mexico in 2004 [31]. These days, when talking to scientists in the biological sciences, I find that I rarely need to explain what hydrogen exchange is. More often than not, the conversation quickly turns to questions about whether HX-MS could be applied to their problems of interest and to proposed collaborations.

This text serves two purposes. For the newcomer, the book introduces the technique to those who either wish to practice HX-MS or those who simply want to better understand the technique. The text provides numerous illustrations of the kinds of questions that HX-MS can answer. For experienced practitioners, the book provides a compendium of more advanced topics. Our goal was to bring together knowledge scattered across the primary and review literature into a single volume.

The book begins with fundamentals, both of hydrogen exchange itself, in Chapter 1, and how a traditional peptide-based experiment is conducted, in Chapter 2. HX-MS is a data-intensive method; data analysis and visualization tools play an essential role. The general requirements for a software platform and several examples of data analysis software are reviewed in Chapter 3. The requirements for method validation and standards for hydrogen exchange measurements are presented in Chapter 4.

The second unit of the book addresses more advanced technical aspects, beginning in Chapter 5 with millisecond hydrogen exchange, often useful for identification of the most dynamic regions of the protein backbone. An essential step in most HX-MS work is the localization of the sites of deuteration by proteolysis of the labeled protein. Chapter 6 reviews the array of different proteases that have been developed for hydrogen exchange. Hydrogen exchange is a kinetic process; Chapter 7 describes the many different ways that hydrogen exchange kinetics can be interpreted. In particular, there is now considerable interest in achieving single-residue resolution through analysis of exchange kinetics at the peptide level. Another way to achieve single-residue resolution is by making use of gas-phase fragmentation and tandem MS. Chapters 8 and 9 address this topic from two different standpoints: fragmentation of the individual proteolytic peptides (middle down) and direct fragmentation of whole proteins in the gas phase (top down). Although there are many exchangeable hydrogens in proteins, it is primarily the amide hydrogens that are measured since these sites retain deuterium strongly enough to be measurable. The C-2 hydrogen atom of the imidazole ring of histidine also exchanges with deuterium, albeit much more slowly than amide hydrogen atoms. The slow rate of exchange means loss of the label is also slow. The stability of the deuterated histidine label makes analysis using proteome-scale approaches possible, as discussed in Chapter 10.

The final portion of the book follows various methods and classes of problems that are tackled using HX-MS. Different hydrogen exchange-based approaches to capture the details of ligand binding are described in Chapter 11. These approaches include methods to map binding sites, track ligand-induced effect, and measure binding affinity and stoichiometry. Drugs are often protein ligands. Chapter 12 introduces us to the kinds of information that can be derived from HX-MS that are informative to the drug discovery process. The theme of drug development continues for the next several chapters. Chapter 13 introduces the problem of comparability: How can a biotherapeutic

protein manufacturer assure that all lots of a protein have the same structure or that the generic version of a protein therapeutic is "highly similar" to the innovator? The theme of therapeutic proteins continues in subsequent chapters. Monoclonal antibody therapeutics is one of the fastest-growing segments of the pharmaceutical industry. A major challenge to developing an antibody is defining the epitope, the site on the antigen that is recognized by the antibody. Chapter 14 reviews the application of HX-MS to define these epitopes. Beyond the therapeutic entity itself, a drug contains a carefully developed formulation of additives, stabilizers, and preservatives. Chapters 15 and 18 review the applications of HX-MS to guide the development of these formulations. Ultimately, the goal is to develop a structural/molecular understanding of how formulations affect protein therapeutics, either in the solid state (Chapter 15) or in solution (Chapter 18).

A problem that continues to challenge the structural biology community is understanding the structure of membrane proteins in their native environments. Chapter 16 describes the application of HX-MS to membrane proteins when the proteins are reconstituted *in vitro* in simulated membrane systems ranging from simple vesicles all the way to purified mitochondria. Chapter 17 explores the application of HX-MS to learn about the behavior of intrinsically disordered proteins. Disordered proteins derive at least some aspect of function from their disordered regions. HX-MS has provided new insights into how these disordered regions function.

I would like to express my gratitude to all of the authors who contributed to this volume. Together, we have attempted to capture and distill the collective wisdom of an active and rapidly growing field. I also extend my appreciation to the editorial and production staff at Wiley; this text would not have been possible without their efforts. Finally, I thank my family for their patience, understanding, and support during the many hours I devoted to this project.

David D. Weis
The University of Kansas
Lawrence, KS, USA

References

[1] Urey, H.C., Brickwedde, F.G., Murphy, G.M. (1932) A hydrogen isotope of mass 2. *Phys Rev*, **39** (1), 164–165.
[2] Hvidt, A., Johansen, G., Linderstrøm-Lang, K., Vaslow, F. (1955) Exchange of deuterium and ^{18}O between water and other substances. 1. Methods. *Comptes rendus des travaux du laboratoire Carlsberg*, **29** (9), 129–157.
[3] Hvidt, A., Johansen, G., Linderstrøm-Lang, K., Vaslow, F. (1955) Exchange of deuterium and ^{18}O between water and other substances. 3. Deuterium exchange of short peptides, Sanger's A-chain and insulin. *Comptes rendus des travaux du laboratoire Carlsberg*, **29** (23), 385–402.
[4] Hvidt, A., Linderstrøm-Lang, K. (1954) Exchange of hydrogen atoms in insulin with deuterium atoms in aqueous solutions. *Biochim Biophys Acta*, **14**, 574–575.
[5] Englander, S.W., Mayne, L., Bai, Y., Sosnick, T.R. (1997) Hydrogen exchange: The modern legacy of Linderstrøm-Lang. *Protein Sci*, **6** (5), 1101–1109.
[6] Schellman, J.A., Schellman, C.G. (1997) Kaj Ulrik Linderstrøm-Lang (1896–1959). *Protein Sci*, **6** (5), 1092–1100.
[7] Englander, S.W. (2006) Hydrogen exchange and mass spectrometry: A historical perspective. *J Am Soc Mass Spectrom*, **17** (11), 1481–1489.
[8] Baldwin, R.L. (2011) Early days of protein hydrogen exchange: 1954–1972. *Proteins*, **79** (7), 2021–2026.
[9] Englander, S.W., Downer, N.W., Teitelbaum, H. (1972) Hydrogen exchange. *Annu Rev Biochem*, **41** (1), 903–924.
[10] Hvidt, A., Nielsen, S.O. (1966) Hydrogen exchange in proteins. *Adv Protein Chem*, **21**, 287–385.
[11] Dyson, F.J. (1997) *Imagined Worlds*, Harvard University Press, Cambridge.

[12] Haggis, G.H. (1957) Proton-deuteron exchange in protein and nucleoprotein molecules surrounded by heavy water. *Biochim Biophys Acta*, **23**, 494–503.
[13] Englander, S.W. (1963) A hydrogen exchange method using tritium and sephadex: Its application to ribonuclease. *Biochemistry*, **2** (4), 798–807.
[14] Sheinblatt, M. (1970) Determination of an acidity scale for peptide hydrogens from nuclear magnetic resonance kinetic studies. *J Am Chem Soc*, **92** (8), 2505–2509.
[15] Molday, R.S., Englander, S.W., Kallen, R.G. (1972) Primary structure effects on peptide group hydrogen exchange. *Biochemistry*, **11** (2), 150–158.
[16] Molday, R.S., Kallen, R.G. (1972) Substituent effects on amide hydrogen exchange rates in aqueous solution. *J Am Chem Soc*, **94** (19), 6739–6745.
[17] Richarz, R., Sehr, P., Wagner, G., Wüthrich, K. (1979) Kinetics of the exchange of individual amide protons in the basic pancreatic trypsin inhibitor. *J Mol Biol*, **130** (1), 19–30.
[18] Wagner, G., Wüthrich, K. (1979) Correlation between the amide proton exchange rates and the denaturation temperatures in globular proteins related to the basic pancreatic trypsin inhibitor. *J Mol Biol*, **130** (1), 31–37.
[19] Wüthrich, K., Wagner, G. (1979) Nuclear magnetic resonance of labile protons in the basic pancreatic trypsin inhibitor. *J Mol Biol*, **130** (1), 1–18.
[20] Rosa, J.J., Richards, F.M. (1979) An experimental procedure for increasing the structural resolution of chemical hydrogen-exchange measurements on proteins: Application to ribonuclease S peptide. *J Mol Biol*, **133** (3), 399–416.
[21] Wagner, G., Wüthrich, K. (1982) Amide proton exchange and surface conformation of the basic pancreatic trypsin inhibitor in solution: Studies with two-dimensional nuclear magnetic resonance. *J Mol Biol*, **160** (2), 343–361.
[22] Kossiakoff, A.A. (1982) Protein dynamics investigated by the neutron diffraction-hydrogen exchange technique. *Nature*, **296** (5859), 713–721.
[23] Verma, S., Pomerantz, S.C., Sethi, S.K., McCloskey, J.A. (1986) Fast atom bombardment mass spectrometry following hydrogen-deuterium exchange. *Anal Chem*, **58** (14), 2898–2902.
[24] Katta, V., Chait, B.T. (1991) Conformational changes in proteins probed by hydrogen-exchange electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom*, **5**, 214–217.
[25] Zhang, Z., Smith, D.L. (1993) Determination of amide hydrogen exchange by mass spectrometry: A new tool for protein structure elucidation. *Protein Sci*, **2** (4), 522–531.
[26] Woods, V.L., Hamuro, Y. (2001) High resolution, high-throughput amide deuterium exchange-mass spectrometry (DXMS) determination of protein binding site structure and dynamics: Utility in pharmaceutical design. *J Cell Biochem*, **84** (S37), 89–98.
[27] Chalmers, M.J., Busby, S.A., Pascal, B.D., *et al.* (2006) Probing protein–ligand interactions by automated hydrogen/deuterium exchange mass spectrometry. *Anal Chem*, **78** (4), 1005–1014.
[28] Pan, J., Han, J., Borchers, C.H., Konermann, L. (2008) Electron capture dissociation of electrosprayed protein ions for spatially resolved hydrogen exchange measurements. *J Am Chem Soc*, **130** (35), 11574–11575.
[29] Rosa, J.J., Richards, F.M. (1981) Hydrogen exchange from identified regions of the S-protein component of ribonuclease as a function of temperature, pH, and the binding of S-peptide. *J Mol Biol*, **145** (4), 835–851.
[30] Pirrone, G.F., Iacob, R.E., Engen, J.R. (2014) Applications of hydrogen/deuterium exchange MS from 2012 to 2014. *Anal Chem*, **87**, 99–118.
[31] Weis, D.D., Kjellen, P., Sefton, B.M., Engen, J.R. (2006) Altered dynamics in Lck SH3 upon binding to the LBD1 domain of *Herpesvirus saimiri* Tip. *Protein Sci*, **15** (10), 2402–2410.