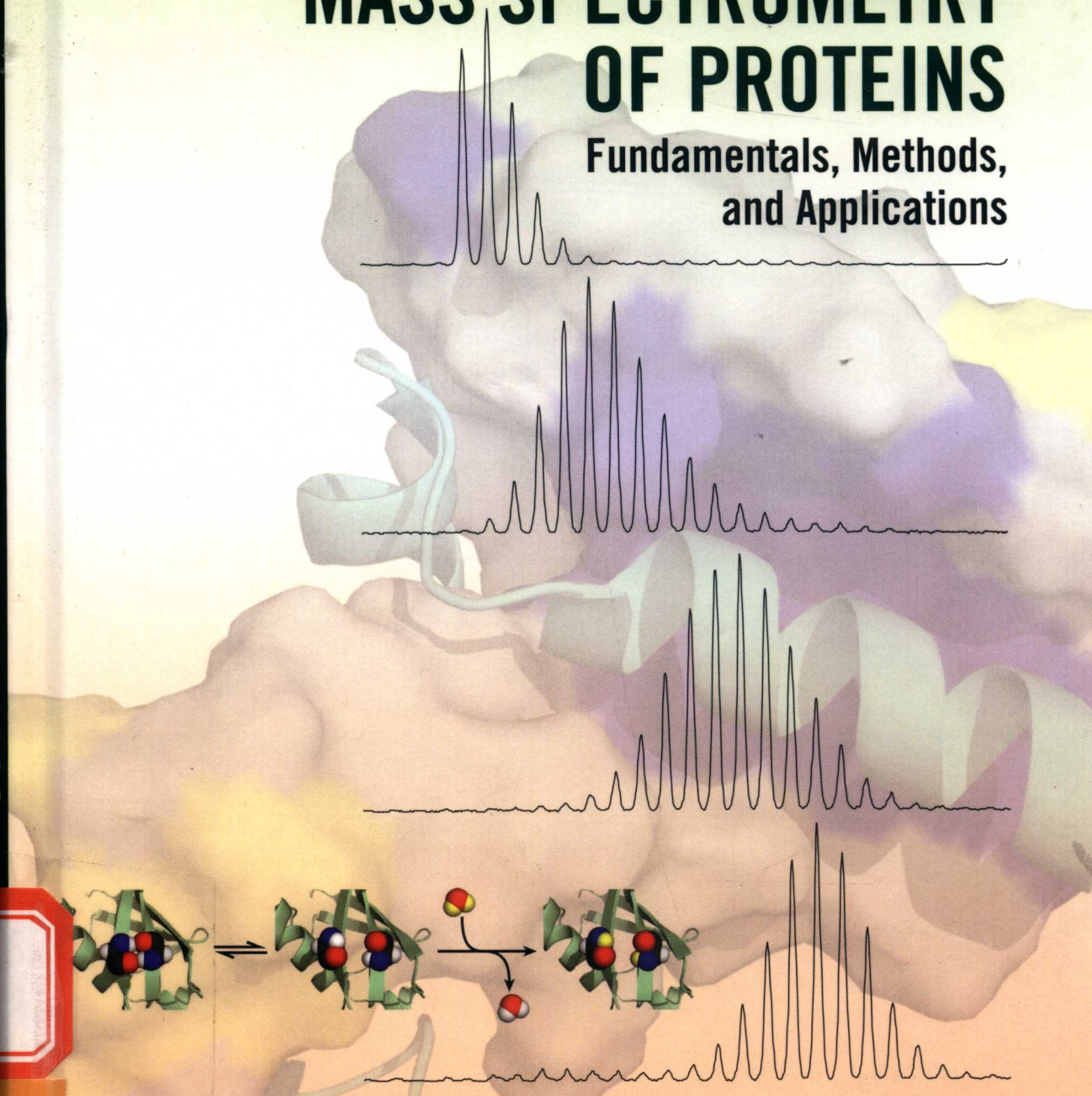
David D. Weis
Editor

HYDROGEN EXCHANGE MASS SPECTROMETRY OF PROTEINS

Fundamentals, Methods, and Applications



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DAVID D. WEIS

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Foreword

Perhaps you opened this book wondering, "What is hydrogen exchange mass spectrometry?" If so, I am happy to welcome another recruit to the field—skip to the next paragraph. Or maybe you already knew what it was and instead opened the book thinking, "Wow, a whole book on that?" If so, yes, and wait until you see what's in here—skip to paragraph three.

The hydrogen exchange (HX) phenomenon is the basis for a method of investigating protein shape, motion, folding, and interactions. In amino acids—and therefore peptides and proteins—there are certain hydrogen atoms that continuously change places with hydrogen atoms in the surrounding solvent. Hydrogen bonds within peptides/proteins and solvent protection afforded by the protein fold can alter HX. If the solvent surrounding a protein (normally H_2O) is replaced with a solvent containing an isotope of hydrogen (deuterium, D_2O, or tritium, T_2O), the isotope in the solvent exchanges into the protein. The exchange can be measured, both to find where it occurred and at what rate. Any measurement method capable of distinguishing between the isotopes of hydrogen can be used.

As you will learn in this book, while HX is quite an old technique (starting in the 1950s), the detection of the exchange with mass spectrometry (MS) is comparatively recent (starting in the early 1990s). In the 1940s and 1950s, the study of proteins was growing rapidly including the first X-ray crystal structures of proteins and an understanding of hydrogen bonding in proteins. These revelations were important to the beginnings of HX and development of exchange theory. Various methods were used over the years to measure the exchange including density, radioactivity, nuclear magnetic resonance, and infrared spectroscopy. Although the atomic weight of the different hydrogen isotopes allows them to be distinguished from each other by MS, analysis of proteins and peptides by MS did not become commonplace until the advent of MALDI and electrospray ionization in the early 1990s. Hydrogen exchange mass spectrometry (HX-MS) as a method is essentially 25 years old. This book will arrive in time for the silver anniversary of the method.

In the beginning, some immediately saw the value of MS and went to great lengths to perform it. Brian Chait's laboratory at the Rockefeller University was the first to report on HX-MS of a protein. A good reason for this was that he had the necessary instrumentation. In the early 1990s, liquid chromatography (LC) and electrospray MS were not widely available. In fact, the first report of measuring HX after proteolytic fragmentation of a labeled protein by David Smith's laboratory at Purdue University used a much older method of ionization, fast atom bombardment, that was not nearly as ideal for HX-MS. It took almost 10 years for LC-MS to become commonplace and routine, and as a result, HX-MS was a method "only for experts" during the early days. The early HX-MS disciples could see clear advantages to MS detection of HX, including the ability to analyze proteins that would not crystallize, characterization of proteins in very dilute solutions or proteins that were only available in small quantities, and analysis of protein motion and folding that could not be done by other biophysical techniques.

I remember well the excitement of David Smith, my Ph.D. advisor, when he organized a journal focus issue devoted to HX-MS (August 1999 issue, *Journal of the American Society for Mass Spectrometry*). The focus issue only contained five articles, but that was critical mass at that time! In an insightful opening Perspective article, Clare Woodward (who by 1999 had been in the field of HX for over 30 years) noted that the field of HX was "on the cusp of a new advancement in methodology,

mass spectrometric determination [of exchange] .... " She further wrote that with HX-MS "the future is bright for important new biological applications" and that "the addition of hydrogen exchange by MS could add very useful, time saving information." She was not wrong—but it was 1999 and things moved slowly in HX-MS at that time. There was hardly enough interest to justify a book on the topic. Further, those outside mass spectrometry were not about to adopt an analytical method they believed was confined to esoteric MS laboratories in the basements of chemistry buildings. HX-MS had a bit of a public relations problem—for good reason; it needed development and refinement. Many people, including myself, toiled throughout the 2000s to make HX-MS easier, more reliable, and more routine and most of all to show that what Clare Woodward had prophesized was right—HX-MS *did* have a bright future for new biological applications.

Fast-forward to 2015 where HX-MS is now routinely used in a wide array of applications, not the least of which is in the biopharmaceutical industry to assist in development and characterization of protein drugs. Many more people—including many protein experts not trained as mass spectrometrists—are performing the method, partially due to the commercialization of an instrument designed for the purpose. While there have been several thousand journal articles about HX-MS since 1990, there has never been a thorough and comprehensive book that collected all the most vital HX-MS information into one place. You now hold such a book in your hands (or have it up on your computer screen). This book is written by some of the top practitioners in the field, both academic and industrial, and is an excellent starting point for learning about HX-MS and delving into the rest of the related literature. I would recommend that anyone in the HX-MS field have this book on their shelf and plan to loan it out to colleagues and newcomers who will also no doubt find it invaluable.

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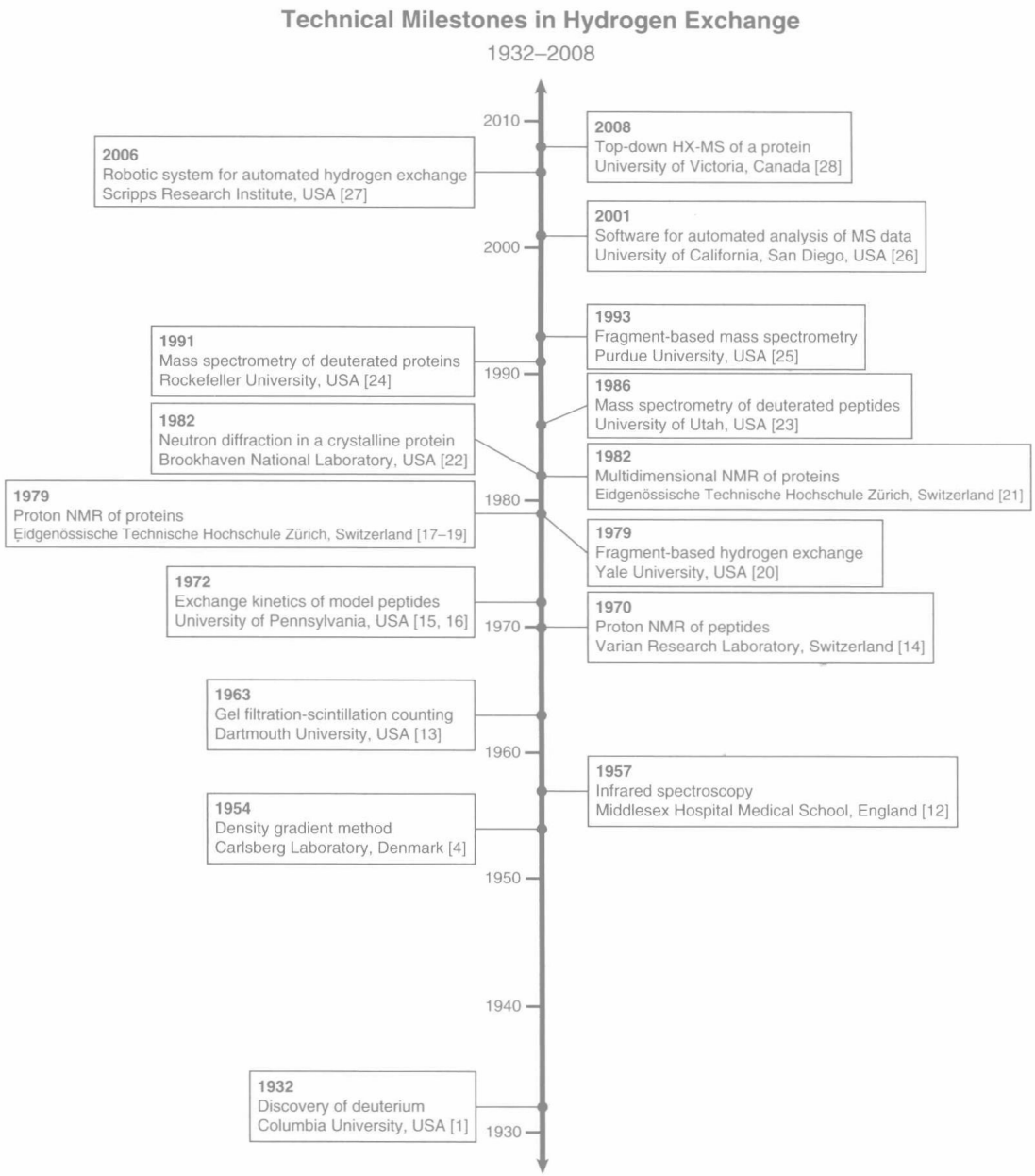
Preface

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.

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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.

A Note about Nomenclature

The technique of amide hydrogen exchange, especially when coupled with mass spectrometry, has been and continues to be described by a number of different terms and acronyms. One of the more arduous tasks that I had as editor of this text was standardization of the terminology across the many chapters of this book written by many different authors. *Hydrogen* is a naturally occurring element composed of three named isotopes: *protium*, *deuterium*, and *tritium* [1]. Hydrogen is unique in that its isotopes have these distinct names, as opposed to, carbon, for example, also having three equally celebrated isotopes that nevertheless are relegated to utilitarian titles: carbon-12, carbon-13, and carbon-14. In this text, the term *hydrogen exchange* is used to describe any mode of isotopic exchange that involves two different isotopes of hydrogen. Thus, the term hydrogen exchange is universal since it refers to any form of isotope exchange from the conventional $^{1}H/^{2}H$ exchange mode all the way to $^{3}H/^{2}H$ exchange, a seemingly exotic and costly enterprise. At the very least, though, the term hydrogen exchange does describe equally well the exchange-in ($^{1}H/^{2}H$) and exchange-out ($^{2}H/^{1}H$) experiments in common practice today as well as some $^{3}H/^{1}H$ experiments that were developed prior to measurement of hydrogen exchange by nuclear magnetic resonance and MS. In addition, we gain in economy through the loss of four syllables allocated to "deuterium." As most of this text is concerned specifically with the amide hydrogen atom, amide is implied except where noted.

Having established hydrogen exchange mass spectrometry as the preferred term to describe the technique, the next question is how to abbreviate it. In particular, what to do about the term *exchange*. The technique is hyphenated: it combines two distinctive techniques [2]. Thus, a hyphen, at least in the abbreviation, seems suitable. Choices such as HE-MS or HEx-MS strike me as both unreadable and unpronounceable. This seems to leave *HX-MS* as the best abbreviation for hydrogen exchange mass spectrometry where the *X* represents *exchange*.

This, then, is the terminology upon which the text has been standardized. I thank a member of the community who swayed my thinking on these points—I have for a long time been a diehard user of *H/D-MS*. My intention here has been to use consistent terminology in the text, not to settle any debate about nomenclature. My apologies to those of you who might find this term like fingernails upon the proverbial chalkboard. While I hope that the choices I have made here reflect a desire for simplicity and clarity, it is possible, perhaps, that they merely reflect my own esthetic sensibilities.

References

[1] International Union of Pure and Applied Chemistry (2014). IUPAC Compendium of Chemical Terminology—the Gold Book. Release 2.3.3.

[2] Hirschfeld, T. (1980) The Hy-phen-ated methods. *Analytical Chemistry*, **52** (2), 297A–312A.

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