PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

PERSPECTIVES

Early days of protein hydrogen exchange: 1954–1972

Robert L. Baldwin*

Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, California 94305

ABSTRACT

Hydrogen exchange (HX) is recognized today as one of the most powerful and versatile tools available to protein scientists, especially for studying protein conformational change. This short history traces the beginnings of the HX method and the basic problems that faced the founders. Protein HX began as a simple idea with a straightforward goal, but the first experiments revealed both the unexpected complexity of the subject and the potential power of the method for probing deep into how proteins work. By 1972, the chemistry of the exchange reaction in peptides began to be well understood, but the challenge of getting and interpreting data on HX for individual peptide NH protons in proteins remained for decades longer.

Proteins 2011; 79:2021–2026.

Key words: Linderstrøm-Lang model; EX1, EX2 exchange; poly-D,L-alanine; α-helix search.

SEARCH FOR THE α-HELIX

Linderstrøm-Lang's motivation for developing the hydrogen exchange (HX) method[1,2] in 1954 was to find hydrogen-bonded structures in proteins. In 1951, when Pauling and Corey proposed the structures of the α-helix[3] and the two β-sheets,[4] there was no experimental evidence supporting them. In fact, Pauling worked out these structures in 1948 when he was on sabbatical at Oxford, and he delayed publishing them for 3 years for this reason.[5] His structures of the α-helix and two β-sheets were very persuasive but how could they be identified and measured in proteins? The first X-ray structure of a protein (myoglobin, Mb) was not published[6] until 1958 (at 4 Å resolution) and the α-helix itself was not seen until 1960 when the 2 Å structure of Mb appeared.[7] Shortly after the proposed structure of the α-helix was published, Perutz[8] found its predicted axial spacing in a published cast film photo of poly-γ-benzyl-L-glutamate, but the axial spacing of the α-helix is not very different from those of other proposed helical structures considered earlier by Bragg et al.[9]

BACKGROUND OF THE DEUTERIUM EXCHANGE METHOD

Linderstrøm-Lang knew key facts about HX before he developed the deuterium exchange method.[10] He knew that a nonaqueous density gradient column could be used to measure a density difference of 1×10^{-6} g/cm^3 between two aqueous droplets, and he knew that NH protons in peptides undergo exchange with H_2O by a mechanism that involves hydrogen bonding, whereas C—H protons do not exchange measurably in the same time frame. He reasoned that, because the hydrogen bonds of α-helices and β-sheets are formed cooperatively, internally H-bonded peptide NH protons in proteins would exchange much more slowly than free peptide NH protons. He believed he could count even the free NH protons in proteins and easily distinguish them from internally H-bonded peptide NH protons. From John Schellman's work in Linderstrøm-Lang's laboratory (the Carlsberg Laboratory) on characteristic differences

Based on a talk given at an 80th birthday symposium for Walter Englander, Philadelphia, April 24, 2010.

*Correspondence to: Robert L. Baldwin, Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305. E-mail: baldwinb@stanford.edu.
Received 14 March 2011; Accepted 14 March 2011
Published online 22 March 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/prot.23039

between optical rotatory dispersion curves of native proteins versus denatured proteins and peptides,[11] Linderstrøm-Lang believed that native proteins almost certainly contain H-bonded backbone structures.

FIRST RESULTS

Linderstrøm-Lang's first paper (1954) on the deuterium exchange method[1] focuses on the method itself and was illustrated with experiments on NH_4Cl. In a second paper, he and coworkers showed they could count correctly the number of free NH protons both in short peptides and in insulin.[2] Their results for insulin[2] revealed that its HX kinetics are highly complex; the kinetics are far from being single-exponential and extend over a much longer time range than with short peptides. Whereas short peptides apparently showed complete exchange in less than a minute (pH 3, 0°C), complete exchange of insulin in the same conditions required at least a day. Increasing the temperature from 0 to 38.6°C or adding 5.2 molal urea speeded up exchange hugely. The isolated A-chain of insulin showed rapid exchange, like short peptides.[2]

EARLY CONNECTIONS BETWEEN HX AND THE MECHANISM OF PROTEIN FOLDING

The first HX experiments on a protein (insulin, 1955)[2] made clear the intimate connection between protein HX and basic concepts of protein folding that were just beginning to be grasped. The proposal that a protein has measurable thermodynamic stability, which depends on temperature and denaturant concentration, was just beginning to be accepted in that period. In 1956, Harrington and Schellman[12] reported that thermal unfolding of ribonuclease A (RNase A) is reversible, and equilibrium is reached rapidly (∼seconds). RNase A stability evidently changed rapidly with temperature in their experiments because, when the unfolding transition zone was reached, there were large changes in the relative amounts of folded and unfolded protein. Thus, RNase A stability was a thermodynamic property because it could be described by equilibrium concentrations of folded and unfolded protein species, although they were yet to be characterized. Earlier experiments by Walter Kauzmann's group at Princeton demonstrated that protein denaturation is basically unfolding of the protein's ordered 3D structure,[13] but these experiments (which began in 1953[13]) measured irreversible denaturation.

The concept that protein denaturation is intrinsically a reversible process, and that equilibrium can be reached between native and denatured forms has a very long history: see the 1948 study of soy bean trypsin inhibitor by Kunitz,[14] who gives a history of earlier work. Slow denaturation (hours) of relatively complex proteins is characteristic of these earlier studies, which were interpreted in terms of a two-state equilibrium between native and denatured forms. A 1951 study of the thermal denaturation of chymotrypsinogen A by Eisenberg and Schwert[15] emphasizes its reversibility and rapidity (∼seconds) but misses the later point that denaturation is produced by unfolding. These authors found a huge activation enthalpy for denaturation and interpreted[15] it as the result of breaking many interactions between the water lattice and the native protein.

The 1950s saw the birth of a new research field, the mechanism of protein folding, and the Carlsberg Laboratory was a central meeting point for this work. Key figures in protein folding work either made repeated short visits to the Carlsberg Laboratory (Chris Anfinsen, Walter Kauzmann) or came as postdoctoral fellows (Bill Harrington, Fred Richards, John Schellman) or as sabbatical visitors (Harold Scheraga). Fred Richards' revolutionary discovery[16] in 1958 that an active enzyme (RNase S) can be generated by mixing together two inactive protein fragments (S-protein and S-peptide) began in1955 when he was an incoming postdoctoral fellow and Linderstrøm-Lang gave him the problem of understanding the mysterious kinetics of the digestion of RNase A by subtilisin[17] when measured by enzyme activity.

Chris Anfinsen's discovery that the free energy of folding drives the protein folding process began in 1957 with a study of the reductive cleavage of the 4 S—S bonds of RNase A,[18] and this work was essentially completed by 1963.[19] With hindsight, his principle might have been guessed from experiments made earlier in the Carlsberg Laboratory. Anfinsen wanted to synthesize a protein (RNase A) in the test tube, and he had to discover how to make the four correct S—S bonds out of 105 possible choices. He found that the free energy of the folding process itself chooses the four correct S—S bonds when refolding/reoxidation is allowed to proceed in reversible conditions. It was well known by 1957 that breaking S—S bonds causes protein unfolding: for example, in 1956 the four S—S bonds of RNase A were oxidized to generate a model for a single-chain, unfolded protein.[12] Adding this knowledge to the knowledge that RNase A undergoes rapidly reversible thermal unfolding[12] makes Anfinsen's principle plausible as early as 1956.

THE "LINDERSTRØM-LANG EQUATION" FOR HX

Today the "Linderstrøm-Lang equation" for protein HX is well known and widely cited. However, the equation used today (which comes from the 1966 review by Hvidt and Nielsen[20]) was based on John Schellman's derivation, which was first published by Hvidt[21] in 1964 (after Linderstrøm-Lang' death). It is based on the

two-step mechanism of HX described below. Hvidt says[21] "The exchange mechanism described here is the one described by Linderstrøm-Lang[22]", and her statement is often taken to mean that he derived the modern equation for protein HX. Her reference[22] is to a 1958 symposium paper[22] by Linderstrøm-Lang that was condensed from a 1957 paper by Berger and Linderstrøm-Lang.[23] Because Hvidt's own paper[21] was published in the Carlsberg Laboratory journal, it is not easily available today and neither is Ref. [22]. The net result is that Ref. [23], which describes the HX kinetics of poly-D,L-alanine (PDLA), is frequently cited as the origin of the Linderstrøm-Lang equation for the HX kinetics of proteins! PDLA is known today to be a random-coil polypeptide.

Berger and Linderstrøm-Lang[23] considered PDLA to be a helix stabilized by interactions between neighboring —CH_3 side chains (with opposing chiral orientations on L and D residues), and so they considered PDLA to be a simple model for a folded protein. Although the two-step exchange mechanism they used is the same as that considered by Hvidt,[21] the equations they gave for HX from a PDLA helix are more complex than the modern equation[20] for protein HX, and they did not consider the helix opening reaction to be rate-limiting[20] for exchange of PDLA. The modern equation[20] is written for a single NH proton and a single-exponential time course. The two-step exchange mechanism given by Hvidt[21] in 1964 is

$$\mathrm{N} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \mathrm{I} \overset{k_3}{\leftrightarrow} [\text{exchanged}] \qquad (1)$$

The closed (N) and open (I) forms of the protein are specified to be exchange-resistant and exchange-accessible, respectively. Various NH protons may take part in a given protein opening reaction. The complete time course for exchange[21] was taken by Schellman from a standard kinetics text.

The commonly used modern equation[20] for protein HX is less general than the complete solution given by Hvidt[21] (her equations 6–9), and the modern equation[20] is based on the steady-state approximation and has a single-exponential time course. The observed HX rate constant ($k_{ex} = 1/\tau$) is given by

$$k_{ex} = k_1 k_3/(k_1 + k_2 + k_3) \qquad (2)$$

$$k_3 = k_{ch}[\text{cat}] \qquad (2a)$$

where [cat] is the catalyst concentration, [OH^-] for base-catalyzed HX and [H^+] for acid-catalyzed HX. There are two important limiting cases[20] of Eq. (2) for base-catalyzed exchange (see below): EX2 with $k_3 \ll k_2$ and EX1 with $k_3 \gg k_2$.

PDLA CONSIDERED AS A STABLE HELIX

Why was PDLA considered to be a stable helix in 1957? In 1952, Elliott, a polymer chemist at the Courtauld Institute, was using polarized infrared radiation to look for β and α forms of synthetic polypeptides in cast films. For a PDLA film cast from aqueous solution, he found an α infrared absorption band and concluded that PDLA exists in an α form.[24] At that time, X-ray fiber photos were used to tell whether a structural protein exists in either an α (helical) or β (extended) form; it was known from earlier work that an α⇔β transition can be produced by mechanical stretching of feather keratin. (These helical and extended forms probably explain how the α-helix and β-sheet got their names.) Berger, who was at the Weizmann Institute, was aware of Elliott's 1952 study of PDLA,[24] and he concluded that Elliott's α form is probably a water-soluble α-helix. He prepared a sample of PDLA (made by the Leuchs polymerization of the mixed D and L N-carboxy-anhydrides) and brought the sample with him to study its HX kinetics in the Carlsberg Laboratory.

In support of a helical structure for PDLA, Berger and Linderstrøm-Lang[23] found that its HX kinetics were slower than those of oxidized RNase A, which was taken then as a standard model for an unfolded polypeptide.[12] Doubts about PDLA being a stable helix appeared fairly soon. From an infrared study of the HX kinetics of PDLA in 1960, Bryan and Nielsen[25] concluded that PDLA is probably a random coil. When Gratzer and Doty[26] studied whether poly-L-alanine (made water-soluble by adding flanking L-glutamate residues) forms a stable helix in water, they also reinvestigated the proposed helix formed by PDLA. They concluded that Elliott's cast film of PDLA probably contained some short runs of L-alanine and D-alanine, long enough to form short helices that gave the observed α infrared absorption band. A 1969 study by Englander and Polsen[27] compared the pH-dependent HX kinetics of PDLA with those of poly-D,L-lysine (known to be a random coil), and the results left no doubt that PDLA is also a random coil.

ACID- AND BASE-CATALYZED HX AND THE BRIDGE MECHANISM OF PROTON TRANSFER

A critical step forward in understanding the mechanism of peptide HX was made in 1959 by Berger et al.[28] who used NMR to demonstrate that HX of the single NH proton of *N*-methyl-acetamide is catalyzed by acid as well as by base. Acid catalysis becomes evident only below pH 3.0 and was generally overlooked in earlier work, although Berger and Linderstrøm-Lang were aware of it in 1957.[23]

Another important step in understanding peptide HX was made in 1964 by Eigen,[29] who found that aqueous acids and bases, including amides, undergo proton transfer by a bridge mechanism. The bridged complex between an acid AH and a base B is formed in a diffusion-controlled reaction, after which the proton shuttles rapidly back and forth between AH and BH (AH•••B ↔ A•••BH). The pK difference between AH and BH determines the probability of whether the proton resides on A or B when the bridged complex breaks apart.

EX1 AND EX2 MECHANISMS OF HX

Tragically, Linderstrøm-Lang died at an early age. Åase Hvidt, his principal research assistant in the early HX studies, then joined the Chemistry faculty of the University of Copenhagen. Sigurd Nielsen, who had been developing an infrared absorbance method for measuring HX kinetics, moved from the Carlsberg Laboratory to the atomic energy research facility at Risø, Denmark. Together they wrote a monumental review[20] of HX research up to 1966. It is cited today especially because Hvidt and Nielsen[20] introduced terms for the EX1 and EX2 mechanisms of protein HX; the two mechanisms are found in the pH range above pH 3 where HX is base-catalyzed. The rate of EX2 exchange is proportional to $K_{op}k_{ch}[OH^-]$ and is base-catalyzed, whereas the rate of EX1 exchange is proportional to k_{op} and is relatively pH-independent [see Eq. (2)]. Hvidt and Nielsen were able to fit existing protein HX data to pH curves that show a transition with increasing pH from EX2 to EX1 exchange. In the EX2 regime (typically below pH 5), log k_{ex} increases linearly with pH whereas in the EX1 regime (typically above pH 8) k_{ex} is nearly pH-independent. As she searched earlier for the proper HX equation to describe protein HX kinetics, Hvidt was puzzled by why the HX kinetics of different proteins depend strongly on pH but always in a similar manner.[21] In 1964, a typical explanation was that protein structures are motile and their motility is pH-dependent,[21] whereas Hvidt suspected that the correct explanation derives from the properties of base catalysis of the chemical step.

^{3}H-GEL FILTRATION METHOD OF MEASURING HX

By 1963, new HX studies employing the deuterium exchange method had almost ceased, despite the many gifted scientists who had come to Linderstrøm-Lang's laboratory to learn the HX method and to learn about new developments in the mechanism of protein folding. The initial goal set by Linderstrøm-Lang was to use HX to identify H-bonded backbone structures in proteins, but in 1960 the 2Å structure of Mb[7] already showed that the future lay in determining X-ray structures of proteins. Although HX studies were uniquely suited to the further goal of analyzing protein conformational changes, the obstacles were disheartening, especially the problem of how to disentangle the overlapping kinetic curves for various peptide NH protons piled together. Also, the deuterium exchange method was time consuming and subject to artifacts in the freeze-drying step.[30] This glum picture of the future of the HX method changed for the better in 1963 when Walter Englander introduced the ^{3}H-gel filtration method.[31] He had been a postdoctoral fellow studying collagen with Bill Harrington at NIH. They wanted to answer the question of whether hydrogen bonding to water is an important factor in explaining the stable helical structure of polyproline II, which does not have internal peptide H-bonds (personal communication, SWE). Englander and Harrington soon concluded that water H-bonding changes too rapidly to study by their HX method. Nevertheless, Englander remembered the intriguing possibilities of HX and decided to find a better way of measuring it. His ^{3}H-gel filtration method[31] was fast (^{3}H-labeled protein could be separated in 2 min from ^{3}H-labeled solvent), sensitive, and free from artifacts. Moreover, in 1963 scintillation counters were readily available for measuring ^{3}H. Water enters the sephadex gel rapidly because of its small size whereas proteins diffuse in slowly, and at equilibrium there is a good separation between the water and protein peaks as they emerge from the gel.

EFFECTS OF SIDE CHAINS AND NEIGHBORING PEPTIDE GROUPS

Before 1972, papers on peptide HX from different laboratories had shown that the rate of the chemical step depends on additional factors besides acid and base catalysis, but it was difficult to disentangle the various factors. The main problem was the use of unblocked peptides, since the charged end groups, especially the positively charged α-NH_3^+ group, were a serious complicating factor. By 1972, various factors were beginning to be well understood. In 1970, Sheinblatt[32] obtained relevant data for individual peptide NH protons in short peptides by using 1D-^{1}H-NMR. He showed that the chemical nature of the adjacent side chain has a major effect on the HX rate and so does the presence or absence of neighboring peptide groups. Moreover, by comparing log k_{ch} values for various adjacent side chains with the pK_a values of the corresponding carboxylic acids, Sheinblatt[32] was able to show that k_{ch} for base-catalyzed HX depends on the alkaline pK_a value of the peptide NH group, as expected from Eigen's bridge mechanism for proton transfer.[29] However, more work would be needed to take account of the major complicating effect of nearby charged end groups.

In 1972, Molday et al.[33] succeeded in quantifying the effects of neighboring peptide groups and the chemical nature of the side chain. To measure k_{ch} values for the various polar side chains they used blocked dipeptides (single amino acid residues flanked by two peptide groups) and they measured rates of both acid- and base-catalyzed HX. Their base-catalyzed k_{ch} values are known colloquially today as 'the Molday parameters'. Although their HX results for the effect of neighboring peptide groups are referenced less often, the effects are even larger. The base-catalyzed k_{ch} for the single peptide NH of *N*-methyl-acetamide increases 100-fold when neighboring peptide groups are added on both sides[33]; additional peptide groups have little further effect. Varying the adjacent side chain gave smaller changes in k_{ch}, typically less than 10-fold. The polar side chain effect was described in 1972 as an electron-withdrawing or an inductive effect.[33,34] Later work showed there is also a "blocking effect" that gives rise to different k_{ch} values for the various nonpolar amino acids. The two peptide NH protons on the left and right sides are affected quite differently by the nature of the side chain.

HX IN 1972

A turning point occurred in 1972 when most of the basic factors affecting the rate of the chemical step of protein HX were well understood, although the effects of nearby charged groups and the "blocking effect" of nonpolar side chains came later. By 1972, much of the earlier mystery surrounding protein HX had dissipated. These developments are reviewed in a short, classic 1972 paper.[30] On the other hand, the nature of protein opening reactions would remain mysterious (and much debated) up to this time. It would be necessary later to obtain NMR assignments of ^{1}H and ^{15}N chemical shifts of peptide NH groups using 2D methods, so that HX kinetics could be measured for individual peptide NH protons.

Inspired by the success of HX in opening up the study of protein conformational change, Printz and von Hippel,[35] Englander and Englander,[36] and their coworkers began to study the opening-dependent HX kinetics of DNA[35] and tRNA[36] as early as 1965. By 1972 (a turning point in the study of protein HX), a lot was already known both about DNA HX[37,38] and RNA HX[39] although much more would be learned in the future, especially by using new NMR approaches.

ACKNOWLEDGMENTS

My view of the early days of protein HX owes much to two sources: (1) discussions over the years with Walter Englander, Neville Kallenbach and John Schellman, and (2) a study leave in 1958-59 in Linderstrøm-Lang's laboratory. I am grateful to Bill Harrington and John Schellman for urging me to take a study leave and go. I thank Walter Englander, George Rose, and John Schellman for their comments on the Ms.

REFERENCES

1. Hvidt A, Johansen G, Linderstrøm-Lang K, Vaslow F. Exchange of deuterium and ^{18}O between water and other substances. I. Methods. C R Trav Lab Carlsberg 1954;29:129–157.
2. Hvidt A, Linderstrøm-Lang K. Exchange of deuterium and ^{18}O between water and other substances. III. Deuterium exchange of short peptides, Sanger's A-chain and insulin. C R Trav Lab Carlsberg 1955;29:385–402.
3. Pauling L, Corey RB, Branson HR. The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain. Proc Natl Acad Sci USA 1951;37:205–211.
4. Pauling L, Corey RB. The pleated sheet, a new layer configuration of polypeptide chains. Proc Natl Acad Sci USA 1951;37:251–256.
5. Eisenberg D. The discovery of the α-helix and β-sheet, the principal structural features of proteins. Proc Natl Acad Sci USA 2003; 100:11207–11210.
6. Kendrew JC, Bodo G, Dintzis HM, Parrish RG, Wyckoff H, Phillips DC. A three-dimensional model of the myoglobin molecule obtained by x-ray analysis. Nature 1958;181:662–666.
7. Kendrew JC, Dickerson RE, Strandberg BE, Hart RG, Davies DR, Phillips DC, Shore VC. Structure of myoglobin. A three-dimensional Fourier synthesis at 2 Å resolution. Nature 1960;185:422–427.
8. Perutz MF. New x-ray evidence on the configuration of polypeptide chains. Nature 1951;167:1053–1054.
9. Bragg L, Kendrew JC, Perutz MF. Polypeptide chain configurations in crystalline proteins. Proc Roy Soc Lond A 1950;203:321–357.
10. Schellman JA, Schellman CG. Kaj Ulrik Linderstrøm-Lang (1896-1959). Protein Sci 1997;6:1092–1100.
11. Linderstrøm-Lang K, Schellman JA. The structure of insulin as compared to that of Sanger's A-chain. Biochim Biophys Acta 1954;15:156–157.
12. Harrington WF, Schellman JA. Evidence for the instability of hydrogen-bonded peptide structures in water based on studies of ribonuclease and oxidized ribonuclease. C R Trav Lab Carlsberg 1956; 30:21–43.
13. Simpson RB, Kauzmann W. The kinetics of protein denaturation. I. The behavior of the optical rotation of ovalbumin in urea solutions. J Am Chem Soc 1953;75:5139–5152.
14. Kunitz M. The kinetics and thermodynamics of reversible denaturation of crystalline soybean trypsin inhibitor. J Gen Physiol 1948;32:241–263.
15. Eisenberg MA, Schwert GW. The reversible denaturation of chymotrypsinogen. J Gen Physiol 1951;35:583–606.
16. Richards FM. On the enzymic activity of subtilisin-modified ribonuclease. Proc Natl Acad Sci USA 1958;44:162–166.
17. Kalman SM, Linderstrøm-Lang K, Ottesen M, Richards FM. Degradation of ribonuclease by subtilisin. Biochim Biophys Acata 1955;16:297–298.
18. Sela M, White ,FH Jr, Anfinsen CB. Reductive cleavage of disulfide bridges in ribonuclease. Science 1957;125:691–692.
19. Epstein CJ, Goldberger RF, Anfinsen CB. The genetic control of tertiary protein structure: Studies with model systems. Cold Spring Harbor Symp Quant Biol 1963;28:439–449.
20. Hvidt A, Nielsen SO. Hydrogen exchange in proteins. Adv Protein Chem 1966;21:287–386.
21. Hvidt A. A discussion of the pH dependence of the hydrogen-deuterium exchange of proteins. C R Trav Lab Carlsberg 1964;34:299–317.
22. Linderstrøm-Lang K. Deuterium exchange and protein structure. In: Neuberger A, editor. Symposium on protein structure. London: Methuen; 1958; pp 23–24.
23. Berger A, Linderstrøm-Lang K. Deuterium exchange of poly-D,L-alanine in aqueous solution. Arch Biochem Biophys 1957;69:106–118.

24. Elliott A. Behaviour of a synthetic polypeptide analogous to protein denaturation. Nature 1952;170:1066–1067.
25. Bryan WP, Nielsen SO. Hydrogen-deuterium exchange of poly-D,L-alanine in aqueous solution. Biochim Biophys Acta 1960;42:552–553.
26. Gratzer WB, Doty P. A conformation examination of poly-L-alanine and poly-D,L-alanine in aqueous solution. J Am Chem Soc 1963;85:1193–1197.
27. Englander SW, Polsen A. Hydrogen-tritium exchange of the random chain polypeptide. Biopolymers 1969;7:379–393.
28. Berger A, Lowenstein A, Meiboom S. Nuclear magnetic resonance study of the protolysis and ionization of N-methyl-acetamide. J Am Chem Soc 1959;81:62–67.
29. Eigen M. Proton transfer, acid-base catalysis, and enzymatic hydrolysis. I. Elementary processes. Angew Chem Int Ed Engl 1964;3:1–19.
30. Englander SW, Downer NW, Teitelbaum H. Hydrogen exchange. Annu Rev Biochem 1972;41:903–924.
31. Englander SW. A hydrogen exchange method using tritium and sephadex: its application to ribonuclease. Biochemistry 1963;2:798–807.
32. Sheinblatt M. Determination of an acidity scale for peptide hydrogens from nuclear magnetic resonance kinetic studies. J Am Chem Soc 1970;92:2505–2509.
33. Molday RS, Englander SW, Kallen RG. Primary structure effects on peptide group hydrogen exchange. Biochemistry 1972;11:150–158.
34. Molday RS, Kallen RG. Substituent effects on amide hydrogen exchange rates in aqueous solution. J Am Chem Soc 1972;94:6739–6745.
35. Printz MP, von Hippel PH. Hydrogen exchange studies of DNA structure. Proc Natl Acad Sci USA 1965;53:363–370.
36. Englander SW, Englander JJ. Hydrogen exchange studies of sRNA. Proc Natl Acad Sci USA 1965;53:370–378.
37. McConnell BM, von Hippel PH. Hydrogen exchange as a probe of the dynamic structure of DNA. I. General acid-base catalysis. J Mol Biol 1970;50:297–316.
38. McConnell BM, von Hippel PH. Hydrogen exchange as a probe of the dynamic structure of DNA. II. Effects of base composition and destabilizing salts. J Mol Biol 1970;50:317–332.
39. Englander JJ, Kallenbach NR, Englander SW. Hydrogen exchange study of some polynucleotides and transfer RNA. J Mol Biol 1972;63:153–169.