

Hydrogen exchange

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The central role played by crystallography and nuclear magnetic resonance in modern macromolecular studies is being supplemented, more and more, by high resolution hydrogen exchange (HX) which can access structural information that the more familiar methods cannot. Hydrogens on the main chain amides of proteins and the hydrogen bonding groups of nucleic acids engage in continuous exchange with solvent hydrogens. HX rates depend on the important details of macromolecular structure, its dynamic behavior, and its thermodynamic parameters. The challenge for the last half century has been to measure the HX signals and decode them in these terms.

That challenge has begun to be met. HX methods have now been used to study structure and conformational changes in proteins and nucleic acids in various physical states — in solution, and in the membrane-bound, crystalline, molten globular and lyophilized states, in many biologically relevant complexes — for example when interacting with GroEL, calmodulin, antibodies, substrates and inhibitors, and redox partners, and even in whole active muscle. HX can access kinetic folding intermediates that fly by in milliseconds and intermediates that exist in infinitesimal quantities as high energy excited states in equilibrium with the native state. HX measurements can illuminate the macromolecular structure as a function of time — the dynamic structural fluctuations that occur even under native conditions, ranging from small local fluctuations up to global reversible unfolding; the measured exchange rates add kinetic and thermodynamic information about these processes.

It was not always that way. Although hydrogen exchange came on the scene 10 years before the first X-ray structures were solved, and HX observations opened the issue of structural dynamics almost 20 years before the onset of molecular dynamics studies, a very long time elapsed before it rose to its present level of capability.

In the 1950s, amid the excitement generated by the discovery of the α -helix and the β -sheet, Kaj Linderström-Lang realized that Pauling's postulated hydrogen bonded structures might be sought experimentally in real proteins by measuring

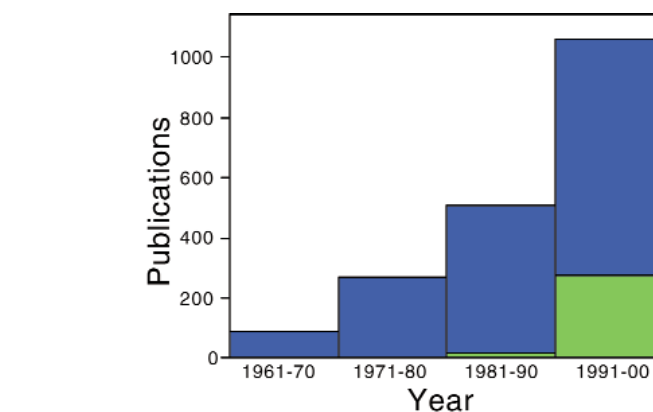


Fig. 1. The increasing role of hydrogen exchange studies in biomolecular studies. The upsurge in contributions to the protein folding field is shown in green.

amide hydrogen exchange rates. To accomplish this Lang and his colleagues at the Carlsberg Laboratories in Copenhagen invented entirely new technologies, based on H-D exchange and its quantitative determination by a sensitive density method. In a remarkable feat of scientific intuition and imagination, Lang was able to infer the mechanisms and write the theoretical equations that still underlie our most modern concepts¹.

In the 1960s the invention of size exclusion chromatography and the commercialization of liquid scintillation technology allowed the development of facile tritium exchange methods that provided excellent accuracy and ease of use². These methods dominated further developments for the next 20 years during which a small band of hardy investigators — most intensively Andreas Rosenberg, Clare Woodward, and Joan and Walter Englander — pressed stubbornly on with the development of basic concepts and calibrations, in spite of a marked lack of interest by the community at large. They were inspired by a vision of the inherent potential of the approach. However the reach of these early studies was limited by the inability to measure hydrogen exchange at significantly high structural resolution.

The tide began to turn about 1980 with the advent of a medium resolution fragmentation, and fragment separation technology based on then newly emerging

HPLC technology, inaugurated by Fred Richards and realized in following work by Joan Englander and her coworkers^{3,4}. In this approach, exchanging hydrogens can be roughly located and their exchange rates measured by stopping the HX reaction at various time points (sample plunged into slow HX conditions at low pH and temperature), fragmenting with an acid protease, quickly separating the fragments by HPLC, and determining their carried tritium label. These methods are just now being extended to high resolution and to large proteins through the explosive development of mass spectrometric technology, which replaces the liquid scintillation analysis for tritium with mass determination of the fragments for their deuterium content. Especially promising is the potential marriage of these methods with HX 'functional labeling' approaches which can be used to selectively label and measure just those sites that actively participate in the function being studied while automatically rejecting the vast number of confusing 'background' hydrogens⁵.

The 1980s also ushered in the modern era of truly high resolution hydrogen exchange⁶ with the development of 2D NMR, driven by the work of Ernst and Wüthrich and their collaborators, and more recently amplified by the multi-dimensional multi-isotopic capabilities pioneered by Bax and others. NMR methods now routinely access H-D exchange in

reasonably small proteins and nucleic acids and thus make available all of the above mentioned information resolved to the individual proton level.

A brief literature search shows the growing utilization of HX methods for biophysical studies and the significant fraction devoted specifically to the protein folding problem since 1990 (Fig. 1). HX was initially introduced to the folding field by Buzz Baldwin with Franz Schmid in 1979⁷, and Baldwin together with Peter Kim described its potential applications during the 1980s. The merger of these ideas with high resolution NMR in 1988 by Baldwin and Roder and their coworkers revealed for the first time the structure of very short lived kinetic folding intermediates^{8,9}, previously thought to be wholly out of reach. This pivotal event opened the floodgates to many further studies that

have rather dramatically elucidated the structure and stability of partially folded kinetic and equilibrium intermediates, which can be inferred only dimly by other available methodologies.

Work now in progress in many laboratories builds on a foundation constructed over a half century of hydrogen exchange development. The remarkable power of HX approaches is now apparent. Future work will be able to mobilize that power to peer ever more deeply into the secrets of biomolecular structure and conformational changes, their dynamics and energetics, the bases of molecular cooperativity, and the principles of structural design, in ways that were unimaginable only a short time ago.

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