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Perspectives in Magnetic Resonance

A dynamic look backward and forward

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ABSTRACT

The 2015 Gunther Laukien Prize recognized solution NMR studies of protein dynamics and thermodynamics. This Perspective surveys aspects of the development and application of NMR spin relaxation for investigations of protein flexibility and function over multiple time scales in solution. Methods highlighted include analysis of overall rotational diffusion, theoretical descriptions of $R_{1\rho}$ relaxation, and molecular dynamics simulations to interpret NMR spin relaxation. Applications are illustrated for the zinc-finger domain Xfin-31, the calcium-binding proteins calbindin D_{9k} and calmodulin, and the bZip transcription factor of GCN4.

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1. Introduction

The investigation of the dynamics of proteins, nucleic acids, and other biological macromolecules has a long history in NMR spectroscopy. The first nuclear spin relaxation measurement for a protein was a one-dimensional natural-abundance ^{13}C R_1 experiment for ribonuclease A reported in 1971 [1]. Of course, theoretical descriptions of spin relaxation essential for interpreting relaxation measurements already were established by the 1970's with key papers establishing the Bloch–Wangness–Redfield (BWR) [2] and Bloch–McConnell [3] equations, which form the basis for theoretical descriptions of Carr–Purcell–Meiboom–Gill (CPMG) [4], $R_{1\rho}$ [5], and exchange spectroscopy [6]. Indeed, by 1970, both CPMG [7] and $R_{1\rho}$ [5] experiments had been used to determine the kinetic rate constant for the chair-to-boat isomerization of cyclohexane. However, the congestion of one-dimensional NMR spectra and the low sensitivity of natural abundance ^{13}C NMR spectroscopy severely limited applications of what was widely known, at least to NMR spectroscopists, to be a potentially powerful technique for investigating the dynamic properties of biological molecules. Despite these limitations, critical aspects of protein statistical mechanics were elucidated, summarized for BPTI in an influential review by Wagner in 1983 [8]. Notably, ^{13}C relaxation measurements demonstrated that certain aromatic rings in BPTI rotate rapidly, showing that the interiors of proteins have considerable plasticity. As one measure of the fundamental importance of NMR spin relaxation measurements for understanding protein properties, investigations of relaxation of aromatic groups continue to be pursued to this day [9].

The situation changed dramatically in the two-year period from 1988 to 1989. First, "inverse" ^{1}H-detected techniques for measuring nuclear spin relaxation of ^{13}C and ^{15}N heteronuclei began to be developed. The first methods by Nirmala and Wagner were based on a double DEPT experiment for ^{13}C and were sensitive enough for ^{13}C natural abundance NMR spectroscopy of small soluble proteins [10,11]. These initial reports were quickly followed by a seminal study of uniformly ^{15}N enriched staphylococcal nuclease using INEPT-based experiments for ^{15}N R_1, ^{15}N R_2, and the heteronuclear {^{1}H}–^{15}N steady-state NOE [12]. The approach for data acquisition and analysis presented in this paper, although highly refined over the past two decades, remains the basic framework for contemporary work.

The developments in 1988–1989 revolutionized and invigorated studies of biological systems by spin relaxation and subsequent developments in experimental methods have enabled investigation of numerous spin types in proteins and nucleic acids [13]. With these advances, site-specific information on multiple time scales can be obtained on large molecules and molecular machines, ultimately up to ~one-half megadalton in pioneering work by Kay and co-workers [14]. Impressive studies of complex systems demonstrate that important information can be obtained from these measurements. At the same time, these experimental methods have diffused out of the laboratories of specialists and are being employed increasingly outside of academic settings [15].

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The main goal of this Perspective is to summarize my own contributions to the development and application of spin relaxation methods, beginning with my introduction to the field as a postdoctoral scientist with Peter E. Wright at The Scripps Research Institute and continuing throughout my career at Columbia University. The 2015 Gunther Laukien Prize Lecture described some aspects of this work and is available at www.enc-conference.org/LaukienRecipients/PastRecipients/. In addition, recent Perspective [16] and Accounts [17] articles have summarized chemical exchange methods and applications of spin relaxation methods to enzymes, respectively. Accordingly, this Perspective focuses on the role of protein flexibility and dynamics in recognition of target molecules by proteins.

2. First results: Xfin-31 and Calbindin D_{9k}

Investigations of the Zn-finger domain Xfin-31 [18–20] and the EF-hand protein calbindin D_{9k} [21,22] provided early experimental insights and illustrate the research concerns and approaches that continue to be central to my research.

Initial studies of the 25-residue Xfin-31 protein ligated with Zn^{2+} used two-dimensional DEPT-based experiments to measure backbone and side chain ^{13}C spin relaxation at natural abundance [18]. The first experimental work focused on the backbone $^{13}C^{\alpha}$ spins, analyzed with the Lipari–Szabo model-free formalism [23,24]. Values of the ^{13}C generalized order parameter, S^2, are shown in Fig. 1. Like the earlier example of staphylococcal nuclease [12], the results showed that bond vectors in secondary structures are highly ordered, with values of the generalized order parameter $S^2 > 0.85$. The termini and loops were more heterogeneously disordered, with the most C-terminal residue having $S^2 = 0.30$. The experimental results subsequently were compared to values calculated from a 100 ps molecular dynamics (MD) simulation in aqueous solution [19]. The MD simulations yielded values of S^2 that were more than 0.10 larger than experimental values only for three residues: Tyr 1, Phe 10, and Lys 24. For the remaining residues, the average difference between experimental and simulated values was 0.013 with a sample deviation of 0.06. The two residues near the N- and C-termini have small secondary ^{13}C shifts [25], suggesting that these residues are fairly disordered in solution and overrestrained in the MD simulation, perhaps reflecting the short trajectory length that could be achieved in 1992.

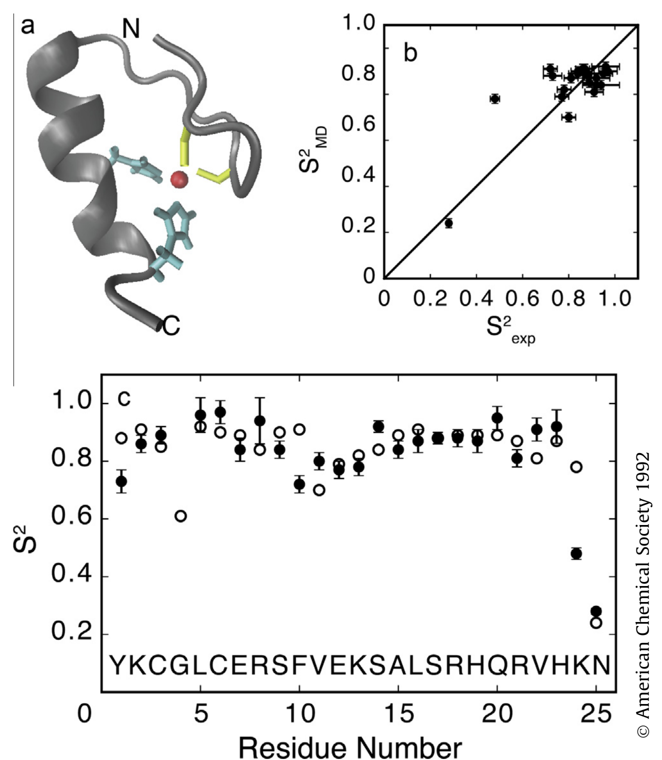

Fig. 1. Conformational flexibility of Xfin-31. (a) Structure of Xfin-31. The protein backbone is depicted in grey. The Zn^{2+} ion is shown as a red sphere; the Zn^{2+} ion is ligated by two Cys and two His residues. The Cys side chains are shown as yellow sticks and the His side chains as cyan sticks. (b) (●) Experimental and (○) simulated values of S^2 for the $^{13}C^{\alpha}$–^{1}H bond vector. Experimental uncertainties were determined by Monte-Carlo simulations. (c) Correlation between experimental and simulated order parameters. Reprinted with permission from J. Am. Chem. Soc., 1992, 114, 9059–9067.

Chemical exchange contributions to R_2 were observed for six of the backbone $^{13}C^{\alpha}$ resonances, five of these are either ligands to the Zn^{2+} ion or residues or adjacent to such residues. Exchange broadening also was observed for side chain $^{13}C^{\alpha}$ resonances of the Zn^{2+} ligand His 19 and His 23 and the adjacent residue Val 22. The fitted values of the chemical exchange broadening R_{ex} are uncorrelated with the squares of the $^{13}C^{\alpha}$ secondary chemical shifts, suggesting that the exchange process is not rapid unfolding of the protein following transient loss of the Zn^{2+} ion. In these initial studies, further characterization of chemical exchange was not possible, but these phenomena sparked an interest that developed into a major aspect of subsequent work.

Comparison between apo and holo forms of the calcium-binding EF-hand protein calbindin D_{9k} demonstrated the importance of NMR investigations of conformational flexibility for understanding molecular recognition by proteins. In collaboration with Walter Chazin and coworkers, backbone ^{15}N relaxation rate constants were measured for the apo, $(Cd^{2+})_1$, and $(Ca^{2+})_2$ states of the protein [21,22]. Calbindin D_{9k} contains two metal-binding EF-hand motifs and Cd^{2+} binds to the C-terminal EF-hand, mimicking the unobservable state in which a single Ca^{2+} ion is bound to that site (because Ca^{2+}, but not Cd^{2+}, binding is cooperative). Subsequently Mäler and coworkers generated an Asn56Ala mutant that blocks binding to the C-terminal EF-hand, and thereby obtaining a $(Ca^{2+})_1$ species with the metal ion occupying the N-terminal EF-hand [26]. As shown in Fig. 2, backbone ^{15}N order parameters show that the N-terminal EF-hand is ordered and the C-terminal EF-hand is highly disordered in the apo state. In contrast, both EF-hands are highly ordered in the holo state. In addition, the C-terminal EF-hand is ordered in both the $(Cd^{2+})_1$ and $(Ca^{2+})_1$ states. In the former case, rigidification is the direct result of ion binding in the C-terminal EF-hand. In the latter case, binding to the N-terminal site rigidifies the C-terminal site as a consequence of structural changes propagated through the β-strand interface between the two EF-hand motifs. The key conclusion is that one contribution to the allosteric phenomenon in calbindin D_{9k} results from rigidification of the C-terminal binding site regardless of which binding site is occupied by the first Ca^{2+} ion.

3. Acquisition and analysis of relaxation data

Method developments in my laboratory for fast (ps-ns) time-scale dynamics in proteins have focused on pulse sequence development and optimization [27–29], error analysis and model-selection in relaxation data analysis [30], determination of overall rotational diffusion tensors [31–33], relationships between relaxation and conformational entropy [34,35], and temperature dependence of intramolecular motions [36–38]. For brevity, only recent results on the analysis of rotational diffusion tensors are discussed herein.

In an early study, the quadric method of Brüschweiler and coworkers [39] was extended to joint analysis of ^{13}C and ^{15}N

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