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Historical Perspective

NMR studies of protein structure and dynamics – A look backwards and forwards

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ABSTRACT

NMR spectroscopy has evolved to become one of the most powerful tools for the study of protein structure and dynamics. Advances over the past decade have greatly extended the methodology to studies of molecules of ever increasing complexity. Herein I provide a short perspective relating the circumstances that led to some of the contributions from my laboratory in this area and highlight how these original experiments, summarized in a Journal of Magnetic Resonance article in 2005 (JMR, 173 193–207), have influenced the current focus of my research.

Interview with the author(s).

A video interview with the author(s) associated with this Historical Perspective and the original article can be found in the online version, at doi:10.1016/j.jmr.2011.08.010.

On this, the occasion of the publication of a 'Special Issue' of JMR highlighting a number of papers that have been published in the Journal, I am particularly honored to write a short perspective on our work using NMR spectroscopy to study both protein structure and protein dynamics. My interest in using NMR as a tool to probe molecular motion extends over the past 25 years to the time when I was a graduate student in Jim prestegard laboratory at Yale. I became enamored by the rich mathematical description that is required for an understanding of the general principles of spin relaxation, by the opportunities to use spin physics to quantify the motion of biomolecules over a broad spectrum of time-scales and by the opportunity to develop new experiments that would facilitate studies of complex biomolecules and perhaps shed light on the important relation between dynamics and function. Later on, as a post-doctoral fellow at the NIH working with Ad Bax and Dennis Torchia, I participated in the development of experiments for measuring protein backbone amide ^{15}N spin relaxation [1] that have subsequently been applied to a great number of different systems [2]. The success of these experiments convinced me of the importance of extending the methodology to side-chain positions in proteins and my laboratory has spent the better part of the past two decades working on this problem in one form or the other, focusing to a large extent on methyl group probes.

Seminal studies of methyl dynamics in a number of proteins such as myoglobin [3], BPTI [4] and the M13 coat protein [5] had already being published in the late 1970s, early 1980s that clearly pointed to the fact that there were significant internal motions in these molecules. Yet these experiments were ^{13}C direct observe and lacked both the resolution and the sensitivity that are necessary for wide applicability. One solution involves recording two-dimensional ^{13}C—^{1}H correlation spectra with the intensity of each cross-peak encoding the ^{13}C relaxation property of interest (T_1, T_2, NOE). However, this is not without problems. ^{13}C relaxation in methyl groups is complex since there are a large number of relaxation interactions that contribute to signal decay, with each carbon multiplet component relaxing differently [6–9]; the relaxation is thus multi-exponential. This differential relaxation further comes into play during magnetization transfer schemes that are a prerequisite for any two-dimensional pulse sequence [10,11]. Such effects must be taken into account in the analysis of relaxation data obtained in this manner because they influence the initial state of the magnetization whose relaxation is to be interrogated. As a consequence of these problems it seemed to me that ^{13}C relaxation (at least in fully protonated proteins) was not the best way to obtain quantitative information about protein side-chain dynamics, although I worked rather diligently on the problem for several years as a post-doctoral fellow at the NIH.

An excellent way of 'obtaining new ideas' is to copy what others in related fields have already done successfully. Thus, I turned to the solids NMR community to provide inspiration for my dynamics studies of protein side-chains. This group had been using the deuteron as a probe of dynamics in a wide range of molecules, including proteins [12] and lipids [13], exploiting the well known fact that the quadrupolar interaction dominates deuterium relaxation [14]. The limiting

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facet of the solids experiments is lack of resolution, often necessitating the production of separate samples for each label. Such a problem can, however, be circumvented in the solution state by exploiting the resolution in ^{13}C, ^{1}H two-dimensional spectra so that all sites can be probed at once. In this approach proteins are prepared using an approximate 50%H_2O/50%D_2O mixture, ensuring a substantial population of $^{13}CH_2D$ methyl isotopomers that are then used to measure ^{2}H relaxation via a magnetization transfer scheme, $^{1}H \rightarrow {}^{13}C \rightarrow {}^{2}H(T) \rightarrow {}^{13}C(t_1) \rightarrow {}^{1}H(t_2)$, which optimizes both sensitivity and resolution [15] (here t_1, t_2 denote indirect and direct acquisition times, respectively, while T is a duration during which deuterium relaxation is monitored). Five different relaxation rates can be measured [16], providing a unique opportunity to cross-validate the relaxation measurements.

Our studies of protein dynamics led us naturally into thinking about excited protein states, conformers that are only fractionally populated and often only transiently so. While traditional structural biology methods have proven powerful for the study of highly abundant conformations (so called ground states), excited conformers frequently escape detection and have thus largely remained 'invisible'. This is a significant limitation of current methodology because excited states play important roles in many biochemical processes, including folding [17], molecular recognition [18], fibril formation [19], enzymology [20,21] and binding [22]. Our inspiration in this area came from fundamental work by some of the major pioneers in the NMR field who showed that millisecond time-scale exchange processes could be probed using experiments that consisted of spin-echo building blocks [23,24]. By changing the spacing between successive refocusing pulses the effective chemical shift difference between exchanging sites is modulated, leading to differences in transverse relaxation rates that can be quantified to extract chemical shifts of the excited state as well as the kinetics and thermodynamics of the exchange process. An important paper by Pat Loria, Mark Rance and Art Palmer in the late 1990s extended the methodology, previously demonstrated for isolated spins, into the realm of protein applications [25]. After the initial ^{15}N protein relaxation dispersion experiments were developed [25,26], Rieko Ishima and Dennis Torchia introduced experiments for probing exchange at ^{1}HN and ^{13}CO sites [27,28] and my laboratory worked out efficient labeling schemes and the corresponding pulse sequences for measuring $^{1}H^{\alpha}$ [29], $^{13}C^{\alpha}$ [30], $^{13}C^{\beta}$ [31] and methyl ^{13}C [32,33] chemical shifts of the excited state.

Typically when we think about a chemical exchange processes we imagine, for example, the evolution of a spin localized to a particular site that changes its precession frequency each time an exchange event occurs. The Bloch–McConnell formalism can then be used to evaluate the effects of the exchange process on lineshapes or relaxation rates [34]. It is sometimes convenient, however, to think about the evolution of magnetization associated with a given multiplet component, as opposed to the sum over all components, such as the TROSY or anti-TROSY line for amide magnetization for example. Under conditions of fractional protein alignment it is straightforward to show that for a system undergoing two-site exchange the effective chemical shift difference between exchanging states is different for TROSY and anti-TROSY magnetization components ($\Delta\omega \pm \pi\Delta D$, where $\Delta\omega$ (rad/s) is the shift difference that would be measured in an unaligned sample and ΔD is the difference in dipolar couplings of amide bond vectors in each state) [35]. It is possible, therefore, to measure residual dipolar couplings in the invisible state by carrying out spin-state selective relaxation dispersion NMR experiments that probe the exchange process one multiplet component at a time.

The large number of restraints that can now be measured for excited protein states (^{15}N, ^{1}HN, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, ^{13}CO and $^{1}H^{\alpha}$ chemical shifts along with ^{1}HN—^{15}N, $^{1}H^{\alpha}$—$^{13}C^{\alpha}$ and ^{1}HN—^{13}CO residual dipolar couplings) forms the basis for the determination of structural models of these elusive conformers [36,37]. Central to these efforts has been the emergence of computational data base protocols for calculating high quality structures from backbone chemical shifts [38–40].

The inherent spin physics of a methyl group that made it difficult to extract robust measures of side-chain dynamics in proteins from ^{13}C relaxation measurements (see above) can be exploited to great use in studies of very high molecular weight proteins. During our (frustrating) attempts to quantify dynamics using $^{13}CH_3$ probes we recognized that certain single quantum transitions, both ^{1}H and ^{13}C, relax very rapidly, while others very slowly [9,41,42]. What we were able to show subsequently was that ^{1}H—^{13}C multiple quantum transitions also exhibit this behavior and that, further, the standard HMQC pulse scheme [43,44] transfers magnetization components via one of two pathways in which either only slowly relaxing transitions or only rapidly decaying coherences contribute [42]. This naturally results in a situation whereby the two pathways are sequestered which is the basic requirement of a 'good' TROSY pulse scheme. ^{1}H—^{1}H cross-relaxation can, however, lead to the interconversion of fast and slowly relaxing transitions, effectively decreasing the isolation between the different pathways. It is thus imperative that experiments be conducted on highly deuterated proteins where only the methyl groups are protonated. Since we had been working with methyl groups as probes of structure and dynamics in proteins on the order of 100 kDa long before we realized that a TROSY effect could be exploited [45] the labeling approaches for preparing the requisite highly deuterated, methyl protonated proteins were already available [46]. The very significant gains in sensitivity and resolution that HMQC-based experiments provide suggested that NMR studies could be extended in a quantitative way to protein systems with molecular weights in the MDa regime. We have been able to exploit this TROSY-effect in studies of the ClpP protease (300 kDa) [47], the proteasome (700 kDa) [48–50] and aspartate transcarbamoylase (300 kDa) [51] and work from other laboratories on very high molecular weight complexes [52,53] suggests that this approach has a bright future.

While it is, of course, impossible to predict what the future of NMR holds in the realm of 'protein structure and dynamics' the immediate directions of my laboratory are clear. The possibility of determining structures of invisible, excited states that play critical roles in biochemical processes but that have been recalcitrant to study using more established methodologies is an exciting one indeed, and we are currently pursuing such studies on a variety of systems that promise to provide insight into both protein folding and misfolding. The role of NMR in relating structure, function and dynamics in supra-molecular machines is becoming increasingly established and holds great promise in providing important insights that are missing from static X-ray or cryo-EM structures. We are currently exploiting methyl-TROSY based approaches to understand the role of dynamics in the function of supramolecular systems, including the proteasome and the nucleosome. There have been many significant advances in 'protein structure and dynamics' since my 2005 JMR article that summarized some of our work in this area. By exploiting spin physics in new and clever ways it is clear that advances will continue to emerge.

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References

[1] L.E. Kay, D.A. Torchia, A. Bax, Backbone dynamics of proteins as studied by ^{15}N inverse detected heteronuclear NMR spectroscopy: application to staphylococcal nuclease, Biochemistry 28 (1989) 8972–8979.

[2] V.A. Jarymowycz, M.J. Stone, Fast time scale dynamics of protein backbones: NMR relaxation methods, applications, and functional consequences, Chem. Rev. 106 (2006) 1624–1671.

[3] R.J. Wittebort, T.M. Rothgeb, A. Szabo, F.R. Gurd, Aliphatic groups of sperm whale myoglob. ^{13}C NMR study, Proc. Natl. Acad. Sci. USA 76 (1979) 1059–1063.

[4] R. Richarz, K. Nagayama, K. Wuthrich, Carbon-13 nuclear magnetic resonance relaxation studies of internal mobility of the polypeptide chain in basic pancreatic trypsin inhibitor and a selectively reduced analogue, Biochemistry 19 (1980) 5189–5196.

[5] G.D. Henry, J.H. Weiner, B.D. Sykes, Backbone dynamics of a model membrane protein: ^{13}C NMR spectroscopy of alanine methyl groups in detergent-solubilized M13 coat protein, Biochemistry 25 (1986) 590–598.

[6] L.G. Werbelow, D.M. Grant, Intramolecular dipolar relaxation in multispin systems, Adv. Magn. Reson. 9 (1977) 189–299.

[7] L.G. Werbelow, A.G. Marshall, Internal rotation and nonexponential methyl nuclear relaxation for macromolecules, J. Magn. Reson. 11 (1973) 299–313.

[8] R.R. Vold, R.L. Vold, Transverse relaxation in heteronuclear coupled spin systems: AX, AX2, AX3, AXY, J. Chem. Phys. 64 (1976) 320–332.

[9] L.E. Kay, D.A. Torchia, The effects of dipolar cross-correlation on ^{13}C methyl-carbon T_1, T_2 and NOE measurements in macromolecules, J. Magn. Reson. 95 (1991) 536–547.

[10] L.E. Kay, T.E. Bull, L.K. Nicholson, C. Griesinger, H. Schwalbe, A. Bax, D.A. Torchia, The measurement of heteronuclear transverse relaxation times in AX3 spin systems via polarization transfer techniques, J. Magn. Reson. 100 (1992) 538–558.

[11] A.G. Palmer, P.E. Wright, M. Rance, Measurement of relaxation time constants for methyl groups by proton-detected heteronuclear NMR spectroscopy, Chem. Phys. Lett. 185 (1991) 41–46.

[12] M.A. Keniry, A. Kintanar, R.L. Smith, H.S. Gutowsky, E. Oldfield, Nuclear magnetic resonance studies of amino acids and proteins. Deuterium nuclear magnetic resonance relaxation of deuteriomethyl-labeled amino acids in crystals and in *Halobacterium halobium* and *Escherichia coli* cell membranes, Biochemistry 23 (1984) 288–298.

[13] R.L. Smith, E. Oldfield, Dynamic structure of membranes by deuterium NMR, Science 225 (1984) 280–288.

[14] A. Abragam, Principles of Nuclear Magnetism, Clarendon Press, Oxford, 1961.

[15] D.R. Muhandiram, T. Yamazaki, B.D. Sykes, L.E. Kay, Measurement of deuterium T_1 and T_{1r} relaxation times in uniformly ^{13}C labeled and fractionally deuterium labeled proteins in solution, J. Am. Chem. Soc. 117 (1995) 11536–11544.

[16] O. Millet, D.R. Muhandiram, N.R. Skrynnikov, L.E. Kay, Deuterium spin probes of side-chain dynamics in proteins. 1. Measurement of five relaxation rates per deuteron in C-13-labeled and fractionally H-2-enriched proteins in solution, J. Am. Chem. Soc. 124 (2002) 6439–6448.

[17] D.M. Korzhnev, X. Salvatella, M. Vendruscolo, A.A. Di Nardo, A.R. Davidson, C.M. Dobson, L.E. Kay, Low-populated folding intermediates of Fyn SH3 characterized by relaxation dispersion NMR, Nature 430 (2004) 586–590.

[18] O.F. Lange, N.A. Lakomek, C. Fares, G.F. Schroder, K.F. Walter, S. Becker, J. Meiler, H. Grubmuller, C. Griesinger, B.L. de Groot, Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution, Science 320 (2008) 1471–1475.

[19] F. Chiti, C.M. Dobson, Amyloid formation by globular proteins under native conditions, Nat. Chem. Biol. 5 (2009) 15–22.

[20] J.S. Fraser, M.W. Clarkson, S.C. Degnan, R. Erion, D. Kern, T. Alber, Hidden alternative structures of proline isomerase essential for catalysis, Nature 462 (2009) 669–673.

[21] D.D. Boehr, D. McElheny, H.J. Dyson, P.E. Wright, The dynamic energy landscape of dihydrofolate reductase catalysis, Science 313 (2006) 1638–1642.

[22] D.M. Korzhnev, I. Bezsonova, S. Lee, T.V. Chalikian, L.E. Kay, Alternate binding modes for a ubiquitin-SH3 domain interaction studied by NMR spectroscopy, J. Mol. Biol. 386 (2009) 391–405.

[23] H.Y. Carr, E.M. Purcell, Effects of diffusion on free precession in nuclear magnetic resonance experiments, Phys. Rev. 54 (1954) 630–638.

[24] S. Meiboom, D. Gill, Modified spin-echo method for measuring nuclear magnetic relaxation times, Rev. Sci. Instrum. 29 (1958) 688–691.

[25] J.P. Loria, M. Rance, A.G. Palmer, A relaxation compensated CPMG sequence for characterizing chemical exchange, J. Am. Chem. Soc. 121 (1999) 2331–2332.

[26] M. Tollinger, N.R. Skrynnikov, F.A.A. Mulder, J.D. Forman-Kay, L.E. Kay, Slow dynamics in folded and unfolded states of an SH3 domain, J. Am. Chem. Soc. 123 (2001) 11341–11352.

[27] R. Ishima, J. Baber, J.M. Louis, D.A. Torchia, Carbonyl carbon transverse relaxation dispersion measurements and ms-micros timescale motion in a protein hydrogen bond network, J. Biomol. NMR 29 (2004) 187–198.

[28] R. Ishima, D. Torchia, Extending the range of amide proton relaxation dispersion experiments in proteins using a constant-time relaxation-compensated CPMG approach, J. Biomol. NMR 25 (2003) 243–248.

[29] P. Lundstrom, D.F. Hansen, P. Vallurupalli, L.E. Kay, Accurate measurement of alpha proton chemical shifts of excited protein states by relaxation dispersion NMR spectroscopy, J. Am. Chem. Soc. 131 (2009) 1915–1926.

[30] D.F. Hansen, P. Vallurupalli, P. Lundstrom, P. Neudecker, L.E. Kay, Probing chemical shifts of invisible states of proteins with relaxation dispersion NMR spectroscopy: how well can we do?, J Am. Chem. Soc. 130 (2008) 2667–2675.

[31] P. Lundstrom, L.E. Kay, Measuring 13Cb chemical shifts of invisible excited states in proteins by relaxation dispersion NMR spectroscopy, J. Biomol. NMR 44 (2009) 139–155.

[32] P. Lundstrom, P. Vallurupalli, T.L. Religa, F.W. Dahlquist, L.E. Kay, A single-quantum methyl ^{13}C-relaxation dispersion experiment with improved sensitivity, J. Biomol. NMR 38 (2007) 79–88.

[33] N.R. Skrynnikov, F.A.A. Mulder, B. Hon, F.W. Dahlquist, L.E. Kay, Probing slow time scale dynamics at methyl-containing side chains in proteins by relaxation dispersion NMR measurements: application to methionine residues in a cavity mutant of T4 lysozyme, J. Am. Chem. Soc. 123 (2001) 4556–4566.

[34] H.M. McConnell, Reaction rates by nuclear magnetic resonance, J. Chem. Phys. 28 (1958) 430–431.

[35] P. Vallurupalli, D.F. Hansen, E.J. Stollar, E. Meirovitch, L.E. Kay, Measurement of bond vector orientations in invisible excited states of proteins, Proc. Natl. Acad. Sci. USA 104 (2007) 18473–18477.

[36] P. Vallurupalli, D.F. Hansen, L.E. Kay, Structures of invisible, excited protein states by relaxation dispersion NMR spectroscopy, Proc. Natl. Acad. Sci. USA 105 (2008) 11766–11771.

[37] D.M. Korzhnev, T.L. Religa, W. Banachewicz, A.R. Fersht, L.E. Kay, A transient and low-populated protein-folding intermediate at atomic resolution, Science 329 (2010) 1312–1316.

[38] Y. Shen, O. Lange, F. Delaglio, P. Rossi, J.M. Aramini, G. Liu, A. Eletsky, Y. Wu, K.K. Singarapu, A. Lemak, A. Ignatchenko, C.H. Arrowsmith, T. Szyperski, G.T. Montelione, D. Baker, A. Bax, Consistent blind protein structure generation from NMR chemical shift data, Proc. Natl. Acad. Sci. USA 105 (2008) 4685–4690.

[39] A. Cavalli, X. Salvatella, C.M. Dobson, M. Vendruscolo, Protein structure determination from NMR chemical shifts, Proc. Natl. Acad. Sci. USA 104 (2007) 9615–9620.

[40] D.S. Wishart, D. Arndt, M. Berjanskii, P. Tang, J. Zhou, G. Lin, CS23D: a web server for rapid protein structure generation using NMR chemical shifts and sequence data, Nucl. Acids Res. 36 (2008) W496–502.

[41] L.E. Kay, J.H. Prestegard, Methyl group dynamics from relaxation of double quantum filtered NMR signals-application to deoxycholate, J. Am. Chem. Soc. 109 (1987) 3829–3835.

[42] V. Tugarinov, P. Hwang, J. Ollerenshaw, L.E. Kay, Cross-correlated relaxation enhanced ^{1}H–^{13}C NMR spectroscopy of methyl groups in very high molecular weight proteins and protein complexes, J. Am. Chem. Soc. 125 (2003) 10420–10428.

[43] A. Bax, R.H. Griffey, B.L. Hawkings, Correlation of proton and nitrogen-15 chemical shifts by multiple quantum NMR, J. Magn. Reson. 55 (1983) 301–315.

[44] L. Mueller, Sensitivity enhanced detection of weak nuclei using heteronuclear multiple quantum coherence, J. Am. Chem. Soc. 101 (1979) 4481–4484.

[45] V. Tugarinov, R. Muhandiram, A. Ayed, L.E. Kay, Four-dimensional NMR spectroscopy of a 723-residue protein: chemical shift assignments and secondary structure of malate synthase G, J. Am. Chem. Soc. 124 (2002) 10025–10035.

[46] N.K. Goto, K.H. Gardner, G.A. Mueller, R.C. Willis, L.E. Kay, A robust and cost-effective method for the production of Val, Leu, Ile (d1) methyl-protonated ^{15}N-, ^{13}C-, ^{2}H-labeled proteins, J. Biomol. NMR 13 (1999) 369–374.

[47] R. Sprangers, A. Gribun, P.M. Hwang, W.A. Houry, L.E. Kay, Quantitative NMR spectroscopy of supramolecular complexes: dynamic side pores in ClpP are important for product release, Proc Natl Acad Sci USA 102 (2005) 16678–16683.

[48] R. Sprangers, L.E. Kay, Quantitative dynamics and binding studies of the 20S proteasome by NMR, Nature 445 (2007) 618–622.

[49] T.L. Religa, R. Sprangers, L.E. Kay, Dynamic regulation of archaeal proteasome gate opening as studied by TROSY NMR, Science 328 (2010) 98–102.

[50] A.M. Ruschak, T.L. Religa, S. Breuer, S. Witt, L.E. Kay, The proteasome antechamber maintains substrates in an unfolded state, Nature 467 (2010) 868–871.

[51] A. Velyvis, Y.R. Yang, H.K. Schachman, L.E. Kay, A solution NMR study showing that active site ligands and nucleotides directly perturb the allosteric equilibrium in aspartate transcarbamoylase, Proc. Natl. Acad. Sci. USA 104 (2007) 8815–8820.

[52] I. Gelis, A.M. Bonvin, D. Keramisanou, M. Koukaki, G. Gouridis, S. Karamanou, A. Economou, C.G. Kalodimos, Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR, Cell 131 (2007) 756–769.

[53] C. Amero, P. Schanda, M.A. Dura, I. Ayala, D. Marion, B. Franzetti, B. Brutscher, J. Boisbouvier, Fast two-dimensional NMR spectroscopy of high molecular weight protein assemblies, J. Am. Chem. Soc. 131 (2009) 3448–3449.