Marius Clore, G: Adventures in Biomolecular NMR

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INTRODUCTION

My NMR career started in 1980 when I joined the Division of Molecular Pharmacology at the Medical Research Council's National Institute of Medical Research (NIMR) located in Mill Hill, London. I had been recruited 18 months previously by the director of NIMR, Sir Arnold Burgen, while still a medical student at University College Hospital Medical School, on the basis of the work I had published on cytochrome oxidase and other metalloproteins, largely involving low temperature transient kinetics. At the time that I was interviewed at NIMR in January 1979 I had no intention of going into NMR but was principally interested in the application of a variety of spectroscopic and kinetic techniques to study the flow of electrons in the respiratory pathway. NIMR, however, did not have the necessary instrumentation, and Sir Arnold Burgen suggested that I take up NMR. Since I had a strong mathematical bent, this was not too difficult, particularly as at the time, the number of NMR experiments that could be carried out on proteins was rather limited. All of this was very fortunate since it enabled me to fully participate in the development of NMR as a significant tool in structural biology and one that could be used to determine the three-dimensional structures of biological macromolecules in solution at atomic resolution.

When I joined NIMR in August 1980, it was quite difficult, with the 270-MHz spectrometer then available to us, to even assign a simple dinucleotide such as NAD. And while it was clear that, in principle NMR could be used to determine three-dimensional structures of proteins, the path to accomplishing that was far from clear or evident. Indeed, I remember a heated discussion with Arnold Burgen when he told me in no uncertain terms that solving structures of proteins by NMR would never be feasible and that I was wasting my time. The progress that NMR has made in the last 30 years is simply astounding and never ceases to amaze, and I suspect that the end is nowhere near in sight. Indeed, just recently we succeeded in determining the three-dimensional structure of a protein known as enzyme I, a 128-kDa dimer, and its 146-kDa complex with its partner protein HPr,[1] which only a few years ago would have been unimaginable. Further, using paramagnetic NMR, an old idea with a new twist, we have shown that it is possible to detect, characterize, and even directly visualize sparsely populated states of macromolecules that are invisible to conventional structural biology methods including conventional NMR spectroscopy and X-ray crystallography.[2,3] In this brief overview, I summarize the developments along the way that I have participated in and that have led to these current advances.

MRC NIMR—FIRST STEPS INTO STRUCTURE DETERMINATION BY NMR (1980–1984)

When I came to NIMR I was assigned a small office, no larger than about 8×8 ft that I shared with Angela Gronenborn. We quickly decided to team up and work together, as our expertise and strengths were clearly very complementary. She had considerably more expertise in wet biochemistry, while I had a greater bent toward instrumentation, technical aspects of NMR, and computation. At that time, Jim Feeney, Gordon Roberts, and Berry Birdsall, located in the same division as us, were very interested in the application of transferred saturation methods to assign the ^{1}H chemical shifts of small ligands bound to dihydrofolate reductase where exchange between free and bound states was in slow exchange on the chemical shift time scale. I quickly extended the formalism to deal with such systems in the context of three-site exchange which provided the background for our future work on the transferred NOE (TRNOE).[4,5]

Angela and I had decided to work on the cyclic AMP receptor protein (CRP), and having gone to Toulouse to learn how to make this protein, we returned, keen to do some NMR experiments. The first thing we did was to carry out transfer of saturation experiments for cAMP and cGMP bound to CRP. These simple 1-D experiments involved obtaining saturation profiles on the free ligand resonances by applying a weak saturation pulse at 20-Hz intervals across the spectrum and recording the intensity of the resonances of the free ligand present in excess. To our surprise we did not observe any transfer of saturation from bound ligand resonances. Instead, we observed what we called TRNOEs. Thus for example, a negative NOE on the H8 resonance of free cAMP was observed upon irradiating the H1′ sugar resonance of cAMP.[6] Since the NOEs observed for cAMP in the absence of protein are very small and positive, these large negative NOE effects had to arise from cross-relaxation between bound ligand protons. In this instance, the TRNOE data indicated unambiguously that cAMP was bound to CRP in the *syn* conformation, in contrast to the crystal structure where cAMP was in the *anti* conformation. A condition of the TRNOE is that exchange between free and bound states is fast, thereby permitting the transfer of information concerning cross relaxation between two bound ligand nuclei from the bound state to the free state by chemical exchange. As a result, negative NOEs on the easily detectable free or observed ligand resonances could be seen following irradiation of other ligand resonances. Rapid chemical exchange usually entails weak binding but cAMP was known to bind tightly to the C-terminal dimerization domain of CRP. The discrepancy between the NMR and crystal data was only resolved over a decade later when a crystal structure of CRP revealed a second cAMP molecule in the *syn* conformation bound to the N-terminal DNA-binding domain.[7] The experimental observation of the TRNOE prompted us to describe the theory of the steady state and time-dependent TRNOE in detail.[4,5] The beauty of the TRNOE is that cross-relaxation rates in the ligand–protein complex are directly proportional to the rotational correlation time of the ligand–protein complex, and

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hence the technique was particularly suitable for large systems as its sensitivity increased as the molecular weight of the protein increased, allowing larger ratios of free over bound ligand to be employed. We subsequently applied the TRNOE both as 1-D and 2-D experiments to a range of problems, including binding of nucleotides,[8] dinucleotides,[9] peptides,[10] and single-stranded DNA[11] to a variety of proteins.

In 1983, NIMR acquired a 500-MHz NMR spectrometer. This was a huge advance over the existing 270-MHz spectrometer and opened up a whole new avenue of research. At that time, we were interested in studying the structure of DNA by NMR and to this end Angela and I synthesized a self-complementary DNA hexamer by old-fashioned solution methods. As the S/N on the 500-MHz spectrometer, while hugely improved compared to the 270-MHz spectrometer, was still relatively low compared to today's (2011) standards and we did not have enough contiguous NMR time to record a 2-D NOE spectrum, we simply recorded a series of 1-D NOE experiments. I took these spectra home and, together with a model of DNA, was able to figure out how to sequentially assign the complete spectrum within about 30 min. We wrote this paper up for *Nature*, who, despite two out of three favorable reviewers' comments, did not deem the work of sufficient general interest, so we published this in the *EMBO J.* in 1983.[12] At roughly the same time, several other groups, including David Kerns at UCSD and Michael Weiss and Martin Karplus at Harvard, also succeeded in assigning the spectra of double-stranded DNA using the same sequential assignment strategy.

THE MAX-PLANCK INSTITUTE FOR BIOCHEMISTRY IN MARTINSRIED (1984–1988)

In October 1984, Angela Gronenborn and I moved to the Max Planck Institute for Biochemistry in Martinsried, Germany, to set up a newly created NMR group. Equipped with our own 500-MHz spectrometer we were now able to make full use of its potential and employ all the tools of two-dimensional NMR spectroscopy then available.

We first continued on our work with DNA. The challenge was to devise an appropriate calculational approach to determine the three-dimensional solution structure of a DNA duplex. My first approach was to make use of the program RESTRAIN, written by David Moss and Iain Tickle at Birbeck, London, for crystallographic refinement, to refine classical B-DNA based on NOE-derived interproton distance restraints.[13] The fundamental problem, however, was that RESTRAIN could only carry out restrained minimization that effectively only allowed the coordinates to sample the closest local minimum. Thus, it was not feasible, for example, to start with an A-DNA structure and end up with B-DNA. It was evident that a far more powerful minimization algorithm was required to sample a much larger range of conformational space. The answer was restrained molecular dynamics. At that time, Axel Brünger had returned to Martinsried following a postdoc with Martin Karplus and brought with him an extension of CHARMM known as CHARMMF, which stood for the **f**ast version of CHARMM because it ran on a Cray supercomputer. In actuality, CHARMMF had significantly diverged from CHARMM and was the precursor of what came to be known as the program XPLOR. Together, Axel and I quickly implemented the incorporation of distance and torsion angle restraints into CHARMMF/XPLOR and showed that we could use these to obtain the solution structure of a small 17mer peptide comprising the DNA binding helix of CRP starting from many different starting configurations.[14] Subsequently, we extended this technique to DNA[15,16] and proteins.[17,18] For DNA we were able to demonstrate that one could arrive at a unique solution, irrespective of whether the starting coordinates were A or B DNA. For proteins, we could readily obtain the correct fold starting from a completely extended strand. At first, the use of restrained molecular dynamics was treated with skepticism but it has subsequently become universally adopted.

With these tools in hand, Martin Karplus suggested that we solve the solution structure of α1-purothionin, a small 45-residue protein related to crambin, the model system we had used to develop the restrained molecular dynamics protocols for NMR-based protein structure determination.[17] We obtained a sample of α1-purothionin from Martha Teeter at Boston University, had complete assignment within about 3 weeks, and a complete structure 3 weeks later.[18] α1-Purothionin was the second protein structure (the first being proteinase inhibitor II from bull seminal plasma by the Wuthrich group[19]) to have been determined de novo without the use of an initial model. The Kaptein group had previously solved the structure of lac repressor headpiece but rather than determine the structure from scratch so to speak, they built an initial model based on the coordinates of the CI repressor of bacteriophage λ and then refined it using the molecular dynamics program GROMOS.[20]

We then proceeded to determine the structures of a number of other small proteins and to improve the efficiency of the structure determination protocols. Rather than make use of a full empirical energy function traditionally used in molecular dynamics simulations, I realized that it was far more efficient to carry out simulated annealing using a very simple target function comprising potentials for the NMR-derived restraints, a quartic van der Waals repulsion term for the nonbonded contacts, and terms to maintain idealized covalent geometry.[21–23] To ensure extensive sampling and high convergence, the simulated annealing calculations start at high temperature followed by slow cooling. The convergence properties of this method were so high that one could even use a completely random array of atoms with no intact covalent geometry of any sort for the starting coordinates, and end up with the correct fold.[23] Even more computationally efficient, given the slow speed of computers at the time, was to combine distance geometry with simulated annealing in a hybrid method.[21] The idea was to carry out a very crude form of distance geometry in which an approximate polypeptide fold was obtained by generating a set of substructures comprising only a small subset of atoms by projection from multidimensional distance space into three-dimensional Cartesian space using a procedure known as embedding, followed by adding the remaining atoms by best-fitting extended amino acids one residue at a time to the substructures, and using the resulting structures as the starting point for real space simulated annealing. All these methods now form the mainstay for computing 3-D structures of macromolecules by NMR.

On the NMR side, we rapidly realized that the resolution of 2-D NMR was simply insufficient to extend NMR-based structure determination of proteins beyond 80–100 residues.

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Together with Hartmut Oschkinat, a postdoc in the lab, and in collaboration with Richard Ernst and Christian Griesinger at the ETH, we recorded the first 3-D NMR spectrum of a protein. It was obvious that any 3-D NMR experiment could simply be obtained by combining two 2-D experiments, leaving out the detection period of the first experiment and the preparation pulse of the second. The 3-D experiment we recorded was a 3-D-NOE-Hartman–Hahn experiment with ^{1}H chemical shifts in all three dimensions. While this experiment clearly demonstrated the potential of 3-D NMR as a means of increasing spectral resolution, it was also clear to us that a far more useful 3-D strategy was one in which a 2-D ^{1}H–^{1}H spectrum was extended into a third dimension by the chemical shift of the directly bonded heavy atom (i.e., ^{13}C or ^{15}N). Hartmut Oschkinat and I actually even recorded a 3-D ^{15}N-separated NOE spectrum on a 10-mM sample of α1-purothionin at natural abundance in 1987 and presented this on a poster at the Ninth European Experimental NMR Conference in 1988. Not surprisingly, we could only see cross-peaks involving the mobile side chain NH_2 groups of Asn and Gln, and further progress in this area would have to wait for uniform ^{15}N labeling.

LABORATORY OF CHEMICAL PHYSICS, NIDDK, NATIONAL INSTITUTES OF HEALTH (1988–1998)

In 1987, Bill Eaton, Ted Becker, and Ad Bax recruited Angela and myself to the NIH. The Office of the Director of the NIH had just set up a structural biology program devoted to AIDS Targeted Antiviral Research. This program had provided the funds to enable the Laboratory of Chemical Physics (LCP) to purchase a 600-MHz spectrometer. We moved to the NIH in 1988 and the next few years saw some very exciting developments in NMR as a result of a highly collaborative environment comprising Ad, Angela, Dennis Torchia, and myself.

We set out to solve the solution structure of interleukin-1β, a key modulator of the immune response, which, at 153 residues, was over 50% larger than any protein structure previously determined by NMR. This project was actually started while we were still at the Max-Planck but it became evident very quickly that 2-D homonuclear NMR simply could not cut it. The key trick to making full use of 3-D NMR for extending spectral resolution was to uniformly label the protein with ^{13}C and ^{15}N. Dennis had developed the technology for doing this on staphylococcal nuclease.[24] The protein of interest had to be expressed in bacteria (e.g., *E. coli*) and labeling was achieved by growing the bacteria in minimal medium supplemented by ^{15} NH_4Cl and $^{13}C_6$-glucose as the sole nitrogen and carbon sources, respectively. We were able to demonstrate quite readily that complete sequential ^{1}H and ^{15}N backbone assignments could be made using the traditional NOE and ^{1}H–^{1}H correlation approach by means of 3-D ^{15}N-separated NOE and homonuclear Hartman–Hahn (HOHAHA) spectra.[25,26] Side chain assignments required 3-D 1H–^{13}C–^{13}C–^{1}H correlation spectroscopy using either a COSY mixing scheme (HCCH-COSY) or isotropic mixing of ^{13}C magnetization (HCCH-TOCSY).[27–29] To connect the sidechain assignments to the backbone then required the use of a 3-D HNCA experiment, developed by Ad, Lewis Kay, and Mitsu Ikura to unambiguously correlate the $^{13}C\alpha$ shift of residue i with the H_N and ^{15}N shifts of residue i as well as $i + 1$.[30] Despite almost complete resonance assignments in hand, interpretation of the 3-D ^{13}C- and ^{15}N-separated NOE spectra necessary to obtain a structure was still difficult. The next obvious step was to increase the dimensionality to four, thereby permitting the originating proton and destination proton for a given NOE to be separated by the chemical shifts of their respective directly bonded heavy atom. Thus, an NOE would be identified by four chemical shift coordinates. The first experiment, developed in conjunction with Ad and Lewis, was a 4-D ^{13}C/^{15}N-separated NOE spectrum which, fortunately, was relatively straightforward.[31] Implementation of the complementary 4-D ^{13}C/^{13}C-separated NOE spectrum, however, proved to be far more challenging due to the presence of a large number of spurious magnetization transfer pathways that can lead to an observable signal. Ad and I eventually ended up with a reasonable scheme through a long and iterative procedure involving a lot of trial and error.[32] With the advent of pulse field gradients now routinely available, elimination of such artifacts is easy and high-quality 4-D ^{13}C/^{13}C-separated NOE spectra can be recorded with ease.[33] Interpreting 4-D spectra, however, was virtually impossible with 2-D paper plots, although such plots could be used quite effectively for 3-D spectra since the number of planes in the ^{13}C or ^{15}N dimension was limited to 64. As a result, Dan Garrett, then a postdoc in the lab and now a permanent staff scientist, wrote the program PIPP that permitted simple computer-graphics-based analysis of multidimensional spectra.[34] With all the relevant spectroscopy and analysis tools in hand, we were now in a position to determine the 3-D structure of interleukin-1β, which was successfully completed in 1991.[35,36]

Following our work on interleukin-1β, we quickly made use of the NMR techniques that had been developed by ourselves and Ad in LCP to solve the 3-D solution structures of a range of interesting biological systems, including several immune modulators (interleukin-8 in 1990,[37] interleukin-4 in 1992,[38] and human MIP-1β in 1994[39]), the first example of a calmodulin–peptide complex in 1992[40] with Mitsu and Ad, and the first protein–DNA complex with Jim Omichinski in 1993.[41] This was subsequently followed up by several other protein–DNA complexes including those with the transcription factor GAGA,[42] the male sex-determining factor SRY[43] and the architectural factor HMG-I/Y,[44] and a number of HIV proteins including the N- and C-terminal domains of integrase[45,46] and the ectodomain of gp41.[47]

In addition to protein structure, we were also interested in dynamics. Lewis, Ad, and Dennis had just developed a series of experiments to measure ^{15}N relaxation data and applied these to staphylococcal nuclease,[48] and we quickly did the same for interleukin-1β.[49] Looking at the data for both proteins I noticed something unusual, namely, that not all the data could be accounted for by the simple Lipari–Szabo model but in fact required an extended formalism that took into account not only very rapid motions on the picosecond time scale but also larger amplitude motions on the nanosecond time scale.[49,50]

At the same time, we were interested in probing structural waters in proteins by NMR, and together with Ad we developed a 3-D ^{15}N-separated ROE experiment to unambiguously locate protons in close proximity to bound water in interleukin-1β.[51]

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During this period I was also interested in developing methods to improve the accuracy and precision of NMR structure determination. At first this involved interpreting as much of the NOE data as possible to increase the number of structurally useful interproton distance restraints per residue, together with obtaining stereospecific assignments and/or side chain torsion angle restraints through the combined interpretation of NOE and ^{3}J coupling data.[36] At the same time we developed methods for direct refinement against ^{3}J-couplings,[52] $^{13}C\alpha/^{13}C\beta$ chemical shifts[52], and ^{1}H chemical shifts.[53] I soon realized that further significant improvements could be made through the use of multidimensional torsion angle databases of mean force derived from very high resolution structures to restrict sampling during simulated annealing refinement to conformations that are likely to be energetically possible by effectively limiting the choices of torsion angles (both backbone and side chain) to conformations that are known to be physically realizable.[54] Along the same lines, we also developed a radius of gyration restraint which resulted in significant improvements in coordinate accuracy (as judged by comparison with crystal structures) by offsetting the tendency of NMR structures to be expanded when refined using only a van der Waals repulsion term.[55]

In 1997, we came to the realization that further significant improvements in accuracy would require experimental data that could provide long-range order since all the usual NMR observables were confined to short-range interactions. This included residual dipolar couplings (RDCs)[56] and ^{15}N-T_1/T_2 relaxation data[57] whose magnitude is dependent on bond vector orientations relative to the alignment and diffusion tensors, respectively. Jim Omichinski, Nico Tjandra, Ad, and I first showed that RDCs measured on a protein–DNA complex and arising from very weak alignment of the DNA in the magnetic field could yield significant increases in backbone accuracy.[56] Subsequently, Ad and Nico showed that weak alignment could be measured routinely by dissolving the sample in a dilute liquid crystalline medium of bicelles,[58] and shortly thereafter Angela and I showed that alignment could also be obtained with a medium comprising filamentous phage.[59] The two media are complementary since bicelles are largely neutral and alignment is almost entirely sterically induced, while for the negatively charged phage, electrostatic effects are also involved. The first extensive application of residual dipolar couplings in a protein structure determination was for the small antiviral protein cyanovirin-N.[60]

LABORATORY OF CHEMICAL PHYSICS, NIDDK, NATIONAL INSTITUTES OF HEALTH (1998–PRESENT)

Over the last 12 years, my work has focused on three complementary areas: first, improving methods of structure calculation from NMR data; second, extending protein NMR structure determination to complexes of increasing complexity and molecular weight; and third exploring spectroscopically invisible, sparsely-populated states using paramagnetic relaxation.

Developments in computational methods have included the incorporation of new experimental observables (not just NMR ones but also from non-NMR sources such as X-ray scattering[1,61]), more advanced database potentials, and improved methods for simulated annealing such as torsion angle and rigid body methods.[62,63] This, in large part through the efforts of my colleague Charles Schwieters, has resulted in the development of the NMR molecular structure determination package Xplor-NIH.[64,65]

Our structural work on complexes has led us to develop what we have called conjoined rigid body/torsion angle dynamics for solving structures in which conformational changes within the individual components of the complex are minimal or confined to selected regions.[62,63] This approach, which largely makes use of intermolecular NOE data and RDCs, was used to determine the first ternary protein–DNA complex by NMR, namely, the 42-kDa ternary Oct1/Sox2/DNA complex,[66] as well as to explore the underlying basis of protein–protein recognition in the soluble complexes of the bacterial phosphotransferase pathway. To date, we have solved eight out of the nine complexes which range from 30 to ~80 kDa in size. These complexes provide a paradigm for understanding how individual proteins can recognize multiple, structural dissimilar partners with no common secondary, tertiary, or quaternary structural features. This is achieved by making use of similarly shaped surfaces with similar residue type distributions.[67,68] Further, this particular set of structures highlights the role of redundancy and side chain conformational plasticity in protein–protein complexes involving multiple interaction partners.

By combining residual dipolar couplings to provide orientational information and small and wide angle X-ray scattering to provide shape and translational information, we were able to solve the structure of the 128-kDa enzyme I and its 146-kDa complex with HPr.[1] This particular work was able to highlight the large interdomain motions that accompany the catalytic cycle in which enzyme I is first autophosphorylated by phosphoenolpyruvate prior to transferring the phosphoryl group to its partner protein HPr.

More recently, we have focused on paramagnetic relaxation enhancement to shed light on sparsely-populated states that are invisible to conventional biophysical and structural techniques (including crystallography and regular NMR spectroscopy).[2,3] Such states of macromolecules, characterized by short lifetimes and high free energies relative to the predominant ground state, often play a key role in many biological, chemical, and biophysical processes. The underlying theory of the PRE for static systems dates back to the 1960s but quantitative use of the PRE for structure determination was thwarted until recently for lack of an appropriate theoretical framework and computational methods to take into account the large conformational space sampled by the paramagnetic label attached to the protein via a linker with multiple bonds. Initially Junji Iwahara and I developed the relevant theory and refinement tools for the PRE as a method of structure determination to provide long-range (up to 35 Å) distance information as a complement to the short-range distance information (<6 Å) afforded by the ^{1}H–^{1}H NOE which provides the mainstay of NMR structure determination. By representing the paramagnetic label by an ensemble of states and taking care to calculate PRE order parameters from the coordinates during the course of structure refinement, we were able to demonstrate that direct refinement against PRE data is feasible and yields increased coordinate accuracy as judged by independent validation against RDCs.[69]

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The finding that the intermolecular PRE was capable of detecting sparsely-populated states was entirely fortuitous. We had been interested in studying the structure of a ternary complex involving the HoxD9 homeodomain, HMGB-1A, and DNA. Despite numerous reports in the literature that HoxD9 and HMGB-1A interacted with one another both in the absence and presence of DNA, Junji and I were unable to detect any NMR evidence for such an interaction. However, since the presence of HMGB-1A was reported to enhance transcriptional activation by HoxD9 we speculated that the effect of HoxD9 bound to DNA was to modulate the distribution of nonspecifically bound HMGB-1A on the DNA, and hence alter the extent of DNA bending induced by HMGB-1A. We never addressed this issue in the end because control experiments on the specific HoxD9/DNA binary complex revealed a totally unexpected finding that opened the door for using the PRE to detect and probe transient sparsely-populated states. Specifically, while the PRE data at low salt were fully consistent with the known structure of the HoxD9/DNA complex, at higher salt (100- and 150-mM NaCl) the PRE data indicated that HoxD9 was sampling multiple sites in multiple orientations on the DNA, despite the fact that the dissociation rate constant is less than 0.01 s^{-1}, the equilibrium dissociation constant is around 1 nM, and the ^{1}H-^{15}N correlation spectrum is that of the specific complex (with essentially no shifts from the heteronuclear single-quantum coherence (HSQC) spectrum at low salt).[70] We were able to demonstrate that this phenomenon was due to the existence of states populated at less than 1% that involved intramolecular translocation (i.e., sliding) of HoxD9 along a DNA molecule, as well as direct intermolecular translocation of HoxD9 from one DNA molecule to another.

The key to using the PRE to detect transient low-population species lies in rapid exchange phenomena, whereby the transverse PRE observed on a major species is modulated by the presence of the minor species.[70] In a two-site exchange system comprising two species A and B that interconvert on a time scale that is fast on the PRE time scale, the observed PRE measured on either resonance will be the population-weighted average of the PRE rates for the two species. Therefore, providing distances between the paramagnetic center and the protons of interest are significantly shorter in the minor species than the major one and the interconversion rate between the two species is fast, the PRE profiles observed on the major species will reveal the footprint of the minor species. The PRE profiles can be analyzed quantitatively to derive structural information if the PRE profile for the major species is either known or can be calculated from a known structure. As the exchange rate decreases, the influence of the minor species on the observed PRE profile for the major species will be reduced until in the slow exchange limit the PRE profile for the major species will be unaffected by the presence of the minor species. Using the PRE we were able to investigate for the first time the dynamic processes involved in the location of a specific cognate DNA-binding site by a transcription factor[70]; the formation of transient encounter complexes on the pathway to stereospecific protein–protein complex formation[71,72]; the sampling of sparsely-populated states involving large scale domain motions in a multidomain protein[73]; and the visualization of transient events involved in amino-terminal auto-processing of HIV-1 protease.[74]

CONCLUDING REMARKS

The last 30 years have seen an incredible number of very exciting developments in biomolecular NMR. It is now possible to solve the structures and study the dynamics of systems that would have been impossible to contemplate even 20 years ago. I suspect that the next 20 years will see many more exciting developments and that NMR will tackle problems that one cannot even imagine today.

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