Teaching Biophysics

Teaching High-Resolution Nuclear Magnetic Resonance to Graduate Students in Biophysics

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ABSTRACT Teaching modern methods of high resolution, multi-dimensional nuclear magnetic resonance (NMR) spectroscopy in the limited time usually available is a challenge because of the breadth and complexity of the subject. Here we present two outlines for lectures on basic principles and biophysical applications of NMR, either in one lecture or in six to twelve lectures. We advocate emphasizing the versatility of NMR, and its numerous applications to biophysical questions. An annotated list of references is provided.

INTRODUCTION

Understanding structure-function relationships lies at the heart of molecular biophysics. Currently, the two most popular and powerful methods for describing the first part of the structure-function equation are x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. High-resolution multidimensional NMR methods are being used routinely to determine the solution conformations of biomolecules. The greatest progress has been made in studies of proteins, followed by nucleic acids, carbohydrates, and other classes of biomolecules. Structure determination by NMR has spread rapidly, as can be shown by perusing the tables of contents of major biochemical journals.

All students of biophysics should have exposure to these methods at some level, if only to enable them to evaluate conclusions based on the application of these methods to systems of interest to the students. It is important for students to know that nuclear magnetic resonance (NMR) is a supremely versatile method, useful for studying molecular motions and interactions, as well as conformations. It is equally important for them to know its limitations.

The purpose of this article is to present a minimum basis set for describing modern NMR methods to nonspectroscopists when time is limited. Very few biophysics programs have the luxury of an entire semester's class devoted to NMR spectroscopy. More typically, 1 or 2 weeks is allocated for lectures about NMR. (An intermediate situation would be a semester course devoted to both x-ray and NMR methods.) One or two weeks of a three-credit course translates into no more than six lectures. Clearly, one must think hard about what can be covered realistically in so few lectures, and one must set realistic goals.

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Decisions about the course content boil down to one key question: What do the students need to know, and when do they need to know it? Quantum mechanics is beautiful, elegant, and powerful; but how much of it can you convey in one lecture? Another example of a difficult choice is: if you devote too much time to describing homonuclear nuclear Overhauser effects (NOEs), the mainstay of structure determination, you will not have time to present the breadth of NMR methods.

The curricula suggested below are designed to be edited according to the time available. We have chosen two typical models: one or two lectures as part of a course in general biophysical chemistry or physical biochemistry, and a few weeks as a major portion of a course on structural methods. The content of a semester-long course on NMR would depend on what other courses are available, probably in the chemistry department (for example, introductory quantum mechanics, density matrix theory, spectroscopy), and which of these courses are reasonable for biophysics students to take.

A semester-long class allows more time for theory, including product operator formalism that will enable students to analyze pulse sequences and phase cycling. However, if you are limited to fewer than a dozen lectures, even purists should overcome their distaste and stick to vector diagrams for following the magnetization. (This effectively limits which pulse sequences can be explained to inversion-recovery (T_1), spin-echo (T_2), and two-dimensional exchange spectroscopy.) Always keep in mind that the interested student, the one who will not be satisfied by flipping a pencil from one axis to another, can opt to pursue further study in quantum mechanics.

If only a few lectures are available, all you can hope to achieve is to provide the students with some vocabulary, give them a sense of the breadth of applications possible, and pique their interest. Wherever possible, show examples from the literature that illustrate practical, interesting results that abound in NMR. Remember that although most students will acquire only a small portion of the knowledge presented,

there will be a few students who absorb it all and will still be unsaturated. Those students can always be told more and given more to learn. They may even ask to work in an NMR laboratory!

In a way, the choice of material to cover is simplified by severe time limitations. When we have attempted very brief coverage of NMR in an undergraduate biophysical chemistry class, in general the students say they enjoy it. A typical comment has been, "At least now I have some idea what all this talk about NMR at meetings and seminars is about." Honesty compels us to report that a minority of students feel it is a waste of time to cover something so superficially.

Below is an outline suitable for one or two lectures, followed by an expanded version suitable for five to ten lectures over a two- to three-week period.

ONE OR TWO LECTURES

How some nuclei behave in an applied magnetic field, and how a one-dimensional NMR spectrum is generated

Concepts that should be covered: macroscopic magnetization; B_0 and B_1; the Larmor equation; a short table of NMR-active nuclei including ω and sensitivity relative to ^1H. Explain how the sample sits in a copper coil that acts as a transmitter and receiver of radiofrequency; explain a simple 90° pulse experiment, and the resultant decaying sinusoidal signal, which is then Fourier transformed into a spectrum. To give the students some feeling for what a Fourier transform is, we give the general equation and explain qualitatively that it translates periodic functions into their frequency components. A simple example is that a sine wave can be described either by tracing it out in time or by giving its characteristic amplitude and frequency.

One-dimensional ^1H spectra of a simple compound and of a more complex compound

The simple spectrum can be used to define chemical shift and other basic features such as intensity, linewidth, and coupling constants. If the more complex spectrum is of a large molecule such as a protein, it affords the opportunity of explaining that the NMR spectrum is sensitive to motions of the molecule: larger molecules → slower motions → broader lines. This relationship segues into the next point: the types of information available from NMR.

Types of information available from NMR/types of NMR

Summarize the information that NMR can provide about primary chemical structure, molecular motions, molecular interactions (including presence of binding, binding constants, exchange rates, reaction rates), conformation, etc.

List the types of NMR experiments: high-resolution; solid-state; one-, two-, three-, four-, n-dimensional; in vivo spectroscopy (cells and whole organisms); imaging.

More about high-resolution NMR—introducing more dimensions

Show the simple and complex one-dimensional spectra again, to illustrate the need for simplifying spectra, either by going to higher field and/or adding more frequency dimensions. It is worth spending time to carefully explain two-dimensional NMR, even if it is at a qualitative level. In our experience, the single most confusing aspect for NMR neophytes is where the second (and higher) dimension originates. Many students start from the misleading assumption that two-dimensional NMR is something akin to two-dimensional chromatography or electrophoresis. If the students "sort of" understand introduction of the second dimension, they will allow that the basic idea can be extended to three and four dimensions. But if they do not see the transition from one to two dimensions, they will give up trying to follow before you even get to biophysical applications.

To assist instructors who may not have access to a spectrometer, we provide a series of figures that have proven useful to us in explaining two-dimensional NMR (see Figs. 1 and 2). The easiest two-dimensional experiment to understand without resorting to quantum mechanics, scalar coupling, and other sophisticated concepts, is exchange spectroscopy. The example shown here demonstrates methyl group exchange in *N,N*-dimethylacetamide, and was suggested by Dr. Ad Bax.

Examples illustrating the breadth of applications of high-resolution NMR to biophysics

Specific references are suggested in the annotated reference list.

1. Magnetic and chemical environment (chemical shift); paramagnetic shifting of selected resonances in a metal-

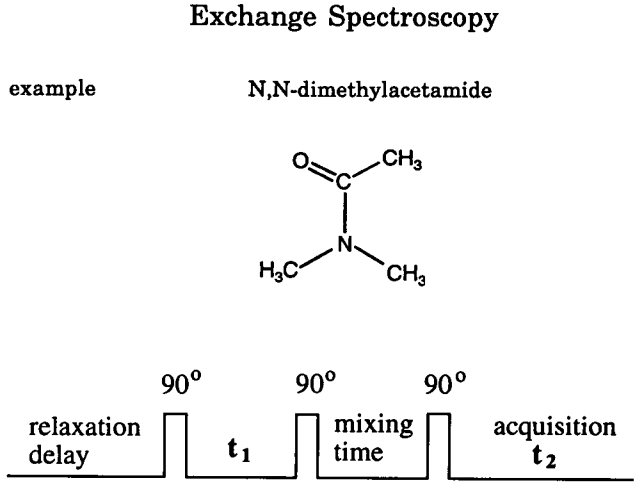

FIGURE 1 Pulse sequence for exchange (or NOE) spectroscopy. (For clarity, phase cycling is omitted.) For the exchanging methyl groups on the nitrogen of *N,N*-dimethylacetamide, protons of the methyl group at one position during t_1 that exchange to the other position during the mixing time will have a different precession frequency during t_2.

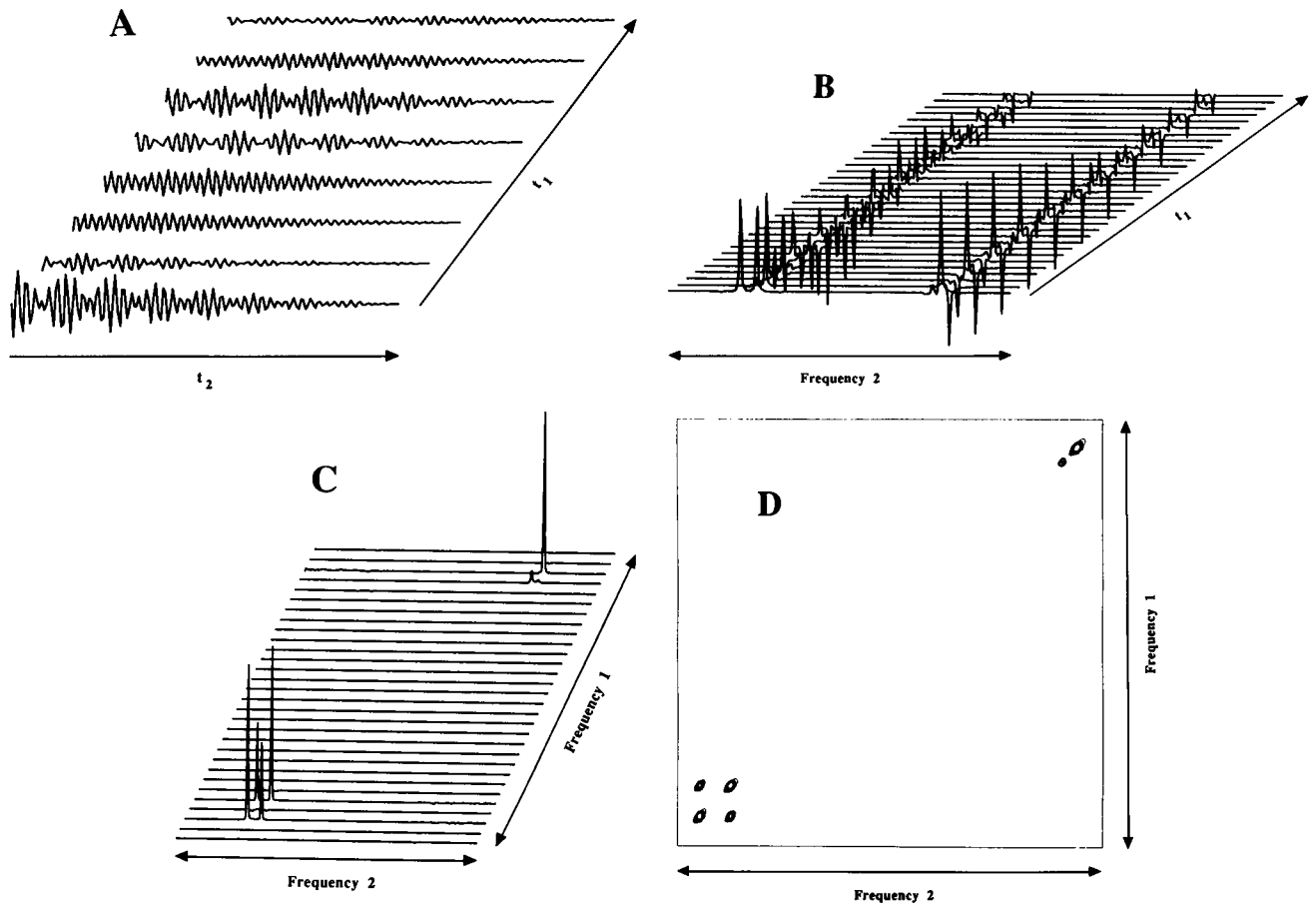

FIGURE 2 These figures are included for the convenience of readers without access to an NMR spectrometer and are meant to illustrate the origin of higher dimensions in NMR spectroscopy. The data were generated with the pulse sequence shown in Fig. 1, using a sample of 15 μl *N,N*-dimethylacetamide in 700 μl *d*-chloroform at 29°C on a Varian UNITY 500-MHz spectrometer. For clarity, not all traces are shown. (*A*) Series of free induction decays recorded in real time (t_2) for incremented values of t_1. This helps students think of two-dimensional experiments as a series of one-dimensional experiments, with different t_1 values. (*B*) Transposed spectra, after first Fourier transform. This helps students visualize that the signals will have some periodic dependence on t_1, which can be revealed by a second Fourier transform with respect to t_1. (*C*) Stacked plot after second Fourier transform. The small peak slightly downfield of the nonexchanging methyl protons arose from an impurity in the sample. (*D*) Contour plot of *C*, to display diagonal and cross-peaks. This will orient students to the usual mode of displaying two-dimensional spectra.

loprotein; broadening of surface amino acid residues of a protein, proton exchange rates in proteins and nucleic acids, pH-titration curves for histidines in proteins.

2. Molecular motions (linewidth, relaxation times), binding
3. Solution conformation, both static and dynamic (protein folding). If time allows, this may be an appropriate place to discuss scalar coupling constants and the use of a Karplus curve to extract dihedral angles.

Advantages and limitations

Discuss sample preparation. The method is nondestructive but requires rather large amounts of relatively pure material, of molecular weight < 30,000 (except under special circumstances). Almost any buffer system can be used; nonprotonated buffers such as phosphate are the simplest, but with deuteration or presaturation it may be possible to use a protonated buffer.

For biological macromolecules, the most severe handicap is restriction to a molecular weight less than ~30,000. Also, the sample must be available in multimilligram quantities, preferably labeled with ^{13}C and ^{15}N and soluble to millimolar concentration. These restrictions can often, but not always, be overcome by expression (for proteins), in vitro synthesis (peptides and oligonucleotides), selective labeling, and varying solvent composition.

To discuss the uncertainties in NOEs introduced by internal motions in molecules, it will be necessary to explain the dependence of the NOE on the correlation time of the internuclear vector. One approach is to give a simplified equation for two spins, showing that the rate of NOE buildup is proportional to the correlation time divided by the sixth power of the distance between the spins. We have found that a qualitative explanation of correlation time is sufficient, for example "the correlation time is a measure of how long a $^{13}C—^{1}H$ bond or $^{1}H—^{1}H$ internuclear vector stays correlated with its original position", with an emphasis on the concept that short correlation times mean fast motions and long correlation times mean slow motions. At this point, the students can see that uncertainties in the cor-

relation time and/or internuclear distance could lead to errors in interpretation of buildup rates in terms of internuclear distances.

Extras

If possible, show students a spectrometer. If the class is too large, take them in smaller groups or only take those who express an interest. A possible homework assignment is for students to find a recent journal article in which NMR spectroscopy is used; summarize the major conclusion(s) reached; and describe how evidence from NMR was used to support those conclusions. Usually, biophysics students already have some research interests, and may be able to find an article that deals with a biological system with which they are already familiar. Seeing how NMR can contribute to understanding a system is a useful reinforcement for the importance of the method.

TWO- TO THREE-WEEK SYLLABUS

How much of this you fit into each lecture depends partly on how many examples you use. Everything discussed above, for the shorter time period, also applies for the longer version. The main differences between the short syllabus and this one is the introduction of more quantitative material (e.g., the Bloch equations and explanations of T_1 and T_2) and more time to discuss specific examples.

Lectures 1 and 2

Give an overview of NMR spectroscopy. This is easier if the class has already covered basic principles of spectroscopy, such as absorption and resonance. One source of basic background material is undergraduate texts in physical chemistry. Some sources are listed in the annotated reference list. Start with the concept that NMR exploits the magnetic properties of atomic nuclei and that detecting the responses of these nuclei to applied magnetic fields provides a wealth of information at the molecular level.

List and briefly describe the range of NMR methods (high-resolution, solid-state, in vivo, imaging) and applications (chemical identity, conformation, molecular motions, exchange processes, metabolism, et al.). Give a few impressive examples. Now that you have established that NMR is worth learning about, present more of the underlying principles. Although we have advocated minimal use of quantum mechanics when time is limited, it is useful to introduce quantization of nuclear spins for spin 1/2. A simple two-spin (four-level) diagram at this point will make it easier to discuss concepts such as scalar coupling and multiple quantum methods later on. Definition of the resonance frequency as the transition frequency between spin levels can serve as a transition back to a vector description, via the Larmor equation. Show a partial listing of "NMR-active" nuclei at a given field strength. Show a diagram of an NMR spectrometer, consisting of a sample in a coil in a magnet, connected to a transmitter and receiver. Correlation of these simplified components to their actual counterparts in a color slide of a high field spectrometer can give students a better sense of the instrumentation.

Lectures 3 and 4

Present a more detailed discussion of NMR, keeping to a semiclassical description when possible: include the Larmor equation, a simple one-pulse experiment, the Bloch equations, and Fourier transformation. Explain longitudinal and transverse relaxation and why they provide information about molecular motions (this requires some discussion of the meaning of a correlation time). Explain the relationship between linewidth and T_2. A nice example to illustrate the utility of linewidth (T_2) in studying binding is the study of spermine and DNA published by Wemmer et al. (1985) (see annotated reference list).

Lectures 5 and 6

Discuss multidimensional NMR. Reprise the one-pulse experiment and Fourier transformation; present a simple two-dimensional experiment. The example of exchange spectroscopy previously described is useful. List the advantages of adding more time/frequency dimensions to an experiment, such as simplifying assignment and revealing direct and relayed connectivities. Introduce ways in which nuclei are coupled: through bonds and/or through space. Introduce cross-relaxation and the nuclear Overhauser enhancement, and how it depends on internuclear distance and correlation time.

Lectures 7 and 8

Explain how to derive solution conformation from NMR spectroscopy, primarily through NOEs, coupling constants (Karplus relations), and comparison with model compounds. Discuss what can be gleaned from chemical shifts. (Nice place to discuss paramagnetic proteins.) Limitations (molecular weight, flexible molecules, uncertainty in the NOEs) and ways to compensate, to be continued in lecture 9. This might be an appropriate time to compare structure determination by x-ray crystallography and NMR spectroscopy.

Lectures 9 and 10+

Show students how it is really done. You are in luck if you can take the students step by step through the assignment of a peptide or an oligonucleotide; show them actual NOE buildup curves; discuss various options for extracting cross-relaxation rates; discuss options for modeling (distance geometry, back calculation, restrained molecular dynamics).

OTHER SUGGESTIONS

1. Take students on a tour of your local NMR facility, so they get an idea of what samples and instruments look like.

Ideally, you could insert a standard sample, demonstrate tuning and shimming, calibrate a 90° pulse, take a simple one-pulse free induction delay, and Fourier transform it before their eyes. A demonstration like this early in the lecture series can help make abstract concepts more concrete. It may be more practical to bring a sample and a probe to class so the students can visualize at least part of the experimental setup.

2. Provide a series of one- and two-dimensional spectra of a simple biomolecule, such as a small peptide. Ask the students to assign the ^1H spectrum. Suggested series of spectra: COSY, DQCOSY, TOCSY, NOESY, ROESY; ^1H-^{13}C HMQC; ^1H-^{15}N HMQC (if possible). Obviously, setting up this exercise will entail several days of spectrometer time, plus the time it takes for your own analysis. Alternatively, an interesting example in the literature (such as *Biochemistry*) could be chosen. An example of this approach, complete assignment for sucrose octa-acetate at 250 MHz, is described in detail by Sanders and Hunter, chapter 10. Guidelines for assignment of proteins, peptides, and oligonucleotides are thoroughly explained in Wuthrich's text.

SUMMARY

Every graduate student in molecular biophysics should know about high-resolution NMR and its importance in understanding structure-function relationships in biomolecules. It is important to emphasize the enormous range of applications for NMR, beyond structure determination. When lecture time is limited, students should at least know what kinds of information the method can supply and its advantages and disadvantages.

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ANNOTATED REFERENCE LIST

General comments

This list is meant to provide references that we have found useful for teaching ourselves and others. It is not meant to be comprehensive, and it cannot be up to date because of the continual outpouring of publications about NMR theory and applications. Older papers generally contain more explanation and detail about methods and underlying principles than do post-1990 papers, although the specific pulse sequences used may not represent the current state of the art.

Many review articles about relevant topics are available, especially on determining protein structures from multidimensional high-resolution NMR. Not all the useful ones are listed here, but all the ones listed are useful.

TEXTS

Freeman, R. 1987. A Handbook of Nuclear Magnetic Resonance. John Wiley and Sons, New York. 312 pp. *Essentially a large glossary, this book provides clear explanations of many terms and ideas utilized in current NMR research.*

Wuthrich, K. 1986. NMR of Proteins and Nucleic Acids. John Wiley and Sons, New York. 292 pp. *This is the standard book on the subject. It explains the how and why of structural analysis of proteins and nucleic acids in a logical progression. Essential for anyone assigning their first peptide, protein, or oligonucleotide.*

Levine, I. 1975. Molecular Spectroscopy. John Wiley and Sons, New York. 497 pp. *This is a good basic graduate-level text on spectroscopic methods, and it is useful for providing quantum mechanics background. Another excellent source for basic background material on spectroscopy in general and magnetic resonance in particular is a standard undergraduate text in physical chemistry, such as Alberty, R. A., and R. J. Silbey. 1992. Physical Chemistry. John Wiley and Sons, Inc., New York, and Atkins, P. W. 1990. Physical Chemistry. 4th ed. W. H. Freeman and Co., New York.*

Campbell, I. D., and R. A. Dwek 1984. Biological Spectroscopy. Benjamin/Cummings Publishing Co., Menlo Park, CA. 464 pp. *Chapter 6 on NMR is an excellent, comprehensive survey of applications to biological problems, including titration of specific amino acid residues.*

Cantor, C. R., and P. R. Schimmel 1980. Biophysical Chemistry. Vol. 2. W. H. Freeman and Co., New York. *Chapter 7 (pp. 349–408) covers general principles of spectroscopy and Chapter 9 (pp. 481–538) describes magnetic resonance. The section on NMR is outdated, but still worth reading because it covers many interesting applications of NMR to biomolecules besides structure determination.*

REFERENCES

The next four texts are written with an emphasis on analysis of small molecules, but they explain NMR phenomena at a level appropriate for graduate students. These, and many other texts available about high-resolution NMR, could serve as primary or supplemental texts for a semester-long course.

Sanders, J. K. M., and B. K. Hunter 1993. Modern NMR Spectroscopy: A Guide for Chemists. 2nd ed. Oxford University Press, New York. 314 pp.

Derome, A. E. 1987. Modern NMR Techniques for Chemistry Research. Pergamon Press, New York. 280 pp.

Harris, R. K. 1987. Nuclear Magnetic Resonance Spectroscopy: A Physicochemical View. Longman Scientific and Technical, Harlow, England. 260 pp.

Friebolin, H. 1991. Basic One- and Two-dimensional NMR Spectroscopy. VCH Publishers, New York. 344 pp.

GENERAL ARTICLES

Bax, A., M. Ikura, L. E. Kay, G. Barbato, and S. Spera. 1991. Multidimensional triple resonance NMR spectroscopy of isotopically uniformly enriched proteins: a powerful new strategy for structure determination. *Ciba Found. Symp.* 161:108–119.

Clore, G. M., and A. M. Gronenborn. 1991. Structures of larger proteins in solution: three- and four-dimensional heteronuclear NMR spectroscopy. *Science (Washington DC).* 252:1390–1399. *Besides providing clear explanations of higher dimension experiments, the authors provide an impressive example of extending the size limit on structure determination.*

Croasmun, W. R., and R. M. K. Carlson, editors. 1987. 2D NMR Spectroscopy: Applications for Chemists and Biochemists. VCH Publishers, New York. 54 pp. *Several chapters in this collection, especially those by G. Gray and by W. Hull, are very straightforward, practical descriptions of pulse sequences and strategies for their use.*

Ernst, R. R. 1992. Nuclear magnetic resonance Fourier transform spectroscopy. (Nobel lecture). *Angew. Chem. Int. Ed. Engl.* 31:805–823.

Jelinski, L. W. 1984. Modern NMR spectroscopy. *Chem. Eng. News.* 5: 26–47. *Although this review article is almost 10 years old, it is still the best choice for background reading for students with no previous knowledge of NMR. With clarity and interesting examples, Dr. Jelinski covers the major areas of NMR: solution, solid-state, two-dimensional, and imaging.*

Kessler, H., M. Gehrke, and C. Griesinger. 1988. 2D NMR spectroscopy: background and overview of the experiments. *Angew. Chem. Int. Ed.*

Engl. 27:490–536. *This article provides a clear summary of most high-resolution methods and applications up to 1988. It explains and uses product-operator formalism in a helpful way.*

Oppenheimer, N. J., and T. L. James, editors. 1989. *Methods in Enzymology* Vol 176. *This volume contains many useful reviews of NMR methods that were state of the art as of 1989. Applications are covered in volume 177 of the same series.*

Wuthrich, K. 1989. Protein structure determination in solution by nuclear magnetic resonance spectroscopy. *Science (Washington DC).* 243:45–50. *Introductory article on how one obtains tertiary structure from NMR data.*

SPECIFIC EXAMPLES

Proteins and peptides

Klevit, R. E., G. P. Drobny, and B. E. Waygood. 1986. Two-dimensional ^{1}H NMR studies of histidine-containing protein from *Escherichia coli*. *Biochemistry.* 25:7760–7781. *This is a three-part series describing the assignment and structure determination of HPr.*

Wand, A. J., D. L. Di Stefano, Y. Feng, H. Roder, and S. W. Englander. 1989. Proton resonance assignments of horse ferrocytochrome C. *Biochemistry.* 28:186–203. *This pair of papers describes an attempt to assign the ^{1}H resonances of a reasonably large protein and obtain the secondary structure.*

Marion, D., M. Zasloff, and A. Bax. 1988. A two-dimensional NMR study of the antimicrobial peptide magainin-2. *FEBS Lett.* 227:21–26. *These very nice spectra of a 23-amino acid residue peptide could be used to explain assignment.*

Protein Folding

Varley, P., A. M. Gronenborn, H. Christensen, P. T. Wingfield, R. H. Pain, and G. M. Clore. 1993. Kinetics of folding of the all-β sheet protein interleukin-1β. *Science (Washington).* 260:1110–1113.

Lecomte, J. T., and C. R. Matthews. 1993. Unraveling the mechanism of protein folding: new tricks for an old problem. *Protein Eng.* 6:1–10. *This is an interesting use of paramagnetic probes.*

Udgaonkar, J. B., and R. L. Baldwin. 1988. NMR evidence for an early framework intermediate on the folding pathway of ribonuclease A. *Nature (Lond.).* 335:694–699.

Roder, H., G. A. Elove, and S. W. Englander. 1988. Structural characterization of folding intermediates in cytochrome *c* by H-exchange labelling and proton NMR. *Nature (Lond.).* 335:700–704.

Oligonucleotides

Wemmer, D. E., and B. R. Reid. 1985. High resolution NMR studies of nucleic acids and proteins. *Annu. Rev. Phys. Chem.* 36:105–137. *Good starting point.*

Gronenborn, A. M., and G. M. Clore. 1985. Investigation of the solution structures of short nucleic acid fragments by means of nuclear Overhauser enhancement measurements. *Prog. NMR Spectrosc.* 17:1–32. *This article covers a broad range of experiments that can be used to analyze oligonucleotides.*

Hare, D. R., D. E. Wemmer, S-H. Chou, G. Drobny, and B. R. Reid. 1983. Assignment of the non-exchangeable proton resonances of d(C-G-C-G-A-A-T-T-C-G-C-G) using two-dimensional nuclear magnetic resonance methods, *J. Mol. Biol.* 171:319–336. *One of the first papers on assigning DNA, this is a very helpful explanation of how to recognize and assign B-DNA.*

Other

Blaszak, J. A., E. L. Ulrich, J. L. Markley, and D. R. McMillin. 1982. High resolution ^{1}H NMR studies of the Nickel(II) derivative of azurin. *Biochemistry.* 21:6253–6258. *This is one of many possible examples of paramagnetic shifts in metalloproteins.*

Berliner, L. J., and J. Reuben. 1993. Biological Magnetic Resonance. Vol. 12. NMR of Paramagnetic Molecules. 440 pp. *This volume contains many examples of paramagnetic effects. The first chapter by G. N. La Mar and J. S. de Ropp is a useful summary of methods.*

Wemmer, D. E., K. S. Srivenugopal, B. R. Reid, and D. R. Morris. 1985. NMR studies of polyamine binding to a defined DNA sequence. *J. Mol. Biol.* 185:457–459. *A clear explanation of using linewidths and NOEs to detect binding between a small ligand and a large molecule, this is a dramatic example of using NMR to get important information about biomolecules without the need to do a complete structure determination.*

LIMITATIONS OF NMR FOR STRUCTURE DETERMINATION

Lane, A. N. 1988. The influence of spin diffusion and internal motions on NOE intensities in proteins. *J. Magn. Reson.* 7:425–439. *This article presents an analysis of some of the processes that affect the accuracy of structure determination by NMR.*

Madrid, M., J. E. Mace, and O. Jardetzky. 1989. Consequences of magnetization transfer on the determination of solution structures of proteins. *J. Magn. Reson.* 83:267–278.

Metzler, W. J., D. R. Hare, and A. Pardi. 1989. Limited sampling of conformational space by the distance geometry algorithm: implications for structures generated from NMR data. *Biochemistry.* 28:7045–7052. *A critique of the distance geometry method as applied to NMR data, this article gives examples of some of the problems with using distance geometry and some of the solutions.*

de Vlieg, J., R. M. Scheek, W. F. van Gunsteren, H. J. C. Berendsen, R. Kaptein, and J. Thomason. 1988. Combined procedure of distance geometry and restrained molecular dynamics techniques for protein structure determination from nuclear magnetic resonance data: application to the DNA binding domain of Lac repressor from *Escherichia coli*. *Proteins.* 3:209–218. *This is an example of molecular dynamics as a refinement procedure for distance geometry calculations.*

COMPARISONS WITH X-RAY CRYSTALLOGRAPHY

Wagner, G., S. G. Hyberts, and T. F. Havel. 1992. NMR structure determination in solution: a critique and comparison with x-ray crystallography. *Annu. Rev. Biophys. Biomol. Struct.* 21:167–198. *This comprehensive review cites many specific examples.*