Applications of NMR in drug discovery

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In the half-century since its discovery, nuclear magnetic resonance (NMR) has become the single most powerful form of spectroscopy in both chemistry and structural biology. The dramatic technical advances over the past 10–15 years, which continue apace, have markedly increased the range of applications for NMR in the study of protein–ligand interactions. These form the basis for its most exciting uses in the drug discovery process, which range from the simple identification of whether a compound (or a component of a mixture) binds to a given protein, through to the determination of the full three-dimensional structure of the complex, with all the information this yields for structure-based drug design.

Modern nuclear magnetic resonance (NMR) techniques provide an enormously powerful set of tools with which to study the interaction of small molecules with proteins. Several recent reviews[1–4] also cover this area and there are also reviews of developments in the more strictly chemical applications within drug discovery – for example in the combination of NMR with high-performance liquid chromatography (HPLC), and in the ability to obtain spectra of compounds using solid-phase synthesis resins[5,6]. Only illustrative references will therefore be provided here. Methodological developments are also reviewed elsewhere[7–11], but particularly important in the present context are:

- The use of residual dipolar couplings in proteins partly oriented in solution to provide additional structural information, increasing the quality of structures obtained by NMR (Ref. 12)
- The exploitation of relaxation effects to obtain narrow resonances from large proteins – the TROSY (transverse relaxation optimized spectroscopy) experiment[13], which will extend the applicability of many of the experiments described here to much larger proteins, up to ~150 kDa
- A range of new solid-state NMR experiments that will faciliate the study of ligands bound to membrane proteins[10,11].

In virtually all the applications that will be discussed here, the appropriate experimental design will depend on how tightly the ligand binds or, more precisely, how rapidly it exchanges between the free and bound states[14] (Box 1).

Screening for binding

One of the early questions in the drug design process is, of course, 'does this compound bind to this protein?' While NMR is by no means a high-throughput technique, there are circumstances in which the use of NMR to screen for binding could be useful, especially as a preliminary to a more detailed study. Almost all the NMR parameters of a ligand could change when it binds to a protein, but not all are equally valuable in screening for binding. Thus, scalar coupling constants between nuclei separated by three bonds, which provide information on the dihedral angle about the central bond, could change if the conformation of the ligand changes on binding. However, these effects are almost always too small to be useful. Changes in chemical shift on binding are almost universal, but the magnitude of the change cannot be predicted, as it depends on the detailed structure of the binding site. The most useful

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Box 1. Exchange regimes and isotope labelling

When studying a protein–ligand complex, the appearance of the nuclear magnetic resonance (NMR) spectrum will depend on how tightly the ligand binds or, strictly, on how rapidly it exchanges between the free and bound states (Fig. I). If binding is relatively weak (typically K_D >10 μM), such that the rate of exchange is fast compared with the difference in NMR parameters (chemical shift, relaxation rate, etc.) between the two states, the observed spectrum will be the weighted average of the spectra of the free and bound ligands, and will thus reflect the changes induced by binding. If the change in the NMR parameter is sufficiently large, it will often be possible to see changes in the averaged spectrum of the ligand at protein concentrations low enough that the complex spectrum of the protein does not interfere. However, if the ligand binds tightly and exchanges slowly, separate spectra will be observed for the bound and free ligands, and information concerning binding will only be obtained by observing the spectrum of the bound ligand directly.

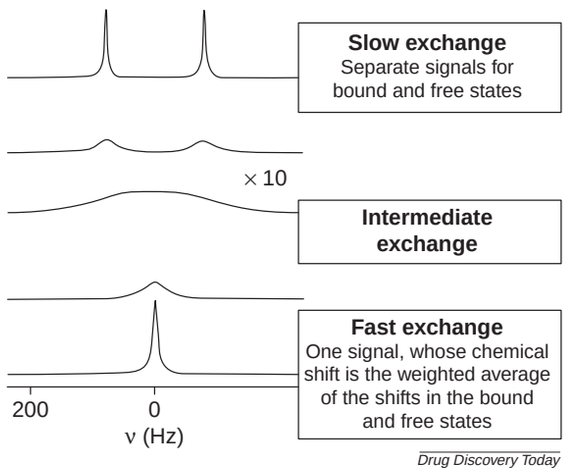

Figure I. *Schematic spectra showing the effects of the rate of exchange of a ligand between free and bound states on the appearance of the spectra.*

The problem of overlap with the protein spectrum can be overcome by the use of labelling with stable isotopes, such as ^{13}C, ^{15}N and/or ^{2}H, together with 'isotope editing' experiments, as illustrated schematically in Fig. II. Isotope labelling of recombinant proteins produced in bacteria or yeast is now routine, and is possible even for proteins produced in mammalian cells; it is almost always easier to label the protein than the ligand. Even when ligand exchange is fast, isotope labelling can often be very helpful.

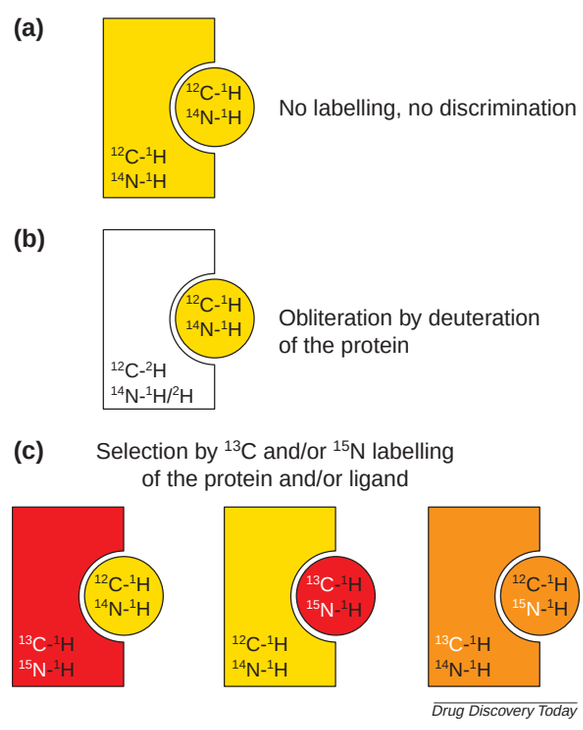

Figure II. *The use of isotope labelling to select signals from either the ligand or the protein when a stoichiometric complex is being observed.*

parameters for use in a binding screen are those that depend on one change that can be predicted with certainty – the translational and rotational diffusion of a ligand bound to a protein will be slower than for the free ligand.

Translational diffusion

The effects on the NMR spectrum of diffusion of molecules through a transient magnetic field gradient set up across the sample can be used to make accurate measurements of translational diffusion coefficients. If a small molecule binds to the protein in fast exchange (see Box 1), its apparent diffusion coefficient will be the weighted average of that in the bound and free states, and the change in apparent diffusion coefficient with concentration can be used to estimate binding constants[15]. For screening purposes, a simpler experiment will often suffice, in which

the field gradient is used to 'edit' the spectrum so as to remove signals from the rapidly diffusing – nonbinding – small molecules, leaving only the signals from the molecules that bind, which can thus readily be identified[16]. This simple and useful experiment has the limitation, common to all experiments carried out under fast exchange conditions, that a sufficiently large proportion of the ligand must be bound for the measured parameter – here the diffusion coefficient – to be clearly different from that of the free ligand. There is therefore a limit to the ligand–protein ratio that can be used.

Rotational diffusion

Rotational diffusion is an important determinant of nuclear relaxation, reflected in the relaxation times T_1 and T_2 (and hence in the resonance linewidth) and, perhaps most usefully, in the ^{1}H-^{1}H nuclear Overhauser effect (NOE). This is familiar, and extremely valuable, as a method for identifying pairs of protons close together in space (see later), but it also depends on the rotational tumbling of the molecule. NOEs in a small, rapidly tumbling, molecule are positive, whereas they are negative in a slowly tumbling molecule such as a protein. Thus, binding of a small molecule to a protein is accompanied by an inversion in sign of NOEs between nuclei in the small molecule. This knowledge has been applied in screening for binding[17], where NOEs were measured from a mixture of ten sugar analogues alone and in the presence of E-selectin, and only one compound showed a change in sign of its NOEs in the presence of the protein, which is indicative of binding. Similarly, Moore and colleagues[18] used either line-broadening effects or the observation of negative NOEs as part of their strategy for the identification of lead compounds by NMR screening of a limited but structurally diverse compound library for binding to a target protein.

Locating the binding site

Having established that a compound binds to the protein of interest, the next question is 'where does it bind?' A simple yet powerful approach to answering this question is that of 'chemical shift mapping'. This requires a knowledge of the structure of the protein (or at least a model based on homology with proteins of known structure), and also the assignment of at least the backbone resonances of the protein. This latter requirement is now not difficult to meet, using ^{13}C and ^{15}N labelling, for proteins up to ~30 kDa, while the additional use of deuteration and the TROSY experiment make it possible to use these techniques for significantly larger proteins[7–9].

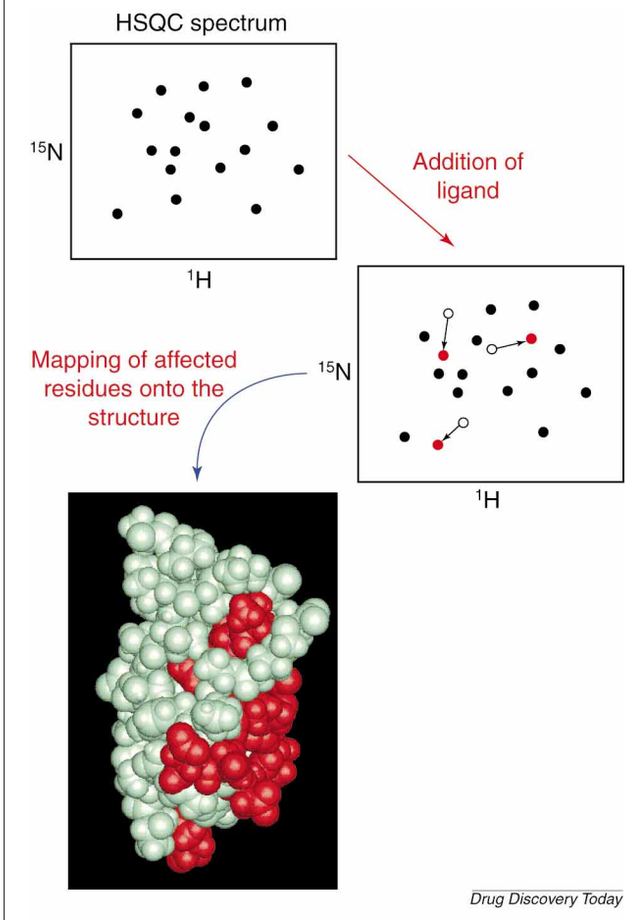

Figure 1. *The principle of 'chemical shift mapping'. Resonances in a nuclear magnetic resonance (NMR) spectrum [usually a ^{1}H-^{15}N homonuclear single quantum correlation (HSQC) spectrum] that are affected by ligand binding are shown on a surface representation of the protein. This commonly defines a 'patch' of surface residues that form the binding site.*

Chemical shift mapping

The concept of chemical shift mapping is illustrated in Fig. 1. In its simplest form, it is based on the ^{1}H-^{15}N HSQC or HMQC (heteronuclear single or multiple quantum correlation) experiments, which yield a two-dimensional spectrum containing one cross-peak for each amide in the protein, at a position characterized by its ^{1}H and ^{15}N chemical shifts. The spectrum therefore contains one signal for each residue except prolines, with additional signals from the side-chain amides of asparagine and glutamine. On addition of the ligand, the signals of those amides whose environments are changed by ligand binding will change position. If the ligand binds relatively weakly, in fast exchange, addition of increasing concentrations of the

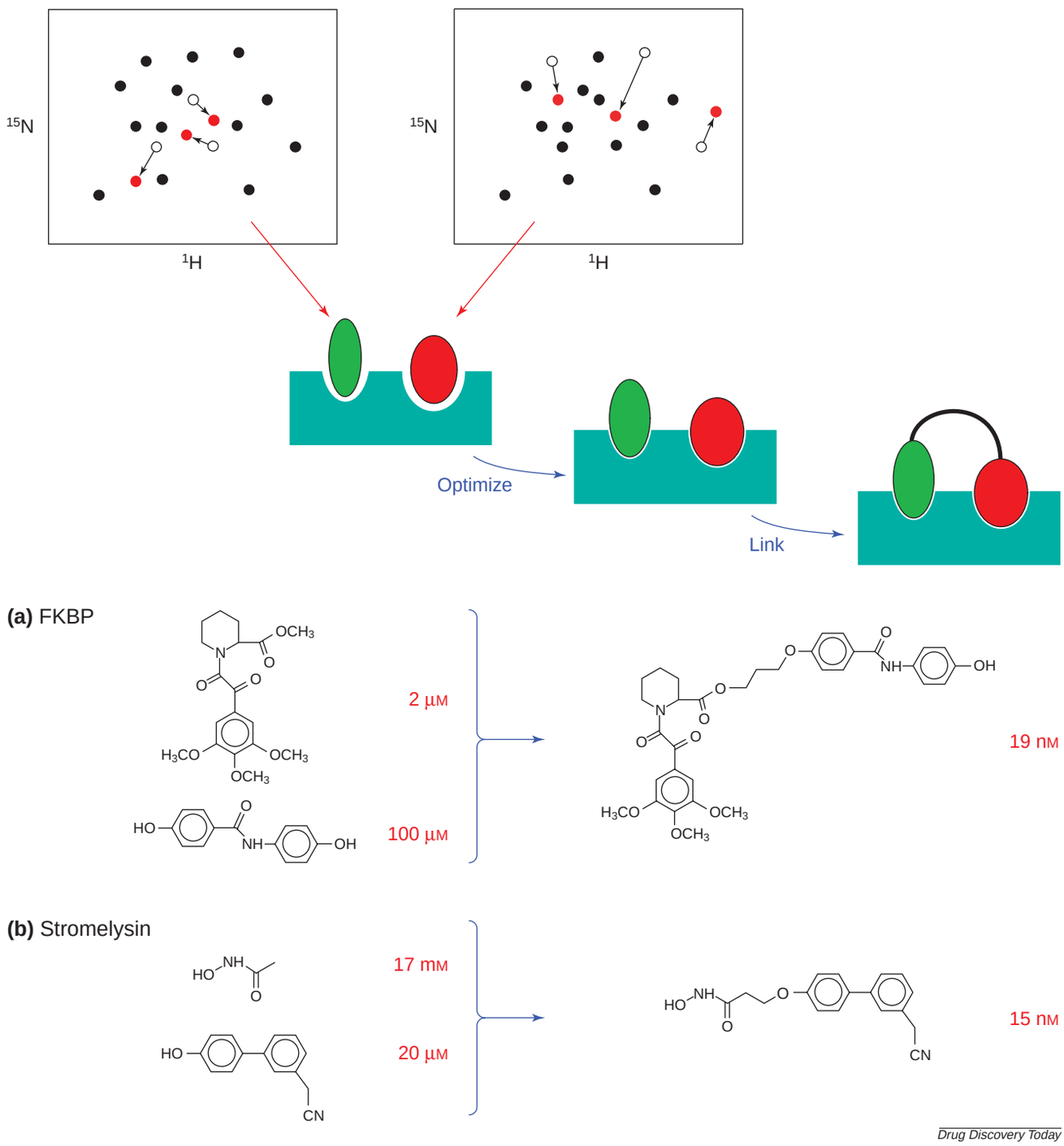

Figure 2. *The principle of structure–activity relationships (SAR) by nuclear magnetic resonance (NMR). The binding sites of two, relatively weakly binding, ligands are defined by chemical shift mapping, and the binding to those sites is optimized. On the basis of knowledge of the relative positions of the two sites, a linker is designed to join the two ligands, yielding a molecule with a much higher affinity. The examples of ligands and their binding affinities (before and after linking) shown in parts (a) and (b) are from the work of Fesik and colleagues[25–27].*

ligand will lead to progressive shifts of the resonances, so that each amide signal can be followed from its position in the free protein to its position in the complex. For tight binding (slow exchange), affected residues will be characterized by the disappearance of the signal from the free protein and the appearance of a signal from the complex. In either event, it is possible to identify from the spectrum all the amide groups whose environment is affected by ligand binding. These will include groups both in residues that make contact with the ligand and in residues that are affected indirectly by ligand-induced changes in protein structure. Although these groups cannot be distinguished on the basis of this experiment, in practice, if the affected residues are mapped onto the protein structure, a clear surface patch of affected residues is generally observed, and this indicates the location of the binding site (Fig. 1).

This approach has been widely used to study both protein–ligand and protein–protein interactions[19–24]. It is generally simple and inexpensive to prepare ^{15}N-labelled protein, and ^{1}H-^{15}N HSQC or HMQC experiments require only a few minutes. This technique therefore provides a rapid and convenient method for the identification of binding sites.

SAR by NMR

An ingenious extension of chemical shift mapping has been developed by Fesik and colleagues and dubbed 'SAR (structure–activity relationships) by NMR'[25–27]. The principle of this method is illustrated in Fig. 2.

^{1}H-^{15}N HSQC spectra are used to screen a library of small molecules to identify molecules that bind to two distinct sites; by combining the rapid HSQC experiment with initial screening of 'batches' of, for example, ten compounds at a time, it is possible to screen several hundred compounds per day. When these 'lead' molecules have been identified, analogues are synthesized and tested to optimize the affinity at the two sites. As only relatively weakly binding compounds are involved at this stage, both the binding constants and the mode of binding can be determined from the HSQC experiment, providing important guidance to the developing SAR. Once two ligands with reasonable affinity have been identified, knowledge of the protein structure, and hence the relative positions of the two sites, is used to design a 'linker' to combine the two relatively weakly binding molecules into one that binds much more strongly. The substantial increases in affinity that can be obtained in this way are illustrated in Fig. 2.

The 'SAR by NMR' method provides a clear demonstration of the value of rapidly available, if limited, structural information from chemical shift mapping, not only in designing the linker in the latter stages of the process, but also in guiding the optimization of the affinity of the lead molecules. The latter is perhaps the most generally applicable use of chemical shift mapping in ligand design and has the potential to make a valuable contribution, provided that the necessary investment of time in obtaining isotopically labelled protein and making resonance assignments is made at an early stage in the project.

Location of binding sites by distance measurements

Much more precise identification of binding sites, in terms of distances between atoms of the protein and those of the bound ligand, are provided by intermolecular NOEs and by paramagnetic relaxation experiments. Because these methods yield interatomic distances, they can be used not simply to locate the binding site but also to 'dock' the ligand into a known or modelled protein structure to obtain a model of the complex. The use of intermolecular NOEs is a particularly powerful approach, and is discussed below in the context of determination of the conformation of the bound ligand and the three-dimensional structure of the protein–ligand complex.

Paramagnetic relaxation experiments are, of course, limited to proteins that have a natural paramagnetic centre such as a transition metal (haem proteins are an obvious example) or that have been specifically labelled with a nitroxide free radical. They depend on the fact that relaxation by the unpaired electron of the paramagnetic centre dominates over other sources of relaxation, and is proportional to the inverse sixth power of the distance between the paramagnetic centre and the nucleus being observed. It is therefore possible to position a ligand bound to a haem protein, for example, quite precisely with respect to the haem Fe. This kind of NMR experiment has a long history[28]; interesting recent examples include studies of substrate binding to cytochrome P450 enzymes[29–32]. It is important to bear in mind that the experiments yield only distance, not angular information. Thus, if they are to be used to compare the binding positions and orientations of a series of ligands, additional information is required. In a recent study of substrate binding to cytochrome P450 2C9, this came from the postulate that the anionic centres of the substrates interacted with a proposed positive charge on the protein[31]. Alternatively, the NMR distances can be used to dock the ligand into a protein structure, the necessary additional information coming from the structural constraints of the binding site[29]. In the case of human cytochrome P450 enzymes, the protein structure is not yet available, and a method has been described in

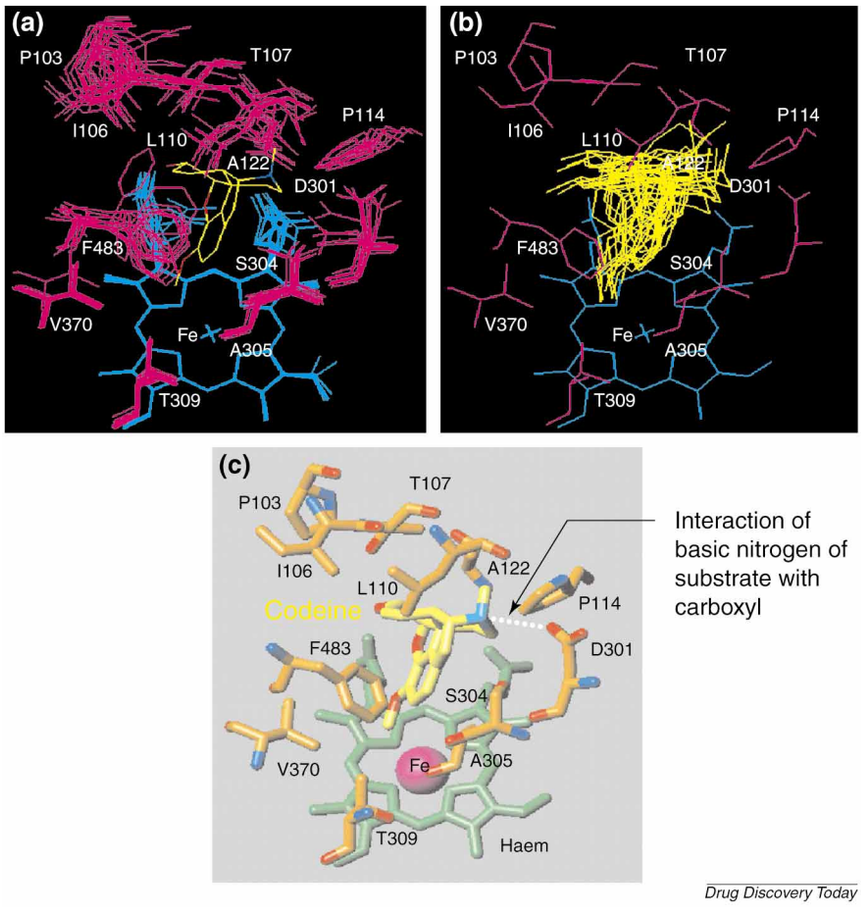

Figure 3. *Determination of the mode of binding of codeine to the active site of cytochrome P450 2D6. Paramagnetic relaxation experiments were used to determine distances from the haem Fe to protons of the bound codeine, and this was combined with homology modelling to obtain models of the enzyme–codeine complex. The calculations yield a set of 13 models consistent with the data (top); for clarity, these are shown (a) with a single structure for codeine and the 13 structures for the amino acid residues in the binding site, and (b) with the 13 structures for codeine and a single structure for the amino acid residues. (c) From this family of structures, a representative structure can be obtained. For details, see Ref. 30.*

which the NMR distances are combined with homology modelling to obtain a structural model of the enzyme–substrate complex[30], as illustrated in Fig. 3.

Conformation of the bound ligand

When the lead compound is conformationally flexible, a knowledge of its conformation when bound to the target molecule can provide valuable information to guide the synthesis of analogues. The best-known NMR approach to determining interatomic distances is the NOE. To use this method to determine the ligand conformation, it is necessary to be able to measure intramolecular NOEs in the bound ligand specifically, in the presence of all the resonances from the protein. As already noted, the design of the experiment required for this will depend on the rate of exchange of the ligand between the free and bound states. If the ligand binds tightly, then isotopic labelling, most easily of the protein, will be required to distinguish their resonances (see Box 1). [^{13}C,^{15}N]-labelled protein can be used in combination with appropriate 'editing' pulse sequences to observe only NOEs between protons that are not attached to ^{13}C or ^{15}N, or alternatively per-deuterated protein can be used. However, if the exchange is fast (expected for K_D values >1–10 μM), the NOEs can be measured from the averaged spectrum of the free and bound ligand. Because the NOEs of the bound ligand have a different sign and a much larger magnitude than those of the free ligand, these measurements can be made with concentrations of ligand in excess of the enzyme concentration.

The transferred NOE

The measurement of NOEs under fast exchange conditions, known as transferred NOEs, has been widely used to determine the conformation of bound ligands[33–35]. In some cases, simple qualitative observation of transferred NOEs can provide useful conformational information – for example, in distinguishing between syn- and anti-type conformations of the glycosidic bonds of nucleotides. More commonly, particularly if a complete description of the conformation of the bound ligand is required, a quantitative analysis of the observed NOEs is necessary. This requires some care to ensure that the observed NOE intensities are not affected by the exchange rate (i.e. that the exchange rate is very fast) or by contributions from indirect magnetization transfer (so-called 'spin diffusion'). If the NOE between two protons is to be safely used as evidence that they are close together in space, it is obviously important to be sure that the magnetization is transferred directly between them, and not via one or more

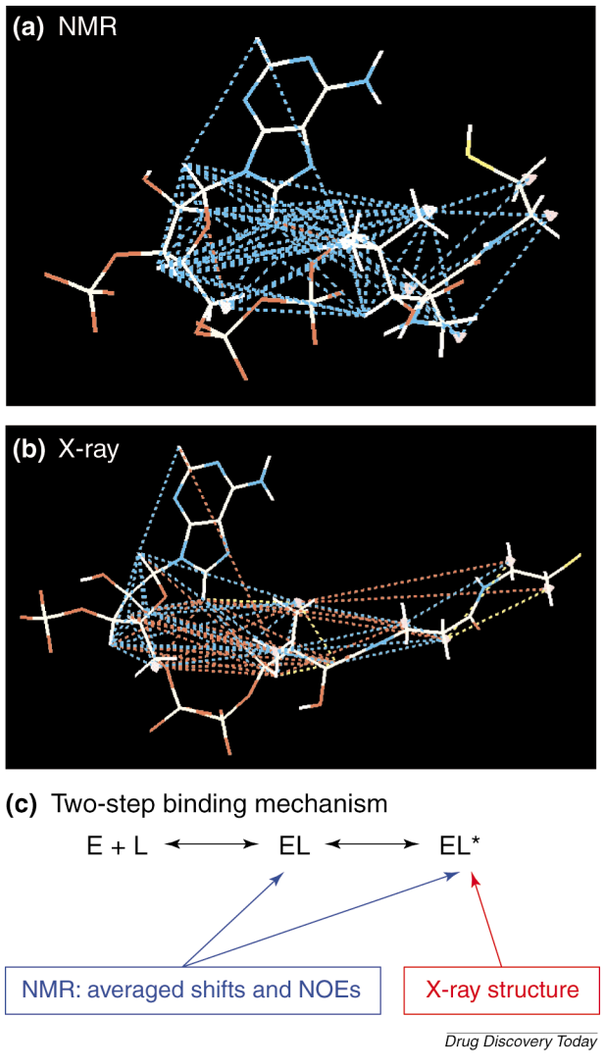

Figure 4. *Determination of the conformation of coenzyme A bound to chloramphenicol acetyltransferase. Comparison of the conformation determined by (a) nuclear magnetic resonance (NMR) and (b) X-ray crystallography shows that the latter is inconsistent with several of the observed ^{1}H-^{1}H nuclear Overhauser effects (NOEs; blue indicates distances consistent with NOEs; red indicates distances inconsistent with NOEs). This apparent discrepancy can be resolved by postulating a two-step binding mechanism (c). For details, see Ref. 35.*

other protons. It is straightforward to establish that the fast exchange condition is satisfied[33,35], and any residual exchange effects can be allowed for by analysis using a combined relaxation and exchange matrix approach[33,35].

Eliminating spin diffusion effects can be more difficult. Spin diffusion within the bound ligand can be allowed for by using a full relaxation matrix. By contrast, effects involving protein protons cannot be dealt with in this way, as the positions of these protons are generally not known. Experimental approaches for dealing with this problem include the combined use of NOEs and rotating-frame NOEs (Ref. 36) to identify spin diffusion effects, and the use of per-deuterated protein[35] or of QUIET-NOESY (Ref. 37) (a technique based on the use of selective pulses) to eliminate them. Several recent papers illustrate the type of information that can be obtained[38–42].

It is important to recognize that the results of transferred NOE experiments, like those of any fast exchange experiment, depend on the correctness of the kinetic model that is (often implicitly) used to interpret the data. The possibility of nonspecific binding can be tested by repeating the experiment in the presence of a high-affinity competing ligand, if one is available. It is commonly assumed that ligand binding is a simple one-step process, E + L ↔ EL and, thus, that the information obtained concerning the ligand conformation relates simply to the EL complex. However, if in fact the kinetic model is even slightly more complex, for example E + L ↔ EL ↔ EL*, but the data are analyzed in terms of the simpler model, erroneous conclusions could be drawn. Under fast exchange conditions, the NMR data for the bound state will be a weighted average of those for EL and EL*, and the bound conformation calculated from them might not correspond to any real state. Several examples have been reported in which the data are not easily reconciled with the simple one-step kinetic model[35,43,44]. For example, transferred NOE studies of the binding of coenzyme A to chloramphenicol acetyltransferase[35] (the enzyme responsible for bacterial resistance to chloramphenicol) revealed that the measured NOEs were not consistent with the conformation of the molecule in the same complex, as determined by X-ray crystallography (Fig. 4). The data could be reconciled by assuming a two-step binding process, in which the ligand conformation differed in the two-states EL ('folded', resembling the structure calculated from the NOEs) and EL* ('extended', more like the crystal structure); for details, see the original paper[35].

Ligands bound to membrane proteins

Recent developments in NMR methods applicable to the solid state have made it possible to extend studies of the conformation of bound ligands to membrane protein systems[3,11]; specific isotopic labelling is crucial for these experiments. Two kinds of information can be obtained. Firstly, the so-called dipolar recoupling experiments provide internuclear distances, for example between two ^{13}C labels, and have been used to determine the conformation

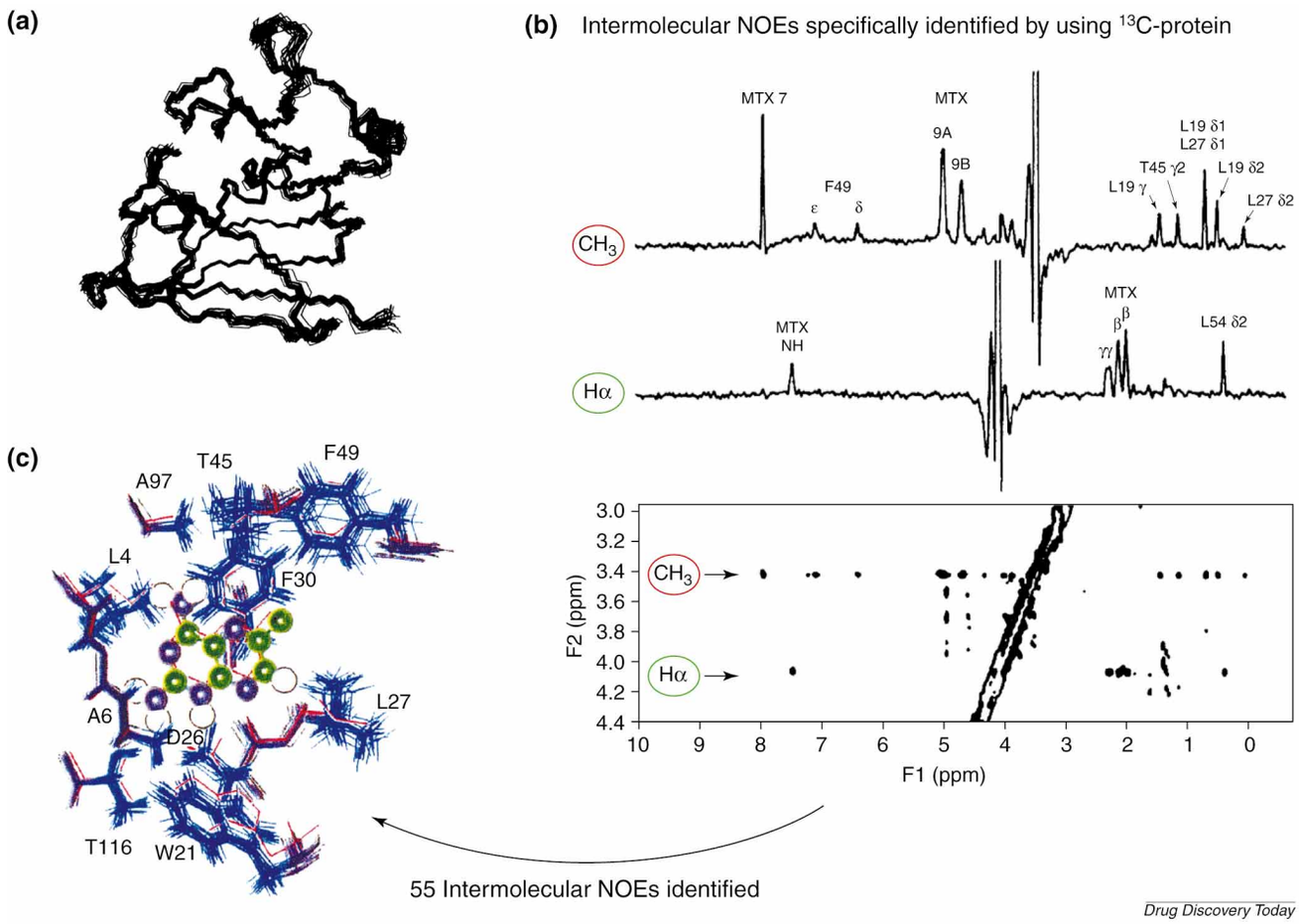

Figure 5. *Determination of the solution structure of the complex of dihydrofolate reductase with methotrexate. (a) The overall structure of the complex is shown as a backbone trace, together with (b) a close-up of the binding of the pteridine ring of the ligand. As in Fig. 3, an ensemble of structures consistent with the data are shown. (c) The spectra illustrate some of the intermolecular ^{1}H-^{1}H nuclear Overhauser effects (NOEs) important in defining the ligand binding. By using ^{13}C-labelled protein in conjunction with 'editing' nuclear magnetic resonance (NMR) experiments, it is possible to observe selective NOEs involving ^{12}C-H (ligand) protons; examples are shown in two- and one-dimensional formats for the N-CH_3 and $C_\alpha H$ of methotrexate, with NOEs to other protons of methotrexate and to protons of the protein. For details, see Ref. 51.*

of an inhibitor bound to the gastric H^+/K^+-ATPase[45] and the conformation of retinal bound to rhodopsin[46]. Secondly, in membrane systems oriented with respect to the magnetic field, quadrupolar nuclei, notably deuterium, can be used to obtain precise information on the orientation of individual C-^{2}H bond vectors with respect to the bilayer, and this has been used to build up a picture of retinal conformation in bacteriorhodopsin and rhodopsin, and the way in which this changes on illumination[47].

The structure and dynamics of the complex

The most detailed information can of course be obtained by determining the full three-dimensional structure of the drug–target complex. This requires essentially complete assignment of the resonances of the protein, which is usually readily achieved, at least for proteins up to ~35 kDa. A variety of NMR experiments are then used to obtain structural constraints, and 'families' of structures are calculated that are consistent with these constraints. Clearly, the larger the number of structurally informative constraints the better defined the structure. The constraints traditionally used have been short-range ones – the ^{1}H-^{1}H NOE, which identifies pairs of protons close together in space (usually within 5Å), and the three-bond scalar coupling, which provides information on the dihedral angle around individual bonds. However, the recent use of residual

dipolar couplings provides additional longer-range structural information that promises to improve the quality of the structures significantly. Descriptions of the structure determination process are given elsewhere[7–9,48,49].

In determining the structure of a protein–ligand complex, where the exact mode of binding of the ligand is of crucial interest, the observation of intermolecular NOEs between protons of the bound drug and protons of the protein is particularly important. (Where the structure of the protein is already known, intermolecular NOEs alone can be used to 'dock' the ligand into the binding site[50].) Depending on the affinity of the ligand, these NOEs can be measured either directly or by means of the transferred NOE experiment although, in general, direct measurements on stoichiometric complexes are to be preferred. Intermolecular NOEs can be selectively observed by using the 'editing' pulse sequences of the kind mentioned above, for example to select for NOEs between a proton attached to ^{13}C or ^{15}N (from the protein) and one attached to ^{12}C or ^{14}N (from the ligand).

As an example, Fig. 5 shows the structure of the complex between dihydrofolate reductase and the anti-cancer drug methotrexate[51]. In this case, 55 intermolecular NOEs were identified, permitting a detailed picture of the mode of binding of the inhibitor in solution. The overall backbone structure of the protein is shown, together with a more detailed picture of the residues surrounding the diaminopteridine ring of the inhibitor. In both cases, the structures are shown as a superposition of a family of structures consistent with the NMR constraints. Both at the level of the backbone and for individual side-chains, it is possible to see regions that are 'less well determined' than others, that differ more than the average between members of the ensemble of structures. A recent analysis provides evidence that these 'ill-defined' regions in the NMR-derived ensemble do correspond to regions undergoing relatively large-amplitude low-frequency motions[52] and, in practice, the regions of the structure that are poorly determined often also show NMR behaviour characteristic of internal motion.

Dynamics

One of the strengths of NMR spectroscopy is its ability to provide a detailed picture of the dynamics of proteins and their complexes with ligands[53]. NMR relaxation experiments can qualitatively demonstrate nanosecond mobility without ambiguity, although detailed description of its nature and frequency is more difficult[53]. Molecular motion in the binding site is of fundamental importance for the kinetics of association and dissociation, and for determining the structural range of ligands that can bind. In this context, motions in the microsecond–milliszxxecond range are probably more important than those in the nanosecond range[54]. Rotating frame relaxation measurements afford a direct approach for measuring dynamics on the microsecond–millisecond timescale[53,55] while, in favourable instances, lineshape analysis enables both rate measurement and characterization of the nature of the motion. An interesting recent example of the latter is the correlated motion of an arginine guanidino group and a ligand carboxylate involved in an ion-pair between methotrexate and dihydrofolate reductase[56].

Several examples have been reported of proteins in which the ligand-binding site is among the most mobile parts of the structure, and becomes more rigid when the ligand binds. Thus, in the intestinal fatty-acid binding protein[57–59], local regions of the structure are less well defined than the remainder and appear by several criteria to be mobile; on the addition of ligand, these regions become structurally better defined and less mobile. One function of this localized mobility could be to allow access of the ligand to a rather deep binding pocket. In different proteins, some residues involved in ligand binding are found to be already restricted in the unligated protein, others are mobile and become restricted only when the ligand binds, and still others show increases in mobility of some residues on complex formation[60–65]. The entropy changes resulting from these changes in local mobility[64–66] could be significant in relation to the overall free energy of binding, and an interesting analysis of the relationship between binding-site mobility and binding energy has been made in the case of phosphopeptide binding to *src*-homology 2 (SH2) domains[64]. In cases where the residues that are mobile are directly involved in ligand binding, a degree of active site mobility could be required to enable the protein to bind a range of ligands, and a knowledge of this mobility could be exploited in the design of new structurally diverse ligands.

Other dynamic processes that are significant in the context of drug design can also influence NMR spectra, notably tautomeric and ionization equilibria. These can only be inferred from X-ray crystallography, as they involve the determination of the position of hydrogen atoms, but can often be studied much more directly by NMR, as illustrated by studies of substrate and inhibitor binding to dihydrofolate reductase[67–69].

Conclusions

The diversity of applications of NMR to drug design described in this review parallels the range of its applications to protein structure in general, and is a testament to the contributions that NMR can make to the drug

discovery process. Screening experiments are relatively quick and, for weakly binding ligands, require minimal quantities of protein. The types of experiments that provide detailed information on the complex are much more informative, but are also more demanding in terms of protein production. However, even for these experiments, developments in protein expression systems mean that it is commonly straightforward to obtain soluble proteins in the quantities required and with the isotope labels needed. For all but the smallest protein, the assignment of the resonances of the protein can be the rate-limiting step. However, if this is undertaken at the very beginning of a programme, the subsequent study of the binding of natural ligands and of lead compounds is generally simple, and detailed structural and dynamic information can be obtained very quickly. The use of solid-state NMR methods to obtain information for drug design is still at a relatively early stage but, with the parallel developments in the expression of membrane proteins, holds out considerable promise for the future. Continuing methodological advances across the range of NMR will improve the precision of the structural information obtained and will extend the range of applicability of the methods, leading to an expansion in the use of NMR as a valuable component in the armoury of the drug designer.

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Collaboration…

Oxford GlycoSciences (OGS; Oxford, UK) has entered into a collaboration with **Bayer AG** (Leverkusen, Germany) to identify disease-associated proteins that are potential targets for the treatment of respiratory diseases, as well as identify clinical markers of disease for use in drug development. Under the terms of the agreement, Bayer has agreed a funding programme for OGS for initially two years, with performance-related milestone payments and royalties to OGS on products developed from the collaboration by Bayer. The two companies will jointly own any intellectual property and OGS will have exclusive rights to commercialize any diagnostic markers arising from the collaboration. Michael Kranda, CEO of OGS, said, 'There is a real need for new therapies in the area of respiratory disease and an opportunity for new franchises to be built. With genome closure now approaching, proteomics is the key technology to leverage gene data.'