Table 12.1 NMR Parameters and Their Applications in Drug Design/Discovery

Parameter	Information Provided Relevant to Drug Design
Chemical shift	Reflects local chemical environment; provides a fingerprint marker of structure (particularly in HSQC spectra)
Coupling constants	Conformational analysis, establishing molecular connectivity
Nuclear Overhauser effect	Determining interproton distances, three-dimensional structures
Relaxation times	Molecular dynamics
Line-shape	Detecting and quantifying chemical exchange processes
Peak intensities	Reflect relative number of nuclei, molecular symmetry
Amide exchange rates / temperature coefficients	Hydrogen bonding or solvent exposure of amide protons

clear that spectral overlap can potentially be a major problem for anything but the simplest of molecules. The development of higher field NMR spectrometers, which effectively provide greater dispersion in the frequency dimension, has contributed significantly to overcoming this limitation and increasing the application of NMR for studying pharmaceutically relevant molecules. In addition to such instrumental developments, methodological advances have also played a key role in extending the use of NMR. Multidimensional NMR methods have revolutionized biomolecular NMR spectroscopy by removing the limitations of a single frequency dimension, leading to the development of 2D, 3D, and 4D spectra.

A simple way of illustrating multidimensional NMR is through reference to heteronuclear correlation spectroscopy, in which two or more separate frequency dimensions are correlated with one another. For example, a particularly valuable 2D experiment is ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC) spectroscopy, in which the resultant spectrum has two frequency axes, corresponding to ^{1}H and ^{15}N frequency dimensions, and one intensity axis. Analogous ^{1}H-^{13}C HSQC spectra are also widely used. Such spectra are normally represented with the intensity axis in contour form so that they may be drawn in two dimensions as a set of contour peaks. Spectral peaks occur for pairs of $^{15}N/^{1}H$ or $^{13}C/^{1}H$ nuclei that are directly bonded to one another, and with each frequency being characteristic for the local chemical environment they represent a relatively simple, but highly characteristic fingerprint of the sample. Figure 12.3 shows the relationship between 1D and 2D spectra for the immunosuppressive drug cyclosporin, and includes a region of both the $^{1}H/^{15}N$ and $^{1}H/^{13}C$ HSQC spectra. In HSQC spectra-overlap problems are alleviated because, even if two protons have the same chemical shift and would hence be overlapped in a 1D spectrum, chances are that the respective heteronuclear signals will not be overlapped, allowing the signals to be resolved in the 2D spectrum. HSQC spectra are widely used in NMR-based drug screening and we will return to them later.

Multidimensional NMR spectra are not restricted to cases where the separate frequency axes encode signals from different nuclear types. Indeed, much of the early work on the development of 2D NMR was performed on cases where both axes involved ^{1}H chemical shifts. The main value in such spectra comes from the information content in cross peaks between pairs of protons. In COSY-type spectra (COSY = Correlation SpectroscopY) cross peaks occur only between protons that are scalar coupled (i.e., within 2 or 3 bonds) to each other, whereas in NOESY (NOE Spectroscopy) spectra cross peaks occur for protons that are physically close in space (<5 Å apart). A combination of these two types of 2D spectra may be used to assign the NMR signals of small proteins and provides sufficient information on internuclear distances to calculate three-dimensional structures. Figure 12.3 includes a panel showing the COSY spectrum of cyclosporin and highlights the relationships between 1D ^{1}H-NMR spectra and corresponding 2D homonuclear (COSY) and heteronuclear (HSQC) spectra.

Homonuclear 2D spectra are generally applicable for the study of proteins up to only approximately 80 amino acids in size. For