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Applications of NMR screening in the pharmaceutical industry

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A major role of NMR in drug discovery over the past 10 years has mainly been in the areas of protein structure determination and the conformation of a bound ligand [1]. While this area is still being heavily investigated, there is a new paradigm beginning to take hold, NMR as a ligand screening tool, which has been termed 'affinity NMR' [2]. This term can be defined as 'the utilization of an NMR observable parameter, to detect a ligand–receptor interaction'. The nature of these 'observables' includes relaxation, chemical shift perturbations, inter-ligand NOE, transfer NOE, PFG diffusion weighted NMR, saturation transfer, NOE pumping and spin label effects.

The use of relaxation as a probe of ligand binding was described some time ago, but its utility as a tool for drug design has only recently been exploited [3]. It is one of the most frequently used NMR experiments for ligand binding studies. The interpretation of the data is straight forward as seen in Fig. 1. A mixture of potential ligands is recorded in the absence and presence of the target protein.

The chemical shift perturbation method 'SAR by NMR' has received much interest [4,5]. Using ^{15}N labeled protein the ligand interaction is seen as a shift in the HSQC spectrum as shown in Fig. 2.

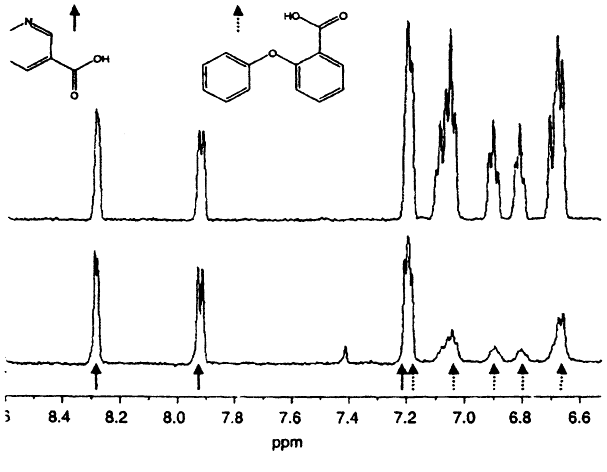

Fig. 1. Relaxation experiement using spin-echo pulse sequence. Dotted lines indicate where a reduction in signal intensity is observed.

The inter-ligand NOE [6] and transfer NOE [7] experiments are interrelated in that the observation of binding of the small molecule is observed by its phase difference with the diagonal in a NOE spectrum. The inter-ligand NOE has the advantage of relaying information concerning the distance between two ligands which are simultaneously bound.

PFG diffusion NMR experiments to observe binding events have recently been shown to be a useful deconvolution tool [8,9]. The concept behind these studies involves the translation motion of a bound ligand being reduced by comparison to compounds not bound, by reducing the translational motion [10]. This experiment can be performed either in a one or two dimensional version called DECODES [11]. The DECODES experiment can be used either to identify bound ligands or to determine binding constants.

The NOE pumping experiments were developed to alleviate some concerns resulting from diffusion experiments [12,13]. These experiments only detect binding ligand signals transferred from target macromolecules or from the ligand to the macromolecule (inverse pumping) by NOE shown in Figs. 3 and 4.

Saturation transfer experiments (STD) have also been quite useful in determination of a bound ligand to a receptor [14–16]. These experiments utilize the magnetization transfer from the protein to the ligand or from the ligand to the protein. In the experiment proposed by Meyer and coworkers, i.e. the steady-state NOE experiment, a difference spectrum is generated from a spectrum recorded with saturation of a protein resonance (or resonances) and a normal spectrum (with off-resonance saturation). A variant of this experiment uses the large reservoir of bulk H_2O magnetization [17]. It is possible to detect via different transfer mechanisms small molecules that interact with a target protein, DNA or RNA fragments.

Recently the application of spin labels has been applied to the problem of orientation of bound frag-

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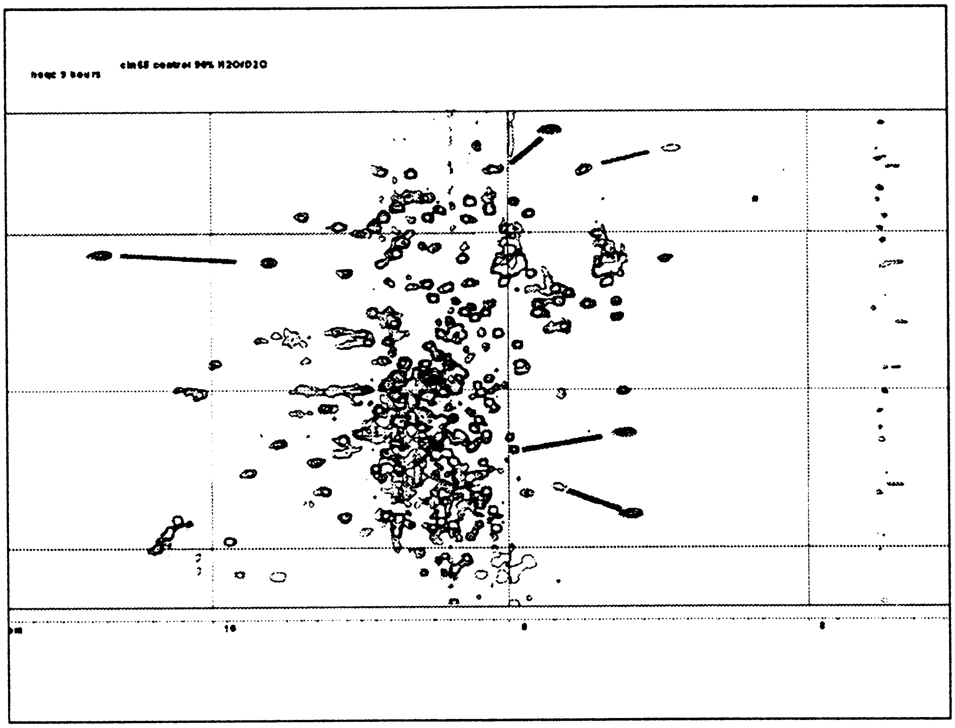

Fig. 2. ^{15}N HSQC spectrum for a protein showing the chemical shift perturbation caused by ligand binding indicated by the broad lines.

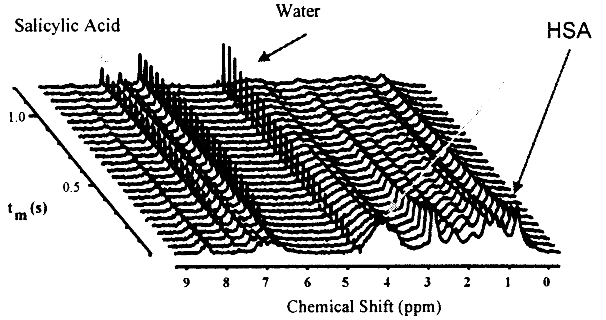

Fig. 3. Stack plot of 1D ^{1}H spectra of HSA with three ligands as the mixing time t_m increases (from 5 ms to 1.2 s in intervals of 0.05 s) at a temperature of 300 K, where $g = 34.8$ g/cm, $\delta = 3$ ms, $T = 50$ ms, $\tau = 3.5$ ms, 512 scans were collected, and the experiment time for each spectrum is 30 to 40 min. Signals from bound ligand salicylic acid and water are indicated.

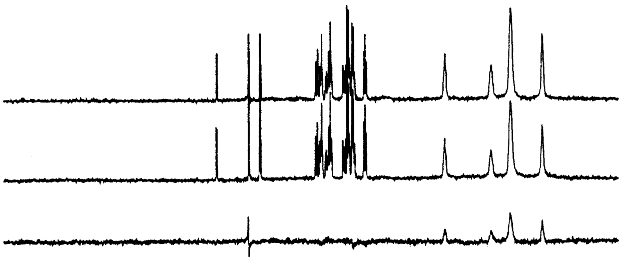

Fig. 4. 1 mM octanoic acid, 1 mM glucose and 20 µM HSA in D_2O: (top) reference spectrum; (middle) RNP spectrum obtained with Fig. 1a; (bottom) difference spectrum of the above two spectra. In each spectrum, 16 transients were accumulated and the experiment time was around 2 min.

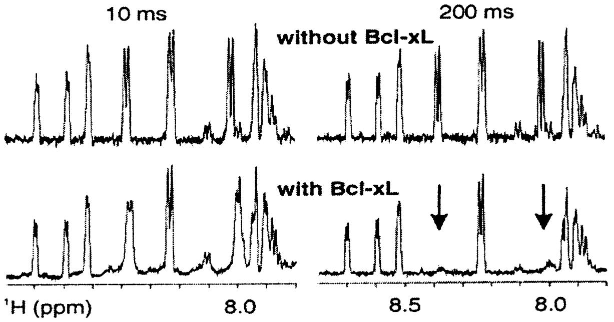

Fig. 5. ^{1}H spectra of a library of eight aromatic compounds in the presence of spin-label **1***, and in the absence (upper trace) and presence (lower trace) of Bcl-xL. The active compound **2** can be easily identified since its two resonances (arrows) are broader and vanish in the presence of Bcl-xL.

ments [18]. This experiment shown in Fig. 5, when applicable, promises to be a powerful drug design tool.

The one dimensional NMR methods can be applied to macromolecules without size limit, without well resolved signals and without isotope labeling. They are applicable to weakly to modestly bound ligands and the results can be obtained relatively fast and the process can be automated.

References

[1] B.J. Stockman, Prog. Nucl. Magn. Reson. Spectrosc. 33 (1998) 109–151.

[2] J.M. Moore, Anal. Biotechnol. 10 (1999) 54–58.

[3] P.J. Hajduk, E.T. Olejniczak, S.W. Fesik, J. Am. Chem. Soc. 119 (1997) 12257.

[4] S.B. Shuker, P.J. Hajduk, R.P. Meadows, S.W. Fesik, Science 274 (1996) 1531–1534.

[5] P.J. Hajduk, D.G. Sheppard, D.G Nettesheim, E.T. Olejniczak, S.B. Shuker, R.P Meadows, D.H. Steinman, G.M. Carrera, P.M. Marcotte, J. Severin, K. Walter, H. Smith, E. Gubbins, R. Simmer, T.F Holzman, D.W. Morgan, S.K. Davidsen, J.B. Summers, S.W. Fesik, J. Am. Chem. Soc. 119 (1997) 5818–5827.

[6] R.E. London, J. Magn. Reson. 141 (1999) 301–311.

[7] B. Meyer, T. Weimar, T. Peters, Eur. J. Biochem. 246 (1997) 705–709.

[8] M. Lin, M.J. Shapiro, J.R. Wareing, J. Am. Chem. Soc. 119 (1997) 5249–5250.

[9] A. Chen, M.J. Shapiro, J. Am. Chem. Soc. 120 (1998) 10258–10259.

[10] P. Stilbs, Prog. Nucl. Magn. Reson. Spectrosc. 19 (1987) 1.

[11] M. Lin, M.J. Shapiro, J.R. Wareing, J. Org. Chem. 62 (1997) 8930–8931.

[12] A. Chen, M.J. Shapiro, J. Anal. Chem. 71 (1999) 669–675.

[13] A. Chen, M.J. Shapiro, J. Am. Chem. Soc. 122 (2000) 414–415.

[14] D. Henrichsen, B. Ernst, J.L. Magnani, W.-T. Wang, B. Meyer, T. Peters, Angew. Chem. Int. Ed. Engl. 38 (1999) 98–102.

[15] M. Mayer, B. Meyer, Angew. Chem. Int. Ed. Engl. 38 (1999) 1784–1788.

[16] J. Klein, R. Meinecke, M. Mayer, B. Meyer, J. Am. Chem. Soc. 121 (1999) 5336–5337.

[17] C. Dalvit, P. Pevarello, M. Tatò, A. Vulpetti, M. Sundström, private communication.

[18] W. Jahnke, L.B. Perez, C.G. Paris, A. Strauss, G. Fendrich, CM. Nalin, J. Am. Chem. Soc. 122 (2000) 7394–7395.