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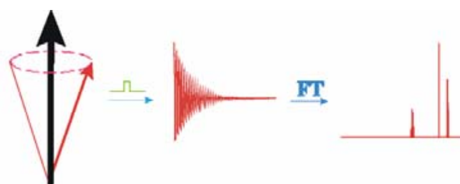

Probing Ligand-Receptor Interactions with NMR

Introducing the Saturation Transfer Difference (STD) Experiment

The detection and understanding of ligand-receptor binding interactions is of paramount importance in understanding biological function. A number of NMR techniques have been developed to (a) detect binding activity and (b) provide structural information on both the binding pocket of the receptor as well as the ligand's binding epitope. Which experiment(s) to use is a multi-faceted question, depending upon many factors such as the affinity of the ligand towards the receptor (size of K_D) as well as the solubility and molecular weight of both ligand and receptor to name but a few. One of the more versatile and robust experiments in this area is known as the saturation transfer difference (STD) experiment (Mayer and Meyer *Angew. Chem. Int. Ed.* **1999**, *38*, 1784-1788). STD can rapidly detect the presence of binding equilibria and also yields information on the ligand binding epitope. It can easily be concatenated with 2D sequences such as HSQC, TOCSY, or NOESY to aid further in understanding the structure of the complex. As a result of these features, STD has found widespread use as a fast and sensitive screening method for binding affinities in mixtures, useful for example in generating leads in the area of drug-discovery.

The STD experiment is performed by first selectively saturating a narrow frequency region of receptor proton resonances where ligand resonances are absent. Due to the typically large molecular weight, spin diffusion is able to "spread" this saturation throughout the receptor spectrum, including the key area of the binding pocket. If binding activity is present, the saturation is transferred to the bound ligand which in turn takes this information into solution for detection once it dissociates. Since the ligands are typically small molecules with long relaxation times, the saturation information is able to persist for a time during which "new", previously unsaturated ligand molecules can also bind with the saturated receptor, increasing the population of saturated ligands in solution. This process is illustrated in Figure 1. If one runs a reference spectrum where an identical saturation train is applied remotely from receptor and ligand resonances and subtracts, the difference is the STD spectrum showing only those signals that have been saturated (receptor + binding epitope). Proton signals from non-binders or those within binding molecules but far removed from the binding pocket are cancelled in the difference spectrum. An example of the STD experiment obtained on our Bruker AV-500 is shown in Figure 2. The mixture used was 0.2 mM human serum albumin with a 50-fold molar excess of both L-tryptophan (binder; K_D = 23 μM at 20° C and pH 7) and sucrose (non-binder) in D_2O. Spectra **1** and **2** are ^1H spectra of sucrose and tryptophan, respectively whereas **3** is the ^1H spectrum of the mixture. Spectra **4**, **5** and **6** are STD results using different versions of the experiment, each clearly showing strong tryptophan signals due to the presence of a tryptophan-albumin complex whereas sucrose signals are absent (compare sharp signals in **4, 5, 6** with **2**). Spectrum **4** is the most basic version of STD showing signals from all protons saturated, including protein. Spectra **5** and **6** were obtained using a 50 ms $T_{1\rho}$ filter at the end of the sequence to eliminate the broad protein background whereas **6** additionally incorporates water suppression into the experiment (in this case excitation sculpting). Of particular note in all the STD spectra in the figure is the fact that the aromatic signals of tryptophan are much more intense than the aliphatic region signals meaning a stronger aromatic contact with the albumin binding pocket.

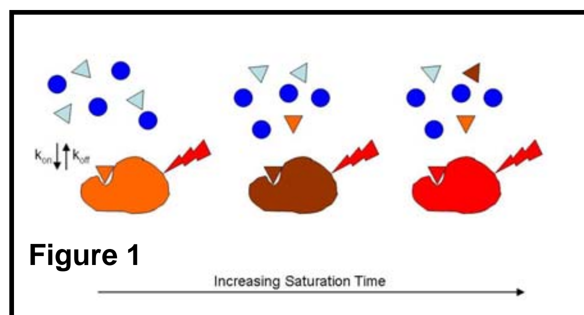

Figure 1

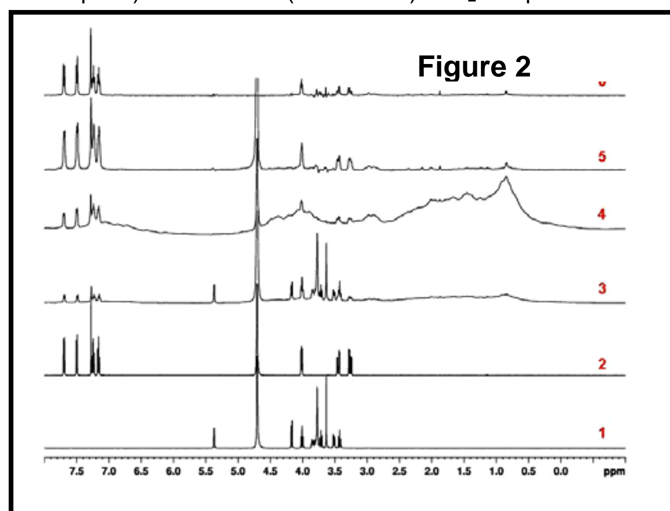

Figure 2

Compared to its competitors, STD has several advantages. One is that there is no upper limit on the size of the receptor. In fact, STD favours heavier receptor molecules at stronger magnetic fields. It can be applied to a broad range of dissociation constants, with the typical range quoted $10^{-8} < K_D < 10^{-3}$ M. The technique yields excellent sensitivity, in part due to the saturation amplification process alluded to and pictured above. As a result, only small amounts of receptor are needed with as little as 0.3 nmol being sufficient (15 μg of a 50 kDa protein!). It is important to remember that in general STD works better for heavier receptor molecules and smaller ligands. Rules of thumb are that the receptor have a molecular weight > 20 kDa and the ligand < 8 kDa.

In summary, STD is a powerful, state-of-the-art, and highly versatile NMR experiment to detect bioaffinity. It is now working beautifully on the ARMRC Bruker AV-500. If your research program involves bioaffinity related questions, I strongly encourage you to discuss with me this experiment and its potential application in your lab.