The NMR application for medicinal screening

Product used : Nuclear Magnetic Resonance (NMR)

The merit of the observation of protein – ligand[*1] interaction by NMR is that one can observe interaction at equilibrium state between association and dissociation in solution. 1D NMR techniques are useful methods that can be used to reveal the target molecule among candidates of many small compounds.

In this context, they are attracting attention as screening [*2] method s of pharmaceutical products.

※1 The ligand is a molecule that specifically binds to a specific receptor (protein).

※2 Screening is select those that show drug efficacy / activity from a large number of candidate compounds.

spin diffusion and saturation transfer

"Spin diffusion" and "Saturation transfer" are keywords in NMR techniques of protein-ligand interaction.

When certain protein signals are saturated , saturation is diffused to all protein signals. This phenomenon is called **Spin diffusion .**

Then the effect of diffusion is transferred to the ligand that binds the protein. This is called **Saturation Transfer**.

The signal intensities of saturated ligand are decreased (other signals are not changed). Schematic explanation of saturation transfer is shown in Fig 1 .Saturation transfer is introduced by chemical exchange between protein-bound and protein-free ligand.
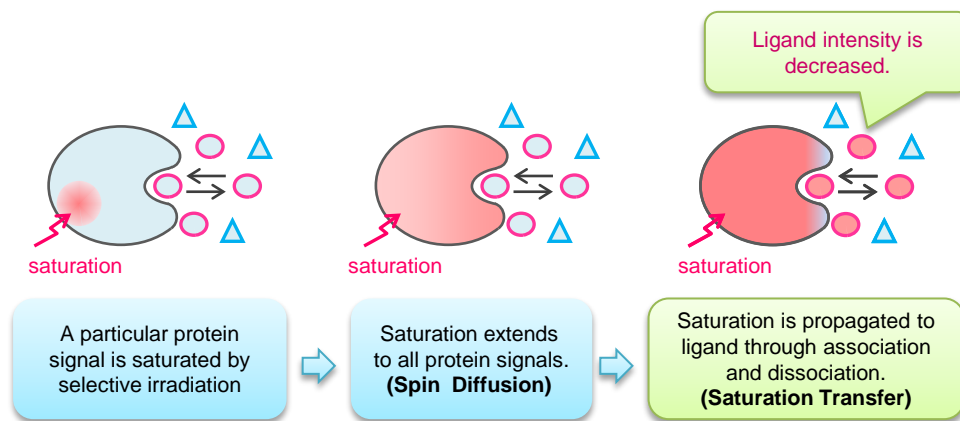

Fig 1：Explanation of saturation transfer

STD (Saturation Transfer Difference)

In STD experiment, one obtains the difference spectrum between a spectrum with saturation and a spectrum without saturation. As a result, signals that show no intensity change are canceled out and only binding ligand signals remain. In other words, STD spectrum shows only ligand signals with binding activity to protein. A schematic explanation of STD is shown in Fig 2. Red-colored area indicates saturation where signals are not observed or are observed with reduced intensity.
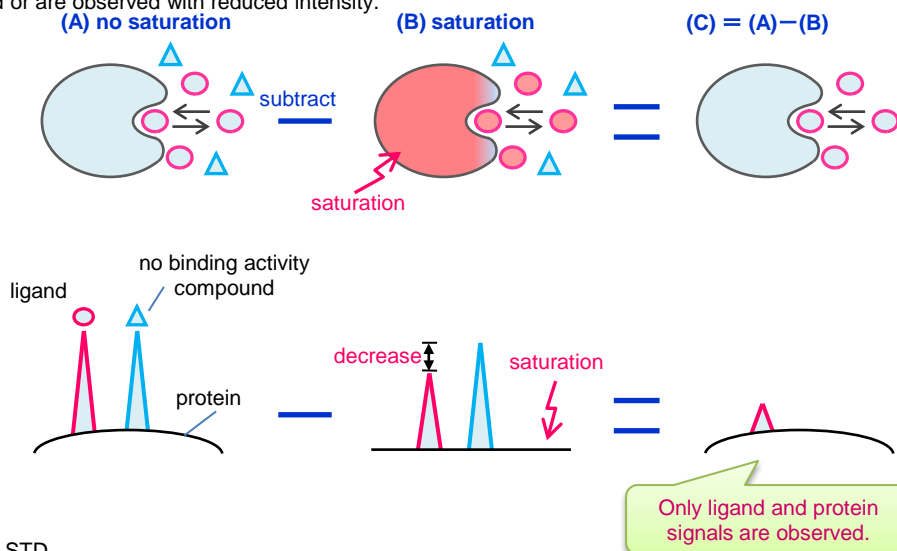

Fig 2：Explanation of STD

STD experiment

STD experiment produces a difference spectrum. The difference spectrum can be obtained by alternately collecting the excited spectrum and the non-excited spectrum in the same experiment. Moreover , protein signals are reduced by $T_1\rho$ filter to make analysis easier.(Fig 3)
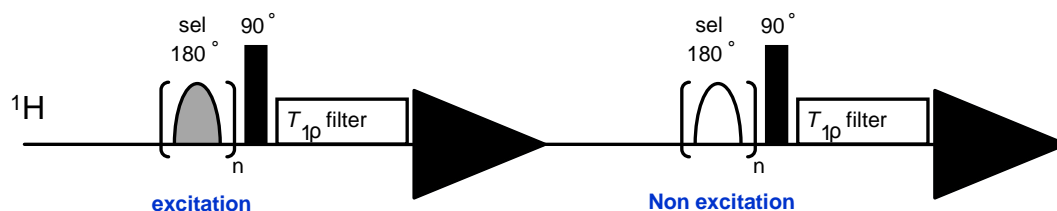

Fig 3：The pulse sequence of STD experiment

Reference：J. Klein, R.Meinecke, M.Mayer, and B.Meyer, *J. Am. Chem. Soc. 1999, 121, 5336-5337.*

STD spectrum

Fig 4. shows the STD spectrum (A) and ^1H spectrum (B) of mixture solution of Human Serum Albumin (HSA), L-(+)-Arginine (Arg) and L-Tryptophan (Trp).
In the STD spectrum, Tryptophan signals which have binding activity to Albumin can be observed, while Arginine signals which have no binding activity are not present.
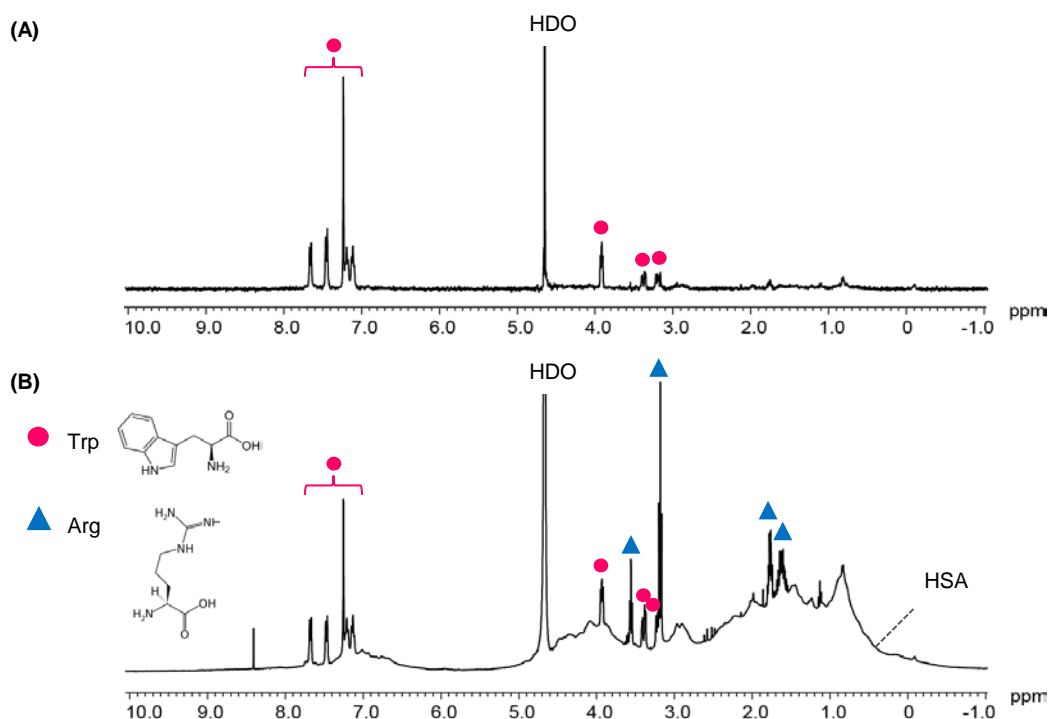

Fig 4：(A) STD spectrum, and (B) ^1H spectrum mixture solution of Albumin 0.1 mM, Trp 2mM, Arg 2 mM /D$_2$O

(A) STD spectrum：64 scans, selective excitation position 0.6 ppm, non excitation position -10ppm, saturation time 8 s, $T_1\rho$ filter 50 ms
(B) ^1H spectrum： 64 scans

console：JNM-ECZ400S+ROYALPROBE

DIRECTION (Difference of Inversion RECovery rates with and without Target IrradiatiON)

DIRECTION[※1] is a NMR technique to analyze the protein-ligand interaction by longitudinal relaxation time.

In general, signal strength in the STD spectrum increases proportionately to the strength of interaction with protein. Thus, it's possible to obtain hints about the part of ligand involved in binding.

However, when ligand's T_1 is short, efficiency of saturation transfer decreases. Therefore, STD spectrum analysis needs to take into consideration the T_1 of each ligand signal.

DIRECTION is the experiment that estimates the efficiency of saturation transfer by probing not the signal intensity but directly the T_1.

DIRECTION is essentially an inversion recovery experiment where T_1 is measured twice with saturation transfer and no saturation transfer.

[1]H signals from ligands that strongly bind to protein show decreased T_1.

DIRECTION experiment

In DIRECTION experiment, T_1 is measured twice with and without presaturation (Fig.5). Analysis of the changes in [1]H's T_1 can identify binding compounds. Protein signals are decreased by T_2 filter.
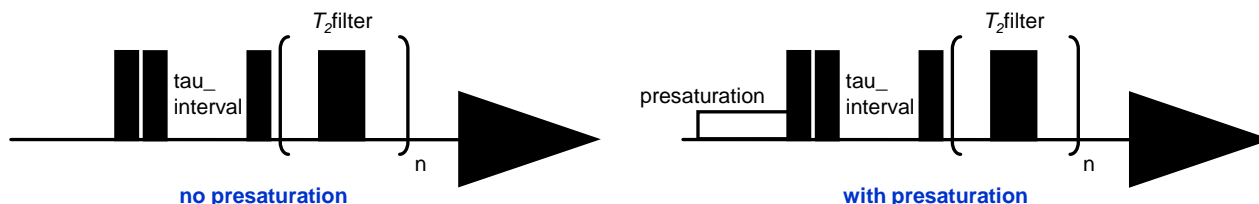

Fig 5 : DIRECTION experiment

Reference : Y. Mizukoshi, A.Abe, T.Takizawa, H.Hanzawa, Y.Fukunishi, I.Shimada and H.Takahashi, *Angew. Chem. Int. Ed. 2012, 51, 1362-1365.*

Results of DIRECTION

Table 1 shows the results of DIRECTION for a mixture solution of 0.1mM HSA , 2mM L-(+)-Arg, and 2mM L-Trp / D_2O.

T_1 of Arginine which has no binding activity shows no significant change when it is measured with and without saturation. On the other hand, Tryptophan's T_1 is significantly decreased . Especially 3.22ppm and 3.24ppm's signals are drastically decreased.

Table 1 : Results of DIRECTION

	assign	CS/ppm	no presaturation T_1/s	with presaturation T_1/s	Change %
1	arg	1.62	0.94	0.96	102.13
2	arg	1.77	0.96	0.94	97.92
3	arg	3.18	1.04	0.99	95.19
4	trp	3.22	1.11	0.62	55.86
5	trp	3.24	1.02	0.60	58.82
6	trp	3.38	1.06	0.70	66.04
7	arg	3.57	3.18	3.24	101.89
8	trp	3.93	1.83	1.28	69.95
9	trp	7.13	1.90	1.41	74.21
10	trp	7.21	1.92	1.41	73.44
11	trp	7.25	2.18	1.49	68.35
12	trp	7.48	1.92	1.22	63.54
13	trp	7.69	1.80	1.27	70.56

DIRECTION :
32 scans, loop number 80, relaxation delay 15 s,
T_2filter 80ms, tau interval 0.1s～16s   8 points

console : JNM-ECZ400S+ROYALPROBE