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Transient NOE-exchange-relay experiment: Application to ligand–protein binding under slow exchange conditions

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Abstract

A new version of one-dimensional ^{1}H experiment has been developed to probe ligand binding to macromolecular targets. The experiment, called transient NOE-exchange relay, is similar to the 'reverse NOE pumping' technique [A. Chen, M.J. Shapiro, J. Am. Chem. Soc. 122 (2000) 414–415]. The T_2 filter is used to erase protein magnetization, and the saturation then spreads from protein to bound ligand (via NOE) and further to a free ligand (via on–off exchange). The ligand signals, monitored as a function of mixing time, present a familiar 'dip' pattern characteristic of transient NOE or transient exchange experiments. In addition to the T_2 filter, we have also implemented a T_1 filter which makes use of the fact that the selective T_1^{-1} rates in macromolecules are much higher than those in small ligands. To model the experiment, complete relaxation and exchange matrix analysis has been invoked. This formalism was further used as a starting point to develop a simplified treatment where the relaxation and exchange components are represented by 2×2 matrix and, in addition, there is a special term responsible for coupling of ligand magnetization to the protein spin bath. The proposed experimental scheme has been tested on a system of peanut agglutinin complexed with Me-β-D-galactopyranoside, which is known to be in a slow exchange regime. The results suggest that the NOE-exchange-relay experiment can be used at the advanced stages of the drug development process to confirm high-affinity ligand binding.

Keywords: Ligand–protein binding; Transient NOE-exchange-relay experiment; NMR screening; Reverse NOE pumping; Saturation transfer difference; Peanut agglutinin

1. Introduction

NMR spectroscopy proved to be a useful screening tool for identification of small molecules that bind to macromolecular targets [1–4]. In the process of drug development, NMR-based screen initially identifies a small number of compounds with modest binding affinity. Based on the obtained results, working compound libraries are constructed in a more focused manner to search for ligands with higher binding affinities. During this iterative procedure, NMR serves as one of the important binding assays. A number of homo- and heteronuclear pulse sequences have been developed with this purpose in mind.

Transient NOE [5] and transient exchange [6] experiments are part of a standard NMR toolkit. In these experiments one component of a spin system is selectively excited; the perturbation then propagates through the system, carried by Overhauser effect or by chemical exchange. Here we describe a simple variant of this experiment where the perturbation is transmitted in a relayed fashion: from a large protein to its bound ligand via NOE and farther on to the pool of free ligand molecules via chemical exchange. Conceptually, the experiment bears strong similarity to the saturation transfer difference experiment [7] and especially to the reverse NOE pumping experiment [8]. Of note, closely related methods have been also developed in the field of magnetic resonance imaging [9,10]. Our specific experimental scheme, however, is distinct from the commonly used differential spectroscopy approaches.

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To analyze the proposed experimental scheme we conducted complete relaxation and exchange matrix analysis [11–13]. We further found a way to simplify this treatment, reducing it to a system of two differential equations. Using the simplified approach, we explored the range of applicability of NOE-exchange-relay experiments. So far practical applications of this class of experiments have been limited to the case of weak binding/fast exchange. We found that such measurements can be also carried out under slow exchange conditions. This has been confirmed experimentally for the model system of peanut agglutinin complexed with Me-β-D-galactopyranoside. The slow exchange in this case plays a role of a limiting step in the NOE-exchange relay. It is envisioned that the described measurement scheme can be helpful at the advanced stages of the screening process where it can be used to identify compounds with relatively tight binding, $K_d < 1$ μM, and exclude false positives [14,15].

2. Pulse sequence

The sequence shown in Fig. 1a is designed for mixture of small ligand and large macromolecular target. The first T_2 filter nullifies the magnetization associated with the protein, while preserving the magnetization of the free ligand (in effect, the filter achieves saturation of the protein magnetization). During the mixing time, τ_{mix}, saturation is transferred from the protein onto a bound ligand and further to the free ligand, causing a decrease in the amount of magnetization associated with the free ligand. As τ_{mix} becomes longer, T_1 relaxation takes over, and the free ligand magnetization is restored to its equilibrium value. The observed signal, taken as a function of τ_{mix}, displays therefore a 'dip' pattern characteristic of transient NOE and transient exchange measurements [5,6].

Prior to the detection, the system is subjected to the second T_2 filter. The purpose of this filter is to eliminate the broad protein background which builds up during the time τ_{mix}. Finally, the 3-9-19 element [16] is inserted in the sequence to suppress the residual HDO signal (the measurements are conducted in D_2O solvent).

Fig. 1b shows a variant of this sequence where the starting T_2 filter is replaced with a T_1 filter. The new filter takes advantage of large selective T_1^{-1} relaxation rates in a big protein. In the presented application, the ligand is a simple monosaccharide with proton resonances lying downfield of 3.4 ppm. The starting selective 180° pulse, of i-SNOB variety [17], inverts the entire spectrum of the ligand as well as portion of the protein spectrum, but not the protein methyl or methylene resonances. Subsequently, during the time τ_1, inverted protein magnetization rapidly recovers (the selective character of protein relaxation is ensured by the presence of unperturbed methyl/methylene magnetization [18]). On the other hand, the magnetization of the free ligand does not change substantially over the same time period. At this point, the application of a hard 180° pulse creates the state where ligand magnetization is close to equilibrium, whereas protein magnetization is, in a good approximation, inverted. The advantage of this preparation scheme is that a uniform (even if incomplete) inversion is achieved for all protons in the protein. The remaining part of the sequence, beginning with τ_{mix}, is identical to Fig. 1a.

3. Theory

For the two-component system at hand, spin evolution during the mixing time τ_{mix} is described by the following system of equations:

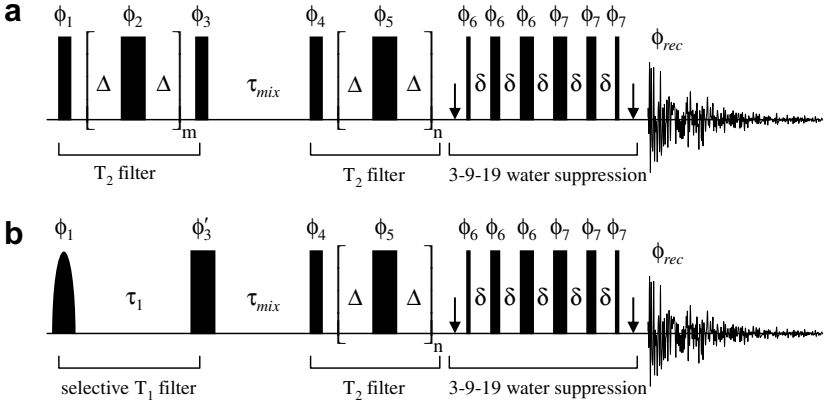

Fig. 1. Transient NOE-exchange-relay experiments. The CPMG T_2 filters are applied with $\Delta = 1$ ms, $m = 10$, $n = 44$. The water suppression element, which serves to eliminate the residual HDO signal, employs (3π/26)–(9π/26)–(19π/26)–(19π/26)–(9π/26)–(3π/26) pulse train [16] with interpulse separation $\delta = 0.37$ ms. Arrows denote the application of sine-bell shaped gradients with duration of 1 ms and amplitude of 10 G/cm. The (excessive) phase cycling is $\phi_1 = -\phi_3 = x$, $\phi_2 = 32(y)32(-y)$, $\phi_4 = 8(x)8(y)8(-x)8(-y)$, $\phi_5 = 4(y)4(-y)4(-x)4(x)4(-y)4(y)4(x)4(-x)$, $\phi_6 = -\phi_7 = 16(x,y,-x,-y)$, $\phi_{rec} = 4(x,-x)4(-y,y)4(-x,x)4(y,-y)$. The variant of the sequence shown in (b) begins with i-SNOB-2 pulse [17] applied at the center of the ligand spectrum, 3.68 ppm, with the duration of 3.3 ms and the power of 0.73 kHz. The subsequent delay τ_1, at the core of the selective T_1 filter, is 50 ms. The phase cycle is identical to the sequence (a), with the exception of $\phi_3' = 32(x)32(y)32(-x)32(-y)$.