HHS Public Access

Author manuscript

J Biomol NMR. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

J Biomol NMR. 2016 December ; 66(4): 221–225. doi:10.1007/s10858-016-0075-4.

Perspective: Revisiting the Field Dependence of TROSY Sensitivity

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Abstract

The discovery of the TROSY effect (Pervushin et al. 1997) for reducing transverse relaxation and line sharpening through selecting pathways in which dipole-dipole and CSA Hamiltonians partially cancel each other had a tremendous impact on solution NMR studies of macromolecules. Together with the methyl TROSY (Tugarinov and Kay 2004) it enabled structural and functional studies of significantly larger systems. The optimal field strengths for TROSY have been estimated to be on spectrometers operating around 900 MHz (21.14 T) for the $^{1}H_{N}$ TROSY (Pervushin et al. 1997) while the aromatic ^{13}C ($^{13}C_{aro}$) TROSY is posited to be optimal at around 600 MHz (14.09 T) (Pervushin 2000; Pervushin et al. 1998b). The initial rational was based on the consideration of where the quadratic B_0 field dependences of the TROSY relaxation rates reach a minimum. For sensitivity consideration, however, it is interesting to estimate which field strengths yield the tallest peaks. Recent studies of ^{15}N-detected TROSYs suggested that maximal peak heights are expected at 1.15 GHz (27.01 T) although the slowest relaxation rates or longest transverse relaxation times T_2 are indeed expected around 900 MHz (21.14 T) (Takeuchi et al. 2016; Takeuchi et al. 2015). This was based on the fact that the heights of Lorentzian lines are proportional to $B_o^{3/2} * T_2 (B_o)$. Thus, multiplying the parabolic $T_2(B_o)$ dependence with the increasing function of $B_o^{3/2}$ shifts the maxima of peak-height field dependence from the T_2 maximum at 900 MHz to higher fields. Moreover, besides shifting the peak height maximum for ^{15}N TROSY, this analysis yields estimates for optimal peak heights for $^{1}H_{N}$ detected TROSY to 1.5 GHz, and to 900 MHz for ^{13}C-detected $^{13}C_{aro}$TROSY as is detailed below. To our knowledge, this aspect of field dependence of TROSY sensitivity has not been in the attention of the NMR community but may affect perspectives of NMR at ultra-high fields.

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Compliance with Ethical Standards

Conflict of Interest: GW receives a honorarium from Springer as Editor in Chief of JBNMR. The authors declare that they have no further conflicts of interest

Keywords

TROSY; aromatic TROSY; sensitivity; ^{15}N detection; ^{13}C detection; field dependence; CSA

Though nuclear magnetic resonance (NMR) has intrinsically low sensitivity compared to other spectroscopic techniques, its slowly relaxing coherences are rich in structural and dynamics information. Therefore, solution NMR has been a widely used approach for structural and dynamics analyses of macromolecules at atomic resolution, especially since NMR can analyze samples at near-physiological conditions. However, with a shift of focus to higher molecular weight (MW) systems, the application of solution NMR has become more challenging, primarily because of the shortening of the transverse relaxation time (T_2) and the associated line broadening of the resonances. In addition to the broader lines, larger systems have a proportionally larger number of resonances within a given spectral width, which hampers unambiguous resonance assignment. Furthermore, as the heights of Lorentzian lines are directly proportional to T_2 (Fig. 1A), the sensitivity of solution NMR spectroscopy without the TROSY effect decreases with higher MW.

Several innovative strategies have been introduced to cope with the short transverse relaxation times of larger systems. These include perdeuteration for reducing dipole-dipole (DD) broadening (Farmer and Venters 1996; Kushlan and LeMaster 1993; LeMaster 1989; Venters et al. 1995), and the use of slowly relaxing methyl resonances for detection (Goto et al. 1999; Lemaster 1990; Markus et al. 1994; Rosen et al. 1996). Subsequently, the introduction of transverse relaxation-optimized spectroscopy (TROSY) has revolutionized solution NMR and enabled studies of significantly larger systems (Loria et al. 1999; Meissner and Sorensen 1999; Pervushin 2000; Pervushin et al. 1998a; Pervushin et al. 1997; Pervushin et al. 1998b; Pervushin et al. 1998c; Pervushin et al. 1998d; Salzmann et al. 1998; Weigelt 1998). The TROSY method offsets a major part of the transverse relaxation by the cross-correlated DD-chemical shift anisotropy (CSA) interference to elongate T_2 (Pervushin et al. 1997). While the principle and anticipation for the cross-correlated relaxation resulting in different line widths of heteronuclear doublet components have also been described in earlier literatures (Farrar and Quintero-Arcaya 1985; Griffey and Redfield 1987) it has only later been recognized as a major tool for improving resolution, and thus sensitivity as well, of protein spectra (Pervushin et al. 1997). This TROSY effect is most efficient in the isolated two-spin systems that have CSA large enough to interfere with DD. In addition, the angle between DD and principal CSA component should be close to parallel, to have an optimal TROSY effect. The “methyl TROSY” effect for methyl groups in deuterated proteins had also a dramatic effect on NMR studies of large proteins. However, it is not derived from the interference of DD-CSA relaxations but from interference between auto- and cross-correlated ^{1}H-^{1}H dipolar relaxations (Tugarinov et al. 2003) and is not be discussed in this perspective.

In the past, the TROSY effect for $^{15}N_H$ or $^{13}C_{aro}$ has primarily been used in ^{1}H-detected multidimensional experiments. However, the long T_2 of TROSY $^{15}N_H$ and $^{13}C_{aro}$ resonances have made these nuclei attractive for direct detection (Takeuchi et al. 2016; Takeuchi et al. 2015). Due to their low gyromagnetic ratio (γ), these spins do not experience much dipolar

broadening from ^{1}H nuclei in remote positions even at non-deuterated conditions. Therefore, we have recently shown that the TROSY effect can be utilized for $^{15}N_H$-detection even if proteins are not deuterated (Takeuchi et al. 2016; Takeuchi et al. 2015). This has important advantages as it opens avenues for studies of systems that cannot be deuterated because they can only be expressed in cells that do not grow at high concentrations of $^{2}H_2O$, such as insect or mammalian cells. Furthermore, this largely eliminates the problem of incomplete amide back exchange encountered when working with large perdeuterated proteins that cannot be refolded. In addition, the T_2 ratio of TROSY vs decoupled non-TROSY coherences is insensitive to the molecular weight of the system for any nuclei (Takeuchi et al. 2016; Takeuchi et al. 2015). Thus, the TROSY strategy significantly pushed the upper limit for systems that solution state NMR can address.

It has been widely postulated that 900 MHz is the optimal frequency for the both $^{15}N_H$ and $^{1}H_N$ TROSY, because the optimal DD-CSA interference give rise to the longest transverse relaxation times T_2 of TROSY components at the magnetic field strength of 21.14 T (900 MHz). As shown in Fig. 1B, the T_2 value has a quadratic dependence on the field strength, in the shape of parabolic T_2 dependence with a vertex at 900 MHz as noted in previous publications (Pervushin 2000; Pervushin et al. 1997). Therefore, the aforementioned assumption is entirely true for minimal line width, which is inversely proportional to T_2. However, we posit that this has to be revisited with respect to sensitivity. For sensitivity, the peak height is what matters and the peak height is not only directly proportional to T_2 but also depends on the strength of the magnetic field by $B_0^{3/2}$.

Therefore, the sensitivity is proportional to the product of $T_2(B_0)$ and $B_0^{3/2}$. The product of the T_2 field dependence with the increasing $B_0^{3/2}$ function (Fig. 1C) shifts the maximum of the peak height significantly to higher field (Fig. 1D, red). We have discussed this previously and have shown that the $^{15}N_H$-deteced TROSY resonance is estimated to be most sensitive at 1.1-1.2 GHz, while the narrowest line widths can be obtained at 900 MHz (Takeuchi et al. 2016; Takeuchi et al. 2015). The difference between the optimal field strength for slowest transverse relaxation and for sensitivity varies depending on parabolic T_2 dependence that reflects the relative contribution of the DD and CSA of each nucleus and the angle between the respective tensors (Fig. 1D, red). The maximum sensitivity of the $^{1}H_N$ TROSY shifts to around 1.5 GHz, which is at significantly higher field than the maximum for $^{15}N_H$ TROSY. This is due to a smaller contribution of CSA relative to DD to ^{1}H relaxation, which makes the tangent of the parabolic T_2 dependence less steep for $^{1}H_N$ than for $^{15}N_H$ and sensitivity is more affected by the B_0 strength. Therefore, it is clear that 900 MHz is only optimal for obtaining longest T_2, and higher field strength near and above a 1 GHz is required to gain the benefit of maximal sensitivity. Relative to 900 MHz, the predicted sensitivity gain of the TROSY components at the optimal field strength of 1.2 GHz is 1.3-fold for $^{15}N_H$ and 1.6-fold at 1.5 GHz for $^{1}H_N$. Compared to 1.2 GHz, the $^{1}H_N$ TROSY sensitivity is expected to increase at 1.5 GHz by a factor of 1.1. On the other hand $^{15}N_H$ TROSY sensitivity is expected to decrease from 1.2 GHz to 1.5 GHz by a factor of 0.82, based on the assumptions described in the Supplement.

This reasoning can also be extended to the $^{13}C_{aro}$ TROSY. The $^{13}C_{aro}$ TROSY effect is reported to be optimal at 600 MHz when considering T_2 (Pervushin et al. 1998b). However,

the maximum signal height of the $^{13}C_{aro}$ TROSY should actually be achieved at around a 1 GHz. The sensitivity gain for the $^{13}C_{aro}$ TROSY at this optimal field strength for sensitivity, as compared to 600MHz is 1.4-fold (Fig. 1D, red).

In all cases, optimal sensitivity of the TROSY resonances is achieved at higher field compared to that for optimal relaxation rates and these are at or above 1 GHz (23.5 T) magnetic field strength. It should also be noted that even though the TROSY selection schemes only recover half of the coherence pathways as compared to the conventional non-TROSY detection, TROSY is the most sensitive detection method at and near the optimal field strength (Fig. 1D, cyan for non-TROSY detection scheme).

Although this perspective is focused on the sensitivity in detecting 1D TROSY resonances for the sake of simplicity, the advantage of higher-field magnets also holds for multidimensional TROSY experiments. For the aromatic ^{13}C TROSY there is essentially no TROSY effect in the ^{1}H dimension, thus the prediction shown in Fig. 1 will also be valid in 2D or 3D experiments. For the $^{15}N_H$ detected and $^{1}H_N$ detected TROSYs of the amide groups the peak heights would depend on the product of the field dependent T_2 curves of ^{1}H and ^{15}N, multiplied with a single $B_o^{3/2}$ curve. This will place the optimum field for 2D amide TROSYs in between 1.1 and 1.5 GHz. A more detailed analysis is beyond the scope of this manuscript, however, the $T_2(B_0)$ of indirectly frequency labeled nuclei and the coherence loss in the pulse scheme also affect the resultant sensitivity. Theoretical estimates clearly show the advantage of higher-field magnets, above 1 GHz, however, in practice, this gain in sensitivity for ^{1}H-detected TROSY can be partly offset by adverse salt effects in high-Q probes at higher field strengths, an effect that is less significant for low γ nuclei (Takeuchi et al. 2016).

Although, the calculations shown in Figure 1 do not take dynamics into account, it is worth simulating the contribution of additional relaxation due to exchange broadening (R_{ex}) for the field dependence of the peak heights. This will be especially true when the chemical exchange is fast on the NMR time scale, where R_{ex} is proportional to the field strength. As expected, the advantage of higher field magnets will be less in magnitude while the field dependencies are largely unchanged (Figure S1). Since the intrinsic relaxation becomes larger for higher molecular weight systems, the contribution of R_{ex} to the signal height will be smaller. The variation of $^{1}H_N$ T_1 with field might also influence the sensitivity; however, $^{1}H_N$ can have rather short T_1 values even at high protein molecular weight, due to a strong coupling between the ^{1}H magnetization of water and the macromolecule (Riek et al. 2002). In addition, there are many experimental options to address this point, such as using relaxation agents that reduce the ^{1}H longitudinal relaxation (Takeuchi et al. 2010) and water management in pulse sequences (Schanda et al. 2005). Likewise, structure dependence of CSA would also affect the optimal B_o values. Nevertheless, our calculation is consistent with previously published experimental data (Takeuchi et al. 2015). The ratio of signal heights of TROSY vs. regular HSQC at two different field strengths closely match the calculation presented here.

This perspective warrants rethinking the maximum field strength limit, the design and development of higher field magnets over 1 GHz and the associated development in probe technology that will allow taking advantage of the increased sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Prof. Ichio Shimada for valuable discussions on this topic.

Funding: This study was supported by the National Institute of Health (Grant GM047467 and EB002026). This work was partially supported by PREST, JST to KT.

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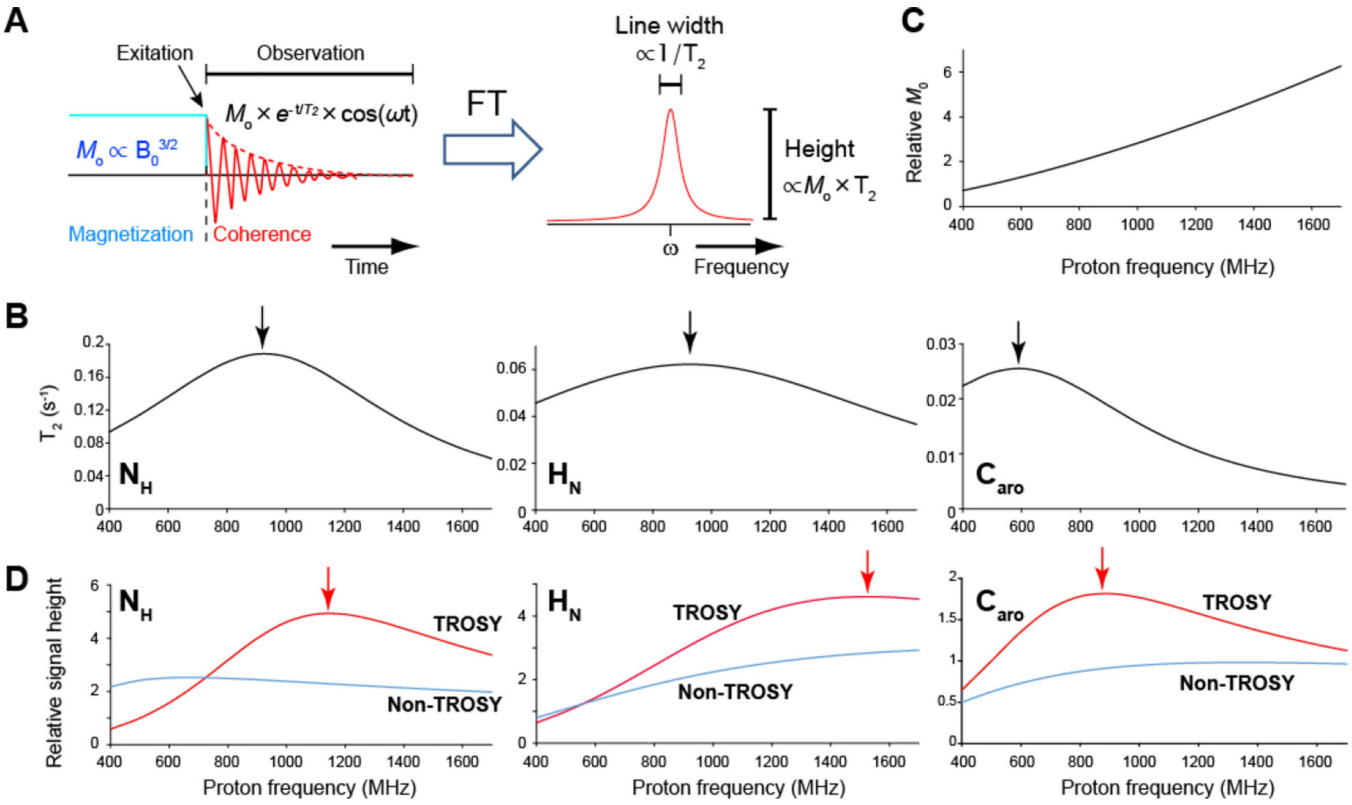

Figure 1. Resolution and sensitivity of TROSY resonances

(A) Relation of T_2 and initial magnetization (M_0) in time domain to resolution (line width) and sensitivity (signal height), respectively, in frequency domain. (B) T_2 of the $^{15}N_H$, ^1H_N, and $^{13}C_{aro}$ TROSY resonances for the protein with rotational correlation time of 20 ns. For the $^{15}N_H$ and ^1H_N TROSY resonances, α-helical part of $^2H^{15}N$ labeled protein is assumed. For the $^{13}C_{aro}$ TROSY resonances, non-deuterated altenately^{13}C-^{12}C labeled protein that is labeled with 3-^{13}C labeled pyruvate was assumed. Detail of the calculation of T_2 are shown below. (C) Field dependence of M_0 relative to that at 500 MHz. (D) 1D Signal height of TROSY (red) and conventional decoupled non-TROSY (cyan) resonances relative to the signal height of TROSY resonances at 500 MHz. The signal height for the non-TROSY resonances are doubled.