J Biomol NMR (2013) 56:285–290
DOI 10.1007/s10858-013-9748-4

ARTICLE

# Biomolecular ligands screening using radiation damping difference WaterLOGSY spectroscopy

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Received: 4 March 2013 / Accepted: 28 May 2013 / Published online: 6 June 2013


**Abstract** Water-ligand observed via gradient spectroscopy (WaterLOGSY) is a widely used nuclear magnetic resonance method for ligand screening. The crucial procedure for the effectiveness of WaterLOGSY is selective excitation of the water resonance. The selective excitation is conventionally achieved by using long selective pulse, which causes partial saturation of the water magnetization leading to reduction of sensitivity, in addition to time consuming and error prone. Therefore, many improvements have been made to enhance the sensitivity and robustness of the method. Here we propose an alternative selective excitation scheme for WaterLOGSY by utilizing radiation damping effect. The pulse scheme starts simply with a hard inversion pulse, instead of selective pulse or pulse train, followed by a pulse field gradient to control the radiation damping effect. The rest parts of the pulse scheme are similar to conventional WaterLOGSY. When the gradient pulse is applied immediately after the inversion pulse, the radiation damping effect is suppressed, and all of the magnetization is inversed. When the gradient pulse and the inversion pulse are about 10–20 ms apart, the radiation damping effect remains active and drives the water magnetization toward +z-axis, resulting in selective non-inversion of the water magnetization. By taking the differences of the spectra obtained under these two conditions, one should get the result of WaterLOGSY. The method is demonstrated to be simple, robust and sensitive for ligand screening.

**Keywords** NMR · Radiation damping · Ligand screening · WaterLOGSY

## Introduction

As a powerful tool for potential lead compounds or ligands screening, nuclear magnetic resonance (NMR) spectroscopy has been increasingly appreciated in academic and industrial application. This is mainly due to the fast development of NMR hardwares, softwares and pulse sequences. All these make NMR a unique approach for monitoring weak molecular interactions at atomic and molecular level without prior knowledge of biomolecular function, and for deriving structural information of the target or receptor macromolecules and the ligands (Pellecchia et al. 2002; Stockman and Dalvit 2002; Meyer and Peters 2003; Lepre et al. 2004).

Generally, NMR techniques for ligand screening can be classified into two categories: receptor-detected approaches and ligand-detected approaches. The former technique can provide structural information of the binding site on the receptor by exploiting differences of NMR parameters, such as chemical shift, or relaxation rates (Shuker et al.

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10858-013-9748-4) contains supplementary material, which is available to authorized users.

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1996). For the receptor of molecular weight exceeding 10 kDa, stable isotope labeling is generally required. The latter category of approaches are based on the detection of small molecular ligands (Chen and Shapiro 1998, 2000; Dalvit et al. 2000, 2001; Meyer and Peters 2003; Gossert et al. 2009; Xia et al. 2010), and many methods have been developed and widely used, such as NOE-pumping (Chen and Shapiro 1998; Chen and Shapiro 2000), saturation transfer difference (STD) (Mayer and Meyer 1999; Ji et al. 2009; Xia et al. 2010) and water-ligand observed via gradient spectroscopy (WaterLOGSY) (Dalvit et al. 2000, 2001; Gossert et al. 2009). Compared with the receptor-detected approaches, the ligand-detected approaches need much smaller amount of unlabelled receptor and do not limited by the receptor size (Pellecchia et al. 2002). Although ligand-detected methods can not provide structure of the binding site, they can provide information about the competitive binding (Dalvit et al. 2001; Cui et al. 2004; Bai et al. 2005) and even can differentiate the specific and non-specific binding (Ji et al. 2009). Therefore, ligand-detective methods have been widely used for ligand screening.

WaterLOGSY is one of the most used ligand-detected ligand screening approach, where the large bulk water magnetization is partially transferred via the protein–ligand complex to the free ligand in a selective manner (Dalvit et al. 2000, 2001). The crucial issues of WaterLOGSY are the selective excitation of the water resonance and the magnetization transfer during the mixing time. They both affect the experimental time, sensitivity, and quality (Gossert et al. 2009). Many improvements have been made to enhance the sensitivity and robustness of WaterLOGSY. The double pulsed field gradient spin-echo has been incorporated which results in superior selective excitation and minimum phase distortion (Shimotakahara et al. 2005). The polarization optimized PO-WaterLOGSY was proposed to enhance the sensitivity (Gossert et al. 2009). It has been reported that incorporation of solvent suppression scheme W5 (Liu et al. 1998) in WaterLOGSY (Furihata et al. 2008) and PO-WaterLOGSY (Gossert et al. 2009) could improve the quality of the spectra.

Radiation damping is a well-known phenomenon associated with strong magnetization and can cause line-shape distortion and other artifacts in NMR experiment, especially when aqueous sample is measured in high field NMR spectrometer (Mao and Ye 1997; Shishmarev and Otting 2011; Krishnan and Murali 2013). A lot of works have been done in order to minimize the effect or make use of the radiation damping, such as water suppression (Price and Arata 1996; Liu et al. 1998; Wang et al. 2010; Cui et al. 2011), experiments with water flip-back (Lippens et al. 1995; Fulton and Ni 1997; Shishmarev and Otting 2011), and measurements of chemical exchange (Chen and Mao 1998; Fan et al. 2011). It was reported that the radiation damping based method could reduce the contribution of direct NOE, but not exchange-relayed NOE, to the measured chemical exchange rates (Fan et al. 2011). Here in this article, we propose an improved WaterLOGSY by utilizing the radiation damping effect to achieve selective excitation of the water resonance (RD-WaterLOGSY). The new method is demonstrated to be sensitive, robust, and convenient for academic and industrial application in ligand screening.

## Materials and methods

The pulse sequence of the proposed RD-WaterLOGSY (Fig. 1) starts with a non-selective 180° pulse followed by a gradient pulse (g1) and a weak gradient pulse (g4), respectively, during the mixing or recovery period ($T_{mix}$) to manipulate the radiation damping effect and to remove any possible artifacts. A water flip-back scheme (Grzesiek and Bax 1993a, b) is used as read pulse to enhance sensitivity and WATERGATE W5 is used for water suppression (Liu et al. 1998; Wang et al. 2010) before data acquisition. The experiments are run in an interleave mode with the gradient g1 applied immediately after the first 180° pulse for the odd scans and after an extra delay Δ for the even scans, respectively. In the odd scan, the successive application of the 180° pulse and the gradient g1 removes the radiation damping (Mao and Ye 1997; Shishmarev and Otting 2011; Krishnan and Murali 2013) and makes this part of the pulse sequence as a conventional inversion-recovery scheme. In the even scan, during the delay Δ, the radiation damping remains active and drives nearly all of the water magnetization toward +z-axis or equilibrium state, while the magnetizations of the other components are less affected (Mao and Ye 1997; Shishmarev and Otting 2011; Krishnan and Murali 2013). Therefore, this part of the pulse sequence works as a selective non-inversion scheme for the water resonance in the even scans. The recommended delay Δ is between 10 and 20 ms, because the recovery time of water magnetization inversed by a 180° hard pulse is usually less than 20 ms on high field spectrometer equipped with cryoprobe (Shishmarev and Otting 2011). As the result, polarizations of the water magnetizations are opposite after the delay Δ for g1-Δ and Δ-g1, respectively. The other parts of the pulse sequence are similar to the general WaterLOGSY (Dalvit et al. 2001). By taking the differences between the odd scans and even scans, one should get similar results as WaterLOGSY, which is easy to achieve in a modern NMR machine (see text version of the pulse sequence in the supporting information).

To validate the performance and efficiency of the proposed RD-WaterLOGSY, a well recognized model

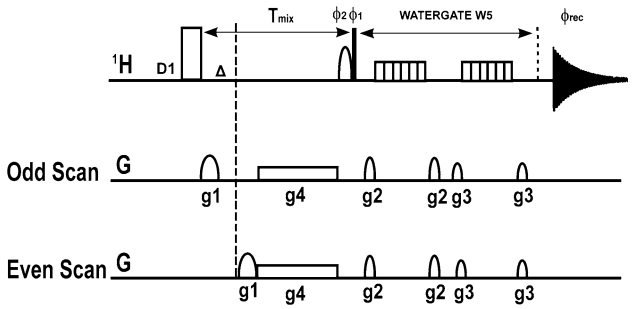


**Fig. 1** Diagramed view of the proposed RD-WaterLOGSY pulse sequence with water flip-back (Gossert et al. 2009) as read pulse and W5 for water suppression (Liu et al. 1998; Wang et al. 2010). The *solid narrow* and *wide opened bars* represent 90° and 180° hard pulses, respectively. A gauss shaped 90° selective pulse of 3.2 ms is applied to the water resonance for the flip-back. The gradient pulse g1, 39.75 G/cm and 3.0 ms, and the 12 ms delay Δ are used to manipulate the radiation damping. The 1.0 ms gradient pulses are used for water suppression with strengths of 18.02 G/cm for g2 and 11.66 G/cm for g3. The strength of g4 is 0.05 G/cm. All gradient pulses are sine shaped. Phase of the 180° is fixed at x. The phase programs for the RF pulses and receiver are $\phi_1 = x, x, -x, -x$; $\phi_2 = -x, x, x, -x$; $\phi_{rec} = x, -x, -x, x$. The mixing time $T_{mix}$ is 1.4 s and the delay D1 (2.0 s) should be long enough allowing most signals to recovery

receptor–ligand sample was prepared (Dalvit et al. 2001; Furihata et al. 2008, 2010). The sample contained 2.0 mM tryptophan (Trp), 2.0 mM glucose (Glu) and 0.1 mM human serum albumin (HSA). The solvent was 90 % $H_2O$/10 % $D_2O$, where $D_2O$ was used to provide the lock signal. All chemicals were purchased from Sigma-Aldrich and used without further purification.

The experiments were performed on Bruker Avance III 600 and 800 spectrometers both equipped with 5 mm triple-resonance cryogenic probe and had the proton frequencies of 599.8 and 800.13 MHz respectively, at 298 K. For comparison, RD-WaterLOGSY, WaterLOGSY (Dalvit et al. 2001) and PO-WaterLOGSY (Gossert et al. 2009) experiments were run at the same set of parameters except for the specific required ones, and all with water flip-back (Dalvit et al. 2001) for sensitivity enhancement and double WATERGATE W5 (Liu et al. 1998; Wang et al. 2010) for water suppression. For PO-WaterLOGSY, a single WATERGATE W3 sandwiched by two 90° hard pulses were used for selective excitation of the water resonance, where the two gradient pulses were anti-polarized (−20, 20 G/cm, 0.8 ms) as suggested (Gossert et al. 2009). A 12.0 ms Gaussian-shaped soft pulse was used for selective excitation of the water resonance in WaterLOGSY. A 3.2 ms Gaussian-shaped selective pulse was used for flip-back. 32 transients were acquired with spectral width of 14 ppm, total mixing time ($T_{mix}$) of 1.4 s, delay Δ of 12.0 ms, and dummy scans of 8. The durations and strengths of the other gradient pulses are given in caption of Fig. 1. The number of data points in the time domain was 32,768 corresponding to an acquisition time of 1.9 s. The recovery delay of 2.0 s (D1) was used for all experiments with exception of 0.0 s for the odd scans of PO-WaterLOGSY. To optimize the radiation damping manipulation delay Δ, six parallel RD-WaterLOGSY experiments were carried out with the delay values of 6.0, 8.0, 10.0, 12.0, 14.0 and 16.0 ms, respectively. Four RD-WaterLOGSY experiments, with D1 values of 0.0, 1.0, 2.0 and 3.0 s, were performed to test the effect of recovery time on the sensitivity.

## Results and discussion

Figure 2 shows the 600 MHz spectra obtained using RD-WaterLOGSY (a), PO-WaterLOGSY (b), and WaterLOGSY (c), respectively. The profiles of the three spectra are identical with exception of the intensities. The strong peaks of the Trp are due to the high binding affinity to HSA. The small negative peaks of Glu may be due to positive NOEs from water. The demonstration indicates that the proposed RD-WaterLOGSY works as perfect as the PO-WaterLOGSY (Gossert et al. 2009) and the conventional WaterLOGSY (Dalvit et al. 2001). The measured peak intensity of Trp and HSA show that RD-WaterLOGSY gives rise to a 1.3 times gain in intensity than PO-WaterLOGSY and WaterLOGSY. We get nearly the same results for PO-WaterLOGSY and WaterLOGSY under current experimental setup. The RD-WaterLOGSY and WaterLOGSY experiments take 3.6 min, about 40 s longer than PO-WaterLOGSY. The 40 s time saving for the PO-WaterLOGSY experiment corresponds to about 13 % gain in signal intensity.

It is known that WaterLOGSY with soft pulse for selective excitation causes partial saturation of the water resonance leading to the reduction of sensitivity. When W3 or W5 with anti-polarized gradients is used for selective excitation, water resonance are selectively refocused and inversed, but the other resonances are dephased, and the large number of pulses and delays cause intensity lost (Dalvit et al. 2001). In addition, no extra delay for the odd scans in the PO-WaterLOGSY experiment may also affect the signal intensity. The RD-WaterLOGSY takes the advantage of radiation damping to achieve selective inversion of the water resonance, and the magnetizations of all other spins, especially those from receptors, are well conserved. These ensure the higher sensitivity for the RD-WaterLOGSY experiment. When high field NMR machine, high sensitive cryoprobe, or highly aqueous sample is used, the radiation damping effect is enhanced, therefore, a better performance of RD-WaterLOGSY experiment is expected. We, therefore, repeated the experiments on an 800 MHz machine equipped with a cryoprobe, where the recovery

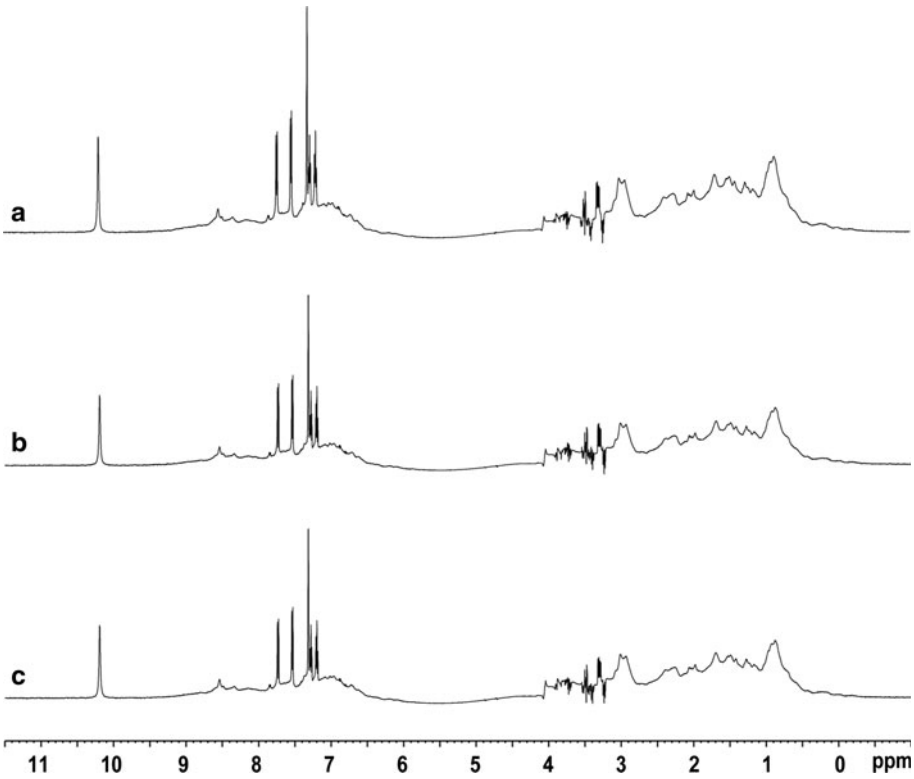


**Fig. 2** 600 MHz spectra acquired using the pulse sequences of RD-WaterLOGSY (*a*), PO-WaterLOGSY (*b*) and WaterLOGSY (*c*), all with water flip-back as read pulse, double W5 for water suppression and mixing time $T_{mix}$ of 1.4 s. The sample contains 0.1 mM HSA as receptor protein, 2.0 mM Trp and 2.0 mM Glu as binding and non-binding ligands, respectively, in solution of 90 % $H_2O$/10 % $D_2O$. The number of scans, dummy scans, and the number of data points in the time domain were 32, 8, and 32,768, respectively. The recovery delay D1 of 2.0 s was used for all experiments with exception of 0.0 s for the odd scans of PO-WaterLOGSY

time D1 was set to 2.5 s instead of 2.0 s as on 600 MHz to compensate the relaxation difference. The results (Fig. 1S) show that the relative peak intensity (Trp) of 1.00 for WaterLOGSY, 1.12 for PO-WaterLOGSY and 1.48 for RD-WaterLOGSY, respectively. We should indicate that the RD-WaterLOGSY experiment may not work when the radiation damping effect is small.

To test the effect of the recovery delay (D1) on the peak intensity, we carried out the RD-WaterLOGSY experiments with four D1 values of 0.0, 1.0, 2.0 and 3.0 s, respectively. Considering the acquisition time of 1.9 s, the total recovery times are 1.9, 2.9, 3.9 and 4.9 s, respectively, for the four experiments. The low field regions of the resulting spectra are shown in Fig. 3. The relative peak intensity is increased from 1.00 (D1 = 0.0 s) to 2.48 (D1 = 1.0 s), 3.16 (D1 = 2.0 s) and 3.26 (D1 = 3.0 s), respectively. This indicates that the recovery time has significant impact on the sensitivity. For the WaterLOGSY type experiments, conservation of the water magnetization is very important. Since the strong radiation damping, the water magnetization can be recovered during the acquisition time (1.9 s). However, magnetization recovery of the labile protons may take longer time. It is those labile protons that contribute significantly to the WaterLOGSY signals via the second mechanism (Dalvit et al. 2001). If the labile protons have different chemical shifts from that of water, their magnetization may be partially dephased during the selective excitation procedure in the PO-WaterLOGSY experiment, resulting in a loss of sensitivity. From above results (Fig. 3), we recommend to use a 2.0 s recovery delay to ensure a better result.

The radiation damping manipulation delay (Δ) is another crucial parameter for the RD-WarerLOGSY experiment. We optimized the manipulation delay by carrying out six parallel experiments with the delay varied from 6.0 to 16.0 ms with a step of 2.0 ms. The experiments were done with recovery delay of 3.0 s and mixing time of 1.4 s and with the above used sample. The results (Fig. 4) show that with a radiation damping manipulation delay of 6.0 ms, reasonable signal intensity could be obtained. The signal intensities increase as longer delays are used, and reach to a maximum steady state between 10.0 and 16.0 ms. This indicates that under radiation damping, the water magnetization in the sample nearly recovers to the equilibrium state on the 600 MHz machine with cryoprobe in about 10.0 ms. Shishmarev et al. has reported that radiation damping field could restore 95 % of the equilibrium water magnetization of a 90 % $H_2O$ sample in a 5 mm sample tube within about 5.0 ms following a 165° pulse (Shishmarev and Otting 2011). We used a 180° pulse in order to get the largest magnetization differences of the water and mobile protons between the odd and even scans, and thus higher sensitivity of the RD-WaterLOGSY experiment. It is known that the radiation damping effect depends on the field strength of the NMR machine, probe and sample (Otting and Liepinsh 1995; Mao and Ye 1997;

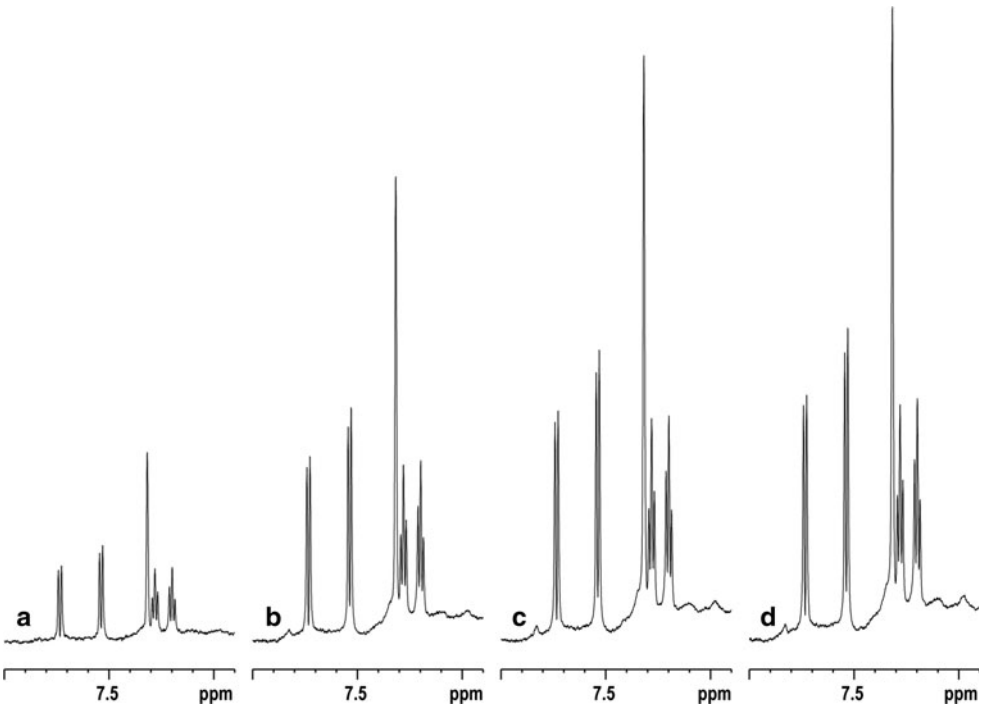


**Fig. 3** Low field regions of 600 MHz spectra acquired using the pulse sequences of RD-WaterLOGSY with the recovery times varied from 0.0 s (*a*), to 1.0 s (*b*), 2.0 s (*c*) and 3.0 s (*d*), respectively. The sample is the same one in Fig. 2. The results indicate a recovery delay of 2.0 s may be best choice for balancing the sensitivity and experimental time

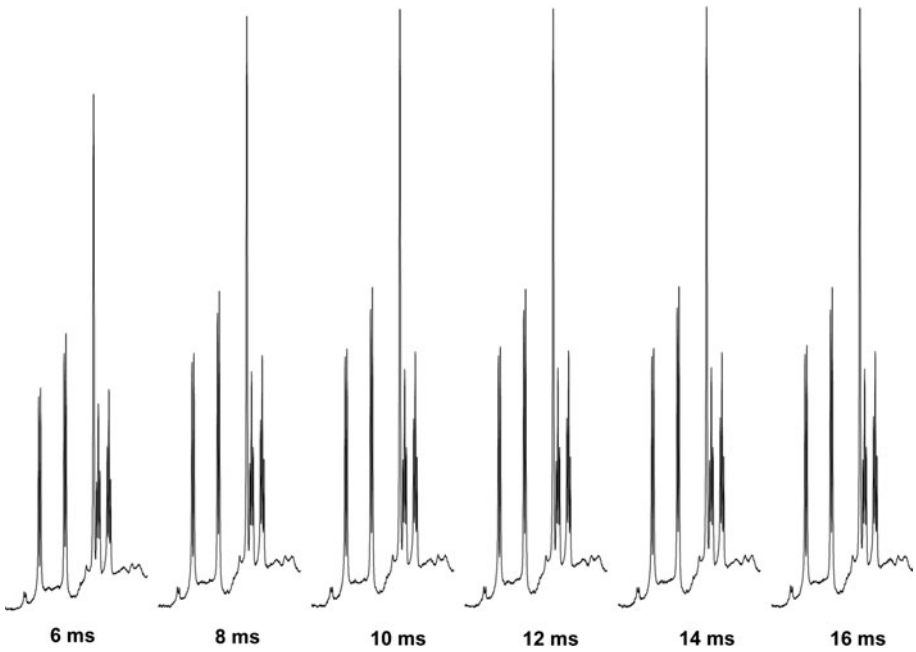


**Fig. 4** Low field regions of 600 MHz RD-WaterLOGSY spectra showing the dependence of signal intensities on the delay Δ (given in the bottom of the spectrum) used to manipulate the radiation damping effect. The experiments were done with recovery delay of 3.0 s and mixing time of 1.4 s using a same sample as Fig. 2. The results show that the intensity reaches to high level when the delay Δ sets at 10.0 ms, but to be on the safe side, a 12.0 ms manipulating delay is recommended

Cui et al. 2011; Shishmarev and Otting 2011). Therefore, the radiation damping manipulation delay should be optimized when using RD-WaterLOGSY on different machines and samples. We find the delay of 12.0 ms may be a good start.

## Conclusion

In conclusion, the RD-WaterLOGSY is proposed and verified with a sample consisting of 0.1 mM HSA, 2.0 mM Trp and 2.0 mM Glu in 90 % $H_2O$/10 % $D_2O$ solution. The new method uses the radiation damping as driving force for selective excitation of the water magnetization. The radiation damping force can be turn "OFF" and "ON", respectively, by simply positing the gradient pulse close to the 180° RF pulse in odd scans and 10–20 ms after the 180° RF pulse in the even scans, resulting in inversion or selective non-inversion of the water magnetization accordingly. The RD-WaterLOGSY experiment runs in an interleaved mode with radiation damping "ON" and "OFF", respectively, which is easy to be achieved on a

modern NMR machine. By taking the differences between the odd scans and even scans, one should get similar results as WaterLOGSY. It is demonstrated that the RD-WaterLOGSY results in about 30 % more sensitive than PO-WaterLOGSY and WaterLOGSY. We expect that the RD-WaterLOGSY will become a favorable method for studying the receptor–ligand interaction and screening the potential leading compounds or drug candidates.

**Acknowledgments** The authors would like to thank the financial support from National Major Basic Research Program of China (2009CB918603, 2013CB910200) and National Natural Science Foundation of China (20875098, 21075132, 21120102038), and the Ministry of Science and Technology of China (2011IM030300).

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