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Triarylmethyl Radical: EPR Signal to Noise at Frequencies between 250 MHz and 1.5 GHz and Dependence of Relaxation on Radical and Salt Concentration and on Frequency

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Abstract

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Dedicated to: Kev Salikhov on the occasion of his 80th birthday.

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In vivo oximetry by pulsed electron paramagnetic resonance is based on measurements of changes in electron spin relaxation rates of probe molecules, such as the triarylmethyl radicals. A series of experiments was performed at frequencies between 250 MHz and 1.5 GHz to assist in the selection of an optimum frequency for oximetry. Electron spin relaxation rates for the triarylmethyl radical OX063 as a function of radical concentration, salt concentration, and resonance frequency were measured by electron spin echo 2-pulse decay and 3-pulse inversion recovery in the frequency range of 250 MHz–1.5 GHz. At constant OX063 concentration, $1/T_1$ decreases with increasing frequency because the tumbling dependent processes that dominate relaxation at 250 MHz are less effective at higher frequency. $1/T_2$ also decreases with increasing frequency because $1/T_1$ is a significant contribution to $1/T_2$ for trityl radicals in fluid solution. $1/T_2 - 1/T_1$, the incomplete motional averaging contribution to $1/T_2$, increases with increasing frequency. At constant frequency, relaxation rates increase with increasing radical concentration due to contributions from collisions that are more effective for $1/T_2$ than $1/T_1$. The collisional contribution to relaxation increases as the concentration of counter-ions in solution increases, which is attributed to interactions of cations with the negatively charged radicals that decrease repulsion between trityl radicals. The Signal-to-Noise ratio (S/N) of field-swept echo-detected spectra of OX063 were measured in the frequency range of 400 MHz–1 GHz. S/N values, normalized by Q , increase as frequency increases. Adding salt to the radical solution decreased S/N because salt lowers the resonator Q . Changing the temperature from 19 to 37 °C caused little change in S/N at 700 MHz. Both slower relaxation rates and higher S/N at higher frequencies are advantageous for oximetry. The potential disadvantage of higher frequencies is the decreased depth of penetration into tissue.

Keywords

cross loop resonator; electron spin relaxation; in vivo imaging; tumbling

1 Introduction

Oxygen concentration (pO_2) is a crucial parameter in physiology and pathophysiology of living organisms. Measurement tools are needed for diagnosis and optimization of treatment strategies for diseases such as cancer and ischemic heart disease [1–4]. In vivo electron paramagnetic resonance (EPR) imaging of O_2 concentration in tissues uses the O_2 effect on the relaxation of paramagnetic probes such as triarylmethyl radicals, lithium phthalocyanine and related compounds [2, 3, 5]. Triarylmethyl-based radicals are promising probes to monitor pO_2 in vivo, because of their sharp, ‘single-line’ EPR spectra and better stability in vivo, compared to the traditional nitroxide-based radicals [6]. Some oximetry studies have been based on the linewidth changes of the spin probes. However, changes in relaxation rates are more sensitive and accurate measure of pO_2 than linewidths [7]. The current method of choice for in vivo oximetry is pulsed EPR measurement of $1/T_1$ of the triarylmethyl radical OX063 [3]. To select an optimum frequency for in vivo oximetry with trityl radicals for animals of varying sizes, information is needed concerning the frequency dependence of experimental signal-to-noise (S/N) and of electron spin relaxation rates.

The clinical goals for in vivo oximetry led to selection of about 250 MHz as a feasible tradeoff of signal intensity and depth of penetration in tissues [8, 9]. Although the eventual goal is human clinical applications, technology development has focused on preclinical studies using mice. These studies could accept less penetration depth if greater signal-to-noise (S/N) could be achieved at a higher RF/microwave frequency. This work investigated the dependence of S/N of OX063 on observation frequency. Most of the experiments were carried out on a locally designed and constructed pulse instrument operated in the ultra-high frequency (UHF) band, which uses an adjustable-frequency cross-loop resonator. EPR signal intensity depends on the number of spins, the resonator filling factor, η , and the resonator quality factor Q . The frequency range from ca. 400 to 1000 MHz was covered with a single resonator that was designed to vary the frequency with constant resonator and sample size. Thus, in this study, the number of spins and η were kept constant. Since resonator Q depends on frequency and on buffer and salt concentration in the aqueous solutions, it was measured for each sample. The S/N results are normalized for change in Q by dividing by Q .

Slower relaxation rates increase the sensitivity to oxygen concentration [3, 10]. Most pulsed EPR measurements suffer from a “dead time” after the end of the pulse during which data cannot be acquired because of the need to protect the detection system from high power pulses, resonator ring-down, switching times of devices, and reflections from impedance mismatches in the RF/microwave system. Thus, changes in spin relaxation rates with frequency could enhance or detract from inherent signal increases with increase in resonant frequency. For in vivo experiments the local concentrations of radicals may vary, so relaxation rates were measured as a function of radical and salt concentrations.

2 Experimental methods

OX063, Tris 8-carboxyl-2,2,6,6-tetra[2-(1-hydroxyethyl)]-benzo(1,2-d : 4,5-d) bis(1,3)-dithiol-4-ylmethyl radical sodium salt (molecular weight 1427 g/mol) was obtained from GE Healthcare. Solutions were prepared in 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid) buffer or phosphate buffered saline (PBS) solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4). To mimic physiological ionic strength, 0.9% wgt/vol of NaCl (154 mM) was included in some solutions in HEPES buffer. Unless otherwise noted, samples were contained in quartz tubes that were de-oxygenated by bubbling with N_2 and flame sealed.

2.1 Spectroscopy

Spin-spin relaxation rates at 250 MHz were measured at the University of Chicago on the previously-described spectrometer [3, 8]. Experiments at 400–1000 MHz were performed at the University of Denver using the variable frequency UHF spectrometer described in [11]. The cross-loop resonator that was used for the multifrequency measurements was designed such that the frequency can be adjusted between about 400–1000 MHz with the same 16-mm OD sample and the same filling factor, thus maintaining all resonator characteristics, except resonance frequency and resonator Q , constant throughout the series of relaxation and S/N measurements. The actual frequency range over which data were obtained for individual samples depended on the height of the liquid in the tube and the dielectric properties of the sample. Frequencies spanned from 420 to 440 MHz at the low end to 980–1020 MHz at the high end. Resonator Q was measured using an HP 8719D network analyzer, which has an operating range of 50 MHz–13.5 GHz. Measurements at 1.5 GHz were performed on the previously described spectrometer at the University of Denver [12].

T_2 was measured by two-pulse spin echo using a $\pi/2 - \tau - \pi - \tau - \text{echo}$ pulse sequence. The initial value of τ was 2400 ns and data were acquired for 1024 60-ns increments of τ . T_1 was measured by inversion recovery with a $\pi - T - \pi/2 - \tau - \pi - \tau - \text{echo}$ pulse sequence. The initial value of T was 1200 ns and data were acquired for 1500 60-ns increments in T . The lengths of the $\pi/2$ pulses were 30 ns at 250 MHz, 80 ns at 400 MHz–1 GHz, and 90 ns at 1.5 GHz. At each frequency the attenuation of the output from the high power amplifier was adjusted to achieve $\pi/2$ and π pulses with the specified length. The fixed τ value in the inversion recovery sequence was 2400 ns at 400 MHz–1 GHz and 1500 ns at 1.5 GHz. Four-step phase cycling was used for the field sweep two-pulse spin echo decay experiment, and eight-step phase cycling was used for the field sweep and inversion recovery experiments. At 400 MHz–1 GHz the spin echo and inversion recovery data were not subjected to background subtraction since the off-resonance baselines were quite flat. For data acquired at 1.5 GHz off-resonance backgrounds were subtracted from the echo decays. S/N was calculated from the amplitude of field-swept 2-pulse echo-detected spectra with a constant τ value of 2400 ns. For the relaxation rate and S/N measurements, five echoes were averaged at each time or field point.

Most of the experiments were performed at ambient temperature, which was about 19 °C. For 700 MHz experiments at 37 °C, a heating tape (Electrothermal[®]) was wrapped around the resonator. The voltage of the heating tape was controlled via a variable autotransformer (Powerstat[®]). A thermocouple was attached to the top of the resonator to monitor the temperature. Layers of cloth and aluminum foil insulated the resonator assembly.

Relaxation rates were calculated by fitting the experimental data to single exponentials. Values of T_1 and T_2 are listed in Tables S1–S5. Based on variations between replicate measurements, the uncertainty in $1/T_1$ and $1/T_2$ is estimated to be less than 8%. Error bars in figures depict one standard deviation.

3 Results

3.1 Frequency dependence of $1/T_2$, $1/T_1$ and $1/T_2-1/T_1$

The spin-lattice relaxation rates, $1/T_1$, for OX063 in aqueous solutions with and without buffer and with or without NaCl are shown in Figure 1 for frequencies ranging from 250 MHz to 9.2 GHz. For all of the solutions studied $1/T_1$ decreased by more than a factor of 2 as frequency is increased over this range. The trityl radical has g values close to the free electron value, so spin rotation and modulation of g anisotropy are relatively ineffective spin-lattice relaxation processes [13]. There is a large proton/deuteron isotope effect on $1/T_1$ for OX063 at 250 MHz [13]. The enhanced spin-lattice relaxation of OX063 at 250 MHz relative to X-band is therefore attributed to modulation of electron-proton dipolar coupling by tumbling of the radical at rates that are comparable to the reciprocal of the 250 MHz resonance frequency. This contribution to $1/T_1$ becomes less effective as frequency increases above 250 MHz. In this motional regime increasing the tumbling correlation time, τ_R , decreases $1/T_1$ [13].

The spin-spin relaxation rates, $1/T_2$, for OX063 in aqueous solutions with and without buffer and with or without NaCl are shown in Figure 2 for frequencies ranging from 250 MHz to 9.2 GHz. $1/T_2$ is less strongly frequency dependent than $1/T_1$. If the radicals were in the rapid tumbling regime, $1/T_1$ would equal $1/T_2$. The motional contribution, $1/T_2-1/T_1$, is plotted as a function of frequency in Figure 3. The concentration dependent contribution to $1/T_2-1/T_1$ is due to collisions. At 250 MHz and low radical concentration $1/T_2-1/T_1$ is very small [3]. However, for all of the solutions studied at frequencies greater than 250 MHz $1/T_2$ is larger than $1/T_1$ even at the lowest radical concentrations, which indicates that there is a significant contribution to $1/T_2$ from incomplete motional averaging of anisotropic interactions. The frequency dependence demonstrates that g anisotropy is a significant contribution to $1/T_2-1/T_1$. Previous work at 250 MHz demonstrated that increasing the tumbling correlation time τ_R , increases $1/T_2$ [13], which is the opposite of the effect of tumbling on $1/T_1$.

3.2 Dependence of relaxation rates on the concentration of OX063

The dependence of relaxation rates on radical concentration (Table 1) is a potentially confounding variable in oximetry [3]. At frequencies between 500 and 1500 MHz the slopes of plots of $1/T_1$ in HEPES buffer with 154 mM NaCl vs radical concentration is 2.1×10^{-2} MHz/mM which is much smaller than the slope of plots of $1/T_2 = 0.15$ MHz/mM under the same conditions (Tables 1, S1 and S3). This observation is consistent with prior reports that $1/T_1$ for trityl radicals at 250 MHz is less strongly dependent on concentration than is $1/T_2$ [3]. The dependence of relaxation on trityl concentration at 500–1500 MHz is similar to that reported previously at 250 MHz (Table 1), which indicates that concentration dependence is independent of frequency. Thus the advantage of monitoring $1/T_1$ rather than $1/T_2$ for oximetry is independent of frequency.

In OX063 and closely related triarylmethyl (trityl) radicals, the molecule is designed to minimize as much as possible the couplings to ^1H or, in the deuterated versions, ^2H . Small unresolved couplings contribute to the inhomogeneously broadened central line in the

spectrum. Although the triaryl methyl radicals are commonly described as exhibiting “single line” or “narrow line” CW EPR spectra, they actually have a very large number of resolved ^{13}C lines because of the large numbers of C atoms in the molecule. Depending on the structure of the trityl, as much as 30% of the signal intensity can be in the resolved ^{13}C sidebands [14]. Details of the hyperfine splitting for OX063 are in the supplementary material (Figures S1 and S2). It is these lines that are the predominant contribution to the concentration-dependence of the relaxation rates. Most collisions are between radicals with different ^{13}C nuclear spin states, and hence collisions cause exchange between different field positions, which contributes to relaxation. The effect of molecular collisions on $1/T_2$ is greater than that on $1/T_1$, because each collision between species of different nuclear spin state causes an increase in $1/T_2$, but only those collisions between species with different electron spin states and different nuclear spin states contribute to $1/T_1$ relaxation.

Bowman and co-workers [14, 15] measured hyperfine splitting of trityl-CH₃, using ENDOR, and calculated the couplings using Gaussian DFT theory. All of the ^{13}C out to the methyl or CH₂CH₂OH substituents could be resolved from the central line in the CW EPR spectra. The isotropic couplings to the 36 methyl protons of trityl-CH₃ are of the order of 1.0 μT . In rigid lattice the anisotropic couplings range from -0.8 to $+1.4$ MHz [15]. The couplings to these substituents create the anoxic width of the center line in the fluid solution spectra. The directly measured T_2 values for several trityl radicals correspond to Lorentzian line widths of less than about 1.0 μT , derivative peak-to-peak, which is smaller than the observed linewidths.

3.3 Dependence of relaxation rates of OX063 on temperature between 19 and 37 °C

For either 0.2 mM or 0.4 mM OX063 in 10 mM HEPES + 154 mM NaCl, increasing the temperature from 19 to 37 °C at 700 MHz increases $1/T_1$ (decreases T_1) by about 16% (Table S2 and S4). This is the direction in which $1/T_1$ is expected to change because of faster tumbling (shorter τ_R) or from increased collision frequencies. For the same solutions the increase in temperature at 700 MHz decreases $1/T_2$ (increases T_2) by about 10%. This is the direction in which $1/T_2$ is expected to change for faster tumbling, but the opposite of that expected for increased collisions. This temperature dependence is small compared with the observed dependence of relaxation rates on frequency at 19 °C.

3.4 Dependence of relaxation rates of OX063 on salt concentration

The concentration of other charged species in solution impacts both the relaxation rates of OX063 and the dependence of the relaxation rates on radical concentration. The effect of ions in solution on relaxation rates is smaller for $1/T_1$ than for $1/T_2$ or $1/T_2 - 1/T_1$ (Figures 1–3). The average slope of plots of $1/T_1$ vs [NaCl] at 700 MHz is 3.2×10^{-5} MHz/mM, which is about a factor of six smaller than the dependence of $1/T_2$ on salt concentration under similar conditions (1.9×10^{-4} MHz/mM). The higher the concentration of ions in solution, the larger the slope of plots of $1/T_2$ vs. concentration of OX063, increasing from 0.11 MHz/mM in water to 0.2 MHz/mM in $10 \times$ PBS at 250 MHz (Table 1). The increase in the collisional contribution to relaxation as the concentration of counter-ions in solution increases is attributed to interactions of cations with the negatively charged radicals which decreases repulsion between trityl radicals.

3.5 Dependence of S/N on resonance frequency

The S/N value was calculated from the two-pulse field-swept echo-detected spectra recorded at frequencies between about 400 and 1000 MHz. The peak height of the echo was divided by the standard deviation of noise in the baseline. Expressions for the frequency dependence of sensitivity in [16, 17] can be rearranged to show that the echo intensity at the output of the cross-loop-resonator is proportional to RF/microwave frequency to the $3/2$ power and to the square root of the resonator Q . The S/N value was therefore normalized by Q , where Q is the quality factor of the detection resonator.

The S/N normalized for Q as a function of frequency for 0.2 and 0.4 mM OX063 solutions in 10 mM HEPES buffer containing 154 mM NaCl are listed in Table S7. The frequency dependence of S/N is plotted in Figure 4. For both radical concentrations, S/N increases as frequency increases. For 0.4 mM OX063 the S/N is 2.0–2.3 times better than for the 0.2 mM sample. The deviation from a factor of 2.0 is an indication of the uncertainty in the measurements.

At a preliminary stage in these measurements, the data obtained at 600 MHz had poorer S/N than the linear trend through adjacent frequencies. Use of a spectrum analyzer with a short wire as antenna on its input revealed that the high noise near 600 MHz was due to interference by the signal from a local television channel. Avoiding frequency regions where communications channels interfere resulted in the final selection of frequencies.

3.6 Dependence of S/N on salt concentration and temperature at 700 MHz

The values of S/N (normalized by Q) at 700 MHz for both 0.2 mM and 0.4 mM OX063 at three salt concentrations and two temperatures are summarized in Table S8. The decrease in resonator Q with increase in RF frequency is due predominantly to eddy current losses in the sample. Over the frequency range from 200 MHz to 1.4 GHz, Gadani et al. [18] showed that the dielectric loss of salt solutions decreased with increasing frequency, and the rate of decrease is larger the larger the salt concentration. Increased RF losses, and reduction in resonator Q with increase in RF frequency due to highly conductive salt solutions is predominantly due to eddy currents rather than dielectric losses [19–22]. Eddy current losses are proportional to the 4th power of the radius of a cylindrical sample [21], so the reduction

in resonator Q is greater for our homogeneous cylindrical samples than would occur for a mouse. Eddy currents are induced by the alternating magnetic field, so some papers call these effects magnetic loss. At constant radical concentration and temperature, S/N is lower at the higher salt concentration (Table S8), which is attributed to the lowering of Q by the salt. The ratio of the S/N at 37 °C to that at 19 °C varied from 0.81 to 1.1, with an average of 0.97 (Table S8). The variation is attributed to experimental uncertainties, and we conclude that the S/N is insensitive to temperature over this narrow interval.

4 Discussion

Relaxation rates increase when salt concentration increases. The change in $1/T_1$ was smaller than in $1/T_2$. One possible explanation is that the cations in the added salt associate with the negatively charged trityl, decreasing the electrostatic repulsion, and thus allowing closer approach of the radicals and enhancing exchange upon collisions. The association of the cations with the anionic trityl also increases the effective size of the radical, slowing its motion, and increasing $1/T_2$. The OX063 was dissolved as the Na^+ salt. At the $\text{pH} = 7.4$ used in these studies, the three carboxylic acids on the para positions of the phenyl rings are essentially fully deprotonated. Salt added to the solution partially compensates the mutual repulsion of OX063 trianions, permitting them to more closely approach one another and collide more frequently. Aggregation of OX063 has been measured at concentrations just above those used in the present studies [23]. Tetramethyl ammonium cation (TMA) was shown to create dimers of OX063 that were detected by mass spectroscopy. DFT calculations revealed a “dome-shaped” stereochemistry of OX063 with a cavity into which TMA fit. The calculations showed “that a single molecule should be able to strongly link two OX063 molecules.” With 30 mM concentrations of OX063, the frozen solution CW spectra exhibit sidebands due to spin-spin interactions. In a small set of previously unpublished comparisons of relaxation rates for trityl- CD_3 , we observed a larger increase in $1/T_2$, but smaller increase in $1/T_1$ when the cation was TMA than for LiCl or NaCl (see Table S9). These trends are consistent with a larger increase in the tumbling correlation time for the trityl in solutions with TMA than with Li^+ or Na^+ .

The literature value for the dissociation constant of Li^+ salts of aliphatic carboxylic acids is ca. 0.1. Approximate calculations, ignoring activity coefficients, indicate that about half of the carboxylic acids (i.e. more than one per trityl) will be associated with a cation in our experiments. Association of Li^+ and Na^+ with the carboxylic acids is likely. However, the Me_4N^+ ion is far less likely to associate with the $-\text{CO}_2^-$ group than the ions with higher charge/radius ratio. Approximating 0.15 M as a simple cubic lattice would put ions ca. 22 Å apart, and the average distance is about 0.554 times this value [24]. Thus, a cation is within a few molecular diameters of every trityl molecule. This may be why the Me_4N^+ ion has a large effect. Although the above comments focus on the cations, note that there is a contribution from the Cl^- present in all of the salt solutions.

5 Summary

S/N (normalized by Q) increases when the frequency increases, in an almost linear relationship. The results presented in this paper show that for OX063, under the conditions

of radical and salt concentration used for in vivo imaging, for a given time following a pulse, echo S/N improves with increase in RF frequency at least up to 1 GHz, which is advantageous for in vivo imaging. $1/T_1$ and $1/T_2$ decrease with increasing frequency in this frequency range. Slower inherent relaxation of the probe in the absence of oxygen provides a larger dynamic range for monitoring enhancement by O_2 , which also is advantageous for imaging. Consequently, selection of an optimum frequency for in vivo imaging depends on the desired depth of penetration for a particular physiological study [9]. Construction of a practical preclinical imager will also depend on practical availability of key spectrometer components.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Microwave frequency dependence of $1/T_1$ for OX063 in pH = 7.4 solution at ambient temperature (19 °C). (●) 0.05 mM OX063 + 154 mM NaCl in 10 mM HEPES, (◆) 0.1 mM OX063 + 154 mM NaCl in 10 mM HEPES, (▲) 0.2 mM OX063 + 154 mM NaCl in 10 mM HEPES, (■) 0.4 mM OX063 + 154 mM NaCl in 10 mM HEPES, (○) 0.2 mM OX063 in 10 mM HEPES, and (△) 0.2 mM OX063 in water.

Fig. 2.

Microwave frequency dependence of $1/T_2$ for OX063 in pH = 7.4 solution at ambient temperature (19 °C). (●) 0.05 mM OX063 + 154 mM NaCl in 10 mM HEPES, (◆) 0.1 mM OX063 + 154 mM NaCl in 10 mM HEPES, (▲) 0.2 mM OX063 + 154 mM NaCl in 10 mM HEPES, (■) 0.4 mM OX063 + 154 mM NaCl in 10 mM HEPES, (○) 0.2 mM OX063 in 10 mM HEPES, and (△) 0.2 mM OX063 in water.

Fig. 3.

Microwave frequency dependence of $(1/T_2 - 1/T_1)$ for OX063 in pH = 7.4 solution at ambient temperature (19 °C). (●) 0.05 mM OX063 + 154 mM NaCl in 10 mM HEPES, (◆) 0.1 mM OX063 + 154 mM NaCl in 10 mM HEPES, (▲) 0.2 mM OX063 + 154 mM NaCl in 10 mM HEPES, (■) 0.4 mM OX063 + 154 mM NaCl in 10 mM HEPES, (○) 0.2 mM OX063 in 10 mM HEPES, and (△) 0.2 mM OX063 in water.

Fig. 4. Microwave frequency dependence of S/N (normalized by $\sqrt{Q}$) for OX063 in 10 mM HEPES buffer at room temperature. (○) 0.2 mM OX063 + 154 mM NaCl in 10 mM HEPES, (△) 0.4 mM OX063 + 154 mM NaCl in 10 mM HEPES. Values for the 0.4 mM solutions were divided two to facilitate comparison.

Tab. 1

Dependence of relaxation rates for OX063 on radical concentration and salt concentration.^a

Relaxation rate	Resonance frequency (MHz)	Parameters held constant	Parameter varied	Impact
1/T ₁	500–1500	10 mM HEPES, 154 mM NaCl	[OX063] = 0.1–0.4 mM	(2.1 ± 0.7) × 10 ⁻² MHz/mM ^b
1/T ₁	250	145 mM NaCl, 37 °C,	[OX063] = 0.1–4.6 mM	3.6 × 10 ⁻² MHz/mM ^c
1/T ₁	700	10 mM HEPES, [OX063] = 0.2 or 0.4 mM, T = 19 or 37 °C	[NaCl] = 0, 77, or 154 mM	3.2 × 10 ⁻⁵ MHz/mM ^d
1/T ₂	500–1500	10 mM HEPES, 154 mM NaCl	[OX063] = 0.1–0.4 mM	0.15 ± 0.01 MHz/mM ^b
1/T ₂	250	Water	[OX063] = 0.2–15 mM	0.11 MHz/mM
1/T ₂	250	0.5 PBS	[OX063] = 0.2–5 mM	0.14 MHz/mM
1/T ₂	250	PBS	[OX063] = 0.2–15 mM	0.15 MHz/mM
1/T ₂	250	145 mM NaCl, 37 °C	[OX063] = 0.1–4.6 mM	0.16 MHz/mM ^c
1/T ₂	250	10 × PBS	[OX063] = 0.2–5 mM	0.20 MHz/mM
1/T ₂	700	10 mM HEPES, [OX063] = 0.2 or 0.4 mM, T = 19 or 37 °C	[NaCl] = 0, 77, or 154 mM	1.9 × 10 ⁻⁴ MHz/mM ^d
1/T ₂	250	PBS, [OX063] = 1 mM	[NaCl] = 0, 77, 154 mM	7.0 × 10 ⁻⁴ MHz/mM
1/T ₂	250	PBS, [OX063] = 2 mM	[NaCl] = 0, 77, 154 mM	1.5 × 10 ⁻³ MHz/mM
1/T ₂	250	PBS, [OX063] = 5 mM	[NaCl] = 0, 77, 154 mM	4.0 × 10 ⁻³ MHz/mM
1/T ₂	250	PBS, [OX063] = 0.5 mM	pO ₂ = 0–21%	6.1 × 10 ⁻² MHz/%O ₂
1/T ₂ –1/T ₁	500–1500	10 mM HEPES, 154 mM NaCl	[OX063] = 0.1–0.4 mM	0.13 ± 0.01 MHz/mM ^b
1/T ₂ –1/T ₁	700	10 mM HEPES, 0.2 or 0.4 mM OX063, T = 19 or 37 °C	[NaCl] = 0, 77, or 154 mM	1.4 × 10 ⁻⁴ MHz/mM ^d

^a pH = 7.4, temperature = 19–21 °C, unless noted.

^b Average at 7 resonance frequencies.

^c Data from ref. [3].

^d Average for two concentrations of OX063 at two temperatures.