Published in final edited form as:

J Magn Reson. 2011 April ; 209(2): 142–148. doi:10.1016/j.jmr.2011.01.003.

Spin-Label Oximetry at Q- and W-Band

W.K. Subczynski^a, L. Mainali^a, T.G. Camenisch^a, W. Froncisz^b, and J.S. Hyde^{a,*}

^aDepartment of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

^bDepartment of Biophysics, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University, Krakow, Poland

Abstract

Spin-lattice relaxation times (T_1 s) of both small water-soluble spin labels in the aqueous phase as well as lipid-type spin labels in membranes increase when the microwave frequency increases from 2 to 35 GHz (Hyde et al., *J. Phys. Chem. B* 108 [2004] 9524–9529). The T_1 measured at W-band (94 GHz) for the water-soluble spin labels CTPO and TEMPONE (Froncisz et al., *J. Magn. Reson.* 193 [2008] 297–304) is, however, shorter than when measured at Q-band (35 GHz). In this paper, the decreasing trends at W-band have been confirmed for commonly used lipid-type spin labels in model membranes. It is concluded that the longest values of T_1 will generally be found at Q-band, noting that long values are advantageous for measurement of bimolecular collisions with oxygen. The contribution of dissolved molecular oxygen to the relaxation rate was found to be independent of microwave frequency up to 94 GHz for lipid-type spin labels in membranes. This contribution is expressed in terms of the oxygen transport parameter $W = T_1^{-1}(\text{Air}) - T_1^{-1}(\text{N}_2)$, which is a function of both concentration and translational diffusion of oxygen in the local environment of a spin label. The new capabilities in measurement of the oxygen transport parameter using saturation-recovery (SR) EPR at Q- and W-band have been demonstrated in saturated (DMPC) and unsaturated (POPC) lipid bilayer membranes with the use of stearic acid (n-SASL) and phosphatidylcholine (n-PC) spin labels, and compared with results obtained earlier at X-band. SR EPR spin-label oximetry at Q- and W-band has the potential to be a powerful tool for studying samples of small volume, ~30 nL. These benefits, together with other factors such as a higher resonator efficiency parameter and a new technique for canceling free induction decay signals, are discussed.

Introduction

The first reports that oxygen can affect EPR spectra of organic free radicals in solutions via liquid-phase Heisenberg exchange are dated about 50 years ago [1–3]. Later, Povich pointed out that oxygen-broadening of the EPR spectra of spin labels can be used to measure dissolved oxygen concentration and diffusion in homogeneous solvents [4,5]. Backer et al. [6] were the first who applied spin-label oximetry to biological systems. Shortly after, the water-soluble spin probe 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxy (CTPO),

© 2011 Elsevier Inc. All rights reserved.

* Author to whom correspondence should be addressed: James S. Hyde, Ph.D., Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226-0509, Phone: (414) 456-4005, Fax: (414) 456-6512, jshyde@mcw.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

which is widely used for spin-label oximetry, was introduced (see Ref. [7] for additional historical details). The first rigorous spin-label oximetric study was carried out by Windrem and Plachy [8], who reported in 1980 profiles of the oxygen diffusion-concentration product across the lipid bilayer membrane obtained from line-broadening measurements of lipid-soluble spin labels. These first applications were based on the oxygen-induced line-broadening of spin-label EPR spectra.

In the beginning of the 1980s, T_1 -sensitive spin-label oximetry methods were developed at the National Biomedical EPR Center at the Medical College of Wisconsin for measurement of the product of the oxygen diffusion constant and the oxygen concentration in membranes [9–11]. T_1 -sensitive methods have significant advantages over T_2 -sensitive methods because T_1 (usually 1 to 10 μ s) is from one to three orders of magnitude longer than T_2 . Additionally, T_1 -sensitive methods can be applied to any system that can be spin-probed or spin-labeled, without the need for a narrow EPR line or the presence of a resolved superhyperfine structure. T_1 -sensitive oximetry methods include saturation recovery (SR) (the absolute T_1 method [11–13]), CW saturation [10,14], passage display [15], and the multiquantum approach [16,17]. Spin-label oximetry can be used as a quantitative method because every collision of oxygen with a spin label contributes to a change in the EPR spectrum in both T_1 and T_2 sensitive methods [10,18–20].

The first oximetry measurements using either T_1 - or T_2 -sensitive methods were made at X-band. The development of loop-gap resonators (LGRs) [21] permitted oximetry measurements to be made at lower microwave frequencies for both analytical samples and small laboratory animals [22,23]. This direction developed into a significant branch of *in vivo* oximetry and imaging in which oxygen concentration and the distribution of oxygen in laboratory animals [24–26] and also in humans [24,27] are measured. These measurements are done at L- and S-band using T_2 -sensitive methods. This direction of research has also stimulated development of new spin labels including deuterated CTPO [28] and trityls [29], as well as other microscopic particulate probes, lithium phthalocyanine [30], coal-derived particles (fusinite) [31], and India ink [32].

The development of new LGRs [33,34] has allowed transfer of the T_1 -sensitive SR oximetry method to higher microwave frequencies, including Q- and W-band, which permits oximetry measurements to be made for very small water-containing samples (~30 nL). In our previous work [35], we reported oximetric data acquired using SR at frequencies from 2 to 35 GHz. Here we present oximetry measurements at Q- and W-band and discuss advantages of spin-label oximetry at these frequencies, especially for biological samples that can only be obtained in limited quantity. In two previous papers [35,36], we showed that (1) the T_1 of water-soluble spin labels depends on microwave frequency (being largest at Q-band), and that (2) the effect of collisions between oxygen and spin-labels on the measured T_1 value is independent of frequency up to 35 GHz. Here we extend SR measurements to W-band, showing also that the T_1 of lipid-type spin labels in membranes exhibits a maximum value at Q-band.

Additionally, we showed that the measured collision rate between the nitroxide moiety and oxygen is the same from 2 to 94 GHz. Although the sensitivity in terms of number of spins required for good SR signals increases markedly with increase in microwave frequency, the concentration sensitivity of SR of spin labels has been found to be substantially independent of microwave frequency. It follows that the best frequency for spin-label oximetry in terms of sensitivity to bimolecular collisions with molecular oxygen is 35 GHz (Q-band). However, W-band has its own advantages over other frequencies, which are discussed.

Materials and Methods

Materials

One-palmitoyl-2-(n-doxylstearoyl)phosphatidylcholine spin labels (n-PC, n = 5, 12, or 16), tempocholine-1-palmitoyl-2-oleoylphosphatidic acid ester (T-PC), dimyristoylphosphatidylcholine (DMPC), and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). The n-doxylstearic acid spin label (n-SASL, n = 5, 10, or 16) and the cholesterol analog, cholestane spin label (CSL), were purchased from Molecular Probes (Eugene, OR). Other chemicals, of at least reagent grade, were purchased from Sigma-Aldrich (St. Louis, MO).

Sample preparation

The membranes used in this work were multilamellar dispersions of lipids (liposomes) containing 0.5 mol% of n-SASL or CSL (in DMPC) or containing 1 mol% n-PC or T-PC (in POPC), and were prepared as described previously [37]. The buffer used for measurements with n-SASL was 0.1 M sodium borate at pH 9.5. To ensure that all carboxyl groups of SASL were ionized in DMPC membranes, a rather high pH was chosen [38,39]. For other measurements, 10 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) and 150 mM NaCl (pH 7.0) were used. The dense liposome dispersion (the loose pellet after centrifugation) with a final spin-label concentration of 1 to 3 mM was used. The structures of water-soluble and lipid-type spin labels used in this work and in our previous Q- and W-band SR measurements [35,36], together with structures of phospholipids, saturated DMPC, and unsaturated POPC, are shown in Figs. 1a and b.

EPR measurements

For EPR measurements, samples were transferred either to a capillary made from the gas-permeable methylpenetene plastic known as TPX (i.d. 0.6 mm; X-band) or to a Teflon tube (i.d. 0.2 mm; Q-band). Quartz capillaries (i.d. 0.15 mm) were used at W-band. At X- and Q-band, samples were equilibrated directly in the resonator with nitrogen or a mixture of nitrogen and air adjusted with a flowmeter (Matheson Gas Products, model No. 7631 H-604) [20]. The same gas was used for temperature control. At W-band, samples were equilibrated with either nitrogen or air at room temperature outside the resonator, transferred to a quartz capillary, and positioned in the resonator of the W-band spectrometer, which is equipped with a temperature-control system. At W-band, we also occasionally used the gas exchange method of removing oxygen, placing the sample into a Teflon sample tube and equilibrating it with the gas directly in the resonator. Results were indistinguishable.

Measurements were made using SR capabilities of X-, Q-, and W-band EPR spectrometers. Spectrometers and LGRs used for Q- and W-band measurements were described earlier [35,36]. For water-soluble spin labels, the duration of the microwave pulse was 0.3 μ s. For lipid spin labels in membrane suspensions, the pulse duration was 0.3 to 1 μ s. All recovery times acquired in these measurements were single exponential in character. For membrane spin labels, motion was sufficiently slow such that the nitrogen nuclear relaxation times were shorter than the electron T_1 values, resulting in strong coupling of the three hyperfine lines. The statement that nuclear relaxation times are sufficiently short arises from the prediction of Yin et al. [40] that if this were not true, multi-exponential recovery times would be observed, contrary to the experiment. Experimental knowledge of nitrogen nuclear relaxation times under the conditions of this experiment does not exist to the best of our knowledge.

For both Q- and W-band SR, the low-field hyperfine line, which is most intense, was observed (Fig. 2; see also Fig. 4 in Ref. [35] for Q-band spectra). At X-band, the central line was observed.

Data acquisition and processing

Typically, 10^6 decays were averaged, half of which were off-line and differenced for baseline correction, with 2048 (X- and Q-band) and 1024 (W-band) data points per decay. Sampling intervals depended on sample, temperature, oxygen tension, and microwave frequency, and were from 1 to 40 ns. Total accumulation time was about 2 to 5 min. Recovery curves were fitted by single, double, and triple exponentials, and compared. Results indicated that for all of the recovery curves obtained in this work, no substantial improvement in the fitting was observed when the number of exponentials was above one, establishing that recovery curves can be analyzed as single exponentials. Fits were based on a damped linear least-squares method, which utilizes the Gauss-Newton minimization procedure to which a scalar factor is included. This helps to “damp” the process toward the minimum and assures convergence in the iterative steps. The damped least-squares method has proven successful for fitting exponential decay curves [41] and EPR spectra [42]. Figure 3 shows typical SR curves for 5-PC in POPC membranes from Q- and W-band measurements in the presence and absence of oxygen. Fits to single exponentials are excellent. Decay time constants were determined with accuracy better than $\pm 3\%$.

Results

T_1 measurements of deoxygenated samples

In Fig. 4, results obtained in the present work at W-band for lipid-type spin labels n-SASL and CSL in saturated DMPC membranes (see Fig. 1a for their structures) are combined with previously obtained measurements from the microwave frequency range 2.5 to 35 GHz [35]. All spin-lattice relaxation times measured at W-band are shorter than when measured at Q-band. These data confirm again the remarkable fact that the values obtained at 94 GHz depart significantly from the trend observed using data obtained at lower frequencies. Plotted data result in smooth curves, lending credence to the reliability of the surprisingly short times obtained at 94 GHz. It can be concluded that the longest obtainable values for T_1 will generally be found at Q-band.

Spin labels CTPO, TEMPONE, and n-SASL, together with saturated DMPC membranes, were used in our previous multifrequency measurements for “historical” reasons. Most earlier SR measurements were obtained from these spin labels in DMPC membranes [11,13,43], which provides reference data. However, the more biologically relevant phospholipid is unsaturated POPC, and the much better lipid-type spin-label analogues are the phospholipid analogues n-PC and T-PC, which can be obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Because of these circumstances, we show in Fig. 5 new data obtained at X-, Q-, and W-band for the phospholipid-type spin labels (5-, 16-, and T-PC) in unsaturated POPC membranes (see Fig. 1a for these structures). As expected, the longest T_1 values were measured at Q-band. Values at X- and W-band were significantly shorter, confirming trends observed for other spin labels in water and membrane environments. The data of Figs. 4 and 5 indicate in all cases that T_1 values are shorter at 94 GHz than at 35 GHz. These data indicate that the observed T_1 dependence on microwave frequency is independent of the structure of the nitroxide moiety, the structure of the environment of the spin label, the polarity of the local nitroxide environment (which changes with the depth in the membrane), the rate and anisotropy of motion, and the temperature. This experimental finding complements our earlier paper [36], showing data only for CTPO and TEMPONE (see Fig. 1b for these structures). The break in the trend of relaxation rate versus microwave

frequency (Figs. 4 and 5; see also Fig. 8 in Ref. [36]) requires further investigation. Hofbauer et al. [44] report unexpectedly short T_{1e} values for 1 mM solution of TEMPO in a 10% glycerol-water mixture at W-band and suggest a mechanism involving dynamic modulation of the g-value.

The oxygen transport parameter

Bimolecular collision rates with oxygen as a function of microwave frequency from 2.54 to 35 GHz using 16-SASL in DMPC bilayers at 27°C were reported by Hyde et al. [35]. Data were expressed in terms of the oxygen transport parameter defined by Kusumi et al. [11] by Eq. (1):

$$W(x) = T_1(\text{Air}, x) - T_1(\text{N}_2, x) \sim D(x)C(x), \quad (1)$$

where T_1 values are the spin-lattice relaxation times of the nitroxide in samples equilibrated with atmospheric air and nitrogen, respectively. $W(x)$ is proportional to the product of the local translational diffusion coefficient $D(x)$ and the local concentration $C(x)$ of oxygen at a “depth” x in the lipid bilayer that is in equilibrium with atmospheric oxygen.

Measurement of the oxygen transport parameter in the same system has been extended to 94 GHz, and all results are presented in Table 1. A new value of $3.02 \mu\text{s}^{-1}$ obtained at 94 GHz is in satisfactory agreement with values reported at lower frequencies. It is concluded that the effect of bimolecular collisions of oxygen on observed spin-label relaxation rates is independent of microwave frequency even at W-band, where spin-lattice relaxation times behave anomalously. To emphasize this independence, we added in Table 1 the values of T_1 s. These values change drastically with microwave frequency, increasing more than five times between 2.54 and 34.6 GHz before decreasing at 94 GHz. These results, together with the fact that the long values of the spin-lattice relaxation times are advantageous for measurement of bimolecular collisions with oxygen, indicate that the best frequency for spin-label oximetry measurements using SR is Q-band.

It is notable that for such a large change of microwave frequency (from 2 to 94 GHz), which causes a five-time increase in the spin-lattice relaxation times of lipid-type spin labels, the oxygen transport parameter measured for the same spin label at the same temperature is invariant of frequency. Thus, the measured spin-lattice relaxation rate (T_1^{-1}) can be related to the oxygen transport parameter, or expressed in frequency-independent oxygen transport parameter units (see Eq. (1)), which we call a frequency-independent ruler or a golden standard for T_1 measurements.

Profiles of the oxygen transport parameter across membranes

One of the achievements of spin-label oximetry is the capability to acquire profiles of the oxygen transport parameter across lipid bilayer membranes. These profiles contain significant structural information that is sensed by the solubility and movement of the small hydrophobic molecule, molecular oxygen [11,43,45–47]. They also permit calculation of a significant membrane parameter—namely, the membrane oxygen permeability coefficient, which connects oxygen flux across the membrane with the difference in oxygen concentration in water on both sides of the bilayer [13,48,49]. In Fig. 6, we compared profiles of the oxygen transport parameter across the unsaturated POPC membrane obtained at X-, Q-, and W-band.

Based on the profiles obtained from X-, Q-, and W-band measurements (Fig. 6), values of the oxygen permeability coefficient across the POPC bilayer were evaluated using methods previously described [13]. These values are listed in Table 2. They are independent of

microwave frequency within an error of 30% (see Ref. [48] for the minimal and maximal evaluations of the oxygen permeability coefficient across lipid bilayer membranes). These data confirm our conclusion that the results of the oximetry measurements are independent of microwave frequency.

Discussion

The W-band capabilities for studying oxygen transport within and across biological membranes at Q-band have been discussed previously [35]. In the present paper, we demonstrate measurement of the oxygen transport parameter in model membranes as a function of the depth in the lipid bilayer (Fig. 6). Suspensions of POPC liposomes for Q-band measurements were prepared in the same way as for X-band measurements. The time of observation at Q-band was similar to that at X-band (~3 min) as was the signal-to-noise ratio (compare SR signals in Fig. 3a and b with those in Ref. [37]). An LGR, with a sample volume of 30 nL, was used for Q-band measurements [35,50], while the sample volume of the LGR used for X-band was 3 μ L [21]. Feasibility is therefore established for experiments on samples with limited sample volumes. For example, all profiles obtained at X-band for calf- [46] and pig-lens [51] lipid membranes were obtained for samples prepared from 50 eyes. Similarly, measurements for cortical and nuclear bovine-lens lipid membranes were based on samples prepared from 100 eyes [47]. Because the sample volume at Q-band is 100 times smaller, reliable profiles can, in principle, be obtained based on samples prepared from one eye.

Some recommendations for handling, degassing, and equilibrating with gas-mixture samples of small size are given in Ref. [52]. Yin and Hyde [53] showed that the use of high observing power in SR EPR experiments does not affect the ability to extract bimolecular collision rates and can increase the signal-to-noise ratio up to ten times. Thus, this approach can be used if the signal-to-noise ratio in samples prepared from biological materials is too low. Another way to increase the signal-to-noise ratio is to increase the repetition rate during data acquisition. This approach should be beneficial for measurements at microwave frequencies at which the spin-lattice relaxation time is shorter—for example, at W-band compared with Q-band.

The results of this paper also demonstrate the ability to make oximetry measurements at W-band. Saturation recovery “concentration sensitivity” has not previously been discussed in the literature. The general conclusion of the present work as well as in previous papers in this series is that SR concentration sensitivity is experimentally found to be independent of microwave frequency. This is one of the significant findings of the present study. However, when sensitivity issues are considered based on the number of spins measured, there is very significant increase in sensitivity as the microwave frequency increases. In the present study, the volume of sample was 20 times less at W-band compared with Q-band, but the SR signal intensity at a fixed accumulation time remained about the same.

A new technique for canceling free induction decay (FID) contamination was introduced for Q-band SR [35] and has been extended to W-band. In X-band, as practiced in this laboratory, the pump and observing power are derived from the same source, and signals are a superposition of FID and SR responses. The technique of 180° pump-phase modulation, which modulates the signal of the FID response but not that of the SR response, permits separation of the superimposed signals. In Q- and W-band spectrometers, the observing and pump powers are derived from separate synthesizers. The synthesizers are phase-locked, but the pump synthesizer is offset from the observe by a small frequency difference, typically 1 kHz. A benefit of this method is that the two frequencies are well within the spin packet width, but cannot drift with respect to each other. With the pump and observed frequencies

separated by this difference, FID signals are effectively averaged away, leaving only the SR signal.

Other significant factors in the experiments presented here are the high resonator efficiency parameter Λ (the field produced by 1 Watt of incident power [54]) and the low resonator quality factor Q . The Λ is between 10 and 20 for Q- and W-band LGRs. The LGR with a water sample has a loaded Q factor of approximately 100. One significant consequence of high Λ and low resonator Q is that the dead time between the end of the pulse and the start of data collection can be reduced because there is less stored energy in the resonator when the pulse ends. Shortening of the dead time is crucial for the discrimination by oxygen transport method, which should allow discrimination of domains with high lipid exchange rates [43].

Acknowledgments

This work was supported by grants EY015526, EB002052, and EB001980 of the National Institutes of Health.

References

1. Deguchi Y. Proton hyperfine spectra of diphenyl picryl hydrazyl. *J. Chem. Phys.* 1960; 32:1584–1585.
2. Haussler KH. Über den einfluß des gelösten sauerstoffs auf die linienbreite der elektron-spin-resonanz in lösung. *Naturwissenschaften.* 1960; 47:251.
3. Edelstein N, Kwok A, Maki AH. Effects of hydrostatic pressure and temperature on spin exchange between free radicals in solution. *J. Chem. Phys.* 1964; 41:3473–3478.
4. Povich MJ. Measurement of dissolved oxygen concentrations and diffusion coefficients by electron spin resonance. *Anal. Chem.* 1975; 47:346–347.
5. Povich MJ. Electron spin resonance oxygen broadening. *J. Phys. Chem.* 1975; 79:1106–1109.
6. Backer JM, Budker VG, Eremenko SI, Molin YuN. Determination of the kinetics of biochemical reactions with oxygen using exchange broadening in the ESR spectra of nitroxide radicals. *Biochim. Biophys. Acta.* 1977; 460:152–156. [PubMed: 192284]
7. Subczynski WK. Spin-label oximetry in biological and model systems. *Curr. Top. Biophys.* 1999; 23:69–77.
8. Windrem DA, Plachy WZ. The diffusion-solubility of oxygen in lipid bilayers. *Biochim. Biophys. Acta.* 1980; 600:655–665. [PubMed: 6250601]
9. Popp CA, Hyde JS. Effects of oxygen on EPR spectra of nitroxide spin-labels probes of model membranes. *J. Magn. Reson.* 1981; 43:294–258.
10. Subczynski WK, Hyde JS. The diffusion-concentration product of oxygen in lipid bilayers using the spin-label T_1 method. *Biochim. Biophys. Acta.* 1981; 643:283–291. [PubMed: 6261814]
11. Kusumi A, Subczynski WK, Hyde JS. Oxygen transport parameter in membranes as deduced by saturation recovery measurements of spin-lattice relaxation times of spin labels. *Proc. Natl. Acad. Sci. USA.* 1982; 79:1854–1858. [PubMed: 6952236]
12. Yin J-J, Hyde JS. Spin-label saturation recovery electron spin resonance measurements of oxygen transport in membranes. *Zeitschrift für Physikalische Chemie Folge.* 1987; 153:57–65.
13. Subczynski WK, Hyde JS, Kusumi A. Oxygen permeability of phosphatidylcholine-cholesterol membranes. *Proc. Natl. Acad. Sci. USA.* 1989; 86:4474–4478. [PubMed: 2543978]
14. Altenbach C, Froncisz W, Hyde JS, Hubbell WL. Conformation of spin-labeled melitin at membrane surface investigated by pulse saturation recovery and continuous wave power saturation electron paramagnetic resonance. *Biophys. J.* 1989; 56:1183–1191. [PubMed: 2558734]
15. Froncisz W, Lai C-S, Hyde JS. Spin-label oximetry: kinetic studies of cell respiration using a rapid-passage T_1 -sensitive electron spin resonance display. *Proc. Natl. Acad. Sci. USA.* 1985; 82:411–415. [PubMed: 2982155]

16. Mchaourab HS, Hyde JS. Dependence of the multiquantum EPR signal on the spin lattice relaxation time. Effect of oxygen in spin-label membranes. *J. Magn. Reson. B.* 1993; 101:178–184.
17. Mchaourab HS, Hyde JS, Feix JB. Binding and state of aggregation of spin-labeled cecropin AD in phospholipid membranes: effects of surface charge and fatty acyl chain length. *Biochemistry.* 1994; 33:6691–6699. [PubMed: 8204604]
18. Hyde JS, Subczynski WK. Simulation of ESR spectra of the oxygen-sensitive spin-label probe CTPO. *J. Magn. Reson.* 1984; 56:125–130.
19. Subczynski WK, Hyde JS. Diffusion of oxygen in water and hydrocarbons using an electron spin resonance spin label technique. *Biophys. J.* 1984; 45:743–748. [PubMed: 6326877]
20. Hyde, JS.; Subczynski, WK. Spin-label oximetry. In: Berliner, LJ.; Reuben, J., editors. *Biological Magnetic Resonance, Vol. 8, Spin Labeling: Theory and Applications.* New York: Plenum; 1989. p. 399-425.
21. Froncisz W, Hyde JS. The loop-gap resonator: a new microwave lumped circuit ESR sample structure. *J. Magn. Reson.* 1982; 47:515–521.
22. Lukiewicz SJ, Lukiewicz SG. *In vivo* ESR spectroscopy of large biological objects. *Magn. Reson. Med.* 1984; 1:297–298.
23. Subczynski WK, Lukiewicz S, Hyde JS. Murine *in-vivo* L-band ESR spin-label oximetry with loop-gap resonator. *Magn. Reson. Med.* 1986; 3:747–754. [PubMed: 3023786]
24. Subczynski, WK.; Swartz, HM. EPR oximetry in biological and model samples. In: Eaton, SS.; Eaton, GR.; Berliner, LJ., editors. *Biological Magnetic Resonance, Vol. 23, Biomedical ESR—Part A: Free Radicals, Metals, Medicine, and Physiology.* Boston: Kluwer; 2005. p. 229-282.
25. Halpern, HJ.; Bowman, MK.; Eaton, GR.; Eaton, SS.; Ohno, K. Low frequency EPR spectrometers: MHz range, EPR imaging and *in vivo* EPR. Boca Raton, FL: CRC Press; 1991. p. 45-63.
26. Khan M, Kwiatkowski P, Rivera BK, Kuppusamy P. Oxygen and oxygenation in stem-cell therapy for myocardial infarction. *Life Sci.* 2010; 87:269–274. [PubMed: 20600148]
27. Williams BB, Khan N, Zaki B, Hartford A, Ernstoff MS, Swartz HM. Clinical electron paramagnetic resonance (EPR) oximetry using India ink. *Adv. Exp. Med. Biol.* 2010; 662:149–156. [PubMed: 20204785]
28. Halpern J, Perik M, Nguyen T-D, Spencer DP, Teicher BA, Lin YJ. Selective isotopic labeling of a nitroxide spin label to enhance sensitivity for T2 oximetry. *J. Magn. Reson.* 1990; 90:40–51.
29. Ardenkjaer-Larsen JH, Laursen I, Leunbach I, Ehnholm G, Wistrand L-G, Petersson JS, Golman K. EPR and DNP properties of certain novel single electron contrast agents intended for oximetric imaging. *J. Magn. Reson.* 1998; 133:1–12. [PubMed: 9654463]
30. Liu KJ, Moussavi M, Norby SW, Vahidi N, Walczak T, Wu M, Swartz HM. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. *Proc. Natl. Acad. Sci. USA.* 1993; 90:5436–5442.
31. Swartz HM, Boyer S, Gest P, Glockner JF, Hu H, Liu KJ, Moussavi M, Norby SW, Vahidi N, Walczak T, Wu M, Clarjson RB. Measurements of pertinent concentrations of oxygen *in vivo*. *Magn. Reson. Med.* 1991; 20:333–339. [PubMed: 1663568]
32. Goda F, Liu JK, Walczak T, O'Hara JA, Jiang J, Swartz HM. *In vivo* oximetry using EPR and India ink. *Magn. Reson. Med.* 1995; 33:237–245. [PubMed: 7707915]
33. Froncisz W, Oles T, Hyde JS. Q-band loop-gap resonator. *Rev. Sci. Instrum.* 1986; 57:1095–1099.
34. Sidabras JW, Mett RR, Froncisz W, Camenisch TG, Anderson JR, Hyde JS. Multipurpose EPR loop-gap resonator and cylindrical TE(011) cavity for aqueous samples at 94 GHz. *Rev. Sci. Instrum.* 2007; 78:034701. [PubMed: 17411204]
35. Hyde JS, Yin J-J, Subczynski WK, Camenisch TG, Ratke JJ, Froncisz W. Spin label EPR T_1 values using saturation recovery from 2 to 35 GHz. *J. Phys. Chem. B.* 2004; 108:9524–9529.
36. Froncisz W, Camenisch TG, Ratke JJ, Anderson JR, Subczynski WK, Strangeway RA, Sidabras JW, Hyde JS. Saturation recovery EPR and ELDOR at W-band for spin labels. *J. Magn. Reson.* 2008; 193:297–304. [PubMed: 18547848]

37. Subczynski WK, Lewis RNAH, McElhaney RN, Hodges RS, Hyde JS, Kusumi A. Molecular organization and dynamics of 1-palmitoyl-2-oleoylphosphatidylcholine bilayers containing a transmembrane α -helical peptide. *Biochemistry*. 1998; 37:3156–3164. [PubMed: 9485469]
38. Egreet-Charlier M, Sanson A, Ptak M, Bouloussa O. Ionization of fatty acids at lipid-water interface. *FEBS Lett*. 1978; 89:313–316. [PubMed: 207576]
39. Kusumi A, Subczynski WK, Hyde JS. Effects of pH on ESR spectra of stearic acid spin labels in membranes: probing the membrane surface. *Fed. Proc*. 1982; 41:1394.
40. Yin J-J, Pasenkiewicz-Gierula M, Hyde JS. Lateral diffusion of lipids in membranes by pulse saturation recovery electron spin resonance. *Proc. Natl. Acad. Sci. USA*. 1987; 84:964–968. [PubMed: 3029766]
41. Laiken SL, Printz MP. Kinetic class analysis of hydrogen exchange data. *Biochemistry*. 1970; 9:1547–1553. [PubMed: 5436143]
42. Pasenkiewicz-Gierula M, Antholine WE, Subczynski WK, Baffa O, Hyde JS, Petering DH. Assessment of the ESR spectra of CuKTSM2. *Inorg. Chem*. 1987; 26:3945–3949.
43. Ashikawa I, Yin J-J, Subczynski WK, Kouyama T, Hyde JS, Kusumi A. Molecular organization and dynamics in bacteriorhodopsin-rich reconstituted membranes: discrimination of lipid environments by the oxygen transport parameter using a pulse ESR spin-labeling technique. *Biochemistry*. 1994; 33:4947–4952. [PubMed: 8161556]
44. Hofbauer W, Earle KA, Dunnam CR, Moscicki JK, Freed JH. High-power 95 GHz pulsed electron paramagnetic resonance spectrometer. *Rev. Sci. Instrum*. 2004; 75:1194–1208.
45. Subczynski WK, Wisniewska A, Hyde JS, Kusumi A. Three-dimensional dynamic structure of the liquid-ordered domain in lipid membranes as examined by pulse-EPR oxygen probing. *Biophys. J*. 2007; 92:1573–1584. [PubMed: 17142270]
46. Widomska J, Raguz M, Dillon J, Gaillard ER, Subczynski WK. Physical properties of the lipid bilayer membrane made of calf lens lipids: EPR spin labeling studies. *Biochim. Biophys. Acta*. 2007; 1768:1454–1465. [PubMed: 17451639]
47. Raguz M, Widomska J, Dillon J, Gaillard ER, Subczynski WK. Physical properties of the lipid bilayer membrane made of cortical and nuclear bovine lens lipids: EPR spin-labeling studies. *Biochim. Biophys. Acta*. 2009; 1788:2380–2388. [PubMed: 19761756]
48. Subczynski WK, Hopwood LE, Hyde JS. Is the mammalian cell plasma membrane a barrier to oxygen transport? *J. Gen. Physiol*. 1992; 100:69–87. [PubMed: 1324973]
49. Widomska J, Raguz M, Subczynski WK. Oxygen permeability of the lipid bilayer membrane made of calf lens lipids. *Biochim. Biophys. Acta*. 2007; 1768:2635–2645. [PubMed: 17662231]
50. Klug CS, Camenisch TG, Hubbell WL, Hyde JS. Multiquantum EPR spectroscopy of spin-labeled arrestin K267C at 35 GHz. *Biophys. J*. 2005; 88:3641–3647. [PubMed: 15749769]
51. Raguz M, Widomska J, Dillon J, Gaillard ER, Subczynski WK. Characterization of lipid domains in reconstituted porcine lens membranes using EPR spin-labeling approaches. *Biochim. Biophys. Acta*. 2008; 1778:1079–1090. [PubMed: 18298944]
52. Subczynski WK, Felix CC, Klug CS, Hyde JS. Concentration by centrifugation for gas exchange EPR oximetry measurements with loop-gap resonators. *J. Magn. Reson*. 2005; 176:244–248. [PubMed: 16040261]
53. Yin J-J, Hyde JS. Use of high observing power in electron resonance saturation-recovery experiments in spin-labeled membranes. *J. Chem. Phys*. 1989; 91:6029–6035.
54. Hyde, JS.; Froncisz, W. Loop gap resonators. In: Hoff, AJ., editor. *Advanced EPR: Applications in Biology and Biochemistry*. Amsterdam: Elsevier; 1989. p. 277-306.

Fig. 1.

a: Chemical structures of lipid-type spin labels together with structures of DMPC and POPC to illustrate approximate locations of nitroxide moieties across the membrane. **b:** Chemical structures of water-soluble spin labels.

Fig. 2.
EPR spectra of lipid-type spin labels in POPC bilayers at W-band (25°C).

Fig. 3.

Representative Q-band (**a, b**) and W-band (**c, d**) SR signals with fitted curves and the residuals (the experimental signal minus the fitted curve) of 5-PC in the POPC membrane obtained for samples equilibrated with nitrogen (**a, c**), 50% air (**b**, Q-band) and 100% air (**d**, W-band). Equilibration with 100% air significantly shortened the T_1 value, and the available pump power was insufficient to saturate the signal. The accumulation time was held constant.

Fig. 4. Microwave frequency dependence of the spin-lattice relaxation rate of stearic acid spin labels (5-, 12-, and 16-SASL) and CSL in saturated DMPC membranes.

Fig. 5. Microwave frequency dependence of the spin-lattice relaxation rate of phospholipid-type spin labels (5-, 16-, and T-PC) in unsaturated POPC membranes. Measurements at X- and Q-band were performed at 29–30°C. Measurements at W-band were performed at room temperature.

Fig. 6.

Profile of the oxygen transport parameter across the POPC membrane obtained at X-, Q-, and W-band. Measurements and equilibrations with gas for X- and Q-band were performed at 29–30°C. Measurements and equilibrations with gas for W-band were performed at room temperature.

Table 1

Spin-lattice relaxation times and oxygen transport parameter vs. microwave frequency obtained from 16-SASL in DMPC bilayers at 27°C.

Freq. (GHz)	S ₁ (2.54)	S ₂ (3.45)	X(9.2)	K(18.5)	Q(34.6)	W (94)
T ₁ (μs)	0.69	0.94	2.54	3.46	3.69	2.05
W(x) (μs ⁻¹)	2.68	2.71	2.67	2.54	2.68	3.02

Table 2

Oxygen parameter coefficient across the POPC bilayer vs. microwave frequency obtained at 29–30°C (X- and Q-band) and at room temperature (W-band).

Freq. (GHz)	X(9.2)	Q(34.6)	W (94)
P _M (cm/s)	112	101	87