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Spin-labeled small unilamellar vesicles with the T_1 -sensitive saturation-recovery EPR display as an oxygen sensitive analyte for measurement of cellular respiration

Laxman Mainali, Jeannette Vasquez-Vivar, James S. Hyde, and Witold K. Subczynski*

Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, USA

Abstract

This study validated the use of small unilamellar vesicles (SUVs) made of 1-palmitoyl-2-oleoylphosphatidylcholine with 1 mol% spin label of 1-palmitoyl-2-(16-doxylstearoyl)phosphatidylcholine (16-PC) as an oxygen sensitive analyte to study cellular respiration. In the analyte the hydrocarbon environment surrounds the nitroxide moiety of 16-PC. This ensures high oxygen concentration and oxygen diffusion at the location of the nitroxide as well as isolation of the nitroxide moiety from cellular reductants and paramagnetic ions that might interfere with spin-label oximetry measurements. The saturation-recovery EPR approach was applied in the analysis since this approach is the most direct method to carry out oximetric studies. It was shown that this display (spin-lattice relaxation rate) is linear in oxygen partial pressure up to 100% air (159 mmHg). Experiments using a neuronal cell line in suspension were carried out at X-band for closed chamber geometry. Oxygen consumption rates showed a linear dependence on the number of cells. Other significant benefits of the analyte are: the fast effective rotational diffusion and slow translational diffusion of the spin-probe is favorable for the measurements, and there is no cross reactivity between oxygen and paramagnetic ions in the lipid bilayer.

Keywords

spin-label oximetry; mammalian cells; oxygen consumption; saturation recovery EPR

1. Introduction

The application of spin-label oximetry in biological systems began with Backer et al. [1] who emphasized that the resolution of the proton superhyperfine structure in the EPR spectrum of a water soluble small spin label is highly sensitive to the concentration of oxygen and can be used as a parameter in measurements of oxygen consumption in mitochondria and cell suspensions. EPR spectra of 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxy (CTPO), with resolved superhyperfine structure, were carefully calibrated for measurements of oxygen consumption in cell suspensions during the cell cycle [2]. These two publications introduced the T_2 -sensitive oximetry method and its application for measurements of cell respiration. Small nitroxide spin labels freely tumbling in water often

*Corresponding Author: Witold K. Subczynski, Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA, Tel: (414) 955-4038, Fax: (414) 955-6512, subczyn@mcw.edu.

exhibit resolved superhyperfine coupling to protons. This resolution tends to disappear in the presence of oxygen because of the line broadening caused by collisions with oxygen. An application of T_2 -sensitive methods to measure oxygen consumption by mammalian and plant cells was reviewed in [3].

T_1 -sensitive oximetry methods were introduced and developed by Hyde and co-workers including saturation-recovery (the absolute T_1 method [4-6]), CW saturation [7, 8], passage display [9], and the multiquantum approach [10, 11]. Heisenberg exchange between a fast-relaxing species (e.g., molecular oxygen) and a slow-relaxing species (e.g., a spin label) leads to faster spin-lattice relaxation of the nitroxide. This is the basis of T_1 -sensitive methods, which have significant advantages over T_2 -sensitive methods because T_1 (usually 1–10 μ s) is 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. 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 [7, 12-14].

A key paper in which a T_1 -sensitive spin-label oximetry method was applied to investigate kinetics of cellular respiration was published in the Proceedings of the National Academy of Sciences in 1985 [9]. The closed chamber oxygen consumption of Chinese hamster ovary (CHO) cells was measured using water soluble CTPO as an oxygen-sensitive spin label and a rapid-passage T_1 -sensitive EPR display. The display exhibited sensitivity to bimolecular collisions of oxygen dissolved in water with CTPO at oxygen concentrations as low as 0.1 μ M. Additionally, this display was linear in dissolved oxygen concentration up to ~ 50 μ M (water saturated with $\sim 30\%$ air).

A novel oxygen sensitive analyte is described here that consists of a dispersion of small unilamellar vesicles (SUVs) prepared from 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) lipids, about 1 mol% of which have been spin-labeled at the 16 position (1-palmitoyl-2-(16-doyleoyl)phosphatidylcholine (16-PC)). In this analyte, the nitroxide moiety of 16-PC is isolated from water-soluble reductants and paramagnetic ions that might interfere with spin-label oximetry measurements. A hydrocarbon environment, which dissolves oxygen very well, always surrounds the nitroxide moieties of the spin-label molecules. Therefore, the partial pressure of oxygen is the only factor that can influence the EPR spectrum of spin-labels in the analyte. The spin label undergoes fast rotational diffusion resulting in a three line spectrum with narrow lines. The concentration of spin labels in the POPC bilayer can be fairly high because the tether to the phospholipid head group restricts lateral diffusion. Such microscopic analytes (~ 30 nm in diameter) are readily and uniformly distributed within the sample, thus giving a rapid response to changes in oxygen partial pressure. Additionally, oxygen sensitivity of the analyte will be increased due to the T_1 value of 16-PC which is about three times longer in POPC SUVs than in water soluble spin-labels used in previous oximetry measurements [9]. We validated the application of the analyte for measurements of cellular respiration of rat dopaminergic neuronal cells in suspension. The successful application of the analyte in quantitative

measures of oxygen consumption in cells represents a significant advance in cellular oximetry measurements

1.1. Outline of theory

Because the collision rate ω between molecular oxygen and the free radical moiety of a spin label is at the heart of spin label oximetry, analyses of the EPR data are based on the Smoluchowski equation,

$$\omega = 4\pi r_o (D_{SL} + D_O) C \quad (1)$$

where r_o is the interaction distance between oxygen and nitroxide radical spin labels (4.5 Å; [15]), and C is the oxygen concentration expressed in molecules per unit volume. An experimental observable, ω_{exp} , is related to ω by Eq. 2

$$\omega_{exp} = 4\pi p r_o (D_{SL} + D_O) C \quad (2)$$

where p is the probability that a spectroscopically observable event occurs when a collision takes place. Usually the diffusion coefficient of oxygen, D_O , is much greater than the diffusion coefficient of the spin label, D_{SL} . However, in water at 37°C, oxygen diffuses only three to four times faster than small water soluble spin labels [16-18]. [In a more viscous environment such as lipid bilayer membranes, the diffusion coefficient of oxygen and the diffusion coefficient of lipid spin labels differ by two to three orders of magnitude (see [13, 19] for more data and discussion)]. Therefore, for lipid bilayer membranes a useful simplification can be made by neglecting the diffusion coefficient of the spin label relative to that of oxygen:

$$\omega_{exp} = 4\pi p r_o D_O C \quad (3)$$

Thus, the experimental observation yields the oxygen diffusion-concentration product, $D_O C$. The interaction distance, r_o , can be adjusted to force the equality of the oxygen diffusion coefficient obtained from measuring the diffusion against a concentration gradient (macroscopic diffusion) and that from the Smoluchowski equation (self diffusion). It was shown for spin labels that this agreement is obtained for $p = 1$ [12-14] which allows quantitative application of the EPR spin label oximetry method.

Because saturation recovery EPR is the most direct way to carry out oximetric studies, we developed a new approach in which saturation recovery measurements of the spin-lattice relaxation time is directly used to monitor oxygen consumption in cell suspensions. We can write for the electron relaxation probability:

$$W(\text{with } O_2) = W(N_2) + p\omega \quad (4)$$

or

$$T_1^{-1}(\text{with O}_2) = T_1^{-1}(\text{N}_2) + 2p\omega \quad (5)$$

For solvents with high viscosity (following eq. (3)) we can write

$$T_1^{-1}(\text{with O}_2) = T_1^{-1}(\text{N}_2) + 8\pi pr_o D_o C \quad (6)$$

Because the effect of molecular oxygen on T_1^{-1} of spin-labels depends on the diffusion-concentration product of oxygen in the solvent surrounding the nitroxide moiety, the location of spin-labels in solvents with high oxygen solubility and high oxygen diffusion coefficients will exhibit more sensitivity to changes in oxygen partial pressure. If the solvent is unchanged and the temperature of measurements fixed (37°C for mammalian cells), equation (6) shows that measured T_1^{-1} (with O₂) value changes linearly with oxygen concentration.

Previous experience has shown that the best solvents for designing a new oxygen sensitive analyte with the T_1 -sensitive SR display are hydrocarbons, which dissolve oxygen four to ten times more than water [20], while the diffusion of oxygen is about as rapid as in water [13]. The spin label undergoes fast rotational diffusion, while translational diffusion is restricted [13]. These favorable properties are enhanced near the center of the phospholipid bilayer.

2. Materials and methods

2.1. Materials

One-palmitoyl-2-oleoylphosphatidylcholine (POPC) and 1-palmitoyl-2-(16-doxylstearoyl) phosphatidylcholine spin-label (16-PC) (see Fig. 1C for structures) were obtained from Avanti Polar Lipids (Alabaster, AL) and 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxy (CTPO) was obtained from Sigma-Aldrich ((St. Louis, MO). Other chemicals, of at least reagent grade, were purchased also from Sigma-Aldrich (St. Louis, MO).

2.2. Preparation of SUVs

The analytes, SUVs, were prepared by sonication of the dispersion of large multilamellar vesicle (LMV) suspensions made of POPC containing 1 mol% of 16-PC. Chloroform solutions of POPC and 16-PC were mixed to attain a desired spin label concentration. A chloroform solution with a final volume of 100 µL was added to the test tube of 1.2 mL of the cell culture medium, RPMI, at room temperature. LMVs were obtained by the standard rapid solvent exchange method [21]. Sonication was performed using a probe tip sonicator (Fisher Scientific, model 550). Five to seven 15 second sonication cycles followed by a 15 second cooling in ice were sufficient to transfer the milky suspension of LMVs into a slightly hazy transparent solution. Sonication produces SUVs approximately 30 nm in diameter. The final concentration of POPC was 25 mg/mL. Thus, ~2.5% of media volume was trapped inside the SUVs. These analytes are uniformly distributed and monitor the average oxygen concentration in the media.

2.3. Cells

Rat dopaminergic neuronal cells, 1RB3AN27 (N27 cells), were grown in RPMI 1640 containing 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) and were maintained at 37°C in a humidified atmosphere of 5% CO₂/95% air. For measurements of oxygen consumption, a known number of cells was mixed with 20 µL of SUVs in RPMI. The final cell suspension contained 750, 1,500, 2,500, or 5,000 cells/µL. After aeration (gentle shaking) the sample was quickly transferred into the closed chamber (quartz capillary with 0.7 mm i.d. and 0.87 mm o.d.), positioned in the loop-gap resonator (preheated to 37°C) of the EPR spectrometer, and SR signals were recorded as a function of time.

2.4. Saturation-recovery EPR measurements and calibration of the analyte

SR EPR signals were obtained at X-band on a home-built spectrometer and loop-gap resonator previously described [22]. Spin-lattice relaxation times, T_1 s, were determined by analyzing the SR signals of the most intense central line obtained in short pulse experiments [22]. Typically, 10^5 – 10^6 decays were acquired with 2,048 data points on each decay with a sampling interval from 2 to 10 ns. The total accumulation time was about one minute. SR signals were fitted by single- and double-exponential functions. Single exponential fits were sufficient.

For calibration a suspension of the analyte was placed in the open chamber (a 0.6 mm i.d. capillary made of gas-permeable methylpentene polymer called TPX [14]). TPX sample tubes permit convenient calibration of the analyte by flowing various mixtures of air and nitrogen gasses, adjusted with flow-meters (Matheson Gas Products, model 7631H-604), over the sample [14]. The same gas was used for temperature control. The calibration was carried out at the same temperature at which oxygen consumption by the cell suspension was measured, namely 37°C.

3. Results and discussion

3.1. Characterization of the oxygen-sensitive analyte (calibration curve)

The analyte illustrated in Fig. 1A was prepared as described in Sect. 2.2. POPC membranes are in the fluid phase in the physiological temperature range, which ensures high solubility of oxygen, especially in the membrane center (Fig. 1D) where the 16-PC nitroxide moiety (Fig. 1C) is located (Fig. 1B and D).

Calibration using known mixtures of gases was the first step in the evaluation of the oxygen sensitivity of the analyte. In this study, T_1 was used as an empirical parameter for determining oxygen concentration (partial pressure) in a solution. For calibration, the SR signals were recorded for analyte suspensions placed in the TPX capillary and equilibrated with 100% nitrogen and air/nitrogen mixture, with increased air content up to 100%. Representative SR signals and fitting curves are shown in Fig. 2A for 100% nitrogen and for 40% air. As can be seen from residuals (the experimental signal minus the fitting curve) in both cases, the single exponential fit was excellent (additional criteria for the quality of a

single-exponential or a double-exponential fit are described in [23]). Other SR signals were also satisfactorily fitted with single exponentials. From SR signals, T_1 values were obtained.

Figure 2B shows the calibration curves (T_1 and T_1^{-1} as a function of oxygen concentration) obtained at 37°C for the 0-100% air-saturated cell culture medium, RPMI. We assumed that RPMI contains about the same amount of dissolved oxygen as air saturated water, 0.22 mM O_2 at 37°C [24]. It is notable that the T_1^{-1} display as a function of oxygen concentration is linear in dissolved oxygen concentration. As shown in Eq. 6, this linearity follows from the Smoluchowski equation under the well-established fact that the Heisenberg interaction between oxygen and the nitroxide moiety of a spin label is a strong interaction, and every collision contributes to the relaxation process. From the linear fit (Fig. 2B), we arrived at a simple equation that relates the measured value, namely $T_1^{-1}(t)$, to the oxygen concentration in the media, $C(t)$ at 37°C:

$$C(t) = 67(T_1^{-1}(t) - 0.618) \mu M, \quad (7)$$

where $T_1(t)$ is in μs . The analyte concentration did not affect the calibration curve (data not shown). Thus, the use of higher analyte concentration can be expected to yield higher sensitivity.

3.2. Stability of the analyte

A significant finding from our experiments is that nitroxides in the analyte are protected from reduction in cell suspension. Swartz et al. [25] reported that the rate of reduction of water soluble nitroxides in cell suspensions dramatically increases under anaerobic conditions (see also [26]). Data presented in Fig. 3A demonstrate that the EPR signal intensity of SUVs labeled with 16-PC does not change in cell suspensions up to 15 hours after anaerobic conditions are reached. This is a great advantage over the molecular spin-label oximetry methods where water soluble spin labels (CTPO) can be reduced by intracellular reductants [25]. Data presented in Fig. 3B also demonstrate that sodium ascorbate at high concentration of 5 mM, which almost completely reduces the EPR signal of the water soluble spin label CTPO within three minutes, decreased the EPR signal of the analyte by 47% after 60 minutes. Also, the water soluble relaxation agent NiEDDA at 1 mM concentration reduced T_1 of CTPO from 0.73 μs to 0.37 μs (Fig. 4A). The effect of NiEDDA at 10 mM concentration on the relaxation time of the analyte (T_1 value of 16-PC) was negligible, T_1 values were reduced from 2.32 μs in the absence of NiEDDA to 2.10 μs in its presence (Fig. 4B). These experiments were performed for thoroughly deoxygenated samples. Data are in agreement with our previous measurements showing that the accessibility of NiEDDA to the membrane center is negligible [27]. These data ensure that the nitroxide moiety is well isolated from water-soluble cellular reductants and paramagnetic ions that might interfere with spin-label oximetry measurements.

The analyte was also stable in a wide range of pH, from 4.0 to 11.0. In addition, the EPR spectrum is not affected in this pH range (data not shown). This is in agreement with previous data in that the structure of the PC lipid bilayers does not change in this pH range

because the ionization of the PC headgroups does not change [28, 29]. This statement is true for n-PC spin labels and cholesterol analog spin labels but not for the widely used stearic acid spin labels (SASLs). The ionization of the carboxyl group of SASLs in the lipid membranes changes just at the physiological pH region where protonated (neutral) and ionized (anionic) forms of SASLs exist, giving two overlapped EPR spectra [30-32]. Thus, changes of pH which can occur during the cell respiration will not affect oximetry measurements.

3.3. Oxygen consumption by rat dopaminergic neuronal cells

Kinetic curves of oxygen consumption by cell suspensions after re-calibration of data with the use of Eq. 7 are shown in Fig. 5A. The calculated oxygen consumption rate was linearly proportional to the number of cells for each measurement (Fig. 5B). In this study, the maximum rate of oxygen consumption by rat dopaminergic neuronal cells in suspension was about 1.8 fmols/min/cell.

4. Concluding Remarks

We introduce here the use of spin-labeled SUVs as an oxygen-sensitive analyte with a T_1 -sensitive SR display to measure the concentration of dissolved molecular oxygen in cell suspensions confined in closed chamber geometry. What are the advantages of this method over already developed and widely applied T_1 - and T_2 -sensitive EPR spin-labeling oximetric methods? Previously described oximetric approaches were based on the use of a small water soluble spin label, mainly CTPO, which tumbles in water very quickly (molecular approach). Two displays were used: an unusual rapid-passage T_1 -sensitive EPR display [9] and a T_2 -sensitive display [2], the superhyperfine structure of the EPR spectrum. In these displays it was assumed that in water the diffusion coefficient of water soluble spin labels was significantly slower than the diffusion coefficient of oxygen, permitting the simplification of Eq. 2 to Eq. 3 to be made. This simplification ensures that measured EPR parameters are proportional to the oxygen diffusion-concentration product. As indicated in the introduction this simplification is not straightforward (oxygen diffuses only about three to four times faster than small water soluble spin labels) and, to relate the EPR observable with oxygen concentration, corrections are made (see [19]). This is especially significant while fitting the data for cellular respiration.

Another assumption was that water soluble spin labels are located in the same environment (cell media, buffer, water). However, these spin labels can penetrate plasma membranes and locate within cellular cytosol where diffusion, as well as diffusion and solubility of molecular oxygen, are different. These influence the measured effect of dissolved oxygen on EPR spectral parameters. Additionally, water soluble spin labels can interact with other paramagnetic molecules and can be reduced by water soluble reducing agents. Inside the cell, these spin labels can serve as electron acceptors for mitochondria when oxygen is already consumed [25]. In the newly designed analyte, these problems were solved or minimized.

The idea of having spin labels always surrounded by the same solvents with high oxygen solubility and high oxygen diffusion coefficients has been already exploited in oxygen-

sensitive microscopic spin-label approaches. Typical examples of microscopic spin-label probes are a few micrometers in diameter, such as BSA-coated paraffin oil particles containing cholestane spin labels [3, 33], or BSA-coated hexane particles containing stearic acid spin labels [34]. In these approaches T_2 -sensitive methods were used (measured parameters were line-width or line-height). A major concern of using these microspheres is the possibility of leaking nitroxides from the microsphere into the media. Another potential concern is leakage, as well as the release of organic liquid from the microspheres. These problems were not investigated in depth. POPC SUVs are stable, and 16-PC spin label is stably anchored within the PC bilayer. Doping SUVs with negatively charged phosphatidylserine should additionally protect analytes against possible clustering and phagocytosis.

The development of new LGRs [35-37] has allowed transfer of the T_1 -sensitive SR-oximetry methods to higher microwave frequencies, including Q-band [36, 38] and W-band [38, 39]. This possibility is very significant for measurements with biological samples that can be obtained only in limited quantities. The sensitive volume of the LGR at Q-band is ~ 30 nL, thus oximetry measurements for the system presented here could be performed for ~ 25 cells (comparing the lowest number of cells measured in the presented data of 750 cells per 1 μ L). This straightforward comparison was possible because the SR concentration sensitivity is experimentally found to be independent of microwave frequency [37, 38, 40, 41]. Two additional advantages of using a higher frequency for T_1 -sensitive SR-oximetry measurements are: (1) the T_1 of spin labels depends on microwave frequency (being largest at Q-band), and (2) the effect of collisions between oxygen and spin-labels on the measured T_1 value is independent of frequency (up to 94 GHz) [37, 38, 42]. Thus, the longest values of T_1 will generally be found at Q-band, noting that long values are advantageous for the measurement of bimolecular collisions with oxygen. The contribution of dissolved molecular oxygen to the spin label relaxation rate is expressed by Eq. 6. The new capabilities in measurement of this contribution using SR EPR at Q- and W-band have also been demonstrated in POPC lipid bilayer membranes and compared with results obtained earlier at X-band [38]. 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. Other approaches that will lead to increased concentration sensitivity include the method of Yin and Hyde [43], who showed that the use of high observing power in saturation-recovery EPR experiments does not affect the ability to extract bimolecular collisions of oxygen with spin-labels and can increase the signal-to-noise ratio up to ten times.

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References

1. Backer JM, Budker VG, Eremenko SI, Molin YN. *Biochim Biophys Acta*. 1977; 460:152–156. [PubMed: 192284]
2. Lai CS, Hopwood LE, Hyde JS, Lukiewicz S. *Proc Natl Acad Sci USA*. 1982; 79:1166–1170. [PubMed: 6280170]

3. Ligeza A, Wisniewska A, Subczynski WK. *Curr Topics in Biophys.* 1992; 16:92–98.
4. Kusumi A, Subczynski WK, Hyde JS. *Proc Natl Acad Sci USA.* 1982; 79:1854–1858. [PubMed: 6952236]
5. Yin JJ, Hyde JS. *Z Phys Chem.* 1987; 153:57–65.
6. Subczynski WK, Hyde JS, Kusumi A. *Proc Natl Acad Sci USA.* 1989; 86:4474–4478. [PubMed: 2543978]
7. Subczynski WK, Hyde JS. *Biochim Biophys Acta.* 1981; 643:283–291. [PubMed: 6261814]
8. Altenbach C, Froncisz W, Hyde JS, Hubbell WL. *Biophys J.* 1989; 56:1183–1191. [PubMed: 2558734]
9. Froncisz W, Lai CS, Hyde JS. *Proc Natl Acad Sci USA.* 1985; 82:411–415. [PubMed: 2982155]
10. Mchaourab HS, Hyde JS. *J Magn Reson.* 1993; 101:178–184.
11. Mchaourab HS, Hyde JS, Feix JB. *Biochemistry.* 1994; 33:6691–6699. [PubMed: 8204604]
12. Hyde JS, Subczynski WK. *J Magn Res.* 1984; 56
13. Subczynski WK, Hyde JS. *Biophys J.* 1984; 45:743–748. [PubMed: 6326877]
14. Hyde, JS.; Subczynski, WK. *Biological Magnetic Resonance.* Berliner, LJ.; Reuben, J., editors. Vol. 8. Plenum Press; New York: 1989. p. 399–425.
15. Windrem DA, Plachy WZ. *Biochim Biophys Acta.* 1980; 600:655–665. [PubMed: 6250601]
16. St Denis CE, Fell CJ. *Can J Chem Eng.* 1971; 49:885.
17. Molin, YN.; Salikhov, KM.; Zamaraev, KI. *Spin Exchange.* Springer-Verlag; New York: 1980. p. 111–115.
18. Ahn MK. *J Magn Reson.* 1976; 22:289–293.
19. Subczynski WK, Hopwood LE, Hyde JS. *J Gen Physiol.* 1992; 100:69–87. [PubMed: 1324973]
20. Linke, WF. *American Chemical Society.* 4th. Washington, DC: 1965. p. 1233–1236.
21. Mainali L, Raguz M, Subczynski WK. *J Phys Chem B.* 2013; 117:8994–9003. [PubMed: 23834375]
22. Yin JJ, Subczynski WK. *Biophys J.* 1996; 71:832–839. [PubMed: 8842221]
23. Subczynski, WK.; Widomska, J.; Wisniewska, A.; Kusumi, A. *Methods in Molecular Biology, Lipid Rafts.* Vol. 398. Humana Press; Totowa: 2007. p. 143–157.
24. Hitchman, ML. *Measurement of Dissolved Oxygen.* John Wiley & Sons, Inc.; New York: 1978.
25. Swartz HM, Chen K, Pals M, Sentjurs M, Morse PD 2nd. *Magn Reson Med.* 1986; 3:169–174. [PubMed: 3959885]
26. Subczynski, WK.; Swartz, HM. *Biomedical ESR Part A: Free Radicals, Metals, Medicine, and Physiology, Biological Magnetic Resonance.* Eaton, SS.; Eaton, GR.; Berliner, LJ., editors. Vol. 23. Kluwer; Boston: 2005. p. 229–282.
27. Raguz M, Mainali L, Widomska J, Subczynski WK. *Chem Phys Lipids.* 2011; 164:819–829. [PubMed: 21855534]
28. Träuble H, Eibl H. *Proc Natl Acad Sci USA.* 1974; 71:214–219. [PubMed: 4521052]
29. Papahadjopoulos D. *Biochim Biophys Acta.* 1968; 163:240–254. [PubMed: 4301073]
30. Egret-Charlier M, Sanson A, Ptak M. *FEBS Lett.* 1978; 89:313–316. [PubMed: 207576]
31. Sanson A, Ptak M, Rigaud JL, Gary-Bobo CM. *Chem Phys Lipids.* 1976; 17:435–444. [PubMed: 11049]
32. Kusumi A, Subczynski WK, Hyde JS. *Fed Proc.* 1982; 41:1394.
33. Ligeza A, Swartz H, Subczynski WK. *Curr Topics in Biophys.* 1994; 18:29–38.
34. Liu KJ, Grinstaff MW, Jiang J, Suslick KS, Swartz HM, Wang W. *Biophys J.* 1994; 67:896–901. [PubMed: 7948703]
35. Froncisz W, Oles T, Hyde JS. *Rev Sci Instrum.* 1986; 57:1095–1099.
36. Sidabras JW, Mett RR, Froncisz W, Camenisch TG, Anderson JR, Hyde JS. *Rev Sci Instrum.* 2007; 78:034701. [PubMed: 17411204]
37. Hyde JS, Yin JJ, Subczynski WK, Camenisch TG, Ratke JJ, Froncisz W. *Phys Chem B.* 2004; 108:9524–9529.

38. Subczynski WK, Mainali L, Camenisch TG, Froncisz W, Hyde JS. J Magn Reson. 2011; 209:142–148. [PubMed: 21277814]
39. Mainali L, Sidabras JW, Camenisch TG, Ratke JJ, Raguz M, Hyde JS, Subczynski WK. Appl Magn Reson. 2014; 45:1343–1358. [PubMed: 25541571]
40. Mainali L, Raguz M, Camenisch TG, Hyde JS, Subczynski WK. J Magn Reson. 2011; 212:86–94. [PubMed: 21745756]
41. Mainali L, Hyde JS, Subczynski WK. J Magn Reson. 2013; 226:35–44. [PubMed: 23207176]
42. Froncisz W, Camenisch TG, Ratke JJ, Anderson JR, Subczynski WK, Strangeway RA, Sidabras JW, Hyde JS. J Magn Reson. 2008; 193:297–304. [PubMed: 18547848]
43. Yin JJ, Hyde JS. J Chem Phys. 1989; 91:6029–6035.

Fig. 1.

Schematic showing the cross section of the oxygen sensitive analyte, which is a small unilamellar vesicle (SUV, A), cross section of the bilayer in the SUV (B), and structures of POPC and 16-PC (C). Location of the nitroxide moiety of 16-PC in the POPC bilayer is indicated by the black dot (B). In (D) the profile of the oxygen transport parameter across the POPC bilayer at 37°C indicates high oxygen diffusion-concentration product in the membrane center compared with water. The system is equilibrated with air at oxygen partial pressure of 159 mm Hg.

Fig. 2.

Steps performed to obtain calibration curves for the oxygen-sensitive analyte. (A) Representative SR EPR signals from the analyte in cell culture medium equilibrated at 37°C with nitrogen and the mixture of 40% air and 60% nitrogen. As can be seen from the residuals (the experimental signal minus the fitted curve), signals can be fitted to a single exponential function in both the absence and presence of molecular oxygen with time constants 1.81 ± 0.01 μs and 0.5 ± 0.01 μs. (B) Oxygen calibration curves. T_1 values (●) and T_1^{-1} values (◆) as a function of oxygen concentration ([O₂]) and oxygen partial pressure (oxygen partial pressure at 100% air equals 159 mm Hg) obtained at 37°C are shown. All measurements were performed for samples positioned in the open chamber (TPX capillary).

Fig. 3.

(A) Kinetic curve for oxygen consumption at 37°C by cell suspension containing 5000 cells/ μL (●) together with EPR signal intensities (▲) measured for 15 hours after oxygen was consumed in the cell suspension. (B) Kinetic curves for chemical reduction of the water soluble nitroxide CTPO (●) and nitroxide in 16-PC protected inside membranes of SUVs (▲) by 5 mM sodium ascorbate. Sodium ascorbate was added to the CTPO solution (1mM) or suspension of SUVs (containing 1 mM concentration of 16-PC) and EPR signal intensities were measured.

Fig.4.

(A) SR signal for 0.1 mM CTPO in water in the absence and presence of 1 mM NiEDDA. Single exponential fits and residuals with time constants of $0.73 \pm 0.002 \mu\text{s}$ and $0.37 \pm 0.002 \mu\text{s}$, respectively, in the absence and presence of NiEDDA. (B) SR signal for 16-PC in the analyte in the absence and presence of 10 mM NiEDDA. Single exponential fits with time constants of $2.32 \pm 0.01 \mu\text{s}$ and $2.10 \pm 0.01 \mu\text{s}$, respectively, in the absence and presence of NiEDDA are shown. All measurements were performed at room temperature for thoroughly deoxygenated samples.

Fig. 5.

(A) Kinetic curves of oxygen consumption at 37°C for rat dopaminergic neuronal cells obtained using Eq. (7). Number of cells per μL is indicated. (B) Rate of oxygen consumption by cells in 1 μL of the medium measured at 37°C. All measurements were performed in closed-chamber geometry.