Supporting information:

Supplementary text:

In the methods section we showed that the enhanced relaxant effect in the bilayer is a product of the local relaxivity and oxygen concentration. We now demonstrate that the products of the average relaxivity and the average oxygen concentration in the bilayer are approximately the average relaxation rate. The definition of the equilibrium partition coefficient is: $K = \left\langle \left[O_2^{bilayer} \right] \right\rangle \Big/ \left[O_2^{bulk} \right]$. The meaning of the average concentration of oxygen in the bilayer is not a simple quantity. The oxygen profile has no abrupt edge: All additional oxygen is assigned to the presence of the bilayer whether or not it is physically within the bilayer. All the oxygen that is in excess (or deficit) over the bulk amount must be added up. It is necessary to carry out an integration in such a way that the integral converges to a finite value as the distance from the bilayer goes to infinity. Therefore, the average quantity in the bilayer must be evaluated as a two-step process:

$$\langle \Delta O_2 \rangle = \frac{1}{L} \int_{-\infty}^{\infty} \left(\left[O_2 \right](x) - \left[O_2^{bulk} \right] \right) dx$$

$$\left\langle \left[O_2^{bilayer} \right] \right\rangle = \langle \Delta O_2 \rangle + \left[O_2^{bulk} \right]$$

The analogue to the integral in three dimensions is $\Delta O_2 = \frac{1}{Vf} \iiint_V \left(\left[O_2 \right](x) - \left[O_2^{bulk} \right] \right) d\tau$,

where the integral is over the volume, V, of the sample and f is the volume fraction of the lipid in the sample. It is interesting that the integration in three dimensions does not require knowing the thickness of the bilayer, but computations on the molecular level in one dimension do. A similar definition for the relaxivity (or the diffusion coefficient) is necessary:

$$\langle \Delta \chi \rangle = \frac{1}{L} \int \left(\chi - \chi^{bulk} \right) dx$$

$$\left\langle \chi^{bilayer} \right\rangle = \langle \Delta \chi \rangle + \chi^{bulk}$$

The analogous definitions for the incremental average rate and the average rate in the bilayer are given in the methods section above. The integrals are well behaved and

include effects from inside or outside the bilayer and are not limited by the value of L. The necessary approximation is achieved using the above definitions and the fact that the relaxation rates are a product of the local oxygen concentration and the relaxivity, which is proportional to the diffusion coefficient. Below, the previous definitions of the integrals are used and the expansion in terms of the relaxivity and the oxygen concentration:

$$\begin{aligned}\langle \Delta\Delta R_1 \rangle &= \frac{1}{L}\int \left(\Delta R_1(x) - \Delta R_1^{bulk} \right) dx \\ &= \frac{1}{L}\int \left(\chi[O_2] - \chi^{bulk}\left[O_2^{bulk}\right] \right) dx \\ &= \frac{1}{L}\int \left(\left(\chi - \chi^{bulk}\right)[O_2] + \chi^{bulk}\left([O_2] - \left[O_2^{bulk}\right]\right) \right) dx\end{aligned}$$

This can be further rearranged

$$\begin{aligned}\langle \Delta\Delta R_1 \rangle &= \frac{1}{L}\int \left(\left(\chi - \chi^{bulk}\right)\left([O_2] - \left\langle \left[O_2^{bilayer}\right] \right\rangle\right) + \left(\chi - \chi^{bulk}\right)\left\langle \left[O_2^{bilayer}\right] \right\rangle \right) dx + \chi^{bulk}\Delta O \\ \langle \Delta\Delta R_1 \rangle &= I_{ovlp} + \chi^{bulk}\Delta O + \Delta\chi \left\langle \left[O_2^{bilayer}\right] \right\rangle\end{aligned} \tag{S1}$$

Where the I_{ovlp} represents the overlap between the deviation of the relaxivity from the bulk and the deviation of the oxygen from the bilayer average:

$$I_{ovlp} = \frac{1}{L}\int \left(\chi - \chi^{bulk}\right)\left([O_2] - \left\langle \left[O_2^{bilayer}\right] \right\rangle\right) dx \tag{S2}$$

We suggest that I_{ovlp} is small relative to the other terms contributing to the average relaxation rates. For example, the integrand of (S2), when x is in the bilayer, is small because the oxygen is near the bilayer value even though $\chi(x)$ may be far from the bulk value. In the aqueous region where the oxygen is far from the bilayer value then χ is near the bulk value, and again the integrand is small.

Retaining the overlap integral, the other quantities in equation (S1) can be written in terms of the average values:

$$\left\langle \Delta\Delta R_1 \right\rangle = I_{ovlp} + \chi^{bulk}\left(\left\langle \left[O_2^{bilayer}\right]\right\rangle - \left[O_2^{bulk}\right]\right) + \left\langle \left[O_2^{bilayer}\right]\right\rangle\left(\left\langle \chi^{bilayer}\right\rangle - \chi^{bulk}\right)$$
$$\left\langle \Delta\Delta R_1 \right\rangle = I_{ovlp} + \left\langle \left[O_2^{bilayer}\right]\right\rangle\left\langle \chi^{bilayer}\right\rangle - \chi^{bulk}\left[O_2^{bulk}\right]$$
$$\frac{\left\langle \Delta\Delta R_1 \right\rangle + \chi^{bulk}\left[O_2^{bulk}\right]}{\Delta R_1^{bulk}} = \frac{I_{ovlp}}{\Delta R_1^{bulk}} + \frac{\left\langle \chi^{bilayer}\right\rangle\left\langle \left[O_2^{bilayer}\right]\right\rangle}{\chi^{bulk}\left[O_2^{bulk}\right]} \tag{S3}$$

The rearrangement relies on the identity that: $\Delta R_1^{bulk} = \chi^{bulk}\left[O_2^{bulk}\right]$

$$\frac{\left\langle \Delta R_1^{bilayer}\right\rangle}{\Delta R_1^{bulk}} = \frac{I_{ovlp}}{\Delta R_1^{bulk}} + \frac{\left\langle \chi^{bilayer}\right\rangle}{\chi^{bulk}}K = \frac{I_{ovlp}}{\Delta R_1^{bulk}} + \left(\frac{\left\langle D^{bilayer}\right\rangle}{D^{bulk}}\right)K$$
$$\frac{\left\langle \Delta R_1^{bilayer}\right\rangle}{\Delta R_1^{bulk}} \approx \left(\frac{\left\langle D^{bilayer}\right\rangle}{D^{bulk}}\right)K \tag{S4}$$

This relates the relaxivities measured in these experiments to the oxygen partition coefficient, K, and to the average diffusion coefficient. When using the fitting parameters obtained in the text for DOPC, and assuming $L = 50A$, we found that $\frac{\left\langle \Delta R_1^{bilayer}\right\rangle}{\Delta R_1^{bulk}} = 1.3$.

We tested whether or not the overlap integral would be small compared to the other terms in the expression for $\frac{\left\langle \Delta R_1^{bilayer}\right\rangle}{\Delta R_1^{bulk}}$. To test this, we adapted a theoretically determined diffusion profile for water[28] in DOPC, which we used to model a diffusion profile for oxygen. The theoretical diffusion profile gives $\frac{\left\langle D^{bilayer}\right\rangle}{D^{bulk}} = 0.32$. From the theoretical diffusion profile and the relaxivity profile, presented in this paper, we estimated an oxygen concentration profile, and determined that $K = 5.1$ for this profile. Using both the diffusion profile and the oxygen concentration profile, the overlap integral was then computed giving: $\frac{I_{ovlp}}{\Delta R_1^{bulk}} = -0.13$. Thus, the overlap integral is a small contribution relative to the product of the diffusion and oxygen profile means, which is approximately

1.4. This example illustrates the contention that the overlap integral is a small contribution to the ratio $\frac{\left\langle \Delta R_1^{bilayer} \right\rangle}{\Delta R_1^{bulk}}$.

Supplementary tables:

Table 1S: ΔH_{p-p} and R_1 data for spin-labeled WALP in DOPC

		N_2	O_2 [c]	NiEDDA[d]
Site	ΔH_{p-p} (Gauss)	R_1 (Mrad/s)	R_1 (Mrad/s)	R_1 (Mrad/s)
-2	2.83(9)[*]	0.305(2)	1.57(2)	0.840(9)
0	3.08(9)	0.379(3)	1.67(2)	0.888(6)
1	3.18(9)	0.334(2)	1.57(2)	0.777(4)
2	3.09(9)	0.301(2)	1.73(2)	0.782(3)
4	3.28(9)	0.302(2)	1.66(2)	0.598(2)
6	3.80(9)	0.300(2)	2.00(3)	0.448(2)
8	3.38(9)	0.353(2)	2.34(4)	--
10	2.88(9)	0.448(6)	3.15(9)	--
12[a]	2.88(9)	0.439(5)	3.27(6)	--
14	3.16(9)	0.406(2)	3.06(5)	--
16	3.72(9)	0.353(2)	2.12(3)	--
18	3.84(9)	0.272(3)	1.64(4)	0.442(3)
20	3.01(9)	0.319(3)	1.56(2)	0.928(4)
22	3.40(9)	0.287(3)	1.46(2)	0.887(9)
22[b]	--	0.288(2)	1.38(2)	--
23	2.82(9)	0.298(2)	1.46(2)	1.16(3)
24	2.70(9)	0.377(2)	1.58(2)	1.06(4)
26	2.90(9)	0.345(2)	1.69(2)	0.88(1)

[*] 2.83 ± 0.09

[a]Measured in addition on DMPC: $R_1^{N_2}$ =0.413(6) , $R_1^{O_2}$ =2.39(6)

DOPS: $R_1^{N_2}$ =0.424(7) , $R_1^{O_2}$ =2.44(6)

[b]This mutant's sequence is `Ac-GWWLALALALALALALALALWC(sl)W-NH2`

[c]Medical Air (21% O_2)

[d] 50 mM NiEDDA

Table 2S: ΔH_{p-p} and R_1 data for spin-labeled WALP in DOPM

		N_2	O_2
Site	ΔH_{p-p} (Gauss)	R_1 (Mrad/s)	R_1 (Mrad/s)
-2	3.17(9)	0.321(3)	1.38(2)
0	3.18(9)	0.354(2)	1.41(2)
1	3.24(9)	0.314(2)	1.48(2)
2	3.35(9)	0.455(4)	1.64(3)
4	3.50(9)	0.301(2)	1.64(2)
6	3.79(9)	0.323(3)	2.35(5)
8	3.80(9)	0.427(4)	2.74(5)
10	3.12(9)	0.432(4)[a]	3.5(3) [a]
12	3.12(9)	0.422(5)[b]	3.18(8)[b]
14	3.43(9)	0.422(3)[a]	3.22(5)[a]
16	4.20(9)	0.370(4)	1.90(3)
18	3.77(9)	0.278(3)	1.75(4)
20	3.25(9)	0.366(2)	1.46(1)
22	2.73(9)	0.303(4)	1.22(2)
23	2.90(9)	0.384(2)	1.56(1)
24	2.58(9)	0.383(2)	1.62(1)
26	2.90(9)	0.331(3)	1.26(2)

[a] average of two values
[b] average of three values

Table 3S: The parameters for the least-squares fits to the different relaxation data, ΔR_{1e}, R_{1e}, and line width, ΔH^{*}_{pp}, $\Delta H^{*Fit}_{pp} = \left(5 - 7 \cdot R^{Fit}_{1e}\right) / 3$. Data shown in figures 4 and 5. The first three rows of fitting data refer to the relaxant-induced relaxation rates, the last four rows are parameters used to fit the relaxant-independent relaxation rates.

Application	Width $a\left(\text{Å}\right)$	Transition Center $\pm l\left(\text{Å}\right)$	Scale $m\left(\text{Mrad/sec - Å}\right)$	Center $\bar{x}\left(\text{Å}\right)$	Rate in Bulk $\Delta R^{bulk}_{1e}\left(\text{Mrad/sec}\right)$ or $R^{bulk}_{1e}\left(\text{Mrad/sec}\right)$	Fit Error[a] Mrad/sec
O_2 in DOPC	4.2(9)	5.0(9)	20(1)	-0.5(2)	1.24(3)	0.1
Ni in DOPC	3.9	12.0	-13.0	-0.5	0.6	0.1
O_2 in DOPM	3.7(9)	7.0(6)	27(2)	-1.6(3)	1.08(6)	0.15
1st S in DOPC	4.2	5.0	2.6	-0.5	0.35	---
2nd S in DOPC	4.2	12..0	-3.0	-0.5	---	---
1st S in DOPM	3.7	7.0	3.0	-1.6	0.35	---
2nd S in DOPM	3.7	14.0	-3.0	-1.6	---	---

[a]Standard Deviation of Fit using equation (8).

Supplementary figures:

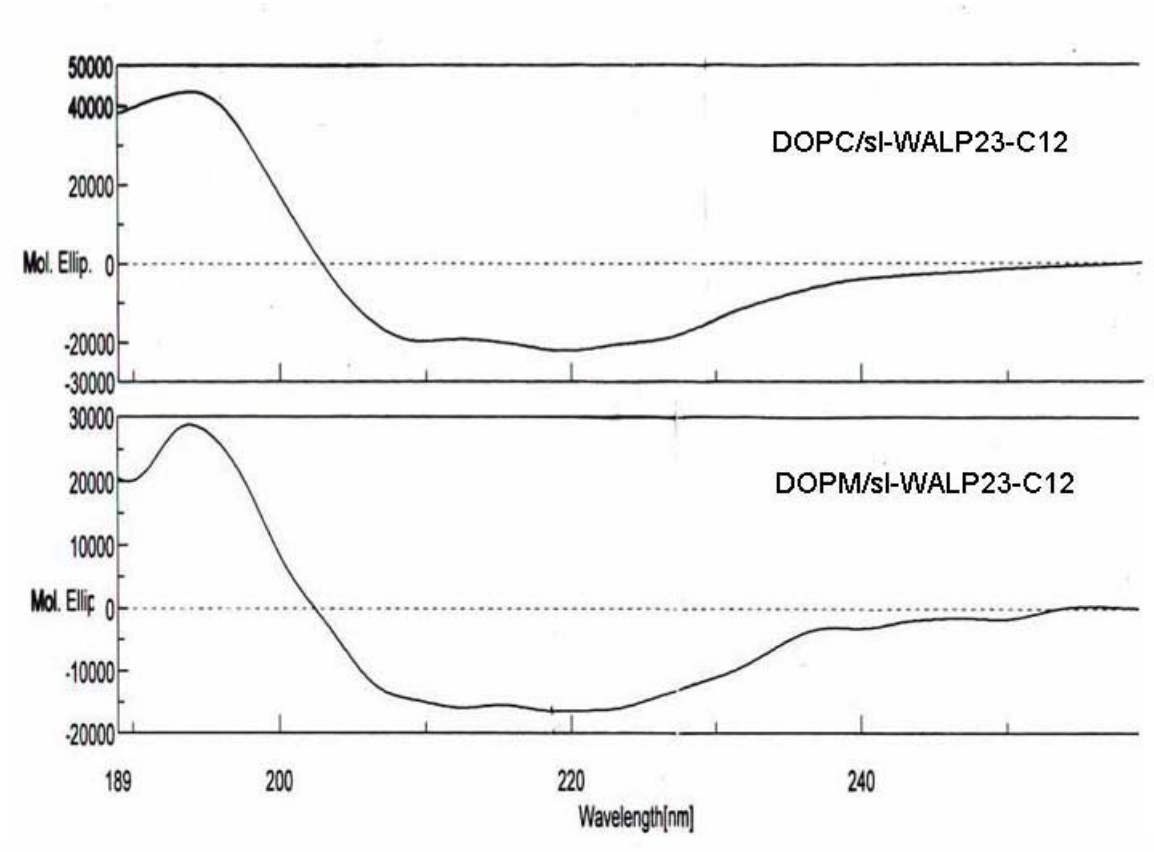

Figure 1S The CD spectra of sl-WALP23-12 peptide in DOPC (top) and DOPM (bottom) bilayers.

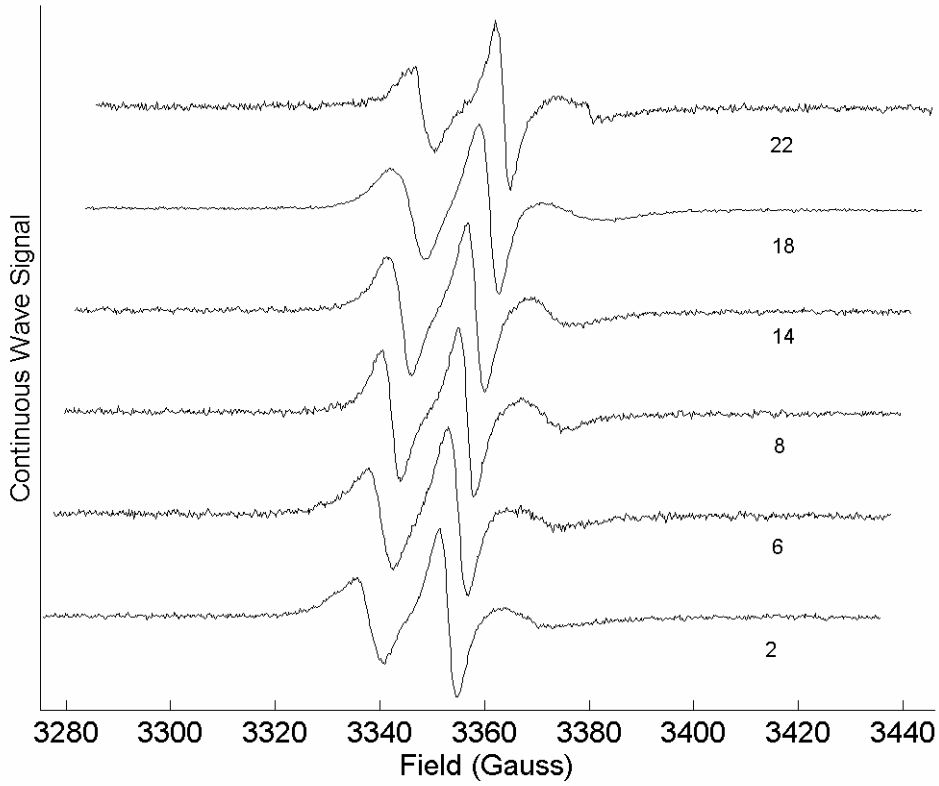

Figure 2S. displays a set of CW spectra for positions 2,6,8,14,18, and 22 of WALP in DOPM at 21°C. The field axis applies to the bottom spectrum; spectra are staggered by 1.5 G, for clarity.