

Interaction of Spin Labeled Lipid Membranes with Transition Metal Ions.

Boris Dzikovski, Vsevolod Livshits and Jack Freed

SUPPLEMENTARY INFORMATION

S1. Effect of metal salts on the main phase transition and pretransition in DMPC bilayers.

The table illustrates more pronounced effect on the main phase transition of DMPC for perchlorate ions compared e.g. to chloride. Also, the data shows similarities between the effects of cations in pairs Mg – Ni and Ca – Cu, as discussed in Results #4.

Table S1. Main phase transition/pre-transition temperatures for multilamellar vesicles of DMPC in the presence of corresponding concentrations of various metal salts.

Salt/concentration	Main phase transition, °C	pretransition, °C
No salt	24.4	15.7-16.0
NaCl, 120 mM	24.8	16.1
NaClO ₄ , 120 mM	24.6	None
NaClO ₄ , 1M	22.6	None
LiCl, 120 mM	24.3	15.5
LiClO ₄ , 120 mM	22.5	None
MgCl ₂ , 120 mM	24.7	15.8
Mg(ClO ₄) ₂ , 120 mM	22.1-22.2	None
Mg(ClO ₄) ₂ , 500 mM	42.2, probably different phase	None
MgSO ₄ , 120 mM	24.7	15.4
NiCl ₂ , 120 mM	24.6	15.4
Ni(ClO ₄) ₂ , 120 mM	22.1	None
Ni(ClO ₄) ₂ , 500 mM	21.5	None
CaCl ₂ , 120 mM	26.2	None
Ca(ClO ₄) ₂ , 100 mM	23.9	None
CuCl ₂ , 120 mM	26.4	None
Cu(ClO ₄) ₂ , 100 mM	24.0	None
CuSO ₄ , 120 mM	27.8	18.6
Zn(ClO ₄) ₂ , 120 mM	24.7	None
ZnSO ₄ , 120 mM	25.9	17.4

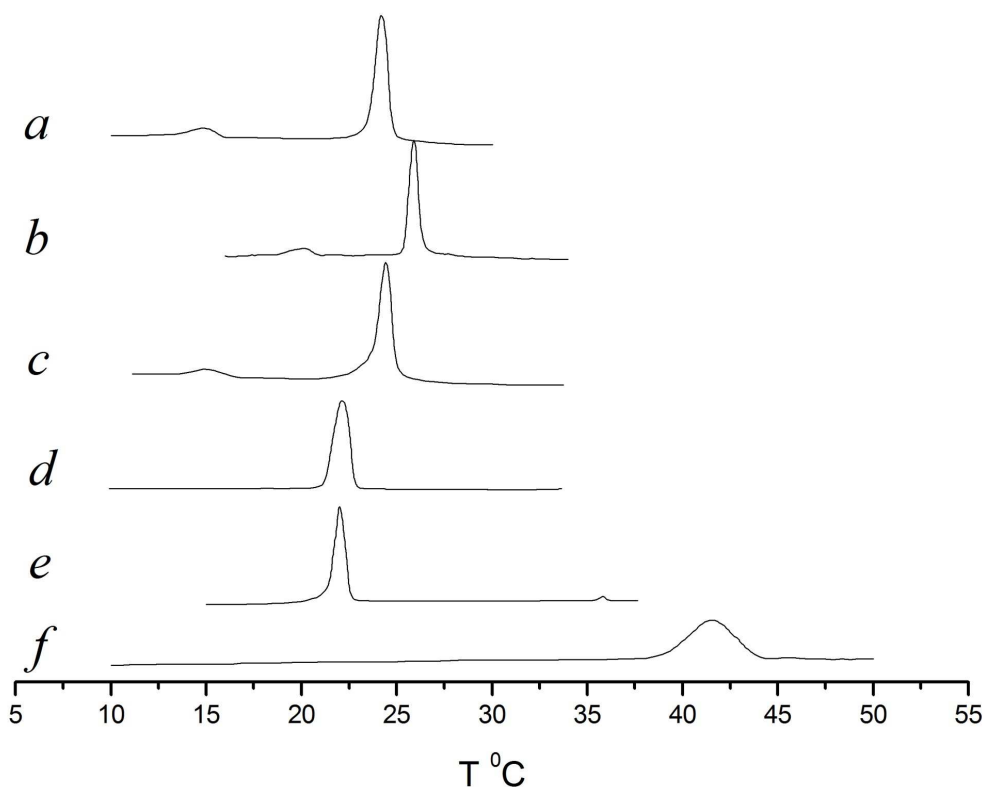


Fig. S1. DSC scans of DMPC in (a) water, (b) 120 mM CaCl_2 , (c) 120 mM NiCl_2 , (d) 120 mM $\text{Ni}(\text{ClO}_4)_2$, (e) 120 mM $\text{Mg}(\text{ClO}_4)_2$, (f) 500 mM $\text{Mg}(\text{ClO}_4)_2$, it is likely that in this case we a change in the phase state of the membrane.

S2. Values of T_2 and T_1 relaxation enhancements for PC spin labels in the DMPC membrane induced by some nickel and copper salts.

Table S2. Dependence on anion of the spin relaxation enhancement parameters : spin-lattice relaxation enhancement, $\Delta(T_1^{-1})$, and additional relaxation broadening, $\Delta\omega$, for nickel salts at concentration of 30 mM and copper salts at concentration of 20 mM in aqueous phase at 39°C. The T_1 values are determined using the simplified approach described in ^{1,2}.

n-PC	Salt	$\Delta(T_1^{-1}) \times 10^{-6} \text{s}^{-1}$	$\Delta\omega \cdot 10^{-6} \text{s}^{-1}$
5-PC	NiCl_2	4.6 ± 0.3	5.3 ± 0.4

5-PC	Ni(ClO ₄) ₂	14.0±0.4	13.6±0.4
14-PC	Cu(ClO ₄) ₂	13.4±0.25	13.1±1.2
14-PC	Ni(ClO ₄) ₂	7.0±0.4	7.0±0.4
14-PC	CuBr ₂		4.8±1
	CuCl ₂		5.1±1
	Cu(NO ₃) ₂		5.1±1
	Cu(CH ₃ COO) ₂		1.76±1

S3. Relaxation Enhancement of TEMPO radical in frozen water/glycerol solutions containing paramagnetic salts.

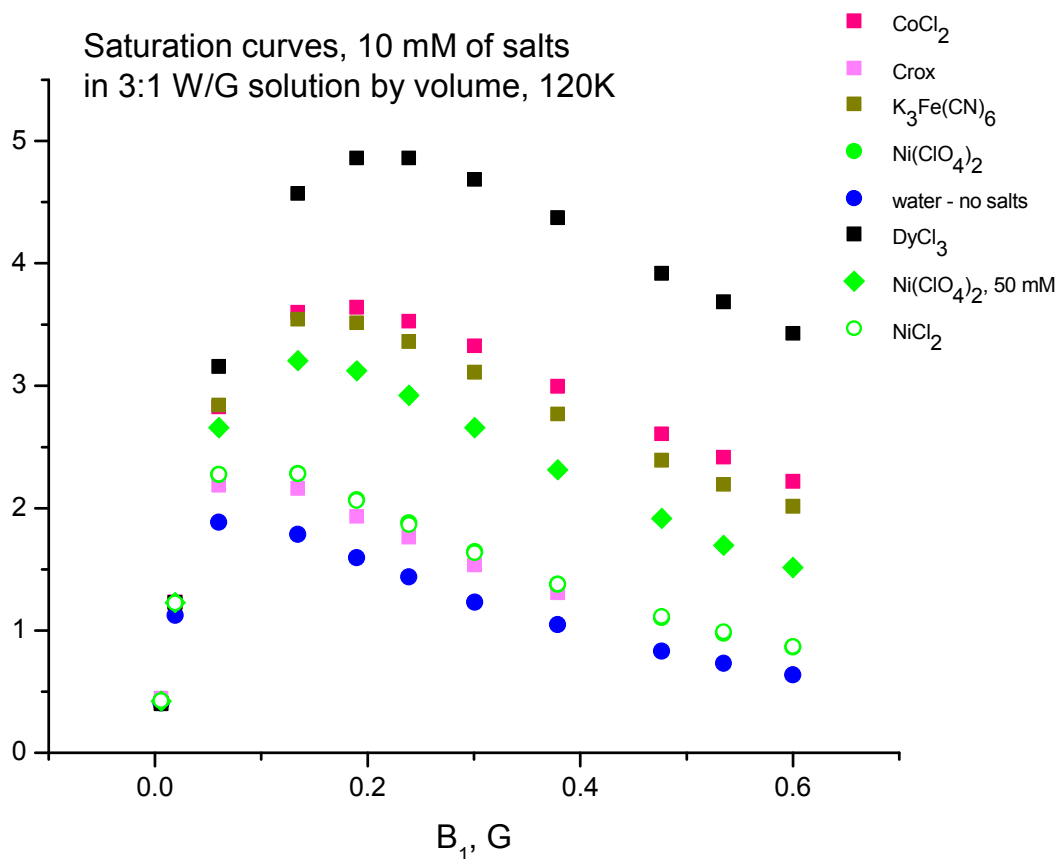


Fig. S2. Saturation curves for 0.4 mM TEMPO radical in frozen water/glycerol solutions containing 10 mM of paramagnetic salts (also a curve for 50 mM of Ni(ClO₄)₂ is shown), T=120K. At this conditions 10 mM of GdCl₃ and MnCl₂ almost completely eliminate saturation (not shown).

S4. Partitioning of paramagnetic ions into the membrane. Is it possible?

Though there is a general consensus that the low-dielectric hydrocarbon interior of the membrane represents a nearly insurmountable barrier for ion transport³ the possibility of partitioning should be taken into consideration, for perchlorates in particular.

Perchlorates are known for their remarkable solubility in organic solvents⁴. For example, the extreme (well over 5M) solubility of LiClO₄ in ethyl ether is widely used in organic synthesis⁵ to increase the polarity of the reaction media. Similar implementation was reported also for less soluble Mg(ClO₄)₂⁶. Unusual solubility of perchlorates in organic solvents is known also for alkaline earth, rare earth, transition metals, lead, silver etc.⁴ Considerable partition coefficients ($\geq 10^{-2}$) between n-octanol and water were reported for both Ni(ClO₄)₂ and Cu(ClO₄)₂⁷.

The concentration dependence of RE, however, is not consistent with a simple two-phase partitioning model. It gives indication of considerable involvement of the membrane surface into the mechanism of relaxation enhancement. It has been shown^{8,9} that the binding of aqueous anions to lipid bilayer membranes increases in order of the Hofmeister series. The factor, which appears to correlate with binding affinity of the anions, is the surface charge density of the ion. Binding to the membrane surface implies some loss of the anion solvation shell. The large anions with low charge density readily lose solvation water and bind to the membrane with the highest affinity. It should be noted that the affinity does not mean specific binding to any specific group at the lipid surface, like Ca²⁺, for example. In a sense, the effect is similar to retaining perchlorates on ion exchange resins due to their cosmotropic/hydrophobic properties⁴.

Partitioning of ionic compounds into the hydrophobic membrane phase still remains a possibility, however, if one takes the salt concentration at the membrane surface as the concentration, which determines the concentration in the membrane phase via partition coefficients.

Unlike permeation through high-dielectric defects, ion partitioning into the membrane is an equilibrium process and can be described in terms of thermodynamics. Hence, estimates based on thermodynamics may give a conclusion if partitioning can explain the observed in the lipid phase concentrations of paramagnetic species and their anion dependence.

The high values of diffusion-concentration products virtually rule out any possibility of a transfer for isolated ions. The total Gibbs energy of ion transfer between aqueous phase and membrane can be written as a sum of electrostatic, neutral and specific contributions^{10, 11}:

$$\Delta G = \Delta G_B + \Delta G_I + \Delta G_D + \Delta G_N + \Delta G_{sp} \quad (3)$$

The first term, ΔG_B , is a difference of the classical Born energy terms¹²:

$$\Delta G_B = \frac{q^2}{2r} \left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_w} \right) \quad (4)$$

which accounts for electrostatic energy required to transfer an ion of a radius r and charge q from the aqueous phase to a membrane (dielectric constants ε_w and ε_m , respectively). Both media are considered as homogeneous continuum.

The Born theory is oversimplified, in particular, because it does not take into account the effects of dielectric saturation caused by strong electric field in the vicinity of an ion. A simple modification of the theory considers the first solvation layer to be completely ordered by electric field of an ion; hence, its dielectric permittivity is equal to its optical limit (n_D^2). Beyond this layer there is a solvent with a bulk dielectric permittivity, ε . Such a one-layer model gives satisfactory results for the ion hydration energies^{13, 14}.

Second electrostatic contribution, ΔG_I , arises from image forces that occur when an ion located close to interface interacts with its own induced image of opposite sign on the other side of the interface. The expression for ΔG_I obtained for a homogeneous dielectric slab of thickness d , dielectric constant ε_m and distance x from one interface is given by¹⁵:

$$\Delta G_I = -\frac{q^2}{4\varepsilon_m} \left\{ \frac{1}{x} + \frac{1}{d} \sum_{n=1}^{\infty} \left[\frac{\alpha^{2n}}{n + x/d} + \frac{\alpha^{2n-2}}{n - x/d} - \frac{\alpha^{2n}}{n + r/d} - \frac{\alpha^{2n-2}}{n - r/d} \right] \right\} \quad (5)$$

where $\alpha = \frac{\varepsilon_w - \varepsilon_m}{\varepsilon_w + \varepsilon_m}$

Flewelling and Hubbell¹⁰ found a satisfactory approximation to (5) for $r \leq 4 \text{ \AA}$, $d \cong 40 \text{ \AA}$,

$r \leq x \leq d/2$:

$$\Delta G_I \cong -\frac{q^2}{2\varepsilon_m r} \left[\frac{r}{2x} + 1.2 \cdot \left(\frac{r}{d} \right) \left(\frac{x}{d} \right)^2 \right] \quad (6)$$

The term ΔG_D corresponds to dipole potential ψ_d ($\Delta G_D = q\psi_d$), which supposedly originates from three sources: surface water molecules, lipid head groups, and ester groups.

In addition to the electrostatic terms there is a neutral (solvophobic) contribution to the Gibbs energy of transfer caused by difference between surface energies at the ion/solvent interfaces for aqueous and membrane environment (see e.g.¹⁶):

$$\Delta G_N = -4\pi \cdot r^2 \cdot \gamma_{w,m} \quad (7)$$

where $\gamma_{w,m}$ is an interface tension between water and membrane. One can get an upper estimate for the absolute value of ΔG_N by approximating the hydrocarbon part of a membrane as a liquid hydrocarbon; then $\gamma_{w,m} \cong 52 \text{ erg/cm}^2$. Alternative (experimental) approach to estimating ΔG_N is the use of solvation energies for a non-polar solute (e.g. inert gas) of the same radius as the ion¹³. We found that both approaches give similar results. Our estimates for the Born term in the total Gibbs energy for a “bare” Ni^{2+} ion give $\sim 1548 \text{ KJ/mol}$. The value totally overwhelms negative contributions from image forces and non-electrostatic interactions and the entire ΔG of the Ni^{2+} transfer of $\sim 1505 \text{ KJ/mol}$ gives an absolutely unrealistic partition coefficient of $\sim 10^{-252}$. Much smaller but also very high ΔG are obtained for bare anions.

The corresponding ΔG values for the transfer of the hydrated Ni^{2+} ions and non-hydrated anions are given in Table 3. It is seen that the barrier for the transfer of hydrated Ni ions into membrane is sharply decreased. However, the ΔG values for Ni^{2+} ion and anions remain too high to be consistent with experiment.

Our results also clearly rule out another possible mechanism of transfer, partition of non-ionic (covalent) species. For perchlorates, showing highest concentrations in the membrane phase, formation of covalent species is extremely unusual, if known at all⁴. They exhibit highest dissociation constants in aqueous solutions and considerable ionic conductivity in organic solvents. On the other hand, a prominent tendency to form covalent compounds is known for chlorides¹⁷, which ranked only in the middle of the observed RE series.

Apparently, transfer of ionic salts into the low- permittivity part of the membrane could occur only in some ionic aggregates. The aggregation of ionic species in low-dielectric media is a well-

known phenomenon. Since transport of charged species is extremely unfavorable due to the high energy penalty arising from the Born term, uncharged aggregates, like ionic triples ($\text{ClO}_4^- \text{Ni}^{2+} (\text{ClO}_4)^-$), are likely to prevail in the membrane phase. As well known, several types of ion pairs can exist in solution: solvent separated, solvent shared or contact ones (see e.g. ¹⁸). An approximate estimate for the electrostatic part of ΔG can be obtained from the polarization of ions in membrane and Coulomb attraction inside the ion triple, which is here considered linear:

$$\Delta G_{w-m}^{ii-d}(2) = (1/\varepsilon_m - 1/\varepsilon_w) \left(\frac{z^2 q^2}{2a} + \frac{2q^2}{2b} \right) - \frac{2zq^2}{\varepsilon_m l} + \frac{q^2}{2\varepsilon_m l} \quad (8)$$

An exact estimate for the value is hardly possible because of unknown values of effective ionic radii and the geometry of the triple. It looks appropriate to use the dipole length of 4.1 Å, obtained by Pottel¹⁹ from dielectric relaxation measurements for the divalent ion salts in water. This value corresponds to the structure of a “solvent shared” ion pair¹⁸ in which the charged oxygen of an anion (perchlorate) enters into the second hydration shell of the Ni^{2+} ion. That gives $\Delta G_{w-m}^{i,i-d}(2) \approx 7.4$ kJ/mol for $a = 4.04$ Å determined for a fully hydrated ion Ni^{2+} and ~ 134 kJ/mol for $a = 3.12$ Å (Table. 3). The contribution to ΔG from image forces is very small in the case of a dipole compared to this value for an ion¹⁶ and should be negligible for a linear ion triple $(-1)(+2)(-1)$.

The neutral contribution (ΔG_N) can be estimated from the surface area of the ion triple. If we take a sum of the surfaces for the hydrated Ni^{2+} ion and non-hydrated ClO_4^- ($b = 2.5$ Å), that gives estimates of $\Delta G_N \cong -88.9$ kJ/mol for $a = 4.04$ Å, and -68.7 kJ/mol for $a = 3.12$ Å. The estimates for the total ΔG_{tr} value, $-80 - +70$ kJ/mol, cover a very broad range. Moreover, the range may be even extended into the positive direction due to the uncertainty in the geometry of the triple (which affects the $\frac{q^2}{2\varepsilon_m l}$ term) and the value $\gamma_{w,m}$, which we initially gave rough (and upper)

estimate as the surface tension at the water/hydrocarbon interface.

Therefore, the estimates, if compared to a specific experimental value of $D_m \cdot c_m$, do not give an unambiguous conclusion on the possibility of partitioning. However, focusing on the trends across the whole series of paramagnetic salts, rather than absolute values of diffusion-concentration products, allows for more certain conclusions.

As seen in figs 3 and 5, the anionic series covers approximately an order of magnitude in the RE between nickel perchlorate and ions with a weaker effect. The difference between perchlorate and sulfate is approximately 6 times in the whole concentration range 0-1M. Just 7.8 kJ/mol (3RT) of the difference in the energy would give 20 times difference in the partition coefficients. If assume partition, the free Gibbs energy of transfer for all salts should hit a narrow range of ~ 5 kJ/mol. Estimates for Gibbs free energies of transfer for ClO_4^- and SO_4^{2-} as 60.7 and 320.5 kJ/mol (Tab. 4) give 200 kJ/mol difference in the free energy of transfer for neutral $\text{Ni}(\text{ClO}_4)_2$ and NiSO_4 . A slightly different approach is using the expression for the electrostatic part of the free Gibbs energy for sulfate, corresponding to Eq. (8), which is

$$\Delta G_{w-m}^{ii-d}(2) = (1/\epsilon_m - 1/\epsilon_w) \left(\frac{z^2 q^2}{2a} + \frac{z^2 q^2}{2b} \right) - \frac{z^2 q^2}{\epsilon_m l} \quad (9)$$

Subtracting Eqs. (9) and (10) gives

$$\Delta G_{\text{NiSO}_4}^{tr} - \Delta G_{\text{Ni}(\text{ClO}_4)_2}^{tr} = (1/\epsilon_m - 1/\epsilon_w) \left(\frac{z^2 q^2}{2b_{\text{SO}_4^{2-}}} - \frac{z^2 q^2}{2b_{\text{ClO}_4^-}} \right) - \frac{z^2 q^2}{\epsilon_m l_{\text{SO}_4^{2-}}} + \frac{2zq^2}{\epsilon_m l_{\text{ClO}_4^-}} + \frac{q^2}{2\epsilon_m l_{\text{ClO}_4^-}} \quad (10),$$

a value that at any sensible choice of b and l values exceeds 200 kJ/mol.

This difference is apparently insurmountable by adjusting neutral (solvophobic) terms and the geometry of the $(\text{ClO}_4)\text{Ni}(\text{ClO}_4)$ triple.

Explaining the experimental values of $D_m \cdot c_m$ product in terms of partition means an assumption that for each salt the sum of very different positive and negative energy terms (each one is hundreds or dozens kJ/mol) yields a total within a narrow range of 5kJ/mol to provide measurable RE values. Moreover, salts of copper, manganese and cobalt similarly affect RE of PC spin labels and, therefore, should have, assuming the partition model, their free Gibbs energy within the same range.

The above does not completely rule out the possibility that some salts, perchlorates in particular, can partition into the membrane. It may affect the phase behavior, but the mechanism responsible for the high observed values of $D_m \cdot c_m$ product should be different.

Table S3

The radius, hydration radius, and free energy of hydration of ions, calculated in terms one-layer model¹⁴

Ion	Ion radius (Å)	Hydrated ion radius (Å)	ΔG_{hydr} (kJ/mol)
Ni ²⁺	0.69	3.12 4.04 ¹⁹	2005
ClO ₄ ⁻	2.5	2.69	180
Cl ⁻	1.81	2.24	270
NO ₃ ⁻	1.79	2.23	275
SO ₄ ²⁻	2.3	2.73	1145
CH ₃ COO ⁻	1.62	2.17	300

Table S4

The calculated contributions to the Gibbs free energy of transfer (kJ/mol) from Born electrostatic (ΔG_B^a), neutral (ΔG_N^b), image forces (ΔG_I^c) terms, and total ΔG values for the hydrated ions, and, in parenthesis, for the “bare” Ni^{2+} ions.

Ion	ΔG_B	ΔG_N	ΔG_I	ΔG
Ni^{2+}	264.4 ^d 353 ^e (1547.8)	-64 ^d -35.7 ^e (-1.9)	-41 (-41)	159.4 ^d 276 ^e (1504.1)
ClO_4^-	99.3 (106.8)	-28.4 (-24.5)	-10.25	60.7 (72)
Cl^-	119.2 (147.5)	-19.7 (-12.8)	-10.25	89.3 (124.45)
NO_3^-	119.7 (149.2)	-19.5 (-12.6)	-10.25	89.95 (126.3)
SO_4^{2-}	391.2 (464)	-29.7 (-20.7)	-41	320.5 (402.3)
CH_3COO^-	123 (164.8)	-18.45 (-10.3)	-10.25	94.3 (144.2)

^a ΔG_B values for the “bare” ions were calculated using Eq. (3); for the hydrated ions – in terms of the “one-layer” model with account for dielectric saturation. ^b ΔG_N values were calculated using Eq. (6). ^c ΔG_I values were calculated using Eq. (5) for the distance from ion to interface, $x=15\text{\AA}$. ^d $a=4.04\text{\AA}$. ^e $a=3.02\text{\AA}$.

S5. A large difference between RE observed as ΔT_1 and ΔT_2 as a sign of dominant dynamic D-D interactions.

As seen in figs 17b and 17c, Gd has much weaker T_1 effect compared to its T_2 effect. For PC labels in DMPC we see (figs. 17) that the GdCl_3 induced broadening is larger than broadening due to the same concentration of $\text{Ni}(\text{ClO}_4)_2$, while $\Delta(1/P)$ in the same system is larger for Ni. Heisenberg exchange contributes equally into both T_1 and T_2 relaxation, i.e. $T_{1,\text{HE}} = T_{2,\text{HE}}$ which

applies for Ni. The observed difference for Gd and Ni requires for Gd $\Delta T_2 \geq 5\Delta T_1$ and cannot be achieved in an assumption of static dipole. On the other hand, for dynamic dipole-dipolar mechanism the T_1 can vary from $2/5 T_2$ for ions with the same Larmor frequency as nitroxide ($\Delta g < 0.01$) to $T_2 \ll T_1$ if $\Delta g \gg 0.01$ ⁷. Indeed, for the case that dynamic D-D relaxation dominates, the T_2 enhancement is given by²⁰:

$$T_{2,dd}^{-1}(\text{dynamic}) = \mu_R^2 \gamma_e^2 \left[\frac{1}{6} J^{(0)}(0) + \frac{1}{24} J^{(0)}(\omega_R - \omega_L) \right],$$

where ω_R and ω_L are the electron Larmor frequencies of the paramagnetic ion and spin label, respectively. In this equation, spectral densities, $J^{(k)}(\omega)$, at the Larmor frequency and above are assumed to be negligible compared with those at low frequency²¹ and therefore are omitted. For ions (e.g., Mn^{2+}) with g -values close to the spin label g -value, such that $(\omega_R - \omega_L)^2 \tau_D^2 \ll 1$, it can be assumed that $J^{(0)}(\omega_R - \omega_L) \approx J^{(0)}(0)$.

For ions with $(\omega_R - \omega_L)^2 \tau_D^2 \gg 1$, on the other hand, the relaxation term involving $J^{(0)}(\omega_R - \omega_L)$ may be neglected.

With the zero-frequency spectral density for translational diffusion given by²⁰:

$J^{(0)}(0) = (48\pi/15^2) n_R / (D_T r_{RL})$, the T_2 -relaxation enhancement becomes

$$T_{2,dd}^{-1}(\text{dynamic}) = C \cdot \mu_R^2 \gamma_e^2 n_R / (D_T r_{RL}),$$

where $C = 2\pi/45$ for $(\omega_R - \omega_L)^2 \tau_D^2 \ll 1$, and $C = 8\pi/15^2$ for $(\omega_R - \omega_L)^2 \tau_D^2 \gg 1$

In contrast, the corresponding expression for the dynamic dipolar enhancement in T_1 relaxation is²⁰:

$$T_{2,dd}^{-1}(\text{dynamic}) = \mu_R^2 \gamma_e^2 \left[\frac{1}{6} J^{(0)}(0) + \frac{1}{24} J^{(0)}(\omega_R - \omega_L) \right]$$

i.e. $T_{1,dd}^{-1} = \frac{2}{5} T_{2,dd}^{-1}$ for $(\omega_R - \omega_L)^2 \tau_D^2 \ll 1$, and $T_{1,dd}^{-1} \ll T_{2,dd}^{-1}$ for $(\omega_R - \omega_L)^2 \tau_D^2 \gg 1$

Therefore, for ions with g -values that differ considerably from that of the spin label ($\Delta g \gg 0.01$), the dynamic dipolar T_1 relaxation is expected to be small. For ions with g -values close to those of the spin label ($\Delta g < 0.01$), the T_1 -relaxation enhancement is two-fifths that of the dynamic dipolar T_2 relaxation rate.

The Δg between nitroxide and Gd is 0.0135 and for this value $T_1/T_2 \approx 0.25$, which is consistent with our observations.

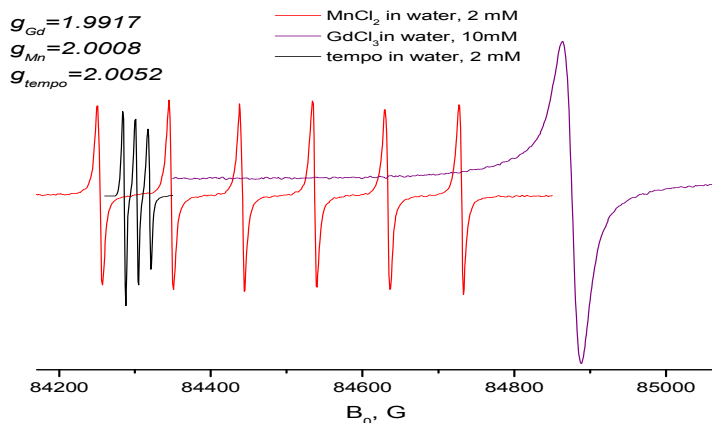


Fig. S3. 236 GHz spectra of 10 mM GdCl_3 , 1 mM of MnCl_2 and 1 mM of TEMPO in water. The difference in the g-factor values for nitroxide and Gd^{3+} is 0.0136, for nitroxide and Mn^{2+} is 0.0044.

S6. WALP peptides – further test for the model

Other membrane embedded compounds that were suggested as an ESR rulers for determination of the immersion depth in the membrane are WALP peptides²². WALP peptides are the membrane spanning hydrophobic α -helical peptides made of alternating alanines and leucines, which are flanked with tryptophans at both termini²³.

Raw cysteine-substituted WALP peptide was made for us by Anaspec (Fremont, CA), then purified by preparative TLC on silica in the Chloroform/Methanol/Water 65:25:4 system and labeled with MTSL (Toronto Research Chemicals) in trifluoroethanol.

As in²² we used a WALP peptide with a cysteine replacement of one of leucines, which was then labeled with MTSL: Ac-GWWLALACALALAWWA-NH₂. It has a length of 24Å. As seen in fig. S4, see also²², the ESR spectrum of this compound in the DMPC membrane is indicative of relatively fast motion of the nitroxide moiety. The length of the cysteine side chain + MTSL tethers gives a distance between the backbone attachment and the nitrogen of the nitroxide group

of 8.1 Å. For the nitroxide oxygen this distance is 9.4 Å. This means that the spin label can be in principle located anywhere in the membrane – just nearly missing to reach the membrane surface. However, some evidence, like a low value of a_{iso} (14.5 G) and very little cholesterol dependence of the spectral lineshape (cholesterol does not reach the hydrophobic core near the membrane center) membrane point at the predominant location deep in the hydrophobic core of the membrane.

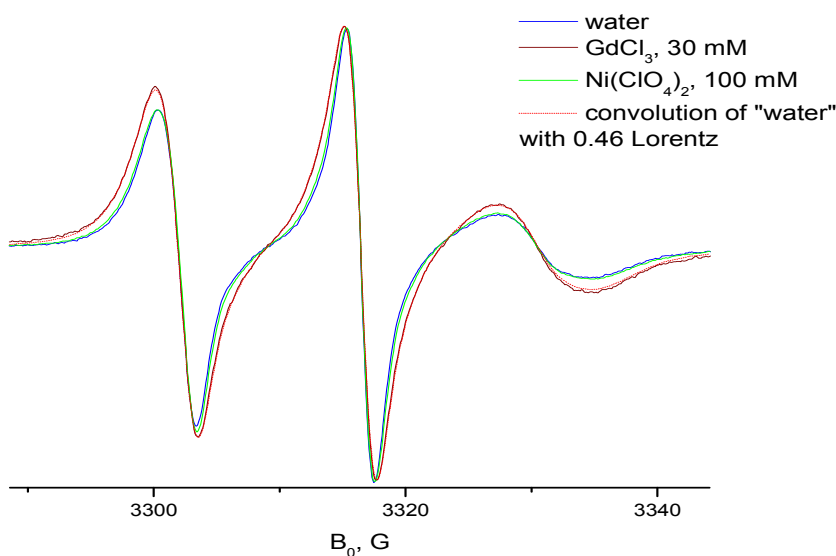


Fig. S4. Broadening of ESR lines of cysteine-substituted and MTSSL labeled WALP16 by $\text{Ni}(\text{ClO}_4)_2$ and GdCl_3 in DMPC at 39°C.

As seen in fig. S4 the difference between GdCl_3 and $\text{Ni}(\text{ClO}_4)_2$ in broadening ESR lines of the reporting nitroxide for this system is much larger than for PC spin labels. If all PC spin labels show substantial broadening by 30 mM of $\text{Ni}(\text{ClO}_4)_2$ which in general has a similar magnitude of broadening by 10 mM of GdCl_3 (fig 17b), the broadening even by 100 mM of $\text{Ni}(\text{ClO}_4)_2$ for spin labeled WALP is very small. The values for 30 mM of GdCl_3 and for 100mM of $\text{Ni}(\text{ClO}_4)_2$ are 0.46 and $\sim 0.06\text{G}$ respectively. That means that SL WALP shows a broadening value for Gd which is about 1/3 of the value observed for 14 PC, while Ni shows more significant drop in broadening for SL WALP vs. PC which is barely detectable at 30 mM. Moreover, a part of the effect for 100 mM $\text{Ni}(\text{ClO}_4)_2$ may be due to a minor fraction of non-aligned peptide²⁴, since even at this small broadening value we cannot obtain good fits with Lorentzian convolution; this may indicate that the broadened fraction of the spectrum has a different lineshape than the main

component. This result demonstrates that if for a membrane-embedded spin-labeled molecule the access of the nitroxide moiety to the membrane surface is fully or partially blocked, the magnetic interaction between the nitroxide and Ni (Heisenberg exchange) is impaired but the interaction with Gd (dynamic dipole) is somewhat less affected.

S7. What our results tell about the membrane viscosity?

The estimates of the average viscosity in the fluid phase of lipid membrane vary. While some data suggest a viscosity of ~ 30 times higher than in water²⁵, there are indications that the hydrophobic core of the fluid phase lipid membrane is similar to a liquid hydrocarbon^{26, 27}. In this latter case the diffusion rate for the vertical fluctuations of the nitroxide moiety can be estimated from translational diffusion rates of free nitroxides in hydrocarbons, which is $\sim 10^{-5} \text{ cm}^2/\text{s}$ ²⁸. At this rate and a traveling range for the nitroxide moiety within the membrane of $\sim 10 \text{ Å}$, the motion of the nitroxide can be considered fast on the ESR time scale. Indeed, 10 mM of NiSO_4 or $\text{Ni}(\text{ClO}_4)_2$ homogeneously broadens the lines of TEMPOL in water by $\sim 0.5\text{G}$. It corresponds to $\Delta\Delta\omega = 1.52 \times 10^7 \text{ s}^{-1}$. At this relaxant concentration the average separation between nickel ions in the solution is $\sim 55 \text{ Å}$ and each molecule of TEMPOL should travel $\sim 1,000 \text{ Å}$ to collide with a nickel ion, assuming the molecular radius for both TEMPOL and hydrated Ni^{2+} ion $\sim 3 \text{ Å}$. Our estimates based on Results sub-section #5 at 10mM $\text{Ni}(\text{ClO}_4)_2$ suggest $\sim 1/20$ - $1/25$ ratio of adsorbed Ni ions to lipid. Using this value we get ~ 36 - 40 Å separation between Ni ions attracted to the membrane surface (if surface area covered by the polar head is taken 64 Å^2). Using these values and taking $\sim 15 \text{ Å}$ for the depth of the area where the tethered nitroxide like 10 PC can freely move in the membrane we get ~ 4.5 - 5×10^{-5} Ni ions in this area of membrane per Å^3 , which corresponds to ~ 75 - 80 mM . Thus, broadening for 10PC in membrane is about 15 times less than that in water with the same effective concentration of Ni ions (e.g. 10 mM of $\text{Ni}(\text{ClO}_4)_2$ in DMPC vs 80 mM of Ni in water). The broadening is linearly related to the exchange constant: $\Delta H = \Delta H_0 + k_{RL}[\text{R}]/g_e$, where ΔH and ΔH_0 are the linewidth in the presence/absence of the relaxant, k_{RL} is the exchange constant, g_e is the gyromagnetic ratio and $[\text{R}]$ is the concentration of the relaxant. The exchange constant is then itself linearly related through the Smoluchowski relation to the diffusion coefficient D_T : $k_{RL} = 4\pi\sigma_{RL}D_T$, where σ_{RL} is a steric factor ~ 1 in homogeneous solutions and may be <1 for interaction of ions and nitroxides at the membrane interface.

This then gives an estimate for the rate of translational diffusion of tethered nitroxides in the membrane as $\geq 1/20$ of this rate in water.

Similar conclusions can be made from our observations on gadolinium effects. We concluded that the mechanism of Gd^{3+} – nitroxide interaction in the spin-labeled membrane is predominantly dynamic dipole-dipolar. The criterion of the dynamic dipolar mechanism prevailing over static is $\tau_D = r_{RL}^2/D_T < T_1$. For Gd this condition cannot be met if we take r_{RL} much larger than the distance of direct contact between nitroxide and adsorbed Gd ion, which can be estimated as $\sim 5\text{\AA}$, and/or high viscosity of the membrane medium. E.g. for $r_{RL} = 10\text{\AA}$ and $D_T = 2 \times 10^{-6} \text{ cm}^2/\text{s}$ $\tau = 5 \times 10^{-9} \text{ s} \gg T_1 \sim 1.4 \times 10^{-10} \text{ s}^{-1}$. However, if we take $r_{RL} = 5\text{\AA}$ and $D_T = 10^{-5} \text{ cm}^2/\text{s}$ $\tau = 2.5 \times 10^{-11} \text{ s} < T_1$. Hence, flexibility of nitroxide tethers which brings ions and nitroxides into closer proximity contributes also into the values of dipole-dipolar RE.

Thus, there are patterns of both Heisenberg exchange and dynamic D-D interactions which we observed in fluid DMPC membranes which suggest relatively low viscosity of the membrane interior.

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