SUPPLEMENTARY INFORMATION

Microsecond Exchange Processes Studied by Two-Dimensional ESR at 95 GHz

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1. EXCHANGE BETWEEN TWO SITES

For two exchanging species one has:¹⁻²

$$\frac{d}{dt} \begin{bmatrix} \widehat{M}_z(1) \\ \widehat{M}_z(2) \end{bmatrix} = L \begin{bmatrix} \widehat{M}_z(1) - \widehat{M}_0(1) \\ \widehat{M}_z(2) - \widehat{M}_0(2) \end{bmatrix}$$
(S1a)

where
$$L = \begin{bmatrix} -k_1 - \frac{1}{T_1(1)} & k_2 \\ k_1 & -k_2 - \frac{1}{T_1(2)} \end{bmatrix}$$
 (S1b)

and
$$\frac{d}{dt} \begin{bmatrix} \widehat{M^{\pm}}(1) \\ \widehat{M^{\pm}}(2) \end{bmatrix} = \begin{bmatrix} -k_1 - i\omega_1 - \frac{1}{T_2(1)} & k_2 \\ k_1 & -k_2 - i\omega_2 - \frac{1}{T_2(2)} \end{bmatrix} \begin{bmatrix} \widehat{M^{\pm}}(1) \\ \widehat{M^{\pm}}(2) \end{bmatrix}$$
 (S1c)

Letting indices j = (1) and (2) correspond to the interbilayer water and the lipid phase respectively:

 $\widehat{M}_z(j)$ and $\widehat{M}_0(j)$ are the longitudinal components of magnetization and its equilibrium value and $M^{\pm}(j) = M_x(j) \pm i \times M_y(j)$, where M_x and M_y are transverse components of magnetization, $T_l(j)$ and $T_2(j)$, are the relaxation times, and ω_j are the Larmor frequencies in each phase. Also, k_l is the rate constant corresponding to nitroxide molecules leaving the water phase to go into the lipid core, and k_2 is the rate constant corresponding to nitroxide molecules leaving the lipid core to go into the water phase (cf. Fig. 11). The partition coefficient of the spin label between these two phases can be expressed as $K_p = k_l/k_2$.

Note that partition coefficients of radicals between phases are routinely estimated by EPR (e.g., Fig.14) but not the rate constants k_1 and k_2 .

The population and the exchange rates are related by $p_i = \frac{k_i}{k_1 + k_2} \equiv \frac{k_i}{k_{ex}}$, so knowing one of the rate constants and the population ratios (via partition coefficients) is enough to determine the exchange rates. Solving equations S1 in order to obtain a 2D ELDOR signal involves, first, obtaining a time-domain signal²:

$$S(\mathbf{t}_1, T_m, t_2) \propto \sum_{jn} \exp(-\lambda_j^* t_2) \times [\exp(-\mathbf{L} T_m)]_{jn} \times \exp(\lambda_n t_1) M_{n0}$$

where L is the matrix defined in Eq.S1b and λ 's are the complex eigenvalues of the 2 x 2 matrix in Eq. S1c. For the case of slow exchange where the spectral lines from each site remain distinct we have $\sqrt{k_1k_2} \ll |(k_1 - k_2) + i(\omega_1 - \omega_2)|$. One can neglect the off-diagonal elements in Eq. S1c and λ_1 and λ_2 are just given by the diagonal elements in Eq. S1c.

The M_{n0} values are proportional to the equilibrium populations of the exchanging states. The diagonal elements of *L* lead to the development of the auto-peaks while the off-diagonal elements of this matrix contribute to the development of cross-peaks. Then, after 2D-FT along the t_1 and t_2 dimensions, the intensities of the auto- and cross-peaks as functions of the mixing time are²⁻³:

Auto peaks (*j*=1,2):

$$I_{jj}(T_m) = \frac{I_{jj}(0) \left[-\left(\Lambda_- - k_j - \frac{1}{T_1(j)}\right) e^{-\Lambda_+ T_m} + \left(\Lambda_+ - k_j - \frac{1}{T_1(j)}\right) e^{-\Lambda_- T_m} \right]}{\Lambda_+ - \Lambda_-}$$
(S2)

Cross peaks (*i*≠*j*):

$$I_{ji}(T_m) = \frac{I_{jj}(0) k_j [e^{-\Lambda_+ T_m} - e^{-\Lambda_- T_m}]}{\Lambda_+ - \Lambda_-}$$
(S3)

Here Λ_+ and Λ_- are the eigenvalues of the *L* matrix:

$$\Lambda_{\pm} = \frac{1}{2} \left\{ k_1 + \frac{1}{T_1(1)} + k_2 + \frac{1}{T_1(2)} \pm \sqrt{\left(k_1 - k_2 + \frac{1}{T_1(1)} - \frac{1}{T_1(2)}\right)^2 + 4k_1k_2} \right\}$$
(S4a)

with

$$\Lambda_{+} - \Lambda_{-} = \sqrt{\left(k_{1} - k_{2} + \frac{1}{T_{1}(1)} - \frac{1}{T_{1}(2)}\right)^{2} + 4k_{1}k_{2}}$$
(S4b)

where $I_{11}(0)$ and $I_{22}(0)$ are the initial intensities of the auto-peaks at $T_m=0$.

Under the assumption of $I_{11}(0) = I_{22}(0) = 1/2$, $k_1 = k_2 = k$ and $T_1(1) = T_1(2) = T_1$ these expressions reduce to²

$$I_{auto}(T_m) = \frac{1}{2}I_{auto}(0)[1 + exp(-2kT_m)]exp(-\frac{T_m}{T_1})$$
(S5a)

and

$$I_{cross}(T_m) = \frac{1}{2} I_{cross}(0) [1 - exp(-2kT_m)] \exp\left(-\frac{T_m}{T_1}\right)$$
(S5b)

leading to Eq. 4 appropriate for the case of proton transfer in Sect. III A.

In the case of lipid-water exchange we have for the partition coefficient:

$$K_p = p_2 / p_1 \approx 28$$

i.e., $p_2 \gg p_1$ and $k_1 \gg k_2 \approx k_1 / 28$ at equilibrium.

Using the latter inequality we obtain:

$$\Lambda_{+} \cong k_{1} + T_{1}(1)^{-1}$$

$$\Lambda_{-} \cong T_{1}(2)^{-1} + k_{2} \equiv [T_{1}(2)^{Ex}]^{-1}$$
So from Eq. S2 one has:

$$I_{11}(T_{m}) \approx I_{11}(0) \{exp [-(k_{1} + T_{1}(1)^{-1})T_{m}]\}$$

and from Eq. S3:

$$I_{12}(T_m) \approx I_{12}(0) \frac{\{exp[-(k_1 + T_1(1)^{-1})] - exp[-T_m/T_1(2)^{Ex}]\}}{k_1 + T_1(1)^{-1} - [T_1(2)^{Ex}]^{-1}}$$
(S7)

(S6)



Supplementary figures

Fig. S1. 95 GHz quasioptical bridge schematic diagram. A quasioptical beam is launched from the transmitter (Tx) waveguide-to-horn transition at the bottom right of the figure, reflected off the wire grid linear polarizer, and directed into a low-loss corrugated waveguide coupled to an induction mode Fabry-Pérot resonator within the main magnet. An orthogonally polarized reflected signal component passes through the first wire-grid polarizer, where it is focused by the mirror onto the receiver (Rx) horn-to-waveguide transition. A third wire-grid polarizer and associated Faraday rotator (at the top of the figure) form an isolator that provides additional discrimination between the signal and reflections of the transmitted pulse. (Adapted with permission from Ref. 4, Fig. 8a, © 2005 John Wiley & Sons, Ltd.).



Fig.S2 The dependence of the intensity of the 2D ELDOR signal (circles) and FID (triangles) for a 0.4 mM solution of trityl radical in water on the magnetic field offset for a pulse length of either 8 ns (blue) or 4 ns (red). In the case of 2D ELDOR the bandwidth allows for simultaneous coverage of lines separated by 25-30 G, which is sufficient for this study.



Fig. S3. Dependence of the ESR linewidth of PDT on its concentration in water at 20 °C. The ESR frequency is 9.4 GHz. The linear fit gives a self-exchange constant of $2.77 \times 10^9 M^{-1}s^{-1}$, determined from the relation $\Delta B=2K_{ex}[PDT]/3\gamma_e$, where ΔB is the half width at half height $\Delta B=0.866\Delta B_{pp}$, γ_e is the electron magnetogyric ratio and the factor 2/3 reflects the fact that only 2/3 of collisions can cause line broadening (cf. Ref. 5).

(S4a)



(S4b)



(S4c)



(S4d)

Fig. S4a-d. 2D ELDOR spectra at $T_m=1.6$ (a) for mixtures of 78 μ M of ¹⁴N-PDT and 48 μ M of ¹⁵N-PDT and (b) 39 μ M of ¹⁴N-PDT and 24 μ M of ¹⁵N-PDT. One can see complete disappearance of cross-peaks

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caused by Heisenberg exchange at these lower concentrations. Contour plots shown for 620 μ M of ¹⁴N-PDT and 380 μ M of ¹⁵N-PDT (c) and 39 μ M of ¹⁴N-PDT and 24 μ M of ¹⁵N-PDT (d).



B

A



С



Fig. S5 Comparison of T_1 decay curves vs. T_m obtained for single component spectra either by integration of the whole 2D ELDOR plot (blue stars) or by summation of the heights of all auto and cross peaks (red squares): A. 2 mM PDT in water; (B) 0.5 mM R[•] at pH = 2.5 when only protonated form is present; (C) 1 mM PD-DTBN in D₂O. One can see the excellent agreement between the two methods; it justifies using peak heights in cases when accurate integration becomes difficult.



Fig. S6. 2D ELDOR spectrum of DTBN in multilamellar vesicles of DMPC at 17 °C. T_m =100 ns. Although most spins are located in the lipid phase, the signal from them is much weaker than the signal from the probe molecules in the water phase.



Fig. S7. Decay of the signal intensity due to T_1 relaxation for two components of the ESR spectrum corresponding to the partition of PD-DTBN between the perdeuterated hydrophobic core and the deuterated water phase in multilamellar vesicles of DMPC at 40 °C. The signal amplitude shown in the ordinate axis is the sum of intensities of all auto-peaks and cross-peaks for each component in relative units. Exponential fits of these decays yield T_1^{lipid} = 272 ns and $T_1^{interbilayer_water}$ = 449 ns. Triangles shows the decay of the PD-DTBN signal in bulk water (D₂O) with $T_1^{bulk_water}$ = 680 ns.

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