Functionalized ¹²⁹Xe as a Potential Biosensor for Membrane Fluidity

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- Electronic Supplementary Information -

1 Experimental details

1.1 NMR setup

Data was acquired using a 9.4 T NMR spectrometer (Bruker Biospin, Ettlingen, Germany) with gradient coils for MRI. A 10 mm inner diameter double-resonant probe for ¹H and ¹²⁹Xe was used for excitation and detection. For all experiments temperature was set to 303 K using the spectrometer's temperature control unit.

1.2 CEST-spectra acquisition

The data points in Fig. 1 of the original manuscript were acquired by irradiating a 5 s saturation pulse ($B_1 = 10 \ \mu T$) at a desired frequency or rather chemical shift and detecting the Xe@solution signal (at 110.7046 MHz) afterwards. Sweeping the saturation pulse over a chemical shift range yields a CEST-spectrum. The integral of the signal after off-resonant irradiation at -74.0 ppm was normalized to 1. Due to exchanging Xe atoms the signal of Xe@solution drops when the saturation pulse "hits" the frequency of Xe@CrA_{ma}@aq or Xe@CrA_{ma}@lipid.

1.3 Hyper-CEST imaging

MR images were acquired using an echo planar imaging (EPI) sequence which was modified for Hyper-CEST¹. Therefore a variable saturation pulse before image acquisition had been implemented. The EPI sequence was set

to a resolution of 32 x 32 pixel and a field of view of 20 x 20 mm² at 20 mm slice thickness. The bandwidth of the detection was 78 kHz centered around 110.7046 MHz (frequency of the detected ¹¹²⁹Xe in solution peak). The minimal possible echo time of 6.42 ms was used. Before image acquisition hyperpolarized ¹²⁹Xe (ca. 16% nuclear spin polarization; gas mixture: 5 % Xe, 10 % N₂ and 85 % He; nat. abundance of ¹²⁹Xe ~ 26%), produced in a custom-designed polarizer², has been bubbled for 15 s at 0.1 SLM (standard liters per minute) via glass capillaries into the solution followed by a 2 s delay allowing all bubbles to collapse. Once the gas mixture had been bubbled into solution a saturation pulse of $|B_1| = 10 \ \mu$ T was irradiated on-resonant (74.0 ppm) on caged Xe (Xe@CrA_{ma}@lipid) with different saturation times t_{sat} varying from 0 – 20 s.

1.4 Sample preparation

To model different chemical environments, we prepared small unilamellar vesicles in buffer solution (10 mM Hepes, 100 mM NaCl, pH 7.3) via extrusion of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (Sigma-Aldrich, Steinheim, Germany). In brief: A thin lipid film was prepared by evaporating a solution of 25 mg DPPC or POPC in 1.6 ml MeOH/CHCl₃ (1:1) on a rotary evaporator at 40 rpm, 25 °C and a final pressure of 13 mbar. Remaining solvents were removed in high vacuum (10^{-3} mbar) over night. After hydration (> 30 min) with 1.0 ml buffer at fluid phase conditions ($25 \circ C$ or $60 \circ C$ for POPC or DPPC respectively) the resulting suspension was subjected to > 5 freeze-thaw cycles (liquid N₂, $25 \circ C$ or $60 \circ C$ water bath). The obtained suspension was pushed > 15 times through polycarbonate membranes (100 nm pore size) using an extruder with heating block ($25 \circ C$ or $60 \circ C$ for POPC or DPPC respectively) (Avanti Polar Lipids, Inc., Alabaster, Alabama, USA) to obtain homogeneous sized small unilamellar vesicles. The size measured with dynamic light scattering was 97 ± 26 nm.

Both compartments of the phantom contained 600 μ L buffer solution with 5 μ M CrA_{ma}, 200 μ M POPC (inner compartment) or DPPC (outer compartment). 0.35 vol% DMSO were in the solution remaining from the 1 mM stock solution of CrA_{ma}. To reduce foaming during gas bubbling 0.1 vol-% Pluronic L81 was added. Both, DMSO and Pluronic L81 did not have a NMR-detectable effect on the small unilamellar vesicles.

1.5 Exchangerates



Supp. Fig. 1 Scheme of the different chemical environments and the Xenon paths. k' is the overall exchange of Xenon atoms which have to travel from a host molecule which itself is embedded into a lipid bi-layer to the surrounding medium to deliver the information of depolarization. The concentration ratio ϕ which is used in the manuscript can be expressed as k_2/k .

1.6 Xe-host:liposome ratio estimation



Supp. Fig. 2 Simplified lipid bi-layer model used for the estimation of the particle ratio.

Using simple geometry one can estimate the number of CrA_{ma} molecules per unilamellar liposome. The measured average diameter of the liposomes was d = 97 nm. The thickness of a lipid bilayer can roughly be estimated as shown by Woodka *et al.*³ with $h \approx 5$ nm. The area of the phosphocholine headgroup of one lipid molecule is roughly a = 0.71 nm². Therefore the number of lipid molecules per liposome is approximately given by

$$N_{\rm tot} = \frac{4\pi (d/2)^2 + 4\pi (d/2 - h)^2}{a} \approx 75124.$$
 (1)

This allows one to estimate the number of liposomes N_{lipo} in the samples which contained 200 μ M phospholipids in a 600 μ L solution as

$$N_{\rm lipo} = \frac{N_{\rm A} \cdot 200 \ \mu \rm M}{N_{\rm tot}} \cdot 600 \ \mu \rm L \approx 9.6 \cdot 10^{11}, \tag{2}$$

in which $N_{\rm A} \approx 6.02 \cdot 10^{23} \text{mol}^{-1}$ is Avogadro's number. The number of $\text{CrA}_{\rm ma}$ molecules in the solution can be estimated to

$$N_{\rm CrA_{ma}} = \frac{N_{\rm A} \cdot 5 \ \mu M}{1} \cdot 600 \ \mu L \approx 1.8 \cdot 10^{15}.$$
(3)

Therefore, the maximum ratio ($N_{\text{CrA}_{\text{ma}}}$: N_{lipo}) is 1875 (if all CrA_{ma} molecules are embedded in the lipid bilayer). Since each of the two liposomes' bi-layers contains 75124/2 lipid molecules (eq. 1), one CrA_{ma} molecule is surrounded by ca. 20 lipid molecules.

2 Data analysis

2.1 Fitting of CEST-spectra using an exponential Lorentzian

The recorded data has been phased, baseline corrected, normalized to signal intensity after off-resonant (-74.0 ppm) saturation and fitted in MATLAB (R2011b) using a superposition of exponential Lorentzians of the form:

$$ExpLor(\delta) = e^{-\frac{2A}{\pi} \cdot \frac{\gamma^2}{4 \cdot (\omega_{\text{res}} - \delta)^2 + \gamma^2}}$$
(4)

In which δ is the chemical shift, γ is related to the width of the resonance in the CEST-spectrum, ω_{res} is the resonance frequency of the functionalized xenon either in solution or lipids and the on-resonant depletion is determined via A:

$$ExpLor(\delta = \omega_{\rm res}) = e^{-\frac{2A}{\pi}}$$
(5)

2.2 Inverse Laplace transform procedure

In the following the analysis of the depolarization processes is described: After subtraction of the noise, which is the arithmetic mean of an arbitrary, non-phantom region in the image, as the first step of the data analysis an initial DT distribution F(s) with a certain resolution (we used 25 points per 10³ s logarithmically spaced; all points = 1) was chosen. The ILT of F(s) which describes the depolarization process best had then to be found. In theory the Bromwich-Integral

$$F(s) = \mathfrak{L}^{-1}\left\{f(t_{\text{sat}})\right\} = \frac{1}{2\pi i} \lim_{\gamma \to \infty} \int_{c-i\gamma}^{c+i\gamma} f(t_{\text{sat}}) \cdot e^{s \cdot t_{\text{sat}}} dt_{\text{sat}}$$
(6)

has to be solved by contour integrating over the complex plane of t_{sat} . The MATLAB routine *rilt.m*⁴, based on CONTIN⁵ uses the Tikhonov regularization⁶ on this very F(s). Therefore

$$V(\alpha) = \sum_{i=1}^{m} \sum_{j=1}^{n} (f(t_i) - e^{-t_i \cdot s_j} \cdot F(s_j))^2 + \alpha^2 \cdot \sum_{j=1}^{n} (2 \cdot F(s_j) - F(s_{j+1}) - F(s_{j-1}))^2$$
(7)



Supp. Fig. 3 Illustration of the numerical inverse Laplace transform (ILT) of the experimental data $f(t_{sat})$ which yields the probability distribution F(s) that is further used to calculate $F(\tau = 1/s)$.

has to be minimized. The first term is the difference of the *m* experimental data points $f(t_i)$ and the (discrete) LT of the guessed $F(s_j)$. This term equals the residual χ^2 . A least squared minimization of the second term, which represents the curvature of F(s), is performed with the weight α^2 . α is the "smoothing parameter" in *rilt.m*. For high alpha values, F(s) will be smooth and one might miss small peaks. For $\alpha = 0$ it will be totally "free" and not at all continuously differentiable. Therefore the data has to have as low noise as possible to be able to choose α as small as possible. For all analyses herein α was set to 0.1. The minimum of $V(\alpha)$ is obtained when F(s) has the least degree of curvature and its LT represents the experimental data well, i. e. χ^2 is minimized as well. The condition for the end of the cycle was, that the difference of $V(\alpha)$ in step p and step p - 1 was below 10^{-3} .

In the next step the resolution of the obtained F(s) was increased via interpolation. Gaussian noise with a standard deviation of 10 % of the maximum value of F(s) was added to F(s) to help prevent the routine from getting trapped in a local minimum. The minimization of this new $V(\alpha)$ was performed as described above.

The whole procedure ended once a specified resolution was obtained. Here we used a final resolution of 100 points per 10^3 s logarithmically spaced. The final F(s) was fitted with one (two for the entire phantom) log-normal distribution(s) from which the depolarization time(s) were obtained.

3 Supplementary figures



Supp. Fig. 4 Raw ¹²⁹Xe Hyper-CEST images (4 averages) at 74.0 ppm of the phantom which were later post processed using an adaptive weights filter with a local quadratic model⁷.



Supp. Fig. 5 T_1 relaxation of the Hyper-CEST signal of the entire phantom (blue diamonds), DPPC (green triangles (down)) and POPC (black triangles (up)) with applying an off-resonant saturation pulse at -74.0 ppm.



Supp. Fig. 6 Depolarization-time profile along row 18 of the phantom of the depolarization-time map in Fig. 4 in the main manuscript.



Supp. Fig. 7 Pixel-wise depolarization analysis of the phantom. The ILT result for each pixel is overlayed with the color coding representing its depolarization time (maximum of the fit). Feel free to zoom in in the electronic version. The inset in each pixel figure is the decaying data for that very pixel for $t_{sat} = 0.001 - 20$ s. The inset's y-axis is in arbitrary units. Mainly at the edges of the compartments multiple maxima are visible sometimes (pixel 19/14 for example).

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