# **Supplementary Information**

# Time-domain analysis of R-type PDLF NMR experiments with RF spatial inhomogeneity

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#### SAMPLE PREPARATION

All the lipids used in this work were acquired from AVANTI LIPIDS as dry powders. The purity of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (POPE), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and perdeuterated DMPC (DMPCd54) was higher than 99%. The brain lipid extract was a chloroform:methanol extract of porcine brain with a designated composition of 9.6 wt% of phosphatidylcholine (PC), 16.7 wt% of phosphatidylethanolamine (PE), 10.6 wt% of phosphatidylserine (PS), 2.8 wt% of phosphatidic acid, 1.6 wt% of phosphatidylinositol (PI) and of remaining undesignated lipids. The multi-lamellar vesicles of POPE and of the brain lipid extract were prepared by simply mixing the lipid powder with water (40 wt% of water) and repeatedly centrifuging and mixing the system using a thin metal rod until the sample was visibly homogeneous. DMPC/DMPCd54 samples were prepared by co-dissolving equal amounts (wt%) of DMPC and DMPCd54 in chloroform. The solvent was then evaporated under nitrogen flow, in a sonicator heatbath above the lipid melting temperatures. To remove any remaining solvent, the resulting lipid film was kept at reduced pressure over night. For hydration, the glass vial containing 3 ml of water. The desiccator was evacuated and the lipid film kept in the resulting high humidity environment for at least six hours. The hydrated films were then filled into Bruker MAS sample inserts of 25  $\mu$ l volume.

## NMR EXPERIMENTS

The experiments were conducted on a Bruker Avance III spectrometer operating at a <sup>1</sup>H Larmor frequency of 400.03 MHz. The <sup>2</sup>H NMR experiments and the <sup>1</sup>H-<sup>13</sup>C R-PDLF NMR experiments were performed by using a broadband static probe and a standard double channel 4 mm MAS solid-state NMR probe, respectively. All R-PDLF experiments were done on samples kept inside 25  $\mu$ l Kel-F disposable high-resolution inserts purchased from BRUKER and using magic angle spinning (MAS) at a frequency of 5 kHz.

## **RF** pulse callibration

RF pulses were calibrated by performing 2D NMR experiments to measure the nutation frequency at fixed RF power levels. The 2D NMR experiments used consisted simply on using a single pulse with increments of the pulse length in the indirect dimension. Fourier transformation in both the direct and indirect dimensions yielded splittings from which the nutation frequency was determined and used to calibrate all RF pulses. The nutation frequency of the resulting power levels for R18<sup>7</sup><sub>1</sub> pulses was verified immediately before and after the R-PDLF experiments were performed.

#### **R-PDLF** experiments

The R-PDLF experiments were performed using  $R18_1^7$  recoupling blocks and rINEPT as the polarization transfer step [1]. The nutation frequencies of the  $R18_1^7$  pulses was 45 kHz. The delays used for the rINEPT block were 1.2 and 2 ms and the rINEPT pulses had a nutation frequency of 78.125 kHz. To optimize the rINEPT step, it is crucial to use multiples of the MAS period for these delays. During acquisition heteronuclear decoupling was performed with SPINAL64 [2]. Depending on the sample, a total number of 8 to 32 points in the indirect dimension were measured with increments of twice the rotation period. For processing the data, the Fourier transform in the direct dimension was done after performing zero filling and apodization (with a simple single-exponential decay) of the free induction decays. The Fourier transform in the indirect dimension was used.



FIG. S1: Measurement of the RF field strength spatial inhomogeneity using a water sample contained in an insert. (A) Imaging of the sample by measuring the <sup>1</sup>H NMR spectrum of the sample under a magnetic field *z*-gradient generated with the *z*-linear shim coil. (B) Indirect dimension spectra showing the nutation frequency accross the different positions in the sample. (C) Experimental RF inhomogeneity profile (circles) and gaussian functions with different width (solid lines). The red line corresponds to the gaussian function used to describe the spatial inhomogeneity in the NMR simulations performed.

# <sup>2</sup>H NMR experiments

For acquiring the <sup>2</sup>H NMR spectrum of the DMPC/DMPCd54 sample, we employed a quadrupole echo sequence [3] with a pulse nutation frequency of 58 kHz, an inter-pulse delay of 40  $\mu$ s and a relaxation delay of 1 s. Signal acquisition was started directly after the second pulse, for a total of 50 ms with dwell time of 0.5  $\mu$ s. For processing the data, we used the maximum of the quadrupole echo acquired as the first point of the time domain signal and discarded the previous points. The <sup>2</sup>NMR spectrum was obtained then by performing a Fourier transform on the time domain signal without using neither zero-filling nor apodization.

#### Measurement of the RF spatial inhomogeneity profile

To determine the RF spatial inhomogeneity of the double channel 4 mm MAS solid-state NMR probe, we used an insert for 4 mm solid-state NMR rotors (purchased from BRUKER) filled with water. The same type of inserts were used for all measurements such that all the samples investigated experienced the same exact RF spatial inhomogeneity. The RF field strength inhomogeneity was measured by applying the method proposed by Odedra and Wimperis [4], namely by using a strong linear magnetic field gradient along the direction of the external magnetic field (by means of the corresponding shimming coil) enabling 1D imaging of the sample and performing the 2D NMR pulse sequence described in the previous section for recording a spatially resolved nutation frequency profile. Fig. S1 shows the RF inhomogeneity profile measured which was fitted with a gaussian lineshape. Such gaussian profile was then used to generate R-PDLF simulation data that was representative of the experimental conditions (details in the next section). Similarly to previous studies [5, 6], the RF profile measured shows a plateau for the RF strength in the central part of the sample (the region of the sample with the highest nutation frequency), and a nearly symmetrical decrease of RF field strength towards the outer parts of the sample. By summing over the Fourier transforms of the spatially resolved slices one observes that the resulting splitting is equal to the maximum splitting i.e. the splitting corresponding to the plateau region. This means that if one performs the same 2D nutation experiment using typical shimming settings that enable high resolution (i.e. narrow <sup>1</sup>H peaks), the splitting observed in the indirect dimension yields the nutation frequency corresponding to the middle part of the sample where the highest nutation frequency is observed. We use this nutation frequency value to calibrate our RF pulses. Note that by simply finding the pulse length for which the magnetization crosses zero after the first maximum yields a lower nutation frequency leading to miscalibration of the RF pulses.

#### NMR SIMULATIONS

The NMR simulations of the R-PDLF experiments were generated with SIMPSON [7]. To account for the RF inhomogeneity in the simulations a MATLAB script was composed that generates SIMPSON input files and SIMPSON call-outs to cover the measured RF inhomogeneity profile of the used double channel 4 mm MAS solid-state NMR probe. Both the dipolar recoupling and the rINEPT blocks were included in the NMR simulations performed. The procedure generated a simulation database of R-PDLF simulations with inclusion of RF inhomogeneity for several dipolar couplings ranging from 0 to 22 kHz in steps of 22 Hz which corresponds to C–H bond order parameter steps of 0.001. For each distinct dipolar coupling value, a total number of 40 simulations were used to cover the RF inhomogeneity profile. Each simulation was performed with the following parameters. The total number of time domain points simulated (indirect dimension) was 128. The number of repulsion angles used was 320 and the number of gamma angles was 6. All the remaining settings were the same as in the experiments performed. The simulation database generated was then used to fit the experimental data. The MATLAB script used and the database generated are accessible for public use in https://github.com/tfmFerreira/inhomogeneous-rf-nmr.git.

### TIME-DOMAIN FITTING AND DEFINITION OF ERROR LIMITS

In order to fit the experimental R-PDLF dipolar modulations, we first determined the root mean square deviation (RMSD) between the experimental data and the full range of NMR simulations generated with distinct order parameter,  $S_{CH}$ , magnitudes (from 0 to 0.5) and used the minimum of the RMSD dependence on order parameter magnitude. We then optimized the RMSD of the resulting simulation by multiplying a single-exponential decay to the simulation data. These two steps were iterated, using the exponential decay of each iteration to fit subsequently the simulation data multiplied by such decay, until no change in the fitted parameters was observed. This is shown for both the DMPC/DMPCd54 and the POPE samples in Figs. S2-S8. For the DMPC/DMPCd54 sample the exponential decay resulted in only a minimal improvement of the RMSD for the  $\omega - 1$  and  $\omega - 2$ . On the other hand, for the  $\omega$  carbon of DMPC and for the  $\beta$  carbon of the POPE sample, multiplication of an exponential function to the data improves the match between the experimental data and the fitted simulation data more significantly, as can be seen in Figs. S2, S3 and S8. After such optimization, the error limits were then defined by considering only data points for which the difference between the simulation (after multiplication of the exponential decay) and experimental values was within a margin of 50 % of the experimental uncertainty - data points that did not fit this requirement were discarded (red points) in order to ensure that the errors were not underestimated. The upper and lower boundaries were then calculated as the limits at which the NMR simulations fall outside the experimental uncertainty augmented by a factor of 1.5 for the points kept (plot at the top right corner in Figs. S2-S8).

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FIG. S2: Schematic illustration of the methodology used to fit the experimental data and to estimate the potential errors of the order parameters determined. (A) The complete simulation database is used to estimate the RMSD with respect to the experimental measurement. The minimum of the RMSD is then used as the most probable order parameter. (B) The matching of the best fit simulation and experimental values is then optimized by multiplying a single-exponential decay to the simulation data which minimises the RMSD further and steps (A) and (B) are iterated until convergence is reached. (C) The result from (B) is then used to determine the potential uncertainties of the estimated order parameter in (A). First, the points for which the difference between simulation and experiment is higher than 50% of the experimental uncertainty are discarded (red) and then the remaining points are used to determine the order parameter boundaries at which simulated data deviates from the experimental data more than 130% of the experimental uncertainty (top right). The data shown corresponds to the analysis of the DMPC carbon peak labelled  $\omega$  in Fig. 5 of the main text.



FIG. S3: The same methodology as in Fig. \$2 but using two-component fits.



FIG. S4: Schematic illustration of the methodology used to fit the experimental data and to estimate the potential errors of the order parameters determined as in Fig. S2 but for the DMPC carbon peak labelled  $\omega - 1$  in Figure 5 of the main text.



FIG. S5: The same as in Fig. S4 but using two-component fits.



FIG. S6: Schematic illustration of the methodology used to fit the experimental data and to estimate the potential errors of the order parameters determined as in Fig. S2 but for the DMPC carbon peak labelled  $\omega - 2$  in Figure 5 of the main text.



FIG. S7: The same as in Fig. S6 but using two-component fits.



FIG. S8: Schematic illustration of the methodology used to fit the experimental data and to estimate the potential errors of the order parameters determined as in Fig. S2 but for the POPE carbon peak labelled  $\beta$  in Figure 4 of the main text.