Supporting Information

Solid-State NMR on Bacterial Cells: Selective Cell-Wall-Signal Enhancement and Resolution Improvement using Dynamic Nuclear Polarization

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Section 1

<u>Experimental conditions for DNP-enhanced ssNMR</u>:

All experiments except the conventional NMR experiment were performed on a Bruker AVANCE™ III 400 MHz NMR system equipped with a 263 GHz gyrotron, a transmission line (corrugated waveguide) and a low temperature (~100 K) triple-resonance 3.2-mm MAS probe. All data were recorded, processed and analyzed using Bruker Topspin 3.0. For 1D ^{13}C-CPMAS experiments, 100 kHz of RF-field strength was applied for the ^{1}H $\pi/2$ pulse and SPINAL-64[1] heteronuclear decoupling. The ^{13}C CP spin-lock was applied at a RF-field strength of 60 kHz and the ^{1}H RF-field strength was adjusted to match a Hartmann-Hahn condition using a linear ramp. CP contact time was set to 0.4 ms. All 1D experiments were performed at a sample spinning frequency of 8.5 kHz unless otherwise stated. The time-domain signals were zero-filled and Fourier transformed without any apodization.

The pulse sequence used for the 2D ^{13}C homonuclear dipolar correlation experiments involving the SPC5[2] recoupling scheme is shown in Fig. S1. The same ^{1}H RF-field strength as for the 1D ^{13}C-CPMAS experiments were used for $\pi/2$ pulse and SPINAL-64 decoupling. MAS frequency was set to 13 kHz. ^{13}C RF-field strength during SPC5 recoupling was 65 kHz. High-power ^{1}H Lee-Goldburg[3] continuous wave (CW) decoupling was applied during the recoupling pulses. The SPC5 mixing time was set to 1.23 ms (n = 4) to observe one-bond correlations. Recycle delay, number of complex t_1 points (spectral width: 65 kHz) and total experiment time on the EC sample were 4 s, 128 (2 ms evolution time) and 4.6 hours for 5 mM TOTAPOL sample and 1 s, 96 (1.5 ms evolution time) and 14.5 hours for 60 mM TOTAPOL sample, respectively. Quadrature detection in the indirect dimension was obtained by States-TPPI. The experimental data were zero-filled and apodized with a cosinebell function.

A 1D ^{13}C-CPMAS experiment using conventional NMR was performed on a Bruker AVANCE™ 400 MHz NMR system equipped with a double-resonance 4-mm MAS probe at a sample temperature of ca. 270 K. All RF-field strengths and processing parameters used were the same as for the 1D DNP-enhanced NMR experiments. MAS frequency was set to 13 kHz.

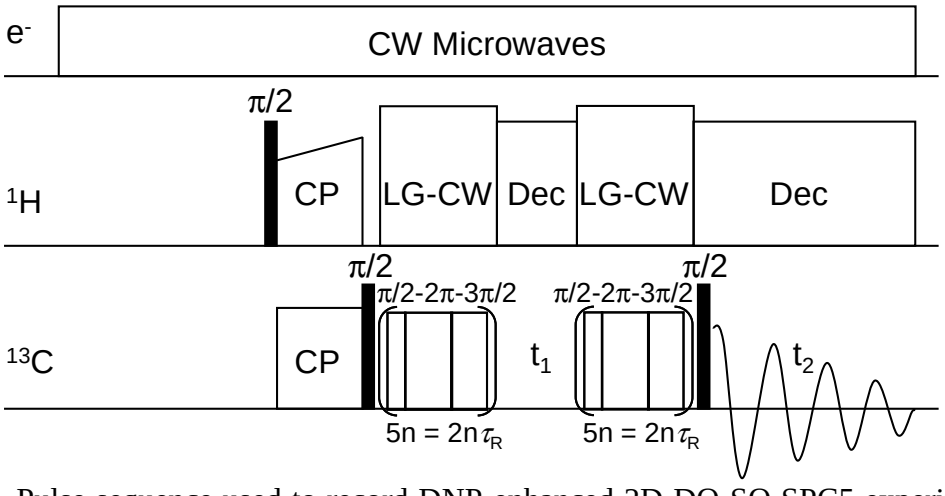

Figure S1. Pulse sequence used to record DNP-enhanced 2D DQ-SQ SPC5 experiments: ^{1}H magnetization is enhanced by a DNP step followed by the application of a standard recoupling pulse sequence.

Section 2

<u>Evaluation of sample re-suspension time in DNP matrix</u>:

For DNP experiments, EC, DC and CWM samples were first re-suspended for a few minutes in a DNP matrix composed of d_6-DMSO/D_2O/H_2O in a volume ratio of 30:60:10 with various concentrations of TOTAPOL, and then centrifuged back to recover the pellet. For each washing step, the volume of DNP matrix used was 5 times larger than the cell or CWM pellet. In order to check that a re-suspension time of a few minutes is long enough for TOTAPOL to uniformly distribute in the entire sample, spectra obtained on a CWM sample washed for one minute and 30 minutes are compared. 40 µL of CWM sample previously rinsed with DNP matrix without TOTAPOL was re-suspended for one minute in 200 µL of the above mentioned DNP matrix containing 1 mM TOTAPOL. The sample was then centrifuged and packed into the rotor. After spectra acquisitions, the same sample was again re-suspended for 30 minutes in the supernatant kept from the previous centrifugation. The sample was centrifuged again and packed back into the rotor. Figure S2 shows the 1D CPMAS spectra of the one-minute and the 30-minutes washed samples. No significant difference can be observed, confirming that a re-suspending time of a few minutes as used throughout this work is long enough to reach equilibrium.

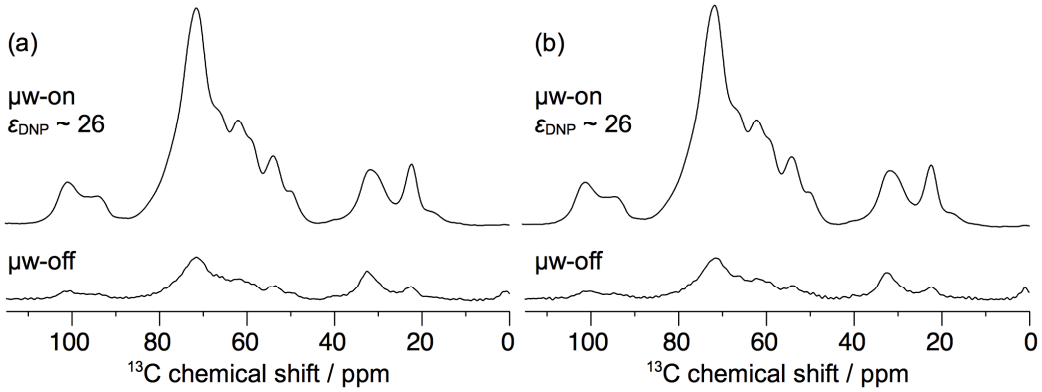

Figure S2. 1D ^{13}C-CPMAS spectra of CWM washed for 1 (a) and 30 (b) minutes in 1 mM TOTAPOL DNP solution with microwave-on (top) and -off (bottom). ε_{DNP} is about 26 in both cases. DNP build-up time constants are in the range 2.5 – 3.2 s for (a) and 2.4 – 3.1 s for (b).

Section 3

DNP build-up time constants on CWM, DC and EC (a full range of Fig. 2):

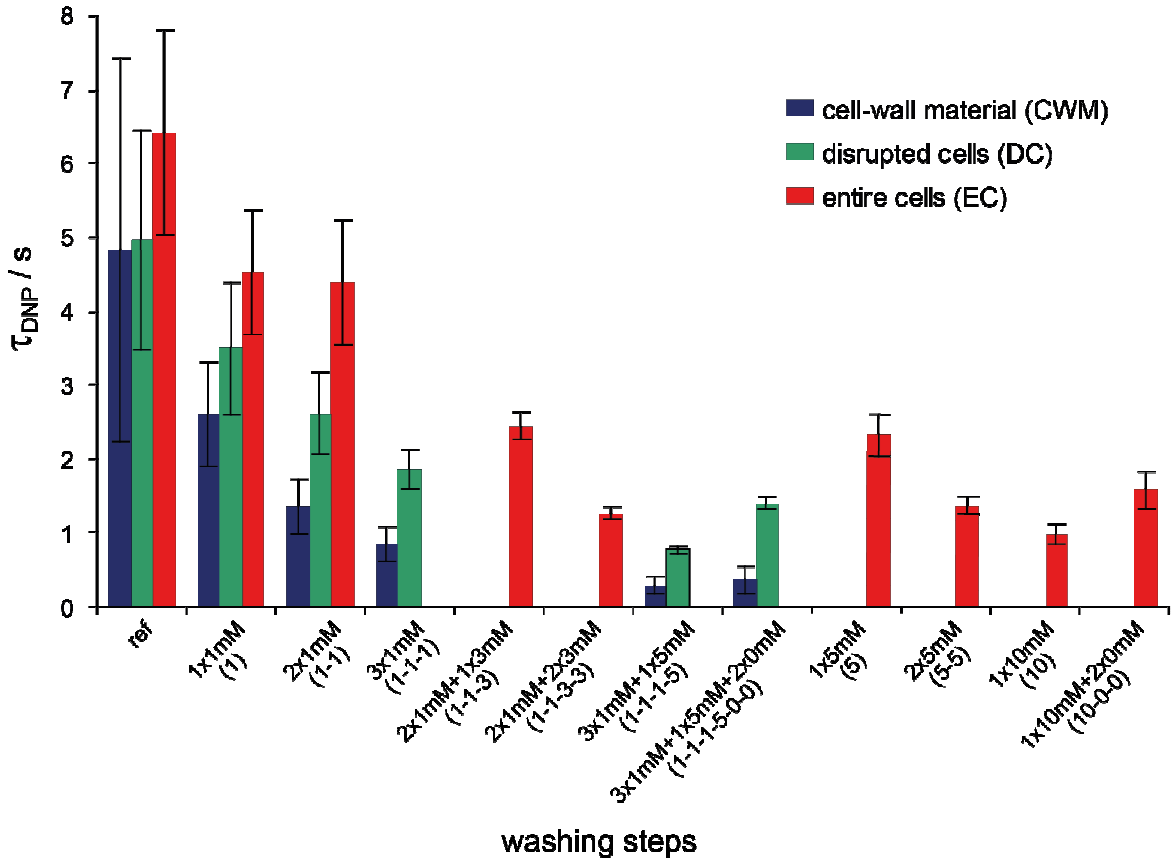

Figure S3. DNP build-up time constants τ_{DNP} averaged over the different cell-wall peaks of the 1D spectra on CWM (blue), DC (green) and EC (red) samples, recorded at different TOTAPOL concentrations. Error bars reflect the heterogeneity of τ_{DNP} over the different peaks of the spectra. Note that very similar results are obtained on EC for the (1-1-3) and the (5) samples, as well as for the (1-1-3-3), the (5-5), and the (10) samples. In each group, the cells were in contact with the same total amount of TOTAPOL despite the different number of washing steps with different concentrations.

Section 4

<u>1D ^{13}C-CPMAS experiments on the EC and DC samples</u>:

Successive washing steps in different DNP matrices were also carried out on the EC and DC samples. The washing procedure was the same as for CWM, which is described in the article, with however different TOTAPOL concentrations.

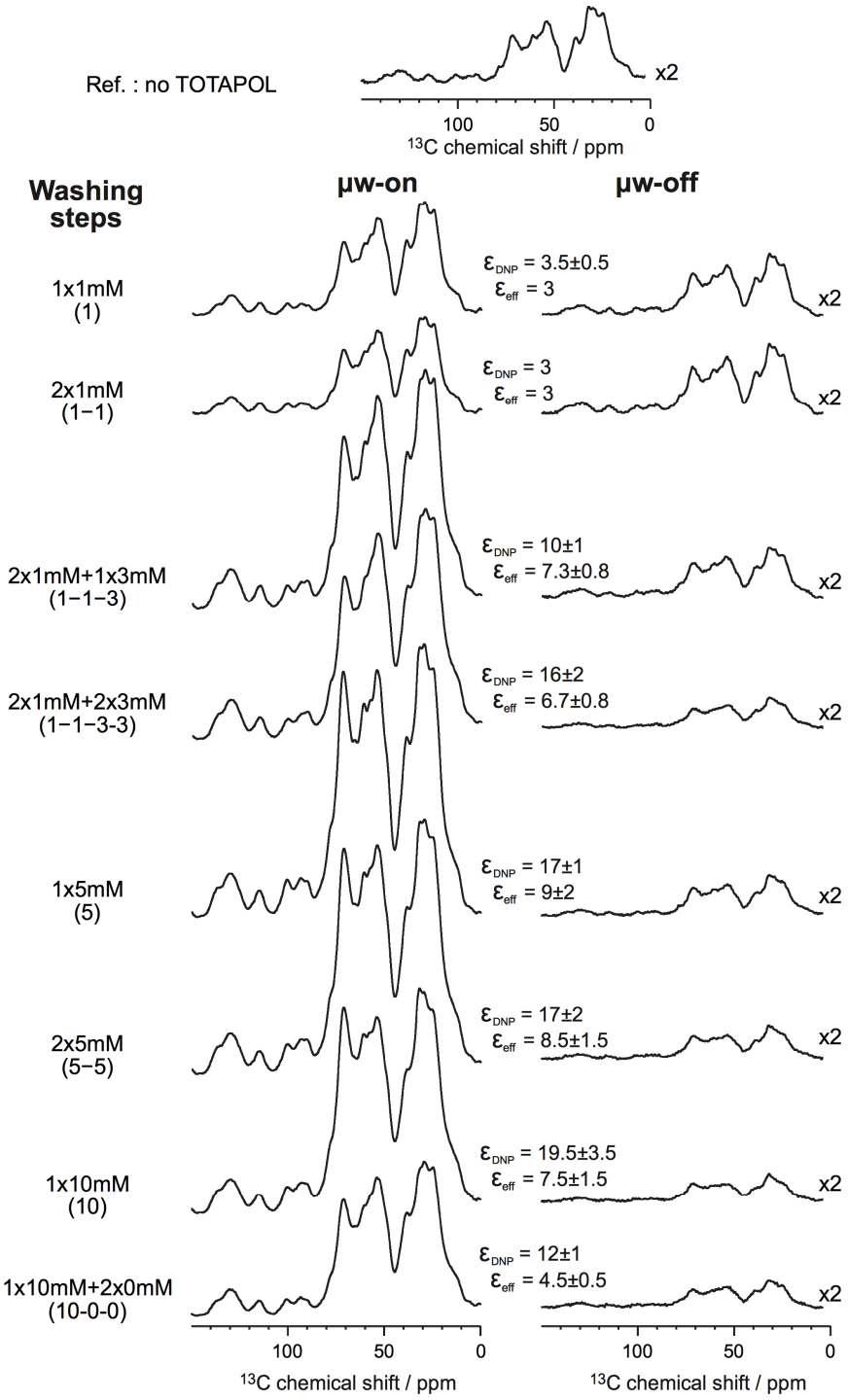

Figure S4. 1D ^{13}C-CPMAS spectra on EC recorded at various TOTAPOL concentrations with (left column) and without (right column) µw irradiations. See caption of Fig. 1 for additional experimental conditions.

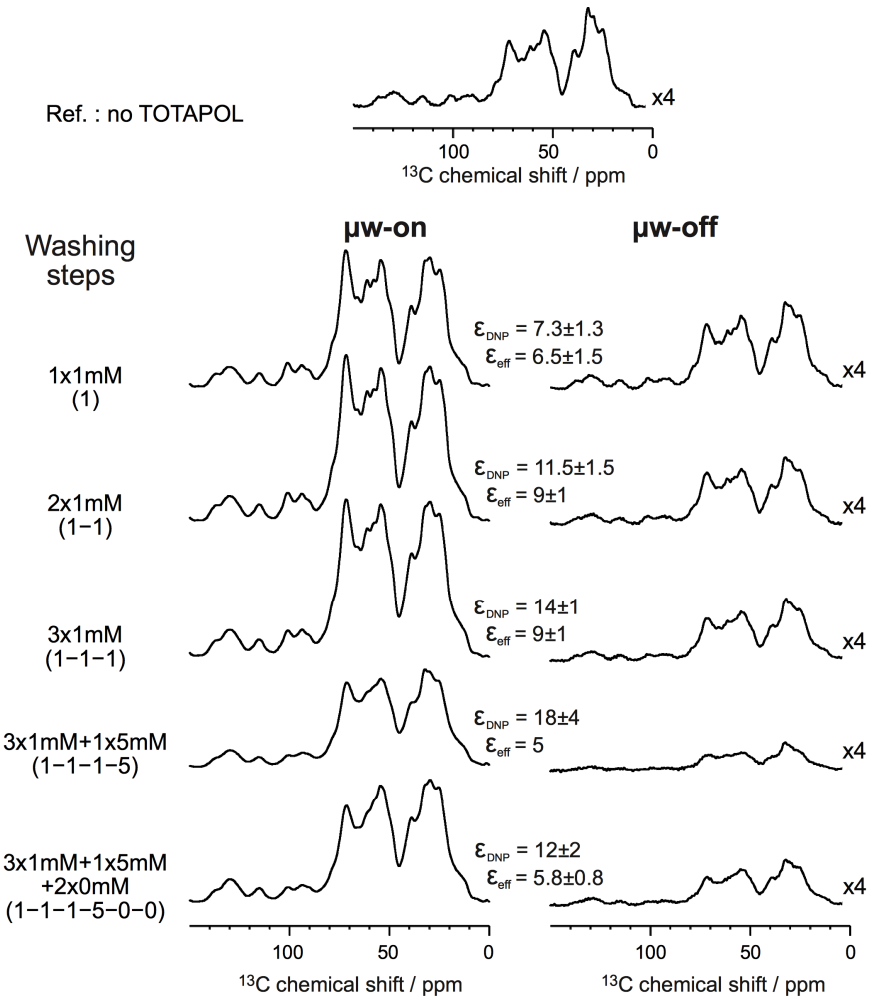

Figure S5. 1D ^{13}C-CPMAS spectra on DC recorded at various TOTAPOL concentrations with (left column) and without (right column) µw irradiations. See caption of Fig. 1 for additional experimental conditions.

Section 5

<u>Propagation of DNP-enhanced magnetization by ^{1}H spin diffusion</u>:

Estimation of the distance over which the DNP-enhanced magnetization propagates is difficult and complex, as it relies on the proton spin-diffusion rate which is not readily available. Pseudo one-dimensional model of ^{1}H spin diffusion accounting for polarization-transfer depth has been developed for the case of amyloid crystals.[4] Estimation of a proper spin-diffusion rate D for our system needs to take into account several aspects when compared to Ref. 4

i) some molecular mobility is still present at 100 K as revealed by the short T_1 time constant measured on entire cells without radical doping (see Fig. 2);

ii) peptidoglycan is a very loose polymer containing a lot of solvent (DNP matrix), which is 90% deuterated, leading to a longer average ^{1}H–^{1}H distance;

iii) the presence of proton-deuterium couplings slows down proton spin-diffusion;[5]

iv) the proton spin-diffusion rate is inversely proportional to the MAS spinning speed in the absence of strong dipolar couplings such as ^{1}H–^{1}H or ^{1}H–^{13}C,[6] which is the case here when considering the high level of deuteration.

All these factors lead to a decrease of the spin-diffusion rate. Precise estimation of D is beyond the scope of this paper. In a rough estimation, we considered a reduction factor of 20 compared to a typical value of D = 0.7 nm^2/ms for polymers at room temperature.[7] Using Eq. (6) in Ref. 4, it leads to a polarization-transfer depth (at half of the original intensity) of 30-50 nm, which is in the order of magnitude of the cell-wall thickness in *B. subtilis*. Considering furthermore that a gradient in TOTAPOL concentration is probably present across the cell wall with higher concentrations at the outer surface, the DNP sensitivity enhancement can roughly be considered as essentially present in the cell wall.

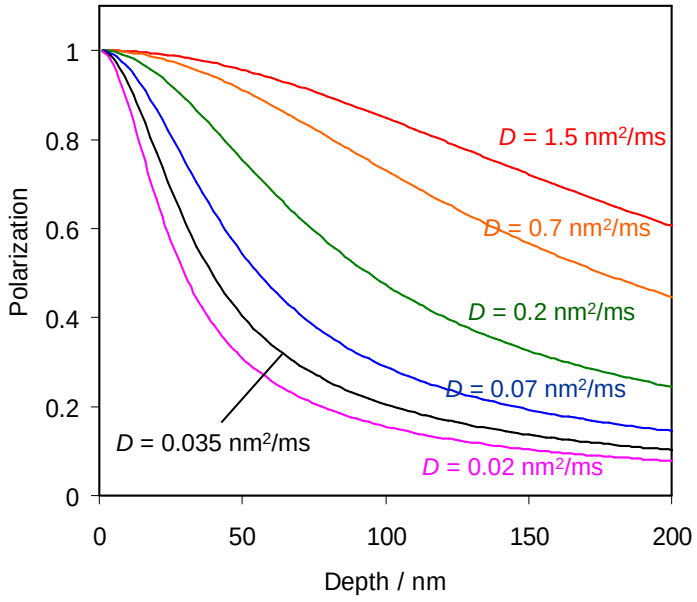

Figure S6. Polarization-transfer depth calculated using Eq. (6) in Ref. 4 with various ^{1}H spin-diffusion rate.

Section 6

<u>Sample stability during long-time storage</u>:

The stability of the EC sample was measured by comparing the spectra of a sample as freshly prepared and after 6-months storage in the freezer at 253 K. No change in the 1D ^{13}C-CPMAS and 2D ^{13}C–^{13}C DARR spectra was observed (Fig. S7). ε_{DNP} and the linewidths were almost unchanged, indicating that all TOTAPOL radicals were still active.

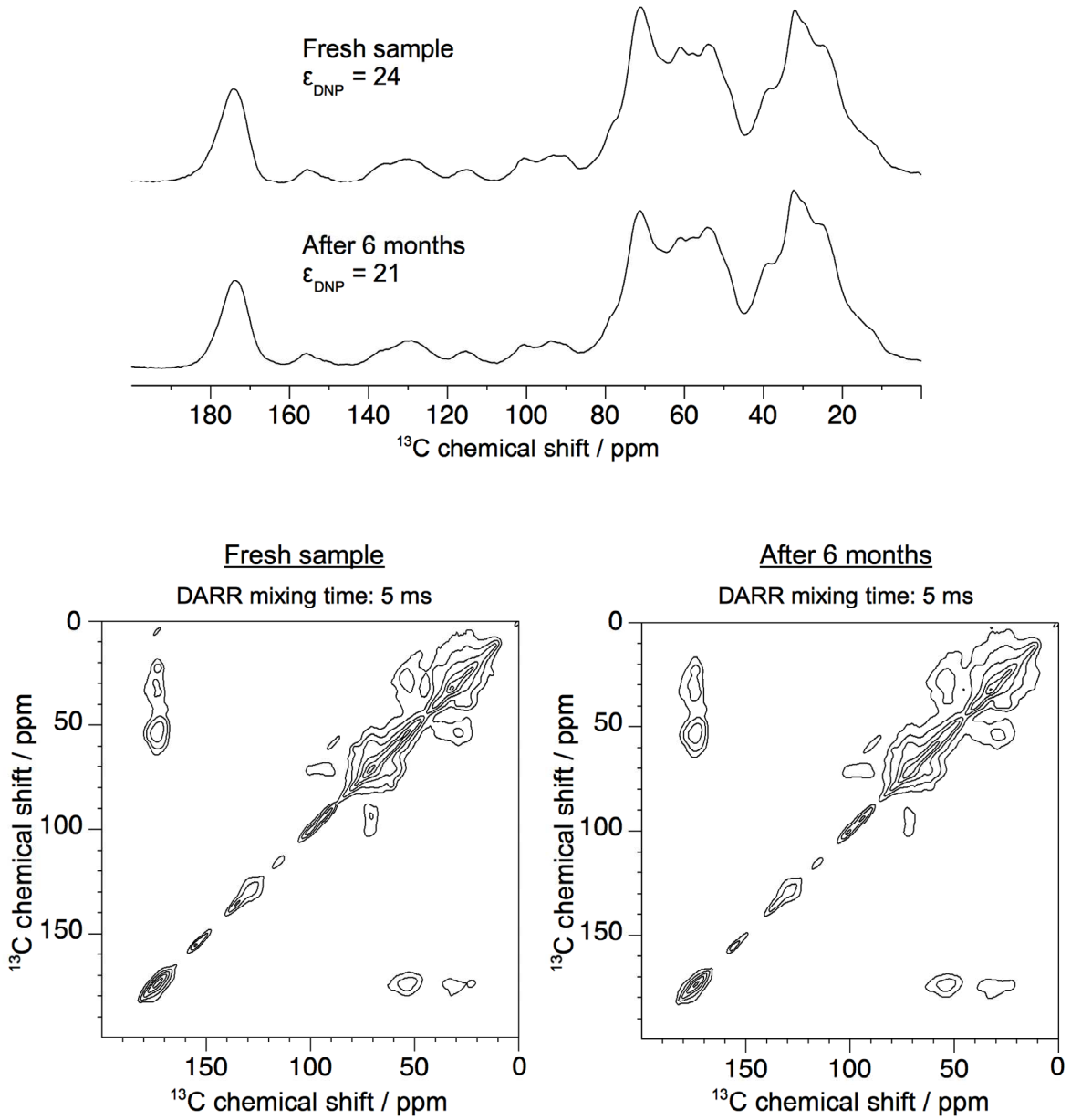

Figure S7. 1D ^{13}C-CPMAS spectra (top) and 2D ^{13}C–^{13}C DARR spectra (bottom) of EC samples. The state of the sample (fresh or 6 months old) is indicated in the figure.

References:

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