Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Material

Quantification of the amount of mobile components in intact stratum corneum with natural-abundance ^{13}C solid-state NMR

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Estimation of the total amount of lipids in SC:

The total molar amount of lipids in 1 g dry SC obtained from Q-INEPT experiments on intact SC at 50 wt% water and 90 °C is 254 μmol ωCH_3, 379 μmol αCH_2 and 150 μmol CHOL 9 (median values in Fig. 8). At this high temperature, the resonance from αCH_2 lipid acyl chain may overlap with the peaks from proteins and therefore is not employed to estimate the amount of lipids. We then assume that:

• The number of mole of CHOL 9 is equal to the number of mole of cholesterol. The number of mole of ωCH_3 includes ωCH_3 in both ceramides and fatty acids. Each ceramide molecule contains two ωCH_3 segments (Fig. 1B).

• The SC lipids comprise ceramides and fatty acids and we here assume they are present in equimolar proportions.[1]

• The mean molecular weight of ceramides is 700 g/mol.[2] The mean molecular weight of fatty acids was calculated to be 349 g/mol based on a fatty acid mixture[3] with composition similar to native SC.[4] This mixture consists of C16:0, C18:0, C20:0, C22:0, C23:0, C24:0, and C26:0 lipids, where the notation C*C*:*D* is used to denote the numbers *C* of carbon atoms and *D* of double bonds of the fatty acid, at mole percent of 1.3, 3.3, 6.7, 41.7, 5.4, 36.8, and 4.7, respectively.

Taken together, we estimate that 1 g dry SC contains 254/3 = 85 μmol fatty acids (corresponding to $85 \cdot 10^{-6} \cdot 349 = 0.030$ g), 254/3 = 85 μmol ceramide (corresponding to $85 \cdot 10^{-6} \cdot 700 = 0.060$ g) and 150 μmol cholesterol (corresponding to $150 \cdot 10^{-6} \cdot 387 = 0.058$ g). The total amount of SC lipids is therefore ca. 0.15 g per 1 g dry SC or 15 wt% of the total weight of dry SC.

Example of changes in chemical composition of the SC mobile lipid domains by adding a hydrophobic chemical:

As an example, a small hydrophobic compound with a molecular weight of 200 g/mol is added to SC at a concentration of 5 wt% or 263 μmol per 1 g of dry SC. The total amount of mobile ωCH_3 and CHOL segments in SC at 40 wt% water and 32 °C is 153 μmol (Table S1). If the added hydrophobic compound is dissolved in SC fluid lipids, the SC fluid lipid domains now contain a high amount of this chemical and their chemical-physical properties are likely not the same as the fluid lipid domains in the samples of SC without the added chemical.

Table S1. Fraction of mobile segments (mol%) in SC at 40 wt% water and 32°C, F_{40W}, over the total mole of the same segment. This fraction is calculated from median value of the molar amount of mobile segments in SC at 40 wt% water and 32 °C, C_{40W} (μmol per 1 g dry SC), and median value of the total molar amount of lipids in SC, C_{all} (μmol per 1 g dry SC).

Segments	ωCH_3	CHOL 9
C_{40W} (μmol)	46	107
C_{all} (μmol)	254	150
F_{40W} (mol%)	18	71

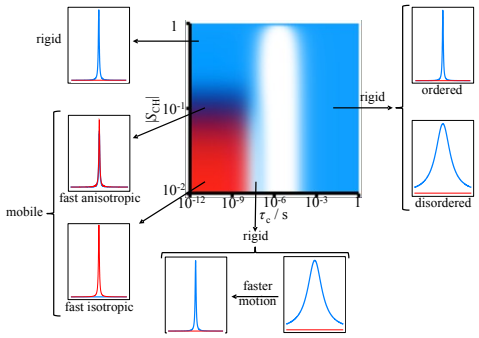

Fig. S1. Theoretical CP (blue) and INEPT (red) signal enhancement as a function of τ_c (rotational correlation time) and $|S_{CH}|$ (C-H bond order parameter) for a CH_2 segment at the experimental conditions used in this study, i.e., 11.72 T magnetic field and 5 kHz MAS (adapted from Nowacka et al.).[5] White indicates the absence of signal for both CP and INEPT. Corresponding lineshapes and intensities of CP (blue) and INEPT (red) signals in different regimes are also shown.

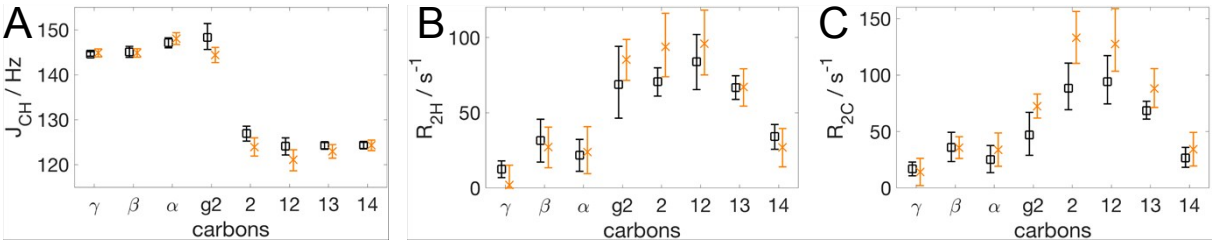

Fig. S2. Results (median and 90% confidence intervals) obtained from Q-INEPT for DMPC L_α phase with 35 wt% water at 27 °C and measured on the 500 MHz (black ☐) and 800 MHz (orange x) spectrometers. (A) ^{1}H–^{13}C through-bond scalar coupling J_{CH}. (B-C) Effective ^{1}H (B) and ^{13}C (C) transverse dephasing rates R_2^H and R_2^C. The J_{CH} values are much lower in the acyl-chains compared to the headgroups as well as the glycerol backbone C_{g2}. The high J_{CH} values are likely due to chemical bond of the ^{13}C with oxygen or nitrogen.[6] The different relaxation rates are lowest for carbons in the outer part of the headgroup and in the end of the acyl-chain, which have faster and more isotropic reorientation than the others carbon in the lipid molecule.[7] We note that the relaxation rates of C_{g2} and some acyl-chain carbons including C_2, C_{12} and C_{13} are lower at the lower Larmor frequency.

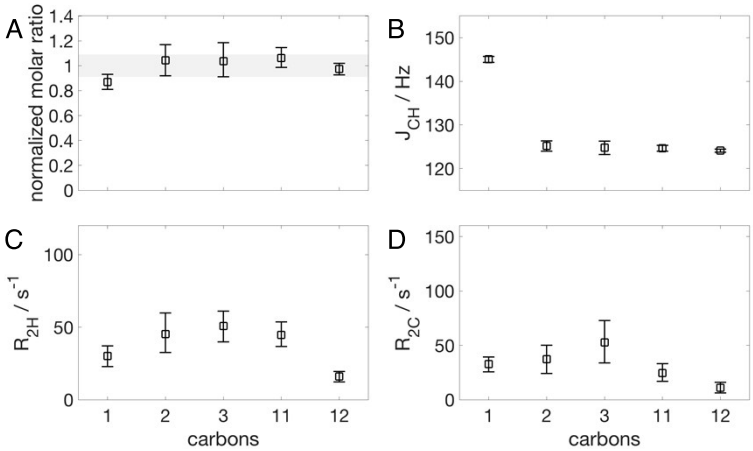

Fig. S3. Results (median and 90% confidence intervals) obtained from Q-INEPT for SDS H_I phase at 35 °C and measured on the 500 MHz spectrometer. (A) Normalized molar ratio. The spread of the normalized molar ratios over different segments is represented by grey shaded area. (B-D) ^{1}H–^{13}C through-bond scalar coupling J_{CH} (B), and effective ^{1}H (C) and ^{13}C (D) transverse dephasing rates, R_2^H and R_2^C. The J_{CH} value of C_1 of SDS is higher than the others due to its bond with oxygen.[6]

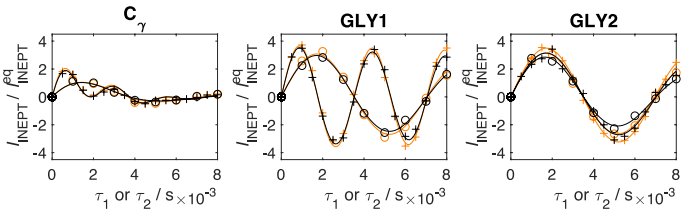

Fig. S4. Experimental (symbol) and fitted (line) data of I_{INEPT}/I_{INEPT}^{eq} of different carbons in DPPC and glycerol for DPPC L_{gel} phase at 35 °C in Q-INEPT experiment obtained on the 500 (black) and 800 MHz (orange) spectrometers. The varied parameters are τ_1 (○□) while τ_2 = 1.2 ms or τ_2 (+) while τ_1 = 1.8 ms.

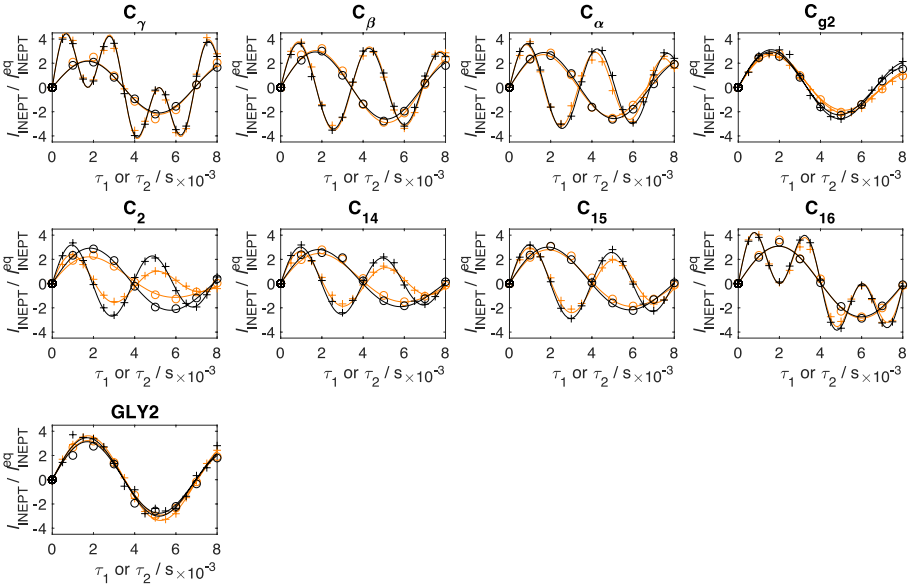

Fig. S5. Experimental (symbol) and fitted (line) data of I_{INEPT}/I_{INEPT}^{eq} of different carbons in DPPC and glycerol for DPPC L_{α} phase at 70 °C in Q-INEPT experiments obtained on the 500 (black) and 800 MHz (orange) spectrometers. The varied parameters are τ_1 (○□) while τ_2 = 1.2 ms or τ_2 (+) while τ_1 = 1.8 ms.

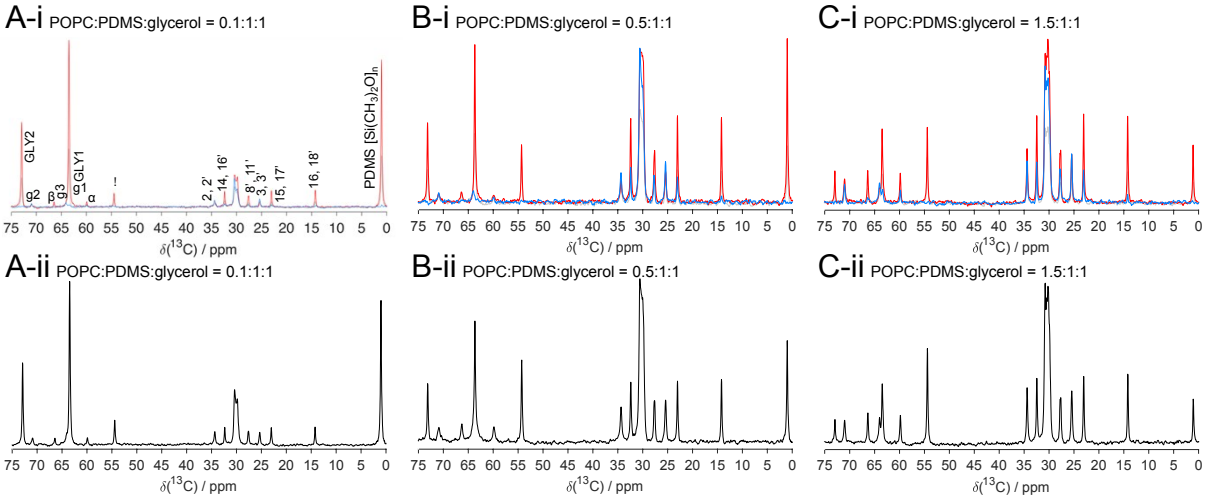

Fig. S6. ^{13}C MAS NMR spectra (DP: grey, CP: blue, INEPT: red) (i) and ^{13}C MAS NMR quantitative DP spectra (Q-DP, recorded at τ_R = 50 s) (ii) for POPC-PDMS-glycerol-water systems at different POPC:PDMS or

POPC:glycerol mixing molar ratios including 0.1 (A), 0.5 (B) and 1.5(C) measured at 32 °C on the 500 MHz spectrometer.

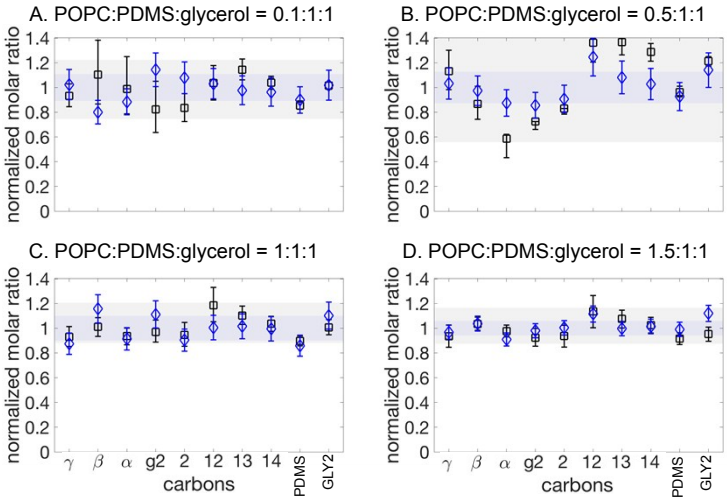

Fig. S7. Normalized molar ratios obtained from Q-INEPT (median (black □) and 90% confidence intervals (grey shaded area)) and Q-DP (median (blue ◊) ± standard deviation (blue shaded area)) for POPC-PDMS-glycerol-water systems at different POPC:PDMS or POPC:glycerol mixing molar ratios including 0.1 (A), 0.5 (B), 1 (C) and 1.5 (D) measured at 32 °C on the 500 MHz spectrometer. The molar ratios of PDMS and glycerol were normalized against the mixing ratios so that their normalized molar ratios are ideally 1 as the lipid. The 90% confidence intervals in Q-INEPT and the standard deviations in Q-DP of POPC refer to the spread of the normalized molar ratios over different segments of lipids. The 90% confidence intervals of PDMS and glycerol in Q-INEPT are calculated from different fitted values of C_{INEPT} obtained from Monte Carlo analysis, whereas the standard deviations of these molecules in Q-DP are from the standard deviations of eight values of the molar ratio of these molecules to each of the lipid segment.

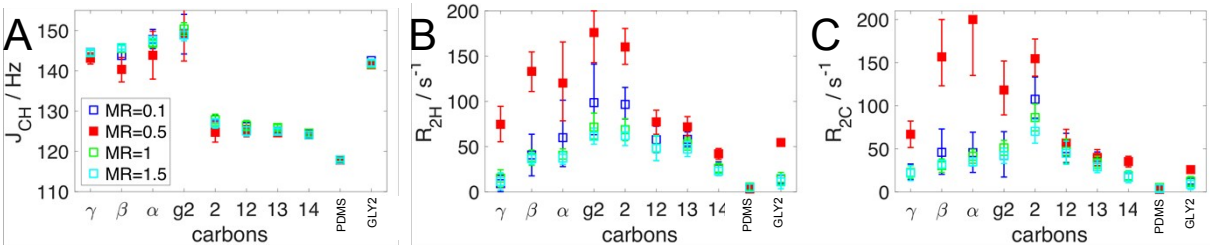

Fig. S8. Results (median and 90% confidence intervals) obtained from Q-INEPT for POPC-PDMS-glycerol-water systems at different mixing molar ratios (MR) of POPC:PDMS or POPC:glycerol measured at 32 °C on the 500 MHz spectrometer: ^{1}H–^{13}C through-bond scalar coupling J_{CH} (A) and effective ^{1}H (B) and ^{13}C (C) transverse dephasing rates, R_2^H and R_2^C.

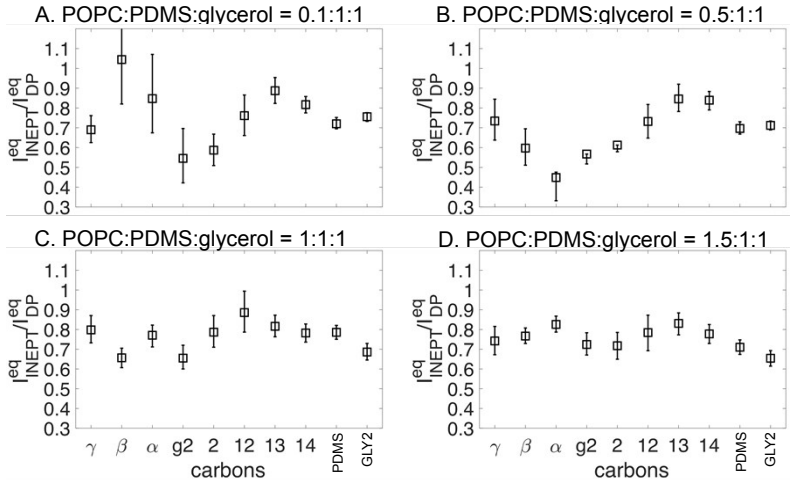

Fig. S9. $I_{INEPT}^{eq}/I_{DP}^{eq}$ ratios (median and 90% confidence intervals) of different segments of POPC, PDMS and glycerol obtained from Q-INEPT and Q-DP in samples of POPC-PDMS-glycerol-water at different POPC:PDMS or POPC:glycerol mixing molar ratios including 0.1 (A), 0.5 (B), 1 (C) and 1.5 (D) measured at 32 °C on the 500 MHz spectrometer.

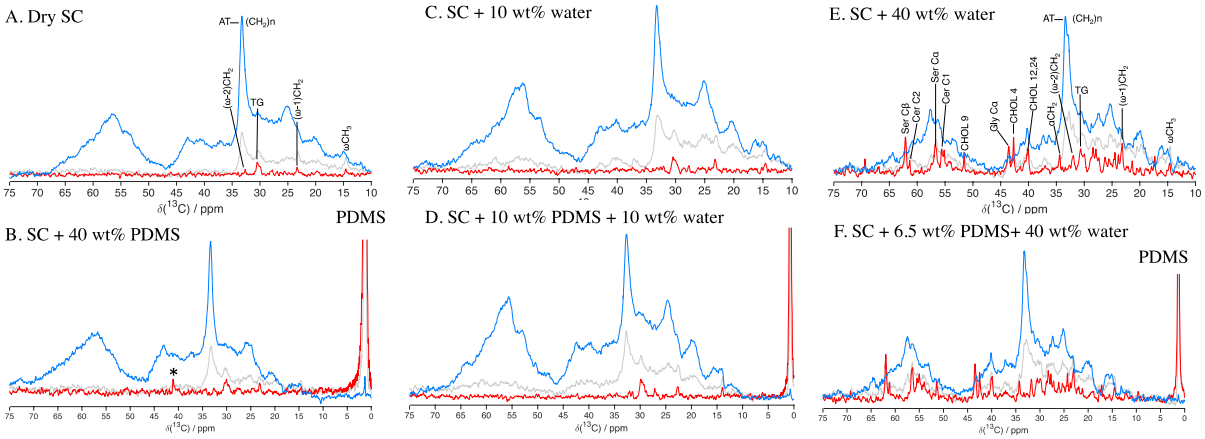

Fig. S10. ^{13}C MAS NMR spectra (DP: grey, CP: blue, INEPT: red) of dry SC (A), SC with 40 wt% PDMS (B), SC with 10 wt% water (C), SC with 10 wt% PDMS and 10 wt% water (D), SC with 40 wt% water (E), and SC with 6.5 wt% PDMS and 40 wt% water (F) at 32 °C. The peak marked with asterisk at ca 41 ppm in sample of SC with 40 wt% PDMS in (B) is a spinning sideband of a very intensive peak of PDMS at ca 1 ppm. The CP peak of PDMS in (B), (D) and (F) is negligible compared to its INEPT and also detected in sample of pure PDMS.

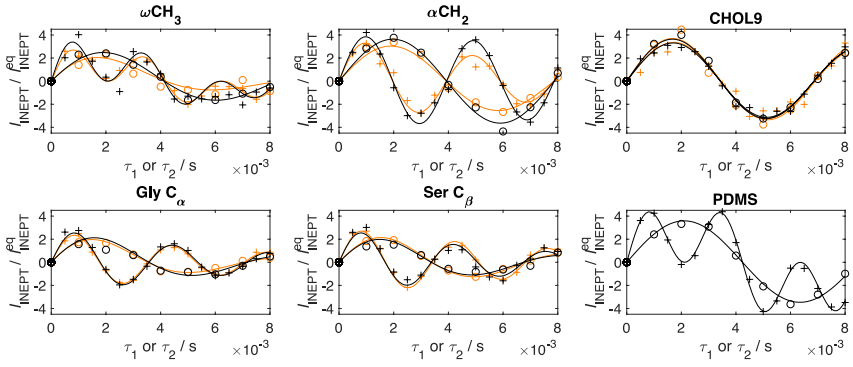

Fig. S11. Experimental (symbol) and fitted (line) data of I_{INEPT}/I_{INEPT}^{eq} of different carbons in SC-PDMS (0.5 wt%)-water (black) and SC-water (orange) systems at 40 wt% water and 32 °C in Q-INEPT obtained on the 500 MHz spectrometer. The varied parameters are τ_1 (○□) while τ_2 = 1.2 ms or τ_2 (+) while τ_1 = 1.8 ms.

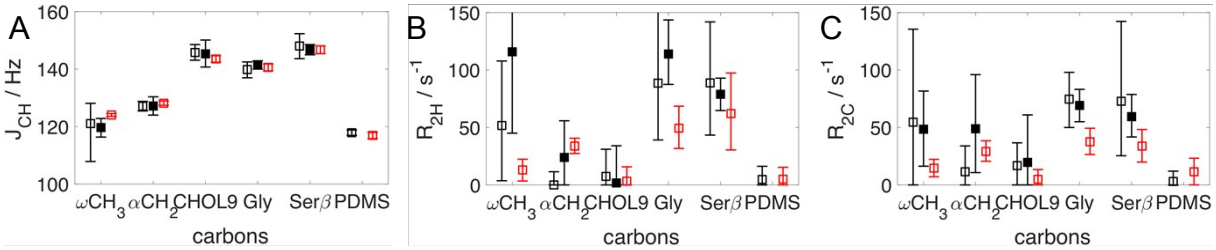

Fig. S12. Results (median and 90% confidence intervals) obtained from Q-INEPT for SC-PDMS (0.5 %wt)-water (□) and SC-water (■) systems at the same 40 wt% water and at 32 °C and for SC-PDMS (0.5 wt%)-water systems at 50 wt% water and 90 °C (□) measured on the 500 MHz spectrometer: ^{1}H–^{13}C through-bond scalar coupling J_{CH} (A), effective ^{1}H (B) and ^{13}C (C) transverse dephasing rates, R_2^H and R_2^C.

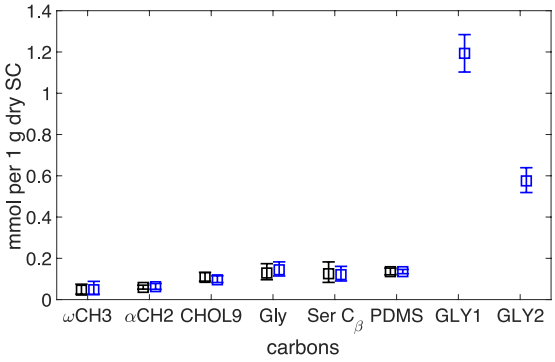

Fig. S13. Amount of mobile segments per 1 g dry SC (median and 90% confidence intervals) in samples of SC + 0.5 wt% PDMS (black) and of SC + 0.5 wt% PDMS + 5 wt% glycerol (based on the total weight of SC and glycerol) (blue) at the same water content of 40 wt%. The amount of glycerol added to the mixture of SC-PDMS is 0.571 mmol per 1 g dry SC which is similar to the amount obtained from Q-INEPT using PDMS as a reference, indicating that the amount of PDMS in the mixture of SC-PDMS is 0.5 wt%. The amount of GLY1 segment herein is not normalized against its number of carbon in the molecule (two GLY1 segments per one glycerol molecule as shown in Fig. 3A).

References

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