Supplemental Information

for

Lipid Dynamics and Protein-Lipid Interactions in 2D Crystals Formed with the β-barrel Integral Membrane Protein VDAC1

Matthew T. Eddy, Ta-Chung Ong, Lindsay Clark, Oscar Teijido, Patrick C. A. van der Wel, Robert Garces, Gerhard Wagner, Tatiana K. Rostovtseva, Robert G. Griffin

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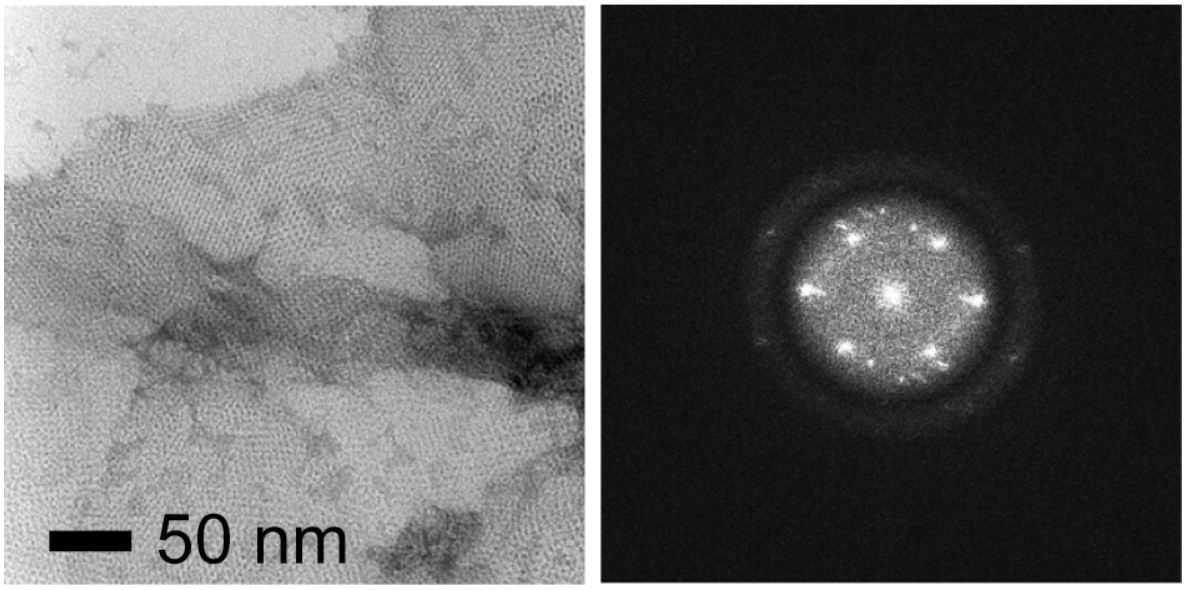

Figure S1. Electron microscopy image (left) and electron diffraction (right) of recombinant human VDAC1 reconstituted into DMPC. The bar on the left corresponds to a length of 50 nm. VDAC1 molecules can be observed as small black dots.

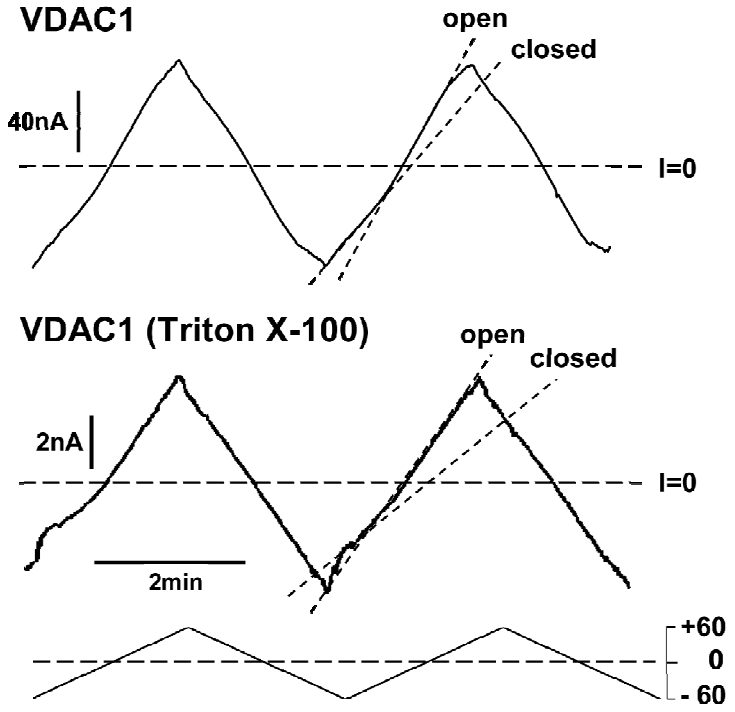

Figure S2. Current traces through multichannel membranes for VDAC1 as a function of the applied voltage wave for VDAC1 from 2D crystals without (top) or with (bottom) the presence of Triton X-100 in the sample buffer. Steeper slopes at low potentials correspond to the "open" channels, whereas the irregular lower slopes at higher potentials correspond to the low conductance of the "closed" states (dotted lines). Dashed lines indicate zero current levels and dotted lines indicate the slopes for the "open" and "closed" states. Membranes in these experiments contained from 15 to 340 channels.

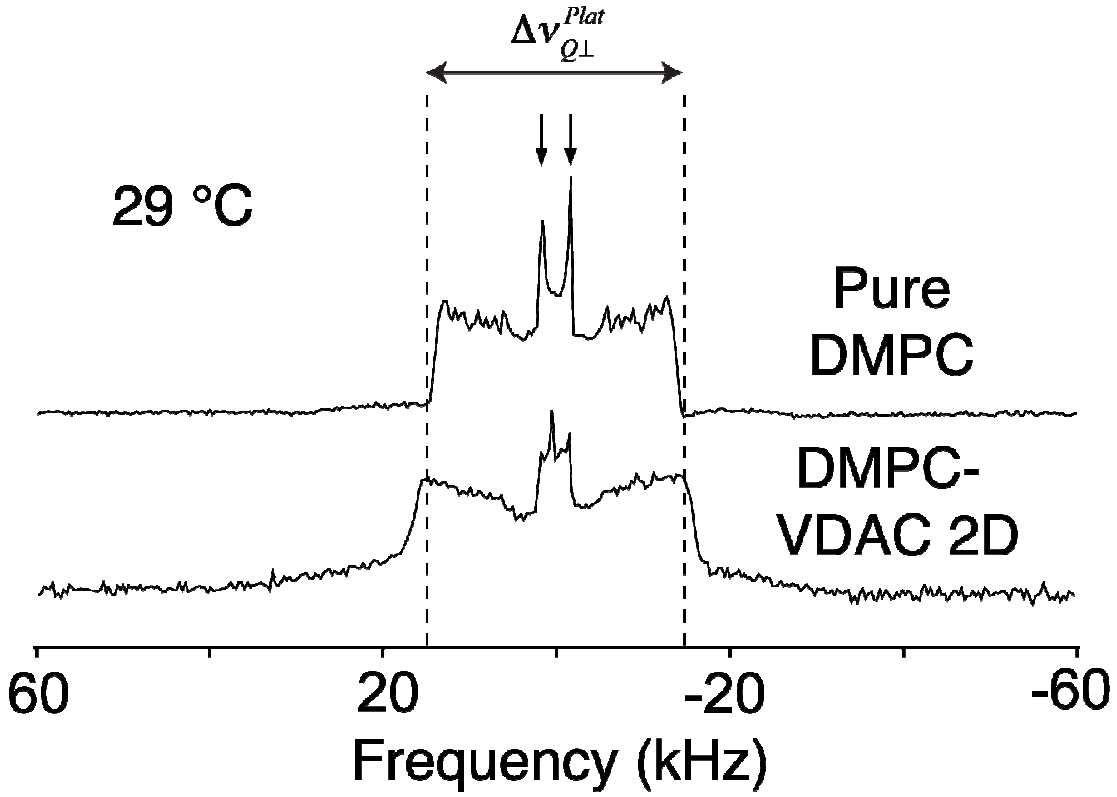

Figure S3. Expansion of ^{2}H NMR spectra of d_{54}-DMPC (top) and VDAC1/d_{54}-DMPC 2D crystals (bottom) at 29 ºC. $\Delta\nu_{Q\perp}^{Plat}$ is shown as the difference between the two outermost dashed lines and $\Delta\nu_{Q\perp}$ for the terminal methyl group of the acyl chain is highlighted by the arrows. For the 2D crystals, $\Delta\nu_{Q\perp}^{Plat}$ is larger at temperatures above and smaller below 20 °C compared to only DMPC while the terminal methyl group appears to show a slightly decreased splitting for the 2D crystals compared to pure DMPC.

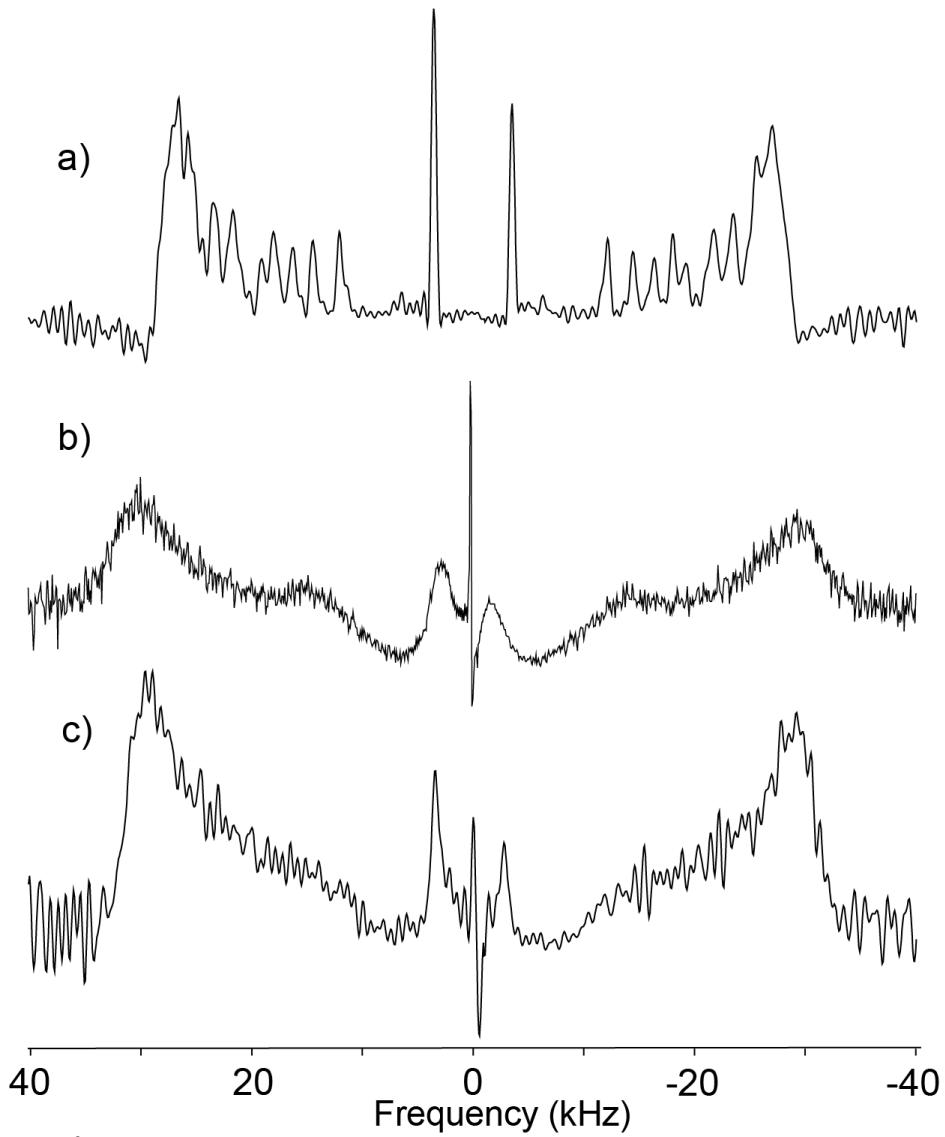

Figure S4. De-Paked ^{2}H NMR spectra of a) d_{54}-DMPC b) VDAC1/d_{54}-DMPC 2D crystals at 27 °C, and c) at 34ºC. De-Pake-ing is a commonly applied mathematical transform that allows one to enhance resolution in spectra of overlapped powder patterns. The de-Paked lines of VDAC1/d_{54}-DMPC show pronounced line broadening, and thus lines from various methylenes in the acyl chain cannot be resolved $\Delta\nu_{Q\perp}^{Plat}$.

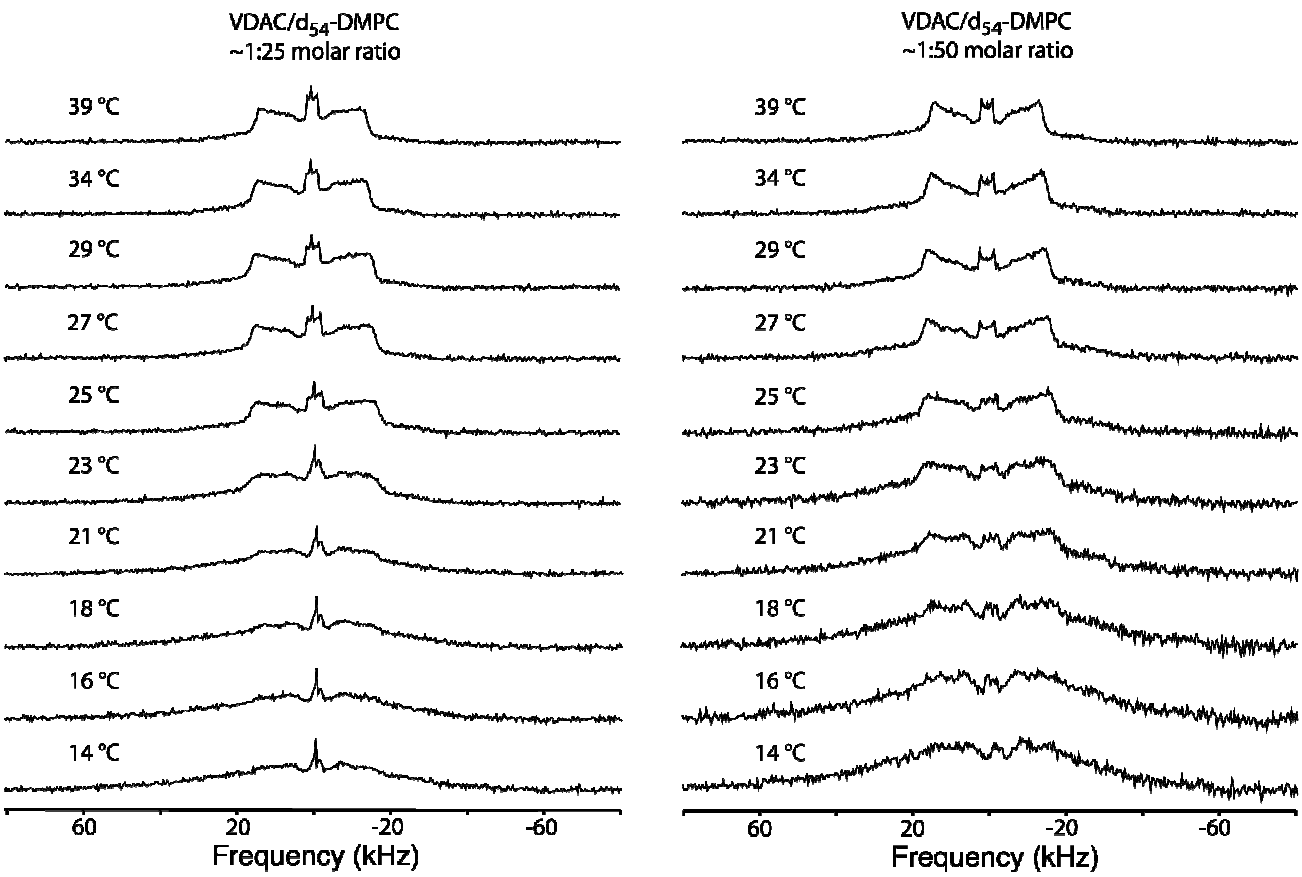

Figure S5. ^{2}H NMR spectra of VDAC1/d_{54}-DMPC ~1:25 protein-to-lipid ratio (left) and ~1:50 (right) as a function of temperature. The protein-to-lipid ratio of 1:25 corresponds with 2D crystals while 1:50 likely corresponds with the formation of liposomes.

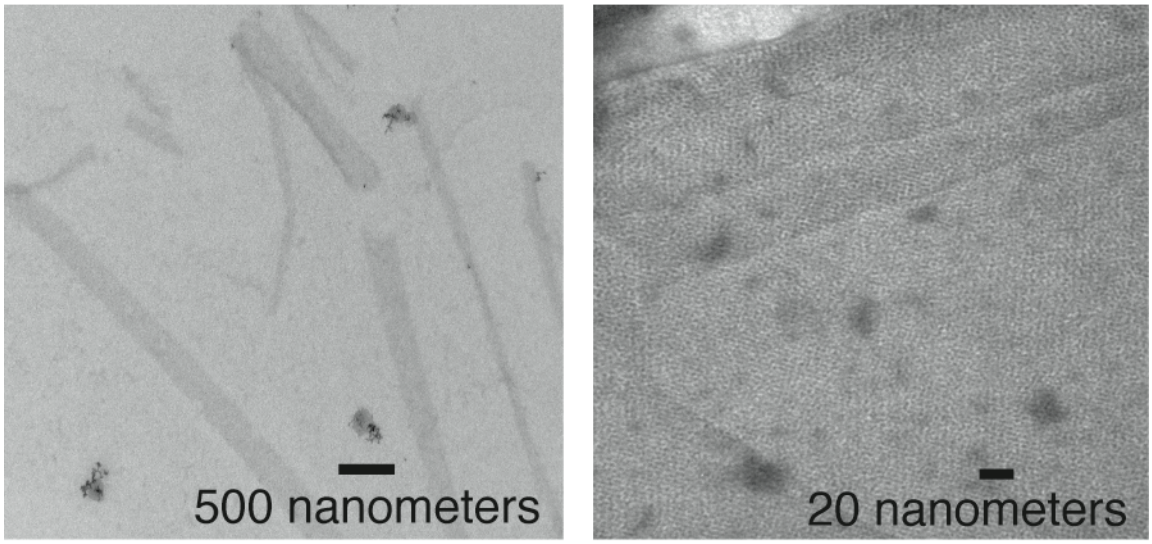

Figure S6. EM images of VDAC1 2D crystals in mixed DMPC/DOPG (80:20 w/w) lipids at a final protein-to-lipid molar ratio of ~1:25. Horizontal bars indicate relative sizes.

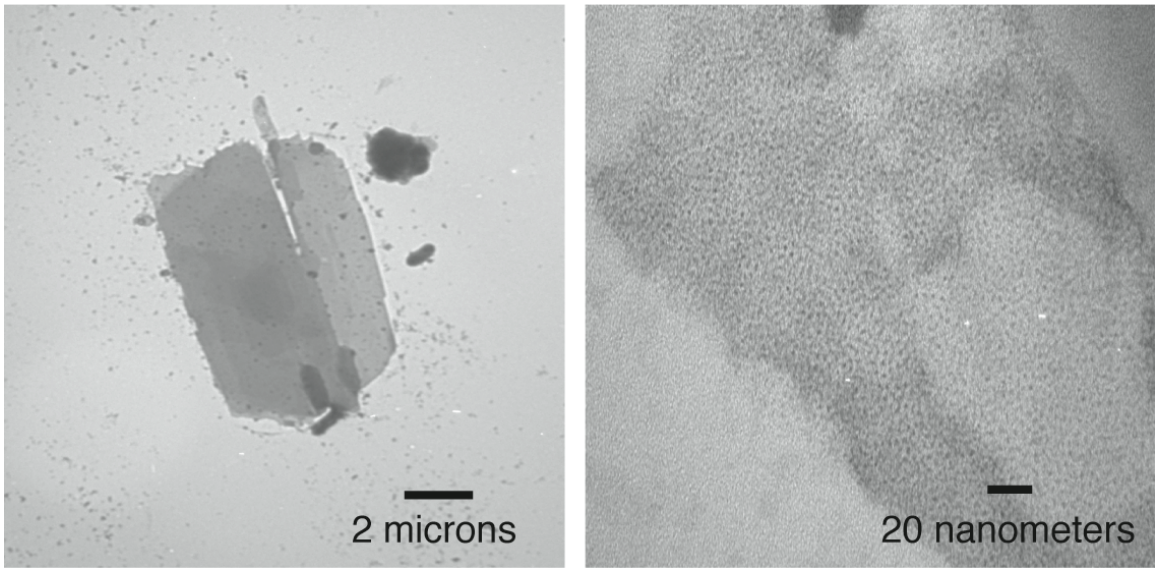

Figure S7. EM images of VDAC1 2D crystals prepared with DPhPC lipids at a protein-to-lipid molar ratio of 1:20. Horizontal bars indicate relative sizes.

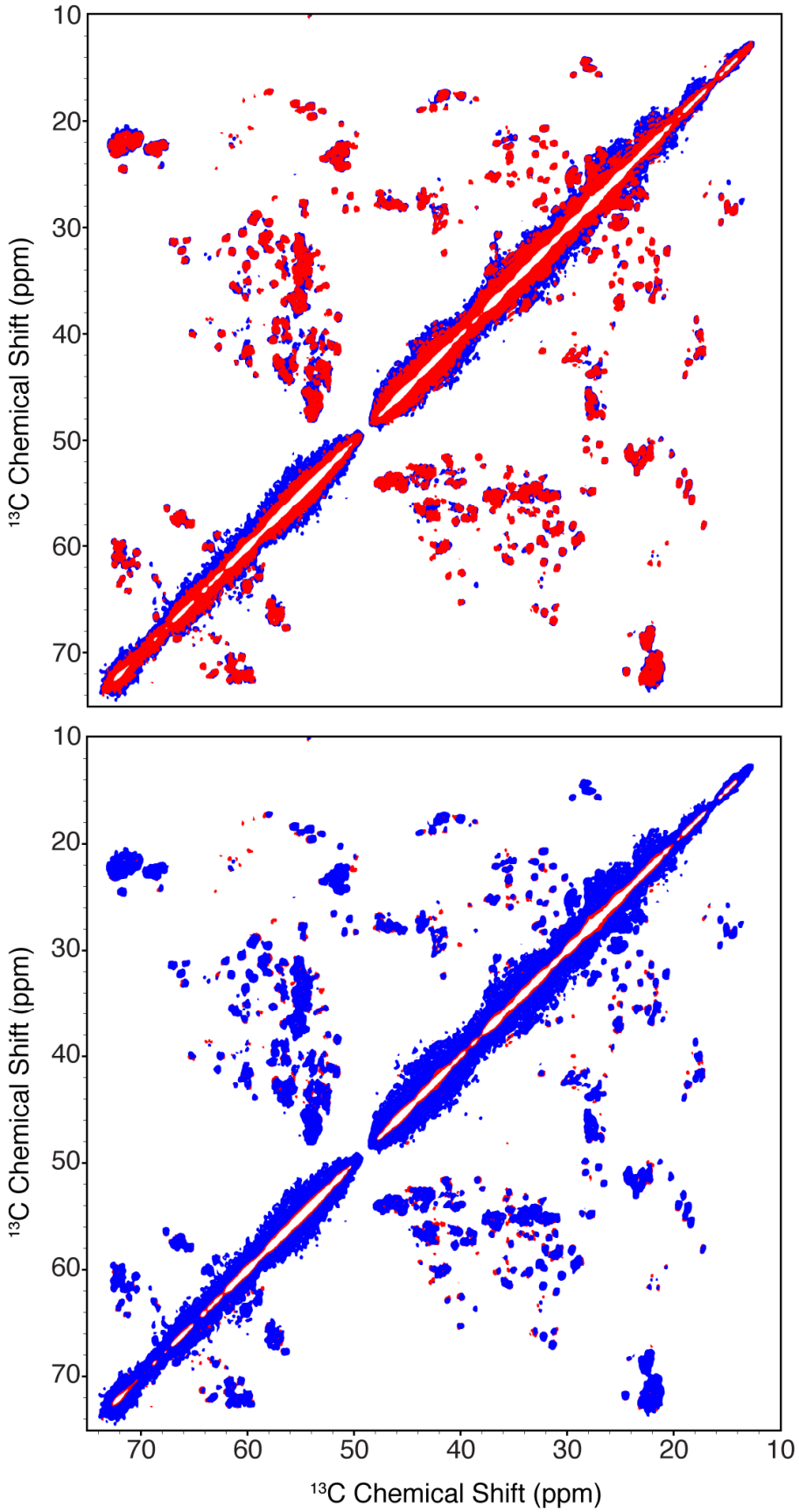

Figure S8. ^{13}C-^{13}C correlation spectra of VDAC1 in DMPC (red) and DMPC:DOPG 80:20 w/w (blue). Spectra were acquired with 1.6 ms RFDR mixing at 20 kHz MAS, 900 MHz ^{1}H field strength. 83 kHz TPPM ^{1}H decoupling was applied during evolution and acquisition periods.

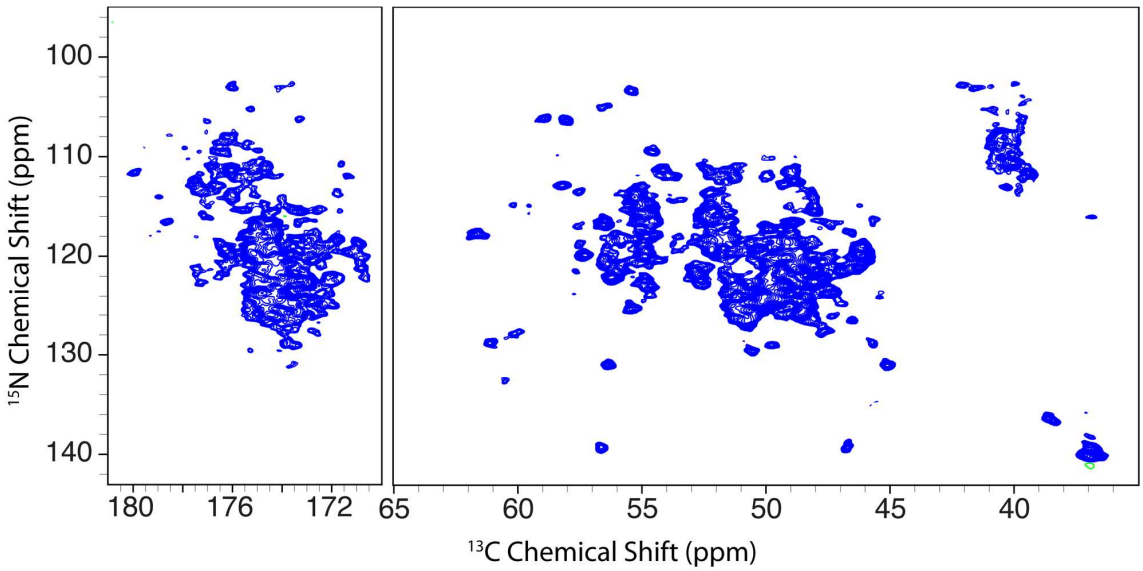

Figure S9. ^{15}N-^{13}C correlation spectrum acquired with 1.6 ms TEDOR mixing on VDAC1 in DPhPC at 900 MHz ^{1}H field strength and 20 kHz MAS frequency. The experiment averaged 32 scans per point for a total experimental time of approximately 8 hours. Peaks near ~35 ppm ^{13}C and 140 ppm ^{15}N are from lysine side chains and folded into the spectrum.