Spherical Nanoparticle Supported Lipid Bilayers for the Structural Study of Membrane Geometry-Sensitive Molecules

Riqiang Fu[3, ‡], Richard L. Gill Jr.[1, ‡], Edward Y. Kim[2], Nicole E. Briley[1], Erin R. Tyndall[1], Jie Xu[1], Conggang Li[4], Kumaran S. Ramamurthi[2], John M. Flanagan[1] & Fang Tian[1*]

[1]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, Hershey, Pennsylvania 17033, United States

[2]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

[3]National High Magnetic Field Laboratory, Tallahassee, Florida 32310, United States

[4]National Center for Magnetic Resonance in Wuhan, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, P. R. China

Supporting Information

Material and Methods

Protein purification and labeling. SpoVM and SpoVM^{R17D} peptides were synthesized and purified to >90% purity by GenScript. The peptides were dissolved at 3 mg/mL in 0.1% HCl solution. Biosynthetically labeled ^{13}C, ^{15}N SpoVM and SpoVM^{P9A} peptides were prepared as previously described.[1]

SSLB preparation. 50 (SNOWTEX-20L) and 100 (MP-1040) nm silicon beads were gifts from Nissan Chemical Industries, Ltd. 1 μm silicon beads (Duke Standards, Catalog Number: 8100) were purchased from Thermo Scientific. SSLBs were made as previously described with slight modifications.[2] Typically, 40 mg silicon beads were prepared by successive washes with ultrapure water, methanol and three times ultrapure water. The beads were then resuspended in 1 mL ultrapure water and mixed with equal volumes of sonicated DMPC liposomes in water (the amount of lipids used was at least four times the calculated amount to form a single bilayer [2]). $CaCl_2$ was added to 2 μM final concentration to accelerate SSLB formation. The suspension was vortexed and incubated at 42 ºC for one hour with occasionally vortexing. SSLBs were collected by centrifugation at 14,000xg for 5 minutes, washed three times with ultrapure water (to remove excess lipids), and resuspended in a 1 mL NMR buffer (16 mM phosphate, pH 6.0, 80 mM NaCl in D_2O) or D_2O solution (for ^{31}P NMR experiments).

NMR sample preparations. For the ^{13}C observed MAS ssNMR experiments, purified ^{13}C, ^{15}N labeled His_6-Sumo-SpoVM and His_6-Sumo-SpoVM^{P9A} were directly eluted

from Ni-NTA column (Qiagen) with 4 mL elution buffer (consisting of 50 mM phosphate buffer, pH 8.0, 500 mM NaCl, 500 mM imidazole) into ~30 mg DMPC-coated 50 nm SSLBs suspended in 4 mL water. Then H-Ulp-1 (His_6-sumo protease) was added to the solution at a molar ratio of ~1:5 (protease to recombinant protein) and incubated at room temperature for 2 hours with agitation. Complete cleavage of His_6-Sumo tag was monitored by SDS-PAGE. Following cleavage, the SpoVM or $SpoVM^{P9A}$ bound SSLBs were collected by centrifugation at 14,000xg for 5 minutes at room temperature and washed once with the NMR buffer. About 25 mg SSLBs were transferred into a thick-wall 3.2 mm MAS rotor (Revolution NMR) by centrifugation and 5 μL NMR buffer (or D_2O solution for ^{31}P NMR experiments) was added to the rotor before it was tightly capped.

For the ^{1}H detected HRMAS experiments, SpoVM or $SpoVM^{R17D}$ peptides in 0.1% HCl solution was mixed with ~30 mg DMPC-coated SSLBs suspected in a 1 mL NMR buffer. After 1 hour incubation at room temperature, the SSLBs were collected by centrifugation at 14,000xg for 5 minutes at room temperature and washed once with the NMR buffer in D_2O. About 20 mg SSLBs and 5 μL D_2O NMR buffer were loaded into a disposable Bruker HRMAS rotor insert. The insert was tightly sealed using a taper and a screw cap (included in Bruker's disposable insert kit) and there was no loss of solvent during several days of experiments, as indicated by little changes in NMR lock signal.

NMR spectroscopy. HRMAS spectra were acquired on a Bruker 500 MHz NMR

spectrometer equipped with a 4 mm HRMAS probe. The instrument temperature setting was at 30 °C, and the resonance position of water indicated that the sample temperature was about 37 °C at 10 kHz MAS spinning speed. The on- and off-saturation spectra were collected in an interleaved manner with a selective pulse at 7.45 and 14.7 ppm, respectively. Selective saturation was accomplished by a Q3 gaussian cascade pulse of 50 ms with a saturation bandwidth of ~50 Hz. A spin-echo sequence with a total delay of 5 ms was employed prior to FID acquisition to suppress broad resonances from silicon beads.[3] A recycle delay of 3 s was used to ensure complete relaxation of lipid resonances. The data were processed and analyzed using Topspin software. The STD spectrum was obtained by subtracting on-resonance and off-resonance spectra. Signal intensity in the STD spectrum was usually less than 2.5% and 1.5% of the reference spectrum (off-resonance spectrum) for the 100 nm and 1 μm SSLBs, respectively.

The ^{13}C-^{13}C correlation spectra were acquired on a Bruker AvanceIII 800 MHz NMR spectrometer using a home-made low-E 3.2 mm triple-resonance MAS probe at 15 ºC (sample temperature) and a sample spinning rate of 10 kHz . The ramped cross polarization (CP) was used to enhance the ^{13}C magnetization with a contact time of 400 μs, during which the ^{1}H spinlock field of 50 kHz was used while the ^{13}C rf amplitude was ramped from 38 to 56 kHz. The ^{13}C 90° pulse length was 4.6 μs. A SPINAL64 decoupling sequence was used for ^{1}H decoupling during the t_1 and t_2 dimensions with a ^{1}H rf amplitude of 78 kHz. The quadrature detection in the t_1 dimension was achieved by TPPI. During the ^{13}C-^{13}C mixing time a weak ^{1}H RF

amplitude of 10 kHz, matched with the sample spinning rate, was applied on the ^{1}H channel to enhance the spin diffusion among ^{13}C spins. The acquisition times for the t_1 and t_2 dimensions were 6 and 8.7 ms, respectively. The data was processed using NMRPipe and analyzed using NMRView. The ^{13}C chemical shifts were referenced to the carbonyl carbon of glycine at 176.4 ppm.

References

(1) Gill, R. L.; Castaing, J. P.; Hsin, J.; Tan, I. S.; Wang, X.; Huang, K. C.; Tian, F.; Ramamurthi, K. S. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, 112, E1908-E1915.

(2) Bayerl, T. M.; Bloom, M. *Biophys. J.* **1990**, *58*, 357-362.

(3) Faulkner, R. A.; DiVerdi, J. A.; Yang, Y.; Kobayashi, T.; Maciel, G. E. *Materials* **2013**, *6*, 18-46.

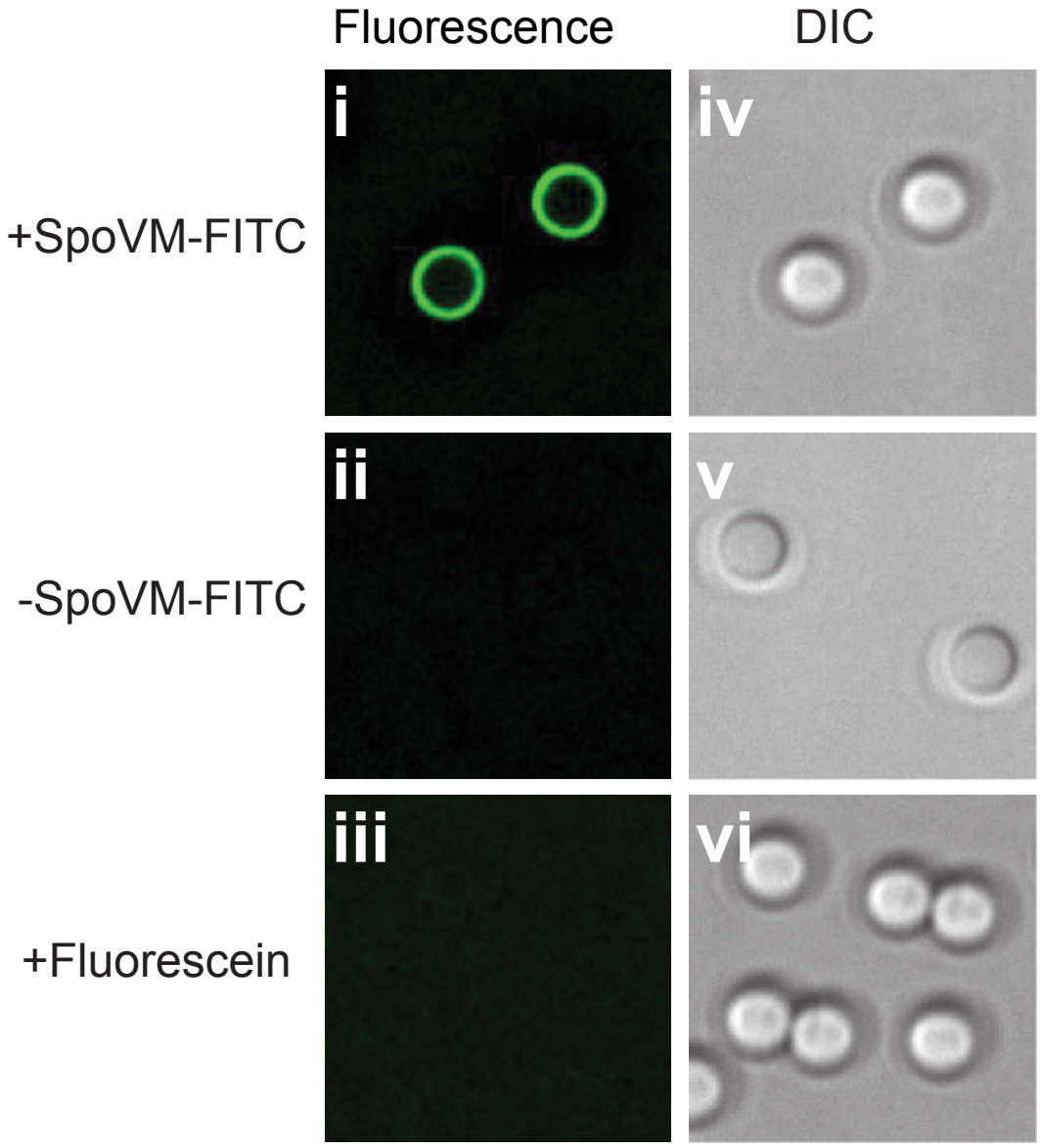


Figure S1: Adsorption of SpoVM onto SSLBs. 2 μm SSLBs with (i) SpoVM-FITC, (ii) without SpoVM-FITC, or (iii) with fluorescein. (i-iii) and (iv-vi) Fields visualized by fluorescence and differential interference contrast (DIC) microscopy, respectively.

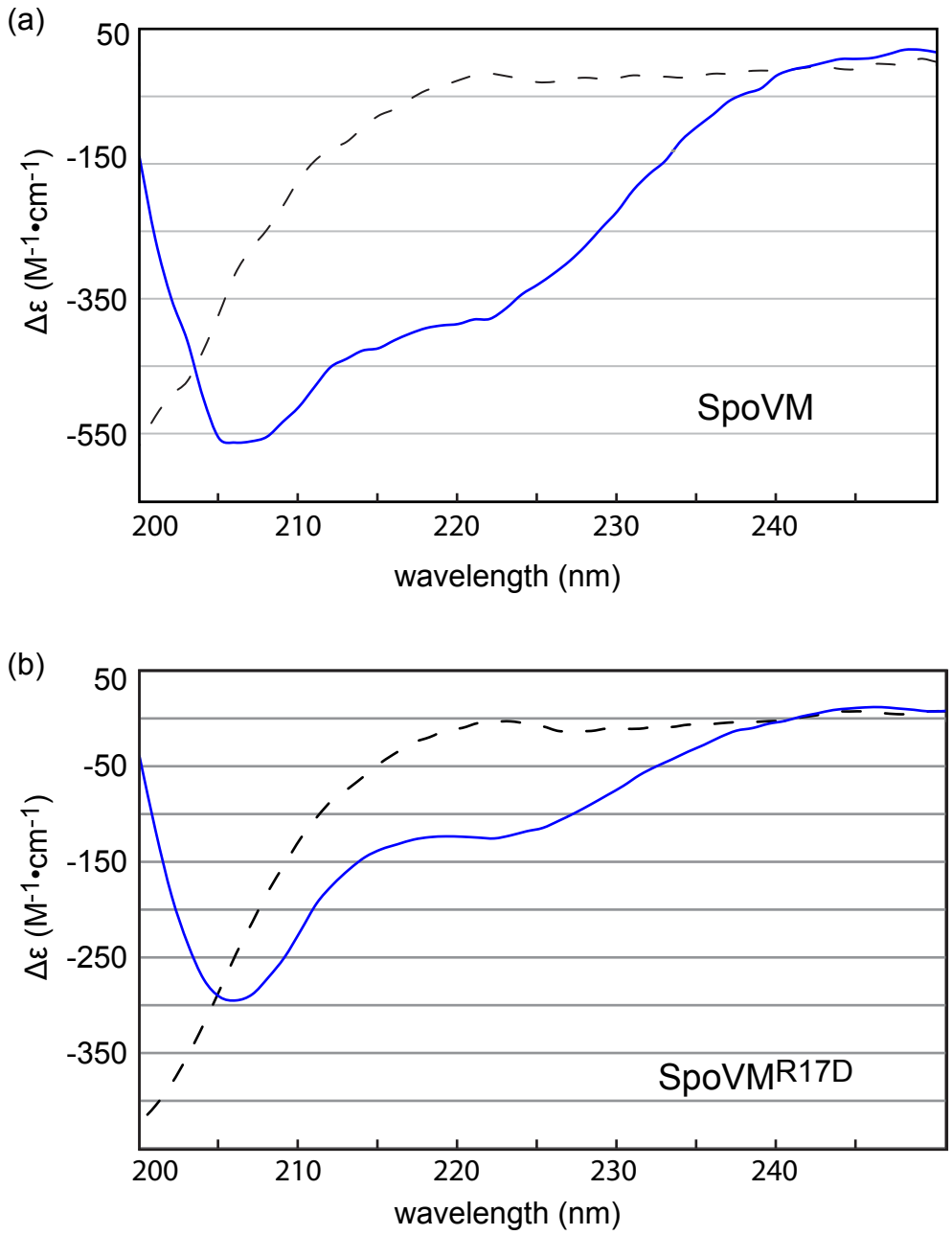
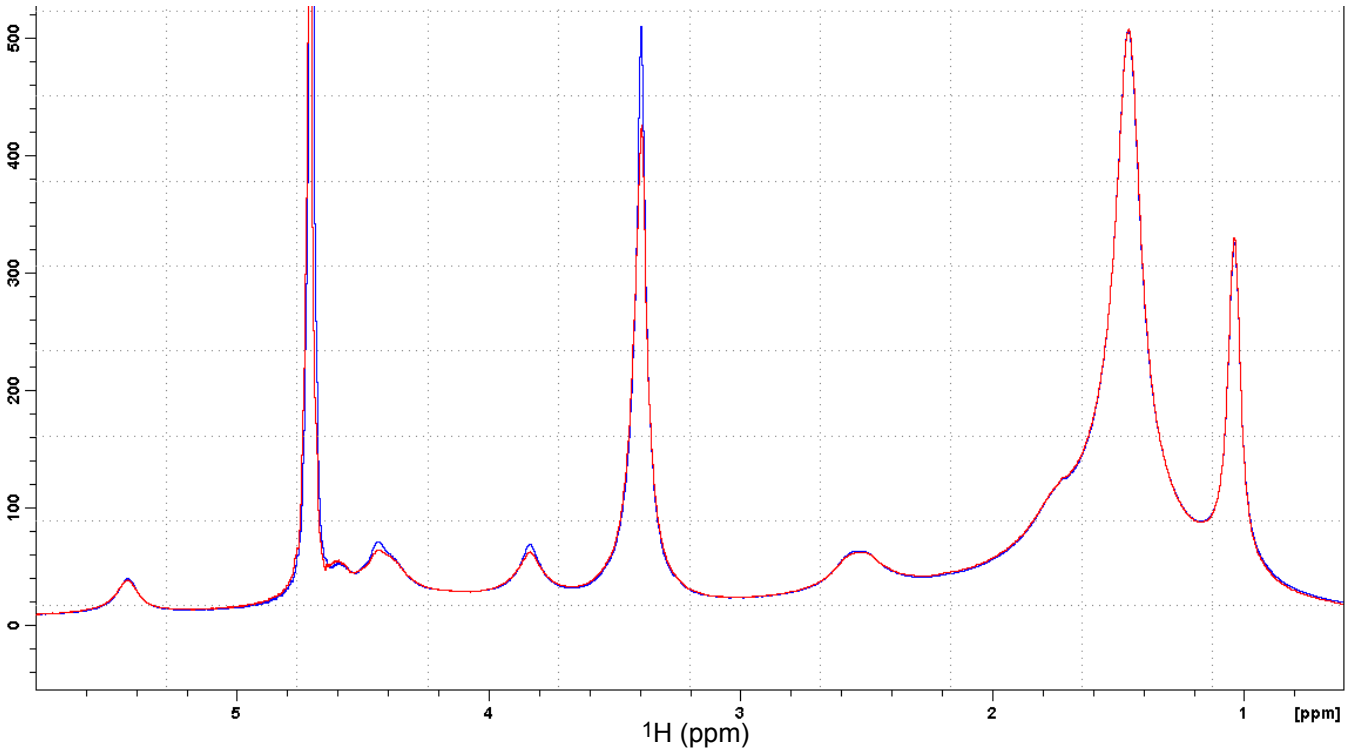


Figure S2: CD spectra of (a) SpoVM (22 μM) and (b) SpoVM^{R17D} (22 μM) in aqueous solution (black) and DMPC liposomes (blue).

(a) Blue: DMPC-coated 100 nm SSLBs
Red: DMPC-coated 100 nm SSLBs + SpoVM (molar ratio, DMPC:SpoVM = ~110:1)



(b) Blue: DMPC-coated 100 nm SSLBs
Red: DMPC-coated 100 nm SSLBs + SpoVM^{R17D} (molar ratio, DMPC:SpoVM^{R17D} = ~110:1)

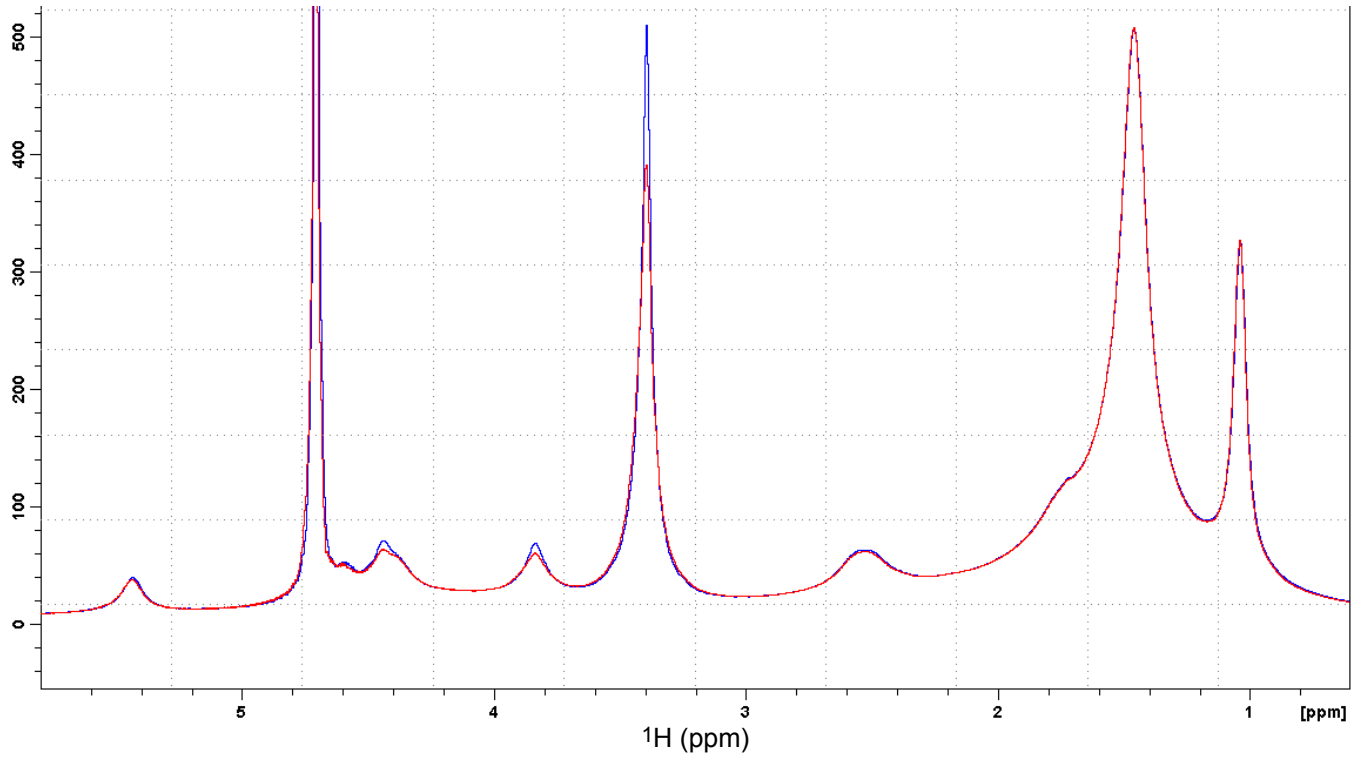


Figure S3: Overlay of 1D ^{1}H HRMAS spectra of DMPC-coated 100 nm SSLBs with and without SpoVM (a) and SpoVM^{R17D} (b). Substantial reductions in peak intensity of choline methyl groups at 3.4 ppm indicated their interactions with the peptides. The spectra were collected on a 500 MHz Bruker NMR spectrometer using a single pulse experiment with 10 kHz spinning speed, 16 scans, and 3 s recycle delay at 30 ^{o}C.

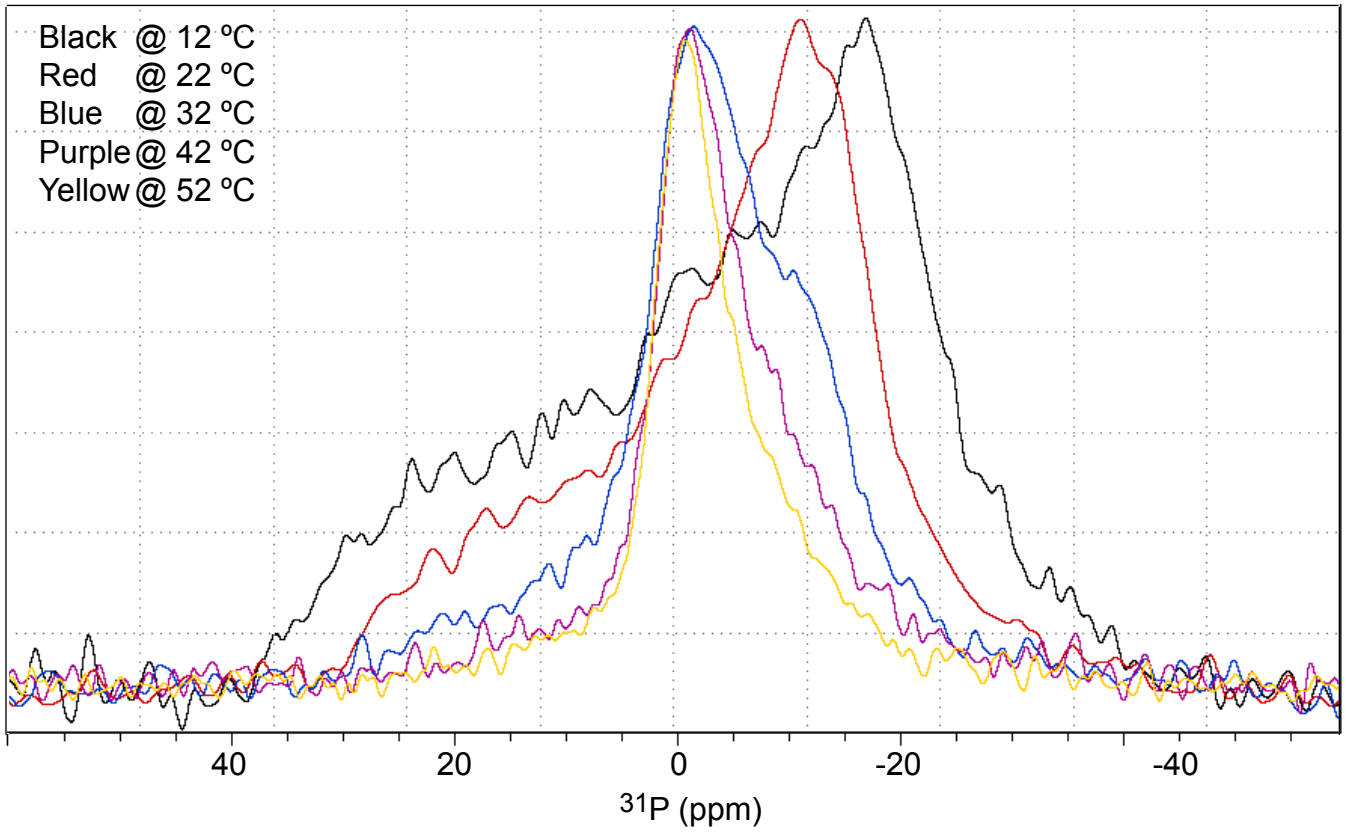


Figure S4: Temperature dependencies of the static ^{31}P NMR spectra for 50 nm DMPC-coated SSLBs. Below the phase transition temperature, the spectrum shows a typical chemical shift powder pattern. As the temperatures increase, the spectra are averaged by both the rotational diffusion and the lateral diffusion of lipids on the surface of silicon beads. At 52 ºC, the lateral diffusion appears to be sufficiently fast to produce an isotropic-like spectrum. This is consistent with the model that lipids form a continuous bilayer diffusing over the surface of silicon beads. When the sample was in the gel phase, the spectrum at 12 ºC does not show an isotropic peak, indicating that no lipids are detached from the beads to form small discoidal structures. This behavior is different from a previous observation (Picard et al. Biophys. J., 1998, 74:857-868), and may suggest that the lipids are less tightly packed in these highly curved 50 nm SSLBs comparing with those in less curved 320 nm beads used in the previous study. The spectra were obtained utilizing a single pulse sequence with proton decoupling during FID acqusition on a 400 MHz Bruker NMR instrument. Each spectrum was collected with 1024 scans and 3 s recyle delay.

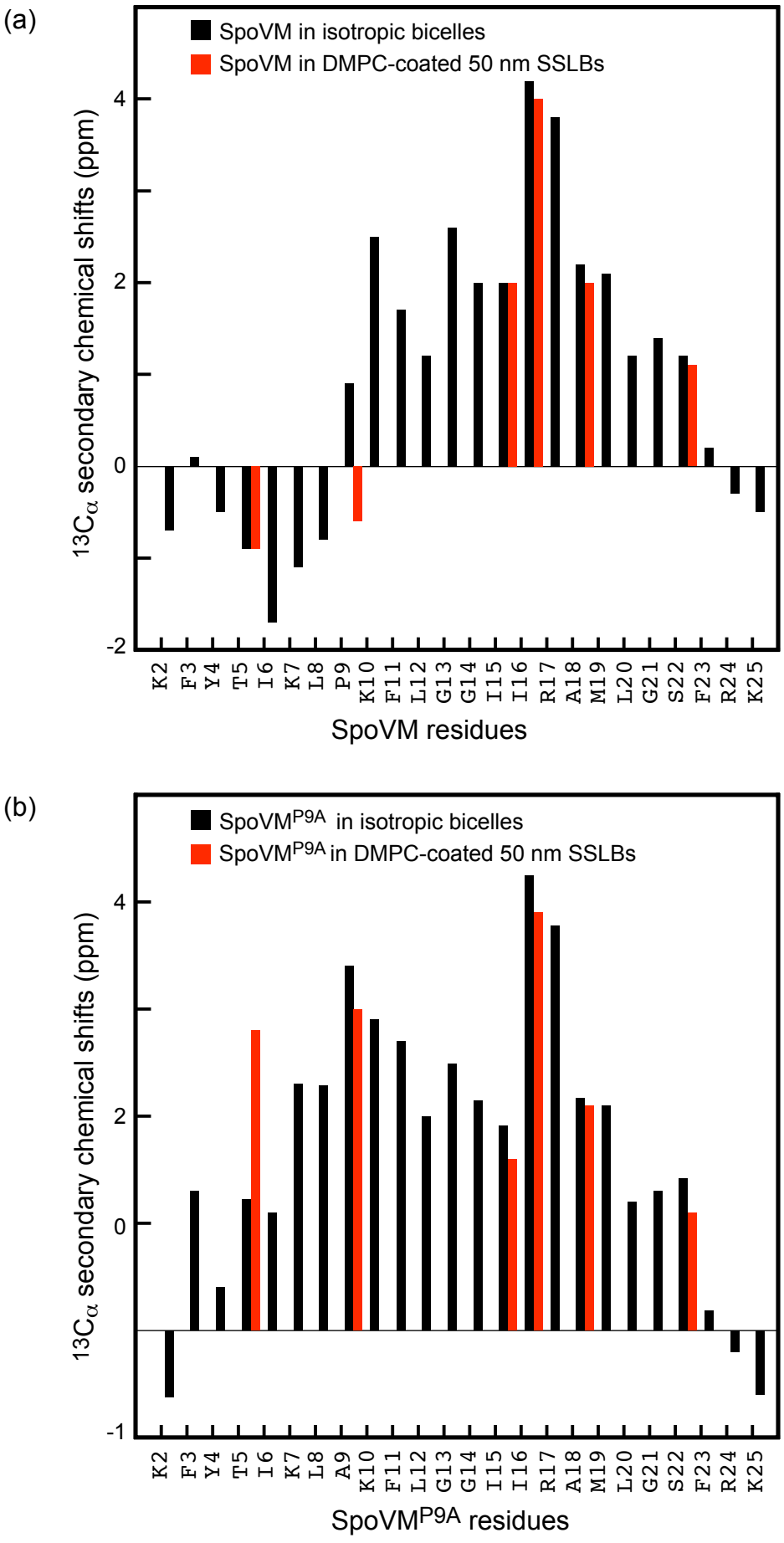


Figure S5: $^{13}C_\alpha$ secondary chemical shifts of SpoVM (a) and SpoVM^{P9A} (b) in isotropic bicelles (black) and DMPC-coated 50 nm SSLBs (red).

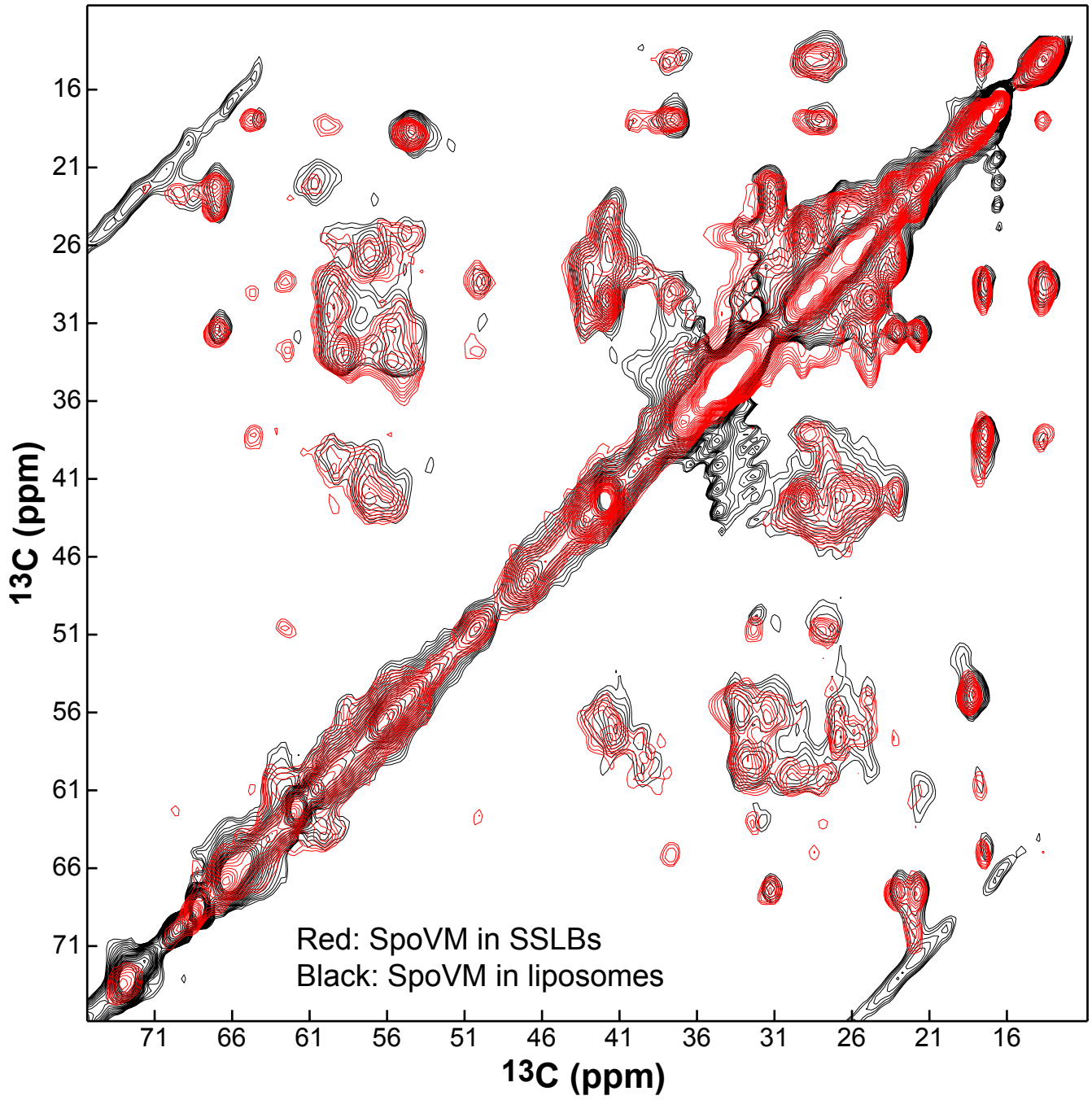


Figure S6: An overlay of SpoVM 2D 800 MHZ ^{13}C-^{13}C correlation spectra in DMPC -coated 50 nm SSLBs (red) at 15 °C and in sonicated DMPC/POPC (2:1 molar ratio) liposomes (black) at -15 °C with 35 ms mixing time. Data were recorded at different temperatures for improved spectral sensitivity.

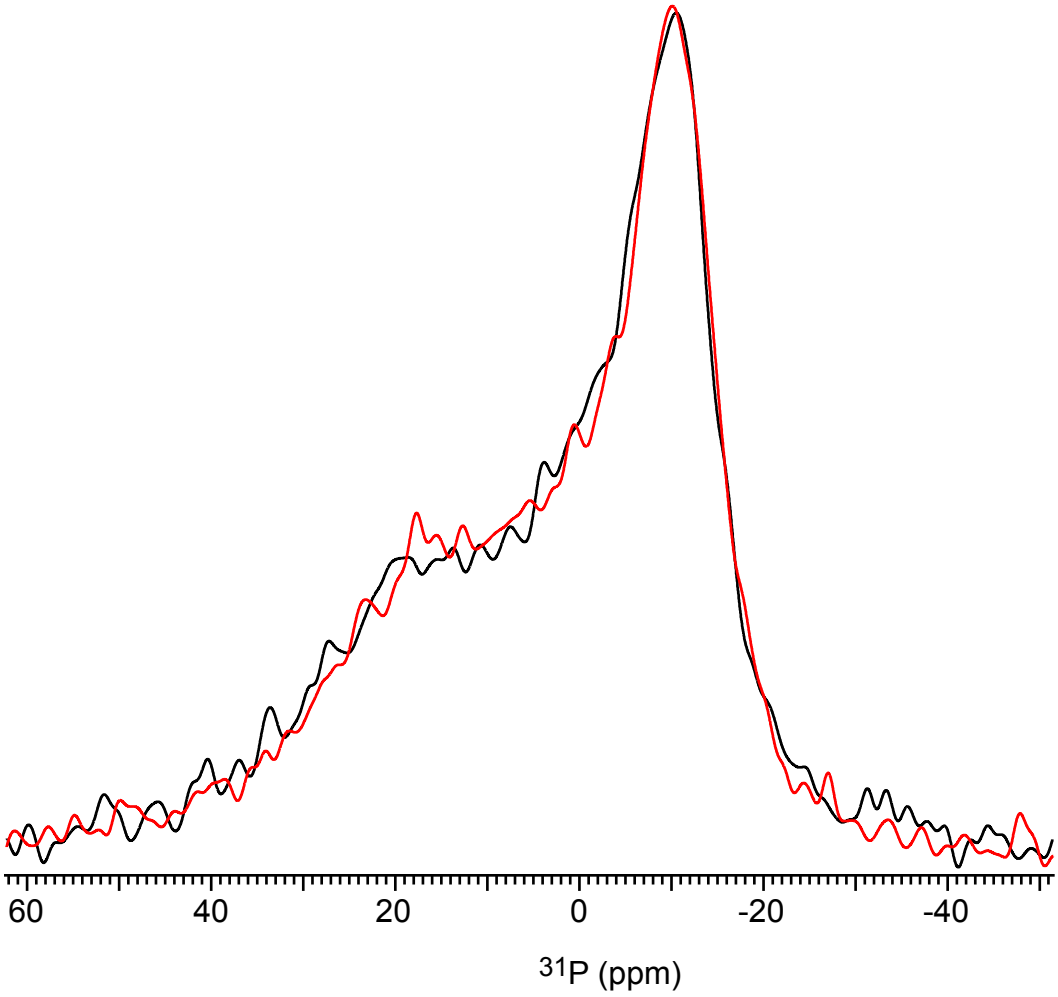


Figure S7: ^{31}P NMR spectra of 100 nm DMPC-coated SSLBs at 23 ºC before (black) and after (red) spinning at 10 kHz for ~5 hours. The spectra show a typical chemical shift powder pattern. The spectra were acquired on a Bruker 500 MHz spectrometer with a HRMAS probe.

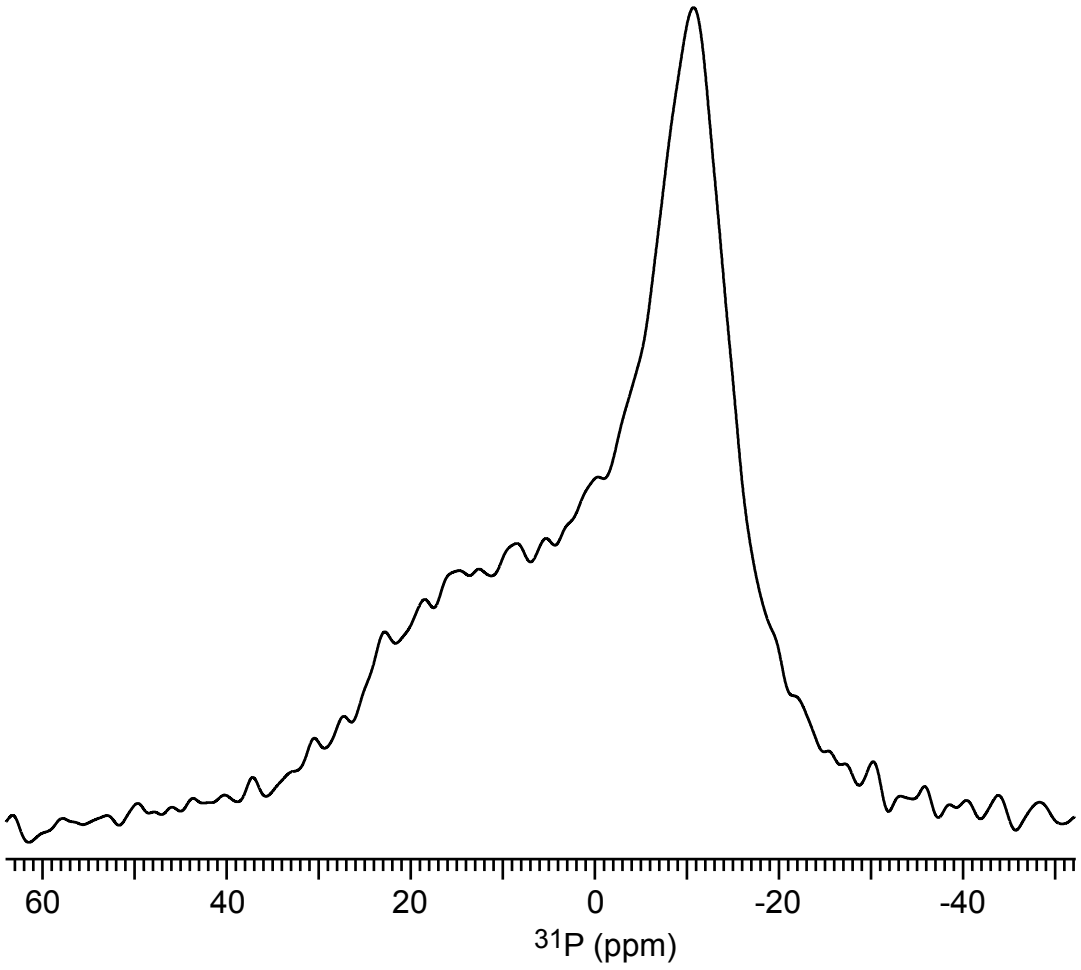


Figure S8: ^{31}P NMR spectrum of 100 nm DMPC-coated SSLBs with SpoVM (peptide:lipids=1:280 molar ratio) at 23 ºC. The spectrum indicates that the binding of SpoVM minimally perturbs the bilayer structure. The spectrum was acquired on a Bruker 500 MHz spectrometer with a HRMAS probe.