Thermal stabilization of DMPC/DHPC bicelles by addition of cholesterol sulfate: SUPPLEMENT

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Supporting Information

Protocols

Bicelle Preparation

q=2.6

Powdered lipids were obtained from Avanti Polar Lipids and 50 mg DHPC was combined with 200 mg DMPC (q=2.6). Cholesterol or cholesterol sulfate was added to 13.4% with respect to moles DMPC. Lipids were diluted to 20% w/v lipids with 90% H_2O and 10% D_2O phosphate buffer (10 mM). The samples were pipetted into an NMR tube and heated to 37 °C until the viscosity visibly increased and were then cooled to 4 °C until there was a decrease in viscosity. The samples were then vortexed and cycled through the 37 °C, 4 °C three more times and allowed to hydrate until all components were soluble, which at 18 °C was 2 days for cholesterol and 4 days for cholesterol

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sulfate. The heating and cooling cycles were repeated before each set of experiments.

q=3.5

Lipids dissolved in chloroform (20 mg/mL) were obtained from Avanti Polar Lipids. DHPC and DMPC were mixed in chloroform at a q=3.5 and 15% total lipids. The samples were dried for 4 hours under an N_2 stream in 25 mL Erlenmeyer flasks to allow the majority of chloroform to evaporate. The samples were lyophilized overnight. Cholesterol or cholesterol sulfate was added at 13.4% with respect to moles DMPC. The samples were resuspended in 10 mM phosphate buffer with 90% H_2O and 10% D_2O. The samples were pipetted into NMR tubes, cycled between 37 °C and 4 °C were hydrated until all components were soluble, which at 18 °C was 2 days for cholesterol and 4 days for cholesterol sulfate. The heating and cooling cycles were repeated before each set of experiments.

Protein Preparation

The ubiquitin cDNA was obtained from Open Biosystems. A 6-histidine tag and a TEV protease site was added by PCR using primers synthesized by Sigma-Genosys. The constructed gene was sub-cloned into a pET-28a vector and expressed in Rosetta *E. coli*. The cell free extract was purified using Ni-affinity FPLC. The ubiquitin was desalted into 10 mM phosphate buffer using a desalting column and lyophilized. Ubiquitin was added to the bicelle mixture to give an approximate concentration of 0.3 mM protein. The constructed gene was sub-cloned into a pET-28a vector and expressed in Rosetta *E. coli* using ^{15}N labeled minimal media.

Bicelles with Ubiquitin

Bicelles were prepared as described with a q=3.5 and 13.6% w/v lipids with 13.4% cholesterol or cholesterol sulfate. They were hydrated at 18 °C in 0.4 mL D_2O with 150 mM NaCl, 25 mM phosphate buffer pH 6.8 for two days. The protein (100 μL) was added to a total concentration of 0.3 mM. The sample was hydrated at 4 °C until all components were soluble, which was an additional two days for cholesterol and and additional 4 days for cholesterol sulfate.

Additional NMR Data

Temperature Dependence of Measured 2H Quadrupolar Splitting (Hz) in q=2.6, 20% w/v DMPC/DHPC Bicelles Doped as Listed			
Temperature	**No Additives**	**Cholesterol**	**Cholesterol Sulfate**
21.7 °C	0.00	0.00	0.00
25 °C	0.00	0.92	13.48
30 °C	5.07	2.17	20.79
35 °C	27.54	34.22	40.02
37 °C	*.*	31.16	39.51
40 °C	34.51	*.*	*.*
45 °C	0.00	38.82	45.78
50 °C	0.00	0.00	64.47
55 °C	0.00	0.00	0.00
. = not measured			

Temperature Dependence of Measured 2H Quadrupolar Splitting (Hz) in q=3.5, 25% w/v DMPC/DHPC/CS	
25 °C	9.9
30 °C	6.2
35 °C	28.9
40 °C	30.0
45 °C	33.8
50 °C	37.5
55 °C	49.8

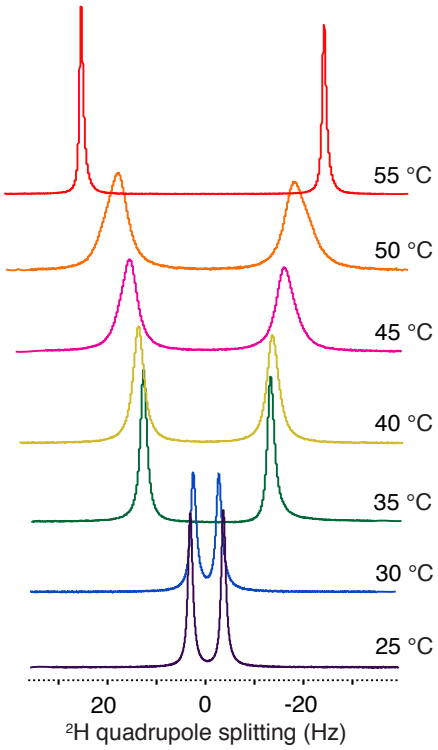

Figure 1: Deuterium quadrupole splitting in 10% D_2O/90% H_2O of q = 3.5 DMPC/DHPC bicelles of total lipid content 25% w/v doped with cholesterol sulfate (CS) to 13.4% of the lipid content. Measurements were made at temperatures of 25 °C to 55 °C in a 400 MHz Bruker DRX instrument. As with the q=2.6 bicelles described by Figure 1 of the paper, CS-doping increases the temperature range of stable alignment. The deviation from the trend at 30 ° C is reproducible, possibly suggesting a phase transition from one aligned phase to another.

^{15}N HSQC RDC Measurements

Measured Splittings (Hz) and RDCs for Given Ubiquitin Sample Type				
Residue†	**Isotropic J-coupling**	**CS-Doped Bicelles 32°C**	**Undoped Bicelles 32°C**	**CS-Doped Bicelles 25°C**
Gln 62-SC	180.0	169.0	177.0	168.0
RDCs:		-11.0	-3.0	-12.0
Gly 75	95.0	82.0	92.0	80.0
RDCs:		-13.0	-3.0	-15.0
Gly 76	93.0	96.0	90.0	96.0
RDCs:		3.0	-3.0	3.0
Ser 20	93.0	94.0	87.0	98.0
RDCs:		1.0	-6.0	5.0
Lys 33	93.0	109.0	91.0	110.0
RDCs:		16.0	-2.0	17.0
Asp 39	93.0	79.0	91.0	80.0
RDCs:		-14.0	-2.0	-13.0
Gln 31-SC	180.0	197.0	186.0	195.0
RDCs:		17.0	6.0	15.0
Asn 60-SC	178.0	193.0	181.0	192.0
RDCs:		15.0	3.0	14.0
Ser 65	94.0	124.0	93.0	123.0
RDCs:		30.0	-1.0	29.0
Gly 47	115.0	92.0	88.0	114.0
RDCs:		-23.0	-27.0	-1.0
Asn 25	93.0	84.0	88.0	83.0
RDCs:		-9.0	-5.0	-10.0
Asn 25-SC	173.0	181.0	176.0	178.0
RDCs:		8.0	3.0	5.0
Gln 2-SC	176.0	192.0	180.0	190.0
RDCs:		16.0	4.0	14.0

Figure 2: Summary of RDCs for select ubiquitin residues. Doublets were taken from the IP spectra of ^{15}N-HSQC-IPAP data taken on an 800 MHz Varian Inova instrument. Parameters used in data acquisition: recycle delay=1.4 s, B_1 field strength=38.5 kHz, decoupling power=1.4 kHz, ^{1}H spectral width=10 kHz, ^{15}N spectral width=3.4 kHz, number of scans=64, acquisition length in ^{1}H dimension=1500, acquisition length in ^{15}N dimension=128. All couplings are from backbone ^{1}H-^{15}N pairs except for those listed as "SC," which are from side chains. † SC peaks originate from the NH_2 group of the side chain of the residue listed. Because this experiment gives 1:0:1 triplets for NH_2 spin systems, the splittings shown for these residues are 2J for the isotropic J-coupling values and 2(J+D) for the aligned values, which have additional residual dipolar coupling.