Supporting Information

Association of the PLIN1 Amphipathic Helix with Membrane Mimetics Shows a Threshold-like Dependence on Phosphatidylglycerol Content

Kunyi He[1] · Zhangjie Liang[1] · Wen Chen[1]*

[1] School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, Guangdong, China

*Corresponding author: Wen Chen

Email: wenchen@scut.edu.cn

ORCID: https://orcid.org/0000-0001-7387-4532

Table of Contents

Experimental Details

Materials

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DMPG), and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) were obtained from Avanti Polar Lipids. Dodecylphosphocholine (DPC) was purchased from Anatrace. Nickel–nitrilotriacetic acid (Ni–NTA) resin (L00666) was obtained from GenScript Biotech Corporation. Ni–NTA biosensors were obtained from Sartorius, while $^{15}NH_4Cl$ and D_2O were purchased from Cambridge Isotope Laboratories. Polycarbonate membranes with a nominal pore diameter of 100 nm were obtained from Avanti Polar Lipids. *Escherichia coli* BL21(DE3) competent cells were obtained from Shenzhen Kangti Biopharmaceutical Technology Co., Ltd. (Cat. No. KTSM104L) and used for recombinant protein expression.

Plasmid construction

The PLIN1 amphipathic-helix construct, designated PLIN1-AH, comprised residues 94–193 of human perilipin 1 (UniProt O60240). This region contains an N-terminal flanking segment, seven complete 11-residue repeats, and a partial C-terminal repeat. The coding sequence was codon-optimized for expression in *Escherichia coli*, synthesized by GenScript, and inserted into pET-28a(+) to generate an N-terminally His-tagged construct. The final plasmid was verified by Sanger sequencing before use.

Sequence analysis and helical-wheel projection

The theoretical molecular mass of PLIN1-AH was calculated from the complete expressed sequence using the ExPASy ProtParam tool and was determined to be 12,580 Da. Helical-wheel projections were generated with the HeliQuest server under an idealized α-helical geometry and were subsequently redrawn for figure preparation without altering residue order, angular position, or physicochemical classification. Residues were grouped as hydrophobic, positively charged, negatively charged, polar uncharged, or glycine/proline, allowing the amphipathic organization of PLIN1-AH and the spatial segregation of its hydrophobic and polar/charged surfaces to be visualized. The arrow labeled μH denotes the direction of the hydrophobic moment vector and identifies the predicted hydrophobic face of the α-helix.

Protein expression and purification

Escherichia coli BL21(DE3) cells harboring the PLIN1-AH expression plasmid were grown overnight in LB medium containing the appropriate antibiotic, diluted into fresh medium to an initial OD_{600} of 0.1, and cultured at 37°C and 220 rpm until the OD_{600} reached 0.6–0.8. Protein expression was induced with 0.3 mM isopropyl β-D-1-thiogalactopyranoside, after which the cultures were incubated for 16 h at 24°C and harvested by centrifugation at 5,000 × *g* for 30 min at 4°C. Uniformly ^{15}N-labeled PLIN1-AH was produced under identical conditions in M9 minimal medium supplemented with $^{15}NH_4Cl$ (1 g/L) as the sole nitrogen source.

Cell pellets were resuspended in lysis buffer (25 mM phosphate, 100 mM NaCl, 20 mM imidazole, 8 M urea, pH 7.4), disrupted by sonication on ice, and incubated overnight at room temperature to solubilize

inclusion bodies and other aggregated protein species. Following centrifugation at 20,000 × *g* for 30 min, the clarified supernatant was incubated with Ni–NTA resin under denaturing conditions to capture the N-terminally His-tagged PLIN1-AH. The resin was washed with wash buffer (25 mM phosphate, 100 mM NaCl, 100 mM imidazole, 8 M urea, pH 7.4), and the bound protein was eluted with the elution buffer (25 mM phosphate, 100 mM NaCl, 500 mM imidazole, 8 M urea, pH 7.4). The eluate was dialyzed against NMR buffer (25 mM phosphate, pH 7.4) to remove urea.

PLIN1-AH was further purified by reversed-phase high-performance liquid chromatography (RP-HPLC) on a C4 column using mobile phase A, composed of acetonitrile and ultrapure water at a volume ratio of 25:75 with 0.1% (v/v) trifluoroacetic acid, and mobile phase B, composed of acetonitrile and isopropanol at a volume ratio of 25:75 with 0.1% (v/v) trifluoroacetic acid. Both mobile phases were filtered through 0.22-μm solvent-compatible membranes and thoroughly degassed before use. The column was equilibrated with 20% mobile phase B for 15 min at a flow rate of 3 mL min^{-1}, after which the sample was loaded and eluted using a linear gradient from 20% to 60% mobile phase B, with absorbance monitored continuously at 214 and 280 nm.

Fractions containing PLIN1-AH were identified by SDS–PAGE, pooled, and lyophilized to remove the volatile components of the RP-HPLC mobile phase. The dried protein was redissolved in denaturing buffer (25 mM phosphate, 100 mM NaCl, 8 M urea, pH 7.4) and was subsequently refolded by dialysis against NMR buffer (25 mM phosphate, pH 7.4). Following refolding, PLIN1-AH was evaluated by SDS–PAGE and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to confirm sample purity and molecular identity, respectively, before use in subsequent experiments.

Preparation of DPC micelles and DMPC/DMPG/DHPC bicelles

DPC was dissolved in NMR buffer to a concentration of 100 mM and gently mixed until a clear, homogeneous solution was obtained. The resulting DPC micelle preparation was used for subsequent NMR sample preparation.

The required lipid stocks were combined in chloroform at the specified molar ratios, dried under a gentle stream of nitrogen, and placed under vacuum for at least 2 h to remove residual solvent. The q value was defined as ([DMPC] + [DMPG])/[DHPC], while the DMPG mole fraction was calculated relative to the total long-chain phospholipid content. For the composition series, the DMPG fraction was varied at a fixed q value of 0.8, whereas for the q series, the DMPC:DMPG molar ratio was maintained at 50:50 and the DHPC concentration was adjusted accordingly. The dried lipid films were hydrated in NMR buffer to a final total long-chain phospholipid concentration of 50 mM and vortexed until clear and homogeneous. The resulting bicelle preparations were used for subsequent NMR sample preparation, and the compositions and concentrations of all formulations are summarized in Table S3.

NMR spectroscopy

Two-dimensional ^{1}H–^{15}N heteronuclear single-quantum coherence (HSQC) spectra were acquired at 37°C on a Bruker Avance 600-MHz spectrometer equipped with a cryogenically cooled probe. Preformed DPC micelles or DMPC/DMPG/DHPC bicelles were mixed with uniformly ^{15}N-labeled PLIN1-AH and adjusted with NMR buffer to 450 μL, after which D_2O was added to a final concentration of 10% (v/v),

yielding a total sample volume of 500 µL. The final samples contained 200 µM PLIN1-AH together with either 60 mM DPC or bicelles containing 15 mM total long-chain phospholipid, with the DHPC concentration determined by the specified q value. Samples were transferred to standard 5-mm NMR tubes and equilibrated for 15 min at 37°C before data acquisition. Each HSQC dataset was acquired with 2,048 complex points in the ^{1}H dimension and 156 complex points in the ^{15}N dimension. To permit direct comparison across conditions, the protein concentration, sample volume, temperature, acquisition parameters, and data-processing settings were held constant within each experimental series.

NMR processing and peak-resolved analysis

NMR datasets were processed in NMRPipe using identical apodization, zero-filling, Fourier-transformation, phase-correction, and baseline-correction procedures within each experimental series, and spectra were analyzed in Sparky. Peak positions used for cross-condition tracking and quantitative analysis are summarized in Tables S1–S2. Weighted chemical shift perturbations (CSP) were calculated according to Equation 1:

$$\mathrm{CSP} = \sqrt{(\Delta\delta_{\mathrm{H}})^2 + (0.154 \times \Delta\delta_{\mathrm{N}})^2} \quad (1)$$

where $\Delta\delta_{\mathrm{H}}$and $\Delta\delta_{\mathrm{N}}$are the chemical-shift differences in the ^{1}H and ^{15}N dimensions, respectively. Box plots summarize the CSP distribution of tracked cross-peaks, with the center line denoting the median, boxes indicating the interquartile range, whiskers extending to 1.5 × the interquartile range, and individual points representing individual quantified resonances. The 0.05 ppm line was included as a visual guide and was not treated as a significance threshold.

Preparation of DMPC/DMPG large unilamellar vesicles

DMPC and DMPG stocks in chloroform were mixed to obtain DMPG mole fractions of 0, 25, 50, 75, or 100 mol%, with the mole fraction calculated relative to total phospholipid. The solvent was removed under a gentle stream of nitrogen, and the resulting lipid films were dried under vacuum for at least 2 h. Films were hydrated in LUV preparation buffer (20 mM HEPES, 140 mM KCl, pH 7.4) to a total phospholipid concentration of 1 mg mL^{-1}, vortexed to generate multilamellar vesicles, and subjected to 5 freeze–thaw cycles. The suspensions were extruded through 100-nm polycarbonate membranes for 15 passes using a mini-extruder maintained at room temperature.

Biolayer interferometry

Recruitment of DMPC/DMPG LUVs to surface-immobilized PLIN1-AH was measured by biolayer interferometry (BLI) using an Octet R8 instrument equipped with Ni–NTA biosensors. All measurements were performed using Ni–NTA biosensors in detergent-free assay buffer (20 mM HEPES, 140 mM KCl, pH 7.4) containing 0.1% fatty-acid-free BSA to minimize nonspecific adsorption. Biosensors were hydrated in assay buffer (20 mM HEPES, 140 mM KCl, pH 7.4) and loaded with His-tagged PLIN1-AH. After a baseline step, biosensors were transferred into LUV suspensions, and association was recorded in real time. PLIN1-AH-loaded biosensors were transferred into LUV suspensions containing 0, 25, 50, 75, or 100 mol% DMPG at a matched total phospholipid concentration of 1 mg mL^{-1}, and association was monitored for 600 s. Blank Ni–NTA biosensors lacking immobilized PLIN1-AH were exposed to

50 mol% DMPG LUVs to assess direct adsorption of the lipid particles to the sensor surface. The complete DMPG-composition series was measured in three replicate BLI experiments under identical experimental and analytical conditions. For each composition, extracted parameters are reported as the mean ± SD of three replicate experiments (n = 3)

Because LUV recruitment can involve multivalent particle–surface interactions and cannot be represented reliably by a simple 1:1 molecular binding model, no molecular equilibrium or kinetic constants (K_D, k_{on}, or k_{off}) were derived. Instead, recruitment was quantified using three operational parameters: the initial association slope calculated over 5–120 s, the endpoint response averaged over 580–600 s, and the area under the association curve from 0 to 600 s. Initial slopes, endpoint responses, and AUC values were calculated separately for each of three independent BLI experiments. Data are presented as individual experimental values with the mean ± SD. Because all five DMPG compositions were measured within each independent experiment, the overall effect of DMPG composition was evaluated by one-way repeated-measures ANOVA with the Greenhouse–Geisser correction. Four planned pairwise comparisons—0 versus 25, 25 versus 50, 50 versus 75, and 50 versus 100 mol% DMPG—were assessed using two-tailed paired t-tests with Holm adjustment for multiple comparisons. Holm-adjusted P values were used to determine statistical significance.

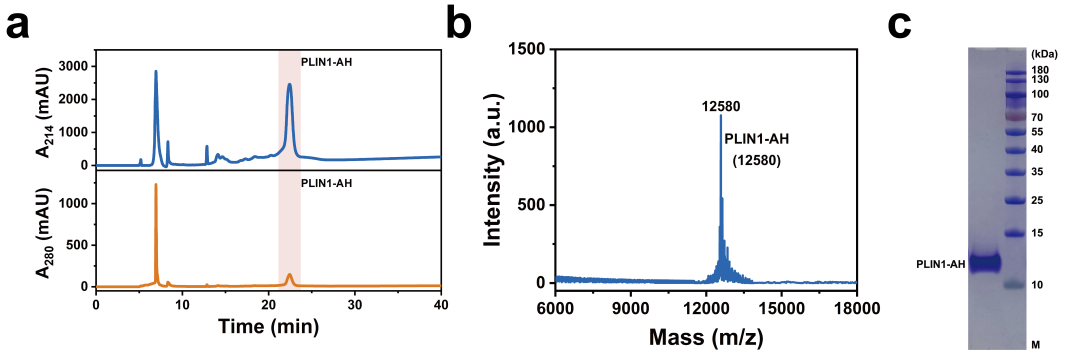

Fig. S1 Purification and analytical characterization of recombinant PLIN1-AH. **a** RP-HPLC profile of recombinant PLIN1-AH monitored at 214 nm and 280 nm, with the shaded region indicating the fraction collected for subsequent analyses. **b** Mass spectrum of purified PLIN1-AH showing a predominant signal at *m/z* 12,580. **c** Coomassie Brilliant Blue-stained SDS–PAGE analysis of the purified PLIN1-AH fraction, showing a predominant band near 12.5 kDa; M, molecular-mass marker

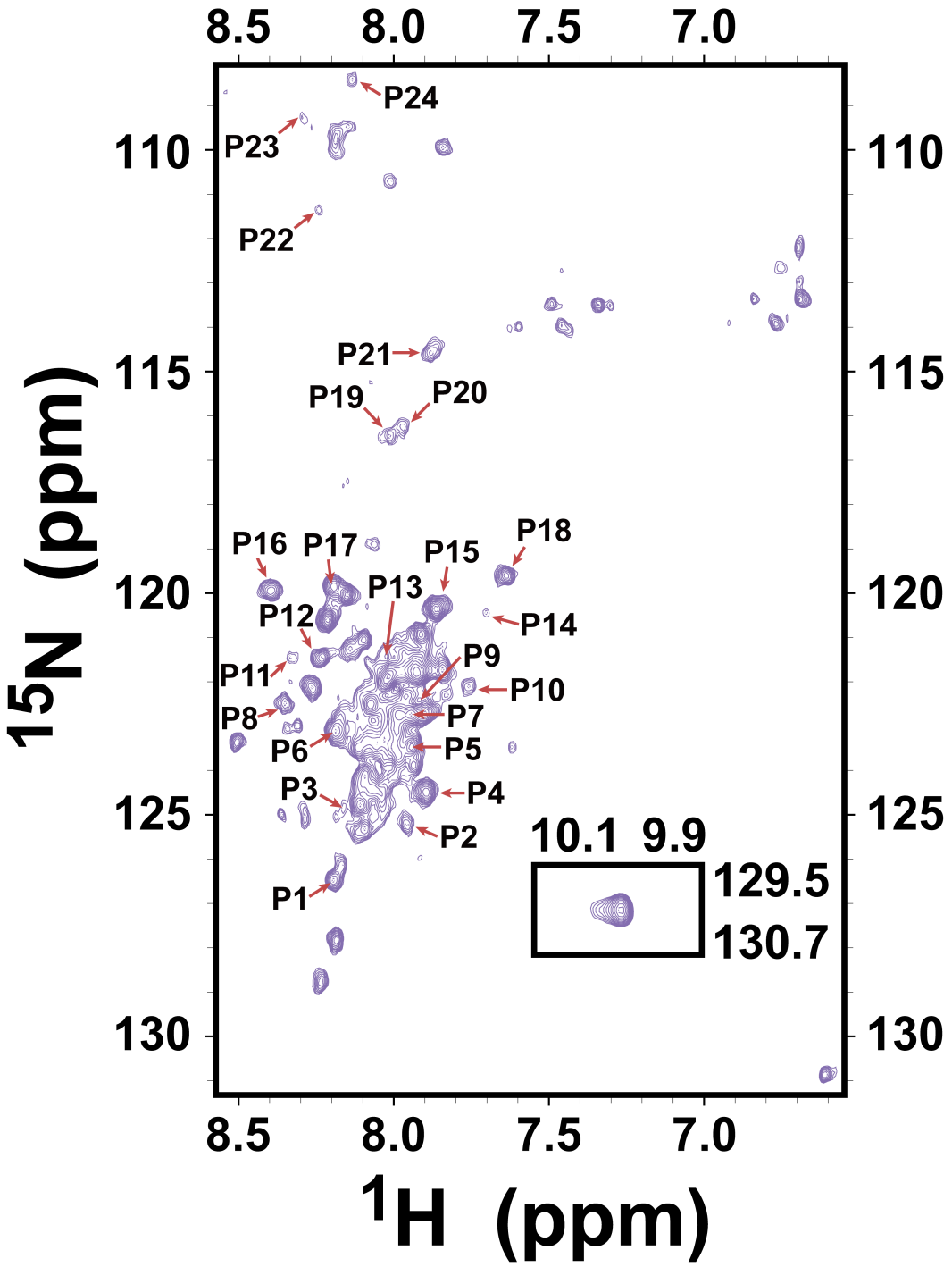

Fig. S2 Selection of PLIN1-AH cross-peaks for weighted CSP analysis. The ^{1}H–^{15}N HSQC spectrum of free PLIN1-AH shows the 24 cross-peaks selected on the basis of reliable one-to-one correspondence across the bicelle spectra. The selected resonances were designated P1–P24 and used for weighted CSP calculations across the DMPG-composition series at q = 0.8 and the q series containing 50 mol% DMPG. Peak identifiers correspond to those reported in Tables S1 and S2

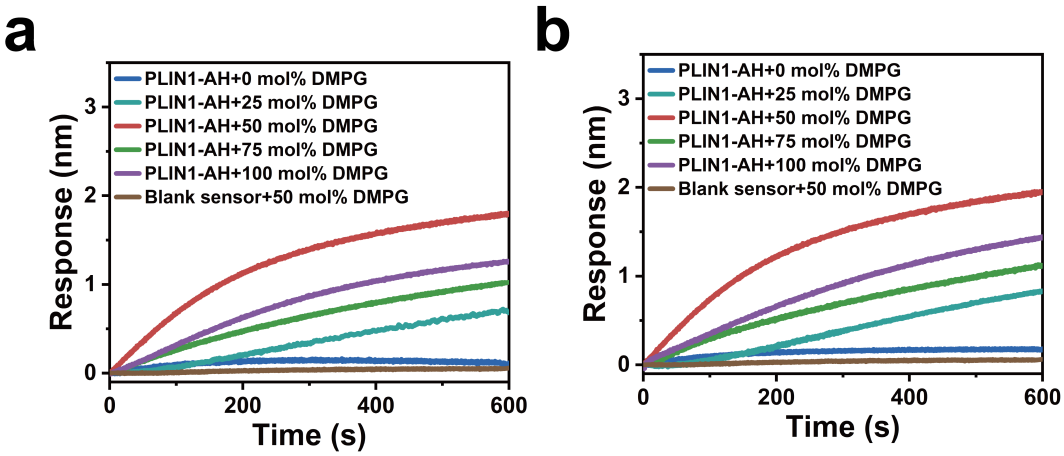

Fig. S3 Additional independent BLI experiments reproduce DMPG-dependent DMPC/DMPG LUV recruitment by immobilized PLIN1-AH. **a** and **b** show association traces from two additional independent experiments performed under the same conditions as the experiment presented in Fig. 3a. In each experiment, PLIN1-AH-loaded Ni–NTA biosensors were exposed to DMPC/DMPG LUVs containing 0, 25, 50, 75, or 100 mol% DMPG at a matched total phospholipid concentration of 1 mg mL^{-1}. Together with the dataset shown in Fig. 3a, all three experiments reproduced the same composition-dependent pattern, with low association responses at 0–25 mol% DMPG and elevated responses at 50–100 mol% DMPG; among the compositions tested, 50 mol% DMPG consistently produced the largest response. Blank Ni–NTA biosensors exposed to 50 mol% DMPG LUVs remained close to baseline, indicating minimal direct vesicle adsorption and supporting PLIN1-AH-mediated LUV recruitment as the principal source of the measured signal

Table S1. Tracked $^{1}H–^{15}N$ HSQC Peaks and Peak-Resolved NMR Parameters of PLIN1-AH in DMPG-Containing Bicelles with Varying DMPG Mole Fractions at $q = 0.8$

Peak ID	Free		0 mol% DMPG			25 mol% DMPG			50 mol% DMPG			75 mol% DMPG			100 mol% DMPG		
	$\delta^{1}H$	$\delta^{15}N$	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP
P1	8.194	126.484	8.192	126.336	0.02288	8.193	126.850	0.05637	8.210	126.205	0.04585	8.204	126.333	0.02531	8.198	126.322	0.02527
P2	7.958	125.213	7.973	125.645	0.06820	7.965	125.294	0.01430	7.958	125.187	0.00400	7.954	125.179	0.00659	7.945	125.198	0.01320
P3	8.164	124.851	8.198	124.691	0.04199	8.207	124.332	0.09076	8.212	124.350	0.09087	8.207	124.336	0.09022	8.209	124.337	0.09105
P4	7.897	124.487	7.881	124.313	0.03121	7.871	124.603	0.03155	7.822	124.863	0.09475	7.827	124.764	0.08197	7.815	124.715	0.08920
P5	7.943	123.469	7.931	123.533	0.01553	7.939	123.427	0.00760	7.940	123.264	0.03171	7.948	123.374	0.01546	7.933	123.365	0.01888
P6	8.187	123.113	8.199	122.890	0.03638	8.237	123.113	0.05000	8.172	122.832	0.04580	8.227	122.884	0.05333	8.242	122.931	0.06173
P7	7.944	122.737	7.949	122.843	0.01707	7.935	122.508	0.03640	7.905	122.494	0.05405	7.906	122.523	0.05030	7.899	122.530	0.05515
P8	8.351	122.487	8.364	122.435	0.01527	8.383	122.389	0.03538	8.327	121.997	0.07918	8.299	121.885	0.10630	8.316	121.962	0.08810
P9	7.905	122.333	7.917	122.333	0.01200	7.907	122.119	0.03302	7.888	121.920	0.06583	7.891	121.954	0.06002	7.890	121.988	0.05521
P10	7.759	122.104	7.753	121.904	0.03138	7.673	121.726	0.10385	7.636	121.635	0.14264	7.642	121.656	0.13583	7.657	121.542	0.13377
P11	8.335	121.428	8.471	121.684	0.14160	8.363	121.329	0.03188	8.288	121.465	0.04734	8.284	121.420	0.05101	8.293	121.612	0.05066
P12	8.234	121.478	8.245	121.253	0.03635	8.273	121.084	0.07213	8.282	120.910	0.09978	8.264	120.884	0.09627	8.250	120.848	0.09833
P13	8.029	121.291	7.991	121.200	0.04050	8.020	121.324	0.01034	8.035	121.507	0.03380	8.030	121.550	0.03990	8.029	121.642	0.05405
P14	7.703	120.440	7.699	120.121	0.04929	7.716	120.064	0.05935	7.694	120.008	0.06713	7.701	120.069	0.05717	7.693	120.046	0.06149
P15	7.864	120.357	7.836	120.153	0.04208	7.867	120.780	0.06521	7.880	120.795	0.06932	7.840	120.616	0.04655	7.875	120.828	0.07336
P16	8.396	119.938	8.397	119.876	0.00960	8.387	119.739	0.03194	8.358	119.230	0.11546	8.359	119.260	0.11077	8.369	119.228	0.11262
P17	8.195	119.860	8.160	119.910	0.03584	8.237	119.811	0.04267	8.275	119.734	0.08232	8.260	119.801	0.06563	8.263	119.809	0.06845
P18	7.638	119.607	7.676	119.396	0.05000	7.657	119.557	0.02050	7.548	119.606	0.09000	7.557	119.757	0.08423	7.565	120.002	0.09502
P19	8.016	116.466	8.003	116.422	0.01466	8.024	116.495	0.00916	8.011	116.792	0.05045	8.044	116.929	0.07660	7.993	116.305	0.03382
P20	7.968	116.235	7.921	116.577	0.07059	7.979	116.241	0.01104	7.952	116.596	0.05785	7.961	116.623	0.06016	7.957	116.430	0.03198
P21	7.876	114.547	7.847	114.344	0.04264	7.786	114.271	0.09953	7.716	114.230	0.16728	7.742	114.269	0.14067	7.747	114.244	0.13718
P22	8.242	111.351	8.269	111.296	0.02830	8.246	111.161	0.02953	8.204	111.078	0.05667	8.213	111.101	0.04820	8.204	111.086	0.05576
P23	8.288	109.330	8.293	108.724	0.09346	8.297	109.554	0.03565	8.274	109.607	0.04490	8.273	109.584	0.04189	8.263	109.597	0.04812
P24	8.134	108.417	8.134	108.229	0.02895	8.181	108.040	0.07470	8.080	108.297	0.05707	8.083	108.492	0.05229	8.079	108.515	0.05703

Weighted CSP are reported in ppm. Peak identifiers P1–P24 correspond to the reference spectrum shown in Fig. S2.

Table S2. Tracked $^{1}H–^{15}N$ HSQC peaks, chemical shifts, and peak-resolved chemical-shift perturbations of PLIN1-AH in bicelles containing 50 mol% DMPG at different *q* values.

Peak ID	Free		*q* = 0.4			*q* = 0.8			*q* = 1.6		
	$\delta^{1}H$	$\delta^{15}N$	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP	$\delta^{1}H$	$\delta^{15}N$	CSP
P1	8.194	126.484	8.185	126.314	0.02768	8.196	126.607	0.01900	8.248	125.929	0.10110
P2	7.958	125.213	7.923	125.269	0.02605	7.938	125.196	0.02020	7.976	125.221	0.01804
P3	8.164	124.851	8.217	124.475	0.07850	8.210	124.334	0.09200	8.231	124.339	0.10347
P4	7.897	124.487	7.794	124.444	0.10321	7.833	124.585	0.06580	7.799	125.050	0.13085
P5	7.943	123.469	7.929	123.438	0.01479	7.960	123.587	0.02490	7.960	123.625	0.02943
P6	8.187	123.113	8.202	122.817	0.04799	8.257	122.944	0.07470	8.211	122.538	0.09174
P7	7.944	122.737	7.923	122.784	0.02221	7.904	122.545	0.04970	7.888	122.406	0.07573
P8	8.351	122.487	8.349	121.926	0.08642	8.320	122.016	0.07890	8.321	121.942	0.08913
P9	7.905	122.333	7.911	122.403	0.01234	7.898	122.079	0.03970	7.876	121.901	0.07257
P10	7.759	122.104	7.635	121.411	0.16360	7.660	121.602	0.12560	7.667	121.450	0.13641
P11	8.335	121.428	8.293	121.777	0.06821	8.293	121.575	0.04770	8.295	121.574	0.04589
P12	8.234	121.478	8.250	121.283	0.03403	8.252	120.968	0.08060	8.250	120.631	0.13142
P13	8.029	121.291	7.983	121.463	0.05308	8.032	121.544	0.03910	8.008	121.578	0.04893
P14	7.703	120.440	7.713	119.976	0.07215	7.756	120.208	0.06390	7.673	119.794	0.10391
P15	7.864	120.357	7.862	120.725	0.05671	7.829	120.390	0.03540	7.849	120.995	0.09939
P16	8.396	119.938	8.382	119.632	0.03916	8.364	119.269	0.10790	8.396	119.215	0.11134
P17	8.195	119.860	8.209	120.000	0.02571	8.222	119.965	0.03150	8.285	119.851	0.09001
P18	7.638	119.607	7.567	120.375	0.13795	7.524	120.077	0.13500	7.613	120.405	0.12541
P19	8.016	116.466	8.007	116.555	0.01640	8.047	116.262	0.04410	7.996	116.326	0.02941
P20	7.968	116.235	7.928	116.003	0.05363	7.993	116.300	0.02690	7.929	116.370	0.04420
P21	7.876	114.547	7.844	114.410	0.02833	7.786	114.341	0.09540	7.866	113.308	0.17107
P22	8.242	111.351	8.252	111.280	0.01482	8.219	111.137	0.04020	8.177	110.970	0.08757
P23	8.288	109.330	8.242	109.475	0.05113	8.267	109.567	0.04210	8.271	109.667	0.05461
P24	8.134	108.417	8.127	108.130	0.04475	8.085	108.934	0.09350	7.934	108.301	0.18080

Weighted CSP are reported in ppm. Peak identifiers P1–P24 correspond to the reference spectrum shown in Fig. S2.

Table S3. Composition of DMPC/DMPG/DHPC bicelle samples used for NMR spectroscopy

Experimental series	Condition	DMPC:DMPG	q	DMPC (mM)	DMPG (mM)	Total long-chain lipid (mM)	DHPC (mM)	^{15}N-PLIN1-AH (µM)	Final volume (µL)
Neutral bicelles	q = 0.4	100:0	0.4	15.00	0	15.00	37.50	200	500
Neutral bicelles	q = 0.8	100:0	0.8	15.00	0	15.00	18.75	200	500
DMPG-composition series	0 mol% DMPG	100:0	0.8	15.00	0	15.00	18.75	200	500
DMPG-composition series	25 mol% DMPG	75:25	0.8	11.25	3.75	15.00	18.75	200	500
DMPG-composition series	50 mol% DMPG	50:50	0.8	7.50	7.50	15.00	18.75	200	500
DMPG-composition series	75 mol% DMPG	25:75	0.8	3.75	11.25	15.00	18.75	200	500
DMPG-composition series	100 mol% DMPG	0:100	0.8	0	15.00	15.00	18.75	200	500
q series	q = 0.4	50:50	0.4	7.50	7.50	15.00	37.50	200	500
q series	q = 0.8	50:50	0.8	7.50	7.50	15.00	18.75	200	500
q series	q = 1.6	50:50	1.6	7.50	7.50	15.00	9.375	200	500

Table S4 Statistical analysis of BLI-derived LUV recruitment parameters

Parameter	Overall	Comparison	Holm-adjusted	Figure symbol
Initial slope	0.000202	0 vs 25 mol% DMPG	0.887200	**ns**
		25 vs 50 mol% DMPG	0.004680	**
		50 vs 75 mol% DMPG	0.000309	***
		50 vs 100 mol% DMPG	0.006090	**
Endpoint response	0.000861	0 vs 25 mol% DMPG	0.002630	**
		25 vs 50 mol% DMPG	0.000672	***
		50 vs 75 mol% DMPG	0.002630	**
		50 vs 100 mol% DMPG	0.001890	**
AUC	0.0000388	0 vs 25 mol% DMPG	0.002030	**
		25 vs 50 mol% DMPG	0.001680	**
		50 vs 75 mol% DMPG	0.002240	**
		50 vs 100 mol% DMPG	0.001300	**