

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

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Abstract

The central amphipathic region of perilipin 1 (PLIN1) contributes to lipid-droplet targeting, but the interfacial properties that govern its association with lipid assemblies remain incompletely defined. Here, we examined human PLIN1 residues 94–193 (PLIN1-AH) by solution nuclear magnetic resonance spectroscopy in dodecylphosphocholine (DPC) micelles and dimyristoyl phosphatidylcholine/dihexanoylphosphatidylcholine (DMPC/DHPC) bicelle-forming assemblies, complemented by bilayer interferometry (BLI) measurements of DMPC/dimyristoyl phosphatidylglycerol (DMPG) large unilamellar vesicle recruitment to immobilized PLIN1-AH. DPC micelles produced extensive spectral reorganization, whereas neutral DMPC/DHPC bicelles caused comparatively modest perturbations. At $q = 0.8$, increasing DMPG from 25 to 50 mol% shifted the NMR response from a low- to a high-response regime, with no further increase at 75 or 100 mol%. At 50 mol% DMPG, increasing q to 1.6 further enhanced the spectroscopic response. Vesicle recruitment showed a corresponding nonlinear composition dependence, reaching its maximum at 50 mol% DMPG among the compositions tested. These results indicate that PLIN1-AH association is sensitive to both membrane mimetic organization and anionic-phospholipid content and support a threshold-like transition around 50 mol% DMPG under the conditions tested.

Introduction

Perilipin 1 (PLIN1) is the predominant lipid-droplet coat protein in adipocytes and a key regulator of stored-lipid mobilization (Sztalryd and Brasaemle 2017). Human PLIN1 contains a conserved N-terminal perilipin–ADRP–TIP47 (PAT) domain, a central region of 11-mer repeats, and a regulatory C-terminal region (Griseti et al. 2024). The 11-mer repeat region acts as a primary and highly reversible lipid-droplet-targeting motif and can adopt amphipathic-helical conformations at lipid interfaces (Ajjaji et al. 2019). The periodic arrangement of hydrophobic, polar, and charged residues generates an amphipathic surface in which hydrophobic side chains can engage the lipid phase, while polar and charged residues remain exposed to phospholipid headgroups and the aqueous environment (Rowe et al. 2016; Giménez-Andrés et al. 2018). This organization enables reversible association with the phospholipid monolayer surrounding lipid droplets. However, how the PLIN1 amphipathic-helix (AH) region responds to differences in the supramolecular organization and chemical composition of lipid interfaces remains incompletely understood.

Membrane mimetics permit protein-lipid interactions to be examined under compositionally defined conditions, although different mimetics reproduce distinct and often coupled interfacial properties. Dodecylphosphocholine (DPC) micelles are small, highly curved assemblies of single-chain amphiphiles (Klöpfer and Hagn 2019). Their dynamic, non-bilayer environment can produce protein conformational and exchange behavior distinct from those observed in phospholipid bilayers (Chipot et al. 2018). Mixture of long-chain phospholipids and short-chain amphiphiles, such as dimyristoyl phosphatidylcholine (DMPC) and dihexanoyl phosphatidylcholine (DHPC), form rapidly tumbling assemblies whose dimensions, morphology, and dynamics depend on the long-chain to short-chain

molar ratio (q), as well as on total amphiphile concentration and temperature (Caldwell et al. 2018; Giudice et al. 2022). Large unilamellar vesicles (LUVs), by contrast, form closed phospholipid bilayers without an exposed rim (Majeed et al. 2021). These membrane mimetics provide complementary interfaces that differ in molecular composition, continuity, curvature, and dynamics, allowing the sensitivity of PLIN1-AH to supramolecular context to be examined without attributing the observed responses to any single physical parameter.

In addition to supramolecular organization, phospholipid headgroup composition can influence AH association by altering interfacial electrostatics, hydration, lipid packing, and lipid–lipid interactions (Cornell 2016). Phosphatidylcholine (PC) is zwitterionic and forms an interface with little net charge, whereas phosphatidylglycerol (PG) carries a net negative surface charge and has distinct headgroup chemistry (Rønneet et al. 2016). DMPC and dimyristoyl phosphatidylglycerol (DMPG) contain the same pair of saturated 14-carbon acyl-chains but differ in their headgroups and net charge (Harmouche and Bechinger 2018). Varying the DMPC/DMPG ratio therefore changes the nominal anionic-lipid fraction while preserving the acyl-chain background, thereby minimizing differences arising directly from the hydrophobic region of the membrane (Molugu et al. 2022). Nevertheless, replacing DMPC with DMPG can simultaneously affect surface electrostatics, headgroup hydration, lipid packing, and aggregate organization. This matched lipid pair consequently provides a controlled system for examining the dependence of PLIN1-AH association on anionic-phospholipid content, without equating DMPG mole fraction with a directly measured membrane surface-charge density.

Here, we investigated how PLIN1-AH, comprising residues 94–193 and encompassing the central 11-mer repeat region, responds to membrane-mimetic organization and DMPG content. Solution nuclear magnetic resonance (NMR) spectroscopy was first used to compare PLIN1-AH in DPC micelles and neutral DMPC/DHPC bicelles. The DMPG fraction was subsequently varied at a fixed q , and the effect of q was examined at a fixed DMPG composition. As a complementary assembly-scale readout, BLI was used to quantify the recruitment of compositionally defined DMPC/DMPG LUVs to immobilized PLIN1-AH. DPC micelles induced extensive reorganization of the PLIN1-AH spectrum, whereas neutral DMPC/DHPC bicelles produced comparatively modest perturbations. Within DMPG-containing bicelles, the NMR response depended on both q and the DMPG fraction. The NMR and BLI measurements together supported a transition from a low-response regime at 0–25 mol% DMPG to an elevated-response regime at 50–100 mol% DMPG, with the largest measured responses at 50 mol% DMPG among the compositions tested. These findings indicate that PLIN1-AH association is sensitive to both membrane-mimetic organization and anionic-phospholipid content and support a threshold-like compositional response under the conditions examined.

Materials and Methods

Detailed materials and methods can be found in the supporting information.

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 $([\text{DMPC}] + [\text{DMPG}])/[\text{DHPC}]$, 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 ^1H – ^{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 ^1H 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.

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 $\pm$ 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 $\pm$ 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.

Results

PLIN1-AH Displays Distinct NMR Responses to DPC Micelles and Neutral DMPC/DHPC Bicelles

The PLIN1-AH construct comprised residues 94–193 of human PLIN1, including seven complete 11-residue repeats, an N-terminal flanking segment, and a partial C-terminal repeat (Fig. 1a). When mapped onto a helical-wheel projection, hydrophobic residues preferentially clustered on one side of the putative helix, whereas polar and charged residues were distributed predominantly on the opposite side. This sequence pattern is consistent with the formation of an amphipathic surface upon helix formation (Fig. 1b). RP-HPLC yielded a major, well-resolved PLIN1-AH peak. Mass spectrometry identified a

dominant ion at m/z 12,580, and SDS-PAGE showed a predominant band at approximately 12.5 kDa, together supporting the expected molecular size and high purity of the protein preparation used in the subsequent experiments (Fig. S1).

To assess the response of PLIN1-AH to DPC micelles, we compared its two-dimensional ^1H - ^{15}N heteronuclear single-quantum coherence (HSQC) spectra in the absence and presence of DPC micelles (Fig. 1c). The spectrum of free PLIN1-AH exhibited limited overall ^1H chemical-shift dispersion, with most cross-peaks forming a compact central cluster and a smaller subset appearing at more dispersed positions. This pattern is consistent with a conformationally heterogeneous protein ensemble containing locally distinguishable chemical environments. Addition of DPC caused extensive spectral reorganization, including the disappearance or pronounced attenuation of numerous free-state resonances and the appearance of signals at new chemical-shift positions. These changes indicate that DPC micelles substantially alter the local chemical environments and exchange behavior of the NMR-visible PLIN1-AH population.

We next examined PLIN1-AH in neutral DMPC/DHPC bicelles at $q = 0.4$ and $q = 0.8$, where q denotes the molar ratio of long-chain DMPC to short-chain DHPC. Increasing q altered the relative abundance of the two amphiphilic components and, consequently, the organization and dynamics of the bicelle assemblies, while retaining phosphocholine as the common headgroup. In the HSQC overlays, the overall pattern of free-state resonances remained recognizable under both conditions, and most traceable cross-peaks exhibited only small, continuous chemical-shift changes (Fig. 1d). Relative to $q = 0.4$, the $q = 0.8$ condition did not produce a separate set of resonances or induce extensive additional spectral reorganization. Thus, within the neutral DMPC/DHPC bicelle system, increasing q from 0.4 to 0.8 caused only modest additional perturbation of the NMR-visible PLIN1-AH ensemble and provided no evidence for a transition to a discrete, spectrally distinct state.

Although DPC and DMPC/DHPC assemblies both present phosphocholine groups, they produced markedly different PLIN1-AH spectral responses. The presence of a common phosphocholine moiety was therefore insufficient to produce comparable NMR behavior, indicating that differences in amphiphile molecular architecture and supramolecular organization also contribute to the response of PLIN1-AH. Under the conditions examined, neutral DMPC/DHPC bicelles produced substantially smaller perturbations than DPC micelles, prompting us to test whether an anionic phospholipid would enhance the response. Because the predicted polar surface of PLIN1-AH contains multiple charged residues, interfacial electrostatics represented one possible contributing factor. DMPG was therefore selected as the anionic counterpart to DMPC: the two phospholipids contain the same pair of C14 acyl chains but differ in headgroups and net charge. This matched pair enabled the DMPG fraction to be varied while maintaining a constant long-chain acyl background, thereby minimizing confounding effects arising directly from differences in acyl-chain length or saturation.

DMPG Composition and Bicelle Organization Jointly Modulate the Response of PLIN1-AH

Neutral DMPC/DHPC bicelles at $q = 0.8$ provide an increased contribution from bilayer-like regions while retaining sufficiently well-resolved and traceable PLIN1-AH cross-peaks, and were therefore selected to examine the dependence of the NMR response on anionic phospholipid content. The mole fraction of DMPG within the long-chain lipid component was set to 0, 25, 50, 75, or 100 mol%, while q was maintained at 0.8. For quantitative comparison, 24 cross-peaks that could be followed unambiguously between free PLIN1-AH and the different lipid-containing conditions were selected and designated P1–P24 (Fig. S2). The same set of resonances was used for weighted chemical-shift perturbation (CSP) analysis across the DMPG-composition and q series.

Most of the selected cross-peaks remained traceable throughout the DMPG-composition series. The spectra obtained at 0 and 25 mol% DMPG were broadly similar, with most resonances displaying relatively small chemical-shift changes. Increasing the DMPG fraction from 25 to 50 mol% produced coordinated and readily discernible displacements of multiple resonances (Fig. 2a). Consistent with the spectral overlays, the weighted CSP distributions at 0 and 25 mol% DMPG showed similarly low median values, whereas the distribution at 50 mol% DMPG shifted upward, with the median exceeding the 0.05 ppm line included for visual comparison (Fig. 2b). At 50 mol% DMPG, the weighted CSP distribution shifted upward, with the median exceeding the 0.05 ppm line included for visual comparison (Fig. 2b). At 75 and 100 mol% DMPG, the CSP distributions remained elevated relative to those at 0 and 25 mol% DMPG but were modestly lower than the distribution observed at 50 mol% DMPG. Thus, under the compositions examined, the PLIN1-AH NMR response did not increase continuously with DMPG content. Instead, the data indicate an apparent transition from a low-response regime at 0–25 mol% DMPG to an elevated-response regime at 50–100 mol% DMPG, placing the transition between 25 and 50 mol% DMPG. The ^1H and ^{15}N chemical shifts and weighted CSP values of the tracked peaks across the DMPG composition series are provided in Table S1.

Because the largest median CSP was observed at 50 mol% DMPG, this composition was selected to examine whether the NMR response also depended on the DMPC/DHPC molar ratio. Bicelles containing 50 mol% DMPG within the long-chain phospholipid component were prepared at $q = 0.4$, 0.8, and 1.6. Most of the selected cross-peaks remained traceable under all three conditions. Increasing q from 0.4 to 0.8 produced only limited additional changes in peak position, whereas substantially larger chemical-shift changes were observed at $q = 1.6$ (Fig. 2c). The corresponding CSP distributions showed a low median at $q = 0.4$, a modest increase at $q = 0.8$, and a pronounced increase in both the median and the upper range at $q = 1.6$ (Fig. 2d).

These results show that, at 50 mol% DMPG, the NMR visible response of PLIN1-AH depends on the long-chain to short-chain phospholipid ratio and becomes substantially greater at $q = 1.6$. This enhanced response is consistent with q -dependent changes in assembly dimensions and interfacial organization.

The corresponding ^1H and ^{15}N chemical shifts and weighted CSP values for the tracked peaks at $q = 0.4$, 0.8, and 1.6 are provided in Table S2.

PLIN1-AH-Mediated LUV Is Greatest at 50 mol% DMPG

To determine whether the composition-dependent NMR response was accompanied by changes in the recruitment of intact lipid assemblies, BLI was used to compare LUVs containing 0, 25, 50, 75, or 100 mol% DMPG within the total phospholipid component. His-tagged PLIN1-AH was immobilized on Ni-NTA biosensors, and the accumulation of LUVs at the sensor surface was monitored during a 600-s association phase. All PLIN1-AH-loaded sensors produced positive, time-dependent responses upon exposure to LUVs, although the response magnitude varied substantially with lipid composition (Fig. 3a). A blank sensor lacking immobilized PLIN1-AH remained close to baseline when exposed to LUVs containing 50 mol% DMPG, indicating minimal direct adsorption of this vesicle preparation to the protein-free Ni-NTA sensor surface under the conditions tested.

The BLI response showed a nonlinear dependence on DMPG content. LUVs containing 0 or 25 mol% DMPG generated comparatively small responses, whereas increasing the DMPG fraction to 50 mol% produced a pronounced increase in response amplitude (Fig. 3a). Among the compositions tested, LUVs containing 50 mol% DMPG generated the largest response. Further increasing the DMPG fraction to 75 or 100 mol% did not produce an additional increase; instead, the responses were modestly lower than the response observed at 50 mol% DMPG while remaining higher than those obtained with 0 or 25 mol% DMPG. This composition-dependent response pattern was observed in three independent experiments using independently prepared LUV samples performed under the same conditions (Fig. S3).

Three descriptive parameters were extracted from the association traces: the initial slope, calculated over 5–120 s as an operational measure of early response accumulation; the endpoint response, averaged over 580–600 s; and the area under the curve (AUC), representing the integrated response over the complete 600-s association phase. Across the three replicate experiments, all three parameters showed the same composition-dependent pattern, with relatively low values at 0 and 25 mol% DMPG, the highest values at 50 mol% DMPG, and modestly lower values at 75 and 100 mol% DMPG (Fig. 3b–d). One-way repeated-measures ANOVA identified a significant effect of DMPG composition on each BLI-derived parameter, including the initial slope, endpoint response, and AUC (Greenhouse–Geisser-corrected $P < 0.001$ for each analysis). Holm-adjusted planned pairwise comparisons further showed that values at 50 mol% DMPG were significantly higher than those at 25, 75, and 100 mol% DMPG for all three parameters ($P_{\text{adj}} < 0.01$, Table S4). Thus, under the conditions tested, increasing the DMPG fraction from 25 to 50 mol% markedly enhanced LUV recruitment to immobilized PLIN1-AH, whereas further enrichment of DMPG provided no additional enhancement.

This composition-dependent BLI profile was broadly consistent with the NMR results, in which the transition from 25 to 50 mol% DMPG was likewise accompanied by a pronounced increase in the PLIN1-AH spectroscopic response. NMR reports changes in the local environments and exchange behavior of the NMR-visible protein population, whereas BLI measures the cumulative accumulation of intact

vesicles at an immobilized protein surface. Although the two readouts should not be interpreted as equivalent measures of binding, their convergence nevertheless supports a transition from relatively weak responses at 0–25 mol% DMPG to elevated responses at 50–100 mol% DMPG under the experimental conditions used.

Discussion

The present study examined how the physicochemical context of a lipid interface influences the association of PLIN1-AH. The distinct NMR responses observed across the membrane-mimetic conditions suggest that PLIN1-AH does not adopt a single, spectroscopically homogeneous lipid-bound conformation. Instead, chemical-shift changes, signal attenuation, and the appearance or disappearance of resonances are consistent with redistribution among solution-visible and interface-associated populations undergoing exchange on different timescales (Ceccon et al. 2022). These observables report the NMR-visible response of PLIN1-AH to the lipid environment but do not by themselves distinguish changes in bound fraction from changes in conformation, residence time, or particle-coupled dynamics. The pronounced spectral reorganization induced by DPC, compared with the modest perturbations produced by neutral DMPC/DHPC assemblies, further shows that presentation of phospholipid headgroup alone is insufficient to determine the response. DPC differs from DMPC/DHPC assemblies in molecular architecture, curvature, hydrophobic-core organization, and dynamics; it should therefore be regarded as a strongly perturbing reference rather than as a closer mimic of native lipid-droplet binding (Chipot et al. 2018).

Introducing DMPG revealed an apparent compositional transition rather than a continuously increasing response to nominal anionic-lipid content. Both the NMR and BLI measurements distinguished a low-response regime at 0–25 mol% DMPG from an elevated-response regime at 50–100 mol% DMPG. Because the composition series was sampled at 25 mol% intervals, the combined NMR and BLI data place the transition between 25 and 50 mol% DMPG. Within this sampling resolution, the reproducibility of the BLI profile across three independent experiments, together with the significantly higher BLI responses at 50 mol% DMPG than at 25, 75, or 100 mol% DMPG, supports 50 mol% DMPG as the operational threshold for entry into the elevated-response regime and as the maximal-response condition within the tested series. Moreover, DMPG mole fraction may not be equated directly with membrane surface-charge density. Replacing DMPC with DMPG can simultaneously alter effective electrostatics, headgroup hydration, lipid packing, and lateral organization (Cornell 2016). The absence of further enhancement above 50 mol% DMPG may therefore reflect saturation of favorable electrostatic contacts, attainment of a sufficient density of productive interfacial sites, or compensating changes in lipid organization at higher DMPG fractions. The present data do not distinguish among these possibilities.

The q -dependent response provides additional evidence that the physical organization of the lipid assembly contributes to PLIN1-AH association. At 50 mol% DMPG, the spectroscopic response was substantially greater at $q = 1.6$ than at $q = 0.4$ or 0.8 . In DMPC/DHPC systems, increasing q can increase

particle lateral dimensions while altering the relative proportions of long-chain-rich and highly curved regions. For approximately disk-like particles, this would correspond to an increased bicelle radius. A larger particle could provide a more extensive contiguous long-chain-phospholipid-rich surface on which the extended PLIN1 amphipathic region forms multiple simultaneous contacts, thereby increasing interfacial residence or stabilizing an interface-associated population. Importantly, this model concerns the continuous binding area available on an individual particle rather than the total surface area in the sample. Increasing q enlarges the lateral dimensions of DMPC/DHPC bicelle and consequently expands the continuous long-chain-phospholipid-rich interface (Mortensen et al. 2018). This provides a direct structural rationale for the markedly greater NMR response observed at $q = 1.6$. The precise dimensions of the DMPG-containing assemblies remain to be established, but the direction of the q -dependent effect is consistent with the established relationship between q and particle lateral size.

The combination of NMR and BLI is an important strength of the study because the two methods interrogate different physical scales of the interaction. NMR detects changes in the local environments and exchange behavior of the solution-visible PLIN1-AH population and is sensitive to transient or heterogeneous association. BLI measures the time-dependent accumulation of intact LUVs at a surface bearing immobilized PLIN1-AH and integrates vesicle recruitment, particle mass, multivalent contact, and surface avidity rather than reporting a simple one-to-one affinity (Wallner et al. 2013). A large weighted CSP could therefore arise without a proportional increase in vesicle recruitment, whereas a large BLI response could be influenced by vesicle size or multivalency. The observation that both readouts reached their largest values at 50 mol% DMPG makes it less likely that the compositional trend arises solely from a method-specific effect. Their convergence links molecular-scale perturbation of PLIN1-AH with assembly-scale lipid recruitment, although the magnitudes of the NMR and BLI responses should not be interpreted as quantitatively equivalent.

Taken together, the data support an interface-selection model in which productive PLIN1-AH association depends on a composite set of interfacial properties. DMPG may increase the probability of electrostatically favorable encounters, whereas the dimensions and organization of the assembly determine whether a sufficiently extended lipid-rich surface is available for multi-residue engagement. Once both requirements are met, the cumulative contribution of multiple weak polar, electrostatic, and hydrophobic contacts along the repetitive amphipathic region could stabilize association without requiring a single, rigid bound conformation. This model is compatible with previous work showing that the long amphipathic regions of perilipins use distributed sequence features to associate with lipid-droplet surfaces (Čopić et al. 2018; Giménez-Andrés et al. 2021).

Several limitations define the scope of this interpretation. Particle size, morphology, surface potential, and lipid mixing were not measured directly, and the current design did not include a complete DMPG-by- q matrix. Dynamic light scattering or small-angle scattering, together with zeta-potential and ^{31}P NMR measurements, would help separate compositional effects from changes in particle organization. Backbone assignment, peak-intensity or relaxation analysis, paramagnetic relaxation enhancement, and mutations targeting charged or hydrophobic residues would further distinguish initial electrostatic

recruitment from subsequent interfacial stabilization. These measurements are best viewed as mechanistic extensions rather than requirements for the present compositional observation.

Finally, bicelles and LUVs are bilayer-based mimetics, whereas native lipid droplets contain a neutral-lipid core surrounded by a phospholipid monolayer. The apparent transition identified here should therefore not be assumed to apply quantitatively to cellular lipid droplets. Testing selected compositions and PLIN1-AH mutants in artificial lipid droplets, followed by full-length PLIN1 localization assays, will establish which principles are retained at a monolayer interface (Caillon et al. 2020). Within these limitations, the combined NMR–BLI analysis shows that PLIN1-AH responds to both anionic-phospholipid content and the organization of the membrane-mimetic assembly, supporting a threshold-like compositional transition rather than a simple linear dependence on DMPG abundance.

Detailed experimental procedures are provided in the Supplementary Information.

Declarations

Author contributions K.H. and W.C. conceived the study and designed the experiments. K.H. performed the experiments. K.H. and Z.L. analyzed the data and prepared the figures. K.H. wrote the original draft. W.C. supervised the study, and reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

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Data Availability The data supporting the findings of this study are provided in the article and its Supplementary Information.

Competing Interests The authors declare no competing interests.

References

1. Ajjaji D, Ben M’barek K, Mimmack ML, et al (2019) Dual binding motifs underpin the hierarchical association of perilipins 1–3 with lipid droplets. *Mol Biol Cell* 30:703–716. <https://doi.org/10.1091/mbc.E18-08-0534>
2. Caillon L, Nieto V, Gehan P, et al (2020) Triacylglycerols sequester monotopic membrane proteins to lipid droplets. *Nat Commun* 11:3944. <https://doi.org/10.1038/s41467-020-17585-8>
3. Caldwell TA, Baoukina S, Brock AT, et al (2018) Low-*q* Bicelles Are Mixed Micelles. *J Phys Chem Lett* 9:4469–4473. <https://doi.org/10.1021/acs.jpcllett.8b02079>

4. Ceccon A, Kubatova N, Louis JM, et al (2022) Global Dynamics of a Protein on the Surface of Anisotropic Lipid Nanoparticles Derived from Relaxation-Based NMR Spectroscopy. *J Phys Chem B* 126:5646–5654. <https://doi.org/10.1021/acs.jpccb.2c03519>
5. Chipot C, Dehez F, Schnell JR, et al (2018) Perturbations of Native Membrane Protein Structure in Alkyl Phosphocholine Detergents: A Critical Assessment of NMR and Biophysical Studies. *Chem Rev* 118:3559–3607. <https://doi.org/10.1021/acs.chemrev.7b00570>
6. Čopič A, Antoine-Bally S, Giménez-Andrés M, et al (2018) A giant amphipathic helix from a perilipin that is adapted for coating lipid droplets. *Nat Commun* 9:1332. <https://doi.org/10.1038/s41467-018-03717-8>
7. Cornell RB (2016) Membrane lipid compositional sensing by the inducible amphipathic helix of CCT. *Biochim Biophys Acta BBA - Mol Cell Biol Lipids* 1861:847–861. <https://doi.org/10.1016/j.bbalip.2015.12.022>
8. Giménez-Andrés M, Čopič A, Antonny B (2018) The Many Faces of Amphipathic Helices. *Biomolecules* 8:45. <https://doi.org/10.3390/biom8030045>
9. Giménez-Andrés M, Emeršič T, Antoine-Bally S, et al (2021) Exceptional stability of a perilipin on lipid droplets depends on its polar residues, suggesting multimeric assembly. *eLife* 10:e61401. <https://doi.org/10.7554/eLife.61401>
10. Giudice RD, Paracini N, Laursen T, et al (2022) Expanding the Toolbox for Bicelle-Forming Surfactant–Lipid Mixtures. *Molecules* 27:7628. <https://doi.org/10.3390/molecules27217628>
11. Griseti E, Bello AA, Bieth E, et al (2024) Molecular mechanisms of perilipin protein function in lipid droplet metabolism. *FEBS Lett* 598:1170–1198. <https://doi.org/10.1002/1873-3468.14792>
12. Harmouche N, Bechinger B (2018) Lipid-Mediated Interactions between the Antimicrobial Peptides Magainin 2 and PGLa in Bilayers. *Biophys J* 115:1033–1044. <https://doi.org/10.1016/j.bpj.2018.08.009>
13. Klöpfer K, Hagn F (2019) Beyond detergent micelles: The advantages and applications of non-micellar and lipid-based membrane mimetics for solution-state NMR. *Prog Nucl Magn Reson Spectrosc* 114–115:271–283. <https://doi.org/10.1016/j.pnmrs.2019.08.001>
14. Majeed S, Ahmad AB, Sehar U, Georgieva ER (2021) Lipid Membrane Mimetics in Functional and Structural Studies of Integral Membrane Proteins. *Membranes* 11:685. <https://doi.org/10.3390/membranes11090685>
15. Molugu TR, Thurmond RL, Alam TM, et al (2022) Phospholipid headgroups govern area per lipid and emergent elastic properties of bilayers. *Biophys J* 121:4205–4220. <https://doi.org/10.1016/j.bpj.2022.09.005>
16. Mortensen HG, Jensen GV, Hansen SK, et al (2018) Structure of Phospholipid Mixed Micelles (Bicelles) Studied by Small-Angle X-ray Scattering. *Langmuir* 34:14597–14607. <https://doi.org/10.1021/acs.langmuir.8b02704>
17. Rønneest AK, Peters GH, Hansen FY, et al (2016) Structure and dynamics of water and lipid molecules in charged anionic DMPG lipid bilayer membranes. *J Chem Phys* 144:144904.

<https://doi.org/10.1063/1.4945278>

18. Rowe ER, Mimmack ML, Barbosa AD, et al (2016) Conserved Amphipathic Helices Mediate Lipid Droplet Targeting of Perilipins 1–3. *J Biol Chem* 291:6664–6678.
<https://doi.org/10.1074/jbc.M115.691048>
19. Sztalryd C, Brasaemle DL (2017) The perilipin family of lipid droplet proteins: Gatekeepers of intracellular lipolysis. *Biochim Biophys Acta BBA - Mol Cell Biol Lipids* 1862:1221–1232.
<https://doi.org/10.1016/j.bbalip.2017.07.009>
20. Wallner J, Lhota G, Jeschek D, et al (2013) Application of Bio-Layer Interferometry for the analysis of protein/liposome interactions. *J Pharm Biomed Anal* 72:150–154.
<https://doi.org/10.1016/j.jpba.2012.10.008>

Figures

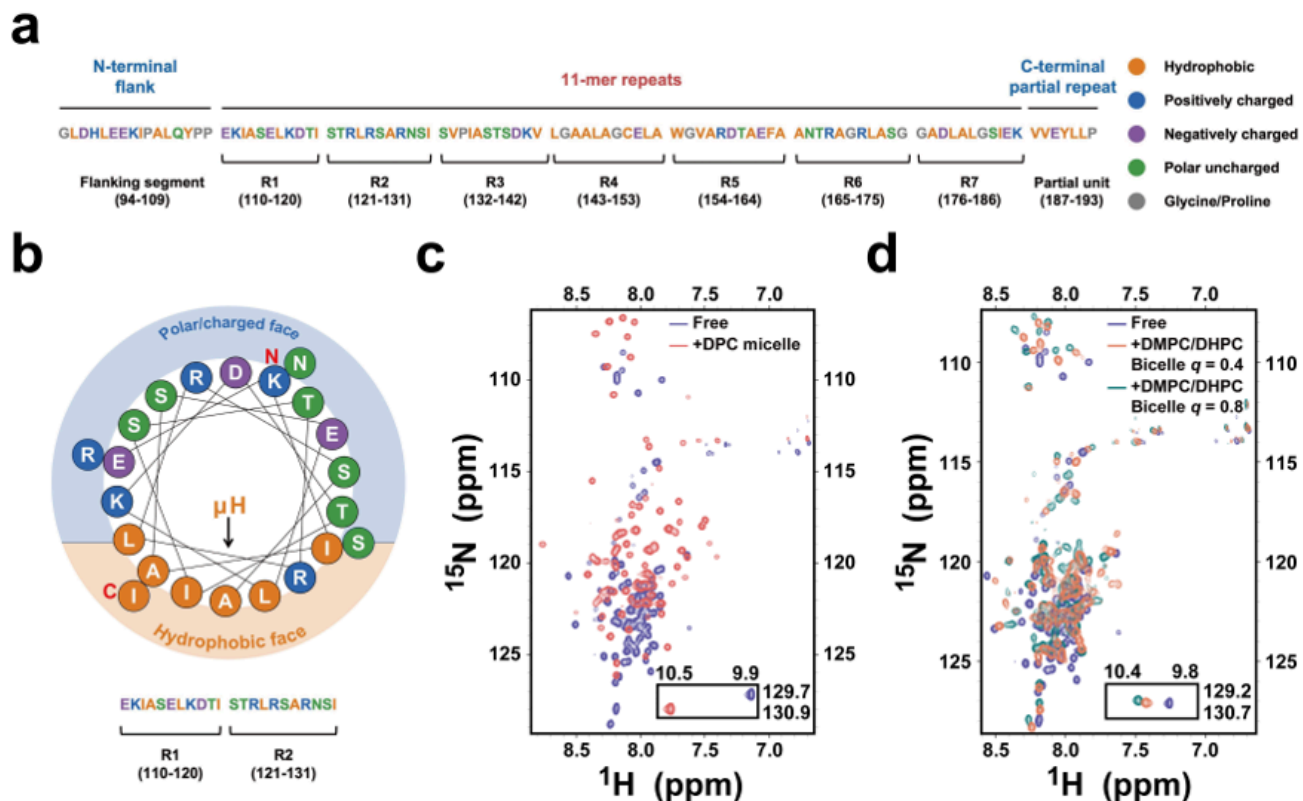


Figure 1

Sequence organization of PLIN1-AH and its distinct NMR responses to DPC micelles and neutral DMPC/DHPC bicelles. **a** Sequence organization of PLIN1-AH, comprising residues 94–193 of human PLIN1. The construct contains an N-terminal flanking segment, seven complete 11-mer repeats (R1–R7), and a C-terminal partial repeat. Residues are colored according to physicochemical class. **b** Helical-wheel projection of residues 110–131, corresponding to two consecutive 11-mer repeats (R1–R2), displayed in an idealized α -helix conformation. Hydrophobic residues cluster predominantly on one side

of the projection, whereas polar and charged residues are distributed mainly on the opposite side. The N and C labels indicate the termini of the displayed sequence segment, and the μH arrow indicates the direction of the hydrophobic moment vector toward the predicted hydrophobic face. **c** Overlay of two-dimensional ^1H - ^{15}N HSQC spectra of uniformly ^{15}N -labeled PLIN1-AH in the free state and in the presence of DPC micelles. **d** Overlay of two-dimensional ^1H - ^{15}N HSQC spectra of free PLIN1-AH and PLIN1-AH in neutral DMPC/DHPC bicelles at $q = 0.4$ and $q = 0.8$, where q denotes the molar ratio of DMPC to DHPC. Insets in **c** and **d** show the downfield tryptophan side chain indole NH resonance region

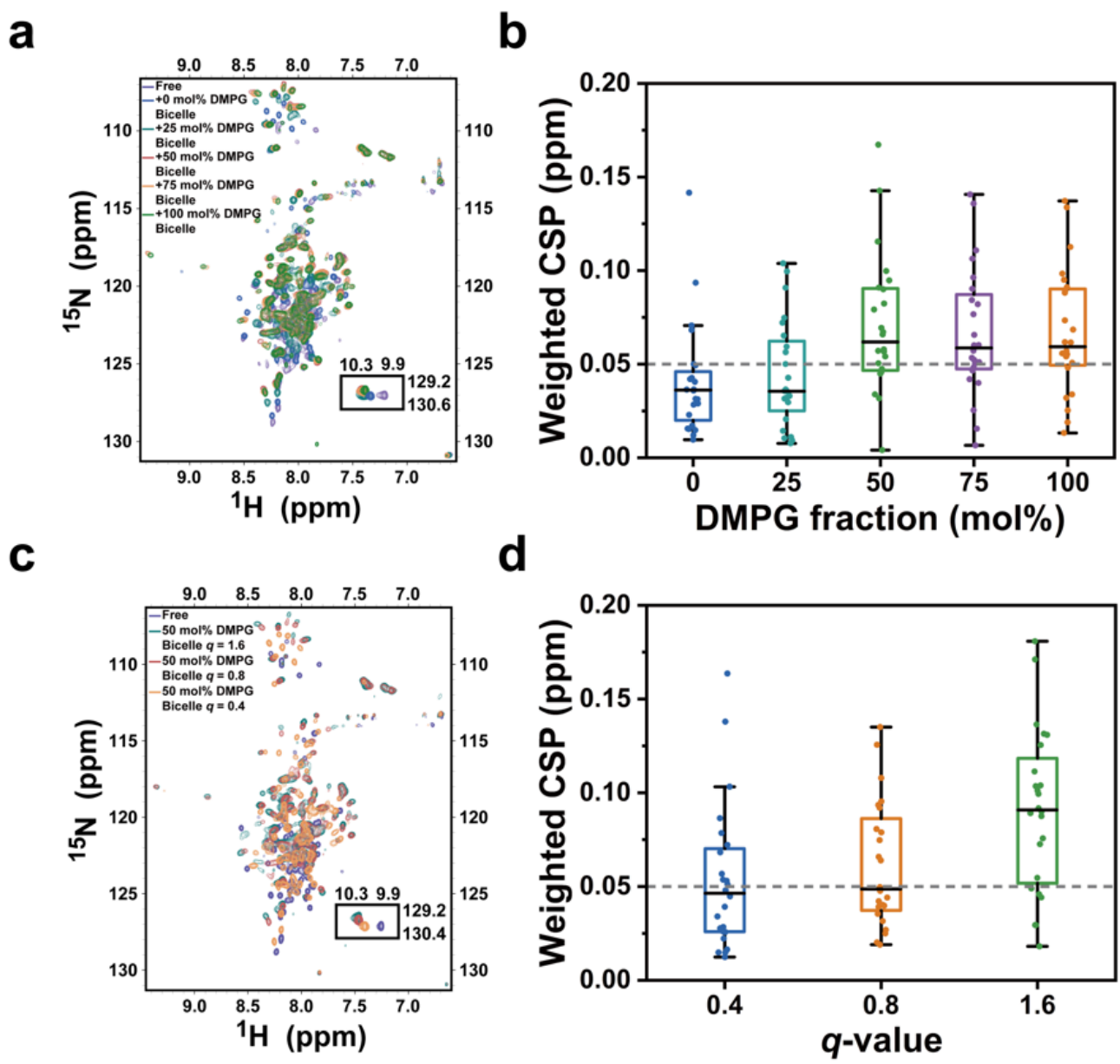


Figure 2

Dependence of the PLIN1-AH NMR response on DMPG mole fraction and bicelle q . **a** Overlay of two-dimensional ^1H - ^{15}N HSQC spectra of uniformly ^{15}N -labeled PLIN1-AH in the free state and in DMPC/DMPG/DHPC bicelle assemblies containing 0, 25, 50, 75, or 100 mol% DMPG within the long-

chain phospholipid component at a fixed $q = 0.8$. Here, q is defined as $([DMPC]+[DMPG])/[DHPC]$. **b** Distributions of weighted CSPs calculated for the same 24 cross-peaks that could be reliably tracked across the DMPG-composition series shown in **a**, using free PLIN1-AH as the reference state. **c** Overlay of two-dimensional $^1\text{H}-^{15}\text{N}$ HSQC spectra of free PLIN1-AH and PLIN1-AH in DMPC/DMPG/DHPC bicelle assemblies containing 50 mol% DMPG within the long-chain phospholipid component at $q = 0.4, 0.8$, or 1.6 . **d** Distributions of weighted CSPs calculated for the same 24 tracked cross-peaks across the q series shown in **c**, using free PLIN1-AH as the reference state. In **b** and **d**, the center line denotes the median, the box represents the interquartile range, and the whiskers extend to 1.5 times the interquartile range. Each point represents an individual tracked cross-peak. Weighted CSPs were calculated as described in the supplementary experimental details. The horizontal dashed line at 0.05 ppm is included solely as a visual guide and is not treated as a significance threshold.

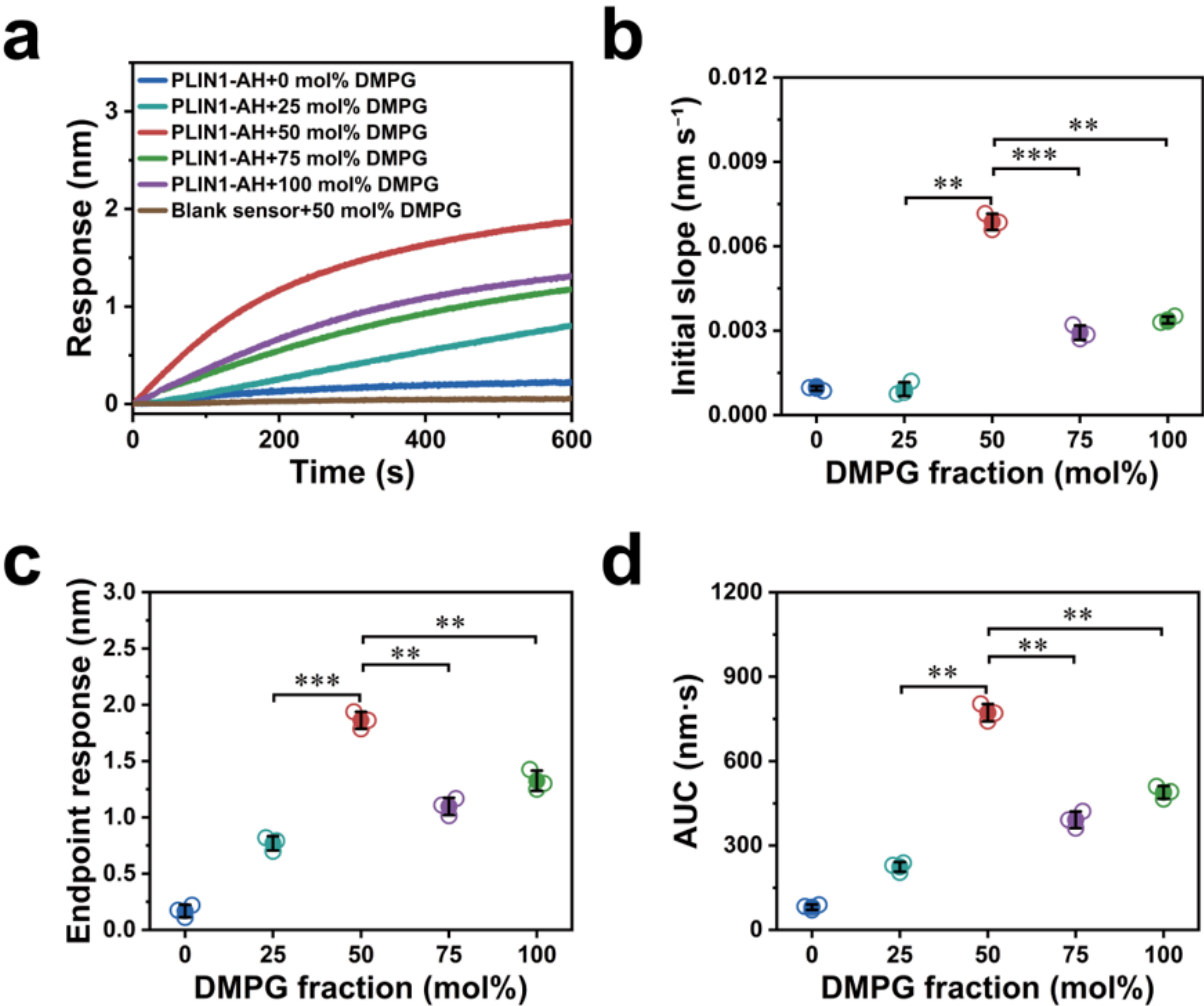


Figure 3

DMPG content modulates the recruitment of DMPC/DMPG LUVs to immobilized PLIN1-AH. **a** Representative BLI association traces obtained by exposing Ni-NTA biosensors bearing immobilized His-tagged PLIN1-AH to LUVs containing 0, 25, 50, 75, or 100 mol% DMPG relative to total phospholipid. All LUV samples were tested at the same total phospholipid concentration. A blank Ni-NTA biosensor

lacking immobilized PLIN1-AH was exposed to LUVs containing 50 mol% DMPG to assess direct adsorption of this vesicle preparation to the sensor surface. Responses were monitored over a 600-s association phase, and the traces shown are representative of one of three replicate experiments performed under the same experimental conditions. **b** Initial slopes calculated over 5–120 s as an operational measure of early BLI response accumulation. **c** Endpoint responses calculated as the mean signal over 580–600 s. **d** AUC integrated from 0 to 600 s. Data in **b–d** are presented as the mean $\pm$ SD of three independent experiments ($n = 3$). For clarity, only the 25 versus 50, 50 versus 75, and 50 versus 100 mol% DMPG comparisons are shown. ****** $P_{\text{adj}} < 0.01$ and ******* $P_{\text{adj}} < 0.001$.

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