

Molecular Flexibility and Electrostatic Interactions Drive the Nanoscale Structure of Coiled-Coil GM130 Protein Condensates

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Golgins are coiled-coil proteins that decorate Golgi *cisternae* with highly specific spatial distribution acting as vesicle tethers through their extended, semiflexible architectures. Among them, GM130 is also known to undergo phase separation, yet the molecular basis of this behavior remains poorly understood. Here, we use atomic force microscopy (AFM) to directly visualize individual GM130 molecules adsorbed onto attractive surfaces. We find that GM130 is highly flexible and undergoes conformational transitions from extended chains to collapsed coils—globular structures that serve as building blocks for the submicrometric two-dimensional and dynamic domains when anchored to lipid bilayers. Molecular-dynamics simulations predict a supramolecular scaffold for GM130 domains composed of coiled-coil intermolecular nodes surrounded by a dispersed phase of intrinsically disordered regions with electrostatic interactions acting as chief drivers of phase-separation. These assemblies are experimentally validated under high-force AFM measurements. Our findings establish the structural basis linking GM130 conformational polymorphism to its phase separation capacity.

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I. INTRODUCTION

Golgins are key regulatory proteins present at the cytoplasmic face membrane of the Golgi apparatus. Predominantly formed by coiled-coil domains, these proteins function as long tethers that capture transport vesicles, bringing them close to the Golgi, facilitating SNARE-mediated membrane fusion and ensuring specificity in vesicular trafficking [1,2]. Beyond this canonical role, golgins have also been proposed to self-assemble into two-dimensional, membrane-bound condensates that help organize the stacked architecture of the Golgi apparatus—a concept known as the “liquid crystal” hypothesis [3]. This model suggests that golgin phase behavior drives spatial compartmentalization and may be regulated during the cell cycle to coordinate Golgi disassembly and reassembly [4,5].

Indeed, several golgins, including GM130, have been observed to undergo phase separation into liquidlike droplets when overexpressed in cells or at high concentrations when purified *in vitro* [6,7]. These supramolecular assemblies display hallmark features of biomolecular condensates, such as fusion, deformability, and dynamic turnover. More recently, GM130 was shown to form RNA-dependent condensates that contribute to Golgi ribbon integrity, with its disordered N-terminal domain playing a key role in RNA-induced phase separation and membrane tethering [8].

The liquidlike behavior of GM130 and related golgins closely resembles that of RNA granules (e.g., P granules, nucleoli, and stress granules) [9–12] and other two-dimensional (2D) condensates, such as those formed by the membrane-bound LAT protein involved in T-cell signaling [13]. However, unlike many canonical condensate-forming proteins [14,15], golgins are predominantly structured with only minor intrinsically disordered regions (IDRs). This raises the question of how coiled-coil-rich architectures can drive dynamic condensation. Recent work on other coiled-coil proteins such as TDP-43 and nucleophosmin has shown that helix-helix interactions can also promote phase separation, suggesting that ordered structures may contribute to condensate formation via alternative mechanisms [16,17].

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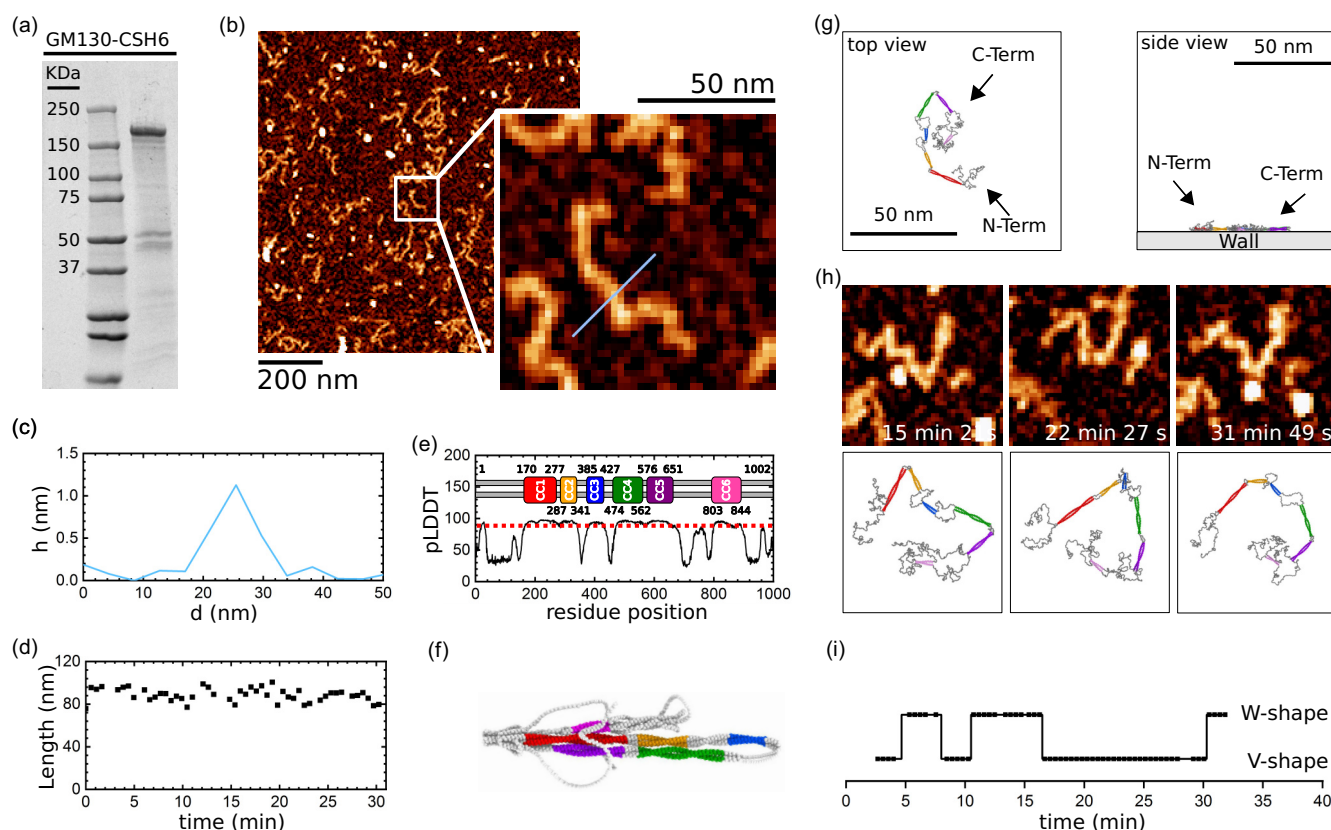


FIG. 1. Single molecule imaging of GM130 dimers adsorbed to mica. (a) SDS-PAGE showing the purified full-length GM130 protein. (b) AFM image of GM130 molecules adsorbed onto freshly cleaved mica in imaging buffer (20 mM Tris, 50 mM KCl, 10 mM MgCl_2 , pH 7.5); the inset shows a magnified region highlighting individual filaments. The blue line in (b) indicates the trace used for the height profile shown in (c). (c) Representative height profile indicating a filament thickness of ~ 1 nm. (d) Time evolution of the contour length of a single GM130 filament ranging between 80 and 100 nm. (e) AlphaFold-derived structural confidence (pLDDT) along the GM130 sequence, revealing predicted rigid segments. The dashed line indicates the 90th percentile of the pLDDT confidence score, serving as a threshold to highlight the most structurally reliable regions. (f) Molecular model of the GM130 dimer predicted by AlphaFold, highlighting rigid domains in color and intrinsically disordered regions (IDRs) in white. (g) Coarse-grained molecular-dynamics simulations of a single GM130 molecule adsorbed onto a surface showing top and side views indicating the N- and C-terms (interaction energy with the wall $\varepsilon = 1.00$ kcal/mol). Top view simulation box: $100 \times 100 \text{ nm}^2$; side view: $100 \times 40 \text{ nm}^2$. (h) Comparison between AFM images (in 20 mM Tris, 50 mM KCl, 10 mM MgCl_2 , pH 7.5) and modeling predictions displaying W- and V- shaped conformations. (i) Time-course analysis of filament morphologies from AFM imaging. Individual filaments were classified as extended, V-shaped, or W-shaped in each frame of the time lapse. The plot shows the number of frames in which each conformation was observed over time, capturing transitions between filament shapes. Related image sequences to (b) can be found in videos S1 and S2; and to (g) and (h) in videos S3 and S4.

To elucidate the molecular basis of GM130 condensation, we integrated atomic force microscopy (AFM) [18] with high-resolution coarse-grained molecular-dynamics (MD) simulations [19]. This multiscale approach allowed us to probe GM130 organization from subresidue interactions to the mesoscale architecture of membrane-bound condensates, providing insight into how structured proteins can support liquidlike, dynamic self-assembly.

II. RESULTS

A. Conformational flexibility of single GM130

Biochemical analysis confirmed successful purification of full-length GM130, yielding a single, well-defined band. The observed band (~ 180 kDa) is in good agreement with the predicted molecular weight of ~ 165 kDa for the full construct, including the contributions of multiple affinity and fluores-

cent tags (mCherry, SNAP, FLAG, and His-tags) [Fig. 1(a)]. Individual GM130 proteins, likely existing as homodimers [20], were adsorbed on mica using standard methods based on Mg^{2+} and K^+ as divalent and monovalent stabilizing cations, respectively [21]. This preparation protocol allowed the observation of two types of conformations, i.e., extended proteins and molten globules [Fig. 1(b); see also Video S1 of the Supplemental Material [22]]. These conformations reflect distinct surface adsorption states. Globular structures correspond to partially adsorbed chains, where only a limited number of segments interact with the mica surface, and the net adsorption energy remains close to the adsorption threshold. In this regime, pronounced conformational fluctuations are observed, including transient spreading and partial desorption events (Fig. S1). In contrast, extended filamentous conformations arise under strong adsorption conditions, where the surface interaction energy per contact exceeds a critical value,

compensating for the entropic penalty of chain confinement and stabilizing a flattened, surface-bound state. Once fully adsorbed, filaments rarely revert to globules on the experimental timescale, consistent with a kinetically stabilized adsorption regime. Quantitative analysis of time-lapse AFM data further shows that both conformations coexist in steady proportions, with the area fraction occupied by globules being approximately 0.4 relative to filaments, and no measurable change in this ratio over time (Fig. S2). The two types of structures were analyzed separately.

An initial topographical analysis revealed that individual GM130 molecules appeared as flexible, filamentous structures distributed across the mica surface [Fig. 1(b), zoom]. Filaments have a typical height of 1 nm [Fig. 1(c)], as expected for the ~ 1 nm crystallographic width of the constitutive α -helices forming the coiled-coils. Due to the well-known tip-sample dilation effect in AFM imaging [23], the apparent filament widths (12–13 nm) exceed the true molecular diameter. Using the measured apparent filament width ($W \approx 12$ –13 nm) and correcting for tip convolution ($R = 10$ nm) yields an intrinsic filament diameter of $d \approx W/(8R) \approx 1.8$ –2 nm, consistent with the height measured by AFM [Fig. 1(c)].

Time-lapse AFM imaging revealed dynamic fluctuations in filament contour length (89 ± 6 nm), reflecting conformational flexibility [Fig. 1(d) and Video S2]. Contour lengths were obtained by tracing each filament along its backbone from end to end and measuring the total path length. GM130 filaments frequently displayed characteristic kinks [Fig. 1(b), zoom], consistent with coiled-coil domains connected by flexible and intrinsically disordered linker regions. This morphology suggests at least five rigid segments, aligning with secondary structure predictions [7,20,24]. This conclusion is supported by quantitative analysis of time-lapse AFM imaging, in which manual backbone tracing of a single filament over 51 consecutive frames revealed an average of 5 ± 1 straight segments per frame, with a maximum of six segments observed (Fig. S3).

AlphaFold-based models of the GM130 monomer further supported this segmented architecture, identifying six discrete rigid regions separated by lower-confidence linkers, as indicated by the pLDDT profile along the sequence [Fig. 1(e)]. Consistent with established pLDDT scores [25], regions scoring < 90 were interpreted as flexible rather than disordered.

To further explore GM130's conformational landscape, coarse-grained simulations at residue resolution-level were performed using the Mpipi-Recharged force field [19], using the dimeric structure predicted by AlphaFold [Fig. 1(f)]. In this model, the intrinsically disordered regions are considered as fully flexible polymers, in which subsequent amino acids are connected by harmonic bonds, while the coiled-coil domains are treated as rigid bodies, preserving the pLDDT confidence scores along the sequence. Simulations yield representative top and side views of GM130 adsorbed on a surface [Fig. 1(g), Video S3]. Notably, the predicted conformational ensemble exhibited strong morphological correspondence with the experimentally imaged filaments, including W- and V-like conformations [Fig. 1(h), Videos S3 and S4]. A quantitative classification of simulated conformations shows an ensemble composed of 16%

V-shaped and 13% W-shaped structures. AFM time-lapse imaging of a single filament captured transitions between V-shaped and W-shaped conformations over time, with a relative ratio of 1.8 between them [Fig. 1(i)]. Although this dataset represents only a single filament and is too limited to derive statistically robust percentages, the relative distribution of shapes is qualitatively consistent with the simulation trends, reinforcing the connection between the experimental and computational ensembles.

Together, these findings support a model in which GM130 behaves as a structurally adaptable molecule capable of sampling a rich configurational ensemble from which dynamic intermolecular interactions may be established.

B. GM130 forms flexible, high-order assemblies through tip-to-tip and lateral coiled-coil interactions

The dynamic properties of GM130 filaments on mica (Fig. 2) indicated the ability of proteins to engage in both tip-to-tip and lateral interactions. Tip-to-tip interactions led to long filaments exceeding the length of a single GM130 dimer, forming higher-order linear assemblies [Fig. 2(a), Video S5]. Head-to-head or tail-to-tail arrangements of GM130 dimers produce only short, dimer-of-dimers assemblies, whereas head-to-tail stacking or coexisting head-to-head and tail-to-tail interactions would allow the addition of multiple dimers, forming the extended filaments observed by AFM. In the Golgi, the C-terminal orientation toward the membrane likely prevents C–C interactions, so these *in vitro* stacking modes may not directly reflect *in vivo* filament assembly.

These structures showed localized discontinuities along their contour, indicative of dynamic organization [Fig. 2(b)]. These discontinuities correspond to the junctions between adjacent filaments within the linear molecular assembly, which fluctuate over time. As an example, contour length analysis of five individual joined filaments yielded an average length of 95 ± 9 nm, consistent with the expected size of a single GM130 dimer and supporting linear oligomerization through tip-to-tip interactions [Fig. 2(b)]. The resulting contour-length distribution reflects both the intrinsic conformational flexibility of the filaments observed in time-lapse imaging [Fig. 1(c)] and minor variability in filament tracing, with the time-averaged length of each filament closely matching the population average.

To strengthen this analysis, additional AFM time-lapse recordings were analyzed. Longer filaments were observed with lengths corresponding approximately to twofold and threefold multiples of this unit. The resulting stepwise increase in contour length supports a model in which GM130 dimers are arranged end-to-end, forming linear oligomers. Consistently, the number of rigid segments increases in discrete steps with filament length, indicating modular addition of GM130 units: Single-, double-, and triple-length filaments, along with their contour-length and segment quantization (Videos S6 and S7, Fig. S4). The observation of comparable filament formation and conformational dynamics in nontagged wild-type GM130 demonstrates that linear assembly is an intrinsic property of GM130 and is not driven by C-terminal tags (Fig. S5).

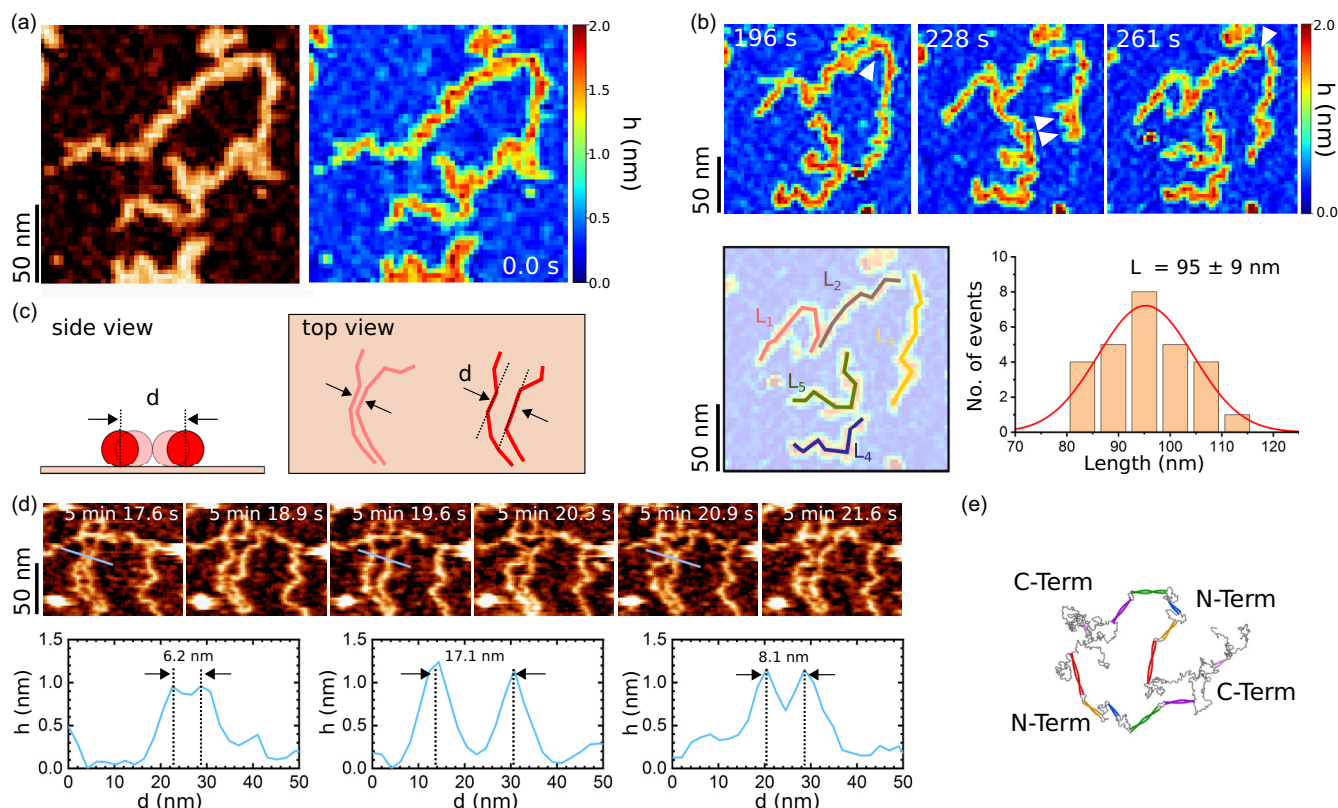


FIG. 2. Intermolecular interactions of GM130 molecules adsorbed to mica. (a) AFM image showing a GM130 filament longer than a single protein, suggesting the formation of multiprotein filaments through tip-to-tip interactions. Image acquired in QITM imaging-mode in imaging buffer (20 mM Tris, 50 mM KCl, 10 mM MgCl₂, pH 7.5). This panel corresponds to a zoomed-in region of the AFM image shown in Fig. 1(b). (b) Contour length fluctuations of a GM130 filament, revealing modularity consistent with five subunits. Histogram across 27 well-resolved filaments, with an average of 95 ± 9 nm. The histogram includes the time-dependent distribution of resolvable filaments per frame. The analysis was restricted to a high-magnification region of the AFM image where lateral resolution and signal-to-noise were sufficient to reliably resolve individual filament contours and their temporal evolution. (c) Schematic representation of lateral coiled-coil interactions (side and top views). (d) AFM images showing adjacent GM130 filaments exhibiting transient lateral alignment and spacing fluctuations. Blue lines indicate the trace used for the height profiles. Imaging buffer is 20 mM Tris, 50 mM KCl, 10 mM MgCl₂, pH 7.5. Topographical height profiles show dynamic, reversible interactions between coiled-coil domains in the nanometer range. (e) Top view of a GM130 dimer simulation adsorbed on a surface showing tip-to-tip contacts among C-terminal and N-terminal domains, as well as lateral contacts between coiled-coils. Simulation box dimensions: top view 100×100 nm; side view 100×40 nm. Protein-surface interaction energy $\varepsilon = 0.35$ kcal/mol. Image sequences related to (a) and (b) can be found in Video S5, and related to (d) in Videos S8 and S9.

Lateral interactions [Fig. 2(c)] were also observed between adjacent GM130 filaments, often characterized by transient alignment and spacing fluctuations between rigid coiled-coil domains, as captured by AFM images. These lateral contacts were generally reversible and exhibited dynamic behavior consistent with weak interactions with an energy scale on the order of $k_B T$ (Fig. S6).

Height profiles further confirmed that the spacing between filaments varied dynamically in the nanometer range [Fig. 2(d), subsecond high-speed AFM Videos S8 and S9], supporting a model in which GM130 can be laterally associated through transient interactions, potentially mediated by exposed coiled-coil regions. In parallel, coarse-grained simulations also evidenced that GM130 multimers are facilitated through tip-to-tip interactions with a strong contribution of contacts between the C-terminal and N-terminal domains [Fig. 2(e), Video S10].

C. Globular states define the molten conformation of GM130

The coexistence of filaments and globules was clearly observed across large fields of view [Fig. 3(a) and Video S11], with zoomed-in views revealing heterogeneity in globule height and flexibility [Fig. 3(b) and Videos S12–S13]. Like filaments, protein globules presented also a very dynamic behavior in terms of shape fluctuations, where linear parts of the protein protruded stochastically in and out the globular unit (Fig. S2 and Video S14).

Thermal energy sustained globule fluctuations for extended periods, lasting tens of minutes, along the experimental observation. Moreover, we visualized dynamic processes such as the merging and separation of GM130 globular structures [Fig. 3(c)]. In some cases, two adjacent globules were observed to undergo fusion, forming a single and larger globular structure. Conversely, other observations captured the splitting of a globule into two smaller ones, indicating a reversible

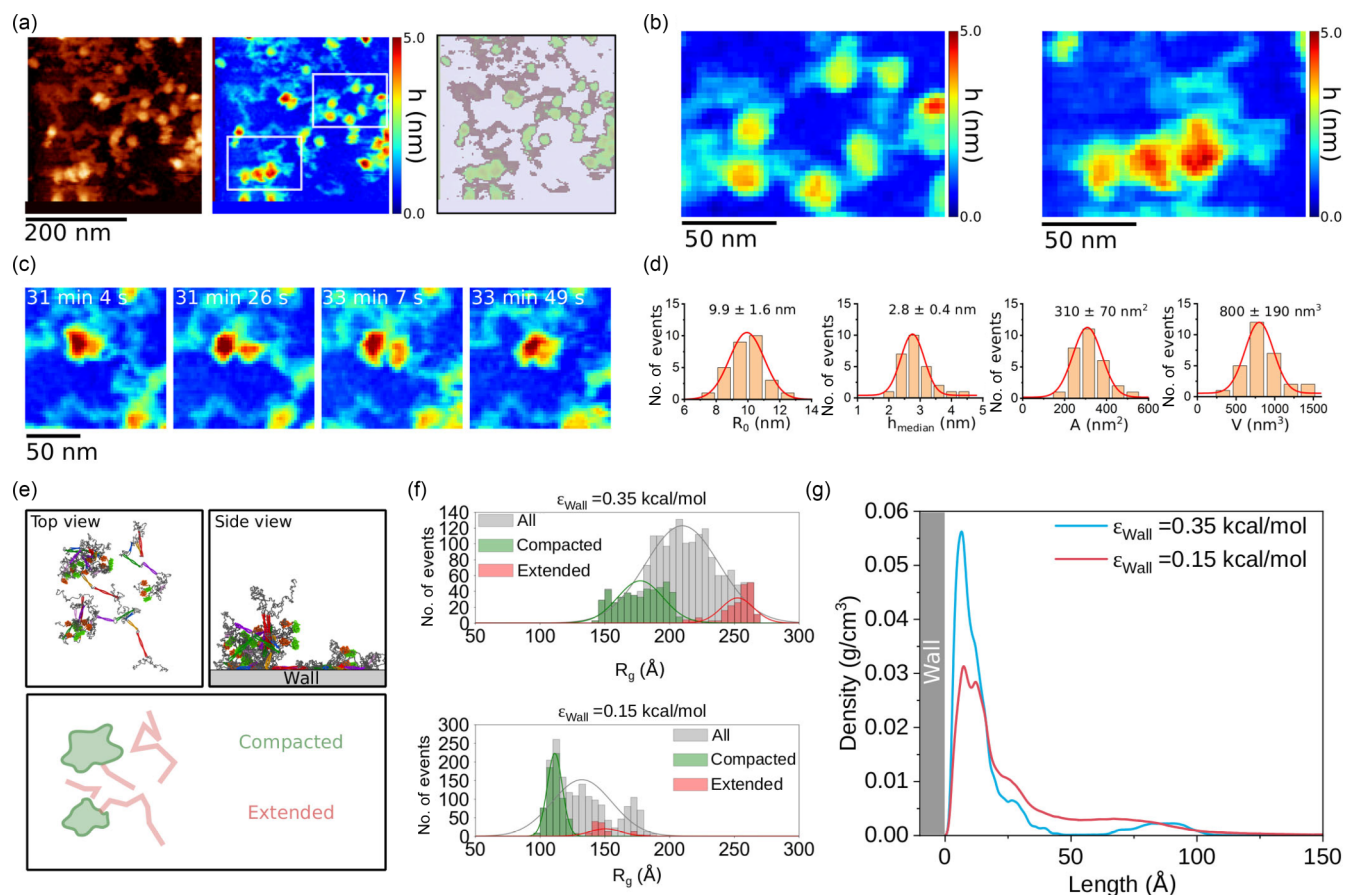


FIG. 3. Globule configuration of GM130 molecules. (a) Representative AFM image showing a large field of view with coexisting GM130 filaments and globular structures, acquired in imaging buffer (20 mM Tris, 50 mM KCl, 10 mM MgCl₂, pH 7.5). Pseudocolor height map and binary mask highlight the position and distribution of globules. (b) Zoom-in of regions in (a) reveal heterogeneity in globule height and flexibility (see Videos S12 and S13). (c) Time-lapse AFM sequence capturing fusion and fission events among GM130 globules, indicative of liquidlike behavior. (d) Quantification of structural parameters of individual globules, including radius (R_0), height (h), projected area (A), and volume (V). R_0 denotes the mean radius of the droplet, obtained from Fourier-mode decomposition of shape fluctuations (see Methods). (e) Representative configurations from coarse-grained MD simulations of two dimers at high surface interaction strength (0.15 kcal/mol, Video S16) from top and side view showing the entire simulation box ($60 \times 60 \times 100$ nm³). Both filamentous and globular forms of GM130 are observed. (f) Radius of gyration histograms of GM130 molecules simulated at decreasing surface interaction strength (0.35 and 0.15 kcal/mol), showing a shift from extended structures to compact, globular conformations. (g) Vertical density profiles of GM130 at different surface interaction strength, highlighting an expanded distribution at 0.15 kcal/mol (red line) consistent with a more extended conformational ensemble. Image sequences related to (a), (b), (c), and (e) can be found in Videos S11, S12, S13, S15, and S16, respectively.

process characteristic of liquidlike behavior [Fig. 3(c), Video S15].

A volume histogram of GM130 globules was generated from the entire AFM field of view using the DBSCAN clustering algorithm (Fig. S2), revealing distinct peaks corresponding to different size populations. The smallest peak is likely to represent individual GM130 dimers. From this population, 29 well-resolved particles with minimal lateral overlap were selected for detailed analysis. Morphological analysis of these GM130 globules revealed unexpectedly flat structures, with heights of $h = 2.8 \pm 0.4$ nm and lateral sizes of $R_0 = 9.9 \pm 1.6$ nm, yielding an internal volume of $V = 800 \pm 190$ nm³ [Fig. 3(d)]. Notably, the volume of the protein chain itself when approximated as a cylinder using the time-averaged contour length $L \approx 97$ nm and thickness $h \approx 2$ nm (Fig. 1) is significantly smaller [$V = \pi(\frac{h}{2})^2 L \approx$

310 ± 30 nm³]. The ratio between the pervaded volume and the chain volume suggests a high internal water content, up to 65%, in line with hydration levels typically reported for biomolecular condensates [26]. This value should be regarded as an upper estimate due to tip convolution effects. The large, pervaded volume of GM130 globules relative to the chain volume, combined with dynamic fusion and fission events observed in time-lapse AFM, suggests that these assemblies are internally reconfigurable and capable of forming interlinked protein chains that contribute to condensate formation.

Representative configurations from Mpipi-Re-charged simulations confirmed the morphological adaptivity of GM130, revealing dynamic transitions between globular and extended states [quantified in Fig. 3(e) and visualized in Video S16] that enable diverse intermolecular contacts. The predicted size of molten globules matched those observed

on mica [Fig. 3(c)]. Coarse-grained simulations showed a broad configurational ensemble, with radius of gyration (R_g) values spanning over 12 nm from ranging compact globules to extended filaments [Fig. 3(f), top]. A reduced interaction strength between the surface and GM130 ($E_{\text{wall}} = 0.15$ kcal/mol) favored globular conformations [Fig. 3(f), bottom], highlighting the effective interaction competition between surface adhesion and compaction. This balance resulted in broader height probability distributions, indicating GM130's intrinsic tendency to form compacted, dynamic structures [Fig. 3(g)].

D. Formation of GM130 protein domains anchored on supported lipid bilayers

The high-water content of GM130 globules, along with their structural flexibility and propensity to interact, suggested that these moltenlike units act as fundamental building blocks of protein condensation [7]. To investigate GM130 self-assembly on supported lipid bilayers (SLBs), benzylguanine (BG) and nitrilotriacetic acid (NTA) lipid linkers were incorporated in the lipid composition. Both lipids have been previously used separately to anchor and orient proteins onto lipid membranes, as well as to control their surface density varying the protein concentration between 0.1 and 1 μM [27]. Inclusion of both lipids in the bilayer allowed protein binding to the SLB at concentrations one order of magnitude lower. When anchored on SLBs at low surface concentrations (~ 10 globules/ μm^2), GM130 formed isolated globules that marked the onset of domain formation (Videos S17–S19 and Fig. S8). As surface coverage increased ($\geq 10^2$ globules/ μm^2), GM130 self-assembled into flat 2D domains with diameters spanning from a few tens to a few hundred nanometers [Fig. 4(a) and Fig. S9].

AFM imaging was performed after extensive rinsing of free protein from the bulk solution; therefore, no additional protein was available to exchange with the membrane after the initial 30 min incubation. The material observed at later time points thus originates from proteins already membrane-anchored via SNAP–BG covalent coupling and His–Ni–NTA coordination. Immediately after rinsing, these molecules exist in a highly dilute, laterally diffusive, gaslike two-dimensional state, where rapid membrane diffusion prevents stable tip engagement and renders them effectively invisible in AFM topography.

Over time, lateral diffusion and intermolecular interactions drive their condensation into denser assemblies with increased local height and compactness, making them detectable by AFM. As captured by time-lapse imaging, GM130 therefore appears sparsely distributed immediately after anchoring and progressively self-assembles into membrane-bound domains [Fig. 4(a) and Fig. S9], reflecting time-dependent reorganization of a preexisting membrane-associated population rather than recruitment from solution.

Nanoindentation experiments after domain formation revealed a two-layer architecture, with GM130 protein domains forming a distinct, mechanically separable layer on top of the underlying lipid bilayer (Fig. S10). Topographical profiles show that these protein assemblies have a consistent height of 5 nm [Fig. 4(b)]. In addition, the height histogram allowed

us to quantify the roughness of both the lipid bilayer and the protein domains, being below 1 nm in both cases [Fig. 4(c)].

E. Dynamic behavior of GM130 2D protein domains anchored to SLBs

The dynamic properties of GM130 self-assembly on lipid bilayers were assessed through the temporal evolution of the 2D flat domains (Video S20). Four primary dynamic traits were observed, i.e., shape adaptability, fusion, rupture, and internal reorganization [Fig. 4(d), Videos S21–24]. Most of the visualized domains displayed boundary contour fluctuations, and some of them were even able to undergo a prominent change in morphology from circular-to-elongated form [Fig. 4(d), top panel], revealing in both cases a high degree of shape compliance. In addition, fusion and rupture events [Fig. 4(d), middle panels], as well as lateral movement of vertical protrusions within the assemblies, were directly observed [Fig. 4(d), bottom panel] indicating internal rearrangement of GM130 proteins.

Mechanically, GM130 assemblies exhibit a low effective line tension ($\lambda = 1$ pN), as determined from contour fluctuation analysis (see Fig. S11 for methodological details). This analysis quantifies the amplitude of boundary shape fluctuations and provides a direct measure of interfacial mechanical properties. Over time, the dynamic morphological flexibility of the two-dimensional flat domains decreased, particularly after 60 min of incubation. This stabilization is visually apparent in the persistence of domain shape and is quantitatively reflected by a reduction in contour fluctuation amplitudes (Fig. S12), indicating a progressive mechanical stiffening and maturation of the protein assemblies. A control construct lacking GM130 formed only globular, nondynamic blobs on the bilayer (Fig. S13 and Video S25).

F. Supramolecular scaffold as revealed by AFM

To reveal the supramolecular structure within the protein condensate [Fig. 4(e)], we varied the AFM setpoint force from 0.25 to 1.5 nN, allowing us to probe the internal organization of GM130 protein domains [Fig. 4(f)]. Under higher forces, the softer regions of the condensate exhibited a decrease in height, while the upper surface revealed resilient nodes. Based on their mechanical stability and spatial arrangement, we hypothesize that these nodes correspond to coiled-coil structures within the condensate [Figs. 4(f) and 4(g)]. To further elucidate such an intermolecular network, we performed simulations of GM130 with SNAP tags anchored on a surface that models the lipid bilayer. Interestingly, GM130 self-assembles laterally to form small domains of similar size to those experimentally found [Fig. 4(h)], which are scaffolded by coiled-coil interactions and transient contacts among the different IDRs of the sequence [Fig. 4(h), Video S26].

G. Condensation of GM130 is driven by electrostatic interactions

To elucidate the molecular interactions driving GM130 condensation, we built contact frequency probability maps across domains in our simulations [28]. At the

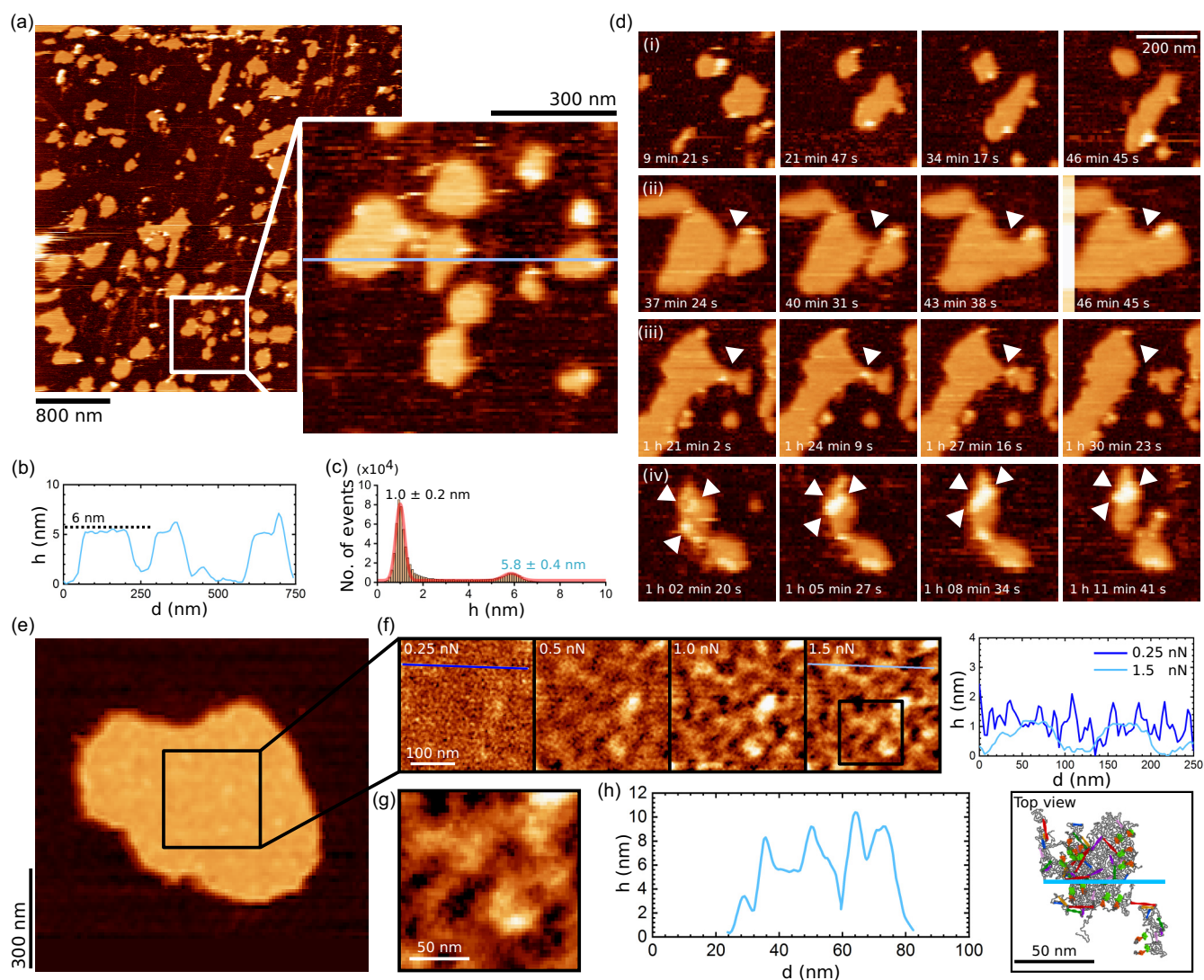


FIG. 4. GM130 protein domains in supported lipid bilayers (SLB). (a) AFM image of GM130 domains formed on SLBs. Bilayers were prepared from POPC:DGS-NTA(Ni):DOPE-PEG2000-BG (94:5:1 mol.%) liposomes formed by extrusion and incubated on freshly cleaved mica. GM130 (20–50 nM) was incubated for 30–60 min in Hepes buffer (20 mM Hepes, 150 mM KCl, 1 mM MgCl_2 , pH 7.3), followed by extensive rinsing prior to imaging with the same buffer. (b) Blue line depicts topography profile shown in (a). (c) Height histogram shows an average value of domain height of 4.8 nm. (d) Basic traits of liquidlike dynamic behavior of GM130 protein domains formed on SLBs. AFM images show morphological adaptability including changes in their conformation from circular to elongated shapes (top panel), as well as the ability of GM130 domains to fuse and rupture (middle panels), and the internal rearrangement of proteins within a domain as revealed by lateral movement of protruding spots marked with arrows (bottom panel). (e) AFM topography of a GM130 condensate reveals a heterogeneous supramolecular structure. (f) Increasing the AFM setpoint force from 0.25 to 1.5 nN revealed internal structural features of GM130 condensates. Softer regions were compressed, whereas resilient surface nodes persisted, suggesting stiffer substructures. These nodes likely correspond to coiled-coil domains within the condensate. The force-dependent AFM indentation depth reflects internal roughness within the domain. (g) Zoomed-in region marked in (f) highlighting resilient nodes. (h) Snapshot of a GM130 domain composed of seven dimers from coarse-grained molecular-dynamics simulation (Video S26). Top and side views are shown (simulation box dimensions: top view 60×60 nm; side view 50×60 nm). Differential surface interactions were applied to mimic bilayer binding, with $\epsilon_{\text{bilayer}} = 7.0$ kcal/mol for the SNAP-tag and $\epsilon_{\text{bilayer}} = 1.5$ kcal/mol for the remainder of the protein. Blue line on the top view snapshot indicates the trace used for the height profile. Height profile consistent with AFM observations, where a compact supramolecular assembly is stabilized by coiled-coil interactions and transient contacts between intrinsically disordered regions (IDRs). Image sequences related to (a) and (d) can be found in Videos S20 and S21–S24, respectively, and Video S26 for (h).

intramolecular level, two major interaction modes emerged [Fig. 5(a)]: (i) antiparallel coiled-coil contacts spanning CC1–CC5, and (ii) disorder-like tip-to-tip interactions between N- and C-terminal domains, including the IDR linker between CC5 and CC6 [Fig. 1(e)]. At the intermolecular level,

key condensate-stabilizing contacts included CC1–CC1, CC1–CC4, CC1–CC5, and CC4–CC5 along with the interactions of the N-terminal domain with the IDR linker connecting CC5 and CC6. Also, additional contributions from CC2–CC4 and CC4–CC5 displayed significantly high contact

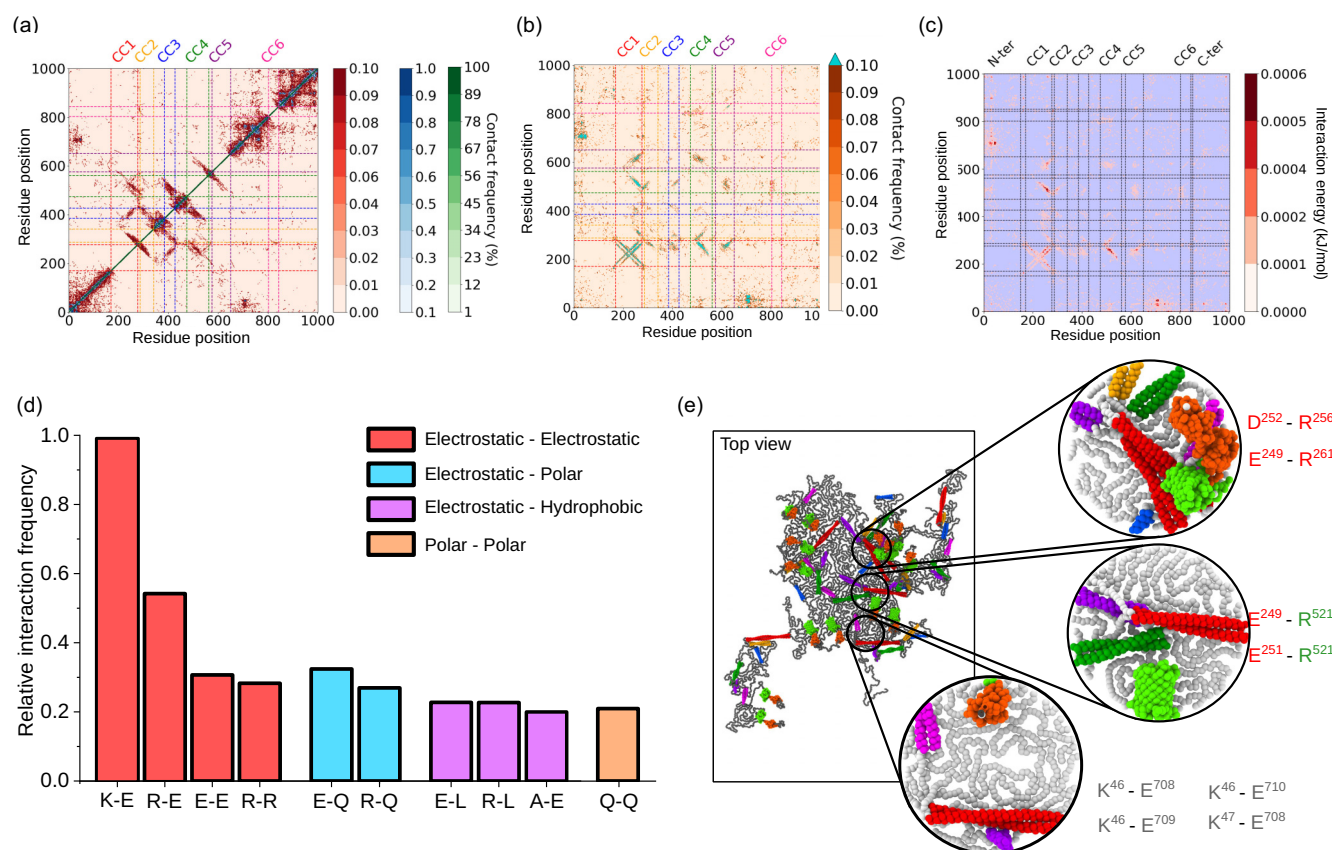


FIG. 5. Key molecular contacts driving phase-separation in GM130. (a) Contact frequency probability of pairwise residue intramolecular contacts of a GM130 dimer attached to a surface through SNAP and mCherry tags mimicking a lipid bilayer at 293 K and physiological salt conditions (150 mM NaCl). The different GM130 coiled-coil domains are specified through colored dashed lines and indicated at the top of the panel. (b) Contact frequency probability of pairwise residue intermolecular interactions of GM130 forming a small condensate as shown in panel (c). (c) Associated intermolecular potential energy to the pairwise residue contacts depicted in panel (b). (d) Most frequent intermolecular contacts found in GM130 small domain renormalized by the total number of the most abundant interactions, K–E. (e) Top-view snapshot from coarse-grained molecular dynamics simulations of a GM130 domain composed of seven dimers (form Video S26) attached to a surface through SNAP tags. The simulation box dimensions are 60×60 nm (top view) and 50×60 nm (side view). Differential bilayer interaction strengths were applied ($\epsilon_{\text{bilayer}} = 7.0$ kcal/mol for the SNAP-tag and $\epsilon_{\text{bilayer}} = 1.5$ kcal/mol for the remainder of the protein). The specific pairwise residue-residue interactions highlighted among the different protein domains represent the highest contact probability interactions predicted by our simulations.

probability [Fig. 5(b)]. To quantify the structural scaffold, contact analysis showed coiled-coil (CC) domains form preferential interaction hubs: whereas CC–CC contacts comprise 33% of all pairwise interactions, they represent 75.7% of the normalized total, confirming their role as nodes. In contrast, interactions among intrinsically disordered regions (IDRs) constitute a less specific, dispersed phase (45.6% of absolute contacts, 15.2% normalized). Interaction energy analysis (see Sec. IIH for details) revealed that CC1–CC1 and CC1–CC4 coiled-coil contacts as well as disorder-like interactions among the 35th–50th residues of the N-terminal with the 675th–710th residues of the CC5–CC6 IDR linker provided the largest interaction increase. Tip-to-tip contacts also supported network connectivity [Fig. 5(c)].

We next examined the dominant residue-level interactions [Fig. 5(d)]. Electrostatic lysine-glutamic acid (K–E) and arginine-glutamic acid (R–E) contacts made the greatest contribution to stability, whereas polar glutamine (Q) and hydrophobic leucine (L) residues also mediated connectivity with charged residues like arginine and glutamic acid. Despite

partial self-repulsion, E–E and R–R contacts were supported by nearby attractive K–E and R–E contacts, creating a dynamic, liquidlike environment. Although arginine, tyrosine, and phenylalanine mediated the strongest individual contacts [Fig. 5(d)], the most frequent and specific contacts were electrostatic, featuring K–E and R–E interactions among the CC1–CC1 (red), CC1–CC4 (red and green), and intrinsically disordered domains (gray) [Fig. 5(e)].

H. GM130 domains disassemble under conditions of high ionic strength that effectively screen electrostatic interactions

To gain deeper insight into the predicted electrostatic interactions from our coarse-grained simulations, we conducted experiments to assess the effect of ionic strength on protein condensation. In our system, condensation is primarily driven by attractive electrostatic interactions between charged residues. Therefore, increasing the ionic strength of the buffer should screen these interactions via dissolved ions, leading to a significant dissolution of the protein condensates. As shown

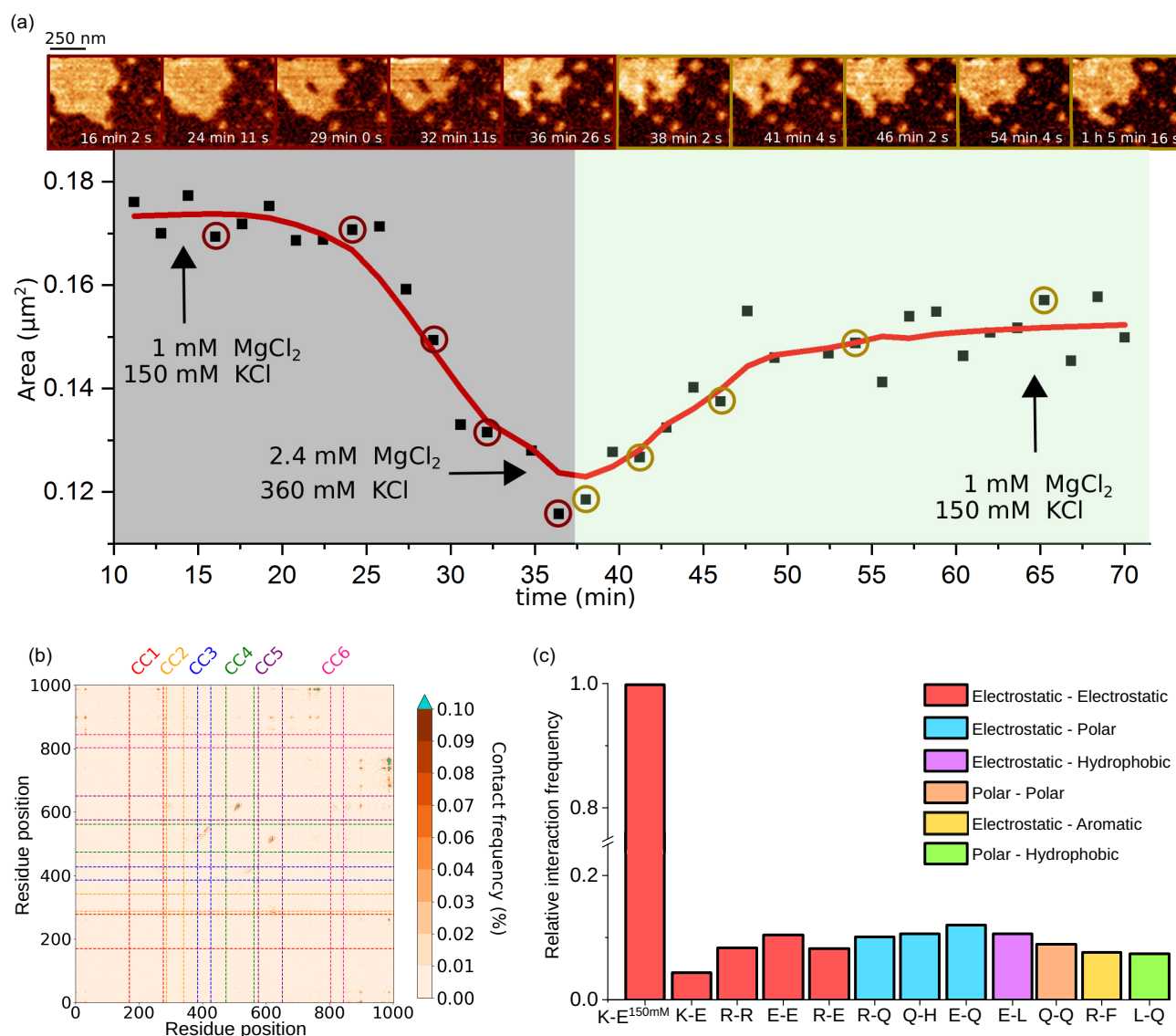


FIG. 6. Ionic strength dynamically regulates GM130 condensate size via reversible electrostatic interactions. (a) AFM time-lapse imaging demonstrates that increasing buffer ionic strength induces a progressive reduction in condensate surface area, accompanied by the formation of small globules that can detach from the larger domain, consistent with electrostatic screening of charged residues. Upon subsequent dilution with distilled water, the condensates gradually recover their original size, highlighting the reversibility of the process. Notably, at elevated ionic strength, GM130 condensates reversibly dissolve into a dilute surface-bound phase, and upon salt reduction, new condensates nucleate at distinct locations, indicating redistribution rather than membrane detachment. The bottom plot shows condensate surface area as a function of time (frame number), illustrating this salt-dependent collapse and recovery. The line is a guide to the eye. AFM imaging conditions correspond to SLBs: Hepes 20 mM, KCl 150 mM, MgCl_2 1 mM, pH 7.3. (b) Contact frequency probability of pairwise residue intermolecular contacts of GM130 proteins attached to a surface through SNAP tags mimicking a lipid bilayer at 293 K and 1 M NaCl. The different GM130 coiled-coil domains are specified through colored dashed lines and indicated at the top of the panel. (c) Most frequent intermolecular contacts found in GM130 at 1 M NaCl renormalized by the total number of K-E interactions at physiological salt conditions reported in Fig. 5(d). Image sequence related to (a) can be found in Video S27.

by AFM, increasing ionic strength results in a marked decrease in the surface area of the protein domains [Fig. 6(a) and Video S27]. Notably, this effect is reversible: upon dilution of the buffer with distilled water, the surface area of the condensates is restored, indicating the reestablishment of electrostatic interactions. This reversibility highlights the dynamic nature of the GM130 condensation process and its sensitivity to ionic conditions, in agreement with our simulations [Figs. 5(d) and 5(e)].

To further confirm this behavior, we repeated GM130 condensate simulations at 1 M NaCl. Phase separation was substantially inhibited, with $\sim 45\%$ fewer intermolecular contacts compared to simulations at 150 mM NaCl [Fig. 6(b)]. Only CC4-CC5 interactions and disorder-like contacts involving the C-terminal and CC5-CC6 linker remained. In particular, the majority of interactions with frequency $> 0.1\%$ vanished at 1 M. Analysis of key intermolecular residue contacts [Fig. 6(c)] revealed a shift from dominant electrostatic

interactions (K–E, R–E) at 150 mM to interactions involving polar residues—such as glutamine (Q)—with abundant sequence partners (e.g., arginine, glutamic acid, histidine, and leucine), cation– π (R–F), and R–R contacts at 1 M, consistent with previous findings [29], and confirming the overall reduction of $\sim 45\%$ of the contacts. Electrostatic screening reduced most charged residue contacts (K–E, R–E, and E–R). This overall decrease in electrostatic contacts confirms that high salt weakens the intermolecular interactions that stabilize GM130 association.

III. DISCUSSION AND CONCLUSIONS

Biomolecular phase separation arises from multivalent intermolecular interactions and depends on sequence composition, interaction chemistry, and molecular architecture. Electrostatic, hydrophobic, π -mediated, and domain-domain interactions can all contribute to condensate formation when sufficient valency and interaction strength are present [30]. Increasing evidence demonstrates that folded, multidomain proteins are also capable of undergoing liquid-liquid phase separation under appropriate conditions [31,32].

GM130 consists almost entirely of structured coiled-coil domains, raising the question of how such ordered architecture supports dynamic condensation. Our findings reveal that GM130 belongs to a growing class of coiled-coil proteins capable of phase separation, including TDP-43, nucleophosmin 1, and TPX2 [16,17,33]. These proteins differ in sequence and domain organization but share the ability to scaffold liquidlike assemblies through coiled-coil multivalency rather than disordered flexibility.

Golgins like GM130 are based on heptad repeat domains, typically forming dimers or tetramers through coiled-coil motifs [1]. The “HPPHPPP” repeat pattern drives their supercoil structure, and small changes in sequence can drastically affect structure and specificity [34]. Intramolecular interaction energies are still weak, typically a few $k_B T$ per residue [35] suggesting that GM130 intermolecular interactions might also be weak, nonspecific, and multivalent among its heptad domains for two-dimensional fluidlike condensation [36]. Our experimental data show that GM130 adopts a range of conformations, from extended rods to collapsed globules (Figs. 1–3), exhibiting high accessibility of configuration space. These globules resemble molten globules: compact, yet dynamic and partially disordered. Their pervaded volume permits molecular mobility and charge exposure, enabling interglobule interactions via electrostatic and steric contacts (Fig. 3), additionally supported by filament tip-to-tip and lateral associations with interaction energies on the order of $k_B T$. This structural polymorphism underlies the ability of GM130 to form multivalent networks, providing a direct mechanistic basis for its phase separation and condensate assembly.

Molten globules serve as the fundamental building blocks of GM130 condensates, which assemble into two-dimensional, liquidlike domains on supported lipid bilayers with the capability to fuse, divide, and flow while maintaining nanometric thickness and structural compactness (Fig. 4). The ~ 5 nm thickness and low roughness suggest coiled-coils align laterally with conformational flexibility.

AFM images registered at increasing forces revealed internal structural heterogeneity: dense interacting nodes interspersed with flexible regions. Molecular dynamics simulations highlight electrostatic contacts—particularly among CC1–CC1, CC1–CC4, and CC1–CC5—as key drivers of self-assembly (Fig. 5). These interactions, predominantly lysine–glutamic acid and arginine–glutamic acid pairs, are sensitive to ionic strength, making the condensates reversible under salt stress (Fig. 6).

Our findings revise the molecular picture of GM130 condensation and offer a mechanistic framework for its role in Golgi organization *in vivo*. Although we replaced the natural anchor GRASP65 with artificial linkers [37,38], the experimental setup preserved key topological features of the endogenous system—namely, spatial confinement and membrane orientation. Strikingly, the reversible rupture and reassembly of these domains under ionic stress mirrors the fragmentation and reconstitution of the Golgi during cell division, where GM130-containing remnants are redistributed and rapidly reassembled post-mitosis [4]. The lability of the electrostatic interactions—strong enough to maintain organization, yet readily reversible—suggests a built-in flexibility that may underlie the Golgi’s capacity for rapid structural transitions across the cell cycle.

In summary, we uncover a previously unrecognized condensation mechanism in the structured Golgi tether GM130, driven by molten-globule intermediates and sequence-specific electrostatic coiled-coil interactions. These dynamic and reversible membrane-bound assemblies reveal a coiled-coil-based phase separation strategy, extending the molecular grammar of condensation beyond disordered proteins and aligning with the organizational needs of the Golgi apparatus.

IV. METHODS

A. Protein purification and concentration measurement

The GM130-mCherry-SNAP-Myc-FLAG-6xHis construct was generated using the human GM130 gene (Origene, RC209641) followed by stacking the various tags using an In-Fusion HD Cloning Kit (Clontech Laboratories). The entire DNA cassette was subsequently moved into the pCI-neo vector (Promega) digested with EcoRI and NotI. Plasmid DNA was used to transfect 30 mL cultures of Expi293 cells (Thermo Fisher) using an ExpiFectamine293 Transfection Kit (Life Technologies) following the manufacturer’s instructions. Cells were cultured in Expi293 Expression Medium at 37 °C with 8% CO₂ and 125 rpm platform shaking. After 30–40 h (depending on efficiency of transfection), cells were pelleted at 500xg for 5 min, washed with PBS, flash frozen in liquid nitrogen, and stored at –80 °C. For purification, cell pellets were thawed and resuspended in 50 mM Hepes pH 7.3, 175 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM TCEP, 0.5 μ g/mL DNase, and a protease inhibitor cocktail (Roche). All subsequent steps were performed at room temperature. Cells were broken using a dounce homogenizer followed by centrifugation (33 000xg for 30 min) to remove cellular debris. The supernatant was incubated with prewashed anti-FLAG affinity resin (Sigma) for 1 h, followed by washes with the same buffer (excluding EDTA) and a 30 min ATP wash

(1 mM ATP with 1 mM MgCl_2). The resin was then washed with 50 mM Hepes pH 7.3, 175 mM NaCl, 1 mM EDTA, 5% glycerol, and 1 mM TCEP before the protein was eluted in fractions using 125 ng/mL of a 3xFLAG peptide (APEXBio). The GM130 protein was desalted into a buffer containing 5 mM Hepes pH 7.3 using a NAP-5 column (GE Healthcare). Protein concentration was determined using a known mCherry protein standard for comparison. To produce the control protein, a construct containing only mCherry-SNAP-6xHis was generated in a pET28a vector (Novagen). The plasmid was expressed in Rosetta2 cells (Novagen) at 37 °C for 4 h with 1 mM IPTG. Cells were resuspended in 25 mM Hepes pH 7.4, 400 mM KCl, 10% glycerol, 0.2 mM TCEP, and 1 mM PMSF and passed through a cell homogenizer (Avestin) followed by centrifugation (140 000xg for 30 min). The supernatant was incubated with NiNTA resin (Thermo Fisher) for 2 h at 4 °C. Afterwards, the resin was washed and the protein was eluted using the same buffer with the addition of 500 mM Imidazole.

B. Liposomes preparation and SLB formation

Liposomes were prepared by extrusion method as previously described [27]. Briefly, lyophilized lipids were dissolved in chloroform solution, mixed in the desired molar ratio [POPC:DGSNTA(Ni):DOPE-PEG2000-BG, 94:5:1 n/n], and dried under a stream of nitrogen before resuspension for at least 30 min in Tris 10 mM, NaCl 175 mM, pH 7.5 at 2 mg/mL total lipid concentration. The resulting multilamellar vesicle solution was extruded through polycarbonate membrane with 50-nm nominal pore diameter (Avanti Polar Lipids) leading to a stock solution of unilamellar vesicles or liposomes. Stock solutions were stored at 4 °C and used for 10 days maximum. SLBs were formed incubating liposomes onto freshly cleaved mica surface diluted at 0.2 mg/mL for 1 h. Excess of vesicles in the bulk was removed and aqueous solution was exchanged by protein buffer Hepes 20 mM, KCl 150 mM, MgCl_2 1 mM pH 7.3.

C. AFM imaging

To investigate GM130 surface organization at different densities, we conducted AFM imaging under two experimental setups: single-molecule adsorption on mica and membrane-bound assembly on supported lipid bilayers (SLBs). For single-molecule imaging, GM130 was incubated at 7.5 nM in K^+ and Mg^{2+} -containing buffer (Tris 20 mM, KCl 50 mM, MgCl_2 10 mM pH 7.5) for 10 minutes on freshly cleaved mica, allowing us to resolve individual proteins in both globular and extended conformations. For SLB experiments, GM130 was incubated at concentrations ranging from 20 to 50 nM for 30 to 60 minutes in Hepes 20 mM, KCl 150 mM, MgCl_2 1 mM pH 7.3., resulting in low (~ 10 globules per μm^2) and intermediate (~ 100 globules per μm^2) surface densities. In both cases, excess protein was removed by rinsing with the same buffer the protein was incubated, and imaging was performed immediately afterward.

AFM data were acquired using two systems: a Nanotec Electrónica microscope (Madrid, Spain) operating in jump mode [39] and a Bruker JPK NanoWizard Ultraspeed2, operating in QI imaging mode as well as in high-speed imaging in

tapping mode. Nanoindentation force spectroscopy was used to probe the mechanical properties and two-layer organization of GM130 assemblies on membranes. Imaging parameters were optimized for each system and image scan size. Acquisition rates ranged from ~ 0.5 s per frame, in the case of high-speed imaging of small scan-areas, to ~ 3 min per frame for standard jump mode of larger scan areas. A list with the image acquisition rate and scan size corresponding to each video was included in SI.

Routine image processing—such as background flattening and height profile extraction—was performed using WSxM software [40], whereas quantitative analyses of protein domain morphology, mechanical properties (e.g., line tension), and dynamic behavior were carried out using custom matlab scripts.

D. Contour shape parametrization and line tension determination

Only isolated protein domains with a minimum size were analyzed. Domains at the edges with an incomplete perimeter lying within the AFM images were excluded. The boundary shape of the selected domains were parametrized using a harmonic series in polar coordinates

$$R(\phi) = R_0 \left[1 + \sum_{n=1}^{\infty} (a_{n,t} \cos(n\phi) + b_{n,t} \sin(n\phi)) \right]$$

truncated at the tenth order. Detailed procedure on the line tension determination using the coefficients $a_{n,t}$ and $b_{n,t}$ are described in the Supplemental Material [41–46].

E. Coarse-grained model for GM130 and simulation details

We carry out molecular-dynamics (MD) simulations using the lammmps software version 2 Aug 2023 [47]. We use the Mpipi-Recharged model to model the proteins with a resolution of one bead per amino acid, i.e., each amino acid is represented by a single bead centered on the C_α atom. The mass of the different beads is given by the specific molecular weight of the given residue. Intrinsically disordered regions are considered as disordered polymers connected by bonds, and globular domains are treated as rigid bodies with the positions given by the atomistic structure taken from AlphaFold or an experimental crystal structure from Protein Data Bank (see the next section for details). The solvent is modeled implicitly by using a screening length for the electrostatic interactions.

The potential energy of the model is given by

$$E = E_{\text{Bonds}} + E_{\text{Electrostatic}} + E_{\text{Hydrophobic}}.$$

Bonded interactions only include bonds between adjacent amino acids given by a harmonic potential as follows:

$$E_{\text{Bonds}} = \sum_{\text{Bonds}} k(r_i - r_0)^2,$$

where $k = 9.6 \text{ kJ/mol } \text{\AA}^2$ is the bond constant, and $r_0 = 3.81 \text{ \AA}$ is the equilibrium length of the bond. Non-bonded interactions include hydrophobic and electrostatic interactions. All the amino acids interact via van der Waals interactions given by the Wang-Frenkel potential,

defined as

$$E_{\text{Hydrophobic}} = \sum_i \sum_{j < i} \epsilon_{ij} \alpha_{ij} \times \left(\left[\frac{\sigma_{ij}}{r} \right]^{2\mu_{ij}} - 1 \right) \left(\left[\frac{3\sigma_{ij}}{r} \right]^{2\mu_{ij}} - 1 \right)^2$$

where

$$\alpha_{ij} = 2\nu(3)^{2\mu_{ij}} \left[\frac{3}{2((3)^{2\mu_{ij}} - 1)} \right]^3,$$

the σ_{ij} is the bead size of each residue, r is the distance between the interacting particles ($i-j$), ϵ_{ij} is the interaction energy, $r_c = 3\sigma_{ij}$ is the cutoff, and μ and ν_{ij} are positive integer numbers that modulate the shape of the potential. In particular, $\nu = 1$ and μ_{ij} ranges from 2 to 12 depending on the specific interacting pair. The values of σ_{ij} are defined from the molecular diameter of the individual amino acids using the Lorentz-Berthelot relation: $\sigma_{ij} = \frac{\sigma_i + \sigma_j}{2}$. The interaction of each possible pair is precisely parameterized to ensure physico-chemical realism. Charged residues also interact via Yukawa potential, given by

$$E_{\text{Electrostatic}} = \sum_i \sum_{j < i} \frac{A_{ij}}{r} e^{-r\kappa},$$

where A_{ij} represents the interaction strength between charged residues and κ is the Debye screening length representing the effect of the salt. The Mpipi-Recharged treats the effect of the solvent in an implicit way by modulating the screening length, but the dependence of κ with the salt concentration is explicit. In particular,

$$\kappa = \sqrt{8\pi\lambda_B c_s},$$

where c_s is the ionic strength, and $\lambda_B = \frac{e^2}{4\pi k_B T \epsilon_0 \epsilon_r}$ is the Bjerrum length, being e the electron charge, k_B the Boltzmann constant, T the temperature, and ϵ_0 the vacuum dielectric permittivity. The relative dielectric permittivity depends on the temperature according to this empirical law

$$\epsilon_r(T) = \frac{5321}{T} + 233.76 - 0.9297 T + 1.417 \times 10^{-3} T^2 - 8.292 \times 10^{-7} T^3.$$

The simulations were carried out at a constant temperature of 293 K (at which $\epsilon_r \approx 80.3$) using a Langevin thermostat for the disordered segments and a rigid *NVT* thermostat (rigid/nvt in the RIGID package of lammmps) for the structured domains, both with a relaxation time of 5 ps. The trajectories of our simulation were obtained by using Verlet integration with a time step of 10 fs. For all the results presented, we performed productions runs of $\sim 2 \mu\text{s}$ upon reaching equilibration ($\sim 0.5 \mu\text{s}$).

F. System setup and wall interaction

In the presented work, we considered different system configurations of GM130 attached to the surface. The initial configuration was built by placing the desired number of protein replicas next to the surface and equilibrating under *NVT* conditions at 293 K. The attachment between the protein

and the surface is modeled through a Lennard-Jones potential between the wall and specific regions of the protein depending on the specific configuration. The interaction is given by

$$E_{\text{wall}} = 4\epsilon \left[\left(\frac{1}{r} \right)^{12} - \left(\frac{1}{r} \right)^6 \right],$$

where r is the distance between the residue and the wall, ϵ is the interaction parameter that depends on the specific configuration, and the cutoff is set to 3.75 nm. The wall potential is applied at both the lower ($z = 0$) and upper ($z = z_{\text{box}}$) periodic boundaries. The simulations were initialized with the condensate near the lower substrate, and with the chosen potential strength, no detachment or diffusion to the upper wall occurred, confining all interactions to the intended interface.

The dimers were built by coupling two replicas of GM130 by the globular domains so that they are attached (please see the next subsection for the details of the sequence and globular domains). The dimer GM130 simulation (Fig. 1, no mCherry or SNAP attached) was placed in an x with the surface placed in the $z = 0$ plane, and with enough space to avoid self-interactions through the periodic boundary conditions. The surface interacts with the full sequence of GM130 with $\epsilon_{\text{single}} = 1.00$ kcal/mol. We simulated two dimers of GM130 (Fig. 2) with an attachment to the surface of $\epsilon = 0.35$ kcal/mol.

In Figs. 3(e)–3(g), the attachment with the surface for the two dimers was $\epsilon = 0.15, 0.35$ kcal/mol. For the full protein complex (GM130-mCherry-SNAP), we simulated seven dimers coupled by the globular domains as indicated above and using different wall interaction strengths depending on the specific surface. For the mica surface, the wall interacts with the whole protein with an interaction of $\epsilon_{\text{mica}} = 1.5$ kcal/mol as indicated in Fig. 3. The bilayer surface has an interaction strength of $\epsilon_{\text{bilayer}} = 7.0$ kcal/mol with the SNAP tag and $\epsilon_{\text{bilayer}} = 1.5$ kcal/mol with the rest of the protein to ensure a proper attachment. The different interaction strengths with the substrate reflect the distinct physical nature of the surfaces modeled. A uniform interaction is applied for the homogeneous mica substrate, whereas the bilayer requires separate parameters for headgroup and tail regions and for different protein domains. For all the explored interactions, the typical lifetime of inter-protein and protein-wall contacts is around of the order of 10^2 – 10^3 time steps in the span of 10^7 time steps (corresponding to the order of microseconds), ensuring reversible interactions throughout the simulations. As a convergence test, we provide Fig. S14 showing a steady potential energy for the whole simulations.

G. Protein sequences and PDBs

The sequence of GM130 fused to SNAP and mCherry is given below:

**MWPQPRLP RPAMSEETRQSKLAAAKKKLREY
QQRNSPGVPTGAKKKKKIKNGSNPETTTSGGCHS
PEDTPKDNAATLQPSDDTVLPGGVPSPGASLTSMAA
SQNHADNVPNLMDETKTFSSTESLRQLSQQLNGL
VCESATCVNGEGPASSANLKDLESRYQQLAVALDSS
YVTNKQLNITIEKLKQQNQEITDQLEEEKKECHQK
QGALREQLQVHIQTIGILVSEKAELQTALAHTQHA**

ARQKEGESEDLASRLQYSRRRVGELERALSASTQ
 QKKADRYNKELTKERDALRLELYKNTQSNEDLKQ
 EKSELEEKLRLVLTKEAGMQLNLEELQKKLEMTE
 LLLQQFSSRCEAPDANQQLQQAMEERAQLEAHLG
 QVMESVRQLQMERDKYAENLKGESAMWRQRMQ
 QMSEQVHTLREEKECSMSRVQELETSLAELRNQM
 AEPPPPPEPPAGPSEVEQQLQAEAEHLRKELEGLAG
 QLQAQVQDNEGLSRLNREQEERLLELERAELWG
 EQAEARRQILETMQNDRTTISRALSQNRELKEQLA
 ELQSGFVKLTNENMEITSALQSEQHVKRELGKKL
 GELQEKLSELKETVELKSQEAQSLQQQRDQYLGH
 LQQYVAAYQQLTSEKEVLHNQLLLQTQLVDQLQQ
 QEAQKGKAVAMARQELQETQERLEAATQQNQQLR
 AQLSLMAHPGEGDGLDREEEEEEEEEEEAVVPQ
 PMPSPEDLESREAMVAFFNSAVASAEQARLRGQ
 LKEQVRRCRLAHLASAQKEPEAAAPAPGTGGDS
 VCGETHRALQGAMEKQLQSRFMELMQEKADLKER
 VEELEHRCIQLSGETDTIGEYIALYQSQRAVLKERH
 REKEEYISRLAQDKEEMKVKLLELQELVLRVGD
 RNEWHGRFLAAQNPADPTSGAPAPQELGAANQ
 QGDLCEVSLAGSVEPAQGEAREGSPRDNPQAQIM
 QLLREMQRPRERPGLGSNCPFFYRADENDEVKI
 TVITRTLMSVSKGEEDNMAIHKFMRFKVHMEGSVN
 GHEFEIEGEGEGRPYEGTQAKLKVTKGGPLPFAW
 DILSPQFMYGSKAYVKHPADIPDYLLKLSFPEGFNWE
 RVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNF
 PSDGPVMQCRTMGWEASTERMYPEDGALKGEIKQ
 RLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVDI
 KLDILSHNEDYTIVEQYERAEGRHSTGGMDLYK
 ALMDKDCMKRTTLDSPGLKLELSGCEQGLHRIIF
 LGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNA
 YFHQPEAIEEFPVPALHHPVFQESFTRQVLWKL
 KVVKFGEVISYSHLAALAGNPAATAAVKTALSGNPV
 PILIPCHRVPQGDLDVGGYEGGLAVKEWLLAHEGH
 RLGKPGLGARPLEQKLISEEDLAANDILDYKDDDDKV
 GSGGSHHHHHH

In the above sequence, GM130 is bolded, mCherry is highlighted in red, SNAP in blue, and the histidine tag that attaches to the wall is marked in green. The rigid structure to simulate GM130 is taken from AlphaFold considering the following rigid domains in residue number: 170-277, 287-341, 385-427, 474-562, 576-651, and 803-844. To simulate a GM130 dimer we coupled two replicas of GM130 by the globular domains as predicted by AlphaFold, using the rigid/nvt (in the RIGID package available in LAMMPS) thermostat that groups homologous CC residues from each monomer. The globular domain of mCherry is given by the 2H5Q PDB

code and the rigid segment goes from residue 1007 to 1242 according to the sequence presented above, while for SNAP we used the PDB code 3KZY, involving residues from 1246 to 1422.

H. Calculation of the contact maps

Intermolecular and intramolecular contact maps are calculated from the trajectories obtained from our simulations. We define a contact as a coordination between two residues inside a given cut-off distance given by $1.2\sigma_{ij}$, where σ_{ij} is the mean excluded volume of the contacting amino acids. Considering that the minimum of the interaction potential of the Mpipi-Recharged is approximately located around $2^{1/6}\sigma_{ij} \approx 1.122\sigma_{ij}$, with the given cutoff we ensure significant binding while neglecting adjacent contacts that may coincide with actual interacting amino acids along the sequence [28]. The contact maps (both intra- and intermolecular) presented in Figs. 5 and 6 are normalized by the number of GM130 replicas and the number of snapshots in the analysed trajectory. The interaction energy is defined as the contact frequency between amino acid pairs weighted by their hydrophobic and electrostatic energy contributions:

$$\text{Interaction energy} = f_{ij}(E_{ij}^{\text{Hydrophobic}} + E_{ij}^{\text{Electrostatic}}).$$

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DATA AVAILABILITY

The data that support the findings of this article are not publicly available. The data are available from the authors upon reasonable request.

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