Stabilization of supramolecular membrane protein–lipid bilayer assemblies through immobilization in a crystalline exoskeleton

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Artificial native-like lipid bilayer systems constructed from phospholipids assembling into unilamellar liposomes allow the reconstitution of detergent-solubilized transmembrane proteins into supramolecular lipid-protein assemblies called proteoliposomes, which mimic cellular membranes. Stabilization of these complexes remains challenging because of their chemical composition, the hydrophobicity and structural instability of membrane proteins, and the lability of interactions between protein, detergent, and lipids within micelles and lipid bilayers. In this work we demonstrate that metastable lipid, protein-detergent, and protein-lipid supramolecular complexes can be successfully generated and immobilized within zeolitic-imidazole framework (ZIF) to enhance their stability against chemical and physical stressors. Upon immobilization in ZIF bio-composites, blank liposomes, and model transmembrane metal transporters in detergent micelles or embedded in proteoliposomes resist elevated temperatures, exposure to chemical denaturants, aging, and mechanical stresses. Extensive morphological and functional characterization of the assemblies upon exfoliation reveal that all these complexes encapsulated within the framework maintain their native morphology, structure, and activity, which is otherwise lost rapidly without immobilization.
All living organisms, from prokaryotes to higher eukaryotes, rely on transmembrane protein systems for a variety of functions including signal transduction, substrate transport, and intramembrane enzymatic catalysis. A significant fraction of polytopic transmembrane proteins act as transporters and are critical for the translocation of large and/or charged substrates in and out of the cell, and understanding their function is imperative to understanding the etiology of many human diseases. Purification of transmembrane proteins and subsequent reconstitution in artificial lipid bilayers—called proteoliposomes—generates metastable systems that are utilized for both structural and functional investigations in which substrate transport can be studied on transporters embedded into their native-like environments. Unfortunately, owing to the patchwork of hydrophobic and hydrophilic surfaces of transmembrane proteins and the dynamic non-covalent nature of liposomes, these assemblies are intrinsically unstable and susceptible to denaturation, precipitation, and loss of function when left at room temperature for even a few hours. Despite years of effort toward stabilizing membrane-bound proteins, only a handful of approaches exist, and discovery of methods that protect them within their native bilayer environment remains an open challenge. Immobilization of biomacromolecular motion by chaperone-like confinement in polymers has emerged as a way to stabilize soluble proteins, but the accessibility of addressable functional groups of transmembrane proteins is reduced from confinement in a lipid matrix and the poor stability of membrane-bound proteins further complicates bioconjugation. Recently, polymeric excipients designed to match the natural distribution of polar residues on proteins or stabilization in amphipathic copolymer-based membrane nanodiscs have advanced the state-of-the-art; however, these coatings are neither removable nor scalable to protect a functional catalytic proteoliposome system. Specifically, a sheddable coating that avoids excipients or chemical functionalization of either protein or lipid would help advance work on these systems tremendously. To that end, we turned to metal–organic frameworks (MOFs), which are a class of crystalline porous coordination polymers constructed from the interlinking between metal centers and monomeric organic ligands. Through thoughtful selection of the organic ligand and metal center, MOFs can be modulated sophisticatedly toward a wide array of applications such as gas separation/sensing/storage, catalysis, and protein stabilization. In recent years, several MOFs displaying zeolitic topologies have been reported, the most ubiquitous being zeolitic-imidazole framework-8 (ZIF-8) and associated structural analogues, all of which are coordination polymers consisting of Zn2+ and 2-methyl imidazole (MIM)13–24. ZIF-8 and its associated (pseudo) polymorphs can form thermodynamically stable crystalline shells by nucleating on biomacromolecules and these systems can withstand high temperatures and pH yet are kinetically labile in the presence of metal binding chelates. ZIF-8 in particular is well known for its ability to nucleate on the surface of colloidal and dissolved biomacromolecules forming a crystalline matrix shell. We suspected that colloidal liposomes, proteoliposomes, and detergent-solubilized transmembrane proteins would nucleate the growth of ZIFs over their surface, and this protective shell would inhibit both protein denaturation and liposome fusion and/or degradation. Further, the kinetic lability of Zn-MIM bond in the presence of high-affinity metal chelators would allow us to recover the encapsulated systems without significant loss of protein function or sample homogeneity. We selected two α-helical polytopic (eight transmembrane α-helices) iron and copper transporters—called IroF/MavN and CopA respectively—as representative examples of metal transport systems and virulence factors in bacteria that cause fatal human diseases. Transition metal transporter proteins are the subject of ongoing research in a number of laboratories and their stabilization serve not only as a proof-of-concept but also in aiding and abetting research into these systems.

In this work, we demonstrate a method for the thermal stabilization of blank 200 nm liposomes, purified transmembrane proteins, and 200 nm transmembrane protein-liposome supramolecular complexes (proteoliposomes) against chemical and thermal stressors through biomimetic nucleation of a pseudopolymorph of ZIF-8 called ZIF-L. We found that encapsulation of the proteoliposome complex generates thermodynamically stable bio-composites that can withstand exposure to high temperatures, aging, and common protein denaturants (Fig. 1). Further, the ZIF-L coatings can be removed to afford pristine proteoliposomes, liposomes, and transmembrane protein–micelle complexes of similar composition, morphology, structure, and catalytic activity to their native counterparts. Finally, our work demonstrates the generalizability and potential ZIF scaffolds have for stabilizing highly sensitive and metastable supramolecular systems.

**Results and discussion**

**Liposome stabilization.** We progressed systematically to demonstrate that each component—the as-prepared liposome, the detergent purified protein, and the proteoliposome system—could be encapsulated and protected by biomolecular nucleation. We prepared 200 nm liposomes by freeze-fracture and extrusion using mixtures of L-α-phosphatidylcholine and *E. coli* total polar lipid extracts, that allow the formation of native-like unilamellar lipid bilayers. While this composition was selected because it provides the best stabilization for our selected transmembrane proteins, the anionic nature of the formulated lipids is useful in promoting ZIF nucleation and growth, as we and others have
after exposure to 80 °C for 5 min (Scale bar liposome (Scale bar line), Lp RT (blue), and Lp 55 °C (dark-pink). Lp@ZIF 55 °C (green), Lp@ZIF RT (brown), Lp pristine (orange-dashed line), Lp RT (blue), and Lp 55 °C (dark-pink). F) TEM micrograph of pristine liposome (Scale bar = 50 nm) and (G) 40 × 16 Lp@ZIF following exfoliation after exposure to 80 °C for 5 min (Scale bar = 100 nm).

![Figure 2 Characterization of artificial lipid bilayers embedded in ZIF.](image)

**A** Biomolecular nucleation of liposomes in ZIF. **B** PXRD spectra of ZIF liposome complexes (Lp@ZIF) and ZIF controls. 40 × 16 Lp@ZIF (red), 40 × 16 ZIF-L (purple), 20 × 16 Lp@ZIF (green), 20 × 16 ZIF-L (yellow), and calculated ZIF-L (orange). **C** SEM micrograph of pristine liposomes exposed at high temperatures (after exfoliation) compared to native and nonencapsulated liposomes. Lp@ZIF 55 °C (green), Lp@ZIF RT (brown), Lp pristine (orange-dashed line), Lp RT (blue), and Lp 55 °C (dark-pink). E) DLS profiles obtained for immobilized liposomes exposed at high temperatures (after exfoliation) compared to native and nonencapsulated liposomes. Lp@ZIF 55 °C (green), Lp@ZIF RT (brown), Lp pristine (orange-dashed line), Lp RT (blue), and Lp 55 °C (dark-pink). F) TEM micrograph of pristine liposome (Scale bar = 50 nm) and (G) 40 × 16 Lp@ZIF following exfoliation after exposure to 80 °C for 5 min (Scale bar = 100 nm).

Fig. 2 Characterization of artificial lipid bilayers embedded in ZIF.

Fluorescence imaging, powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM) analysis of the Lp@ZIF formulation as well as controls were carried out (Fig. 2B–D and Supplementary Figs. 1, 2). Intriguingly, XRD patterns obtained for both conditions, including controls, are consistent with the formation of ZIF-L, a crystalline phase with a leaf-like morphology that explains the highly faceted nanoparticles observed in the micrographs in Fig. 2C, D39. Nitrogen isotherms of both lipidosome loaded composites and controls reveal low porosity (Supplementary Fig. 2C and Supplementary Table 1), which is consistent with ZIF-L40. Finally, thermogravimetric analysis (TGA) of 20 × 16 Lp@ZIF and 40 × 16 Lp@ZIF both display a mass loss (~18%) at 200 °C, which has been attributed to solvent loss in prior work with ZIF-L (Supplementary Fig. 2D)41.

The protective capacity of the ZIF-L coating toward the liposomes was evaluated on samples suspended in water and stressed at RT for 48 h and 55 °C for 15 min—this latter temperature being above the phase transition temperature of the liposomes. After stressing, the ZIF-L shell was dissolved in ethylenediaminetetraacetic acid (EDTA; 50 mM)—a process we refer to as exfoliation (Fig. 1)—and these solutions were analyzed by dynamic-light scattering (DLS) to assess liposomal size distribution. As controls, we stressed nonencapsulated liposomes following the same experimental conditions. As expected, the unprotected liposomes showed a significant increase in size and polydispersity (average diameter: 604.8 nm; PDI: 0.449; Supplementary Table 2) resulting from membrane fusion and lipidosome aggregation (Fig. 2E, lower two traces) under both conditions. Conversely, we were happy to find that the monodisperse nature of freshly extruded blank liposomes (average diameter: 141.2 nm; PDI: 0.138) was retained for the ZIF-L coated composites (average diameter: 123.2 nm; PDI: 0.179; Fig. 2E, top two traces). Preservation of the original liposomal morphology in ZIF-L immobilized samples was further confirmed by transmission-electron microscopy (TEM, Fig. 2F, G). While the stressed ZIF-L liposomes showed liposomes that were indistinguishable from freshly extruded pristine samples, unprotected liposomes showed altered morphology as a consequence of extensive membrane rupture and fusion (Supplementary Fig. 3). In light of the observed stabilization, Lp@ZIF composites were thermally stressed at 80 °C and subsequent analysis by DLS and TEM imaging revealed that the original morphology and size distribution was largely retained (average diameter: 119.3 nm; PDI: 0.231; Supplementary Fig. 4). To test the ability of ZIF encapsulation in protecting from aging, Lp@ZIF was dried and the obtained hydrated suspension left on the bench top for 20 days at room temperature. Following exfoliation, TEM characterization revealed that the liposomes retained their original size distribution and morphology (Supplementary Figs. 3, 4). In contrast, the nonencapsulated liposomes kept in solution fused and/or aggregated within 2 days (Supplementary Fig. 3F–I). Interestingly, both formulations discussed above (20 × 16 and 40 × 16) provided outstanding protection against thermal stress and aging following liposome immobilization in ZIF-L composites (Fig. 2, and Supplementary Figs. 3, 4).

Finally, we and others have previously reported that different polymorphs of ZIF can be produced by increasing the concentration of ligand and metal22,30. This is potentially useful as different polymorphs can impart greater or less protection and the kinetics of dissolution can be very different, which may be useful in drug delivery applications. We were able to find conditions that resulted in the growth of the ZIF-8 sod (sodalite) topology (Supplementary Fig. 5). Though, qualitatively, we noticed it was more time consuming to exfoliate the ZIF-8...
Mechanism of ZIF growth. In reactions with ZIF and its associated polymorphs, we and others have observed that the initial interaction occurs between the biomolecule surface and the zinc ions and this interaction has been proposed as an important indicator for a biomimetic crystallization process on the surface of biomacromolecules²⁶,³⁰. We were able to confirm qualitatively that zinc can bind to the negative surface of liposomes, which have a ~16.9 mV ζ potential (Supplementary Fig. 7A, B) from the presence of anionic phospholipids, by observing zinc-lipid complexes by inductive coupled plasma mass spectrometry. When the lipids were doped with 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), a cationic lipid, we observed less or even no Zn²⁺ interaction with the liposome (Supplementary Fig. 7C, D).

Cationic surface charge has been shown to reduce the encapsulation yield or even prevent crystal growth, although there are strategies to overcome this, and we are actively studying this. Kinetics of nucleation, particle growth³⁸, crystallization, and the morphology of the particles were investigated in situ via synchrotron-based small-angle and wide-angle X-ray scattering (SAXS/WAXS) techniques. We investigated four different samples (20 × 16 Lp@ZIF, 20 × 16 ZIF-L, 40 × 16 Lp@ZIF, and 40 × 16 ZIF-L) using a stopped flow device to initiate the rapid mixing of the reagents (mixing time <100 ms). See the SAXS section in methods for full experimental details, and Supplementary Figs. 8-12 and Supplementary Tables 5, 6 for experimental setup and results. The injection of the aqueous precursors solutions (Zn²⁺, MIM, liposomes) into a micromixer triggered the SAXS acquisition system data collection (time resolution: 100 ms). Data for the 20 × 16 conditions are summarized in Fig. 3—see the Supplementary Information for 40 × 16 results. To investigate the kinetics of nucleation and growth, we monitored the time evolution of SAXS patterns (Fig. 3A, B) and of the invariant Q̃ (Fig. 3C, see Methods for details). Q̃ is related to the Porod invariant of the scattering curve and is sensitive to changes in particle volume fraction and electron density contrast. An increase of Q̃ over time indicates the formation of particles/agglomerates within the investigated volume of the sample. A plateau in the time series of Q̃ values indicates stationary conditions. The time evolution of Q̃ is reported in Fig. 3C in the “Time-resolved Small Angle X-Ray Scattering (SAXS)” section of Methods. The increase of Q̃ related to the particle growth is observed 0.8 and 0.6 s after the mixing of precursors for samples 20 × 16 Lp@ZIF and 40 × 16 Lp@ZIF, respectively. In the control samples, the particle growth is observed at 4 s (20 × 16 ZIF-L) and 2.6 s (40 × 16 ZIF-L) after mixing the precursors. The plateau of Q̃ related is reached 25 and 5 s after the mixing of precursors for samples 20 × 16 Lp@ZIF and 40 × 16 Lp@ZIF, respectively. In the control samples, the plateau is reached 40 s (20 × 16 ZIF) and 25 s (40 × 16 ZIF) after mixing the MOF precursors.

The crystallization kinetics were monitored by following the integrated intensity of the (200) ZIF-L diffraction peak (5.25 nm⁻¹; Fig. 3D, E). In the presence of liposome, the added mixture of Zn and MIM initially produced an amorphous phase for both the 20 × 16 and 40 × 16 conditions between 0.1–120 and 0.1–50 s respectively. The initial formation of an amorphous phase is consistent with what we have observed with viral nanoparticles and other proteins. Importantly, compared to the control samples, we observed faster crystallization for Lp@ZIF particles (e.g., 20 × 16 Lp@ZIF crystallization is 15 times faster than the pure ZIF-L particles; See below). These data demonstrate that the presence of liposomes templates a faster nucleation, growth, and crystallization of ZIF-L when compared with the control conditions.

By fitting the SAXS patterns 120 s after the mixing of the reagents (Fig. 3F), we observed that the presence of the liposome induced the formation of plate-like particles with a thickness of 30–50 nm. Conversely, in the absence of liposomes, ZIF particles with an average size larger than 100 nm and no sharp size distribution were observed. Thus, a role of the biomacromolecules in the final crystal morphology was determined. From these data, we conclude that the liposomes act as seeding agents for the MOF growth, altering their growth kinetics as well as the ultimate morphology of the crystalline particles that are produced and that liposome MOF bio-composites are formed via a biomimetic mineralization process.

Stabilization of purified transmembrane proteins. We selected two different transmembrane proteins, both of which are poorly stable at room temperature, to demonstrate the broad utility of our approach. IroT/MavN is a transmembrane protein found in Legionella pneumophila (L. pneumophila), a thin, flagellated, gram-negative bacteria responsible for Legionnaires’ disease. IroT mediates iron sequestration as an essential micronutrient from host cell, allowing ltx1 L. pneumophila to replicate in a host-derived vacuole within the infected macrophages. IroT topology is characterized by eight transmembrane (TM) helices and a long C-terminal domain. The structure and substrate translocation modality in IroT are active areas of research, but much has been gleaned from reconstituting IroT in artificial lipid bilayer systems and performing representative transport assays. IroT was shown to act as a Fe²⁺/H⁺ antiporter that allows Fe²⁺ acquisition into the vacuole from the host cell for pathogen survival. The second protein selected is a copper P₁b₁-type ATPase from E. coli (CopA), a TM primary active pump, and part of the P-type ATPase superfamily, that utilize energy generated by ATP hydrolysis to drive Cu⁺ transport across biological membranes against electrochemical gradients. These catalytically driven pumps constitute an essential system to drive the selective translocation and export of Cu⁺ ions, thereby controlling the intracellular Cu⁺ levels. Their activity tightly balances the biogenesis and integrity of copper centers in vital enzymes to nontoxic intracellular copper levels. The CopA structure is characterized by the existence of an 8 TM helices membrane domain (M-domain) connected to large cytosolic domains (N-, P-, and A-domains) responsible for ATP hydrolysis, phosphorylation and energy transduction, allowing Cu⁺ translocation across the membrane. As a result of their critical involvement in essential iron and copper metabolism, both IroT and CopA homologs have been identified as key virulence factors in bacterial pathogens.

TM proteins, including IroT and CopA, are commonly extracted from membranes and purified as detergent micellar complexes for solubilization in aqueous environments. The detergent molecules surround the hydrophobic regions of the protein in the micelles, which helps avoid aggregation, precipitation, and unfolding in water. Though they are more stable, these proteinaceous assemblies still require unique environmental conditions to remain fully active—e.g., long-term storage at ~80 °C, constant refrigeration for analysis, etc. Since this strategy is employed in the typical workflow for incorporating TM proteins in liposomes, and naked TM proteins are extremely prone to denaturation, we suspected simply nucleating the ZIF over the detergent–protein supramolecular complex would...
improve the likelihood of retaining protein function in high yields following exfoliation. We thus solubilized and purified IroT in Cymal-7 (7-Cyclohexyl-1-Heptyl-β-D-Maltoside) micelles and CopA in micelles prepared with DDM (n-Dodecyl-β-D-Maltopyranoside) and applied our synthetic strategy, developed above, to produce ZIF-L composites (Fig. 4A). Crystals were isolated by centrifugation, washed, and allowed to dry at RT for 12 h. As-obtained crystals were characterized by SEM and showed a star-shaped morphology (Fig. 3B–E and Supplementary Fig. 13A, B) and PXRD of both protein–micelle composites and controls again showed the phases to be ZIF-L (Fig. 4F). TGA analysis of 20 × 16 IroT@ZIF and 20 × 16 control revealed high thermal stability as shown in Supplementary Fig. 13C, where the 20 × 16 IroT@ZIF is characterized by an ~20% mass loss at 200 °C, attributed to loss of solvent (Supplementary Fig. 13C).41. Further, nitrogen isotherms of 20 × 16 IroT@ZIF reveal no measurable porosity, whereas 40 × 16 ZIF-L shows a BET surface of 385 m²g⁻¹ (Supplementary Table 1).

Quantification of encapsulation efficiency was determined by SDS-PAGE gel densitometry. Supernatants obtained during the washing of ZIF-L bio-composites and exfoliated protein–detergent complexes were run in tandem with either IroT or CopA pristine standards of varying concentrations. We found the encapsulation efficiency to be quantitative—no residual protein was found in the supernatant after the encapsulation procedure. Indeed, after encapsulation, isolation of the final protein@ZIF product, and subsequent exfoliation, resulted in almost 75% recovery, regardless of the protein or metal-to ligand ratio used (Supplementary Fig. 14A–C). To determine the integrity of IroT or CopA detergent–protein micelles after biomolecular nucleation, two properties were analyzed to benchmark the protective effect of immobilization: monodispersity analysis by size-exclusion chromatography (SEC) for IroT; and catalytic metal transport activity assessed by metal-stimulated ATP-hydrolysis, for CopA. SEC is a good proxy for testing the stability of the generated IroT–detergent complexes as the absence of aggregation is an indicator of the integrity and stability of the protein–detergent assembly. On the other hand, for purified Cu⁺ P-type ATPases, we can measure the rate of ATP hydrolysis in the presence of selected metal substrate to show persistence of structure and function, as ATP hydrolysis and Cu⁺ transport in CopA are tightly coupled. To verify CopA functionality in detergent micelles (or upon incorporation in proteoliposomes) the Cu⁺-dependent stimulation of ATPase activity was determined by photometric quantification.

![Fig. 3 X-Ray Diffraction Kinetics Study of Biomimetic ZIF Formation.](image-url)

Time evolution of SAXS patterns (background subtracted) from time-resolved SAXS synthesis of (A) 20 × 16 Lp@ZIF. 10 s (magenta), 20 s (orange), 30 s (yellow), 40 s (green), 50 s (blue), 60 s (dark-blue), 80 s (light-purple), and 120 s (dark-purple). (B) 20 × 16 ZIF measured at 10 s intervals. 10 s (pink), 20 s (magenta), 30 s (dark-orange), 40 s (light-orange), 50 s (green), 60 s (blue), 80 s (dark-blue), and 120 s (purple). (C) Time evolution of invariant Q calculated from 0.1 to 0.6 nm⁻¹ of the time-resolved SAXS patterns in (A, B) and corresponding to the synthesis of 20 × 16 Lp@ZIF (black dots) and 20 × 16 ZIF-L (red dots). The dashed lines are plotted to highlight the starting time of the Q increase. (D) Time evolution of the integrated intensity of (200) diffraction peak of ZIF-L (5.25 nm⁻¹) calculated from time-resolved SAXS synthesis 20 × 16 Lp@ZIF and (E) 20 × 16 ZIF-L. In the insets, selected diffraction patterns highlighting the time-evolution of the (200) diffraction peak of ZIF-L (5.25 nm⁻¹) are reported. Color scheme for (D): 2 min (black), 5 min (red), 10 min (green), and 15 min (blue); and (E) 2 min (black), 29 min (red), 32 min (green), and 35 min (blue), and 40 min (purple). Time zero is referred to the end of the precursors mixing. F) SAXS patterns (background subtracted and averaged) and fitted data 120 s after mixing the precursors of 20 × 16 Lp@ZIF (black dots) and 20 × 16 ZIF-L (red dots). The theoretical Porod power law (kq Q = q⁻⁴) is plotted for comparison.
of released inorganic phosphate (P) generated by catalytic ATP-hydrolysis using Malachite green. Upon stressing, immobilized samples were exfoliated and immediately characterized by either SEC or metal-dependent ATP-hydrolysis assays. The nonencapsulated IroT samples showed increased polydispersity when incubated at RT, as evidenced by development of an asymmetric elution peak shoulder in SEC and almost complete aggregation after exposure to 55 and 80 °C for a few minutes (Fig. 4G and Supplementary Fig. 15A). This is to be expected, as these proteins are extremely prone to aggregation at even low temperature. In contrast, for the encapsulated samples, exposure to RT for 48 h and 55 °C for 15 min had little impact on the monodispersity of IroT. SEC analysis of the exfoliated IroT samples show a single elution peak (elution volume = 12.0 mL), closely corresponding to non-stressed and refrigerated IroT controls (12.0 mL) as shown in Fig. 4G. Incredibly, even exposure to 80 °C (5 min) produced minimal aggregation (Supplementary Fig. 15A).

Resilience to thermal stress was subsequently investigated on the CopA-DDM micelle complexes by analyzing the catalytic substrate-dependent ATP hydrolytic activity after exposing the various samples suspended in water to a range of denaturing temperatures. Cu$^{+1}$-dependent stimulation of ATPase rates for non-stressed CopA, in the presence of Mg$^{2+}$/ATP, revealed characteristic catalytic hyperbolic Michaelis–Menten-type dependency as a function of Cu$^{+1}$, confirming that the purification in detergent micelles maintains CopA in a functional form ($K_M$, Cu$^{+1} = 0.12 ± 0.02$ μM; $V_{max}$ of 7.1 ± 0.2 nmol (mg min)$^{-1}$). However, upon thermal stress at RT, 55 and 80 °C, the CopA-DDDM...
catalytic activity was completely abolished, with <10% residual activity at RT. In contrast to unprotected protein, stressed samples of CopA encapsulated in ZIF retained the characteristic Michaelis–Menten dependency of their ATPase activity after thermal stress and exfoliation (Fig. 4H). Analysis of CopA@ZIF bio-composite ATPase activities at saturating Cu⁺ concentrations revealed that CopA retained >80% (6.3 nmol Pi mg⁻¹ min⁻¹) of its maximal ATPase activity upon stress at RT (Fig. 4I), >60% at 55 °C (4.7 nmol Pi mg⁻¹ min⁻¹; Fig. 4I) and at >42% at 80 °C (3.1 nmol Pi mg⁻¹ min⁻¹; Supplementary Fig. 14D). Accordingly, analysis of the $K_M$, $Cu(I)$ values at RT indicated that upon stress unaltered catalytic parameters are preserved by ZIF encapsulation (Supplementary Fig. 14E).

It is noteworthy that formulation conditions are an important aspect of stability. CopA was encapsulated under both metal–ligand ratios discussed above (20 × 16 and 40 × 16) and, interestingly, IroT-cymal-7 micelles showed better stabilization with the 40 × 16 formulation, while the 20 × 16 formulation was most effective at enhancing the thermal stability of CopA-DDM micelles. Thus, formulation optimization is an important parameter to be screened for optimal bio-composite protection depending on the protein topology and protein–detergent micelle structure (Supplementary Fig. 14D–F).

Encapsulation of proteinaceous materials has been widely used to increase stability of moieties against chemical stressors, such as organic solvents and chaotropic agents. Motivated by such reports, CopA@ZIF and IroT@ZIF were chemically stressed using SDS, a commonly used protein denaturant. Briefly, the samples were incubated for 30 min in a solution consisting of 0.1% SDS. Crystals were harvested by centrifugation, washed 5x with ultrapure water, exfoliated, and immediately characterized by SEC analysis (IroT) or Cu⁺-dependent ATP-hydrolysis assays (CopA). As shown in Supplementary Fig. 15, immobilization in ZIF affords retention of monodispersity and activity for both encapsulated TM proteins, while nonencapsulated control samples are fully denatured and inactive in the presence of 0.1% SDS. These results also suggest that a population of proteins are at least partly exposed to the MOF surface, accounting for the modest (~15%) loss of functionality.

Proteoliposome stabilization. Our analysis demonstrates that immobilization in new ZIF composites allow for stabilization of both pristine liposomal vesicles as well as protein–detergent micellar complexes, providing a 3D scaffold that can morph around complex and chemically diverse biomolecular assemblies providing protection against stressors. In light of the versatility of the approach, we sought to determine if our ZIF encapsulation strategy could protect even more complex and metastable supramolecular entities such as proteoliposomes. Purified IroT and CopA were reconstituted in unilamellar liposomes via freeze–thaw and extrusion through 200 nm filters, followed by liposome destabilization by detergent addition and subsequent detergent removal by Biobeads resin. Protein incorporation was subsequently quantified by SDS-PAGE following removal of excess detergent-solubilized protein from the proteoliposomes by ultracentrifugation with subsequent protein quantification of the soluble and proteoliposome fractions conducted by gel densitometry. We subsequently biomimetically mineralized proteoliposomes with IroT or CopA embedded in the lipid bilayer in ZIF (Fig. 5A–C and Supplementary Figs. 16, 17). In a typical experiment, 6.25 mg mL⁻¹ of proteoliposomes (protein concentration is 0.25 mg mL⁻¹; TEM of typical samples shown in Fig. 5B) were encapsulated in ZIF at ambient conditions mixing the proteoliposome complexes with a solution of MIM and zinc acetate dihydrate using M-buffer as solvent. Crystals were harvested by centrifugation after 18 h of aging at RT, washed with ultrapure water and allowed to dry at RT for 12 h (Fig. 5C). SDS-PAGE gel densitometry revealed protein recovery after exfoliation with no detectable traces of protein in the supernatants (Supplementary Fig. 16A). In the presence of sodium chloride, TCEP or DTT, and MOPs the crystal morphology appears as aggregates of star-like shape chunks (Supplementary Fig. 16B–H) of ZIF-L. The encapsulation and exfoliation process, which were optimized in the prior two studies, was very straightforward for proteoliposome assemblies to generate IroTPL@ZIF and CopA-PL@ZIF bio-composites. Following exfoliation, we found the proteoliposome size and shape were not altered compared to freshly extruded proteoliposomes by TEM and DLS analyses, even after heat exposures, regardless of the ZIF formulation utilized for immobilization (Fig. 5E, Supplementary Fig. 17, and Supplementary Tables 3, 4).

In addition to the size and morphology preservation, activity assays performed on the ZIF immobilized CopA proteoliposomes confirmed that the MOF shell thermally enhances these delicate systems enabling them to resist temperatures that would otherwise promote loss of function (Fig. 5F–I). Indeed, proteoliposome formulations of CopA are so thermally unstable they lose 90% of their catalytic activity in 48 h at 4 °C (Supplementary Fig. 18).

To demonstrate that the encapsulated proteoliposomes not only resist prolonged periods of no refrigeration but also enhances stability towards physical/mechanical stressors, prepared PL@ZIF samples were shipped across America in a padded envelope through the United States Postal Service. PL@ZIF bio-composites in water were placed in a standard cushioned mailer and shipped across the United States, from Dallas, Texas to Rhode Island and back again. They were then left at room temperature for 2 months following shutdown of our laboratories during the 2020 SARS-CoV-2 pandemic. By the time we had opened the package, the water had completely evaporated. Nonetheless, after exfoliation, the catalytic activity and liposome morphology were similar to the pristine counterparts (Supplementary Fig. 19) in contrast to controls, which we know degrade within a day at room temperature or 2 days under refrigerated conditions.

Lipid bilayers are the core building unit of cell membranes, which serve as the main line of action between the outside and the inside environments of the cells and organelles. Given their structural complexity, researchers have been motivated to develop simpler model systems to understand the molecular processes associated with cellular membrane dynamics and investigate protein-mediated solute translocation across lipid bilayers. Proteoliposomes are a powerful tool that mimic cellular membranes. By virtue of tuning the vesicle size and the lipid and protein composition, proteoliposomes have become instrumental to the study both prokaryotic and complex eukaryotic cell membranes, and proteins embedded into them, including TM transporter proteins. Despite their utility, proteoliposomes are delicate systems that require unique conditions to maintain their functionality that have long imposed obstacles for their handling/transport and hindering their usefulness for the better understanding of the modus operandi of TM proteins. Taking advantage of the high thermal and aequous stabilities of ZIF-L, blank liposomes, detergent-solubilized proteins, and proteoliposome complexes no longer require constant refrigeration and repeated extrusion to maintain their intrinsic structure, monodispersity and functionality over long incubation times. Further, we show that immobilization in ZIF-L enables the as-prepared bio-composites to be exposed to chemical denaturants and temperatures above their lipid bilayer phase transition without structural and/or functional changes. Finally, we have shown that...
bimolecular nucleation is an effective process to preserve supramolecular membrane protein–lipid ‘blyer’ assemblies against conditions that, without encapsulation, would easily impair their structural and functional integrity.

**Methods**

**Expression and purification of MavN/IroT.** MavN/IroT protein was expressed recombinantly in *E. coli* BL21 Gold(DE3) cells using a pET-52b (+) plasmid (Genscript Inc.) encoding the codon-optimized *Legionella pneumophila* iroT sequence (strain Philadelphia 1/ATCC 33152). The expression was induced at 18 °C for 19 h, and the cells were harvested by centrifugation (20 min, 4 °C, 20,000 × g). The membranes were isolated upon harvesting the cells and resuspending them in buffer (20 mM Tris/HCl pH 7, 500 mM NaCl, 1 mM dithiothreitol (DTT), 0.05% (w/v) 7-cyclohexyl-1-heptyl-β-D-maltoside (Cymal-7) to remove any bound impurities. The bound protein was eluted by using a linear imidazole gradient (0–100%) of a mixture of the wash and elution buffer (20 mM Tris/HCl pH 8, 500 mM NaCl, 35 mM imidazole, 1 mM dithiothreitol (DTT), 0.05% (w/v) 7-cyclohexyl-1-heptyl-β-D-maltoside (Cymal-7) to remove any bound impurities. The bound protein was eluted by using a linear imidazole gradient (0–100%) of a mixture of the wash and elution buffer (20 mM Tris/HCl pH 8, 500 mM NaCl, 35 mM imidazole, 1 mM dithiothreitol (DTT), 0.05% (w/v) 7-cyclohexyl-1-heptyl-β-D-maltoside (Cymal-7)). SEC was carried out on IroT encapsulated in ZIF and exfoliated, to determine the monodispersity of protein encapsulated in ZIF upon stress.

Reconstitution of MavN/IroT in proteoliposome small unilamellar vesicles (SUVs). All the buffer solutions utilized in proteoliposome preparation were treated with Chelex resin (Biorad) to remove metal contaminants. Purified IroT was reconstituted at a protein-to-phospholipid ratio of 1:25 (w/w) as reported earlier. *

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Expression and purification of CopA protein. For the recombinant expression of CopA, a pET-52b (+) expression plasmid with an N-terminal Strep Tag II encoding the codon-optimized CopA homolog from E. coli (Strain K12; UniProt accession number P25553; generated by GenScript Inc.). The synthetic DNA insert encoding for CopA was generated using the primers reported in Supplementary Table 8 and cloned in the pET-52b (+) Staphylococcus aureus restriction sites (GenScript Inc.). E. coli expression was performed by transforming the expression plasmid into E. coli BL21 Gold (DE3) competent cells (Agilent Technologies). The overnight preculture was inoculated into fresh TB media supplemented with 1% (v/v) glycerol and grown at 37 °C for 15 h at 200 rpm (LambDAir 1510). The overnight preculture was pelleted after incubation (20 min, 4 °C, 16,000 × g) and resuspended with 2× EDTA-free protease inhibitor cocktail (Thermo Scientific) in ultrapure water. After centrifugation (45 min, 4 °C, 16,000 × g), the resulting supernatant was desalted to a final lipid concentration of 25 mg mL⁻¹. The complete detergent removal was achieved by Bio-Beads exchange after 1, 2, 14, and 16 h. The proteoliposomes were collected by ultracentrifugation at 16,000 × g at 4 °C in a Sorvall mX120 Micro-Ultracentrifuge. The proteoliposome pellets were resuspended in buffer containing 20 mM MOPS/NaOH pH 7, 100 mM NaCl in 1 mL TCEP (treated with Chelex) to a final lipid concentration of 25 mg mL⁻¹ (Irot = 1 mg mL⁻¹). Incorporation of Iot in proteoliposomes was determined by SDS-PAGE (4–15% Tris-Glycine Mini-PROTEAN gels, BioRad) through analysis of the supernatant and the resuspended proteoliposome pellets. Flash-frozen proteoliposomes were stored at −80 °C prior to encapsulation in ZIF.

Determination of specific ATPase activity of CopA-detergent micelles encapsulated in ZIF upon exfoliation. CopA protein immobilized in ZIF, stressed and subsequently exfoliated was buffer exchanged to remove EDTA by passing through a 5 mL Hitrap desalting column (GE Healthcare). ATPase assays were performed using solutions prepared in Chelex treated MilliQ water. Protein concentration upon exfoliation was determined by SDS-PAGE densitometric analysis parallel with the ATPase assays. CopA solutions were prepared in a 9:1 well plate format. The 96-well plate carrier wells were filled with a mixture of ϵ-MOPS/NaOH pH 7, 100 mM NaCl, 1 mM EDTA (ATPase assays). ATPase assays were carried out for the protein encapsulated in ZIF and stressed with different temperatures (25, 55, and 80 °C) or protein denaturant (0.1% SDS (w/v)).

Preparation of Lp@ZIF. Two metal-to-ligand ratios were used for the encapsulation of blank liposomes. The lower ratio (20 : 16) encapsulation was performed as follows: 40 μL of a 12.5 mg mL⁻¹ stock solution of blank liposome were added to a mixture of 4326 μL of M-buffer (20 mM MOPS, 100 mM NaCl, and 1 mM TCEP pH = 7) and 334 μL of 3 M 2-methylimidazole. After thoroughly mixing, 100 μL of 1 M zinc acetate dihydrate were added to the solution. Immediately after adding the mixture, the mixture went from colorless to cloudy. The solution was incubated for 18 h at RT and centrifuged at 2071 × g for 15 min. The resulting pellet was washed twice with ultrapure water. After washing, the pellet was either left to dry at RT or

Desiccator. The following day, lipids were hydrated and suspended 1 mM TCEP NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-021-22285-y | www.nature.com/naturecommunications

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used as is. Accordingly, controls consisting of pristine ZIF were prepared by combining 4366 μL of M-buf (20 mM MOPS, 100 mM NaCl, and 1 mM TCEP pH = 7) and 800 μL of 1 M zinc acetate dihydrate. The mixture was incubated for 18 h at RT, centrifuged at 2071 × g for 15 min and the supernatant collected for further characterization. The ZIF pellet was washed twice with ultrapure water, and either dried at RT or used as is. For the higher metal to ligand ratio (40:16) added of 1.5 mg mL⁻¹ of ZIF Lp composite was incubated for 18 h at RT, centrifuged at 2071 × g for 15 min and the supernatant collected for further characterization. The ZIF pellet was washed twice with ultrapure water, and either dried at RT or used as is.

Preparation of IroT@ZIF. A batch of freshly prepared IroT@ZIF was incubated in 0.1% sodium-dodecyl sulfate (SDS) for 30 min, and thoroughly washed five times with ultrapure water. The pellet was harvested by centrifugation at 2071 × g for 15 min, immediately exfoliated using a mixture of 20 mM MOPS, 100 mM NaCl, 1 mM TCEP, 0.05 M EDTA pH = 7.5, and CYMAL-7 (0.05% (w/v)) and characterized through SEC. Controls included non-stressed pristine –80 °C refrigerated and non-encapsulated stressed IroT.

Preparation of CopA@ZIF. Two concentration ratios of zinc acetate dihydrate and 0.05% (w/v) DDM were used (20 × 16 and 40 × 16). For the 20 × 16 condition, 1000 μL of 0.5 mg mL⁻¹ CopA stock was combined with 3333.3 μL of 3 M 2-methylimidazole and 3370 μL of 20 mM MOPS, 100 mM NaCl, 1 mM DTT pH = 7 and 0.05% (w/v) DDM. The resulting aqueous solution was thoroughly mixed, and 100 μL of 1 M zinc acetate dihydrate was added. For the 40 × 16 ratio, 1000 μL of 0.5 mg mL⁻¹ stock solution of purified CopA was combined with 1066.7 μL of 3 M 2-methylimidazole and 2733.3 μL of 20 mM MOPS, 100 mM NaCl, 1 mM DTT pH = 7 and 0.05% (w/v) DDM. The resulting aqueous solution was thoroughly mixed, and 200 μL of 1 M zinc acetate dihydrate was added. CopA@ZIF crystals were harvested by centrifugation at 2071 × g for 15 min. The obtained crystals were washed twice with ultrapure water. The resulting bio-composites were kept as a suspension by leaving a layer of ultrapure water (~200 μL). Supernatants from first wash were collected and used for determination of encapsulation efficiency.

Preparation of CopA-PL@ZIF proteoliposome bio-composites. 500 μL of 12.5 mg mL⁻¹ CopA proteoliposome stock were combined with 246 μL M-buf (20 mM MOPS, 100 mM NaCl, and 1 mM TCEP pH = 7), and 214 μL of 3 M 2-methylimidazole. After carefully mixing, 40 μL of 1 M zinc acetate dihydrate was added. Immediately after addition of the zinc solution, a milky suspension was obtained. The reaction was aged at RT for 18 h, centrifuged at 2071 × g for 15 min, washed twice with water and twice with methanol to remove unreacted precursors and either used as is or left drying at RT. The reaction was aged at RT for 18 h, centrifuged at 2071 × g for 15 min, washed twice with water and twice with methanol to remove unreacted precursors and either used as is or left drying at RT.
CopA-PL@ZIF temperature stressing. Freshly extruded CopA proteoliposomes were encapsulated as formerly mentioned and used for temperature stressing following identical experimental guidelines as for blank liposomes and IrO4 detergent–protein micelles discussed above. After stressing, samples were immediately exfoliated and characterized by performing ATP-hydrolysis activity assays.

Cy5 liposome encapsulation. Cy5 fluorescence dye was encapsulated into liposomes prepared with E. coli polar lipids and L-α-phosphatidylcholine 3:1 ratio (w/w) (Avanti Polar Lipids). Liposomes were diluted to 12.5 mg mL⁻¹. Final lipid concentration with M-buffer (20 mM MOPS, 100 mM NaCl, and 1 mM TCEP pH = 7). Cy5 was added to a final concentration of 200 nM and encapsulated to the liposome lumen by freeze–thaw membrane fracture followed by extrusion through PC membranes with decreasing pore sizes (1 µm, 400 nm, and 200 nm), similar to the liposome preparation protocol. Excess Cy5 was removed by three successive ultracentrifugation and resuspension steps with M-buffer at 160,000 × g for 45 min. A Sorvall mX120+ Micro-Ultracentrifuge for 45 min. The Cy5-encapsulated liposome mixture was finally resuspended to the initial volume with the M-buffer for ZIF immobilization.

Lp@ZIF encapsulation efficiency determination. Both 20 × 16 and 40 × 16 Lp@ZIF composites were prepared for determination of encapsulation efficiency. Briefly, the 20 × 16 Lp@ZIF composite was prepared by mixing 4326 μL of M-Buffer, 534 μL of 3 M MIM, 40 μL of 12.5 mg mL⁻¹. Then, 100 μL of 1 M zinc acetate dihydrate was added to the mixture. On the other hand, the 40 × 16 Lp@ZIF composite was prepared by 3693 μL of M-Buffer, 1067 μL of 3 M MIM, 40 μL of 12.5 mg mL⁻¹. Further, 200 μL of 1 M zinc acetate dihydrate was mixed with into the solution. Composites were then allowed to sit at RT for 18 h. Supernatants were collected upon centrifugation at 4000 × g for 10 min. Emission spectra were then measured on the collected supernatants. Samples were placed in a sub-micro quartz cell (Starna Cells) and emission spectra collected from 640–700 nm with 1 nm increments at 25 °C in a spectrophotometer (Horiba scientific Fluoromax-4) at an excitation wavelength of 620 nm, using an excitation and emission slit width of 5 nm.

Cationic liposome preparation. For liposome preparation with cationic lipids possessing positive polar heads, lipid composition was selected as follows: E. coli polar lipids: 1,2-dioleoyl-3-trimethylammonium propane (DOTAP): L-α-phosphatidylcholine in a 1:2:1 ratio (w/w) (Avanti Polar Lipids). Liposomes were prepared following the same protocol as for liposomes with negative polar heads.

Zn²⁺ liposome binding assay. Liposome samples were diluted to 12.5 mg mL⁻¹ final lipid concentrations with the M-buffer and freshly extruded through PC membranes with decreasing pore sizes (1 µm, 400 nm, and 200 nm) prior to the experiment followed by three freeze–thaw cycles. Each liposome sample was separately treated with 1 M Zn(acetate)₂ or Zn(OH)₂ stock solution to set the final Zn²⁺ concentration to 20 mM and 40 mM and incubated at RT for 1 h to allow Zn²⁺ binding and then incubated by three successive rounds of ultracentrifugation and resuspended with M-buffer steps (160,000 × g, 4 °C, 45 min) in a Sorvall mX120+ Micro-Ultracentrifuge. Zn²⁺ bound liposomes were digested with 35% HNO₃ for 48 h. Samples were then diluted to 2% HNO₃ and Zn²⁺ bound to the liposome measured with inductively-coupled plasma mass spectrometry (ICP-MS).

Material characterization. Surface morphology was investigated using a Zeiss Supra 40 scanning electron microscope at 2.5 kV and a working distance of 6–10 nm. Each sample was sputtered with ~40 Å layer of gold. Proteoliposomes and blank liposomes were observed before and after encapsulation on a JEOL JEM-1400 plus transmission-electron microscope of 120 kV.

Surface area measurements were carried out on a Micrometrics ASAP 2020 surface area analyzer by nitrogen adsorption under 77 K. Surface areas were assessed through Brunauer–Emmett–Teller (BET) method and pore sizes calculated by a non-localized density functional theory with a carbon slit pore model. Samples were activated prior to surface area measurements by soaking with MeOH and drying them under vacuum for 24 h. The MeOH solution was decanted and replaced with dichloromethane. Samples were dried again under vacuum for an additional 24 h. Prior to analysis samples were degassed at 120 °C under vacuum for 12 h.

PXRD data was measured using a Rigaku SmartLab X-ray diffractometer equipped CuKα (1.54060 Å) at 30 mA and 40 kV. Samples were activated through MeOH and DCM soaking, dried under vacuum for 24 h, and degassed under N₂ for 12 h. Phase identification was done using the ZIF phase analysis software developed by Carraro et al. Data for each individual PXRD collected was uploaded into the software and analyzed from 6° to 39° (2θ). Dynamic-light scattering was measured with a Malvern Panalytical Zetasizer Nano ZS. Measurements were collected using a disposable cuvette at RT with a 633 nm laser source, a medium refractive index of 1.33, a material refractive index of 1.51, and a scattering angle of 179°.

TGA thermal stabilities were tested using a TA Instruments SDT Q600 Analyzer, from 30 to 800 °C, under a N₂ atmosphere, with a heating rate of 5 °C min⁻¹.

Sample shipping. Samples were pipetted into sturdy glass vial into a single bubble padded UPSF mailer and shipped via ground service from Dallas, Texas to a recipient in the state of Rhode Island, USA. Upon arrival and subsequent storage for two months at RT, the proteoliposomes encapsulated in ZIF–L were exfoliated and ATPase assays were carried out on the proteoliposomes refilled in M buffer (20 mM MOPS pH = 7, 100 mM NaCl, 1 mM DTT), after ultracentrifugation (Sorvall mX120+ Microcentrifuge, min, 4 °C, 160,000 × g) to remove EDTA. Control assays were done on the shipped and exfoliated proteoliposomes.

Time-resolved small-angle X-ray scattering (SAXS). Time-resolved SAXS have been collected at the SAXS beamline (ELETRRA synchrotron light source) at Elettra. The operation at photon energy of 6 keV covering the range of momentum transfer, q = 4π sin(θ)/λ, between 0.1 and 7.2 nm⁻¹. We monitored the kinetics of the nucleation and growth of MOF particles by using a commercial stopped flow apparatus (SF4, Bio-Logic, Grenoble, France) especially designed for Synchrotron Radiation SAXS investigations. Three independently driven syringes were filled respectively with the Zn(acetate)₂, the 2-methylimidazole and the liposome solutions. With three step-motors we controlled the volumes mixed in a microcomer that was subsequently injected in a horizontal 1 mm quartz capillary placed in the X-ray beam (Supplementary Fig. 8A). The volume ratio between the three solutions was set to maintain the final concentrations used for the syntheses in batch.

For each experiment, a total volume of 1 mL was injected in the microcomer and then, the solution was transferred to a 1 mm capillary horizontally oriented (Supplementary Fig. 8A). The start of the mixing sequence is triggered from the X-ray data-acquisition system, which took images with a time resolution of 100 ms (detector: Pilatus 3M1, Dectris Ltd, Baden, Switzerland; sample to detector distance: 1260 mm, and determined with a silver behenate calibration sample). All the experiments were performed at RT. The ligand solution was measured to record the background that was subtracted from the data sets. The resulting two-dimensional images were radially integrated to obtain a 1D pattern of normalized intensity versus scattering vector q⁻². With this set-up, we investigated the nucleation and the growth of the samples 20 × 16 Lp@ZIF, 20 × 16 ZIF, 40 × 16 Lp@ZIF, 40 × 16 ZIF and the crystallization kinetics of 40 × 16 Lp@ZIF, 40 × 16 ZIF.

The crystallization kinetics of the samples 20 × 16 Lp@ZIF and 20 × 16 control were investigated by manually filling the precursors into a vertically positioned glass capillary (Supplementary Fig. 8B). This was necessary because of the rapid flocculation of the particles that inhibit their detection in the horizontally mounted stop-flow capillary (Supplementary Fig. 8C). The precursors (total volume: 1 mL) were pre-mixed in an Eppendorf tube and mixed following the same procedure described for the preparation of the batch samples. After mixing the precursors, the solution was transferred in a 1.5 mm capillary using a syringe. Then, the capillary was sealed with parafilm and mounted vertically on the SAXS sample holder. The measurement started 120 s after the mixing of the precursors. Every 8 s, a SAXS pattern was collected and three different vertical positions of the capillary were investigated over time. This means that a specific vertical position was measured every 24 s. The scan over three different positions were performed to simultaneously investigate the bulk of the solution and the precipitate that accumulates at the bottom of the capillary over time.

Data analysis was performed using the software package Igor Pro (IGOR Pro 7.0.8.1, WaveMetrics, USA). The Invariant Q is related to the Pof Q invariant of the scattering curve and is defined as:

\[ Q = \frac{\int dq q^2 \times I(q)}{\int dq} \quad (1) \]

where \( q_1 = 0.1 \text{ nm}^{-1} \) and \( q_2 = 0.6 \text{ nm}^{-1} \). The increase of Q over time describes the formation of agglomerates/particles within the investigate volume. A plateau in the time series of Q values, indicates stationary conditions. To evaluate the morphology of the growing particles, the SAXS patterns were fitted. For the plate-like morphology, a simplified model for quasi-infinite large plates with the thickness T was used. This model is based on the Cauchy integral and the asymptotic behavior of the scattering being proportional to \( 1/q^2 \) in the q-range \( q \ll 1/T \) due to the large (infinite) lateral size of the plate-like particles. The form factor \( P(q) \) is:

\[ P(q) = \frac{1}{\left(1 + \frac{q^2}{\xi^2}ight)^2} \quad (2) \]

with \( \xi \) as thickness correlation parameter. \( \xi \) can be related to plate thickness T with \( T = \sqrt{2\xi} \), if compared with the Guinier approximation for quasi-infinite plates. The integrated scattering intensity I(200) is defined as:

\[ I(200) = 4q_2 \int dq_1 \times I(q) \quad (3) \]

where \( q_1 = 5 \text{ nm}^{-1} \) and \( q_2 = 5.4 \text{ nm}^{-1} \).
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F.C.H., S.S.A, and N.S.A. contributed equally to this paper. Primary paper writing and editing was done by F.C.H., G.M., and J.J.G. Blank liposome, IroT, and IroTPL encapsulation, stressing, was done by F.C.H. IroT expression, purification, proteoliposome preparation, SECs of IroT/IroTPL were performed by S.S.A. CopA expression purification, proteoliposome preparation, and ATPase activity assays were done by N.S.A. CopA/CopA-PL encapsulation and stressing were done by Y.H.W. SDS was done by F.C.H., S.S.A, N.S.A., and Y.H.W. DLS was done by F.C.H., S.S.A., and Y.H.W. TEMs were taken by O.R.B. SEMs were taken by M.A.L. and O.R.B. PXRDs were done by F.C.H. and M.A.L. TGA was done by A.D.S. Nitrogen sorption and BET analysis were done by S.D.D. The synthesis of Lp@ZIF was independently verified and SAXS/WAXS experiments were performed by F.C., H.A, and P.F. SAXS/WAXS data was interpreted by F.C., H.A., and P. F. Funding was raised by R.A.S, G.M., and J.J.G.

Competing interests
The authors declare no competing interest.

Additional information

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