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Article

Native Mass Spectrometry of Membrane Protein Lipid and Ligand Complexes Using *de novo* Designed WRAP Domains

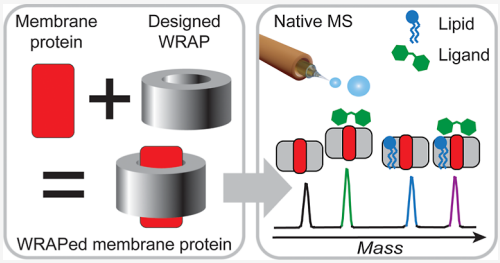
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ABSTRACT: Membrane proteins engage in dynamic transient interactions with surrounding lipids along with their substrates. Capturing these concomitant contacts often remains a challenging problem. Here, we demonstrate that generative deep learning-designed WRAP domains can preserve weak interactions of membrane proteins in native mass spectrometry. Using WRAP-fused GlpG, AqpM, and OmpA as model systems, we show that lipids can bind in multiple positions inside the WRAP, allowing for their characterization by native mass spectrometry. We show that lipid interactions can be altered by changing the WRAP structure. We use the approach to show that WRAP−OmpA binds phosphatidylethanolamine lipids within cavities formed at the protein−WRAP interface, while simultaneously accommodating weak chitobiose ligand binding. These findings establish WRAPs as versatile vehicles to probe ligand and lipid interactions of membrane proteins.



INTRODUCTION

Membrane proteins generally exist in a complex environment, embedded in the lipid bilayer with hydrophilic surfaces exposed to the cytoplasm, periplasm, or cell exterior. Within the bilayer, the protein surface is coated with a shell of loosely associated lipids, which are in dynamic exchange with the surrounding bulk lipids, along with a distinct subset of lipids that bind at specific sites.[1,2] As a result, they often engage in multiple simultaneous interactions with the lipids in the bilayer and their surface-exposed ligands or substrates. Capturing these interactions has become increasingly important in recent years for understanding the mechanisms underlying the folding, oligomerization, and function of membrane proteins.

Native mass spectrometry has emerged as a useful tool to capture membrane protein−lipid complexes.[3] Membrane proteins are ionized while embedded in detergent micelles or other membrane mimetics, or inserted into lipid vesicles.[4−6] This protective environment is then removed through controlled collisions with gas molecules inside the vacuum region of the mass spectrometer to release the intact complexes for mass analysis.[6−9] Soft-landing approaches have demonstrated that the tertiary and quaternary structures of membrane proteins remain essentially intact throughout the process.[10] Similarly, tightly bound lipids or ligands remain attached during complex release.[11−13] However, weak interactions with lipids or ligands are more sensitive to collisional activation and may therefore be distorted or lost during micelle or vesicle removal.[14] As a result, the detection of labile ligand interactions has remained an open challenge in native MS.

As a possible solution, we turned to protein design. Deep learning has recently been used to design a solubilizing protein domain (WRAP) around the membrane-facing region of the target membrane protein.[15] The WRAP sequence is fused to its target membrane protein and provides a stably folded cage around the transmembrane region (TM). The inner surface of the WRAP domain is sterically and hydrophobically matched to the respective membrane protein, whereas the outer surface is characterized by a hydrophilic salt bridge network that ensures high stability and solubility. WRAPed membrane proteins are not membrane-inserted during translation and can readily be purified as soluble proteins.[15] We therefore speculated that WRAPs may enable the preservation of labile ligand interactions with membrane proteins for native MS.

RESULTS

WRAPs Can Transport Membrane Proteins from Solution to the Gas Phase

First, we evaluated the use of WRAPs as vehicles for membrane proteins in native MS. As target, we selected the *Escherichia coli* membrane protease GlpG, which has been characterized extensively by native MS in detergent-solubilized form.[16,17]

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GlpG acquires a high number of charges when released from DDM micelles at high activation energy (trap voltage 150 V), indicating partial unfolding (Figure 1a).[18] Importantly, GlpG still retains a cardiolipin (CDL) molecule after detergent extraction, indicating a specific, functional lipid interaction.[16]

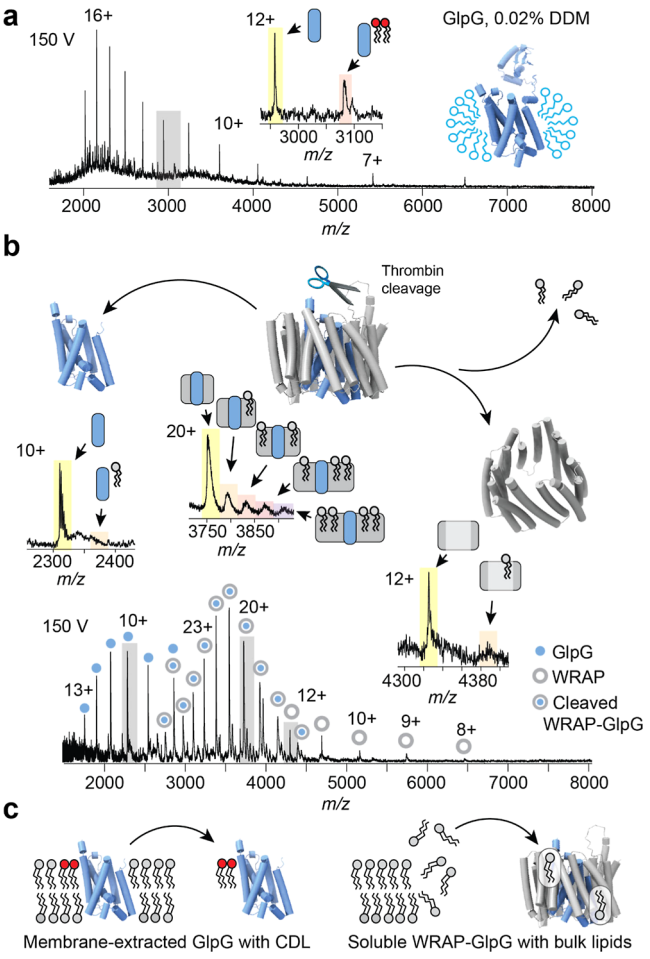


Figure 1. WRAPed GlpG binds membrane lipids. (a) Native mass spectrum of full-length GlpG released from DDM micelles at a trap voltage of 150 V shows highly charged protein ions and a specifically retained CDL adduct. (b) Mass spectra of the cleaved fusion protein show adducts consistent with lipid binding to the noncovalent GlpG–WRAP complex. Dissociation of the complex releases highly charged GlpG and lower-charged WRAP, and concomitant loss of lipid adducts. Charge states highlighted with gray shading are shown as zoom in the insets. The predicted structure is shown as an inset with GlpG in light blue and the WRAP domain in gray. (c) Membrane-extracted GlpG retains CDL bound in the membrane, whereas WRAP–GlpG traps bulk lipids solubilized during purification.

We then subjected GlpG fused to an N-terminal WRAP domain via a polyglycine linker with a thrombin cleavage site to native MS analysis. The fusion protein could be analyzed without detergent and produced a single narrow charge state distribution which was sensitive to chemical unfolding, as expected for a soluble, globular protein (Figure S1a,b). Next, we performed enzymatic cleavage of the linker between WRAP and GlpG. Mass spectrometric analysis of the enzymatically cleaved WRAP–GlpG protein at an activation energy of 100 V showed mainly the intact WRAP–GlpG complex (Figure S1c). Raising the activation energy to 150 V, which is commonly used for detergent removal, resulted in three distinct ion populations, corresponding in mass to the intact fusion protein, free GlpG, and the empty WRAP domain. At 200 V, we observed loss of signal for the fusion protein, and increased signal for highly charged GlpG and lowly charged WRAP (Figure S1c). We conclude that GlpG remains noncovalently bound by WRAP after cleavage and can be dissociated and unfolded in the gas phase, analogous to the release of a native membrane protein from a detergent micelle (Figure S1d).

We observed that the noncovalent WRAP–GlpG complex retains a low amount of adducts with an average size of 730 Da, in good agreement with the average mass of an *E. coli* membrane lipid. Interestingly, lipids are not observed for free GlpG or empty WRAP, suggesting that they might only interact with the intact GlpG–WRAP protein. However, we did not detect any adducts with a mass of 1.4 kDa that would indicate a bound CDL molecule (Figure 1b). This finding suggests that the protein is not inserted into the membrane, as this would likely have resulted in CDL retention. We speculate that the low-intensity adducts are lipids that are solubilized when bacteria are lysed during purification and subsequently become trapped by the GlpG–WRAP fusion protein (Figure 1c). This process would imply that lipid binding to WRAPed membrane proteins is largely driven by abundance instead of lipid type.

Lipids Bind to Membrane Proteins Inside the WRAP

Having established that WRAPs can transport and preserve membrane proteins in the gas phase, we further investigated their ability to interact with lipids. For this purpose, we selected Aquaporin M from *Methanothermobacter marburgensis* (AqpM) and the transmembrane domain of the outer membrane protein A from *E. coli* (OmpA) which excludes the soluble peptidoglycan-binding domain involved in dimerization, fused to N-terminal WRAP domains. Both proteins could be purified from the soluble fraction of *E. coli* expression cultures and buffer-exchanged into ammonium acetate in the absence of detergent. Native WRAP–AqpM and WRAP–OmpA can be detected without a need for extensive activation. Mass spectra show that both proteins exhibit up to three high-intensity adducts of ca. 730 Da each, consistent with the presence of copurified lipids (Figure 2a).[15] Lipid binding does not differ notably between charge states, unlike what has been reported for protein-detergent systems, which may be affected by charge redistribution upon detergent dissociation.[18,19] We speculate that the even distribution of charged residues on the WRAP surface promotes stable gas-phase charge configurations that prevent lipid dissociation (Figure S2a).

Dissociating lipids from detergent-solubilized membrane proteins usually requires an additional activation step.[20] WRAPed proteins can be dissociated directly in the ion trap. Indeed, all adducts from WRAP–OmpA could be completely stripped off at an activation energy of 150 V (Figure S2b). To identify the adducts, we isolated the 13+ ion with a single adduct and recorded MS/MS spectra at a trap voltage of 100 V. Dissociating the complex resulted in multiple singly charged peaks in the low m/z range consistent with sodiated phosphatidylethanolamine (PE) with acyl chains of 16–18 carbons (Figure S2c). Sodium adduction to the dissociated lipids may be promoted by the high density of acidic residues on the WRAP domain and the WRAP–OmpA interface (Figure S2a). PE is the most abundant lipid in the bacterial membrane, suggesting that PE adducts are not selectively

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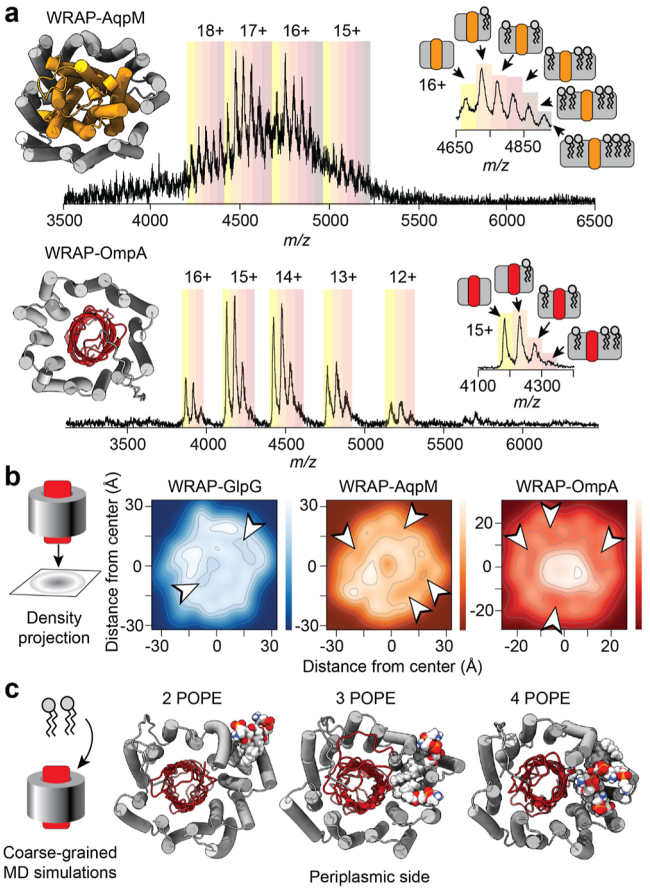


Figure 2. The transmembrane domains of WRAPed membrane proteins bind lipids. (a) Native mass spectra of WRAP−AqpM (top) WRAP−OmpA (bottom) show prominent additional peaks corresponding to up to three adducts. The predicted structures are shown as insets with the WRAP domains in gray. (b) Atom density projections along the transmembrane axis of WRAP−GlpG, WRAP−AqpM, and WRAP−OmpA indicate loose packing between the WRAP domain and AqpM or OmpA, but to a lesser extent for GlpG. Arrows indicate areas with low atom density. (c) CG−MD simulations of WRAP−OmpA with POPE clusters in solution show insertion of the lipids on the periplasmic side of the protein. Representative snapshots of simulations with two, three, or four POPE molecules are shown. WRAP is colored in gray, OmpA in red, and lipids are rendered as spacefill.

retained but reflect a stochastic association. However, PE is a major component of the outer membrane where OmpA is located, making it a likely interaction partner.

To test whether the lipids may be wedged between WRAP and the TM barrel of OmpA, we employed an MS-based detergent competition assay.[21] Briefly, the membrane protein−lipid complex is exposed to high concentrations of the detergent nonyl glucoside (NG) and analyzed by native MS. Lipids in exposed positions are easily displaced by the detergent and sequestered in the empty micelles, whereas lipids in protected positions, such as high-affinity binding sites, remain bound. Mass spectra of WRAP−OmpA incubated with 0.5% NG recorded under gentle conditions show broad peaks, as expected for detergent binding. When the excess detergent was removed through activation in the gas phase, the lipid adducts remained essentially unaffected (Figure S3a,b). We conclude that the copurified PE lipids are not sensitive to detergent competition, consistent with binding inside the WRAP domain (Figure S3c).

The fact that WRAP−AqpM and WRAP−OmpA display more pronounced lipid adducts than WRAP−GlpG suggests that they differ in their ability to accommodate lipids inside their WRAPs. To investigate this possibility, we computed the atom densities of the proteins and plotted the results as a density projection along the transmembrane axis (Figure 2b). WRAP−GlpG shows a relatively even atom density. WRAP−AqpM and WRAP−OmpA show a decreased density between membrane protein and WRAP, indicating free space that can potentially be occupied by lipids.

To confirm that lipid binding to wrapped membrane proteins is sterically feasible, we performed coarse-grained molecular dynamics simulations (CG−MD) of WRAP−GlpG and WRAP−OmpA in solution in the presence of two, three and four POPE molecules diffused in an aqueous medium (Figure 2c). These simulations show that both WRAP−OmpA and WRAP−GlpG can accommodate up to four POPE molecules able to bind in the interface between WRAP and the membrane protein. In all cases, lipids were found to insert transiently and interact with the protein via their acyl chains and headgroups. In most replicas of the WRAP−GlpG simulations, the POPE molecules bind near transmembrane helices 1−4 and orient toward the cytoplasm (Figure S4a). This site is able to accommodate up to four POPE molecules and has been previously identified as a cardiolipin binding site in GlpG.[16] Superimposition of GlpG simulated in the membrane[16] with the WRAP−GlpG shows good agreement between the poses of two POPE molecules and the native CDL ligand at the primary site (Figure S4b). Secondary binding sites for POPE have also been observed in a subset of replicas. These sites are located near transmembrane helices 5 and 6, and near helices 3 and 6, in the vicinity of the catalytic helix and facing the periplasm (Figure S4a).[22]

WRAP−OmpA and WRAP−AqpM are also able to accommodate multiple POPE lipids in CG−MD simulations. For WRAP−AqpM, lipid clusters insert on the periplasmic side (Figure S4c). For WRAP−OmpA, lipids in most replicas tend to be trapped within the WRAP helices 11−14 and not interact with the OmpA interface, adopting a random orientation instead (Figure S4d). However, in a subset of replicas, POPE molecules diffuse toward the WRAP−OmpA interface and bind to OmpA. Two POPE interaction sites can be identified: The primary site is located near the transmembrane strands 5, 6, and 8 and can accommodate up to four POPE molecules (Figure 2c), and the secondary binding site, which is occupied less frequently by 1−2 POPE, is located near strands 1−4 (Figure S4d). The headgroups of lipids in the primary site face the periplasmic side of the protein, but do not interact with the periplasmic loops. Lipids rather bind to the transmembrane strands, as would happen in a membrane. POPE binds more persistently to WRAP−AqpM and WRAP−OmpA than to WRAP−GlpG, with some interactions lasting for 60% of the simulation time.

The combined observations from nMS and MD raise the question of whether lipids inside the WRAP exhibit features of native lipid interactions in the membrane. MD simulations of OmpA in model bilayers show lipids interacting with a ring of tyrosine and tryptophan residues below the membrane surface on the periplasmic side, and with basic residues facing the outer leaflet, but no high-affinity binding sites.[23−25] This interpretation is supported by the fact that detergent-purified

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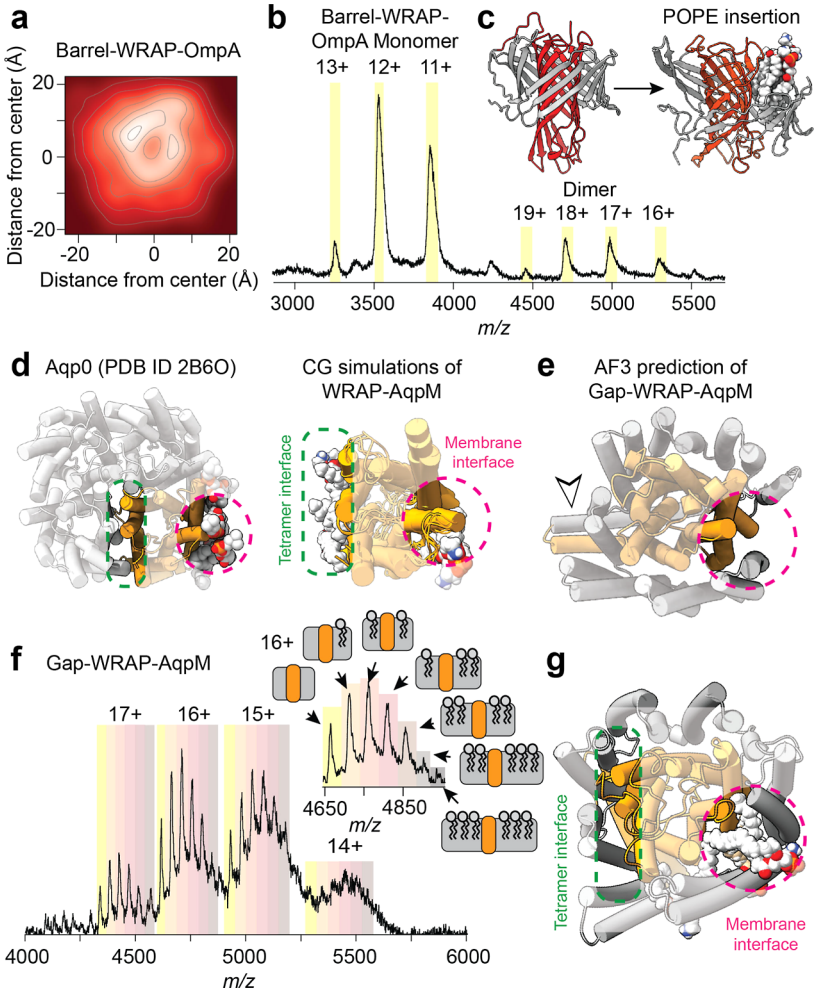


Figure 3. Modulating the WRAP structure affects lipid binding. (a) The density projection of OmpA with a β-barrel WRAP shows no discernible gaps between WRAP and target protein. (b) The native mass spectrum of barrel−WRAP−OmpA reveals no lipid adducts as well as a small amount of dimers. (c) CG simulations of barrel−WRAP−OmpA with three POPE molecules indicate that lipid insertion disrupts the WRAP structure. WRAP and OmpA are colored gray and red, respectively, in a representative simulation snapshot. (d) The 2D crystal structure of Aqp0 in a bilayer shows native lipid interactions at the membrane-facing side of the tetramer (pink circle), as well as a lipid-free subunit interface (green circle). Lipids are shown as spacefill models on a single subunit (orange) for clarity. In CG simulations of WRAP−AqpM, lipids occupy both sides of the AqpM monomer. An overlay of four simulations is shown, with the WRAP removed for clarity. (e) AF3 model of the Gap−WRAP−AqpM protein, where the last helix of the WRAP is attached to the C-terminus of AqpM (arrow), leaving a gap that exposes helices 3, 4, and 9 of the membrane-facing side of the AqpM monomer (pink circle). The WRAP domain is colored gray and the AqpM monomer orange. (f) nMS spectrum of Gap−WRAP−AqpM recorded under identical conditions as WRAP−AqpM (Figure 2a) shows lower charge states and well-resolved lipid adducts. Insert: The 16+ charge state of Gap−WRAP−AqpM is shown, with higher-intensity peaks for the second and third lipid adduct than the same charge state for WRAP−AqpM (Figure 2a). (g) Periplasmic view of representative snapshots of CG simulations with Gap−WRAP−AqpM and 3 POPE molecules show lipid binding to the membrane-facing side of the AqpM monomer (pink circle).

OmpA does not retain lipid adducts.[26] In the CG-MD simulations of WRAP−OmpA with POPE, we detect instances of these interactions, with 8 of 16 simulated lipids across four replicates clustering around tyrosines 55, 85, and 168, as well as arginine 103 (Figure S4e). The data suggest that lipids can engage in native-like interactions with the OmpA surface, but unlike in the bilayer simulations, these interactions are localized and not distributed around the transmembrane domain. We conclude that the placement of the lipids is influenced by the presence of interaction sites on the target protein, but also by the spacing and flexibility afforded by the WRAP, which dictates where the lipids can insert.

WRAP Modifications Affect Lipid Binding

The fact that lipids can easily insert into the helical WRAPs prompted us to ask whether WRAPs can be designed to exclude lipids. For this purpose, we tested OmpA with a rigid β-barrel-WRAP that closely matches the surface of the OmpA transmembrane domain as judged by the density projection (Figure 3a). When prepared under identical conditions as WRAP−OmpA, this variant (termed barrel-WRAP−OmpA) retained no lipid adducts (Figure 3b). Interestingly, the barrel-WRAP variant also forms dimers in nMS. The soluble loops of OmpA have been suggested to contribute to dimer formation,[25] hence the dimers in nMS would be compatible with less steric hindrance due to the smaller β-barrel compared to the helical WRAP. CG simulations suggest that lipid insertion disrupts the contacts between β-strands, disrupting the WRAP (Figure 3c). We conclude that WRAPs can be designed to exclude lipids, however, this requires closely matching WRAP and target protein surface with minimal flexibility.

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We then considered the opposite scenario, i.e. inclusion of additional space, using the already lipid-rich WRAP−AqpM. AqpM forms a native tetramer in the membrane, with one side of each monomer exposed to the surrounding bilayer and the other engaged in tight interactions with the two neighboring subunits. 2D crystals of the homologue Aqp0 from sheep lens tissue show extensive lipid binding on the membrane side, whereas the subunit interfaces are lipid-free (Figure 3d).[27] In CG-MD simulations of WRAP−AqpM, lipids accumulate at two places inside the WRAP: near helices 3, 4, and 9 of the membrane-facing side of AqpM, and near helices 1 and 2 of the subunit interface (Figure 3d). While the first site is similarly occupied by lipids in the Aqp0 crystal structure,[27] lipids at the second site are an artifact of WRAPing the monomeric protein.

The presence of lipids at physiological and nonphysiological sites prompted us to include additional space at the membrane-facing side by modifying the WRAP structure. We chose a variant where the last helix of the WRAP domain (helix 16) is located on the C-terminus of the AqpM sequence, and helix 15 of the WRAP is fused directly to the N-terminus of AqpM.[15] This variant, which we termed "Gap−WRAP−AqpM", closely matches the molecular weight and amino acid composition of the intact WRAP−AqpM (Table S1), but exposes helices 3, 4, and 9 of the natively membrane-facing side of AqpM (Figures 3e and S5a). In nMS, Gap−WRAP−AqpM yields well-resolved spectra with lower charge states compared to WRAP−AqpM (Figure 3f). The lipid binding pattern is also shifted, with the second and third adduct having a notably higher intensity in Gap−WRAP−AqpM than in WRAP−AqpM (Figures 2a and 3f). Both observations were found to be consistent across multiple protein preparations (Figure S5b). We conclude that the change to the WRAP structure affects the appearance of the fusion protein in nMS.

To investigate whether the changes in the mass spectra reflect a change in lipid interactions, we performed CG-MD simulations of Gap−WRAP−AqpM in the presence of 3 POPE molecules. As expected, we observe clustering of the POPE molecules at the membrane-facing side of the monomer. Across 12 replicas, only one lipid was bound at the subunit interface (Figure S5c). The lipids were found to insert not only into the gap in the WRAP domain, but also behind the first two helices of the WRAP (Figure S5d). While the intact WRAP remained relatively static over the course of the simulations, the Gap−WRAP displayed some rearrangements, notably partially closing the gap around AqpM, which trapped the bound lipids (Figure S5e). We speculate that the greater flexibility compared to the intact WRAP generally improves the accessibility of the membrane interface of the AqpM monomer and helps to direct lipid binding. In addition, the WRAP helix on the AqpM C-terminus collapsed onto the periplasmic surface of the protein (Figure S5d). Furthermore, the ability of the Gap−WRAP to rearrange likely increases the contact with the AqpM surface, which compacts the protein and may thus contribute to its lower charge states in nMS. Importantly, these data do not exclude other structural rearrangements or lipid interactions. However, our observations indicate that nMS can help to guide WRAP design for protein−lipid complexes.

WRAPs Enable Detection of Concomitant Lipid−Ligand Interactions with MS

The fact that we can detect complexes between WRAPed membrane proteins and lipids under gentle MS conditions suggests that the system can be used to probe simultaneous ligand and membrane contacts. To explore this possibility, we selected chitobiose (GlcNAcβ1−4GlcNAc), a disaccharide present on the Ecgp glycoprotein that is expressed by brain microvascular endothelial cells.[28] Ecgp acts as a receptor for OmpA which recognizes the chitobiose moieties, and in this manner mediates *E. coli* invasion in bacterial meningitis.[29] Computational simulations and *in vivo* studies have shown that loops 1, 2, and 3 of OmpA bind 1−2 chitobiose molecules.[30]

We previously used native MS to show ligand binding to WRAPed proteins.[15] In line with these observations, addition of excess chitobiose to WRAP−OmpA resulted in several additional peaks corresponding in mass to protein-saccharide complexes (Figure 4a). However, chitobiose and lipid binding

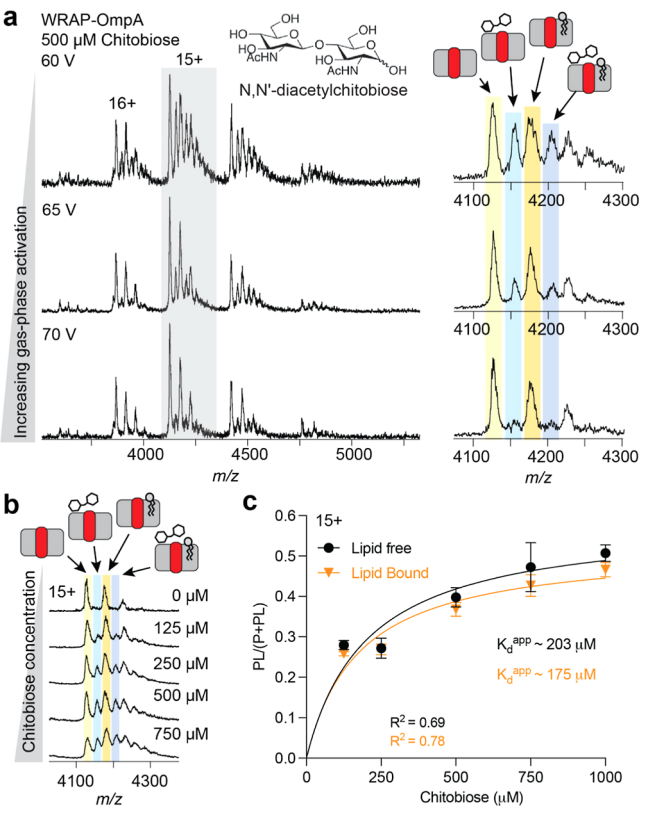


Figure 4. MS captures chitobiose binding to WRAP−OmpA−lipid complexes. (a) Mass spectra of WRAP−OmpA with chitobiose recorded at increasing trap voltages show facile dissociation of the saccharide ligand, but not the lipid adduct. A zoom of the 15+ charge state is shown to the right. (b) Zoom of the 15+ charge state at increasing chitobiose concentrations shows that increasing ligand binding can be monitored by native MS. (c) Plotting the ratio of PL to P + PL for WRAP−OmpA and WRAP−OmpA−PE binding to chitobiose for the 15+ charge state produces near-identical binding curves. Error bars indicate standard error of the mean (n = 4).

could not be distinguished directly, since two chitobiose molecules are similar in mass to a phospholipid. To determine complex stoichiometries, we took advantage of the fact that sugars bind relatively weakly in the gas phase whereas lipids require significant activation.[20,31,32] Collisional activation of the WRAP−OmpA−chitobiose complexes revealed that the second and fourth adduct peaks were removed near-completely at moderate trap voltages (60−70 V). The third and fifth adduct peak, on the other hand, did not show a comparable reduction in signal intensity (Figure 4a). These observations allow us to assign the second peak as a bound chitobiose, the

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third peak as a bound lipid, and the fourth peak as a complex with both a chitobiose and a lipid molecule. Notably, the trap voltages that dissociate the chitobiose complexes are below the range commonly required to dissociate detergent micelles from membrane proteins.[7] Therefore, these labile interactions would essentially be inaccessible without the use of WRAPs as membrane protein vehicles.

Since we can resolve simultaneous lipid and chitobiose binding, we asked whether both interactions affect each other. As outlined above, the copurified PE molecules likely resemble native membrane interactions with regard to both lipid type and location, making the insights potentially valid in the biological system. Molecular docking of a chitobiose molecule into the predicted structure of the WRAP−OmpA−lipid complex confirms ligand binding to the disordered loops L1−L3.[30] Plotting the fraction of intact chitobiose complexes with and without bound PE as a function of trap voltage shows essentially identical stabilities for the chitobiose complexes with WRAP−OmpA and with WRAP−OmpA plus one PE (Figure S6a). Next, we titrated WRAP−OmpA with chitobiose and monitored the change in signal intensities for the different ligand and lipid populations (Figure 4b). We then calculated the ratios of chitobiose-free and chitobiose-bound protein at each concentration. Plotting the PL/(PL + P) ratios as a function of ligand concentration shows no pronounced difference between chitobiose binding to WRAP−OmpA or to WRAP−OmpA with one PE adduct (Figure 4c). We also detect a notable difference in apparent K_Ds between charge states (Figure S6b). Close inspection of the spectra shows that while the intensity of the lipid adducts is comparable between charge states, the chitobiose adduct intensity is notably lower for the 16+ than the 15+ charge state (Figure 4a). This observation suggests that the sugar ligands, but not the lipid adducts, partially dissociate after desolvation as the more highly charged ions experience more collisional activation.

The highly similar stabilities and binding curves for chitobiose binding to lipid-bound and lipid-free WRAP−OmpA suggest that PE does not affect chitobiose binding. To contextualize this finding, we considered the native topology of OmpA. Chitobiose binds to the extracellular side of OmpA in the outer *E. coli* membrane during pathogen-host contacts. The outer leaflet is composed mainly of lipopolysaccharides, whereas the inner leaflet is rich in PE, which interacts nonspecifically with the periplasmic side of OmpA.[33] Consistent with the lipid distribution in the outer membrane, our CG−MD simulations show spontaneous PE insertion on the periplasmic side of the WRAP complex (Figure 2c). Since these lipids are therefore far removed from the chitobiose binding site on the extracellular side, they are unlikely to affect ligand binding (Figure S6c).

DISCUSSION

Several vehicles for membrane protein purification and characterization have been developed, ranging from bespoke detergents to nanodiscs and lipid vesicles.[34] Many of these approaches have been successfully interfaced with nMS, providing unique insights into the interaction between proteins and membranes, and each has specific advantages. For example, detergents can be optimized to support noncovalent complexes,[35] reconstitution into nanodiscs and vesicles provides a precisely tunable bilayer environment,[36,37] and vesicles can be derived directly from the native membrane.[38] A common feature of all nMS vehicles is the requirement for removal in the gas phase through collisional activation,[6,7,39] with the possible exception of entire proteins binding to nanodiscs, which can be determined from mass defects of the intact disc.[40]

We previously showed that WRAP domains can be used for membrane protein structure determination, but their potential for analysis of membrane protein−lipid complexes remained unexplored.[15] We therefore turned to nMS, which is a powerful technique for the interrogation of lipid interactions. Using WRAPs as vehicles, we show the release of target proteins in the gas phase, demonstrate lipid binding inside the WRAPs, and quantify concomitant lipid and ligand binding. WRAPs offer two advantages over other vehicles: they do not need to be stripped off in the gas phase, preserving weakly bound ligands, and they are easily modified, which raises the possibility to control interactions through targeted design.

Like other membrane protein vehicles used for nMS, WRAPs also have some limitations. For example, nMS can inform about the effect of lipids on membrane protein stability, detect lipid-mediated oligomerization, or identify native lipid binding partners.[11,41,42] However, all of these aspects are complicated when using WRAPs:

Lipids stabilize membrane protein ions via electrostatic and hydrophobic interactions, which can be determined from collision-induced unfolding profiles.[11,43] These subtle changes would likely be obscured by the presence of the WRAPs, which are stabilized by extensive salt-bridge networks, and also directly interact with the lipids.

Many oligomeric membrane proteins, including GPCRs and antiporters, assemble with the help of specific membrane lipids.[13,41] WRAP−mediated assembly under lipid-free conditions would favor specific oligomeric states that are normally regulated only after insertion into the membrane. Therefore, the current study focuses on lipid and ligand interactions of monomeric proteins as well as isolated protein subunits.

Based on our observations for OmpA and GlpG, lipid binding inside the WRAP involves nonspecific retention of membrane lipids during purification. Although MD simulations reveal similarities to native interactions, such as binding to aromatic and basic residues, it is likely that WRAP flexibility and spacing affect the type and pose of the bound lipids. Even though lipid binding can potentially be directed by changing the WRAP structure, these systems remain artificial. More targeted strategies, for example designing WRAPs for protein−lipid complexes from MD simulations, in combination with cell-free expression and loading with specific lipids, offer a potential way forward.

CONCLUSIONS

Our results highlight the potential of WRAPed membrane proteins for the study of lipid and ligand interactions under gentle native mass spectrometry conditions. By stabilizing transient contacts that are otherwise lost during conventional sample preparation, WRAPs provide new opportunities to dissect the interplay between membrane composition, protein folding, and functional ligand recognition. Future work may focus on rationally designing WRAP architectures with tailored lipid pockets or dynamic interfaces to better mimic native bilayers. Integrating this strategy with computational modeling, structural MS workflows, and functional assays could expand its applicability to diverse membrane protein classes, facilitating mechanistic insight and aiding drug discovery efforts.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.6c15293.

Supplementary methods Table S1: protein sequences Figure S1: mass spectra showing release of GlpG from WRAP Figure S2: MS/MS of WRAP−OmpA lipid adducts Figure S3: detergent competition assay for lipid binding to WRAP−OmpA Figure S4: MD snapshots of WRAP−lipid complexes Figure S5: nMS spectra and MD snapshots of WRAP−AqpM variants Figure S6: quantification and MD snapshots of WRAP−OmpA−chitobiose complexes (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lee, A. G. How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta Biomembranes* **2004**, *1666*, 62−87.

(2) Sych, T.; Levental, K. R.; Sezgin, E. Lipid-Protein Interactions in Plasma Membrane Organization and Function. *Annu. Rev. Biophys.* **2022**, *51*, 135−156.

(3) Bechara, C.; Robinson, C. V. Different modes of lipid binding to membrane proteins probed by mass spectrometry. *J. Am. Chem. Soc.* **2015**, *137*, 5240−5247.

(4) Barrera, N. P.; Di Bartolo, N.; Booth, P. J.; Robinson, C. V. Micelles protect membrane complexes from solution to vacuum. *Science* **2008**, *321*, 243−246.

(5) Hopper, J. T. S.; et al. Detergent-free mass spectrometry of membrane protein complexes. *Nat. Methods* **2013**, *10*, 1206−1208.

(6) Chorev, D. S.; et al. The use of sonicated lipid vesicles for mass spectrometry of membrane protein complexes. *Nat. Protoc.* **2020**, *15*, 1690−1706.

(7) Laganowsky, A.; Reading, E.; Hopper, J. T. S.; Robinson, C. V. Mass spectrometry of intact membrane protein complexes. *Nat. Protoc.* **2013**, *8*, 639−651.

(8) Landreh, M.; Costeira-Paulo, J.; Gault, J.; Marklund, E. G.; Robinson, C. V. Effects of Detergent Micelles on Lipid Binding to Proteins in Electrospray Ionization Mass Spectrometry. *Anal. Chem.* **2017**, *89*, 7425−7430.

(9) Reading, E.; et al. The role of the detergent micelle in preserving the structure of membrane proteins in the gas phase. *Angew. Chem. Int. Ed.* **2015**, *54*, 4577−4581.

(10) Fan, J.; De'Ath, C.; Eriksson, L.; von Hallerstein, C.; Persson, L. J.; Oluwole, A. O.; Naseeb, N.; Qureshi, A.; Mesoy, S.; Seeley, L. T.; et al. Membrane proteins retain native architecture through native ESI and soft-landing. *bioRxiv* **2026**.

(11) Laganowsky, A.; et al. Membrane proteins bind lipids selectively to modulate their structure and function. *Nature* **2014**, *510*, 172−175.

(12) Marcoux, J.; et al. Mass spectrometry reveals synergistic effects of nucleotides, lipids, and drugs binding to a multidrug resistance efflux pump. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9704−9709.

(13) Yen, H.-Y.; Hopper, J. T. S.; Liko, I.; Allison, T. M.; Zhu, Y.; Wang, D.; Stegmann, M.; Mohammed, S.; Wu, B.; Robinson, C. V. Ligand binding to a G protein-coupled receptor captured in a mass spectrometer. *Sci. Adv.* **2017**, *3*, No. e1701016.

(14) Mehmood, S.; et al. Charge reduction stabilizes intact membrane protein complexes for mass spectrometry. *J. Am. Chem. Soc.* **2014**, *136*, 17010−17012.

(15) Mihaljević, L.; Kim, D. E.; Bandawane, P. D.; Eisenach, H. E.; Borst, A. J.; Courbet, A.; Weidle, C.; Carr, K. D.; Bettin, E.; Liu, Q.; et al. Membrane protein solubilization and structure determination using de novo−designed proteins. *Science* **2026**, *393*, No. eadr3817.

(16) Abramsson, M. L.; Corey, R. A.; Skerle, J. L.; Persson, L. J.; Anden, O.; Oluwole, A. O.; Howard, R. J.; Lindahl, E.; Robinson, C. V.; Strisovsky, K.; et al. Engineering cardiolipin binding to an artificial membrane protein reveals determinants for lipid-mediated stabilization. *eLife* **2025**, *14*, RP104237.

(17) Hellwig, N.; et al. Native mass spectrometry goes more native: investigation of membrane protein complexes directly from SMALPs. *Chem. Commun.* **2018**, *54*, 13702−13705.

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(18) Yen, H.-Y.; et al. Electrospray ionization of native membrane proteins proceeds via a charge equilibration step. *RSC Adv.* **2022**, *12*, 9671−9680.
(19) Patrick, J. W.; Laganowsky, A. Generation of Charge-Reduced Ions of Membrane Protein Complexes for Native Ion Mobility Mass Spectrometry Studies. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 886−892.
(20) Landreh, M.; et al. Controlling release, unfolding and dissociation of membrane protein complexes in the gas phase through collisional cooling. *Chem. Commun.* **2015**, *51*, 15582−15584.
(21) Bolla, J. R.; et al. A Mass-Spectrometry-Based Approach to Distinguish Annular and Specific Lipid Binding to Membrane Proteins. *Angew. Chem. Int. Ed.* **2020**, *59*, 3523−3528.
(22) Bondar, A.-N. Mechanisms by Which Lipids Influence Conformational Dynamics of the GlpG Intramembrane Protease. *J. Phys. Chem. B* **2019**, *123*, 4159−4172.
(23) Bond, P. J.; Sansom, M. S. P. Membrane protein dynamics versus environment: simulations of OmpA in a micelle and in a bilayer. *J. Mol. Biol.* **2003**, *329*, 1035−1053.
(24) Deol, S. S.; Bond, P. J.; Domene, C.; Sansom, M. S. P. Lipid-protein interactions of integral membrane proteins: a comparative simulation study. *Biophys. J.* **2004**, *87*, 3737−3749.
(25) Ortiz-Suarez, M. L.; Samsudin, F.; Piggot, T. J.; Bond, P. J.; Khalid, S. Full-Length OmpA: Structure, Function, and Membrane Interactions Predicted by Molecular Dynamics Simulations. *Biophys. J.* **2016**, *111*, 1692−1702.
(26) Marcoux, J.; et al. Mass spectrometry defines the C-terminal dimerization domain and enables modeling of the structure of full-length OmpA. *Structure* **2014**, *22*, 781−790.
(27) Gonen, T.; et al. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. *Nature* **2005**, *438*, 633−638.
(28) Prasadarao, N. V.; Wass, C. A.; Kim, K. S. Endothelial cell GlcNAc beta 1−4GlcNAc epitopes for outer membrane protein A enhance traversal of Escherichia coli across the blood-brain barrier. *Infect. Immun.* **1996**, *64*, 154−160.
(29) Datta, D.; et al. Interaction of E. coli outer-membrane protein A with sugars on the receptors of the brain microvascular endothelial cells. *Proteins* **2003**, *50*, 213−221.
(30) Pascal, T. A.; et al. Experimental validation of the predicted binding site of Escherichia coli K1 outer membrane protein A to human brain microvascular endothelial cells: identification of critical mutations that prevent E. coli meningitis. *J. Biol. Chem.* **2010**, *285*, 37753−37761.
(31) Bolivar, E. G. B.; et al. Submicron Emitters Enable Reliable Quantification of Weak Protein−Glycan Interactions by ESI-MS. *Anal. Chem.* **2021**, *93*, 4231−4239.
(32) Bui, D. T.; Kitova, E. N.; Han, L.; Mahal, L. K.; Klassen, J. S. Quantifying Protein−Glycan Interactions Using Native Mass Spectrometry. *Mass Spectrom. Rev.* **2026**, *45*, 800−828.
(33) Shearer, J.; Jefferies, D.; Khalid, S. Outer Membrane Proteins OmpA, FhuA, OmpF, EstA, BtuB, and OmpX Have Unique Lipopolysaccharide Fingerprints. *J. Chem. Theory Comput.* **2019**, *15*, 2608−2619.
(34) Thoma, J.; Burmann, B. M. Fake It 'Till You Make It-The Pursuit of Suitable Membrane Mimetics for Membrane Protein Biophysics. *Int. J. Mol. Sci.* **2021**, *22*, 50.
(35) Alker, K.; Behnke, J.-S.; Urner, L. H. Recent Advances in Detergent Chemistry and Handling Support Membrane Protein Analysis Including Lipid Interactions. *Chem.—Eur. J.* **2025**, *31*, No. e202501549.
(36) Kostelic, M. M.; Zak, C. K.; Jayasekera, H. S.; Marty, M. T. Assembly of Model Membrane Nanodiscs for Native Mass Spectrometry. *Anal. Chem.* **2021**, *93*, 5972−5979.
(37) Panda, A.; et al. Direct determination of oligomeric organization of integral membrane proteins and lipids from intact customizable bilayer. *Nat. Methods* **2023**, *20*, 891−897.
(38) Chorev, D. S.; et al. Protein assemblies ejected directly from native membranes yield complexes for mass spectrometry. *Science* **2018**, *362*, 829−834.
(39) Marty, M. T.; Hoi, K. K.; Gault, J.; Robinson, C. V. Probing the Lipid Annular Belt by Gas-Phase Dissociation of Membrane Proteins in Nanodiscs. *Angew. Chem.* **2016**, *55*, 550−554.
(40) Townsend, J. A.; Marty, M. T. What's the defect? Using mass defects to study oligomerization of membrane proteins and peptides in nanodiscs with native mass spectrometry. *Methods* **2023**, *218*, 1−13.
(41) Gupta, K.; et al. The role of interfacial lipids in stabilizing membrane protein oligomers. *Nature* **2017**, *541*, 421−424.
(42) Gault, J.; et al. Combining native and 'omics' mass spectrometry to identify endogenous ligands bound to membrane proteins. *Nat. Methods* **2020**, *17*, 505−508.
(43) Landreh, M.; Marklund, E. G.; Uzdavinys, P.; Degiacomi, M. T.; Coincon, M.; Gault, J.; Gupta, K.; Liko, I.; Benesch, J. L. P.; Drew, D.; et al. Integrating mass spectrometry with MD simulations reveals the role of lipids in Na+/H+ antiporters. *Nat. Commun.* **2017**, *8*, 13993.

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