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Review Article

Mass spectrometry of intact membrane proteins: shifting towards a more native-like context

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Integral membrane proteins are involved in a plethora of biological processes including cellular signalling, molecular transport, and catalysis. Many of these functions are mediated by non-covalent interactions with other proteins, substrates, metabolites, and surrounding lipids. Uncovering such interactions and deciphering their effect on protein activity is essential for understanding the regulatory mechanisms underlying integral membrane protein function. However, the detection of such dynamic complexes has proven to be challenging using traditional approaches in structural biology. Native mass spectrometry has emerged as a powerful technique for the structural characterisation of membrane proteins and their complexes, enabling the detection and identification of protein-binding partners. In this review, we discuss recent native mass spectrometry-based studies that have characterised non-covalent interactions of membrane proteins in the presence of detergents or membrane mimetics. We additionally highlight recent progress towards the study of membrane proteins within native membranes and provide our perspective on how these could be combined with recent developments in instrumentation to investigate increasingly complex biomolecular systems.

Introduction

Integral membrane proteins comprise approximately one-third of the human proteome [1] and undertake key roles in signal and energy transduction, cell adhesion, molecular transport, and enzymatic catalysis [2]. Such functions are carefully regulated by post-translational modifications and biomolecular interactions that fine-tune protein activity in response to internal and external stimuli. When these mechanisms fail, the subsequent dysregulation of membrane protein function can induce a wide range of pathological conditions including cardiovascular, central nervous system, and immune disorders. Accordingly, certain classes of membrane proteins (e.g. G-protein coupled receptors [GPCRs] and ion channels) have been extensively employed as biological targets within drug discovery programs investigating such indications [3–5].

Understanding how endogenous binding partners and drugs impact the structures and function of membrane proteins is a key step towards elucidating protein structure–function relationships. Advances in experimental and computational techniques have enabled access to hitherto elusive knowledge of membrane proteins' working mechanisms and their interactions with other biomolecules. These methodological advancements range from developments in membrane protein crystallisation [6] and solubilisation methods [7] to high-resolution cryo-electron microscopy [8] and tools for predicting protein structure [9,10]. In parallel, native mass spectrometry (native MS) has emerged as a powerful technique that can provide unique insights [11–16]. Native MS analysis of purified membrane proteins provides direct information regarding purity, folding, modifications, oligomeric state, and sub-unit interactions in homo- or heterooligomers. Owing to the possibility of monitoring protein-ligand interactions with molecular-level

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precision, native MS has developed into a valuable tool for high-throughput drug screening platforms [17,18].

By integrating native MS alongside other structural techniques, deep insights into the relationship between protein sequence, structure, dynamics, and function can be derived. The ease of coupling native MS with ion-mobility and hydrogen-deuterium exchange MS enables access to information regarding protein folding, conformation, and dynamics [19–23]. Similarly, combining native MS directly with cryo-EM via selective deposition using electrospray ionisation offers additional benefits of increasing the scope and throughput of structural determination [24–26]. Native MS can serve as a preliminary tool to screen for the best protein homologues and optimal purification conditions for subsequent interrogation by other structural techniques [27,28]. Additionally, native MS enables the detection of lipids bound to membrane proteins [29,30] – offering valuable insights into the critical role of lipids in membrane protein structures and functions.

There has been a multitude of excellent, in-depth reviews focused on the study of membrane protein structure by native MS [15,31–33]. Herein we focus on recent studies investigating interactions between membrane proteins and endogenous ligands captured throughout the purification process and the dynamics of membrane proteins in signalling pathways observed directly in the native membrane. Finally, we will draw attention to the native top-down MS approach that combines native MS with top-down proteomics in a single experiment and give our perspective on how native top-down can be used to study complex signalling pathways in native membranes.

Native mass spectrometry of detergent-purified and detergent-free membrane proteins

A fundamental challenge associated with handling membrane proteins outside of their cellular context is to maintain their native fold in aqueous buffers. This is often achieved using detergents, which mimic the phospholipid bilayer [34,35]. Detergents can drastically impact the structure and function of solubilised membrane proteins [36], and an optimal detergent for a biophysical technique must be determined on a case-by-case basis [37]. Fortunately, there exists a wide range of detergents with varying properties to select from, with both commercial and specialist detergents offering assorted head groups and chain chemistries. Many detergents typically used to maintain protein stability and function are not always suitable for native MS measurements. For example, DDM (*n*-dodecyl β-D-maltoside) and LMNG (lauryl maltose neopentyl glycol) are often considered the preferred detergents for protein extraction and purification from cellular membranes as they create a stable environment for proteins [38]. However, these surfactants form relatively larger micelles which can only be removed from the protein in the gas phase using extensive collisional activation [39,40]. Therefore, when using DDM or LMNG in native MS measurements, it can be difficult to liberate the protein from the micelle without inducing ligand and/or subunit dissociation. By contrast, native MS-friendly detergents such as the charge-reducing C8E4 and LDAO, considered to provide a harsher environment for proteins, can be removed at lower collisional energies, assisting in the preservation of non-covalent interactions between membrane proteins and their binding partners. However, such detergents may be less effective at maintaining the protein's fold in solution. There is a delicate balance between activation energies and preserving interactions of membrane proteins in native MS analyses, so care must be taken in selecting the detergents for MS analyses [41,42]. Table 1 exemplifies the variety of detergents used for protein solubilisation and subsequent native MS analyses over the past 3 years. We hope that a future database presenting 'reference' mass spectra of membrane proteins characterised to date, along with suitable experimental conditions and instrument settings can guide the community in selecting optimal detergents suitable for new protein targets.

Excitingly, new detergents with modified headgroups, linkers, and chain configurations continue to emerge. A notable example in this regard is the development of a 'tailored' OGD-based detergent that combines the membrane solubilisation and protein extraction efficacy of DDM with the ease of protein release from C8E4 micelles [43]. Synthesis of such hybrid detergents containing two different headgroups [44] can foster the creation of detergents with predictable biophysical properties, paving the way for an ideal detergent tailored towards a given membrane protein target and downstream analysis technique. Nevertheless, an optimal detergent for solubilising and stabilising a given membrane protein target is currently difficult to predict.

Detergent-purified proteins can also be reconstituted into other membrane mimetics, such as bicelles, amphiphilic polymers, liposomes and nanodiscs, for native MS analyses [45,46]. However, the initial exposure to detergents may impact the observed oligomeric state of the protein, as well as strip away structurally or functionally important endogenous lipids and ligands. In those cases, adopting a completely 'detergent-free' strategy is desirable. To this end, membrane proteins can be directly solubilised by synthetic polymers, including SMA and DIBMA, forming the so-called native nanodiscs [47,48]. This detergent-free strategy is highly appealing since the protein is maintained in a lipid bilayer composed of its own native lipids. However, releasing intact protein complexes from SMALPs is

Table 1 Selected list of membrane proteins purified in various detergents and analysed by native MS in recent years (2020–2022)

Membrane protein	Detergent used for purification	Detergent used for native MS analysis	References
		2022	
Kirbac3.1	DDM	C8E4	[79]
Aqp0		C8E4	[12]
Aqp0	OG	C8E4	[56]
OmpF, FepA, BtuB	OG	OG	[65]
TqsA	DDM	LDAO, C8E4	[80]
FhaC	OG	OG	[81]
GCGR	DDM, CHS	OGDs G1	[82]
Glut5 PfHT1 GlpG NTR1	DDM DDM DDM LMNG	DDM DDM DDM, LDAO DDM, NM, DDTM, LDAO, C8E4, C12E8, LMNG, OGNG, Fos-choline-12, -14, -16, HEGA-11, MEGA-10, sucrose monodecanoate	[83]
NHA2	DDM, CHS	C12E9	[84]
β1AR	DDM	DDM/Foscholine16/cholesteryl hemisuccinate	[85]
MraY MurJ	DDM	DDM, OG, C8E4, LDAO, LDAO	[48]
NIP2;1	DDM, DM	LDAO	[86]
PA2880	DDM	DDM	[87]
PatAB	DDM	OGD G1	[88]
PMP22	Empigen BB, DM	C12E9	[59]
Rho and opsin	LMNG	LMNG	[54]
SthK	DDM	OGNG	[89]
TRAAK TREK2	C10E5	C10E5	[90]
		2021	
A2aR GCGR	LMNG G1, CHS	LMNG G1/CHS	[72]
AKCR1	LMNG, CHA	DDM	[91]
BAM complex	DDM	C8E4	[68]
bR, AR3	OG	OG	[30]
Kir3.4 Kir3.2	C10E5	C10E5	[92]
LptDE	DDM	C8E4	[67]
MFSD2A	DDM, CHS	DDM	[93]
RAGE	Triton X-100	Triton X-100	[94]
Rhodopsin	CHAPS	C8E4	[95]
SusCD	LDAO	C8E4	[74]
TmrAB	DDM	DDM	[96]
TolC	OG	OG	[97]
tOmpA, OmpAAG, OmpTrans2, OmpTrans3	DDM	DDM	[98]
Wzc	LMNG	C8E4, LDAO	[57]
		2020	
AceI	DDM	LDAO, C8E4, DDM, OGNG	[66]
AcrB	DDM	DDM, Triton X-100	[99]
AmtB	DDM	OGD	[100]
AqpZ MATE AmtB BAM NTSR1 OmpT	DDM and OGDs	OGDs	[43]
CD9, CD81	DDM	DDM	[101]
Cytochrome bc1 complex	GDN	GDN	[102]

Continued over

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Table 1 Selected list of membrane proteins purified in various detergents and analysed by native MS in recent years (2020–2022) (Continued)

Membrane protein	Detergent used for purification	Detergent used for native MS analysis	References
DGAT1			[103]
EmbB	DDM	C12E8, DDM	[104]
FapF, FapD	C8E4	C8E4	[76]
FptA	OG	OG	[105]
Get1/2	C12E9, Cymal-5/6, DM, UDM, DDM, LMNG, Cymal-5-NG	UDM, LMNG, C12E9, Cymal-5-NG	[106]
HAS	DM	DM	[107]
K2p4.1	C10E5	C10E5	[108]
Lci1	OG	LDAO	[109]
NHE9	DDM	DDM	[110]
OmpF	OG	OG	[111]
PSH	NG, LDAO	NG, LDAO	[66]
LeuT	DDM	OG	
MurJ	DDM	LDAO	
Semisweet	DDM	C8E4	[78]
DHODH	LDAO	LDAO	
CB1	LMNG	DDM, foscholine, CHS	
Beta-1 AR	DDM	DDM, foscholine, CHS	
OmpF	OG	C8E4	
AqpZ	DDM	C8E4	
AmtB	DDM	C8E4	
TSPO	DDM	C8E4	
V-ATPase	DDM	LMNG	[60]

nontrivial, potentially due to low charge acquisition by proteins embedded in the nanodiscs [49], the heterogeneity of the system, and strong interactions between the protein–lipid complex and the scaffold polymer. Over the past decade, significant efforts have been dedicated to optimising the protocols to eject samples from these membrane mimetics and in several instances, it was possible to capture protein-lipid interactions at even lower activation energies than those used to remove detergent micelles [33]. Additionally, selection of the appropriate membrane mimetic can have a significant impact on the observed results; for example, the proportion of PMP22 dimer was shown to be different in detergent micelles or when reconstituted into SCOR bicelles that better mimic the cholesterol and sphingolipid-rich native membrane environment (PMP22 is further discussed in a later section) [50]. Nanodiscs, that consist of a lipid bilayer surrounded by membrane scaffolding protein, have also been gaining popularity recently. Using nanodiscs with a 50:50 distribution of two different lipids allowed the investigation of lipid selectivity for two membrane proteins, AmtB and AqpZ [51]. AqpZ displayed the preference for PG over PE, and PC over either lipid; while AmtB was shown to be broadly enriched in bound PG, with a few tightly bound PC molecules. More complex mixed lipid nanodiscs that mimic various biological membranes were also recently investigated [52]. In combination with methodological improvements, such as controlling the stability of membrane protein nanodiscs by adding charge manipulation reagents [53], these developments could lead to even more widespread use of nanodiscs in native MS in the near future. Nevertheless, the possibility of scrutinising membrane proteins directly solubilised and purified in the complete absence of traditional detergents continues to gain interest. For example, a recent study demonstrates that SMA copolymer can selectively solubilise and maintain bacteriorhodopsin and archeerrhodopsin-3 in a correctly folded conformation, unlike detergents [30]. Further method developments, such as supercharging or new activation methods, can further improve the analysis of SMALP-solubilised membrane proteins. An exciting detergent-free method has recently emerged that involves the ejection of lipid vesicles derived directly from native membranes [54,55]; this is discussed in more detail in a later section.

Probing covalent modifications and non-covalent interactions of membrane proteins

Although the use of native MS in probing the interactions of membrane proteins has been well described in recent reviews [32,33], here we highlight the recent observations made in the past 3 years.

Covalent modifications

While native MS (not coupled with doing multiple rounds of MS/MS), does not provide localisation information on covalent modifications to proteins, it can still play a role in the characterisation of individual proteoforms. Such measurements can enable the effects of PTMs on protein interactions to be directly identified. For example, Harvey et al. [56] employed native MS to study post-translational modifications of AQP0, the most abundant membrane protein in the eye lens. By performing surface-induced dissociation on doubly phosphorylated tetramers, they discovered that these forms contain exclusively singly phosphorylated monomers, despite the presence of two phosphorylation sites on each monomer. In a different study, native MS was used to monitor the number of phosphorylations on Wzc, a protein involved in EPS regulation in Gram-negative bacteria, to complement high-resolution structural studies [57]. The non-phosphorylated form of the protein assembled into octamers while the multiply phosphorylated form was monomeric, indicating a potential regulatory mechanism for glycan polymerisation and translocation.

On the other hand, coupling native MS with tandem MS (MS^n), known as native top-down MS, can enable the identification and characterisation of specific proteoforms and post-translational modifications. In an elegant study, Ro et al. [58] recently coupled nanodiscs with native top-down MS and gained valuable insights into the active form of particulate methane monooxygenase, pMMO by determining the stoichiometry of copper ions bound to each subunit and detecting post-translational modifications.

Non-covalent interactions

Protein–protein

A more common application of native MS is in the investigation of non-covalent biomolecular interactions, such as protein–protein interactions. Fantin et al. [59] compared the oligomerisation of PMP22 between the wild-type protein and disease-associated mutants and detected decreased proportions of dimer for mild and moderate disease phenotypes but enhanced dimerisation for the severe disease mutants. By employing ion-mobility MS, the authors observed greater gas-phase stability of dimers but lower stability of monomers of all mutants compared with the wild-type protein with respect to unfolding. This led to a proposed pathophysiological mechanism in which disease-associated mutants are more likely to aggregate, resulting in extensive mistrafficking. As another example, native MS has been extremely useful for confirming the subunit composition of the V1 region of the mammalian V-ATPase [60].

Protein–lipid

Lipids can influence the structure and function of membrane proteins. For example, while some oligomeric membrane proteins contain strongly bound inter-subunit interfaces, other weakly oligomerise and readily dissociate into monomers or lower oligomeric forms upon extraction from their native lipid environment. It has been suggested [61] that in these cases, specific lipid interactions may be required for oligomeric assembly in the phospholipid bilayer. One approach to elucidate the effect of lipids on membrane proteins is to titrate a detergent-purified, fully delipidated sample with the most abundant lipids in their native membrane and quantify the relative binding affinities [62]. The specificity of bound ligands can then be ascertained by systematic titration with a delipidating detergent such as NG as demonstrated for the lipid II flippase MurJ [41]. In another study, MraY dimer, the first integral membrane enzyme in the biosynthesis of bacterial cell wall peptidoglycan, was found to be stabilised by endogenous lipids [63]. Further investigation by MS/MS and functional assays revealed the identity of these ligands as substrate and product of MraY-catalysed reaction (Figure 1). Furthermore, Patil et al. [64] found densities at the cavity formed by the transmembrane helices of human GPR158. PE and PI lipids were identified by native MS and lipidomics and remodelled into the EM structure with high confidence and their roles were ascribed to stabilising the dimer interface. More recently, native MS combined with chemical crosslinking was used to probe the organisation of bacterial outer membrane proteins and lipids [65]. The pattern of lipid crosslinks captured by native MS amongst various OmpF mutants reflects that the outermost leaflet of the outer membrane is enriched with lipopolysaccharides while the inner leaflets are mainly composed of phospholipids.

Protein–drug/antibiotics

Another interesting area of research is the interaction of membrane proteins with drugs or antibiotics. Using native MS, the antibiotic chlorhexidine was shown to induce a change in the oligomeric state of the chlorhexidine efflux pump AceI [66]. Chlorhexidine was also shown to cause an increase in the formation of the functional dimeric form of AceI. In 2021, Fiorentino et al. [67] investigated the mechanism by which an antibacterial peptide thanatin impairs LPS translocation through LptDE complex. Native MS confirmed the simultaneous binding of thanatin and LPS to LptDE, ruling out inhibition by competitive binding. This result, in combination with hydrogen-deuterium exchange MS and molecular dynamics simulations data, suggested a mechanism whereby thanatin obstructs LPS transport by

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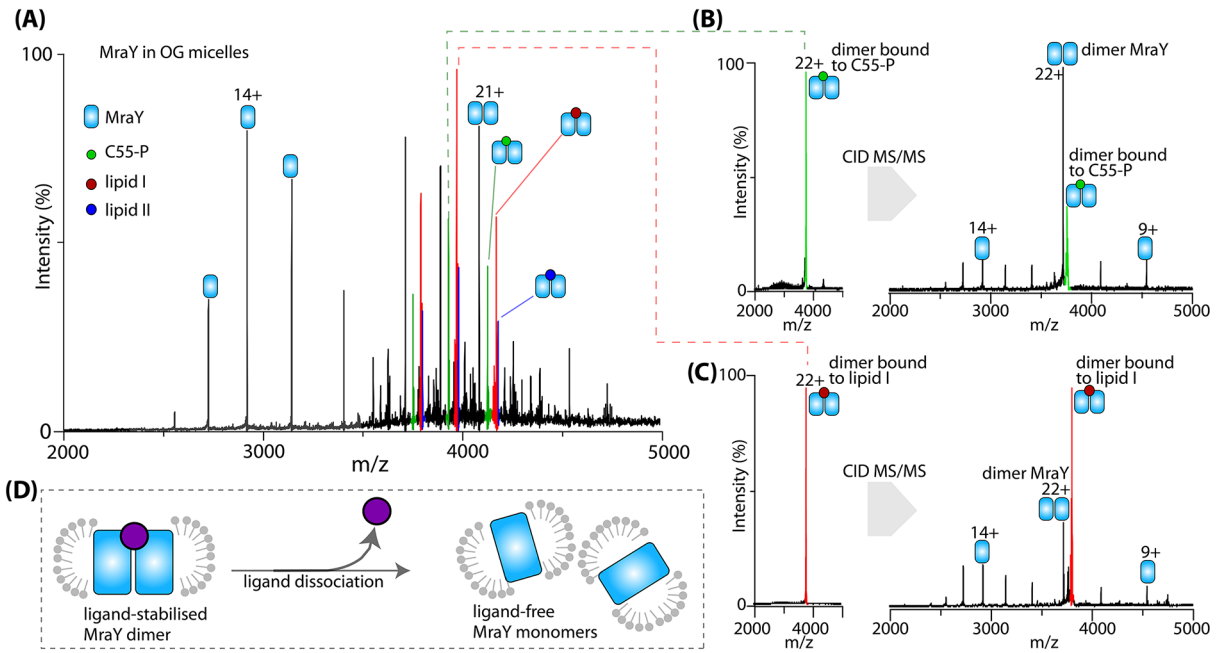

Figure 1. Native mass spectrometry of purified MraY

(**A**) Mass spectrum of MraY released from OG (octylglucoside) detergent micelles. Peaks in the spectrum correspond to the MraY monomer, the ligand-free dimer, and the dimer bound to undecaprenyl phosphate (C55P), lipid I and lipid II. (**B** and **C**) Dimer-bound to C55P (B) and lipid I (C) were separately isolated and subjected to collision-induced dissociation (CID) to liberate MraY protomers. (**D**) Schematic depiction of a ligand stabilised MraY dimer in detergent micelles. Removal of bound ligand caused the dimer to dissociate. Data reproduced from [63].

stabilising the inactive form of LptDE. In another study, a combination of native MS and MD simulations enabled the elucidation of the lipid-binding preference of the Bam complex [68]. The study revealed that the Bam complex preferentially binds cardiolipin and that the latter enhances its interaction with darobactin, a peptidomimetic antibiotic.

Protein–metal ion

One type of interaction that is challenging to observe by native MS is the binding of proteins to non-volatile salt ions, such as Na^+. Such ions have been previously shown to regulate the assembly of several membrane protein complexes [69]. In native MS, the presence of even relatively low concentrations of salts results in signal suppression, loss of resolution and spectra that are often too difficult to interpret. Consequently, native MS typically employs volatile salts, such as ammonium acetate, that are readily removed upon gentle activation [70]. Nevertheless, there are instances where metal ions are necessary cofactors for protein stability and enzymatic reactions. One way to study the effect of non-volatile salts on protein structure and stability is to use emitters with submicron tip diameters that form smaller electrospray droplets and thus greatly reduce the number of observed protein–salt adducts [71]. This approach was recently used by [72] to study the effects of sodium ions on two GPCRs: a Class A GPCR, A2aR, and a Class B GPCR, GCGR. For A2aR, native MS revealed enhanced Na^+ binding in the presence of antagonists and suppressed Na^+ binding in the presence of agonists. This result is consistent with previous structural studies that showed the presence of an allosteric Na+ binding pocket in inactive conformations of Class A GPCRs, which is absent in the active conformations. For GCGR, a more stable conformation was observed in NaCl/Tris buffer compared to ammonium acetate, as indicated by the lower charge state distribution and enhanced binding of the stabilising negative allosteric modulator, NNC0666, observed in the former buffer. These results highlight the applicability of native MS to study membrane proteins from physiologically relevant salt buffers. More recently, another native MS approach was developed to interrogate the propensity of β_1-adrenergic receptor coupling to G_s and G_i. Upon capturing the G-protein-coupling complexes in presence of different ligands, it was observed that endogenous zinc ions specifically stabilise the complexes between G-proteins and receptors. Overall, this study uncovered a novel role for metal ions in modulating the selectivity of G-protein coupling [73].

Protein–ligand/Enzyme–substrate

To probe the interactions with ligands, in one study Gray et al. [74] combined native MS analysis with other structural tools to identify the finite size of the substrate molecules (approximately 2.5 kDa) that are imported by the SusCD complex, a major fructooligosaccharides uptake system in Bacteroidetes. Native MS was also used to study the interactions of membrane proteins, involved in peptidoglycan biosynthesis pathway, with lipid-like substrates. The key step in the synthesis of peptidoglycan is the translocation of lipid II from the inner to the outer leaflet of the plasma membrane. The flippase MurJ is thought to play a dominant role over FtsW in this process. A previous native MS study revealed a stronger lipid II binding preference for MurJ over FtsW, and that the lipid II binding to MurJ is reduced in the presence of cardiolipins, which is suggestive of lipid-regulated precursor translocation [75]. Similarly, an increase in the binding affinities of MraY towards C55P, MurG towards lipid I, and MurJ towards lipid II were observed using native MS recently, suggesting that the affinity of each membrane enzyme for its native substrate promotes their trajectory along the peptidoglycan pathway [63].

Transferring membrane proteins into the mass spectrometer: a shift from isolated solubilised protein target towards endogenous proteoform complexes within their native membrane context

When using even the most 'native-like' membrane mimetic, there is always a risk of experimental artefacts caused by the extraction of the protein from its native membrane environment. Recently, membrane proteins and their complexes have been released into the mass spectrometer directly from their native membrane, marking a significant shift towards native MS of membrane proteins in the most native-like contexts. In 2018, Chorev et al. [55] used native MS to investigate protein complexes directly from native membrane vesicles. This technique named sonicated lipid vesicles MS (SoLVe MS) was successful in revealing a range of subunit interactions and lipid preferences for endogenous proteins that are abundant in the membrane. Several of the observed complexes were not previously reported, highlighting the potential of SoLVe MS. For example, the Bam complex with either a second BamE subunit or up to three cardiolipin molecules was observed, providing more structural insight about this complex. The interactions between SecYEG translocon and an F-type ATPase were also observed directly.

In a very recent study, Chen et al. [54] ejected retinal rod segments (ROSs) directly from the native bovine disc membrane into the mass spectrometer under controlled light conditions to uncover signalling events of a GPCR (rhodopsin) in the context of its own native membrane (Figure 2). Crucially, all components of the signalling pathway were observed in real-time and without any extensive sample pre-treatments. The study found that one of the key steps in the pathway, the hydrolysis of retinal, was significantly slower in native membranes compared to LMNG detergent micelle. In addition, the presence of PE lipids in ROS membranes was shown to be important for the regeneration of rho species following the original activation by light. Unsaturated PC lipids were observed to be enriched around rho shortly after light activation, shedding light on the role of lipid microenvironments for signalling facilitation. The ability to observe all the steps of the signalling pathway directly from the native membranes enabled kinetic studies in the presence of exogenously added downstream effector molecules, highlighting the potential application of this methodology in drug discovery workflows.

Despite the numerous advantages and a recently published protocol [76], the practical application of SoLVe MS remains challenging. The main obstacle is that the identities of various complexes observed in the spectrum are difficult to achieve through mass alone. Various other techniques exist that can be combined with native MS to further characterise species observed in SoLVe MS spectra. Native top-down mass spectrometry employing high energy collision- (CID HCD), electron- (ECD, ETD and EID) and photon- (IRMPD and UVPD) based activation techniques can be used to break covalent bonds (while preserving non-covalent interactions in some cases) to obtain information about protein sequence and identify proteoforms. Recent advances in this field have been outlined in a recent review [31]. In addition, co-purified lipids and other small molecules often need to be identified. A traditional approach to this problem is to perform lipidomics on the co-purified lipids in a separate experiment to native MS [77]; however, while this method can be very informative, the direct protein–lipid binding information is lost during the extraction step. The recent advances in MS instrumentation enabled the development of a hybrid method termed 'nativeomics' (also known as native top-down), this allows observation and identification of protein-bound lipids and ligands in a single experiment [78]. For instance, the application of this workflow to an outer membrane protein TSPO allowed previously unidentified electron density in the crystal structure of this protein to be assigned as PE lipid species [78].

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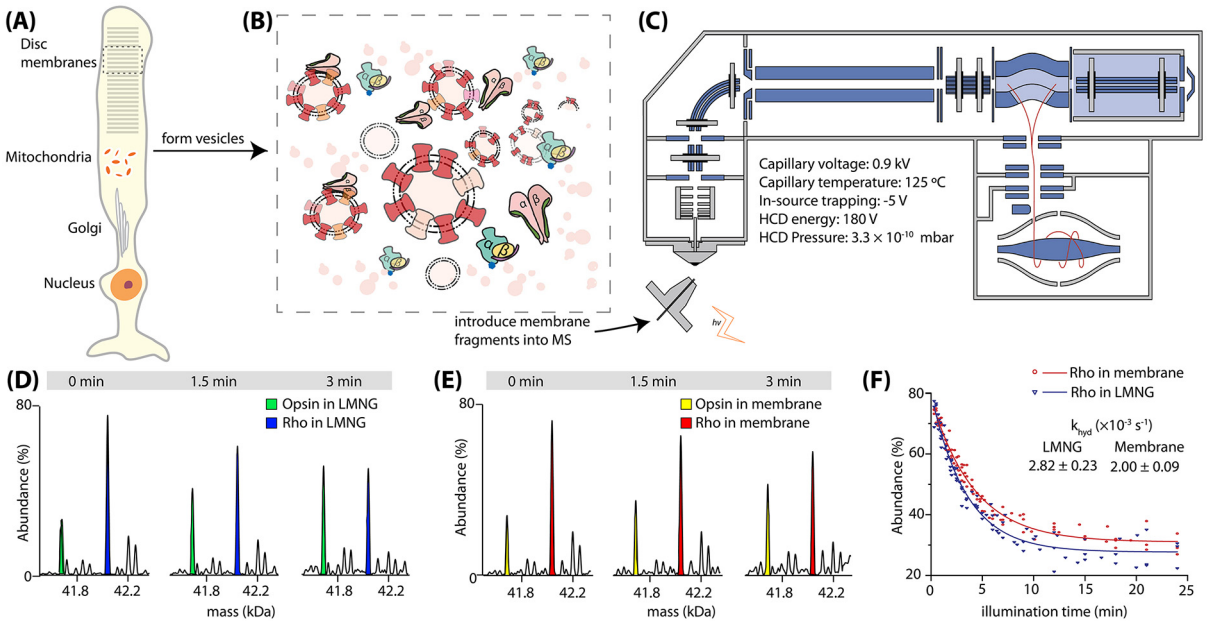

Figure 2. Rhodopsin signalling cascade in the native membrane captured by mass spectrometry

(**A–C**) Disc membranes of rod cells (A) are homogenised to form a heterogeneous distribution of vesicles (B) that are introduced directly into the mass spectrometer (C), a UHMR equipped with an LED light source. (**D,E**) Changes in the population of rho and opsin in LMNG (blue and green, respectively) and in the native membrane vesicles (red and yellow, respectively; Individual spectra are shown as zero-charge plots with illumination times stated. (**F**) Relative abundance of rho as a function of illumination time in LMNG micelles (blue) or native membranes (red). Figures reproduced from [54].

Future perspectives and concluding remarks

Native MS is a versatile and rapid technique for obtaining non-covalent interactions of membrane proteins that are often difficult or outright intractable to other techniques. It can be used to study membrane proteins in a varied range of membrane mimetic systems, notably in their own native lipid bilayer. Advances in the synthesis and commercialisation of mild and native MS-compatible detergents will continue to be relevant for investigating protein–drug/lipid binding where highly purified samples are required. Increasing the repertoire of activation regimes that enables release of intact oligomers from lipid bilayer nanodiscs will allow selective disruptions of the nanodiscs with minimal dissociation of the encapsulated protein complexes. Such an advance will pave the way for releasing intact proteins from more complex biological membranes and tissues for which strong lipid–protein interactions must be overcome.

Recent advances in native MS have enabled protein assemblies to be released from intact lens tissue [12]. For such a method to be widely applicable, it will be critical to identify novel sample preparation strategies and develop gas phase activation techniques that ensure efficient but selective dissociation. Tagging of endogenous proteins for enrichment with affinity binders and the development of new affinity probes to enrich the target proteins over other noninteracting members of the proteome will enhance the signal-to-noise ratio in applying this method to investigations of specific pathways. It will be interesting to be able to analyse various proteoforms in a healthy versus diseased state in a single experiment to accelerate diagnosis, monitor disease progression, and perform drug screening in the native context of their protein targets – towards this goal, native top-down holds great promise.

Summary

- In the modern era of structural biology, native MS has emerged as a powerful tool for defining the molecular sociology of a variety of membrane protein targets including transporters, channels, and receptors. Starting off with detergents and membrane mimetics, this area of research is now going through a transformational period towards more native environments.

- Although still in its infancy, analysis of complexes directly from native membranes by native MS has already managed to capture several key signalling events. However, the full potential and widespread use of this approach are yet to happen.
- In addition to optimising sample preparation strategies, there is a clear need for new instrumentation, which combines sensitivity, dynamic mass range, resolution, and access to diverse ion activation techniques including HCD, ECD, ETD, IRMPD and UVPD.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

A2aR, adenosine 2A receptor; C8E4, tetraethylene Glycol Monooctyl Ether; CID, collision induced dissociation; Cryo-EM, cryogenic electron microscopy; DDM, *n*-dodecyl β-D-maltoside; DIBMA, diisobutylene-maleic acid; ECD, electron-capture dissociation; EID, electron-induced dissociation; ETD, electron-transfer dissociation; GCGR, glucagon receptor; GPCR, G-protein-coupled receptor; HCD, higher-energy collisional dissociation; HDX, hydrogen-deuterium exchange; IRMPD, infrared multiple photon dissociation; LDAO, *n*-Dodecyl-N,N-Dimethylamine-N-Oxide; LMNG, lauryl maltose neopentyl glycol; MS, mass spectrometry; NG, nonylglucoside; OG, octylglucoside; OGD, oligoglycerol detergents; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SMA, styrene maleic anhydride; SMALPS, styrene-maleic acid lipid particles; SoLVe-MS, sonicated lipid vesicle mass spectrometry; UVPD, ultraviolet photodissociation; XL-MS, cross-linking mass spectrometry.

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