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Chemical additives enable native mass spectrometry measurement of membrane protein oligomeric state within intact nanodiscs

James E. Keener¹, Dane Evan Zambrano¹, Guozhi Zhang¹, Ciara K. Zak¹, Deseree J. Reid¹, Bhushan S. Deodhar¹, Jeanne E. Pemberton¹, James S. Prell², and Michael T. Marty^{1,*}

¹Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721 USA

²Department of Chemistry and Biochemistry, University of Oregon, Eugene, OR 97403 USA

Abstract

Membrane proteins play critical biochemical roles but remain challenging to study. Recently, native or nondenaturing mass spectrometry (MS) has made great strides in characterizing membrane protein interactions. However, conventional native MS relies on detergent micelles, which may disrupt natural interactions. Lipoprotein nanodiscs provide a platform to present membrane proteins for native MS within a lipid bilayer environment, but prior native MS of membrane proteins in nanodiscs has been limited by the intermediate stability of nanodiscs. It is difficult to eject membrane proteins from nanodiscs for native MS but also difficult to retain intact nanodisc complexes with membrane proteins inside. Here, we employed chemical reagents that modulate the charge acquired during electrospray ionization (ESI). By modulating ESI conditions, we could either eject the membrane protein complex with few bound lipids or capture the intact membrane protein nanodisc complex—allowing measurement of membrane protein oligomeric state within an intact lipid bilayer environment. The dramatic differences in the stability of nanodiscs under different ESI conditions opens new applications for native MS of nanodiscs.

Graphical Abstract:

*Corresponding Author mtmarty@email.arizona.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website and contains supplemental methods, discussions, figures, and tables.

Notes

The authors declare no competing financial interests. MetaUniDec software can be downloaded at <http://unidec.chem.ox.ac.uk>.

Introduction

Membrane proteins play important biochemical roles and make up the majority of drug targets.¹ Many membrane proteins function as protein complexes,² but it can be challenging to determine their stoichiometries within lipid environments, especially because specific lipids may influence the oligomeric state.³ Recently, native or nondenaturing mass spectrometry (MS) has made substantial contributions to understanding membrane protein oligomerization and lipid interactions.^{3–6} Conventionally, membrane proteins are solubilized in detergent micelles, and collisional activation inside the mass spectrometer removes the detergent to leave a “naked” membrane protein complex with any bound ligands or lipids.^{7,8} Mass analysis of the noncovalent complex reveals the stoichiometry of the membrane protein complex. However, detergents may disrupt membrane protein structure, function, and interactions.^{9,10}

To present membrane proteins in a more natural lipid environment, we have employed nanodiscs for native MS.¹¹ Nanodiscs consist of a nanoscale lipid bilayer surrounded by two membrane scaffold protein (MSP) belts.^{12,13} Nanodiscs are particularly well-suited for native MS due to their monodispersity, homogeneity, and optimal size. Numerous examples have shown that nanodiscs provide a more natural environment than detergent micelles that better preserves membrane protein stability and function.^{14–21}

However, nanodiscs do not efficiently release naked membrane proteins under conventional native MS conditions and are also not stable enough to detect the intact nanodisc with the membrane protein inside. Initial native MS of membrane protein nanodiscs required exceptionally high collisional activation to liberate membrane proteins.²² Subsequent studies with higher-resolution instrumentation revealed that low levels of activation eject membrane proteins with an annulus of bound lipids, but the intact nanodisc complex was not observed.^{23,24}

To address these limitations, we hypothesized that chemical charge manipulation reagents would modulate the stability of nanodiscs during native MS. Charge manipulation reagents are added at low concentrations to solution prior to ESI and often do not substantially influence protein structure in bulk solution.^{25–27} During ESI, they exert their influence by either increasing (supercharging) or decreasing (charge reducing) the charge acquired by the analyte.²⁸ Although the mechanisms of supercharging and charge reduction are still debated,

charge reduction has been shown to stabilize native-like protein complexes.^{29–31} Conversely, supercharging reagents can destabilize complexes.^{32–34}

Here, we discovered that nanodiscs respond to charge manipulation reagents and ionization polarity in unexpected ways that allow us to modulate the stability of nanodiscs to either eject membrane proteins or preserve the intact membrane protein nanodisc complex for native MS (Figure 1). Importantly, preserving the intact membrane protein nanodisc complex enables measurement of membrane protein oligomeric state without having to eject the membrane protein complex from the lipid bilayer. We considered several potential mechanistic explanations of these unusual observations and found that the mechanisms of supercharging are complex, eluding explanation by a single chemical or physical effect.

Methods

Materials

Propylene carbonate was purchased from Arcos Organics at 99.5% purity. 4-vinyl-1,3-dioxolan-2-one was purchased from Alfa Aesar at 99% purity. Glycerol carbonate was purchased from either Tokyo Chemical Industry Co., Inc. or CarboSynth (San Diego, CA) at >90% purity. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG) lipids were purchased from Avanti Polar Lipids. Ammonium acetate, Amberlite XAD-2, myoglobin, and sodium cholate were purchased from Sigma Aldrich. Membrane scaffold proteins were expressed in *E. coli* and purified by immobilized metal affinity chromatography (IMAC) as previously described.^{24,35} MSP1E3D1T2 scaffold protein was created by adding two additional threonine residues to the N-terminal region of MSP1E3D1 as previously described.²⁴ TEV protease was added to remove the polyhistidine tags from all MSPs. Membrane proteins AmtB and AqpZ were expressed and purified as previously described.^{23,24} Briefly, HIS-MBP-TEV-AmtB and AqpZ-TEV-GFP-HIS were expressed in *E. coli* and purified by IMAC and size exclusion chromatography (SEC) using a Superdex 200 16/600 (GE Healthcare) with buffers containing 0.025% dodecyl-maltoside (DDM) from Anatrache.

Nanodisc Assembly

Nanodiscs were assembled as previously described.^{23,24,36} Briefly, nanodiscs without membrane proteins were assembled by solubilizing POPC, POPG, or a mixture of POPC and POPG³⁷ in sodium cholate. MSP1D1T1(–)²⁴ was added to the lipids, and detergent was removed by addition of Amberlite XAD-2 hydrophobic beads. MSP1D1T1(–) is based on the conventional MSP1D1, which is engineered from human ApoAI and creates nanodiscs with a 9.7 nm diameter.¹² The (–) indicates that the polyhistidine purification tag has been removed, and the T1 variant has an additional threonine residue inserted near the N-terminus.²⁴ We chose this MSP variant because MSP1D1(–) has been the most extensively characterized by native MS, and the T1 variant has been engineered so that it is not isobaric with 29 POPC molecules, which complicates mass assignment with conventional MSP1D1(–). Nanodiscs were purified by SEC using a Superose 6 Increase 10/300 column (GE Healthcare) into 0.2 M ammonium acetate at pH 6.8.

Membrane protein nanodiscs were assembled by adding purified AmtB or AqpZ to cholate-solubilized POPC and a 1:1 mixture of MSP1E3D1(–) and MSP1E3D1T2(–).²⁴ MSP1E3D1(–) is similar to MSP1D1(–) but has three additional helices, which lead to formation of larger 12.8 nm nanodiscs.¹² We chose the larger MSP1E3D1 scaffold because it is more suitable for the larger AmtB and AqpZ oligomeric complexes. The T2 variant has two additional threonine residues near the N-terminus. The mixture of the MSP1E3D1(–) and the T2 variant was used to encode the stoichiometry of the MSP belt,²⁴ as described below. Following detergent removal, membrane protein nanodiscs were purified by IMAC and SEC. The polyhistidine tags were then removed by TEV protease and again purified by IMAC and SEC. Nanodiscs were concentrated to 1–10 μ M using a 100 kDa molecular weight cut-off concentrator (EMD Millipore) prior to native MS. Triplicate assemblies were measured to assess the reproducibility of the results.

Supercharging and Charge Reduction

Preliminary experiments with empty POPC nanodiscs optimized the concentration of charge manipulation reagents for maximum effect without significant loss of MS quality. For charge reduction, samples were mixed 9:1 v/v with 400 mM imidazole (IM) with the pH adjusted to 7 using acetic acid for a final concentration of 40 mM.²⁹ Controls were performed with addition of ammonium acetate to demonstrate that charge reduction effects were not caused by the slightly higher ionic strength or slight dilution of the analyte. For supercharging, samples were mixed 19:1 v/v with neat propylene carbonate (PC),³⁸ 4-vinyl-1,3-dioxolan-2-one (4V),³³ or glycerol carbonate (GC)³² to give a final concentration of 5% supercharging reagent by volume.

Mass Spectrometry

Nano-electrospray ionization (nESI) was performed using homemade borosilicate needles pulled with a P-1000 micropipette puller (Sutter Instrument, Novato, CA). Mass spectrometry was performed using a Q-Exactive HF quadrupole-Orbitrap mass spectrometer equipped with the Ultra-High Mass Range (UHMR) research modifications (Thermo Fisher Scientific).³⁹ Detailed instrumental parameters were used as previously described.^{24,36} Key instrumental parameters included 0.9–1.3 kV for capillary voltage, 200 °C for capillary temperature for samples run in positive ion mode, and 175–200 °C for negative ion mode. Scans were collected from 2,000–25,000 m/z at a target resolution of 15,000 with 10 microscans summed into one scan. The in-source trapping voltage applied in the injection flatpole was increased from 0–300 V in 20 V increments with 1- or 2-minute acquisitions at each step. For AqpZ nanodiscs, an additional 50 V of source fragmentation was applied to aid desolvation.

Mass Spectrometry Data Analysis

Native mass spectra were deconvolved using MetaUniDec as previously described.³⁶ The mass range was extended to an upper limit of 350 kDa, and the charge range was increased to 1–35. The average mass was measured as the weighted average of peaks above 50% relative abundance. Deconvolved spectra were summed across all collision voltage steps as previously described to provide a full picture of all species observed during dissociation.²³

Assignment of membrane protein nanodisc spectra relied on two techniques. First, we determined the stoichiometry of MSP using mixed MSP belts that differ in mass by 202 Da.²⁴ Because the belts mix randomly, complexes with one MSP produced doublet peaks, and complexes with two MSPs produced triplet peaks. Second, we used macromolecular mass defect analysis to assign the stoichiometry of the membrane protein complex.²³ Each nanodisc population contained a distribution in the number of lipids per nanodisc, which caused a series of peaks separated by the lipid mass. Macromolecular mass defect analysis divides the measured mass by the lipid mass and plots the remainder of the division (Figure 2). Peaks with different numbers of lipids but the same protein content have the same mass defect value, which helps to visualize and identify protein components. Mass defect values follow modular arithmetic and fall between 0 and 1. After determining the stoichiometry of MSP from the peak shape, we subtracted the known mass defect of the MSP belts. The remaining mass defect was caused by the membrane protein oligomers.

For example, the AmtB monomer has a mass of 42,261 Da and POPC has a mass of 760.076 Da. Because $42,261/760.076 = 55.60$, the AmtB monomer has a mass defect of 0.60. Because $0.60 \times 3 = 1.80$ and the integer part is lost during modular arithmetic, the AmtB trimer has a mass defect of 0.80 (Figures 2A and 2C).²³ MSP1E3D1(–) and MSP1E3D1T2(–) have mass defects of 0.45 (29,982 Da) and 0.71 (30,184 Da), respectively, so a mixed nanodisc containing one of each will have an additional mass defect of 0.16. Thus, a complex with AmtB trimer and two mixed MSP belts will have a mass defect of 0.96 (Figures 2B and 2D). In practice, nanodisc masses tend to be slightly larger than predicted from protein and lipid masses alone,^{23,40,41} likely due to incomplete removal of salt and/or other cosolutes. For example, MSP_{LH}AmtB₃ (Figure 2B and 2D) has a predicted mass defect of 0.96 but a measured mass defect of 0.06, which is around 76 Da (0.025%) higher than the predicted mass.

Results and Discussion

Supercharging and Charge Reduction of Empty Nanodiscs

Prior literature has shown that supercharging reagents generally destabilize noncovalent complexes toward unfolding or dissociation during native MS while charge reducing reagents are stabilizing.^{29–34} Charge reducing reagents are thought to remove charge from the analyte due to their basicity, causing less Coulombic repulsion between charges on the ion and thus greater stability.^{30,42,43} Supercharging reagents are thought to either increase the charge acquired by the analyte, which destabilizes complexes in the gas phase by increasing Coulombic repulsion,^{28,44,45} or to destabilize the complex in the electrospray droplet, leading to unfolding and subsequent acquisition of greater charge during electrospray due to higher surface area of the unfolded protein.^{32,46}

Thus, we predicted that nanodisc ions would be stabilized by charge reduction and destabilized by supercharging. We first tested this prediction using “empty” nanodiscs without membrane proteins. We assembled empty nanodiscs with MSP1D1T1(–)²⁴ belts and either POPC, POPG, or a 50/50 POPC/POPG mixture. Native mass spectra were collected for controls with no additives and with added IM, 4V, PC, and GC. Representative mass spectra and deconvolved mass distributions are shown in Figures S-1 and S-2. To investigate

their gas-phase stability, we examined how the average mass and charge of the nanodiscs changed as a function of collision voltage for triplicate nanodisc assemblies (Figure 3). SEC analysis revealed no measurable solution-phase changes to the Stokes diameter of empty nanodiscs upon incubation with the supercharging reagents, which demonstrates that charge manipulation reagents do not significantly disrupt the structure of nanodiscs in bulk solution.

In positive ion mode, we observed that addition of IM, a charge reducing reagent, increased the average number of lipids in empty POPC nanodisc ions (Figure 3A) while decreasing the average charge (Figure 3C), consistent with charge reduction stabilizing the nanodisc. On the other hand, addition of supercharging reagents 4V and PC showed lower average mass, indicating destabilization of the nanodisc by dissociation of lipids. Both MSP belts remained bound to the complex with 4V and PC. In contrast, GC was extremely destabilizing and dismantled the nanodisc into single MSPs with few bound lipids under the same instrumental conditions (Figure S-3). Interestingly, the average charge state of nanodiscs produced with supercharging reagents under low activation conditions was lower than that of controls with no additive (Figure 3C). This is consistent with a mechanism whereby ejection of charged lipids from supercharged nanodisc ions results in immediate loss of both mass and charge from the nanodiscs, even upon minimal activation.

In negative ionization mode, we observed that POPC nanodiscs are generally more stable to lipid loss (Figure 3B). We were unable to resolve peaks with GC in negative mode for empty nanodiscs. The only reagent that resulted in measurable destabilization was PC, and PC was less destabilizing in negative mode than in positive mode. With all reagent conditions, charge states for the nanodisc ions were substantially lower in negative mode than positive mode (Figure 3D), consistent with prior observations that negative mode leads to lower absolute charge states.^{47–49} The observation that nanodiscs with added 4V are stable in negative mode but unstable in positive further suggests that the effects of charge manipulation reagents are caused during or after ESI and not in bulk solution.

To evaluate how the lipid charge influences the effects of charge manipulation on empty nanodiscs, we evaluated nanodiscs with either 50% or 100% POPG.³⁷ At neutral pH, POPC is zwitterionic, but POPG is anionic. The 50% and 100% POPG nanodiscs were generally stabilized by charge reduction and negative mode (Figures S-4 and S-5). In positive mode, supercharging reagents did not have significant effects on POPG nanodiscs but were destabilizing for POPC/POPG. As with empty POPC nanodiscs, charge manipulation reagents had minimal effects in negative mode. In all cases, the charge states were lower in negative mode, indicating that the primary stabilizing effect is the lower charge of negative ionization rather than the chemistry of the lipid head groups. Compared to 100% POPC nanodiscs, the charges in positive mode were lower for all reagents with 100% POPG and lower still for 50% POPG, in agreement with past results with no additives.³⁷ It is not clear why addition of POPG lipids lowers charge states, but it may be due to the large number of anionic lipids loaded in the nanodiscs. Interestingly, the lower charge states for 100% and 50% POPG nanodiscs do not seem to prevent dissociation or eliminate the stabilizing effects of imidazole in positive mode.

These results are consistent with POPC being more likely than POPG to carry a positive charge due to the quaternary ammonium in the head group of POPC. Because the lipids are noncovalently bound in the nanodisc, charged lipids are more labile to dissociation upon ion activation due to Coulombic repulsion. In positive mode, supercharging reagents thus have a larger destabilizing effect on nanodiscs with POPC lipids because a greater number of lipids can become charged and labile to dissociation. These data show that head group chemistry can affect the stability of nanodisc ions toward lipid dissociation, especially under supercharging conditions in positive ion mode.

Supercharging Membrane Protein Nanodiscs

Based on the effects on empty nanodiscs, we predicted that 1) charge reducing reagents would stabilize membrane protein nanodiscs against loss of lipids and scaffold protein in positive mode; 2) supercharging reagents would destabilize membrane protein nanodiscs in positive mode; and 3) negative mode would be generally stabilizing. We tested these predictions by assembling POPC nanodiscs with trimeric AmtB or tetrameric AqpZ. MSP1E3D1(–), an MSP that creates 13 nm nanodiscs, was used to accommodate the larger membrane protein complexes.¹² To aid in interpretation of the spectra, we mixed conventional MSP1E3D1(–) with a variant containing two additional threonine residues, MSP1E3D1T2(–), in a 1:1 ratio.²⁴ Control experiments indicated that empty MSP1E3D1(–) nanodiscs behaved similarly to empty MSP1D1T1(–) nanodiscs (data not shown). Positive mode native MS of both AmtB and AqpZ nanodiscs without chemical additives reproduced earlier findings²³ where the membrane protein complexes were ejected with an annulus containing tens of bound lipids (Figures 4A and 5A).

Unlike empty nanodiscs, positive mode with IM showed no stabilization of AmtB nanodiscs (Figure 4B). Furthermore, negative mode spectra with either no additive or IM (Figures 4F and 4G) were challenging to interpret because the data had low signal and a high baseline. The limited peaks that were observed were ambiguous and could not be definitively assigned. Thus, negative mode with no additive or IM was not useful for these membrane protein nanodiscs.

Unexpectedly, supercharging reagents in positive mode had a range of different effects. PC was stabilizing in positive mode (Figure 4C). Although some dissociation products were observed with one or no remaining MSP belts, nearly intact complexes were observed at high abundance with two MSP belts, the AmtB trimer, and over 100 lipids. The stoichiometry of the MSPs was confirmed by the presence of triplet peaks,²⁴ and the trimeric stoichiometry of AmtB was confirmed with macromolecular mass defect analysis as described above.²³ Some spectra with 4V in positive mode showed evidence of nanodiscs with two MSP belts, but most of the signal was due to ejected AmtB trimer with many bound lipids, similar to no additive and IM (Figure 4D).

GC, on the other hand, dramatically destabilized the nanodisc complex in positive mode, leading to ejection of AmtB trimer with only a few bound lipids (Figure 4E). Even with no added collision voltage, the minimum levels of activation were enough to release AmtB from the nanodisc. Increasing levels of activation yielded stripped complexes without lipids but also dissociation of the trimer into monomers and dimers (Figures 2A and 2C).

Despite the dramatic differences in positive mode stability, each supercharging reagent showed very similar results and nearly complete stabilization of the nanodiscs in negative mode (Figures 4H, 4I, and 4J). We observed membrane protein nanodisc complexes near 300 kDa. As with PC in positive mode, triplet peaks confirmed the presence of two MSP belts (Figure S-6), and mass defect analysis confirmed the presence of AmtB trimer (Figures 2B and 2D). Each complex contained around 150 lipids. A bimodal distribution was often observed, which may suggest the loss of a small number of lipids as has been previously observed with empty nanodiscs.³⁷ Molecular modeling of AmtB trimer (PDB ID: 4NH2)⁵⁰ in POPC MSP1E3D1 nanodiscs using Charmm-GUI^{51,52} predicted a stoichiometry of 154 lipids for fully packed nanodiscs. Thus, we expect that these nanodiscs are nearly fully intact and have at most lost a small number of lipids. Remarkably, these nearly intact nanodisc complexes were stable up to 300 V of collisional activation, demonstrating the robust stabilization of membrane protein nanodiscs against dissociation with negative mode and cyclic carbonate reagents (Figure S-7).

Similar results were observed for AqpZ, demonstrating that these unusual observations are not specific to AmtB. IM did not show significant differences compared to controls with no additives, and 4V showed similar behavior to PC. Thus, data are shown for no additive, PC, and GC (Figures 5 and S-8). Like AmtB, AqpZ in positive mode is stabilized by PC and destabilized by GC. Addition of GC caused dissociation into monomers, dimers, and trimers (Figure S-9). Although the relative ratios of monomer, dimer, and trimer dissociation products varied between samples, there were generally more dimer products than have previously been observed in collisional dissociation of AqpZ in detergent micelles and nanodiscs.^{23,53,54} Interestingly, the average charge of dissociated dimers is around +8, which is similar to dimer fragments of AqpZ in detergent micelles produced by surface induced dissociation,⁵⁵ but it is unclear whether this is due to symmetric or asymmetric dissociation. In negative mode, both PC and GC are stabilizing and result in nearly completely intact nanodiscs (Figures 5E and 5F).

These data reveal that chemical additives combined with different ionization polarities can open new dissociation pathways for macromolecular complexes in the mass spectrometer. Whereas conventional conditions lead to ejection of the membrane protein oligomer with many bound lipids, supercharging reagents enable a range of dissociation products—ranging from the fully intact nanodisc to the nearly naked membrane protein ejected from the nanodisc. A summary of the effects of charge manipulation reagents on different types of nanodiscs is provided in Table S-1. These techniques illustrate unique new ways to manipulate the stability of nanodisc complexes that will enable future native MS of nanodiscs and other biomolecular complexes.

Considering Potential Mechanisms of Supercharging

Although prior literature has shown some examples of complexes showing no changes or even lower charge upon addition of supercharging reagents,³² the stabilizing effects of supercharging reagents have not been previously described in the literature and warranted some mechanistic consideration. As described above, we ruled out major solution-phase effects prior to ESI such as denaturing because SEC data showed no significant effects from

incubation with supercharging reagents and the results were polarity dependent; for example, GC was destabilizing in positive mode but stabilizing in negative mode from the same starting solution. Therefore, charge manipulation reagents act on nanodiscs during or after ESI.

Because stability of analytes in the presence of supercharging reagents is thought to play an important role in supercharging mechanisms,⁴⁶ we performed thermal stability analysis on nanodiscs with and without membrane proteins in the presence of different supercharging reagents. As described in the Supporting Discussion, PC, 4V, and GC are thermally destabilizing, but all analytes show a similar stability profile (Figure S-10A and Table S-2). Thus, thermal stability alone does not explain the observed differences between the effects of charge manipulation reagents on nanodiscs with and without membrane proteins.

Because PC and 4V behave differently than GC despite their chemical similarity, we considered some of their physical and chemical properties that have been proposed to play a role in supercharging mechanisms.³⁸ As described in the Supplemental Methods and Discussion, we measured the surface tension for PC and GC mixed into water or ammonium acetate solution (Figure S-10B) and calculated gas-phase basicity for all reagents (Table S-3). Differences in these values for the different reagents were minor. The only physical parameters investigated that exhibited large differences among the reagents were the boiling point and dielectric constant, but these do not directly explain the observed differences between positive and negative mode or between nanodiscs with and without membrane proteins. Thus, the influence of supercharging and charge reducing reagents on stability of biomolecular complexes during native ESI-MS is likely influenced by multiple physical and chemical factors.⁵⁶ Further research will be required to unravel the complex mechanisms underlying the stability differences.

Conclusions

We have shown that charge manipulation reagents have substantial and unexpected influences on the stability of nanodiscs during native ESI-MS. Empty nanodiscs are stabilized against dissociation by charge reduction and negative mode, but membrane protein nanodiscs are stabilized by supercharging reagents in negative mode. By measuring the mass of the intact nanodisc complex, we can measure the oligomeric state of membrane proteins within a lipid bilayer environment, eliminating potential distortions introduced by collision induced dissociation. Importantly, GC stabilizes membrane protein nanodiscs in negative mode but destabilizes in positive mode, improving the ejection of membrane protein complexes from the nanodisc. This approach opens up new directions in applying nanodiscs to study membrane proteins with native MS and may also prove useful for other membrane mimetics,¹¹ including styrene-maleic acid lipid particles⁵⁷ or natural membranes.⁵⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Deconvolved native mass spectra reveal that addition of supercharging reagent glycerol carbonate stabilizes membrane protein nanodiscs for native MS in negative mode but is destabilizing in positive mode.

Figure 2. Macromolecular mass defect analysis of AmtB nanodiscs with added GC in positive mode (A, C) and added 4V in negative mode (B, D). The deconvolved mass distributions are colored in A and B by the normalized mass defect values. C and D show intensities of the same spectra as mass vs. mass defect. Mass defect values are annotated for the intact AmtB trimer; AmtB monomer and dimer fragments; MSP1E3D1(–) (MSPL for light) and MSP1E3D1T2(–) (MSPH for heavy); and combinations thereof.

Figure 3.

The average mass (A, B) and charge (C, D) versus collision voltage for empty POPC nanodiscs with 40 mM IM (blue), no additive (black), 5% 4V (orange), and 5% PC (red) in both positive (A, C) and negative (B, D) ionization modes. Error bars show the standard deviation of three replicate nanodisc assemblies.

Figure 4.

Deconvolved zero-charge mass spectra in positive (A-E) and negative (F-J) ionization mode for AmtB nanodiscs summed over 0–300 V collision voltage with no additive (A, F), IM (B, G), PC (C, H), 4V (D, I), and GC (E, J). Pictograms show the stoichiometry of AmtB and MSP. The numbers of lipids annotate the most abundant peak of the distribution. Figure S-7 shows raw and deconvolved spectra at different collision voltages.

Figure 5.

Deconvolved zero-charge mass spectra in positive (A-C) and negative (D-F) ionization mode for AqpZ nanodiscs summed over 0–300 V collision voltage with no additive (A, D), PC (B, E), and GC (C, F). Pictograms show the stoichiometry of AqpZ and MSP. The numbers of lipids are annotated for the most abundant peak of the distribution. Figure S-8 shows raw and deconvolved spectra at different collision voltages.