Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Information

Native mass spectrometry of proteoliposomes containing integral and peripheral membrane proteins

Yun Zhu,[1,†] Sangho D. Yun,[1,†] Tianqi Zhang,[1] Jing-Yuan Chang,[1] Lauren Stover,[1] and Arthur Laganowsky[1,*]

[1] Department of Chemistry, Texas A&M University, College Station, TX 77843
[†] These authors contributed equally to this work.
[*] Corresponding author: ALaganowsky@chem.tamu.edu

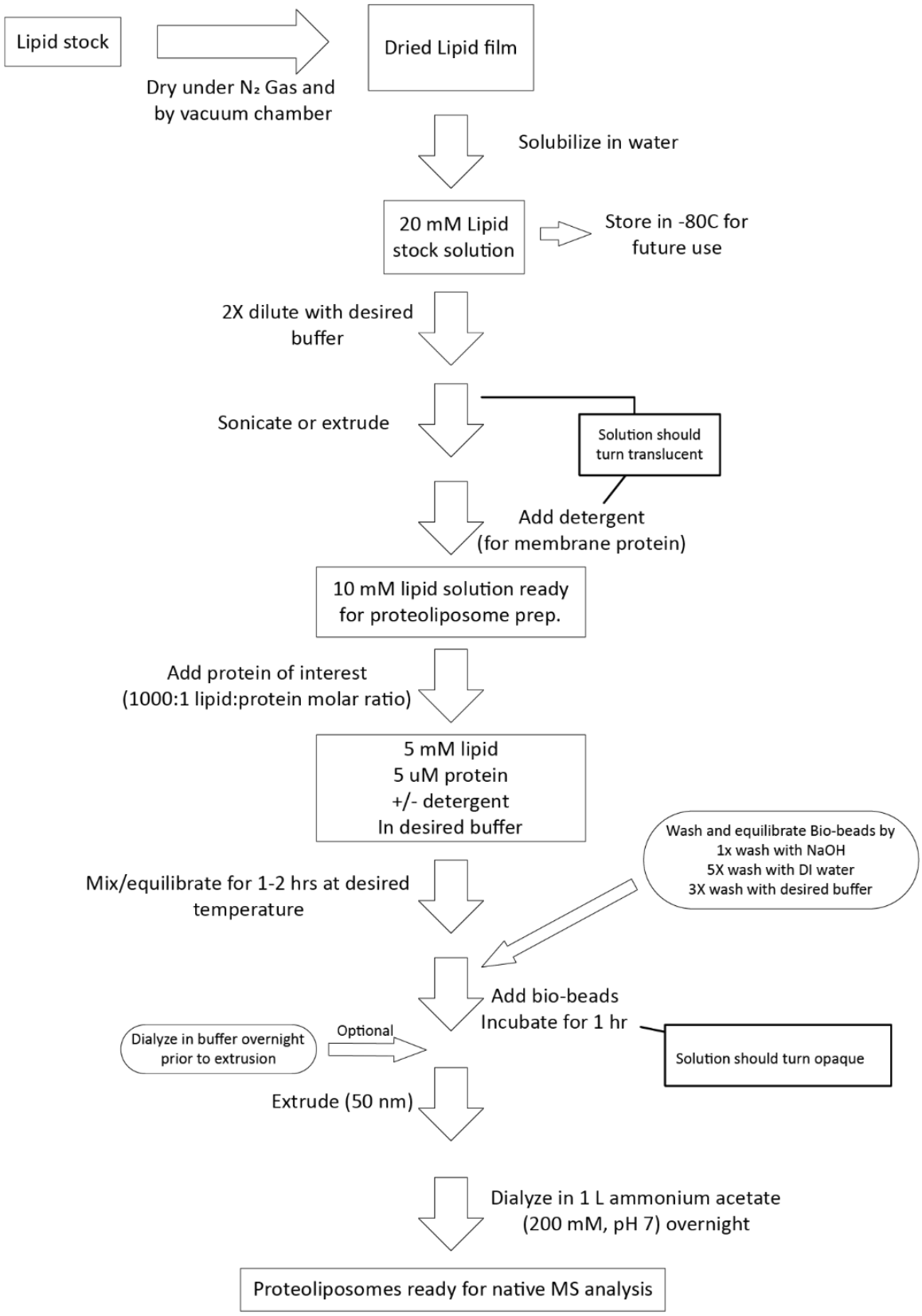

Figure S1. **Detailed flowchart for the preparation of proteoliposome for native MS studies.**

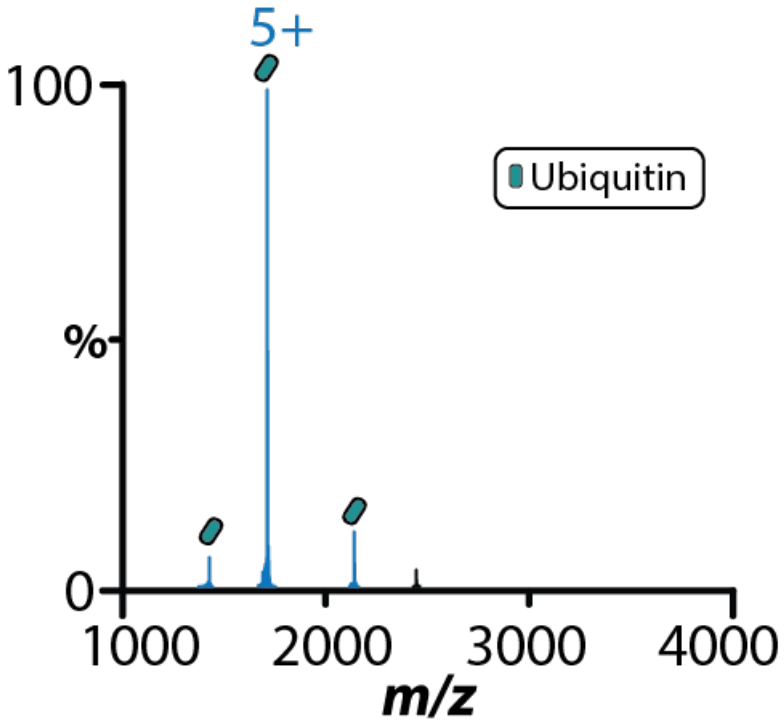

Figure S2. Native mass spectrum for Ubiquitin in 200 mM Ammonium Acetate.

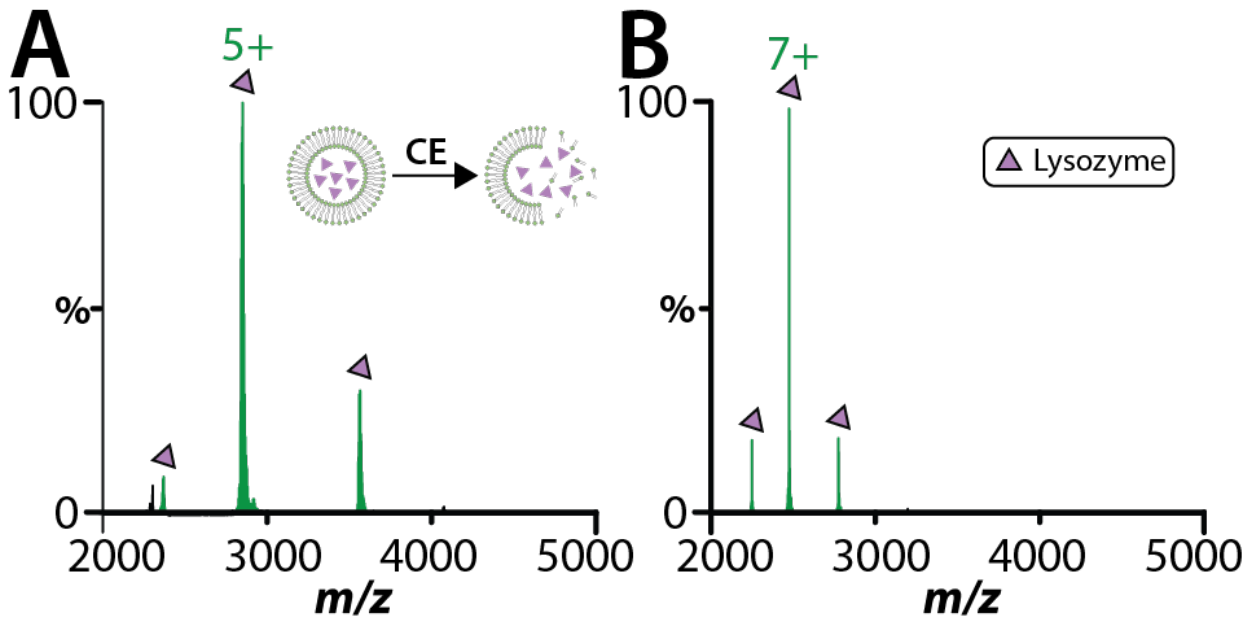

Figure S3. Comparison between Lysozyme encapsulated in POPC SULs versus in solution. A) Lysozyme ejected from POPC liposome. B) Lysozyme in 200 mM ammonium acetate.

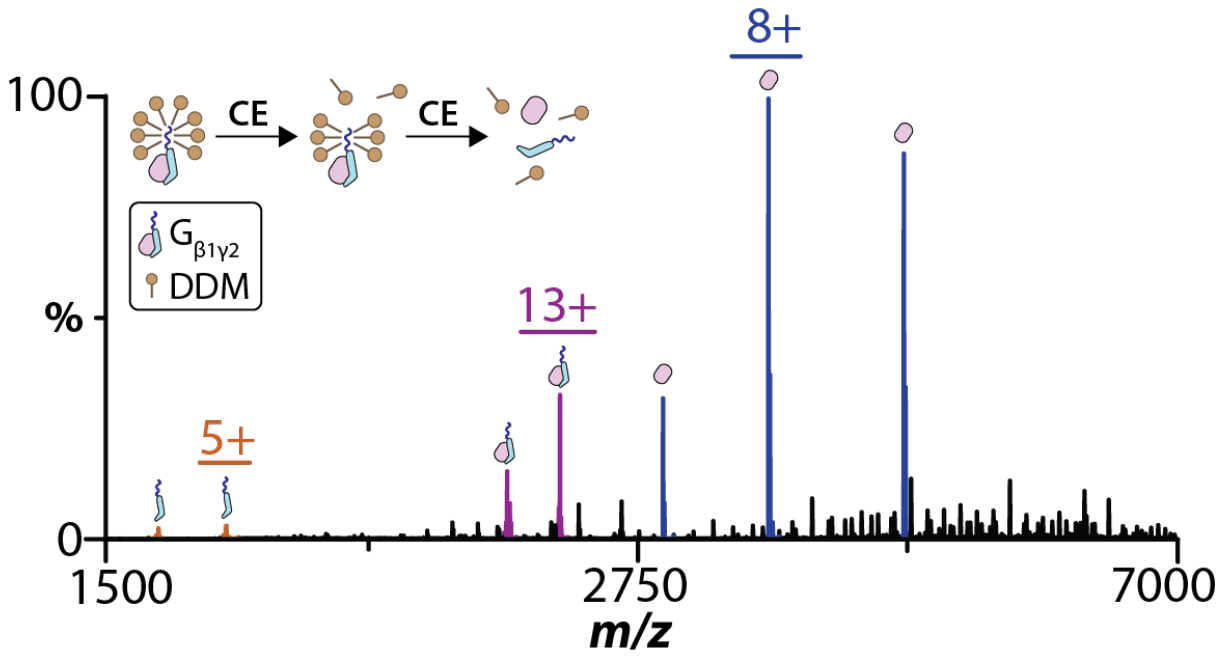

Figure S4. Dissociation of the $G_{\beta1\gamma2}$ complex using higher collisional energy. $G_{\beta1\gamma2}$ complex in ammonium acetate supplemented with DDM. The complex was dissociated using a CE of 80 eV and CID of 60 eV.

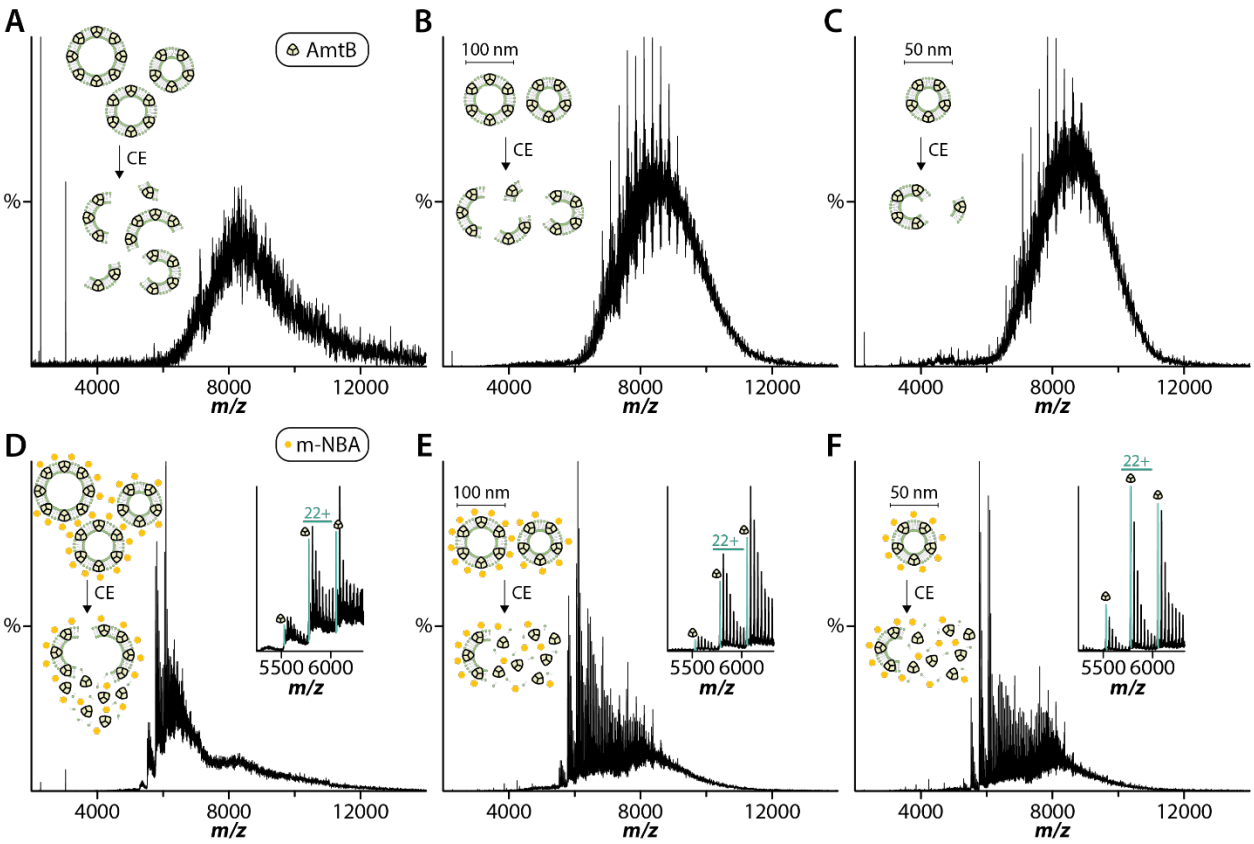

Figure S5. AmtB incorporated into POPC liposomes with and without extrusion. A-C) MS analysis of AmtB from unextruded and extruded vesicles. Mass spectra were obtained using a CE of 50 eV and CID of 50 eV. D-F) MS analysis of AmtB from unextruded and extruded vesicles with the addition of m-NBA. Mass spectra were obtained using a CE of 50 eV and CID of 20 eV.

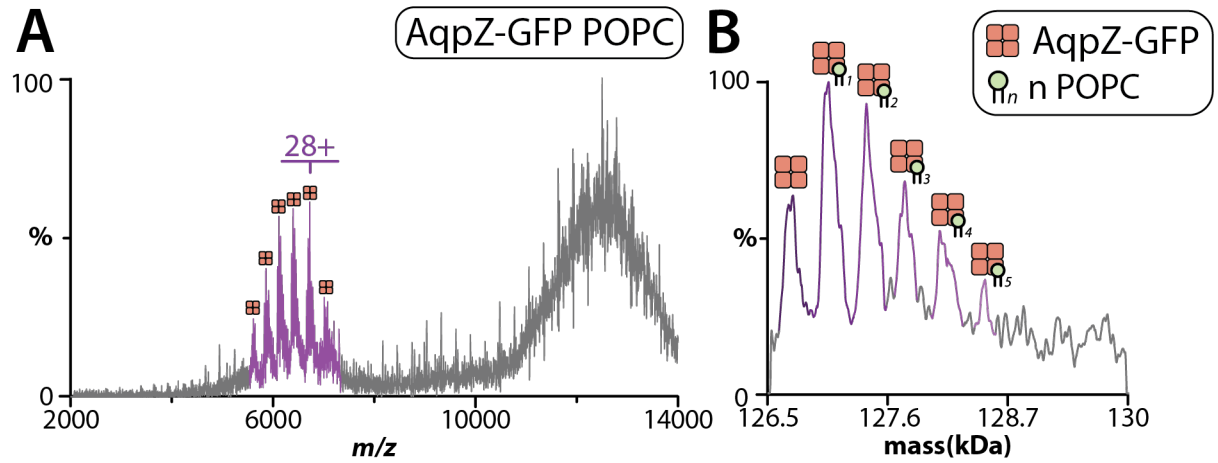

Figure S6. AqpZ-GFP incorporated into POPC liposomes. A) Mass spectrum of AqpZ-GFP ejected from liposomes. B) Deconvolution of the mass spectrum shown in panel A along with masses of the adducts.

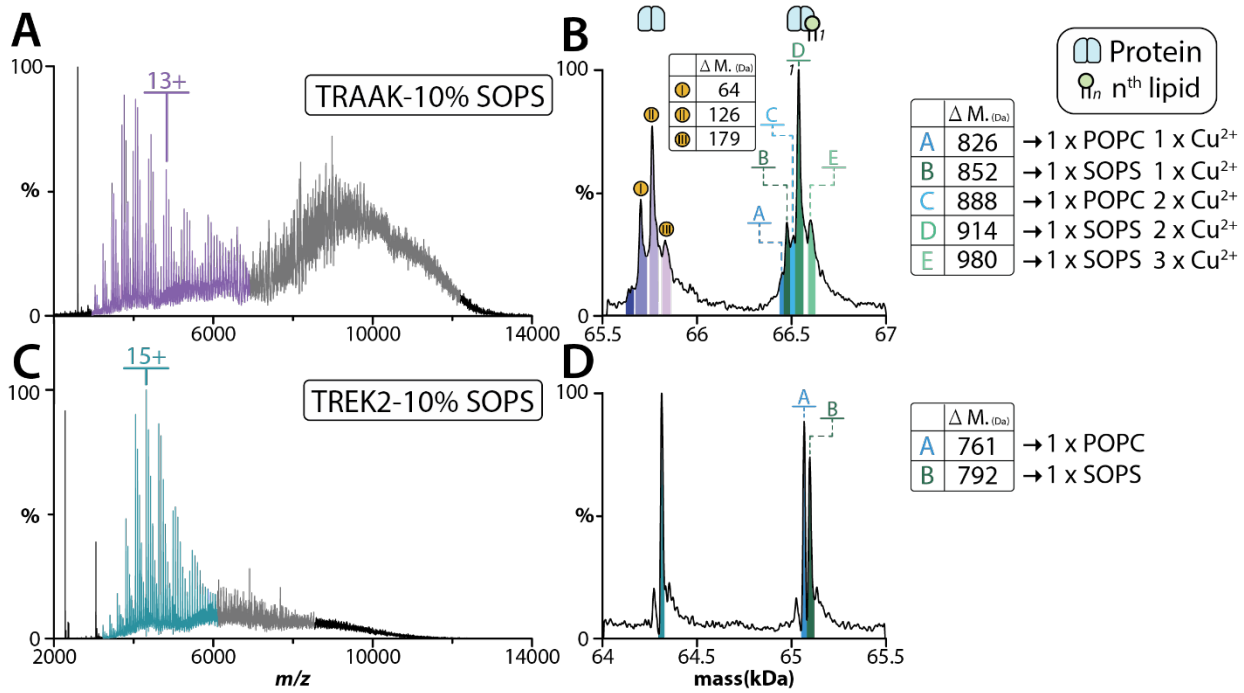

Figure S7. Comparison of TRAAK and TREK2 reconstituted in POPC liposomes containing 10% SOPS. A-B) TRAAK in 10% SOPS. C-D) TREK2 in 10% SOPS.

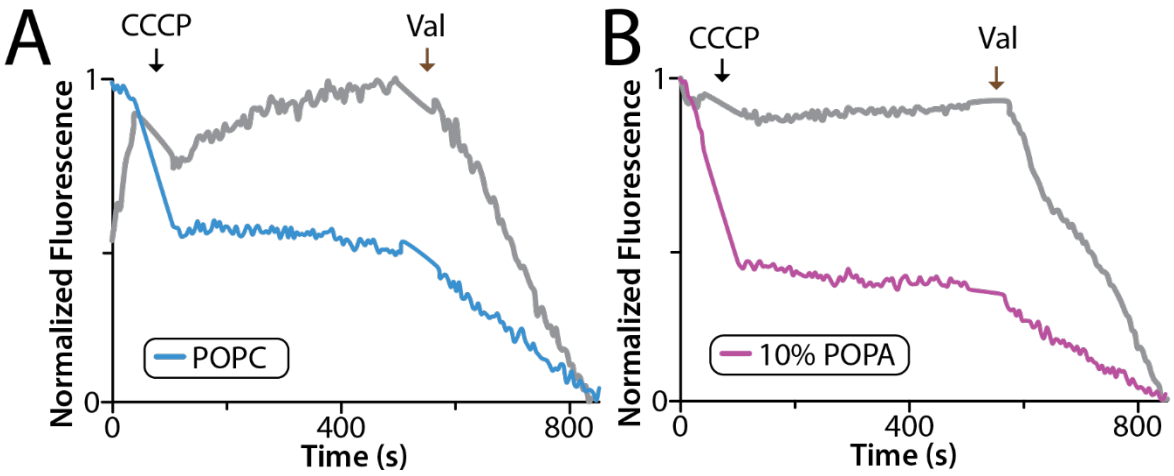

Figure S8. Functional assay with TRAAK. Shown are the traces for TRAAK in A) POPC liposomes and B) POPC liposomes containing 10% POPA.

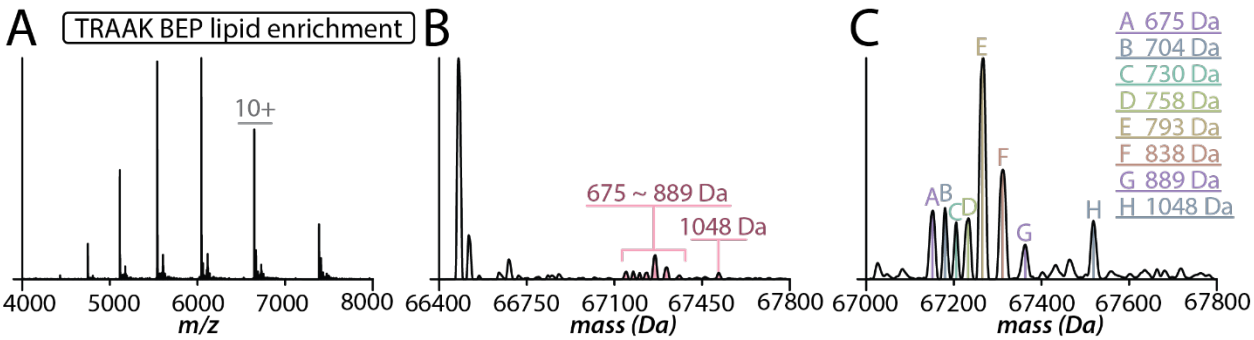

Figure S9. TRAAK enriches lipids from a brain polar extract in detergent. The lipid extract enrichment protocol has previously been described.[1] A) Native mass spectrum of TRAAK-BEP. B) Deconvoluted spectrum of A. C) Zoom of the first lipid bound states. Different masses are labeled A-H.

Table S1. Q-Exactive UHMR instrument parameters.

Parameters	*Values*
m/z range	1000-15000
Resolution	25000
In-source collision induced dissociation (eV)	10.0-70.0
HCD Collision Energy (eV)	70.0-120.0
Source temperature	200-250
Desolvation Voltage (V)	-20
Capillary voltage (kV)	1.1-1.2
Source DC offset (V)	10.0-21.0
Inject flatapole DC (V)	15
Inter flatapole Lens (V)	13
Bent flatapole DC (V)	14.0-18.0
Transfer multipole DC (V)	3
Pressure (mbar)	6

Table S2. Q-Exactive EMR instrument parameters for the analysis of membrane proteins.

Parameters	*Values*
m/z range	2000-16000
Resolution	35000
In-source CID (V)	40-80
In-Source CE (eV)	50-80
Source temperature	250
Capillary voltage (kV)	1.2-1.4
Source DC offset (V)	25-30
Inject flatapole DC (V)	12.0-13.0
Inter flatapole lens (V)	7.0-8.0
Bent flatapole DC (V)	9.0-10.0
Transfer multipole DC (V)	5.0-8.0
C-Trap Entrance Lens Tune Offset (V)	3.0-5.0
Pressure (mbar)	6

Table S3. Q-Exactive EMR instrument parameters for the analysis of $G_{\beta 1\gamma 2}$.

Parameters	*Values*
m/z range	1600-10000
Resolution	8750
In-source CID (V)	35
In-Source CE (eV)	20
Source temperature	200
Capillary voltage (kV)	1.6
Source DC offset (V)	25
Inject flatapole DC (V)	12
Inter flatapole lens (V)	8
Bent flatapole DC (V)	5.0-6.0
Transfer multipole DC (V)	3
C-Trap Entrance Lens Tune Offset (V)	2.0-3.0
Pressure (mbar)	5

Table S4. Q-Exactive EMR instrument parameters for the analysis of soluble proteins.

Parameters	*Values*
m/z range	1000-10000
Resolution	8750
In-source CID (V)	0-50
In-Source CE (eV)	0-80
Source temperature	200
Capillary voltage (kV)	1.5
Source DC offset (V)	25
Inject flatapole DC (V)	18
Inter flatapole lens (V)	8
Bent flatapole DC (V)	3
Transfer multipole DC (V)	8
C-Trap Entrance Lens Tune Offset (V)	2
Pressure (mbar)	5

Table S5. Theoretical and measured masses for the $G_{\beta 1\gamma 2}$ complex.

Protein	Theoretical Mass (Da)	Observed Mass (Da)	ΔDalton
$G_{\beta 1}$	39177	39176	1
$G_{\gamma 2}$	10478.6	10478	0.6
$G_{\beta 1\gamma 2}$	49655.6	49659	3.4

Table S6. Expected Post Translational Modifications for $G_{\beta 1\gamma 2}$ complex.

	PTM	*ΔDalton*
Gβ1	N-acetylation	42
	Removal of initiating Met	-131
Gγ2	N-acetylation	42
	Removal of initiating Met	-131
	Methylation	14
	Geranylgeranylation	272
	CAAX Cleavage	-297.4

Supplementary References

1. Y. Zhu, M. T. Odenkirk, P. Qiao, T. Zhang, S. Schrecke, M. Zhou, M. T. Marty, E. S. Baker and A. Laganowsky, Combining native mass spectrometry and lipidomics to uncover specific membrane protein-lipid interactions from natural lipid sources, *Chem Sci*, 2023, **14**, 8570-8582.