The effects of detergent micelles on lipid binding to proteins in electrospray ionization mass spectrometry

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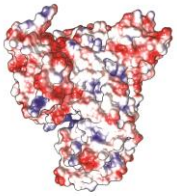
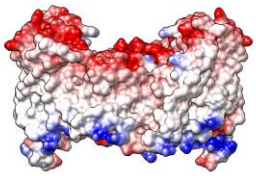
Supplementary Table 1. Hydrophobic, hydrophilic, and total solvent-accessible surface areas (SASAs) and number of charged residues of the proteins used in this study. Calculations were performed used gmx sasa, a tool from GROMACS (1, 2), with the default solvent probe radius 0.14 nm and the pdb structures of the proteins.

				
Protein	**Ubiquitin**	**MaSp1-NT**	**BSA**	**NapA**
(pdb ID)	(1UBQ)	(4FBS)	(3CX9)	(4BWZ)
Basic/Acidic residues	12/11	5/8	99/97	50/56
Area (nm^2 | percent)				
Total	45.4 | 100	57.6 | 100	292.9 | 100	308.7 | 100
Hydrophobic	8.9 | 19.6	17.4 | 30.2	76.9 | 26.2	160.4 | 51.9
Polar	15.8 | 34.9	33.0 | 57.3	64.5 | 22.0	46.9 | 15.2
Positive (Basic)	11.1 | 24.4	2.5 | 4.3	71.9 | 24.5	52.6 | 17.0
Negative (Acid)	9.5 | 20.9	4.7 | 8.1	79.6 | 27.1	48.8 | 15.8
Positive/Negative	1.166	0.533	0.904	1.078

(1) Eisenhaber F, Lijnzaad P, Argos P, Sander C, & Scharf M (1995) J. Comput. Chem. 16, 273-284
(2) M.J. Abraham, D. van der Spoel, E. Lindahl, B. Hess, and the GROMACS development team, GROMACS User Manual version 2016.1, www.gromacs.org (2016)

Supplementary Figures 1-4

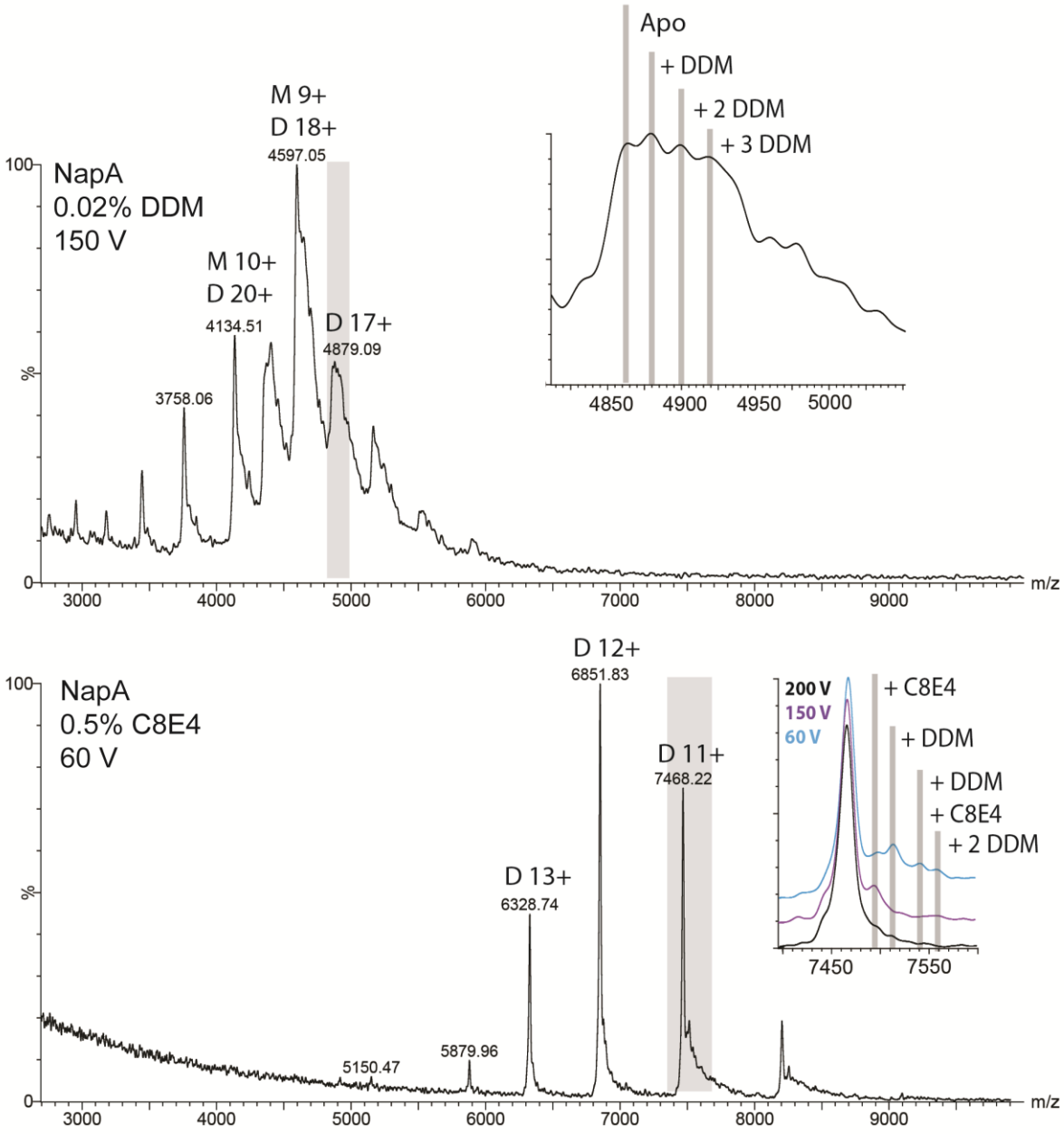


Figure S1. Release of NapA from DDM (top) and C8E4 micelles (bottom). Although the DDM concentration is 10-fold lower than that of C8E4, its removal requires significantly more activation energy (150V, enough to initiate dissociation of the NapA dimer), yet, significant amounts of DDM adducts are retained. C8E4, on the other hand, dissociates easily, even revealing bound DDM retained from earlier purification steps.

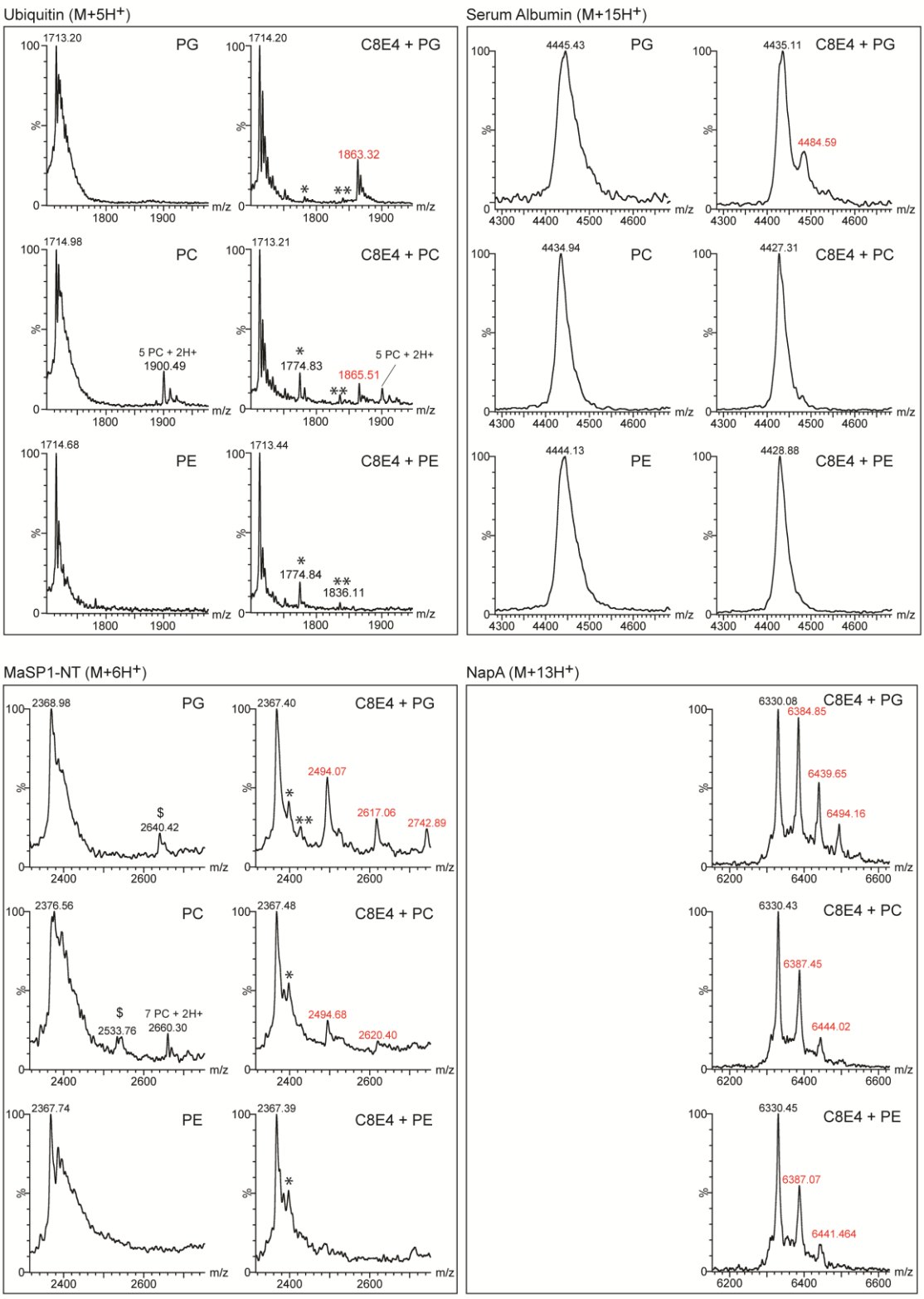


Figure S2. BSA, ubiquitin, and MaSP1-NT show robust dependence on C8E4 for lipid binding, but exhibit differential lipid binding preferences. *m/z* values of lipid adduct peaks are labelled in red. * and ** indicate protein ions with one or two detergent adducts, respectively. $ denotes unassigned peaks. Spectra of detergent-free NapA could not be recorded.

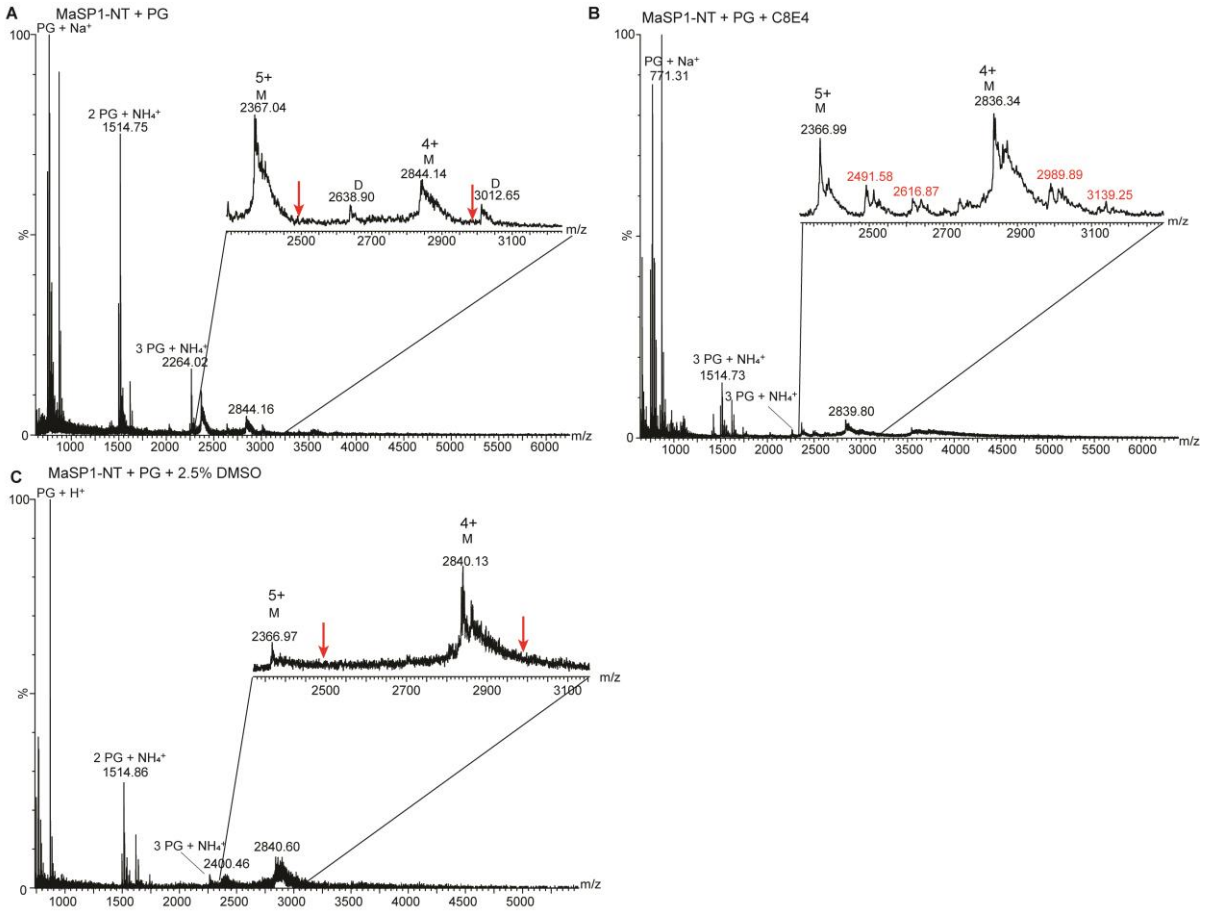


Figure S3. (A) In the absence of C8E4, free lipids can be readily detected by ESI-MS. (B) After addition of C8E4, the amount of free lipids remains unaffected, but lipid binding to MaSP1-NT can be observed. (C) Adding 2.5% DMSO (final concentration) instead of C8E4 similarly reduces protein charge, but does not promote lipid binding. Red arrows indicate the theoretical *m/z* of 1:1 complexes between POPG and MaSP-1-NT. “D” denotes MaSP1-NT dimers. Red arrows indicate the expected *m/z* values of MaSp1-NT-PG complexes.

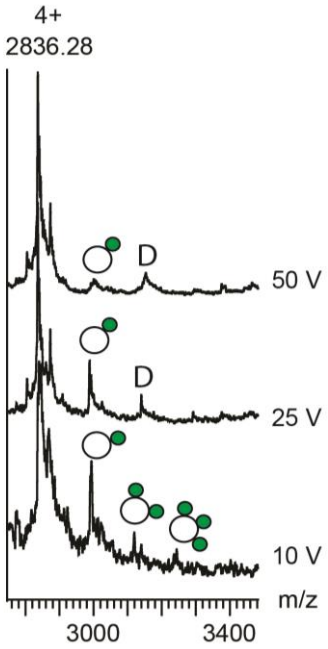


Figure S4. Complexes between MaSP1-NT and POPG are disrupted at collision energies above 10 V. “D” indicates MaSP1-NT dimers.