

Supplementary Information to

## **Electrospray ionization of native membrane proteins proceeds via a charge equilibration step**

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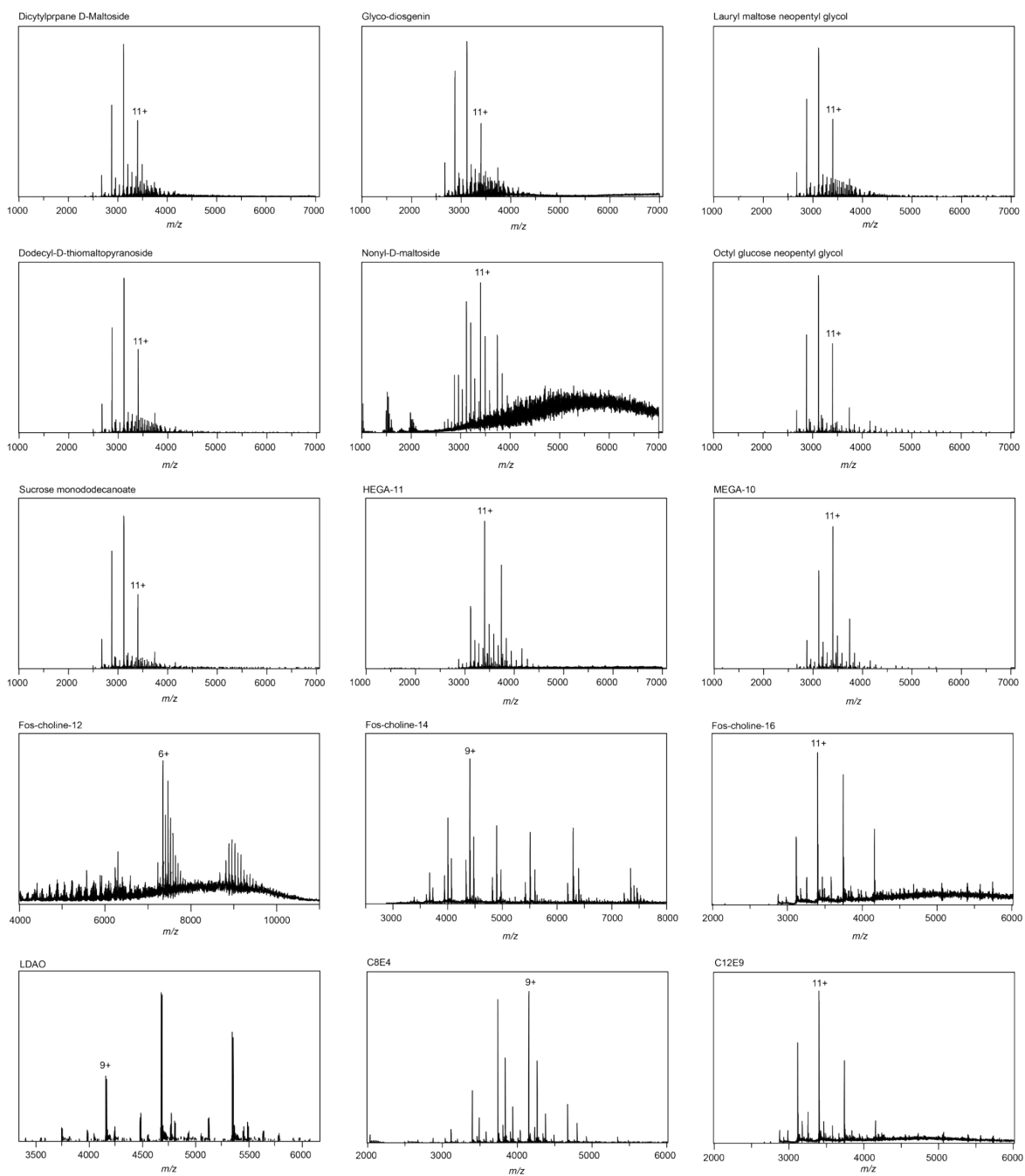
**Supplementary Table 1. Total surface area (SA), solvent-accessible solvent area (SASA), and membrane-accessible surface area (MASA) of Glut5, NTR1, and PfHT1.** A maximum distance of 2.5 Å between surface residues and either solvent or lipids was used as cut-off for accessibility, which results in a significant overlap between SASA and MASA. All values are given in Å<sup>2</sup>.

Protein	SA	SASA	MASA	MASA/SASA
Glut5	213.637	132.121	114.474	0.74
NTR1	162.669	89.532	87.651	0.98
PfHT1	204.845	167.565	125.031	0.86

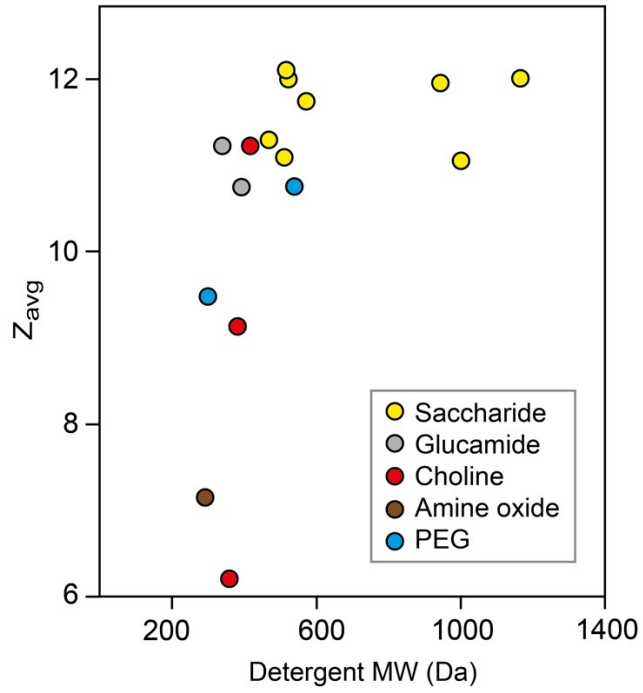
**Supplementary Table 2. CMCs of the detergents used in this study.** Values were taken from Anatrace (<https://www.anatrace.com>).

Detergent	CMC
DDM	0.17 mM
Dioctylpropane D-Maltoside	0.036 mM
Glyco-diosgenin	0.018 mM
Lauryl maltose neopentyl glycol	0.01 mM
Dodecyl-D-thiomaltopyranoside	0.05 mM
Nonyl-D-maltoside	6 mM
Octyl glucose neopentyl glycol	1.02 mM
Sucrose monododecanoate	0.3 mM
HEGA-11	35 mM
MEGA-10	7 mM
Fos-choline 12	1.5 mM
Fos-choline 14	0.12 mM
Fos-choline 16	0.013 mM
LDAO	2 mM
C8E4	8 mM
C12E9	0.05 mM

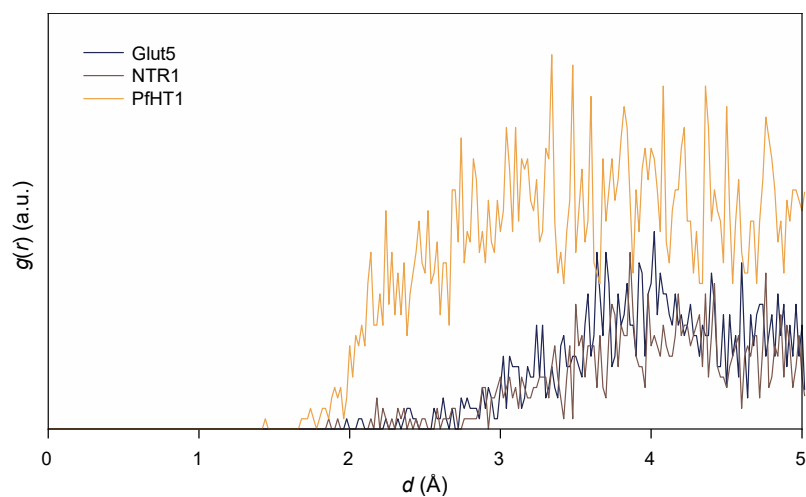




**Figure S2.** Spectra of NTR1 in saccharide, glucamide, and fos-choline detergents. Average charges are plotted in Figure 2 A.



**Figure S3.** Plotting the average charge of NTR1 as a function of detergent molecular weight does not reveal a clear correlation.



**Figure S4.** Radial distributions [ $g(r)$ ] of lipids calculated from the protein surface. Glut5 and NTR1 show a maximum just below 4 Å, whereas PfHT1 shows a plateau starting at around 3 Å. This difference is due to the different lipids surrounding the proteins. The differences in amplitude are irrelevant for this study.