GPI-anchor synthesis in plants – a glycobiology perspective

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Supplementary Methods

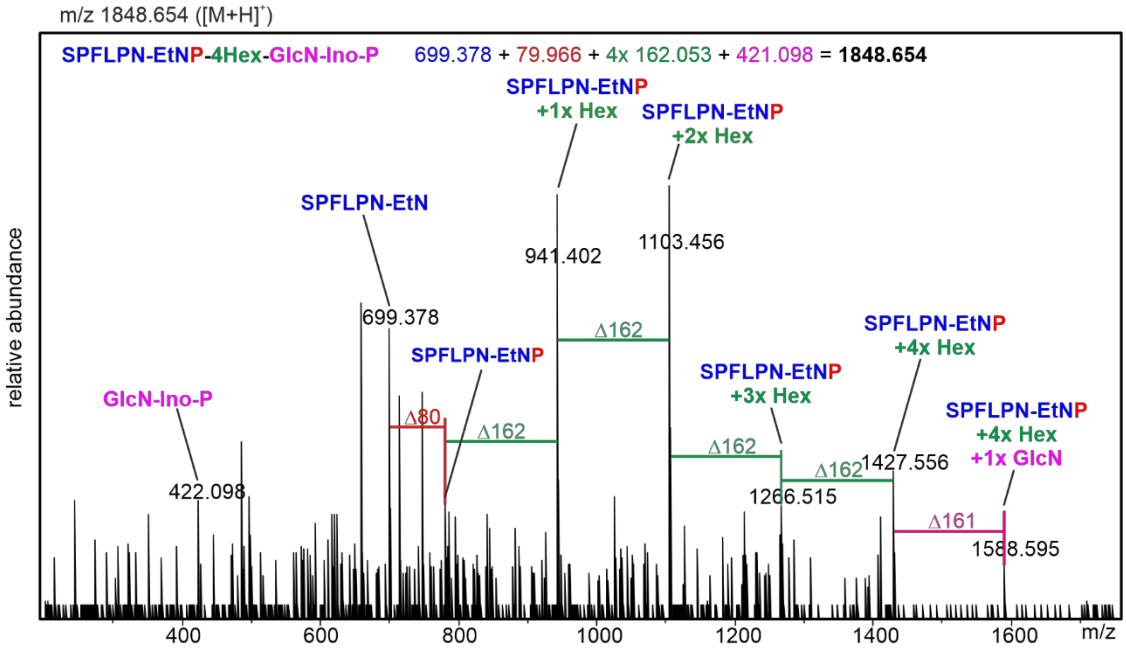

Figure S1. LC-ESI-MS/MS analysis of the C-terminal peptide from *N. benthamiana* RFP-COB1. RFP-COB1 was transiently expressed by agroinfiltration of leaves, captured by binding to RFP-Trap resin and subjected to PI-PLC and trypsin digestion. A representative analysis from two biological replicates is shown.

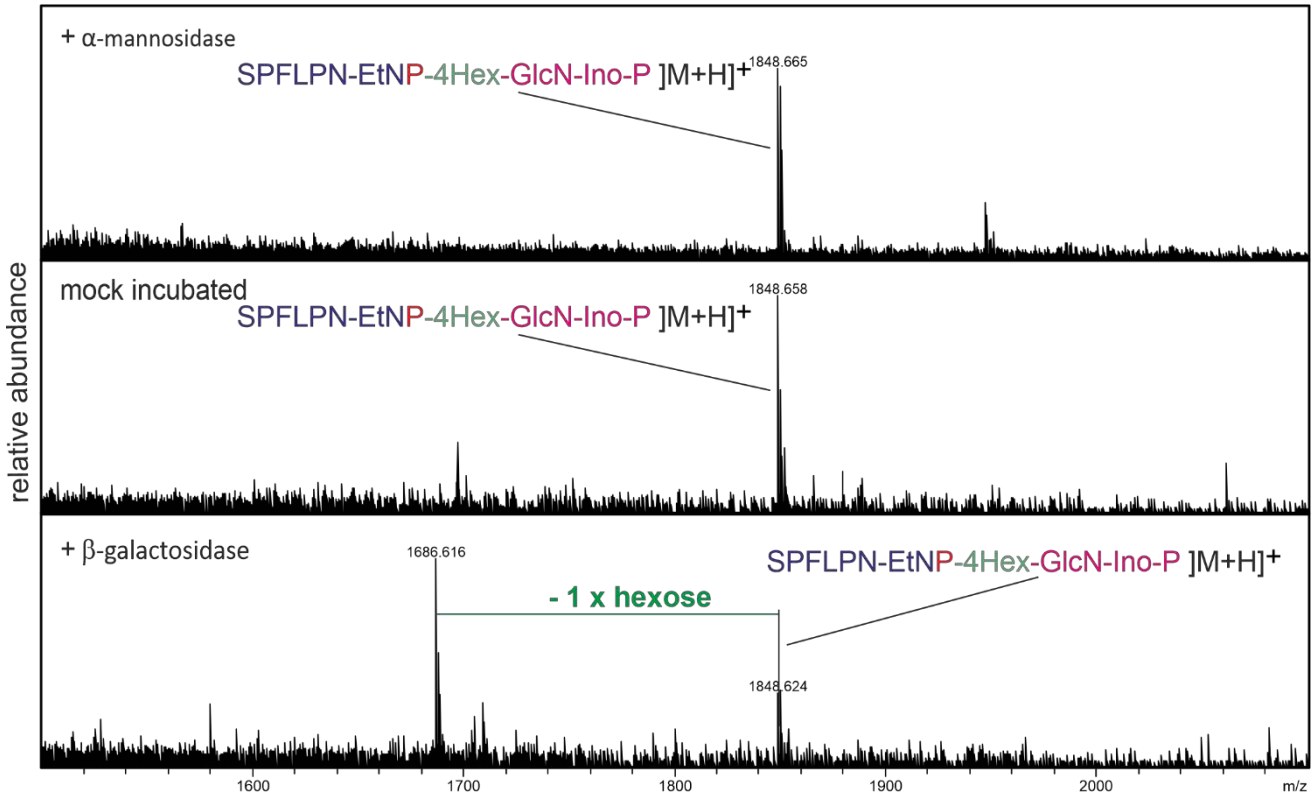

Figure S2. LC-ESI-MS analysis of α-mannosidase or β-galactosidase digested GPI-anchor derived from *N. benthamiana* expressed RFP-COB1. The β-galactosidase digestion was carried out two times with identical results. A representative analysis is shown.

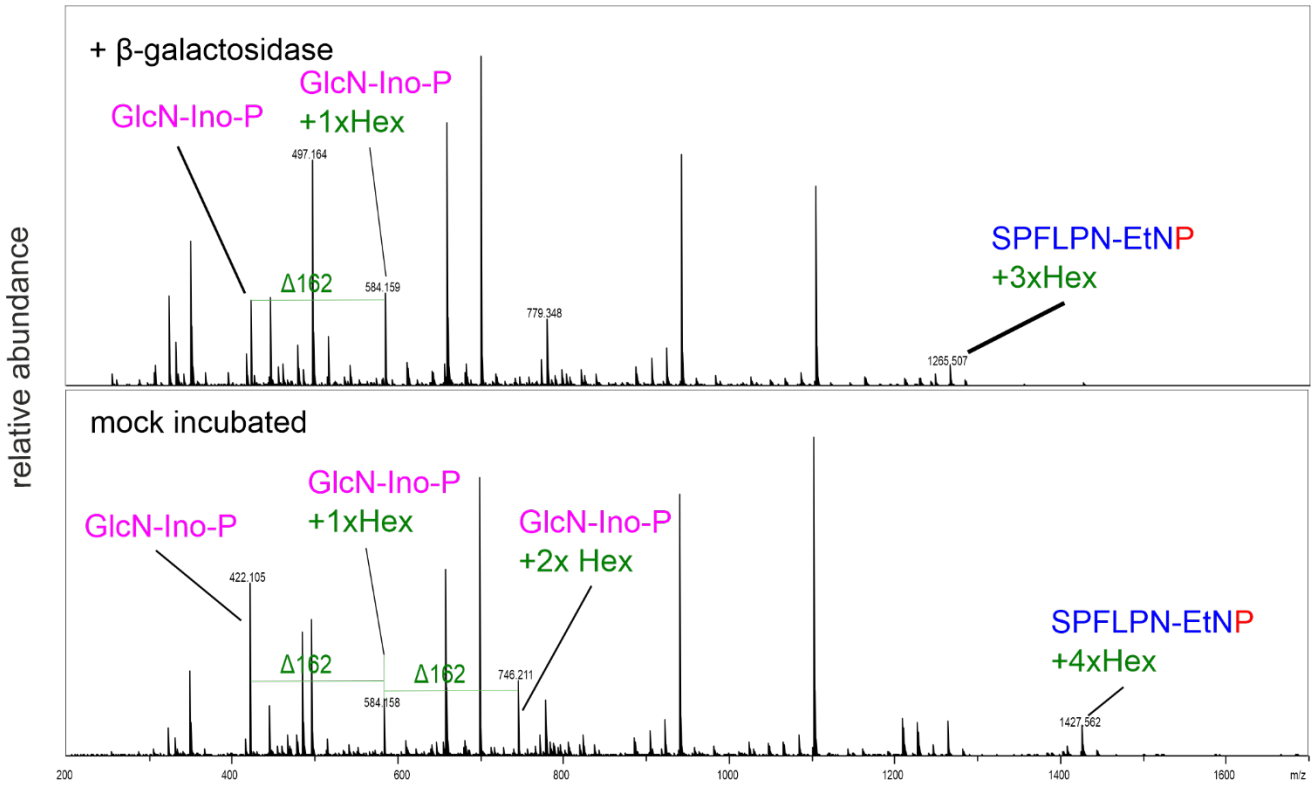

Figure S3. LC-ESI-MS/MS analysis of β-galactosidase digested and mock-incubated GPI-anchor derived from RFP-COB1 expressed in *A. thaliana* seedlings. A representative analysis from two technical replicates is shown.

Supplementary Methods

The C-terminal peptide of COBRA_ARATH was amplified from genomic DNA of *A. thaliana* using primers cob1_5F (5'-TATAGGATCCCCTTTTCTTCCCAACGGTGGTTCC-3')/cob1_6R (5'-TATACTCGAGTTAGGCAGAGAAGAAGAAAAAGAC-3') and cloned into a modified version of the pPT2-vector (Strasser *et al.* 2005) containing the Arabidopsis ubiquitin10-promotor and N-terminal mRFP. Reporter proteins were either purified from leaves of *N. benthamiana* following agroinfiltration or from 14-day old seedings of stably transformed *A. thaliana*. Plant samples were snap frozen, grinded and the membrane fraction collected by centrifugation in 100 mM Tris, 25 %(w/v) sucrose and 5 % (v/v) glycerol buffer (pH 7.4) at 65000 g for 1 h at 4 °C. After washing in 100 mM Tris, 150 mM NaCl and 0.5 mM EDTA buffer (pH 7.4), the membrane pellet was resuspended in 20 mM Tris, 10 mM NaCl, 0.1 mM $CaCl_2$, 1% (v/v) Phosphoinositide Phospholipase C (PIPLC, P6466, Thermo Fisher Scientific) (pH7.4) and incubated for 15 min. After cleavage of lipids from RFP-GPI, the protein was purified using RFP trap (Chromotek), separated by SDS-PAGE, excised from the gel, reduced, S-carbamidomethylated and subjected to tryptic digest as described previously (Kolarich and Altmann 2000). The resulting GPI-bearing peptide fragment was analyzed via LC-ESI-MS/MS using a Bruker Maxis 4G Q-TOF instrument. For enzymatic deglycosylation of the GPI anchor, either β-galactosidase from *Aspergillus oryzae* (Zeleny *et al.* 1997) or α-mannosidase from *Canavalia ensiformis* (M7257, Sigma-Aldrich) was used. RFP-GPI was purified once from *A. thaliana* and two times from *N. benthamiana*.

References

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Zeleny, R., Altmann, F. and Praznik, W. (1997). A capillary electrophoretic study on the specificity of beta-galactosidases from *Aspergillus oryzae*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Canavalia ensiformis* (jack bean). Anal Biochem. 246, 96-101.