Membrane-anchored substrate binding proteins are deployed in secondary TAXI transporters

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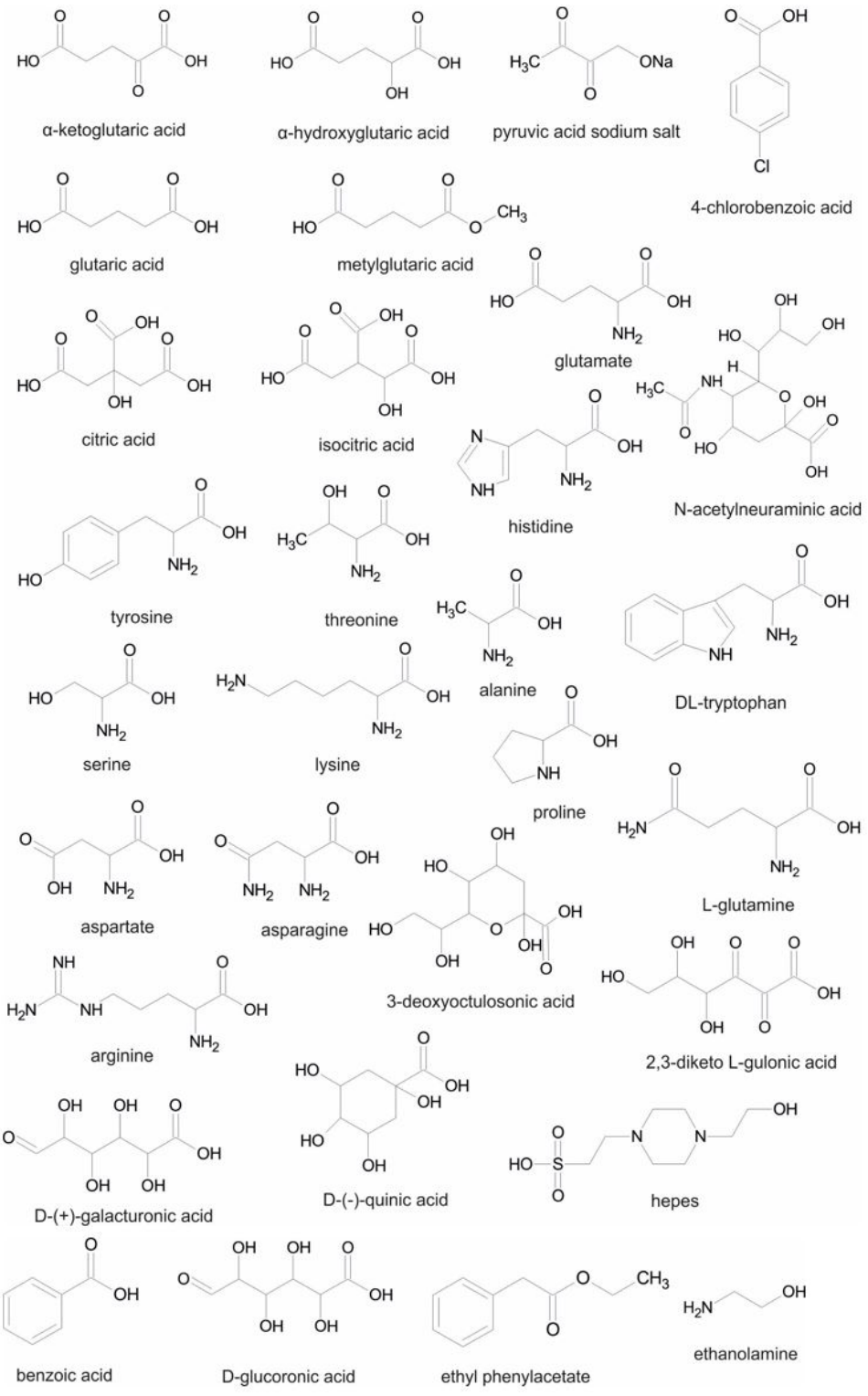
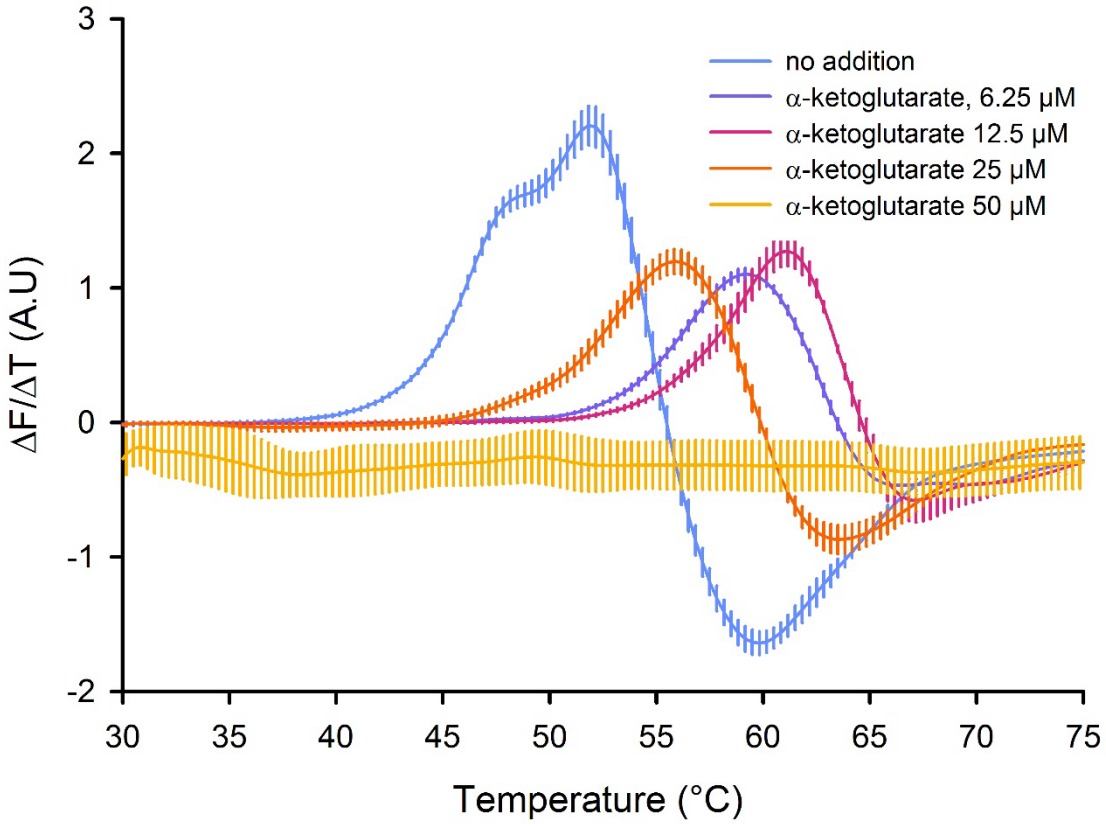
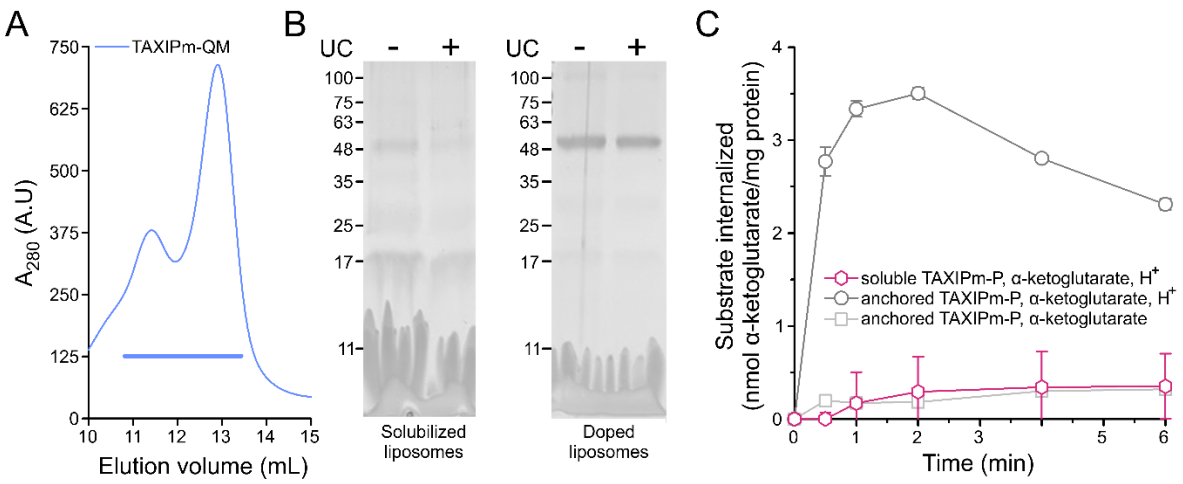
Supplementary Figures



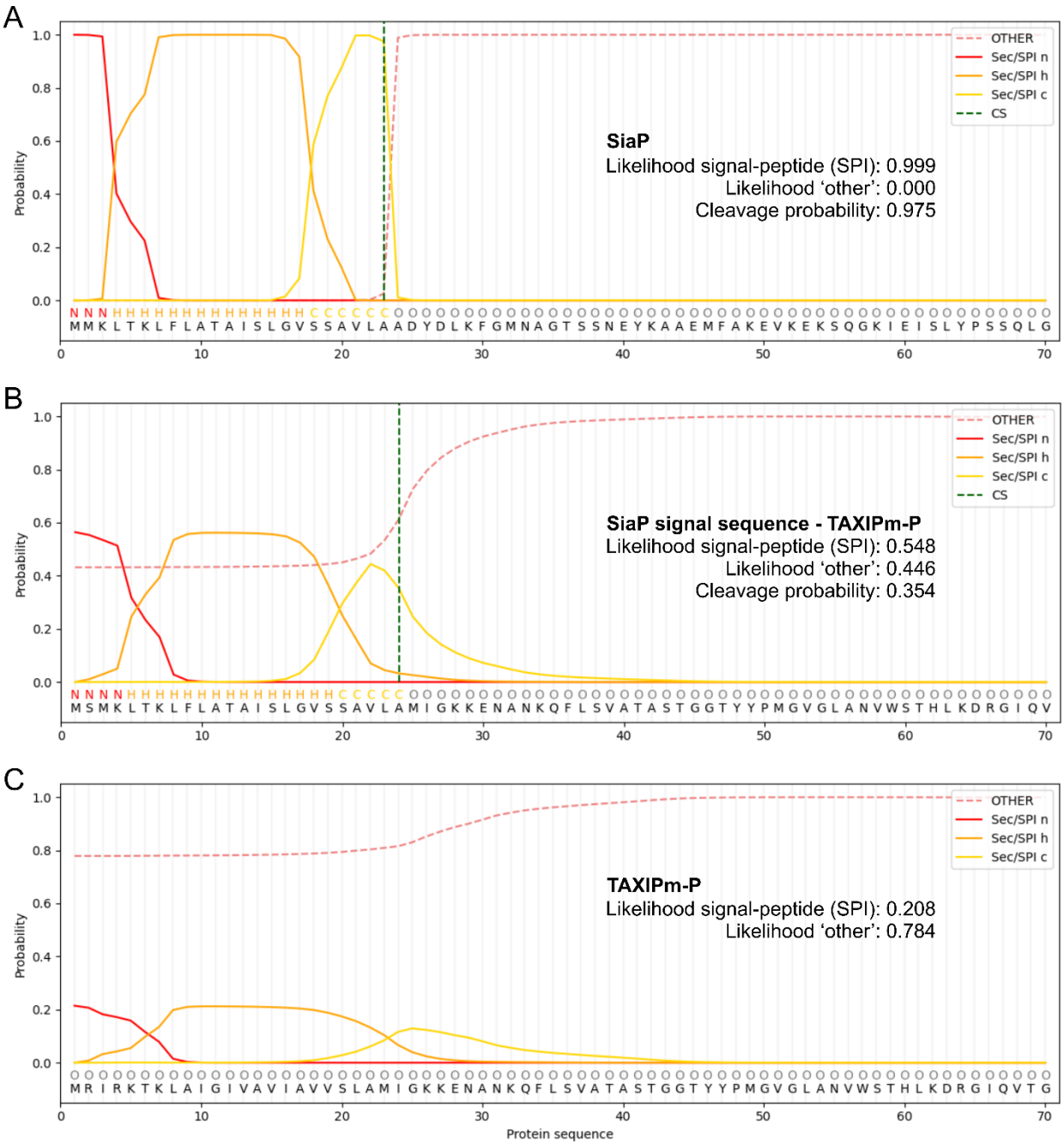
Supplementary Fig. 1: Composition of the compound library used for TAXIPm-P deorphanization.



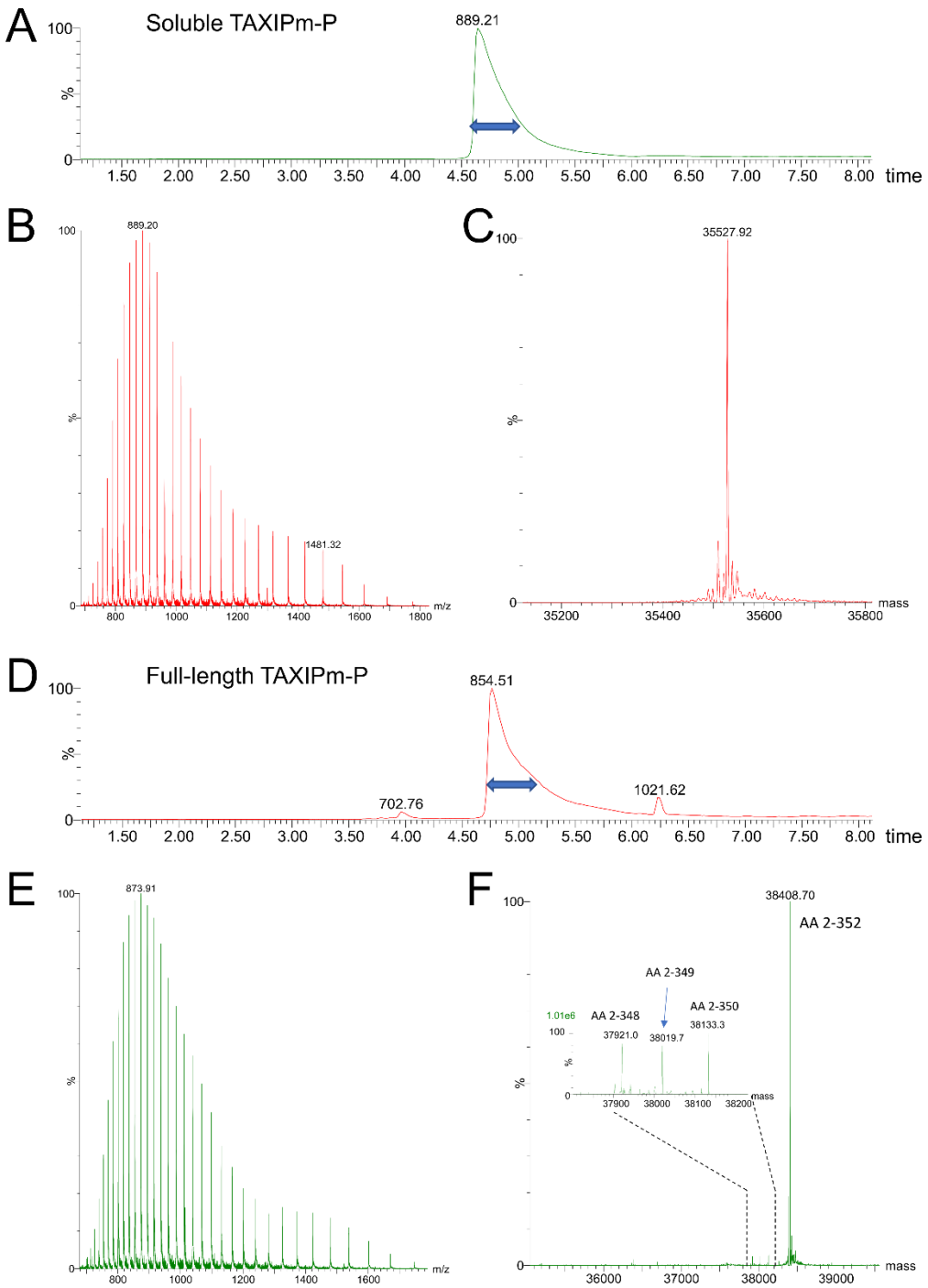
Supplementary Fig. 2: Differential scanning fluorimetry of TAXIPm-P without any addition or in the presence of increasing concentrations of α-ketoglutarate. The peak in the first derivative of the curve indicates the melting temperature. Curves represent average values of triplicates with standard errors.



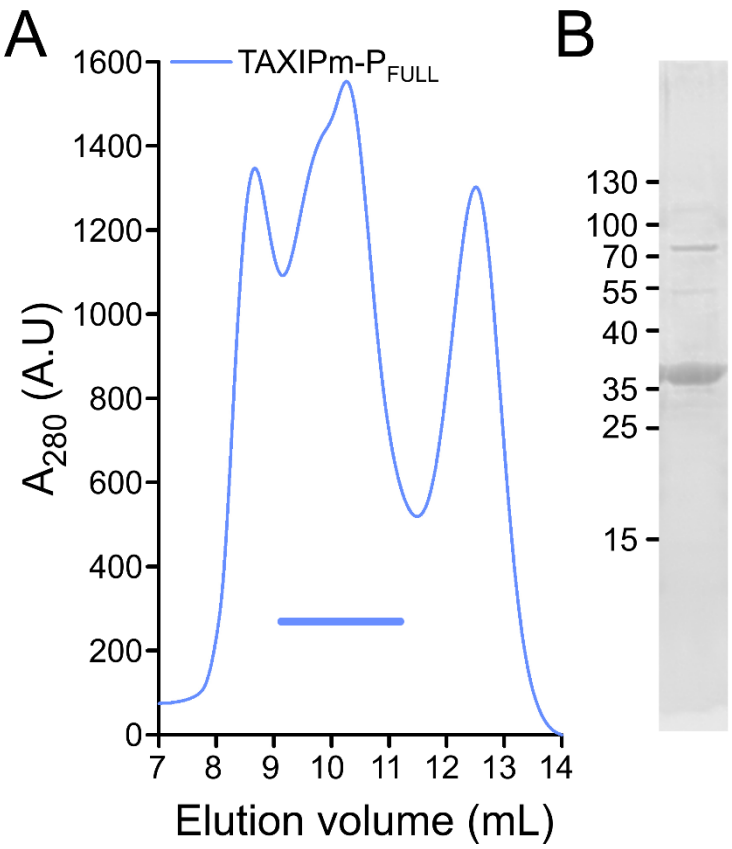
Supplementary Fig. 3: (A) Size-exclusion chromatography of TAXIPm-QM in 0.2% (w/v) DM. Thick line below the curve indicates the fractions pooled and used for reconstitution. **(B)** Assessment of the reconstitution efficiency of TAXIPm-QM. TAXIPm-QM was reconstituted using detergent-solubilized liposomes (left panel) or detergent-doped liposomes (right panel). Coomassie-stained SDS-PAA-gel of DDM-solubilized proteoliposomes before (minus) ultracentrifugation and the supernatant following ultracentrifugation (plus). **(C)** Time-dependent uptake of 10 µM [^{14}C]-α-ketoglutarate into TAXIPm-QM proteoliposomes at 20 °C in the presence of 5 µM soluble TAXIPm-P (pink trace). Gray traces indicate reference data obtained for proteoliposomes containing TAXIPm-QM and membrane-anchored TAXIPm-P. H^+, and α-ketoglutarate refer to inwardly-directed proton- (out: pH 6.0; in: pH 7.5), and α-ketoglutarate-gradients (out: 10 µM; in: 0 µM; symmetrical pH 7.5 unless otherwise stated).



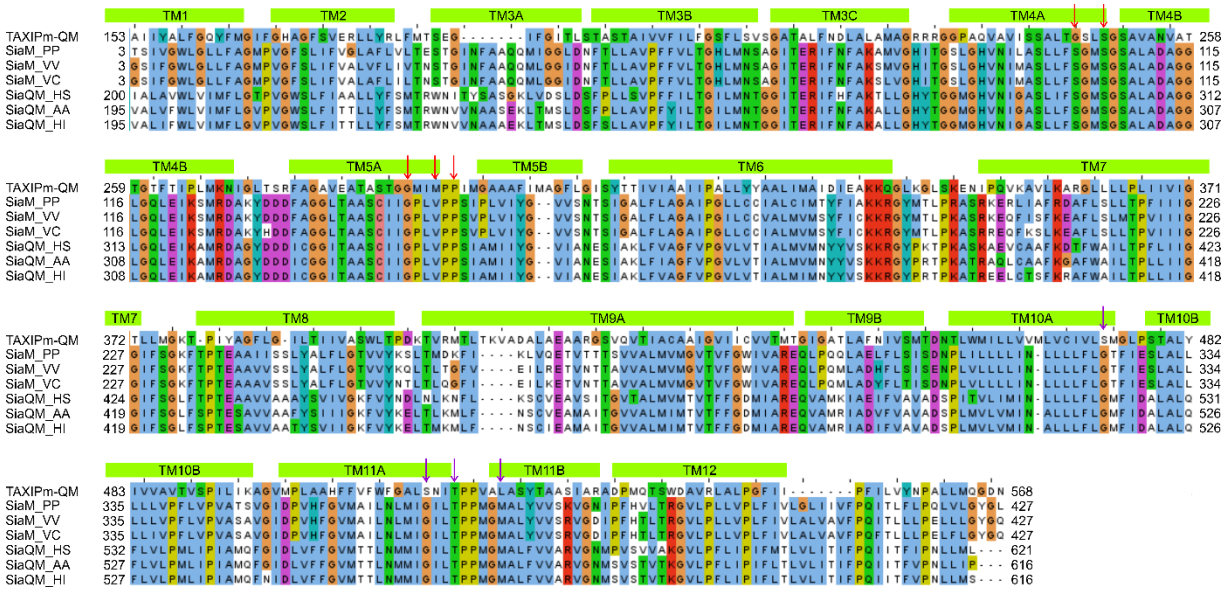
Supplementary Fig. 4: SignalP 6.0 analysis of **(A)** SiaP from *H.* influenzae, **(B)** TAXIPm-P expressed with the signal sequence derived from SiaP, and **(C)** TAXIPm-P. The likelihood for a signalpeptidase I signal sequence and a sequence that cannot be assigned to known signal peptides (designated 'other') are indicated in the figure, together with the predicted cleavage probabilities.



Supplementary Fig. 5: Intact protein mass analysis of soluble TAXIPm-P and full-length TAXIPm-P. **(A, C)** Chromatograms. **(B, E)** Summed-up spectra. **(C, F)** Deconvoluted spectra. The experimentally determined mass of soluble TAXIPm-P (35527.90 Da) is in good agreement of the expected mass (35527.75 Da). The experimentally determined mass of full-length TAXIPm-P (38408.70 Da) in in good agreement with the mass expected for the protein with its N-terminal Methionine excised (38539.57 Da). Some very low signals fit to C-terminally truncated species.



Supplementary Fig. 6: (A) Size-exclusion chromatography of TAXIPm-P in 0.05% (w/v) Triton X-100. Thick line below the curve indicates the fractions pooled and used for further analysis. **(B)** SDS-PAGE analysis of the SEC-peak fraction.



Supplementary Fig. 7: Protein sequence alignment. The sequence of TAXIPm-QM was aligned with the sequences of SiaM from *Photobacterium profundum* (PP; Q6LPW1), *Vibrio vulnificus* (VV; HAS8545205.1), *Vibrio cholerae* (VC; WP_255122496.1), and the sequences of SiaQM from *Histophilus somni* (HS; WP_249961534.1), *Aggregatibacter actinimycetemcomitans* (AA; MBN6066990.1), and *Haemophilus influenzae* (HI; WP_048953901.1). The amino acid identity of TAXIPm-QM with these proteins ranges from 19.4 to 23.1%. Protein sequences were aligned using Clustal Omega. Red and purple arrows indicate residues involved in the potential Na^+ coordination sites as assigned by Davies *et al.* (Davies *et al.*, 2022). Green boxes indicate the position of the transmembrane segments for SiaM from *P. profundum* as assigned by Davies *et al.* (Davies *et al.*, 2022).