Supplementary Material (ESI) for Metallomics

Absolute quantification of transferrin in blood samples of harbour seals using HPLC-ICP-MS

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Sample preparation

All sample handling and manipulation was carried out under cooled conditions (4 °C) inside a class 100 clean bench, with the aim to minimise contaminations and sample degradation, respectively.

Since the absolute quantification requires a uniform iron stoichiometry of the different Tf glycoforms, an iron saturation procedure was developed based on a method described by del Castillo Busto *et al.* [27, 32]. Briefly, 100 µL serum were mixed with 5 µL of a 500 mM Na_2CO_3 solution as well as 5 µL of a 10 mM $FeCl_3$ solution followed by an incubation time of 30 minutes at room temperature. To improve the long term stability of the chromatographic column as well as to remove additional interfering matrix components from the serum samples, the original method was further optimised by the implementation of a lipoprotein precipitation step similar to the one described by Jeppsson *et al.* [50]. Therefore, 5 µL of the precipitation reagent (2 g $MgCl_2$ and 1 g dextransulphate in 10 mL H_2O) were added to the iron saturated sample. After a further incubation time of additional 30 minutes at 4 °C, the sample was centrifuged for 10 minutes with 18000 *g* at 4 °C. 100 µL of the supernatant were diluted with 400 µL of a 20 mM, pH 6.5 Bis-Tris buffer followed by an additional centrifugation step (conditions as mentioned before) for 5 minutes. The resulting supernatant was stored under cooled conditions until its further investigation. This combined sample preparation procedure was published by Grebe *et al.* [24].

Table S-1: Compilation of the average retention times and peak area RSDs (n=3) obtained during the repetitive, highly resolved separation of Tf glycoforms using anion exchange HPLC-ICP-MS for the certified reference serum ERM-DA470k/IFCC

peak no.	average retention time [min]	retention time RSD [%]	peak area RSD [%]
S_2	9.36	0.24	5.59
S_3	10.37	0.17	1.35
S_4	11.94	0.24	1.84
S_5	13.44	0.18	5.23
S_6	14.93	0.50	0.48

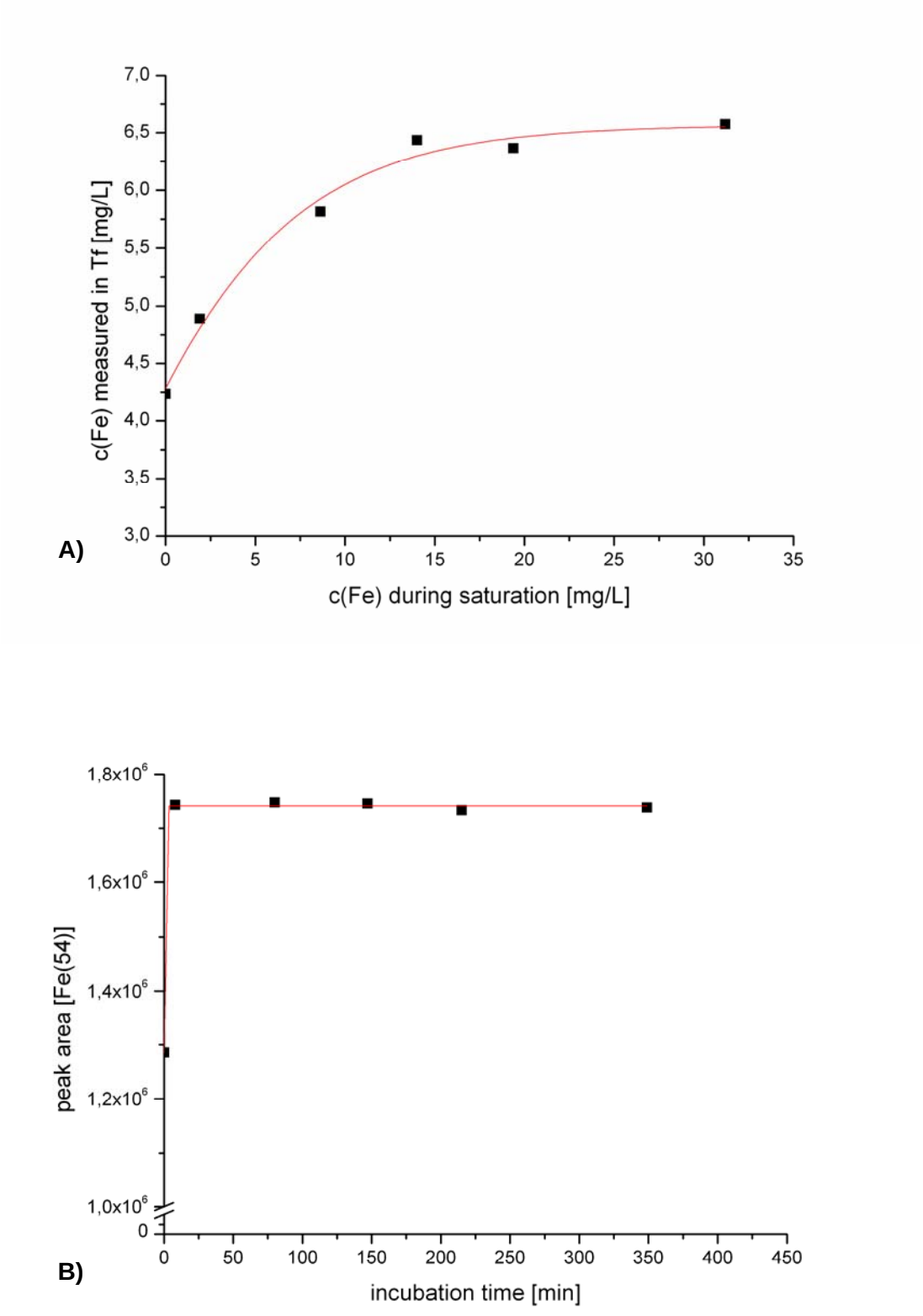

Figure S-1

A) Evaluation of the optimal iron concentration in the reaction mixture to allow an uniform iron saturation **B)** Investigation of different incubation times on the Tf iron saturation.