Electronic Supplementary Material (ESI) for Dalton Transactions.
This journal is © The Royal Society of Chemistry 2018

Supporting information

Methods:

cIEF (UV-visible detection):

The separations were carried out at 25°C with an optical detection at 280 nm. Capillary conditioning was performed by rinsing the capillary with acetic acid (Chemical mobilizer, Sigma Aldrich) for 5 min at 50 psi followed by milliQ water (2 min, 50 psi) and then cIEF gel (Beckman) for 5 min at 50 psi. The method ended by submerging both capillary inlet and outlet in milliQ water. Anolyte consisted of 200 mM phosphoric acid (Sigma Aldrich) and catholyte consisted of 300 mM sodium hydroxide (Sigma Aldrich). Transferrin sample (15 µL of approximately 10 $mg.mL^{-1}$) was mixed with 200 µL of cIEF gel, 15 µL of carrier ampholyte (40% Roti®lyte 4-7, Carl Roth GmbH), 25 µL of a 500 mM arginin solution (Cathodic Stabilizer, Sigma Aldrich) and 1.5 µL of the needed pI marker solution (pI markers 7.0, 5.5 and 4.1 from Beckman and pI markers 6.6 and 6.2 from Sigma Aldrich).

For the separation procedure, the capillary is filled with the mixture previously described by applying a high pressure rinse mode (25 psi) for 99 seconds. Then, a voltage of 25 kV is applied for 25 min to focus the ampholytes and samples; the cathode is placed in the NaOH solution (catholyte) and the anode in the phosphoric acid solution (anolyte). Since the sample is introduced into the capillary by entirely filling it with a mixture of ampholytes and samples, the injected sample amount can easily be controlled by adjusting the sample concentration in the mixture. Once the focalization step completed, the voltage is increased up to 30 kV and the catholyte is replaced by the chemical mobilizer (Acetic Acid, 350 mM). The disruption of the pH gradient caused by the chemical mobilizer leads to its mobilization and the samples and pI markers can then be detected when passing the 200 µm window of the capillary cartridge.

Between each analysis, the capillary is rinsed with a 4.5 M urea solution and milliQ water in order to ensure the cleanliness of the capillary and to avoid protein aggregation.

The linearity of the pH gradient was checked before the analysis and was monitored at least once a day. The linearity of the gradient was determined by plotting the pI of the markers versus their migration time. Typically a determination factor $R^2 = 0.995$ or higher was obtained showing the good linearity of the pH gradient. This linearity ensures the accuracy of pI estimation, especially when the cIEF method is used for analysis of samples with unknown pI.

CE-ICP-MS: The capillaries were preconditioned by rinsing with BGE before use at 25 psi for 5 min. The CE system was provided with a tailor-made capillary cartridge support designed for the adaptation of an external detector, i.e. a X SeriesII ICP-MS (Thermo). Both pieces of apparatus were coupled by a commercial interface using a parallel path micro-nebulizer (Mira Mist CE, Burgener, Mississauga, Canada) specially designed for capillary electrophoresis. A make-up liquid (HNO_3 2% and ethyl alcohol absolute 10%) was introduced by means of a syringe pump (11 Pico Plus, Harvard Apparatus, Holliston, MA) at a nominal flow rate of 8 $\mu L.min^{-1}$ to improve the signal stability by (i) decreasing the surface tension of the water droplets and the size of the droplets and (ii) providing

the nominal flow rate for the nebulizer. Samples were injected at the capillary inlet over a period of 4s at a constant pressure of 2 psi. Separations were performed at +7 kV, 25 °C and a constant pressure of 1.2 psi to avoid capillary clogging. The voltage value was chosen with respect to the Ohm's law and to avoid for temperature rises larger than 1 °C during the experiments. The buffer was changed every run to avoid the effects of electrolysis. Before the injection of each sample, the following washing procedure was applied: 10 mM NaOH, 20 psi for 1 min; water 20 psi for 1 min, then buffer electrolyte 20 psi for 1 min. NTA as well as T*f* were present in both the BGE and the samples. Thorium concentration (1 µM) remained constant in all experiments. The peak areas were determined using an exponentially modified gauss function by the Levenberg-Marquardt algorithm. The Origin Pro2017 software (OriginLab Corporation, Northampton MA, USA) was used to fit the electropherograms.

cIEF-ICP-MS: The capillaries were preconditioned by rinsing with acetic acid (Sigma) for 5 min at 50 psi followed by milliQ water (2 min, 50 psi) and then cIEF gel (Beckman) for 5 min at 50 psi. Anolyte consisted of 200 mM phosphoric acid (Sigma Aldrich) and catholyte consisted of 200 mM NH_4OH (Sigma Aldrich). Transferrin sample (50 µL of approximately 1 mg.mL^{-1}) was mixed with 200 µL of cIEF gel, 50 µL of carrier ampholyte (40% Roti®lyte 4-7, Carl Roth GmbH), 25 µL of a 500 mM Arginin solution (Cathodic Stabilizer, Sigma Aldrich). No pI marker was used due to the impossibility to detect them by ICP-MS.

For the separation procedure, the capillary was filled with the mixture previously described by applying the high pressure rinse mode (25 psi) for 15 min. Then, a voltage of 20 kV was applied for 45 min to focus the ampholytes and samples; the cathode was placed in the NH_4OH solution (catholyte) and the anode in the phosphoric acid solution (anolyte). Once the focalization step completed, the capillary was coupled with the ICP-MS and the make-up liquid (NH_4OH 200 mM) corresponded to the catholyte reservoir. The coupling making the chemical mobilization impossible the pH gradient was mobilized by pressure. A voltage of 20 kV and a 1 psi pressure are applied for 60 min. The capillary was rinsed between each analysis with a 4.5 M urea solution, a 10 mM NaOH, and milliQ water in order to ensure the cleanliness of the capillary and avoiding protein aggregation.

Calculation method of the stability constant between Tf and Th:

According to the thermodynamic data available, $Th(NTA)_2^{2-}$ is the major species in solution (100 %) at pH = 7.0 and C_{NTA} = 10 µM. Therefore, the equilibrium below is only considered (charges are omitted):

$$Th(NTA)_2 + Tf \rightleftharpoons ThTf + 2NTA, \quad (1)$$

K^* the conditional constant defined as:

$$K^* = \frac{[ThTf][NTA]^2}{[Tf][Th(NTA)_2]} \quad (2)$$

At the inflexion point of the sigmoidal function, the [Th(NTA)$_2$] and [ThT*f*] concentrations are equal,[1] therefore Eq.(2) can be simplified to:

$$K^* = \frac{[NTA]^2}{[Tf]} = \frac{\left(C_{NTA}/\alpha\right)^2}{C_{Tf}}, \quad (3)$$

with C_{NTA} and C_{Tf} the total concentration of ligand, the coefficient α is defined as:

$$\alpha = \frac{C_{NTA}}{[NTA^{3-}]} = 1 + 10^{pka_4}[H^+] + 10^{pka_4+pka_3}[H^+]^2 + 10^{pka_4+pka_3+pka_2}[H^+]^3 + 10^{pka_4+} \\ [H^+]^4 \quad (4)$$

where pka_i stands for the four stepwise dissociation constants of nitrilotriacetic acid. Within our experimental conditions (pH = 7.0), $log_{10}\alpha$ is equal to 2.68 ± 0.04.

From Eq.(3) and the constant of formation of Th(NTA)$_2$ ($log\ K_{Th(NTA)_2}$ = 30.26), the conditional constant relative to the formation of ThT*f* can be deduced:

$$log_{10}K^*_{ThTf} = log_{10}K^* + log_{10}K_{Th(NTA)_2} \quad (5)$$

Based on electropherograms obtained at pH 7 (Figure 2), the relative areas between the species Th(NTA)$_2$ and ThT*f* were calculated for an increasing concentrations of protein. The intersection point has been found to be [T*f*] = (1.3 ± 0.1) µM corresponding to a conditional binding constant of $log_{10}K^*_{ThTf} =$ 20.92 ± 0.19.

It was previously shown that the bicarbonate ions drive nitrilotriacetate anions out of the transferrin-Fe^{3+}-NTA ternary complex.[2] The same behavior is expected for the transferrin-Th^{4+}-NTA complex. Therefore, the concentration of bicarbonate ions must be taken into account in order to compare our results with other published data obtained at different carbonate concentrations. This correction is applied by the means of the bicarbonate-independent binding constant (K^*_1):[3]

$$log_{10}K^*_1 = log_{10}K^*_{PuTf} + log_{10}\alpha_c, \quad (6)$$

where α_c is the fractional saturation of the human apo-transferrin-binding sites with bicarbonate according to the equation:

$$\alpha_c = K\frac{\left[HCO_3^-\right]}{1 + K\left[HCO_3^-\right]}, \quad (7)$$

with K_c the binding constant of bicarbonate with transferrin $log_{10}K$ = 2.23 ± 0.18 (mean value between the binding constant of bicarbonate with transferrin at the C-terminal site ($log_{10}K_c$ = 2.66 ± 0.07) and the one of bicarbonate at the N-terminal site ($log_{10}K_N$ = 1.8 ± 0.3)).[4]

At the equilibrium with atmosphere, the concentration of bicarbonate can be easily determined and as a result, $log_{10}\alpha_c =$ –2.27. After correction due to the presence of bicarbonate in the solution, the conditional binding constant was recalculated $log_{10}K^{*}_{ThTf}$ = 18.65 ± 0.19. In the absence of bicarbonate, the affinity of transferrin for thorium at pH 7 was of the same order of magnitude than for iron. Despite the larger ionic radius of Th^{4+} compared to Fe^{3+}, the results showed that T*f* had a strong affinity for thorium, but lower than for Pu (log K = 25.0).[5]

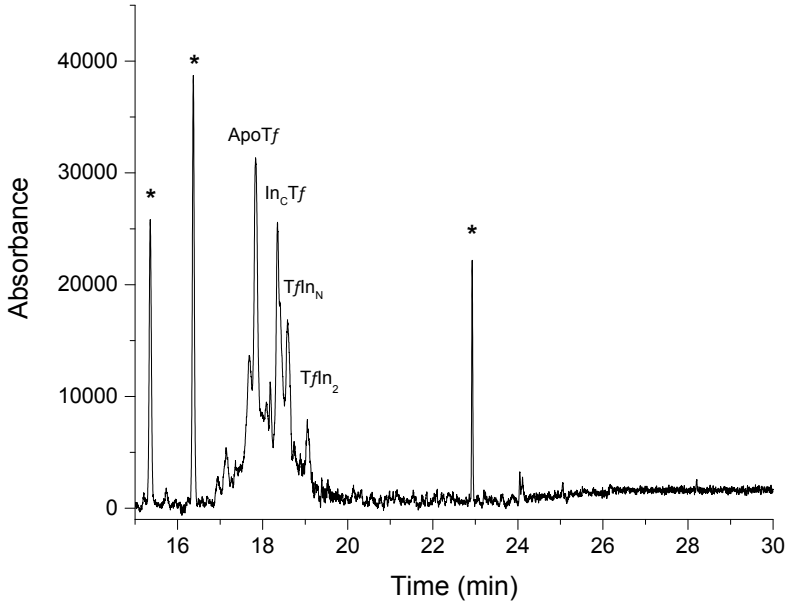

Figure 1S: cIEF electropherogram of transferrin in presence of 1 equivalent of indium, * pI marker. Conditions: focalization 25kV, 20 min; mobilization 30kV, 15 min. The four molecular forms are labelled on the figure, UV detection at 280 nm.

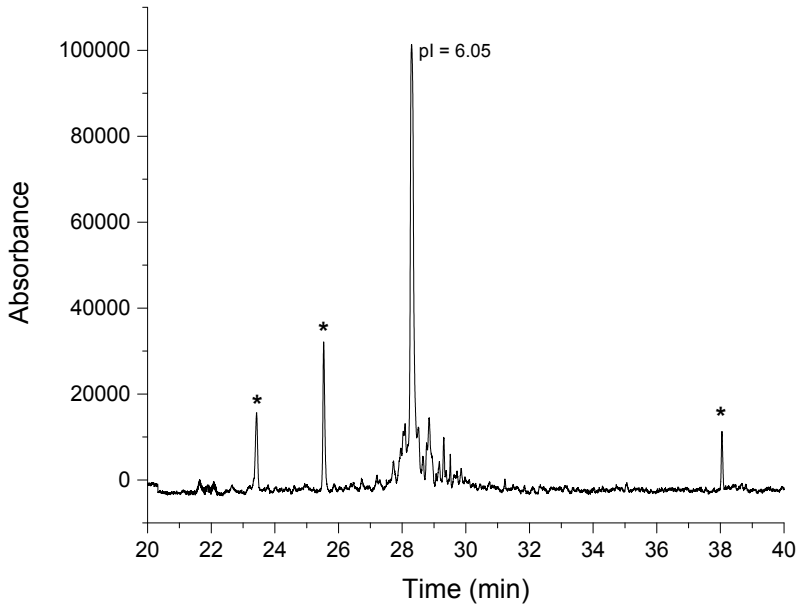

Figure 2S: cIEF electropherogram of transferrin in presence of 1 equivalent of thorium, * pI marker. Conditions: focalization 25kV, 20 min; mobilization 30kV, 15 min, UV detection at 280 nm.

Reference:

(1) Bonin, L.; Aupiais, J.; Kerbaa, M.; Moisy, P.; Topin, S.; Siberchicot, B. *RSC Adv.* **2016**, *6*, 62729-62741.

(2) Schlabach, M. R.; Bates, G. W. *J. Biol. Chem.* **1975**, *250*, 2182-2188.

(3) Harris, W. R.; Chen, Y.; Wein, K. *Inorg. Chem.* **1994**, *33*, 4991-4998.

(4) Harris, W. R. *Biochem.* **1985**, *24*, 7412-7418.

(5) Sauge-Merle, S.; Lemaire, D.; Evans, R. W.; Berthomieu, C.; Aupiais, J. *Dalton Trans.* **2017**, *46*, 1389-1396.