Electronic Supplementary Material (ESI) for Chemical Communications


# Supplementary material

## Ferritin and Metallothionein: Dangerous Liaisons

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**Supplementary Experimental Details:**

*Reaction of ferritin with MTs*: Horse spleen ferritin (56 mg/ml) was obtained from Sigma-Aldrich and passed down a Sephadex G-25 column to remove any free iron. Aqueous solutions of proteins were prepared using water purified through the Milli-Q system. Ferritin (0.1 mg/ml, 21 μM in Fe) was incubated at room temperature with each Zn-MTx preparation (1 μM) in the presence of an excess of ferrozine. The experiments were carried out in anaerobic conditions in a glove box and the solutions transferred to screw-cap quartz cuvettes. The development of the iron(II)-ferrozine complex was followed by UV-visible spectroscopy using a Thermospectronic UV300 spectrophotometer against reference containing solutions containing Milli-Q water. Spectra were recorded during 24 h. The concentrations of $[Fe(ferrozine)_3]^{4-}$ after 24 h were calculated from the UV-Vis absorbance values at 562 nm using $\varepsilon^{562}$= 27.900 $mM^{-1}cm^{-1}$.

To determine the Zn(II) concentration, the resulting Ft-MTx solutions were filtered and then exhaustively dialyzed at 4 ºC for two days against several changes of Milli-Q water using a dialysis bag with a molecular weight cut-off of 5.000 Da. After 48 h, the water of the dialysis reservoirs (100 ml) were collected and used to determine the Zn(II) concentration by atomic absorption spectrometry. Likewise the Zn(II) concentration of the dialyzed solution was also determined by atomic absorption spectrometry.

Two blank experiments were performed at the same conditions either in the absence of ferritin or the Zn-MT complex. In the absence of Zn-MT, the ferritin-ferrozine solution did not show any absorption at 562 nm in the UV-Visible spectrum. Likewise, anaerobic solutions of the Zn-MTx species did not change over time as corroborated by ESI-MS.

*Incubation of ferritin with Tiron*: To a purified sample of horse spleen ferritin 1 mg/ml), as that used for the reactions with the distinct Zn-MTs, Tiron (1,2-dihydroxy-3,5-benzenedisulfonate) was directly added up to a final concentration of 0.1 M. The resulting solution was gently stirred during 24 h. The resulting solution did not show absorption bands in the 400-800 nm range in the UV-Visible spectrum after 24h, indicating the absence of any Fe-Tiron complex. Addition of Zn-MTx (10 μM) to the starting solution did not produce the appearance of any absorption band in the UV-vis spectra. Formation of Fe-Tiron species do not occur from ferritin preparations incubated either in the presence or absence of Zn-MT complexes.

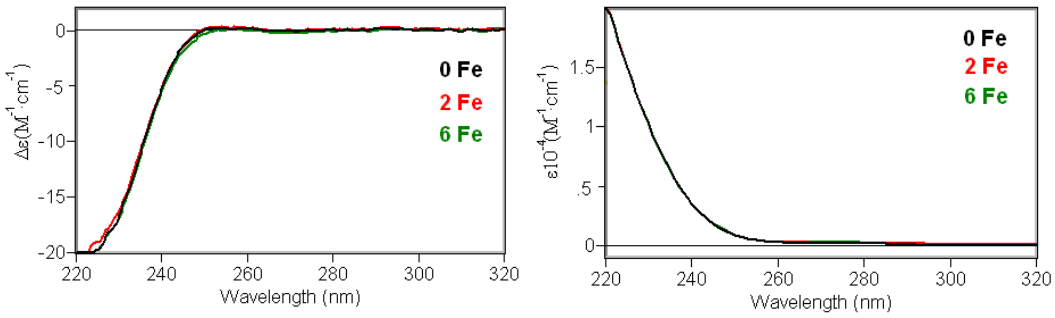
**Supplementary Figures:**

**Fig. S1.** CD and UV-Vis spectra recorded during the addition of several $Fe^{II}$ eq to a 20 μM solution of recombinant Zn-Cup1. The total coincidence of the successive spectra indicates that the Zn-Cup1 preparation remains invariant in presence of excess $Fe^{II}$.

**Fig. S2.** ESI-MS data corresponding to distinct stages of the reaction of the recombinant mouse $Zn_7$-MT1 with different equivalents of Fe(III). The observed species are collected in the Table shown in Figure 1b.

**Fig. S3**. ESI-MS data, at the +4 charge state, corresponding to distinct stages of the reaction between the recombinant mouse Zn-MT complexes of MT1, MT2 and MT3 and horse spleen Ft at different reaction times: (a) t= 0 h (showing the initial Zn-complexes present in each preparation); (b) t= 5 h (note here that while MT1 and MT3 give rise to undermetalated species, MT2 shows a drastic decrease of the concentration of the $Zn_7$-MT complex – note the change in the Y axis), and (c) t= 24 h in the case of MT1. The table shows the differences observed between the theoretical and the observed masses of the underloaded Zn-MT1 species, which are concordant with the formation of disulphide bonds between the non-coordinating Cys residues. Furthermore, the 20 Da difference between the theoretical and the observed masses of the apo-MT1 suggests the total oxidation of its 20 Cys residues (Gehrig *et al.*, Protein Science, (2000), 9, 395-402).

Fig. S4 Native polyacrylamide gel electrophoresis (7%) stained with Coomassie brilliant blue R250. Lanes 1-3 correspond to the mixtures of Zn-MTs and Ft after 24h. Lanes 4-7 correspond to purified Ft and the distinct Zn-MTx (1, 2 and 3, respectively) protein controls.

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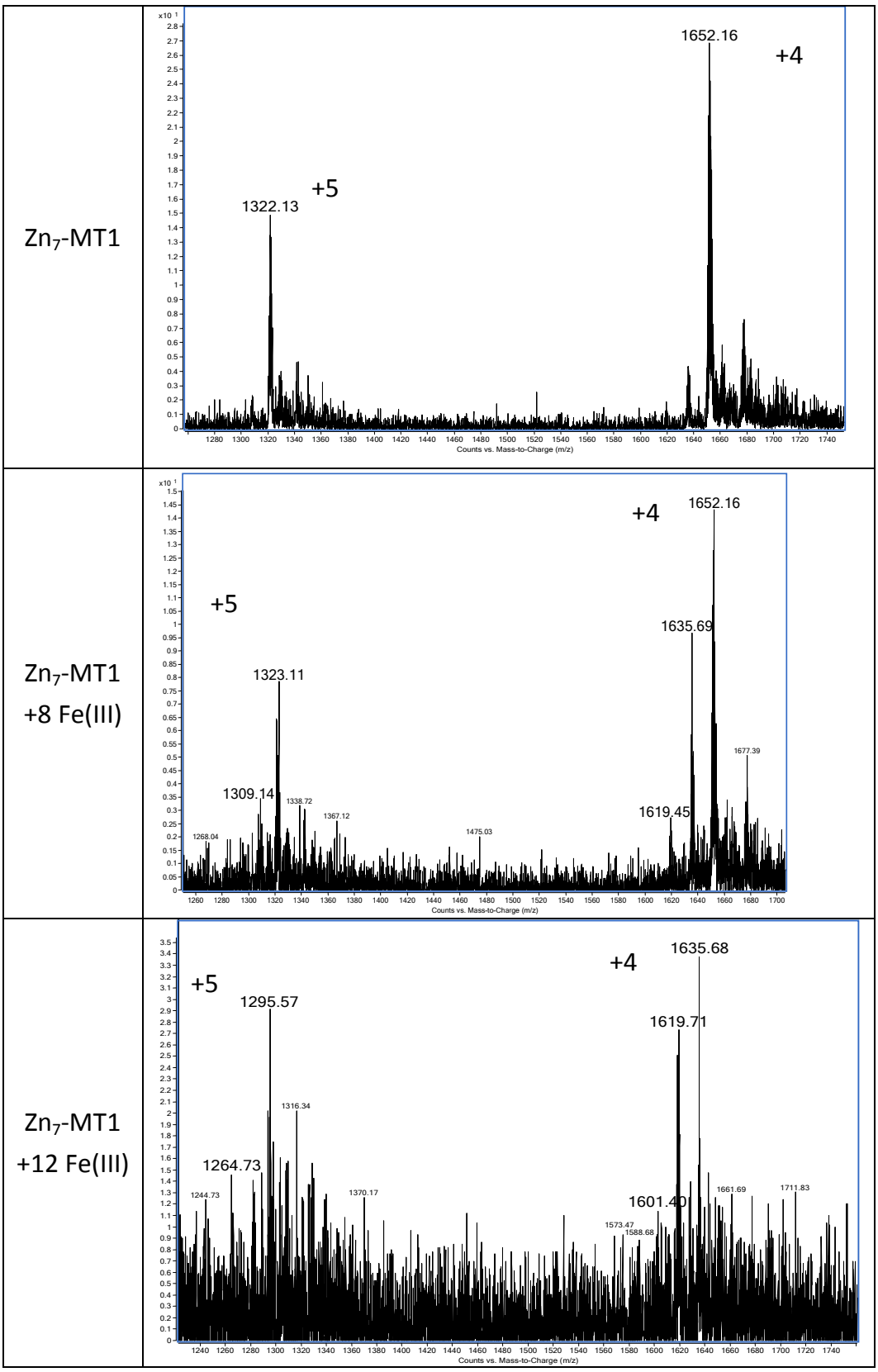


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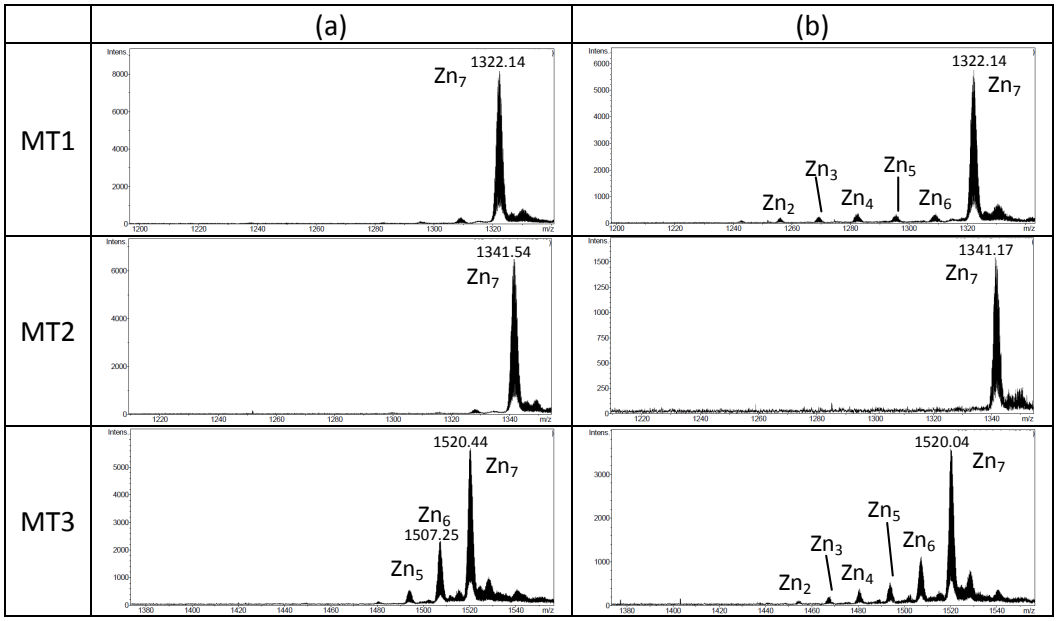
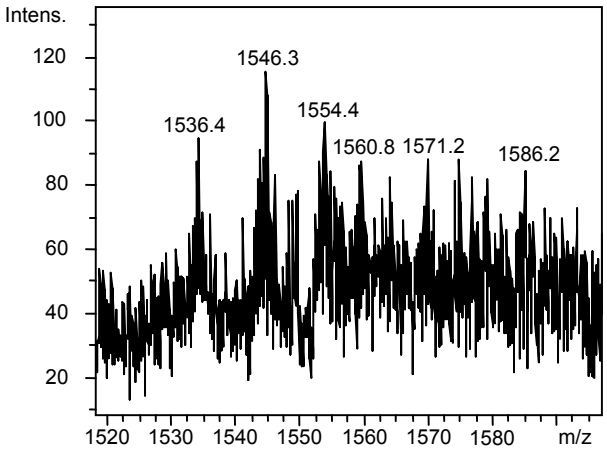



(c)




| | Theoretical | Experimental |
|---|---|---|
| Apo-MT1 | 1541.6 (6162.2) | 1536.4 (6141.6) |
| Zn1-MT1 | 1557.4 (6225.6) | 1554.4 (6213.6) |
| Zn2-MT1 | 1573.3 (6289.0) | 1571.2 (6280.8) |
| Zn3-MT1 | 1589.1 (6352.4) | 1586.2 (6340.4) |

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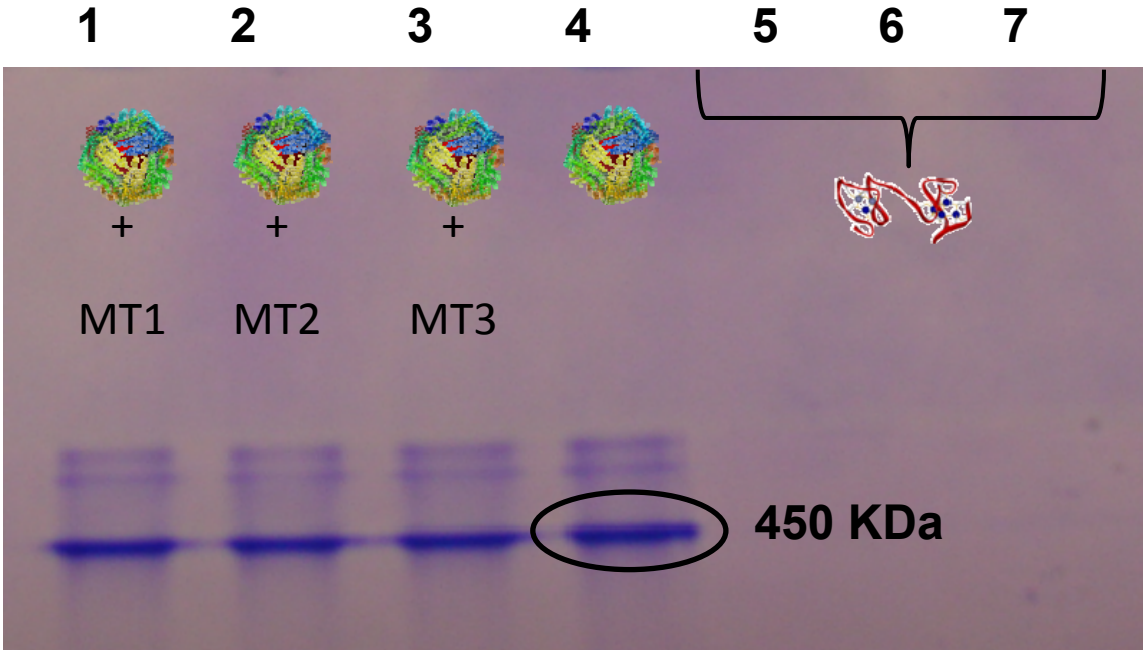


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