

Supplementary Material

to

**Sulfide ions as modulators of metal-thiolate cluster size
in a plant metallothionein**

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Sulfide ions as modulators of metal-thiolate cluster size: Supplementary Material

Vector pGtMT (modified pGEX-4T-1)

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Sulfide ions as modulators of metal-thiolate cluster size: Supplementary Material

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Amino acid sequence of expressed fusion protein

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H N Met L G G C P K E R A E I S Met L E G A V L D I R Y G V S R I A Y S K D F E
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E T L V Met G V A S G K T Q F E G A E Met G F G A E N D G C K C G S N C T C N P
C T C K
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GST

Thrombin

TEV

cicMT2

BamH1 ggatcc

XmaI cccggg

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Sulfide ions as modulators of metal-thiolate cluster size: Supplementary Material

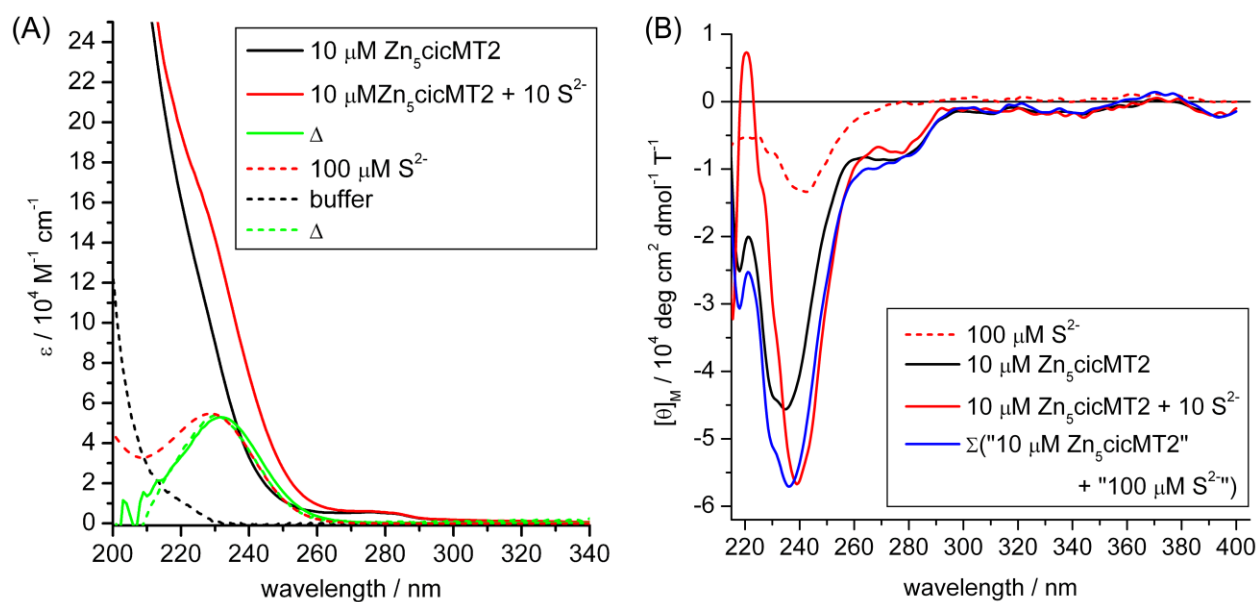


Fig. S1 Influence of sulfide ions on the spectroscopic features of Zn₅cicMT₂. (A) UV spectra of Zn₅cicMT₂ in absence (black solid line) and presence of 10 equiv. of sulfide ions (red solid line). The green solid line is the delta-spectra obtained by subtraction of the Zn₅cicMT₂ absorption from the absorption of Zn₅cicMT₂ in presence of 10 equiv. of sulfide. To analyse the contribution of free sulfide ions, a spectrum of a 100 μM Na₂S solution was recorded (red dashed line). The delta spectrum of the latter spectrum and the buffer (10 mM Tris-HCl, pH 7.3, black dashed line) results in the calculated green dashed spectrum. Both green spectra are virtually identical indicating the absence of interaction between Zn₅cicMT₂ and sulfide ions under these conditions. (B) MCD spectra of Zn₅cicMT₂ in absence (black) and presence of 10 equiv. of sulfide ions (red). The red dashed spectrum was recorded with a solution containing 100 μM sulfide ions in form of Na₂S, the blue spectrum is the sum of the black and red dashed spectra.

Sulfide ions as modulators of metal-thiolate cluster size: Supplementary Material

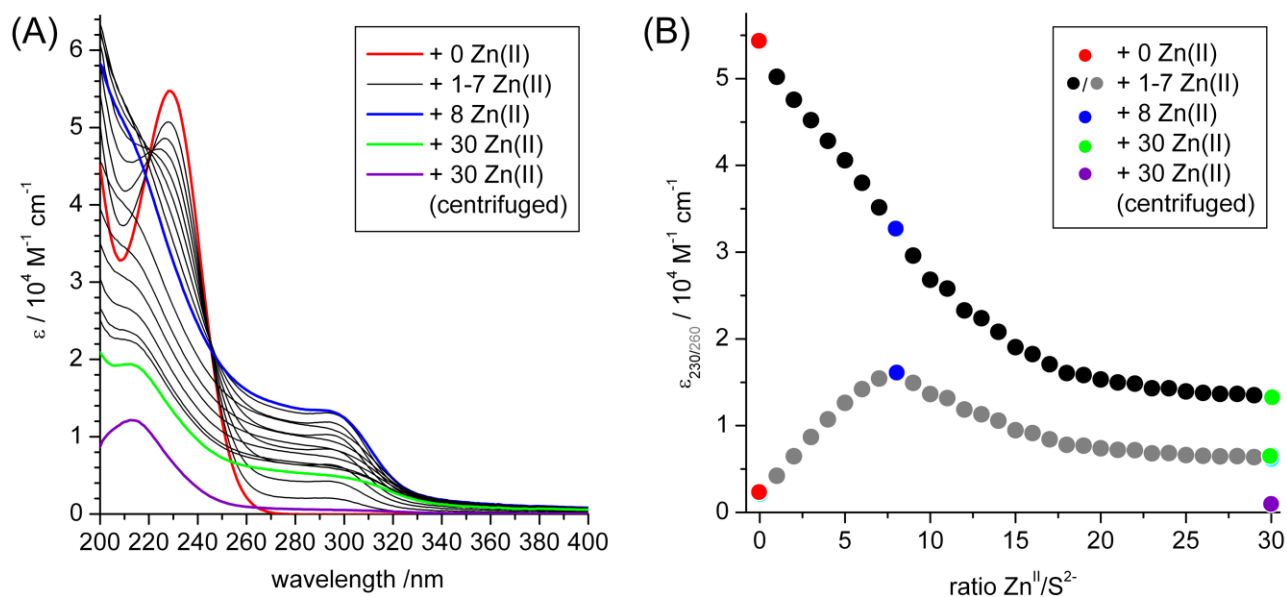


Fig. S2 (A) UV spectra of the titration of a 100 μM Na₂S solution with increasing amounts of Zn(II) ions. Certain spectra are highlighted in colour according to the legend. (B) Molar absorptivity plots at 230 (black and coloured dots) and 260 nm (gray and coloured dots) from the spectra shown in (A). The red spectrum in (A) is the same as in Fig. S1A.

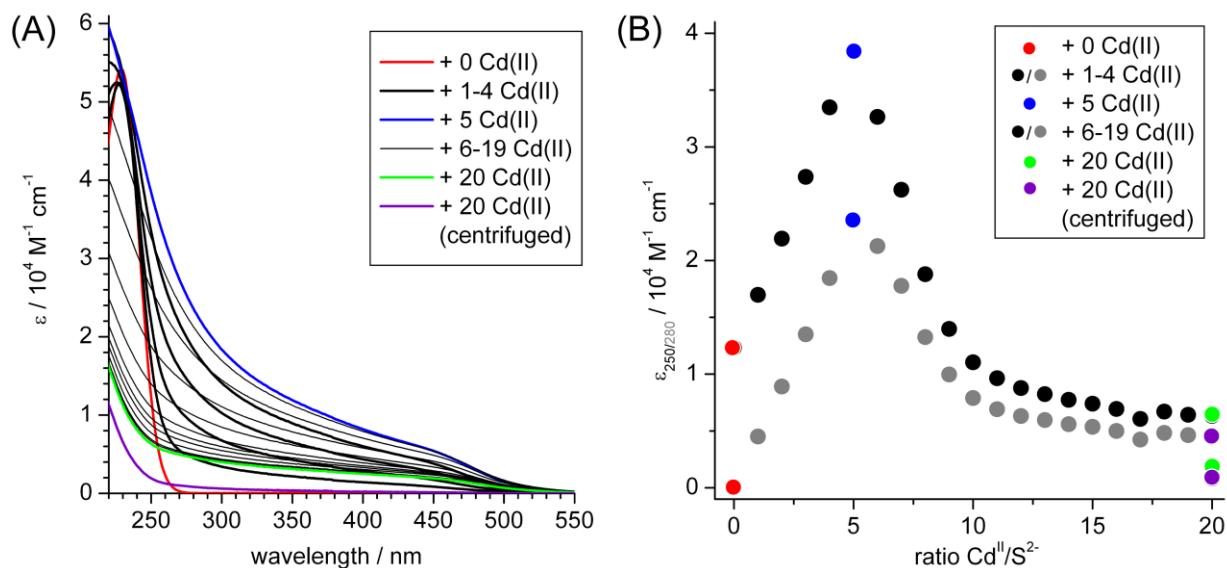


Fig. S3 (A) UV spectra of the titration of a 100 μM Na₂S solution with increasing amounts of Cd(II) ions. Certain spectra are highlighted in colour according to the legend. (B) Molar absorptivity plots at 250 (black and coloured dots) and 280 nm (gray and coloured dots) from the spectra shown in (A).

Sulfide ions as modulators of metal-thiolate cluster size: Supplementary Material

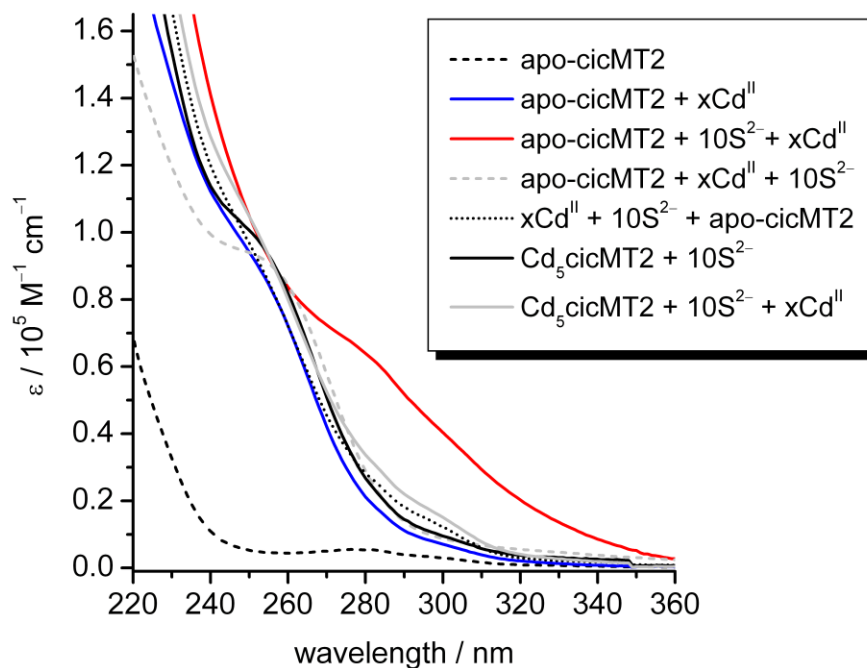


Fig. S4 Probing the mechanism of cluster formation with incorporated sulfide ions. Starting from apo-cicMT2 the order of MT, Cd^{II} , and sulfide addition was varied. Apparently, only when apo-cicMT2 was first incubated with sulfide ions and only then titrated with increasing amounts of Cd^{II} (red spectra), formation of sulfide containing Cd-thiolate clusters was observed.

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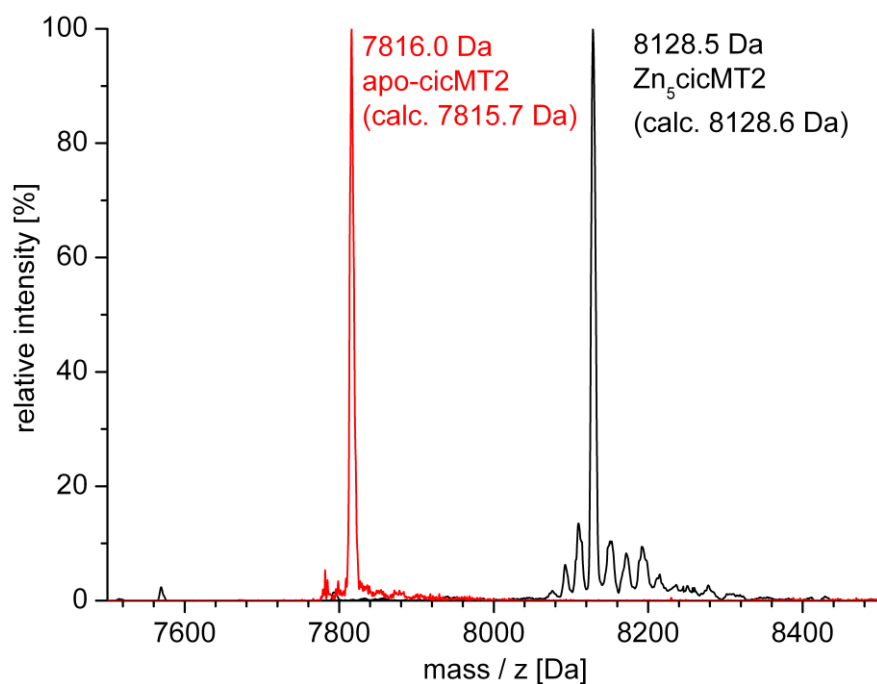


Fig. S5. Deconvoluted ESI⁺-MS spectra of Zn₅cicMT2 from GST-thrombin-TEV-cicMT2 cleaved with TEV protease measured at acidic pH (pH 2, red line) and neutral pH (pH 7, black line), with a normalized intensity of 100 %. Additional peaks of the sample at pH 7 arise from Na⁺-adducts of the Zn₅-form and to a minor extent also from of the Zn₄-form.