Supplementary Material

to

METAL ION RELEASE FROM METALLOTHIONEINS:

PROTEOLYSIS AS AN ALTERNATIVE TO OXIDATION

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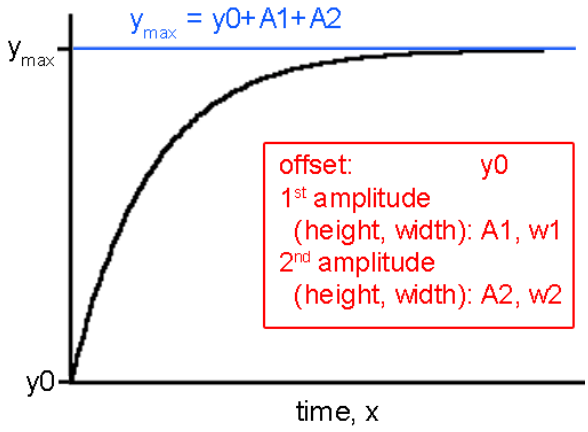
Fitting of Zn^{II}-release data in Origin with equations for exponential association kinetics

Depending on the plot form obtained from the Zn^{II}-MT / PAR metal ion exchange reaction two different equations were used to extrapolate the data to equilibrium conditions, if necessary:

1) two-phase exponential association



$$y = y0 + A1 \cdot (1 - e^{-x/w1}) + A2 \cdot (1 - e^{-x/w2})$$

2) three-phase exponential association

$$y = y0 + A1 \cdot (1 - e^{-x/w1}) + A2 \cdot (1 - e^{-x/w2}) + A3 \cdot (1 - e^{-x/w3})$$

All fittings were performed with Origin® 7.

Calculation of apparent binding constants, K_{app}, from PAR data

$$K_{app,MT} = \frac{[ZnBS]\cdot[PAR]^2}{[BS]\cdot[Zn(PAR)_2]}\cdot 10^{-6}\cdot K_{app,Zn(PAR)_2}$$

$K_{app,Zn(PAR)_2}$ (pH 7.4) = $10^{13.49}$ M^{-2}

1) All Zn^{II} binding sites (ZnBS) in the respective MT are treated as non-interacting with equal K_{app}

Example: Zn_6E_c-1 (control, 1st measurement)

A_{500nm} = 0.11954

$[Zn(PAR)_2] = A_{500nm} / \varepsilon_{Zn(PAR)_2}$ = 0.11954 / 0.065 μM^{-1} cm^{-1} = 1.8391 μM

[PAR] = $[PAR]_o - 2*[Zn(PAR)_2]$ = 100 μM – 2*1.8391 μM = 96.3218 μM

[ZnBS] = $[ZnBS]_o - [Zn(PAR)_2]$ = 9 μM – 1.8391 μM = 7.1609 μM

[BS] = $[Zn(PAR)_2]$ = 1.8391 μM

$\Rightarrow K_{app,MT}$ = $10^{11.78}$ M^{-1}

2) All ZnBS of (partially) released Zn^{II} ions have the same K_{app}

All other ZnBS have much higher K_{app} and are not affected

Example: Zn_6E_c-1: 1.14 eq Zn^{II} are released, hence only 2 of the 6 ZnBS are affected by PAR

[ZnBS] = $[ZnBS]_o - [Zn(PAR)_2]$ = $^{9\ \mu M}/_6$ * 2 – 1.8391 μM = 1.1609 μM

$\Rightarrow K_{app,MT}$ = $10^{10.99}$ M^{-1}

3) If more than 1 eq Zn^{II} is released by PAR:

This Zn^{II} is much weaker bound and not considered

a) All remaining Zn^{II} binding sites (ZnBS) in the respective MT have the same K_{app}

Example: Zn_6E_c-1: 1.21 eq Zn^{II} are released, hence only 5 of the 6 ZnBS are considered for the calculation, i.e. a Zn_5E_c-1 species

1 eq Zn^{II} ≡ 9 μM / 6 = 1.5 μM Zn^{II}

1.5 μM Zn^{II} ≡ A_{500nm} = 1.5 * 0.065 = 0.0975

A_{500nm} = 0.11954 – 0.0975 = 0.02204

$[Zn(PAR)_2] = A_{500nm} / \varepsilon_{Zn(PAR)_2}$ = 0.02204 / 0.065 μM^{-1} cm^{-1} = 0.3391 μM

[PAR] = $[PAR]_o - 2*[Zn(PAR)_2]$ = 100 μM – 2*1.5 μM – 2*0.3391 μM = 96.3218 μM

[ZnBS] = $[ZnBS]_o - [Zn(PAR)_2]$ = 9 μM – 1.5 μM – 0.3391 μM = 7.1609 μM

[BS] = $[Zn(PAR)_2]$ = 0.3391 μM

$\Rightarrow K_{app,MT} = 10^{13.25}\ M^{-1}$

b) Only K_{app} of the ZnBS of the remaining partially released Zn^{II} ion is calculated

All other filled ZnBS have much higher K_{app} and are not affected

Example: the Zn_5E_c-1 species from a), hence only 1 of the 5 remaining ZnBS is affected by PAR

[ZnBS] = $[ZnBS]_o - [Zn(PAR)_2]$ = $^{9\ \mu M}/_{6}$ * 1 – 0.3391 μM = 1.1609 μM

$\Rightarrow K_{app,MT} = 10^{12.46}\ M^{-1}$

Calculation of first-order rate constant k of Zn^{II} release

Integrated law of first-order reaction:

$$\ln\frac{[\mathrm{ZnBS}]_t}{[\mathrm{ZnBS}]_0} = -k \cdot t$$

Formation of the $Zn(PAR)_2$ complex is followed with UV/vis spectroscopy, and hence the observed absorption increase is a measure for the Zn^{II} release from the Zn^{II} binding sites (ZnBS) in the respective MT:

$[\mathrm{ZnBS}]_0 \sim A_{max} - A_0$ and $[\mathrm{ZnBS}]_t \sim A_{max} - A_t$

(in our measurements, A_0 equals 0).

Hence:

$$\ln\frac{A_{max} - A_t}{A_{max} - A_0} = -k \cdot t$$

For the determination of the first-order rate constant, $\ln\frac{A_{max} - A_t}{A_{max} - A_0}$ (or $\ln(A_{max}-A_t)$) is plotted against time resulting in a straight line with the slope –k.

In the experiments presented here, straight lines were generally obtained for the data points between 2.5 and 100 min. The first faster Zn^{II} release step cannot be monitored with the UV/vis instrument available due to the limited number of data points (0, 0.5, 2.5 min, etc). Fittings were performed with Origin® 7.

SUPPLEMENTARY FIGURES

Figure S1. Competition experiment of PAR with musMT3 (A) and E_c-1 (B) followed by UV/vis spectroscopy at 500 nm under the different conditions indicated. All solutions, except for the control experiment, contain additionally 1 mM GSH. Data fitting (lines) to obtain the equilibrium absorption values A_{max} were performed with equations for exponential association kinetics as described in detail above in the Supplementary Material.

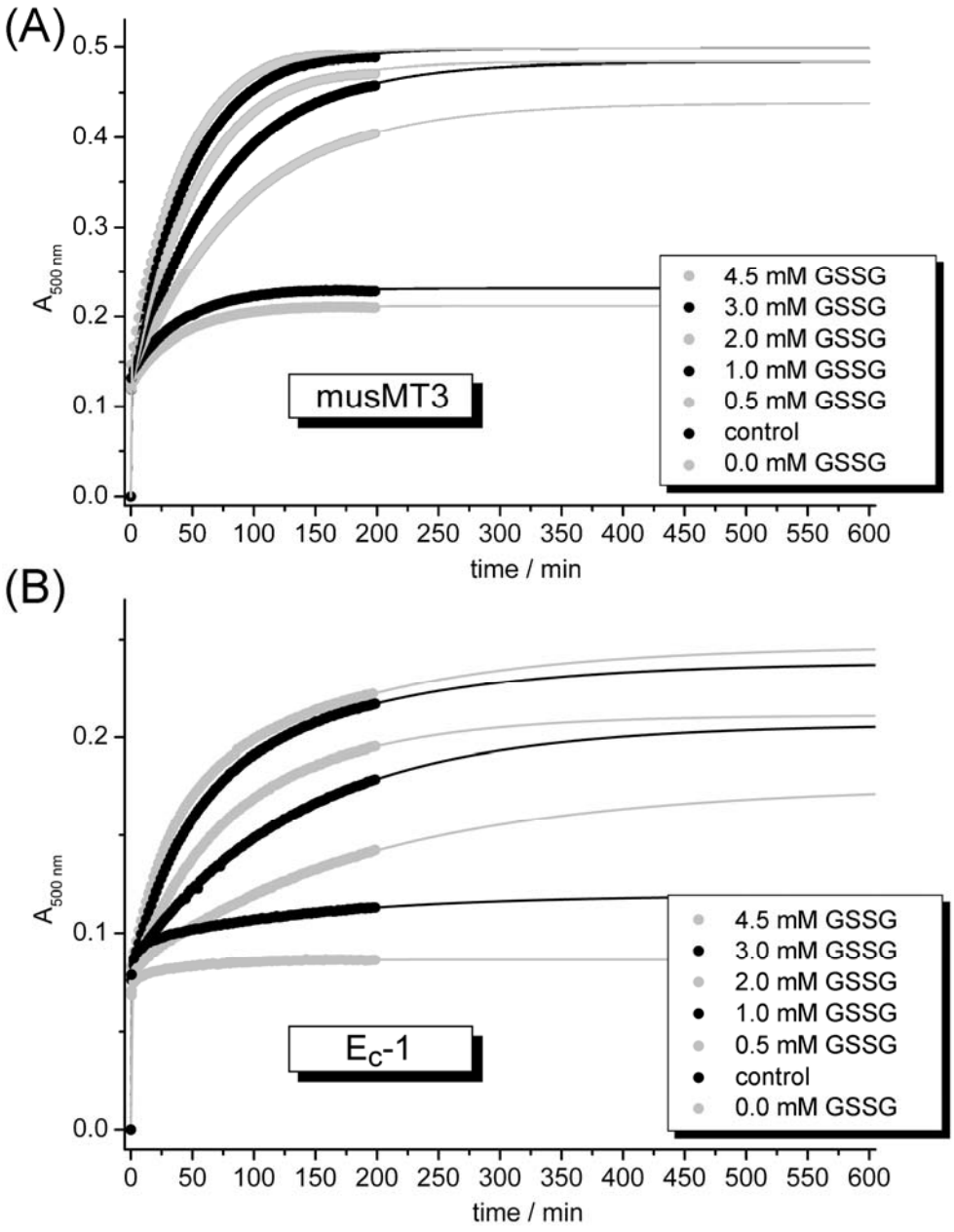


Figure S2. Amino acid sequences of the different MTs studied here. Cys-rich regions are highlighted with black boxes and potential proteinase K cleavage sites indicated by red vertical bars.

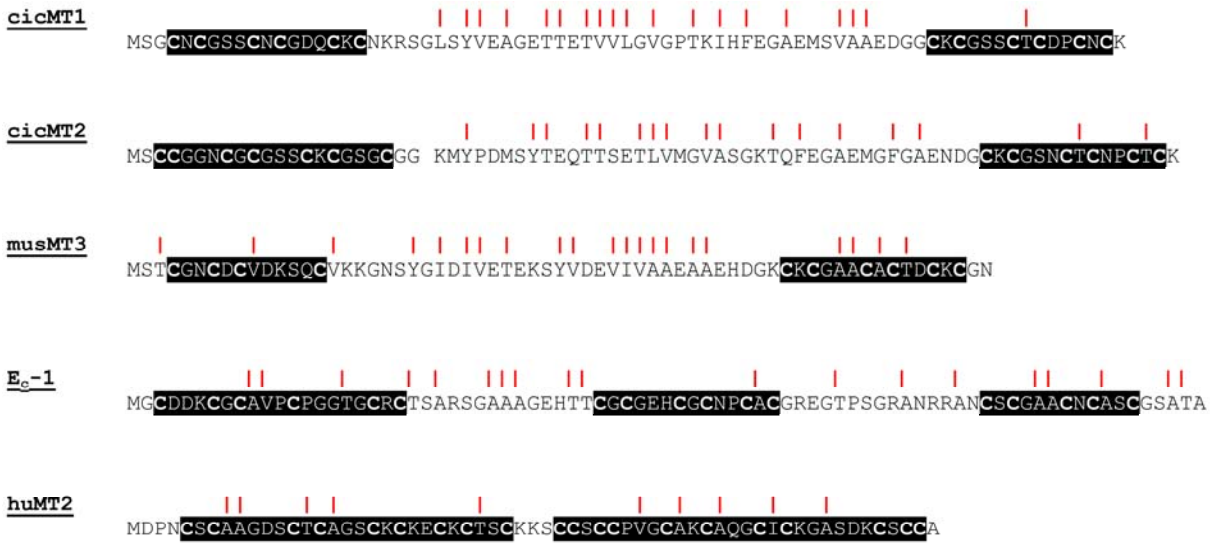


Figure S3. Competition experiment of PAR with E_c-1, γ-E_c-1, and β_E-E_c-1 followed by UV/vis spectroscopy at 500 nm under control conditions. Evidently, a 1:1 mixture of γ-E_c-1 and β_E-E_c-1 results in the same absorption values (meas.) as the sum of the values obtained with the individual domains (calc.).

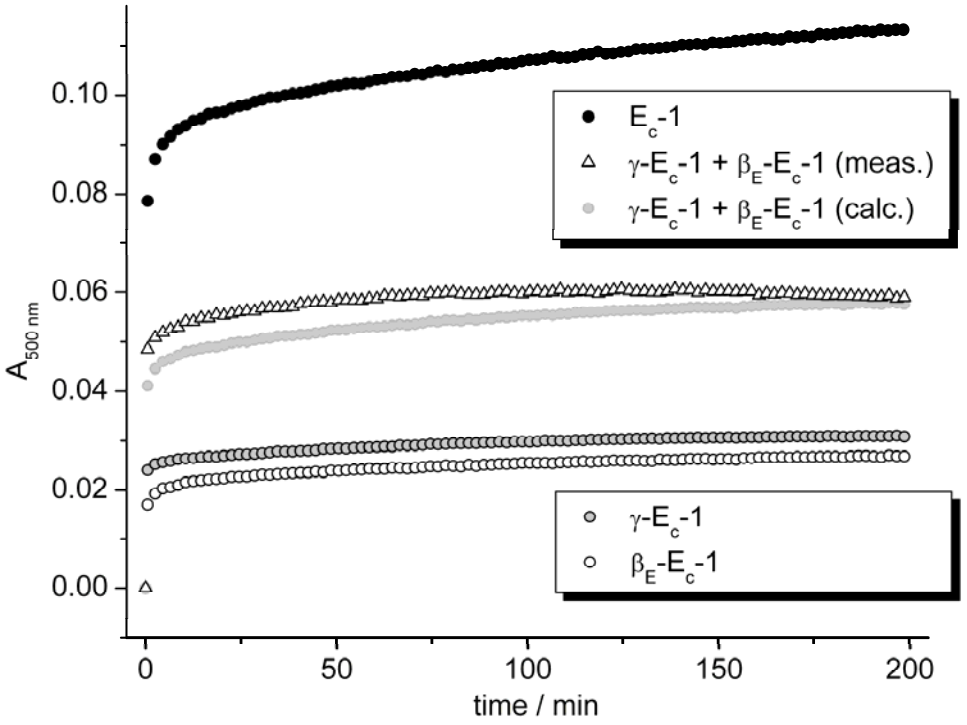


Figure S4. Percentage and equivalents of Zn^{II} ions released per individual domain (fitted equilibrium data) based on two Zn^{II} ions in γ-E_c-1 and 4 Zn^{II} ions in β_E-E_c-1 for the different conditions tested. Compare also Fig. 7 in the main text.

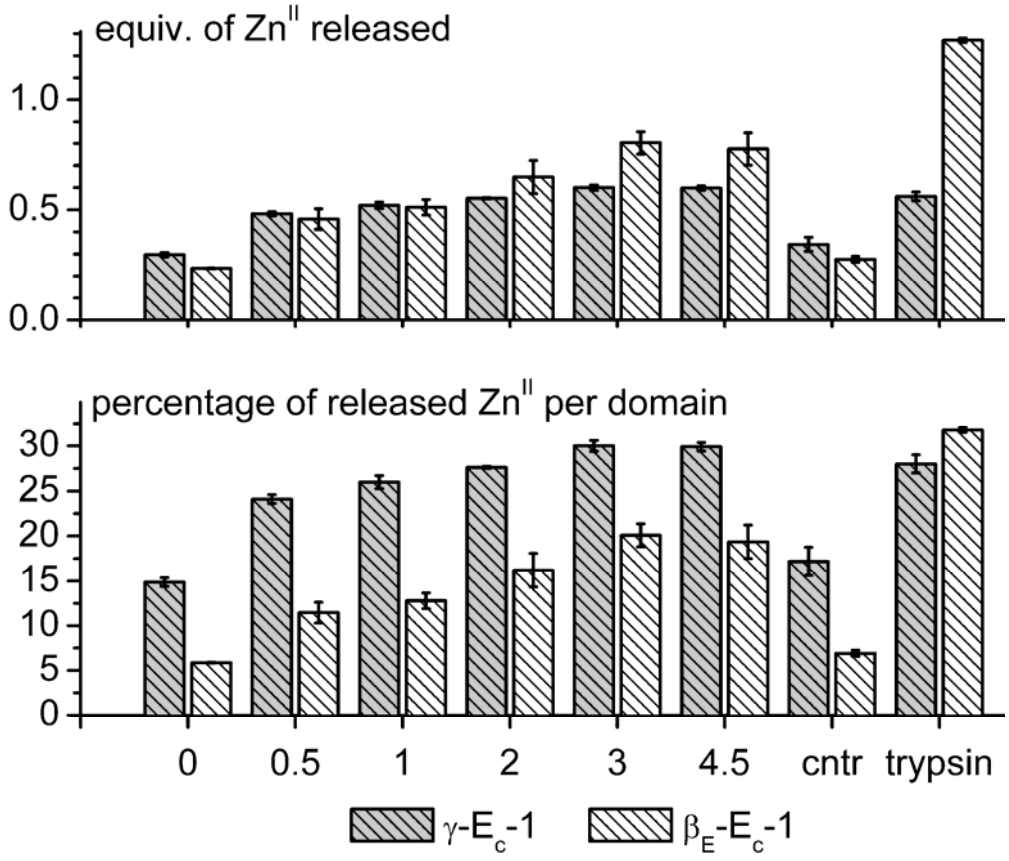


Figure S5. Summary of Zn^{II} release data for E_c-1, γ-E_c-1, and β_E-E_c-1 at equilibrium under control and oxidizing conditions (1 mM GSH/4.5 mM GSSG) as well as upon proteolytic cleavage with trypsin. To allow better comparison with the full-length E_c-1 protein, the sum of Zn^{II} release values of the individual domains is also displayed.