Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

to

Non-coordinative metal selectivity bias in human metallothioneins metal-thiolate clusters

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Supplementary Table 1. Zn-to-MT and Cu-to-MT ratios obtained after the reaction of 15 µM Zn_7MT in 25 mM Tris/HCl, 50 mM NaCl, pH 8.0 with 4 Cu^{2+} equivalents determined using ICP-MS.

	$[Zn^{2+}]/[MT]$	$[Cu^{+}]/[MT]$
MT-3	4.31 ± 0.79	3.59 ± 0.12
MT-2	4.14 ± 0.17	3.74 ± 0.18
ΔT5 MT-3	3.82 ± 0.21	3.69 ± 0.16
P7SP9A MT-3	4.39 ± 0.16	3.93 ± 0.08
ΔT5-P7SP9A MT-3	4.80 ± 0.17	4.00 ± 0.10
E23K MT-3	4.37 ± 0.26	3.72 ± 0.21
E23KG24E MT-3	3.89 ± 0.48	3.39 ± 0.24
Δ55-60 MT-3	3.93 ± 0.13	3.74 ± 0.27
ΔT5-P7SP9A-E23K MT-3	4.25 ± 0.17	3.78 ± 0.09
ΔT5-P7SP9A-Δ55-60 MT-3	4.30 ± 0.48	3.97 ± 0.15
ΔT5-P7SP9A-E23K-Δ55-60 MT-3	4.02 ± 0.30	4.40 ± 0.10
ΔT5-P7SP9A-E23KG24E-Δ55-60 MT-3	4.19 ± 0.33	4.46 ± 0.03

Supplementary Table 2. Retention times and areas under curves from size-exclusion chromatograms obtained upon the reaction of 5 μM Zn_7MT-2 or Zn_7MT-3 with 0, 4, or 8 Cu^{2+} equivalents, incubated aerobically for 1 h or 24 h in 25 mM Tris/HCl, 50 mM NaCl, pH 8.0, monitored at 220 and 260 nm.

	Cu(I) equivalents	Absorbance, nm	Equilibration Time	Retention Time, ml	Area Under Curve, ml*mAU
MT-2	0	220	1 h	13.313	111.6628
			24 h	13.295	109.5448
	4	220	1h	13.742	92.7420
			24h	13.774	74.6572
		260	1 h	13.773	35.4257
			24 h	13.779	30.7852
	8	220	1h	14.080	39.0801
			24h	14.451	8.2408
		260	1 h	14.045	20.0178
			24 h	14.005	2.6550
MT-3	0	220	1 h	12.157	133.0609
			24 h	12.166	130.9959
	4	220	1h	12.315	98.2759
			24h	12.331	76.8766
		260	1 h	12.323	20.1665
			24 h	12.323	17.7844
	8	220	1h	12.492	67.0252
			24h	12.869	26.0590
		260	1 h	12.491	32.8353
			24 h	12.750	4.3013

Supplementary Table 3. Cysteine-to-MT ratios and Zn-to-MT ratios after protein reconstitution. Cysteine-to-MT ratios were determined by reaction with 2,2-dithiodipyridine in 0.2 M sodium acetate/1m M EDTA (pH 4.0) using ε_{343} = 7,600 M^{-1} cm^{-} while Zn-to-MT ratios were determined by ICP-MS.

	[SH]/[MT]	$[Zn^{2+}]$/[MT]
MT-3	20.97 ± 0.19	7.41 ± 0.07
MT-2	17.89 ± 0.13	6.85 ± 0.15
ΔT5 MT-3	17.23 ± 0.12	7.65 ± 0.12
P7SP9A MT-3	19.26 ± 0.11	7.10 ± 0.21
ΔT5-P7SP9A MT-3	21.11 ± 0.59	7.05 ± 0.04
E23K MT-3	17.71 ± 0.17	7.21 ± 0.30
E23KG24E MT-3	17.03 ± 0.28	7.63 ± 0.09
Δ55-60 MT-3	21.27 ± 0.06	7.22 ± 0.03
ΔT5-P7SP9A-E23K MT-3	20.46 ± 0.02	7.39 ± 0.22
ΔT5-P7SP9A-Δ55-60 MT-3	20.46 ± 0.07	7.13 ± 0.14
ΔT5-P7SP9A-E23K-Δ55-60 MT-3	16.87 ± 0.04	6.46 ± 0.04
ΔT5-P7SP9A-E23KG24E-Δ55-60 MT-3	17.93 ± 0.12	7.26 ± 0.07

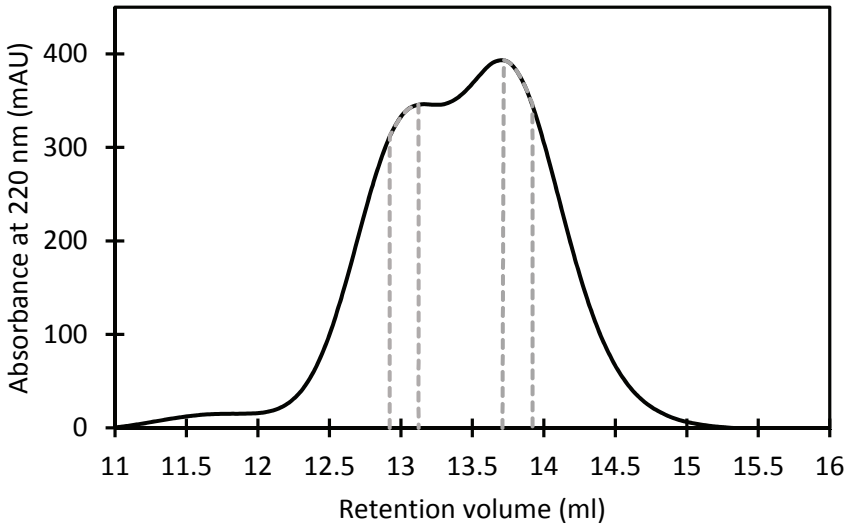
Supplementary Table 4. Apparent initial Cu(I) binding rates to MT derived from Cys-Cu(I) LMCT obtained from stopped-flow kinetic traces upon the reaction of 2.5 μM Zn_7MT-2, Zn_7MT-3, or Zn_7MT-3 mutants in 25 mM Tris/HCl, 50 mM NaCl, pH 8.0 with 4 Cu^{2+} equivalents, monitored at 260 nm at 25 ^{o}C.

	Apparent initial rate [μM Cu]/[μM MT] * s^{-1}
MT-3	0.30 ± 0.04
MT-2	0.06 ± 0.01
ΔT5 MT-3	0.34 ± 0.06
P7SP9A MT-3	0.11 ± 0.02
ΔT5-P7SP9A MT-3	0.10 ± 0.01
Δ55-60 MT-3	0.14 ± 0.02
E23K MT-3	0.30 ± 0.02
E23KG24E MT-3	0.36 ± 0.02
ΔT5-P7SP9A-Δ55-60 MT-3	0.09 ± 0.00
ΔT5-P7SP9A-E23K MT-3	0.09 ± 0.01
ΔT5-P7SP9A-E23K-Δ55-60 MT-3	0.05 ± 0.00
ΔT5-P7SP9A-E23KE24K-Δ55-60 MT-3	0.06 ± 0.00

Supplementary Table 5. Lifetime measurements of the emissive bands at 425 nm and 575 nm in $Cu(I)_4Zn_4$MTs obtained from the reaction of 10 µM Zn_7MT samples in 25 mM Tris/HCl, 50 mM NaCl, pH 8.0 with 4 Cu^{2+} equivalents recorded at 77 K, fitted using a single exponential decay function.

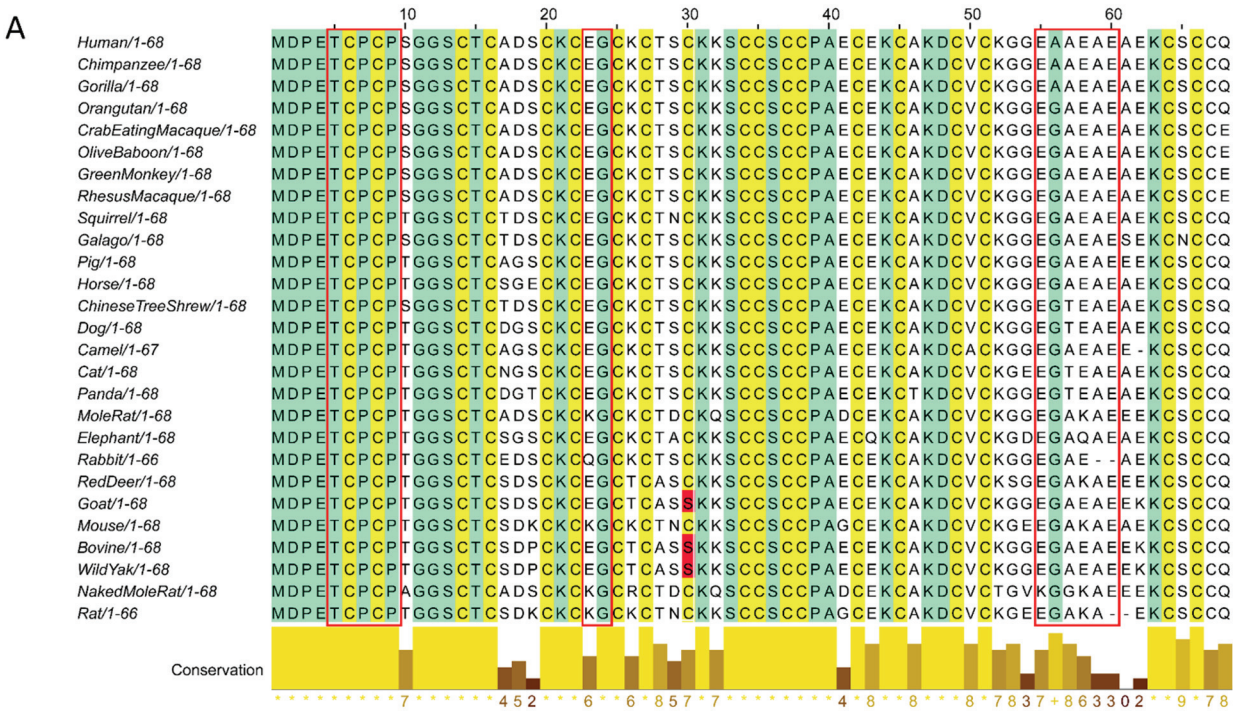
MT sample	τ (µs)	
	425 nm	575 nm
MT-3	46.5 ± 0.5	129.5 ± 2.5
MT-2	45.5 ± 1.5	131.0 ± 6.0
ΔT5 MT-3	45.0 ± 2.0	132.0 ± 2.0
P7SP9A MT-3	47.5 ± 0.5	132.5 ± 0.5
ΔT5-P7SP9A MT-3	47.0 ± 1.0	132.5 ± 1.5
E23K MT-3	46.5 ± 0.5	130.0 ± 1.0
E23KG24E MT-3	45.0 ± 0.0	128.0 ± 3.0
Δ55-60 MT-3	45.0 ± 1.0	130.0 ± 4.0
ΔT5-P7SP9A-E23K MT-3	46.5 ± 1.5	130.0 ± 3.0
ΔT5-P7SP9A-Δ55-60 MT-3	48.5 ± 0.5	131.0 ± 4.0
ΔT5-P7S-9A-E23K-Δ55-60 MT-3	46.5 ± 0.5	132.0 ± 1.0
ΔT5-P7SP9A-E23KG24E-Δ55-60 MT-3	48.0 ± 0.0	132.5 ± 0.5

Supplementary Fig. 1. Size exclusion chromatogram elution profile of a mixture of 5 µM Zn_7MT-2 and 5 µM Zn_7MT-3 upon reaction with 20 µM Cu^{2+} incubated for 1 h in 25 mM Tris/HCl, 50 mM NaCl, pH 8.0 at 25 °C, monitored at 220 nm. Analysis of the metal content of MT-2 and MT-3 peaks after SEC has been subsequently determined by ICP-MS on samples collected at the center of each elution peak (dotted line).



	[Cu]/[Cu+Zn]
MT-3	0.380 ± 0.004
MT-2	0.211 ± 0.005

Supplementary Fig. 2: Sequence alignment and sequence conservation scores plot for selected mammalian MT-3 (A) and MT-2 (B) proteins generated with Jalview. The conserved 20 cysteine metal coordinating residues are highlighted in yellow and the other conserved residues for each isoform are highlighted in light green. Serine residues for which a mutation to Cys is reported as conflict in the deposited sequences (UniProt) are highlighted in red. Conserved amino acid signature sequence features in MT-3 for which mutants have been generated and characterized in this study are highlighted in red boxes (A) with the corresponding positions in MT-2 highlighted in blue boxes.



Supplementary Figure 3. SDS-PAGE of purified MTs (1.4 µg) upon mBrB modification, visualized by staining with Coomassie Brilliant Blue R-250 (left) and visualized directly (without staining) by fluorescence imaging (right) (1: MT-3; 2: ΔT5 MT-3; 3: P7SP9A MT-3, 4: ΔT5-P7SP9A MT-3; 5: E23K MT-3; 6: E23KG24E MT-3; 7: Δ55-60 MT-3; 8: ΔT5-P7SP9A-E23K MT-3, 9: ΔT5-P7SP9A-Δ55-60 MT-3; 10: ΔT5-P7SP9A-E23K-Δ55-60 MT-3; 11: ΔT5-P7SP9A-E23KE24K-Δ55-60 MT-3; and 12: MT-2).