

**The type 4 metallothionein from *Brassica napus* seeds folds in a metal-dependent fashion
and favours zinc over other metals**

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Supplementary Information

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-1510 TATTTTAAATATTATTAGTTTATTTTCGTTATTAATAACCTATTATAAGTTTGTATTTTATTTTAGATTTAAA
-1435 AAATGAATTTAAGATAATTTTAAATATTCTTAAACACTCGTATTTGCCATATCAATACTATGTACAAAATGAC
                                         CuRE   Skn-1
-1360 ATTTTACACGGAAAAATGATATTCAAATATGTATATGATATATGGTGTAAAGTTTGTATTGAAAATCT
      HMRE-forward border
-1285 GCATAATAITTGAATTATCTATTTTGAATATTGAAACATAGATTTTATGAAAATAATATTTTCATGTGTTGTAA
      HMRE-reverse border
-1210 TTTATTTATGGTTCATAATATATTTTACTTAGAATATTTATATTAATTTAAAGTTAACATATCTTTTATATAC
-1135 ATATATATGCTCATTACTTATAGATCCATTTAGGTGTATCAATAGGCTCAAAACCAAATACTTAAATGCGATTA
-1060 ATGAATTTTATTAATTCCTTGTAATAAATAGGAGTATTTTGAAAAAATGTAACTTTCATTAAGAGTATAATTTG
-985 GGAATGATCTGGTATTGATTACCGTTAAATTAGTCTAGGATGTTGAACCGAACCAACCCGCTAAACCAAGAT
-910 TTTGGTGAGAAGTTTGGTTCGATTTGGCAGGTTTATTTAAAAATCTGGTTTTTCAGTTCAATTAGGTAAACGTTAG
-835 TTTTCAGTAAAATTACCTGAAATCACCCGAGTTTAAACCGAATTTTAAACCAAATATAATCAAACCTAAAAACGGT
-760 TAATTTAGGTAAAAATTAATAAATATCCAAAATACCGAACCAACCAACATTTTTCCAGTTTTTTTATGCGA
-685 GATTTTGGTTAAACCTAACTACCCGAATGCCGAACCTACCTACCCGACTACCCTAACCATTTGCCGAATTTAAATC
-610 AATCATATTAGCTTATTAATTTGGGCTTAACATAAATATACAAAACATCTTACCTTTTAATTAAGATCATAAA
-535 AATGGTCCAATGTATTTAAACATAAATCAACTAATAATCTGGTTTGGCATAATGGTTGAGCATATGGTTAGATA
-460 TGATTTGAAACTGGGTGTGATGTCCACTATCTATAGATTTTGTAGAATGAATTTTTTATTTTTTATTAATTAA
-385 TTAATAATGTAGATGTCATGTCAGTGTATCATTTGAATAATGGAGATACGTCAATGTATGTATAAACATAAATAT
      Skn-1                               MeJA-RE
-310 TTTTGTAGTGTATAAAATTTAAATATATTTTGTGATATATGTTTTAACATATGATTCGTGTGTTTAGCACTGAT
-235 TCGGACCGCGACTCTTTATTGATGTATGAATTGGCCAATCTTTT TTTGTGATGGTGTGTTACGTAGCACA
                                         SA-RE
-160 TTTGAACTCATGAAGCTTCGTCAACAATCAAGTGAGACGAGAAGCTACCACATGGCACATGCTACCCAACATAGA
      MeJA-RE
-85  GCCACGTGCACGGCCCATCGTCTATATCTATATATGATCATGTGAAAGACGGGAGTAGAAGAAAAGAGAAA
      ABRE                               5'UTR Py-rich stretch
-14  GCGAGGTAAAAGAAATG
  
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Fig. S1 The sequence of the putative promoter region of BnMT4 derived from NCBI database (acc. no. LOC10638850). The putative start codon is underlined. The following potential *cis*-elements were identified using the PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plant-care/html/>) (Lescot *et al.*, 2002) database: ABRE (abscisic acid response element), MeJA-RE (methyl jasmonate response element), SA-RE (salicylic acid response element), Skn-1 (element responsible for endosperm specific expression), 5'UTR Py-rich stretch (pyrimidine rich stretch responsible for conferring high level of transcription), CAAT and TATA boxes.

Additional putative metal response elements were identified manually: CuRE (copper response element) (Quinn and Merchant, 1995), HMRE (high metal responsive element) (Qi *et al.*, 2007; Ren and Zhao, 2009).

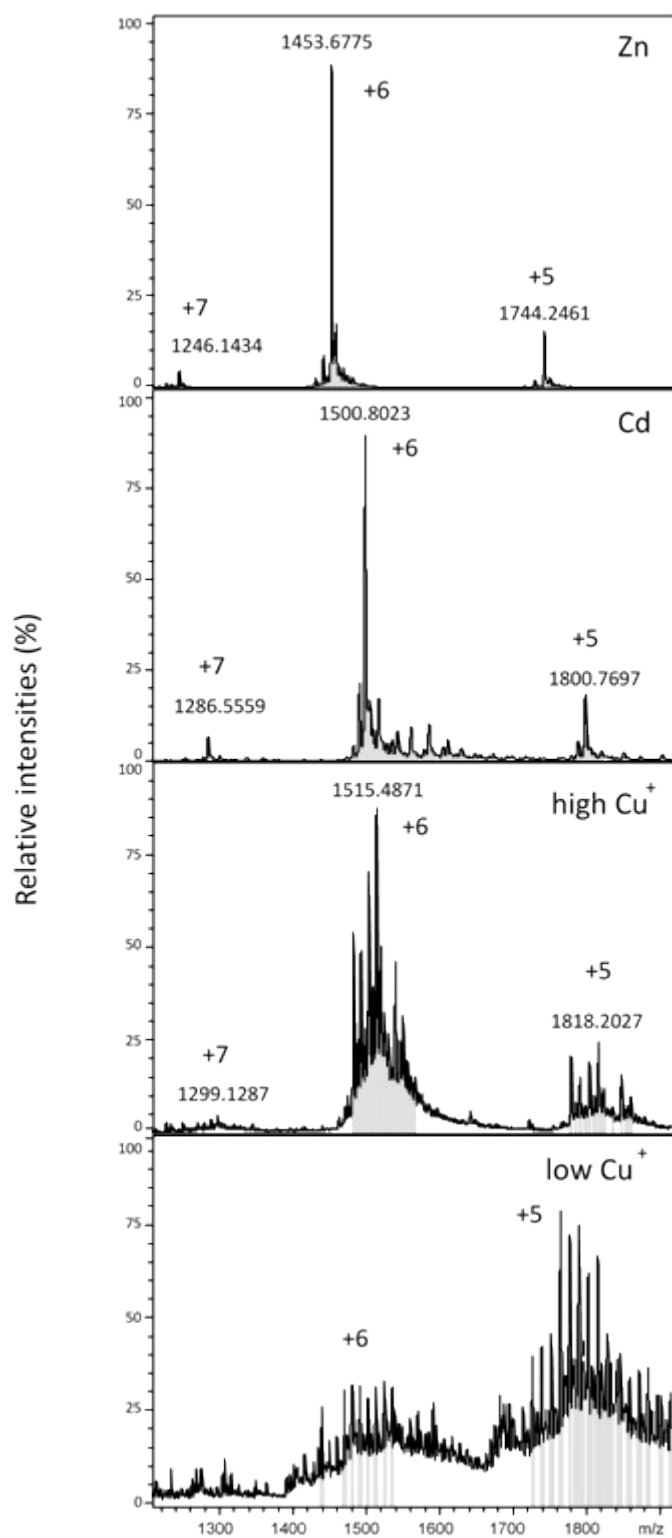


Fig. S2 Raw ESI mass spectra of Zn(II)-, Cd(II)- and Cu(I)-BnMT4 complexes at native pH (10 mM NH_4HCO_3 , 10% MeOH).

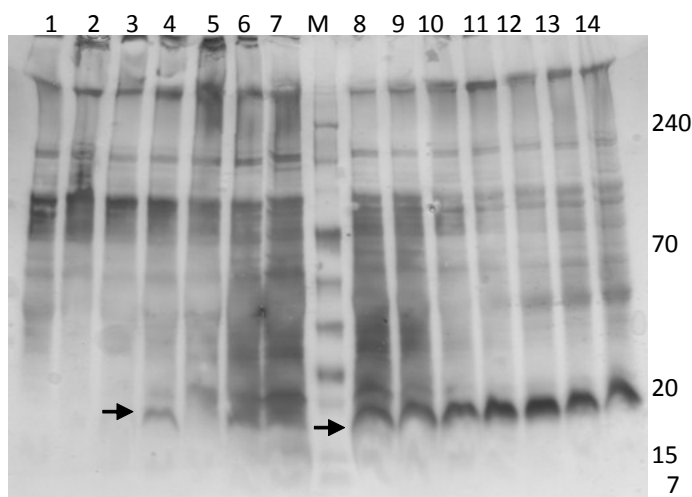


Fig. S3 SDS-PAGE analysis of crude cell lysate of *E. coli* BL21 cells transformed with pET-BnMT4 construct, grown in medium containing 0.5 mM ZnSO₄ without (1-7) or with (8-14) IPTG. Cells were harvested every hour for seven hours and proteins were isolated as described in materials and methods. Arrows indicate the positions of BnMT4 protein. M – Perfect Colour Protein Ladder (Eurx, Gdańsk, Poland).

Table S1. Calculated and experimental masses of species observed in +6 charge state ESI-MS for Zn- and Cd-BnMT4 at native and acidic pH.

	Species	Calculated MW	Experimental MW
Zn-BnMT4 native pH	Zn ₆ -BnMT4	8717.35	8716.11
	Zn ₅ -BnMT4	8653.85	8650.39
	Zn ₄ -BnMT4	8590.35	8585.71
Cd-BnMT4 native pH	Cd ₈ -BnMT4	9219.55	9218.97
	Cd ₇ -BnMT4	9109.15	9109.37
	Cd ₆ -BnMT4	8998.75	8998.80
	Cd ₅ Zn ₁ -BnMT4	8951.85	8951.86
Zn-BnMT4 acidic pH	Apo form	8336.35	8336.10
	Zn ₁ -BnMT4	8399.85	8399.00
Cd-BnMT4 acidic pH	Apo form	8336.35	8336.34
	Cd ₁ -BnMT4	8446.75	8442.06
	Cd ₂ -BnMT4	8557.15	8556.90
	Cd ₃ -BnMT4	8667.55	8667.30
	Cd ₄ -BnMT4	8777.95	8778.06
	Cd ₅ -BnMT4	8888.35	8888.52

Table S2. Calculated and experimental masses of species observed in +6 charge state ESI-MS for Cu-BnMT4 at neutral and acidic pH. The calculated molecular weights refer to the pure Cu⁺-bound species.

	Species	Calculated MW	Experimental MW	Cal. MW-Exp. MW
Cu-BnMT4 normal aeration level native pH	Me ₅ -BnMT4	8648.85	8638.90	9.95
	Me ₈ -BnMT4	8836.35	8826.86	9.49
	Me ₉ -BnMT4	8898.85	8889.99	8.86
	Me ₁₀ -BnMT4	8961.35	8953.64	7.71
	Me ₁₁ -BnMT4	9023.85	9016.87	6.98
	Me ₁₂ -BnMT4	9086.35	9081.64	4.71
	Me ₁₃ -BnMT4	9148.85	9145.56	3.29
	Me ₁₄ -BnMT4	9211.35	9214.28	-2.93
Cu-BnMT4 reduced aeration level native pH	Me ₉ -BnMT4	8898.85	8896.08	2.77
	Me ₁₀ -BnMT4	8961.35	8959.62	1.73
	Me ₁₁ -BnMT4	9023.85	9022.14	1.71
	Me ₁₂ -BnMT4	9086.35	9085.86	0.49
	Me ₁₃ -BnMT4	9148.85	9149.28	-0.43
Cu-BnMT4 acidic pH	Cu ₈ -BnMT4	8836.35	8832.36	3.99
	Cu ₉ -BnMT4	8898.85	8896.08	2.77
	Cu ₁₀ -BnMT4	8961.35	8959.62	1.73
	Cu ₁₁ -BnMT4	9023.85	9022.14	1.71
	Cu ₁₂ -BnMT4	9086.35	9085.86	0.49

Table S3. Calculated and experimental masses of species observed in ESI-MS for Zn-BnMT4 at different values of pH. Predominant species at particular pH is marked in bold.

	Species	Calculated MW	Experimental MW
pH 7.27	Zn₆-BnMT4	8717.35	8716.11
	Zn ₅ -BnMT4	8653.85	8650.39
	Zn ₄ -BnMT4	8590.35	8585.71
pH 5.05	Zn₆-BnMT4	8717.35	8715.59
	Zn ₅ -BnMT4	8653.85	8652.37
	Zn ₄ -BnMT4	8590.35	8588.27
	Zn ₃ -BnMT4	8526.85	8524.24
pH 4.48	Zn ₆ -BnMT4	8717.35	8716.33
	Zn ₅ -BnMT4	8653.85	8653.03
	Zn₄-BnMT4	8590.35	8589.61
	Zn ₃ -BnMT4	8526.85	8526.08
	Zn ₂ -BnMT4	8463.35	8462.87
pH 4.14	Zn ₆ -BnMT4	8717.35	8715.44
	Zn ₅ -BnMT4	8653.85	8652.58
	Zn ₄ -BnMT4	8590.35	8589.44
	Zn ₃ -BnMT4	8526.85	8526.10
	Zn ₂ -BnMT4	8463.35	8463.04
	Zn ₁ -BnMT4	8399.85	8300.43
	Apo form	8336.35	8336.43
pH 3.50	Zn ₁ -BnMT4	8399.85	8399.22
	Apo form	8336.35	8336.16

Table S4. Calculated and experimental masses of species observed in ESI-MS for Cd-BnMT4 at different values of pH. Predominant species at particular pH is marked in bold.

	Species	Calculated MW	Experimental MW
pH 6.61	Cd ₇ -BnMT4	9109.15	9109.28
	Cd₆-BnMT4	8998.75	8999.04
	Cd ₅ Zn ₁ -BnMT4	8951.85	8950.50
	Cd ₅ -BnMT4	8888.35	8888.53
pH 5.40	Cd ₇ -BnMT4	9109.15	9109.17
	Cd₆-BnMT4	8998.75	8998.76
	Cd ₅ Zn ₁ -BnMT4	8951.85	8951.46
	Cd ₅ -BnMT4	8888.35	8888.15
pH 4.21	Cd ₇ -BnMT4	9109.15	9109.14
	Cd₆-BnMT4	8998.75	8998.86
	Cd ₅ -BnMT4	8888.35	8888.46
pH 3.76	Cd ₆ -BnMT4	8998.75	8998.68
	Cd₅-BnMT4	8888.35	8888.40
	Cd ₄ -BnMT4	8777.95	8777.88
pH 3.51	Cd ₆ -BnMT4	8998.75	8999.15
	Cd₅-BnMT4	8888.35	8888.92
	Cd ₄ -BnMT4	8777.95	8778.00
pH 3.06	Cd ₆ -BnMT4	8998.75	8998.84
	Cd ₅ -BnMT4	8888.35	8888.56
	Cd ₄ -BnMT4	8777.95	8777.97
	Cd ₃ -BnMT4	8667.55	8667.38
	Cd ₂ -BnMT4	8557.15	8556.91
	Cd ₁ -BnMT4	8446.75	8446.71
	Apo form	8336.35	8336.06