Electronic Supplementary Material (ESI) for Metallomics.

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

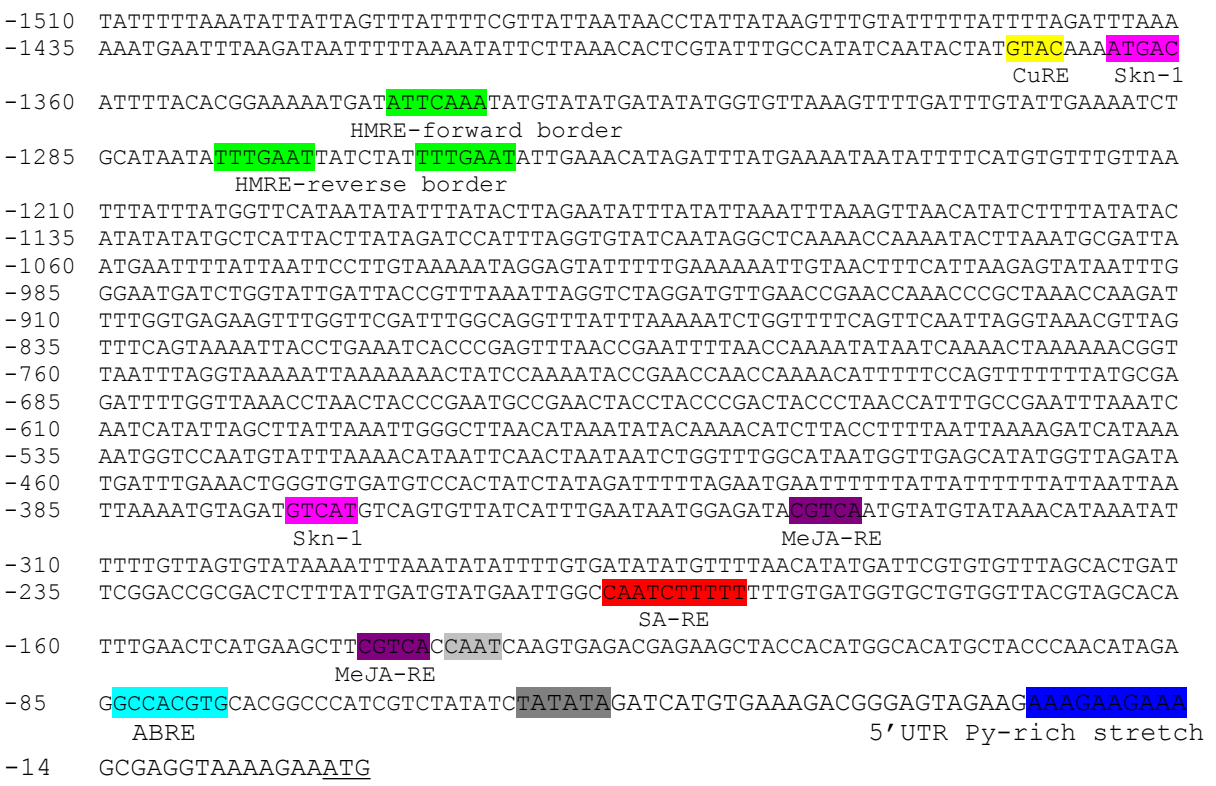

Fig. S1 The sequence of the putative promoter region of BnMT4 derived from NBCI database (acc. no. LOC10638850). The putative start codon is underlined. The following potential *cis*-elements were identified using the PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plant-care/html/) (Lescot *et 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).

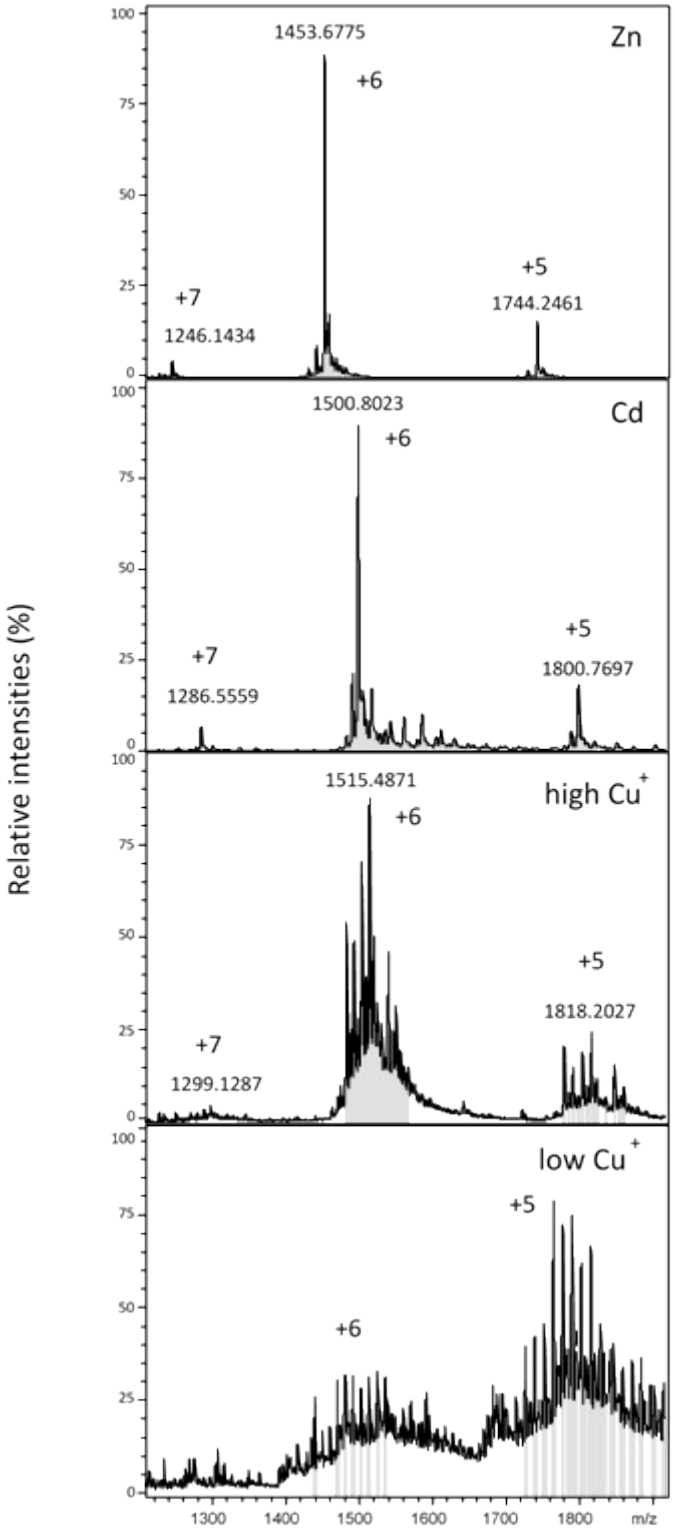

Fig. S2 Raw ESI mass spectra of Zn(II)-, Cd(II)- and Cu(I)-BnMT4 complexes at native pH (10 mM NH_4HCO_3, 10% MeOH).

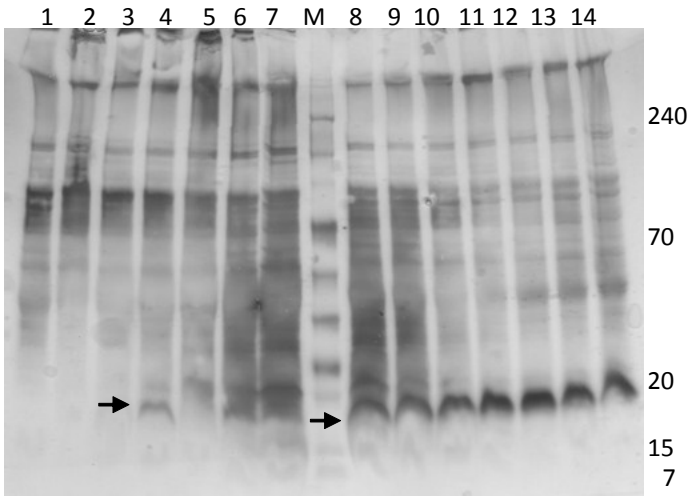

Fig. S3 SDS-PAGE analysis of crude cell lysate of *E. coli* BL21 cells transformed with pET-BnMT4 construct, grown in medium containing 0.5 mM $ZnSO_4$ without (1-7) or with (8-14) IPTG. Cells were harvested every hour for seven hours and proteins were isolated as described in materials and methods. 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_5Zn_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	**Zn_6-BnMT4**	**8717.35**	**8716.11**
	Zn_5-BnMT4	8653.85	8650.39
	Zn_4-BnMT4	8590.35	8585.71
pH 5.05	**Zn_6-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
	Zn_4-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
	Cd_6-BnMT4	**8998.75**	**8999.04**
	Cd_5Zn_1-BnMT4	8951.85	8950.50
	Cd_5-BnMT4	8888.35	8888.53
pH 5.40	Cd_7-BnMT4	9109.15	9109.17
	Cd_6-BnMT4	**8998.75**	**8998.76**
	Cd_5Zn_1-BnMT4	8951.85	8951.46
	Cd_5-BnMT4	8888.35	8888.15
pH 4.21	Cd_7-BnMT4	9109.15	9109.14
	Cd_6-BnMT4	**8998.75**	**8998.86**
	Cd_5-BnMT4	8888.35	8888.46
pH 3.76	Cd_6-BnMT4	8998.75	8998.68
	Cd_5-BnMT4	**8888.35**	**8888.40**
	Cd_4-BnMT4	8777.95	8777.88
pH 3.51	Cd_6-BnMT4	8998.75	8999.15
	Cd_5-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**