3D structure of a Pseudomonas MT: Supplementary Information

Supplementary Information

to

A histidine-rich *Pseudomonas* metallothionein with a disordered tail displays higher binding capacity for cadmium than zinc

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Figure S1. Amino acid sequence alignment of MTs from different *Pseudomonas* species and strains. Cys residues are highlighted in black, His residues in grey. 1) *P. fluorescens* strain Pf0-1 (UniProt¹ accession number: Q3K9Q2), 2) *P. fluorescens* R124 (K0X1F5), 3) *P. fluorescens* SBW25 (C3K8N4), 4) *P. fluorescens* BRIP34879 (L7HBL7), 5) *P. fluorescens* BBc6R8 (V7E9L0), 6) *P. aeruginosa* PAO1 (Q9I1X5), 7) *P. putida* KT2440 (Q88HU1), 8) *P. fluorescens* Q2-87 (J2EKT7), 9) *P. fluorescens* BTR19 (A0A1Q5X1W4), 10) *S. elongatus* PCC 7942 (P30331).



Figure S2. Graphical representation of results obtained from the D^2P^2 (Database of Disordered Protein Predictions)² for PflQ2 MT.



Figure S3. UV spectra of titration of the apo-form of PflQ2 MT (A, B) and sh_PflQ2 MT (C, D) with the indicated metal ions.



Figure S4. Deconvoluted ESI(+) mass spectra for Zn^{II} and Cd^{II} species of PfIQ2 MT (A: M_{calc} 9253.20 Da (Cd₄), 9140.7 9 Da (Cd₃); B: M_{calc} 8999.7 Da (Zn₃)) and sh_PfIQ2 MT (C: M_{calc} 6199.92 Da (Cd₄), 6087.51 Da (Cd₃), D: M_{calc} 6250.16 Da (Zn₃, ¹³C, ¹⁵N labelled protein)).



Figure S5. A) Log values for average apparent binding constants (I=321 mM) for Zn₄ (dark grey), Zn₃ (light grey), Cd₄ (red), Cd₃ (orange) species of PflQ2 MT (WT) and sh_PflQ2 MT (shWT) as well as its mutants. The reference value for Zn₄SmtA (I=93 mM) is taken from the literature.³ B) Amount of metal ion equivalents removed by 5F-BAPTA during the experiment.



Figure S6. Ensemble of the 20 lowest-energy structures of Cd₄PflQ2 MT. Residues 51-81 of the C-terminal tail are coloured in blue.



Figure S7. Analysis of chemical shift differences of Cd₄ species of PflQ2 MT and sh_PflQ2 MT for A) backbone, B) C^{α} , and C) C^{β} .

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Figure S8. Analysis of "Zn₄" species of sh_PflQ2 MT: A) [¹⁵N,¹H]-HSQC spectrum, B) Deconvoluted ESI(+) mass spectrum (M_{calc} 5946.42 Da (Zn₃), 6011.8 Da (Zn₄)).



Figure S9. Deconvoluted ESI(+) mass spectrum of Zn^{II} -to-Cd^{II} exchange of sh_PflQ2 MT (M_{calc} 6199.92 Da (Cd₄)).



Figure S10. Proton-decoupled 1D ¹¹³Cd spectrum at 320 K of a sample containing a mixture of ¹¹³Cd₄-(*) and ¹¹³Cd₃sh_PflQ2 MT (•).



Figure S11. A) Deconvoluted ESI(+) mass spectrum ($M_{calc}.6161.95$ Da (Cd₃, ¹⁵N labelled protein), B) 1D ¹¹³Cd spectrum (320 K, note that due to high concentrations needed for the measurement of ¹¹³Cd spectra, background formation of additional cadmium peaks with chemical shifts corresponding to Cd₄ species are always observed), C) [¹⁵N,¹H]-HSQC spectrum, and D) long-range [¹⁵N,¹H]-HSQC spectrum ($J(^{15}N, ^{1}H) = 22.75$ Hz; 310 K) of ¹³C, ¹⁵N labelled ¹¹³Cd₃sh_PflQ2 MT.



Figure S12. Comparison of backbone dynamics and chemical shift differences between Cd₄- and Cd₃sh_PflQ2 MT: A) ¹⁵N{¹H}-NOE values for Cd₄ (full circles) and Cd₃ (empty circles). B) Backbone chemical shift perturbation (values that could not be determined are indicated by o). Chemical shift differences for C^{α} (C) and C^{β} (D).



Figure S13. Chemical shift perturbation differences between Zn₃- and Cd₃sh_PflQ2 MT for the backbone (A), C^{α} (B) and C^{β} (C). Cysteine residues are shown with black bars while data points that could not be derived unambiguously are indicated by o.





Figure S14. Comparison of backbone dynamics and chemical shift differences for Cd₄- and Zn₃sh PflQ2 MT: A) ¹⁵N{¹H}-NOE values for Cd₄ (full circles) and Zn₃ (empty circles). B-D) Chemical shift changes for the backbone (B), C^{α} (C), and C^{β} (D).



Figure S15. Difference UV spectra of the titration of A) apo-sh_PflQ2 MT (see Figure S6C for the original spectra) and B) apo-A44H-sh_PflQ2 MT with Cd^{II} ions. In both cases, a bathochromic shift of the absorption (around 260-265 nm) that is indicative of Cd^{II} -thiolate cluster formation is observed upon addition of the 3rd and of the 4th equivalent of Cd^{II} .



Figure S16. Assignment of C^{β}/H^{β} correlations of Cys residues in [¹³C,¹H]-HSQC spectra for A) Cd₃and B) Zn₃sh PflQ2 MT. For the Zn₃ species, C47 was not observed in the [¹³C,¹H]-HSQC spectrum.



Figure S17. [¹¹³Cd, ¹H]-HSQC-TOCSY of ¹¹³Cd₃sh_PflQ2 MT (320 K, J(¹¹³Cd ¹H)=35 Hz).



Figure S18. [¹¹³Cd,¹H]-HSQC-TOCSY of ¹¹³Cd₄sh_PflQ2 MT (310 K, J(¹¹³Cd ¹H)=40 Hz).



Figure S19. Deconvolved ESI(+) mass spectra of Cd₄ species of His-to-Asn mutants (M_{calc} . 6064.24 Da (Cd₃), 6177.04 Da (Cd₄)).



Figure S20. Comparison of the proton-decoupled 1D 113 Cd spectra of Cd₄ species of sh_PflQ2 MT (shWT) and its His-to-Asn mutants at 320 K.



Figure S21. Comparison of Cd₄ species of sh_PflQ2 MT and its H11N mutant: A) Backbone chemical shift changes (o symbol depicts values that could not be determined) B) $^{15}N{^{1}H}$ -NOE values for sh_PflQ2 MT (full circles) and its H11N mutant (empty circles).



Figure S22. Comparison of Cd₄ species of sh_PflQ2 MT and its H22N mutant: A) Backbone chemical shift changes (o symbol depicts values that could not be determined) B) $^{15}N{^1H}$ -NOE values for sh PflQ2 MT (full circles) and its H22N mutant (empty circles).



Figure S23. Comparison of Cd₄ species of sh_PflQ2 MT and its H48N mutant: A) Backbone chemical shift differences (values that could not be determined are indicated by **o**) B) $^{15}N{^1H}$ -NOE values for sh PflQ2 MT (full circles) and its H48N mutant (empty circles).



Figure S24. Plot of $C^{\epsilon 1}$ chemical shifts for the four histidine residues in ¹⁵N, ¹³C labelled Cd₄sh_PflQ2 MT with decreasing pH.

	Equation	$\delta_{obs} = \frac{\delta_{HA} + \delta_A \cdot 10^{pH-pK_a}}{1+10^{pH-pK_a}}$		
	Residue	H11	H22	H48
$^{1}\mathrm{H}^{\epsilon 1}$	δ_{HA}	7.63476 ± 0.00537	7.68905 ± 0.00929	7.65545 ± 0.02164
	δ_A	8.54295 ± 0.00606	8.58688 ± 0.01146	8.53726 ± 0.01534
	pK ₁	6.58873 ± 0.01691	6.46612 ± 0.03253	7.39965 ± 0.04444
	Reduced Chi-Sqr	1.33E-04	4.41E-04	0.00123
	R-Square(COD)	0.99913	0.99706	0.9901
	Adj. R-Square	0.99899	0.9966	0.98858
$^{13}C^{\epsilon 1}$	δ_{HA}	138.81136 ± 0.02043	139.37344 ± 0.03692	138.81622 ± 0.03801
	δ_A	136.30922 ± 0.0233	137.00155 ± 0.04901	136.41352 ± 0.02778
	p <i>K</i> ₁	6.57362 ± 0.02362	6.3706 ± 0.05252	7.34241 ± 0.02897
	Reduced Chi-Sqr	0.00195	0.00756	0.00395
	R-Square(COD)	0.99832	0.99277	0.99576
	Adj. R-Square	0.99806	0.99165	0.99511
	Average pK_1	6.58	6.42	7.37

Table S1. Fitting data for determination of pK_a values of histidine residues for Cd₄sh_PflQ2 MT.



Figure S25. Structure comparison of Zn_4SmtA (red backbone, Zn^{II} are represented as grey spheres)³ and Cd₄sh_PflQ2MT (black backbone, Cd^{II} are represented as orange spheres).



Figure S26. A) Plot of thiolate-to-Zn^{II} charge transfer band at 230 nm for the titration of apo-sh_PflQ2 (\bullet) and apo-A44H-sh_PflQ2 MT (Δ) with Zn^{II} ions; B) Overlay of [¹⁵N, ¹H]-HSQC spectra of Cd₄A44H-sh_PflQ2 (black) and Zn₄A44H-sh_PflQ2 MT (red).



Figure S27. A) Deconvolved ESI(+) mass spectrum of Cd₄A44H-sh_PflQ2 MT (M_{calc} 6265.98 Da (Cd₄)). B) The plot of thiolate-to-Cd^{II} charge transfer band at 250 nm for the titration of apo-sh_PflQ2 (\bullet) and apo-A44H sh PflQ2 MT (Δ) with Cd^{II} ions.



Figure S28. Comparison of proton-decoupled 1D ¹¹³Cd spectra of different Cd^{II} species of sh_PflQ2 MT and its A44H mutant.



Figure S29. Comparison of Cd₄ species of sh_PflQ2 MT and its A44H mutant: A) ${}^{15}N{}^{1}H$ -NOE values for sh_PflQ2 MT (full circles) and its A44H mutant (empty circles). B-D) Chemical shift changes for the backbone (B), C^{α}(C), and C^{β}(D) (values that could not be determined are indicated by **o**).



Figure S30. [113 Cd, 1 H]-HSQC-TOCSY spectrum of 113 Cd₄A44H-sh_PflQ2 MT (T=320 K, J(113 Cd 1 H)= 40 Hz).



Figure S31. Zinc ion transfer to apo-alkaline phosphatase at t = 20 min.

	GGA	TCC	GAG	AAC	CTT	TAC	TTC	CAA									
	Ban	nHI	Е	N	L	Y	F	Q									
DNA sequence of PflQ2 MT	AAC	GAG	TTA	CGT	TGC	GGC	TGC	CCG	GAT	TGC	CAC	TGC	AAG	GTA	GAT	CCG	
Optimized DNA sequence	AAC	GA <mark>A</mark>	CTG	CGT	TGC	GGC	TGC	CCG	GAT	TGC	CAC	TGC	AA <mark>A</mark>	GT <mark>T</mark>	GAT	CCG	
Translation	N	Е	L	R	С	G	С	Р	D	С	н	С	к	v	D	Р	
	GAG	CGC	GTA	TTC	AAT	CAC	GAC	GGC	GAA	GCC	TAT	TGC	AGC	CAG	GCC	TGT	GCC
	GAG	CGC	GT <mark>G</mark>	TTC	AAT	CAC	GAC	GGC	GAA	GCC	TAT	TGC	AGC	CAG	GCC	TGT	GCC
	Е	R	v	F	N	н	D	G	Е	A	Y	С	S	Q	A	С	A
	GAG	CAG	CAC	CCC	AAC	GGC	GAG	CCC	TGT	CCG	GCG	CCG	GAT	TGC	CAT	TGT	GAG
	GAA	CAG	CAC	CCT	AAC	GGC	GAG	CCG	TGT	CCG	GCG	CCG	GAT	TGC	CAT	TGT	GAG
	Е	Q	н	Р	N	G	Е	Р	С	Р	A	Р	D	С	н	С	Е
	CGC	AGC	GGC	AAG	GTC	GGT	GGG	CGG	GAC	ATT	ACC	AAC	AAT	CAA	CTG	GAC	GAG
	CGC	AGC	GGC	AA <mark>A</mark>	GT <mark>T</mark>	GGT	GG <mark>C</mark>	CG <mark>C</mark>	GAC	ATT	ACC	AAC	AAT	CAA	CTG	GAC	GAG
	R	s	G	к	v	G	G	R	D	I	т	N	N	Q	L	D	Е
	GCC	CTG	GAA	GAA	ACC	TTT	CCA	GCC	AGT	GAC	CCG	ATT	TCT	CCC	TGA		
	GCC	CTG	GAA	GAA	ACC	TTT	CCA	GCC	AGT	GAC	CCG	ATT	AGC	CCG	TGA		
	A	L	Е	Е	т	F	Р	A	s	D	Р	I	s	Р	stor	>	
	CCC	GGG															
	Xma	ιI															

Figure S32. Codon usage for PflQ2 MT and its optimization for expression in E. coli.

Table S2. List of primers used for the construction of coding sequences.

DEVT7 E1 ford	GCG AAT TGG ATC CGA GAA CCT TTA CTT CCA AAA CGA
JZEKI/_FI_IWU	ACT GCG TTG CGG CTG CCC GGA TTG
DEVT7 DO FON	GCC GTC GTG ATT GAA CAC GCG CTC CGG ATC AAC TTT
JZEKI/_KZ_IEV	GCA GTG GCA ATC CGG GCA GCC
DEVT7 D2 row	GTT AGG GTG CTG TTC GGC ACA GGC CTG GCT GCA ATA
JZEKI /_KJ_ICV	GGC TTC GCC GTC GTG ATT GAA C
DEVT7 DA roy	GCT GCG CTC ACA ATG GCA ATC CGG CGC CGG ACA CGG
J2EK17_K4_I6V	CTC GCC GTT AGG GTG CTG TTC G
DEVT7 D5 roy	CGT CCA GTT GAT TGT TGG TAA TGT CGC GGC CAC CAA CTT
JZERT /_RJ_ICV	TGC CGC TGC GTC ACA ATG G
12FKT7 R6 rev	GTC ACT GGC TGG AAA GGT TTC TTC CAG GGC CTC GTC
J2LK17_K0_ICV	CAG TTG ATT GTT GG
I2FKT7 R7 rev	GTT CCC CCC GGG TCA CGG GCT AAT CGG GTC ACT GGC
J2LK1/_K/_ICV	TGG AAA G
12FKT7 sh R4 rev	GCT CAC AAT GGC AAT CCG GCG CCG GAC ACG GCT CGC
<u>52LIC17_511_IC4_IC7</u>	CGT TAG GGT GCT GTT CG
J2EKT7_sh_R5_rev	GTT CCC CCC GGG TCA GCT GCG CTC ACA ATG GCA ATC C
	GCT CAC AAT GGC AAT CCG GAT GCG GAC ACG GCT CGC
J2EKT7_sh_R4_A44H_rev	CGT TAG GGT GCT GTT CG
J2EKT7_sh_H11N_fwd	GCT GCC CGG ATT GCA ACT GCA AAG TTG ATC
J2EKT7_sh_H11N_rev	GAT CAA CTT TGC AGT TGC AAT CCG GGC AGC
J2EKT7_sh_H22N_fwd	CGC GTG TTC AAT AAC GAC GGC GAA GC
J2EKT7_sh_H22N_rev	GCT TCG CCG TCG TTA TTG AAC ACG CG
J2EKT7_sh_H48N_fwd	GCG CCG GAT TGC AAT TGT GAG CGC AG
J2EKT7_sh_H48N_rev	CTG CGC TCA CAA TTG CAA TCC GGC GC



Figure S33. Plasmid map of pGEX-4T-1-MT expression vector.



Figure S34. Deconvoluted ESI(+) mass spectra of apo-PflQ2 MT (WT) and its mutants. (WT (M_{calc} 8812.56 Da), shWT (M_{calc} 5759.28 Da), H11N, H22N, H48N (M_{calc} 5736.24 Da), A44H (M_{calc} 5825.34 Da)).

Table S3. Metal-ligand connectivities of Cd_4 and Cd_3 cluster in sh_PflQ2 MT. Connectivities obtained from [¹¹³Cd, ¹H]-HSQC-TOCSY spectra are marked in black, those resulting from structure calculations in red. For the involvement of H36 in coordination see the main text.

Cd_4	Cd(A) (713.8 ppm)	Cd(B) (615.4 ppm)	Cd(C) (585.8 ppm)	Cd(D) (535 ppm)
	C5	C10	C12	H36
	C28	C42	C28	C7
	C32	C47	C42	C32
	C10	C49	C10	C49
Cd_3	Cd(A) (695 ppm)	Cd(B) (676 ppm)	Cd(D) (562 ppm)	
	C5	C10	H36	
	C28	C42	C49	
	C12	C47	C32	
	C32	C49	C7	

Table S4. Details of the structure calculation and geometry of Cd₄PflQ2 MT, Cd₄sh_PflQ2 MT, Zn_3sh_PflQ2 MT and Cd_4A44H-sh_PflQ2 MT.

	Cd ₄ PflQ2 MT	
Input data for structure calculation		
NOE distance restraints		
total	1634	
short-range, i-j<2	1215	
medium range, 1>i-j>5	180	
long-range, i-j≥5	239	
Structure statistics, 20 conformers		
CYANA target function value $(Å^2)$	0 78-0 82 (average: 0 80)	
XPLOB energies (kcal/mol)	TOTAI : -1316 25 + 42 79	NOF: 23 36 + 3 71
Xi Loix energies (kear/nor)	1011421310.23 - 42.77	NOL: 25.50 ± 5.71
Ramachandran plot analysis (Procheck)	residues: all	residues: 3-50
Residues in favored regions (%)	78.5	85.3
Residues in additional allowed regions (%)	18.5	12.6
Residues in generously allowed regions (%)	1.5	2.2
Residues in disallowed regions (%)	1.5	0
Root mean square deviation to the average (Å)	residues: all	residues: 3-50
Average backbone RMSD to mean	10.708 ± 2.755	0.215 ± 0.033
Average heavy atom RMSD to mean	10.953 ± 2.771	0.660 ± 0.089
	Cd₄sh_PflQ2 MT	
Input data for structure calculation		
NOE distance restraints		
total	1143	
short-range, i-j<2	881	
medium range, 1>i-j>5	111	
long-range, i-j≥5	151	
Structure statistics, 20 conformers		
CYANA target function value ($Å^2$)	0.48-0.52 (average: 0.52)	
XPLOR energies (kcal/mol)	TOTAL: -907.88 ± 53.45	NOE: 9.14 ± 2.13
Ramachandran nlot analysis (Procheck)	residues: all	
Residues in favored regions (%)	75 6	
Residues in additional allowed regions (%)	19.5	
Residues in generously allowed regions (%)	49	
Residues in disallowed regions (%)	0.0	
residues in disultaned regions (70)	0.0	
Root mean square deviation to the average (Å)	residues: all	residues: 5-49
Average backbone RMSD to mean		
	0.781 ± 0.165	0.502 ± 0.134
Average heavy atom RMSD to mean	$\begin{array}{c} 0.781 \pm 0.165 \\ 1.316 \pm 0.194 \end{array}$	$\begin{array}{c} 0.502 \pm 0.134 \\ 0.965 \pm 0.140 \end{array}$

	Zn ₃ sh_PflQ2 MT	
Input data for structure calculation		
NOE distance restraints		
total	1354	
short-range, i-j<2	1030	
medium range, 1>i-j>5	110	
long-range, i-j≥5	214	
Structure statistics, 20 conformers		
CYANA target function value ($Å^2$)	0.61-0.72 (average: 0.69)	
XPLOR energies (kcal/mol)	-1092.44 ± 60.34	NOE: 20.61 ± 6.57
Ramachandran plot analysis (Procheck)	residues: all	
Residues in favored regions (%)	85.4	
Residues in additional allowed regions (%)	14.6	
Residues in generously allowed regions (%)	0	
Residues in disallowed regions (%)	0	
Root mean square deviation to the average (Å)	residues: all	residues: 5-49
Average backbone RMSD to mean	0.957 ± 0.242	0.443 ± 0.066
Average heavy atom RMSD to mean	1.629 ± 0.248	0.973 ± 0.090
	Cd4A44H-sh_PflQ2 MT	
Input data for structure calculation		
NOE distance restraints		
total	1485	
short-range, i-j<2	1099	
medium range, 1>i-j>5	120	
long-range, i-j≥5	266	
Structure statistics, 20 conformers		
CYANA target function value $(Å^2)$	0.750.96 (average: 0.86)	
XPLOR energies (kcal/mol)	-887.93 ± 64.79	NOE: 19.02 ± 6.21
Ramachandran plot analysis (Procheck)	residues: all	
Residues in favored regions (%)	80.5	
Residues in additional allowed regions (%)	17.1	
Residues in generously allowed regions (%)	0	
Residues in disallowed regions (%)	2.4	
Root mean square deviation to the average (Å)	residues: all	residues: 5-49
Average backbone RMSD to mean	0.909 ± 0.174	0.373 ± 0.068
Average heavy atom RMSD to mean	1.630 ± 0.184	0.933 ± 0.103

References

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