Supplemental Data

Structure and metal binding properties of ZnuA, a periplasmic zinc transporter from *Escherichia coli*

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Table S1. Important interatomic distances and angles. Only distances less than 3.60 Å are included in the table.

Ligand atoms	ZnZnuA *form I*	ZnZnuA *form II*	CoZnuA	apoZnuA
Metal-to-ligand distances				
$O^{\varepsilon 1}$(E59)	2.02 (1.96)[1]	1.95 (2.04)	1.98 (2.15)	- (-)
$N^{\varepsilon 2}$(H60)	2.03 (1.98)	1.97 (2.02)	2.00 (2.00)	- (-)
$N^{\varepsilon 2}$ (H143)	2.08 (2.05)	2.02 (2.07)	2.21 (2.19)	- (-)
$N^{\varepsilon 2}$ (H207)	2.03 (2.12)	2.04 (1.97)	1.94 (1.93)	- (-)
H_2O			3.11 (3.21)	- (-)
E59				
$O^{\varepsilon 1}$ – M	2.02 (1.96)	1.95 (2.04)	1.98 (2.15)	- (-)
$O^{\varepsilon 1}$ – $N^{\varepsilon 2}$ (H143)	3.41 (3.28)	3.21 (3.36)	3.43 (3.56)	3.44 (3.22, 3.56)
$O^{\varepsilon 1}$ – $N^{\varepsilon 2}$ (H207)	3.27 (3.28)	3.24 (3.15)	3.24 (3.45)	3.53 (-)
$O^{\varepsilon 1}$ – H_2O	3.41 (3.39)	- (3.47)	- (-)	- (-)
$O^{\varepsilon 2}$ – H_2O	2.80 (2.69)	2.77 (2.76)	2.84 (2.55)	- (-)
His60				
$N^{\varepsilon 2}$ – M	2.03 (1.98)	1.97 (2.02)	2.00 (2.00)	- (-)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H143)	3.27 (3.38)	3.33 (3.32)	3.44 (3.41)	- (-)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H207)	3.28 (3.24)	3.26 (3.42)	3.43 (3.39)	- (-)
$N^{\varepsilon 2}$– H_2O	- (-)	- (3.29)	3.08 (3.19)	- (3.26)
$N^{\delta 1}$ – H_2O(1)	- (2.84)	3.08 (2.75)	2.70 (2.65)	- (3.26)
$N^{\delta 1}$ – H_2O(2)	- (-)	- (-)	- (3.58)	- (-)
$N^{\varepsilon 2}$ – CO(M85)	- (-)	- (-)	- (-)	3.34 (3.28)[2]
His143				
$N^{\varepsilon 2}$ – M	2.08 (2.05)	2.02 (2.07)	2.21 (2.19)	- (-)
$N^{\varepsilon 2}$ – $O^{\varepsilon 1}$(E59)	3.41 (3.28)	3.21 (3.36)	3.43 (3.56)	3.44 (3.22, 3.56)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H60)	3.27 (3.38)	3.33 (3.32)	3.44 (3.41)	- (-)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H207)	3.42 (3.46)	3.36 (3.33)	3.39 (3.23)	- (-)
$N^{\delta 1}$ – O(N141)	2.90 (2.97)	2.90 (2.90)	2.99 (3.19)	2.84 (3.02)
$N^{\delta 1}$ – CO(D208)	3.37 (3.37)	3.44 (3.32)	3.27 (3.26)	- (-)
$N^{\delta 1}$ – H_2O	3.25 (3.40)	3.30 (3.29)	3.39 (3.30)	- (-)
His207				
$N^{\varepsilon 2}$ – M	2.03 (2.12)	2.04 (1.97)	1.94 (1.93)	- (-)
$N^{\varepsilon 2}$ – $O^{\varepsilon 1}$(E59)	3.27 (3.28)	3.24 (3.15)	3.24 (3.45)	3.53 (-)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H60)	3.28 (3.24)	3.26 (3.42)	3.43 (3.39)	- (-)
$N^{\varepsilon 2}$ – $N^{\varepsilon 2}$ (H143)	3.42 (3.46)	3.36 (3.33)	3.39 (3.23)	- (3.61)[3]
$N^{\delta 1}$ – $O^{\varepsilon 1}$(E256)	3.35 (3.30)	3.10 (3.62)	- (3.18)	3.10 (2.90, 3.16[3])
$N^{\delta 1}$ – $O^{\varepsilon 2}$(E256)	2.70 (2.73)	2.79 (2.73)	2.62 (2.78)	2.49 (-)
Tetrahedral Angles				
E59-Zn-H60	112.0 (110.0)	106.0 (105.3)	94.3 (100.3)	
E59-Zn-H143	112.4 (109.5)	108.0 (109.9)	110.1 (109.0)	
E59-Zn-H207	107.3 (106.9)	108.9 (103.6)	111.3 (115.7)	
H60-Zn-H143	105.2 (113.9)	112.8 (108.6)	109.8 (109.0)	
H60-Zn-H207	107.5 (104.4)	109.0 (117.8)	120.8 (119.4)	
H143-Zn-H207	112.5 (111.8)	111.9 (111.2)	109.5 (103.2)	

[1]Data for molecule B are shown in parentheses
[2]Only if flipped by 180º

Table S2. Comparison of metal – ligand distances in various ZnZnuA structures.

Ligand atoms[1]	ZnZnuA *form I*	ZnZnuA *form II*	CoZnuA	ZnZnuA Chandra	ZnZnuA Li	*Syn*-ZnZnuA
$O^{\varepsilon 1}$(E59)	2.02 (1.96)[2]	1.95 (2.04)	1.98 (2.15)	-	1.86	-
$N^{\varepsilon 2}$(H60)	2.03 (1.98)	1.97 (2.02)	2.00 (2.00)	1.90 (2.00)	2.03	2.00
$N^{\varepsilon 2}$ (H143)	2.08 (2.05)	2.02 (2.07)	2.21 (2.19)	2.07 (2.07)	2.05	2.10
$N^{\varepsilon 2}$ (H207)	2.03 (2.12)	2.04 (1.97)	1.94 (1.93)	2.00 (2.06)	2.08	2.10
H_2O	-	-	3.11 (3.21)	2.27 (2.29)	-	2.30

[1]Based on *Eco*-ZnuA numbering
[2]Data for molecule B are shown in parentheses

Figure S1. Circular dichroism spectra of apoZnuA, 5 μM (bold solid line) in 20 mM potassium phosphate, pH 7.5, 20 mM NaF) with excess of (A) Cd^{2+} 125 μM (dashed line), (B) Mn^{2+} 25 μM (dashed line), (C) Co^{2+} 25 μM (dashed line).

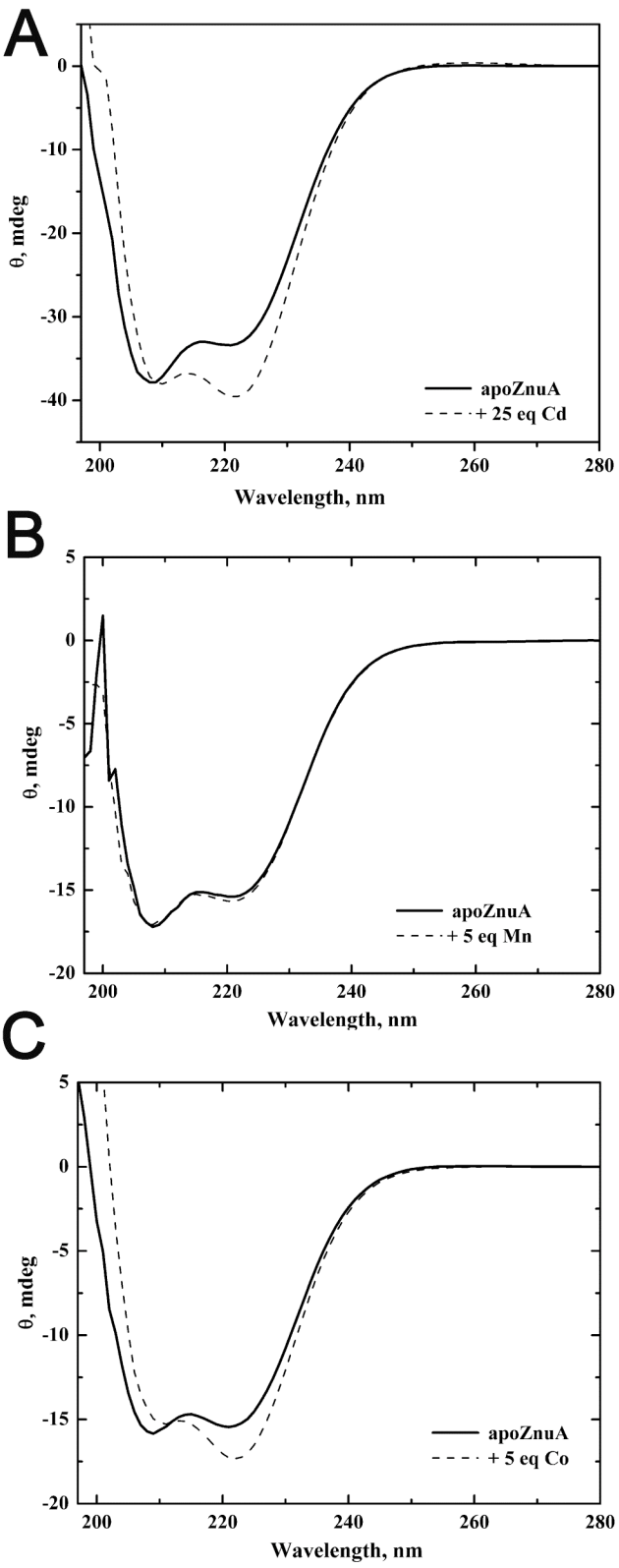


Figure S2. Zinc exchange between ZnZnuA and apo MF. ApoZnuA was fully loaded with Zn^{2+}, excess metal was removed by 5 successive rounds of concentration/dilution and fully metallated ZnZnuA was incubated with increasing concentration of MF for 1 h. Protein and MF were separated on a Superdex 75 gel filtration column. Zinc and protein concentration were measured to calculate the extent of metal transfer between *Eco*-ZnuA and MF.

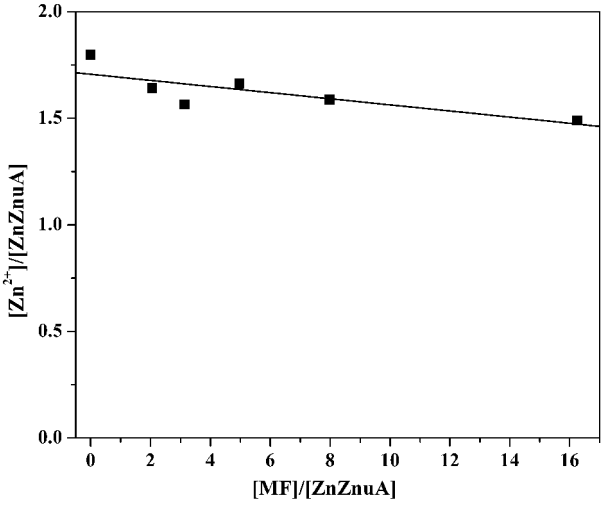


Figure S3. UV-Vis difference spectrum of 2-CoZnuA minus 1-CoZnuA. The spectral feature at 506 nm with $\varepsilon \approx 8$ $M^{-1}cm^{-1}$ is assigned to the second Co^{2+} binding site in *Eco*-ZnuA that is predicted to be six-coordinate.

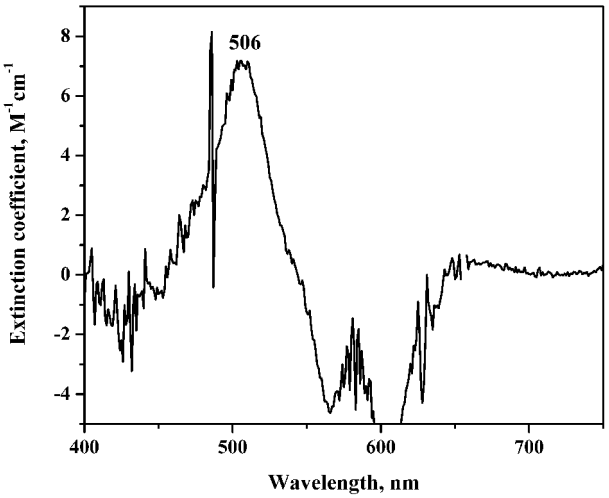


Figure S4. Fits to unfiltered Zn EXAFS data for 1-Zn and ZnCo ZnuA and corresponding Fourier transforms. Solid lines represent experimental data, while open diamonds represent the best fits, summarized in Table 3.

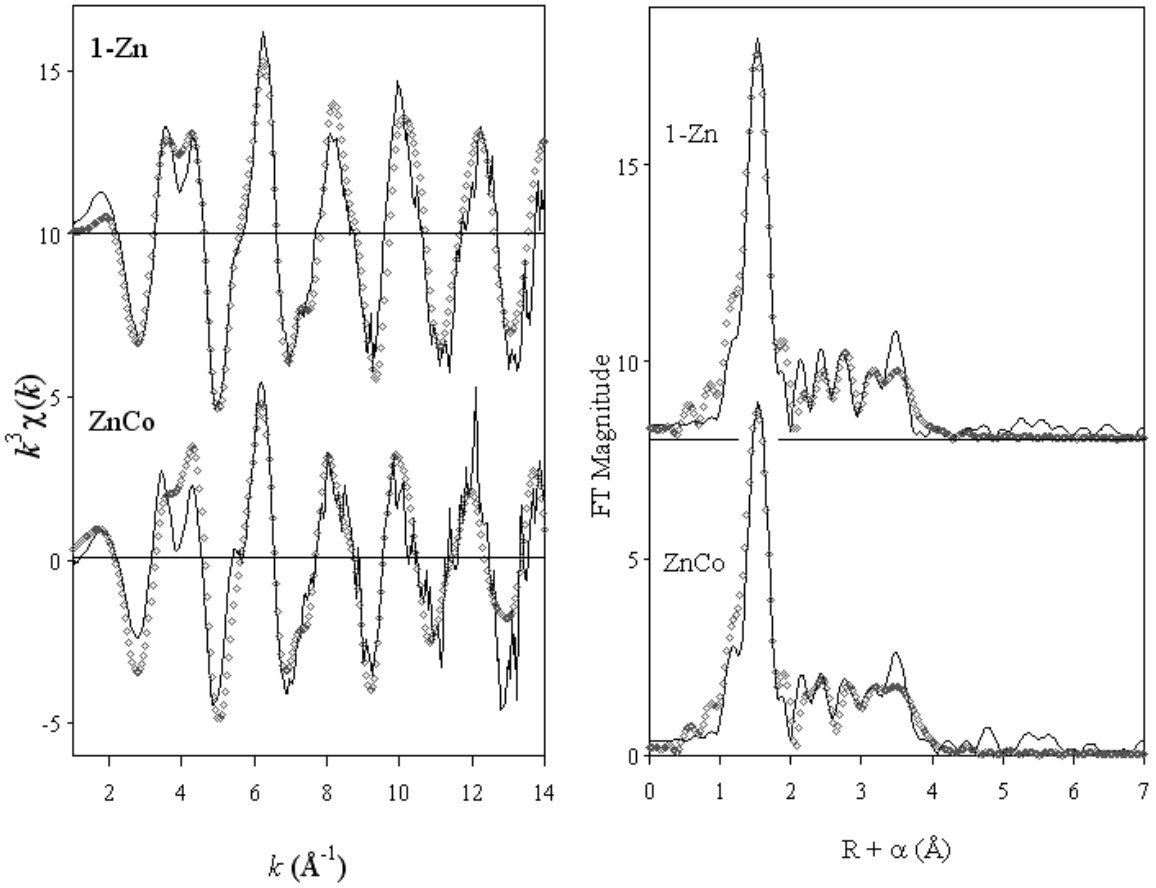
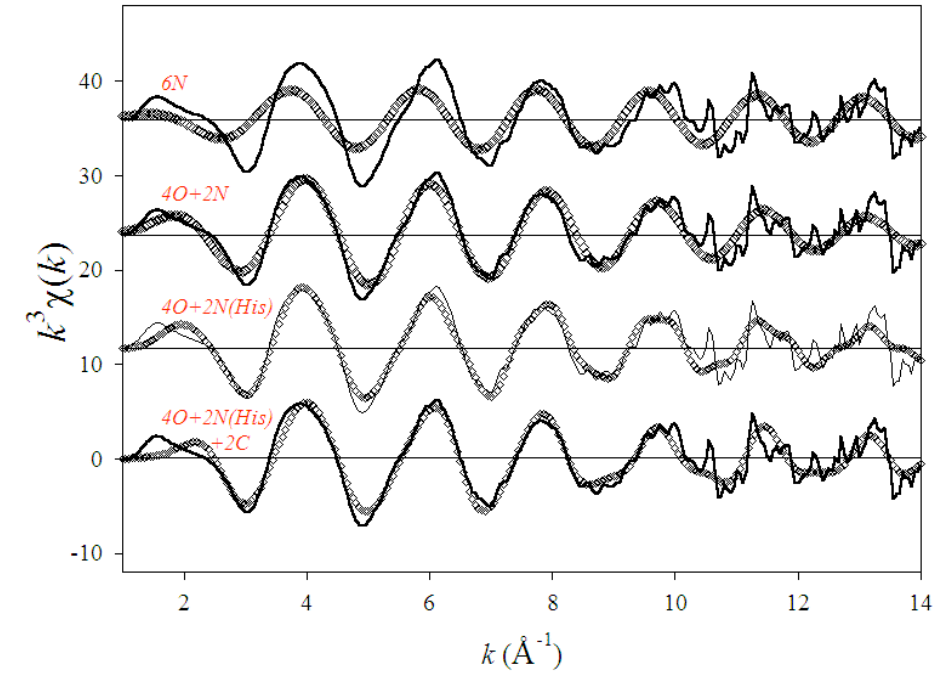


Figure S5. Fits to unfiltered Co EXAFS data for ZnCoZnuA and corresponding Fourier transforms. Solid lines represent raw data, while open diamonds represent the best fits; the order of presentation corresponds to that in Table 3.



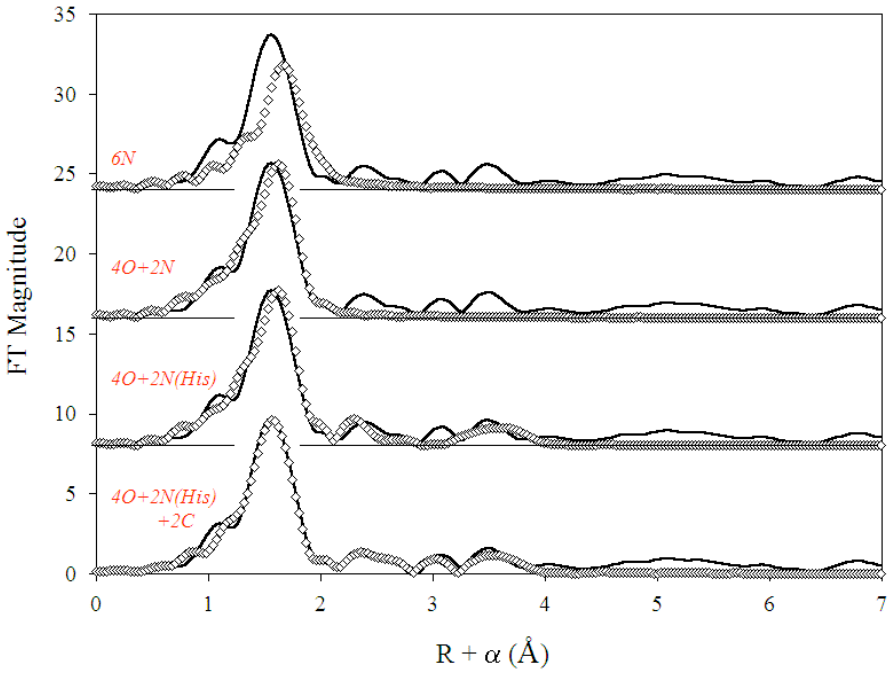


Figure S6. Changes in ANS fluorescence intensity at 510 nm of 0.5 mM ANS solution upon addition of various metal ions. All the spectra have been corrected for ANS fluorescence by subtracting the fluorescence of an ANS solution of equivalent volume and concentration. Experimental conditions: λ_{ex} = 380 nm, λ_{em} = 510 nm, T = 23 °C, buffer, 50 mM HEPES, pH 7.5, 200 mM NaCl.

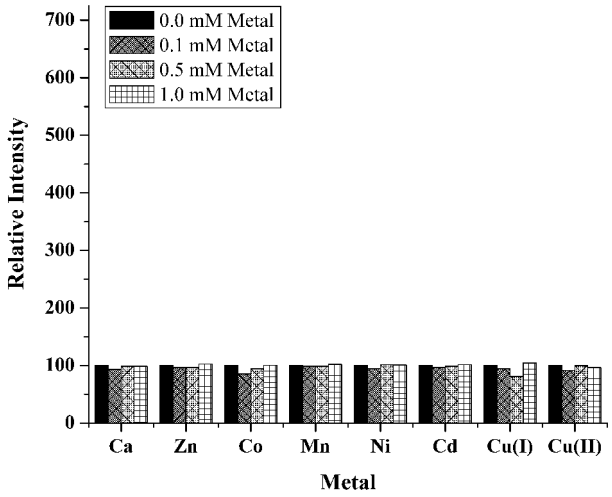


Figure S7. Comparison of *Eco*-ZnuA to other related MBRs. *Eco*-ZnuA (molecule ***A***, red), *Syn*-ZnuA (green), *Spn*-PsaA (blue) and *Tpa*-TroA (grey). Metals are colored accordingly. The His-rich loop and the C-terminus are labeled The arrow points to the position of helix α6 and the asterisk marks the position of the β6α6 loop. All the structures have the position of α6 and β6α6 similar to that in *Eco*-ZnuA containing two Zn^{2+} ions.