Structure *16*

Supplemental Data

Insights into the Mode of Action of a Putative

Zinc Transporter CzrB in *Thermus thermophilus*

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Supplemental Experimental Procedures

Materials

BL21 cells and Dnase (Lot 1339311) were purchased from Invitrogen (Carlsbad, CA). Ampicillin (Lot 060573), Luria Bertani broth (Lot 020845), sodium chloride (Lot 060877), and glacial acetic acid (Lot 041410) were obtained from Fisher Scientific (Pittsburgh, PA). Isopropyl ß-D-1-thiogalactopyranoside (Lot 11106300), lysozyme (Lot 10683700), and dithiothreitol (Lot 1067 4000) were from American Bioanalytical (Natick, MA). Rnase (Lot 124101816), Ni-NTA agarose (Lot 11230934), Factor Xa removal resin (Lot 12194722) were supplied by Qiagen (Valencia, CA). Tris buffer (Lot 49240B) was obtained from BioRad (Hercules, CA). Triton X-100 (Lot 106F-0052), calcium chloride (Lot 68H06341), and sodium azide (Lot 118H2514) were bought from Sigma (St. Louis, MO). Imidazole (Lot 33271711 494), sodium formate (Lot 1103535 13304122), glycerol (Lot 40959/1 293), polyethylene glycol monomethyl ether 2000 (Lot 449313/1 21504221), and di-potassium hydrogen phosphate (Lot 429860/1 20502) were from Fluka (Buchs, Switzerland). Amicon Ultra-4 concentrators (5,000 MWCO) were purchased from Millipore (Bedford, MA). Dialysis tubing (3,500 MWCO; Lot 3223607) was from Spectra / Por (Rancho Dominguez, CA). Factor Xa protease (Lot N67059-2) was obtained from Novagen (EMD Chemicals, Inc., San Diego, CA). Bradford Albumin Standard (2 mg/mL in 0.9 % aqueous NaCl solution containing sodium azide; Lot GH97262) and Coomassie Plus^{TM} Protein assay (Lot FH71183) were from Pierce (Rockford, IL). For crystallization trials VDXm plates, Hampton Screen I and II (Lots 04169940 and 05039921), polyethylene glycol 4000 (Lot 260522) were obtained from Hampton Research (Aliso Viejo, CA). Zinc chloride (Lot B18339) and potassium phosphate monobasic (Lot D22083) were purchased from Baker Analyzed (Phillipsburg, PA). Ammonium sulfate (Lot 124-1316) was from Jenneile Chemical Company (Cincinnati, OH) and deuterium oxide (99.96 % D_20; Lot 21768LO) was from Aldrich (Milwaukee, MN). Water, with a resistivity of >18 MΩ cm, was purified by using a Milli-Q Water System (Millipore, Bedford, MA). The system consists of a carbon filter cartridge, two ion exchange filter cartridges, an organic removal cartridge, and a final filter (Sterile Millipore, millipak 40, lot F6BN22683).

Supplemental References

Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., 3rd, Snoeyink, J., Richardson, J.S., and Richardson, D.C. (2007). MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Research *35*, W375–383.

Sreerama, N., and Woody, R.W. (2000). Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. Analytical Biochemistry *287*, 252–260.

Table S1. Geometry of Zinc-Binding Residues in $CzrB_{sf}$ Analyzed by MolProbity (Davis et al., 2007)

Residue	State, Chain	High B (Å²)	Ramachandran Φ (°), ψ (°)	Rotamer chi1 (°), chi2 (°), chi3 (°)	C_β Deviation (Å)
His 31	apo, chain A	33.3	Favored (9.14%) -145.3, 176.8	3.6% 76, 116.9	0.113
	apo, chain B	27.0	Favored (26.25%) -152.5, 166.9	22.5% 71.2, 91.9	0.092
	zinc-	4.3	Favored (5.07%) -148.4, -176.8	3.1 % 66.1, 237.5	0.118
Asp 32	apo, chain A	36.8	Favored (25.58%) 55.1, 41.5	22.2% 203.9, 13.5	0.08
	apo, chain B	27.3	Favored (8.84%) 56.6,52.9	33.5% 193.4,29.7	0.121
	zinc-	8.4	Favored (4.73%) 43.4, 54.4	28.1% 196.6, 30.3	0.141
His 47	apo, chain A	30.7	Favored (46.53%) -97.0, 132.8	19.4% 279.7, 60.1	0.053
	apo, chain B	27.1	Favored (43.99%) -96.8,134.3	20.1% 288.7,239.7	0.021
	zinc-	6.9	Favored (58.52%) -105.5, 129.7	26.7% 297.5, 189.4	0.07
His 60	apo, chain A	31.2	Favored (91.91%) -62.6, -46.0	28.9% 173.5, 246.5	0.027
	apo, chain B	30.9	Favored (91.66%) -62.2,-46.2	57.2% 168.1,65.8	0.066

	zinc-	6.2	Favored (75.28%) -69.7, -33.9	51.4% 188.5, 259.8	0.056
His 82	apo, chain A	29.9	Favored (27.38%) -107.4, 109.8	88.7% 304.1, 296.9	0.066
	apo, chain B	32.3	Favored (31.75%) -103.6, 112.5	85.2% 307.8, 285	0.044
	zinc-	10.6	Favored (36.86%) -104.8, 114.3	58% 170.5, 61.7	0.043
Glu 84	apo, chain A	35.8	Favored (22.98%) -131.5, 132.6	86% 178.7, 182, 175.4	0.107
	apo, chain B	35.1	Favored (28.55%) -145.5, 143.3	11.2% 56.2, 186.8, 52.2	0.012
	zinc-	11.8	Favored (68.57%) -134.6, 155.3	63.7% 297.1, 184.8, 131.9	0.033

Table S2. Results of Analysis of CD Spectra from Apo-$CzrB_{sf}$ Before/After Lyophilization Using the CDPro Suite (Sreerama and Woody, 2000)

Method	**a-helices**	**β-strands**	**turns**	**disordered**
CDSSTR	0.163 / 0.071	0.306 / 0.35	0.26 / 0.242	0.269 / 0.329
CONTINLL	0.196 / 0.177	0.297 / 0.307	0.237 / 0.229	0.269 / 0.288
SELCON3[a]	- / 0.238	- / 0.328	- / 0.111	- / 0.169
Average	0.18 / 0.16	0.3 / 0.33	0.25 / 0.2	0.27 / 0.26

[a]The SELCON3 method failed to process the data obtained using protein before lyophilization.

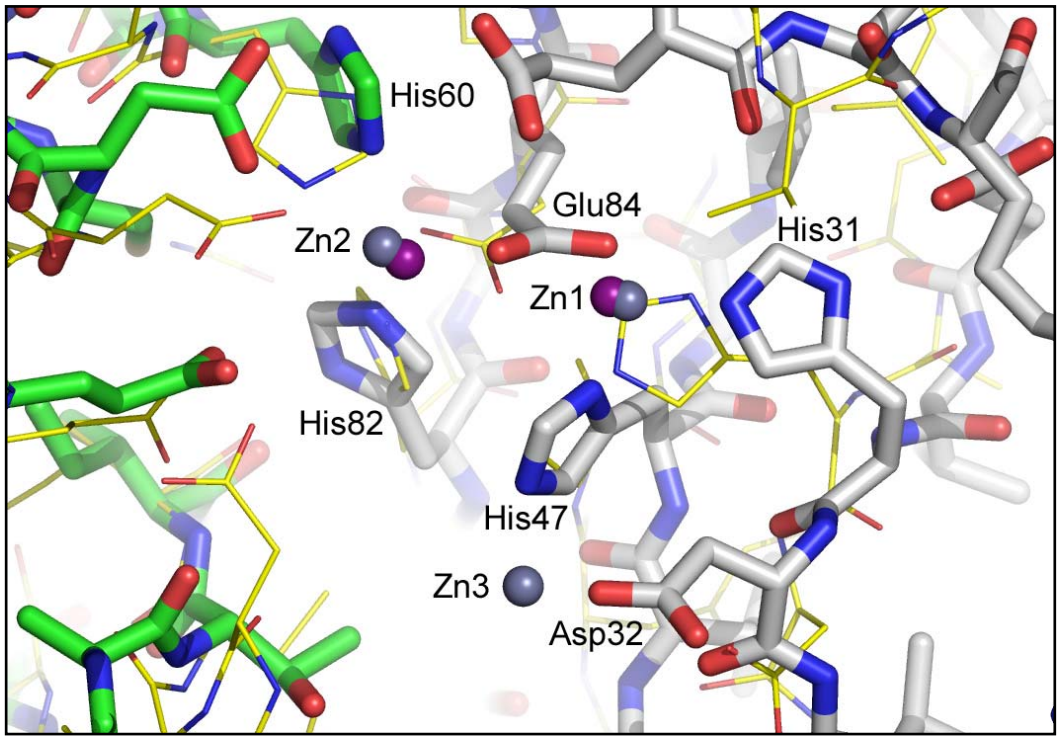

Figure S1. Overlay of Zinc-$CzrB_{sf}$ (thick sticks, gray and green carbons) and YiiP (thin sticks, yellow carbons) at Homologous Zinc Binding Sites

The same view is shown as in Figure 5. Zinc ions in $CzrB_{sf}$ and in YiiP are colored bluish gray and purple, respectively. The distance between common zinc ions in $CzrB_{sf}$ and in YiiP is 4.9 Å and 4.2 Å, respectively. Notable differences between the two structures include i) Glu84 in $CzrB_{sf}$ which corresponds to Asp285 in YiiP and ii) His47 in $CzrB_{sf}$ which corresponds to Ile245 in YiiP. The latter may be accounted for by a 3-residue frame shift along strand β2 in YiiP as described in the text and in Figure S2.

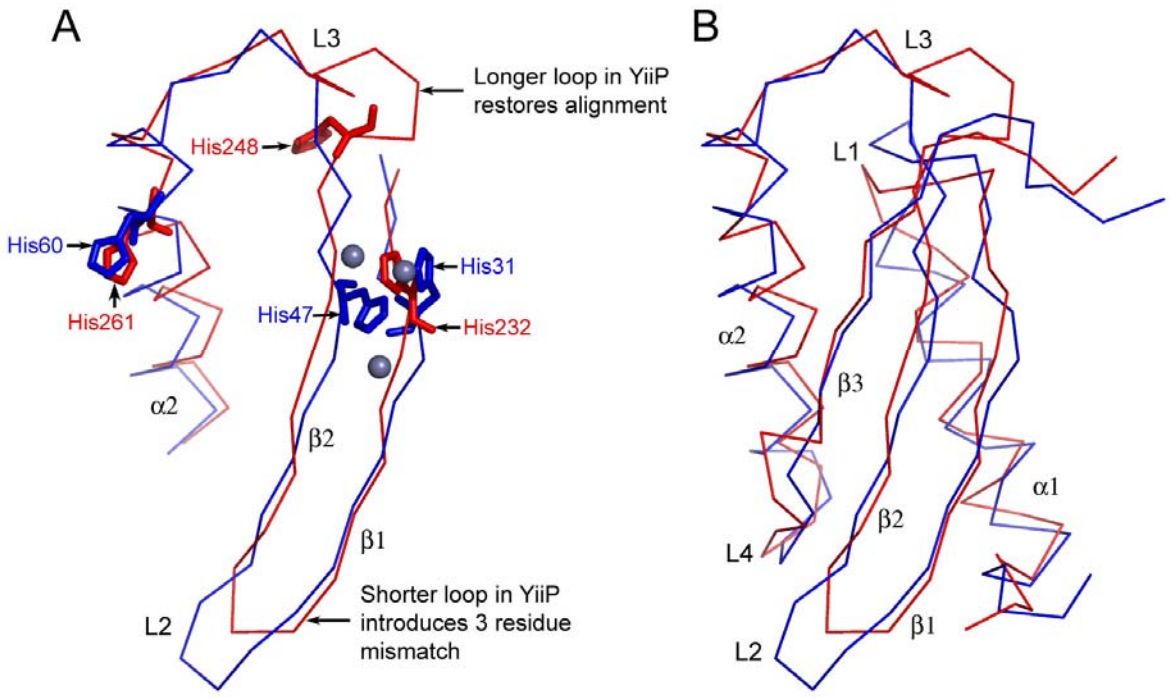

Figure S2. An Overlay of the C_α Trace of Zinc-$CzrB_{sf}$ (blue) and YiiP (red) in the Region That Includes Strands β1 and β2 and Loops L2 and L3 (A) and for the Full-lLength Zinc-$CzrB_{sf}$ (B)

The zinc-coordinating residues His31, His47 and His60 in $CzrB_{sf}$ and sequence homologous residues in YiiP are shown in stick representation. Zinc atoms in zinc-$CzrB_{sf}$ are shown as bluish gray spheres. The structural coincidence of residues homologous to His31 and His60 in YiiP is obvious. However, His47 in $CzrB_{sf}$ and its sequence homologue His248 in YiiP are displaced along the β2 strand by ~3 residues. Interestingly, L2 is shorter whilst L3 is longer by about 3 residues in YiiP compared to $CzrB_{sf}$. This suggests that the sequence was incorrectly threaded into the electron density of YiiP beginning at L2. The frame shift of 3 residues that ensued was likely corrected subsequently by a second misthreading at L3.

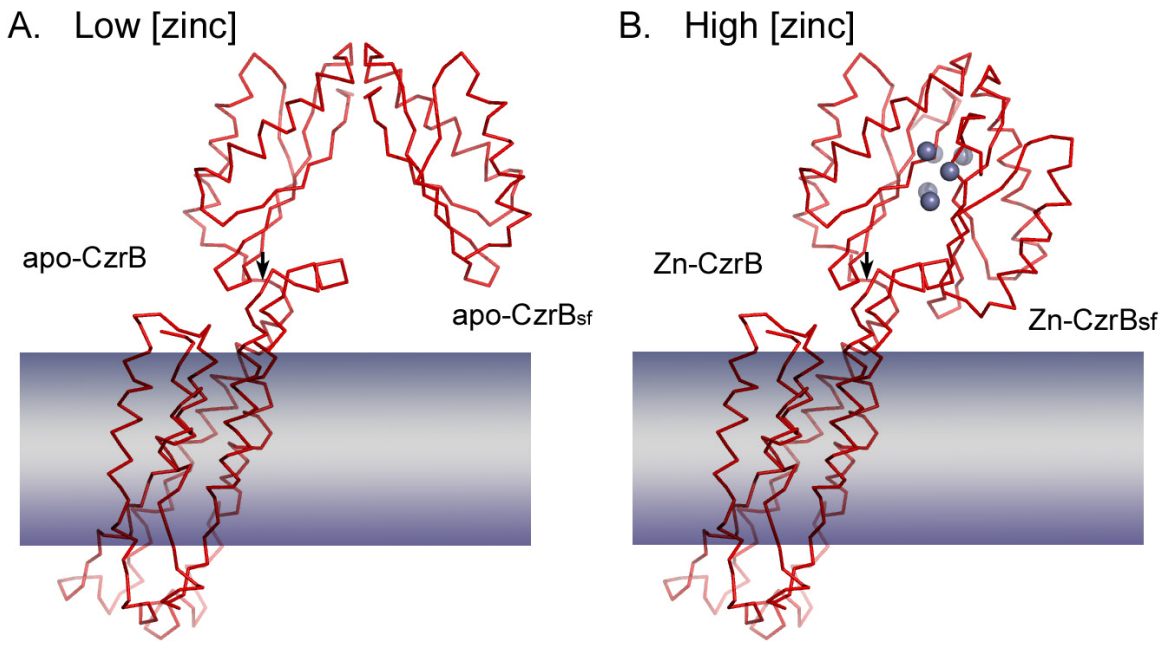

Figure S3. A Model to Explain How $CzrB_{sf}$ Can Render CzrB Inactive as a Zinc Transporter Through CzrB-$CzrB_{sf}$ Heterodimer Formation

Based on the structure of the apo-$CzrB_{sf}$ dimer in Figure 2 and the model in Figure 8 we propose that $CzrB_{sf}$ displaces one full-length CzrB protomer from the apo form of the dimer and produces the heterodimer shown in **A**. Upon zinc binding to the apo-heterodimer (**B**) the $CzrB_{sf}$ is entirely free to rotate into position next to its partner in the full-length monomer without the need for a bending motion at the hinge (arrow) between the cytosolic and transmembrane domains of the latter. Failure to rotate at the hinge means that the site for metallochaperone docking (vicinity of arrow, see Figure 8) does not materialize and the heterodimer is rendered inactive as a zinc transporter. In the heterodimer therefore zinc binding is effectively uncoupled from the creation of a chaperone docking site. Zinc ions are shown as bluish gray spheres. The membrane is represented as a shaded rectangle the upper surface of which faces the cytoplasm.