Electronic Supplementary Information for

Metal-binding Properties of *Escherichia coli* YjiA, a Member of the Metal Homeostasis-Associated COG0523 Family of GTPases

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Supplementary Results

Zn(II)-binding induces a space group transition in YjiA crystals. The space group of apo WT YjiA was reported as *C*2 previously.[1] We also collected diffraction data on apo E37A,C66A,C67A YjiA, which we could assign to the space group *C*2 as well (data not shown). Diffraction data of both Zn(II)-soaked WT YjiA and Zn(II)-soaked E37A,C66A,C67A YjiA, however, could be indexed either in space group $P2_1$ or in space group *C*2. The data statistics are nearly identical for the two space groups and do not exclude either possibility. When the data are processed in the space group $P2_1$, analysis of the Patterson function using phenix.xtriage[2] reveals an off-origin peak that is 79.2 % of the origin peak for WT YjiA and 73.8% for E37A,C66A,C67A YjiA, indicating the presence of pseudo-translational symmetry. This pseudo-translational symmetry corresponds to the crystallographic symmetry observed in the *C*2 crystal lattice.

We therefore independently refined the structure in both space groups. As the data to parameter ratio is approximately the same for both space groups ($P2_1$: 35507 unique reflections for about 4900 protein atoms or a ratio of 7.2; *C*2: 16967 reflections for about 2450 protein atoms or a ratio of 6.9), the free *R*-factor from refinement should be an unbiased criterion to assess space group assignment. For Zn(II)-soaked WT YjiA we obtained a free *R*-factor of 26.8 after refinement in the space group $P2_1$ and a free *R*-factor of 29.3 after refinement in the space group *C*2.

From these results, we concluded that data of both Zn(II)-soaked WT YjiA and Zn(II)-soaked C66A,C67A,E37A YjiA should be assigned to the space group $P2_1$. Although the crystal lattice also supports the assignment of the space group *C*2, the resulting crystallographic symmetry would be imperfect as a result of subtle structural changes, most likely induced by

Zn(II)-binding. In particular, a Zn(II) ion would be bound on the 2-fold axis that is crystallographic in the *C*2 lattice, as opposed to the $P2_1$ lattice. Furthermore, several loop regions involved in Zn(II)-binding exhibit different conformations in the two protomers in the asymmetric unit. Thus, all data indicate that Zn(II)-binding induces small changes in the crystal lattice, reducing crystallographic symmetry but keeping the overall crystal lattice intact.

The resulting structure of Zn(II)-soaked WT YjiA refined in the space group $P2_1$ is very similar to that of apo WT YjiA (PDB ID 1NIJ [1]), with C_α r.m.s.d. values of 1.111 Å and 0.915 Å for the two protomers of the Zn(II)-soaked structure, respectively. Furthermore, the arrangement of molecules in the crystal lattices of Zn(II)-soaked WT YjiA and of apo WT YjiA is nearly identical despite the different space groups (Figure S3). In particular, the two protomers in the asymmetric unit of Zn(II)-soaked WT YjiA (space group $P2_1$) are related by a crystallographic symmetry operation for apo WT YjiA (space group *C*2).

Table S1: PCR primers used for cloning and mutagenesis.[a]

Primer Name	Primer Sequence
YjiA forward	5'GGAGAC**CATATG**AACCCGATTGCAGTTAC3'
YjiA reverse	5'GCCCAT**CTCGAG**TTACTTCCTCAACCC3'
YjiA C66A,C67A forward	5'CTGACCAACGGCTGCATCgctgctTCGCGCTCCAACGAGC3'
YjiA C66A,C67A reverse	5'GCTCGTTGGAGCGCGAtgctgcGATGCAGCCGTTGGTCAG3'
YjiA E42A forward	5'GATTGAAAACGAATTCGGCccAGTCTCTGTTGATGATC3'
YjiA E42A reverse	5'GATCATCAACAGAGACTggGCCGAATTCGTTTTCAATC3'
YjiA E37A forward	5'CAAGATTGCCGTGATTGcAAACGAATTCGGCGAAG3'
YjiA E37A reverse	5'CTTCGCCGAATTCGTTTgCAATCACGGCAATCTTG3'

[a] Restriction enzyme sites are shown in bold. Mutations are shown in lowercase. The E42A,C66A,C67A and E37A,C66A,C67A mutants were constructed using the C66A,C67A YjiA-pEt24b vector as a template and the E42A and E37A primers, respectively.

Table S2: Calculated and observed molecular masses (MM) for WT YjiA and mutants, as determined by ESI-MS.

Protein	Calculated MM (Da)	Observed MM (Da)
WT YjiA	35659.6	35660.0
YjiA C66A,C67A	35595.5	35596.0
YjiA E37A,C66A,C67A	35537.5	35538.0
YjiA E42A,C66A,C67A	35537.5	35538.0

Table S3: Summary of gel filtration chromatography results of WT YjiA with added metals or nucleotides.[a]

Added Metal/ Nucleotide	Calculated MW (kDa)	Relative Peak Area[b]	Assignment
Apo	30 ± 2	100	Monomer
2 eq. Ni(II)	31.2 ± 0.4	21.5	Monomer
	316 ± 5	78.5	Oligomer
2 eq. Zn(II)	38.4 ± 0.7	11.0	Monomer
	440 ± 40	89.0	Oligomer
2 eq. Co(II)	36.5 ± 0.5	14.5	Monomer
	350 ± 10	85.5	Oligomer
2 eq. GDP[c]	29.1 ± 0.1	91.0	Monomer
	62 ± 2	9.0	Dimer
2 eq. GTP[c]	28.6 ± 0.1	92.0	Monomer
	61.3 ± 0.8	8.0	Dimer

[a]Errors are standard deviations of at least two replicates. The expected molecular weights are: WT YjiA monomer: 35.7 kDa; WT YjiA dimer: 71.3 kDa; WT YjiA tetramer: 142.6 kDa. In cases where the dimeric and oligomeric species were not well resolved they are summarized as "oligomer".

[b]The relative peak areas varied by < 4.3 % of the total peak area.

[c]Samples contained 400 μM GDP or GTP in running buffer.

Table S4: Summary of gel filtration chromatography results of mutant YjiA with added metals and nucleotide.[a]

Protein	Added Metal/ Nucleotide	Calculated MW (kDa)	Relative Peak Area[b]	Assignment
E37A,C66A,C67A YjiA	**Apo**	29.2 ± 0.2	100	Monomer
	2 eq. Ni(II)	29.2 ± 0.8	92.5	Monomer
		63 ± 2	7.5	Dimer
	2 eq. Zn(II)	29.6 ± 0.2	31.0	Monomer
		590 ± 20	69.0	Oligomer
	2 eq. Co(II)	27.7 ± 0.6	100	Monomer
E42A,C66A,C67A YjiA	**Apo**	29.7 ± 0.4	94.0	Monomer
		66 ± 1	6.0	Dimer
	2 eq. Ni(II)	29.48 ± 0.02	29.5	Monomer
		600 ± 50	70.5	Oligomer
	2 eq. Zn(II)	29.5 ± 0.1	90.0	Monomer
		66.4 ± 0.2	10.0	Dimer
	2 eq. Co(II)	29.22 ± 0.03	70.0	Monomer
		286 ± 7	30.0	Oligomer

[a]Errors are standard deviations of at least two replicates. The expected molecular weights are: E37A,C66A,C67A and E42A,C66A,C67A YjiA monomer: 35.5 kDa; E37A,C66A,C67A and E42A,C66A,C67A YjiA dimer: 71.1 kDa; E37A,C66A,C67A and E42A,C66A,C67A YjiA tetramer: 142.2 kDa. In cases where the dimeric and oligomeric species were not well resolved they are summarized as "oligomer".

[b]The relative peak areas varied by < 4.3 % of the total peak area.

Table S5. Crystallographic data collection and refinement statistics.

	Zn(II)-soaked WT YjiA[a]	Zn(II)-soaked E37A,C66A,C67A YjiA
Data collection		
Space group	$P2_1$	$P2_1$
Unit cell parameters		
a, *b*, *c* (Å)	56.30, 68.87, 77.83	56.07, 68.44, 77.70
α, β, γ (°)	90, 104.09, 90	90, 104.23, 90
Resolution (Å) [b]	30.0 – 2.57 (2.61 – 2.57)	75.3 – 2.05 (2.09 – 2.05)
Unique reflections [b]	35529 (1535)	35715 (1784)
R_{sym} (%) [b]	7.9 (41.2)	8.5 (59.0)
$\langle I \rangle / \sigma(\langle I \rangle)$ [b]	18.3 (3.3)	18.6 (2.9)
Completeness (%) [b]	97.0 (84.6)	98.5 (98.8)
Multiplicity [b]	3.8 (3.6)	6.5 (5.7)
Wavelength (Å)	1.1808	0.9795
Refinement		
Resolution range (Å)	30.0 – 2.57	75.3 – 2.05
R_{cryst}/R_{free}	0.220/0.262	0.203/0.238
No. of non-hydrogen atoms		
protein	4821	4895
Zn	5	3
sulfate	10	10
water	43	256
Average isotropic *B*-factors (Å^2)		
protein	71.7	43.8
Zn (internal sites)	92.0	–
Zn (bridging site)	70.2	36.9
Zn (surface sites)	89.5	74.0
sulfate	111.0	65.3
water	58.3	42.0
r.m.s.d.[c] bond lengths (Å)	0.002	0.008
r.m.s.d.[c] bond angles (°)	0.56	0.73
Ramachandran statistics (% residues)		
Favored	96.2	97.5
Allowed	3.9	2.5
Disallowed	0.0	0.0
Rotamer outliers (%)	1.2	1.0

[a] For this data set, Bijvoet pairs were treated as separate reflections
[b] Values in parentheses indicate highest resolution bin
[c] r.m.s.d. is root mean squared deviation

Figure S1. Electrospray ionization mass spectra of WT YjiA. Observed mass spectra (top spectrum) and reconstructed masses (bottom spectrum) of WT YjiA after incubating with 5 equivalents of metal. WT YjiA is observed binding two (35786 Da), three (35849 Da) and four (35912 Da) zinc ions. Also observed are the sodiated (+22 Da) and potassiated (+28 Da) peaks for each species.

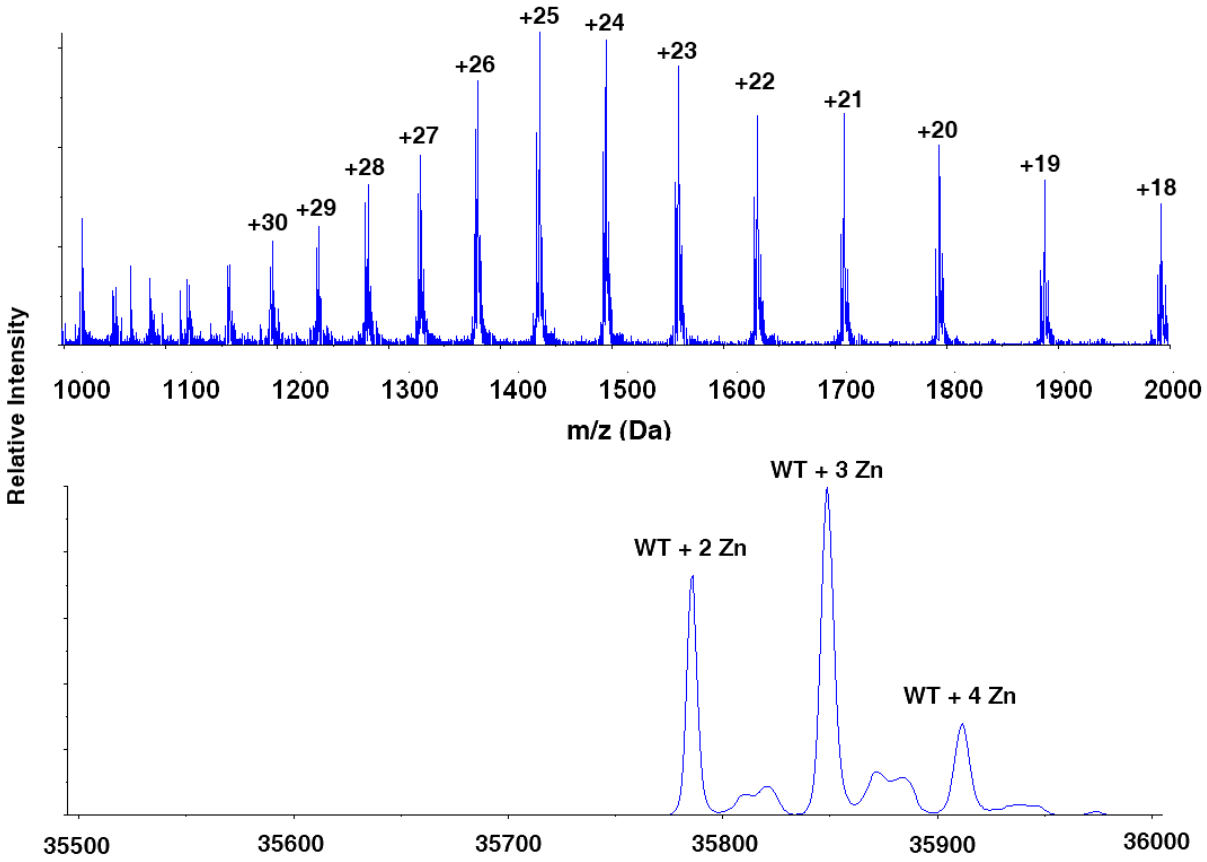
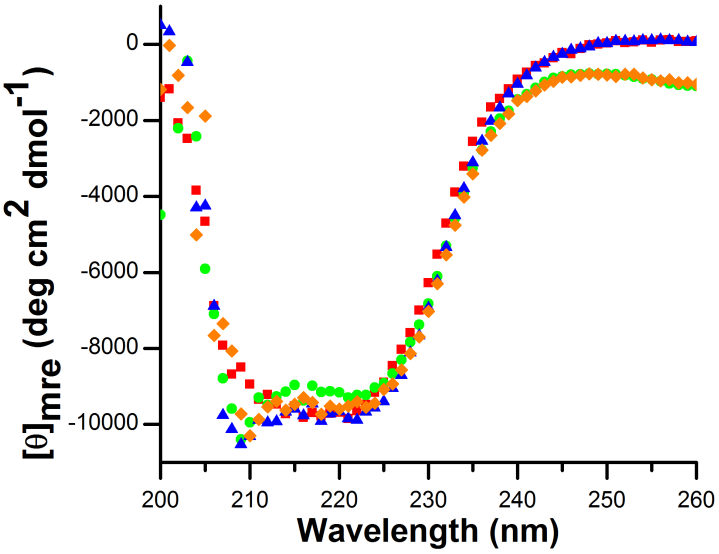


Figure S2. Circular dichroism spectra of WT YjiA and two YjiA triple mutants. (**Top Graph**) The circular dichroism spectrum of 20 μM apo-WT YjiA (red squares) possesses the general characteristics consistent with a mixed αβ protein, in agreement with the published crystal structure of YjiA.[1] Analysis of the spectrum using the K2D3 program[3] indicates the presence of 22% α-helix and 26% β-sheet. Upon the addition of 100 μM $NiCl_2$ (green circles), 100 μM $ZnSO_4$ (blue triangles), or 200 μM $CoSO_4$ (orange diamonds), an increase in negative ellipticity near 208 nm is observed, suggesting a conformational change in the protein. However, K2D3 analysis did not indicate a major change in the overall content of α-helix and β-sheet (for + Ni(II), 23% α-helix, 25.0% β-sheet; for + Zn(II), 22% α-helix, 25.0% β-sheet; for + Co(II), 21% α-helix, 26.0% β-sheet). (**Bottom Graph**) CD Spectra of WT YjiA (red squares) compared to E37A,C66A,C66A YjiA (blue triangles) and E42A,C66A,C67A YjiA (green circles).



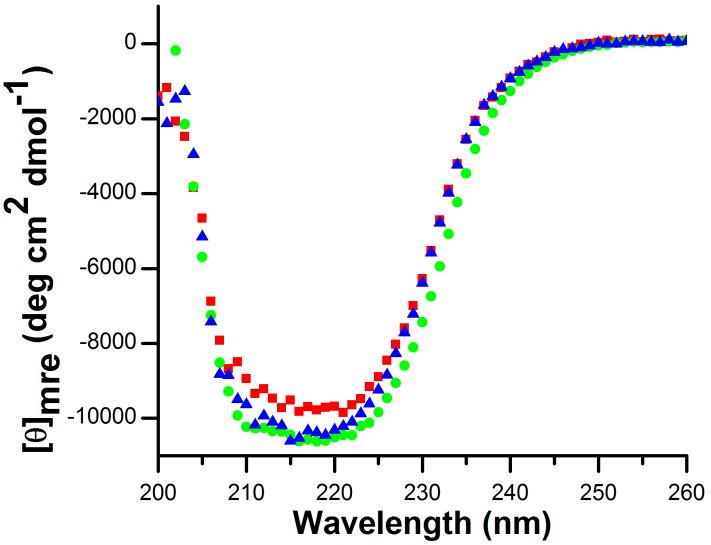


Figure S3. Comparison of crystal lattices of apo-WT YjiA (blue, PDB ID 1NIJ [1]) and Zn(II)-soaked WT YjiA (asymmetric unit in yellow and green, remaining protomers in gray). The crystal lattices of the two structures are almost identical.

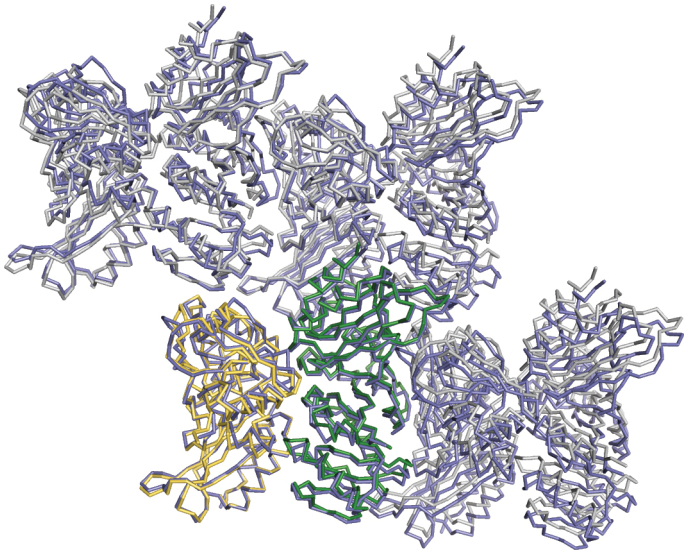


Figure S4. The Walker A motif (P-loop) adopts multiple conformations in YjiA. Two distinct conformations (yellow and green carbons) are observed in the structures of Zn(II)-soaked WT YjiA and of Zn(II)-soaked E37A,C66A,C67A YjiA. One of the conformations (yellow) resembles the conformation observed in apo WT YjiA (pink carbons, PDB ID 1NIJ [1]). The Walker A motif is also in proximity to the metal-binding site with bound Zn(II) (purple sphere).

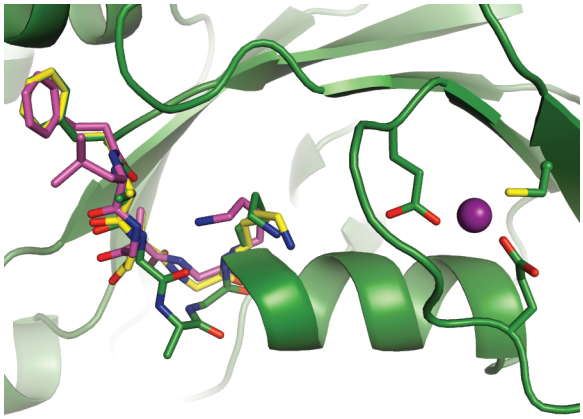


Figure S5. Crystal structure of Zn(II)-soaked E37A,C66A,C67A YjiA. **(A)** Overall structure of the two E37A,C66A,C67A YjiA protomers in the asymmetric unit, colored in pink and orange ribbons. The crystal structure of Zn(II)-soaked WT YjiA is superimposed (coloring as in Figure 5A). **(B)** Close-up view of the bridging Zn(II)-binding site. This site is still occupied with Zn(II) (purple sphere), as indicated by the Zn anomalous difference Fourier map contoured at 5 σ (yellow mesh). Zn(II)-coordinating residues are labeled. **(C)** Comparison of the internal sites of Zn(II)-soaked WT YjiA (green carbons) and Zn(II)-soaked E37A,C66A,C67A YjiA (pink carbons). For the triple mutant, F40 occupies the space left open by the mutations and the lack of Zn(II). A $2F_o-F_c$ simulated annealing composite omit map (blue mesh) is contoured around F40 at 1.0 σ. The side chain of E42 is truncated to the C_β in E37A,C66A,C67A YjiA due to lack of electron density. **(D)** and **(E)** Close-up views of the surface Zn(II)-binding sites. Crystallographically related YjiA molecules are shown with gray carbons. Map and coloring as in **(B)**.

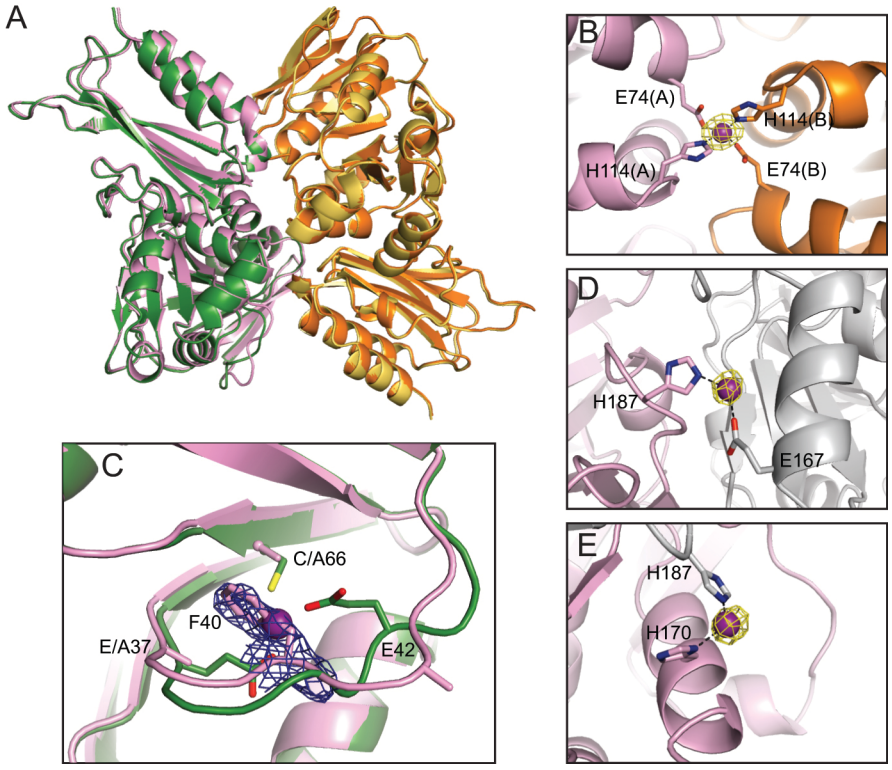
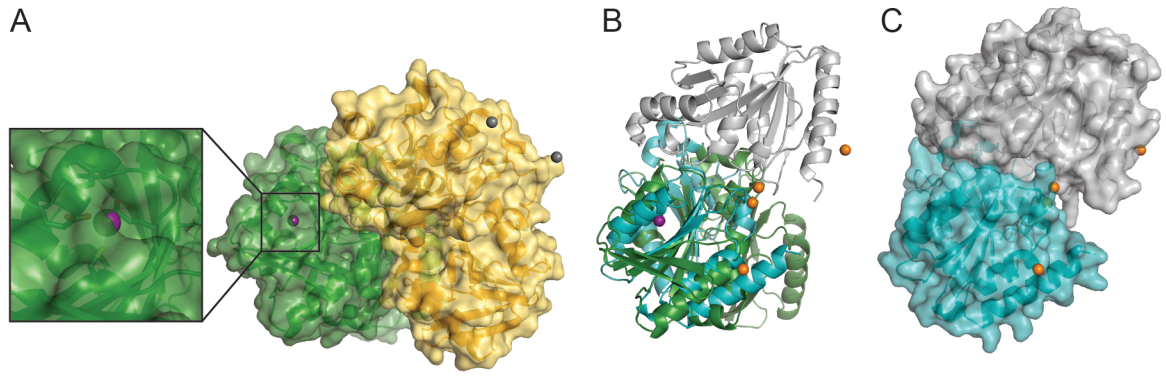


Figure S6. Comparison of the metal-binding sites of YjiA and HypB. (**A**) The internal metal-binding site of YjiA is located in a solvent-accessible pocket of the protein beneath the surface of the protein. YjiA protomers in the asymmetric unit are shown in surface representation in green and yellow, with ribbons shown underneath. Bound Zn(II) in the internal site is shown as a purple sphere, additional Zn(II) ions are shown as gray spheres. The inset shows a close-up view of the internal Zn(II)-binding site. (**B**) Superimposition of a YjiA protomer and a HypB dimer (PDB ID 2HF8[4]), shown in the same orientation as in panel (**A**). HypB is colored in cyan and gray and HypB-bound Zn(II) ions are shown as orange spheres. Although YjiA and HypB share structural homology in the GTPase domain, the Zn(II)-binding sites of HypB are located on the protein surface and do not resemble the internal Zn(II)-binding site in YjiA. (**C**) The HypB Zn(II)-binding sites are located directly on the surface of the protein. HypB is colored and oriented as in panel (**B**).



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