Crystallographic snapshots of active site metal shift in *E. coli* fructose 1,6-bisphosphate aldolase

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Running title: *Ligand binding induced metal shift in EcFBA*

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Supplementary Figure Legends

Figure S1. Sequence alignment of class II FBAs from various organisms. *Ec*FBA (GI: 16130826) is from *E. coli*; *Cj*FBA (GI: 919260097) from *Campylobacter jejuni*, *Mt*FBA (GI: 1000901037) from *Mycobacterium tuberculosis, Gl*FBA (GI: 159119664) from *Giardia lamblia*, and *Sa*FBA (GI: 447054585) from *Staphylococcus aureus.* An asterisk (*) denotes the conserved three His and one Glu residues coordinating the active site metal ion. Secondary structures of core $(\alpha/\beta)_8$ barrel fold of FBAs are shaded in pink: β-strand in pink triangle and α-helix in pink circle. Representative variable loop regions close to the C-terminal side of the core $(\alpha/\beta)_8$ barrel are shaded in blue, salmon, and yellow rectangles.

Figure S2. Structure comparison of FBAs. A) *Ec*FBA structure (NAT2). A) The NAT1 and NAT2 structures of *Ec*FBA are well conserved (Table S3). B) *Gl*FBA structure (PDB ID: 3GB6). C) *Sa*FBA structure (PDB ID: 4TO8). D) Superimposed structures of *Ec*FBA, *Gl*FBA, and *Sa*FBA. Overall structure of core $(\alpha/\beta)_8$ barrel and two long protruded helices involved in dimerization was well conserved in FBAs. However, the loops connecting the $(\alpha/\beta)_8$ barrel showed differences in structures. The representative variable loop regions in Fig. S1 are shaded in circles or ovals with the same color in each structure. The catalytic metal-binding site in each protomer was also represented in red circles.

Figure S3. A) Crystal structure of active site of substrate fructose 1,6-bisphosphate-bound *Gl*FBA (PDB ID: 3GB6). B) Crystal structure of active site of apo *Gl*FBA (PDB ID: 3GAK). C) Crystal structure of active site of citrate-bound *Sa*FBA structure (PDB ID: 4TO8). Zn1 and Zn2 are marked with red and green circles, respectively.

Fig. S1

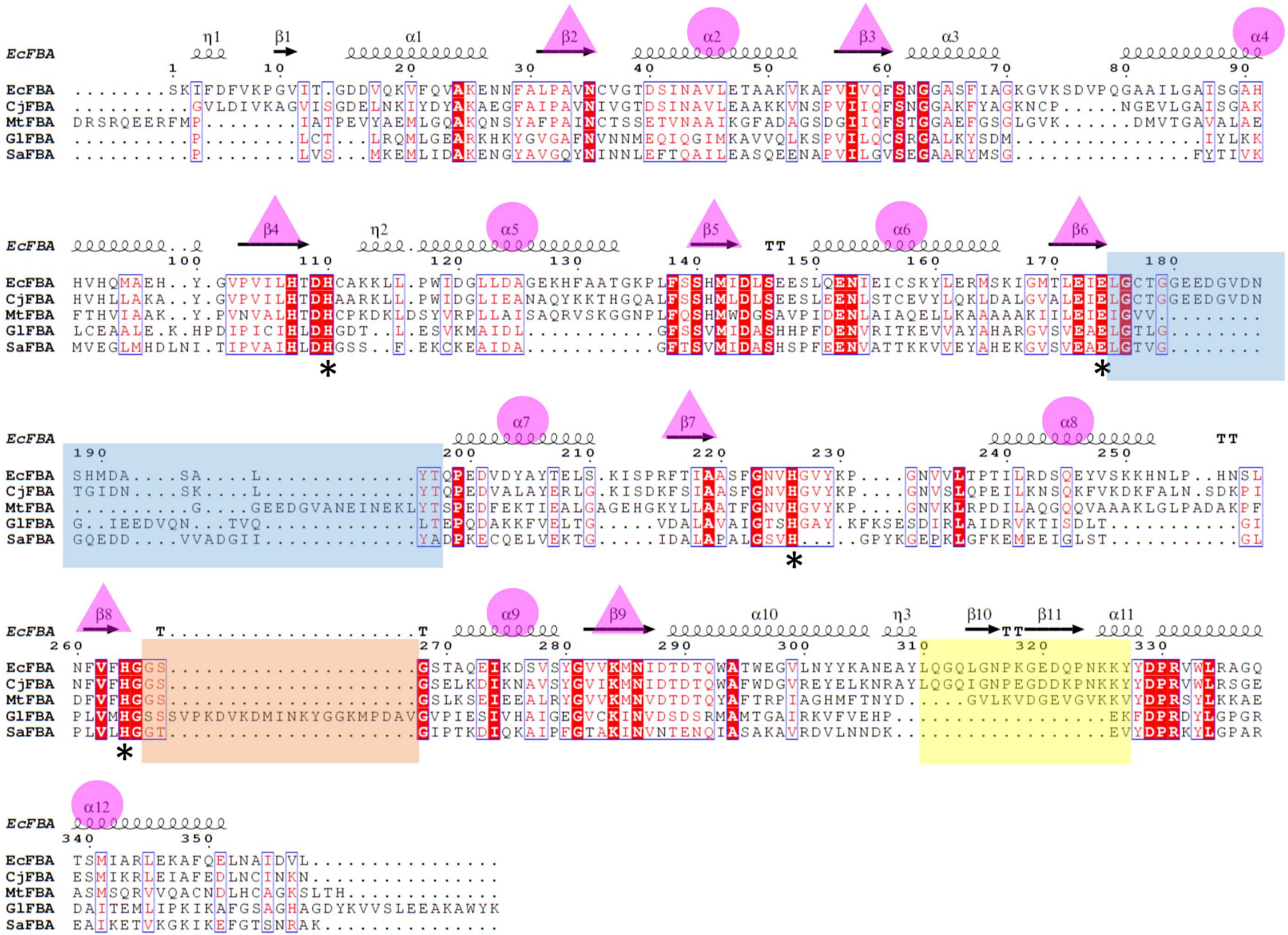

EcFBA
CjFBA
MtFBA
GlFBA
SaFBA
η1 β1 α1 β2 α2 β3 α3 α4
β4 η2 α5 β5 α6 β6
α7 β7 α8
β8 α9 β9 α10 η3 β10 β11 α11
α12

Fig. S2

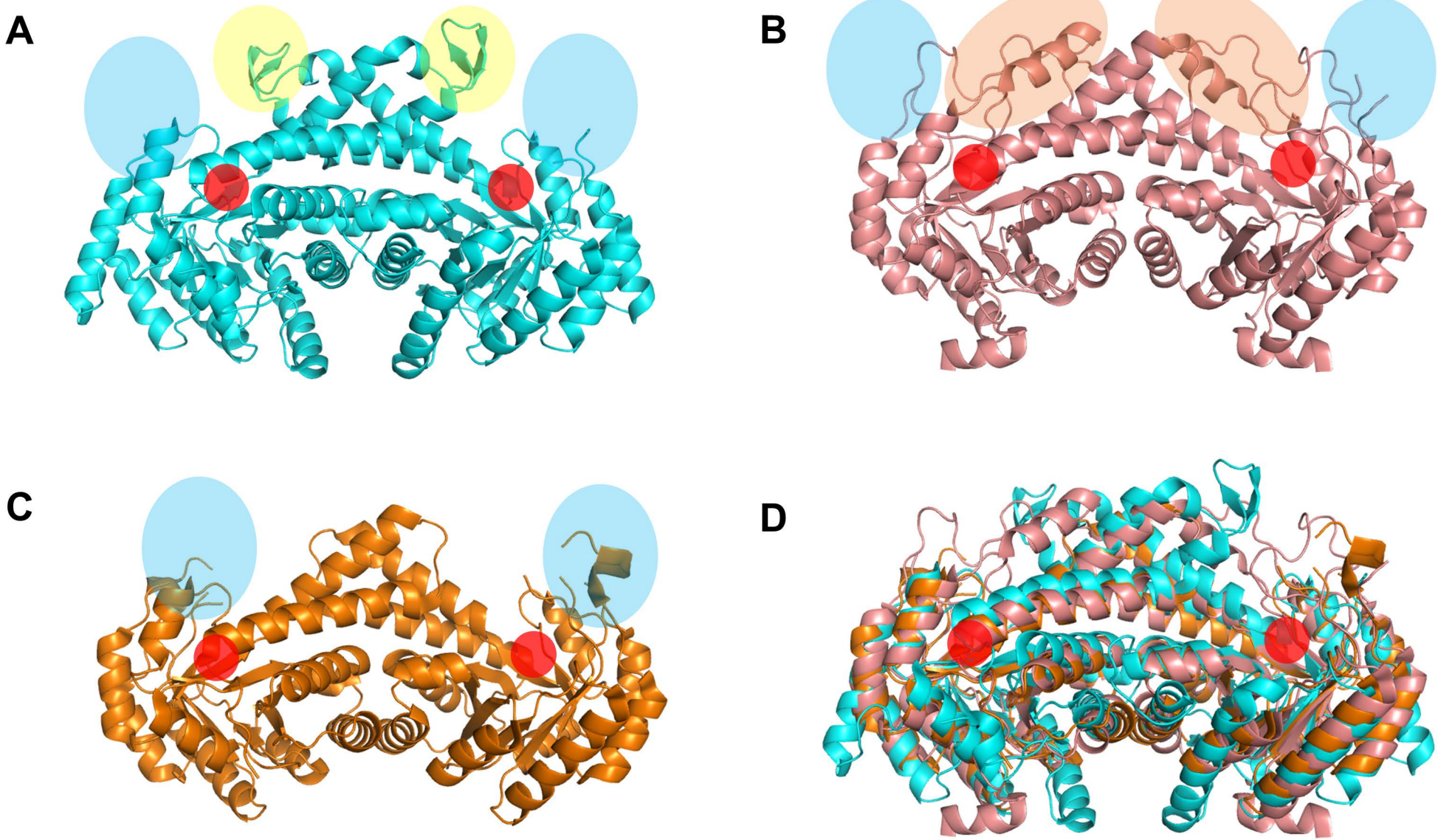
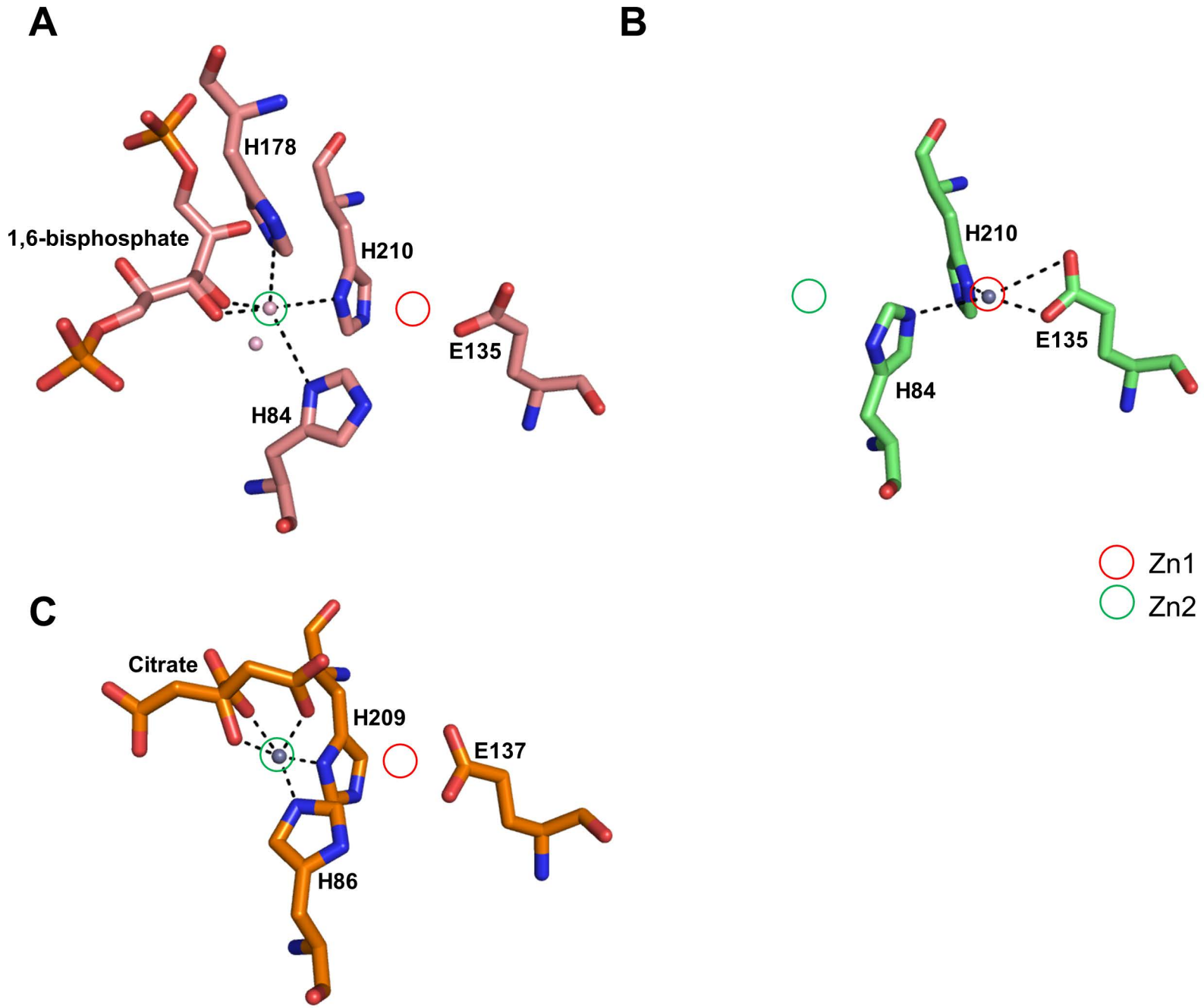


Fig. S3


A
H178
1,6-bisphosphate
H210
E135
H84
B
H210
E135
H84
Zn1
Zn2
C
Citrate
H209
E137
H86

Supplementary Tables

Table S1. Data collection and refinement statistics

Data	NAT1 (1 Zn^{2+} at Zn1, and 3 His)	NAT2 (2 Zn^{2+} at both Zn1 and Zn2, and 2 His)	Citrate-bound (1 Zn^{2+} at Zn2, and 2 His)	Tris-bound (1 Zn^{2+} at Zn2, and 2 His)
Data collection				
Protein Data Bank code	5GK3	5GK4	5GK6	5HK7
Beamline				
Wavelength (Å)	PAL-5C	PAL-5C	PAL-5C	PAL-5C
	0.979	0.979	0.979	0.979
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Unit-cell parameters				
a, b, c (Å)	72.3,72.8, 73.4	73.1, 72.9, 72.5	72.0, 73.0, 73.0	72.4, 72.7, 73.1
α, β, γ (°)	90.0, 103.1, 90.0	90.0, 103.2, 90.0	90.0, 104.1, 90.0	90.0, 103.2, 90.0
Resolution (Å)	50.00-1.80	50.00-2.00	50.00-1.80	50.00-1.80
Total reflections	357228	175378	405243	506459
Unique reflections	68416	50406	67229	67264
Completeness (%)	99.9(100.0)	99.8(100)	98.9(99.7)	97.7(96.3)
Redundancy	5.2(5.2)	3.5(3.4)	6.0(5.3)	7.5(7.4)
Mean I/σ(I)	36.7(5.2)	33.8(12.8)	29.9(2.3)	38.7(5.1)
Rmerge (%)	5.4(42.4)	4.7(13.9)	14.5(62.6)	6.2(48.8)
Refinement				
Resolution (Å)	35.7 (1.8)	36.4 (2.0)	32.4 (1.8)	36.3 (1.8)
Number of reflection (Fo>0 σ(Fo))				
Working set	68389	50375	67208	67235
Free R set	3458	2561	3403	3408
R/Rfree (%)c	15.5/ 18.9	15.2/ 20.0	15.7/ 19.4	16.2/ 19.8
No. atoms				
Protein	5,162	5,098	5,173	5,124
Ligand/ion				
Zn ion	2	4	2	2
Glycerol	3	2	2	1
Di(hydroxyethyl)ether	1	1	1	1
Acetate ion	N/D	N/D	N/D	N/D
Citric acid	N/D	N/D	2	N/D
Tris	N/D	N/D	N/D	2
Water	448	374	442	291
Mean B factors (Å2)				
Main-chain atoms	20.8	21.3	25.9	19.6
Side-chain atoms	26.5	26.7	32.7	24.5
Ligand				
Zn ion	20.5	31.5	26.8	19.2
Glycerol	35.5	47.1	N/D	33.4
Di(hydroxyethyl)ether	25.5	27.3	41.5	26.4
Acetate ion	N/D	N/D	N/D	N/D
Citric acid	N/D	N/D	38.7	N/D
Tris	N/D	N/D	N/D	28.9
waters	30.4	27.3	39.0	24.5
R.m.s. deviations				
Bond lengths (Å)	0.02	0.02	0.024	0.02
Bond angles(o)	1.81	1.84	2.00	2.00
Ramachandran plot (%)				
Favored regions	96.8	98.0	97.3	97.5
Allowed regions	2.6	1.7	1.9	1.8
Outlier regions	0.6	0.3	0.8	0.6

Values in parentheses are for the highest resolution shell.

$R_{merge} = \sum_{hkl} \sum_i \left| \left(I_i(hkl) \right) - \langle I(hkl) \rangle \right| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the mean intensity of *i*th observation of symmetry-related reflections *hkl*. $R_{free} = \sum_{hkl} \left| |F_{obs}| - |F_{calc}| \right| / \sum_{hkl} |F_{obs}|$, where F_{calc} is the calculated protein structure factor from the atomic model (R_{free} was calculated with a randomly selected 5% of the reflections).

Table S2. Metal ion occupancy and coordination of His residues in *Ec*FBA structures

Dataset	Protomers	Calculated metal occupancy at each site		Interpreted metal occupancy at each site	
		Zn1 occupancy	Zn2 occupancy	Zn1 occupancy	Zn2 occupancy
NAT1	Protomer A	0.67	n/a	0.7	n/a (less than 0.3)
	Protomer B	0.72	n/a	0.7	n/a (less than 0.3)
NAT2	Protomer A	0.48	0.68	0.4	0.6
	Protomer B	0.60	0.62	0.5	0.5
Citrate-bound	Protomer A	n/a	0.75	n/a (less than 0.2)	0.8
	Protomer B	n/a	0.73	n/a (less than 0.3)	0.7
Tris-bound	Protomer A	n/a	0.80	n/a (less than 0.2)	0.8
	Protomer B	n/a	0.79	n/a (less than 0.2)	0.8

*Ec*FBA is previously shown to have mutually exclusive two metal binding sites (Blom *et al.*, 1996). Firstly, the metal occupancy of Zn1 and Zn2 was calculated separately. The combined metal occupancy of Zn1 and Zn2 was slightly higher than 1.0. The interpreted metal occupancy was the adjusted occupancy to have the combined occupancy of Zn1 and Zn2 as 1.0. The other metal occupancy was rounded to the first decimal value.

Table S3. RMSD values (Å) between FBA structures

	NAT2	Citrate-bound	TRIS-bound	*Ec*FBA (1DOS)
NAT1 (dimer vs dimer)	0.10 in 631 a.a.	0.16 in 631 a.a.	0.12 in 630 a.a.	0.33 in 608 a.a.