1 **SUPPLEMENTARY DATA**

2 **Supplementary Table S1. Primers used in this study.**

Primer name	Sequence (5'-3')
H99A-F	GATGGCCAATGGCTTCGTGGCCAAGCTCGAATCGGTC
H99A-R	GCCACGAAGCCATTGGCCATCAAAAAATCCAGCGCGC
C110S-F	GTCAATGCCTTCGTCGCCTCCCATCACCCCAACAG
C110S-R	GAGGCGACGAAGGCATTGACCGATTCGAGCTTGTGCAC
H118A-F	CACCCCAACAGCGCCCAGGCCTCGGTGCCGTTCCTG
H118A-R	GCCTGGGCGCTGTTGGGGTGATGGCAGGCGACGAAG
E133A-F	CCGCTGCCATAGCGCGGTGGCGCTGGAAGACCGCGATG
E133A-R	GCCACCGCGCTATGGCAGCGGTCGCAGATCAGGAAC
C125S-F	CTCGGTGCCGTTCCTGATCTCCGACCGCTGCCATAGC
C125S-R	GAGATCAGGAACGGCACCGAGTGCTGGGCGCTGTTG
C128S-F	GTTCCTGATCTGCGACCGCTCCCATAGCGCGGTGGAG
C128S-R	GAGCGGTCGCAGATCAGGAACGGCACCGAGTGCTG
C165S-F	CTGGAAGTGCACGGCCTGTCTGCCAAGTGCGCGGCTG
C165S-R	GACAGGCCGTGCACTTCCAGTGTCTGTGCCTGCGGTTG
C168S-F	CACGGCCTGTGTGCCAAGTCCGCGGCTGCGGGTTG
C168S-R	GACTTGGCACACAGGCCGTGCACTTCCAGTGTCTGTG
K75A-F	CTGGACTGGGTGCGCGAAGGCGCGGGTGTGGGCGCCGAC
K75A-R	GCGCCTTCGCGCACCCAGTCCAGCAGCTCGTAGGCCTTGAC
R43K-F	CTTGCAGCGAACGCGGCCTGAAGCTGACGCCGATC
R43K-R	TTCAGGCCGCGTTCGCTGCAAGCGCGCTCTACCGCAC
R43A-F	CTTGCAGCGAACGCGGCCTGGCGCTGACGCCGATC
R43A-R	GCCAGGCCGCGTTCGCTGCAAGCGCGCTCTACCGCAC
T45A-F	CGAACGCGGCCTGCGGCTGGCGCCGATCCGCGCCAAC
T45A-R	CCAGCCGCAGGCCGCGTTCGCTGCAAGCGCGCTC
R48A-F	CTGCGGCTGACGCCGATCGCCGCCAACGTACTGCGGCTG
R48A-R	GCGATCGGCGTCAGCCGCAGGCCGCGTTCGCTGCAAG
K63A-F	GATGCCGGCAAACCGGTCGCGGCCTACGAGCTGCTG
K63A-R	GCGACCGGTTTGCCGGCATCGGCGATCAGCCGCAG
Y65A-F	CGGCAAACCGGTCAAGGCCGCCGAGCTGCTGGACTG
Y65A-R	GCGGCCTTGACCGGTTTGCCGGCATCGGCGATCAGC
Y86A-F	CGACGCCCCGCCAACGGTGGCCCGCGCGCTGG
Y86A-R	GCCACCGTTGGCGGGGCGTCGGCGCCCACACCCTTG
R87A-F	GCCCCGCCAACGGTGTACGCCGCGCTGGATTTTTTGATG
R87A-R	GCGTACACCGTTGGCGGGGCGTCGGCGCCCACAC
K100A-F	GGCCAATGGCTTCGTGCACGCGCTCGAATCGGTCAATG
K100A-R	GCGTGCACGAAGCCATTGGCCATCAAAAAATCCAGC
N105A-F	CACAAGCTCGAATCGGTCGCTGCCTTCGTCGCCTG
N105A-R	GCGACCGATTCGAGCTTGTGCACGAAGCCATTG
F-P_{2976L}-FAM	CCACCCGCCAATGAAGGCAGCGTGG
R-P_{2976L}	GGGGTGTCGGCCTTGTGCAATGCGCG

3

4 **Supplementary Table S2. Oligos used in this study.**

Oligos	Oligo sequence (5'-3')
F-P_{0267}-FAM	GGCGAGTTGTTATACAGTAACATTTCTAG
R-P_{0267}	CTAGAAATGTTACTGTATAACAACTCGCC
F-P_{2472}-FAM	AAGTGTTATAAAGTAACACTAGTCGCG
R-P_{2472}	CGCGACTAGTGTTACTTTATAACACTT
F-P_{3788}-FAM	ATTGATACGTTATCACATTGCGCCCCGCGCT
R-P_{3788}	AGCGCGGGGCGCAATGTGATAACGTATCAAT
F-EcZur-box-FAM	AGAAGTGTGATATTATAACATTTCATGACTATG
R-EcZur-box	CATAGTCATGAAATGTTATAATATCACACTTCT
F-P_{2976}-FAM	TTAGGGCCACGCGGTTGCGGAGACTATTACACCGGCGCGTGATGAGATCACATCACGTG
R-P_{2976}	CACGTGATGTGATCTCATCACGCGCCGGTGTAATAGTCTCCGCAACCGCGTGGCCCTAA
F-P_{0267}-Biotin	GGCGAGTTGTTATACAGTAACATTTCTAG
R-P_{0267}	CTAGAAATGTTACTGTATAACAACTCGCC
F-P_{2472}-Biotin	AAGTGTTATAAAGTAACACTAGTCGCG
R-P_{2472}	CGCGACTAGTGTTACTTTATAACACTT
F-P_{3788}-Biotin	ATTGATACGTTATCACATTGCGCCCCGCGCT
R-P_{3788}	AGCGCGGGGCGCAATGTGATAACGTATCAAT

5

6 **Supplementary Table S3. Metal content analysis by ICP-MS.**

Protein	Molar ratio of zinc to protein	Molar ratio of nickel to protein
XcZur (purified)	0.89 ± 0.012	0.096 ± 0.0026
XcZur-Δ16 (purified)	0.92 ± 0.018	0.067 ± 0.0044
H99A (purified)	0.85 ± 0.076	-
C125S (purified)	0.15 ± 0.012	-
XcZur (EDTA-treated)	0.87 ± 0.016	-
XcZur-Δ16 (EDTA-treated)	0.92 ± 0.018	-

7

8 **Supplementary Table S4. The metal ligands of XcZur homologs and XcZur.**

Structure	PDB	Divalent metal	Metal ligands
CjFur	4ETS	Zn	C105, C108, C145, C148, D101, E120, H137
CjFur	6D57	Zn	C105, C108, C145, C148
HpFur	2XIG	Zn	C102, C105, C142, C145, E90, H97, H99, E110, H96, D98, E117, H134,
FtFur	5NBC	Mn, Zn	C93, C96, C133, C136, H33, E81, H88, H90, E101
FtFur	5NHK	Fe, Zn	C93, C96, C133, C136, H33, E81, H88, H90, E101
VcFur	2W57	Zn	H33, E81, H88, H90, E101, H71, H87, D89, E108, H125
MgFur	4RB3	Mn	H33, E81, H88, H90, E101, H87, D89, E108, H125
MgFur	4RAZ	Mn	H33, E81, H88, H90, E101, H87, D89, E108, H125
MgFur	4RB1	Mn	H33, E81, H88, H90, E101, H87, D89, E108, H125
MgFur	4RB2	Mn	H33, E81, H88, H90, E101, H87, D89, E108, H125
PaFur	1MZB	Zn	H32, E80, H89, E100, H86, D88, E107, H124
RlMur	5FD6	Zn	H36, H74, E84, H91, H93, H90, D92, E111, H128
CjPerR	6DK4	Mn, Zn	C89, C92, C132, C135, H30, D78, H84, H86, D97
SpPerR	4I7H	Ni, Zn	C104, C107, C144, C147, H4, H6, N15, H19, H97, H99
BsPerR	2FE3	Zn	C96, C99, C136, C139
BsPerR	2RGV	Zn	C96, C99, C136, C139
LiPerR	5NL9	Zn	H36, D84, H90, H92, D103
ScZur	3MWM	Zn	C90, C93, C130, C133, H84, H86, E105, H122, D65, C79, H85, H87
MtZur	2O03	Zn	C103, C106, C143, C146, H80, H82, E101, H118, D62, C76, H81, H83
EcZur	4MTD	Zn	C86, C89, C126, C129, H77, C88, H96, E111, H89
ScNur	3EYY	Zn	H86, H88, H90, E101
XcZur	7DH7	Zn	C125, C128, C165, C168
XcZur	7DH8	Zn	C125, C128, C165, C168, H99, C110, H118, E133

9 Metal ligands in site 1, site 2 and site 3 are highlighted in red, blue and green, respectively.

10

11 **Supplementary Table S5. The position (corresponding to XcZur) and frequency of metal**
12 **ligands in XcZur homologs.**

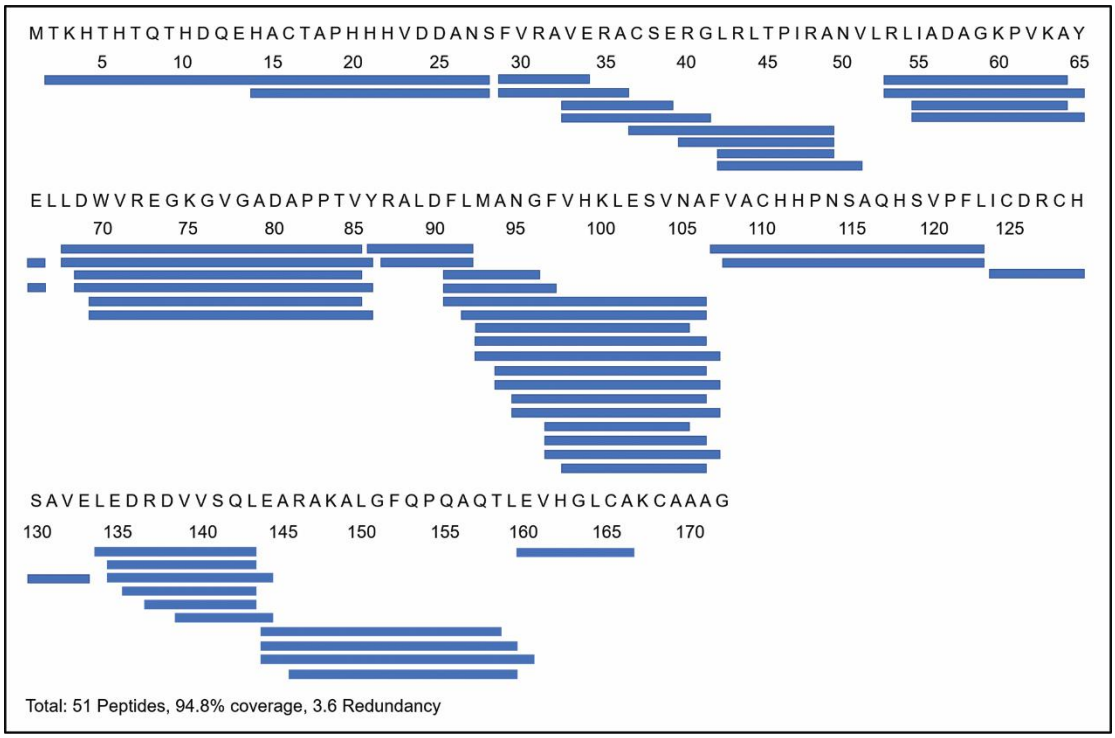
Residue position	Zur group (Frequency)	PerR group (Frequency)	Fur group (Frequency)	Total (Frequency)
H21	0	1	0	1
V23	0	1	0	1
A32	0	1	0	1
A36	0	1	0	1
P61	0	2	5	7
H99	4	0	0	4
L101	0	0	2	2
C110	2	0	0	2
H111	1	2	5	8
P113	2	0	0	2
A116	1	1	0	2
Q117	2	2	5	9
H118	5	1	5	11
S119	2	0	6	8
F122	3	2	6	11
C125	4	3	3	10
C128	4	3	3	10
E133	3	2	5	10
V140	2	0	6	8
Q157	2	0	6	8
C165	4	3	3	10
C168	4	3	3	10

13

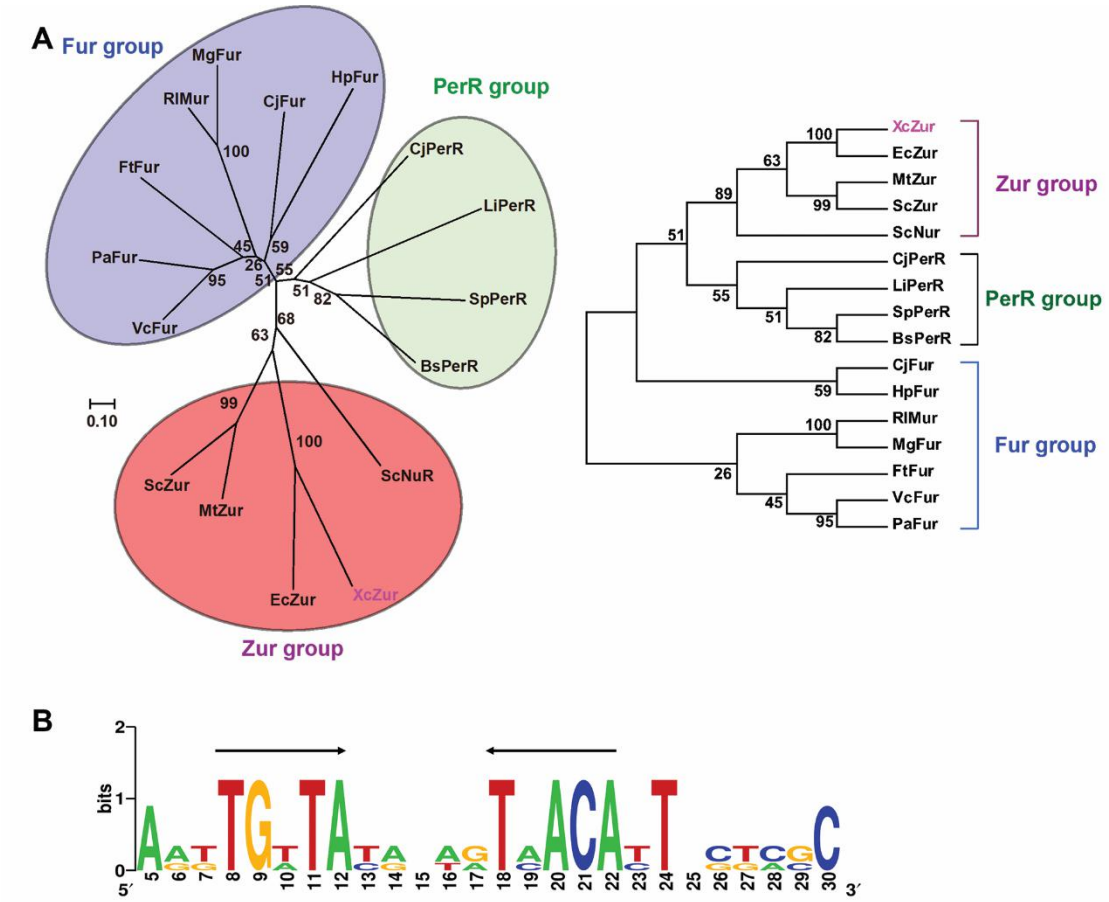
14 **Supplementary Table S6. The deuterium-labeling rate of XcZur, XcZur+Zn,**
15 **XcZur+Zn+DNA in HDX-MS and solvent accessibility of Apo and Holo XcZur.**

HDX-MS #Deut		#Deut-1min			#Deut-5min			Solvent Accessibility	
Peptide	Sequence	XcZur	XcZur +Zn	XcZur +Zn +DNA	XcZur	XcZur +Zn	XcZur +Zn +DNA	Apo XcZur	Holo XcZur
14-28	HACTAPHHHVDDANS	3.25	2.36	2.00	3.13	2.33	1.41	-	-
29-34	FVRAVE	0.75	1.00	0.67	1.48	1.70	1.12	223.15	-
33-39	VERACSE	1.58	2.01	1.50	2.39	2.41	1.96	315.90	559.90
40-49	RGLRLTPIRA	2.52	2.96	2.30	2.83	3.25	2.42	380.17	857.57
53-66	RLIADAGKPVKAYE	2.98	3.60	2.84	3.56	4.73	3.44	787.79	857.09
68-86	LDWVREGKGVGADAPPTVY	7.31	8.10	6.38	8.03	8.51	6.10	1196.03	-
70-86	WVREGKGVGADAPPTVY	6.23	6.61	5.17	6.53	6.59	4.95	1103.36	-
86-92	YRALDFL	0.50	1.09	0.86	0.78	1.40	1.07	296.18	365.79
91-96	FLMANG	0.43	0.60	0.43	0.57	0.75	0.56	69.41	188.73
93-106	MANGFVHKLESVNA	3.96	4.24	3.41	4.32	4.77	3.98	492.95	579.47
97-106	FVHKLESVNA	2.85	2.88	2.21	3.01	3.19	2.41	432.70	416.19
107-123	FVACHHPNSAQHSVPFL	3.43	3.68	2.79	3.32	3.81	2.77	752.46	819.79
124-133	ICDRCHSAVE	1.74	1.60	1.36	1.93	1.78	1.58	342.80	543.73
137-143	RDVVSQL	2.62	2.47	2.27	2.74	2.58	2.32	428.14	557.66
144-159	EARAKALGFQPQAQTL	7.04	6.14	5.05	7.01	6.41	5.14	754.68	1424.37
160-166	EVHGLCA	1.45	1.06	0.88	1.47	1.12	0.98	112.43	456.75

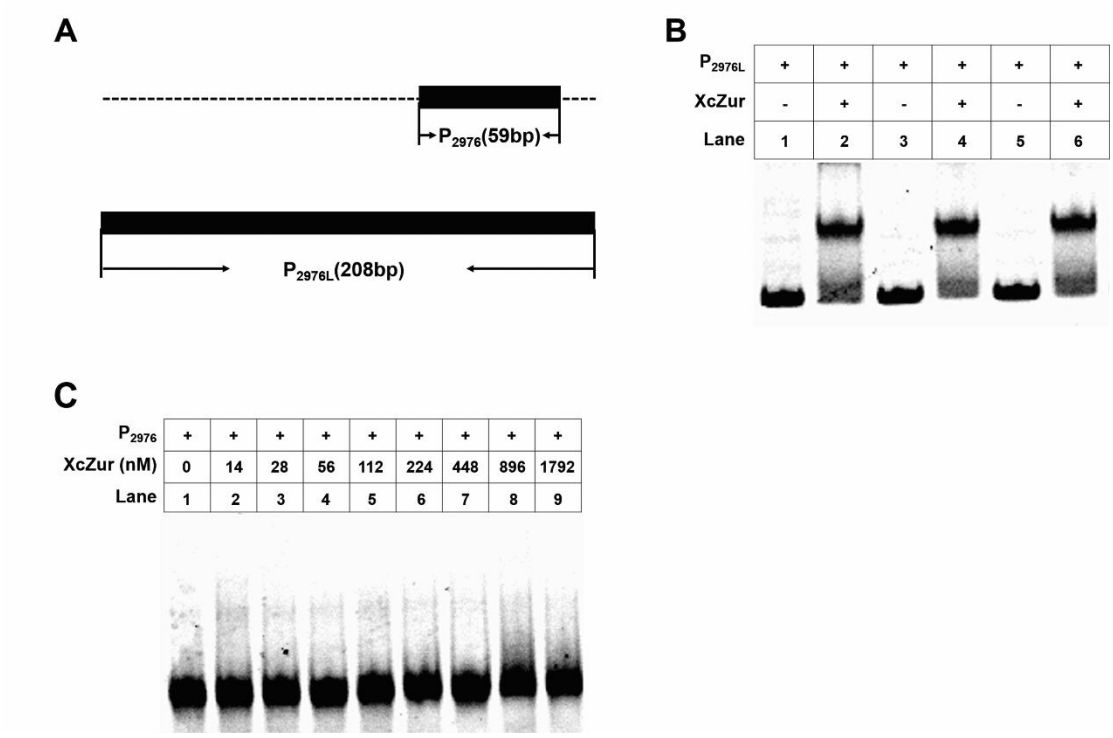
16 Solvent accessibility was calculated by using CCP4MG.
17



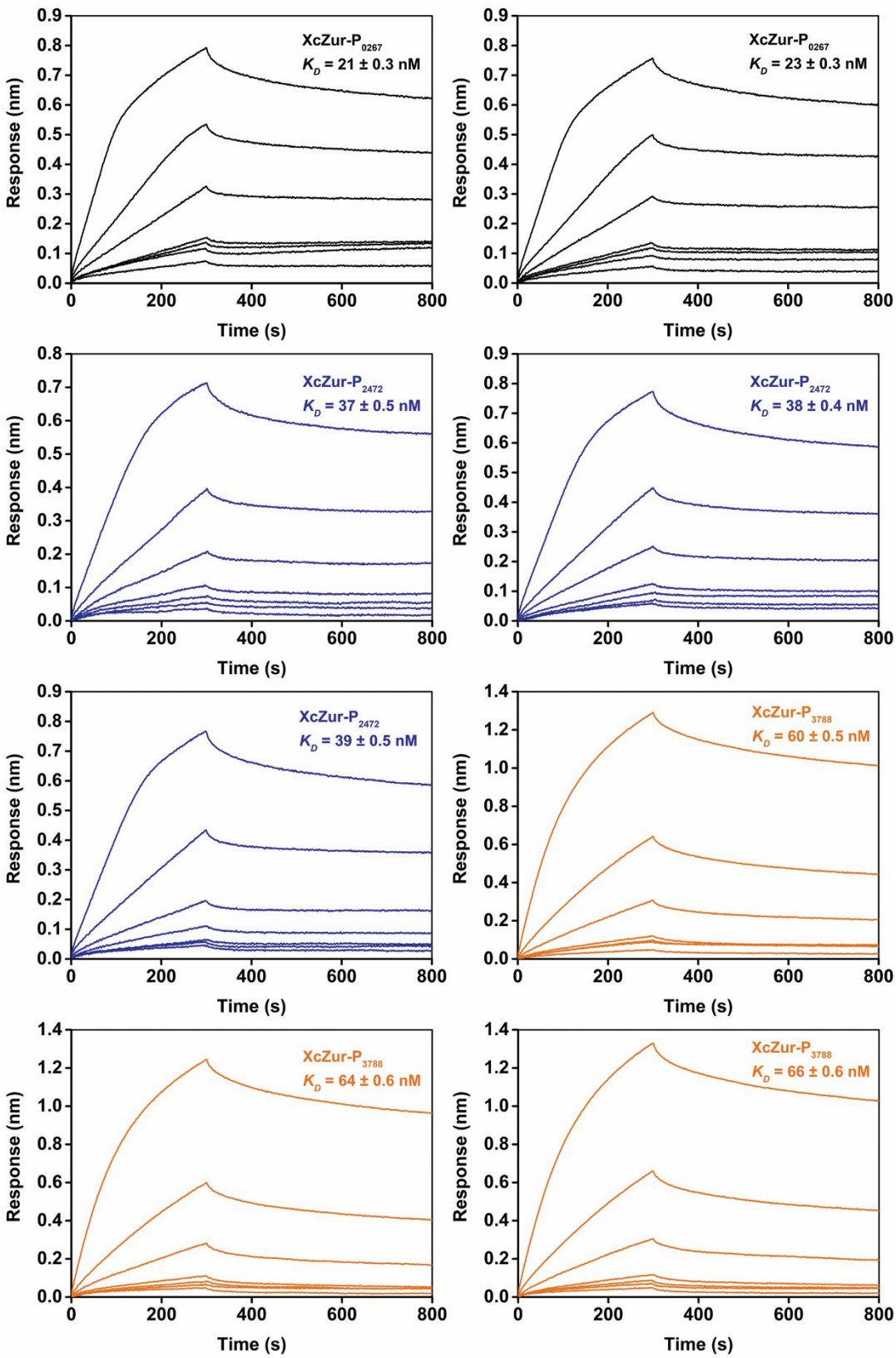
Supplementary Figure S1. **Sequence coverage map of XcZur in HDX-MS.** The 51 peptides identified by HDX-MS are described as blue bands and aligned with the corresponding XcZur sequence. The peptides cover 94.8% of the XcZur sequence.



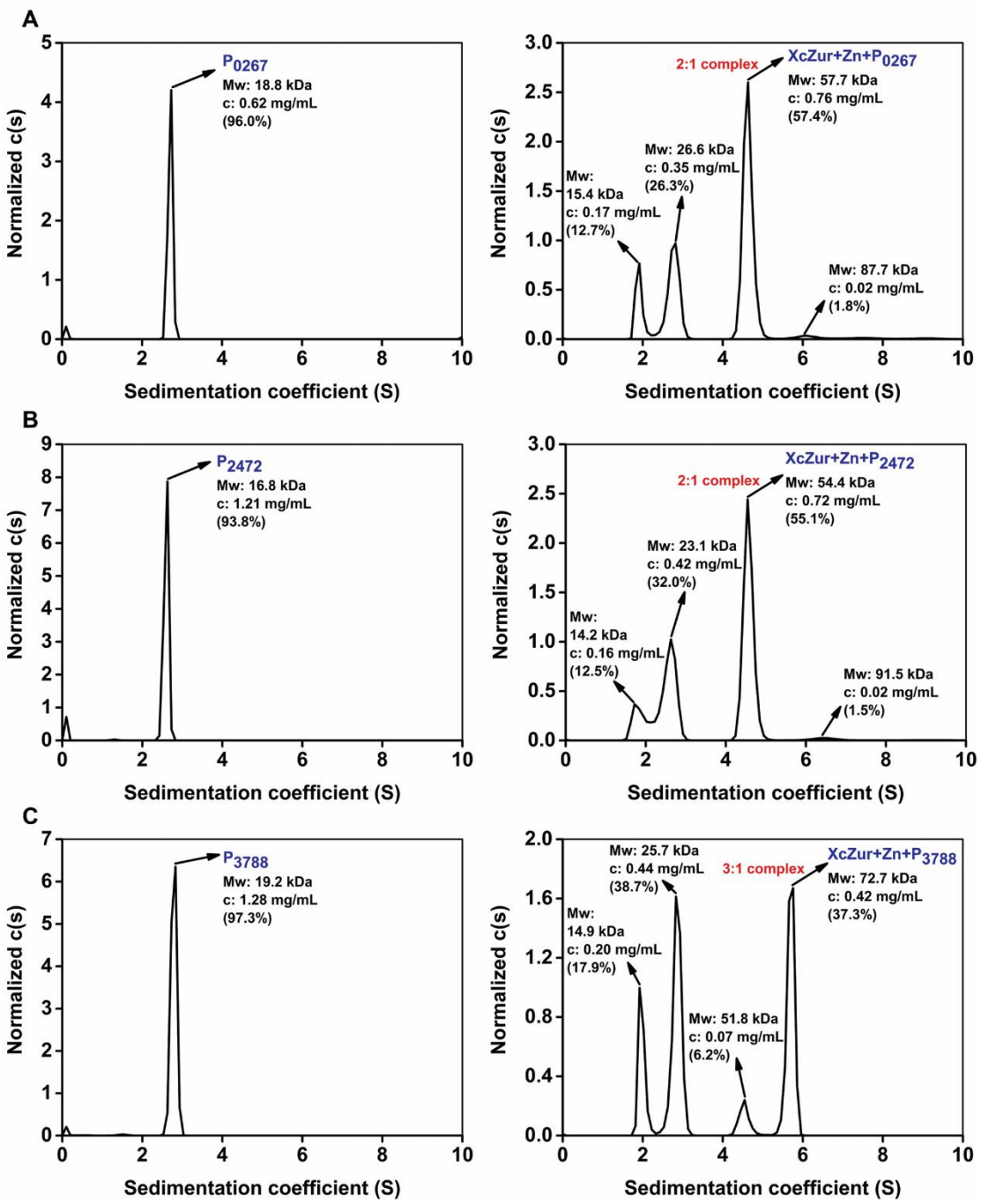
Supplementary Figure S2. Evolutionary relationships of Fur family proteins and conservation of XcZur box. (**A**)Phylogenetic tree of Fur family proteins. The evolutionary tree is shown in a three-clade radiation style (left panel) and squarish-corner style (right panel). The tree is drawn to scale and percentages of replicate trees where corresponding taxa clustered together in the bootstrap test (10,000 replicates) are shown. The XcZur is categorized into the Zur group (red circle). (**B**) Consensus sequences for XcZur binding sites, P_{0267}, P_{2472} and P_{3788}. Consensus sequences were calculated by using WebLogo (http://weblogo.berkeley.edu) and the palindromic sequence was highlighted with arrows.



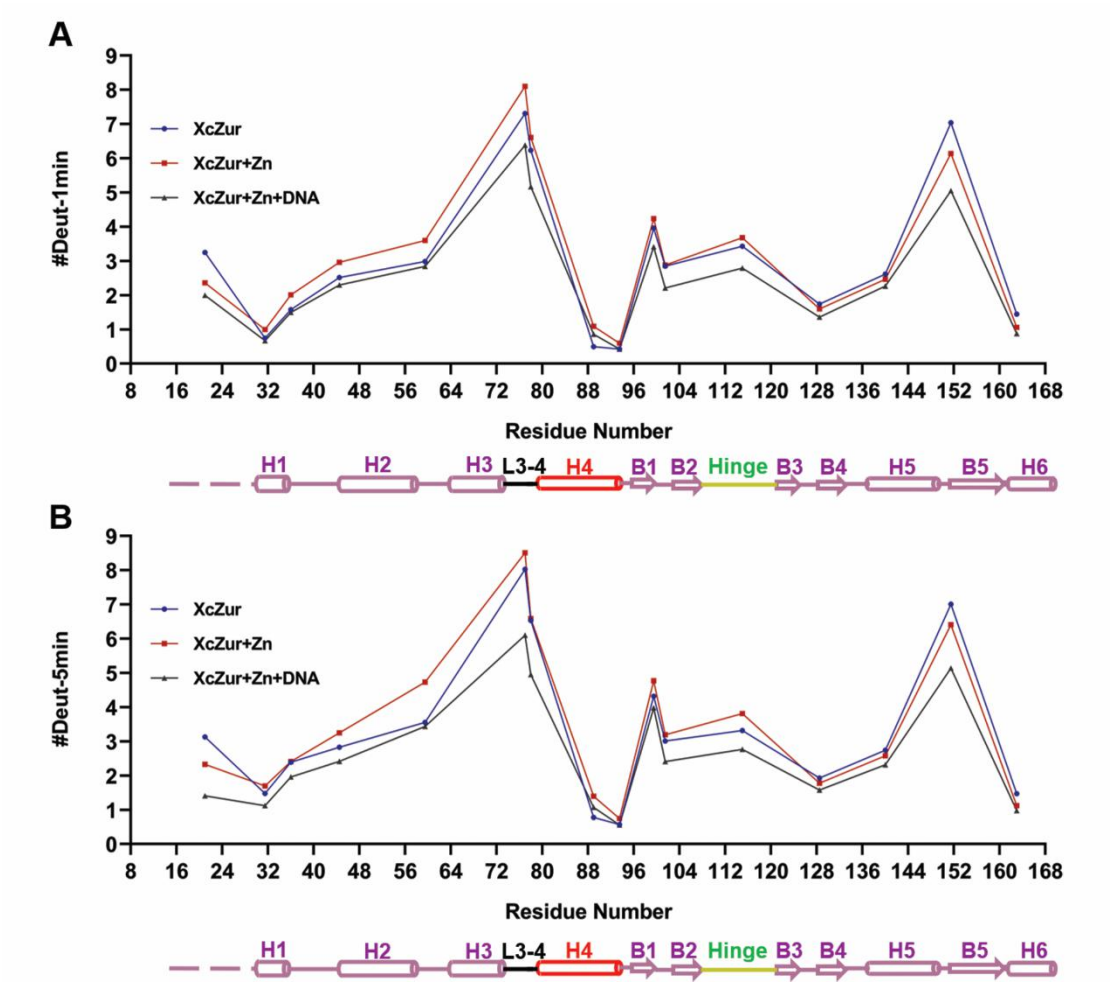
Supplementary Figure S3. **EMSA of XcZur binding to the promoter region of *XC2976*.** (**A**) Two fragments from the *XC2976* promoter used in our EMSA studies. (**B**) EMSA of XcZur binding to P_{2976L}. (**C**) EMSA of XcZur binding to P_{2976}.



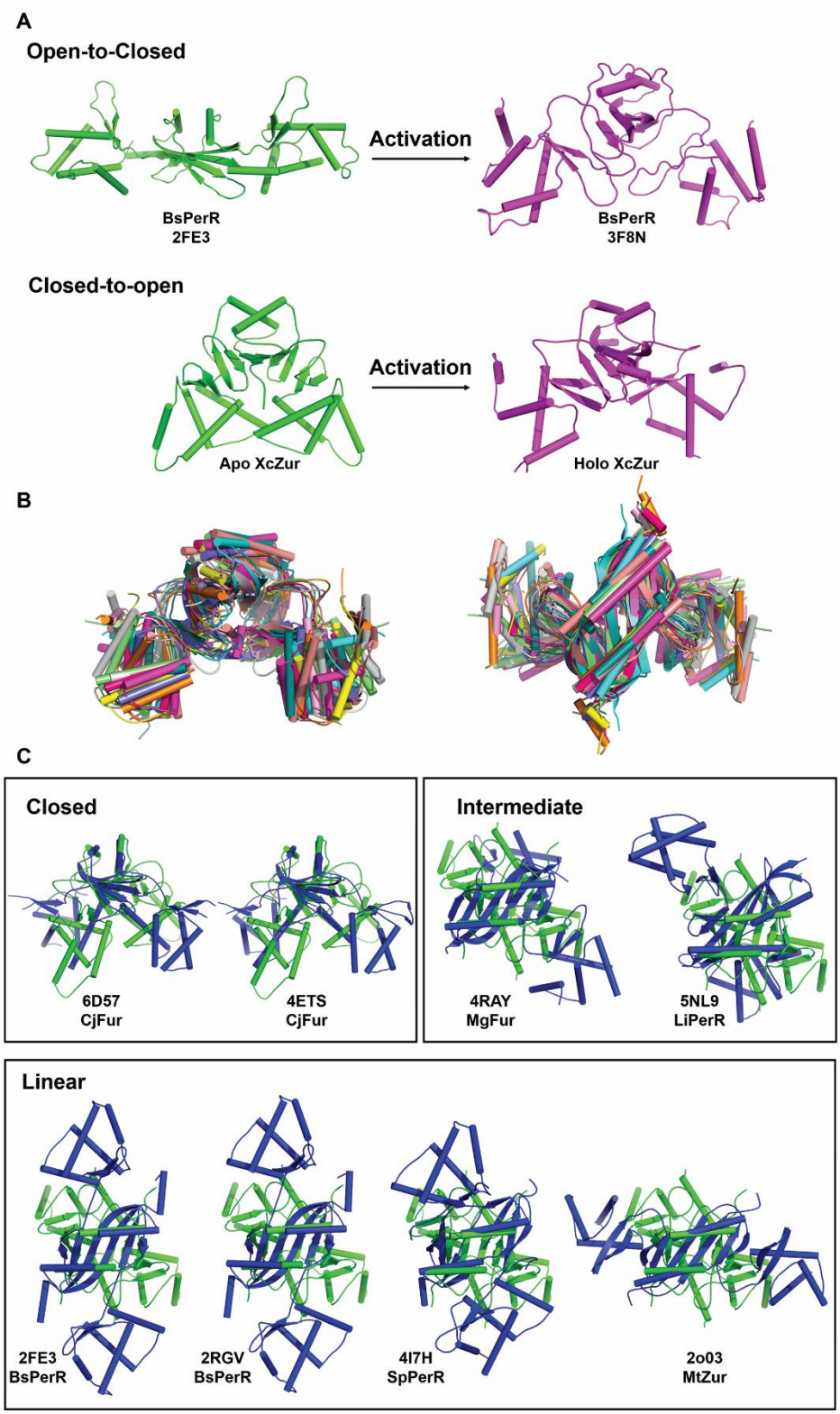
Supplementary Figure S4. Affinity determination of XcZur binding with target DNAs by BLI (Related to Figure 2E). Association and dissociation curves for XcZur with P_{0267}, P_{2472} and P_{3788} were colored by black, blue and orange, respectively. The calculated K_D were shown. Each experiment was repeated three times and similar results were obtained.



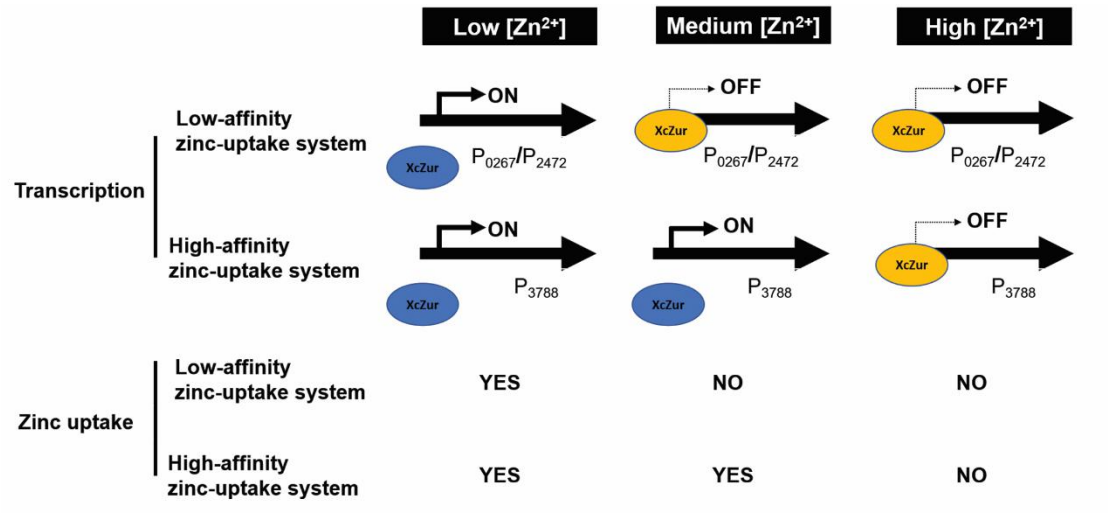
Supplementary Figure S5. Sedimentation coefficient distribution c(s) of target DNA and XcZur-DNA complexes determined by SV-AUC. (**A**) Sedimentation coefficient distribution c(s) of P_{0267} and XcZur-P_{0267} complex determined by SV-AUC. (**B**) Sedimentation coefficient distribution c(s) of P_{2472} and XcZur-P_{2472} complex determined by SV-AUC. (**C**) Sedimentation coefficient distribution c(s) of P_{3788} and XcZur-P_{3788} complex determined by SV-AUC. The calculated molecular weight, concentrations of the components (c) and stoichiometry of the XcZur-DNA complex are indicated. The weight fractions are also shown in the parentheses.



Supplementary Figure S6. In solution evidence for zinc-mediated conformational changes of XcZur. (**A**) HDX-MS analysis of XcZur, XcZur+Zn, XcZur+Zn+DNA at 1 min. Secondary structure elements are labeled at the bottom of the figure. (**B**) HDX-MS analysis of XcZur, XcZur+Zn, XcZur+Zn+DNA at 5 min.



Supplementary Figure S7. The inactive and active conformations of Fur proteins. (**A**) Comparison of the activation mechanisms between the open-to-closed (BsPerR) model and the closed-to-open (XcZur) model. (**B**) The conserved caliper-like active conformation of Fur family proteins. (**C**) The three forms of the inactive conformations of Fur proteins: the closed form, the linear form, and the intermediate form between the two. The structure of apo XcZur aligned with each homolog structure was colored in green.



Supplementary Figure S8. A proposed model of XcZur-mediated zinc uptake.