

Supporting Online Material

Molecular Basis of Selectivity and Zeptomolar Sensitivity in Metal Ion Recognition by CueR

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Materials and methods

In vitro Run-Off Transcription Assay

Metal concentrations of an acetonitrile solution of $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ and aqueous solutions of AgNO_3 , $\text{K}[\text{Au}(\text{CN})_2]$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and HgCl_2 (all ACS grade reagents) were analyzed by ICP-AES. Purification of CueR and pUC19/*copA* was carried out as described previously (1). The *copA* transcription template was prepared by the polymerase chain reaction (PCR) using the (-48) and (-47) pUC sequencing primers (New England Biolabs) and pUC19/*copA* and was labeled by incorporation of $[\alpha\text{-}^{32}\text{P}]\text{dATP}$. The PCR fragment was then digested with EcoRI and Aval to make a 315-bp fragment, P_{copA} , as the DNA template. Run-off transcription reactions were performed as previously described (2), except that 4 nM labeled P_{copA} was used as the DNA template and 1.0 mM sodium cyanide (NaCN) (ACS reagent) was used to buffer metal concentrations. The Cu^{1+} stock solution was kept under N_2 atmosphere until just before the reaction and the reduced metal state was maintained by 1.0 mM dithiothreitol (DTT) in the reaction solution. Transcription was initiated by the addition of ribonucleotide

substrates mixed with [α - 32 P]UTP (no dinucleotide initiator was necessary) and terminated after 11 min. The labeled RNA levels were normalized against labeled DNA template and were expressed as a percentage of induction relative to the maximum activation by Cu^{1+} .

CueR crystallization and structure determination

Prior to crystallization of copper-bound CueR, the protein (1~2mg/ml) was mixed with 10 mM DTT and an equimolar amount of $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ (in CH_3CN). In an attempt to crystallize the metal-activated protein-DNA ternary complex, a 3-fold molar excess of duplex DNA derived from the CopA promoter sequence was included in the crystallization experiment, but was not present in the crystal. CueR crystals were grown at 18°C by vapor diffusion in hanging drops equilibrated against 1.0 M sodium citrate and 0.1 M sodium cacodylate (pH 6.5). Crystals of silver-bound CueR were grown in the same manner except that the protein/DNA/metal mixture was incubated for 1 hour at 22°C prior to crystallization and contained a 5-fold molar excess of AgNO_3 , 5 mM DTT, and a 1.5-fold molar excess of duplex DNA. Crystals of derivatized CueR were obtained by soaking Ag-CueR crystals in crystallization solution containing 1 mM $\text{K}[\text{Au}(\text{CN})_2]$ for 2 days. For data collection, crystals were transferred in a single step to crystallization solution supplemented with 12% ethylene glycol for 1~2 minutes and flash-cooled in liquid nitrogen.

All data sets were collected at 100 K using synchrotron radiation. Data were processed using MOSFLM (3) or XDS (4) and scaled with SCALA (5). CueR crystals belong to space group $\text{P2}_12_12_1$ ($a = 59 \text{ \AA}$, $b = 66 \text{ \AA}$, $c = 82 \text{ \AA}$) and contain one dimer in the asymmetric unit. The structure was determined by SAD methods from a gold soaked

CueR crystal. Data were collected just above the theoretical gold L_{III} absorption edge in 15° segments using inverse beam techniques. One gold site was identified in an anomalous difference Patterson map. Refinement of the first site in SHARP (6) led to the identification of a second site. Heavy atom refinement and SAD phasing with 2 sites in SHARP followed by solvent flattening yielded an excellent electron density map from which the entire structure could be traced. Model building was carried out in O (7) and alternated with cycles of simulated annealing, positional, and individual temperature factor refinement in CNS (8). The Au-CueR structure was used as a model for subsequent structure determination and refinement of the Ag-CueR and Cu-CueR structures. Metal ions were identified by strong peaks in anomalous difference Fourier and F_o-F_c difference maps. The Cu¹⁺ and Ag¹⁺ atoms were refined at full occupancy whereas the Au¹⁺ atoms were refined at 60% occupancy. For each CueR structure, residues 115-119 of one monomer and residues 128-135 at the C-termini of both monomers are disordered and not included in the final model. All residues in the final model are found within the most favored or allowed regions in the Ramachandran plot.

ZntR crystallization and structure determination

The ZntR protein was overexpressed and purified as described previously (2). For crystallization, ZntR (4 mg/ml) was mixed with 5 mM DTT, a 5-fold molar excess of ZnSO₄, and a 1.5-fold molar excess of duplex DNA designed around the ZntA promoter sequence. Hanging drop vapor diffusion at 18°C yielded three different Zn-ZntR crystal forms. Form I crystals were obtained in 40% ammonium sulfate, 20 mM NaCl, 10 mM MgCl₂, and 0.1 M MES (pH 6.5) and diffracted to 2.5Å resolution. They belong to space

group $I4_122$ ($a = b = 83.8 \text{ \AA}$, $c = 180 \text{ \AA}$) and contain one monomer in the asymmetric unit. Form II crystals were grown in 0.52 M Na/K tartrate and 0.08 M HEPES (pH 7.5) and diffracted to 2 \AA . They belong to space group $C222$ ($a = 85 \text{ \AA}$, $b = 171 \text{ \AA}$, $c = 42 \text{ \AA}$) and contain a dimer in the asymmetric unit. Crystal form III grew in 0.1 M Na_2HPO_4 , 0.1 M MES (pH 6.5), 20 mM NaCl, and 10 mM MgCl_2 , belongs to space group $P2_12_12_1$ ($a = 42 \text{ \AA}$, $b = 47 \text{ \AA}$, $c = 119 \text{ \AA}$) and contains a dimer in the asymmetric unit. Form III crystals were easily reproducible and diffract strongly and thus were used in the initial ZntR structure determination and all subsequent structural analysis.

A heavy atom derivative was obtained by soaking Form III ZntR crystals in crystallization solution containing 0.5 mM K_2PtCl_4 for 14 hours. Crystals were cryoprotected by a quick transfer to crystallization solution supplemented with 35% glycerol followed by flash cooling in liquid nitrogen. To maximize the anomalous scattering from the platinum, a derivative data set was collected just above the theoretical Pt L_{III} absorption edge in 15° segments using inverse beam techniques. All data were processed with MOSFLM and scaled using SCALA. The platinum derivative data set was scaled against a native data set using SCALEIT (5) and two platinum sites were located. Heavy atom refinement and SIRAS phasing with two sites were carried out in SHARP, and a readily interpretable electron density map was obtained after solvent flattening. The electron density map revealed that only a fragment of ZntR had crystallized which was later confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry, and was the result of proteolysis occurring in the crystallization drop. Model building was carried out in O and was interspersed with cycles of simulated annealing, positional, and individual temperature factor refinement in

CNS. Once most of the structure had been built, the resolution was extended using native data to 1.9 Å. Two Zn²⁺ atoms and a phosphate ion were modeled in at each binding site at full occupancy. The final model contains residues 43-136 for one monomer and residues 45-133 for the other monomer, four Zn²⁺ atoms, two phosphate ions, one hydrated magnesium ion, and 236 water molecules. All residues are within the most favored or allowed regions of the Ramachandran plot. Structure determination of the other two crystal forms was performed by molecular replacement in AMORE (5) by using the ZntR dimer as a search model. All crystal structures reveal identical conformations of the truncated form of ZntR and contain two zinc atoms and a sulfate or phosphate ion (assignment based on crystallization components) at each metal binding site.

Supplemental References

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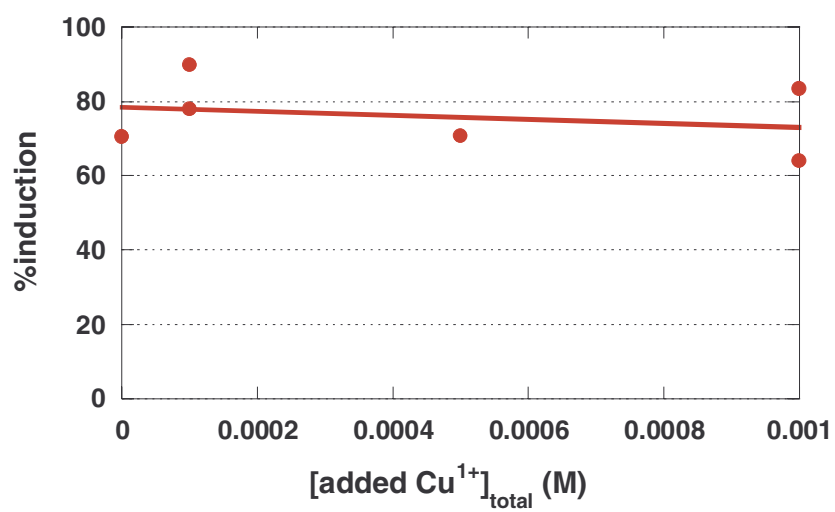


Figure S1

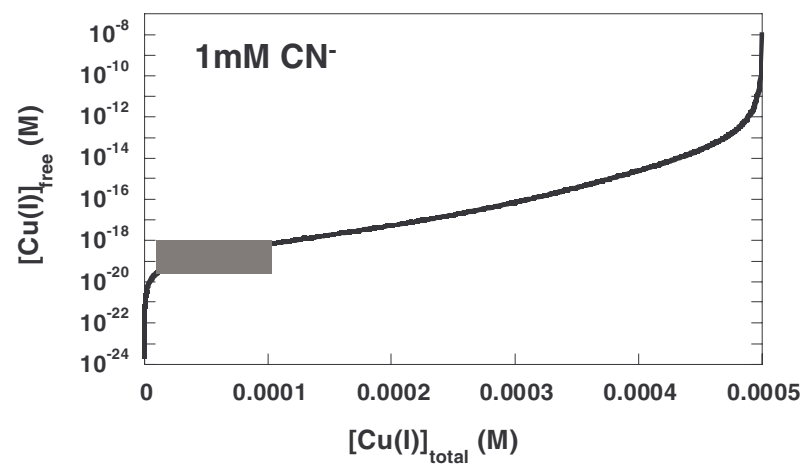
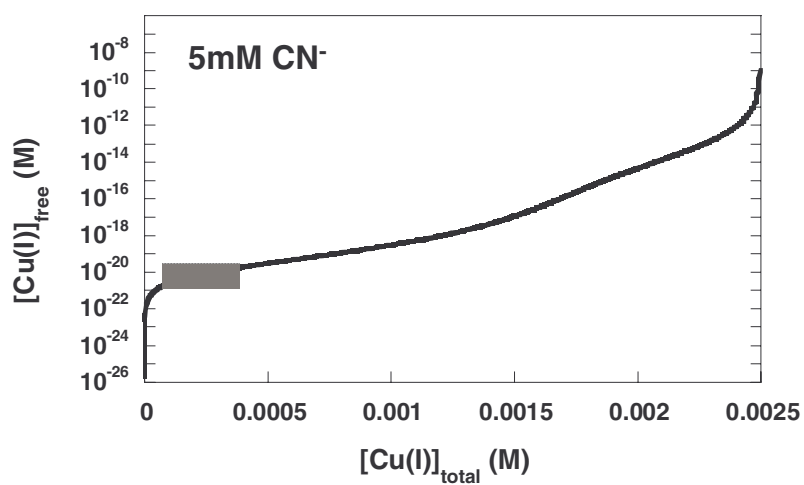
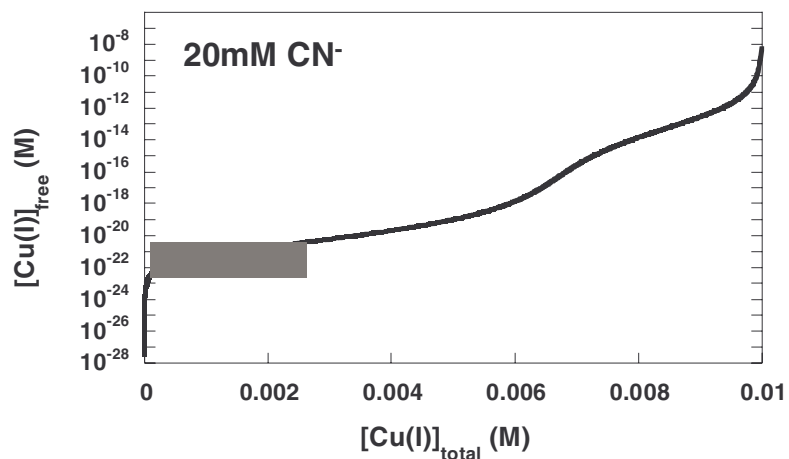


Figure S2

Supplemental Figure Captions

Figure S1. CueR run-off transcription assays with P_{copA} , showing that, at physiologically relevant concentrations, GSH is not capable of buffering CueR transcription over a wide range of total Cu^{1+} concentrations. The incubation buffer was treated with Chelex to remove exogenous metal. $[\text{CueR}] = 50 \text{ nM}$, $[\text{RNAP}] = 5 \text{ nM}$, and $[\text{GSH}]_{\text{total}} = 10 \text{ mM}$.

Figure S2. Free Cu^{1+} concentrations controlled by various concentrations of CN^- at pH 8.0. The area highlighted in grey represents the metal buffering range used in the experiments shown in Fig. 1B.

Table S1. Data collection and refinement statistics

	Pt-ZnIR	Native ZntR	Au-CueR	Cu-CueR	Ag-CueR
Data collection					
Detector type/source	MAR-CCD/ APS	MAR-345/ SSRL	MAR-CCD/ APS	MAR-CCD/ APS	MAR-CCD/ APS
Wavelength (Å)	1.068	0.97	1.0358	1.000	1.000
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
Resolution (Å)	2.7	1.9	2.5	2.2	2.07
Measured reflections	46,624	50,994	76,958	82,696	107,567
Unique reflections	6821	18,897	11,501	15,854	20,248
Completeness (%) ^a	98.0 (99.7)	99.2 (99.0)	98.3 (96.1)	95.8 (97.2)	99.3 (91.9)
R _{sym} (%) ^{ab}	4.0 (11.8)	6.6 (28.5)	4.8 (21.4)	5.5 (25.4)	4.2 (23.0)
R _{meas} (%) ^{ac}	4.7 (13.8)	8.1 (35.4)	5.7 (28.7)	6.6 (31.2)	5.0 (29.5)
Redundancy	6.8 (6.7)	2.7 (2.7)	6.7 (4.0)	5.2 (4.7)	5.3 (3.8)
I/σ(I)	12 (6.2)	8.4 (2.6)	12.6 (3.2)	10.9 (3.0)	10.8 (3.1)
Phasing					
Phasing power (acentric) ^d	1.23		1.82		
R _{collis} (acentric) ^e	0.845		0.737		
Figure of merit	0.37		0.35		
Figure of merit after density modification	0.93		0.77		
Refinement					
Resolution (Å)		29-1.9	27-2.5	24-2.2	24-2.071
Number of reflections: working set/test set		917/17,949	780/10,067	1923/26,900	1382/18835
R-factor ^f		18.3	21.0	21.2	21.9
R _{free} ^g		21.4	25.3	25.0	25.9
Protein atoms		1455	1974	1978	1974
Water molecules		236	64	150	135
Other atoms		15	2	2	2
R _{m,s.d.}					
Bond lengths (Å)		0.004	0.007	0.006	0.005
Bond angles (°)		1.2	1.1	1.1	1.0
Average B-factor (Å ²):					
Main chain		20	43	34	34
Side chain		24	46	38	39
Solvent		36	50	48	44

^aNumbers in parenthesis represent values in the highest resolution shell.

^b $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$, where I =observed intensity, and $\langle I \rangle$ =average intensity obtained from multiple measurements.

^c R_{meas} as defined by (9)

^dPhasing power=r.m.s. ($|F_h|/|E|$), where $|F_h|$ =heavy atom structure factor amplitude and E =residual lack of closure error.

^e $R_{\text{collis}} = \sum (|F_h(\text{obs})| - |F_h(\text{calc})|) / \sum |F_h(\text{obs})|$, where $|F_h(\text{obs})|$ =observed heavy atom structure factor amplitude and $|F_h(\text{calc})|$ =calculated heavy atom structure factor amplitude.

^f R -factor= $\sum ||F_o| - |F_c|| / \sum |F_o|$, where $|F_o|$ =observed structure factor amplitude and $|F_c|$ =calculated structure factor amplitude.

^g R_{free} : R-factor-based on 7% of the data excluded from refinement

Table S1: Data collection, phasing, and refinement statistics for the CueR and ZntR structures. The CueR structure was solved by SAD phasing from a Ag-CueR crystal that was soaked in $\text{K}[\text{Au}(\text{CN})_2]$. The Au^{1+} replaces the Ag^{1+} at the metal binding site but is only present at 60% occupancy. Consequently, the Au-CueR structure represents an average of Ag^{1+} and Au^{1+} forms of the protein. The ZntR fragment crystallized in three different space groups and the structure was solved using SIRAS phases obtained from a platinum derivative. ZntR structures from all three space groups were found to be identical and thus only the highest resolution structure from the $\text{P}2_12_12_1$ crystal form is reported here.