Electronic Supplementary Material (ESI) for RSC Advances.

Cadmium Adsorption *E. coli* with Surface Displayed CadR

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S1 Expression and purification of CadR, TC21 and TC68

The plasmid pet28a was bought from *Sangon Biotech Co.* with a His_6 tag at C-terminal. The construction of TC21-pet28a and TC68-pet28a were carried out by sub-cloning with PCR primer P1, P2, P3, P4 and digestion sites of NcoI and XhoI. (Table S1)

Table S1 Primers for the construction of TC21-pet28a and TC68-pet28a

Primers	Sequences
P1 (forward)	CATGCCATGGAGATTGGCGAACTGGCCAAA
P2 (reverse)	CCGCTCGAGTTCCAGTTGCTGCAGAATAGC
P3 (forward)	CATGCCATGGTGCGTGACTCTCCTGACGATTCC T
P4 (reverse)	CCGCTCGAGGTGGCCGTGAGAGCGACCAA

Then the plasmids were transformed into *E. coli* BL21 (DE3) cells. A single colony was cultured in 10mL LB media at 37℃ overnight containing 50 mg L^{-1} kanamycin. Then these overnight culture was diluted(1:100) in 1000 mL LB media with 50mg L^{-1} kanamycin and grown at 37℃ until an OD_{600} reached 0.6-0.8. Then 0.5 mM IPTG (isopropyl-beta-D-1-thioglactopyranoside) was added and proteins were expressed with a further incubation at 16℃ for 20h. Cells were harvested by 4000rpm centrifugation at 4℃, stored at -80℃ before further use. Cell pellet was re-suspended in Buffer A (10 mM Tris, 100 mM NaCl, 10 mM 2-Mercaptoethanol, pH=7.4) with 1mM PMSF. After sonication, the lysate was centrifuged at 4℃ for 30 min and the supernatant was filtered with sterile 0.22 μm filters. The filtrate was loaded onto a 5mL HisTrap column (GE Healthcare) on ice, which was then washed with 25mL buffer A and the protein was eluted with linear gradient increased Buffer B (Buffer A with 500 mM imidazole) fraction with FPLC (AktapurifierTM 10, GE Helthcare) at 4℃. The elution fractions containing CadR, TC21 or TC68 were concentrate by minipore and exchanged the buffer to Buffer C (20 mM Tris, 50 mM NaCl, 10 mM 2-Mercaptoethanol, pH=7.4) with a HiTrap Desalting column (GE Healthcare). After dialysis the protein was concentrated again and loaded onto Superdex-200 column (Superdex-200 10/300 GL, GE Healthcare) with Buffer C without 2-Mercaptoethanol. After this purification step the proteins were single bands on SDS gel (Figure S1) and the protein concentrations were measured by NanoDrop (Thermo scientific, Nano Drop 2000). The proteins were then flush-freezed with liquid nitrogen and stored at -80℃ for future usage. The protein for ITC measurements needs a further purification by dialyzing in Buffer C without 2-Mercaptoethanol with 10 mM EDTA to remove the metal ions chelated with CadR in the media, then with the desalting column to remove the EDTA from the system.

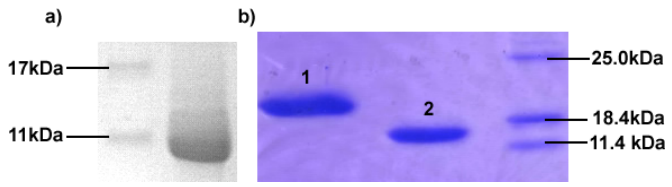

Figure S1 SDS-PAGE for the purified protein: a), TC68, 10.7 kDa; b-1), CadR, 18.7 kDa; b-2), TC21, 15.3 kDa

S2 Electrophoretic mobility shift assays for proteins towards the metal ions

The gel shift assay followed the protocol reported[1].

S3 ITC measurements

The ITC experiments were measured with ITC 200 (GE Healthcare) in a buffer of 20 mM Tris, 50 mM NaCl and pH=7.4. The 400 μL sample cell was filled with 50μM protein solution and stirred constantly at 800 rpm. The

syringe was filled with 250μM cadmium nitrate solution and titrated into the sample cell in one 0.4μL injection followed by 2.0μL injections at 120 seconds intervals. The binding data were processed with Origin 7.0 software (OriginLab, MA, USA) by least squares regression to a model corresponding to one set of identical independent binding sites. Figure S2 and S3 are titration results of CadR and TC68 respectively. The binding constants were in the figures. N is the binding ratio of cadmium ions to CadR, K is the association constants, ΔH and ΔS are the enthalpy and entropy change respectively.

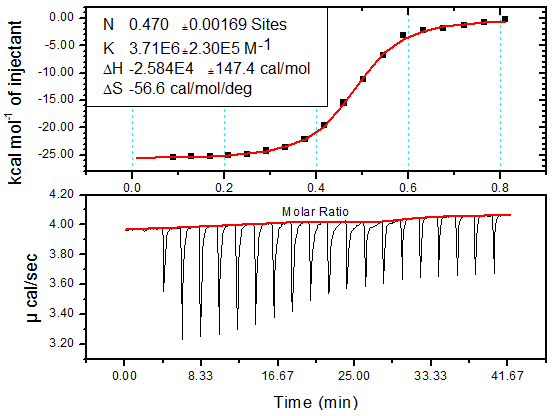

Figure S2 ITC of CadR

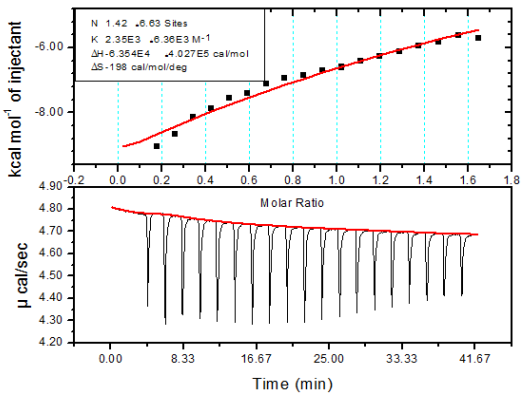

Figure S3 ITC of TC68

S4 The control for the ITC analysis

We used the several proteins as the control of ITC. First, we wanted to test the availability of Tris buffer (20 mM Tris, 50 mM NaCl, Ph 7.4) in the titration. Figure S8 is the titration of Cd^{2+} to buffer.

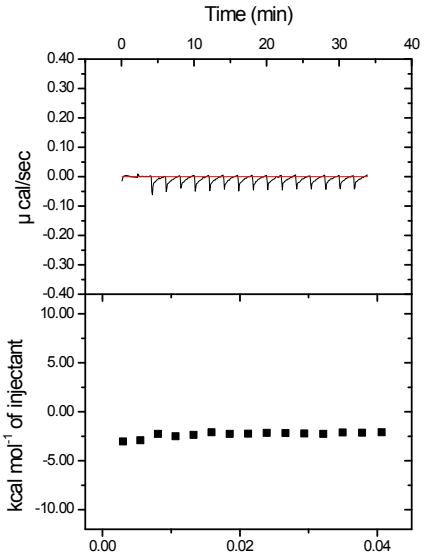

Figure S4 ITC of buffer

Besides, we used two other proteins with His-tag as the control of ITC. The first one is the idsA [3], another is hMsrA[4] (S9).

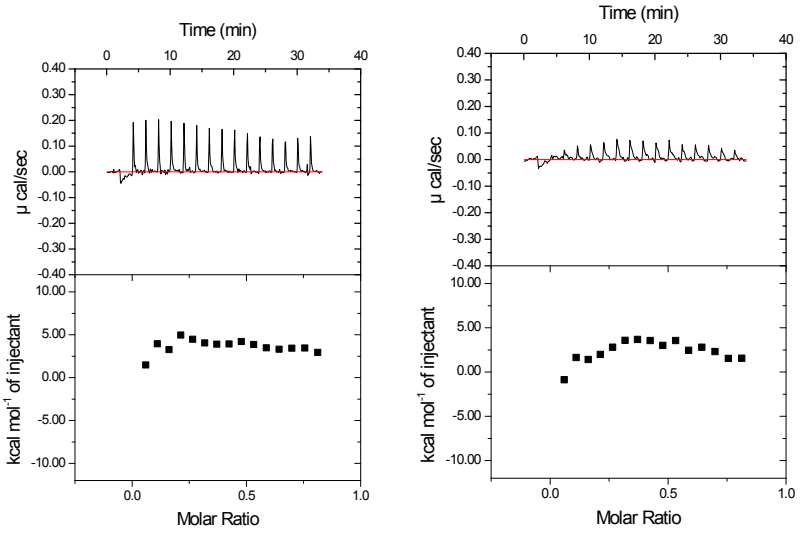

Figure S5 ITC of idsA and ITC of hMsrA

Also, I used a protein MDM2[5] , with GST tag for further control.

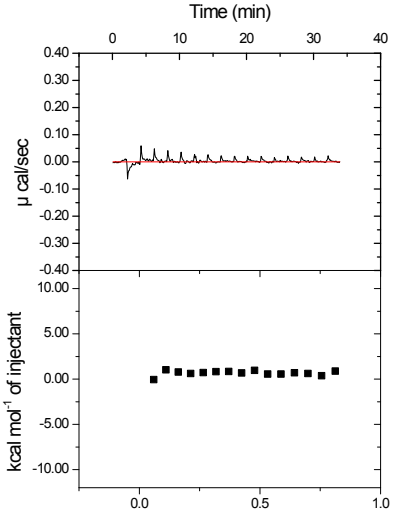

Figure S6 ITC of MDM2-GST

S5 The analysis of binding site of CadR towards cadmium ions

We first mutated the three cysteine residues to serine residues. With site-directed mutations we got three mutated plasmids of C-77-S, C-112-S and C-119-S (Table S2 for mutation primers, Figure S7 for the SDS-PAGE of mutated proteins). We purified the three mutated proteins with similar protocols stated in S1 and performed the ITC measurements with them. Figure S8, S9 and S10 show the results of their binding.

Table S2 Primers for the construction of mutated constructs

Primers	Sequences
P77-F (forward)	TCCTGACGATTCCTCCGGTTCTGTAAACGCT
P77-R (reverse)	GAGGAATCGTCAGGAGAGTCACGCAGGC
P112-F (forward)	TTGAACTGCGCCGTCGTTCTAATGCGCAGGG T
P112-R(reverse)	GAACGACGGCGCAGTTCAACCAGCTCGTCC
P119-F (forward)	AGGGTGCGGAATCCGCTATTCTGCAGCAA
P119-R(reverse)	GATTCCGCACCCTGCGCATTACAACGACG

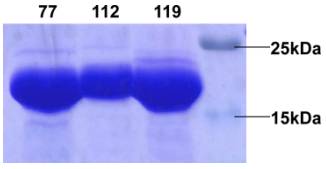

Figure S7 SDS-PAGE for the mutated protein

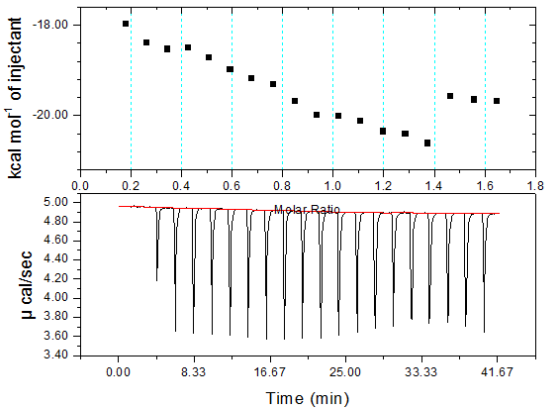

Figure S8 ITC of C-77-S

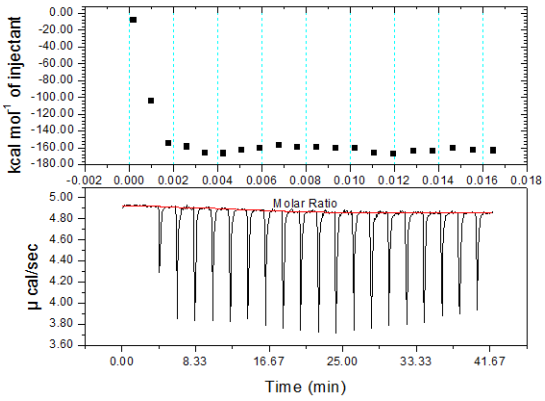

Figure S9 ITC of C-112-S

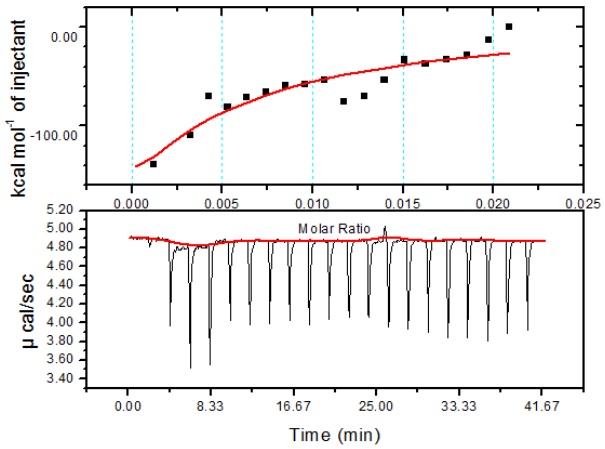

Figure S10 ITC of C-119-S

S6 Construction of the surface displayed bacteria

The plasmid of pBAD-OmpA was graciously provided by Prof. Zhao's lab at Peking University[2]. Through sub-cloning, we inserted the sequence encoding CadR, TC21, TC68 and TC68/21 into the digested pBAD-OmpA chain using the PCR primers of P1, P3, P5, P6 (Table S1, S3) with digestion sites as KpnI and XhoI. As a control, we also inserted the sequence into the digested pBAD chain using primers of P1, P2, P3, P4 with digestion sites as NcoI and XhoI.

Table S3 Primers for the construction of surface displayed plasmid and its controls

Primers	Sequences
P5 (forward)	CGGGGTACCATGAAGATTGGCGAACT
P6 (forward)	CGGGGTACCCTGCGTGACTCTCCT

S7 Analysis of SDS-PAGE and Western blotting

The fusion proteins were expressed in *E. coli* strain DH10B. Cells were grown in LB medium containing ampicillin (100 μg mL^{-1}) for overnight at 37℃. After 1 : 100 dilution in LB medium containing ampicillin (100 μg mL^{-1}), the culture was grown to an OD_{600}=0.6~0.8. Protein expression was induced by the addition of arabinose to the final concentration of 0.5% and incubated at 30 ℃ overnight. Surface displayed *E. coli* cells were harvested by centrifugation (5000 rpm, 8 min) and re-suspended in PBS buffer (pH 7.4). After sonication, the rudimental bacteria was removed by low-speed centrifugation (6000 g, 10 min, 4 ℃). Then the cell membrane fraction was collected by a further high-speed centrifugation (14000 rpm, 30 min, 4 ℃). For Western blotting analysis, the proteins were transferred onto polyvinylidene difluoride membranes (Bio-rad) at 250 mA, 4 ℃ for 2 hours. After blocking at room temperature for 1 hour in skim milk buffer (5% skim milk in 1 x TBST), the membranes were treated with 1 : 1000 dilutions of monoclonal anti FLAG-tag (Santa Cruz) as primary antibody for overnight at 4 ℃ followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Santa Cruz) at room temperature for 2 hours. Antibodies were detected with ECL reagents (Pierce).

S8 Immunofluorescence microscopy analysis

To confirm the display of proteins on the E. coli surface, immunofluorescence labeling of cells was performed using the FLAG-tag as described below. Surface displayed cells were fixed with 4% (v/v) formaldehyde/PBS (pH7.4) for 1.5 h and incubated in PBS (pH 7.4) containing 1% (w/v) horse blood serum for 30 min prior to immunostaining. Mouse monoclonal anti FLAG-tag antibody (Santa Cruz) was used as the primary antibody at a dilution rate of 1 : 200. A mixture of cells and the antibody was incubated at room temperature for 4 hours. The cells were then washed with PBS (pH7.4) three times. FITC-conjugated goat anti-mouse IgG antibody (Santa Cruz) diluted at a 1 : 300 was then reacted with the cells using a rotator for 1.5 hours at room temperature. After washing with PBS (pH 7.4), the cells were suspended in appropriate volume of PBS (pH 7.4) and observed by fluorescent microscopy (Olympus, GX51).

S9 Bioadsorption of cadmium and other metal ions using the surface displayed cells

60 μM different metal ions were added in LB medium after 2 hours' induction. Cells were then harvested from LB medium by centrifugation (5000 rpm, 10 min) and washed by water for at least three times. The cells were lypophilized for dry weight and digested by nitric acid with microwave followed by Inductively Coupled Plasma mass spectrometry (ICP-MS, Thermo, Bremen, Germany).

S10 Plate sensitivity assays of surface displayed *E. coli*

The *E. coli* (DH10B) strains containing CadR-OmpA, TC21-OmpA, TC68-OmpA plasmids and its negative control without OmpA motif were grown in LB medium as in S7 overnight. The numbers of bacterial cells from each strain of bacterial samples were normalized to approximately 5 x 10^8 CFU mL^{-1} with fresh LB and followed by nine 10-fold serial dilutions. Then 5 μL of each cell dilutes (from 10^5 to 10^0 dilution) were spotted onto the LB agar plates containing ampicillin (50 μg mL^{-1}), arabinose (0.5%) and cadmium ions at different concentrations. In the suppression experiments, the arabinose induced bacterial samples were mixed with melting solid medium (LB, 1.2% Agar, 0.5% arabinose, 37 ℃) to approximately 5 x 10^8 CFU mL^{-1}. Then the mixtures were uniformly poured over 12-well plates. After the medium solidified, 5 μL of Cd^{2+} with different concentrations were spotted onto the agar plates. The plates were further incubated at 37 ℃ for about 18h.

References

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