Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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An Exceptionally Selective Lead(II)-Regulatory Protein from *Ralstonia Metallidurans*: Development of A Fluorescent Lead(II) Probe

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Chemicals and Buffers

All buffers are prepared using metal-free reagents and water that has been purified by a MilliQ purificaition system (Millipore). Lead stock solution is purchased from Aldrich as atomic absorption standards (1005 μg/ml Pb in 0.9 wt % HNO_3). All the other metals were purchased from Aldrich, Fisher or STREM with >99.9% purity.

Construction, Expression and Purification of *Ralstonia metallidurans* PbrR691. The PbrR691 gene, including its stop codon, was cloned into pET30b between the *Nde*I and *BamH*I sites and transformed with *E. coli* BL21(DE3) onto LB-agar plates containing kanamycin (50 μM). Overnight pre-cultures were grown aerobically at 37 °C and shaken at 200 rpm, which was then used to inoculate 1 L of LB medium and kanamycin (50 μM). The cells were grown until the OD_{600} was 0.6. IPTG (1 mM) was added and the cells were grown overnight (~10 h) at room temperature. The cells were harvested by centrifugation and stored at -80 °C. All subsequent steps were performed at 4 °C. The cell pellet was resuspended in 30 ml of lysis buffer (10 mM Tris [pH 7.34], 300 mM NaCl, 10 mM 2-mercaptoethanol (BME), 10% glycerol), disintegrated by sonication, and centrifuged at 12000 rpm for 20 minutes. The protein in the supernatant was purified by Heparin affinity column (Hiprep 16/10 Heparin FF, Amersham Bioscience) with a 200 ml linear gradient from 0.0 to 1.0 M NaCl in TRIS buffer (10 mM Tris [pH 7.34], 5mM BME). PbrR691 protein was eluted with 0.3–0.4 M NaCl. Peak fractions were pooled and concentrated before applied to the Superdex-200 Gel filtration column with a running condition of 500 mM $NaNO_3$ in TRIS buffer for further purification. The purified PbrR691 fractions were concentrated to ~2 ml and were >90 % pure as estimated by a 12 % SDS-PAGE gel.

Synthetic Oligonucleotides. Oligodeoxynucleotides were synthesized on an Applied Biosystems 392 DNA synthesizer. The pyrrolo-C-containing oligonucleotides were prepared by incorporation of Pyrrolo-C-CE phosphoramidite (Glen Research) at the modified positions during solid-phase synthesis, followed by deprotection and purification by denaturing polyacrylamide gel electrophoresis. Concentrations of the oligonucleotides were estimated by UV at 260 nm. The 2-Aminopurine-based (Glen Research) oligonucleotides were synthesized in the same way.

Fluorescent Measurements. The fluorescent measurements were performed at 15 °C with the use of a FluoroMax-3 (JOBIN YVON Inc.).

The Job's Method. A series of solution are prepared such that the sum of total metal ion (Pb^{2+}) and ligand (PbrR691 dimer) molar concentrations is kept constant:

$$[\text{Metal}]_{\text{total}} + [\text{L}]_{\text{total}} = \text{Constant}$$

If the absorbance of a solution at a given wavelength is plotted against mole fractions of metal or ligand, the maximum absorbance yields a value for x_{max}, which corresponds to the stoichiometry of the species formed in solution. Thus, an x_{max} value of 0.5 would correspond to the formation of a 1:1 complex. The experiment was carried out under conditions that are identical to those of fluorescent experiments.

Isothermal Titration Calorimetry. The ITC experiment was conducted with the VP-ITC instrument (MicroCal LLC. Northhampton, MA). Both fluorescent and ITC measurements were done at the Biophysical Core Facility at the University of Chicago.

Metal Content Analysis By using a 10 K cutoff membrane, 2 ml of PbrR691 protein (50 μM) was dialyzed against 2 L of lead(II) incubating buffer [500 μM $Pb(NO_3)_2$, 100 mM $NaNO_3$, 20 mM Tris-HNO_3, 5% glycerol, pH 7.0] for 2 hours. The lead(II)-loaded protein was then dialyzed with 2 L of washing buffer (100 mM $NaNO_3$, 20 mM Tris-HNO_3, 5% glycerol, pH 7.0) for 2 hours. This step was repeated three times to completely get rid of

the non-specific bound Pb^{2+} in the sample. For EDTA washed sample, the sample was dialyzed against EDTA buffer (100 μM EDTA, 100 mM $NaNO_3$, 20 mM Tris-HNO_3, 5% glycerol, pH 7.0) for 2 hours. These samples were submitted to the STAT Analysis Facility of Illinois for ICP-MS measurements.

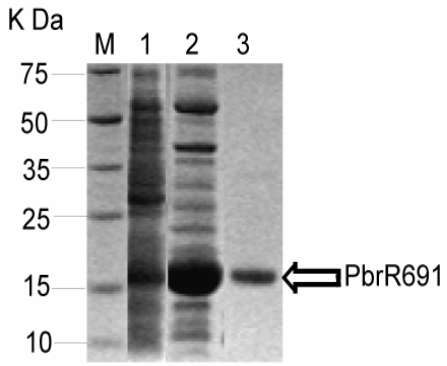

Figure S1. SDS-PAGE gel of the *Ralstonia metallidurans* PbrR691 protein expressed with *E. coli*. Lanes: lane M, molecular mass marker; lane 1, induced cell extract; lane 2, pooled fractions from Heparine column; lane 3, fraction from Superdex-200 (gel-filtration).

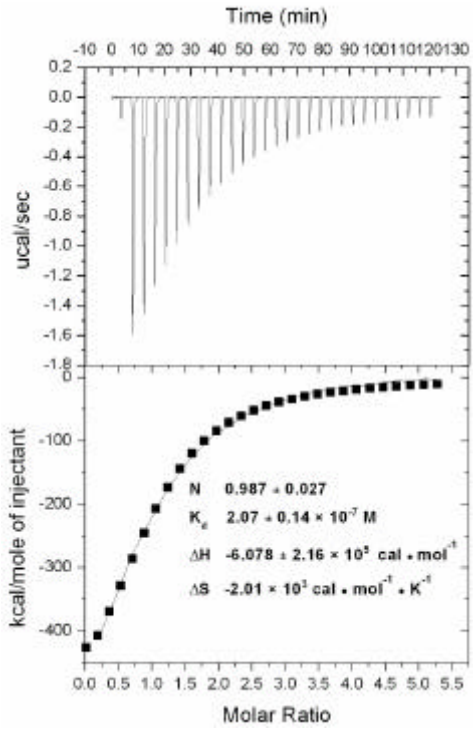

Figure S2. Calorimetric titration of Pb^{2+} binding to PbrR691. *Top,* raw data from titration of 2.0 µM of PbrR691 with 10 µM standard Pb^{2+} solution. *Bottom,* plot of integrated heat versus the Pb^{2+}/ PbrR691(dimer) ratio. The ITC experiment was conducted at 25 ºC with constant stirring of the solution in the cell at 310 rpm.