Supporting Information

Reagents. Potassium antimonyl tartrate was purchased from Aldrich (Milwaukee, WI). Sodium-*m*-arsenite, sodium arsenate, potassium phosphate (monobasic), ampicillin, sodium chloride, anti-FLAG M2-agarose affinity gel, sodium dodecyl sulphate (SDS), sodium phosphate, isopropyl β-D-1-thiogalactopyranoside (IPTG), dimethyl sulfoxide (DMSO), and the protease inhibitor cocktail were obtained from Sigma (St. Louis, MO). 2-amino-2-hydroxymethyl-propane-1,3-diol hydrochloride (Tris-HCl), sodium chloride, tris(2-carboxyethyl)phosphine and ethylenediaminetetraacetic acid (EDTA) were purchased from Fisher Scientific (Fair Lawn, NJ). OH-PCB reference standards for the compounds listed in table 3.1 were purchased from AccuStandard (New Haven, CT). Urea, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), and glycerol were purchased from BDH/VWR (Bridgeport, NJ). Maltose was purchased from Acros (Pittsburg, PA). QIAquick gel purification kit and QIAprep DNA isolation kit were purchased from Qiagen (Valencia, CA). All restriction endonucleases, amylose resin and agarose were from New England Biolabs (Beverly, MA). *PfuTurbo* Hotstart DNA polymerase and T4 DNA ligase were from Stratagene (La Jolla, CA). Luria Bertani (LB) broth and agar were from Difco (Detroit, MI). Potassium chloride was obtained from J. T. Baker (Phillipsburg, NJ). Ni-NTA purification kit was obtained from BD Biosciences (Palo Alto, CA). 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulphonic acid (IAEDANS) was purchased from Molecular Probes (Eugene, OR). Sodium phosphate dibasic heptahydrate was purchased from Mallinckrodt (Paris, KY). Enhanced green fluorescent protein was purchased from Clontech (Palo Alto, CA). Bovine serum albumin (BSA) was from Pierce (Rockford, IL). Ampligase DNA ligase and T4 polynucleotide kinase were from Epicentre (Madison, WI). The PRO Bacterial Expression System, including plasmid pPROTet.E133, bacterial strain DHSaPRO, and TALON Metal Affinity chromatography kit was

from CLONTECH Laboratories (Palo Alto, CA). 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM), acrylodan, and tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Molecular Probes (Eugene, OR). Tris(hydroxymethyl)aminomethane (Tris), nucleotides, and all other reagents were purchased from Sigma (St. Louis, MO). For CRP studies all restriction endonucleases, T4 DNA ligase, *taq* DNA polymerase, Luria Bertani (LB) Broth, LB agar, DNA mass ladder were purchased from Gibco-BRL (Gaithersburg, MD). All oligonucleotides (primers) employed for PCR were synthesized by Operon Technologies (Alameda, CA) and are shown in Table 1. *Pfu* polymerase (Stratagene, La Jolla, CA) was used in all mutagenic PCR reactions. All chemicals were reagent grade or better and were used as received. All aqueous solutions were prepared with 18-MΩ deionized reverse-osmosis water obtained by a Milli-Q water purification system (Millipore, Bedford, MA). Arsenite solutions were prepared fresh daily using 100 mM sodium bicarbonate.

Bacterial strains and plasmids. The expression vector pFLAG-MAC was purchased from Sigma (St. Louis, MO). The pEGFP vector was obtained from Clontech Laboratories (Palo Alto, CA). One Shot® TOP10 competent cells were purchased from Invitrogen (Carlsbad, CA) and bacterial strain *E. coli* DH5α was from Stratagene (La Jolla, CA). Expression vector pRSET cloning kit was obtained from Invitrogen, Inc. (Carlsbad, CA). Expression vector pMal-p4E was purchased from New England Biolabs (Ipswich, MA).

Media and growth conditions. Cells were grown with shaking (250 rpm) at 37 °C in LB medium unless otherwise noted. Ampicillin (75 μg/mL) was added as required. Bacterial colonies were grown on LB agar plates, containing the corresponding antibiotic, at 37 °C.

Apparatus. Polymerase chain reactions (PCR) were performed on a Perkin Elmer Gene Amp PCR system 2400 (Norwalk, CT). Centrifugation was performed in a Beckman J2-MI centrifuge (Palo Alto, CA) and a Micro Speed Fuge SFR 11K (Savant Instruments, Farmingdale, NY). Lysis of the bacterial cells was achieved with a Sonic Dismembrator Model 550 (Fisher Scientific). Protein expression and purification was examined by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques. Fluorescence was monitored with a POLARstar Optima microplate based multi-detection reader from BMG LABTECH (Durham, NC) equipped with a high energy xenon flash-lamp. Disposable Fluorolux™ Flat Bottom 96-well microplates, used for all fluorescence experiments, were purchased from DYNEX Technologies (Chantilly, VA). All experiments were conducted at room temperature unless otherwise specified. All fluorescence intensities reported are background subtracted and are an average of a minimum of three replicates.

Construction of plasmid pSD2003 for expression of ArsR. All molecular biology procedures were performed following standard protocols.[1] The *arsR* gene sequence was amplified by the polymerase chain reaction (PCR) from pSD601.[2] The *Sph*I and *BamH*I restriction sites were inserted at the 5' and 3' end of the gene, respectively, during amplification. The following oligonucleotides were used for the PCR: GGTGGTGCATGCATGTTGCAACTAACACCACTT (1); GGTGGTTGGATCCATGCAGACAGCCTTACTGCT (2). The underlined bases encode for the restriction enzymes cleavage sites, *Sph*I in 1 and *BamH*I in 2. The PCR was carried out with the thermocycler parameters of 95 °C for 1 min, 64 °C for 1 min, and 72 °C for 2 min, for a total of 30 cycles. The PCR amplified fragment was isolated on an agarose gel and purified using Qiagen Gel Purification Kit. The *arsR* gene was digested with *Sph*I and *Bam*HI and then

cloned into the pEGFP vector. The gene sequence encoding for the whole ArsR-EGFP fusion protein was amplified, inserting the *Xho*I restriction site in the 5' end and the *Bgl*II restriction site in the 3' end. The following oligonucleotides were used for the PCR: GGTGGTCTCGAGATGTTGCAACTAACACCACTT (3); ACAACAACAACAACAGATCTTTACTTGTACAGCTCG (4). The underlined bases encode for the restriction endonucleases cleavage sites, *Xho*I in 3 and *Bgl*II in 4. The PCR was carried out with the thermocycler parameters of 95 °C for 1 min, 65 °C for 1 min, and 72 °C for 2 min, for a total of 30 cycles. The PCR product was isolated on a 1% agarose gel and purified using QIAgen Gel Extraction Kit. The *arsR-egfp* gene was digested with *Xho*I and *Bam*HI restriction endonucleases and then cloned into the pFLAG-MAC vector to yield plasmid pSD2003. DNA sequencing was carried out to verify the fusion construct. The new plasmid contains the amp^R gene that confers resistance to ampicillin, the FLAG affinity tag, the *arsR* gene, and the *egfp* gene.

Transformation of *E. coli* strain DH5α. The plasmid pSD2003 was transformed into *E. coli* DH5α maximum efficiency competent cells using conventional protocols.[1] Single colonies of the transformed bacteria, isolated on Luria Bertani agar plates containing ampicillin, were grown in Luria Bertani broth for plasmid isolation. Restriction digestion analysis with *Xho*I and *Bgl*II enzymes followed by a 1% agarose gel electrophoresis was employed to confirm the presence of plasmid pSD2003. A glycerol stock from a single positive colony was prepared and stored at -80 °C until further use.

Preparation and isolation of the ArsR-EGFP fusion protein. The bacteria were grown in 250 mL of LB broth containing 50 μg/mL ampicillin at 37 °C until it reached an absorbance of 0.5 -

0.6 at 600 nm. The culture was induced with 1.0 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h with shaking at 30 °C. The cells were then harvested by centrifuging at 4 °C for 30 min at 10000 rpm. Media was discarded and cells were resuspended in TBS buffer (50 mM Tris HCl, 150 mM NaCl, pH 7.4). Sonication was performed on the cells to release the expressed protein. A protease inhibitor cocktail was added prior to sonication to prevent proteolysis.

Purification of the ArsR-EGFP fusion protein. After sonication, the crude extract was separated from the debris by centrifugation at 4 °C for 20 min at 8000 rpm. The crude extract was purified using an anti-FLAG M2 affinity column according to the manufacturer's instructions. The purity of the eluted fractions was checked by using SDS-PAGE employing 12.5% polyacrylamide PhastGels (Pharmacia Biotech, Uppsala, Sweden). The gels were developed by following the silver staining method. The purified protein was dialyzed three times against 100 mM sodium bicarbonate, pH 8.0 at 4 °C. The protein concentration was obtained using the BioRad Quick Start Bradford Protein Assay (Hercules, CA) with BSA as the standard. The ArsR-EGFP protein was stored at 4 °C for up to 8 months. All protein dilutions were performed using 100 mM sodium bicarbonate containing 10 mM dithiothreitol (DTT).

Calibration curve for ArsR-EGFP fusion protein. A calibration curve was constructed by serial dilutions of the stock protein in 100 mM sodium bicarbonate containing 10 mM DTT. A volume of 100 μL of the protein was loaded into a 96-well microtiter plate, in quadruplicates. The fluorescence intensity was measured in the POLARstar Optima microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 510 nm.

Dose-response curve of ArsR for arsenite. Fluorescence emission was obtained for a fixed amount of EGFP protein both in the presence of sodium-*m*-arsenite and in the absence of it. Equal parts of 5 x 10^{-7} M of the EGFP protein and 1 x 10^{-4} M sodium-*m*-arsenite were mixed in a test tube and transferred to a microtiter plate for measurement. The EGFP blank (no arsenite) was prepared with 100 mM sodium bicarbonate buffer containing 10 mM DTT instead. Measurements were performed in quadruplicates at an λ_{ex} = 485 nm and an λ_{em} = 510 nm. To verify that arsenite does not affect the intrinsic fluorescence of wild-type EGFP in solution, 1 × 10^{-4} M of arsenite was mixed with EGFP, after which the fluorescence intensity of the protein was measured. The obtained results show absence of change in the fluorescence of EGFP, indicating that arsenite does not interact with it (data not shown).

Selectivity studies of ArsR. Dose-response curves were generated for the different anions in a similar manner to the previously described experiments. Different concentrations of the different anions were prepared by serial dilution from a 1 x 10^{-3} M stock in 100 mM sodium bicarbonate containing 10 mM DTT. Equal parts of 5 x 10^{-7} M ArsR-EGFP fusion protein was mixed with the corresponding concentration of the inducer in a test tube. Four 200 μL aliquots were transferred to separate wells of a 96-well microtiter plate followed by immediate reading. The fluorescence intensity was measured at an excitation wavelength of 485 nm and an emission wavelength of 510 nm.

Construction of pHbpR-A-MBP plasmid. For all cloning and culturing steps, cells were grown in LB supplemented for selection as described below. Cell cultures were grown at 37 °C, with shaking at 250 rpm. Initially, the gene for the A-domain of HbpR (*hbpR-A*) was amplified by PCR using the procedure provided by the supplier of the Pfu polymerase. The primers used were HbpR-A-BamHI (5'-GGTGGTGGATCCATGAAATCAAATAAAAATAATAGC-3') and HbpR-A-EcoRI (5'-GGTGGTGGATTCCGCCCACATTTCGGCGGGCTTCGC-3'). The template DNA used was pHYBP109 and was obtained from our previous work.[3] PCR reaction conditions were carried out as described by the supplier's protocol. Briefly, the reaction mixture contained final concentrations of 2.5 μM for each primer and 25 ng/μL of template DNA. The temperature program used for amplification consisted of an initial denaturation step of 95 °C for 2 min, followed by 30 cycles of 95 °C for 1 min, 65 °C for 2 min, and 72 °C for 2 min and a final elongation step of 72 °C for 5 min.

The resulting PCR product was purified by agarose gel electrophoresis, and the corresponding DNA band (617 bp) was excised from the gel and purified using the QIAquick Gel Extraction Kit. The purified PCR product, in tandem with the vector $pRSET_A$, was then digested with the restriction enzymes BamHI and EcoRI following the supplier's protocol. The digested fragments were purified by agarose gel electrophoresis, co-purified using the QIAquick Gel Extraction Kit, and ligated using T4 ligase following the supplier's protocol. The resulting ligation product was transformed into TOP10F' cells, which were grown on LB agar with ampicillin (100 μg/mL) and tetracycline (15 μg/mL) for selection.

Transformation colonies were grown overnight in LB media with ampicillin and tetracycline. Plasmid from these cultures was isolated using the QIAprep kit, digested with BamHI and EcoRI, and analyzed by agarose gel electrophoresis for the correct plasmid, $pRSET_A$-

-hbpR-A. The purified plasmid possessing bands of the correct size was sequenced for confirmation. For transformants containing plasmids with the correct sequence, glycerol stocks were prepared and stored at -80 °C.

To prepare a plasmid for expression of the A-domain of HbpR (HbpR-A) as a fusion to maltose binding protein (MBP), the plasmids pRSET$_A$-hbpR-A and pMal-p4E were digested with BamHI and HindIII. The resulting products were purified by agarose gel electrophoresis, co-isolated from the gel using the QIAquick Gel Extraction Kit and ligated using T4 ligase as above. The resulting product was transformed into TOP10F' cells and grown on LB agar with ampicillin and tetracycline for selection. As above, the transformants were analyzed for the correct plasmid, pMal-p4E-hbpR-A, and candidates were sent for confirmation by DNA sequencing. Transformants containing the correct plasmid were preserved as glycerol stocks at -80 °C.

Expression and Purification of HbpR-A-MBP. For expression of HbpR-A-MBP, a 2.0 mL culture of TOP10F' cells containing pMal-p4E-hbpR-A was grown overnight in LB media supplemented with ampicillin (100 μg/mL) and tetracycline (15 μg/mL). Two 500 mL expression cultures in LB media were inoculated the following day using the overnight cultures and grown to an OD_{600} of 0.4-0.5, and protein expression was induced by the addition of 1 mM IPTG. Expression was carried out overnight at 37 °C. Following expression, the cultures were centrifuged to a pellet (10,000 x g, 20 min, 4 °C), and the supernatant was discarded. The isolated cell pellet was used to prepare purified HbpR-A-MBP fusion protein.

The cell pellet was resuspended in lysis buffer (20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, pH 7.4) and placed on ice. Cells were lysed on ice by pulsed sonication (10 s on, 10 s

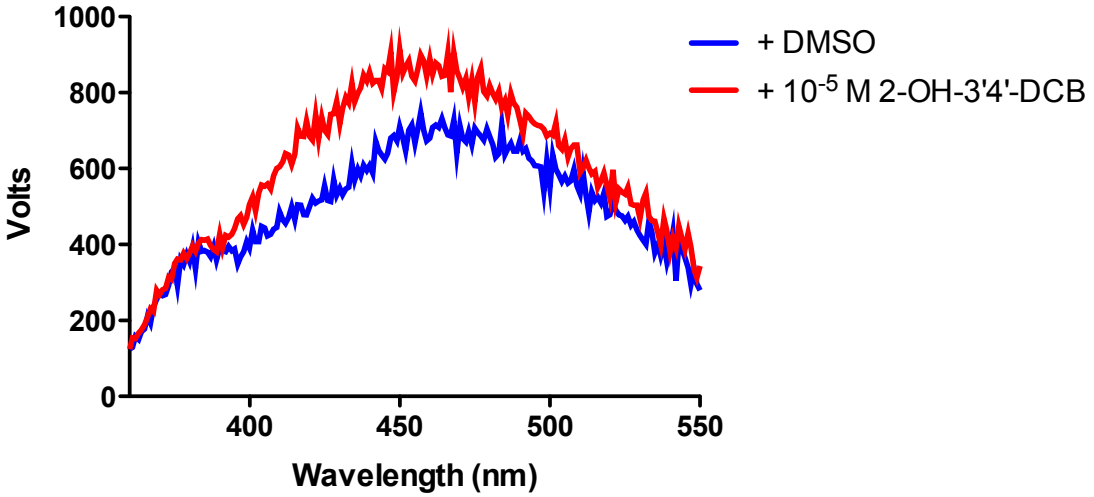
off, 10 min total), and the resulting suspension was immediately centrifuged (10,000 x g, 20 min, 4 °C) to separate the protein-containing supernatant from the cellular debris. The supernatant was added to a gravity-flow column containing 2 mL of amylose resin equilibrated according to the supplier's instructions, and the flow-through fraction was collected. The column was washed with 16 mL of lysis buffer, and the fraction was collected. Finally, the fusion protein was eluted from the column in 9 aliquots of 0.5 mL of lysis buffer containing an increasing concentration of maltose (1 mM for fraction 1, 2 mM for fraction 2, 5 mM for fraction 3, and 10 mM for fractions 4-9). All purification fractions were analyzed by SDS-PAGE electrophoresis to determine the purity of the expressed protein. Fractions containing significant impurities were discarded, and purified protein was combined and dialyzed in 1 L of labeling buffer (20 mM HEPES, pH 7.2) overnight at 4 °C.

Labeling HbpR-A-MBP with IAEDANS. The thiol-reactive, environmentally-sensitive fluorophore IAEDANS was used for protein labeling. The X-ray crystal structure of the HbpR-A protein is not known yet. There are six cysteines in HbpR-A at position 45, 161, 168, 175, 187, and 195, where as MBP has no cysteines. Most likely all of these cysteines will be conjugated with IAEDANS. However, without the knowledge of the structure of the protein we can not predict which cysteine may experience the change in confirmation upon ligand binding to yield the change in the fluorescence intensity of the fluorophore.

Protein modification was carried out according to the Invitrogen Molecular Probes handbook for thiol-reactive probes. Briefly, the protein was denatured and possible disulfide bonds were reduced by reacting 3.0 mL of the protein in labeling buffer containing 1 mM TCEP and 6 M urea for 2 h at 4 °C with stirring. Following this reaction, 10 μL of a 2.3 x 10^{-2} M stock of

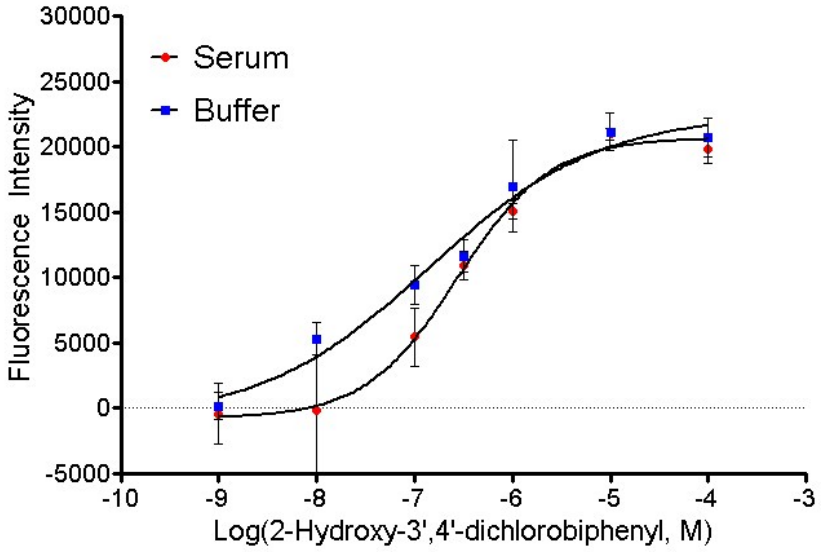
IAEDANS in DMSO was added to the same vial, resulting in a 10-fold molar excess of IAEDANS with respect to the number of cysteines present. This mixture was allowed to react in an amber glass vial in the dark overnight at 4 °C with stirring. Following the labeling reaction, the solution was dialyzed extensively at 4 °C to remove any free IAEDANS from solution and to gradually remove urea and allow protein refolding. The reaction solution was placed inside of a 10,000 molecular weight cut-off, 10 mL dialysis cassette and dialyzed in an initial dialysis buffer (20 mM HEPES, 2 M urea, 0.2 mM EDTA, pH 7.4) for 7 h at 4 °C with mixing in the dark. Following this, consecutive buffer changes were performed by removing half of the dialysis buffer and replacing with dialysis buffer with no urea, halving the previous urea concentration. Each dialysis step was carried out for at least 4 h. This was repeated 5 times, and then a final dialysis was carried out replacing the entire volume of dialysis buffer. The thoroughly dialyzed protein solution was then removed from the dialysis cassette, placed in an amber glass vial, and stored at 4 °C.

Fluorescence Emission Spectra of HbpR-A-MBP-IAEDANS. HbpR-A-MBP labeled with IAEDANS was used to generate fluorescence emission spectra by adding 100 μL of protein in 20 mM HEPES buffer (pH 7.4) with 10% DMSO (v/v) to a quartz microcuvette and measuring fluorescence with a PTI fluorometer (Edison, NJ). This assay was performed in the presence and absence of OH-PCBs.



Fluorescence Assays of OH-PCBs in Buffer. For fluorescence assays in buffer, stock solutions of selected OH-PCBs were prepared at a concentration of 1 x 10^{-2} M in DMSO. From these stock solutions, standards were prepared by serial dilution in DMSO resulting in a range of concentrations from 1 x 10^{-2} M to 1 x 10^{-8} M. Using these DMSO standards, assay standards were prepared by diluting each DMSO standard 1:10 into assay buffer (10 mM HEPES, pH 7.5), resulting in assay standards with a range of concentrations from 1 x 10^{-3} M to 1 x 10^{-9} M. An assay blank was also prepared by preparing a 10% (v/v) solution of DMSO in assay buffer. To perform assays of the compounds, 180 µL of the HbpR-A-MBP-IAEDANS solution at a concentration of 1 x 10^{-7} M was mixed with 20 µL of assay standards, as well as a blank sample, in triplicate. The fluorescence was measured using a BMG Labtech Polarstar Optima microtiter plate fluorimeter. Data were plotted using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) with a three-parameter sigmoidal curve fitting equation, and apparent binding constants were derived from this equation. Detection limits were determined as the concentration tested that produced a signal intensity that is at least 3 standard deviations above the blank signal.

Detection of Model Compound in Human Serum. 2-Hydroxy-3′,4′-dichlorobiphenyl was used to evaluate the assay in human serum. This compound was prepared in a concentration range of 1 x10^{-4} to 1x 10^{-9} M in serum and buffer. The assay was performed by following the procedure described above. The fluorescence was measured using a BMG Labtech Polarstar Optima microtiter plate fluorimeter. Data were plotted using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).



PCR and Cloning of CRP. The native CRP gene was amplified using PCR from the genomic DNA of *E. coli* based on the sequence of Aiba *et al*.[4] The PCR mix contained 250 µM of each dNTP, 25 pmol of each primer (CRPF-Hind3 and CRPR-BamHI, see Table 1), 2.5 units of taq polymerase, and 1 µL of a saturated suspension of E. *coli* JM109 as the template in 50 µL total volume. The polymerase was added to the other reagents after a 5 min incubation of the PCR mix at 94 °C. The cycling parameters were 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 90s for a total of 35 cycles. The resulting product was confirmed by gel electrophoresis on a 1% agarose gel. The 600-bp band was excised from the gel and purified with the QIAgen gel

extraction kit. The purified PCR fragment was cut with the restriction enzymes *Hind*lll and *BamH*I and cloned into the plasmid pPROTet.E133. The resulting plasmid, pCRPTet, was transformed into the bacterial strain DH5αPRO. The plasmid was then sequenced to confirm its identity, the fact that it was in-frame, and that it contained all three native cysteine residues.

PCR-Based Site-Directed Mutagenesis. The technique of combined chain reaction (CCR) was used to produce the CRP mutants.[5,6] The plasmid pCRPTet was used as the template for mutagenesis. A typical mutagenesis reaction would contain the template, two mutagenic oligonucleotides, and the forward and reverse primers (CRPF-Hind3 and CRPR-BamHI) (Table 1). The mutagenic oligos were first forward phosphorylated using T4 polynucleotide kinase and heat inactivated before being used in CCR. The CCR mix would contain approximately 50 ng pCRPTet, 100 pmol of each phosphorylated mutageic oligo, 5 units Ampligase DNA ligase, 2.5 units pfu polymerase, 0.5 mM NAD, and 250 μM of each dNTP in a volume of 100 μL. The CCR reaction was carried out by first incubating for 5 min at 94 °C, then cycling 35 times through the sequence: 94 °C for 45 s, 55 °C for 1 min, 72 °C for 5 min. A final incubation of 7 min at 72 °C was used to fully elongate any pieces which were left short. The CCR reaction was then purified using a QIAgen PCR purification kit before electrophoresis on a 1% agarose gel. The 600-bp band was excised and cloned into the pPROTet.E133 plasmid, transformed into DH5αPRO, and sequenced as described above.

Expression and Purification of Native and Mutant CRP. Cloning of the CRP gene into the plasmid pPROTet.E133 allowed for the inducible expression of CRP by adding anhydrotetracycline (aTe) to the media. In addition, the protein is expressed with an N-terminal

6xHis tag so that the protein could be purified using metal affinity chromatography. Bacteria containing the CRP genes in plasmid pPROTet.E133 were grown overnight in 6 mL of TB broth supplemented with 34 μg/mL chloramphenicol and 50 μg/mL spectinomycin at 37 °C with shaking (250 rpm). The following morning the saturated overnight culture was used to inoculate 333 mL of LB/Cm/spec and the culture was grown for 4 h (37 °C w/shaking) to an OD_{600} of ~0.5. Anhydrotetracycline was then added to the media to give a final concentration of 100 ng/mL. Incubation was continued for another 3 h. The culture was then centrifuged at 6000 rpm at 25 °C for 5 min and the media discarded. The bacterial cell pellet was resuspended in 10 mL of buffer (50 mM sodium phosphate, 300 mM NaCI, pH 7.0, with 0.75 mg/mL lysozyme) and allowed to sit at room temperature for 20 min to lyse the cells. After a 5 min incubation with 0.5 mg/mL DNase I to thin the solution, the cell extract was centrifuged at 20,000 x g for 20 min to pellet the insoluble material. The clarified supernatant was then applied to the TALON column according to the manufacturer's instructions for combined batch/column purification. An extra column wash containing 10 mM imidazole was used before elution of the bound protein to remove other proteins adhering to the column. Purity of CRP was confirmed by running on a 12.5% polyacrylamide PhastGel (Pharmacia Biotech, Uppsala, Sweden) followed by silver staining. Protein concentration was determined using the BCA Protein Assay.

Labeling CRP with Cysteine-Selective Fluorophores. Purified CRP (wild type or mutant) was reacted with cysteine-selective fluorophores immediately after purification on the TALON column. The buffer (50 mM sodium phosphate, 300 mM NaCI, 150 mM imidazole, pH 7.0) was not exchanged before reaction. The sample was introduced into a reaction vial and stirred magnetically while a 10-fold molar excess of the reducing agent

TCEP was added. After approximately 1 min of reaction, a 20-fold molar excess of fluorophore (in DMF or DMSO) was added slowly to prevent denaturation of the protein. The reaction was allowed to proceed in the dark at room temperature for 2-3 h. The conjugate was then purified on a polyacrylamide 6000 desalting column using PBS buffer (150 mM NaCI, 20 mM sodium phosphate, 10 mM EDTA, pH 7.0) to elute. The presence of the CRP-fluorophore conjugate was confirmed by SDS-PAGE on a PhastSystem. The number of fluorophore molecules attached to each CRP molecule was determined by measuring the absorbance spectra of the conjugates and determining the concentration of the fluorophore based on the molar absorptivity using ε_{419}=20,000 (acrylodan) and ε_{384}=33,000 (CPM). The ratio was then determined by comparison to the conjugate concentration from a BCA protein assay.

Dose-response Curve for cAMP. A dose-response curve for cAMP was constructed by incubating 100 µL of the CRP-fluorophore conjugate with 100 µL cAMP in 200 µL assay buffer (50 mM Tris-HCI, pH 7.0) in a test tube. The contents were shaken in the dark for 30 min. A sample (200 µL) from each tube was transferred to a 96-well clear-bottom microtiter plate (Cat. # 6321, Dynex Technologies, Chantilly, VA) using appropriate filters for each fluorophore. The total fluorescence of each well was the sum of 10 reading cycles of the plate. The concentrations of CRP-fluorophore conjugates used were: wild- type conjugates, 5.5 x 10^{-7} M; CRPC178-acrylodan, 1.5 x 10^{-7} M.

Selectivity study. Selectivity of the system was tested by performing the assay as described above using different cyclic nucleotides. The most closely related structurally, cGMP, cIMP, and 2'3'-cAMP show some of the largest responses.

Table 1. Primers used in this study for the cloning and mutagenesis of CRP and for the experiments of CRP binding to its consensus sequence.

Name	Sequence	Mutation
CRPF-Hind3	NNN NNN AAG CTT GTG CTT GGC AAA CCG	none
CRPR-BamHI	NNN NNN GGA TCC TTA ACG AGT GCC GTA	none
CRPC18S	TTC TTG TCT CAT TCC CAC ATT CAT AAG TAC	Cys18→Ser
CRPC92S	GCG AAA ACC GCC TCT GAA GTG GCT GAA	Cys92→Ser

References

(1) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*; 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1996.

(2) Feliciano, J.; Liu, Y.; Daunert, S. *Biotechnology and Bioengineering* **2005**, *in press*.

(3) Turner, K.; Xu, S.; Pasini, P.; Deo, S.; Bachas, L.; Daunert, S. *Anal Chem* **2007**, *79*, 5740.

(4) Aiba, H.; Fujimoto, S.; Ozaki, N. *Nucleic Acids Research* **1982**, *10*, 1345.

(5) Bi, W.; Stambrook, P. J. *Nucleic Acids Research* **1997**, *25*, 2949.

(6) Bi, W.; Stambrook, P. J. *Analytical Biochemistry* **1998**, *256*, 137.