Supplemental Methods S1

CfrA-His$_6$ expression and purification and anti-CfrA antibody production

A DNA fragment encompassing the *cfrA* ORF was amplified by PCR from *Synechocystis* genomic DNA, using the oligonucleotides cfrANde and cfrAXho. This fragment was *NdeI-Xho*I digested and cloned into pET24a(+) plasmid digested with the same enzymes (Novagen) to generate pET24-CfrA plasmid. Exponentially growing *E. coli* BL21 cells transformed with pET24-CfrA were treated with 0.5 mM of isopropyl ß-D-1-thiogalactopyranoside for 4 h. For purification of CfrA-His$_6$ protein, cells were collected, resuspended in buffer A (20 mM sodium phosphate, pH 7.4, 0.5 M NaCl, 5 mM imidazole) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and disrupted by sonication. The lysate was centrifuged at 18.000 x *g* for 20 min. CfrA-His$_6$ from the supernatant was purified by Ni-affinity chromatography using HisTrapHP column (GE Healthcare) and following the manufacturer's instructions. Elution was performed with a linear gradient (5-500 mM imidazole) in buffer A. Fractions with CfrA-His$_6$ were pooled, concentrated using Centrifugal Filter Units (Amicon Ultra-15 3 kDa) (Millipore), and subjected to gel filtration chromatography using a Hiload 16/60 Superdex 75 gel filtration column (GE Healthcare) running on an AKTA FPLC system. Fractions containing purified CfrA-His$_6$ protein until electrophoretic homogeneity were pooled, concentrated and quantified in a NanoDrop 1000 spectrophotometer (Thermo scientific) using the extinction coefficient of CfrA-His$_6$ calculated with ExPASy-ProtParam tool.

Anti-CfrA antiserum was obtained according to standard immunization protocols by injecting one milligram of purified CfrA-His$_6$ protein in rabbits.

Supplemental Methods S2

Immunoprecipitation assays

Superparamagnetic beads with recombinant Protein A (Dynabeads Protein A from Invitrogen) were washed twice with Hepes 50 mM pH 7 buffer using a magnetic holder for microtubes. Two volumes of immune or pre-immune serum (100 µl) was added to the beads suspension and the tubes were incubated at 4ºC for 2 h with rotation. Beads were washed with Hepes 50 mM pH 7 buffer and antibodies were crosslinked to beads using BS^3 (Thermo Scientific) following the instructions of the supplier.

Crude cell extracts were obtained from the Pars-cfrA strain after 24 h of treatment with arsenite using glass beads in MES buffer (25 mM Mes pH 6.5, 5 mM $CaCl_2$, 10 mM $MgCl_2$ and 20% (v/v) glycerol) supplemented with Complete Protease Inhibitor Cocktail EDTA-free (Roche). Soluble proteins were recovered after a 25 min centrifugation at

20,000 *g*. A volume of crude extract equivalent to 100 OD_{750} was used for each immunoprecipitation assay. Crude extracts were incubated with antibodies-crosslinked Dynabeads overnight at 4ºC with rotation. Dynabeads were then washed twice with the same buffer and the precipitated material was eluted with standard SDS protein buffer without ß-mercaptoethanol and heating at 50ºC for 10 min.

LC-MS/MS protein identification

Protein samples were precipitated with a TCA:acetone mix. The protein precipitate was resuspended with a 0.2% RapiGest solution (Waters) in 50 mM ammonium bicarbonate. 5 mM DTT was added and incubated for 30 min at 60°C. Then, Iodoacetamide (IAA) was added to a final concentration of 10 mM by incubating 30 min in the dark at room temperature. Trypsin digestion was done at 37°C overnight. Trypsin was quenched with formic acid and samples were injected into the liquid chromatography equipment with tandem mass spectrometry (LC-MS/MS).

The analysis was performed on a triple TOF quadrupole (5600 plus from Sciex) equipped with a nano electrospray source, coupled to a nano HPLC (Eksigent). The software used for the control of the equipment, as well as for the acquisition and processing of data, was Analyst TF 1.7. The peptides were first loaded into a trap column (Acclaim PepMap 100 C18, 5 µm, 100 Å, 100 µm id × 20 mm, Thermo Fisher Scientific) isocratically in 0.1% formic acid/5% acetonitrile (v/v) at a flow of 3 $\mu l \cdot min^{-1}$ for 10 min. Subsequently, they were eluted in a reverse phase analytical column with the "emitter" already incorporated (New Objective PicoFrit column, 75 µm id x 250 mm, packed with Reprosil-PUR 3 µm), using a linear gradient of 5–35% of solvent B for 60 min at a flow of 250 $nl \cdot min^{-1}$. Solvent A was 0.1% formic acid (v/v) and B, acetonitrile with 0.1% formic (v/v). A standard (digest of *E. coli* Beta galactosidase) was used to self-calibrate and control the equipment's sensitivity and chromatographic conditions.

The source voltage was selected at 2600 V and the heater temperature was maintained at 100°C. Gas 1 was used at 15 psi, gas 2 at zero, and curtain gas at 25 psi. For protein identification experiments, the acquisition was carried out with a DDA (Data Dependent Acquisition) method, consisting of a TOF-MS with a sweep window of 400-1250 m/z, acquisition time of 250 ms, followed by 50 MS/MS with a sweep window of 230-1500 m/z, acquisition time of 65 ms and with a cycle time of 3.54 s.

For protein identification, ProteinPilot v5.0.1 software (Sciex) was used, with a Paragon method, with trypsin as enzyme and IAA as alkylating agent of cysteines, using as protein

database the reference proteome of *Synechocystis* in FASTA format from Uniprot.org merged with the Sciex database of contaminants.