SUPPORTING INFORMATION

for

Synthesis and Application of an Environmentally Insensitive Cy3-Based Arsenical Fluorescent Probe to Identify Adaptive Microbial Responses Involving Proximal Dithiol Oxidation

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General Methods: ^{1}H and ^{13}C NMR spectra were recorded using a Varian (Agilent) Inova spectrometer operating at 11.7 tesla (ν_o = 500 MHz for ^{1}H and 125 MHz for ^{13}C). Mass spectra of synthesized compounds were recorded using a Thermo Finnigan LCQ DecaXP Mass Spectrometer. Absorption spectra were recorded using a Nicolet Evolution 300 uv/vis spectrophotometer (Thermoelectron Corp). A FluoroMax-2 fluorometer (HORIBA Jobin Yvon Inc.) was used to measure fluorescence emission spectra. An ISS K2 fluorometer was used to measure fluorescence polarization (excitation: green light laser (532 nm) and appropriate filters for Cy3 detection, Em: 575 nm (50 nm bandwidth)). Reagents were obtained from Sigma-Aldrich, unless otherwise noted, and used without further purification. Calmodulin with four cysteines engineered at positions 6, 7, 10, 11 in helix A was expressed and purified as previously described;[1] calmodulin with a single cysteine inserted at position 110 was expressed and purified as previously described.[2] Thioredoxin (Trx) from *Escherichia coli* (UniProtKB B7UMN6) and bovine serum albumin (UniProtKB P02769) (≥ 98%) were from Sigma-Aldrich.

Synthesis of TRAP_Cy3 and characterization:

Compound 1: The unsymmetrical cyanine dye was synthesized as previously described.[3] After the reaction, the solvent was removed and the solid residue was purified by chromatography on silica gel using methanol in methylene dichloride as eluent (gradient of 0 – 10 %) with an 80 % yield. ^{1}H NMR ($CDCl_3$): δ 1.71 (d, *J* = 3.0 Hz, 12H), 1.93 (m, 2H), 1.98 (m, 2H), 2.10 (m, 2H), 2.18 (m, 2H), 3.09 (t, *J* = 6.5 Hz, 2H), 3.82 (t, *J* = 6.5 Hz, 2H), 4.27(m, 4H), 6.92 (d, *J* = 13.5 Hz, 1H), 7.14-7.41 (m, 9H), 7.68 (m, 2H), 7.79 (m, 2H), 8.38 (t, *J* = 13.5 Hz, 1H). ESI/MS: m/z calcd for $C_{39}H_{44}N_3O_5S^+$ 666.3, found 666.3.

AsCy3: Compound **1** (200 g, 0.3 mmol) was added into trifluoroacetic acid containing mercuric oxide 1.275 g, 6 mmol) at room temperature. After 48 hours with stirring, 4 mL of methanol was added to the mixture and refluxed for 0.5 hour. Solvent was then removed and the residue was dried under high vacuum. The purple solid was suspended in dry N-methylpyrrolidinore (3 mL) with arsenic trichloride (500 μL, 6 mmol), diisopropylethyl amine (151 μL, 1.6 mmol), and catalytic palladium acetate (1 mg). The reaction was stirred at room temperature for 8 hours, at which point 5 mL phosphate buffer (pH 7) and 1, 2- ethanedithiol (262 μL, 6.4 mmol) were added into the reaction mixture. The product was extracted with CH_2Cl_2 (3 × 30 mL), dried over Na_2SO_4, evaporated, and then purified by chromatography on silica gel using methanol in methylene dichloride as eluent (gradient of 0 – 5 %) with an 5 % yield. ^{1}H NMR ($CDCl_3$): δ 1.71 (d, *J* = 3.5 Hz, 12H), 1.90 (m, 2H), 1.97 (m, 2H), 2.07 (m, 2H), 2.15 (m, 2H), 3.05 (t, *J* = 6.5 Hz, 2H), 3.16 (m, 4H), 3.39 (m, 4H), 3.81 (t, *J* = 6.5 Hz, 2H), 4.20 (m, 4H), 6.97 (d, *J* = 13.5 Hz, 1H), 7.12-7.33 (m, 5H), 7.60 (m, 3H), 7.70 (m, 2H), 7.80 (m, 2H), 8.36 (t, *J* = 13.5 Hz, 1H). ESI/MS: m/z calcd for $C_{43}H_{50}As_2N_3O_5S_3^+$ 998.1, found 998.1.

TRAP_Cy3 (AsCy3_2): ^{1}H NMR ($CDCl_3$): δ 1.68 (bs, 12H), 1.93 (m, 2H), 2.00 (m, 2H), 2.06 (m, 2H), 2.15 (m, 2H), 3.06 (t, *J* = 6.5 Hz, 2H), 3.20 (m, 2H), 3.42 (m, 2H), 3.82 (t, *J* = 6.5 Hz,

2H), 4.22 (t, J = 13.5 Hz, 2H), 4.31 (t, J = 10.0 Hz, 2H), 7.02 (d, J = 10.0 Hz, 1H), 7.14-7.34 (m, 6H), 7.59 (s, 1H), 7.68 (m, 3H), 7.80 (m, 2H), 8.37 (t, J = 13.5 Hz, 1H). ^{13}C NMR ($CDCl_3$): 22.6, 24.6, 25.6, 25.9, 27.8, 28.0, 37.5, 42.0, 44.0, 44.6, 48.3, 48.6, 50.4, 105.3, 105.5, 110.5, 110.7, 121.9, 123.0, 123.9, 125.9, 128.8, 131.3, 133.8, 134.0, 140.0, 140.5, 140.7, 141.8, 142.8, 150.9, 168.2, 173.0, 173.6, ESI/MS: m/z calcd for $C_{41}H_{47}AsN_3O_5S_3^+$ 832.2, found 832.2.

AsCy3_3: ^{1}H NMR ($CDCl_3$): δ 1.70 (s, 6H), 1.72(s, 6H) 1.89 (m, 2H), 1.98 (m, 2H), 2.12 (m, 4H), 3.09 (t, J = 6.5 Hz, 2H), 3.18 (m, 2H), 3.42 (m, 2H), 3.82 (t, J = 6.5 Hz, 2H), 4.19 (t, J = 6.0 Hz, 2H), 4.25 (t, J = 6.0 Hz, 2H), 6.85 (d, J = 13.5 Hz, 1H), 7.10-7.43 (m, 6H), 7.58 (s, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.69 (m, 2H), 7.82 (m, 2H), 8.37 (t, J = 13.5 Hz, 1H). ^{13}C NMR ($CDCl_3$): 22.3, 24.2, 25.4, 25.5, 27.8, 28.0, 37.2, 42.0, 43.7, 44.4, 48.2, 48.7, 49.7, 104.4, 106.0, 110.5, 110.0, 121.8, 122.9, 123.8, 125.4, 128.8, 131.3, 133.7, 133.9, 140.0, 140.2, 140.6, 141.7, 142.9, 150.9, 168.3, 172.4, 174.1, ESI/MS: m/z calcd for $C_{41}H_{47}AsN_3O_5S_3^+$ 832.2, found 832.2.

2D NMR Structure Determination

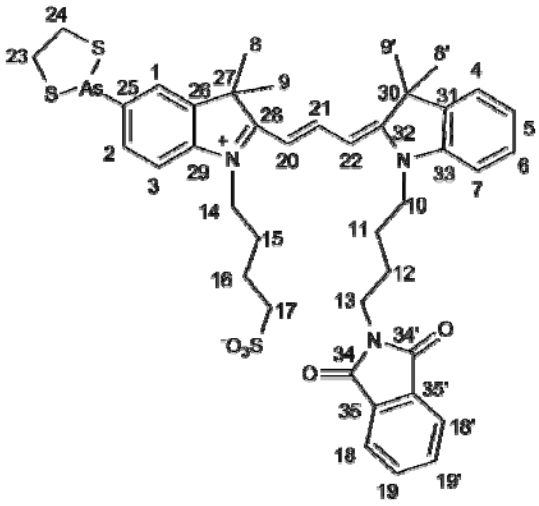
Figure S1: *Determination of structures of TRAP_Cy3 (Figure S1a) and AsCy3_3 (Figure S1b).* NMR spectroscopy was used to determine the position of the ethanedithiol stabilized arsenic on the indole ring of the cyanine dye, essentially as previously described.[4]

1-D ^{1}H, 2-D ^{1}H-^{1}H NOESY and phase-sensitive double quantum filtered COSY, and 2-D ^{1}H-^{13}C HSQC and HMBC spectra were recorded at 20 °C on a 500 MHz Varian (Agilent) Inova spectrometer. Samples were dissolved in $CDCl_3$ (Cambridge Isotope Laboratories) containing 0.05% tetramethylsilane as an internal reference (TMS, ^{1}H and ^{13}C δ = 0.00 ppm). HMBC spectra were optimized for J_{CH} = 8 Hz, with a filter element for one-bond couplings tuned to J_{CH} = 140 Hz. All spectra were recorded with 1024 (HSQC), 2048 (HMBC, COSY), or 4096 (NOESY) complex data points over ^{1}H spectral widths of 16-17 ppm in the direct dimension. In the indirect ^{1}H dimension of COSY and NOESY spectra, 1024 and 896 complex points, respectively, were collected over a 12.0 ppm spectral width. Indirect ^{13}C dimensions in HSQC and HMBC spectra were collected with 512 and 1024 complex points, respectively, over spectral widths of 120 ppm (HSQC) and 200 ppm (HMBC). The mixing time in NOESY spectra was 200 ms. Spectra were recorded with relaxation delays of 1.0 to 1.5 s.

Data was processed with Felix (FelixNMR, Inc.). 1-D ^{1}H experiments were processed without apodization. COSY experiments were processed with sine-bell apodization (shift = 0^o) in both dimensions. ^{1}H-^{13}C HSQC experiments were processed with sine-bell apodization (shift = 45^o) in both dimensions. HMBC experiments were processed with cosine-bell apodization in the direct dimension and sine-bell squared apodization (shift = 0^o) in the indirect dimension. NOESY experiments were processed with cosine-bell apodization in both dimensions. All experiments were processed with two-times zero filling in each dimension.

COSY and HSQC spectra were used to assign spin systems in each compound. Each spin system was extended into neighboring ring carbons not bearing protons using 2-D ^{1}H-^{13}C HMBC spectra. HMBC correlations from the N-alkyl substituent protons to indole ring carbons seen in common by nearby ring protons indicated perforce that they were part of the same N-alkylindole system. A NOESY correlation from the N-alkyl substituent to the nearest alkene proton of the linker was also important in confirming the correct assignments. The figures show the COSY (top tier) and HMBC (bottom tier) spectra and the critical correlations allowing identification of spin systems and determination of the correct structure for TRAP_Cy3 and AsCy3_3. The critical correlations are also indicated in Tables S1 and S2 for TRAP_Cy3 and AsCy3_3, respectively.

Figure S1a: TRAP_Cy3



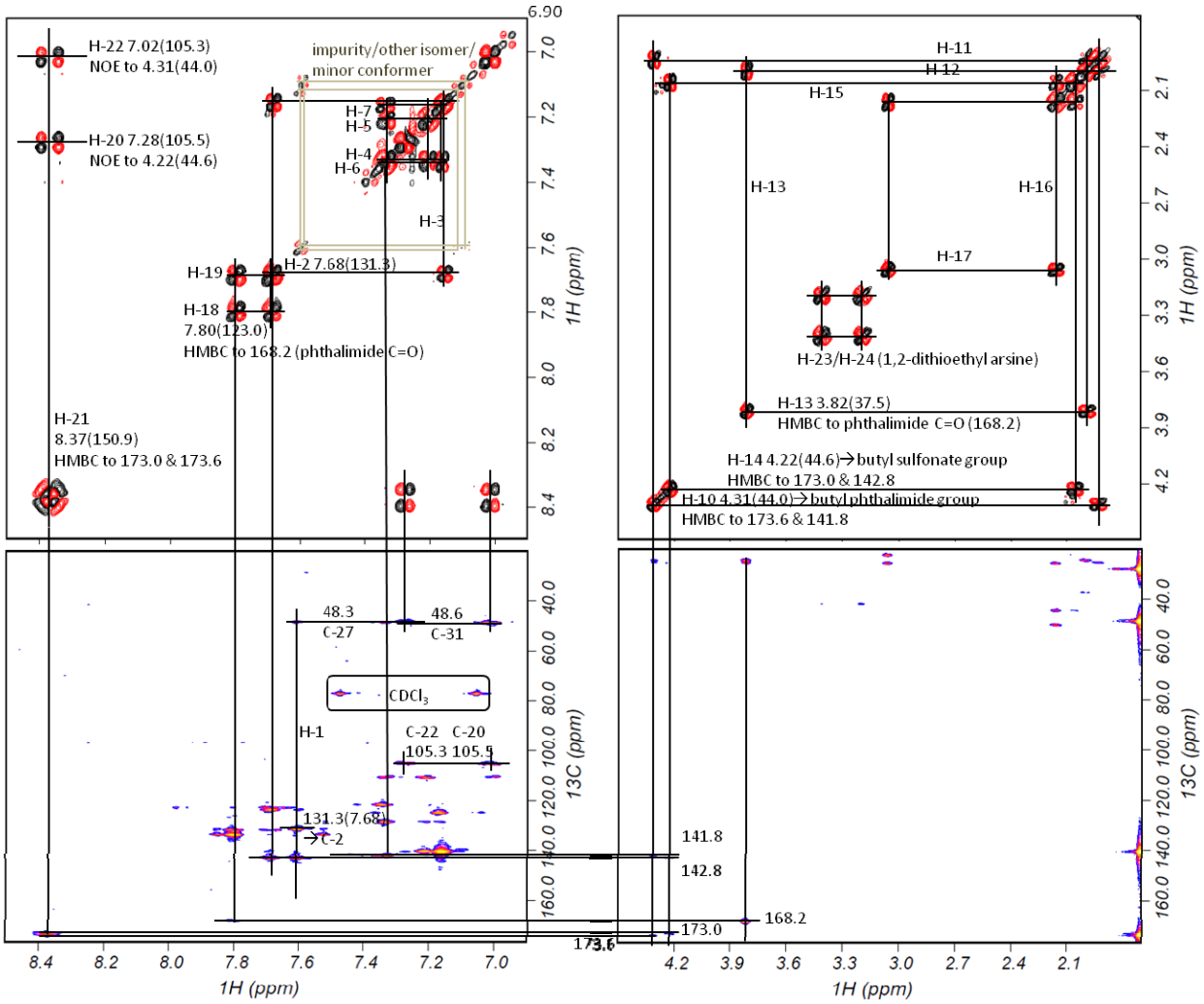
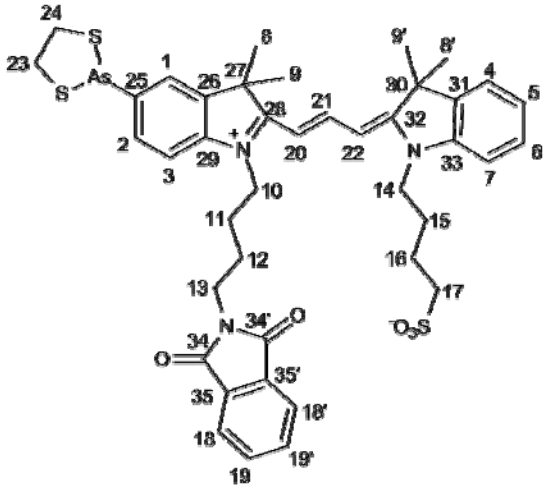


Figure S1b: AsCy3_3



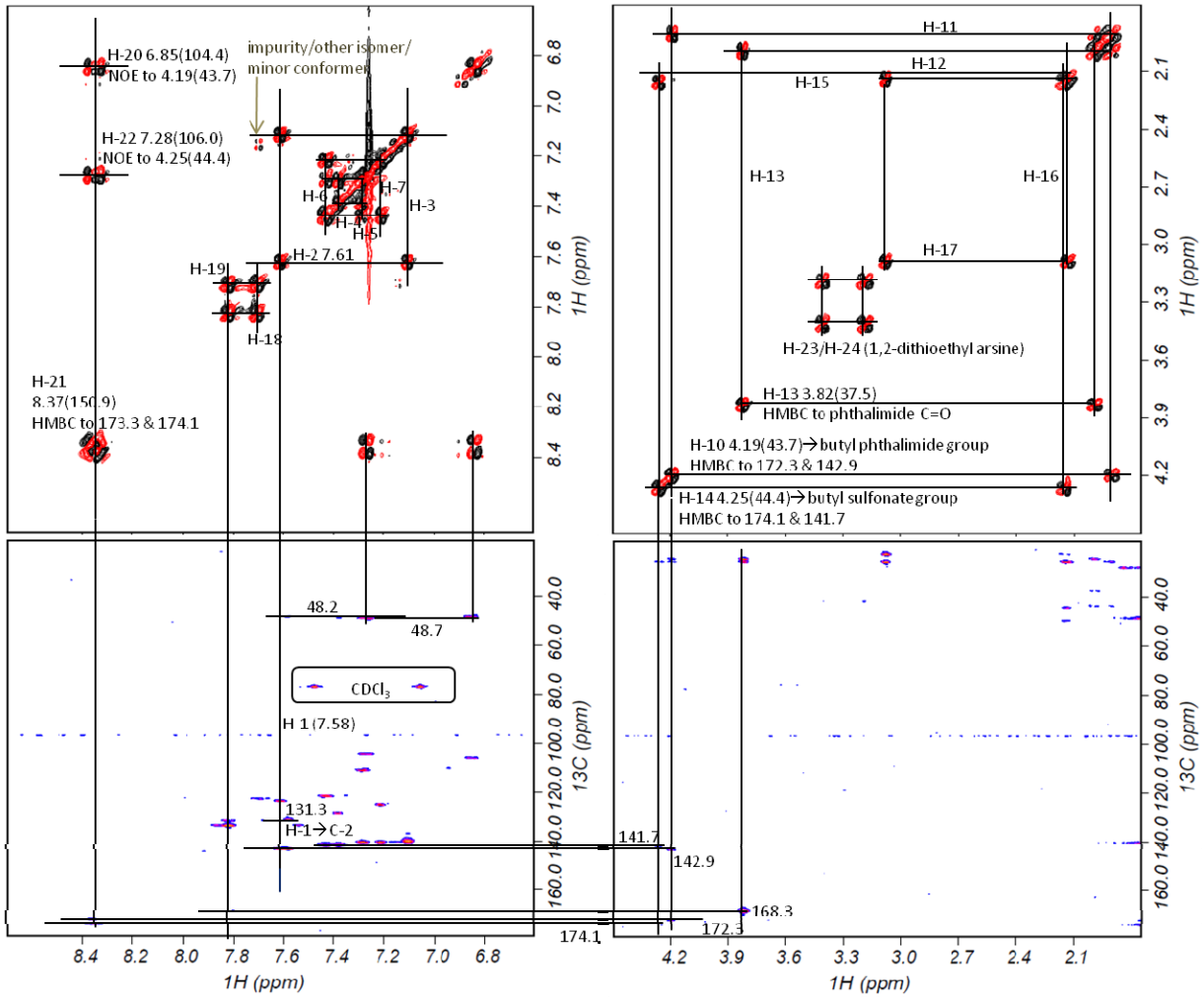


Table S1a: Chemical shifts for TRAP_Cy3 (butyl sulfonate on indole ring with EDT stabilized arsenic) in $CDCl_3$ at 20 °C.

Carbon	$\delta^{13}C$	Hydrogen	$\delta^{1}H$	key HMBC (C) and NOESY (H) cross peaks
C-1	123.9	H-1	7.59	H-1 → C-2,C-27,C-29
C-2	131.3	H-2	7.68	H-2 → C-1,C-29
C-3	110.5	H-3	7.16	
C-4	121.9	H-4	7.32	H-4 → C-30,C-33
C-5	125.9	H-5	7.20	
C-6	128.8	H-6	7.34	H-6 → C-33
C-7	110.7	H-7	7.14	
C-8/8'/9/9'	28.0	H-8/8'/9/9'	1.68	
C-10	44.0	H-10	4.31	H-10 → C-32,C-33
C-11	24.6	H-11	1.93	
C-12	25.6	H-12	2.00	
C-13	37.5	H-13	3.82	
C-14	44.6	H-14	4.22	H-14 → C-28,C-29
C-15	25.9	H-15	2.06	
C-16	22.6	H-16	2.15	
C-17	50.4	H-17	3.06	
C-18/18'	123.0	H-18/18'	7.80	
C-19/19'	133.8	H-19/19'	7.67	H-19 → C-34
C-20	105.5	H-20	7.28	H-20 → C-22,C-27,H-14
C-21	150.9	H-21	8.37	
C-22	105.3	H-22	7.02	H-22 → C-20,C-30,H-10
C-23/24	42.0	H-23/24	3.20/3.42	
C-25	140.0			
C-26	140.7			
C-27	48.3			
C-28	173.0			
C-29	142.8			
C-30	48.6			
C-31	140.5			
C-32	173.6			
C-33	141.8			
C-34/34'	168.2			
C-35	134.0			

Table S1b: Chemical shifts for AsCy3_3 (butyl phthalimide on indole ring with EDT stabilized arsenic) in $CDCl_3$ at 20 °C.

Carbon	$\delta^{13}C$	Hydrogen	$\delta^{1}H$	key HMBC (C) and NOESY (H) cross peaks
C-1	123.8	H-1	7.58	H-1 → C-2,C-27,C-29
C-2	131.3	H-2	7.61	H-2 → C-1,C-29
C-3	110.5	H-3	7.10	
C-4	121.8	H-4	7.38	H-4 → C-30,C-33
C-5	125.4	H-5	7.28	
C-6	128.8	H-6	7.43	H-6 → C-33
C-7	111.0	H-7	7.21	
C-8/9	28.0	H-8/9'	1.70	
C-8'/9'	27.8	H-8'/9'	1.72	
C-10	43.7	H-10	4.19	H-10 → C-28,C-29
C-11	24.2	H-11	1.89	
C-12	25.4	H-12	1.98	
C-13	37.2	H-13	3.82	
C-14	44.4	H-14	4.25	H-14 → C-32,C-33
C-15	25.5	H-15	2.12	
C-16	22.3	H-16	2.13	
C-17	49.7	H-17	3.09	
C-18/18'	122.9	H-18/18'	7.82	
C-19/19'	133.7	H-19/19'	7.69	H-19 → C-34
C-20	104.4	H-20	6.85	H-20 → C-22,C-30,H-10
C-21	150.9	H-21	8.37	
C-22	106.0	H-22	7.28	H-22 → C-20,C27,H-14
C-23/24	42.0	H-23/24	3.18/3.42	
C-25	140.0			
C-26	140.2			
C-27	48.2			
C-28	172.4			
C-29	142.9			
C-30	48.7			
C-31	140.6			
C-32	174.1			
C-33	141.7			
C-34/34'	168.3			
C-35	133.9			

Cell Permeability of TRAP_Cy3 and AsCy3_3: *Shewanella* W3-18-1 cells were incubated with 0.5 μM TRAP_Cy3 for 30 minutes at RT. In order to remove unbound TRAP_Cy3, cells were washed (pelleted) seven times prior to lysis in 25 mM HEPES (pH 7.7), where washing steps 2 and 3 included 5 mM N-ethyl maleimide (NEM) and washing steps 4 and 5 included 2.5% w/v bovine serum albumin. Cells were lysed by six successive freeze-thaw cycles involving freezing in liquid nitrogen followed by thawing at a 37 °C water bath prior to centrifugation at 17,200 x g for 25 min to remove cell debris. The lysis buffer contained 25 mM HEPES (pH 7.6). Protein concentrations of lysates were measured using a BCA assay kit (Pierce) prior to freezing samples at -80°C for storage.

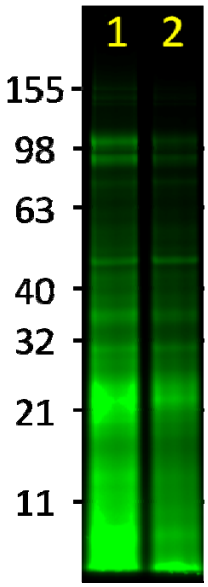


Figure S2: *Live-Cell Labeling of Proximal Cysteines*. Bacterial cells (*Shewanella* W3-18-1) were incubated in the presence of 0.5 μM TRAP_Cy3 (lane 1) or AsCy3_3 (lane 2) for 30 minutes prior to cell lysis and resolution of labeled proteins by SDS-PAGE. Protein loads were 20 μg per lane. Positions of molecular mass standards are indicated. λ_{ex} = 530 nm; λ_{em} = 570 nm.

Photoproperties of TRAP_Cy3: Spectra were measured in 25 mM HEPES (pH 7.7) with 5% DMSO (v/v) for both TRAP_Cy3 and compound **1**. The fluorescence quantum yield (Φ) was calculated using Rhodamine 6G as standard (Φ =0.95 in 1N H_2SO_4).[5]

$$\Phi_S = \Phi_R [F_S / F_R] * [OD_R / OD_S] * [n_S / n_R]^2 \qquad \text{Eq. S1}$$

Subscripts S and R respectively indicate the sample and reference fluorophore, F is the integrated area under the corrected fluorescence emission spectrum, A is the absorbance (optical density), and n is the refractive index of the solvent. In all cases fluorescence excitation was at 525 nm. In aqueous solution, the quantum yields for TRAP_Cy3 and compound **1** are 5% and 4%, respectively (Φ in increases upon protein binding by approximately six-fold for cyanine dyes).[6]

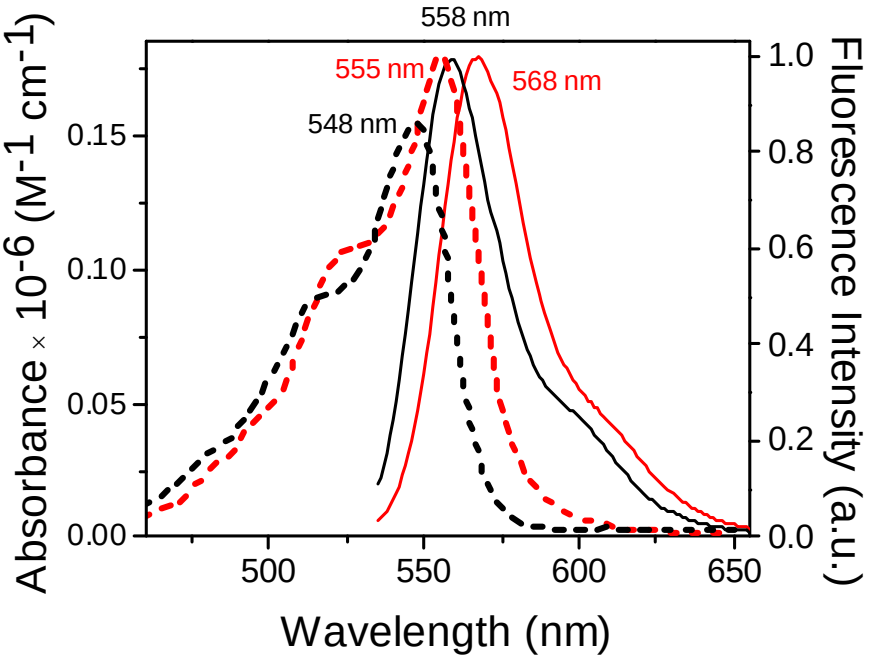


Figure S3: *Spectroscopic Properties*: Absorption (dashed line) and emission (solid line) spectra for TRAP_Cy3 (black) and compound **1** (red) (1 µM) in 5 % (v/v) DMSO in 25 mM HEPES buffer (pH = 7.7) (λ_{ex} = 525 nm). Emission maxima are indicated above spectra.

Albumin Sequence and MS/MS Identification of TRAP_Cy3 Bound Peptides

```
1    MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPF   60  P02768   ALBU_HUMAN
1    MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPF   60  P02769   ALBU_BOVIN
     **********:**************:*****:**********:**.******:********

61   EDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEP  120  P02768   ALBU_HUMAN
61   DEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEP  120  P02769   ALBU_BOVIN
     ::********:*************  .*:**********:**.**:*******:****** ****

121  ERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLF  180  P02768   ALBU_HUMAN
121  ERNECFLSHKDDSPDLPKLK-PDPNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLY  179  P02769   ALBU_BOVIN
     *******.****.*:**:*  *: :.:*  *: :*:.*  ********************:

181  FAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAV  240  P02768   ALBU_HUMAN
180  YANKYNGVFQECCQAEDKGACLLPKIETMREKVLASSARQRLRCASIQKFGERALKAWSV  239  P02769   ALBU_BOVIN
     :*::*:..* ***** **.******:: :*::  ****:***:***:********:***:*

241  ARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLK  300  P02768   ALBU_HUMAN
240  ARLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLK  299  P02769   ALBU_BOVIN
     *****:******.**:**********.**********************:***:******

301  ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYAR  360  P02768   ALBU_HUMAN
300  ECCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSR  359  P02769   ALBU_BOVIN
     ***:***************:* :* :**.*:***.*.******* ****.*** *****:*

361  RHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFE  420  P02768   ALBU_HUMAN
360  RHPEYAVSVLLRLAKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFE  419  P02769   ALBU_BOVIN
     ***:*:* :****** **:***:***  *** **:.***::* **:**********: **

421  QLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVV  480  P02768   ALBU_HUMAN
420  KLGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLI  479  P02769   ALBU_BOVIN
     :**** *****:****:***************.*****::**.:**::****:******::

481  LNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTL  540  P02768   ALBU_HUMAN
480  LNRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTL  539  P02769   ALBU_BOVIN
     **:***********::******************  ******* *: : **********

541  SEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLV  600  P02768   ALBU_HUMAN
540  PDTEKQIKKQTALVELLKHKPKATEEQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLV  599  P02769   ALBU_BOVIN
     .:.*:***********:********:****:**::*.***:*** ******:*** ** ***

601  AASQAALGL  609  P02768   ALBU_HUMAN
600  VSTQTALA-  607  P02769   ALBU_BOVIN
     .::*:**.
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Figure S4*: Primary sequence alignment of human and bovine albumin*. Conserved (*) or homologous (. or :) amino acids are indicated. Positions of 34 cysteines that form disulfide bonds are indicated in blue.

Table S2. LC_MS/MS Identification of Peptides that Bind TRAP_Cy3 in BSA[a]

Identified Peptide Sequence	Counts (Control)	Counts (Sample)	Cysteine Binding Sites
K.VHKECCHGDLLECADDRADLAK.Y	0	23	268, 269, 276
K.TPVSEKVTKCCTESLVNRRPCFSALTPDETYVPK.A	0	11	499, 500, 510
K.SHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAK.D	0	10	312, 339
K.VHKECCHGDLLECADDRADLAKYICDNQDTISSK.L	0	10	268,269,276,288
K.DAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSR.R	0	10	339
K.VHKECCHGDLLECADDRADLAK.Y	0	7	269, 276
R.LAKEYEATLEECCAKDDPHACYSTVFDKLK.H	0	7	383, 384, 392
R.MPCTEDYLSLILNRLCVLHEK.T	0	7	484
K.TVMENFVAFVDKCCAADDKEACFAVEGPK.L	0	7	581
K.LKPDPNTLCDEFKADEKKFWGK.Y	0	6	147
R.MPCTEDYLSLILNRLCVLHEK.T	0	6	471
K.TVMENFVAFVDKCCAADDKEACFAVEGPK.L	0	6	582, 590
K.TVMENFVAFVDKCCAADDKEACFAVEGPK.L	0	6	582
K.ECCDKPLLEK.S	0	5	301, 302
K.VGTRCCTKPESER.M	0	5	460, 461
K.LKPDPNTLCDEFKADEKKFWGK.Y	0	5	147
K.DDPHACYSTVFDKLKHLVDEPQNLIK.Q	0	5	392
K.TVMENFVAFVDKCCAADDKEACFAVEGPK.L	0	5	581, 590
K.LKPDPNTLCDEFKADEKK.F	1	4	147
K.TVMENFVAFVDKCCAADDK.E	1	4	582

[a]The most abundant identified peptide sequences are listed in Table S1, in which the binding sites by the TRAP_Cy3 is highlighted in red. An increase of more than 4-fold due to TRAP_Cy3 labeling comparing to control sample without TRAP_Cy3 labeling, were considered as confident probe-modified peptides. Peptides were identified from LC-MS/MS data using the SEQUEST algorithm[7] (version 27, revision 12) following searching all MS/MS spectra against bovine serum albumin precursor (gi|418694), following data formatting using Extract_MSn (version 3.0) in Bioworks Cluster 3.2 (Thermo Fisher Scientific, Cambridge, MA). Search parameters were: 0.1 Da tolerance for precursor ion masses and 1 Da for fragment ion masses, a maximum of 2 missed tryptic cleavages, dynamic oxidation of methionine (+15.9949 Da), dynamic N-ethyl-

maleimide (NEM) modification of cysteine (+125.0477 Da), and dynamic iodoacetamide (IAM) modification of cysteine (+57.0215Da). The false discovery rate (FDR) at the unique peptide level is <1%. For each identified spectrum, MS Generating-Function (MSGF) scores were generated by computing rigorous p-values (spectral probabilities). If mass measurement error is less than 5 ppm, identification is restricted within fully tryptic peptides with MSGF score < 1E-8 and partially tryptic peptides with MSGF score < 1E-10. If mass measurement error is larger than 5 ppm but picked for fragmentation as non-monoisotopic peaks, only fully tryptic peptides with a MS-GF score < 1E-10 were accepted.

Growth Limitations Affect Redox Status of Proximal Cysteines:

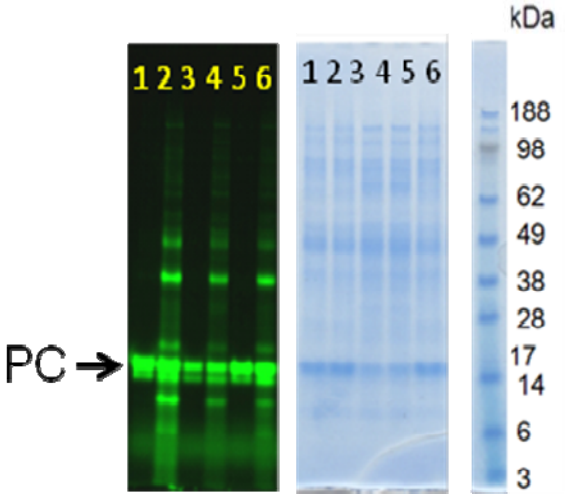

Figure S5: *Growth Limitations Affect Redox Status of Proximal Cysteines.* Fluorescence image (left) and protein stain (right) following SDS-PAGE of lysates prepared from *Synechococcus* sp. PCC7002 grown in chemostats at 30 °C with either limiting carbon (lanes 1 and 2 grown in the presence of 7.7 mM $NaHCO_3$) or limiting nitrogen (lanes 3 and 4 grown in the presence of 0.9 mM NH_4Cl). In comparison, cells were grown with abundant carbon or nitrogen (i.e., 40 mM $NaHCO_3$ (pH 7.5) and 17 mM NH_4Cl) (lanes 5 and 6). In all cases light levels (600 µmoL m^{-2} sec^{-1}) were constant. DOT varied between 8% (no limitations), 10% (nitrogen limited), and 29% (carbon limited). All growth media contained the following: 8 mM KCl, 2 mM $CaCl_2$, 40 mM $MgSO_4$, 0.55 mM H_3BO_3, 020 µM $MnCl_2$, 2 µM $ZnCl_2$, 0.05 µM $CoCl_2$, 0.2 µM Na_2MoO_4, and 0.00001 mM $CuSO_4$, and 0.1 mM EDTA.

Cysteine-Reactive Probes Target Thylakoid Membranes

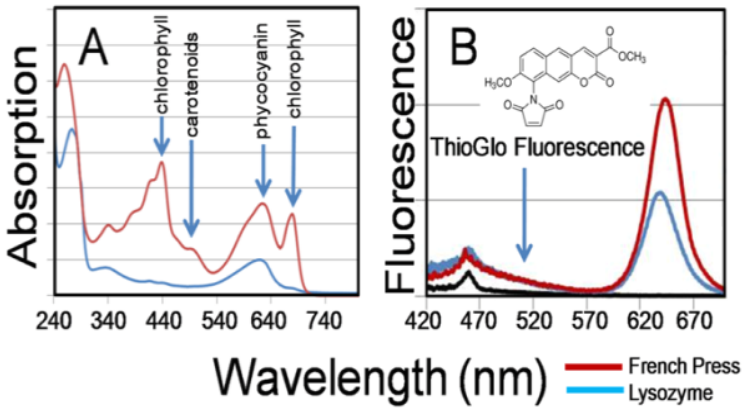

Figure S6. *Cysteine-Reactive Probes Target Thylakoid Membranes***.** Conventional thiol-directed probe ThioGlo selectively binds reactive cysteines in preparation enriched in thylakoid membranes (red) relative to total cellular lysate (blue). Lysates were prepared from *Synechococcus sp. PCC7002* using either a French Press (red) or sonication (blue), following growth as described in the legend to Figure 5 (lanes 5 and 6).

Amino Acid Sensitivities to Reactive Oxygen Species

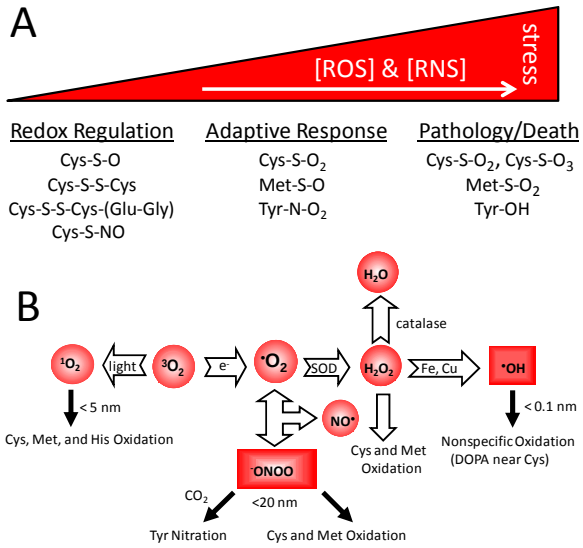

Figure S7: *Relationships Between Oxidative Stress and Expected Post-translational Modifications*. Proposed hierarchical levels of regulation involving differences in reactivity between cysteines, methionines, and tyrosines (panel A) to generated reactive oxygen species (ROS) and reactive nitrogen species (RNS) (panel B) that are consistent with a central regulatory role for disulfide bond formation between proximal cysteines in modifying cellular metabolism to minimize the oxidation of Met, Tyr, and irreversible oxidation states of cysteine that are associated with pathology and cell death.

Peptide Enrichment Following Live-Cell Labeling by TRAP_Cy3:

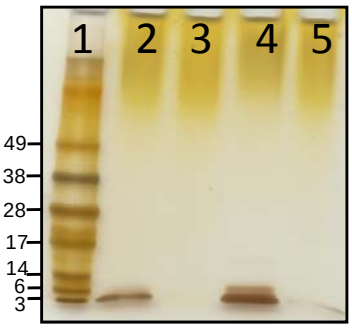

Figure S8: *Peptide Enrichment Following Affinity Capture and EDT Release*. Lysates obtained before (lanes 3 and 5) or following (lanes 2 and 4) live-cell labeling of *Synechococcus* sp. PCC7002 with TRAP_Cy3 (100 µM) were alkylated (10 mM iodoacetamide and 10 mM N-ethylmaleimide) and following addition of ethanedithiol (1 mM) were affinity purified using thiolproyl Sepharose™ 6B resin (as in Figure 2). Following extensive washing, retained protein was subjected to trypsin digestion. Eluted peptides were analyzed using SDS-PAGE, and visualized using silver stain. Optimal results require quantitative alkylation of cellular thiols. Molecular mass marker (lane 1).

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