SUPPLEMENTAL DATA:

FIGURES:

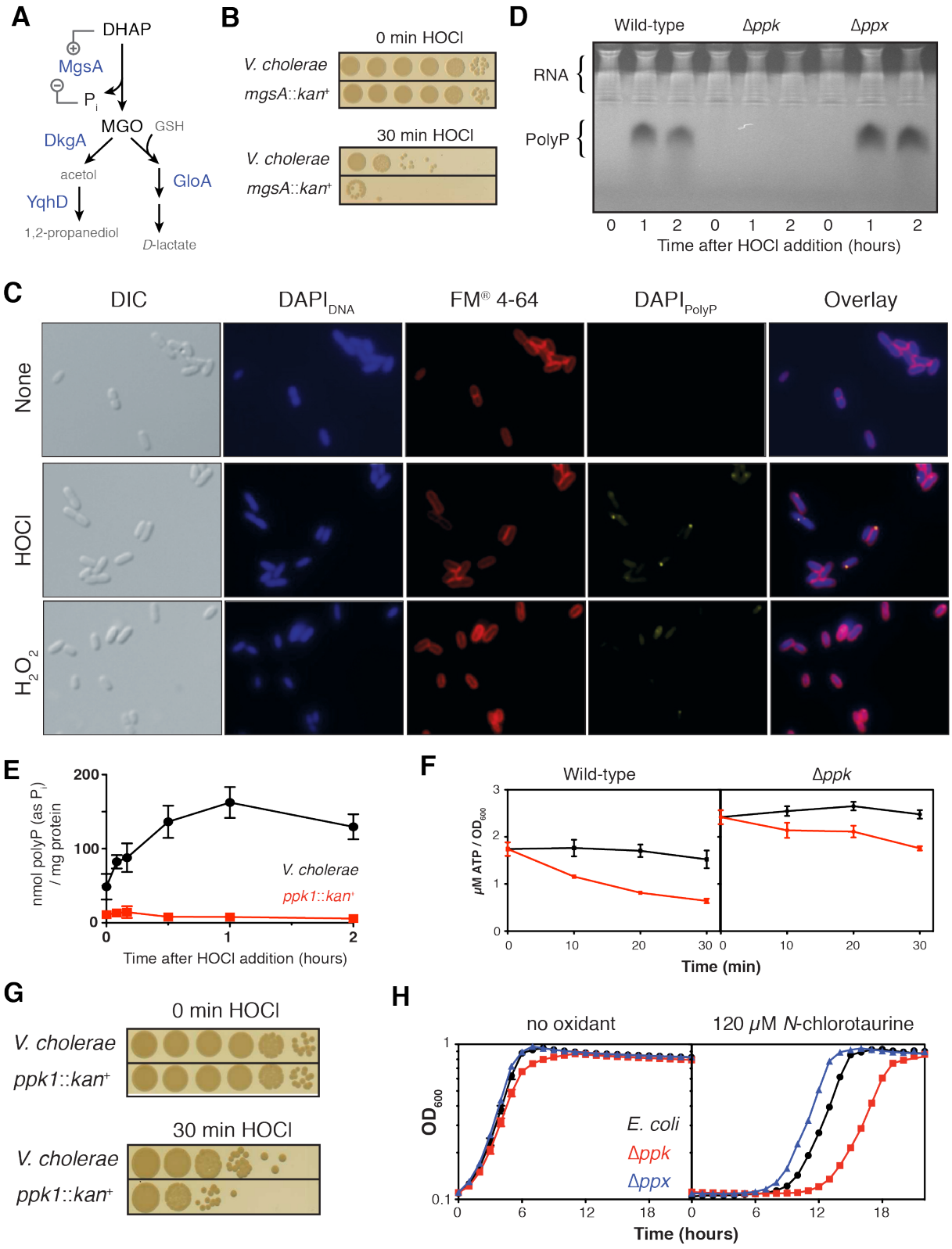

Figure S1, Related to Figure 1. ATP-derived PolyP Protects Against HOCl.

(A) Methylglyoxal (MGO) biosynthesis and detoxification pathways in *E. coli*. Abbreviations: DHAP, dihydroxyacetone phosphate; P_i, inorganic phosphate; GSH, reduced glutathione.

(B) Exponentially growing *V. cholerae* were incubated with 550 μM HOCl, serially diluted and spot-titered.

(C) *E. coli* MG1655 was grown to log phase, then incubated in MOPS medium containing no additive (None), 2 mM HOCl, or 2 mM H_2O_2 for 30 min. Cells were stained with DAPI and FM® 4-64 and visualized by differential interference contrast (DIC) microscopy. DNA ($DAPI_{DNA}$), cell membranes (FM® 4-64), and polyP granules ($DAPI_{polyP}$) were visualized by fluorescence microscopy. Overlay shows results for all strains.

(D) *E. coli* strains were grown to log phase in MOPS medium, then treated with 1 mM HOCl. PolyP was separated on TBE-Urea PAGE gels (Bio-Rad) and visualized by negative DAPI staining.

(E) PolyP content of *V. cholerae* after addition of 550 μM HOCl (mean ± SD).

(F) Cellular ATP levels in MG1655 (Wild-type) and the Δ*ppk* mutant grown to log phase, then treated with 0 (black) or 1 mM (red) HOCl; ATP concentration is expressed as μM ATP per OD_{600} of cell culture (mean ± SD).

(G) Exponentially growing *V. cholera* were incubated with 550 μM HOCl, then diluted and spot-titered.

(H) *E. coli* wild-type (black circles), Δ*ppk* (red squares), and Δ*ppx* (blue triangles) strains were grown at 37°C with aeration in MOPS medium with or without 120 μM *N*-chlorotaurine. Growth was monitored by A_{600} (mean ± SD).

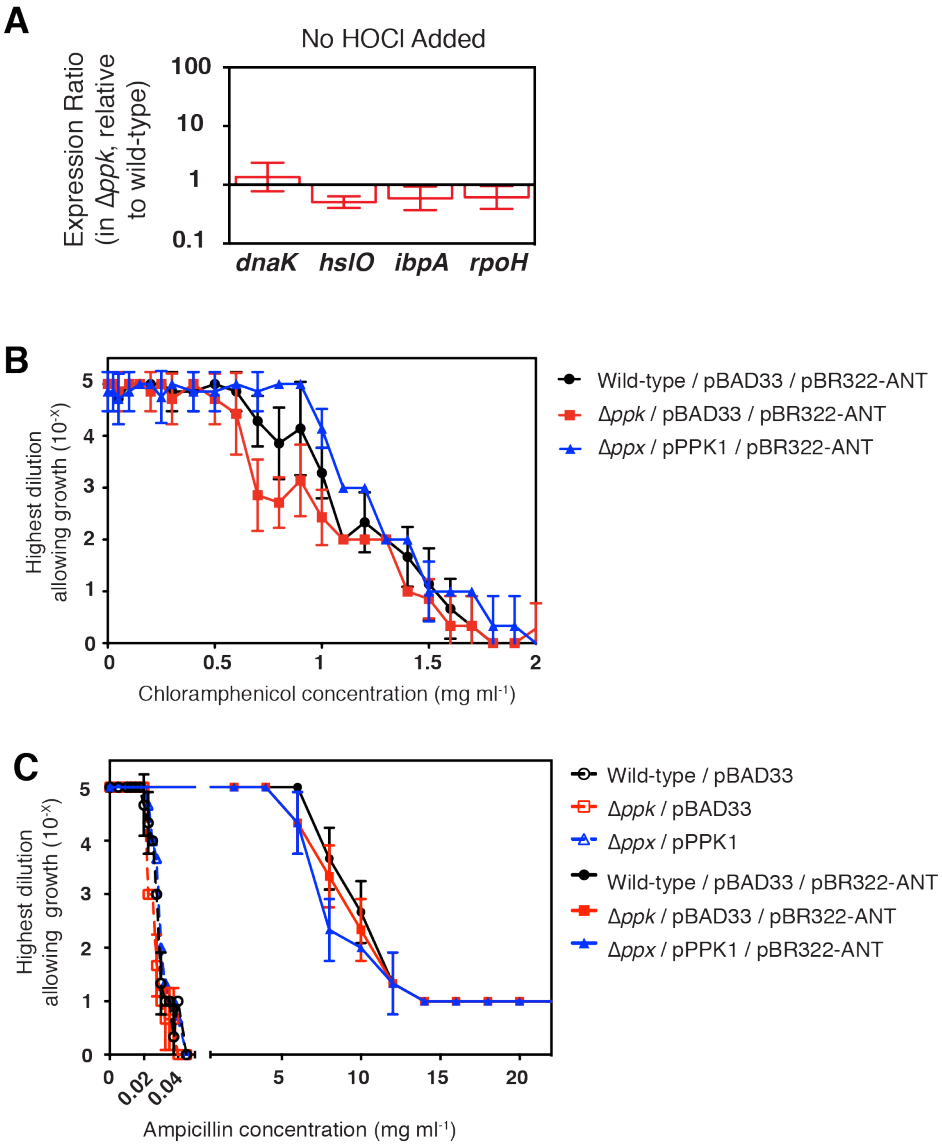

Figure S2, Related to Figure 2. PolyP is a Protein-Protective Chaperone *In Vivo*.

(A) Relative expression of heat shock genes in wild-type and Δ*ppk* strains in the absence of HOCl stress was measured by qRT-PCR (mean ± SD).

(B) and (C) Plasmid-bearing *E. coli* containing no polyP (red squares), wild-type (black circles), or higher than normal levels of polyP (blue triangles) were grown to log phase, serially diluted, spot-titered on agar containing different concentrations of chloramphenicol (B) or ampicillin (C) and scored for growth (mean ± SD).

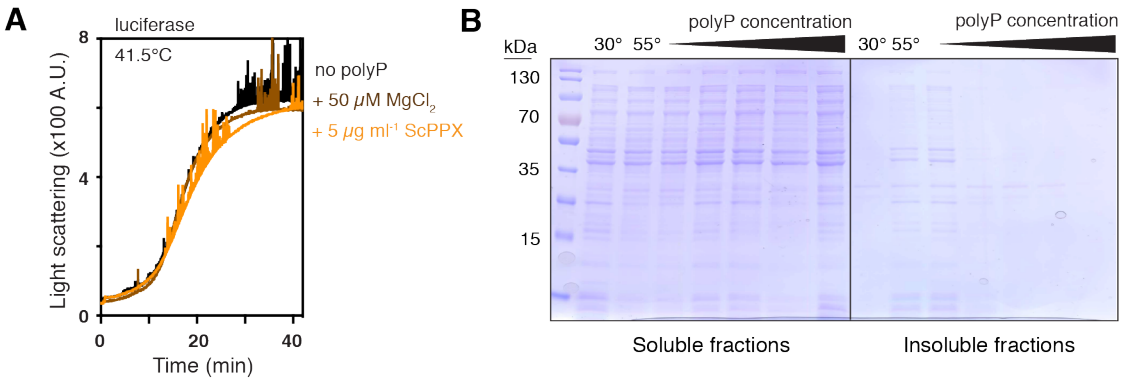

Figure S3, Related to Figure 3. PolyP is a Protein-Protective Chaperone *In Vitro*.

(A) Thermal aggregation of luciferase upon its dilution into pre-warmed buffer (black) or buffer containing 50 μM $MgCl_2$ (brown) or 5 μg ml^{-1} ScPPX (orange).

(B) Crude lysates of *E. coli* MG1655 were incubated 30 minutes at 30° or 55°C, with 0, 0.2, 1, 2, 6, or 10 mM polyP. Soluble and insoluble fractions were separated and examined by SDS-PAGE.

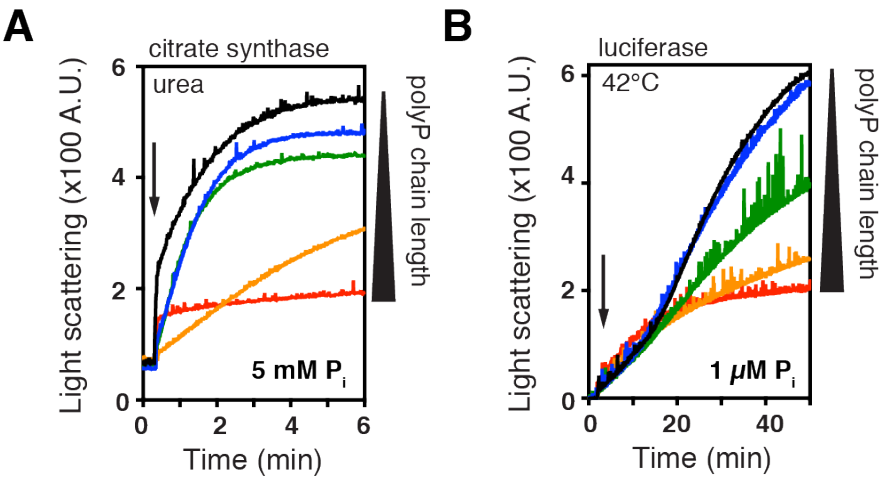

Figure S4, Related to Figure 4. PolyP Chain Length Influences Chaperone Activity

Aggregation of (A) urea-denatured citrate synthase or (B) thermally-denatured luciferase upon their dilution into buffer containing no polyP (black) or the indicated concentrations of different length polyP: 14-mer (blue), 60-mer (green), 130-mer (orange), or 300-mer (red). Concentrations were determined in terms of total phosphate concentration. Arrows indicate time of protein addition.

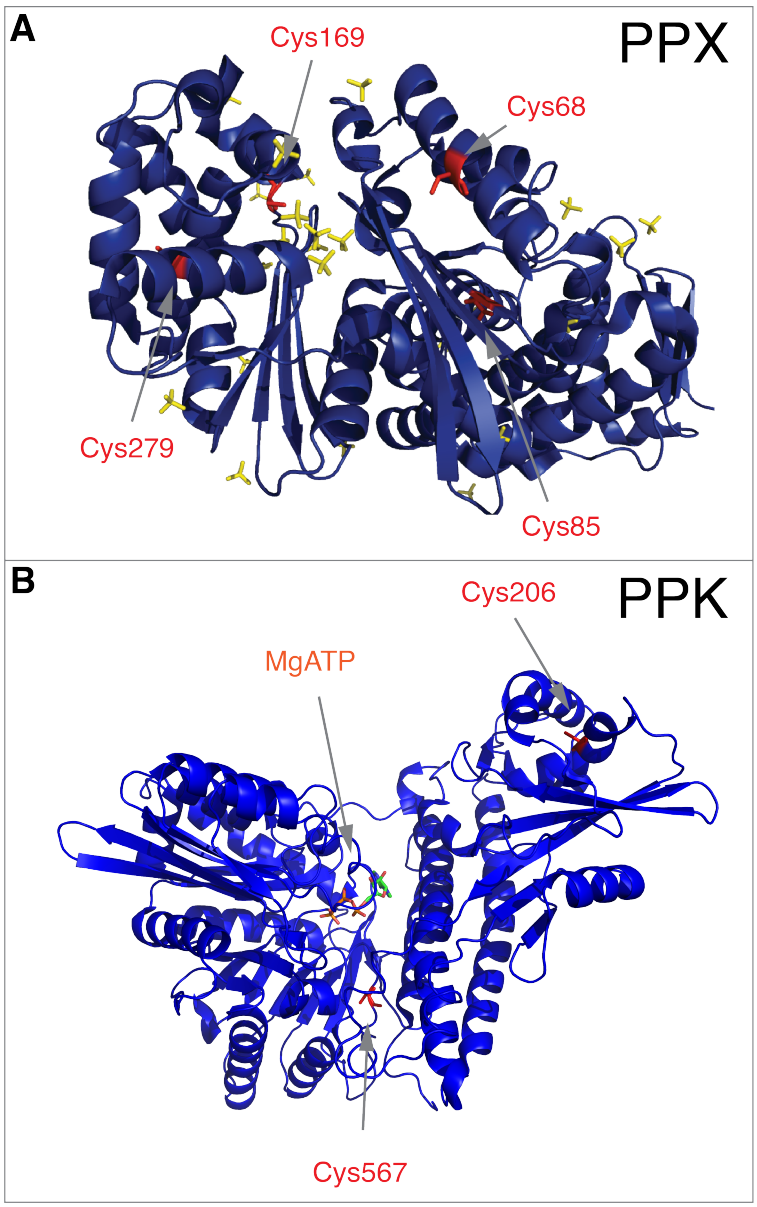

Figure S5; Related to Figure 5. PPX is a Redox-Regulated Enzyme.

(A) The structure of *E. coli* PPX monomer (PDB 1U67) (Alvarado et al., 2006) is shown. Cysteine residues are indicated in red, sulfate ions (thought to indicate the region of PPX to which polyP binds) are indicated in yellow. Cys169 is located directly within the predicted polyP binding site of PPX.

(B) The structure of a monomer of PPK (PDB 1XDO) (Zhu et al., 2005). Cysteine residues are indicated in red and MgATP in the active site is shown as sticks. The highly conserved Cys567 is located directly within the predicted active site of PPK.

SUPPLEMENTAL TABLES:

Supplemental Table S1, Related to Figure 5. Cysteine Oxidation State of PPX *In Vitro*.

no oxidant added[a]

Cysteine	IAM (-SH)	NEM (-SOH)[b]	$-SO_2H$	$-SO_3H$	no modification
68	22[c]	16	0	1	0
85	42	33	0	1	0
169	24	14	0	1	0
279	2	3	0	0	0

10:1 *N*-chlorotaurine

Cysteine	IAM (-SH)	NEM (-SOH)	$-SO_2H$	$-SO_3H$	no modification
68	25	17	0	0	0
85	25	32	0	12	0
169	7	7	2	24	2
279	1	3	0	1	0

[a] PPX incubated aerobically without reducing agent; resulting in the observed proportion of NEM-modified (reversibly oxidized) cysteine residues in the "no oxidant" PPX treatment.

[b] Includes all DTT-reversible cysteine oxidations.

[c] Spectral counts for PPX peptides containing cysteine residues with the indicated modifications identified by LC MS/MS (MS Bioworks).

Table of Strains and Plasmids.

Strain name	Marker(s)[a]	Relevant Genotype	Source[b]
E. coli strains:			
XL1-Blue	Tc^R Nx^R	*endA1 gyrA96*(Nx^R) t*hi-1 recA1 relA1 lac glnV44* F'[::Tn10(*tet*$^+$) *proAB*$^+$ *lacI*$_q$ Δ(*lacZ*)M15] *hsdR17*(r_K^- m_K^+)	Stratagene
BL21(DE3)		F^-, *ompT gal dcm lon hsdSB*(r_B^- m_B^-) λ(DE3 [*lacI lacUV5*-T7 gene 1 *ind1 sam7 nin5*])	Novagen
BB7224	Sm^R Km^R	F^-, λ^-, $e14^-$, [*araD139*]$_{B/r}$ Δ(*argF-lac*)*169 flhD5301* Δ(*fruK-yeiR*)*725*(*fruA25*) *relA1 rpsL150*(Sm^R) *rbsR22* Δ(*fimB-fimE*)*632*(::IS1) *ptsF25 zhf*::Tn*10*(Tc^S) *suhX401 deoC1 araD*$^+$ *rpoH*::*kan*$^+$	(Tomoyasu et al., 2001)
MG1655		F^-, λ^-, *rph-1 ilvG*$^-$ *rfb-50*	(Blattner et al., 1997)
MJG154	Cm^R	MG1655 *mgsA*::*cat*$^+$	
MJG155	Cm^R	MG1655 *yqhD*::*cat*$^+$	
MJG176		MG1655 Δ*mgsA*	
MJG177		MG1655 Δ*yqhD*	
MJG184	Cm^R	MG1655 *dkgA*::*cat*$^+$	
MJG187		MG1655 Δ*dkgA*	
MJG208	Km^R	MG1655 *ppk::kan*$^+$	
MJG224		MG1655 Δ*ppk*	
MJG308	Cm^R	MG1655 *ppx*::*cat*$^+$	
MJG315		MG1655 Δ*ppx*	
MJG356	Cm^R	MG1655 / pBAD33 (*cat*$^+$)	
MJG360	Cm^R	MG1655 Δ*ppk* / pBAD33 (*cat*$^+$)	
MJG361	Cm^R	MG1655 Δ*ppx* / pPPK1 (*ppk*$^+$ *cat*$^+$)	
MJG491	Sm^R Km^R Ap^R	BB7224 / pBAD18 (*bla*$^+$)	
MJG492	Sm^R Km^R Ap^R	BB7224 / pPPK7 (*ppk*$^+$ *bla*$^+$)	
MJG513	Sm^R Km^R	BB7224 *ppx*::*cat*$^+$	

	Cm^R		
MJG514	Sm^R Km^R Cm^R Ap^R	BB7224 *ppx*::*cat*$^+$ / pBAD18 (*bla*$^+$)	
MJG515	Sm^R Km^R Cm^R Ap^R	BB7224 *ppx*::*cat*$^+$ / pPPK7 (*ppk*$^+$ *bla*$^+$)	
MJG531	Sm^R Km^R Ap^R	BB7224 / pDNAKJ2 (*dnaKJ*$^+$ *bla*$^+$)	
AMS346	Cm^R Sp^R Tc^R Ap^R	MG1655 / pBAD33 (*cat*$^+$) / pBR322-ANT (*aadA*$^+$ *tet*$^+$ *bla*$^+$)	
AMS348	Cm^R Sp^R Tc^R Ap^R	MG1655 Δ*ppk* / pBAD33 (*cat*$^+$) / pBR322-ANT (*aadA*$^+$ *tet*$^+$ *bla*$^+$)	
AMS353	Cm^R Sp^R Tc^R Ap^R	MG1655 Δ*ppx* / pPPK1 (*ppk*$^+$ *cat*$^+$) / pBR322-ANT (*aadA*$^+$ *tet*$^+$ *bla*$^+$)	
<u>*V. cholerae* El Tor strains:</u>			
C6706*lacZ*	Sm^R		(Cameron et al., 2008)
EC2798	Sm^R Km^R	*mgsA*-TnFGL3::*kan*$^+$	(Cameron et al., 2008)
EC21753	Sm^R Km^R	*ppk1*-TnFGL3::*kan*$^+$	(Cameron et al., 2008)
<u>Plasmids:</u>			
pET-15b	Ap^R	N-terminal His_6 tag overexpression vector	Novagen
pET-21b(+)	Ap^R	C-terminal His_6 tag overexpression vector	Novagen
pKD3	Cm^R Ap^R	*cat*$^+$ chloramphenicol resistance cassette donor	(Datsenko and Wanner, 2000)
pKD46	Ap^R	λ Red recombinase$^+$	(Datsenko and Wanner, 2000)
pCP20	Cm^R Ap^R	Flp recombinase$^+$	(Datsenko and Wanner, 2000)
pBAD18	Ap^R	cloning vector with P_{BAD} arabinose-inducible promoter	(Guzman et al., 1995)

pBAD33	Cm^R	cloning vector with P_{BAD} arabinose-inducible promoter	(Guzman et al., 1995)
pBAD43	Sp^R Ap^R	cloning vector with P_{BAD} arabinose-inducible promoter	(Stewart et al., 1999)
pBR322	Tc^R Ap^R	cloning vector	(Bolivar et al., 1977)
pBR322-ANT	Sp^R Tc^R Ap^R	*aadA*$^+$	
pPPK1	Cm^R	*ppk*$^+$	
pPPK2	Ap^R	*ppk*$^+$ translational fusion to C-terminal His_6 tag for protein purification	
pPPK7	Ap^R	*ppk*$^+$	
pDNAKJ2	Ap^R	*dnaKJ*$^+$	
pScPPX2	Ap^R	*S. cerevisiae PPX1*$^+$ translational fusion to N-terminal His_6 tag for protein purification	
pEcPPX	Km^R	*ppx*$^+$	(Alvarado et al., 2006)

[a] Abbreviations: Tc^R, tetracycline resistance; Nx^R, nalidixic acid resistance; Cm^R, chloramphenicol resistance; Ap^R, ampicillin resistance; Km^R, kanamycin resistance; Sp^R, spectinomycin resistance; Sm^R, streptomycin resistance; Tc^S, tetracycline sensitivity.

[b] Unless otherwise indicated, all strains and plasmids were generated in the course of this work.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Bacterial Strains and Growth Conditions

All DNA manipulations were carried out in *E. coli* XL1-Blue (Stratagene). The identity of inserts in all plasmids constructed during this study was confirmed by sequencing (GENEWIZ, Inc. or GenScript, Inc.). Null mutations in *E. coli* MG1655 (Blattner et al., 1997) and BB7224 (Tomoyasu et al., 2001) were constructed as previously described (Baba et al., 2006; Datsenko and Wanner, 2000; Silhavy et al., 1984). *V. cholerae* El Tor C6706*lacZ* and its *ppk1*-TnFGL3::*kan*$^+$ and *mgsA*-TnFGL3::*kan*$^+$ derivatives were from the defined transposon insertion library of Cameron *et al.* (Cameron et al., 2008), and were provided by Victor DiRita (University of Michigan). *E. coli* and *V. cholerae* were grown at 37°C in lysogenic broth (LB; Fisher) or in MOPS minimal medium (Teknova) containing 0.2% glucose, 1.32 mM K_2HPO_4, and 10 μM thiamine. Unless indicated, chemicals were from Fisher or Sigma-Aldrich. Short-chain heterogeneous sodium polyP (Type 45 sodium phosphate glass) was from Sigma-Aldrich or Acros Organics. Long-chain heterogeneous sodium polyP was from KeraFAST. Defined-length polyphosphates were a gift from Dr. Toshikazu Shiba (Regenetiss, Japan). *N*-chlorotaurine was synthesized before each use (Peskin and Winterbourn, 2001).

Sequence Analysis and Primer Design

Gene and protein sequences were obtained from the Integrated Microbial Genomes database (http://img.jgi.doe.gov). PCR and sequencing primers were designed with Web Primer (http://www.yeastgenome.org/cgi-bin/web-primer). qRT-PCR primers were designed with Primer3 0.4.0 (http://frodo.wi.mit.edu/primer3/input.htm) for the following genes: *rrsD*, 5' AAG AAC TTA CCT GGT CTT GAC ATC 3' and 5' CAG TTT ATC ACT GGC AGT CTC CTT 3'; *ibpA*, 5' TGC TAT TGG ATT TGA CCG TTT G 3' and 5' CGG CAC GTT ATA CGG AGG GTA GCC G 3'; *hslO*, 5' TGC CGC AAC ATG ACC AAT TAC ATC GC 3' and 5' TCA CCA TCA AAC TTC AGC GTA GCG GT 3'; *dnaK*, 5' ACA GCA CCC GTA AGC AGG TTG AAG AA 3' and 5' TGG

GCG ATT TCC ATC AGT TTC TGG GA 3'; *rpoH*, 5' GAT TTG ATT CAG GAA GGT AAC ATC G 3' and 5' GTT TTA CGC AGG TTG AAG AAC AGT 3'; *sulA*, 5' CGG GCT TAT CAG TGA AGT TGT CTA T 3' and 5' CTG GCT AAT CTG CAT TAC TTT CGT T 3'.

Strain Construction

In-frame replacements of complete coding sequences with chloramphenicol resistance cassettes (Datsenko and Wanner, 2000) were constructed for the following genes in *E. coli* MG1655, using the indicated primers: *mgsA*: 5' ATA AGT GCT TAC AGT AAT CTG TAG GAA AGT TAA CTA CGG ATG TAC ATT ATG GTG TAG GCT GGA GCT GCT TC 3' and 5' TGG CGA GAA AAC CGT AAG AAA CAG GTG GCG TTT GCC ACC TGT GCA ATA TTA CAT ATG AAT ATC CTC CTT AG 3'; *yqhD*: 5' GGC AGA TCG TTC TCT GCC CTC ATA TTG GCC CAG CAA AGG GAG CAA GTA ATG GTG TAG GCT GGA GCT GCT TC 3' and 5' TAA GTC TGG ACG AAA TGC CCG AAA ACG AAA GTT TGA GGC GTA AAA AGC TTA CAT ATG AAT ATC CTC CTT AG 3'; *dkgA*: 5' ACA CCT CAC CGG AGC CTG CTC CGG TGA GTT CAT ATA AAG GAG GAA CGT ATG GTG TAG GCT GGA GCT GCT TC 3' and 5' TGA AAA GTC CGG TAG CGG AAC ATT ACC GCC ACC GGG AGA ATT TGC ATG TTA CAT ATG AAT ATC CTC CTT AG 3'; *ppx*: 5' TGG CGA TTT ATG ACT ACA TCA AAT CAC TCG AAC AAC CTG AAT AAC CCT ATG GTG TAG GCT GGA GCT GCT TC 3' and 5' AAG TGC CTG AAT AAT GCG GGC CGA CAT TTC TCG TCG GCC CGC AAA GTA TTA CAT ATG AAT ATC CTC CTT AG 3'. The presence of the desired alleles was confirmed by PCR amplification of each locus. P1*vir* transduction (Silhavy et al., 1984) was used to move the *ppk*::*kan*$^{+}$ allele from the Keio collection (Baba et al., 2006) into MG1655 and to move the *ppx*::*cat*$^{+}$ allele from MJG308 into BB7224. Chloramphenicol and kanamycin resistance cassettes were resolved where indicated to generate in-frame deletions (Datsenko and Wanner, 2000). The presence of the desired alleles was confirmed by PCR amplification and sequencing.

The *ppk* gene (2,067 bp) plus 20 bp of upstream sequence was amplified from *E. coli* MG1655 genomic DNA with primers 5' ACC GGT ACC AAA ACG GAG TAA AAG TGG TAA TGG G 3' and 5' CTT AAG CTT TTA TTC AGG TTG TTC GAG TGA TTT G 3' and cloned into the *Kpn*I and *Hin*dIII sites of plasmid pBAD33 to yield plasmid pPPK1. The *ppk* gene was subcloned from pPPK1 into the *Kpn*I and *Hin*dIII sites of plasmid pBAD18 to yield plasmid pPPK7.

The *dnaKJ* locus (3,136 bp) plus 19 bp of upstream sequence was amplified from *E. coli* MG1655 genomic DNA with primers 5' ACC GGT ACC ATA TAG TGG AGA CGT TTA GAT GGG 3' and 5' AGA TCT AGA TTA GCG GGT CAG GTC GTC AAA G 3' and cloned into the *Kpn*I and *Xba*I sites of plasmid pBAD18 to yield plasmid pDNAKJ2.

The spectinomycin resistance gene (*aadA*), encoding aminoglycoside 3' adenyltransferase (ANT), was amplified from pBAD43 (Stewart et al., 1999) using primers 5’ AAG CTT ATG CGC TCA CGC AAC TGG TCC AGA ACC 3’ and 5’ AAG CTT ATT ATT TGC CGA CTA CCT TGG TGA TC 3’ and cloned into the *Hin*dIII site of plasmid pBR322 (Bolivar et al., 1977) to yield plasmid pBR322-ANT. Strains MJG356 (MG1655 / pBAD33), MJG360 (Δ*ppk* / pBAD33), and MJG361 (Δ*ppx* / pPPK1) were transformed with pBR322-ANT to yield strains AMS346, AMS348, and AMS353, respectively.

In Vivo ATP Measurements

ATP measurements were carried out as described (Yang et al., 2002) with minor modifications. MG1655 wild-type and Δ*ppk* deletion cells were grown in MOPS medium at 37°C to an OD_{600} of approximately 0.5, at which point cells were treated with 1 mM HOCl. At the indicated time points, 100 µl bacterial culture was added to 900 µl boiling 40 mM HEPES (pH 7.8), 4 mM $MgSO_4$ and rapidly shaken for 4 min at 99°C. After boiling, the samples were transferred on ice, and the total ATP content was determined using a luciferase activity assay. For this, 50 µl samples were transferred in triplicate in a 96 well plate format, 150 µl assay buffer (140 µM

luciferin, 0.1 μM luciferase, 0.1 mg ml^{-1} BSA in 100 mM KH_2PO_4 pH 7.8, 25 mM glycylglycine, 0.2 mM EDTA) was added, and bioluminescence was recorded for 2 min.

PolyP Measurements

PolyP granules were visualized by fluorescent microscopy as described (Aschar-Sobbi et al., 2008), with modifications as follows. *E. coli* cultures were prepared as for HOCl survival assays, stained 10 min at room temperature with 50 μg ml^{-1} 4'-6-diamidino-2-phenylindole (DAPI) and 1 μg ml^{-1} N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM® 4-64) (Molecular Probes), then fixed to glass slides using poly-L-lysine and Citifluor mountant media (Ted Pella, Inc.). Cells were visualized by differential interference contrast (DIC) microscopy, and DNA, polyP granules, and cell membranes were visualized by fluorescence microscopy using the 100X oil immersion objective of an Olympus BX61 upright microscope (Olympus America, Inc.) controlled by the Metamorph Basic software package (v. 7.7.2.0) (Molecular Devices, Inc.). DNA was visualized by DNA-DAPI fluorescence (λ_{ex} = 387 ± 11 nm, λ_{em} = 440 ± 40 nm), polyP granules were visualized by polyP-DAPI fluorescence (λ_{ex} = 420 ± 40 nm, λ_{em} = 535 ± 30 nm) (Aschar-Sobbi et al., 2008), and cell membranes were visualized by FM® 4-64 fluorescence (λ_{ex} = 560 ± 25 nm, λ_{em} 607 ± 34 nm).

Cellular polyP content was also visualized by gel electrophoresis and negative DAPI staining as described (Smith and Morrissey, 2007). Samples were prepared for gel analysis by resuspending 1 mL of *E. coli* culture in 50 μl 50 mM Tris-HCl (pH 8), boiling 10 min, centrifuging 1 min at 16,100 x g, and diluting supernatants 1:1 in 6X DNA loading dye (Promega).

Intracellular polyP levels were quantified as previously described (Ault-Riche et al., 1998), with modifications as follows. A Biomek® FX fluid handling robot (Beckman Coulter) was used to automate polyP extraction, digestion, and measurement. *E. coli* strains were grown at 37°C with aeration in 50 ml MOPS medium (in 300 ml Klett flasks) to an optical density of 40 - 50 Klett units (OD_{600} ~ 0.35), tracking growth with a Klett-Summerson colorimeter. HOCl was added to 1

mM final concentration and 2 ml samples were collected by centrifugation, resuspended in 0.5 ml GITC Lysis Buffer (4 M guanidinium isothiocyanate, 50 mM Tris-HCl [pH 7]), incubated at 95°C for 5 min, then stored at -80°C. Protein concentration was determined by Bradford assay (Bio-Rad). PolyP was precipitated by addition of 0.5 ml 95% ethanol and 30 µl 10% SDS, then bound to 10 µl glassmilk (0.1 g ml^{-1} silicon dioxide in 6 M guanidine-HCl) (Boyle and Lew, 1995). Samples were transferred to AcroPrep™ Advance 96 filter plates (Pall), rinsed with 5 mM Tris-HCl [pH 7.5], 50 mM NaCl, 5 mM EDTA, 50% ethanol, and then dried by vacuum to remove residual ethanol. PolyP was eluted with 50 mM Tris-HCl (pH 8), and degraded to P_i using the yeast exopolyphosphatase ScPPX (Wurst and Kornberg, 1994). P_i concentration was measured using a colorimetric Na_2MoO_4 / malachite green assay (Carter and Karl, 1982). PolyP concentrations were expressed in terms of individual P_i units, normalized to mg of protein in the original sample.

ScPPX Purification

The *PPX1* gene (1,194 bp) was amplified from *S. cereviseae* genomic DNA with primers 5' GTC TAG ACA TAT GTC GCC TTT GAG AAA GAC GG 3' and 5' GAA TTC GGA TCC TCA CTC TTC CAG GTT TGA GT 3' and cloned into the *NdeI* and *Bam*HI sites of plasmid pET-15b to yield the N-terminally His_6-tagged ScPPX expression plasmid pScPPX2. ScPPX was overproduced from pScPPX2 in *E. coli* BL21(DE3) (Novagen). A single colony of the overexpressing strain was resuspended in 5 ml of LB broth, and 1 ml of this suspension was inoculated into 1 liter of LB broth containing ampicillin. Cultures were grown overnight at 37°C without shaking. To induce expression, these cultures were incubated at 37°C with shaking (200 rpm) for 30 min, then IPTG was added to 1 mM, and cultures were incubated for an additional 4 h at 37°C with shaking. Cells were harvested by centrifugation (20 min at 4000 rpm) and resuspended in 50 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole (pH 7.4). Lysozyme (1 mg ml^{-1}) and Benzonase (50 U ml^{-1}, Merck) were added and cells were incubated 30 min on ice.

Cells were broken by sonication (2 cycles of 2 min at 5 sec on, 5 sec off on ice). Cell lysate was cleared by centrifugation (20 min at 15,000 g at 4°C), filtered through a 0.8 µm syringe filter (Gelman), and applied to a 5 ml nickel-charged HiTrap Chelating HP column (GE Biosciences) at 4 ml min^{-1} with a P1 peristaltic pump. The column was rinsed with 50 ml 50 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole (pH 8), then with 50 ml 50 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole (pH 8). ScPPX was eluted with 50 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole (pH 8), and fractions containing ScPPX were concentrated to 1 mg ml^{-1}, dialyzed against 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 10% (v/v) glycerol, and stored at 4°C. Specific activity, determined in 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 50 mM ammonium acetate, but otherwise as described below for *E. coli* PPX, was 535 ± 105 µmol P_i min^{-1} mg $ScPPX1^{-1}$.

PPK and PPX Purification

PPK was purified as described (Zhu et al., 2003), with minor modifications. The *ppk* gene (2,067 bp) without the stop codon was amplified from *Escherichia coli* MG1655 genomic DNA with primers 5' ATG CAT ATG GGT CAG GAA AAG CTA TAC ATC G 3' and 5' CTT AAG CTT TTC AGG TTG TTC GAG TGA TTT G 3' and cloned into the *Nde*I and *Hin*dIII sites of plasmid pET-21b to yield the C-terminally His_6-tagged PPK expression plasmid pPPK2. PPK was overproduced from pPPK2 in *E. coli* BL21(DE3) (Novagen); 5 ml of an overnight culture of the overexpressing strain was inoculated into 1 liter of protein expression medium (12 g l^{-1} tryptone, 24 g l^{-1} yeast extract, 4% glycerol [vol / vol], 178 mM potassium phosphate [pH 7]) and incubated at 37°C with shaking to OD_{600} = 1. The culture was cooled to 20°C, then induced with 150 µM IPTG and incubated overnight at 20°C with shaking. Cells were harvested by centrifugation (10 min @ 6,000 g @ 4°C), resuspended in 50 mM Tris-HCl (pH 7.5), 10% sucrose (w / vol), 300 µg ml^{-1} lysozyme, then incubated 45 min on ice followed by 10 min at 37°C. Insoluble material was removed by centrifugation (10 min @ 16,100 g @ 4°C), the

supernatant was removed, and the pellet was resuspended by sonication in 50 mM Tris-HCl (pH 7.5), 10% sucrose (w / vol), 5 mM $MgCl_2$, 30 U ml^{-1} Benzonase (Merck) with Complete Mini, EDTA-free protease inhibitor cocktail (Roche). PPK was extracted from membranes by adding solid KCl to 1 M, then adding 1/10 volume 1 M Na_2CO_3 and stirring 30 min at 4°C. The resulting solution was sonicated for 2 min (5 sec on, 5 sec off) and cell debris was removed by centrifugation (1 h @ 20,000 g @ 4°C). The supernatant was immediately diluted 1:1 with distilled water, then applied to a 5 ml nickel-charged HiTrap Chelating HP column (GE Biosciences) equilibrated with 50 mM HEPES, pH 7.5, 0.5 M NaCl, 15% glycerol, 50 mM imidazole. PPK was eluted with a 17 column-volume linear gradient to 0.5 M imidazole, using an ÄKTA FPLC (Amersham Pharmacia). Fractions containing pure PPK were pooled and dialyzed overnight against 20 mM HEPES, pH 8, 150 mM NaCl, 15% glycerol, 1 mM EDTA, 5 mM DTT and stored at -80°C.

The *E. coli* PPX overexpression vector pEcPPX was a gift from David Sanders (Purdue University) (Alvarado et al., 2006), and PPX was purified by anion exchange and hydroxyapatite affinity chromatography. PPX was overproduced from pEcPPX in *E. coli* BL21(DE3) (Novagen); a single colony of the overexpressing strain was resuspended in 5 ml of LB broth, and 1 ml of this suspension was inoculated into 1 liter of LB broth containing kanamycin. Cultures were grown overnight at 37°C without shaking. To induce expression, these cultures were incubated at 37°C with shaking (200 rpm) for 30 min, then IPTG was added to 1 mM, and cultures were incubated for an additional 2.5 h at 37°C with shaking. Cells were harvested by centrifugation (20 min @ 4,000 rpm), resuspended in 10 ml HEPES buffer (50 mM; pH 8) containing 50 mM NaCl, 1 mM EDTA, 2 mM DTT, and 10% (w/v) glycerol, then lysed by two passages through a French press (19,000 psi). The lysate was clarified by centrifugation (1 h @ 20,000 g @ 4°C) and passage through a 0.8 µm syringe filter, then applied to a 5 ml HiTrap Q anion exchange column (GE Biosciences). Proteins were eluted with a 30 column-volume linear gradient to 1 M NaCl using an ÄKTA fast pressure liquid chromatography (FPLC) system (Amersham

Pharmacia), and fractions containing PPX were pooled and concentrated to 5 ml using an Amicon Ultra 30-K MWCO centrifugal concentrator. They were then dialyzed against 50 mM HEPES (pH 8), 50 mM NaCl, 10% glycerol, 1 mM DTT, 5 mM K_2HPO_4 and applied to a 69 ml hydroxyapatite column (Bio-Rad). PPX was eluted with a 3 column-volume linear gradient to 400 mM K_2HPO_4, and fractions containing pure PPX were pooled, concentrated, dialyzed against 50 mM HEPES / KOH (pH 8), 50 mM NaCl, 10% (w/v) glycerol, 1 mM EDTA, 1 mM DTT, and stored at -80°C.

PPK and PPX Activity Assays

PPK and PPX activity were assayed as described (Akiyama et al., 1993; Tzeng and Kornberg, 2000), with minor modifications as follows. Oxidized PPK samples were prepared by incubating PPK for 15 min at 37°C in 20 mM sodium phosphate (pH 8), 150 mM NaCl, 15% (v/v) glycerol with or without the indicated molar ratios of *N*-chlorotaurine. Oxidants were removed with P-30 gel chromatography columns (Bio-Rad). Forward (polyP-generating) reaction mixtures contained 50 mM HEPES (pH 7.5), 50 mM ammonium sulfate, 5 mM $MgCl_2$, and 10 nM PPK. After equilibration (1 min) at 37°C, reactions were started by addition of ATP to 10 mM, and aliquots were taken at 1 min intervals, stopping the reaction by mixing with 4 volumes of GITC Lysis Buffer. PolyP content of reaction aliquots was assayed as described above for *in vivo* polyP measurements, and specific activities were calculated as μmol polyP formed (in individual P_i units) min^{-1} mg PPK^{-1}. Reverse (ATP-generating) reaction mixtures contained 50 mM HEPES (pH 7.5), 50 mM ammonium sulfate, 2 mM MgADP, and 50 nM PPK. After equilibration (1 min) at 37°C, reactions were started by addition of polyP to 500 μM, and aliquots were taken at 30 sec intervals, stopping the reaction by incubating 5 min at 99°C. The ATP content of PPK reaction aliquots was measured using luciferase (Lundin, 2000) and specific activities were calculated as μmol ATP formed min^{-1} mg PPK^{-1}.

Oxidized PPX samples were prepared by incubating PPX for 15 min at 37°C in 50 mM potassium phosphate (pH 8), 175 mM KCl containing the indicated molar ratios of *N*-chlorotaurine or H_2O_2. Oxidants were removed with P-30 gel chromatography columns (Bio-Rad). Reaction mixtures contained 50 mM HEPES (pH 8), 175 mM KCl, 1 mM $MgCl_2$, and 1 nM PPX. After equilibration (1 min) at 37°C, reactions were started by addition of polyP to 200 μM, and aliquots were taken at 1 min intervals, stopping the reaction by mixture with 0.7 volume of Buffer A (4 parts 2 N HCl, 3 parts 0.1 M Na_2MoO_4). Free phosphate was measured with the Na_2MoO_4 / malachite green colorimetric assay (Carter and Karl, 1982), and specific activities were calculated as μmol P_i released min^{-1} mg PPX^{-1}.

Mass Spectrometry for Cysteine Thiol Status.

PPX, with or without *N*-chlorotaurine oxidation (as above), was prepared for mass spectrometry by differential thiol trapping. PPX samples (20 μg) were precipitated with trichloroacetic acid (TCA), then incubated 1 hr at room temperature in pH 8.5 denaturing alkylating buffer (DAB) (200 mM Tris-HCl, 6 M urea, 10 mM EDTA, 0.5% sodium dodecylsulfate [SDS]) containing 100 mM iodoacetamide (IAM) to irreversibly alkylate reduced cysteine residues. After reduction in DAB (pH 8.5) containing 100 mM DTT, samples were further modified by incubation for 1 hr in DAB (pH 7) containing 100 mM *N*-ethylmaleimide to irreversibly modify any cysteine which had been reversibly oxidized in the original sample. Cysteine modification status was determined by LC-MS/MS of tryptically-digested samples (MS Bioworks, Ann Arbor, MI).

***In Vivo* Thiol Trapping of PPX.**

E. coli BL21 containing plasmid pEcPPX was grown at 37°C in MOPS medium to OD_{600} = 0.4 - 0.5. PPX expression was induced with 50 μM IPTG for 30 min, then cells were treated with 1 mM HOCl. Before and after treatment, 2 ml aliquots were lysed in the presence of 10% trichloroacetic acid (TCA) to prevent further thiol oxidation. After 30 min incubation on ice,

precipitated proteins were pelleted by centrifugation (13,000 rpm, 20 min, 4°C). The protein pellet was resuspended in denaturing alkylating buffer (DAB, 6 M Urea, 200 mM Tris-HCl [pH 8.5], 10 mM EDTA, 0.5% w/v SDS) supplemented with 100 mM IAM to irreversibly alkylate all reduced cysteines and incubated 1 hr at 25°C with shaking. Samples were again precipitated with TCA to remove unbound IAM and pelleted by centrifugation. For differential thiol trapping with PEG-maleimide, protein pellets were resuspended in DAB buffer supplemented with 10 mM DTT and incubated for 1 h at 25°C to reduce oxidized cysteines. Excess DTT was removed by TCA precipitation and centrifugation. All newly accessible cysteines were then modified with 10 mM of the thiol-specific alkylation reagent PEG-maleimide, which adds 2 kDa to every modified cysteine. Samples were incubated for 1 hr at 25°C, again precipitated with TCA to remove unbound PEG-maleimide, and then suspended in reducing loading buffer. As controls, purified PPX protein samples were reduced with DTT, then alkylated with either IAM or PEG-maleimide, as described above. All samples were separated by SDS-PAGE and visualized by western blot using polyclonal anti-PPX antibodies (Pacific Immunology).

Crude Cell Lysate Preparation

E. coli strains were incubated overnight in 30 mL LB at 37°C with shaking (200 rpm), then subcultured into 2 L LB and grown at 37°C with shaking to OD_{600}= 0.6-0.7. Cells were harvested by centrifugation and resuspended in 25 mL ice-cold 50 mM Tris-HCl (pH 7.5), 150 mM NaCl. Cells were then spun down again (10 min @ 8,000 rpm @ 4°C) and resuspended in 10 mL ice-cold buffer. Phenylmethanesulfonylfluoride (PMSF) was added to 1 mM and the cells were broken with a French press (2 passages at 20,000 psi). Cell debris was spun out (5 min @ 3,000 g) and aliquots of the supernatant were stored at -80°C. Before use, lysates were thawed on ice, spun down (5 min @ 16,100 g), and exchanged into 10 mM potassium phosphate (pH 7.5) using 7K MWCO Zeba Spin Desalting Columns (Thermo Scientific). Lysates were spun again (20 min @ 16,100 g @ 4°C) to remove any remaining insoluble material before use.

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