# Supporting Information

*Reagents* — All restriction enzymes and DNA modifying enzymes were obtained from New England BioLabs (Beverly, MA). *Taq* DNA polymerase was from Promega (Madison, WI). QIAprep spin plasmid mini-prep kit, QIAEX II gel purification kit, and QIAquick PCR purification kit were purchased from Qiagen (Valencia, CA). Various oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA). Unless otherwise specified, all chemicals were obtained from Sigma (St. Louis, MO).

*Bacterial strains, plasmids and cell growth — E. coli* BL21 (DE3) and DH5α were obtained from Novagen (Madison, WI) and the Media Preparation Facility of the University of Illinois Biochemistry Department (Urbana, IL), respectively. *Streptomyces wedmorensis* was obtained from ATCC (Manassas, VA) and *Streptomyces luridus* was obtained from the National Center for Agricultural Utilization Research (Peoria, IL). Vectors pET-28a(+) and pET-15b(+) were purchased from Novagen (San Diego, CA) and were used for cloning and expression of N-terminal $His_6$-tagged FomC and DhpG. When necessary, kanamycin and ampicillin were added at 50 μg/mL and 100 μg/mL, respectively. *S. wedmorensis* and *S. luridus* were grown aerobically at 30 °C in malt-yeast-glucose (MYG) broth (4 g/L yeast extract, 10 g/L malt extract and 4 g/L glucose) with constant shaking (250 rpm). The corresponding solid medium ISP2 was obtained from Difco (Franklin Lakes, NJ).

*S. wedmorensis fomC* and *S. luridus dhpG* cloning — *S. wedmorensis* and *S. luridus* were grown in 3 mL ISP2 medium for 24 hours at 30 °C and genomic DNA was prepared using Wizard® genomic DNA purification kit (Promega, Madison WI). The *S. wedmorensis fomC* gene was PCR-amplified using a forward primer 5'-ATATGCTACAT*ATG*AGTCCGC GCCGTCC-3' (NdeI restriction site is underlined; the sequence corresponding to the first 17 bp of *fomC* is italicized and the start codon GTG is changed to ATG) and a reverse primer 5'-AGTCAGTCAAGCTT*TCAGGAACACCTCATGAG*-3' (HindIII restriction site is underlined, and the sequence corresponding to the last 18 bp of *fomC* is italicized). A mixture of *Taq* DNA polymerase (Promega, Madison WI) and *pfu* Turbo DNA polymerase (Stratagene, La Jolla, CA) with a unit ratio of 1:1 was used to minimize potential point mutations introduced by PCR under standard conditions. Failsafe™ 2x premix buffer G from EPICENTRE Biotechnologies (Madison, WI) was used due to its advantage in amplifying fragments from a genome with high GC content. The PCR program is 3 min at 98 °C followed by 20 cycles of 10 sec at 98 °C, 1 min at 60 °C and 2 min at 72 °C, and a final elongation of 8 min at 72 °C. The PCR product was digested with NdeI and HindIII and purified using gel purification kit purchased from Qiagen. The purified product was ligated into the NdeI- and HindIII-digested vector pET-28a(+). *S. luridus dhpG* was PCR-amplified similarly using primers 5'-GCGGCCGCCAT*ATG*AGCCGGCCCCTGGCG-3' (NdeI restriction site is underlined; the sequence corresponding to the first 18 bp of *DhpG* is italicized) and *5'*-GCGGCCGCGGATCC*ACGTCAGGGGCAGCAC*-3' (BamHI restriction site is underlined; the sequence corresponding to the last 16 bp of *dhpG* is italicized). The PCR product was digested by NdeI and BamHI and subjected to the ligation reaction with the digested pET-15b(+) vector. The sequences of the coding regions were confirmed at the Biotechnology Center of University of Illinois using the Big Dye™ Terminator sequencing method and an ABI PRISM 3700 sequencer (Applied Biosystems, Foster City, CA).

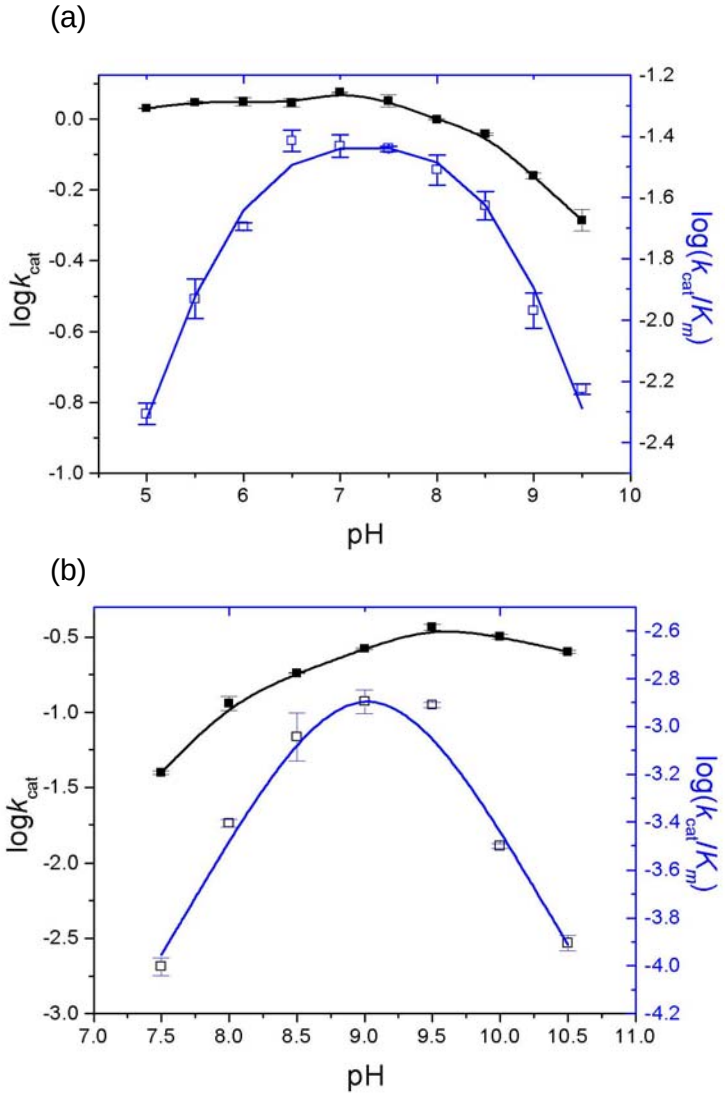


Fig. S1. (a) pH dependence of steady state kinetic parameters of FomC, log$k_{cat}$ (■), log$k_{cat}/K_m$ (□), for PnAA reduction with PnAA and NADPH as substrates. (b) pH dependence of steady state kinetic parameters of FomC, log$k_{cat}$ (■), log$k_{cat}/K_m$ (□), for HEP oxidation with HEP and $NADP^+$ as substrates. For assays at different pH values, the reactions were performed in universal buffers. Universal buffer I (25 mM acetate/MES/HEPES/borate plus 200 mM NaCl) with pH 5.0-9.5 was used for determining the kinetic parameters for PnAA reduction. Universal buffer II (25 mM HEPES/glycine plus 100 mM NaCl) with pH 7.5-10.5 was used for determining the kinetic parameters for HEP oxidation. To construct the pH profiles, the kinetic parameters $k_{cat}$ and $k_{cat}/K_m$ for PnAA and HEP were determined at each pH, and the pH dependence of $Y$ ($k_{cat}/K_m$) was fitted to a bell-shaped curve described by equation 1, where $H$ is the proton concentration, $K_1$ and $K_2$ are the dissociation constants for the groups that ionize at low and high pH, respectively, and $Y_H$ is the pH-independent plateau value of $Y$ at intermediate pH. The pH profile for $k_{cat}$ was constructed in a point-to-point manner.

$$\log Y = \log[Y_H /(1 + H / K_1 + K_2 / H)] \qquad \text{(Eq.1)}$$

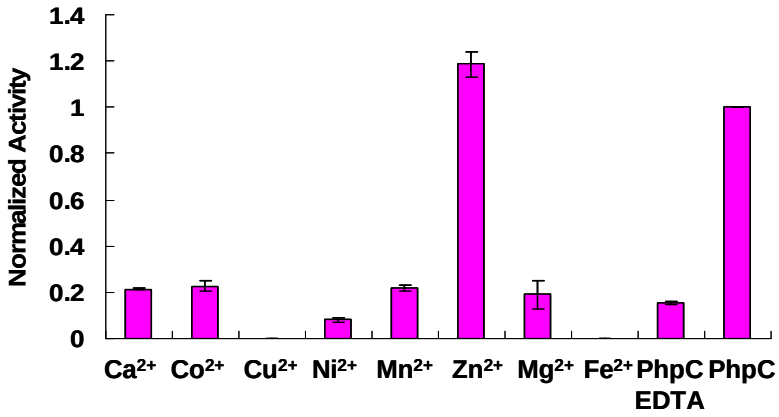


Fig. S2. Determination of the metal identity of PhpC. EDTA was used to chelate the metal from the active enzyme. After removing the free EDTA in excess, different divalent metals were mixed with the inactivated PhpC. The activity of PhpC reconstituted by divalent metals was assayed and compared with that of the holo form PhpC as a control.

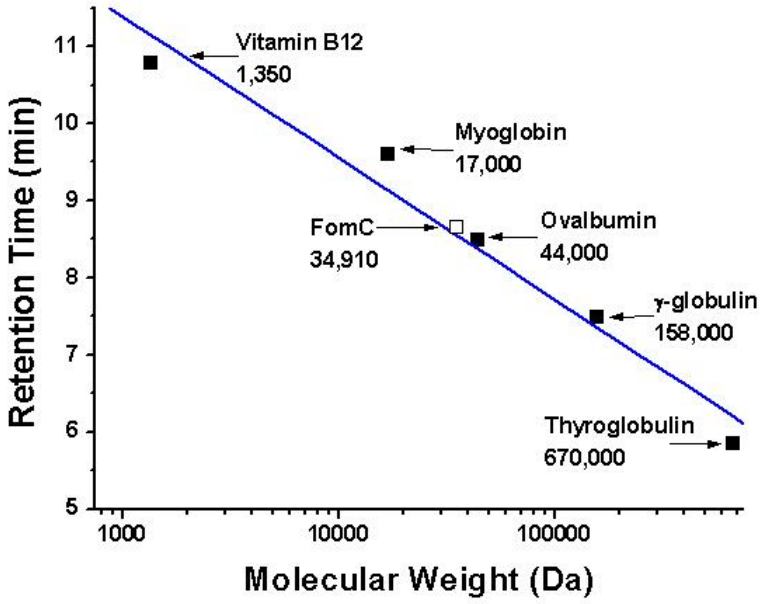


Fig. S3. HPLC size exclusion chromatography to determine the quaternary structure. The standard proteins are represented by closed square. The retention time of FomC sample (represented by open square) was fitted to the standard curve.

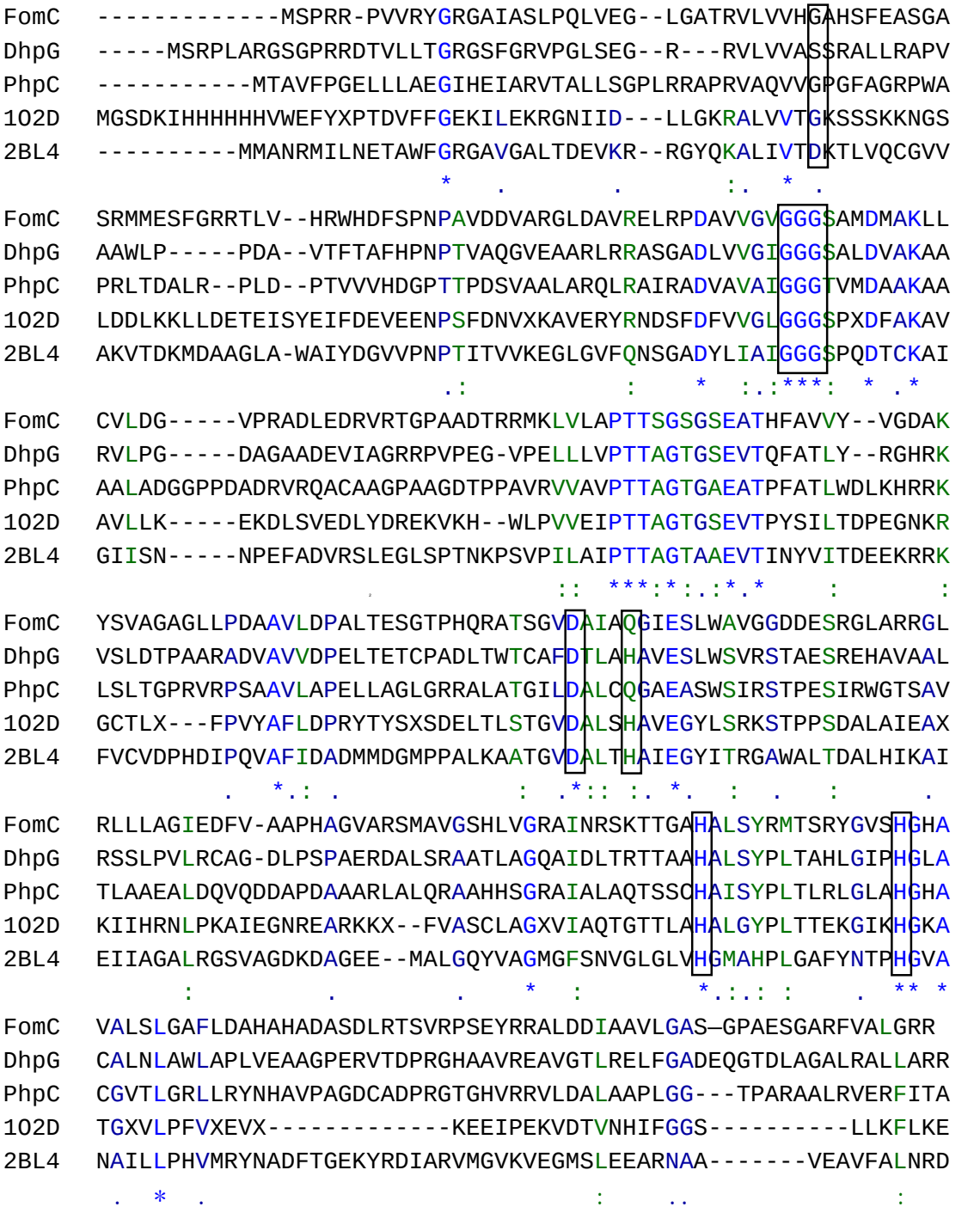


Fig. S4. Multiple sequence alignment. Key residues are indicated by boxes. 1O2D: alcohol dehydrogenase from *Thermotoga maritime*; 2BL4: 1, 2-propanediol oxidoreductase from *E. coli*; NAD(P)H-preferring residues: Gly35 (FomC), Ser41 (DhpG), Gly40 (PhpC); NAD(P)H recognition residues: Gly91, Gly92, Gly93 (FomC), Gly92, Gly93, Gly94 (DhpG), Gly94, Gly95, Gly96 (PhpC); Metal coordinating residues: Asp189, Gln193, His257, His271 (FomC), Asp189, His193, His257, His271 (DhpG), Asp199, Gln203, His268, His282 (PhpC).

| Gene ID | BLAST Homolog | Identity |
|---|---|---|
| | *Amycolatopsis orientalis* | |
| CZA382.04 | serine--glyoxylate transaminase (*Syntrophomonas wolfei*, 383 aa) | 37% |
| CZA382.05 | aminotransferase, class I and class II (*Chloroflexus aurantiacus*, 386 aa) | 36% |
| CZA382.06 | nucleotidyl transferase (*Carboxydothermus hydrogenoformans*, 237 aa) | 32% |
| CZA382.07 | *N*-acetylglutamate synthase and related acetyltransferase (*Thermobifida fusca*, 269 aa) | 24% |
| CZA382.08 | transposase (*Streptomyces coelicolor*, 281 aa) | 26% |
| | *Azoarcus sp*. BH72 | |
| azo2697 | glycosyltransferase (*Oceanobacter sp.*, 346 aa) | 32% |
| azo2701 | CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase (*Congregibacter litoralis*, 391 aa) | 33% |
| azo2702 | glycosyl transferase, family 2 (*Pseudomonas putida*, 1162 aa) | 33% |
| azo2703 | TPR repeat (*Methylobacillus flagellatus*, 700 aa) | 36% |
| azo2704 | flagellin domain protein (*Verminephrobacter eiseniae*, 501 aa) | 55% |
| | *Frankia alni* ACN14a | |
| FRAAL6372 | aldehyde dehydrogenase PhpJ (*Streptomyces viridochromogenes*, 466 aa) | 47% |
| FRAAL6373 | hypothetical protein | |
| FRAAL6374 | Permeases of the major facilitator superfamily (*Brevibacterium linens BL2*, 452 aa ) | 27% |
| FRAAL6375 | PhpD (*Streptomyces viridochromogenes*, 443 aa) | 51% |
| FRAAL6376 | putative D-3-phosphoglycerate dehydrogenase PhpE (*Streptomyces viridochromogenes*, 336 aa) | 62% |
| | *Delta proteobacterium* MLMS-1 | |
| MldDRAFT_5199 | lic-1 operon protein (*Haemophilus influenzae*, 267 aa) | 19% |
| MldDRAFT_5200 | thymidylate kinase (*Salinispora tropica*, 203 aa) | 26% |
| MldDRAFT_5187 | transposase, IS5 family (*Azoarcus sp.*, 345 aa) | 32% |
| MldDRAFT_5188 | transposase, IS5 family (*Azoarcus sp.*, 299 aa) | 22% |
| MldDRAFT_5189 | transposase, IS204/IS1001/IS1096/IS1165 family protein (*Alkalilimnicola ehrlichei*, 421 aa) | 68% |
| | *Bacteroides fragilis* YCH46 | |
| BF2575 | glycosyl transferase, family 2 (*Geobacter uraniumreducens*, 316 aa) | 28% |
| BF2579 | putative cholinephosphotransferase (*Bacteroides vulgatus*, 428 aa) | 32% |
| BF2580 | putative carbamoylphosphate synthase large subunit short form (*Anabaena variabilis*, 428 aa) | 38% |
| BF2581 | nucleotide sugar transaminase (*Bacteroides thetaiotaomicron*, 369 aa) | 76% |
| BF2582 | putative lipopolysaccharide biosynthesis protein (*Bacteroides thetaiotaomicron*, 494 aa) | 43% |

Table S1. Open reading frame analysis of the clusters containing PPM, PPD and ADH.