Supplemental Material

Expression and purification of PqqE grown aerobically and isolated from inclusion bodies under aerobic conditions. 100 mL starter cultures of E. coli BL21 (DE3) cells containing pET24b-pqqE were grown overnight aerobically at 37 ºC in LB media containing 50 µg/mL of kanamycin (kan). After approximately 16 h, 15 mL of the starter cultures were transferred to 4 L Erlenmeyer flasks containing 2 L of LB media with 50 µg/mL of kan. The flasks were left to shake aerobically at 37 ºC for approximately 3-4 h. Once the OD\textsubscript{600} reached 0.7, 0.1-1.0 mM of IPTG and 5 mg/L of Fe(NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{2}6(H\textsubscript{2}O) were added to the flasks and the cultures left to shake at 37 ºC for 4 h. After 4 h, the cells were harvested via centrifugation and frozen at -80 ºC until further use (cell paste yields were approximately 28 g per 8 L of LB).

The cell paste was reconstituted on ice with 200 mL of 50 mM Tris (pH 7.9), 1 mM dithiothreitol (DTT), 300 mM NaCl, 5 µL of Benzonase nuclease (Novagen) and lysed via sonication (power at 30% for 3 x 15 min). After lysis, the cells were centrifuged at 15,000 x g at 4 ºC for 20 min. When the cells were grown and induced in this manner, PqqE was found solely in insoluble inclusion bodies. They could be dissolved in a concentrated urea solution (50 mM Tris, 1 mM DTT, 300 mM NaCl, 8 M Urea, pH 7.9) and then refolded via dialysis in a low ionic strength buffer (50 mM Tris, 1 mM DTT, pH 7.9). After dialysis, the refolded apo-enzyme was purified via strong anion exchange (Q-sepharose). The Q-sepharose was equilibrated with 50 mM Tris, 1 mM DTT, pH 7.9, and a linear gradient from 0-400 mM KCl was used to elute PqqE from the column. Under these conditions, PqqE eluted at approximately 200 mM KCl. The protein was then
dialyzed overnight into 50 mM Tris (pH 7.9), 1 mM DTT, 300 mM NaCl and concentrated to approximately 10 mg/mL using a 30 kDa Amicon membrane (Millipore). The protein was then further purified by gel filtration (Sephacryl S-300). The protein was finally concentrated down to approximately 10 mg/mL using a 30 kDa Amicon membrane, divided into 200 µL aliquots, frozen in liquid nitrogen and stored at -80 ºC until further use.

Expression and purification of PqqE grown anaerobically and isolated aerobically from soluble supernatants. Initial starter cultures were grown in an identical manner to those described above. After 16 h, the starter cultures were transferred to 2 L Erlenmeyer flasks containing LB with kan and equipped with a rubber septum. The head space of the flasks was purged with Argon and left to grow anaerobically at 30 ºC for approximately 8 h. Once the OD$_{600}$ reached 0.1, 0.1 mM IPTG and 5 mg/L Fe(NH$_4$)$_2$(SO$_4$)$_2$6(H$_2$O) were added to the flasks and the head space purged with argon. The cultures were left to grow anaerobically at 18 ºC overnight. The next day, the cells were harvested via centrifugation and frozen at -80 ºC until further use (cell paste yields were approximately 4-6 g per 10 L of LB).

The cell paste was reconstituted on ice with 200 mL of 50 mM Tris (pH 7.9), 5 mM DTT, and 5 µL of benzonase nuclease. The cells were then lysed on ice at 0 ºC via sonication (power at 30% for 3 x 15 min). After lysis, the cells were centrifuged at 15,000 x g at 4 ºC for 20 min. The supernatants of PqqE grown and lysed in this manner were dark brown-red in color and rapidly precipitated unless stabilized with a large
excess (5 mM) of DTT. After removal of insoluble material, the enzyme was then purified and stored as described above.

**Anaerobic reconstitution of apo-PqqE.** PqqE isolated aerobically was made anaerobic by several vacuum and argon purge cycles. The enzyme was then brought into the anaerobic chamber and mixed with a ten-fold excess of DTT, followed by stepwise additions of 5x molar excess of ferrous/ferric ions, and 10x molar excess of sulfide ions. The buffer conditions were 50 mM Tris (pH 7.9), 1 mM DTT, 300 mM NaCl. The protein solutions were left to incubate anaerobically at room temperature for approximately 1 h, and then desalted over a PD-10 gel filtration column (GE Healthcare).

**Synthesis and purification of S-adenosyl-L-methionine (SAM).** Procedures for the expression and isolation of cell lysates containing SAM synthetase are described by Park et al. (13). In brief, *E. coli* TB1 (pUC18: sam2) was grown at 37 ºC overnight in LB media containing 50 µg/mL ampicillin. The cells (about 20 g from 8 L) were harvested via centrifugation and reconstituted in 75 mL of 100 mM Tris (pH 8.0) and 1 mM EDTA. Lysozyme (Sigma) was added to the mixture for a final concentration of 50 µg/mL and left to incubate at room temperature for 30 min. Phenylmethanesulfonyl fluoride (0.1 mM) was added to the solution and the cells lysed via sonication in an ice bath. The cell lysates were cleared via centrifugation and the supernatants stored at -20 ºC until further use.

The synthesis and purification of SAM were carried out using a similar procedure to that outlined by Walsby et al. (14), with a few minor modifications. A typical 10 mL
reaction mixture contained 100 mM Tris (pH 7.9), 1 mM EDTA, 50 mM KCl, 26 mM MgCl₂, 13 mM ATP, 10 mM methionine, 8 % β-mercaptoethanol, and 2 mL of SAM synthetase lysate. The reactions were left stirring vigorously overnight at room temperature in the dark. After approximately 16 hrs the reactions were quenched with 1 mL of 1.0 M HCl and then centrifuged at 4 °C at 15,000 x g for 20 min. The lysates were then purified via FPLC (BioRad) using an UNO S-6 (BioRad) cation exchange column. A linear gradient from 0-1 M HCl was used, and SAM eluted at approximately 0.4 M HCl. The fractions containing SAM were pooled and lyophilized to dryness. After the samples had been completely dried, they were brought into the glove box and anaerobically reconstituted with 50 mM Tris (pH 7.9) and 1 mM DTT. Samples were distributed into 50 µL aliquots, stored in glass vials equipped with a rubber cap, brought out of the glove box and immediately frozen in liquid nitrogen. The samples could be stored at -80 °C for several months before use. Final yields for SAM from this procedure were approximately 5-15 % (calculated from the known ε₂₆₀ = 15,400 M⁻¹cm⁻¹) (15).

Methods and instrumentation for high-resolution mass spectral analyses of PqqA and reaction mixtures. Peptides were analyzed using an Agilent 1200 series liquid chromatograph that was connected in-line with an LTQ Orbitrap XL hybrid mass spectrometer equipped with an Ion Max electrospray ionization source (ESI; Thermo Fisher Scientific, Waltham, MA). The LC was equipped with C₈ guard (Poroshell 300SB-C8, 5 µm, 2.1 × 12.5 mm, Agilent) and analytical (75 × 0.5 mm) columns and a 100 µL sample loop. Solvent A was 0.1 % formic acid in water and solvent B was 0.1 % formic acid in acetonitrile (v/v). Sample solutions contained in 0.3 mL polypropylene
snap-top vials sealed with rubber septa caps were loaded into the Agilent 1200 autosampler compartment prior to analysis. Approximately 50 to 100 picomoles of analyte were injected onto the column. Following sample injection, analyte trapping was performed for 5 min with 99.5 % A at a flow rate of 90 µL/min. The elution program consisted of a linear gradient from 35 % to 95 % B over 34 min, isocratic conditions at 95 % B for 5 min, a linear gradient to 0.5 % B over 1 min, and then isocratic conditions at 0.5 % B for 14 min, at a flow rate of 90 µL/min. The column was maintained at 35 ºC. The connections between the LC column exit and the mass spectrometer ion source were made using PEEK tubing (0.005" i.d. × 1/16" o.d.). External mass calibration was performed prior to analysis using the standard LTQ calibration mixture containing caffeine, the peptide MRFA, and Ultramark 1621 dissolved in 51 % acetonitrile/25 % methanol/23 % water/1 % acetic acid solution (v/v). The ESI source parameters were as follows: ion transfer capillary temperature 275 ºC, normalized sheath gas (nitrogen) flow rate 25 %, ESI voltage 2.0 kV, ion transfer capillary voltage 33 V, and tube lens voltage 125 V. Mass spectra were recorded in the positive ion mode over the range m/z = 500 to 2000 using the Orbitrap mass analyzer, in profile format, with a full MS automatic gain control target setting of 5 × 10⁵ charges and a resolution setting of 6 × 10⁴ (at m/z = 400, FWHM). Mass spectra were processed using Xcalibur software (version 4.1, Thermo). High-resolution mass spectra of PqqA (found: m/z 2632.52 (C₁₂₄H₁₉₈N₃₂O₃₁ + H⁺), calculated: 2631.49 (C₁₂₄H₁₉₈N₃₂O₃₁) confirmed the identity of the intact peptide.