Supplementary Materials for

Paradigm Shift for Radical S-Adenosyl-L-methionine Reactions: The Organometallic Intermediate Ω is Central to Catalysis

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Materials and Methods

$^{[13]}$C$_{10},^{[15]}$N$_{5}$-Adenosine 5’-triphosphate sodium salt and adenosine-2,8-D$_{2}$-1’,2’,3’,4’,5’,5’-D$_{6}$-5’-triphosphate sodium salt solutions were purchased from MilliporeSigma/ISOTEC® Stable Isotopes. $^{[15]}$N-Methionine was purchased from Cambridge Isotope Labs, Inc. (96–98%, NLM 752). Iron-57 (93-95%) was purchased from Cambridge Isotope Labs, Inc. (FLM-1812PK). All unlabeled adenosine 5’-triphosphate and L-methionine were purchased from MilliporeSigma.

HydG Preparations

[FeFe]-hydrogenase maturase HydG preparations were performed as described previously, with minor modifications. Briefly, the HydG gene (hydG from Clostridium acetobutylicum) cloned into a pCDF DUET™-1 vector allowed for expression of HydG with an N-terminal His$_{6}$ tag in BL21-(ΔISCR) E. coli cells. After inoculation from overnight starter cultures, cell culture growth in phosphate buffered LB media (9 L in six 2.8 L Fernbach flasks), supplemented with kanamycin (30 µg/mL) and streptomycin (50 µg/mL) antibiotics, glucose (5 g/L), and iron in the form of ferric ammonium citrate (4.2 g total in 9 L) and maintained at 37°C with 200 rpm shaking, exhibited good over-expression of HydG upon induction with IPTG (0.5 mM) at an OD$_{600}$ of 0.5 AU via comparatively high protein concentrations on SDS-PAGE. After transfer to 4°C, this culture was sparged overnight (14 - 16 h) with N$_{2}(g)$ until cell pellets (~30 - 40 g) were harvested the next day and stored at -80°C.

To isolate HydG with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box (Coy Laboratories, Grass Lake, MI). At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis was performed at 4°C in Buffer A (50 mM HEPES, 250 mM KCl, 5% glycerol, 10 mM imidazole, pH 8.0) in the presence of lysozyme (180 µg/mL), phenylmethylsulfonyl fluoride (PMSF, 180 µg/mL final, added in 1 mL MeOH), DNase (50 µg/mL), RNase (50 µg/mL), 0.5% Triton™ X-100 (v/v), and MgCl$_{2}$ (2 mg/mL). After centrifugation (38,000 x g, 60 min., 4°C), the HydG clarified lysate was purified via FPLC using a step gradient into Buffer B (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 250 mM KCl, 5% glycerol, 500 mM imidazole, pH 8.0) on a Ni$^{2+}$-affinity column (5 mL). The purified enzyme solution was buffer exchanged into Buffer C (50 mM HEPES, 50 mM KCl, 5% glycerol, pH 8.0) before reconstitution.

To increase [4Fe-4S] cluster content in HydG, an iron-sulfur cluster reconstitution was performed at 4°C as described elsewhere. Briefly, purified HydG (100 µM) was incubated with DTT (5 mM) for 5 minutes in Buffer C, before sodium sulfide (0.6 mM final concentration, from a 50 mM stock) and ferric chloride (0.6 mM final concentration, from a 10 mM stock) were added, respectively, over the course of an hour. After a 3.5 hour incubation at 4°C, the reconstitution solution was centrifuged (26,000 x g, 10 min., 4°C), gel filtrated, and concentrated to yield a final iron content of 7.8 ± 0.1 irons/protein. EPR spectroscopy confirmed [4Fe-4S]$^{+}$ cluster presence in photoreduced enzyme.

Lysine 2,3-aminomutase Preparations

Lysine 2,3-aminomutase (LAM) preparations were performed as described previously to yield a final iron content of 3.9 ± 0.1 irons/protein. EPR spectroscopy confirmed [4Fe-4S]$^{+}$ cluster presence in photoreduced enzyme.
OspD and OspA (substrate) Preparations

The gene encoding OspD (OSCI_3660007 from Oscillatoria sp. PCC 6506) cloned into a modified pCDF DUET™-1 vector allowed for expression of OspD protein with a TEV protease cleavable N-terminal His tag in BL21-(AIscR) E. coli cells. After inoculation from overnight starter cultures, cell culture growth in terrific broth media (1.2% tryptone, 2.4% yeast extract, 0.5% glucose and 89 mM phosphate; 6 L in six 2.8 L Fernbach flasks) was supplemented with spectinomycin (50 µg/mL) and kanamycin (34 µg/mL) antibiotics and maintained at 37°C with 200 rpm shaking. At an OD<sub>600</sub> of 1.2, the cultures were chilled on ice for 15 minutes and supplemented with ferrous ammonium sulfate (FAS, 0.25 mM), induced with IPTG (1 mM), and incubated for over 24 h at 16°C, with shaking at 220 rpm and N<sub>2</sub> sparge until cell pellets (~40 g) were harvested the next day and stored at -80°C.

To isolate OspD with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box. At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis at ~21°C in Buffer A (50 mM HEPES, 150 mM KCl, 10% glycerol (w/v), pH 8.0) in the presence of imidazole (1 mM), fresh DTT (2 mM, added 5 min prior to lysis), MgCl<sub>2</sub> (2.5 mg/mL), lysozyme (0.18 mg/mL), Triton™ X-100 (1% w/v), PMSF (2.1 mM), and DNase and RNase (0.18 mg/mL each). After centrifugation (38,000 x g, 60 min., 4°C), the OspD clarified lysate was purified via gravity-flow on a Ni-NTA agarose column (5 mL) pre-equilibrated with Buffer A with the following procedure: 1) a 10 CV wash step with Buffer A, 2) a 10 CV wash with 15% Buffer B (50 mM HEPES, 150 mM KCl, 10% glycerol (w/v), 100 mM imidazole, pH 8.0), 3) elution of pure OspD in 75% Buffer B. Pure fractions (confirmed by SDS-PAGE) were concentrated, gel filtered (Sephadex G25 resin column, 75 mL) in Buffer A, and re-concentrated with Amicon 10 kDa MWCO spin filters.

To increase [4Fe-4S] cluster content in OspD, an iron-sulfur cluster reconstitution was performed at 4°C as described elsewhere. Briefly, purified OspD (100 µM) was incubated with DTT (5 mM) for 5 minutes in Buffer A, before ferric chloride (0.6 mM final concentration, from a 10 mM stock) and sodium sulfide (0.6 mM final concentration, from a 50 mM stock) were added, respectively, over the course of an hour. After a 2 hour incubation at 4°C, the reconstitution solution was centrifuged (26,000 x g, 10 min., 4°C), gel filtrated, and concentrated to yield a final iron content of ~4.0 ± 0.2 irons/protein. EPR spectroscopy confirmed [4Fe-4S]<sup>+</sup> cluster presence in photoreduced enzyme.

The gene encoding OspA (OSCI_3660009 from Oscillatoria sp. PCC 6506) in pET28 was used to produce the peptide substrate OspA with a thrombin-cleavable N-terminal His<sub>e</sub> tag, which was prepared as described previously. If the lysis and purification steps were performed aerobically, the OspA protein was made anaerobic through several Schlenk line nitrogen/vacuum cycles.

PFL-AE and PFL (substrate) Preparations

PFL-AE preparations were performed as described previously<sup>2</sup> with minor modifications. Briefly, the PFL-AE gene (pflA from E. coli) cloned into a pCAL-n-EK vector allowed for expression of PFL-AE protein without a tag in BL21(DE3)pLysS E. coli cells. After inoculation from overnight starter cultures, cell culture growth in LB media (9 L in six 2.8 L Fernbach flasks) was supplemented with ampicillin (50 µg/mL) antibiotic and maintained at 37°C with 200 rpm shaking. At an OD<sub>600</sub> of ~0.3, the cell culture was supplemented with glucose (5 g/L); at an OD<sub>600</sub> of ~0.8, the cell culture induced with IPTG (0.25 mM) and supplemented with FAS (0.20 mM) and L-cysteine (0.20 mM), wherein the temperature was reduced to 30°C and the pH maintained (every
30 min.) between pH 7.2-7.5 with NH₄OH (6 M) or HCl (6 M) as necessary, until the pH no longer showed a significant change (~4 - 5 h). Additional FAS and L-cysteine were added (0.40 mM, final), and the culture was transferred to 4°C and sparged overnight (14 - 16 h) with N₂(g) until cell pellets (~50 - 60 g) were harvested the next day and stored at -80 °C.

To isolate PFL-AE with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box. At a ratio of ~1- 1.5 mL of buffer per 1 g of cell pellet, lysis was performed at 4°C in buffer (50 mM Tris, 100 mM NaCl, 5% w/v glycerol, 1% Triton™ X-100, 10 mM MgCl₂, 1.0 mM dithiothreitol (DTT), pH 7.5) in the presence of lysozyme (0.32 mg/mL), PMSF (2 mM), DNase I and RNase A (0.01 mg/mL). After centrifugation (38,000 x g, 60 min., 4°C), the PFL-AE clarified lysate was purified as previously described except a lower concentration of NaCl was used in the purification buffer (50 mM Tris, 100 mM NaCl, 5% w/v glycerol, 1.0 mM dithiothreitol (DTT), pH 7.5). As the final cluster content determined by atomic absorbance spectroscopy was 3.9 ± 0.1 or 4.0 ± 0.1 Fe/protein, iron-sulfur cluster reconstitution was not needed. EPR spectroscopy confirmed [4Fe-4S]⁺ cluster presence in photoreduced enzyme.

PFL preparations were performed as described previously with minor modifications. The PFL gene (pfl from E. coli) cloned into the pKK plasmid vector allowed for expression of PFL protein without a tag in BL21(DE3)pLysS E. coli cells. PFL was grown, lysed, and purified as described previously with the following exceptions: 1) during growth, induction with IPTG (0.2 mM) was immediately followed by a temperature drop to 30°C and 14 – 16 hours of additional incubation, 2) during lysis and purification, DTT was not present in buffers (Buffer A: 20 mM Tris, pH 7.2; Buffer B: 20 mM Tris, 500 mM NaCl, pH 7.2; Buffer C: 20 mM Tris, 1 M (NH₄)₂SO₄, pH 7.2). If the lysis and purification steps were performed aerobically, the PFL protein was made anaerobically through several Schlenk line nitrogen/vacuum cycles.

PoyD and PoyA (substrate) Preparations

The gene encoding PoyD (poyD from Candidatus Entotheonella factor TSY1) cloned into a modified pCDF DUET™-1 vector allowed for expression of PoyD protein with a TEV protease cleavable N-terminal His tag in BL21-(∆ISCR) E. coli cells. After inoculation from overnight starter cultures, cell culture growth in terrific broth media (1.2% tryptone, 2.4% yeast extract, 0.5% glycerol and 89 mM phosphate; 6 L in six 2.8 L Fernbach flasks) was supplemented with spectinomycin (50 µg/mL) and kanamycin (34 µg/mL) antibiotics and maintained at 37°C with 200 rpm shaking. At an OD₆₀₀ of 1.2, the cultures were chilled on ice for 15 minutes and supplemented with FAS (0.25 mM), induced with IPTG (1 mM), and incubated for over 24 h at 16°C, with shaking at 220 rpm and N₂(g) sparge until cell pellets (~40 g) were harvested the next day and stored at -80°C.

To isolate PoyD with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box. At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis at ~21°C in Buffer A (50 mM HEPES, 150 mM KCl, 10% glycerol (w/v), pH 8.0) in the presence of imidazole (1 mM), fresh DTT (2 mM, added 5 min prior to lysis), Triton™ X-100 (1% w/v), PMSF (180 µg/mL final, added in 1 mL MeOH), DNase and RNase (0.18 mg/mL each) was performed via sonication (15 sec. pulses, 59 sec. rest, 60% amplitude, 5 min. total pulse time). After centrifugation (38,000 x g; 60 min.; 4°C), the PoyD clarified lysate was purified via FPLC using a HisTrap™ column (5 mL) pre-equilibrated with Buffer A with the following procedure: 1) a 10 CV wash step with Buffer A, 2) a 10 CV wash with 25% Buffer B (50 mM HEPES, 150 mM KCl, 10% glycerol (w/v), 100 mM imidazole, pH 8.0), 3) elution of pure PoyD in 100% Buffer B. Pure fractions
(confirmed by SDS-PAGE) were gel filtered (Sephadex G25 resin column, 75 mL) in Buffer A and concentrated with Amicon 15 kDa MWCO spin filters.

To increase [4Fe-4S] cluster content in PoyD, an iron-sulfur cluster reconstitution was performed at 4°C as described elsewhere. Briefly, purified PoyD (100 µM) was incubated with DTT (5 mM) for 5 minutes in Buffer A, before ferric chloride (0.6 mM final concentration, from a 10 mM stock) and sodium sulfide (0.6 mM final concentration, from a 50 mM stock) were added, respectively, over the course of an hour. After a 2 hour incubation at 4°C, the reconstitution solution was centrifuged (26,000 x g, 10 min., 4°C), gel filtered, and concentrated to yield a final iron content of 3.6 ± 0.1 irons/protein. EPR spectroscopy confirmed [4Fe-4S]⁺ cluster presence in photoreduced enzyme.

The gene encoding PoyA (poyA25 gene from Candidatus Entotheonella factor TSY1) cloned into a modified pET-28b vector allowed for expression of PoyA protein with a TEV protease cleavable fusion with an N-terminally His₆-tagged maltose binding protein (MBP) in BL21(DE3) E. coli cells. Though full length PoyA cannot be expressed without co-expression of PoyD epimerase and has very limited unepimerized production as an MBP fusion, the truncation variant PoyA25 can be produced in good yields. After inoculation from overnight starter cultures, cell culture growth in LB (or TB) media (1 L a 2.8 L Fernbach flask) was supplemented with kanamycin (25 µg/mL) antibiotic and maintained at 37°C with shaking (~220 rpm) to an OD₆₆₀ of 0.7 – 1.0 for the LB media or an OD₆₆₀ of 1.5 – 2.0 in the TB media. The cultures were chilled to 16 – 20°C, induced with IPTG (1 mM), and incubated overnight at 16 – 20°C while shaking (~220 rpm). To harvest the cells, the cultures were centrifuged (12,000 x g, 10 min., 4°C) and the wet cell pellets were immediately frozen in liquid nitrogen and stored in -80°C.

As PoyA does not contain an FeS cluster, all lysis and purification steps were performed aerobically. To cleave the MBP solubility tag, purification of PoyA required a double Ni-affinity purification with a TEV cleavage step in between. Isolation of PoyA started with cell lysis by sonication (10x 10 sec on, 10 sec off, 70% amplitude 6.4 mm probe QSonica Q700 sonicator) in 4 mL per gram cell pellet cold Buffer P (100 mM NaPO₄, 300 mM NaCl, 10% glycerol, 5 mM imidazole, pH 8.0) supplemented with protease inhibitor cocktail (cOmplete Protease Inhibitor Cocktail tablets, Roche) and 1 mg/mL lysozyme treatment for 1 h prior to sonication. After sonication, the cell lysate was centrifuged (27,000 x g, 30 min., 4°C) and the clarified lysate supernatant was bound to cOmplete His-tag Purification Resin (Roche) for 1 h before being transferred to a gravity flow column and treated with the following wash steps: 1) 4x 5 CV Buffer P; 2) 1x 5 CV Buffer P with 20 mM imidazole; and 3) 3x 1 CV Buffer P with 250 mM imidazole to elute PoyA. Fractions with the His₆-MBP-PoyA identified by SDS-PAGE were pooled, concentrated (Amicon Ultra 10 kDa MWCO filter units), and buffer exchanged into Buffer T (50 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 8.0) using a PD-10 BioRad desalting column (load 2.5 mL, elute 3.5 mL). TEV cleavage at room temperature overnight with a ratio of 1:100 of His₆TEV-pro tease to His₆-MBP-PoyA (expressed and purified as previously described) removed the His₆-MBP tag, as monitored by SDS-PAGE. Once complete, this cleavage reaction was supplemented with NaCl (150 mM), centrifuged to clarify any precipitant that formed, and the supernatant was passed over a cOmplete His Purification resin twice to isolate PoyA. The flowthrough and first wash (1 CV Buffer T) were pooled and concentrated to give relatively pure PoyA, which was then supplemented with 10% glycerol and flash frozen in liquid nitrogen prior to storage at -80°C. As this substrate protein doesn’t coordinate FeS clusters, reconstitution was not needed. If the lysis and purification steps were performed aerobically, the PoyA protein was made anaerobic through several Schlenk line nitrogen/vacuum cycles.
Anaerobic RNR-AE and RNR (substrate) Preparations

From *E. coli*, the gene encoding RNR-AE was isolated using primers 5'-TAC ATA TGA ATT ATC ATC AGT ACT ATC CTG TCG-3' (forward) and 5'-CTA AGC TTT CAT CGC AAA TGA ACC ACC -3' (reverse) which imparted restriction enzyme cleavage sites for NdeI and HindIII restriction enzymes. This RNR-AE gene (*nrdG* from *E. coli*) cloned into a pCAL-n-EK vector allowed for expression of the 17 kDa RNR-AE protein without a tag in BL21(DE3)pLysS *E. coli* cells. After inoculation from overnight starter cultures, cell culture growth in minimal media (9 L in a 14 L fermenter) supplemented with ampicillin (50 µg/mL) and chloramphenicol (50 µg/mL) antibiotic and FAS (0.3 mM) was maintained at 37°C with 200 rpm shaking. Upon induction at OD$_{600}$ of 0.5 with IPTG (0.5 mM), the temperature was dropped to 25°C and 2.5 hours later to 4°C, wherein this culture was sparged overnight (14 - 16 h) with N$_2$(g) until cell pellets (~20 g) were harvested the next day and stored at -80°C.

To isolate RNR-AE with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box. At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis at 4°C in buffer (50 mM Tris, 200 mM NaCl, 5% glycerol, pH 8.5, 1% Triton™ X-100, 10 mM MgCl$_2$) was supplemented with PMSF (1 mM), lysozyme (8 mg/40 mL), and DNase and RNase (0.1 mg per 40 mL). After 15 minutes on ice, the cells were chemically lysed for 1 h, with stirring. The lysate solution was centrifuged (38,000 x g, 30 min., 4°C) and the supernatant was subjected to two ammonium sulfate cuts (0 – 20% ammonium sulfate and 20 – 60% ammonium sulfate). After centrifugation of the last cut (38,000 x g, 30 min., 4°C), the RNR-AE was found in the pellet which was solubilized on ice in Buffer R (50 mM Tris, 200 mM NaCl, 1 mM DTT, pH 8.5) and loaded at 3 mL/min via a chilled Superloop (50 mL) onto a Superdex 75 AP5 column (~800 mL column volume). The RNR-AE eluted between 575 mL and 750 mL and the pooled fractions were concentrated (Amicon YM-10 spin filter) and reloaded onto the same column that had been equilibrated in Buffer R. The RNR-AE fractions with the best iron content as illustrated by the A$_{426}$ : A$_{280}$ ratio (~0.160 – 0.150) eluted between 700 mL and 720 mL and were pooled and concentrated (on ice).

To increase [4Fe-4S]$^+$ cluster content in RNR-AE, an iron-sulfur cluster reconstitution was performed at 4°C as described elsewhere.$^2$ Briefly, purified RNR-AE (100 µM) was incubated with DTT (5 mM) for 20 minutes in buffer (100 mM Tris, 200 mM NaCl, pH 8.5), before sodium sulfide (0.5 mM final concentration, from a 50 mM stock) and ferric chloride (0.5 mM final concentration, from a 10 mM stock) were added, respectively, over the course of two hours. After a 2.5 hour incubation at 4°C, the reconstitution solution was centrifuged (38,000 x g, 10 min., 4°C), gel filtrated, and concentrated to yield a final iron content of 3.5 ± 0.2 iron/ protein. EPR spectroscopy confirmed [4Fe-4S]$^+$ cluster presence in photoreduced enzyme.

From *E. coli*, the RNR gene was isolated using primers 5'-TAC ATA TGA CAC CGC ATG TGA TGA AAC CAG ACG-3' (forward) and 5'-CTA AGC TTT TAA CCT ATC TGC CCA TTC CCC AAA -3' (reverse) which imparted restriction enzyme cleavage sites for NdeI and HindIII restriction enzymes. The RNR gene (*nrdD* from *E. coli*) cloned into a pCAL-n-EK vector allowed for expression of RNR protein without a tag in BL21(DE3)pLysS *E. coli* cells. After inoculation from overnight starter cultures, cell culture growth, in LB media (4.5 L in three 2.8 L Fernbach flasks) supplemented with ampicillin (50 µg/mL) and maintained at 37°C with 250 rpm shaking, exhibited good over-expression of RNR upon induction with IPTG (1 mM) at an OD$_{600}$ of 0.5 AU via comparatively high protein concentrations on SDS-PAGE. After induction, cultures were grown with shaking for an additional 3 hours; the cells were centrifuged (12,000 x g, 10 min., 4°C) and the cell pellet (~25 g) was flash frozen in liquid nitrogen and stored at -80°C.
As RNR does not contain FeS cluster content, all lysis and purification steps were performed aerobically. At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis in buffer (20 mM Tris, 1 mM DTT, 10 mM MgCl₂, 1% Triton™ X-100, 5% glycerol, pH 7.8) in the presence of PMSF (1 mM), DNase and RNase (0.5 mg each). After centrifugation (38,000 x g, 30 min., 4°C), the supernatant was loaded onto an Accell Plus™ QMA Ion exchange AP5 column (~600 mL) that had been equilibrated with Buffer A (20 mM Tris, 1 mM DTT, pH 7.8) at 3 mL/min. The purification steps included an 300 mL isocratic step with Buffer A, followed by a linear gradient from 0% into 100% Buffer B (20 mM Tris, 500 mM NaCl, 1 mM DTT, pH 7.8) over 900 mL, and ended with a 300 mL isocratic step in 100% Buffer B; all steps were performed at 5 mL/min. Fractions collected between ~1000 and 1500 mL were pooled and concentrated (Amicon YM-80 spin filters) and buffer exchanged into Buffer C (20 mM Tris, 1 mM DTT, 1M (NH₄)₂(SO₄), pH 7.8). The protein solution was briefly centrifuged and, using a superloop (50 mL), the supernatant was loaded at 1 mL/min onto a Phenyl Sepharose HR 16/10 column (~20 mL column volume) equilibrated with Buffer C. This second stage of the RNR purification had three steps: 1) equilibration in Buffer C for 50 mL, 2) linear gradient from 0% to 100% Buffer A (20 mM Tris, 1 mM DTT, pH 7.8) over 50 mL and 3) a final wash step with Buffer A (50 mL); all steps were performed at 1 mL/min. The RNR protein that eluted between ~90 and 105 mL was collected and reapplied to the same Phenyl Sepharose column that had been equilibrated into Buffer C and the identical purification procedure was repeated. The RNR that eluted between ~90 and 105 mL from this final purification was concentrated. As this substrate protein doesn’t coordinate FeS clusters, reconstitution was not needed. If the lysis and purification steps were performed aerobically, the RNR protein was made anaerobic through several Schlenk line nitrogen/vacuum cycles.

**Spore Photoproduct Lyase (SPL) Preparations**

SPL preparations were performed as described previously⁹ with minor modifications. Briefly, the splB gene from *Clostridium acetobutylicum*, cloned into a pET14b vector allowed for expression of SPL protein with a N-terminal His₆ tag in Tuner(DE3)-pLysS *E. coli* cells. After inoculation from overnight starter cultures, cell culture growth in phosphate buffered LB media (9 L in 2.8 L Fernback flasks) supplemented with chloramphenicol (34 µg/mL) and ampicillin (100 µg/mL) antibiotic and FAS (0.3 mM) and maintained at 37°C with 200 rpm shaking exhibited good over-expression of SPL upon induction with IPTG (1 mM) at an OD₆₀₀ of 0.8 AU. Grown for 3 more hours before transfer to 4°C, this culture was then sparged overnight (14 - 16 h) with N₂(g) until the cell pellet (~35 g) was harvested the next day and stored at -80°C.

To isolate SPL with minimal cluster loss, all lysis and purification steps were performed in an anaerobic Coy vinyl glove box. At a ratio of ~2 mL of buffer per 1 g of cell pellet, lysis was performed at 4°C in buffer (20 mM sodium phosphate pH 7.5, 350 mM NaCl, 5% glycerol, 10 mM imidazole) in the presence of 1% Triton™ X-100 (w/v), MgCl₂ (10 mM), PMSF (1 mM), lysozyme (0.5 mg/cell g), DNase I and RNase A (< 1 mg/cell g). After centrifugation (38,000 x g, 60 min., 4°C), the SPL clarified lysate was purified via FPLC on two 1 mL in-line HisTrap column with the following step gradient: 1) 0% Buffer B (20 mM sodium phosphate pH 7.5, 350 mM NaCl, 5% glycerol, 500 mM imidazole) wash for 5 CV, 2) 5% Buffer B for 5 CV, 3) 50% Buffer B to elute SPL. After brief centrifugation, the enzyme solution was gel filtrated (Sephadex G-25 resin column, 75 mL) and immediately reconstituted to increase [4Fe-4S] cluster content in SPL; this iron-sulfur cluster reconstitution was performed at 4°C as described elsewhere. Briefly, purified SPL (204 µM) was incubated with DTT (5 mM) for 5 minutes in buffer (20 mM sodium phosphate pH 7.5, 350 mM NaCl, 5% glycerol), before ferrous ammonium sulfate (1.2 mM final concentration, from a 10
mM stock) and sodium sulfide (1.2 mM final concentration, from a 10 mM stock) were added, respectively, over the course of an hour. After a 2-hour incubation at 21°C, the reconstitution solution was centrifuged, desalted (Sephadex G-25 resin column, 75 mL) into buffer (20 mM sodium phosphate pH 7.5, 350 mM NaCl, 5% glycerol), and concentrated (Amicon 30 kDa MWCO centrifugation filters) to yield a final iron content of 3.2 ± 0.2 iron/s. EPR spectroscopy confirmed [4Fe-4S]²⁺ cluster presence in the photoreduced enzyme.

The R-spore photoproduct (R-SP) was prepared as previously described.9-12

SAM Preparation
Unlabeled SAM and the following labeled SAMs ([¹³C₆₅,N₇-Ado]-SAM, [adenosyl-2,8-D₂-1',2',3',4',5',5''-D₆]-SAM, and [¹⁵N-(amino)]-SAM) were synthesized from L-methionine and ATP (labeled as needed to achieve the indicated labeled SAM) and purified as previously described.13-14 Lyophilized SAM was reconstituted in a degassed 100 mM Tris, pH 7.0 – 8.1 buffer and brought up to a pH between 7.0 and 8.0 in an anaerobic (<1 ppm O₂) environment.

For preparation of 5′-labeled SAMs (including [5′,5′'-D₃]-SAM), synthesis from methionine and the corresponding labeled ATPs were according to previously published methods;15 specifically 5′-labeled ATP ([5′,5′'-D₃]-ATP) was synthesized from the corresponding [5′,5′'-D₃]-ribose based on established procedures.16 For a 10 mL reaction, 8 mL of SAM synthetase prepared as described previously15 was mixed with 1 mL of 130 mM ATP (pH 7.50) 1 mL of 250 mM L-methionine (pH 7.5), and 10 µL of inorganic pyrophosphatase (1 unit/µL). The reaction mixture was incubated for 1 h at 30°C in a water bath and the progress was monitored using an HPLC. The reaction was quenched by filtration through a 1 kDa MWCO filter. The resulting small molecule fraction was diluted with ten volumes of 0.2 M sodium acetate pH 4.0, and loaded onto an Amberlite CG-50 column (50 mL, Ammonium form). The column was washed with 500 mL of 0.2 M sodium acetate and eluted by a linear gradient (125 x 125 mL) of 0 - 0.5 M hydrochloric acid. The fractions containing SAM were identified based on HPLC, pooled, and adjusted to pH 6.0 using ammonium hydroxide. The samples were then lyophilized and the residue was dissolved in water. The resulting solution was then loaded to a Biogel P2 column (20 mL) and SAM was eluted using water. Typically, SAM eluted after 2-3 column volumes. The fractions containing SAM were then combined and lyophilized.

RFQ Sample Preparation
For all RFQ experiments, the same sample preparation was carried out in an anaerobic Coy chamber. Generically, a solution of each radical SAM enzyme in the appropriate buffer was placed in an EPR or NMR tube and photoreduced by illumination with a 500 W halogen lamp for 1 h in an ice water bath; if the radical SAM enzyme substrate was a protein (PFL-AE and RNR-AE) or peptide (OspA and PoyA), solutions of these substrates were simultaneously photoreduced employing the identical method as for the enzyme. If the enzyme was not prepared in a Tris buffer, then some Tris buffer (50 mM, final) was added as a sacrificial electron donor. After photoreduction, and except where specifically stated otherwise, the radical SAM enzyme solution was loaded into one syringe, while SAM (1 - 5 mM) was added to the substrate solution and loaded into the second syringe. RFQ was performed as soon as possible after photoreduction completion. In general, the RFQ sample preparation for each enzyme was nearly identical, however the details for each radical SAM enzyme can be found in the following paragraphs.

A HydG stock solution containing 220 µM HydG, 200 µM 5-deazariboflavin, 50 mM Tris, and 5 mM DTT in buffer (50 mM HEPES, 300 mM KCl, 5% glycerol, pH 8.0) was photoreduced in
conjunction with a separate substrate mixture containing 2 mM tyrosine, 50-200 µM 5-deazariboflavin, and 50 mM Tris in the same buffer (50 mM HEPES, 300 mM KCl, 5% glycerol, pH 8.0). After photoreduction, SAM (5.5 mM) was added to the tyrosine solution, unless otherwise noted, and this set-up yielded a sample with the following concentrations: 110 µM HydG, 1 mM tyrosine, 125-200 µM 5-deazariboflavin, 2.5 mM DTT, 2.25 mM SAM.

A LAM stock solution containing 1.2 mM LAM, 10 mM cysteine, 1 mM ferrous ammonium sulfate, 15% glycerol, 1.2 mM pyridoxal 5'-phosphate (PLP) was incubated at 37°C for 4 h prior to photoreduction in 42 mM EPPS, pH 8.0 buffer. This stock solution was combined with 200 µM 5-deazariboflavin and photoreduced for one hour. A separate substrate solution containing 40 mM L-lysine, 50-200 µM 5-deazariboflavin was photoreduced for at least 15 minutes. After photoreduction, SAM (5.5 mM) was added to the substrate solution, unless otherwise noted. This set-up yielded a sample with the following concentrations: 450 µM LAM, 20 mM L-lysine, 125-200 µM 5-deazariboflavin, 2.25 mM SAM.

A PFL-AE stock solution containing 550 µM PFL-AE, 200 µM 5-deazariboflavin and 1 mM DTT in buffer (100 mM Tris, 200 mM KCl, pH 7.6) was photoreduced in conjunction with a separate PFL mixture containing 770 µM PFL, 50-200 µM 5-deazariboflavin, 10 mM oxamate, and 1 mM DTT in the same buffer (50 mM Tris, 100 mM KCl, pH 7.6). After photoreduction, SAM (5.5 mM) is added to the PFL solution, unless otherwise noted. The protein concentrations used were such as to achieve a ratio of PFL-AE: PFL of 1:1.4 after mixing. This set-up yielded a sample with the following concentrations: 275 µM PFL-AE, 385 µM PFL, 125-200 µM 5-deazariboflavin, 5 mM oxamate, 1 mM DTT, 2.25 mM SAM in buffer (100 mM Tris, 200 mM KCl, pH 7.6).

A PoyD stock solution containing 200 µM PoyD, 200 µM 5-deazariboflavin, 50 mM Tris, 10 mM DTT in buffer (50 mM HEPES, 150 mM KCl, 10% glycerol, pH 8.0) was photoreduced in conjunction with a separate PoyA25 mixture containing 300 µM PoyA, 100-200 µM 5-deazariboflavin, 50 mM Tris, and 10 mM DTT in the same buffer. After photoreduction, SAM (3 mM) was added to the PoyA solution, unless otherwise noted, to yield a sample with the following concentrations: 100 µM PoyD, 150 µM PoyA, 150-200 µM 5-deazariboflavin, 50 mM Tris, 10 mM DTT, 1.5 mM SAM.

A RNR-AE stock solution containing 500 µM RNR-AE, 200 µM 5-deazariboflavin, and 1 mM DTT in buffer (100 mM Tris, 200 mM NaCl, pH 8.5.) was photoreduced in conjunction with a separate RNR mixture containing 500 µM RNR, 200 µM 5-deazariboflavin, 10 mM sodium formate, and 1 mM DTT in the same buffer (100 mM Tris, 200 mM NaCl, pH 8.5). After photoreduction, SAM (5.5 mM) was added to the RNR solution, unless otherwise noted, and this set-up yielded a sample with the following concentrations: 250 µM RNR-AE, 250 µM RNR, 200 µM 5-deazariboflavin, 1 mM DTT, 2.25 mM SAM in buffer (100 mM Tris, 200 mM NaCl, pH 8.5).

A SPL stock solution containing 550 µM SPL, 200 µM 5-deazariboflavin, and 5 mM DTT in buffer (20 mM sodium phosphate, 350 mM NaCl, 5% glycerol, pH 7.5) was photoreduced. A separate substrate mixture containing 770 µM SP substrate, 10 mM DTT, and SAM (5.5 mM) was prepared in the same buffer. After photoreduction, SAM (5.5 mM) was added to the substrate
solution, unless otherwise noted to yield a sample with the following concentrations: 275 µM SPL, 385 µM R-SP, 100 µM 5-deazariboflavin, 5.5 mM DTT, 2.25 mM SAM.

Rapid Freeze-Quench Experiments

Rapid freeze-quench (RFQ) experiments were performed with a System 100 apparatus from Update Instrument. In general, each RS enzyme, photoreduced in the presence of 5-deazariboflavin to generate the catalytically relevant $[4\text{Fe-4S}]^+$ cluster, was loaded into one loop while the corresponding substrate and SAM (substrate+SAM) mixture was loaded into the other loop, with the aim of achieving a post-mixing ratio between 1:20 and 1:1 of RS enzyme: substrate. However, two additional mixing procedures were also examined with PFL-AE: 1) (PFL-AE+SAM) + PFL, and 2) (PFL-AE+PFL) + SAM. In all cases, samples were loaded in an anaerobic chamber and, despite the RFQ instrument location outside of an anaerobic environment, strict anaerobic technique was maintained for all samples. Both enzyme loop and substrate loop tubing(s) were each connected to respective syringes that had been washed several times with a dithionite solution (100 mM, in water) and then dried with N$_2$ gas before loading. Each enzyme or substrate solution was preceded and followed by a small plug of N$_2$ gas and a dithionite solution (100 mM, in water) to create the following set-up: dithionite solution, N$_2$ gas, sample (enzyme or substrate/SAM solution), second N$_2$ gas, second dithionite solution. The N$_2$ gas was introduced in the loops between the dithionite and the sample solutions to prevent dithionite contamination of the samples; in addition to minimizing the likelihood of potential protein oxidation, the dithionite solution helped to ensure all sample solutions exited the loop into the mixing chamber and onto the copper wheels. The mixture was quenched by spraying onto two rotating copper wheels cooled to liquid nitrogen temperatures after 500 ms mixing times, though a range of times between 250 ms and 1 sec were tested. The frozen powder was collected in a funnel and packed into precision Q-band tubes (2.5 mm OD) for EPR and ENDOR analysis.

EPR and ENDOR Measurements

X-band CW EPR spectroscopy was conducted on a Bruker ESP 300 spectrometer equipped with an Oxford Instruments ESR 910 continuous helium flow cryostat. Typical experimental parameters were at 40 K, 9.37 GHz, 1.99 mW microwave power, 100 kHz modulation, and 8 G modulation amplitude. EPR simulation were performed with QPOW.  

The 35 GHz CW ENDOR measurements employed 100 kHz field modulation and dispersion mode detection under rapid passage conditions at 2 K. $^1$H CW ENDOR spectra employed broadening of the RF to 100 kHz to improve signal-to-noise. For a single molecular orientation and for nuclei with nuclear spin of I = $\frac{1}{2}$ ($^1$H, $^{15}$N), the ENDOR transitions for the $m_s = \pm \frac{1}{2}$ electron manifolds are observed, to first order, at frequencies, where $v_n$ is the nuclear Larmor frequency, and A is the orientation-independent hyperfine coupling. For I $\geq$ 1 ($^{14}$N, I = 1), the two ENDOR lines are further split by the orientation-dependent nuclear quadrupole coupling (P) into 2I lines given by equation:

$$I = \frac{1}{2}: \nu_\pm = v_n \pm \frac{A}{2}$$  \hspace{1cm} (1)$$  

$$I \geq 1: \nu_\pm = v_n \pm \frac{A}{2} \pm \left( \frac{3P(2M_s) - 1}{2} \right)$$  \hspace{1cm} (2)$$

where P is a parameter that characterizes this coupling (see ref.$^{23}$).
EPR of isotopically labeled \( \Omega \) with PFL-AE

\(^{56}\text{Fe and }^{57}\text{Fe PFL-AE:} \)

Fig. S3 (top panel) shows the EPR spectrum of \( \Omega \) with \(^{57}\text{Fe} \) labeled PFL-AE and non-labeled PFL-AE. The EPR spectrum of \(^{57}\text{Fe-}\Omega\) shows the most distinguishable broadening between \( g_\parallel \) and \( g_\perp \) in comparison to \(^{56}\text{Fe-}\Omega\). The EPR spectrum of \(^{56}\text{Fe-}\Omega\) is best simulated with \( g_\parallel = 2.035 \), \( g_\perp = 2.004 \), and EPR line-width [40, 26, 26] MHz for the unresolved hyperfine coupling. The EPR spectrum of \(^{57}\text{Fe-}\Omega\) is simulated with the same spin Hamiltonian as \(^{56}\text{Fe-}\Omega\) except with varied \(^{57}\text{Fe}\) hyperfine coupling to account for the broadening pattern. As shown in Fig. S3 (bottom panel), a singly coupled \(^{57}\text{Fe}\) nuclei with hyperfine coupling \( A = [30, 35, 30] \) MHz marginally matches the broadening, while the simulation of two equivalents of \( A^{(57}\text{Fe}) = [30, 35, 30] \) MHz, or one large coupled \( A^{(57}\text{Fe}) = [30, 35, 30] \) MHz in addition to two small coupled \( A^{(57}\text{Fe}) \sim [20, 20, 20] \) MHz closely match the broadening pattern; although the latter two simulations do not distinguish each other. The simulation shows more than one \(^{57}\text{Fe}\) nuclei is required to reproduce the broadening pattern, which suggests the spin density resides within a multi-iron cluster, namely \([4\text{Fe-4S}]^{3+}\) of PFL-AE consistent with our previous \(^{57}\text{Fe}\) ENDOR analysis.

EPR and ENDOR of \( \Omega \) prepared with \(^{1/2}\text{H SAM:} \)

The loss of \(^{1}\text{H ENDOR signals from } \Omega \) upon deuteration of SAM is described in the main text. As a parallel measurement, Fig. S4 shows the EPR spectrum of \( \Omega \) with \(^{1}\text{H, [D}_8\text{-ado]}\)-SAM ([adenosyl-2,8-D$_2$-1',2',3',4',5',5''-D$_6$]-SAM), and \([5',5''-\text{D}_2\text{-ado}]-\text{SAM ([adenosyl-5',5''-D}_2\text{-SAM}]). The EPR spectrum of \([\text{D}_8\text{-ado}]-\text{SAM and } [5',5''-\text{D}_2\text{-ado}]-\text{SAM display roughly the same line-width at } g_\parallel = 2.035 \), and both are noticeably narrower than \(^{1}\text{H-SAM}, which indicates the } \Omega \text{ Fe spin center specifically interact with the } 5'-\text{H/D nuclei of SAM. This confirms the presence of the direct Fe-(5'-C) bond as discussed in main text.}
Fig. S1
The previously accepted mechanism for RS enzymes, involving reductive cleavage of SAM to generate methionine and the 5'-dAdo•, followed directly by H atom abstraction from substrate. Here, the substrate from which the H atom is abstracted is represented by “R” (red). The [4Fe-4S] cluster is shown as spheres (yellow, sulfur; rust, iron)
Fig. S2
EPR spectra of Ω as freeze-quenched at 500 ms. **Top**, pre-mixed PFL and SAM solution, is rapidly mixed and freeze-quenched with PFL-AE solution. **Bottom**, pre-mixed PFL-AE and PFL solution, is rapidly mixed and freeze-quenched with SAM solution. **EPR conditions**: microwave frequency, 9.374 GHz (top), 9.375 GHz (bottom); modulation amplitude, 10 G; $T = 40$ K.
Fig. S3
Normalized EPR spectra of Ω with all the radical SAM enzymes examined. Left, as freeze-quenched at 500 ms; Right, after annealing 1 min at 150 K. RFQ mixing condition: pre-mixed substrate (PFL, tyrosine, PoyA, RNR, OspA, α-lysine, and R-SP) and SAM solution, is rapidly mixed and freeze-quenched with radical SAM enzyme solution (PFL-AE, HydG, PoyD, RNR-AE, OspD, LAM, and SPL, respectively). In addition to the Ω signal, variable amounts of a second, free-radical-like signal, which is lost during brief (1 min) annealing at ~150 K, can be seen in each of these enzymes; the origin of this second signal has yet to be identified. As noted in main text, HydG, and to a lesser extent LAM, spectra contain a feature from Cu(II) introduced by Cu freezing wheels. EPR conditions: microwave frequency, 9.375 GHz; modulation amplitude, 10 G; T = 40 K.
Fig S4.
Normalized representative EPR spectra of [4Fe-4S]$^+$ and ([4Fe-4S]$^+$+SAM) cluster for PFL-AE in comparison with spectra of freeze-quenched samples, which show that the formation of $\Omega$ accompanies the complete loss of the cluster signal for each of the enzymes studied: PFL-AE, HydG, PoyD, RNR-AE, OspD, LAM, and SPL. Note, the ‘derivative-shaped’ feature to low field of the $\Omega$ signal, ~3250 G, is from Cu contaminant derived from the Cu wheels on which the RFQ samples are frozen. *EPR conditions:* microwave frequency, 9.375 GHz; modulation amplitude, 10 G; T = 12 K.
**Fig. S5**
EPR spectrum of product Gly radical formed upon annealing RNR-AE Ω as indicated, overlaid with spectrum of hand-quenched enzyme with radical. The Gly radical in RNR-AE has a considerably longer $T_1$/slower relaxation than that in PFL-AE, and as a result the hyperfine-split doublet in both the annealed RNR-AE RFQ sample and hand-quenched sample are highly saturated at any usable power at 40 K, unlike the reported spectra for PFL-AE;⁴ at 77 K, where the unsaturated signal with resolved hyperfine doublet from the hand-quenched sample is seen, the signal from the RFQ/annealed sample is too weak to study. As in ref 4, residual signal from Ω has been subtracted. It was not possible to determine whether the extremely sharp signal with low integrated intensity, which overlays the glycyl radical signal, is present before annealing or forms as a minority byproduct during annealing. *EPR conditions:* microwave frequency, 9.375 GHz; power, 2 mW; modulation amplitude, 10 G.
**Fig. S6**

**Top,** the EPR spectra of Ω formed with $^{57}$Fe labeled PFL-AE (red); $^{56}$Fe PFL-AE (black) and simulation (black dash), $g_{\parallel} = 2.035$, $g_{\perp} = 2.004$, LW$_\parallel$ = 40 MHz, LW$_\perp$ = 26 MHz. **Bottom,** spectrum of Ω - $^{57}$Fe (red) and simulations with varied $^{57}$Fe hyperfine couplings as indicated. RFQ mixing condition: pre-mixed PFL with SAM solution is rapidly mixed with PFL-AE solution and freeze-quenched. **EPR conditions:** microwave frequency, 9.375 GHz; modulation amplitude, 10 G; $T = 12$ K. As noted in main text, this figure and the simulation confirm that the spin of Ω is localized on the Fe-S cluster. In response to a reviewer question, we note that no attempt is made to precisely fit the signal, which likely contains a slight distribution in parameters originating in a slight spread in geometries. The origin of the small, variable signal primarily to high field of Ω, which is not seen in any of the other samples of any of the enzymes (eg. Fig 3) is not known.
Fig. S7
X-band EPR spectra of $\Omega$ for PFL-AE/PFL with $^1$H-SAM (black), [D$_8$-ado]-SAM ([adenosyl-2,8-D$_2$-1',2',3',4',5',5''-D$_6$]-SAM, blue), and [5',5''-D$_2$-ado]-SAM ([adenosyl-5',5''-D$_2$-SAM], red), showing the distinct narrowing of the $g_{||}$ feature upon uniform and specific deuteration. The apparent shift of that feature upon deuteration is a consequence of different microwave frequencies. 

**RFQ mixing condition:** pre-mixed PFL with SAM solution is rapidly mixed with PFL-AE solution and freeze-quenched. **EPR conditions:** microwave frequency 9.373 GHz ($^1$H SAM), 9.375 GHz ([D$_8$-ado]-SAM), 9.375 GHz ([5',5''-D$_2$-ado]-SAM); modulation amplitude, 10 G; $T = 40K$. 
Fig. S8

35 GHz CW $^{15}$N/$^{14}$N ENDOR of $\Omega$. $^{15}$N EDNOR shows $A^{(15)}(N) \sim 5.6$ MHz corresponding to the hyperfine coupling of the “anchor” amine group of adenosyl methionine. Based on the nuclear g factor ratio $g_n^{(14)}(N)/g_n^{(15)}(N) = A_n^{(14)}(N)/A_n^{(15)}(N) = |0.71|$, $A^{(14)}(N) \sim 4$ MHz is calculated which is further split by quadrupole coupling, denoted 3P (see equation 2), as marked by the goalpost. RFQ mixing condition: pre-mixed substrates, PFL and SAM, solution, is rapidly mixed and freeze-quenched with PFL-AE solution. ENDOR conditions: microwave frequency, 35.042 GHz; scan rate, 0.5 MHz/s; scan direction, reverse; $T = 2$ K.
Table S1. Overview of radical SAM enzymes examined in this study. Iron content is reported as irons/monomer.

| Enzyme | Iron Content | Substrate     | SAM role  |
|--------|--------------|---------------|-----------|
| HydG   | 7.8 ± 0.2    | tyrosine      | co-substrate |
| LAM    | 3.9 ± 0.1    | alpha-lysine  | co-factor   |
| OspD   | 4.0 ± 0.2    | OspA peptide  | co-substrate |
| PFL-AE | 3.9 ± 0.1    | PFL protein   | co-substrate |
| PoyD   | 3.6 ± 0.1    | PoyA peptide  | co-substrate |
| RNR-AE | 3.5 ± 0.2    | RNR protein   | co-substrate |
| SPL    | 3.2 ± 0.2    | DNA           | co-factor   |
SI References

1. Duffus, B. R.; Ghose, S.; Peters, J. W.; Broderick, J. B. J. Am. Chem. Soc. 2014, 136, 13086-13089.
2. Byer, A. S.; McDaniel, E. C.; Impano, S.; Broderick, W. E.; Broderick, J. B. Methods Enzymol. 2018, in press.
3. Morinaka, B. I.; Vagstad, A. L.; Helf, M. J.; Gugger, M.; Kegler, C.; Freeman, M. F.; Bode, H. B.; Piel, J. Angew. Chem. Int. Ed. 2014, 53, 8503-8507.
4. Horitani, M.; Shisler, K. A.; Broderick, W. E.; Hutcheson, R. U.; Duschene, K. S.; Marts, A. R.; Hoffman, B. M.; Broderick, J. B. Science 2016, 352, 822-825.
5. Broderick, J. B.; Henshaw, T. F.; Cheek, J.; Wojtuszewski, K.; Smith, S. R.; Trojan, M. R.; McGhan, R. M.; Kopf, A.; Kibbey, M.; Broderick, W. E. Biochem. Biophys. Res. Comm. 2000, 269, 451-456.
6. Nnyepi, M. R.; Peng, Y.; Broderick, J. B. Arch. Biochem. Biophys. 2007, 459, 1-9.
7. Freeman, M. F.; Gurgui, C.; Helf, M. J.; Morinaka, B. I.; Uria, A. R.; Oldham, N. J.; Sahl, H.-G.; Matsunaga, S.; Piel, J. Science 2012, 338, 387-390.
8. Tropea, J. E.; Cherry, S.; Waugh, D. S. Methods Mol. Biol. 2009, 498, 297-307.
9. Silver, S. C.; Chandra, T.; Zilinskas, E.; Ghose, S.; Broderick, W. E.; Broderick, J. B. J. Biol. Inorg. Chem. 2010, 15, 943-955.
10. Chandra, T.; Broderick, W. E.; Broderick, J. B. Nucleosides, Nucleotides, & Nucleic Acids 2009, 28, 1016-1029.
11. Chandra, T.; Silver, S. C.; Zilinskas, E.; Shepherd, E. M.; Broderick, W. E.; Broderick, J. B. J. Am. Chem. Soc. 2009, 131, 2420-2421.
12. Chandra, T.; Broderick, W. E.; Broderick, J. B. Nucleosides, Nucleotides, & Nucleic Acids 2010, 29, 132-143.
13. Walsby, C. J.; Hong, W.; Broderick, W. E.; Cheek, J.; Ortillo, D.; Broderick, J. B.; Hoffman, B. M. J. Am. Chem. Soc. 2002, 124, 3143-3151.
14. Walsby, C. J.; Ortillo, D.; Broderick, W. E.; Broderick, J. B.; Hoffman, B. M. J. Am. Chem. Soc. 2002, 124, 11270-11271.
15. Iwig, D. F.; Booker, S. J. Biochemistry 2004, 43, 13496-13509.
16. Scott, L. G.; Geierstanger, B. H.; Williamson, J. R.; Hennig, M. J. Am. Chem. Soc. 2004, 126, 11776-11777.
17. Kim, S. H.; Perera, R.; Hager, L. P.; Dawson, J. H.; Hoffman, B. M. J. Am. Chem. Soc. 2006, 128, 5598-5599.
18. Lin, Y.; Gerfen, G. J.; Rousseau, D. L.; Yeh, S.-R. Analytical Chemistry 2003, 75, 5381-5386.
19. Aitha, M.; Moller, A. J.; Sahu, I. D.; Horitani, M.; Tierney, D. L.; Crowder, M. W. J. Inorg. Biochem. 2016, 156, 35-39.
20. Nilges, M. J. Electron Paramagnetic Resonance Studies of Low Symmetry Nickel(i) and Molybdenum(v) Complexes. Part I: Computer Simulation of Electron Paramagnetic Resonance Spectra. Part II: Electron Paramagnetic Resonance Studies of Nickel(i) Triphenylphosphine Complexes. Part III: Electron Paramagnetic Resonance Studies of a Molybdenum(v) Thiocyanate Complex. Ph.D. Thesis, University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, 1979.
21. Maurice, A. M. Acquisition of Anisotropic Information by Computational Analysis of Isotropic EPR Spectra. Ph.D. Thesis, University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, 1982.

22. Hoffman, B. M.; DeRose, V. J.; Ong, J. L.; Davoust, C. E. J. Magn. Reson. **1994**, *110*, 52-57.

23. DeRose, V. J.; Hoffman, B. M. *Methods Enzymol.* **1995**, *246*, 554-589.