Supplementary Information for:

X-ray and EPR Characterization of the Auxiliary Fe-S clusters in the Radical SAM Enzyme PqqE

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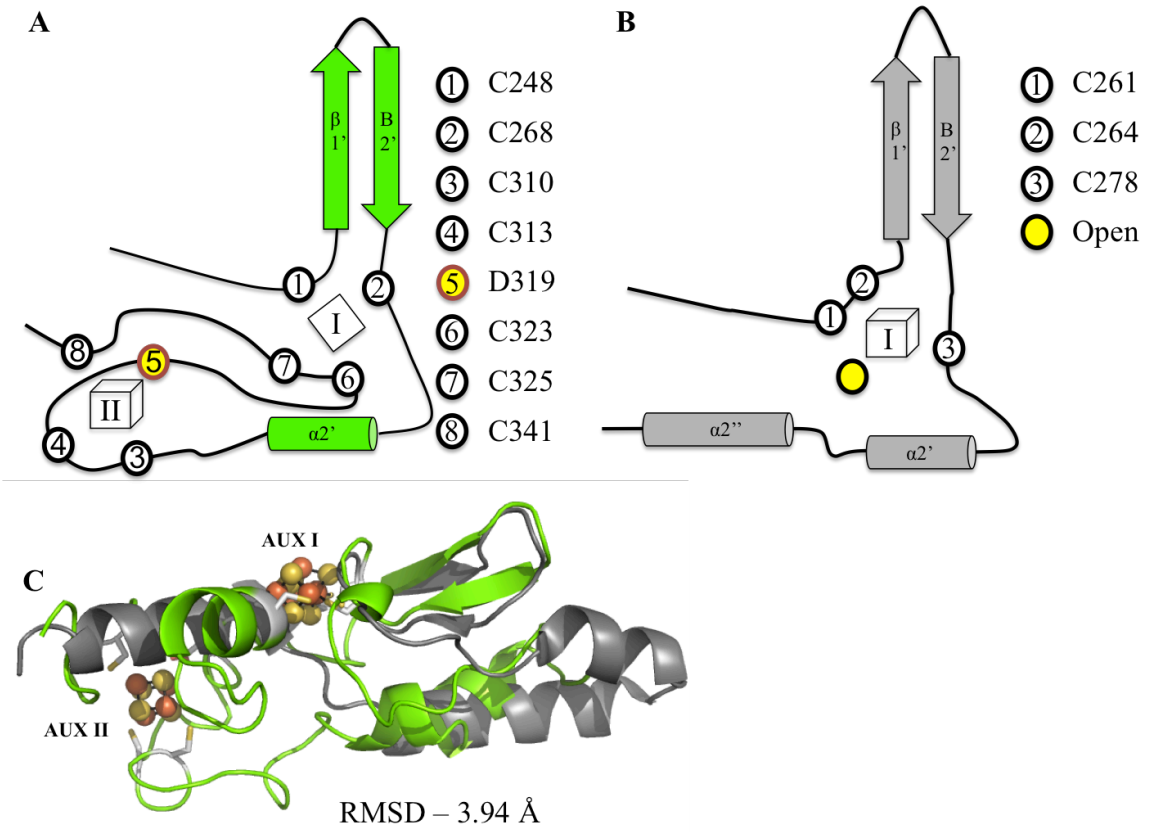

Figure S1: Structural similarity of *Me*PqqE and MoaA (1TV7[1]). Secondary topology of *Me*PqqE (A) and MoaA (B) show the ligation of auxiliary cluster(s). AuxI and Aux II are labeled as I and II. Ligating residues are represented by circles and corresponding residue numbers are shown to the right of each topological rendering. Asp 319 is shown for *Me*PqqE by a number 5 and yellow highlighting. The yellow circle on MoaA represents the open coordination site and point of GTP binding. (C) Structural alignment of the SPASM domains of *Me*PqqE (green) and MoaA (grey; RMSD 3.94 Å).

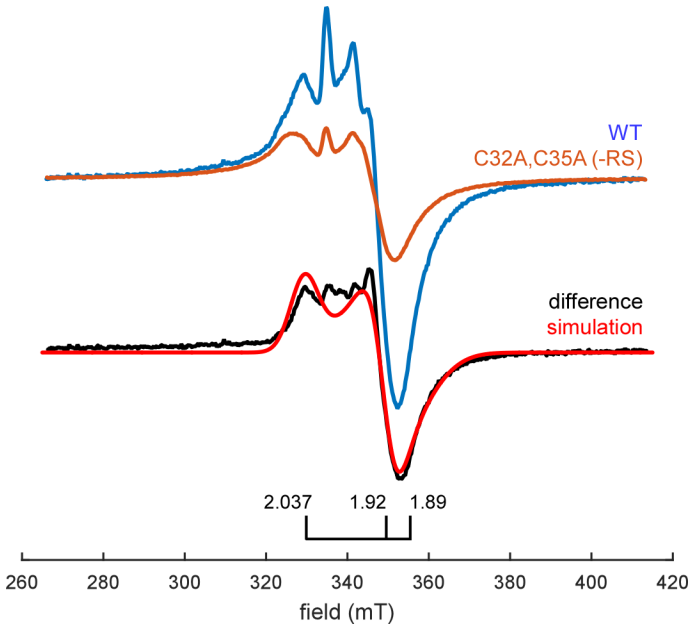

Figure S2. Continuous-wave EPR of dithionite reduced reconstituted wild-type PqqE and PqqE - RS. Spectra are scaled arbitrarily in order to generate a reasonable difference spectrum that should correspond to the EPR signal of the RS cluster (black trace). From this difference, the contribution from the $[2Fe–2S]^{+}$ cluster is also removed by subtracting the 60 K spectrum from each trace. We note that the simulation (red trace) does not accurately predict the spectral intensity seen between g_1 and g_2 (330 mT–350 mT). Subtle lineshape changes are possible when dipolar interactions between the three clusters are turned off as mutagenesis prevents one cluster from forming. Importantly, the simulation of the RS cluster signal does not account for the spectral intensity observed at fields lower than that corresponding to $g > 2.037$ in the spectrum of wild-type PqqE and PqqE - RS.

Table S1. g-Values for Cys_3Asp Clusters and for Auxiliary $[4Fe–4S]^+$ Clusters in Radical SAM Enzymes

$[4Fe–4S]^+$	cluster ligation	$g_{1,2,3}$-values	E° (mV vs SHE)	ref
aconitase w/ *cis*-aconitate w/ *trans*-aconitate w/ nitroisocitrate		2.06, 1.93, 1.86 2.04, 1.85, 1.78 2.01, 1.88, 1.80 2.04, 1.87, 1.77		[2,3]
Clusters w/ Cys_3Asp Ligation				
Dark-operative protochlorophyllide reductase NB-cluster	Cys_3Asp	5.1, 2.3, 1.92		[4]
8Fe Fd III	Cys_3Asp	5.27, 2.34, 1.62		[5]
Pf. ferredoxin	Cys_3Asp	2.10, 1.87, 1.80 5.68, 1.35, 1.09 5.05, 2.61, 1.72	–405	[6]
FNR IscA	Cys_3Asp	2.05, 1.94, 1.89		
Auxiliary Clusters in RSE				
AlbA AuxI	Cys_3	2.02, 1.92		[7]
anSME AuxI AuxII	 Cys_4 Cys_4			[8]
BioB [2Fe–2S]	 Cys_3Arg			
BtrN AuxI w/ DOIA and SAM	Cys_4[12]	1.99, 1.99, 1.83	–760	[9,10]
HydG AuxI AuxI w/ Cys_{ex} + EDTA AuxI w/ CN^-	 Cys_3(?) Cys_3Cys_{ex} Cys_3CN	 2.03, 1.92, 1.88 2.06, 1.90, 1.87 2.09, 1.94, 1.93		[11] [12] [13]
LipA AuxI	 Cys_3Ser	 2.05, 1.91 (I)		[14]
MiaB AuxI	 Cys_3	 2.06, 1.94[19]	 –405	[15,16]
MoaA AuxI w/o 5'-GTP AuxI w/ 5'-GTP	 Cys_3 Cys_3GTP	 2.062, 1.911 2.063, 1.897		[17]
NifB AuxI AuxII Aux(?)		 2.062, 1.917, 1.875 2.039, 1.923, 1.886 2.058, 1.985, 1.909		[18]
PqqE no attribution RS AuxI $[2Fe2S]^+$ AuxI $[4Fe4S]^+$ AuxII	 Cys_3 Cys_4 Cys_4 Cys_3Asp	 2.06, 1.96, 1.91 2.034, 1.918, 1.895 2.0049, 1.958, 1.906 2.053, 1.922, 1.895 not observed		[19] this work this work this work

RimO AuxI ...AuxI		 2.05, 1.94	 –390 (II) –470 (III)	 [16] [20]
SkfB WT C117A C121A C124A C380A C385A C387A		 2.04, 1.93 2.03, 1.92 2.03, 1.93		[21]
Tte1186 AuxIa AuxIb AuxIIa AuxIIb		 2.063, 1.932, 1.880 2.022, 1.951, 1.900 2.050, 1.926, 1.887 2.085, 1.940, 1.867		[22]
TWY1 AuxI ...AuxI + SAM	 Cys_3 Cys_3	 2.054, 1.926, 1.842 2.055, 1.932, 1.828		[23]
(I) Cicchillo noted that this auxiliary cluster in LipA was difficult to reduce in high yield using dithionite. (II) Addition of SAM was shown to induce formation of an S = 3/2 species with features at g = 5.3, 3.3, and 1.0.[21] (III) PFE performed on mutant RimO lacking RS cluster.				

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