

Supplementary Information for:

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

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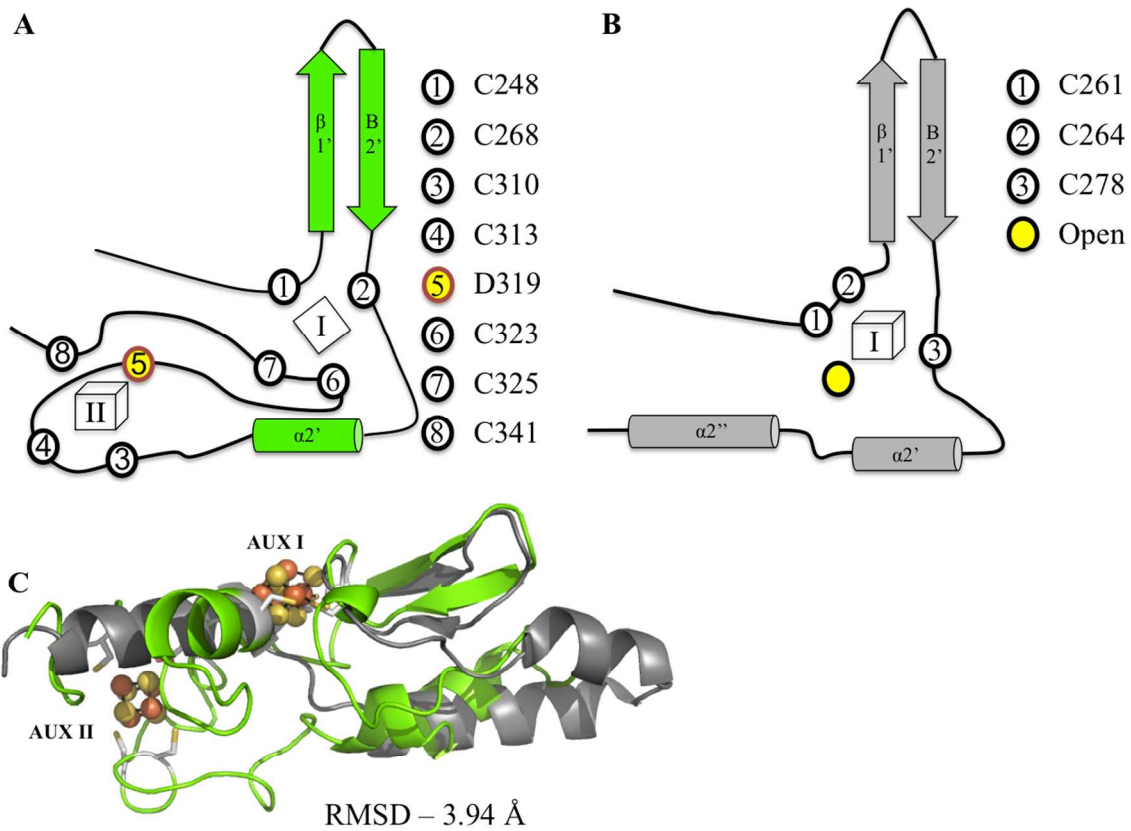


Figure S1: Structural similarity of *MePqqE* and *MoaA* (1TV7¹). Secondary topology of *MePqqE* (A) and *MoaA* (B) show the ligation of auxiliary cluster(s). AuxI and Aux II are labeled as I and II. Ligating residues are represented by circles and corresponding residue numbers are shown to the right of each topological rendering. Asp 319 is shown for *MePqqE* 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 *MePqqE* (green) and *MoaA* (grey; RMSD 3.94 Å).

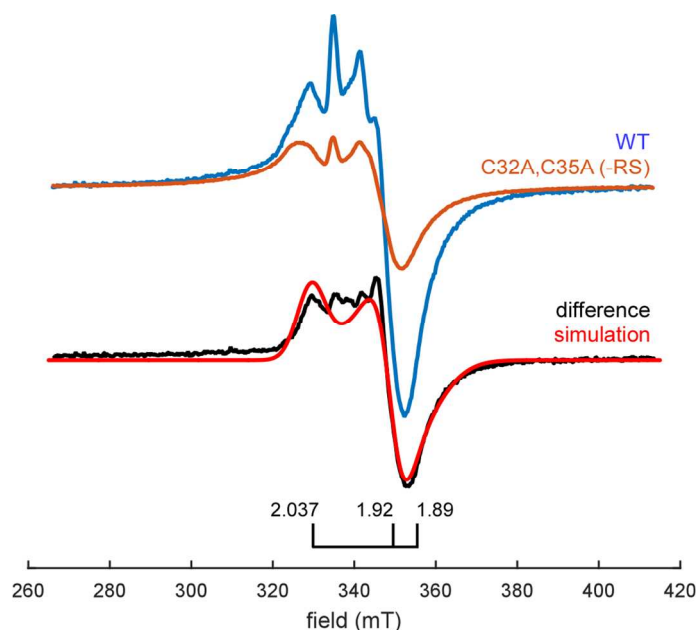


Figure S2. Continuous-wave EPR of dithionite reduced reconstituted wild-type PqqE and PqqE - RS. Spectra are scaled arbitrarily in order to generate a reasonable difference spectrum that should correspond to the EPR signal of the RS cluster (black trace). From this difference, the contribution from the $[2\text{Fe}-2\text{S}]^+$ 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 Cys₃Asp 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/ <i>cis</i> -aconitate w/ <i>trans</i> -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/ Cys₃Asp Ligation				
Dark-operative protochlorophyllide reductase NB-cluster	Cys ₃ Asp	5.1, 2.3, 1.92		4
8Fe Fd III	Cys ₃ Asp	5.27, 2.34, 1.62		5
<i>Pf.</i> ferredoxin	Cys ₃ Asp	2.10, 1.87, 1.80 5.68, 1.35, 1.09 5.05, 2.61, 1.72	-405	6
FNR IscA	Cys ₃ Asp	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 ₃ Arg			
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 ₃ Cys _{ex} Cys ₃ CN	2.03, 1.92, 1.88 2.06, 1.90, 1.87 2.09, 1.94, 1.93		11 12 13
LipA AuxI	Cys ₃ Ser	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 ₃ GTP	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 ₃ Asp	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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