Electronic Supplementary Information

Probing the role of the bridging C509 between $[Fe_4S_4]$ cubane and $[Ni_pNi_d]$ centre in the A-Cluster of Acetyl-CoA Synthase

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Experimental Procedure

Materials*:* Restriction enzymes were purchased from New England Biolabs. The pQE-60 expression vector was purchased from Novagen. *Moorella thermoacetica* strain 39073 was purchased from ATCC. *E. coli* strain JM109 was from Agilent Technologies. *Pfu Turbo* polymerase and *E. coli* strain XL1-Blue were from Stratagene. Platinum *Pfx* DNA polymerase was purchased from Invitrogen. T4 DNA ligase, Wizzard® Genomic DNA Purification Kit and Wizzard® Plus SV Mini-Prep DNA Purification System were from Promega. Buffers, purification reagents and other chemicals were purchased from Sigma-Aldrich, unless otherwise stated. The oligonucleotide primer pairs below, labeled P1-P9, were synthesized and sequenced by Shanghai Invitrogen Biotech Co. Ltd.

P1 [5'-GGCGGACATGTCTGATTTTGATAAAATC-3'(F)
5'-GGCGGAGATCTCATAATGGGATCCATGG-3'(R)]
P2 [5'-CTCCTGCGTCCTCGCCCAGTCCTTTGCCC-3' (F),
5'-GGGCAAAGGACTGGGCGAGGACGCAGGAG-3' (R)]
P3 [5'-CTCCTGCGTCCTCCACCAGTCCTTTGC-3' (F),
5'-GCAAAGGACTGGTGGAGGACGCAGGA -3' (R)]
P4 [5'-CTCCTGCGTCCTCTCCCAGTCCTTTGC-3' (F),

5'-GCAAAGGACTGGGAGAGGACGCAGGAG-3'(R)]

P5 [5'-CTCCTGCGTCCTCGTCCAGTCCTTTGC-3' (F),

5'- GCAAAGGACTGGACGAGGACGCAGGAG-3'(R)]

P6 [5'-CCTCGCCCAGGCCTTTGCCCCCAACGCTGTGTGTATTG-3' (F),

5'-CAATACACACAGCGTTGGGGGCAAAGGCCTGGGCGAGG-3'(R)]

Mutagenesis and cloning of acsB*:* Genomic DNA of *Moorella thermoacetica* was isolated using the DNA isolation kit as described.[S1] Gene *acsB* encording ACS-α subunit was cloned using the genomic DNA with primer pair P1 and the plasmid pLHK05 was isolated as reported.[S2] Site-directed mutagenesis of *acsB* was performed using the QuikChange™ Site-Directed Mutagenesis Kit (Strategene) with plasmid pLHK05 as template. Genes *C509A*, *C509H, C509S, C509V* were constructed similarly, using primers P2, P3, P4, and P5 respectively. Mutant gene *Δbridge* (*C509A/H516A/S511A*) was constructed using the P6 primer pair with C509A as template. In each case, the resulting PCR products were transformed into *E. coli* XL-1 Blue cells (Strategene). Transformed cells were selected on ampicillin plates. The cloned regions of the plasmids were sequenced (Shanghai Invitrogen Biotech Co. Ltd) to verify the fidelity of the PCR reactions; Proteins corresponding to the genes above will be referred to C509A, C509H, C509S, C509V, Δbridge, respectively.

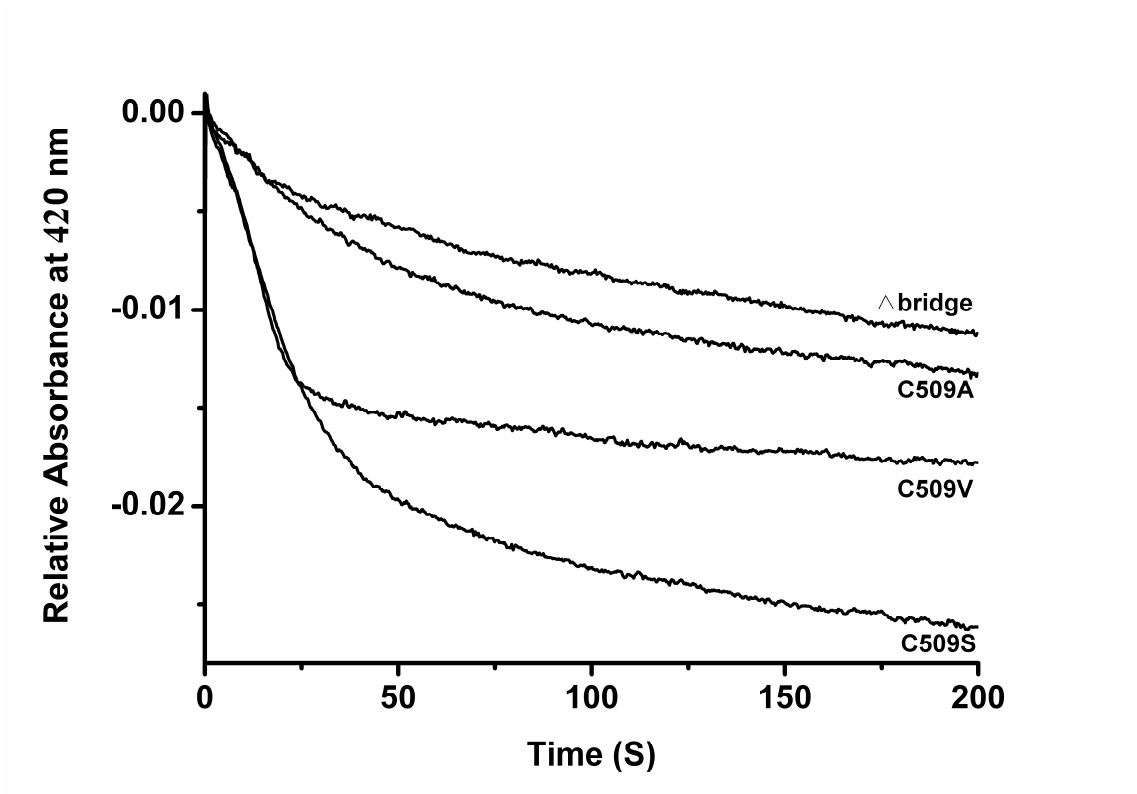
Protein Purification*: E. coli* strain JM109 cells were transformed with each plasmid; cells were grown in 10 L of Begg's media at 37 ^{o}C under anaerobic conditions, induced with 0.5 mM IPTG, harvested anaerobically and then frozen in liquid N_2. All protein purifications and manipulations were performed in an inert atmosphere (N_2) glove box (MBraun). Cells were thawed, suspended in anaerobic Buffer A (50 mM Tris pH 8.0 plus 1 mM sodium dithonite), and sonicated as described.[S2,S3] The supernatant fraction of the cell extract was loaded onto a Ni-NTA agarose column (2.6 x 15 cm) that had been equilibrated in Buffer A. After washing with 5 column volumes of buffer A containing 10 mM imidazole, the protein was eluted using Buffer A plus 100 mM imidazole. Combined fractions were concentrated and passed through a column of Sephadex-G25 equilibrated in 50 mM Tris-HCl pH 8.0. The resulting proteins were found to be at least 90% pure based on the densitometry of denaturing polyacylamide gels stained with Coomasssie Brilliant Blue. Aliquots were frozen in liquid N_2. For

Ni-activation, protein aliquots in 50 mM Tris-HCl buffer pH 8.0 were incubated with 4 molar equivalents of $NiCl_2$ per mol α protein, and then subjected to a column of Sephadex G-25 (equilibrated in Buffer A) to remove excessive $NiCl_2$.[S2,S3]

M. thermoacetica cells were grown in a 10 L bioreactor and harvested as described.[S4,S5] The Corrinoid protein (CoFeSP) and methyltransferase (MeTr) from *M. thermoacetica* cells were purified as reported.[S4,S5] Ti(III) citrate, Co^{+}FeSP and methylated Co^{3+}FeSP were prepared as described.[S4] Protein concentrations were determined by the Bradford method.[S6]

Characterization of Proteins*:* Proteins were activated with $NiCl_2$ and then subjected to a PD10 column to remove excessive nickel ions. The metal content of proteins was determined by overnight digestion in 1M HNO_3 (Trace Metal Grade). Digested samples were diluted to 0.2 M HNO_3 for analysis with a thermo X7 ICPMS (Thermo Electron Corp., USA). UV/Vis absorption spectra were recorded using a HP8453 (Hewlett-Packard, Palo Alto, CA, USA) UV/vis spectrophotometer. Pre-steady-state stopped-flow experiments were performed at 25^{o}C with an SF-61 DX2 Double-Mixing Stopped-Flow instrument (Hi-Tech Limited, UK) installed in a glove box (Braun, MB100 with an O_2 analyzer to assure that the O_2 concentration was < 1 ppm). Methyl group transfer was monitored at 390 nm.

Kinetics of $[Fe_4S_4]^{2+}$ Reduction : The stopped-flow kinetics of reduction in variants, inculding Δ bridge, C509A, C509V and C509S, were preformed as described.[S4] The reduction reaction was monitored at 420 nm and the results were shown as in SF1.



SF1. Reduction of ACS variants with Ti-citatrate (1.0 mM) in Tris-HCl pH 8.0. Concentrations of variants were 40 μ M for each before mixing.

References

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S6. M.M. Bradford, *Anal Biochem*., 1976, **72**, 248-254.