Supplementary Materials for

X-ray Absorption Spectroscopy Reveals an Organometallic Ni-C bond in the CO-treated form of Acetyl-CoA Synthase

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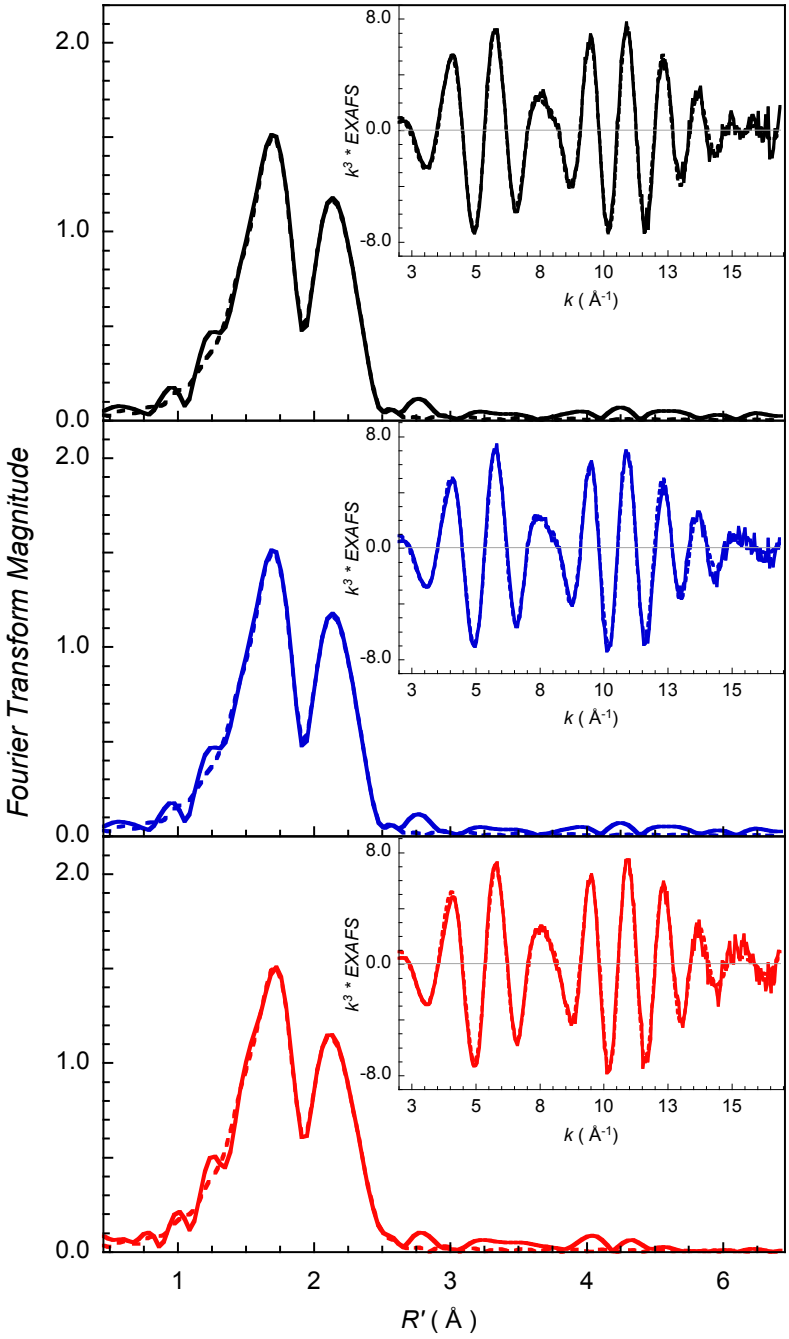

Fig. S1. FEFF fits to the Fe K-edge EXAFS data for the three forms of ACS. FEFF fits to the Fe K-edge EXAFS data for A_{OX} (—), A_{RED} (—) and A_{NiFeC} (—). Non-phase shift corrected Fourier Transform and their corresponding EXAFS (inset) (k = 2-17 Å^{-1}). Data (—), Fit (----)

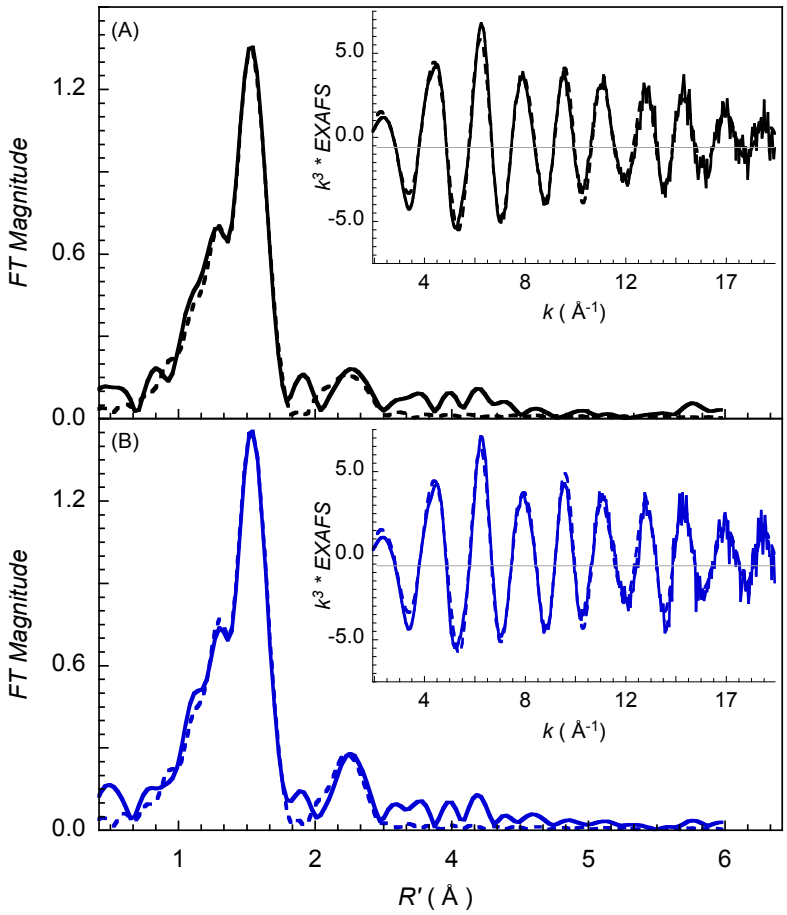

Fig. S2. FEFF fits to the Ni K-edge EXAFS data for the A_{OX} and A_{RED} forms of ACS. FEFF fits to the Ni-EXAFS data for A_{OX} (A) and A_{RED} (B). Non-phase shift corrected Fourier Transform and their corresponding EXAFS (inset) (k = 2-18.6 Å^{-1}). Data (—), Fit (----)

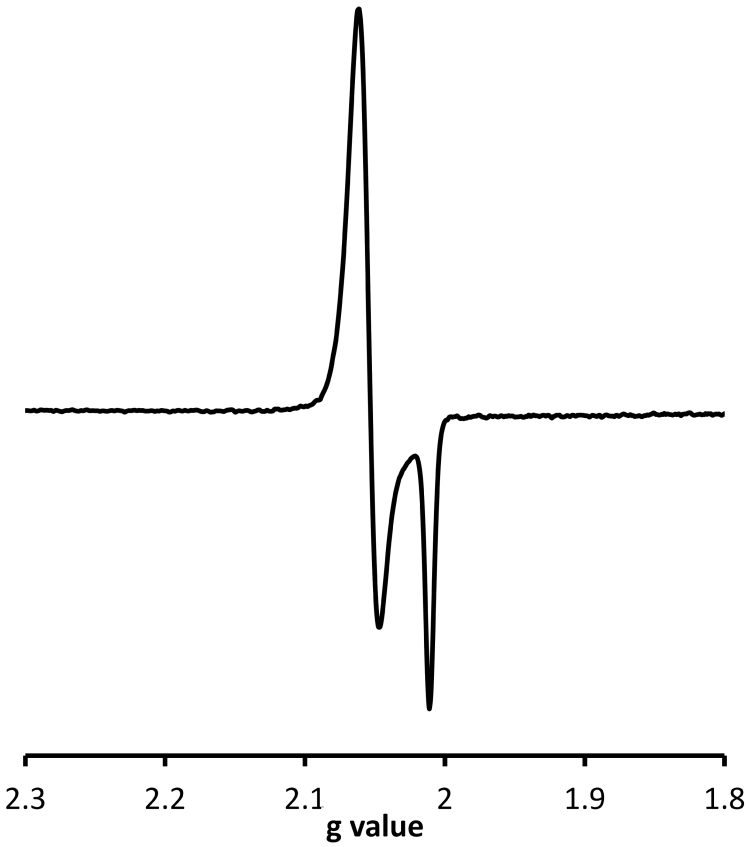

Fig. S3. EPR spectrum of the A_{NiFeC} sample used in these studies.

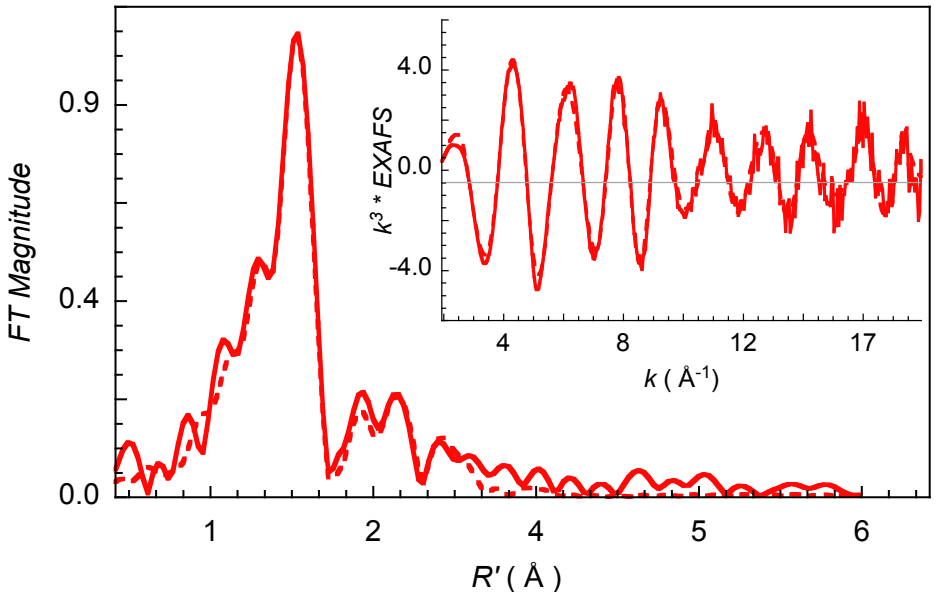

Fig. S4. FEFF fits to the Ni K-edge EXAFS data for the CO-bound form of ACS. FEFF fits to the Ni-EXAFS data for A_{NiFeC}. Non-phase shift corrected Fourier Transform and their corresponding EXAFS (inset) (k = 2-18.6 Å^{-1}). Data (—), Fit (----)

Table S1. Fe K-edge EXAFS Curve-Fitting Results for ACS

ACS	Coordination/Path	R(Å)[a]	σ^2(Å^2)[b]	ΔE_0 (eV)	*F*[c]
A_{OX}	4 Fe-S	2.30	486	-7.64	0.27
	2.5 Fe-Fe[d]	2.73	459		
	1 Fe-C	2.94[e]	182		
A_{RED}	4 Fe-S	2.30	512	-7.49	0.29
	2.5 Fe-Fe[a]	2.73	484		
	1 Fe-C	2.94[e]	402		
A_{NiFeC}	4 Fe-S	2.30	466	-7.45	0.34
	2.5 Fe-Fe[a]	2.73	479		
	1 Fe-C	2.96[e]	204		

[a]The estimated standard deviations for the distances are in order of ± 0.02 Å. [b]The σ^2 values are multiplied by 10^5. A / indicates that the σ^2 value was linked to the previous path. [c]Error is given by $[\Sigma[(\chi_{obsd} - \chi_{calcd})^2 k^6]/(N_{data} - N_{var})]$. [d]The data can accommodate 3 Fe-Fe contributions with slightly worse Error values. The bond distances and rest of the fit parameters remain unchanged. [e]The Fe-C component results from the cysteine carbon. It has a very small contribution to the EXAFS data. The S_0^2 factor was set at 1.

Table S2. Previously published EXAFS data on ACS

	Lindahl 1995[1]		Lindahl 1998[2]	Cramer 2003[3]		Svetlitchnyi 2004[a 4]	Schrapers 2016[5]	
	As-iso	Reduced	Phen-treated	As-iso	Ti^{3+}-Treated	Oxidized	Oxidized	Reduced
Ni-N	(2) 1.89	(2) 1.89	(2) 1.87	(1.5) 1.89	(1.5) 1.89	(2) 1.86	(1-2) 1.98	(1-2) 1.99
Ni-S	(2) 2.19	(2) 2.19	(2) 2.20	(2.5) 2.19	(2.5) 2.19	(2.5) 2.17	(2) 2.2	(2) 2.24
Ni-O/S						(0.5) 2.32	(1) 2.60[b]	(1) 2.62[b]
Ni-Fe					(0.5) 2.80	(0.5) 2.71	(0.5) 2.69	(0.5) 2.64
Ni-Ni				(1) 2.96	(1) 2.97	(1) 2.89	(0.5) 2.91	(0.5) 2.98

[a]The average Ni-ligand distance is reproduced here. [b]This is a Ni-S interaction.

Table S3. The DFT calculated Löwdin Spin Densities of A_{NiFeC}

The spin-densities reported in Table S3 shows that the $[Fe_4S_4]^{2+}$ cluster has converged to the correct ground state for both A_{OX} and A_{NiFeC} with two Fe(III) and two Fe(II) centers that are antiferromagnetically coupled with the Fe_4S_4

	Löwdin Spin Population	
	A_{OX}	A_{NiFeC}
Ni_p	0.09	0.49
Ni_d	0.00	0.25
Fe_1	3.10	3.04
Fe_2	-2.99	2.98
Fe_3	-3.08	-3.00
Fe_4	2.99	-3.00
Four Sulfide of 4Fe4S	0.02	0.02
Bridging Cysteine	0.05	-0.01
Remaining Cysteine	-0.18	0.19
Protein backbone ligand	0.01	0.06
H_2O/CO	0.01	-0.04

Table S4. The DFT calculated Molecular Orbital Contributions of the valance orbitals of A_{NiFeC} resulting in the Ni K-pre-edge transition.

	0a -> 284a	0a -> 285a	0b -> 273b	0b -> 284b	0b -> 285b
Ni_P, D orbitals only	12.1	11.1	34.0	14.1	12.8
Ni_d, D orbitals only	0.3	0.6	17.1	0.3	0.7
4Fe4S cluster	0.8	2.8	21.5	0.2	1.9
CO ligand	58.3	69.1	2.9	53.2	67.8
Bridging Cysteine ligand	2.3	7.1	15.2	1.8	7.2
Remaining Cysteine ligands	0.1	0.2	1.0	0.1	0.2
N atoms of amidate, N only	0.1	0.1	1.5	0.1	0.2
Remaining amidate ligand	26.0	9.1	6.5	30.2	9.4

The Mulliken MO populations reported above shows that the orbitals involved in the Ni K-pre-edge transition (the two alpha and three beta LUMO orbitals) are strongly delocalized over the two Ni centers, the bridged S(Cys) groups and the $[Fe_4S_4]^{2+}$ cluster. TD-DFT contribution to the pre-edge reported in Table S4 are at least 5% or higher of the total TD-DFT intensity. This includes a contribution from orbitals with strong Ni_d d orbital contribution (both alpha and beta). Due its square planar nature, the sum of total contribution from these orbitals is < 9% consistent with dominant quadrupole character.

EXAMPLE of input file for optimization of A_{NiFeC}:

```
# GO NiFeC active site, fully optimized with 6 alpha carbons fixed. 1RU3 structure modified
#
! UKS BP86 normalscf opt SlowConv grid4 nofinalgrid PAL8

%output PrintLevel Normal
       Print[P_MOs] 1
       Print[P_Overlap] 1
       end

%cosmo epsilon 7
      end

%basis basis TZVP
      end

%scf
maxiter 1500
DIISMAXEq 20
directresetfreq 1
end

%geom constraints
   {C 5 C}
   {C 8 C}
   {C 2 C}
   {C 11 C}
   {C 34 C}
   {C 14 C}
 end
end

%Method SpecialGridAtoms 28
       SpecialGridIntAcc 7
       end

* xyz -3 2
Ni        0.00000       0.00000       0.00000 newgto "TZVPP" end
Ni        0.00000       0.00000       2.82804 newgto "TZVPP" end
C        -6.51393      -5.56487      -4.68891
C        -6.60172      -4.14251      -4.15938
S        -5.31721      -3.10937      -5.08857
C         0.76978      -3.80939      -2.68858
C         0.28694      -3.11375      -1.41892
S        -0.20223      -1.41714      -1.75705
```

C	-7.18390	-5.95800	0.35811
C	-7.02274	-4.52585	-0.17754
S	-6.26650	-3.29453	0.87864
C	-4.47599	3.63365	0.97393
C	-3.84846	3.19072	-0.33406
S	-5.18753	2.67710	-1.54032
C	-2.55577	-3.82648	5.95519
C	-1.19308	-3.92884	5.26364
O	-0.40470	-4.77327	5.61856
N	-0.91479	-3.06907	4.27833
C	0.36952	-3.12693	3.58124
C	1.31907	-2.19056	4.27660
O	2.19730	-2.61815	5.08062
C	0.17332	-2.77789	2.08957
S	-1.05832	-1.51810	1.76660
N	1.14315	-0.89609	4.06580
C	2.03907	0.03476	4.75946
C	1.69369	1.46001	4.41951
O	2.22011	2.41710	4.98436
N	0.71909	1.59032	3.53884
C	0.29906	2.87770	3.04102
C	-1.00375	3.35598	3.71827
O	-1.59725	4.34369	3.29102
C	0.03602	2.66820	1.59704
S	-1.12052	1.25711	1.59317
N	-1.48668	2.65324	4.73868
C	-2.71577	3.09439	5.39597
Fe	-4.68959	-1.85456	-3.38446
Fe	-5.03546	-2.03894	-0.74710
Fe	-4.79855	0.41194	-1.85949
Fe	-2.60137	-1.17873	-1.68762
S	-3.67251	-0.32912	0.01842
S	-3.23070	0.00263	-3.64902
S	-3.46332	-3.33303	-2.00004
S	-6.45496	-0.97382	-2.21453
C	1.36905	0.94782	-0.56037
H	-7.22969	-6.17859	-4.18301
H	-5.53013	-5.94916	-4.51756
H	-6.72019	-5.56795	-5.73884
H	-6.39141	-4.12960	-3.11034
H	-7.58400	-3.74504	-4.30789
H	1.05374	-4.81499	-2.45833
H	1.61249	-3.28277	-3.08535
H	-0.01835	-3.81812	-3.41223
H	1.07817	-3.11243	-0.69861
H	-0.56288	-3.64409	-1.04285

H	-7.64922	-6.56762	-0.38804
H	-7.79314	-5.94340	1.23761
H	-6.22147	-6.35965	0.59755
H	-8.00094	-4.17028	-0.42574
H	-6.33564	-4.62812	-0.99138
H	-3.70658	3.92543	1.65787
H	-5.03708	2.82436	1.39240
H	-5.12696	4.46361	0.79418
H	-3.19712	2.36147	-0.15247
H	-3.29088	4.00329	-0.75088
H	-2.62725	-4.57623	6.71522
H	-3.33322	-3.97353	5.23489
H	-2.65888	-2.85815	6.39860
H	-1.58023	-2.39114	4.01980
H	0.78450	-4.11273	3.61099
H	-0.11985	-3.66919	1.57525
H	1.10641	-2.38026	1.74878
H	3.04808	-0.16230	4.46289
H	1.92813	-0.10342	5.81469
H	1.04610	3.61856	3.23581
H	0.93730	2.42854	1.07248
H	-0.35519	3.53621	1.10875
H	-1.02076	1.84325	5.04867
H	-3.07295	3.98543	4.92335
H	-2.51652	3.29349	6.42824
H	-3.45742	2.32713	5.31743
O	1.97417	1.64999	-0.98184

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References and Notes:

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