Supporting Information

Nicolet et al. 10.1073/pnas.1302388110

Conserved Radical *S*-Adenosyl-L-Methionine FeS Cluster Stability in the Glove Box

The structure presented in Fig. S2 was obtained using 5′-deoxyadenosine instead of *S*-adenosyl-L-homocysteine (SAM) after in vitro FeS cluster reconstitution. No DTT was added either for crystallization or for crystal flash cooling. When crystals are frozen within days after crystallization, the conserved cluster consists of a mixture of Fe_4S_4 and Fe_3S_4 species as indicated by the progressive disappearance of the electron density corresponding to the unique iron atom (not shown). If crystals are frozen within weeks only the Fe_3S_4 cluster species is present. This indicates that in our crystals this cluster is relatively stable in our anaerobic chamber, both under the Fe_4S_4 and Fe_3S_4 forms. This result shows that the radical SAM cluster and the second cluster of HydE have very different stabilities when exposed to low-level oxygen. In this crystal, the second cluster site is occupied by a mixture of states including species **3** (major) and **4** (minor). The relatively high stability of the Fe_3S_4 form of the radical SAM cluster most likely results from the low level of oxygen in the glove box.

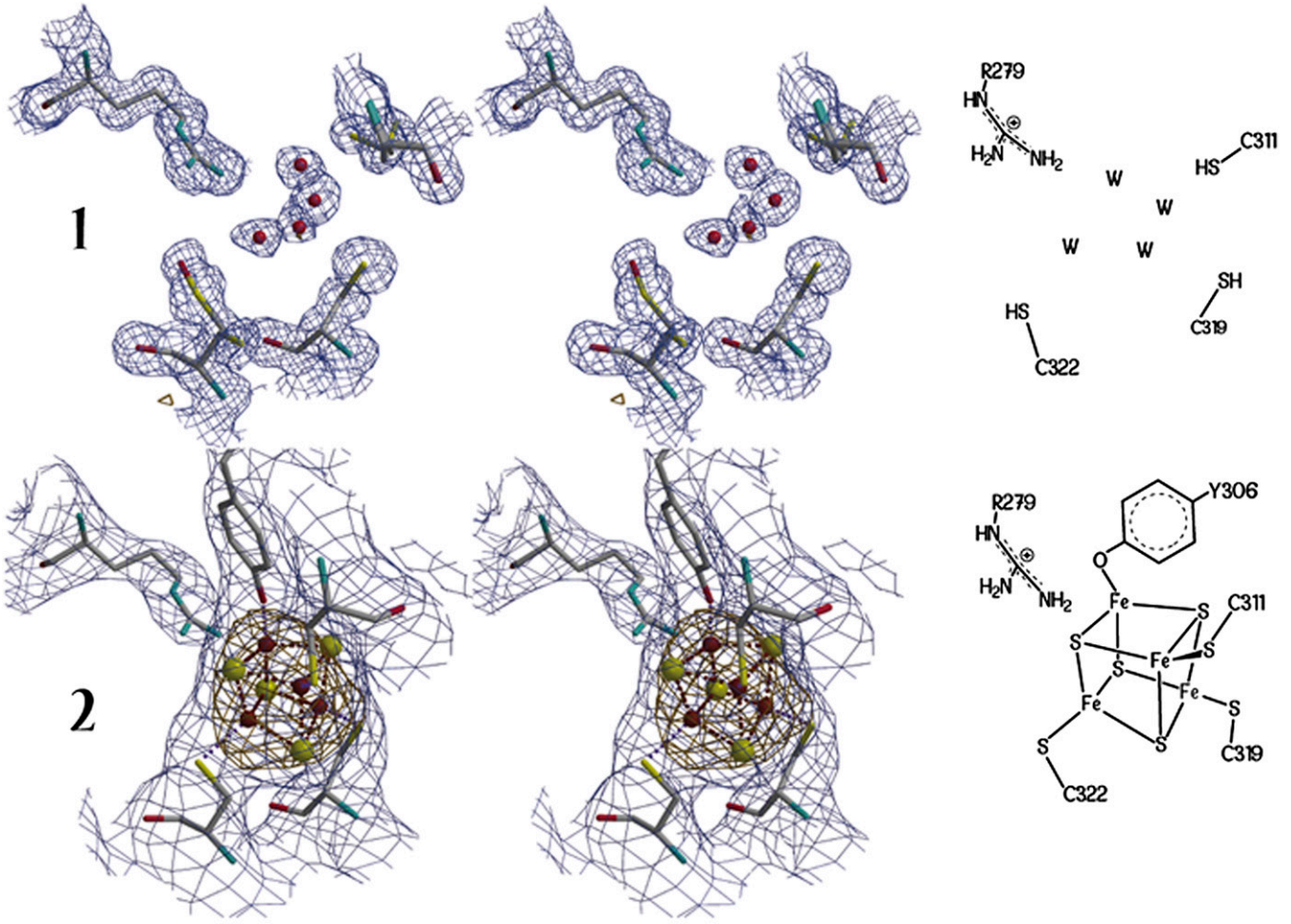

Fig. S1. (Continued)

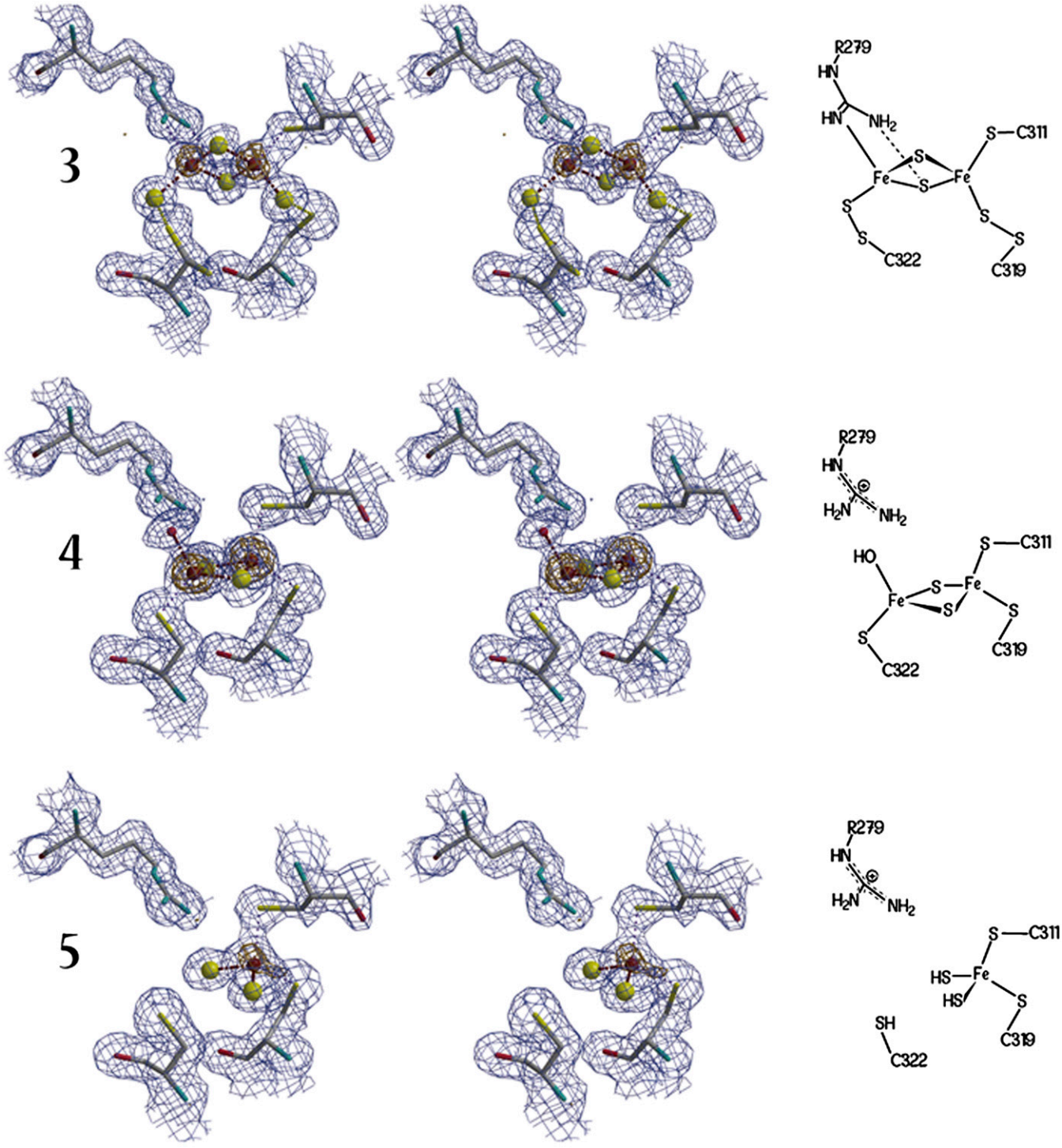

Fig. S1. (Continued)

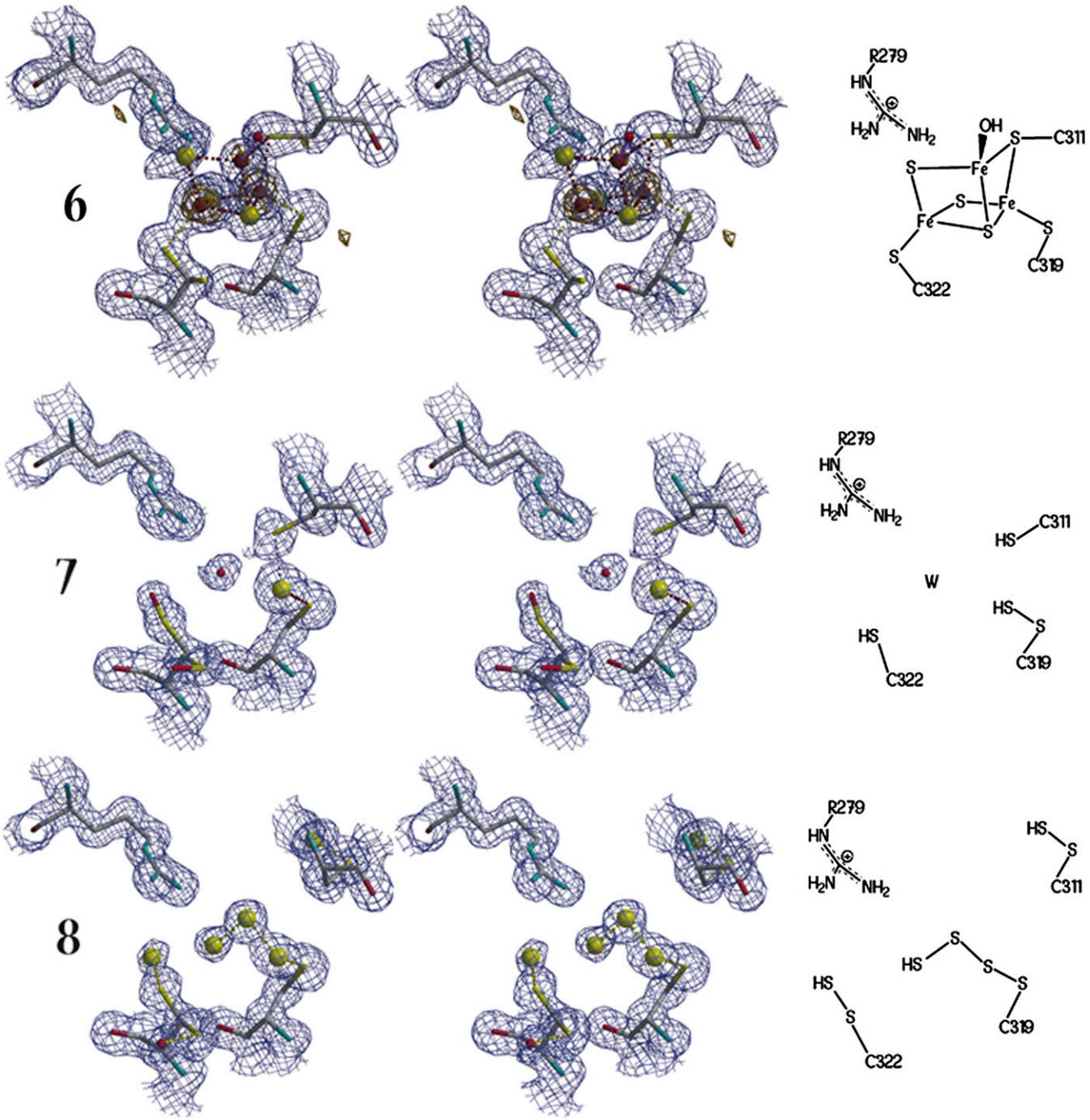

Fig. S1. Stereoviews of the X-ray structures of species **1** to **8** represented by their atomic model and corresponding (2*Fo-Fc*) electron density maps shown as a blue mesh and contoured at the 1 σ level. The anomalous difference (Δ_{anom}) electron density map (depicted as an orange mesh, contoured at the 3.5 σ level) shows the position of the iron atom. Sulfur atoms corresponding to both sulfide ions and cysteine persulfide sulfane are depicted as yellow spheres, whereas iron atoms are represented by brown spheres. On the right, a schematic view of each structure is presented.

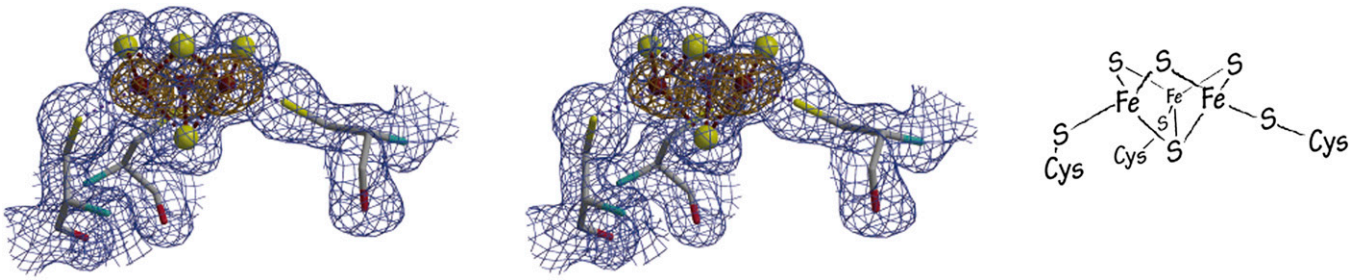

Fig. S2. Radical SAM cluster of HydE in its Fe_3S_4 form at 1.7 Å resolution. Stereoview of the atomic model and corresponding (*2Fo-Fc*) electron density maps (shown as a blue mesh and contoured at the 1 σ level). The anomalous difference (Δ_{anom}) electron density map (depicted as an orange mesh, contoured at the 3.5 σ level) shows the positions of the iron atoms.

Table S1. Summary of the crystallization conditions and crystal treatment before flash cooling

	Species							
Sample preparation conditions	1	2	3	4	5	6	7	8
Crystallization condition								
DTT 5 mM	Yes	—	—	—	Yes	Yes	—	—
NaCl	—	Yes	—	—	—	—	—	—
Cryo condition								
DTT 5 mM	Yes	—	—	Yes	Yes	Yes	—	—
Na_2S 1 mM	—	—	—	—	—	—	—	Yes
$Fe(NH_4)_2(SO_4)_2$ 1 mM	—	—	—	—	—	Yes	—	Yes
Further soak	—	—	—	—	NaBr 100 mM	—	—	—
Approximate time between crystallization and crystal flash cooling	2 mo	2 wk	2 wk	6 mo	1 mo	1 mo	4 mo	4 mo

Table S2. Data collection and refinement statistics

Dataset	K11 (ref. 1)	Fe_4S_4	F6	H4	M10	M1 (ref. 1)	S18	R16 (ref. 2)
Data collection								
FeS species	1	2	3	4	5	6	7	8
PDB code	3CIW	4JY8	4JXC	4JY9	4JYD	4JYE	4JYF	3IIX
Beamline	ID14-eh1	PXI	ID14-eh1	ID14-eh2	ID23-eh2	ID23-eh2	ID29	ID29
Cell parameters, Å	50.97, 79.23, 85.94	82.3, 82.3, 217.19	51.13, 78.73, 86.21	51.25, 78.95, 86.14	50.60, 78.70, 85.96	51.17, 79.86, 86.66	50.64, 78.76, 86.09	51.05, 78.92, 86.19
Space group	$P2_12_12_1$	$P6_522$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Wavelength, Å	0.93	0.976	0.934	0.933	0.873	0.873	0.976	0.976
Resolution, Å	1.35	2.9	1.45	1.60	1.71	1.70	1.45	1.25
Rsym	0.056 (0.422)	0.051(0.571)	0.061 (0.368)	0.054 (0.262)	0.063 (0.350)	0.090 (0.334)	0.053 (0.452)	0.045 (0.407)
I/σ	16.5 (2.8)	28.33 (4.08)	16.28 (3.06)	18.20 (4.88)	9.13 (2.09)	17.7 (4.3)	13.75 (2.61)	14.87 (2.10)
Completeness, %	99.4 (96.7)	99.8 (98.6)	87.7 (41.0)	98.4 (93.9)	96.0 (85.1)	99.3 (99.0)	99.1 (96.4)	95.1 (76.8)
Multiplicity	4.2 (3.1)	10.4 (10.5)	3.7 (2.8)	3.6 (3.1)	2.1 (1.2)	5.6 (5.3)	3.7 (3.3)	3.2 (1.4)
Refinement statistics								
R_{cryst}	0.136	0.177	0.124	0.102	0.189	0.136	0.135	0.139
R_{free}	0.172	0.241	0.169	0.156	0.251	0.180	0.166	0.166
No. of reflections	72,284	18,306	52,356	43,857	35,223	37,369	58,494	88,534
Work set	68,447	17,399	49,547	41,496	33,334	35,372	55,375	83,859
Test set	3,837	907	2,809	2,361	1,879	1,997	3,119	4,675
rms deviation from ideal geometry								
Bonds, Å	0.012	0.014	0.012	0.021	0.016	0.014	0.013	0.012
Angles, °	1.682	1.921	1.705	2.072	1.843	1.682	1.723	1.675
No. of nonhydrogen atoms								
Protein	2,831	2,697	2,830	2,776	2,795	2,816	2,815	2,877
Water molecules	433	4	331	354	441	421	382	470
Iron atoms	4	8	6	6	5	7	4	4
Others	192	24	197	205	190	202	193	197

Numbers in parentheses correspond to the highest resolution shell.

1. Nicolet Y, et al. (2008) X-ray structure of the [FeFe]-hydrogenase maturase HydE from Thermotoga maritima. *J Biol Chem* 283(27):18861–18872.
2. Nicolet Y, Amara P, Mouesca JM, Fontecilla-Camps JC (2009) Unexpected electron transfer mechanism upon AdoMet cleavage in radical SAM proteins. *Proc Natl Acad Sci USA* 106(35):14867–14871.