

Reduction of C₁ Substrates to Hydrocarbons by the Homometallic Precursor and Synthetic Mimic of the Nitrogenase Cofactor

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MATERIALS AND METHODS

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Natural abundance ^{12}CO (99.5% purity) was purchased from Praxair (Danbury, CT). All isotope-labeled compounds ($\geq 98\%$ isotopic purity) were purchased from Cambridge Isotopes (Andover, MA). A Fe/S/Mo/V standard solution was purchased from Inorganic Ventures (Christiansburg, VA). Anaerobic manipulations were performed in a Vacuum Atmospheres (Hawthorne, CA) Omni-lab glovebox with an argon atmosphere operating at < 2 ppm O_2 . Solvents were dried over columns containing Q-5 and molecular sieves and were sparged with argon and stored over molecular sieves in the glovebox. Triethylammonium tetrafluoroborate $[\text{Et}_3\text{NH}(\text{BF}_4)]$,¹ samarium(II) diiodide tetrahydrofuran adduct $[\text{SmI}_2(\text{THF})_2]$,² and the Fe_6^{RHH} -cluster $([\text{Et}_4\text{N}]_4[\text{Fe}_6\text{S}_9(\text{SEt})_2])$ ³ were prepared as described previously.

Protein Purification and L-Cluster Extraction. An *Azotobacter vinelandii* strain expressing His-tagged NifEN was grown as described elsewhere.^{4,5} Published methods were used for the purification of these nitrogenase proteins.^{4,5} The L-cluster was extracted into dimethylformamide (DMF) from NifEN using a previously described method.^{6,7}

Cluster Concentration Determination. Aliquots of clusters were digested for 30 min in 20% HNO_3 at 100°C . Metal ion concentrations were then determined via inductively-coupled plasma–optical emission spectroscopy (ICP–OES) using an iCAP 7200 DUO system (Thermo Fisher Scientific Inc., Waltham, MA). Samples were calibrated against diluted Fe/S/Mo/V standard solutions. The concentration of the 8Fe L-cluster was determined by dividing the corresponding Fe concentration by eight.

Assays of CN^- , CO and CO_2 Reduction by Clusters. The $\text{SmI}_2(\text{THF})_2$ stock solution was prepared by dissolving solid $\text{SmI}_2(\text{THF})_2$ in dry tetrahydrofuran (THF). The reaction buffer was prepared by dissolving $\text{Et}_3\text{NH}(\text{BF}_4)$ in dry dimethylformamide (DMF), followed by addition of 1.5% v/v triethylamine (Et_3N). A stock solution of the Fe_6^{RHH} -cluster $([\text{Et}_4\text{N}]_4[\text{Fe}_6\text{S}_9(\text{SEt})_2])$ was prepared by dissolving crystals of the cluster in dry DMF. Assays were performed in 10 mL assay vials with crimped butyl rubber serum stoppers. Each assay of CN^- -reduction contained, in a total volume of 1 mL, 100 mM $\text{Et}_3\text{NH}(\text{BF}_4)$, 100 mM tetrabutylammonium cyanide $[\text{Bu}_4\text{N}(\text{CN})]$, 2 μM Fe_6^{RHH} - or L-cluster, and 20 mM $\text{SmI}_2(\text{THF})_2$. Each assay of CO- or CO_2 -reduction contained the same composition as that of CN^- -reduction, except for the omission of $\text{Bu}_4\text{N}(\text{CN})$ and replacement with 10 psi of either CO or CO_2 in the headspace. The negative controls contained the same composition as the assays, except for the omission of either the

cluster or the substrate. The reaction was initiated with the addition of Fe_6^{RHH} - or L-cluster, followed immediately by injection of SmI_2 via a syringe. All assays and controls were incubated in a 30°C water bath shaker for 10 min before the headspace samples were taken and examined for hydrocarbon formation (see “*Gas Chromatograph Analysis of Hydrocarbons*” below). The stability of the Fe_6^{RHH} -cluster under assay conditions was established by inserting the cluster upon completion of the assay into a cofactor-deficient form of the catalytic component of Mo-nitrogenase, which resulted in a semi-artificial enzyme that was capable of C_2H_2 reduction.⁸ The C_2H_2 -reducing activity of thus-generated enzyme was 95% relative to that generated upon insertion of the Fe_6^{RHH} -cluster undergoing the same treatment except for the absence of substrate.

Gas Chromatograph (GC) Analysis of Hydrocarbons. The hydrocarbon products CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , C_4H_8 , C_4H_{10} , C_5H_{10} and C_5H_{12} were measured on an activated alumina column (Grace, Deerfield, IL) in an SRI 8610C GC (SRI Instruments, Torrance, CA) equipped with a flame ionization detector (FID), which was held at 55°C for 1 min, heated to 200°C at 12.5°C/min, and held at 200°C for another 3.6 min. The quantities of all products were determined as described previously^{9,10} by using a purchased gas mixture containing ~15 ppm of each hydrocarbon compound (Praxair, Geismar, LA).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. For GC-MS experiments with isotope labels, natural abundance $\text{Bu}_4\text{N}(^{12}\text{CN})$, ^{12}CO and $^{12}\text{CO}_2$ were replaced by Na^{13}CN , ^{13}CO and $^{13}\text{CO}_2$, respectively, in the activity assays (see “*Assays of CN^- , CO - and CO_2 -Reduction by Clusters*” above). The hydrocarbon products were identified by GC-MS using a Thermo Trace 1300 GC coupled to an ISQ-QD MS (Thermo Fisher Scientific Inc., Waltham, MA). The identities of CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , C_4H_8 , C_4H_{10} , C_5H_{10} and C_5H_{12} were confirmed by comparing their masses and retention times with those of Praxair standard alkane and alkene gas mixtures (Praxair, Geismar, LA). A total of 250 μL gas was injected into a split/splitless injector operated at 125°C in splitless mode. A 1 mm ID liner was used to optimize sensitivity. Gas separation was achieved on a Restek (Bellafonte, PA) PLOT-QS capillary column (0.320 mm ID x 30 m length), which was held at 40°C for 2 min, heated to 180°C at 10°C/min, held at 180°C for 1 min, heated to 220°C at 40°C/min, and finally held at 220°C for 2 min. The carrier gas, helium (He), was passed through the column at 1.1 mL/min. The mass spectrometer was operated in electron impact (EI) ionization and selected ion monitoring (SIM) mode.

Table S1. Yields of hydrocarbon formation by L- and Fe_6^{RHH} -clusters

		C_1 Substrate					
		CN^-		CO		CO_2	
Cluster		L-cluster	Fe_6^{RHH} -cluster	L-cluster	Fe_6^{RHH} -cluster	L-cluster	Fe_6^{RHH} -cluster
<i>Hydrocarbon Products:</i>							
Methane	CH_4	392 ± 51	240 ± 32	112 ± 11	49 ± 4	21 ± 7	7.6 ± 1.6
Ethene	C_2H_4	27 ± 2	23 ± 17	2.2 ± 0.2	2.7 ± 0.5	0.4 ± 0.1	0.5 ± 0.2
Ethane	C_2H_6	33 ± 4	24 ± 3	19 ± 2	10 ± 1	2.8 ± 0.8	1.5 ± 0.5
Propene	C_3H_6	16 ± 2	13 ± 1	1.4 ± 0.1	2.0 ± 0.7	0.2 ± 0.03	0.4 ± 0.1
Propane	C_3H_8	8.1 ± 0.9	5.6 ± 0.8	4.2 ± 0.1	2.8 ± 0.7	0.5 ± 0.1	0.4 ± 0.1
1-Butene	C_4H_8	3.7 ± 1.0	2.6 ± 0.1	0.22 ± 0.03	0.20 ± 0.08	0.04 ± 0.01	0.02 ± 0.01
n-Butane	C_4H_{10}	1.7 ± 0.4	1.2 ± 0.2	0.59 ± 0.05	0.48 ± 0.02	0.11 ± 0.01	0.08 ± 0.04
1-Pentene	C_5H_{10}	0.63 ± 0.03	0.40 ± 0.05	0.03 ± 0.01	0.03 ± 0.01	0	0
n-Pentane	C_5H_{12}	0.33 ± 0.04	0.30 ± 0.07	0.09 ± 0.01	0.07 ± 0.02	0	0
Yield in $\mu\text{mol HC}/\mu\text{mol cluster}$. HC = hydrocarbons							

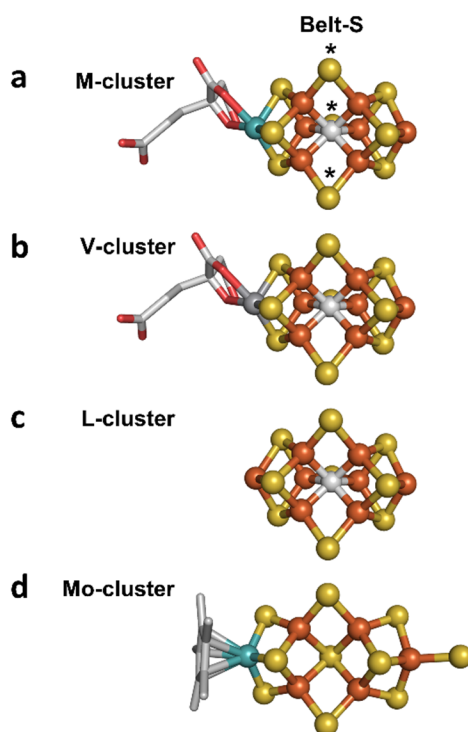


Figure S1. Structural models of the (a) M-, (b) V-, (c) L- and (d) Mo-clusters. PDB entries 3U7Q¹¹ (M-cluster), 3PDI¹² (L-cluster), XAS/EXAFS data from refs. 13, 14 (V-cluster) and data from ref. 15 (Mo-cluster) were used to generate these models. Atoms are colored as follows: Fe, orange; S, yellow; C, light gray; H, red; Mo, cyan; V, dark gray. The belt-S atoms in the M-cluster are indicated by * in a.

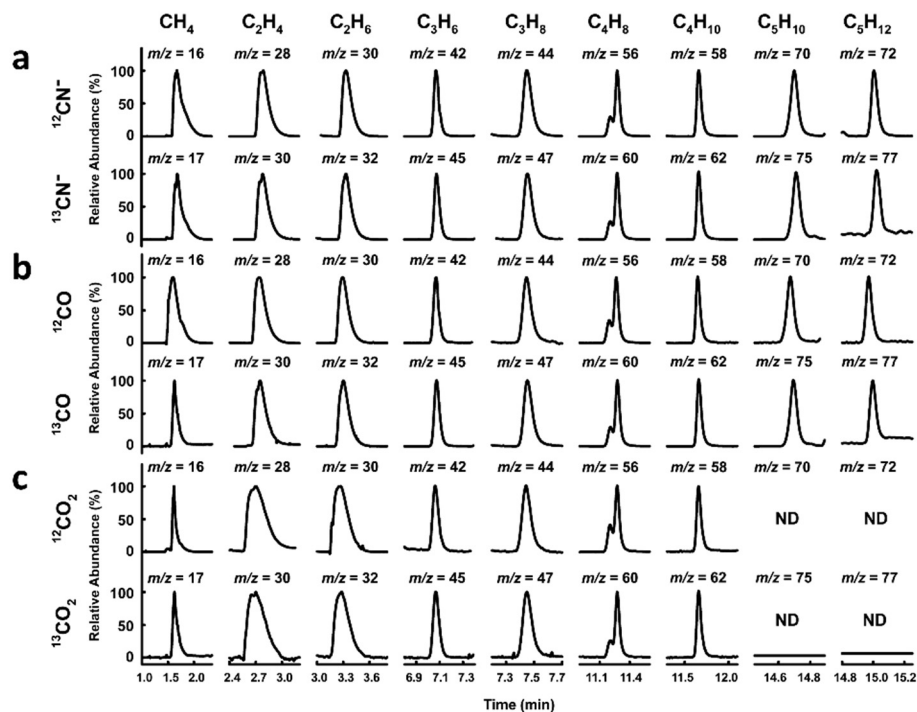


Figure S2. GC-MS analysis of hydrocarbons generated from the reduction of (a) CN^- , (b) CO and (c) CO_2 . Products were generated from the ^{12}C - or ^{13}C -labeled CN^- (a), CO (b) and CO_2 (c). The mass-to-charge (m/z) ratios at which the products were traced are indicated in the figure.

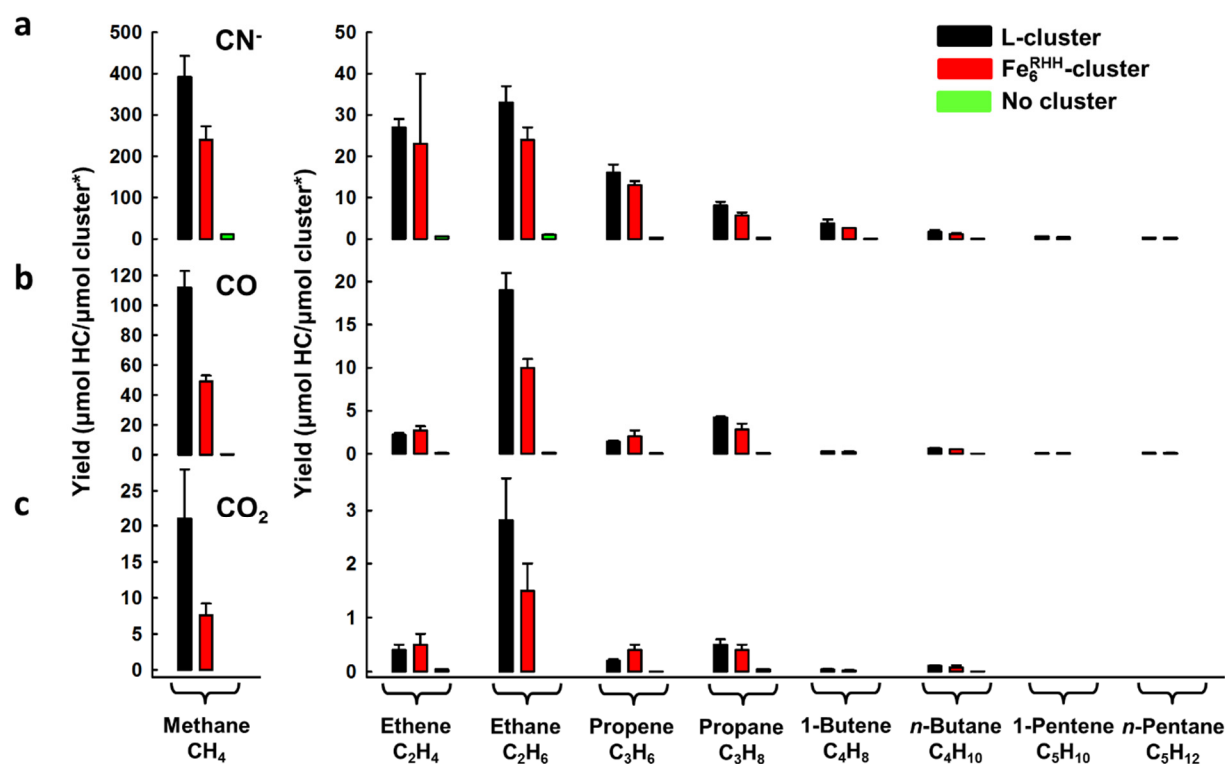


Figure S3. Yields of hydrocarbon formation by L- and Fe_6^{RHH} -clusters from the reduction of (a) CN^- , (b) CO and (c) CO_2 . Shown are the products formed by assays containing the L-cluster (black bars) and the Fe_6^{RHH} -cluster (red bars), respectively, and controls containing the same components of the assay except for the absence of the cluster (green bars).

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