

Supporting Information

Catalytic reduction of CN^- , CO and CO_2 by nitrogenase cofactors in lanthanide-driven reactions **

Chi Chung Lee, Yilin Hu,* Markus W. Ribbe*

EXPERIMENTAL SECTION

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Natural abundance ^{12}C O (99.5% purity) was purchased from Praxair (Danbury, CT). All isotope-labeled compounds ($\geq 98\%$ isotopic purity) were purchased from Cambridge Isotopes (Andover, MA).

Protein purification and cofactor extraction. *Azotobacter vinelandii* strains expressing His-tagged NifEN, MoFe protein and VFe protein were grown as described elsewhere.^[1,2] Published methods were used for the purification of these nitrogenase proteins.^[1,2] The L-, M- and V-clusters were extracted into *N*-methylformamide (NMF) from 1.0 g of NifEN, MoFe protein and VFe protein, respectively, using a previously described method.^[3,4]

Reduction of CN^- , CO and CO_2 by isolated clusters in reactions driven by SmI_2 . A samarium (II) iodide (SmI_2) stock solution was prepared by dissolving 404.2 mg of solid SmI_2 in 10 mL of dry tetrahydrofuran (THF). 2,6-lutidinium triflate (Lut-H) was produced by mixing triflic acid with 2,6-lutidine as described elsewhere.^[5,6] The reaction medium was made by dissolving 100 mM of LutH in dry dimethylformamide (DMF) and adjusting the pH of the solution to 9.0 with triethylamine. The reaction of CN^- reduction contained, in a total volume of 25 mL of the Lut-H/triethylamine reaction medium, 20 mM SmI_2 , 0.45 μmole of NMF-extracted/isolated L-, V- and M-clusters, and 100 mM tetrabutylammonium cyanide $[(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_4\text{N}(\text{CN})]$. The reaction of CO or CO_2 reduction was of the same composition, except that tetrabutylammonium cyanide was replaced by 100% CO or CO_2 that was bubbled into the reaction medium before the addition of SmI_2 and isolated clusters. The negative controls were prepared with the same reaction mixture, except for the omission of either the isolated cluster or the reductant. All reactions were run at ambient temperature and pressure for varying lengths of time. For gas chromatograph-mass spectrometry (GC-MS) experiments with isotope labels, natural abundance $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_4\text{N}(^{12}\text{CN})$, ^{12}CO and $^{12}\text{CO}_2$ were replaced by $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_4\text{N}(^{13}\text{CN})$, ^{13}CO and $^{13}\text{CO}_2$, respectively (also see below).

Activity analysis of CN^- , CO- and CO_2 -reduction by isolated clusters. The products CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , 1- C_4H_8 and *n*- C_4H_{10} were measured by gas chromatography (GC) on an activated alumina column (Grace, Deerfield, IL), which was held at 55°C for 2 min, heated to 200°C at 15°C/min, and held at 200°C for another 2 min. For the SmI_2 -driven reactions, the reaction vials were heated to 40°C to better vaporize the hydrocarbon products. The quantities of all products were determined as described previously^[7,8] by using a purchased Scott (Air Liquide America Specialty Gases LLC, Plumsteadville, PA) gas mixture containing ~15 ppm of each hydrocarbon compounds. The detection thresholds for the products were, in nmole/ μmole cluster, 0.8 (CH_4), 0.7(C_2H_4), 0.9 (C_2H_6), 0.6 (C_3H_6), 0.9 (C_3H_8), 1.1 (1- C_4H_8) and 1.3 (*n*- C_4H_{10}).

Gas chromatograph-Mass Spectrometry (GC-MS) Analysis. The hydrocarbon products were identified by GC-MS using a Thermo Trace 1300 GC coupled to an ISQLT MSD (Thermo Electron North America LLC, Madison, WI). The identities of CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , 1- C_4H_8 and *n*- C_4H_{10} were confirmed by comparing their masses and retention times with those of the Scott standard *n*-alkane and 1-

alkene gas mixture (Plumsteadville, PA). A total of 50 μ L 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 1 min, heated to 220°C at 10°C/min, and held at 220°C for another 3 min. The carrier gas, helium (He), was passed through the column at 1.0 mL/min. The mass spectrometer was operated in electron impact (EI) ionization and selected ion monitoring (SIM) mode.

REFERENES:

1. C. C. Lee, Y. Hu, M. W. Ribbe, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 9209–9214.
2. Y. Hu, A. W. Fay, M. W. Ribbe, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 3236.
3. A. W. Fay, M. A. Blank, C. C. Lee, Y. Hu, K. O. Hodgson, B. Hedman, M. W. Ribbe, *J. Am. Chem. Soc.* **2010**, *132*, 12612–12618.
4. A. W. Fay, M. A. Blank, C. C. Lee, Y. Hu, K. O. Hodgson, B. Hedman, M. W. Ribbe, *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 7787–7790.
5. R. R. Schrock, R. M. Kolodziej, A. H. Liu, W. M. Davis, M. G. Vale, *J. Am. Chem. Soc.* **1990**, *112*, 4338–4345.
6. R. Cordone, W. D. Harman, H. Taube, *J. Am. Chem. Soc.* **1989**, *111*, 2896–2900.
7. C. C. Lee, Y. Hu, M. W. Ribbe, *Science* **2010**, *329*, 642.
8. Y. Hu, C. C. Lee, M. W. Ribbe, *Science* **2011**, *333*, 753–755.

SUPPLEMENTARY TABLE

Table S1. Activities of product formation by M-, V- and L-clusters in the reactions of CN⁻, CO and CO₂ reduction.

Cluster		M-cluster			V-cluster			L-cluster		
Substrate		CN ⁻	CO	CO ₂	CN ⁻	CO	CO ₂	CN ⁻	CO	CO ₂
Product*										
Methane	CH ₄	7157 ± 821	1297 ± 143	806 ± 98	6173 ± 514	1223 ± 131	1166 ± 129	8276 ± 914	2414 ± 214	1523 ± 143
Ethene	C ₂ H ₄	1592 ± 134	148 ± 17	13 ± 1.7	1437 ± 117	165 ± 17	17 ± 1.9	884 ± 107	124 ± 16	21 ± 2.9
Ethane	C ₂ H ₆	1373 ± 151	365 ± 44	20 ± 1.7	1149 ± 98	291 ± 33	29 ± 3.5	1205 ± 187	501 ± 49	40 ± 5.1
Propene	C ₃ H ₆	182 ± 21	61 ± 8	0.7 ± 0.1	143 ± 13	48 ± 4.0	1.2 ± 0.13	68 ± 11	66 ± 13	1.7 ± 0.23
Propane	C ₃ H ₈	226 ± 32	102 ± 11	1.0 ± 0.1	197 ± 25	84 ± 10	1.3 ± 0.21	149 ± 24	151 ± 16	2.3 ± 0.22
1-Butene	C ₄ H ₈	41 ± 6.1	10 ± 0.8	0	51 ± 8.1	9.1 ± 0.13	0	7.0 ± 1.3	17 ± 1.5	0
<i>n</i> -Butane	C ₄ H ₁₀	52 ± 6.0	27 ± 2.1	0	67 ± 7.9	25 ± 3.3	0	13 ± 1.7	24 ± 2.9	0
Carbon monoxide	CO	--	--	545 ± 86	--	--	580 ± 93	--	--	664 ± 87
Hydrogen	H ₂	13900 ± 904	5260 ± 591	5404 ± 512	11070 ± 1073	4730 ± 431	4950 ± 379	14030 ± 1137	4773 ± 587	4677 ± 519
TON	C-based	15	3.0	1.4	13	2.7	1.8	13	4.5	2.3

*Activities are background-corrected expressed in nmol product per assay.

SUPPLEMENTARY FIGURE

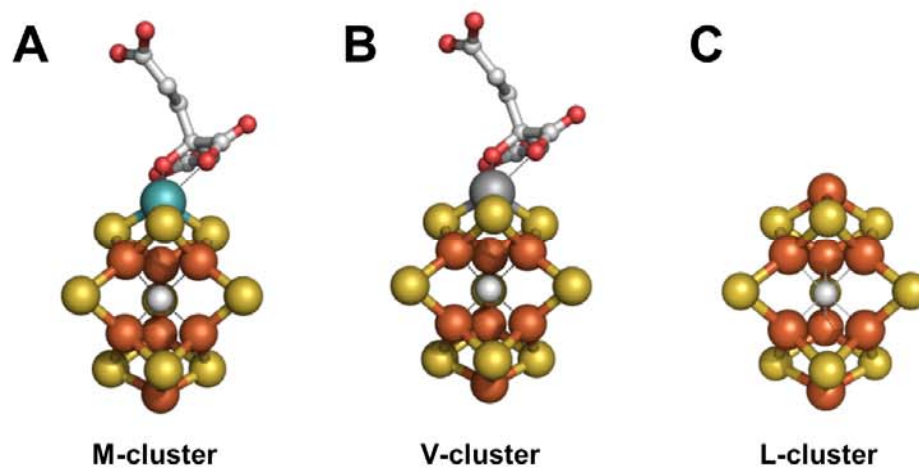


Fig. S1. Nitrogenase cofactors. Shown are the structural models of (A) M-cluster, (B) V-cluster, and (C) L-cluster. PYMOL was used to generate this figure. Atoms are colored as follows: Fe, orange; Mo, blue green; V, dark grey; S, yellow; C, light grey; O, red.

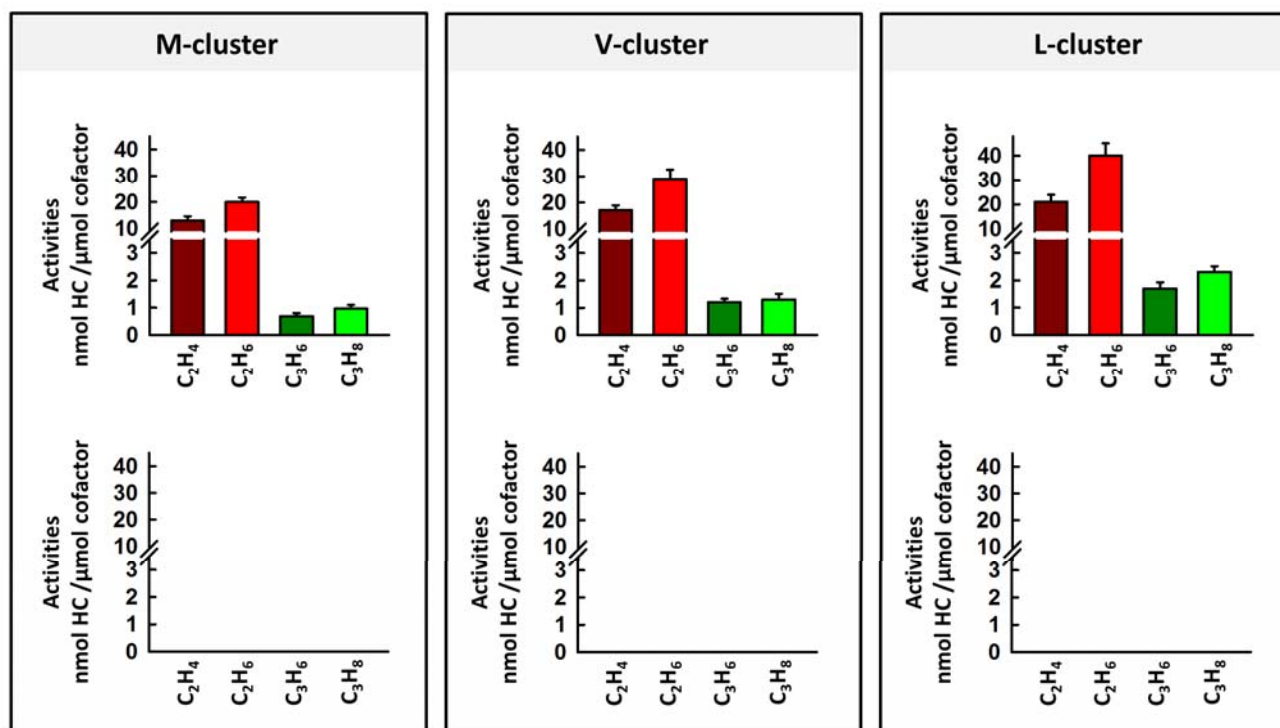


Fig. S2. Reduction of CO₂ (upper) and 0.4 % CO (lower) to C2 and C3 hydrocarbons (HC) by nitrogenase cofactors. The CO concentration used in the experiments (0.4 %) represents the maximum amount of CO produced from CO₂ reduction overnight.