Structure and Reactivity of an Asymmetric Synthetic Mimic of Nitrogenase Cofactor
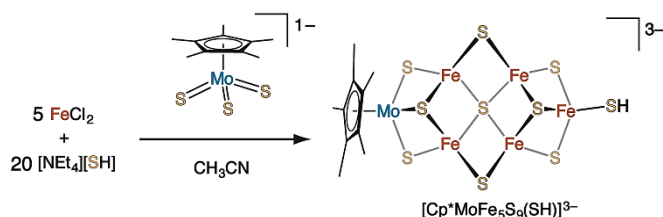
Kazuki Tanifuji, Nathaniel Sickerman, Chi Chung Lee, Takayuki Nagasawa, Kosuke Miyazaki, Yasuhiro Ohki,* Kazuyuki Tatsumi,* Yilin Hu,* and Markus W. Ribbe*

Abstract: The Mo-nitrogenase catalyzes the ambient reduction of N_2 to NH_3 at its M-cluster site. A complex metallocofactor with a core composition of $[MoFe_7S_9C]$, the M-cluster can be extracted from the protein scaffold and used to facilitate the catalytic reduction of CN^-, CO, and CO_2 into hydrocarbons in the isolated state. Here we report the synthesis, structure, and reactivity of an asymmetric M-cluster analog with a core composition of $[MoFe_5S_9]$. Designated the Mo-cluster, this analog is the first synthetic example of an M-cluster mimic with Fe and Mo positioned at the opposite ends of the cluster. Moreover, the ability of the Mo-cluster to reduce C_1 substrates to hydrocarbons suggests the feasibility to develop nitrogenase-based, biomimetic approaches to recycle the C_1 waste into fuel products.

The molybdenum (Mo)-dependent nitrogenase catalyzes the ambient reduction of dinitrogen (N_2) to ammonia (NH_3), a key step in the global nitrogen cycle, at its active cofactor site (designated the M-cluster).[1-3] Arguably the most complex metallocluster identified to date in biological systems, the M-cluster consists of $[MoFe_4S_3]$ and $[Fe_4S_3]$ subclusters that are bridged by three μ_2-sulfides and one μ_6-carbide; additionally, it is coordinated by R-homocitrate, an organic compound, at the Mo end (Figure S1).[4,5] The M-cluster can be studied within the protein matrix or upon extraction into an organic solvent, such as N-methylformamide (NMF) or dimethylformamide (DMF), wherein the M-cluster retains its $[(R\text{-homocitrate})MoFe_7S_9C]$ composition and structural integrity.[6,7] This distinct molybdenum-iron-sulfur (MoFeS) cluster has been unequivocally established as the site of enzymatic N_2 reduction, a feat that involves the breaking of one of the strongest bonds in Nature under ambient conditions. Recently, the M-cluster was shown to enable the reduction of C_1 substrates, such as CN^- and CO, to hydrocarbons in either protein-bound or solvent-extracted state.[8-11] Interestingly, products generated from the enzymatic

reduction of C_1 substrates are limited to short-chain hydrocarbons, such as CH_4, C_2H_4, and C_3H_8, likely due to the constricted space within the active site of the protein that limits the carbon-chain growth.[11] In contrast, when the solvent extracted M-cluster is combined with a strong reductant and an appropriate proton source in water- or solvent-based buffer systems, it can generate a larger product profile of longer hydrocarbons (up to C_4 products) from a broadened spectrum of C_1 substrates (now including CO_2),[8-10] demonstrating the inherent catalytic properties and improved product range of this type of clusters in performing Fischer-Tropsch-like reactions[12,13] on their own.

Attracted by the unique characteristics of the M-cluster, synthetic chemists have sought to synthesize biomimetic analogs to reproduce the structural, spectroscopic, and functional properties of this complex cofactor.[14] Notable examples of synthetic multi-iron clusters[15-17] include $[Fe_6S_9(S^tBu)_2]^{4-}$, $[(edt)_2Mo_2Fe_4S_9]^{4-/3-}$ (edt=ethane-1,2-dithiolate), and $[Fe_4S_3\{N(SiMe_3)_2\}\{SC(NMe_2)_2\}]_2(\mu_6\text{-}S)\{\mu\text{-}N(SiMe_3)_2\}_2$, which underscore advances in the development of synthetic analogs with certain structural features of the nitrogenase cofactor. In particular, the successful synthesis of clusters with an $M_2Fe_6S_8$ or $M_2Fe_6S_9$ core (M=Mo, V) marks significant progress in developing heterometallic mimics of the nitrogenase cofactor.[14] In the meantime, a major challenge has remained for efforts toward generating compounds that exhibit the same asymmetry of the M-cluster structure and the same ability as this cluster to catalyze C–C coupling reactions. Previously, we have synthesized a half-sandwich W(VI) trisulfido complex, $[Cp^*WS_3]^-$ ($Cp^*=\eta^5$- pentamethylcyclopentadienyl)[18] and demonstrated that addition of $[PPh_4][Cp^*MoS_3]$ (PPh_4^-=tetraphenylphosphonium) to a mixture of 5 equivalents of iron(II) chloride ($FeCl_2$) and 20 equivalents of tetraethylammonium hydrosulfide ($[NEt_4][SH]$) in dry acetonitrile (CH_3CN), which resulted in the formation of a black suspension that contained the Mo-cluster, a synthetic compound with a composition of $[Cp^*MoFe_5S_9(SH)]^{3-}$ (Figure 1). Subsequent workup and crystallization of this compound by slow diffusion of diethyl ether (Et_2O) into a concentrated CH_3CN
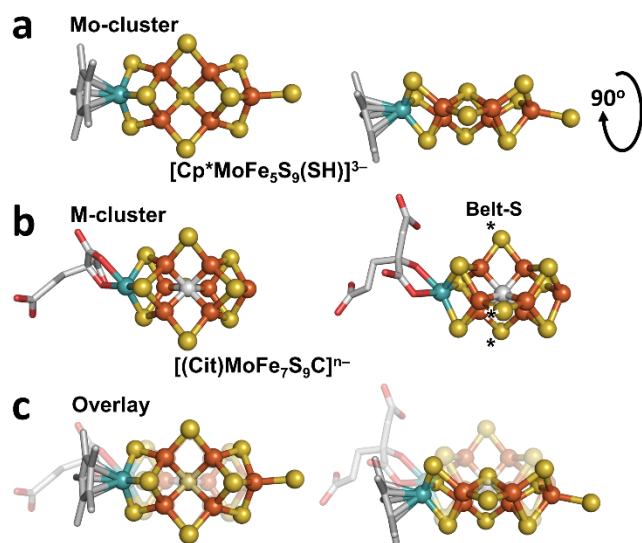
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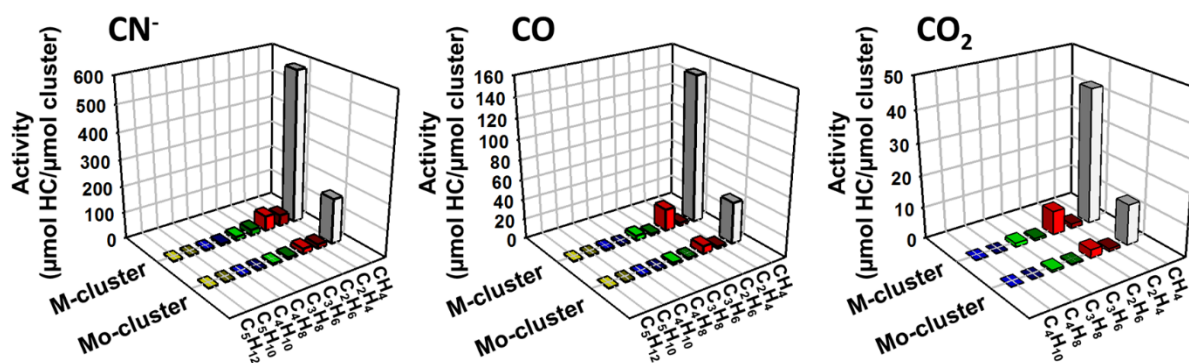
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Figure 1. Synthesis of Mo-cluster. Addition of 1 equivalent of $[Cp^*MoS_3]^-$ ($Cp^* = \eta^5$-pentamethylcyclopentadienyl) to a mixture of 5 equivalents of $FeCl_2$ and 20 equivalents of $[NEt_4][SH]$ in CH_3CN resulted in the formation of $[Cp^*MoFe_5S_9(SH)]^{3-}$ (designated the Mo-cluster). For details see Supporting Information, Experimental Section.

Figure 2. Structural models and overlay of M- and Mo-clusters. The ball-and-stick models of (**a**) Mo- and (**b**) M-clusters, and (**c**) an overlay of the two structures in top (*left*) and side (*right*) views. PDB entry 3U7Q[9] and crystallographic data from this study were used to generate these models. Atoms are colored as follows: Fe, orange; S, yellow; Mo, cyan; O, red; C, light gray. The belt-S atoms in the M-cluster are indicated by * in b.

structures of the two clusters places the unique μ_4-S atom of the Mo-cluster in a similar position to that of the μ_6-C^{4-} ion within the M-cluster (Figure 2c), highlighting a rough structural homology of the Mo-cluster as representative of one face of the M-cluster while implying a loose functional analogy of the former to the latter. Specifically, the three pairs of Fe atoms coordinated to the μ_6-C^{4-} ion, which are bridged by three so-called belt-S atoms in the M-cluster (see Figure 2b, *), have been proposed to be the sites of catalysis in models of cofactor reactivity.[21,22] Structural analysis of a CO-bound conformation of Mo-nitrogenase provided further support for this hypothesis, pointing to a possible mechanistic relevance of sulfur displacement to the activation of belt-S-bridged Fe sites in the M-cluster.[23,24] By analogy, the two pairs of Fe atoms coordinated by the μ_4-S atom, which are further bridged by two belt-S-like atoms in the Mo-cluster (Figure 2a), may also facilitate substrate binding and catalysis in a similar manner to that of their counterparts in the homologous M-cluster.

Recent reports have demonstrated the ability of isolated nitrogenase cofactors to catalytically generate hydrocarbons in the presence of a proton source [2,6-lutidinium triflate (Lut-H)], a strong reductant [samarium(II) diiodide (SmI$_2$); $E^{0\prime}$ = −1.55 V *vs.* SCE in tetrahydrofuran (THF)], and a C$_1$ substrate (CN$^-$, CO or CO$_2$).[8] Given the structural similarities between the Mo- and M-clusters, we reasoned that the Mo-cluster could also facilitate hydrocarbon formation in the same assay system. Indeed, a variety of hydrocarbons were produced from the reduction of C$_1$ substrates upon addition of a THF solution of SmI$_2$ to a DMF solution containing the Mo- or M-cluster and triethylamine (Et$_3$N)-buffered triethylammonium tetrafluoroborate (HNEt$_3$[BF$_4$], pK_a=9.0 in DMSO). For both Mo- and M-clusters, assays with CN$^-$ (Figure 3, *left*) and CO (Figure 3, *middle*) as substrates yielded products CH$_4$, C$_2$H$_4$, C$_2$H$_6$, C$_3$H$_6$, C$_3$H$_8$, C$_4$H$_8$, C$_4$H$_{10}$, C$_5$H$_{10}$ and C$_5$H$_{12}$; whereas assays with CO$_2$ (Figure 3, *right*) as a substrate yielded products CO, CH$_4$, C$_2$H$_4$, C$_2$H$_6$, C$_3$H$_6$, C$_3$H$_8$, C$_4$H$_8$ and C$_4$H$_{10}$. Remarkably, the reactions catalyzed by both Mo- and M-clusters demonstrated strong turnover numbers (TON, μmol total carbon atoms in products/μmol cluster) of (*i*) 282 and 914, (*ii*) 73 and 225, and (*iii*) 24 and 68, respectively, when CN$^-$, CO, and CO$_2$ were supplied as the C$_1$ substrates (Figure 4a, b; Table S1). The TONs of CN$^-$-, CO- and CO$_2$-reduction by M-cluster increased by 61, 75 and 49-fold, respectively, from the previously reported TONs of the same reactions,[8] which resulted from titration experiments that lowered the optimum amount of clusters required for the assays, as well as stabilizing effects of a higher pH of the optimized

solution allowed black crystals of the Mo-cluster to be isolated in 54% yield. The Mo-cluster was then characterized by elemental, mass, UV/vis, EPR and ^{1}H NMR analyses, as well as cyclic voltammetry and X-ray crystallography, which revealed analogous yet distinct spectroscopic, electronic and structural properties of the synthetic Mo-cluster as compared to those of the biogenic M-cluster (see Supporting Information, Experimental Section).
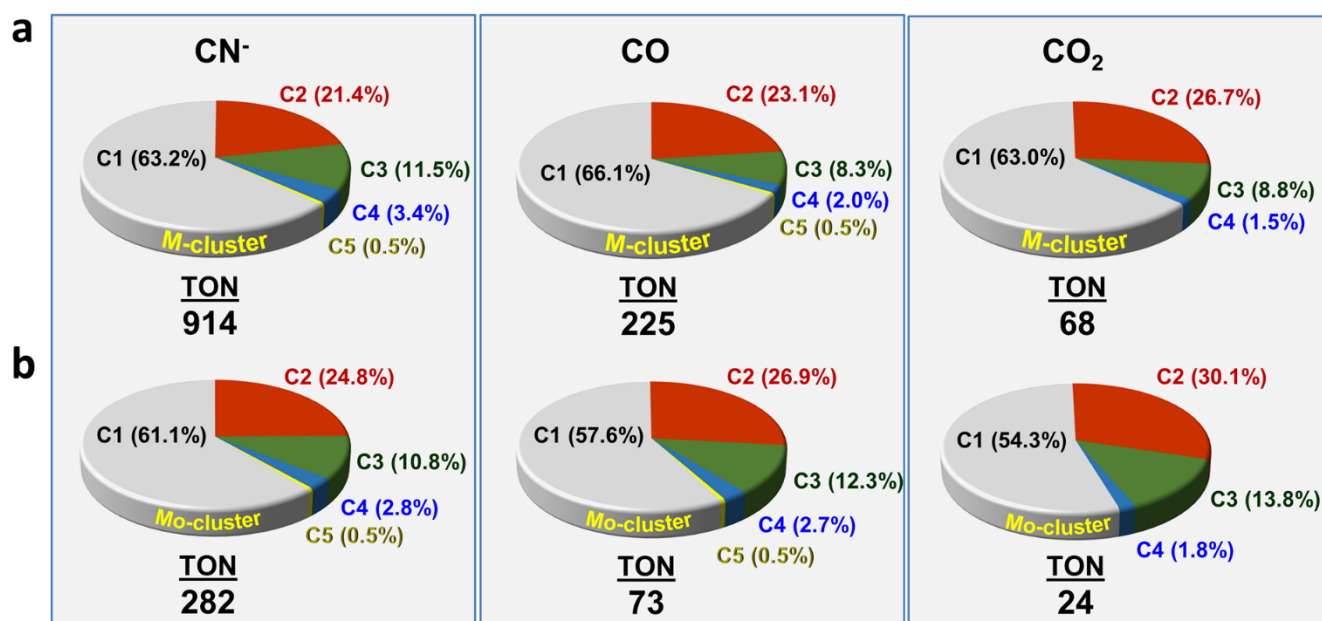
Excitingly, single crystal X-ray diffraction analysis revealed an asymmetric [MoFe$_5$(μ_2-S)$_6$(μ_3-S)$_2$(μ_4-S)]$^{3-}$ core of the Mo-cluster, with one terminal position of the cluster occupied by a Mo center of similar geometry to that in the precursor [Cp*MoS$_3$]$^-$ and the other terminal position occupied by a Fe atom capped with a terminal –SH group (Figure 1; Figure 2a). Like the M-cluster (Figure 2b), the Mo-cluster (Figure 2a) possesses a "face" with μ_3-S and μ_2-S atoms coordinated to Fe atoms, although the absence of two additional faces renders it resemblant to a partially "collapsed" M-cluster. An overlay of the

Figure 3. Hydrocarbon formation by M- and Mo-clusters. Activities of product formation from the reduction of CN$^-$ (*left*), CO (*middle*) and CO$_2$ (*right*) by M- and Mo-clusters. Also see Supporting Information, Table S1.

Figure 4. Product profiles of hydrocarbon formation by M- and Mo-clusters. The total amounts of hydrocarbons generated by (a) M- and (b) Mo-clusters from the reduction of CN^- (*left*), CO (*middle*) and CO_2 (*right*) were set as 100%, respectively, and the percentages of individual products were determined accordingly for each cluster. Please also refer to Figure 5S for $C_{>1}/C_1$ product ratios.

buffer system on the clusters. More importantly, the observed activities of the Mo-cluster established this compound as the first example of a synthetic cofactor mimic with the ability to efficiently convert C_1 substrates to hydrocarbons.

The synthetic Mo-cluster displayed TONs that were on average ~33% of the values determined for the biogenic M-cluster in reactions of CN^--, CO- and CO_2-reduction (Figure 4a, b; Table S1). The reduced activity of the Mo-cluster may be attributed, in part, to the absence of two belt-S-bridging Fe atoms (see Figure 1 and Figure 2a), which have been implicated i n substrate binding and catalysis.[3] Additionally, contrary to the Mo center in the M-cluster, the Mo center in the Mo-cluster is coordinatively saturated and sterically encumbered by the Cp* ligand, which could render Mo inaccessible and prevent it from carrying out certain functions in catalysis. Other than a change in the overall activity, the Mo-cluster also displayed a different substrate profile than the M-cluster in the reduction of C_1 substrates. Despite a clear preference of both clusters in producing CH_4 from C_1 substrates, the M-cluster appeared to bias the reactions further toward the formation of CH_4; whereas the Mo-cluster seemed to favor C-C coupling, or the formation of $>C_1$ hydrocarbons (Figure S5). Such a discrepancy underlines the structural-functional impact on the reactivities of the two clusters, which could originate from a number of architectural features of this type of clusters. The "collapsed" belt-S region in the Mo-cluster, for one, could account for the altered activity of this cluster given the potential catalytic relevance of this region. The "substitution" of a μ_4-S atom for the μ_6-C^{4-} ion, for another, may also contribute to the modified reactivity of the Mo-cluster, as a role of μ_6-C^{4-} ion in stabilizing the cluster and/or fine-tuning its reactivity has been proposed through theoretical and synthetic studies of the cofactor.[25] Together, these observations provide a rough outline of the structural criteria for this type of clusters to perform the Fischer-Tropsch-like synthesis of hydrocarbons. It is interesting to note that, in the presence of a fixed amount of chemical reductants, the reaction time appears to be rather short for product formation by both the M- and Mo-clusters. As such, future studies will focus on assessing the catalytic mechanism at low temperatures using spectroelectrochemical methods. Moreover, deuterated acids will be employed to evaluate the kinetic isotope effect and deuterated product distribution to provide further insights into the reaction mechanism.

While the mechanistic details of the reaction are yet to be revealed, the current study establishes the Mo-cluster as one of the most faithful structural mimics of the M-cluster to date, but perhaps more importantly, it provides the initial proof that synthetic mimics of the nitrogenase cofactor are capable of converting the greenhouse gas CO_2 to renewable hydrocarbon fuels under ambient conditions. Efforts along this line, together with the exciting development of functional synthetic analogs that can either bind and/or activate N_2 or other nitrogenase substrates—including sulfido- or thiolate-bridged diiron complexes, as well as single Fe complexes with a C anchor[21,26-30]—could collectively contribute to a better mechanistic understanding of the nitrogenase cofactor. Further, the utility of the synthetic approach to the Mo-cluster may be explored for the generation of a library of synthetic analogs with various heterometals, which could lead to the identification of novel heterometallic clusters with desirable catalytic capabilities in the future.

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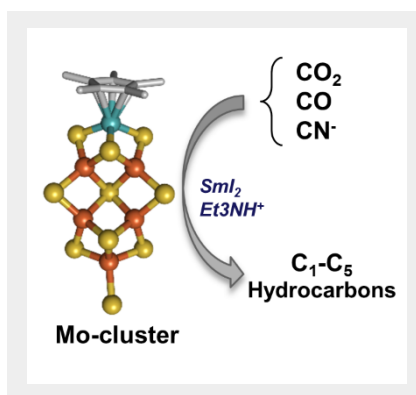[30]   S. E. Creutz, J. C. Peters, *J. Am. Chem. Soc.* **2015**, *137*, 7310-7313.

Entry for the Table of Contents

COMMUNICATION

A synthetic analog (Mo-cluster) of the nitrogenase cofactor (M-cluster) is capable of reducing C_1 substrates to hydrocarbons, suggesting the possibility to develop nitrogenase-based, biomimetic approaches to recycle the C_1 waste into useful fuel products.

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