

Ambient Conversion of CO₂ to Hydrocarbons by Biogenic and Synthetic [Fe₄S₄] Clusters

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Abstract:

The Fe protein of nitrogenase contains a redox active [Fe₄S₄] cluster that plays a key role in electron transfer and substrate reduction. Here we show that the Fe protein of *Methanosarcina acetivorans* can reduce CO₂ and CO to hydrocarbons under ambient conditions. Further, we demonstrate that this reactivity is inherent to [Fe₄S₄] clusters, showing the ability of a synthetic [Fe₄S₄] compound to catalyse the same ambient reaction in solutions. Theoretical calculations suggest a reaction mechanism involving an aldehyde-like intermediate that gives rise to hydrocarbon products upon proton-coupled electron transfer and concomitant removal of water molecules. These results provide a framework for mechanistic investigations of FeS-based activation and reduction of CO₂ and CO while facilitating potential development of FeS catalysts capable of ambient conversion of CO₂ and CO into fuel products.

Iron-sulphur (FeS) proteins are crucial for a wide variety of biological functions, such as electron transfer, enzyme catalysis, iron homeostasis, DNA synthesis, and gene regulation¹⁻⁵. Designated the iron (Fe) protein, the reductase component of nitrogenase is a homodimeric protein containing a subunit-bridging [Fe₄S₄] cluster⁶⁻⁸, and it serves as an obligate electron donor for its catalytic partner during catalysis. Recently, the Fe protein of *Azotobacter vinelandii*, a soil bacterium, was shown to act as a reductase on its own and catalyse the ambient reduction of CO₂ to CO under *in vitro* or *in vivo* conditions via the redox change of its [Fe₄S₄] centre; however, further reduction past CO was not detected in this case⁹. This observation has piqued our interest in further exploring the reactivity of the nitrogenase Fe protein toward CO₂ and addressing the question of whether the Fe protein can reduce CO₂ beyond CO into hydrocarbon products. The Fe protein homologs from methanogenic organisms are interesting subjects of study along this line of pursuit, given the high concentration of CO₂ in the habitat of these organisms and their metabolic adaptation to CO₂ reduction. Moreover, the relatively surface-exposed location of the [Fe₄S₄] cluster in the Fe protein⁶⁻⁸ points to the possibility that [Fe₄S₄] clusters that exist freely in solutions may have the same CO₂-reducing activity as their protein-bound counterpart. Understanding the reactivity of the [Fe₄S₄] cluster toward CO₂ is important, as it would enable identification of simple FeS clusters as catalysts that mimic the function of the industrial Fischer-Tropsch process¹⁰ in carbon fuel production under ambient conditions.

Here we show that the Fe protein of *Methanosarcina acetivorans* is capable of ambient reduction of CO₂ and CO to hydrocarbons. We further demonstrate that this reactivity is inherent to [Fe₄S₄] clusters, as a synthetic [Fe₄S₄] compound can catalyse the same reaction in solutions. Our theoretical calculations suggest a reaction mechanism involving an aldehyde-like intermediate,

thereby providing a useful framework for mechanistic investigations of FeS-based CO₂ reduction and future development of strategies to recycle CO₂ into valuable chemical commodities.

Results

Reduction of CO₂ and CO by protein-bound [Fe₄S₄] clusters. To examine the reactivity of methanogen Fe proteins toward CO₂, we expressed the Fe protein of *Methanosarcina acetivorans* in *Escherichia coli*¹¹ and conducted comparative CO₂ reduction assays of this Fe protein with the Fe protein of *A. vinelandii*. Like its *A. vinelandii* counterpart (designated NifH^{Av}), the Fe protein of *M. acetivorans* (designated NifH^{Ma}) was capable of reducing CO₂ to CO in the presence of a strong reductant, europium diethylenetriaminepentaacetic acid (Eu^{II}–DTPA; $E^{0'} = -1.14$ V at pH 8)¹². In the case of NifH^{Av}, the yield of CO increased when the concentration of Eu^{II}–DTPA was increased from 10 to 100 mM (Fig. 1a). In the case of NifH^{Ma}, however, while the formation of CO increased slightly upon an increase of the concentration of Eu^{II}–DTPA from 10 to 20 mM, it decreased concomitantly with an increase in the formation of C₁–C₃ hydrocarbons when Eu^{II}–DTPA concentration was increased beyond 20 mM, reaching a maximum hydrocarbon yield of 3.9 nmol reduced C/nmol cluster at 100 mM Eu^{II}–DTPA (Fig. 1a). Gas chromatography-mass spectrometry (GC-MS) analysis further confirmed that these hydrocarbon products originated from CO₂, showing the characteristic fragmentation patterns and expected mass shifts of C₁ (CH₄), C₂ (C₂H₄, C₂H₆) and C₃ (C₃H₆, C₃H₈) products by +1, +2 and +3, respectively, upon substitution of ¹³CO₂ for ¹²CO₂ (Fig. 1b). Interestingly, despite an increase in the hydrocarbon/CO ratio with increasing concentrations of Eu^{II}–DTPA, the total carbon yield calculated on the basis of both CO and hydrocarbons remained approximately the same (~5 nmol reduced C/nmol cluster) when ≥20 mM Eu^{II}–DTPA was supplied to the NifH^{Ma}-catalysed reaction (Fig. 1a), suggesting a reductant concentration-dependent re-distribution of electrons between CO- and hydrocarbon-formation in the NifH^{Ma}-catalysed reaction (Supplementary Fig. 1). In contrast,

hydrocarbon products could not be detected in the NifH^{Av}-catalysed reaction (Fig. 1a), highlighting the difference between NifH^{Ma} and NifH^{Av} in their reactivities toward CO₂.

The same discrepancy between NifH^{Ma} and NifH^{Av} was observed when CO was supplied as a substrate, as C₁-C₄ hydrocarbons were only detected in the reaction catalysed by NifH^{Ma} (Fig. 1c). The reduction of CO to hydrocarbons by NifH^{Ma}, like that of CO₂ to hydrocarbons, increased dramatically with increasing Eu^{II}-DTPA concentrations, reaching a maximum turnover number (TON) of 29.5 at 100 mM Eu^{II}-DTPA (Fig. 1c). GC-MS analysis provided evidence that these hydrocarbons were derived from the substrate CO, showing the expected fragmentation patterns and mass shifts of the C₁ (CH₄), C₂ (C₂H₄, C₂H₆), C₃ (C₃H₆, C₃H₈) and C₄ (C₄H₈, C₄H₁₀) products when ¹²CO was replaced by ¹³CO (Fig. 1d). Notably, the TONs of the reactions of CO-reduction were consistently higher than those of the reactions of CO₂-reduction at varying concentrations of Eu^{II}-DTPA, with the biggest difference between the two (by 7.5-fold) observed at 100 mM Eu^{II}-DTPA (Fig. 2a, b). Moreover, there was a shift toward formation of longer hydrocarbon chains when CO-reduction was compared with CO₂-reduction, although both reactions showed a tendency toward formation of longer hydrocarbon products with increasing concentrations of Eu^{II}-DTPA (Fig. 2a, b). Despite these differences, the reduction of CO and CO₂ by NifH^{Ma} displayed similar hydrocarbon product distributions (Fig. 2a, b), suggesting routing of the reduction of CO₂ via CO or CO-derived intermediates. It is plausible, therefore, that the differential reactivities of NifH^{Ma} and NifH^{Av} toward CO₂ originate from differential binding affinities of these proteins to the intermediate CO, with NifH^{Ma} having a much stronger affinity for CO than NifH^{Av}, which facilitates the reduction of CO and formation of C-C bonds by NifH^{Ma} while permitting the release of CO as an end product by NifH^{Av}. The observation that the product yield of NifH^{Ma} saturated quickly (at ~0.5% CO) with increasing CO concentrations provides support for a high affinity of NifH^{Ma} for CO (Supplementary Fig. 2). Additionally, the ability of the oxidized NifH^{Av} to catalyse the conversion

of CO to CO₂⁹—a feature resembling the CO/CO₂ interconverting enzyme, carbon monoxide dehydrogenase¹³⁻¹⁵—was not duplicated in the case of the oxidized NifH^{Ma} (Supplementary Fig. 3a), which could further contribute to the differential reactivities of these two Fe proteins toward CO and CO₂. Such a difference likely reflects a difference in the redox potentials of NifH^{Ma} and NifH^{Av}, with the [Fe₄S₄]^{1+/2+} couple of the former protein ($E^{\circ'} = -395$ mV) showing a significantly lower redox potential than that of the latter protein ($E^{\circ'} = -301$ mV) (Supplementary Fig. 3b, c). Indeed, when driven by 200 mM dithionite ($E^{\circ'} \approx -430$ mV at pH 7)¹⁶, NifH^{Ma} was capable of reducing CO to hydrocarbons at low yields, with a product profile resembling that generated in the presence of Eu^{II}–DTPA; whereas no hydrocarbon could be detected in the reaction catalysed by NifH^{Av} under the same conditions (Supplementary Fig. 4a, b). Importantly, the fact that CO can be reduced by NifH^{Ma} at a redox potential mimicking that in the cell suggests a potential physiological relevance of this reaction.

Reduction of CO₂ and CO by synthetic [Fe₄S₄] clusters. The observation of ambient conversion of CO₂ and CO to hydrocarbons by a protein-bound [Fe₄S₄] cluster (Supplementary Fig. 5a) has led to the question of whether the reactivity toward these substrates is an inherent catalytic feature of the [Fe₄S₄] clusters. To address this question, a synthetic [PPh₄][Fe₄S₄(SCH₂CH₂OH)₄] compound (designated [Fe₄S₄]^{Syn}) containing a [Fe₄S₄] core and four cysteine-like, β-mercaptoethanol (BME) thiolate ligands (Supplementary Fig. 5b)^{17,18}, was tested for its ability to reduce CO₂ and CO. Driven by 20 mM Eu^{II}–DTPA in an aqueous buffer, [Fe₄S₄]^{Syn} was capable of generating up to C₂ hydrocarbon products (i.e., CH₄, C₂H₄, C₂H₆) hydrocarbons at TONs of 0.3 and 0.4, respectively, in the reactions of CO₂- and CO-reduction (Fig. 2a, b). Substitution of 20 mM samarium(II) iodide (SmI₂; $E^{\circ'} \approx -1.5$ V in DMF)¹⁹ along with a proton source, triethyl-

ammonium tetrafluoroborate [Et₃NH(BF₄)], in the organic solvent dimethylformamide (DMF) for Eu^{II}–DTPA in an aqueous buffer resulted in substantial increases of hydrocarbon formation to TONs of 15.8 and 89.7, respectively, in the [Fe₄S₄]^{Syn}-catalysed reactions of CO₂- and CO-reduction while pushing both reactions toward the formation of up to C₄ hydrocarbon products (i.e., CH₄, C₂H₄, C₂H₆, C₃H₆, C₃H₈, C₄H₈, C₄H₁₀) (Fig. 2a, b). Remarkably, the maximum TONs of [Fe₄S₄]^{Syn} in the reactions of CO₂- and CO-reduction were 4- and 3-fold, respectively, of those of the biogenic [Fe₄S₄] cluster in NifH^{Ma} (Fig. 2a, b), likely due to the improved accessibility of the reaction site in solutions of [Fe₄S₄]^{Syn} clusters that increased the overall yields of hydrocarbons in the reactions of CO₂- and CO-reduction. Compared to the activity of hydrocarbon formation by [Fe₄S₄]^{Syn}, the CO₂- and CO-reducing activities of iron(III) chloride (FeCl₃) solutions containing equimolar Fe to that of the [Fe₄S₄]^{Syn} cluster were only 7% and 4%, respectively, in the presence of sodium sulphide (Na₂S); and 2% and 2%, respectively, in the absence of Na₂S (Supplementary Fig. 6a, b), providing strong, albeit indirect proof that the activity of [Fe₄S₄]^{Syn} did not originate from small Fe/S fragments/constituents.

Proposed pathway of CO₂ reduction by the [Fe₄S₄] cluster. Density functional theory (DFT) calculations, which provided mechanistic insights into the reaction of CO reduction by vanadium nitrogenase²⁰, were performed on the [Fe₄S₄] cluster, leading to the proposal of plausible mechanisms of [Fe₄S₄]-cluster-catalysed CO₂/CO reduction (see Methods and Supplementary Notes 1-3; also see Supplementary Fig. 7). One mechanism involves protonation and dissociation of a thiolate ligand, which permits C-C coupling at the same Fe centre of the cluster (Fig. 3; also see Supplementary Figs. 8a and 9a). Binding of CO₂ to the cluster is only slightly exothermic (Fig. 3a, step 1), which might contribute to the moderate turnover efficiency of this catalyst. Favourable coordination of

CO₂ to one Fe atom of the free cluster, like that observed in the case of the protein-bound cluster⁹, occurs when the cluster is present in the all-ferrous oxidation state with a $S=0$ spin state (Supplementary Note 3). Protonation of the coordinated CO₂ species, followed by proton-coupled electron transfer, then initiates cleavage of a water molecule and leaves the cluster in a CO-bound, [Fe₄S₄]⁺ state (Fig. 3a, steps 2, 3). The reaction pathway becomes branched at this point. In one branch, the reaction continues with dissociation of CO (Fig. 3a, step 4) and re-reduction of the cluster (Fig. 3a, step 5). Dissociation of CO is energetically disfavoured, as is the re-reduction of the cluster, which is consistent with the low CO₂-reducing activity of the cluster. In the other branch, the reaction proceeds with protonation-induced dissociation of the thiolate ligand at the Fe atom coordinated by CO (Fig. 3b, step 6), followed by proton-coupled electron transfer to the Fe-coordinated CO moiety, forming an aldehyde-like, iron-formyl species (Fig. 3b, step 7). Subsequently, this moiety could undergo an overall exothermic series of proton-coupled electron transfer steps (see Supplementary Fig. 9a), which, coupled with dissociation of a water molecule, eventually lead to a reactive cluster-bound CH₃ group (Fig. 3b, steps 8-11). Subsequent electron transfer and proton transfer from a free thiol molecule readily yield CH₄ and restore the cluster to its fully thioethanolate-coordinated form (Fig. 3b, step 12).

Several mechanistic branching points are possible that involve coordination of a second CO molecule when a primary C₁ species is still bound to the cluster. One likely pathway for C-C bond formation involves coordination of CO at the CH₃-bound Fe site, which is exothermic by ca. -14 kcal/mol and allows for an exothermic migratory insertion, yielding an iron-acetyl intermediate (Fig. 3c, steps 14, 15). A subsequent series of reduction and proton-transfer steps, which is overall highly exothermic (see Supplementary Fig. 9a), results in dissociation of a water molecule and formation of an Fe-bound ethyl moiety (Fig. 3c, steps 15-19). Note that, for the dissociation of

water to occur, the cluster has to be formally present in the all-ferrous $[\text{Fe}_4\text{S}_4]^0$ state (Fig. 3c, step 18, transfer of two electrons). Subsequent exothermic proton-coupled electron transfer readily yields a cluster-bound ethyl species (Fig. 3c, steps 17, 18). Analogous to the formation of the C_1 species, ‘recombination’ of the cluster with a thiol molecule results in the formation of C_2H_6 (Fig. 3c, step 19), and restores the cluster to the $[\text{Fe}_4\text{S}_4]^0$ or $[\text{Fe}_4\text{S}_4]^+$ state, depending on whether an additional electron is simultaneously transferred (Fig. 3c, steps 20a, 20b). It seems energetically plausible for the cluster to employ a similar mechanism for the formation of longer carbon chains: CO could coordinate to an ethyl-bound Fe atom with a reaction energy of ca. -12 kcal/mol, followed by another round of migratory insertion, which would be exothermic by ca. -9 kcal/mol. Due to multiple competing reaction branches, however, the probability for this chain elongation event to occur would be substantially lower than for the C_2 mechanism (e.g., dissociation of the carbon species), which is in line with the experimentally observed bias toward short-chain product formation (Fig. 2).

Other than the mechanism proposed above, which involves removal of the thiolate ligand (Fig. 3a, step 6) for the subsequent C-C coupling via migratory insertion at a single Fe site (Fig. 3c, step 14), an alternative mechanism is conceivable for the fully ligated cluster, which allows the pathway to proceed through coordination events at two neighbouring Fe centres (Fig. 4; Supplementary Figs. 8b and 9b). The two mechanisms share the same pathway for CO reduction (Figs. 3a and 4a, steps 1-5) and analogous pathways for CH_4 formation except for variations of energetics in the presence of the thiolate ligand (Fig. 3b, steps 6-12; Fig. 4b, steps 6-11). In the latter mechanism, however, the second CO molecule can be reduced to a CH_2 species by a sequence of proton/electron transfer steps (Fig. 4c, steps 14-17), strongly resembling the reduction of the first CO molecule. The resulting, highly reactive CH_2 moiety can then be combined with the

CH₃ moiety at the proximal Fe atom to form an Fe-bound ethyl species with an energy barrier of only ~7 kcal/mol (Fig. 4c, step 18). Exothermic proton or coupled proton/electron transfer and ethane formation close the cycle (Fig. 4c, steps 19a, 19b). Coordination of a second CO molecule to the ethyl-bound cluster is energetically feasible and could explain the formation of longer carbon chains following the same sequence of events that lead to the formation of the C₂ species (Fig. 4c, steps 13-18), although the chain extension process would occur at a significantly reduced efficiency due to the multitude of competing reactions.

Based on these calculations, a constantly high supply of electrons would steer the reactivity of the cluster from CO dissociation (Figs. 3a and 4a, steps 4, 5) towards CO reduction (Fig. 3b, steps 6-12; Fig. 4b, steps 6-11), which is corroborated by the experimentally observed increase of the hydrocarbon/CO ratio in response to increasing amounts of reductant (Fig. 1a). Moreover, consistent with the calculation-based proposal of an aldehyde-like intermediate, [Fe₄S₄]^{Syn} was shown to reduce formaldehyde (CH₂O) at a yield of 16 nmol CH₄/nmol cluster (Fig. 5a), and GC-MS analysis further demonstrated the expected mass shift and fragmentation pattern of the product CH₄ upon substitution of ¹³CH₂O for ¹²CH₂O, thereby confirming that the carbon in CH₄ originated from CH₂O (Fig. 5b-e). Of the CO reduction events, however, C-C coupling has to compete with protonation of the CH₃ moiety, which can occur for every intermediate and which would consequently generate intermediates for CH₄ formation (Figs. 3b, 4b), thereby further increasing the yield of CH₄.

The ability of [Fe₄S₄] clusters to catalyse the ambient reduction of CO₂ and CO to hydrocarbons could have implications for the prebiotic evolution of organic molecules, as previous studies have suggested a role of Fe- or FeS-containing minerals around underseas hydrothermal vents in forming

hydrocarbons and other organic molecules in the presence of CO₂²¹⁻²⁴. It has been demonstrated that CO can react with CH₃SH on coprecipitated FeS and NiS to form carboxylic acids, carbohydrates and amino acids²⁵⁻²⁸, suggesting a prebiotic CO fixation pathway that could be correlated to the emergence of primordial lipids, sugars and peptides/proteins on Earth (see Supplementary Discussion). By analogy, the reduction of CO by FeS clusters in a speculated, highly reducing early atmosphere might represent another prebiotic route of CO activation, which generates small alkenes and alkanes as carbon and/or electron sources for certain methane- and ethene-assimilating organisms^{29,30}. It is plausible, therefore, that the activity of NifH^{Ma} in reducing CO₂ to hydrocarbons may represent an evolutionary relic of the function of FeS proteins in methanogens. Given the CO₂-rich habitats of many methanogenic organisms, the differential abilities of the methanogenic and non-methanogenic organisms to handle CO₂ may account for the differential reactivities of the [Fe₄S₄] clusters in NifH^{Av} and NifH^{Ma} toward CO₂. While the impact of protein environments on the reactivities of these biogenic [Fe₄S₄] clusters awaits further investigation, the reaction pathway proposed for the [Fe₄S₄] cluster serves as a good starting point for further exploration of the mechanism of FeS-based ambient reduction of CO₂, which will in turn facilitate future development of FeS catalysts that convert the greenhouse gas CO₂ into useful fuel products.

Methods

Materials. Unless otherwise specified, all chemicals were purchased from Thermo-Fisher Scientific (Waltham, MA) and Sigma-Aldrich (St. Louis, MO). Natural abundance ¹²CO (99.9% purity) and ¹²CO₂ (99.98% purity) were purchased from Praxair (Danbury, CT). Isotopologues ¹³CO (99% isotopic purity) and ¹³CO₂ (99% isotopic purity) were purchased from Cambridge Isotope Laboratories (Andover, MA). An Fe/S/Mo/V standard solution for metal content determination was purchased from Inorganic Ventures (Christiansburg, VA). Air-free manipulations were performed in a Vacuum Atmospheres (Hawthorne, CA) Omni-lab glovebox with an argon atmosphere operating at <2 ppm O₂. Water was purified using a

Barnstead E-Pure water purification system (Thermo Scientific). Organic solvents were dried over columns containing Q-5 and molecular sieves and sparged with Ar; the solvents were stored over molecular sieves in the glovebox. Triethylammonium tetrafluoroborate ($[\text{Et}_3\text{NH}(\text{BF}_4)]$), samarium(II) diiodide tetrahydrofuran adduct ($[\text{SmI}_2(\text{THF})_2]$), and the $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ cluster ($[\text{Ph}_4\text{P}]_2[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]$) were prepared as described earlier^{18,31,32}.

Cell growth and protein purification. An *Azotobacter vinelandii* strain expressing a non-tagged, *nifH*-encoded Fe protein of the Mo-nitrogenase (designated NifH^{Av}) was grown as described elsewhere^{33,34}. An *Escherichia coli* strain expressing a His-tagged, *nifH*-encoded Fe protein of the Mo-nitrogenase of *Methanosarcina acetivorans* (designated NifH^{Ma}) was grown as reported earlier¹¹. Published methods were used for the purification of NifH^{Av} and NifH^{Ma} from their respective expression strains^{11,33,34}.

Redox potential determination. Redox titration was performed at ambient temperature in a glove box under anaerobic conditions. A series of NifH^{Ma} samples were prepared in a 20 mM Tris–HCl buffer (pH 8.0) at a protein concentration of 2.5 mg/mL. Redox mediator dyes, including methyl viologen, benzyl viologen, safranin O and phenosafranin, were added at a final concentration of 10 μM to the series of NifH^{Ma} protein samples. Subsequently, these samples were first reduced by excess sodium dithionite (DT; $\text{Na}_2\text{S}_2\text{O}_4$) and then subjected to oxidative titration with 1 mM potassium ferricyanide. Reduction potentials were monitored with a combination electrode comprising a platinum working electrode and a saturated Ag/AgCl reference electrode (Microelectrodes, Inc., Bedford, NH), which were used to report potentials relative to the standard hydrogen potential (SHE). After achieving the desired potential, a 200 μL aliquot of each mixture was transferred to a capped quartz cuvette, and the transition between the $[\text{Fe}_4\text{S}_4]^{1+/2+}$ couple was monitored at 420 nm by UV/vis spectroscopy.

Assays of CO- and CO₂-reduction with Fe proteins. The *in vitro* CO- and CO₂ reduction assays were carried out in 9.4 mL assay vials with crimped butyl rubber serum stoppers. Each assay contained, in a total volume of 1.0 mL, 500 mM Tris–HCl (pH 10.0, for CO₂ reduction assays) or 250 mM Tris–HCl (pH 8.0, for CO reduction assays), 0.5 mg Fe protein (NifH^{Av} or NifH^{Ma}), and either 200 mM dithionite or increasing concentrations (10, 20, 40 or 100 mM) of europium(II) diethylenetriaminepentaacetate (Eu^{II} –DTPA). The optimum concentration of CO (0.53%) or CO₂ (100%) that yielded the maximum amount of product was determined via titration against increasing concentrations of CO or CO₂ and added to the headspace of the respective assay for activity analysis. For reactions of CO reduction, assays were assembled by repeatedly flushing and exchanging the buffer solution (without Eu^{II} –DTPA and protein) with 100% Ar, followed by the addition of 0.53% CO; whereas for reactions of CO₂ reduction, assays were assembled by repeatedly

flushing and exchanging the buffer solution (without Eu^{II} -DTPA and protein) with 100% CO_2 , and equilibrating for 30 min until pH stabilized at approximately 8.1. The reaction was initiated by the addition of Fe protein, followed immediately by the addition of Eu^{II} -DTPA and incubation with continuous shaking at 30°C until the reaction was complete (300 min) before it was quenched by the addition of 100 μL of 30% trichloroacetic acid. Subsequently, headspace samples were taken to examine the production of CO and hydrocarbons.

Assays of CO oxidation with Fe proteins. The *in vitro* CO oxidation assays were carried out under 100% CO in 9.4 mL assay vials with crimped butyl rubber serum stoppers. Each assay contained, in a total volume of 1.0 mL, 250 mM Tris-HCl (pH 8.0), 10 mg NifH^{Av} or NifH^{Ma} , and 20 mM IDS. All assays were assembled by repeatedly flushing and exchanging the buffer solution (without IDS and protein) first with 100% Ar and then with 100% CO, followed by equilibration for 30 min. The reaction was initiated by the addition of Fe protein, followed immediately by the addition of IDS and incubation with continuous shaking at 30°C until the reaction was complete (300 min). Subsequently, 100 μL concentrated hydrochloric acid (HCl) was added to each assay to release the dissolved CO_2 before the headspace sample was examined for the production of CO_2 .

Assays of CO- and CO_2 -reduction with $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ clusters and SmI_2 . The $\text{SmI}_2(\text{THF})_2$ stock solution was prepared by dissolving solid $\text{SmI}_2(\text{THF})_2$ in dry 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 $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ cluster ($[\text{Ph}_4\text{P}]_2[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]$) was prepared by dissolving crystals of the cluster in dry DMF. Assays were performed in 9.4 mL assay vials with crimped butyl rubber serum stoppers. Each assay contained, in a total volume of 1 mL, 100 mM $\text{Et}_3\text{NH}(\text{BF}_4)$, 2 μM $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$, and 20 mM $\text{SmI}_2(\text{THF})_2$. In addition, the headspace of each assay contained 100% CO or CO_2 , which was determined as the optimum concentration that yielded the maximum amount of products via titration against increasing concentrations of CO or CO_2 . The negative controls had 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 $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$, followed immediately by injection of SmI_2 via a syringe. All assays and controls were incubated in a 30°C water bath shaker until the reaction was complete (10 min) before the headspace samples were taken and examined for hydrocarbon formation. The CO-reduction controls with FeCl_3 and $\text{FeCl}_3/\text{Na}_2\text{S}$ lacked $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ and instead contained FeCl_3 that contained equimolar Fe to that of $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ in the absence or presence of Na_2S at a 1:1 molar ratio to FeCl_3 . The reaction was initiated with the addition of FeCl_3 or $\text{FeCl}_3/\text{Na}_2\text{S}$, followed immediately by injection of SmI_2 via a syringe and incubation in a 30°C water bath

shaker until the reaction was complete (10 min) before the headspace samples were taken and examined for hydrocarbon formation.

Assays of CO- and CO₂-reduction with [Fe₄S₄]^{Syn} and Eu^{II}-DTPA. The Eu^{II}-DTPA stock solution was prepared by dissolving equimolar amounts of europium(II) chloride (EuCl₂) and diethylenetriaminepentaacetic acid (H₅DTPA) to a final concentration of 200 mM in 1M Tris-HCl (pH 8.0) buffer. Assays were performed in 9.4 mL assay vials with crimped butyl rubber serum stoppers. Each assay for CO₂ reduction contained, in a total volume of 1 mL, 500 mM Tris-HCl (pH 9.8), 20 mM Eu^{II}-DTPA, and 5 μM [Fe₄S₄]^{Syn}, with 100% CO₂ in the headspace. This solution was allowed to sparge under CO₂ for 30 min until it reached saturation, when the pH of the solution stabilized at 7.8. Each reaction for CO reduction contained, in a total volume of 1 mL, 50 mM Tris-HCl (pH 7.8), 20 mM Eu^{II}-DTPA, and 5 μM [Fe₄S₄]^{Syn}, with 100% CO in the headspace atmosphere. The negative controls had the same composition as the assays, except for the omission of either the cluster or the substrate. The reaction was initiated by the addition of [Fe₄S₄]^{Syn}, followed immediately by injection of Eu^{II}-DTPA via a syringe. All assays and controls were incubated in a 30°C water bath shaker until the reaction was complete (60 min) before the headspace samples were taken and examined for hydrocarbon formation.

Quantification of CO and CO₂. The amount of CO or CO₂ generated in the *in vitro* assays was determined through headspace analysis using a Thermo Scientific Trace 1300 Gas Chromatography with flame-ionization detector (GC-FID), in which the detector was interfaced with a methanizer (Thermo Electron North America LLC). CO or CO₂ in the headspace sample was separated on a TG-BOND Msieve 5A column (30 m × 0.32 mm ID × 30 μm film; Thermo Electron North America LLC), which was held at 45°C for 1 min before it was heated to 110°C at a rate of 25°C/min. Subsequently, CO or CO₂ was hydrogenated at 350°C by the methanizer, and the resulting CH₄ was measured by GC-FID. The amount of CO or CO₂ was determined based on a linear standard curve ($R^2 \geq 99.5$) that was derived from the measurement of varying amounts of CO or CO₂ using the same instrument. The detection limit for CO or CO₂ was 2 ppm.

Quantification of hydrocarbons. The hydrocarbon products CH₄, C₂H₄, C₂H₆, C₃H₆, C₃H₈, 1-C₄H₈, *n*-C₄H₁₀, 1-C₅H₁₀ and *n*-C₅H₁₂ 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). From each headspace sample, a total of 250 μL gas was injected into the GC. The heating program for separating the gaseous products in the Eu^{II}-DTPA-driven, aqueous assays was as follows: held at 55°C for 1 min; heated to 180°C at a rate of 12.5°C/min; and then held at 180°C for 0.6 min. The heating program for

separating the gaseous products in the SmI₂-driven, DMF-based assays was as follows: held at 55°C for 1 min; heated to 200°C at 12.5°C/min; and then held at 200°C for 3.6 min. The quantities of all products were determined as described previously^{35,36} using a purchased gas mixture containing ~15 ppm of each hydrocarbon compound (Praxair, Geismar, LA).

Gas chromatography–mass spectrometry (GC–MS) analysis. Isotopic CO- and CO₂-reduction assays were prepared in the presence of ¹²CO/¹³CO and ¹²CO₂/¹³CO₂, respectively, for GC–MS analysis. The reaction products were then analysed by GC-MS using a Thermo Scientific Trace 1300 GC system coupled to a Thermo ISQ QD (Thermo Electron North America LLC) by comparing its mass and retention time with those of the ¹²CO or ¹³CO standard (≥99.9% purity), C₁–C₅ alkane hydrocarbon standard (containing 15 ppm of each gas), or a C₁–C₅ alkene hydrocarbon standard (containing 15 ppm of each gas). For each sample or standard, a total of 250 μL gas was injected into a split/splitless injector operated at 150°C in split mode with a split ratio of 10. Gas separation was achieved on a HP-PLOT/Q+PT column (30 m × 0.32 mm ID × 20 μm film; Agilent Technologies North America LLC), which was held at 30°C for 3 min, heated to 200°C at a rate of 15°C/min, and held at 200°C for 5 min. The rate at which the carrier gas, helium (He) passed through the column was held at 0.7 mL/min for 4 min, increased to 1.4 mL/min at a rate of 0.5 mL/min, and held at 1.4 mL/min for the duration of the run. The mass spectrometer was operated in electron impact (EI) ionization mode. The fragmentation patterns of the products were compared to standards and are consistent with those deposited in the NIST database (<http://webbook.nist.gov>).

Density functional theory calculations. Density functional theory (DFT) calculations of the mechanism of CO₂ reduction were carried out with the DFT programs in the Turbomole package, version 7.0³⁷. Atomistic models of [Fe₄S₄](SCH₂CH₂OH)₄ ([Fe₄S₄]^{Syn}) (see Lewis model in Supplementary Fig. 7a) and its carbon species-bound intermediates were built with Molden³⁸ and all atoms were allowed to relax fully during structure optimisations. Solvent effects were calculated implicitly by the conductor-like solvent screening model COSMO³⁹ as implemented in Turbomole, with a dielectric constant of ε = 37 and a solvent radius of 2.65 Å to represent the solvent DMF. The models were treated as open-shell systems in the unrestricted Kohn-Sham framework. Structural optimisations were performed with the TPSS functional⁴⁰ and a def2-TZVP^{41,42} basis set assigned to all model atoms. Computational time was reduced by utilizing the resolution-of-the-identity approximation^{43,44}. Antiferromagnetic coupling in the FeS cluster was accounted for by the broken symmetry approach⁴⁵⁻⁴⁷. All intermediates were optimised for at least three different spin states (*S* = 1/2, 3/2, 5/2 for systems with an odd number of electrons, and *S* = 0, 1, (2), 4 for those with an even number) in order to obtain low-energy solutions. All protonation energies were obtained

by considering the optimised cluster structure (TPSS, def2-TZVP, COSMO $\epsilon=37$) and explicitly calculating the deprotonation energy of Et_3NH^+ —the proton donor used in the DMF-based, cluster-catalysed reactions—in DMF (see Supplementary Fig. 7b). The energies of all coordinating and dissociating species, including $\text{HSCH}_2\text{CH}_2\text{OH}$ (Fig. 3, step 6), were calculated with the same settings. The energy of $\text{HSCH}_2\text{CH}_2\text{OH}$ was also used to calculate the energy for re-ligating the cluster, where $\text{HSCH}_2\text{CH}_2\text{OH}$ simultaneously acted as a proton donor (Fig. 3, steps 20a, 20b). Reduction energies were first calculated by assuming transfer of a free electron with zero kinetic energy. In order to obtain approximate redox free energies that better describe the energetics of the system, the resulting energies were then corrected with the reported experimental electrode potential of SmI_2 , $E^{0'} = -1.5 \text{ V vs. SHE}$, following a previously described procedure^{48,49}. This value was adapted to the DMF solvent with a previously reported value⁵⁰ for the relative electrode potential, $\Delta E (\text{SHE}) = -4.34 \text{ V}$. The initially obtained reduction energies were transformed into redox free energies by adding the redox free energy of the reductant half reaction: $\Delta G^0 = -n F (E^{0'} - \Delta E (\text{SHE}))$ (n , the number of electrons; F , Faraday constant)^{48,49}. For SmI_2 , this value is -65.5 kcal/mol.

Data availability. The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Y.H. planned the research, M.T.S., C.J.H., N.S.S., C.C.L., K.T., and Y.O. performed the experiments, and Y.H. prepared the manuscript with suggestions of Y.O.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper.

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FIGURES

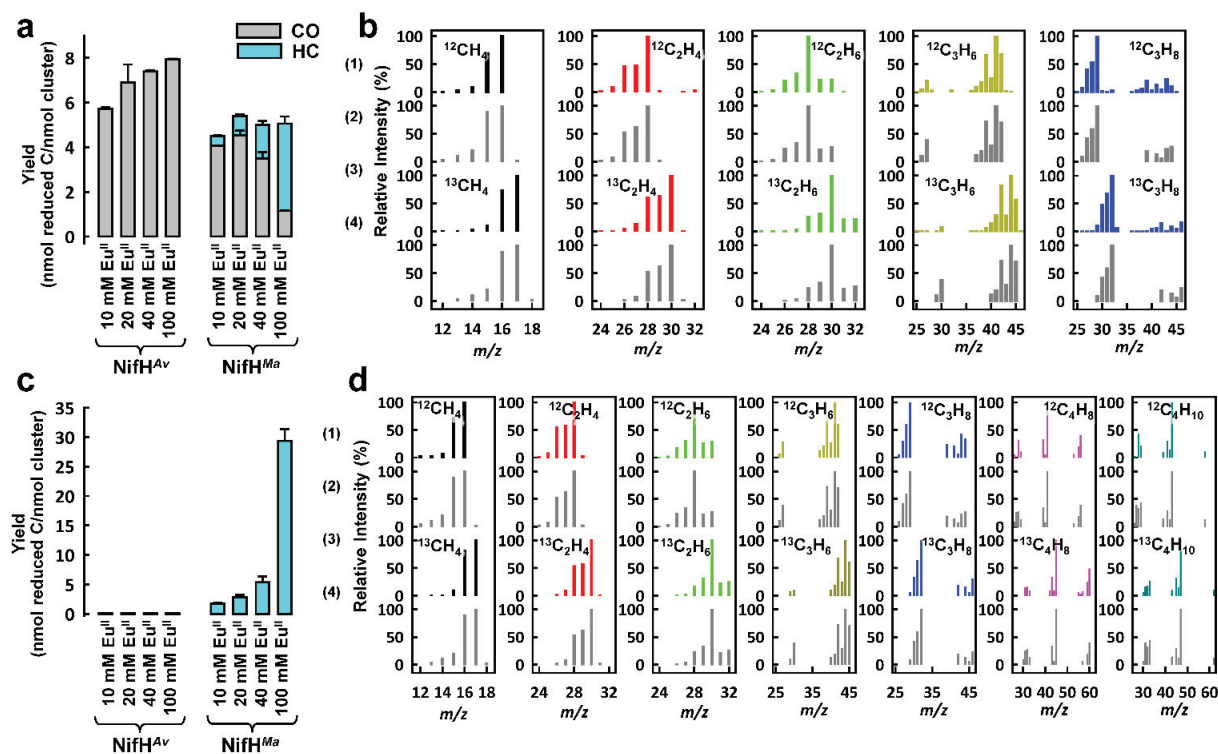


Fig. 1 | Reduction of CO₂ and CO by protein-bound [Fe₄S₄] clusters. (a, c) Yields of CO (grey) or hydrocarbons (cyan) generated from the reduction of CO₂ (a) or CO (c) by NifH^{Av} or NifH^{Ma} at increasing Eu^{II}-DTPA concentrations. Yields were calculated based on nmol reduced C in CO or hydrocarbons per nmol cluster. HC, hydrocarbons; Eu^{II}, Eu^{II}-DTPA (b, d) GC-MS analysis of the hydrocarbon products generated from the reduction of ¹²CO₂ (b, 1), ¹³CO₂ (b, 3), ¹²CO (d, 1) or ¹³CO (d, 3) by NifH^{Ma}, shown in comparison with the fragmentation patterns of the corresponding ¹²C (b, d, 2) or ¹³C (b, d, 4) labelled hydrocarbon standards. Colour code for GC-MS experimental data (1, 3): CH₄, black; C₂H₄, red; C₂H₆, green; C₃H₆, yellow; C₃H₈, blue; C₄H₈, pink; C₄H₁₀, dark green. The corresponding hydrocarbon standards (2, 4) are shown in grey. Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.

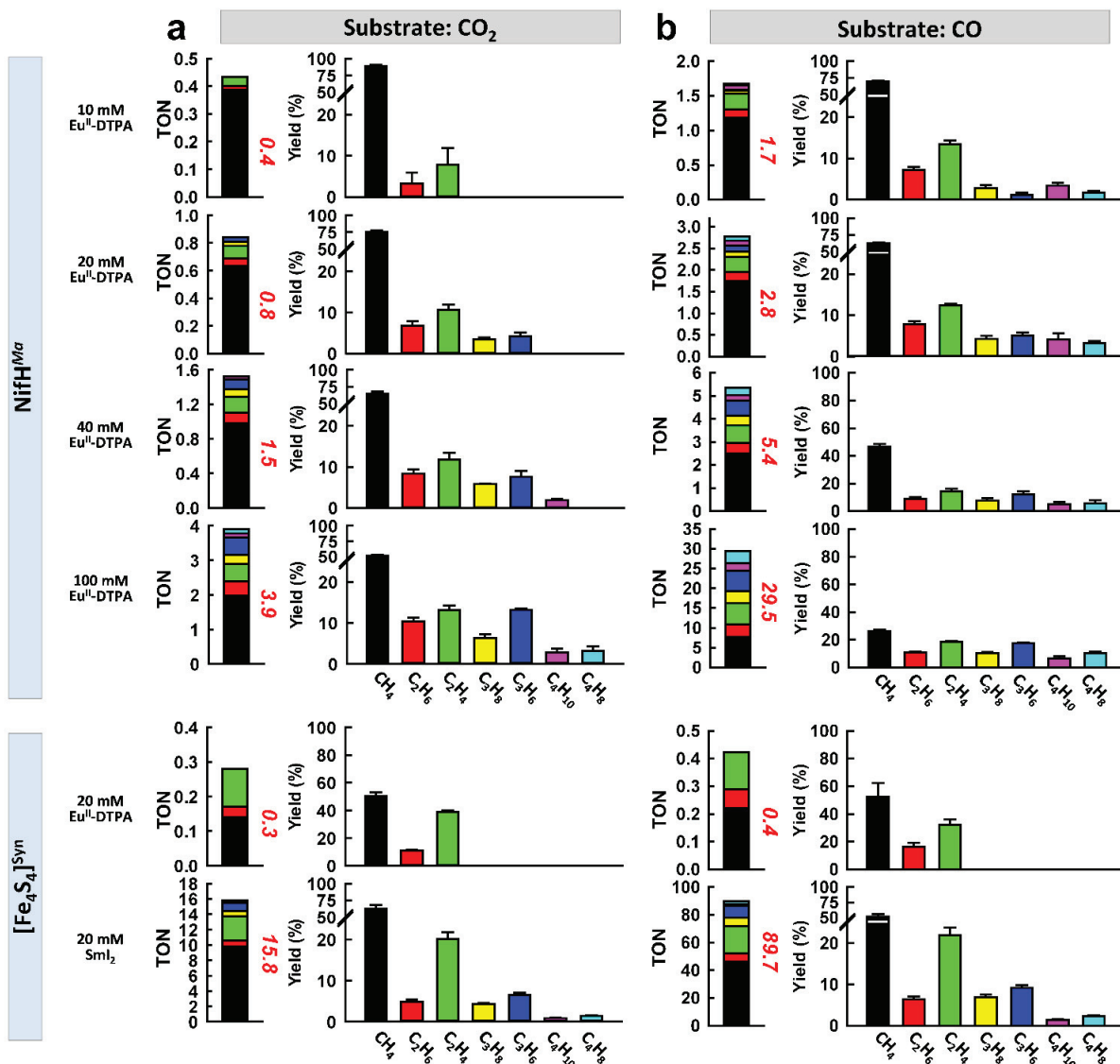


Fig. 2 | Formation of hydrocarbons by protein-bound and synthetic [Fe₄S₄] clusters. Turnover numbers (TONs) calculated based on all hydrocarbons (left charts) and percentage yields of individual hydrocarbon products (right charts) generated from the reduction of CO₂ (a) or CO (b) by NifH^{Ma} at increasing Eu^{II}-DTPA concentrations, or by [Fe₄S₄]^{Syn} in the presence of Eu^{II}-DTPA or SmI₂. TONs, or total nmol reduced C in all hydrocarbons per nmol cluster, are shown in red fonts next to the stacked bars (a, b, left charts). Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.

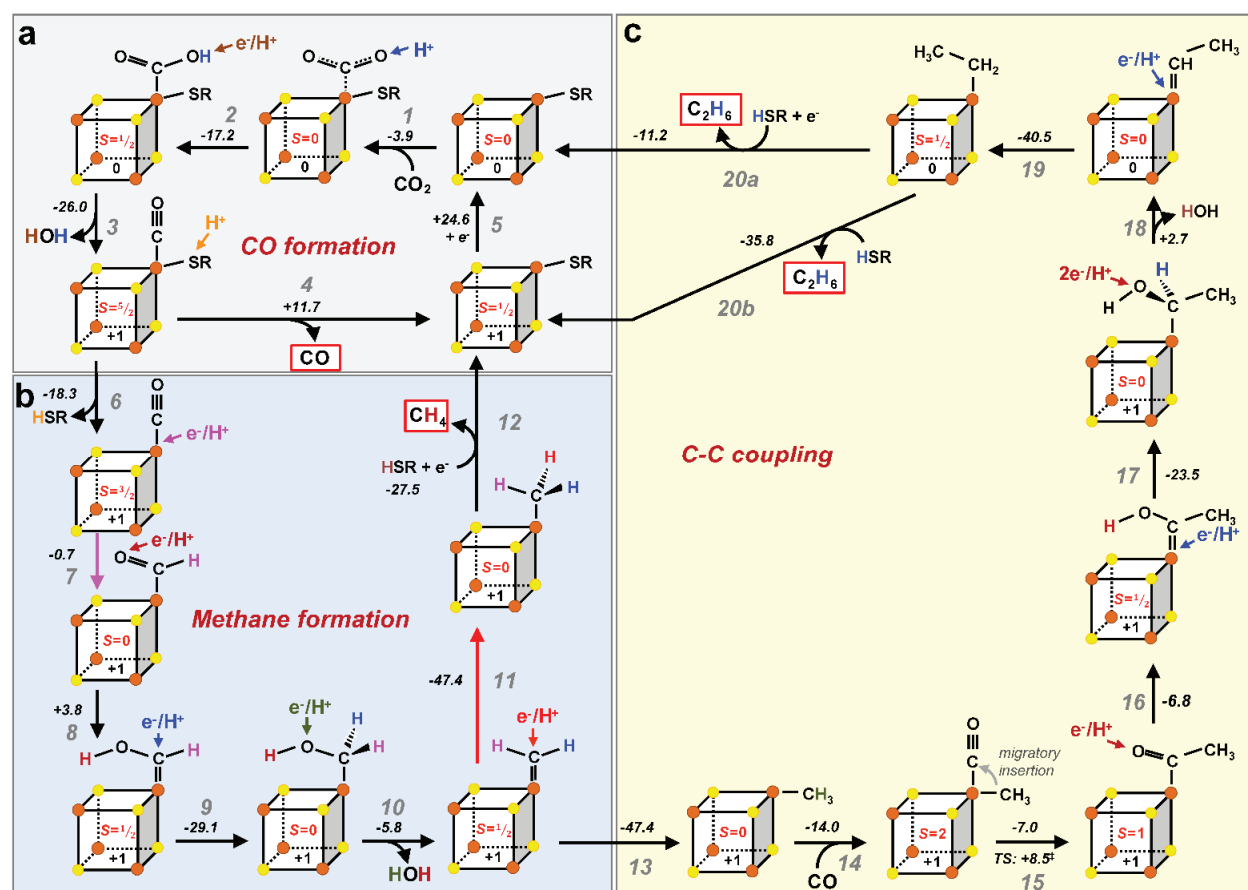


Fig. 3 | Proposed reaction pathway of CO₂ reduction catalysed by the [Fe₄S₄] cluster. Shown is the energetically plausible pathway derived from DFT calculations. Cluster ligand (-SR, SC₂H₄OH⁻ or HSR, HSC₂H₄OH) is indicated as needed for the purpose of simplicity. The calculated reaction energy (kcal/mol) is indicated for each step (above or next to the arrow). **(a)** Coordination of CO₂ to a [Fe₄S₄]⁰ cluster and release of CO upon reduction of CO₂. **(b)** Reduction of a [Fe₄S₄]⁺-cluster-bound CO moiety to CH₄ via the formation of an aldehyde-like intermediate. **(c)** Formation of a C-C bond via migratory insertion that involves a [Fe₄S₄]⁺-cluster-bound -CH₃ species and a second CO molecule coordinated to the same Fe atom. See Supplementary Fig. 8a and 9a for the transition state and energy landscape of the proposed mechanism. TS, transition state.

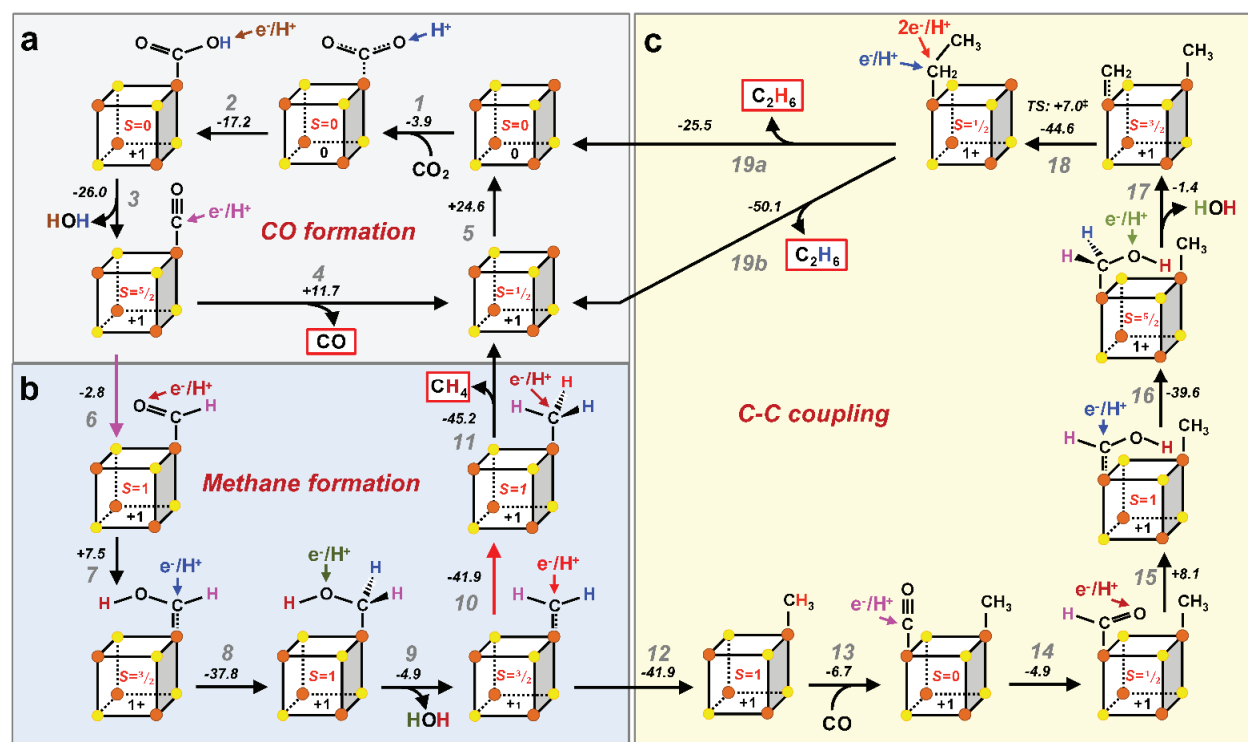


Fig. 4 | Alternative reaction pathway of CO_2 reduction catalysed by the $[\text{Fe}_4\text{S}_4]$ cluster. Shown is the energetically plausible pathway derived from DFT calculations. The calculated reaction energy (kcal/mol) is indicated for each step (above or next to the arrow). Unlike the mechanism proposed in Fig. 3, this pathway doesn't involve dissociation of a thiolate ligand and proceeds by coordination events at two neighbouring Fe centres. Note that the thiolate groups are not depicted in the figure for the purpose of simplicity. **(a)** Coordination of CO_2 to a $[\text{Fe}_4\text{S}_4]^0$ cluster and release of CO upon reduction of CO_2 . **(b)** Reduction of a $[\text{Fe}_4\text{S}_4]^+$ -cluster-bound CO moiety to CH_4 via the formation of an aldehyde-like intermediate. **(c)** Formation of a C-C bond via coupling between a $[\text{Fe}_4\text{S}_4]^+$ -cluster-bound $-\text{CH}_3$ species with a reactive, bridged Fe- CH_2 intermediate that is derived from the second bound CO. See Supplementary Figs. 8b and 9b for the transition state and energy landscape of the proposed mechanism. TS, transition state. Other than the two plausible mechanisms presented in Figs. 3 and 4, reaction pathways involving protonation of the cluster sulphur atoms were also considered (Supplementary Figs. 10-12). However, given the initial strong endothermic coordination of CO_2 and structural destabilization of the cluster upon sulphur protonation, we strongly favour the mechanisms presented in Figs. 3 and 4.

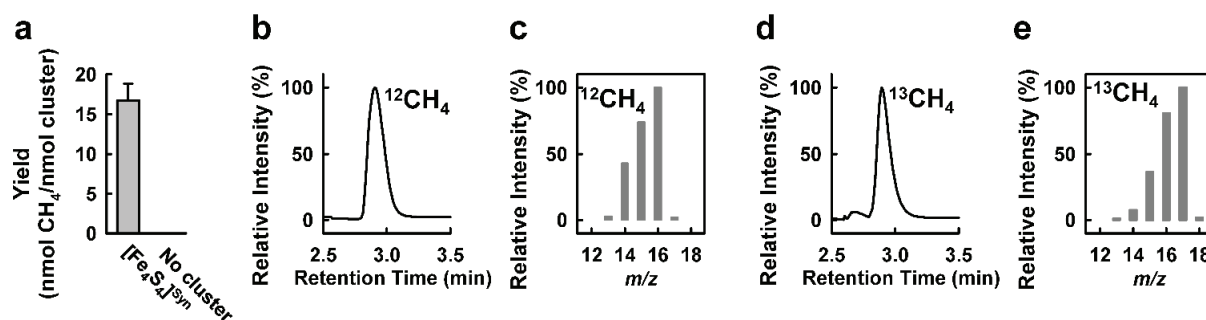


Fig. 5 | Reduction of CH_2O to CH_4 by synthetic $[\text{Fe}_4\text{S}_4]$ cluster. Turnover number (TON) calculated based on CH_4 generated from the reduction of CH_2O (a), and GC-MS traces (b, d) and fragmentation patterns (c, e) of $^{12}\text{CH}_4$ and $^{13}\text{CH}_4$, respectively, generated from the reduction of $^{12}\text{CH}_2\text{O}$ (b, c) and $^{13}\text{CH}_2\text{O}$ (d, e). The experiment that determined the product yield was performed three times ($n=6$), and data from this experiment is presented as mean $\pm$ s.d.

Supplementary Information

Ambient Conversion of CO₂ to Hydrocarbons by Biogenic and Synthetic [Fe₄S₄] Clusters

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Supplementary Notes

Supplementary Note 1: Transition states for the steps of C-C bond formation. For the rate-determining steps of the mechanisms depicted in Figs. 3 and 4, possible transition states were identified by optimizing distance-constrained structures along the expected reaction coordinates (C-C bond formation), followed by calculating the Hessian using the NumForce script provided with the Turbomole package and visualizing the normal modes with Jmol. Promising guess structures were then optimised with the statpt script provided with Turbomole, employing an eigenvector-following approach. Both transition states reported herein (Supplementary Fig. 8) show exactly one imaginary frequency, and the associated normal modes are consistent with the expected reaction coordinates. The transition states were further verified by calculating the reaction paths leading to the formation of product and reactant.

Supplementary Note 2: Other considered reaction pathways involving protonation of the cluster sulphur atoms. Protonation of the sulphur atoms of the all-ferrous [Fe₄S₄] cluster is exothermic by -14.3 kcal/mol. However, this protonation event disrupts and opens up the compact cubane structure by weakening the bond between the newly formed SH group and the proximal Fe atom (Supplementary Fig. 10), which provides an avenue for cluster destruction and contradicts our experimental findings of sustained catalytic turnover. Moreover, the x-ray crystallographic structure of the all-ferrous Fe protein further argues against disruption of its [Fe₄S₄] cluster, as the cluster remains intact in the all-ferrous state¹. Nevertheless, we considered possible mechanistic routes starting from this unlikely cluster geometry for the sake of completeness (Supplementary Fig. 11).

Coordination of CO₂ to the protonated cluster is highly endothermic by +54.9 kcal/mol, (highlighted as a red box in Supplementary Fig. 11, step 2), and the subsequent reduction of its unlikely reaction product is exothermic by -37.0 kcal/mol (Supplementary Fig. 11, step 3). Taken together, these two initial steps of this reaction route would still be endothermic. However, we still considered plausible reaction events that occur subsequent to these initial steps. As can be seen from the scheme in Supplementary Fig. 11, reaction steps analogous to those proposed in Figs. 3 and 4 could in principle occur, which involve protonation of the CO₂ moiety, dissociation of an H₂O molecule, and consecutive e⁻/H⁺ transfers, yielding an Fe-bound CH₃-species upon dissociation of an additional H₂O molecule (Supplementary Fig. 11, step 11). In analogy to the mechanisms presented in Figs. 3 and 4, further electron/proton transfer steps result in exothermic methane formation (Supplementary Fig. 11, step 12). In two alternative scenarios, a second CO molecule can either coordinate to the Fe atom that is already bound with a CH₃ species (exothermic by -18.6 kcal/mol, Supplementary Fig. 11, step 14), thereby resembling the mechanism proposed in Fig. 3, or to the neighbouring Fe atom (exothermic by -4.3 kcal/mol, Supplementary Fig. 11, step 15), which corresponds to the scheme presented in Fig. 4. The first route, however, results in a dead-end; specifically, the migratory insertion step depicted in Fig. 3 cannot occur in the case of the open cluster, as structural optimisation of an activated complex leads to the re-formation of the end product of step 14. The second alternative, on the other hand, proceeds analogously to the mechanism depicted in Fig. 4, thereby corroborating the originally considered reaction steps.

In addition to the mechanism discussed in Supplementary Fig. 11, we considered the energetics of proton and coupled electron/proton transfer steps to various intermediates of the main mechanisms presented in Figs. 3 and 4. The results are shown in Supplementary Fig. 12, which

again indicate substantial cluster distortion and unfavourable energetics caused by protonation of the cluster sulphur atoms.

Taken together, given the initial strong endothermic coordination of CO₂ and structural destabilization of the cluster upon sulphur protonation, we strongly favour the mechanisms presented in Figs. 3 and 4 to those involving the protonation of cluster sulphur atoms.

Supplementary Note 3: Coordination of CO₂ to the cluster. As reported previously for the coordination of CO₂ to the [Fe₄S₄] cluster of the *A. vinelandii* Fe protein², we only observed exothermic binding when CO₂ was directly coordinated to one of the Fe atoms of the all-ferrous [Fe₄S₄]⁰ cluster in a singlet spin state (*S*=0). Alternative scenarios for different charge and spin states, or alternative binding sites, such as ligand and cluster S atoms, or sites exposed by opening of the cluster, resulted in strongly endothermic reaction energies. Previous EPR spectroscopic data suggest that the cluster can indeed adopt a *S*=0 spin state³. With the DFT approach described herein, the *S*=0 state of the ligand-free cluster is slightly less stable than the *S*=4 state (by ~4-5 kcal/mol). At ambient temperatures, the cluster might be represented best by a mixture of spin states. For all other forms that can be formally considered as existing in the all-ferrous state, the low-spin solution is more stable than the *S*=4 state [i.e., -2 to -3 kcal/mol for the CO₂-bound form (see Figs. 3 and 4), -5 to -6 kcal/mol for the CO₂H-bound form (see Figs. 3 and 4), and ~ -10 kcal/mol for the CHCH₃-bound cluster (see Fig. 4, where the most favourable spin state is *S*=1)].

Supplementary Discussion

The ambient reduction of CO to hydrocarbons is highly interesting because of its industrial potential and its plausible relevance to prebiotic chemistry. Here, we compare the reactivities of biogenic and synthetic [Fe₄S₄] clusters toward CO with those of several other notable systems, such as (1) the nitrogenase-based catalysts, including the catalytic component of nitrogenases (e.g., MoFe and VFe proteins) and the nitrogenase cofactors (e.g., M- and V-clusters); (2) the Fe/S-based catalysts, including those generated by synthetic approaches (e.g., synthetic FeS clusters) and those associated with prebiotic chemistry (e.g., Fe/S precipitates); and (3) the non-Fe/S catalysts, including homogeneous molecular catalysts (i.e., synthetic compounds) and heterogeneous surface catalysts (e.g., the Fischer-Tropsch catalysts).

Fe protein (Fe₄S₄) vs. nitrogenase (cofactor)

Fe protein vs. nitrogenase. The V-nitrogenase is the most active enzyme known to convert CO to hydrocarbons under ambient conditions⁴. Using its Fe protein component (VnfH^{4v}) to deliver electrons to its catalytic VFe protein component (VnfDGK^{4v}) in the presence of ATP and dithionite, the holo V-nitrogenase of *A. vinelandii*^{4,5} averages approximately 14 turnovers of CO per minute in an *in vitro* assay and produces more than 50 times the yield of hydrocarbons relative to that produced by NifH^{Ma}. In contrast, the holo Mo-nitrogenase of *A. vinelandii* reduces CO to hydrocarbons at a rate that is approximately 800-fold lower than that the V-nitrogenase when it utilizes its Fe protein component (NifH^{4v}) to deliver electrons to its catalytic MoFe protein component (NifDK^{4v}) in the presence of ATP and dithionite in an *in vitro* assay⁵. Under improved conditions, such as upon substitution of D₂O for H₂O in the assay⁵, the yield of hydrocarbon formation by Mo-nitrogenase is comparable to that by NifH^{Ma}. In addition, certain variants of Mo-nitrogenase have been reported to achieve 20-30 turnovers over the course of the reaction⁶, which is comparable to that of NifH^{Ma}.

The higher activity of the holo nitrogenase than its Fe protein component in CO reduction is not surprising, given the intricate two-component mechanism employed by nitrogenase that renders it efficient in transferring electrons to the cofactor site, rendering the cofactor in a sufficiently low redox potential for substrate binding and reduction. Moreover, the high-nuclearity metallocofactors likely provide more reaction sites than the smaller $[\text{Fe}_4\text{S}_4]$ clusters, which could further account for a higher activity of these clusters in catalysing CO reduction. The disadvantages of this system, however, are the complexity of the reaction mechanism and the ATP-dependent nature of the reaction, making it difficult to use this system for mechanistic investigations of CO reduction, as well as potential applications for industrial use in the future. In comparison, the Fe protein (i.e., NifH^{Ma}) is a unique, stand-alone catalyst that can work in an ATP-independent manner, which makes it a simplified template for mechanistic investigations of FeS-based CO activation, as well as future development of strategies for ambient conversion of CO and CO_2 into useful chemical commodities.

Fe₄S₄ cluster vs. cofactor. Other than the holo enzyme systems, simplified systems that are ATP-free and consist of only reductants and the cofactors isolated from the Mo- and V-nitrogenases (i.e., the M- and V- clusters) have been shown to reduce CO to hydrocarbons⁷⁻⁹. Both Eu^{II} -DTPA and SmI_2 are effective in driving the reduction of CO to hydrocarbons by these systems. In the presence of Eu^{II} -DTPA, the hydrocarbon yields of both M- and V- clusters are similar to that of $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ under the same reaction conditions⁷. In the presence of SmI_2 , the hydrocarbon yields of both M- and V-clusters are improved, and a recent study shows a TON of greater than 200 for the M-cluster¹⁰, which is approximately twice the TON of $[\text{Fe}_4\text{S}_4]^{\text{Syn}}$ reported in this work. The corresponding number for the V-cluster-catalysed CO reduction under the same reaction conditions has not been reported so far.

The higher activity of the isolated cofactor than the $[\text{Fe}_4\text{S}_4]$ cluster in this reaction again illustrates the effectiveness of the complex, high-nuclearity metallocofactors in CO reduction, possibly due to the presence of more reaction sites on these larger clusters. However, the disadvantage of this system is the laborious process to isolate sufficient amounts of cofactors from the nitrogenase enzymes. In this light, the current report is unique in demonstrating the surprising ability of a much more accessible, synthetic $[\text{Fe}_4\text{S}_4]$ cluster to drive difficult reactions of CO_2 - and CO-reduction at reasonable yields prior to any optimisation, suggesting this system as a potential candidate for future development of strategies of FeS-based conversion of CO and CO_2 to hydrocarbons under ambient conditions.

Fe₄S₄ cluster vs. other Fe/S-based catalysts

Fe₄S₄ cluster vs. synthetic FeS-based catalysts. While a large number of synthetic or biomimetic FeS-based clusters have been reported in the literature¹¹⁻¹⁴, only a few of them have been described for their abilities to activate small molecules. These handful of examples include the sub-stoichiometric reduction of acetylene to ethylene by a synthetic $[\text{Fe}_4\text{S}_4]$ cluster¹⁴, and the catalytic reduction of hydrazine to ammonia by $[\text{MoFe}_3\text{S}_4]$ and $[\text{VFe}_3\text{S}_4]$ clusters^{15,16}. In addition, a report from 1992¹⁷ describes the $2e^-$ -reduction of CO_2 by a $[\text{Mo}_2\text{Fe}_6\text{S}_8(\text{SEt})_9]^{3-}$ catalyst to a product which then reacts with a thioester (CH_3COSEt in this case) to produce a β -ketoacid (RCOCOO^-) and SEt^- . The scarcity of early examples for CO_2/CO reduction to hydrocarbons highlights the difficulty of these reactions to occur under ambient conditions. Recently, in light of the discovery of the reactivity of nitrogenase toward CO, two synthetic nitrogenase cofactor mimics—namely, a $[\text{Et}_4\text{N}]_4[\text{Fe}_6\text{S}_9(\text{SEt})_2]$ cluster (synthesized by the Holm group)¹⁸ and a $[\text{Cp}^*\text{MoFe}_5\text{S}_9(\text{SH})]^{3-}$ cluster (synthesized by the Tatsumi group)¹⁰, which represent a

homometallic and a heterometallic analogue of the cofactor, respectively—were examined for their abilities to reduce CO and CO₂ to hydrocarbons. Interestingly, these two cofactor mimics demonstrated activities of hydrocarbon production from CO- and CO₂-reduction that were comparable with, but lower than those of the nitrogenase cofactors under the same reaction conditions^{10,19}. These results point to the possibility that more reports along this line will surface in the near future, which could help bridge the gap of our current knowledge on the reactivities of synthetic FeS-based catalytic systems toward CO₂ and CO.

Fe₄S₄ cluster vs. Fe/S-containing precipitates. In their seminal work published in 1997, Huber and Wächtershäuser show that CO can react with CH₃SH on coprecipitated NiS and FeS to form CH₃COOH and H₂S, with CH₃-CO-SCH₃ being the potential intermediate of this reaction²⁰. This result suggests a prebiotic CO fixation pathway that might be employed by the earliest organisms living around the hyperthermal vents underneath the deep oceans. Based on this work, a series of follow-up studies using CO as a carbon feedstock and/or an electron donor show that the product profile of this reaction can be substantially extended to include long chain carboxylic acids, carbohydrates (i.e., hydroxyl acids) and amino acids (when CN⁻ or NH₄⁺ is used as a co-reactant), with each of them correlated to the emergence of primordial lipids, sugars and peptides/proteins, respectively, on Earth²¹⁻²⁴. By analogy, the reduction of CO by FeS clusters in a speculated, highly reducing early atmosphere might represent another prebiotic route of CO activation, which generates small alkenes and alkanes as carbon and/or electron sources for certain methane- and ethene-assimilating microorganisms^{25,26}. It has been postulated that in the Archean era, there was a methane- and CO-rich atmosphere and anoxic oceans inhabited by iron- and sulphur-metabolizing microbes, such as purple- and green-sulphur bacteria²⁷. It is plausible that these microorganisms could produce small hydrocarbons via secondary metabolic pathways that parallel that reported in the case of *A. vinelandii*²⁸, which were then assimilated by microorganisms utilizing hydrocarbons for cell growth.

There are some notable differences between these prebiotic synthesis systems and the [Fe₄S₄] cluster-based system reported in this work. One, the reaction described in our work is, in essence, a reductive coupling reaction of CO or CO₂ on the [Fe₄S₄] cluster (see mechanisms depicted in the main text); whereas the general reaction described in the pre-biotic synthesis models involves the capture of CO on a given carbon backbone through carbonylation, followed by further reaction of the carbonyl moiety to generate carboxylic acids or amino acids. Consequently, hydrocarbons have seldomly been reported as products generated by these models of pre-biotic synthesis. Two, the reaction reported in this work utilizes a homogeneous catalyst (i.e., the [Fe₄S₄] cluster); whereas the primordial model systems are essentially semi-heterogeneous, where substrates are activated on a slurry of material particles. Three, our reaction is driven by low reduction potentials delivered by strong reductants; whereas the prebiotic synthesis reactions are powered by high temperatures (above the boiling point) and often high pressures, and they are allowed to proceed over a much longer reaction timeframe²⁰⁻²³.

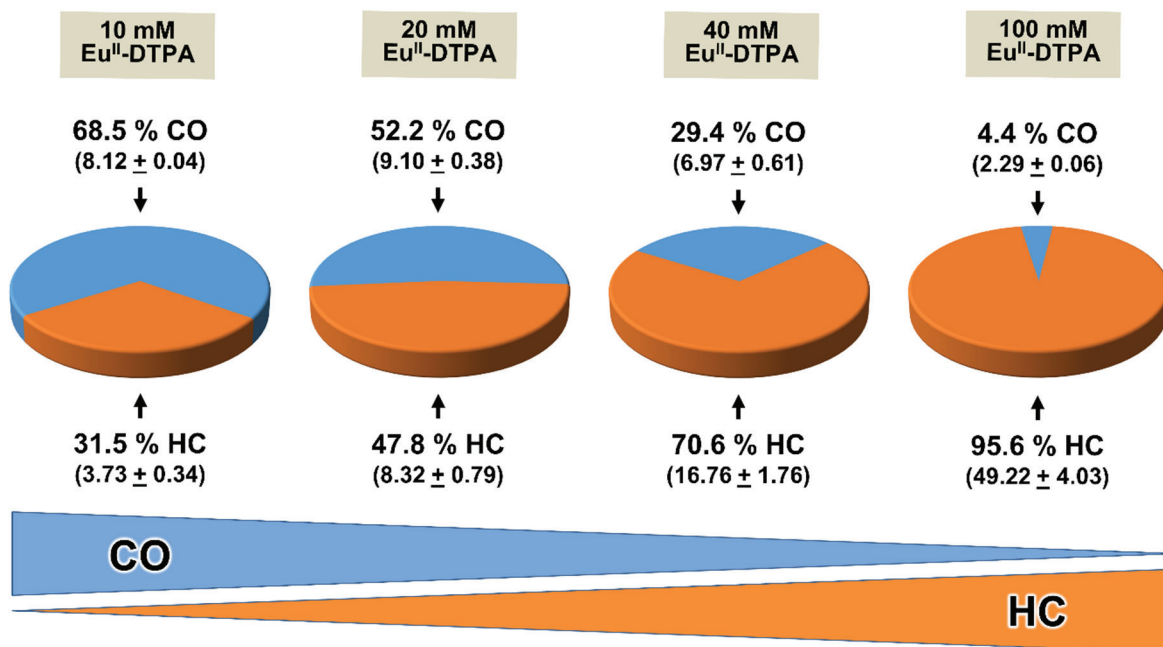
Fe₄S₄ cluster vs. non-Fe/S catalysts

Fe₄S₄ cluster vs. homogeneous non-Fe/S catalysts. Several notable attempts have been made to convert the inactive carbon oxides to useful chemical compounds by combining these molecules with reactive or pre-activated organic compounds, such as the poly- and cyclic carbonate syntheses from CO₂^{29,30} and the Monsanto acetic acid synthesis from CO³¹. In addition, synthetic compounds have been explored for their abilities to directly transform CO and CO₂ into hydrocarbons. One such example was reported by Hou et al. in 2006, where tetranuclear lanthanide polyhydrido

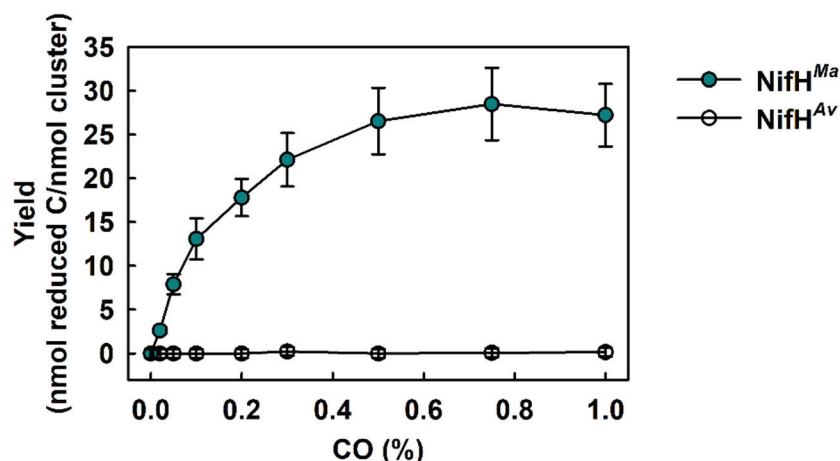
clusters were used for selective formation of C_2H_4 from CO^{32} . This type of reaction is remarkable in that it achieves selective C-C coupling and cleavage of C-O bond at ambient temperature and pressure; however, the reactions are not catalytic because of the absence of a H^+ source in these systems and, consequently, the need to remove O atoms from CO in the forms of Ln_4O_4 and Si-O-Si species, resulting in the conversion of the catalysts into dead-end by-products, i.e., Ln_4O_4 clusters ($Ln = Y$ or Lu).

Fe₄S₄ cluster vs. heterogeneous non-Fe/S catalysts. The best-known example of the heterogeneous catalysis of CO reduction is the Fischer-Tropsch (FT) process, which combines CO with H_2 into hydrocarbons. There are a number of points that distinguish the FT process³³⁻³⁵ with the reaction reported in this work. One, our reaction occurs at ambient temperature and pressure; whereas the FT process, which is a gas-solid two-phase reaction, typically requires high temperature and pressure. Two, contrary to the reaction described in this report, the reactivity and product selectivity of the FT process vary greatly depending on the reaction conditions and the choice of catalysts. Three, the reaction catalysed by the $[Fe_4S_4]$ cluster utilizes protons as the hydrogen source; whereas the reducing power of the FT process is provided in the form of molecular hydrogen. Other than the FT process, alternative strategies for the conversion of CO and CO_2 to hydrocarbons, such as the electrochemical reduction of these carbon oxides, have also been explored to circumvent the requirement of high temperature and pressure for the 2-phase catalysis. The products generated by these alternative approaches are mainly methane and ethene, and they typically require a metal surface, such as copper, for catalysis to occur^{36,37}. While this electrode-based approach has the shortcomings in needing a relatively large overpotential for preferential hydrocarbon production and having a relatively low selectivity of products comparing to FT synthesis, it bears potential in supplying a continuous flow of electrons that may be combined with the $[Fe_4S_4]$ cluster-based reaction to generate a continuous system for ambient conversion of CO and CO_2 to hydrocarbons.

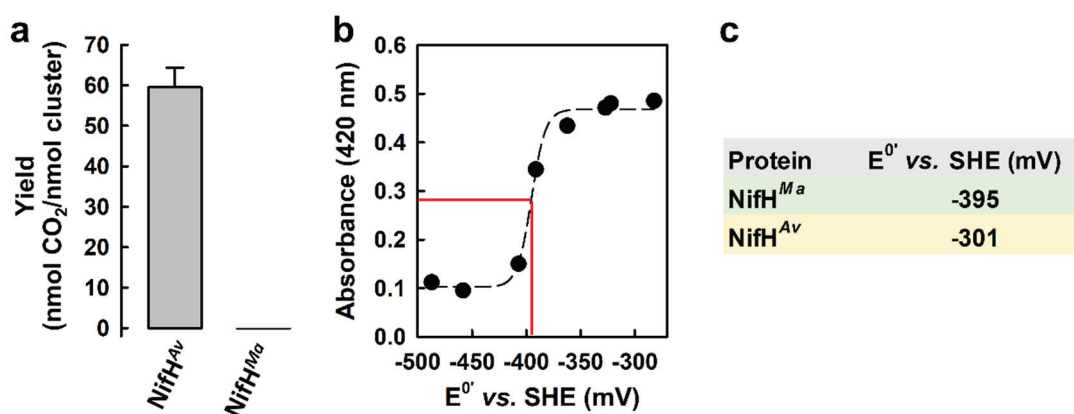
Supplementary Figures



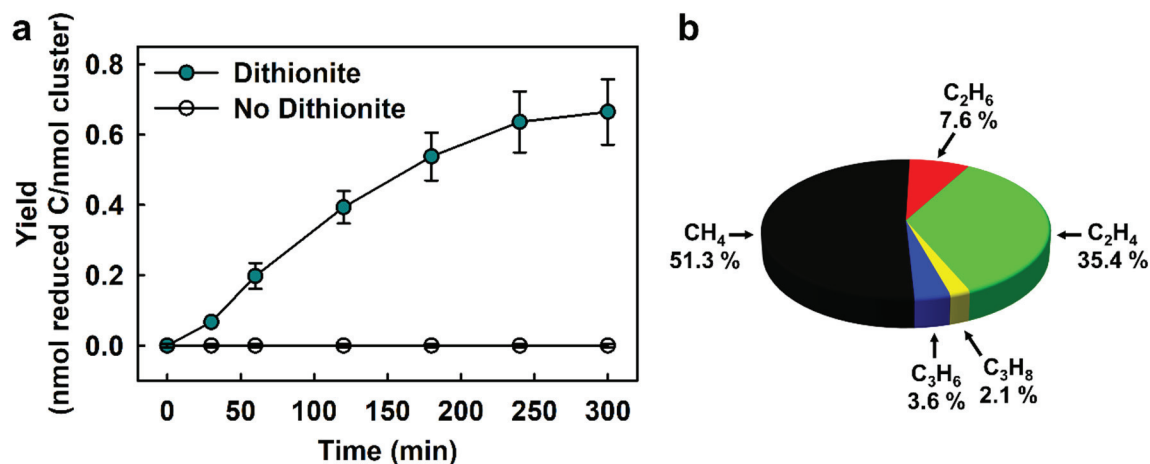
Supplementary Fig. 1 | Product distribution of CO₂ reduction in the presence of increasing amounts of reductant. Data are taken from Fig. 1a. The activity of CO or hydrocarbon (HC) formation from the reduction of CO₂ by NifH^{Ma} is expressed as nmol electrons that appear in CO or HC per nmol cluster and shown in parentheses. The percentage distributions of electrons in CO and HC were calculated based on these numbers. The total amount of electrons that appear in products (both CO and HC) increases concomitantly with a shift toward HC formation when CO₂ is reduced by NifH^{Ma} in the presence of increasing Eu^{II}-DTPA concentrations. Experiments that determined the product yields (Fig. 1a) were performed three times (n=6), and data from these experiments are presented as mean±s.d.



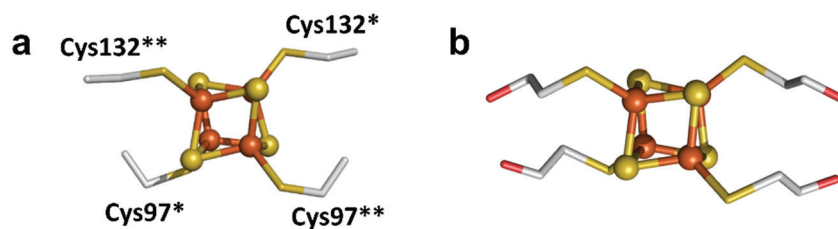
Supplementary Fig. 2 | Titration of product yield *versus* CO concentration. Shown are the yields of hydrocarbons generated from increasing amounts of CO by NifH^{Av} or NifH^{Ma}. Yields were calculated based on nmol reduced C in hydrocarbons per nmol cluster. Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.



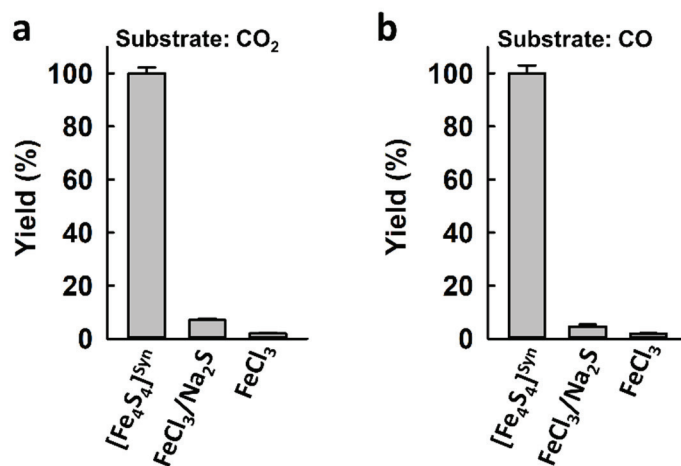
Supplementary Fig. 3 | Redox properties of protein-bound [Fe₄S₄] clusters. (a) Yields of CO₂ generated from the oxidation of CO by NifH^{Av} or NifH^{Ma} in the presence of 20 mM IDS. (b) Titration of the +1/+2 redox couple of the [Fe₄S₄] cluster of NifH^{Ma}. The oxidation of the cluster from the +1 to the +2 state was monitored by the absorbance at 420 nm. (c) Redox potentials of the +1/+2 couples of the [Fe₄S₄] clusters of NifH^{Ma} and NifH^{Av}. The potential of NifH^{Ma} was determined as described in b, and the potential of NifH^{Av} was determined in a previous work². The redox titration experiment was performed 3 times, and the representative result is shown in b. Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.



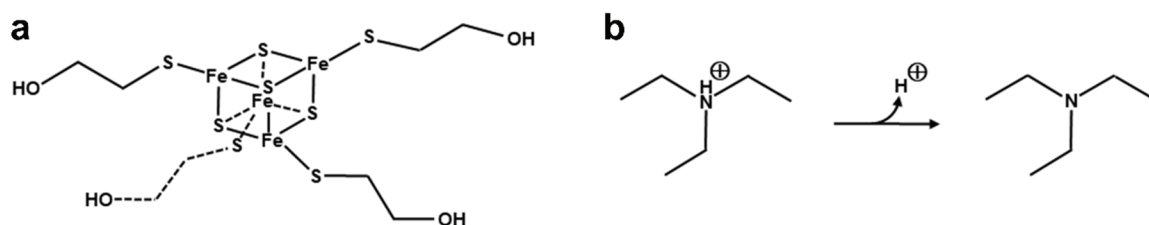
Supplementary Fig. 4 | Reduction of CO to hydrocarbons by protein-bound [Fe₄S₄] clusters using dithionite as a reductant. (a) Time-dependent formation of hydrocarbons from CO reduction by NifH^{Ma}. (b) Distribution of hydrocarbons generated by NifH^{Ma} from the reaction of CO reduction. Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.



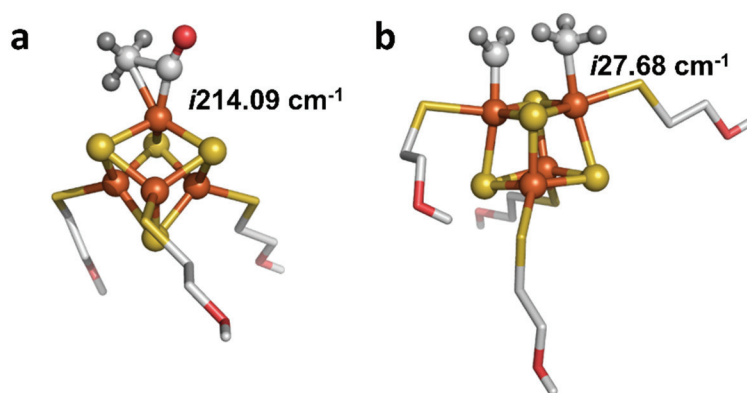
Supplementary Fig. 5 | Structures of protein-bound and synthetic [Fe₄S₄] clusters. Shown are x-ray crystal structures of (a) NifH^{Av}-associated [Fe₄S₄] cluster with four protein-bound Cys ligands³⁸ and (b) synthetic [PPh₄][Fe₄S₄(SCH₂CH₂OH)₄] compound (designated [Fe₄S₄]^{Syn}) with four β-mercaptoethanol thiolate ligands.^{39,40} Element colour code: Fe, orange; S, yellow; C, light grey; O, red.



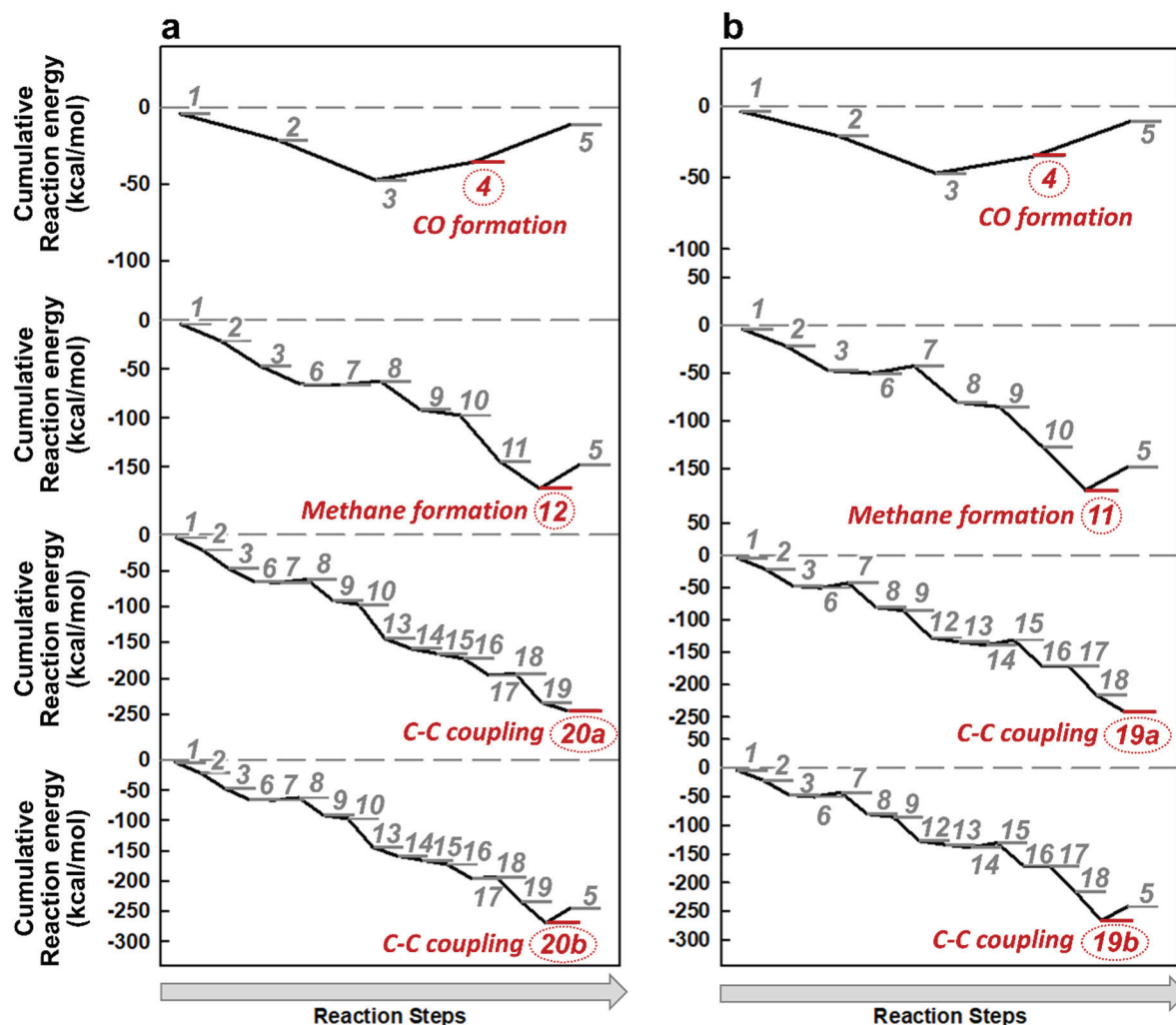
Supplementary Fig. 6 | Formation of hydrocarbons by [Fe₄S₄]^{Syn} and Fe-containing controls. Shown are the percentage yields of hydrocarbons generated from the reduction of (a) CO₂ or (b) CO by [Fe₄S₄]^{Syn}, or by Fe₃Cl₃ in the presence and absence of Na₂S. The experiments were conducted in 20 mM SmI₂, and the hydrocarbon yield of the [Fe₄S₄]^{Syn}-based reaction was set as 100%. Experiments that determined the product yields were performed three times (n=6), and data from these experiments are presented as mean±s.d.



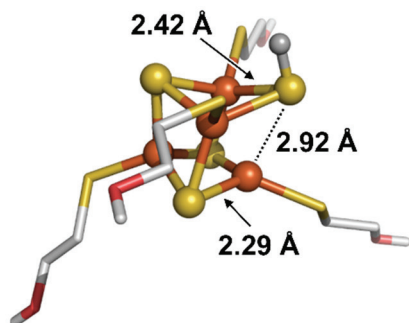
Supplementary Fig. 7 | Structures of the catalyst and proton source for DFT calculations. (a) Lewis Structure of the [Fe₄S₄] cluster considered in the computational investigation. (b) Deprotonation reaction of NH₄⁺ considered for calculations of all protonation energies.



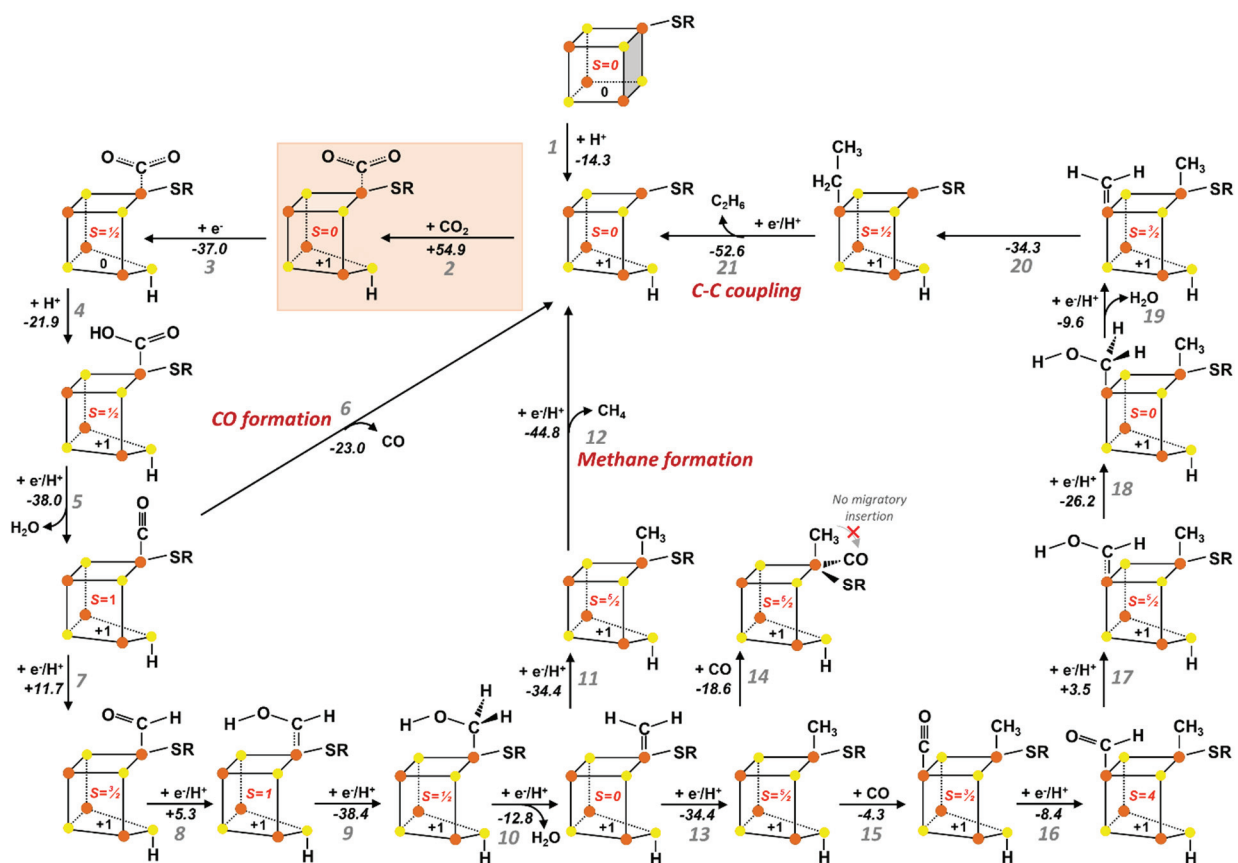
Supplementary Fig. 8 | Transition states for the proposed mechanisms. Shown are the transition states (TPSS/def2-TZVP) for (a) migratory insertion (see Fig. 3, step 15) and (b) CH₂-CH₃ bond formation (see Fig. 4, step 18). Element colour code: Fe, orange; S, yellow; C, light grey; O, red; H, grey.



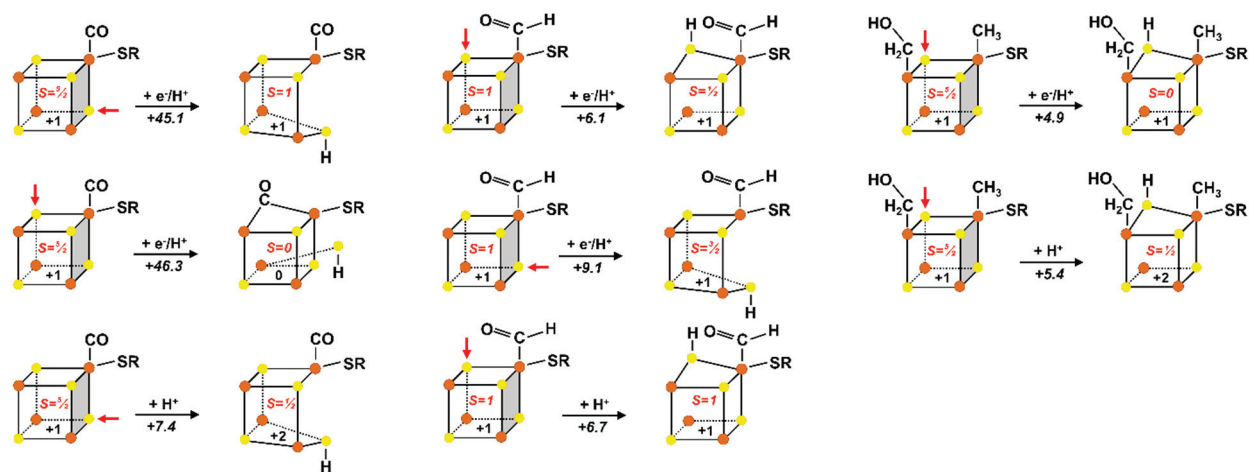
Supplementary Fig. 9 | Cumulative reaction energies of CO₂ reduction pathways catalysed by the [Fe₄S₄] cluster. Shown are the energies for the pathways of CO formation, methane formation and C-C coupling that are depicted in (a) Fig. 3 and (b) in Fig. 4. The steps of CO formation, methane formation and C-C coupling are indicated by red circles.



Supplementary Fig. 10 | Protonation of the $[\text{Fe}_4\text{S}_4]$ cluster. Shown is the optimised structure (TPSS/def2-TZVP, COSMO $\epsilon=37$) of the all-ferrous $[\text{Fe}_4\text{S}_4]$ cluster protonated at one of its S atoms. Protonation leads to significant distortion of the cluster and consequently opens up the cubane. Element colour code: Fe, orange; S, yellow; C, light grey; O, red; H, grey.



Supplementary Fig. 11 | Reaction mechanism starting with the protonation of one of the S atoms of the $[\text{Fe}_4\text{S}_4]$ cluster. Reaction energies were derived from the energies of the structurally optimised compounds (TPSS/def2-TZVP, COSMO $\epsilon=37$). Cluster models were considered fully coordinated by $\text{SC}_2\text{H}_4\text{OH}^-$. Only the ligand of the open Fe site is depicted. SR, $\text{SC}_2\text{H}_4\text{OH}^-$.



Supplementary Fig. 12 | Examples of endothermic proton or electron/proton transfer reactions to the sulphur atoms of the $[\text{Fe}_4\text{S}_4]$ cluster. Reaction energies were derived from the energies of the structurally optimised compounds (TPSS/def2-TZVP, COSMO $\epsilon=37$). Cluster models were considered fully coordinated by $\text{SC}_2\text{H}_4\text{OH}^-$. Proton or electron/proton transfer is indicated by a red arrow. Only the ligand of the open Fe site is depicted. SR, $\text{SC}_2\text{H}_4\text{OH}^-$.

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