

Supporting Information

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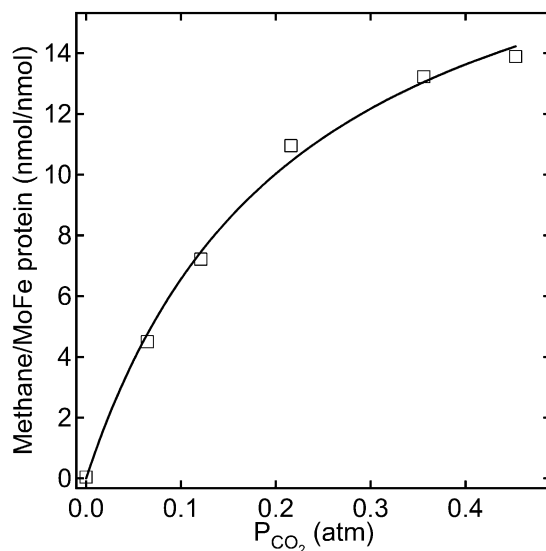


Fig. S1. Dependence of CH_4 formation on partial pressure of CO_2 . Methane production is shown under different partial pressure of CO_2 catalyzed by $\alpha\text{-}^{70}\text{Ala}/\alpha\text{-}^{195}\text{Gln}$ MoFe protein. The concentration of MoFe protein was 0.5 mg/mL, and Fe protein was 3 mg/mL. The reactions were incubated at 30 °C for 20 min. The data were fit to the Michaelis–Menten equation, yielding V_{max} of 21 nmol CH_4 /nmol MoFe protein and K_m of 0.23 atm of CO_2 .

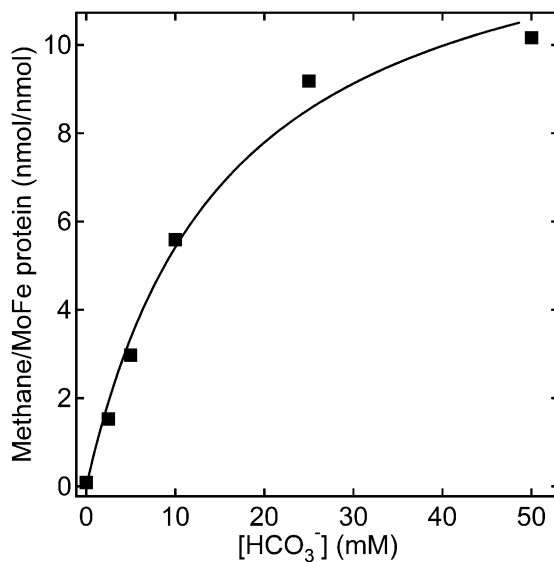


Fig. S2. Dependence of CH_4 formation on concentration of HCO_3^- . The amount of CH_4 formed as a function of the concentration of HCO_3^- is shown for the $\alpha\text{-}^{70}\text{Ala}/\alpha\text{-}^{195}\text{Gln}$ MoFe protein. The assay contained 3 mg/mL Fe protein and 0.5 mg/mL MoFe protein. The assay was at 30 °C for 20 min. The data were fit to the Michaelis–Menten equation, yielding V_{max} of 14 nmol CH_4 /nmol MoFe protein and K_m of 16 mM HCO_3^- .

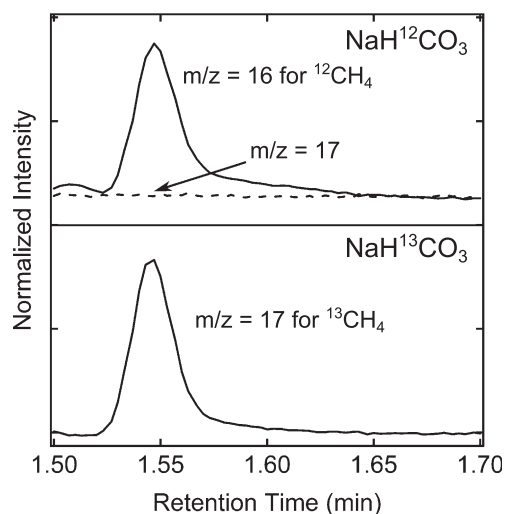


Fig. S3. GC-MS analysis of methane. A portion of the gas mixture of an assay with α -70^{Ala}/ α -195^{Gln} MoFe protein and CO₂ was separated by gas chromatography, with detection by mass spectrometry. H¹²CO₃⁻ (Upper) or H¹³CO₃⁻ (Lower) were used as substrate. The peak eluting at 1.55 min was analyzed for molecular ion peak of ¹²CH₄ or ¹³CH₄ with a mass over charge ratio (*m/z*) of 16 or 17, as indicated.

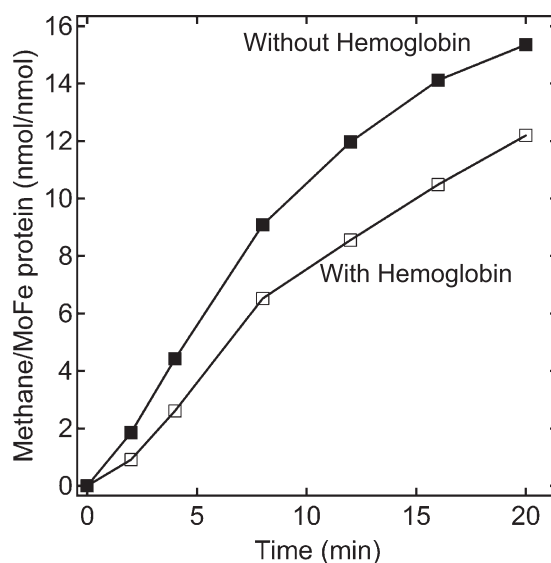


Fig. S4. Effect of hemoglobin on CH₄ formation. The formation of CH₄ for the α -70^{Ala}/ α -195^{Gln} MoFe protein is shown as a function of time either without (■) or with 0.3 mg/mL hemoglobin (□). The partial pressure of CO₂ was 0.45 atm, the concentration of MoFe protein was 0.5 mg/mL, and Fe protein was 3 mg/mL. The reactions were conducted at 30 °C.

