SUPPORTING INFORMATION

Intermediate P* from Soluble Methane Monooxygenase Contains a Diferrous Cluster

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Table S1. Characteristics of the reaction cycle intermediates of the *M. trichosporium* diferrous MMOH-MMOB single turnover cycle.[a,b]

Intermediate	Nature of the diiron cluster	Evidence
H^{red}	$Fe^{II}Fe^{II}$ cluster, ferromagnetically coupled	Characteristic EPR signal at g = 16 Mössbauer spectrum in a strong applied magnetic field shows two sites with 1:1 site ratio
O	$Fe^{II}Fe^{II}$ cluster, ferromagnetically coupled with O_2 bond in the active site	Same spectral features as **H^{red}**. The apparent rate constant for decay of the g = 16 signal is independent of the O_2 concentration, implying that an irreversibly formed intermediate occurs between **H^{red}** and **P*** or **P**.
P*	Shown here to be an $Fe^{II}Fe^{II}$ cluster, but distinct from **O**	Mössbauer spectra demonstrate that the cluster is diferrous; it lacks, however, the g = 16 EPR signal. A small change in the exchange coupling between **P*** and **H^{red}** or **O** may account for the disappearance of the g = 16 signal. The decay rate constant for **O** is much faster than the formation rate constant for **P**, indicating that there must be an intermediate between **O** and **P**.
P	Cis μ-peroxo $Fe^{III}Fe^{III}$ cluster with the antiferromagnetically coupled irons	The optical spectrum of **P** at 700 nm can be observed. It has much faster formation than decay rate constants, and it breaks down to yield **Q**. The Mössbauer spectrum of **P** shows that the irons are ferric and antiferromagnetically coupled. The isomer shift of **P** is larger than that of **H^{ox}**.
Q	Bis-μ-oxo $Fe^{IV}Fe^{IV}$ cluster, antiferromagnetically coupled	Mössbauer spectra reveal an antiferromagnetically coupled Fe^{IV} Fe^{IV} cluster comprising high-spin Fe^{IV} sites. EXAFS analysis reveals a short Fe-Fe distance, consistent with a diamond core structure. The kinetics of the reaction can be monitored using the intense yellow color of **Q**. **Q** forms with the same rate constant as **P** decays. The rate constant for **Q** decay depends on the concentration of added substrate, suggesting that it is the reactive intermediate of the cycle. The **Q** reaction with methane shows a deuterium kinetic isotope effect of ~50 suggesting that a tunneling reaction mechanism dominates.
R	Too short-lived to be trapped. Thought to be an $Fe^{III}Fe^{IV}$-OH cluster with a substrate radical bound nearby	$^{1}H,^{2}H,^{3}H$-Chiral ethane shows ~70 % racemization during reaction with **Q** to form ethanol, requiring a radical intermediate with a very short lifetime. Similar results were obtained using radical clock substrates and by observing the desaturation chemistry.
T	$Fe^{III}Fe^{III}$ cluster with product bound to the cluster or nearby	Substrates that form chromophoric products show that product formation is faster than product release, thereby requiring a discrete product complex. All rate constants for the formation and decay of intermediates between **H^{red}** and**R** exceed the turnover number for methane and other substrates, implying a product complex with rate limiting product release.

[a] Compiled from references [1-12]

[b] Comparable intermediates have been described for the *Methylococcus capsulatus* Bath soluble methane monooxygenase system. [13]

Supplemental Results

Fourier Transform Treated Spectra of Figure 7. Briefly, a Mössbauer spectrum is the convolution of a source line shape [a Lorentzian of ~0.12 mm/s full width with our current ^{57}Co(Rh) source] with the spectrum of the absorber. By the convolution theorem, the Fourier transform of a Mössbauer spectrum is the product of the transforms of the source (known) and the absorber (the desired quantity). By removing the source contribution in the time-domain and back-transforming we obtain, in the ideal case, the spectrum of the absorber as it would appear if the source would not contribute to the line width. In practice, experimental noise requires filtering the data in the time domain, which yields some distortion of the lines; for details see references. [14,15] The Fourier transform treated spectra presented here (Figure S1) yield rather precise line positions. A line that is substantially broadened by heterogeneities (e.g. a distribution in ΔE_Q) will narrow very little by this treatment. The colored arrows indicate the positions of the two lines belonging to each identified species; this analysis shows that all species present have been identified.

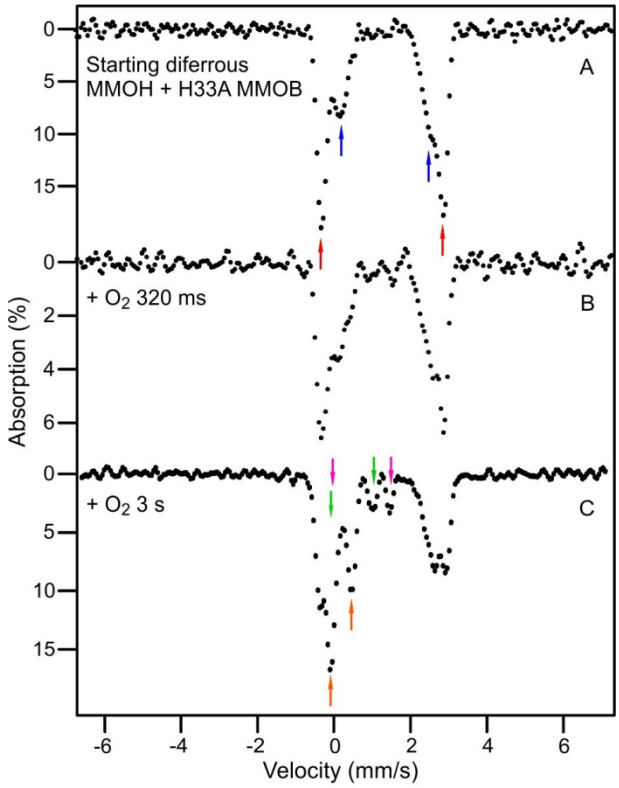

Figure S1. Fourier transform treated data of the spectra shown in Figure 7 (panels A, B, and C) of the main text. The colored arrows mark the two lines of each assigned quadrupole doublet. Sites 1 and 2 of $\mathbf{H}^{\mathbf{red}}$ (red and blue, respectively); intermediates **P** (magenta) and **Q** (orange) and $\mathbf{H}^{\mathbf{ox}}$ (green). Intensities are obtained from fitting the original spectra of Figure 7 using the constrained line positions. The spectrum of (A) shows that site 2 (blue arrows) is heterogeneously broadened and contributes a single (broadened) doublet rather than two doublets with sharp lines and different quadrupole splittings.

Supplemental References

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