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Authors: Subhasree Kal and Lawrence Que

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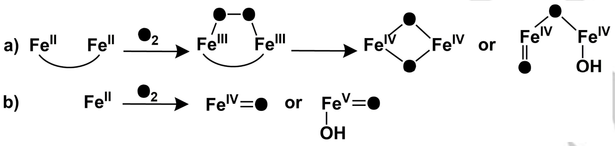
Activation of a nonheme Fe(III)–OOH by a second Fe(III) to hydroxylate strong C–H bonds: possible implications for sMMO

Subhasree Kal and Lawrence Que, Jr.*[a]

Abstract: Nonheme iron oxygenases contain either mono-iron or diiron active sites, and the role of the second iron in the latter enzymes is a topic of particular interest, especially for soluble methane monooxygenase (sMMO). Herein we report the activation of a nonheme Fe^{III}–OOH intermediate in a synthetic mono-iron system using $Fe^{III}(OTf)_3$ to form a high-valent oxidant capable of effecting cyclohexane and benzene hydroxylation within seconds at -40 °C. Our results show that the second iron acts as a Lewis acid to activate the iron-hydroperoxo intermediate, leading to the formation of a powerful Fe^V=O oxidant - a possible role for the second iron in sMMO.

Nonheme iron oxygenases form a major class of oxygen activating metalloenzymes.[1] It can be further divided into two subgroups based on the number of iron atoms in the active site: monoiron and diiron enzymes.[2,3] Both iron atoms in the active sites of diiron enzymes are responsible for activating O_2. Soluble methane monooxygenase (sMMO) belongs to this subgroup and is one of the best–studied diiron enzymes.[4,5] sMMO performs the challenging oxidation of methane (C–H BDE = 105 kcal/mol) to form methanol and does so via a diiron(III)-peroxo species that is converted to the diiron(IV)-oxo oxidant that cleaves the strong C–H bond of methane (Scheme 1). On the other hand, in mono-iron enzymes, a single iron atom is responsible for activating O_2 (often with the help of cofactors or substrate) to form the active oxidant (Scheme 1).[2,6] This raises the intriguing question - what is the role of the second iron in diiron enzymes?



Scheme 1. Proposed active oxidants for a) di-iron and b) mono-iron nonheme enzymes. (The hydroxide moieties bound to the high-valent iron centers are proposed to be derived from water.)

Lewis acidic metal ions can be used for tuning the redox potentials of metal-oxygen intermediates. For example, redox-inactive metal ions have been shown to increase the $Fe^{III/IV}$ redox potential of mononuclear nonheme Fe^{IV}=O species.[7,8] Similarly, in the oxygen evolving complex of photosystem II, Ca^{2+} is proposed to raise the redox potential of the manganese oxo cluster.[9–11] Lewis acids can also assist in cleaving O–O bonds in peroxo(hydro) intermediates to form high-valent metal-oxo species. For $[(TMC)Fe^{III}\text{-}\eta^2\text{-}O_2]^+$, Lewis acids such as Sc^{3+} and Y^{3+} induce cleavage of the O–O bond to form the $[(TMC)Fe^{IV}(O)]^{2+}$.[12,13] Recently, we have demonstrated that Lewis acidic Sc^{3+} activates the $[Fe^{III}(\beta\text{-BPMCN})(OOH)]^{2+}$ intermediate to form a reactive Fe^V=O oxidant that cleaves strong C–H bonds within seconds at -40 °C.[14] Fe^{3+} and Fe^{2+} are generally known for their redox properties but can potentially act as Lewis acids as well. However, neither Fe^{3+} nor Fe^{2+} has to date been reported to activate Fe^{III}-peroxo or hydroperoxo species. Here we report the activation of the $[Fe^{III}(\beta\text{-BPMCN})(OOH)]^{2+}$ intermediate by a second iron(III) ion to form a strong oxidant that hydroxylates both cyclohexane and benzene efficiently.

$[Fe^{II}(\beta\text{-BPMCN})(CH_3CN)_2]^{2+}$ (**1,** BPMCN = *N,N'*-bis(pyridyl-2-methyl)-*N,N'*-dimethyl-*trans*-1,2-diaminocyclohexane) (Figure 1) is a nonheme iron complex that catalyzes olefin oxidation with H_2O_2, but can only perform stoichiometric oxidation of cyclohexane (Table 1).[15] In 2018, we have shown that redox-inactive Lewis acid Sc^{3+} activates this system to form a powerful oxidant that can efficiently catalyze cyclohexane oxidation within seconds at 25 °C.[14] Additionally, under these conditions it is able to carry out catalytic electrophilic substitution of benzene and benzene analogs with electron-withdrawing substituents. Both redox-inactive Lewis acidic Sc^{3+} and perchloric acid activate the $[Fe^{III}(\beta\text{-BPMCN})(OOH)]^{2+}$ intermediate **(2)** (Figure S1) to comparable extents, supporting the role of Sc^{3+} as a strong acid in activating an Fe^{III}–OOH species.

In this paper we explore the effect of adding $Fe^{II}(OTf)_2$ or $Fe^{III}(OTf)_3$ to the **1**/H_2O_2 reaction mixture containing cyclohexane or benzene as substrate. In the absence of any additive, the oxidation of cyclohexane by **1** and 10 eq 90% H_2O_2 affords 0.5 eq cyclohexanol and 0.6 eq cyclohexanone. The use of $Fe^{II}(OTf)_2$ as an additive does not affect the amount of cyclohexane oxidized relative to that obtained in its absence, but the alcohol-to-ketone (A/K) ratio is increased (Figure 1). Furthermore, with benzene as substrate, no phenol formation is detected upon $Fe^{II}(OTf)_2$ addition, just like **1** in the absence of any additive. These results suggest that Fe^{2+} cannot activate the **1**/H_2O_2 combination for catalysis.

In significant contrast, the addition of 2 eq $Fe^{III}(OTf)_3$ relative to **1** to the reaction mixture containing cyclohexane as substrate results in 7.5 turnovers (TON) of cyclohexanol, a 15-fold increase compared to that obtained without additives (Table 1 and Figure 1). Additionally, very little cyclohexanone (0.1 TON) is formed, resulting in a remarkably large alcohol-to-ketone (A/K) ratio of 75 (Table 1). Furthermore, unlike for $Fe^{II}(OTf)_2$, benzene is converted to phenol in the presence of $Fe^{III}(OTf)_3$ with a turnover number (TON) of 5.4. As shown in Figure 1, the yields of cyclohexanol and phenol grow with increasing $[Fe^{3+}]$, plateau at 2-3 eq $Fe^{III}(OTf)_3$, and then decrease beyond 3 eq $Fe^{III}(OTf)_3$. The latter behavior might be attributed to unproductive side reactions involving $Fe^{III}(OTf)_3$ and H_2O_2. These observations demonstrate that $Fe^{III}(OTf)_3$ is able to activate the **1**/H_2O_2 combination to generate a powerful oxidant that can perform both cyclohexane and benzene hydroxylation.

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[a] S. Kal, Prof. Dr. L. Que, Jr.
Department of Chemistry
University of Minnesota, Twin Cities
207 Pleasant Street SE, Minneapolis, MN 55455
E-mail: larryque@umn.edu

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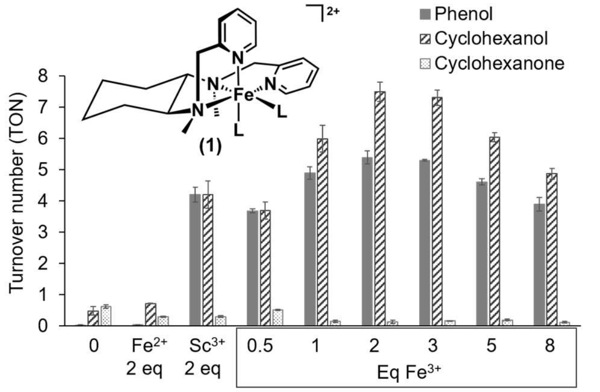


Figure 1. Effect of $Fe^{III}(OTf)_3$ on cyclohexane and benzene oxidation reactions catalyzed by **1** (L = CH_3CN) and comparisons with $Sc^{III}(OTf)_3$ and $Fe^{II}(OTf)_2$. Reaction conditions: **1** (0.7 mM), 1000 eq cyclohexane or 100 eq benzene, 10 eq 90% H_2O_2 in CH_3CN at room temperature.

Control experiments show that neither $Fe^{III}(OTf)_3$ nor $Fe^{II}(OTf)_2$ alone leads to the observed reactivity under similar reaction conditions (Table S1). When the results of $Fe^{III}(OTf)_3$ activation of the **1** + H_2O_2 system are compared to those previously reported for Sc^{3+} and $HClO_4$ activation,[14] it is clear that Fe^{3+} is more effective than either Sc^{3+} or $HClO_4$ in substrate oxidation (Table 1 and Figure 1). For benzene hydroxylation, a turnover number of 5.4 is obtained with 2 eq Fe^{3+}, which is about 30% higher than found for $Sc^{III}(OTf)_3$ or $HClO_4$. For cyclohexane oxidation with 2 eq $Fe^{III}(OTf)_3$, a TON of 7.5 is observed for cyclohexanol, which is almost two-fold higher than that reported for $Sc^{III}(OTf)_3$ or $HClO_4$. Furthermore, the A/K ratio for $Fe^{III}(OTf)_3$ increases five-fold relative to that observed for $Sc^{III}(OTf)_3$ due to the lower yield of cyclohexanone.

A product kinetic isotope effect (PKIE) of 2.1(1) is observed for the competitive oxidation of *c*-C_6H_{12} versus *c*-C_6D_{12} in the presence of 2 eq Fe^{3+}. This result is similar to those found for cyclohexane hydroxylation by the **1**/H_2O_2 combination in the presence of either $Sc^{III}(OTf)_3$ or $HClO_4$ and points toward the formation of quite a powerful oxidant that is less discriminating in its preference for cleaving C–H versus C–D bonds (Table 1). The high A/K ratio suggests that the alkyl radical formed in this reaction must be quite short-lived and immediately rebounds to the oxygen attached to the iron center after the initial H-atom abstraction by the iron-based oxidant.

For the hydroxylation of benzene, an inverse product kinetic isotope effect of 0.9 is observed in the presence of Fe^{3+}, comparable to values found for both Sc^{3+} and $HClO_4$ (Table 1). These results support an electrophilic aromatic substitution mechanism involving a metal-based electrophile.[16–18] Additionally, the oxidant formed in the presence of Fe^{3+} can oxidize electron-poor benzene analogs such as nitrobenzene, bromobenzene and trifluorotoluene, as revealed by the appearance of chromophores of the corresponding Fe^{III}–OAr products, which are blue shifted relative to that of **3** (Figure S2). These results suggest that the oxidant is quite a potent electrophile. For both cyclohexane and benzene oxidation, essentially quantitative ^{18}O incorporation from $H_2{}^{18}O_2$ is observed, which is confirmed by the complementary experiment using $H_2{}^{18}O$ (~ 2% ^{18}O incorporation detected from $H_2{}^{18}O$). No incorporation from O_2 is observed, unlike in the absence of any additive (Table 1). This observation is similar to what has been observed for Sc^{3+} or $HClO_4$. In a competitive oxidation between benzene and cyclohexane, it is observed that hydroxylation of benzene is favored by 10-fold over that for cyclohexane, as found for Sc^{3+} and $HClO_4$. Cumulatively, these results suggest that $Fe^{III}(OTf)_3$ behaves mechanistically similar to $Sc^{III}(OTf)_3$ and $HClO_4$ in forming a metal-based oxidant; however, $Fe^{III}(OTf)_3$ is more effective than either $Sc^{III}(OTf)_3$ or $HClO_4$ in converting cyclohexane to cyclohexanol and benzene to phenol.

Table 1. Comparing the oxidative reactivity of the **1**/90% H_2O_2 combination with different acid additives.[a]

	No Additive	2 eq Sc^{3+}	2 eq $HClO_4$	2 eq Fe^{3+}
TON cyclohexanol (A)	0.5(1)	4.2(4)	4.0(2)	7.5(3)
TON cyclohexanone (K)	0.6(1)	0.3(1)	0.1(1)	0.10(5)
A/K[b]	0.8	14	40	75
PKIE[c] (*c*-C_6H_{12} vs *c*-C_6D_{12})	5(1)	2.5(2)	2.0(1)	2.1(1)
% H_2O_2 converted into cyclohexane products	11	45	41	76
% ^{18}O in $C_6H_{11}OH$ from $H_2{}^{18}O_2$/$H_2{}^{18}O$ [d]	34/10[15]	97/1[14]	98/2	100/2
TON phenol	0	4.2(2)	4.0(3)	5.4(2)
PKIE (C_6H_6 vs C_6D_6)	–	0.9	0.9	0.9
k_2 for Fe^{III}–OOH decay @ -40 °C (M^{-1} s^{-1})		6.7(4) x 10^2	5.5(1) x 10^2	2.7(2) x 10^2

[a] All reactions at room temperature under air with 10 eq 90% H_2O_2; TON (turnover number) = moles of product / moles of **1**. [b] A/K = TON alcohol / TON ketone. [c] PKIE = product kinetic isotope effect based on the yields of cyclohexanol and cyclohexanol-*d*11. [d] ^{18}O incorporated into cyclohexanol when $H_2{}^{18}O_2$ is used or when the reaction is carried out in the presence of $H_2{}^{18}O$ (see SI for further details).

At -40 °C, **1** reacts with H_2O_2 to form the hydroperoxo-iron(III) species **2** with a λ_{max} of 545 nm (Figure S1).[14] Addition of $Fe^{III}(OTf)_3$ to **2** in the presence of benzene as the substrate elicits within seconds the formation of a blue-colored chromophore corresponding to the $[(\beta\text{-BPMCN})Fe^{III}(OPh)]^{2+}$ species **3** (λ_{max} = 620 nm, ε_M ~ 3600 $M^{-1}cm^{-1}$)[19,20] (Figure 2a). On the other hand, the use of cyclohexane as substrate allows us to monitor the effect of $Fe^{III}(OTf)_3$ on accelerating **2** decay, as the corresponding Fe^{III}–OR product does not have a visible chromophore. Under the latter conditions, intermediate **2** decays within 10 s upon addition of 8 eq $Fe^{III}(OTf)_3$, whereas it takes 90 min for **2** to decay without $Fe^{III}(OTf)_3$. Notably, the decay rate of **2** in the presence of cyclohexane matches that for the formation of **3** in the presence of benzene. These observations strongly suggest the formation of a common oxidant from the reaction of **2** with $Fe^{III}(OTf)_3$ that is responsible for the hydroxylation of both benzene and cyclohexane (Figure 2). As observed for the reaction of **1** with $Sc^{III}(OTf)_3$ or $HClO_4$, increasing $[Fe^{3+}]$ accelerates the reaction rates proportionately, further connecting Fe^{3+} addition with the formation of the oxidant (Figure 2 and

Table S2). However, the decay rate of **2** or formation rate of **3** with $Fe^{III}(OTf)_3$ is three-fold slower than corresponding rates upon the addition of Sc^{3+} or $HClO_4$. With 8 eq Sc^{3+} or $HClO_4$, the rate of formation of **3** is 2.6(1) s^{-1}, whereas the rate is 0.9(1) s^{-1} for 8 eq Fe^{3+}. The second order rate constants derived from varying [Lewis acid] are 6.7(4) x 10^2 M^{-1} s^{-1} for Sc^{3+} and 2.7(2) x 10^2 M^{-1} s^{-1} for Fe^{3+} at -40 °C. Thus, the nature of the Lewis acid affects the activation of **2** to form the active oxidant responsible for benzene and cyclohexane hydroxylation.

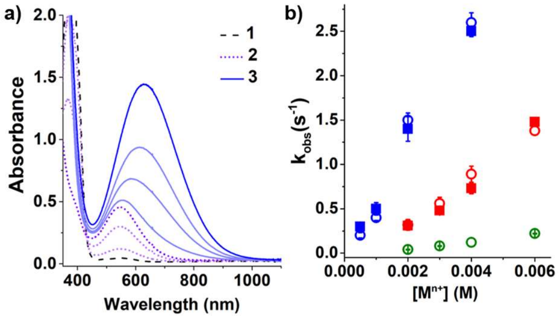


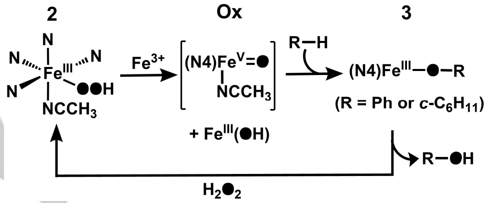
Figure 2. a) Formation of **3** upon addition of Fe^{3+} to **2,** which is formed by reacting H_2O_2 (20 eq) with **1** (0.5 mM) at -40 °C in CH_3CN. Dashed black trace **1,** dotted purple trace **2**, solid blue trace **3**. b) M^{n+} (Sc^{3+}, Fe^{3+}, Fe^{2+}) concentration dependence on rates of **3** formation or **2** decay at -40°C. Sc^{3+} (blue); Fe^{3+} (red) and Fe^{2+} (green). Open circles for benzene and filled squares for cyclohexane.

In contrast, addition of $Fe^{II}(OTf)_2$ to **2** in the presence of benzene simply results in the decay of **2** without forming **3**, indicating that it does not activate **2**. However, the rate of **2** decay is found to depend on [Fe^{2+}], affording a second order rate constant of 4.5(3) x 10^1 M^{-1} s^{-1}, which is six-fold slower than the decay rate for **2** upon addition of $Fe^{III}(OTf)_3$ in the presence of cyclohexane (Figure 2b). We suggest that this reaction corresponds to the oxidation of $Fe^{II}(OTf)_2$ by **2**. Indeed, when this reaction is monitored beyond the initial 100-s time period where rapid decay of **2** occurs, a small amount of **3** can be observed spectroscopically to form over the next 1000 s (Figure S3). This outcome likely derives from the reaction of the nascent Fe^{3+} formed *in situ* with residual **2** to form a small amount of **3**.

A further comparison of the differing effects of $Fe^{III}(OTf)_3$ and $Fe^{II}(OTf)_2$ is presented in Figure S4, in which H_2O_2 is added to a solution containing **1**, benzene and either $Fe^{III}(OTf)_3$ or $Fe^{II}(OTf)_2$ at -40 °C, instead of adding $Fe^{II}(OTf)_2$ or $Fe^{III}(OTf)_3$ to a solution of pre-formed **2** and benzene at -40 °C as presented in earlier paragraphs. For $Fe^{III}(OTf)_3$, exponential formation of **3** occurs within 100 s (Figure S4a). However, for $Fe^{II}(OTf)_2$, a small amount of **2** is observed to form initially, followed by a much slower appearance of **3** over 1000 s with an A_{620} value corresponding to less than 0.2 eq **3** formed (Figure S4b). This sequence of spectroscopic changes demonstrates that $Fe^{III}(OTf)_3$ interacts with **2** to form the oxidant for benzene hydroxylation, whereas $Fe^{II}(OTf)_2$ must first be oxidized to Fe^{3+} by **2** before any phenol can be formed.

The Lewis acidities of $M^{n+}(H_2O)_x$ ions decrease in the order Fe^{3+} (2.2) > Sc^{3+} (4.3) > Fe^{2+} (9.5),[21] with Fe^{2+} being much less Lewis acidic than either Fe^{3+} or Sc^{3+}. Thus, it is not surprising to find Fe^{2+} is unable to convert **2** into an oxidant that is capable of hydroxylating benzene or cyclohexane. Furthermore, among the three ions, only $Fe^{II}(OTf)_2$ can undergo one-electron oxidation and deactivate **2** by reduction. In contrast, $Fe^{III}(OTf)_3$ can act as a strong Lewis acid like $Sc^{III}(OTf)_3$ to activate **2**, generating a powerful oxidant that can perform both cyclohexane and benzene oxidation. In fact, $Fe^{III}(OTf)_3$ appears to be more effective than $Sc^{III}(OTf)_3$, affording higher yields of oxidized products as well as 5-fold higher selectivity for cyclohexanol over cyclohexanone at room temperature. However the rate at which $Fe^{III}(OTf)_3$ reacts with **2** to form the active oxidant at -40 °C is only one-third as fast as that for $Sc^{III}(OTf)_3$, clearly suggesting some differences in the way these two metal ions interact with **2**.

In Scheme 2, we propose that the Lewis acidic Fe^{3+} interacts with the distal oxygen atom of the hydroperoxo ligand of **2** to facilitate the heterolytic cleavage of the O–O bond to form the powerful $Fe^V(O)$ oxidant (**Ox**). Such a potent oxidant rationalizes the hydroxylation of cyclohexane with a very high A/K ratio, a relatively low PKIE of ~ 2 and the hydroxylation of benzene with an inverse KIE of 0.9.



Scheme 2. Proposed mechanism for the activation of intermediate **2** by $Fe^{III}(OTf)_3$ for the oxidation of benzene and cyclohexane by **1**/H_2O_2.

An alternative possibility for the activation of **2** is the homolysis of its O–O bond to form $[(\beta\text{-BPMCN})Fe^{IV}(O)]^{2+}$ and •OH, which is inconsistent with our experimental observations. Hydroxyl radicals cannot give rise to the selective cyclohexane hydroxylation chemistry we observe here, reducing the probability of this pathway, but some Fe^{IV}=O complexes reported to date are capable of oxidizing cyclohexane.[22] Furthermore, $[(\beta\text{-BPMCN})Fe^{IV}(O)]^{2+}$, the other homolysis-derived product, may be activated by formation of an $Fe^{IV}(O)\bullet\bullet\bullet Fe^{III}(OTf)_3$ adduct, by analogy to the Lewis acid adducts of Fe^{IV}=O complexes investigated by Fukuzumi and Nam, which have been found to exhibit enhanced electron transfer properties.[7,8] We have tested this hypothesis by adding $Fe^{III}(OTf)_3$ to a solution containing $[(\beta\text{-BPMCN})Fe^{IV}(O)]^{2+}$ (Figure S5). When done in the presence of benzene, no formation of phenol was observed. Similarly, addition of $Sc^{III}(OTf)_3$ to a solution containing $[(\beta\text{-BPMCN})Fe^{IV}(O)]^{2+}$ in the presence of benzene did not elicit its hydroxylation, excluding the likelihood of Lewis acid activation of the $Fe^{IV}(O)$ unit to generate a powerful electrophilic oxidant. Therefore, we eliminate $Fe^{IV}(O)M^{III}$ (M = Fe or Sc) adducts or $Fe^V(O)$ generated from $Fe^{IV}(O)$ and Fe^{III} as possibilities, and **Ox** is assigned to be an $Fe^V(O)$ species formed by Fe^{3+}-assisted heterolytic cleavage of the O–O bond of **2**.

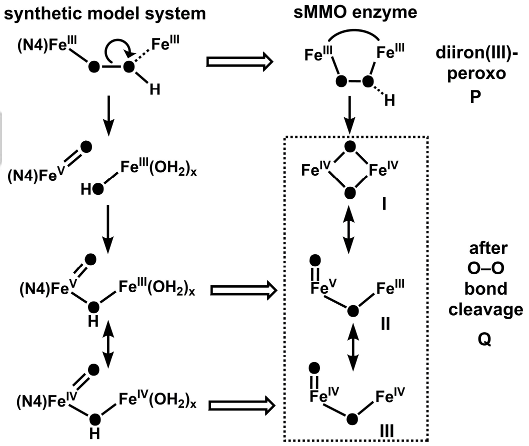
We have compared our results with those of previously reported nonheme iron oxidants capable of oxidizing cyclohexane (Table 2). For all other cases listed in this table, the high-valent iron oxidants can be directly observed and decay upon addition of cyclohexane at rates dependent on substrate concentration. However, for our experiments with [Fe(β-BPMCN)OOH]$^{2+}$, the oxidant **Ox** cannot be observed directly, because the formation of **Ox** is slower than its subsequent reaction with substrate. The rate of **Ox** formation thus represents the lower limit for the rate of substrate oxidation, which is why the decay rate of **2** in the presence of cyclohexane matches the rate of **3** formation with benzene, even though hydroxylation of benzene is favored 10-fold over that of cyclohexane in mixed-substrate competition experiments. To compare the rates listed for **2** in the bottom half of Table 2 with previously reported rates for cyclohexane oxidation by high-valent iron oxidants listed in the upper half of Table 2, we have calculated first-order rate constants for those in the top half assuming the presence of 1 M cyclohexane in the reaction solution. Thus the rate found for the reaction of **2** with 8 eq Sc^{3+} (2.5 s^{-1}) is comparable to that of [Fe^{V}(O)(O$_2$CR)(PyNMe$_3$)]$^{2+}$ (2.8 s^{-1}), the fastest nonheme iron system for cyclohexane hydroxylation documented to date,[23] followed by **2** with 8 eq of Fe^{3+} (0.7 s^{-1}), which is in turn two-fold faster than *S* = 1 [Fe^{IV}(O)(Me$_3$NTB)]$^{2+}$ (0.25 s^{-1})[24] and *S* = 2 [Fe^{IV}(O)(TQA)]$^{2+}$ (0.37 s^{-1})[25] the two most reactive Fe^{IV}(O) complexes characterized thus far. It should be noted that the rates associated with **2** are dependent on [Sc^{3+} or Fe^{3+} or H^{+}]; hence increasing the concentrations of these additives results in higher rates, potentially allowing substrate oxidation by **2** to be faster than by [Fe^{V}(O)(O$_2$CR)(PyNMe$_3$)]$^{2+}$. From a comparison of the products formed by these systems, it is clear that the **2**/Fe^{3+} combination affords the highest alcohol/ketone product ratio, making the oxidant formed by this combination a fast and highly selective oxidant for hydroxylating cyclohexane.

Table 2. Decay rates of nonheme iron intermediates involved in cyclohexane oxidation and their alcohol/ketone (A/K) ratios.

	k_2 (M^{-1}s^{-1}) @ -40 °C	A/K ratio	Ref
[Fe^{V}(O)(TAML)]$^{-}$	0.00026	—	[26]
[Fe^{IV}(O)(Me$_3$NTB)]$^{2+}$ (*S = 1*)	0.25	—	[24]
[Fe^{IV}(O)(TQA)]$^{2+}$ (*S = 2*)	0.37	ketone only	[25]
[Fe^{V}(O)(O$_2$CR)(PyNMe$_3$)]$^{2+}$	2.8	5	[23]
	k_{obs} (s^{-1})		
[Fe^{III}(β-BPMCN)(OOH)]$^{2+}$ (**2**)	$t_{1/2}$ ≈ 1 h	0.8	[14]
+ 8 eq Sc^{III}(OTf)$_3$	2.5	14	[14]
+ 8 eq HClO$_4$	2.3	40	[14]
+ 8 eq Fe^{III}(OTf)$_3$	0.7	75	This work
[Fe^{III}(PyNMe$_3$)(OOH)]$^{2+}$ + HBF$_4$•OEt$_2$	0.02	4.0^{a}	[27]

Abbreviations used: TAML = tetraazamacrocyclic ligand; Me$_3$NTB = tris(benzimidazolyl-2-methyl)amine; TQA = tris(quinolyl-2-methyl)amine; PyNMe$_3$ = 3,6,9-trimethyl-3,6,9-triaza-1(2,6)-pyridinacyclodecaphane.
^{a} HOTf used in place of HBF$_4$•OEt$_2$

The observations we have described above may also shed some light on how the diiron center of soluble methane monooxygenase (sMMO) could act to hydroxylate methane. The efforts of Lipscomb[5,28,29] and Lippard[4,30] have shown that reduced sMMO has a diiron(II) active center that reacts with O$_2$ to form a diiron(III)-peroxo intermediate called **P**, which in turn converts into **Q**, the diiron(IV) oxidant responsible for methane hydroxylation (Scheme 1 and 3). The conversion of **P** to **Q** has been demonstrated to exhibit a pH dependence implicating a water-derived proton with a pK$_a$ ~ 7.6 that plays a role in O–O bond cleavage.[31,32] Parallels may be drawn between the structures of the protonated **P** intermediate and the Fe^{III}-adduct with **2** postulated in Scheme 3. In the latter case, we have proposed that Lewis acidic Fe^{3+} facilitates heterolytic O–O bond cleavage to form an oxidant **Ox** formulated simply as [(L)Fe^{V}=O]$^{3+}$. However, it is possible that the Lewis acidic Fe^{III} center could interact with the nascent [(L)Fe^{V}=O]$^{3+}$ species to form an O=Fe^{V}–OH–Fe^{III} adduct. This structure would then be related to the high-valent diiron structures in sMMO formed after O–O bond cleavage as shown in the sMMO column in Scheme 3. At present, there is resonance Raman evidence supporting the diamond core structure **I** for sMMO-**Q**,[33] but recent EXAFS studies favor the open core structure **III**.[34,35] The putative O=Fe^{V}–O(H)–Fe^{III} species **II** proposed in this study can be thought of as an electromer of **I** and **III** and provides a mechanism to concentrate the oxidizing power of **Q** onto one iron center in order to cleave the very strong C–H bond of methane.[34,35] Hence, the second iron in diiron sMMO can have an additional role as a Lewis acid that is positioned in the active center to activate the O–O bond and form a highly reactive oxidant.



Scheme 3. Parallels between the activation of **2** by Fe^{III}(OTf)$_3$ and the proton-activated diiron(III)-peroxo intermediate **P** of soluble methane monooxygenase. **I**, **II** and **III** are the three plausible structures proposed for **Q**.

In summary, this work shows that Fe^{III} can act as a Lewis acid and activate a synthetic nonheme Fe^{III}–OOH intermediate **2** to form a powerful electrophilic oxidant **Ox** that can perform the hydroxylation of benzene and cyclohexane within seconds even at -40 °C. Such activation of an iron(III) peroxo or hydroperoxo intermediate using another iron(III) center is unprecedented in bioinspired nonheme iron chemistry and raises the possibility that the second iron in soluble methane monooxygenase may be required not only for its redox capabilities but additionally to act as a strong Lewis acid to form the powerful oxidant **Q** for the hydroxylation of methane.

Experimental Section

See the supporting information for experimental details.

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Keywords: Bioinspired iron oxidation • Iron(III) hydroperoxo • Nonheme iron • C–H activation • Benzene hydroxylation

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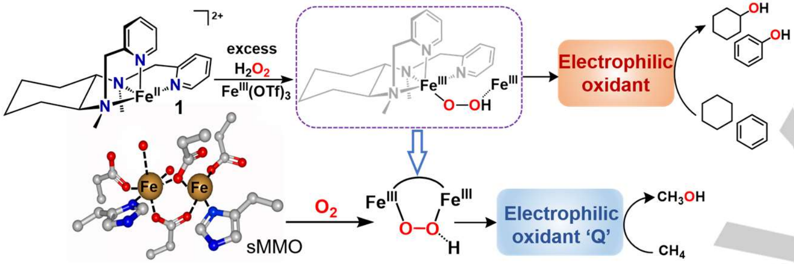
COMMUNICATION

Lewis acidic Fe(III) activates Fe(III)-OOH: A mononuclear nonheme Fe(III)-OOH species can be activated by Lewis acidic $Fe^{III}(OTf)_3$ to form a potent oxidant that can hydroxylate cyclohexane and benzene within seconds at -40 °C. These observations may provide useful insight into the chemistry required for the diiron enzyme sMMO in its conversion of methane to methanol.



*Subhasree Kal, Lawrence Que, Jr.**

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Activation of a nonheme Fe(III)–OOH species by a second Fe(III) to hydroxylate strong C–H bonds: possible implications for sMMO