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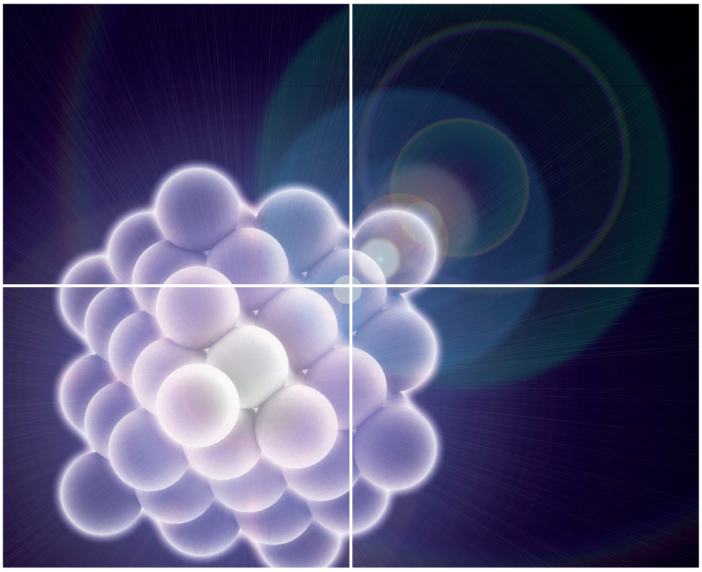

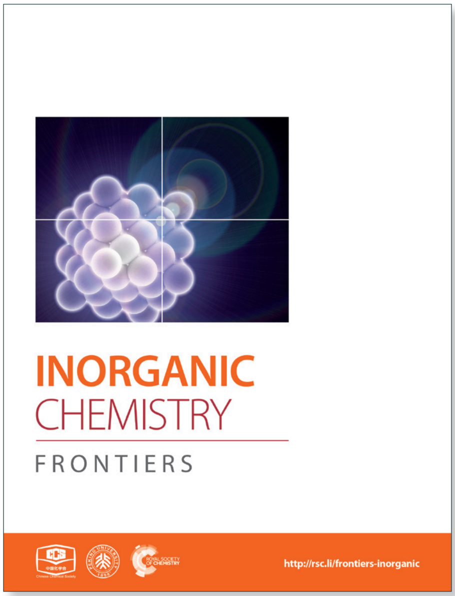

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COMMUNICATION

The Q, Compound Q is Finally Deciphered

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Methane monooxygenases (MMOs) activate the high energy C-H bond of methane and convert it to methanol with high selectivity and under physiological conditions. Despite decades of efforts focusing on elucidating the structure, function and mechanism of soluble MMOs, the structure of a key intermediate (called compound Q) remained unknown. This article highlights a recent report by Banerjee et. al. which not only firmly establishes the core-structure of Q, but also provides significant insight into its formation, reaction with methane and eventual decay.

Methane is the primary component of natural gas; it is relatively abundant and could potentially serve as a practical substitute for petroleum-based fuels. However, its gaseous form makes its storage and transportation challenging. Moreover, the gas may leak from wells or through pipelines and act as a potent greenhouse gas. One solution to these challenges, is to convert methane to liquid methanol, which is not only easier to store/transport but also serves as a raw material for several chemicals and polymers. The commercial process for methanol production involves industrial cracking of *syn* gas, which is a multi-step energy-consuming process.[1] In contrast, methane monooxygenase (MMO) enzyme from methanotrophic bacteria utilizes inexpensive and readily available oxygen to oxidize the high energy C-H bond (104 kcal/mol) of methane to produce methanol, under ambient conditions, without producing over-oxidized products such as formaldehyde or CO_2. Two types of MMOs have evolved to perform this reaction: a membrane-bound particulate MMO present in most methanotrophs and a soluble MMO expressed in some methanotrophs under copper-limiting conditions.[2] Although, there is some debate regarding the catalytic active site of the particulate MMO as to whether it is mono-, di- or tri-nuclear copper center, the catalytic centre in the hydroxylase subunit of soluble MMO (called MMOH) is unquestionably characterized as a carboxylate-bridged diiron unit (Fig. 1). The oxidative chemistry performed at these catalytic sites, especially the latter, has been investigated extensively over the last 20 years.[3] Nevertheless, the chemical structure of the key oxidizing species that reacts with methane and cleaves the high energy C-H bond, called compound Q for MMOH, remained unclear. A recent report by Banerjee et. al. solved this missing piece in the puzzle of MMOH mechanism by unequivocally establishing the structure of Q as a diamond-core bis-μ-oxo Fe^{IV} Fe^{IV} cluster.[4]

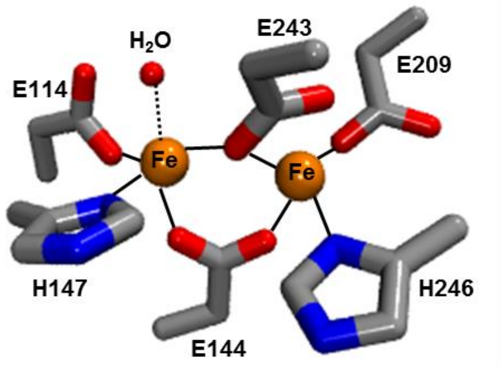

Fig. 1 Diiron active site structure of reduced *Mc* MMOH (PDB: 1FYZ). The amino acid residues as shown in stick representation while iron and water molecules are shown as spheres.

The compound Q was first detected by transient kinetics in a single-turnover reaction between di-ferrous MMOH and oxygen[5] (Fig. 2). The experiment revealed the formation of three intermediates named P, Q and T. While many spectroscopic studies have identified the intermediates P and T as μ-peroxodiiron(III) and μ-oxodiiron(III) respectively , the structure of compound Q, which displays broad absorbance bands around 330 nm and 430 nm, has been much difficult to pinpoint, because it does not exhibit any EPR signal, attributable to exchange coupled high-valent $Fe^{IV}Fe^{IV}$ form (as confirmed later by Mössbauer spectroscopy[6]). Additionally, the decay rate of compound Q increased linearly with methane concentration suggesting it to be an activated form of MMOH that directly catalysed methane oxidation.[5] Thus, it was important to elucidate the exact structure of Q in order to understand how MMOH catalyses methane oxidation and to design more robust and less expensive artificial catalysts to carry out the same reaction. Even though the oxidation and spin states of the compound Q were fully characterized *via* UV-Vis, EPR and Mössbauer spectroscopies, its core structure remained ambiguous. To address this issue, an extended x-ray absorption fine structure (EXAFS) study on Q revealed a short Fe-Fe separation of 2.47 Å and two short Fe-O bonds of slightly different lengths. It was proposed that these structural

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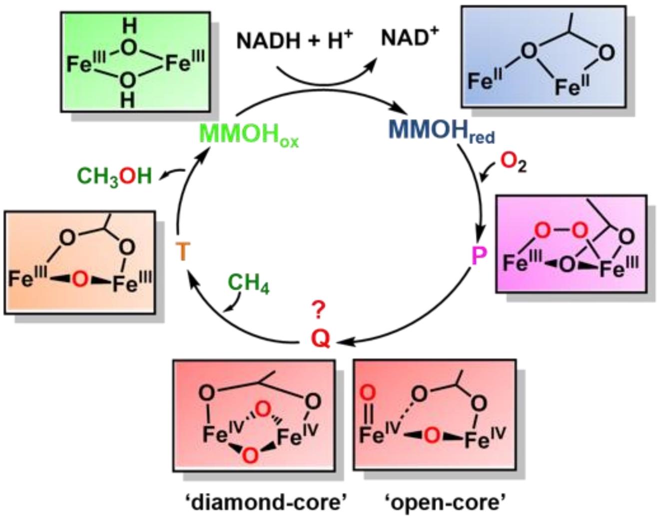

Fig. 2. Simplified mechanism of methane oxidation in MMOH. The reduced diiron center ($MMOH_{red}$) binds an oxygen molecule (in red) to form a peroxy intermediate (P), which then cleaves the O-O bond to form Q. The compound Q is the key oxidizing species in MMOH and could possess either closed 'diamond-core' or 'open-core' structure. It reacts with methane to oxidize the high energy C-H bond to form methanol converting itself to intermediate T, which finally decomposes to the oxidized form of the enzyme ($MMOH_{ox}$).

characteristics could best be explained by the presence of two asymmetric single-atom oxygen bridges between the irons forming a diamond-shaped core.[7] This proposal was further supported by comparison with spectroscopic data of structurally characterized synthetic model complexes as well as oxygen evolving complex of photosystem II, which exhibited an $M_2(\mu\text{-}O)_2$ geometry and metal distances ranging between 2.5 to 2.9 Å. However, because the EXAFS data only definitively reflected the presence of two short Fe-O bonds, other core structures for compound Q were also conceivable. As a result of this uncertainty, the 'diamond-core' hypothesis came under scrutiny by several experimental and computational observations. For example, high-level density functional theory (DFT) calculations could not reproduce the short Fe-Fe distance of compound Q as predicted by EXAFS studies. [8] Furthermore, a synthetic model, possessing $[Fe^{IV}_2(\mu\text{-}O)_2]$ diamond core and anti-ferromagnetic coupling similar to the compound Q, exhibited an Fe-Fe distance of 2.73 Å. Even though the irons in the synthetic model differed in spin-state and ligation from Q, the Fe-Fe distance was clearly longer.[9] Finally, conversion of a synthetic complex with a valence-delocalized $[Fe^{3.5}(\mu\text{-}O)_2Fe^{3.5}]^{3+}$ diamond-core structure into a complex with a valence-localized $[HO\text{–}Fe^{III}\text{–}O\text{–}Fe^{IV}\text{=}O]^{2+}$ open core resulted in over a million-fold faster aliphatic C–H bond cleavage. This activity enhancement, presumed to be resulting from the formation of a terminal oxo-iron(IV) moiety with high oxidizing capability and conversion of the low-spin (S=1) centre to a high-spin (S=2) centre, further raised the question of the viability of diamond core as the structure of the key oxidizing species of compound Q.[10]

As a result, the structure of compound Q remained a mystery for many years. The definite characterization of the core structure of compound Q in MMOH, in principle, could be possible using resonance Raman (RR) spectroscopy, as it can detect molecular vibrations from the stretching/bending of Fe-O bonds providing clues to the Fe-O structure and bonding. However, there were several challenges associated with such an experiment, for example (1) relatively weak Raman enhancement of signals demanded high sample concentration and (2) photolysis of the compound Q resulted in destruction of the intermediate before Raman spectra could be collected. Banerjee et al. overcame these obstacles using a custom-designed time-resolved RR instrument[11] that continuously mixed the reduced MMOH with oxygen and substrate (methane or furan) in a flowing system. By recording at a specific time after mixing in the flowing system, the RR spectrum of the short-lived compound Q could be averaged for hours at high concentration and without photolysis. By comparing the spectra generated using oxygen-16 isotope ($^{16}O_2$) with that using oxygen-18 isotope ($^{18}O_2$), they isolated vibrations due to Fe-O bonds in MMOH from vibrations due to protein backbone or the solvent. On mixing di-ferrous MMOH with oxygen under this experimental set-up, they observed a vibration at 690 cm^{-1} which disappeared on addition of substrates methane/furan and exhibited a large deuterium kinetic isotope effect when CD_4 was used as the substrate, suggesting the vibration to be due to compound Q. Since the frequency of the compound Q vibration was much lower than typically observed for Fe(IV)=O species (850 cm^{-1}), but much higher than reported for Fe(IV)-OH species (565 cm^{-1}), this finding ruled out the possibility of an 'open' structure. Furthermore, the compound Q vibration at 690 cm^{-1} was similar to that of other structurally characterized synthetic models that bear diamond-core $(Fe^{IV}\mu\text{-}O)_2$ structures (666-674 cm^{-1}), making it possible to define the structure of compound Q as a 'closed-diamond' core (Fig. 2). In order to probe the origin of the two oxygen atoms in compound Q, which could either be from an activated oxygen molecule or the solvent water molecule, the authors used a mixed isotopic form of dioxygen ($^{16}O^{18}O$) and observed a new frequency in the spectrum at 673 cm^{-1}. The exact positioning of this feature, midway between those of $^{16}O_2$ (690 cm^{-1}) and $^{18}O_2$ (654 cm^{-1}) incorporated Q, could only be explained as a tetratomic vibration arising from a diamond-core structure in which both ^{16}O and ^{18}O atoms of the dioxygen ($^{16}O^{18}O$) molecule have been incorporated. The experiment suggests that the O-O bond in the peroxo- (intermediate P) cleaves to form the compound Q. However, the mechanism of O-O bond cleavage, whether homolytic or heterolytic, remains uncertain. The energy constraints of the system, in fact, favors the homolytic cleavage of O-O bond for MMOH, in a stark contrast to heme-containing cytochrome P450s which also activates oxygen to oxidize aliphatic C-H bonds but cleaves O-O bond heterolytically to form the key oxidizing species heme compound I.[12]

Overall, this study makes significant progress towards not only elucidating the mechanism of methane oxidation but also understanding structure-function relationship of other oxygen activating diiron enzymes, such as ribonucleotide reductase which generates an intermediate 'X' possessing a relatively short Fe^{III} Fe^{IV} bond length similar to the intermediate 'Q'.[13] The study also opens up several questions in the field of bioinorganic chemistry regarding the reactivity and formation of compound Q. For example, why are biomimetic models exhibiting diamond-core structure like compound Q not reactive enough to oxidize methane? Potential cause may include factors such as low-spin state of synthetic models[9-10] or the lack of hydrogen bonding provided by glutamate in synthetic models.[14] Furthermore, what properties of MMOH causes the peroxo-species (intermediate P) to spontaneously convert to compound Q whereas the peroxodiiron(III) units of most other diiron enzymes do not appear to access such high-valent intermediates?[9] Potential causes may include water molecules in the active site of MMOH which may help to polarize and activate the bound peroxide.[15]

Like many important discoveries in science and engineering, Banerjee et al. were able to achieve a milestone in methane oxidation chemistry by working extremely hard (the experiments required a whopping 30g of highly purified MMOH), developing and optimizing novel experimental setup (that of continuously mixing and flowing time-resolved RR spectroscopy) and meticulously performing and analysing the data. The work addresses a key issue that puzzled researchers in the MMO field for many years and provides a strong basis for understanding nonheme diiron enzymes and making efficient catalysts for applications in C-H bond oxidation.

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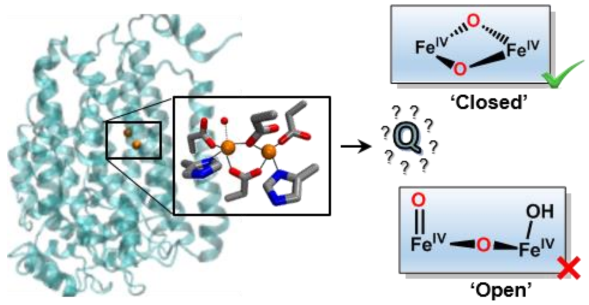

The elusive compound Q in methane monoxygenase has been characterised and firmly established as a diamond-core bis-µ-oxo Fe^{IV} Fe^{IV} cluster.