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Mimicking Heme Enzymes in the Solid State: Metal−Organic Materials with Selectively Encapsulated Heme

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Supporting Information

ABSTRACT: To carry out essential life processes, nature has had to evolve heme enzymes capable of synthesizing and manipulating complex molecules. These proteins perform a plethora of chemical reactions utilizing a single iron porphyrin active site embedded within an evolutionarily designed protein pocket. We herein report the first class of metal−organic materials (MOMs) that mimic heme enzymes in terms of both structure and reactivity. The MOMzyme-1 class is based upon a prototypal MOM, HKUST-1, into which catalytically active metalloporphyrins are selectively encapsulated in a "ship-in-a-bottle" fashion within one of the three nanoscale cages that exist in HKUST-1. MOMs offer unparalleled levels of permanent porosity and their modular nature affords enormous diversity of structures and properties. The MOMzyme-1 class could therefore represent a new paradigm for heme biomimetic catalysis since it combines the activity of a homogeneous catalyst with the stability and recyclability of heterogeneous catalytic systems within a single material.

Heme proteins represent one of the most diverse classes of metallo-enzymes in nature and are ubiquitous to all organisms.[1−4] This class of protein participates in diverse catalytic chemistry ranging from relatively simple electron transfer to complex monooxygen reactions. This broad catalytic diversity is achieved using a single type of heme macrocycle (iron protoporphyrin IX) selectively encapsulated within a protein cavity which allows ligand access to the fifth and sixth coordination sites of the central iron. The protein structure also provides functionally distinct distal and proximal pockets as well as specific pathways leading from solution to the heme active site that allows for size selectivity of the substrate.[5,6] Metal−organic materials (MOMs) that are based upon multiple polyhedral cages[7−11] offer excellent platforms for the development of MOM-based heme biomimetic catalytic systems since these polyhedral MOMs share two common structural features with heme proteins: large pockets (cages) which can accommodate a catalytic metalloporphyrin and access channels which connect the bulk solvent to various other cages within the porous material. We herein report the first class of MOMs that mimic heme enzymes in terms of both structure and reactivity. The MOMzyme-1 class (Metal Organic Material enzyme) is based upon a prototypal MOM, HKUST-1, into which catalytically active metalloporphyrins are selectively encapsulated in a "ship-in-a-bottle" fashion within one of the three polyhedral cages that exist in HKUST-1. MOMs offer unparalleled levels of permanent porosity and their modular nature affords enormous diversity of structures and properties. The MOMzyme-1 class therefore represents a new paradigm for heme biomimetic catalysis since it combines the activity of a homogeneous catalyst with the stability and recyclability of heterogeneous catalytic systems within a single material.

The catalytic diversity of heme proteins is an ongoing target for biomimetic chemistry and a wide array of systems have been developed to capture the salient catalytic features of heme proteins with limited success including porphyrin encapsulated sol−gels,[12,13] clay-like layered materials,[14,15] synthetic zeolites,[16,17] detergent micelles,[18] and polymer films.[19,20] Although these materials exhibit catalytic activity reminiscent of heme proteins, they lack structurally tunable distal and proximal heme pockets limiting their usefulness in heme biomimetic chemistry. In the case of MOMs, it has been previously demonstrated that it is possible to encapsulate free base and metalated cationic porphyrins into the large cages of a zeolite-like metal organic framework (rhoZMOF) using a 'ship-in-a-bottle' approach.[21] Although this system exhibited limited biomimetic activity toward mono-oxygenation, the porphyrin lacked orientational specificity. Thus, this system did not possess the requisite distal and proximal heme pockets found in proteins. This effort inspired the current system in which selective encapsulation of catalytically active porphyrins within specific cages associated with the HKUST-1 framework has been achieved thereby creating functionalizable and orientationaly specific proximal and distal heme pockets as well as substrate selective access channels to and from the porphyrin active sites. As such, the new materials retain many of the critical catalytic features associated with heme enzymes and promise the potential for the development of bioinspired materials spanning a wide range of catalytic chemistry.

HKUST-1, formed through the assembly of benzene-1,3,5-tricarboxylate anions and copper(II)[22] or zinc(II)[23] cations, is well-suited to serve as a platform for heme biomimetic chemistry since its topology affords three distinctly different polyhedral cages capable of entrapping guest molecules (Figure 1, top right and bottom and Figure 2). Indeed HKUST-1 selectively encapsulates polyoxometallate anions and exhibits size selective catalysis of ester hydrolysis.[24] In the case of the MOMzyme-1 systems reported herein, a metalloporphyrin (either Fe(3+) tetrakis(4-sulphonatophenyl)porphyrin, Fe4SP, or Mn(3+) tetrakis(4-sulphonatophenyl)porphyrin, Mn4SP) has been encapsulated within the octahedral cage that is most suited to serve

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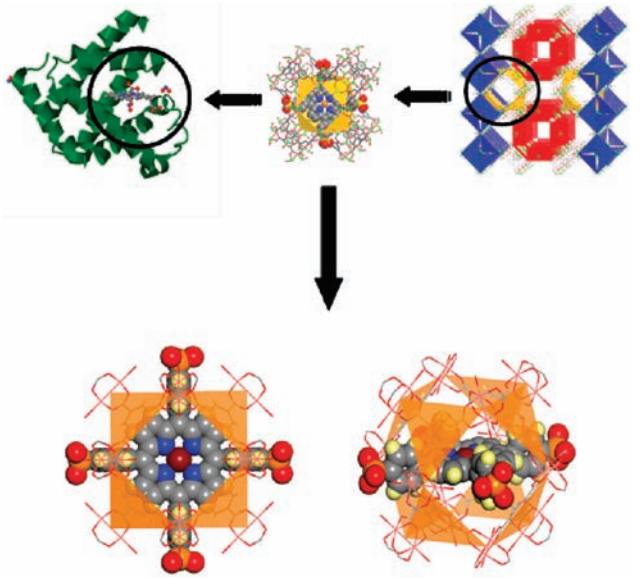

Figure 1. (Top) Illustration of the similarities in overall structural paradigm between heme proteins (left) and the porphyrin encapsulated HKUST-1 MOMzyme-1, right. The diagram of HKUST-1 highlights the three distinct polyhedral cages that make up its structures. (Bottom) Diagram showing two equivalent orientations of the Mn(III)4SP within the octahemioctahedral cage of HKUST-1(Cu, Zn). The structure illustrates the open access of the porphyrins central metal ion.

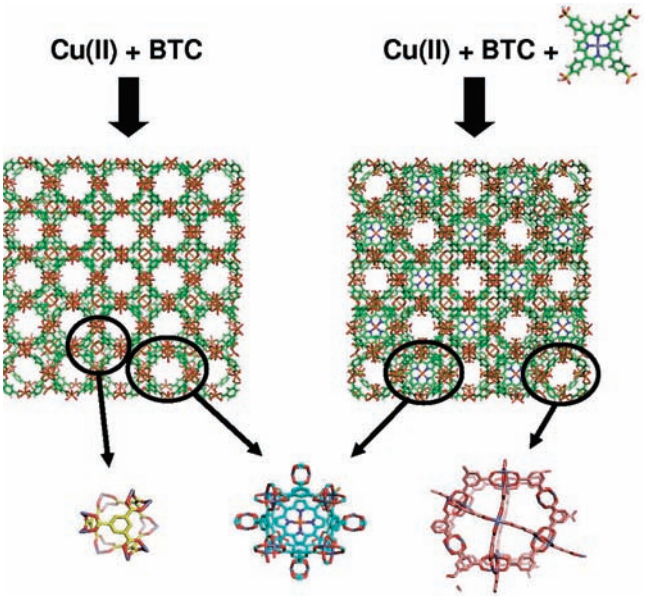

Figure 2. Diagram illustrating the encapsulation of metalloporphyrins within the octahemicatohedral cages of HKUST-1. Also illustrated are the other cavities associated with the framework.

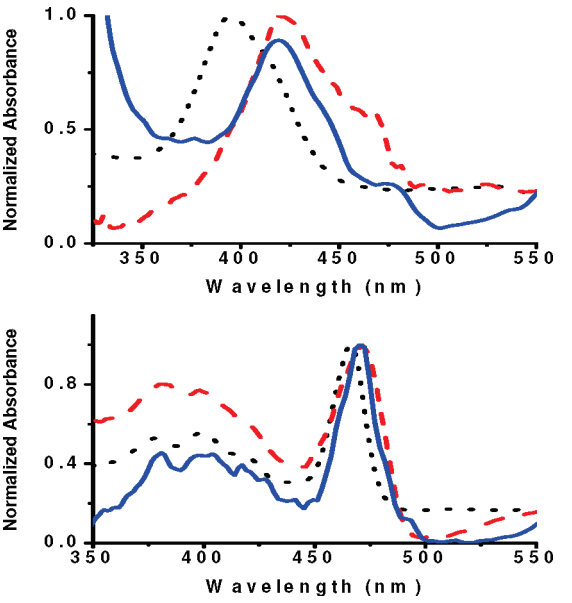

Figure 3. Normalized single crystal absorption spectra (derived from specular reflectance data) for M4SP@HKUST-1(Cu) (solid line), M4SP@HKUST-1(Zn) (dashed line), and solution optical spectrum (dotted line) for Fe(3+)4SP (top panel) and Mn(3+)4SP (bottom panel).

as a host for a metalloporphyrin based upon cage size and symmetry (Figure 1, bottom middle), while the remaining cavities allow small molecules to reach the active site for catalysis much like channels in heme proteins. The new materials are designated Fe4SP@HKUST-1(Cu or Zn) or Mn4SP@HKUST-1(Cu or Zn). Crystal structures of these MOMzymes were determined through single-crystal X-ray diffraction and were found to be isostructural with HKUST-1 (see Supporting Information for full details). These structures have the porphyrin benzenesulfonic acid peripheral groups oriented through four of the six square windows of the octahemioctahedral cages (Figure 1). It is this penetration of the benzenesulfonic acid groups of the porphyrin into neighboring cages that locks the porphyrin into a well-defined orientation within the cage. The axial sites on both planes of the porphyrin are therefore necessarily exposed to the access channels of the framework since they lie directly above and below the other two square windows of the cage (Figure 1). The porphyrin ring can occupy one of three equivalent orientations within the cavity due to cavity symmetry (Figure 1). Although the porphyrin ring in each cavity possesses a specific orientation, the orientations between cavities vary leading to static (as opposed to dynamic) disorder throughout the crystal. The porphyrin planes are clearly resolvable as the D_{4h} symmetry of the porphyrin's core is a subgroup of the cage symmetry and the core is located on the symmetry plane and axes. The porphyrin loading was estimated using both X-ray data (site occupancy refinement of metal atom) and spectroscopically (see Supporting Information) to be between 33% and 66% depending on reaction conditions suggesting that 1/3 to 2/3 of octahemioctahedral cages are occupied by porphyrin. The extent of porphyrin loading can be controlled by the amount of porphyrin present during the synthesis. The maximal loading was found to be ~66% (cavity loading) at saturating porphyrin concentrations. The presence of porphyrin molecules in the HKUST-1 framework reduces the experimentally observed surface area after activation at 85 °C in vacuo from 1663 m^2/g for HKUST-1(Cu) to 980 m^2/g for the Mn(3+)4SP HKUST-1(Cu).

The optical spectrum of metallopophyrins provides important information regarding oxidation and spin state of the central metal, the hydrophobicity of the macrocycle pocket, and the metalation state of the porphyrin. The single crystal optical absorption spectra (derived from specular reflectance data) of

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Table 1. Summary of Kinetic Results for the Degradation of H_2O_2 by the Fe4SP@HKUST-1(Cu) Materials and Model Systems

material	rate of H_2O_2 degradation (μM ABTS s^{-1} μM^{-1} of heme)	% [ABTS] converted per mole of heme (relative to hhMb)
Met Horse Heart Myoglobin (solution)	3.2	100
Microperoxidase-11 (solution)	3.6	52
Fe4SP (solution)	1.1	50
HKUST-1(Cu)	0	0
6 mg Fe4SP@HKUST-1(Cu)	0.3	50
4 mg Fe4SP@HKUST-1(Cu)	0.3	41
Fe4SP@HKUST-1(Cu)	0.1	60
Once Recycled Fe4SP@HKUST-1(Cu) Three Recyclings	0.09	55

both Fe4SP@HKUST-1(Cu or Zn) or Mn4SP@HKUST-1(Cu or Zn) are displayed in Figure 3. The spectra of Fe4SP@HKUST-1(Cu or Zn) exhibits a Soret maximum at ~419 nm, while the corresponding Soret maximum of Fe(3+)4SP in buffer-ethanol solution is found to be 394 nm (characteristic of six coordinate high-spin ferric iron). The bathochromic shift of the encapsulated Fe(3+)4SP in the MOMzyme frameworks is similar to that of Fe(3+)4SP in the presence of zwitterionic surfactants where the Soret maximum shifts to ~416 nm.[25] Thus, the spectral results are consistent with the encapsulated Fe(3+)-4SP retaining a six coordinate high-spin ferric iron experiencing a more hydrophobic environment relative to aqueous solution. The optical spectra of the Mn4SP@HKUST-1(Cu, Zn) also display a slight bathochromic shift of the Soret band relative to that of the porphyrin solution (467 nm for Mn(3+)-4SP in solution versus ~471 nm for the Mn4SP@HKUST-1(Cu, Zn)) also consistent with the hydrophobic nature of the HKUST-1 cavity. The fact that the single crystal optical spectra of the encapsulated porphyrins are nearly identical between M4SP@HKUST-1(Cu) and M4SP@HKUST-1(Zn) (M = Fe(3+) or Mn(3+)) indicates that the electrostatic environment of the binding pockets is similar between the two frameworks.

One of the most important catalytic reactions performed by heme proteins is monooxygenation of organic substrates.[26,27] The general mechanism for heme monoxygenation proceeds through a high-valent Fe(IV)═O intermediate which is highly oxidizing. This intermediate can be arrived at through either a ferrous heme in the presence of molecular oxygen (e.g., cytochrome P450 (CYP) class of proteins) or through ferric enzymes in the presence of a peroxide (e.g., peroxidase class of heme enzymes).

As a probe for heme protein biomimetic capacity of the new MOMzymes, the peroxidase activity of the material was assayed using 2,2′ azinodi(3-ethylbenzthiazoline)-6-sulfonate (ABTS) as a redox indicator by monitoring the rate of increase in absorbance at 660 nm (ε = 12 mM^{-1} cm^{-1} for $ABTS^{+\bullet}$) subsequent to the addition of peroxide.[27] The results of the assay are summarized in Table 1 and Figure 4 and are compared to the catalytic activity of microperoxidase-11 (MP-11), horse heart myoglobin (hhMb), and Fe4SP in solution. The data reported in Table 1 indicate that

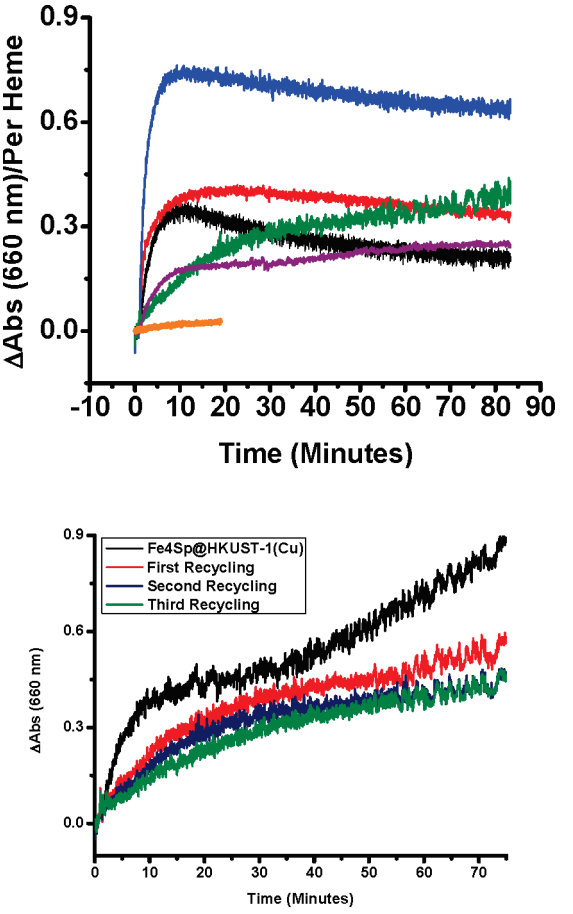

Figure 4. Representative kinetic traces for the reaction of Fe4SP@HKUST-1(Cu) with H_2O_2 and ABTS. (Top panel) Overlay of the catalytic traces for Fe4SP in ethanol (black), horse heart metMyoglobin (blue, in 50 mM phosphate buffer, pH 6.5), Microperoxidase-11 (red, in 50 mM phosphate buffer, pH 6.5), 8 mg of HKUST-1(Cu) (orange), and two different weights of Fe4SP@HKUST-1(Cu) (purple, 4 mg; green, 6 mg in ethanol). (Bottom panel) Overlay of kinetic traces for 4 mg of Fe4SP@HKUST-1(Cu) in the presence of ABTS and H_2O_2 and traces for three subsequent catalytic cycles.

the initial rate for $ABTS^{+\bullet}$ formation by the Fe4SP@HKUST-1(Cu) material is lower than observed for MP-11, hhMb, or Fe4SP (all in solution), while the maximum yield of $ABTS^{+\bullet}$, relative to hhMb, is comparable to that of MP-11 and Fe4SP in solution. The hhMb, MP-11, and Fe4SP were selected as preliminary benchmark systems as each displays peroxidase activity with increasing levels of structural complexity.

The lower initial rate for $ABTS^{+\bullet}$ formation, relative to the three benchmark systems, is due to the fact that substrate molecules must diffuse into/out of the channels of the HKUST-1(Cu) framework within the bulk material. However, %ABTS conversion is comparable to both MP-11 and Fe4SP. The significant percent conversion demonstrates several important features of the new material: (1) the axial positions of the encapsulated porphyrins are accessible to small molecules diffusing from solution into the HKUST-1(Cu) framework, (2) the Fe4SP remain catalytically active within the framework, (3) the larger ABTS substrate still has access to the encapsulated active sites, and (4) successive turnovers can take place without significant degradation of the porphyrin macrocycles (in contrast to free Fe4SP or hhMB in solution).

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One of the most significant limitations of homogeneous catalysts involving monooxygenation is the survivability of the catalyst. For metalloporphyrin systems, the intermediates present during catalysis (both ferryl and porphyrin $\pi^{\bullet+}$ in the case of iron porphyrin) are reactive and interact with other porphyrin macrocycles in solution, thus, rendering them inactive. In the case of proteins such as hhMb, excess H_2O_2 results in protein cross-linking and heme inactivation after successive turnovers. The ability of the Fe4SP@HKUST-1(Cu) material to isolate the catalytic centers within cavities and minimize catalytic degradation is illustrated in Table 1 (and Figure 4). Recovery and recycling results in retention of ~33% of the initial rate of $ABTS^{\bullet+}$ formation while the maximal production of $ABTS^{+\bullet}$ remains at ~66% of the initial catalysis run after three rounds of catalyst recycling (catalytic run-collection, washing and drying of the crystalline material followed by the next catalytic cycling). The initial loss of activity is likely due to the presence of guest molecules within the framework (possibly solvent or solvent breakdown products) that degrade the porphyrin catalyst but are consumed during the initial turnover cycle. No significant reduction in catalyst activity or percent ABTS conversion is observed after the initial catalytic cycle.

Whereas HKUST-1 type nets provide the platforms for the prototypal MOMzymes described herein, it is unlikely that they are the only nets suitable for porphyrin encapsulation or that they will offer optimal performance. There already exists a plethora of existing MOMs[7] that are based upon polyhedral building blocks and many of these materials exhibit higher surface area and pore size. The prototypal MOMzymes described herein suggest the feasibility of custom-designing the right MOM for the right substrate and the right metalloporphyrin combination. For example, the proximal and distal heme pockets within the MOMzyme could be functionalized through derivatization of the organic linkers making up the MBBs or through modification of the porphyrin ring. In addition, the dimensionality of the substrate access channels can also be modulated through the design of the organic linkers. The ability to functionalize the discrete porphyrin cavities provides an opportunity to develop unique solid state MOMzyme type materials that can span the range of heme protein catalytic chemistry including the extensive range of stereospecific monooxygenation reactions associated with the cytochrome P450 class of enzymes, dioxygen reduction (cytochrome oxidase-like single crystal fuel cells), nitric oxide production, and even photoactivated directional electron transfer (artificial photosynthesis).

■ ASSOCIATED CONTENT

Supporting Information. Crystallographic details, and methodologies for synthesis and catalytic assays are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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