

Supporting Information available: Ultraviolet absorption and fluorescence spectra of the conjugates and representative transient absorption spectra and transient decays.

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- [7] Data provided as Supporting Information.
- [8] Detailed information concerning the synthesis and characterization of triplex-forming conjugates will be reported separately. The UV thermal dissociation and circular dichroism spectra of **2–5** are consistent with the assignment of triplex structures. In the case of **1**, the structure may be an end-capped hairpin rather than a triplex.
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## Electrocatalytic O<sub>2</sub> Reduction by Synthetic Analogues of the Heme/Cu Site of Cytochrome Oxidase Incorporated in a Lipid Film\*\*

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Over 95 % of dioxygen consumed by an aerobe is used in respiration,<sup>[1]</sup> whereby O<sub>2</sub> is reduced by four electrons (4e) to two molecules of H<sub>2</sub>O. In most aerobes the reduction occurs at a heterodimetallic Fe/Cu site (Figure 1A) of membrane-bound heme/Cu terminal oxidases (cytochrome c or ubiquinol

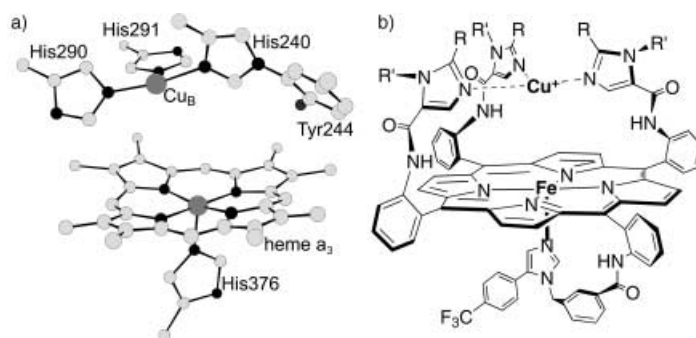
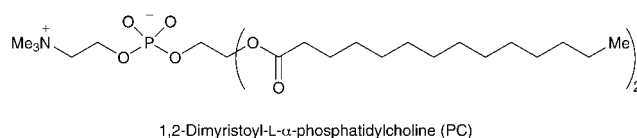


Figure 1. A) The heme/Cu site of bovine cytochrome oxidase;<sup>[5]</sup> the C atoms are light gray and the N and O atoms are black. B) The catalysts in the reduced form without probable exogenous ligands<sup>[6]</sup> (the NH series: R = Pr, R' = H; the NMe series: R = H, R' = CH<sub>3</sub>).

oxidases).<sup>[2,3]</sup> Coupling of this exergonic reaction to proton translocation against the electrochemical transmembrane gradient<sup>[2,4]</sup> maintains the proton-motive force that drives ATP synthesis.

The O<sub>2</sub>-reducing heme/Cu site has long been a target for biomimetic chemists.<sup>[7]</sup> A number of synthetic Fe porphyrins with various degrees of structural similarity to the heme/Cu site when adsorbed on a graphite electrode catalyze the electrochemical 4e reduction of O<sub>2</sub>, although rarely at physiological potentials (namely those of cytochrome c and ubiquinol). Electrocatalytic O<sub>2</sub> reduction is the only known method to probe the reactivity of synthetic heme/Cu analogues under catalytic and biologically relevant conditions. This technique is conceptually similar, and in some respects superior, to spectrophotometric and polarographic assays traditionally used to quantify the activity of cytochrome oxidase.<sup>[8]</sup> Previously, biomimetic O<sub>2</sub> reduction have been probed only by electrode-adsorbed heme/Cu mimics. However, studies of adsorbed heme/Cu analogues, while experimentally simple, limits the potential of such systems to unravel the structure/function relationship within this enzymatic site, and thus to contribute to understanding the enzyme. Firstly, cytochrome oxidase reduces O<sub>2</sub> under diffusion-limited electron delivery, whereas the electron-transfer rate to an electrode-adsorbed catalyst usually far exceeds the catalytic turnover frequency (TOF). Secondly, the high concentration of redox sites in films of adsorbed catalysts creates an environment which both differs notably from that of a protein matrix and depends strongly on the overall redox state of the film.<sup>[6b]</sup>

Herein we report on electrocatalytic O<sub>2</sub> reduction by a series of synthetic heme/Cu analogues (Figure 1B) incorporated into a matrix of 1,2-dimyristoyl-L- $\alpha$ -phosphatidylcholine (PC), which is a major constituent of mitochondrial mem-



branes. Among the known synthetic porphyrins, those employed in the present study most faithfully mimic the heme/Cu site.<sup>[6]</sup> High dilution of the redox centers by, and relatively

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facile diffusion of the catalysts in, PC films eliminates complications associated with the use of adsorbed catalysts and permits biomimetic reduction of  $O_2$  under more biological conditions of diffusion-limited electron flux to isolated catalytic sites.

Among several matrixes screened only PC was found suitable.<sup>[9,10]</sup> In all the experiments performed the amount of PC at the electrode surface was identical ( $375 \text{ nmol cm}^{-2}$ ), with a catalyst loading between 3 and 0.7 mol %.

The electrochemical behavior of the catalysts in the PC matrix under anaerobic conditions was studied by cyclic voltammetry. Diffusion-limited charging of the catalyst/PC film is evident from the downward curvature of the peak current ( $i_p$ ) versus scan rate ( $v$ ) graph (Figure 2A)<sup>[11]</sup> at  $v > 5 \text{ mV s}^{-1}$ . The constant peak separation ( $\Delta E_p \sim 70 \text{ mV}$ )<sup>[9]</sup> at scan rates faster than  $100 \text{ mV s}^{-1}$  indicates facile heterogeneous electron transfer between the catalyst and the electrode. As expected in thin-layer voltammetry,<sup>[12]</sup> the  $\Delta E_p$  value approaches 0 V at slow scan rates.<sup>[9]</sup> This observation suggests that the redox state of the catalyst in a PC matrix does not significantly affect the film's morphology and/or ionic composition, an effect that accounts for the scan-rate-independent non-zero peak separation of an adsorbed catalyst (Figure 2B).<sup>[13]</sup>

Reduction of  $O_2$  by catalyst/PC films was studied by rotating ring-disk voltammetry<sup>[12]</sup> (RRDV) in pH 7 buffered aqueous electrolytes saturated with air. The catalyst/PC film was deposited on an edge-plane graphite disk, whose potential was scanned. The Pt ring electrode was set at a sufficiently oxidizing potential so that any  $H_2O_2$  released by the catalytic film would be oxidized and generate a ring current (Figure 3). Catalysis starts at about 250 mV, which is comparable to the  $Fe^{III}/Fe^{II}$  potential observed under anaerobic conditions (Figure 2B). Application of a more reducing potential than  $-100 \text{ mV}$  increases the catalytic currents. This result indicates that the overall rate of  $O_2$  reduction in this regime is limited by a redox-based process, which becomes more facile at lower potentials. At  $E_{\text{disk}}$  values less than  $-100 \text{ mV}$  the current reaches a plateau, thus revealing a change of the turnover-determining step (TDS) to a nonredox process. The zero ring current (the lower trace) throughout the potential range demonstrates that the FeCu/PC film does not release detectable amounts of  $H_2O_2$  into the electrolyte (the fraction of the 4e pathway exceeds 99 %).

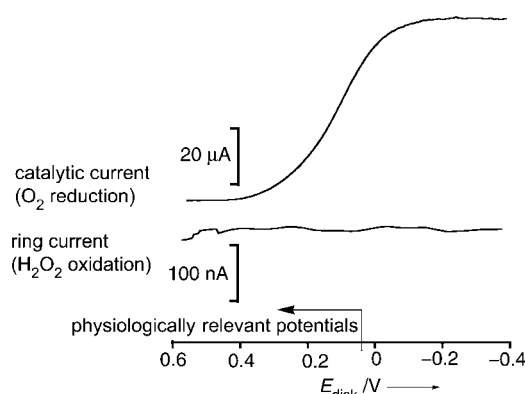


Figure 3. A linear sweep voltammogram of the FeCu catalyst (3 % mol)/PC film in an air-saturated electrolyte at the electrode rotation frequency ( $\omega$ ) of 200 rpm. The ring collection efficiency 15 %.<sup>[9]</sup> Note the different scale for the ring and disk (catalytic) currents. Physiological potentials are determined by the midpoint potentials of the physiological reductants of terminal oxidases, cytochrome c (ca. 200 mV) and ubiquinol (ca. 50 mV).

The catalytic currents  $i_{\text{cat}}$  at physiological potentials are independent of  $O_2$  flux, which was modulated by changing the electrode rotation frequency  $\omega$  (Figure 4) or the bulk  $O_2$

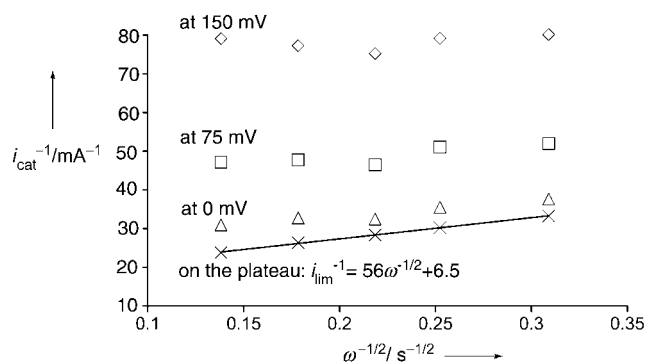


Figure 4. Dependence of the catalytic currents  $i_{\text{cat}}$  on  $O_2$  flux (controlled by the electrode rotation frequency  $\omega$ ) as a function of the applied potential.<sup>[9]</sup> The solid line is a linear least-squares fit to the equation shown.

concentration. Such independence is a commonly used criterion for a charge-transfer-limited catalytic rate.<sup>[14]</sup> Consistent with this assertion is the fact that the  $i_{\text{cat}}$  values in this regime ( $\leq 100 \mu\text{A cm}^{-2}$ ) are comparable to the independently measured rate of charge propagation through the film under a similar potential gradient (for example,  $70 \mu\text{A cm}^{-2}$  at 150 mV).<sup>[9]</sup> Hence, at potentials above about 75 mV the reduction of  $O_2$  by our biomimetic assembly proceeds under diffusion-limited electron flux.

The linear dependence of the inverse limiting (plateau) currents  $i_{\text{lim}}^{-1}$  on  $\omega^{-1/2}$  indicates that TOF is no longer charge-transfer limited at potentials below  $-100 \text{ mV}$ . As a result, the slope of the  $i_{\text{lim}}^{-1}$  versus  $\omega^{-1/2}$  graph provides a means of assessing the redox stoichiometry of the catalysis. This slope is similar to that measured for  $O_2$  reduction on Pt, and is half the slope for  $O_2$  reduction on graphite.<sup>[9]</sup> This observation confirms the RRDV measurements (Figure 3) that show the catalyst/PC film does not release significant amounts of partially reduced oxygen species. Since the maximum TOF of the catalyst/PC system (the inverse intercept of the  $i_{\text{lim}}^{-1}$

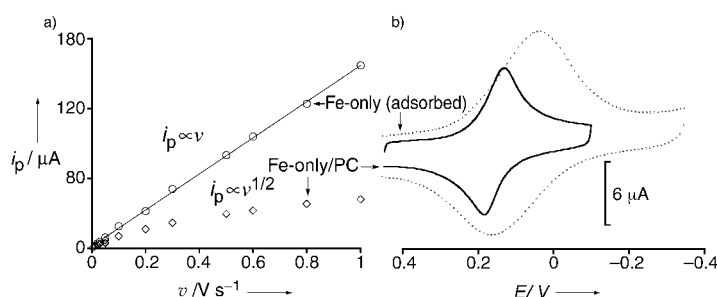


Figure 2. The electrochemical behavior under  $N_2$  of the Fe-only catalyst in a PC film (0.7 % mol) and the same amount of the catalyst adsorbed on graphite ( $2.5 \text{ nmol cm}^{-2}$ ): A) peak current  $i_p$  versus scan rate  $v$ ; B) cyclic voltammograms ( $v = 50 \text{ mV s}^{-1}$ ; all potentials are versus the normal hydrogen electrode, NHE).

versus  $\omega^{-1/2}$  graph, ca.  $0.7 \text{ mA cm}^{-2}$ ) is comparable to the rate of substrate transfer within the PC film (ca.  $0.8 \text{ mA cm}^{-2}$ ),<sup>[9]</sup> the latter process is the plausible TDS at the plateau.<sup>[14]</sup> In contrast, the TOF of an electrode-adsorbed catalyst is limited by the rate of the catalytic cycle, not charge or substrate transfer, at all potentials.<sup>[6b]</sup>

As expected for catalysis under limited electron availability, the PC-dispersed catalyst degrades more than 100 times faster than the electrode-adsorbed analogue during  $\text{O}_2$  reduction at 100 mV. Intra- or intermolecular oxidation of the organic ligand is likely in a PC matrix when other reductants ( $\text{Fe}^{\text{II}}$ ,  $\text{Cu}^{\text{I}}$ , or the electrode) are inaccessible to complete the  $\text{O}_2$  reduction cycle. Indeed, the very low stability of the Cu-free, NMeFe-only catalyst in the PC matrix appears to account for the lack of a well-defined catalytic wave in this system.<sup>[9]</sup> This result contrasts with only minor differences in the catalytic properties of the FeCu and Fe-only complexes adsorbed on the electrode.<sup>[6b]</sup>

In summary we presented a biomimetic system that reproduces a key aspect of terminal oxidases reactivity. The synthetic heme/Cu analogue dispersed in a PC matrix catalyzes  $\text{O}_2$  reduction: 1) at physiological potentials, 2) under diffusion-limited electron flux, and 3) without releasing partially reduced oxygen by-products into the bulk medium. In contrast to electrode-adsorbed systems,<sup>[6b]</sup> notable differences in the catalytic behavior of the FeCu and Cu-free analogues in a PC matrix illustrate the benefit engendered by an additional readily accessible reductant ( $\text{Cu}^{\text{I}}$ ) within the catalyst molecule. This system mimics the proposed role of  $\text{Cu}_\text{B}$  as an electron reservoir at the catalytic site. The use of a PC matrix should allow meaningful studies of the catalytic properties, under steady-state turnover, of heme/Cu analogues containing a phenol moiety. In cytochrome oxidase, a tyrosine residue (Tyr244 in Figure 1 A) is involved in catalysis when the availability of electrons is limited<sup>[15]</sup> (for example,  $\text{O}_2$  reduction by a mixed-valence enzyme). Whereas such conditions are inaccessible for an electrode-adsorbed functional analogue, they are realized for a PC-dispersed complex.

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## Taking Nanocasting One Step Further: Replicating CMK-3 as a Silica Material



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*Dedicated to Professor Dieter Fenske  
on the occasion of his 60th birthday*

The replication of nanoscale structures by a direct templating process has been used in recent years in several creative ways for the synthesis of carbon replicas of zeolites<sup>[1]</sup> or ordered mesoporous carbons, such as CMK-1<sup>[2]</sup> or SNU-1.<sup>[3]</sup> Such processes rely on the fact that an ordered pore system, provided by the zeolite or ordered mesoporous silica, can be filled with a carbon precursor which is pyrolyzed and the silica leached with NaOH or HF solution. However, the technique is difficult to apply to the synthesis of framework compositions other than carbon, since the leaching of the silica typically also affects the material which is filled into the silica pore system. This problem could possibly be circumvented by not using the silica as the mold, but to instead go one step further and use the mesoporous ordered carbons as templates, which could then easily be removed by combustion or other techniques, as suggested recently.<sup>[4]</sup> On the macroscale, that is, for the production of photonic crystals, similar approaches are well known, where latex spheres are used as templates which can be removed by calcinations.<sup>[5]</sup> Also carbon black has been used as a “template”, for instance to synthesize mesoporous zeolite single crystals, in which the pores, however, are disordered.<sup>[6]</sup>

In a first attempt to show the feasibility of using ordered mesoporous carbon to synthesize ordered mesoporous oxides, we decided to template mesostructured silica by using an ordered mesoporous carbon. Although this brings one only back to the starting point, that is, a mesoporous silica, it

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