RSC Advances

Electronic Supplementary Information for

Probing Structural and Catalytic Characteristics of Galactose Oxidase Confined in Nanoscale Chemical Environments

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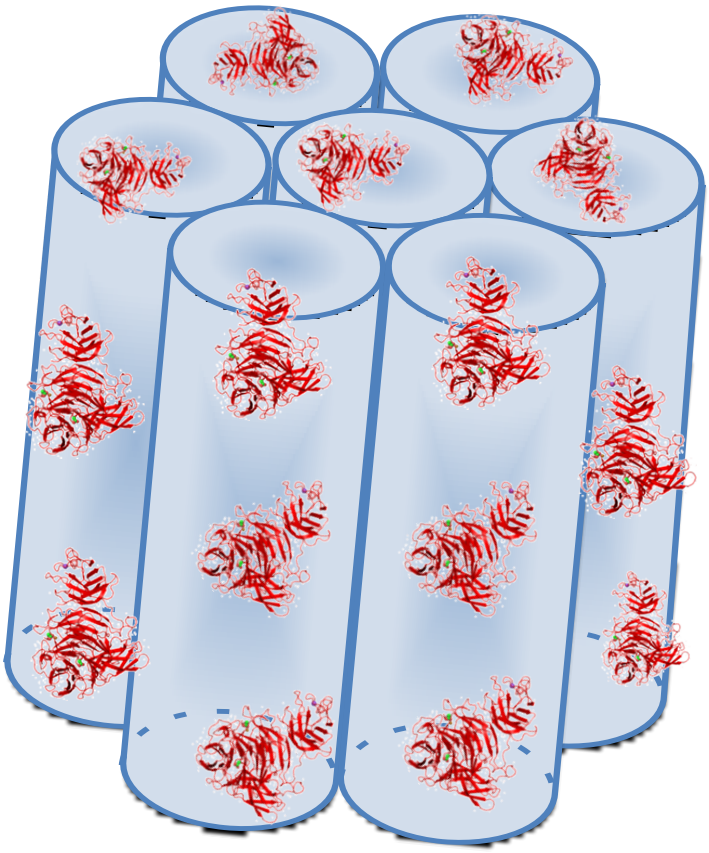

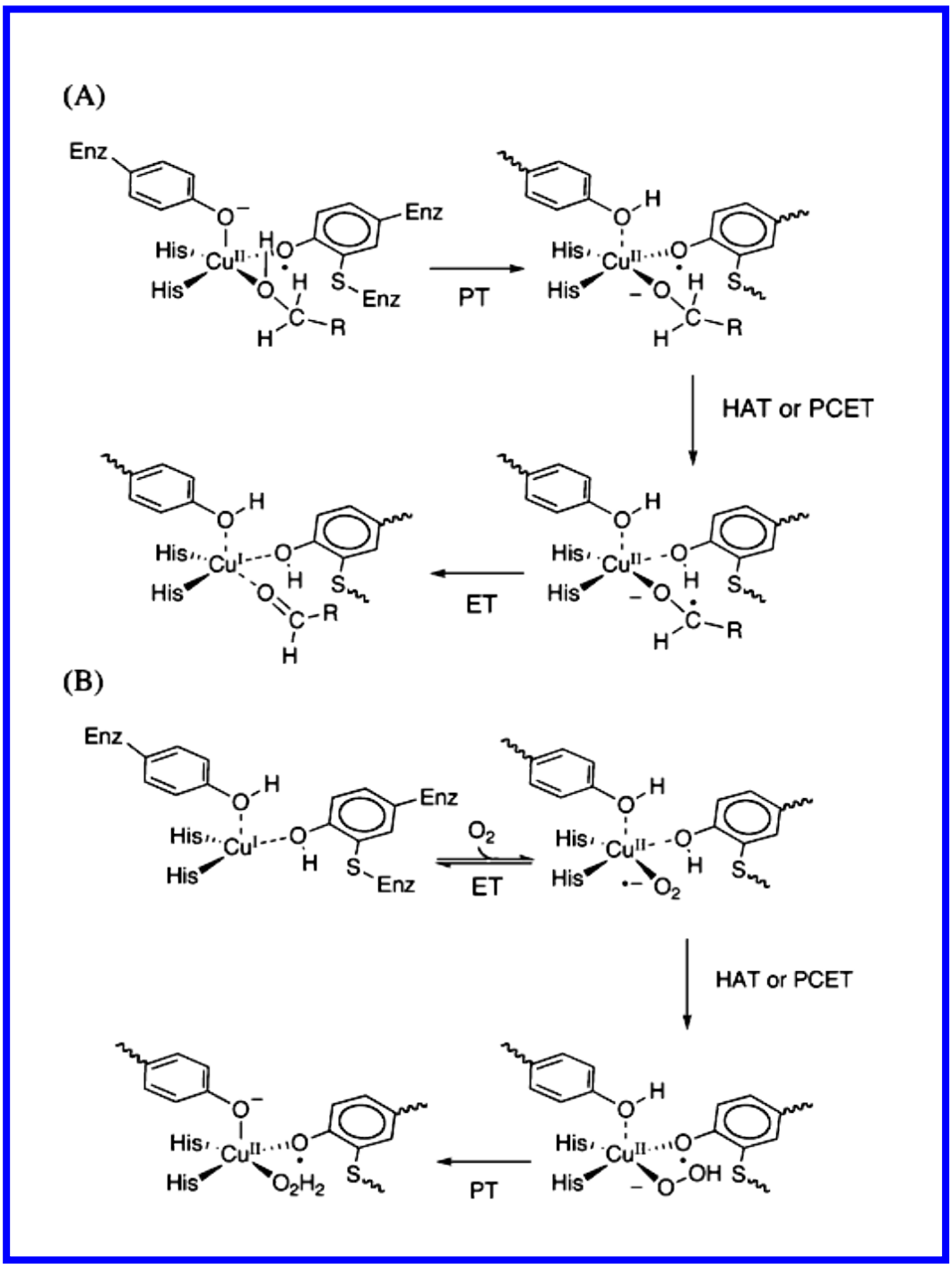

FIGURE S1. The catalytic mechanisms of galactose oxidase. (A) The reductive half-reaction involves three steps: i) PT: Proton transfer from substrate, RCH_2OH, to an active site base, Tyr495; ii) PCET: to the radical center,; and iii) ET: Electron transfer to Cu(II). (B) The oxidative half reaction also involves three steps: i) electron transfer from Cu(I) to coordinated oxygen, giving metal bound superoxide; ii) Hydrogen atom transfer (HAT) or PCET: The metal bound superoxide abstracts a hydrogen atom from the hydroxyl group of the Tyr272, producing hydroperoxide and Tyr•272; and iii) A proton is transferred from Tyr495 to the metal bound hydroperoxide, producing hydrogen peroxide and reoxidized enzyme. For the detals see: Humphreys et al. *J. Am. Chem. Soc.* **2009**, *131*, 4657-4663.

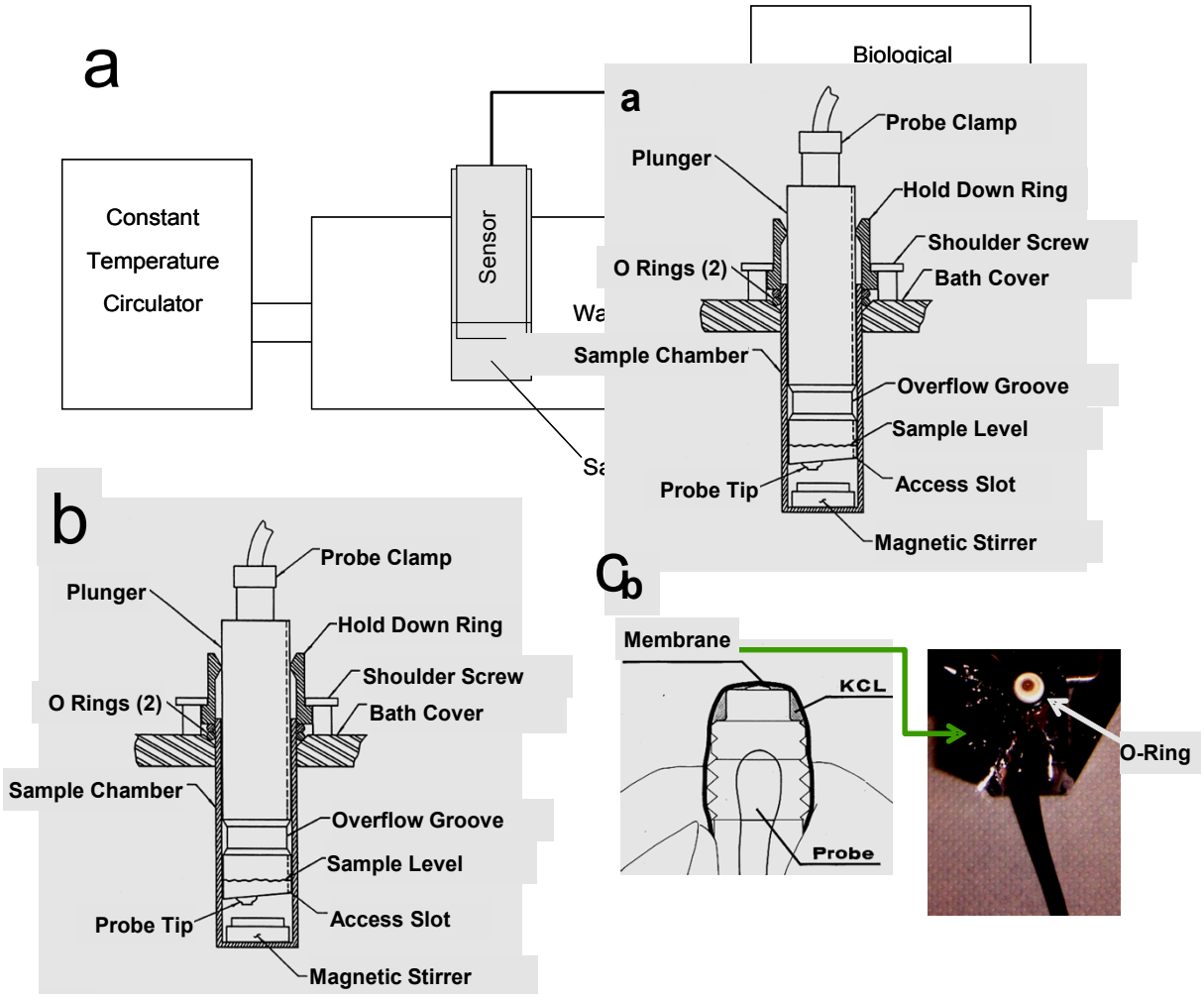

FIGURE S2. (a) Schematic diagram of dioxygen monitoring system. The dioxygen probe (sensor) is inserted into the chamber containing a sample. The chamber is maintained at a constant temperature by the water bath, which is connected to the constant temperature circulator. The monitor displays %AIR based on 100 % AIR corresponding to the concentration of dissolved dioxygen in air-saturated distilled water. (b) and (c) Schematic diagram of the sample chamber assembly (b) and the dioxygen probe covered with a Teflon membrane (c).

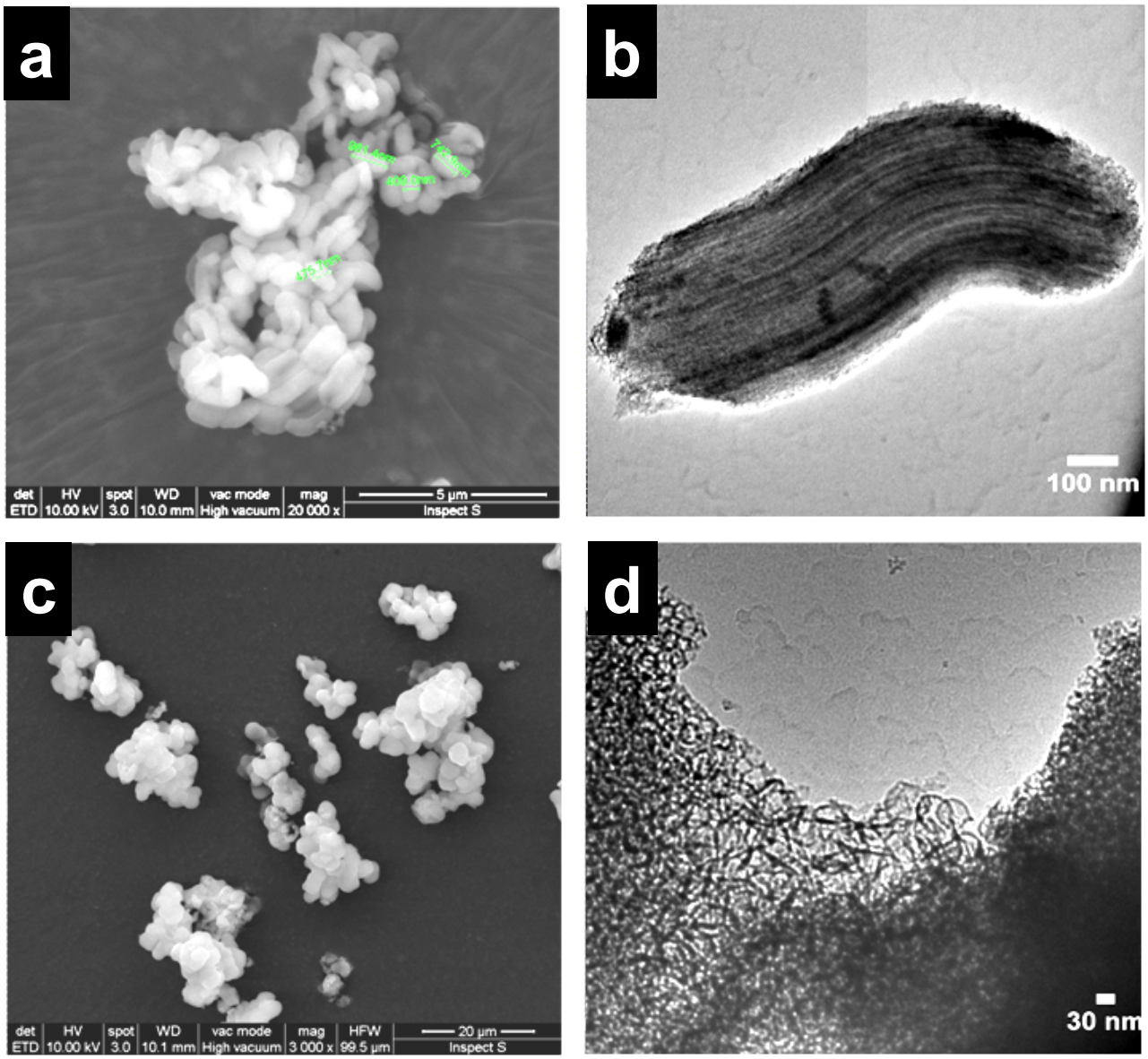

FIGURE S3. SEM and TEM micrographs of SBA-15-ROD (a, b) and MCF (c, d). The SEM image (a) reveals that the SBA-15 particles are rod-shaped, and the TEM image (b) shows that the ordered channels are running in parallel. MCF does not have a well-defined morphology as seen from the SEM image (c), but the TEM image (d) shows that MCF has a disordered foam-like structure with large pores. Scale bars: (a) 5 µm, (b) 100 nm, (c) 20 µm, and (d) 30 nm.

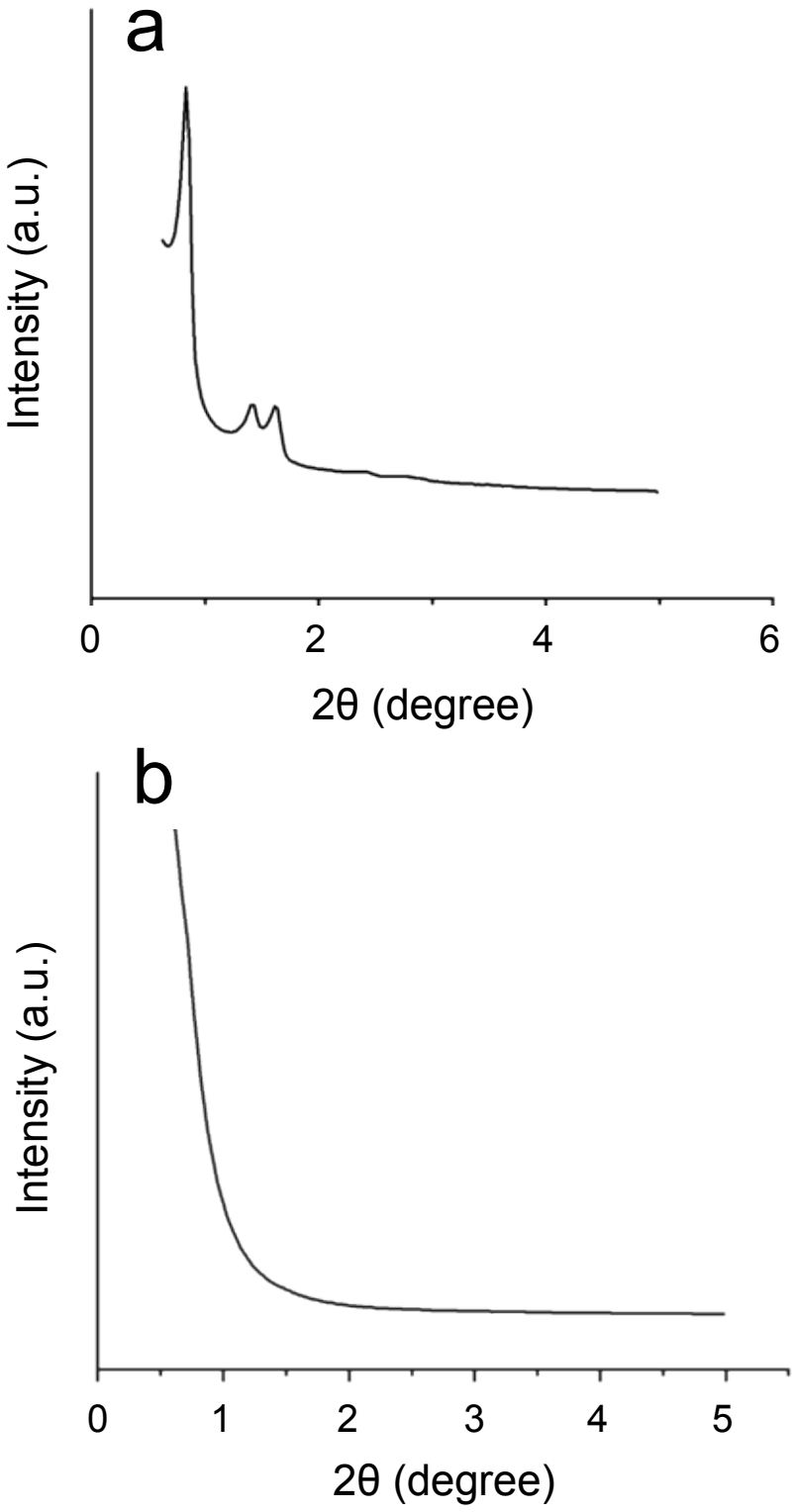

FIGURE S4. Powder XRD patterns of the MPS samples used for GAOX immobilization. (a) SBA-15-ROD with a hexagonally ordered pore structure. The three peaks at 2θ = 0.84, 1.41, and 1.62° are indexed as (100), (110), and (200) reflections associated with p6mm hexagonal symmetry. (b) MCF has disordered pore structures, and therefore there is no defined diffraction peak observed.

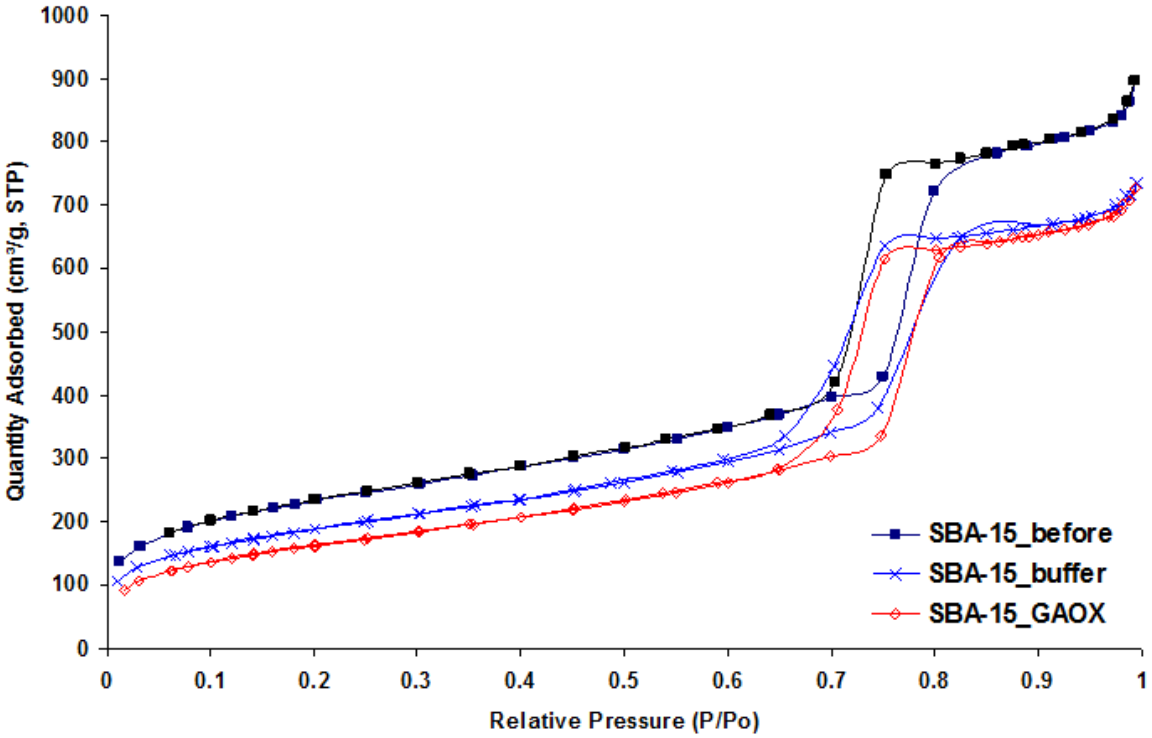

FIGURE S5. Nitrogen adsorption-desoprtion isotherms of the SBA-15-ROD samples before and after GAOX immobilization. SBA-15_before (black): SBA-15 before the immobilization (calcined SBA-15), SBA-15_buffer (blue): sample after stirring SBA-15 alone in a buffer solution. SBA-15_GAOX (red): the conjugate of SBA-15 and GAOX. As required, the samples of SBA-15_buffer and SBA-15_GAOX were degassed at 40 °C under vacuum for 12 hours before recording the isotherms.

TABLE S1. Comparison of Surface Area and Total Pore Volume of SBA-15 Samples before and after Stirring in a Buffer Solution or Immobilization with GAOX.

Sample	S_{BET} (m^2/g)	V_{tot} (cm^3/g)
SBA-15	840	1.26
SBA-15-buffer	682	1.05
SBA-15-GAOX	592	1.03

S_{BET}: Total surface area, V_{tot}: Total pore volume

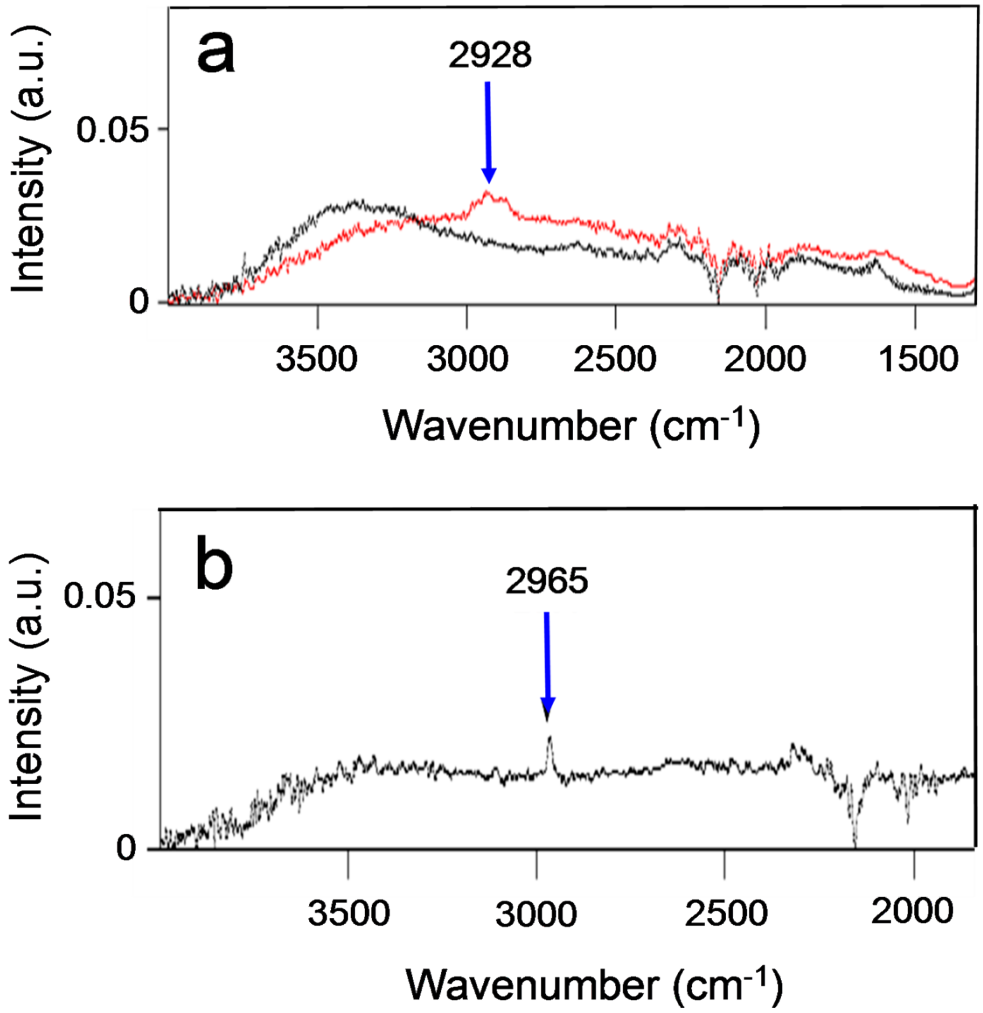

FIGURE S6. FT-IR spectra of MCF before (black line) and after (red line) functionalization with amine (a) and methyl (b) groups. The IR signals are relatively weak, but the chemical functionalization is characterized by the new peaks at 2928 and 2965 cm^{-1} respectively, rising from the C-H stretching mode appears at.

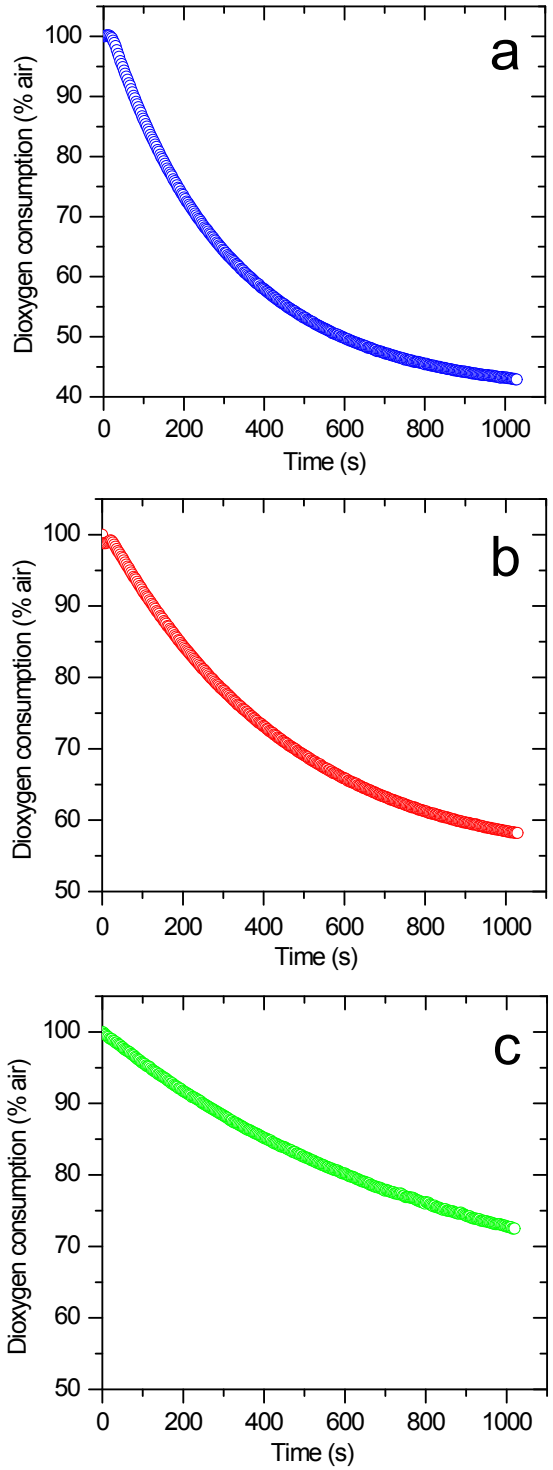

FIGURE S7. Kinetic responses of enzyme activity to substrate (galactose) monitored by dioxygen consumption: (a) free GAOX, (b) SBA-15-ROD-GAOX (enzyme physically confined) and (c) MCF-GAOX (enzyme covalently attached). Free or immobilized enzyme was mixed with phosphate buffer solutions (100 mM, pH 7.0) containing galactose (200 mM) and $K_3[Fe(CN)_6]$ (1 mM). The 100 % AIR corresponds to the concentration of dissolved dioxygen in air-saturated water.