

**A hydrogen/oxygen hybrid biofuel cell comprising an electrocatalytically active
nanoflowers/laccase-based biocathode**

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Characterizations

FE-SEM samples were prepared by depositing 2 μl of each laccase-metal hybrid suspension onto the silicon wafer, followed by sputter-coating with platinum. For TEM analysis, a suspension droplet was deposited onto the copper grid and dried at room temperature. TEM images were taken with JEOL JEM 2100F transmission electron microscope at 200 kV. For X-ray diffraction (XRD) analysis, 50 mg of laccase was dissolved in 100 ml of 0.01 M PBS (pH 7.4) prior to the addition of 0.667 ml of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), manganese acetate tetrahydrate ($\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$), and zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) aqueous solutions. Each precipitate was collected onto the membrane filter (0.2 μm), followed by washing with deionized water and drying in the oven at 70 $^\circ\text{C}$. Crystalline structures of dried powder samples were analyzed with Bruker D2 Phaser XRD instrument with Cu-K α radiation ($\lambda = 0.15405 \text{ nm}$). Elemental composition of hybrid suspension samples was analyzed with X-ray photoelectron spectroscopy (XPS) with a Thermo Scientific ESCALAB-250Xi spectrometer with monochromatic Al-K α radiation.

Synthesis of laccase-metal hybrid suspensions

All laccase-metal hybrid suspensions were prepared as described by Zare and coworkers [1]. 20 μl of metal aqueous solution was added to 3 ml of 0.01 M phosphate buffered saline (PBS) containing different amount of laccase (0.5 mg ml^{-1} , 1.0 mg ml^{-1} , and 2.5 mg ml^{-1}) and incubated at room temperature for 3 days. After 3 days, light blue precipitates were formed for all laccase-

Cu samples whereas white-colored suspensions were observed for laccase-Mn and laccase-Zn hybrid suspensions.

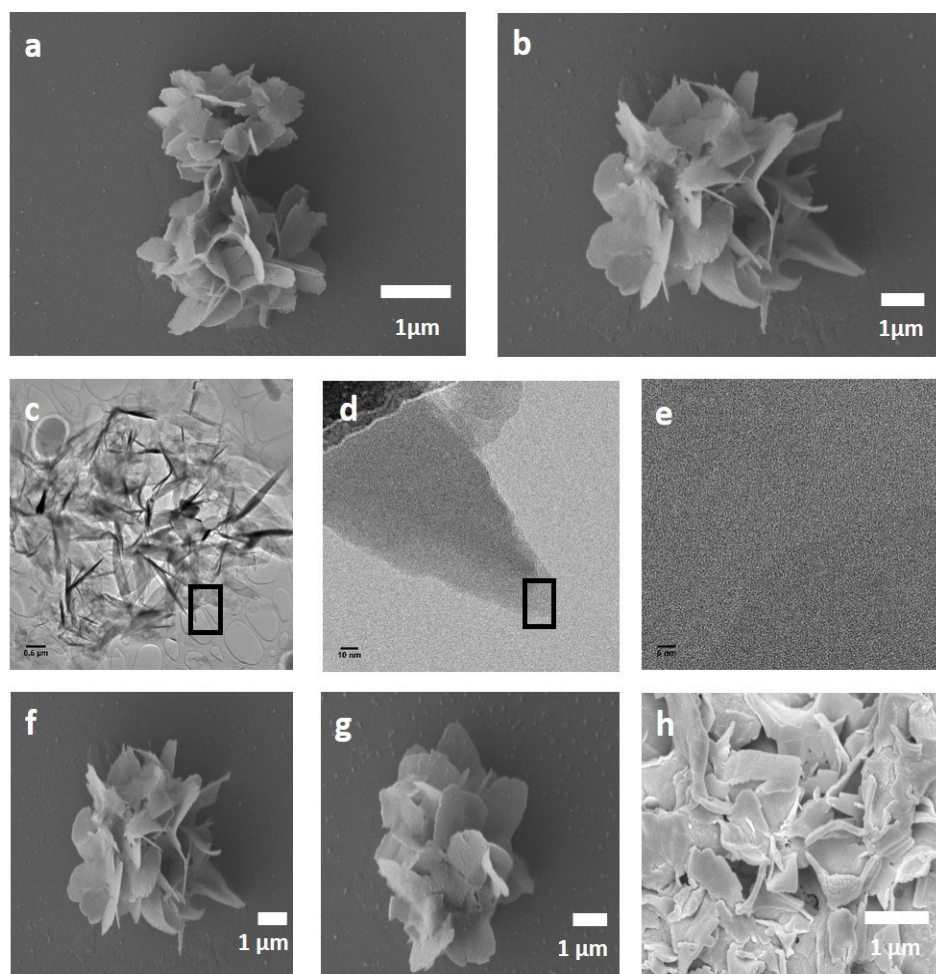


Fig. S1. Laccase-Mn hybrid suspensions. **a**, FE-SEM image of 2.5 mg ml⁻¹ laccase-Mn hybrid suspensions. **b**, FE-SEM image of suspensions. **c-e**, TEM images of laccase-Mn hybrid nanoflowers. **f-h**, Laccase-Mn suspensions in different concentrations of laccase. 0.5 mg ml⁻¹ (**f**), 1.0 mg ml⁻¹ (**g**), 2.5 mg ml⁻¹ (**h**).

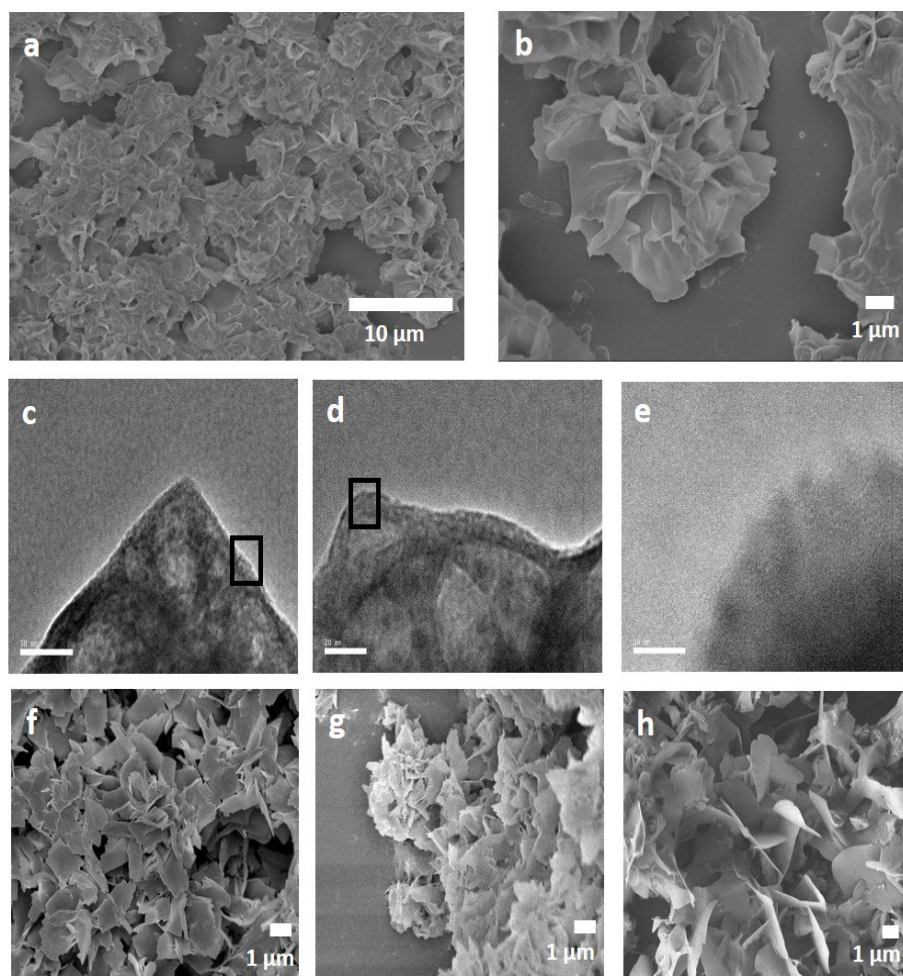


Fig. S2. Laccase-Zn hybrid suspensions. **a**, FE-SEM image of 2.5 mg ml⁻¹ laccase-Zn hybrid suspensions. **b**, FE-SEM image of suspensions. **c-e**, TEM images of laccase-Zn hybrid nanosuspensions. **f-h**, Laccase-Zn suspensions in different concentrations of laccase. 0.5 mg ml⁻¹ (**f**), 1.0 mg ml⁻¹ (**g**), 2.5 mg ml⁻¹ (**h**).

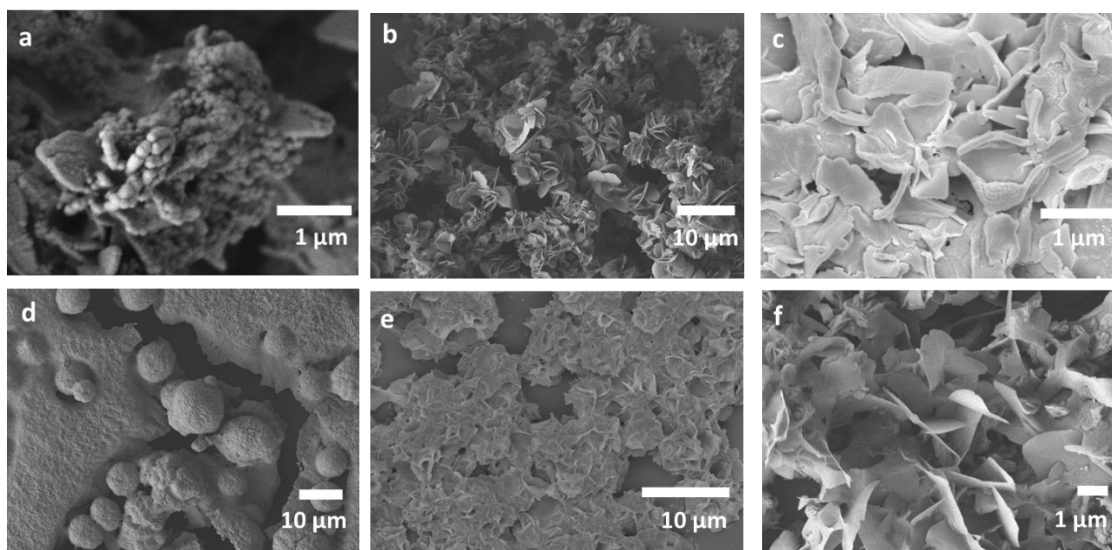


Fig. S3. Morphology of 2.5 mg ml^{-1} laccase-Mn and laccase-Zn hybrid suspensions as a function of time. **a-c**, 2.5 mg ml^{-1} laccase-Mn hybrid suspensions at 1 h (**a**), 5 h (**b**), and 76 h (**c**). **d-f**, 2.5 mg ml^{-1} laccase-Zn hybrid suspensions at 1 h (**d**), 5 h (**e**), and 76 h (**f**).

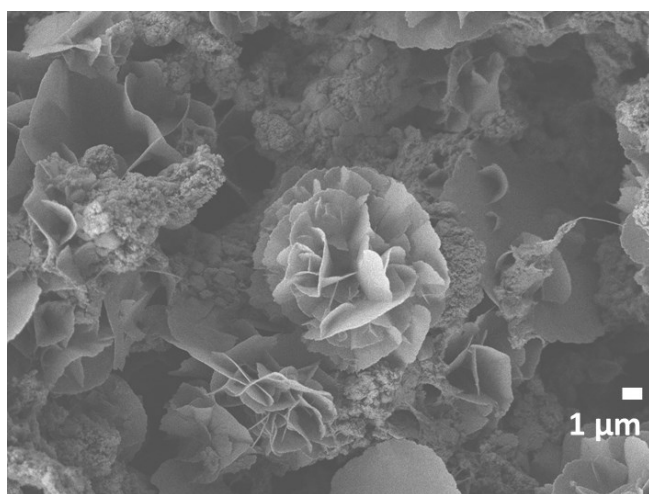


Fig. S4. FE-SEM image of laccase-Cu hybrid suspensions combined with carbon nanotubes (CNTs).

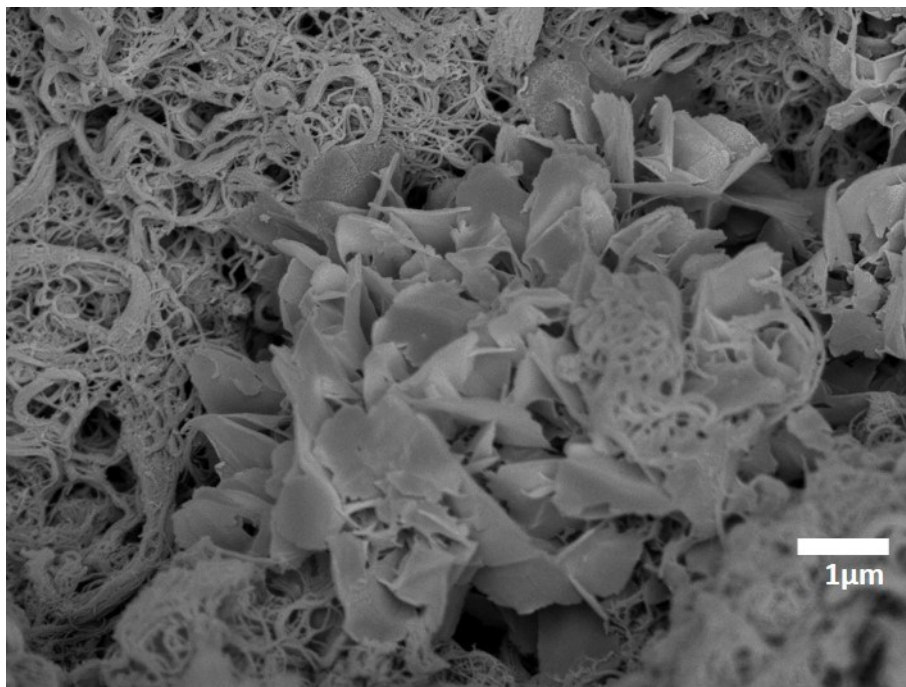


Fig. S5. FE-SEM image of laccase-Mn hybrid suspensions combined with carbon nanotubes (CNTs).

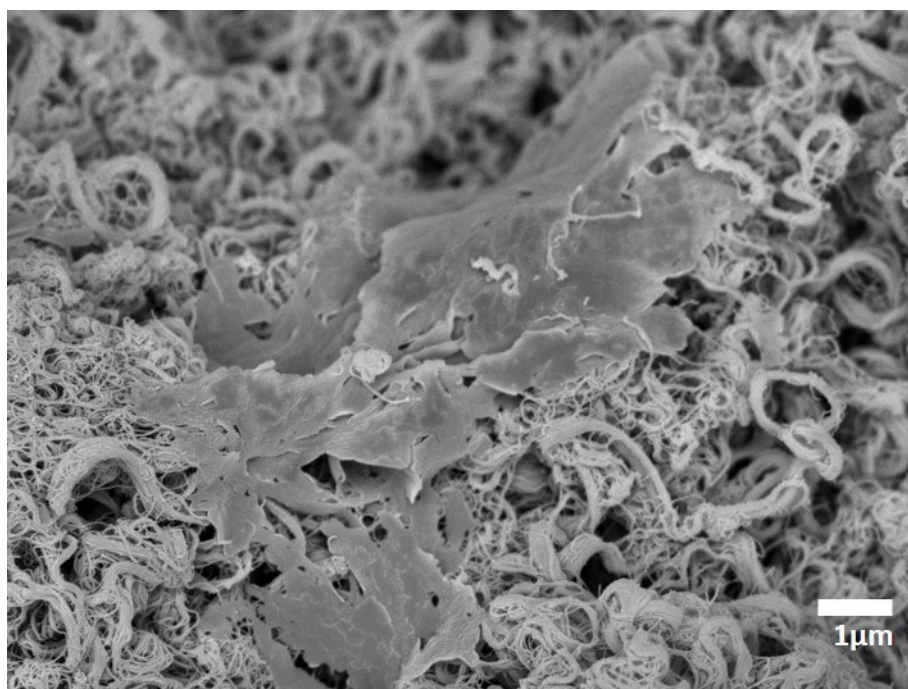


Fig. S6. FE-SEM image of laccase-Zn hybrid suspensions combined with carbon nanotubes (CNTs).

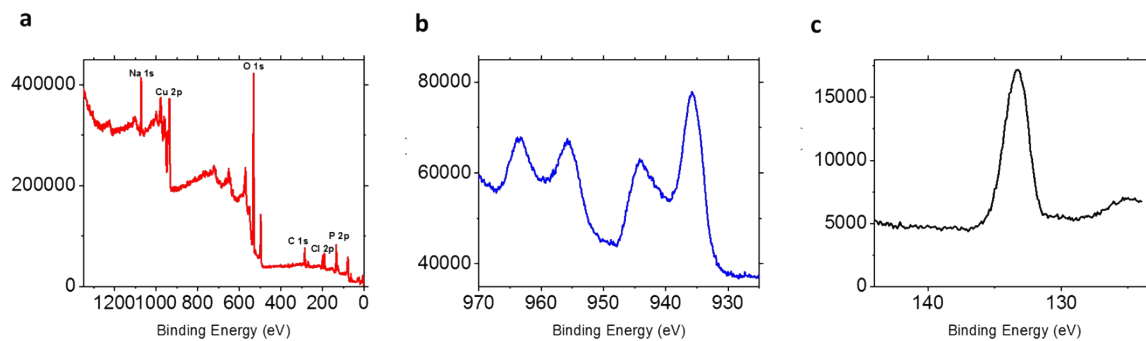


Fig. S7. XPS spectrum of Laccase-Cu nanoflowers with survey scan (a), Cu 2p (b), and P 2p peaks (c).

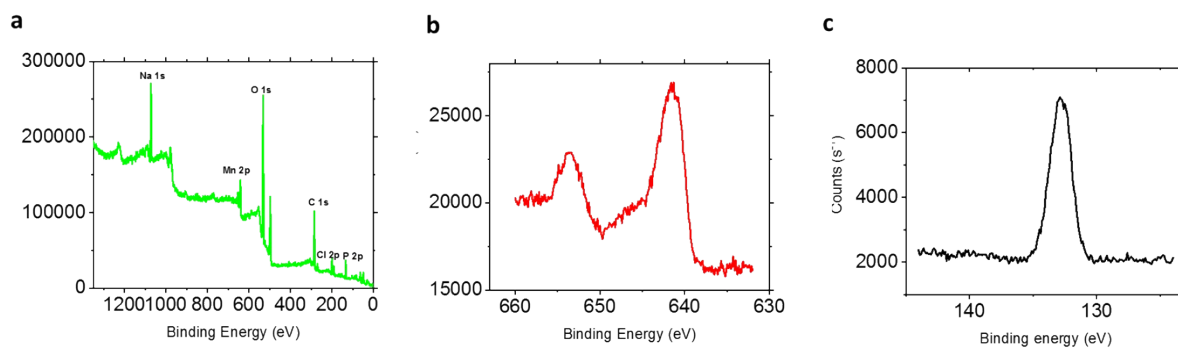


Fig. S8. XPS spectrum of Laccase-Mn suspensions with survey scan (a), Mn 2p (b), and P 2p peaks (c).

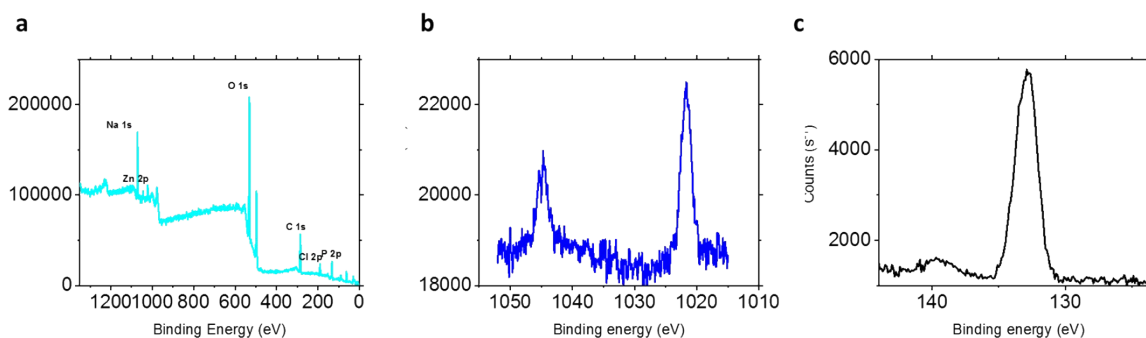


Fig. S9. XPS spectrum of Laccase-Zn suspensions with survey scan (a), Zn 2p (b), and P 2p peaks (c).

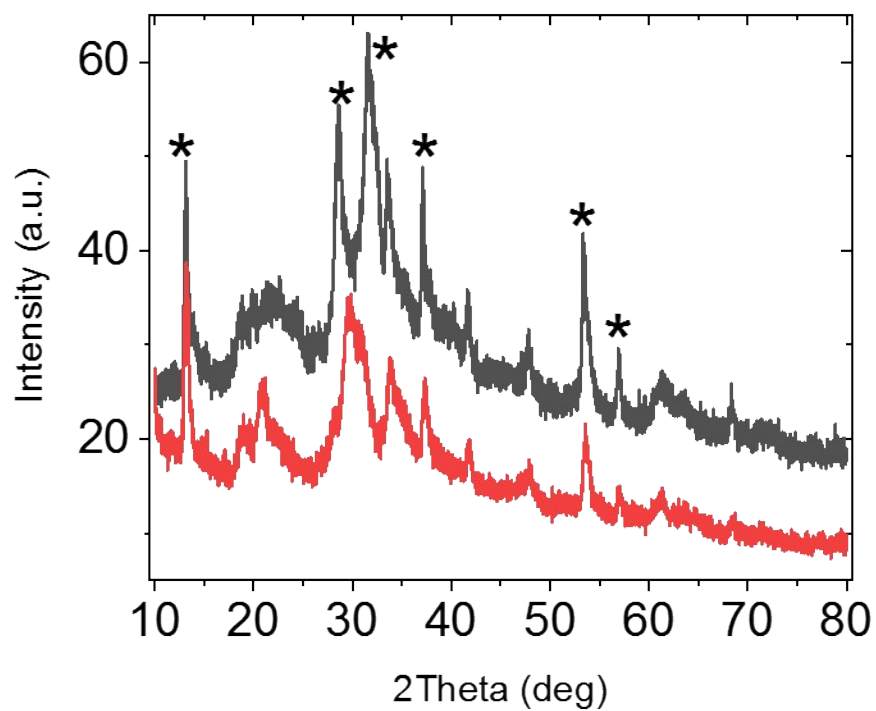


Fig. S10. XRD spectra of 2.5 mg ml⁻¹ Laccase-Cu nanoflowers (black) and PBS-Cu mixture (red). Asterisk corresponds to the Cu₃(PO₄)₂ peaks.

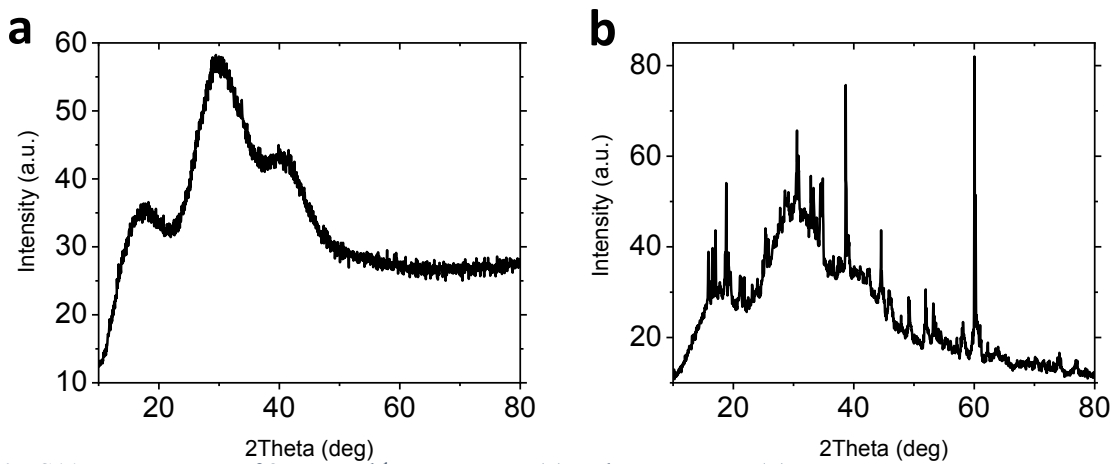


Fig. S11. XRD spectra of 2.5 mg ml⁻¹ Laccase-Mn (a) and Laccase-Mn (b).

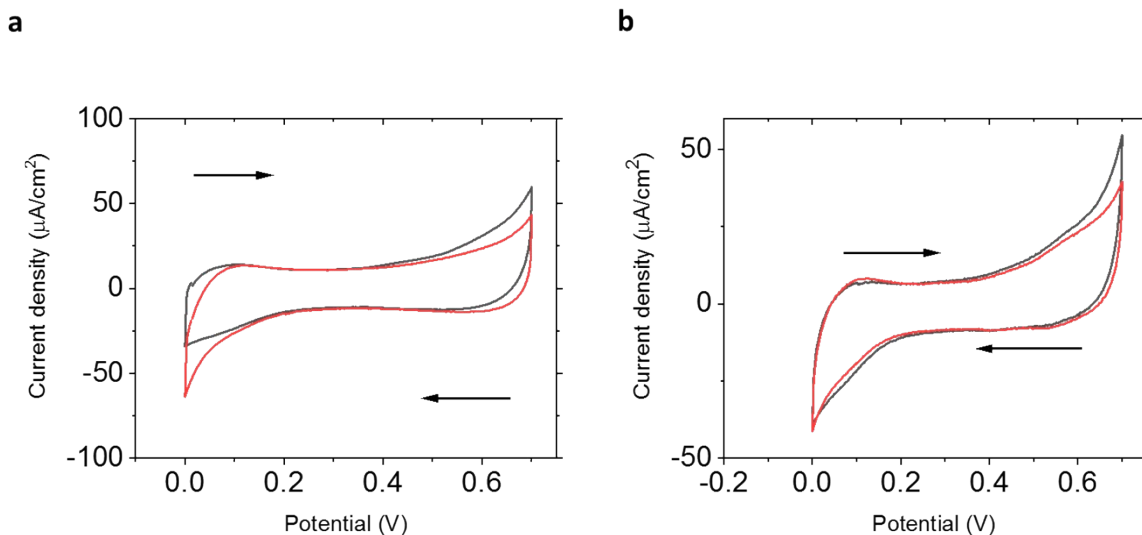


Fig. S12. Cyclic voltammetry measurement of MWCNT/2.5 mg ml⁻¹ Laccase-Mn (scan rate: 2 mV s⁻¹) modified GC in Ar (black) and O₂ (red) atmosphere (a), and MWCNT/1.0 mg ml⁻¹ Laccase-Zn (scan rate: 5 mV s⁻¹) in Ar (black) and O₂ (red) atmosphere (b).

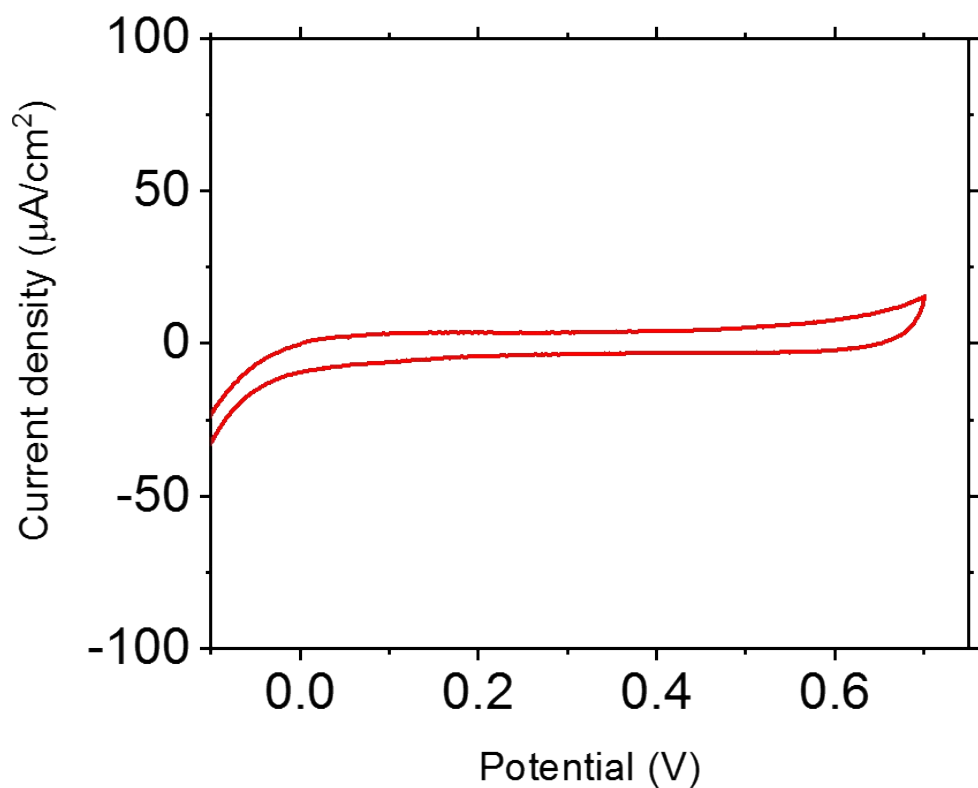


Fig. S13. Cyclic voltammetry (CV) of MWCNT/2.5 mg ml⁻¹ free laccase modified GC electrode in O₂ environment (scan rate: 5 mV s⁻¹).

References

- [1] J. Ge, J. Lei, R. N. Zare, Protein-inorganic hybrid nanoflowers, *Nat. Nanotechnol.*, 2012, **7**, 428-432