Supporting Information

Highly active, stable and self-antimicrobial enzyme catalysts prepared by biomimetic mineralization of copper hydroxysulfate

Zhixian Li, Yi Ding, Shaomin Li, Yanbin Jiang, Zheng Liu, Jun Ge**

Materials

Chemicals including H_3BO_3, $Na_2B_4O_7$ and $CuSO_4$ were purchased from Alfa Aesar. Albumin from bovine serum (BSA), glucose oxidase (GOx), horseradish peroxidase (HRP), *Candida antarctica* lipase B (CALB), cytochrome c (Cyt c), 2,2"-azino-di-3-ethylbenzothiazoline sulfonic acid-6-diammonium salt (ABTS), 4-nitrophenyl butyrate, hydrogen peroxide, fluorescein isothiocyanate (FITC) were purchased from Sigma Aldrich.

Activity Assay

For the Cyt c activity assay, 20 μL of 200 μg/mL Cyt c or Cyt c@CHNs solution and 50 μL of 0.3% H_2O_2 were added into 930 μL of borate buffer solution (pH 7.4, 50 mM) containing 0.5 mM ABTS. The increase in absorbance at 405 nm was measured for 1 min. The enzyme activity was calculated from the slope of absorbance versus time curve.

For the HRP activity assay, 20 μL of 10 μg/mL HRP or HRP@CHNs solution and 50 μL of 0.3% H_2O_2 were added into 930 μL of borate buffer solution (pH 7.4, 50 mM) containing 0.5 mM ABTS. The increase in absorbance at 405 nm was measured for 1 min. The enzyme activity was calculated from the slope of absorbance versus time curve.

For the GOx activity assay, 20 μL of 20 μg/mL GOx solution or GOx@CHNs solution and 100 μL of 100 μ g/mL HRP solution were added to 880 μL of the substrate borate buffer solution (pH 7.4, 50 mM), containing 100 mM glucose and 0.5 mM ABTS) to initiate the reaction. The enzyme activity was calculated from the slope of absorbance versus time curve.

For the CALB activity assay, 3 μL of pNPB was first dissolved in 1 mL acetone and then diluted with 20 mL borate buffer solution (pH 7.4, 50 mM) containing 0.125% (v/v) Triton X-100.The increase in absorbance was detected at 348 nm after adding 20 μL of the enzyme solution (200 μg/mL) or CALB@CHNs solution to 980 μL of the substrate solution.

The activity assays were carried on the SHIMADZU UV-2450 spectrophotometer at room temperature.

Lineweaver–Burk plot

The Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.[1] K_m *and* V_{max} were determined by Lineweaver–Burk plot, which procedure was described elsewhere for each enzyme.[2] k_{cat} was calculated from V_{max}/[enzyme].

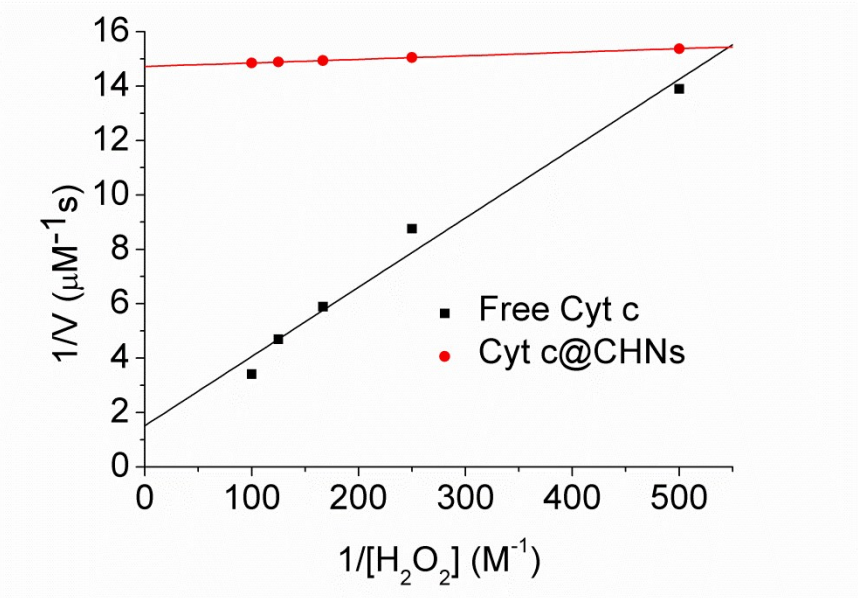

Figure S1 The Lineweaver–Burke plots of Cyt c and Cyt c@CHNs.

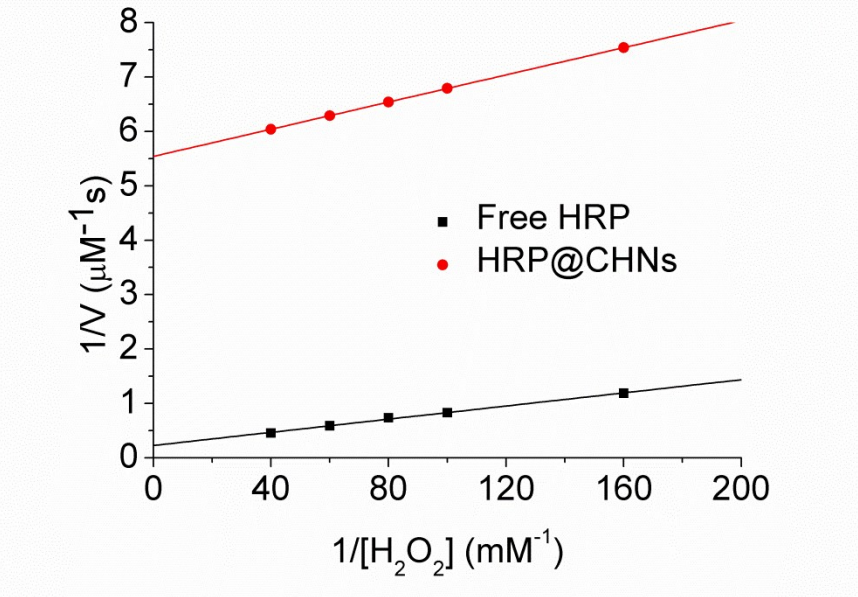

Figure S2 The Lineweaver–Burke plots of HRP and HRP@CHNs.

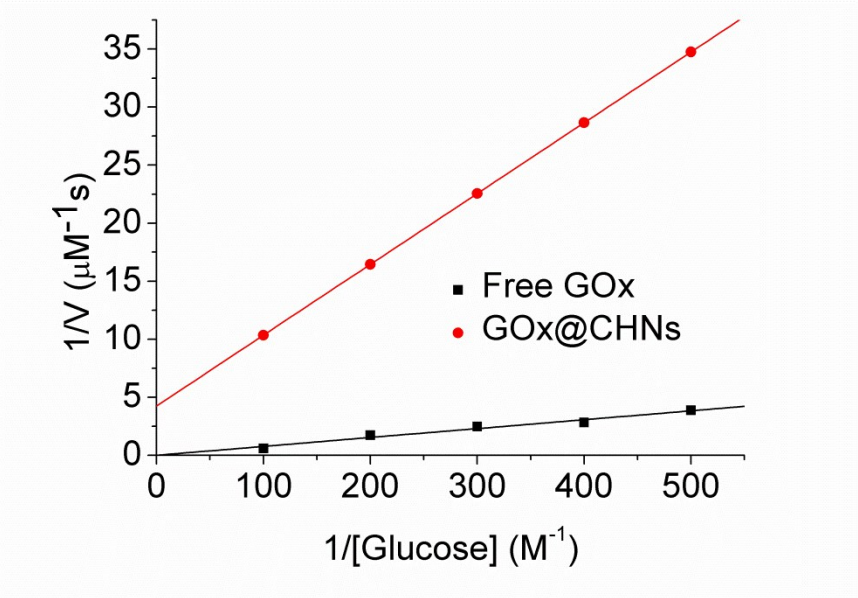

Figure S3 The Lineweaver–Burke plots of GOx c and GOx@CHNs.

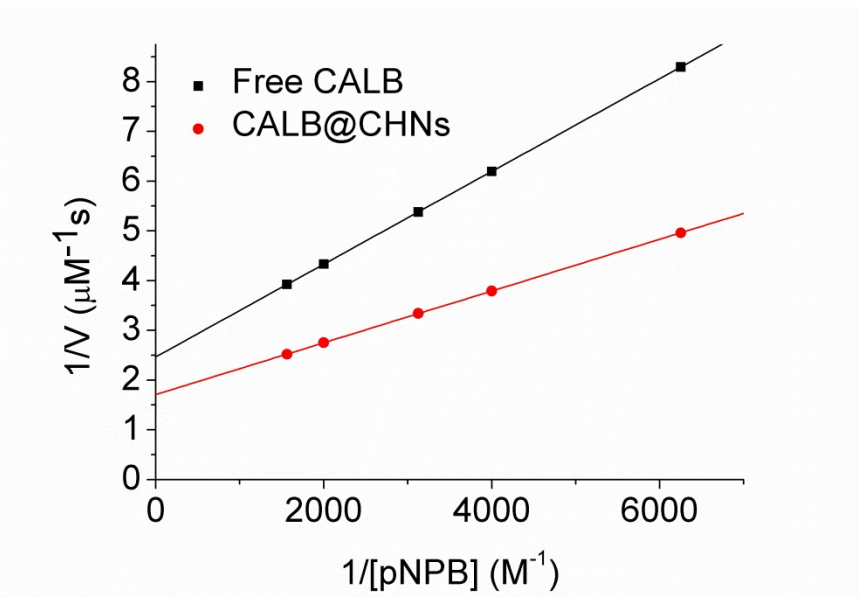

Figure S4 The Lineweaver–Burke plots of CALB and CALB@CHNs.

Antibacterial Test

The tests were performed using *E. coli* according to the revised wet plating protocol.[3] Briefly, 50 μL 20 mg/mL silica, HRP@CHNs, CHNs, CuO or Cu nanoparticle powder suspended PBS Buffer (10 mM, pH 7.4) solution were mixed with 50 μL *E. coli* (cell density was $2*10^8$–$8*10^8$ cfu/mL) suspended PBS Buffer (10 mM, pH 7.4). After the incubation for in a water-saturated atmosphere for a specific time, 20 μL samples were withdrawn and the dilutions in PBS were spread on agar plates containing N-media. Following growth for 2 h, survival was calculated from the cfu.

References

[1] H. Lineweaver, D. Burk, *J. Am. Chem. Soc.* **1934**, *56*, 658-666.

[2] a) K. Blank, J. Morfill, H. Gumpp, H. E. Gaub, *J. Biotechnol* **2006**, *125*, 474-483; b) F. J. Lyu, Y. F. Zhang, R. N. Zare, J. Ge, Z. Liu, *Nano Lett.* **2014**, *14*, 5761-5765; c) Y. F. Zhang, Y. Yong, J. Ge, Z. Liu, *Acs Catal.* **2016**, *6*, 3789-3795.

[3] C. Molteni, H. K. Abicht, M. Solioz, *Appl. Environ. Microb.* **2010**, *76*, 4099-4101.