Electronic Supplementary Information

Ionic Strength-Modulated Catalytic Efficiency of a Multienzyme Cascade Nanoconfined on Charged Hierarchical Scaffolds

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Materials and General Methods

All chemicals and solvents were purchased from Sigma-Aldrich or Acros Organics, and used without further purification unless otherwise noted. Glucose oxidase (GOx from *Aspergillus niger*, 160 kDa, pI = 4.2) from Sigma Co. was used as received. Horseradish peroxidase (HRP, activity >200 units/mg, 44.0 kDa) and *o*-dianisidine were purchased from Aladdin Inc. (Shanghai, China). *β*-D-Glucose from Beijin J&K Scientific Inc. (China) was used without further purification. All aqueous solutions were prepared with deionized distilled water obtained from a Milli-Q water-purifying system (18 MΩ cm). UV-vis absorption spectra of sample solutions were collected by a UV-3150 spectrophotometer (Shimadzu Corporation, Japan). The fluorescence spectra were obtained from RF-5301PC fluorescence spectrophotometer. The zeta potential was measured using a Zeta-Plus instrument (Brookhaven Instruments Corporation, USA) at room temperature. The data represent the averages ± standard deviations. All tests were performed in triplicate.

Synthesis of DPE-PPE$^+$

DPE-PPE$^+$ was synthesized following our previously reported procedure.[S1] In brief, the synthesis procedure was described as follows: (1) Synthesis of dendritic polyethylene macromonomers terminated by iodine group (DPEI) by chain walking polymerization (CWP); (2) Based on DPEI and the 1,4-Bis[3-(*N,N*-diethylamino)-1-oxapropyl]-2-iodo-5-[(trimethylsilyl)ethynyl] benzene, synthesis of dendritic polyethylene-cationic poly(*p*-phenylene ethynylene) (DPE-PPE) by palladium/copper-catalyzed Sonogashira cross-coupling; (3) Synthesis of its cationic polymer DPE-PPE$^+$ through quaternization process of DPE-PPE.

Determination of the Maximum Amount of GOx Adsorbed in the DPE-PPE$^+$

The maximum amount of enzyme GOx adsorbed in the DPE-PPE$^+$ was determined by the change

of intrinsic GOx tryptophan (Trp) fluorescence, and Trp fluorescence emission was excited at 280 nm and recorded in the range 300−420 nm. GOx solution was added to the DPE-PPE$^+$ systems and the reaction solution was incubated for 45min at 4 °C. By this step, DPE-PPE$^+$/GOx was achieved through electrostatics interactions.

Enzymatic Activity Assay for DPE-PPE$^+$/GOx/HRP

The activity of GOx was measured spectrophotometrically by the *o*-dianisidine-peroxidase method. For preparing enzyme cascade on DPE-PPE$^+$, the GOx (0.025U/mL) and HRP (0.02U/mL) were assembled with DPE-PPE$^+$ nanostructures in stoichiometric ratio at 4 °C for 45 min. The GOx-HRP cascade activity on DPE-PPE$^+$ was measured in a phosphate buffer (pH 7.2 containing 0.15M NaCl) and in presence of 0.1 M Glucose and 2 mM *o*-dianisidine by monitoring the increase in absorbance at 436 nm.

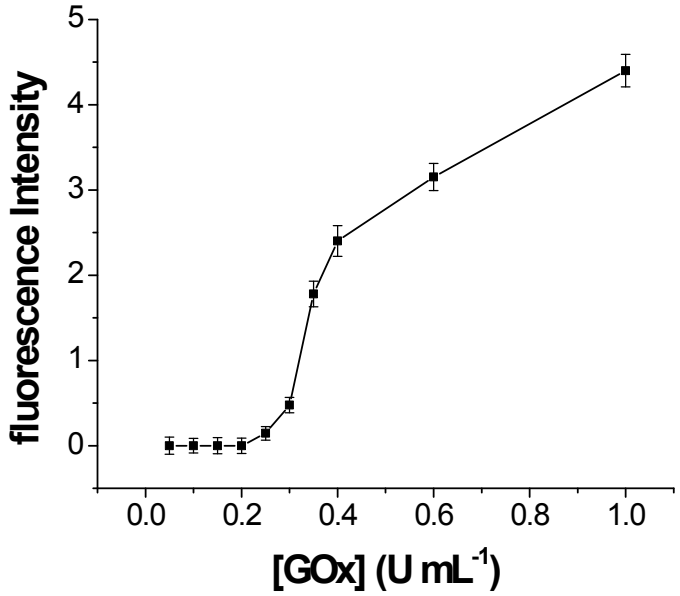

Fig. S1 Tryptophan fluorescence intensity at 340 nm as a function of the concentration of GOx in the presence of DPE-PPE$^+$.

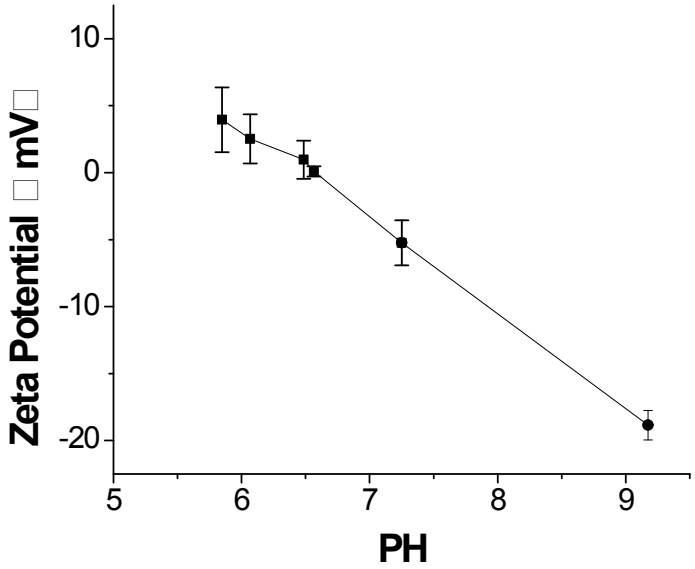

Fig. S2 Zeta Potential of HRP as a function of pH.

Notes and references

S1 L. Zhang, Q. H. Yin, H. Y. Huang and B. X. Wang, *J. Mater. Chem. B*, 2013, **1**, 756–761.