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 Beijing 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. 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 45 min 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-HPR 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.

![Graph](image-url)

**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