SUPPORTING INFORMATION

for

"Turn-On" Fluorescence Determination of β-Glucosidase Activity Using Fluorescent Polymer Nanoparticles Formed from Polyethylenimine Cross-Linked with Hydroquinone

Jinshui Liu,*,† Huijuan Bao,† Chenfu Liu,‡ Fangfei Wu† and Feng Gao*,†

[†]College of Chemistry and Materials Science, Anhui Key Laboratory of Chemo/Biosensing, The Key Laboratory of Functional Molecular Solids, Ministry of Education, Anhui Laboratory of Molecule-based Materials, Anhui Normal University, Wuhu 241000, China

[‡]School of Pharmaceutical Sciences, Gannan Medical University, Ganzhou 341000, China

*Corresponding authors

*E-mail: jsliu@ahnu.edu.cn

*E-mail: fgao@mail.ahnu.edu.cn

Optimization of catalytic hydrolysis conditions

The effect of the pH value on the catalytic hydrolysis conditions was investigated (Figure S13). It was found that the fluorescent intensity of the detection system increased gradually as the pH increased from pH 3.5 to 6.5, then decreased sharply as the pH increased from pH 6.5 to 8, and results indicated pH = 6.5 gave the best fluorescence enhancement, which was selected for the following experiments. As the incubation time was prolonged, the fluorescence intensity increased gradually (Figure S14). Considering hydrolysis efficiency, therefore, 60 min was chosen as the incubation time. The effect of enzyme hydrolysis temperatures on the fluorescence intensity were investigated (Figure S15), and 40 °C yielded the highest fluorescence intensity and was used as the enzyme hydrolysis temperature.



Figure S1 Fluorescence emission spectra for 0.33 g L⁻¹ hyperbranched polyethylenimine (PEI), 1.5 μ M hydroquinone, and a mixture of 1.5 μ M hydroquinone and 0.33 g L⁻¹ PEI. The solutions were in phosphate-buffered saline at pH = 8.2.



Figure S2 Fluorescence decay curves for the fluorescent polymer nanoparticles (FPNs)



Figure S3 X-ray photoelectron spectroscopy (XPS) survey spectra of fluorescent polymer nanoparticles (FPNs).



Figure S4 High-resolution X-ray photoelectron spectroscopy (XPS) spectra of the C 1s of the fluorescent polymer nanoparticles (FPNs).



Figure S5 High-resolution X-ray photoelectron spectroscopy (XPS) spectra of the N 1s of the fluorescent polymer nanoparticles (FPNs).



Figure S6¹H NMR spectra of PEI (A) and FPNs (B).



Figure S7 UV-vis absorption spectra for hydroquinone (360 μ M), PEI (11.1 g L⁻¹), and FPNs (360 μ M hydroquinone and 11.1 g L⁻¹ PEI).



Figure S8. Effect of daylight on the fluorescence intensity of fluorescent polymer nanoparticles (FPNs)



Figure S9 Fluorescence intensity at 510 nm for a solution containing 0.33 g L^{-1} hyperbranched polyethylenimine and 1.5 μM hydroquinone plotted against time.



Figure S10 Effect of pH on the fluorescence intensity at 510 nm of a solution containing 0.33 g L^{-1} hyperbranched polyethylenimine and 1.0 μ M hydroquinone.



Figure S11 UV-vis absorption spectra for β -glucosidase (1000 U L⁻¹), β -arbutin (500 μ M) plus β -glucosidase (1000 U L⁻¹), hydroquinone (170 μ M), and β -arbutin (500 μ M).



Figure S12 Transmission electron microscopy images of the FPNs prepared by hydrolysis products crosslink with PEI.



Figure S13 Fluorescence intensities at 510 nm found for fluorescent polymer nanoparticles formed in solutions at different pH values. Concentrations: polyethylenimine 0.33 g L⁻¹; β -arbutin 50 μ M; β -glucosidase 35 U L⁻¹.



Figure S14 Fluorescence intensities at 510 nm found for fluorescent polymer nanoparticles after different hydrolysis times. Concentrations: polyethylenimine 0.33 g L^{-1} ; β -arbutin 50 μ M; β -glucosidase 35 U L^{-1} .



Figure S15 Fluorescence intensities at 510 nm for fluorescent polymer nanoparticles formed at different temperatures. Concentrations: polyethylenimine 0.33 g L^{-1} ; β -arbutin 50 μ M; β -glucosidase 35 U L^{-1} .



Figure S16 Photographs of β -arbutin/polyethylenimine systems containing β -glucosidase at concentrations of (1) 0 U L⁻¹, (2) 6 U L⁻¹, (3) 15 U L⁻¹, and (4) 48 U L⁻¹. The photographs were taken while the samples were illuminated with light at a wavelength of 365 nm.

Materials	Methods	Detection limit (U/L)	References
A triple-signaling fluorescent	Fluorometric	1.0	1
probe			
Glucometer-based assay	Glucometer	87.3	2
Enzyme-triggered click	Fluorometric	0.456	3
chemistry			

Table S1 Comparison of different methods for the determination of β -Glucosidase Activity.

REFERENCES

(1) Li, Y.; Wang, H.; Li, J.; Zheng, J.; Xu, X.; Yang, R., Simultaneous intracellular beta-D-glucosidase and phosphodiesterase I activities measurements based on a triple-signaling fluorescent probe. *Anal. Chem.* **2011**, *83* (4), 1268-74.

(2) Jin, M. Y.; Zhang, T.; Yang, Y. S.; Ding, Y.; Li, J. S.; Zhong, G. R., A simplified and miniaturized glucometer-based assay for the detection of beta-glucosidase activity. *J. Zhejiang Univ.*, *Sci.*, *B* 2019, *20* (3), 264-272.

(3) Wang, L.; Ma, J.; Cheng, X.; Li, Z.; Sun, L.; Zeng, Z.; Jiang, H., Determination of β-Glucosidase Activity Based on Enzyme-Triggered Click Chemistry. *Youji Huaxue* 2018, *38* (10), 2775-2779.