A Label-free Optical Sensing Platform for Beta-glucosidase Activity Using Protein-inorganic Hybrid Nanoflowers

Ziping Liu
Northeast Normal University
Shasha Liu
Northeast Normal University
Decai Gao (gaodc493@nenu.edu.cn)
Northeast Normal University https://orcid.org/0000-0002-6167-3634
Yanan Li
Northeast Normal University
Ye Tian
Jilin Province Product Quality Supervision Testing Insititute
Edith Bai
Northeast Normal University

Research Article

Keywords: Optical sensing platform, Dual-signal readout, β-Glu, Protein-inorganic hybrid NFs, Inhibitor screening

Posted Date: June 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-556583/v1

Abstract

In this work, a convenient, label-free and dual-signal readout optical sensing platform for the sensitively and selectively determination of beta-glucosidase (β-Glu) activity was reported using protein-inorganic hybrid nanoflowers [BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$] possessing peroxidase-mimicking activity. The nanoflowers (NFs) were facilely synthesized through a self-assembled synthesis strategy at room temperature. The as-prepared NFs could catalytically convert the colorless and non-fluorescent Amplex Red into colored and highly fluorescent resorufin in the presence of hydrogen peroxide via electron transfer process. β-Glu could hydrolyze cyanogenic glycoside, using amygdalin (Amy) as a model, into cyanide ions (CN^-), which can subsequently efficiently suppress the catalytic activity of NFs, accompanied with the fluorescence decrease and the color fading. The concentration of CN^- was controlled by β-Glu-triggered enzymatic reaction of Amy. Thus, a sensing system was established for fluorescent and visual determination of β-Glu activity. Under the optimum conditions, the present fluorescent and visual bimodal sensing platform exhibited good sensitivity for β-Glu activity assay with a detection limit of 0.33 $U \cdot L^{-1}$. The sensing platform was further applied to determinate β-Glu in real samples and satisfactory results were attained. Additionally, the label-free optical sensing system can potentially be a promising candidate for β-Glu inhibitors screening.

Introduction

bata-Glucosidase (β-Glu, EC 3.2.1.21), as one of the main glycosidases, is capable of specifically and efficiently catalyzing the cleavage of β-glycosidic linkages in alkyl and aryl β-D-glucopyranosides as well as disaccharides and other glucose-containing molecules [1-4]. β-Glu widely exists in organisms and plays crucial roles in a variety of biological processes, such as biotransformation, secondary metabolism, plants defense against pathogens and glucoside ceramide catabolism in human tissues and cell walls [5,6]. In medical diagnosis, β-Glu has been shown to be closely correlated with metabolic diseases such as diabetes, neonatal necrotizing enterocolitis, bacterial or viral infections, Gaucher's disease and cancer [7,8]. In food industry, β-Glu is very crucial to remove the glycoside moieties of glycosylated flavonoids in soybean products, and thus the flavonoids can be absorbed by the intestine and provide beneficial health effects [9,10]. β-Glu is also quite important for biotransforming the constituents and improving the flavors of beverages [11]. In agriculture science, β-Glu is a useful biological indicator for assessing soil quality [12-14]. In environmental research, β-Glu activity is found to be intimately related to the phytoplankton blooms breakdown in the eutrophic lakes during the spring algal bloom [15]. Therefore, it is of significance for the development of reliable and sensitive approaches for β-Glu activity assay.

Traditional assay for the determination of β-Glu activity is based on the transformation of the artificial substrate *p*-nitrophenyl-β-D-glucopyranoside (pNPG) into *p*-nitrophenol, which subsequently measured by spectrophotometrically [16]. However, this method is not sufficiently sensitive owing to the intrinsic drawbacks of colorimetric methods [9]. Besides, the substrate used in this method is very unstable, and a large number of chemical reagents that are unfriendly to the environment are required [13]. Fluorescence-

based strategies are considered promising substitutes for analysis field because of their high sensitivity, easy operation, fast response as well as high-throughput screening [17-20]. During the past two decades, numerous fluorescent sensing approaches have been reported for the determination of β-Glu activity [21.22]. For example, a variety of β-glucoside derivatives such as 7-hydroxycoumarin [23], fluorescein [13] and nitrophenols [24] are employed to measure β-Glu activity. Although these methods have shown great promise, they are always requiring complicated processing procedures that are time- and labour-intensive, susceptible to poor reproducibility, uneconomical and professional operator in well-equipped laboratories [25]. Hence it is still highly desirable to develop reliable, cost-effective and environmentally friendly innovative fluorophore-based assays for sensitive and selective sensing of β-Glu activity.

Nanomaterials with enzyme-imitating catalytic activities hold great potential applications in a wide range of fields due to their unique fascinating catalytic properties, stable catalysts and economical efficiency [26-28]. It is an ideal alternative for nature enzymes which often suffer from intrinsic drawbacks including costly of preparation and purification, catalytic activity susceptible to environmental conditions and low operational and low storage stabilities [29-31]. The potential convenience and merits of the nanozymes deserves further and fully explored. Among the reported nanozymes, the advanced organic-inorganic hybrid NFs with flowerlike nanostructures exhibit extraordinary peroxidase-mimicking catalytic activity thanks to their hierarchical structures which possess a large surface area. It is favorable to expose more active sites and has a better promoting effect on the contact of the nanomaterials with the tested substances [32]. Based on the previous literatures, the NFs on the basis of copper can function as the similar efficient catalysts as Fenton-like reagents in catalyzing the substrates to generate colored products [33].

Enlightened by the aforementioned above, in our study, we try to design a convenient, label-free and environmental friendly sensing platform for fluorescent and visual determination of β-Glu activity based on the integration of bovine serum albumin (BSA) and inorganic copper (II) compound hybrid nanoflower [BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$]. And we expected the proposed cost-effective dual-signal readout optical sensing system could be utilized to the β-Glu assay in real samples.

Experimental

Materials

Bovine serum albumin (BSA), β-Glu, Amplex Red, dimethylsulfoxide (DMSO), copper sulfate pentahydrate ($CuSO_4 \cdot H_2O$), Amy, horseradish peroxidase (HRP), acid phosphatase (ACP), glucose, L-cysteine (L-Cys), glucose (Glu) and protamine (Prot) and glucose oxidase (GOX) were purchased from Sigma-Aldrich reagent Co., Ltd. (St. Louis, MO, USA). H_2O_2, sodium hydroxide (NaOH) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium phosphate dibasic anhydrous (Na_2HPO_4), sodium phosphate monobasic anhydrous (NaH_2PO_4) and sodium phosphate (Na_3PO_4) were bought from Aladdin Reagent Co., Ltd. (Shanghai, China). Hydrochloric acid, acetonitrile and the other reagents were purchased from Beijing Chemicals (Beijing, China). The ultrapure water (18.2

MΩ·cm^{-1}) used throughout the experiments was obtained from a Millipore water purification system. All chemicals used in our work were at least analytical grade and used as received without any further purification.

Apparatuses

A RF-6000 fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) equipped with a xenon lamp using right-angle geometry and 1 cm quartz cells was used to record the fluorescence spectra. The centrifugation process was carried out with a Sorvall Legend Micro 21R refrigerated centrifuge (Thermo Scientific, USA). All pH measurements were carried out with a PB-10 pH meter (Sartorius, Germany). A HHS-11-4S thermostatic bath device (Baidian, Shanghai, China) was also used in the incubation experiments.

Preparation of Protein-inorganic Hybrid NFs

The BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid NFs were perfectly synthesized on the basis of pioneering work [27,28,34]. Typically, 200 mmol·L^{-1} $CuSO_4$ were injected into 50 mmol·L^{-1} PBS (pH 6.8) containing 0.1 mg·mL^{-1} BSA and 1690 μL of ultrapure water, followed by standing at room temperature for 12 h. The as-prepared NFs were obtained by centrifugation at 10000 rpm for 10 min, and then purified via washing three times using ultrapure water. The collected turquoise precipitation was redispersed in 200 μL PBS and kept at 4 °C for the follow-up experiments.

Sensing of β-Glu Activity

Specifically, a series different concentration of β-Glu solution was prepared and kept at 4 °C. 150 μL of NFs solution, 50 μL of Amy solution (20 mmol·L^{-1}) and 50 μL different concentration of β-Glu were then mixed thoroughly and kept for 1 h at room temperature for the enzymatic reaction. Afterwards, 20 μmol·L^{-1} Amplex Red and 200 mmol·L^{-1} H_2O_2 were added to the above reaction solution. The mixture was diluted to 1.5 mL with PBS and incubated for 90 min. The fluorescence spectrums were recorded from 565 nm to 800 nm with an excitation wavelength of 550 nm and emission wavelength of 585 nm. The slits for excitation and emission were 5 nm.

β-Glu Activity Assay in Real Samples

The proposed method was used to detect β-Glu activity in human serum, water and soil samples to evaluate its practicality in different medium. The fresh drug-free human blood samples were supplied by the affiliated hospital of Northeast Normal University (Changchun, China) hospital and all the blood samples were from healthy volunteers through venipuncture. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the writing of informed consent for all samples was obtained from human subjects. The water samples were from tap water in our laboratory. The soil samples frozen at -20 ℃ were provided by a local biogeochemical lab and all the soils were collected from the surface layer (0-20 cm). Some necessary pretreatment processes for these samples

were performed as previously reports [3,28,35]. The human serum samples processing steps were as follows: the blood samples were standing for 12 h to get the upper serum. Then the samples were segregated by adding acetonitrile (V_{blood} to $V_{acetonitrile}$ was 1 to 1.5) and centrifuged at 10000 rpm for 10 min. The samples were diluted 100 folds with ultrapure water before analysis. The tap water samples were filtered through a 0.45 μm membrane to remove the probably existing impurities. The soil samples processing steps were as follows: after removing the plant roots and other plants residues, the samples were sifted through a 2 mm sieve. 5 g soil and 30 mL ultrapure water was fully mixed, followed by collecting the supernatant via centrifuging at 10000 rpm for 10 min and filtered. A known amount of β-Glu was added for the preparation of spiked samples. These prepared samples were measured using the aforementioned detection procedures for β-Glu activity assay.

Results And Discussion

The Strategy for the Sensing of β-Glu

The principle of the designed sensing platform for β-Glu detection was shown in Scheme 1. As illustrated in Scheme 1 (A), the BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanohybrids possessing peroxidase-like activity were facilely obtained through the spontaneously self-assembly reaction in phosphate-buffered saline (PBS) an room temperature. In the presence of hydrogen peroxide (H_2O_2), BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ can catalyze the oxidation of Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) to resorufin (7-hydroxy-3H-phenoxazin-3-one), which is accompanied with the fluorescence enhancement and the corresponding color change from colorless to dark magenta [Scheme 1 (B)] [36]. While β-Glu and cyanogenic glycoside amygdalin (Amy) were added, the catalytic oxidation process was blocked by the enzymatic hydrolysate cyanide ions (CN^-), which can greatly restrain the catalytic activity of BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanozyme [Scheme 1 (C)] [31]. Meanwhile, the fluorescence decreased and the color changed from dark magenta to pink. Therefore, the changing of fluorescence intensity and hue parameters produced the corresponding signals to accurately detect β-Glu. Owing to the convenient, cost-effective and environmental friendly, it is expected that the present fluorescent and visual sensing system can be utilized to the β-Glu assay in real samples.

Peroxidase-like Activities of BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$

We investigated the peroxidase-like catalytic properties of BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanohybrids via acting BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ on Amplex Red solution (fluorogenic and chromogenic substrate) and H_2O_2 to initiates a redox reaction. As displayed in Fig. 1, the control experiments suggested that there are no marked fluorescence emission peaks between 565 nm and 800 nm with the single Amplex Red solution (curve a) and Amplex Red solution containing NFs (curve b) or H_2O_2 (curve c), separately. While NFs, Amplex Red and H_2O_2 were mixed, the mixed solution exhibited a remarkable fluorescence enhancement from the very low fluorescence background level that was almost flat (curve d). The fluorescence of the NF-Amplex Red-H_2O_2 system is significantly much higher than that of Amplex Red (a), NF-Amplex Red

system (b) and H_2O_2-Amplex Red system (c), revealing that the BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ NFs possessed excellent peroxidase-mimicking catalytic activity. The fluorescence is derived from the oxidation product of Amplex Red that is known as resorufin [37,38]. The maximum fluorescence emission wavelength of the reaction mixture is around 584 nm at the excitation wavelength of 550 nm. Meanwhile, as displayed in the inset of Fig. 1, it can be observed inset that the colorless Amplex Red was rapidly oxidized to a typical dark magenta product, which can be easily discernible by the naked eyes.

We tested the fluorescence emission spectra of the reaction in which different concentrations of H_2O_2 were catalyzed by series of certain amount of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ in order to further investigate the catalytic performance of the NFs. As depicted in Fig. 2 (A), the fluorescence emission intensity steadily increased as the increasing concentrations of H_2O_2 from 0.05 to 200 $mmol \cdot L^{-1}$. And a good linearity was founded between the fluorescence emission intensity and the H_2O_2 concentration [Fig. 2 (B) inset]. The above results confirmed the satisfactory peroxidase-like catalytic performance of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ nanohybrids. Moreover, the incubating time was studied since it takes a crucial role during the enzymatic catalytic process. It can be seen from Fig. 3 that the fluorescence emission intensity enhanced as the increasing incubating time within a certain range, and reached a plateau at about 90 min that indicating the catalytic reaction was finished within 90 min.

As we all known, the catalytic performance of enzyme is closely associated with the reaction conditions. In this work, we first studied the influence of pH on the enzyme catalytic properties of the as-prepared NFs. As shown in Fig. 4, when the pH changed from 4.0 to 9.0, both the nanoflower catalytic activity and the HRP catalytic activity are higher in alkaline medium than in acidic medium using Amplex Red as substrate. But overall the NFs had better catalytic capacity when compared with HRP in the pH range of 4.0 to 9.0. The effect of temperature on the enzyme catalytic properties of the obtained NFs was subsequently tested from 4 ℃ to 50 ℃. From Fig. S1, it can be found that the NFs possess stronger ability of resistance to high temperature. Furthermore, we carried out a control experiment to contrast the stability of the NFs and HRP. The results in Fig. S2 suggested that the NFs catalytic activity basically unchanged in one month, but the HRP almost no longer exhibited catalytic activity only within 75 h, which is accordance with the previous work [27]. The above experimental results indicated that the as-prepared nanozyme have advantage over the nature enzyme HRP and hold potential application in practice.

Optimization of the Sensing Platform

In order to acquire better performance for sensing β-Glu, we optimized the experimental parameters including incubation time, pH values, temperature and salt concentration. As displayed in Fig. 5 (a), the fluorescence emission intensity was found gradually decreased with the increasing of reaction time and reached a plateau at around 55 min [Fig. 5]. Therefore, 55 min was chosen for the further study. As exhibited in Fig. 5 (b), it can be observed that the fluorescence readout declined from pH 4.0 to pH 8.0 and increased from pH 8.0 to pH 10.0, the minimum value was appeared at pH 8.0. So 8.0 was chosen as the optimal pH value for β-Glu sensing platform. As shown in Fig. 5 (c), the fluorescence readout decreased from pH 4 ℃ to 25 ℃ and increased from 25 ℃ to 45 ℃, the minimum value was found at 25 ℃. Thus,

the optimal reaction temperature for β-Glu sensing platform is 25 ℃. The salt concentration showed no obvious effect on the sensing platform, and we chose 50 mmol·L^{-1} NaCl for the subsequent experiments in this work.

Fluorescence Sensing of β-Glu

Under the optimum experimental conditions, further experiments were performed for β-Glu assay. Fig. 6 (a) recorded the fluorescence emission spectra of NFs-H_2O_2-Amplex Red-Amy system recorded from 565 nm to 800 nm at an excitation wavelength of 550 nm when addition of different concentrations of β-Glu activities from 0 to 1500 U·L^{-1}.

We can noticeably found that the fluorescence emission intensity at 585 nm can be regulated by the β-Glu concentrations and decreased step by step until a platform, and the color of the mixed solution gradually becomes shallow from dark magenta to light magenta at the same time [Fig. 6 (c)]. Additionally, it can be calculated that from Fig. 1 that the fluorescence emission intensity at 584 nm decreased to 33.8% of the original fluorescence intensity of NFs-Amplex Red-H_2O_2 system. A quality linear range for β-Glu activity was established between fluorescence intensity ratio (I/I_0) and β-Glu activity concentration form 0.5 U·L^{-1} to 1500 U·L^{-1} (0.5, 10, 20, 50, 100, 300, 500, 700, 900, 1100, 1300 and 1500 U·L^{-1}). The linear fitting equation is I/I_0 = 0.992-0.0002 [β-Glu] (U·L^{-1}) with a correlation coefficient (R^2) of 0.997. The limit of detection (LOD) defined by the 3σ equation (signal-to-noise ratio of 3) was calculated to be 0.33 U·L^{-1}, which is comparable to LODs of the previous published approaches that are summarized in Table S1, suggesting that the present sensing method has satisfactory sensitivity. In addition, the sensing strategy displayed excellent detection range in comparison with the existing β-Glu assay methods. It should be further noted that the influence of single Amy or β-Glu had a negligible effect on the NFs-H_2O_2-Amplex Red system (Fig. 7).

Under the optimized reaction conditions, the selectivity and interference of the protein-inorganic hybrid nanoflower-based sensing platform were assessed by carrying out the experiment of various common coexisting substances that consist of several potential interfering enzymes (ACP and GOX), biological materials (L-cysteine, glucose and protamine) and inorganic ion (K^+, Na^+, Mg^{2+} Ca^{2+}, NO^{3-} and Cl^{-1}). The concentration of coexisting ACP is 1.0 μU·mL^{-1}. The concentration of coexisting GOX is 1.0 μg·mL^{-1}. The concentration of coexisting L-cysteine, glucose and protamine is 1.0 μmol·L^{-1}, respectively. The concentration of coexisting K^+, Na^+, Mg^{2+} Ca^{2+}, NO^{3-} and Cl^{-1} is 1.0 μmol·L^{-1}, respectively. A relative error of ±5.0% is considered to be acceptable. As exhibited in Fig. 8, the protein-inorganic hybrid NF-based sensing system showed excellent specifcity for β-Glu determination, and the presence of these coexisting substances didn’t interfere the proposed sensing system for the β-Glu activity assay.

Sensing of β-Glu in Real Samples

To assess the reliability of the proposed sensing system, we applied it to the measurement of β-Glu in real samples of human serum samples, water samples and soil samples. To obtain spiked real samples, a

series of three different fixed concentrations of β-Glu were added into the human serum samples, water samples and soil samples. Then, a recovery study was carried out on these real samples β-Glu assay and the results were given in Table S2. It can be seen that the average β-Glu recoveries for the human serum samples, water and soil samples were in the range from 96.2-104%. The relative standard deviations (RSDs, n=3) are lower than 5.0%. The above results reveal the proposed sensing platform held potential application for β-Glu activity assay.

Conclusions

In summary, a novel convenient and efficient label-free sensing platform was developed for highly sensitive and selective determination of β-Glu activity on the basis of inorganic-organic hybrid NFs in this study. β-Glu can catalyze the Amy hydrolysis to yield CN^-, which can hinder the redox reaction between Amplex Red and H_2O_2 activated by the peroxidase-like NFs that can be facilely prepared through spontaneous self-assembled synthesis strategy at room temperature in PBS. The decrement of the systematic fluorescence intensity was found to be correlated linearly to the β-Glu activity. Interestingly, the process triggered color change that can be can be observed with the naked eye, making it possible for designed a dual signal readout sensing platform. We believe this low-cost optical sensing platform combined with simple working principle will be applied in various applications in the medical, environmental and agricultural fields. Additionally, it can potentially be a promising candidate for β-Glu inhibitors screening.

Declarations

Author Declarations

Funding

The authors gratefully acknowledge the financial support of the National Key R&D Program of China (2019YFA0607301), the China Postdoctoral Science Foundation (Nos. 2018M631850 and 2020T130088), and the Natural Science Foundation of Jilin Province, China (YDZJ202101ZYTS104).

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

Data Availability

All data generated or analyzed during this study are included in this manuscript and the Attach File “Supporting Information” section.

Authors Contributions

Ziping Liu: Conceptualization, Methodology, Investigation, Data curation, Writing-original draft. **Shasha Liu:** assisted with data collection and data analysis. **Decai Gao:** Resources, Supervision, Funding acquisition. **Yanan Li:** assisted with data collection and data analysis. **Ye Tian:** assisted with data collection and data analysis. **Edith Bai:** Resources, Supervision, Funding acquisition. All authors read and approved the final manuscript.

References

1. Bi YH, Zhu C, Wang ZY, Luo HZ, Fu RP, Zhao XJ, Zhao XJ, Jiang L (2019) Purification and characterization of a glucose-tolerant β-glucosidase from black plum seed and its structural changes in ionic liquids. Food Chemistry 274: 422-428.
2. Liu JS, Bao HJ, Liu CF, Wu FF, Gao F (2019) “Turn-on” fluorescence determination of β-glucosidase activity using fluorescent polymer nanoparticles formed from polyethylenimine cross-linked with hydroquinone. ACS Appl Polym Mater 1: 3057-3063.
3. Liu ZP, Tian Y, Han Y, Bai E, Li YN, Xu ZW, Liu SS (2019) A “turn off-on” fluorescent nanoprobe consisting of $CuInS_2$ quantum dots for determination of the activity of β-glucosidase and for inhibitor screening. Microchim Acta 186: 806.
4. Zeng ZH, Mizukami S, Kikuchi K (2012) Simple and real-time colorimetric assay for glycosidases activity using functionalized gold nanoparticles and its application for inhibitor screening. Anal Chem 84: 9089-9095.
5. Cairns JRK, Mahong B, Baiya S, Jeon JS (2015) β-Glucosidases: Multitasking, moonlighting or simply misunderstood? Plant Science 241: 246-259.
6. Chen GY, Zhang H, Yang FQ (2021) A simple and portable method for β-Glucosidase activity assay and its inhibitor screening based on a personal glucose meter. Analytica Chimica Acta 1142: 19-27.
7. Moreno JLGC, Torronteras AR, Gonzalez MDR, Palomares LI, Valverde DB, Laguna JR, Rubio AD, Lopez-Barea J (2016) The β-glucosidase assay: a new diagnostic tool for necrotizing enterocolitis.

Sensitivity, specificity, and predictive values. Eur J Pediatr 175: 931-941.

8. Zhang WJ, Oehrle M, Prada CE, Schwartz IVD, Chutipongtanate S, Wattanasirichaigoon D, Inskeep V, Dai M, Pan D, Sun Y, Setchell KDR (2017) A convenient approach to facilitate monitoring Gaucher disease progression and therapeutic response. Analyst 142: 3380-3387.
9. Strahsburger E, de Lacey AML, Marotti I, DiGioia D, Biavati B, Dinelli G (2017) In vivo assay to identify bacteria with β-glucosidase activity. Electron J Biotechnol 30: 83-87.
10. Ningtyas DW, Hati S, Prakash S (2021) Bioconversion and bioaccessibility of isoflavones from sogurt during *in vitro* digestion. Food Chemistry 343: 128553.
11. de Ovalle S, Brena B, González-Pombo P (2021) Influence of beta glucosidases from native yeast on the aroma of Muscat and Tannat wines. Food Chemistry 346: 128899.
12. Gil-Sotres F, Trasar-Cepeda C, Leiros MC, Seoane S (2005) Different approaches to evaluating soil quality using biochemical properties. Soil Biol Biochem 37: 877-887.
13. Stege PW, Messina GA, Bianchi G, Olsina RA, Raba J (2010) Determination of β-glucosidase activity in soils with a bioanalytical sensor modified with multiwalled carbon nanotubes. Anal Bioanal Chem 397: 1347-1353.
14. Sazawa K, Kuramitz H (2015) Hydrodynamic voltammetry as a rapid and simple method for evaluating soil enzyme activities. Sensors 15: 5331-5343.
15. Chróst RJ, Overbeck J (1990) Substrate-ectoenzyme interaction: significance of β-glucosidase activity for glucose metabolism by aquatic bacteria. Arch Hydrobiol Beih Ergebn Limnol 34: 93-98.
16. Trasar-Cepeda C, Leiro's MC, Gil-Sotres F Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. (2008) Soil Biol Biochem 40: 2146-2155.
17. Wang XZ, Hou T, Dong SS, Liu XJ, Li F (2016) Fluorescence biosensing strategy based on mercury ion-mediated DNA conformational switch and nicking enzyme-assisted cycling amplification for highly sensitive detection of carbamate pesticide. Biosens Bioelectron 77: 644-649.
18. Zhou JW, Zou XM, Song SH, Chen GH (2018) Quantum dots applied to methodology on detection of pesticide and veterinary drug residues. J Agr Food Chem 66: 1307-1319.
19. Yan X, Kong DS, Jin R, Zhao X, Li HX, Liu FM, Lin YH, Lu GY (2019) Fluorometric and colorimetric analysis of carbamate pesticide via enzymetriggered decomposition of Gold nanoclusters-anchored MnO_2 nanocomposite. Sensors & Actuators: B Chemical 290: 640-647.
20. Zhang XB, Liu CH, Wang HH, Wang H, Li ZP (2015) Rare earth ion mediated fluorescence accumulation on a single microbead: an ultrasensitive strategy for the detection of protein kinase activity at the single-cell level. Angew Chem Int Ed 54: 15186-15190.
21. Komatsu T, Kikuchi K, Takakusa H, Hanaoka K, Ueno T, Kamiya M, Urano Y, Nagano T (2006) Design and synthesis of an enzyme activity-based labeling molecule with fluorescence spectral change. J Am Chem Soc 128: 15946-15947.
22. Kwan DH, Chen HM, Ratananikom K, Hancock SM, Watanabe Y, Kongsaeree PT, Samuels AL, Withers SG (2011) Self-immobilizing fluorogenic imaging agents of enzyme activity. Angew Chem Int Ed 50:

300-303.

23. Watanabe A, Suzuki M, Ujiie S, Gomi K (2016) Purification and enzymatic characterization of a novel β-1,6-glucosidase from *Aspergillus oryzae*. J Biosci Bioeng 121: 259-264.
24. Yan S, Wu G (2011) Prediction of michaelis-menten constant of beta-glucosidases using nitrophenyl-beta-D-glucopyranoside as substrate. Protein Pept Lett 18: 1053-1057.
25. Serdiuk IE, Reszka M, Myszka H, Krzyminski K, Liberek B, Roshal AD (2016) Flavonol-based fluorescent indicator for determination of β-glucosidase activity. RSC Adv 6: 42532-42536.
26. Ge J, Lei JD, Zare RN (2012) Protein-inorganic hybrid nanoflowers. Nat Nanotechnol 7: 428-432.
27. Huang YY, Ran X, Lin YH, Ren JS, Qu XG (2015) Self-assembly of an organic-inorganic hybrid nanoflower as an efficient biomimetic catalyst for self-activated tandem reactions. Chem Commun 51: 4386-4389.
28. Kong DS, Jin R, Zhao X, Li HX, Yan X, Liu FM, Sun P, Gao Y, Liang XS, Lin YH, Lu GY (2019) Protein-inorganic hybrid nanoflower-rooted agarose hydrogel platform for point-of-care detection of acetylcholine. ACS Appl Mater Interfaces 11: 11857-11864.
29. Barber J (2009) Photosynthetic energy conversion: natural and artificial. Chem Soc Rev 38: 185-196.
30. Zhou Z, Klupp Taylor RN, Kullmann S, Bao HX, Hartmann M (2011) Mesoporous organosilicas with large cage-like pores for high efficiency immobilization of enzymes. Adv Mater 23: 2627-2632.
31. Lien CW, Unnikrishnan B, Harroun SG, Wang CM, Chang JY, Chang HT, Huang CC (2018) Visual detection of cyanide ions by membrane-based nanozyme assay. Biosensors and Bioelectronics 102: 510-517.
32. Gao JJ, Liu H, Pang LY, Guo K, Li JQ (2018) Biocatalyst and colorimetric/fluorescent dual biosensors of H_2O_2 constructed via hemoglobin-$Cu_3(PO_4)_2$ organic/inorganic hybrid nanoflowers. ACS Appl Mater Interfaces 10: 30441-30450.
33. Jiao J, Xin X, Wang X, Xie Z, Xia C, Pan W (2017) Self-assembly of biosurfactant-inorganic hybrid nanoflowers as efficient catalysts for degradation of cationic dyes. RSC Adv 7: 43474-43482.
34. Gimenez C, Climent E, Aznar E, Martinez-Manez R, Sancenon F, Marcos MD, Amoros P, Rurack K (2014) Towards chemical communication between gated nanoparticles. Angew Chem Int Ed 53: 12629-12633.
35. Liu ZP, Lin ZH, Liu LL, Su XG (2015) A convenient and label-free fluorescence “turn off-on” nanosensor with high sensitivity and selectivity for acid phosphatase. Analytica Chimica Acta 876: 83-90.
36. Wang T, Xiang YQ, Liu XX, Chen WL, Hu YG (2017) A novel fluorimetric method for laccase activities measurement using Amplex Red as substrate. Talanta 162: 143-150.
37. Wang SR, Fu BS, Wang JQ, Long YL, Zhang XE, Peng S, Guo P, Tian T, Zhou X (2014) Novel Amplex Red oxidases based on noncanonical DNA structures: property studies and applications in microRNA detection. Anal Chem 86: 2925-2930.

38. Summers FA, Zhao BZ, Ganini D, Mason RP (2013) Photooxidation of Amplex Red to resorufin: implications of exposing the Amplex Red assay to light, in: E. Cadenas, L. Packer (Eds.). Hydrogen Peroxide and Cell Signaling, 1-17 Pt A.

Figures

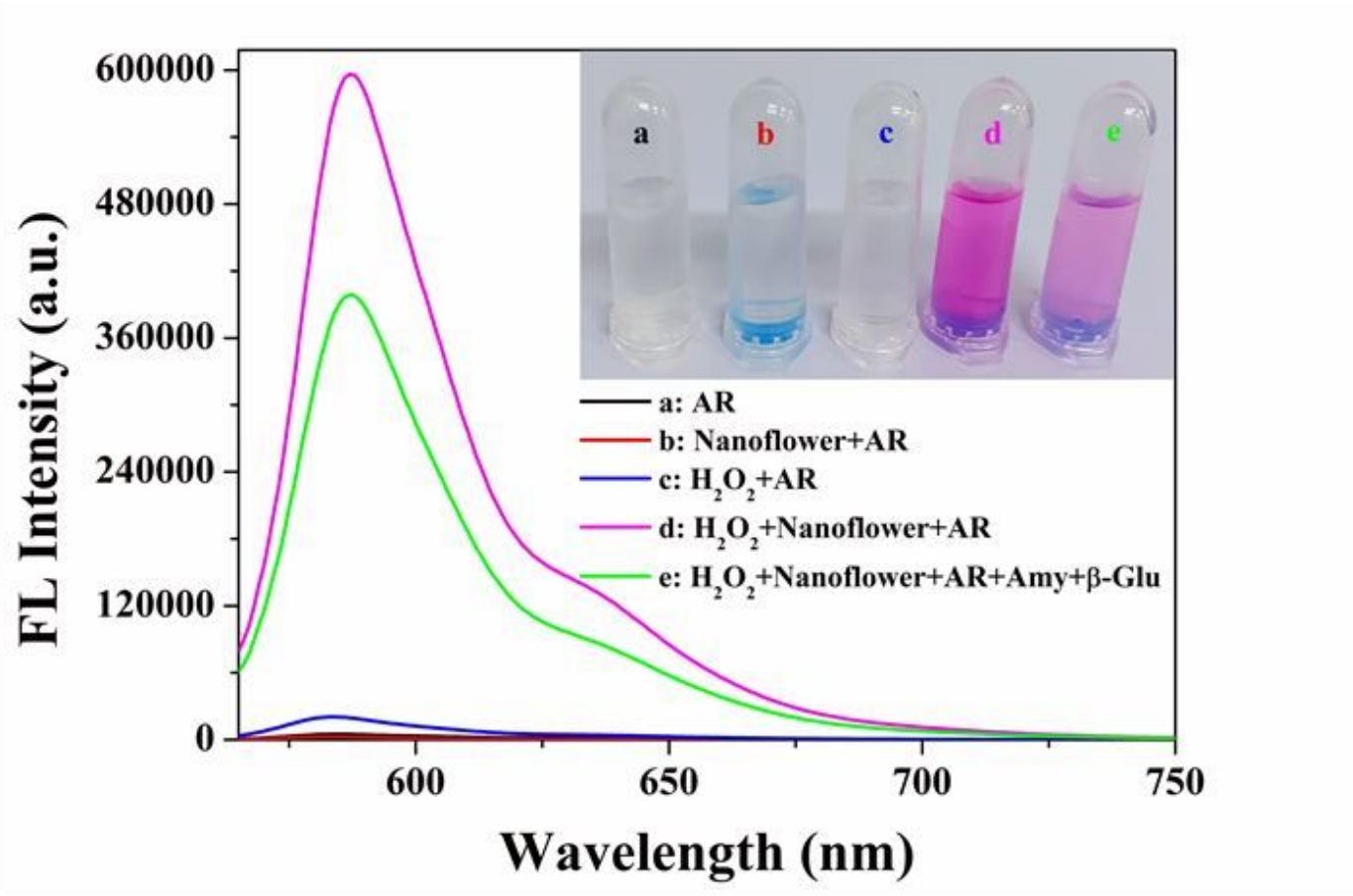

Figure 1

Fluorescence emission spectra of different systems: AR blank control (a), NFs + AR (b), H2O2 + AR (c), AR + NFs + H2O2 (d), and AR + NFs + H2O2 + β-Glu + Amy (e). The inset shows the corresponding color change in response. FL is short for fluorescence and AR is short for Amplex Red

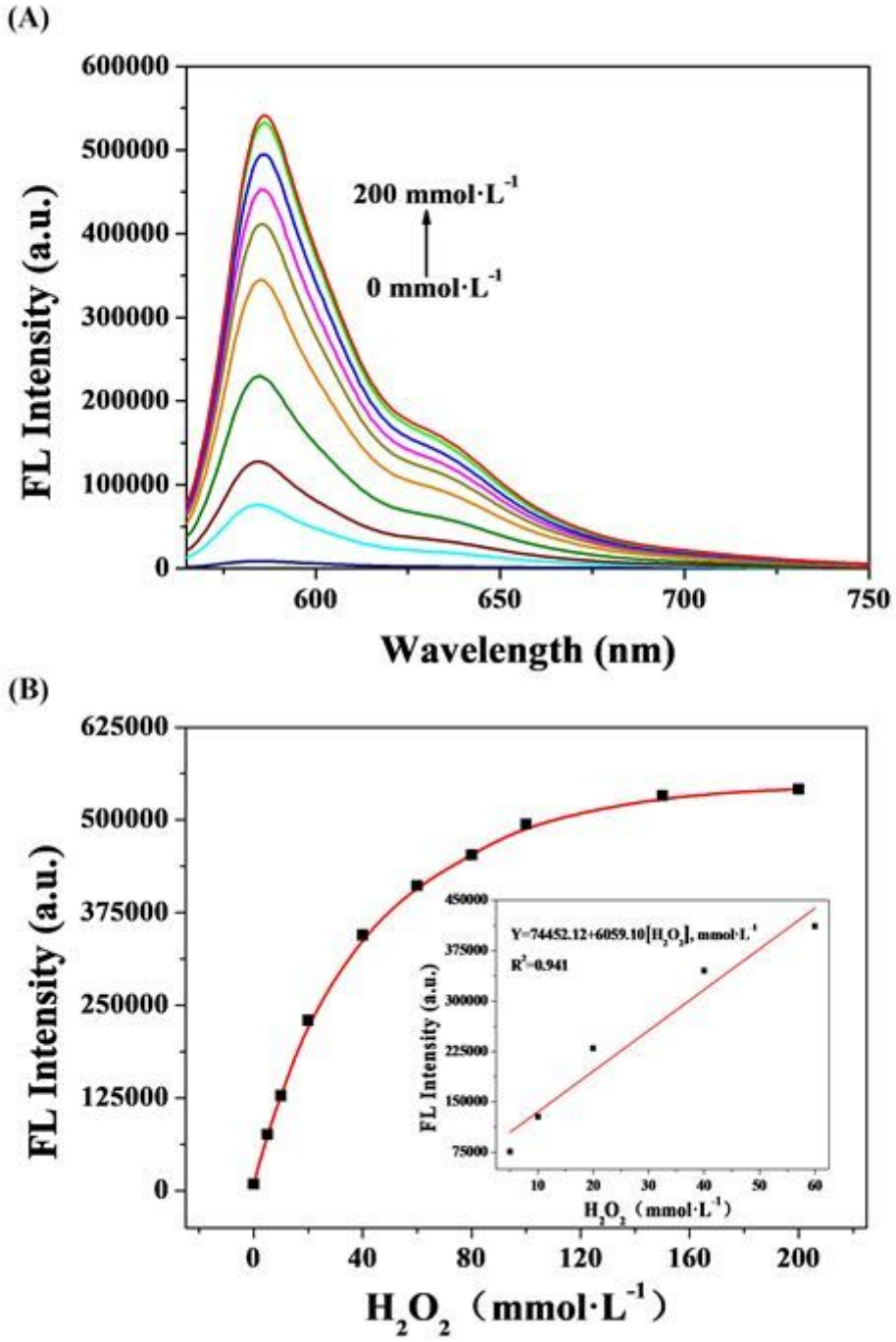

Figure 2

(A) Fluorescence emission spectra of different concentration of H2O2 (from 0 to 200.0 mmol·L-1). (B) Different concentrations of H2O2 changes lead to changes in fluorescence intensity. The inset shows good linearity between different concentrations of H2O2 (5, 10, 20, 40 and 60 mmol·L-1) and fluorescence intensity

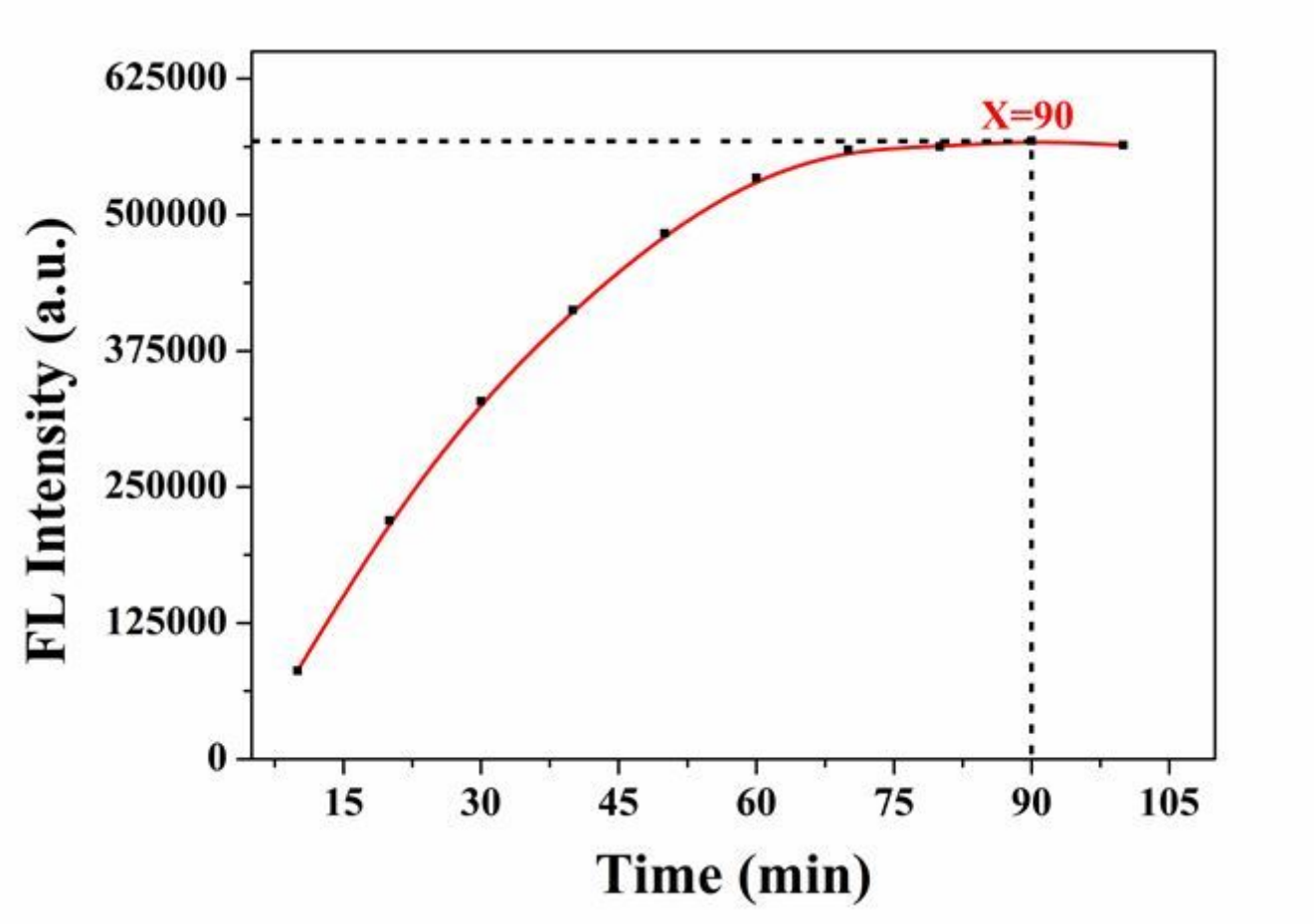

Figure 3

Effect of incubating time on the catalytic performance of the NFs

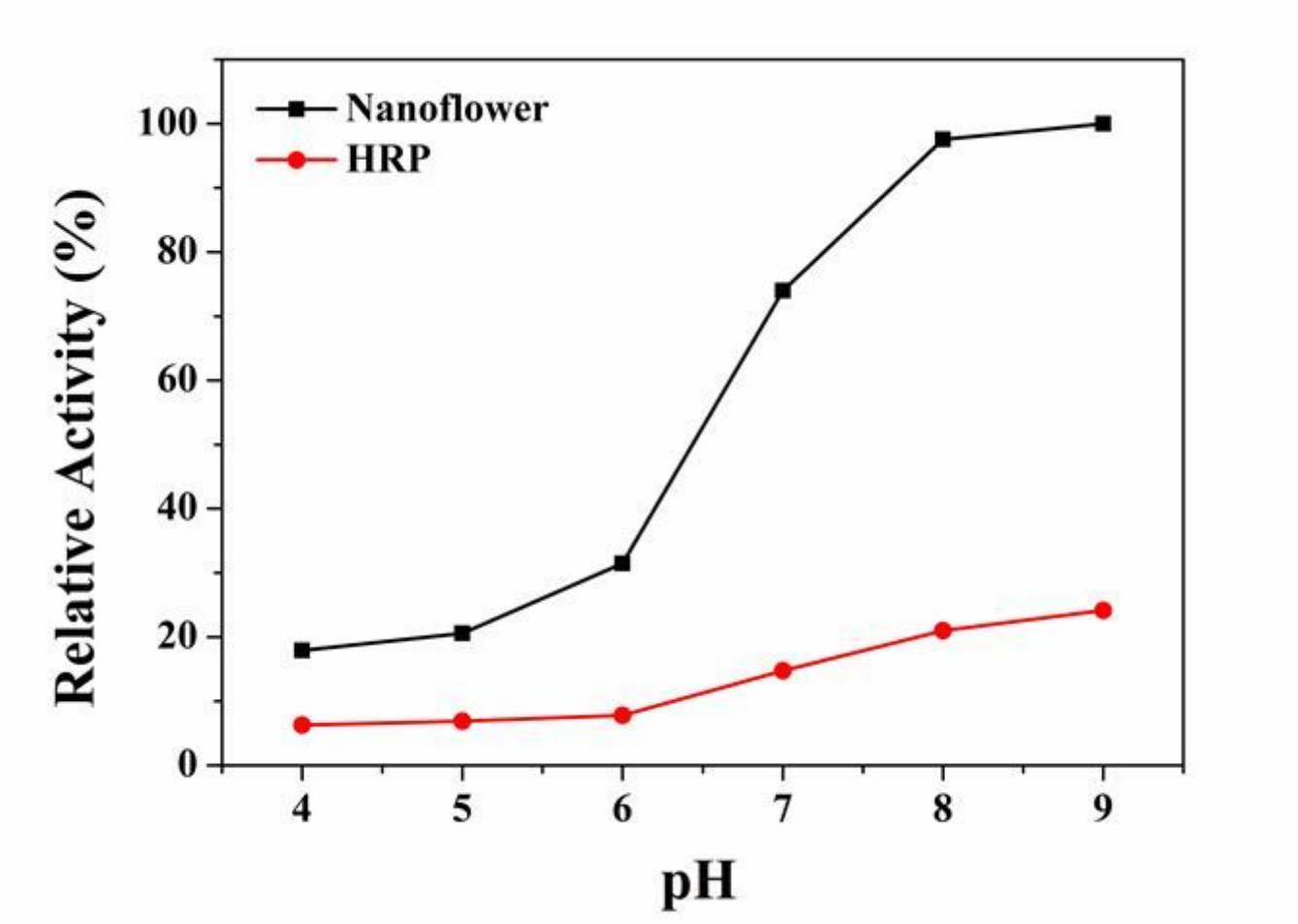

Figure 4

Effects of pH on the catalytic activities of NFs (red curve) and free HRP (black curve)

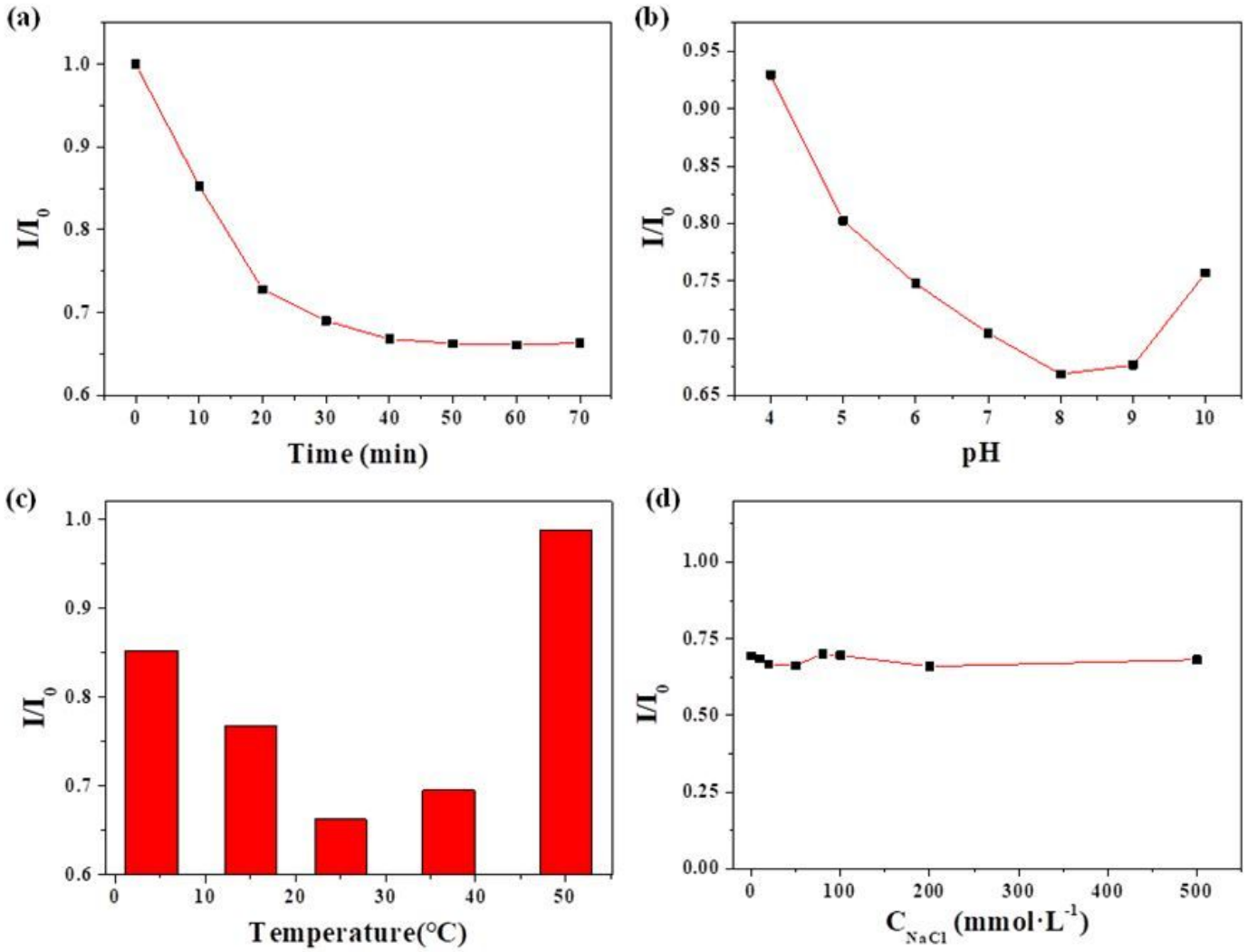

Figure 5

Effects of reaction time (a), reaction pH (b), reaction temperature (c) and reaction salt concentration (d) on the performance of the present β-Glu sensing system. I and I0 represented the fluorescence emission intensity of NFs-H2O2-Amplex Red-Amy in the presence and absence β-Glu, respectively

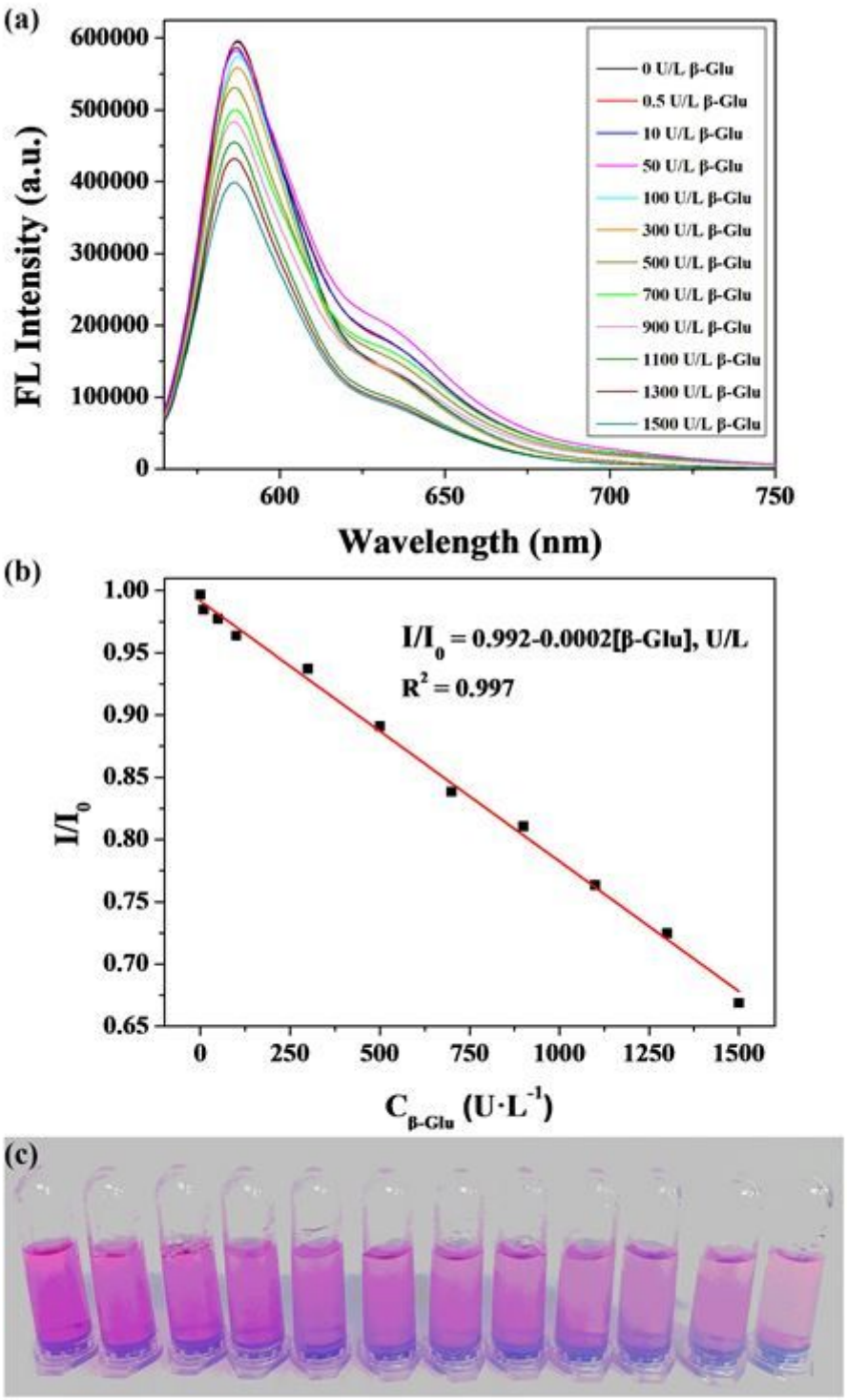

Figure 6

(a) Fluorescesnce emission spectrum of the NFs-H2O2-Amplex Red-Amy sensing system for β-Glu activity. (b) The plot shows the linear relation between the β-Glu activity and the fluorescence intensity ratio (I/I0, I and I0 represented the fluorescence emission intensity of NFs-H2O2-Amplex Red-Amy in the presence and absence β-Glu, respectively). (c) Corresponding photographs of catalytic reaction systems of different β-Glu activity under sunlight

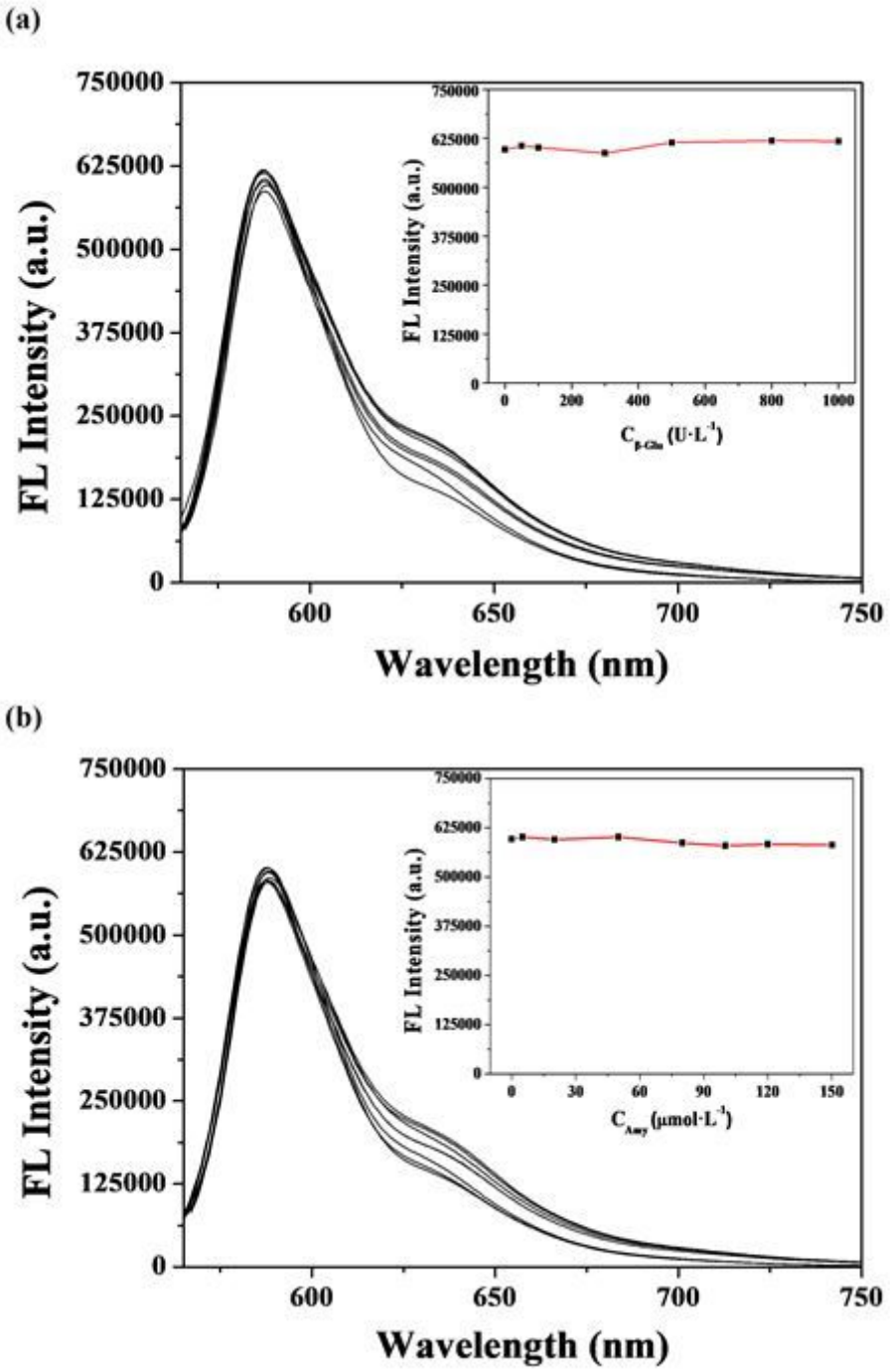

Figure 7

Effect of different concentration of (A) β-Glu and (B) Amy on the FL intensity of NFs-Amplex Red-H2O2 system

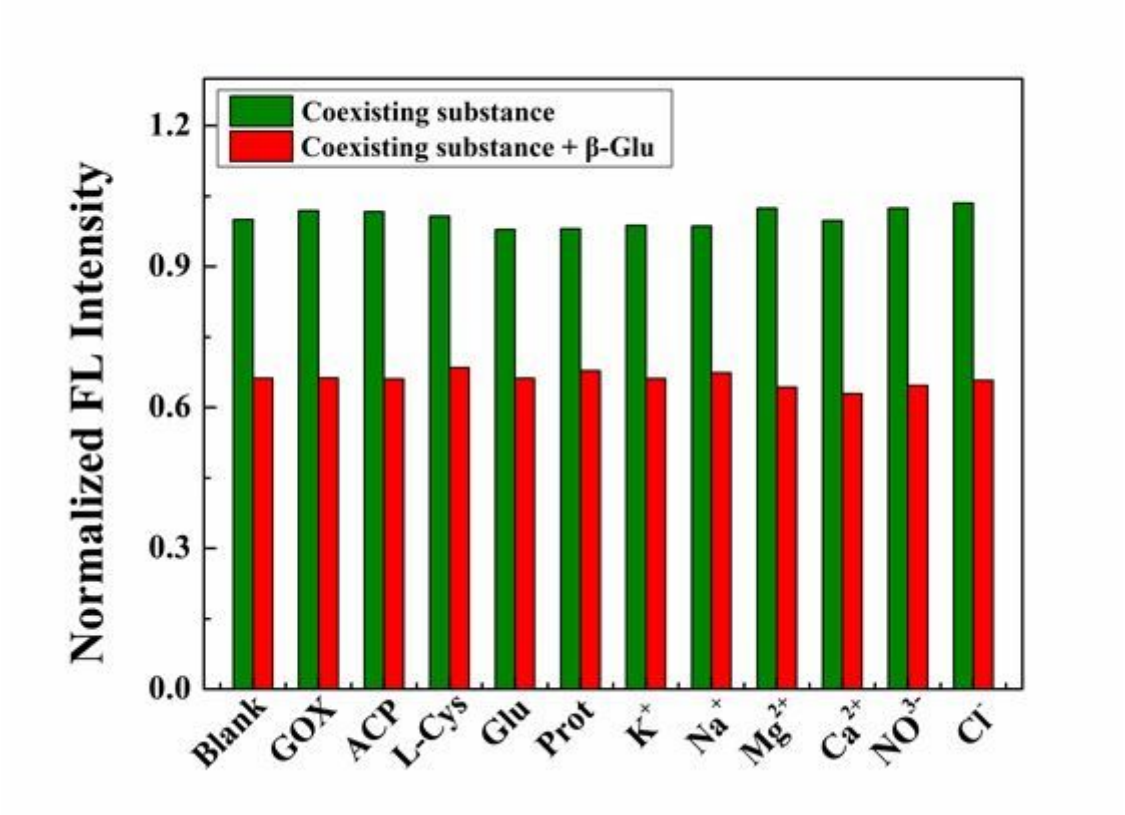

Figure 8

Fluorescence signal response of the NFs-H2O2-Amplex Red-Amy system to β-Glu (1500 U·L-1) or the coexisting substances and the mixture of the coexisting substances with β-Glu

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx
- SupportingInformation.docx
- scheme1.jpg