



Communication

Self-assembled nanozyme complexes with enhanced cascade activity and high stability for colorimetric detection of glucose

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ABSTRACT

Construction of multi-enzyme complexes not only can help expand the understanding of biological mechanisms, but also holds great promise in biosynthesis, biosensing and biomedicine. Herein, a hybrid multi-enzyme system, CeO₂/glucose oxidase (GOx) nanocomplex was developed via self-assembly and exhibited excellent catalytic activity toward cascade reactions. Compared to mixed GOx and CeO₂, the nanocomplexes displayed enhanced efficiency, mainly attributed to the minimal diffusion of intermediate in the nanocomplexes system. Moreover, the nanocomplexes exhibited outstanding long-term stability and excellent recyclability. Benefiting from these merits, a highly sensitive and selective biosensor for colorimetric detection of glucose was constructed based on CeO₂/GOx nanocomplexes. Such a self-assembled nanozyme complex offers a simple and efficient example to build spatially confined multi-enzyme systems to potentiate their applications in energy conversion, detoxification and bioanalysis

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Cellular regulatory programs usually require the orchestration of multi-enzyme processes to carry out a consecutive series of biochemical reactions, executing various exquisite life processes such as energy conversion, biosynthesis, detoxification and signal conduction [1,2]. To be specific, different types of enzyme are spatially confined in organelles or self-assembled into complexes [3,4]. Such close confinement helps to entrap the intermediates on the confined multi-enzyme systems and minimize the diffusion of intermediates [3–5]. As a result, the conversion of intermediates into product is significantly accelerated, and side reactions are greatly inhibited, thus remarkably enhancing the overall efficiency and specificity [3–7]. Construction of these spatially confined multi-enzyme systems not only provides deep insight to physiological and pathological mechanisms but also is of great importance for biosynthesis, bioanalysis and biomedicine [3,4]. Recently, a host of strategies, including liposomes encapsulating [8], polymersomes entrapping [1], chemical crosslinking [9,10] and covalent conjugation [11,12], have been exploited to arrange multiple enzymes into a confined space for the construction of artificial multi-enzyme complexes. Although promising, there are still several obstacles remaining which may impede the practical applications. For example, synthesis of these multi-enzyme

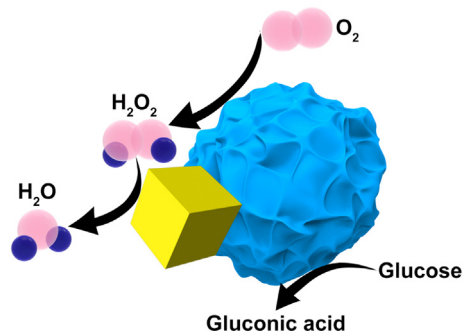
complexes involves tedious preparations and multiple steps, leading to the loss of enzyme activity and further decreasing the catalytic efficiency [12–15]. Therefore, it remains greatly challenging to develop a simple and efficient strategy to build spatially confined multi-enzyme complexes.

Owing to their robust stability and easy synthesis, nanozymes have attracted numerous interests and been regarded as a promising alternative to natural enzymes [16–22]. Among various nanozymes, CeO₂ is of particular interest because of its exceptional oxygen-storage capacity, mixed oxidation state (Ce(III) and Ce(IV)), and high catalytic reactivity [23–27]. Through switching between Ce(III) and Ce(IV), CeO₂ exhibits strong redox behavior and display mimetic properties of multiple enzymes such as oxidase, catalase, superoxide dismutase and peroxidase [28,29]. More significantly, the high specific surface area contributes to rich surface Ce atoms and offers numerous active sites for interaction with biomolecules, making nanoceria as a promising building block for construction of multicomponent and multifunctional biomaterials [30–33]. In this regard, self-assembly of CeO₂ and natural enzymes could be a simple and highly promising strategy to construct highly efficient multi-enzyme nanocomplexes.

In this work, taking self-assembled CeO₂/glucose oxidase (CeO₂/GOx) as an example, we demonstrate the feasibility to co-confine CeO₂ and enzymes in a restricted space to form hybrid nanozyme complexes. As shown in Scheme 1, in the hybrid nanozyme complexes, glucose oxidase can catalyze the oxidation

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Scheme 1. Schematic illustration of the cascade reaction catalyzed by hybrid CeO_2/GOx complexes.

of glucose with the simultaneous generation of H_2O_2 , and at the same time, H_2O_2 can be immediately degraded by CeO_2 with minimal diffusion. As a result, CeO_2/GOx exhibits improved catalytic efficiency when compared to mixed GOx and CeO_2 nanoparticles. In addition, the hybrid nanozyme complex displays excellent recyclability and long-term stability. Benefiting from these merits, the hybrid nanozyme complexes were utilized to construct a colorimetric glucose biosensor with high selectivity and sensitivity. Our demonstration of self-assembled CeO_2/GOx hybrid nanozyme complexes offers a vivid and simple example of efficient multi-enzyme systems, holding great promise in biosensing, biosynthesis and biomedicine.

CeO_2 nanoparticles were prepared according to a previously reported method with a facile hydrothermal synthesis [25]. Transmission electron microscopy (TEM) was used to investigate the structure and morphology of the obtained CeO_2 nanoparticles. As shown in Fig. 1a, it can be clearly seen that the CeO_2 nanoparticles distributed homogeneously with a narrow size distribution about 4.5 nm. High-resolution TEM image (Fig. S2 in Supporting information) further shows a well-defined crystalline fringe of CeO_2 nanoparticles and that the interplanar spacing is 0.32 nm, corresponding to the d spacing of (111) plane. Energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD) were also used to investigate the structure and composition of CeO_2 nanoparticles, as presented in Figs. S4 and S7 (Supporting information), respectively. All these results indicate that CeO_2 nanoparticles were successfully synthesized. Then, the obtained nanocrystals were assembled with GOx and TEM were performed to characterize the morphology of negatively-stained GOx and CeO_2/GOx nanocomplexes. For TEM observation, GOx and CeO_2/GOx solution was respectively dropped onto a carbon-coated copper grid and dried in air at room temperature, followed by

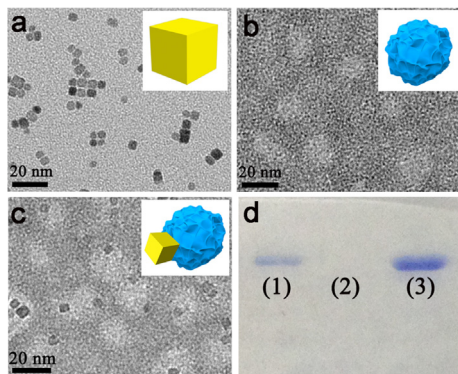


Fig. 1. TEM images of (a) CeO_2 , (b) glucose oxidase (negatively stained) and (c) CeO_2/GOx nanocomplexes (negatively stained). (d) SDS-PAGE gels of (1) CeO_2/GOx nanocomplexes, (2) CeO_2 and (3) GOx.

negatively staining with 2% phosphotungstic acid. It is obvious in Fig. 1b that the GOx displays an excellent dispersity with an average size about 15 nm. The image of CeO_2/GOx nanocomplexes (Fig. 1c) shows GOx are assembled to the surface of CeO_2 nanoparticles, and these two build units are interacted tightly to form well-integrated nanocomplexes. The SDS-PAGE result (Fig. 1d) shows identical mobilities for GOx and CeO_2/GOx nanocomplexes, further indicating the integrality of nanocomplexes. Taken all together, these results confirmed the successful assembly of CeO_2/GOx nanocomposites.

To investigate the catalytic properties of CeO_2/GOx nanozyme complexes, a classic colorimetric reaction was conducted whereby ABTS serves as a chromogenic reagent. With the cascade catalytic reaction, produced $\text{ABTS}^{\bullet+}$ exhibits a strong absorbance at 420 nm. As is shown in Fig. 2a, when there is only GOx alone in the solution, the reaction system shows no obvious color change as well as negligible absorbance variation at 420 nm. Similar results were obtained for the solution containing only CeO_2 nanoparticles, indicating that only GOx or CeO_2 nanoparticles alone cannot catalyze this cascade reaction. In the presence of CeO_2/GOx nanozyme complexes, the reaction solution containing CeO_2/GOx nanocomposites displays a remarkable absorbance variation, and the color of the solution system quickly changes to deep green in a few minutes. Even though the solution containing mixed CeO_2 and GOx also displays an absorbance variation at 420 nm, the intensity change is much lower than that of the system containing nanocomplexes, indicating that the catalytic activity of CeO_2/GOx

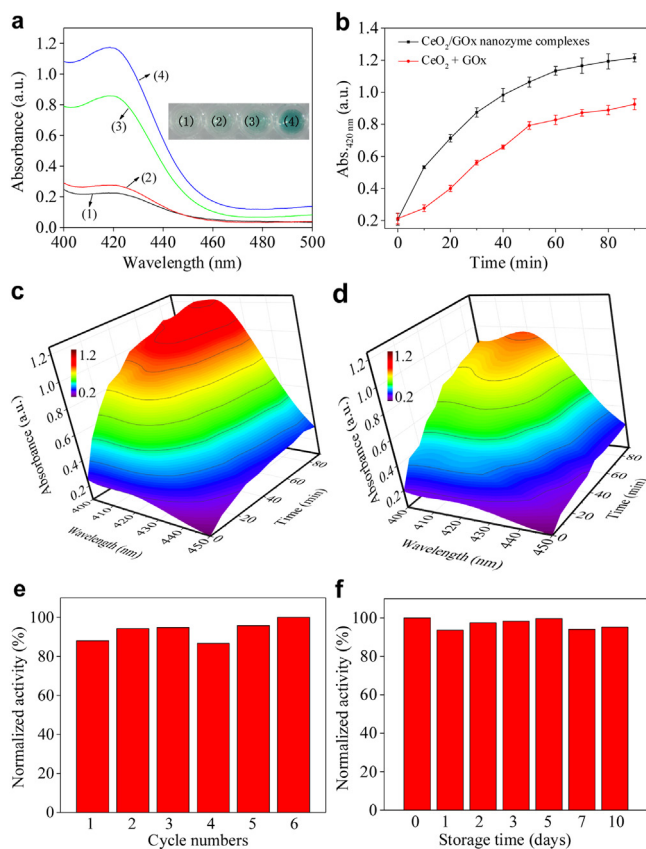


Fig. 2. (a) UV-vis absorption spectra of the reaction systems followed by the addition of (1) CeO_2 only, (2) GOx only, (3) the mixture of CeO_2 and GOx, (4) CeO_2/GOx nanozyme complexes. The corresponding photographs are presented in the insets. (b) Time-dependent absorbance intensity at 420 nm of the reactions solutions containing the mixture and nanozyme complexes. Time-dependent UV-vis absorption spectra of the reaction solutions containing (c) CeO_2/GOx nanozyme complexes and (d) the mixture. Cascade catalytic activity of the CeO_2/GOx nanocomplexes with different (e) cycle numbers and (f) storage time.

nanozyme complexes is much higher than that of mixed CeO₂ and GOx. Furthermore, time-dependent absorption intensity changes at 420 nm were measured to record the cascade reaction process. As presented in Fig. 2b, the absorption intensity of the solution containing CeO₂/GOx nanozyme complexes increases much faster than that of the solution containing mixed CeO₂ and GOx, indicating that the nanocomplexes exhibit a faster reaction rate compared to mixtures. A similar result was obtained when time-dependent absorbance spectra was analyzed and compared, as shown in Figs. 2c and d. The fast reaction rate of nanocomplexes can be ascribed to nanoscale spatial confinement of intermediates. To be specific, for the CeO₂/GOx nanozyme composites, since CeO₂ and GOx were binding closely together, the *in situ* generated H₂O₂ by GOx would easily react with the surrounding CeO₂ nanoparticles and be degraded with minimal diffusion. While for the mixed CeO₂ and GOx, the generated H₂O₂ needed to diffuse with a long distance to interact with CeO₂. Such a long diffusion distance decreases the catalytic rate for the cascade reactions, and moreover, H₂O₂ is likely to undergo self-decomposition without reaction with CeO₂, further decreasing the catalytic efficiency. These findings suggest that CeO₂/GOx nanozyme complexes display enhanced catalytic activity to the cascade reaction.

Stability especially long-term stability and recyclability is recognized as one of the most significant features. Enzymes with high stability can not only meet the requirements of practical use but also ameliorate the process economy of industrial enzymatic catalysis [11,34]. Therefore, the multi-enzyme with high stability is of critical importance for practical applications. The recycling experiments of CeO₂/GOx were carried out in the way that nanocomplexes were concentrated through centrifugation after every cycle, followed by washing twice with buffer solution and finally being redispersed for further use. As shown in Fig. 2e, only a little decrease of the catalytic activity was observed after 5 cycles. That is to say, the activity of the nanozyme composites maintained at a relatively high level after several reaction cycles. Probably, this slight decrease may be due to the loss of the nanocomplexes during centrifugation. In addition, the long-term stability CeO₂/GOx was also investigated. More specifically, the nanozyme complexes were stored at about 25 °C, and the catalytic activity to cascade reaction was measured during 10 days as presented in Fig. 2f. It can be observed that CeO₂/GOx nanocomplexes exhibited high catalytic efficiency even after storage for 7 days, indicating that the CeO₂/GOx nanocomplexes possess excellent long-term stability. Overall, the CeO₂/GOx nanocomplexes display excellent recyclability for several times and outstanding long-time stability over an extended period, holding great promise in practical applications.

Glucose plays an important role in biosystems as the energy source and metabolic intermediate of living cells [21]. The aberrant concentration of glucose is closely related to the occurrence and

development of many diseases such as high blood pressure, diabetes and cancer [35–37]. Therefore it is very important for the quantitative detection of glucose. To demonstrate the applications of CeO₂/GOx nanocomplexes, a high-performance biosensor was built for the colorimetric detection of glucose with high sensitivity and selectivity. In the presence of glucose, the reaction solution exhibits a characteristic absorbance spectrum with a peak at 420 nm. Moreover, with the increased concentration of glucose, absorbance intensity at 420 nm increases gradually and shows a positive correlation to glucose concentration. Fig. 3a shows that the absorbance at 420 nm was proportional to the concentration of glucose and that the biosensor can detect glucose solution with concentration as low as 25 μmol/L. As shown in the inset, it is obviously that with the increase of glucose concentration, the reaction solution exhibits a remarkable color change from colorless to deep green, suggesting that the concentration of glucose can be read out with the naked eye. These results indicate that CeO₂/GOx nanocomplexes-based biosensor can be utilized for the colorimetric detection of glucose with high sensitivity. Furthermore, the selectivity of CeO₂/GOx nanocomplexes-based biosensor was tested under the same conditions using fructose, galactose, mannose, maltose, lactose and saccharose as contrast. As indicated in Fig. 3b, all of these glucose analogues generate negligible absorbance variation. In contrast, there was an evident absorbance variation in the reaction system with glucose, demonstrating that the CeO₂/GOx nanocomplexes-based biosensing platform also displays high selectivity towards glucose. All these results demonstrated that the developed colorimetric method exhibited high sensitivity and selectivity toward glucose, exhibiting great potential in applications such as biosensing, fundamental research and disease diagnosis.

In summary, a hybrid nanozyme complex was developed through self-assembly of CeO₂ nanoparticles and GOx and displayed excellent catalytic activity toward cascade reactions. CeO₂ nanoparticles exhibit peroxidase-mimicking catalytic activity which can catalyze the H₂O₂ generated by GOx. Compared to mixed CeO₂ and GOx, the nanocomplexes showed much improved catalytic efficiency because of the minimal diffusion of intermediates. Moreover, CeO₂/GOx nanocomplexes also exhibited robust recycle stability and excellent long-term stability, holding great promise in practical applications. A colorimetric biosensor for glucose detection was built based on CeO₂/GOx nanocomplexes, and showed high sensitivity and selectivity, holding great promise in fundamental research and diagnosis. Given the multi-enzyme mimicking properties of CeO₂ nanoparticles and its general interaction with proteins, CeO₂ can be assembled with other enzymes to various multi-enzyme nanocomplexes, leading to the development of a new family of hybrid nanozyme complexes.

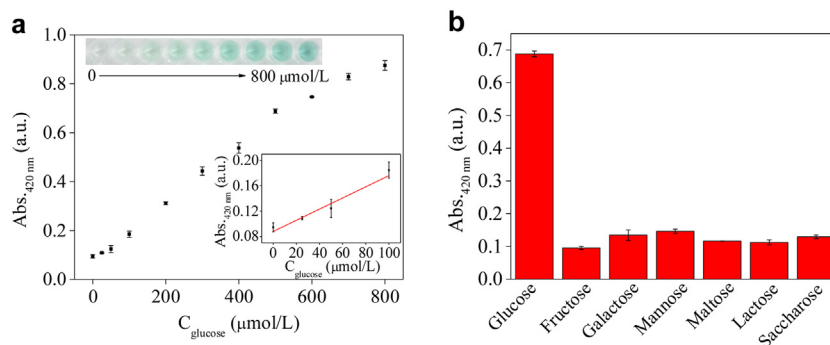


Fig. 3. (a) Absorbance of the reactions solution at $\lambda = 420$ nm with different glucose concentration. The insets show the corresponding photographs (top left) and the initial linear response (low right). (b) Selectivity assays for CeO₂/GOx-based glucose biosensors. The concentrations of glucose and its analogues are all kept at 1 mmol/L.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ccllet.2018.12.021>.

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