Short communication

Dual confinement of high–loading enzymes within metal–organic frameworks for glucose sensor with enhanced cascade biocatalysis

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ABSTRACT

The intrinsically fragile nature and leakage of the enzymes is a major obstacle for the commercial sensor of a continuous glucose monitoring system. Herein, a dual confinement effect is developed in a three dimensional (3D) nanocage-based zeolite imidazole framework (NC-ZIF), during which the high-loading enzymes can be well encapsulated with unusual bioactivity and stability. The shell of NC-ZIF sets the first confinement to prevent enzymes leakage, and the interior nanocage of NC-ZIF provides second confinement to immobilize enzymes and offers a spacious environment to maintain their conformational freedom. Moreover, the mesoporosity of the formed NC-ZIF can be precisely controlled, which can effectively enhance the mass transport. The resulted GOx/Hemin@NC-ZIF multi-enzymes system could not only realize rapid detection of glucose by colorimetric and electrochemical sensors with high catalytic cascade activity (with an 8.3-fold and 16-fold enhancements in comparison with free enzymes in solution, respectively), but also exhibit long-term stability, excellent selectivity and reusability. More importantly, the based wearable sweatband sensor measurement results showed a high correlation (>0.84, P < 0.001) with the levels measured by commercial glucometer. The reported dual confinement strategy opens up a window to immobilize enzymes with enhanced catalytic efficiency and stability for clinical-grade noninvasive continuous glucose sensor.

1. Introduction

Enzymes as the natural biocatalysts, have a wide variety of applications in pharmaceutical, medical, chemical and food industries owing to their high efficiency, specificity and selectivity (Green and Elisseeff, 2016; Klibanov, 2001). However, due to the intrinsically fragile nature, enzymes usually degrade/denature in high temperatures, rigorous pH, or long-term operation, which significantly impedes their practical industrialization (Klibanov, 2001). Although immobilization of the enzymes within inorganic supports (e.g., mesoporous silica (Fried et al., 2013; Hartmann and Jung, 2010; Hudson et al., 2008), sol-gel matrices (Gill and Ballesteros, 1998), hydrogels (Lee et al., 2007; Lee and Yuk, 2007; Sheldon, 2007) and porous carbon materials) have been widely utilized to enhance the enzymes stability in storage and operational conditions, the substantial challenges, including low loading efficiency and the denaturation of enzymes because of incompatible embedded process (Sun et al., 2019), remain to be overcome.

Metal–organic frameworks (MOFs) composed of organic ligands and...
metal containing nodes (Furukawa et al., 2013; Huang et al., 2020; Zhou et al., 2012), possessing high surface area, designable functionality and robust structure (Furukawa et al., 2013; Xiao et al., 2016; Zhou et al., 2012), exhibit excellent compatibility with biomacromolecules (Chen et al., 2019; Feng et al., 2019; Liu et al., 2017; Liang et al., 2015, 2016; Liao et al., 2017; Lyu et al., 2014; Morris et al., 2008; Pang et al., 2018; Tang et al., 2015, 2021; Wang et al., 2017; Zhang et al., 2018a, b; Zhu et al., 2017). These merits endow MOFs with huge potentials to host enzymes (Chen et al., 2012; Feng et al., 2015, 2019; Ikezoe et al., 2012; Li et al., 2016; Liang et al., 2015; Shieh et al., 2015; Wu et al., 2015), which greatly eases the aforementioned problems caused by traditional inorganic solids. However, most of the reported MOFs have restricted pore size smaller than 2 nm, which is unfavorable to the mass transport and conformational retainment of the native enzymes, thus weakening the bioactivity of enzymes. Additionally, the packed enzymes within the common MOFs trend to leaching under long-term operational conditions due to weak interaction between the enzymes and MOFs (the metal nodes and organic units are saturated coordination) (An et al., 2019). Therefore, a more efficient and universal approach is still needed to enhance stability but retain bioactivity for confining enzymes within MOFs.

Herein, we report a dual-confinement strategy that can not only doubly confine high-loading enzymes within nano-aggregated zeolite imidazole framework (NC-ZIF), but also enable them with a high activity and stability. Different from the enzymes confined within conventional ZIFs (enzymes@ZIF), the obtained enzymes@NC-ZIF could exhibit good mass transport and retain excellent conformational freedom of enzymes, due to the enhanced mesoporosity in NC-ZIF. More importantly, by adjusting the etching times, we can realize the precise regulation of the mesoporosity in NC-ZIF to optimize the performance of packed enzymes. Catalytic tests demonstrate that the enzymes@NC-ZIF with ~4 nm mesopores performs an 8.3-fold and 16-fold enhancements in comparison with the enzymes@ZIF, revealing the perspiration glucose is high-correlated to the blood glucose.

2. Materials and methods

All Materials and reagents, characterization, the positron annihilation lifetime measurement, synthesis of partial catalysts are detailed in Supporting Information.

2.1. Synthesis of the enzymes@NC-ZIF

GOx/Hemin@NC-ZIF was synthesized by sacrificial template strategy as follows: Co(NO₃)₂·6H₂O (0.273 g), Zn(NO₃)₂·6H₂O (0.278 g) and 2-methyl imidazole (0.303 g) were dissolved in 3.75 ml, 3.75 ml and 7.5 ml methanol under ultrasound for 5 min at room temperature, respectively. After forming homogeneous solution, 2-methyl imidazole methanol solution was mixed with Co(NO₃)₂·6H₂O methanol solution slowly by syringe. Next, 75 mg GOx and 60 mg Hemin were added into the above mixed solution and keeping ultrasonic for 5 min. Then, Zn(NO₃)₂·6H₂O methanol solution was further added into the above-mentioned solution by syringe under ultrasound for 10 min. Subsequently, the suspensions were centrifuged at 11,000 rpm for 3 min, and the precipitates were dispersed in deionized water-ethanol solution by syringe under ultrasound for 10 min. Afterward, the ultrasound centrifuged products were washed with methanol three times and cleaned by centrifugation. Finally, the as-prepared GOx/Hemin@NC-ZIF was freeze-dried for further use.

The same method was conducted for other enzymes, where Hemin (60 mg), GOx (75 mg), GOx (75 mg) and β-Gal (75 mg) were added instead of Hemin and GOx.

2.2. Colorimetric detection of glucose

TMB (20 μL, 0.6 mM) and GOx/Hemin@NC-ZIF (20 μL, 2 mg/mL) were added into a 96-well plates containing PBS buffer (140 μL, 10 mM, pH 7.2). Equal amounts of GOx/Hemin@ZIF or free GOx and Hemin were used instead of GOx/Hemin@NC-ZIF as a control. The catalytic reaction was initiated by adding glucose (20 μL, 1 mM). The mixed solution was incubated at room temperature for 30 min and collected. Finally, the catalytic cascade bioactivity was detected at 652 nm using a THERMO Varioskan Flash spectrophotometer and evaluated through tracing the production of H₂O₂.

2.3. Colorimetric biosensor of glucose

TMB (20 μL, 0.6 mM) and GOx/Hemin@NC-ZIF (20 μL, 2 mg/mL) were added into a 96-well plates containing PBS buffer (140 μL, 10 mM, pH 7.2). Then, 20 μL glucose solution with different concentrations was added, accompanied by the color changed from colorless to blue. The mixed solution was incubated at room temperature for 30 min and collected. Finally, the catalytic cascade bioactivity was detected at 652 nm using a THERMO Varioskan Flash spectrophotometer.

2.4. Electrochemical characterization of GOx/Hemin@NC-ZIF electrodes

The electrodes were modified as follows: the glassy carbon electrodes (GCE, 3.0 mm) were consecutively polished with 1.0 and 0.5 μm alumina slurry and cleaned with deionized water, followed by sonication in 1:1 nitric acid, ethanol and deionized water for 3 min, respectively. Then, the electrodes were dried under room temperature. 20 mg biocatalysts were dispersed in 4 mL of deionized water/Nafton (ν/ν = 19:1) by sonication for 40 min. Then, 10 μL well-dispersed GOx/Hemin@NC-ZIF (5 mg/mL) was drop-casted on the surface of glassy carbon electrode and dried at room temperature (noted as GOx/Hemin@NC-ZIF GCE).

The electrochemical characterization was performed with a CHI 760 electrochemical workstation (Shanghai Chenhua, China) in a three-electrode system with a sample volume of 50 mL PBS buffer (10 mM, pH 7.2) electrolyte. A GOx/Hemin@NC-ZIF GCE served as the working electrode, Ag/AgCl was used as reference electrode, and graphite carbon was used as counter electrode. CVs were measured in the potential range from 0 to 0.80 V vs. Ag/AgCl reference electrode under a sweep rate of 50 mV s⁻¹ in O₂-saturated electrolyte. The electrolyte was continuously stirred during measurements with a magnetic stirring bar at a speed of 300 rpm.

2.5. Electrochemical glucose sensor in perspiration

The electrochemical biosensor of Glucose was performed on a CHI 760 electrochemical workstation (Shanghai Chenhua, China) in a three-electrode system with a sample volume of 10 mL perspiration as electrolyte. Carbon paste with GOx/Hemin@NC-ZIF was prepared and screen-printed on a flexible PET substrate as the working electrode, carbon paste and Ag/AgCl paste were used as counter electrode and reference electrode, respectively. Amperometric measurement was carried out under an applied potential of 0.6 V.

2.6. In situ analysis of sweat glucose

Subject’s forehead was wiped and cleaned with alcohol swab before wearing the sensor. Next, the subject was asked to wear a sweatband embedded with the all-integrated sensing system and then bike for 30 min at a constant workload leg-cycle ergometer (Shuhua Upright Ergometer Exercise Bike, SH-B8901U1). Cycling protocol included a 5 min ramp-up and a 25 min biking at a power of 150 W. Data were directly recorded and transmitted to the mobile phone via Bluetooth.
3. Results and discussion

3.1. Characterization of the catalysts

Fig. 1a presents the schematic for the fabrication of GOx and Hemin within NC-ZIF. The ZIF-67 rhombododecahedron with an average diameter of 280 nm was firstly synthesized through a bottom-up method (Fig. 1b, Supporting Information, Fig. S1). Subsequently, GOx and Hemin were uniformly adsorbed on the surface of ZIF-67 (denoted as ZIF-67@GOx/Hemin) due to the well coordination between the Co cations and the carbonyl group of the proteins. Then the ZIF-67@GOx/Hemin was used as template for the ZIF-8 epitaxial growth to obtain a well-defined core-shell composite (denoted as ZIF-67@GOx/Hemin@ZIF-8) (Fig. 1c). Further, ZIF-67@GOx/Hemin@ZIF-8 was transformed into a three dimensional nanocage-based zeolite imidazole framework with GOx and Hemin dually confined inside (denoted as GOx/Hemin@NC-ZIF) by a phase transformation under H2O and ethanol continual etching at room temperature (Fig. 1e). Representative TEM images acquired at different reaction time reveal that a small nanocage was initially emerged in the center of the ZIF-67 (Supporting Information, Fig. S2). Then, the nanocage would become larger gradually with the increased etching time, accompanied with an enhanced strain (Jasuja et al., 2013; Liu et al., 2017). The generated strongly strain would contribute to a series of stably stacked nanoflakes within the cage, while the unstable part of ZIF-67 was selectively etched. Meanwhile, the dissociated Co2+ would partly exchange with the Zn2+ of ZIF-8 layer to form a unique Zn/Co ZIF shell (Fang et al., 2020; Han et al., 2016; Zhang et al., 2017). As shown in Fig. 1c–e, no nanocage was existed in GOx/Hemin@NC-ZIF initially. However, after reaction for 24 h, a nanocage with diameter of 190 nm can be observed and its diameter was increased to 268 nm at 48 h. The generated nanocage can be further demonstrated by the discrete element distribution of N, Co, Zn and Fe on a line scan and EDS mapping images (Fig. 1f and g). Also, the Fe element is mainly concentrated in the nanocage, whereas Zn is existed in the shell of NC-ZIF, which further verify Hemin is successfully encapsulated within NC-ZIF.

Confocal laser scanning microscopy (CLSM) demonstrates that fluorescein isothiocyanate labelled GOx (FITC-GOx) of FITC-GOx/Hemin@NC-ZIF is mainly embedded in NC-ZIF (Fig. 2a). However, by physically mixing FITC-GOx with Hemin@NC-ZIF (details see the supporting information), the resulted FITC-GOx is only distributed on the surface of NC-ZIF (Fig. 2b). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) experiments were designed to demonstrate the specific location of enzymes. The GOx/Hemin@NC-ZIF shows a clear indicative band in 79 KD, corresponding to the molecular weight of monomeric GOx, suggesting the enzymes are successfully confined within NC-ZIF. But no band is observed in the GOx on Hemin@NC-ZIF after cleaning and digestion, indicating the interaction between the Hemin@NC-ZIF surface and the GOx molecules is rather weak, further suggesting no GOx is adsorbed on the surface of Hemin@NC-ZIF after washing (Supporting Information, Fig. S8). The Fourier transform infrared spectroscopy (FTIR) reveals the stretches characteristic of GOx at 1610-1700 cm\(^{-1}\) and 1500-1580 cm\(^{-1}\), corresponding to amide I and amide II (Barth, 2007; Jackson and Mantsch., 1995) (Fig. 2c). These results well demonstrate that the GOx and Hemin are successfully encapsulated within NC-ZIF.
Positron annihilation (PA) technique was adopted to further evaluate the specific location of enzymes relative to the NC-ZIF. The positron lifetime spectra were measured in vacuum with five lifetime components, which are $\tau_1$ (corresponding intensity of $I_1$), $\tau_2$ ($I_2$), $\tau_3$ ($I_3$), $\tau_4$ ($I_4$) and $\tau_5$ ($I_5$) by using LTv9 program for solid ZIF, NC-ZIF, Hemin@NC-ZIF and GOx/Hemin@NC-ZIF. Given that the electron density of ZIF is quite low, the positronium (Ps) is prone to form in the open volume regions. Therefore, the $\tau_1$ and $\tau_2$ are originated from the positron annihilation with free electrons and the spin-singlet positronium (p-Ps, mean lifetime (around 125 ps) much shorter than 1 ns) annihilation. The three long lifetime components ($\tau_3$, $\tau_4$ and $\tau_5$) that longer than 1 ns correspond to the spin-triplet positronium (o-Ps) annihilation in pores of different sizes. From the intensity comparison of three samples, $I_5$ decreases from 2.2% to 0.8% and the long lifetime ($\tau_5$) component diminishes after loading Hemin and GOx, suggesting that the free space in NC-ZIF becomes smaller, which further verifies that NC-ZIF-confined Hemin and GOx composites are indeed formed (Supporting Information, Fig. S9; Table S1-2). Based on a spherical infinite potential well model (Tao–Eldrup model) (Tao, 1972), the average radius ($R$) of free-volume holes could be estimated from the o-Ps lifetime ($\tau_5$), $\tau_4$ and $\tau_3$. The results reveal that NC-ZIF and enzymes@NC-ZIF possess 3–4 nm mesopores and the solid ZIF 1.1 nm micropores (Fig. 2d), agreeing well with the pore sizes distribution obtained from $N_2$ adsorption-desorption isotherm experiments (the mesopores are centered at 4.0 nm for NC-ZIF and the micropores are centered at 1.1 nm for solid ZIF; Fig. 2e, Supporting Information, Fig. S10). The specific surface area of NC-ZIF, Hemin@NC-ZIF and GOx/Hemin@NC-ZIF are estimated to be 1859 m$^2$g$^{-1}$, 1176 m$^2$g$^{-1}$ and 407 m$^2$g$^{-1}$, respectively (Supporting Information, Fig. S11). The large surface area of NC-ZIF is extremely beneficial for high-loading enzymes. X-ray diffraction (XRD) pattern of the GOx/Hemin@NC-ZIF shows no obvious changes in comparison with initial pattern of NC-ZIF, further verifying that the confinement of Hemin or GOx has negligible effects on the formation of NC-ZIF crystals (Fig. 2f).

Inductively coupled plasma-mass spectroscopy (ICP-MS), X-ray photoelectron spectroscopy (XPS) and Thermogravimetric analysis (TGA) measurement were further used to quantify the enzymes in GOx/Hemin@NC-ZIF. ICP-MS result reveals that the Fe content of GOx/Hemin@NC-ZIF is about 1.66 wt% (Supporting Information, Table S3) and XPS analysis reveals the Fe content on the surface of GOx/Hemin@NC-ZIF is about 1.5 at% (Fig. 2g and Table S4), both indicating the existence of a large amount of enzymes in NC-ZIF. TGA analysis further demonstrates that the weight loss of GOx is about 15.9 wt% over the whole evolution process (Fig. 2h), as further demonstrated by Bradford assay (20 wt%; Supporting Information, Fig. S15), indicating the high loading efficiency of enzymes in NC-ZIF, which is higher
than most of the reported MOF-encapsulated enzymes (Chen et al., 2019; Lyu et al., 2014; Shieh et al., 2015; Wei et al., 2019; Zhou et al., 2014) (Supporting Information, Table S5).

Moreover, this dual-confinement strategy was applied to other proteins (e.g., β-Gal). As shown in Fig. S16, a series of characterization analysis (TEM, SEM, PXRD, FTIR, CLSM, and SDS-PAGE) all confirm that the proteins were successfully confined within NC-ZIF, indicating the good generality of our strategy.

3.2. Cascade reactions conducted in GOx/Hemin@NC-ZIF systems

To explore the peroxidase-like activity of GOx/Hemin@NC-ZIF, 3,3’,5,5’-tetramethylbenzidine (TMB) was chosen as the peroxidase substrate. Fig. S18 shows that GOx/Hemin@NC-ZIF can oxidize colorless TMB to a typical blue oxTMB (monitored by absorption at 652 nm) in the presence of H$_2$O$_2$, indicating the peroxidase-like activity of GOx/Hemin@NC-ZIF. Furthermore, the absorbance changes of TMB were investigated at different pH (pH 3.0–11.0) and temperatures (20–100 °C). Experiment results reveal that the optimized pH and temperature are 4.0 and 50 °C (Supporting Information, Fig. S19). According to the fitted Michaelis-Menten curves and Lineweaver-Burk plots, the $V_{\text{max}}$ was calculated as 26.4 × 10$^{-8}$ M s$^{-1}$ for TMB and 113.8 × 10$^{-8}$ M s$^{-1}$ for H$_2$O$_2$, indicating the higher peroxidase-like activity of GOx/Hemin@NC-ZIF, exceeding free Hemin and many reported catalysts (Alizadeh et al., 2018; Gao et al., 2007; Qin et al., 2013; Wang et al., 2017) (Supporting Information, Fig. S20-21; Table S6).

The enzymatic cascade reaction was firstly performed in chromogenic system based on the bifunctional GOx/Hemin@NC-ZIF catalyst. As shown in Fig. 3a and Fig. S22a-b, GOx/Hemin@NC-ZIF catalyzed the glucose oxidation by molecular oxygen to produce gluconic acid and H$_2$O$_2$, and then the generated H$_2$O$_2$ catalyzed the oxidation of TMB resulting in a colored product oxTMB. However, no color changes were observed in Hemin@NC-ZIF and GOx@NC-ZIF, indicating that the necessity of integrating both GOx and Hemin within NC-ZIF for the cascade reactions. GOx/Hemin@NC-ZIF shows a 1.7-fold and 8.3-fold enhancements in the catalytic activity in comparison with GOx/Hemin@ZIF and free GOx/Hemin (Fig. 3d, Supporting Information, Fig. S22c). The absorbance of oxTMB gradually increases with the glucose concentration in GOx/Hemin@NC-ZIF system. A good linear relationship between the absorbance of oxTMB and glucose concentrations is established in the range of 1–20 mM with a limit of detection (LOD) of 10 μM, surpassing to most of the reported colorimetric glucose sensors (Fig. 3b-c, Supporting Information, Table S7). Moreover, the correlation between mesoporosity and activity of the GOx/Hemin@NC-ZIF was explored. Fig. S23 exhibits a volcano relationship between catalytic efficiency and mesoporosity, suggesting the optimal catalytic ability can be obtained after etching for 48 h.
3.3. Stability, reproducibility and selectivity of glucose sensor

The stability of the formed GOx/Hemin@NC-ZIF was explored under various denaturing conditions, such as high temperature, urea, DMF and DMSO. Fig. 3e shows that the GOx/Hemin@ZIF and GOx/Hemin@NC-ZIF retain 80.1% and 72.1% bioactivity after exposing to 80 °C, while only 9.6% for free GOx/Hemin. Urea, a chaotropic agent, can induce loss of bioactivity by unfolding proteins (Monera et al., 1994). The bioactivity of urea-treated GOx/Hemin@ZIF and GOx/Hemin@NC-ZIF maintain 82.3% and 84.6%, respectively. In contrast, free GOx/Hemin decreases to 27%. GOx/Hemin@ZIF and GOx/Hemin@NC-ZIF still maintain 79.1% and 70.7% of its prior activity after exposing to DMF with 87.2% and 90.1% to DMSO. In comparison, the activity of free GOx/Hemin dramatically reduces when exposing to these organic solvents, exhibiting a high stability of GOx/Hemin@NC-ZIF for glucose sensor. This remarkably enhanced stability can be attributed to the dual confinement of enzymes. The shell of NC-ZIF as exoskeleton provides the first protective effect for the encapsulated enzymes, and the interior nanocage affords additional protection, which can significantly increase enzymes stability under operations.

In addition, the reproducibility of GOx/Hemin@NC-ZIF was measured with the same modification. As shown in Fig. S25a, the GOx/Hemin@NC-ZIF exhibits no significant loss of bioactivity for 10 cycles, the relative standard deviation (RSD) is about 2.4%. Also, no detectable changes in the SEM, TEM images and XRD spectra after 10 catalytic cycles, further indicating the good durability and reproducibility of GOx/Hemin@NC-ZIF for catalytic reaction (Supporting Information, Fig. S24). Furthermore, the selectivity of the GOx/Hemin@NC-ZIF was evaluated for the responses of the common interfering substances, including fructose, ascorbic acid, dopamine, uric acid, maltose and lactose. Fig. S25 shows that only glucose produces remarkable distinction in tests, suggesting a high selectivity for glucose sensor.

3.4. Glucose detection in perspiration

We also investigated the electrocatalytic glucose detection in conventional three-electrode system based on the GOx/Hemin@NC-ZIF. As shown in Fig. S27a, the anode peak current gradually increases along with the glucose concentration from 0 to 100 mM, indicating GOx/Hemin@NC-ZIF can oxidize glucose. Fig. S27b shows that GOx/Hemin@NC-ZIF exhibits a 16 times higher cascade reaction activity than free GOx/Hemin, indicating the synergetic effect between the two enzymes is significantly enhanced after confining in NC-ZIF. Moreover, we fabricated a miniaturized, portable and all-integrated glucose sensor, which has a great potential to replace the traditional, complex and large electrochemical instruments for point-of-care applications (POC). As shown in middle part of Fig. 3f, GOx/Hemin@NC-ZIF was used as the working electrode (WE), carbon paste and Ag/AgCl paste were served as the counter electrode (CE) and the reference electrode (RE), respectively. The results show the current continuously increases with the glucose concentration in perspiration. A good linearity range is also established from 50 to 600 μM with a LOD of 2 μM under an applied potential of 0.6 V (Fig. S27), which are better than the reported electrochemical glucose sensors based on the GOx@ZIF-8 (NiPd) (Wang et al., 2017), GOx-PANI (Uang and Chou, 2003) and single walled carbon nanotubes (Gao et al., 2011; Yu et al., 2014) (Supporting Information, Table S8). The exceptionally catalytic activity can be attributed to: 1) the large mesoporous in NC-ZIF that can capture more substrates and facilitate the substrate/product molecules diffusion and electronic transport; 2) the commodious microenvironment of the NC-ZIF can maintain the conformational freedom for released enzyme molecules from the tightly confined ZIF-67, which is favorable to remain their bioactivity. Following the ex situ analysis of perspiration, we also performed real time human sweat analysis using the fully integrated wearable device. As illustrated in Fig. 3f, a subject was asked to wear a sweatband embedded with the all-integrated sensing system during exercise. Real-time result is then wirelessly transmitted to a smartphone and displayed in a screen of the mobile phone. Fig. 3g and Fig. S28a show that the device measurement results (black dots) are well correlated with the commercial glucose meter results (red dots) in 2 weeks, with a high correlation coefficient of 0.846 (P < 0.001), indicating GOx/Hemin@NC-ZIF can retain high catalytic activity and stability in long-term operation.

4. Conclusions

In conclusion, we have developed a general and moderate dual confinement strategy that can controllably confine a series of enzymes in nanocage-based zeolite imidazole framework with a high-loading, high catalytic activity and stability. The obtained GOx/Hemin@NC-ZIF exhibits an excellent catalytic cascade reaction efficiency in colorimetric and electrochemical glucose biosensor. Moreover, the GOx/Hemin@NC-ZIF-based wearable sweatband sensor exhibits capabilities for long-term quantitative analysis and continuous real-time monitoring perspiration glucose. Although the GOx/Hemin@NC-ZIF provides a promising design for the flexible sensors, they are currently only available for sweat testing. The future research is expected to evaluate other bodily fluids to facilitate personalized and real-time physiological and clinical investigations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113695.

References
