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Robust Single-Molecule Enzyme Nanocapsules for Biosensing with Significantly Improved Biosensor Stability

Dhanjai, Xianbo Lu,* Lingxia Wu, Jiping Chen, and Yunfeng Lu

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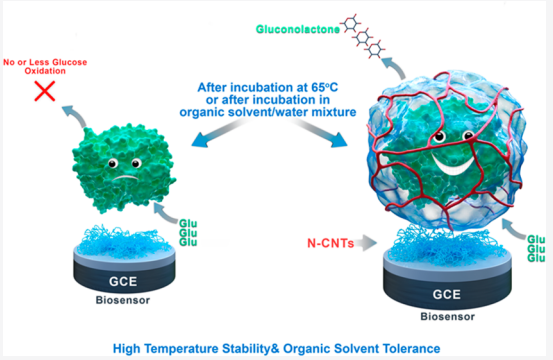
ABSTRACT: The present study demonstrates the use of highly stable single-molecule enzyme nanocapsules (SMENs) instead of traditional native enzyme as biorecognition element in enzyme-based biosensors. The main purpose of this study is to resolve the major obstacle and challenge in the biosensor field, i.e., the poor stability of enzyme-based biosensors, including thermal stability, organic solvent tolerance, long-term operational stability, etc. Highly active and robust SMENs of glucose oxidase (GOx, as a model enzyme) were synthesized (nGOx) using an in situ polymerization strategy in an aqueous environment. The particle-size distribution, transmission electron microscopic (TEM) images, and UV−vis spectral characterization revealed the formation of a thin polymer layer around each enzyme molecule. The polymer shell effectively stabilized the GOx enzyme core while enabling rapid substrate transportation, resulting in a new class of biocatalytic nanocapsules. Multiple covalent attachments between a thin polymer layer and an enzyme molecule strengthened the encapsulated GOx molecule. Encapsulation created a favorable microenvironment to avoid any structural dissociation at high temperature and helped to retain essential water during the organic solvent operation. The present work reports a study implementing nGOx SMENs as highly stable nano(bio)sensors for point-of-care diagnostic applications. Prepared nGOx SMENs manifested significantly improved thermal stability (even at 65 °C) and organic solvent tolerance without any compromise in biocatalytic activity. For example, the native GOx-based biosensor lost its catalytic activity for glucose after 4 h of incubation at high temperature (65 °C), while the nGOx/N-CNTs-Chi/GCE nano(bio)sensor maintained ∼56% of its original catalytic activity for glucose oxidation. The proposed SMENs-based nano(bio)sensors with robust stability in variable working environment could promote the development and applications of biosensors in point-of care diagnostics, biomedical detection, wearable devices, implantable equipment, and biofuel cells.



Proteins act as natural catalysts for biological reactions and exhibit enzymatic properties. In fact, enzymes are basically protein molecules that act as efficient bioreactors with significant selectivity and specificity. However, enzymes are highly sensitive to the external working environment (temperature, solvent, pH, etc.) and possess short shelf life, which consequently limits their applications in different fields.[1,2] Any improvement in increasing enzyme stability and shelf life is very crucial to broadening their applications.[3] Reportedly, encapsulating enzyme molecules within a permeable polymer shell significantly enhances their stability[4] without compromising their bioactivity. Enzyme nanocapsules are composed of a protein molecule enclosed within a very thin permeable and porous polymer layer that allows easy diffusion of substrate molecules to the active center of the enzyme.[5,6] Literature reports synthesis and fabrication of organophosphorus hydrolase (OPH) enzyme nanocapsules for detoxification and decontamination of organophosphate contaminants.[7] A typical synthesis procedure involves conjugation of an enzyme molecule with acryl functional groups followed by in situ polymerization. Similarly, catalase nanocapsules (nCATs) were prepared for tobacco smoke-free radicals scavenging.[8] In recent times, such single-enzyme/protein nanocapsules have been applied in a variety of biomedical research studies.[9−12] A bibliographic search suggested that, until now, such single-molecule protein (enzyme, DNA, RNA) nanocapsules were only applied to drug/protein delivery, organophosphate detoxification, scavenging of tobacco smoke-free radicals, etc. However, there are no reports available regarding their use as biosensors. Recent years have seen tremendous growth in developing enzymatic biosensors for health care and environmental monitoring.[13−15] However, different working environments such as a change in temperature, organic solvents, humidity, pH, etc. lead to a loss of bioactivity of enzymatic

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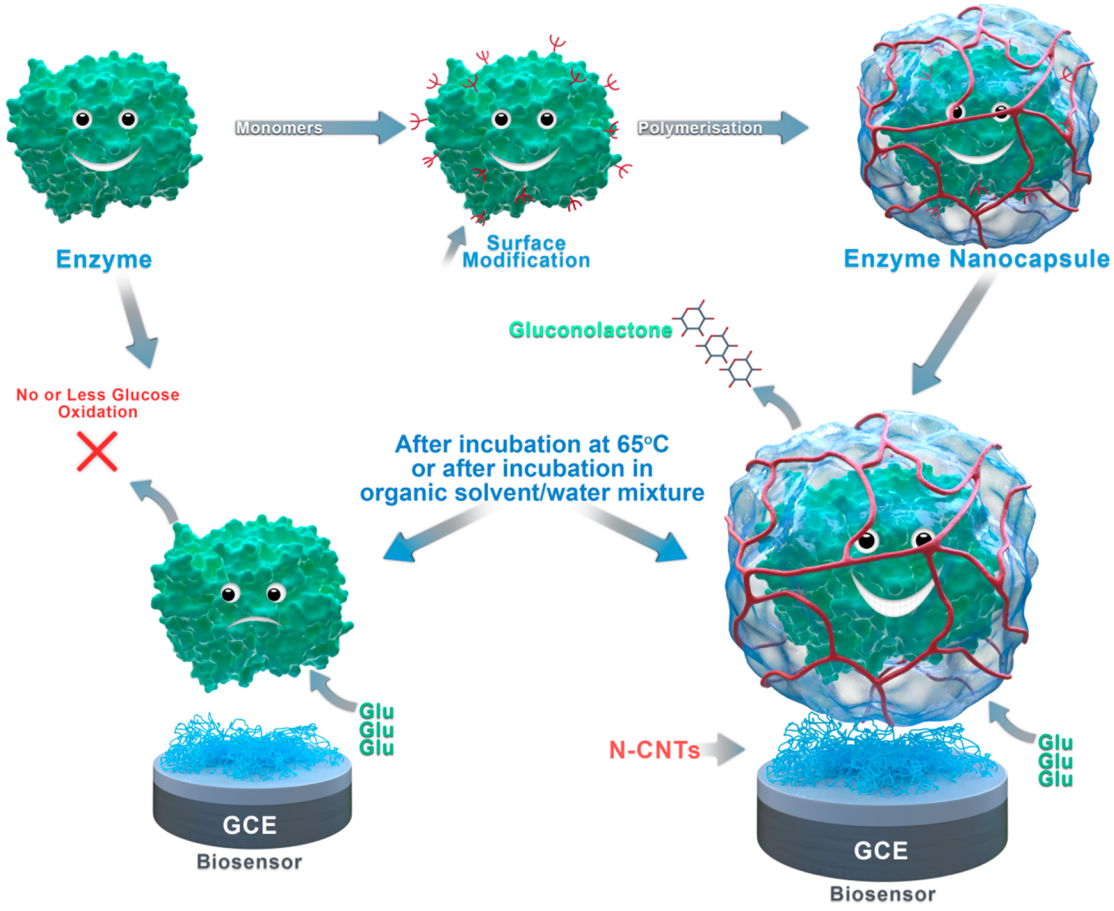
Scheme 1. Schematic Illustration of Preparation, Application, and Improved Stability of SMENs-Based Biosensor for Glucose Detection



biosensors despite considerable sensitivity and selectivity. Consequently, low stability of the enzyme molecules has become one of the major difficulties for their application in diagnostic systems. Thus, it is highly imperative to develop robust enzymatic biosensing systems with high sensitivity and improved stability irrespective of the working conditions.

Considering the biomedical applications of highly stable protein nanocapsules and in order to resolve the core technical problem of enzymatic biosensors stability, the present work has been aimed to prepare single-molecule enzyme nanocapsules (SMENs) of glucose oxidase (GOx) with significantly improved stability. Preparation of SMENs of GOx (nGOx) was accomplished by simulating the principle of a cell reactor and encapsulating the enzyme molecules within a semipermeable polymer membrane. The strategy of preparing highly active and robust enzyme nanocapsules involved a simple two-tier in situ polymerization method. In the first step, polymerizable vinyl/acryloyl groups were engineered on the enzyme (protein) surface by acryloxylation,[16,17] while the second step involved subsequent in situ polymerization in an aqueous environment encapsulating acryloxylated enzyme molecules. Each single-enzyme nanocapsule was composed of a protein (enzyme) core and a thin permeable polymer shell, which effectively stabilized the interior enzyme. Reportedly, the thin polymer shell was highly permeable for rapid substrate transportation, resulting in a novel class of biocatalytic nanocapsules with outstanding activity and stability.[18] The biocompatible microenvironment provided by the nanocapsules was used to resolve the problems of poor stability, low activity, and inactivation of enzyme molecules.[19−23] Enhanced tolerance of enzyme molecules to the external environmental effects resulted in an improved enzymatic biosensor sensitivity with significantly better stability and shelf life.

In recent times, heteroatom-doped nanomaterials have been applied to immobilize enzymes for biosensing purposes.[24−26] For instance, N-doped carbon nanotubes (N-CNTs) have been considered as an extraordinary support material for biosensor fabrication because of their improved structural and electrical properties. Moreover, their excellent electrocatalytic conductivity, large surface area, and biocompatibility significantly contribute to the ease of biosensor preparation as compared to pristine CNTs.[27,28] Because of the similarities in atomic size and valence electron states of N and C atoms, N-CNTs readily form a C−N bond. The extra valence electrons of N provide additional π electrons to the graphitic plane of carbon, which means that N doping crafts an electron-donor state in the conduction band, enabling CNTs to act as n-type conductors. Basically, the strong electronegativity of N creates permanent dipoles in C−N, which leads to enhanced electrical and catalytic properties of N-CNTs versus pristine CNTs or any other form of undoped carbon nanomaterials. Further-

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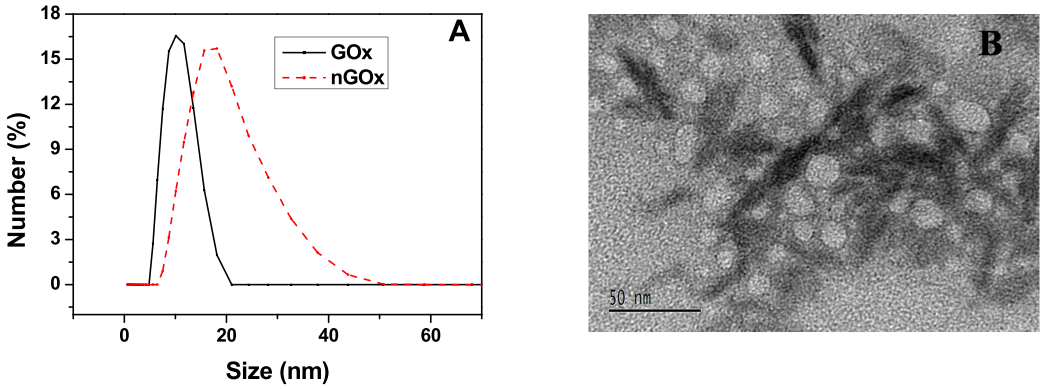

Figure 1. (A) Particle-size distribution of native GOx and nGOx molecules; (B) TEM image of nGOx nanocapsules.

more, it creates defects as well as functional group anchor sites on the CNT surface, making it a promising material in electrochemical biosensing.[29−31] The present work attempts to immobilize enzyme nanocapsules (nGOx) into three-dimensional conductive network of N-CNTs by intercalation assembly and mesoporous trap, so as to reduce the long-range electron transmission between the enzyme and the electrode.[32] The efficiency of the signal exchange/capture and the nanoscale amplification effect of N-doped CNTs have been used to improve the sensitivity and detection limit of the biosensor. Basically, by regulating the surface structure of nanocapsules and their enrichment effect on the specific substrates, a highly stable and sensitive nano(bio)sensor has been developed. The nanobiocomposite of enzyme nanocapsules and N-doped carbon nanotubes has been used to fabricate a glassy carbon electrode (GCE) to prepare a sensitive, robust, and highly stable nano(bio)sensor for glucose detection, as shown in Scheme 1. As a proof of concept, the present study demonstrates SMENs for application in enzyme-based biosensors for resolving the major obstacle and challenge in the biosensor field, i.e., the stability of enzyme-based biosensors (thermal stability, organic solvent tolerance, long-term stability, etc.). As a new promising nanotechnology with significantly improved biosensor stability in different working environments, the proposed SMENs-based nano(bio)sensor strategy would inevitably attract the general interest of chemists and promote the development of analytical fields, biomedical detection fields, wearable devices, implantable equipment, biofuel cells, etc.

EXPERIMENTAL SECTION

A detailed discussion regarding the preparation of SMENs and the construction of nano(bio)sensors has been provided in the Supporting Information. The procedure for preparation of SMENs includes conjugation with acryloyl groups and in situ polymerization methods (Figure S1).

RESULTS AND DISCUSSION

Structural and Morphological Characterizations of SMENs. Particle-size distribution and zeta-potential determination of nGOx and native GOx molecules were achieved by the dynamic light scattering (DLS) method. The native GOx molecules exhibited a negative charge of approximately −9.6 mV with a particle-size distribution centered at ∼10.1 nm. The average particle-size distribution of nGOx molecules was obtained around 15.6 nm, confirming the successful formation of a thin polymer layer of ∼2−3 nm thickness around each GOx molecule (Figure 1A). The complete intensity distribution plot (Figure S2A) showed that 95.2% peak intensity was observed for the first peak, indicating that most of the enzyme molecules are individually encapsulated. The nGOx molecules showed a less negative charge of approximately −6 mV due to encapsulation by the polymer layer. Figure 1B exhibits a TEM image of prepared nGOx nanocapsules indicating their spherical morphology.

The UV−visible spectra of GOx exhibited a characteristic peak near 277 nm and a couple of peaks at 372 and 447 nm corresponding to the oxidized flavin group (Figure S2B).[33,34] The characteristic absorption peaks of GOx remained in nGOx nanocapsules, suggesting that the encapsulated enzyme maintained its secondary structure and preserved the native GOx bioactivity. Furthermore, the TEM image of preprepared N-CNT showed a characteristic bamboo-like structure of N-doped carbon nanotubes with a length of several micrometers and diameters in the range of 20−50 nm (Figure S2C).

Enzymatic Bioactivity of SMENs. Recent years have seen significant effort to develop multiple strategies for encapsulation and immobilization of enzyme molecules. However, a considerable decrease in enzymatic bioactivity after encapsulation/immobilization remains a major problem that can be attributed to the steric hindrance and substrate-transport resistance. Therefore, in this work the single-enzyme molecules were separately encapsulated, resulting in the highly stable SMENs, but not at the expense of enzyme bioactivity. SMENs possess comparable bioactivity to native enzyme molecules. The enzymatic bioactivity of nGOx nanocapsules and GOx enzyme molecules was evaluated by the colorimetric method. In a typical reaction, the enzyme molecules within the nanocapsules oxidized glucose in the presence of O_2 at a reaction temperature of 35 °C and pH 5.1 (50 mM sodium acetate buffer). This reaction generated H_2O_2 as a byproduct, which in-turn oxidizes *o*-dianisidine (dye) in the presence of peroxidase enzyme (POD), resulting in an increment in color intensity.[35] The change in absorbance per minute (ΔA/min) was measured at 500 nm. This reaction has been expressed by eqs i and ii:

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{nGOx or GOx}} \text{D-glucono-1,5-lactone} + H_2O_2 \quad \text{(i)}$$

$$H_2O_2 + o\text{-dianisidine (reduced)} \xrightarrow{\text{POD}} o\text{-dianisidine (oxidized)} \quad \text{(ii)}$$

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The obtained results demonstrate that the constructed nGOx nanocapsules maintained ~86% of the native GOx bioactivity for glucose oxidation after encapsulation. The main advantage of the prepared nGOx nanocapsules was that the thin, permeable polymer layer provided free accessibility to enzyme substrate, which was evident from the obtained catalytic bioactivity.

Catalytic Activity of SMENs. *Immobilization of SMENs for Effective Electron Transfer.* The fabrication and sensing scheme for SMENs-based biosensors is shown in Figure S3. The nGOx and GOx-based nano(bio)sensors were constructed by a drop-casting method. Figure 2A exhibits the cyclic voltammograms of nGOx immobilized on bare GCE and different modified electrodes in phosphate-buffer solution (PBS) (100 mM, pH 7.01). There were no obvious peaks obtained for enzyme active centers at fabricated nGOx/GCE and nGOx-Chi/GCE electrodes, illustrating very weak or no electron transfer. However, immobilization of nGOx on N-CNTs-Chi/GCE resulted in the appearance of characteristic quasi-reversible peaks for FAD/$FADH_2$ reaction. The results indicated effective immobilization and catalytic electron transfer between the enzyme active center of GOx enzyme inside the nanocapsule and the transducer. This further demonstrated that the nGOx retained the catalytic behavior of native GOx and could be effectively immobilized onto the N-CNTs-Chi/GCE for fabrication of a catalytic nano(bio)-sensor.

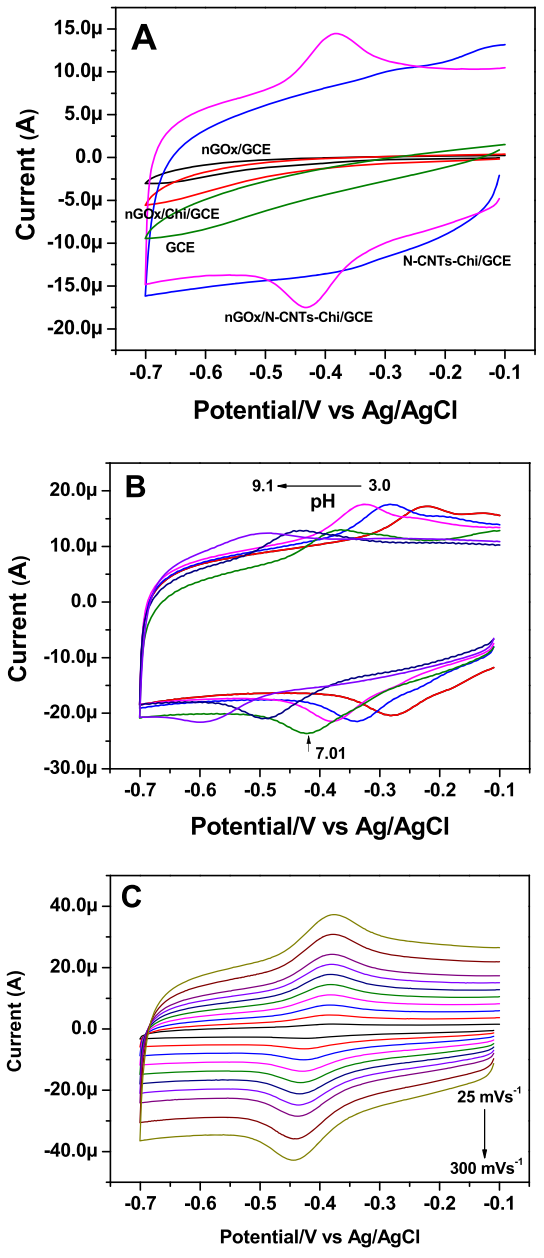

Figure 2. (A) CV of different electrodes in N_2-saturated 0.1 M phosphate buffer (pH 7.01) at 100 mV s^{-1} scan rate; (B) CV of nGOx/N-CNTs-Chi/GCE in 0.1 M N_2 saturated phosphate buffer at different pH values and (C) at different scan rates.

Furthermore, the effect of different pH values on the behavior of fabricated nGOx/N-CNTs-Chi/GCE nano(bio)-sensor was investigated. Figure 2B shows cyclic voltammograms of nGOx/N-CNTs-Chi/GCE in different pH ranges from 3 to 9. The fabricated biosensor showed well-defined redox peaks at almost all pH values, indicating high stability of the nGOx/N-CNTs-Chi/GCE nano(bio)sensor in a varying pH range; however, pH 7.01 was selected as optimum for further studies.

Cyclic Voltammetric Studies. Cyclic voltammetry (CV) of nGOx/N-CNTs-Chi/GCE was performed in nitrogen-saturated 0.1 M PBS at different scan rates (25−300 mV s^{-1}) (Figure 2C). Corresponding current responses for cathodic current (I_{pc}) and anodic current (I_{pa}) were plotted against the scan rate, as shown in Figure S4. The cathodic and anodic peak currents exhibited a linear relationship with scan rate (ν), indicating the surface-controlled electrode process and effective immobilization of nGOx for efficient electron transfer between the active enzyme center of nGOx nanocapsules and the electrode. The formal potential of nGOx/N-CNTs-Chi/GCE was about −0.4 V, which was consistent with previous literature results for native GOx molecule-based biosensors.[32,36] These results further confirmed that the GOx molecules retained their secondary structure and basic behavior, preserving the biocatalytic activity inside the nGOx nanocapsules.

Electrocatalysis of Glucose Molecules. The developed nGOx/N-CNTs-Chi/GCE biosensor was applied to study the electrocatalysis of glucose in air (oxygen)-saturated PBS (0.1M, pH 7.01). Figure 3A exhibits CVs of nGOx/N-CNTs-Chi/GCE in N_2-saturated PBS and in the presence/absence of glucose in air-saturated PBS. The redox peaks in the absence of glucose with a high cathodic current and a diminished anodic current corresponds to excellent catalytic activity for oxygen reduction reaction (ORR). However, a small quasi-reversible peak with almost equal anodic and cathodic current in N_2-saturated PBS was observed, corresponding to the GOx(FAD) and GOx($FADH_2$). The formal peak potential (−0.43 V) was observed to be consistent with previous studies.[32] On addition of 1 mM glucose, the nano(bio)sensor catalyzed glucose oxidation and consumed the available oxygen, resulting in the decrement of ORR peak current according to the following equations:[36]

$$\text{nGOx-FAD} + \text{glucose} \leftrightarrow \text{nGOx-FADH}_2 + \text{gluconolactone} \quad \text{(iii)}$$

$$\text{nGOx-FADH}_2 + \text{O}_2 \rightarrow \text{nGOx-FAD} + \text{H}_2\text{O}_2 \quad \text{(iv)}$$

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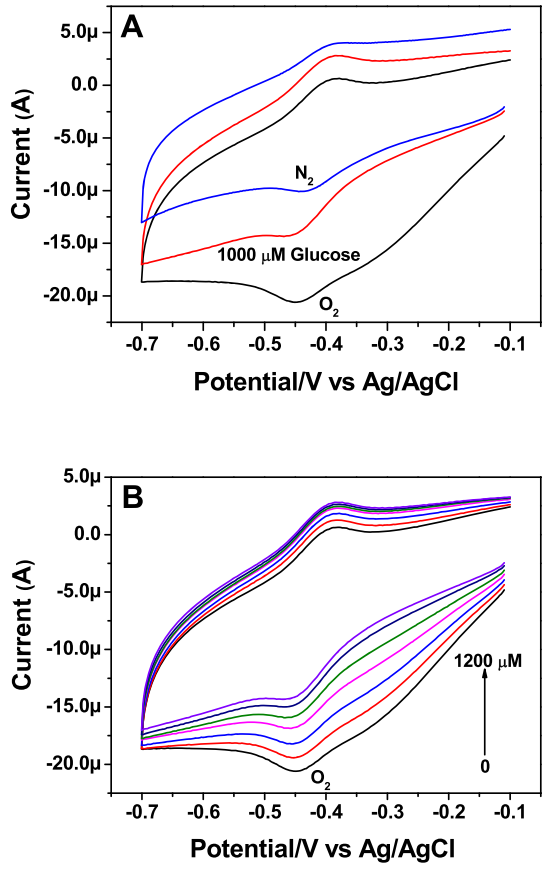

Figure 3. CV of nGOx/N-CNTs-Chi/GCE (A) in N_2-saturated PBS and in the presence/absence of glucose in air-saturated PBS and (B) at 100 mV s^{-1} in the presence of different glucose concentrations from 0 to 1200 μM in air-saturated PBS.

The cathodic peak current corresponding to ORR decreased linearly with the addition of glucose concentrations, as shown in Figure 3B. The nGOx/N-CNTs-Chi/GCE nano(bio)sensor effectively catalyzed glucose oxidation and ORR, performing the basic catalytic functions of native GOx molecules. This observation confirmed that nGOx nanocapsules retained native biocatalytic activity and can be successfully applied as biosensing elements.[36,37]

Stability of SMENs-Based Biosensor in Different Working Environments. Nanocapsules-based nGOx/N-CNTs-Chi/GCE nano(bio)sensors were investigated for their stability in different working environments using CV, and the performances were compared with native GOx-based biosensors. With comparable bioactivity and electrocatalytic activity, the nGOX-based nano(bio)sensor exhibited excellent stability against denaturation and deactivation at high temperature and showed greater organic solvent tolerance.

Thermal Stability. High temperature dissociates flavin cofactors from the enzyme centers, resulting in a conformational change and loss of native bioactivity.[38] Moreover, at high temperature, native GOx enzyme molecules tend to lose their essential water, leading to loss of their biological functions. Therefore, thermal stability is an important aspect for a nGOx/N-CNTs-Chi/GCE nano(bio)sensor to justify its applications in health monitoring and point-of-care diagnostics. To investigate thermal stability, a fabricated nGOx/N-CNTs-Chi/GCE nano(bio)sensor and a native GOx-based biosensor were incubated at different temperatures (45, 55, and 65 °C) for 1 h (60 min) and used to detect glucose before and after incubation using CV. As shown in the deactivation curve (Figure 4A), the thermal endurance for the biosensing activity

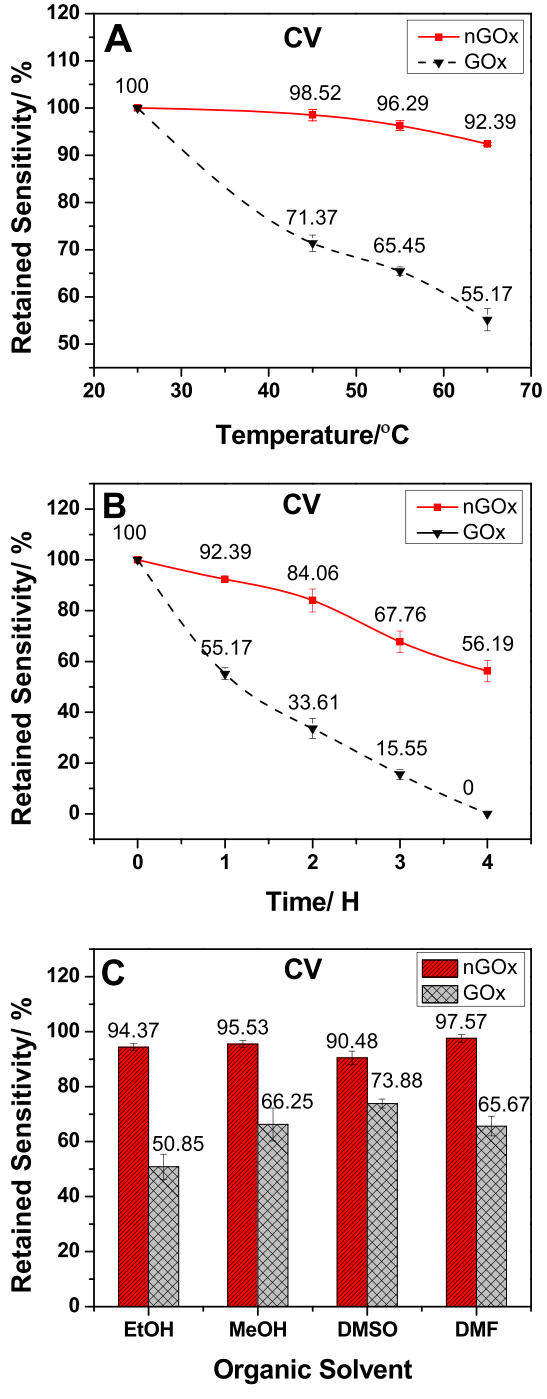

Figure 4. Relative sensitivity of nGOx/N-CNTs-Chi/GCE and GOx/N-CNTs-Chi/GCE after incubation (A) for 1 h at different temperature; (B) at 65 °C for 1, 2, 3, and 4 h; (C) for 1 h in the mixture of organic solvents and water (50:50) using CV.

of the nGOx/N-CNTs-Chi/GCE nano(bio)sensor was considerably improved because of the encapsulation of the GOx molecule within a thin polymer layer. Herein, the catalytic activity obtained at room temperature (25 °C) was treated as 100%. The native GOx-based biosensor showed fast and considerable loss in catalytic activity with the increase in

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temperature and retained only ∼55% of the original catalytic activity after incubation at 65 °C. However, the nGOx/N-CNTs-Chi/GCE nano(bio)sensor exhibited significantly enhanced stability and maintained ∼92% of its original catalytic activity for glucose oxidation (Figure 4A). The nGOx nanocapsules provided a suitable microenvironment for functioning of enzyme molecules even at high working temperature.[5,18] Furthermore, fabricated nano(bio)sensors were incubated at 65 °C over different time spans (i.e., 1, 2, 3, and 4 h) to study the operational thermal stability at high temperature. The deactivation curves for catalytic bioactivity for native GOx- and nGOx-based biosensors have been demonstrated in Figure 4B. The native GOx-based biosensor showed a fast and continuous loss in its catalytic activity for glucose. Moreover, it completely lost its activity at high temperature (65 °C) after 3−4 h of incubation due to denatured free GOx molecules, as observed by CV. On the other hand, because of the protected GOx molecules, the nGOx/N-CNTs-Chi/GCE nano(bio)sensor maintained ∼56% of its original catalytic activity for glucose oxidation even after 4 h of incubation (Figure 4B). The improved thermal stability of the nGOx/N-CNTs-Chi/GCE nano(bio)sensor can be attributed to the multiple covalent attachments between the thin polymer shells and the enzyme molecules. These covalent attachments hindered the thermal fluctuations inside the nanocapsules, restricting any conformational changes and thus protecting the enzyme molecules from denaturation at high temperature.[5,9,18]

Organic Solvent Tolerance. Preserving the enzyme catalytic activity in a polar organic solvent environment is a difficult and key task for their application in industrial and biomedical devices. Organic solvents tend to compete with essential water to form a hydrogen bond with protein structures, resulting in conformational changes and thus loss of activity. Improved solvent tolerance can be achieved using protein engineering but with reduced catalytic activity.[39] As-prepared nGOx-based nano(bio)sensors exhibited greater polar solvent tolerance without compromising their catalytic activity. To investigate the stability against the organic solvent environment, fabricated nano(bio)sensors were exposed to a water−organic solvent mixture (50:50) for 1 h. The catalytic activity of nano(bio)sensors was investigated before and after the incubation using CV method. Herein, the catalytic activity obtained before the incubation is treated as 100%, and the results for after the organic solvent incubation are presented relative to this (Figure 4C).

As shown in Figure 4C, the nGOx/N-CNTs-Chi/GCE nano(bio)sensor exhibits enhanced enzymatic stability and demonstrates only a little loss in catalytic activity for glucose oxidation after organic solvent incubation as compared to the native GOx-based biosensor. For instance, the nGOx/N-CNTs-Chi/GCE nano(bio)sensor retained about 94% and 97% of its original activity after incubation in ethanol (EtOH) and *N*,*N*-dimethylformamide (DMF), respectively. In contrast to this, the native GOx-based biosensor exhibited only 50% and 65% of its original catalytic activity (Figure 4C) after incubation in EtOH and DMF, respectively. The nGOx-based nano(bio)sensor also demonstrated better catalytic activity after incubation in methanol (MeOH) and dimethyl sulfoxide (DMSO) as compared to the native GOx-based biosensor. The nGOx/N-CNTs-Chi/GCE nano(bio)sensor showed better performance in terms of residual catalytic bioactivity for glucose after incubation in organic solvents. The considerable increase in organic solvent tolerance can be attributed to soft polymer shells, which helped in retaining the hydrophilic environment and protected the essential water, enabling the GOx molecules to efficiently display their biological functions.[7,39] Moreover, the multiple covalent attachments between the thin polymer layer and the enzyme molecule strengthened the encapsulated GOx molecules.[7,40,41]

Operational Stability. Operational stability of the nano-(bio)sensors was evaluated by performing continuous CV measurements from cycle 1 to cycle 500. As shown in Figure S5, the nGOx/N-CNTs-Chi/GCE nano(bio)sensor maintained 97% and 95.33% of initial peak current after the 300th and 500th cycles, respectively. This strong operational stability performance of the nGOx/N-CNTs-Chi/GCE nano-(bio)sensor can be ascribed to the excellent loading ability of nGOx onto the N-CNTs-Chi/GCE, where thin polymer-layered nanocapsules prevented the leaching of nGOx molecules during the electrode operations.

Amperometric Studies for Catalytic Oxidation of Glucose. Amperometric (I−t) response characteristics of the nGOx/N-CNTs-Chi/GCE nano(bio)sensor for catalytic oxidation of glucose were investigated. In general, for a specific enzymatic glucose biosensor, the working potential for glucose determination can be either negative or positive, which depends on the different sensing mechanisms (i.e., detecting the decrease of O_2 concentration or the increase of produced H_2O_2 intermediate upon the addition of glucose). In our study, the potential used for amperometric catalytic oxidation of glucose is 0.45 V. It is evident from Figure 5A that there is a sharp and well-defined amperometric response upon each addition of glucose, even at low concentrations. It showed no hindrance for the glucose substrate to reach the active center of the GOx enzyme for catalytic oxidation inside the nGOx nanocapsules. Moreover, it demonstrated that the thin polymer layer was highly permeable to the glucose substrate. nGOx nanocapsules immobilized onto N-CNTs provided a favorable microenvironment for glucose oxidation and established fast electron transfer between the GOx enzyme and the electrode. The amperometric response of the nGOx/N-CNTs-Chi/GCE nano(bio)sensor reached 95% of the steady-state current within 6 s, which further confirmed the fast electro-oxidation of glucose at the nGOx/N-CNTs-Chi/GCE electrode.

At low concentrations, the amperometric current response was observed to be proportional to glucose substrate concentrations, which showed the characteristics of the first-order reaction (Figure 5). However, beyond 1740 μM glucose concentrations, the current response started to decrease, which at further higher concentrations became saturated and followed the zero-order reaction kinetics.[42] These characteristic enzymatic reactions confirmed that there was no considerable decrement in biological activity of GOx enzyme molecules inside the nanocapsules; instead, nanocapsules further empowered them with more stability and endurance in different working environments. The I−t current response increased linearly with glucose concentrations, exhibiting a linear relationship over the concentration range of 10−1740 μM (Figure 5B), which further demonstrated the high sensitivity of the fabricated nano(bio)sensors. The obtained limit of detection (LOD) was 6.92 μM. The excellent bioelectrochemical performance of nGOx/N-CNTs-Chi/GCE was due to the favorable microenvironment provided by the nanocapsules to retain enzymatic activity with improved stability and catalytic endurance.

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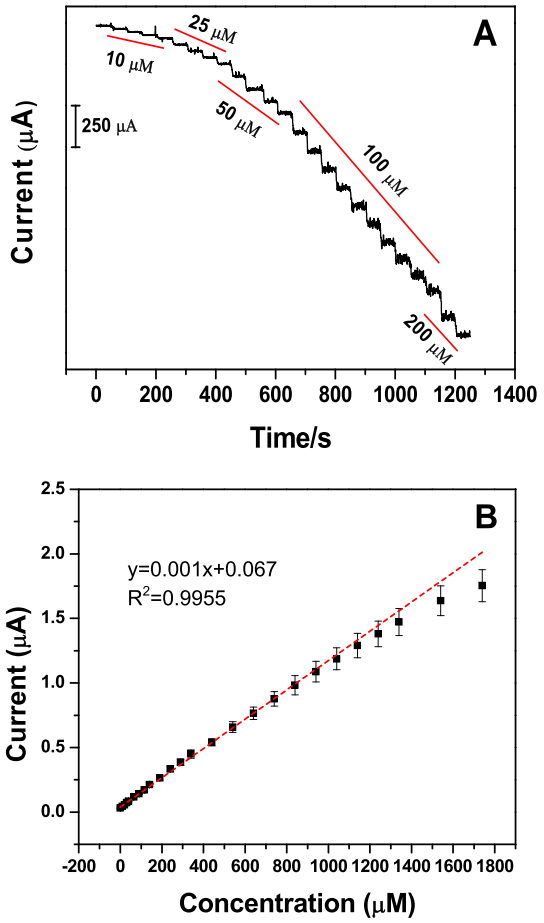

Figure 5. (A) I−t curves of nGOx/N-CNTs-Chi/GCE for successive addition of glucose concentrations into a stirring solution of air-saturated 0.1 M phosphate buffer (pH 7.01); (B) corresponding calibration plot for glucose concentrations up to 1740 μM.

CONCLUSIONS

The present work reports the very first study implementing single-molecule enzyme naocapsules as a highly stable biosensing platform that bridges the gap of activity loss of enzyme biosensors during practical applications in varying working environments. The thin polymer layer around the enzyme molecule was observed to be highly permeable to glucose substrate, which facilitated substrate transportation. The multiple covalent attachments between the thin polymer layer and the enzyme molecule strengthened the encapsulated GOx molecules, resulting in highly stable nano(bio)sensors. The constructed nano(bio)sensor exhibited good catalytic bioactivity for glucose oxidation with enhanced stability at high temperature and in the presence of polar organic solvents. The nGOx/N-CNTs-Chi/GCE nano(bio)sensor retained ∼92% of its original catalytic activity for glucose oxidation after incubation at 65 °C for 1 h. Similarly, the nano(bio)sensor maintained ∼94% of its original bioactivity after incubation in the EtOH/water (50:50) mixture. This work promises to be a paradigm shift in the development of highly stable nano(bio)-sensors for diverse applications with authentic results irrespective of the working environment. For example, traditional biosensors can only be used in the aqueous phase because they are unstable in organic solvents, and they cannot detect some targets with low solubility in the aqueous phase. The SMENs-based biosensor can be used in the mixed phase of water/organic solvent, and the detection of these targets can be easily realized by taking advantage of the good solubility of these targets in organic solvents. Thus, the proposed SMENs nano(bio)sensors exhibited significantly improved stability over traditional enzymatic biosensors, which could potentially be applied in point-of-care diagnostics, biomedical detection, wearable devices, implantable equipment, biofuel cells, etc.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b05466.

Materials; preparation of single-molecule enzyme nanocapsules (SMENs), conjugation with acryloyl groups, and in situ polymerization; material characterizations; construction of nano(bio)sensor; amperometric procedure; procedure for single-molecule enzyme nanocapsules (SMENs) preparation; UV−visible spectra of native GOx and nGOx molecules; TEM image of N-CNTs; illustration for construction and sensing of nGOx-based nano(bio)sensor; plots of cathodic I_{pc} and anodic I_{pa} vs scan rate for SMENs-based biosensor; and operational stability of SMENs-based biosensor by CV (PDF)

AUTHOR INFORMATION

Corresponding Author

Xianbo Lu − *CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China*; Email: xianbolu@dicp.ac.cn

Authors

Dhanjai − *CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China*

Lingxia Wu − *CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China*

Jiping Chen − *CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China*; orcid.org/0000-0001-9404-1687

Yunfeng Lu − *Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California 90095, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.9b05466

Author Contributions

D. and L.W. performed the experiments. X.L., J.C., and Y.L. developed the idea, guided the whole work, and acquired the funding for the research. D. wrote the original manuscript, and X.L. helped in polishing the manuscript.

Notes

The authors declare no competing financial interest.

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