Au/In$_2$O$_3$ Nanocubes Based Label-free Aptasensor for Ultrasensitive and Rapid Recognition of Cardiac Troponin I


Abstract: Cardiac Troponin I (cTnI) is a preferred biomarker to diagnose acute myocardial infarction which is one of the leading risks to health globally due to its short term. However, clinical analyzers are difficult to achieve its on-site quantitative detection. A novel label-free aptasensor was constructed to realize ultrasensitive and rapid recognition of cTnI. A nanocubic AuNPs/In$_2$O$_3$ composite was designed to provide synergistic effects of abundant active sites and signal magnification for aptamers grafting. Relying on a conductance-dependence strategy, this aptasensor can achieve the quantitative detection within 10 min, which is much faster than state-of-the-art analyzers, as well as exhibiting an ultrawide linear range of 0.1–1000 ng/mL and a low detection limit of 0.06 ng/mL with an excellent selectivity in the analysis of human serum.

Keywords: Au/In$_2$O$_3$ nanocubes · label-free aptasensor · rapid detection · cardiac troponin I · high sensitivity

1 Introduction

As reported by World Health Organization (WHO), cardiovascular diseases (CVDs) have already been the highest cause of death globally, claiming ca. 17.1 million lives each year [1, 2]. Among them, ca. 4/5 CVDs deaths are resulted by the acute myocardial infarction (AMI) of heart attacks and strokes which often happen suddenly and fast cause irreversible injury due to the myocardial necrosis, producing great difficulty in emergency treatment [3, 4]. Nowadays, thrombolytic therapy served as the main treatment method to efficiently decrease the mortality, however, the operation time plays a decisive role in the success of life saving [5]. Hence, the fast and accurate diagnosis of AMI is vital for the decision of therapy strategy.

For clinical AMI diagnosis, electrocardiography, coronary angiography and serum analysis for myocardial markers are the main test methods [6]. Electrocardiography is most applied among various AMI diagnostic tools, and patients who assessed for a ST-segment elevation are classified as a high-risk for AMI [7–8]. Nevertheless, quite a few AMI patients show the non-diagnostic electrocardiography or ambiguous reading in testing [9]. Coronary angiography is a traumatic detection method [10]. Although it can visualize the location of the infarction that provides much more accurate results, it is unable to judge the disease cause and non- obstructive arteriosclerotic lesions [10, 11]. Serum analysis to determine the cTnI is confirmed as a sensitive measurement with the high specificity to full-cause AMIs, even enabling to indicate the minor myocardial damage [12, 13]. Currently in clinical diagnosis, the detection of cTnI is mainly relied on the solid-phase chromatography immunoassay and the enzyme-linked immunoosorbent assay (ELISA) in hospital [14]. However, both of above detection principles have some limitations [15]. The former method takes approximately 15 minutes, but the results are only semi- quantitative to hardly provide an accurate value [16]. Although ELISA technique enables to provide accurate data of the cTnI level, it requires at least one hour for the enzyme binding and the support of expensive and large instrument [17–19].

Using the similar immune principle, electrochemical biosensor is advanced owing to its quantitative ability, high sensitivity, miniaturization ability and less sampling [20, 21]. However, these antibody-based electrochemical systems are facing challenges of poor stability, high cost and accuracy [22]. In addition, the long-term binding

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reaction between antibody and antigen as well as the complex operation limit its practical application [23]. In order to address these issues, the aptamer biosensor using the specific DNA or RNA strands for cTnI capture is gradually developed to accelerate the assay response [24,25]. Normally, only the aptamer binding cannot produce enough signal intensity to ensure the accurate recognition of the target, thereby the electrode material often serving as the signal amplifier is the key to determine the performance of the aptasensor [26]. In this case, various nanomaterials have rapidly emerged to confirm the promotion efficiency in nanoscale. Among all kinds of these materials, the metal oxide nanomaterial enables to present superior stability, specific surface area and abundant oxygen groups [27,28]. Indium oxide (In$_2$O$_3$) has a large electroactive specific surface area and can provide more active sites, which contributes to catalytic activity. In addition, In$_2$O$_3$ has low electron affinity, which can provide a natural channel for the transmission of electrons [29]. Thus, it can show good catalytic activity. Besides, it is usually served as the main material for the field-effect transistor biosensor and supercapacitors [30,31]. Our detection strategy is based on the electron transfer effect, so we chose In$_2$O$_3$ as the modified material. However, it can hardly provide the effective sites for the binding of the aptamer because of the saturated in bonds [32–34]. Therefore, giving the condition for the aptamer bonding on the In$_2$O$_3$ surface is the prerequisite for the construction of an In$_2$O$_3$ based aptasensor for cTnI detection.

In this work, we developed a nanocubic Au/In$_2$O$_3$ material for the label-free detection of cTnI. The well-defined nanocubic In$_2$O$_3$ crystals were first built through coupling the hydrothermal and calcination routes, serving as the skeleton for the further growth of Au nanoparticles (Figure 1). Then relying on a low-speed chemical reduction method, monodispersed Au nanoparticles with the size of ca. 50 nm were in-situ assembled on the whole surfaces of In$_2$O$_3$, which dramatically improved the conductivity to two orders of magnitude verified by the broadband dielectric impedance. This nanocomposite was applied as a transducer to immobilize thiol-functionalized DNA aptamers via the self-assembled mechanism without any labels, achieving the target-specific binding of cTnI with high sensitivity and low limit of detection within 10 min. Besides, its high accuracy has been confirmed by using different cases of human serum.

2 Experimental

2.1 Reagents and Materials

All the drugs used were analytically pure grade or better. Hexamethylenetetramine (HMTA), Indium chloride tetrahydrate (InCl$_3$·4H$_2$O, 99.99%) were purchased from Aladdin, polyvinylpyrrolidone (PVP, 99.5%) was obtained from Aldrich, Potassium ferricyanide (K$_4$[Fe(CN)$_6$]·3H$_2$O), Potassium hexacyanoferrate (K$_4$[Fe(CN)$_6$]·3H$_2$O) were brought from Sigma Aldrich, potassium chloride (KCl) was obtained from Yonghua chemical Co., Ltd. The oligonucleotide (Tro4: 5’-HS-(CH$_2$)$_n$-CGT GCA GTA CGC CAA CCT TTC TCA TGC GCT GCC CCT CT T-A’3’). (CN)$_6$-3H$_2$O) were synthesized and purified from Sangon Biotech (Shanghai) Co., Ltd. Human cardiac troponin I (cTnI), human cardiac troponin C (cTnC), human cardiac troponin T (cTnT) and carcinoembryonic antigen (CEA) were obtained from Shanghai Linc-Bio Science Co., Ltd. DNA immobilization buffer: 10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl (pH 7.4). Antigen dilution buffer: 0.1 M phosphate buffered solution (PBS, pH 7.4) with 0.1 M KCl. All aqueous solutions were prepared with deionized water (≥18.2 MΩ, Smart2Pure 6, Thermo Fisher Scientific, USA). The human serum was obtained from Nanjing Drum Tower Hospital (Nanjing, China) with ethical approval.

2.2 Synthesis of Au/In$_2$O$_3$ Cubic Crystals

0.1 M aqueous indium chloride solution (InCl$_3$·4H$_2$O) was prepared and then added into 0.75 M hexamethylene diamine (HMTA) solution slowly with continuous stirring. The hybrid solution was allowed to stir for 10 minutes in order to mix the two solutions thoroughly. The solution obtained was transferred into the magnetic stirring hydrothermal kettle and kept inside the Teflon liner maintained at 100°C for 9 h with continuous stirring (200 rpm). After being cooled down to room temperature,
separated through centrifugation, washed by ultrapure water and ethanol for several times, we can get a white powdery solid, which is proved to be In(OH)$_3$. Then the prepared In(OH)$_3$ particles were dried for 6 h in hot air oven at 80°C, and calcinations were carried out for 2 h in Muffle furnace at 300°C. Finally, Indium oxide (In$_2$O$_3$) nanoparticle of regular morphology was obtained. Then, the In$_2$O$_3$ (0.2 g) were added into 10 mM HAuCl$_4$ solution (2 mL). After stirring for sufficient time, 10 mM ascorbic acid was injected at a low speed to reduce HAuCl$_4$. After the reaction completed, the hybrid solution was allowed to centrifuge with ultrapure water and ethanol for three times. The obtained powdery solid was dried at 80°C.

2.3 Electrochemical Measurements

All electrochemical experiments were performed on the CHI660E electrochemical workstation (Shanghai Chenhua Instrument Co.Ltd., China). The impedance tests of In$_2$O$_3$ and Au/In$_2$O$_3$ were operated by Broadband Dielectric Spectrometer. The cyclic voltammetry (CV) tests were performed in a 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 0.1 M KCl, 10 mM K$_4$Fe(CN)$_6$, 10 mM K$_2$Fe(CN)$_6$. The differential pulse voltammetry (DPV) measurements were performed in a 0.1 M phosphate buffered saline (PBS, pH 7.4) containing 0.1 M KCl, 5 mM K$_4$Fe(CN)$_6$, 5 mM K$_2$Fe(CN)$_6$. and electrochemical impedance spectroscopy (EIS) measurements were operated in the presence of a 5 mM K$_4$Fe(CN)$_6$/K$_2$Fe(CN)$_6$, (molar ratio is 1:1)-mixture as a redox probe solution with the frequency changed from 0.1 Hz to 1000 kHz with signal amplitude of 5 mV at the 0.05 V potential. A three-electrode system consisting of a gold disk electrode working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (saturated KCl) electrode was used as reference electrode.

2.4 Impedance Test of the Materials

Alternating current impedance spectra test was operated on the Broadband Dielectric Spectrometer, a Concept 80 system (Novocontrol, Germany) at 25°C. The powdered flake, with a thickness of ~1.5 mm and a diameter of 16.0 mm, was prepared using ~0.5 g of the In$_2$O$_3$ sample under a static pressure of 5 MPa for 1 min, which was then sandwiched with gold electrodes with an ac frequencies span of 1 to 10$^7$ Hz.

2.5 Detection Strategy of cTnI

The fabrication process of the specific cTnI biosensor developed was illustrated in Figure 1. The DNA probe was modified with a 3’terminal thiol, which can combine firmly with Au through the Au–S bond [35]. Thus, the DNA probe can be stably covalently attached to the surface of a gold disk electrode. In the absence of cTnI, the exchange of electrons generated by [Fe(CN)$_6$]$_{3-}$ oxidation in the solution could result in a current response. With the increase of cTnI concentrations, the peak current (I$_p$) of [Fe(CN)$_6$]$^{3-}$ oxidation was decreased. This is due to the charge disturbance induced by the negatively-charged [Fe(CN)$_6$]$_{3-}$ and negatively-charged cTnI. According to the change of I$_p$, the concentration of cTnI added can be detected exactly.

3 Result and Discussion

3.1 Structure Evolution of the Au/In$_2$O$_3$ Nanocubes

According to our previous work, we have demonstrated that the geometric edge of the nanocubic crystals enables to provide a higher catalysis than other sites [36]. Hence, we expect to obtain a regular shape of In$_2$O$_3$ in nanoscale. In order to achieve this goal, the In(OH)$_3$ (Indium hydroxide) was first proposed to build a well-defined geometric morphology as the precursor for In$_2$O$_3$ preparation. Thus, we carefully investigated the structure evolution of In(OH)$_3$ over time during its whole crystallization period. As shown in Figure 2A, it can be observed that the In(OH)$_3$ crystals only formed the irregularly shaped crystals at the initial 3 hours. If prolonging the reaction time, the crystals gradually appeared a feature of fuzzy nanocube without clear edges and corners (Figure 2B). Till 9 hours, well-defined nanocubic In(OH)$_3$ crystals had been prepared with the smooth surface and average size.

![Fig. 2. FESEM images of the In(OH)$_3$ prepared at different hydrothermal reaction time, respectively: A) 3 h, B) 6 h, C) 9 h, D) 12 h; E) The In(OH)$_3$ prepared with NaOH; F) The In(OH)$_3$ prepared with HMTA.](image-url)
of ca. 800 nm (Figure 2C). However, further increasing the hydrothermal time would cause a heavy aggregation of crystals, producing too big particles composed by the intergrowth of smaller cubes (Figure 2D).

The formation of In(OH)$_3$ requires In$^{3+}$ ions to combine with OH$^-$ ions in the solution. Hence, the influence of source of OH$^-$ ions was further investigated through the addition of different alkali. The strong alkaline substance, NaOH was first added into the system. As shown in Figure 2E, large pieces of irregularly nanoparticles were observed, which was owing to the strong ionization property of NaOH. In this case, OH$^-$ ions in the solution reached a very high concentration instantly, which was not conducive to the slow and orderly growth of crystals. In order to slow down the ionization rate of OH$^-$ ions, HMTA was chosen to act as the reactive base, which decomposed slowly in heating aqueous solution to yield ammonia and formaldehyde [37]. The decomposition process provided a slow and controllable supply of OH$^-$ ions, which combined with In$^{3+}$ ions to form a well-defined nanocubic In(OH)$_3$ (Figure 2F).

The crystal growth is often referred to a phase transition process which induces the target element crossing the interface into the crystal lattice [38]. In this process, temperature and reactant concentration will greatly affect the driving force of crystallization. Therefore, we also studied the influences of temperature and concentration on the crystal morphology. Through experiments, the preferred temperature and reactant concentration were confirmed to 100°C and 0.1 M, respectively (Figure S1 and S2, Supporting Information).

In order to obtain In$_2$O$_3$, the In(OH)$_3$ was required to calcine at a high temperature. The TGA results in Figure 3D presented that the total weight loss of the precursor was about 16% from 10°C to 800°C. The main weight loss occurred in the range of 280 to 290°C indicated the removal of bound water, and the decomposition of the In(OH)$_3$ framework happened at about 280°C [39]. The weight loss showed to be stable at the temperature beyond 300°C, which confirmed the complete decomposition of In(OH)$_3$ crystals. The process can be expressed by a following equation:

$$2\text{In(OH)}_3 = \text{In}_2\text{O}_3 + 3\text{H}_2\text{O} \quad (1)$$

According to the chemical equation theory, the weight loss ratio should be 16.72%, and the actual weight loss ratio is consistent with the theoretical value within the error norm. As mentioned above, we expect to maintain the original cubic morphology of the In(OH)$_3$ after In$_2$O$_3$ formation to increase the catalytic activity. Therefore, the calcination conditions were required to carefully investigated. First, the crystal was calcined at 200°C for 2 hours, which showed rare change of the cubic shape (Figure 3A). Then we increased the temperature to 300°C, the crystal can still survive from the thermal treatment to keep its original smooth surface (Figure 3B).

However, when reaching 400°C, the crystal was observed to collapse so that the cubic shape began to disappear (Figure 3C). Obviously, the geometric surface of the crystal was damaged with the increasing of temperature, proving that the temperature control is essential to maintain the crystal morphology. During the whole calcination process, the thermal oxidation process from In(OH)$_3$ precursor to In$_2$O$_3$ was very mild owing to a low temperature rising rate (2°C·min$^{-1}$). The removal of bound water was slow and smooth with the low temperature rising, avoiding the structure collapse from the inner of the cubic crystal [40]. Meanwhile, the In(OH)$_3$ had sufficient oxygen atoms to directly connect with In$^{3+}$, avoiding the destroy from the external oxygen source in air.

In order to further investigate the crystal structures of the In(OH)$_3$ and In$_2$O$_3$, X-Ray diffraction (XRD) analysis were performed in Figure 3E. All diffraction peaks of the In(OH)$_3$ coincide with the standard spectra of cubic indium hydroxide (JCPDS-76-1464). The observed diffraction peaks (211), (222), (400), (440) and (622) of the prepared In$_2$O$_3$ are consistent with those of the standard XRD diffraction patterns of JCPDS-71-2195, confirming the successful preparation of In$_2$O$_3$ nanoparticles [41]. Meanwhile, the crystals calcined at different temperature were also characterized (Figure 3F). The results indicated that only till the calcination temperature reached 300°C, the In$_2$O$_3$ crystals were created, which is consistent with the TGA results.

As well known, In$_2$O$_3$ material is a semi-conductor to show a poor conductivity which will greatly block the electron transfer to reduce the biosensing performance. On the purpose of enhancing the conductivity, AuNPs were chosen to assist In$_2$O$_3$ for overcoming this deficiency [42]. Besides, to sufficiently use the In$_2$O$_3$ surfaces, an excessive In$^{3+}$ was added to produce the positive charge surrounding the In$_2$O$_3$ faces to push the adsorption of [AuCl$_4$]$^-$ via an electrostatic force. The FESE results (Figure 4A and B) showed that many gold nanoparticles enabled to successfully grow on all surfaces of In$_2$O$_3$. 

![Fig.3. FESEM images of the In$_2$O$_3$ nanocubes prepared at different calcining temperatures for 2 h, respectively: A) 200°C, B) 300°C, C) 400°C; D) TGA curve of the synthesized In(OH)$_3$ nanocubes; E) XRD patterns of the In(OH)$_3$, and In$_2$O$_3$; F) XRD patterns of the products calcined at different temperatures.](image-url)
nanocubes. Furthermore, combing with the EDX results (Figure 4C to E), it can be verified that these nanoparticles were consisted of Au element with a uniform distribution. As shown in Figure 4F, once AuNPs was deposited on the In$_2$O$_3$ crystal, two peaks at 38.2° and 44.4° which represented the (111) and (200) planes of Au were observed, confirming the formation of AuNPs [43].

In order to evaluate the improvement of the AuNPs in conductivity, we measured the impedance of the In$_2$O$_3$ and Au/In$_2$O$_3$ nanocrystals by a broadband dielectric spectrometer. The powder samples were pressed into a flake, with a thickness of ~1.5 mm and a diameter of 16.0 mm. This test was carried out in the air at 25°C, which represents the intrinsic character of the material itself. Thus, this result represents the conductivity of the powder material. As shown in Figure 4G, with the introduction of Au nanoparticles, the conductivity of the In$_2$O$_3$ nanocubes was significantly improved. The value of the charge transfer resistance ($R_{ct}$) has dramatically dropped from 142 kΩ to 1.25 kΩ after covering AuNPs.

More nanostructure details were then characterized by HRTEM. As revealed in Figure 5A, the interplanar spacing of Au nanoparticles on the surface (Figure 5B) was measured by lattice fringe to present the values of approximately 0.23 nm and 0.2 nm, which respectively corresponded to the (111) and (200) planes of Au. It was also in accord with the conclusion from the above XRD results (Figure 4F) for the crystalline planes. The well-defined diffraction pattern in Figure 5C indicated the polycrystalline nature of this nanocomposite. Besides, it is worth mentioning that the Au element (Figure 5D to F) was uniformly distributed on all faces of the In$_2$O$_3$ instead of diffusion into the cubic interior due to the effects of the created positive charge surface of the In$_2$O$_3$ nanocubes.

Then, the chemical composition and element valances of the prepared In$_2$O$_3$ before and after the Au deposition were studied by XPS measurements. The survey spectra of the In$_2$O$_3$ and Au/In$_2$O$_3$ powders indicated that the prepared nanocomposite was mainly composed of In, O and Au elements without other impurity elements (Figure 6A). Moreover, Figure 6B and C revealed that two main In3d peaks located at 451.58 (3d $^{3/2}$) and 443.98 eV (3d $^{5/2}$) and O1s peaks at 529.1 and 530.5 eV, which corresponding to lattice oxygen and dissociated oxygen, were rarely changed. This demonstrated that there were no ionic or covalent bonds between AuNPs and In$_2$O$_3$ to produce any influence on the oxidation state of In$_2$O$_3$ nanocubes. Furthermore, the Au4f peaks located at 83.7 and 87.4 eV are derived from the core-level emissions of Au4f $^{7/2}$ and Au4f $^{5/2}$, respectively [37]. This result confirmed that the synthesized AuNPs on the In$_2$O$_3$ surface were classified as the elemental state (Figure 6D).
3.2 Electrochemical Behaviors of the Au/In$_2$O$_3$ Nanocubes

The electrochemical redox ability of the as-prepared nanocomposite, which is determined by the electrocatalytic activity and conductivity, was evaluated through its CV performance [44,45]. We compared the results of the bare, In$_2$O$_3$ and Au/In$_2$O$_3$ modified electrodes in the same indicator solution (0.1 M KCl, 10 mM Fe(CN)$_6^{3-}$). In Figure 7A, we can find that, after the deposition of In$_2$O$_3$, both strengths of the oxidation and reduction peaks were increased. This indicates that the catalytic activity of the In$_2$O$_3$ modified electrode has been enhanced. Nevertheless, if we applied Au/In$_2$O$_3$ nanocubes as the electrode material, the peaks would reach higher levels which were stronger than those of In$_2$O$_3$ and bare electrodes to indicate the promotion ability of AuNPs. Further investigation of these three electrodes was performed by EIS technique to quantify the electron transfer resistance ($R_t$) in solution. Figure 7B presents the Nyquist plots of the bare, In$_2$O$_3$-modified and Au/In$_2$O$_3$-modified gold electrodes. The $R_t$ values of these three electrodes were calculated to be 101.13, 159.25 and 72.01 Ω, respectively. Above results proved that the introduction of AuNPs enabled to improve not only the conductivity but also electrocatalysis. Meanwhile, we found that the introduction of the aptamer has hardly changed the electrode.

![Image](48x101 to 289x188)

![Image](48x292 to 289x387)

Fig. 7. Electrochemical characterization diagrams of the materials. A) provides the CV diagrams of the bare, In$_2$O$_3$-modified, Au/In$_2$O$_3$-modified and Apta-Au/In$_2$O$_3$-modified gold electrodes in the presence of 10 mM of [Fe(CN)$_6^{3-}$] containing 0.1 M of KCl; B) shows the EIS diagrams of the bare, In$_2$O$_3$-modified, Au/In$_2$O$_3$-modified and Apta-Au/In$_2$O$_3$-modified gold electrodes in 5 mM [Fe(CN)$_6^{3-}$] indicator solution containing 0.1 M KCl.

Fig. 8. A) DPVs of Au/In$_2$O$_3$-modified electrodes in the presence of cTnI with different concentrations (0.1-2000 ng/mL); B) Linear calibration curves for the current response of cTnI.

In this equation [46], $n$ is the number of electrons transferred in the redox reaction process, $D_0$ is the molecular diffusion coefficient, $I_p$ is the peak current of the redox reactions, $C_o^*$ is the concentration of the probe molecules, $v$ is the scan rate and $A$ is the effective specific surface area of electrodes. Obviously, the effective specific surface area ($A$) is proportional to $I_p/v^{1/2}$ in this equation. Due to the fact that $n$, $D_o$ and $C_o^*$ are constant values, the effective surface area of the prepared electrodes could be obtained according to the linear fitting in Figure S4B. Among these three calibration lines, the Au/In$_2$O$_3$ one has the largest slope, indicating the largest surface area to arouse stronger electrochemical response. Based on the known area of the bared electrode (0.0314 cm$^2$), the effective surface areas of the In$_2$O$_3$ and Au/In$_2$O$_3$ electrodes are calculated as 0.643 cm$^2$ and 0.685 cm$^2$, respectively. Above evidences demonstrate that the introduction of AuNPs enables to significantly enhance the conductivity and slightly improve the surface area of In$_2$O$_3$, producing a synergistic effect to greatly improve the electrocatalysis.

Then the Au/In$_2$O$_3$ nanocubes based electrode was constructed as an aptasensor by the self-assembly of a thiol grafted DNA single strand. The $K_d$ value of this strand was lower than the antibody, suggesting that the binding affinities of selected aptamers were stronger than that of the antibody [24]. In order to discuss the further investigated through CV characterization in the kinetic control mechanism during the detection of cTnI, this as-prepared aptasensor was presence of 1 ng/mL of cTnI. Figure S4C presented that both peak currents in redox enhanced with the increase of the scan rate. The values of the peak currents followed a linear dependence with the root of the scan rate [47], verifying that the detection process was dominated by a diffusion-controlled reaction to closely associate with the target concentration (Figure S4D). In this case, the as-prepared biosensor is capable of the quantitative detection of cTnI.
Table 1. Comparison of the proposed aptasensors with other methods in the determination of cTnI.

<table>
<thead>
<tr>
<th>Detection Materials</th>
<th>Linear range (ng/mL)</th>
<th>Detection limit (ng/mL)</th>
<th>Time (min)</th>
<th>References</th>
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<td>75</td>
<td>[50]</td>
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<td>Screen-printed Carbon Electrode</td>
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<td>[51]</td>
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<td>0.2</td>
<td>70</td>
<td>[52]</td>
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3.3 Optimization of the Test Time

The combination of cTnI and aptamer requires a certain amount of time, which determines the detection time. In order to shorten the detection time as much as possible, we investigated the influence of binding time for cTnI and aptamer on current response by the DPV method. In a target solution containing 1ng/mL cTnI, the differential pulse voltammetry (DPV) test were performed every 2 minutes. As shown in Figure S5A and B, the largest change of the current signal occurred at 8 minutes, and after 10 minutes, the current intensity no longer changed significantly. This indicates that the binding sites of the aptamer have reached almost saturation through the combination of cTnI since 10 minutes. Therefore, we proved the optimum detection time of 10 minutes which provided enough capture time of the aptamer to cTnI for generating an effective current change.

3.4 Biosensing Performance of the As-prepared Aaptasensor

The biosensing of the Au/In$_2$O$_3$ nanocubes based aptasensor for the detection of cTnI was evaluated by the DPV measurement [48]. Figure 8A shows the current responses of the as-prepared aptasensor after the combination of cTnI with different concentrations during the electrochemical oxidation of [Fe(CN))$_6$$.^{3–4+}$. With the increase of the cTnI concentration, the $I_{pa}$ of [Fe(CN)$_6$$]^{3–4+}$ oxidation decreased significantly. On the one hand, the combination of cTnI increases the resistance of the electrode surface, which is negative to the electron transfer. On the other hand, the charge perturbation induced by the negatively-charged [Fe(CN)$_6$$]^{3–4+}$ and negatively-charged cTnI also results in the decrease of the $I_{pa}$. As calibration (Figure 8B), with the increase of the concentration of cTnI from 0.1 to 1000 ng/mL, the $I_{pa}$ decrease is corresponding to a linear relationship between the $I_{pa}$ and $\ln$[cTnI(ng/mL)]. The linear regression equation is $I_{pa}$ (μA) = (6.219 ± 0.27) log[cTnI (ng/mL)] + 15.46 ± 1.08 (R$^2$ = 0.992). The detection limit can reach to 0.06 ng/mL.

Electrochemical method is one of the commonly used methods to detect cTnI. The electrode materials, such as Au nanoparticles, carbon and In$_2$O$_3$, always determine the performance. As shown in Table 1, some sensors including In$_2$O$_3$ Nanoribbon and screen-printed carbon electrode enable to exhibit the very low detection limit, however their linear ranges are very narrow, which can hardly satisfy the clinical requirement. Compared with the reported cTnI biosensors, our aptasensor exhibits a much higher sensitivity and lower detection limit. It is worth mentioning that the concentration of cTnI in normal healthy human beings is usually below 0.4 ng/mL and concentration higher than 2.0 ng/mL implies an increased danger for serious heart diseases, the linear range of 0.1–1000 ng/mL in this work is reasonable and may be used in the accurate diagnosis of AMI at early stage. Besides, most of the biosensors require at least one hour for the detecting. However, the aptasensor we established enable to accurately recognize the cTnI in human serum with 10 mins, showing great potentials on the rapid and on-site diagnosis of cardiovascular diseases.

3.5 Selectivity, Reproducibility and Stability of the As-prepared Biosensor

Selectivity is a vital parameter to evaluate the accuracy of biosensors. Therefore, the common interfering substances, such as CEA, cTnC and cTnT, were selected to investigate the selectivity of the as-prepared aptasensor. As shown in Figure 9A, with the successive additions of various interfering substances (10 ng/mL CEA, cTnT and cTnC), the current variations were inconspicuous (1 μA, 2.3 μA and 1.6 μA). When cTnI was then added, this biosensor can provide a strong current response (15.21 μA). The results demonstrate that our prepared biosensor can effectively recognize the cTnI with a good selectivity.

![Fig. 9. A) DPV signal changes (ΔI) for the biosensor after being injected with different antigens (cTnI, CEA, cTnT and cTnC); B) The reproducibility of the sensor in 1 ng/mL of cTnI solution.](Image)
Subsequently, the reproducibility of the sensor was verified by the DPV measurements for [Fe(CN)$_6$]=$^{1/4}$ oxidation at eight independent aptasensors, and the results are shown in Figure 9B. It can be found that a relative standard deviation (RSD) of 8.96% was obtained to indicate the good reproducibility. Moreover, we also confirmed that, after 30 times CV scanning cycles, both the oxidation and reduction peaks exhibited slight changes (Figure S3). This result verified that the state of the modified electrode has not changed significantly, which indicates that the bond between the electrode surface and the material is very stable. Meanwhile, it could also be verified that the Au/In$_2$O$_3$-based biosensor can maintain excellent electrochemical stability in the detection process.

3.6 Detection of cTnI in Human Serum

The detection of cTnI using human serum was operated in the Nanjing Drum Tower Hospital. [Fe(CN)$_6$]=$^{1/4}$ and KCl were added into PBS as the indicator solution, which concentrations are 5 mM and 0.1 M, respectively. Then, add 1 mL serum samples into 39 mL indicator solution. We can obtain the final detection system after mixing evenly by rotor. The applicability of this aptasensor for detecting serum samples was examined by adding different concentrations of cTnI into the mixture solution. Three serum cases with different cTnI concentrations were applied to evaluate the recovery ability of the as-prepared aptasensor. As shown in Table 2, the recovery of spiked samples was 88.7–112% (n = 3), indicating that the approach has the potential for practical applications in the early diagnosis of AMI.

4 Conclusion

In this work, we have successfully designed a cube-shaped Au/In$_2$O$_3$ nanocomposite based label-free aptasensor for ultrasensitive and quick recognition of cTnI. Precise control of the In$_2$O$_3$ nano structure was achieved by adjusting the reaction conditions, and the regular cubic In$_2$O$_3$ was obtained. Through electrostatic attraction strategy, we have successfully obtained nano-gold on the surface. The regular cubic structure and the surface-coated gold nanoparticles are beneficial for the simultaneous increase of its catalytic area and active site, resulting in a synergetic promotion of the conductivity and electrocatalysis. Due to the promotion of nano-composite, the as-prepared biosensor exhibited a wide linear range of 0.1–1000 ng/mL and an ultralow detection limit of 0.06 ng/mL. In order to shorten the detection time, we chose the aptamer which can bind faster with the antigen as the biological probe. Only within 10 min, this aptasensor enabled to accurately recognize the cTnI in human serum, showing great potentials on the rapid and on-site diagnosis of cardiovascular diseases. In addition, we used the [Fe(CN)$_6$]=$^{1/4}$ solution as the indicator, which simplified the experimental operation in a large extent. By designing different sequences of the aptamer, this detection strategy is promising to shorten the detection period for more physiological indicators.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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