Phenylboronic acid derivative-modified (6,5) single-wall carbon nanotube probes for detecting glucose and hydrogen peroxide

Yunfan Qiao, Rushi Zhao, Min Zhang, Hongyang Zhang, Yuerong Wang and Ping Hu

In this paper, we presented a new method for constructing near-infrared fluorescence probes and their applications in detecting glucose and hydrogen peroxide \( (\text{H}_2\text{O}_2) \). We used purified (6,5) single-wall carbon nanotubes (SWCNTs) separated by a polyethylene glycol/dextran aqueous two-phase system as the basis for near-infrared probes. Different phenylboronic acids were used for non-covalent modification of SWCNTs (6,5). Glucose was detected by the specific binding of the boronic acid group with cis-diol. Hydrogen peroxide was detected by horseradish peroxidase (HRP) combined with phenylboronic-acid-modified SWCNTs. The results revealed that the fluorescence intensity of purified SWCNTs was significantly enhanced without other chiral nanotube interactions compared to that of the raw SWCNT material. The fluorescence responses of 3-carboxy-5-nitrophenylboronic acid-modified purified CNTs could be used to effectively measure glucose in the concentration range from 0.01 mM to 0.50 mM with an interval linear index of \( R^2 = 0.996 \) (LOD = 1.7 \( \mu \text{M} \), S/N = 3). The detected \( \text{H}_2\text{O}_2 \) with the 3-aminobenzeneboronic acid-modified (6,5) SWCNT with HRP was in the concentration range from 5.0 \( \mu \text{M} \) to 40 \( \mu \text{M} \) with an interval linear index of \( R^2 = 0.997 \) (LOD = 0.85 \( \mu \text{M} \), S/N = 3). Moreover, this sensor exhibited a strong anti-interference effect without biological matrix fluorescence effects above 1000 nm wavelength. Thus, the proposed novel near-infrared fluorescence probes employed for reducing the interference of the biomatrix in this region are expected to be used in subsequent biosensor investigations.

1. Introduction

Single-wall carbon nanotubes (SWCNTs) have a stable and strong fluorescence in the near-infrared (NIR) region.\(^1,2\) In view of this special advantage, SWCNTs have been widely used as NIR optical sensors for detecting glucose,\(^3\) nitric oxide,\(^4\) nitroaromatics,\(^5\) and proteins\(^6-7\) by monitoring the fluorescence of SWCNTs after selective binding to the target analytes even at the single-molecule level.\(^8-10\) The NIR fluorescence probe provides a unique advantage over conventional fluorophores, which typically bleach and fluoresce in the ultraviolet or visible region. SWCNT NIR probes can overcome the challenges encountered because biological tissues are optically transparent and have minimal auto-fluorescence in the NIR wavelengths.\(^11\) Different chiral SWCNTs have varied absorption wavelengths,\(^12\) which results in uncontrollable interference during the application of unsorted SWCNTs for NIR detection because of the presence of different chiral CNTs. Hence, separation and purification of chiral SWCNTs are necessary for their application to detect biomolecules.

Diabetes is a metabolic disorder syndrome, which can cause mellitus-induced heart disease, kidney failure, or blindness due to insulin deficiency and is reflected by high blood glucose concentrations.\(^13\) Blood glucose monitoring is necessary for treatment and prevention of this disease. Enormous efforts have thus been invested to develop glucose biosensors to determine and monitor the level of glucose in the blood. These methods mainly depend on electrochemistry,\(^14\) optical techniques,\(^15-17\) spectroscopy,\(^17,18\) and field-effect transistors (FETs).\(^19\) Among them, optical techniques have been widely applied because of simple operation, quick response and low cost for further applications.\(^20\) However, most of these optical techniques are based on visible-region fluorescence, which may lead to interference signals from the biological matrices during further applications. By contrast, the probe having stable and strong fluorescence in the NIR region can overcome this defect. This leads to an attractive approach for the study of living cells.

---

\(^*\)Shanghai Key Laboratory of Functional Materials Chemistry, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China. E-mail: huping@ecust.edu.cn

\(^*\)Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China. E-mail: zhangm@ecust.edu.cn

\(\dagger\) Electronic supplementary information (ESI) available. See DOI: 10.1039/c8ra09272a
remodeling, and apoptosis. H$_2$O$_2$ plays an important regulatory role in many biological processes such as immune cell activation, vascular protection, and apoptosis. H$_2$O$_2$ plays an important regulatory role in many biological processes such as immune cell activation, vascular protection, and apoptosis.

In vivo. Boronic acids are excellent molecular receptors for saccharides and glycoproteins$^{21-23}$ in electrochemical$^{21-25}$ and fluorescence$^{26-28}$ sensors. Boronic acids can bind to the 1,2-cis-diol structure of glucose and modulate the signal response of sensors. A recent report showed that novel magnetic composites functionalized by boronic acids to form sandwich-like nanostructures can be applied to determine human platelet-derived growth factor. The method could achieve a remarkable detection limit of 0.22 FM.$^{29}$ As a reactive oxygen species, hydrogen peroxide (H$_2$O$_2$) is a key analyte because of its wide applications in biology, biomedicine, food security, and environmental protection.$^{30-32}$ H$_2$O$_2$ is commonly considered as a biomarker that can reflect human health. H$_2$O$_2$ not only induces cytotoxic effects but also acts as a signal molecule that regulates different biological processes such as immune cell activation, vascular remodeling, and apoptosis. H$_2$O$_2$ plays an important regulatory role in health and disease diagnosis. Given that H$_2$O$_2$ is a byproduct of many enzymatic reactions, its concentration can be used as an indicator to track biochemical processes.$^{33-35}$ Therefore, an accurate and sensitive method must be established for detecting H$_2$O$_2$ in the cell environment.

To date, several analytical techniques have been proposed for H$_2$O$_2$ detection including spectrometry,$^{36}$ fluorometry,$^{37,38}$ chromatography,$^{39,40}$ cell imaging,$^{41,42}$ and electrochemical methods.$^{43,44}$ Among these methods, fluorometry is an H$_2$O$_2$ detection strategy characterized by simplicity, easy operation, rapid response time, high sensitivity, and excellent selectivity. Fluorometry using fluorescent nanomaterials functionalized with a natural enzyme horseradish peroxidase (HRP) was found to be an attractive method for H$_2$O$_2$ sensing.$^{45}$ However, the fluorescence in the visible region is easily disturbed by the biological matrix, which greatly restricts the potential application of this approach for in vivo detection.

In vivo and in-time detection of biomarkers by biosensors is the goal for bioanalysis, and the biocompatibility of CNTs is gradually being explored. For example, SWCNTs have been used as optical probes for plants and small animals.$^{45-47}$ In view of the lack of biological fluorescence interference in the NIR regions of SWCNTs, SWCNT-based NIR optical sensing of glucose and H$_2$O$_2$ has great potential for in vivo sensors. Here, we report the first application of purified single chiral SWCNTs (6,5) by aqueous two-phase extraction for preparation of NIR probes. An NIR fluorescence sensor for glucose detection was prepared by non-covalently binding phenylboronic acid to the sidewalls of SWCNTs. Then, we functionalized HRP on this sensor by utilizing HRP’s cis-diol structure to establish a good measurement detector of H$_2$O$_2$ (Scheme 1).

2. Experimental section

2.1 Materials

Polyethylene glycol (PEG, 6 kDa) was purchased from Alfa Aesar. Dextran (DEX, 70 kDa) was purchased from TCI. Sodium dodecyl sulfate (SDS) was acquired from Sigma-Aldrich. Horseradish peroxidase was purchased from Shanghai Titan Scientific Co., Ltd. Phenylboronic acid was purchased from Aladdin. Various phenylboronic acid derivatives (3-nitrophenylboronic acid (3NPBA), 4-nitrophenylboronic acid (4NPBA), 2-carboxybenzenecboronic acid (2CPBA), 3-carboxy-phenzenecboronic acid (3CPBA), 4-carboxybenzenecboronic acid (4CPBA), 3-amino-phenenzecboronic acid (3AMBA), 3-carboxy-5-fluorobenzenecboronic acid (5F3CPBA), 3-carboxy-5-nitrophenylboronic acid (5N3CPBA), 5-carboxy-2-chlorobenzene boronic acid (2Cl5CPBA) 3-amino-4-methylbenzene boronic acid (3AAMPBA), 4-carboxy-3-fluorobenzenecboronic acid (4CF3PBA), 9-anthracenecboronic acid (9ANBA), 9-phenanthrenecboronic acid (9PHBA), 4-methyl-1-naphthalenecboronic acid (4M1NPBA)] were purchased from Aladdin. 4-Chlorophenyl boronic acid (4CYBA) and 3-acetylphenyboronic acid (3ACPBA) were purchased from Adamas-beta (chemical structures are in Table S1).

Aultrapure water (18.2 MΩ cm) was used throughout the experiment.

2.2 Instruments and characterization

UV/Vis/NIR spectra of all SWCNT solutions were obtained by using a SHIMADZU UV-2600 scanning spectrophotometer. The NIR fluorescence of the SWCNT solutions was measured using a QM/TM fluorescence spectrometer (Photon Technology Canada Inc.). Sample excitation was from a 560 nm photodiode laser. The SWCNT dispersion was centrifuged in a GT 16-WS ultrasonication centrifuge obtained from Thermo Fisher Scientific. The samples were put in a quartz fluorescence cuvette with 10 mm optical path length. The samples were excited by light with a wavelength of 560 nm, and the emission spectrum was recorded in the range from 900 to 1300 nm.

2.3 Separation of (6,5) SWCNT

First, 1 mg of SWCNT raw material was dispersed in 1 mL of 2 wt% sodium deoxycholate (SDC) solution. The samples were sonicated in an ice water bath for 1 h at 225 W and then ultracentrifuged for 1 h at 18g and 19 °C. After centrifugation, the supernatant was collected to obtain a uniformly dispersed SWCNTs–SDC dispersion. Then, 20 mL of 15 wt% PEG and 20 mL of 15 wt% DEX were mixed in a 50 mL centrifuge tube. After reaching equilibrium, the upper phase and the lower phase were collected for further separation.

A volume of 10 μL of the SWCNTs–SDC dispersion and 18 μL of 10 wt% SDS solution were added to 250 μL of 15 wt% PEG and 250 μL of 15 wt% DEX ATP system and then centrifuged for 30 s at 5000 rpm and room temperature after mixing. Then, 4 μL of MgCl$_2$ (ionic strength, $I = 0.5$ mol kg$^{-1}$) was added and centrifuged under the same conditions. The bottom (B) phase was collected, and 250 μL of the new upper phase was added to B to obtain a new ATP system. The amounts of 10 wt% SDS, 2 wt% SDC, and 0.05 M HCl that were added to the aqueous two-phase system were adjusted to make the upper phase olive in color. The bottom phase BB was collected, and 250 μL of the new upper phase was added to BB to obtain a new ATP system. The amounts of 10 wt% SDS, 2 wt% SDC, and 0.05 M HCl that were added to the aqueous two-phase system were adjusted to make the top (T) phase purple in color. The top phase BBT was the purified SWCNT (6,5) sample. The purified samples were heated and cleaned to remove the salts and the acids during...
2.5 Method of phenylboronic acid-functionalized SWCNT sensor for H2O2 detection

A PBA-HRP-SWCNT sensing system was established using the following procedures. Phenylboronic acids were dissolved in DMSO at 0.1 M concentration. Ten μL of the phenylboronic acid solution was added to 190 μL of the purified SWCNT solution. The concentration of the purified SWCNTs (6,5) was 0.06 mg mL\(^{-1}\) after calculation (Fig. S1†). The mixture solution was incubated for 30 min at room temperature.

Ten μL of the glucose solution dissolved in 1 M PBS buffer solution (pH 7.4) was added to 190 μL of the prepared SWCNT solution. A total of 17 kinds of incubated phenylboronic acid-functionalized SWCNT solutions were needed to make the final glucose concentration to 0.2 mM. After quick mixing, the fluorescence spectra of the mixture solution were consistently monitored at 30 min intervals at room temperature.

2.6 Fluorescence assay for glucose

A fluorescence glucose assay was performed using the following procedures. Different amounts of glucose were dissolved in 1 M PBS buffer solution (pH 7.4). We chose 5-nitro-3-carboxyphenyl boronic acid-functionalized SWCNTs as the glucose sensor. The method of glucose detection was the same as that described in Section 2.4. A fluorescence assay was conducted on potential interfering substances under the same conditions.

2.7 Fluorescence assay for H2O2

Different amounts of H2O2 were dissolved in 1 M PBS buffer solution (pH 7.4). We chose 3-aminophenylboronic acid-functionalized SWCNTs as the H2O2 sensor. The method of H2O2 detection was the same as that described in Section 2.5. We used HeLa cells in real sample detection of H2O2. The HeLa cells were cultured in a culture vessel containing 15 mL of DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin. The vessel was placed in a humid incubator under an atmosphere consisting of 95% air and 5% CO2 at 37 °C. When the cells reached confluence, the number of HeLa cells was counted using microscopy and calculated as 3.2 × 10\(^5\). All cells were collected through centrifugation (1000 rpm, 5 min) and dispersed in 4 mL of PBS solution for the subsequent fluorescence test. Ten μL of pretreated cell solution samples was added to 190 μL of the incubated HRP phenylboronic acid-functionalized SWCNT (6,5) solution and reacted for 30 min. Then, the fluorescence intensity was measured.

3. Results and discussion

3.1 Comparison between unsorted CNTs and purified SWCNTs (6,5)

The UV-VIS-NIR spectra of unsorted SWCNTs and purified SWCNTs (6,5) are shown in Fig. 1. When SWCNTs are sorted by the aqueous two-phase system, other chiral SWCNTs except for (6,5) are nearly excluded in the UV-VIS-NIR spectrum.
absorption spectrum, except for the characteristic E₁₁ (at 560 nm) and E₂₂ peaks (at 990 nm) of SWCNTs (6,5), the absorption peaks of other chiral carbon nanotubes nearly disappear. At 560 nm excitation, the emission spectra of the two fluorescence patterns differed greatly. Purified SWCNTs were superior to unsorted CNTs. The possible reason is that other chiral carbon tubes significantly affected the quenching of SWCNTs (6,5). After separation of CNTs, the NIR fluorescence intensity was significantly enhanced; hence, we chose purified SWCNTs for fabricating the probes.

Fig. 2 shows the fluorescence spectra of CNTs combined with phenylboronic acid derivatives. The purified and unsorted CNTs exhibited significantly varied fluorescence intensities. For example, for 5-nitro-3-carboxyphenylboronic acid-functionalized two kinds of CNTs, although the phenylboronic acid derivatives exerted a quenching effect on both CNTs according to fluorescence spectroscopy, the fluorescence intensity recovery greatly varied after glucose was added. In view of the sensitivity and intensity of the fluorescence signal, the purified CNTs offered a great advantage. The possible reason is that for unsorted CNTs, other chiral carbon nanotubes could quench SWCNTs (6,5), and this effect is markedly clear such that the fluorescence intensity of the carbon nanotubes varies greatly. Other chiral CNTs not only quenched the fluorescence of the carbon nanotubes but also combined with phenylboronic acid derivatives, thereby further reducing the sensitivity and stability of the carbon nanotubes as sensors. This phenomenon is the main reason for the use of purified CNTs in the following experiment. The effects of other chiral carbon nanotubes were reduced because of the separation of the carbon nanotubes, and the fluorescence performance of the sensors greatly improved.

3.2 Screening of phenylboronic acid derivatives

Phenylboronic acids can reversibly form stable phenylboronic complexes with cis-diols, which allow the detection of a wide range of saccharide markers. A generally accepted hypothesis is that aromatic boronic acids are conjugated on the surface of SWNTs through π–π interactions between the hydrophobic sidewall of SWCNTs and the aromatic moiety of boronic acids, which affects the fluorescence intensity of SWCNTs. The 1,2-cis-diol structure of glucose can combine with phenylboronic acids and modulate the fluorescence intensity of SWNTs when glucose is added. Strano and coworkers® used different phenylboronic acids combined with CNTs wrapped with different surfactants to detect saccharides. Although they performed excellent screening on the phenylboronic acid derivative-modified carbon tubes, the carbon tubes were not completely purified and the fluorescence was not improved. No study has considered the differences in the binding ability of phenylboronic acid and the fluorescence effects of purified CNTs. In this study, we used purified SWCNTs (6,5) for detecting glucose. As shown in Fig. 3, the CNTs were quenched in the NIR region after their sidewalls combined with phenylboronic acid derivatives through π–π stacking interactions.
Fig. 3 Screening of the reactivity of SWCNTs (6,5) with 17 phenylboronic acid derivatives and the fluorescence response of the 17 phenylboronic acid–functionalized purified SWCNTs (6,5) to glucose. (a) Fluorescence intensity change (red) of purified SWCNTs (6,5) after the addition of 17 different phenylboronic acid derivatives. (b) Fluorescence intensity change (blue) of purified SWCNTs (6,5) after the subsequent addition of 0.2 mM glucose to the 17 phenylboronic acid derivative–functionalized purified SWCNT (6,5) complexes shown in (a). Excitation at 560 nm and emission wavelength at 1010 nm.

Fig. 3a shows the fluorescence response to the addition of various phenylboronic acid derivatives calculated by Fig. S2.† Ten phenylboronic acids quenched the fluorescence intensity of purified CNTs. Among them, the phenylboronic acid derivatives double-substituted on the phenyl ring (except 4-fluoro-3-carboxyphenylboronic acid) induced a quenching effect on SWCNTs (6,5). For mono-substituted phenylboronic acid derivatives, nitro-substitution increased the fluorescence recovery of CNTs. In these phenylboronic acid derivatives, 5-nitro-3-carboxyphenylboronic acid-functionalized SWCNTs (6,5) displayed good fluorescence recovery, as shown in Fig. 2b. Thus, we chose this sample for linear analysis and selectivity experiment for the sensor.

3.3 Sensitivity and kinetics of the fluorescent SWCNT glucose sensors

We measured the linear response range of the phenylboronic acid derivatives combined with the SWCNT sensing system. As shown in Fig. 4, the fluorescence intensities of 5-nitro-3-carboxyphenylboronic acid–functionalized SWCNTs (6,5) were highly sensitive to glucose, and they increased as the analyte concentration increased. We used the emission peak wavelength at 1010 nm of (6,5) to calculate the intensity change \( \frac{I - I_0}{I_0} \) and establish the calibration curve (Fig. 4b).

The relationship between the analyte and the available docking sites for glucose can be described as follows:

\[
\text{PBASWCNT} + \text{GLU} \rightleftharpoons \text{GLUPBASWCNT} \quad (1)
\]

The calibration curve can be described by a kinetic adsorption model, in which G is the analyte glucose, S is an available recognition site on the SWCNT sensor, and SG is the product of this reaction. The equilibrium for this reaction can be modeled as an instantaneous reaction and described by the following equilibrium constant:

\[
K = \frac{[SG]}{[S][G]} \quad (2)
\]

\( S_{\text{tot}} \) denotes the total recognition sites of the free and occupied sites.

\[
S_{\text{tot}} = [SG] + [S] \quad (3)
\]

We can use eqn (2) and (3) to derive the following expression for \( S_{\text{tot}} \) in terms of the glucose concentration \([G]\):

\[
S_{\text{tot}} = [SG] + [S] = [SG] + \frac{[SG]}{K[G]} = [SG] \left( 1 + \frac{1}{K[G]} \right) = [SG] \left( \frac{K[G] + 1}{K[G]} \right) \quad (4)
\]

If we assume that the sensor response \( i.e., \) the normalized intensity change \( \frac{I - I_0}{I_0} \text{ ratio} \), we find that

\[
\frac{I - I_0}{I_0} = \alpha \frac{[SG]}{S_{\text{tot}}} + B = \alpha \frac{K[G]}{K[G] + 1} + B \quad (5)
\]

With regard to the response to glucose addition, the phenylboronic acid derivative on the sidewall of the carbon tube bound to the cis-diol structure of glucose after glucose was added to the phenylboronic acid-modified CNTs, resulting in fluorescence recovery of CNTs. In these phenylboronic acid derivatives, 5-nitro-3-carboxyphenylboronic acid-functionalized SWCNTs (6,5) displayed good fluorescence recovery, as shown in Fig. 2b. Thus, we chose this sample for linear analysis and selectivity experiment for the sensor.
where $B$ is the background, and $\alpha$ is the proportionality factor. Given that the curve is linear in the glucose concentration range from 10 $\mu$M to 500 $\mu$M, $K[G] + 1$ can be approximated as 1. Thus, eqn (5) can be approximated as

$$\frac{I - I_0}{I_0} = \alpha K[G] + B$$  \hspace{1cm} (6)

Fitting the curve in Fig. 4b indicated that a good linear relationship ($R^2 = 0.996$) existed between the fluorescence intensities and the glucose concentrations in the range from 10 $\mu$M to 500 $\mu$M as $\alpha K = 3.93$ and $B = 0.47$ with 1.7 $\mu$M detection limit ($S/N = 3$). This value was calculated by adding the sensor response for the addition of only PBS solution to thrice the standard deviation and then using the fit to eqn (6) to calculate the concentration.

3.4 Selectivity of the fluorescent SWCNT glucose sensors

Selectivity is a key parameter in assessing the performance of fluorescent chemosensors and is particularly important in the blood serum samples of biological samples with complex matrices. We examined the fluorescence responses of SWCNTs toward potential interfering substances coexisting in various amino acids, inorganic salts, and uric acid. As shown in Fig. 5, these substances only minimally affected (either by fluorescence quenching or enhancement) the SWCNT fluorescence.

3.5 Real sample detection of glucose

To investigate the potential practical applications of this method, we detected glucose in human serum. Pretreated serum solution samples (10 $\mu$L) were added to 190 $\mu$L of the prepared SWCNT (6,5) sensor system and reacted for 30 min. Then, the fluorescence intensity was measured. Table 1 shows the glucose concentrations obtained by our proposed method.

3.6 Determination of H$_2$O$_2$ by phenylboronic acid derivative-modified SWCNT (6,5) probe

As a glycoprotein, HRP has been proven to contain 21.8% carbohydrates, due to which it can be combined with boronic acid groups. Due to the specific binding of HRP to H$_2$O$_2$, we prepared a PBA-HRP-SWCNT sensor for H$_2$O$_2$ detection. On the basis of the preliminary screening of phenylboronic acid derivatives, five phenylboronic acid derivatives were selected for further analysis of H$_2$O$_2$ detection. Considering the fluorescence quenching of the purified SWCNTs by phenylboronic acid, as shown in Fig. 2, HRP did not significantly affect the 3AMBA-modified CNT fluorescence, whereas the fluorescence changed significantly after the addition of H$_2$O$_2$, as shown in Fig. 6. The variation in NIR fluorescence of SWCNTs was due to electron transfer when H$_2$O$_2$ was combined with PBA-HRP-

![Fig. 4](image_url)  
(a) Fluorescence spectra of the 5-nitro-3-carboxyphenylboronic acid-functionalized SWCNTs (6,5) at various glucose concentrations. (b) Linear plots of the reduced intensity of the SWCNT (6,5) fluorescence as linear relationship of glucose concentration, $R^2 = 0.996$. $I_0$ and $I$ are the corresponding fluorescence intensities of SWCNTs in the absence and presence of the analyte. Excitation at 560 nm and emission wavelength at 1010 nm.

![Fig. 5](image_url)  
Fig. 5 SWCNT-based system for various potential interfering substances. The concentrations of various inorganic salts (from left to right) are 0.16, 5.6, and 0.04 mM. The concentrations of various amino acids (from left to right) are 6, 6, 14, 8, and 30 $\mu$M. The concentrations of various nucleosides (from left to right) are 0.2, 200, and 0.1 nM. The concentrations of uric acid and glucose are 12 and 200 $\mu$M, respectively. The concentrations of potential interfering substances correspond to 25-fold dilution of those in serum considering the proper concentration of glucose detected by this sensor.
SWCNT. The combination of HRP and different derivatives of PBA-modified SWCNTs showed significant impact on the fluorescence intensity of SWCNTs. We assumed that the intensity change was due to the structural differences in the PBA derivatives. Although the four other phenylboronic acid derivative-modified SWCNTs (6,5) presented stronger fluorescence changes than 3AMBA after HRP addition, their fluorescence response to the analyte did not reach the level of signal variation.

To utilize phenylboronic acid-functionalized purified CNTs, HRP was combined with the probe to detect H$_2$O$_2$. The purified CNTs showed a good fluorescence signal and sensitivity to hydrogen peroxide (Fig. 7a). The unsorted CNTs exhibited nearly no response to hydrogen peroxide. The possible reason is that the ability of other chiral CNTs to bind PBA-HRP was stronger than that of SWCNTs (6,5), leading to the lack of quenching of the fluorescence signal at this wavelength after the addition of hydrogen peroxide. Another possible reason is that the signal variation was too small to recognize its change. In a previous research, it was reported that H$_2$O$_2$ affected the optical properties of CNTs due to its oxidation effect on SWCNTs.$^{10}$ In this paper, the introduction of H$_2$O$_2$ to PBA-SWCNT did not have significant fluorescence changes, as shown in Fig. 7b. We assumed that the boronic acid group that modified the SWCNT sidewall hindered the redox reaction. Meanwhile, boronic acid might also inhibit the dissolved oxygen in water from having an oxidation effect on SWCNTs. With the aid of HRP, H$_2$O$_2$ could be recognized by the sensor.

### 3.7 Sensitivity of the fluorescent SWCNT H$_2$O$_2$ sensors

The linear response range of the PBA-HRP-CNT sensing system was measured with the purified CNT probe. As shown in Fig. 8, a good linear relationship

\[ \frac{I - I_0}{I_0} = 0.0081C_{(H_2O_2)} + 0.1007, \quad R^2 = 0.997 \]

existed between the fluorescence intensities and the concentrations of H$_2$O$_2$ in the range from 5 μM to 40 μM with detection limit of 0.85 μM (S/N = 3). The fluorescence intensity was quenched with increasing H$_2$O$_2$ concentration. As we assumed, H$_2$O$_2$
might change the conformation/structure of HRP and PBA-HRP-SWCNT. The change in the surrounding environment of SWCNTs may result in fluorescence quenching. The high concentration of hydrogen peroxide could lead to complete fluorescence quenching of CNTs, which is the main constraint of this sensor. Various H₂O₂ determination results based on nanomaterial biosensors are listed in Table 2. It demonstrates that the 3AMBA-HRP-SWCNT sensor has an advantage for H₂O₂ determination. It was suggested that HRP in the functionalized SWCNTs provided an active site that was more conducive to the detection of H₂O₂. Additionally, the fluorescence emission affected by this combination in the NIR region exhibited a large Stokes shift. This advantage allowed the sensor to be less affected by interference in real samples, suggesting that the sensor could be applied for biosensing.

### 3.8 Real sample detection of H₂O₂

Table 3 shows the H₂O₂ concentrations obtained by our proposed method. The concentration of H₂O₂ in HeLa cells was determined to be lower than the detection limit of this method. Thus, the feasibility of the method was verified by the cell culture solution after H₂O₂ addition. The result shows that the method proposed in this study has good performance with respect to H₂O₂ detection in real samples because of small interference in the NIR fluorescence region, suggesting that this method is promising for glucose determination in medical diagnosis and biological analysis.

### 4. Conclusion

In conclusion, we demonstrated that purified SWCNTs (6,5) can be used as a raw material of potential sensors and probes in the NIR region. The separation of carbon tubes greatly enhanced the fluorescence signal of CNTs of the same concentration. The high concentration of hydrogen peroxide could lead to complete fluorescence quenching of CNTs, which is the main constraint of this sensor. Various H₂O₂ determination results based on nanomaterial biosensors are listed in Table 2. It demonstrates that the 3AMBA-HRP-SWCNT sensor has an advantage for H₂O₂ determination. It was suggested that HRP in the functionalized SWCNTs provided an active site that was more conducive to the detection of H₂O₂. Additionally, the fluorescence emission affected by this combination in the NIR region exhibited a large Stokes shift. This advantage allowed the sensor to be less affected by interference in real samples, suggesting that the sensor could be applied for biosensing.

Table 2  Comparison of the nanomaterial-based sensors for the detection of H₂O₂

<table>
<thead>
<tr>
<th>Sensors</th>
<th>Methods</th>
<th>Detection limit/µM</th>
<th>Linear range/µM</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRP-BMIM·BF₄/SWCNTs/CFUME</td>
<td>Electrochemical</td>
<td>0.13</td>
<td>0.49–10.2</td>
<td>50</td>
</tr>
<tr>
<td>Nitrogen-doped CNTs</td>
<td>Electrochemical</td>
<td>0.5</td>
<td>Not reported</td>
<td>51</td>
</tr>
<tr>
<td>AuNPs/4-CA</td>
<td>SERS</td>
<td>0.08</td>
<td>0.1–20</td>
<td>52</td>
</tr>
<tr>
<td>HTP-AuNCs</td>
<td>SERS</td>
<td>0.07</td>
<td>0.1–2.53</td>
<td>36</td>
</tr>
<tr>
<td>dLys-AgNCs</td>
<td>Fluorescence</td>
<td>0.2</td>
<td>0.8–200</td>
<td>53</td>
</tr>
<tr>
<td>HRP-MWCNT</td>
<td>Fluorescence</td>
<td>1.2</td>
<td>Not reported</td>
<td>28</td>
</tr>
<tr>
<td>dsDNA-SWCNT</td>
<td>NIR absorption</td>
<td>Not reported</td>
<td>29–5900</td>
<td>54</td>
</tr>
<tr>
<td>3AMBA-HRP-SWCNT</td>
<td>NIR fluorescence</td>
<td>0.85</td>
<td>5–40</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 3  H₂O₂ detection in HeLa cell suspension

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added H₂O₂/µM</th>
<th>Found H₂O₂/µM</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>11.8</td>
<td>118</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>20.4</td>
<td>102</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>28.2</td>
<td>94.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 8  (a) Fluorescence spectra of the 3AMBA-HRP-functionalized SWCNTs (6,5) at various H₂O₂ concentrations. (b) Linear plots of the reduced intensity of the SWCNT (6,5) fluorescence as linear relationship of H₂O₂ concentration, R² = 0.997. I₀ and I are the fluorescence intensities of the SWCNTs in the absence and presence of analytes, respectively.
concentration measurements and anti-interference in complex environments. Moreover, we briefly studied the fluorescence response of phenylboronic acid derivatives combined with SWCNTs (6,5) and enzymes, and we selected 3AMBA-HRP-functionalized SWCNTs (6,5) as the sensor for detecting hydrogen peroxide. Given that biological tissues are optically transparent and minimally auto-fluorescent in the NIR wavelengths, this study provides a new stable and robust NIR fluorescence sensor material for subsequent biosensor investigations.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Dr Geyou Ao for her critical comments on the manuscript. This research was financially supported by the National Natural Science Foundation of China (No. 81573397) and Shanghai Natural Science Foundation (No. 15ZR1409400).

References


