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A new biosensor for glucose determination in serum based on up-converting fluorescence resonance energy transfer

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ABSTRACT

In this work, a new glucose sensor based on up-converting fluorescence resonance energy transfer (UC-FRET) was developed. Up-converting phosphors (UCPs, $NaYF_4$: Yb, Er), which were covalently labeled with Concanavalin A (ConA), were used as the energy donor with thiolated β-cyclodextrins (SH-β-CDs) functionalized gold nanoparticles as the energy acceptor. Due to the combination between ConA and SH-β-CDs, the energy donor and the acceptor were brought to close proximity, resulting in the quenching of the fluorescence of UCPs by gold nanoparticles. In the presence of glucose which competed with SH-β-CDs towards the binding sites of ConA, the biosensor (UCPs–ConA–SH-β-CDs-Au) was decomposed and the energy donor was separated from the acceptor. Therefore, the fluorescence of UCPs was restored dependent on the concentration of glucose. The increase of UCPs fluorescence intensity was proportional to glucose concentration within the range from 0.4 μM to 10 μM in aqueous buffer, with a limit of detection (LOD) of 0.043 μM. A same linear range of glucose concentration was obtained in a human serum matrix (which was pretreated and thus contained no glucose) with a slightly higher LOD (0.065 μM). The glucose sensor was applied to real human serum samples with the results consistent with that of a classic hexokinase (HK) method, indicating that the UC-FRET biosensor was competent for directly sensing glucose in serum samples without optical interference, which benefited from the near infrared (NIR) excitation nature of UCPs. The results of this work suggested that the UC-FRET technique could be a promising alternative for detecting biomolecules in complex biological sample matrixes for diagnostic purposes.

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1. Introduction

Glucose is the most significant energy source in human metabolism processes, and the concentration of glucose in blood is considered as an indicator to human health conditions. The development of reliable methods for monitoring blood glucose has long been an interesting topic. Up to now, various methods for glucose determination have been proposed, mainly based on fluorescence spectrophotometry and electrochemical signal transduction. Electrochemical methods are simple and fast and have been widely used in glucose sensing (Xiao et al., 2009; Yuan et al., 2010; Zhu et al., 2010), but the signal is likely to be affected by those coexisting reductive substances such as ascorbate or ureate in serum or urine samples (Tang et al., 2008). As a highly sensitive and non-destructive technique, fluorescence has also been extensively utilized in glucose sensing with various principles and designs. A straightforward and commonly seen model is the competition between glucose and other carbohydrate derivatives such as dextran or mannoside towards ConA molecules, which are labeled with fluorescent dyes or fluorescent proteins (Meadows and Schultz, 1988; Lee and Perez-Luna, 2005; Aslan et al., 2005; Russell and Pishko, 1999; Ballerstadt and Schultz, 2000). A fluorophore-tagged glucose oxidase was also applied for glucose assay (Sierra et al., 1997, 2000). In addition, some boronic acid derivatives were constructed as fluorescent probes for glucose determination (James et al., 1994, 1996). A common concern of these sensors and probes is the rather severe photobleaching of the fluorescent molecules under the excitation of UV/vis spectral region (Frangioni, 2003). To solve this problem, some inorganic fluorophores including quantum dots (QDs, Yuan et al., 2009; Tang et al., 2008; Wu et al., 2010; Freeman et al., 2009) and fluorescent Au nanoclusters or nanodots also have been used in glucose sensing lately (Shiang et al., 2009; Jin et al., 2011). Such efforts have made the assays more robust and reli-

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able. Nonetheless, there is still an important issue related with the down-converting fluorescence, in which the fluorophores are normally excited with UV/vis light. Within this excitation window, the interference of auto-fluorescence and/or scattering light from biological substances always arise and cannot be ignored, which limits the use of fluorescence in the assay of biomolecules, especially in complicated biological matrixes.

Therefore, it is still meaningful to explore new techniques and methods for determining biomolecules like glucose, directly in complicated biological matrixes such as serum. In the past few years, anti-Stokes phosphors have attracted increasing attentions in bioassay. Due to their photophysical feature, which is a visible emission under near-infrared excitation, they are capable of circumventing the problem of autofluorescence and scattering light, enabling highly sensitive determination of target molecules in biological matrixes without optical interferences (Liu et al., 2008, 2010, 2011; Dong et al., 2009; Kim et al., 2007; Kumar and Zhang, 2010; Rufaihah and Zhang, 2008).

Up-converting phosphors (UCPs) are a kind of promising anti-Stokes fluorophore because of their excellent photophysical and chemical properties which include high photoluminescence efficiency, narrow emission peak, low toxicity, good chemical stability and photostability. Hence, UCPs have found broad applications in bioassay in recent years. In particular, UCPs are employed as energy donors in fluorescence resonance energy transfer (FRET)-based assays. FRET is a nonradiative energy transfer process from a fluorescent donor molecule to an acceptor in close proximity (1–10 nm) via long-range dipole–dipole interactions (Sapsford et al., 2006). As the energy transfer rate is inversely proportional to R^6, where R is the distance between the donor and the acceptor (Pickup et al., 2005), FRET has been recognized as an exceptionally sensitive method and has been widely used in bioassays (Wang et al., 2002; Oswald et al., 2000). In addition to the above mentioned merits of UCPs, the very large Stokes shift (>100 nm) also makes them promising energy donors, since in this circumstance the excitation overlap of the donor and the acceptor can be avoided and thus the co-excitation of the donor–acceptor pair is thoroughly eliminated (Schmid et al., 2001; Willard et al., 2001). The earliest reports on FRET using UCPs as the energy donor appeared in 2005 (Wang et al., 2005; Kuningas et al., 2005). Thereafter, the UC-FRET technique was developed in several fields such as protein detection and DNA assay (Rantanen et al., 2007, 2008; Kuningas et al., 2006; Zhang et al., 2006; Wang et al., 2009).

So far, however, there have been relatively rare reports on employing UCPs in FRET-based methods for the determination of biomolecules with clinical significance in complicated biological sample matrixes (Kuningas et al., 2007). Herein in this work, we have developed a UC-FRET-based biosensor for directly determining glucose in human serum samples. Rare earth (Yb, Er) codoped $NaYF_4$ nanocrystal, which is regarded as one of the most efficient up-converting materials due to the low phonon energy of $NaYF_4$ as a host matrix (Heer et al., 2004; Wang et al., 2011), is adopted as the energy donor. Au nanoparticles are used as the energy acceptor, which are tagged to thiolated β-cyclodextrins. The assay is based on the competition between thiolated β-cyclodextrins and glucose towards ConA. This new biosensor is able to selectively respond to glucose in serum, with a high sensitivity and strong ability to resist background interference.

2. Materials and methods

2.1. Materials

All chemicals were of analytical grade and were used as received without further purification. Concanavalin A (ConA, type IV), polyacrylic acid (average molecular weight is 1800) and 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC·HCl) were purchased from Sigma–Aldrich. Glucose oxidase was the product of Amresco Corporation. Morpholineethanesulfonic acid (MES) was obtained from Biosharp Corporation. The rest chemical reagents were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity). Human serum samples were obtained from three volunteers and collected in Zhongnan Hospital, Wuhan University School of Medicine.

2.2. Instrumentation

The crystal phase of $NaYF_4$:Yb, Er nanoparticles was identified by a Brucker D8 Discover X-Ray Diffractometer (XRD) with 2θ range from 10° to 70° at a scanning rate of 4° per minute, with Cu Ka irradiation (k = 1.5406 Å). The sizes and morphologies of PAA–UCPs and Au nanoparticles were characterized by a JEM-2010 transmission electron microscope (TEM) operated at 200 kV. A 980 nm CW laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) was used as the excitation source with the power being set at 500 mW for all upconverting fluorescence experiments. The upconverting fluorescence spectra were recorded on a DCS200PC Photon Counting (Beijing Zolix Instruments Co., Ltd.) with single-photon sensitivity through an Omni-λ300 monochromator (Beijing Zolix Instruments Co., Ltd.). The FT-IR spectra of PAA–UCPs nanoparticles and SH-β-CDs were measured on a Magan-IR™ Spectrometer 500 (Nicolet, Madison, WI, USA) with the KBr pellet technique. The UV–vis absorption spectra of gold nanoparticles modified with SH-β-CDs and UCPs–ConA bioconjugates were acquired using a TU-1900 UV–vis spectrophotometer (Beijing Purkinje General Instrument, China). An automatic Biochemistry Analyzer (Aeroset™, USA) was used for glucose assay with a hexokinase method.

2.3. Synthesis of $NaYF_4$: Yb, Er nanoparticles modified with polyacrylic acid

In a typical synthesis procedure, lanthanide oxides Y_2O_3 (0.195 mmol), Yb_2O_3 (0.05 mmol) and Er_2O_3 (0.005 mmol) were dissolved in hot nitric acid (65 °C) to acquire $Ln(NO_3)_3$, and the solvent was evaporated after 6 h reaction. The as-obtained nitrate salts were added to a solution containing 900 mg polyacrylic acid. And then another aqueous solution containing 0.21 g NaF (F^-/Ln^{3+} = 10) was added dropwise to the above solution under vigorous stirring. After stirring at room temperature for 20 min, the mixture (with a total volume of 36 ml, $V_{ethanol}$:V_{water} = 1:1) was transferred into a 50 ml Teflon autoclave and heated to 240 °C. After 10 h hydrothermal treatment, the autoclave was cooled down to room temperature naturally, and then a precipitate was obtained by centrifuging. The precipitate was washed with water and absolute ethanol three times respectively and finally dried under vacuum.

2.4. Preparation of UCPs–ConA bioconjugates

Concanavalin A (ConA, 1 mg) was dissolved in 1 ml Tris–HCl buffer (pH = 7.4, 10 mM) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM) to obtain a solution (1 mg/ml), which was then stored at 4 °C. To activate the surface carboxylic groups of UCPs, EDC·HCl (3 mg) and Sulfo-NHS (9 mg) were added to 5 ml MES buffer (0.1 M, pH = 6.1) containing 3 mg UCPs. Then the mixture was stirred for 2 h at room temperature. After centrifuging, the obtained activated UCPs were washed with water and Tris–HCl buffer once respectively in order to remove excess small molecules, and then they were re-dispersed in 2 ml Tris–HCl buffer. Subsequently, the activated UCPs and the ConA solution were mixed together to react for 2 h with gentle stir at 4 °C. The UCPs–ConA bioconjugates

were obtained from the solution by centrifuging and washing with Tris–HCl buffer three times to remove free ConA. The final bioconjugates were dissolved in 2 ml Tris–HCl buffer.

2.5. Synthesis of mono-6-thio-β-CDs (SH-β-CDs)

6-OTs-β-CDs were prepared by the reaction of *p*-tosyl chloride with β-CDs in dry pyridine according to references (Melton and Slessor, 1971; Tang et al., 2005). In a typical synthesis procedure, 18 g β-CDs were dissolved in 180 ml dry pyridine. Under vigorous stirring, 20 ml dry pyridine containing 3.024 g *p*-tosyl chloride was added dropwise to the above solution at 0 °C. The mixture was kept at 0 °C for 12 h, followed by stirring at room temperature for two days. Pyridine was removed by evaporation until a yellow solid was obtained. 40 ml DMF solvent was added to dissolve the yellow solid, and then 12 ml acetone was added to the solution to acquire a white precipitate. The precipitate was filtered and washed with water, followed by recrystallization from hot water three times. Finally, 2.6 g 6-OTs-β-CDs were obtained with 16% yield.

2.0 g 6-OTs-β-CDs and 2.2 g thiourea were mixed in 100 ml solution containing 80% methanol (v/v) and 20% (v/v) water. The mixture was refluxed for two days at 85 °C and evaporated under vacuum. Then the obtained white solid was dispersed in 30 ml methanol and stirred for 1 h. The precipitate was filtered and dissolved in 70 ml 10% NaOH (w/w) and kept at 50 °C for 5 h. After that, 10% HCl (v/v) solution was added to adjust the pH value of the solution to 2.0, and then 25 ml 1, 2-dichloroethane was added to the solution and stirred for one day. After removing 1, 2-dichloroethane, a white solid was obtained. Finally, the white solid was recrystallized from hot water three times and 0.83 g SH-β-CDs were obtained with a yield of 48.3%.

2.6. Preparation of SH-β-CDs modified Au nanoparticles

50 ml aqueous solution containing 0.01% $HAuCl_4$ and 30 mg SH-β-CDs was heated to reflux. Subsequently 0.75 ml trisodium citrate (1%) was injected quickly to the boiling solution (Liu et al., 2001; Grabar et al., 1995; Chen and Chang, 2004). One minute later, the color of the reaction changed to deep red. The mixture was heated under reflux for 30 min and then set aside to cool down to room temperature naturally. The free SH-β-CDs molecules were separated from the SH-β-CDs-Au conjugates by selective diffusion through a semipermeable membrane. The concentration of the as-prepared Au-NPs was calculated to be approximately 3.0 nM.

2.7. Disposal of the serum sample

2 ml human serum containing 0.2 mg glucose oxidase (with a specific activity of 100 μmol/min/mg) was incubated for 18 h at 37 °C to remove inherent glucose in the serum. And then the pH of the serum was adjusted to 8.5 using diluted NaOH solution to destroy the activity of glucose oxidase, which irreparably loses its catalytic activity under the condition of pH < 3 or pH > 8 (Keilin and Hartree, 1948). The pretreated serum with the pH value being adjusted to 7.4 was used as a biological matrix in the following experiments.

2.8. Up-converting fluorescence experiments

In a typical FRET assay in aqueous solution, 300 μL SH-β-CDs-Au (0.9 nM) was added to 160 μL UCPs–ConA (0.24 mg/ml) and the mixture was incubated at room temperature for 1.0 h to ensure the complete fluorescence quenching. Thereafter, varying concentrations of glucose were individually introduced to the UCPs–ConA–SH-β-CDs-Au complexes and each reaction was diluted with Tris–HCl buffer (10 mM, pH 7.4) to a final volume of 1 ml. Then, the mixtures were incubated for another 1 h at room temperature to achieve the recovery of UCPs fluorescence. The reaction mixture was then subject to fluorescence measurements with the excitation of 980 nm. The emission intensity at 547 nm was used for glucose quantification. To examine the specificity of the FRET sensor, some other biomolecules and metal ions were added into the biosensor in place of glucose with the same experimental conditions. For the glucose detection using human serum as the matrix, 100 μL pretreated serum (containing no glucose) was added in each reaction and the final volume was compensated with Tris–HCl buffer to 1 ml. An identical detection procedure as in the aqueous solution was then followed.

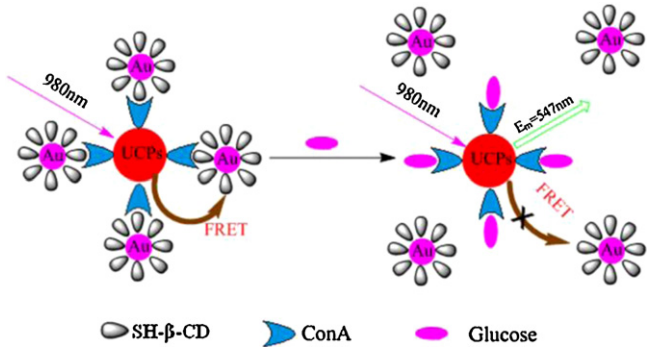

Fig. 1. Schematic illustration of the principle of the glucose biosensor based on UC-FRET.

In the process of glucose determination in real serum samples, 100 μL pretreated human serum was used as the assay medium. After the construction of the UCPs–ConA–SH-β-CDs-Au complexes as described above, 1 μL fresh human serum sample containing glucose was introduced and the mixture was diluted with Tris–HCl buffer to 1 ml and was incubated for 1 h. The up-converting fluorescence of the mixture was measured and the glucose concentration was determined using the calibration curve obtained in pretreated serum. The results were compared with that of the hexokinase method (Bondar and Mead, 1974), a widely used clinical method in hospitals for glucose assay. Standard addition experiments were further conducted in the real serum samples, in which different amounts of external glucose were added and the recoveries were calculated.

3. Results and discussion

3.1. Principle of the glucose biosensor based on up-converting fluorescence resonance energy transfer

The principle of the glucose biosensor based on UC-FRET is schematically illustrated in Fig. 1. Concanavalin A is a plant lectin with four binding sites for glucose or mannose through the specific molecular recognition. Glucose sensors are usually based on the competitive binding to the ConA molecules of either glucose or labeled carbohydrates containing α-D-glucopyranosyl subunits, such as dextran, mannoside or glycated protein. β-Cyclodextrin is a kind of carbohydrate containing seven α-D-glucopyranosyl units, thus it has the potential affinity towards ConA. In our design, UCPs labeled with ConA were adopted as energy donors, and SH-β-CDs modified Au nanoparticles were used as energy acceptors. Through the specific combination between SH-β-CDs and ConA, the energy donor and the acceptor could be brought to a close distance enabling the FRET process. In this situation, the fluorescence emission of UCPs was quenched by Au nanoparticles. In the presence of glucose which possesses higher affinity to ConA than SH-β-CDs, the biosensor, i.e., the UCPs–ConA–β-SH-CDs-Au com-

plex, was disassembled and thus the energy donor and the acceptor were separated, blocking the FRET process. Therefore, the fluorescence of UCPs was restored in a glucose concentration-dependent manner.

3.2. Characterization of $NaYF_4$: Yb, Er nanoparticles modified with polyacrylic acid

The crystal phase of the as-synthesized $NaYF_4$: Yb, Er nanoparticles was identified by XRD patterns (Fig. S1A). It can be seen that the main crystal phase of the UCP materials is hexagonal phase, combined with a little amount of cubic phase. It is known that the photoluminescence efficiency of hexagonal phase is about one order of magnitude higher than that of cubic phase. Therefore the as-prepared UCPs would have a relatively high photoluminescence efficiency. The existence of PAA molecules on the surface of UCPs was verified with FT-IR spectra (Fig. S1B). Compared to the spectrum of unmodified $NaYF_4$: Yb, Er nanoparticles (curve a), the FT-IR spectrum of PAA/$NaYF_4$: Yb, Er (curve b) shows unique absorption peaks of methylene asymmetric C–H stretching (2945 cm^{-1}), C=O stretching vibration (1720 cm^{-1}), C–O stretching (1195–1273 cm^{-1}) and carboxylate asymmetric and symmetric COO^- stretching (1570–1394 cm^{-1}). Owing to the abundant carboxyl groups on the surface, the PAA–UCPs possess good water-solubility and reactive sites for subsequent ConA labeling. The TEM image (Fig. S1C) exhibits spherical morphology of the PAA modified UCP materials with a diameter distribution from 30 nm to 60 nm, and it also reveals good monodispersibility of the nanoparticles in aqueous solution.

3.3. UV–vis characterization of UCPs–ConA bioconjugates

The successful preparation and purification of UCPs–ConA conjugates are illustrated by UV–vis spectra (Fig. S2). The spectrum of the last filtrate of the purification (curve a) shows no obvious absorption, indicating that the last filtrate did not contain free ConA or UCPs and that the purification was quite complete. Pure ConA shows a typical protein absorption peak at 278 nm (curve c), and the labeling product exhibits an absorption peak at 273 nm (curve d). Further comparing the spectrum of the product with that of pure UCPs colloid (curve b), it is safe to conclude that ConA molecules have been successfully conjugated to UCPs.

3.4. Characterization of SH-β-CDs and SH-β-CDs modified Au nanoparticles

The FT-IR spectra of β-CDs, Au-SH-β-CDs and SH-β-CDs were recorded and compared (Fig. S3A). Through the comparison between the spectra of β-CDs (curve a) and SH-β-CDs (curve c), the successful thiolation of β-CDs can be confirmed since curve c shows a unique absorption peak at SH stretching vibration 2505 cm^{-1}. The characteristic SH stretching vibration absorption peak disappears after the conjugation between SH-β-CDs and Au nanoparticles (curve b), confirming that SH-β-CDs were modified to the surface of Au through Au–S covalent bonds. The surface plasmon resonance absorption peak of the as-prepared SH-β-CDs-Au nanoparticles can be observed at 528 nm (Fig. S3B). The TEM image of the SH-β-CDs modified Au particles (Fig. S3C) shows an average diameter of approximate 18 nm and excellent monodispersibility in aqueous solution.

3.5. Fluorescence quenching of UCPs–ConA by SH-β-CDs-Au

According to the principle of fluorescence resonance energy transfer (FRET), when the emission spectrum of the energy donor overlaps well with the absorption spectrum of the energy acceptor, and the distance between the donor and the acceptor is in the range of ca. 1–10 nm, the fluorescence of the donor could be quenched effectively by the acceptor. In our system, $NaYF_4$: Yb, Er nanoparticles have bright green emission (peaking around 547 nm) excited by a near-infrared 980 nm diode laser, which has a good match to the absorption spectrum of SH-β-CDs-Au. Owing to the specific combination between SH-β-CDs and ConA, the distance between the donor and the acceptor was close enough leading to the occurrence of FRET. As a result, the fluorescence emission of UCPs was notably quenched by Au. As shown in Fig. 2A, the fluorescence intensity of UCPs–ConA (0.24 mg/ml) decreased gradually with the addition of increasing amounts of SH-β-CDs-Au. And the fluorescence quenching was linearly related to the concentration of the quencher from 0 to 0.9 nM (Fig. 2B). To acquire optimal fluorescence quenching efficiency in the subsequent glucose sensing, 0.9 nM of SH-β-CDs-Au was used with the concentration of UCPs–ConA being fixed at 0.24 mg/ml.

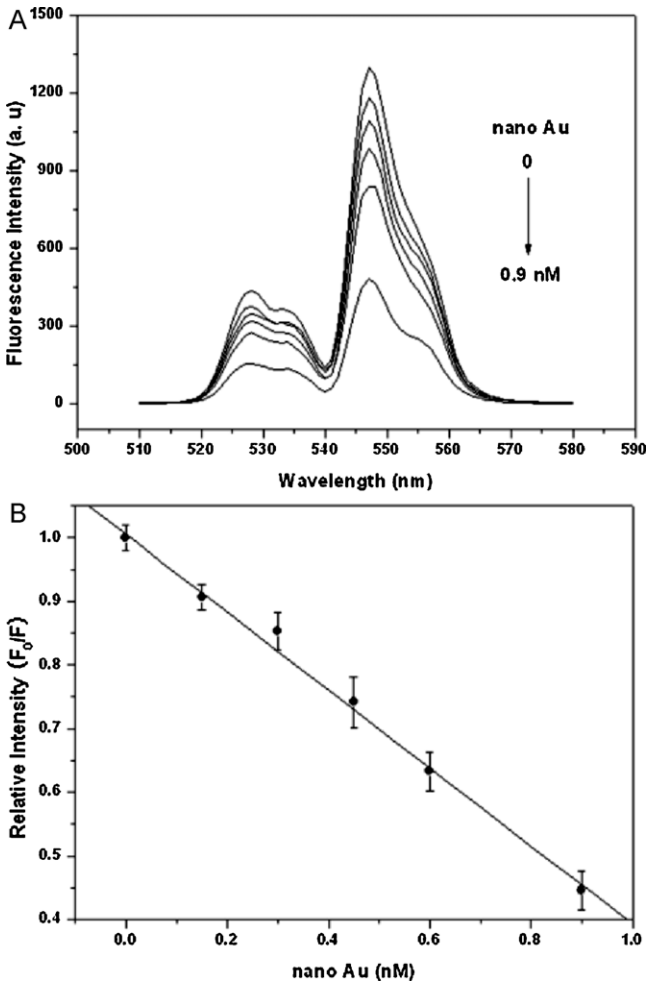

Fig. 2. (A) Up-converting fluorescence spectra of 0.24 mg/ml UCPs–ConA in the presence of different concentrations of SH-β-CDs-Au (0, 0.15, 0.3, 0.45, 0.6, 0.9 nM). (B) Linear plot of F/F_0 versus the concentration of SH-β-CDs-Au within 0–0.9 nM, where F and F_0 represent the fluorescence intensity of UCPs–ConA (at 547 nm) in the presence and in the absence of SH-β-CDs-Au, respectively. Data were presented as average (±SD) from three independent measurements. Excitation wavelength: 980 nm.

3.6. Glucose determination with the UC-FRET biosensor in aqueous solution

Upon the introduction of glucose into the UCPs–ConA–SH-β-CDs-Au system, the formation of UCPs–ConA–glucose complexes

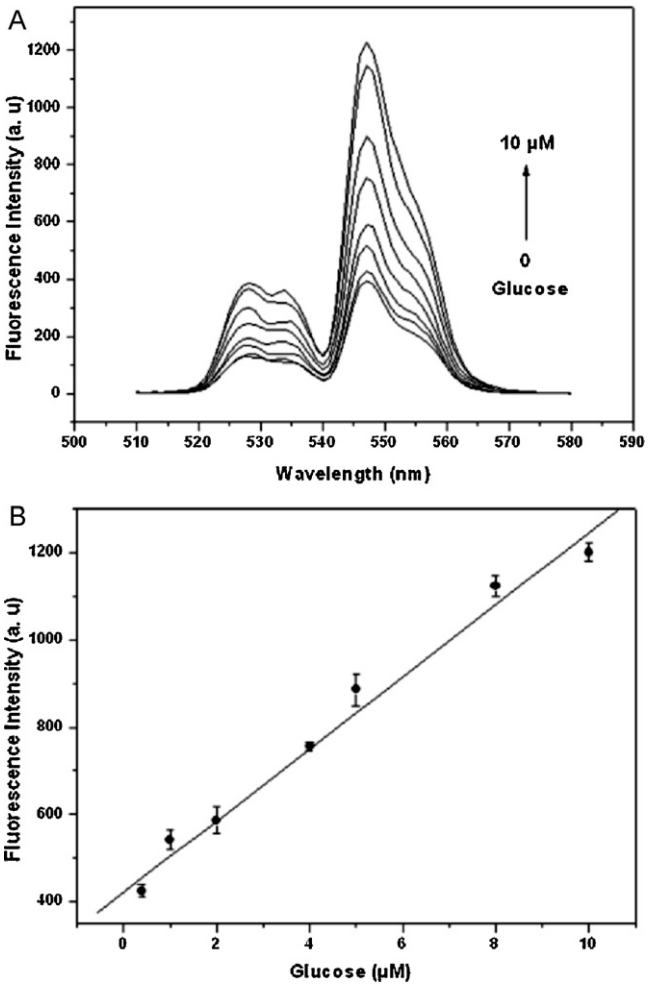

Fig. 3. (A) Fluorescence recovery of the biosensor with the introduction of varying amounts of glucose (0, 0.4, 1, 2, 4, 6, 8, 10 μM) in Tris–HCl buffer (pH 7.4). (B) The linear relationship between the fluorescence intensity at 547 nm and glucose concentration within the range of 0–10 μM, in Tris–HCl buffer. Data were presented as average (±SD) from three independent measurements. Excitation wavelength: 980 nm.

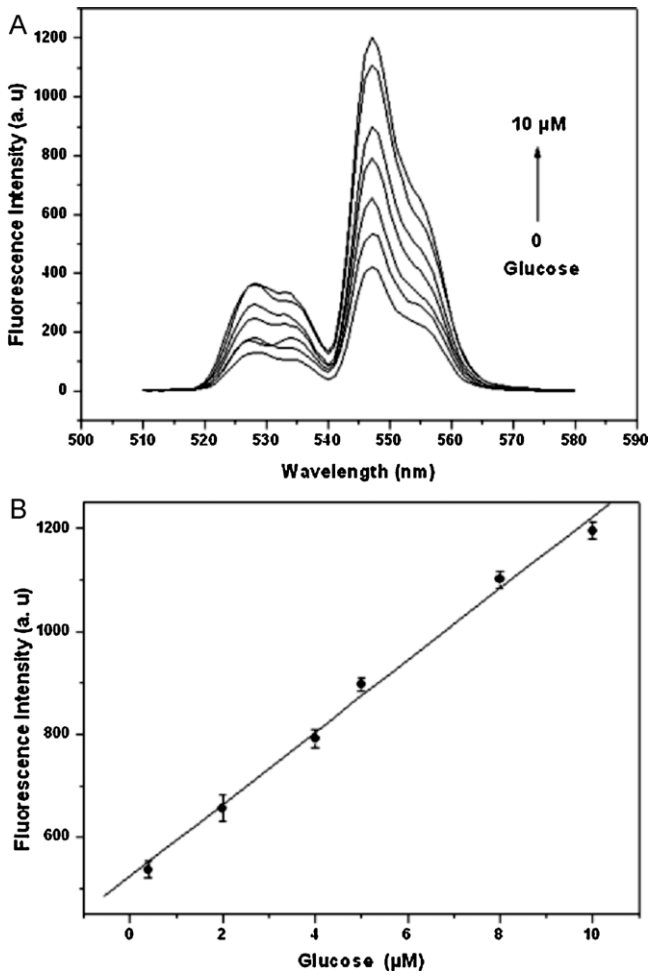

Fig. 4. (A) Fluorescence recovery of the biosensor with the introduction of different concentrations of glucose (0, 0.4, 2, 4, 5, 8, 10 μM) in pretreated serum (10-fold diluted with Tris–HCl buffer, pH 7.4). (B) The linear relationship between the fluorescence intensity at 547 nm and glucose concentration within the range of 0–10 μM, in 10-fold diluted pretreated serum. Data were presented as average (±SD) from three independent measurements. Excitation wavelength: 980 nm.

separated UCPs–ConA away from the quencher, Au nanoparticles, resulting in the disappearance of FRET and the restoration of the donor fluorescence. The up-converting fluorescence intensity of the biosensor gradually increased with increasing the amount of glucose (Fig. 3A). A linear relationship was found between the fluorescence intensity and glucose concentration within the range from 0.4 μM to 10 μM (Fig. 3B). The determination limit was calculated as 0.043 μM according to the $3s_b/m$ criterion, where m is the slope for the range of the linearity used and s_b the standard deviation of blank ($n = 7$). As compared to those currently available methods for glucose determination, the sensitivity of this UC-FRET method is quite competitive, that is, the LOD is lower than that of most literatures, which reported LOD values ranging from 0.05 μM to 0.1 mM (Tang et al., 2008; Yuan et al., 2009; Wu et al., 2010; Freeman et al., 2009; Jin et al., 2011; Shiang et al., 2009).

3.7. Interferences of coexisting biological species

To assess the selectivity of the UC-FRET biosensor for glucose, the response of the sensor towards other sugars, metal ions, amino acid and protein were investigated. The concentrations of the sugars were 10 times higher than glucose, and the concentrations of the other inspected species were approximate to normal levels in human serum. To the above constructed biosensor, the inspected species were added individually, the fluorescence intensities of which were compared to that of the biosensor (denoted as blank). As shown in Table 1, none of these substances caused obvious fluorescence alteration of the sensor. The relative deviations of fluorescence ($F - F_0/F_0$, where F and F_0 were the fluorescence intensities of the biosensor with and without the interferential substances, respectively) were within the range of ±6%. The results have shown a pronounced selectivity of the sensor for glucose.

3.8. Glucose determination in pretreated human serum

In order to estimate whether this method can work in complicated biological matrixes, the biosensor was tried for glucose determination in a human serum medium. To preclude the influence of inherent glucose in serum, they were removed through disposal of the serum sample with glucose oxidase. Since the specific activity of the glucose oxidase was 100 μmol/min/mg, the inherent glucose in the 2 ml human serum sample must have been effectively depleted by 0.2 mg glucose oxidase after 18 h reaction (Swoboda and Massey, 1965). The enzyme was then deactivated to avoid the influence on the subsequent glucose determination. The up-converting fluorescence of UCPs–ConA in the 10-fold diluted pretreated serum was first compared with

Table 1
Responses of the UC-FRET biosensor to other biomolecules and metal ions.

Investigated substance	Concentration (mol L^{-1})	Fluorescence intensity (average ± SD)[a]	Relative deviation (%) $[(F-F_0/F_0)] \times 100$[b]
Sensor	–	385 ± 14.2	0
Na^+	1.0×10^{-3}	370 ± 15.7	−3.9
K^+	1.0×10^{-3}	360.7 ± 16.6	−6.3
Mg^{2+}	1.0×10^{-4}	376.5 ± 11.3	−2.2
Ca^{2+}	1.0×10^{-3}	374.6 ± 10.8	−2.7
Zn^{2+}	1.0×10^{-5}	380 ± 18.2	−1.3
Cu^{2+}	1.0×10^{-6}	375.8 ± 4.8	−2.4
D-Fructose	1.0×10^{-4}	395.8 ± 16.2	2.8
Maltose	1.0×10^{-4}	400 ± 20.5	3.9
Sucrose	1.0×10^{-4}	409.6 ± 15.6	6.4
L-Cysteine	1.0×10^{-5}	403.1 ± 12.9	4.7
BSA	1.0×10^{-6}	395.4 ± 10.5	2.7
Glucose	1.0×10^{-5}	1216.6 ± 37.5	216

[a] The data are results from three independent measurements.
[b] F_0 and F represent the fluorescence intensity of the sensor in the absence and in the presence of the investigated substance, respectively.

Table 2
Determination of glucose in three real human serum samples.

Sample no.	UC-FRET method (mM)	RSD[a] (%) $n=3$	Hexokinase method (mM)[a]	RSD[b] (%) $n=3$	Added (μM)	Found (μM)	Recovery (%)
1	4.42	3.1	4.3	1.3	2	6.35	96.5
2	6.27	2.7	6.32	0.5	2	8.32	103
3	8.58	6.2	8.2	0.2	1	9.63	105

[a] RSD for the UC-FRET method.
[b] RSD for the hexokinase method.

that in the aqueous solution (Fig. S4). It shows that the fluorescence of the donor in these two assay media were nearly the same, indicating that the numerous substances in the serum had no obvious effect on UCPs fluorescence. The UCPs–ConA–SH-β-CDs-Au sensor was constructed in the 10-fold diluted pretreated serum, to which varying amounts of external glucose were then added and allowed for reaction and fluorescence measurements. The glucose-induced fluorescence enhancement was found to be similar with that in the aqueous solution (Fig. 4A). Significantly, a linear calibration curve for glucose in the range of 0.4–10 μM was also obtained in the serum medium. Such a linear dynamic range is wide enough for monitoring the blood glucose level. The detection limit was calculated as 0.065 μM, slightly higher than that in the aqueous buffer. The results demonstrated high robustness of the UC-FRET sensor in the serum, which can be attributed to the substantive power of up-converting phosphors to resist background interferences in complicated biological sample matrixes.

3.9. Glucose sensing in real human serum samples

To validate the biosensor in the analysis of real samples, it was then used to detect intrinsic glucose in human serum. Considering the normal glucose level in human serum and the linear range of our method, 1 μL of fresh human serum was added to the UCPs–ConA–SH-β-CDs-Au sensor, using the pretreated and 10-fold diluted serum as the assay medium. As list in Table 2, the glucose concentrations detected with this UC-FRET sensor were consistent with that measured by the hexokinase method. The relative standard deviations (RSD) of the proposed method were at a low level (from 2.7% to 6.2%), demonstrating a good reproducibility. The practical applicability of the proposed method was further verified through standard addition experiments, with the recoveries of glucose in the three serum samples ranging from 96.5% to 105%. Therefore, this newly developed UC-FRET-based biosensor has shown an attractive prospect of glucose determination in biological samples and is likely to be used in clinical assay.

4. Conclusions

A new and facile glucose biosensor based on UC-FRET has been developed, utilizing up-converting phosphors ($NaYF_4$: Yb, Er) as the energy donor and Au nanoparticles as the energy acceptor. Through the specific molecular recognition between ConA and SH-β-CDs, which were tagged to the donor and the acceptor respectively, the fluorescence of UCPs was quenched by Au nanoparticles. Resulting from the competition between glucose and β-SH-CDs towards the binding sites of ConA, the fluorescence of UCPs was restored dependent on the concentration of glucose, which built the foundation of glucose quantification. A linear range for glucose concentration (0.4–10 μM) was found both in an aqueous solution and in a serum medium, which proved that the UC-FRET technique was capable of eliminating background interferences in complex biological sample matrixes. Quite low a limit of detection was acquired in both the two assay media, i.e., 0.043 μM in aqueous buffer and 0.065 μM in serum, showing that the sensitivity of the proposed method is higher than those currently available methods for glucose determination. The biosensor was used to detect glucose in real human serum samples with satisfactory results. Therefore, the UC-FRET-based biosensing would have potential use in clinical determination or diagnosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.07.057.

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