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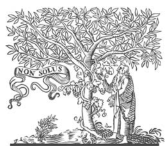

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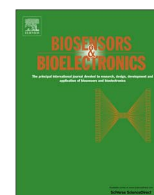

A double responsive smart upconversion fluorescence sensing material for glycoprotein

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ABSTRACT

A novel strategy was developed to prepare double responsive smart upconversion fluorescence material for highly specific enrichment and sensing of glycoprotein. The novel double responsive smart sensing material was synthesized by choosing Horse radish peroxidase (HRP) as modal protein, the grapheme oxide (GO) as support material, upconversion nanoparticles (UCNPs) as fluorescence signal reporter, N-isopropyl acrylamide (NIPAAM) and 4-vinylphenylboronic acid (VPBA) as functional monomers. The structure and component of smart sensing material was investigated by transmission electron microscopy (TEM), Scanning electron microscopy (SEM), X-ray photoelectron spectroscopic (XPS) and Fourier transform infrared (FTIR), respectively. These results illustrated the smart sensing material was prepared successfully. The recognition characterizations of smart sensing material were evaluated, and results showed that the fluorescence intensity of smart sensing material was reduced gradually, as the concentration of protein increased, and the smart sensing material showed selective recognition for HRP among other proteins. Furthermore, the recognition ability of the smart sensing material for glycoprotein was regulated by controlling the pH value and temperature. Therefore, this strategy opens up new way to construct smart material for detection of glycoprotein.

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1. Introduction

Recently, sensors have been utilized widely in clinical diagnosis, food analysis and bioprocess monitoring. Sensors, as analytical device for detecting analytes, usually include recognition element, transducer element and read-out device. According to the transducer types, sensors can be classified chemical sensors, thermal sensors, fluorescence sensors and electrochemical sensors and so on (Ronkainen et al., 2010; Haupt and Mosbach, 2000; Kriz et al., 1995). Yang et al. (2014) reported the microfluidic electrochemical DNA sensors for the detection of single-nucleotide polymorphisms. The fluorescence sensors process can directly convert an event into a fluorescence signal and have attracted increasing attention due to their rapid response and high sensitivity (Wu and Chiu, 2013; Shen et al., 2012). Ma and co-workers constructed the fluorescence sensors for the detection of protein tyrosine kinase-7 and a potential cancer biomarker (AGR2) based on iridium (III) and G-quadruplex (Lin et al., 2015; Wang et al., 2016a). At present, upconversion nanoparticles (UCNPs) have shown great potential as fluorescence probes in biological science (Cheng et al., 2011; Liu et al., 2011; Yang et al., 2012; Alonso-Cristonbal et al., 2015; Zhu et al., 2012; Kumar et al., 2009; Wang et al., 2016b). Compared with traditional fluorescence dye and quantum dots (QDs), sensors based on UCNPs have plenty of advantages, such as low toxicity, good photostability, long lifetime, and importantly, little background autofluorescence, making them application in a number of areas (Zhang et al., 2012a; Guo et al., 2015, 2016).

The smart materials with stimuli-responsive ability can reversible change dimensions of their structure depending on temperature (Chen et al., 1995; Zhang et al., 2012b; Kawamura et al., 2014; Li et al., 2014), pH (Ma et al., 2014; Li et al., 2015, 2014; Zhang et al., 2013) and light (Takashima et al., 2012) stimulation from the environment. The smart materials based on changes in the hydrophilicity of polymer networks have potential applications in the drug delivery systems and fabrication of sensors. As one of the smart materials, thermo-sensitivity materials prepared with N-isopropyl acrylamide (NIPAAM) can change the dimensions of their structure response to temperature changes, which because NIPAAM as a well-known thermo-sensitivity compound undergoes a reversible hydrophilic-hydrophobic phase transition

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at the lower critical solution temperature (LCST) of 32 °C. When the temperature reaches LCST, NIPAAM changes from a hydrophilic, coil state to a hydrophobic, collapsed state (Li et al., 2014a; Sun et al., 2014). The boronate affinity materials as another smart material have attracted increasing attention for detection and enrichment of glycoprotein owing to the reversible covalent bonds between bornate and molecules containing cis-diol groups such as glycans or glycoproteins in an alkaline aqueous solution, whereas such bonds dissociate when the medium transform to the acidic solution (Wang et al., 2013; Li et al., 2013; Ye et al., 2014; Li et al., 2014b; Wang et al., 2014; Stepheneson-Brown et al., 2015; Nishiyabu et al., 2011). However, the studies on smart materials response to pH and temperature simultaneously are less (Man et al., 2015; Zhang et al., 2014).

Glycoproteins, which are one of the most important post-translational modifications, play a critical role in a variety of biological activities and they have been used as disease biomarkers and therapeutic targets for clinical diagnostics (Krishnamoorthy and Mahal, 2009; Pan et al., 2013; Ohtsubo et al., 2006). At present, the methods based on mass spectrometry (MS) have been proven to be a useful tool for the analysis of glycoproteins, but it is still a challenge to directly determine glycoproteins without any enrichment process because of the low abundance of glycoprotein and their poor ionization efficiency during mass spectrometric analysis (Alley et al., 2013; Nie et al., 2013; Dell et al., 2001). Therefore, to develop an effective, facile and highly sensitive method for detection and enrichment of glycoproteins is of a great importance.

To date, antibodies are the most commonly recognition elements for the sensing systems because of its high specificity. However, antibodies are high cost and low stability, which limited their further application (McConnell et al., 2014). Therefore, to develop stability and low cost recognition element is urgently needed. Molecular imprinting has been considered as an important method to create recognition sites which are spatially and chemically complementary to the template. Due to their excellent mechanical and chemical stability, low cost, ease of preparation and reusable, molecularly imprinted polymers (MIPs) have been the most promising recognition element and have been extensively used in separation, sensing and drug delivery (Lofgreen et al., 2011; Tan et al., 2013). The strategy that MIPs combined with smart materials has a wide range of application.

Herein, we present a novel smart upconversion fluorescence sensing material response to pH and temperature for glycoprotein, where UCNPs as transducer element and MIPs with response to pH and temperature as recognition elements. Graphene oxide (GO) is covalently decorated with functional groups involving hydroxyl and carboxyl on the basal plane or at the edges of the thin sheet of grapheme. GO have been used in different areas due to their ultrahigh specific surface area and excellent property in electrical, thermal and mechanical (Zhu et al., 2010; Wang et al., 2011; Song et al., 2010). In this study, Horseradish peroxidase (HRP) was chosen as target protein to investigate the properties of the smart sensing material. The proposed smart material has potential application in biomedicine and clinical diagnostics.

2. Materials and methods

2.1. Materials and chemicals

All reagents were analytical grade at least. HRP (molecular weight (MW) 44 kDa, isoelectric point (pI) 6), Ovalbumin (OVA, MW 45 kDa, pI 4.7) and Bovine serum albumin (BSA, MW 67 kDa, pI 4.9) were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). $Y(CH_3COO)_3 \cdot 4H_2O$ (99.9%), $Yb(CH_3COO)_3 \cdot 4H_2O$ (99.9%), $Er(CH_3COO)_3 \cdot xH_2O$ (99.9%), polyacrylic acid (PAA, M = 1800), diethylene glycol (DEG) were purchased from Sigma Aldrich (St Louis, USA). Oleic acid (OA, 90%), 1-octadecene (ODE, 90%), GO was obained from Xianfeng nano (Nanjing, China). VPBA and NIPAAM were purchased from Alfa Aesar Co. Ltd. (Massachusetts, USA). 3-meracaptopropionic acid (MBA), ammonium perulfate (APS) and N,N,N,N-tetramethylenediamine (TEMED) were provided by Aladdin (Los Angeles, USA).

2.2. Characterizations

Ultraviolet absorbance at a wavelength of 279 nm was recorded on a Cary 50-Bio Ultraviolet-visible (UV–vis) spectrometer. Fluorescence measurements were performed on a Hitachi F-2500 fluorescence spectrometer connected with an external 980 nm diode laser (1 W, continuous wave with 1 m fiber) as the excitation source. Scanning electron microscopy (SEM) images were obtained on a Hitachi SU1510 microscope. Transmission electron microscopy (TEM) was obtained by a 2010 FEF microscope. Energy-dispersive X-ray photoelectron spectroscopic (XPS) measurements were performed on PHI-5000 Versaprobe. Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) in KBr were recorded in a Vector 22 FT-IR spectrophotometer (Bruker, Germany).

2.3. Preparation of UCNPs

UCNPs were prepared according to the previous literature (Li and Zhang, 2008). Y $(CH_3COO)_3$ (0.78 mmol), Yb $(CH_3COO)_3$ (0.2 mmol) and Er $(CH_3COO)_3$ (0.02 mmol), 6 mL OA and 17 mL ODE were added into a 100 mL flask and the mixture solution was heated to 160 °C, to form a transparent solution. The mixture solution was cooled down to room temperature naturally. Methanol (10 mL) solution with NaOH (2.5 mmol) and NH_4F (4 mmol) was slowly dropped into the flask and stirred for 30 min To removal of methanol, the solution was slowly heated, degassed at 100 °C for 10 min Subsequently, the solution was heated to 300 °C and kept for 1 h at argon protection. After the resulted solution was cooled down naturally, UCNPs were obtained via centrifugation, and washed with ethanol for three times.

PAA-UCNPs were synthesized according to the previous method with a modified procedure (Naccache et al., 2009). PAA (300 mg) was mixed with DEG (30 mL) in a 100 mL flask. The mixture solution was heated to 110 °C. Toluene (3 mL) solution with hydrophobic UCNPs (100 mg) was added, and maintained at 110 °C for 1 h under argon atmosphere. Then the mixture solution was heated to 240 °C and kept for 1 h. The resulted solution was cooled down to room temperature naturally, the PAA-UCNPs were collected from the resulted solution with the excess dilute hydrochloric aqueous solution, and washed three times with water.

2.4. Synthesis of the smart sensing material

UCNPs (20 mg) and GO (3.5 mg) were dispersed in deionized water (10 mL) by ultrasonication and stirred for 30 min Then, HRP (10 mg), NIPAAM (100 mg), VPBA (25 mg) and MBA (40 mg) were added to the mixture, which was incubated 1.5 h under stirring for pre-polymerizaiton. The oxygen was removed by nitrogen bubbling for 10 min The polymerizaiton was initiated by APS (10 mg) and TEMED (100 μL, 5%, v/v), and then polymerization was carried out at 25 °C for 20 h. The smart sensing material was collected via centrifugation and washed with 0.5% (w/v) SDS −0.5% (v/v) HAc and doubly distilled water in order, which was repeated several times until no template was detected by UV–vis spectrophotometry. Finally, the control (non-imprinted polymer, NIP) was prepared using the same procedure except addition of the template protein.

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