Biosensors and Bioelectronics 100 (2018) 201–207

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Supramolecular nano-sniffers for ultrasensitive detection of formaldehyde

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ARTICLE INFO

Keywords:
Quantum dots
FRET
Formaldehyde detection
Fluorescence quenching

ABSTRACT

Supramolecular nanoparticle hybrids for biosensing of analytes have been a major focus due to their tunable optical and surface properties. Quantum dots-Gold nanoparticle (QDs-GNP) based FRET probes involving turn on/off principles have gained immense interest due to their specificity and sensitivity. Recent focus is on applying these supramolecular hybrids for enzyme operated biosensors that can specifically turn-on fluorescence induced by co-factor or product formed from enzymatic reaction. The present study focuses on locking and unlocking the interaction between QD-GNP pair leading to differential fluorescent properties. Cationic GNPs efficiently quenched the anionic QD fluorescence by forming nanoparticle hybrid. Quenching interaction between QD-GNP pair was unlocked by NADH leading to QD fluorescence turn-on. This phenomenon was applied for the successful detection of formaldehyde using NAD^+ dependent formaldehyde dehydrogenase. The proposed nano-sniffer could successfully detect formaldehyde from 0.001 to 100000 ng/mL (R^2 = 0.9339) by the turn off-turn on principle. It could also detect formaldehyde in fruit juice and wine samples indicating its stability and sensitivity in real samples. The proposed nanoprobe can have wide applications in developing enzyme biosensors in future.

1. Introduction

Supramolecular nanoparticle hybrids for biosensing of analytes have been a major focus due to their tunable optical and surface properties. Advances in nanoparticle synthesis, immobilization, and fluorescence resonance energy transfer (FRET) phenomenon has led to the design of numerous nano probes for ultrasensitive detection of analytes in complex matrices (Akshath et al., 2012a, 2012b; Chen et al., 2013; Costa-Fernández et al., 2006). Quantum dots (QDs) based FRET probes involving turn-on/off principle have gained immense interest due to their specificity and sensitivity (Clapp et al., 2005; Willner et al., 2007). QDs are colloidal nano crystalline semiconductors which possess unique optical properties due to quantum confinement effects making them a superior tool for optical biosensors (Algar et al., 2010; Freeman and Willner, 2012; Akshath and Bhatt, 2016a). Also, QDs are size-tunable, and their narrow emission spectra reduce the donor spectral leakage. Their broad absorption spectra offers them as suitable candidates for spectral overlap based FRET from donor to acceptor (Akshath et al., 2012a, 2012b; Chen et al., 2013; Clapp et al., 2006; Medintz et al., 2003). Quantum dot-dye hybrids are extensively studied for biosensing of various analytes. However, photo-instability of the dyes, is a major problem in real time biosensing that makes them unsuitable candidates for enzyme biosensors (Cai et al., 2006; Clapp et al., 2004; Geissler et al., 2010; Gustafsson et al., 2014; Sadhu et al., 2010; Wu et al., 2010; Xu et al., 2006). In this direction, many groups have attempted gold nanoparticle induced quenching of QD fluorescence and subsequent turn-on of fluorescence for metal ion detection (Dyadyusha et al., 2005; Nikoobakht et al., 2002; Pons et al., 2007; Samanta et al., 2014; Xue et al., 2012; Choi et al., 2012; Lowe et al., 2011; Ren et al., 2014). Recently, use of these supramolecular hybrids for enzyme operated biosensors that can specifically turn-on fluorescence induced by co-factor or product formed from enzymatic reaction have been reported (Akshath and Bhatt, 2016b).

There are many reports on successful use of nano-hybrids for monitoring enzyme catalysis (Freeman et al., 2010; Freeman and Willner, 2008; Gill et al., 2008a; Qian et al., 2015; Akshath and Bhatt, 2016a; Shi et al., 2007, 2006; Tang et al., 2017). To design successful nanoprobe for enzyme monitoring, effective enzyme immobilization and nanoparticle induced enzyme inhibition are of major concern. Wang et al. (2016) used QD-GNP probe as a fluorescence switch for “turn-on-off-on” based detection of ovotransferrin. For detection of protease using QD nanohybrids, many groups have used FRET phenomenon based on QD-peptide-dye probes that are cleaved upon protease action leading to modulation of fluorescence (Clapp et al., 2008; Gill et al., 2008b; Kim and Kim, 2012; Medintz et al., 2006). Ron Gill et al., designed QD-enzyme hybrids that can detect

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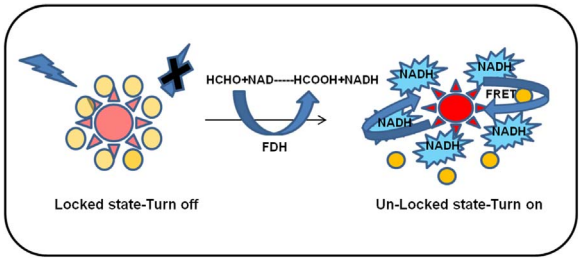
http://dx.doi.org/10.1016/j.bios.2017.09.010
Received 29 May 2017; Received in revised form 5 September 2017; Accepted 7 September 2017
Available online 09 September 2017
0956-5663/



Scheme 1. Proposed nano-sniffer unlocking as a result of the enzyme reaction. FDH: Formaldehyde dehydrogenase; Turn off: QD: GNP supra-hybrid; Turn on: FRET between NADH and QD.

glucose and acetyl choline esterase inhibitors using hydrogen peroxide sensitive QDs. Many reports are available where QD is conjugated with dye functionalized DNA, protein or peptides that can serve as FRET probes for sensing and imaging (Chiu and Huang, 2009; Smith et al., 2006; Zhang et al., 2011a, 2011b; Zhou, 2012).

However, reports on designing of optical sensors using dehydrogenase based reactions with supramolecular hybrids are scarce. It has been previously reported that electrostatic interaction of GNPs with QDs can efficiently quench their fluorescence (Xue et al., 2012; Han et al., 2007). To exploit this phenomenon, we designed a nano- sniffer consisting of QD-GNP hybrid and a co-factor based dehydrogenation reaction to unlock the interaction leading to differential fluorescent properties as a result of FRET (Scheme 1). Formaldehyde was selected as the experimental analyte to prove the above principle. Formaldehyde is a food contaminant and is known for its carcinogenic (Group I) and potent neurotoxic activity (IARC Monographs, 2006). Formaldehyde addition to food commodities such as wine, noodles, beers, milk, etc. and potential serious health effects at higher accumulated concentrations is reported (IARC Monographs, 2006). There is, therefore, a need to develop 'non-conventional' techniques for rapid and sensitive detection of formaldehyde.

Classically, formaldehyde is detected by high-performance liquid chromatography (HPLC) (Wu et al., 2003), Chemiluminescence (Akshath et al., 2012a, 2012b), gas sensors (Bianchi et al., 2007), whereas potentiometric methods based on immobilized formaldehyde dehydrogenase/alcohol oxidase have also been reported (Korpan et al., 2010). Recently, nanoparticle probes for colorimetric/optical based detection are reported (Wang et al., 2013; Zeng et al., 2014). In an earlier study, we designed a QD based optical probe using non-classical cofactors for the detection of formaldehyde (Akshath and Bhatt, 2016a).

To the best of our knowledge, this is the first report of using an enzyme based reaction to unlock the interaction of supramolecular nanoparticle hybrid and its application in detection of an analyte of interest. The proposed study overcomes drawbacks in existing methods regarding specificity, sensitivity, and stability in real samples. Table 1 summarizes sensitivity of proposed method in comparison with existing reports.

Table 1
Summarizes various detection methods for formaldehyde in comparison with present study.

State-of-art techniques	Detection limit	Reference
HPLC	0.2 μg/mL	Wu et al. (2003)
GC	0.24–16 μg/mL	Bianchi et al. (2007)
Fluroimetry	3 ng/mL	Helaleh MIH et al. (2001)
Calorimetric methods	0.02–4 μg/mL	OSHA/NIOSHA (1989, 1994)
Flow injection system	0.8 ng/mL	Piyanete et al. (2004)
Enzymatic methods	10 μg/mL	Korpan et al. (2014)
Nano-hybrids	152 ppb	Naseer Iqbal et al. (2014)
Au NPs coupled Tollen's reagent	50 nM	Zeng et al. (2014)
Turn-on fluorescence	**1 pg/mL In fruit juice & Wine samples**	**Proposed work**

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2. Experimental

2.1. Materials & methods

Cadmium acetate [Cd $(CH_3COO)_2$], gold chloride trihydrate, 2-Mercapto ethanolamine, mercaptopropionic acid, sodium borohydride ($NaBH_4$), tellurium, formaldehyde, formaldehyde dehydrogenase and NADH & NAD^+ were procured from Sigma Chemicals, St. Louis, USA. All reagents used were of analytical grade and acquired from standard suppliers. Zinc acetate, ferrous sulfate, manganese chloride, sodium chloride, potassium chloride and mercuric chloride were obtained from Sigma Chemicals. All reagents used were of synthesis or analytical grade acquired from standard suppliers and used without any further purification. For analysis of real samples, water, wine, and fruit juice were procured from the market and used directly without any pre-treatment. The instruments used were: UV–Vis spectrophotometer (UV-1601, Shimadzu, Japan) for analysis of spectral changes during synthesis of CdTe QDs, Spectrofluorimeter (RF-5301 PC, Shimadzu, Japan) for photoluminescence measurements. Transmission electron microscope (TEM) studies were carried out using a Jeol 2100, Japan Make, operating at 100 kV accelerating voltage. Samples were prepared by depositing a drop of GNPs on a carbon grid and were dried in vacuum. Zeta-Potential analysis of synthesized CdTe QDs and cationic GNPs was performed using microtrac Zeta-potential analyzer.

2.2. Synthesis of CdTe QDs

For the synthesis of CdTe QD emitting at 540–580 nm, borohydride reduction method was adopted as reported by Li et al. (2007) and our earlier report, with slight modification (Akshath and Bhatt, 2016a; Li et al., 2007). In brief, 0.02 M Cd- $(CH_3COO)_2$ was dissolved in 25 mL of double distilled water and mixed with 0.05 M of MPA. After degassing the solution, pH was adjusted to 9.2 using NaOH. In a separate reaction, sodium hydrogen telluride (NaHTe) synthesized by reacting 0.03 M of $NaBH_4$ and 0.01 M of Te in ice-cool water. We observed the metal dissolved leaving a faint pinkish-colored solution. NaHTe was added drop wise to the above-synthesized solution until the solution turned orange. The solution refluxed at 100 ± 2 °C for 180 min after which both absorption and emission spectra was recorded.

2.3. Synthesis of cationic gold nanoparticles

Mercapto ethanolamine coated cationic gold nanoparticles were synthesized as reported by Niidome et al. (2004). Briefly, 400 μL of cysteamine solution (213 mM) was added to 40 mL of $HAuCl_4$ (1.42 mM) and mixed for 20 min at room temperature. After 20 min, 10 μL of freshly prepared ice-cold $NaBH_4$ (10 mM) was added under vigorous stirring in the dark. The solution was allowed to continue at RT for 60 min, and the resulting wine-red solution was kept at 4 °C until further use. The GNPs were characterized using UV–Vis absorption spectra, zeta potential and TEM.

2.4. Design of nano-sniffer for formaldehyde detection

2.4.1. Studies on interaction of QDs with GNPs

To study the interaction of anionic QDs and cationic GNPs, varying