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Ultrasensitive free bilirubin detection in whole blood via counting quantum dots aggregates at single nanoparticle level

Wenfeng Zhao*,[1], Chenghua Zong[1], Tian Lei[1], Wentao Tian, Mimi Sun, Xiaojun Liu, Qingquan Zhang, Hongwei Gai*

School of Chemistry and Materials Science, Jiangsu Key Laboratory of Green Synthetic Chemistry for Functional Materials, Jiangsu Normal University, Xuzhou 221116, PR China

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ABSTRACT

Here we report a versatile strategy for detecting bilirubin by harnessing the cationic polymer polyethylenimine induced aggregation of human serum albumin-modified quantum dots. These aggregates are easily discriminated from the individual QD by single molecule spectral imaging microscopy and correlated to the bilirubin concentration. The proposed method can selectively detect bilirubin with a detection limit as low as 0.6 nM. Remarkably, this method has been successfully applied to the trace detection of free bilirubin in human whole blood and serum samples with satisfactory results.

1. Introduction

Bilirubin, a bioactive yellowish compound produced from the normal heme catabolism [1], is an important clinical diagnostic marker for liver function and lung cancer [2]. It can be found in two forms, unconjugated (free bilirubin, FB) and direct (conjugated to human albumin) [3]. It is reported that approximately 95% of bilirubin is in the unconjugated form in healthy adult individuals [4]. The FB is able to permeate the blood-brain barrier and can be neurotoxic [5]. The high level of FB has been involved in neurotoxicity, permanent brain damage, and even death in severe cases [6]. Hence the FB level might be a better parameter to assess the risk caused by kernicterus in jaundice, brain damage thalassemia and acute bilirubin encephalopathy [7]. Owing to the biological and clinical significance, detection of FB in biological fluids has gained great interest.

To date, several methods have been developed for the determination of FB, including the peroxidase test, high performance liquid chromatography, fluorescence and electrochemical sensors [8–14]. The peroxidase test is not accurate since the method relies on the intrinsic light absorption of bilirubin which supposing that only one single bilirubin specie is present in the blood samples. However, it is well know that photoisomers could constitute up to 30% of the total bilirubin [12,15,16]. Chromatographic and sensors methods are reliable but require cumbersome sample pre-treatment procedures, and suffer from low sensitivity and low sample throughput.

Recently, fluorescence-based single molecule detection (SMD) systems become more attractive and reliable due to their high sensitivity and high throughput. They have been developed for different analytes, especially those of great biological interest, like single DNA quantification, counting proteins and detection of single copies of virus [17–24]. However, SMD systems have achieved limited success. The major problem is that the rapid photobleaching of the organic dyes or fluorescent proteins hamper quantification of a single molecule [17,20,22]. The use of quantum dots (QDs), which offers remarkable brightness and photostability can potentially mitigates these concerns and allows for the development of improved SMD techniques [25]. Furthermore, the inherent properties of transient fluctuations in intensity of individual fluorophore (i.e. blinking) complicate the study of dynamics at the molecular level [26–28]. The other problem is that it is still difficult for low-abundance targets derivatization with a fluorophore and to be discriminated subsequently in the complex mixture.

In a previous publication, we have demonstrated a sensitive, rapid, non-derivatization technique for protein detection based on counting the degree of aggregation of QDs [29–31]. The splitting of spectra indicates that QDs aggregates are formed which provides a simple technique for protein quantification. However, wide adoption of this method is still limited due to the following reasons: (1) the QDs aggregates are assembled through the binding between the biotin-QDs with streptavidin and thus, it can be used only to quantify the biotinylated protein in the biological samples; (2) a relatively narrow

* Corresponding author.
E-mail addresses: gai@jsnu.edu.cn, zhaowf@jsnu.edu.cn (W. Zhao), gai@jsnu.edu.cn (H. Gai).
[1] These authors contributed equally to this work.

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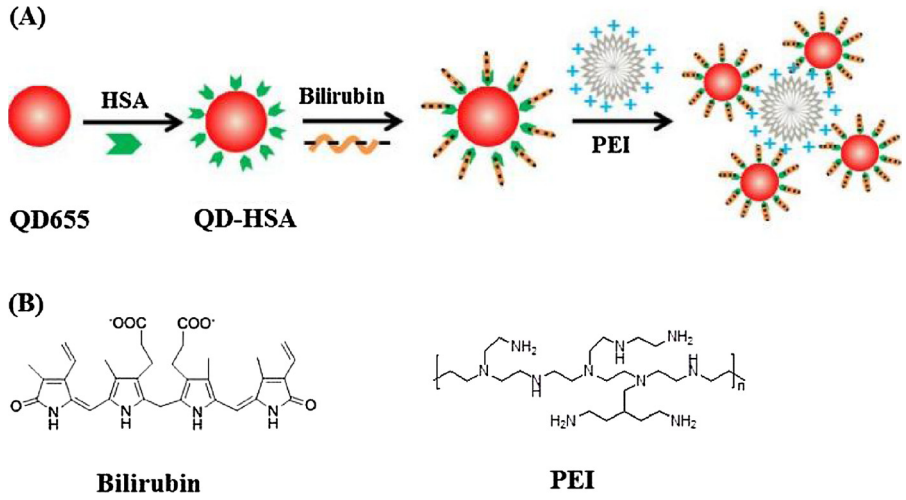

Scheme 1. (A) Schematic illustration for assay of bilirubin based on the PEI induced of QDs aggregation; (B) Chemical Structures of Bilirubin and PEI.

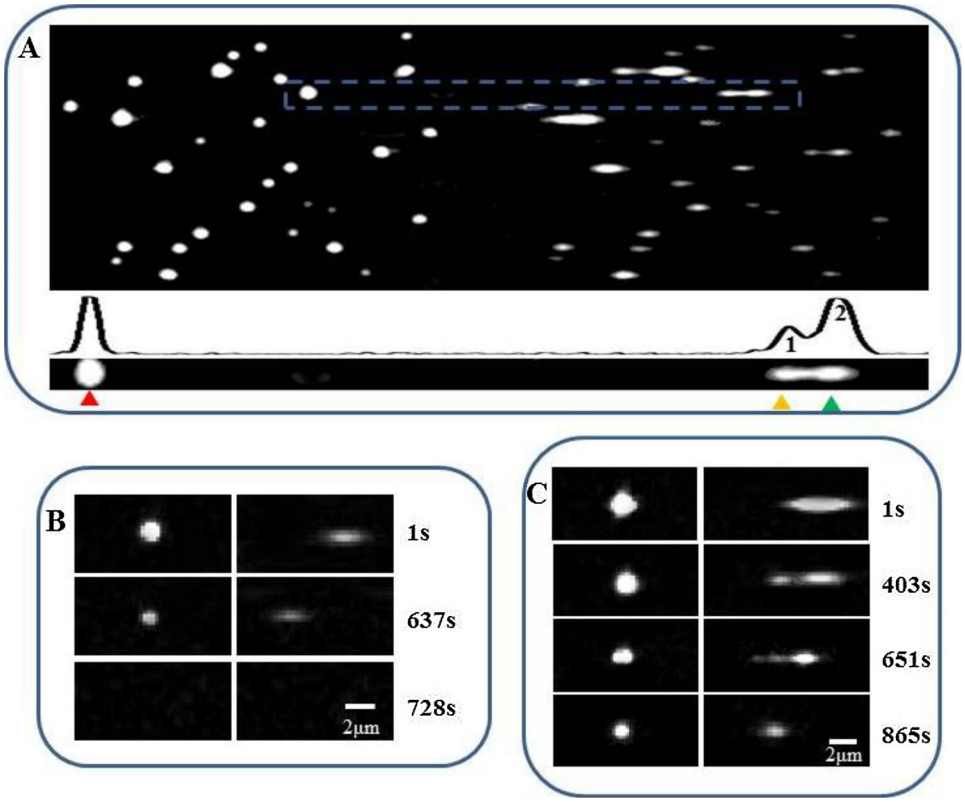

Fig. 1. (A) Typical spectral imaging of QDs-HSA with bilirubin in 200 μM PEI with enlarged zeroth-spot and first-order streak and the corresponding intensity profile; (B) spectral imaging of a single QD; and (C) spectral imaging of QDs aggregates. The spots at the left side are the zeroth-order images and the steaks at the right side are the first-order spectral images.

dynamic range are obtained since the degree of aggregates QDs are used to correlate the biotinylated protein concentration. Hence, development of a versatile strategy is needed to induce QDs aggregation for target detection for future application. As a typical flocculant, polyelectrolytes are widely used to turn off the stability of the nanoparticle and thus initiate flocculation [32]. Polyethylenimine (PEI) is a pH sensitive polyelectrolyte, which has high adsorption capacity and high solubility in pure water [33–37]. Bilirubin could be negatively charged due to the dissociation of the carboxyl groups (−COOH) and protons (Scheme 1). The surface of PEI polymer is cationic under appropriate pH below its pKa (the pKa of PEI is 10–11) [35], making it a good candidate for attraction of anionic target molecules. Inspired by this, we proposed a new method for ultrasensitive detection of FB based on the PEI induced QDs aggregations.

Here, we have used the HSA modified QDs (QDs-HSA) as a biorecognition element to detect FB through forming the QDs-HSA-FB complex. The complexes are then induced to aggregate upon addition of PEI, a cationic polyelectrolyte. The mechanisms of the designed biosensors and optimization of main factors, evaluation of the performance, and application to analysis of FB in human whole blood and serum samples were studied in detail.

2. Experimental

2.1. Materials and measurements

Carboxyl quantum dots 655 (QDs 655) were purchased from Invitrogen/Molecular Probes (Eugene, OR). Human serum albumin (HSA) and bilirubin were purchased from Heowns Biochemical Technology Co., Ltd (Tianjin, China). Polyethyleneimine (PEI, Mw