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# CEA fluorescence biosensor based on the FRET between polymer dots and Au nanoparticles†

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**Polymer dots were employed as luminophors to design a fluorescence biosensor for carcinoembryonic antigen (CEA) with high sensitivity and selectivity; the increased fluorescence intensity is proportional to CEA concentration in the range of 0.1–10 ng mL$^{-1}$.**

Organic dyes are extensively employed as fluorophores; however, they often suffer from low absorptivity and poor photostability, which prevents their application in high-throughput screening and ultrasensitive assays.[1,2] Another frequently used fluorophore is inorganic semiconductor quantum dots (QDs), which show a number of superiorities over conventional organic dyes, such as improved brightness and photostability.[3–5] However, QDs also have some drawbacks. First, they are not bright enough for many photon-starved applications because of low emission rates, blinking, and a significant fraction of nonfluorescent dots.[6] Second, toxicity caused by leaching of heavy metal ions may harm the biological samples.[7] Difficulty in surface modification is another problem.[8] Recently, a novel class of fluorescent nanoparticles, polymer dots, have received great attentions.[9–11] These nanoparticles are made from conjugated polymer monomers, whose abundant π-electrons lead to strong fluorescence emission. Thus, in previous work they have been adopted as energy-acceptors in fluorescence resonance energy transfer (FRET) systems to engender or amplify signals.[12–14] Water-soluble polymer dots have desirable characteristics for biomedical research, including an apparently high fluorescence quantum yield and good photostability, a tunable size with flexible and convenient modification, and excellent biocompatibility without toxicity. Such nanoparticles have been successfully applied in tumor targeting and specific cell imaging.[15,16] Nevertheless, research on polymer dots is still in its early stages, though prospects are assuredly attractive.[11]

Au nanoparticles (Au-NPs) represent a class of materials with a high extinction coefficient and a broad absorption in the visible range.[17] Au atoms on the surface possess unoccupied orbitals, which facilitate nucleophiles to donate electrons.[18,19] FRET between Au-NPs and strong electron donors can occur within a restricted distance. Oh *et al.* studied the quenching effect of Au-NPs on the fluorescence of QDs.[20] Subsequently, many similar biosensors linking QDs and Au-NPs together have been designed.[21–23] For instance, an aptamer-linked biosensor was designed for multiple analysis of adenosine and cocaine simultaneously.[24] In addition, based on the fact that protease can cleave the corresponding peptide, a peptide-linked biosensor was applied for the sensitive detection of protease.[25]

In the present work, we introduce polymer dots into a FRET-based biosensor for the analysis of specific proteins, while Au-NPs are used as efficient quenchers. By constructing a sandwich-like structure assisted with the hybridization of DNA sequences, the quenching effect of Au-NPs on polymer dots was studied. Based on the fact that the carcinoembryonic antigen (CEA), a tumor maker,[26,27] would bind to its aptamer[28,29] and destroy the sandwich-like structure, resulting in the recovery of fluorescence, a sensitive and selective CEA detection method can been developed.

The principle of the biosensor is shown in Fig. 1. First, poly(9,9-dioctylfluorenyl-2,7-diyl) dots (PFO dots) were modified with an ssDNA, which is partially complementary with CEA aptamer. Meanwhile, the Au-NPs were also modified

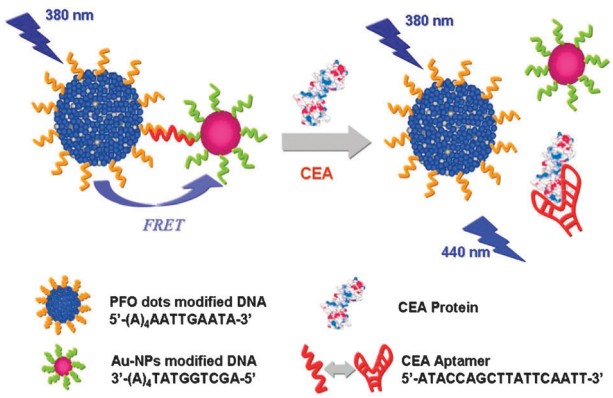


**Fig. 1** Schematic illustration of FRET between PFO dots and Au-NPs for CEA analysis.

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with another partial-aptamer-complementary ssDNA. The presence of CEA aptamer links the PFO dots with Au-NPs together through the hybridization of ssDNA, and formed a sandwich structure. Effective fluorescence resonance energy transfer between the PFO dots and Au-NPs was observed and a low fluorescence signal was detected in such a state. In the presence of CEA, the CEA-binding aptamer preferred to form the CEA–aptamer complex *in lieu* of the aptamer–DNA duplex.[28,29] As a result, the aptamer is released and the Au-NPs quenchers depart away from the PFO dots, leading to the recovery of fluorescence. Accordingly, the CEA protein can be quantitatively detected through variation of fluorescence intensity.

To investigate the quenching effect of Au-NPs on the fluorescence of PFO dots, different concentrations of Au-NPs were added into a solution containing 1 ppm PFO dots and 50 nM CEA aptamer. As shown in Fig. 2, the fluorescence intensities sharply decreased with the increase of Au-NPs concentration until a limiting quenching coefficient of 80% was reached. An Au-NP concentration of 20 nM was used in subsequent experiments.

The incubation time will affect the ssDNA hybridized with each other, which also affects the fluorescence of the system. As shown in Fig. 3 unlike prompt quenching models such as organic dyes and grapheme,[30] the sufficient quenching of PFO dots by Au-NPs needs a minimum incubation time of 30 min to observe maximum fluorescence quenching. A time of 30 min was subsequently adopted in this study.

For CEA detection, various concentrations of CEA were added into the fluorescence-suppressed mixture solution for a certain time with a slight shaking, followed by fluorescence measurement. The recovery fluorescence intensity shows a good linear relationship (eqn (1)) with the logarithm of CEA concentration in the range of 0.1–10 ng $mL^{-1}$ (Fig. 4; results in triplicate).

$$I = 79.12 \log C + 268.15;\ R = 0.9975 \qquad (1)$$

where $I$ represents fluorescence intensity, $C$ represents the CEA concentration and $R$ is the related coefficient.

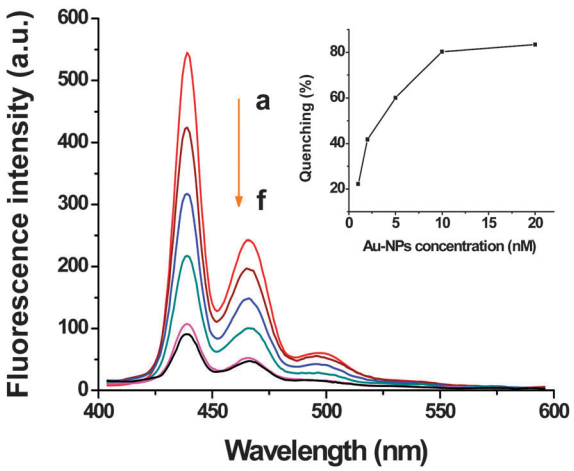


**Fig. 2** Fluorescence of PFO dots (1 ppm) quenched by various concentrations of Au-NPs: a–f: 0, 1, 2, 5, 10, 20 nM. Inset: quenching percentage at different Au-NPs concentrations. *Conditions*: Buffer: 20 mM HEPES, pH = 7.4, incubation time: 30 min.

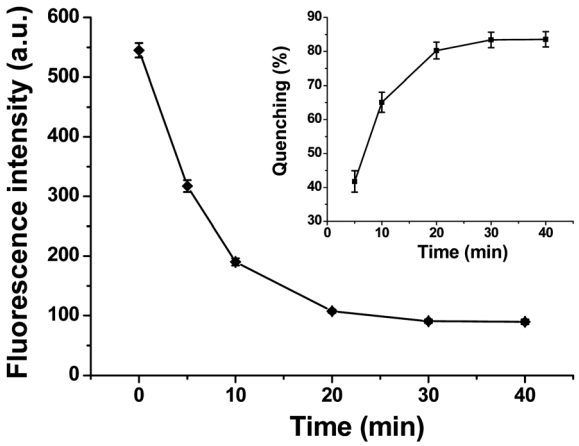


**Fig. 3** Fluorescence intensity of PFO dots (1 ppm) quenched by Au-NPs (20 nM) at different incubation times. Inset: the relationship between the quenching rate and the incubation time. *Conditions*: Buffer: 20 mM HEPES, pH = 7.4. Each sample was measured in triplicate.

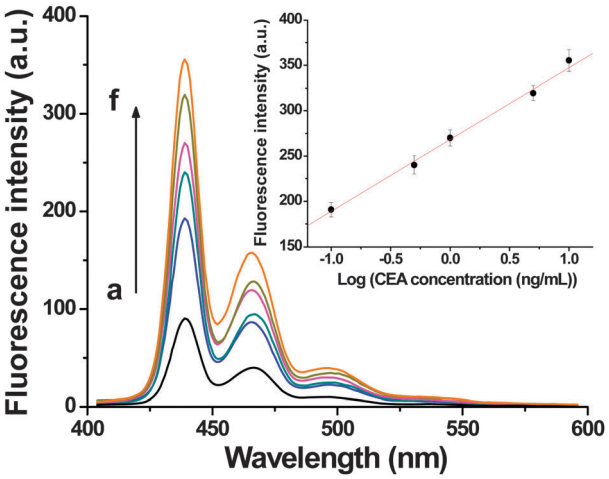


**Fig. 4** Spectra of fluorescence at different CEA concentrations. Inset: linear relationship between fluorescence intensity and logarithm of CEA concentration. *Conditions*: pH 7.4, 20 mM HEPES buffer solution; 1 ppm PFO dots; 20 nM Au-NPs; a–f: 0, 0.1, 0.5, 1, 5, 10 ng $mL^{-1}$. Each sample was measured in triplicate.

Compared with former methods utilizing QDs and corresponding quencher,[22,24] the present method shows a lower detection limit, which can be attributed to the high brightness of PFO dots. Additionally, the functional particles were remeasured a week later in parallel tests, and results showed that the given CEA concentration (2 ng $mL^{-1}$) can be correctly detected (relative error $<5\%$), indicating the particles have good stability.

To ensure the specificity of this system for CEA protein detection, three kinds of proteins, AFP, BSA and thrombin, were selected as interferents. Under identical conditions, the four proteins were added into the fluorescence-suppressed mixture solution separately and the recoveries of fluorescence intensity were measured. By comparing the values of $(F - F_0)/F_0$ from the different proteins, ($F$ and $F_0$ correspond to the fluorescence intensity of the fluorescence-suppressed complex after and before adding targeted protein), CEA can be clearly

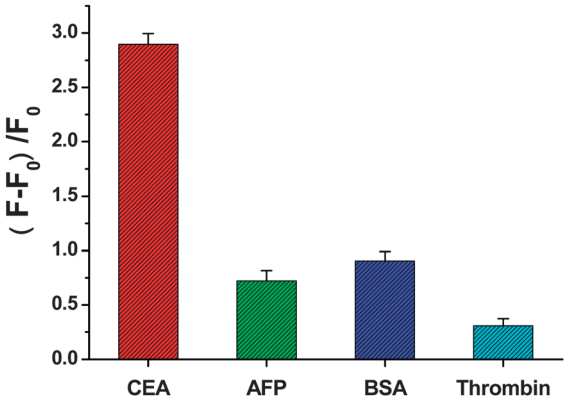


**Fig. 5** Selectivity of the proposed biosensor. Protein concentrations were 10 ng $mL^{-1}$.

distinguished from other proteins (Fig. 5). Such results indicate that the proposed method has good selectivity.

In summary, this work demonstrates a new assay based on FRET between PFO dots and Au-NPs for CEA detection. By means of hybridization using ssDNA, the fluorescence of PFO dots was effectively suppressed by Au-NPs. However, the presence of CEA destroys the fluorescence-suppressed complex, resulting in an enhancement of fluorescence intensity. Accordingly, CEA can be quantifiably detected with high sensitivity owing to the ultrabright luminophor of PFO dots. Meanwhile, as the CEA aptamer was employed as the linker within the sandwich-like structure, very high selectivity was provided. Overall, the unique combination of high sensitivity and good selectivity of this assay indicates its promising potential for the clinical determination of CEA.

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