Teaching enzymes to generate and etch semiconductor nanoparticles

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Abstract

The traditional fluorogenic enzymatic assays broadly employed in bioanalysis are based on the biocatalytic cleavage of bonds between presynthesized organic fluorescent molecules or fluorescent semiconductor nanoparticles (SNPs), so called quantum dots (QDs) and quenching moieties.[1] Usually, they suffer from insufficient quenching of fluorophores by quenchers and nonspecific adsorption on surfaces resulting in high background signals.[2] We pioneered enzymatic assays in which formation of CdS QDs *in situ* is modulated by biocatalytic processes. The first group of assays employs enzymatic production of S^{2-} ions leading to formation of CdS QDs in the presence of Cd^{2+} ions ($Cd^{2+} + S^{2-} = CdS$).[3] The second group of QDs-generating fluorogenic enzymatic assays developed by us relies on modulating the growth of CdS QDs with the products of biocatalytic transformation.[4] Enzymatically generated CdS QDs show homogeneous size distribution with the medium diameter of 2 nm.[3,4] The size of the resulting SNPs is controlled by the nature of capping agents such as citrate, orthophosphate, L-cystein, glutathione, etc. The advantages of biocatalytic modulation of QDs over employment of traditional organic chromogenic and fluorogenic enzymatic substrates, include lower background signals, higher quantum yield, reduced photo-bleaching and lower costs.

We demonstrated the use of the peroxidase-mimicking DNAzyme (peroxidase-DNAzyme) as general and inexpensive platform for development of fluorogenic assays that do not require organic fluorophores.[5] The system is based on the affinity interaction between the peroxidase-DNAzyme bearing molecular beacon and the analyte (DNA or low-molecular weight molecule), which changes the folding of the hairpin structure and consequently the activity of peroxidase-DNAzyme. Hence, in the presence of the analyte the peroxidase-DNAzyme structure is disrupted and does not catalyze the aerobic oxidation of L-cysteine to cystine. Thus, L-cystein is not removed from the system and the fluorescence of the assay increases due to the in situ formation of fluorescent CdS QDs. The capability of the system as a platform for fluorogenic assays was demonstrated through designing model geno- and aptasensor for the detection of a tumor marker DNA (**Figure 1**) and a low-molecular weight analyte, adenosine 5´triphosphate (ATP), respectively.

We developed an innovative photoelectrochemical process (PEC) based on graphite electrode modified with electroactive polyvinylpyridine bearing osmium complex (Os–PVP). The system relies on the *in situ* enzymatic generation of CdS QDs. Alkaline phosphatase (ALP) catalyzes the hydrolysis of sodium thiophosphate (TP) to hydrogen sulfide (H_2S), which in the presence of Cd^{2+} ions yields CdS SNPs. Irradiation of SNPs with the standard laboratory UV-illuminator (wavelength of 365 nm) results in photooxidation of 1-thioglycerol (TG) mediated by Os–PVP complex on the surface of graphite electrode at applied potential of 0.31 V vs.Ag/AgCl. **(Figure 2)** A novel immunoassay based on specific enzyme linked immunosorbent assay (ELISA) combined with the PEC methodology was developed. Having selected the affinity interaction between bovine serum albumin (BSA) with anti-BSA antibody (AB) as amodel system, we built the PEC immunoassay for AB. The new assay displays a linear range upto 20 ng mL^{-1} and a detection limit of 2 ng mL^{-1} (S/N = 3) which is lower 5 times that of the traditional chromogenic ELISA test employing p-nitro-phenylphosphate.

We observed for the first time enzymatic etching of CdS QDs. Fluorescence of semiconductor CdS QDs is modulated irreversibly by the enzymatic reaction catalyzed by horseradish peroxidase (HRP). We detected blue-shifts of corresponding fluorescence peak for CdS QDs and decrease in the intensity of the fluorescence signal. During the study of this phenomenon it was found out that CdS QDs are enzymatically oxidized by hydrogen peroxide resulting in formation of sulfate ions and etching of the initial SNPs (confirmed by electron microscopy) according to **Figure 3**. Formation of sulfate ions was confirmed by two independent analytical methods. This oxidation reaction occurs also when CdS QDs are adsorbed on the surface of polyvinyl chloride microspheres. This study indicates that CdS QDs act as a substrate for HRP. In order to characterize etching of QDs different techniques were employed e.g. fluorescence technique, transmission electron microscopy and wide field fluorescence microscopy. In order to validate our assay we applied it to detection of hydrogen peroxide in tap and rain water.

It should be noted that the novelty of the reported sensing strategy lies on the use of inexpensive compounds for the development of fluorimetric bioanalytical systems. In comparison with other reported fluorogenic assays based on pre-synthesized QDs modified with recognition elements, our assays require neither any synthetic procedures for chemical modification of QDs nor any organic fluorogenic enzymatic substrates.

References

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Figures

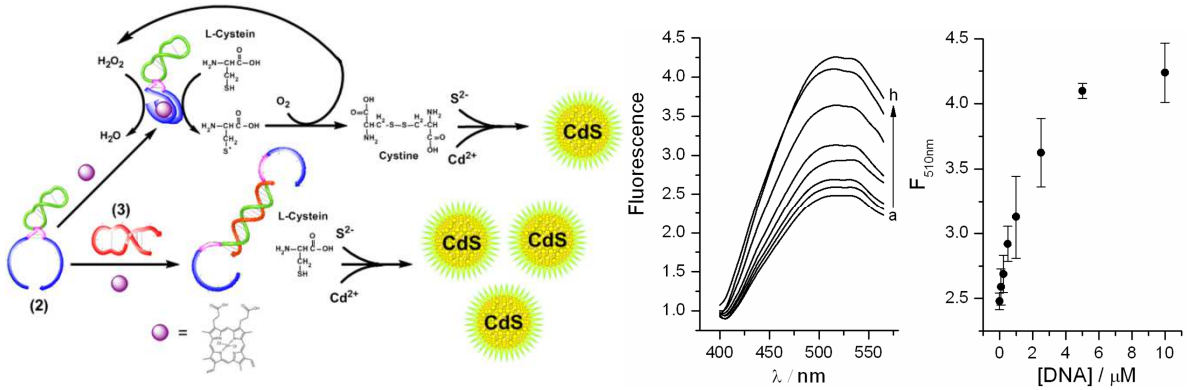

Figure 1. DNA detection through peroxidase-DNAzyme modulated growth of CdS QDs *in situ*.

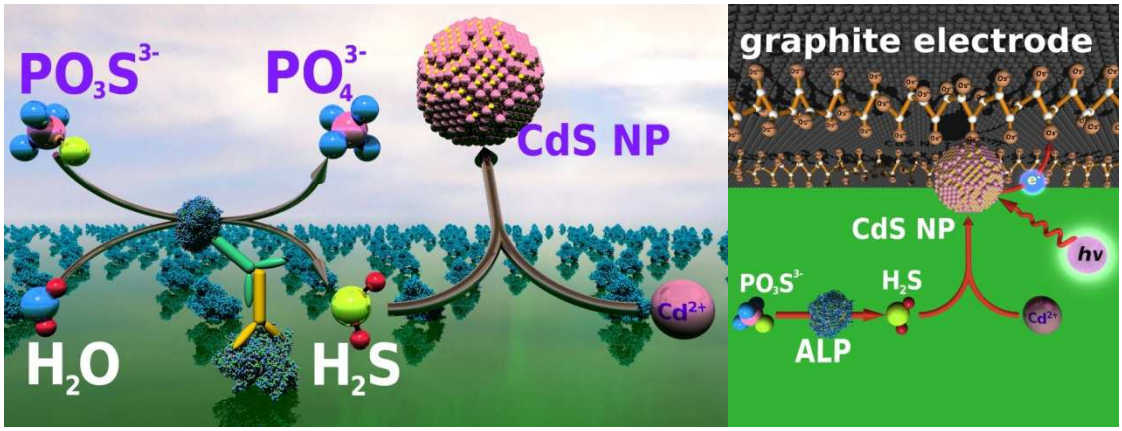

Figure 2. Photoelectrochemical immunosensors based on enzymatic formation of CdS QDs by alkaline phosphatase (ALP) and detection of photocurrent.

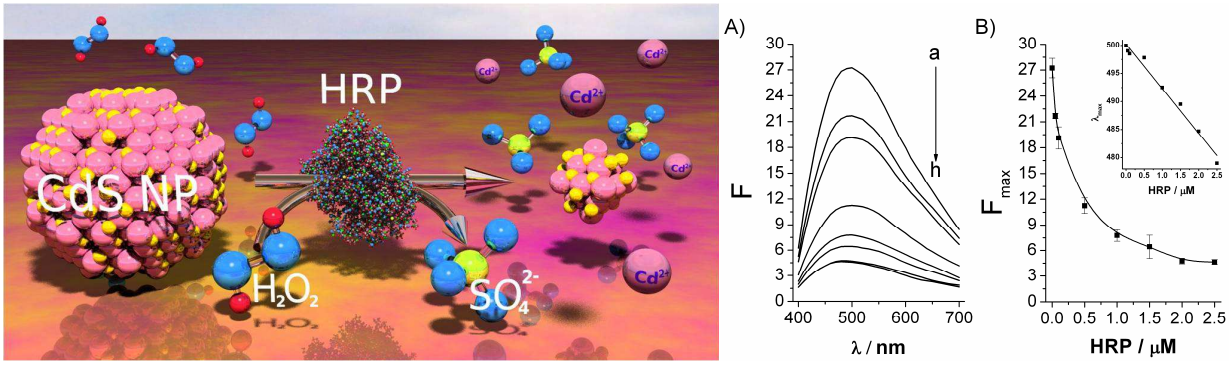

Figure 3. Biocatalytic etching of CdS NPs by horseradish peroxidase (HRP).