Enzymatic Growth of Quantum Dots for Activity Assays

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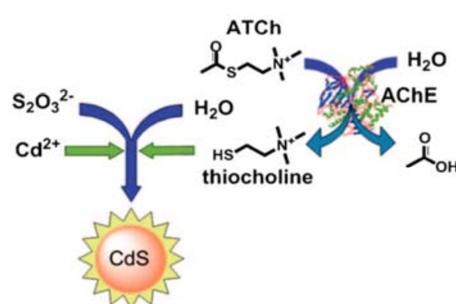
Nanoparticles (NPs) of different nature, for instance, semiconductor and metal NPs are very broadly utilized as labels to read out biorecognition events by optical, electrochemical and other physical methods [1]. In most cases, pre-synthesized metal NPs were linked to recognition elements proteins, polysaccharides, antibodies, RNA and DNA aptamers, DNA oligonucleotides which have affinity for respective target analyte molecules such as proteins, DNA fragments, small organic and inorganic molecules, cations and anions and so on [2,3]. The key advantage of semiconductor NPs is their intrinsic capacity to become photoexcited to produce electron/hole couples, which can recombine to yield fluorescent emission of light. The size, nature and environment of semiconductor NPs define the wavelength and intensity of emitted light [2]. The emission of light by these NPs is explained by quantum effects, therefore they are referred to in the literature as quantum dots (QDs).

We developed three new analytical assays for detection of enzymatic activities in which generation of fluorescent CdS NPs was induced by products of bio-catalytic reactions. The first assay for enzymatic activity of acetylcholine esterase (an enzyme that participates in the termination of the synaptic transmission by breaking down acetylcholine at cholinergic synapses) is shown in Scheme 1. This enzyme breaks the artificial substrate acetylthiocholine (ATCh) to acetate and thiocholine. The latter catalyzes decomposition of $S_2O_3^{2-}$ according to equation: $S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + H_2S$. The resulting hydrogen sulfide interacts with Cd^{2+} to yield CdS NPs: $Cd^{2+} + H_2S \rightarrow CdS + 2H^+$. We found out that the formation of CdS NPs in this system obeys auto-catalytic mechanism.



Scheme 1. *Enzymatic generation of CdS QDs for the detection of AChE activity.*

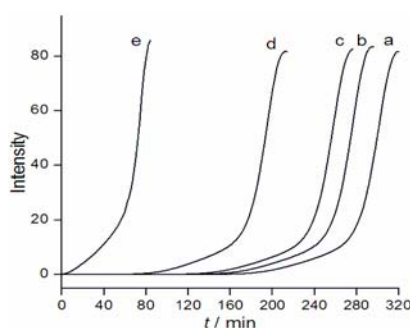
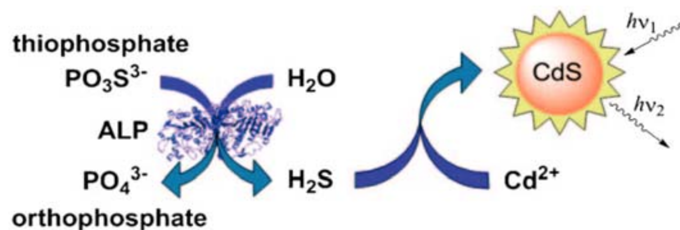


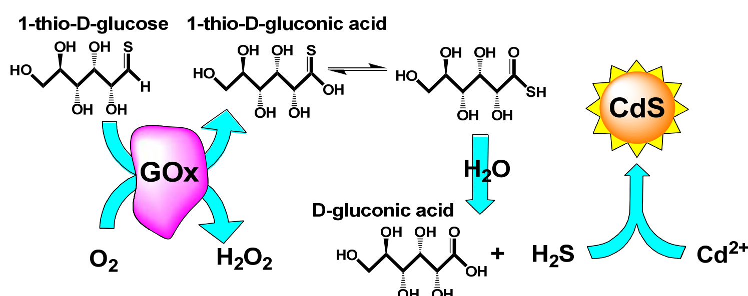
Figure 1. *Evolution of the fluorescence intensity of the CdS QDs formed with different concentrations of AChE: a) 0 mU mL^{-1}; b) 25 mU mL^{-1}; c) 50 mU mL^{-1}; d) 100 mU mL^{-1} and e) 250 mU mL^{-1}.*

The second assay was designed to detect enzymatic activity of alkaline phosphatase (ALP) see Scheme 2 and Figure 2A. This enzyme finds wide application in bioanalysis as a label in enzyme linked immunosorbent assays. ALP hydrolyzes thiophosphate to orthophosphate and H_2S. The latter reacts immediately with cadmium cations to give CdS QDs. When these CdS QDs are excited at 360 nm a strong fluorescence signal was observed.



Scheme 2. Enzymatic generation of CdS QDs for the detection of ALP activity.

The third analytical assays was developed to detect catalytic activity of glucose oxidase (GOx). We used 1-thio-D-glucose as a substrate for GOx. This enzyme oxidizes 1-thio-D-glucose to D-gluconic acid and H_2S according to Scheme 3 and Figure 2B.



Scheme 3. *Enzymatic generation of CdS QDs for the detection of glucose oxidase activity.*

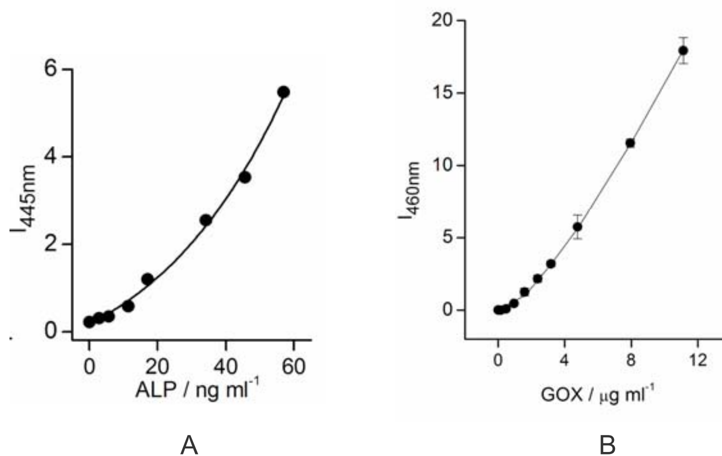


Figure 2. *(A) Calibration curve for ALP; (B) Calibration curve for GOx.*

References

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