Application of the enzymatic product-mediated stabilization of *in situ* produced CdS quantum dots: serum paraoxonase, acetylcholine esterase and glutathion reductase detection

Gaizka Garai-Ibabe, Laura Saa and Valeri Pavlov

CICbiomaGUNE, Parque Tecnologico de San Sebastian, Paseo Miramón 182, 20009, Donostia, Spain
ggarai@cicbiomagune.es

Abstract

Detection of analytes in biosensing is carried out by interaction of biological recognition elements (DNA, enzymes, antibodies and so on) with analytes of interest. The event of recognition is transduced and amplified to yield a signal measurable by physical techniques such as UV-Vis and fluorescence spectroscopy, Raman spectroscopy, electrochemistry etc.
Semiconductor inorganic fluorescent nanoparticles, known as quantum dots (QDs), have been extensively used in recent years as labels for antibodies, DNA and small molecules in bioanalytical affinity assays and imaging.[1,2,3]The advantages of QDs over traditional organic fluorophores include higher quantum yield, reduced photo-bleaching and higher extinction coefficient. Until now QDs based assays relied on pre-synthesized semiconductor NPs. However, the drawbacks of such assays used to be the high background signals caused by the nonspecific adsorption of decorated QDs on surfaces or poor quenching of donor couples. Thus we believed that the generation of QDs *in situ* can address these drawbacks of relevant analytical systems by decreasing the background signal.
Here we introduce a novel concept in bioanalysis and apply it to the rapid, highly sensitive and inexpensive fluorimetric detection of different enzymes with biomedical interest: serum paraoxonase-1(PON-1)(Figure 1), acetylcholine esterase (AChE) (Figure 2) and glutathione reductase (GR)(Figure 3 3).[4,5,6]

References

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Figures

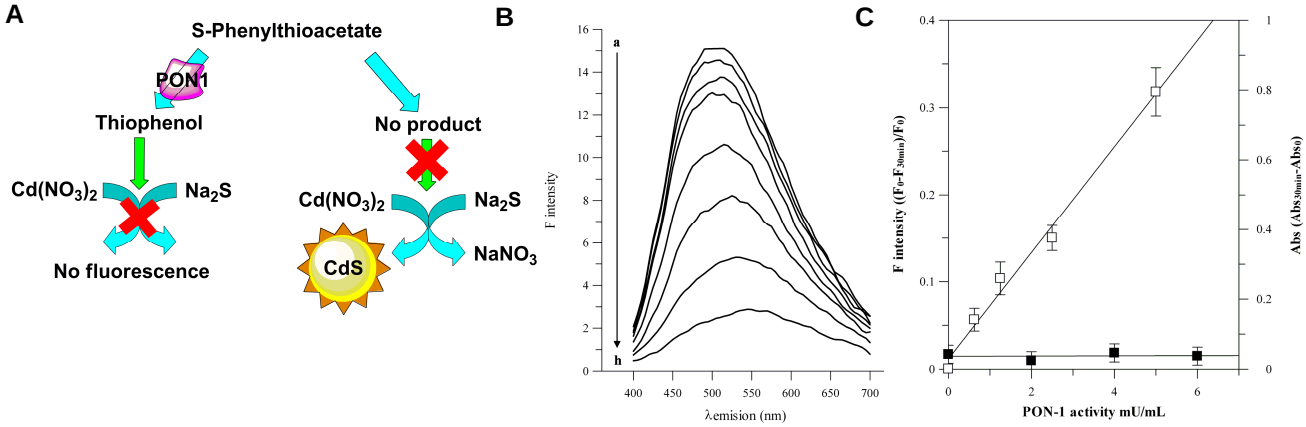

Figure 1. A) Detection of PON1 activity by the enzymatic modulation of growth of fluorescent CdS QDs. B) Emission spectra of CdS QDs formed in the system containing PTA (2mM), Na_2S (0.3 mM), Na_3PO_3S (0.9 mM), $Cd(NO_3)_2$ (1.25 mM) and various concentrations of rHuPON1: a) 0 mU mL^{-1}; b) 0.625 mU mL^{-1}; c) 1.25 mU mL^{-1}; d) 2.5 mU mL^{-1}; e) 5 mU mL^{-1}; f) 10 mU mL^{-1}; g) 20 mU mL^{-1}; h) 40 mU mL^{-1}. C) Calibration curve of rHuPON1, measured by the enzymatic modulation of growth of fluorescent CdS QDs, □, and the standard PA method, ■.

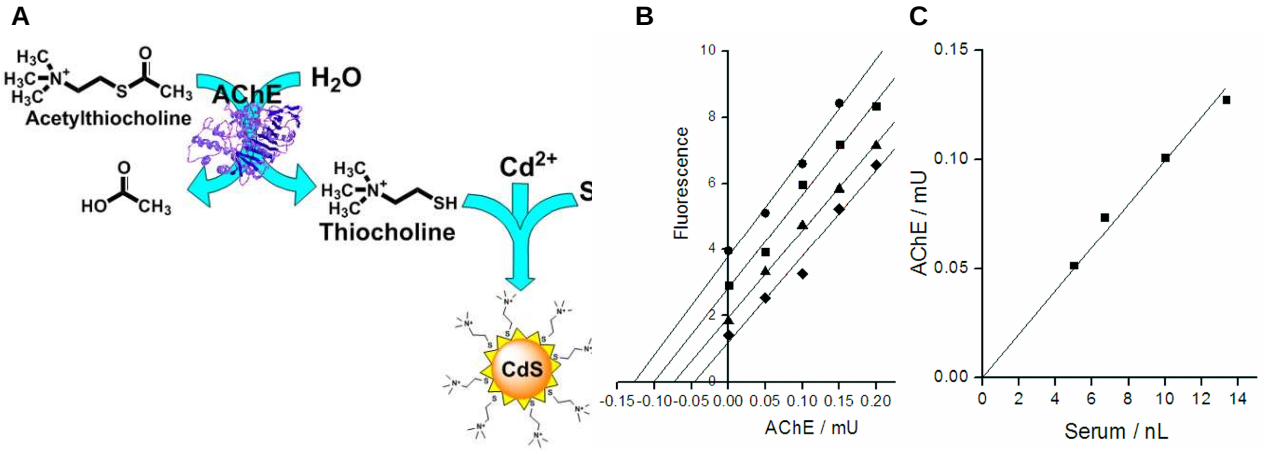

Figure 2. A)Detection of AChE activity by the enzymatic product-mediated stabilization of fluorescent CdS QDs B) Fluorescence intensities of the formed CdS QDs in the system containing different concentrations of added standard solution of AChE, ATCh (0.5 mM), Na_2S (0.1 mM), $Cd(NO_3)_2$ (1.25 mM) and varying volumes of human serum a) 5 nL; b) 6.7 nL; c) 10 nL; d)13.3 nL. C) Dependence of the calculated AChE activity on serum volume.

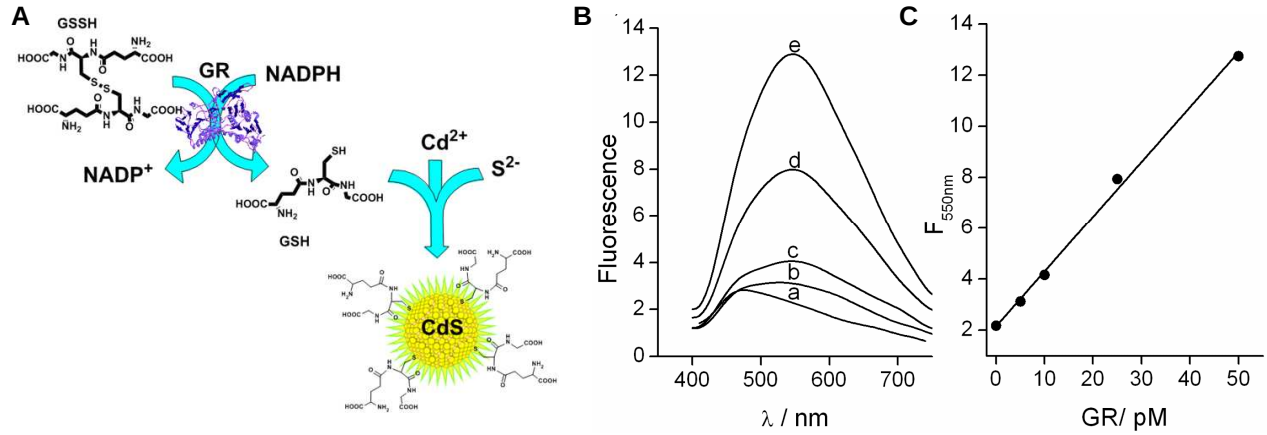

Figure 3. A) Detection of GR activity by the enzymatic stabilization of fluorescent CdS B) Emission spectra of CdS QDs formed in diluted human serum (1:1000) containing NADPH (17.5 μM), Na_2S (0.1 mM), $Cd(NO_3)_2$ (1.25 mM) and different concentrations of GR: a) 0 pM; b) 5 pM; c) 10 pM; d) 25 pM; e) 50 pM;. C) Calibration curve of GR at λ=550 nm.