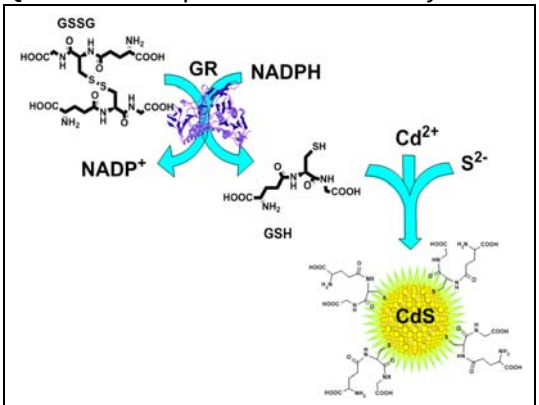
Biosensing Based on Enzymatic Modulation of Growth of Nanoparticles

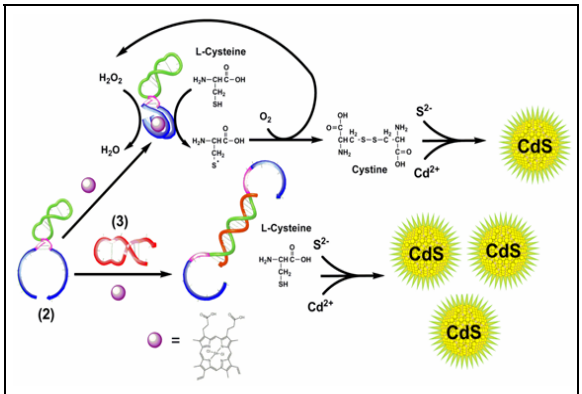
Valery Pavlov, Ruta Grinyte, Laura Saa, Gaizka Garrai
CIC BiomaGUNE, Paseo Miramon 182, San Sebastian, Spain
vpavlov@cicbiomagune.es

Abstract
Semiconductor nanoparticles (SNPs) can be very conveniently employed in biosensing for signal transduction. Their chemical and physical properties are defined by three dimensional structure of NPs, therefore very slight changes in shape and size lead to drastic variation in absorption and emission spectra. We pioneered enzymatic assays in which formation of CdS nanoparticles according to the equation $Cd^{2+} + S^{2-}$ = CdS is caused by biocatalytic processes yielding S^{2-} ions.[1] 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 through inhibition or enhancement. This novel concept has been applied by us to a number of assays, including, the highly sensitive and inexpensive detection of reduced glutathione (GSH), over its oxidized form (GSSG), and glutathione reductase (GR) in human serum.[2] This new fluorogenic bioanalytical system is based on the GR-mediated stabilization of growing CdS nanoparticles (**Scheme 1**). The sensitivity of this new assay is 5 pM of GR, which is 3 orders of magnitude better than other fluorogenic methods previously reported in the literature. We also managed to show how the growth of fluorescent CdS can be modulated by the DNAzyme having peroxidase activity. The system is based on the affinity interaction between the peroxidase-DNAzyme bearing hairpin sequence and the analyte (DNA or low molecular weight molecule), which changes the folding of the hairpin structure and consequently the activity of peroxidase-DNAzyme[3] (**Scheme 2**).



Scheme 1. Detection of PON1 activity by the enzymatic inhibition of growth of fluorescent CdS QDs.

Recognition events, such as specific interaction of target analytes with a number of enzymes or affinity interaction in enzyme-linked immunosorbent assays, may lead to formation of fluorescent quantum dots with photocatalytic activity. Our latest experimental results confirm that CdS QDs, grown under the influence of products of biocatalytic transformations, are photo-catalysts enhancing oxidation of commercially available enzymatic chromogenic substrates such as 3,3',5,5'-tetramethylbenzidine (TMB) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The photocatalysis did not take place under ambient laboratory light but occurred only under irradiation with a standard UV lamp, resulting in high repeatability. We applied this colorimetric method to analytical assays in which the growth of SNPs in situ is modulated by products of enzymatic reactions catalyzed by GOx and GR. The detection limits demonstrated by two developed chromogenic assays were comparable with or better than those of corresponding fluorogenic assays relying on registration of emission light arising from CdS NPs. Due to stability of 3,3',5'5-tetramethylbenzidine diimine the read-out signal was very stable and standard deviation was quite small.[4]



Scheme 2. DNA detection through peroxidase-DNAzyme modulated growth of CdS QDs *in situ.*

References

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[2] G. Garai-Ibabe, L. Saa, V. Pavlov, Anal. Chem., **85**, (2013) 5542.
[3] G. Garai-Ibabe, M. Möller, L. Saa, R. Grinyte, V. Pavlov, Anal. Chem., **86** (2014) 10059.
[4] R. Grinyté, G. Garai, L. Saa, V. Pavlov, Anal. Chem. In Press.