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Application of *in situ* synthesis of fluorophores to bioanalytical assays

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Bioanalytical enzymatic systems producing fluorescent NPs employ sensitive techniques such as fluorescence spectroscopy to register the read out signal as. A number of analytical assays for detection of enzymatic activities in which generation of fluorescent CdS NPs was induced by products of bio-catalytic reactions have been developed: Acetylcholine esterase breaks the artificial substrate acetylthiocholineto acetate and thiocholine. The latter catalyzes decomposition of S₂O₃²⁻ to H₂S. The resulting hydrogen sulfide interacts with Cd²⁺ to yield CdS NPs. Alkaline phosphatase hydrolyzes thiophosphate to orthophosphate and H₂S. The latter reacts immediately with cadmium cations to give fluorescent CdS NPs. Glucose oxidase (GOx) catalyzes oxidation of 1-thio-β-D-glucose by oxygen to yield finally hydrogen sulfide. The assay consists of the generation of CdS QDs in the presence of GOx, 1-thio-β-D-glucose and cadmium nitrate. GOx oxidizes the substrate 1-thio-β-D-glucose to 1-thio-β-D-gluconic acid, which is hydrolyzed to D-gluconic acid and H₂S. The latter reacts with Cd²⁺ to produce fluorescent CdS QDs. The rate of hydrogen peroxide reduction with sodium thiosulfate can be significantly enhanced by horseradish peroxidase (HRP) yielding H₂S as one of the reaction products. Interaction of enzymatically produced H₂S with Cd²⁺ ions results in the formation fluorescent CdS NPs. Additional enzymatic assays for methionine gamma-lyase, serum paraoxonase and other enzymes have been developed. Enzymatic generation of fluorescent semiconductor nanoparticles *in situ* allows development of relatively inexpensive, simple and available assays. This technique offers alternatives to conventional chromogenic and fluorogenic organic enzymatic substrates which usually are not stable and expensive.

Biography

Valery Pavlov obtained his PhD degree in Chemical Engineering in January 2005 from the University Rovira I Virgili (Spain). Since October he continued his postdoctoral study at the Chemistry Department of the University of Heidelberg (Germany). In February 2007, he joined the new research institute CIC BiomaGUNE in San Sebastian as a group leader. His research interests include enzymatic generation of metal and semiconductor nanoparticles, production of new recombinant mutated enzymes, and optical bioanalytical assays.

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