Development of ultrasensitive bioanalytical assays based on metal and semiconductor nanoparticles.

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Abstract

Signal enhancement is an important part in the development of bioanalytical assays. All previously developed strategies for sensitivity improvement in bioanalysis can be devided in molecular biology and nano approaches. In the first category enzyme amplification techniques is widely used to improve sensitivity of several assays. The principle of enzymatic enhancement is based on the presence of primary enzyme label which acts on a substrate whose product catalyses a secondary cycling enzyme reaction. Nano signal amplification methods are based on using of nanomaterials of different nature. Semiconductor and metal nanoparticle (NPs) are extensively used as carriers decorated with recognition molecules or as donor or quencher of FRET pairs. The application of nanomaterials and molecular biology technology for highly sensitive detection of the targets undetectable by traditional methods is of great challenge.

We developed two ultrasensitive analytical assays based on unconventional combination of enzymatic reactions with CdS quantum dots (QDs) and gold NPs.

The first method for the detection of ascorbic acid (AA) is based on DNAzyme cleaving its DNA substrate in the presence of Cu^{2+} and AA [1]. The detection system consists of two DNA strands which bind to each other via Watson-Crick and Hoogsteen hydrogen bonding (Figure 1). The substrate DNA labeled with fluorophore is loaded on the surface of gold NPs and form FRET pair. The cleavage of substrate DNA in close vicinity to the gold surface liberates fluorescein-DNA fragments producing strong fluorescence enhancement. The catalytic function of gold clusters employed in this approach significantly improved detection limit of AA.

The second method for the detection of various analytes (antigens, antibodies) is based on the employing of enzymatically generated CdS QDs in alkaline phosphatase (ALP) immunoassay [2]. p-Nitrophenyl phosphate (pNPP), used as a substrate, in the presence of ALP is catalytically cleaved, giving p-nitrophenol (pNP) and phosphate ions as the products of the reaction (Figure 2). When Cd^{2+} and S^{2-} ions are added to the reaction mixture, phosphate stabilized fluorescent nanocrystals of CdS are formed, giving a rise to the emission. The sensitivity of this novel assay was significantly improved in comparison with the standard method based on colorimetric p-nitrophenyl phosphate assay.

References

[1] N. Malashikhina, V. Pavlov; *Biosens. Bioelectron.*, **33**, (2012), 241.
[2] N. Malashikhina, G. Garai-Ibabe, V. Pavlov; *Anal. Chem.,*(2013), Paper In Press.

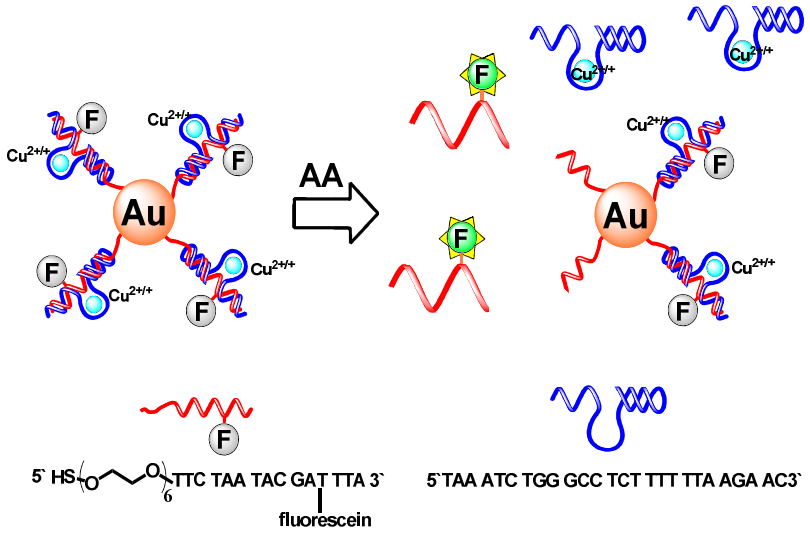

Figure1. Fluorometric assay for detection of ascorbic acid based on DNAzyme and gold nanoparticles modified with fluorophore labeled DNA substrate.

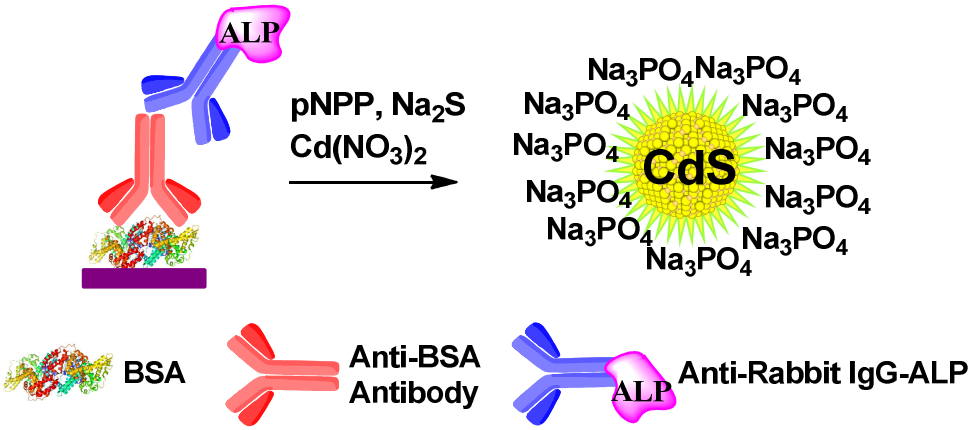

Figure 2. Detection of anti BSA antibody based on enzymatic growth of CdS QDs.