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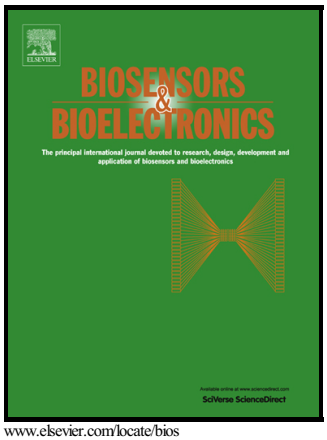

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Nanomaterial-Based Cocaine Aptasensors

Ahad Mokhtarzadeh[1], Jafar Ezzati Nazhad Dolatabadi[2*], Khalil Abnous[1], Miguel de la Guardia[3], Mohammad Ramezani[1*]

[1]*Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran.*

[2]*Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran.*

[3]*Department of Analytical Chemistry, University of Valencia, Dr. Moliner 50, 46100, Burjassot, Valencia, Spain.*

[*]The corresponding author, E-mail: ezzatij@tbzmed.ac.ir, Tel: +98 41 33367914, Fax: +98 41 33367929 & Ramezanim@mums.ac.ir, Tel: +98 51 37112470; Fax: +98 51 38823251.

Abstract

Up to now, many different methods have been developed for detection of cocaine, but most of these methods are usually time-consuming, tedious and require special or expensive equipment. Therefore, the development of simple, sensitive and rapid detection methods is necessary. In the last decade, aptamers have been used as a new biosensor platform for detection of cocaine in different samples. Aptamers are artificial single-stranded DNA or RNA oligonucleotids capable of binding to specific molecular targets with high affinity and if integrated to nanomaterials, it may lead in precise methods for cocaine detection in the common laboratories. In this review, recent advances and applications of aptamer-based biosensors and nanobiosensors, have been updated, paying attention to the use of fluorescence, colorimetric and electrochemical techniques for the detection and quantitative determination of cocaine.

Key words: Cocaine; Aptasensors; Nanosensors; Optical; Colorimetric Assays; Electrochemical.

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1. Introduction

Cocaine addiction is a serious worldwide public health problem and is the second most used illegal substance in both, Europe and United States, after cannabis (Karila et al., 2010). Like other illegal drugs, detection of cocaine is a key tool in various fields such as police apprehensions, fight against drug trafficking and clinical diagnosis. Most of the cocaine detection and quantification methods are usually time-consuming and require special or expensive equipment (Wen et al., 2011). Hence, it would be highly suitable to develop rapid, accurate, simple, and sensitive methods for cocaine detection. Recently, aptamer-based biosensors (aptasensors) have been introduced as new alternative methods to overcome these limitations (Kang et al., 2011).

Aptamers are short DNA or RNA fragments capable of specific binding to target molecules with high affinity (Brody and Gold, 2000). In comparison with antibodies, aptamers exhibit great potential for application in diagnosis and medicine. Briefly, the production of antibodies with

high affinity is costly and time consuming. Therefore, preparation of antibodies for any target molecule is not easy. But high affinity, high degree of specificity, high stability, simple labelling, ability of directional amplification by PCR and low production cost are examples of aptamers advantages over antibodies (Citartan et al., 2012; Jayasena, 1999). Thus, they can be considered as one of the most promising diagnostic and therapeutic tools (Citartan et al., 2012).

During the recognition of the target molecules, aptamers usually provide well-defined three-dimensional (3D) structures that could be applied for the generation of aptasensors (Jiang et al., 2012). Since the discovery of first aptamer in 1990, various aptamers have been evaluated *in vitro* against many classes of molecules such as proteins (Çalık et al., 2010), toxins (Tang et al., 2006), ATP (Huizenga and Szostak, 1995), and even virus (Chen, F. et al., 2009) and cell surface markers (Shangguan et al., 2006). Therfore, aptamer-based biosensors and nanobiosensors have been widely studied due to their high selectivity and sensitivity to almost all types of ligand (Hamula et al., 2006; Song et al., 2008).

In this review; we have paid special attention to the recent advances in aptasensors developed for cocaine detection. We have classified nanomaterial- based cocaine aptasensors according to their signal-harvesting methods, including optical and electrochemical approaches.

2. Aptamer-based cocaine biosensors

A biosensor is a compact analytical device which can be used for the detection and quantitative measurement of an analyte by combining a biological component (a recognition layer) with a physicochemical detector (a transducer). The recognition layer can be composed of antibodies, enzymes, microorganisms, organelles or cells which respond to the presence of the specific analyte. The transducer changes the biological recognition response into the desired format of signal. Biosensors that employ aptamers as a recognition element are called aptasensors

(Dolatabadi et al., 2011; Ezzati Nazhad Dolatabadi and De La Guardia, 2014; Jamali et al., 2014). To improve the detection system, chemical modification and labelling of DNA and RNA aptamers with reporter molecules can be carried out without affecting their affinity on target molecules (Chambers et al., 2008; Ebrahimi et al., 2013; Ebrahimi et al., 2012). Moreover, aptamers can be designed to withstand repeated cycles of denaturation and renaturation, which lead to the possibility of regeneration of the immobilized biocomponent function for repeated use. Besides, they can be easily labelled for diagnostic applications (Hong et al., 2012). Aptasensor-based systems are constantly being developed with various detection methods including electrochemistry (Yunhui, 2010), fluorescence (He et al., 2010), chemiluminescence (Li, Y. et al., 2011), colorimetry (Zhang, J. et al., 2008), surface-enhanced raman scattering (SERS) (Chen, J. et al., 2008), surface plasmon resonance (SPR) (Munoz et al., 2011), and quartz crystal microbalance measurements (Halámek et al., 2005).

2.1. Nanomaterial-based optical aptasensors

The use of nanoparticles in the construction of aptasensors provides tremendous advantages based on either the capability of quantum dots to provide fluorescence signals or on the magnetic properties of some nanoparticles. In general, nanomaterial-based optical aptasensors are transducer of aptamer recognition events to physically measurable signal involving the use of nanoparticles. Optical detection methods like fluorescence, chemiluminescence (CL), colorimetry, SERS and SPR are extensively adopted for signal harvesting in nano-aptasensors due to their ease of use and high sensitivity. Also, fluorescence, magnetic resonance imaging (MRI), and SERS are appropriate for *in vivo* sensing, displaying great potential for practical applications. Besides, nanomaterials usually amplify the originally transduced weak signals by

several orders of magnitude in aptasensors, which make the nanomaterials-based optical aptasensors highly sensitive (Feng et al., 2014; Golub et al., 2009; Liu, Y. et al., 2010; Wang, G. et al., 2010). In this section, for good understanding of nanomaterials-based cocaine optical aptasensors, the highlights and prominent examples were summarized, based on various detection methods such as fluorescence, chemiluminescence, colorimetry, and SPR.

2.1.1. Fluorescence-based cocaine aptasensor and nanosensors

The recognition events between aptamers and target molecules occur when change in the aptamer conformation takes place. Upon practical design, such conformational modification may be used for varying the local environment of fluorophores or nanomaterials, and consequently altering the emission properties of fluorescent systems. Fluorescence methods could potentially employed in determination of bio-interactions in aqueous environment owing to the flexible ways in quantitative analysis with high sensitivity and wide response range. Narrow absorption, broad emission and photobleaching are examples of problems associated with traditional organic dyes. Therefore, functionalized nanomaterials with fluorescence emission have been widely applied to probe dynamic biological processes using electron-transfer quenching or fluorescence resonance energy transfer (FRET) as photophysical probing mechanisms, by which new horizons have opened for signaling assistance in aptasensor (Feng et al., 2014; Golub et al., 2009; Jamali et al., 2014; Liu, Y. et al., 2010; Wang, G. et al., 2010). Optical-based biosensors usually used FRET for detection of different molecules such as nucleic acids, proteins, peptides and small molecules. The optimization of energy donor and acceptor fluorophore in FRET-based aptasensor is critical for development of desired biosensor. There are a large number of donor and acceptor fluorophores such as autofluorescent proteins, organic dyes and inorganic nanostructures which could be used in FRET-based biosensors. Quantum dots (QDs), graphene

oxide (GO) and gold nanoparticles (AuNPs) are fluorescent inorganic reporters, which widely used as donor or acceptor fluorophore due to their excellent fluorescent properties, including high spectral resolution, resistance to photobleaching and extremely sharp emission bands (Dolatabadi et al., 2011; Jamali et al., 2014).

The first cocaine fluorescence-based aptasensor was reported in 2001 by Stojanovic et al. which was an aptamer-based FRET biosensor with a limit of detection (LOD) of 10 μM (Stojanovic et al., 2001). Another cocaine aptasensor based on FRET was reported by the Huang et al. (Huang et al., 2011). Their method was based on a strand-displacement polymerization with application of a primer with FAM labelled on its 5' end as donor fluorophore and a Cy5- labelled hairpin probe on its 3' end as acceptor fluorophore. Primer was designed as complementary to 3' end of hairpin probe and cocaine aptamer was integrated into 5' end of hairpin probe. In the absence of cocaine, the primer was unable to bind to the 3' end of hairpin probe, and the Cy5 label does not emit any fluorescence. However, in the presence of cocaine, hairpin probe would open and primer could bind to the exposed stem of hairpin probe. This reaction brings FAM and Cy5 into close proximity, which results in fluorescence resonance energy transfer. They obtained a LOD of 200 nM for cocaine in about 16 min reaction period (Huang et al., 2011).

QDs are fluorescent semiconductors which contain elements of Groups II–VI, III–V and IV–VI. QDs offer a number of advantages over conventional dyes for sensing events such as high quantum yield, exceptional photochemical stability and broad absorption spectra (Jamali et al., 2014; Jamieson et al., 2007). Based on these advantages and their luminescent potential, QDs have been widely used for the development of biosensor platforms (Frasco and Chaniotakis, 2009). For example, a sensitive and specific FRET-based QD-aptasensors for cocaine detection was reported by Zhang and Johnson. They employed streptavidin-functionalized 605QDs, a biotinylated oligonucleotide, a Cy5-labelled oligonucleotide and a cocaine aptamer. In the first

step, cocaine aptamer was sandwiched by a Cy5-labelled oligonucleotide and biotinylated oligonucleotide. In the next step, this sandwich hybrid was assembled on the surface of a 605QD to form the 605QD/aptamer/Cy5 complex (Fig. 1A). In this complex, the 605QD act as a FRET donor to transfer energy to Cy5. In the presence of cocaine, Cy5-labelled oligonucleotide dissociated from complex and lead to decrease of Cy5 fluorescence due to the lack of energy transfer between 605QD and Cy5 (Zhang, C.-Y. and Johnson, 2009).

Zhou et al. (2012) developed another optical aptasensor using a FITC-labeled aptamer. In the presence of cocaine, a double stranded T junction between a partial single strand aptamer and a short hairpin structure could be formed. In this system, double-strand specific DNA minor groove binder, Hoechst 33342 dye, could be selectively bind to T junction structure and bringing it near the FITC dye. During excitation at 360 nm, minor groove binder-based energy transfer (MBET) could be formed between Hoechst 33342 (as donor) and FITC dye (as acceptor). As a result of this energy transfer, a green fluorescence emission peak could be recorded at 520 nm (Fig. 1B). This fabrication showed a LOD of 0.2 μM for cocaine (Zhou et al., 2012).

AuNPs have unique electrical and optical properties such as high absorption coefficient, scattering flux, highly SPR, luminescence and conductivity. The unique properties of AuNPs making them as an attractive signal transducer for biosensor fabrication (Hutter and Maysinger, 2013; Jeong et al., 2014). Based on fluorescence quenching ability of AuNPs, Shi et al. have developed a new aptasensor composing of GO and AuNPs as the fluorophore and the quencher, respectively. Two parts of cocaine aptamer were covalently attached onto GO and AuNPs surfaces. In this system, GO and AuNPs act as an electron donor and acceptor pair, respectively. In the presence of cocaine, a three-way junction structure could be formed and bring AuNPs near to the GO surface and lead to fluorescence quenching of GO (Fig. 1C). This fabrication showed

low LOD (0.1 mM), high specificity and required minimal amount of reagents (1 mL) (Shi et al., 2013).

Another interesting feature of AuNPs is large surface-to-volume ratio. These fine large available surface areas enable them to adsorb very diverse bio-macromolecules onto their surfaces. Based on this property, a cocaine aptasensor was fabricated *via* application of rolling circle amplification method. In this platform, Au magnetic beads modified with nanoparticles and cocaine aptamers were immobilized onto AuNPs surfaces through Au-S linkage. In the absence of cocaine, this aptamer could be hybridized with a short DNA strand (cDNA) to form DNA double helix on AuNPs. When cocaine was present in solution, cocaine specifically bound to aptamer resulting in the displacement of cDNA from the AuNPs. In the next step, cDNA was directly used to originate rolling circle amplification (RCA) as primer. The end products of RCA were partially complemented with sequence of molecular beacons. At this stage, molecular beacons hybridize with the products of RCA and lead to opening of molecular beacons structure to generate fluorescence signals (Fig. 2). The LOD of this method was 0.48 nM (Ma et al., 2011).

In a similar manner, cocaine aptamer was cleaved into tow fragments; a single strand-probe (ss-probe) and a hairpin-probe (HP). In the presence of cocaine in sample solutions, both probes would join with cocaine to form a tripartite structure. Formation of tripartite complex led to the opening of a hairpin probe and consequent hybridization to a primer. Primer extension process could be triggered by the KF polymerase and the dNTPs. Replication proceeding by polymerase enzyme leads to displacement of ss-probe and cocaine. After intercalation of SYBR Green I (SG) into the new DNA double helix, fluorescence signals generation occurred using suitable excitatory illumination (Fig. 3A). It was shown that such method could detect as low as 2 nM of cocaine in a closed tube (He et al., 2010).

In recent years, GO nanosheets have been introduced as efficient bioanalytical platform for the protein assays, DNA sensing, metal ions and small molecules detection. GO has shown fascinating properties for use in biosensors because of its unique characteristics such as intrinsic photoluminescence, extremely large surface area, easy surface modification, strong mechanical strength, and good water dispersibility (Wang, Y. et al., 2011). Most GO-based optical biosensor platforms depends on the highly quenching capacity of GO and preferential binding of GO to single-strand DNA over duplex DNA. Thus, GO could quench the fluorescence of dyes conjugated to ssDNA (Kasry et al., 2012; Liu, X. et al., 2012). Based on these properties, a novel label-free aptasensor was developed by Qiu et al. They used a hairpin probe (HP) encompass a cocaine aptamer and the region especially considered to hybridize with the primer. In the absence of cocaine, HP structure remained intact and primer could not attach into complement sequence and polymerase elongation would not happen and consequently, 5'-single-stranded end of HP adsorbed on GO platform. In this state, GO act as the quencher by quenching SG fluorescence properties. On the other hand, if cocaine present in sample, it could interact with HP probe and consequently change its conformation, which leads to exposing the 3'-single-stranded end of the HP to hybridize with the primer. Then, polymerase elongation is triggered by KF polymerase and generated a double strand DNA (dsDNA). Since dsDNA has week affinity for GO, SG-stained dsDNA could not efficiently quenched by GO leading to recordable fluorescence signal (Fig. 3B). This simple and convenient method showed high sensitivity and selectivity for cocaine detection with LOD of 190 nM (Qiu et al., 2013).

2.1.2. Colorimetric cocaine aptasensors

Metal nanoparticles, which have size-/distance-dependent optical properties, are perfect candidates for colorimetric assays. For instance, the color of colloidal AuNPs is sensitive to its

aggregation/dispersion because of changing in the inter-particle plasmon coupling and resulting surface plasmon band shift. Moreover, AuNPs extinction coefficient is over 1000 times higher than that of organic dyes, which offer high sensitivity for AuNPs-based colorimetric aptasensors (Ghosh and Pal, 2007; Rex et al., 2006). Consequently, assembly/disassembly of nanoparticles can be regarded as novel indicator of colorimetric assay. Therefore, colorimetric aptasensors can be used for detection of cocaine.

First colorimetric cocaine probe with LOD of 2 μM has been reported by Stojanovic and Landry (Stojanovic and Landry, 2002). Liu and Lu fabricated aptasensors using cocaine induced disassembly of AuNP aggregates. They created AuNPs aggregates using a 5'-thiol-modified DNA (5' Coap Au), a 3'-thiol-modified DNA (3' Coap Au), and a linker DNA (Linker Coc) molecule. The Linker Coc contained three parts: the first segment (in green), the aptamer sequence for cocaine, the second segment (in gray) is hybridized with the 5-nt on the 5' Coap AuNPs, and finally the third segment (in purple) is hybridized with a 3' Coap AuNPs. During addition of cocaine, the aptamer binds to cocaine owing to its conformation change. As a result, 3' Coap AuNPs dissociated from the 5' Coap AuNPs, which leads to disassembly of the aggregates. In this state, the system colour changes from purple to red (Fig. 4A). The rate of color change depends on the concentration of cocaine. The results of this study indicated that this method could be used for cocaine quantification in the range of 50 to 500 μM (Liu, J. and Lu, 2006). In a similar study, Zhang et al. demonstrated a colorimetric method using AuNPs and engineered DNA aptamers. In this approach, an anticocaine aptamer (ACA) was cleaved into two flexible ssDNA fragments, ACA-1 and ACA-2, with a little modification in sequence for avoiding inter-strand binding. In the presence of 20 mM NaCl, AuNPs could be aggregated and created a red to blue colour change. In the absence of cocaine, free ACA-1 and ACA-2 with highly negative charge interact with AuNPs and lead to retain the red colour upon salt-induced

aggregations (Fig. 4B). Interestingly, in the presence of cocaine, ACA-1 and ACA-2 would be interacting with cocaine and form a tertiary conformation which does not have affinity for AuNPs and consequently resists salt-induced aggregation. The result showed that this method could detect as little as 2 μM cocaine (Zhang, J. et al., 2008). In another study, Zou et al. used a similar method for cocaine detection but they cleaved cocaine aptamer into three fragments. They indicated that this aptasensor could detect 100 μM cocaine in human urine or filtered serum (Zou et al., 2012).

Many different types of fingerprint sensors and identification apparatus fingerprints have been reported (Mathiassen et al., 2007). After touching the surface by the fingers, excreted sweat of eccrine glands together with oily substances such as sebum deposited on the surface of fingers form an impression of the fingers ridge pattern. This impression known as a latent fingerprint (LFP) which usually contains the secretions, dead skin cells, skin oils and residues of different chemicals and their metabolites in the human body. These can be used for identification of drugs and drug metabolites, secreted chemicals and residues of explosives for the forensic purposes (Hazarika and Russell, 2012; Li, K. et al., 2013). Based on this idea, Li et al. reported a novel and very attractive nanoplasmonic approach for preparation of high-resolution dark-field microscopy (DFM) images of LFP for detection of cocaine in LFPs. In fabricated aptasensors, cocaine aptamer was divided into two flexible ssDNA fragments which were then attached to different AuNPs *via* Au-S bound. In the presence of cocaine, it acts as linker molecule and could reassemble the two fragment of ssDNA into intact aptamer conformation, resulting in the aggregation of the AuNPs probes. This aggregation resulted in red-to-blue colour alteration of AuNPs. For detection of cocaine in LFPs, fingers contaminated with cocaine and LFPs of the fingers were collected and then incubated with aptamer-bound AuNPs and finally observed by DFM. As depicted in figure 4C, with the increase of cocaine concentration from 150 ng to 30 μg,

high quantity of AuNPs aggregated. Thus, more red coloured dots could be observed with gradual disappearance of green-coloured dots (Li, K. et al., 2013).

DNAzymes and RNAzymes are examples of allosteric aptamers which are widely used for the construction of biosensors. In these platforms, they could act as either signal readouts or recognition elements (Bohunicky and Mousa, 2011; Chambers et al., 2008). G-quadruplex motifs are made up repetitive G-rich and could act as quadruplex DNAzyme. In the presence of hemin, G-quadruplex can form G-quadruplex/hemin complex which exhibit peroxidise-like activity similar to horseradish peroxidise. Therefore, horseradish peroxidises mimicking DNAzymes could be used as a platform for fabrication of colorimetric biosensors. According to this strategy, a new colorimetric aptasensor was developed using the combination of aptamer and peroxidase-mimicking DNAzyme. In this work, the sequences of a DNAzyme (G-quadruplex sequence) and cocaine aptamer were integrated into a single sequence for creation of a HP. Due to base pairing in this structure; their specific functions can be blocked. Introduction of cocaine leads to opening of the HP structure to form cocaine/aptamer complex and releasing of G-quadruplex sequence. Addition of hemin resulted in self-assembly of cage complete G-quadruplex sequence into active DNAzyme which can catalyze colorimetric reaction of 4,4′-diamino-3,3′,5,5′-tetramethylbiphenyl-H_2O_2. The LOD of 5 μM was reported for this method (Nie et al., 2013).

2.1.3. Chemiluminescence-based cocaine aptasensors

Chemiluminescence assay is one of the attractive analytical methods due to high sensitivity, fast reaction, low cost, and simple manipulation. However, only a few Chemiluminescence aptasensors have been reported for the detection of small molecules such as cocaine. Li and his co-workers reported a novel Chemiluminescence method using AuNPs-functionalized magnetic

microbeads (MB-AuNPs) for the detection of cocaine. In the first stage, cocaine aptamer was immobilized on the surface of MB-AuNPs. In the next step, aptamers were hybridized with the signal DNA on the double-functional gold nanoprobes (DF-AuNPs) which was previously modified with horseradish peroxidase (HRP). After introduction of cocaine, a competition occurred for the aptamer between signal DNA and cocaine. This reaction led to dissociation of DF-AuNPs from the MB-AuNPs/aptamer/DF-AuNPs complex, which could be employed for Chemiluminescence measurement (Fig. 5). The Chemiluminescence signal strength of luminol-H_2O_2-HRP system depends on cocaine concentration (Li, Y. et al., 2011). Available optical-based cocaine apta-nanosensors have been demonstrated in Table 1.

Overall, as we demonstrated, fluorescence, colorimetry and chemiluminescence are three types of widespread methods for cocaine aptasensors. Fluorescence is one of the most common signal transduction modes, including both labeled and label-free modes. Colorimetric detection is the simplest sensing procedure and can be visible to the naked eye. Therefore, many colorimetric aptasensors for small biomolecules such as cocaine are extensively designed, mainly involving AuNPs and the formation of HRP-DNAzyme with hemin. Chemiluminescence has also been developed widely in recent years owing to its simple instrumentation and wide calibration ranges. However, few of optical sensors can be applied in the biological samples. It is because of two features. First aspect was due to low concentration of various analysts in biological samples for detection. The second one is the complex environments of biological samples, which will interfere with detection, even resulting in false positive signals. Therefore, enormous efforts have been performed to improving the selectivity and sensitivity of optical aptasensors for cocaine.

2.2. Electrochemical-based cocaine aptasensor

Electrochemical-based biosensors are a device that couples a biological material to an electrode transducer (Saei et al., 2013). Electrochemical- based aptasensor has some advantages over optical based ones. Unlike optical instruments that are usually expensive and not portable, electrochemical-based aptasensors are more feasible for on-field applications. They require less quantity of target molecules and organic or non-organic catalysts for detection due to the possibility of coupling them to target aptamer complex and amplifying the sensing process. Moreover, detection of target molecule without any need for fluorescent labels as an implementation reduced the cost of the equipment and makes them more reusable by washing off the target molecule (Saei et al., 2013; Zuo et al., 2007). In recent years, several electrochemical aptasensors have emerged using electrochemical impedance spectroscopy (EIS), square-wave voltammetry (SWV), differential pulse voltammetry (DPV), chronocoulometry and other techniques as new diagnostic tools for the detection of cocaine (Li, X. et al., 2008; Zhang, D.-W. et al., 2012b). For example, Golub et al. designed three aptasensors based on electrochemical, photoelectrochemical, and SPR methods for detection of cocaine. To achieve this goal, they cleaved cocaine aptamer into two segments. While one segment was assembled onto gold electrode using Au-S bond, the other segment was attached to the surface of the platinum NPs (Pt-NPs), Au-NPs and CdS-NPs. Addition of cocaine can trigger the formation of supramolecular complexes between aptamer- functionalized-NPs and the gold electrode surface. In this case, when aptamer- functionalized Pt-NPs were used, electrocatalytic reduction of H_2O_2 amplified in the presence of cocaine. The LOD of this platform was determined to be around 1×10^{-5} M. The use of aptamer- functionalized Cds-NPs for the photoelectrochemical estimation of cocaine concentration can detect 1×10^{-6} M of cocaine. And finally, using aptamer-

functionalized Au-NPs for the SPR determination of cocaine (Fig. 6A) indicated that this system can detect 1×10^{-6} M (Golub et al., 2009).

Most of the EIS-based aptasensors focused on protein detection. However, recently several studies used EIS aptasensors for detection of cocaine. In these sensing platforms, the recognition of cocaine by aptamer-modified gold electrodes changed the electron transfer resistance (Ret) which is strongly associated with cocaine concentration. In this electrochemical sensing platform, cocaine aptamer usually cleaved into two or three fragments that can be self-assembled into a supramolecular cocaine-aptamer fragments complex in the presence of cocaine (Golub et al., 2009; Zhang, D.-W. et al., 2012b). For instance, Zhang et al. developed a label-free aptasensor based on the formation of a supramolecular aptamer fragments/cocaine complex for detection of cocaine with high sensitivity and specificity. In this platform, anti-cocaine aptamer fragments (Cx and Cy) were used for molecular recognition of cocaine. The 5' or 3' ends of Cx and Cy fragments were chemically modified with a thiol group and then attached to gold surface by Au-S bond to form aptamer/cocaine complex. Formation of this complex was monitored by EIS in the presence of $[Fe(CN)_6]^{3-/4-}$. The results showed that Au/Cx5S/cocaine/Cy aptasensor has good sensitivity for cocaine detection with the LOD range of 0.1–20 μM (Zhang, D.-W. et al., 2012b). In another study, which was reported by the same group, they used electrochemical aptasensor based on enzyme- linked aptamer assay (ELAA). In this work, anti-cocaine aptamer was cleaved into two fragments (CoS and CoB); one fragment (CoS) was attached to a gold electrode surface and another fragment (CoB) was labelled with biotin at 3' end. Biotinylated fragment could be detected with streptavidin-HRP (SA-HRP). During the binding of cocaine, the HRP-labelled aptamer fragment/cocaine complex could be formed on the gold electrode surface and reduction of hydroquinone in the presence of hydrogen peroxide (H_2O_2) was monitored by

DPV. The results showed that this approach could effectively detect the cocaine in the dynamic range of 0.1 M to 50 M with a LOD of 20 nM (Zhang, D.-W. et al., 2012a).

Enhancing sensitivity and minimizing unwanted background noise are critical for obtaining low LOD in biosensors (Das et al., 2007). Redox recycling systems can be used for enhancing detection capability of electrochemical biosensor. Signal amplification is achieved by homogeneous electron transfer between redox compounds in a cyclic manner. The repetition of this cycle leads to multiple heterogeneous electron transfer events for each target molecule which could amplify the signal transduction (Bergren and Porter, 2007). The first redox cycling study was reported by Sanderson and Anderson in 1985 and nowadays, this system have been used in various electrochemical aptasensors (Sanderson and Anderson, 1985). According to this method, Jiang et al. reported a highly sensitive electrochemical cocaine aptasensor using graphene/AuNP nanocomposites. The working electrodes were prepared by electrodeposition of AuNPs and GO nanosheets on the surface of screen printed carbon electrodes (SPCE). On the other hand, cocaine aptamer was cleaved into two fragments; one fragment (SH-C1) was attached onto AuNPs/GO/SPCE with S-Au bond while another fragment was labelled with biotin (Biotin-C2). In the next step, cocaine and biotin-C2 were added to form a sandwich of biotin-C2/cocaine/C1/AuNPs/GO/SPCE complex. Finally, streptavidin-conjugated alkaline phosphate was added to the sensing system *via* biotin streptavidin conjugation. For detection of cocaine concentration, aminophenylphosphate substrate and co-reactant NADH were added into sensing surface where surface-captured ALP enzymes initiated the hydrolysis of p-AP to the electroactive *p*-aminophenol (*p*-AP) substrate. Generated *p*-AP molecules could be electrochemically oxidized to *p*-quinone imine (*p*-QI) near the electrode surface. In the presence of NADH, *p*-QI was reduced back to *p*-AP which led to an amplification of redox cycling process between *p*-QI and *p*-AP (Fig. 6B). Generated current response indirectly

depended on the amount of cocaine attached to the electrode surface. The LOD of this assay was 1 nM of cocaine in sample (Jiang et al., 2012).

Various reagentless and reusable electrochemical aptasensors have been reported for detection of thrombin in blood Serum (Xiao et al., 2005) and detection of ATP (Zuo et al., 2007) in soil and foodstuffs (Lubin et al., 2006). For rapid sensing of cocaine in unmodified and undiluted blood serum, a microfluidic electrochemical aptamer-based sensor (MECAS) chip was developed by Swensen et al. The MECAS chip was contained in a platinum counter electrode (CE) with a 750-nl detection chamber, a platinum electrode as reference electrode (RE) and three gold working electrodes (WE). The 5' and 3 ' ends of cocaine aptamer were modified with thiol group and redoxable methylene blue (MB) moiety, respectively. Then, aptamer was conjugated to WE *via* Au-S bond. During cocaine binding, aptamer could be folded into proper conformation leading to efficient electron transfer from MB tag to the electrode. Changes in peak reduction current was measured by alternating current voltammograms (ACV) and monitored by a computer-controlled data acquisition system. The attained results demonstrated a continuous, real-time (~1 minute time resolution) monitoring system for cocaine at physiologically-relevant concentrations, low micromolar concentrations directly in unmodified blood serum (Swensen et al., 2009).

Finally, Sheng et al. investigated an artificial 3D DNA structure as a new ultrasensitive electrochemical cocaine aptasensor. To prepare this system, they employed combination of five DNA probe: three thiolated DNA probes (P1, P2, and P3) (36-nt), one 55-nt DNA probe (P4) and one 39-nt cocaine aptamer probe (P5). The P4 probe was composed of three identical fragments in which parts of 17-nt could specifically bind to the relative 5′-end of the P1, P2 and P3 probes. The P5 aptamer probe sequence was fully complementary to the 3′-end of the 13-nt parts of three thiolated probes which allowed for the DNA duplex formation at the distal end of

the probes. In the presence of an Au electrode, triangular pyramid frustum DNA nanostructure (TPF DNA) could form onto Au electrode surfaces ("Close" state of the DNA nanostructure). In this structure, $Fe(CN)_6^{3-/4-}$ could not access the Au electrode surface for efficient electron transfer. In presence of cocaine in solution, cocaine could interact with P5 probe (cocaine aptamer) and lead to disassembly of TPF DNA nanostructure, resulting in an "Open" DNA nanostructure. In this state, $Fe(CN)_6^{3-/4-}$ could efficiently interact with Au surface and electron transfer was recorded by Faradaic impedance response (Fig. 6C). The result of this investigation indicated that this ultrasensitive electrochemical platform could detect the cocaine concentration ranging from 1.0 nM to 2.0 μM with LOD of 0.21 nM (Sheng et al., 2014).

A summary of the available reports on cocaine aptasensors based on electrochemical methods are provided in Table 2.

3. Summary and Conclusions

Aptamers are good candidates for designing lab-on-a-chip sensors, especially for rapid, easy, and sensitive detection of targeting molecules. In the case of drug detection, these attributes are very helpful to rapidly find drug in custom cargo, preventing smuggling drug into country as well as recognition of addicts. The three dimensional stability of aptamers offers feasibility in different conditions, that may not be presented by alternative antibody-based biosensors. Among drugs, cocaine abuse is regarded as a major global challenge that takes both time and expenses in order to detect and fight against it. So, it can be suggested that most recent fabricated aptasensor for cocaine detection which are presented in this review are highly promising as control and diagnostic tools.

4. Future Perspectives

The present state of the art of nanomaterials-based aptasensors showed some limitation such as the lack of intrinsic properties and functional moieties in some of the nanomaterials and the difficulty in bioconjugation chemistry. To overcome these obstacles, researchers are trying to increase the advantages of nanomaterials by improvement of the available procedures. Considering the fast expansion in the preparation of versatile nanomaterials, we predict that integration of these nanomaterials in aptasensors will drive various advances in bioanalytical systems for clinical, environmental, food and industrial applications. It seems that some of these fabrications have potential to be marketed in the near future.

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Figure legends

Fig. 1. Some fluorometric aptasensors developed for cocaine determination. **1A)** Principle of signal-off single QD-based aptameric sensor for cocaine detection. In the absence of cocaine, Cy5 fluorescence was detected due to FRET between 605QD and Cy5. While the presence of cocaine led to the formation of complex structure of a cocaine-aptamer complex, and the subsequent abolishment of FRET between 605QD and Cy5, the decrease of Cy5 signal signified the presence of cocaine, **1B)** Schematic illustration of MBET aptamer sensors for cocaine detection on UDA modified PDMS surface and **1C)** Schematic illustration of another cocaine sensor. Adapted from published papers (Shi et al., 2013; Zhang, C.-Y. and Johnson, 2009; Zou et al., 2012).

Fig. 2. Schematic outline of fluorescence biosensor to detect cocaine based on signal amplification under RCA and the separation by magnetic beads reducing the background signal. Reprinted with permission from (Ma et al., 2011).

Fig. 3. Fluorescently-labeled aptasensors proposed for cocaine determination. **3A)** Structure of hairpin-probe, ss-probe, and the complex of hairpin-probe/cocaine/ss probe (A) and Schematic illustration of cocaine sensing strategy based on strand displacement amplification (B) and **3B)**

Schematic representation of the label-free fluorescent aptamer-based sensor method for cocaine detection based on GO and ICSDA. Adapted from (He et al., 2010) and (Qiu et al., 2013).

Fig. 4. Colorimetric aptasensors proposed for cocaine determination. **4A)** Schematic representation of the colorimetric detection of cocaine based on cocaine-induced disassembly of nanoparticle aggregates linked by a cocaine aptamer **4B)** Scheme for the engineered cocaine aptamer and the visual detection of cocaine based on the red-to-blue color change of gold nanoparticles and **4C)** Principle of nanoplasmonic imaging of LFPs and identification of cocaine in LFPs by DFM. Adapted from the published works of (Li, K. et al., 2013; Liu, J. and Lu, 2006; Zhang, J. et al., 2008).

Fig. 5. Schematic diagram for the chemiluminescence detection of cocaine using gold nanoprobes and functionalized magnetic microbeads. Reprinted with permission from (Li, Y. et al., 2011).

Fig. 6. Electrochemical aptasensors developed for the determination of cocaine. **6A)** Electrochemical (Path A), photoelectrochemical (Path B), and SPR (Path C) analysis of cocaine through the self-assembly of supramolecular complexes of Pt-NPs-, CdS-NPs-, or Au-NPs-functionalized aptamer subunits and Au-surfaces functionalized with the second aptamer subunit in the presence of cocaine, **6B)** Illustration of the aptamer-based, sensitive EC detection of cocaine on graphene/AuNP modified electrode through redox-recycling signal amplification, and **6C)** Schematic illustration of the cocaine sensing based on TPF_{DNA} nanostructure assembled on Au electrode. Adapted from (Golub et al., 2009; Jiang et al., 2012; Sheng et al., 2014)

Table. 1 Available optical- based cocaine aptasensors.

Detection methods	Strategy	LOD	Linear range	References
Fluorescence	Aptamer-based folding fluorescent sensor	~10μM	Not reported	(Stojanovic et al. 2001)
Fluorescence	Minor groove binder based energy transfer (MBET)	0.2 μM	0 to 10 μM	(Zhou et al. 2012)
Fluorescence	Combining GO and AuNPs	0.1 μM	10^{-3} to 5× 10^{-6} M	(Shi et al. 2013)
Fluorescence	Strand displacement amplification based aptameric sensor	1 nM	2.0 nM to 200 μM	(He et al. 2010)
Fluorescence	Isothermal circular strand-displacement amplification	190 nM	0.2μM to 100μM	(Qiu et al. 2013)
Fluorescence	Rolling circle amplification	0.48 nM	1.0 nM to 50.0 nM	(Ma et al. 2011)
Fluorescence	Strand-displacement polymerization and FRET	200 nM	200 nM to 200 μM	(Huang et al. 2011)
Fluorescence	Single quantum-dot-based aptameric nanosensor for cocaine	0.5 μM	Not reported	(Zhang and Johnson 2009)
Fluorescence	AuNPs and progressive dilution	2.1 μM	Not reported	(Ge et al. 2012)
Fluorescence	Isothermal polymerization/nicking enzyme/DNAzyme cascade machinery	1× 10^{-18} M	Not reported	(Wang et al. 2013)
Fluorescence	Integrated signal transduction-based autonomous aptameric machine	5 μM	Not reported	(Deng et al. 2012)
Colorimetry	Aptamer-based colorimetric probe	2 μM	Not reported	(Stojanovic and Landry 2002)
Colorimetry	Colorimetric sensor based on aptamers and nanoparticles	50 μM	50 to 500 μM	(Liu and Lu 2006)
Colorimetry	AuNPs and engineered DNA aptamers	2 μM	20 nM to 200 μM	(Zhang et al. 2008)
Colorimetry	Aptamer functionalized microcantilever sensors	5 μM	25 to 500 μM	(Kang et al. 2011)

Colorimetry	Optical detection of cocaine	100 μM	0 to 200 μM	(Zou et al. 2012)
Colorimetry	Latent fingerprints	150 ng	150 ng to 30 μg	(Li et al. 2013)
Chemiluminescence	Chemiluminescence method using AuNPs	0.48 nM	1.0 nM to 10.0 nM	(Li et al. 2011)
Electrochemiluminescence	Electrochemiluminescence "sandwich" biosensor	3.7×10^{-12} mol L^{-1}	0.01nM to 1.0 nM	(Cai et al. 2011)

Table. 2 Available electrochemical and other methods based cocaine aptasensors.

Detection method	Strategy	LOD	Linear range	References
Cyclic voltammetry (CV)	Engineered aptamer-pendant DNA tetrahedral structure	33 nM	100 nM to 1 mM	(Wen et al., 2011)
CV	Electrochemical, photoelectrochemical, and SPR	1.0 to10 µM	Not reported	(Golub et al., 2009)
DPV	Enzyme-linked aptamer assay (ELAA)	20 nM	0.1 to 50µM	(Zhang et al. 2012a)
DPV	Graphene/AuNP nanocomposites	1 nM	1 to 500 nM	(Jiang et al., 2012)
DPV	AuNP-modified electrode	0.3 µM	1.0 to 150 µM	(Hua, M. et al., 2010)
DPV	Solid-state probe based electrochemical aptasensor	0.1 µM	0.1 to 38.8 µM	(Du et al., 2010)
DPV	Electrochemical aptasensor based on Klenow fragment (KF) polymerase reaction	0.097 µM	0.2 to 5.0 µM	(He et al., 2011)
CV, Electrochemical impedance spectroscopy (EIS) and DPV	Electrochemical aptamer biosensors based on QDs	30 nM	0.1 to 1.0 µM	(Hua, Mei et al., 2011)
SWV and CV	Modified gold nanoparticle	0.5 µM	1.0 to 15 µM	(Li et al., 2008)
Alternating current voltammograms (ACV)	Microfluidic electrochemical aptamer-based sensor (MECAS) chip	1 µM	10.0 to 100 µM	(Swensen et al., 2009)
Faradaic impedance response (FIS)	Reversible DNA nanostructure	0.21 nM	1.0 nM to 2.0 µM	(Sheng et al., 2014)
Electrochemical and fluorescence	Surface-enhanced raman scattering spectroscopy	1 µM	1 to 3200 µM	(Chen et al., 2008)
CZE-LIF	Metal cation mediated-capillary electrophoresis (MCM-CZE)	5 µM	Not reported	(Deng et al., 2012)
Solid-phase extraction	Selective solid-phase extraction using an aptamer-based sorbent	0.1µg/mL	Not reported	(Madru et al., 2009)
Biological nanopore embedded in a microchip	Biological nanopores on a chip	1 µM	1.0 to 100 µM	(Kawano et al., 2011)

Highlights:

- Aptamers are artificial single-stranded DNA or RNA oligonucleotids capable of binding to specific molecular targets.
- Aptamers have been used as a new biosensor platform for detection of cocaine.
- Application of nanomaterials in optical and electrochemical aptasensor for cocaine detection has been reviewed.

Figure 1

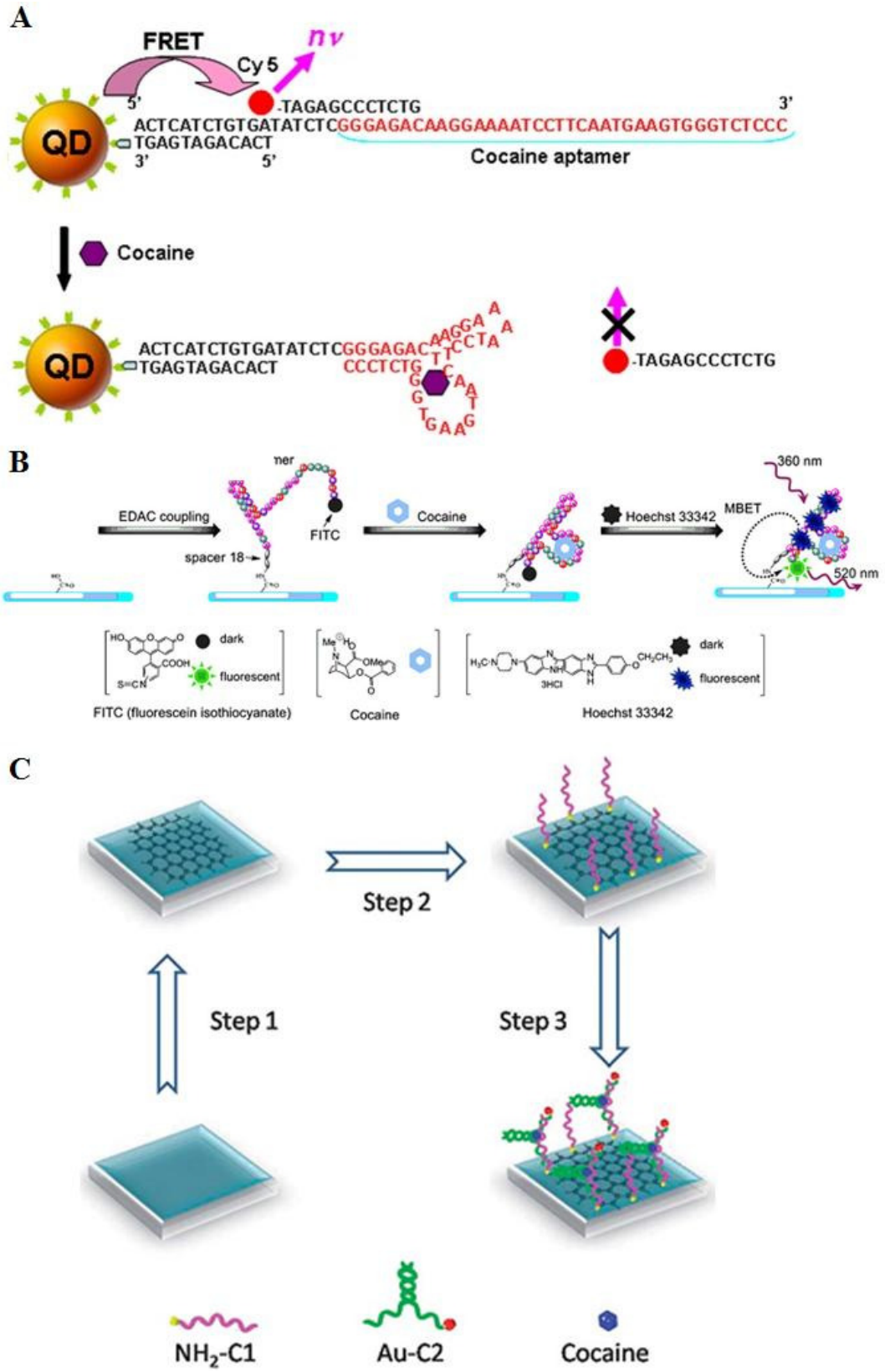

Fig. 1

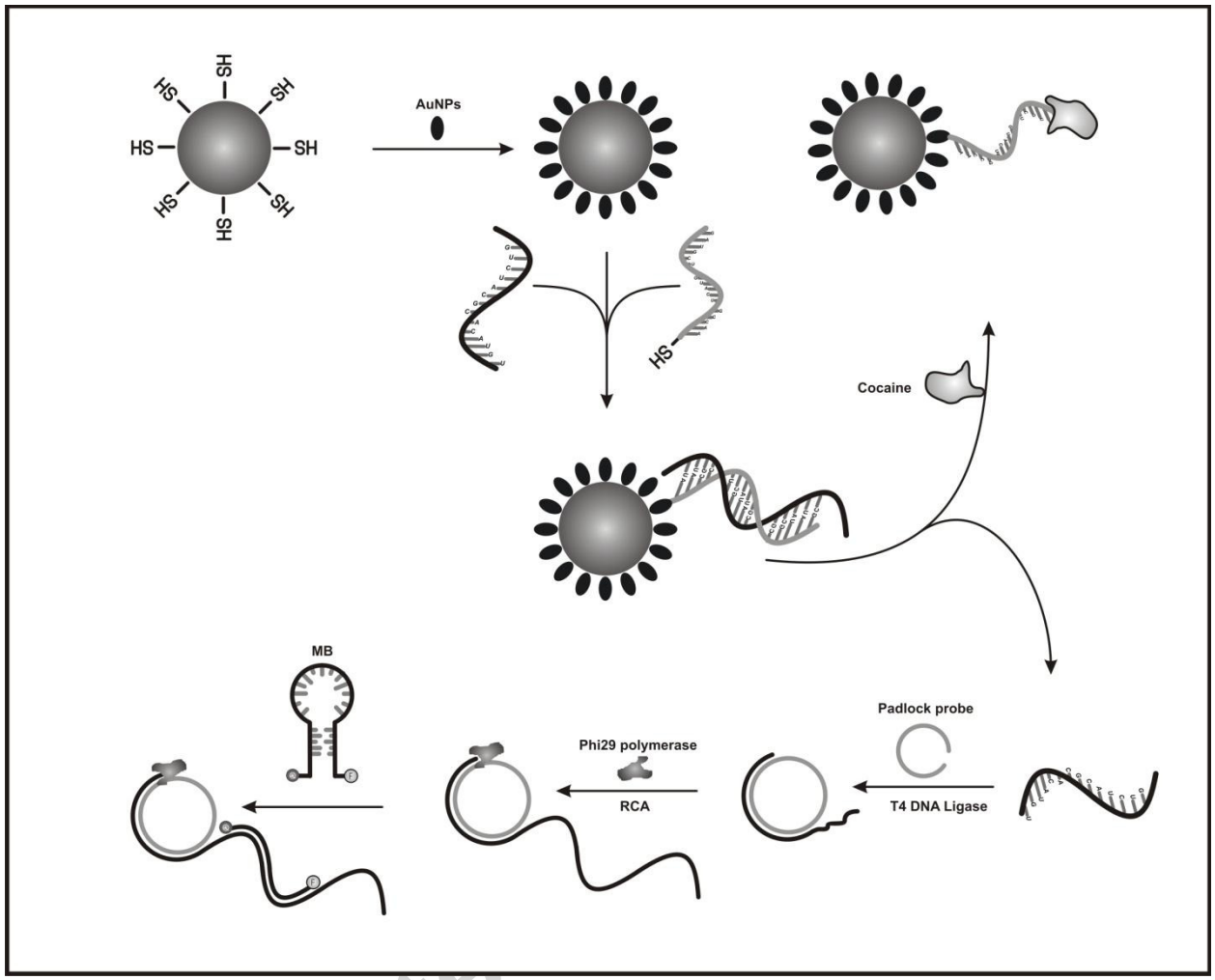

Fig . 2

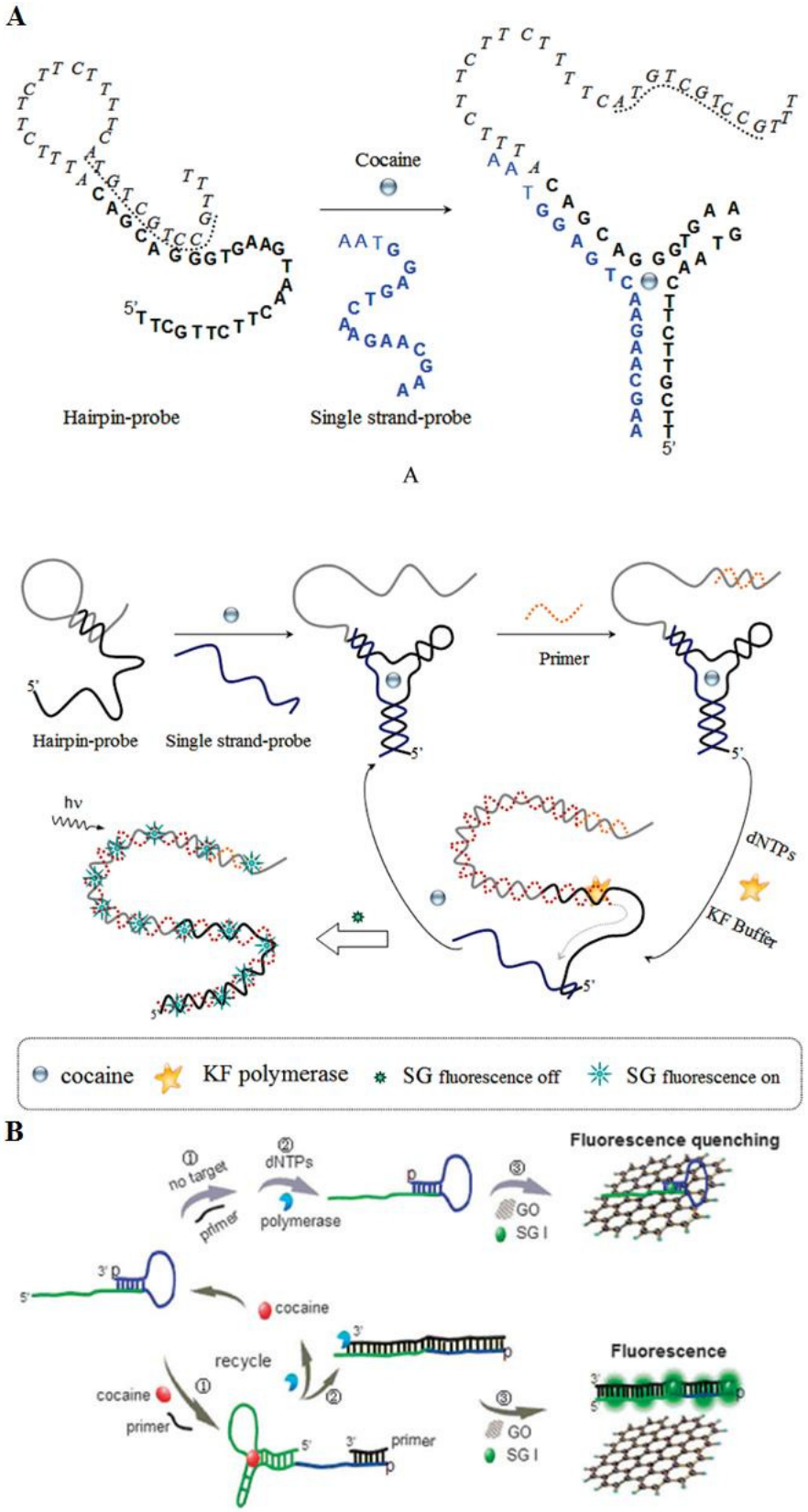

Fig. 3

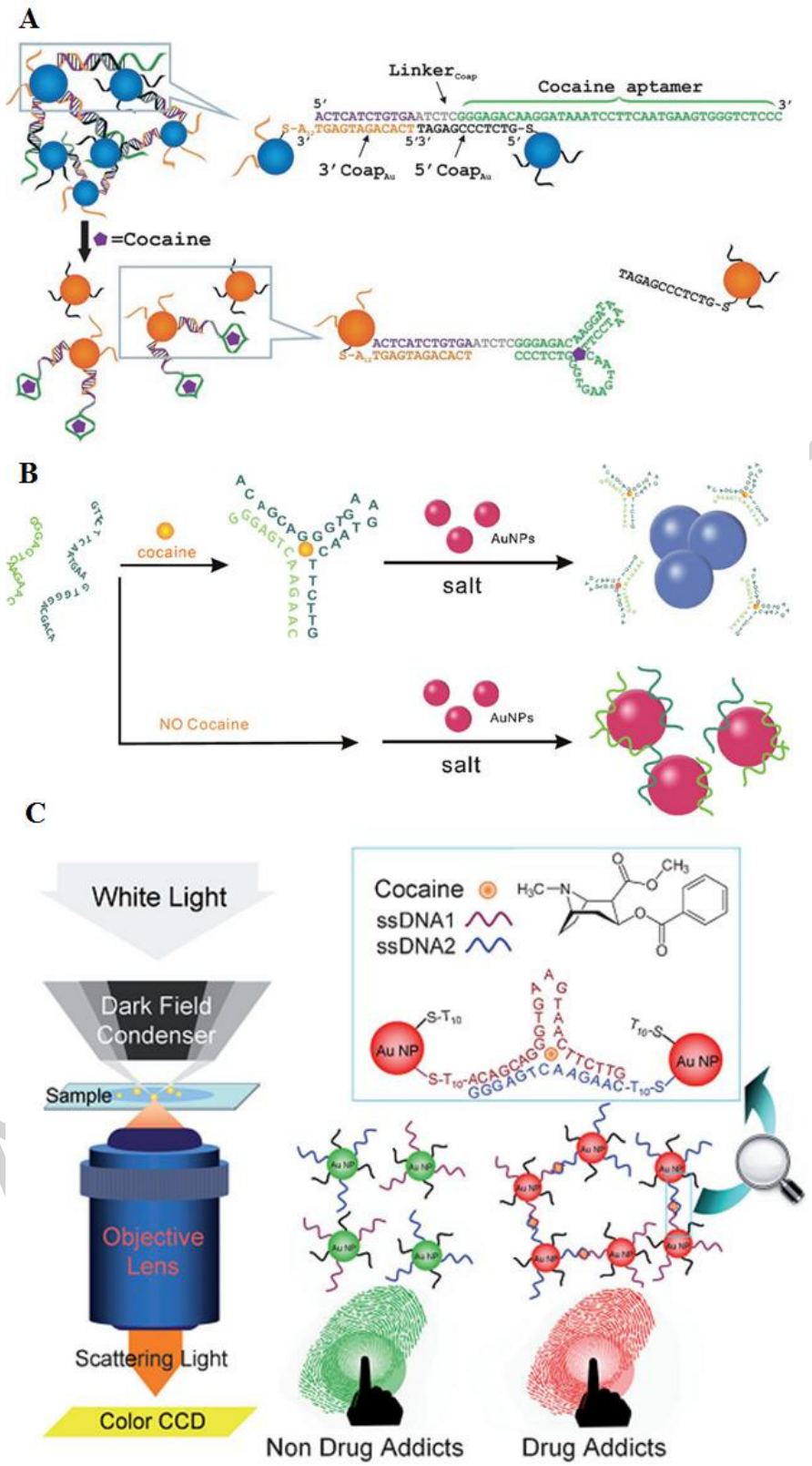

Fig. 7

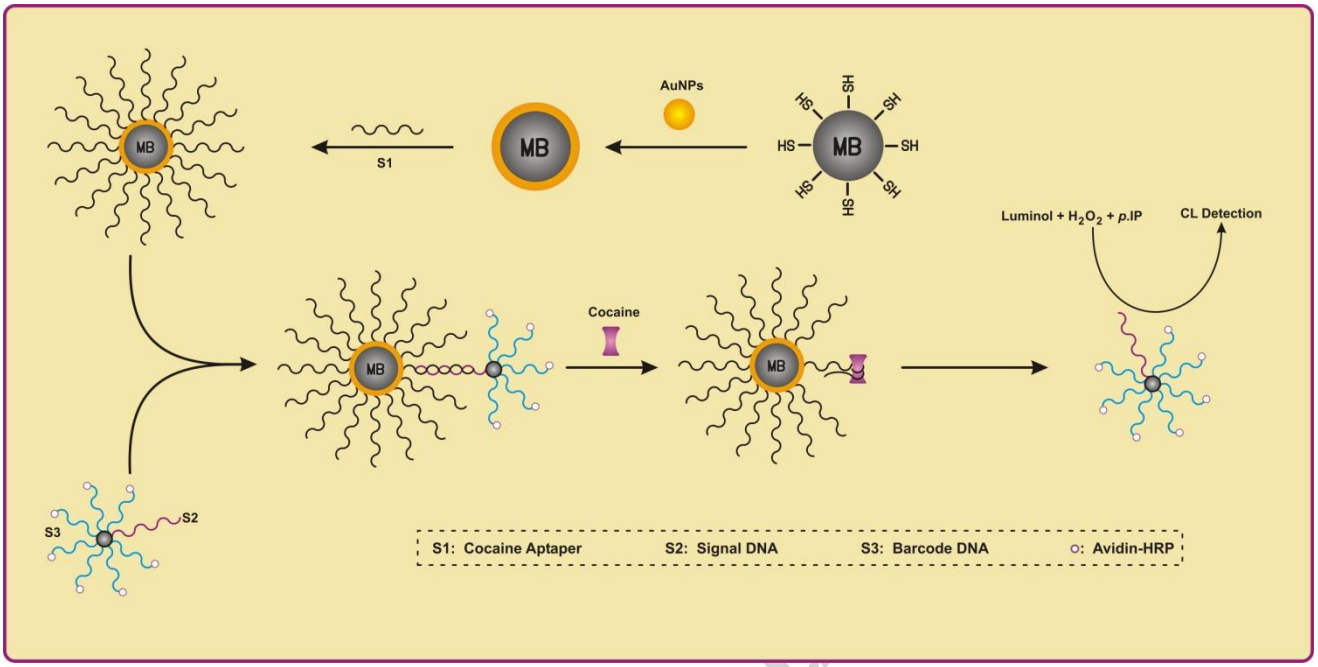

Fig. 5

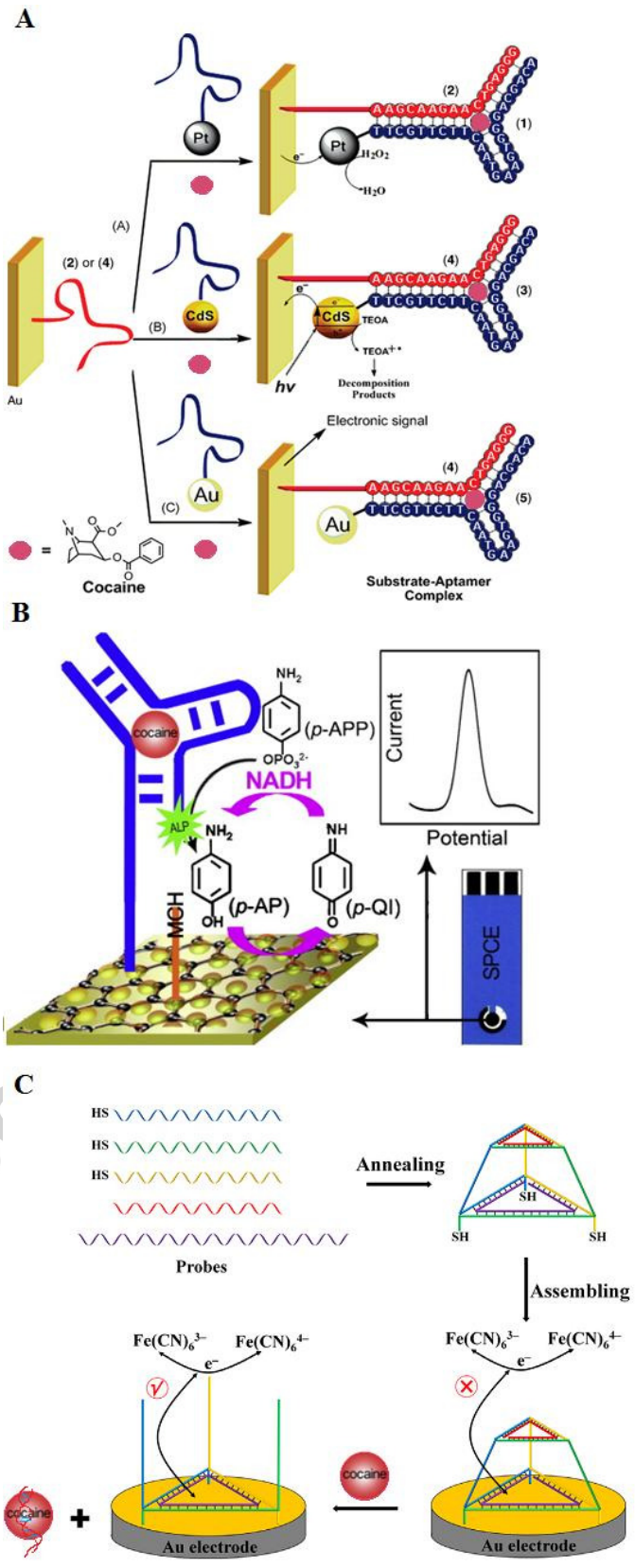

Fig. 6