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A versatile biosensing platform coupling CRISPR–Cas12a and aptamers for detection of diverse analytes

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Abstract

Rapid and sensitive detection of various analytes is in high demand. Apart from its application in genome editing, CRISPR–Cas also shows promises in nucleic acid detection applications. To further exploit the potential of CRISPR–Cas for detection of diverse analytes, we present a versatile biosensing platform that couples the excellent affinity of aptamers for broad-range analytes with the collateral single-strand DNA cleavage activity of CRISPR–Cas12a. We demonstrated that the biosensors developed by this platform can be used to detect protein and small molecule in human serum with a complicated background, i.e., the tumor marker alpha fetoprotein and cocaine with the detection limits of 0.07 fM and 0.34 μM, respectively, highlighting the advantages of simplicity, sensitivity, short detection time, and low cost compared with the state-of-the-art biosensing approaches. Altogether, this biosensing platform with plug-and-play design show great potential in the detection of diverse analytes.

Keywords:

CRISPR–Cas12a, aptamer, biosensing platform, diverse analyte, alpha fetoprotein detection, cocaine detection

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1. Introduction

The development of a biosensing platform that is simple, efficient, reliable, and inexpensive is of paramount importance for bioanalytical applications in diverse areas, including (but not limited to) scientific research, environmental monitoring, food safety, and clinical diagnosis [1, 2]. Because the currently available biosensors do not always meet the increased detection requirements [3, 4], new biological tools must be broadly deployed to develop a general biosensing platform, and this endeavor should accelerate the construction of diverse biosensors.

Prokaryotic CRISPR–Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) immune systems have recently facilitated enormous advances in biotechnological tools [5]. One of the most well-known applications is genome editing, which relies on the ability of RNA-guided Cas proteins to target and cleave specific nucleic acid sequences [6, 7]. In addition to genetic manipulation [8-11], the collateral single-stranded DNA (ssDNA) cleavage (also known as 'trans cleavage') activity of some Cas proteins has allowed the development of cost-effective, portable nucleic acid diagnostic tools [12-15]. For example, these CRISPR–Cas biosensing systems have been used for the applications of pathogen/virus detection and genotyping, cancer mutation detection, and single-nucleotide polymorphism identification [16, 17]. By combining the ssDNA-cleavage ability of activated Cas12a protein and the allosteric effects of allosteric transcription factors, we recently developed the simple, fast,

high-throughput, and supersensitive CaT-SMelor© platform for the detection of small molecules [18], extending the detection scope of CRISPR–Cas-based biosensing systems. However, this Cas12a-based platform are still incapable of detecting other analytes, such as proteins.

To overcome this limitation and extend the detection scope of the powerful CRISPR–Cas system, we proposed to couple the collateral ssDNA cleavage activity of Cas12a to a type of recognition element that can bind a broad range of target analytes. Aptamers are short synthetic single-stranded nucleic acid sequences that can bind to a broad range of targets, including metal ions, chemical compounds, proteins, cells, and whole microorganisms [19, 20], with dissociation constants (K_D) usually ranging from pico- to nanomolar [21, 22]. As a distinct class of recognition elements, aptamers show several advantages, including a prolonged shelf life, low batch-to-batch variation, low/no immunogenicity, and the flexibility to incorporate chemical modifications for enhanced stability and better targeting affinity [20]. Aptamers for a particular analyte are routinely developed *in vitro* with a very defined iterative procedure known as the 'systematic evolution of ligands by exponential enrichment' (SELEX) [23, 24]. Therefore, aptamers meet the criterion of potentially recognizing an unlimited range of analytes [19, 20], and we tend to couple the collateral ssDNA cleavage activity of Cas12a protein and the excellent affinity of aptamers to develop a biosensing platform for the detection of a broad range of analytes.

A clinic tumor marker alpha-fetoprotein (AFP) and a recreational drug cocaine were chosen as analytes for the construction of novel biosensor using the newly developed biosensing platform. AFP is a 70-kDa oncofetal glycoprotein which is gradually recognized biomarker for hepatocellular carcinoma and germ cell tumors [25]. The blood AFP is barely detectable in the healthy adults. Statistically, the AFP concentration in the serum is less than 25 ng/mL (357 pmol/L) in a healthy individual [26]. The reliable detection of AFP is significant for the early clinical diagnosis and the long-term treatment of cancer patients. Cocaine, also known as benzoylmethylecgonine, is a strong stimulant used mostly as an illegal recreational drug [27]. Drug abuse is a serious global public health problem and a contributing factor to severe social problems. To control drug abuse problem and curtail its use, a reliable cocaine test is highly sought after for illegal drug screening by law enforcement and employment background check.

In this study, we combined the collateral cleavage activity of Cas12a with the excellent affinity of aptamers to generate the biosensing platform to detect a broad range of analytes. As a proof of concept, novel biosensors of AFP and cocaine were constructed by the platform. The linear detection ranges of the AFP and cocaine biosensors were 0.24–977 fM and 0.5–15 μM, respectively, fulfilling the requirement for real-life applications. Moreover, when benchmarked against the state-of-the-art ones, our biosensors showed the promising characteristics of robustness, plug-and-play, modularity, and high sensitivity in testing authentic samples. Overall, the developed biosensing platform not only extends the detection scope of CRISPR–

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Cas, but also shows great potential utility in biosensing diverse analytes. It presents a new strategy for developing biosensors.

2. Materials and methods

2.1 Materials and reagents

Aptamers and primers were chemically synthesized by Beijing Tsingke Biotech Co. Ltd (Beijing, China) (Table S1). Beaver Beads™ Streptavidin (1 μm in average diameter) was purchased from Beaver (Suzhou, China). Alpha fetoprotein (AFP) and immunoglobulin E (IgE) were purchased from Shanghai Linc-Bio Science Co., Ltd (Shanghai, China). Immunoglobulin G (IgG), bovine serum albumin (BSA), human serum albumin (HSA), human serum, cocaine hydrochloride, benzoylecgonine, methyl ecgonine, adenosine, uridine, and other chemicals for buffers and solvents were purchased from Sigma-Aldrich, Inc. (Shanghai, China). TB Green™ Premix Ex Taq™ and UltraPure DNase/RNase-free Distilled Water were purchased from Takara Biotechnology Co. Ltd (Dalian, China). HiScribe T7 Quick High Yield RNA Synthesis Kit, Standard Taq Buffer and RNase inhibitor were purchased from NEW ENGLAND BioLabs Inc. (Ipswich, UK). RNA Clean & Concentrator™-5 was purchased from Zymo Research (Irvine, CA). All chemicals and reagents used were of analytical grade and prepared with deionized water (18 MΩ cm) from Milli-Q® Water Purification System (Millipore, Billerica, MA, USA).

2.2 Cas12a protein expression and purification

Cas12a (LbCas12a) from Lachnospiraceae bacterium ND2006 was expressed and

purified as our previous study [18, 28, 29]. Briefly, *Escherichia coli* BL21(DE3) was transformed with the pET28TEV-LbCas12a expression plasmid and grown overnight in Luria–Bertani (LB) medium at 37 °C with shaking at 150 rpm until the exponential growth phase. Protein expression was then induced with 100 μM isopropyl-β-D-thiogalactoside (IPTG) and the cells cultured at 16 °C for 12 h. The cells were harvested by centrifugation at 5,000 × g. For protein purification, the cell pellets were resuspended in lysis buffer (50 mM Tris-HCl [pH 7.4], 200 mM NaCl, 2 mM dithiothreitol, 5% glycerol) supplemented with EDTA-free protease inhibitors (Sigma-Aldrich, Roche Diagnostics), and then lysed with ultrasonication. The lysate was loaded onto a HisTrap FF column (GE Healthcare) and washed with a gradient of imidazole concentrations. The peak fractions were collected and desalted with dialysis. The solution was then loaded onto a HiTrap Q HP column (GE Healthcare), and the peak fractions were collected, pooled, and concentrated. The concentrated solution was loaded onto a HiLoad 16/600 Superdex 200 pg column for fast protein liquid chromatography (AKTA Explorer 100; GE Healthcare). The gel filtration fractions were analyzed with SDS-PAGE and the protein concentrations were determined with the Bradford method. The purified product was dissolved in storage buffer (20 mM Tris-HCl [pH 7.5], 1 M NaCl, 50% glycerol) and stored at −80 °C until use.

2.3 Aptamer–dsDNA and crRNA preparation

The oligonucleotides used in this work are listed in Table S1. The aptamer–dsDNA (AFP aptamer–dsDNA or cocaine aptamer–dsDNA) was prepared by mixing the

synthesized oligonucleotides aptamer-F (AFP aptamer-F or cocaine aptamer-F), dsDNA-F and dsDNA-R in 1 × PBS Buffer with a final ratio of 1:1:1. The DNA solution was then incubated at 95 °C for 5 min and gradually cooled (2 °C/min) to room temperature. The final concentration of aptamer–dsDNA was 5 μM.

To prepare the template for crRNA synthesis, two paired oligonucleotides containing a T7 priming site (Table S1 online) were synthesized and annealed in 1 × *Taq* DNA Polymerase PCR Buffer (Thermo Fisher Scientific). The crRNA was then transcribed with the HiScribe™ T7 Quick High Yield RNA Synthesis Kit and purified with RNA Clean & Concentrator™-5. The resulting crRNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Throughout all the experiments, RNase-free materials and conditions were used.

2.4 Biosensor configuration and sensing.

The biosensor consists of three parts: streptavidin-labeled MBs, biotin–ssDNA and aptamer–dsDNA. First, 20 μL of 10 mg/mL MBs was washed with 1 × PBS containing Tween 20 (PBST) Buffer to remove the residual NaN_3 protection solution. Then, 20 μL of 15 μmol/L biotin–ssDNA in Millipore water was added to the MB solution and mixed well for 30 min at room temperature. The MBs were then washed twice with buffer to remove excess biotin–ssDNA. The aptamer–dsDNA (5 μmol/L, 60 μL) was added to the solution and mixed well at room temperature for 20 min. After the sample was washed three times with buffers to remove excess aptamer–dsDNA, the MBs–ssDNA–aptamer–dsDNA solution was dispersed in 500 μL of 1 × PBST buffer. The MBs contained in each 20 μL aliquot of this solution were used in

this work.

To assess the feasibility of the platform, different concentrations of analyte (AFP or cocaine, 2 μL) were incubated with the biosensor at room temperature for 20 min. Each solution was then separated with a magnetic rack, and 10 μL of the supernatant was transferred into a new centrifuge tube for quantification with qPCR or the Cas12a-based FQ-labeled reporter system.

To optimize the design of the biotin–ssDNA, biotin–ssDNAs were designed to optimize the complementary site on the aptamer (six biotin–ssDNAs for the AFP aptamer and four for the cocaine aptamer) by considering GC content and annealing temperature. Biosensors with different complementary sites were configured. Then 2 μL of analyte (AFP or cocaine) was incubated with the sensor at room temperature for 20 min. The resulting solutions were analyzed with qPCR. To optimize the concentration of aptamer–dsDNA, 2.5, 5, 10, 20, 40, or 60 μL of 5 μmol aptamer–dsDNA was bound to the MBs, as described above. The sensing system was then incubated with or without the analyte for 20 min. The aptamer–dsDNA concentration in the supernatant was detected with qPCR. To optimize the incubation time, the kinetics of aptamer–dsDNA release after the addition of the AFP were examined. After the biosensor was mixed with AFP samples, the supernatants were removed at different time intervals and quantified with the Cas12a-based FQ-labeled reporter system.

2.5 Quantification of aptamer–dsDNA with qPCR

Using the dsDNA as the target sequence, primers were designed using with the

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Primer-BLAST software. RT-qPCR was performed as previously described [30, 31] with 1 × TB Green™ Premix Ex Taq™ (TaKaRa), 200 nmol of primer pair, and 2 μL of diluted supernatant in a 20 μL reaction volume on the LightCycler® 480 System (Roche Diagnostics).

2.6 Collateral cleavage and florescence detection

In the CRISPR–Cas12a cutting system, NEB CutSmart® Buffer was used as the reaction buffer. For the collateral cleavage assay, an equimolar ratio of Cas12a and crRNA was premixed with the FQ-labeled reporter in NEB CutSmart® Buffer and then distributed in a 384-well plate for the subsequent experiment. The diluted supernatant containing the aptamer-dsDNA was added to the reporter system, and the florescent signal was detected every 1.5 min with an EnSpire™ Multimode Plate Reader (PerkinElmer, Inc.,USA) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm.

2.7 Analysis of AFP in human serum

To demonstrate the analytical reliability and practical application of the biosensing platform, we performed recovery experiment. First, human serum was diluted 100 times with PBST buffer and stored at −20 °C for the AFP detection assay. Human serum samples were then spiked with different concentrations of AFP. Each diluted sample (2 μL) was added to 20 μL of the biosensor system and incubated for 10 min. To construct the calibration curve, the AFP samples were diluted by gradient, and the dsDNA in the supernatant was detected with the Cas12a-based FQ-labeled reporter system. The changes in the fluorescence intensity with time were measured, and the

slope of the fluorescence curve in the linear region between 0 and 8 min (normally) represented the ssDNA trans-cleavage rate of CRISPR–Cas12a. The linear relationship between the trans-cleavage rate (or slope) and the AFP standard concentration was obtained.

To confirm the clinical applicability of the biosensing platform, 20 clinical human serum samples were analyzed with the Elecsys AFP II kit (Roche Company, Switzerland) on an Elecsys 2010 electrochemiluminescence instrument (Roche Company). The samples were then used for a the Cas12a-based platform analysis. Before measurement, the blood samples were diluted approximately 10,000-fold until the concentration of AFP was within the linear range.

2.8 Analysis of cocaine in human serum

Human serum was diluted 10-fold with PBST buffer and used as the standard serum samples. Serum samples with different cocaine concentrations were then prepared by spiking the sera with exogenous cocaine. The samples were analyzed with both HPLC and the cocaine biosensor. Cocaine was detected with a C18 Hypersil (160 mm × 5.0 mm, 2 mL/min) column with H_2O as the eluent and detected at 230 nm with a UV detector (Agilent 1260 Infinity II LC System). The procedure for cocaine analysis with the biosensor was the same as that used for the AFP biosensor.

2.9 Data analysis

All experiments and assays were repeated at least three times. The data in all figures are expressed as means ± SD. Microsoft Excel 2016, Origin 8, and GraphPad Prism version 6.0 were used for the data analysis.

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3. Results

3.1 Design of the biosensing platform

The principle of the biosensing platform is shown in Figure 1. Briefly, an aptamer–double-stranded DNA (dsDNA) complex is constructed by fusing an aptamer and a specific dsDNA containing a Cas12a protospacer adjacent motif (PAM) sequence. The partial aptamer region of this aptamer–dsDNA complex complements to a biotin-labeled ssDNA (biotin–ssDNA), which was already fixed to the streptavidin-coated magnetic beads (MBs). Therefore, the entire biotin–ssDNA–aptamer–dsDNA complex is anchored to the MBs. When the target analyte is presented, it interacts with the aptamer and releases the aptamer–dsDNA complex from the MBs, owing to the stronger binding affinity of the aptamer towards its target analyte rather than to its complementary sequence. After the magnetic separation, the target analyte bound aptamer–dsDNA cannot be moved away, and then interacts with the added Cas12a–crRNA complex to activate the nonspecific ssDNA collateral cleavage activity of Cas12a (activated Cas12a). Activated Cas12a cleaves the fluorophore quencher (FQ)-labeled ssDNA probe (reporter) to amplify and output the input signal, allowing the target analyte to be quantified by measuring the fluorescent signal.

3.2 Validation of the signal transduction process

After the design, we validated its feasibility of the biosensing platform. To evaluate the influence of fusing an aptamer to dsDNA on the activation of the nonspecific

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collateral cleavage activity of Cas12a, we recombinantly expressed and purified the Cas12a protein (Fig. S1 online) and assembled an AFP aptamer (AP273 [32]) with a 59-bp dsDNA (Fig. S2 online). We then tested the collateral ssDNA cleavage activity of Cas12a in response to this aptamer–dsDNA complex *in vitro*. When plotting the recorded fluorescence intensity against the different concentrations of dsDNA with and without aptamers, we found that the fluorescence intensity dose-dependently increased as the dsDNA concentration increased, either with or without the AFP aptamer (Fig. 2a and Fig. S3a online). Consistent with the Michaelis–Menten plot of the collateral ssDNA cleavage activity of Cas12a activated by dsDNA [12], the slopes of the fluorescence intensity (cleavage rate, Fig. S3b and c online) showed a linear relationship with the concentration of both types of dsDNA at 0–8 min ($R^2 > 0.99$; Fig. S3d and e online). Furthermore, by comparing the triggered cleavage rates between dsDNA with and without AFP aptamer, we concluded that the modification of the dsDNA (linked with an aptamer) did not significantly affect the activation of the nonspecific collateral cleavage activity of Cas12a (Fig. 2b).

Next, we bound the aptamer–dsDNA to the biotin–ssDNA anchored on MBs via Watson–Crick base pairing (Fig. S2b online), and tested whether AFP interacted with the aptamer to trigger the release of the aptamer–dsDNA. We added different concentrations of AFP, and removed the unreleased aptamer–dsDNA with magnetic separation. We then determined the free aptamer–dsDNA with real-time quantitative PCR (qPCR), and observed that the concentration of released aptamer–dsDNA was linearly associated with the logarithm of the AFP concentration in the range 78–

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2,500 fM (R^2 > 0.99; Fig. 2c). Finally, we tried to couple the Cas12a-based FQ-labeled reporter system to output the detected AFP signal. With the increasing concentration of AFP, we obtained the increasing fluorescent signal as well in a time-course (Fig. 2d and Fig. S4 online). These results demonstrated the promises of the biosensing platform.

3.3 Optimization of the biosensing platform

To streamline the workflow for development of biosensors using this biosensing platform, we further optimized the biosensing platform by taking the AFP aptamer as example. To determine the optimal biotin–ssDNA sequence for capturing the aptamer–dsDNA complex, we designed and tested six ssDNAs paired with different regions of the AFP aptamer, with a G+C content of approximately 55% and an annealing temperature of 40 °C (11–13 bp) (Fig. S5 and Table S2 online). After the aptamer–dsDNA complex was on the biotin–ssDNA coated MBs and then incubated with excess AFP, we determined the signal-to-noise ratio (S/N) of the released aptamer–dsDNA by qPCR. Compared with the other biotin–ssDNAs, com3 gave the highest S/N ratio (Fig. 3a). We then chose 100 nM of biotin labeled com3 to attach aptamer–dsDNA based on the theoretically calculated binding capacity of the streptavidin-coated MBs. Next, we determined the optimal concentration of aptamer–dsDNA, and found that exceeding 100 nM did not increase the S/N ratio any more (Fig. 3b), thus we adopted 100 nM of aptamer–dsDNA to configure the AFP biosensor. We also determined the optimal reaction time for the incubation with AFP, and observed that the release of aptamer–dsDNA plateaued after approximately 8 min

(Fig. 3c). Therefore, we selected an incubation time of 10 min to ensure that the reaction reached equilibrium. Under these optimized conditions, AFP was detected by fluorescence readout (Fig. S6a and b online). We found the direct correlation between the slope of the fluorescence intensity (i.e., cleavage rate) and the AFP concentration ($R^2 > 0.99$) with the limits of detection (LOD) and linear detection range were 0.07 fM and 0.24–977 fM, respectively (Fig. 3d). Blood AFP is barely detectable in the healthy adults (< 25 ng/mL, 357 pmol/L) [26], but the sensitivity of the exemplary AFP biosensor developed by this platform fulfilled the requirements for practical applications. Moreover, the overall operation time of biosensors constructed by the platform is < 20 min (Fig. 3e).

3.4 Performance of the optimized AFP biosensor

To evaluate the performance of the optimized AFP biosensor, we first tested its specificity. In contrast to the effect of AFP, there was a nearly negligible luminescence response in the presence of different analogues, even at 10-fold higher concentrations (Fig. 4a, Fig. S7a and b online). To investigate the validity of the AFP biosensor in detecting authentic samples, we spiked human serum with trace amounts of AFP and characterized the accuracy, precision, and recovery of its detection (Fig. 4b, Fig. S8a and b online). The impressive precision (1.41%–5.77%) ensures that the biosensors developed by this platform are robust and stable for practical applications. The good recovery (99.60%–105.02%) of the biosensor indicates its excellent utility for quantitative analyses. We also benchmarked the performance of the optimized AFP biosensor against the state-of-the-art biosensors constructed by other approaches.

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The results indicate that our biosensors showed the best sensitivity ever reported (Table 1). Finally, we compared the output data of the AFP biosensor with the detection data of human serum in clinical diagnoses. These results further demonstrated the promising robustness and sensitivity of the AFP biosensor in real-life analyses (Fig. 4c). Impressively, the overall detection time is only 18 min for our developed AFP biosensor in real-life application (Fig. 4d).

Table 1 Benchmarking the AFP biosensor against other methods for AFP detection.

Method	LOD	AOM (Y/N)	TC	Cost[1]	Ref.
Fluorescence aptasensor	0.16 ng mL^{-1}	Y	50 min	NA	S Xu et.al. [33]
Resonance light scattering aptasensor	0.94 ng mL^{-1}	N	>30 min	NA	F Chenet.al. [34]
Surface-enhanced Raman spectroscopy	50 pg mL^{-1}	N	130 min	NA	Q Wanget.al. [35]
Electrochemical aptasensor	3.1 fg mL^{-1}	N	30 min	NA	M Cui et.al. [36]
Enzyme-linked immunosorbent assay	6 pg mL^{-1}	Y	>200 min	>10	Sigma-Aldrich Co. LLC.
Electrical chemiluminescent immunoassay (Clinical method)	0.61 ng mL^{-1}	N	18 min	>1.3	Roche Diagnostics GmbH

Cas12a-based biosensing platform	0.07 fM ($4.9\ ag\ mL^{-1}$)	Y	18 min	<0.35	This study

Note: Six representative biosensing approaches for AFP detection, as well as the commercial kits, were selected and compared with the developed AFP biosensor. Here we chose those approaches with the lowest detection limits reported in the last five years. LOD, limit of detection; AOM, availability of microplate; TC, time required (min/reaction);[1]$/reaction.

3.5 Plug-and-play modularity of the biosensing platform

Many aptamers have been identified to respond to diverse molecules [37]. To demonstrate the broader applicability of the biosensing platform, another aptamer, MNS-4.1, with the equilibrium dissociation constant (K_D) of 0.4 μM for the recreational drug cocaine, was chosen to develop a cocaine biosensor. The plug-and-play workflow of the biosensor construction was shown as Figure 5a, and we just need is replacement of the AFP aptamer to cocaine aptamer and optimization the biotin–ssDNA for linking the aptamer–dsDNA and streptavidin-coated MBs. Therefore, we optimized the biotin–ssDNA (Fig. S9a and b online), and configured the novel cocaine biosensor (Fig. 5a). We characterized the linear detection range as 0.47–15,000 μmol/L, with an estimated LOD of 0.34 μmol/L (Fig. 5b and Fig. S11 online). We also tested the specificity of this cocaine biosensor. Like the AFP biosensor, the cocaine biosensor showed a high selectivity (Fig. S10 online), again indicating the stability of the biosensing platform. We also tested the performance of our developed cocaine biosensor for the detection of human serum and urine with

spiked cocaine. Both results for the biosensor agreed well with a high-performance liquid chromatography (HPLC) analysis (Fig. 5c and Fig. S11 online). These results demonstrate that this biosensing platform can be used extensively to develop diverse biosensors by coupling different aptamers.

4. Discussion and conclusion

Development of a novel biosensing platform for the convenient detection of diverse analytes will infuse the detection field with new energy. Since CRISPR–Cas-based detection systems offer remarkable advantages in the detection of nucleic acids, including its sensitivity, accuracy, speed, etc [12-15], we considered that the CRISPR–Cas-mediated nucleic-acid detection system could be regarded as an excellent general signal output module. If coupling it to an appropriate signal recognition and signal transduction module, its detection scopes might be extended beyond nucleic acids. For example, we recently expended the detection scopes of Cas12a by developing an *in vitro* biosensing platform CaT-SMelor to sensitively quantify small molecule [18]. However, this platform still unable to detect other analytes such as proteins. For further expending its detection scopes of Cas12a, in the present work, we developed a novel biosensing platform by coupling the collateral ssDNA cleavage activity of Cas12a with the excellent affinity of aptamers for a broad range of target analytes. In theory, this biosensing platform should allow the detection of a broader range of analytes. As proof of concept, we demonstrated the construction of two biosensors for detection of different kinds of analytes, i.e., tumor marker AFP

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and a recreational drug cocaine (Figs. 4 and 5). As a mother platform, it showed great potential utility for delivering many potential biosensors.

As we were completing this work, the functional DNA coupled with CRISPR–Cas12a for the detection of ATP, Na^+, Pb^{2+}, exosome and pathogenic bacterium has been reported [38-40]. We here emphasized that these work all used single strand DNA as activator to trigger the collateral ssDNA cleavage activity of Cas12a. Doudna et al. has confirmed that single strand DNA is less efficient than dsDNA to the collateral cleavage activity of Cas12a [12]. Unlike to these reports, dsDNA were used in our biosensing platform, ensuring the super-sensitivity of the constructed biosensors. Moreover, to our knowledge, no CRISPR–Cas-based system has been used to detect a protein analyte until now. Our biosensing platform showed excellent performance in real-life human serum AFP detection when compared with state-of-the-art biosensing approaches (Table 1), demonstrating its great potential in clinical diagnosis. Using this AFP biosensor, as little as 1 μL of blood and as short as 18 min are sufficient for the real-life analysis (Fig. 4c and d). The sensitivity, robustness and rapidity of the developed biosensor should ascribe to the ingenious configuration of the biosensing platform, which has many advantages over other approaches, including its convenient operation, rapid signal transformation and amplification, and applicability to 96-well and 384-well workstations for high-throughput assays. Moreover, by a carefully cost accounting, either developing biosensors by this platform or applying the generated biosensors for real-life detection is much cheaper than the use of other approaches (Table 1 and Table S3 online). The

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biosensing platform therefore is extremely affordable.

One of the significant advantages of the biosensing platform is its high modularity with a plug-and-play operation. This characteristic allows the rapid development of diverse biosensors by coupling the cognate aptamers. Here, we employed a cocaine aptamer to replace the AFP aptamer, and easily constructed a novel cocaine biosensor (Fig. 5). The cocaine aptamer in our construction was the same as that used in the study by Xiang et. al [32], who coupled this cocaine aptamer to an electrochemical detector (personal glucose meter). In comparison, the LOD of our cocaine biosensor was more than one order of magnitude lower than the LOD achieved in that study, further demonstrating the excellent performance. In addition, we have well-characterized the robust performance of both the AFP and cocaine biosensors in human serum, which not only contains large amounts of other proteins [41], but also has a very complex chemical composition [42]. The good specificity, accuracy, and stability of the AFP biosensor highlight the reliability of this platform for the development of other biosensors. Given that aptamers have high affinity and specificity towards a wide range of target molecules [19], and can be retrieved from the existing literature or routinely enriched from a large random nucleic acid pool by the process of SELEX [37, 43], many biosensors could be constructed using this platform to meet the various detection requirements. Also, we outlook that conjugating this platform with strip for visual and device-free detection may be feasible for convenient real-life application.

In summary, we have demonstrated a novel biosensing platform, in which CRISPR–Cas12a-based nucleic acid detection is linked with an aptamer to achieve the simple, sensitive, low-cost, and plug-and-play detection of many target analytes other than nucleic acids, such as AFP and cocaine in the present work. This platform therefore offers a novel route to develop biosensors for the *in vitro* detection of diverse analytes. The modular design of this platform, in particular, accelerates the process of biosensor development. This work is a starting point for more general and promising applications using the biosensing platform.

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Author contributions

Lixin Zhang, Gao-Yi Tan and Weishan Wang conceived and supervised the project. Xiangxiang Zhao, Shanshan Li and Guang Liu designed and performed the experiments. Xiangxiang Zhao and Weishan Wang analyzed the data. Zhong Wang performed the clinical sample test. Zhiheng Yang, Quanwei Zhang, Mindong Liang, Jiakun Liu, Zilong Li, Guoliang Zhu, Xinye Wang and Lan Jiang participated in this

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work. Weishan Wang, Xiangxiang Zhao, Lixin Zhang, Gao-Yi Tan and Yaojun Tong wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1 Schematic diagram of biosensing platform.

Fig. 2 Validation of the biosensing platform. (a) Plot of fluorescence intensity against the concentration of dsDNA linking with an AFP aptamer. (b) Readouts obtained for the dsDNA with and without AFP aptamer. Concentrations of dsDNA were 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 nmol/L. The relationship between dsDNA without aptamer and output (slope of the fluorescence intensity, i.e., cleavage rate) is shown in Fig. S3c and the relationship between dsDNA with aptamer and output is shown in Fig. S3e. (c) Released aptamer–dsDNA complex in response to AFP quantified with qPCR. (d) Readouts of fluorescent signal by the biosensing platform in response to AFP. The background signal (control) has already been subtracted from the values displayed on the graph. Data are the means and standard deviations (SD) of three independent replicates.

Fig. 3 Optimization of the biosensing platform using the AFP biosensor as example. (a) Optimizing the biotin-labeled ssDNA for linking aptamer–dsDNA and streptavidin-coated MBs. (b) Determination of the aptamer–dsDNA concentration used to construct the AFP biosensor. (c) Determination of the incubation time for the release of aptamer–dsDNA. Here 1.9 fM and 122 fM of AFP were used. (d) Linear

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relationship between the AFP concentration and the slope of the fluorescence intensity (i.e., cleavage rate). (e) Optimized flow chart of the sensing and detecting steps and the times required. For a–d, the background signal (control) has already been subtracted from the values displayed in the graph. Data are the means and SD of three independent replicates.

Fig. 4 Performance of the optimized AFP biosensor. (a) Cleavage rate readout of AFP biosensor in the presence of AFP (10 pmol/L), bovine serum albumin (BSA, 100 pmol/L), human serum albumin (HSA, 100 pmol/L), immunoglobulin E (IgE, 100 pmol/L), or immunoglobulin G (IgG, 100 pmol/L). (b) Performance of the biosensor in detecting AFP in human serum. (c) Gathering the readout data of our AFP biosensor and the clinic diagnosis results. 20 anonymized clinical samples were used. (d) Detection process with AFP biosensor. For a–c, the background signal (control) has already been subtracted from the values displayed on the graphs. Data are the means and SD of three independent replicates.

Fig. 5 Development of cocaine biosensor using the biosensing platform. (a) Plug-and-play workflow for developing diverse biosensors. Here, a cocaine aptamer was chosen as the example. (b) Linear relationship between output (i.e., cleavage rate) and cocaine concentrations. Plot of the fluorescence level and linear trend of fluorescence signal against the concentration of cocaine are shown in Figure S11a and b, respectively. (c) Gathering the detection results from our cocaine biosensor and HPLC. Human serum was spiked with cocaine at concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 μmol/L, respectively. For a–c, Data are the means and SD of three

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independent replicates.



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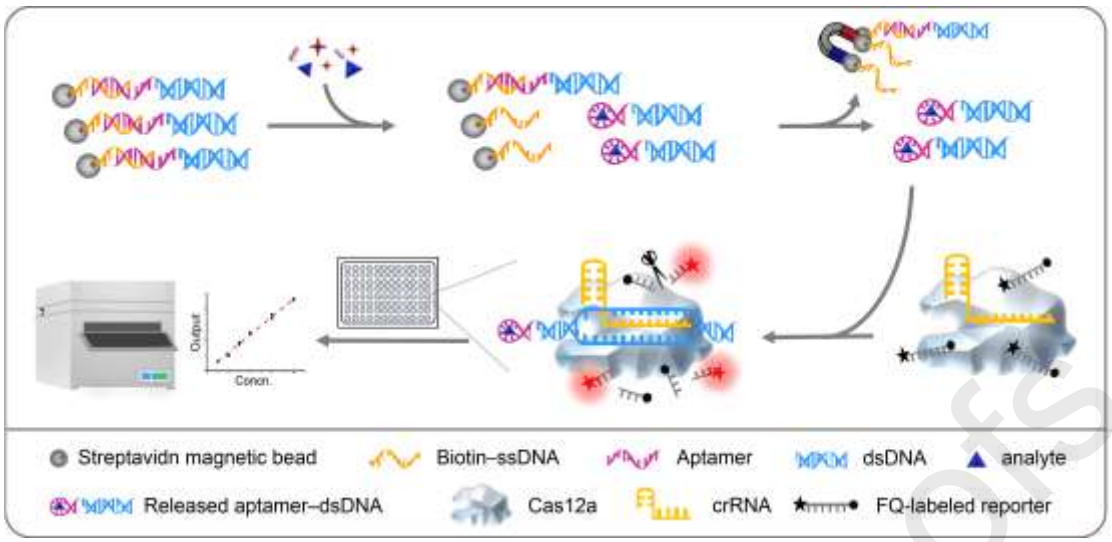
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pharmaceuticals and chemicals in streptomyces or photosynthetic bacteria.

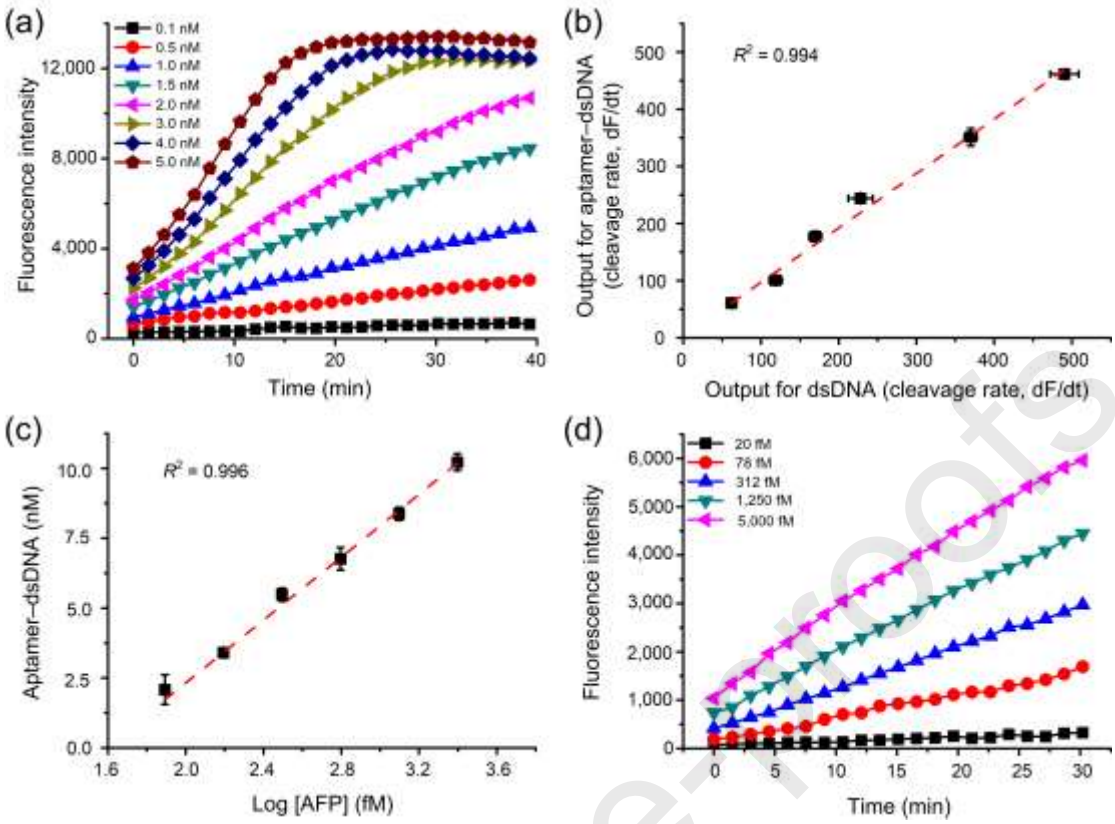


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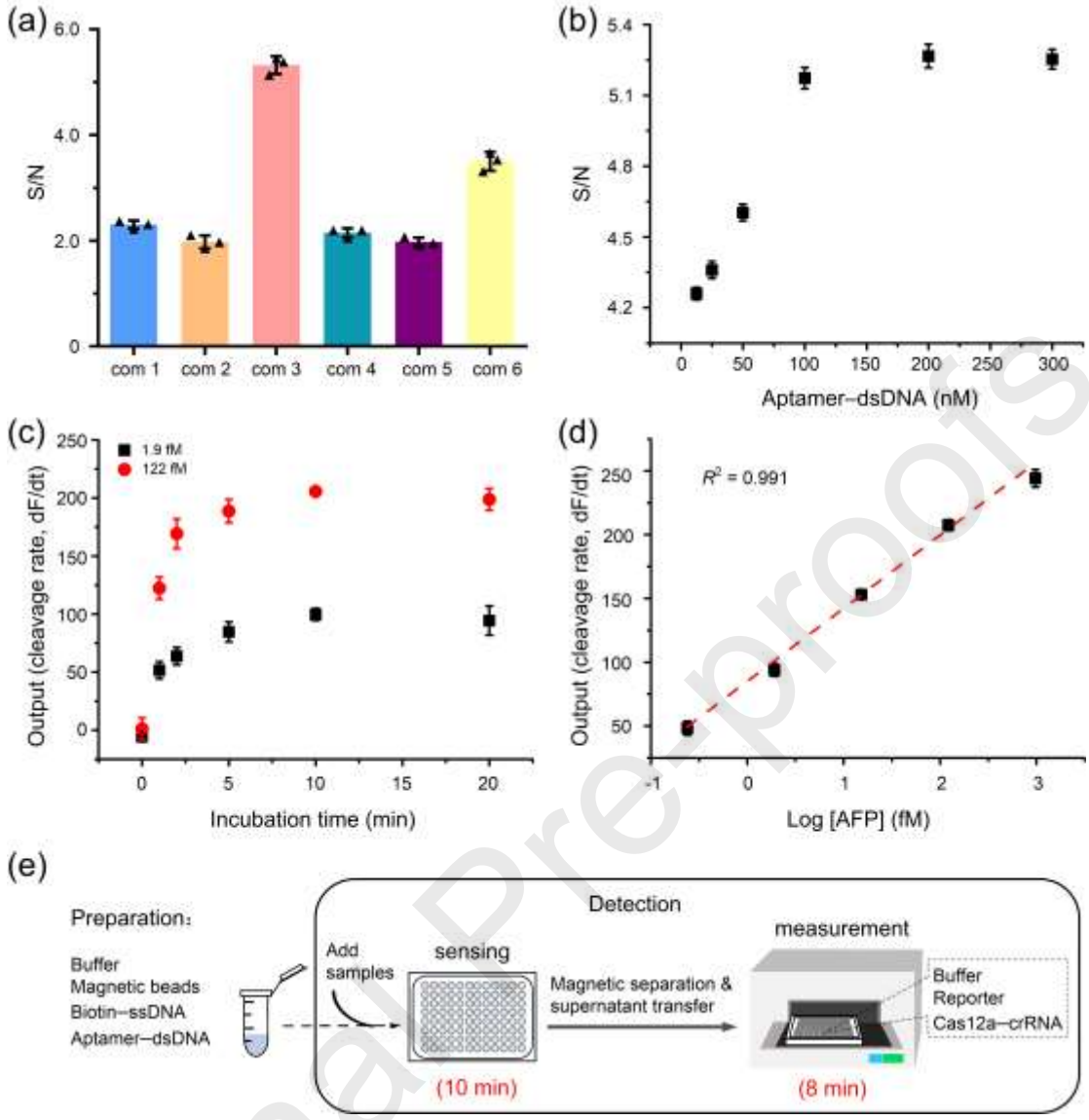
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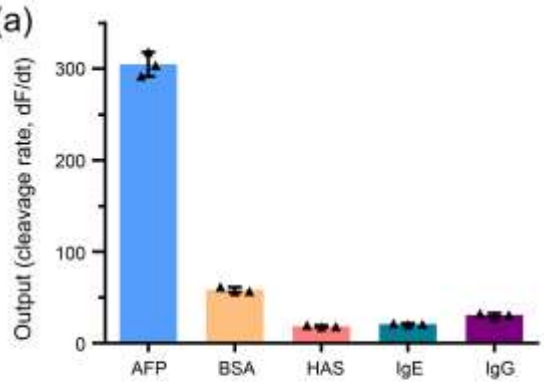


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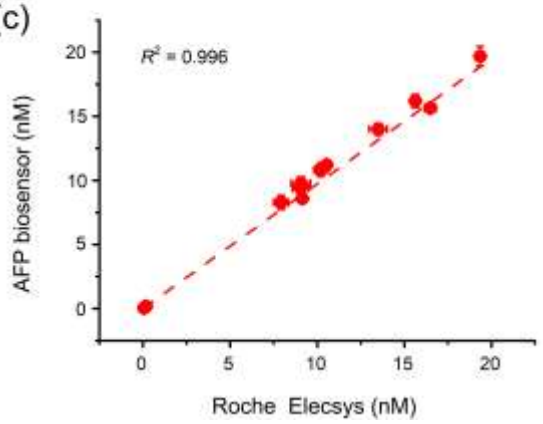
(a)



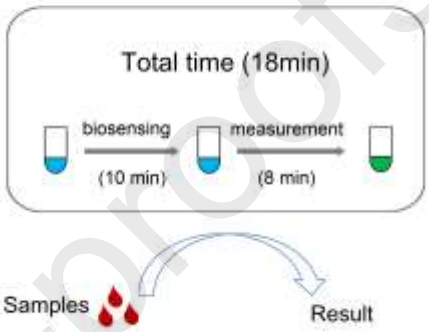
(b)

Sample	Spiked (ng/mL)	Detection (ng/mL)	Recovery (%)	RSD (%)
1	1	1.03	103.16	4.24
2	5	5.02	100.39	1.41
3	20	19.98	99.91	5.01
4	50	49.80	99.60	3.51
5	150	156.55	104.36	1.56
6	400	420.08	105.02	5.77

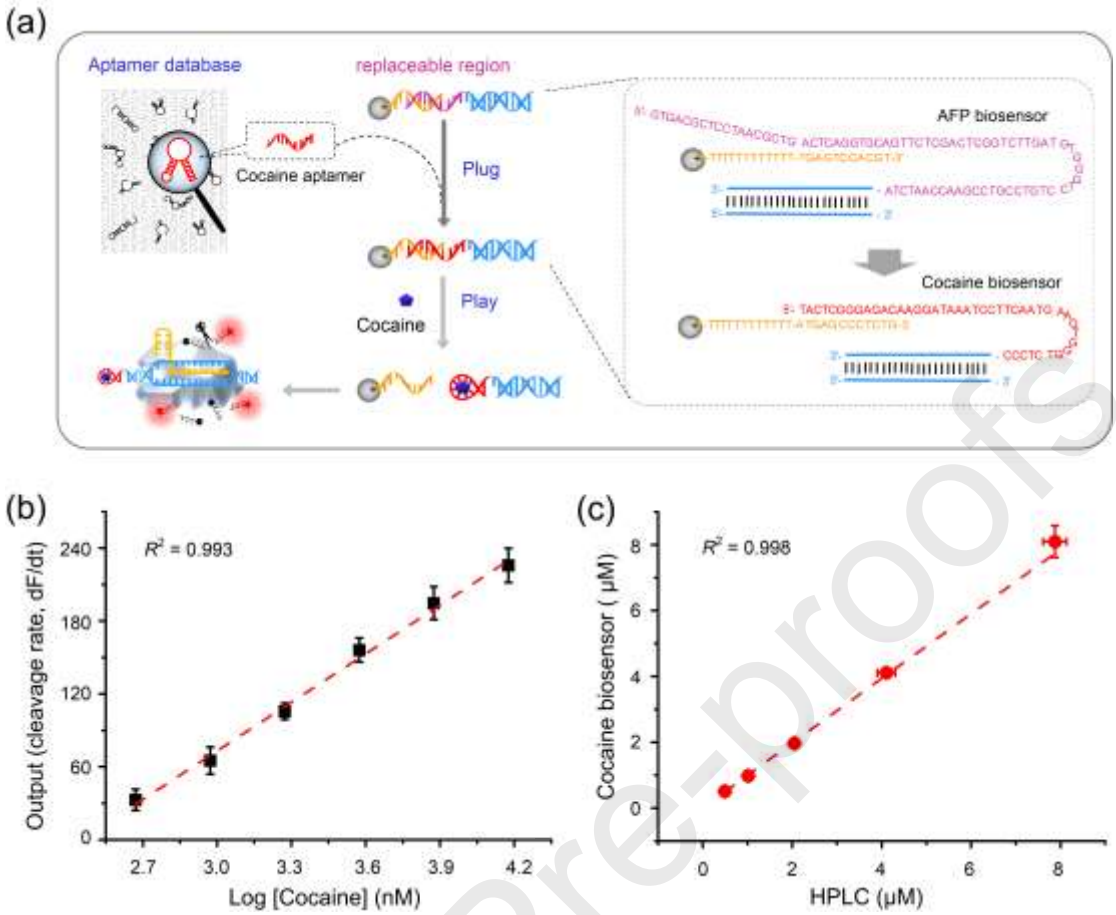
(c)



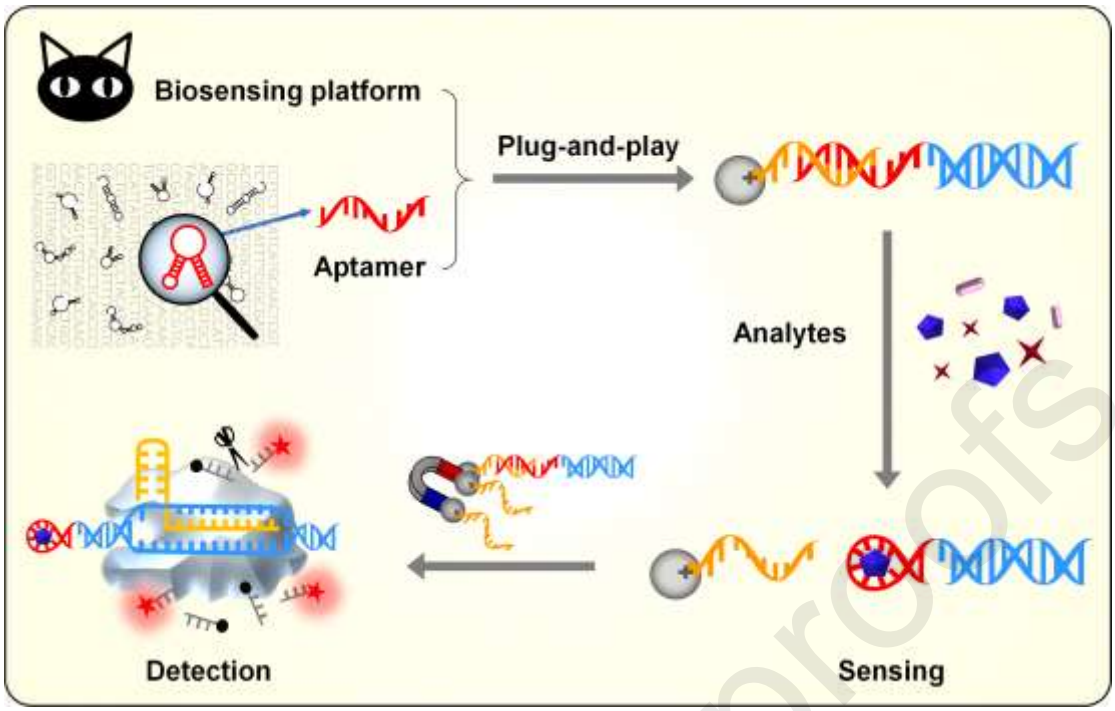
(d)



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