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CRISPR/Cas14 provides a promising platform in facile and versatile aptasensing with improved sensitivity

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ABSTRACT

CRISPR is reshaping biosensing technology due to its programmability, sensitivity, and specificity. Most current CRISPR-based biosensors are developed based on Cas12 and Cas13, while the biosensing potentials of the newly discovered Cas14 have not been fully elucidated yet. Herein, a fluorometric biosensor named HARRY (highly sensitive aptamer-regulated Cas14 R-loop for bioanalysis) was developed. The diblock ssDNA is designed to contain the activator sequence of Cas14 and the aptamer sequence of specific targets. In the absence of targets, the ssDNA activates Cas14a, then the Cas14a *trans*-cleavages the fluorescent reporter, causing fluorescence enhancement. In the presence of the targets, ssDNA-target assembly is formed *via* aptamer interaction, resulting in the inhibition of Cas14a activation. HARRY can detect ATP, Cd^{2+}, histamine, aflatoxin B1, and thrombin with detection limits at the low-nanomolar level, which shows improvement compared with Cas12a-based aptasensors in sensitivity and versatility. We reasoned that the improvement is derived from the ssDNA specificity of Cas14a and found that the detection limit of HARRY is correlated to the binding affinities of aptamers. This study unlocks the potential of Cas14a in versatile aptasensing, which may inspire the development of CRISPR-based biosensors from the Cas14a branch.

1. Introduction

CRISPR/Cas is a powerful tool for gene editing [1–4]. With the born of CRISPR-based diagnostics in 2017, the application field of CRISPR/-Cas technology has quickly extended to biosensing [5–8]. The Zhang group invented a Cas13a-enabled analytical platform for the sensitive detection of nucleic acids and named this platform SHERLOCK, short for Specific High Sensitivity Enzymatic Reporter UnLOCKing [9]. The Doudna group discovered that target binding could trigger the activation of Cas12a and developed a method for rapid and sensitive DNA testing [10]. With the integration of functional nucleic acids and isothermal amplification, CRISPR-based biosensors have been applied in the detection of versatile analytes, including viral and bacterial nucleic acids [11], metal ions [12], small organic molecules [13], proteins [14,15], and microorganisms [16]. In particular, the CRISPR-based aptamer sensors have attracted substantial research interest, because it is recognized as the best way to extend the target scope of CRISPR-based sensing from nucleic acids to non-nucleic-acid analytes [17]. Since most of the meaningful analytes in clinical medicine, food safety, and environmental protection are in the form of non-nucleic-acid, exploring the strategy of CRISPR-aptasensing integration is essential for increasing the versatility of CRISPR-based biosensing.

Most of the previous reports adopted an indirect strategy to realize the CRISPR-aptasensing integration. Shen and co-workers designed a pathway where the aptamer-target binding caused the conformational change of a DNA probe, exposing the recognition domain of RNA polymerase, and resulting in the release of RNA to trigger Cas13a activation [16]. Similarly, our group transduced the DNA-protein binding into the release of DNA, which functioned as the activator of Cas12a [14]. Methods with indirect strategy typically contain a step of releasing

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DNA/RNA activators, which typically involves signal amplifications, imparting them good sensitivity with detection limits down to the sub-nanomolar level [18]. However, due to the release of the DNA/RNA activator involving additional nucleases and treatments, indirect methods are typically multistep and cumbersome. Alternatively, there is a direct strategy where an ssDNA functions as both the activator and aptamer, thereby the target binding directly inhibits the hybridization between the ssDNA and the guide RNA in Cas enzymes. For example, a few reports have used this strategy for ATP sensing [13,19]. The direct strategy bypasses the main drawbacks of the indirect one, which is more applicable in point-of-care testing (POCT); however, they are less sensitive than the methods adopting the indirect strategy. One of the reasons is that they were developed based on Cas12a, which has low specificity and sensitivity towards ssDNA [10,20,21]. In this situation, the interferent nucleic acids in biological samples could cause the unspecific activation of Cas12a, resulting in high background and low specificity for aptasensing. Additionally, some aptamers exhibit low affinities towards the corresponding targets, which caused the insignificant conformational change that cannot be resolved by Cas12a, reducing the sensitivity in aptasensing. Therefore, there is a great need to discover new ways to improve the performances of CRISPR-based aptasensing.

As the smallest reported Cas enzyme, Cas14 has attracted substantial research interest [22,23]. Cas14 is an RNA-guided programmable nuclease, while it is unique that the recognition between Cas14 and target DNA does not dependent on the protospacer adjacent motif (PAM), presenting Cas14 as the only PAM-independent DNA-targeted Cas that has been discovered. Under the guidance of a small guided RNA (sgRNA), Cas14 can recognize ssDNA that is complementary to the recognition arm of sgRNA, forming an R-loop structure that possesses indiscriminate ssDNA cleaving activity [24]. Different from Cas12a, the recognition of Cas14 towards ssDNA is highly specific and sensitive, which has been applied for detecting single-nucleotide polymorphism (SNP) genotyping and pathogen discriminations [23,25]. The Lv group has explored the application of Cas14 in the indirect sensing of non-nucleic acid targets, which enabled the sensitive detection of antibiotics with low nanomolar sensitivity [26]. However, the use of metal isotopes and LC-MS decreased its simplicity. Wu et al. developed a CRISPR/Cas14-based aptasensor for the detection of microcystin-LR with nanomaterials-assisted fluorescence generation, achieving excellent sensitivity in environmental monitoring [27]; however, the use of complementary DNA to block the binding capacity of the aptamer could induce signal loss in the target recognition. Additionally, the versatility of CRISPR/Cas14 in aptasensing has never been explored. Therefore, it is worth attempting to develop a cost-effective, rapid, and direct Cas14-based biosensor for versatile aptasensing.

Herein, we developed a Cas14-empowered fluorometric aptasensing platform entitled HARRY (highly sensitive aptamer-regulated Cas14 R-loop for bioanalysis) for the detection of non-nucleic-acid targets. The diblock ssDNA was designed to contain the aptamer sequence, and the corresponding sgRNA was synthesized and embedded into Cas14. By engineering the ssDNA and its corresponding sgRNA, HARRY can detect versatile analytes including ATP, Cd^{2+}, histamine, aflatoxin B1 (AFB1), and thrombin. This study shows that the activity of Cas14a can be directly inhibited by versatile non-nucleic-acid molecules through sequence engineering, and the resulting sensing platform demonstrated that Cas14a possesses high potential in developing CRISPR-based aptasensors.

2. Methods and materials

2.1. Materials

All the DNA and RNA were synthesized, HPLC-purified, and supplied by Sangon Biotech (Shanghai, China), and their sequences are listed in Table S1. Cas14a and its corresponding reaction buffer (HOLMES Buffer 1) were obtained from Tolo Biotech (Shanghai, China). $CdSO_4$, histamine, and ATP were purchased from Aladdin (Shanghai, China). Aflatoxin B1 (AFB1), Fumonisin B1 (FB1) and Ochratoxin A (OTA) are obtained from Yuanye Biotechnology Company (Shanghai, China). Biological molecules, including human IgG, trypsin, chymotrypsin, and thrombin were supplied by BBI (Shanghai, China). The ELISA kit for AFB1 detection and the colorimetric kit for ATP quantification were obtained from Sangon Biotech. The water used in this research was produced by Millipore's Milli-Q system and treated by DEPC (Sangon Biotech) before use.

2.2. The analysis of targets using HARRY

According to the manufacturer's instructions, Cas14a (50 nM, 20 μL) and corresponding sgRNA (50 nM) were mixed in the HOLMES Buffer 1 at 25 °C for 10 min. Then, the FQ-reporter was added to the solution and mixed in an ice bath. In the final solution, the concentrations of Cas14a-sgRNA and FQ-reporter are 25 nM and 1 μM, respectively. This solution is named CRISPR reporting mix (CRM), and it can be stored at 4 °C for at least 3 days.

For the detection of targets, 20 μL of HOLMES Buffer 1 was mixed with 5 μL of Aptavator (20 nM), followed by adding 5 μL of the target. After incubating at 37 °C for 10 min, 20 μL of CRM was added and mixed gently, and then incubated at 25 °C for 50 min in a dark place, followed by heat deactivation at 80 °C for 10 min. The fluorescence was measured by a Tecan M200 Pro (Switzerland) plate reader (λ_{ex} = 480 nm, λ_{em} = 525 nm, 20 reads per well). The procedures for real sample analysis were similar, except that 5 μL of the testing sample was added instead of 5 μL of standard solutions of the target.

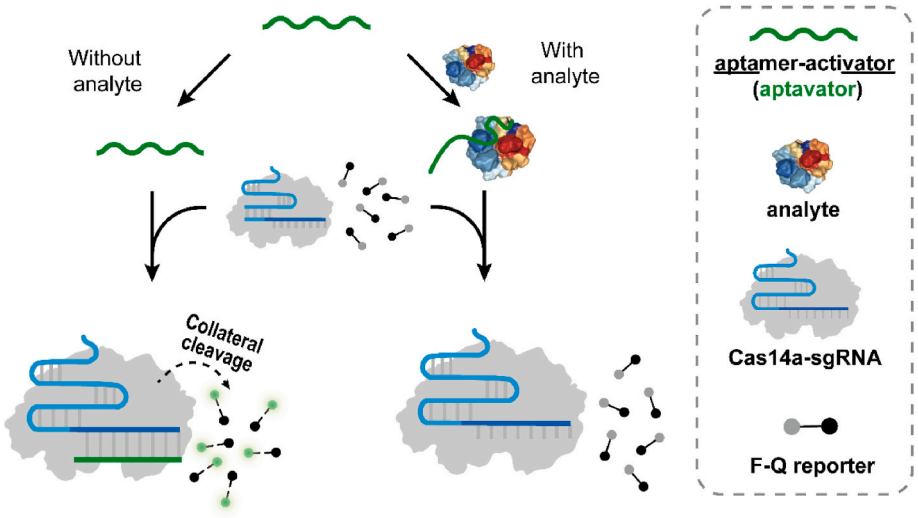
2.3. The analysis of ATP using Cas12a

The protocol was generally designed according to previous reports [13,19], and the concentrations of Cas and nucleic acids were reset according to Section 2.2. Cas12a (50 nM, 20 μL) and corresponding crRNA (50 nM) were mixed in the NEBuffer 2.1 at 25 °C for 10 min. Then, the FQ-reporter was added to the solution and mixed in an ice bath, and this solution is called CRM-Cas12a. For the detection of ATP, 20 μL of NEBuffer 2.1 was mixed with 5 μL of the Aptavator (20 nM), followed by adding 5 μL of the ATP solution. After incubating at 37 °C for 10 min, 20 μL of CRM-Cas12a was added and mixed gently, and then incubated at 25 °C for 50 min in a dark place, followed by heat deactivation at 80 °C for 10 min. The fluorescence was measured by a Tecan M200 Pro (Switzerland) plate reader (λ_{ex} = 480 nm, λ_{em} = 525 nm, 20 reads per well).

3. Results and discussion

3.1. The principle of Cas14a-based aptasensor

As shown in Scheme 1, a DNA strand (denoted in green color) functions as both the aptamer and the activator of Cas14a, so it was named an "Aptavator". (1) In the absence of targets (the *left* pathway), the Aptavator was recognized by the Cas14a-sgRNA ribonucleoprotein, forming a stable R-loop structure to cleave nearby ssDNA [23]. To visualize the activation of Cas14a, the ssDNA probe (F-Q) modified by a FAM and BHQ1 at both ends respectively was used. When F-Q is intact, the fluorescence of the FAM is quenched; however, when the activated Cas14 cleaves and shreds the F-Q, the FAM and BHQ1 will be separated, resulting in fluorescence enhancement [23,28,29]. (2) In the presence of targets (the *right* pathway), the Aptavator is tightly bound to the targets because of its high affinity towards the targets. During this process, the Aptavator will be stabilized in a specific conformation, decreasing its affinity to complementary nucleic acids. In this situation, the bound Aptavator could hardly hybridize with the sgRNA complexed in Cas14a, interfering with the formation of the R-loop and inhibiting the activation



Scheme 1. Principle of the biosensor. The green strand is a combination of the aptamer of a specific analyte and the activator of Cas14a. The Aptavator triggered the activation of Cas14 (the *left* pathway), and the corresponding analyte inhibited this process (the *right* pathway).

of Cas14a. As a consequence, F-Q is not cleaved maintaining the low fluorescence of the system.

In this strategy, the formation of the R-loop structure of Cas14a-sgRNA-ssDNA and the activation of nuclease activity is regulated by non-nucleic-acid targets via an aptamer-functionalized ssDNA activator, therefore, this method is referred to as HARRY (Highly sensitive Aptamer-Regulated Cas14 R-loop for bioanalYsis").

3.2. The verification of HARRY in ATP sensing

To confirm the feasibility of HARRY, control experiments were conducted and the results are shown in Fig. 1a. Comparing the red and blue curves, the CRISPR reporting mix (CRM, a solution consisting of Cas14a, buffer, sgRNA, and F-Q) showed low fluorescence; however, significant enhancement in the fluorescence was found as the Aptavator was introduced. This intensive response is derived from the Aptavator-induced activation of Cas14a, where the Aptavator hybridized with the Cas14a-sgRNA ribonucleoprotein forming an R-loop structure, which caused the collateral cleavage of the ssDNA in F-Q and separated the FAM and BHQ1. This Aptavator contains the aptamer sequence of ATP; hence ATP was introduced to test its influence on the fluorescent signal. By comparing the red and black curves, it was found that the addition of ATP caused a fluorescent decrease, which indicates that the ATP interfered with the Cas14a activation by binding to the Aptavator.

To further confirm the principle of HARRY, the kinetics of fluorescence changes were recorded. As shown in Fig. 1b, a rapid fluorescence enhancement in the FAM channel was found when the Aptavator was introduced into the CRM, and the enhancement lasted for nearly 1 h, causing hundreds of folds of increase. As a comparison, the sample added with ATP showed a slow enhancement (blue curve), which demonstrates that the addition of ATP hindered the association between the Aptavator and Cas14a-sgRNA ribonucleoprotein. In another sample, the ATP was introduced into the system after the Aptavator was mixed with CRM for 30 min (green curve), and it was found that the rapid enhancement in fluorescence immediately slowed down as the ATP was added. Notably, a concentrated ATP solution was added in this experiment, and it caused a less than 1/25 increase in the volume, thus the dilution-derived fluorescent decrease could not cause such significant changes in fluorescence. Based on the results, it was confirmed that the binding between ATP and the Aptavator decreased the association

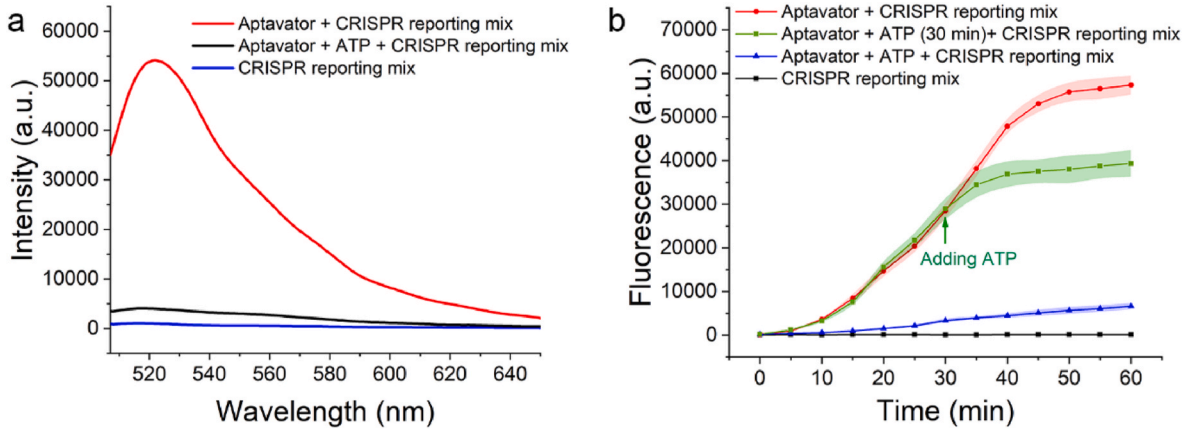


Fig. 1. The verification of HARRY in the detection of ATP. (a) The fluorescent spectra of the differently mixed samples. The blue curve: the CRISPR reporting mix (CRM); the red curve: the mixture of CRM and the Aptavator; the black curve: the Aptavator mixed with ATP, followed by CRM addition. (b) The fluorescence kinetics of samples adding CRM under different sample additions. The black curve: CRM; the red curve: the mixture of CRM and the Aptavator; the blue curve: Aptavator mixed with ATP, followed by CRM addition; the green curve: the mixture of CRM and the Aptavator, while the ATP was added at 30 min. Shades represent the errors during the three replicate measurements. The experiments were conducted as described in Section 2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

between the Aptavator and Cas14a-crRNA, thus verifying the principle of HARRY.

3.3. The versatility of HARRY

By changing the aptamer sequence, diverse targets can be recognized, thus the aptamer has been recognized as a versatile element in the detection of metal ions, small molecules, peptides, proteins, and microorganisms [30–34]. HARRY is an aptasensor so we were curious whether it had high versatility for the analysis of a broad range of targets. The Aptavator contacting each aptamer of Cd^{2+}, histamine, aflatoxin B1 (AFB1), and thrombin was designed (see Table S1), and their feasibility in triggering HARRY was investigated. Notably, most of the aptamer sequences are longer than the length necessary for sgRNA-DNA complementary, to avoid the influence of different sgRNA-DNA affinity, we chose the domains with similar CG% as recognition domains and designed the sgRNA accordingly (detailed in Table S1).

As shown in Fig. S1, all the Aptavators caused significant fluorescence enhancement when they were introduced into the CRM, which confirmed that all these Aptavators were capable of activating Cas14a. It was also found that the final intensities varied, which could be ascribed to the sequence-derived differences in Aptavator-sgRNA affinities. The Cd^{2+}, histamine, AFB1, and thrombin were added to the corresponding reaction system, and their fluorescence intensities were normalized according to the corresponding control group supplemented with buffer. As shown in Fig. 2a, all of the targets caused fluorescent decreases, which indicated the versatility potential of HARRY.

Cross-reaction analyses were conducted to investigate whether the fluorescence decrease was the result of the specific interaction of targets and corresponding aptamers. As shown in Fig. 2b, a significant fluorescence decrease was observed when there was a match between the target and the Aptavator. These results indicate that the fluorescence decrease was target-specific, thus confirming the versatility of HARRY in the sensing of different targets.

3.4. The performances of HARRY in biosensing

As shown in Fig. 3a, the fluorescent intensities in the FAM channel gradually decreased as the amount of ATP increased, and there was a good linear relationship between the ATP concentrations and fluorescent intensities (Fig. 3b). The linear range of HARRY in ATP sensing is 100–2000 nM, and a low limit of detection (LOD) of 80 nM was obtained. Compared with other CRISPR-based aptasensors targeting ATP [13,19], HARRY showed outstanding sensitivity, which was derived from both the high sensitivity of Cas14a in ssDNA detection and the stable binding between the target and the Aptavator.

Notably, compared to the CRISPR/Cas12-based aptasensor targeting ATP [19], HARRY demonstrated a large improvement in sensitivity (80 nM vs. 400 nM). To avoid the errors derived from reagents, instruments, and operators, we tested the responses of Cas14a and Cas12a in the addition of various concentrations of ATP. The crRNA for Cas12a and the sgRNA for Cas14a were designed such that they could recognize the same aptamer-containing ssDNA, and additionally, the Cas12a and Cas14a reaction mixes were prepared with the same concentrations of Cas enzymes, RNA, and F-Q. As depicted in Fig. 3c, the ssDNA caused fluorescence enhancement in both systems, while the group of Cas14a exhibited higher enhancement, which is in accordance with previous reports that Cas14a is more sensitive in ssDNA sensing [25]. In the next step, ATP was introduced into both systems, and the fluorescence after the ATP addition was normalized according to the corresponding intensities in Fig. 3c. As shown in Fig. 3d, the Cas12a group was insensitive in the low concentrations of ATP, and a significant change in fluorescence was recorded as the amount of ATP was raised to 500 nM. In comparison, the Cas14a group was much more sensitive to the ATP, where 50 nM of ATP caused a fluorescent decrease with a statistical significance. The above tests were conducted in different buffers according to manufacturers' instructions (Cas12a in NEBuffer 2.1 and Cas14a in HOLMES Buffer 1), to reveal the influence of buffers, the activities of two Cas enzymes in different buffers were investigated. We found that the activity of Cas12a decreased in HOLMES Buffer 1, and similarly, the activity of Cas14a decreased in NEBuffer 2.1 (Fig. S2). In HOLMES Buffer 1, the LOD for Cas12a in ATP detection was 900 nM (Fig. S3). Therefore, compared with HARRY, Cas12a shows less sensitivity for ATP detection in both HOLMES Buffer 1 and NEBuffer 2.1, indicating that Cas14a shows higher performances for ATP aptasensing.

Cas14a can discriminate ssDNA with single nucleotide variations which have been applied in the detection of single nucleotide polymorphism [22–24]. Therefore, the activation of Cas14a depends on the highly specific hybridization between ssDNA and the sgRNA, like a set of matched keys and locks, while Cas12a has neither a high resolution nor high sensitivity in ssDNA detection. The conformation of the aptamer was changed on interaction with the analyte, which reduced the affinity between the RNA complexed in Cas enzymes and DNA [12,26,29]. According to our results and former reports, this reduction in affinity affects both the Cas14a and Cas12a systems, but its impact on Cas14a is higher than that of Cas12a. We believe that the improvement in sensitivity is rooted in the high specificity of Cas14a-sgRNA ribonucleoprotein to ssDNA, which is an important advantage of Cas14a in ssDNA biosensing.

Since HARRY has high versatility in biosensing, its performance in

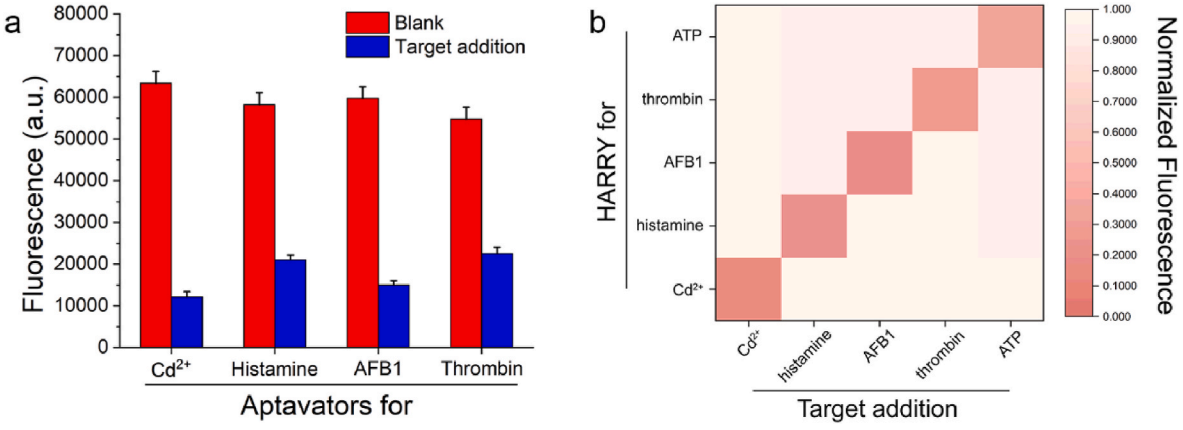


Fig. 2. The versatility of HARRY. (a) The fluorescence of samples mixed with matched Aptavators and CRM (red columns), and the responses after introducing 1 nM of corresponding targets (blue columns). (b) The test of cross-reactions by adding 1 nM of Cd^{2+}, histamine, AFB1, and thrombin, respectively. Error bars indicate the standard deviation of three replicate detections. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

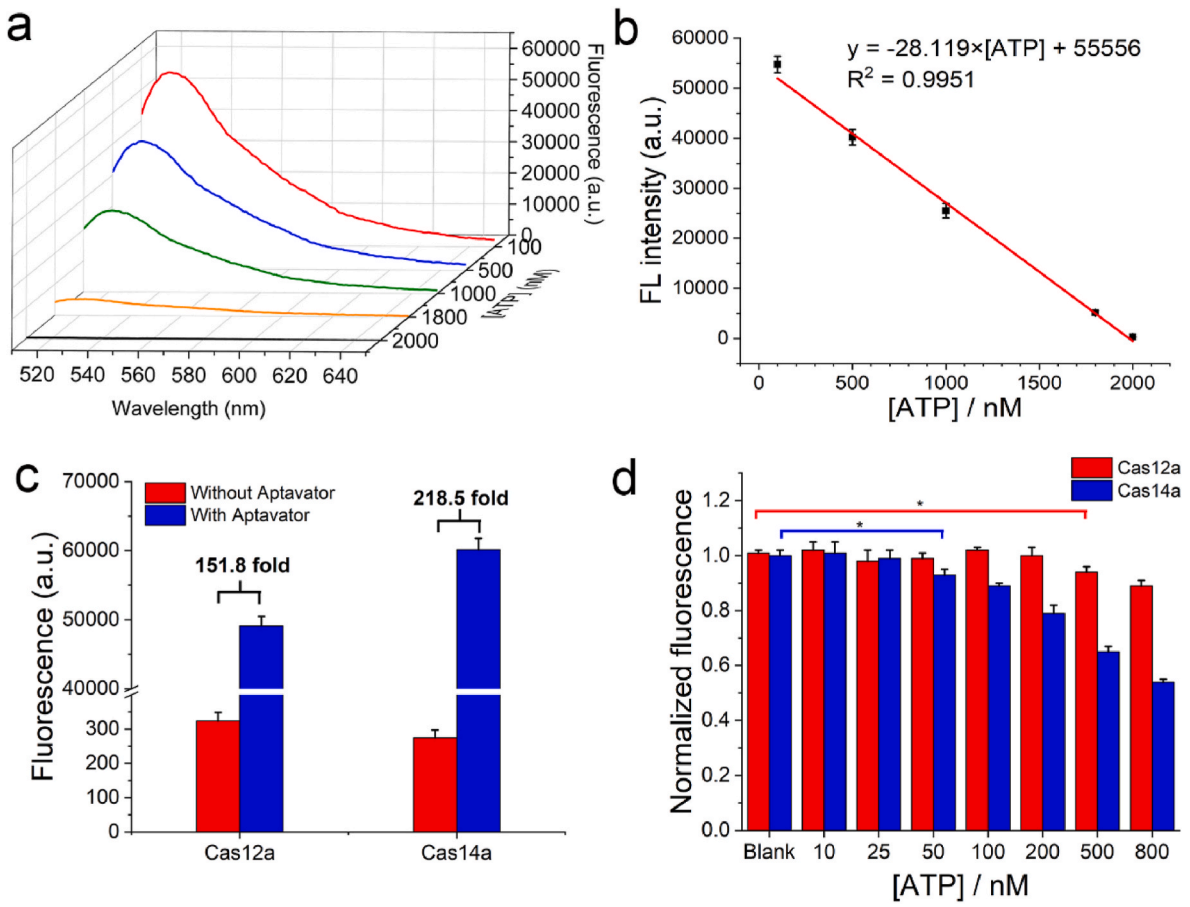


Fig. 3. The analytical performance of HARRY in ATP analysis. (a) The fluorescent spectra of the HARRY system after the introduction of different concentrations of ATP. (b) The relationship between the fluorescence intensities in the FAM channel and the ATP concentrations. (c) The fluorescent responses of Cas12a and Cas14a in the detection of the same amount of Aptavator. Red columns represent the signal without Aptavator addition, and blue columns represent the signal with Aptavator addition. (d) The fluorescence of sensing systems is based on Cas12a (red columns) and Cas14a (blue columns) in the sensing of ATP. Error bars indicate the standard deviation of three replicate detections. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the detection of Cd^{2+}, histamine, AFB1, and thrombin was tested. As shown in Fig. 4, as an increased amount of these targets were added into HARRY, the fluorescence decreased. We found that there were satisfactory linear relationships between the concentrations of the targets and the signal responses, resulting in dynamic ranges of 5–1500 nM in Cd^{2+} detection, 40–1500 nM in histamine detection, 20–3000 nM in AFB1 detection, and 40–2000 nM in thrombin detection. We found that HARRY can be applied in the sensitive detection of Cd^{2+}, histamine, AFB1, and thrombin with LODs of 4 nM, 30 nM, 16 nM, and 36 nM, respectively. These low nanomolar level LODs are satisfactory in the field of direct CRISPR sensing, but they are higher than a few works involving multiple amplifications, which possess LODs down to the subnanomolar level [35,36]. The reason can be ascribed to the "direct strategy" adopted in our work, where the association between the analyte and aptavator directly inhibits the activation of Cas14 without sophisticated signal transductions. Despite this, it should be noticed that the sensitivity of HARRY is higher than that of most Cas12a-based aptasensors [19,37], which is derived from the application of Cas14a as an effector.

The sensitivity of HARRY towards different targets varied, and we speculated that this diversity could be ascribed to the differences in binding affinities and molecular weights: 1) higher target-aptamer binding caused more potent inhibition of ssDNA-derived Cas14a activation; 2) larger molecular weights caused higher steric hindrance to prevent the approaching between crRNA and ssDNA. Fig. 4e indicated the relationship between the sensitivity and the reported binding affinity (K_D) [38–42], and Fig. 4f showed the relationship between the sensitivity and the molecular weights of targets. It was observed that the LOD exhibited a decreasing trend as the binding affinities increased, while the correlation between molecular weights and LOD was low. Therefore, HARRY is more sensitive in the detection of a target with a stronger aptamer binding affinity, while the role of binding affinity in the sensitivity still needs to be elucidated.

The selectivity of HARRY was tested, and the results were presented in Fig. S4. The potential interferents, including GTP, UTP, CTP, ADP, and AMP were selected based on chemical similarities, and the concentrations of the interferents were 5 times higher than that of the targets. As shown in Fig. S4, the samples added with interferents exhibited high fluorescence similar to the blank one, while only ATP caused a significant fluorescence decrease. Similar results were obtained in the analysis of Cd^{2+} (Fig. S5a), histamine (Fig. S5b), AFB1 (Fig. S5c), and thrombin (Fig. S5d), indicating that by changing the Aptavator sequences and the corresponding sgRNA, HARRY possessed good specificity to the designed targets.

3.5. HARRY in real sample analysis

The feasibility of HARRY in real sample applications was tested by analyzing Cd^{2+} in the lake water, AFB1 in peanuts, and ATP in human serum. The samples were pretreated and diluted (detailed in Supplementary Material), followed by being subjected to HARRY analyses. As shown in Fig. S6, the fluorescence of water and human serum samples without spiking was not decreased significantly compared with that of samples with buffer only, indicating that HARRY was not significantly

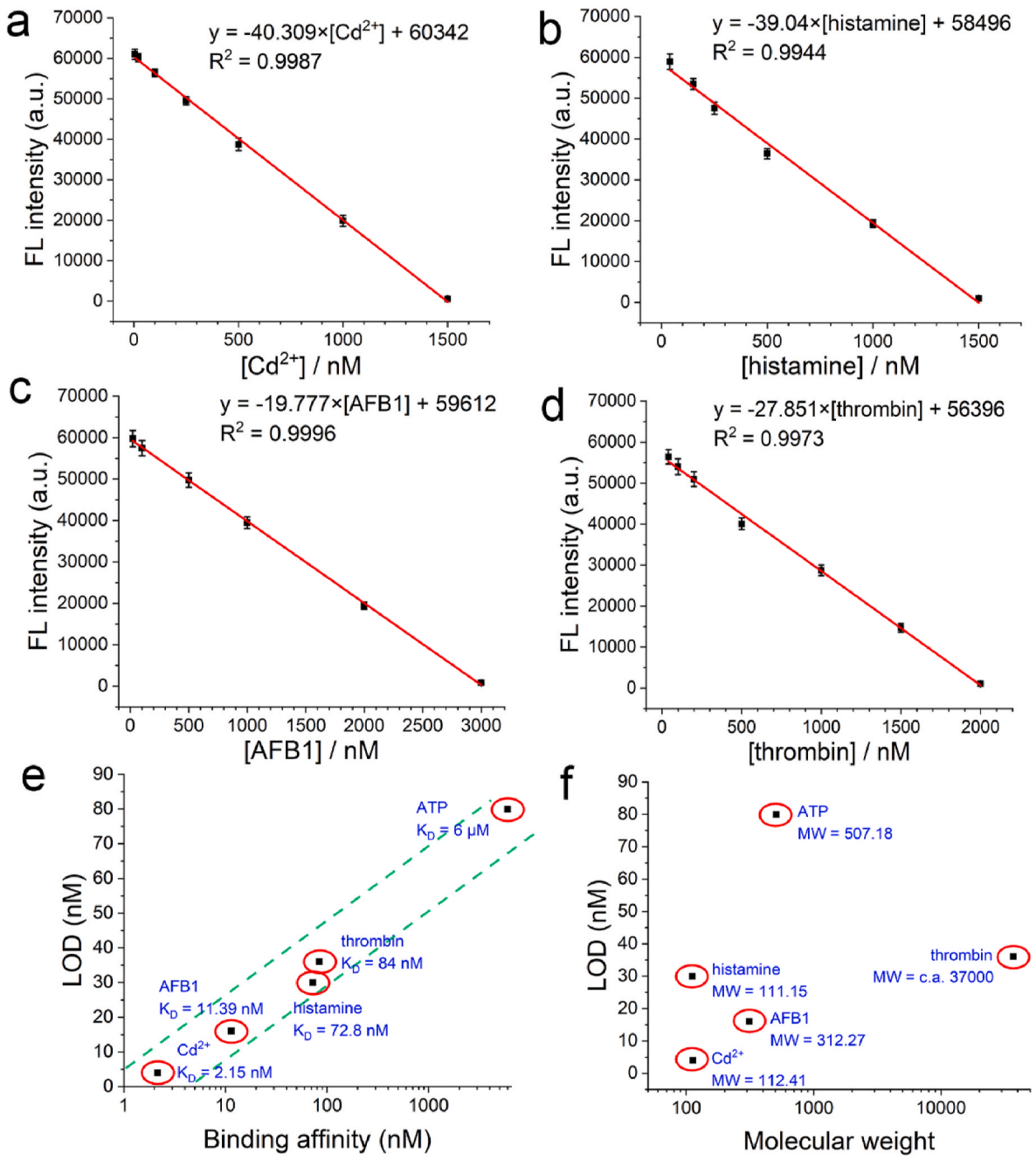


Fig. 4. The analytical performances of HARRY in the sensing of other targets. (a), (b), (c), and (d) represent the relationships between the fluorescence intensities of HARRY and concentrations of Cd^{2+}, histamine, AFB1, and thrombin, respectively. (e) The relationship between the LOD and the reported binding affinities of aptamers towards their corresponding targets. (f) The relationship between the LOD and the molecular weights of the corresponding targets. Error bars indicate the standard deviation of three replicate detections.

affected by interfering substances in the real matrix. The fluorescence of the peanut sample decreased when compared with the buffer group (Fig. S6), which may be derived from the presence of AFB1, or the influence of interfering substances, depending on the results of recovery. The recoveries after spiking of standard solutions and the RSD were presented in Table 1, and it can be found that the recoveries of Cd^{2+} analyses distributed from 102.5% to 105.3%, that of AFB1 analyses ranged from 96.0% to 102.8%, and that of ATP analyses distributed from 96.4% to 103.3%. The highest RSD derived from six paralleled determinations was 2.5%, which demonstrated the stability of HARRY. Notably, the recovery of AFB1 in peanut samples ranged in a satisfactory range, the decrease in the blank sample without spiking is attributed to the effect of the original AFB1 in the peanut sample. To further validate the accuracy of HARRY in real sample analysis, these samples were further analyzed using independent methods (Cd^{2+} by ICP-MS, AFB1 by ELISA, and ATP by colorimetric kit, detailed in Supplementary material). As shown in Fig. S7, the concentrations analyzed by HARRY were in accordance with commercialized kits, which further demonstrate the accuracy of Cas14a in the aptasensing of versatile analytes.

4. Conclusion

In this work, an aptamer-assisted CRISPR biosensor named HARRY was developed based on the Cas14 effector. When there is an interaction between the Aptavator and the target, the Aptavator will be folded, which renders the Aptavator losing its ability for Cas14a activation. Our

Table 1
The performances of HARRY in real sample analyses.

Analytes	Samples[a]	Content (nM)	Spiked (nM)	Detected (nM)	Recovery (%)[c]	RSD (n = 6, %)
Cd^{2+}	Water, Yangcheng Lake	n.d.[b]	100	103.6	103.6	2.3
			200	204.9	102.5	1.8
			400	421.3	105.3	2.1
AFB1	Peanut	40.6	20	59.8	96.0	2.4
			40	79.2	96.5	2.2
			60	102.3	102.8	2.5
ATP	Human serum	n.d.	100	96.4	96.4	1.9
			200	206.5	103.3	1.7
			400	387.8	97.0	2.0

[a] Samples were pretreated as described in Supplementary Material, and the dilution factors for water, peanut, and human serum were 3, 5, and 5, respectively.
[b] n.d.: not detected.
[c] Recovery: values are calculated by ([Detected] - [Content])/[Spiked] × 100%.

study revealed that HARRY was sensitive in sensing aptamer-recognizable analytes, with wide molecular weight distribution ranging from metal ions to proteins. By altering the Aptavator sequences and the corresponding sgRNA, HARRY successfully detected ATP, Cd^{2+}, histamine, AFB1, and thrombin. The low nanomolar sensitivities presented a significant improvement as compared to the Cas12a-based aptasensors, and the superiority of Cas14a could be attributed to its high resolution and sensitivity when compared with Cas12a. Moreover, HARRY exhibited good robustness and specificity that could be applied in real sample analysis, challenged by lake water, peanut extracts, and human serum. Interestingly, our results revealed that certain correlations exist between the LOD of HARRY and the affinity of aptamers, while the clear correlation and underlying mechanisms remain to be discovered. Comparing the cost between HARRY and Cas12a-based methods, the difference mainly lies in the crRNA, in which Cas14a needs longer ones; however, we believe that the cost can be significantly reduced by using commercialized T7 transcription kits to produce crRNA. In summary, this work revealed the possibility of using Cas14a for aptasensing, and we envision that the analytical applications of Cas14a will be further studied.

Credit author statement

Bin Zhou: Visualization, Investigation, and Writing-Reviewing. **Runlin Yang:** Resources and Data curation. **Muhammad Sohail:** Writing-Reviewing and Editing. **Xiaoxue Kong:** Reviewing and Editing. **Xing Zhang:** Visualization. **Ninghua Fu:** Investigation, Supervision, and Editing. **Bingzhi Li:** Editing, Supervision and Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

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