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A ligation-driven CRISPR–Cas biosensing platform for non-nucleic acid target detections†

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Herein, we describe a CRISPR–Cas12a sensing platform activated by a DNA ligation reaction for the sensitive detection of non-nucleic acid targets, including NAD^+, ATP and polynucleotide kinase (PNK). In this design, the DNA ligation reaction triggered by these biomolecules generates DNA duplexes, which can activate the nuclease activity of Cas12a to produce amplified fluorescence signals. As a result, this work provides an alternative strategy to expand the applicability of the CRISPR–Cas system into the detection of non-nucleic acid biomolecules.

As an RNA-guided adaptive immune system widely present in bacteria and archaea, the CRISPR-Cas system can resist the invasion of viruses and plasmids.[1–3] Under the guidance of the guide RNA, the Cas protein can recognize the target nucleic acids with high selectivity and subsequently cut them efficiently,[4–6] which has been repurposed as a versatile and powerful toolkit for gene editing,[7–9] gene regulation,[10,11] bioimaging,[12,13] disease treatment[14,15] and other fields. With the discovery of the *trans*-cleavage activity of Class II type V and VI Cas proteins,[16–18] the potential of the CRISPR-Cas system in molecular diagnostics has attracted much research attention. By leveraging the extraordinarily high *trans*-cleavage activity for signal amplification, CRISPR-based diagnostics, such as DETECTR,[17] SHERLOCK,[19] HOLMES[20] and CONAN,[21] have been developed for the detection of genomic loci with attomolar sensitivity and single-base specificity. Recently, the great potential of the CRISPR-Cas system in the sensitive analysis of microRNA biomarkers has also been demonstrated.[22–25] The CRISPR-Cas system has revolutionized the nucleic acid molecular diagnostic technology to a certain extent, exhibiting great potential in disease screening and epidemic prevention.[26] Moreover, its applicability in the sensitive analysis of non-nucleic acid analytes (*e.g.*, small molecules and proteins) is also worthy of anticipation. By coupling the CRISPR-Cas12a system with bacterial allosteric transcription factors, Liang *et al.* developed a CRISPR-based biosensing platform for the sensitive detection of tetracycline, *p*-hydroxybenzoic acid and uric acid.[27] With the use of functional nucleic acids, such as an aptamer and DNAzyme, for target recognition, the other groups expanded the application of the CRISPR-Cas system to the sensing of protein biomarkers,[28] bacterial pathogens,[29] ATP and Na^+.[30] These studies suggested the value of the superior signal amplification capacity of the CRISPR-Cas system in the amplified detection of non-nucleic acid targets, whereas research studies on this subject are still underdeveloped.

In nature, many enzymatic reactions need the participation of cofactors to take place, which provides an effective strategy for the development of specific bioassays for these biomolecules. For instance, the commercial magnesium assay kit takes advantage of the specific requirement of glycerol kinase for magnesium ions. Therefore, by rationally coupling with specifically enzymatic processes, CRISPR-based bioassays are expected to be established for a variety of biomolecules. In the CRISPR-Cas12a system, Cas12a recognizes the protospacer adjacent motif (PAM) in the non-target strand (NTS) and relies on the base pairing between CRISPR RNA (crRNA) and the target strand (TS) to unwind the dsDNA target.[31,32] Therefore, if a gap is introduced into the TS, the unwinding of the dsDNA target should be prevented, failing to activate the nuclease activity of Cas12a. DNA ligase can catalyse the ligation reaction of gaps in dsDNA in the presence of its cofactors (NAD^+ or ATP), which means that the function of the dsDNA target can be restored by the DNA ligation reaction. Based on this assumption, we sought to construct CRISPR-based bioassays for NAD^+ and ATP by rational integration with the DNA ligation reaction.

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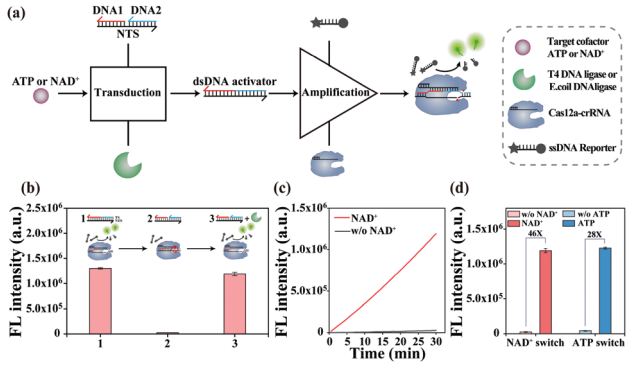


Fig. 1 (a) Schematic illustration of the CRISPR–Cas12a sensing platform activated by the DNA ligation reaction for the detection of NAD^+ and ATP. (b) The effect of the gapped TS on the fluorescence response of the sensing system. The concentration of DNA is 50 nM. (c) The fluorescence response before and after adding 0.5 μM NAD^+ to the system containing the gapped dsDNA and *E. coli* DNA ligase. (d) The off-to-on response of the fluorescence signal triggered by NAD^+ or ATP in the sensing system.

As shown in Fig. 1a, NAD^+ (or ATP)-activated DNA ligation catalyses the conjunction of the gap to form a complete dsDNA that can be recognized by the Cas12a–crRNA complex, leading to the activation of the *trans*-cleavage activity of Cas12a. Using the fluorescent ssDNA as the signal reporter, these molecules (NAD^+ or ATP) can be detected by the amplified fluorescence signals.

To confirm the feasibility of this strategy, we first investigated the effect of a gap on the function of the dsDNA target. In this work, we expressed and purified the Cas12a protein from *Lachnospiraceae bacterium* ND2006 (LbCas12a, Fig. S1, ESI†). The intact dsDNA target could effectively activate the *trans*-cleavage activity of LbCas12a, resulting in the generation of fluorescence signals by the cleavage of the fluorescent ssDNA reporters (Fig. 1b). Next, we prepared several dsDNA targets with a gap in TS (sequences are displayed in Table S1, ESI†) and tested their capacity to activate the *trans*-cleavage activity of LbCas12a. When the dsDNA target contained a gap at the PAM-distal position in TS (8 nt), the *trans*-cleavage signal declined 98% compared with the control without a gap (Fig. S2, ESI†). Therefore, introducing a gap into the dsDNA target at a specific position in TS can efficiently eliminate its ability to activate LbCas12a. This phenomenon might be associated with the fact that the incomplete R-loop between crRNA and gapped TS cannot activate the nuclease activity of LbCas12a. With NAD^+ as the cofactor, *E. coli* DNA ligase catalysed the ligation of the gap and generated the product of an intact dsDNA target (Fig. S3, ESI†) that enables the activation of LbCas12a. As a result, a strong fluorescence signal comparable to that of the control dsDNA target was observed (Fig. 1b). Therefore, with the use of the gap-containing dsDNA and *E. coli* DNA ligase for signal conversion, a CRISPR-based assay for the amplified detection of NAD^+ can be constructed (Fig. 1c). Similarly, using T4 DNA ligase with ATP as the cofactor, a CRISPR-based assay for ATP could also be fabricated (Fig. S4, ESI†). The ratios of signal-to-background in these CRISPR-based assays for NAD^+ and ATP were determined to be 46 and 28, respectively (Fig. 1d). Therefore, by integration with the DNA ligation reaction, CRISPR-based assays for biomolecules, including NAD^+ and ATP, have been established.

After optimizing the experimental conditions (Fig. S5–S8, ESI†), we tested the sensitivity and specificity of the CRISPR-based assays for the detection of NAD^+ and ATP. As shown in Fig. 2a, the fluorescence intensity gradually increased with increasing dosage of NAD^+. A good linear relationship between the fluorescence signals (an enhanced intensity compared to the background, ΔF) and the concentration of NAD^+ was obtained in the range from 0.01 nM to 200 nM with a limit of detection (LOD) of 10 pM (3σ, Fig. 2b). Moreover, the analogues of NAD^+, including its reduced state NADH, the homologous coenzyme NADP, and its reduced state NADPH, caused negligible responses, suggesting the excellent specificity of this assay (Fig. 2d). Similarly, with the use of T4 DNA ligase, the enhanced fluorescence intensity had a linear correlation with an ATP concentration in the range of 0.5–100 nM with a LOD of 50 pM (Fig. 2c), which is four orders of magnitude lower than that of the aptamer-regulated CRISPR-Cas12a assay.[30] The analogues of ATP, such as adenosine monophosphate (AMP), adenosine diphosphate (ADP), uridine triphosphate (UTP) and guanosine triphosphate (GTP), induced only a slight fluorescence response (Fig. 2d). Collectively, these results suggest that the proposed CRISPR-based assays have good sensitivity and selectivity.

Next, we further extended the application of this method to the detection of polynucleotide kinase (PNK) activity. PNK can catalyse the transfer of γ-phosphate from ATP to the 5′-OH group of nucleic acid to make it phosphorylate, which plays a

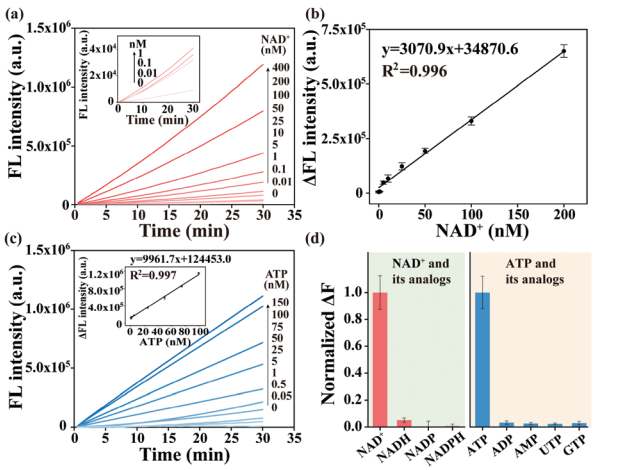


Fig. 2 The sensitivity and selectivity evaluation of the sensing system for the detection of NAD^+ and ATP detection. (a) The fluorescence response of the sensing system toward NAD^+ at different concentrations (0–400 nM). (b) The linear relationship between the fluorescence signals and NAD^+ concentration (0.01–200 nM). (c) The fluorescence response of the sensing system toward ATP at different concentrations (0–150 nM). The inset shows the linear relationship between the enhanced fluorescence intensity and ATP concentration (0.5–100 nM). (d) The normalized enhanced fluorescence signals of NAD^+, ATP and their corresponding analogues. The concentration of each small molecule is 100 nM.

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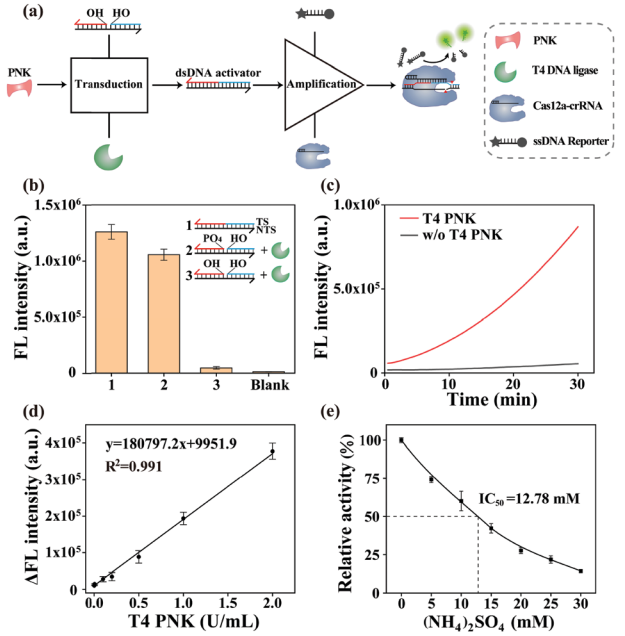


Fig. 3 (a) Schematic illustration of the CRISPR–Cas12a sensing platform for the activity assay of PNK. (b) The effect of 5′-hydroxylation of DNA1 on the fluorescence response of the sensing system. (c) The fluorescence responses before and after adding 5 U mL^{-1} T4 PNK to the system containing 5′-hydroxylated DNA1 and T4 DNA ligase. The concentration of DNA is 50 nM. (d) The linear relationship between the enhanced fluorescence intensity and T4 PNK concentration (0.001–2 U mL^{-1}). (e) Inhibition assay of T4 PNK activity by $(NH_4)_2SO_4$. The concentration of T4 PNK is 1 U mL^{-1}.

vital role in the process of DNA recombination, replication and repair.[33–35] DNA ligase can only catalyse the connection of 5′-phosphate and 3′-hydroxy termini to form a phosphodiester bond. When the 5′-phosphate of DNA1 was replaced with a hydroxyl group, DNA ligase cannot catalyse the ligation reaction. PNK can catalyse the 5′-phosphorylation of DNA1 with the assistance of ATP so that the DNA ligation reaction catalysed by the ligase can continue. Based on this principle, we developed a new method for PNK activity sensing based on CRISPR-Cas12a (Fig. 3a). As a proof-of-concept, T4 PNK was chosen as an example in this work. As shown in Fig. 3b, when the 5′-end of DNA1 was hydroxylated, the fluorescence response dramatically decreased compared to the 5′-phosphorylated DNA1. Once T4 PNK was used to process the 5′-hydroxylated DNA1, the fluorescence signal was significantly restored (Fig. 3c). The above results indicate that T4 PNK can phosphorylate the 5′-end of DNA1, initiate the subsequent DNA ligation reaction, and finally activate the *trans*-cleavage activity of Cas12a to generate an amplified fluorescence signal.

The fluorescence response of the sensing system toward T4 PNK at different concentrations was tested. As shown in Fig. 3d, a linear relationship between the enhanced fluorescence intensity and T4 PNK concentration was obtained in the range of 0.001–2 U mL^{-1} with a LOD of 0.0005 U mL^{-1}, which is lower than many previously reported methods (Table S2, ESI†). Next, an inhibitor of T4 PNK, $(NH_4)_2SO_4$, was used to test whether this method can be applied to evaluate the inhibitory effects on T4 PNK activity. Before this, this inhibitor was verified to have negligible effects on the DNA ligation reaction and the *trans*-cleavage of LbCas12a (Fig. S9, ESI†). As shown in Fig. 3e, the relative activity of T4 PNK gradually decreased as the concentration of $(NH_4)_2SO_4$ increased. The IC_{50} value of $(NH_4)_2SO_4$ was calculated to be 12.78 mM, approximate to the reported value in previous studies.[36]

Next, the potential application of this sensing system in detecting the target analyte in complex biological samples was evaluated. To this end, the activity of PNK in HeLa cells was detected by this sensing method. As shown in Fig. 4a, compared with the blank control (lysis buffer), the lysates from 6×10^3 HeLa cells induced a drastically enhanced fluorescence signal (ΔF), while the cell lysates treated with $(NH_4)_2SO_4$ or denatured cell lysates produced only a low fluorescence signal. This result indicates that the fluorescence signal induced by the cell lysates is specifically originated from the activity of PNK rather than other components. As shown in Fig. 4b, with an increment in the number of HeLa cells, the fluorescence signal caused by the lysates increased successively, and the PNK from the lysates of 28 cells could be detected by this assay (3σ). Collectively, these results imply that the proposed assay has potential applications in biochemical research and clinical diagnosis.

In summary, we developed a CRISPR-Cas12a sensing platform based on DNA ligation reaction for the ultrasensitive detection of non-nucleic acid targets, including small biological molecules (NAD^+ and ATP) and proteins (PNK). In this system, the DNA ligation reaction was used as a "signal converter" to convert non-nucleic acid targets into programmable nucleic acid sequences capable of activating the nuclease activities of the CRISPR-Cas12a system for signal amplification. Such a rational integration has achieved the detection of non-nucleic acid targets with good sensitivity and specificity. The versatility of this strategy has been demonstrated by the specific detection of NAD^+ and ATP with picomolar sensitivity and the sensitive analysis of PNK activities in cell lysates. This CRISPR-Cas12a sensing platform relies on a fluorescence spectrophotometer to record the signals, affecting its applicability under point-of-care settings. To address this limitation, further research efforts are

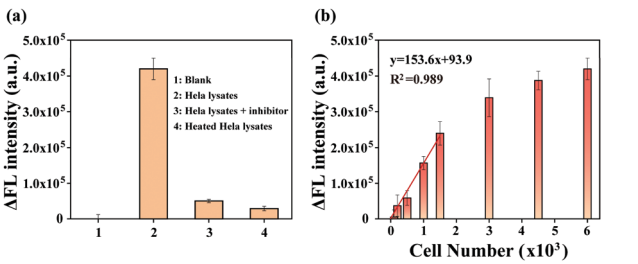


Fig. 4 (a) The fluorescence signals of the proposed assay in response to cell lysates under different conditions. The lysates were extracted from 6×10^3 HeLa cells; the concentration of the inhibitor $(NH_4)_2SO_4$ is 30 mM. (b) The fluorescence signals of the cell lysates from different numbers of HeLa cells.

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needed to convert the *trans*-cleavage effect into colorimetric signals in a homogeneous detection system. Generally, this work has presented an effective strategy to expand the applicability of the CRISPR-Cas system to detect non-nucleic acid targets, which should facilitate the more widespread application of CRISPR-Cas systems in biochemical research and clinical diagnosis.

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Conflicts of interest

There are no conflicts of interest to declare.

Notes and references

1 H. Deveau, J. E. Garneau and S. Moineau, *Annu. Rev. Microbiol.*, 2010, **64**, 475–493.
2 P. Horvath and R. Barrangou, *Science*, 2010, **327**, 167–170.
3 F. V. Karginov and G. J. Hannon, *Mol. Cell*, 2010, **37**, 7–19.
4 J. E. Garneau, M. E. Dupuis, M. Villion, D. A. Romero, R. Barrangou, P. Boyaval, C. Fremaux, P. Horvath, A. H. Magadan and S. Moineau, *Nature*, 2010, **468**, 67–71.
5 C. R. Hale, P. Zhao, S. Olson, M. O. Duff, B. R. Graveley, L. Wells, R. M. Terns and M. P. Terns, *Cell*, 2009, **139**, 945–956.
6 L. A. Marraffini and E. J. Sontheimer, *Nat. Rev. Genet.*, 2010, **11**, 181–190.
7 W. Y. Jiang, D. Bikard, D. Cox, F. Zhang and L. A. Marraffini, *Nat. Biotechnol.*, 2013, **31**, 233–239.
8 B. Shen, J. Zhang, H. Y. Wu, J. Y. Wang, K. Ma, Z. Li, X. G. Zhang, P. M. Zhang and X. X. Huang, *Cell Res.*, 2013, **23**, 720–723.
9 H. Y. Wang, H. Yang, C. S. Shivalila, M. M. Dawlaty, A. W. Cheng, F. Zhang and R. Jaenisch, *Cell*, 2013, **153**, 910–918.
10 L. S. Qi, M. H. Larson, L. A. Gilbert, J. A. Doudna, J. S. Weissman, A. P. Arkin and W. A. Lim, *Cell*, 2013, **152**, 1173–1183.
11 M. L. Maeder, S. J. Linder, V. M. Cascio, Y. F. Fu, Q. H. Ho and J. K. Joung, *Nat. Methods*, 2013, **10**, 977–979.
12 M. E. Tanenbaum, L. A. Gilbert, L. S. Qi, J. S. Weissman and R. D. Vale, *Cell*, 2014, **159**, 635–646.
13 B. H. Chen, L. A. Gilbert, B. A. Cimini, J. Schnitzbauer, W. Zhang, G. W. Li, J. Park, E. H. Blackburn, J. S. Weissman, L. S. Qi and B. Huang, *Cell*, 2014, **156**, 373.
14 C. Z. Long, J. R. McAnally, J. M. Shelton, A. A. Mireault, R. Bassel-Duby and E. N. Olson, *Science*, 2014, **345**, 1184–1188.
15 C. E. Nelson, C. H. Hakim, D. G. Ousterout, P. I. Thakore, E. A. Moreb, R. M. C. Rivera, S. Madhavan, X. F. Pan, F. A. Ran, W. X. Yan, A. Asokan, F. Zhang, D. S. Duan and C. A. Gersbach, *Science*, 2016, **351**, 403–407.
16 A. East-Seletsky, M. R. O'Connell, S. C. Knight, D. Burstein, J. H. D. Cate, R. Tjian and J. A. Doudna, *Nature*, 2016, **538**, 270–273.
17 J. S. Chen, E. B. Ma, L. B. Harrington, M. Da Costa, X. R. Tian, J. M. Palefsky and J. A. Doudna, *Science*, 2018, **360**, 436–439.
18 S. Y. Li, Q. X. Cheng, J. K. Liu, X. Q. Nie, G. P. Zhao and J. Wang, *Cell Res.*, 2018, **28**, 491–493.
19 J. S. Gootenberg, O. O. Abudayyeh, J. W. Lee, P. Essletzbichler, A. J. Dy, J. Joung, V. Verdine, N. Donghia, N. M. Daringer, C. A. Freije, C. Myhrvold, R. P. Bhattacharyya, J. Livny, A. Regev, E. V. Koonin, D. T. Hung, P. C. Sabeti, J. J. Collins and F. Zhang, *Science*, 2017, **356**, 438–442.
20 S. Y. Li, Q. X. Cheng, J. M. Wang, X. Y. Li, Z. L. Zhang, S. Gao, R. B. Cao, G. P. Zhao and J. Wang, *Cell Discovery*, 2018, **4**, 20.
21 K. Shi, S. Xie, R. Tian, S. Wang, Q. Lu, D. Gao, C. Lei, H. Zhu and Z. Nie, *Sci. Adv.*, 2021, **7**, eabc7802.
22 T. Zhou, R. Huang, M. Q. Huang, J. J. Shen, Y. Y. Shan and D. Xing, *Adv. Sci.*, 2020, **7**, 1903661.
23 R. Bruch, M. Johnston, A. Kling, T. Mattmuller, J. Baaske, S. Partel, S. Madlener, W. Weber, G. A. Urban and C. Dincer, *Biosens. Bioelectron.*, 2021, **177**, 112887.
24 Y. Sha, R. Huang, M. Q. Huang, H. H. Yue, Y. Y. Shan, J. M. Hu and D. Xing, *Chem. Commun.*, 2021, **57**, 247–250.
25 S. Peng, Z. Tan, S. Y. Chen, C. Y. Lei and Z. Nie, *Chem. Sci.*, 2020, **11**, 7362–7368.
26 D. S. Chertow, *Science*, 2018, **360**, 381–382.
27 M. D. Liang, Z. L. Li, W. S. Wang, J. K. Liu, L. S. Liu, G. L. Zhu, L. Karthik, M. Wang, K. F. Wang, Z. Wang, J. Yu, Y. T. Shuai, J. M. Yu, L. Zhang, Z. H. Yang, C. Li, Q. Zhang, T. Shi, L. M. Zhou, F. Xie, H. Q. Dai, X. T. Liu, J. Y. Zhang, G. Liu, Y. Zhuo, B. C. Zhang, C. L. Liu, S. S. Li, X. K. Xia, Y. J. Tong, Y. W. Liu, G. Alterovitz, G. Y. Tan and L. X. Zhang, *Nat. Commun.*, 2019, **10**, 3672.
28 Y. F. Dai, R. A. Somoza, L. Wang, J. F. Welter, Y. Li, A. I. Caplan and C. C. Liu, *Angew. Chem., Int. Ed.*, 2019, **58**, 17399–17405.
29 J. J. Shen, X. M. Zhou, Y. Y. Shan, H. H. Yue, R. Huang, J. M. Hu and D. Xing, *Nat. Commun.*, 2020, **11**, 267.
30 Y. Xiong, J. J. Zhang, Z. L. Yang, Q. B. Mou, Y. Ma, Y. H. Xiong and Y. Lu, *J. Am. Chem. Soc.*, 2020, **142**, 207–213.
31 I. Strohkendl, F. A. Saifuddin, J. R. Rybarski, I. J. Finkelstein and R. Russell, *Mol. Cell*, 2018, **71**, 816–824.
32 S. Stella, P. Alcon and G. Montoya, *Nature*, 2017, **546**, 559–563.
33 C. B. Ma and E. S. Yeung, *Anal. Bioanal. Chem.*, 2010, **397**, 2279–2284.
34 L. Lin, Y. Liu, J. Yan, X. S. Wang and J. H. Li, *Anal. Chem.*, 2013, **85**, 334–340.
35 S. F. Liu, J. J. Ming, Y. Lin, C. F. Wang, C. B. Cheng, T. Liu and L. Wang, *Biosens. Bioelectron.*, 2014, **55**, 225–230.
36 X. Y. Li, Y. C. Du, Y. N. Pan, L. L. Su, S. Shi, S. Y. Wang, A. N. Tang, K. Kim and D. M. Kong, *Chem. Commun.*, 2018, **54**, 13841–13844.

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