Electronic Supporting Information

In situ generation of electrochemiluminescent DNA nanoflowers as signal tag for mucin 1 detection based on a strategy of target and mimic target synchronous cycling amplification

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Experimental section

Instrumentation

A Model MPI-E ECL analyzer (Xi'an Remax Electronic Science & Technology Co.Ltd., Xi'an, China) was employed to detect ECL signals. A Model CHI 660A electrochemistry workstation (Shanghai Chenhua Instruments, Shanghai, China) was used to perform electrochemical impedance spectroscopy (EIS). All the measurements above were proceeded with a three-electrode system, among which glassy carbon electrode (GCE, 4 mm in diameter) as working electrode, platinum wire electrode as counter electrode, and Ag/AgCl (with saturated KCl) electrode as reference electrode. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed by a Model DYY-8C electrophoretic device (Beijing WoDeLife Sciences Instrument Company, Ltd.). In order to characterize diverse materials, a scanning electron microscope (SEM, Hitachi, Tokyo, Japan) and UV-vis spectrophotometer (Shimadzu, Tokyo, Japan) was employed in the research.

Reagents and Samples

Mucin 1 (MUC1, 100 ng/mL) and laminin (LN) were bought from Shanghai North Connaught Biotechnology Co. Ltd. (Shanghai, China). *N*-(Aminobutyl)-*N*-(ethylisoluminol) (ABEI), doxorubicin (Dox), tris(2-carboxyethy)phosphine hydrochloride (TCEP) and bovine serum albumin (BSA) were provided by Sigma-Aldrich (St. Louis, MO, USA). Thrombin (TB), alpha fetal protein (AFP) and carcino-embryonic antigen (CEA) were purchased from Biocell (Zhengzhou, China). Deoxyribonucleoside triphosphates (dNTPs) were provided by Takara Biotechnology Company Ltd. (Dalian, China). $AgNO_3$, $FeCl_3$, NaCl, H_2O_2 (30%), glutaric dialdehyde (GA, 50%), polyvinylpyrrolidone (PVP, K30, M_W = 40 000) and ethylene

glycol (EG) were supplied by Chemical Reagent Co. Ltd. (Chongqing, China). Deionized water was used throughout the process of the research and all reagents were of analytical grade. The human sera samples were obtained from Daping Hospital (Chongqing, China).

The oligonucleotides (synthesized and purified by Sangon, Inc. (Shanghai, China)) are exhibited in Table S1, the underlined part stands for the aptamer sequence of MUC1 and **P** stands for the phosphate group.

Table S1. Oligonucleotides used in this work

Synthetic oligonucleotide	Sequence (5′-3′)
HP1	GGG GGC AGT TGA TCC TTT GGA TAC CCT GGG GAT CAA CTG C
HP2	GCA GTT GAT CCG GAT GCA GTT GAT CCT GGT GCC AAC CAG GAT CAA CTG CAT CCG GAT CAA
S_1	**SH-$(CH_2)_6$**-TAA ATG GTG GAA AGG GGT TTT GAT CCG GAT GCA GTT GAT CCT GGT TGG C
S_2	CAG GAT CAA CTG CAT CCG GAT CAA AAC CCA TAA ACA TAA AA
S_3	CCT TTC CAC CAT TTA
Padlock probe	**P**-ATC TAA CTT TGC CAA CCA GGA TCA ACT GCA TCC GGA TCA AAA CCC CTT TCC ACC ATT TAA AAG TTA GAT GCT GCT GCA GCG ATA CGC GTA TCG CTA TGG CAT ATC GTA CGA TAT GCC GCA GCA GC

DNA enzymes are listed as follows: T4 DNA ligase (T4, Takara Biotechnology Company Ltd. Dalian, China); T7 Exonuclease (T7 Exo), phi29 DNA polymerase (phi29, Thermo Fisher Scientific, Inc. Waltham, MA, USA). Furthermore, various kinds of buffers applied in the research are listed as follows:

(1) 0.1 M phosphated buffered solution (PBS, containing 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4 and 2 mM $MgCl_2$, pH 8.0) was used as working buffer solution for ECL detection;

(2) 1× TE buffer (containing 10 mM Tris-HCl, 1.0 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) was used to dissolve and store all oligonucleotides;

(3) DNA hybridization buffer (containing 10 mM Tris-HCl, 1.0 mM EDTA, and 1.0 M NaCl, pH 7.0) was used as buffer solution for DNA hybridization;

(4) 1× T7 Exo buffer (containing 50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 1 mM dithiothreitol (DTT), pH 7.4);

(5) 1× T4 buffer (containing 66 mM Tris-HCl, mM $MgCl_2$ and 10 mM DTT, pH 7.8);

(6) 1× Phi29 buffer (containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$ and 4 mM DTT, pH 7.5).

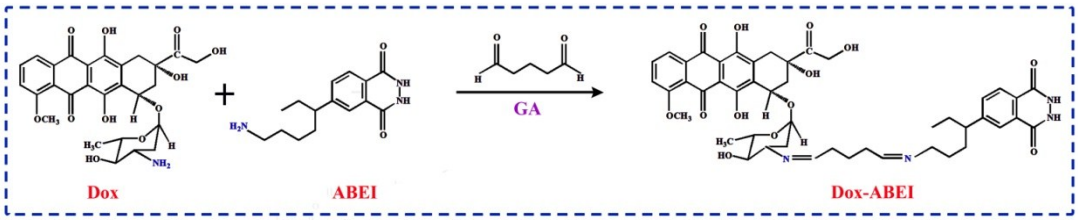
Procedure of Ag Nanowires (Ag NWs) Preparation

Ag NWs were prepared according to the reported literature[1] with some alteration. Briefly, 0.17 g PVP was added into 10 mL of 0.1 mM $FeCl_3$ in EG under tempestuous magnetic stirring. About 30 min later, 10 mL of 0.1 M $AgNO_3$ solution in EG was put into the solution above. Subsequently, the mixture was transferred to a Teflon-lined stainless-steel autoclave and kept at 160 °C for 3 h. Centrifuged and washed with ethanol and deionized water, then the obtained Ag NWs were dispersed in 4 mL deionized water ultimately.

Preparation of the Dox-ABEI Compounds

The Dox-ABEI compounds were gained according to the literature[2] with some slight modification. Concretely, 1.0 mL of 10 mM ABEI solution and 0.5 mL of 10 mM Dox solution were mixed together, and kept stirring for a short while. Afterwards, 0.5 mL of GA (1%) was added into the mixture above and stirred slowly overnight

away from the irradiation of light. The preparation process was exhibited in Scheme S1 and relative UV-vis absorption spectra were shown in Fig. S1. The Dox-ABEI complex was used directly without further purification.



Scheme S1. Route for synthesis of the Dox-ABEI compounds.

Pretreatment of DNA Strands

HP1 (2 μM), HP2 (2 μM) and corkscrew-like padlock probe (2 μM) were obtained through annealing, namely heating corresponding DNA sequences to 95 °C for 5 min and then cooled to ambient temperature automatically. S_1 (2.0 μM) was mixed with S_2 (2.2 μM) and S_3 (2.2 μM) fully, then an annealing treatment was executed. TCEP (1.0 mM) was added to avoid the formation of disulfide bonds of the SH-S1, then the three-strand DNA duplex (as capture probe) was formed finally. Besides, different concentrations of MUC1 and HP1 (2.0 μM) were mixed together for 1 h to obtain a mixture of aptamer-MUC1 complex.

Fabrication of the Modified Electrode

At first, the GCE was polished with 0.3 and 0.05 μm alumina slurry sequentially, then washed with deionized water and alcohol. Whereafter, 7 μL of as-prepared Ag NWs solution was laid on the electrode surface to form an uniform film through naturally dried at room temperature. Subsequently, 10 μL of capture probe was immobilized on the Ag NWs/GCE overnight, then blocking with 0.5% BSA for 40

min.

Target and Mimic Target Synchronous Cycling Amplification Process

Target and mimic target synchronous cycling amplification process was executed in 100 μL of homogeneous solution (abbreviated as *sample solution*): 10 μL of a mixture of aptamer-MUC1 complex, 10 μL HP2 (2.0 μM), 10 μL T7 Exo (30 u/mL), 1 μL 1× T7 Exo buffer and 69 μL DNA hybridization buffer were mixed together and incubated at 25 °C for 100 min in a thermostat container, the reaction system was finally terminated by a treatment at 80 °C for 10 min.

Measurement Procedure

Firstly, 10 μL of padlock probe (100 nM) and 100 μL of *sample solution* were mixed fully. Toehold-mediated strand displacement recycling amplification process was triggered with the incubation of 10 μL the above solution on the surface of capture probe/Ag NWs/GCE for 2 h. Next, 10 μL mixture of T4 and 1× T4 buffer was dropped onto the resultant electrode surface at 37 °C for 1 h to connect the 5′-end and 3′-end of the padlock probe. Afterwards, RCA procedure was executed by adding 10 μL mixed solution of 100 u/mL phi29, 1× phi29 buffer and 1.0 mM dNTPs at 30 °C for 24 h. Subsequently, the resultant electrode was incubated with 10 μL of prepared Dox-ABEI compounds for 2 h at room temperature, then rinsing with deionized water. Lastly, ECL measurements were carried out in 0.1 M phosphate buffer solution (PBS, pH 8.0) containing 2 mM H_2O_2 with the potential ranging from 0.2 V to 0.8 V, the photomultiplier tube and scan rate were set at 800 V and 100 mV/s, respectively.

PAGE analysis

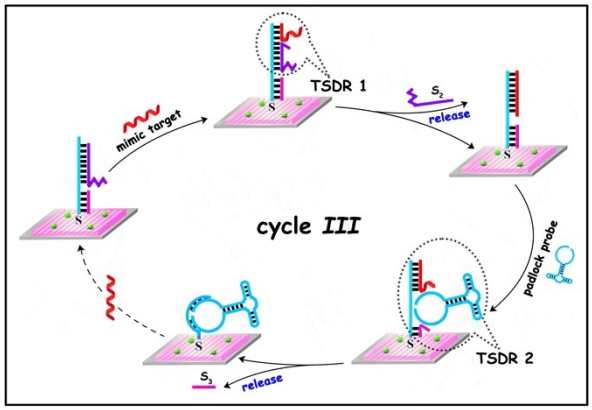
Concerning PAGE, different samples of the proposed DNA structures were put into the notches with newly prepared 16% non-denatured polyacrylamide, and electrophoresis were executed in 1× TBE buffer at the potential of 120 V. After dyeing with ethidium bromide for 20 minutes, electrophoresis images were obtained through a Molecular Imager Gel Doc XR+ with Image Lab software.

Results and discussion

Design Principle of Cycle III

Cycle *III* was designed depend on two toehold-mediated strand displacement reactions (TSDRs), which were first explored by Yurk et al.[3] involves the extraction of a single-stranded DNA from a DNA duplex by an invading strand starting from the unpaired region (toehold). Upon hybridizing with the toehold region, the invading strand attaches to one end of the template and displaces the original shorter complementary strand of the DNA duplex driven thermodynamically by entropy without the assistance of any enzymes at room temperature.[4,5] As exhibited in Scheme S2 in the supporting information, thiolated (-SH) capture probe was immobilized on the Ag nanowires modified glass carbon electrode (GCE) through Ag-S binding to obtain the sensing interface followed by blocking the surface with BSA. Capture probe contains three parts: SH-S_1 as template probe (the green sequence), S_2 as assistant probe (the purple sequence) and S_3 as protection probe (the pink sequence). The sensing probe is designed in such a way that it contains two toehold regions. The first toehold region (6-nt), which can hybridize with the mimic target and initiate the first TSDR, is located at the 3′-terminus of the S_1. The second toehold region (4-nt),

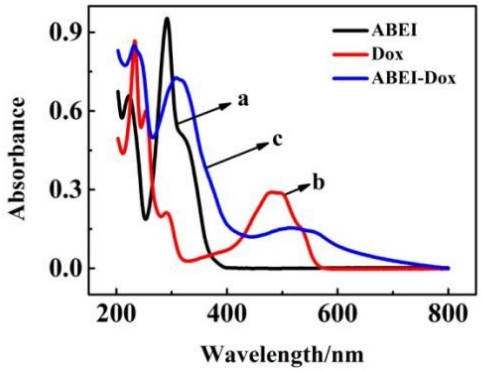
which can hybridize with padlock probe initiate the second TSDR, is positioned in the middle of the SH-S_1. Meanwhile, mimic target was released to participate the recycling process. Moreover, the second toehold region and the 5′-terminus of the SH-S_1 are initially blocked by S_2 and S_3 respectively, to inhibit the TSDRs triggered by the padlock probe in the absence of the target MUC1.



Scheme S2. Sketch map of cycle *III*.

Characterization of the Dox-ABEI Compounds

Usually, UV-vis analysis (showed in Fig. S1) were applied to characterize Dox-ABEI compounds. Apparently, the UV-vis absorption spectra of both ABEI (curve a) and Dox (curve b) possessed a characteristic absorption peak at 230 nm or so, which rooted in the UV absorption of -NH_2 group[6]. However, the UV absorption of -NH_2 in Dox-ABEI compounds (curve c) appeared a slight red shift compared with that of ABEI, and its intensity declined apparently compared with that of Dox, because GA consumed -NH_2 validly. Moreover, ABEI and Dox owned different characteristic absorption peaks at 292 nm and 490 nm, but the absorption spectra of Dox-ABEI compounds emerged a new absorption band at 550 nm approximately, which was related to the Dox.



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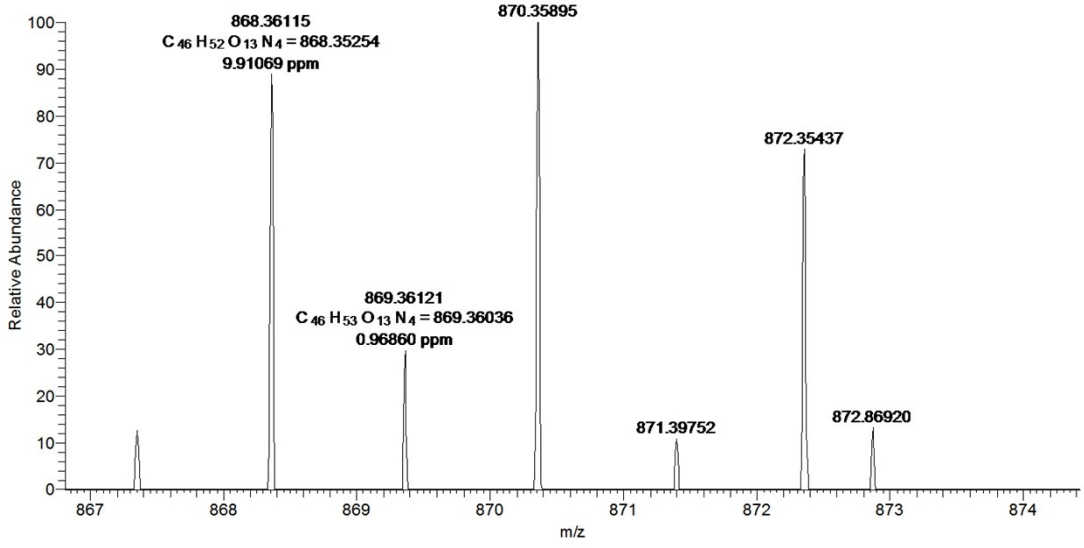
186 **Fig. S1**. UV-vis absorption spectra of (a) ABEI, (b) Dox and (c) Dox-ABEI compounds.

187 In order to further confirm the successful preparation of Dox-ABEI compounds,
188 High Resolution Mass Spectrometry (HRMS) detection was executed to analyze the
189 component of rough Dox-ABEI complex. As we can see from Fig. S2, a component
190 with Mr of 868.36115 was observed, which was very close to the theoretical Mr of
191 Dox-ABEI compounds ($C_{46}H_{52}O_{13}N_4$, Mr = 868.36), indicating the generation of
192 Dox-ABEI compounds. However, the other component with Mr of 869,36121,
193 870.35895, 871.39752, 872.35437 and 872.86920 were also observed in Fig 2. as the
194 products were not purified in the whole experimental process.



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196 **Fig. S2** HRMS spectrum of Dox-ABEI compounds.

EIS Analysis of Stepwise Modifications of the Aptasensor

In the interest of delving the interfacial properties of the modified electrode surfaces, stepwise fabrication of the biosensor was performed by EIS analysis in 5.0 mM $[Fe(CN)_6]^{3-/4-}$, the potential was set at 220 mV and the frequency was ranged from 10^{-2} to 10^6 Hz. The semicircle diameter of EIS is equal to R_{et} in the Nyquist plots. In Fig. S4, a small semicircle was observed on the bare GCE (curve *a*), showing a fairly low electron-transfer resistance. The R_{et} declined after the immobilization of Ag NWs, because Ag NWs have enlarged the effective surface area of the electrode and acclerated electron transfer (curve *b*). After the Ag NWs/GCE was incubated with capture probe, the increase of the semicircle diameter indicated capture probe was successfully immobilized *via* Ag-S binding[11] and it blocked the electron transfer (curve *c*). Subsequently, the resultant electrode was blocked with BSA, the R_{et} value increased overtly owing to BSA, a biomacromolecule with poor electroconductivity and large steric hindrance, which hindered the electron transfer to a great extent (curve *d*). Then, the R_{et} further enhanced with the introduction of padlock probe and *sample solution*, which was attributed to the immobilization of negatively charged padlock probe on the resultant surface (curve *e*). After that, the R_{et} value boosted sharply with the execution of RCA reaction as a consequence of the suppression of the formation of the long DNA sequences (curve *f*). The semicircle diameter showed a further increment after the immobilization of ABEI-Dox complex (curve *g*).

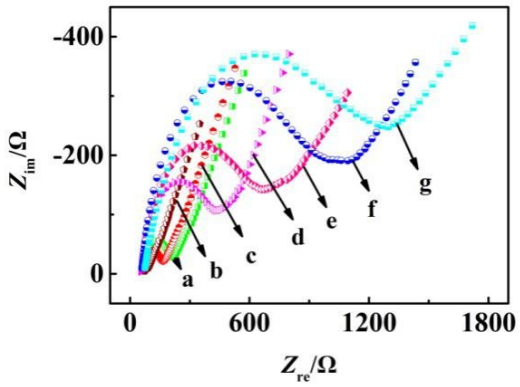

Fig. S4. EIS analysis of the modified electrode at different phases: (a) GCE, (b) Ag NWs/GCE, (c) capture probe/Ag NWs/GCE, (d) BSA/capture probe/Ag NWs/GCE, (e) the electrode *d* executed the toehold-mediated strand displacement recycling amplification process, (f) the electrode *e* executed RCA process and (g) the resultant electrode immobilized with Dox-ABEI compounds.

Optimization of Experimental Conditions

For the sake of achieving optimal performance of the proposed method for MUC1 detection, two vital factors containing the concentration of H_2O_2 and the incubation time of Dox-ABEI compounds were discussed successively. It is worth mentioning that the ECL intensities were estimated at different experimental conditions and treated as the performance index of the detection method, the concentration of MUC1 was remained at 10 pg/mL. The effect of H_2O_2 concentration on the ECL intensity in the PBS was first studied from 0.5 to 3.25 mM. The data in Fig. S5 (A and B) indicated that the ECL intensity augment rapidly with the increasement of H_2O_2 concentration from 0.5 to 3.0 mM, and then leveled off (the incubation time of Dox-ABEI compounds was 3 h). Accordingly, 3.0 mM H_2O_2 was chosen as the optimal concentration in our protocol. Subsequently, the incubation time of Dox-ABEI compounds from 60 to 135 min with a time interval of 15 min was

explored (shown in Fig. S5 (C and D)). Evidently, the ECL intensity raised gradually before it got a peak value when the incubation time was 2 h, indicating that 2 h was appropriate for further study (the concentration of H_2O_2 was 3.0 mM).

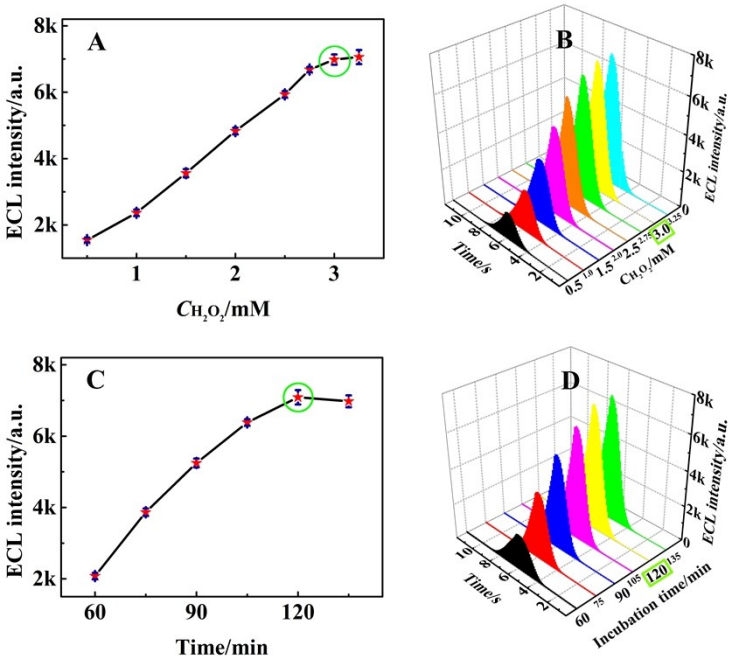

Fig. S5. Optimization of (A and B) the concentration of H_2O_2 (the incubation time of Dox-ABEI compounds was 3 h) and (C and D) incubation time of Dox-ABEI compounds (the concentration of H_2O_2 was 3.0 mM). The optimums were labeled with green circle. Error bars, standard deviation (SD), $n = 3$.

Table S2. Contrast between current work and some relative researches for MUC1 detection

Test method	Sensing range	Detection limit	Reference
EC	10^{-3}-1 μM	0.827 nM	12
Fluorescence	0.8-39.7 μM	250 nM	13
Fluorescence	0.04 -10 μM	28 nm	14
ECL	10^{-3}-10^{3} pg/mL	0.62 fg/mL	15
ECL	10^{-3}-10^{4} pg/mL	0.23 fg/mL	This work

Table S3. Practical application of the fabricated biosensor in sera samples (n = 3)

Order	Added	Found	RSD/%	Recovery/%
1	5 ng/mL	5.051 ng/mL	5.31	101.02
2	0.5 ng/mL	0.515 ng/mL	3.76	103.00
3	50 pg/mL	48.970 pg/mL	2.07	97.94
4	5 pg/mL	5.000 pg/mL	2.71	100.00
5	0.5 pg/mL	0.499 pg/mL	5.04	99.80
6	50 fg/mL	50.300 fg/mL	4.23	100.60

Notes and references

1 J. Han, S. Yuan, L. N. Liu, X. F. Qiu, H. B. Gong, X. P. Yang, C. C. Li, Y. F. Hao and B. Q. Cao, *J. Mater. Chem. A*, 2015, **3**, 5375.

2 S. B. Xie, Y. W. Dong, Y. L. Yuan, Y. Q. Chai and R. Yuan, *Anal. Chem.*, 2016, **88**, 5218.

3 B. Yurke, A. J. Turberfield, A. P. Mills, F. C. Simmel, and J. L. Neumann, *Nature*, 2000, **406**, 605.

4 D. Y. Zhang and E. Winfree, *J. Am. Chem. Soc.*, 2009, **131**, 17303.

5 K. Shi, B. T. Dou, C. Y. Yang, Y. Q. Chai, R. Yuan and Y. Xiang, *Anal. Chem.*, 2015, **87**, 8578.

6 J. P. M. Almeida, A. Y. Lin, E. R. Figueroa, A. E. Foster and R. A. Drezek, *Small*, 2015, **11**, 1453.

7 X. Chen, G. M. Jiang, Z. L. Wang, S. N. Hong, Y. Y. Zhang, Y. H. Guo, H. Chen, J. N. Wang and R. J. Pei, *Anal. bioanal. chem.*, 2016, **408**, 683.

8 Q. Jiang, C. Song, J. Nangreave, X. W. Liu, L. Lin, D. L. Qiu, Z. G. Wang, G. Z. Zou, X. J. Liang, H. Yan and B. Q. Ding, *J. Am. Chem. Soc.*, 2012, **134**, 13396.

9 C. M. Alexander, M. M. Maye and J. C. Dabrowiak, *Chem. Commun.*, 2011, **47**, 3418.

10 D. Agudelo, P. Bourassa, G. Bérubé and H. A. Tajmir-Riahi, *Int. J. Biol. Macromol.*, 2014, **66**, 144.

11 R. R. Gao, S. Shi, Y. Zhu, H. L. Huang and T. M. Yao, *Chem. Sci.*, 2016, **7**, 1853.

12 C. Y. Deng, X. M. Pi, P. Qian, X. Q. Chen, W. M. Wu and J. Xiang, *Anal. Chem.*, 2017, **89**, 966.

13 A. K. H. Cheng, H. P. Su, Y. A. Wang and H. Z. Yu, *Anal. Chem.*, 2009, **81**, 6130.

14 Y. He, Y. Lin, H. W. Tang and D. W. Pang, *Nanoscale*, 2012, **4**, 2054.

15 X. Y. Jiang, H. J. Wang, H. J. Wang, Y. Zhuo, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2017, **89**, 4280.