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Selection and colorimetric application of ssDNA aptamers against metamitron based on magnetic bead-SELEX

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2.Materials & Methods

Affinity tests for candidate by Ultrafiltration Method

(1) Candidate aptamers were prepared into DNA solutions with concentrations of 10 nm, 20 nm, 40 nm, 60 nm, 80 nm, 100 nm, 150 nm and 200 nm using binding buffer. They were heated and denatured at 95 ° C for 6 min, then quickly transferred to ice for 10 min and put at room temperature for 5 min.

(2) 100 μL of the above solutions was absorbed respectively in a 1.5 ml centrifuge tube, 100 ml of 20 μM MTM solution was added and mixed well. Then the mixture was incubated at 25 ° C for 30 min.

(3) After the incubation, pipetted the reaction solution into the ultrafiltration tube (ultra-0.5 centrifugal filter (10 KD)), used the high-speed centrifuge to adjust the rotating speed to 12000 rpm, centrifuged for 5 min, and finally the filtrate was collected. In the filtration process, ssDNA that failed to bind to the target and ssDNA-MTM complex produced by ssDNA that can bind to the target cannot pass through the filter element (molecules less than 10 KD). Therefore, the filtrate contained only MTM solution that failed to react with ssDNA. The MTM that can react with candidate ssDNA can be determined by measuring the concentration of benzophenone in the filtrate.

(4) Established the standard concentration curve of MTM. MTM has a maximum absorption peak at 312 nm. Set MTM solutions with concentrations of 0, 10, 20, 40, 60, 80, and 100 μM, and the absorbance values at 312 nm were recorded respectively to establish the standard concentration curve of MTM concentration. Therefore, the corresponding MTM concentration can be obtained by measuring the absorbance value of the filtrate in step (3) at 312 nm.

(5) According to the dissociation constant formula, $y = B_{max} \cdot X/(K_d+X)$, Y is the concentration of MTM, X is the concentration of candidate aptamer and B_{max} is the maximum binding concentration. The data obtained in the above process can be fitted by origin 8.0 software, and the fitted K_d is the dissociation constant K_d of candidate aptamers and benzophenone.

Preparation of AuNPs

Traditionally[1], the AuNPs solution was prepared by sodium citrate reduction of HAuCl4 solution. First, all the glassware used in the experiment was cleaned using freshly prepared 1:3 (v/v) HNO3/HCl solution, then rinsed in ultrapure water thoroughly and dried at 50 °C. 10.5 mL of 1% (w/v) trisodium citrate solution was added to the boiling solution of HAuCl4·4H2O (100 mL, 0.03%, w/w) quickly and continued to heat and stirred for 30 min to synthesize AuNPs. During this period, the color of the solution gradually changed from colorless to gray, blue, dark purple to wine red. Then stop heating and stir automatically until the solution is completely cooled to room temperature. Finally, the cooled AuNPs solution was filtered with 0.22 μm ultrafiltration membranes, and the filtrate was stored in dark glass bottle at 4 °C for further use.

Optimization of the experimental conditionals

For the better performance in sensitivity and selectivity of this colorimetric detection assay, the experimental conditions including concentration of NaCl, concentration of the aptamer, pH of the system, temperature of incubation were optimized. Each experiment was repeated for at least three times.

For the optimization of NaCl concentration, different volumes (0, 5, 10, 15, 20, 25, 30, 35, 40, and 45μL) of 2 M NaCl standard solution were added respectively to 1.5 mL centrifugation tubes

containing 200 μL AuNPs. The final concentration of NaCl in each tube was set at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 mm respectively, and MOPS buffer was used to supplement the system to 500 μL. After 30 minutes of incubation at 25°C, the absorbance at 650 nm (A_{650}) and 520 nm (A_{520}) of each solution were measured. The above steps were repeated three times. The average value of the absorbance ratio A_{650}/A_{520} was plotted as the ordinate, which could reach the maximum value when AuNPs was completely aggregated by the NaCl solution, while the NaCl concentration was the abscissa. The minimum concentration of NaCl that fully aggregated AuNPs was chosen as the optimal concentration.

To obtain the optimal concentration of aptamer, The aptamer with different final concentration (0, 4, 6, 8, 10, 12, 14, 16, and 18 nM) was added to the sample system contained 1μM MTM (A) and the blank system without MTM (A_0) respectively, then the system was supplemented with MOPS buffer. All systems were incubated for 30 min and then added 200 μL AuNPs, and incubated for 30 min to fully combine aptamer and AuNPs. Finally, added the optimized concentration of NaCl. After incubating for 10 min, A_{650}/A_{520} of the sample system (A) and the blank system (A_0) were obtained and the value ΔA ($\Delta A=A-A_0$) at each aptamer concentration was calculated. The concentration that induced the maximum value of ΔA was chosen as the optimal aptamer concentration.

To optimize the pH of system, considering the instability of MTM under alkaline conditions, set the pH of the MOPS buffer of the colorimetric detection system in the above optimization step to 3, 4, 5, 6 and 7 respectively, and then the optimized concentration of aptamer and MTM were added to the system. The experimental steps and determination methods are the same as the above step of the optimization of aptamer concentration. The pH enabled the ΔA to reach the maximum value was selected as the optimal pH of the system.

Set the incubation temperature of the reaction system to 20 ° C, 25 ° C, 30 ° C, 35 ° C and 40 ° C respectively. The optimization operation is the same as the above steps. The incubation temperature corresponding to the maximum value of ΔA is the optimal temperature of the colorimetric detection system.

Result

Characterization of colorimetric assay based on AuNPs

The concentration of AuNPs solution can also be calculated according to Lambert-Beer's law and the UV absorbance at 450 nm, and the formula is $c = A_{450} / \varepsilon_{450}$,[2] where c is the concentration of AuNPs solution; A is the UV absorbance of AuNPs solution at 450 nm; ε is the molar extinction coefficient of AuNPs; B is the average diameter of free AuNPs. Molar extinction coefficient of 20 nm AuNPs（ε_{450}) is about 5.41×10^{-8} $M^{-1}\bullet cm^{-1}$. Therefore, the concentration of AuNPs solution in this experiment is 4.332×10^{-4}M.

107 **Table S1** The main experimental instruments

Apparatus name	Specification	Manufacturer
Electronic analytical balance	BS124S	Saidoris scientific instrument (Beijing) Co., Ltd
Constant temperature magnetic stirrer	08 - 2G	Shanghai meiyingpu Instrument Manufacturing Co., Ltd
pH meter	PHS-2F	Shanghai Yidian Scientific Instrument Co., Ltd
Millipore-MilliQ Barnstead	Milli-Q Advantage A109	Millipore，USA
High-speed desktop refrigerated centrifuge	Centrifuge 5424	Eppendorf
Constant temperature incubator	ThermoStat Plus	Eppendorf
Micro pipette gun	Different ranges	Eppendorf
Centrifugal tube	1.5 mL	Thermo Fisher Scientific
96 well micro medium	F605033	Shenggong Bioengineering (Shanghai) Co., Ltd
Magnetic separation frame	16 holes	Shanghai Dadi chemical products Co., Ltd
Horizontal electrophoresis instrument	PowerPac Basic	Bio-Rad
Full temperature shaking incubator	HZQ-F160	Suzhou Peiying Experimental Equipment Co., Ltd
PCR instrument	T100 Thermal Cycler	Bio-Rad
Electric constant temperature water bath	XMTE-8112	Shanghai Jinghong Experimental Equipment Co., Ltd
ultramicro spectrophotometer	NanoDrop 2000	Thermo Fisher Scientific
Circular dichroic chromatograph	CD/J-815	JASCO
Field emission transmission electron microscope	TALOS F200X	FEI
Nano Particle Analyzer	Zetasizer Nano S	Malvern
Multifunctional enzyme labeling instrument	Infinite M200 Pro	Tecan Trading AG

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Table S2 The conditions for aptamer selection

Screening rounds	Molar amount of the library (pmol)	Reverse screening content		Positive screening MTM magnetic beads (μL)	Incubation time (min)
		carboxyl magnetic beads (μL)	MBZ magnetic beads (μL)		
1	500	100	—	100	60
2	500	—	100	100	60
3	500	—	100	100	60
4	500	—	100	100	60
5	500	—	100	100	60
6	500	—	100	100	60
7	500	—	100	100	60
8	100	—	100	100	60
9	100	—	100	100	60
10	100	—	100	100	60

Table S3 PCR reaction system

Type	Concentration (μM)	Volume (μL)
ssDNA template	—	3
Forward primer	0.5	1
Reverse primer	500	1
2×HiFiTaq PCR StarMix with Dye	—	25
ddH_2O	—	22

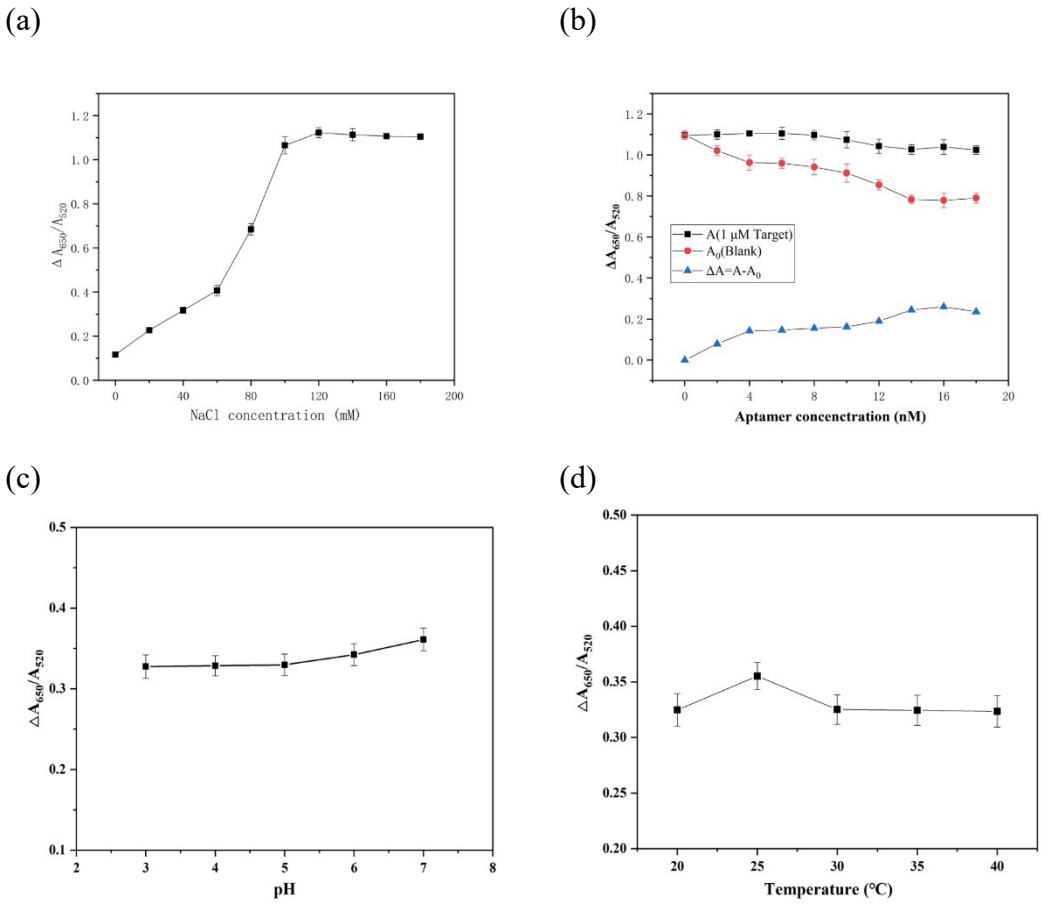
Table S4 PCR reaction cycle

Process	Temperature (°C)	Time (min)
Pre denaturation	95	5
denaturation	95	0.5
annealing	57	0.5
extend	72	0.5
ddH_2O	72	5

Fig S1 The homology analysis of metamitron aptamer

Table S5 The relative standard deviation (σ) of the instrument

No.	1	2	3	4	5	6	7	8	σ
A_{650}/A_{520}	0.124	0.124	0.124	0.123	0.123	0.123	0.123	0.123	4.71×10^{-4}



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124 **Fig S2** Optimization of experimental conditions. (a) Optimization of NaCl concentration; (b)
125 optimization of aptamer concentration. (c)Optimization of pH; (d) optimization of temperature

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Table S6 Determination of spiked in tap water, river water, lake water, and artificial urine (n=3). Experimental conditions: MOPS (10 nM, pH=7.0), 16 nM Aptamer, 120mM NaCl, 200 mL AuNPs, and 100, 200 and 300 nM MTM

Sample	Spiked concentration（nM）	Mean found concentration (nM)	Recovery (%)	RSD (%)
Tap water	100	102.04	102.04	3.34
	200	202.43	101.22	1.75
	300	301.48	100.49	1.77
River water	100	107.83	107.83	2.46
	200	203.21	101.61	1.19
	300	304.91	101.64	1.59
Lake water	100	102.73	102.73	2.76
	200	204.99	102.49	1.46
	300	305.85	101.95	1.21
Urine	100	91.71	91.71	2.49
	200	192.06	96.03	1.11
	300	286.21	95.40	3.48

References

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