Supporting Information

Simultaneous and Visual Detection for Multiple Dopes by Aptamer/AuNPs Sensor

Xuancheng Fu †,[a,b] Miaomiao Gou †,[c] Yong Zhang†,[d] Hao Su,[a] Haotian Zhao,[a] Chang Liu,[a,b] and Jing Han[e]**

[a] School of Sport Science, Beijing Sport University, Beijing 100084, China.

[b] Institute of Anti-Doping in China, Beijing Sport University, Beijing 100084, China.

[c] The Fifth Medical Center, Chinese PLA General Hospital, Beijing 100853, China.

[d] The Second Medical Center, Chinese PLA General Hospital, Beijing 100853, China.

[e] Beijing National Laboratory for Molecular Sciences, Institute of Chemistry Chinese Academy of Sciences, Beijing, 100190, China

Email: changliu.epigenetic@gmail.com

jinghan@iccas.ac.cn

MATERIALS AND METHODS

Materials and Instruments. All organic solvents were obtained from Beijing Chemical Works and used as received. Dexamethasone, salbutamol, and ractopamine were purchased from ehrenstorfte. Glucose, fructose, salidroside, tetrachloroauric(III) acid trihydrate, and sodium citrate were purchased from J&K Scientific Ltd. All chemicals were used without further purification. All of the oligonucleotides used in this paper were synthesized by Sangon Biotechnology Inc. (Shanghai, China). The UV-Vis absorption spectra were recorded via a Thermo Scientific Varioskan LUX. The dynamic light scattering (DLS) was recorded with Malvern Zetasizer Nano ZS90. Hitachi HT7700 transmission electron microscopy (TEM) was employed to determine the morphology of AuNPs before and after aggregation.

Dex aptermer sequence: 5′ -ACACGACGAGGGACGAGGAGTACTTGCCAACGA TAACGTCGTTGGATCTGTCTGTGCCC-3′

Sal aptermer sequence: 5’-AGCAGCACAGAGGTCAGATGT TGTAAAACTTAATCAGCGATTCTCTATGTCTGCAATTACCCTATGCGTGCTA CCGTG-3’

Rac aptermer sequence: 5’-AGTGCGGGC-3’

Preparation of AuNPs. The citrate-protected gold nanoparticles were prepared according to reported procedures. Briefly, 50 mL of 1 mM HAuCl4 was brought to boil with vigorous stirring. Sodium citrate (38.8 mM, 5 mL) was rapidly poured to the vortex of the solution, which result a color change of solution from pale yellow to a wine-red solution. After continuously boiling for 10 min, the solution was cooled to room temperature. Finally, the AuNPs was obtained by filtering through a 0.45-μm membrane

filter, and characterized byUV/Vis spectrometry, dynamic light scattering (DLS), and transmission electron microscopy (TEM).

Optimization of NaCl Concentration. 20 μL milli-Q water was mixed with 70 μL AuNPs. Then, different concentration of NaCl solution (0, 0.5, 1, 1.5, 2, 3, 5 M) at the volume of 10 μL was added. After co-incubated for 5 min in 96-well plate, the A_{650}/A_{520} ratio in absorption spectrum was recorded. As shown in Figure S1, 0.5 M NaCl could induce the total aggregation of AuNPs. Therefore, 0.5 M of NaCl was selected as the suitable salt concentration.

Optimization of Dxm-aptamer, Sal-aptamer, and Rac-aptamer Concentration during single dope detection. 10 μL mill-Q was pre-mixed with different concentration of aptamer (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM) at a volume of 10 μL. Then, 70 μL AuNPs were added into solution, and co-incubated for 15 min. Finally, NaCl (0.5 M, 10 μL) were added intro each well and co-incubated for another 5 min. According to the results of A650/A520 ratio from absorption spectrum, the minimum concentration of aptamers to prevent the aggregation of AuNPs was the optimal salt concentration.

Procedures of detection of Dxm, Sal and Rac during single dope detection. Aptamer (10 μL) and target dope (10 μL) were mixed and incubated for 20 min. Then, 70 μL AuNPs were added into supernatant. Finally, NaCl (0.5 M, 10 μL) was added to mixtures, following the monitoring using UV/Vis spectromer.

Optimization of mixed-aptamers Concentration during multi-dopes detection. Dxm-aptamer (10 μM), Sal-aptamer (10 μM), and Rac-aptamer (10 μM) were pre-mixed with 1:1:1 (v/v/v). 10 μL milli-Q water was pre-mixed with different concentration of mix-aptamer (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM; 10 μL). Then, the AuNPs (70 μL) was added and incubated for 15 min. After the introduction of NaCl solution (0.5 M, 10 μL) and co-incubation, the absorption spectrum was recorded via spectrophotometer. The

concentration of mix-aptamers, which could totally prevent the aggregation of AuNPs, was the suitable concentration.

Procedures of detection of Dxm, Sal and Rac during multi-dopes detection. Similar to the process of "Procedures of detection of Dxm, Sal and Rac during single dope detection", target dopes (10 μL) were added into the solution of mixed-aptamers (10 μL). After 20 min co-incubation, AuNPs (70 μL) was added and incubated for another 15 min. Then, NaCl (0.5 M, 10 μL) was added into the mixtures, following 5 min incubation. All results were recorded by UV/Vis spectromer and camera.

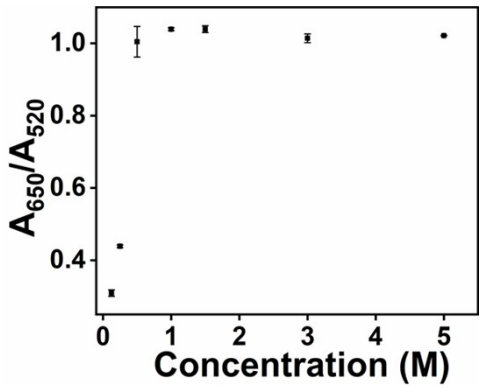

Figure S1. The $A_{650}/_{520}$ nm of supernatant under various concentrations of NaCl.

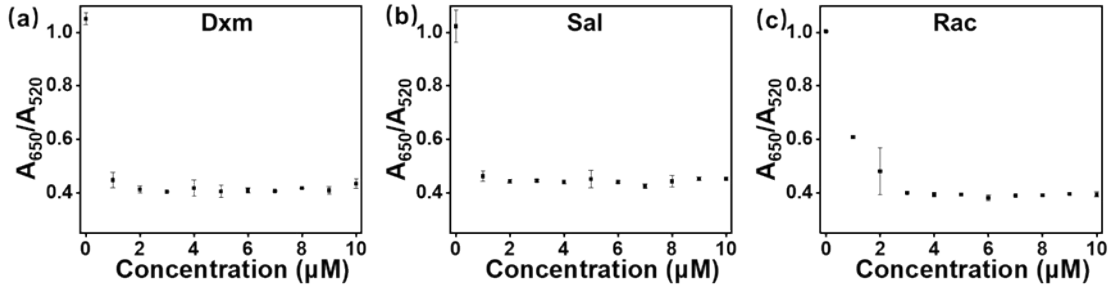

Figure S2. The $A_{650}/_{520}$ ratio of supernatant under various concentrations of aptamer: (a) Dxm, (b) Sal and (c) Rac.

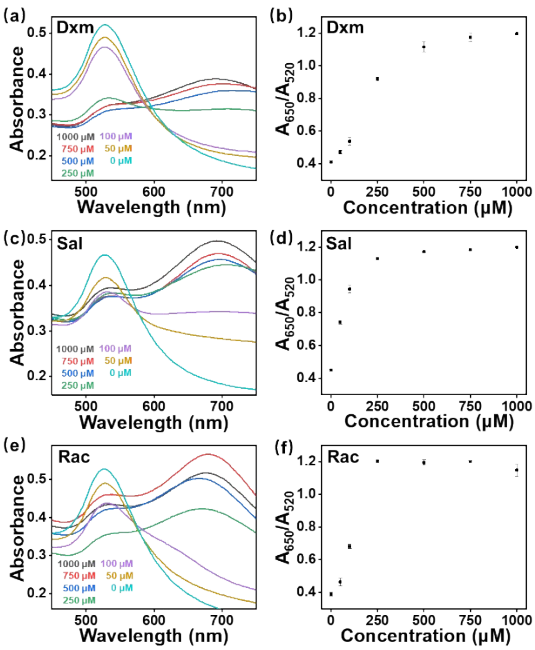

Figure S3. Single-functional Aptamer/AuNPs sensor for dopes detection. Scanning spectroscopy after adding different concentrations of (a) Dex, (c) Sal and (e) Rac and the corresponding (b) Dex, (d) Sal and (f) Rac A650/520 ratios.

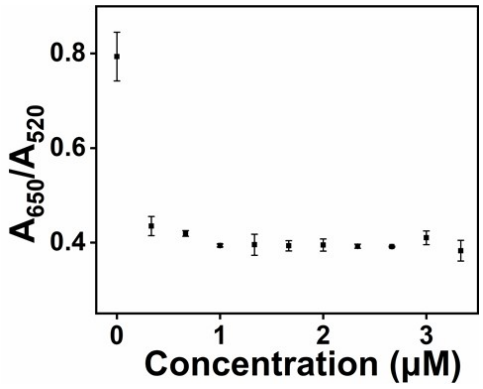

Figure S4. The $A_{650}/_{520}$ ratio of supernatant under various concentrations of the mixed solution of Dxm, Sal and Rac aptamers.

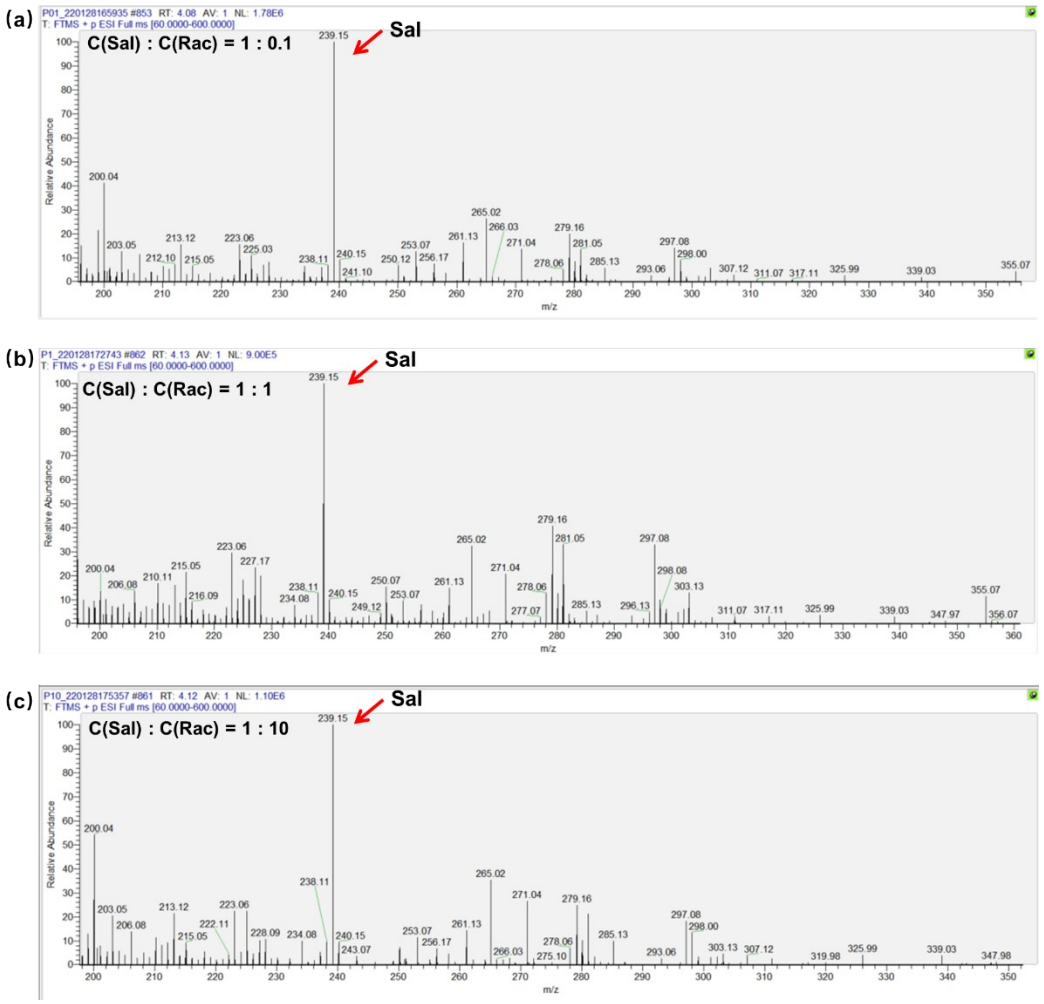

Figure S5. LC-MS results of Sal and Rac in different ratio. The concentration of Sal was set as 10 μM.

Method	Linear range (μM)	Limit of Detection (μM)	Reference
MWCNTs-modified Pencil Electrode	0.15–100	0.09	42
Differential Pulse Voltammetry	26-122	7.6	43
GO/GCE Differential Pulse Voltammetry	0.1–50	0.076	44
edge plane pyrolytic graphite electrode	0.07-100	0.06	45
Multifunctional Aptamer/AuNP Biosensor	0.1-10	0.053	This Work

Table S1. Comparison of majority reported methods for the detection of Dxm.

Method	Linear range (μM)	Limit of Detection (μM)	Reference
Spectrophotometry	1.73-20	0.17	46
Chromatography	1.73–5.00	0.29	47
Capillary electrophoresis	70–300	10	48
MWCNT/GCE	0.80–10	0.20	49
Multifunctional Aptamer/AuNP Biosensor	0.5-100	0.13	This Work

Table S2. Comparison of majority reported methods for the detection of Sal.

Method	Linear range (μM)	Limit of Detection (μM)	Reference
Chemiluminescence	0.059-0.296	0.014	50
High-performance Liquid Chromatography	200-20000	50	51
Electrochemistry	0.1–380	50	52
Gas chromatography–mass Spectrometry	0.073-0.591	0.059	53
Multifunctional Aptamer/AuNP Biosensor	0.05-10	0.011	This Work

Table S3. Comparison of majority reported methods for the detection of Rac.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Dxm 1	0.1	0.102	102	12.3
Dxm 2	1	0.920	92	2.27
Dxm 3	10	11.1	111	12.8
Sal 1	0.5	0.510	102	3.77
Sal 2	1	0.990	99	3.76
Sal 3	10	10.0	100	3.06
Rac 1	0.1	0.118	118	2.08
Rac 2	1	0.93	93	3.05
Rac 3	10	11.3	113	2.32

Table S4. Recoveries and RSD results based on multifunctional aptamer/AuNPs sensor for determination of Dxm, Sal and Rac in urine samples.