Supporting Information

Turn-on Fluorescent Aptasensing for Determination of Serotonin via Target-Induced Knot Displacement at Corona

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1. Experiment

1.1 Reagents and apparatus

Serotonin (5-HT), dopamine hydrochloride (DA) and L-ascorbic acid (AA) were purchased from Sigma-Aldrich (USA). Anti-5-HT aptamer-FAM sequence and six ss-DNAs (including ss-DNA-a-Dabcyl, ss-DNA-b-Dabcyl, ss-DNA-c-Dabcyl, ss-DNA-d-Dabcyl and ss-DNA-e-Dabcyl, random ss-DNA-Dabcyl) were synthesized by Sangon Biotechnology Inc. (China, Table S1). Norepinephrine (NE) and L-3,4-dihydroxyphenylalanine (L-DOPA) were obtained from Wokai Chemical Reagent Co., Ltd (China). 10 mM PBS (containing 0.0018 M NaH_2PO_4, 0.0082 M Na_2HPO_4, 0.10 M KCl, 0.1 M NaCl and 0.005 M $MgCl_2$, pH 7.40) was used as DNA incubation solution. A Millipore Milli-Q water (18.2 MΩ·cm) was used in this work.

FluoroMax-4 (HORIBA, USA), an electrophoresis chamber (Shanghai Titan Scientifc Co., Ltd., China), circular dichroism spectrometer (CD, Applied Photophysics Ltd., UK) were used.

Table S1. Oligonucleotide sequences used in the experiments

Oligonucleotide	sequence (5′→3′)
Anti-5-HT aptamer-FAM	FAM-$(CH_2)_6$-CGA CTG GTA GGC AGA TAG GGG AAG CTG ATT CGA TGC GTG GGT CG
ss-DNA-a-Dabcyl	GCC TAC CAG TCG -$(CH_2)_6$-Dabcyl
ss-DNA-b-Dabcyl	CAG CTT CCC CTA -$(CH_2)_6$-Dabcyl
ss-DNA-c-Dabcyl	TCA GCT TCC CCT -$(CH_2)_6$-Dabcyl
ss-DNA-d-Dabcyl	CAG CTT CCC CTA TTT TTT TTT TTT TTT-$(CH_2)_6$-Dabcyl
ss-DNA-e-Dabcyl	TCA GCT TCC CCT TTT TTT TTT TTT TTT-$(CH_2)_6$-Dabcyl
Random ss-DNA-Dabcyl	ACT TTG TTT GGT-$(CH_2)_6$-Dabcyl

1.2 Preparation of the FAM-ds-DNA-Dabcyl biocomplex

The biocomplexes was fabricated according to the reference.[1] Briefly, 6 μL anti-5-HT aptamer-FAM, 18 μL ss-DNA-Dabcyl and 1976 μL 1× PBS were added into a 2 mL tube with a typically final concentration 30 nM of anti-5-HT aptamer-FAM and 90 nM of ss-DNA-Dabcyl. The mixture complex was incubated for 1 h at 37 ºC using a

mixed incubation instrument. After that, the FAM-ds-DNA-Dabcyl biocomplex was obtained without others handle step.

1.3 Fluorescence measurement for 5-HT detection

The obtained FAM-ds-DNA-Dabcyl biocomplex was incubated with different concentrations of 5-HT for 30 min at 37 ℃. The fluorescence emission was recorded on the FluoroMax-4 (HORIBA, USA) with an excitation wavelength of 465 nm and slit of 5 nm. The concentration of 5-HT was quantified by the increased FL intensity at 515 nm, $\Delta I = I - I_0$, where I_0 and I is the FL intensity in the absence and presence of 5-HT, respectively.

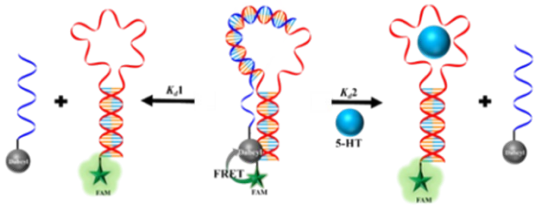
1.4 Calculation for the quenching constant K_{SV}

The quenching constant K_{SV} was calculated according to the Stern-Volmer equation (eq. S1),

$$I_0/I = 1 + K_{SV}C \quad \text{(S1)}$$

where I_0 and I are the FL intensities before and after the addition of the ss-DNA-Dabcyl, K_{SV} is the quenching constant, and C is the ss-DNA-Dabcyl concentration.

1.5 Determination of dissociation constants (K_d)



$$K_{d1} = \frac{[anti-5-HT\ aptamer-FAM][ss-DNA-Dabcyl]}{[FAM-ds-DNA-Dabcyl\ biocomplex]} \quad \text{(S2)}$$

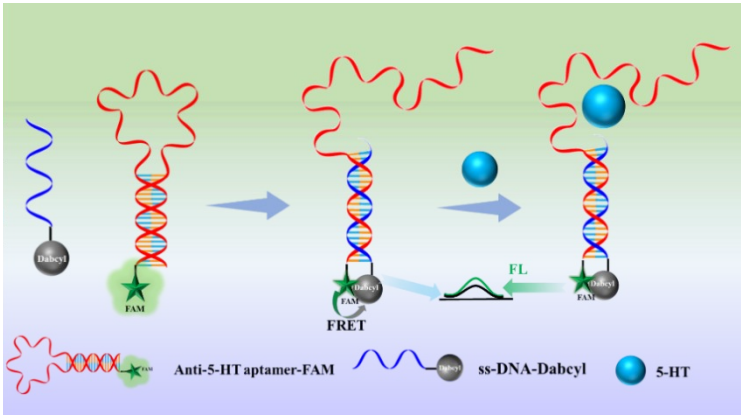
$$K_{d2} = \frac{[ss-DNA-Dabcyl][5-HT \bullet anti-5-HT\ aptamer-FAM]}{[FAM-ds-DNA-Dabcyl\ biocomplex][5-HT]} \quad \text{(S3)}$$

$$K_d = \frac{K_{d1}}{K_{d2}} \quad \text{(S4)}$$

The K_d was calculated by the equation S4 using the obtained K_{d1} and K_{d2}, in which K_{d1} is dissociation constant of FAM-ds-DNA-Dabcyl biocomplex to the ss-DNA-Dabcyl and anti-5-HT aptamer-FAM (eq. S2), while K_{d2} is dissociation constant between the FAM-ds-DNA-Dabcyl biocomplex and 5-HT (eq. S3). Namely, K_{d1} was calculated using the equation S2 by fluorescence quenching method. Upon target 5-HT binding, the anti-5-HT aptamers will dissociate from the formed FAM-ds-DNA-Dabcyl

biocomplex. The K_{d2} was calculated using the equation S3 in the presence of increasing concentrations of 5-HT.

1.6 Schematic diagram for the terminal hybridized tactic



Scheme S1 Schematic diagram of the proposed turn-on fluorescent aptasensing for determination of serotonin via terminal hybridized approach.

2. Results:

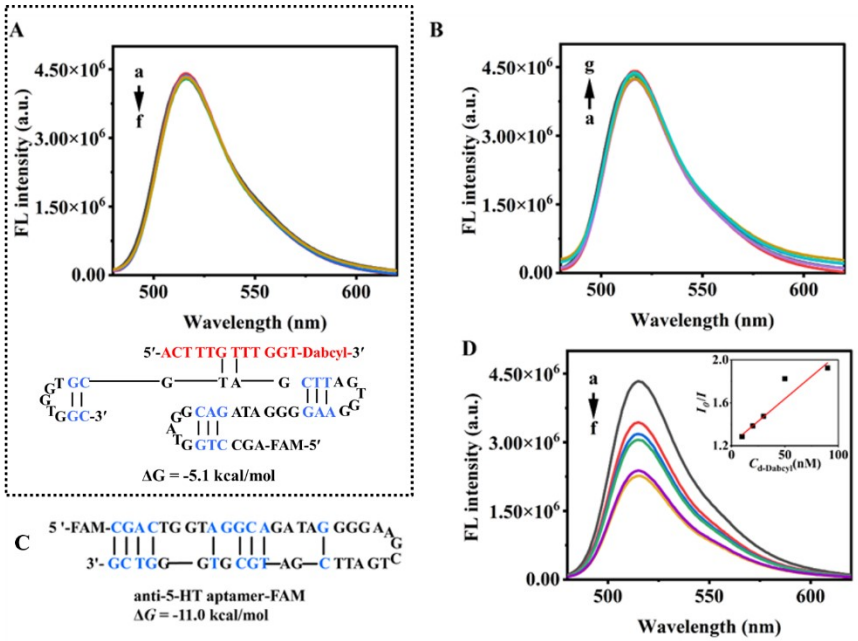


Fig. S1. (A) FL spectra of 30 nM anti-5-HT aptamer-FAM to random ss-DNA-Dabcyl concentration (0, 10, 20, 30, 50 and 90 nM, respectively). Inset, anti-5-HT aptamer-FAM hybridized with random ss-DNA-Dabcyl. (B) FL spectra of anti-5-HT aptamer-FAM reacted with different concentration of 5-HT (a-g, 0, 1, 10, 100, 500 nM, 1.0 μM and 10 μM). (C) Anti-5-HT aptamer-FAM self-hybridization. (D) FL spectra of 30 nM anti-5-HT aptamer-FAM to ss-DNA-d-Dabcyl concentration (0, 10, 20, 30, 50 and 90 nM, respectively). Inset, the relationship between I_0/I and ss-DNA-d-Dabcyl concentration.

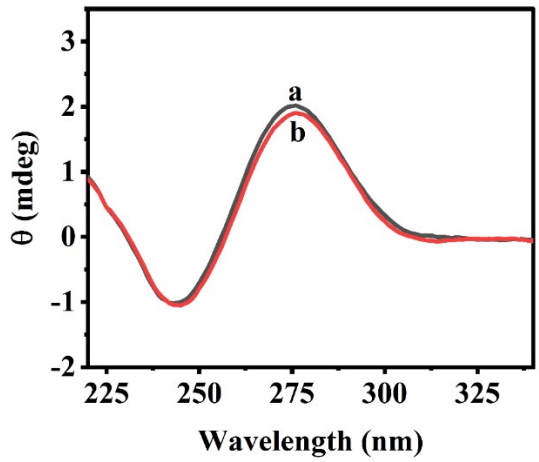


Fig. S2. CD spectra of 3 μM FAM-ds-DNA-a-Dabcyl in the (a) absence and (b) presence of 30 μM 5-HT in 10 mM PBS (pH 7.40).

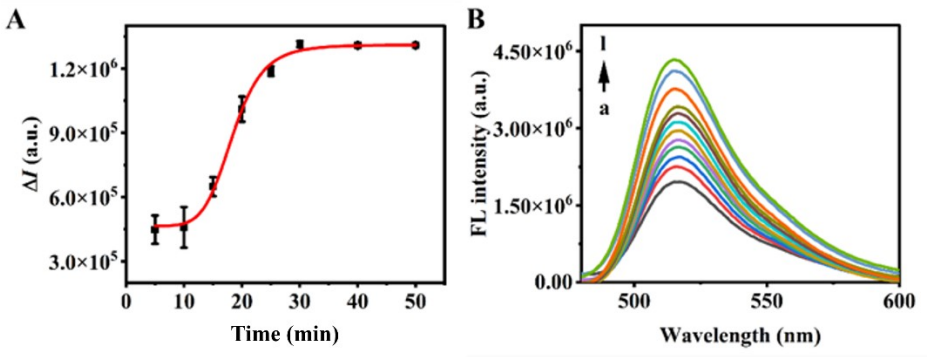


Fig. S3. (A) The relationship between FL intensity and incubation time with 100 nM 5-HT for FAM-ds-DNA-d-Dabcyl knot biocomplex. (B) FL spectra of FAM-ds-DNA-d-Dabcyl reacted with different concentrations of 5-HT (a-l, 0, 0.5, 1, 5, 10, 30, 80, 50,100, 500 nM, 1.0 μM and 10 μM).

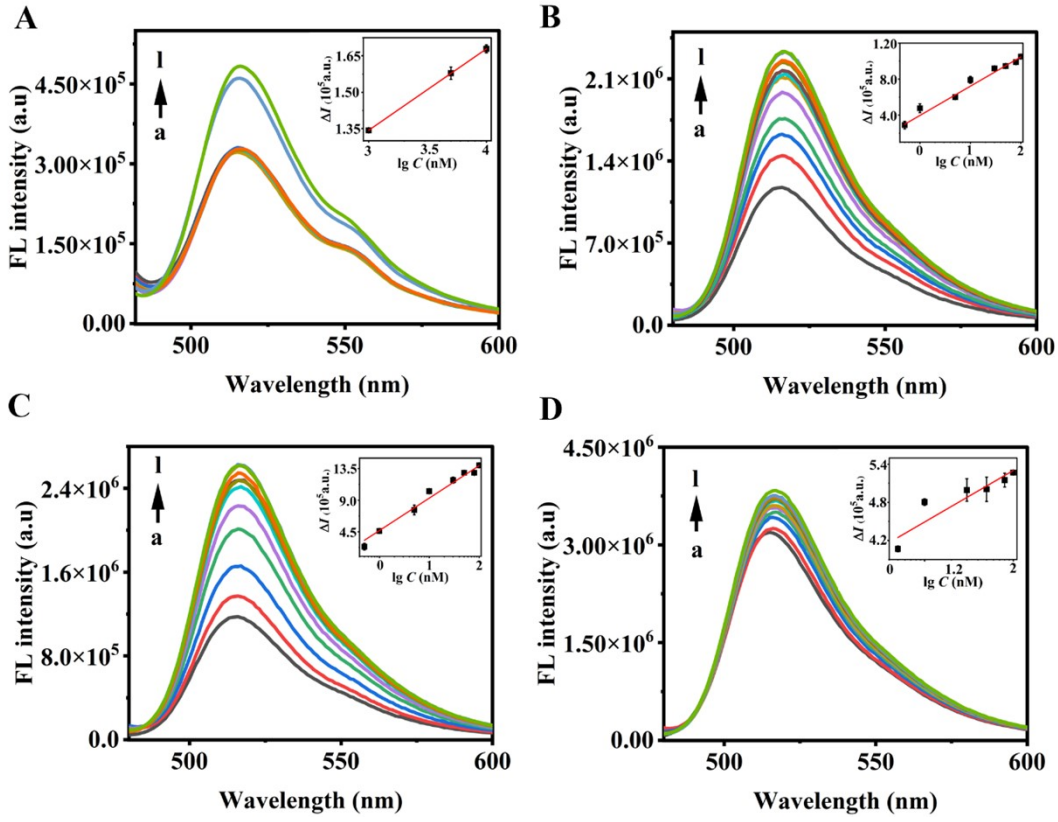


Fig. S4. FL spectra of FAM-ds-DNA-Dabcyl reacted with different concentrations of 5-HT (a-l, 0, 0.5, 1, 5, 10, 30, 50, 80, 100, 500 nM, 1.0 μM and 10 μM), (A) FAM-ds-DNA-a-Dabcyl (B) FAM-ds-DNA-b-Dabcyl (C) FAM-ds-DNA-c-Dabcyl (D) FAM-ds-DNA-e-Dabcyl (inset: calibration curve).

Table S2. Comparison of analytical performance of five FAM-ds-DNA-Dabcyl bio-complex for 5-HT

ds-DNA	Linear range	Detection limit
FAM-ds-DNA-a-Dabcyl	1-10 µM	3 µM
FAM-ds-DNA-b-Dabcyl	0.5-100 nM	0.2 nM
FAM-ds-DNA-c-Dabcyl	0.5-100 nM	0.5 nM
FAM-ds-DNA-d-Dabcyl	0.5-100 nM	0.1 nM
FAM-ds-DNA-e-Dabcyl	5-100 nM	45 nM

Table S3. Comparison of the reported methods for determination of 5-HT

Analytical method	Sensing mechanism	Linear range	Detection limit	References
Electrochemistry	5-HT-apt-Fc	1-100 µM	0.3 µM	(1)
Electrochemistry	Aptamer-MB	1pM-10 nM	0.017 fM	(2)
Optical images based on liquid crystal	CTAB/aptamer	1- 1000 nM	1.68 nM	(3)
Fluorescence	DNA aptamer /SWCNT	0.1-1 µM	-	(4)
Fluorescence	ssDNA−SWCNT	-	100 µM	(5)
Fluorescence	ssDNA-SWCNT	0.1-50 µM	-	(6)
Fluorescence	FAM-ds-DNA-d-Dabcyl	0.5-100 nM	0.1 nM	This work

MB: methylene blue, **Fc:** ferrocene**, CTAB**: cationic surfactant hexadecyl trimethyl-ammonium bromide, **SWCNT**: single-walled carbon nanotube.

Table S4. The controls levels of neurotransmitters in artificial cerebrospinal fluid

Group	aCSF(nM)	References
NE	0.98 ± 0.09	(7)
DA	3.30 ± 3.40	(7)
L–DOPA	3.81	(8)
5–HT	3.30 ± 3.40	(9)

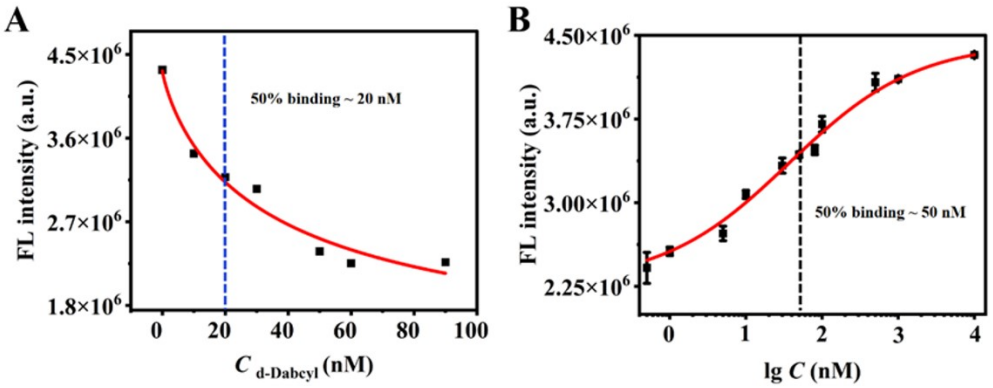


Fig. S5. Determination of binding constants (K_d) for anti-5-HT aptamer-FAM to 5-HT. (A) The relationship between FL intensity of 30 nM anti-5-HT aptamer-FAM reacted with different concentrations of ss-DNA-Dabcyl concentration (0, 10, 20, 30, 50, 60 and 90 nM, respectively). (B) The relationship between FL intensity of FAM-ds-DNA-d-Dabcyl reacted with different concentrations of 5-HT (0, 0.1, 5, 10, 30, 80, 50,100, 500 nM, 1.0 μM and 10 μM). For example, 50% binding between anti-5-HT aptamer-FAM and ss-DNA-d-Dabcyl means the FL intensity of anti-5-HT aptamer-FAM of decrease to 50% of its original FL intensity.

Table S5. Comparison of anti-5-HT aptamer dissociation constants (K_d) value reported by different methods.

Method	K_d	**References**
Field-effect transistors	30 nM	(10)
Fluorescence	127 nM	(11)
Fluorescence	6.3 μM	(6)
Fluorescence	0.307 μM	(4)
Fluorescence	2.3 nM	This work

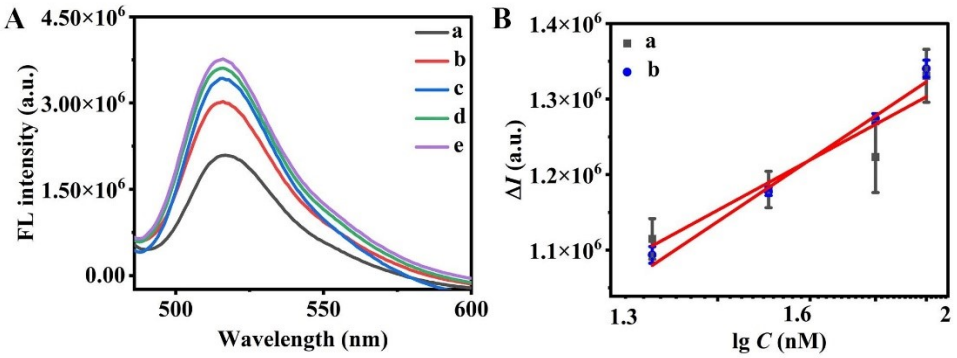


Fig. S6. (A) FL spectra of the FAM-ds-DNA-d-Dabcyl knot biocomplex in aCSF containing different concentrations of 5-HT (a-e, blank, 30, 50, 80 and 100 nM), (B) The relationship between the increased FL intensity and 5-HT concentrations in (a) 10 mM PB (pH 7.40) and in (b) an aCSF.

Table S6. The recovery **(%)** of 5-HT in artificial cerebrospinal fluid

Sample	**Added (nM)**	**Found (nM)**	**Recovery (%)**
1	30	31.6 ± 2.3	105.3 ± 7.6
2	50	49 ± 1.0	98 ± 2.0
3	80	79.4 ± 1.9	99.2 ± 2.3
4	100	100.1 ± 2.4	100.1 ± 2.4

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