Electronic supplementary information

A high affinity dopamine aptamer: implications of library diversity and negative selections

Yi Yu[1,2], Yuanli Li[2] and Juewen Liu[2]*

1. School of Laboratory Medicine, Hubei University of Chinese Medicine, 16 Huangjia Lake West Road, Wuhan, 430065, PR China
2. Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada
Email: liujw@uwaterloo.ca

Table S1. The DNA sequences used in this work

Names	Sequences (from 5′ to 3′)
Library	GGAGGCTCTCGGGACGAC-**N36**-GTCGTCCCGATCACTTGAATGGTCT
cDNA-bio	GTCGTCCCGAGAGCCATA/3BioTEG/
Forward primer	GGAGGCTCTCGGGACGAC
Reverse primer	CTGTGATTCAGAGCATCGGGACG
Bio-reverse primer	/5Biosg/CTGTGATTCAGAGCATCGGGACG
P5-506	AATGATACGGCGACCACCGAGATCTACACACTGCATAACACTCTTTCCC TACACGACGCTCTTCCGATCTTTACGATTGCAGCATCGGGACG
P7-702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTGACTGGAGT TCAGACGTGTGCTCTTCCGATCTGGAGGCTCTCGGGACGAC
DA-T	GACGACTCCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAAGTCGTC
DA-A	CGACGCCAGTTTGAAGGTTCGTTCGCAGGTGTGGAGTGACGTCG
DA-A′	GACGACTCCAGTTTGAAGGTTCGTTCGCAGGTGTGGAGTGAAGTCGTC
FAM-DA-T	/56-FAM/CTCTCGACGACTCCAGTTTGTAGGTTCG TTCGCAGGTGTGGAGTGAAGTCGTC
FAM-DA-A	FAM-/56-FAM/CTCTCGACGACTCCAGTTTGAA GGTTCGTTCGCAGGTGTGGAGTGAAGTCGTC
Quencher-cDNA	AGTCGTCGAGAG-BHQ
Control	GACGACGAGTATGCGAGTGGAACACGAATGAAATGGGTCGTC

Note: /3BioTEG/ is biotinylation at the 3′-end with extended spacer, /5Biosg/ is biotinylation at the 5′-end. /56-FAM/ is a 5′ carboxyfluorescein label. BHQ is a Black Hole Quencher

Table S2. Negative selection conditions for the previous dopamine selection by the Stojanovic group.

Round	Target concentrations (Number of elutions)	Countertarget concentarions (Number of elutions)
1-2	100 μM (3)	No counter selection
3-4	100 μM (3)	Serotonin 100 μM (8)
5	100 μM (3)	No counter selection
6	100 μM (3)	Serotonin 200 μM (16)
7	100 μM (3)	Serotonin 200 μM (16)
8	100 μM (3)	Tyrosine 100 μM (16)
9	100 μM (3)	Tyrosine 200 μM (16)
10	100 μM (3)	Tyrosine 200 μM (16)
11	50 μM (3)	L-Dopa 200 μM (8)
12	50 μM (3)	L-Dopa 200 μM (16)
13	50 μM (3)	No counter SELEX
14-17	20 μM (3)	Serotonin 100 μM (16)
18	20 μM (3)	Serotonin 20 μM (20)

A

```
Round 9: total reads 20604
R9-1  GACGAC ATGGGAGGATGCAAATCCGGCAACGAGAGTACTTAT GTCGTC 43 reads
R9-2  GACGAC GGGGAGAAATATGTTTTTCGGCAGCTCAAGCACTTC GTCGTC 42 reads
R9-3  GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAA GTCGTC 30 reads
R9-4  GACGAC TCCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAA GTCGTC 13 reads
R9-5  GACGAC GCTGGGGGGCTTAGGGCCTTGTGTATAACAATGGGG GTCGTC 9 reads
R9-6  GACGAC TCCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGTA GTCGTC 7 reads
R9-7  GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGGA GTCGTC 5 reads
R9-8  GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGCA GTCGTC 3 reads
R9-9  GACGAC GGGGAGAAATATGTTTTTCGGCAGCTCAAGCCCTTC GTCGTC 3 reads

Round 12: total reads 23205
R12-1 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAA GTCGTC 5962 reads
R12-2 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGGA GTCGTC 559 reads
R12-3 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGCA GTCGTC 237 reads
R12-4 GACGAC TCCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGTA GTCGTC 170 reads
R12-5 GACGAC TCCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAA GTCGTC 111 reads
R12-6 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGCGTGGAGTGAA GTCGTC 27 reads
R12-7 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGTA GTCGTC 26 reads
R12-8 GACGAC CGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAA GTCGTC 21 reads
R12-9 GACGAC TGCAGTTTGTAGGTTCGTTCGCAGGTGTGGAGTGAG GTCGTC 21 reads
```

B

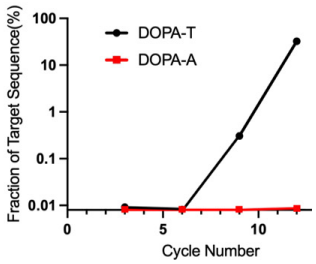

Figure S1. The second dopamine selection experiment in this work. Dopamine concentration was 100 μM from rounds 1-9 and then decreased to 20 μM from rounds 10-12. (A) Alignment of the top 9 sequences from round 9 and round 12. The core aptamer motif is highlighted in yellow. In round 9, only five out of the 9 sequences were the aptamer and the enrichment was still low, and by round 12, all the top 9 sequences are the aptamer and the top sequence reached nearly ¼ of the library. All these top sequences have a thymine in that variable position. (B) The growth of the two aptamer motifs at round 3, 6, 9 and 12. Dopa-A was observed only at round 12 with 2 copies.

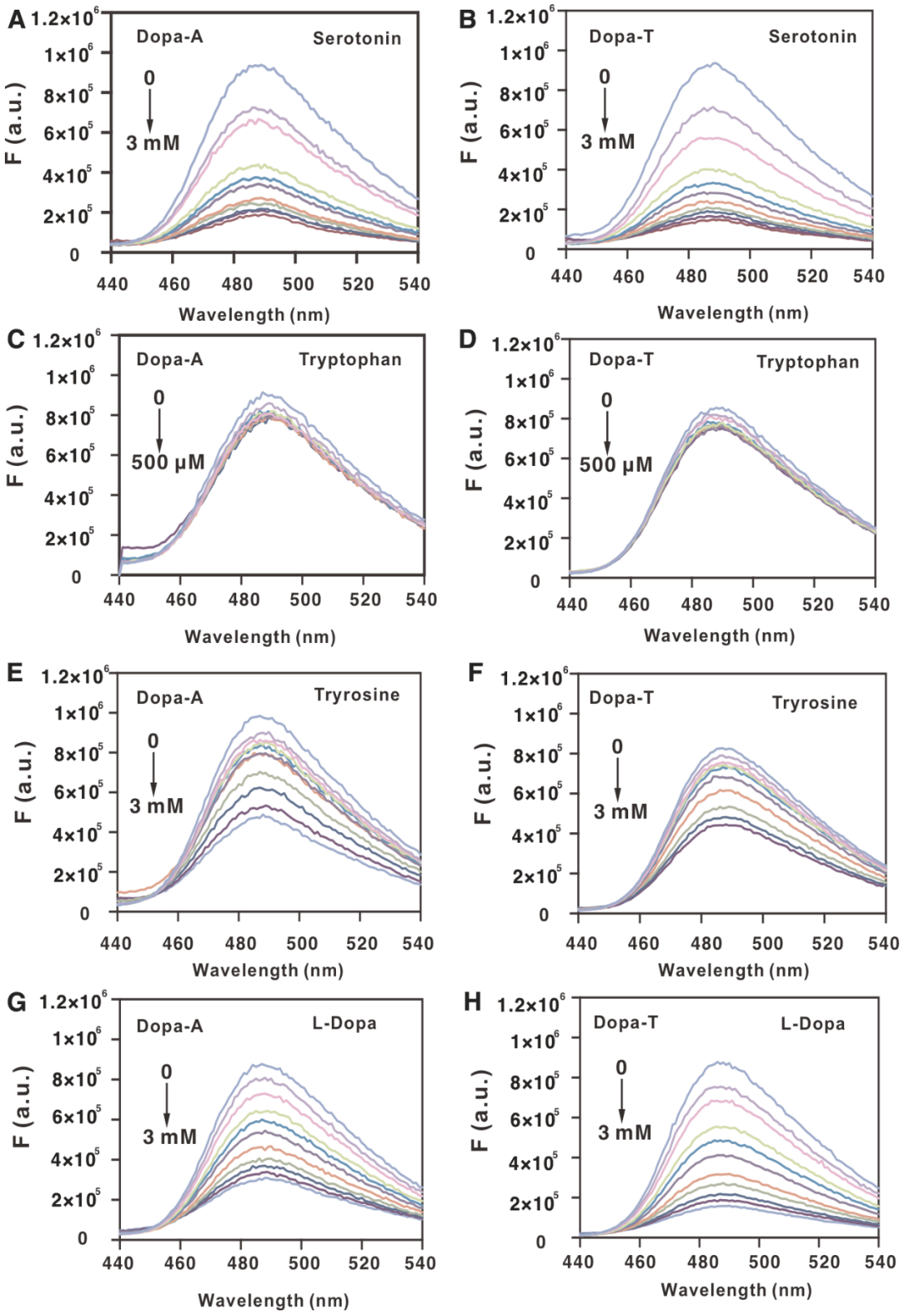

Figure S2. Fluorescence spectra of the aptasensors based on Dopa-A (A, C, E, G) and Dopa-T (B, D, F, H) in the presence of different concentrations of serotonin, tryptophan, tyrosine, or L-DOPA.