Electronic Supplementary Material (ESI) for Analyst.

NIR quantum dot construction of fluorescence anisotropy signal amplification biosensor for sensitive rapid and separation-free detection of dopamine in serum

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Supporting Information

1.Materials and Reagents

1-octadecene (ODE, tech.90%),1-Octanethiol (OT, tech.98%), Selenium powder (≥99.99%), n-Octylamine (tech.99%), polyacrylic acid (PAA,M.W~2000) were purchased from Aladdin Industrial Corporation. Silver acetate (AgAc), tetrachloroethylene, acetone, n-hexane, trichloromethane, anhydrous ethanol, borax, boric acid, ethyl acetate, dimethyl formamide (DMF), tetramethylammonium hydroxide (TMAH), hydrochloric acid, dimethyl sulfoxide (DMSO), hydrogen peroxide, nitric acid, potassium dihydrogen phosphate, potassium chloride, sodium dihydrogen phosphate, sodium chloride were purchased from China National Pharmaceutical Co.,Ltd. Tris and agarose were purchased from Jiru Biotechnology Group Corporation. N-(3-Dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC·HCl) was purchased from Shanghai Medpep Company Limited. Nucleic acid aptamer sequences were purchased from Shanghai Sangon Biotech Co., Ltd. The list of oligonucleotide sequences is in Table T3.

We use UV--3600 UV-Vis-NIR spectrophotometer (Shimadzu) to acquire UV absorption spectra. Analytical Balance Model ML204 (Mettler) was used to weigh. NIR fluorescence emission spectra were acquired by a Fluorolog-3 fluorescence spectrophotometer (HORIBA Jobin Yvon Inc). Malvern Zetasizer Nano ZS nanoparticle size and surface potential analyzer (Malvern Instruments Ltd) was used to measure the hydrated particle size and zeta potential. A DYY-Ⅲ-8B electrophoresis instrument (Beijing Liuyi Instruments) was used for electrophoresis experiments with 1% agarose gel. JEM-2100(UHR) (JEOL Japan Electronics) transmission electron microscopy was used at an accelerating voltage of 200kV to acquire picture. Bruka D8 Advanced X-ray powder diffraction (SMART APEX CCDII) was used to acquire powder diffractograms with a scan range of 2-80°and a scan speed of 0.5°/min. X-ray photoelectron spectroscopy (XPS) information was obtained by ESCALAB 250Xi (Thermo Fisher Scientific). IRIS Intrepid Ⅱ XSP type full spectrum inductively coupled plasma emission spectrometer ICP-AES (Thermoelectric Elements Inc, USA) was used for elemental analysis.

2. Experimental Section

Synthesis of OPA

DMF was added in a round bottom flask. PAA and EDC·HCl were added to the flask and dissolved with stirring at room temperature. After homogenization of the solution add n-octylamine drop by drop and react at room temperature for 12 hours. After the reaction, the DMF in the reaction solution was spin-dried. the remaining reaction material was dissolved with acetone and washed with water to obtain a white solid. Ethyl acetate was added to dissolve the solid, and then TMAH was added to extract. The extracted liquid was adjusted to pH=2 with hydrochloric acid, at which time a white solid was precipitated and washed with water to pH=6. The white solid was dissolved with ethanol and then vacuum dried to obtain a white powder, which is OPA[1].

Optimization of DFAP-SAB

In order to optimize the constructed fluorescence biosensor, we optimized the number of bases of complementary DNA strands, the concentration of streptavidin, the incubation temperature and time of the probes with the targets. Keep the concentration of DFAP probe at 50 nM. First, we tested the effect of cDNAs ranging from 11 to 17 bases in length. The fluorescence anisotropy values of the biosensors constructed with different base numbers were measured separately without the addition of the target dopamine, and then the target dopamine was added to the probes and incubated, and the fluorescence anisotropy values were measured again. Then we tested the effect of the concentration of streptavidin on the detection signal. After the probe bound to the complementary DNA to form a double chain body, we added different concentrations of streptavidin to form a fluorescence complex and detected the fluorescence anisotropy values separately. Then add the target dopamine and incubate for a period of time, and measure the fluorescence anisotropy values again.

3. Supplementary Figures

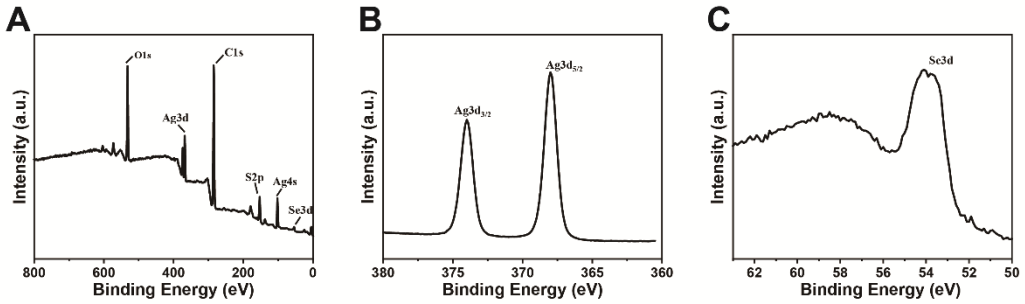

Fig. S1 XPS characterization of hydrophobic Ag_2Se QDs: (A) XPS survey spectrum. (B)The corresponding high-resolution XPS spectra of Ag. (C) The corresponding high-resolution XPS spectra of Se.

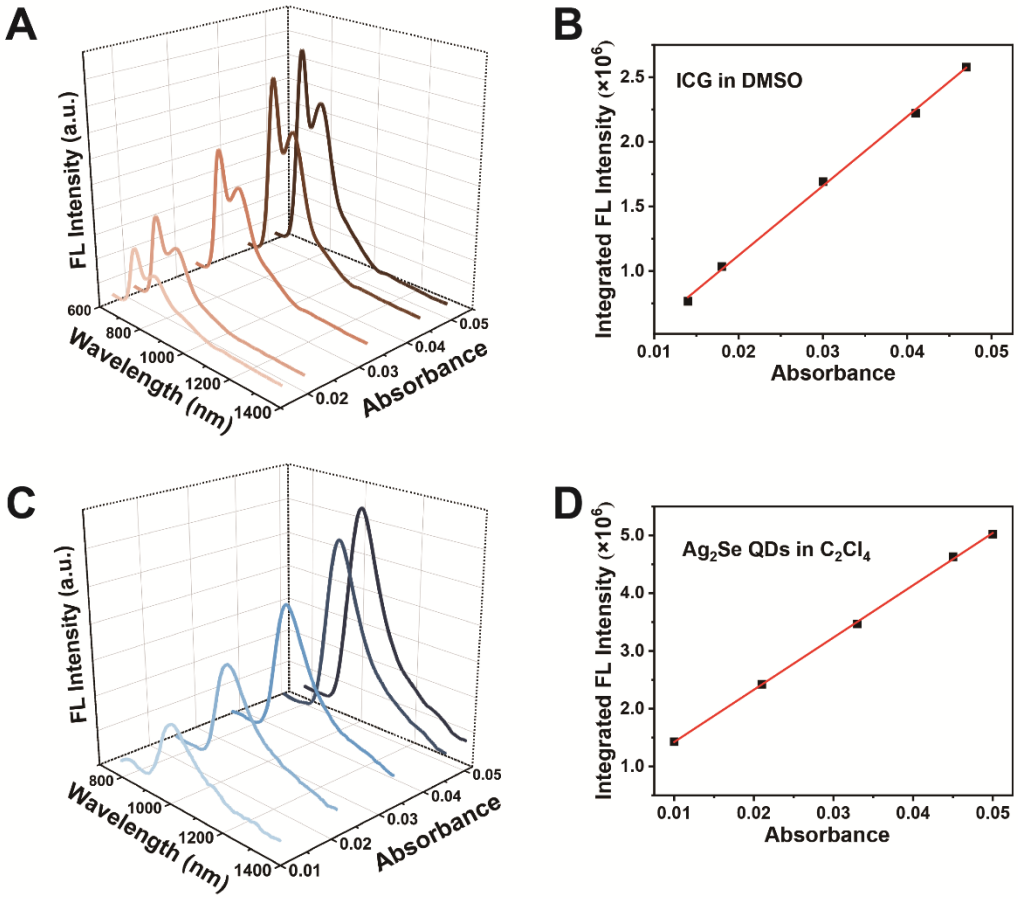

Fig. S2 Measurement of hydrophobic quantum yields: (A)Fluorescence emission spectra of ICG, and the integrated fluorescence intensity versus absorbance of ICG in DMSO. (B)Fluorescence emission spectra of the as-prepared Ag_2Se QDs, and the integrated fluorescence intensity versus absorbance of the as-prepared Ag_2Se QDs in C_2Cl_4.

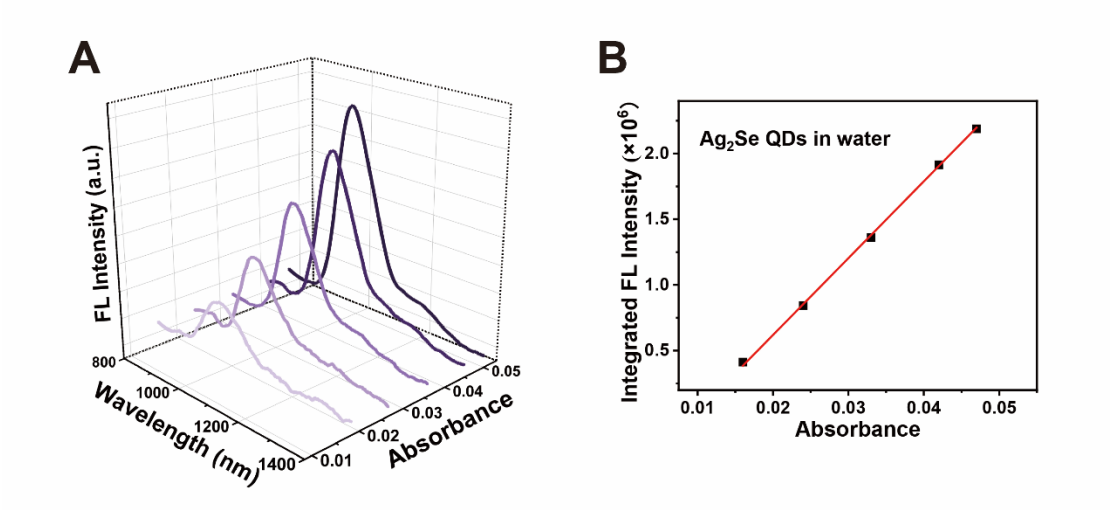

Fig. S3 Measurement of hydrophilic quantum yields.

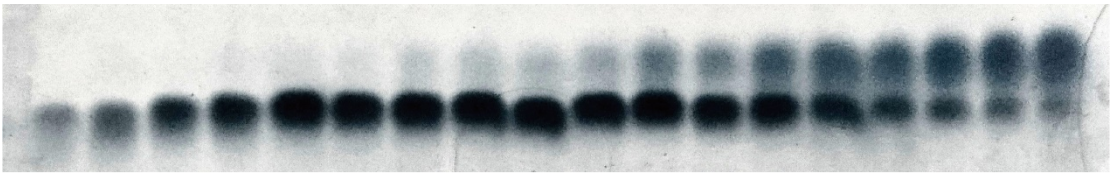

Fig. S4 Results of agarose gel electrophoresis after purification by separation column: The bottom row is quantum dots; the top row is empty micelles.

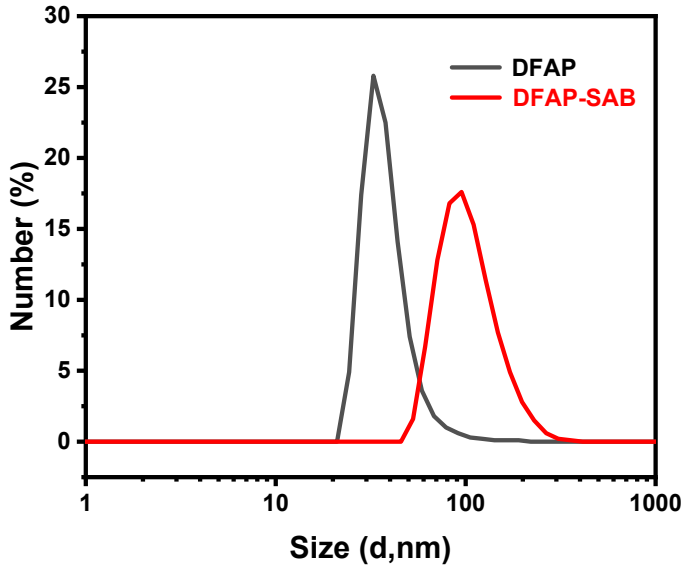

Fig. S5 The hydrated particle size of DFAP and DFAP-SAB

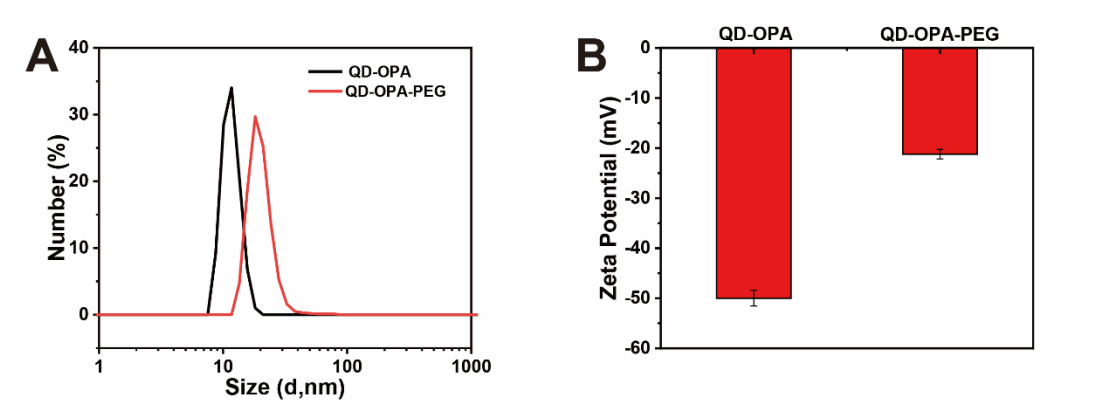

Fig. S6 Connect PEG: (A) Hydration particle size before and after binding of PEG. (B) Potential before and after binding of PEG.

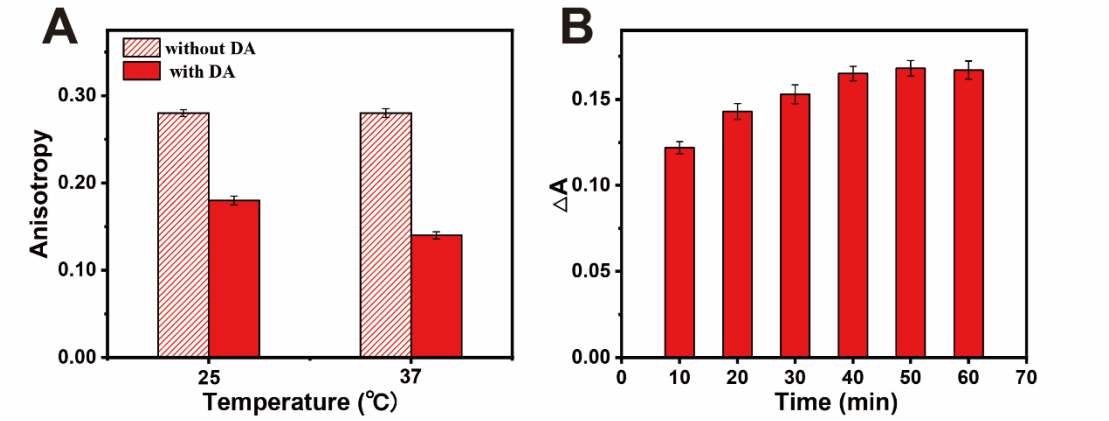

Fig. S7 Incubation conditions of the target to be measured and the DFAP-SAB.

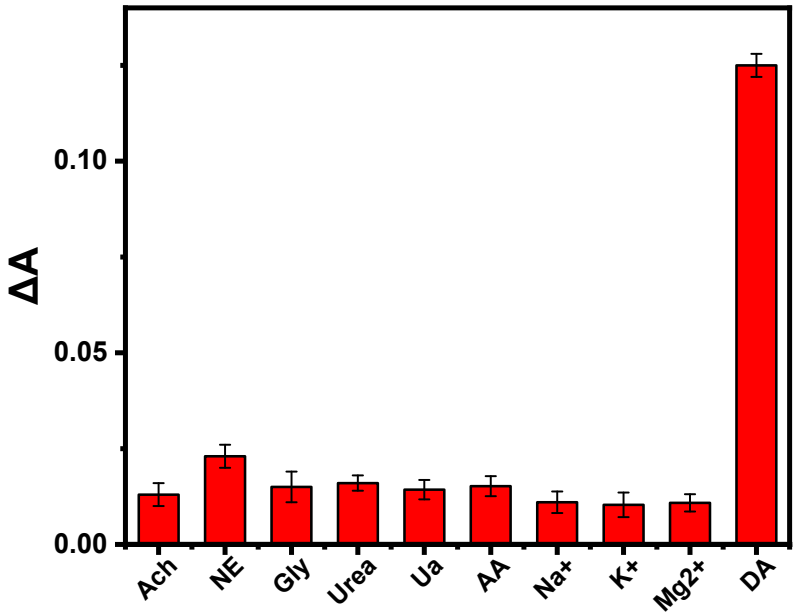

Fig. S8 Specificity of DFAP detection probes: Fluorescence anisotropy values of different neurotransmitters after incubation with DFAP probes. (Ach: acetylcholine, NE: norepinephrine, Gly: glycine, Urea: urea, Ua: uric acid, AA: ascorbic acid, Na^+: NaCl, K^+: KCl, Mg^{2+}: $MgCl_2$, DA: dopamine)

Table T1. The probe is compared with the recently reported DA sensor

Method	Linear range (mol/L)	Detection limit (mol/L)	Detection time	Refs
Colorimetric	1.0×10^{-7}-7.5×10^{-6}	3.1×10^{-8}	10min	2
HPLC	– –	2.0×10^{-10}	6h	3
Electrochemistry	5.0×10^{-7}- 1.2×10^{-4}	1.0×10^{-8}	– –	4
Chemiluminescence	1.0×10^{-8}- 7.0×10^{-7}	2.3×10^{-9}	3min	5
SERS	1.0×10^{-11}- 1.0×10^{-5}	2.3×10^{-12}	2h	6
Proposed	5.0×10^{-8} - 3.0×10^{-6}	1.2×10^{-8}	40min	This work
Graphene QDs	2.5×10^{-7} – 5.0×10^{-5}	9.0×10^{-8}	4h	7
CdTe0.5S0.5/ZnS QDs	2.63×10^{-6} – 2.63×10^{-5}	6.6×10^{-9}	3h	8
Silicon Nanoparticles	5.0×10^{-9} - 1.0×10^{-5}	3.0×10^{-10}	3h	9

Table T2. ICP-AES results of the as-prepared Ag_2Se QDs

Sample	Ag	Se
Concentration (mg/L)	**0.59**	**0.09**
Relative atomic ratio	**6.56**	**1**

Table T3. The list of oligonucleotide sequences

Name	Sequence (5′ to 3′)
DNA	COOH-GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGATATGGGCCAGCACA GAATGAGGCCC
cDNA	Biotin-GCGCACACAGACAG
cDNA	Biotin-GGGCCTCATTCTGT
cDNA11	CACACAGAGACTTTTTT- Biotin
cDNA13	CGCACACAGAGACTTTTTT- Biotin
cDNA15	GGCGCACACAGAGACTTTTTT- Biotin
cDNA17	CTGGCGCACACAGAGACTTTTTT- Biotin

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