Supplementary Information

PolyA-Tail-Mediated Assembly of a Gold Nanoparticle-Based Turn-on Fluorescent Aptasensor for Ultrasensitive Detection of Perfluorooctanoic acid

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Experimental Section:

1. Materials and Reagents

Anhydrous sodium dihydrogen phosphate (NaH_2PO_4), anhydrous disodium hydrogen phosphate (Na_2HPO_4) and disodium citrate dihydrate were purchased from Sangon Biotech (Shanghai, China). Perfluorooctane sulfonate (PFOS) was obtained from J&K Chemical (Beijing, China). Sodium tetrachloroaurate dihydrate ($NaAuCl_4 \cdot 2H_2O$), perfluoroheptanoic acid (PFHA), perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUnDA) and perfluododecanoic acid (PFDoA) were obtained from Aladdin (Shanghai, China). Hydrochloric acid (HCl), nitric acid (HNO_3), tris(hydroxymethyl)aminomethane (Tris Base) and perfluorooctanoic acid (PFOA) were supplied by Sinopharm Chemical Reagent Co. (Shanghai, China). The sequences of the PFOA aptamer and its complementary DNA (FAM-cDNA), provided by Sangon Biotech (Shanghai, China), are listed in Table S1. Ultrapure water was obtained from Wahaha Group Co. Ltd. (Hangzhou, China). All chemicals were of at least analytical grade and used without further purification.

2. Instrumentation

Transmission electron microscopy imaging was conducted on a JEOL JEM-2100 instrument at an accelerating voltage of 200 kV. UV–vis absorption spectroscopy was performed with a Shimadzu UV-3600PLUS spectrophotometer. Fluorescence spectra were acquired using a Hitachi F-7000 fluorescence spectrometer. Dynamic light scattering measurements for particle size determination were carried out on a Malvern

Instruments Nano ZS Zetasizer.

3. Synthesis of PFOA aptamer-modified AuNPs (AuNP-Apt)

AuNPs were synthesized according to previously reported methods[S1, S2]. Briefly, 100 mL of chloroauric acid solution (1/10000, w/w) was placed in a round-bottom flask and heated with stirring in a magnetic heating mantle. After the solution was stirred uniformly and brought to a boil for 10 minutes, 2 mL of freshly prepared 1% (w/w) sodium citrate was rapidly added. The mixture gradually turned wine-red and was heated for an additional 10 minutes. Following heating, stirring was continued for 15 minutes before allowing the solution to cool to room temperature. Finally, the solution was diluted to 100 mL with pure water to obtain a wine-red AuNP suspension. All glassware used in the AuNP synthesis was pre-treated by soaking in aqua regia (a 3:1 volume mixture of concentrated hydrochloric acid and concentrated nitric acid) overnight, followed by thorough rinsing with ultrapure water and drying. **Caution:** aqua regia is highly corrosive and strongly oxidizing, and may evolve toxic fumes. All handling was performed in a certified fume hood with appropriate personal protective equipments. Wastes were collected and disposed of as hazardous oxidizing acidic waste according to institutional regulations.

To conjugate the aptamer to the AuNPs, 20 μL of the aptamer solution (10 μmol L^{-1}) was added to 400 μL of the prepared AuNP solution in a 1.5 mL centrifuge tube. The mixture was gently vortexed and then incubated in a refrigerator at -80°C for 18 minutes. Following incubation, the solution was centrifuged at 15,000 rpm for 30 minutes at 4°C. The supernatant was carefully removed, and the pellet was resuspended

in an equal volume of a washing solution containing 10 mM phosphate buffer (pH 7.4) and 0.1 M NaCl. This centrifugation-washing cycle was repeated twice more to ensure the complete removal of unbound aptamers. Finally, the obtained AuNP-Apt conjugate was redispersed in PBS (0.3 M NaCl, 10 mM phosphate buffer, pH 7.4) and stored at 4°C in the dark until use.

AuNP-Apt conjugates via thiol-gold bonding were prepared following a previously reported protocol with modifications[S3]. Briefly, 400 μL of as-prepared AuNPs was mixed with 20 μL of thiol-modified aptamer (5′-SH-C6-Aptamer-3′, same molar concentration as Group A). The mixture was incubated at room temperature for 12 h with gentle shaking to allow Au–S bond formation. Subsequently, phosphate buffer (0.1 mol L^{-1}, NaH_2PO_4–Na_2HPO_4, pH 7.4) and SDS solution (1%) were added to final concentrations of 0.01 mol L^{-1} and 0.01%, respectively. NaCl solution (0.2 mol L^{-1}) was then added slowly to a final concentration of 0.01 mol L^{-1}. The resulting conjugates were purified by centrifugation at 10 000 rpm for 15 min, and the supernatant was collected for DNA quantification. The pellet was resuspended in 400 μL of PBS (10 mmol L^{-1}, pH 7.4) and washed twice under the same conditions. All supernatants were collected and combined for subsequent determination of unbound DNA. The final pellet was resuspended in PBS and stored at 4 °C prior to use.

AuNP-Apt conjugates via direct adsorption were prepared following a previously reported protocol with modification[S4]. Briefly, 400 μL of as-prepared AuNPs was mixed with 20 μL of unmodified aptamer solution (same molar concentration as Groups A and B). The mixture was incubated at room temperature for 2 h with gentle shaking.

The resulting conjugates were purified by centrifugation at 10 000 rpm for 15 min, and the supernatant was collected for DNA quantification. The pellet was resuspended in 400 μL of PBS (10 mmol L^{-1}, pH 7.4) and washed twice under the same conditions. All supernatants were collected and combined for subsequent determination of unbound DNA. The final pellet was resuspended in PBS and stored at 4 °C prior to use.

4. Sample Preparation from fish tissues

Live crucian carp (*Carassius auratus*) and perch (*Lateolabrax japonicus*) were purchased from a local supermarket. The fish were deboned, cut into small pieces, and homogenized into a paste using a grinder. The homogenate was transferred into glass sample bottles, sealed, labeled, and stored at -18 °C until analysis.

Sample extraction procedures were performed according to the Chinese National Food Safety Standard GB 5009.253—2016 with minor modification. Specifically, 5 g of the fish sample (accurately weighed to 0.01 g) was placed into a 50 mL centrifuge tube, followed by the addition of 50 mL of water. The mixture was vortexed for 1 minute, after which 10 mL of acetonitrile and 30 μL of hydrochloric acid were added and shaken for 10 minutes. Subsequently, 2 g of sodium chloride was added, and shaking was continued for another 10 minutes. The mixture was then centrifuged at 5000 rpm for 10 minutes. The upper acetonitrile layer was transferred to a clean tube and concentrated under a gentle stream of nitrogen in a 45 °C water bath until the volume reached approximately 4 mL. Finally, the solution was passed through a 0.22 μm organic filter membrane, and the filtrate was collected for subsequent analysis.

5. Determination of PFOA

The detection probe was assembled by mixing 350 μL of Tris-HCl buffer, 30 μL of complementary strand cDNA (1 μmol L^{-1}), and 120 μL of AuNP-Apt solution (6 nmol L^{-1}) in a 2-mL centrifuge tube, followed by overnight incubation at room temperature. For detection, 200 μL of the probe was added to 200 μL of either PFOA standard (at varying concentrations) or fish sample extract, making a total volume of 400 μL with Tris-HCl buffer. After 60 minutes of incubation, the fluorescence was measured. Selectivity was evaluated by replacing PFOA with structural analogues—PFOS (50 μmol L^{-1}), PFHpA, PFNA, PFUnDA, and PFDoA (each at 100 μmol L^{-1})—while keeping all other procedures identical.

6. Quality Control and Precision Evaluation

A certified reference material of PFOA in water was obtained from Beijing Weiye Metrology Standard Materials Research Center (Beijing, China). Quality control (QC) samples were independently prepared at three concentration levels (100, 150, and 200 nmol L^{-1}) from a separately prepared PFOA stock solution, which was distinct from that used for the calibration standards. For the evaluation of intra-batch precision, each QC level was analyzed six times within the same day. For the evaluation of inter-batch precision, each QC level was analyzed once per day over seven consecutive days.

7. Calculation of the Förster Radius (R_0):

The Förster radius was calculated using the following equation:

$$R_0(\text{Å}) = 0.2108 \times (\kappa^2 n^{-4} \Phi_D J)^{1/6}$$

where κ^2 is the orientation factor (2/3, assuming random orientation), n is the refractive index of the medium (1.33 for water), Φ_D is the fluorescence quantum yield of the donor (0.92 for FAM), and J(λ) is the spectral overlap integral between the area-normalized FAM emission spectrum and the AuNP molar extinction coefficient spectrum (ε_{520}= 2.7 × 10^8 M^{-1} cm^{-1} for 13 nm AuNPs). The spectral data were obtained from Fig. 2C. The calculated overlap integral J(λ) was defined as:

$$\int \overline{F}(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

where $\overline{F}(\lambda)$ is the area-normalized donor emission spectrum and $\varepsilon_A(\lambda)$ is the molar extinction coefficient spectrum of the acceptor (AuNP). The FAM emission spectrum (510–550 nm) was area-normalized by dividing each intensity value by the integrated spectral area ($\int F(\lambda)d\lambda$ = 86,985), yielding F̄(λ) = F(λ) / 86,985, with an emission peak at 520.4 nm. The AuNP UV–Vis absorption spectrum was converted to molar extinction coefficient using the known value ε_{520}=2.7 × 10^8 M^{-1} cm^{-1} for 13 nm AuNPs at 520 nm, and the measured absorbance at 520 nm (A_{520} = 0.946), giving a conversion factor of ε_{520} / A_{520} = 2.7 × 10^8 / 0.946 = 2.85 × 10^8 M^{-1} cm^{-1}, such that $\varepsilon_A(\lambda)$ = A(λ) 2.85 × 10^8 M^{-1} cm^{-1}. The product $\overline{F}(\lambda)\varepsilon_A(\lambda)\lambda^4$ was evaluated point-by-point across 510–550 nm and integrated numerically using the trapezoidal rule, yielding:

$$J(\lambda) = 1.64 \times 10^{19}\ M^{-1}\ cm^{-1}\ nm^4$$

The Förster radius (R_0) was calculated as 25.3 nm using the above equation.

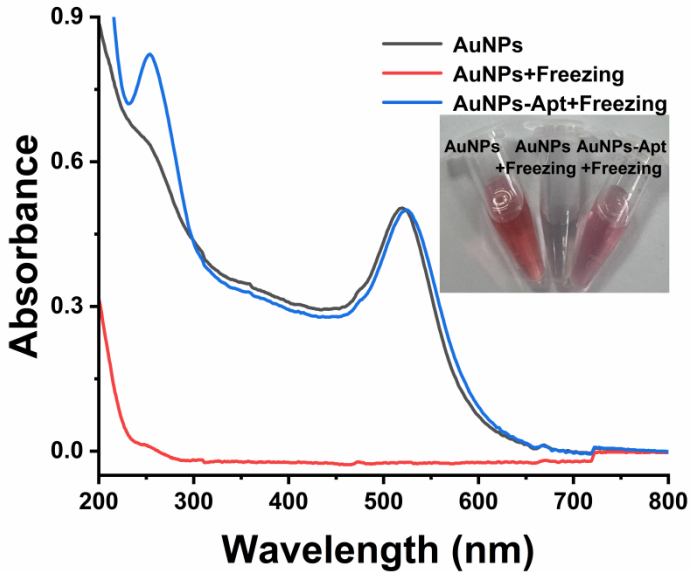

Fig. S1. UV-vis absorption spectra of AuNPs with different treatments. Inset: Corresponding photographs of the AuNPs.

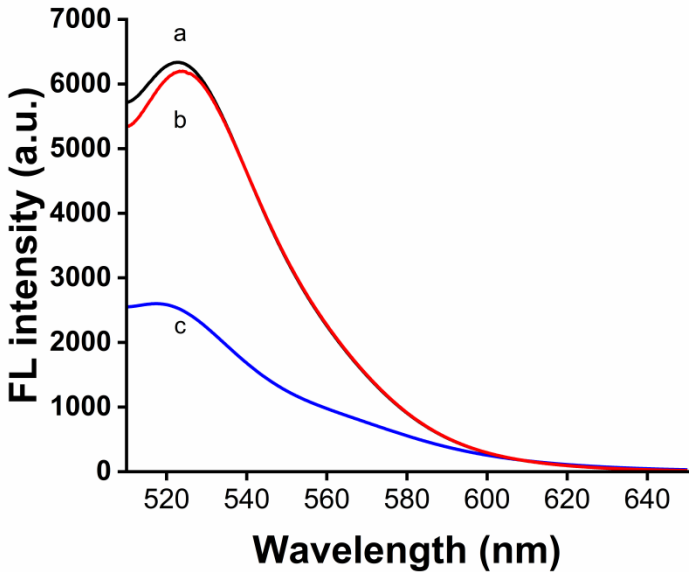

Fig. S2. FL intensity of FAM-cDNA (60 nmol L^{-1}) (a), FAM-cDNA (60 nmol L^{-1}) + AuNP (6 nmol L^{-1}) (b), and FAM-cDNA (60 nmol L^{-1}) + AuNP-Apt (6 nmol L^{-1}) (c).

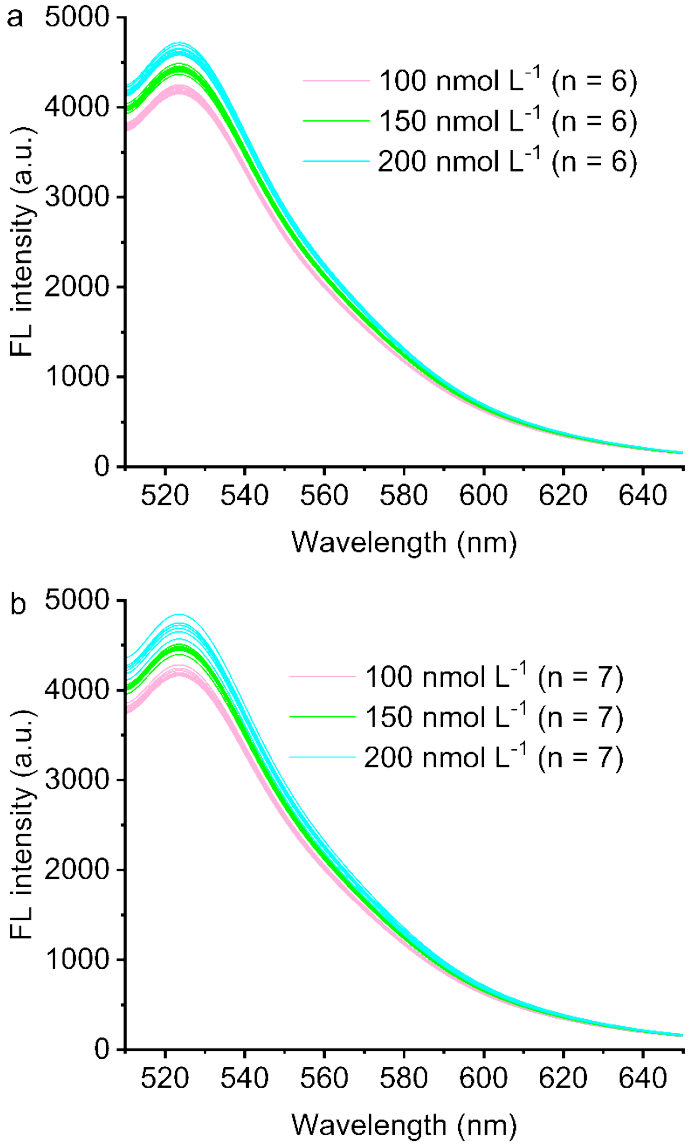

Fig. S3. Fluorescence spectra of replicate measurements for intra-batch (a) and inter-batch (b) reproducibility evaluation at three concentration levels (100, 150, and 200 nmol L^{-1}) of QC sample.

Table S1

Sequences of the involved oligonucleotides.

Name	Sequence
PFOA aptamer	5’-AAA AAA AAA AAA AAA TCT CGG GAC GAC GGC GTG GGG TGG TAG GCT GTA AAG GGG GTC GTC GTC CC-3’
Complementary DNA	5’ -GTC GTC CCG AGA G-FAM-3’

Table S2

Comparison of the developed method with other reported fluorescence methods for the determination of PFOA.

Recognition Materials	Linear range	LOD (nmol L^{-1})	Real Samples	Reference
PDI-MOF	10-50 nmol L^{-1} (paper, SPE); 0-20 μmol L^{-1} (solution)	3.1 (paper) 1680 (solution)	Tap water, drinking water	S5
Erythrosin + B-CTAB	0.05-10 μmol L^{-1}	11.8	Tap water, river water	S6
TGA-capped CdTe QDs	0.02-1.0 μmol L^{-1}	8.6	Tap water, river water	S7
UiO-66-$N(CH_3)_3^+$@SRB	0-3 μmol L^{-1}	220	No real samples	S8
MIP-coated CdTe@CdS QDs	0.25-15 μmol L^{-1}	25	Tap water, river water	S9
Amine-functionalized Ln-MOF	0-36 μmol L^{-1}	23.1	Tap water	S10
Fluorescein-labeled aptamer	0-75 μmol L^{-1}	170	Wastewater effluent	S11
Aptamer-modified AuNPs	5-200 nmol L^{-1}	1.3	Fish	This work

Table S3

Comparison of different types of analytical method for PFOA detection

Analytical Methods	Linear range	LOD (nmol L^{-1})	Real Samples	Reference
DMSPE + UHPLC-MS/MS	0.05-2000 ng L^{-1}	2.66×10^{-5}-9.66×10^{-5}	River water	S12
In-tube SPME + LC-MS	50-5000 ng L^{-1}	0.00362	Tap water, river water	S13
HPLC-MS/MS	10-25000 ng L^{-1}	0.0242 (LLOQ)	Cooking oil, pig adipose tissue	S14
CD-AuNPs colorimetry	-	170 (α-CD), 156 (β-CD), 204 (γ-CD)	Lake water	S15
Hf-WO_3/C electrochemistry	70–300000 nmol L^{-1}	18.3	Soil, water, spoiled vegetables, fruits	S16
PANI-CHT/SPCE electrochemistry	5–150 μg·L^{-1}	2.61	Water sample	S17
Graphene electrochemical sensor	0.05–500.0 μmol L^{-1}	10.4	Spiked soil, water, spoiled vegetables, and fruit samples	S18
Aptamer-modified Au NPs	5-200 nmol L^{-1}	1.3	Fish	This work

Table S4

Intra- and inter-batch reproducibility of the proposed aptasensor.

QC sample concentration (nmol L^{-1})	Measured (mean ± SD)	Accuracy (%)	RSD (%)
Intra-batch (n = 6)			
100	92.01 ± 7.72	92.0	8.4
150	141.93 ± 8.65	94.6	6.1
200	188.79 ± 10.72	94.4	5.7
Inter-batch (n = 7)			
100	99.26 ± 10.87	99.2	10.9
150	145.39 ± 13.11	96.9	9.0
200	206.83 ± 24.38	103.1	11.8

RSD = Relative standard deviation

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