Electronic Supplementary Information

A Fluorescent Conjugated Polymer-based Ratiometric Aptasensor for Highly Specific and Robust Detection of Perfluorooctanoic Acid

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Experimental section

Chemicals and materials

Poly[(9,9-bis(6′-N,N,N-trimethylammonium)hexyl)-fluorenylene phenylene dibromide] (PFP) was synthesized according to the published procedures.[1] Perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorobutyric acid (PFBA), perfluorobutanesulfonic acid (PFBS), and octanoic acid (OA) were purchased from Sigma-Aldrich. The high-performance liquid chromatography (HPLC)-purified oligonucleotide was synthesized by Sangong Biotechnology (Shanghai, China). The sequences of the nucleic acids used in the study are as follows: Apt-FAM, 5′-FAM-GGCGTGGGGTGGTAGGCTGTAAAGGGGGTC-3′. Ultrapure water was obtained from a Milli-Q system (Millipore, Milford, MA, USA).

Ratiometric fluorescence aptasensor for sensitive detection of perfluorooctanoic acid

Apt–FAM was diluted in 1× Tris–EDTA buffer to 10 μM, and then diluted with HEPES buffer (20 mM HEPES, 1 mM NaCl, pH 7.4) to a final concentration of 1 μM. Subsequently, 0.5 μL of Apt–FAM solution was mixed with the reaction mixture (total 19 μL containing different concentrations of PFOA and 18.5 μL of HEPES buffer), and incubated for 10 min at room temperature. After incubation, 0.5 μL of PFP (100 μM) was added to initiate the fluorescent conjugated polymer-based ratiometric aptasensing reaction.

Fluorescence measurements

Fluorescence spectra were recorded on an FS5 fluorescence spectrophotometer (Edinburgh Instruments Ltd., UK) with an excitation wavelength of 380 nm and an emission range of 400–650 nm. The limit of detection (LOD) is determined based on the $3\sigma/k$ method, where σ is the standard deviation of y-intercepts of regression line and *k* is the slope of the calibration curve.

Analysis of tap water samples

To evaluate the practical applicability of the ratiometric aptasensor, tap water was collected from Shaanxi University of Science and Technology (Xi'an, Shaanxi, China) and used for testing. The samples were spiked with the known concentrations of PFOA to prepare the standard solutions. The PFOA-spiked samples were mixed with Apt-FAM in HEPES buffer (20 mM HEPES, 1 mM NaCl, pH 7.4), and incubated for 10 minutes at room temperature. After the incubation, PFP (2.5 μM) was added, and the resulting fluorescence color changes were recorded by using a smartphone for visual analysis.

SUPPLEMENTARY RESULTS

Optimization of experimental conditions.

To achieve optimal assay performance, we systematically optimized several experimental parameters including the concentrations of PFP and Apt–FAM and the incubation time required for PFOA binding to Apt–FAM. The effect of each parameter is evaluated by monitoring $(I_{525}/I_{420})/(I_{525}/I_{420})_0$, where (I_{525}/I_{420}) and $(I_{525}/I_{420})_0$ denote the FRET ratios measured in the presence and absence of PFOA, respectively.

The concentration of PFP is a critical factor in the detection system and should be optimized. As shown in Fig. S1 the $(I_{525}/I_{420})/(I_{525}/I_{420})_0$ value enhances with the increasing PFP concentration from 0 to 2.5 μM, and then decreases at higher PFP concentrations. Thus, 2.5 μM is selected as the optimal PFP concentration for subsequent experiments.

The effect of Apt–FAM concentration on the assay performance is examined well. As shown in Fig. S2 the $(I_{525}/I_{420})/(I_{525}/I_{420})_0$ value improves with the increasing concentration of Apt–FAM from 0 to 25 nM, followed by a decrease beyond 25 nM. Therefore, 25 nM Apt–FAM is chosen as the optimal concentration for subsequent experiments.

The influence of incubation time on the binding interaction between PFOA and Apt–FAM is investigated. As shown in Fig. S3 the $(I_{525}/I_{420})/(I_{525}/I_{420})_0$ value enhances with the incubation time from 3 to 10 min, and reaches a plateau thereafter. Thus, 10 min is selected as the optimal incubation time for the assay.

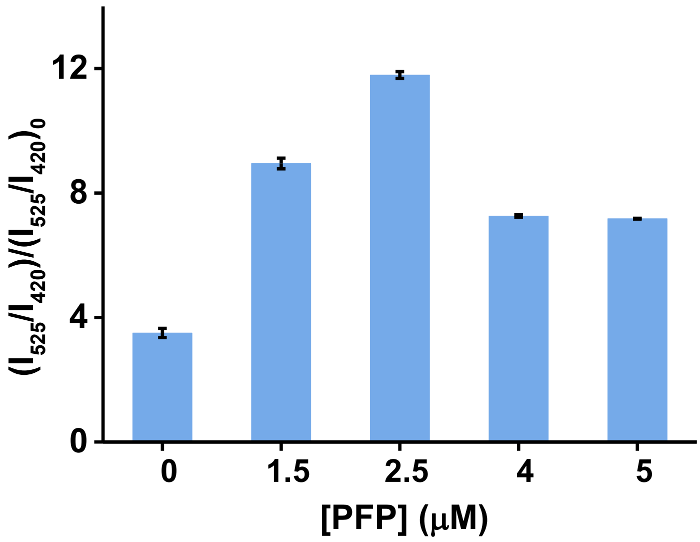

Fig. S1 Variance of the *$(I_{525}/I_{420})/(I_{525}/I_{420})_0$* value with different concentrations of PFP.

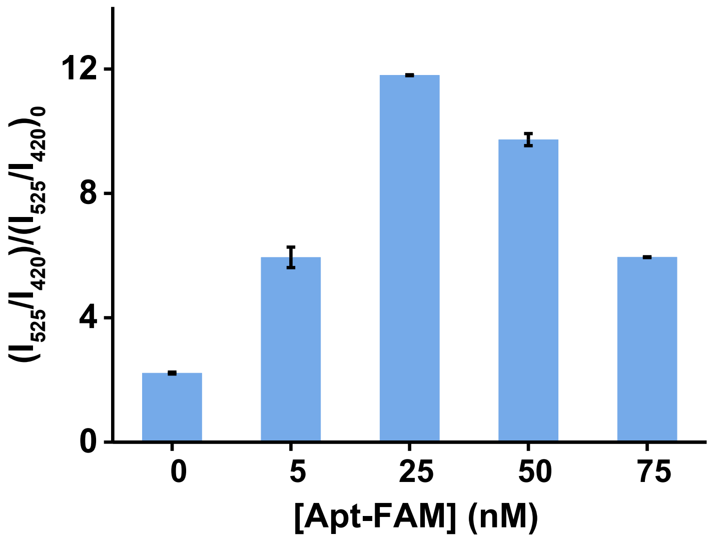

Fig. S2 Variance of the *$(I_{525}/I_{420})/(I_{525}/I_{420})_0$* value with different concentrations of PFP.

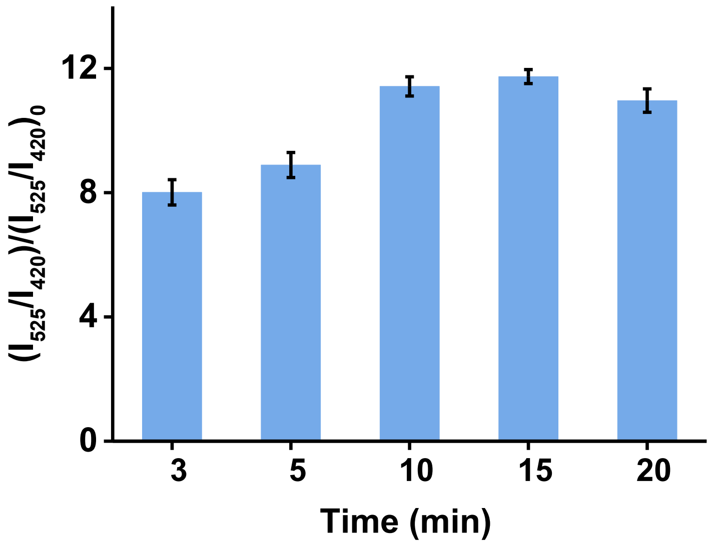

Fig. S3 Variance of the $(I_{525}/I_{420})/(I_{525}/I_{420})_0$ value with different incubation time.

Emission spectra of the PFP system with the increasing concentrations of PFOA

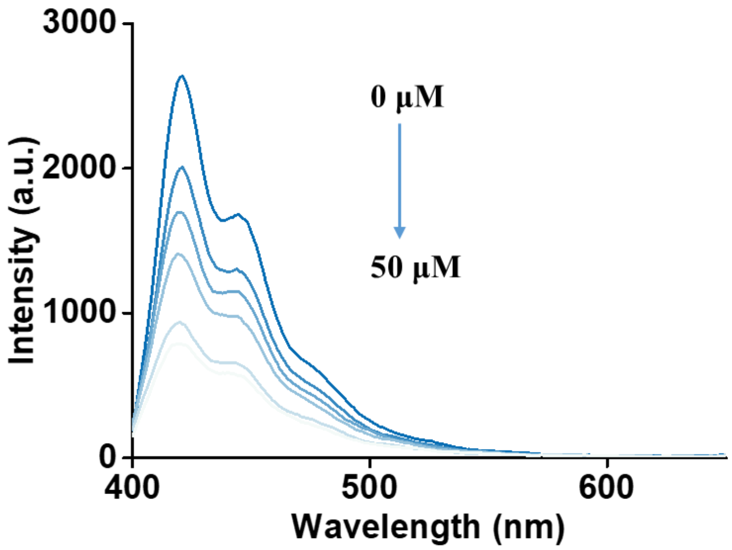

Fig. S4 Emission spectra of the PFP system in response to the increasing concentrations of PFOA (0 – 50 μM). λ_{ex} = 380 nm.

Table S1. Comparison of the sensing for PFAS assay.

Sensing probes	Detection mode	Selectivity against SDS/SDBS	LOD (nM)	Ref.
DNA aptamer/conjugated polymer	ratiometric	Yes	27 (PFOA)	This work
Perylene diimide derivatives	turn off	Yes	28 (PFOS)	2
metal-organic frameworks	turn off	N/A	45.9 (PFOA)	3
porous zinc-based metallacage	turn off	N/A	61.81 (PFOA)	4
AuNCs/lysine-calix[4]arene-propoxy	turn off	N/A	1820 (PFOA)	5
metal-organic frameworks	turn off	N/A	111 (PFOA)	6
indium(III) chloride complex	turn off	N/A	500000 (PFHpA)	7
Guanidinocalix[5]arene	turn on	N/A	26.4 (PFOA)	8
fluorescent macrocycle	turn on	N/A	47.3 (PFOS)	9
metal–organic framework	turn on	N/A	120 (PFOA)	10
cationic deep cavitands	turn on	N/A	130 (PFOS)	11
multi-head cationic siloxane	turn on	N/A	2700 (PFOS)	12
conjugated polymer	ratiometric	N/A	2.86 (PFOA)	13
conjugated polymer	ratiometric	No	6.12 (PFOA)	14
carbon dots	ratiometric	No	27.8 (PFOS)	15
DNA aptamer	turn off	N/A	170 (PFOA)	16

Table S1:(continued)

Sensing probes	Detection mode	Selectivity against SDS/SDBS	LOD (nM)	Ref.
DNA aptamer/polyfluorene	electrochemil-uminescence	N/A	1.97×10^{-6} (PFOA)	17
DNA aptamer/Cas12a	turn on	N/A	8.21×10^{-5} (PFOA)	18
DNA aptamer/oxidase-Like Nanozyme	colorimetric and fluorescence dual-modality	N/A	14.3 (PFOA)	19

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