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Supporting Information

Construction of a novel fluorescent DNA aptasensor for the fast-response and sensitive detection of copper ions in industrial sewage

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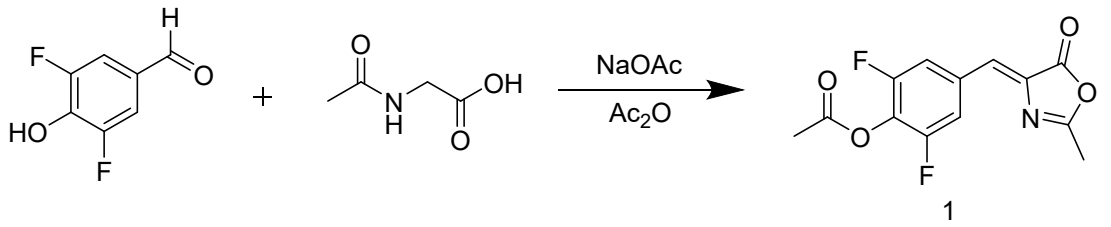
Content

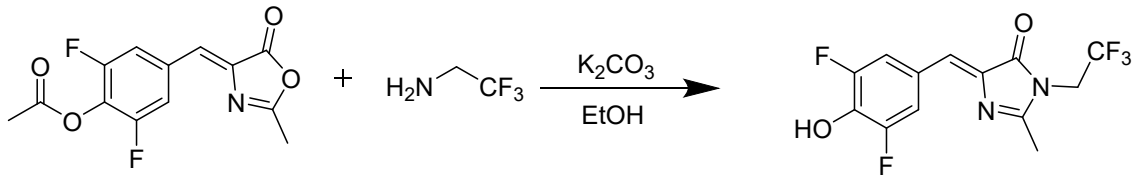
1. Synthesis and Characterization

1.1 Synthesis of (Z)-2, 6-difluoro-4-((2-methyl-5-oxooxazol-4(5H)-ylidene) methyl) phenyl acetate(1)[1]



N-Acetylglycine (1.469 g, 12.65 mmol), anhydrous sodium acetate (1.037 g, 12.65 mmol), 2,6-difluoro-4-hydroxybenzaldehyde (2.00 g, 12 mmol), and acetic anhydride (12 mL) were stirred at 120 °C for 4 h. After the reaction was cooled to room temperature, then the resulting crude solid was washed with a small amount of cold ethanol, hot water dried to afford the primary product which was recrystallized from ethanol, yielding a yellow solid of Compound **1** (2.44 g, yield 72%), without purification and characterization for the next step.

1.2 Synthesis of DFHBI-1T (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl) -1H-imidazol-5(4H)-one



Compound **1** (500 mg, 1.78 mmol) was refluxed with 4.3 mL of absolute ethanol, (264.75 mg, 2.67 mmol) of 2,2,2-Trifluoroethylamine, and 370 mg of potassium carbonate for 4 h. The reaction mixture was a cooled to room temperature. The solvent was evaporated and the mixture was redissolved in a 1:1 mixture of ethyl acetate and 500 mM sodium acetate at pH 3.0. The organic layer was separated and dried with

anhydrous sodium sulfate. The solvent was removed under reduced pressure and the reaction mixture was purified by column chromatography (CH_2Cl_2: EtOH = 10:1) to afford 142 mg (yield 25%) of DFHBI-1T as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 7.99 (d, J = 8.7 Hz, 2H), 7.03 (s, 1H), 4.56 (q, J = 9.3 Hz, 2H), 2.41 (s, 3H).

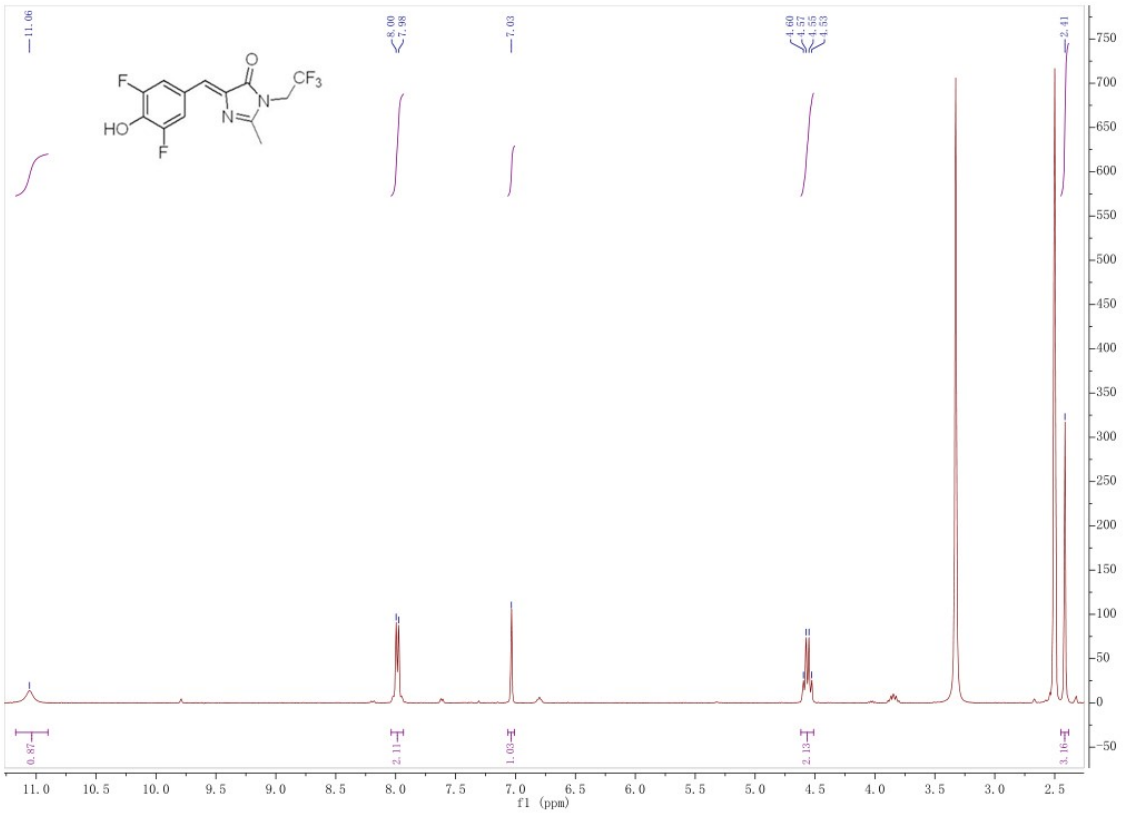


Figure S1. ^{1}HNMR spectrum of DFHBI-1T.

2.Supplemental Tables and Figures

2.1. Supplemental Tables.

Table S1. Sequences and secondary structures of DNA oligomers applied in this study*.

Oligonucleotide	Sequence (5' - 3')	Structure type
$S2T3_{AT}$-GC	CTTAGTAGGGATGATGCGGCAGTGGGCTTCATCTAT ATAAGATGAGGGGACTAAG	Single strand
$S2T3_{AT}$-GC-M1	CTTAGTAGCCATGATGCGGCAGTGGGCTTCATCTAT ATAAGATGAGGGGACTAAG	Single strand
$S2T3_{AT}$-GC-M2	CTTAGTAGGGATGATGCGGCAGTGGGCTTCATCTAT ATAAGATGACCCGACTAAG	Single strand
P2-$S2T4_{AT}$	TAGTAGGGATGATGCGGCAGTGGGCTTCATCTATAT AAGATGAGGGGACTA	Single strand
ssDNA1	CCAGTTCGTAGTAACCC	Single strand
ssDNA2	GGGTTACTACGAACTGG	Single strand
ssDNA3	GAAAATGAGAGACTCAGCTAAGCAAGATAG	Single strand
ssDNA4	GGGAATGAGGGACTCGGCTGGGCAGGATGG	Single strand
ds26	CAATCGGATCGAATTCGATCCGATTG	Duplex
H-Telo	GGGTTAGGGTTAGGGTTAGGG	G-quadruplex
HT	TTGGGTTAGGGTTAGGGTTAGGGA	
Kras	AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG	
Hras	TCGGGTTGCGGGCGCAGGGCACGGGCG	

* dsDNA was obtained from the hybridization between ssDNA1 and ssDNA2.

Table S2. Detection performance of previously reported methods.

Detection methods	Detection linear range	Detection Limits	Reference
Colormetric probe	1.8-200 μM	1.8 μM	1
Reflectance chemosensor	0.78-160 μM	0.612 μM	2
Spectrophotometric method	2-400 μM	0.556 μM	3
Colorimetric probe	10-45 μM	0.56 μM	4
Nanosensor	0.5-80 μM	0.5 μM	5
Fluorescent probe	8.75-20 μM	6.7 μM	6

Table S3. The ICP-OES for the real content of raw sewage-1.

Element	Coefficient of dilution	Values of instrument	Final content	Concentration（mol/L）	Element	Coefficient of dilution	Values of instrument	Final content	Concentration（mol/L）
Yb	1	0.0001	<0.05	5.78×10^{-10}	Hf	1	0.0090	<0.05	5.04×10^{-8}
Ti	1	0.0001	<0.05	2.09×10^{-9}	Er	1	0.0094	<0.05	5.62×10^{-8}
Sc	1	0.0001	<0.05	2.22×10^{-9}	Zr	1	0.0110	<0.05	1.21×10^{-7}
Y	1	0.0001	<0.05	1.12×10^{-9}	Mo	1	0.0114	<0.05	1.19×10^{-7}
Gd	1	0.0003	<0.05	1.91×10^{-9}	Ge	1	0.0134	<0.05	1.85×10^{-7}
Ho	1	0.0004	<0.05	2.43×10^{-9}	Se	1	0.0151	<0.05	1.91×10^{-7}
Cd	1	0.0005	<0.05	4.45×10^{-9}	Tl	1	0.0160	<0.05	7.83×10^{-8}
La	1	0.0007	<0.05	5.04×10^{-9}	Mn	1	0.0192	<0.05	3.49×10^{-7}
V	1	0.0007	<0.05	1.37×10^{-8}	Te	1	0.0199	<0.05	1.56×10^{-7}
Pd	1	0.0007	<0.05	6.58×10^{-9}	As	1	0.0223	<0.05	2.98×10^{-7}
Ga	1	0.0008	<0.05	1.15×10^{-8}	W	1	0.0328	<0.05	1.78×10^{-7}
Be	1	0.0008	<0.05	8.88×10^{-8}	Ba	1	0.0381	<0.05	2.77×10^{-8}
Nb	1	0.0009	<0.05	9.69×10^{-9}	Li	1	0.0435	<0.05	6.27×10^{-6}
Co	1	0.0009	<0.05	1.53×10^{-8}	Al	1	0.0443	<0.05	1.64×10^{-6}
Cu	1	0.0010	<0.05	1.57×10^{-8}	Rh	1	0.0046	<0.05	4.47×10^{-8}
Zn	1	0.0010	<0.05	1.53×10^{-8}	Sn	1	0.0060	<0.05	5.06×10^{-8}
Ag	1	0.0011	<0.05	1.01×10^{-8}	Pr	1	0.0065	<0.05	4.61×10^{-8}
Pt	1	0.0012	<0.05	6.15×10^{-9}	Eu	1	0.0072	<0.05	4.74×10^{-8}
Ir	1	0.0012	<0.05	6.24×10^{-9}	Ru	1	0.0077	<0.05	7.62×10^{-8}
Tm	1	0.0013	<0.05	7.70×10^{-9}	Bi	1	0.0078	<0.05	3.73×10^{-8}
Tb	1	0.0016	<0.05	1.01×10^{-8}	Lu	1	0.0079	<0.05	4.52×10^{-8}
Ni	1	0.0017	<0.05	2.90×10^{-8}	Cr	1	0.1016	0.1016	1.95×10^{-6}
Ce	1	0.0023	<0.05	1.64×10^{-8}	P	1	0.2022	0.2022	4.53×10^{-6}
Dy	1	0.0027	<0.05	1.66×10^{-8}	Fe	1	0.3149	0.3149	5.64×10^{-6}
Ta	1	0.0029	<0.05	1.60×10^{-8}	Ca	100	0.5635	56.35	1.41×10^{-3}
Pb	1	0.0031	<0.05	1.50×10^{-8}	Sr	1	0.7243	0.7243	8.27×10^{-6}
Sm	1	0.0034	<0.05	2.26×10^{-8}	K	100	0.9252	92.52	2.37×10^{-3}
Au	1	0.0034	<0.05	1.73×10^{-8}	Si	1	1.1488	1.1488	4.09×10^{-5}
Sb	1	0.0041	<0.05	3.37×10^{-8}	B	1	1.2901	1.2901	1.19×10^{-4}
Hg	1	0.0043	<0.05	2.14×10^{-8}	Mg	1	6.7872	6.7872	2.79×10^{-4}
In	1	0.0044	<0.05	2.83×10^{-8}	Na	100	19.942	1994.2	8.67×10^{-2}
Nd	1	0.0087	<0.05	6.03×10^{-8}					

Table S4. The ICP-OES for the real content of raw sewage-2.

Element	coefficient of dilution	Values of instrument	Final content	Concentration （mol/L）	Element	coefficient of dilution	Values of instrument	Final content	Concentration （mol/L）
Y	1	5.6×10^{-5}	<0.05	6.29376×10^{-10}	Ta	1	0.009806	<0.05	5.41947×10^{-8}
Ti	1	6.92×10^{-5}	<0.05	1.44526×10^{-9}	Eu	1	0.010937	<0.05	7.19696×10^{-8}
Sc	1	0.000122	<0.05	2.71477×10^{-9}	Nd	1	0.011418	<0.05	7.91817×10^{-8}
Yb	1	0.000189	<0.05	1.09276×10^{-9}	Er	1	0.013753	<0.05	8.22548×10^{-8}
V	1	0.000284	<0.05	5.56819×10^{-9}	Pr	1	0.015069	<0.05	1.06937×10^{-7}
Ho	1	0.000309	<0.05	1.87395×10^{-9}	P	1	0.018752	<0.05	6.05404×10^{-7}
Gd	1	0.000822	<0.05	5.22871×10^{-9}	Tl	1	0.019646	<0.05	9.6162×10^{-8}
Cd	1	0.000979	<0.05	8.70937×10^{-9}	W	1	0.02345	<0.05	1.27582×10^{-7}
La	1	0.001056	<0.05	7.59915×10^{-9}	Mn	1	0.024079	<0.05	4.38294×10^{-7}
Be	1	0.001063	<0.05	1.17967×10^{-7}	Hf	1	0.0274	<0.05	1.53588×10^{-7}
Ce	1	0.001081	<0.05	7.71539×10^{-9}	In	1	0.032751	<0.05	2.8524×10^{-7}
Tm	1	0.001462	<0.05	8.6519×10^{-9}	Te	1	0.032779	<0.05	2.56885×10^{-7}
Mo	1	0.00147	<0.05	1.53221×10^{-8}	Hg	1	0.005763	<0.05	2.87443×10^{-8}
Pb	1	0.001535	<0.05	7.40936×10^{-9}	Ag	1	0.006127	<0.05	5.67991×10^{-8}
Au	1	0.001978	<0.05	1.00416×10^{-8}	Pt	1	0.006174	<0.05	3.1648×10^{-8}
Rh	1	0.002073	<0.05	2.01428×10^{-8}	Co	1	0.006588	<0.05	1.11787×10^{-7}
Sn	1	0.002245	<0.05	1.89288×10^{-8}	Ru	1	0.008421	<0.05	8.33184×10^{-8}
Ir	1	0.002446	<0.05	1.27287×10^{-8}	Ge	1	0.008586	<0.05	1.18433×10^{-7}
Dy	1	0.002479	<0.05	1.52534×10^{-8}	Bi	1	0.008668	<0.05	4.14771×10^{-8}
Pd	1	0.002536	<0.05	1.56089×10^{-8}	Al	1	0.062041	0.062041	2.29934×10^{-6}
Tb	1	0.002551	<0.05	1.60533×10^{-8}	Ba	1	0.06711	0.06711	4.88677×10^{-7}
Zr	1	0.002764	<0.05	3.03028×10^{-8}	Fe	1	0.08713	0.08713	1.56035×10^{-6}
As	1	0.003056	<0.05	4.07824×10^{-8}	Li	1	0.129932	0.129932	1.87195×10^{-5}
Sm	1	0.003179	<0.05	2.11374×10^{-8}	Sr	1	0.752605	0.752605	8.58942×10^{-6}
Cr	1	0.003186	<0.05	6.12736×10^{-8}	Ca	100	0.917754	91.7754	0.002289805
Zn	1	0.003921	<0.05	5.99722×10^{-8}	K	100	0.956576	95.6576	0.002446611
Se	1	0.003974	<0.05	5.03674×10^{-8}	Cu	1	2.667	2.667	4.19736×10^{-5}
Ga	1	0.004449	<0.05	6.38143×10^{-8}	B	1	7.09706	7.09706	0.000656467
Ni	1	0.005086	<0.05	8.66594×10^{-8}	Si	1	7.61469	7.61469	0.00027113
Lu	1	0.005471	<0.05	3.12703×10^{-8}	Mg	1	7.67772	7.67772	0.000315891
Nb	1	0.006763	<0.05	7.27988×10^{-8}	Na	100	20.6423	2064.23	0.089792074
Sb	1	0.0349	<0.05	2.86769×10^{-7}					

Table S5. The detection for Cu^{2+} in raw sewage-2.

Samples	Theoretical value(μM)	Real value (μM)	Recovery (%)	RSD (%)
Raw sewage-2	0.5	0.49	98.1	0.23
	1.5	1.48	98.4	0.13
	10	9.76	97.6	0.89
	20	19.64	98.2	0.22
	30	29.87	99.6	0.12

2.2. Supplemental Figures.

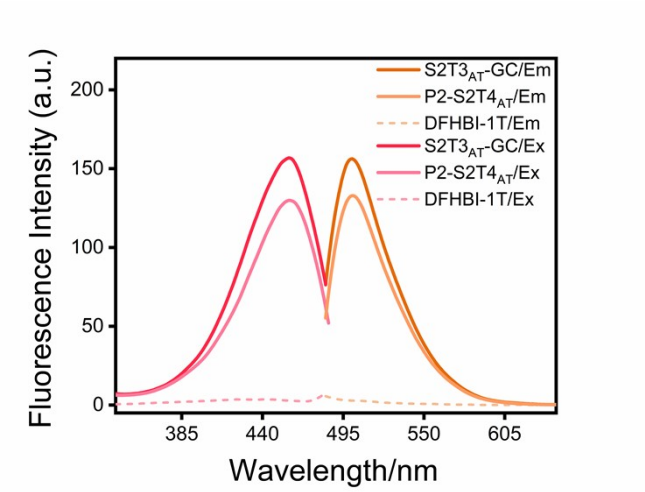


Figure S2. The fluorescence excitation and emission spectrum of 5 μL DFHBI-1T (200 μM) with or without 4 μL DNA oligomers (100 μM) that dissolved into the reaction buffer. The fluorescent intensity values ranging from 340 to 640 nm were recorded.

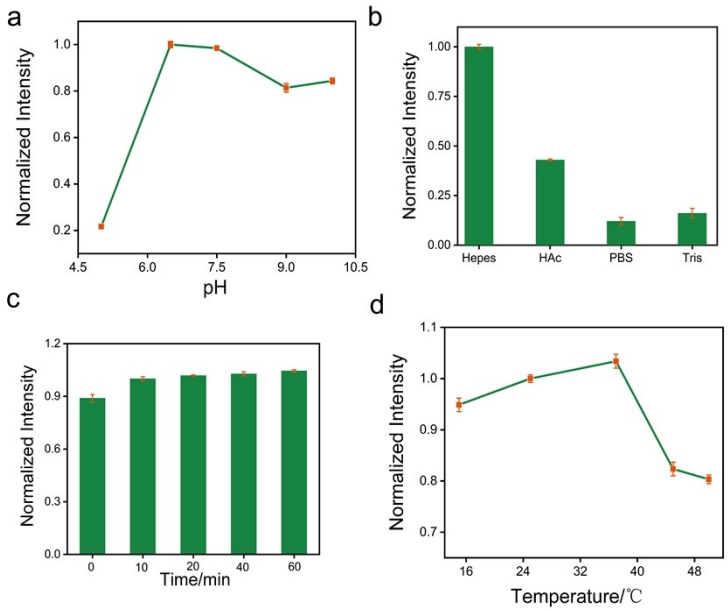


Figure S3. Fluorescence assays for optimizing the binding effect of S2T3$_{AT}$-GC and DFHBI-1T. The fluorescence intensity of the mixture contained 4 μL DNA oligomers (100 μM) and 5 μL DFHBI-1T (200 μM) at 502 nm were measured by adjusting different conditions including pH, reaction buffer, incubation time and temperature.

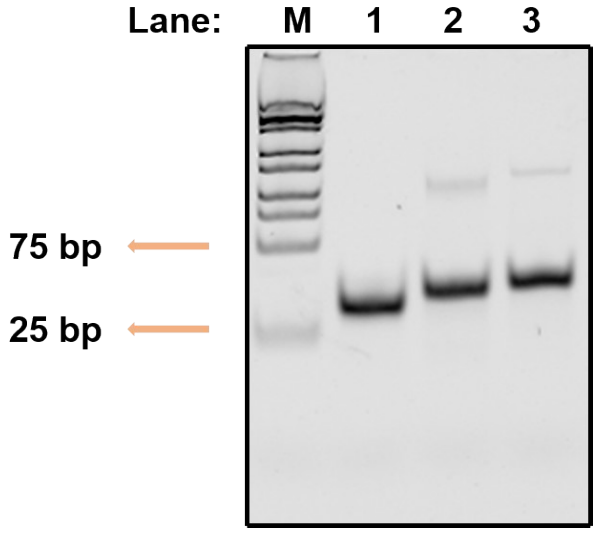


Figure S4. The PAGE analysis of DNA oligomers for 1 h, 90 V electrophoresis migration in 1×TB buffer. Especially, Lane M: 25 bp Marker; Lane 1: S2T3$_{AT}$-GC; Lane 2: S2T3$_{AT}$-GC-M1; Lane 3: S2T3$_{AT}$-GC-M2. All DNA oligomers are 1 μM.

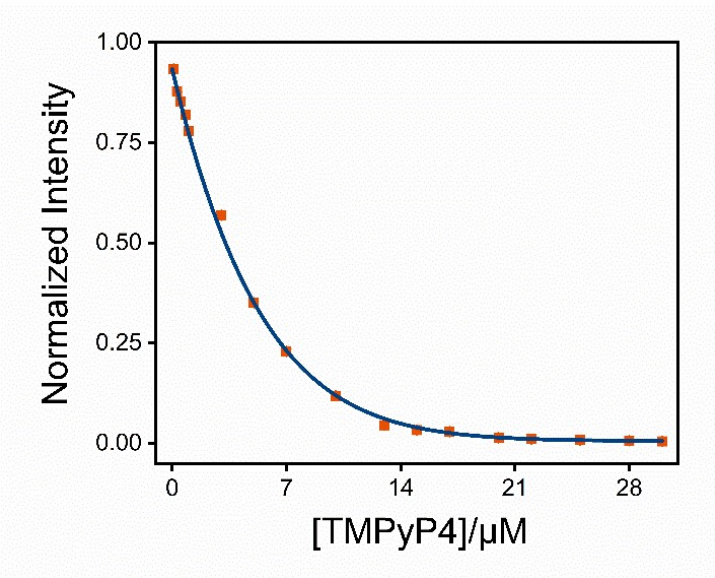


Figure S5. The fluorescence competition experiment between TMyP4 and S2T3$_{AT}$-GC/ DFHBI-1T. Various concentrations of TMPyP4 (0.1-30 μM) were mixed with 4 μL DNA oligomers (100 μM) and 5 μL DFHBI-1T (200 μM).

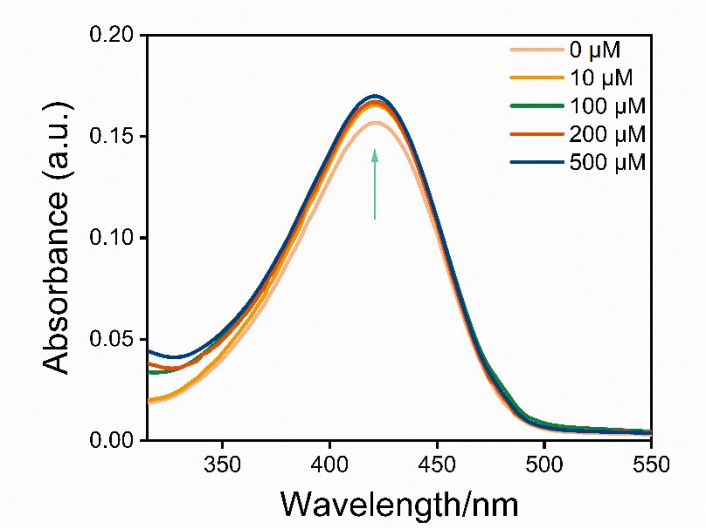


Figure S6. UV-vis spectra of DFHBI-1T/Cu (II). The UV-vis analysis of DFHBI-1T/Cu (II). 10 μL DFHBI-1T (200 μM) was mixed with different concentrations of Cu^{2+}.

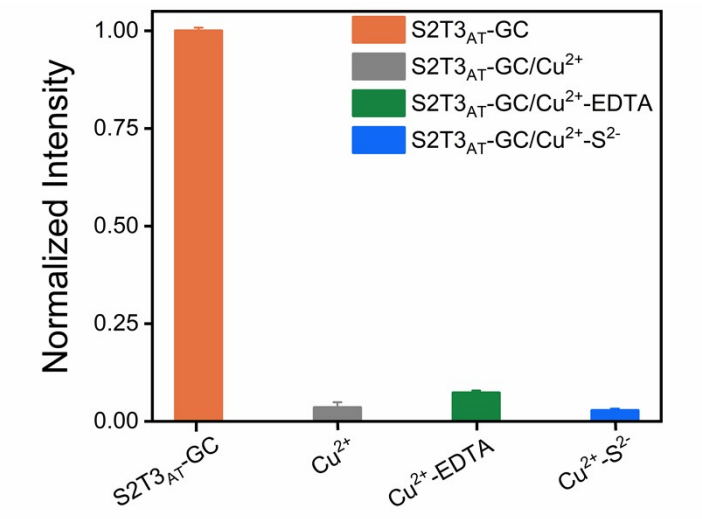


Figure S7. Fluorescence determination of $S2T3_{AT}$-GC/DFHBI-1T with or without the mixture of 500 μM Cu^{2+} and 1000 μM EDTA or the mixture of 500 μM Cu^{2+} and 1000 μM Na_2S.

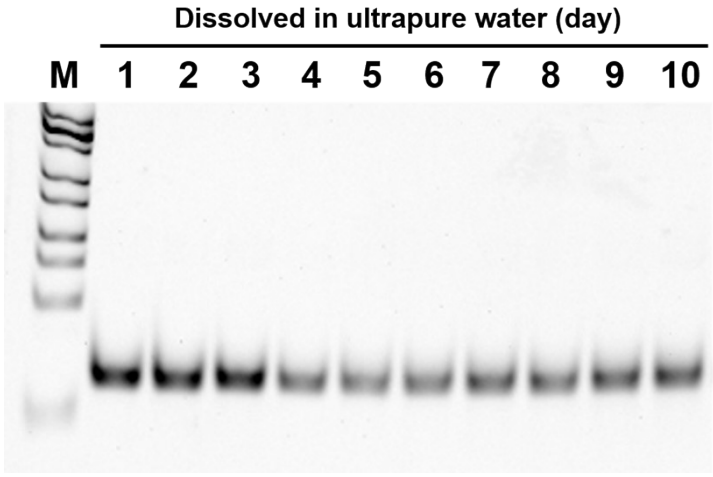


Figure S8. The PAGE analysis of $S2T3_{AT}$-GC which dissolved in the ultrapure water for 10 days interval for 1 h, 90 V electrophoresis migration in 1×TB buffer.

3. References

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