Supporting Information

Aptamer Functionalized DNA Hydrogel for Wise-stage Controlled Protein Release

Chen Liu, Jialun Han, Yuxuan Pei and Jie Du*

State Key Laboratory of Marine Resource Utilization in South China Sea, College of materials and chemical engineering, Hainan University, Haikou 570228, P. R. China. E-mail: dujie@hainu.edu.cn

Detection method for the concentration of thrombin

Method for detection the changed concentration of thrombin was based on graphene oxide-based fluorescent aptasensor. The mechanism was showed in Figure S1.

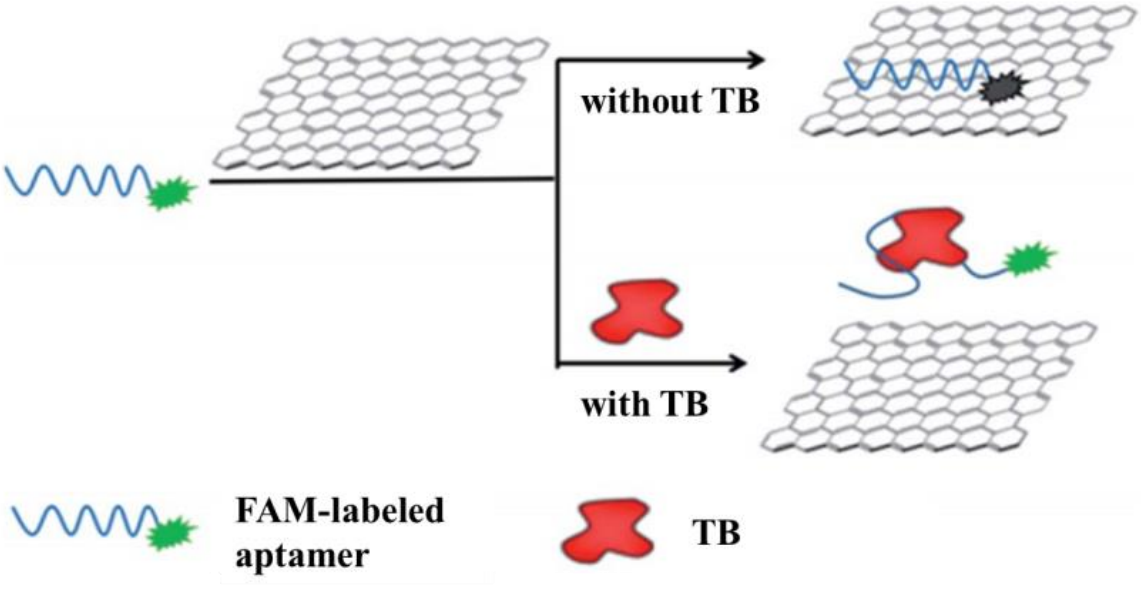

Figure S1. The detection mechanism of graphene oxide-based fluorescent aptasensor for thrombin protein.

Briefly, 1 ml PBS solution containing 20 nM FAM-modified aptamer (5′ –GGTTGGTGTGGTTGG-FAM-3′) and 9 μg ml^{-1} graphene oxide (GO) was firstly detected by RF-6000 fluorescence spectrometer (Shimadzu Co., Japan). FAM-modified aptamer was adsorbed on the surface of GO via π-π stacking interactions. So the fluorescence of FAM was efficiently quenched by GO via FRET between GO and FAM. Then, thrombin with different concentrations of (2, 4, 6, 8 and 10 nM) were added into above solution successively and incubated for 30 min. Based on the specific binding between aptamer and thrombin, the aptamer chain detached from GO surface. Therefore, the quenched fluorescence was recovered as showed in Figure S1. Based on this mechanism, the standard curve was obtained by recording absorbance spectra. By changing the concentration of TB, the fluorescence recovery degree was definitely different as showed in Figure S2. The standard curve showed a good linear relationship between TB concentration (nmol/L) and recovered relative fluorescence value ($\frac{F - F_0}{F_0}$). According to this method, the amount of protein absorbing and releasing could be characterized carefully.

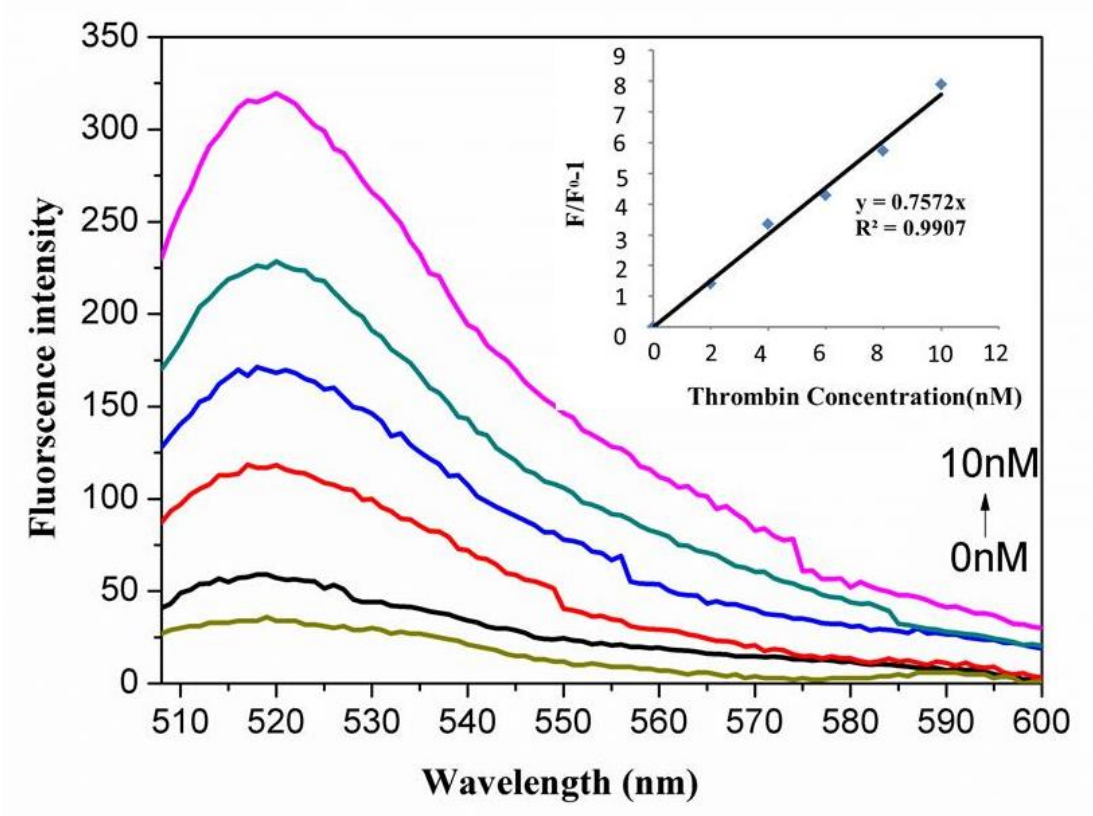

Figure S2. The recovered fluorescence intensity influenced by adding TB with concentrations of (2, 4, 6, 8 and 10 nM) and the linear relationship between TB concentration (nmol L−1) and relative fluorescence value ($\frac{F - F_0}{F_0}$).

Agarose gel electrophoresis analysis

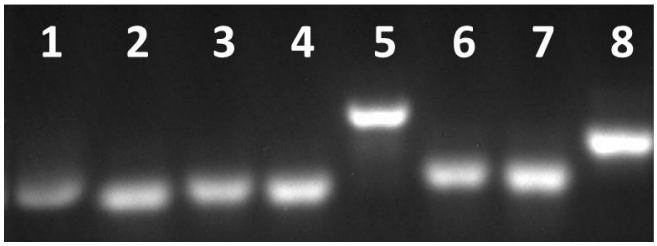

Figure S3. 3% agarose gel electrophoresis analysis. Lane 1, 2 ,3 and 4 were the single-stranded DNAs, X1, X2, X3 and X4, respectively. Lane 5 was the X-DNA; lane 6 and 7 were the single-stranded DNAs, L1 and L2. Lane 8 was the L-DNA.

Table S1. The sequences and the strand length of all oligonucleotides.

Name	Sequence (from 5′ to 3′)	Length (nt)
L1	AGTCCGTGTCTATTCGCATGAGAATTCCATTCACCGTAAGCTAGGATC	48
L2	AGTCCGTGGATCCTAGCTTACGGTGAATGGAATTCTCATGCGAATAGA	48
X1	CACGGACTGCACCGATGAATAGCGGTCAGATCCGTACCTACTCG	44
X2	CACGGACTCGAGTAGGTACGGATCTGCGTATTGCGAACGACTCG	44
X3	CACGGACTCGAGTCGTTCGCAATACGGCTGTACGTATGGTCTCG	44
X4	CACGGACTCGAGACCATACGTACAGCACCGCTATTCATCGGTGC	44
S-DNA	AGTCCGTGGGTTGGTGTGGTTGG	23
FAM-labeled S-DNA	AGTCCGTGGGTTGGTGTGGTTGG-FAM	23
FAM-labeled S-DNA with scrambled “sticky ends”	TCAGTAGCGGTTGGTGTGGTTGG-FAM	23
Controlled S-DNA	AGTCCGTGACGCTACCATACCTA	23
CS8	CCAACCAC	8
CS12	CCAACCACACCA	12
CS15	CCAACCACACCAACC	15
DABCYL-labeled CS15	DABCYL-CCAACCACACCAACC	15

The DNA hydrogels with different concentrations of X-DNA and L-DNA

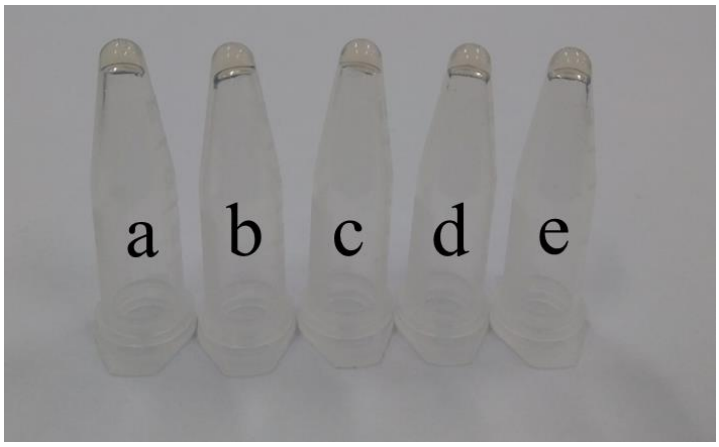

Figure S4. The DNA hydrogel with different concentrations of X-DNA and L-DNA. (a) X: 400 μM, L: 800 μM; (b) X: 500 μM, L: 1000 μM; (c) X: 600 μM, L: 1200 μM; (d) X: 700 μM, L: 1400 μM; (e) X: 800 μM, L: 1600 μM, respectively. The concentration of S-DNA was fixed at 200 μM.

The grafting degree of S-DNA

The grafting degree of the FAM-labeled S-DNA incorporated DNA hydrogels was determined by fluorescence spectroscopy at 522 nm. The prepared DNA hydrogel samples were immersed into DNA buffer and then centrifuged at 1500 rpm for 10 min. The unreacted S-DNA were washed out from hydrogel and in supernatant. The supernatant and a blank solution (containing the same initial aptamer concentration during preparation) were diluted to the same volume in DNA buffer. The fluorescence values at 522 nm were measured to calculate the amount of grafted S-DNA. The grafting degree of S-DNA was calculated through dividing the amount of X-DNA "sticky ends" by the amount of grafted S-DNA. Figure S5 showed the relative fluorescence intensity in supernatant and blank solution. The grafting amount of S-DNA could be obtained from the relative fluorescence values at 522 nm. And the detailed data was showed in Table S2.

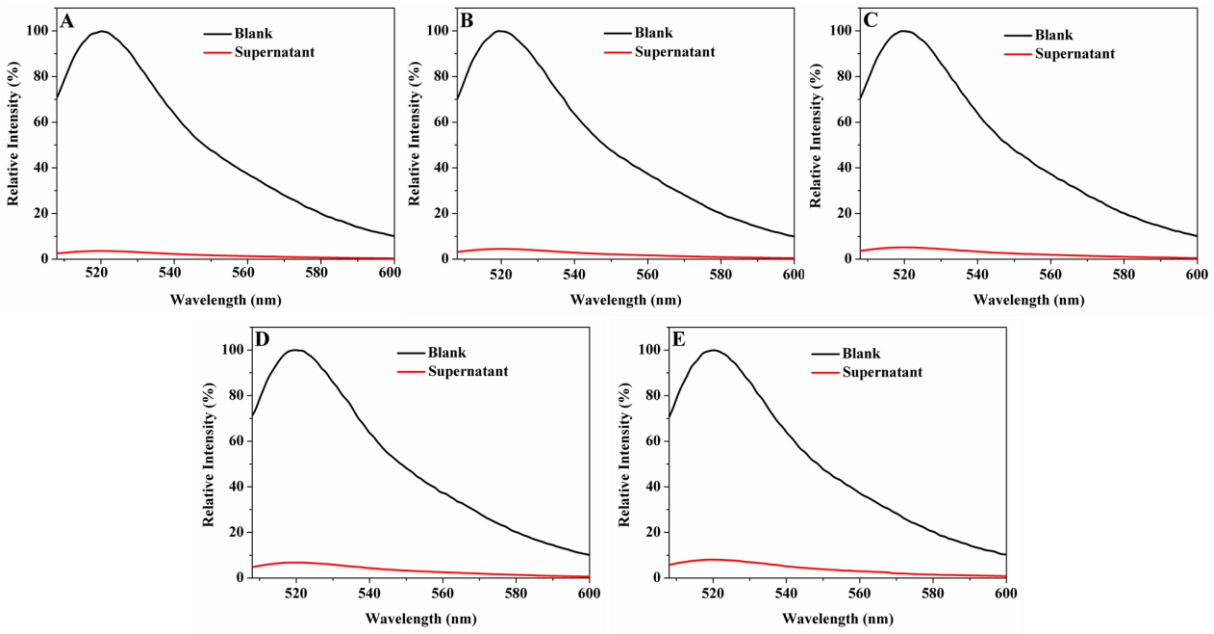

Figure S5. Determination of the grafting amount of S-DNA by fluorescence spectroscopy. The initial amount of added S-DNA was respectively at (A) 0.2 nmol, (B) 0.4 nmol, (C) 0.6 nmol, (D) 0.8 nmol, (E) 1.0 nmol. Their fluorescence values were detected at 522 nm and the amount of grafted S-DNA was calculated through the difference of fluorescence intensity between the supernatant and blank solution.

Table S2. The grafting degree of S-DNA.

Sample No.	The amount of initial added S-DNA (nmol)	The amount of grafted S-DNA (nmol ± SD)	The grafting degree of S-DNA (relative to X-DNA) (±SD) (%)
1	0.2	0.19 ±0.013	1.6 ±0.11
2	0.4	0.38 ±0.016	3.2 ±0.13
3	0.6	0.57 ±0.022	4.7 ±0.18
4	0.8	0.75 ±0.029	6.2 ±0.24
5	1.0	0.92 ±0.025	7.7 ±0.21

The comparison of interaction force between aptamer-TB complex and aptamer-CS duplex

As we know, the number of base pairs directly affects the strength of intermolecular hybridization. Thus, the length of the CSs has a significant influence on its competitive capability. Here, the CSs with 8, 12, and 15 nucleotides were discussed respectively to compare the interaction force between aptamer-TB complex and aptamer-CS duplex. 3'-DABCYL-labelled CSs was utilized to hybridize with 5'-FAM-labeled aptamer. Because of the Forster Resonance Energy Transfer (FRET) effect, the emission of FAM-labelled aptamer was quenched by DABCYL. When TB was added, the change of fluorescence intensity could be detected by fluorospectro photometer to compare the interaction force between two conjugates.

As showed in Figure S6A, when the length of the CSs was 8 nucleotides, DABCYL-labelled CS8 was utilized to hybridize with the aptamer (15 nucleotides) to form a quenched duplex. After TB adding to the quenched duplex, the fluorescence intensity was significantly recovered. It demonstrated that, the interaction force of aptamer-TB was stronger than that of aptamer-CS8. The result showed in Figure S6B further verified the above conclusion. It means that CS8 was hard to trigger the release of TB. Similarly, CS12 and CS15 were utilized to hybridize with the aptamer. Figure S6C and 6D demonstrated that the interaction force of aptamer-TB was smaller than that of aptamer-CS12. Figure S6E and 6F showed that the interaction force of aptamer-TB was also smaller than that of aptamer-CS15. Therefore, the CS12 and CS15 could easily trigger the release of TB.

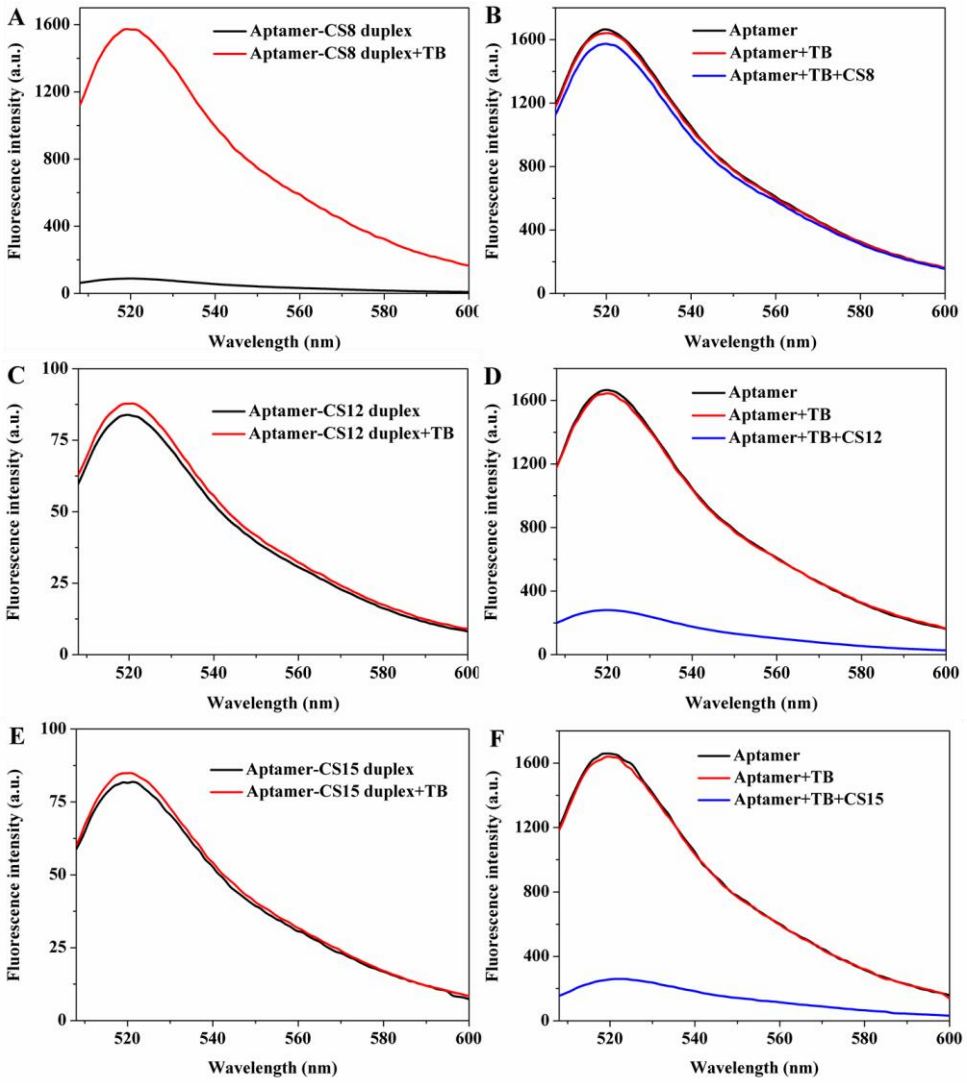

Figure S6. Comparison of the interaction force between the aptamer-TB complex and aptamer-CS duplex.

The amount of CSs that diffused into hydrogel

The amount of the FAM-labeled CS15 diffused into hydrogel could be also determined by fluorescence spectroscopy at 522 nm. The DNA hydrogel samples with captured TB were immersed into CSs buffer solution. Then, the hydrogel simples were washed by using DNA buffer and centrifuged at 1500 rpm for 10 min. The unreacted CSs were washed out from hydrogel and in supernatant. The supernatant and a standard solution (containing the same concentration as that of initial added CSs) were diluted to the same volume in DNA buffer. Their fluorescence emission was measured to calculate diffused amount. Figure S7 and Table S3 showed the percentage of CSs that diffused into hydrogel. The results demonstrated that CSs can easily diffused into hydrogel with a high retention rate. As showed in Table S3, the higher amount of added CSs, the lower percentage of CSs diffusing into hydrogel.

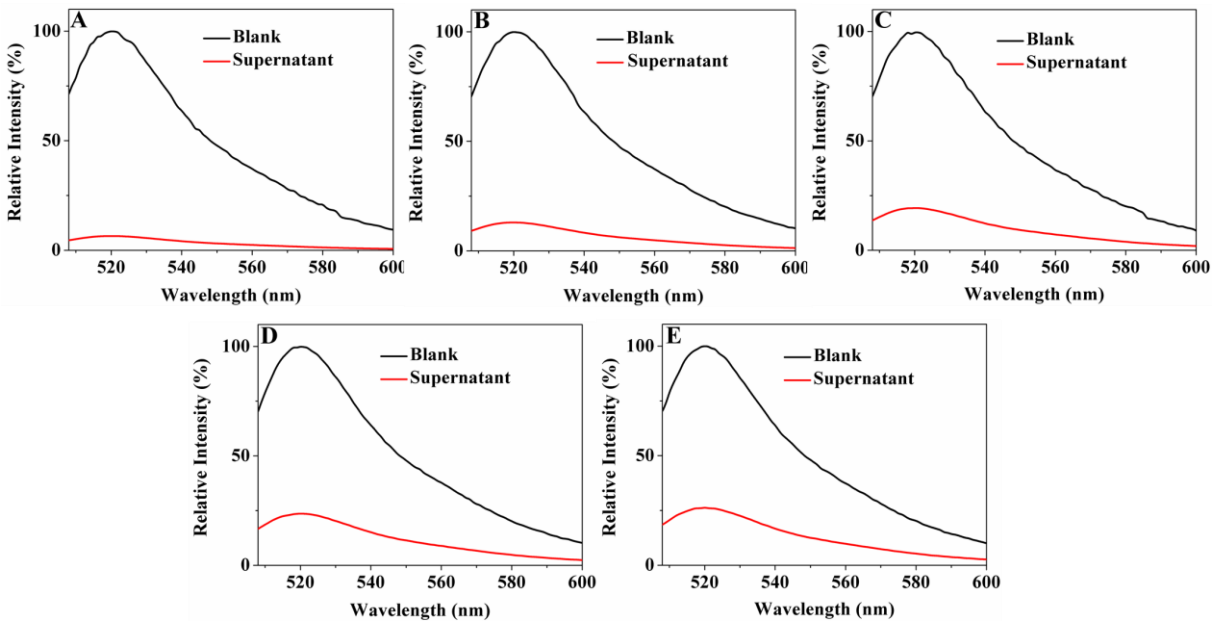

Figure S7. The percentage of CS15 diffused into hydrogel. The initial amount of added CSs was at (A) CSs:S-DNA=1/5, (B) CSs:S-DNA=2/5, (C) CSs:S-DNA=3/5, (D) CSs:S-DNA=4/5, (E) CSs:S-DNA=1. The amount of S-DNA was kept at 0.2 nmol. Their fluorescence values were detected at 522 nm and the percentage of CSs diffused into hydrogel was calculated through the difference of fluorescence intensities between the supernatant and blank solution.

Table S3. The percentage of CS15 that diffused into hydrogel.

Sample No.	The amount of added CSs (CSs:S-DNA, S-DNA was kept at 0.2 nmol)	The percentage of CSs that diffused into hydrogel (±SD) (%)
1	1/5	93.5 ±1.7
2	2/5	87.2 ±2.2
3	3/5	80.7 ±2.6
4	4/5	76.4 ±2.7
5	1	73.7 ±3.1