Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Rational Design and Development of a Universal Baby Spinach-Based Sensing Platform for Detection of Biomolecules

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Experimental Section

1. Reagents and apparatus

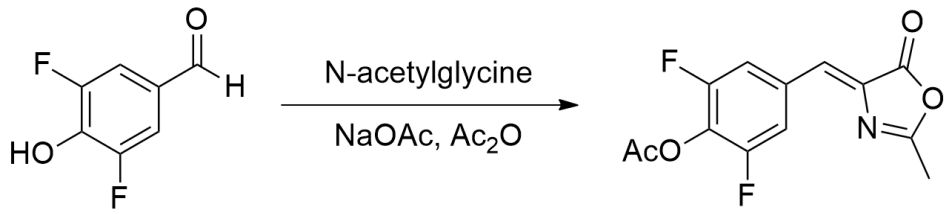
All the DNA/RNA with the sequences listed in Table S1 were purchased from Genewiz Biotechnology Co., Ltd. (Suzhou, China) with PAGE purification. Human-alpha-Thrombin was bought from Haematologic Technologies, Inc. (USA). $HgCl_2$ was purchased from Sigma-Aldrich, Inc. (USA). Tris-HCl buffer (20 mM Tris-HCl, 100 mM NaCl, 20 mM KCl, and 5 mM $MgCl_2$, pH 7.5) was used in this work as reaction buffer. All the stock and buffer were prepared using nuclease-free ultrapure distilled water (Thermo Fisher Scientific, USA).

N-Acetylglycine and 4-hydroxy-3,5-difluorobenzaldehyde were bought from Energy Chemical Co., Ltd. (Shanghai, China). Anhydrous sodium acetate (NaOAc) and potassium carbonate (K_2CO_3) were purchased from J&K Scientific Co., Ltd. (Beijing, China). Acetic anhydride (Ac_2O) and 40% aqueous methylamine were obtained from Sigma-Aldrich, Inc. (USA).

Fluorescence spectra (480-580 nm) were scanned on HORIBA FluoroMax-4 fluorescence spectrophotometer (Japan) with an excitation wavelength of 450 nm. Band-pass of 10 nm and 5 nm were used for excitation and emission slits, respectively, in fluorescence measurements.

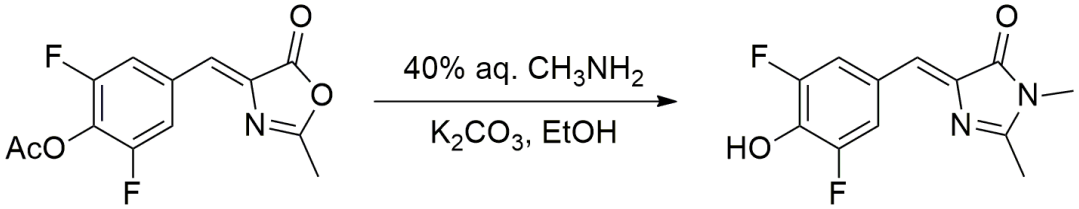
2. Synthesis.

2.1 Synthesis of (Z)-2, 6-difluoro-4-((2-methyl-5-oxooxazol-4(5H)-ylidene)methyl) phenyl acetate (Compound 1)



Compound 1 was synthesized with the previously reported method.[1] N-Acetylglycine (667 mg, 5.70 mmol), anhydrous sodium acetate (467 mg, 5.70 mmol), 4-hydroxy-3,5-difluorobenzaldehyde (900 mg, 5.70 mmol) and 4 mL of acetic anhydride were stirred at 110 ℃ for 2 h. After the reaction cooled to room temperature, 20 mL of cold ethanol was added. Then the resulting mixture was allowed to stir overnight at 4 ℃. After that, the yellow precipitate was filtered and washed with 10 mL of cold ethanol and hot water. The crude solid was dried to afford yellow Compound 1.

2.2 Synthesis of (Z)-4-(3, 5-difluoro-4-hydroxybenzylidene)-1, 2-dimethyl-1H-imidazol-5(4H)-one (DFHBI)



Compound 1 (1 g, 3.56 mmol), potassium carbonate (K_2CO_3) (350 mg, 2.53 mmol), ethanol (20 mL) and 40% aqueous methylamine (2 mL) were stirred at 85 ℃ for 4 h. Then the reaction was allowed to cool down at room temperature. Orange precipitate was formed while cooling down, which was filtered next and washed three times with 10 mL cold ethanol each time. After that, the precipitate was re-dissolved in a 1:1 mixture of ethyl acetate and 500 mM sodium acetate (pH 3.0). The organic layer was separated, dried with anhydrous sodium sulfate and solvent was removed under reduced pressure to afford DFHBI as a bright yellow solid (612 mg, yield 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (m, 2H), 6.89 (s, 1H), 3.09 (s, 3H), 2.35 (s, 3H).

3. Fluorescence detection of miRNA-21, thrombin and Hg^{2+}

For miRNA-21 detection, the Baby Spinach and DNA repressor oligonucleotides were dissolved by nuclease-free ultrapure distilled water to get a stock solution of 100 μM. Baby Spinach was heated at 95 °C for 1.5 min and then quickly cooled down on ice before use. Under the optimized conditions, 100 nM Baby Spinach, 100 nM DNA repressor and miRNA-21 with the concentration of 0 to 100 nM were mixed and incubated at 37 °C for 30 min in Tris-HCl buffer. Then DFHBI was added to make the final volume at 100 μL. The resulted solutions were allowed to incubate at room temperature for another 30 min and followed by fluorescence measurement. Three independent replicates were measured and standard deviation was calculated as error bar. The same procedures were also used for thrombin and Hg^{2+} detection.

4. Preparation of cell lysates

Authenticated HeLa cells with no mycoplasma contamination were seeded in DMEM-Glutamax-I (Gibco) with 10% heat-inactivated fetal bovine serum (Gibco) and 1X antibiotic-antimycotic (Gibco). The incubator was in humidified atmosphere with 5% CO_2 and the incubation temperature was kept at 37 °C. After growing to about 70% confluence, the cells were washed twice with phosphate buffered saline (PBS, pH 7.4, Thermo) and trypsinized in 75 cm^2 flasks (Corning) using TrypLE Express (Thermo Fisher Scientific). Next the collected cells were centrifuged for 5 min at 500×g and washed three times with PBS. Then the cells were disrupted by incubating for 10 min on ice in 500 μl cell lysis buffer (20 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$ and 0.5% Nonidet P-40, pH 7.5). Finally, the lysate was centrifuged at 15,000×g for 10 min at 4 °C, and the supernatant which contains the cell lysate was collected. The above-prepared cell lysates were diluted 20 times with Tris-HCl buffer and used as the analysis medium. Different concentrations of

miRNA-21 and thrombin were added into the diluted cell lysates. The detection procedure was the same as that for miRNA and thrombin detection in Tris-HCl buffer.

5. Fluorescence detection of Hg^{2+} in seawater.

Seawater used in this work was collected from Sai Kung (Hong Kong), which was diluted 20 times with Tris-HCl buffer and used as the analysis medium. Different concentrations of Hg^{2+} were added into the diluted seawater. The detection procedure was the same as that for Hg^{2+} detection in Tris-HCl buffer.

Supplementary Materials

Table S1. Sequences of the oligonucleotides used in this work.

Name*	Sequence
Baby Spinach	5′-GGUGAAGGACGGGUCCAGUAGUUCGCUACUGUUGAGUAGAGUG UGAGCUCC-3′
miRNA-21	5′-UAGCUUAUCAGACUGAUGUUGA-3′
TM-miRNA-21[1]	5′-UAGCUUAUCAGACAGAUAUUGA-3′
miRNA-125b	5′-UCCCUGAGACCCUAACUUGUGA-3′
miRNA-765	5′-UGGAGGAGAAGGAAGGUGAUG-3′
miRNA-197	5′-CGGGUAGAGAGGGCAGUGGGAGG-3′
miRNA-432	5′-UCUUGGAGUAGGUCAUUGGGUGG-3′
miRNA-149	5′-AGGGAGGGACGGGGGCUGUGC-3′
miRNA-5196	5′-AGGGAAGGGGACGAGGGUUGGG-3′
Repressor 1	5′-ACACTCTACTCAACATCAGTCTGATAAGCTACCCGTCCT-3′
Repressor 2	5′-CACTCTACTCAACATCAGTCTGATAAGCTACCCGTCCT-3′
Repressor 3	5′-ACTCTACTCAACATCAGTCTGATAAGCTACCCGTCCT-3′
Repressor 4	5′-CTCTACTCAACATCAGTCTGATAAGCTACCCGTCCT-3′
Repressor 5	5′-CTCTACTCAACATCAGTCTGATAAGCTACCCGTC-3′
Repressor 6	**5′-CTACTCAACATCAGTCTGATAAGCTACCCGTC-3′**
Repressor 7	5′-ACTCAACATCAGTCTGATAAGCTACCCGTC -3′
Repressor 8	5′-TCAACATCAGTCTGATAAGCTACCCGTC-3′
Repressor T1	5′-CTCAACAGGTTGGTGTGGTTGGACCCGTC-3′
Repressor T2	5′-TCAACAGGTTGGTGTGGTTGGACCCGTC-3′
Repressor T3	**5′-CTCAACAGGTTGGTGTGGTTGGACCCGT-3′**
Repressor T4	5′-TCAACAGGTTGGTGTGGTTGGACCCGT-3′
Repressor T5	5′-TCAACAGGTTGGTGTGGTTGGACCCG-3′
Repressor T6	5′-CAACAGGTTGGTGTGGTTGGACCCGT-3′
Repressor T7	5′-CAACAGGTTGGTGTGGTTGGACCCG-3′
Repressor T8	5′-CAACAGGTTGGTGTGGTTGGACCC-3′
Repressor H1	5′-ACACTCTACTTCTTTCTTCCCCTTGTTTGTTGCCGTCCTT-3′
Repressor H2	**5′-CACTCTACTTCTTTCTTCCCCTTGTTTGTTGCCGTCCTT-3′**
Repressor H3	5′-ACACTCTACTTCTTTCTTCCCCTTGTTTGTTGCCGTCCT-3′
Repressor H4	5′-CACTCTACTTCTTTCTTCCCCTTGTTTGTTGCCGTCCT-3′

*Repressor 6, Repressor T3 and Repressor H2 are chosen for miRNA-21, thrombin and Hg^{2+} detection, respectively, which are highlighted in bold; target recognition elements are underlined in repressor sequences.

[1]Two bases-mismatched miRNA-21.

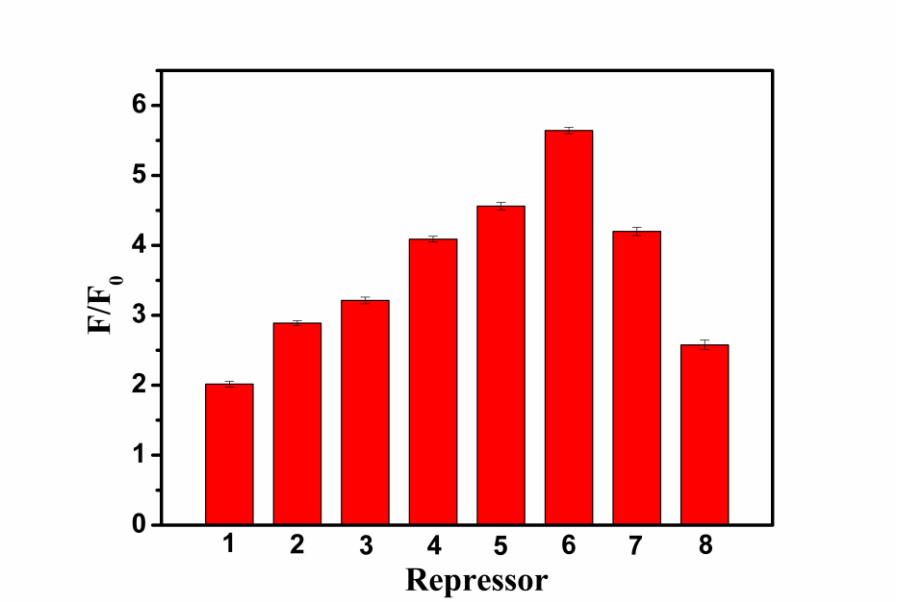


Fig. S1 Optimization of the repressor sequence for miRNA-21 detection. The concentration of miRNA-21 is 100 nM, Baby Spinach is 100 nM, DFHBI is 10 μM. F and F_0 are the fluorescence intensity of the system with and without miRNA-21, respectively. According to the figure, repressor 6 was chosen for further investigation to ensure the highest signal-to-background ratio.

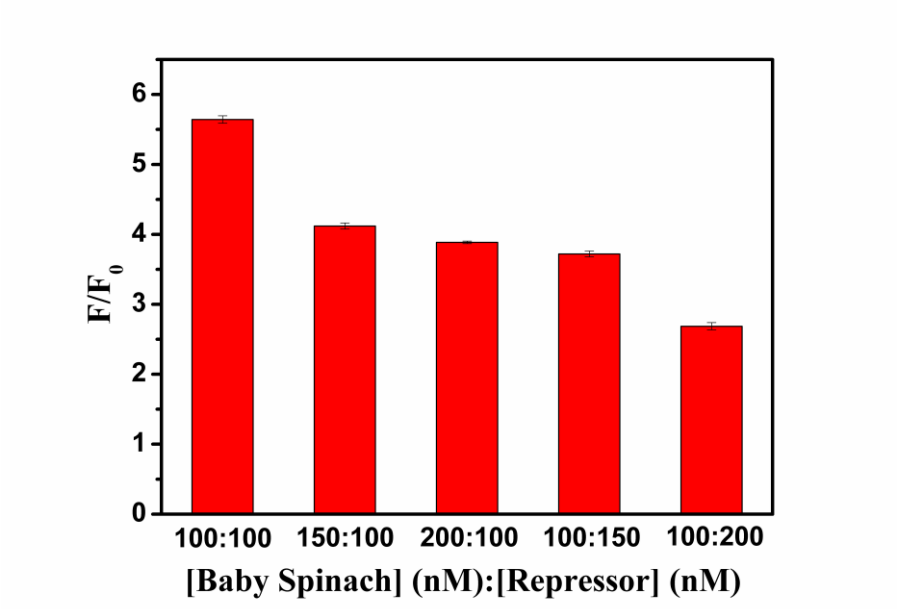


Fig. S2 Optimization of the concentration of Baby Spinach and repressor strand for miRNA-21 detection. The concentration of miRNA-21 is 100 nM, DFHBI is 10 μM. F and F_0 are the fluorescence intensity of the system with and without miRNA-21, respectively. According to the figure, 100 nM Baby Spinach and 100 nM repressor strand were chosen for further investigation to ensure the highest signal-to-background ratio.

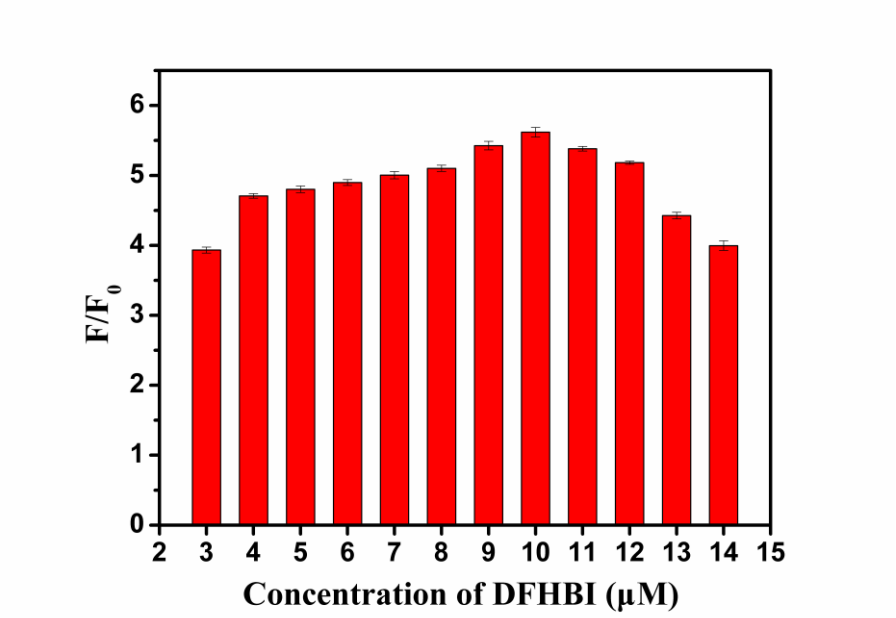


Fig. S3 Optimization of the concentration of DFHBI for miRNA-21 detection. The concentration of miRNA-21 is 100 nM, Baby Spinach is 100 nM, repressor strand is 100 nM. F and F_0 are the fluorescence intensity of the system with and without miRNA-21, respectively. According to the figure, 10 μM DFHBI was chosen for further investigation to ensure the highest signal-to-background ratio.

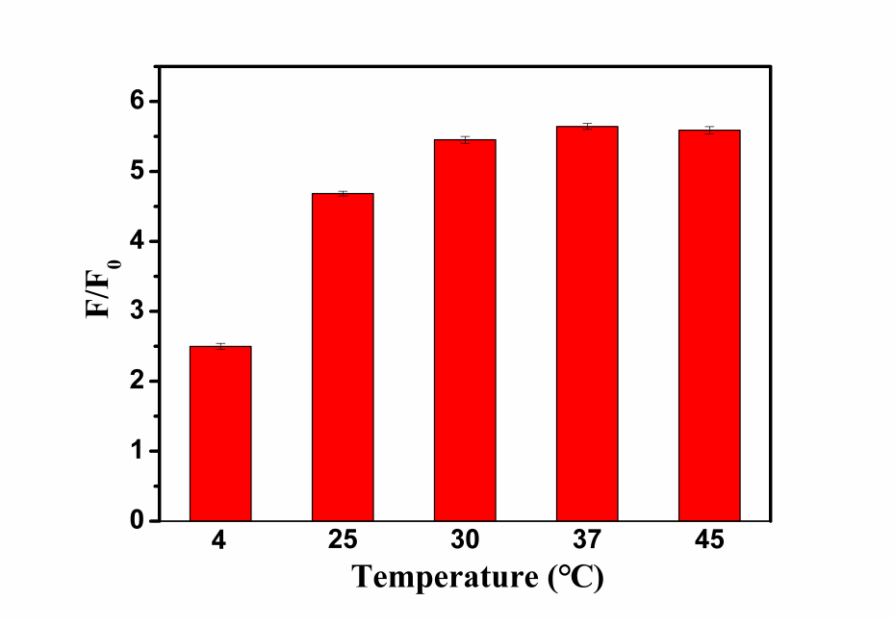


Fig. S4 Optimization of the incubation temperature for hybridization of miRNA-21 and UBSP_miR21. The concentration of miRNA-21 is 100 nM, Baby Spinach is 100 nM, repressor strand is 100 nM, DFHBI is 10 μM. F and F_0 are the fluorescence intensity of the system with and without miRNA-21, respectively. According to the figure, 37 ℃ was chosen for further investigation to ensure the highest signal-to-background ratio.

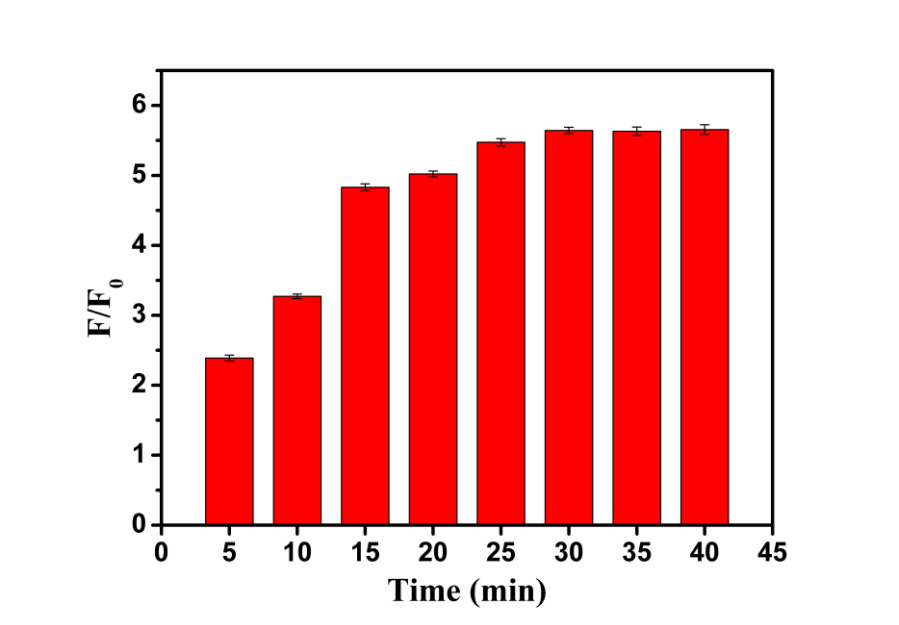


Fig. S5 Optimization of the incubation time for hybridization of miRNA-21 and UBSP_miR21. The concentration of miRNA-21 is 100 nM, Baby Spinach is 100 nM, repressor strand is 100 nM, DFHBI is 10 μM. F and F_0 are the fluorescence intensity of the system with and without miRNA-21, respectively. According to the figure, 30 min was chosen for further investigation to ensure the highest signal-to-background ratio.

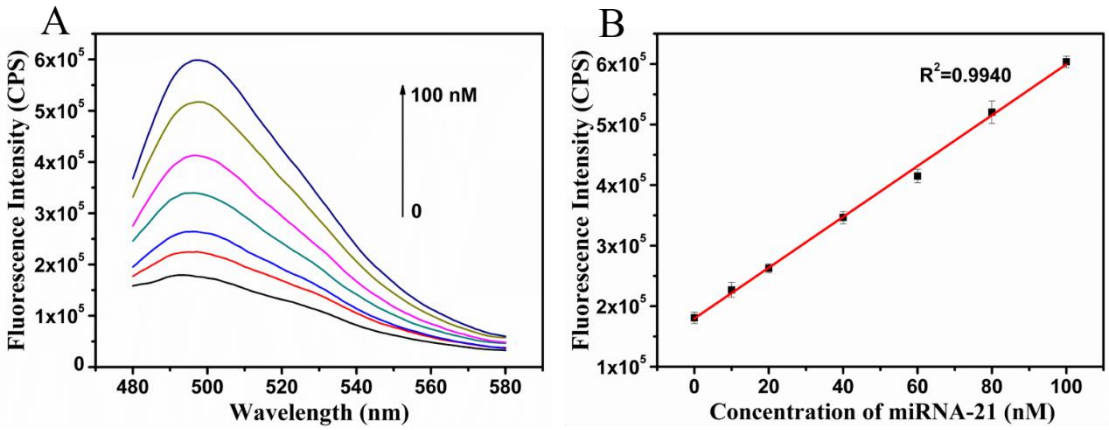


Fig. S6 MiRNA-21 detection in cell lysate by using UBSP_miR21. (A) Fluorescence spectra of the system with different concentrations of miRNA-21 (0-100 nM); (B) Scatter plot of fluorescence intensity as a function of the concentrations of miRNA-21 (0-100 nM).

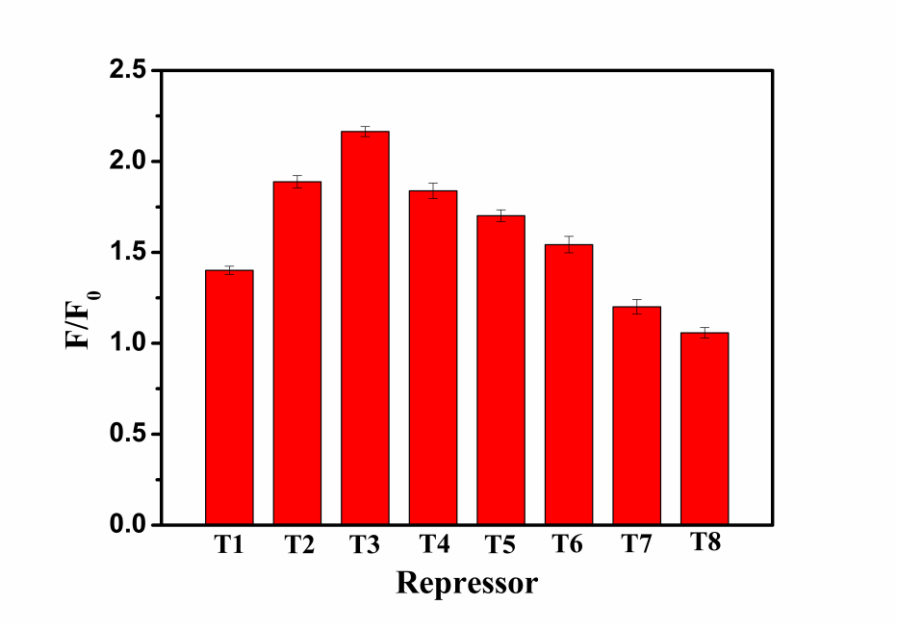


Fig. S7 Optimization of the repressor sequence for thrombin detection. The concentration of thrombin is 250 nM, Baby Spinach is 100 nM, DFHBI is 10 μM. F and F_0 are the fluorescence intensity of the system with and without thrombin, respectively. According to the figure, repressor T3 was chosen for further investigation to ensure the highest signal-to-background ratio.

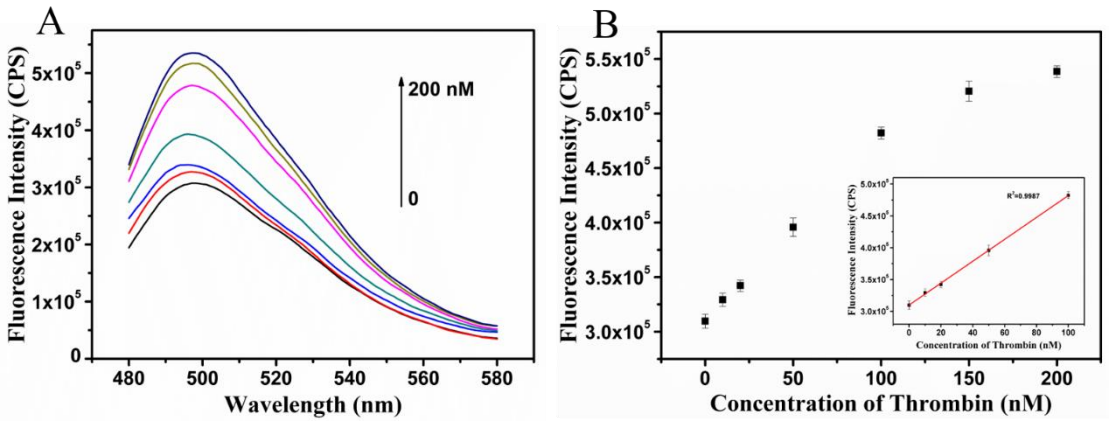


Fig. S8 Thrombin detection in cell lysate by using UBSP_Thrombin. (A) Fluorescence emission spectra of the system with different concentrations of thrombin (0-200 nM); (B) Scatter plot of fluorescence intensity as a function of the concentrations of thrombin (0-200 nM). Inset: Linear relationship in the concentration range from 0-100 nM thrombin.

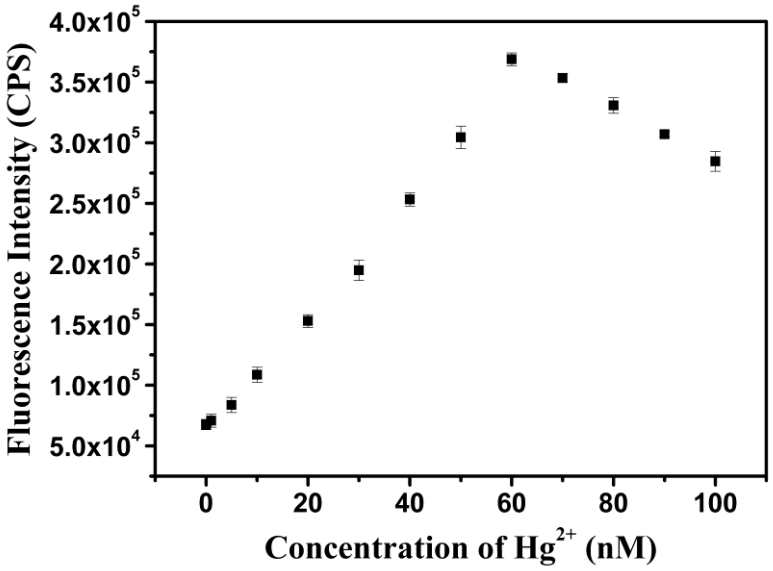


Fig. S9 Hg^{2+} detection in Tris-HCl buffer by using UBSP_Hg^{2+}. Scatter plot of fluorescence intensity as a function of the concentrations of Hg^{2+} (0-100 nM).

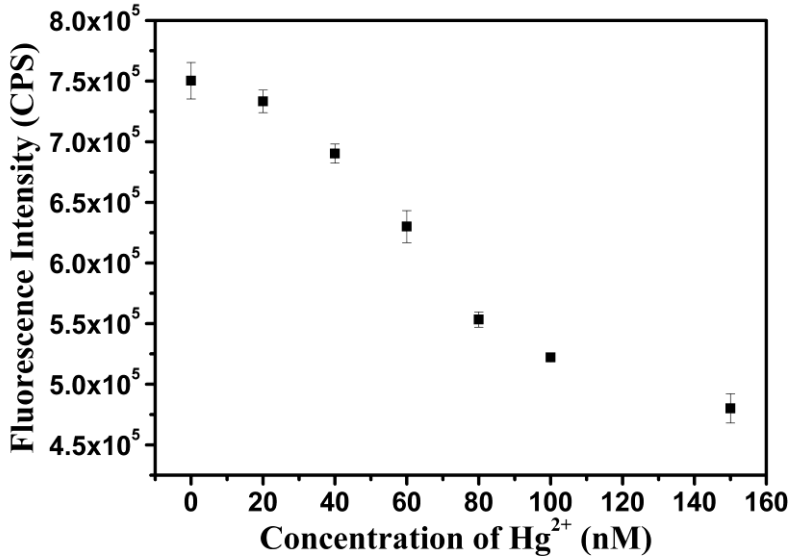


Fig. S10 Scatter plot of fluorescence intensity of Baby Spinach-DFHBI complex as a function of the concentrations of Hg^{2+} (0-150 nM). The concentration of Baby Spinach is 100 nM, and DFHBI is 10 μM.

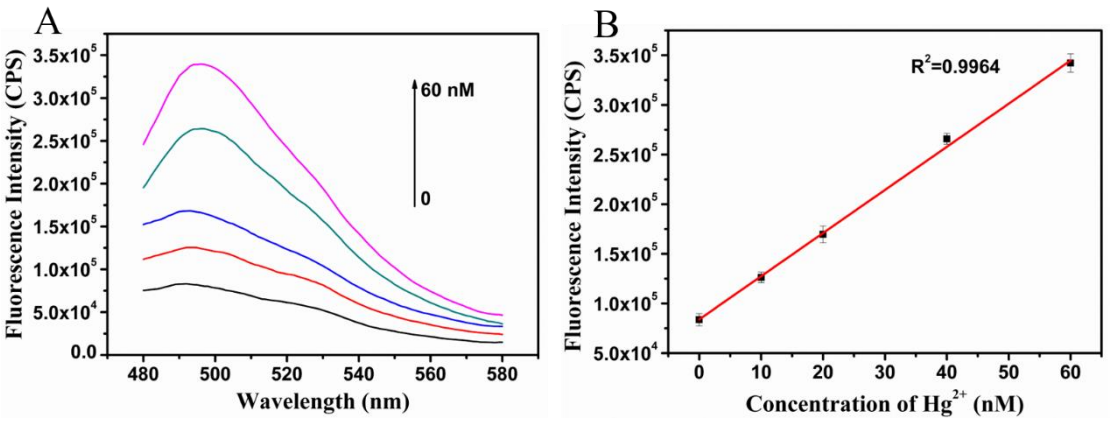


Fig. S11 Hg^{2+} detection in seawater by using UBSP_Hg^{2+}. (A) Fluorescence spectra of the system with different concentrations of Hg^{2+} (0-60 nM); (B) Scatter plot of fluorescence intensity as a function of the concentrations of Hg^{2+} (0-60 nM).

Reference:

1 J. S. Paige, K. Y. Wu and S. R. Jaffrey, *Science*, 2011, **333**, 642.