Electronic Supplementary Material (ESI) for Analytical Methods.

Selection and characterization of Thioflavin T aptamers for the development of light-up probes

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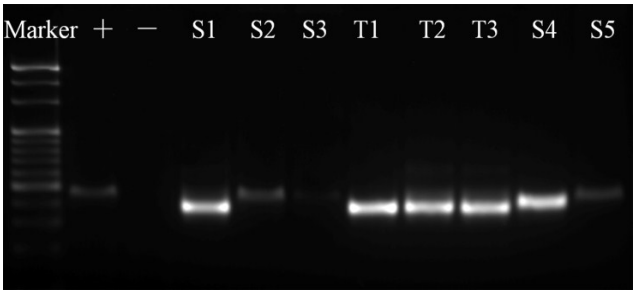


Figure S1. The elution profile by agarose gel electrophoresis for the eighth round of selection. Brighter electrophoresis bands were observed for the fractions eluted by ThT solution (T1~T3) than those eluted by elution buffer.

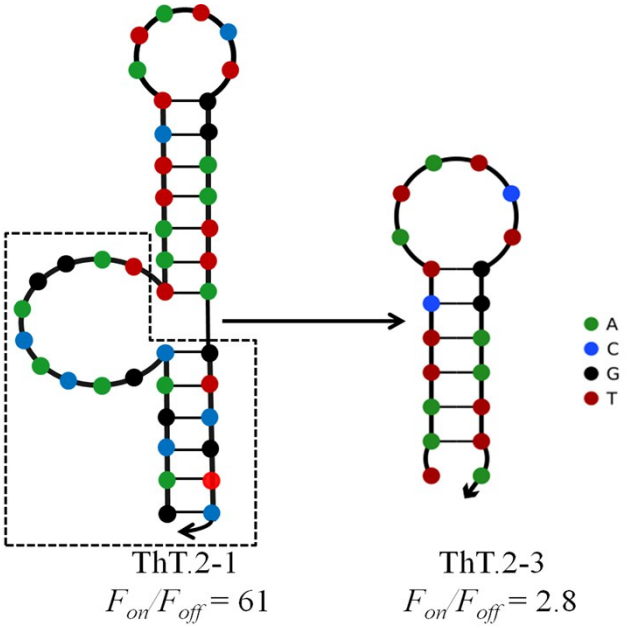


Figure S2. The second structure of ThT.2-1 and ThT.2-3. The fluorescence intensity (excitation = 425 nm and emission = 490 nm) of ThT (1 μM)-DNA (6.5mg/L, equal to 1 μM for ThT.2-1) was measured in binding buffer (20 mM Tris-HCl buffer, pH=7.4).

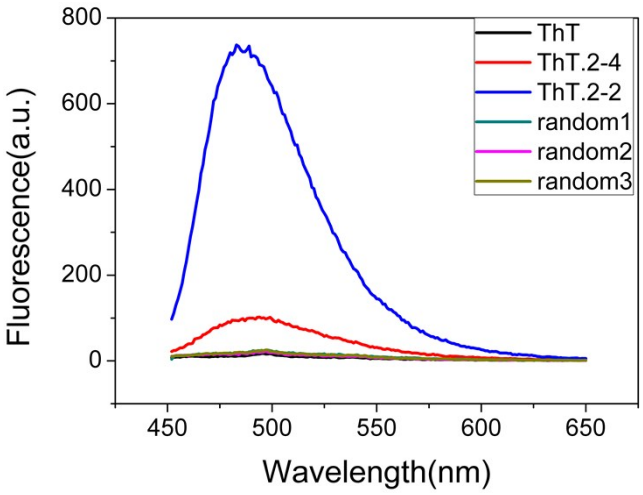


Figure S3. Enhancement in fluorescence for random sequences compared with ThT.2-2. The fluorescence intensity (excitation = 425 nm) of ThT (2 μM)-DNA (1 μM) was measured in binding buffer (20 mM Tris-HCl buffer, pH = 7.4).

The random sequences and aptamers used were as below.

random1: GCGACGACGTAAGCGTTGGGGCACCCGGTCGC 32bp

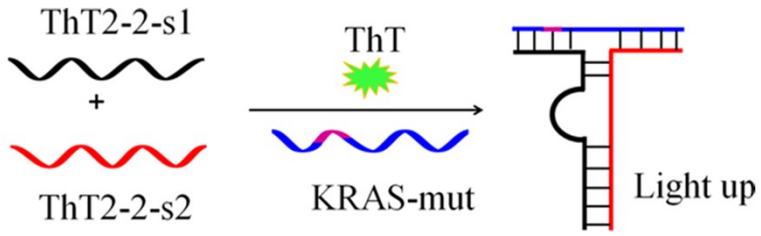
random2: CCCTTAATCCCCTATAATAAATTTACCTTCCTCCGCAAT 39bp

random3: CTCTCTCCTTTCTCCTGTACATCCTCTTTCCTCTC 35bp

ThT.2-4: GACGACATATGGAACTTTTCTCTTATCGTCGTC 33bp

ThT.2-2: GACGACGACACAGGATTAATCTTATTAGTCGTC 33bp

Scheme S1. The design of the "light up" biosensor for the detection of KRAS 13 codon mutation. The purple section represents the mutation site.



The principle of the biosensor is shown in Scheme S1. Here, we engineered ThT.2-2 (a truncated selected aptamer for ThT) into a nucleic acid sensing probe by dividing it into two fragments. Each part was extended to act as the probe with target binding arm, which is complementary to the target DNA. The two split aptamers as the probes were designed for the detection of a hot mutation at 13 codon of KRAS. The core structure of ThT.2-2 could be restored only in the presence of KRAS 13 codon mutation. The fluorescence intensity changed slightly in the presence of wild-type KRAS because of the mismatched bases when being complemented to the binary probes. The enhancement in fluorescence was determined by comparing the intensity of the fluorescence emissions at 490 nm in different solutions. The signal-to-background ratio was calculated as F_{on}/F_{off}, where F_{on} is the fluorescence of the two probes-ThT in the presence of KRAS sequence, and F_{off} is the fluorescence of the two probes-ThT in the absence of KRAS sequence.

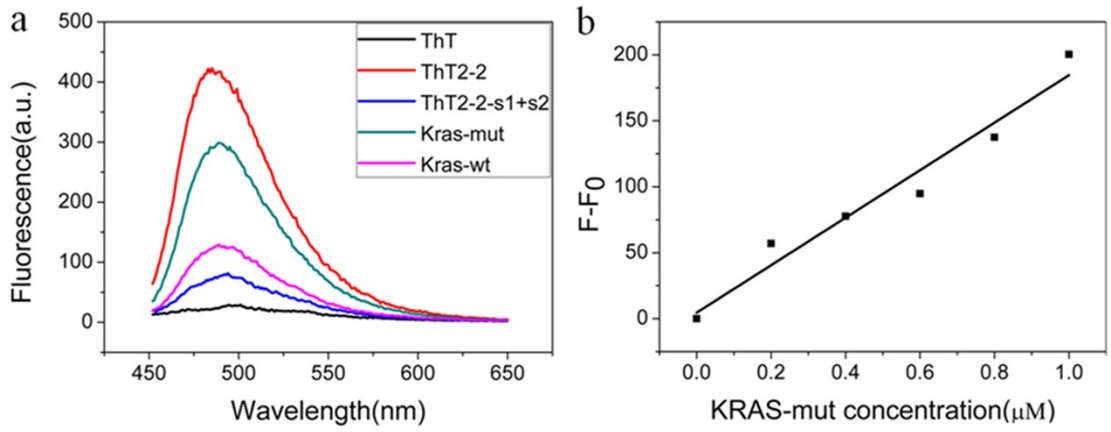


Figure S4. a. Fluorescence intensity in the presence of 1:1 ratio of KRAS sequence to binary probes. [ThT]=1 μM, [DNA]=1 μM. b. Change of fluorescence intensity in different concentrations of KRAS-mutation.

The sequences used for designing biosensors for the detection of KRAS mutation in 13 codon. The mutation sites are underlined.

ThT2-2-s1: GACGACGACACAGGATTAATT ACATCACCAGC

ThT2-2-s2: GGCACTCTTGCCATTAGTCGTC

KRAS-wt: GCTGGTGGCGTAGGCAAGAGTGCC

KRAS-mut: GCTGGTGATGTAGGCAAGAGTGCC