Electronic Supplementary Information

belonging to the article

Moderate stability of scissor double fluorescent triple helix molecular switch for ultrasensitive Biosensing of crop transgene

Xiuling Chu,[‡a] Desong Zhu,[‡*b] Min Liu,[c] Lingrang Kong,[d] and Shiyun Ai [b]

[a] *Shandong Taian Ecological Environment Monitoring Center, Taian 271000, P. R. China.*

[b] *Key Laboratory of Agricultural Film Application of Ministry of Agriculture and Rural Affairs, College of Chemistry and Material Science, Shandong Agricultural University, Taian 271018, P. R. China.*

[C] *Shandong Qingdao Ecological Environment Monitoring Center, Qingdao 266000, P. R. China.*

[d] *State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Taian 271000, P. R. China.*

Corresponding author:
E-mail: zhudesong@sdau.edu.cn; Fax: +86 538 8242251; Tel: +86 538 8241163.

Results:

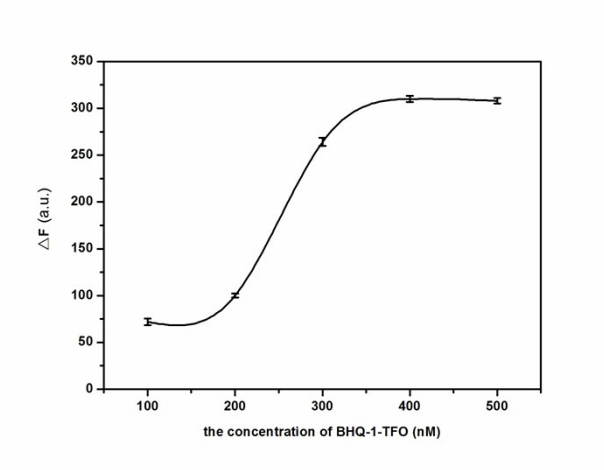

Fig. S1 The effect of BHQ-1-TFO concentration.

Experimental conditions: 10 mM HAc-Ac- buffer (pH 5.0), 300 mM $NaNO_3$, 100 nM SB-3, (100-500) nM (BHQ-1-TFO-3)-3, 100 nM Bt gene, 1.5 h of self-assembly at 40 °C, 0.8 h of binding at 40 °C.

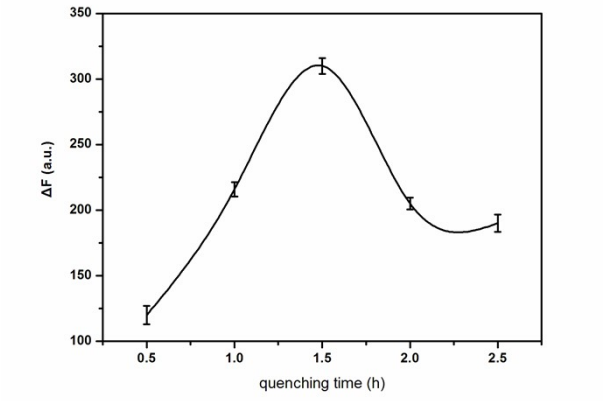

Fig. S2 The effect of quenching time.

Experimental conditions: 10 mM HAc-Ac- buffer (pH 5.0), 300 mM $NaNO_3$, 100 nM SB-3, 400 nM (BHQ-1-TFO-3)-3, 100 nM Bt gene, (0.5-2.5) h of quenching at 40 °C, 0.8 h of binding at 40 °C.

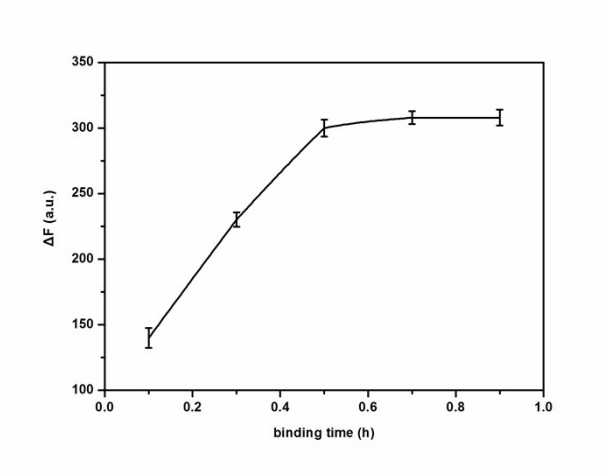

Fig. S3 The effect of binding time.

Experimental conditions: 10 mM HAc-Ac- buffer (pH 5.0), 300 mM $NaNO_3$, 100 nM SB-3, 400 nM (BHQ-1-TFO-3)-3, 100 nM Bt gene, 1.5 h of self-assembly at 40 °C, (0.1-0.9) h of binding at 40 °C.

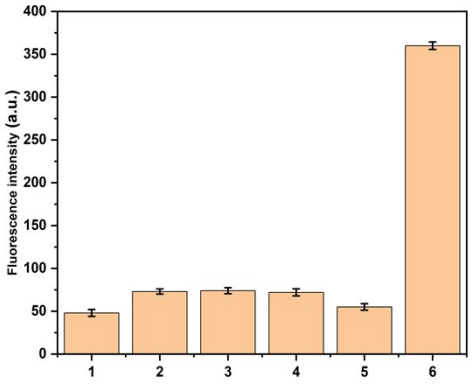

Fig. S4 The specificity of the proposed method.

(1) The control experiment without Bt gene (2) Single-base mismatched DNA (SM-DNA-1), (3) SM-DNA-2, (4) SM-DNA-3, (5) three-base mismatched DNA (TM-DNA), (6) Bt gene.

Experimental conditions: 10 mM HAc-Ac- buffer (pH 5.0), 300 mM $NaNO_3$, 100 nM SB-3, 400 nM (BHQ-1-TFO-3)-3, 100 nM (single-base mismatched DNA, three-base mismatched DNA and Bt gene), 1.5 h of self-assembly at 40 °C, 0.8 h of binding at 40 °C.

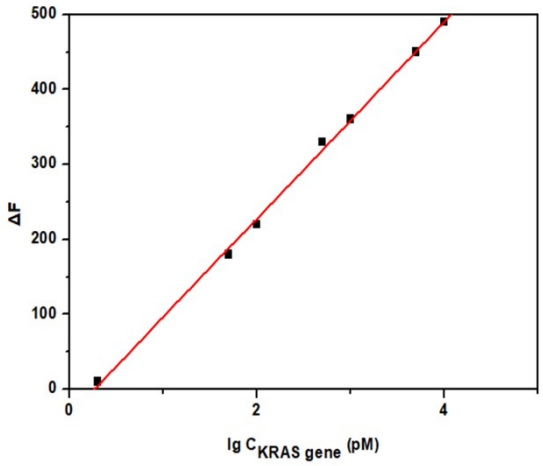

Fig. S5 The relationship between fluorescence intensity and the concentration of KRAS gene.

Experimental conditions: 10 mM HAc-Ac- buffer (pH 5.0), 300 mM of $NaNO_3$, 100 nM SB-3, 400 nM (BHQ-1-TFO-2)-3, (2 pM to 10 nM) KRAS gene, 1.5 h of self-assembly at 40 °C, 0.8 h of binding at 40 °C. The error bars showed the standard deviation of three replicate determinations.

Table S1. Sequences of oligonucleotides in this study

Name	Sequences (5'-3')
SB-1	TTTTTTTTTTTTTT*GAGGTGCTGCCGCTGCCGAAGTGCGCTGG T*TTTTTTTTTTTTT
SB-2	CCCCCCCCCCCCCCC*GAGGTGCTGCCGCTGCCGAAGTGCGCTG GT*CCCCCCCCCCCCCC
SB-3	TCTCTCTCTCTCTC*GAGGTGCTGCCGCTGCCGAAGTGCGCTG GT*CTCTCTCTCTCTCT
(BHQ-1-TFO-1)-1	AAAAAA
(BHQ-1-TFO-2)-1	AAAAAAAA
(BHQ-1-TFO-3)-1	AAAAAAAAAA
(BHQ-1-TFO-4)-1	AAAAAAAAAAAA
(BHQ-1-TFO-5)-1	AAAAAAAAAAAAAA
(BHQ-1-TFO-1)-2	GGGGGG
(BHQ-1-TFO-2)-2	GGGGGGGG
(BHQ-1-TFO-3)-2	GGGGGGGGGG
(BHQ-1-TFO-4)-2	GGGGGGGGGGGG
(BHQ-1-TFO-5)-2	GGGGGGGGGGGGGG
(BHQ-1-TFO-1)-3	AGAGAG
(BHQ-1-TFO-2)-3	AGAGAGAG
(BHQ-1-TFO-3)-3	AGAGAGAGAG
(BHQ-1-TFO-4)-3	AGAGAGAGAGAG
(BHQ-1-TFO-5)-3	AGAGAGAGAGAGAG
Bt gene	ACCAGCGCACTTCGGCAGCGGCAGCACCTC
SM-DNA-1	ACCAGCCCACTTCGGCAGCGGCAGCACCTC
SM-DNA-2	ACCAGCGCACTTCGGGAGCGGCAGCACCTC
SM-DNA-3	ACCAGCGCACTTCGGCAGCGGCAGCTCCTC
TM-DNA	ACCAGCCCACTTCGGCTGCGGCAGCACGTC

T-MB	**TCTCTCTCTC***GAGGTGCTGCCGCTGCCGAAGTGCGCTGGT* **GAGAGAGAGA**
KRAS gene	AG CTG GTG GCG TAG GCA AGA G

(The underlined regions in SB (SB-1, SB-2 and SB-3) denote the two homopyrimidine strands at both ends (the components of the scissor DFTHMS). The italics areas regions in (SB-1, SB-2 and SB-3 as well as T-MB) denote the complementary sequences of Bt gene. The underlined regions in SM-DNA and TM-DNA denote the mismatched bases. The boldface regions in T-MB (traditional molecular beacon) denote the complementary sequence of the neck.