

Parallel [TG(GA)₃]_n-homoduplexes/thioflavin T: An intense and stable fluorescent indicator for label-free biosensing

Qiang Liu,^a Shaochun Jing,^a Mei Liu,^b Yan Jin,^a Baoxin Li^{a*}

^aSchool of Chemistry & Chemical Engineering, Key laboratory of Analytical Chemistry for Life Science of Shaanxi Province, Shaanxi Normal University, Xi'an 710062, China

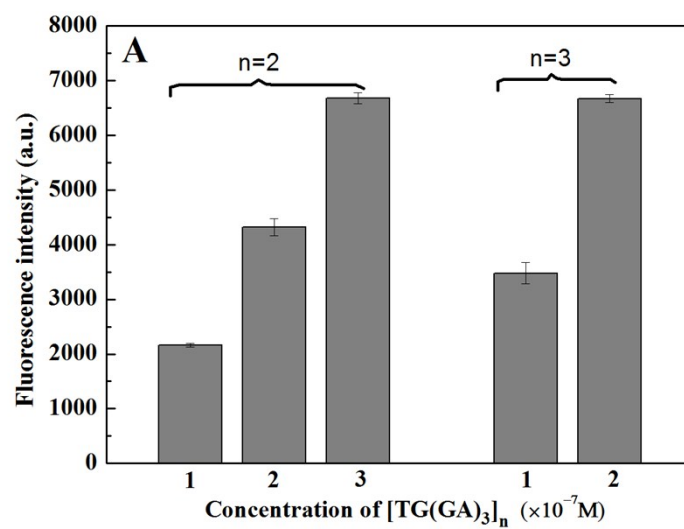
^bCollege of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China

*Fax: +86-29-81530727. E-mail: libaoxin@snnu.edu.cn (B. Li)

Table S1 Sequence of the used oligonucleotides

Name	Sequence (5'-3')
(GA) ₁₀	GA GA GA GA GA GA GA GA GA GA GA
(GA) ₁₀ TG	GA GA GA GA GA GA GA GA GA GA GA TG
(GA) ₁₀ TGTG	GA GA GA GA GA GA GA GA GA GA GA TGTG
TG(GA) ₁₀	TG GA GA GA GA GA GA GA GA GA GA GA
TGTG(GA) ₁₀	TGTG GA GA GA GA GA GA GA GA GA GA GA
TG(GA) ₁₀ GT	TGGA GA GA GA GA GA GA GA GA GA GA GT
TG(GA) ₁₀ TG	TGGA GA GA GA GA GA GA GA GA GA GA TG
[TG(GA) ₅] ₂	TG GAGAGAGAGA TG GAGAGAGAGA
(GA) ₅	GAGAGAGAGA
(GA) ₅ TG	GAGAGAGAGA TG
TG(GA) ₅	TG GAGAGAGAGA
TG(GA) ₅ TG	TG GAGAGAGAGA TG
[(GA) ₅ TG] ₂	GAGAGAGAGA TG GAGAGAGAGA TG
[(GA) ₅ TG] ₂ TG	GAGAGAGAGA TG GAGAGAGAGA TG TG
TG[GA] ₂ TG[GA] ₂	TG GAGA TG GAGA
TG[GA] ₃ TG[GA] ₃	TG GAGAGA TG GAGAGA
TG[GA] ₄ TG[GA] ₄	TG GAGAGAGA TG GAGAGAGA
TG[GA] ₅ TG[GA] ₅	TG GAGAGAGAGA TG GAGAGAGAGA
TG[GA] ₆ TG[GA] ₆	TG GAGAGAGAGAGA TG GAGAGAGAGAGA
TG[GA] ₇ TG[GA] ₇	TG GAGAGAGAGAGAGA TG GAGAGAGAGAGAGA
TG[GA] ₈ TG[GA] ₈	TG GAGAGAGAGAGAGAGA TGGAGAGAGAGAGAGAGA
TG[GA] ₉ TG[GA] ₉	TG GAGAGAGAGAGAGAGAGA TG GAGAGAGAGAGAGAGAGA
TG[GA] ₁₀ TG[GA] ₁₀	TG GAGAGAGAGAGAGAGAGAGA TG GAGAGAGAGAGAGAGAGAGA
[TG(GA) ₃] ₂	TGGAGAGA TGGAGAGA
[TG(GA) ₃] ₃	TGGAGAGA TGGAGAGA TGGAGAGA
[TG(GA) ₃] ₄	TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA
[TG(GA) ₃] ₅	TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA
[TG(GA) ₃] ₆	TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA TGGAGAGA

C-[TG(GA) ₃] ₃	TCT CTC CAT CTC TCC ATC TCT CCA
G4	AGGG TTAGGG TTAGGG TTAGGG TTAGGG
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
ps-Template	TCTCTCCATCTCTCCATCTCTCCATCTTGACTCTCAACATCAGTC TGATAAGCTA-phosphate
G4-Template	CCCTAACCCCTAACCCCTAACCCCTTCTTGACTCTCAACATCAGTCT GATAAGCTA-phosphate



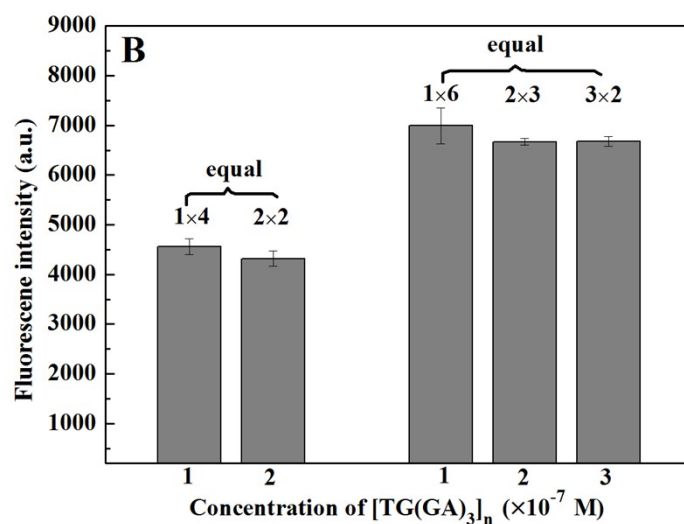


Figure S1. (A) The fluorescence intensity variation of $[\text{TG}(\text{GA})_3]_n/\text{ThT}$ with the increasing concentration of $[\text{TG}(\text{GA})_3]_n$; (B) Comparison of the fluorescence intensity of $[\text{TG}(\text{GA})_3]_n/\text{ThT}$ when $C \times n$ (where C is the concentration of $[\text{TG}(\text{GA})_3]_n$, and n is the number of $\text{TG}(\text{GA})_3$ motifs) is equal.

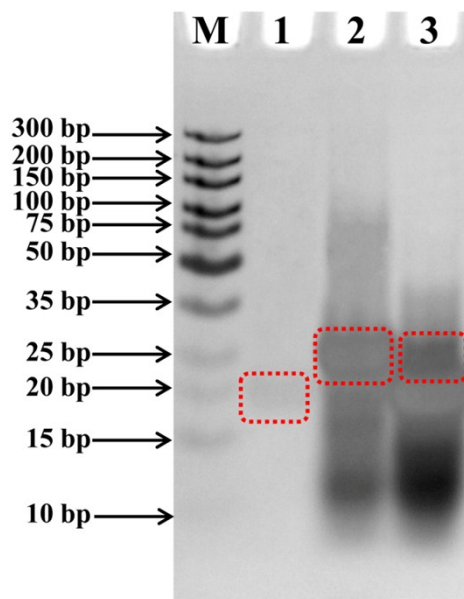


Figure S2 Polyacrylamide gel electrophoresis verification of the structure of parallel $[\text{TG}(\text{GA})_3]_3$ homoduplex in 5 mM Tris-HCl (7.4), 20 mM Mg^{2+} buffer. Lane M: Marker; Lane 1: C- $[\text{TG}(\text{GA})_3]_3$; Lane 2: The mixture of C- $[\text{TG}(\text{GA})_3]_3$ and $[\text{TG}(\text{GA})_3]_3$; Lane 3 : $[\text{TG}(\text{GA})_3]_3$.

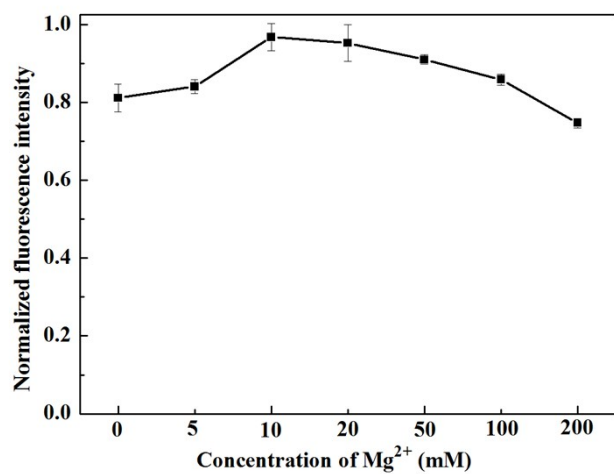


Figure S3 The fluorescence intensity change of [TG(GA)₃]₃/ThT with the increase concentration of Mg²⁺. Experimental condition: 100 nM DNA strands, 20 μM ThT in 5 mM Tris-HCl (pH=7.4).

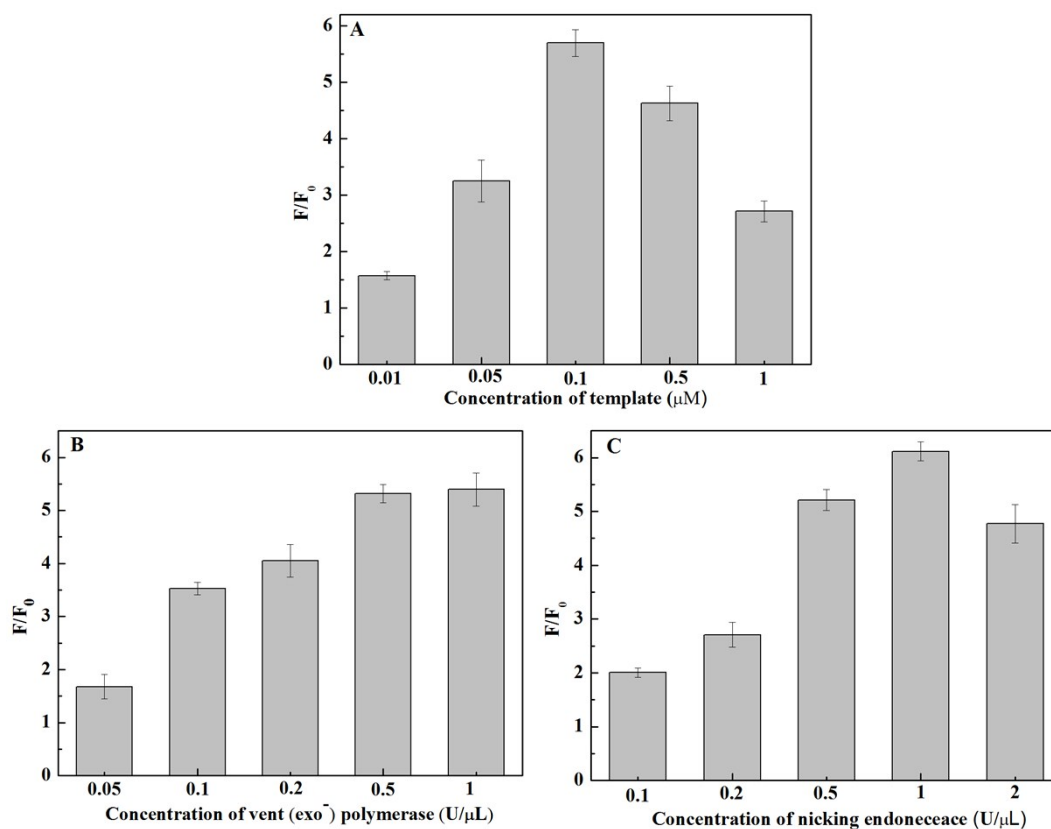


Figure S4. Effects of EXPAR reaction conditions on the sensitivity of $[\text{TG}(\text{GA})_3]_3$ system: (A) concentration of template, (B) amount of Vent (exo-) polymerase, (C) amount of Nt.BstNBI.

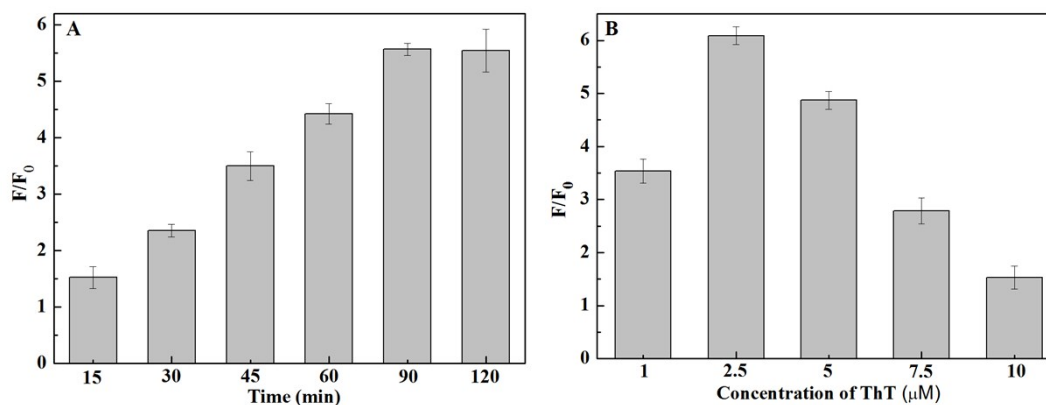


Figure S5. Effects of time (A) and ThT concentration (B) on the sensitivity of $[\text{TG}(\text{GA})_3]_3/\text{ThT-EXPAR}$ system.

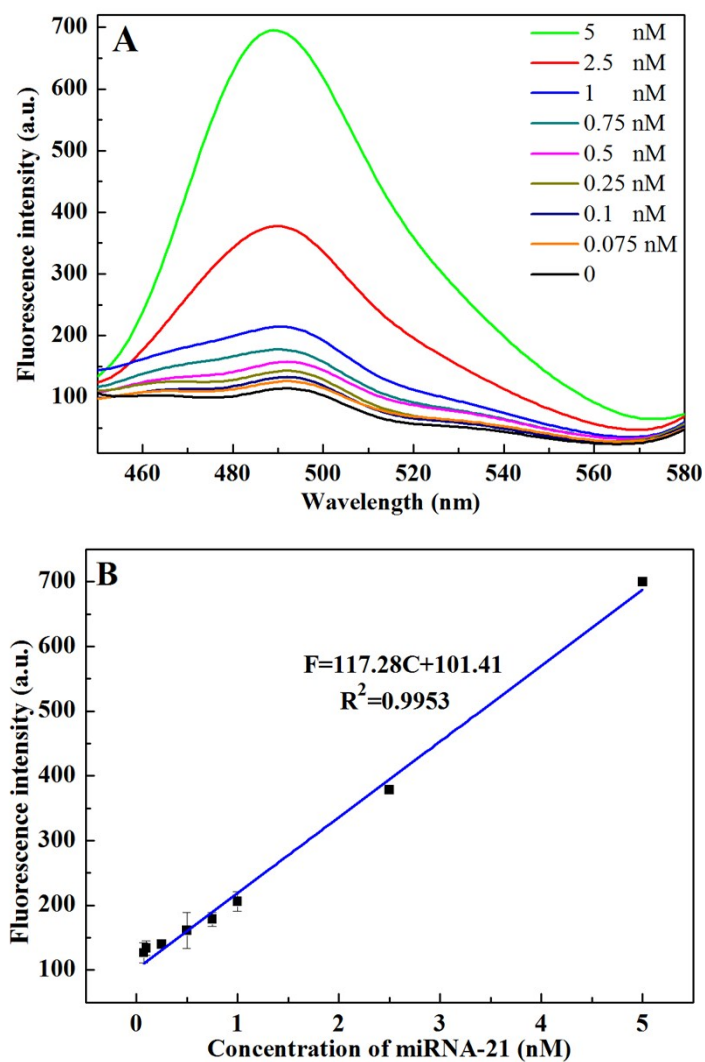


Figure S6. (A) Fluorescence spectra of the $[TG(GA)_3]_3/ThT-EXPAR$ strategy system in the presence of miRNA-21 with concentrations from 0 to 5 nM; (B) The calibration curve between fluorescence intensity at 490 nm and miRNA-21 concentrations.

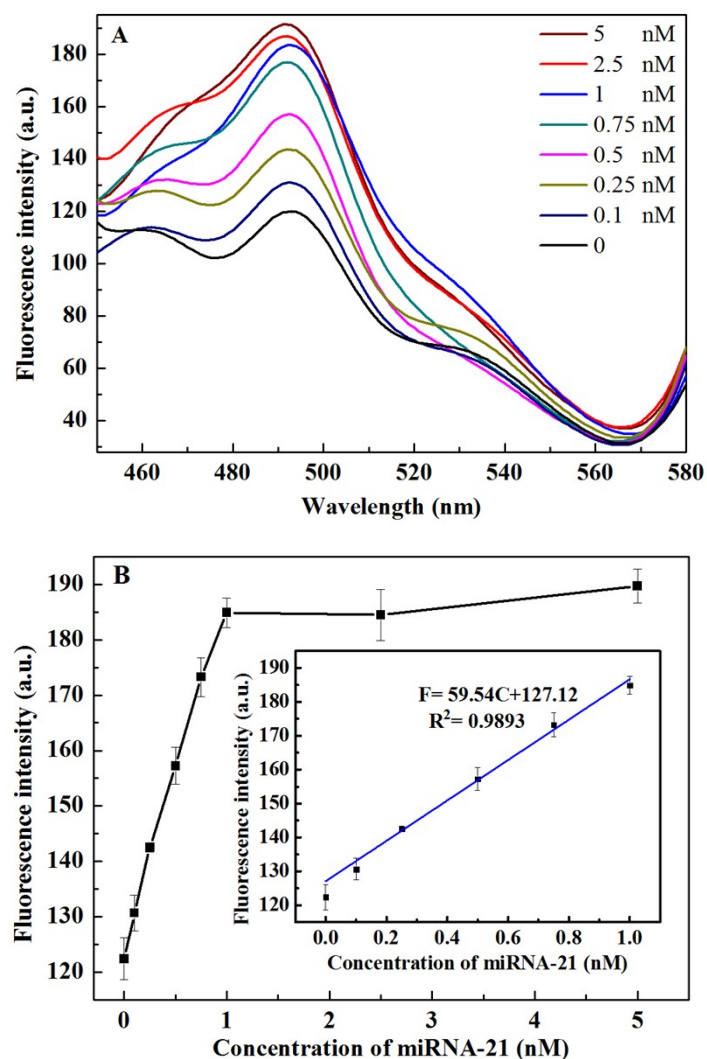


Figure S7. (A) Fluorescence emission spectra of G₄/ThT-EXPAR strategy in the presence of miRNA-21 concentrations from 0 to 5 nM; (B) The calibration curve between fluorescence intensity and the concentration of miRNA.