Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Smart tailor-made G-clip reporter for sensitive detection of G triplet containing sequence

Liang-Yuan Cai, Ji Nie, Yi-wei Zhang, Fang-Ting Zhang, Ying-Lin Zhou, Xin-Xiang Zhang**

Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry, Peking University, Beijing 100871, China

Experimental Section

Reagents

All DNA oligonucleotides (listed in Table S1) were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China). Vent (exo-) DNA polymerase, 10 ×ThermoPol Buffer (200 mM Tris-HCl, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, 1% Triton X-100, pH 8.8), Nt.BstNBI and 10 × NEB 3.1 buffer (500 mM Tris-HCl, 1000 mM NaCl, 100 mM $MgCl_2$, 1 mg/mL BSA, pH 7.9) were obtained from New England Biolabs (Beverly, MA). Deoxynucleotide solution mixture (dNTPs) was provided by Takara Biotechnology Co. Ltd (Dalian, China). Hemin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in dimethylsulfoxide (DMSO) as 5 mM stock solution. TMB-2HCl (TMB: 4,4'- diamino- 3,3',5,5'- tetramethylbiphenyl) was purchased from Ameresco (USA). Other reagents were at least of analytical reagent grade. All solutions were prepared with ultrapure water purified with a Milli-Q purification system (Branstead, MA, U.S.A).

Assay Based on G-Clip Strategy

A total 14 μL of target DNA and R4 was mixed in 1 ×ThermoPol Buffer and heated at 95 ℃ for 5 min and slowly cooled down (0.05 ℃/s) to 25 ℃ on TC-512 Gradient PCR (TECHNE, UK). The above annealing procedure lasted about 30 minutes. After that, 6 μL hemin was added to form the colorimetric solution containing different concentrations of target, 2 μM Reporter, 15 μM hemin and 1 x ThermoPol buffer. A 4 μL of the above solution dropped into 96-well plate was mixed with 100 μL/well TMB−H_2O_2 working solutions (0.12 mg/mL TMB·2HCl, 18 mM H_2O_2, 0.1 M NaH_2PO_4–Na_2HPO_4, pH 6.0) to start chromogenic reaction. After incubating for 3 min in dark at room temperature, 50 μL/well 2 M H_2SO_4 was added to terminate the reaction. Then the absorbance was recorded as (A_{450}-A_{620}), a dual wavelength mode by Thermo Scientific Multiskan FC Microplate Photometer (USA).

Circular Dichroism Spectrometry Analysis

CD spectra (220-320 nm) were recorded on a Jasco J-810 spectra polarimeter (JASCO, Japan) at a rate of 100 nm/min with a 1 cm path length quartz cell at 25 ℃.

Isothermal Exponential Amplification Reaction (EXPAR) for target

The mixtures for EXPAR were prepared separately as part A and part B. Part A consisted of the template X'-X', dNTP mixture, NEB buffer 3.1 and the target DNA. Part B consisted of vent (exo-) DNA polymerase, Nt.BstNBI, and ThermoPol buffer. Parts A and B were mixed immediately before being placed in PCR machine. The EXPAR was performed at 58 ℃ in a volume of 10 μL solution containing X'-X' (200 nM), dNTPs (375 μM), vent (exo-) DNA polymerase (0.05 U/μL), Nt.BstNBI (0.4 U/μL), 1× ThermoPol buffer, 0.5× NEB buffer 3.1 and the target DNA with different concentrations. After incubating for 50 min, the reaction was quenched at 4 ℃. The reaction product was subsequently used for the G-clip based assay.

Table S1 DNA sequences used in this work

Name	Sequence (5' to 3')
TS Mutant	ATTAGGATAGATATACGGGTTCAGG
R0	GGGTGGGTTGGGGTATATCTATCCTAAT
R1	GGGTGGGTTGGGCTATATCTATCCTAAT
R2	GGGTGGGTTGGGCAATATCTATCCTAAT
R3	GGGTGGGTTGGGCATTATCTATCCTAAT
R4	GGGTGGGTTGGGCATAATCTATCCTAAT
R5	GGGTGGGTTGGGCATATTCTATCCTAAT
TS Normal	GGGCCATAGGATATACGGTTCAGG
Random	GGAATAACATGACCTGGATGCA
Single-base mismatch	ATTACGATAGATATACGGGTTCAGG
C Region	GTATATCTATCCTAAT
G Region	GGGTGGGTTGGG
Template X′-X′	CCTGAACCCGTATATCTATCCTAATAACAGACTCTCCTGAACCCGTATATCT ATCCTAATA-P

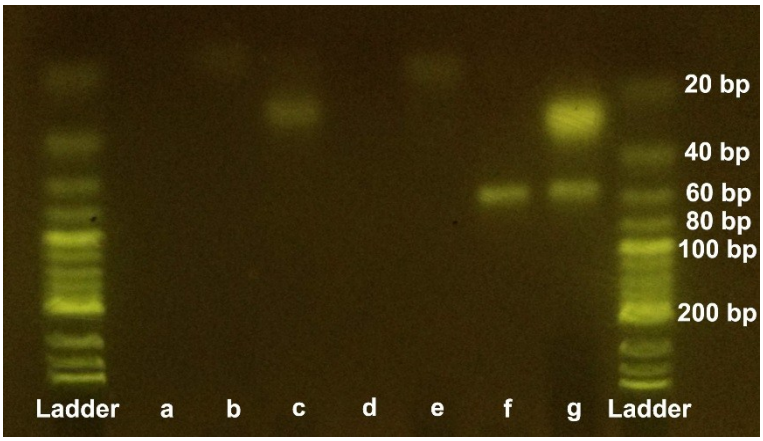

Figure S1 Analysis of the G-clip based assay by 2.5% agarose gel electrophoresis. All samples are prepared in reaction buffer. Lane a: 1 μM target; Lane b: 2 μM R4; Lane c: 1 μM target and 2 μM R4; Lane d: EXPAR initiation solution (100 pM target, 200 nM template X'-X', 375 μM dNTPs, 0.05 U/μL vent (exo-) DNA polymerase, 0.4 U/μL Nt.BstNBI, 1× ThermoPol buffer, 0.5× NEB buffer 3.1); Lane e: EXPAR initiation solution with 2 μM R4; Lane f: EXPAR final solution; Lane g: EXRAR final solution with 2 μM R4.

Agarose gel electrophoresis has been done to verify the formation of target/reporter complex and EXPAR product. Lane a, b, c are the electrophoresis results for target, reporter (R4) and target/reporter complex respectively. Target, an ssDNA, exhibits no observable band. There is a light-colored band in the lane for reporter, which migrates above 20bp marker, for the reporter R4 exists in a hairpin structure in room temperature (simulated by the software DNAMAN). An obvious band is found for target/reporter complex, which migrates between 20 and 30 bp markers. These results demonstrate the effective binding of target and reporter. Lane d is the result for EXPAR initial solution, where the concentration of target is 100 pM, and lane e is for EXPAR initial solution with reporter. In EXPAR initial solution, no band for target/reporter complex is observed, which means the concentration of initial target is too small to be detected. Lane f is the electrophoresis result for the sample after EXPAR, and lane g is for the EXPAR final solution with reporter added. The band migrating similar to 60 bp marker in lane f is the 62 bp dsDNA produced in EXPAR process. Besides the band for 62 bp dsDNA, Lane g shows a far brighter band in the position of target/reporter complex, indicating that target is effectively amplified through EXPAR.

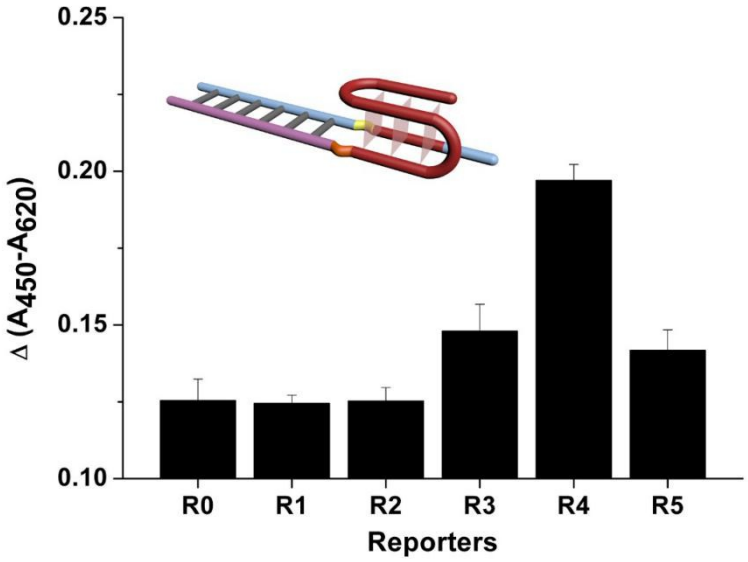

Figure S2 Optimization of the number of unpairing bases between G region and C region in G-clip reporter. We tested six G-clip reporters, namely R0 to R5, with different number of mismatched bases to the target, ranging from 0 to 5 respectively. ΔA means the signal change in the presence and absence of target. Concentrations: $C_{reporter}$=4 μM; C_{target}=250 nM. Error bars represent SDs of three independent experiments.

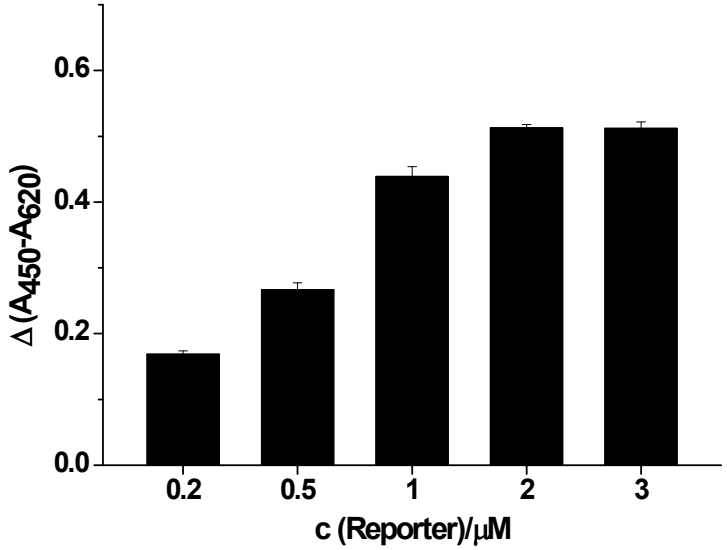

Figure S3 Optimization of the concentration of reporter. ΔA means the signal change in the presence and absence of target. The concentration of the target is 1 μM. Error bars represent SDs of three independent experiments.

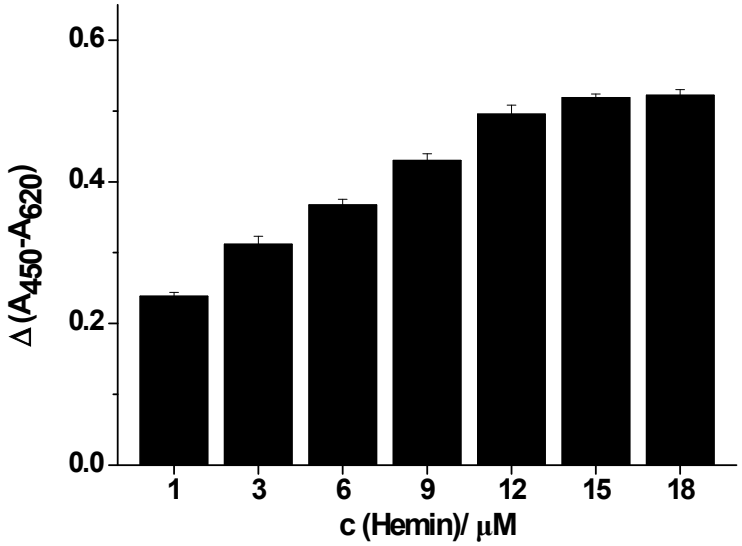

Figure S4 Optimization of hemin concentration. Concentrations: C_{target}=1 μM, C_{R4}=2 μM. Error bars represent SDs of three independent experiments.

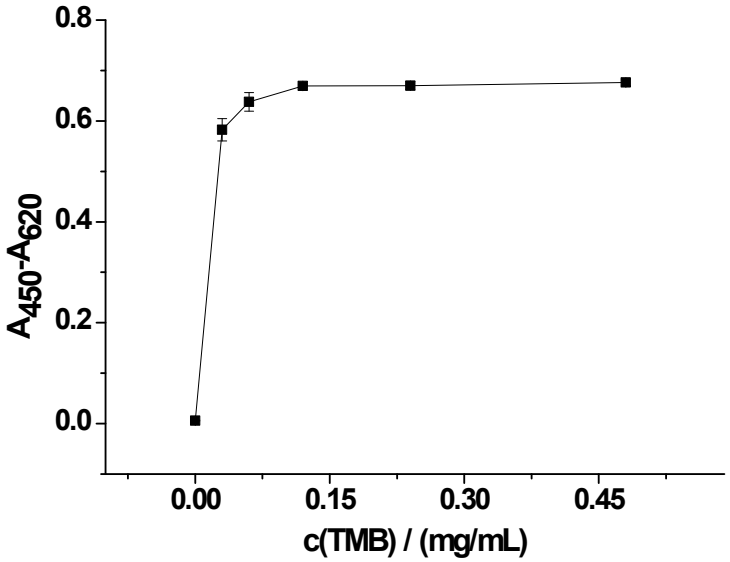

Figure S5 Optimization of TMB concentration. The experiments are done in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0). $C_{H_2O_2}$, C_{target}, C_{R4} and C_{hemin} are 18 mM, 1 μM, 2 μM, and 15 μM respectively. Error bars represent SDs of three independent experiments.

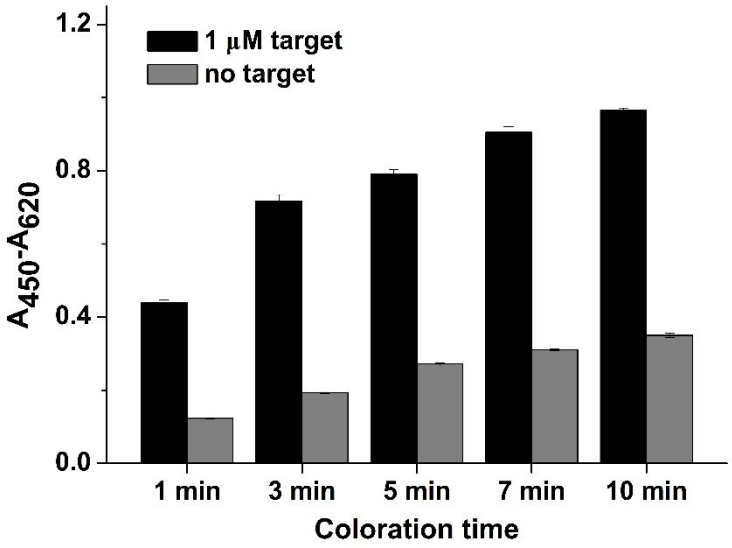

Figure S6 Optimization of coloration time of the TMB. The experiments are done in 0.1 M NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0). C_{TMB}, $C_{H_2O_2}$, C_{hemin} and C_{R4} are 0.12 mg/mL, 18 mM, 15 μM, and 2 μM respectively. Error bars represent SDs of three independent experiments.