Supporting Information

DNA-hosted Hoechst dyes: application for label-free fluorescent monitoring of endonuclease activity and inhibition

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Experimental Section

Reagents and materials. The oligonucleotides used in this study were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) with the following sequences: **Hairpin**: 5'-GCGAATTCCCCCGAATTCGC-3'. 5×PBS buffer (5×2.7 mM KCl，5×2 mM KH_2PO_4，5×136 mM NaCl，5×10 mM Na_2HPO_4，pH=7.4) was prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. Endonucleases including EcoRI, BamHI and HindIII were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. All chemicals used in this work were of analytical grade and obtained from commercial sources and directly used without additional purification.

Instrumentation. Fluorescence was measured in a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) using a black 384 well microplate (Fluotrac 200, Greiner, Germany).

Assays for EcoRI endonuclease activity using the Hairpin-Hoechst solution. The Hairpin-Hoechst solution (Hairpin, and Hoechst 33342 were used) was prepared in PBS buffer, and the mixture was incubated for 10 min at room temperature. For the fluorescent "off" detection of EcoRI endonuclease, an aliquot of the tested EcoRI or control samples or Mill-Q water (as blank sample) was added to the Hairpin-Hoechst solution. The final concentration of Hairpin and Hoechst 33342 was 0.1 μM and 0.001 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 40 min at 37°C and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 360 nm).

Assays for EcoRI endonuclease inhibition with pyrophosphate using the Hairpin-Hoechst solution. 40 U/mL EcoRI endonuclease was firstly treated with various pyrophosphate concentrations for 5 min. The Hairpin-Hoechst solution (Hairpin, and Hoechst 33342 were used) was prepared in PBS buffer, and the mixture was incubated for 10 min at room temperature. For the fluorescent "on" monitoring of EcoRI endonuclease inhibition with pyrophosphate, an aliquot of the tested EcoRI was added to the Hairpin-Hoechst solution. The final concentration of Hairpin and Hoechst 33342 was 0.1 μM and 0.001 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 40 min at 37°C and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the luminescence intensity (excited at 360 nm).

Data analysis. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was employed to perform the data processing. Each sample was repeated in duplicate, and data were averaged. The DNA structures used in this study were predicted by the popular structure-prediction program Mfold (http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form).

Table S1 Structures predicted by Mfold for **Hairpin**

dG = -6.89 dH = -61.20 dS = -175.11 T_m = 76.3 ℃

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         10
|        C
GCGAATTC C
CGCTTAAG C
^        C
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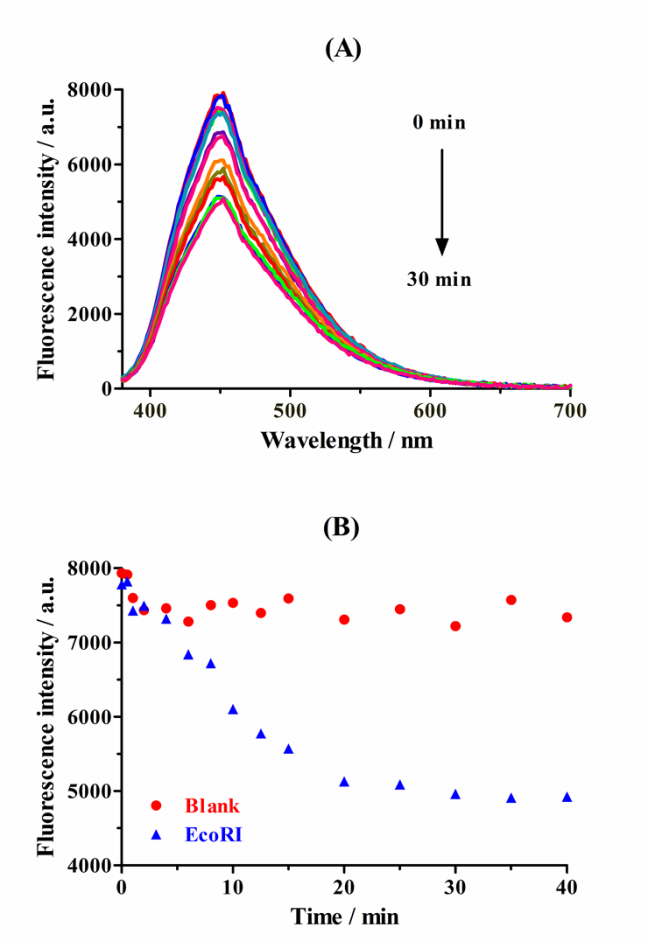

Figure S1 Kinetics investigation of fluorescence response of Hairpin-Hoechst solution to EcoRI endonuclease.