Supplementary Material (ESI) for Chemical Communications

Ultrasensitive fluorescence polarization DNA detection by target assisted exonuclease III-catalyzed signal amplification

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Supporting Information

Experimental Section

Materials: Exonuclease III (Exo III) was used for cleavage a blunt 3' terminus (Fermentas Life Sciences). The nucleic acids were synthesized using a standard procedure, and purified by reverse-phase HPLC from Songon Inc. (Shanghai, China). Unless otherwise noted, all samples were prepared using distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA).

Fluorescence polarization measurement: Fluorescence polarization was measured in a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) with an excitation wavelength at 485 nm and an emission wavelength at 528 nm using a black 384 well microplate (Fluotrac 200, Greiner, Germany). The target assisted Exo III-catalyzed amplification was performed in 100 μL of 1 × reaction buffer (66 mM Tris-HCl, 0.66 mM $MgCl_2$, pH 8.0) which contains 40 nM dye-labeled DNA probe, varying concentrations of DNA target (pcDNA, mcDNA or radDNA) and 25 units of exonuclease III at 37°C (there was a 30-min time-lapse between the addition of the target and the addition of the Exo III for the completion of target hybridization). The fluorescence polarization was begun immediately after the addition of Exo III, following a 60-min period and a 3-min interval at 37°C. In this work, each sample was repeated twice.

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. To determine the initial velocities (v_0) at different concentrations of Probe-Target hybrid, the raw ΔFP data were normalized and multiplied by the concentration of Probe-Target. The curves were fitted to a first order exponential, and the initial velocities were obtained from the slope at t = 0. Using the standard *Michaelis-Menten* equation by non-linear least squares regression, the parameters, including maximum cleavage velocity (V_{max}), Michaelis constant (K_M) were determined.

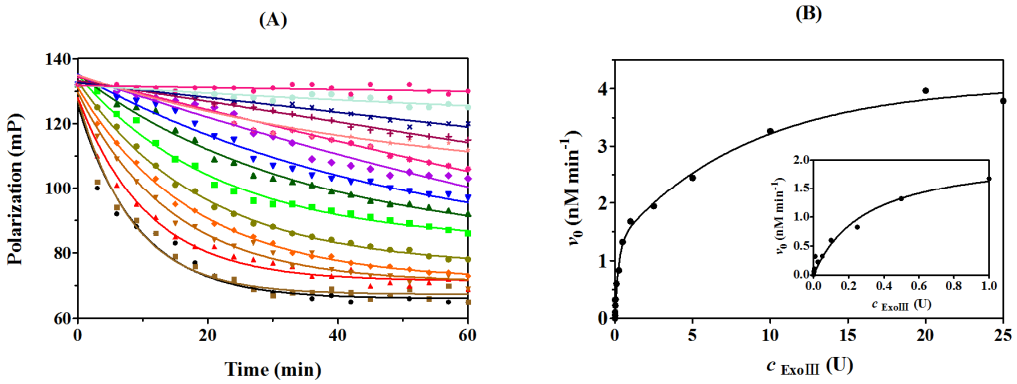

Figure S1. (A) Progress curves at different concentrations of Exonuclease III (0, 0.001, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.25, 5, 10, 20, 25 U); (B) Plots of the initial velocities for the studied concentrations of Exonuclease III. 40 nM probe and 100 nM target were used.