Supporting Information

Ratiometric fluorescent probe: a sensitive and reliable reporter for CRISPR/Cas12a-based biosensing platform

Qiang Liu,[a] Mei Liu,[b] Yan Jin,[a] and Baoxin Li[a]*

[a]Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education; Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, China

[b]College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China

Table S1 Sequence of the used oligonucleotides[a]

Name	Sequence (5'-3')
TaqMan probe	BHQ-TTA TT-FAM
R-5	TAMRA-TTA TT-FAM
R-8	TAMRA-TTA TTA TT-FAM
R-11	TAMRA-TTA TTA TTA TT-FAM
target DNA1	CAG GTA AAC ACA CAA ACC TT
gRNA	UAA UUU **CUA CUA** AGU **GUA GA**U AAG GUU UGU GUG UUU ACC UG
target DNA2	TAG CTT ATC AGA CTG ATG TTG A
template	AAG GTT TGT GTG TTT ACC TG **TCT TGA CTC** TCA ACA TCA GTC TGA TAA GCT A-phosphate

[a] Target DNA1 was used as target in CRISPR/Cas12a-based biosensing, and target DNA2 was used as target in the EXPAR-CRISPR/Cas12a-based biosensing. In template, the red boldface letters are the recognizing sequence of target DNA2, the purple boldface letters are the recognizing sequence of nickase and the green purple boldface letters are the complementary sequences of DNA1(activator).

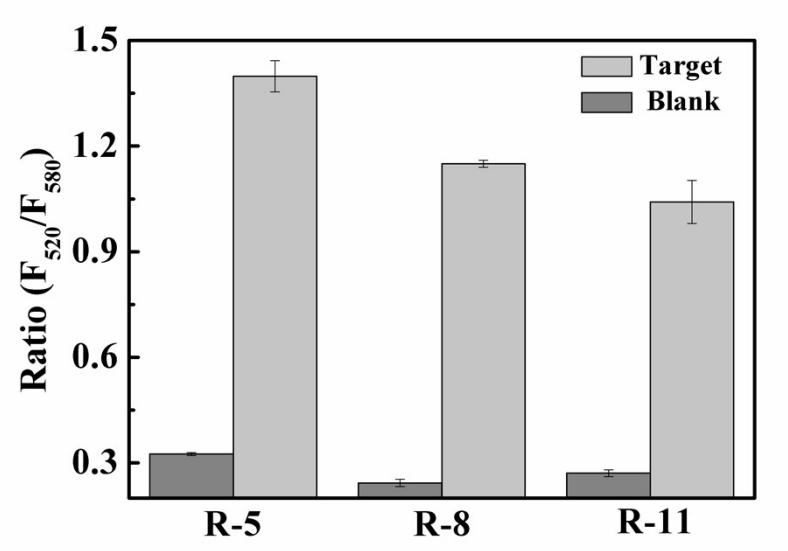

Figure S1. Effect of the ssDNA length of ratiometric probe on the CRISPR-Cas12a biosensing system. Experimental conditions: 5 μL 100 nM gRNA, 5 μL 50 nM Cas12a, 5 μL 1 μM probe, 5 μL 0.25 nM target DNA1 in 25 μL Rnase-free water for reaction 1 h, and add 50 μL buffer for fluorescence test.

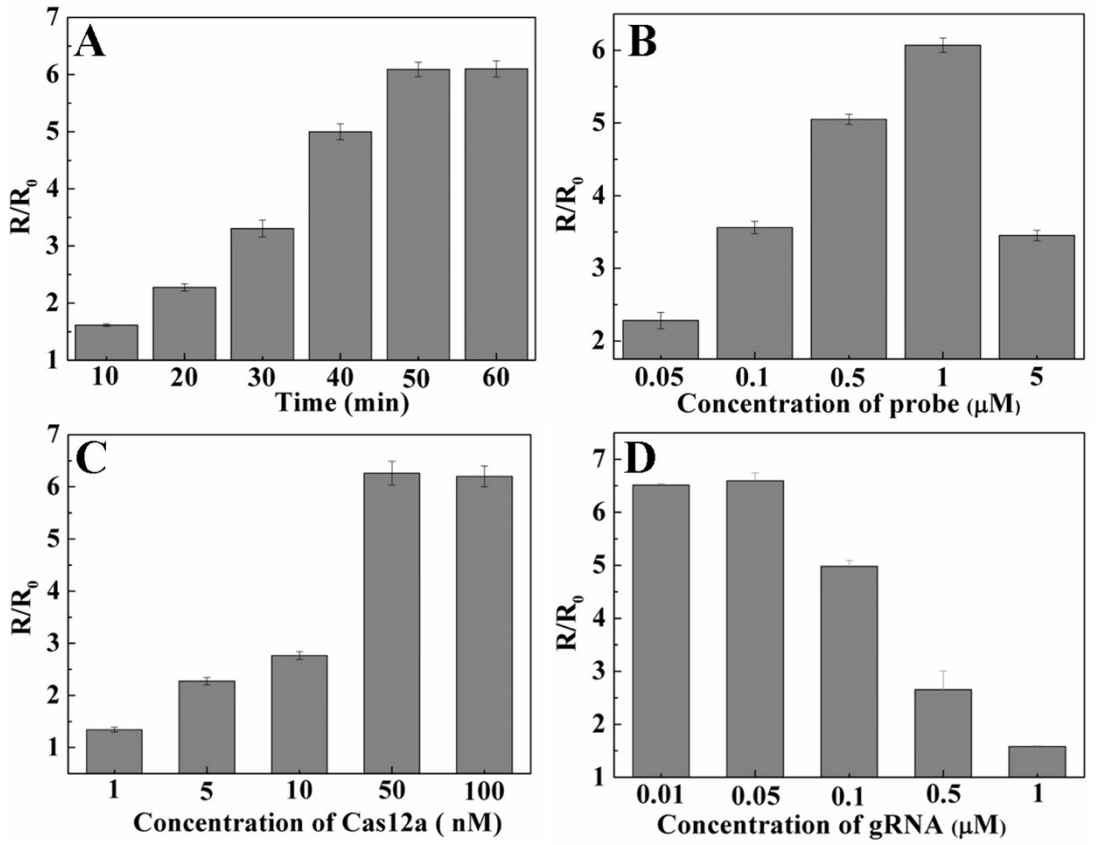

Figure S2. Effect of incubation time (A), probe concentration (B), Cas12a concentration (C) and gRNA concentration (D) on the CRISPR-Cas12a sensing system response.

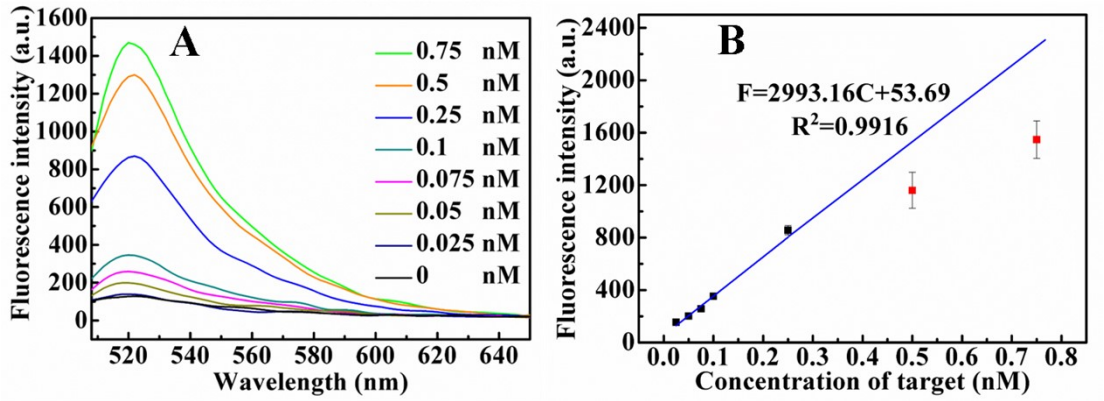

Figure S3. Fluorescence spectra (A) and calibration curve (B) of the sensing system in the presence of increasing target DNA concentrations using TaqMan probe as a reporter.

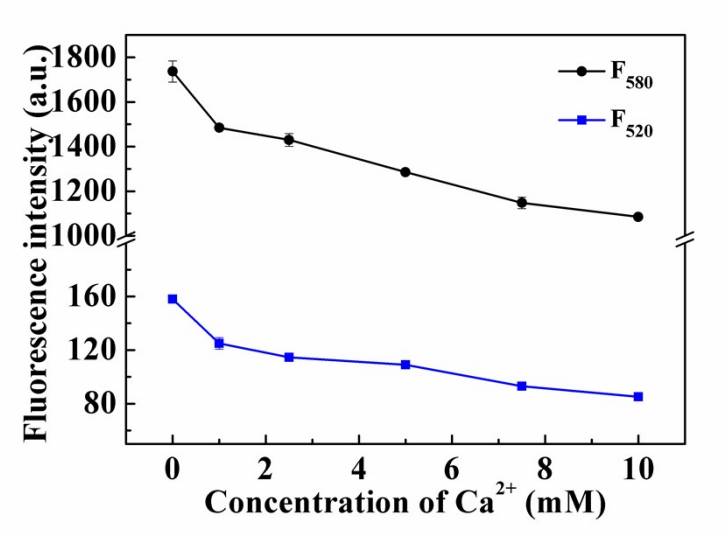

Figure S4. Effect of different concentrations of Ca^{2+} on the fluorescence intensity of ratiometric probe in 20 mM Tris-HCl buffer (7.4) containing 5 μL ratiometric probe (1 μM).

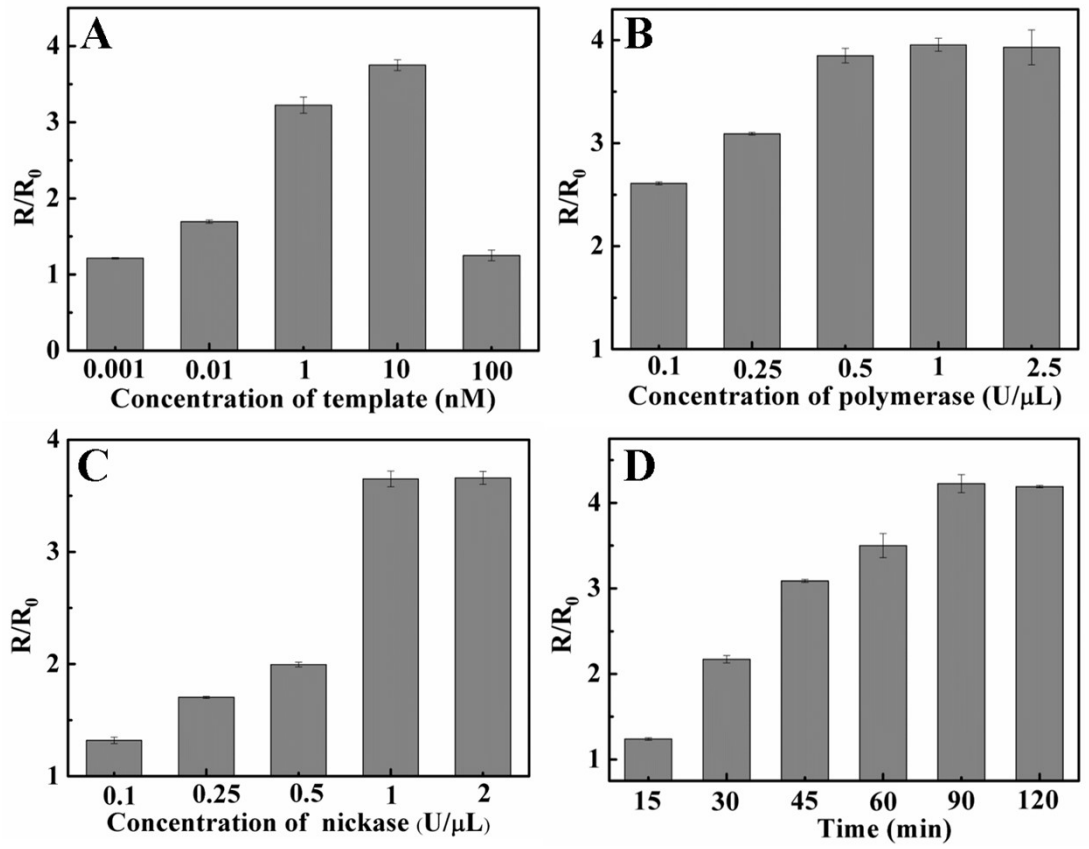

Figure S5. Effect of template concentration (A), polymerase concentration (B), nickase concentration (C) and time (D) on ESDA-CRSPR/Cas12a ratiometric biosensing system.

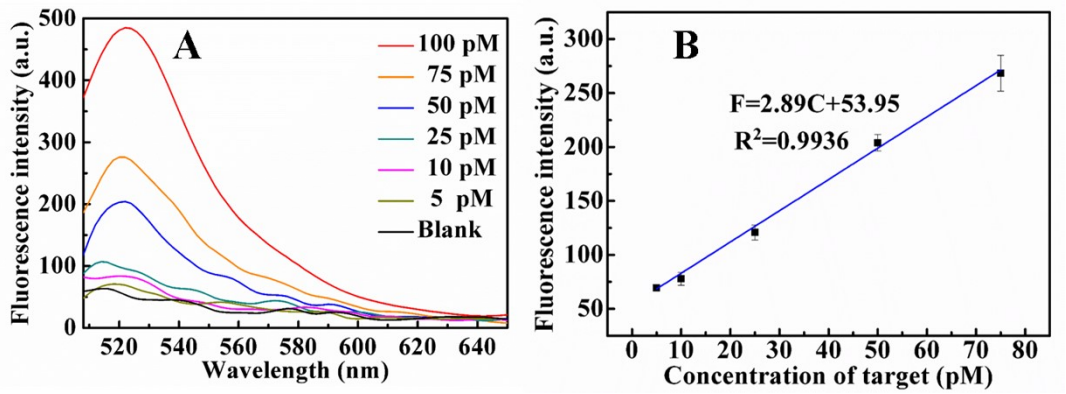

Figure S6. Fluorescence spectra (A) and calibration curve (B) of the ESDA-CRISPR/Cas12a sensing system in the presence of increasing target DNA concentrations using TaqMan probe as signal reporter.