Supplementary Material

Sensitive and Versatile Fluorescent Enzymatic Assay of Nucleases and DNA Methyltransferase Based on Supercharged Fluorescent Protein

Zhuoliang Liu,[a] Chunyang Lei,[a,b] Honghua Deng,[a] Guoyan Lu,[a] Yan Huang,[a*] and Shouzhuo Yao[a]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P. R. China

[b] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China

*Corresponding Authors. Tel.: +86-731-88821626; Fax: +86-731-88821848;
E-mail: huangyan.hnu@gmail.com

Materials

The DNA sequences were:

DNA	Sequence
P1(ss30)	5’-GTGAGCAAGGGTCAGACTGATCGGAAGTTC-Eclipse-3’
P1’(ss30’)	5’-GTGAGCAAGGGTCAGACTGATCGGAAGTTC-3’
[a]P2	5’-GTGAGCAAGAGTCAGACTGAT**GAATTC**CAC-Eclipse-3’ 3’-CACTCGTTCTCAGTCTGACTA**CTTAAG**GTG-5
[b]P3	5’-GTGAGCAAGAGTCAGACTGATGAGATCCAC-Eclipse-3’ 3’-CACTCGTTCTCAGTCTGACTACTCTAGGTG-5’
P3’	5’-GTGAGCAAGAGTCAGACTGATGAGATCCAC -3’ 3’-CACTCGTTCTCAGTCTGACTACTCTAGGTG-5
ss8	5’-GAATTCCAC-Eclipse-3’
ss8’	5’-GAATTCCAC -3’

[a]The letters in bold is the recognition site of EcoRI；

[b]The underline part is the recognition site of Dam and DpnI.

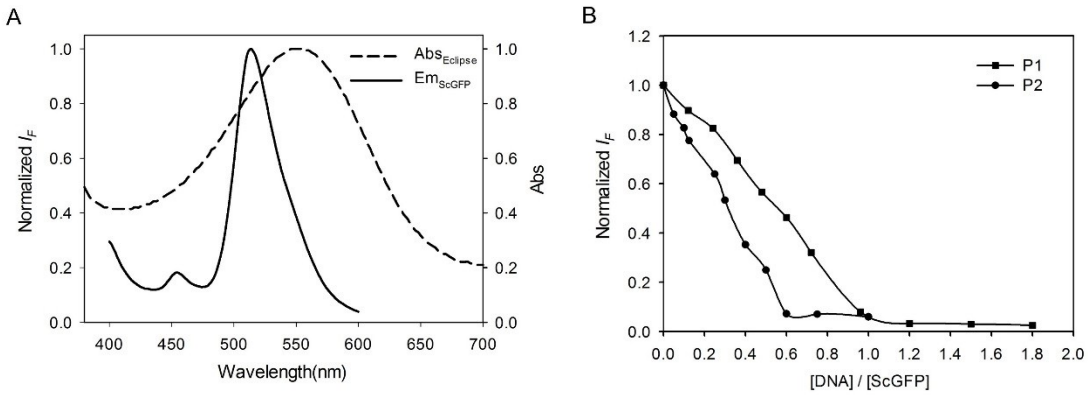

Fig.S1. (A) Fluorescence emission spectrum of ScGFP and absorption spectrum of Eclipse in buffer (10 mM Tris, 100 mM NaCl and pH 7.4). (B) Fluorescence titration curves for the complexation of ScGFP with P1 and P2, respectively.

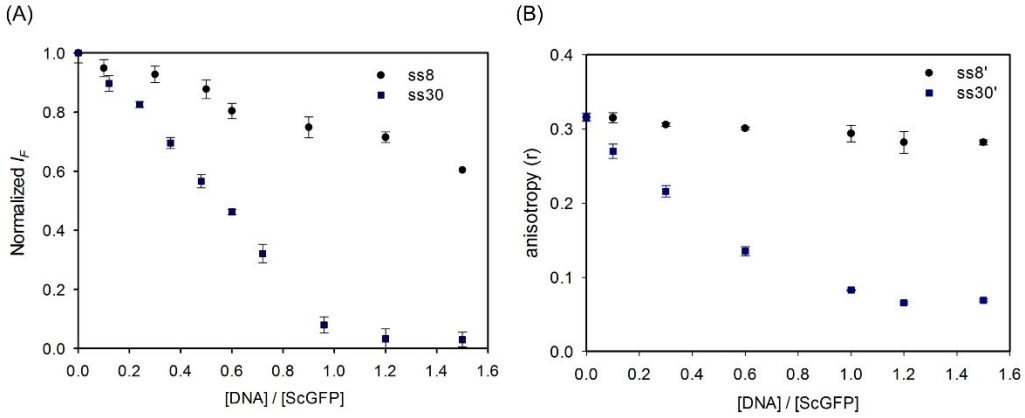

Fig.S2 (A) Fluorescence titration for the complexation of ScGFP with ss8 and ss30, respectively. (B) Fluorescence anisotropies titration for the complexation of ScGFP with ss8' and ss30', respectively. The probe, ss8' (ss30'), the same sequence as ss8 (ss30) but without labelling.

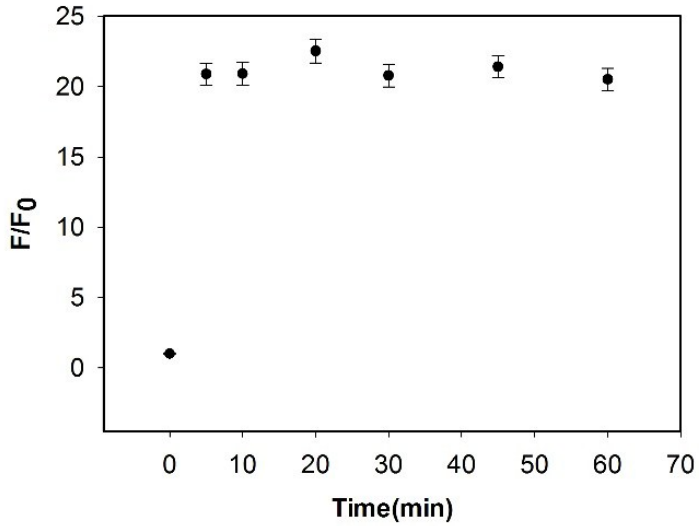

Fig.S3. Relative fluorescence signal changes *versus* different S1 cleavage times. F_0 and F are fluorescence intensities of ScGFP incubated with P1 and S1-treated P1, respectively.

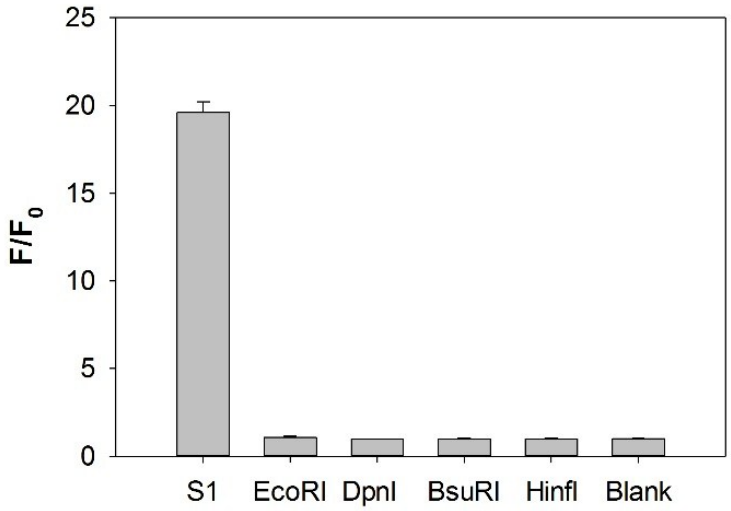

Fig.S4. Selectivity of the ScGFP-based biosensing platform for S1 nuclease activity assay. The concentration of S1 nuclease is 2 U/mL, and the concentrations of EcoRI, DpnI, BsuRI and HinfI are all 500 U/mL. Error bars show the standard deviation of three experiments.

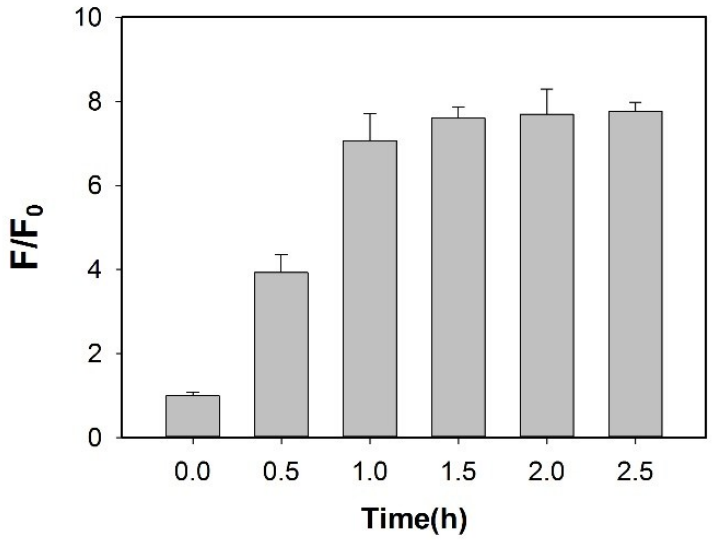

Fig.S5. Relative fluorescence signal *versus* different EcoRI cleavage times. F_0 and F are fluorescence intensities of ScGFP incubated with P2 and EcoRI-treated P2, respectively.

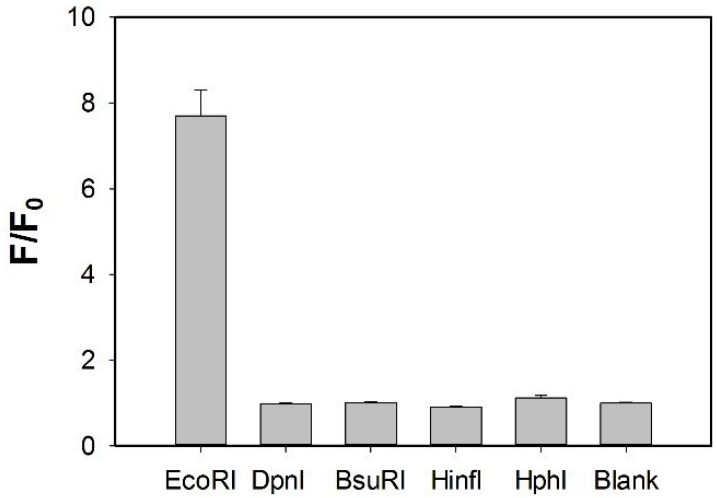

Fig.S6. Selectivity of the ScGFP-based biosensing platform for EcoRI activity assay. The concentration of EcoRI is 400 U/mL, and the concentrations of DpnI, BsuRI, HphI, HinfI are 500 U/mL. Error bars show the standard deviation of three experiments.

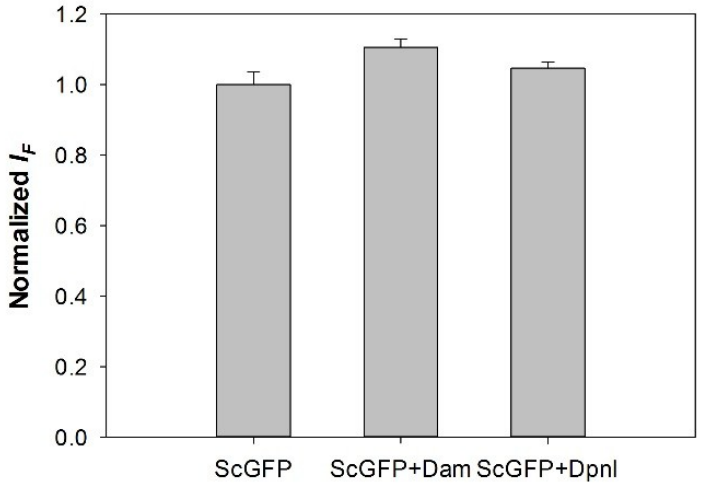

Fig.S7. Relative fluorescence intensity of ScGFP (100 nM) in the presence of Dam MTase or DpnI in buffer solution (10 mM Tris, pH 7.4). [Dam]=40 U/mL, [DpnI]=40 U/mL.

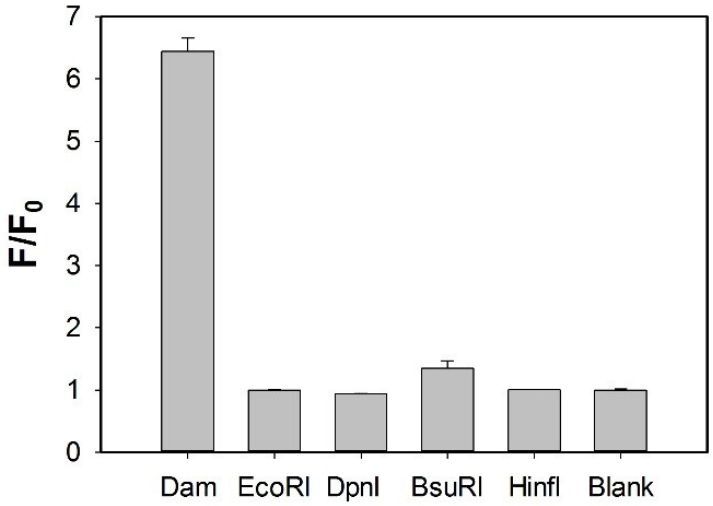

Fig.S8. Selectivity of the ScGFP-based biosensing platform for Dam MTase activity assay. The concentration of Dam MTase is 100 U/mL, and the concentrations of EcoRI, DpnI, BsuRI, Hinfl are 500 U/mL. Error bars show the standard deviation of three experiments.

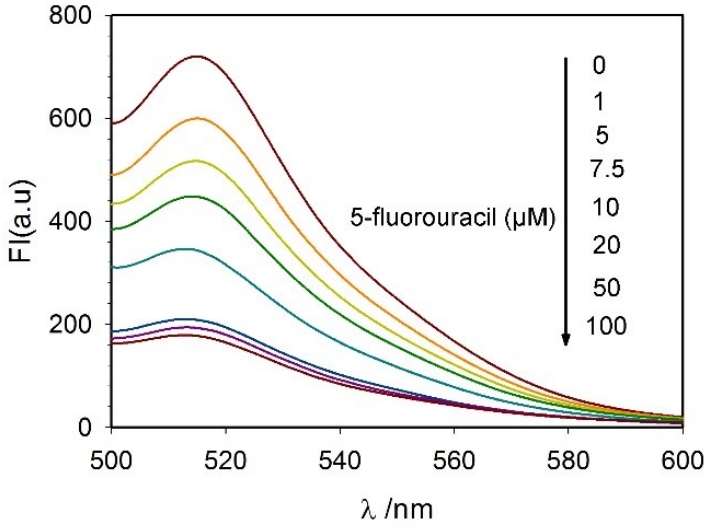

Fig.S9. Assay of the inhibition of Dam MTase, with 5-fluorouracil as the inhibitor of Dam MTase. Fluorescence spectra of ScGFP in the presence of P3 incubated with DpnI/Dam MTase and various concentrations of 5-fluorouracil.

Table S1. Comparison between the proposed ScGFP-based method and reported methods for S1 nuclease analysis.

Material	Method	Detection limit (U/mL)	Detection time (min)	Reference
AuNPs(+)	Colorimetry	4.3	30	[1]
Conjugated Polymer	Fluorescence	0.003	Not mentioned	[2]
CuNPs	Fluorescence	0.3	20	[3]
Ag cluster	Fluorescence	1.0	30	[4]
Molecular beacon	Fluorescence	3.0	10	[5]
ScGFP	Fluorescence	0.002	6	This work

References

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