Electronic Supplementary Information (ESI)

Homo-FRET enhanced ratiometric fluorescence strategy for exonuclease III activity detection

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Table S1 The sequence information.

Names	Sequence (from 5' to 3')
D2	Cy3- GCA CTA AGT CGC TGT
$D1_{12}$	Cy3- GAC TGA TGT TGA
A_{12}	Cy5- ACA GCG ACT TAG TGC TCA ACA TCA GTC
$D1_{15}$	Cy3- GCA CTA GAC TGT TGC
A_{15}	Cy5- ACA GCG ACT TAG TGC GCA ACA GTC TAG TGC
$D1_{18}$	Cy3- GCA CTA GAC TGA TGT TGC
A_{18}	Cy5- ACA GCG ACT TAG TGC GCA ACA TCA GTC TAG TGC
$D1_{24}$	Cy3- GCA CTA GCA CTA GAC TGA TGT TGC
A_{24}	Cy5- ACA GCG ACT TAG TGC GCA ACA TCA GTC TAG TGC TAG TGC

Förster distance calculation

The Förster distances R_0 for the various dye pairs were calculated using the equation (S1):

$$R_0(nm) = 0.02108\left[\kappa^2 n^{-4} \Phi_D J(\lambda)\right]^{1/6} \quad \text{(S1)}$$

where n is the refractive index (set to 1.33 for 1xTris-HCl buffer), ΦD is the fluorescence quantum yield (QY) of the donor and κ^2 is the dipole orientation factor of the dyes. This was generally considered to be 2/3 due to the random assembly and movement of the DNA strands across the ensemble. And the overlap integrals $J(\lambda)$ can be calculated by the equation (R2):

$$J(\lambda)\left(nm^4 M^{-1} cm^{-1}\right) = \int I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \quad \text{(S2)}$$

where $I_D(\lambda)$ is the normalized donor emission spectrum, $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor in units of $M^{-1}cm^{-1}$, and λ is the wavelength in units of nm.

Table S2 Selected photophysical and FRET properties of the fluorophores used to create the D1-D2-A construct.

Fluorophores	Quantum yield	Extinction coefficient ($M^{-1}cm^{-1}$)	λ_{max} absorption	λ_{max} emission	R_0 in nm/$J(\lambda)$ in $nm^4M^{-1}cm^{-1}$		
					D1(Cy3)	D2(Cy3)	A(Cy5)
D1(Cy3)	0.14	150 000	550 nm	570 nm	-	$4.2/2.07e^{15}$	
D2(Cy3)	0.21	150 000	550 nm	570 nm	$4.6/2.34e^{15}$	-	$5.0/4.13e^{15}$
A(Cy5)	0.27	250 000	650 nm	670 nm	-	-	

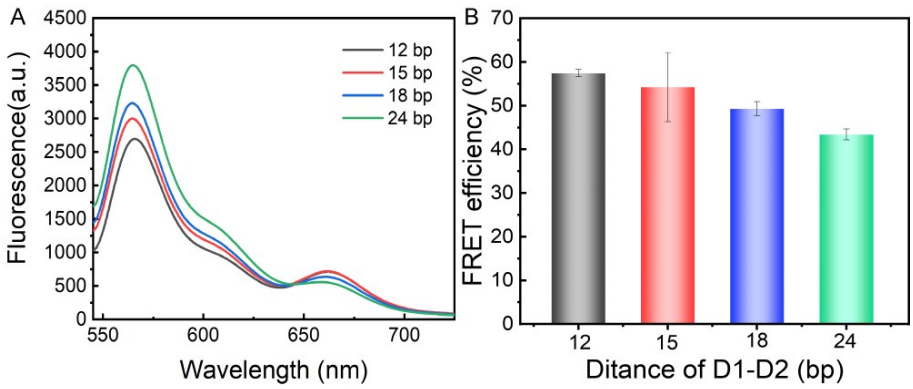

Figure S1 Fluorescence emission spectra (A) and FRET efficiency (B) of D1-D2-A constructs with different distances of D1-D2.

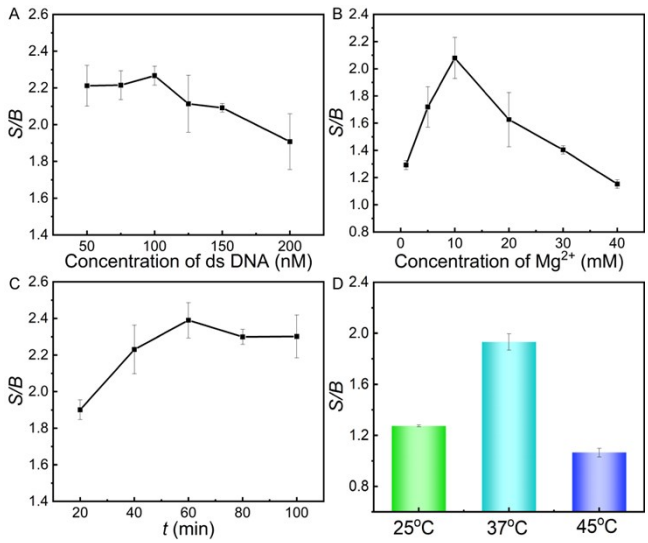

Figure S2 Optimization of detection conditions for Exo III activity. The relationship between the concentration of D1-D2-A probe (A) or Mg^{2+} (B) and *S*/*B*. Optimization of the incubation time (C) and temperature (D) of D1-D2-A probe and Exo III. The concentration of Exo III was 5 U/mL.

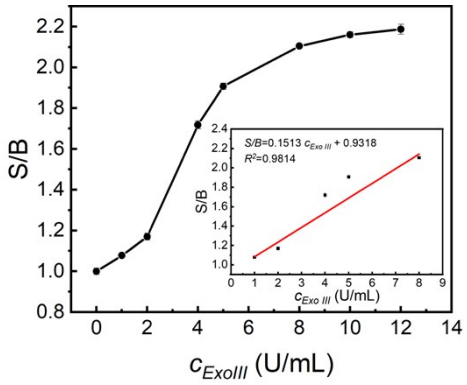

Figure S3 The relationship between the *S/B* versus Exo III concentrations (0, 1, 2, 4, 5, 8, 10, 12 U/mL) of D1-D2-A construct in 10 % FBS. Inset: the linearity of *S/B* with respect to Exo III concentrations.

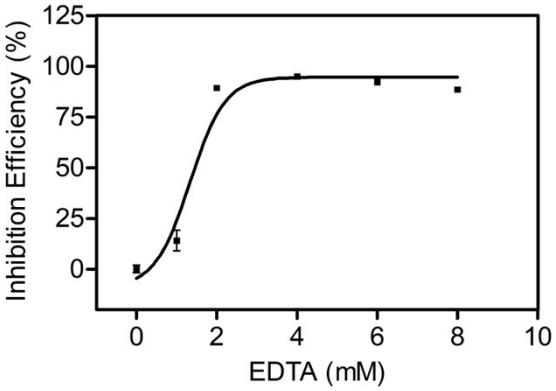

Figure S4 The inhibition efficiency of different concentrations of EDTA (0, 1, 2, 4, 6, 8 mM) on Exo III activity. The concentration of Exo III was 5 U/mL.

Table S3 Comparison of recently reported methods for Exonuclease III detection.

Method	**Linear range (U/mL)**	**Detection limit (U/mL)**	**Signal output mode**	**Reference**
Fluorescence	0.25-8	0.17	Ratiometric	This work
Fluorescence	0-100	4.42	Ratiometric	1
Fluorescence	0.01-0.5	0.001	Single wavelength	2
Fluorescence	0.04-8	0.01	Single wavelength	3
Fluorescence	0-10	0.5	Single wavelength	4
Fluorescence	0.02-10	0.02	Single wavelength	5
Fluorescence	0.05-2	0.02	Single wavelength	6
Luminescence	0-25	1	-	7
Chemiluminescence	-	4.8	-	8
Electrochemical	1-100	-	-	9
Bioluminescence	0.1-5	0.05	-	10

References

1 W. Hu, H. Z. Zhao, J. Jing and X. L. Zhang, *New J. Chem.*, 2018, **42**, 16630-16634.

2 X. Liu, Y. F. Wu, X. Wu and J. X. Zhao, *Analyst*, 2019, **144**, 6231-6239.

3 X. Wu, J. Chen and J. X. Zhao, *Analyst*, 2014, **139**, 1081-1087.

4 X. F. Jiang, H. S. Liu, F. Y. Khusbu, C. B. Ma, A. Ping, Q. L. Zhang, K. F. Wu and M. J. Chen, *Anal. Biochem.*, 2018, **555**, 55-58.
5 J. Ge, Z. Dong, D. M. Bai, L. Zhang, Y. Hu, D. Y. Ji and Z. H. Li, *New J. Chem.*, 2017, **41**, 9718-9723.
6 H. Zhang, Z. Lin and X. Su, *Talanta*, 2015, **131**, 59-63.
7 H. Z. He, W. I. Chan, T. Y. Mak, L. J. Liu, M. Wang, D. S. Chan, D. L. Ma and C. H. Leung, *Methods*, 2013, **64**, 218-223.
8 K. M. Burkin, O. L. Bodulev, A. V. Gribas and I. Y. Sakharov, *Enzyme Microb. Technol.*, 2019, **131**, 109419.
9 H. K. Lee, J. Heo, S. Myung, I. S. Shin and T. H. Kim, *Electroanalysis*, 2017, **29**, 1749-1754.
10 Q. Xu and C. Y. Zhang, *Chem. Commun.*, 2014, **50**, 8047-8049.