Electronic supplementary information

An ultrasensitive fluorometric platform for S1 nuclease assay based on cytochrome c

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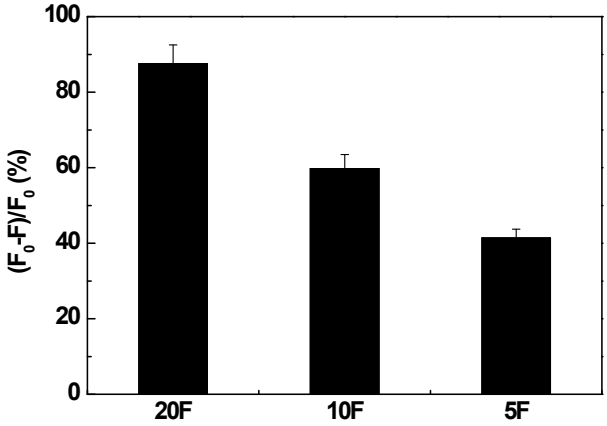

Fig. S1 Comparison of the fluorescence quenching efficiency of *Cyt c* to ssDNA with different lengths. The 5F, 10F, and 20F, denote FAM-labeled ssDNA containing 5, 10, and 20 bases, respectively. Concentration: 5F, 40 nM; 10F, 40 nM; 20F, 40 nM; *Cyt c*, 0.16 μM. Excitation: 480 nm.

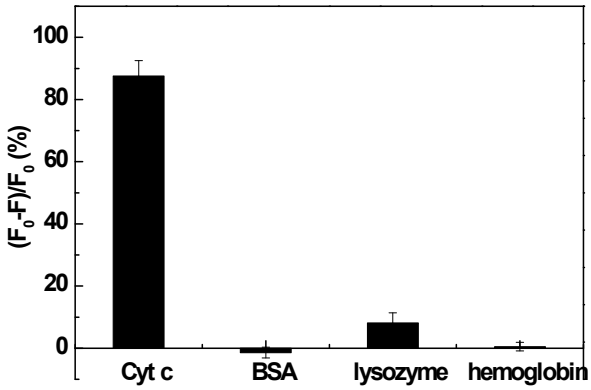

Fig. S2 Comparison of the fluorescence quenching efficiency of different proteins to 20F. Concentration: 20F, 40 nM; *Cyt c*, 0.16 μM; BSA, 0.16 μM; lysozyme, 0.16 μM; hemoglobin, 0.16 μM. Excitation: 480 nm.

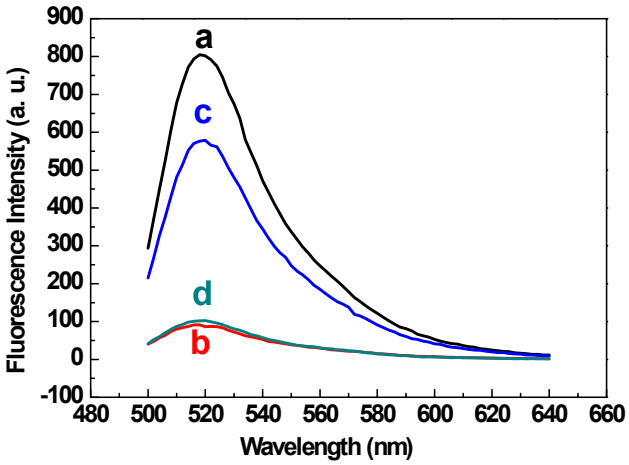

Fig. S3 Fluorescence emission spectra of 20F under different conditions: (a) 20F; (b) 20F + *Cyt c*; (c) 20F + S1 nuclease (with Zn^{2+}) + *Cyt c*; (d) 20F + S1 nuclease (without Zn^{2+}) + *Cyt c*. Concentration: 20F, 40 nM; *Cyt c*, 0.16 μM; S1 nuclease, 3.2×10^{-2} units/mL, , Zn^{2+}, 2 mM. Excitation: 480 nm.

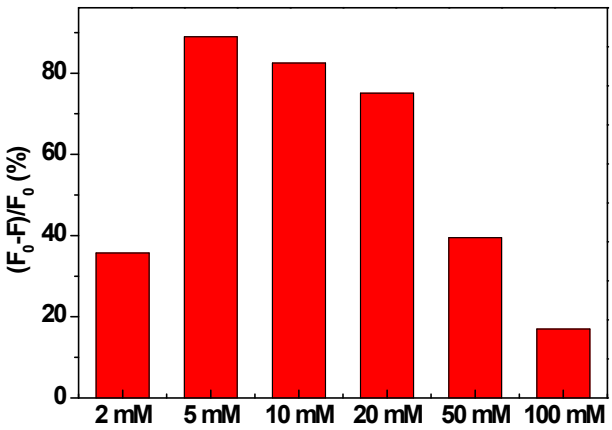

Fig. S4 Comparison of the fluorescence quenching efficiency of *Cyt c* to 20F in different concentration of Tris-HCl buffer. Concentration: 20F, 40 nM; *Cyt c*, 0.16 μM. Excitation: 480 nm.

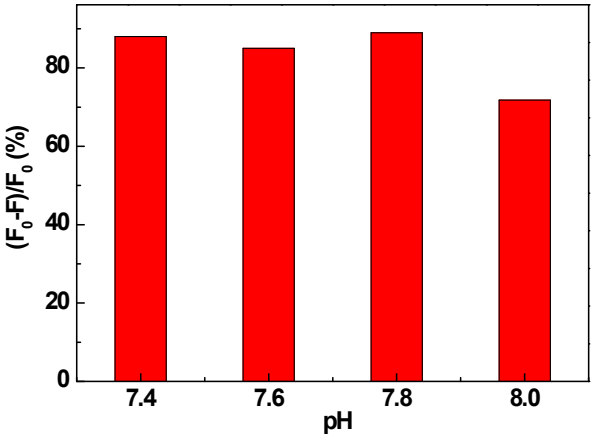

Fig. S5 Comparison of the fluorescence quenching efficiency of *Cyt c* to 20F in different pH value of Tris-HCl buffer. Concentration: Tris-HCl, 5 mM; 20F, 40 nM; *Cyt c*, 0.16 μM. Excitation: 480 nm.

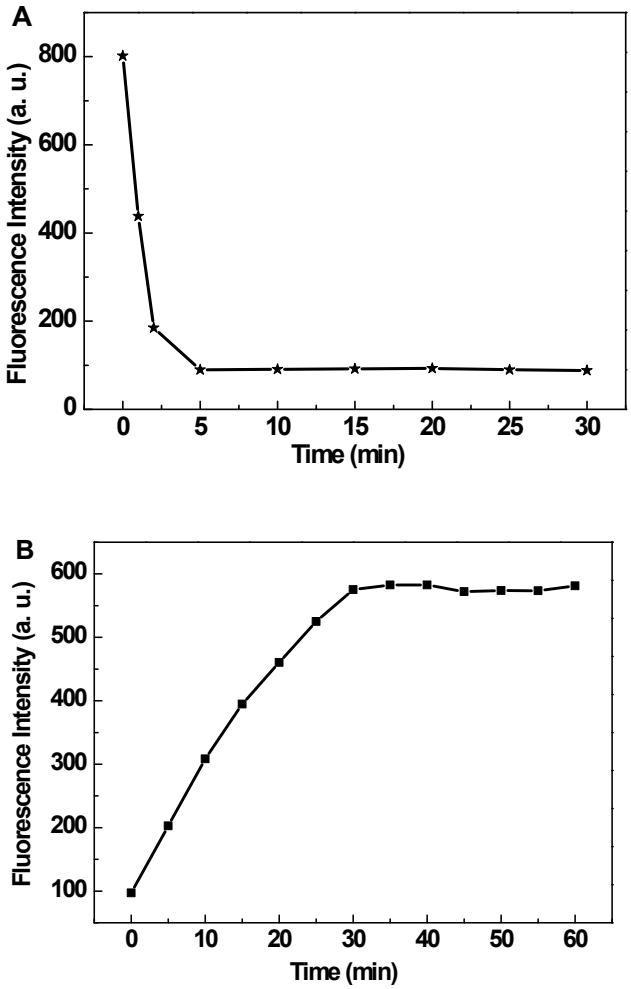

Fig. S6 (A) Fluorescence quenching of 20F in Tris-HCl buffer by *Cyt c* as a function of time. (B) Fluorescence increasing of the biosensor by S1 nuclease as a function of time. Concentration: 20F, 40 nM; *Cyt c*, 0.16 μM; S1 nuclease, 3.2×10^{-2} units/mL. Excitation: 480 nm.

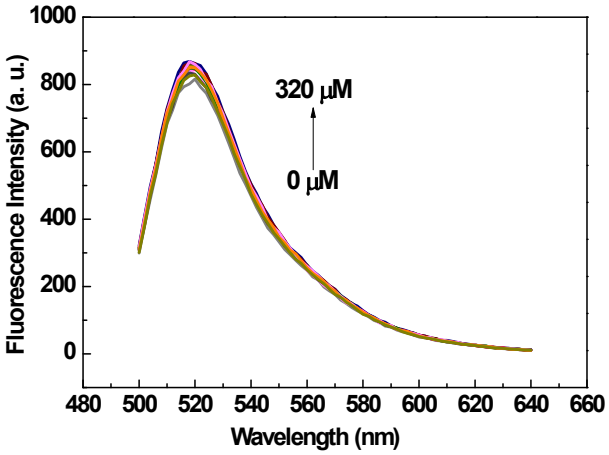

Fig. S7 Fluorescence emission spectra of 20F in the presence of increasing amount of ATP. Concentration: 20F, 40 nM. Excitation: 480 nm.

Table S1. Proteins studied in the current investigation.

Protein	Molar mass (kDa)	pI
Cytochrome c (*Cyt c*)	12.2	10.0-10.5
Bovine serum albumin (BSA)	66	4.7
Lysozyme	14.3	11.0
Hemoglobin	64.5	6.8