

Supporting Information for

Ratiometric Detection of Zn²⁺ using DNAzyme-based Bioluminescence Resonance Energy Transfer Sensors

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Supplemental Figures

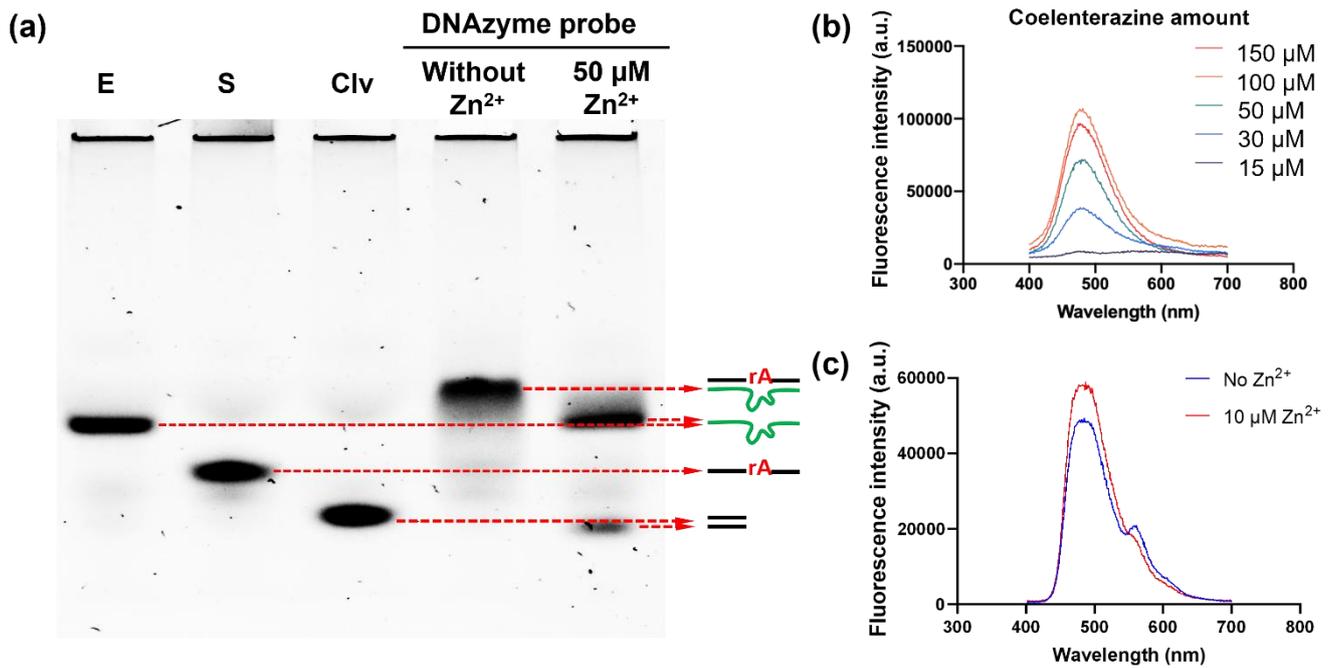


Figure S1. *In vitro* visualization of the BRET system.

(a) 12% native PAGE visualizing the formation of the double stranded DNAzyme probe and its activity in the presence of Zn^{2+} ions. The gel was stained with SYBR Gold for visualization. Lane 1: Cy3 labelled E strand; Lane 2: Cy3 and biotin dual modified S strand; Lane 3: Synthesized cleaved product with Cy3 labeling; Lane 4: DNAzyme probe without Zn^{2+} ; Lane 5: DNAzyme probe with $50 \mu M Zn^{2+}$ in sample ($40 \mu l$, $1/5$ of the total reaction volume). Red lines point to the scheme of potential structures of each size of bands shown on the right side of the gel. (b) Spectra of bioluminescence generated at 30 seconds after introducing different concentration of coelenterazine. (c) Spectra of the DNAzyme-BRET sensor without or with $50 \mu M Zn^{2+}$ in sample.

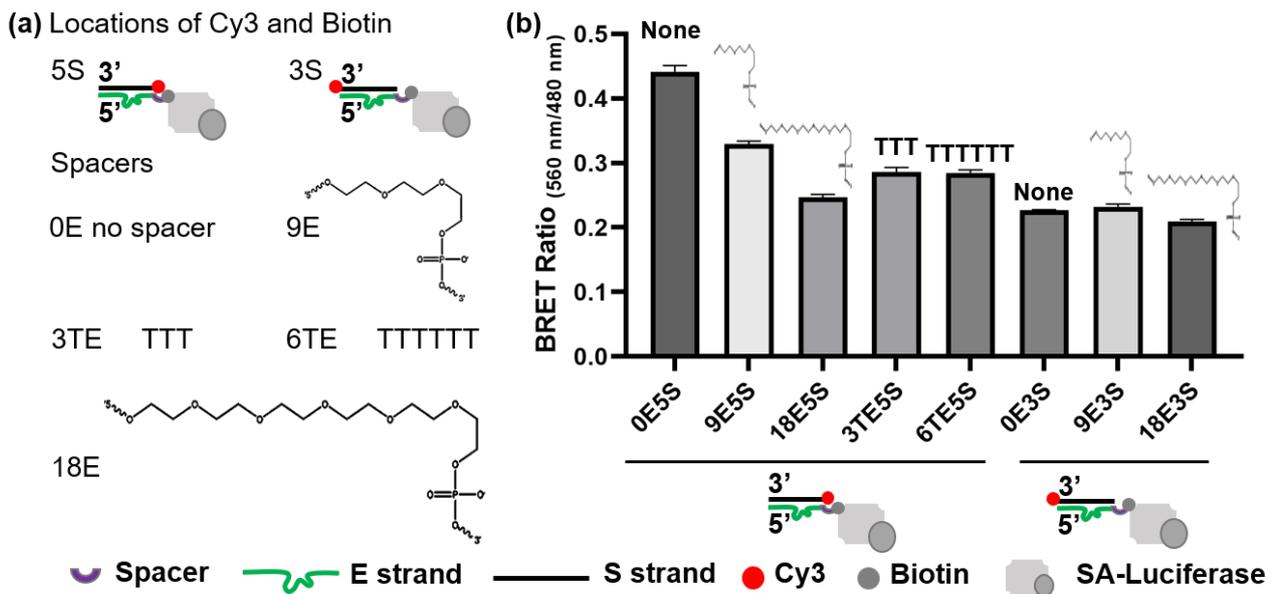


Figure S2. Optimization of the spacer in the single BRET systems.

BRET ratio comparison between different positions of the Cy3 acceptor and different lengths of linkers between the DNAzyme and biotin modification. (a) The information of different linkers: 3S, Cy3 located at the 3' terminal of the S strand; 5S, Cy3 located at the 5' terminal of the S strand. 0E, 9E, 18E, 3TE, 6TE, represent different spacers between the end of the E strand and the biotin modification for SA-luciferase conjugation. 0E, no spacer; 9E, triethylene glycol spacer; 18E, 18-atom hexa-ethyleneglycol spacer; 3TE, trithymidylate spacer; 6TE, hexathymidylate spacer. (b) The BRET ratio (560 nm/480 nm) demonstrates the comparison of BRET efficiency between the sensors with different linkers and Cy3 locations.

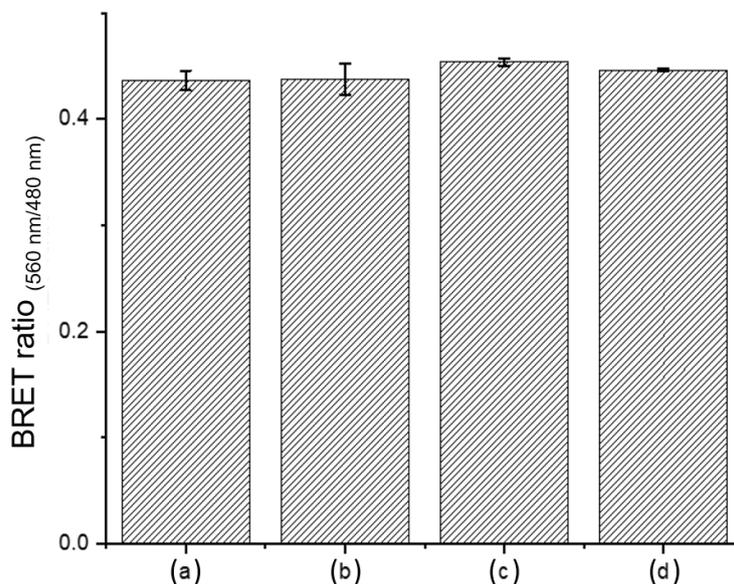


Figure S3. Comparison of cool down techniques for annealing the DNAzyme strands.

After heating up to 95°C for 5 minutes, the DNAzyme strands were cooldown with (a) room temperature (60 minutes); (b) 4°C fridge (60 minutes); (c) 4°C fridge (45 minutes) then -20°C freezer (15 minutes); (d) room temperature (30 minutes) then 4°C fridge (30 minutes).

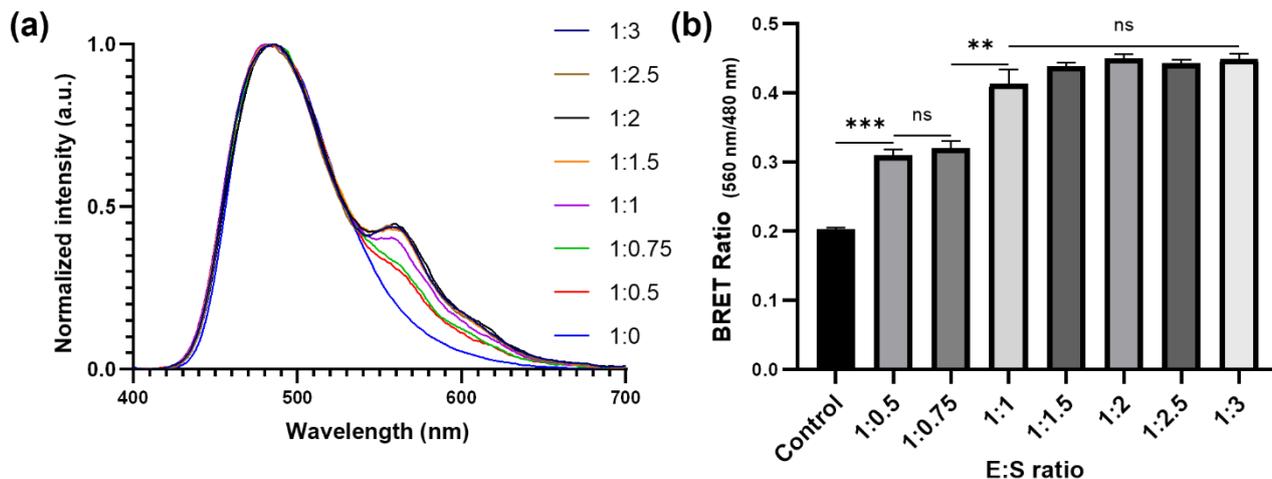


Figure S4. Titration of E and S strand ratio.

(a) Spectra of the different ratios between E and S strands when forming the DNAzyme probe. (b) BRET ratio (560 nm/480 nm) show the titration of influence from different ratios between E and S strands when forming the DNAzyme probe. Unpaired student-t tests were used for statistical analysis. **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$.

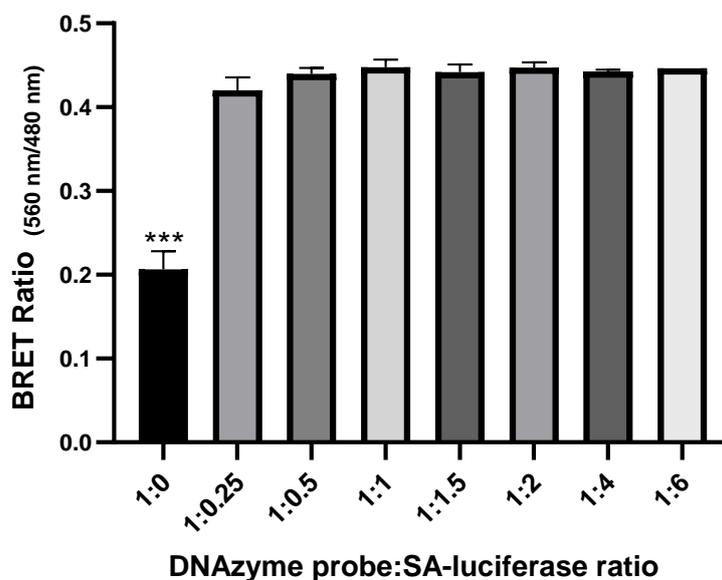


Figure S5. Titration of different ratios between DNAzyme probe (ES) and SA-luciferase.

The BRET ratio from the group of 1:0 DNAzyme probe:SA-luciferase ratio is significantly lower than other groups ($p = 0.0002$ between the 1:0 and 1:0.25 group). There was no significant difference between other groups according to unpaired student-t test ($p > 0.05$).

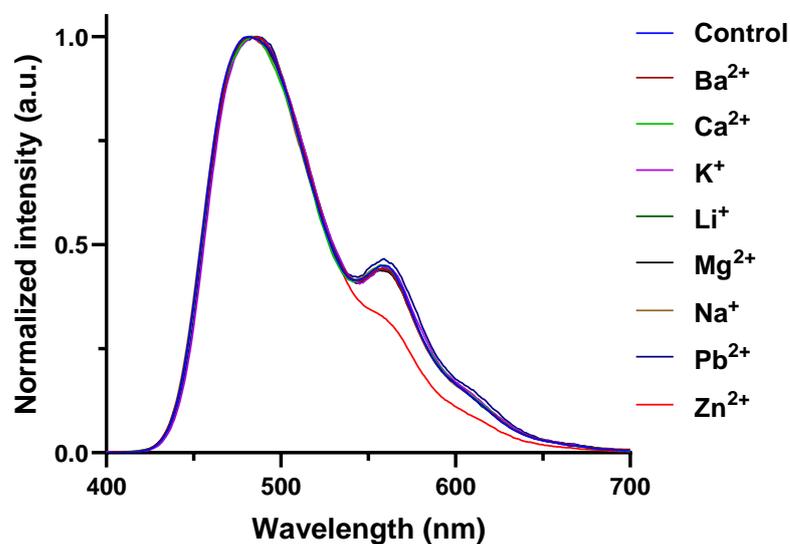


Figure S6. Fluorescence spectra for selectivity assay of the sensor. 40 μL test samples in PBS buffer were incubated with 25 μM of various metal ions.

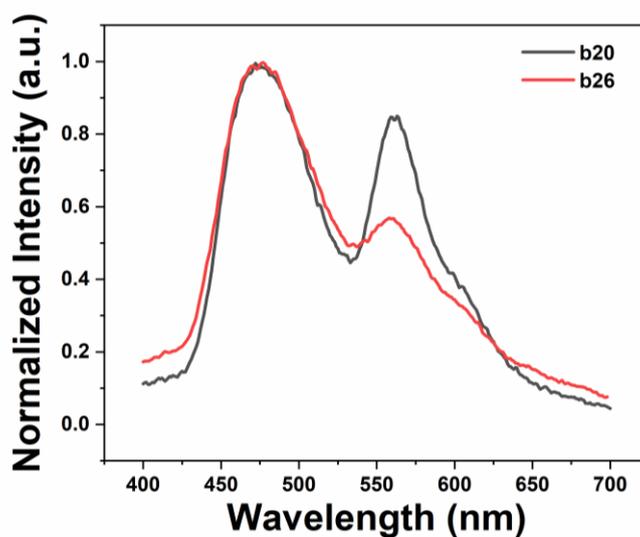


Figure S7. The length of S strand influence on the BRET intensity. Fluorescence spectra showed a higher emission pick with 20 bases S strand (b20) than 26 bases S strand (b26), indicating that a 20 bases S strand has a higher BRET than a 26 bases S strand.

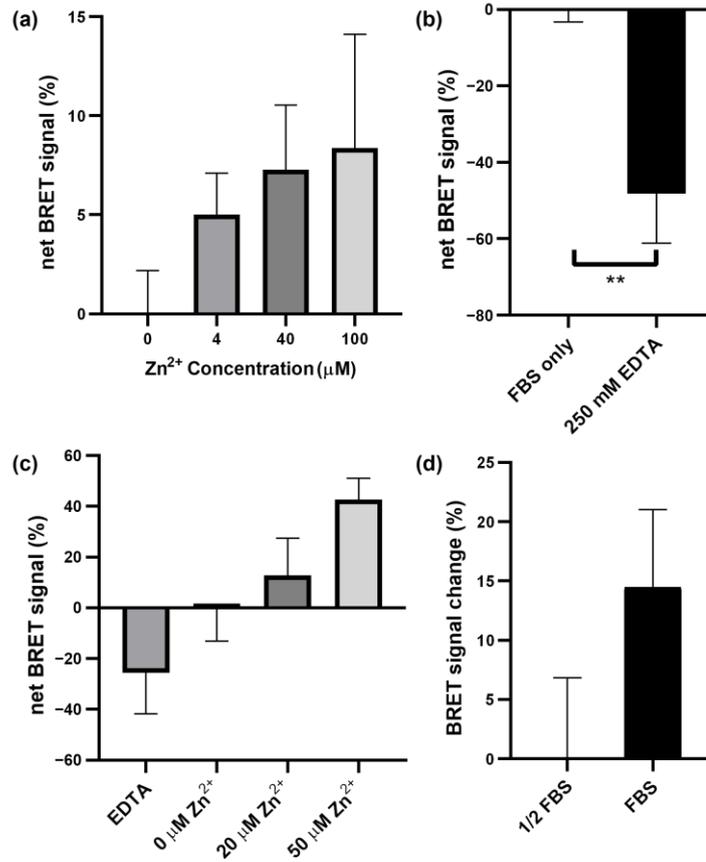


Figure S8. Zn²⁺ sensing in fetal bovine serum (FBS).

(a) Zn²⁺ sensing in 1:2 diluted FBS; (b) adding 250 mM EDTA in the 1:2 diluted FBS increased the BRET ratio; (c) Zn²⁺ sensing in undiluted FBS; (d) a comparison of BRET signal change between different dilutions of FBS.

Supplemental Tables

Table S1. A comparison of advantages and limitations between techniques for Zn²⁺ detection.

Techniques	Example	Advantages	Limitations
Spectrometry technologies	Inductively coupled plasma mass spectrometry [28–32]	Available for most metal ions	Need sophisticated instruments and well-trained technicians, Not suitable for POC detection
Fluorescence-based sensors	Small molecule sensors [33–38]	Can be used in POC settings	Limited selectivity, Not generalizable for other metal ions
	Protein based sensors [91]		Laser excitation requires specific equipment which generates high autofluorescence background and photobleaching
	DNAzyme based sensors [43,70–77]	Highly selective and generalizable	
Chemiluminescence resonance energy transfer sensors	DNAzyme based CRET sensor [85]	Do not require excitation light source, Rapid and portable metal detection	Rely on H ₂ O ₂ , and difficult to apply in various biological samples
Bioluminescence resonance energy transfer sensors	Small molecule sensors [89]	Biocompatible, Low background interference and minimal photobleaching	Limited selectivity, Not generalizable for other metal ions
	Protein based sensors [88]		Short light emission time, need to detect immediately
	DNAzyme based sensors (This work)	All advantages listed above	

Table S2. DNA sequences used in this study.

Name	Sequence (5'-3')
8-17 0E	TCCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT/3Bio/
8-17 3TE	TCCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGTTTT/3Bio/
8-17 6TE	TCCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGTTTTTTTT/3Bio/
8-17 9E	TCCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT/iSp9//3Bio/
8-17 18E	TCCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT/iSp18//3Bio/
8-17 S 5'Cy3	/5Cy3/ACTCACTATrAGGAAGAGATGGA
8-17 S 3'Cy3	ACTCACTATrAGGAAGAGATGGA/3Cy3Sp/
8-17 E 3'Cy3	CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT/3Cy3Sp/
8-17 S 5'bio-3'Cy3 (20 bases S)	/52-Bio/ACTCACTATrAGGAAGAGATG/3Cy3Sp/
8-17 S 5'bio-3'Cy3 (26 bases S)	/52-Bio/ACTCACTATrAGGAAGAGATGGACGTG/3Cy3Sp/
8-17 E 5'Cy3	/5Cy3/CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
8-17 S 5'Cy3-3'bio	/5Cy3/ACTCACTATrAGGAAGAGATG/3Bio/
8-17 S Cleaved 3'Cy3	AGGAAGAGATG/3Cy3Sp/

Table S3. Equations and definitions used for analyzing BRET signals.

Symbol	Definition or calculation for the symbol
BRET ratio (560 nm/480 nm)	Fluorescence intensity at 560 nm/480 nm in the normalized and smoothed spectra curve
R	BRET ratio (560 nm/480 nm) with different concentrations of Zn ²⁺ or EDTA
R0 *	The averaged BRET ratio (560 nm/480 nm) in groups that did not spike in Zn ²⁺ or EDTA
Net BRET signal [% over basal]	$[(R0-R)/R0] \times 100$

* For the comparison between different dilutions of serum sample, which all did not add in additional Zn²⁺, we used the averaged BRET ratio (560 nm/480 nm) in lowest diluted group to serve as R0 for calculating the BRET signal changes [% over 1/2 diluted serum] with the same equation.

Table S4. Metal concentrations in serum samples.*

Metal ions	Reported amount	Human Serum	10% Serum spiked 0 μ M ZnCl ₂	10% Serum spiked 40 μ M ZnCl ₂	10% Serum spiked 100 μ M ZnCl ₂	10% Serum spiked 200 μ M ZnCl ₂
Na	137 mM [103]	148.0 mM	159.7 mM	156.4 mM	161.3 mM	161.4 mM
Mg	850 μ M [104]	149.7 μ M	28.0 μ M	24.6 μ M	33.2 μ M	28.5 μ M
Ca	2.2 mM [105]	2.2 mM	309.8 μ M	237.7 μ M	295.9 μ M	290.8 μ M
Fe	10.74 to 30.43 μ M [106–109]	37.8 μ M	21.5 μ M	18.4 μ M	24.4 μ M	20.5 μ M
Cu	15.9 μ M [110,108]	14.1 μ M	3.1 μ M	3.1 μ M	3.2 μ M	3.2 μ M
Zn	15 μ M [110,108]	26.3 μ M	15 μ M	44.0 μ M	115.6 μ M	222.7 μ M
Rb	2.8 μ M [108]	0.5 μ M	0.2 μ M	0.2 μ M	0.2 μ M	0.2 μ M

* The metal concentrations in human serum were detected with ICP-MS. All the concentrations were shown as mean value. 10% Serum were diluted with PBS (without Ca²⁺ and Mg²⁺), which mimicked the condition we used for BRET sensor-based detections, and then diluted an additional 250-fold with 2% HNO₃ before testing. The concentration of metals in 10% serum sample represents the concentration in the PBS buffer. Other ions such as Al, Mn, Co, Ni, As, Sr, Zr, Mo, Ag, Cd, Ba, Tl, and Pb were also tested, but they were either below the limit of detection or did not show concentration differences with different dilutions of serum samples.