

## Electronic Supplementary Material (ESI)

### Gold Nanocluster-Based Fluorescent Probe for Simultaneous Sensing of pH and Temperature and Its Application to Cellular Imaging and Logic Gate

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#### Abstract

In the section of supporting information, we include detailed information associated with the use of single-emission BSA-AuNCs for temperature sensing, the measurement of activation energy of BSA-AuNCs, the use of FITC/BSA-AuNCs for temperature sensing in a N<sub>2</sub> saturated solution or in the presence of Tween 20, the reusability of FITC/BSA-AuNCs for temperature sensing, simultaneous sensing of temperature and pH changes by FITC/BSA-AuNCs, the sensing of human trypsin by FITC/BSA-AuNCs, and cytotoxicity of FITC/BSA-AuNCs

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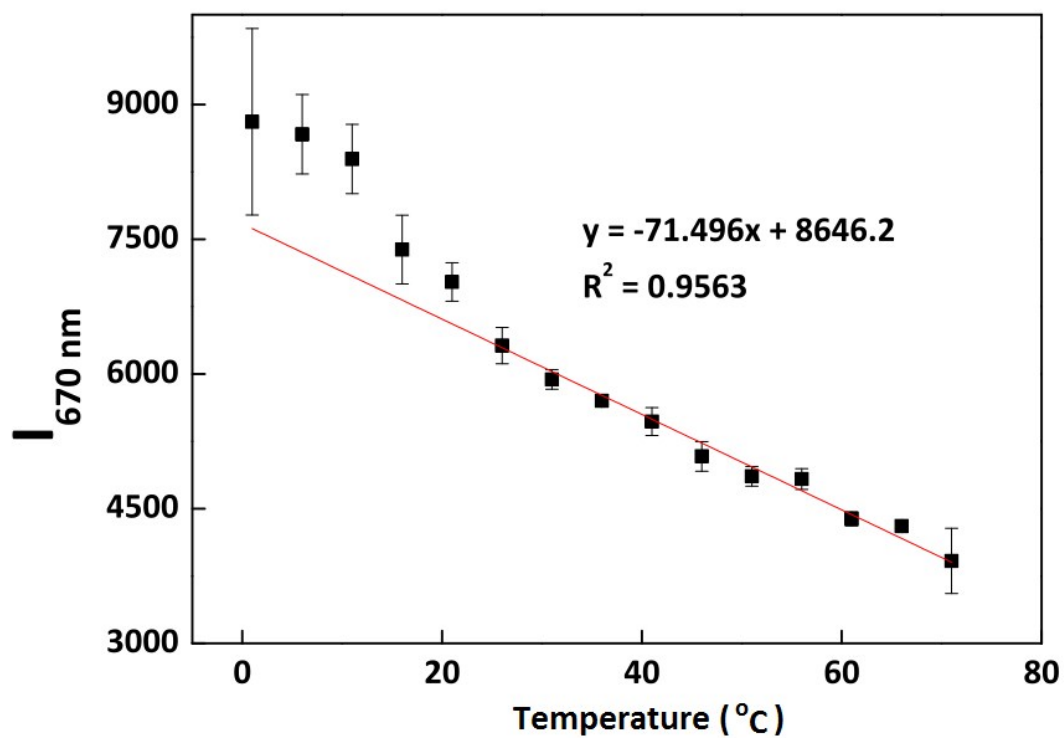
**Fig. S5.** The reusability of FITC/BSA-AuNCs.

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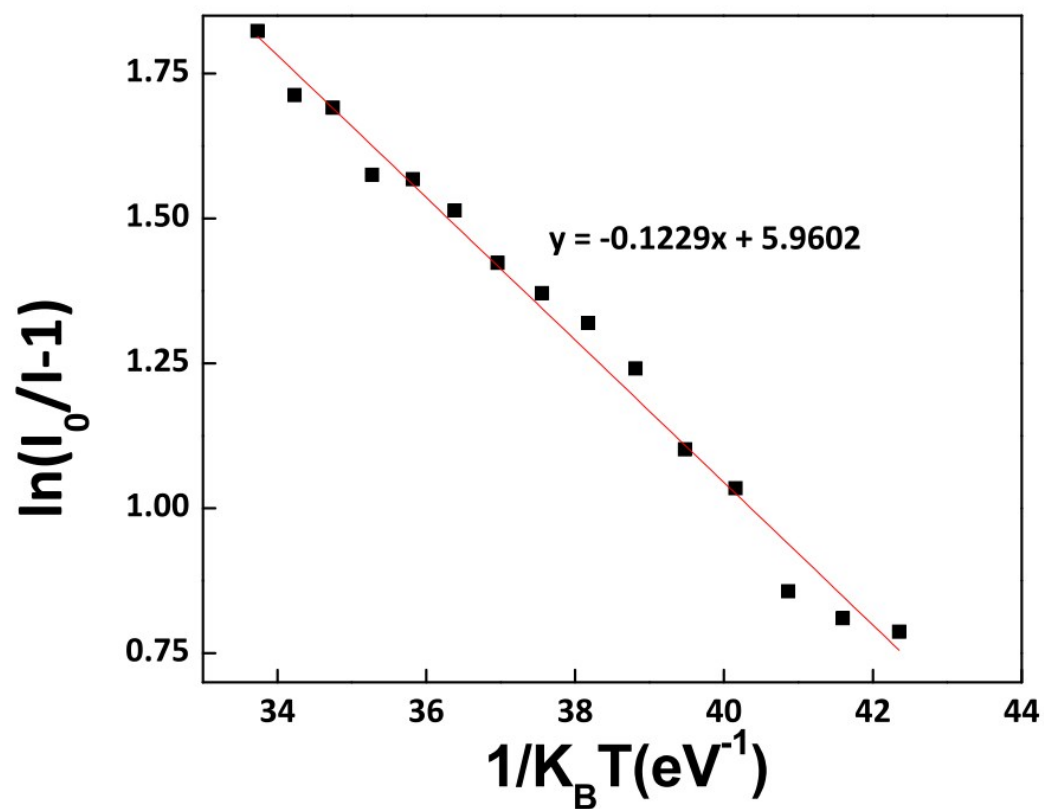
**Fig. S7.** The selectivity of FITC/BSA-AuNCs towards human trypsin.

**Fig. S8.** Determination of human trypsin by FITC/BSA-AuNCs.

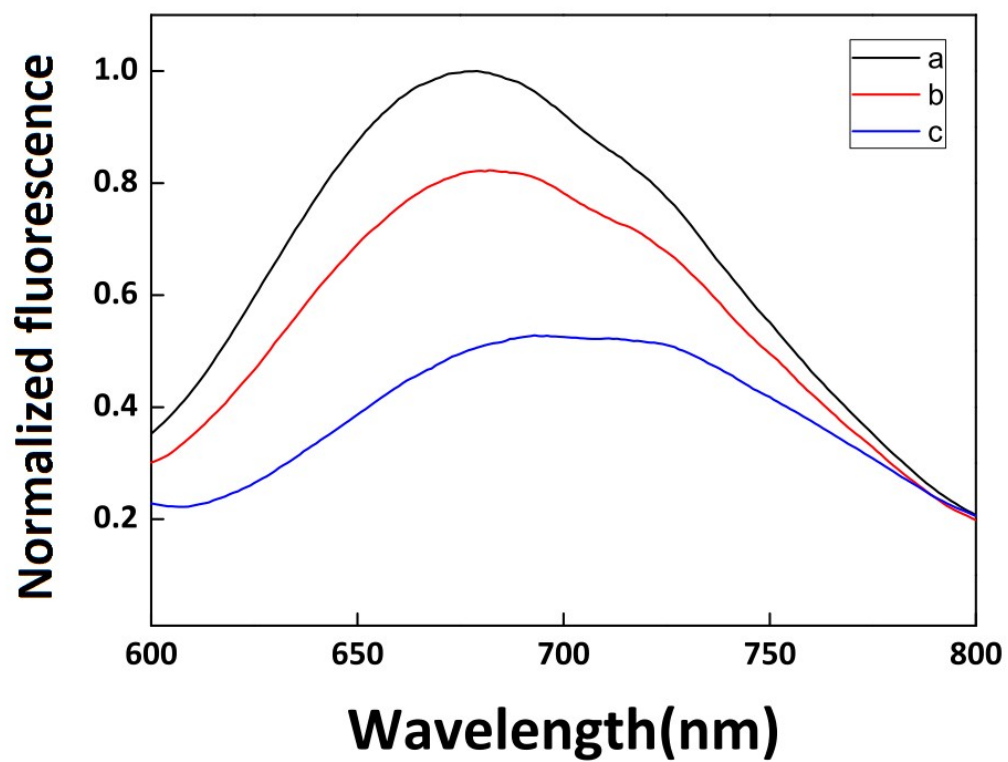
**Fig. S9.** MTT assay for FITC/BSA-AuNCs.



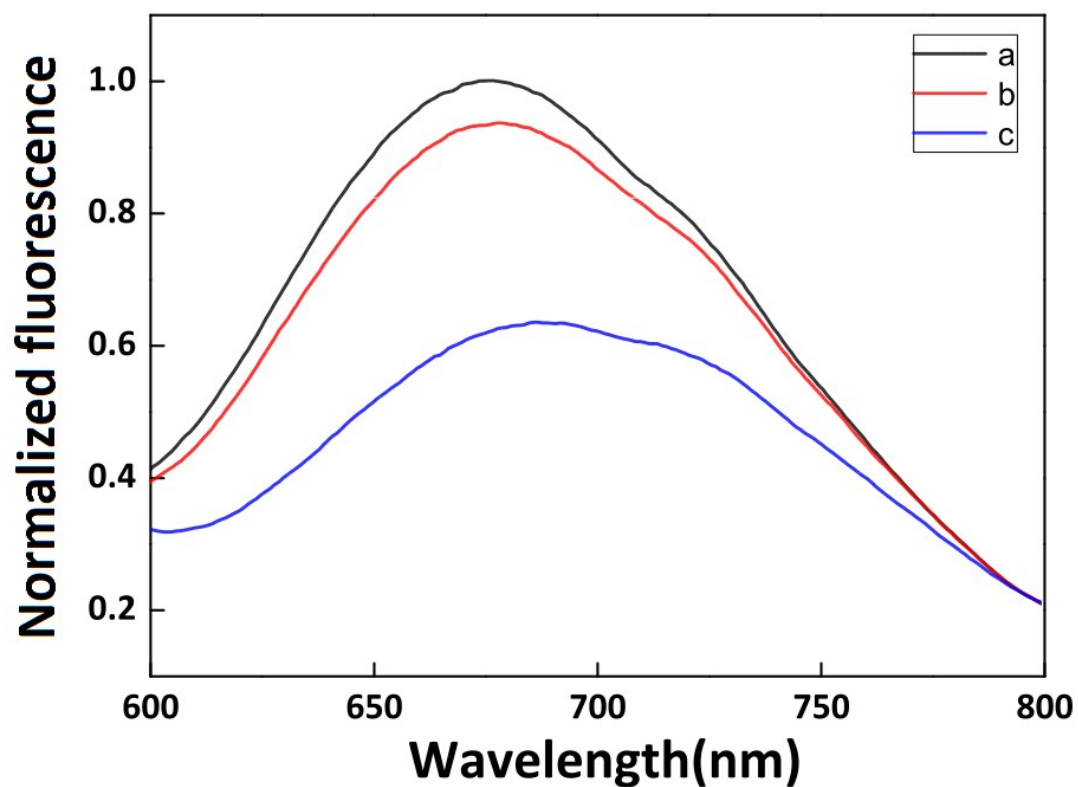
**Fig. S1.** A plot of the fluorescence intensity of BSA-AuNCs at 670 nm versus temperature. FITC/BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0). The error bars represent standard deviations based on three independent measurements.



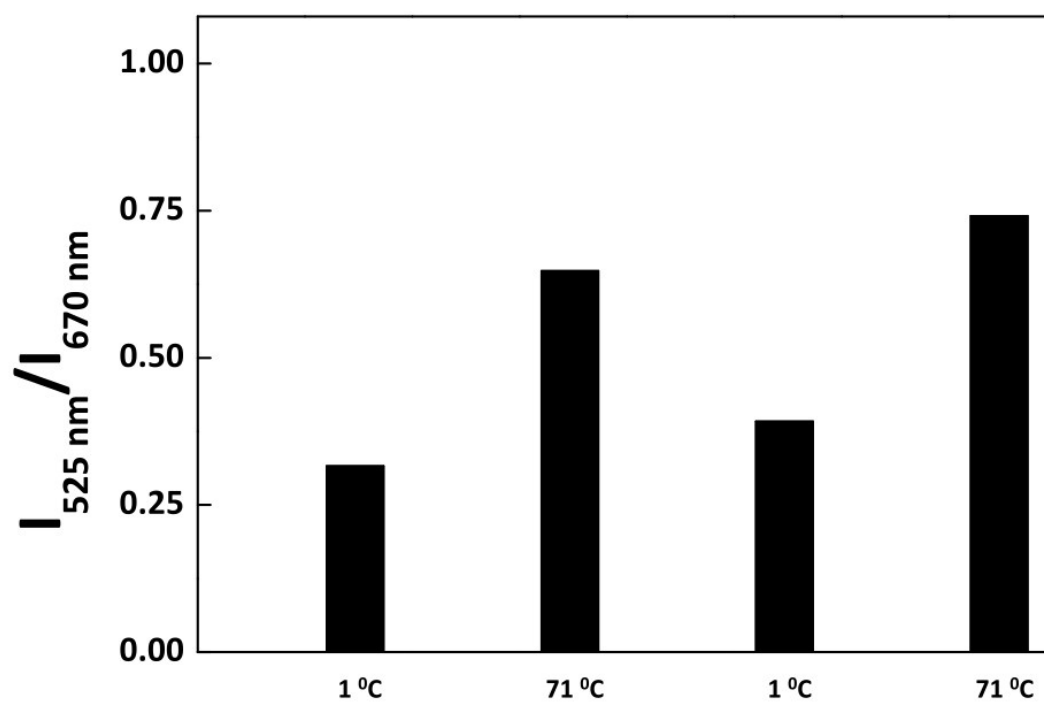
**Fig. S2.** Arrhenius plot for BSA-AuNCs. BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0)



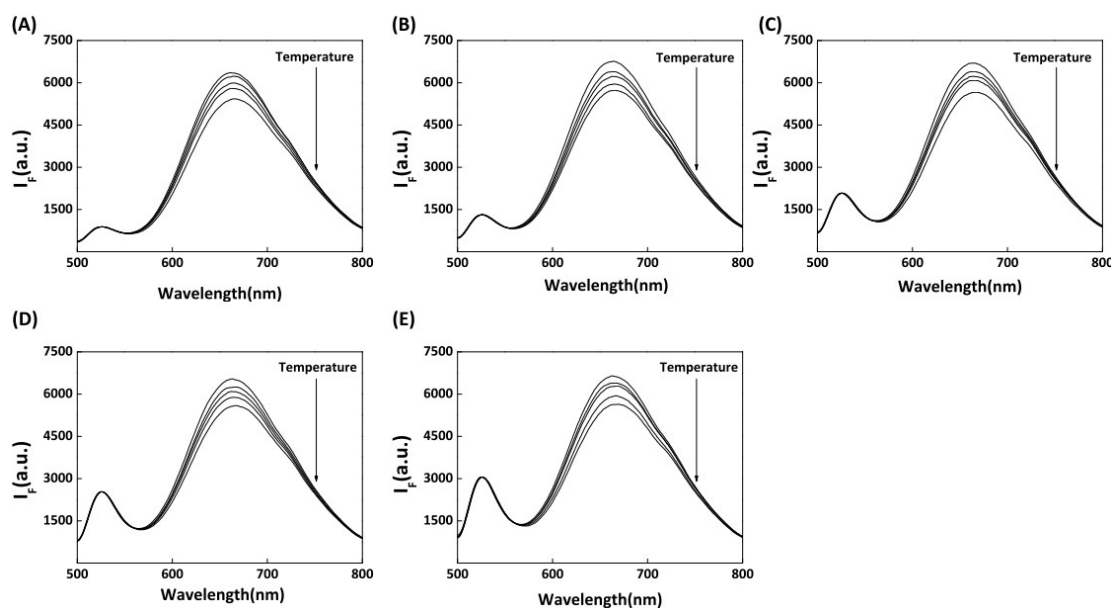
**Fig. S3.** Fluorescence spectra of FITC/BSA-AuNCs (a) 25, (b) 40, and (c) 86 °C in a N<sub>2</sub>-saturated solution. FITC/BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0).



**Fig. S4.** Fluorescence spectra of FITC/BSA-AuNCs (a) 25, (b) 40, and (c) 86 °C in the presence of 1% Tween 20. FITC/BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0) containing 1% Tween 20.

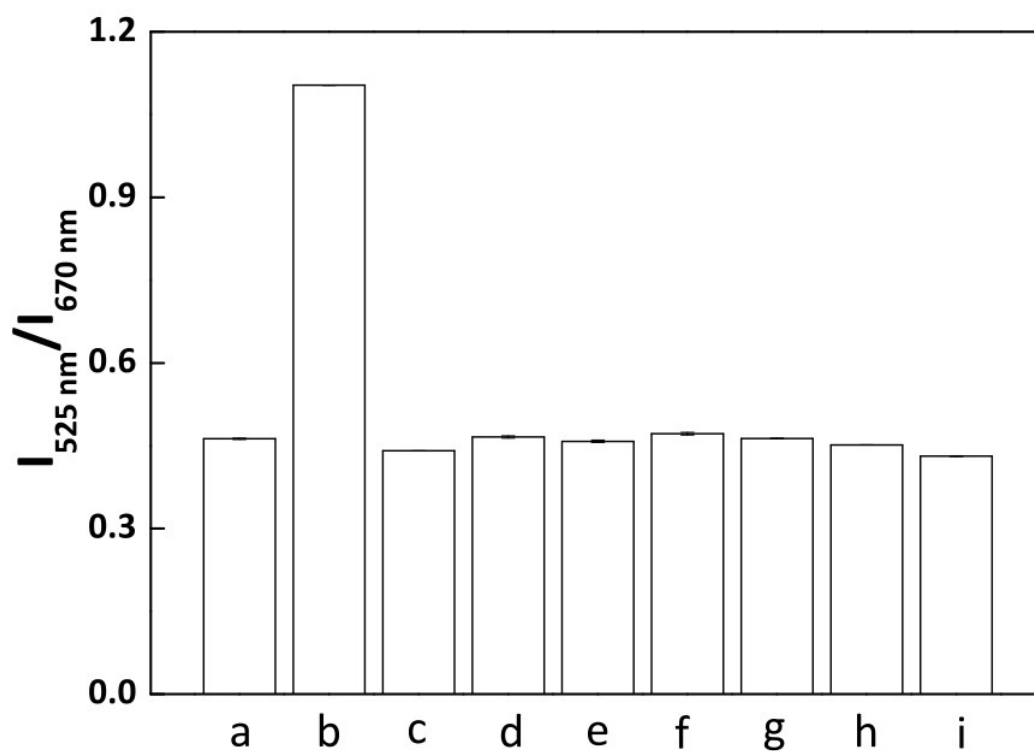


**Fig. S5.** The  $I_{525\text{ nm}}/I_{670\text{ nm}}$  value obtained from the continuous change in temperature. FITC/BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0).

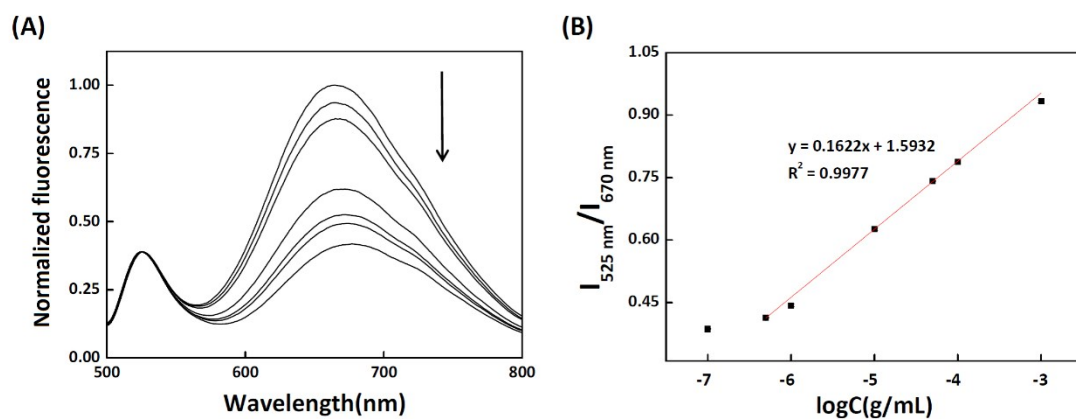


**Fig. S6.** Fluorescence spectra of FITC/BSA-AuNCs at pH (A) 6.0, (B) 6.5, (C) 7.0, (D) 7.5, and (E) 8.0. in the presence of increasing temperature. The arrow indicate the signal change with increasing the temperature (21, 26, 31, 36, and 41°C)

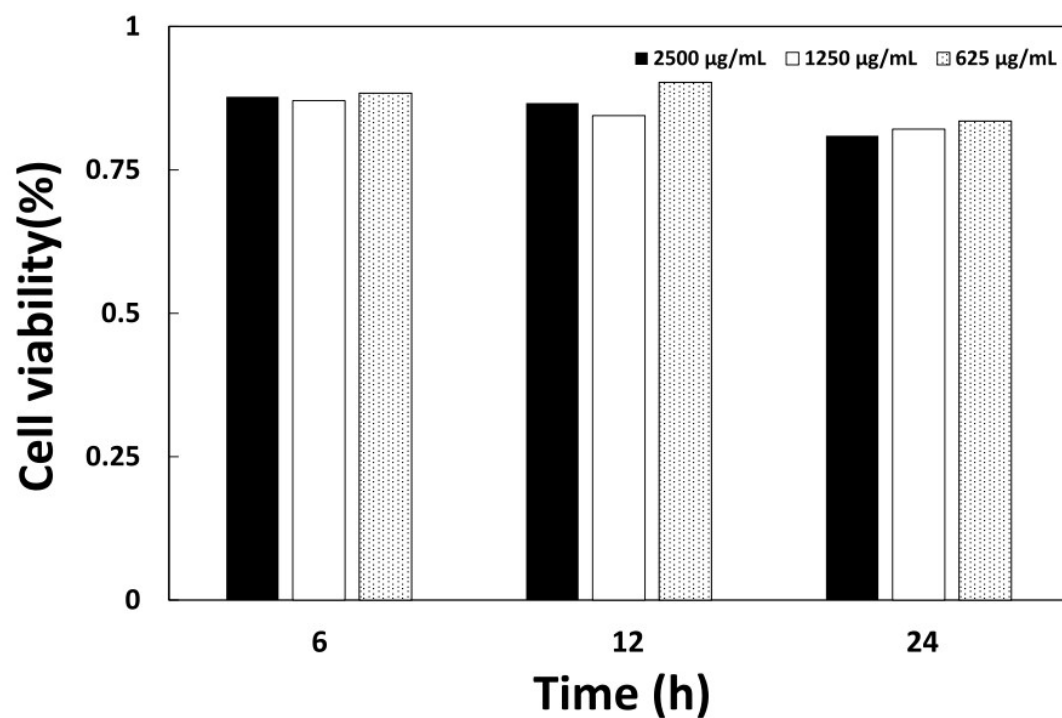




**Fig. S7.** The  $I_{525\text{ nm}}/I_{670\text{ nm}}$  value of FITC/BSA-AuNCs obtained before (a) and after (b-i) the addition of 10  $\mu\text{g/mL}$  (b) human trypsin, (c) BSA, (d) lysozyme, (e) glutathione, (f) glucose oxidase, (g) pepsin, (h) thrombin, and (i) hemoglobin. FITC/BSA-AuNCs were prepared in 10 mM phosphate buffer (pH 9.0). The incubation time is 30 min.



**Fig. S8.** (A) Fluorescence spectra and (B) the  $I_{525\text{nm}}/I_{670\text{nm}}$  values of FITC/BSA-AuNCs obtained from the addition of human trypsin-spiked urine samples. The arrow indicates the signal changes as increases in analyte concentration (1000, 100, 50, 10, 1, 0.5, and 0.1  $\mu\text{g/mL}$ ). Inset: a plot of the  $I_{525\text{nm}}/I_{670\text{nm}}$  value versus the spiked concentration of human trypsin. The error bars represent standard deviations based on three independent measurements.



**Fig. S9.** The value (%) of cell viability obtained from MTT proliferation tests. HeLa cells were incubated with different concentrations of FITC/BSA-AuNCs for 6, 12, and 24 h. In the control samples, the value of cell viability was set to 100%.