Supplementary Material (ESI) for Analyst

Supporting Information

A new specific fullerene-based fluorescent probe for trypsin

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Optimization of experimental conditions

Effect of pH values

In the experiment, the pH of the medium has a great effect on fluorescence intensity. In Fig. S1, the relative fluorescence intensity enhanced gradually with pH increase in the range of 5.00-7.50, while no obvious fluorescence changed in pH 7.50-9.0, so pH 8.0 was selected as an optimal parameter. Also, comparing with HEPES-NaOH, Tris-HCl, and KH_2PO_4-Na_2HPO_4 buffer solution systems, KH_2PO_4-Na_2HPO_4 was more sensitive. Thus, this phosphate buffer solution was chosen in our experiments.

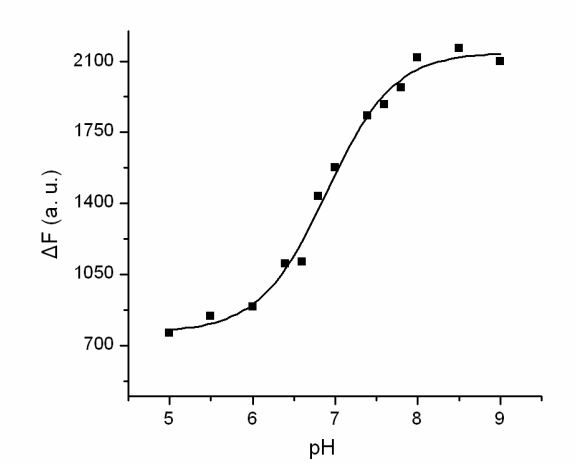

Fig. S1. Effects of pH values (C_{60}-FL concentration: 7.86 nM, trypsin: 40 μg mL^{-1}).

Effect of the concentration of PBS buffer solution

The concentration of PBS may affect the sensitivity of trypsin detection. Therefore, it is necessary to optimize the concentration of PBS buffer. Fig. S2 showed the influence of different concentrations of PBS buffer solution on fluorescence intensity. When the concentration of PBS is at 20 mM, the greatest changes in fluorescence signals appeared. So 20 mM PBS buffer solution was chosen as the optimal concentration in our analytical system.

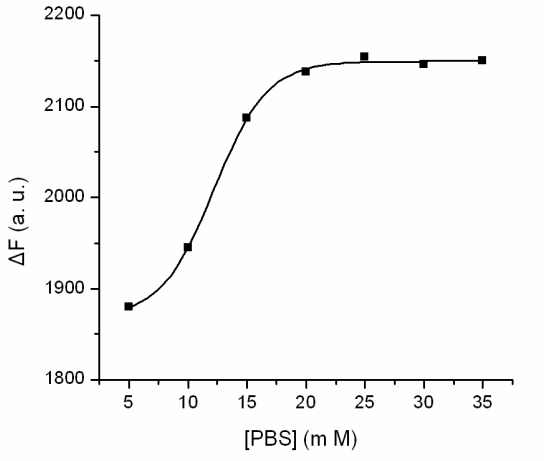

Fig. S2. The effects of different concentration of PBS buffer solution on fluorescence intensity (C_{60}-FL concentration: 7.86 nM; trypsin: 40 μg mL^{-1}).

Effect of temperature

In our experiment, we also detected the effect of temperature. The result showed that the temperature had no significant influence on the determination in Fig. S3. So the experiment was carried out at the room temperature.

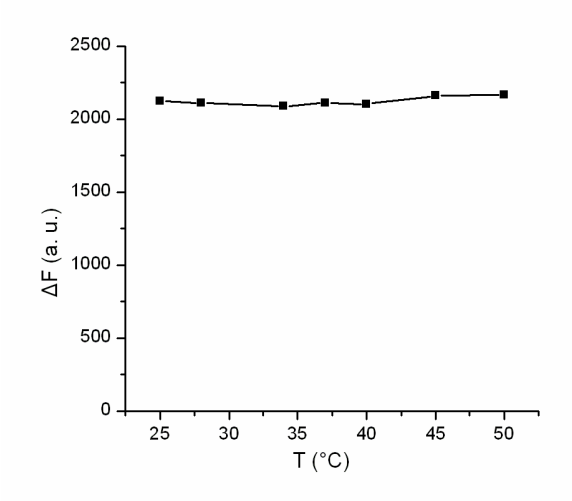

Fig. S3. Effect of temperature (C_{60}-FL concentration: 7.86 nM; trypsin: 40 μg mL^{-1}).

Effect of reaction time

The effect of reaction time on the fluorescence intensity was shown in Fig. S4. As can be seen, the fluorescence intensity of the system didn't change with different reaction time. This indicated that the reaction between the probe and trypsin was rapid.

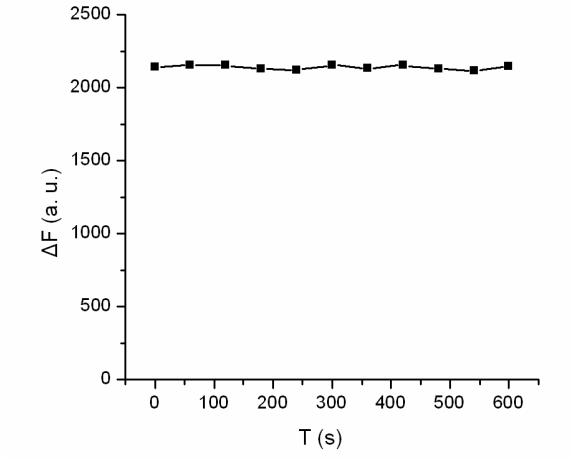

Fig. S4. The changes of fluorescence intensity with the increased response time after introducing 40 μg mL^{-1} trypsin.

Enzyme assay:

IC_{50} was calculated from inhibited percentages of different concentrations of probe to trypsin by the IC_p calculation program. The results showed in Table S1.

Table S1. The inhibition percentage of the probe to trypsin.

Probe Concentration (nM)	3.93	9.83	19.7	197
Inhibition percentage (%)	65.4	65.9	67.5	75.1