Electronic Supplementary Material (ESI) for Analyst.
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Supporting information

Highly sensitive and selective SERS detection of caspase-3 during cell apoptosis based on target-induced hotspot effect

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1. Synthesis of the AuNPs

The AuNPs were synthesized based on the heating reduction method. Briefly, 2.0 mL of $HAuCl_4$ (1.0 wt %) solution was added to 200 mL of deionized water and heated to boiling under vigorous stirring followed by quickly adding 2.0 mL of trisodium citrate (1.0 wt%) solution. The reaction was under stirring for another 40 min. Then, the solution was cooled to room temperature and centrifuged at 9,000 rpm for 10 min. The as-prepared AuNPs were stored at 4°C in the refrigerator for further use.

2. UV-vis spectra of nanosensor for different concentrations of caspase-3

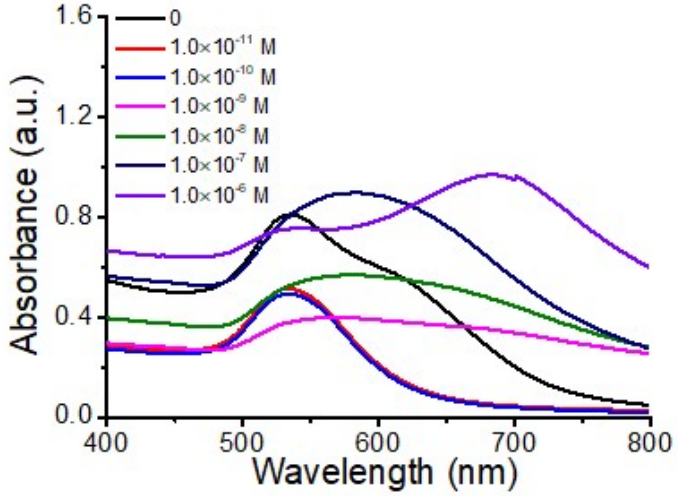

Fig. S1. UV–vis spectra of the nanosensor incubated with different concentrations of caspase-3.

3. Optimization of the reaction temperature

The most important factor for optimization is temperature of the reaction solutions, which have a significant impact on the interaction between peptide and caspase-3. Therefore, the intensity of SERS signal of the nanosensor was investigated at various temperature. Fig. S2b illustrates the effect of temperature values ranging from 20 to 45℃ on the SERS signal intensity produced by caspase-3 (1.0×10^{-7} M) with the greatest SERS intensity at 998 cm^{-1} ocurring at 37℃. As a result, we selected 37℃ as the optimum temperature.

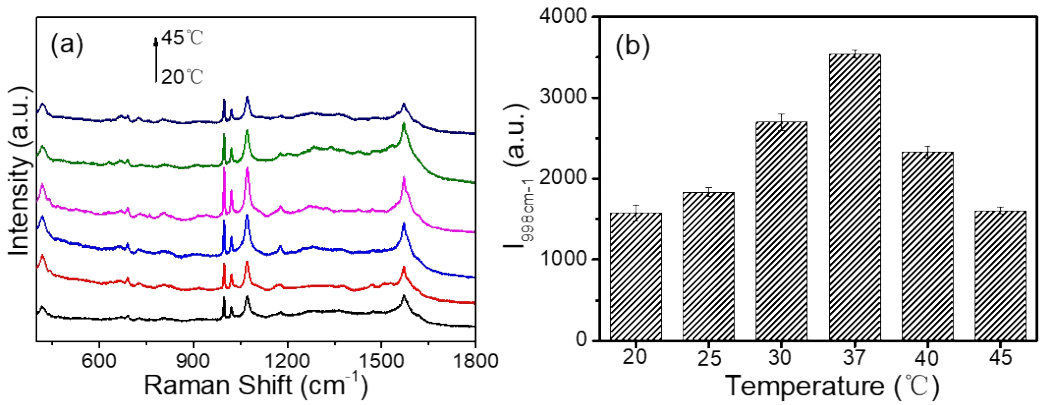

Fig. S2. (a) SERS spectra of the nanosensor at different temperatures (from bottom to top are 20, 25, 30, 37, 40, 45℃), while the reaction time kept at 90 min. (b) A plot of SERS intensity at 998 cm^{-1} with the temperature changes.

4. Optimization of the reaction time

Condition optimization for quantifying caspase-3 level was carried out. The incubation time for SERS intensity of the nanosensors at 998 cm^{-1} in the presence of caspase-3 (1.0×10^{-7} M) was assessed in Fig. S1a. It is noted that SERS intensity at 998 cm^{-1} is sharply increased within the first 90 min. When the reaction time is 90 min, the SERS intensity of 998 cm^{-1} reaches the maximum value (Fig. S1b). Thus, 90 min is chosen as the optimum incubation time.

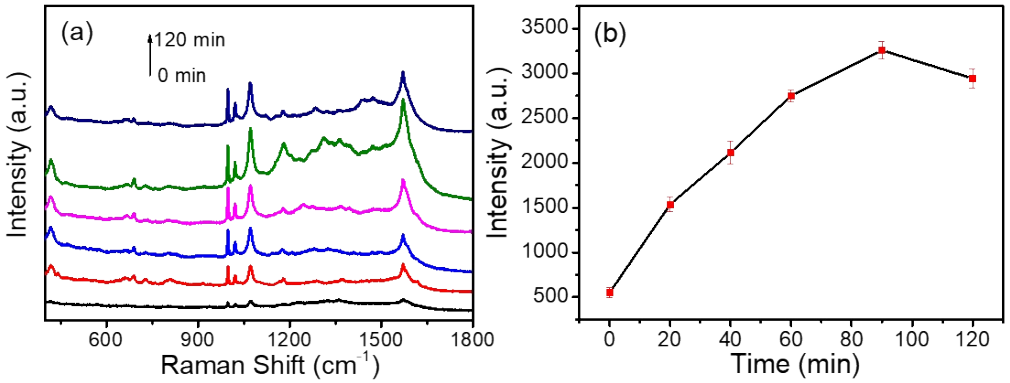

Fig. S3. (a) SERS spectra of the nanosensor at different reaction time (from bottom to top are 0, 20, 40, 60, 90 and 120 min, respectively) (b) A plot of the SERS intensity of the nanosensor at 998 cm^{-1} along with reaction time.

5. The interference of complex cellular environments

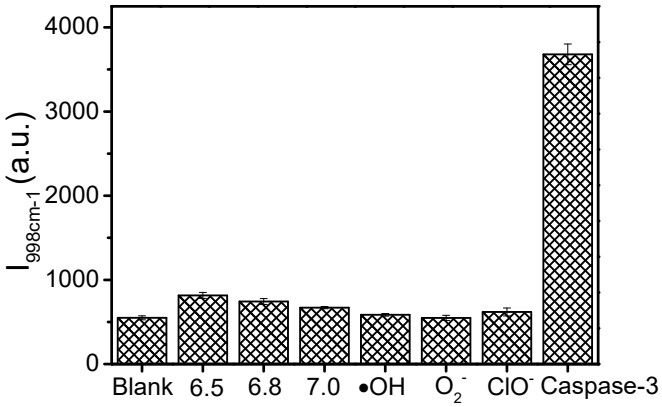

Fig. S4. SERS response of 998 cm^{-1} to different pH and various ROS, and caspase-3 (1.0×10^{-6} M).

6. Stability test of the SERS nanosensor

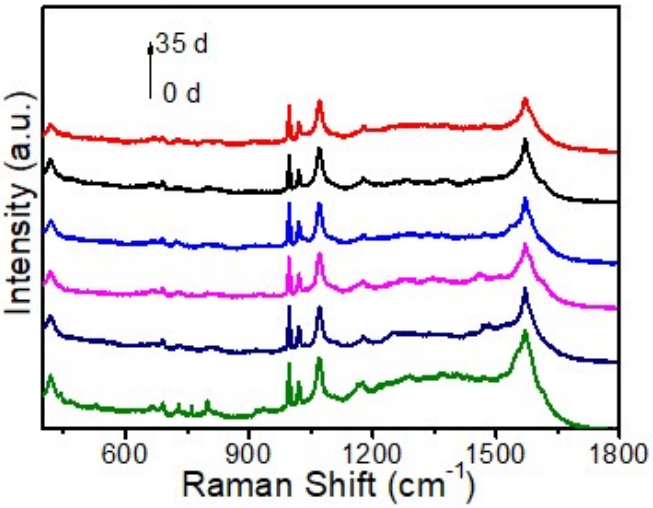

Fig. S5. SERS spectra of the nanosensor kept in natural conditions for different days (from bottom to top are 0, 7, 14, 21, 28, 35 d).

7. Reproducibility of SERS detection

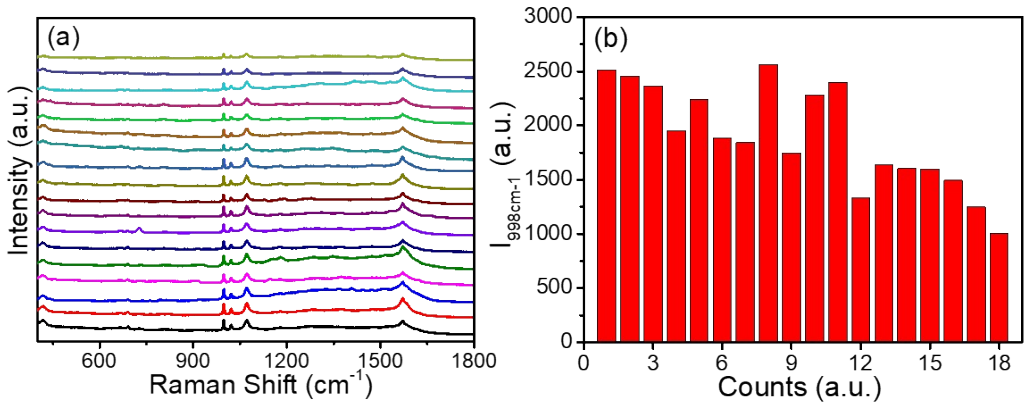

Fig. S6. (a) SERS spectra from randomly selected 18 points on the same sample. (b) Corresponding signal intensities at 998 cm^{-1}.

8. Evaluation of apoptosis by calcein AM/PI co-staining

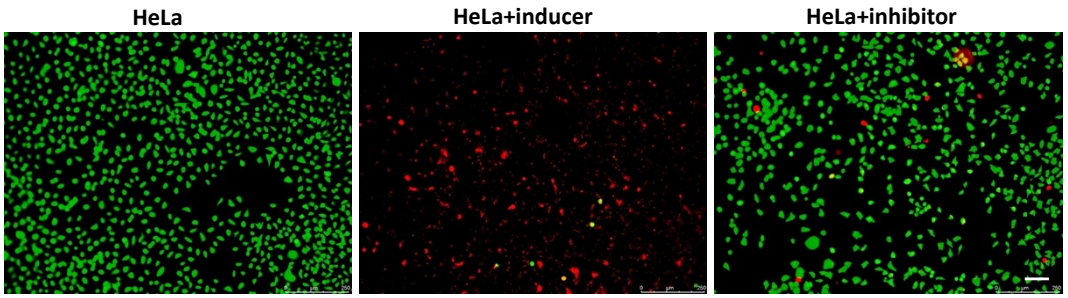

Fig. S7. Calcein AM/PI co-staining of HeLa cells, HeLa cells in the presence of inducer STS and inhibitor Z-DEVD-FMK. Scale bar is 80 μm.

9. Cytotoxicity test of nanosensors

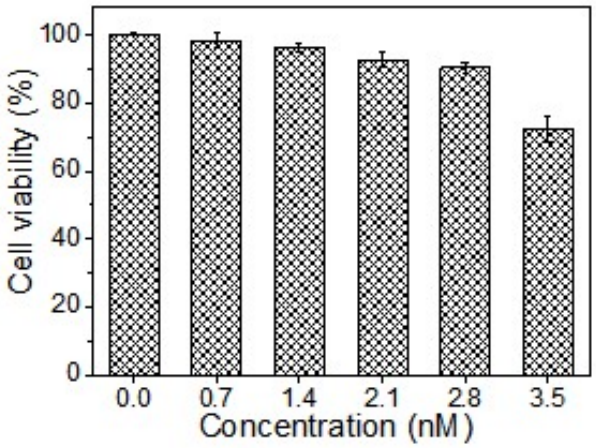

Fig. S8. Cell viabilities of HeLa cells processed with different concentrations of nanosensors for 24 h.

10.Endocytosis of nanosensors

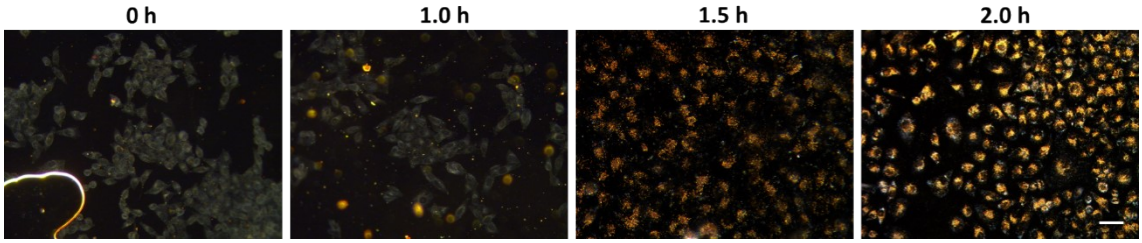

Fig. S9. Dark-field images of HeLa cells incubated with 0.28 nM nanosensor for 0, 1.0, 1.5 and 2.0 h (scale bar = 40 μm).

Table.S1 Assay results of detecting caspase-3 in living cells using the SERS nanosensor and commercial colorimetric method.

Cell types	Commercial colorimetric method (nM)	SERS-based assay (nM)
BNL	0.027±0.014	0.028±0.002
HepG2	0.069±0.023	0.071±0.007
HeLa	0.128±0.016	0.133±0.017
MCF-7	2.154±0.091	2.171±0.328