Electronic Supplementary Information

Single Fluorescent Probe for the Multiple Analyte Sensing: Efficient and Selective Detection of CN⁻, HSO₃⁻ and extremely alkaline pH

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Figures S12. Cell cytotoxic effect of IECBT (10 μM) after upon addition of CN⁻ on Hela cells. CN⁻ concentrations were varied as following: (1) control; (2) 0.01 μM; (3) 0.1 μM; (4) 1 μM; (5) 5 μM; (6) 30 μM. Data are expressed as mean values standard error of the mean of six independent experiments.

1. The solvent dependence in the detection process of CN⁻

The solvent of a system is often considered as a significant influencing factor on interactions. The effect of different solvent conditions on the fluorescence properties of the system was investigated (Figure S4). From Figure S4, we could find that IECBT was stable and displayed the best response for CN⁻ in DMSO. So, in the subsequent UV-vis and fluorescence experiments, DMSO was selected as a testing system to investigate the spectral response of IECBT to CN⁻.

2. Cell cytotoxicity assay

An MTT assay was performed to test the cytotoxicity of IECBT as well as the cell viability after upon addition of CN⁻ on Hela cells. HeLa cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS (fetal bovine serum), 100 mg·mL⁻¹ penicillin, and 100 μg·mL⁻¹ streptomycin in a 5% CO₂, water saturated incubator at 37 °C. Before the experiment, healthy HeLa cells (5 × 10⁵) were plated into 96 well microtiter plates (Nunc) for 16 h, followed by the addition of different concentrations of IECBT (0 to 10 μM). The cells were then incubated
at 37 °C in an atmosphere of 5% CO₂ and 95% air for 24 h. After incubation, the solutions were aspirated and replaced by DMEM (180 μL), followed by the addition of 5 mg·mL⁻¹ MTT solution (20 μL, final concentration of 0.5 mg·mL⁻¹) and incubated for 4 h. Unreacted dye was removed by aspiration; the insoluble formazan crystals were dissolved by adding dimethyl sulfoxide (200 μL) to each well and shaken for 10 min and measured spectrophotometrically in an ELISA reader at a wavelength of 490 nm. To evaluate the cytotoxicity of IECBT after upon addition of CN⁻ on Hela cells, the cells were also treated as previously described, except cells incubated with IECBT (10 μM) for 30 minutes were treated with a varying concentrations of CN⁻ (0, 0.01, 0.1, 1, 5, 30 μM) at 37 °C for 90 minutes. The cells were then washed with PBS (pH = 7.4), followed by analysis via MTT assays. Cells incubated with IECBT (10 μM) in a culture medium without CN⁻ were used as the control. Each group had six samples, and the spectrophotometer was calibrated to zero absorbance using culture medium without cells. The relative cell viability (%) related to the control groups was calculated as follows:

\[
\text{Cell viability} = \left[ \frac{A_{490}(\text{sample})}{A_{490}(\text{control})} \right] \times 100 \%
\]

Where \(A_{490}(\text{sample})\) is the absorbance value of IECBT or IECBT-CN treated cells, and \(A_{490}(\text{control})\) is the absorbance value of cells as control groups.

3. Culture of HeLa cells for intracellular imaging

To observe the subcellular distribution of IECBT, about \(1 \times 10^5\) HeLa cells in growth medium (2 mL) were seeded on a 35 mm diameter round glass Petri dish and incubated for 48 h in a 5% CO₂ atmosphere. The medium was then removed. The cells were first incubated with IECBT (10 μM) dissolved in acetonitrile/water (4/6, v/v) for 30 min. The free IECBT was removed by washing the cells three times with PBS. The cells were then fixed with 4% paraformaldehyde (300 μL) for 8
89 minutes at room temperature and treated with DAPI (1 mg mL⁻¹) for an additional 15 min. The
90 medium was removed and the cells were rinsed with PBS (pH = 7.4) many times. Fluorescence
91 images were collected on a ZEISS LSM 880 confocal laser scanning microscope with a 200×
92 objective lens. DAPI was excited at 405 nm and its blue emission was collected in the 425-475 nm
93 detection range; IECBT was excited at 488 nm and its red emissions were collected in the 500-
94 600 nm detection range.
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