Supporting Information

An Ultrasensitive D-A Fluorescent Probe for Dual-Mode Uranyl Detection in Environmental and Biological Systems

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Materials

phenylboronic 4-(Diphenylamino) acid (CAS: 201802-67-7, 98%), 4-Bromo-2hydroxybenzaldehyde (CAS: 22532-62-3, AR, 98%), potassium carbonate (CAS: 584-08-7, 99%) and Benzoyl hydrazine (CAS: 613-94-5, AR, 98%) were purchased from Macklin (Shanghai, China). Tetrakis(triphenylphosphine) palladium (CAS: 14221-01-3, AR, 98%), 2,6-Pyridinedimethanamine (CAS: 34984-16-2, AR, 98%) were purchased from Bide Pharmatech (Shanghai, China). Toluene (CAS: 108-88-3, AR, 99.5%) was purchased from Kaixin Chemical (Hengyang, China). Ethanol (CAS: 64-17-5, AR, 99.5%), methanol (CAS: 67-56-1, AR, 99.5%), petroleum ether (CAS: 8032-32-4, AR, boiling range 60-90 °C), dichloromethane (CAS: 75-09-2, AR, 99.5%), ethyl acetate (CAS: 141-78-6, AR, 99.5%), dimethyl sulfoxide (DMSO, CAS: 67-68-5, AR, 99.5%) and tetrahydrofuran (THF, CAS: 109-99-9, AR, 99.5%) were purchased from Anergy Chemical (Anhui, China). Stock solutions of anions were prepared from the corresponding sodium salts. The 0.1 M Tris-HCl buffer solution (pH 2 - 10) was prepared by mixing 0.1 M Tris(hydroxymethyl) aminoethane solution and 0.1 M HCl solution with appropriate ratios. TPA-BH stock solution $(4 \times 10^{-4} \text{ M})$ was prepared by dissolving 0.1 g TPA-BH in THF. Deionized distilled water (18.2 M Ω) was used for all the experiments. All chemicals and solvents were at least of analytical grade unless otherwise stated and used as received without further purification.

Apparatus

The ¹H NMR and ¹³C NMR spectra were recorded using a Bruker AVANCE NEO (Bruker

Magnetic Resonance, Germany). High-resolution mass spectra (HRMS) data were acquired on an LCMS-IT-TOF (SHIMADZU, Japan). Scanning electron microscopy (SEM) images were captured using a Zeiss Sigma 300 microscope (Zeiss, German). The elemental composition of TPA-BH was analyzed using an energy dispersive X-ray spectrometer (EDS) (Zeiss, German). Powder X-ray diffraction (PXRD) patterns were measured on an Ultima IV X-ray diffractometer (Rigaku, Japan). Fourier transform infrared (FT-IR) spectra were recorded on a Spectrum Two Fourier transform infrared spectrometer (PerkinElmer, USA). Fluorescence spectra were obtained using an FL-4500 spectrofluorometer (HITACHI, Japan). Fluorescence lifetime measurements were performed on an FLS1000 (Edinburgh, UK). Dynamic light scattering (DLS) experiments were conducted using a Malvern Zetasizer Nano ZS90 (Malvern, UK). The concentration of UO₂²⁺ in actual water samples was determined by ICP-MS (Agilent 7700s, USA). Confocal imaging of HeLa cells was performed using a Leica TCS SP5 II laser confocal microscope (Leica, Germany).

• Synthesis of TPA-P1

Tetrakis(triphenylphosphine) palladium (4.0 mmol, 0.462 g), potassium carbonate (24.0 mmol, 3.32 g), 4-(Diphenylamino) phenylboronic acid (9.6 mmol, 2.78 g) and 4-Bromo-2-hydroxybenzaldehyde (8.0 mmol, 1.61 g) were dissolved in a mixture of 40 mL toluene, 40 mL water and 20 mL anhydrous ethanol. The reaction was conducted under nitrogen atmosphere at 85 °C with continuous stirring for 24 h. Upon cooling to ambient temperature, the crude mixture was subjected to liquid-liquid extraction with ethyl acetate (3×50 mL). The combined organic layers were concentrated under reduced pressure using a rotary evaporator, followed by purification through silica gel column chromatography with a gradient eluent system (petroleum ether/ethyl acetate = 20:1, v/v). The target compound was obtained as a pale-yellow solid in 74% yield.

• Synthesis of TPA-BH

TPA-P1 (1.0 mmol, 0.365 g), benzoyl hydrazine (1.2 mmol, 0.163 g) and 20 mL of anhydrous ethanol were added to a round-bottom flask. The mixture was heated under reflux at 80 °C for 8 hours with continuous stirring. After cooling to room temperature, the crude product was recrystallized using ethanol and subsequently filtered to obtain the purified TPA-BH as a yellow powder with a yield of approximately 90%. HRMS [m/z]: 484.20195 [M]⁺. ¹H NMR (600 MHz, DMSO-d6): δ = 12.13 (s, 1H), 11.43 (s, 1H), 8.66 (s, 1H), 7.95 (d, J = 6.9 Hz, 2H), 7.70 - 7.48 (m, 6H), 7.34 (dd, J = 8.4, 7.3 Hz, 4H), 7.23 (dd, J = 8.0, 1.8 Hz, 1H), 7.20 (d, J = 1.8 Hz, 1H), 7.09 (dd, J = 13.8, 7.4 Hz, 6H), 7.02 (d, J = 8.7)

Hz, 2H) ppm. ¹³C NMR (151 MHz, DMSO-d6): δ =163.25, 158.39, 148.56, 147.81, 147.39, 142.95, 133.31, 133.12, 132.47, 130.64, 130.15, 129.05, 128.16, 128.13, 124.92, 123.98, 123.22, 117.85, 117.81,113.98 ppm.



Fig. S2. ¹H NMR spectra of TPA-BH in DMSO-d6.







Fig. S4. Powder X-ray diffraction (XRD) spectrum of TPA-BH.



Fig. S5. (a) Fluorescence emission spectra of 10 μ M TPA-BH in different solvents (with 90% water fraction); (b) Comparison of fluorescence intensity of TPA-BH before and after addition of UO₂²⁺ in different solvents (with 90% water fraction).



Fig. S6. Excitation and emission spectra of TPA-BH ($f_w = 90\%$).



Fig. S7. Change in fluorescence intensity of TPA-BH (10 µM) after addition of 1 µM of (a) metal ions and (b) anions.

| | | | - | • | | | | _ |
|-------------------|-----------------|--------------------|--------------|-------------------|--------------|------------------|--------------|---|
| metal ions | K _{SV} | metal ions | $K_{\rm SV}$ | metal ions | $K_{\rm SV}$ | metal ions | $K_{\rm SV}$ | _ |
| UO2 ²⁺ | 14.204 | Hg^{2+} | 0.071 | Pb ²⁺ | 0.002 | Mg^{2+} | 0.068 | - |
| Fe ³⁺ | 0.299 | K^+ | 0.114 | Ca^{2+} | 0.060 | Ba ²⁺ | 0.032 | |
| Zn^{2+} | 0.243 | Al ³⁺ | 1.098 | $\mathrm{NH_4^+}$ | 0.064 | Cu^{2+} | 4.081 | |
| Cr ³⁺ | 0.118 | Ni ²⁺ | 3.758 | Fe ²⁺ | 3.075 | Li ⁺ | 0.001 | |
| Be^{2+} | 0.017 | Mn^{2+} | 0.781 | Na^+ | 0.055 | Cd^{2+} | 0.005 | |

Table S1. The Stern-Volmer Quenching Constants (K_{SV}) of metal ions.

Note: The unit of K_{SV} is 10⁶ M⁻¹. The formula for the Stern-Volmer quench constant is $F_0/F = 1 + K_{SV}$ [Q], where K_{SV} is the Stern-Volmer burst constant, [Q] is the concentration of metal ions, and F_0 and F were the fluorescence intensities of TPA-BH and TPA-BH + UO₂²⁺ respectively.



Fig. S8. (a) Variation of fluorescence intensity of TPA-BH with the concentration of UO_2^{2+} (0-0.10 μ M); (b) Stern-Volmer plot of fluorescence quenching of TPA-BH against UO_2^{2+} (0-0.10 μ M), insert: linear Stern-Volmer plot of UO_2^{2+} in the low concentration (0-0.05 μ M) range.

| Sample | $\lambda_{ex}/\lambda_{em}~(nm)$ | $\tau_1(ns)$ | A_1 | $\tau_2(ns)$ | A_2 | $\tau_{aver}\left(ns\right)$ | χ^2 |
|--|----------------------------------|--------------|-------|--------------|-------|------------------------------|----------|
| TPA-BH | 370/504 | 0.674 | 89.1% | 0.674 | 10.9% | 0.674 | 0.991 |
| TPA-BH+UO ₂ ²⁺ (1000:1) | 370/504 | 0.573 | 90.5% | 6.31 | 9.50% | 1.12 | 0.993 |
| TPA-BH+UO ₂ ²⁺ (100:1) | 370/504 | 0.556 | 88.4% | 4.83 | 11.6% | 1.05 | 0.995 |
| TPA-BH+UO ₂ ²⁺ (10:1) | 370/504 | 0.822 | 76.5% | 3.68 | 23.5% | 1.49 | 0.999 |

Table S2. Time-resolved fluorescence spectroscopy data of 10 μ M TPA-BH before and after the addition ofdifferent concentrations of UO22+.

Supporting Information



Fig. S9. SEM images (a) and EDS elemental distribution mapping (b) of TPA-BH $+UO_2^{2+}$.



Fig. S10. The particle size distribution of 10 μ M TPA-BH in a solution of THF/H₂O (f_w =90%) without UO₂²⁺ (a) and with the addition of 1 μ M UO₂²⁺ (b).



Fig. S11. CCK-8 assay for testing the viability of HeLa cells.

| Material | Specificity Application | | LOD | Ref. |
|----------|----------------------------------|------------------------|------------|------|
| TPE-BSA | 1 equiv. of metal ions | Drinking water | 9.3 ppb | [24] |
| HNU-50 | 1 amin of mataliana | Nuclear industry | 2.86 ppb | [40] |
| | r equiv. or metar ions | wastewater/ Seawater | | |
| YTU-100 | 1 equiv. of metal ions Tap water | | 1.07 ppb | [41] |
| USC-001 | 5 amin af matal is no | River water/ | 11.0 mm | [42] |
| | 5 equiv. of metal lons | Cell imaging | 11.9 ppo | |
| | | Lake water/ | | |
| TPE-SA | 1 equiv. of metal ions | River water/ Drinking | 2.6 ppb | [43] |
| | | water | | |
| | 30 equiv. of metal ions and | Simulated nuclear | 0.01(4 1 | [45] |
| TPE-EDC | anions | wastewater / Seawater | 0.0164 ppb | |
| TDA DII | 10 equiv. of metal ions; 500 | River water/ Seawater/ | 0.0417 mmh | This |
| і г А-ВП | equiv. of anions | Cell imaging | v.v417 ppp | work |

 Table S3 Comparison of present work with literature reports.