**Electronic Supplementary Material**

**Gold Nanocluster-based Fluorescence Sensing Probes for Detection of Dipicolinic Acid**

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**Additional Experimental Details**

**Reagents and materials**
Chemicals and materials were obtained from Riedel–de Haën (Seelze, Germany), Sigma–Aldrich (St. Louis, MO, USA), Mallinckrodt (St. Louise, MO, USA), Fluka (Basel, Switzerland), J.T. Baker (Philipsburg, NJ, USA), Showa (Tokyo, Japan), and Merck Millipore (Billerica, MA, USA). Culture media for growing bacteria including tryptic soy broth and yeast extract were purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). *Bacillus cereus* (BCRC 17427) was obtained from Bioresource Collection Research Center (BCRC) (Hsinchu, Taiwan).

**Instrumentation**
Fluorescence spectra were obtained using a Horiba Jobin Yvon Flouromax-3 spectrofluorometer (Edison, NJ, USA). A Varian Cary 50 UV-Vis spectrophotometer (Melbourne, Australia) was used to collect ultraviolet-visible (UV-Vis) absorption spectra of the samples. The lifetime of the fluorescence nanoparticles was measured using a Protrustech Uni-Probe spectrophotometer (Taipei, Taiwan) equipped with a pulsed laser (λ= 405 nm). FluoFit software version 4.5 was used for data analysis. A high-resolution JEM-2100F transmission electron microscope (HR-TEM) (Tokyo, Japan) was used to observe the morphology and size distribution of the as-prepared nanoprobes, while ImageJ (1.48 v) software was used to estimate the particle size distribution. A JEOL JSM-7401F FE scanning electron microscopy (SEM) (Tokyo, Japan) equipped with energy-dispersive X-ray spectroscopy (EDX) was used to observe the morphology of the vegetative cells and spores of bacteria and the elemental composition. Mass spectra were acquired using a Bruker Daltonics Autoflex III Smartbeam time-of-flight mass spectrometer (Bremen, Germany) equipped with a solid laser (λ= 355 nm).

**Characterization of DPA released from the spore lysate**
To examine if the as-prepared spore lysate containing DPA, the spore lysate was characterized by laser desorption/ionization mass spectrometry. That is, the as-prepared spore lysate (1 μL) was mixed with tetramethylammonium hydroxide (TMAH) (20 mM, 1 μL) prepared in methanol. The resultant mixture (1 μL) was deposited and dried on a sample plate. The dried sample was introduced into a laser desorption mass spectrometer that was operated by the reflectron positive ion mode (cut-off mass: m/z 150). Each mass spectrum was acquired from1000 laser shots.
Additional Figures

**Figure S1.** (a) UV-Vis absorption spectra of Au@GSH NCs (0.1 mg mL\(^{-1}\)) in the absence (red) and presence of Cu\(^{2+}\) (50 µM) (blue). (b) HR-TEM image of the Au@GSH NC-Cu\(^{2+}\) complexes. Scale bar: 5 nm.

**Figure S2.** Lifetime measurement of Au@GSH NCs (0.4 mg mL\(^{-1}\)) in the absence (black) and presence of Cu\(^{2+}\) (20 µM) (red), \(\lambda_{\text{ex}} = 405\) nm, \(\lambda_{\text{em}} = 650\) nm, pulse width = 49 ps, average power = 0.11 mW, signals synchronized by external trigger source).
**Figure S3.** (a) Fluorescence spectra of Au@GSH NCs (0.2 mg mL$^{-1}$, 30 µL) in the presence of different metal ions (10 µM, 30 µL) ($\lambda_{ex}$= 396 nm) and their (b) corresponding bar graphs by plotting ($F_0$-$F$)/$F_0$ versus different metal ions. $F$ and $F_0$ stand for fluorescence intensity at the wavelength of 615 nm with and without the addition of the metal ions. Error bars indicated standard deviation from three replicates.

**Figure S4.** (a) Fluorescence spectra of the samples (95 µL) containing DPA with different concentrations (0, 0.04202, 0.06003, 0.08575, 0.12250, 0.17500 and 0.25000 µM) by using concentrated AuNCs@GSH-Cu$^{2+}$ (5 µL) as sensing probes prepared in PBS buffer (pH 5.8). (b) Corresponding plot by plotting ($F$-$F_0$)/$F_0$ versus the concentration of DPA ($\lambda_{ex}$= 396 nm). $F$ and $F_0$ stand for the fluorescence intensity at the wavelength of 615 nm with and without the addition of DPA. The error bars represent the standard deviation from three replicates.
**Figure S5.** SEM images of (a) the *B. cereus* vegetative cells and (b) lyophilized *B. cereus* spores. Scale bar: 1 µm. (c) EDX spectrum of the lyophilized *B. cereus* spores. The pink square marked in the inset shows where the result was acquired. (Inset scale bar: 5 µm).
Figure S6. Laser desorption mass spectra of (a) TMAH (20 mM, 1 µL), (b) DPA (1 mM, 1 µL) with addition of TMAH (20 mM, 1 µL), and (c) the B. cereus spore lysate (1 µL) with addition of TMAH (20 mM, 1 µL).

Figure S7. (a) Fluorescence spectra of the lysate sample (40 µL) derived from B. cereus spores spiked with DPA (10 µL) at different concentrations (0, 4.20174, 6.0025, 8.575, 12.25, 17.5 and 25 µM) by using the Au@GSH NC-Cu²⁺ complexes as sensing probes. (b) The resultant calibration curve by plotting fluorescence intensity ((F-F₀)/F₀) versus the concentration of DPA. F and F₀ stand for fluorescence intensity at 615 nm (λ_ex = 396 nm) with and without the addition of DPA. (Inset) Plot obtained from the linear dynamic range. The error bars represent the standard deviation derived from three replicates.
**Table S1.** Comparison of the LODs of DPA obtained in the previous reports and the current work.

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<tr>
<th>Fluorescent probe</th>
<th>LOD (nM)</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>[Eu(EDTA)(H$_2$O)$_3$]</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>(Tb/DPA@SiO$_2$-Eu/GMP)</td>
<td>1000</td>
<td>2</td>
</tr>
<tr>
<td>FA: Tb–EDTA</td>
<td>8.2</td>
<td>3</td>
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<tr>
<td>FA: Eu–EDTA</td>
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</tr>
<tr>
<td>Eu(BTC)</td>
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<tr>
<td>Tb/Eu-MOFs</td>
<td>4.55</td>
<td>5</td>
</tr>
<tr>
<td>Tb-micelle</td>
<td>54</td>
<td>6</td>
</tr>
<tr>
<td>Erichrome Black T-Eu$^{3+}$</td>
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<td>7</td>
</tr>
<tr>
<td>RB-Eu-BTC</td>
<td>3200</td>
<td>8</td>
</tr>
<tr>
<td>BSA–AuNPs@Tb–AMP</td>
<td>17.4</td>
<td>9</td>
</tr>
<tr>
<td>Au@GSH NC-Cu$^{2+}$</td>
<td>~19</td>
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</table>

**AMP:** Adenosine 5’-monophosphate; **BSA:** Bovine serum albumin; **BTC:** Benzene-1,3,5-tricarboxylic acid; **EDTA:** Ethylenediaminetetraacetic acid; **FA:** Fluorapatite; **GMP:** Guanosine monophosphate; **MOF:** Metal-organic framework; **RB:** Rhodamine-derived molecule.

**References**