Electronic Supporting Information

Water switched aggregation/ disaggregation strategies of a coumarin-naphthalene conjugated sensor and its selectivity on Cu$^{2+}$ and Ag$^{+}$ ions along with cell imaging study on human osteosarcoma cells (U-2 OS)

Ashish Kumar$^a$, Surajit Mondal$^a$, Kumari Somlata Kayshap$^a$, Sumit Kumar Hira$^b$, Partha Pratim Manna$^c$, Wim Dehaen$^d$ and Swapan Dey$^a$$^*$

$^a$ Department of Applied Chemistry, Indian Institute of Technology (ISM), Dhanbad 826004, Jharkhand, India, swapan@iitism.ac.in

$^b$ Department of Zoology, The University of Burdwan, Burdwan, West Bengal-713104, India.

$^c$ Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi-221005, India.

$^d$ Department of Chemistry, KU Leuven, Celestijnenlaan 200F, 3001, Leuven, Belgium.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Table of content</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$^1$H NMR Spectra of R1.</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>$^1$H NMR Spectra of R1:Cu$^{2+}$.</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>$^{13}$C-NMR Spectra of R1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Mass spectra of R1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Mass spectra of R2 (R1:Ag$^+$)</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Mass spectra of R2 (R1:Cu$^{2+}$)</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>FT-IR of R1</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Comparative studies of R1 with different metal ions.</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Fluorescent titration curve of R1 with Cu$^{2+}$ and Ag$^+$ ions.</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>Binding Constant calculation</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>Calculation of limit of detection (LOD)</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>Quantum yield calculation</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>Proliferation and cytotoxicity of U-2 OS cells.</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>Viability and hemolysis of RBC in presence of R1</td>
<td>10</td>
</tr>
<tr>
<td>14</td>
<td>Viability and hemolysis of RBC in presence of R1</td>
<td>11</td>
</tr>
</tbody>
</table>
Fig. S1: $^1$H-NMR spectra (CDCl$_3$, 400 MHz) R1:

Fig. S2: $^1$H-NMR spectra (CDCl$_3$, 400 MHz) R1:Cu$^{2+}$:
Fig. S3: $^{13}$C-NMR spectra (CDCl$_3$, 400 MHz) R1:
Fig. S4: ESI-MS of R1: m/z: calculated for C$_{24}$H$_{23}$N$_3$O$_2$: 417.1511, Found: 418.1583 [M+H$^+$, 50], 440.141 [M+Na$^+$, 100], 857.3092 [2M+Na$^+$, 98].

Fig. S5: ESI-MS of R2 (R1:Ag$^+$): m/z: calculated for C$_{24}$H$_{23}$N$_3$O$_3$: 401.1739, Found: 402.1897 [M+H$^+$, 20], 424.1626 [M+Na$^+$, 51].
**Fig. S6:** ESI-MS of R2 (R1:Cu²⁺): m/z: calculated for C_{24}H_{23}N_{3}O_{3}+ Cu²⁺ +H⁺: 481.0869, Found: 481.0750 [M+Cu²⁺+H⁺].

**Fig. S7:** FTIR spectra of R1

**Fig. S8:** Comparative studies of R1 (5.4 µM) with different metal ions (10.0 equiv.)
Fig. S9: (a) fluorescence titration curve of R1 with continuously increased concentration of Cu$^{2+}$ ions (b) with Ag$^+$ ions.

Fig. S10: Ratiometric analysis of complex using fluorescence titration data by using Job’s plot (a) with Cu$^{2+}$ (b) with Ag$^+$. 
**Fig. S11:** Binding constant calculation was calculated using fluorescence spectra of complex R1:Cu$^{2+}$

![Graph showing the binding constant calculation for R1:Cu$^{2+}$](image1)

**Fig. S12:** Binding constant calculation was calculated using fluorescence spectra of complex R1:Ag$^{+}$

![Graph showing the binding constant calculation for R1:Ag$^{+}$](image2)
Calculation of limit of detection (LOD):
The detection limit of R1 was calculated via fluorescence titration of R1:Cu$^{2+}$ and R1:Ag$^+$. The standard deviation was calculated by taking 10 different values of fluorescence intensity of chemosensor R1. The limit of detection (LOD) was calculated using the following equation.

$$\text{LOD} = K \times \text{SD}/S$$

Where, $K = 2$ or $3$ (we take 2 in this case); SD is the standard deviation (0.52) and S is the slope of the calibration curve.

From the linear fit graph slope of the complex R1:Cu$^{2+}$ is $1.2 \times 10^8$ and Complex R1:Ag$^+$ is $2.3 \times 10^7$. After calculation of LOD using the abovementioned formula, and of the values were found as $8.1 \times 10^{-9}$ M and $44.0 \times 10^{-9}$ M for Cu$^{2+}$ and Ag$^+$ ions, respectively.
Fig. S13: Limit of Detection (LOD) of R1 (a) against Cu²⁺ metal ions (b) against Ag⁺ ions.

Quantum yield calculation:

Fluorescence quantum yields (Φ) were calculated using the equation given below, using 1 quinoline sulfate (Φ₁ = 0.55 in water in 0.5 % H₂SO₄) as standard.

\[ \Phi_u = \Phi_s \frac{I_u}{I_s} \frac{A_s}{A_u} \left( \frac{\eta_u}{\eta_s} \right)^2 \]

Where, \( \Phi_u \) and \( \Phi_s \) are the fluorescence quantum yields of the sample and standard, \( I_u \) and \( I_s \) are the integrated emission intensities of the sample and standard, \( A_u \) and \( A_s \) are the absorbance of the sample and standard at the excitation wavelength (400 nm), and \( \eta_u \) and \( \eta_s \) are the refractive indices of the sample and standard solutions, respectively.

Bio-imagine analysis

The cell (U-2 OS) proliferation assay was carried out using different concentrations of the chemosensor R1 in presence or absence of Cu(ClO₄)₂ and AgNO₃ (10, 25, 50, and 100µM) for 48h. The cells were grown in the presence of R1 or Cu(ClO₄)₂ or AgNO₃ alone or in combination for 48h. Absence of any detectable loss in proliferation in U-2 OS cells indicates both probe and its metallation derivatives are tolerant to U-2 OS cell
growth in vitro. The cell proliferation remained unaffected by the compounds suggesting its cytocompatibility. Tumor cell proliferation reached >85% at a concentration 100µm indicating the compounds were safe for possible biological uses.

![Graphs showing proliferation and cytotoxicity](image)

**Fig. S14:** Proliferation of U-2 OS cells in presence of Cu$^{2+}$ (A) & Ag$^+$ (C) contained Probe R1. Graphs show Cu$^{+2}$ (B) & Ag$^+$ (D) contained R1 Probe on cytotoxicity of U-2 OS cells.

Direct cytotoxicity studies of the probe, Cu(ClO$_4$)$_2$ (Fig.S14B) or AgNO$_3$ (Fig.S14D) either alone or in combination against the U-2 OS cells were performed. The compounds are not cytotoxic to the cells at a concentration of 100µm. Percent cytotoxicity was less than 5% which is considered as non-significant. Taken together, these data suggest that the above compounds are safe and non-toxic to live cells (Fig. S14B & D).

The in vitro blood compatibility of the R1 was determined by % hemolysis & % viability of lymphocytes & monocytes. Metallation of the coumarin based receptor (R1) was also found to be tolerant to peripheral blood mononuclear cells (lymphocytes and monocytes), which constitutes the major fraction of the mononuclear white blood cells (WBC), comprising T, B, NK cells as well as monocytes and dendritic cells. Cell viability data suggests that like U-2 OS cells, PBMC was also unaffected by the compounds with minimum loss of cell viability at highest concentration (100µm), which was found to be
not significant (Fig. S15 A). Like PBMC, human RBC remains unaffected by R1. The hemolysis experiment has been conducted at different concentrations (10, 25, 50, & 100 µM) of the samples and the % hemolysis caused by the R1 probe was presented in (Fig. S15 B).

![Graph A: Viability of human lymphocytes & Monocytes](image1)

![Graph B: Hemolysis of RBC](image2)

**Fig. S15:** Viability of human lymphocytes & Monocytes (A) and hemolysis of RBC (B) in presence of R1

**References**