Quantitative evaluation and *in vivo* visualization of bioaccumulation of Hg$^{2+}$ into rotifer by novel aggregation-induced emission fluorogen nanoparticles

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Supporting information

1. Response of AIE to Hg $^{2+}$ in salt water

The photoluminescence (PL) intensity of AIE with Hg$^{2+}$ affected by the salinity, reaction time and concentrations of AIE and Hg$^{2+}$.

**Salinity effect:** The effect of PL intensity with different salinity in AIE and Hg$^{2+}$ reaction is shown in Fig. S1. With fixed AIE and Hg$^{2+}$ concentrations at 10 μM and at time elapse of 30 min, the fluorescence intensity decreased with increasing salinity from 0 to 35.

![Image](image_url)

Fig. S1. Photoluminescence (PL) intensity of Hg$^{2+}$ and AIE at the concentration of 10 μM at salinity of 0, 5, 10, 15, 20, 25, 30, 35 after 30 min.

**Time effect:** The effect of time elapse on PL intensity associated with AIE and Hg$^{2+}$ reaction is shown in Fig. S2. With fixed AIE and Hg$^{2+}$ concentrations at 10 μM and time elapse at 1, 5, 15, 30 and 45 min, the fluorescence intensity increased with reaction time during the initial 30 min, and reached a plateau at 30 min. A slight decrease was observed from 30 min to 45 min.
Fig. S2. Photoluminescence (PL) intensity of AIE concentration of 10 μM and Hg$^{2+}$ concentration of 10 μM at different time elapse of 1, 5, 15, 30, 60 and 90 min.

2. Response of rotifer to Hg$^{2+}$ in salt water
An acute toxic experiment was conducted to further determine the effect of Hg$^{2+}$ toxicity on rotifer B. plicatilis. Fig. S3 shows the survival rate of rotifer after 1-h incubation at different Hg$^{2+}$ concentrations, followed by 24-h recovery in clean water. After incubation with 1 μM Hg$^{2+}$, 85% rotifers survived, but rotifer survival rates reduced to 40.7% and 15.6% after Hg$^{2+}$ incubation at 2.5 μM and 5 μM, respectively (Fig. S4).

Fig. S3. Survival rate of rotifer after 1 h incubation at different Hg$^{2+}$ concentrations (1, 2.5, 5 μM) followed by recovery in clean water for 24 h.
3. AIEgen nanoparticles characterization

Fig. S5. Particles size of the nano-aggregated of 10 μM m-TPE-RNS in CH$_3$CN-water mixture with 60% water fraction measured by DLS [1].

4. Scanning confocal microscope configuration
5. Master curve for PL intensity with Hg\textsuperscript{2+} concentration

The effect of PL intensity with the function of Hg\textsuperscript{2+} concentration is shown in Fig. S7. With fixed AIE concentration at 10 μM and interaction time at 30 min, the fluorescence intensity increased with increasing the concentration of Hg\textsuperscript{2+} (1, 5, 10, 20 and 30 μM), and reached a plateau at 10 μM of Hg\textsuperscript{2+}. A linear relationship between fluorescence intensity and Hg\textsuperscript{2+} concentration was observed when the ratio of Hg\textsuperscript{2+} and AIE was less than 1.

![Figure S6: Scanning confocal microscope configuration for fluorescence intensity imaging of the samples. 100x objective is mounted onto the scanner and its position is shifted to scan the samples.](image)

![Figure S7: Photoluminescence (PL) intensity of Hg\textsuperscript{2+} (1, 5, 10, 20, 30 μM) at the AIE concentration of 10 μM at salinity 20 and time elapse time 30 min.](image)
8. Bioaccumulation of Hg$^{2+}$ at different rotifer densities

Fig. S8. Bioaccumulation of Hg$^{2+}$ (HgCl$_2$, 5 µM) at different rotifer densities (mg/mL) within 20 min. Lines from the linear curve fit with the equations presented.

9. Bioaccumulation of Hg$^{2+}$ at different rotifer densities

Fig. S9: Fluorescent image of Hg$^{2+}$ in rotifers using AIEgens with small magnification. Bar = 50 µm.
10. Detail FL spectrum obtained from the spectral analysis system

(a)

(b)

Fig. S10. The PL spectra of points corresponding to (a) Fig 8 (c) and (b) Fig 8 (d) in the main text.

References