Supporting information

Monitoring and quantification of the complex bioaccumulation process of mercury ion in algae by a novel aggregation-induced emission fluorogen

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Response of AIE to Hg²⁺

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

1. Time effect: The effect of PL intensity with the function of Hg^{2+} concentration is shown in Fig. S1. With fixed AIE and Hg^{2+} concentrations at 10 μ M and the reaction time varied at 1, 5, 15, 30, 60 and 90 min, the PL intensity increased with reaction time during the initial 30 min, and reached saturation at 30 min. A slight decrease was shown from 30 min to 90 min. So, 30 min was selected this study.



Fig. S1. PL intensity of AIE concentration of 10 μ M and Hg²⁺ concentration of 10 μ M at different time elapse of 1, 5, 15, 30, 60 and 90 min.

2. AIE concentration effect: The effect of PL intensity with the function of AIE concentration is shown in Fig. S2. With the fixed Hg²⁺ concentration at 10 and 20 μ M, respectively, and reaction time at 30 min, the fluorescence intensity increased with increasing the concentration of AIE (0.5, 1, 2.5, 5, 10, 20 and 30 μ M) and reached saturation as the ratio of AIE and Hg²⁺ was close to 1. A linear relationship between fluorescence intensity and AIE concentration was observed when the ratio of AIE and Hg²⁺ was less than 1, as shown in Fig. S2 (a) and (b).



Fig. S2. PL intensity of AIE (0.5, 1, 2.5, 5, 10, 20, 30 and 40 μ M) with Hg²⁺ of (a) 10 μ M and (b) 20 μ M, respectively, at the time elapse of 30 min. The lines in the plots show the linear fitting curves when the ratio of AIE and Hg²⁺ concentrations is less than 1.

3. Hg²⁺ concentration effect: The effect of PL intensity with the function of Hg²⁺concentration is shown in Fig. S3. With the fixed AIE concentration at 10 μ M and interaction time at 30 min, the fluorescence intensity increased with increasing the concentration of Hg²⁺ (1, 5, 10, 20 and 30 μ M), and reached saturation at 10 μ M Hg²⁺. A linear relationship between fluorescence intensity and Hg²⁺ concentration was

observed when the ratio of Hg^{2+} and AIE was less than 1.



Fig. S3. Photoluminescence (PL) intensity of Hg^{2+} (1, 5, 10, 20 and 30 μ M) at AIE concertation of 10 μ M and time elapse of 30 min. The line in the plot shows the linear fitting curve when the ratio of AIE and Hg^{2+} concentrations is less than 1.

Fluorescent images of the algae cell with Hg²⁺

4. Fluorescent images of the algae cell: The chlorophyll in the algae cell can be excited by 488 nm laser diode with a red emission peak at around 680 nm, as shown in Fig. S4. Fig. S4(a) shows the large unicellular organisms , up to 100 μ M in length, possess an eye spot photo-receptor, enabling Euglena to perceive light and swim towards it using its single flagellum for motility or by a unique so-called euglenoid locomotion [1]. Fig. S4(b) shows that the chlorophyll in the cell excited by 488 nm laser diode will having a red emission, as demonstrated in our studies as well.



Fig. S4: (a) Physiology of Euglena gracillis [1] and (b) the chlorophyll in the Euglena gracillis cell excited by 488 nm laser diode with a red emission.

5. Fluorescent images of the algae cell with different concentration of Hg²⁺: m-TPE-RNS shows intense emission around 480 nm before reacting with Hg²⁺ (without Hg²⁺ or with low concentration of Hg²⁺, such as 1 uM of Hg²⁺). The emitted 480 nm will serve as the excitation source for the chlorophyll in the algae cell for a red emission at 680 nm. With 1 uM of Hg²⁺ and AIE added in the algae solution, when the solution was excited at 355 nm, a high PL intensity still existed at 480 nm with mainly **blue color**, as shown in Fig. S5(a). Then, the emitted 480 nm again excites the chlorophyll in the Euglena gracillis cell with a red emission of 680 nm with mainly **red color**, as shown in Fig. S5(a). With the increase of Hg²⁺ concentration, the PL intensity at the emission of 480 nm decreases and finally disappears. So, with high concentration of Hg²⁺ inside, for example, 20 μ M Hg²⁺, as shown in Fig. S5(b), the 480 nm emission is disappeared. So, the chlorophyll in the Euglena gracillis cell does not have any excitation sources for emission. The whole cell only emits light around 595 nm with mainly orange and red colors, as shown in Fig. S5(b).



Fig. S5: The fluorescence images of m-TPE-RNS in algae cells with (a) 1 μ M Hg²⁺ and (b) 20 μ M Hg²⁺ for 2 hours. (a) shows red color in the chlorophyll and blue color in the rest of cell and (b) shows orange and red colors in the whole cell.

Reference

[1] O'Neill, E. C., Trick, M., Hill, L., Rejzek, M., Dusi, R. G., Hamilton, C. J., Zimba,
P. V., Henrissat, B., & Field, R. A. (2015). The transcriptome of Euglena gracilis reveals unexpected metabolic capabilities for carbohydrate and natural product biochemistry. Molecular BioSystems, 11(10), 2808-2820.