Electronic Supplementary Information (ESI)

Dual detection of bioaccumulated Hg^{2+} based on luminescent bacteria and aggregation-induced emission

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Table of Contents

Materials and Chemicals

The 2-(3-oxo-2, 3-dihydro-1*H*-inden-1-ylidene) malononitrile was prepared according to the literature.[1] 1,3-Indanedione was purchased from Acros; Malononitrile was purchased from Alfa-Aesar; Sodium acetate trihydrate, *N*, *N*, *N*'-trimethylethylenediamine, 4-(*N*, *N*-diphenylamino)benzaldehyde, iodomethane, $CoCl_2$, $CuCl_2$, $FeCl_3$, $MgCl_2$, and $AgNO_3$ were purchased from Energy Chemical; Toluene，$HgCl_2$ was purchased from Guangzhou Chemical Reagent Factory; $ZnCl_2$ was purchased from Alfa; $NaCl_2$ and $CaCl_2$ was purchased from Rechjoint Chemical; $PdCl_2$ was purchased from Zhejiang Metallurgical Research Institute Co., Ltd; Dimethyl sulfoxide, Methanol, Nickel(II) perchlorate hexahydrate, $PbCl_2$, $CdCl_2$ and $MnCl_2$ were purchased from Aladdin; Etanol, $CDCl_3$ and d_6-DMSO were purchased from Sigma-Aldrich; Pyridine was purchased from J&K; THF was distilled from sodium under dry nitrogen prior to use; Ultra pure water was supplied by Milli-Q Plus System (Millipore Corporation, United States); The *P. Phosphoreum* was purchased from ShenZhen HuaJu Scientific Instrument Co., LTD.

Equipment and Methods

UV-Vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. The fluorescence lifetime was measured using a Hamamatsu Compact Fluorescence Lifetime Spectrometer C11367. The absolute fluorescence quantum yield was measured using a Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (LSM 710, Zeiss, Germany). Fluorescence images were obtained on the fluorescence optical microscope (Zeiss Axio Vert. A1). The bioluminescence of *P. phosphoreum* was measured on BioTech Cytation 5. The OD_{600} was measured on a Shimadzu UV-2600 spectrophotometer and a microplate reader (Tecan Infinite M200 PRO). ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Bruker AV 500 NMR spectrometer. High resolution mass spectra (HRMS) were recorded on a GCT Premier CAB048 mass spectrometer operated in MALDI-TOF model. The reflection data for single crystals were collected at room temperature on a Gemini A Ultra diffractometer with graphite monochromatized Mo Kα radiation (λ = 0.71073 Å). Size was measured on Dynamic light scattering (ZSE,

Malvern.

Synthesis of 2-AFN

4-(diphenylamino) benzaldehyde (273 mg, 1.0 mmol), 2-(3-oxo-2, 3-dihydro-1*H*-inden-1-ylidene) malononitrile (194 mg, 1.0 mmol) and *N, N, N'*-trimethylethylenediamine (402 μL, 4.0 mmol) was dissolved in MeCN (10 mL). The mixture was stirred at 60 ºC for 12 h. After cooling down to room temperature, the red solid was filtered off, washed with MeCN, and dried under reduced pressure, 2-AFN was obtained. (247 mg, yield 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.45 (d, J = 7.5 Hz, 1H), 7.89 (d, J = 9.0 Hz, 2H), 7.71 (d, J = 7.0 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.30 (t, J = 8.5 Hz, 4H), 7.20 (d, J = 7.5 Hz,4H), 7.11-7.05 (m, 4H), 4.00 (d, J = 6.0 Hz, 2H), 3.56 (s, 3H), 2.71 (s, 2H), 2.33 (s, 6H); 13C NMR ($CDCl_3$, 125 MHz): δ 188.1, 161.1, 159.5, 158.5, 150.3, 147.0, 138.4, 135.7, 134.0, 132.1, 131.4, 129.5, 129.1, 125.6, 124.0, 123.7, 123.4, 120.2, 118.0, 114.8, 81.8, 56.6, 50.6, 45.6, 39.9, 30.3. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{36}H_{32}N_5O$, 550.2607; found, 550.2605.

Synthesis of 2-AFN-I

2-AFN (275 mg, 0.5 mmol) was dissolved in THF (20 mL). Following, CH_3I (62 μL, 1.0 mmol) was added under the condition of ice bath. The reaction mixture was stirred for 10 h. When yellow solid was precipitated, the reaction mixture was quenched with pyridine. The resulting solid were filtered off, washed with THF and dried under reduced pressure. Finally, 2-AFN-I was obtained (304 mg, yield 88%). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.37 (d, J = 9.0 Hz, 1H), 7.87 (d, J = 10 Hz, 2H), 7.79 (d, J = 9.0 Hz, 1H), 7.70 (s, 2H), 7.40 (t, J_1 = 18 Hz, J_2 = 9.0 Hz, 4H), 7.16 (t, J_1 = 24 Hz, J_2 = 8.5 Hz, 6H), 6.94 (d, J = 10.0 Hz, 2H), 4.25 (s, 2H), 3.59 (s, 5H), 3.15 (s, 9H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 187.0, 160.0, 158.8, 157.6, 149.7, 146.3, 137.6, 134.9, 134.6, 132.8, 131.6, 129.8, 128.4, 125.4, 124.4, 123.7, 122.8, 119.1, 117.3, 114.5, 82.4, 67.0, 61.0, 52.7, 45.8. HRMS (ESI): m/z $[M - I]^+$ calcd for $C_{37}H_{34}N_5O$, 564.2758; found, 564.2757.

Bacteria culture

P. phosphoreum was cultured under sterile conditions in Petri dishes, containing agar for stock cultures. The dishes were incubated for 20 h at 18 ± 1 ºC, and the luminescent single colonies were then selected by means of visual observation in the dark. An aliquot of 5 mL of aqueous medium was inoculated with one single colony and after shaking at 200 rpm for 20 h at 18 ± 1 ºC, the bacteria were harvested by centrifuging

(7100 rpm for 5 min). After the removal of the suspension, 3% NaCl aqueous solution was added to the EP tube. The optical density of the suspension was determined by spectrophotometric assay at 600 nm and a stock suspension (1.5 absorbance units equivalent to around 4.0×10^7 CFU mL^{-1}) was prepared.

Bioluminescence assay

The bacteria were cultured in test tubes with shaking (200 rpm) at 18 ± 1 ºC for 20 h. Then, the bacteria were harvested by centrifuging (7100 rpm for 5 min). Standard solutions of chlorides of heavy metals prepared in aqueous solution were added to bacteria suspension placed in a 96-well plates, and the bacterial luminescence was measured and recorded after 10 min.

Photoluminescence assay

The bacteria were cultured in test tubes with shaking (200 rpm) at 18 ± 1 ºC for 20 h. Then, the bacteria were harvested by centrifuging (7100 rpm for 5 min). After the removal of the suspension, 980 μL of 3% NaCl aqueous solution was added to the EP tube. After being dispersed, followed by the addition of 10 μL 2-AFN-I and different concentrations of $HgCl_2$ to the EP tube, 100 μL of the suspension was dispersed in 96-well plates. The detection of Hg^{2+} by 2-AFN-I was conducted using a microplate reader (Tecan Infinite M200 PRO) at a wavelength of 590 nm.

Photoluminescence imaging

The bacteria were cultured in test tubes with shaking (200 rpm) at 18 ± 1 ºC for 20 h. Then, the bacteria were harvested by centrifuging (7100 rpm for 5 min). After the removal of the suspension, 980 μL of 3% NaCl aqueous solution was added to the EP tube. After being dispersed, followed by the addition of 10 μL $HgCl_2$ and 10 μL 2-AFN-I to the EP tube, the bacteria were incubated at room temperature for desired time intervals, and then 2 μL of the bacteria solution were added on the glass slide and covered by a coverslip. The confocal lasing scanning microscopy (CLSM) images were obtained on the confocal microscope (Zeiss 710). λ_{ex} = 405 nm, λ_{em} = 520−650 nm.

Bacterial growth test

Bacteria suspensions (~4×10^7 CFU mL^{-1}) treated with different concentrations of 2-AFN-I and $HgCl_2$ were seeded in 96-well plates and cultured for 24 h on a shaking incubator (200 rpm) at 18 ºC. During the culture process, the optical density at 600 nm (OD_{600}) of each well was measured at different times.

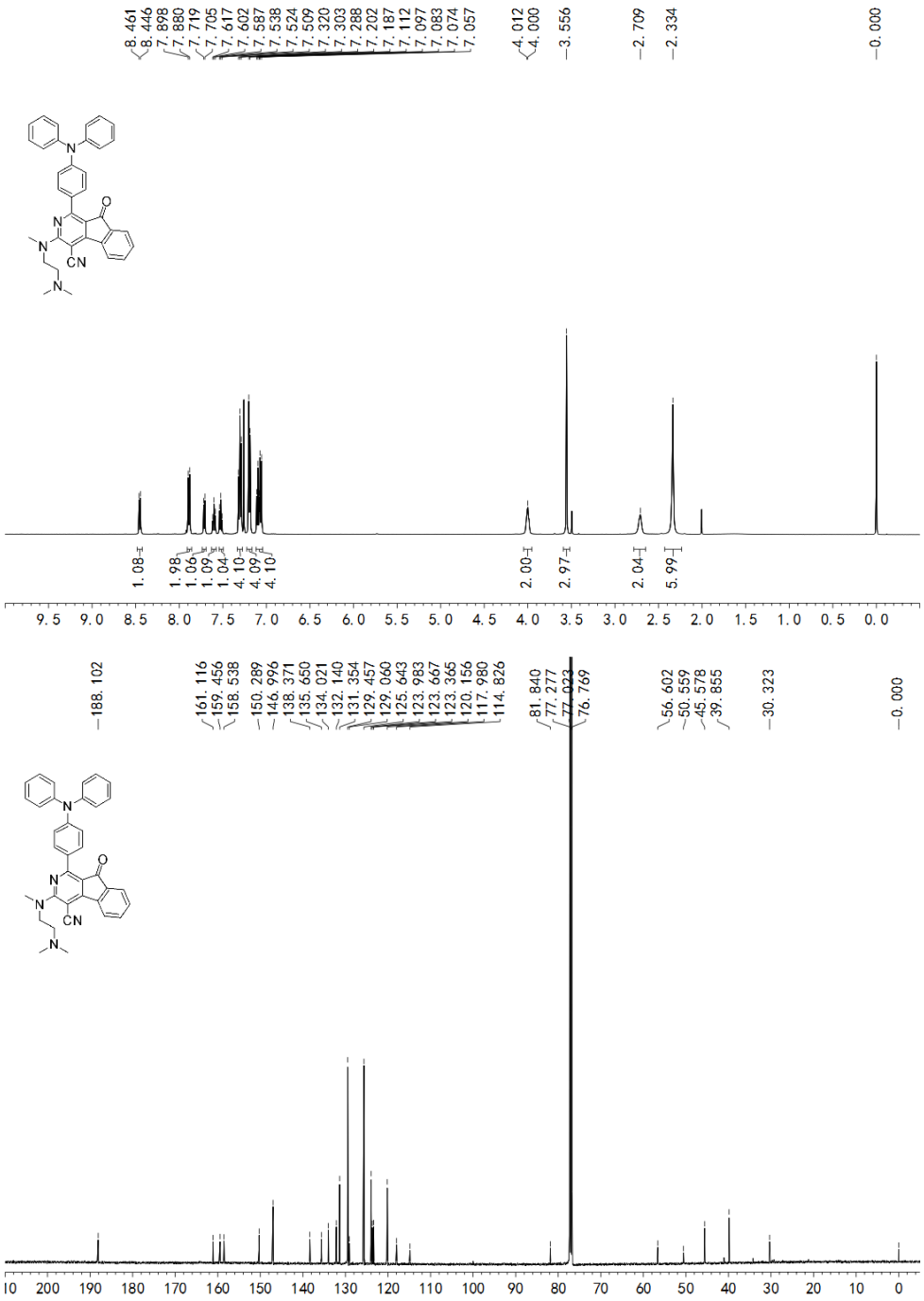

Fig. S1 ^{1}H and ^{13}C NMR spectra of 2-AFN in $CDCl_3$.

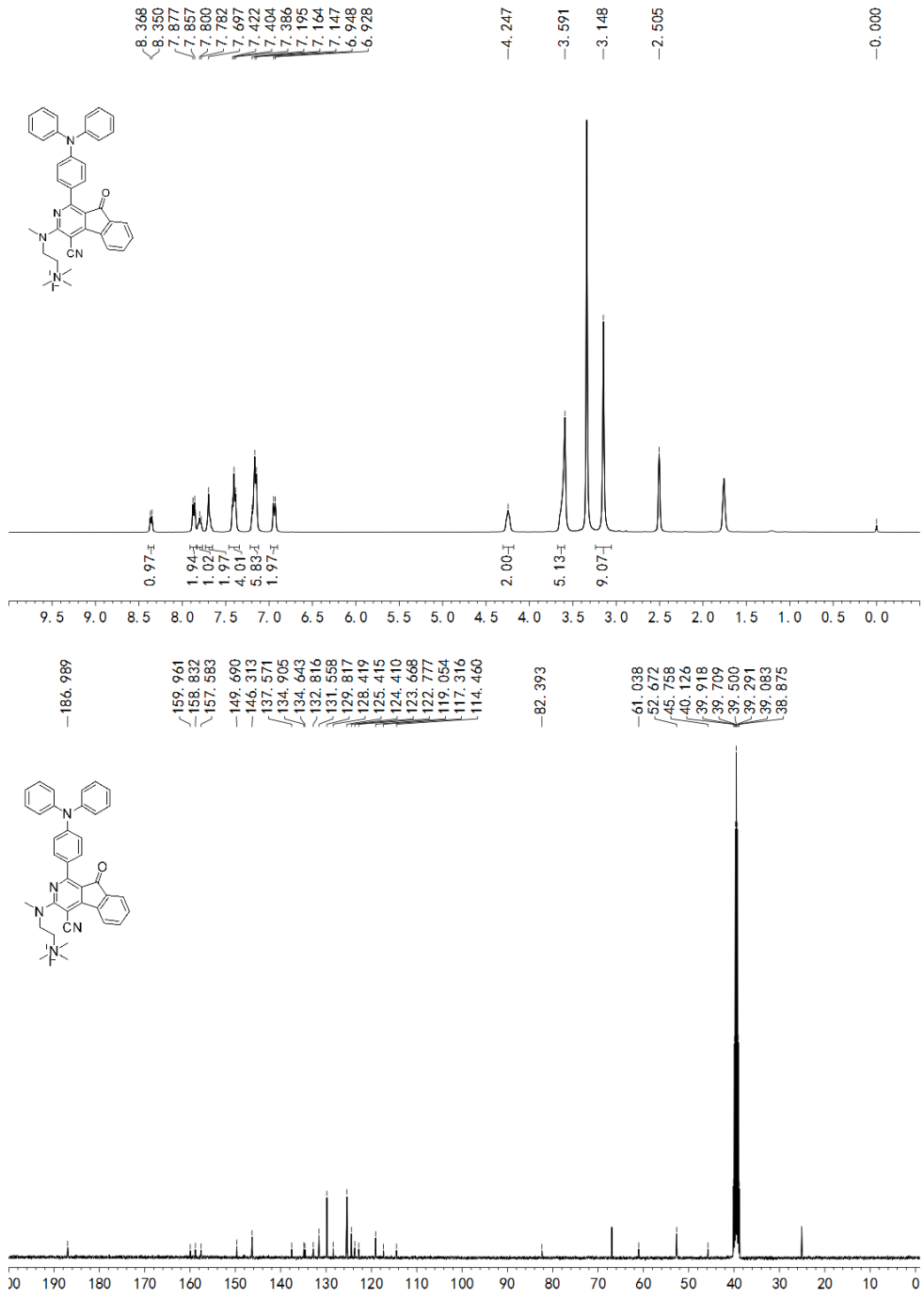

Fig. S2 The ^{1}H and ^{13}C NMR of 2-AFN-I in d_6-DMSO.

Table S1. Photophysical properties of compound 2-AFN, 2-AFN-I and 2-AFN-$HgCl_2I$ in solution (Soln) and solid (Solid) states.

	Soln[a]					
	λ_{ab} [nm][c]	λ_{em} [nm][d]	Φ_f [%][e]	τ (ns)[f]	k_r [10^6 s^{-1}][g]	k_{nr} [10^8 s^{-1}][h]
2-AFN	415	616	1.7	1.04	16.35	9.45
2-AFN-I	413	575	0.5	2.39	2.09	4.16
2-AFN-$HgCl_2I$	403	586	0.9	2.88	3.13	3.44

	Solid[b]					
	λ_{ab} [nm][c]	λ_{em} [nm][d]	Φ_f [%][e]	τ (ns)[f]	k_r [10^6 s^{-1}][g]	k_{nr} [10^8 s^{-1}][h]
2-AFN	407	579	4.8	4.12	11.65	2.31
2-AFN-I	419	615	3.0	6.24	4.81	1.55
2-AFN-$HgCl_2I$	415	595	5.0	8.03	6.23	1.18

a) 2-AFN in THF solution with a concentration of 10^{-5} M; 2-AFN-I and 2-AFN-$HgCl_2I$ in aqueous solution with a concentration of 10^{-5} M; b) Thin solid film; c) Maximum absorption wavelength; d) Maximum emission wavelength; e) Absolute quantum yield; f) Average fluorescence lifetime; g) Radiative relaxation rate $k_r = \Phi/\tau$; h) Non-radiative relaxation rate $k_{nr} = (1-\Phi)/\tau$.

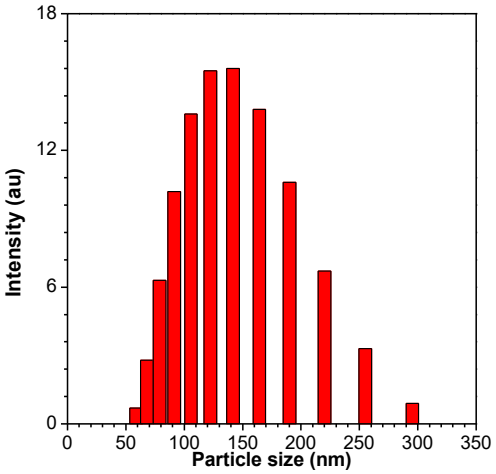

Fig. S3 Particle size distribution of 2-AFN in THF/water mixture (1:99, v/v) measured by dynamic light scattering with a mean diameter of 129.9 nm and a PDI of 0.149.

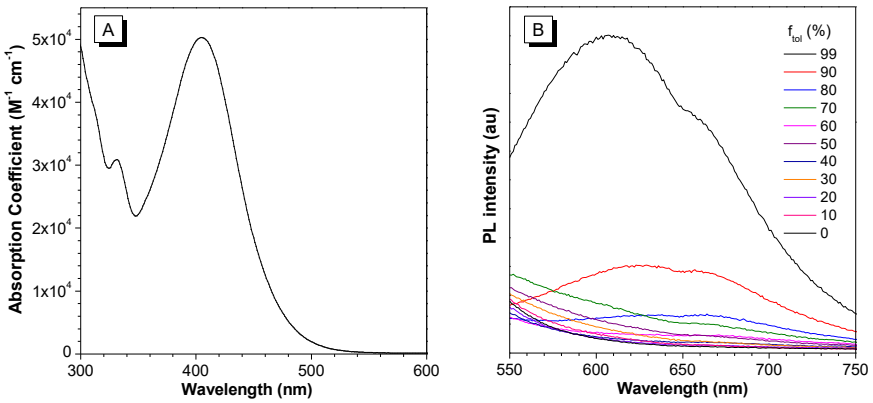

Fig. S4 (A) The UV−Vis absorption spectra of 2-AFN-I in DMSO solution and (B) the PL spectra of 2-AFN-I in DMSO/toluene mixtures with different toluene fractions (f_{tol}). [2-AFN-I] = 10 μM; λ_{ex} = 404 nm.

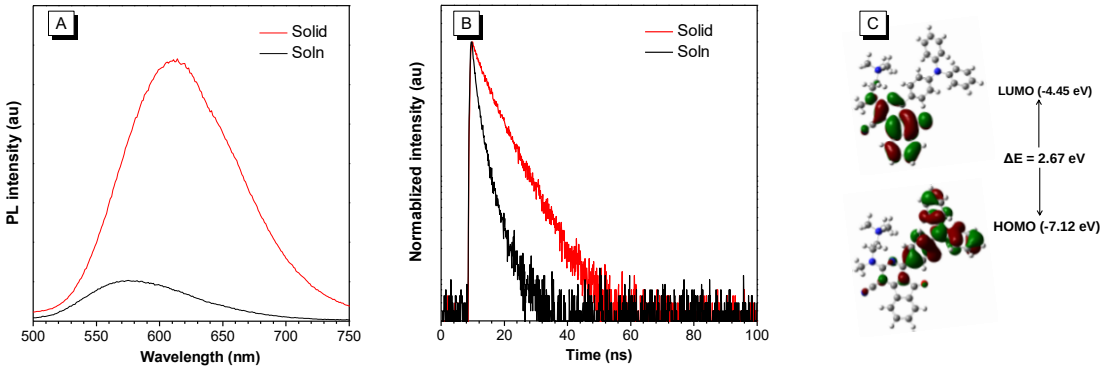

Fig. S5 (A) PL spectra of 2-AFN-I in the solid (Solid) and in aqueous solution (Soln) state. (B) Fluorescence decay spectra of 2-AFN-I in solid (Solid) and aqueous solution (Soln) states. [2-AFN-I] = 10 μM. (C) Molecular orbital amplitude plots of HOMO and LUMO of 2-AFN-I (the iodide ion was omitted).

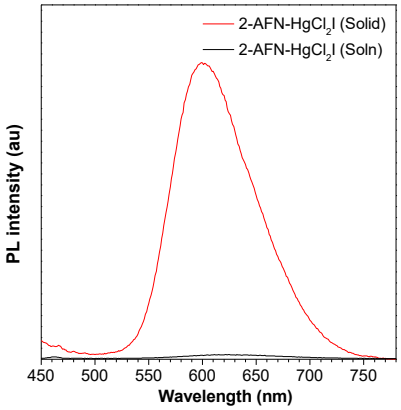

Fig. S6 (A) The PL spectra of 2-AFN-$HgCl_2I$ in the solid (Solid) and in aqueous solution (Soln) state. [2-AFN-$HgCl_2I$] = 10 μM; λ_{ex} = 415 nm.

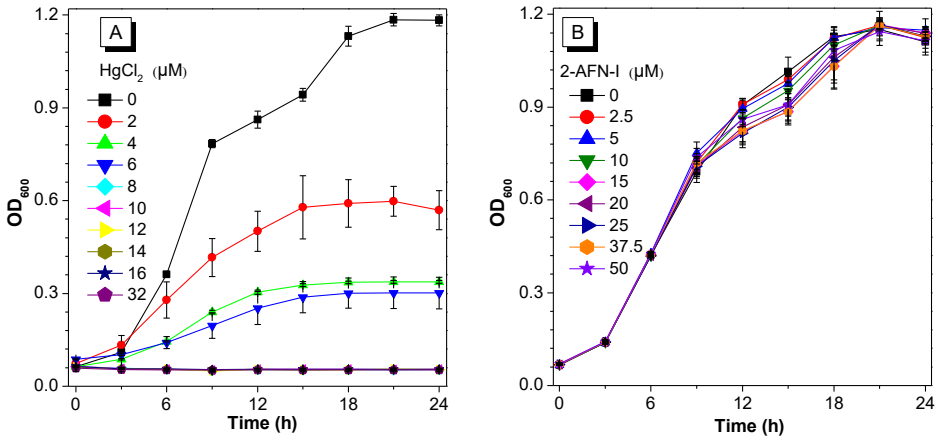

Fig. S7 The growth curves of *P. Phosphoreum* incubated with different concentrations of (A) $HgCl_2$ and (B) 2-AFN-I for 24 hours.

References

1. Y. Shang, Y. Wen, S. Li, S. Du, X. He, L. Cai, Y. Li, L. Yang, H. Gao and Y. Song, *J. Am. Chem. Soc.*, 2007, **129**, 11674-11675.