Supplementary Information for:

Self-assembling nanoprobes that display twodimentional fluorescent signals for identification of surfactants and bacteria

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1. Materials, Instruments and Synthesis

1.1. Materials

Compound **PI-1** was prepared by the established literature procedure^[1]. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. *E. coli DH5a* strains were purchased from invitrogen. Standard strains of *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 10145), *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 12228), *Enterococcus faecalis* (*E. faecalis*, ATCC 29212), *Staphylococcus aureus* (*S. aureus*, ATCC 25923), *and Bacillus cereus* (*B. cereus*, 1.1626) were purchased from China General Microbiological Culture Collection Center (CGMCC). *Agrobacterium rhizogenes* (*A. rhizogenes*, ATCC 15834), *Citrobacter freundii* (*C. freundii*, ATCC 8090), and *Bacillus subtilis* (*B. subtilis*, ATCC 6633) were purchased from American type culture collection. All water used was from a Millipore water purification system with a minimum resistivity of 18.0 M Ω · cm.

1.2. Instruments

¹H-NMR spectra was recorded on Bruker 400 spectrometer with Chemical shifts reported in ppm and coupling constants (J) reported in Hz. UV-vis absorption spectra were obtained on an Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer equipped with a Single Cell Peltier temperature controller. The fluorescence imaging was performed by using OLYMPUS IX73 fluorescence microscope with UV excitation (320-350 nm filter). Particle sizes were measured on a Malvern Zetasizer Nano Series.

1.3. References

1. H. N. Kim, E. H. Lee, Z. C. Xu, H. E. Kim, H. S. Lee, J. H. Lee, J. Yoon, *Biomaterials*. 2012, 33, 2282.

2. Experimental Procedures

2.1 Detection of surfactants

A solution of **PI-1** stock in DMSO (2.0 mM, 5 μ L) was added into 0.995 mL of HEPES (20 mM, pH = 7.4) containing different concentrations of SDS, SDBS, TX-100, BS-12, and DTAB, repectively. After vortex shaking for 1 min, the resultant fluorescence was measured on a spectrofluorometer ($\lambda_{ex} = 345$ nm).

2.2 Bacterial culture and staining

The 9 kinds of bacteria were cultured in LB liquid medium (5 mL) at 37 °C until OD₆₀₀ is about 1.0. The bacterial cells were collected by centrifuging (12000 rpm, 3 min) and washed with PBS buffer (10 mM, pH 7.2) twice. Then the bacterial pellets were suspended in PBS solution to an absorbance of 0.5 at 600 nm. We mixed the bacteria (995 μ L) with probe solution (5 μ L) and put the mixture at room temperature for 1 min for the fluorescent spectra measured ($\lambda_{ex} = 345$ nm). Five repeated experiments were carried out for each bacteria incubated with the probe.

2.3 Monitoring of bacterial growth curve

The bacterium (*E. coli DH5a* or *B. subtilis*) was cultured at 37 °C overnight. And then 1mL culture was transferred in LB medium (100 mL) and shook at 37 °C. 2 mL *E. coli DH5a* solution was collected at different time (0 min, 70 min, 110 min, 170 min, 210 min, 250 min, and 280 min), and 2 mL *B. subtilis* solution was collected at different time (0 min, 70 min, 110 min, 140 min, 170 min, and 210 min). The optical density of 1 mL bacterial collection of different time were tested at 600 nm, repectively. And another 1mL bacterial collection was centrifuged at 12000 rpm for 3 min. The bacterial pellets were washed with PBS buffer (10 mM, pH 7.2) once and then suspended in 1 mL PBS solution. After mixing with **PI-1** (10 μ M) and stay for 1 min, fluorescent spectra were measured with $\lambda_{ex} = 345$ nm.

3. Supplementary Figures



Fig. S1 Absorption spectral of PI-1 (10 μ M) in DMSO or in HEPES (20 mM, pH = 7.4).



Fig. S2 Absorption spectral analysis of **PI-1** (10 μ M) upon the addition of SDBS, SDS, BS-12, TX-100 and DTAB, respectively. [surfactants] = 20 mM.



Fig. S3 Fluorescence spectra of **PI-1** (10 μ M) incubated with *E. coli DH5a* (a) and *B. subtilis* (b) at different time.



Fig. S4 ¹H-NMR of PI-1 in DMSO_{d6}.

Table S1 Details of bacteria used in this study

No.	Name of bacteria	Gram
1	Bacillus subtilis	positive
2	Staphyloccocus aureus Rosenbach	positive
3	Enterococcus faecalis	positive
4	Bacillus cereus	positive
5	Staphylococcus epidermidis	positive
6	Pseudomonas aeruginosa	negative
7	Agrobacterium rhizogenes	negative
8	Citrobacter freundii	negative
9	Escherichia coli DH5α	negative