

Supporting Information

Sulfonate-Functionalized Tetraphenylethylenes for Selective Detection and Wash-Free Imaging of Gram-positive bacteria (*Staphylococcus aureus*)

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1. Chemicals and Reagents

4-Hydroxybenzophenone and benzophenone were purchased from Sigma Aldrich (India). TiCl_4 was purchased from Spectrochem Pvt. Ltd. Mumbai (India). All other common chemicals and solvents of AR grade were obtained from different commercial suppliers and were used without further purification. All ultrapure water used was collected from Millipore water system and purged with N_2 for 15 min prior to use.

2. Instruments and Measurements

NMR spectra were recorded on Bruker Avance (300 or 400 MHz) NMR spectrometer. Mass spectra were obtained from Agilent 6400B LC-MS (ESI). HRMS spectra were recorded on Q-TOF LC-MS (6545 Q-TOF LC-MS, Agilent) using ESI as ion source. Fluorescence spectra were taken on a JASCO FP-6300 spectrofluorimeter; the slit width was 2.5 nm for both excitation and emission. UV-Visible spectra were taken on a JASCO V-550, the bandwidth and data pitch were set as 1 nm. Field emission scanning electron microscopy (FESEM) images were recorded on a Quanta 250 FEG (FEI make). Optical micrographs were carried out on inverted microscope, IX-51 (Olympus) with fluorescence attachment. SHIMADZU UV-2450 UV-Visible spectrophotometer (SHIMADZU Corp., Japan) was used for finding optical density of bacteria.

3. Materials & Methods

a. General procedure

THF was dried over sodium and freshly distilled before use. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates (60F-254) using UV light (254 or 365 nm) for visualization. Stock solutions of probe **1** and probe **2** (1 mM) were prepared in THF and water, respectively. Deionized water (Milli-Q, 18 M Ω) was used as per requirements for dilution purpose.

b. Bacterial sampling

Five cultures of different classes of bacteria, namely *S. aureus* RN4220, *B. subtilis* MTCC441, *E. coli* DH5 α , *P. aeruginosa* MTCC741, *M. smegmatis* MC2 155 were selected for the study. These cultures were routinely grown on Tryptic Soy Broth (Becton-Dickinson and Company, USA) for *S. aureus* and *B. subtilis*, Luria Bertani broth (Hi-Media, India) for *E. coli* and *P. aeruginosa*, and Middlebrook 7H9 broth base (Hi-Media, India) for *M. smegmatis*, respectively. Agar was added as per requirement of routine culture maintenance. At OD₆₀₀ ~0.5 of the grown cells were harvested. OD of bacterial cultures were measured by UV-Visible spectrophotometer and the colony-forming unit (CFU) was obtained by McFarland standards.¹ The sample 1 was half-diluted to obtain bacterial samples at lower concentrations.

Table S1. Bacterial samples, corresponding OD value at 600 nm, and approximate CFUs for *S. aureus*.

Sample No.	1	2	3	4	5
McFarland Standard No.	0.5	1	2	3	4
Absorbance at 600 nm	0.08-0.1	0.257	0.451	0.582	0.669
Cell density (10^8 CFU/mL) approx.	1.5	3.0	6.0	9.0	12.0

c.

Procedure for fluorescence and UV-Vis measurements

1 mM stock solution of probe **1** and probe **2** were freshly prepared before each experiment. The grown bacterial cells were harvested by centrifuging at 4 °C, washed twice with sterilized phosphate-buffered saline (1X PBS, pH 7.4) solution and used for fluorescence and UV-Vis measurements. The solutions prepared were incubated for 15 min before recording the fluorescence and UV-Vis spectra. For fluorescence measurements the excitation wavelength was set to 345 nm and emission spectra were recorded in the range of 346 to 650 nm. UV-Vis spectra were recorded from 200-800 nm. All the experiments were performed at room temperature.

e. Cell imaging with phase-contrast and fluorescence microscopy

Cells from overnight grown culture were used to inoculate the secondary culture media and finally the young grown cells were harvested. At $OD_{600} \sim 0.5$ of the grown cells were harvested by centrifugation at 4 °C and washed twice with sterilized PBS solution. The washed cells were suspended in an appropriate volume of sterile PBS. Different bacterial sets were prepared with and without probe **2**, incubated for 15 min at room temperature and directly viewed under an Olympus IX51 inverted microscope, combining the phase-contrast system and the fluorescence system.

f. Method for growth kinetics study for *S. aureus*

S. aureus RN4220 strain was allowed to grow in Tryptic Soy Broth (TSB), overnight at 37 °C with shaking at 120 rpm. This culture was used for inoculating (1% inocula) three conical flasks having 100 ml TSB (for control, kanamycin and probe **2**) each, to an OD_{600} of ~ 0.05 . Each of the three cultures were allowed to grow at 37 °C with shaking (at 120 rpm) and OD_{600} of the cultures were measured after every 30 min. At 2 h, one set of culture was treated with kanamycin (10X concentration of MIC i.e. 19.5 $\mu\text{g/mL}$) and another set was treated with probe **2** (10X concentration of MIC i.e. 234.3 $\mu\text{g/mL}$) and OD_{600} of the cultures were measured every 30 min for 8 h. The third set served as control.

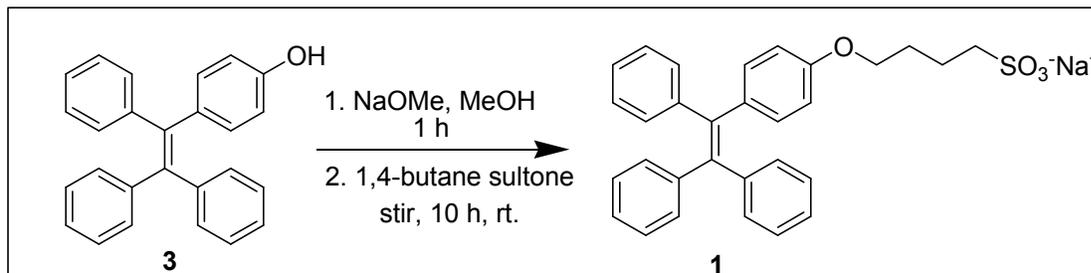
g. SEM analysis

For analysis of samples by SEM, the cultures (the control and the one treated with probe **2**) were taken at 2 h after the treatment with probe **2**, drop casted on a carbon wafer and analysis was carried out.

4. Synthesis

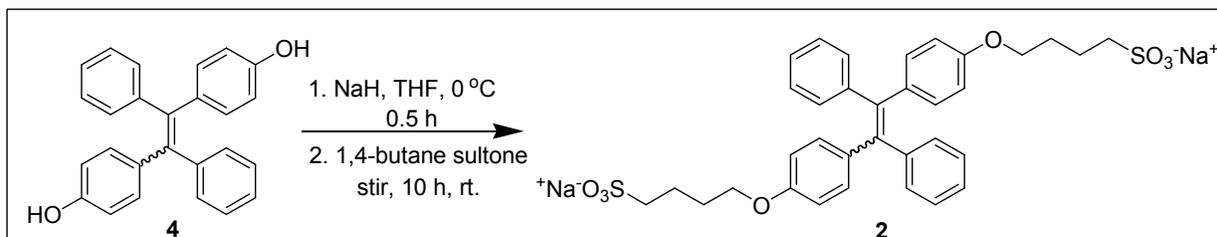
a. 4-(1,2,2-Triphenylvinyl)phenol (**3**) and (*E/Z*)-1,2-bis-(4-hydroxyphenyl)-1,2-diphenylethylene (**4**) were synthesized using a reported procedure of McMurry coupling.²

b. Synthesis of sodium 4-(4-(1,2,2-triphenylvinyl)phenoxy)butane-1-sulfonate (TPE-SO₃⁻, **1**)³



In a 100 mL round-bottom flask were taken 350 mg (1.0 mmol) of **3** in 5 mL of anhydrous methanol under nitrogen. The mixture was stirred until all solids disappear. A solution of NaOMe (81 mg, 1.5 mmol) in 5 mL of methanol was added dropwise and stirred for 1 h, causing the colorless solution to turn pink-red. Into the solution was added 1,4-butanediol sulfonate (204 mg, 1.5 mmol) in 2 mL of methanol. The mixture was vigorously stirred for 8 h, during which time product was precipitated out from the solution. The product was collected by filtration and washed with methanol and acetone twice to afford a white solid. The crude product was purified by Sephadex column chromatography (eluent: 1:1 methanol-water) to afford probe **1** (350 mg, 70% yield). ¹H NMR (CDCl₃, 300 MHz), δ (TMS, ppm): 1.55-1.86 (m, 4H), 2.8 (t, 2H, *J* = 5.6 Hz), 3.47 (m, 2H), 6.42 (d, 2H, *J* = 8.0 Hz), 6.78-7.01 (m, 17H); ¹³C NMR (CDCl₃, 75 MHz), δ (TMS, ppm): 21.4, 28.3, 50.9, 67.2, 113.6, 126.27, 126.35, 127.6, 127.7, 131.3, 132.5, 136.1, 140.1, 140.4, 143.8, 157.2; ESI-MS (-ve mode): *m/z* 483 (M-Na⁺); HRMS (ESI, -ve mode): calculated for C₃₀H₂₈O₄S⁻ [M]: 483.1636, found 483.1632.

c. Synthesis of TPE-di-sulfonate (probe **2**)



In a 25 mL round-bottom flask, NaH (44 mg, 1.1 mmol, 60% in oil) was suspended in dry THF (3 mL) under nitrogen atmosphere and a solution of compound **4** (182 mg, 0.5 mmol) in dry THF (2 mL) was added in ice cold condition, and the mixture was stirred for 30 min during which the colorless solution turned yellowish. To this reaction mixture, 1,4-butane sultone (164 mg, 1.2 mmol) was added and stirred vigorously for 10 h at room temperature. The completion of reaction was monitored by TLC. The reaction was quenched with small amount of ice-chips. The

reaction mixture was concentrated in vacuum to afford crude product. It was first triturated with diethyl ether (2 x 5 mL), the product was taken up in EtOAc (10 mL) and the inorganic residue was discarded. The product was washed with chilled brine solution (2 mL), dried over anhydrous Na₂SO₄ and concentrated to afford probe **2** as pale yellow solid (221 mg, 65% yield).

¹H NMR (D₂O, 400 MHz), δ (TMS, ppm): 1.35-1.53 (m, 8H), 2.25-2.52 (m, 4H), 2.84-3.01 (m, 4H), 6.15-6.305 (m, 4H), 6.75-7.02 (m, 14H); ¹³C NMR (*d*₆-DMSO, 100 MHz), δ (TMS, ppm): 19.04, 25.50, 52.26, 65.43, 111.73, 112.37, 126.15, 127.30, 129.05, 130.30, 141.85, 154.15, 154.87; ESI-MS (-ve mode): *m/z* (*Z* = 2) 317.100 (M-Na⁺)/2; HRMS (ESI, -ve mode): calculated for C₃₄H₃₄O₈S₂²⁻ [M/2]²⁻: 317.0853, found 317.0855.

5. Fluorescence response of probe **1** upon treatment of *S. aureus*

Probe **1** was found to be sparingly soluble in water and its AIE characteristic was determined by carrying out fluorescence studies in variable ratio of water-THF solvent system. It was found that probe **1** starts fluorescing at and above 95% H₂O-THF solvent system and shows strong fluorescence at 95% H₂O-THF due to its AIE property. Hence 94% water-THF was selected for bacterial detection study.

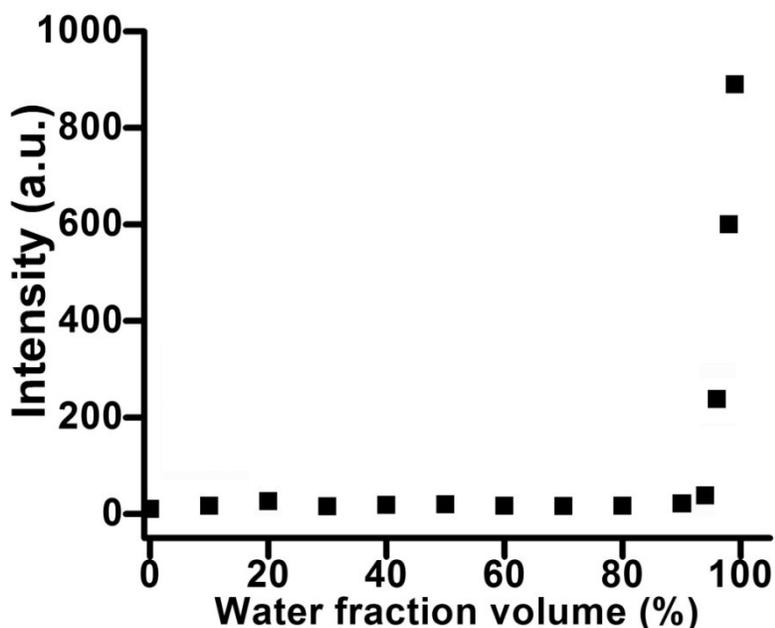


Figure S1. A plot of fluorescence intensity of probe **1** (30 μM) against variable proportions of H₂O-THF mixture (λ_{ex} 345 nm, λ_{em} 460 nm). The plot revealed that probe **1** starts showing strong AIE property at and above 95% H₂O-THF solvent system.

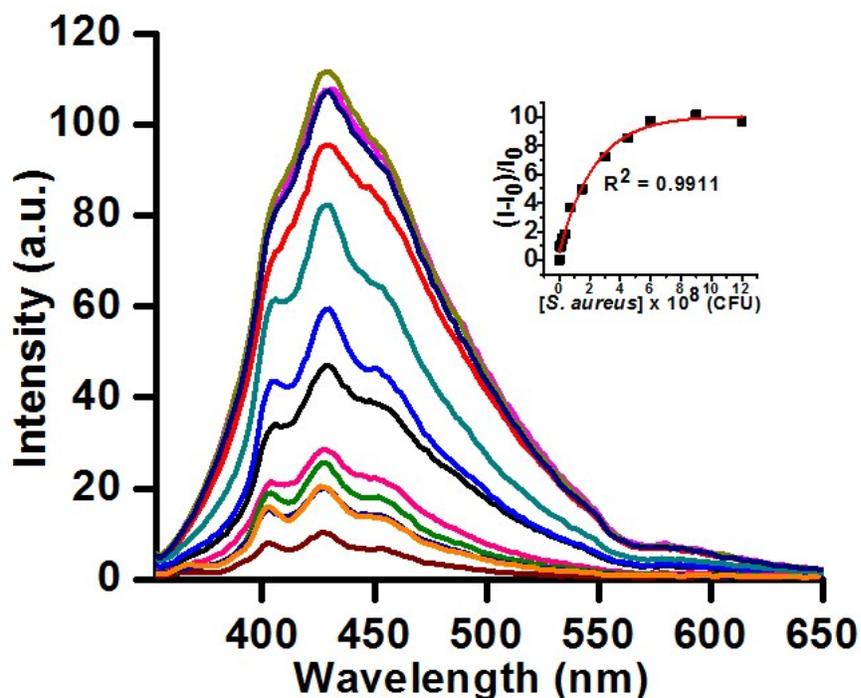


Figure S2. Fluorescence response of probe 1 (30 μM), upon addition of increasing concentration of *S. aureus* in 94% H_2O -THF system [λ_{ex} 345nm; λ_{em} 428 nm]. Inset is a plot of increment in fluorescence intensity against the increasing concentration of bacteria.

6. Solubility profile of probe 2

Probe 2 was tested for solubility in water and up to 22 mg per mL was found to be completely soluble in 1 mL water (which equals to 30 mM).

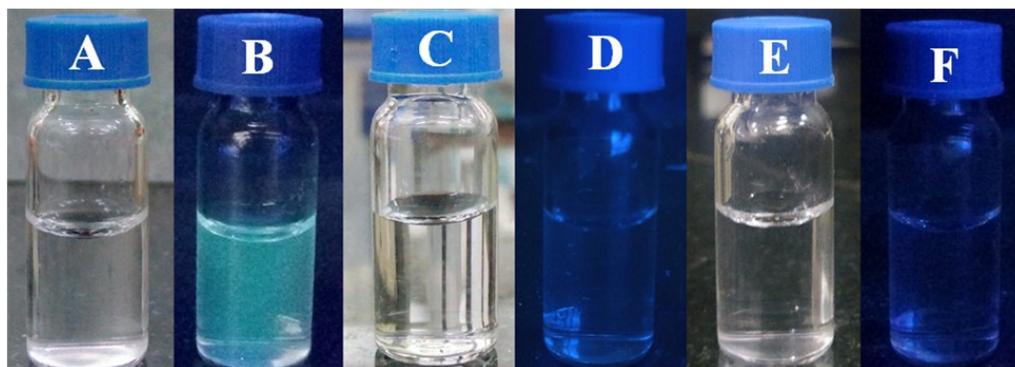


Figure S3. Solutions of probe 2 [30 mM (A & B), 1 mM (C & D) and 30 μM (working solution - E & F) at room light and UV light, respectively].

7. LOD of probe 2 in the detection of *S. aureus*

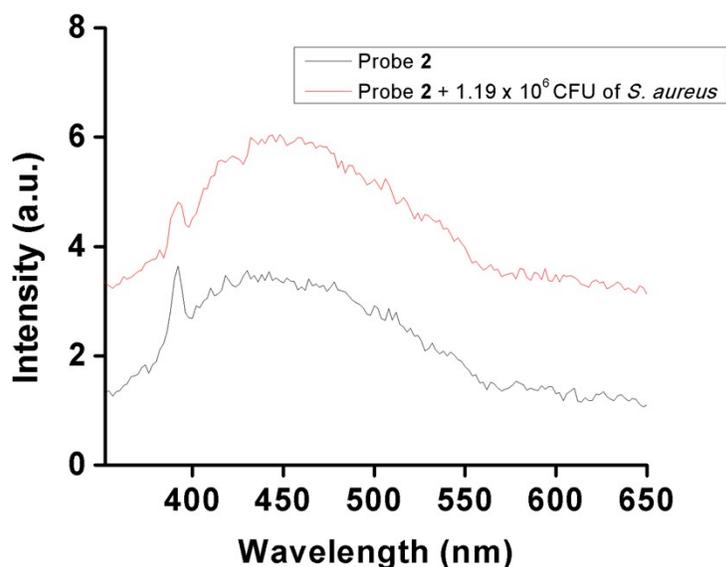


Figure S4. Fluorescence spectra of probe **2** upon addition of *S. aureus* (1.19×10^6 CFU), showing a signal-to-background ratio more than three. From this the LOD of probe **2** for the detection of *S. aureus* was estimated as 1.19×10^6 CFU.

8. UV-Vis response of probe 2 upon treatment of *S. aureus*

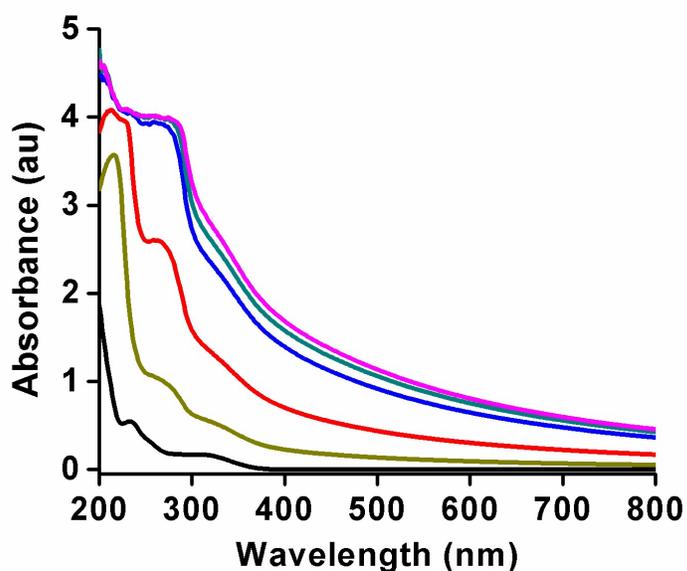


Figure S5. (A) UV-Vis response of probe **2** (30 μ M) upon addition of increasing concentration of *S. aureus*.

9. Graph for optical density and relative intensity against the bacterial samples (*S. aureus*)

The increase in fluorescence intensities with the number of bacterial colonies gives the same trend as obtained from OD₆₀₀ values using standard UV-Vis methods (Figure S6). This indicates that the present fluorimetric technique can also be used in quantifying bacterial colony (for Gram-positive bacteria). In particular, fluorimetric technique allows to detect and quantify bacteria at a much lower concentration wherein UV-Vis methods fail to produce any stable data because of its poor sensitivity.

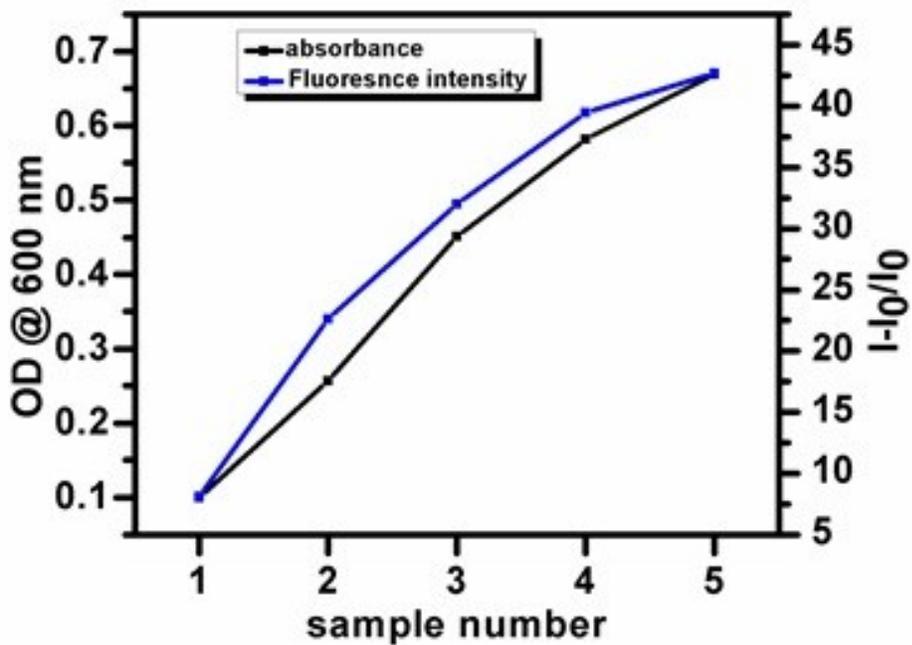
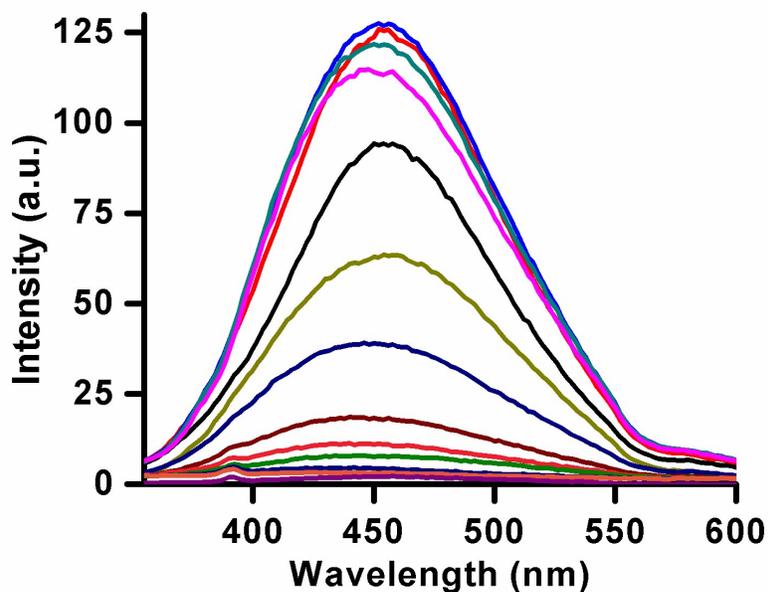


Figure S6. A comparative plots of OD₆₀₀ and fluorescence intensities [(I-I₀)/I₀] vs variable concentrations of *S. aureus* (refer to Table S1).

10. Fluorescence response of probe 2 upon treatment of *B. subtilis*

(A)



(B)

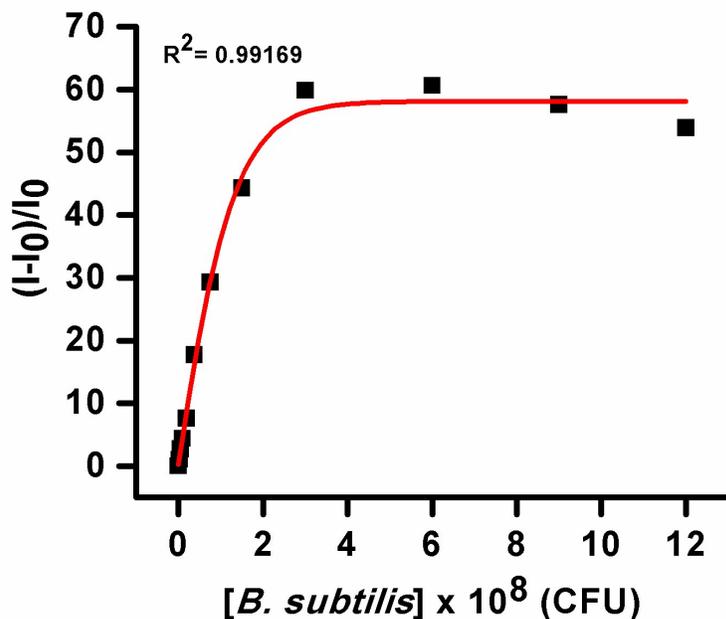


Figure S7. (A) Fluorimetric response of probe 2 (30 μM) upon addition of increasing concentration of *B. subtilis* [λ_{ex} 345 nm]. (B) A plot of increment in fluorescence intensity $[(I-I_0)/I_0]$ against the increasing concentration of *B. subtilis*.

11. LOD of probe 2 in the detection of *B. subtilis*

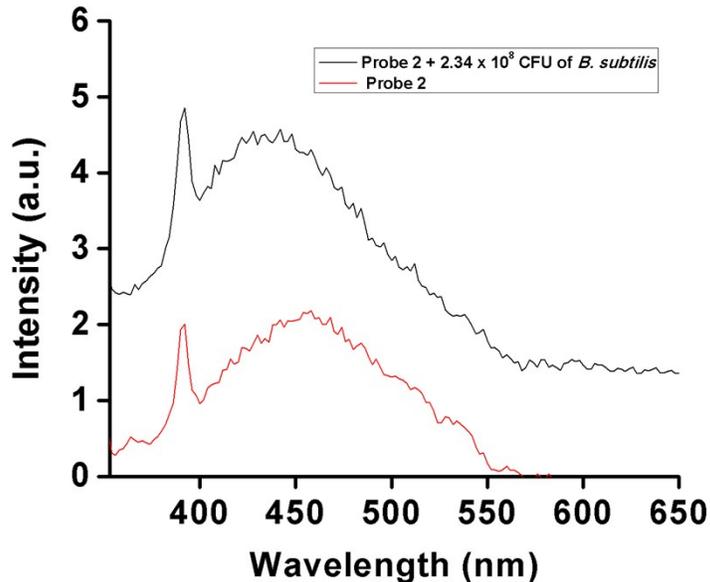


Figure S8. Fluorescence spectra of probe 2 upon addition of *B. subtilis* (2.34×10^6 CFU), showing a signal-to-background ratio more than three. From this the LOD of probe 2 for the detection of *B. subtilis* was estimated as 2.34×10^6 CFU.

12. UV-Vis response of probe 2 upon treatment of *B. subtilis*

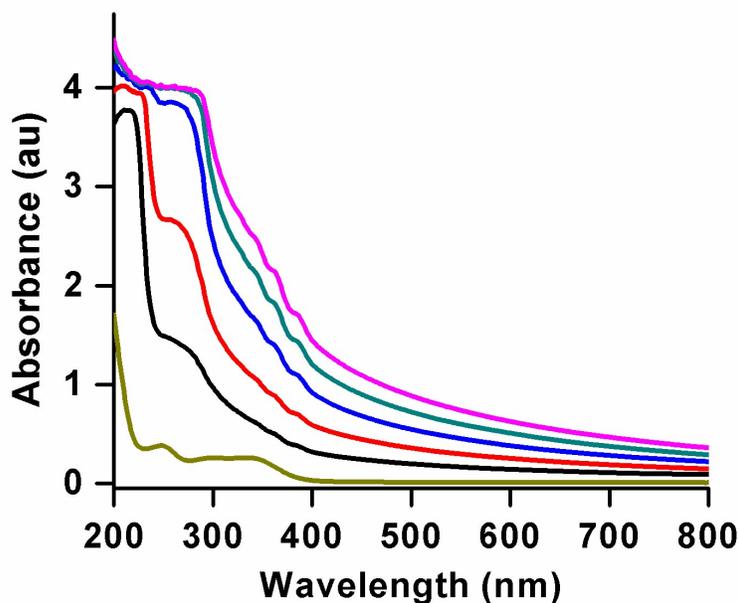
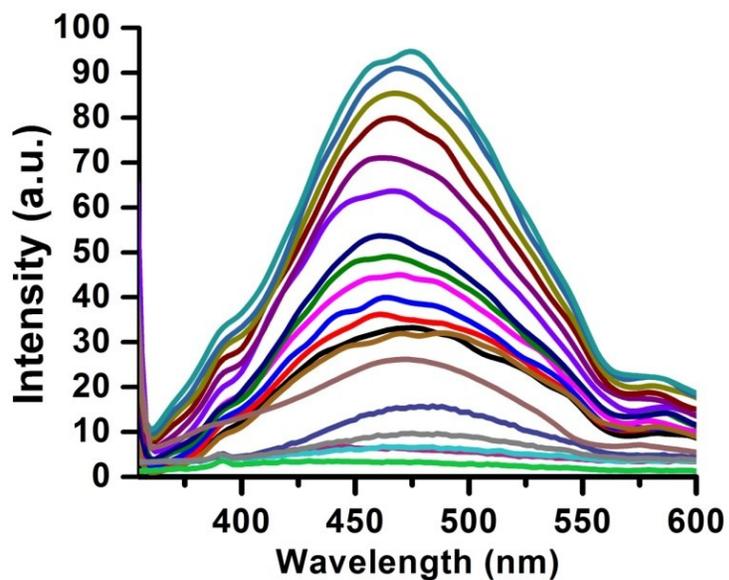


Figure S9. (B) UV-Vis response of probe 2 (30 μM), upon addition of increasing concentration of *Bacillus subtilis*.

13. Fluorescence response of probe 2 against LTA

(A)



(B)

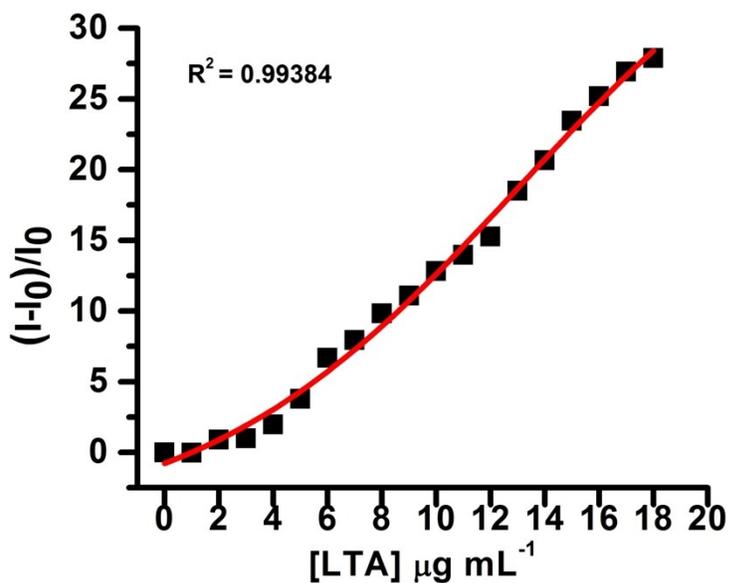


Figure S10. (A) Fluorimetric response of probe 2 (100 μM), upon addition of increasing concentration of LTA [λ_{ex} 345nm]. (B) A plot of increment in fluorescence intensity $[(I - I_0)/ I_0]$ against the increasing concentration of LTA. The regression coefficient was found to be 0.9938.

14. Antimicrobial activity study of probes 1 and 2 with *S. aureus*

Spot test



Figure S11. Spot test for probe 1 (left) and probe 2 (right). The study revealed that no significant inhibition zone was developed for probe 1 but probe 2 showed strong inhibition zones on addition of 5 μ L and 10 μ L of both probes (1 mM) to bacterial colonies of *S. aureus* and incubating them for 16 h at 37 °C.

15. SEM images

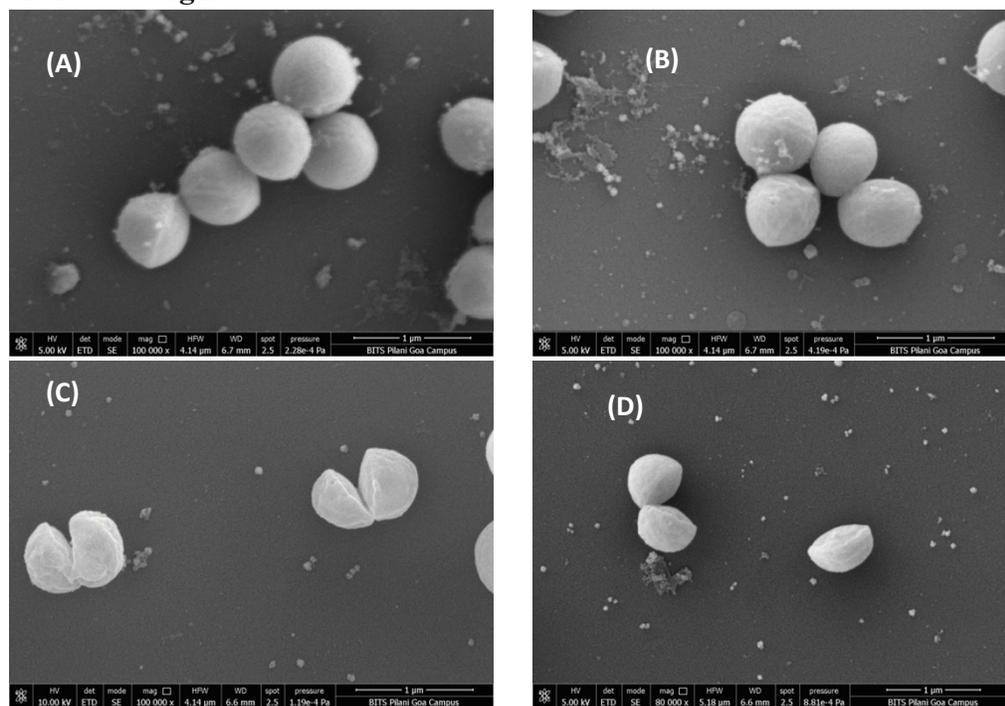


Figure S12. Some more SEM images of *S. aureus* (A) & (B) before and (C) & (D) after treatment with probe 2. Scale bar is 1 μ m. Colony of healthy bacteria were observed in images A and B, and images C and D showed surface defects and inhibition of cell division.

16. Growth kinetics of *S. aureus*

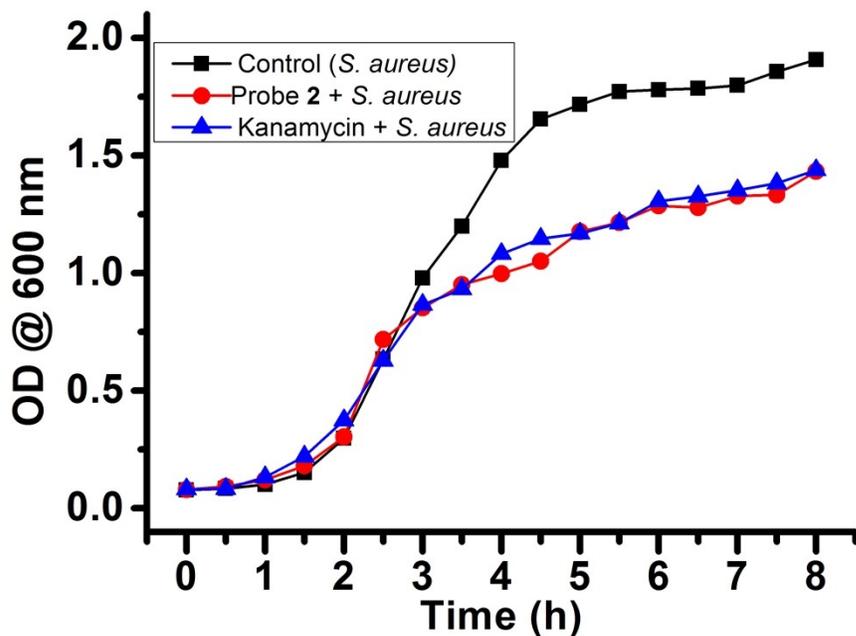
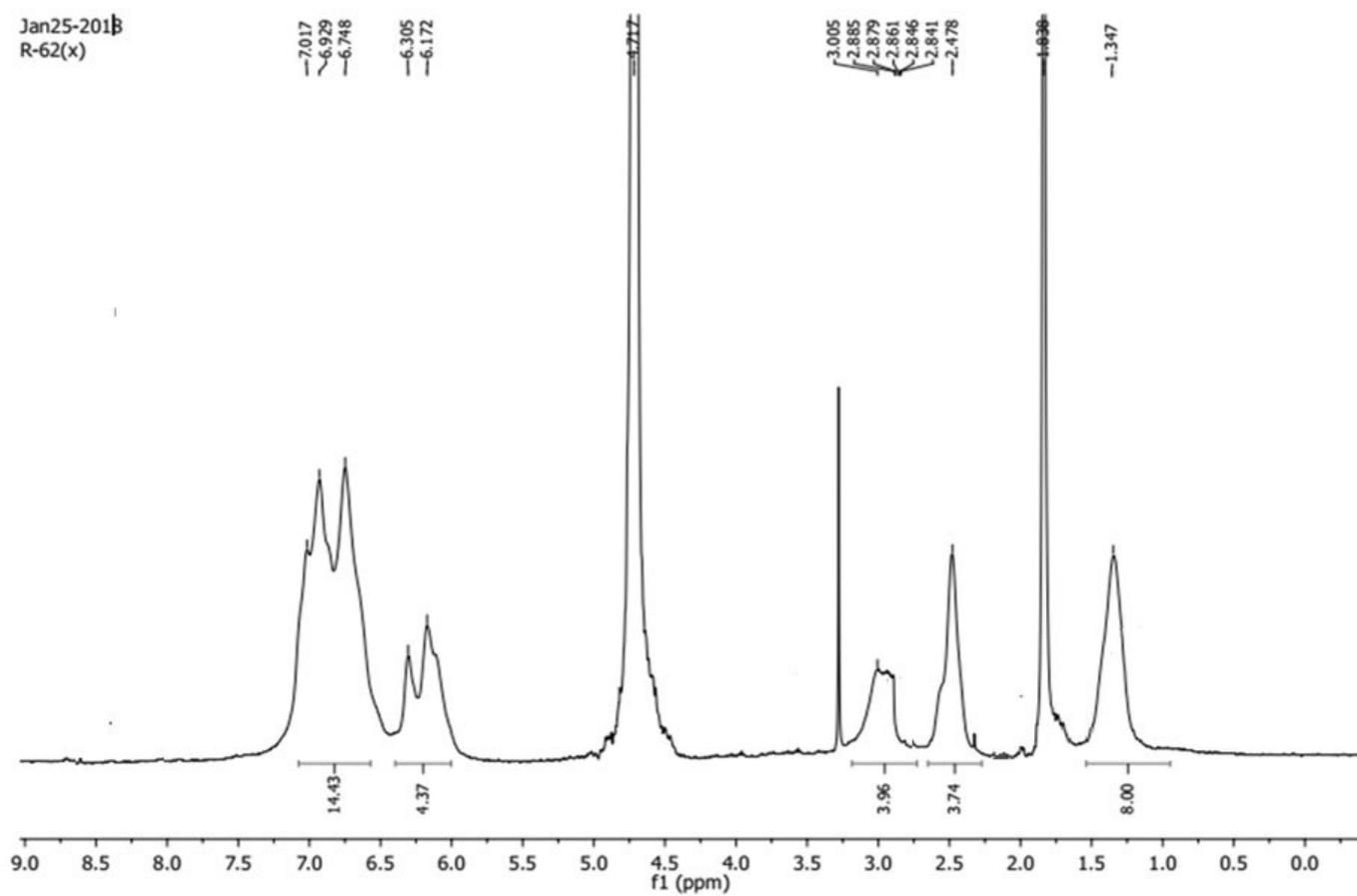


Figure S13. Growth kinetics of *S. aureus* against probe 2. Kanamycin was used as a standard antibacterial agent.

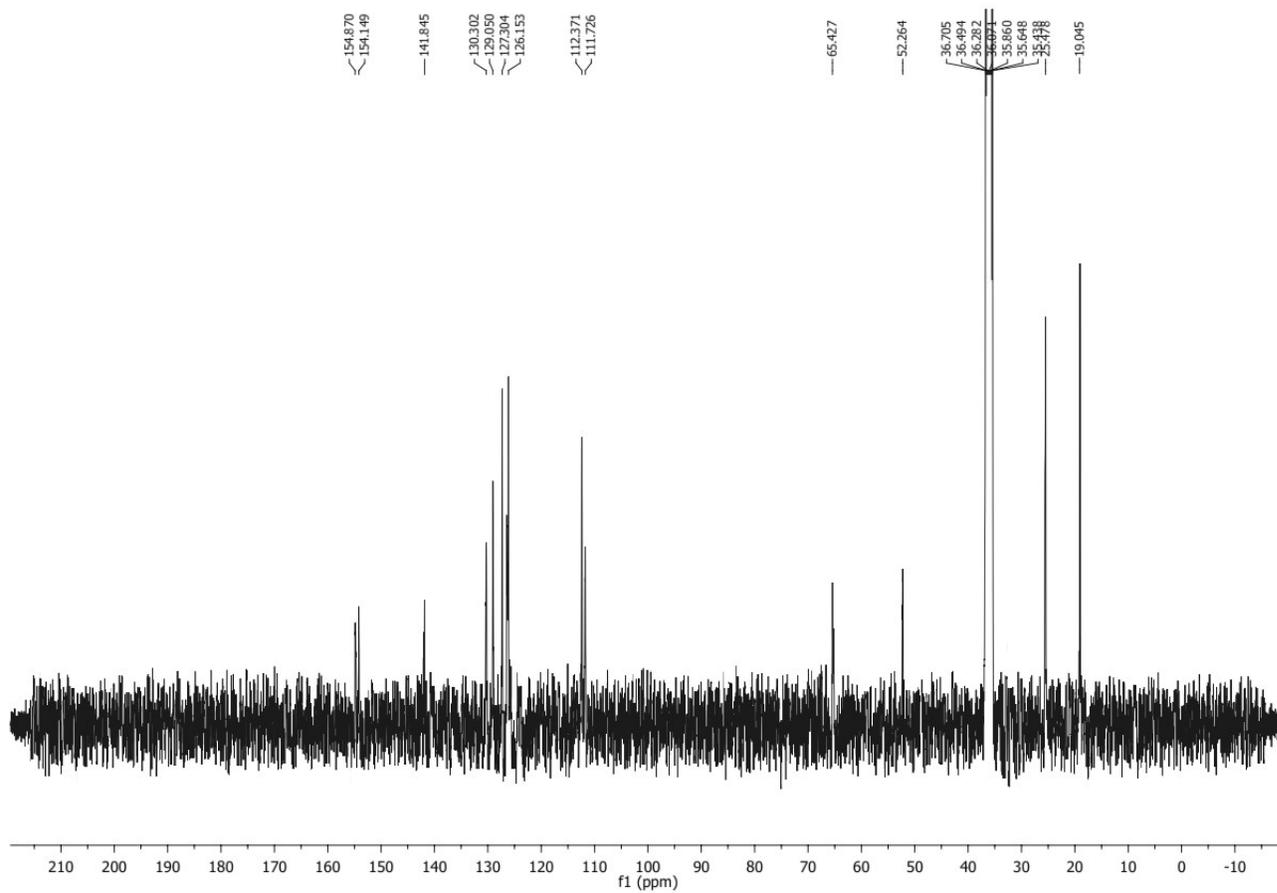
17. References

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¹H NMR for probe 2



¹³C NMR for probe 2



ESI-MS spectrum for probe 2

