Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Dynamic Layer-by-Layer Films on Nanofiber Membrane: A Platform for Ultra-sensitive Bacterial Concentration Detection

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1. Materials and instruments

2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT, 97%, HPLC), azodiisobutyronitrile (AIBN, 99%) were purchased from Aladdin chemical Co. inc. (Shanghai, China). 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 99%) and polyacrylic acid (PAA, M.W. 3000) were purchased from Macklin. PVA-*co*-PE nanofibrous membranes were prepared according to our previous method. *N*,*N*-Dimethylformamide (DMF, 99.7%), n-hexane (99.7%) and iso-propyl alcohol (IPA, 99.7%), sodium hydroxide (NaOH, 99.7%), sodium chloride (NaCl, 99.7%) and agar-agar (99.7%) were purchased from Sinopharm Chemical Reagent Co.,Ltd. (Shanghai, China), The *Escherichia coli* ATCC 9027 and *S. aureus* ATCC 6538 strains were purchased from Nanjing Bianzhen biological technology Co., Ltd..

Fourier transform infrared spectroscopy (FT-IR, BRUKER I27000), UV-Vis spectroscopy (SHIMADZU UV-2700), analytical scanning electron microscopy (SEM, JEOL Ltd JSM-IT300A), a single fiber contact angle meter (KRUSS DSA30S), gel permeation chromatography (GPC, TDA MAX305), X-ray photoelectron spectroscopy (XPS, 250XI), zetasizer nano serier (Nano ZS, ZEN3600), fluorescence spectrophotometer (Lengguang Tech. F97PRO), electrostatic potentiometer (ME275, Monroe Inc., USA) and confocal laser scanning microscope (CLSM, TCS SP5II) were used to characterize the membranes.

2. Synthesis of the fluorescent copolymer-p(DMAEMA-*co*-BODIPY)

The electropositive copolymer-p(DMAEMA-*co*-BODIPY) (PDB) was synthetized by reversible addition-fragmentation chain transfer (RAFT) polymerization. 2-(Dimethylamino)ethyl methacrylate (200 mmol/mL) and boron dipyrromethene monomer (BODIPY, 20 mol/mL), azodiisobutyronitrile (AIBN, 0.8 mmol/mL), 2-methyl-2-[(dodecylsulfanylthiocarbonyl) sulfanyl]propanoic acid (DDMAT, 2.0 mmol/mL) was mixed in *N*,*N*-Dimethylformamide (DMF), the mixture was further degassed by bubbling nitrogen for 30 min and the reaction was conducted at 60 ^{o}C for 12 h to get the fluorescent copolymer. The copolymer was purified using *n*-hexane as precipitant and abbreviated as PDB.

GPC was used to detect the molecular weight and Polymer dispersity index (PDI) of PDB. ^{1}H NMR and FTIR were also used to characterize the molecular structure of PDB. The UV absorption and fluorescence emission spectrum of PDB were detected by UV-Vis spectroscopy and fluorescence spectrophotometer.

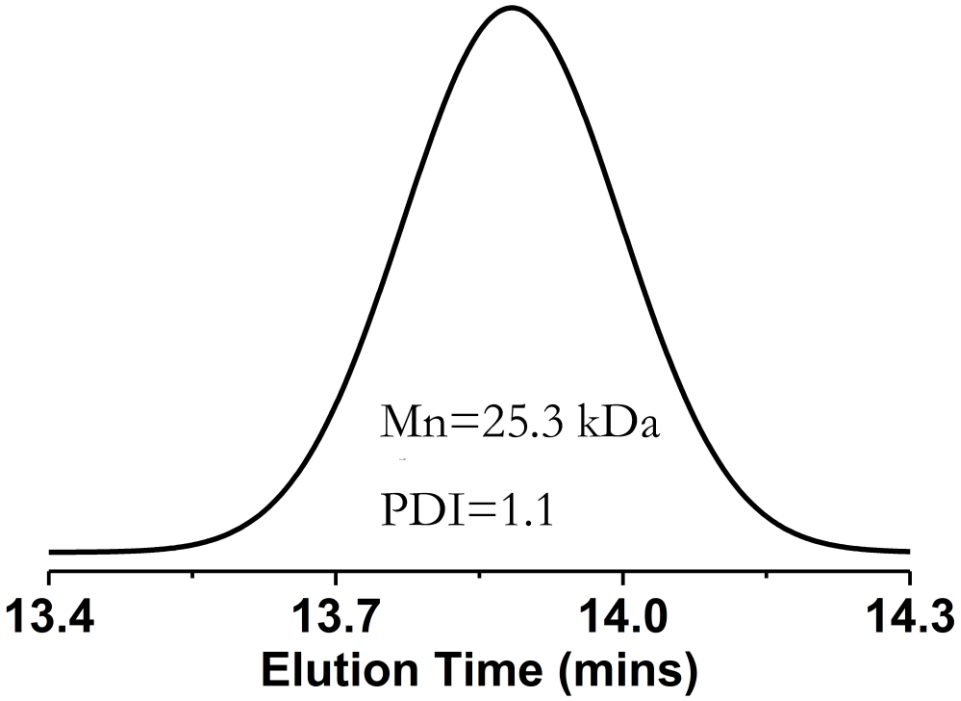

Figure S1. GPC of PDB

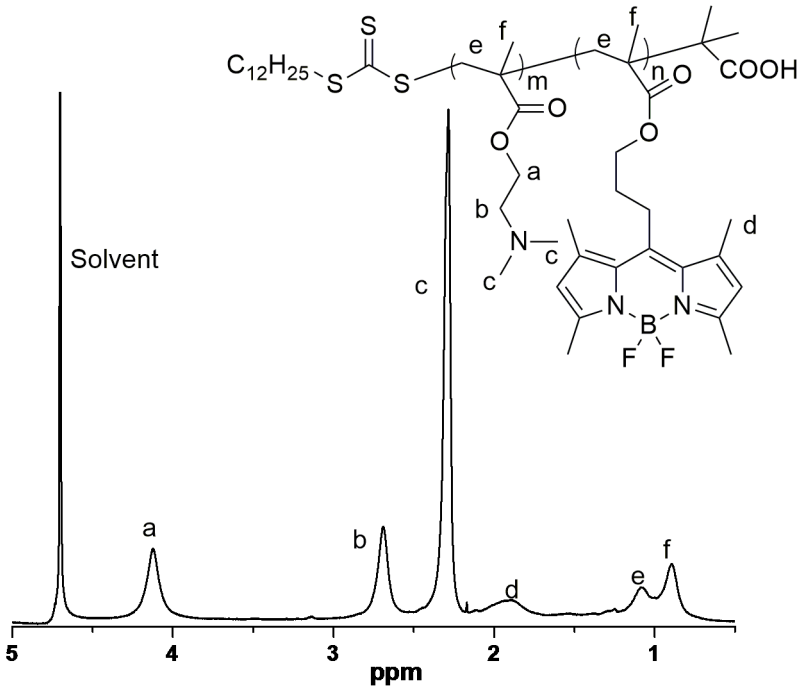

Figure S2. ^{1}H NMR of PDB

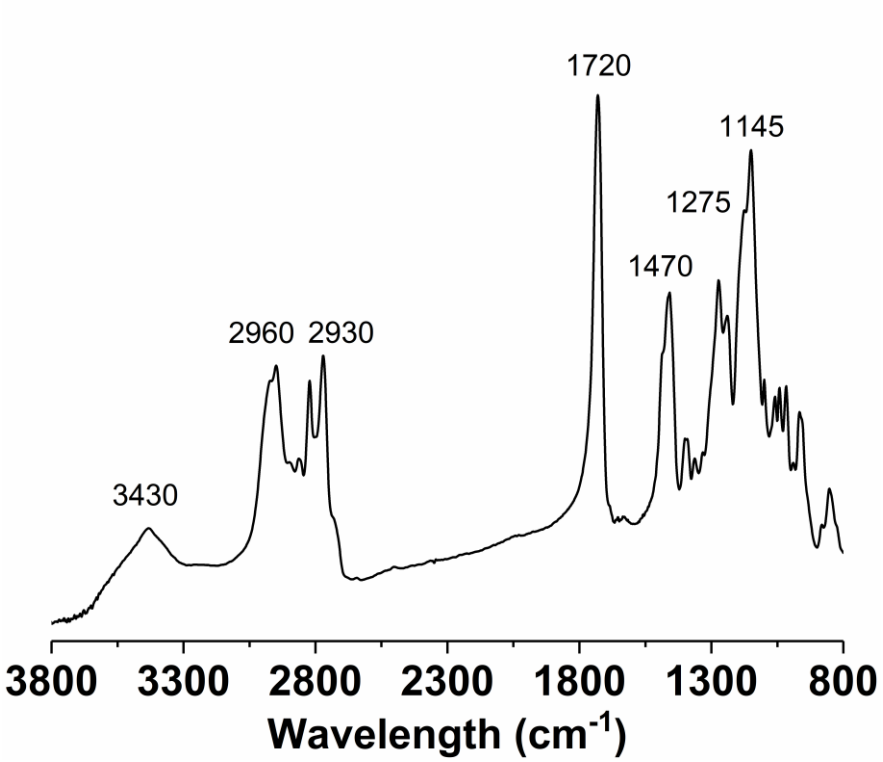

Figure S3. FTIR spectrum of PDB

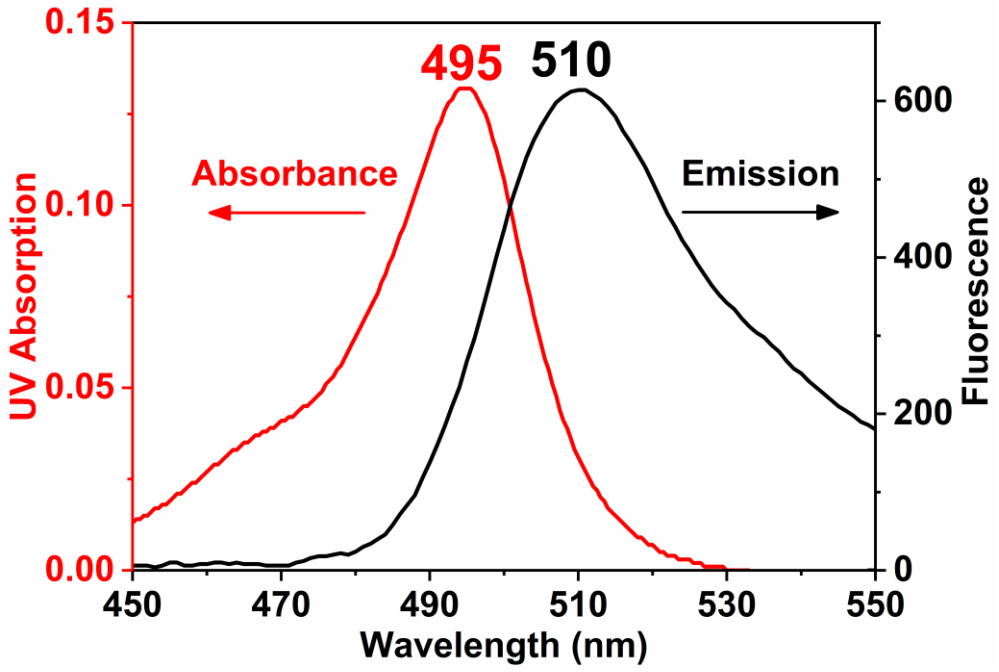

Figure S4. The UV absorption (red line) and fluorescence emission spectrum (black line) of PDB. The maximum absorption wavelength was 495 nm and the maximum emission wavelength was 510 nm.

3. Fabrication of PDB/PAA LBL Films

The PDB/PAA LBL films were fabricated using NFMs as substrates. Before use,

the substrate were cleaned in NaOH solution (1.0 mg/mL), rinsed with deionized (DI) water thoroughly, and dried in air. To introduce PDB, the substrates were immersed in a 0.2 wt % PDB solution for 10 min, washed with DI. Then they were immersed in a 0.2 wt % aqueous solution of PAA (pH 3.0) for 10 min to assemble the PAA. Films with various thicknesses were fabricated by repeating the deposition cycles. After the predetermined bilayer numbers were reached, the films were dried in the air. And, the films were characterized by Zeta potential, X-ray photoelectron spectroscopy (XPS), fourier transform infrared spectrometer (FT-IR) and fluorescence spectrophotometer.

Common fabric membrane also had been used as the substrate; however, the polymers could not self-assembly on common fabric membrane.

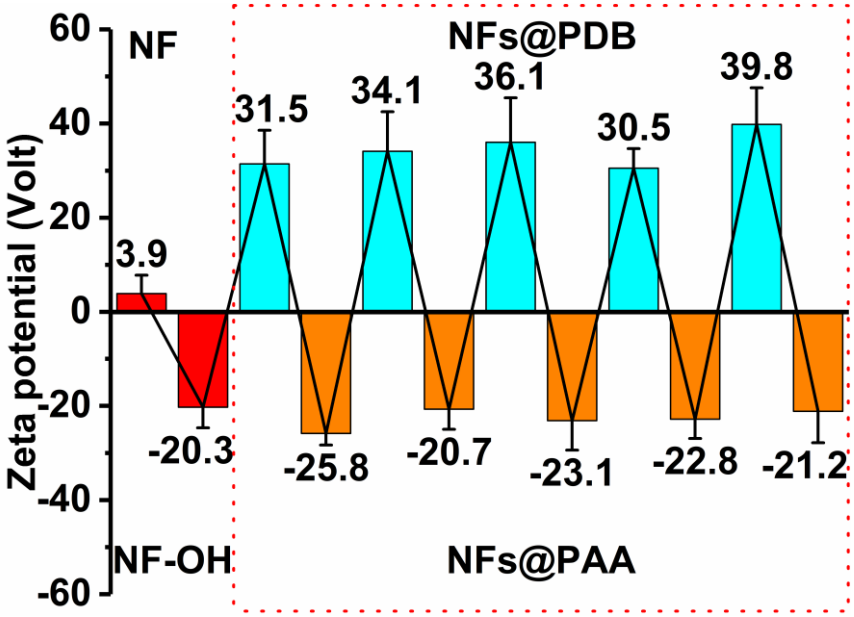

Figure S5. Surface Zeta potential reversion in the LBL process.

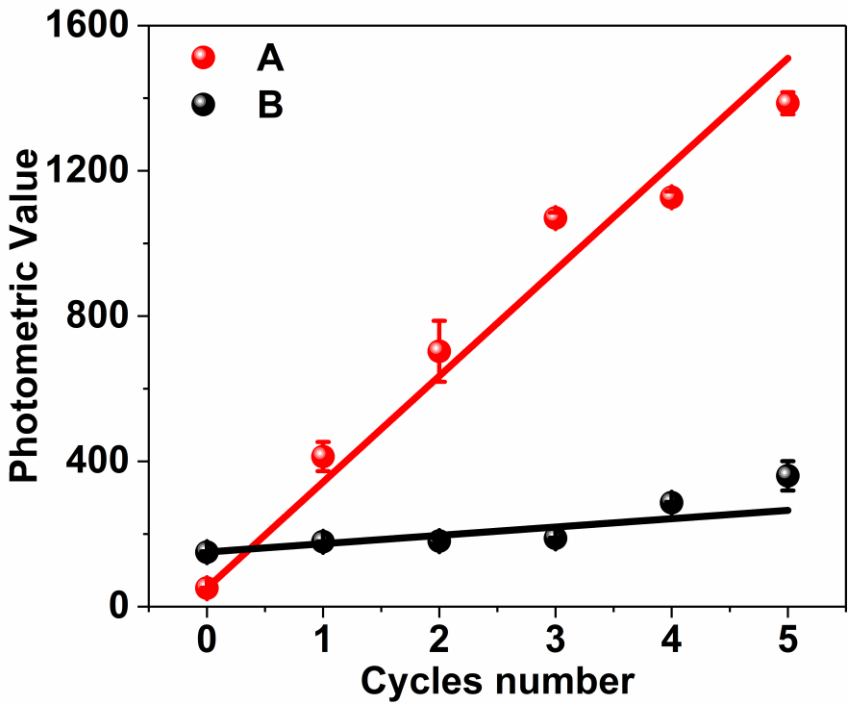

Figure S6. Assembly of PDB/PAA LBL films monitored by fluorescence intensity increase at 510 nm on the surface of NFM (A) and common fabric membrane (B).

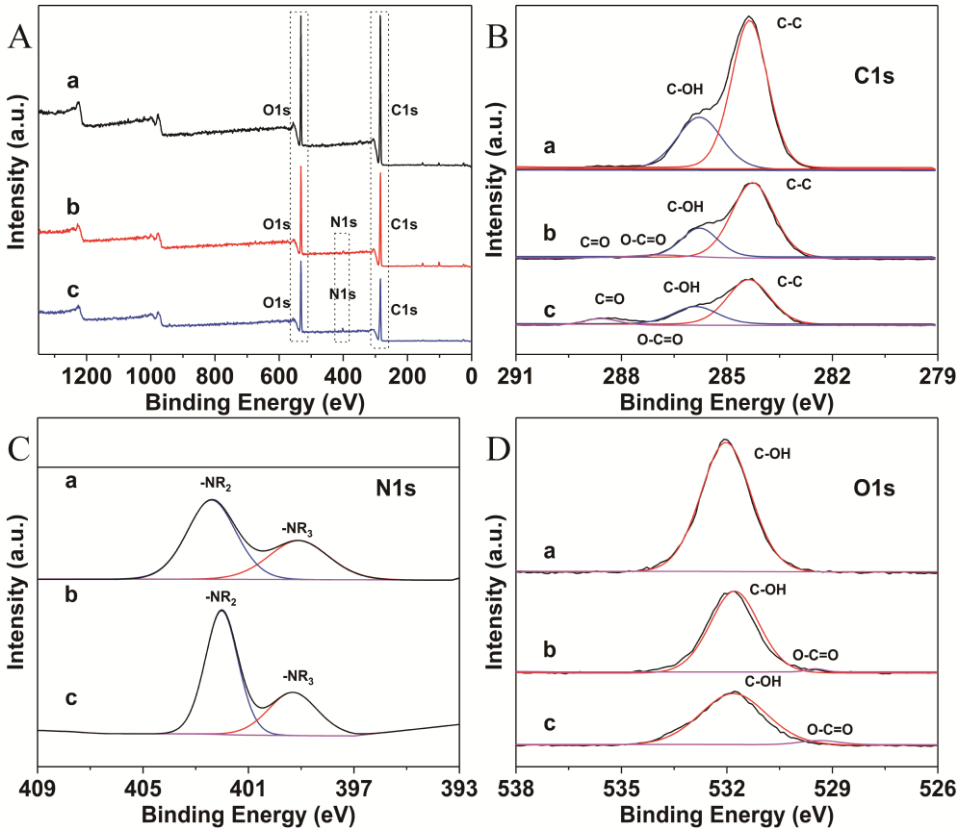

Figure S7. XPS of NFMs (a), NFM@PDB (b) and NFM@PDB/ PAA (c): (A) wide scan spectra, (B) C1s, (C) N1s and (D) O1s spectra.

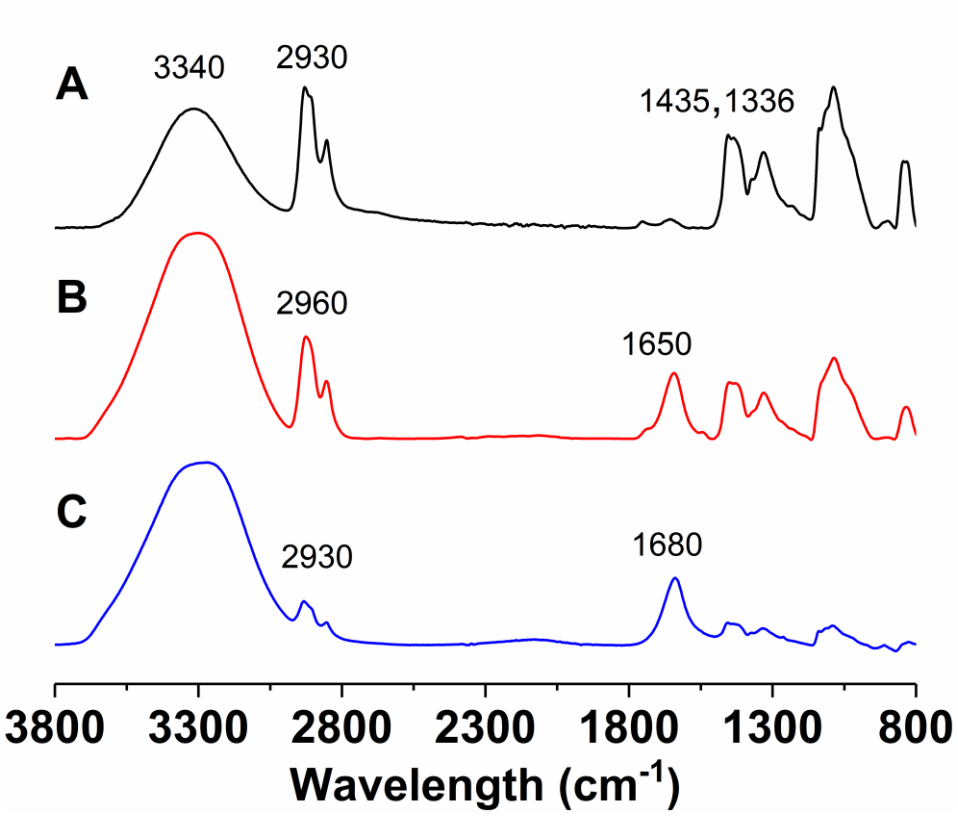

Figure S8. FT-IR spectra of PDB and FNFM. A: NFM; B: NFM@PDB; C: NFM@PDB/PAA.

4. The detection of fluorescence quenching

In order to confirm the effect of bacteria on fluorescence intensity of PDB, the change of the fluorescence density with time was detected using fluorescence spectrophotometer. PDB solution (0.1 mg/L) and the mixed liquid of S. aureus (1.0×10^5 CFU/mL) and PDB (0.1 mg/L) were samples.

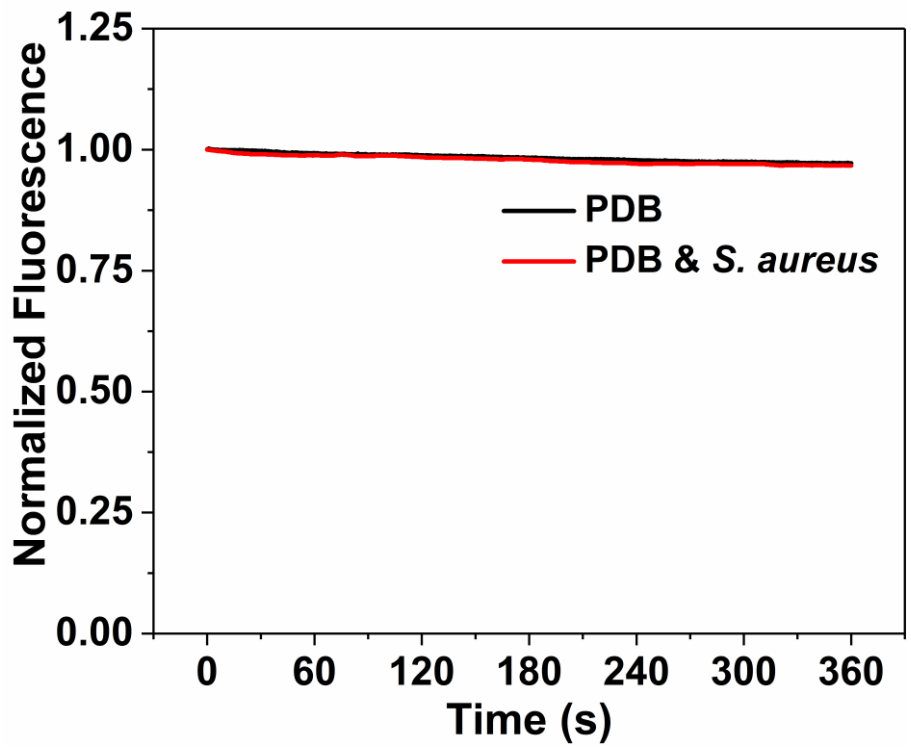

Figure S9. The change of the fluorescence density with time at 510 nm.

5. Fluorescence microscopic observation

Confocal laser scanning microscope (CLSM) was used to observe the fluorescence intensity of FNFM surface with argon laser; the maximum excitation wavelength was 488 nm and the maximum emission wavelength was 510 nm. The surface fluorescence of FNMF with different bilayers had been observed to visual the assembly process, pure NFM as negative control. In order to confirm the bacteria responses, FNFMs were soaked in the bacterial suspension (OD600=0.1) at different time intervals (1, 2, 3, 4 and 5 min), deionized water as negative control.

Fluorescence microscope was also be used to observe the bacteria before and after co-culture with PDB for 5 min, 488 nm as excitation wavelength.

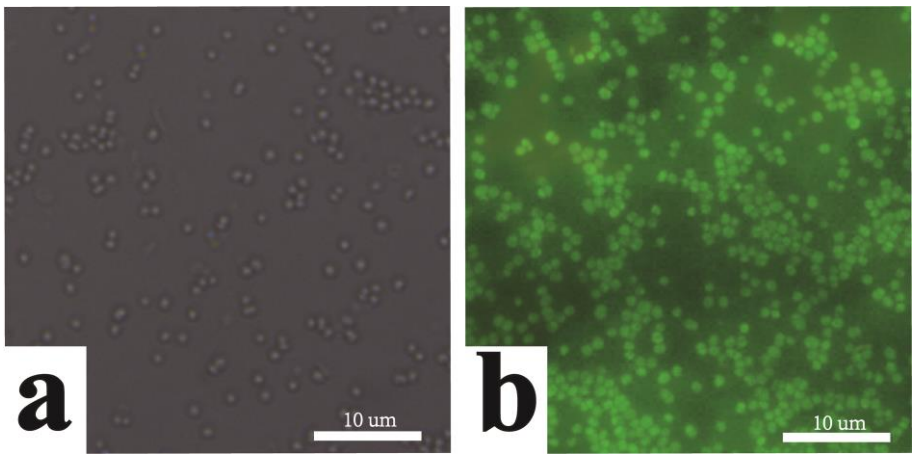

Figure S10. Fluorescence microscope images of *S. aureus* before (a) and after (b) co-culture with PDB 5 min.

6. Bacterial detection of FNFM

E. coli ACTT 8099 and *S. aureus* ATCC 6538 were used as model bacteria. *E. coli* and *S. aureus* were grown overnight in Luria-Bertani (LB) broth (beef extracts 3g/L, peptone 5 g/L, NaCl 5 g/L) at 37 ^{o}C and shaking with 120 r/min subsequently diluted using LB broth to get the working concentration.

Fluorescence spectrophotometer was used to detect the fluorescence intensity of FNFM surface for quantitative detection of bacterial concentration. Bacterial suspensions were prepared with different concentration (10, 10^2, 10^3, 10^4 and 10^5 CFU/mL) to calibrate the liner relationship between bacterial concentration ($C_{bacteria}$) and reducing ratio of fluorescence intensity (k value). Surface fluorescence intensity was detected in different time (1, 2, 3, 4 and 5 min) to determine k value when FNFMs were immersed in the bacterial suspensions. At last, we had built a liner relationship between k value and $C_{bacteria}$.

7. Practical samples analysis

Lakes water and tap water were used to evaluate detection activity of FNFM against practical samples. FNFMs were immersed in the water samples, and then surface fluorescence intensity was detected in different time (1-5 min) to determine *k* values and then bacterial concentration could be calculated using the linear equation between *k* values and $C_{bacteria}$.

The lake water was diluted 100 times and tap water was diluted 10 times using

ultrapure water. The diluted samples were inoculated on the surface of solid medium extracts (beef 3g/L, peptone 5 g/L, NaCl 5 g/L, agar 15 g/L). After culturing for 24 h, the bacterial density on the surface of the culture dish was observed to evaluate the bacterial concentration.

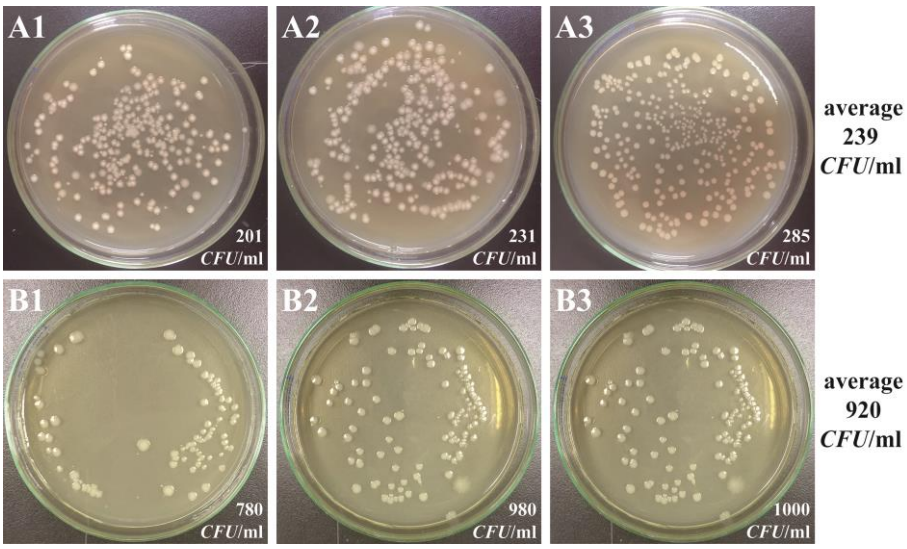

Figure S11. The bacterial concentration of tap water (A) and lake water (B).