Electronic Supplementary Information (ESI)

Bioinspired silica nanocomposite for enhanced Multidrug-resistant Bacteria treatment and Wash-free Imaging

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Table of Contents

1. Experimental Section

1.1 Materials. 1-Butyl-3-vinylimidazolium bromide was purchased from Energy Chemical (Shanghai, China). Azobis(isobutyronitrile)(AIBN), tetraethyl orthosilicate(TEOS), anhydrous sodium acetate, formaldehyde, ammonia aqueous solution, 1,3-indandione, malononitrile, resorcinol, 4-(diphenylamino)benzaldehyde, *N*,*N*,*N*'- trimethylethylene diamine were obtained from Aladdin Reagents Co. Ltd. (Shanghai, China). All the chemicals are analytical grade and used without further purification. Tryptone, yeast extract, NaCl, beef extract, and agar powder were purchased from Solarbio Science & Technology Co. Ltd. (Beijing, China). SYTO green was purchased from Thermo Fisher Scientific. *Staphylococcus aureus* (*S. aureus*) and enteropathogenic *Escherichia coli* (*E. coli) (EPEC)* were bought from BeNa Culture Collection Co., Ltd. (Beijing, China). *E. coli* and *MRSA* were obtained from Guangdong Microbial Culture Collection Center (Guangdong, China).

1.2 Characterization. ^{1}H NMR spectra were recorded with Bruker 500 MHz spectrometer with $CDCl_3$ and DMSO-d_6 as solvents. The morphology of nanoparticles was determined using transmission electron microscopy (JEM-2100F, JEOL). UV−vis absorption spectra were recorded at room temperature using a spectrophotometer (U2910, Hitachi). Fluorescent spectra were collected using fluorescence spectrophotometer (F7100, Hitachi). Fluorescence images were obtained with confocal laser scanning microscopy (LSM 700, Zeiss, Germany). Hydrodynamic diameter of nanocomposite and zeta potentials were measured at room temperature on zetasizer (Nano ZS90, Malvern). Gel permeation chromatography (GPC, TOSOH HLC-8320) was used to characterize the poly(ionic liquid). Nitrogen adsorption-desorption analysis was performed using Micromeritics ASAP 2020 Plus. All the samples were degassed overnight under vacuum before measurement.

1.3 Experimental methods

Synthesis of silica nanospheres. Two types of silica nanospheres were prepared under

the Stöber process condition following literature procedures.[1] Briefly, resorcinol (0.15 g) and formaldehyde (37 wt%, 0.21 mL) were added to the solution containing ammonia aqueous solution (28 wt%, 3.0 mL), deionized water (10 mL) and ethanol (70 mL). The mixture was vigorously stirred for 6 h at room temperature to form a homogeneous solution; then 0.6 mL of TEOS was added into the solution and stirred for 8 min before another addition of resorcinol (0.4 g) and formaldehyde (37 wt%, 0.56 mL). The solution was stirred for another 2 h at room temperature. The as-synthesized RF@RF-SiO2@RF composites were collected by centrifugation. Subsequently, the pellet was washed twice with ethanol and dried at 50 °C. Finally, the rough mesoporous silica hollow spheres (R-MSHSs) were obtained and harvested after calcination at 550 °C for 5 h in the air. Smooth silica hollow spheres (S-SHSs) were synthesized in a similar way by adding 1.4 mL of TEOS without the second addition of resorcinol and formaldehyde.

Preparation of Poly(ionic liquid)s. Poly(3-butyl-1-vinylimidazolium bromide) used in this study was synthesized according to the reported procedure.[2] Briefly, 1-butyl-3-vinyl imidazolium bromide (1.1 g, 4.76 mmol), AIBN (0.033 g, 3 wt %), and ethanol (2.21 g) were mixed and stirred at 60 °C for 12 h under nitrogen. The obtained product was purified by precipitation in acetone to remove the unreacted raw materials and washed three times with acetone. The obtained PILs was dried in a vacuum oven at 60 °C for 24 h and characterized by Gel permeation chromatography (GPC) (Table S1).

Preparation of AFN-I. AFN-I was synthesized via a two-step reaction as reported in the reference.[3] AFN was firstly prepared via a one-pot reaction in MeCN. Briefly, 2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene)malononitrile (194 mg, 1.0 mmol) prepared according to the literature, [4] 4-(diphenylamino) benzaldehyde (273 mg, 1.0 mmol), and N,N,N'- trimethylethylenediamine (402 μL, 4.0 mmol) were co-dissolved in MeCN (10 mL). The mixture was stirred at 60 ℃ for 12h. After cooling down to room temperature, the resulting red solid was collected, washed with MeCN, and dried in the fume hood, from which the compound AFN was obtained (247 mg, yield 70%). AFN further reacted with CH_3I to obtain the compound AFN-I. Typically, the AFN (275 mg, 0.5 mmol) was dissolved in THF (20 mL). CH_3I (62 μL,1.0 mmol) was added into the

above solution under the condition of ice bath as followed. The reaction mixture was stirred for 10h at room temperature. Yellow solids were observed to precipitate and the reaction was quenched with pyridine. The precipitates were filtered off and washed with THF. The final compound was dried and obtained (304 mg, yield 88%).

Nanocomposite Formation. Specifically, R-MSHSs and S-SHSs were added into ILs (200 μM) or PILs (0.4 μM) solution and mixed for 24 h at room temperature, respectively. The mixture were centrifuged and washed with ultrapure water for three times. R-ILs, R-PILs, S-ILs and S-PILs were then obtained and resuspended in water with final concentration of 300 $\mu g \cdot ml^{-1}$. The silica nanospheres (0.6 mg/ml) were dispersed in water by ultrasonication, and then mixed with PILs (0.8 μM) and AFN-I (15μL, 1mM) solution. The mixture was stirred for 24h at room temperature. The nanocomposite R-P-AFN and S-P-AFN were obtained by centrifugation and washed with ultrapure water for three times.

Loading capacity of nanocomposite. The loading efficiency of nanocomposite was evaluated by measuring the UV-vis absorbance of ILs/PILs. The amount of ILs and PILs in the supernatant was determined by recording the absorbance at wavelength of 225 nm according to the following equation:

$$n = \frac{(C_0 - C_{eq})\,V}{m} \qquad (1)$$

where n – absorption [mg g^{-1}], C_0 – initial concentration of ILs/PILs [mg mL^{-1}], C_{eq} – equilibrium concentration after absorption [mg mL^{-1}], m – weight [g].

Bacteria culture and imaging. *Escherichia coli* (*E. coli), Staphylococcus aureus* (*S. aureus*), and their corresponding multidrug-resistant (MDR) strains, i.e. enteropathogenic *E. coli* (EPEC) and methicillin-resistant *S. aureus* (MRSA), were cultured in the liquid Luria Broth (LB) medium and grown up to exponential phase at 37°C with a shaking speed of 200 rpm, respectively. The concentration was determined by measuring the optical density at the wavelength of 600 nm (OD_{600}). Bacteria cells (OD_{600} = 1.0) were then harvested by centrifugation at 6000 rpm and washed twice with

phosphate-buffered saline (PBS). They were further incubated with SYTO green for 20 min at room temperature in dark. After being washed twice with PBS, the cells were suspended in a 500 μL nanocomposite solution. The SYTO-stained bacteria without the addition of nanocomposites were used as the control group. Then the solution was transferred to glass slides for bacteria imaging. Fluorescence images were recorded using a Nikon AX confocal laser scanning microscope (Nikon, Japan).

Antibacterial activity. The antimicrobial activities of ILs and PILs against bacteria were investigated using the Clinical and Laboratory Standards Institute broth microdilution method. Briefly, the antibacterial materials were dispersed in deionized water and diluted at set intervals using the nutrient broth. The diluted bacterial solution (1 x 10^7 CFU mL^{-1}) and antibacterial materials were incubated at 37°C for 24h. After incubation, the bacterial turbidity was determined by measuring the absorbance at 600 nm using a multifunctional microplate reader. The minimum inhibitory concentration (MIC) values were obtained as the concentration of the measured agents at which at least 50% of bacteria strains were inhibited. All the tests were performed and measured in triplicates.

The antibacterial activity of nanocomposites was also determined using the spread plate method. Typically, the nanocomposites containing ILs or PILs were added to the bacterial suspension (1x10^7 CFU·mL^{-1}). After incubation at 37 °C for 24h, the mixture was taken out and diluted with an appropriate dilution factor. Then 100 μL of the dilution was transferred to the agar plate, followed by incubation at 37 °C for 16 h. The number of colony-forming units (CFU) on the agar plate was counted and the bacterial viability was calculated by the following equation:

$$Bacterial\ viability\ (\%) = \frac{N_{test}}{N_{control}} \times 100\%$$

where $N_{control}$ – the number of viable bacterial colonies in CFU in the control group, N_{test} – the number of viable bacterial colonies in CFU in the test group.

Measurement of Fluorescence Spectra. Bacteria were cultured at 37 °C overnight. They were then harvested by centrifuging at 6000 rpm for 5 min and washed with PBS twice to remove the culture medium. The collected cells were resuspended in ultra-pure

water and diluted to a series of concentrations. AFN-I was added into above bacteria solution to keep the final concentration at 10 μM and the mixture was incubated for 10 min in dark. Fluorescence spectra of solution were then measured.

2. Figures

2.1 UV-vis spectrum of ionic liquids.

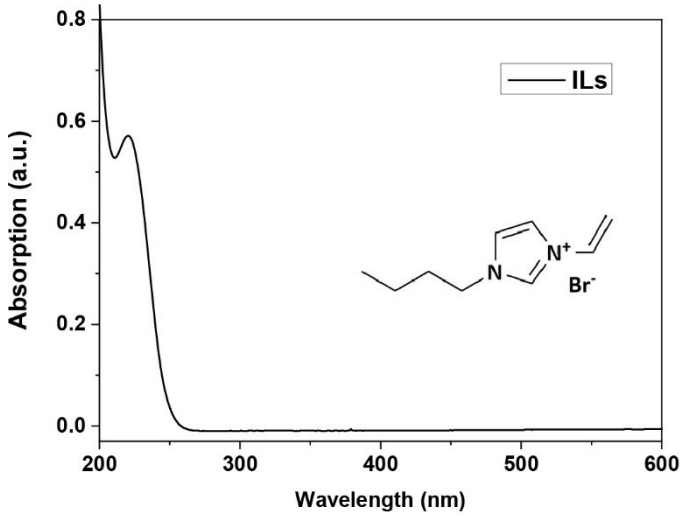

Fig. S1. UV-vis spectrum of 1-butyl-3-vinylimidazolium bromide (ILs) at a concentration of 10 μM in water.

2.2 Characterization of poly(3-butyl-1-vinylimidazolium bromide) (PILs).

Table S1. The GPC spectra of PILs prepared via free radical polymerization

Sample	M_n	M_w	M_p	M_z	Polydispersity
PILs	10036	16323	14041	24775	1.6264

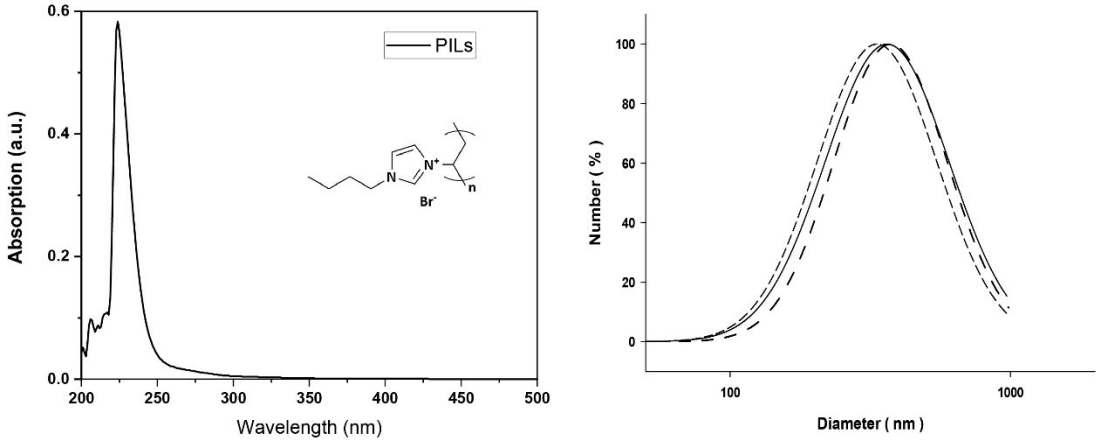

Fig. S2. (A) UV-vis spectrum of PILs in EtOH/CH_2Cl_2 (v/v = 1:1) solution at a concentration of 10μM. (B) Hydrodynamic diameter of PILs in aqueous solution at a concentration of $1x10^{-3}$ mol L^{-1}.

2.3 Characterization of AFN-I

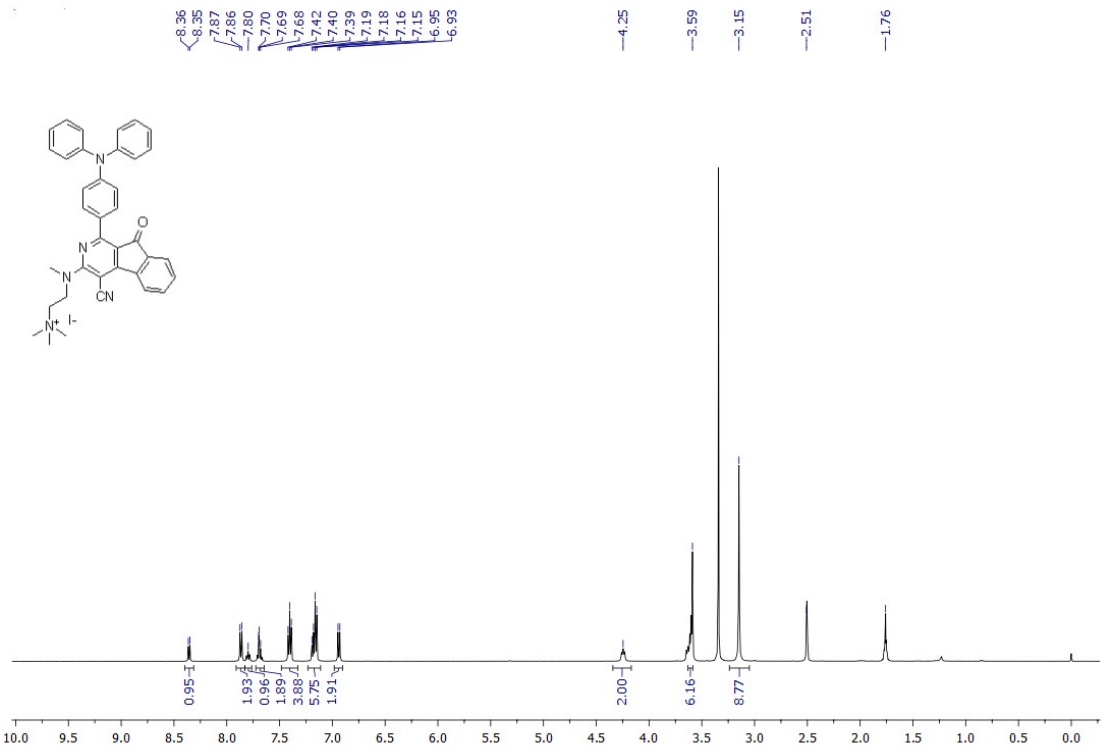

Fig. S3. ^{1}H NMR spectra of AFN-I in DMSO-d_6.

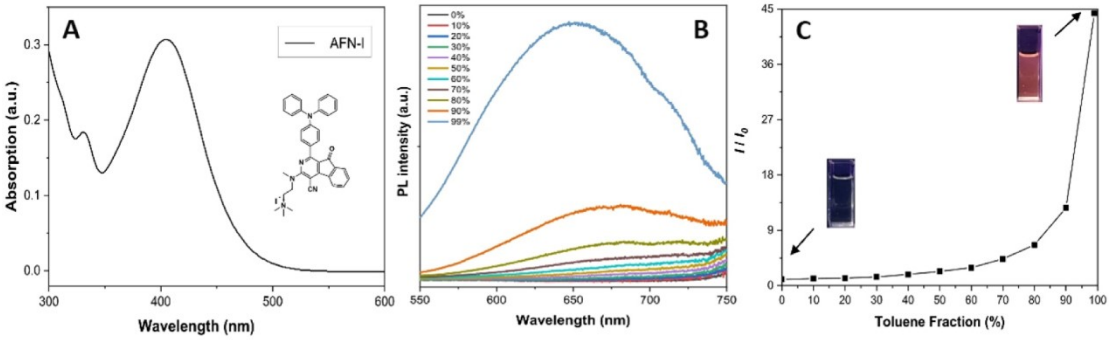

Fig. S4. (A) UV-vis spectra of AFN-I in DMSO solution. (B) PL spectra of AFN-I in DMSO/toluene mixture with different toluene fractions (λ_{ex} = 404 nm). (C) The plot of relative emission intensity (I/I_0) of AFN-I versus the toluene fraction (I_0 was collected at wavelength of 653 nm). Insets are photographs of AFN-I (10 μM) in DMSO and DMSO/toluene mixture taken under excitation wavelength of 365 nm.

2.4 Characterization of nanocomposites

Table S2. Hydrodynamic diameter of nanocomposite sample

Sample	Diameter (nm)
R-MSHSs	275.0 ± 2.48
S-SHSs	229.0 ± 1.64
R-PILs-AFN-I	285.7 ± 1.42
S-PILs-AFN-I	255.7 ± 2.57

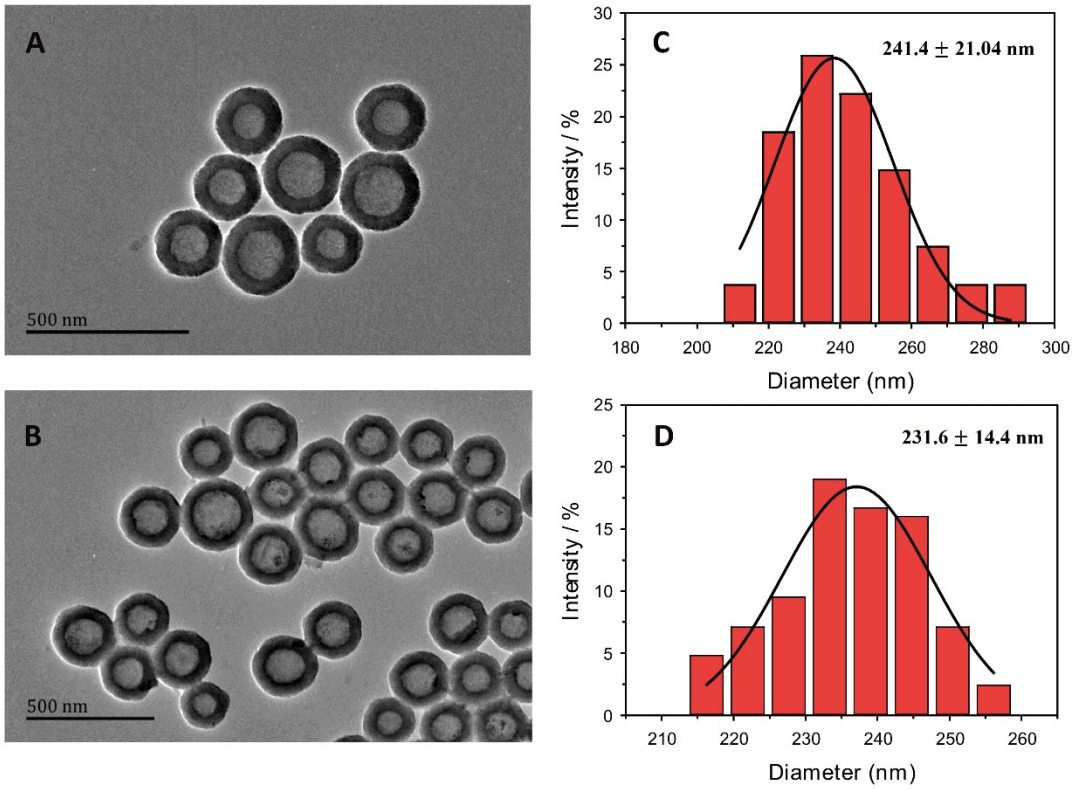

Fig. S5. TEM images of R-P-AFN (A), S-P-AFN (B) and the corresponding diameter distribution of R-P-AFN (C), and S-P-AFN (D).

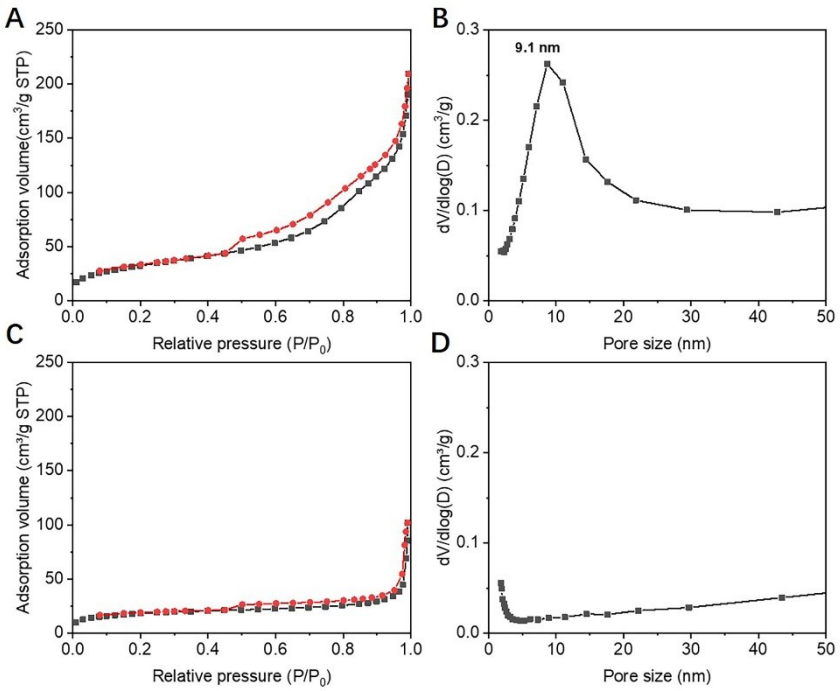

Fig. S6. Nitrogen sorption isotherm and pore size distribution curve of R-MSHSs (A,B) and S-SHSs (C,D).

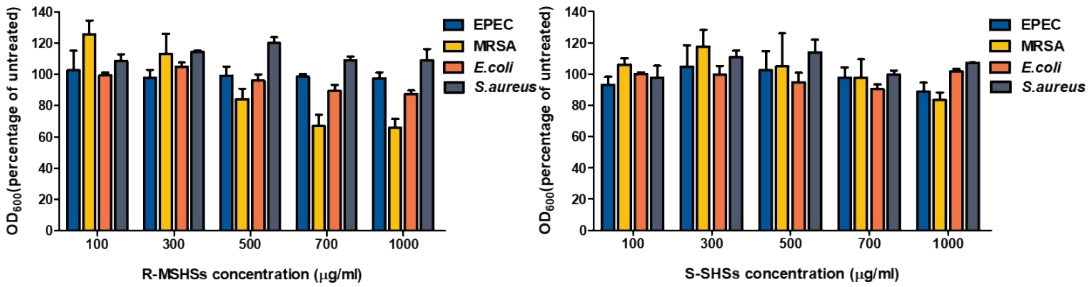

Fig. S7. Dose dependent toxicity of silica nanospheres against bacteria.

Table S3. Antibacterial activity of ILs and PILs

Antimicrobial agents	Minimum Inhibitory Concentration (mM)			
	EPEC	*MRSA*	*E. coli*	*S. aureus*
ILs	40.7	29.8	216.3	51.5
PILs	0.034	0.05	0.40	0.02

* The MIC values were determined as the lowest concentrations of the tested agent that inhibited the growth of *E. coli, S. aureus, EPEC and MRSA* by 50%, respectively. All data are presented as the average of three replicates (n=3).

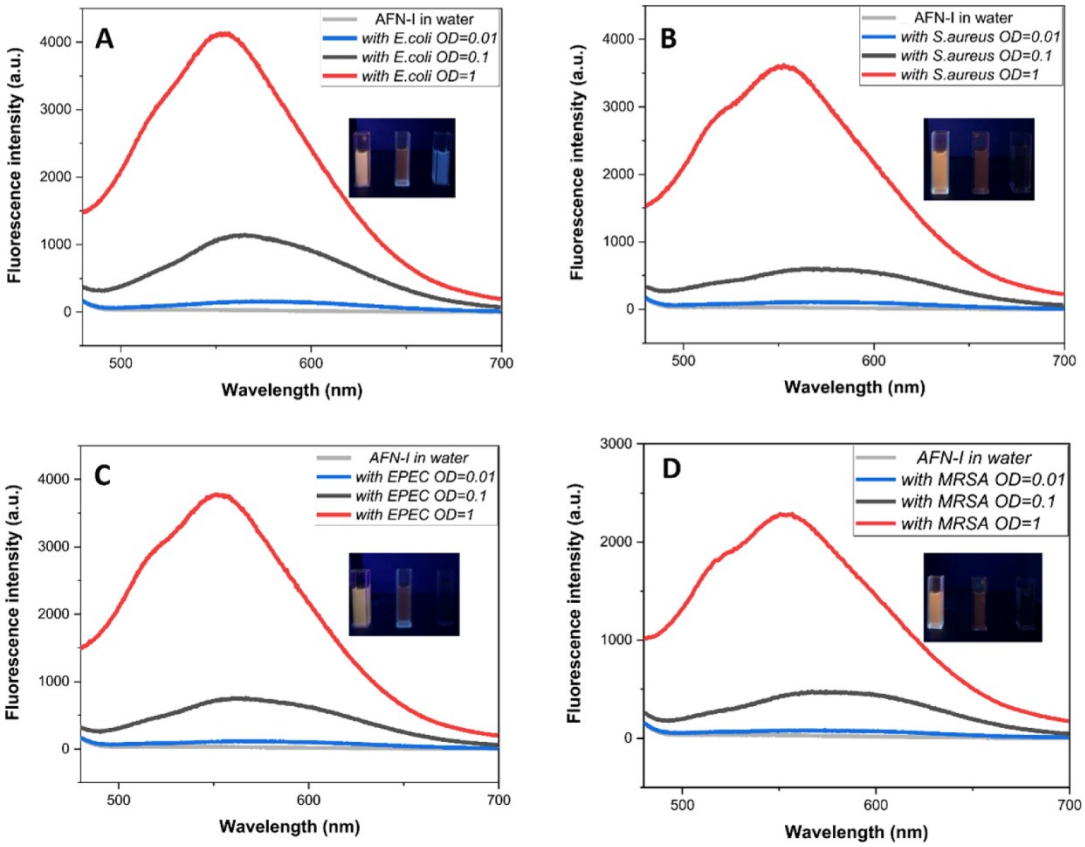

Fig. S8. Fluorescence spectra of AFN-I after incubation with *E. coli* (A) *S. aureus* (B) *EPEC* (C) *MRSA* (D) at different concentrations. Inserts: Photographs of different concentrations of *E. coli*, *MRSA*, *S. aureus*, *EPEC* mixed with AFN-I [10 μM] taken under 365 nm irradiation.

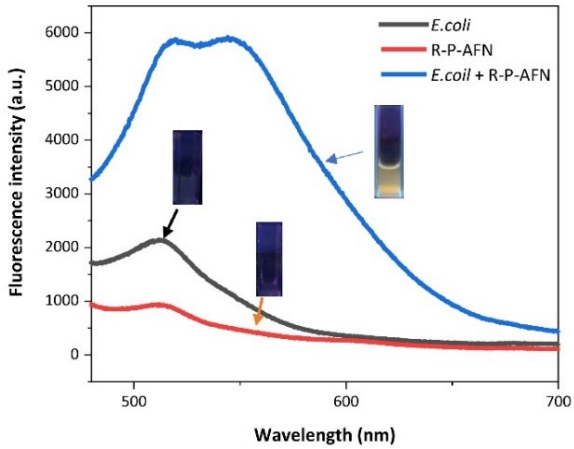

Fig. S9. Fluorescence spectra of bacteria *E. coli*., R-P-AFN and their mixture (*E. coli.*, 1x10^9 CFU mL^{-1}, R-P-AFN 0.3mg/mL). Insets: photographs of *E. coli*, R-P-AFN in water and *E. coli* with R-P-AFN taken under 365 nm irradiation.

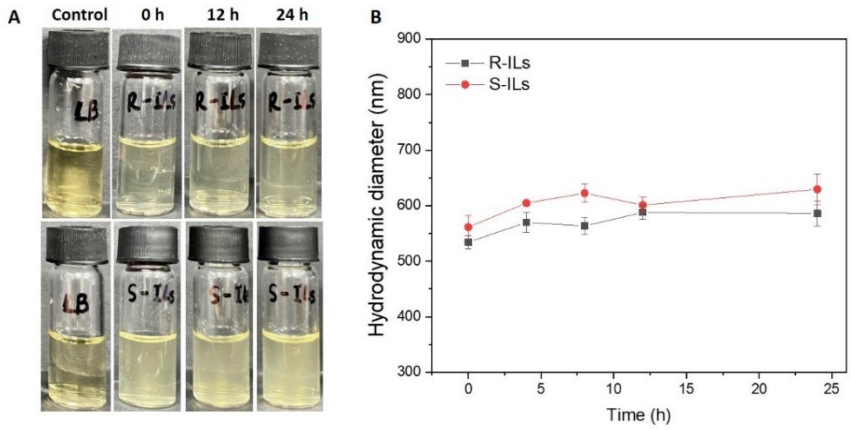

Fig.S10. Photograph of nanocomposites in LB medium (A) and their corresponding hydrodynamic diameter (B).

References

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