

Supporting information

Aggregation-Induced Emission of 2D Protein Supramolecular Nanofilm with Emergent Functions

Ruirui Liu ^a, Jiangbo Jing ^b, Song Zhang ^b, Ke Wang ^c, Bin Xu ^{* b}, Wenjing Tian ^b & Peng
Yang^{*a}

^aR. Liu, Prof. P. Yang, Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710119, China

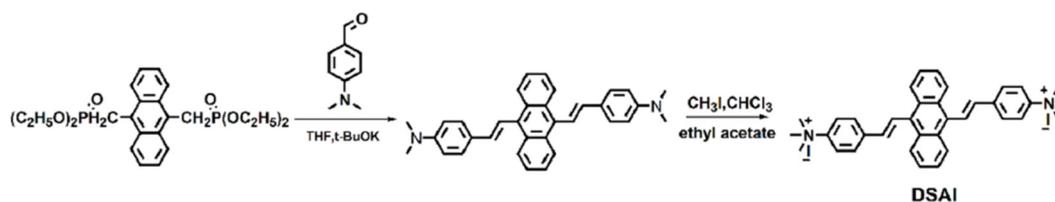
^bJ. Jing, S. Zhang, Prof. B. Xu, Prof. W. Tian, State Key Laboratory of Supramolecular Structure and Materials, Jilin University, Qianjin Street No. 2699, Changchun 130012 China,

^cProf. K. Wang, School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an 710061, China

Experimental Procedures

1. Synthesis of a 9,10-distyrylanthracene with two ammonium groups (DSAI).

DSAI was synthesized by a literature method (Scheme S1).^[1, 2]



Scheme S1. The synthesis of DSAI.

2. The preparation and characterization of DSAI@PTL coating.

2.1 The preparation of the DSAI@PTL buffer.

In order to obtain the DSAI@PTL, the phase transition buffer of DSAI@lysozyme was freshly prepared by mixing the stock buffer of lysozyme (2 mg/ml in 10 mM HEPES buffer at pH 7.2), DSAI (0.1 mg/ml dispersed in water) and TCEP buffer (50 mM TCEP in 10 mM HEPES buffer at pH 5.0) at the volume ratio of 1:0.2:1. Unless otherwise specified, the typical condition for lysozyme buffer is 2 mg/ml lysozyme in 10 mM of HEPES buffer, pH 7.2 and the typical condition for TCEP buffer was 50 mM TCEP in 10 mM of HEPES buffer, pH=5.0 (adjust the pH by 5 M NaOH). A lysozyme concentration higher than 2 mg/ml was not utilized since the concentration of 2 mg/ml was enough to offer a dense and smooth nanofilm on a surface that had a 50 nm in thickness and fully covered the underlying substrate. The assembly of lysozyme and DSAI was initiated spontaneously upon the mixing of them with TCEP added, and the DSAI@PTL product in the form of 2D DSAI@PTL nanofilm was formed at the solution surface after 10-60 minutes.

2.2 The attachment of DSAI@PTL nanofilm via the lift-up transferring.

The preparation strategy was given in **Figure 1A**. In brief, the DSAI@PTL solution was dropped on a hydrophobic surface such as a Petri dish substrate to ensure the solution with a certain thickness. Then the solution on the substrate was incubated in a humid environment for a given time (generally for 60 min) at room temperature. Then, a free-floating DSAI@PTL nanofilm that covered completely the whole solution surface area could be formed at the air/liquid interface. By seriously transferring the substrate with the DSAI@PTL solution held into a tank with ultrapure water inside, the DSAI@PTL nanofilm was then floated on the surface of the ultrapure water. The target substrate was lifted from the bottom of the tank to pass through the DSAI@PTL nanofilm. During this process, the DSAI@PTL nanofilm was inherently transferred onto the substrate surface. In the lift-up process, a pH higher than 5 in the TCEP buffer before mixing with DSAI@lysozyme was recommended since a pH lower than 5 easily produced a very thin nanofilm that was hardly observed by eyes. The coated material was then dried at room temperature.

2.3 The attachment of DSAI@PTL nanofilm via in situ soaking.

The preparation strategy was given in **Figure 1B**. Simple soaking is enough to transfer efficiently the DSAI@PTL nanofilm onto the immersed target substrate surface to form a solid-supported nanofilm. For this aim, the target material was fully contacted with the freshly prepared DSAI@PTL buffer, and after incubating for a given time (typically within 5-50 min), a conformal PTL nanofilm was stably attached onto the immersed solid surfaces. The coated material was then taken out and rinsed by clean water to wash away remaining native lysozyme and other salts. The coated material was then dried by nitrogen for next purpose.

2.4 The attachment of the DSAI@PTL nanofilm via the contact-printing.

The preparation strategy was given in **Figure 1C**. In this method, the DSAI@PTL nanofilm was firstly floated on the ultrapure water surface, and then transferred onto an agarose gel (prepared by dissolving 1% wt agarose in boiling water and cooling the solution to room temperature at ambient conditions) surface with a defined shape by the lift-up transferring technique (2.3). For transferring the DSAI@PTL nanofilm onto a target substrate, the DSAI@PTL nanofilm-coated gel stamp was conformly touched with the underlying substrate, and after a gentle pressure on the stamp for 5 seconds, the gel stamp was seriously peeled off from the substrate. In this way, the DSAI@PTL nanofilm originally carried by the gel stamp would be transferred onto the underlying target substrate.

2.5 The attachment of the DSAI@PTL nanofilm via direct writing or spraying.

The preparation strategy was given in **Figure 1D**. In this method, the DSAI@PTL buffer could be written by a writing brush or sprayed on the substrate through a watering pot and after that the DSAI@PTL nanofilm could be patterned easily through a templates. For this aim, the freshly prepared DSAI@PTL solution was infused into a pen or a spray bottle, and then one can use the pen to write any security label on a substrate surface with designed pattern. By placing the templates on the substrate to be modified, one can use a spray bottle to spray any type of security label on the substrates. In this process, due to the rapid phase transition process, the patterned DSAI@PTL nanofilm could be formed onto the substrate after spraying. If the substrate is allowed, the reaction residue can be washed away by ultra-pure water. In this method, pH maintained at 5 in the TCEP buffer before mixing with DSAI@lysozyme (2 mg/ml lysozyme, concentration of DSAI dependent on requirement as show in **Figure S4**) was recommended since under this condition the system will maintain a stable and non-separated state for several days.

3. Antimicrobial test for the DSAI@PTL coating on glass slides.

E.coli and *S.aureus* were selected as model bacteria to test the antibacterial activities of the DSAI@PTL nanofilm. Firstly, bacterial strains were cultivated in a MHB medium for 16 hr at 37°C, and then the overnight bacterial suspension was harvested by centrifugation, washed with PBS, and then diluted to concentrations of 10⁴⁻⁵ CFU/mL. *C.albicans* was similarly cultivated in YM broth medium for 18 hr at 28°C to OD₆₀₀ = 1.0. The three test inoculums were diluted to a final concentration of about 5×10⁵ CFU/mL. With sterilized 35 mm Petri dish as the container, 10 µL of the bacteria and fungi PBS suspension were then spread over each glass slide coated with the DSAI@PTL (prepared by a strategy shown in **Figure 1B**), respectively, which were then covered with another slide coated with the DSAI@PTL and gently pressed to spread the inoculums over the entire surface of the slides. The inoculated slides were incubated at 37°C (bacteria) or 28°C (fungi) for 2 hr. Then 2 ml PBS were added into the 35 mm Petri dishes to wash off the survived microbes, and the washing PBS was 10-fold diluted and plated for colony counting. Killing ratios were calculated by the formula: Killing ratio = (control CFU – treated CFU) / control CFU × 100%. This test was independently repeated at least in triplicate.

4. Congo red staining test.

The DSAI@PTL-coated glass slides (prepared by the strategy shown in **Figure 1B**) were immersed in the Congo red solution (1 mg/ml) for 1 hr. As the control experiment, glass slide treated by native lysozyme (2 mg/ml) and the PTL-coated glass slide (prepared by the strategy shown in **Figure 1B**) were respectively immersed in the Congo red solution (1 mg/ml) for 1 hr. After that, all of glass slide were taken out and rinsed several times with the deionized water and dried directly by nitrogen.

5. Thioflavin T (ThT) staining test.

The fluorescent dye ThT was used to investigate the formation of amyloid structure during the phase transition process. The lysozyme (2.0 mg/ml) was dissolved in HEPES buffer (10 mM, pH 7.4) containing 100 µM of ThT. After the mixture

was incubated in the dark for 60 min, equivoluminal TCEP buffer (50 mM pH at 4.5) was added. The emission spectra at 484 nm of the samples were successively measured by fluorescence spectrophotometer with the excitation wavelength at 440 nm. The bandwidths of excitation and emission slits were set as 5 nm.

6. ANS staining test.

1-Anilino-naphthalene 8-sulfonate (ANS) as a hydrophobic fluorescent probe was used to explore the exposure of the hydrophobic residues during the phase transition process. The lysozyme (2.0 mg/ml) was dissolved in HEPES buffer (10 mM, pH 7.4) containing 200 μ M of ANS. After the mixture was incubated in the dark for 30 min, equivoluminal TCEP buffer (50 mM pH at 4.5) was added. The emission spectra at 480 nm of the samples were successively measured by fluorescence spectrophotometer with the excitation wavelength at 398 nm. The bandwidths of excitation and emission slits were set as 5 nm.

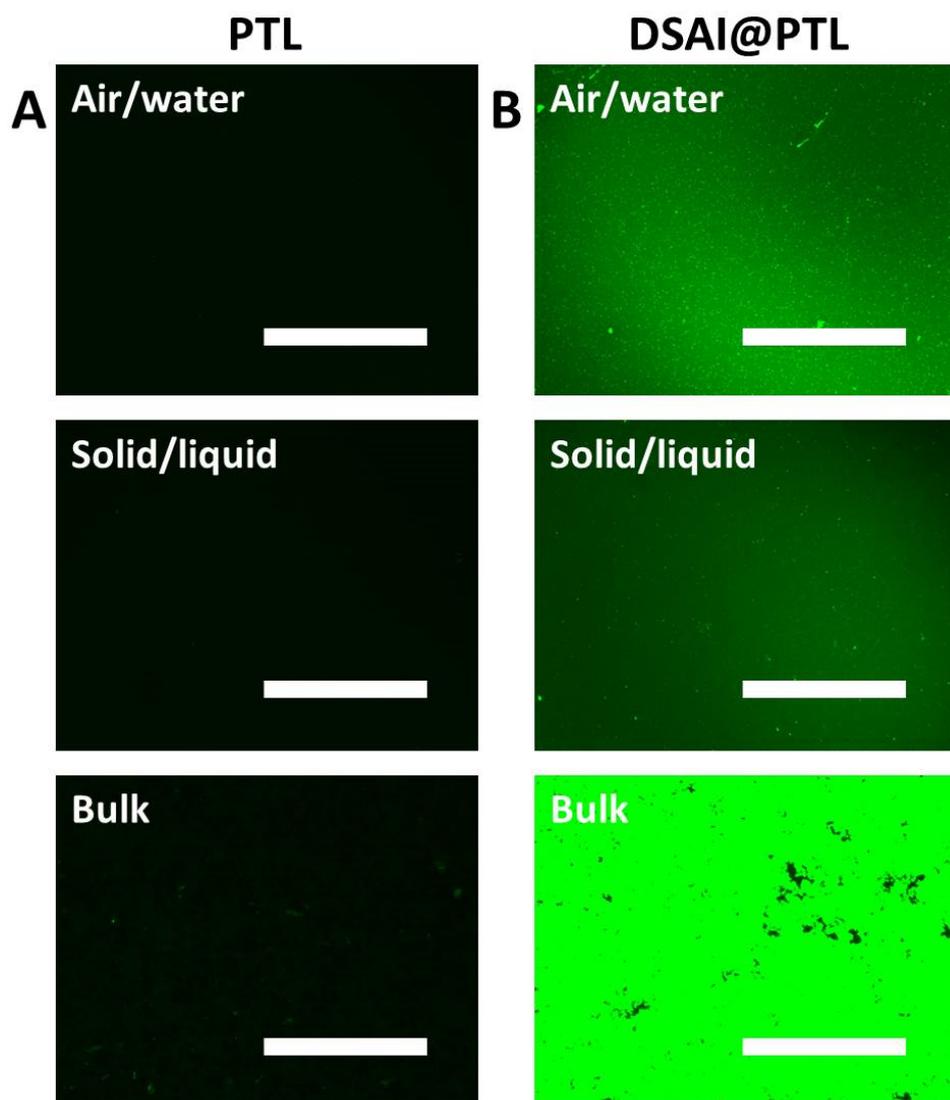


Figure S1. Fluorescence difference between the PTL and DSAI@PTL materials. Confocal laser scanning images of (A) the PTL nanofilms (at air/water interface, solid/liquid interface, respectively) and the PTL bulk product; (B) the DSAI@PTL nanofilms (at the air/water interface or solid/liquid interface, respectively) and the DSAI@PTL bulk product. The scale bar is 300 μm .

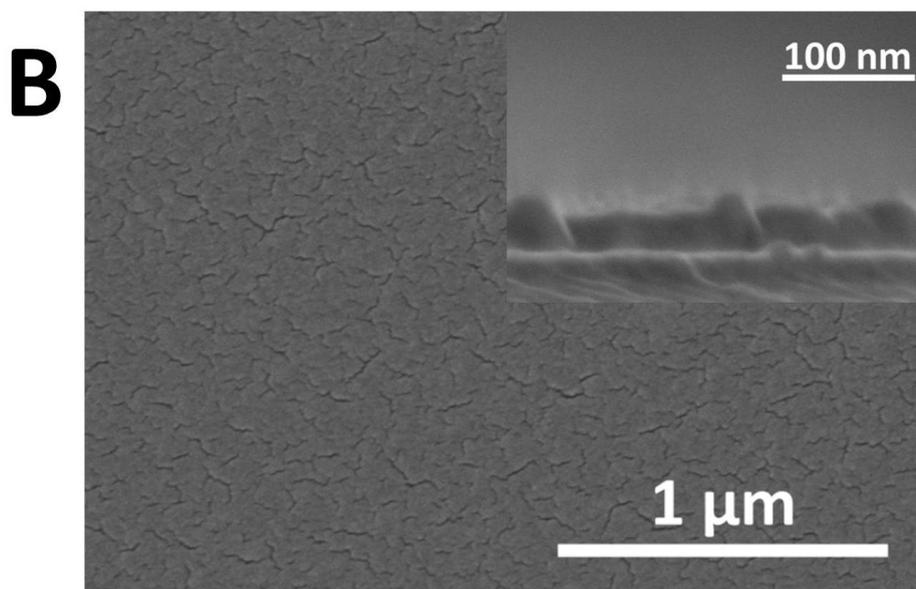
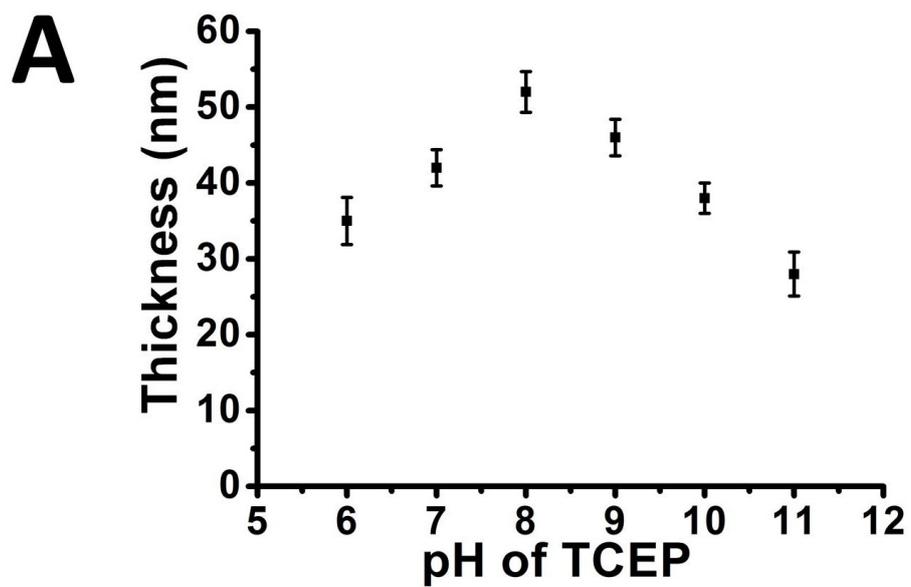


Figure S2. The effect of the pH of TCEP buffer on the thickness of the DSAI@PTL nanofilm. In (A), the nanofilms were prepared with 2 mg/ml lysozyme and 50 mM TCEP at different pH. (B) SEM image of the DSAI@PTL nanofilm by using the condition of 2 mg/ml lysozyme and 50 mM TCEP at pH 6.0. Inset in (B) was the cross-section of the DSAI@PTL nanofilm. 0.1 mg/ml DSAI was used in (A-B), and the volume of lysozyme: TCEP: DSAI ratio was 10:10:2.

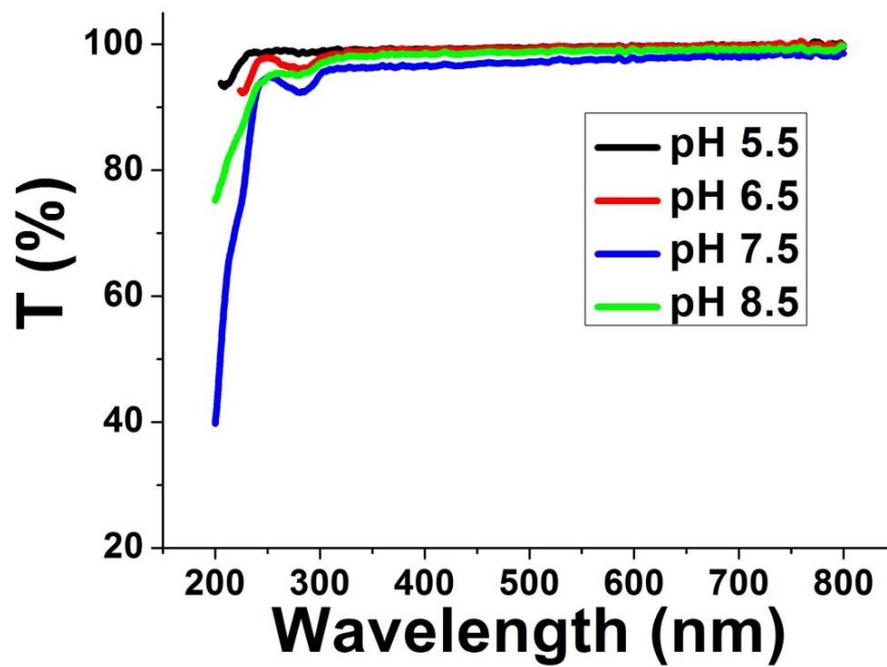


Figure S3. The optical transmittance of the DSAI@PTL nanofilm on glass. These nanofilms were prepared by the strategy given in **Figure 1B** with 2 mg/ml of lysozyme and 50 mM of TCEP at different pH.

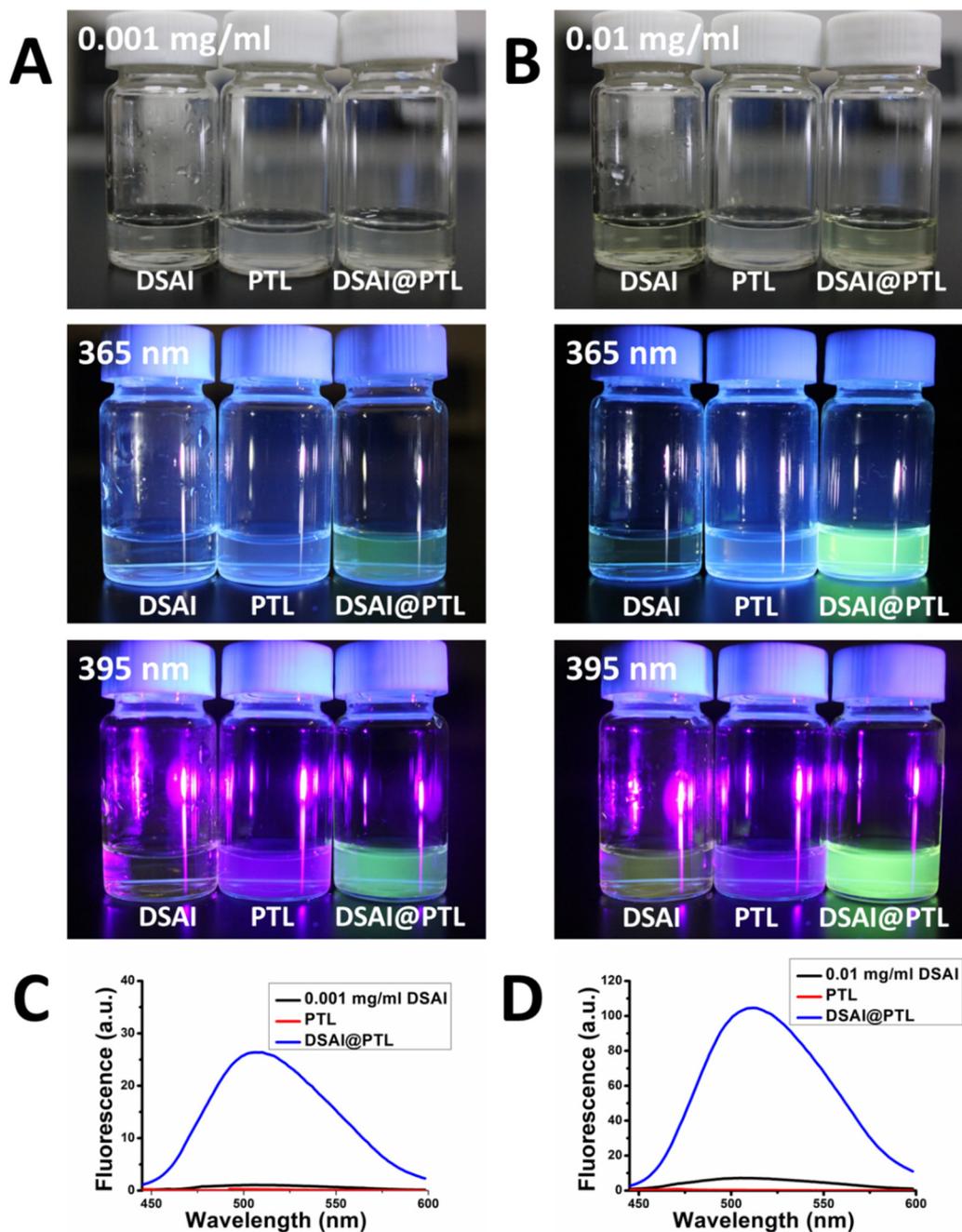


Figure S4. Photographic images (A, B) and corresponding fluorescent spectra (C, D) of free DSAI, free PTL and DSAI@PTL solutions under ambient light (top), 365 nm UV lamp (middle) and 395 nm UV lamp (bottom) respectively. (A) 0.001 mg/ml DSAI. (B) 0.01 mg/ml DSAI. (C) The fluorescence spectra of free DSAI, free PTL and DSAI@PTL solution shown in (A). (D) The fluorescence spectra of free DSAI, free PTL and DSAI@PTL shown in (B). (A, C) 0.001 mg/ml DSAI. (B, D) 0.01 mg/ml.

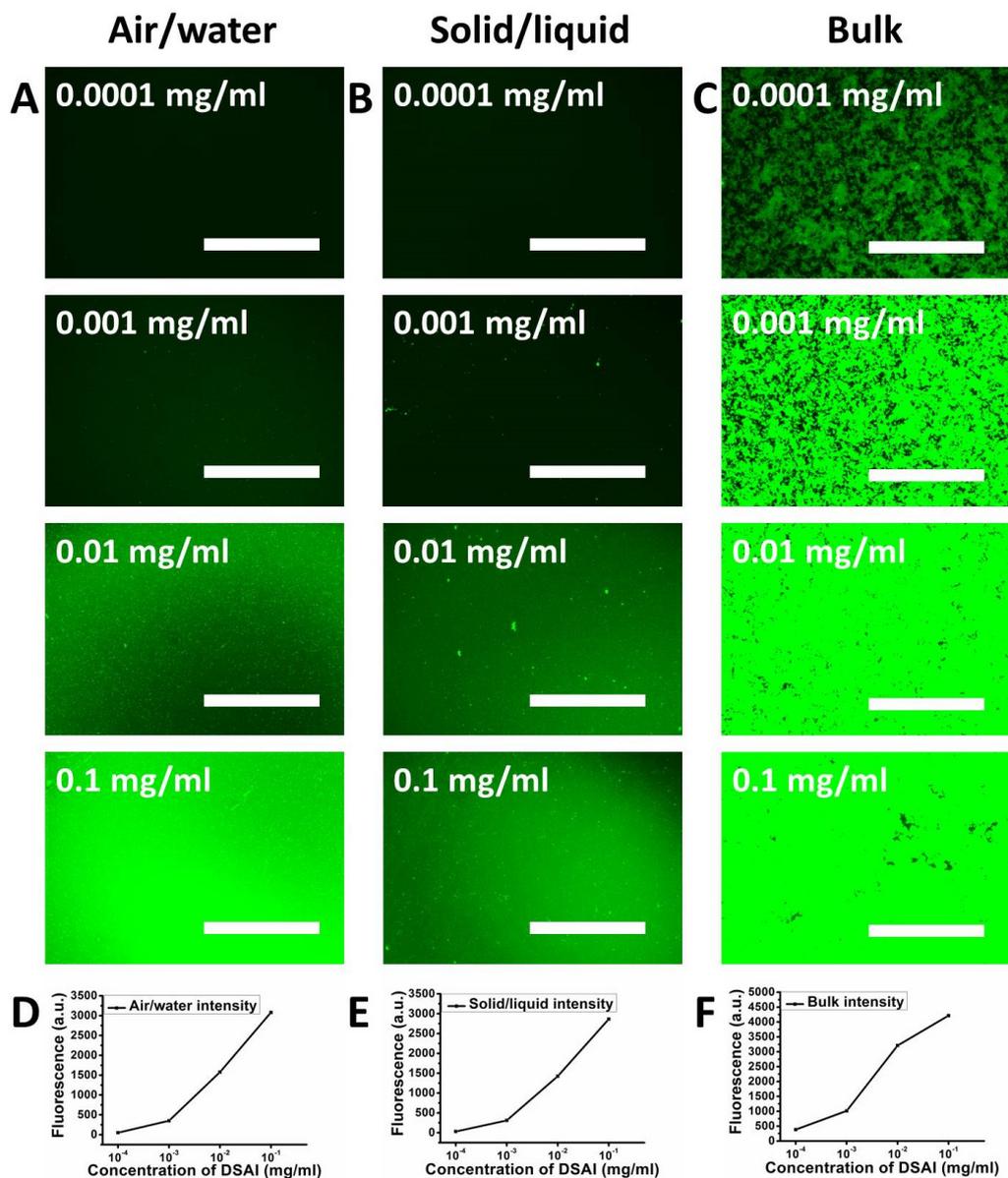


Figure S5. The effect of DSAI concentration on the fluorescence of the DSAI@PTL materials. (A-C) Confocal laser scanning images of the DSAI@PTL materials at (A) air/water interface or (B) solid/liquid interface, and (C) the PTL bulk product. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 7.0) and different concentration of DSAI were used in these cases. The volume ratio was 10:10:2. (D-F) The effect of the DSAI concentration on the fluorescence intensity shown in (A-C). The scale bar is 300 μm .

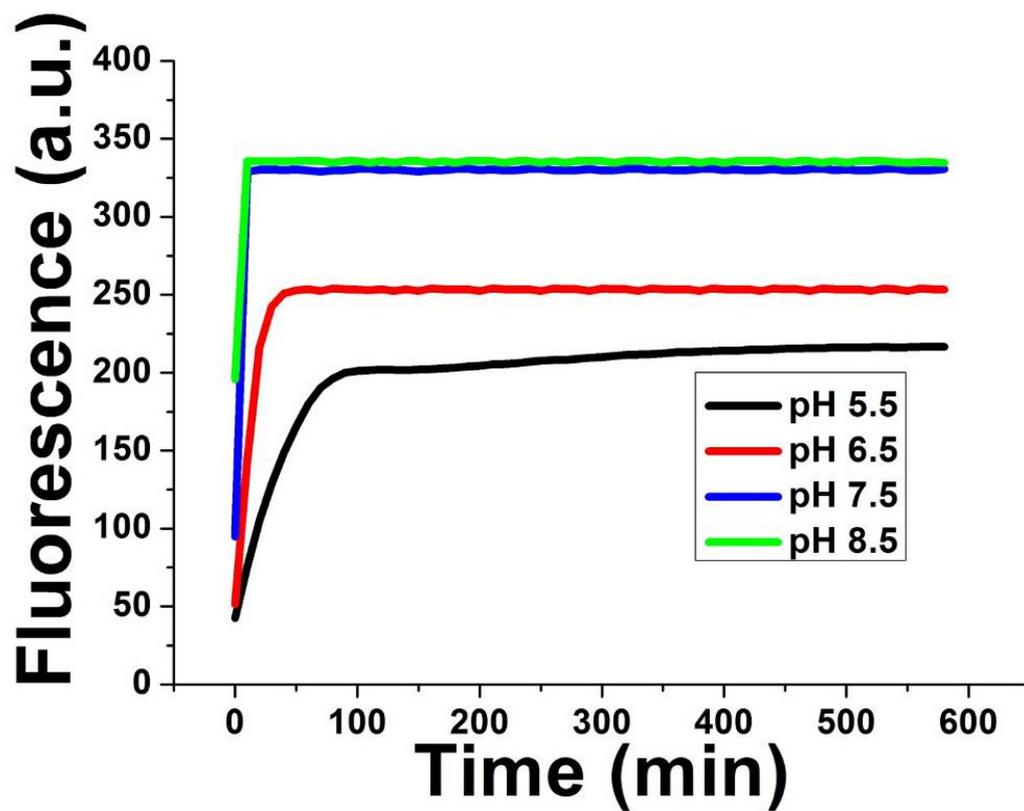


Figure S6. The change of the fluorescence intensity of DSAI@PTL buffer at 520 nm as a function of the phase transition time at four different pH values of TCEP. 2 mg/ml of lysozyme, 50 mM of TCEP and 0.1 mg/ml of DSAI were used in these cases. The volume ratio was 10:10:2.

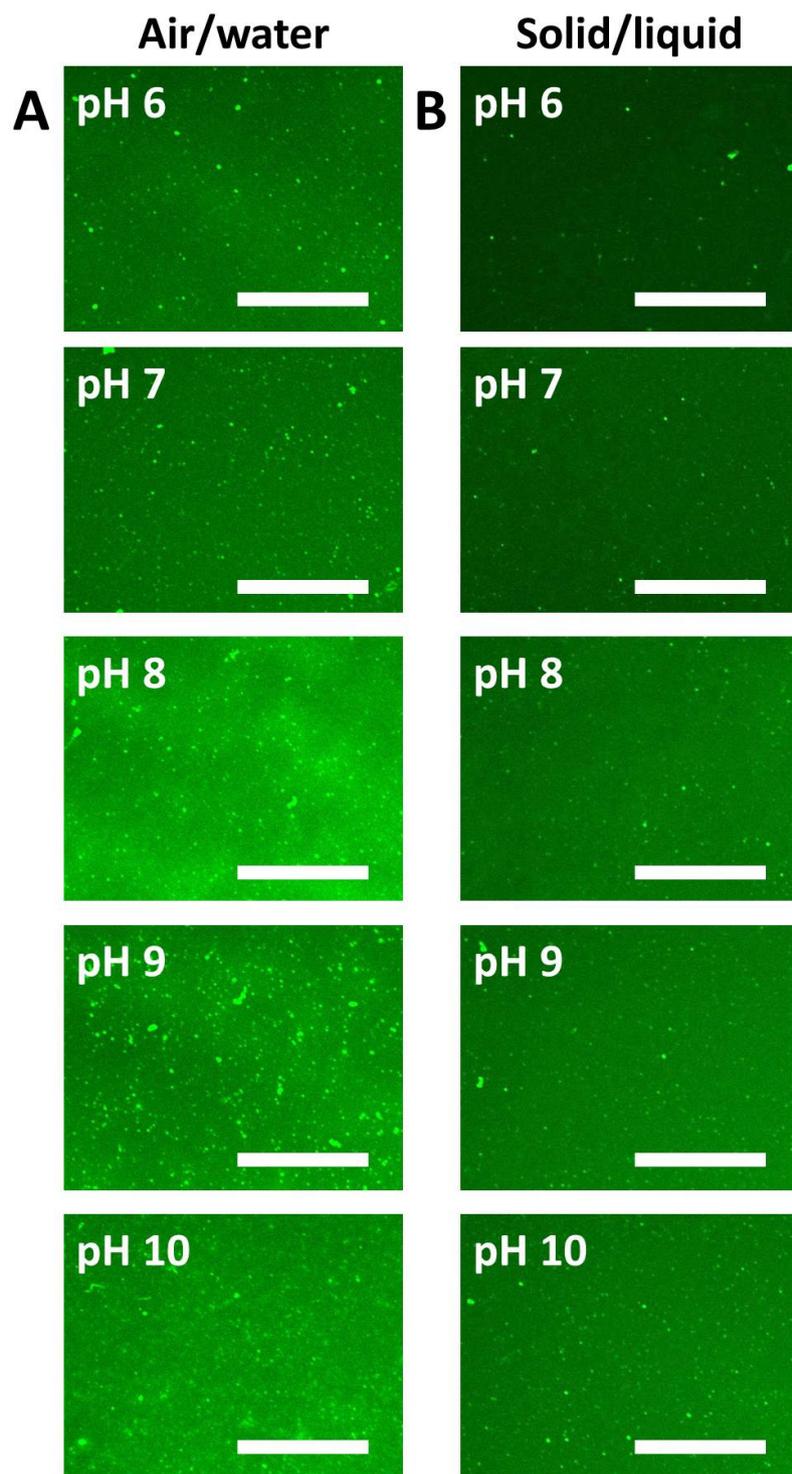


Figure S7. The effect of pH of TCEP buffer on the fluorescence of the DSAI@PTL nanofilm. (A-B) Confocal laser scanning images of the DSAI@PTL nanofilms at (A) the air/water interface, and (B) solid/liquid interface. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 7.0) and 0.1 mg/ml of DSAI were used in these cases. The volume ratio was 10:10:2. The scale bar is 300 μm .

A	Concentration	Quantum yield (%)	Fluorescence lifetime
DSAI@PTL solution	0.1 mg/ml DSAI	17.8	2.46 ns
	2 mg/ml lysozyme 50 mM TCEP pH 5.0		7.04 ns
DSAI@PTL film	0.1 mg/ml DSAI	11.2	1.81 ns
	2 mg/ml lysozyme 50 mM TCEP pH 6.5		4.79 ns

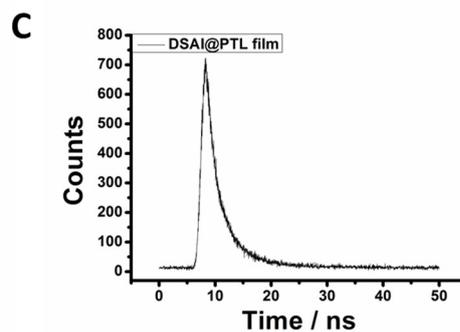
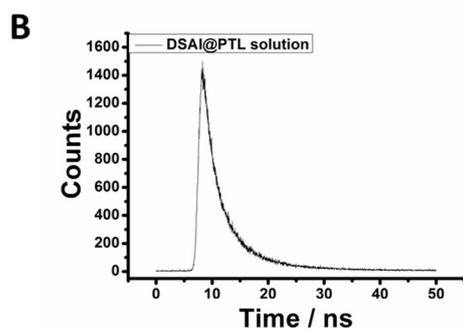


Figure S8. (A) Photophysical characterizations for the DSAI@PTL solution and hybrid film at room temperature. (B) Luminescence decay curve of the DSAI@PTL solution. (C) Luminescence decay curve of the DSAI@PTL hybrid film.

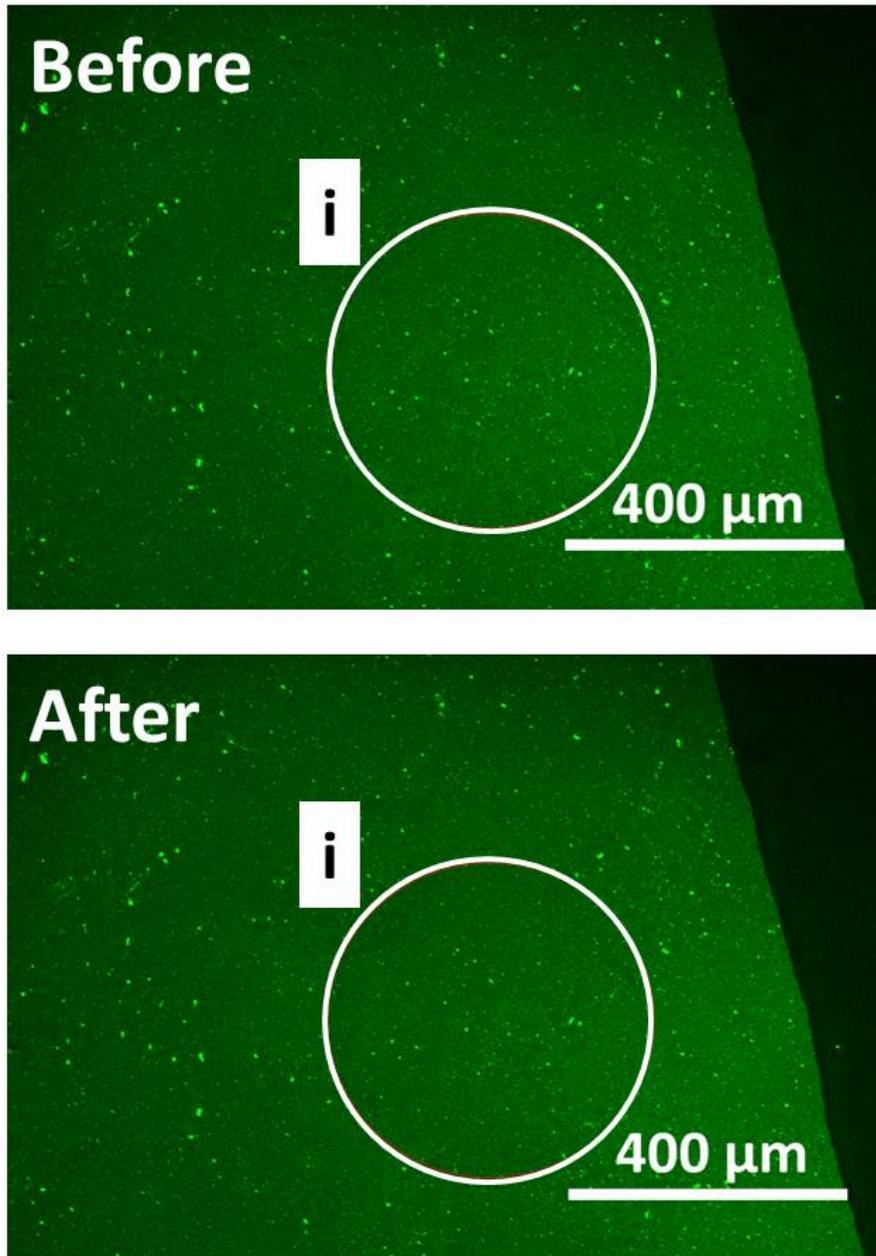


Figure S9. Confocal laser scanning images of the DSAI@PTL nanofilm after 405 nm laser irradiation treatment for 20 seconds (photobleaching process). The white circle area (i) was the irradiation region by 405 nm laser.

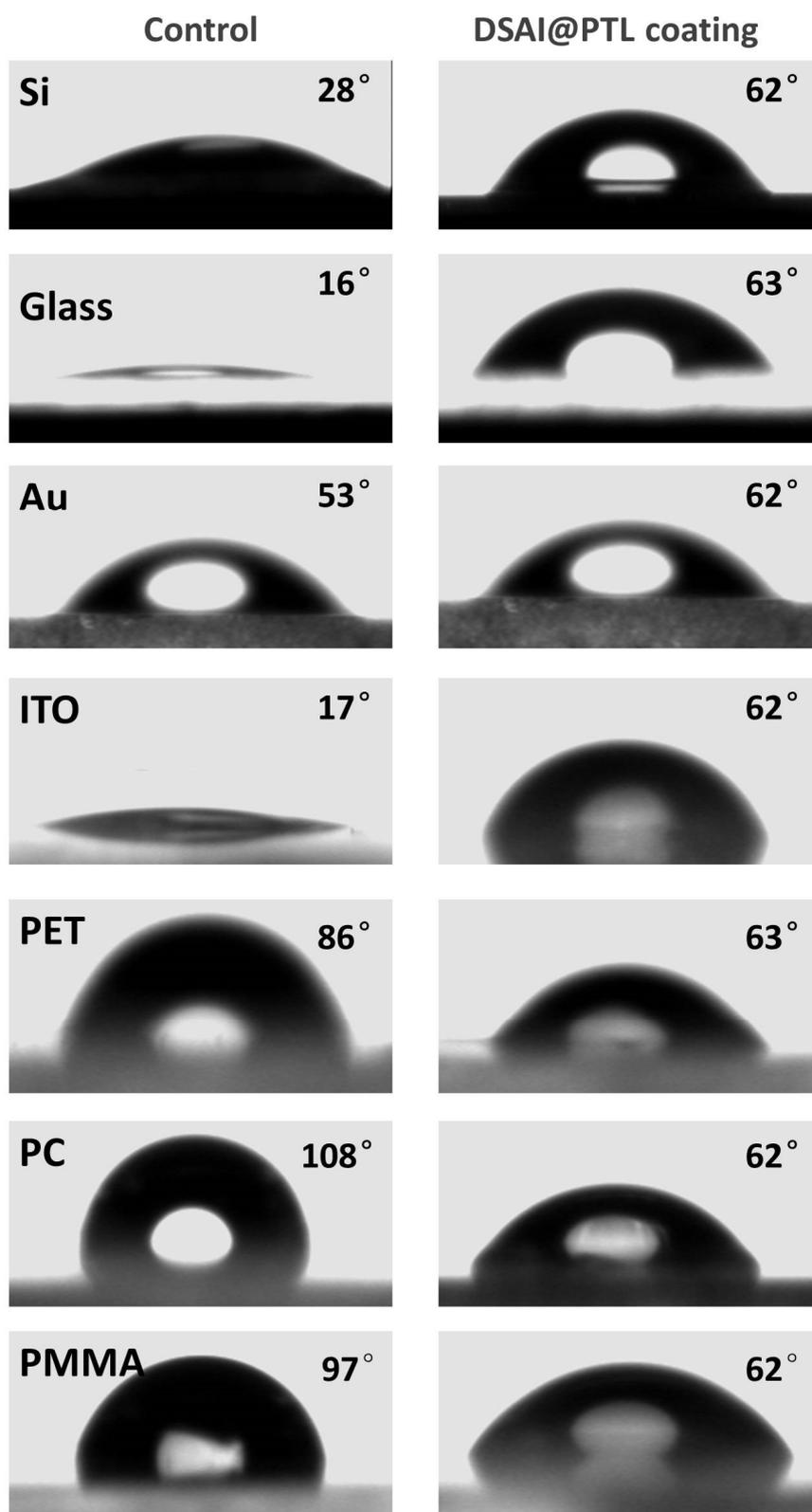


Figure S10. Pictures of water droplets on several bare substrates (left), the DSAI@PTL nanofilm-coated substrates (right). The DSAI@PTL nanofilm was prepared by the strategy given in **Figure 1B**. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 7.0), and 0.1 mg/ml of DSAI were used in these cases. The volume ratio was 10:10:2.

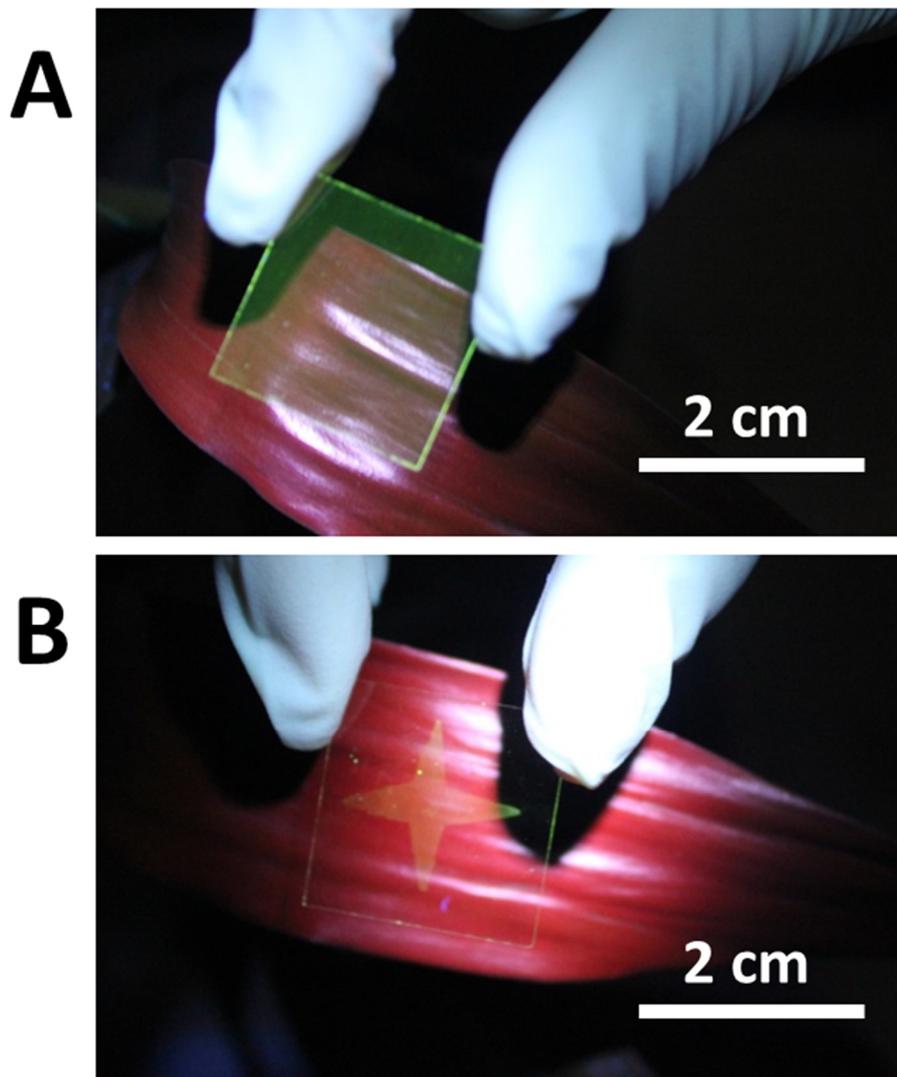


Figure S11. (A) Photographic image to show the fluorescence of the DSAI@PTL nanofilm coated on the glass under UV irradiation. (B) Photographic image displaying the fluorescence of the star-shape DSAI@PTL nanofilm coated on the glass substrate under UV irradiation. The nanofilm and pattern shown in (A) and (B) did not undergo 3M adhesive tape testing and ethanol treatment, which showed the similar morphology to the results shown in **Figure 2B, C** in the manuscript, thus indicating the robustness of the nanofilm under these treatments.

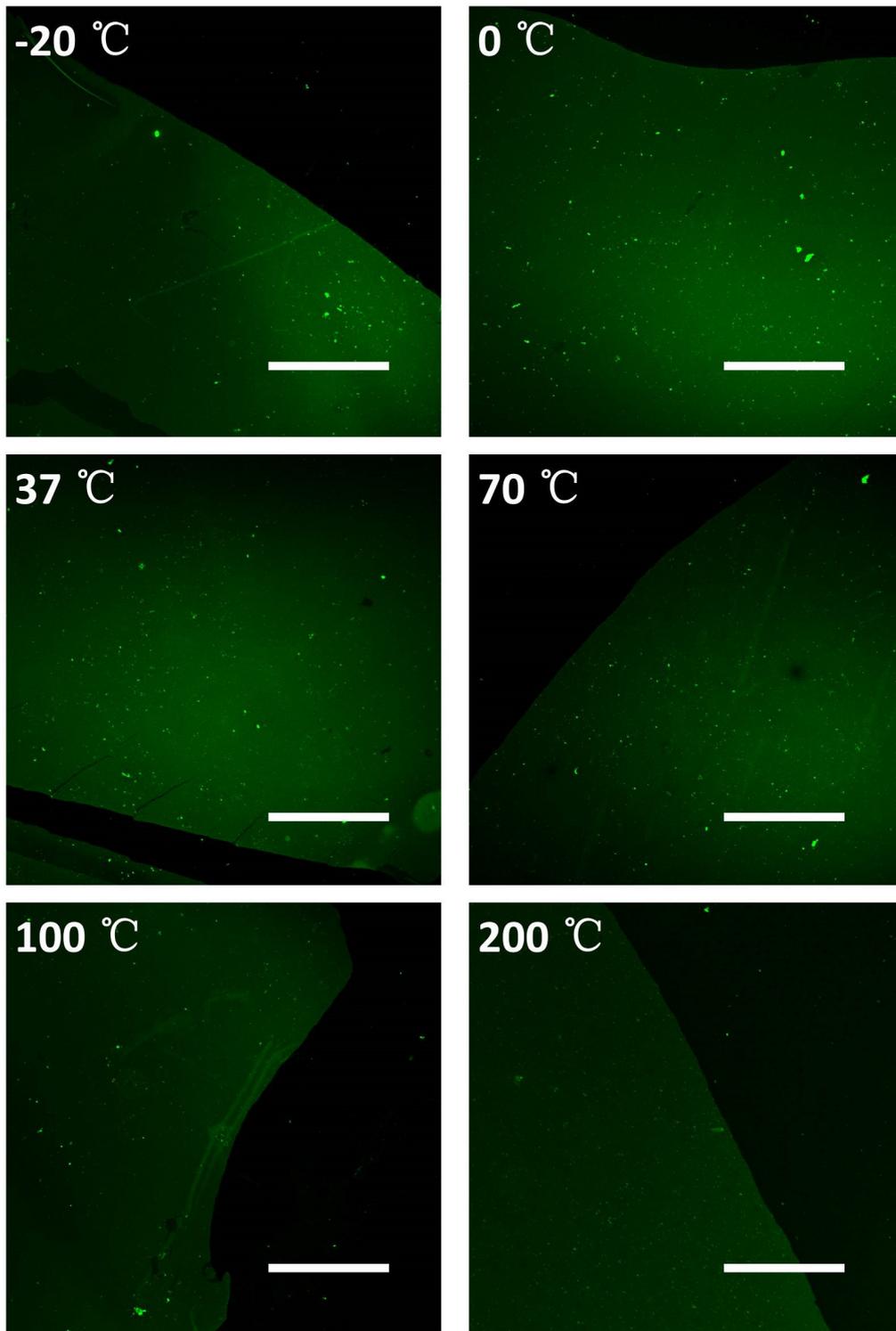


Figure S12. The thermostability tests of the confocal laser scanning images of DSAI@PTL nanofilm by cooling or heating the hybrid film at different temperature conditions for 2 hr. The scale bar is 300 μm .

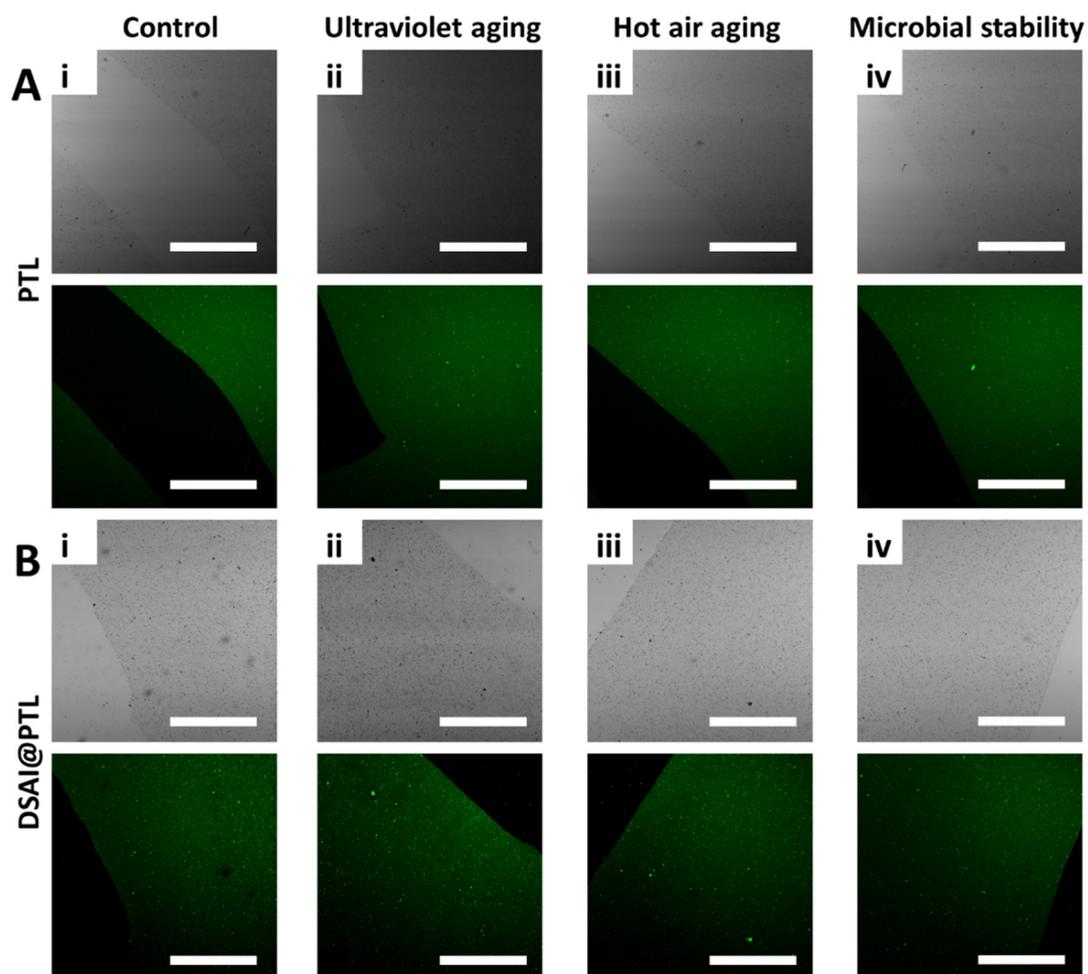


Figure S13. Weather resistance test of PTL film (A) and DSAI@PTL film (B). The fluorescence photographs of PTL films were obtained by ThT staining. Hot air aging test: we placed PTL film and DSAI@PTL film into oven at 80°C for 60 days; Ultraviolet aging test: we placed PTL film and DSAI@PTL film under the ultraviolet lamp (Power for 9 W and samples from the light source 5 cm distance) for 60 days; Microbial environmental corrosion test: we placed PTL film and DSAI@PTL film into the microorganism breeding environment. In this test, the containers containing *E.coli* (about 5×10^5 CFU/mL) and *S.aureus* (about 5×10^5 CFU/mL) were placed in the incubator at 37 °C, and the film was further placed in the test containers. The DSAI@PTL nanofilm was prepared by the strategy given in **Figure 1B**. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 7.0), and 0.1 mg/ml of DSAI were used in these cases. The volume ratio was 10:10:2.

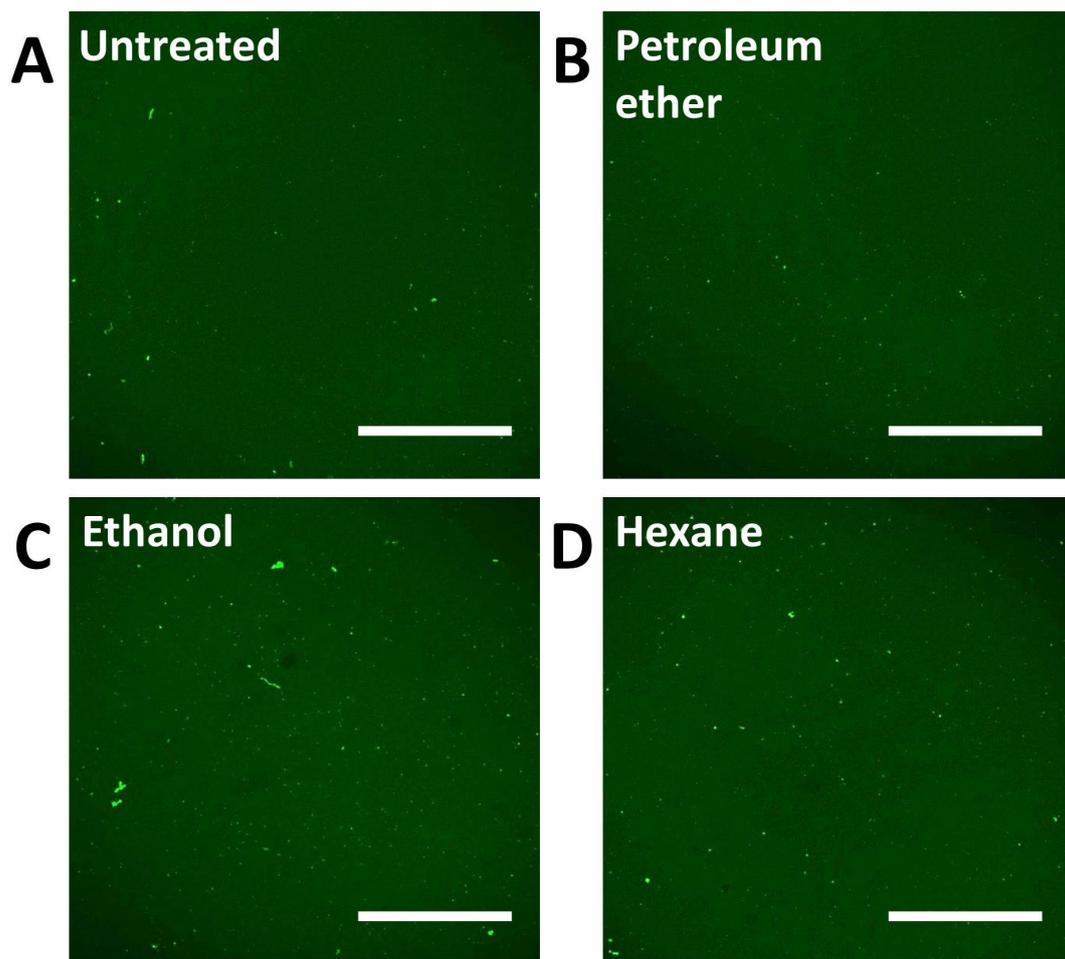


Figure S14. The stability tests of the DSAI@PTL nanofilm after the treatment by organic solvents for 2 hr. The DSAI@PTL hybrid film was directly formed on the glass surface. 2 mg/ml of lysozyme, 50 mM of TCEP and 0.1 mg/ml of DSAI were used in these cases. The volume ratio was 10:10:2. The scale bar is 300 μ m.

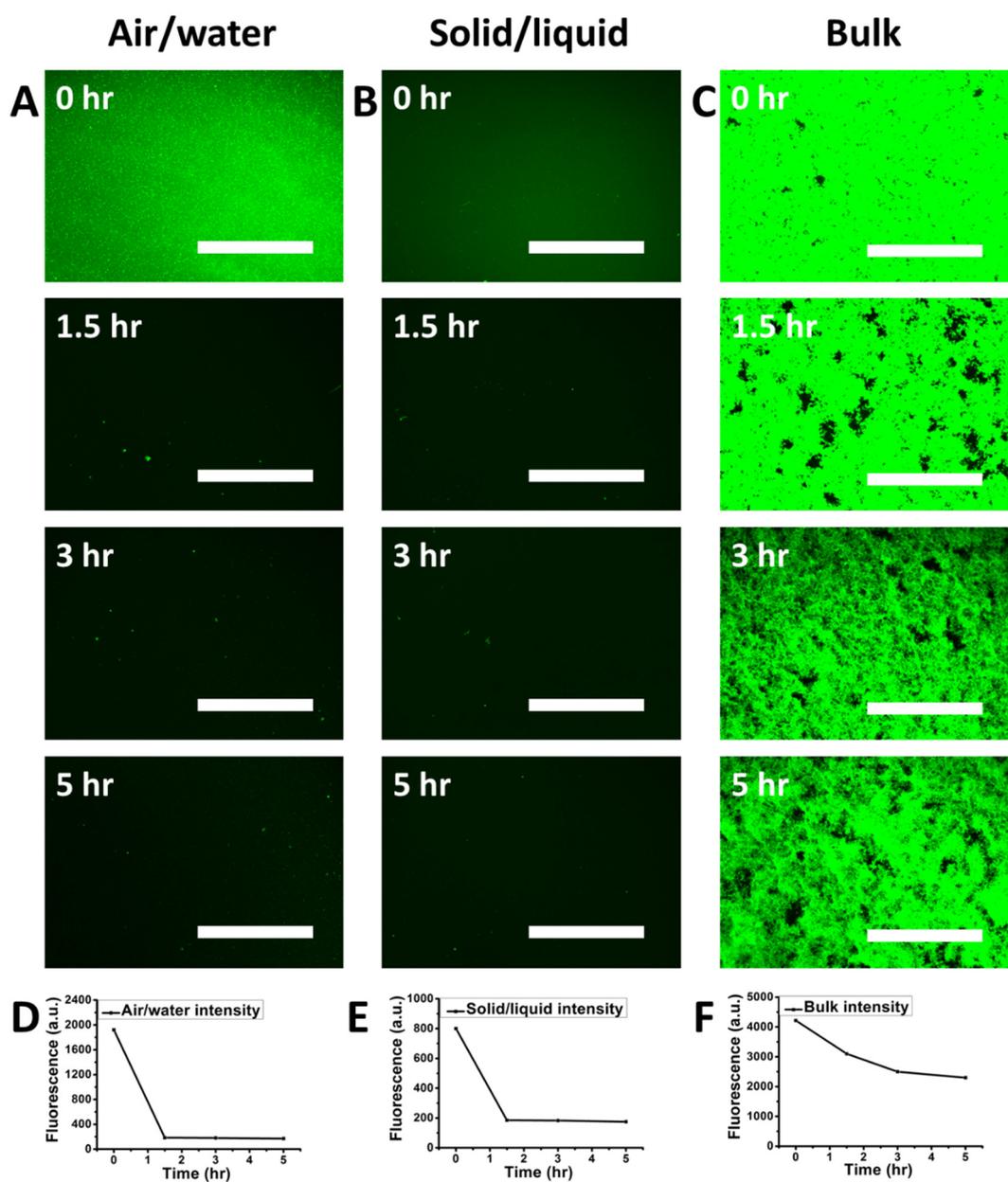


Figure S15. The release of AIE molecules from the DSAI@PTL. (A) Confocal laser scanning images of the DSAI@PTL nanofilm formed at the air/water interface after the immersion in deionized water with different time. (B) Confocal laser scanning images of the DSAI@PTL nanofilm formed at the solid/liquid interface after immersion in deionized water with different time. (C) Confocal laser scanning images of the DSAI@PTL bulk after the immersion in deionized water with different time. The scale bar is 300 μm . (D-F) The change of the fluorescence intensity shown in (A-C). The scale bar is 300 μm .

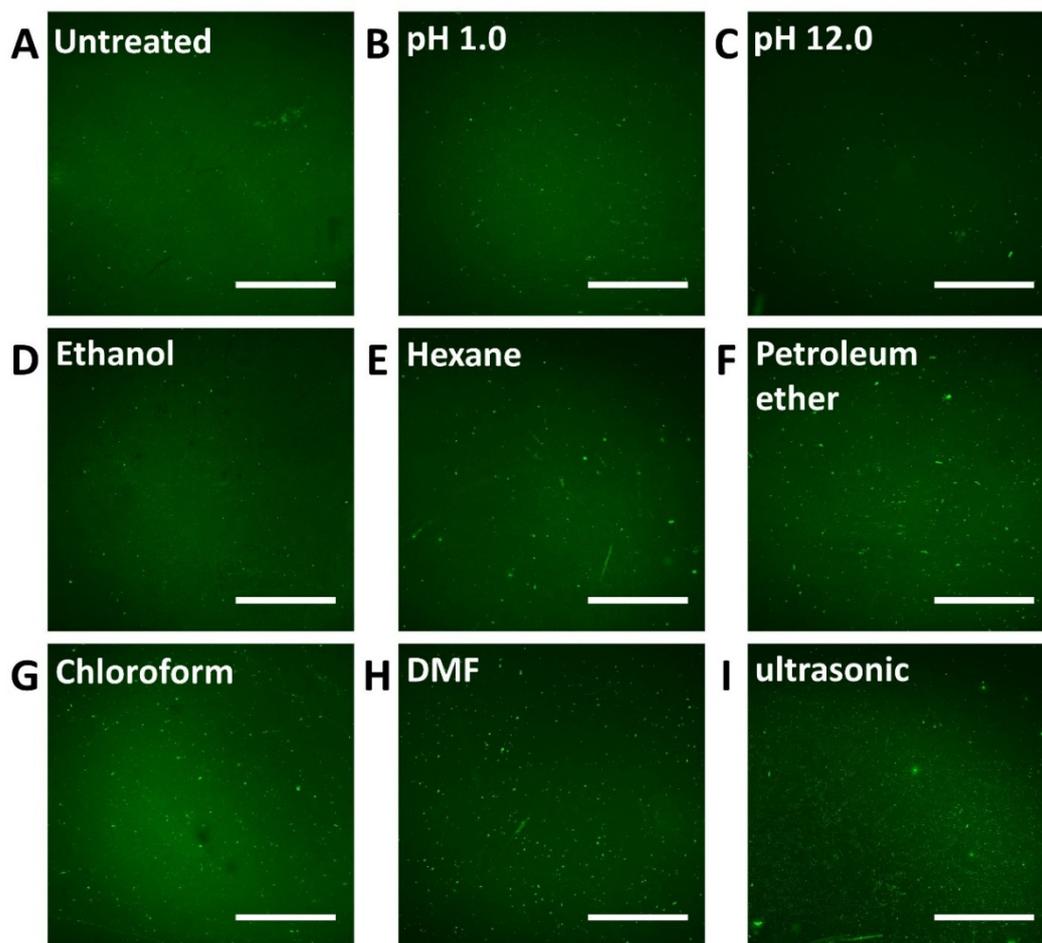


Figure S16. The stability tests of the fluorescence of the DSAI@PTL film after crosslinking through glutaraldehyde towards organic solvents, extreme pH conditions (for 2 hr) and ultrasonic. (A-I) Confocal laser scanning images of the DSAI@PTL nanofilms treated by extreme pH conditions for 2 hr (B, C), organic solvents (D to H) and ultrasonic treatment for 15 minutes (I). The DSAI@PTL film prepared by the strategy given in **Figure 1B**. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 7.0) and 0.1 mg/ml of DSAI were used in these cases. The volume ratio is 10:10:2. The scale bar is 300 μm .

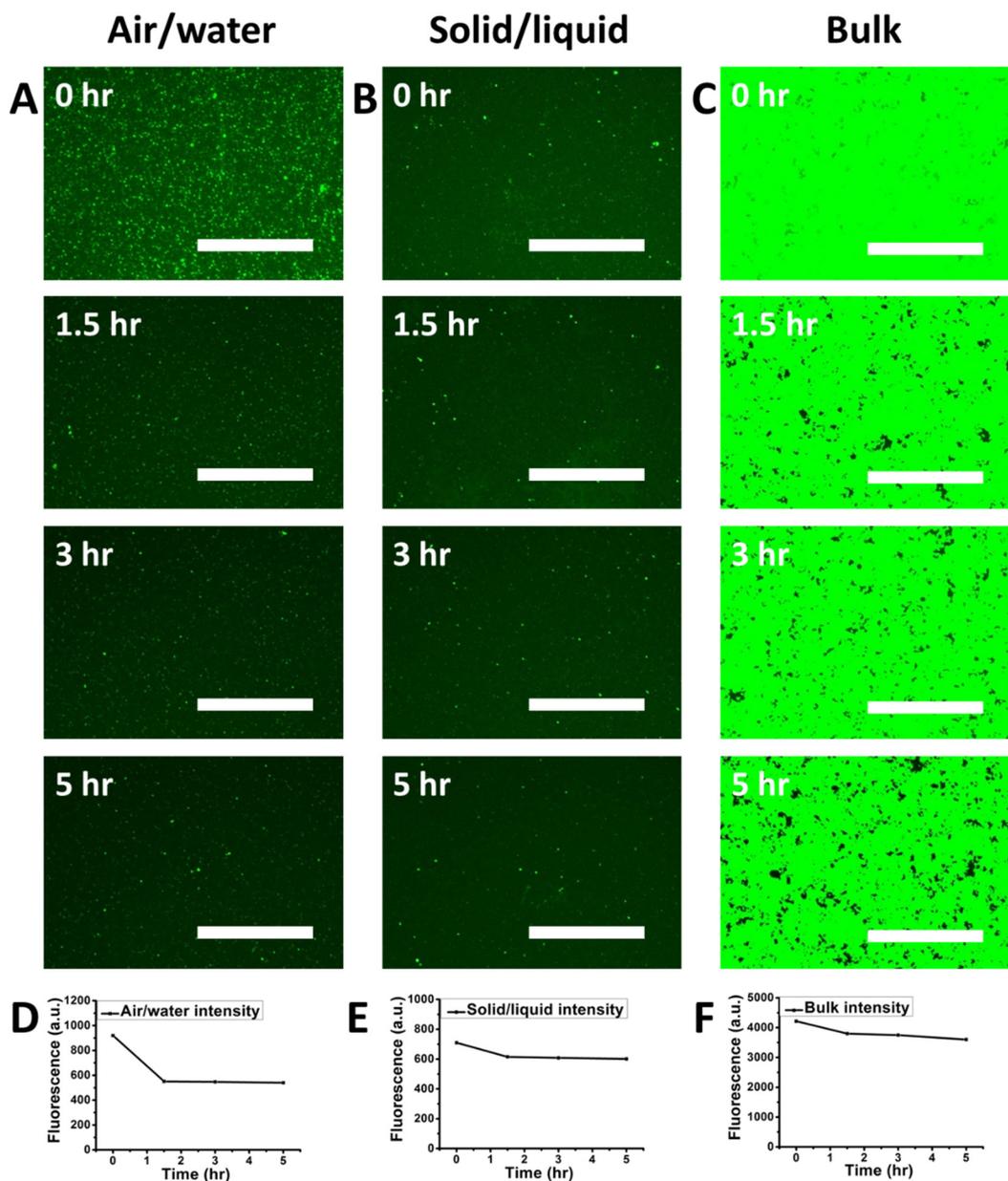


Figure S17. The release inhibition of AIE molecules from the DSAI@PTL by glutaraldehyde crosslinking. Confocal laser scanning images of the cross-linked DSAI@PTL immersed in deionized water for different time. (A) The cross-linked DSAI@PTL nanofilm directly formed at the air/water interface. (B) The cross-linked DSAI@PTL nanofilm directly formed at the solid/liquid interface. (C) The cross-linked DSAI@PTL bulk. The scale bar is 300 μm . (D-F) The change of the fluorescence intensity shown in (A-C). The scale bar is 300 μm .

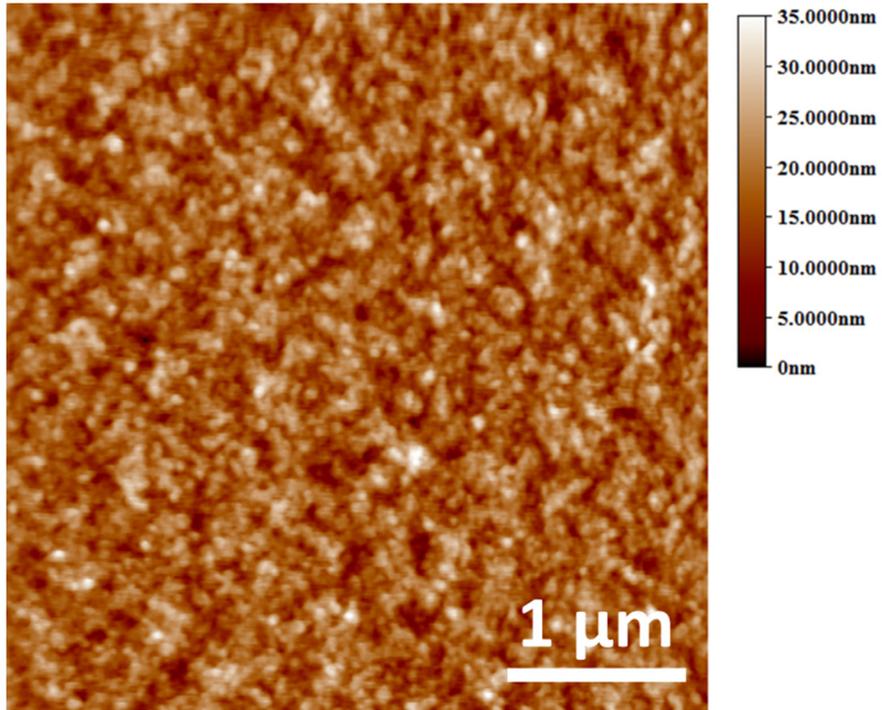


Figure S18. AFM image of the DSAI@PTL nanofilm coated on the surface of silicon wafer.

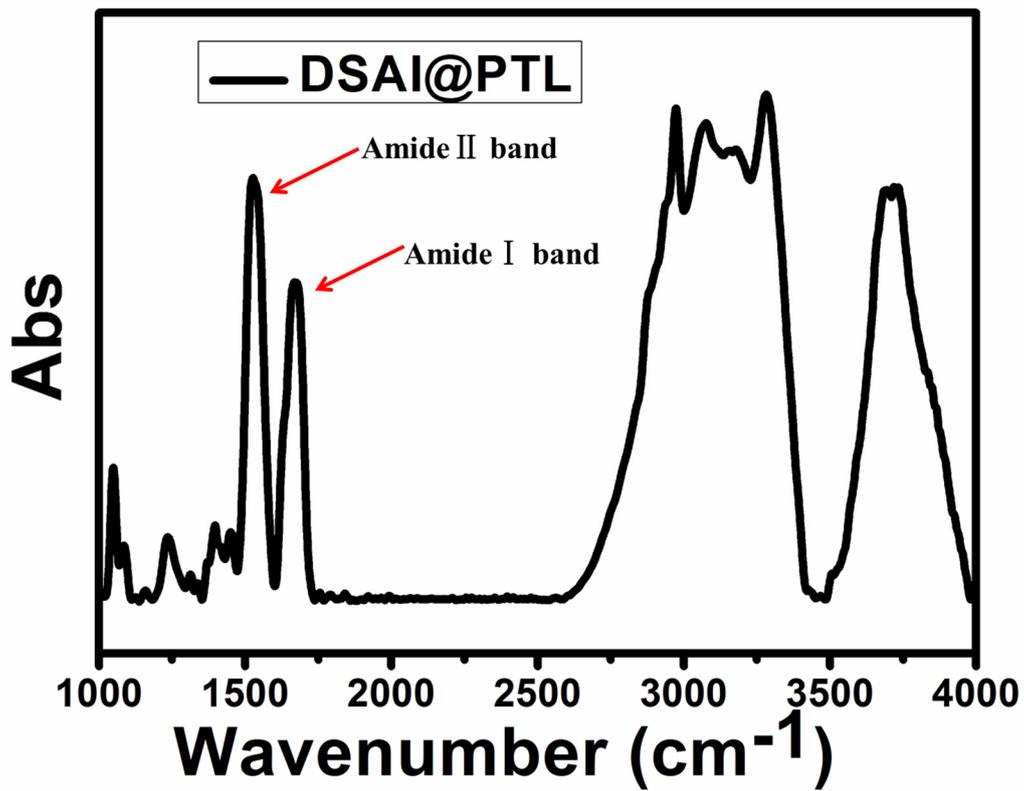


Figure S19. FTIR characterization of the DSAI@PTL nanofilm.

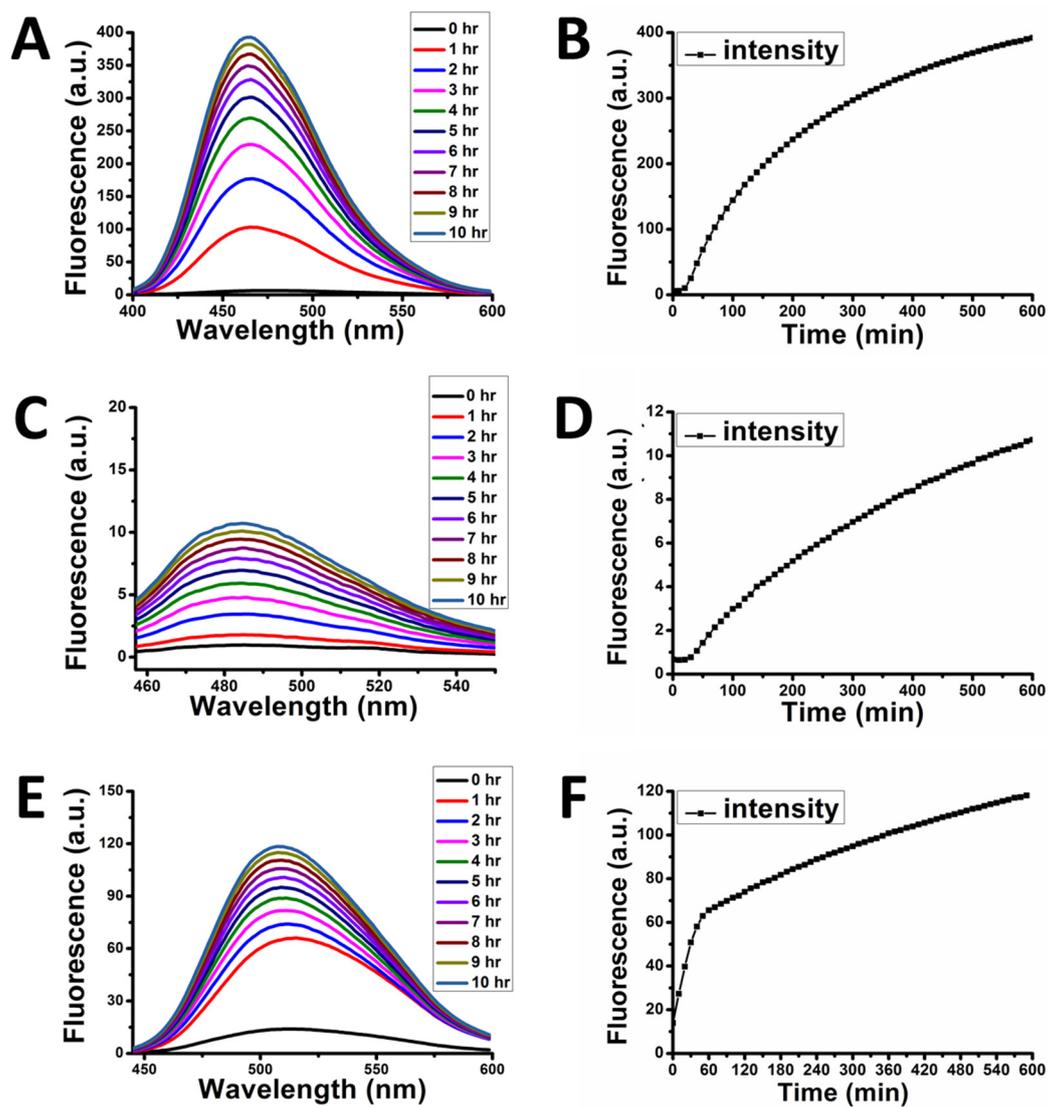


Figure S20. The fluorescent spectra of the phase transition buffer at different phase transition time after binding with ANS, ThT or DSAI. 2 mg/ml of lysozyme, 50 mM of TCEP (pH 4.5), 200 μ M of ANS, 100 μ M of ThT, 133 μ M of DSAI were used in these cases. (A, B) Binding with ANS. (C, D) Binding with ThT. (E, F) Binding with DSAI.

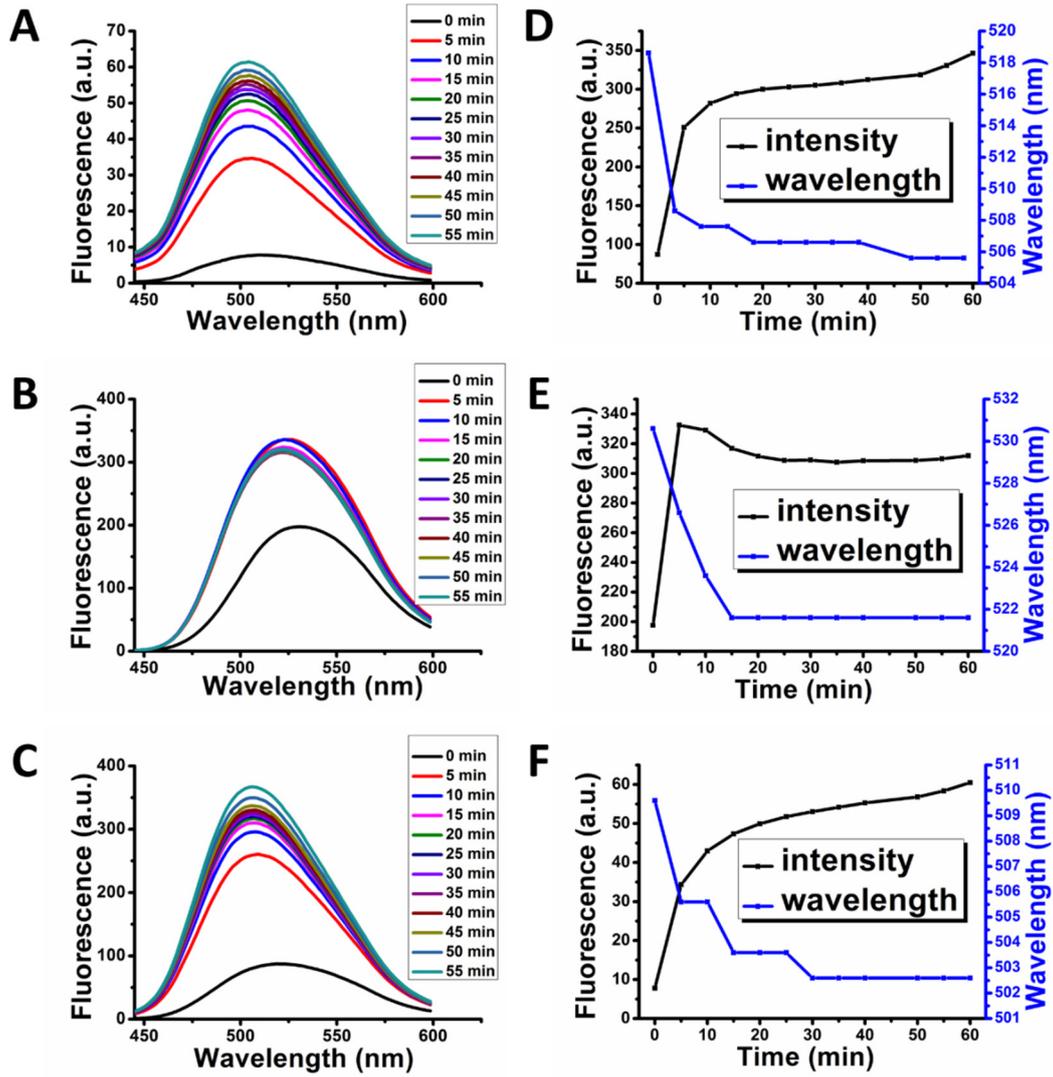


Figure S21. The change of intensity and wavelength fluorescence spectra of the phase transition buffer at different phase transition time after binding with DSAI at different concentration of DSAI. 2 mg/ml of lysozyme and 50 mM of TCEP (pH 7.5) were used in these cases (volume ratio lysozyme: TCEP: DSAI=10: 10: 2). (A, D) DSAI=0.01 mg/ml. (B, E) DSAI=0.1 mg/ml. (C, F) DSAI=1 mg/ml.

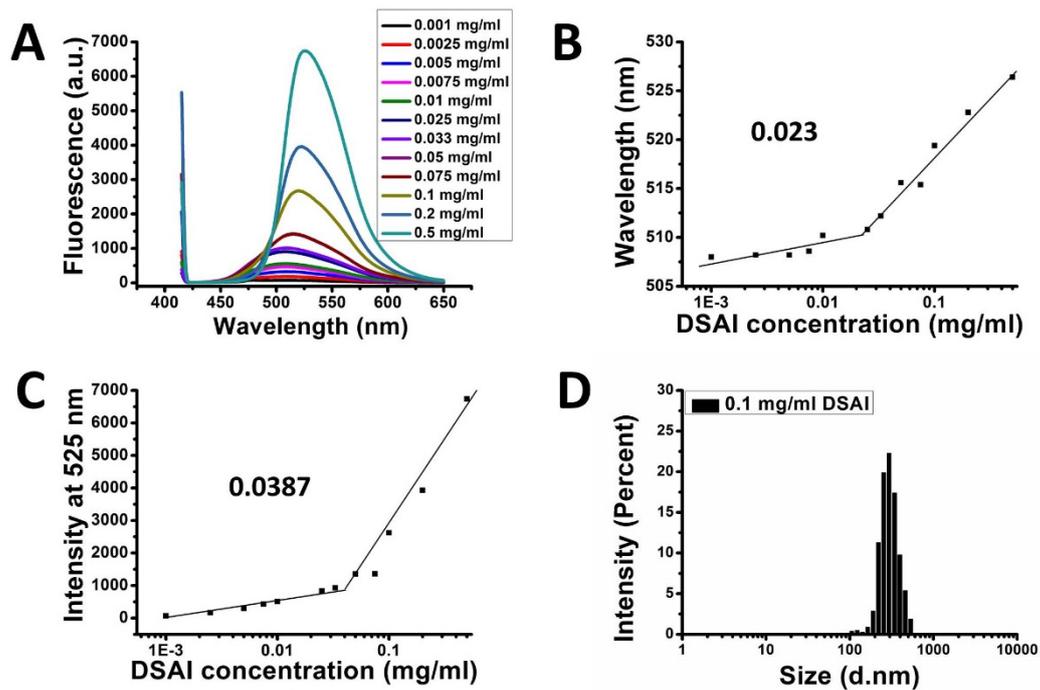


Figure S22. (A) The fluorescence spectra of free DSAI at different concentration. (B) Plot of maximum wavelength as the function of the concentration of DSAI. (C) Plot of fluorescence emission intensity at 525 nm in (A) as the function of DSAI concentration. (D) The DLS size distribution in the solution of 0.1 mg/ml DSAI. There was no detectable size distribution signal in the solution of 0.01 mg/ml DSAI. From (B, C) the critical micelle concentration (CMC) of DSAI was estimated in 0.02-0.04 mg/ml.

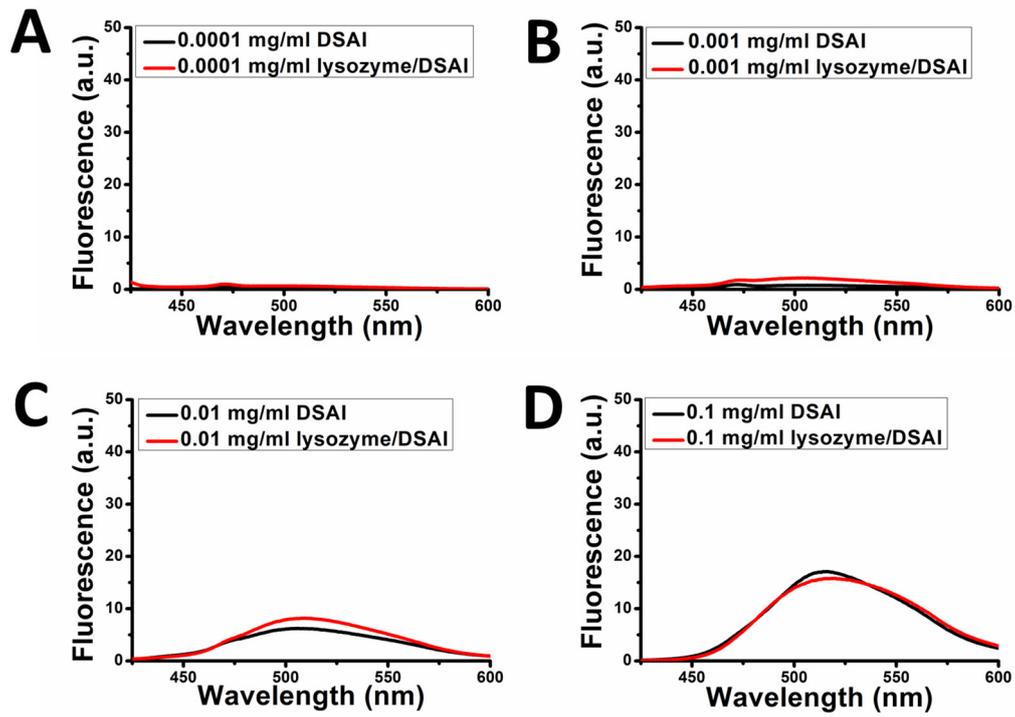


Figure S23. The comparison of the fluorescence spectra of free DSAI and the mixture of native lysozyme and DSAI at different concentration of DSAI. (A) 0.0001 mg/ml DSAI. (B) 0.001 mg/ml DSAI. (C) 0.01 mg/ml DSAI. (D) 0.1 mg/ml DSAI. 2 mg/ml native lysozyme was used in these cases.

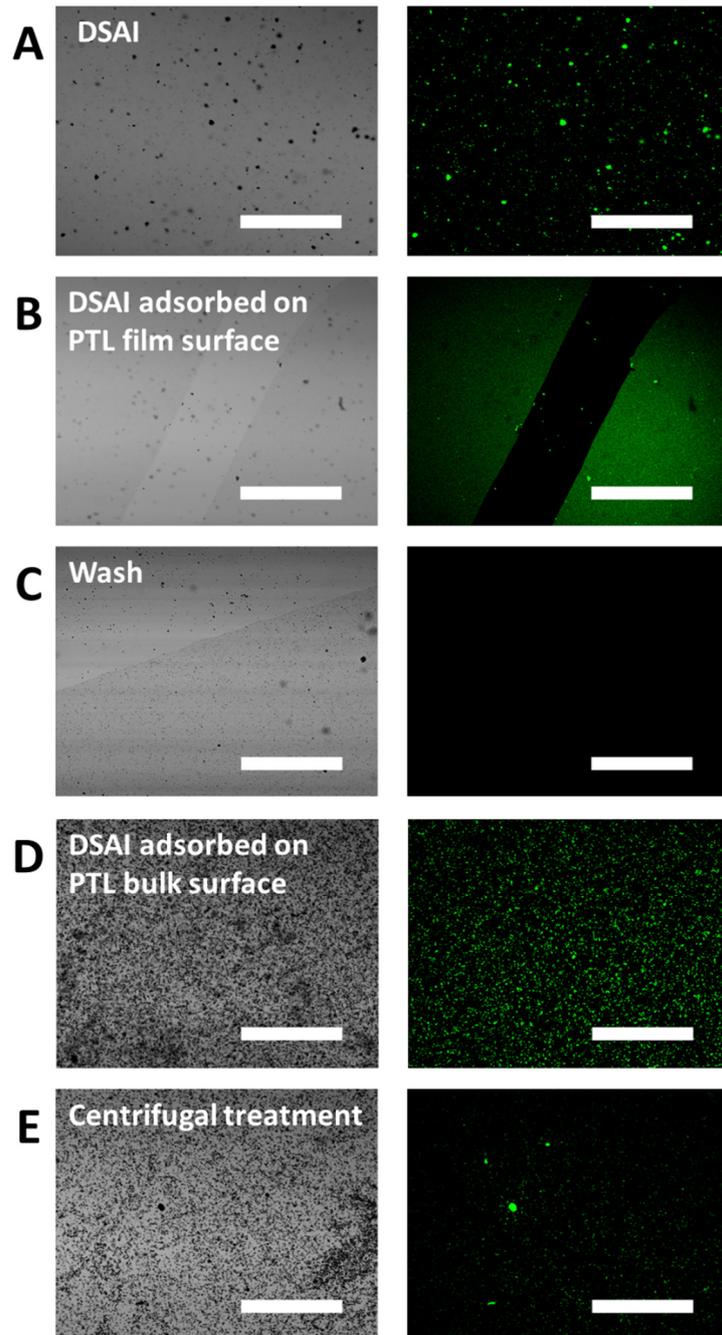


Figure S24. Optical pictures (left) and confocal laser scanning images (right) on the (A) free 0.1 mg/ml DSAI; (B) the PTL nanofilm coated on the glass after the immersion in the 0.1 mg/ml DSAI for 2 hr and (C) then rinsed with deionized water for 5 minutes; (D) the PTL bulk after the immersion in the 0.1 mg/ml DSAI for 2 hr and (E) then centrifugal rinsed several times. The scale bar is 300 μm .

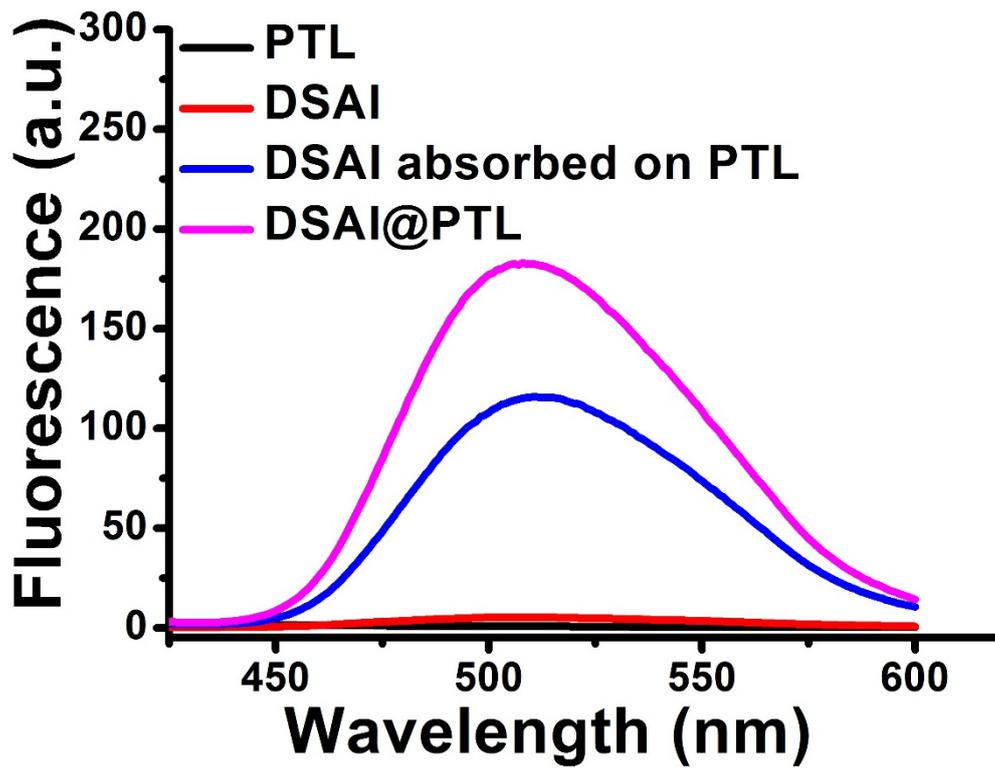


Figure S25. Fluorescence spectra of PTL by DSAI staining.

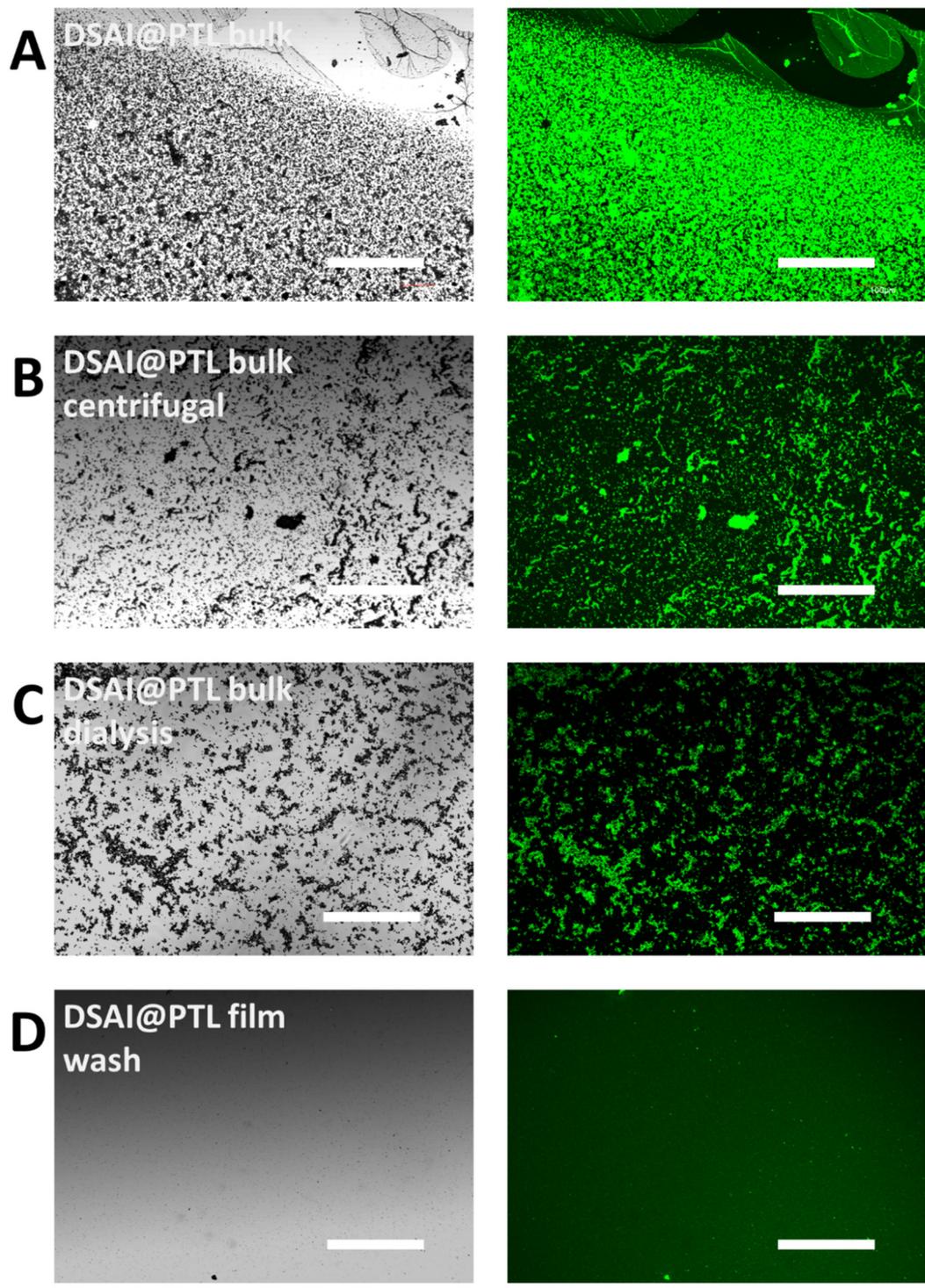


Figure S26. Optical pictures (left) and confocal laser scanning images (right) on the (A) the DSAI@PTL bulk, (B) the DSAI@PTL bulk after rinsing for several times by deionized water centrifuge, (C) the DSAI@PTL bulk after the dialysis for 10 days, (D) the DSAI@PTL nanofilm rinsed with deionized water for 5 minutes. The scale bar is 300 μm .

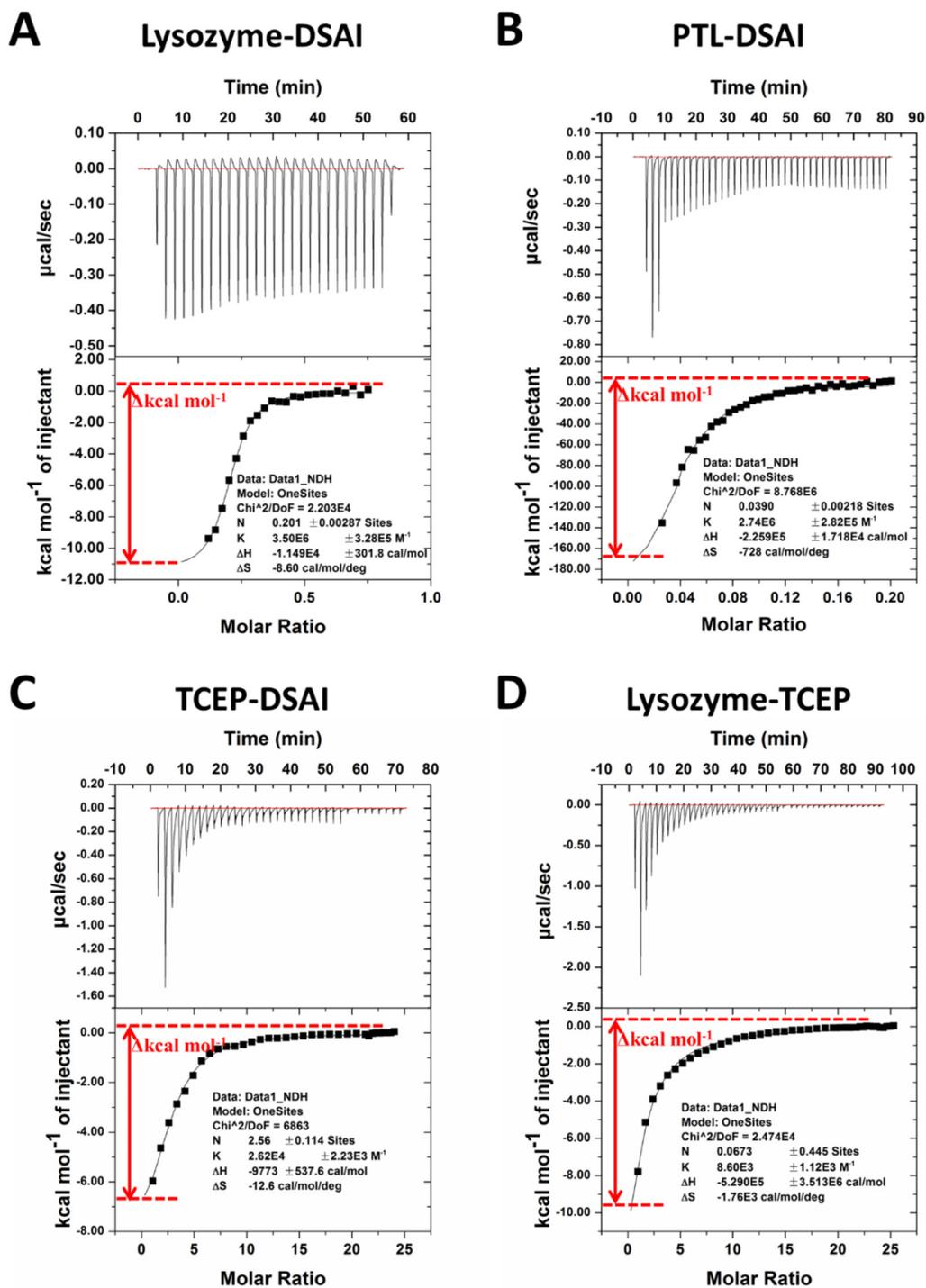


Figure S27. Isothermal Titration Calorimetry (ITC) results for the interaction of (A) DSAI and lysozyme; (B) DSAI and PTL; (C) DSAI and TCEP; (D) lysozyme and TCEP. DSAI 0.0328 mM; TCEP 5 mM pH 5.0; lysozyme 0.0357 mM. The PTL was equivalent volume mixing with 0.0714 mM lysozyme and 10 mM TCEP (pH 5.0), incubation for 1 hr.

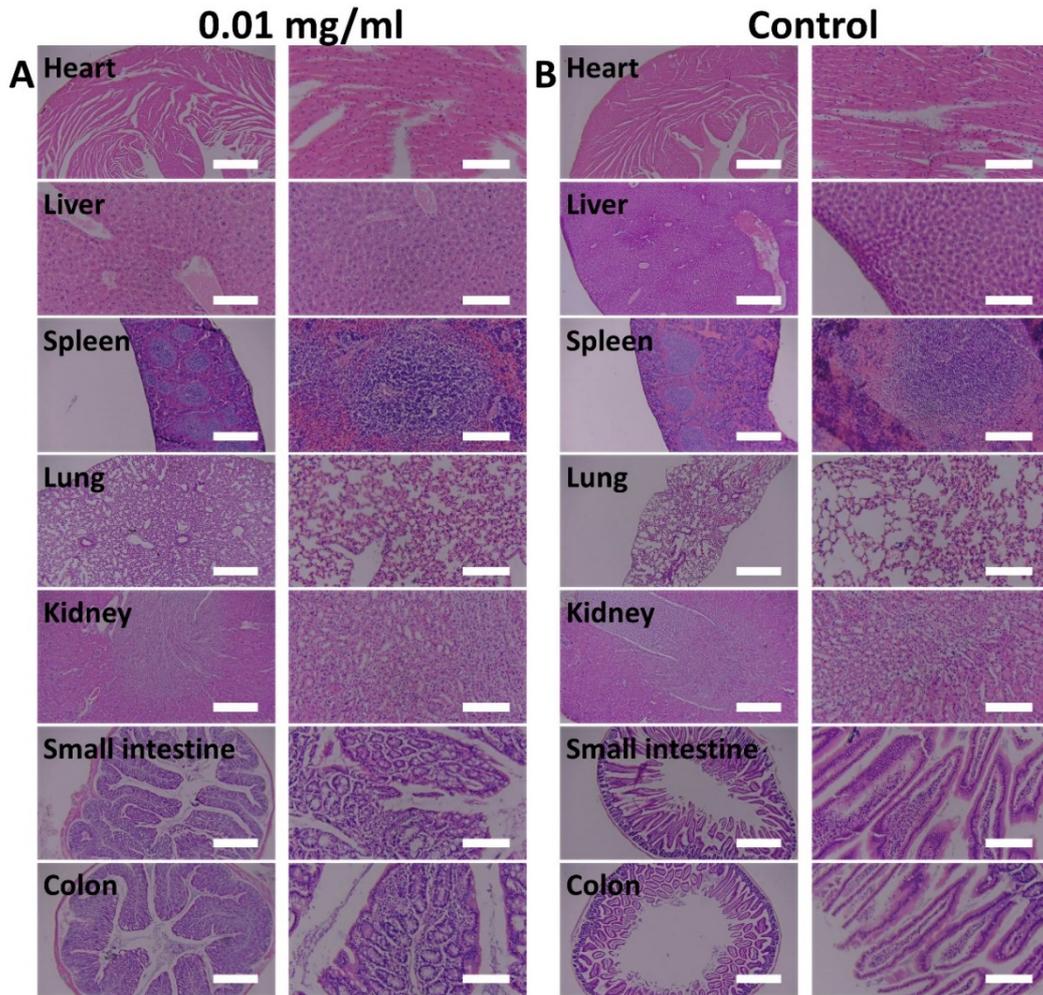


Figure S28. Representative photograph of pathological tissue sections stained with H&E staining from mice. (A) 0.01 mg/ml DSAI. (B) control. The scale bar is 500 µm (left) and 100 µm (right). In **Figure S29-32**, the myocardial cells are clear and well-organized, with no bleeding, necrosis, or inflammatory exudation. Structure of hepatic lobule is clear, neat, does not appear degeneration or necrosis of liver cells, while not observed in neutrophils, lymphocytes and macrophages infiltration. Red pulp of the spleen and white pulp neat clear structure, abnormal pathological spleen venous sinus is not at the same time. The alveolar structure was orderly and clear, no bronchial and alveolar dilation and atrophy were observed, and no degeneration of alveolar epithelial cells was observed. No inflammatory cell infiltration was observed around bronchi and alveoli. Glomerular and renal tubular structure neat clear, did not see degeneration, hemorrhage, or necrosis. The small intestine and colon neat clear structure, did not see degeneration, hemorrhage, or necrosis.

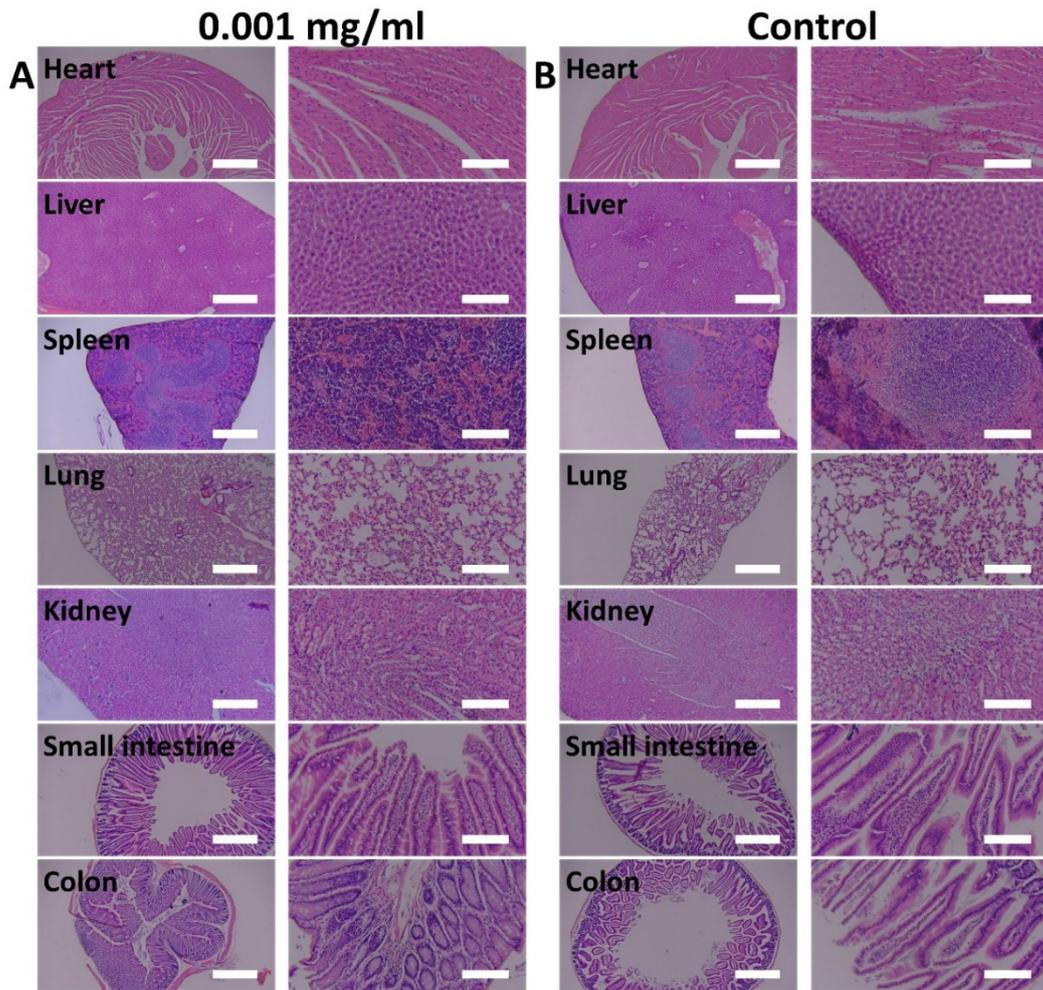


Figure S29. Representative photograph of pathological tissue sections stained with H&E staining from mice. (A) 0.001 mg/ml DSAI. (B) control. The scale bar is 500 μ m (left) and 100 μ m (right).

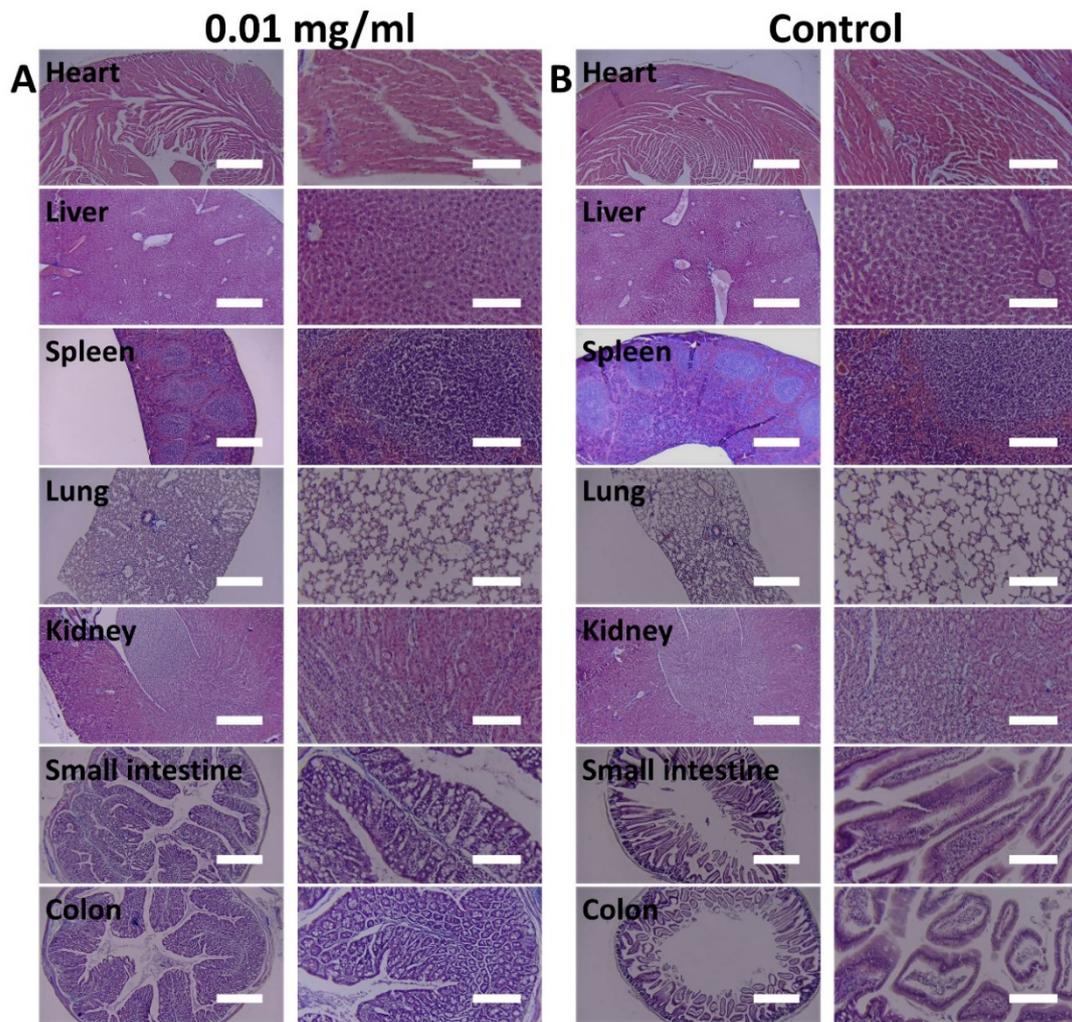


Figure S30. Representative photograph of pathological tissue sections stained with **Masson** staining from mice. (A) 0.01 mg/ml DSAI. (B) control. The scale bar is 500 μ m (left) and 100 μ m (right).

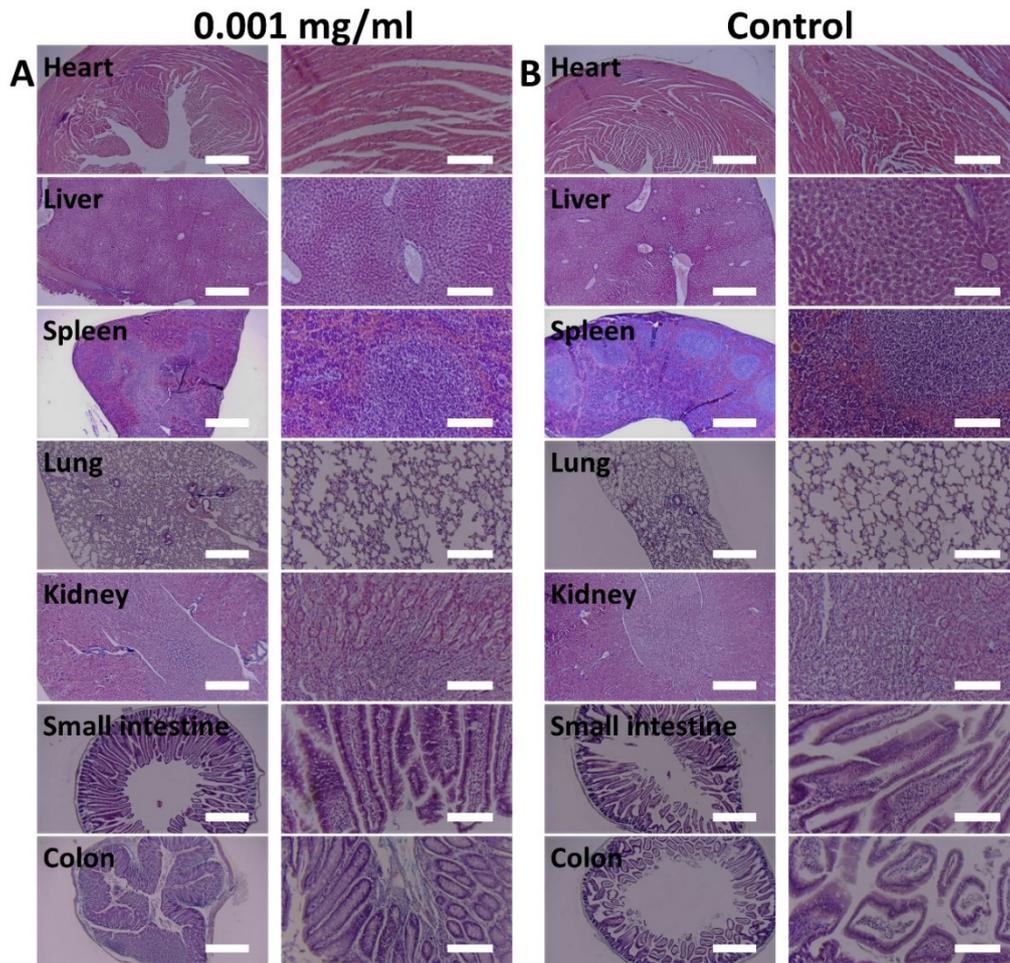


Figure S31. Representative photograph of pathological tissue sections stained with **Masson** staining from mice. (A) 0.001 mg/ml DSAI. (B) control.

The scale bar is 500 μ m (left) and 100 μ m (right).

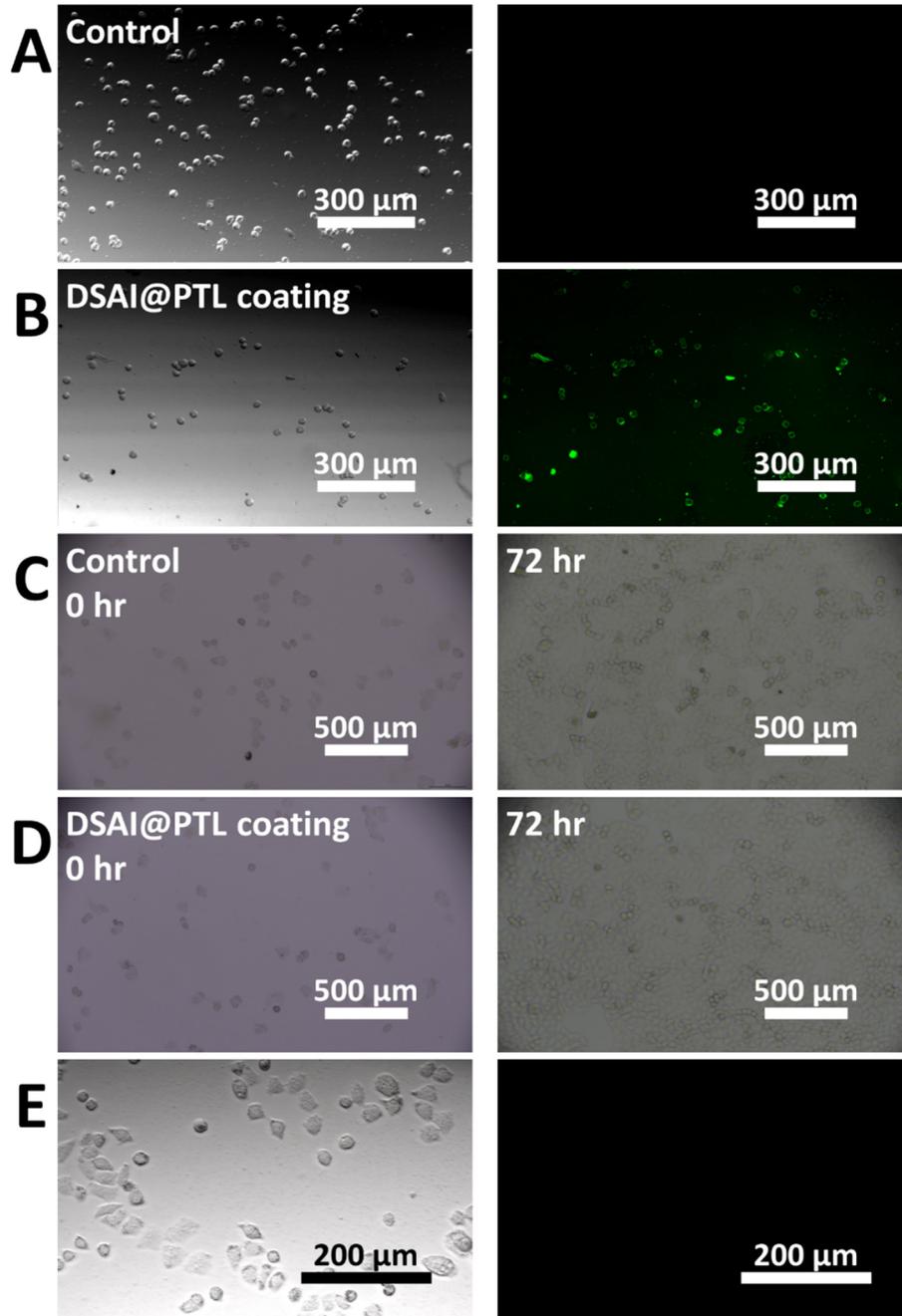


Figure S32. Optical pictures (left) and confocal laser scanning images (right) on the HeLa cells: (A) bare HeLa cells. (B) the DSAI@PTL-coated HeLa cells. Proliferation assay of (C) bare HeLa cells and (D) the DSAI@PTL-coated HeLa cells. (E) Optical pictures (left) and confocal laser scanning images (right) on the DSAI@PTL-coated HeLa cells after PI (Propidium Iodide) dyeing treatment. The PI is a popular red-fluorescent nuclear and chromosome counterstain. Since it is not permeant to live cells, it is then commonly used to detect dead cells in a population. The inability of PI to achieve the cell staining in (E) indicated that cell viability after the interaction with the DSAI@PTL remained normal.

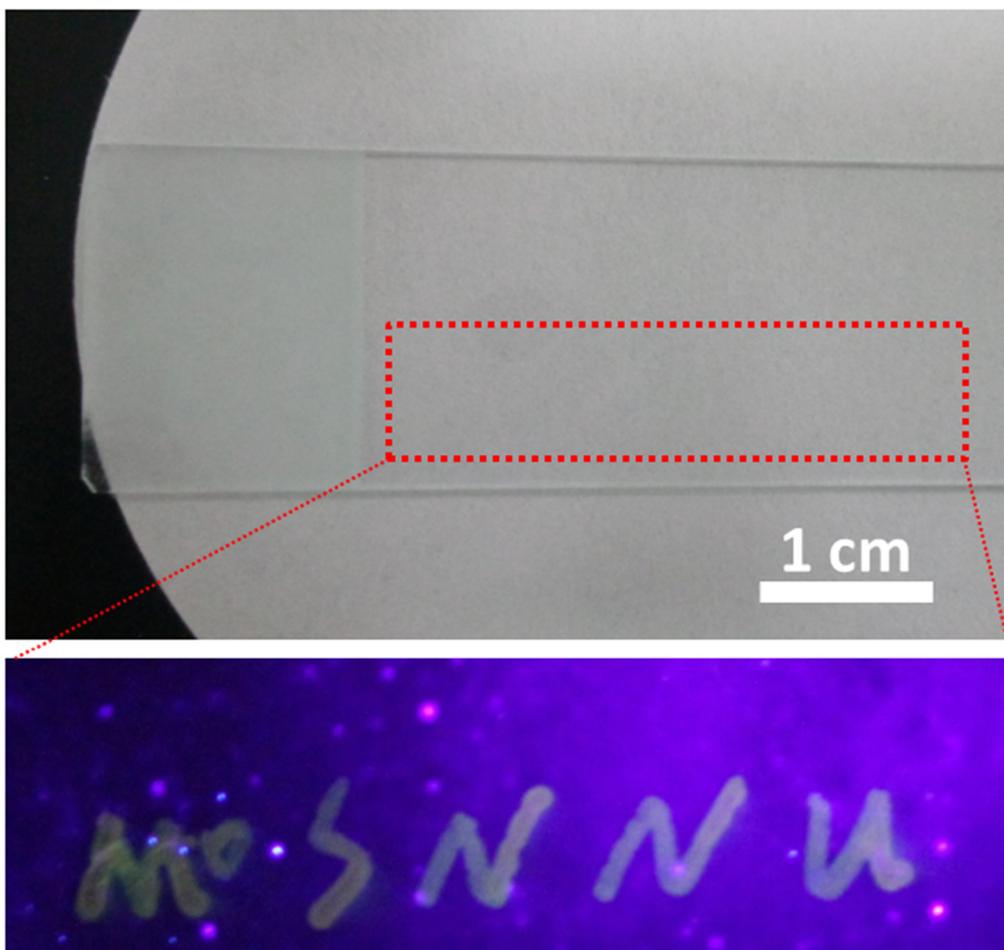


Figure S33. DSAI@PTL written on the glass as invisible ink under ambient light (up) and UV lamp (down). 2 mg/ml of lysozyme, 50 mM of TCEP (pH 5.0) and 0.01 mg/ml of DSAI were used in these cases (volume ratio lysozyme: TCEP: DSAI=10: 10: 2).

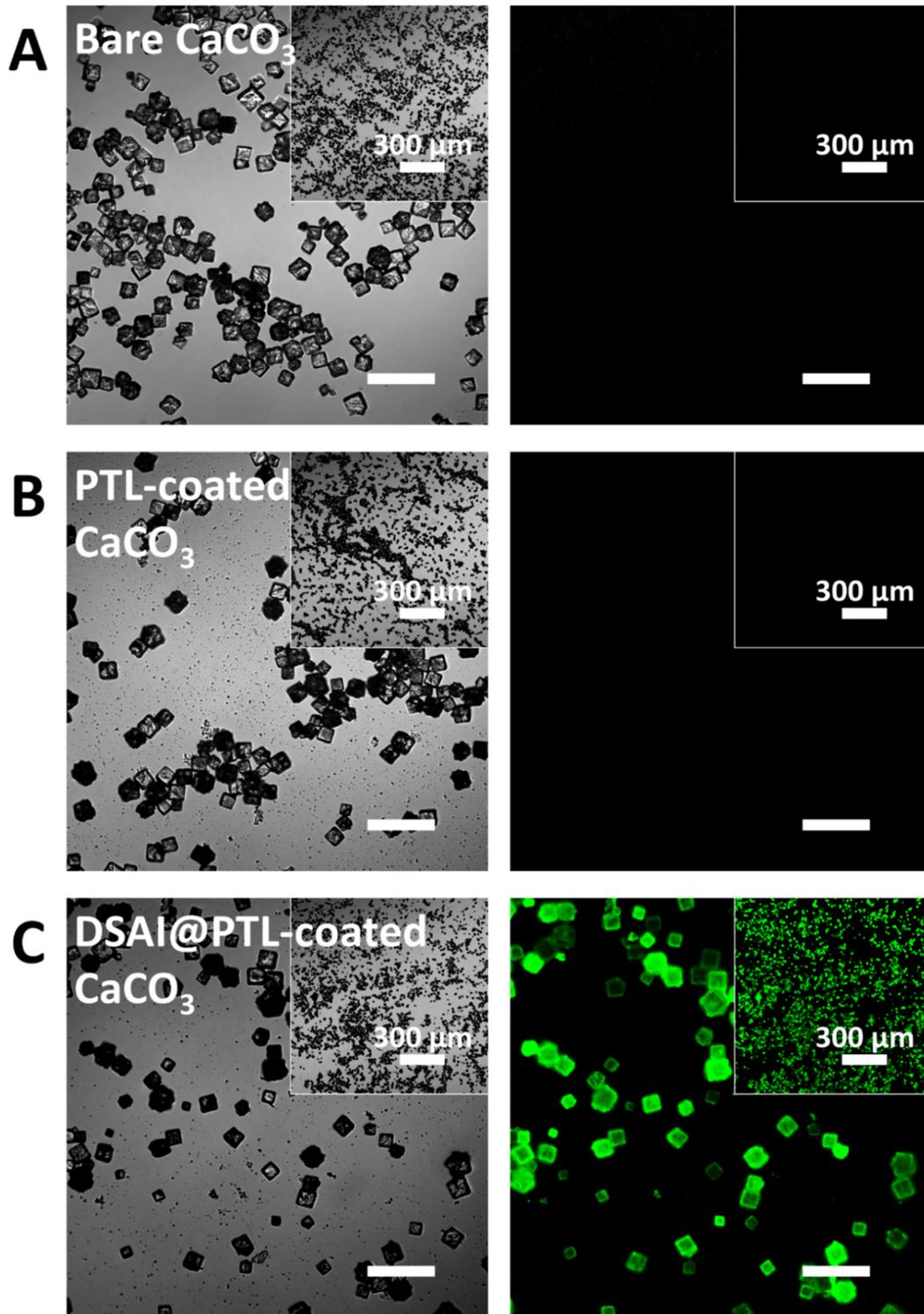


Figure S34. Optical pictures (left) and confocal laser scanning images (right) on the 10 μm DSAI@PTL-coated CaCO_3 particles: (A) Bare 10 μm CaCO_3 particles. (B) The PTL-coated 10 μm CaCO_3 particles. (C) The DSAI@PTL-coated 10 μm CaCO_3 particles. The scale bar is 50 μm .

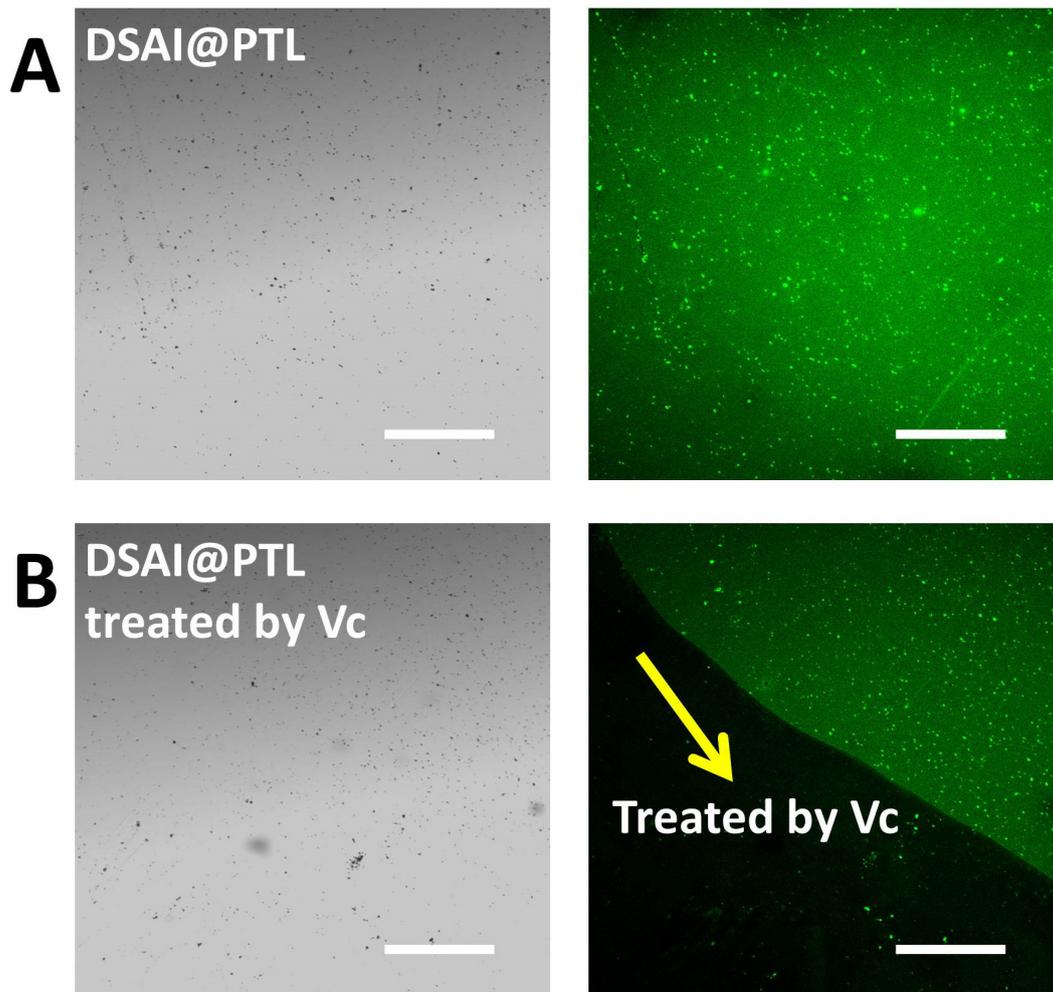


Figure S35. Optical pictures (left) and confocal laser scanning images (right) on (A) the DSAI@PTL nanofilm-coated on the glass, and (B) after rinsing in 500 mM Vitamin C (as indicated by the yellow arrow) for 5 min. All nanofilms were prepared directly on the solid/liquid interface. The scale bar is 300 μm .

Reference

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