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Supplementary information

Experimental details

Materials and methods

Bacteria strains Escherichia coli *(E. coli,* DH5α™) and Staphylococcus aureus *(S. aureus,* ATCC #25923*)* were obtained from Life Technologies and American Type Culture Collection respectively. All chemicals were purchased from Merck Millipore, except 2,2,2-Trifluoroethanol (TFE) and silver nitrate ($AgNO_3$) which were purchased from Sigma-Aldrich. Silicon wafers were purchased from Latech Scientific Supply (Singapore). Polypropylene (PP) films were purchased commercially from Landmark Plastics (Singapore).

Self-assembly of GPG-AG3 protein nanofibers

Expression and purification of GPG-AG3 proteins was performed as previously reported.[7] Lyophilized GPG-AG3 proteins were weighed and dissolved in deionized water (dH_2O) at 4°C. Subsequently, TFE was added to protein solution to induce the formation of nanofibers. The protein solution (final concentration of 20 μM) containing 30% TFE was allowed to incubate at 60°C for 3 days before finally cooled to room temperature.

Synthesis of silver nanoparticles using GPG-AG3

Silver nitrate ($AgNO_3$, 100 mM) was added to the protein nanofibers solution prepared previously. The mixture was shaken on a rocker for 1 h. Subsequently, 1 mM NaCl was then added to solution and further incubated for 24 h at room temperature. The mixture was then dialyzed in dH_2O to remove the excess of $AgNO_3$ and NaCl. The final solution was stored at 4°C for further use.

Adsorption of silver-coated GPG-AG3 nanofibers on polypropylene (PP) films

Untreated PP films were cut into 8mm circular disks using an 8mm biopsy punch. 200 μL of silver-coated GPG-AG3 solutions were dropped directly onto the PP films and allowed to evaporate in air overnight. Varying concentrations of silver-coated GPG-AG3 solutions (i.e., 1, 5, 10, 15 and 20 μM) were used to prepare coatings of varying silver content on identical PP films.

The coated samples were then rinsed in ddH_2O to remove any unbound proteins and dried in nitrogen gas.

Atomic force microscopy (AFM)

GPG-AG3 protein nanofibers were subjected to atomic force microscopy (AFM) to examine the morphology of the self-assembled GPG-AG3 protein nanofibers. 1 μL of the fiber-formed protein solution was dropped onto a piece of untreated silicon wafer and allowed to dry in air. Imaging was performed dry using atomic force microscopy (Veeko, Dimension V), with accompanying Nanoscope 7.3 software, in tapping mode at a scan rate 0.7 Hz with a Si cantilever at 25°C.

Field Emission Scanning Microscopy (FESEM) and Transmission Electron Microscopy (TEM)

Protein-coated PP samples were visualized using Field Emission Scanning Microscopy (JOEL, FESEM, 6340F), at an accelerating voltage of 5 kV and an emission current of 12 mA. Images were acquired at random positions of the films (i.e., near the edge and at the center of the films). The thresholds of the acquired SEM images were adjusted to 50% of the maximum intensities, before converting to binary images. The white areas (i.e., areas covered by proteins) were expressed as percentages of the total areas to yield the percentage coverages of protein on the PP films. For transmission electron microscopy (TEM), an emission current 105 mA and an accelerating voltage of 200 kV were used to visualize the samples.

UV-VIS and FTIR

The presence of silver nanoparticles on GPG-AG3 protein fibers was confirmed using UV-Vis Spectrometer 2501PC. The spectrum was collected over a wavelength range of 300 − 900 nm with a resolution of 0.5 nm using water as reference. FT-IR spectra were recorded on a Fourier transform infrared spectrometer (PerkinElmer) with a DGTS detector.

Antibacterial assays

The antibacterial properties of the PP films containing 1, 5, 10, 15 and 20 μM of silver-coated proteins were evaluated using gram-negative *Escherichia coli* (*E. coli)* and gram-positive

Staphylococcus aureus (S. aureus). Approximately 10^7 colony forming units (CFU) of bacteria were spread on 2×YT (Yeast Extract Tryptone) agar plates. Coated PP films were placed in direct contact with the bacteria and incubated overnight at 37°C. The "inhibition zone" was defined as the bacteria-free zone. The widths of the inhibition zone (i.e, distances between the bacteria lawns and the PP films) were measured manually using ImageJ. For each sample, at least 15 measurements were taken and the means ± SD were reported.

The growths of both bacteria strains in liquid media in the presence of protein-treated PP films were evaluated. Bacteria cultures were grown to log phase (i.e., optical density OD_{600nm} 0.6 – 0.8) and subsequently re-innoculated at 1:100 dilution into 4 mL of 2×YT media. Cultures were immediately supplemented with PP films containing varying amounts of silver-coated proteins and agitated at 250 rpm at 37°C. Bacteria growth was monitored by measuring the optical density at 600 nm (OD_{600nm}) at regular time intervals for up to 24 h.

Evaluation of stability of protein coatings

Protein-coated PP films were subjected to rigorous washing to determine the stability of the coatings. Briefly, samples were immersed in 10 ml of ddH2O, PBS or 2xYT and agitated at 225rpm for 24h at 25°C. Washed samples were air-dried before subjected to FTIR and UV-VIS analysis to determine the residual protein and silver content.

Statistical analysis

For all experimental data, results represented as means ± SEM of 3 independent experiments. The statistical significance of differences was determined using ONE-WAY ANOVA. Differences were taken to be statistically significant at $P \leq 0.05$.

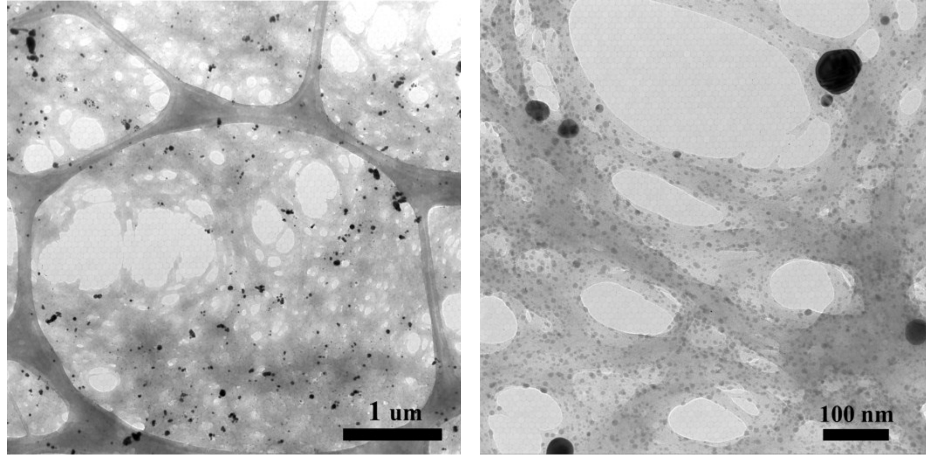


Figure S1. TEM images of Ag-protein fibers after stored in water in 4 ℃ for 1 week.

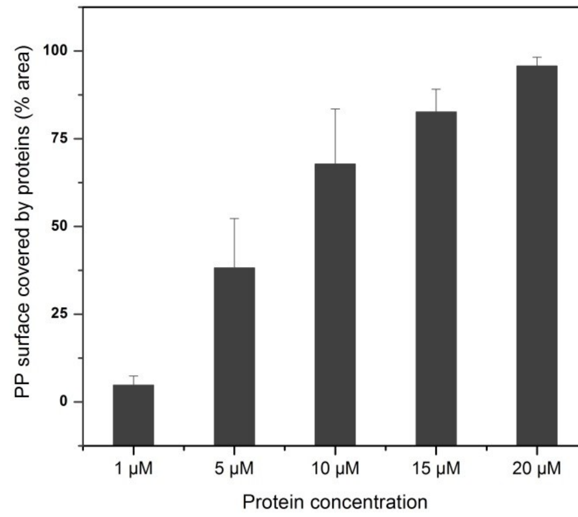
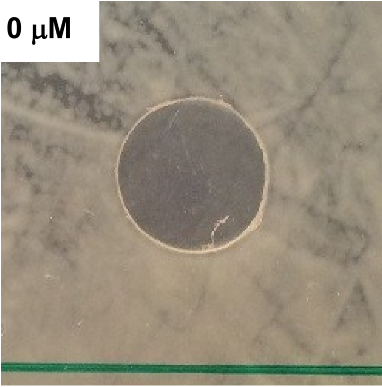
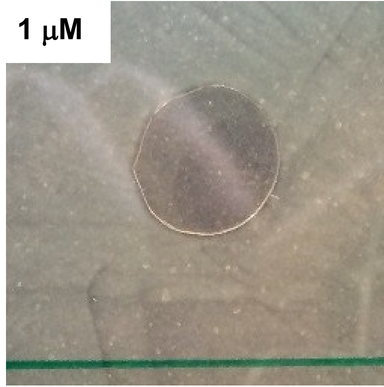
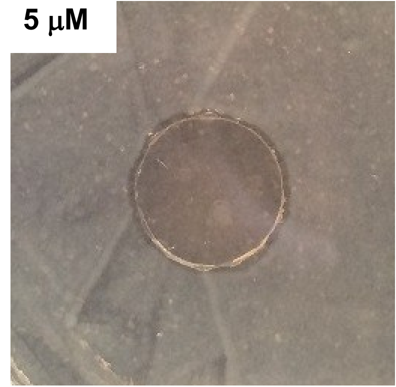


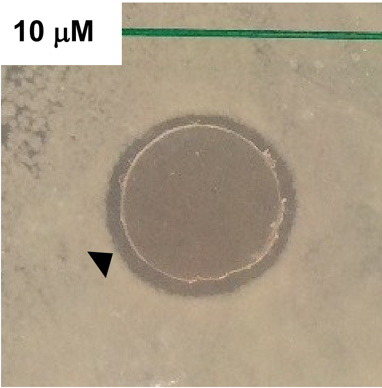
Figure S2. Percent surface coverage of PP films when treated with varying concentrations of silver-bound GPG-AG3.

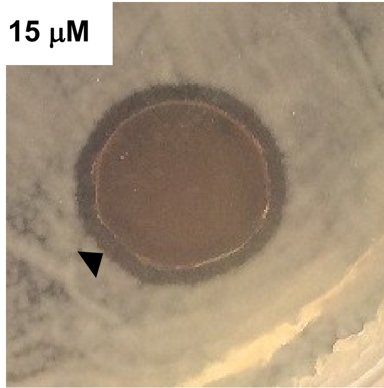
E. coli

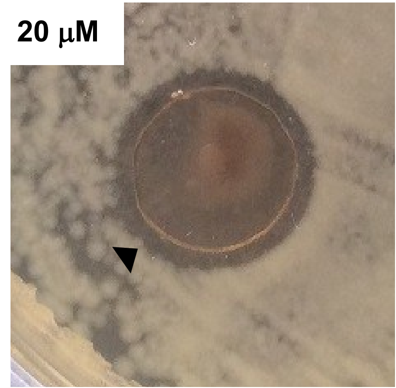












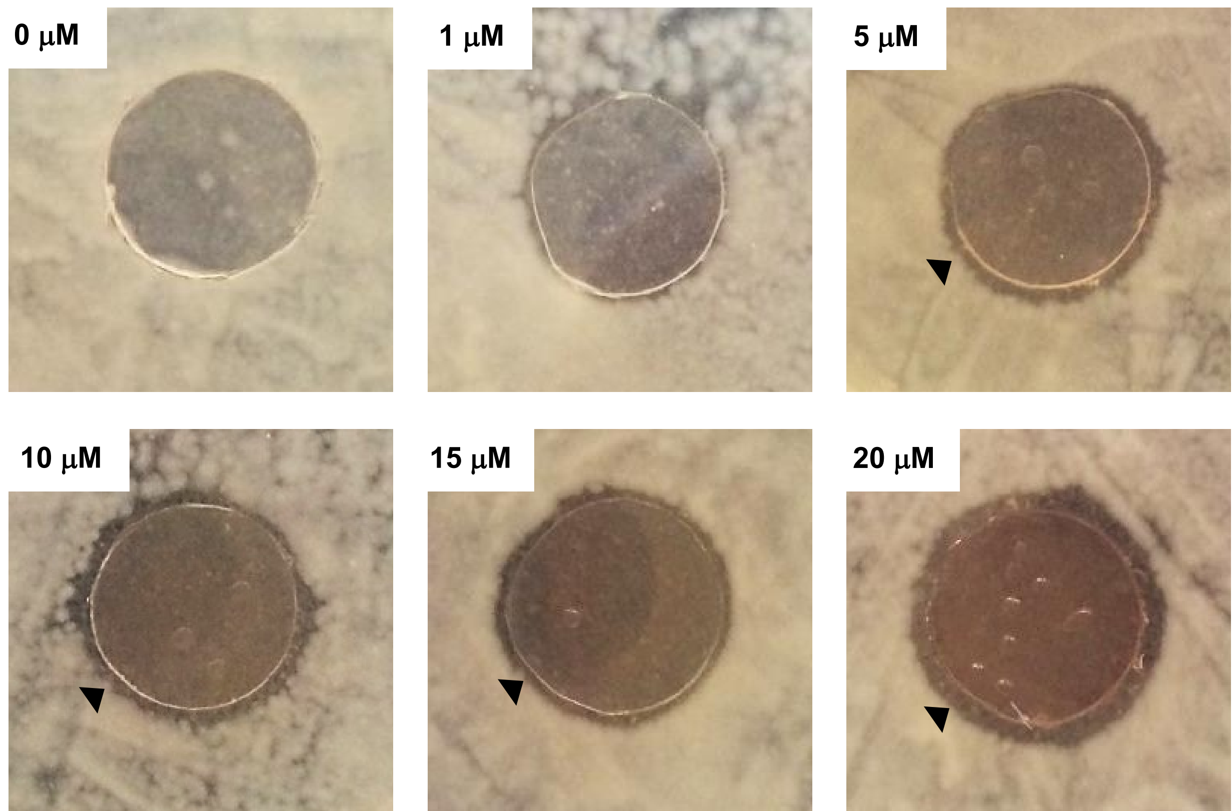


Figure S3. Magnified images of the bacterial inhibition zones for PP films containing varying concentrations of silver-coated GPG-AG3 fibers for *E. coli* and *S. aureus*. Black arrows mark the edges of the inhibition zones.

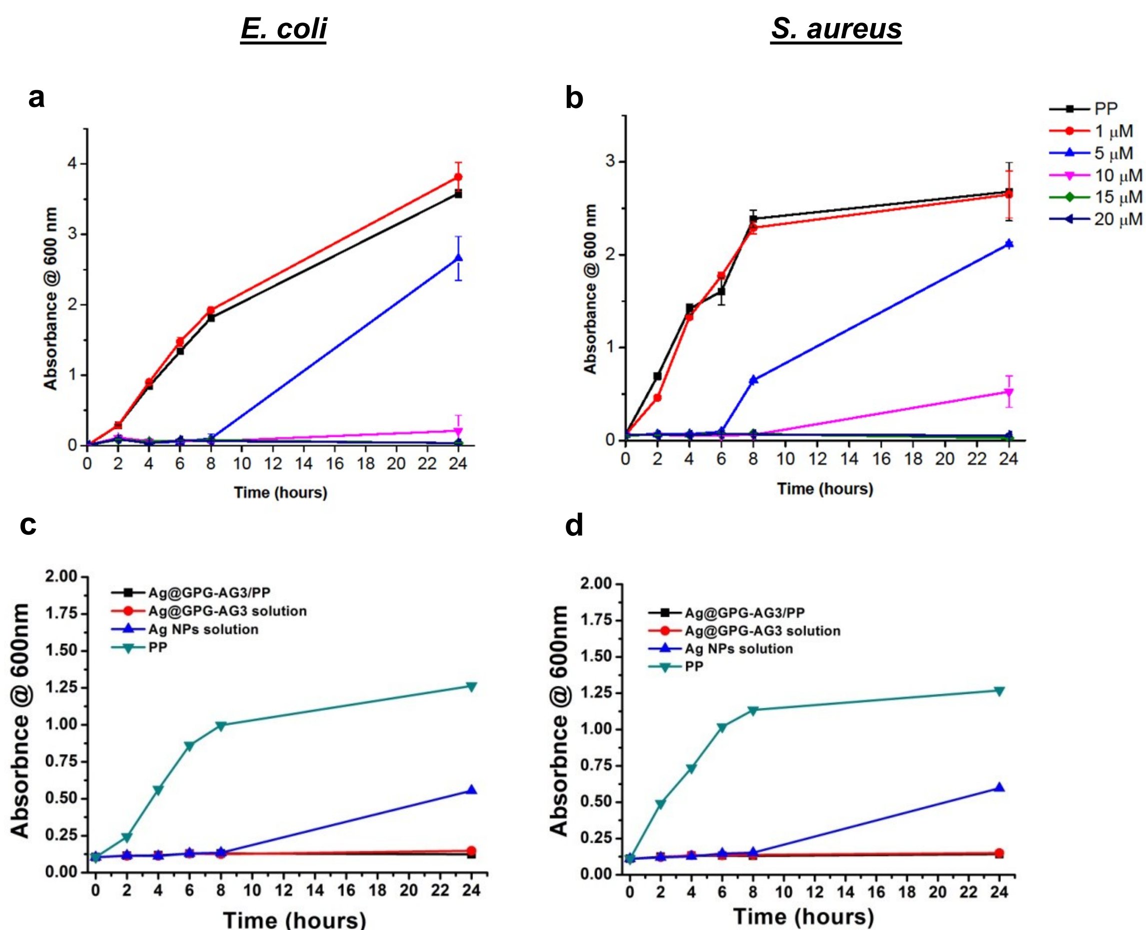


Figure S4. (a – b) Growth curves of *E. coli* and *S. aureus* in the presence of PP films coated with varying concentrations of silver-coated protein nanofibers. (c – d) Growth curves of *E. coli* and *S. aureus* in the presence of coated PP film (20μM, black curve), silver-coated GPG-AG3 (20 μM, red curve), silver nanoparticles (20 μM, blue curve) and blank PP film control (green curve).

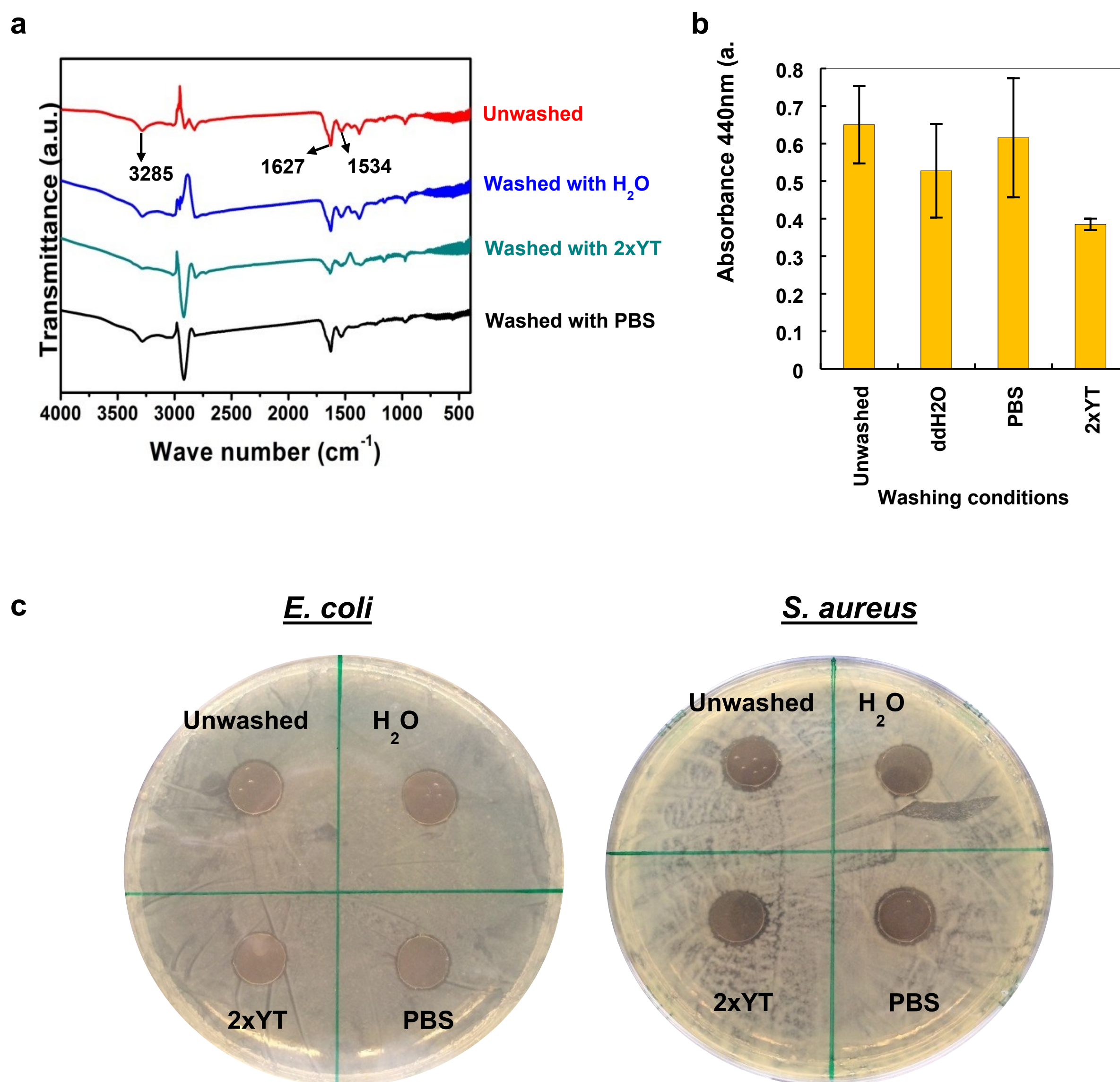


Figure S5. (a) FTIR spectra of silver-protein coated PP films (20 μM) before and after washing in water, PBS and 2×YT for 24h at 25°C. (b) Absorbance values @440nm of the unwashed and washed samples determined using UV-VIS. a.u. represent arbitrary units. Error bars are S.D. (c) Agar disks showing the presence of inhibition zones of washed samples for *E. coli* and *S. aureus* respectively.

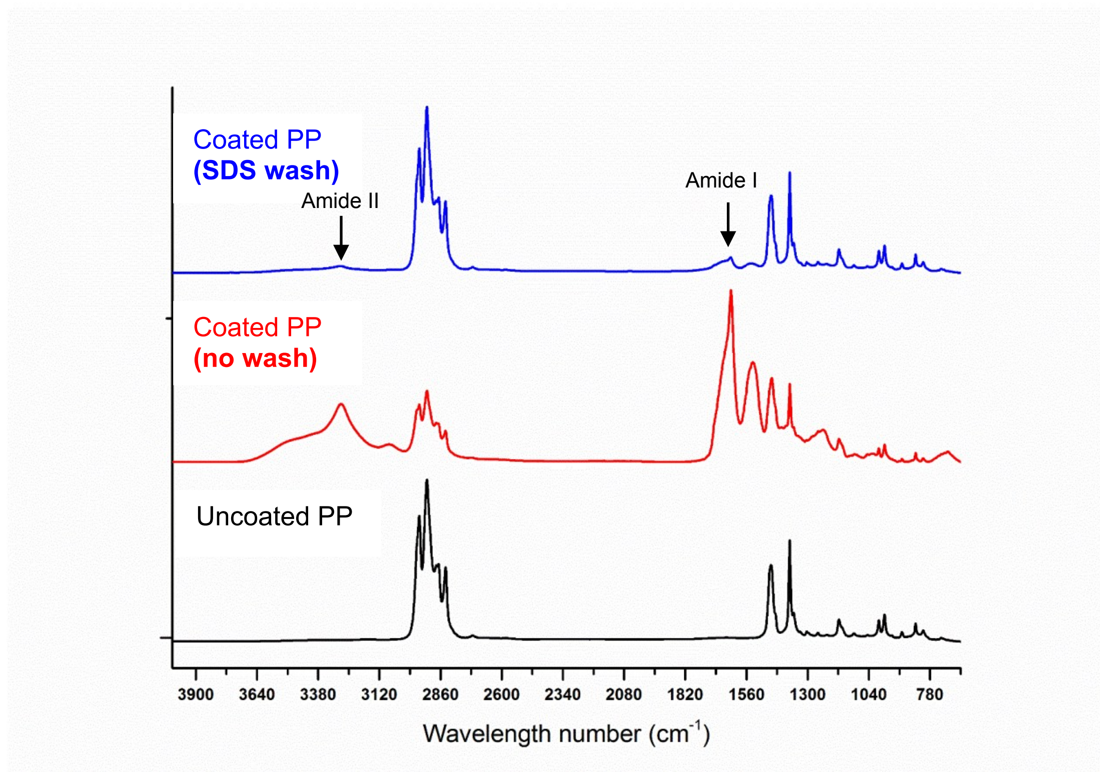
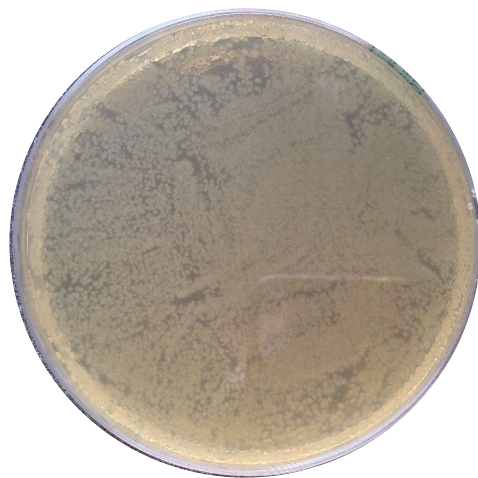


Figure S6. FTIR spectra of protein-coated PP films subjected to 2% SDS washing at 70°C for 1 h (blue curve). Intensities of amide I and II peaks were dramatically reduced after washing, confirming that the proteins were physically adsorbed onto PP.

Uncoated PP film



Protein-coated PP film

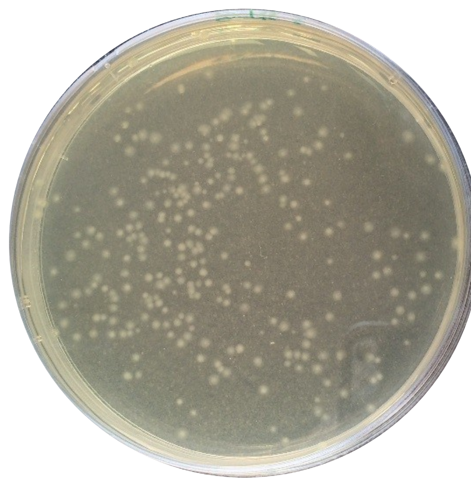


Figure S7. Representative images of agar plates used for CFU counting for uncoated PP film control (left) and protein-coated PP film (20 uM; right).

Protein Concentration (μM)	**Width of inhibition zone (mm)**	
	E. coli	***S. aureus***
1	N.D.	N.D
5	0.59 ± 0.08	0.46 ± 0.14
10	0.64 ± 0.10	0.82 ± 0.10
15	1.11 ± 0.12	0.87 ± 0.18
20	1.16 ± 0.13	0.93 ± 0.18

Table S1 Widths of inhibition zones for *E. coli* and *S. aureus*