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Synthesis and functionalization of scalable and versatile 2D protein films via amyloid-like aggregation

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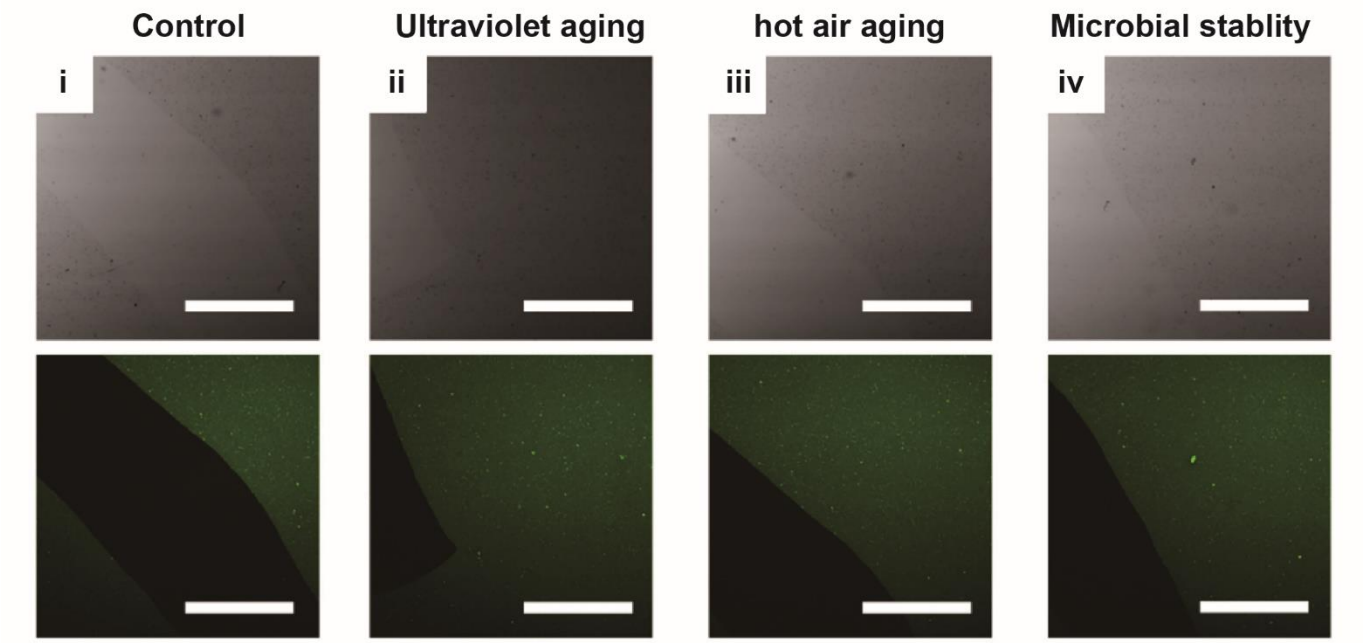
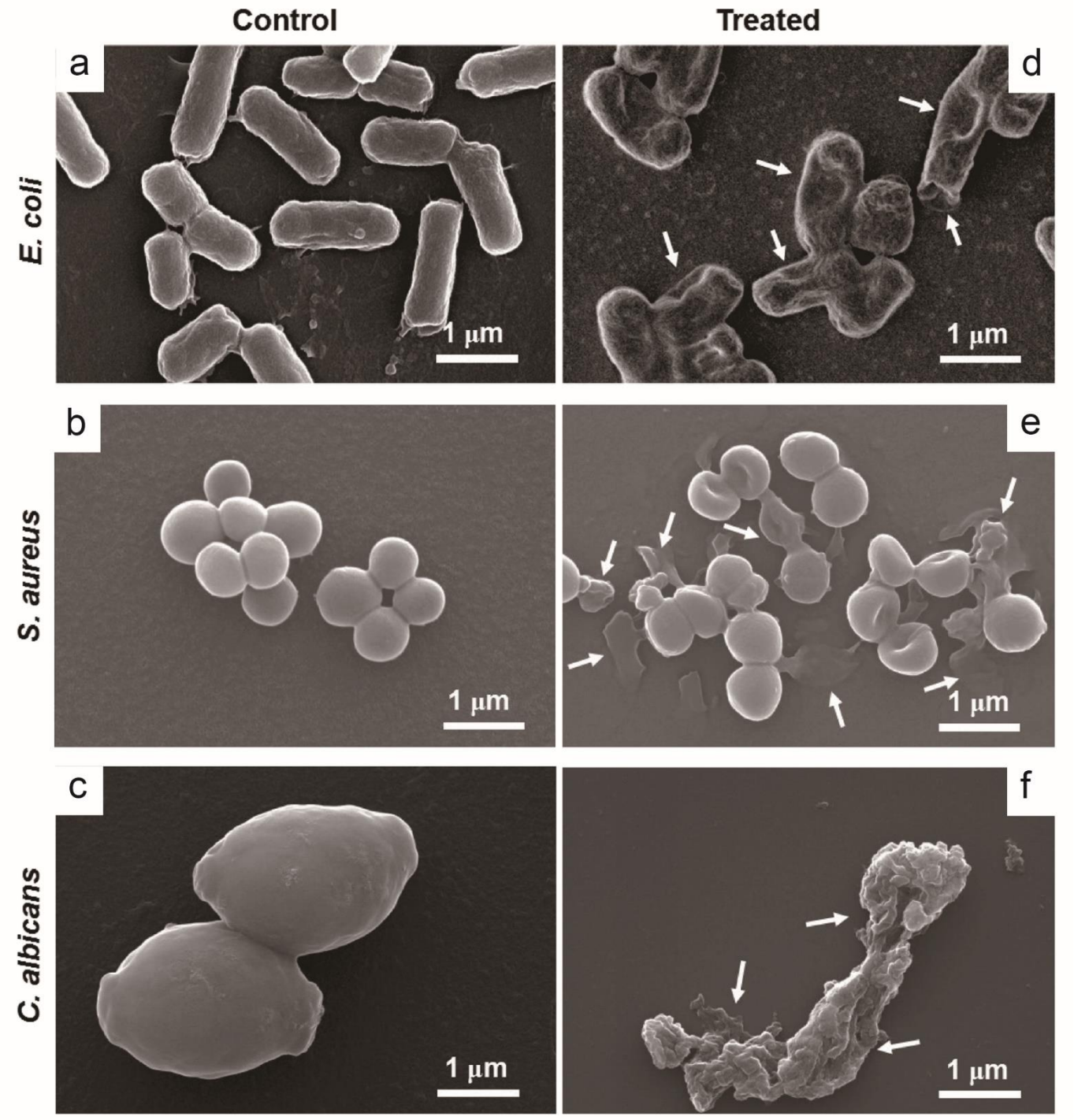
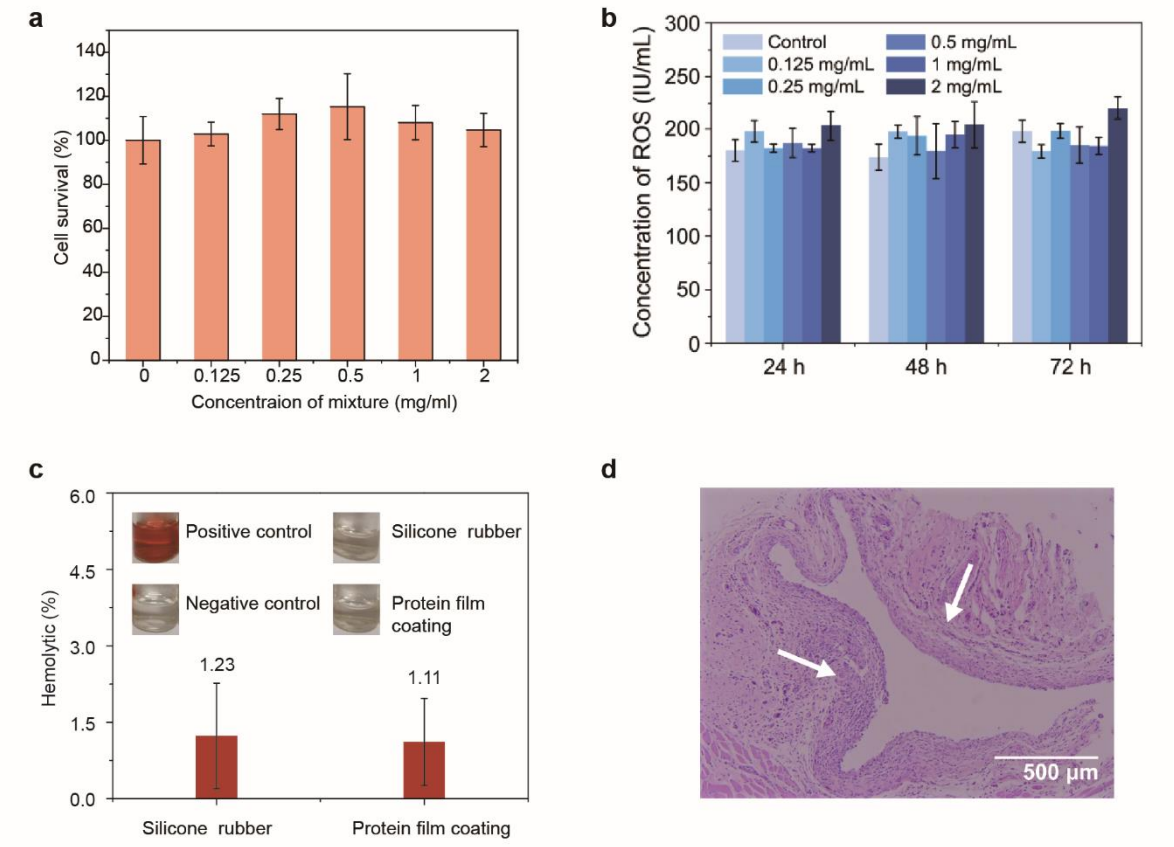


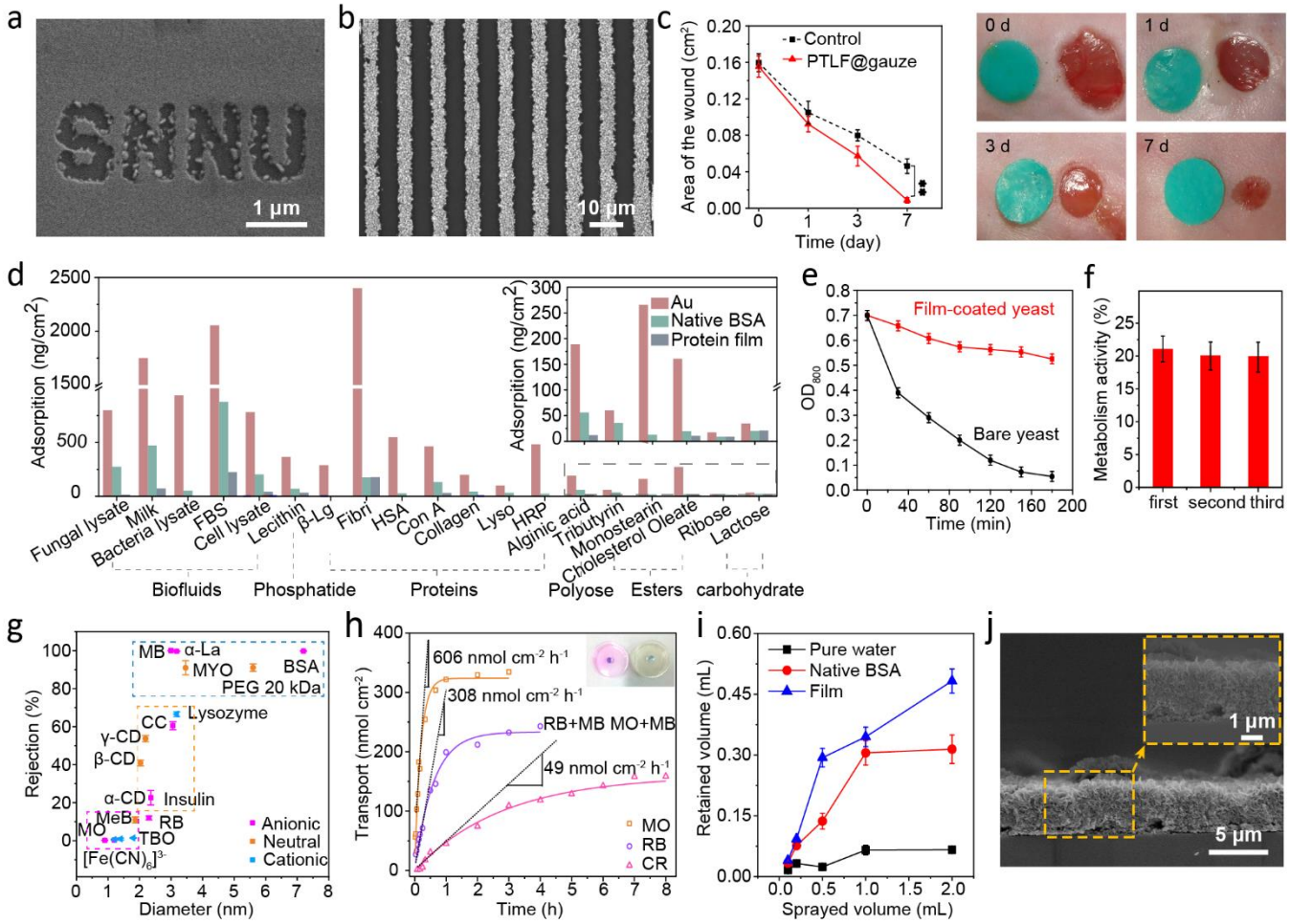
Supplementary Fig. 1 | Weather resistance test of lysozyme film. The bright field and fluorescence images show lysozyme films stained with ThT. The films were subjected to various aging tests to assess their weather resistance. The hot air aging test involved placing the film in an oven at 80°C for 60 days, while the ultraviolet aging test involved exposing the film to a 9 W ultraviolet lamp at a distance of 5 cm for 60 days. The microbial environmental corrosion test involved placing the film in a microorganism breeding environment containing E. coli (about 5×10^5 CFU/mL) and S.aureus (about 5×10^5 CFU/mL) in incubator at 37 °C. Panels adapted with permission from ref.[1], RSC.



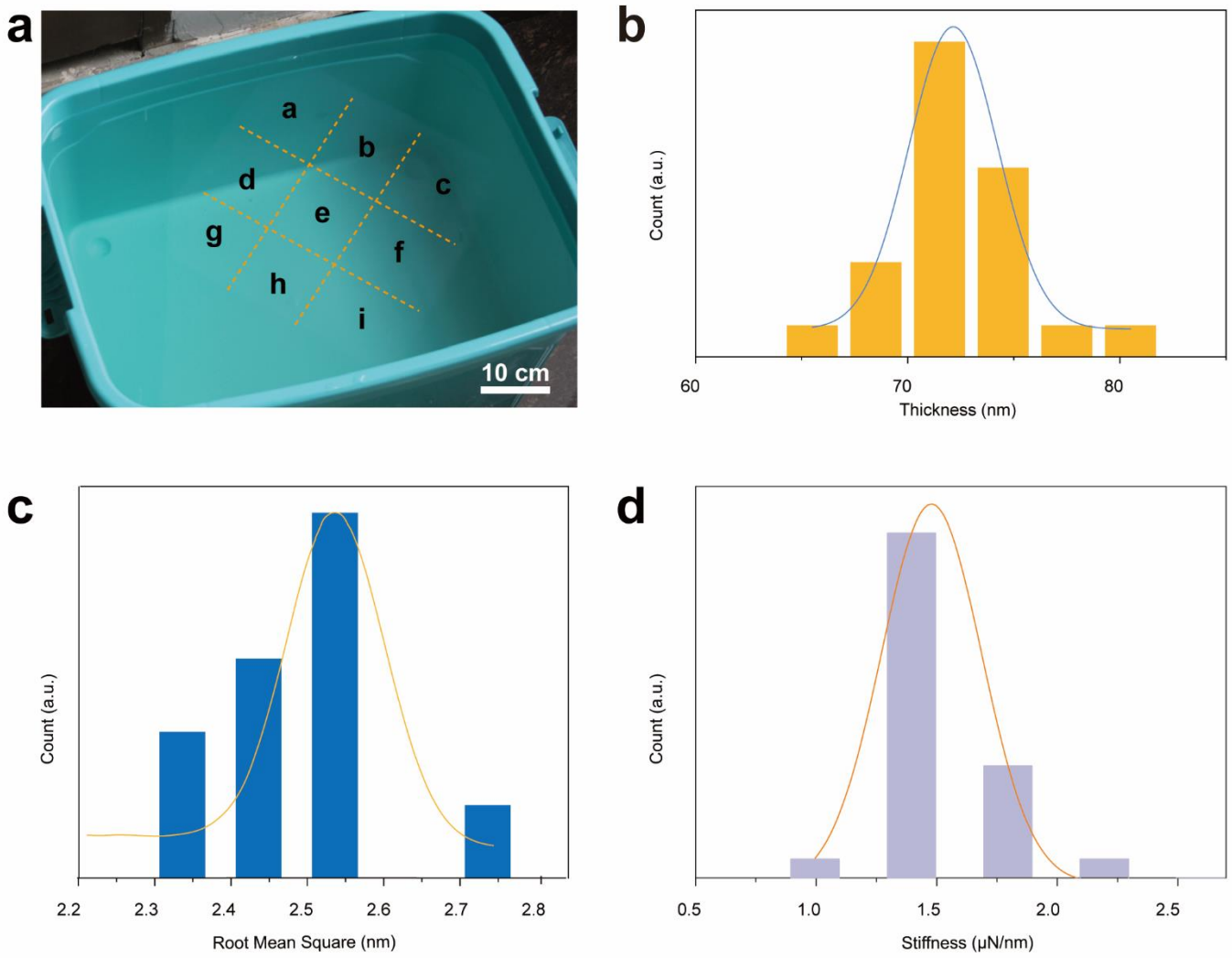
Supplementary Fig.2 | SEM images of an antimicrobial test conducted on the surface of lysozyme film. a-c, SEM images of the control group without the lysozyme coating after exposure to three types of microbes. **d-f**, SEM images of the treated group with the film-coated surface after exposure to three types of microbes. The white arrows in panels (**d-f**) indicate that the cell walls of the microbes were either deformed or destroyed on the lysozyme film surface, suggesting a potential perturbation of the cell membrane by lysozyme. Panels adapted with permission from ref.[2], RSC.



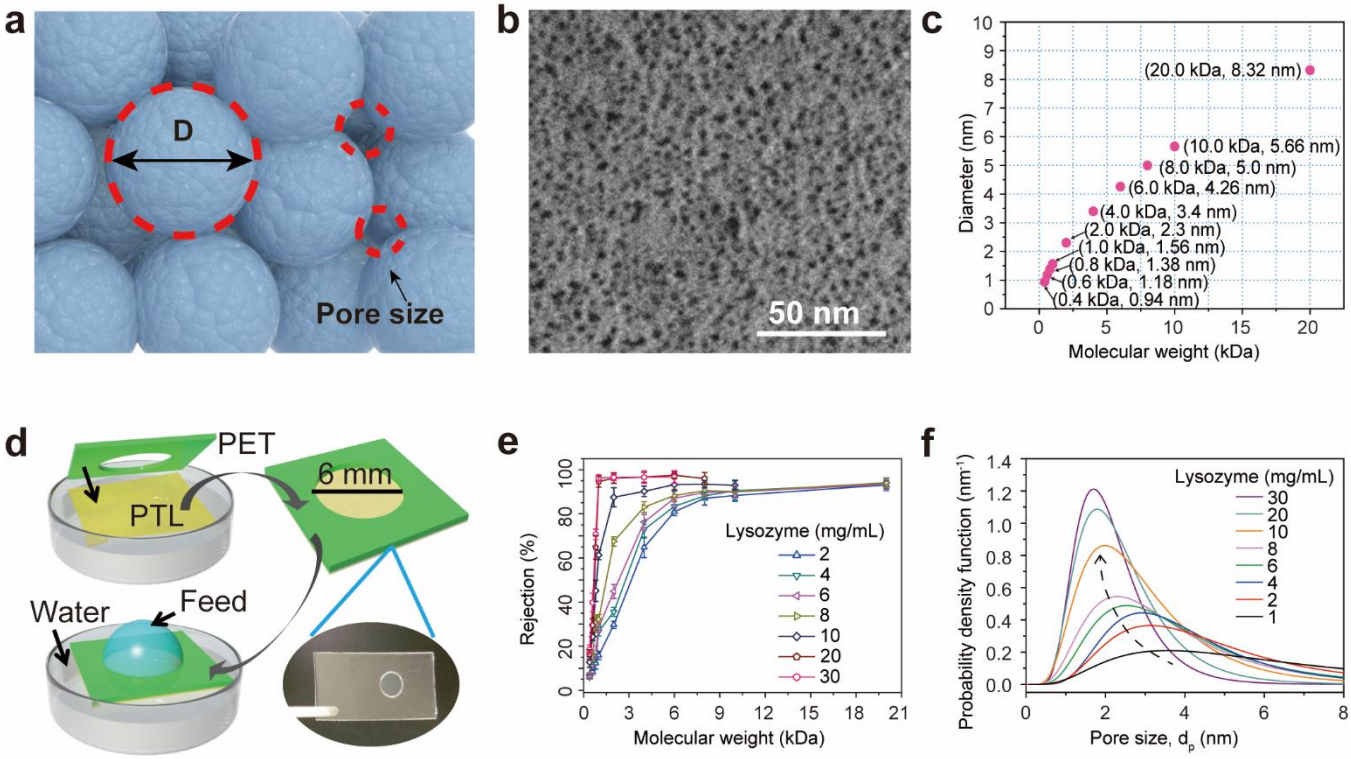
Supplementary Fig. 3 | Biocompatibility of 2D protein film. a, Cell survival of 3T3 mouse fibroblast cells treated with different concentrations of 2D BSA film. **b**, Measurement of reactive oxygen species (ROS) content in 3T3 cells using enzyme-linked immunosorbent assay (ELISA) after treatment with varying concentrations of 2D BSA film. **c**, Hemolysis assay performed on silicone rubber coated with the protein film. The PTB coating showed a low hemolytic effect on red blood cells. In the inset optical photographs, the positive control depicts ruptured red blood cells releasing a large amount of hemoglobin, while the negative control shows no ruptured red blood cells in normal saline. The silicone rubber and protein film coating demonstrated no significant red blood cell rupture. **d**, Histopathologic evaluation of tissue biocompatibility revealed that the capsule (white arrows) consisted mostly of fibroblastic proliferation associated with collagen deposition, without any active inflammatory reaction or necrosis present. Panels adapted with permission from ref.[3], Wiley.



Supplementary Fig. 4 | Applications of pure 2D protein films. a-b, Micro/nanofabrication. SEM image of the nanopatterning on Cu surface (**a**) after electron beam lithographic etching by using protein film as the resist. SEM image of the patterned Ag layer (**b**) by electroless deposition (ELD) of Ag on the patterned protein film. **c**, Protein film-coated wound dressings toward anti-infection and wound healing. The change of size and macroscopic appearance of the wounds post-surgery treated by gauze coated with lysozyme film (PTLF@gauze). **d**, Antifouling coating. The adsorption amount of different molecules on bare Au chip, native BSA adsorption layer, or PTB-coated Au chips. **e-f**, Artificial spores. Protective effect of PTL-coated yeast against the zymolyase (**e**). Repeated measurements of the metabolic activity of a protein film-immobilized yeast layer (**f**). **g-h**, Molecular separation and dialysis. Separation performance for neutral and ionic compounds (**g**). Time-dependent transport of dyes and the transport rate (**h**). Optical photographs to show the film rejects large molecules (methyl blue, MB) while allowing small molecules (methyl orange, MO and rhodamine B, RB) to pass through.[4] **i**, Pesticide deposition enhancement. Retained volume of pure water, native BSA, or the as-prepared PTB solution on a lotus leaf surface after spraying.[5] **j**, Biomineralization. The FE-SEM images of hydroxyapatite cross-section on the protein film after incubation for 2 weeks. Panels adapted with permission from **a, b**, ref.[6], Wiley; **c**, ref.[7] , RSC; **d**, ref.[3], Wiley; **e**, **f**, ref.[8], Wiley; **J**, ref.[9], Wiley.



Supplementary Fig. 5 | Characterization of homogeneity of 2D protein film. a, Photo image showing nine regions of the protein film, labeled as a, b, c, d, e, f, g, h and i, for analyzing the homogeneity. **b**, The thickness frequency distribution of protein films measured by SEM, coefficient of variation=4.5%. **c**, The root mean square (RMS) frequency distribution obtained from AFM measurements, coefficient of variation=10.9%. **d**, The stiffness frequency distribution from nanoindentation measurements, coefficient of variation=14.4%. Panels adapted with permission from ref.[10], Wiley.



Supplementary Fig. 6 | The regulation of pore size of 2D protein film. a, Schematic of the pores resulting from the packing of oligomer particles. The pore diameter (*d*) and particle size (*D*) have a relationship of d = D (2−√3)/√3 = 0.1547 D. **b**, TEM bright-field image of the protein film. **c**, Stokes radii of PEG with different molecular weight. **d**, Schematic of the PEG permeation analysis. The film is transferred to a PET substrate with a pore size of 6 mm. **e**, The rejection of PEGs with different molecular weight by the films prepared at different protein concentration. **f**, The pore size distribution of the 2D lysozyme film shown by a probability density function curve.

Supplementary Table 1 | 2D protein film-based composite films and their applications.

References	Functional block	Protein	New property	Application
[11]	Tin ion	Lysozyme	Electrocatalytic	Electrocatalytic
[12]	Silver nanoparticle	Lysozyme, BSA, α-amylase, collagen, keratin, pepsin, and egg albumin	Electrical conductivity sensitive to slight vibrations	Stealth Information Transmission
[13]	Graphene	Lysozyme	Photothermal effect	Photoactuation
[14]	PEG	Lysozyme	Antifouling	Seal dentinal tubules towards dentin hypersensitivity treatment
[15]	Perfluorooctanoyl fluoride	Lysozyme	Superhydrophobicity	Protein Crystallization
[16]	Carnauba wax	Lysozyme	Superhydrophobicity	Packaging and blood-repelling materials
[17]	Sodium alginate	Lysozyme	High tenacity	Controlling the crack development of the conductive layer
[18]	β-cyclodextrin	Lysozyme	Strong adsorption to uranium ions	Extract uranium ions from aqueous
[1]	9,10-distyrylanthracene	Lysozyme	Stable fluorescence	Anti-counterfeiting
[19]	C-terminal Amelogenin	Lysozyme	Oriented arrangement of amorphous calcium	Enamel Remineralization
[20]	Plant extracts and ZnO	BSA	High ultraviolet absorption	Sunscreen
[21]	Laponite	BSA	High hygroscopicity	Smart window
[22]	ε-Polylysine	BSA	Effective antibacterial activity	Antibacterial coating
[23]	Cyclosporin A	Lactoferrin	Increasing the bioavailability of cyclosporin A	Treatment for dry eye syndrome

Supplementary Table 2 | Encapsulation of different functional blocks into 2D protein film

Proteins	Functional block	Procedure
Lysozyme	Graphene	Mix the following three solutions in equal volume and incubated at 30 °C for 8 hours. After that, adjust the pH of the mixture to ~2.2 and further incubate the system at 90 °C for at 6 hours: GO aqueous dispersion (3 mg/ml) Lysozyme aqueous dispersion TCEP aqueous solution (pH 4.5, 50 mM)
Lysozyme	Sodium alginate	Mix the following two solutions and incubate the system at room temperature for 2

Proteins	Functional block	Procedure
		hours: 1 ml of lysozyme HEPES buffer solution (8 mg/ml, pH 7.4) 1 ml of sodium alginate aqueous solution (2 mg/ml) 2 ml of TCEP aqueous solution (pH 7.0, 50 mM)
Lysozyme	9,10-distyrylanthracene (DSAI)	Mix the following three solutions at a volume ratio of 1 : 0.2 : 1 and incubate the system at room temperature for 2 hours: Lysozyme (2 mg/ml in 10 mM HEPES buffer at pH 7.2) DSAI (0.1 mg/ml dispersed in water) TCEP buffer (50 mM TCEP in 10 mM HEPES buffer at pH 5.0).
Lysozyme	C-terminal Amelogenin (C-AMG)	Mix the following three solutions in equal volume and incubate the system at room temperature for 2 hours: Lysozyme/C-AMG solution (Lysozyme: 2 mg/ml, pH=7.4; C-terminal Amelogenin: 1 mg/ml) TCEP (50 mM, pH=5.8)
BSA	Laponite	BSA-glycerol dispersion (100 mg of BSA, 100 mg of glycerol, 5 ml of water) Laponite (Lap) dispersions (add 0, 12, 60, 120, or 180 mg of Lap powder into 12 ml of pure water under stirring at 600 rpm for 1 h). Mix the BSA-glycerol and Lap dispersions and stir at 100 rpm for 5 h at room temperature. Add 0.5 ml of TCEP aqueous solution (50 mM, pH 4.0) into BSA/Lap dispersion, and cast the system into a polytetrafluoroethylene (PTFE) mold, and a transparent film can be obtained after incubating at room temperature for 12h.
BSA	ε-Polylysine	Mix the following three solutions in equal volume and incubate the system at room temperature for 2 hours: BSA aqueous solution (10 mg/ml) TCEP aqueous solution (50 mM at pH 4.5) ε-Polylysine aqueous solution (0.1-0.5 g/ml)
Lactoferrin	Cyclosporin A	Mix the following four solutions at a volume ratio of 15:15:15:n (n = 1, 2, 3, 4, 5, 6), and then incubated in a humid environment (generally for 6–12 h) at room temperature.

Proteins	Functional block	Procedure
		Lactoferrin (7 mg/ml, pH 6.2),
		Hyaluronic acid solution (6/9/12 mg/ml),
		TCEP solution (50 mM TCEP, pH 7.0),
		Cyclosporin A solution (7.5 mg/ml in aqueous ethanol 50% by volume)

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