

Electronic Supplementary Material**Silver ion-catalysis synthesized protein imprinted polyacrylamide/calcium alginate hydrogel film with high adsorption, recognition, and antibacterial properties**

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Text S1 Antimicrobial properties

Confocal laser scanning microscopy (CLSM, TCS SP8, Germany) was employed to analyze the live/dead imaging of imprinted (MIP) and non-imprinted (NIP) hydrogel membranes in order to evaluate their antimicrobial properties. Sterilized hydrogel membrane slices (2 cm) were placed in 12-well plates, and 400 μL of a bacterial suspension (*Staphylococcus aureus*, *Escherichia coli*, 1×10^8 CFU/mL) diluted in medium was added to each well, followed by incubation for 24 h. After incubation, the samples were washed at least three times with PBS. The samples were then stained with propidium iodide (PI) and Hoechst 33258 dye for 30 min at room temperature, in the dark. To fix the bacteria, 2.5% glutaraldehyde was added and the samples were incubated for 4 h. After fixation, the bacteria were dehydrated with a graded ethanol series. Live and dead cells were observed under CLSM at excitation/emission wavelengths of 488–514 nm for live cells and 352–461 nm for dead cells. The MIP and NIP hydrogel membrane sheets were sterilized front and back by the UV irradiation for 30 min. Bacterial colonies were picked from solid agar medium and placed in 10 mL of liquid bacterial nutrient solution and incubated in a shaker at 37 °C for 12 h, then diluted with liquid medium buffer solution to the target concentration. The sample membrane was added to 3 mL of bacterial solution in a 12-well plate and incubated in a constant temperature incubator for 24 h. The plate was removed and diluted to 10^{-6} coated plate, incubated in a constant temperature incubator for 24 h. Calculation of the inhibition rate (Y) is based on the number of colonies grown, which can be expressed as follows:

$$Y/\% = \frac{N_0 - N_1}{N_0} \times 100 \quad (\text{S1})$$

where N_0 is the number of colonies in the blank control group and N_1 is the number of colonies in the experimental group.

Text S2 Adsorption kinetics models

In order to evaluate the kinetic mechanism of the adsorption as well as the rate-determining step of the adsorption process, the pseudo-first-order and pseudo-second-order models were used to fit the adsorption data. The pseudo-first-order kinetic model and the pseudo-second-order kinetic model are given below as Eqs. (S2) and (S3), respectively:

$$Q_t = Q_e (1 - e^{-k_1 t}) \quad (\text{S2})$$

$$Q_t = \frac{Q_e^2 K_2 t}{1 + Q_e K_2 t} \quad (\text{S3})$$

where Q_e and Q_t are capacities of BSA adsorbed on films at equilibrium and at time t , respectively, and both K_1 and K_2 are rate constants of adsorption.

Text S3 Adsorption isotherm models

Experimental data were fitted into the Langmuir model and the Freundlich isotherm model, shown by Eqs. (S3) and (S4), respectively. Equation (S4) is expressed as follows:

$$Q_e = \frac{Q_m K_L c_e}{1 + K_L c_e} \quad (\text{S4})$$

where Q_e ($\text{mg} \cdot \text{g}^{-1}$) is the adsorbed BSA at adsorption equilibrium, Q_m ($\text{mg} \cdot \text{g}^{-1}$) is the maximum capacity, c_e ($\text{mg} \cdot \text{L}^{-1}$) is the BSA concentration, and K_L is the Langmuir constant related to the affinity of binding sites. Equation (S5) is expressed as follows:

$$Q_e = K_F c_e^{1/n} \quad (\text{S5})$$

where K_F is the Freundlich constant ($\text{L} \cdot \text{g}^{-1}$) and n is the Freundlich exponent obtained from the liquid phase adsorption isotherm.

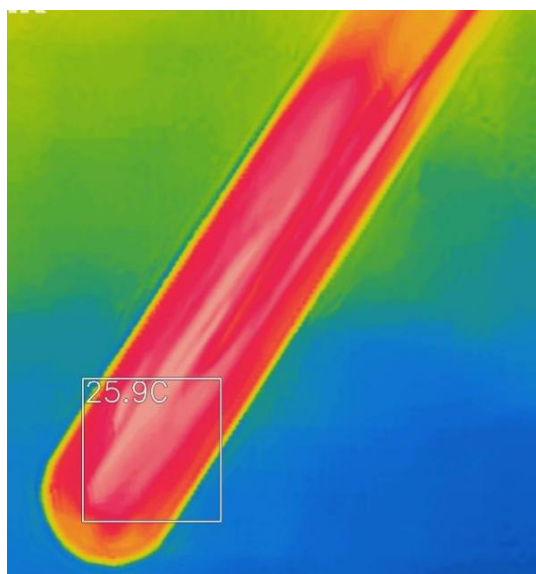


Fig. S1 Infrared image at near room temperature (25.9 °C).

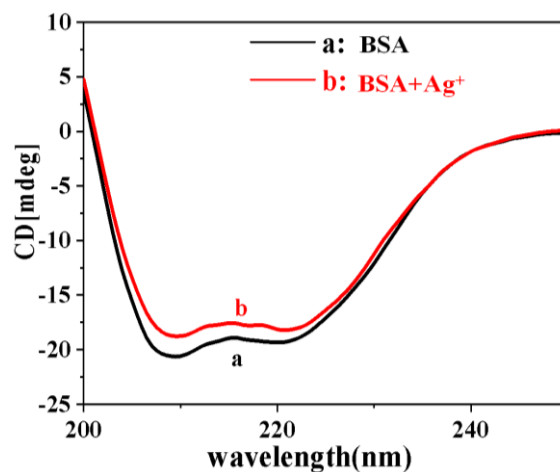


Fig. S2 Circular dichroism spectra of the pure BSA solution and BSA solution with $0.48 \text{ mmol}\cdot\text{L}^{-1} \text{ Ag}^+$.

Table S1 Formulation data for investigating the effect of the silver ion concentration on the polymerization time and temperature

Sample	m/g						
	AM	MBA	NaAlg	AgNO ₃	APS	NaHSO ₃	H ₂ O
1	3.15	0.064	0.210	0	0.0014	0.0025	20
2	3.15	0.064	0.210	0.0003	0.0014	0.0025	20
3	3.15	0.064	0.210	0.0006	0.0014	0.0025	20
4	3.15	0.064	0.210	0.0012	0.0014	0.0025	20
5	3.15	0.064	0.210	0.0018	0.0014	0.0025	20
6	3.15	0.064	0.210	0.0024	0.0014	0.0025	20

Table S2 Synchronized fluorescence peaks of tyrosine and tryptophan

$c(\text{Ag}^+)$ /($\text{mmol}\cdot\text{L}^{-1}$)	Tyr		Trp	
	Fluorescence intensity/a.u.	Peak location/nm	Fluorescence intensity/a.u.	Peak location/nm
0	21076	312	35931	346
0.08	20691	311	35618	346
0.16	20289	312	34324	346
0.24	19626	312	33009	346
0.32	18510	312	31635	346
0.40	17989	312	30942	346
0.48	17556	312	30315	346
0.56	17090	312	29627	346
0.64	16559	312	28887	346