Supporting Information

Polymer membrane with glycosylated surface by a chemo-enzymatic strategy for protein affinity adsorption

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Experiment section

Enzyme was recombinant overexpresed from *E. coli*.

The recombinant E. coli cells were incubated and harvested by centrifugation at 7000 × g for 10 min after cultivation in 100 ml of LB broth. The cells were then suspended in 20 ml of 10 mM PBS (pH 6.0) and sonicated for 20 min. The cell-free extract was obtained by centrifugation at 17 000 × g for 10 min. The cell-free extract was put on a column (1 ml) equilibrated with 10 mM sodium phosphate buffer (pH 7.4). The column was washed with 10 bed volumes of the initial buffer, and the enzyme was eluted with a linear gradient of sodium chloride in the same buffer. Fractions containing β-galactosidase were pooled and concentrated.

Determination of glycosyl binding density (BD) and reaction efficiency (RE) of OH for the glycosylated membrane.

Briefly, a sample of glycosylated membrane was immersed in 3.0 mL of FITC-RCA_{120} (containing 10 μg/mL protein) for 120 min at 25 °C. A piece of POEGMA-modified MPPM was used as control. The membrane adsorbed FITC-RCA_{120} was then rinsed extensively with PBS buffer solution. A fluorescence spectrophotometer (RF-5300-PC, Shimadzu, Japan) was used to measure the concentration of FITC-RCA_{120} in PBS solution (Emission/Excitation of FITC: 488/518 nm). The glycosyl BD (nmol/cm^2) and RE of OH were compared based on the data of fluorescent emission spectra and calculated by the following equations:

$$BD = \frac{(C_0 - C_1) \times V - C_w \times V_w}{M \times A} \quad (1)$$

(C_0: concentration of the FITC-RCA_{120} solution before adsorption; C_1: concentration of the FITC-RCA_{120} solution after adsorption; C_w: concentration of the washed FITC-RCA_{120} solution; V: volume of the initial FITC-RCA_{120} concentration; V_w: volume of the washing buffer solution; M: molecular weight of the FITC-RCA_{120}; A: the area of the membrane (4.91 cm^2))

$$RE = \frac{360BD}{142GD} \times 100\% \quad (2)$$

(360: molecular weight of OEGMA; 142: molecular weight introduced after introducing

galactose residues; *BD*: the glycosyl BD of galactose, *GD*: the GD of POEGMA)

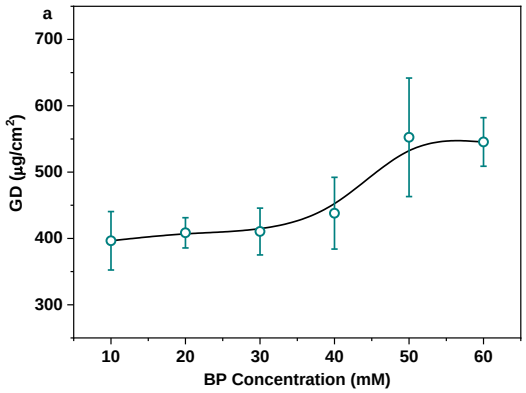
Characterization of the membrane surface.

Chemical composition of the membrane surface was characterized by FT-IR/ATR accessory (Nexus 470, ZnSe crystal, 45º). Thirty two scans were taken for each spectrum at a resolution of 4 cm^{-1}.

FESEM (, Sirion-100, FEI, USA) was used to capture the surface morphologies of MPPM at an acceleration voltage of 25.0 kV after the samples were sputtered with a thin gold layer.

WCA of the membrane surface was determined with a CTS-200 system (Mighty Technology Pvt. Ltd., China) at room temperature by sessile drop method. Briefly, a water drop (2 μL) was carefully dropped onto the top membrane surface with a microsyringe, and then images of the water droplet were recorded and WCA was calculated with the specific software. At least five different surface locations of each sample were measured and the averaged value was presented.

Solid surface fluorescence spectroscopy (SSFS) measurements of the membrane surface were carried by fluorescence spectrophotometer (RF-5300-PC, Shimadzu, Kyoto, Japan) equipped with a solid sample holder accessory (P/N 204-26836-01). The cell plane where the membranes were fixed was set at 45° to the excitation beam. The fluorescence emission at 500 ~ 600 nm was measured with an excitation light of 488 nm and a bandwidth of 5 nm.



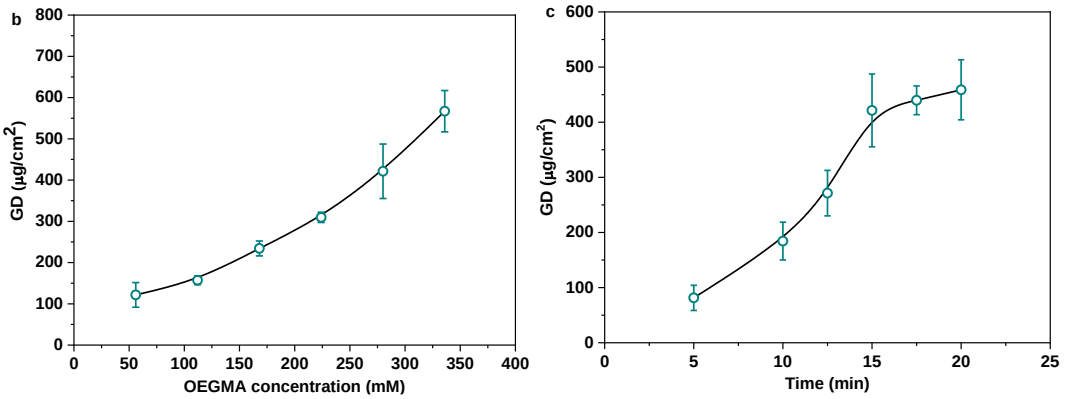


Figure S1. (a) Influence of BP concentration on the POEGMA GD (OEGMA concentration = 280 mM, UV irradiation time = 15 min); **(b)** Influence of the OEGMA concentration on the POEGMA GD (BP concentration = 40 mM, UV irradiation time = 15 min); **(c)** Influence of UV irradiation time on the POEGMA GD (BP concentration = 40 mM, OEGMA concentration = 280 mM). (All experiments were repeated six times.)

The POEGMA GD is correspondingly varied with BP concentration, monomer concentration and UV irradiation time (Figure S1~S3). The effect of BP concentration on the POEGMA GD is shown in Figure S1 (a). Increasing the BP concentration brought slowly enhancement of the POEGMA GD because more active sites were generated. However, the increase of active sites on the membrane surface also may increase the surface density of the propagating chain radicals, which will lead to intensive termination reactions and prevent further growth of the grafted chains. Therefore, the POEGMA GD stopped increasing at high BP concentration. Besides, it can be seen from Figure S1 (b), OEGMA concentration has a great influence on the POEGMA GD. The POEGMA GD increased significantly with the increase of OEGMA concentration. This could be ascribed to the increased chances of molecular collision probability between the active sites and the monomers on the membrane surface. UV irradiation time also has influenced the POEGMA GD largely (Figure S1 (c)). It was found that the POEGMA GD increased obviously with the UV irradiation time at first and then remained almost constant when the radiation time exceeded 15 min. This phenomenon was due to the fact that the adsorbed amount of BP on membrane surface was

approximately limited. Consequently, the active sites on the membrane surface could not increase infinitely. That is, 15 min was enough for surface activation of the membranes.

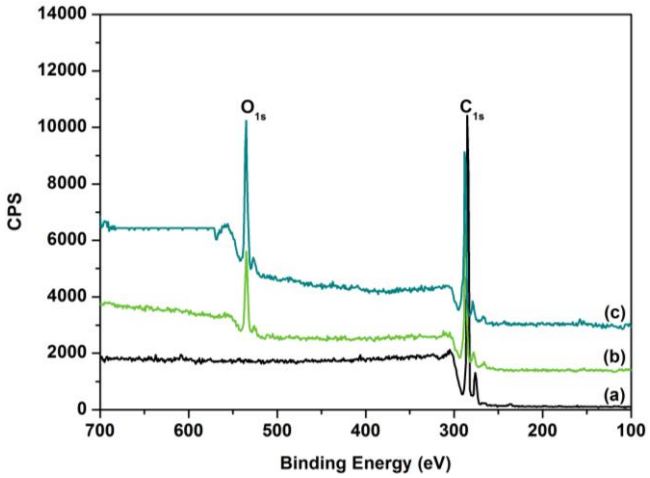


Figure S2. Survey XPS spectra of C1s and O1s of (a) MPPM, (b) the POEGMA-modified MPPM (POEGMA GD = 350 μg/cm2), and (c) the galactose-immobilized MPPM (POEGMA GD = 350 μg/cm2, galactose BD = 1.603 nmol/cm^2).

Table S1 Elemental composition of the surface of nascent MPPM, the POEGMA-modified MPPM (POEGMA GD = 350 μg/cm^2) and the galactose-immobilized MPPM (POEGMA GD = 350 μg/cm^2, galactose BD= 1.603 nmol/cm^2).

	C (%)	O (%)
MPPM	100	0
POEGMA-modified MPPM	76.51%	17.56%
galactose-immobilized MPPM	67.72%	24.87%

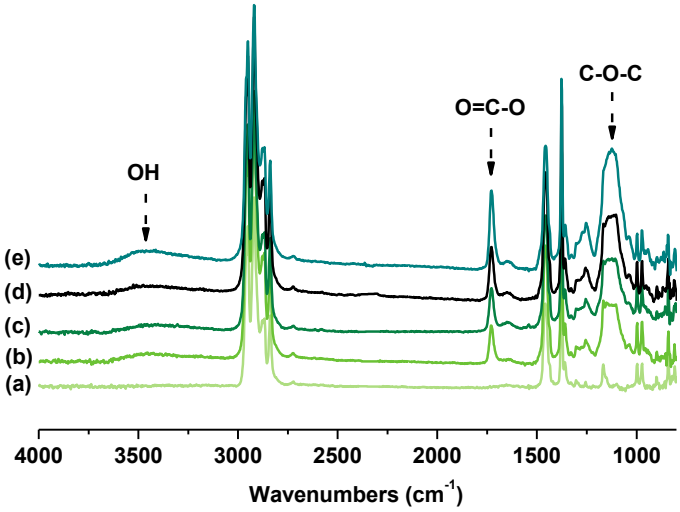


Figure S3. FT-IR/ATR spectra of (a) MPPM, (b) the POEGMA-modified MPPM (POEGMA GD = 350 μg/cm^2), and (c ~ e) the galactose-immobilized MPPM (POEGMA GD = 350 μg/cm^2, galactose BD = 0.831 nmol/cm^2, 1.6031 nmol/cm^2, 5.410 nmol/cm^2).

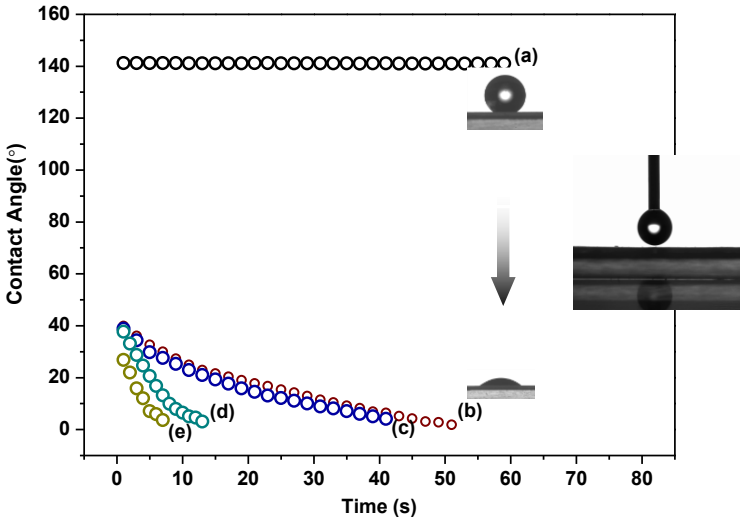


Figure S4. Time dependence of WCA on: (a) MPPM, (b) the POEGMA-modified MPPM (POEGMA GD = 350 μg/cm^2), and (c ~ e) the galactose- immobilized MPPM (POEGMA GD = 350 μg/cm^2, galactose BD = 0.831 nmol/cm^2, 1.6031 nmol/cm^2, 5.410 nmol/cm^2). (All experiments were repeated three times.)

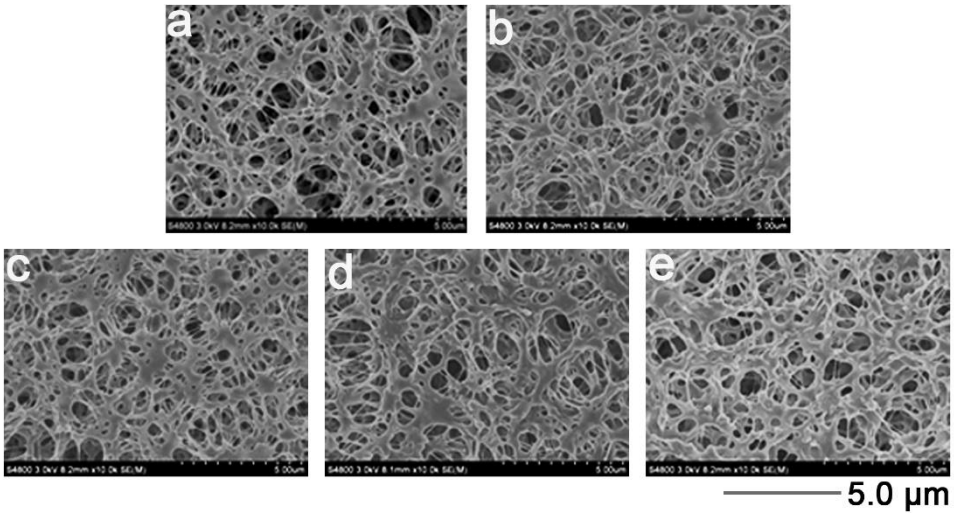
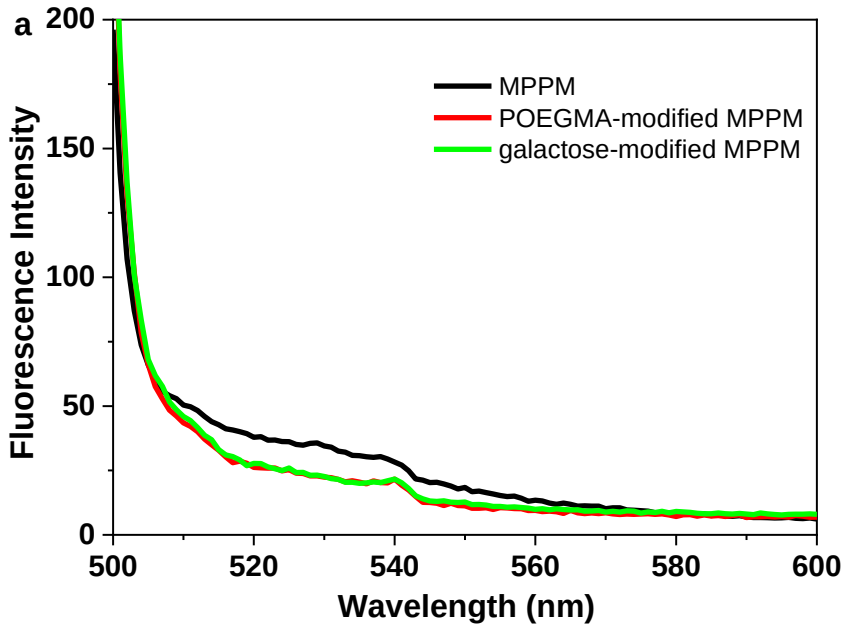


Figure S5. SEM images of the membranes surfaces before and after modification. (a) The nascent MPPM. (b) The POEGMA-modified MPPM (POEGMA GD = 350 μg/cm^2). (c ~ e) The galactose-immobilized MPPM (POEGMA GD = 350 μg/cm^2, glycosly BD = 0.831 nmol/cm^2, 1.6031 nmol/cm^2, 5.410 nmol/cm^2).



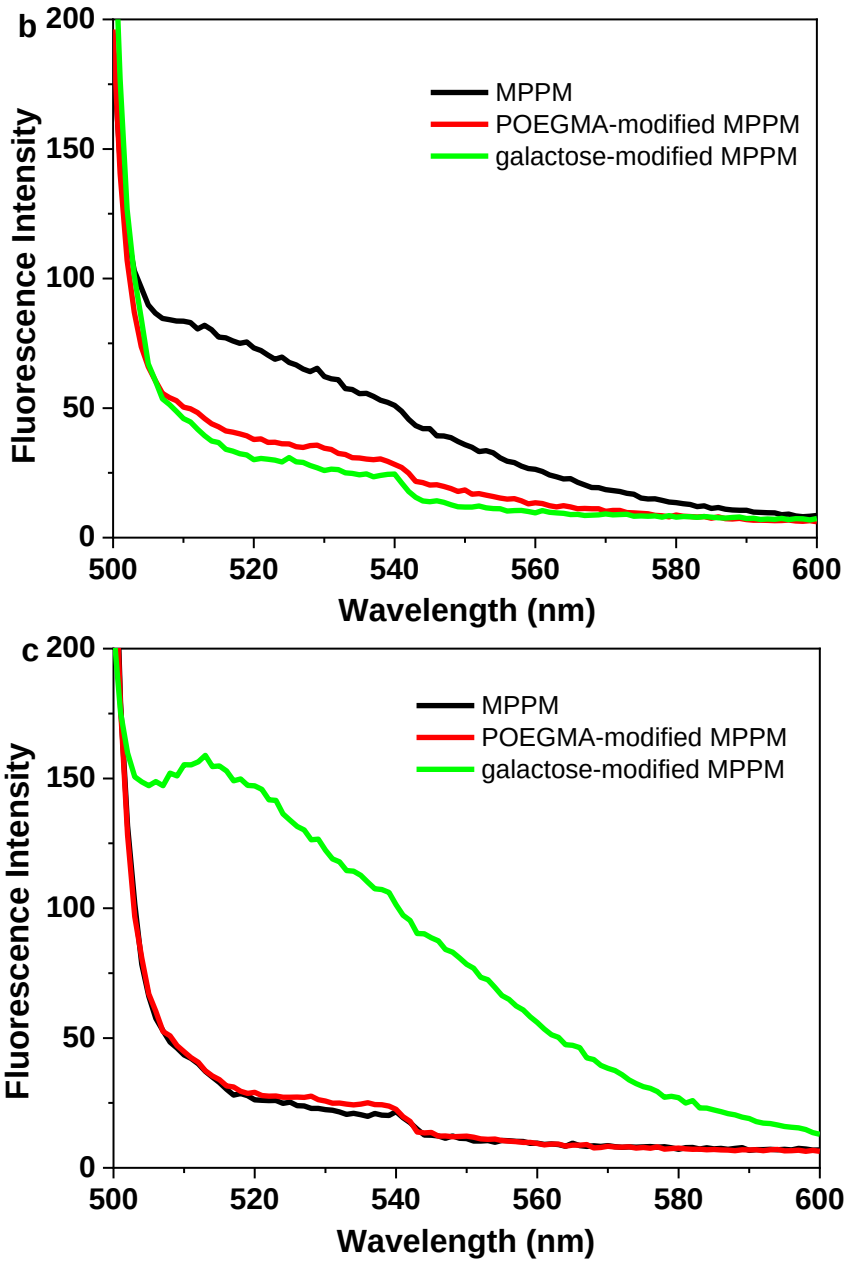


Figure S6. Typical SSFS spectra of the membranes with (a) FITC-BSA, (b) FITC-Con A, and (c) FITC-RCA_{120} adsorption (POEGMA-modified MPPM: POEGMA GD = 350 μg/cm^2; galactose-modified MPPM: POEGMA GD = 350 μg/cm^2, glycosyl BD = 1.603 nmol/cm^2.). (All experiments were repeated three times.)

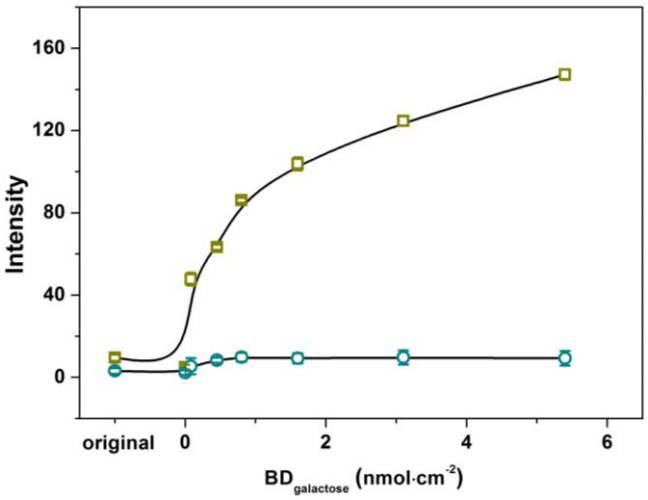


Figure S7. Influence of glycosyl BD (POEGMA GD = 350 μg/cm², glycosyl BD= 0.08 nmol/cm², 0.44 nmol/cm², 0.80 nmol/cm², 1.60 nmol/cm², 3.63 nmol/cm², 5.41 nmol/cm²) on the fluorescence intensity of FITC-RCA_{120} adsorbed (□) or desorbed (○) on membrane surfaces measured with CLSM. Original and 0 on the horizontal axis represent the nascent and the POEGMA-modified MPPM, respectively. (All experiments were repeated three times.)

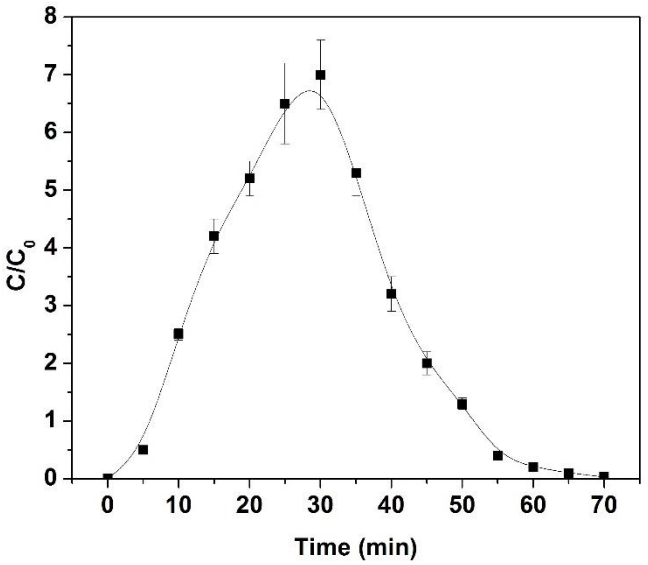


Figure S8. Elution curve of RCA_{120} adsorbed on the glycosylated membrane with 1 M galactose solution. (All experiments were repeated three times.)