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Surface Glycosylation of Polymer Membrane by Thiol–Yne Click Chemistry for Affinity Adsorption of Lectin

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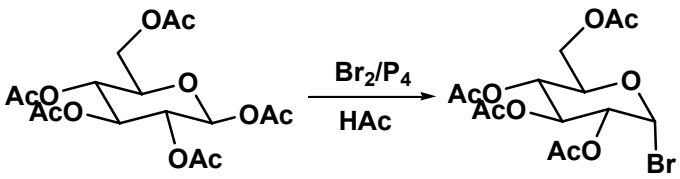
General method and materials

Unless otherwise noted, analytical grade chemicals and solvents were purchased and used without further purification. MPPM was a commercial product from Membrana GmbH (Germany) with an average pore size of 0.20 μm and a relatively high porosity of about 75 %. ^{1}H NMR and ^{13}C NMR (500MHz) analyses were done on a Bruker Avance DMX 500 spectrometer, with the chemical shift reported in ppm and referenced to signals from residual protons of the solvents. HRMS analysis was carried out on a GCT Premier CAB170 (TOF MS EI^{+}). ATR/FT-IR spectra were collected using a Nicolet FT-IR/Nexus 470 spectrometer equipped with an ATR accessory (ZnSe crystal, 45º). Sixteen scans were taken for each spectrum at a resolution of 4 cm^{-1}. XPS measurements were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer, USA) with Al Kα radiation (hν = 1486.6 eV). In general, the X-ray anode was run at 250 W and the high voltage was kept at 14.0 kV with a detection angle at 54°. The base pressure of the analyzer chamber was about 5×10^{-8} Pa. Binding energies were calibrated by using the containment carbon (C 1s = 284.6 eV). The data analysis was carried out by using XPS Peak4.1 software. Morphology of the membrane surface was characterized by a field-emission scanning electron microscopy (SEM, Sirion-100, FEI, USA) after the samples were sputtered with a thin gold layer. Fluorescent images were captured by fluorescence microscopy (Nikon ECLIPES Ti-U, Japan). Blue light was used to excite the fluorescing membrane. The filters were chosen to detect the fluorescence ranging. Photo-reaction were conducted using a UV light system, equipped with a 500 W high pressure mercury bulb with a light intensity of 3 mW·cm^{-2} measured with a ZQJ-254 calibrated radiometer.

Synthesis of 1-Bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside



5.8 mL dry bromine was added to a solution of red phosphorus (3 g) in 30 mL acetic acid. The reaction mixture was stirred for 1 hour at 0 °C. Then, 20 g 1,2,3,4,6-penta-Oacetyl-β-D-glucopyranoside was dissolved in the resultant mixture and stirred overnight at room temperature. 60 mL $CHCl_3$ was added and then poured into 500 mL cool water. The resultant solution was washed by a saturated sodium bicarbonate solution and deionized water, respectively. The combined organic layers were dried over magnesium sulfate, filtered and concentrated to dryness under reduced pressure. The resultant oil was purified by crystallization in aether. 1-Bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside was obtained as a white crystal (15.2 g, 72.3%). mp 86-87 °C. $[\alpha]^{20}_D$= + 190.9 ($CHCl_3$). IR (ν_{max}, KBr, cm^{-1}) 1745 (C=O), 1381 (CH_3), 1230 (C-O-C), 1055 (C-O-C), 1051 (C-O-C), 683 (C-Br). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.04-2.11 (s, 12H, C*H*$_3$), 4.12 (m, 1H, C*H*), 4.3 (m, 1H, C*H*), 4.8 (m, 2H, C*H*$_2$), 5.2 (m, 1H, C*H*), 5.6 (m, 1H, C*H*), 6.6 (d, ***J=4.0 Hz***, 1H, C*H*). ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm): 170.6 (*C*=O), 170.0 (*C*=O), 169.9 (*C*=O), 169.6 (*C*=O), 98.1 (*C*H), 86.8 (*C*H), 72.3 (*C*H), 70.7 (*C*H), 70.3 (*C*H), 67.4 (*C*H$_2$), 61.1 (*C*H$_2$O), 20.8 (3×*C*H$_3$), 20.7 (*C*H3). HRMS (TOF MS EI+) m/z: calcd for [$C_{14}H_{19}O_9Br$] 410.0212, found 410.0229.

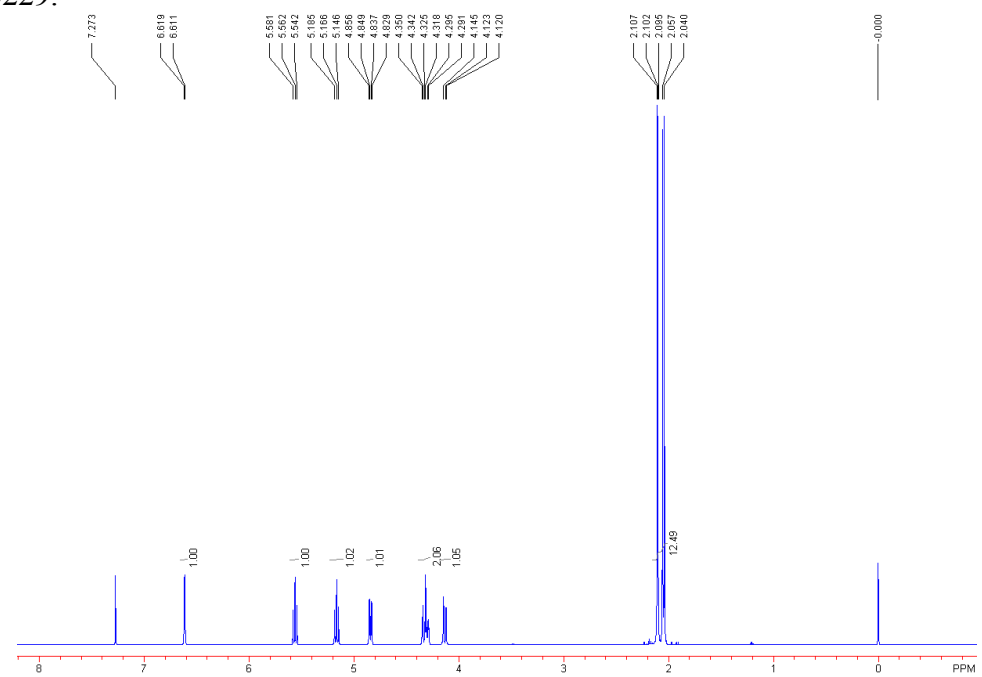


Figure S1 ^{1}H NMR spectrum of 1-bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside

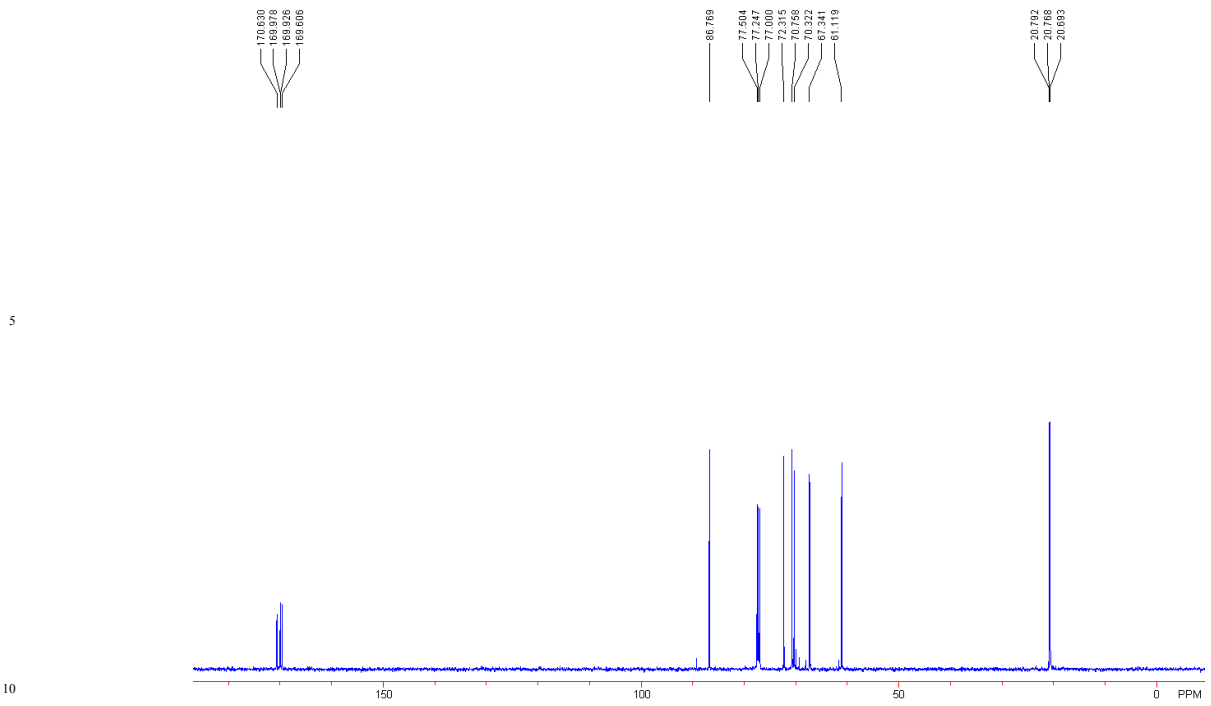
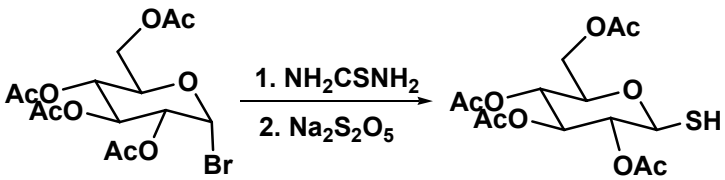


Figure S2 ^{13}C NMR spectrum of 1-bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside

Synthesis of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside thiol



1-Bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (4.53 g, 11 mmol) and sulfocarbamide (0.84 g, 11 mmol) were added into 5 ml dry acetone. After refluxing 1 hour, a white solid was obtained and subsequently filtrated. The solid was recrystallized in isopropanol. A white flocculent crystal can be collected. Then, the resultant compound (2.435 g, 5 mmol) was added into a solution of sodium metabisulfite (0.475 g, 2.5 mmol) in 30 mL $CHCl_3/H_2O$ (V/V, 1/1) mixed solvent and then refluxed for 1 hour. After cooling down till room temperature, the organic layer was separated and the water layer was washed with $CHCl_3$ (3×50 ml). The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness under reduced pressure. Purification by recrystallization in isopropanol afforded 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside thiol (1.61 g, 88.5%) as a white crystal. mp 111-113 °C. $[\alpha]^{20}_D$= + 7.3 ($CHCl_3$). IR (ν_{max} KBr, cm^{-1}): 2582 (S-H) 1745 (C=O), 1381 (CH_3), 1230 (C-O-C), 1055 (C-O-C), 1051 (C-O-C). ^1H NMR (500 MHz, $CDCl_3$) δ(ppm): 2.02-2.12 (4s, 12H, C*H*$_3$), 2.32 (d, 1H, S*H*), 3.73 (m, 1H, C*H*), 4.12-4.14 (dd, 1H, C*H*), 4.25 (t, 1H, C*H*), 4.55 (t,

1H, CH_2), 4.98 (t, 1H, C*H*), 5.11 (t, 1H, C*H*), 5.20 (t, ***J=9.9 Hz***, 1H, C*H*). ^{13}C NMR (500 MHz, $CDCl_3$) δ(ppm): 170.8 (*C*=O), 170.2 (*C*=O), 169.8 (*C*=O), 169.5 (*C*=O), 78.9 (*C*H), 76.5 (*C*H), 73.7 (*C*H), 68.4 (*C*H), 68.3 (*C*H), 62.1 (CH_2), 21.0 (CH_3), 20.9 (2×CH_3), 20.7 (CH_3). HRMS (TOF MS EI+) m/z: calcd for [$C_{14}H_{20}O_9S$] 364.0828, found 364.0836.

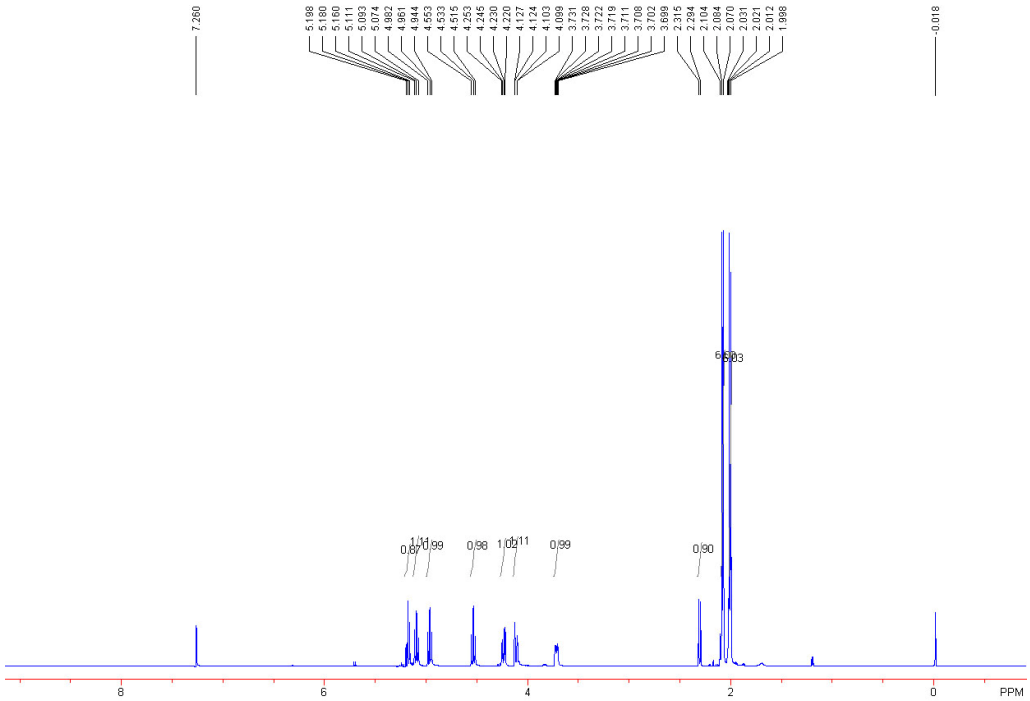


Figure S3 ^{1}H NMR spectrum of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside thiol

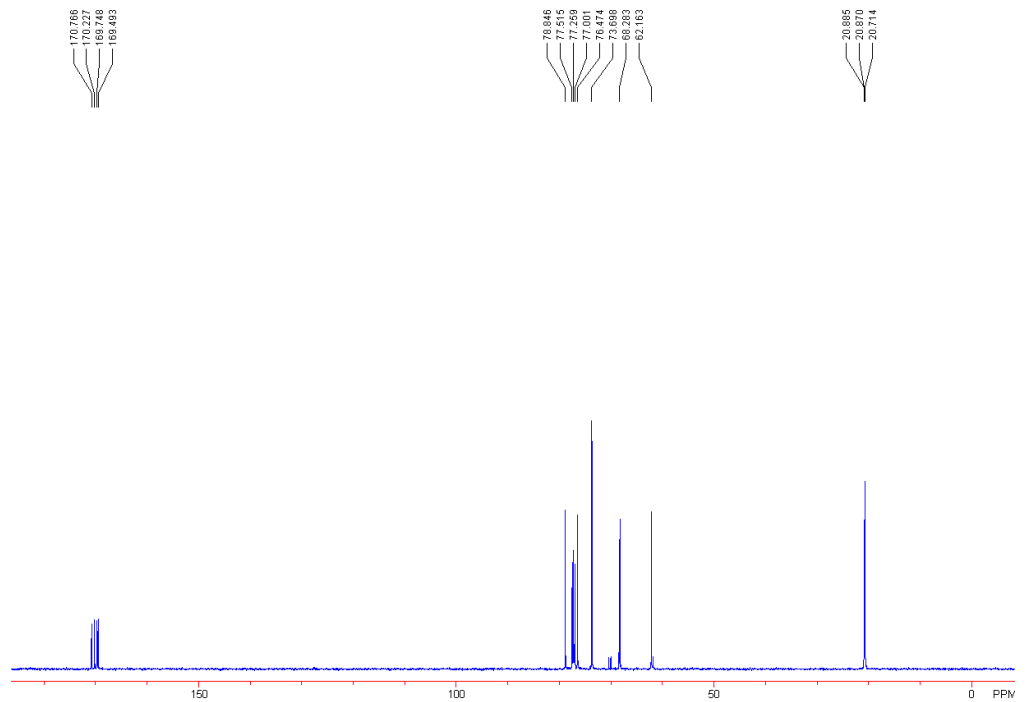


Figure S4 ^{13}C NMR spectrum of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside thiol

Glycosylation Procedure

As shown in Scheme 1, the polyAA-grafted membranes were prepared by photoinduced graft polymerization of AA on the MPPM surface using a UV system. Firstly, a MPPM sample was soaked in a solution of benzophenone (BP) in heptane for 60 min. Thereafter, the sample was taken out, dried in air and subsequently immersed into petri dish containing AA solution in water. UV irradiation was carried out and then, the sample was taken out of the reaction chamber, rinsed extensively with acetone and water, and dried. The grafting degree of polyAA can be well adjusted by the initiator concentration, monomer concentration and UV irradiation time. Thereafter, the polyAA-grafted MPPM was pre-wetted by soaking in ethanol for 5 min and in PBS (pH = 6.47) solution for another 2 h, respectively. The membrane was submerged in PBS solution. Subsequently the excessive EDC, NHS and propargylamine were added and reacted for 24 h. The alkyne-modified membrane was washed and dried. The "yne"-functionalized membrane was further glycosylated by thiol–yne click reaction in the presence of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl thiol of 50% in THF. The reaction was initiated by 2 wt.% benzophenone with irradiation of UV light (λ_{max} = 365 nm, 3 $mW \cdot cm^{-2}$) for 30 min. After that, the membrane was washed extensively with THF and dried. The deprotection of acetyl groups on glucose pendants were carried out by dipping the membrane in 5 mg/mL sodium methoxide/methanol for 2 h at room temperature. A large excess of methanol and water was used to rinse the glycosylated membrane. Then, the membrane was dried under vacuum. The glycosyl density on the membrane surface was determined by adjusting the grafting degree of polyAA. The glycerol modified membrane was fabricated by the reaction between the 1-thioglycerol and the "yne" functionalized surface. The amount of glycerol covalently immobilized on the membrane surface was determined by the change of weight.

Colorimetric Titration

The glycostylated MPPM were cut into piece and placed in a Schlenk tube. A solution of trifluoroacetic acid (4 M in water) was added. The mixture was held at 120 °C for 4 h, and then the solution was transferred to a flask. The membrane was washed with methanol, and the solution was collected and evaporated. Then, 1 mL of water and 0.5 mL of a phenol solution (5% in water) was added into the flask. Subsequently, 5 mL of sulfuric acid (98%) were added quickly. The mixture was stirred at 30 °C for 30 min and analyzed by UV-visible spectroscopy (UV-2450, Shimazu) at 485 nm. The concentration was determined by reference to a calibration curve with glucose as the standard.

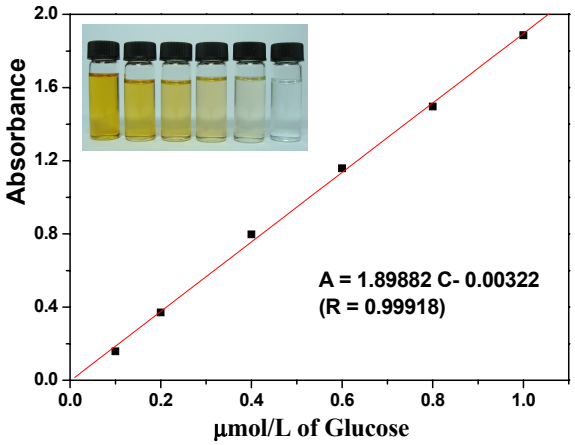


Figure S5 Colorimetric images and standard curve by the phenol-sulfuric acid colorimetric method

Dynamic adsorption behavior of proteins on the glycosylated MPPMs

The membrane (dm = 25 mm) was cut into small pieces, wet with ethanol for 2 h, and washed extensively with HEPES buffer solution (pH 7.5). Then, the wet membrane pieces were incubated into 3 mL of Con A solution (0.2 mg/mL) at 25 °C. Subsequently, absorbance of Con A solution at 280 nm was measured every half an hour by UV-visible spectroscopy (UV-2450, Shimazu). A calibration curve was established for the determination of Con A concentration (Figure S6).

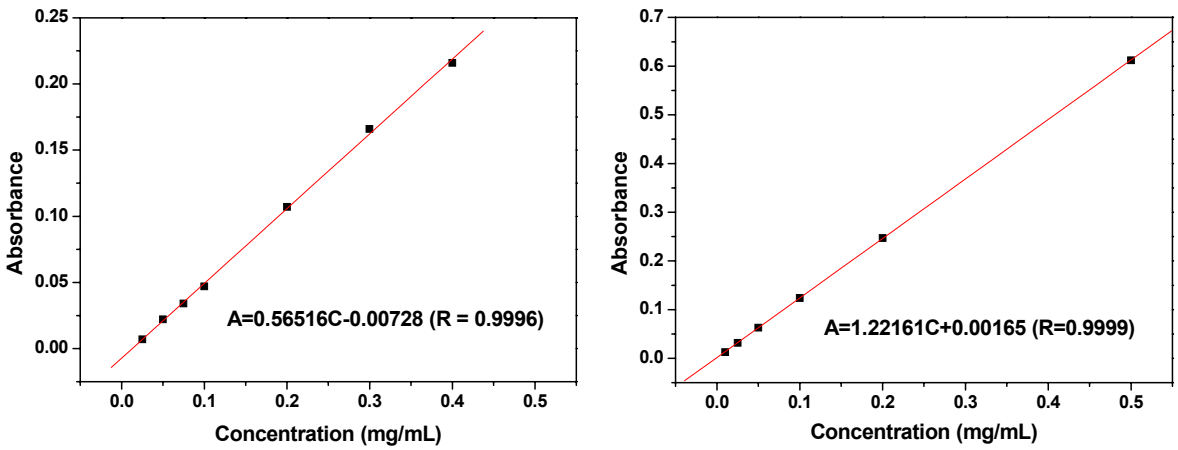


Figure S6 Standard curve of BSA (left) and Con A (right) at 280 nm.

The amount of Con A adsorbed on the glycosylated MPPM was then calculated from the change of Con A concentration. The amount of adsorbed BSA on the glycosylated MPPM was also determined according to the above procedure.

Characterization of surface glycosylation process

Chemical variations of the membrane surface are clearly demonstrated by FT-IR/ATR spectra (Figure S7).

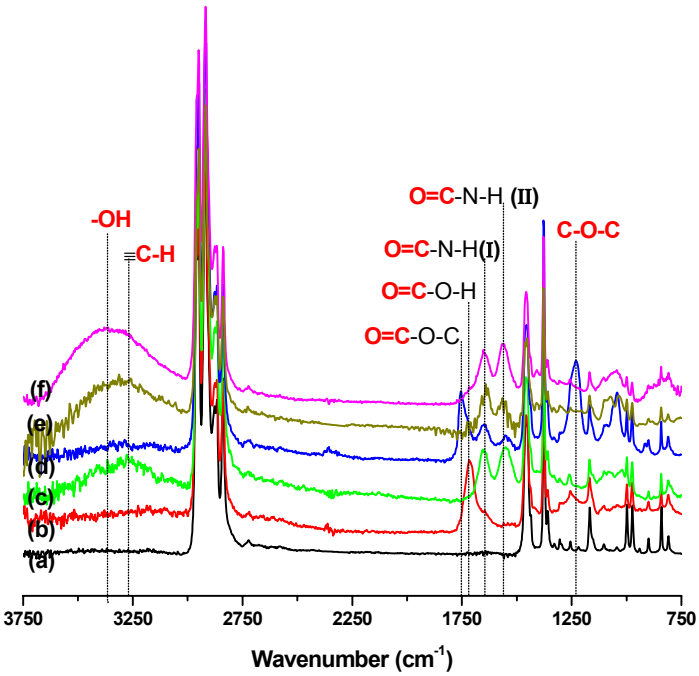


Figure S7 ATR/FT-IR spectra of the MPPMs: (a) native, (b) polyAA-modified , (c) yne-modified, (d) acetyl-glucose modified, (e) glycerol-modified and (f) glycosylated.

Compared with the native MPPM (Figure S7(a)), the polyAA-grafted MPPM (Figure S7(b)) exhibits an absorption peak at 1709 cm^{-1}, which can be assigned to the C=O stretching aroused by the carbonyl group of poly(acrylic acid). When propargylamine reacts with the -COOH group of AA, amide groups are formed on the membrane surface (Figure S7(c)). Correspondingly, the stretching vibration of carbonyl group at 1709 cm^{-1} disappears completely, accompanied by the appearance of typical absorption peaks for the amide bond I (at 1650 cm^{-1}) and II (at 1538 cm^{-1}), as well as the acetylenic hydrogen (at 3280 cm^{-1}). After glycosylation by thiol–yne click chemistry, an intense absorption at 1750 cm^{-1} and 1225 cm^{-1}, which are ascribed to the C=O and C-O-C stretching vibrations from the acetyl-protected groups, appeared clearly (Figure S7(d)). After deprotection of the acetyl groups of glucose pentaacetate, the absorption peaks at 1750 cm^{-1} and 1225 cm^{-1} disappear completely (Figure S7(f)), demonstrating the absolute deprotection. A broad band appears at 3200 cm^{-1}-3500 cm^{-1}, which is ascribed to the free hydroxyl groups of glucose pendants. In addition, the glycerol modified surface shows the existence of hydroxyl group with the strong peak at 3200 cm^{-1}-3500 cm^{-1} after the thiol–yne click chemistry (Figure S7(e)).

Chemical changes of the membrane surfaces were analyzed by XPS to follow the glycosylation process (Figure S8). For the native MPPM, a major peak at 284.6 eV is ascribed to the binding energy of C1s. Upon polyAA-grafting, an additional peak at 534.0 eV, which is corresponding to the binding energy of O1s, is detected from the membrane surface. After the covalent binding of propargylamine,

the peak of N1s at 401.0 eV appears which can be ascribed to the formation of amide groups. Upon thiol–yne click reaction and subsequent deprotection of acetyls, the appearance of S2p doublet structure and S2s at 162.8 eV and 226.9 eV is a clear evidence of thiol glucose pendants bound to MPPMs as a sulfide. According to the changes of chemical components in Table S1, after the glycosylation of surface, the obvious increase of relative intensity of O1s and the ratio decrease of N1s/O1s indicate the immobilization of glucose pendants. Furthermore, the efficiency of thiol–yne click reaction can be also obtained by the components ratio of S2p/N1s on the surface with a value of 56.5%. We tried to improve the efficiency by increasing the irradiation time to 1 h. Unfortunately, the 'click' efficiency was not correspondingly raised. This caused by the heterogeneous reaction. Moreover, it is possible that the reaction did not proceed to the bis-addition product for every alkyne groups but stopped after the first sugar unit addition leading to the formation of carbon-carbon double bond. In fact, the steric hindrance of glucose pendants increased the difficulty of bimolecular addition reaction with alkyne. On the other hand, it is well known that XPS is a surface analytical tool sensitive to the atomic composition of the outermost several nanometers of sample surface. Therefore, the heterogeneous microporous surface and the detection limit (~ 0.1%) of XPS make this semi-quantitative analysis technology of XPS unavailable to detect the inner pores structure. In addition, the S signal was likely attenuated by the overlying grafting chains, which resulted in the deviation in our case.

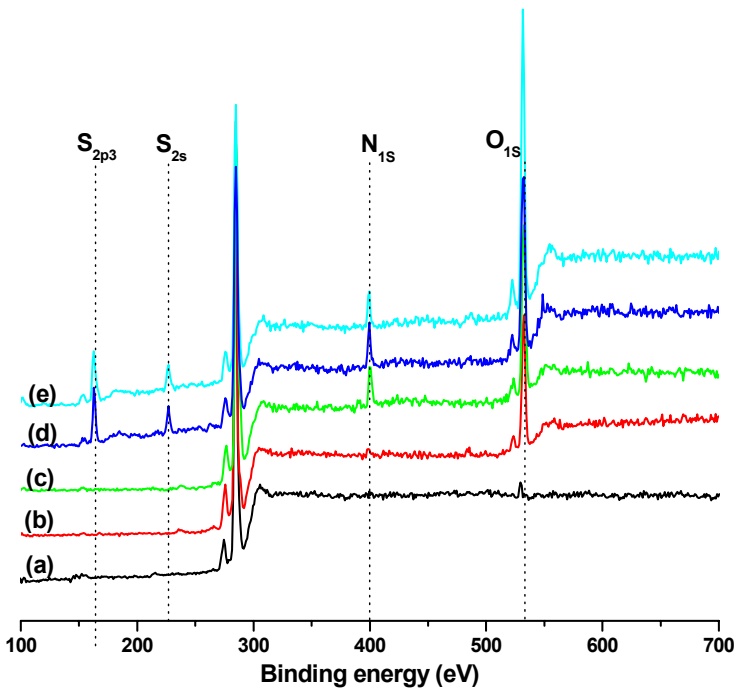


Figure S8 XPS spectra of the MPPMs: (a) native, (b) polyAA-modified, (c) yne-modified, (d) glycerol-modified, and (e) glycosylated.

Table S1 XPS analysis of chemical components on the modified membranes.

	Relative intensity (%)			Components ratio		
Elements	C_{1S}	O_{1S}	N_{1S}	S_{2P3}	N_{1S}/O_{1S}	S_{2P3}/N_{1S}
MPPM (a)	100	–	–	–	–	–
PolyAA-modified (b)	87.8	12.2	–	–	–	–
Yne-modified (c)	79.9	14.5	5.7	–	0.393	–
Glycerol modified (d)	76.1	14.67	4.39	4.84	0.299	1.10
Glycosylated (e)	65.6	24.1	4.78	5.42	0.198	1.13

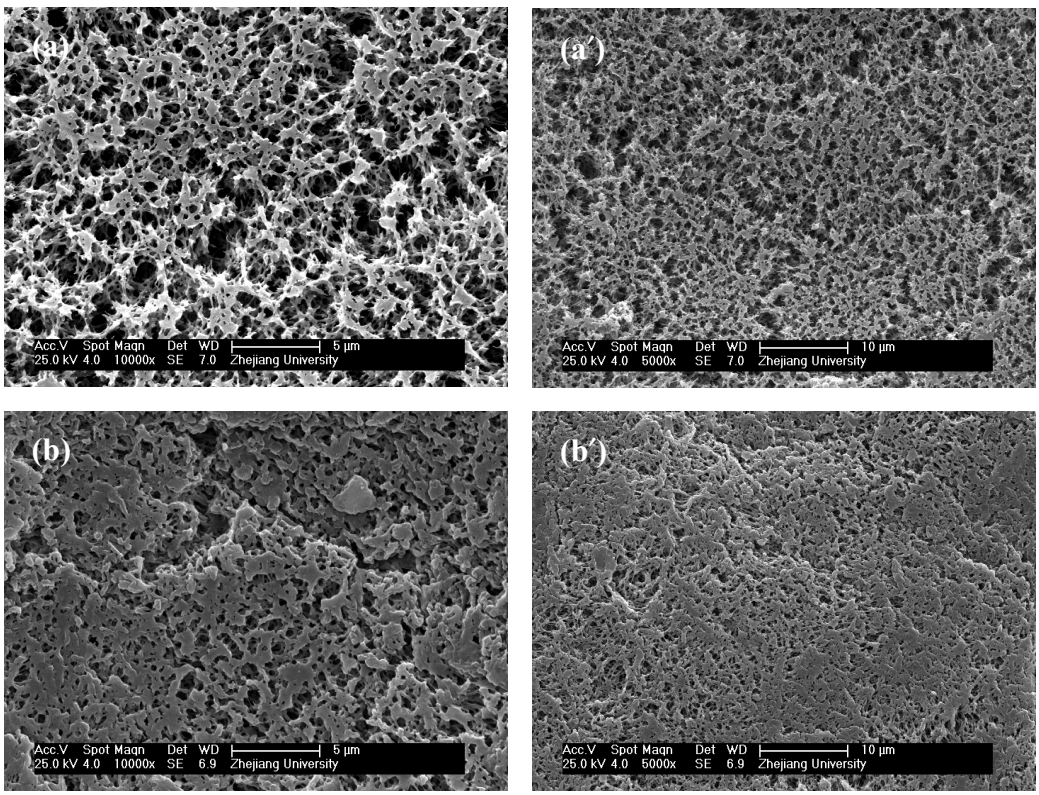


Figure S9 FESEM images for (a, a′) nascent MPPM, (b, b′) glycosylated MPPM. (a, b) ×10,000, (a′, b′) ×5,000.

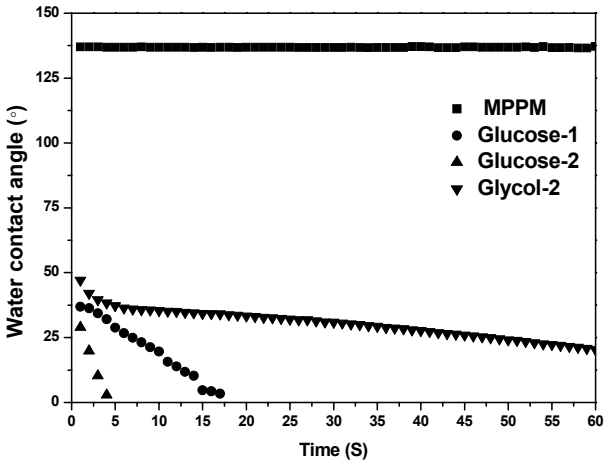


Figure S10 Water contact angle for the membrane surfaces: ■ MPPM; ● Glucose-1 (glycosylated MPPM with GD = 0.807 μmol·cm^{-2}); ▲ Glucose-2 (glycosylated MPPM with GD = 3.15 μmol·cm^{-2}); ▼Glycerol-2 (glycerol modified MPPM with a density = 3.75 μmol·cm^{-2}).