Supplementary Information

Biomimetic nanochannels for the discrimination of sialylated glycans via a tug-of-war between glycan binding and polymer shrinkage

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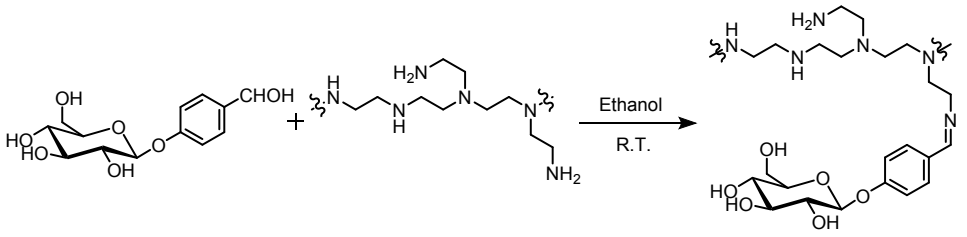
Section 1. Materials and instruments

4-Formylphenyl-β-D-glucopyranoside, deuterated dimethylsulfoxide (DMSO-d6), deuterium oxide (D_2O), (3-aminopropyl) trimethoxysilane (ATMS), 5-Aminofluorescein was purchased from Beijing Innochem Co., Ltd. (China). Branched polyethyleneimine (PEI, M_W = 10000), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) were purchased from Aladdin Co., Ltd. (China). Porous anodic alumina (PAA) membrane (with 80-100 nm aperture, and 60 μm thickness) were purchased from Hefei Pu-Yuan Nano Technology Ltd. (China). Polyethylene terephthalate (PET) foils of 12 μm thickness were irradiated at the linear accelerator UNILAC (GSI, Darmstadt) with swift heavy ions having an energy of 11.4 MeV per nucleon with 10^6 ions/cm^3 ion tracks in the center. Various saccharides, and sialylated glycan models used in experiments were purchased from TCI (Shanghai) Development Co., Ltd. (China) and SugarsTech, Ltd. (Qingdao, China). Other solvents and reaction reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All the reaction solvents were dried by molecular sieves for 24 hours prior to use, and water was purified by Milli-Q system (18.2 MΩ·cm).

All NMR spectra were recorded on Bruker Avance III 400M NMR spectrometer (Bruker Corp., Germany). All current-voltage curves were measured by a Keithley 6487 picoammeter (Tektronix Inc., US). Dynamic adsorption experiments were conducted on a quartz crystal microbalance with dissipation monitoring (QCM-D, Q-Sense E4 System, Biolin Scientific Corp., Sweden). Atomic force microscopy (AFM) investigation was conducted using a JPK NanoWizard Ultra Speed AFM&inVia Raman system. Isothermal titration microcalorimetry (ITC) experiment was conducted on a MicroCal ITC 200 system (Malvern Panalytical Ltd., UK). Electrochemical impedance experiments were carried out on a CHI 760E electrochemical workstation (CH Instruments, Shanghai, China). The bio-layer interferometry measurement was conducted on an ForteBio Octet K2 System (Molecular Devices, LLC., USA). Scanning electron microscope (SEM) image of the base side of PET nanochannel was obtained from JSM 7800F (JEOL Ltd, Japan) after a spray gold processing. The SEM image of the tip side was directly obtained from HITACHI SU8220 in beam deceleration mode.

Section 2. Synthesis, preparation and characterization

2.1 Synthesis and characterization of the Glc-PEI polymer



Scheme S1. The synthesis of the Glc-PEI polymer

As shown in the Scheme S1, 4-formylphenyl-β-D-glucopyranoside (0.5g, 1.76 mmol) was added into a solution of branched polyethyleneimine (Mw = 10000) (1 g, 23.2 mmol) in anhydrous ethanol (20 mL), and the mixture was stirred at room temperature for 24 h. The resultant solution was dialyzed in ethanol/water mixture for 5 days. After being freeze-dried, the Glc-PEI polymer was obtained. ^{1}H NMR experiment were conducted by using D_2O as solvent at 25 °C, and the ^{1}H NMR spectrum is shown in Fig. S1.

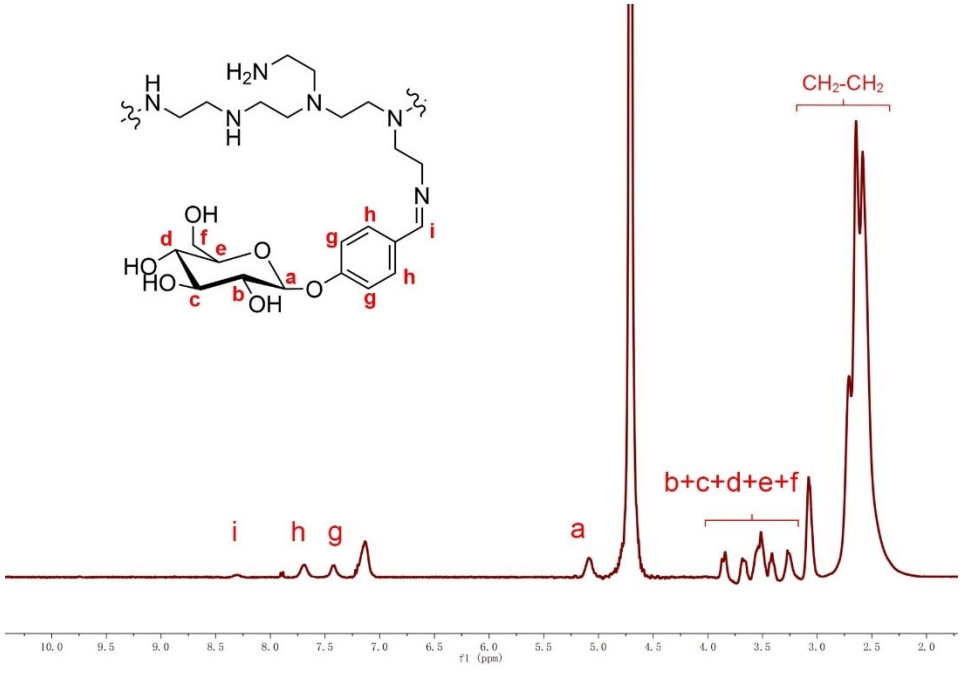
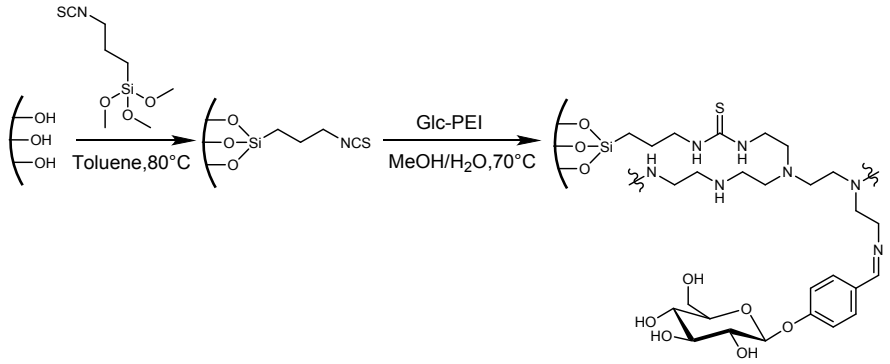


Fig. S1. ^{1}H NMR of Glc-PEI in D_2O.

2.2 Preparation and characterization of the Glc-PEI-modified nanochannels system



Scheme S2. The preparation of the Glc-PEI-modified PAA nanochannels.

Specifically, a porous anodic alumina (PAA) membrane (25 mm in dimeter) was treated with oxygen plasma at 200 W for 10 min to generate hydroxyl groups on surface. After rinsing with excess water and ethanol, the PAA membrane was dried in an oven at 60 ºC for 1 h. Then, the PAA membrane was immersed in a mixture of 0.5 mL 3-(triethoxylsilyl) propyl isothiocyanate (prepared according to the reference [1]) and 20 mL toluene at 80 ºC for 6 hours to afford chemically bonded isothiocyanate (-NCS) group on surface. The PAA membrane was rinsed with toluene and ethanol for three times respectively, to remove the remaining 3-(triethoxylsilyl) propyl isothiocyanate. After dried under a flow of nitrogen gas, the PAA membrane with -NCS groups was immersed in 20 mL Methanol/H_2O solution with 0.5g Glc-PEI at 60 ºC for 24 hours. Then the Glc-PEI-modified nanochannels membrane was obtained after rinsing with water and a drying process with nitrogen gas. The X-ray photoelectron spectroscopy (XPS) experiment and Thermogravimetric Analysis (TGA) were used to characterize the PAA membrane before and after the modification of the Glc-PEI polymer, the results were shown in Fig. S2 and Fig. S3, respectively.

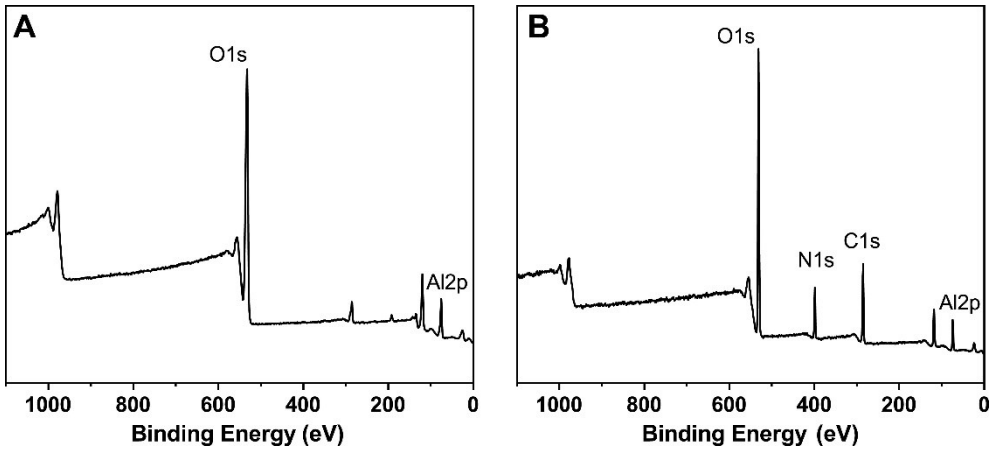


Fig. S2. XPS wide-scan spectrum of the PAA membrane (A) and the Glc-PEI-modified PAA membrane (B).

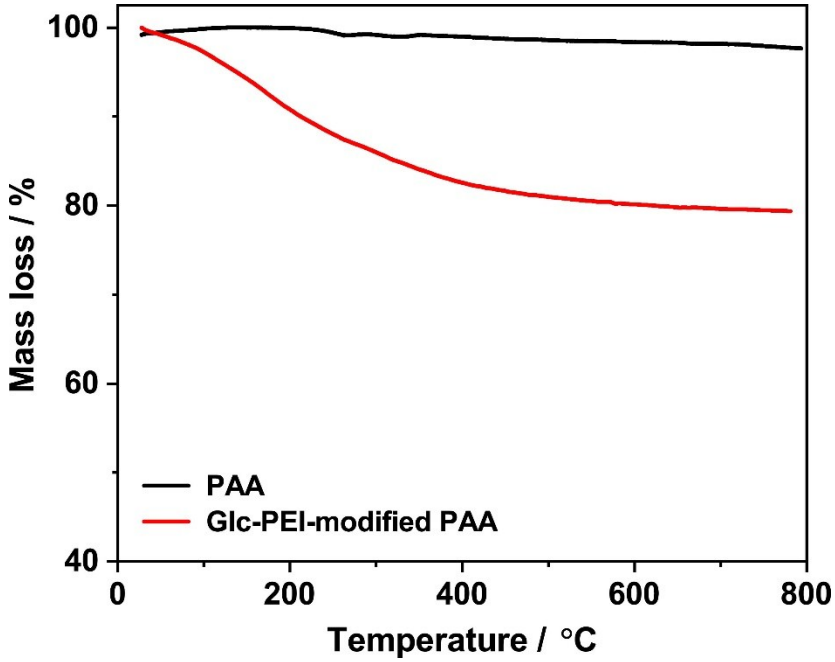


Fig. S3. Thermogravimetric analysis (TGA) of the PAA membrane and the Glc-PEI-modified PAA membrane.

2.3 Preparation and characterization of the Glc-PEI-modified PET conical nanochannels

The conical nanochannels was fabricated according to the method developed by Apel *et al.* and some improved steps, as shown in Fig. S4.[2-4] Briefly, the multiple ion-irradiated PET foil was mounted between two chambers of an home-made electrochemical cell in which it serves as an isolation valve between two chambers. One chamber was filled with etching solution (9 M NaOH), the other was filled with stopping solution (1M HCOOH + 1M KCl). The etching process was performed at 35 °C. During the etching process, the potential of 1 V was applied to be used to monitor the etching process, where the transmembrane ion current could be observed as soon as the nanopores opened. Before the pores was open, the ion current remains zero. Then the etching was stopped when the ion current reached a certain desire value. And the channel was washed with stopping solution to neutralize the etching solution. Finally, the multiple nanochannels membrane was immersed in water to remove residual salts. The resultant PET membrane was characterized by SEM, as shown in Fig. S5.

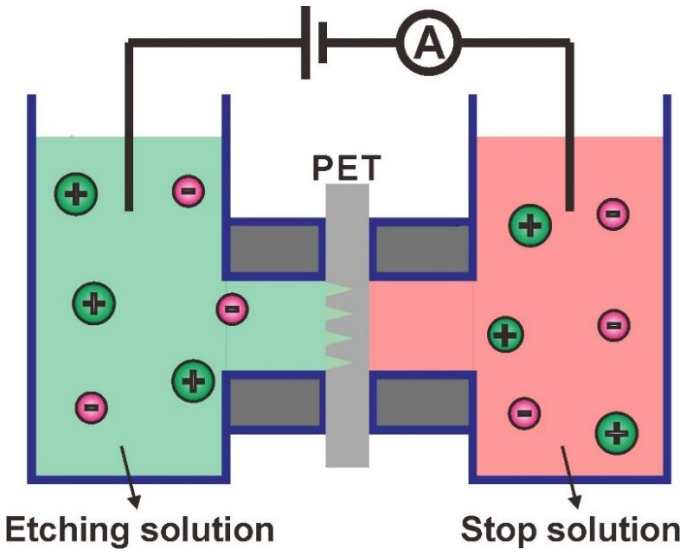


Fig. S4. The schematic of the home-made electrochemical device for the etching of the PET conical nanoporous membrane

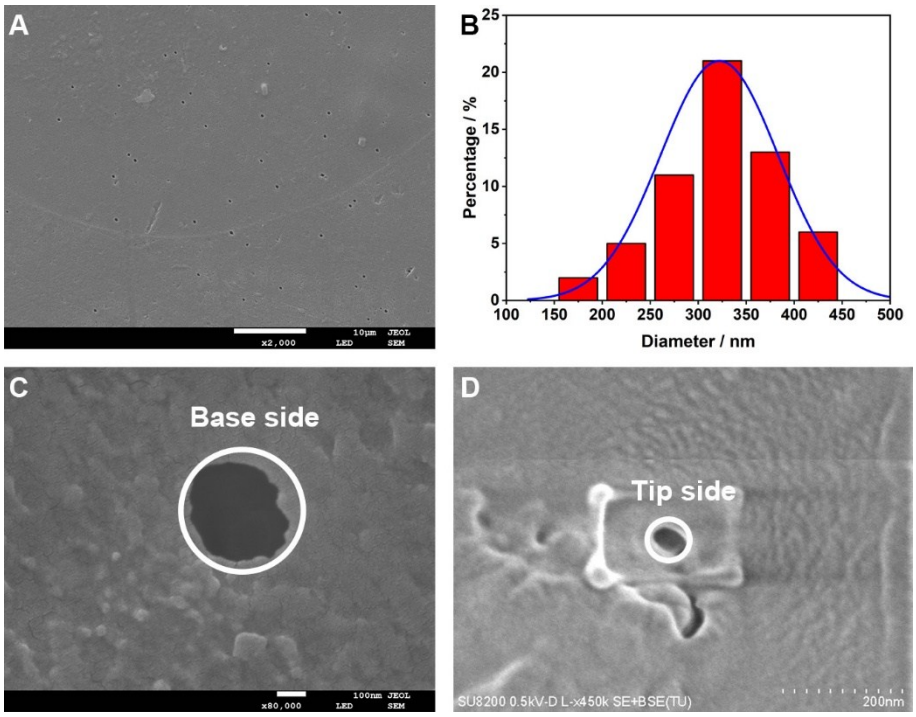
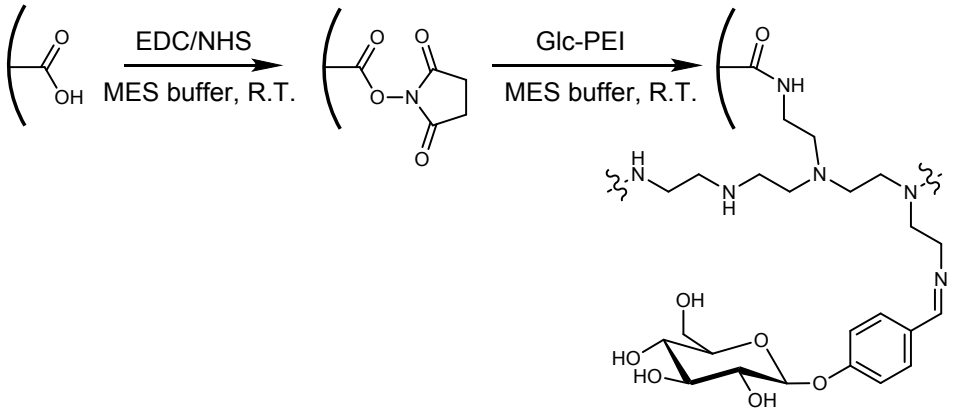


Fig. S5. Scanning electron microscopy (SEM) image of the PET conical nanochannels. (A) The large-scale SEM image of the PEI conical nanochannels membrane. (B) The diameter distribution of the large opening (base side) of the conical nanochannels, and the average diameter is about 323 nm. (C) The large opening (base side) of a single conical nanochannel. (D) The small opening (tip side) of a single conical nanochannel, the diameter is approximatively 32 nm.

The carboxyl group was generated on nanochannel inner wall, as a gift of the chemical etching process. The PET nanochannels membrane was transferred into 4 mL MES buffer solution (0.1 M, pH 5.5) solution containing 30 mg EDC and 18 mg NHS for 1 hour. After activation, the membrane was washed with the MES buffer solution. And the membrane was transferred into Glc-PEI solution (10 mg/mL) in MES buffer overnight to allow the covalent coupling between the amine-reactive ester with the amino residue of Glc-PEI. Then the membrane was washed with the MES buffer solution and water to remove residual chemicals. Fig. S6 shows the transmembrane ion current of PET conical nanochannels before and after being modified with the Glc-PEI polymer, which clearly indicates the successful modification of Glc-PEI in the nanochannels, causing the decrease of ion flux through nanochannels. In addition, the presence of N1s peak in XPS wide-scan spectrum also indicates the successful modification of Glc-PEI in the PET conical nanochannels membrane (Fig. S7).



Scheme S3. The preparation of the Glc-PEI-modified PET nanochannels.

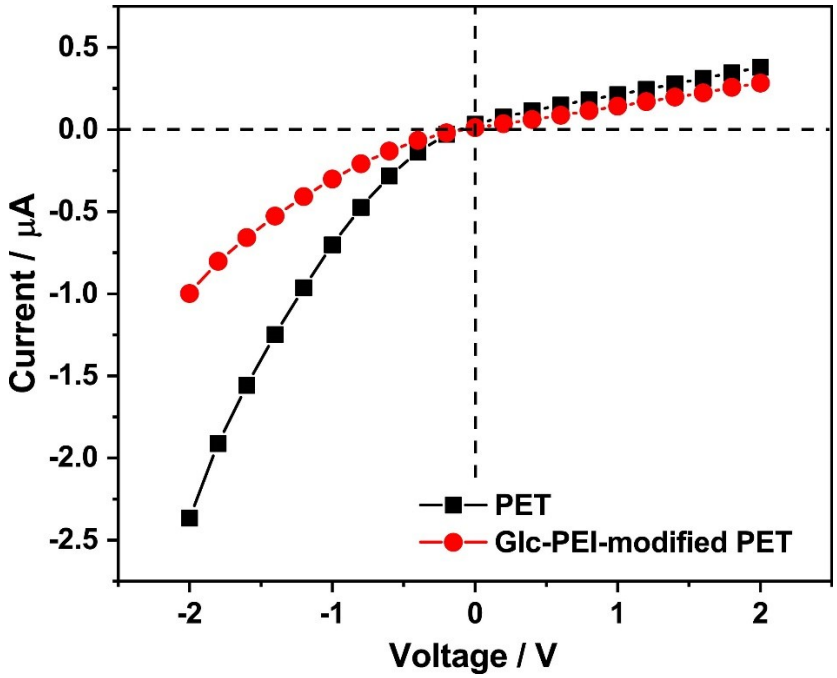


Fig. S6. The transmembrane ion current of the PET conical nanochannels before and after being modified with the Glc-PEI polymer. These results clearly indicate the successful modification of Glc-PEI in the nanochannels, causing the decrease of ion flux through nanochannels.

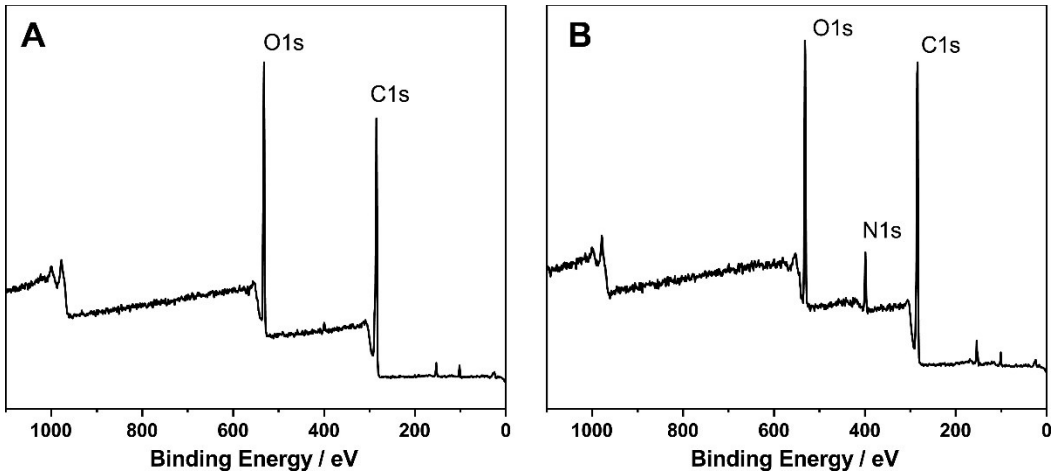


Fig. S7. XPS wide-scan spectrum of bare PET conical nanochannels membrane (A) and the Glc-PEI-modified PET membrane (B).

Section 3. Transmembrane ion current measurements.

The transmembrane ion current was measured by using a Keithley 6487 picoammeter (Keithley Instrument, Cleveland, Ohio, United States). Ag/AgCl electrodes were used to apply a transmembrane potential across the film. The obtained Glc-PEI-modified PAA membrane (The diameter is 25mm) was cut into several small pieces. The small piece of PAA membrane was mounted between the two chambers of a home-made electrochemical cell (effective aperture is 5 mm). Both chambers of the conductivity cell were filled with 0.01m KCl solution. The transmembrane potential used in this work was a scanning voltage varied from -0.2 V to +0.2 V with a 21 s period. Various analytes solution with different concentration was prepared from 0.01 M KCl solution. Prior to each test, the analyte solution was injected into both chambers, and the transmembrane ion current was recorded after 10 min. the whole test process was repeated three times using three pieces of Glc-PEI-modified PAA membrane, that is, each current value at one concentration was repeated three times to obtain the average current value.

For Glc-PEI-modified PET membrane, several pieces of PET conical nanochannels membrane were prepared simultaneously under the same condition, and were modified with Glc-PEI polymer at the same time. We chose three pieces of Glc-PEI-modified PET membrane to parallelly perform the ionic current measurements.

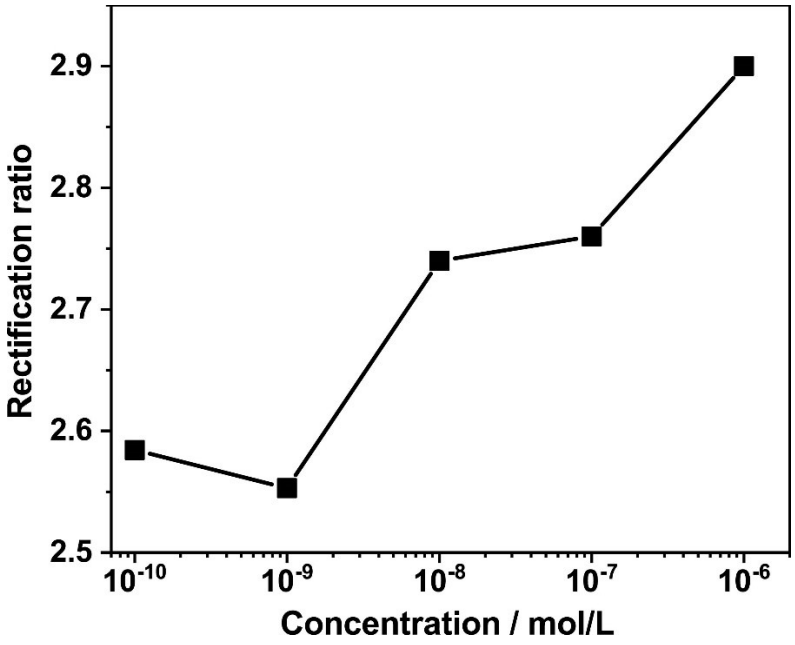
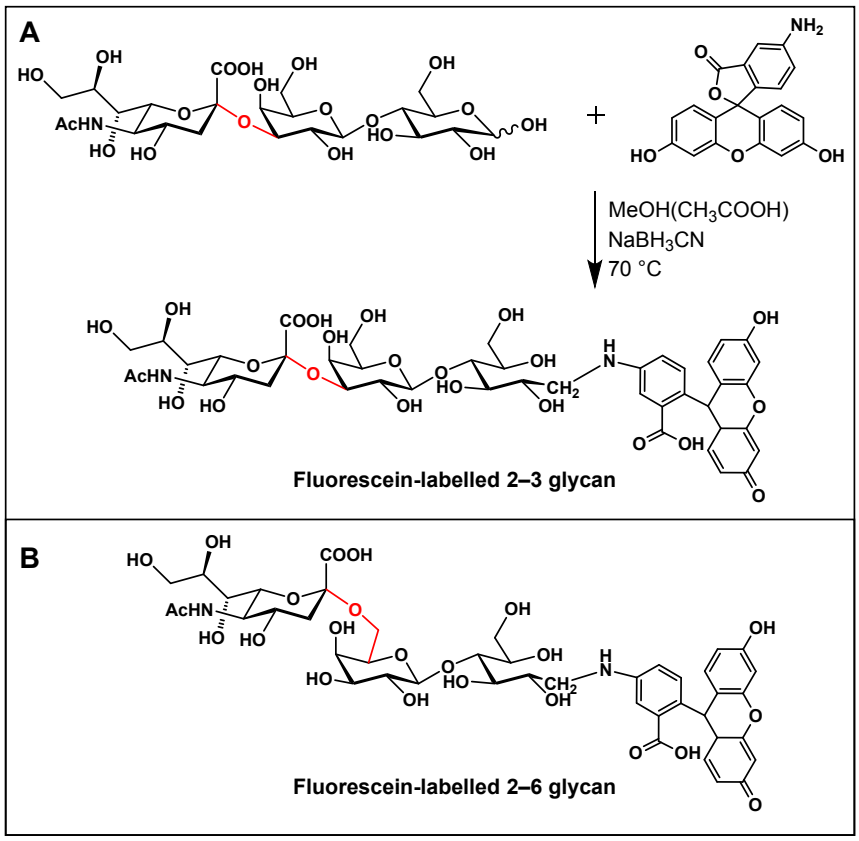


Fig. S8. The relationship of ionic current rectification ratio ($|I_{-2V}|/|I_{+2V}|$) of the Glc-PEI-modified PET conical nanochannels membrane with the concentration of 2−6 glycan. The rectification ratio is a ratio of the current value at -2V and the current value at +2V.

Section 4. LSCM investigation

4.1 Synthesis and characterization of the Fluorescein-labelled sialylated glycans



Scheme S4. Synthesis of the Fluorescein-labelled sialylated glycans.

The Fluorescein-labelled sialylated glycans were synthesized according the derivative method of oligosaccharides. [5,6] A solution of 4% (v/v) acetic acid in methanol was prepared firstly. 10 mg of 2−3 glycan (1 eq), 11 mg of 5-aminofluorescein (2 eq), and 4.8 mg of sodium cyanoborohydride (5 eq) were dissolved in 2 mL of pre-prepared reaction solution. The reaction mixture solution was sealed in a 10 ml centrifuge tube, and were heated (70 °C) for 6 h to complete the reaction. Then, 1 mL of water was added into the reaction mixture to quench excess sodium cyanoborohydride. The product Fluorescein-labelled 2−3 glycan was purified by a semi-preparative HPLC on C18 column (particle size: 5 μm, 10 × 250 mm) with a linear gradient of the binary solvent system of water (solvent A) and acetonitrile (solvent B) and UV detection at 365 nm. The Fluorescein-labelled 2−6 glycan was synthesized through the same method. The products were characterized by MS, as shown in Fig. S9.

Fluorescein-labelled 2−3 glycan: MS: m/z Calcd. for $C_{43}H_{54}N_2O_{23}$: 966.2312; Found: 967.3159 $[M+H]^+$.

Fluorescein-labelled 2−6 glycan: MS: m/z Calcd. for $C_{43}H_{54}N_2O_{23}$: 966.2312; Found: 967.3183 $[M+H]^+$.

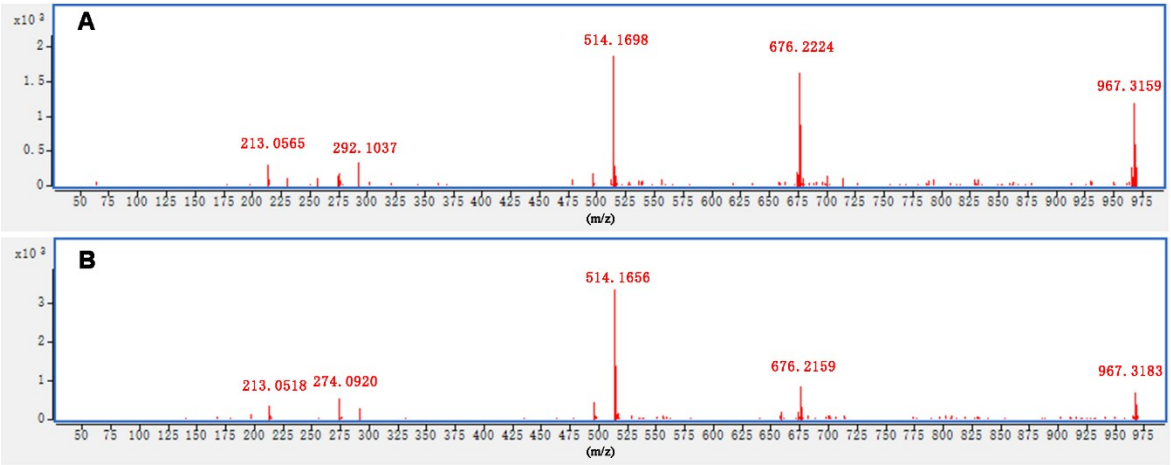


Fig. S9. The MS spectra of the Fluorescein-labelled 2−3 glycan (A), and the Fluorescein-labelled 2−6 glycan (B).

4.2 LSCM observation

Prior to LSCM observation, a piece of PET conical nanochannels membrane grafted with the Glc-PEI polymer on the inner wall of nanochannels (denoted as Glc-PEI-modified PET) was immersed in an aqueous solution of the Fluorescein-labelled sialylated glycan (1mg/mL) for 1 hour. The Glc-PEI-modified PET membrane was rinsed thoroughly with Milli-Q water, then the LSCM observation was performed.

Fig. S10 shows the obtained LSCM images. Clearly, the fluorescent features of Glc-PEI-modified PET membrane that was treat by Fluorescein-labelled 2−3 glycan were much brighter and denser than that treated by the Fluorescein-labelled 2−6 glycan.

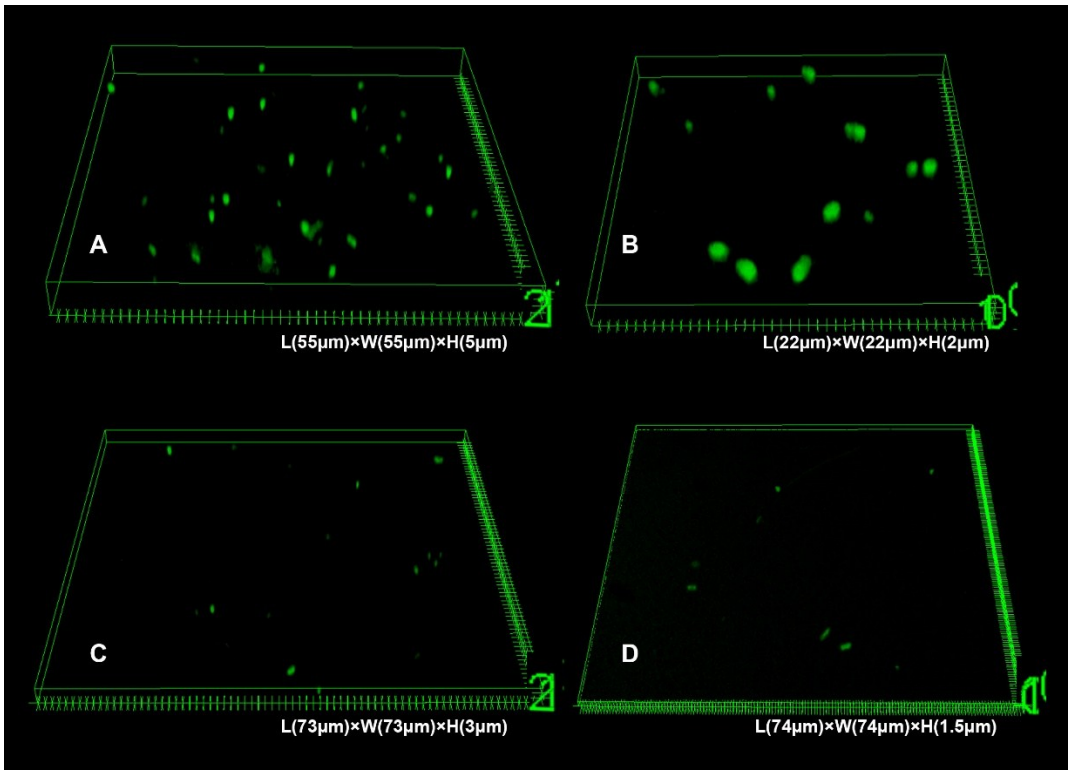


Fig. S10. LSCM observation of the Glc-PEI-modified PET conical nanochannels membrane upon interaction with the Fluorescein-labeled glycans. (A, B) 3D reconstructed LSCM images of the Glc-PEI-modified PET membrane upon interaction with the Fluorescein-labelled 2−3 glycan. (C, D) 3D reconstructed LSCM images of the Glc-PEI-modified PET membrane upon interaction with the Fluorescein-labelled 2−6 glycan.

Section 5. Electrochemical Measurements

Electrochemical impedance measurements (EIS) were carried out on a CHI 760E electrochemical workstation. First, the cleaned gold electrode was modified with the mercaptoacetic acid by immersing the electrode into 2 mL aqueous solution containing 40 mg mercaptoacetic acid for 10 hours. Then the electrode was rinsed with excess water and transferred into 2 mL MES buffer solution (0.1M, pH 5.5) containing 15 mg EDC and 9 mg NHS for 1 hour. Finally, the Glc-PEI-modified gold electrode, as the working electrode, was obtained by further immersing the electrode into 2 mL aqueous solution containing 0.2 g Glc-PEI for 24 hours and a subsequent rinsing with excess water and a drying process with N_2 flow. A conventional three-electrode cell was used, the reference electrode is a saturated Ag/AgCl, the counter electrode is platinum wire. The cell was enclosed in a grounded Faraday cage. The impedance measurements were performed in 0.1 M KCl solution in the electrochemical cell in the presence of a $K_3[Fe(CN)]_6$:$K_4[Fe(CN)_6]$ 1:1 mixture (5 mM) as a redox probe. The experimental conditions were as follows: open-circuit potential, 0.3 V; alternative voltage, 5 mV; frequency range, 10 kHz–0.1Hz. The impedance spectra were plotted in the form of Nyquist plots. The experimental impedance spectra were fitted using electronic equivalent circuits in order to derive the electron-transfer resistance (R_{et}) values by virtue of the software ZView (version 2.1c).

Section 6. ITC experiments

ITC analysis was employed to investigate the interaction of Glc-PEI with various saccharides. The experiment was performed by Microcal ITC 200. The Glc-PEI (0.6 mg·mL^{-1}) and various saccharides (5 mmol·mL^{-1}) were dissolved in ultrapure Milli-Q water sufficiently to prepared work solutions. After first titration with 0.4 μL saccharides solution, a series of 2 μL of saccharides solution was injected into the 200 μL Glc-PEI solution with a time interval of 2 min at 25 ºC. All experiments were repeated twice.

Section 7. QCM-D experiments

The Au coated sensors (frequency: 4.95 MHz ± 50 kHz, diameter: 14 mm) used were cleaned according to the standard protocol: the sensors were washed in a mixture solution of Milli-Q water,

$NH_3 \cdot H_2O$ and H_2O_2 in a proportion of 5:1:1 (v/v/v) at 75 ºC for 5 min. The clean sensors were rinsed by Milli-Q water and dried under N_2 flow. Then, by using the same modifying method with gold electrode in electrochemical measurements (Section 5), the Glc-PEI polymer was grafted onto the sensor surface, and produced the polymer layer on sensor surface.

All the QCM-D measurements were taken at 25 ºC by Q-Sense E4 system (Biolin Scientific Corp. Sweden). Both the dynamic adsorption and dissipation curves of different saccharides (Neu5AC, 2−3 glycan and 2−6 glycan) with same mass concentration of about 16 $mg \cdot mL^{-1}$ on Glc-PEI-modified sensors were recorded by Q-sense software and then analyzed by Q-tools software.

Section 8. Quantum chemistry calculation

Quantum chemistry calculation was conducted to describe the potential interaction modes of Glc-PEI with sialic acid-containing disaccharides, Neu5Acα2−3GalβMP (2−3-linked disaccharide) and Neu5Acα2−6GalβMP (2−6-linked disaccharide). A Glc-PEI fragment with two grafted receptor Glc was used to mimic the polymer and reduce the calculation complexity. As shown in Fig. S11a, the binding of the Glc-PEI fragment with the 2−3-linked disaccharide also shows a double-locking interaction mode via multiple H-bonding interactions between OH groups of two receptor Glc units and OH groups of the 2−3-linked disaccharide. Meanwhile, two amine groups also involved the hydrogen bonding interaction with the disaccharide. In comparison, the 2−6-linked disaccharide only binds one receptor Glc unit via four hydrogen bonds in a potential interaction model obtained using quantum chemistry calculation, accompanied by the involvement of adjacent amine groups of the PEI chain (Fig. S11b). These results indicate the Glc-PEI polymer have a much stronger binding to the 2−3-linked disaccharide, further confirming the recognition and binding reference of Glc-PEI towards the α2−3 linkage.

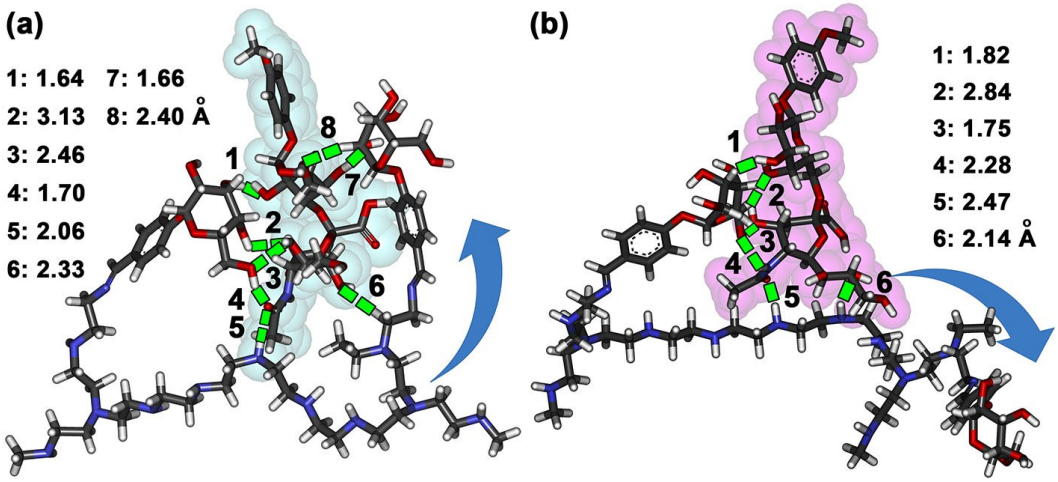


Fig. S11. Potential interaction model of the Glc-PEI fragment with Neu5Acα2−3GalβMP (a) and Neu5Acα2−6GalβMP (b) obtained from quantum chemistry calculation (Gaussian, DFT, B3LYP, at the 3-21G level). H-bonds with different lengths are indicated using green dashed lines.

Section 9. NMR experiments

The NMR experiments were conducted to study the binding interactions between the glucopyranoside (Glc component of Glc-PEI) with 2−3 glycan and 2−6 glycan. The ^{1}H NMR and ^{1}H-^{1}H COSY of the glucopyranoside alone and in the presence of 2−3 glycan and 2−6 glycan were recorded by using DMSO-d_6 as solvent at 25 ºC, and the analysis was presented in the following.

As shown in Fig. S12, the analysis on partial ^{1}H NMR spectra shows that the OH protons of the glucopyranoside shifted upfield obviously (red region, Fig. S12), when it reacted with 2−3 glycan. Meanwhile, a few OH protons from Neu5Ac, Gal and Glc unit of 2−3 glycan also presented evident shift (blue region, Fig. S12). This result means that a multiple H-bonding interaction mode occurred among multiple OH groups of the glucopyranoside and three saccharides of 2−3 glycan. In addition, when the glucopyranoside mixing with 2−6 glycan, similar OH protons shift of the glucopyranoside were observed (red region, Fig. S13, S14), implying the glucopyranoside acted the recognition receptor to bind 2−6 glycan. Whereas, the shifted OH numbers of three saccharides of 2−6 glycan were much less than those of 2−3 glycan (blue region, Fig. S13 and Fig. S14). Thus, these results demonstrated at the molecular level the glucopyranoside possess the strong binding preference toward 2−3 glycan over 2−6 glycan.

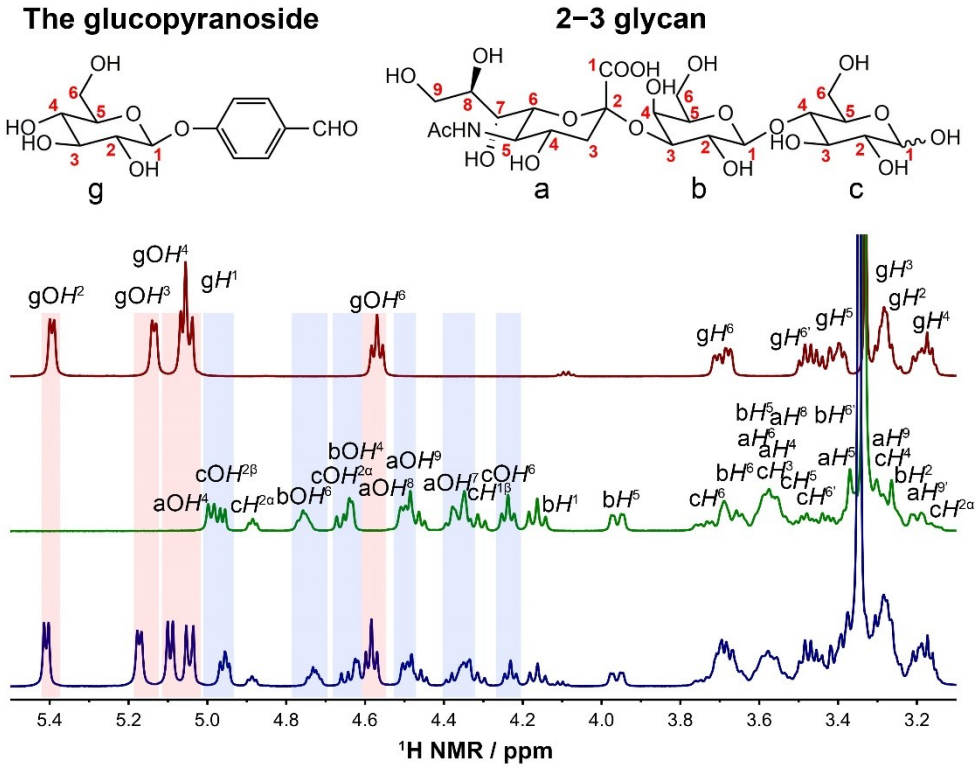


Fig. S12. The chemical structures of the glucopyranoside unit and 2−3 glycan, and partial ^{1}H NMR spectra of the glucopyranoside (purple curve), 2−3 glycan (green curve), and their mixture (2:1 in molar ratio, blue curve). For ease of clear assignments, “g” denotes the glucopyranoside, “a”, “b”, “c” denotes “Neu5Ac”, “Gal”, “Glc” unit of 2−3 glycan respectively.

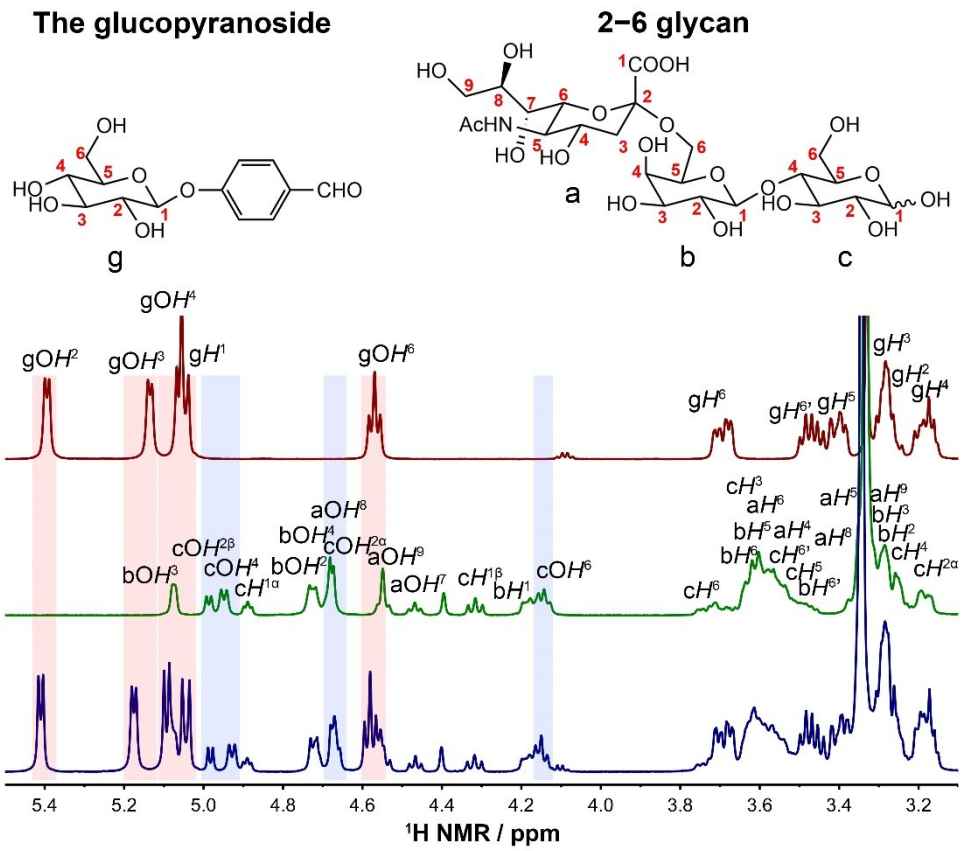


Fig. S13. The chemical structures of the glucopyranoside unit and 2−6 glycan, and partial ^{1}H NMR spectra of the glucopyranoside (purple curve), 2−6 glycan (green curve), and their mixture (2:1 in molar ratio, blue curve). For ease of clear assignments, “g” denotes the glucopyranoside, “a”, “b”, “c” denotes “Neu5Ac”, “Gal”, “Glc” unit of 2−6 glycan respectively

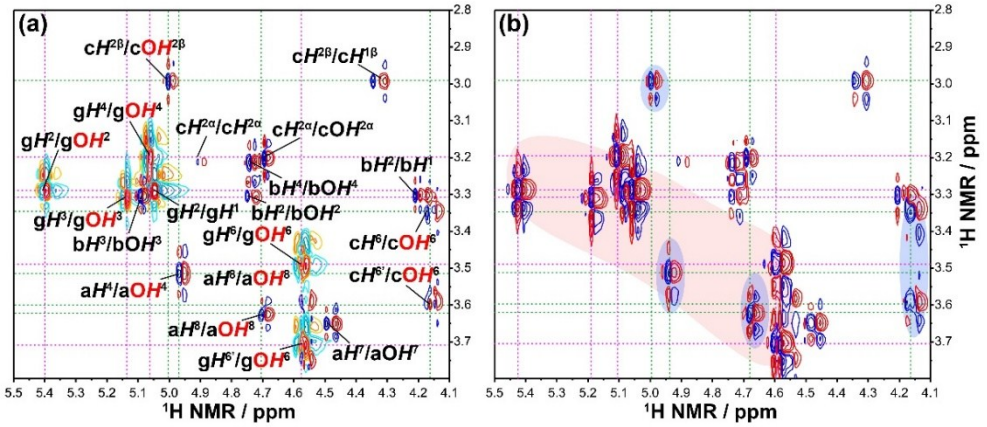


Fig. S14. Stack spectra of partial ^{1}H-^{1}H COSY spectrum of the glucopyranoside (orange-cyan contours) and (a) 2–3 glycan (red-blue contours), and (b) the partial ^{1}H-^{1}H COSY spectrum of their mixture (2:1 in molar ratio).

Section 10. Bio-layer interferometry (BLI) measurements

The binding affinities between the Glc-PEI polymer and different glycans were further measured by bio-layer interferometry measurement using the Octet K2 System (ForteBio) and amine reactive 2nd-generation (AR2G) biosensors. Briefly, the Glc-PEI polymer were immobilized onto the carboxy-terminated biosensor surface through the coupling reaction between carboxyl group and amino residue of polymer with the help of EDC/NHS in MES buffer (0.1M, pH 5.5) solution. Then, the biosensors were exposed to different concentrations of glycan aqueous solution, followed by the dissociation in water. The data were analysed and the equilibrium dissociation constants (K_D) were determined using software provided by ForteBio (Data Analysis 11.0).

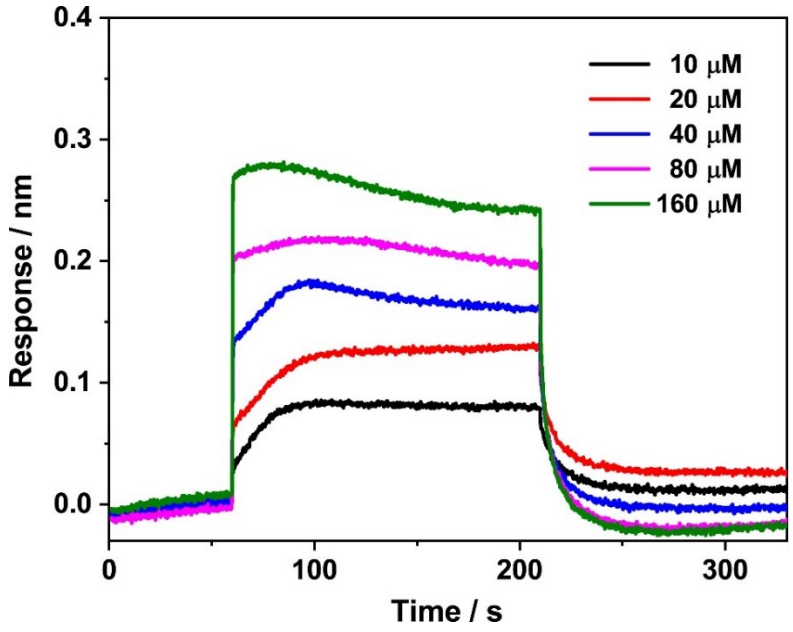


Fig. S15. Sensorgrams of the Glc-PEI polymer binding to 2−6 glycan recorded by BLI.

Section 11. References

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