Electronic Supplementary Information

Disaccharide-driven macroscopic properties transition: From molecular recognition to glycopeptide enrichment

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Supporting information

Part 1. Material and instruments

Silica wafer was purchased from Monocrystalline Silicon. Co. Ltd (Luoyang, China). Aspartame, 3,5-bis(trifluoromethyl)-phenylthiourea, 2-Acetoxyisobutyryl bromide, 3-aminopropyl trimethoxy-silane (ATMS), N,N,N',N",N"-pentamethyldiethylenetriamine (Me_6TREM), acryloyl chloride, CuBr, N-isopropyl acrylamides were obtained from Sigma-Aldrich (Germany). CuBr was recrystallized before being used. *N*-isopropyl acrylamide was recrystallized in n-hexane for three times. Chromatographically pure acetone and methanol were purchased from TEDIA (USA). DMF, toluene, dichloromethane were obtained from Sinopharm Chemical Reagent Co. Ltd and dried for 24 hours before use. Lactose, maltose, sucrose, and trehalose were purchased from TCI corp. (Japan). Human serum immunoglobulin G and ammonium bicarbonate (ABC) were ordered from Sigma-Aldrich (St. Louis, MO). Formic acid (FA) was obtained from Acros Organic (Geel, Belgium). Trypsin was purchased from Promega (Madison, MI). Ammonium hydroxide was obtained from Fluka. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Fluorescence spectra were recorded on a PerkinElmer LS55 fluorescence spectrophotometer. Disaccharides adsorptions experiment was conducted on a Quartz Crystal Microbalance (QCM) with Dissipation monitoring (Q-Sense E4 System, Biolin Scientific Corp. Sweden). The glycosylated asparagine building block was incorporated into target glycopeptide using Fmoc-based peptide synthesis on the Wang resin through solid-supported peptide synthesis (CS-Bio Peptide Synthesizer CS 136XT (CA, USA). ^{1}H NMR spectra were recorded on a Varian Mercury VX-300MHz. Mass spectra was obtained with a Finnigan LCQ Advantage mass spectrometer with a Nano Electrospray Ionization-Quadrupole Time-of-Flight Mass Spectrometer (ESI-Q-TOF MS) (Waters, Manchester, UK). Atomic Force Microscopy (AFM) investigation was conducted on a flat mica substrate using Multimode 8 AFM (Bruker, USA) in a tapping mode. X-ray Photoelectron Spectroscopy (XPS) was obtained with a VG Multilab 2000. The FI-IR was performed with a Bruker Vertex 80V FT-IR spectrometer.

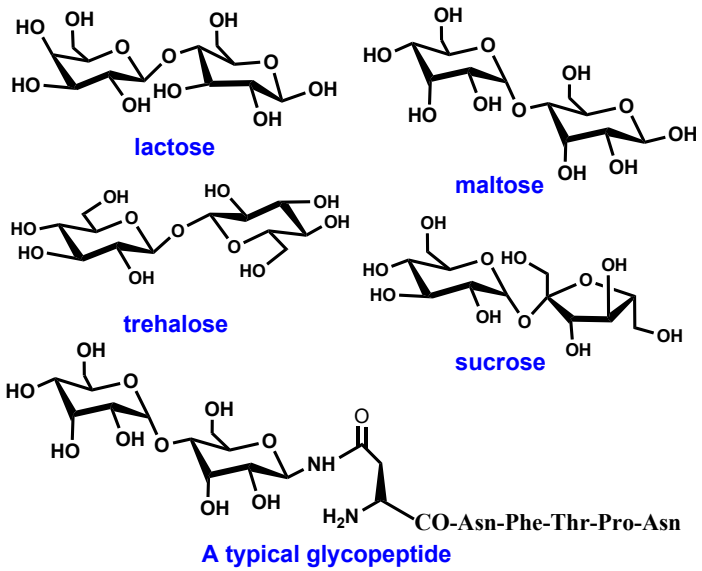
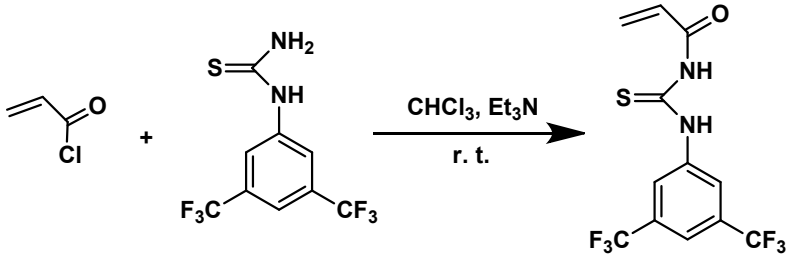


Fig. S1 Chemical structures of lactose, sucrose, maltose, trehalose and a typical glycopeptide

Part 2. The synthesis of functional units.

2.1 Synthesis of acryloyl-3,5-Bis(trifluoromethyl)phenylthiourea (PT)

Et_3N (1.1 g, 10 mmol) was added to a solution of 3,5-Bis(trifluoromethyl)phenylthiourea (2.88 g, 10 mmol) in 30 mL dry $CHCl_3$, the mixture was stirred for 10 min, then acryloyl chloride (0.906 g, 10 mmol) was added dropwise to this mixture at ambient temperature, and continued to stir for 24 hours. The mixture was washed with saturated sodium chloride solution for three times, and the organic layer was dried over anhydrous sodium sulphate overnight. After filtration and evaporation of solvent, the crude product was purified on a silica gel column, with elution of CH_2Cl_2/CH_3OH (100:1), to give target compound as yellow semi-oil.

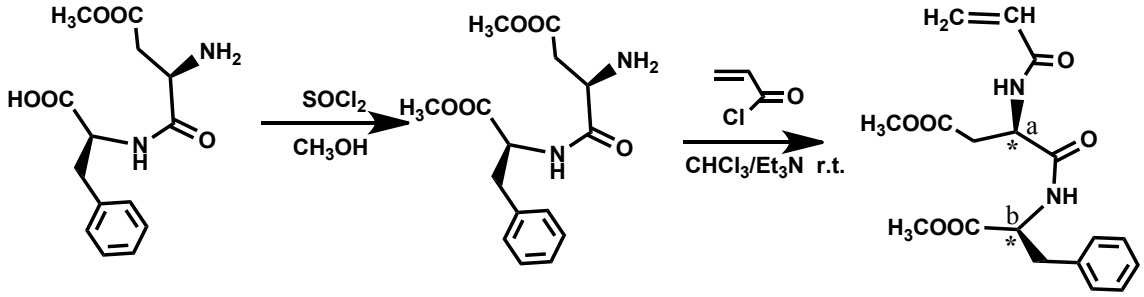


^{1}H NMR (500 MHz, DMSO-d_6) δ 12.69 (s, 1H, CON*H*), 11.87 (s, 1H, CON*H*), 8.42 (d, J = 1.4 Hz, 2H, Ph-*H*), 8.00 (s, 1H, Ph-*H*), 6.66 (dd, J = 17.0, 10.2 Hz, 1H, C=C*H*), 6.50 and 6.06 (dd, J = 17.0, J =10.2 1.6 Hz, 2H, C=CH_2). MADLI MS: m/z calcd for $C_{12}H_8F_6N_2OS$: 342.26; found: 343.06 [M+H].

2.2 Synthesis of acryloyl N-α-L-aspartyl-L-phenylalanine di-methyl ester (DF)

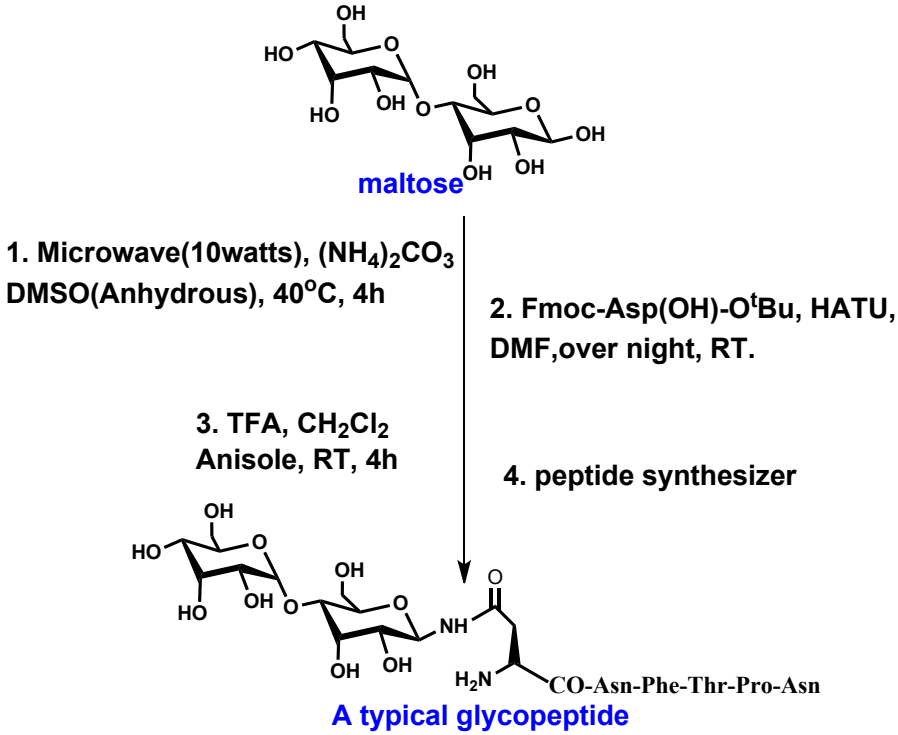
Thionyl chloride (6 mL) was added dropwise to a CH_3OH solution of N-α-L-aspartyl-L-phenylalanine methyl ester (2.94 g, 10 mmol) in ice brine bath. After addition, the reaction mixture was stirred for 3 hours at room temperature. Most of solvent was evaporated under reduced pressure to gain the semi-oil residue. Then 40 mL fresh methanol was added to dissolve this residue and evaporated again under reduced pressure. Repeat this process for three times to remove the extra thionyl chloride. Et_3N (1.01 g, 10 mmol) was added to a solution of N-α-L-aspartyl-L-phenylalanine di-methyl ester hydrochloride (1.724 g, 5 mmol) in 60 mL dry $CHCl_3$, the mixture was stirred for 8 min, then acryloyl chloride (0.453 g, 5 m mol) was added dropwise

to this mixture at ambient temperature, and continued to stir for 24 hours. The mixture was washed with saturated sodium chloride solution for three times, and the organic layer was dried over anhydrous sodium sulphate overnight. After filtration and evaporation of solvent, the pure product was obtained as white powder.



^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (dd, J = 22.5, 7.9 Hz, 2H, CON*H*), 7.37 – 7.06 (m, 5H, Ph-*H*), 6.25 and 6.11 (dd, J_1= J_2=17.1 Hz, 2H, C=C*H*), 5.64 and 5.61 (dd, J = 10.0, 2.3 Hz, 1H, C=CH_2), 4.75 (td, J = 8.3, 5.6 Hz, 1H, *H_a), 4.45 (td, J = 8.2, 5.7 Hz, 1H, *H_b), 3.59 (d, J = 6.1 Hz, 6H, 2OCH_3), 3.07-2.89 (m, 2H, C*H*CO), 2.73-2.69 (m, 2H, C*H*-Ph). MADLI MS: m/z calcd for $C_{18}H_{22}N_2O_6$: 362.38; found: 385.14 $[M+Na]^+$.

2.3 synthesis of a typical peptide



The automated micro wave tube was charged with reducing sugar maltose **1** (0.5 g), ammonium carbonate (2.5 g, 5 fold excess) and anhydrous DMSO (15 ml) [1]. The tube was sealed and place under automated microwave at 40°C for 4 hours under cooling the vessel. The reaction tube was taken off and the reaction mixture was transferred into a round bottom flask. The reaction mixture was freeze dried for overnight to remove excess of ammonia and DMSO to afford β-glycosyl amine (**2**) as colorless hygroscopic solids in excellent yields. These amines were used without further purification. To a stirred suspension of **2** (1.2 g, 3.5 mmol) and HATU (2.6 g, 7 mmol) in

anhydrous DMF (20 ml) was added Fmoc-Asp(OtBu)-OH (2.17 g, 5.3 mmol) in anhydrous DMF(10 ml). The reaction mixture was stirred at room temperature for 4 hours. The solvent was removed under reduced pressure. The crude product was purified by HPLC (High performance liquid chromatograph) using 50% MeOH in water as an eluent to afford **3**. To a 50 ml round-bottom flask was added **3** (1 g, 1.5 mmol), anhydrous DCM (20 ml), TFA (20 ml) and anisole (2 ml). The action mixture was stirred at room temperature under nitrogen atmosphere for 4hours. The mixture was concentrated under pressure to afford a white colored solid. The crude product was purified by HPLC using 30% MeOH in water as an eluent to afford a white solid 4. Then, the glycosylated asparagine building block 4 was incorporated into target glycopeptide using Fmoc-based peptide synthesis on the Wang resin.

HPLC analysis

Measurement:	Peak Area	Run Time:	11 min
Calculation Type:	Percent	Wavelength:	220 nm
Flow rate:	1ml/min	Inj.Vol:	10 uL
Buffer A:	0.1% TFA in water	Buffer B:	0.1%TFA in Acetonitrile
Column:	Kromasil 100-5C18, 4.6mmX250mm, 5 micron		
Gradient (linear):	5%-30% buffer B in 11min		

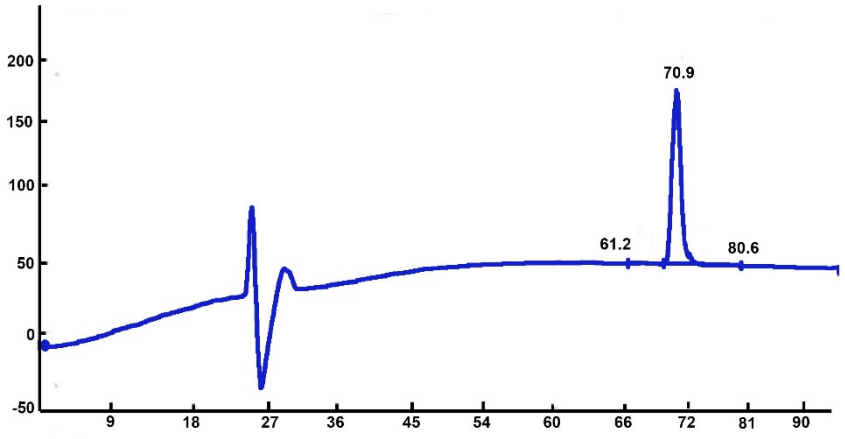


Fig. S2 the chromotagram ot the glycopeptide

Rank	Time	Name	Conc.	Area
1	8.173		0.3375	5511
2	8.617		98.42	1607063
3	8.810		1.248	20372
Total			100	1632946

MS analysis

Expected MS: 1029.8

Flow rate: 0.2ml/min Run Time: 1min

Buffer A: 0.1% HCOOH in water Buffer B: 0.1% HCOOH in Acetonitrile

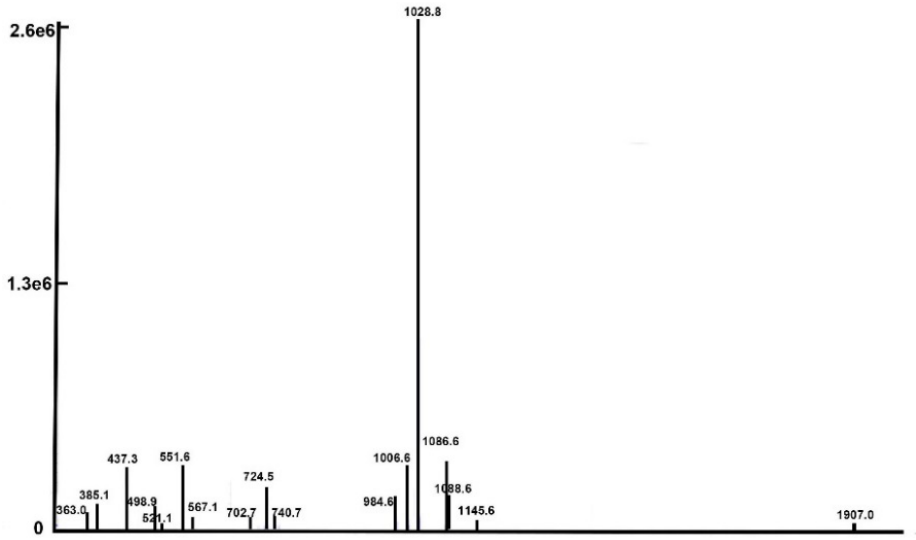


Fig. S3 the MS spectrum of glycopeptide

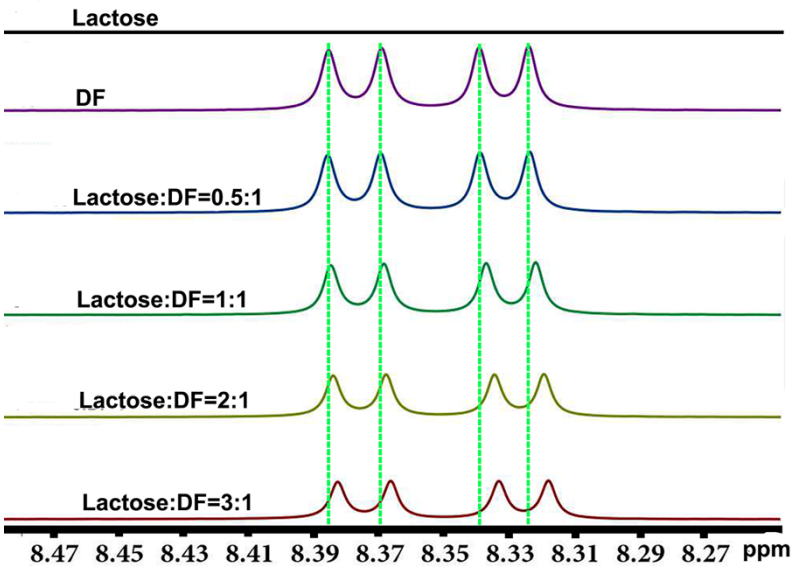


Fig. S4 Partial **^{1}H NMR** spectra (δ 8.47-8.27 ppm) for the addition of lactose to DF in d_6-DMSO at 20 ºC . The concentration of the monomer-complex solution is 5 mM.

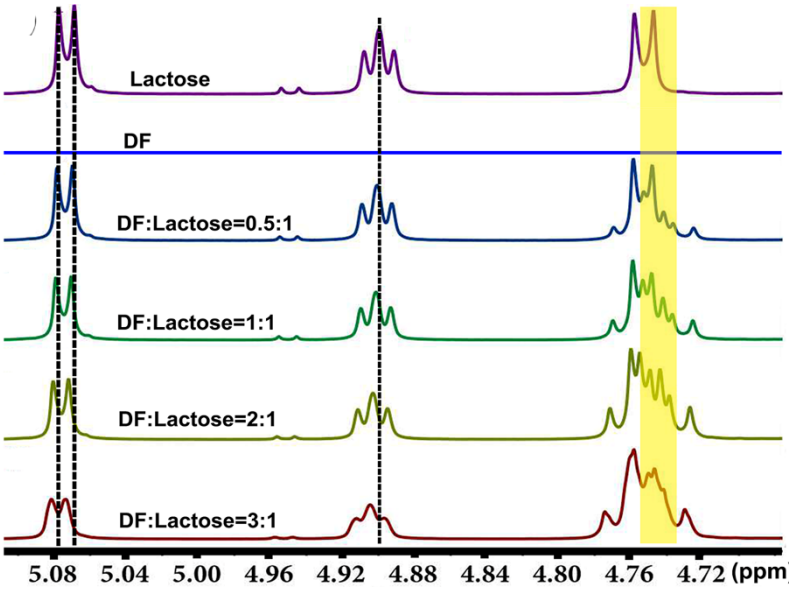


Fig. S5 Partial **^{1}H NMR** spectra (δ 5.08-4.72 ppm) for the addition of DF to lactose in d_6-DMSO at 20 °C. The concentration of the monomer-complex solution is 5 mM.

As shown in Fig. S4 and Fig. S5, both **^{1}N MMR** spectra showed clear indications of binding between the monomer DF and lactose.

Part 3. Preparation of PNI-*co*-DF-*co*-PT@SiO_2 through surface-initiated the surface-initiated atomic transfer radical polymerization (ATRP)[3]

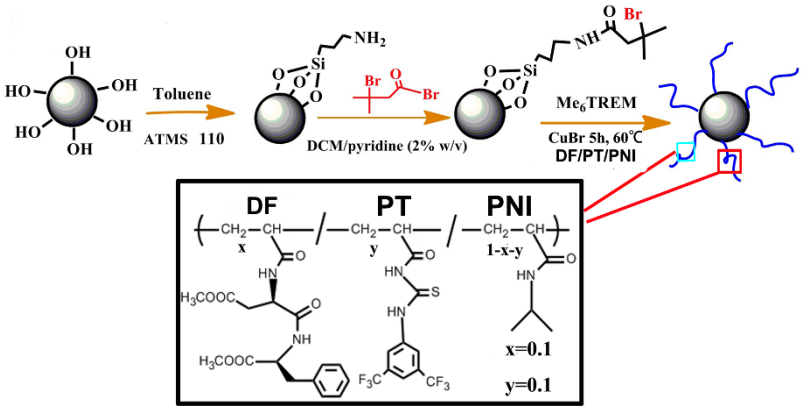


Fig. S6 Synthesis route to PNI-*co*-DF-*co*-PT@SiO_2 and chemical structure of the three component copolymer

The synthesis of aminopropyl silica was synthesized according to reference[2]. The prepared aminoprpyl silica (5 g) was suspended in 120 ml of dichloromethane that contained pyridine (2% w/v), followed by 1 ml of 2-Bromoisobutyryl bromide at 0 °C. The mixture was stirred for 1 hour at this temperature then at room temperature for 8 hours. The reaction was stopped and the resulting silica was filtered and rinsed by dry dichloromethane. Copolymerization of PNI-*co*-DF-*co*-PT was achieved by adding the obtained silica (4.5 g) with the initiator grafted on the surface in a degassed solution of PNI (1.628 g), DF (0.652 g), PT (0.616 g) (10 mol % DF, 10 mol % PT against PNI.) in a mixture of H_2O (15 mL), MeOH (15 mL) and DMF (8 mL) containing CuBr

(0.032 g, 0.23 mmol) and pentamethyl diethylene triamine (PMDETA, 0.16 mL) in a three-neck round-bottom flask. The reaction was mechanically stirred for 5 hours at 60 °C, under the protection of nitrogen atmosphere. Finally, the PNI-*co*-DF-*co*-PT@SiO_2 was filtered and washed by methanol, and dried at 40 °C for 48 hours before use.

Part 4.1 Characterization of PNI-*co*-DF-*co*-PT@SiO_2

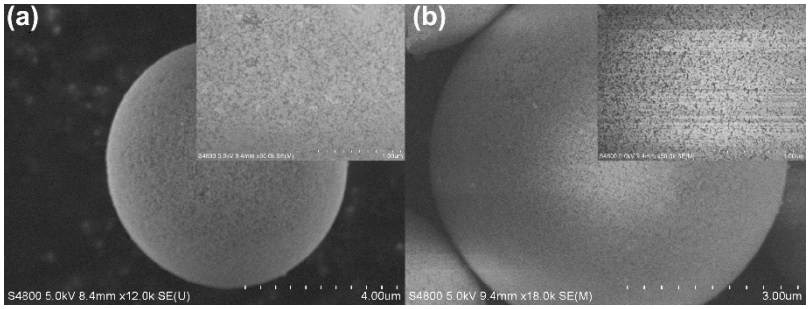


Fig. S7 SEM characterization of SiO_2 (left) and polymer modified SiO_2 (right).

Characterization of the prepared PNI-*co*-DF-*co*-PT@SiO_2 materials was performed for morphology and composition analyses. SEM images revealed that the PNI-*co*-DF-*co*-PT@SiO_2 exhibited a similar morphology to that of naked silica material. A BET surface area of 74.5 $m^2.g^{-1}$ and a pore volume of 0.47 $cm^3.g^{-1}$ were derived from the N_2 adsorption experiment. The pore diameter reduced from 120 nm to 80 nm. Thus, these data indicated the coating with polymers on the silica surface and the pores in the polymer@SiO_2 were not blocked.

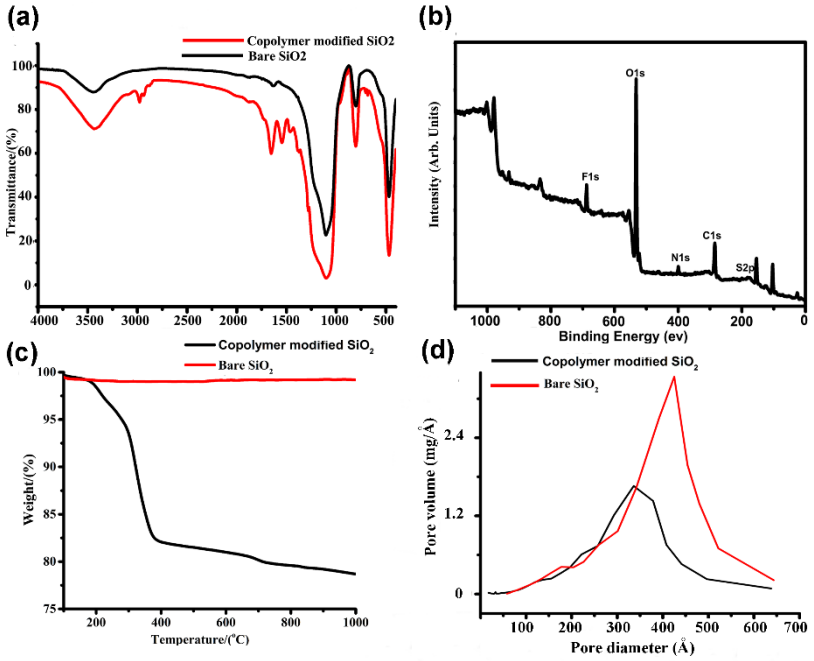


Fig. S8 Chemical analysis of polymer modified SiO_2 with FT-IR (a), XPS (b), TGA (c) and (d) BET techniques.

We further characterized the surface of PNI-*co*-DF-*co*-PT@SiO_2 by FT-IR and X-ray photoelectron spectrum (XPS) to obtain the chemical composition of the grafted polymers. The characteristic stretching and bending vibrations at 3437 cm^{-1}, 2978 cm^{-1}, 2937 cm^{-1}, corresponded to N-H, aliphatic C-H and CH_3O-, implying that the dipeptide aspartame was incorporated into the polymer chains (Fig. 9a). The bands CF_3 and C=S may be were merged into each other because Fluoroalkyls (C-F) stretch and Thiocabonyl (C=S) appeared in the ranges 1400-1000 and 1160-

1100, respectively. In addition, the surface composition of polymer@SiO_2 were further measured by XPS. The XPS spectrum of the resulting microspheres showed five distinct chemical species: nitrogen, oxygen, carbon, sulphur and fluorine (Fig. 9b). This result revealed the existence of monomer PT. From the results of TGA (Fig. 9c), a weight loss of 21.14% occurred for polymer@SiO_2 microspheres and 0.11% for bare SiO_2, which suggested that this three component polymer brushes modified SiO_2 have been successfully fabricated.

Part 4. Thesis of Preparation of PNI-*co*-DF-*co*-PT copolymer film on silicon substrate through ATRP

A clean silicon substrate was immersed in aqueous NaOH (0.1 mol•L^{-1}) for 8 minutes and subsequently in HNO_3 (0.1 mol•L^{-1}) for 15 minutes to generate hydroxyl groups on silicon wafer. After the substrate had been washed with an excess of water and dried under a flow of nitrogen, it was heated to reflux in toluene that contained 5 wt% amino-propyltrimethoxysilane (ATMS) for 4 hours to obtain chemically bonded $–NH_2$ groups on the surface. The surface was rinsed with toluene and dichloromethane to remove remaining ATMS, dried under a flow of nitrogen gas, and immersed in dry dichloromethane that contained pyridine (2% w/v). The polymerization initiator bromoisobutyryl bromide was added dropwise into the solvent containing the silicon substrate at 0 ^oC, and the mixture was left for 1 hour at this temperature, then at room temperature for 12 hours. The silicon substrate was cleaned with dichloromethane, and dried under a nitrogen flow. Copolymerization of PNI-*co*-DF-*co*-PT was achieved by immersing the silicon substrate with the initiator grafted on the surface in a degassed solution of PNI (0.814 g), DF (0.326 g), PT (0.308 g) (10 mol % DF, 10 mol % PT against PNI.) in a mixture of H_2O (3 mL), MeOH (3 mL) and DMF (7 mL) containing CuBr (0.032 g, 0.23 mmol) and pentamethyl diethylene triamine (PMDETA, 0.16 mL) for 5 hours, at 60 ^oC. After polymerization, the substrates were washed by DMF, methanol, H_2O, respectively and dried under a flow of nitrogen. Under this condition, the thickness of film was between 60 nm.

Part 4.1 AFM PeakForce QNM Microscopy[4]

PeakForce QNM (Quantitative NanoMechanics) (Bruker Instruments) enables quantative measurement of nano-scale material properties such as modulus, adhesion, deformation and dissipation. Because Peak Force Tapping Mode controls the force applied to sample by the tip, sample deformation depths are small and the effect of the substrate on the measured modulus is decreased. All the quantitative measurements were performed using standard RTESP for PeakFore QNM. The Tip radius of the probe was evaluated by scanning a tip characterizer sample (Ti roughness sample from Veeco) and the Tip Qualification function in NanoScope Analysis sofware. The morphology and modulusof the three-component copolymer modified Si wafer was obtained through PeakForce QNM Microscopy, in which the spring constant was 0.2387 N/m and Tip radius was 10.02. after being immerged in lactose solution (20mM.L^{-1}) for 30 mins, followed the removal of any remaining excess liquid by a N_2 flow, the morphology and modulus was also recorded, allowing for the comparison with that of original film. The procedure was also same for maltose.

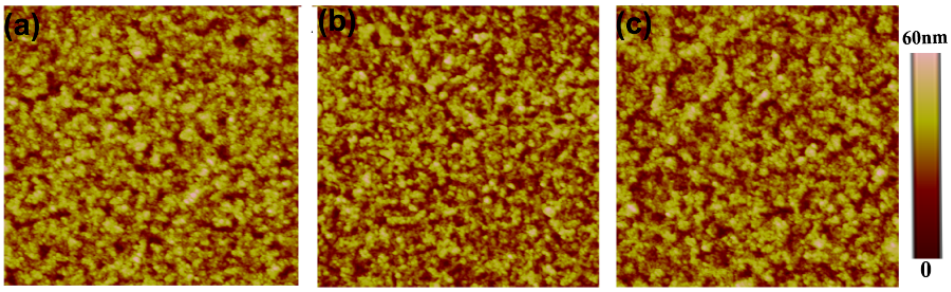


Fig. S9 The AFM image of copolymer film before (a) and after being treated by maltose (b) or lactose (c)

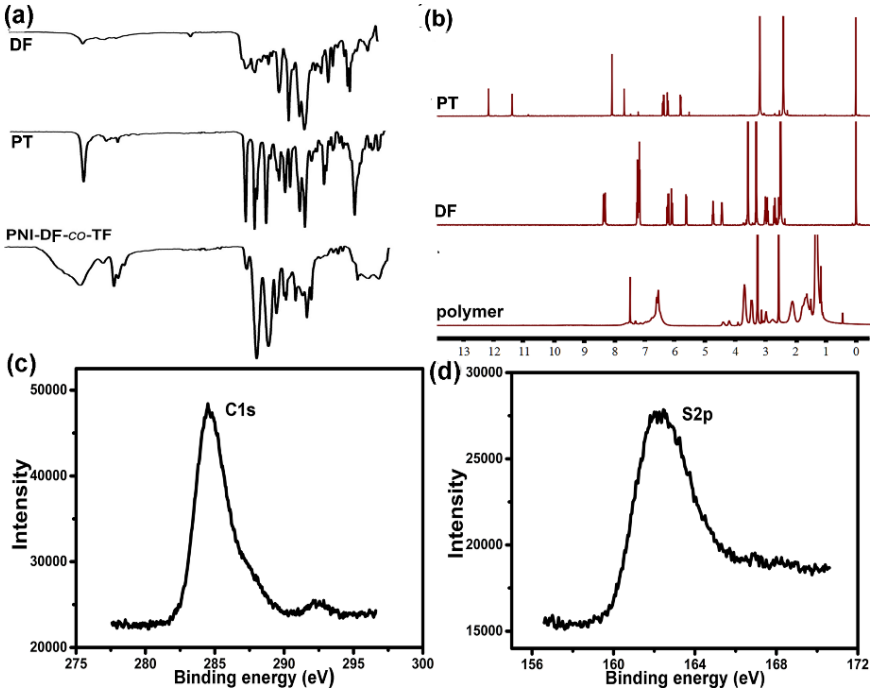


Fig. S10 (a) FI-IR spectrum of DF, PT and the copolymer PNI-*co*-DF-*co*-PT modified silicon wafer; (b) NMR of DF, PT and the copolymer PNI-*co*-DF-*co*-PT; (c) and (d) XPS spectrum of the copolymer PNI-*co*-DF-*co*-PT modified silicon wafer.

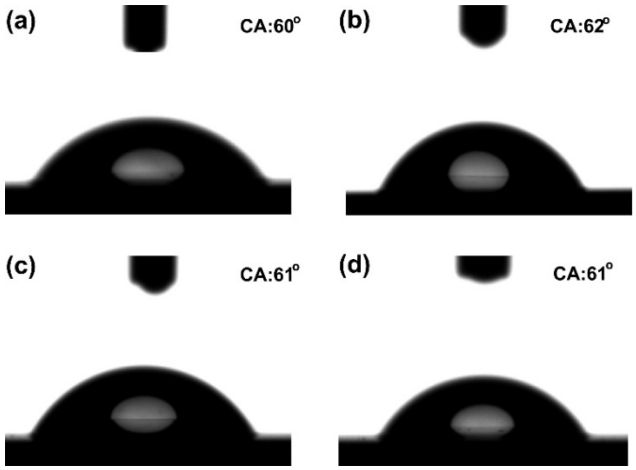


Fig. S11 (a) Profile of water droplet on the PNI film and profiles of water droplets on the PNI film modified silicon substrates treated with maltose (b), sucrose (c) and lactose (d).

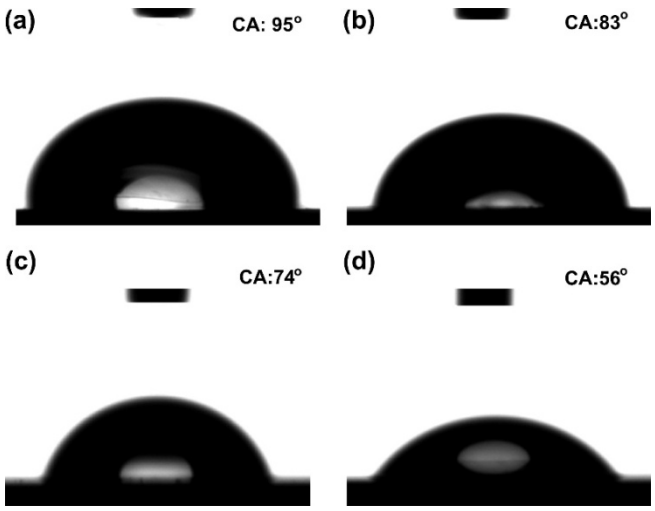


Fig. S12 Profile of water droplet on the copolymer PNI-*co*-DF film and profiles of water droplets on the copolymer modified silicon substrates treated with maltose (b), sucrose (c) and lactose (d).

Part 4.2 Adsorption experiments of different disaccharides[5]

QCM resonator was grafted three-component copolymer through surface-initiated atom transfer radical polymerization**.** Subsequently, the copolymer modified QCM resonator was washed with water for four times and dried by nitrogen gas. All QCM measurements were performed at 20 ^{o}C using QCM-Sence E4 system (Sweden). Prior to binding assays between copolymer modified sensor and disaccharide solution, QCM channels and tubes were washed by ultrapure water. Disaccharide (20 mmol) solution were injected into channels at a flow rate of 100 ml.min^{-1}. In the experiment of glycopeptide adsorptions on the three-component copolymer modified Au resonator, the quality of glycopeptide was same with that of maltose solution. All binding curves were recorded by Q-Sense software and analysed by Q-Tools.

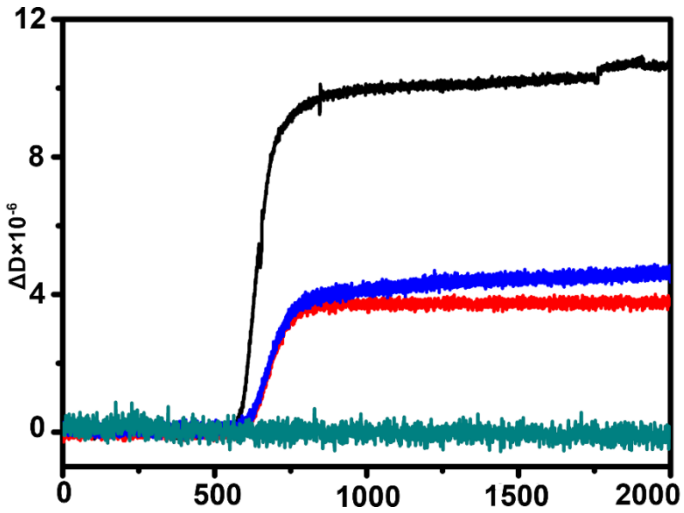


Fig. S13 Time-dependent dissipation curves of lactose adsorption on four kinds of copolymers on Au resonator, black line-PNI-*co*-DF-*co*-PT, blue-PNI-*co*-DF, red-PNI-*co*-PT, green line-PNI

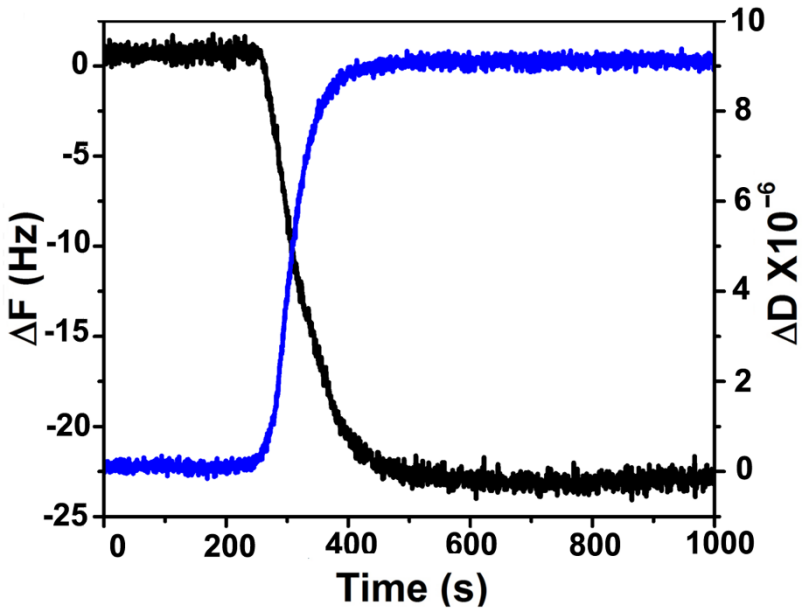


Fig.S14 Time-dependent frequency (black) and dissipation (blue) curve of N-acetyl-D-lactosamine absorbed on the copolymer PNI-*co*-DF-*co*-PT modified Au resonators.

Part 5. Trypsin digestion of glycoproteins

1 mg IgG protein was dissolved in denaturing buffer containing 8 M urea and 50 mM ammonium bicarbonate. Protein containing solution was treated with 50 mM dithiothretiol for 45 min at 56 ^{o}C. Then 50 mM iodoacetamide was added for alkylation and the mixture was incubated in the dark for 30 min at room temperature. The resulting solution was diluted to 10 folds with 50 mM ammonium bicarbonate and digested with trypsin. Digestion was stopped with 50 ml 10% FA.

Part 5.1 Enrichment of glycopeptides

For glycopeptide enrichment, 2 mg of PNI-*co*-DF-*co*-PT materials were packed into GELoader tips. After conditioning and equilibrating with 50 mM NH_4HCO_3 and CH_3CN/50mM ABC (85:15 (v/v), 10μl dried IgG tryptic digests were redissolved with CH_3CN/50 mM ammonium bicarbonate (85:/15 (v/v) and loaded into packed copolymer-based microcolumn. The column was washed CH_3CN/50 mM ammonium bicarbonate (72:28 (v/v) and IgG glycopeptides were detached from column with 50 mM NH_4HCO_3. The eluted solutions were desalted with 50 mM C18 materials before MS analysis. For C18 desalt, 1 mg of C18 materials were packed into GELoader tips. The microcolumns were conditioned and equilibrated with 20 ml 0.1% FA aqueous solution and eluted with 10 ml CH_3CN/H_2O (50:50(v/v) containing 0.1% FA.

Part 5.2 MS analysis

Peptide mixtures were analyzed with nano electrospray ionization-quadrupole time-of-flight-mass spectrometer (ESI-Q-TOF MS) (Waters, Manchester, UK). Desalted peptides were injected to nano ESI source using a Waters Acquity nano LC system (Milford, MA, USA). Full scan MS data were obtained in the mass range 600 to 2000.

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