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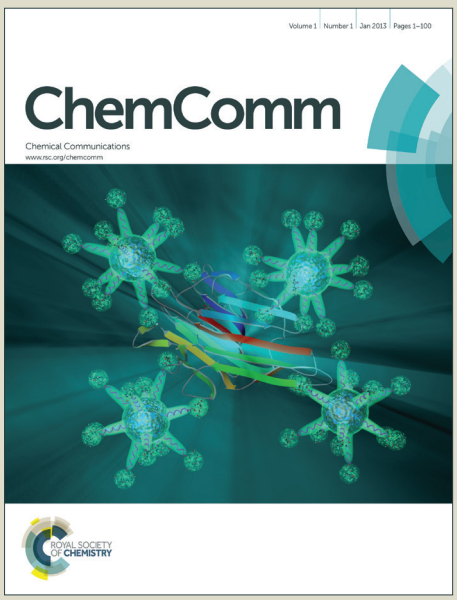

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Disaccharide-driven macroscopic properties transition: From molecular recognition to glycopeptide enrichment

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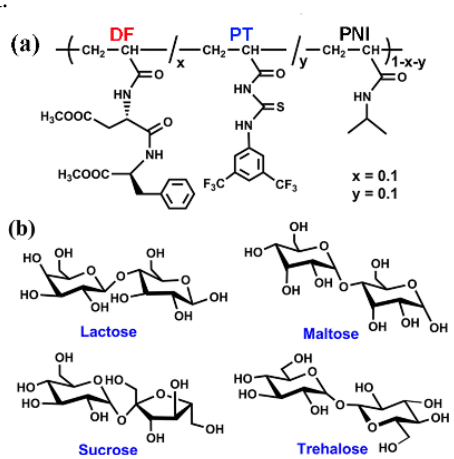
We reported a three-component smart polymer, which could discriminate disaccharide homologues and translate the recognition signals into distinct differences in macroscopic properties (i.e. wettability and adhesion force) of materials. With these features, we further showed its application in glycopeptide enrichment.

Carbohydrate recognition has attracted much attention due to the growing awareness of the crucial roles of carbohydrates in diverse biological processes, including neuronal development,[1] fertilization,[2] immune surveillance[3] and inflammatory responses.[4] Subtle variations in saccharide structures and stereo-arrangement of hydroxyl groups make it challenging to recognize carbohydrates with high selectivity.[5] Efforts to develop artificial carbohydrate receptors have mainly been focused on monosaccharide substrates,[6-13] which are usually implemented by hydrogen nuclear magnetic resonance (^{1}H NMR) spectroscopy or other spectroscopic methods. Compared with monosaccharides, oligosaccharides contain a wealth of biological information due to diverse saccharide compositions, branch structures and linkage types, which, however, are more difficult to study. In this respect, disaccharide recognition represents a key step for better understanding the biofunctions of complex oligosaccharides and glycoconjugates. Until now, only a few well-designed tricyclic or boron-based receptors show good chemoselectivities among disaccharide homologues at the single molecular level.[14-21] On the other hand, practical applications, e.g. chromatographic separation[22] and glycopeptide enrichment, raise higher requirements to directly amplify the recognition signals into drastic changes in macroscopic properties of materials[23], which represents a more challenging direction for carbohydrate recognition.

In this study, taking advantages of a "recognition-mediating-functional main chain" designing strategy, we developed a three-component copolymer thin film, which was comprised of a dipeptide recognition unit, a phenylthiourea mediating unit and a flexible poly(N-isopropylacrylamide) (abbreviated as PNI) backbone, as illustrated in Scheme 1a. This copolymer film could discriminate different disaccharides (i.e. lactose, sucrose, maltose and trehalose (Scheme 1b)), and the disaccharide recognition signals were successfully transformed to the changes in macroscopic properties of materials including surface wettability and adhesion force. These features further facilitated the application to glycopeptide enrichment.



Scheme 1 Chemical structures of three-component copolymer (a) and four typical disaccharides (b).

Here, a methyl esterified dipeptide α-L-Asp-L-Phe (abbreviated as DF) was selected as the saccharide recognition unit, and phenylthiourea (PT) functioned as mediating unit that helped combining disaccharides through directional hydrogen bonding (H-bonding) interaction. Firstly, the binding capacities of DF and PT units towards various disaccharides were investigated through fluorescent titration experiment. As illustrated in Table 1, DF exhibited obvious discrimination among these disaccharides. The largest association constant (*K*ass) of 1.57×10^5 L mol^{-1} was obtained when DF interacted with lactose, which was evidentially larger than that with sucrose (*K*ass: 1.05×10^5 L mol^{-1}) and maltose (*K*ass: 8.76×10^4 L mol^{-1}), and five-fold larger than that with trehalose (*K*ass: 2.91×10^4 L mol^{-1}). By comparison, PT did not show apparent capacity to distinguish these substrates, accompanied by *K*ass values ranging from 6.14×10^4 to 2.59×10^4 L mol^{-1}. When α-Asp-Phe-

thiourea (DFT) was used, the binding capacities increased substantially, resulting in second-order *K*ass values. This indicated that the introduction of thiourea group could significantly strengthen the binding with disaccharides through synergetic hydrogen bonding interactions. This has further been verified by ^{1}H NMR titration experiments (Fig. S4 in ESI) and quantum chemistry calculation (Fig. 1a), in which the hydrogen bonds were depicted as green dot lines.

Table 1. Association constants (*K*ass) of functional monomer DF or PT with various disaccharides at 20 °C.

Saccharides	*K*ass [L mol^{-1}] [a]		
	α-Asp-Phe (DF)	Phenyl thiourea (PT)	α-Asp-Phe-thiourea (DFT)
D-Lactose	1.57×10^5	4.31×10^4	K_1: 1.27×10^5 K_2: 2.87×10^3
D-Sucrose	1.05×10^5	6.14×10^4	K_1: 1.14×10^5 K_2: 1.30×10^4
D-Maltose	8.76×10^4	2.70×10^4	K_1: 9.33×10^4 K_2: 3.87×10^4
D-Trehalose	2.91×10^4	2.59×10^4	K_1: 8.53×10^4 K_2: 2.04×10^4

[a] *K*ass were obtained from fluorescent titration experiments according to the intensity changes at the maximum emission peaks.

Then DF and PT units were incorporated into the PNI main chain through a surface-initiated atomic transfer radical polymerization. As shown in Fig. 1b, the original film was hydrophobic with a contact angle (CA) of 93 ± 1°. After being immersed in a lactose solution (concentration: 20 mM) for 30 minutes, followed by the removal of any remaining excess liquid by a N_2 flow, the film became hydrophilic with a stable CA of about 45 ± 2° . By comparison, the CAs were 75 ± 2° and 63 ± 2° after the film were treated with maltose and sucrose solutions, respectively, both of which were much larger than that induced by lactose. However, through the same treatment procedure, no obvious ΔCA changes (less than 2°) for the reference film pure PNI and a much narrower ΔCA change for another reference copolymer film (denoted as PNI-*co*-DF) towards to different disaccharides (Fig. S11, S12 in ESI) indicating the indispensability of the mediating unit. Moreover, these results were further validated by the disaccharide adsorption experiment on three reference polymer films (i.e. PNI-*co*-DF, PNI-*co*-PT and PNI), which were monitored by a quartz crystal microbalance (QCM). As shown in Fig. 1c, the adsorption of lactose was chosen as an example, which exhibited a strong adsorption on PNI-*co*-DF-*co*-PT film and induced a frequency change (Δ*f*) of 24.5 Hz. That corresponded to an adsorption quantity of 433 ng cm^{-2}. Compared with the notable frequency change on PNI-*co*-DF-*co*-PT film, the frequency changes caused by lactose were much lower on PNI-*co*-DF (Δ*f*: 10 Hz) and PNI-*co*-PT (Δ*f*: 9.6 Hz) films, while no evidential adsorption was observed on pure PNI surface. These results showed that dipeptide and phenylthiourea units synergistically participated in the interaction of the copolymer film with disaccharides.

In addition, surface stiffness and the adhesion force of the copolymer film were strongly influenced by the treatment of disaccharide solution, which was monitored by atomic force microscopy (AFM) in PeakForce QNM mode.[24-25] Fig. 2a-2c displayed the DMT Young's modulus images of the copolymer film before and after immersion in a maltose or lactose solution for 30 minutes, significant change of Young's modulus was observed, which indicated that the film became softer after disaccharide treatments. In particular, the average Young' modulus decreased from the initial value of about 100 MPa to about 50 MPa when the polymer film was treated with lactose, which was much lower than that after maltose treatment with a value of about 70 MPa. Moreover, the adhesion force of polymer surface also underwent notable changes (Fig. 2g-2i) before and after treatment with disaccharides. The average adhesion force of the original copolymer film was about 3.5 nN and it increased to 5 nN after the copolymer film was treated by lactose, which was higher than 4 nN by maltose. These results implied that the synthesized copolymer got softer and stickier after treatment with disaccharides, which will facilitate glycopeptide enrichment.

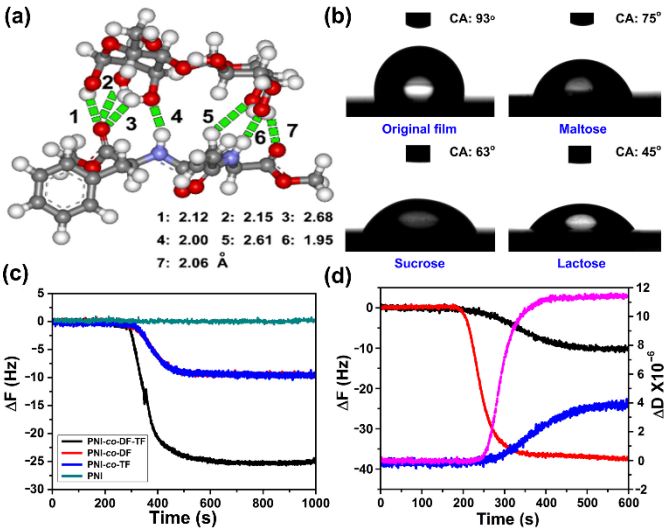


Fig. 1 (a) Quantum chemistry calculation for the interaction between DF and lactose. Hydrogen bonds were depicted by green dot lines; (b) profiles of droplets on the PNI-*co*-DF-*co*-PT modified silicon substrates treated with maltose, sucrose, and lactose solutions, respectively; (c) the comparison of lactose adsorption on Au resonators modified with different polymers (concentrations: 20 mM), (d) time-dependent frequency (black and red) and dissipation (blue and purple) curves of maltose and glycopeptide with maltose appendence absorbed on the copolymer PNI-*co*-DF-*co*-PT modified Au resonators, maltose: black and blue lines, glycopeptides: red and purple lines.

To explore potential application of this film on glycopeptide enrichment, we did the adsorption experiment using a synthesized glycopeptide, in which a maltose residue was appended onto the peptide main chain (peptide sequence see ESI†). Fig. 1d displayed the frequency and dissipation changes of the copolymer film caused by the adsorption of glycopeptide. Notably, the PNI-*co*-DF-*co*-PT film exhibited an intensive adsorption toward glycopeptide, the frequency finally decreased up to -40 Hz (red line). Furthermore, we also tested the affinity of this copolymer to N-acetyl-D-lactosamine (Fig. S14), which was an important an important constitute of glycans lied in IgG protein. This copolymer exhibited strong interaction towards N-acetyl-D-lactosamine and caused a frequency change (Δ*f*) of 22.7 Hz, corresponding to an adsorption quantity of 401 ng cm^{-2}. These results addressed the great potential of this copolymer film in glycopeptide enrichment. More interestingly, the adsorption of glycopeptide also brought substantial increase in dissipation (represents the viscoelasticity of film) than the individual maltose, which indicated that polymer chains became much relaxed

after interaction with glycopeptide. On the other hand, significant difference in surface wettability could become a positive factor in practical chromatographic separation, because hydrophilic surface could further promote the adsorption of polar saccharides, whereas hydrophilic one would repel the permeation of non-polar solutes.

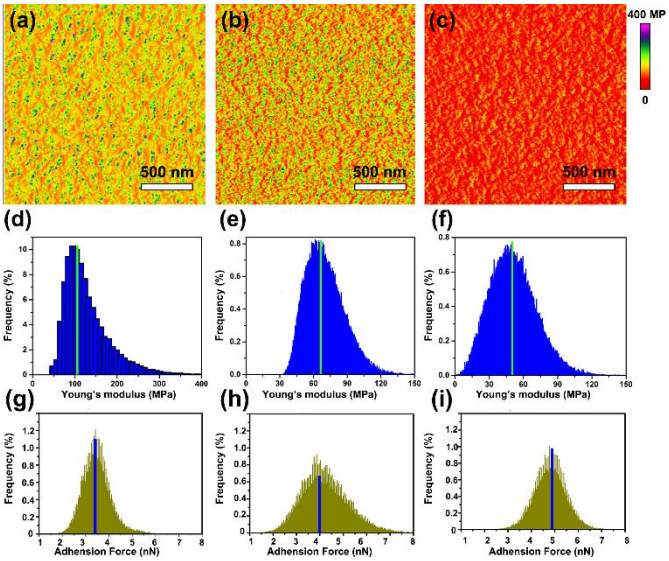


Fig. 2 PeakForce QNM AFM images (a-c) of copolymer film before (a) and after interacting with maltose (b) or lactose(c) solution. Distributions of Young's modulus (d-f) and Adhesion force (g-i) corresponding to the images shown in a-c. Scale bars: 500 nm.

Surface wettability and adhesion force are fundamentally macroscopic properties of materials, which strongly influence adsorption dynamics and mass-transfer process at liquid/solid interfaces.[26]Taking advantages of the dramatic changes in wettability and adhesion force, PNI-*co*-DF-*co*-PT grafted silica microspheres was further employed to enrich glycopeptides, which is the first and crucial step for the analysis of protein glycosylation that dominates cellular signal transduction, cell adhesion and also associates with many diseases.[27-30] Nevertheless, capture of glycopeptides from the complicated bio-samples is still a huge challenge in proteomics. As shown in Fig. 3a, b, after treatment with C18 and commercial enrichment material Sepharose, an abundance of non-glycopeptide signals (indicated by blue dashed boxes) almost dominated the mass spectrum, implying the poor selectivity of Sepharose. In contrast, the copolymer-grafted materials (the detailed characterization data see Fig. S8 in ESI) exhibited high enrichment selectivity toward glycopeptides. Up to 18 glycopeptide signals from human serum immunoglobulin G (IgG) were detected, attributed to the removal of majority of non-glycopeptides. These results clearly demonstrated the copolymer-based material could be served as enrichment material for glycopeptides oriented to glycoproteome analysis due to its strong affinity with oligosaccharides.

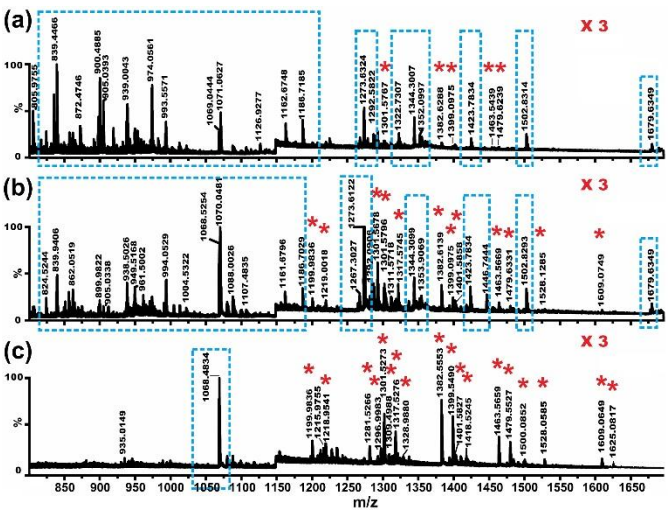


Fig. 3 Nano electrospray (Nano ESI) Q-TOF mass spectrum of IgG tryptic digests enriched with C18 (a), sepharose (b) and smart copolymer modified silica microspheres (c) materials, respectively. Glycopeptides were labelled with ***** and their m/z values. And the non-glycopeptides were labelled with their **m/z** values, indicated by blue dashed boxes. X 3 means the MS spectrum protruding from the base line is magnified for 3 times.

Conclusions

In summary, through synergetic hydrogen bonding interaction, the three-component copolymer material exhibited distinct adsorption behaviors, surface wettability and adhesion force transition toward different disaccharides. Furthermore, as a typical example, these smart features were successfully applied in glycopeptide enrichment. This provides much enlightenment for the application of smart polymer in saccharide separation and proteomics.

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Notes and references

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