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Carbohydrate Sensing Using Water-Soluble Poly(methacrylic acid)-*co*-3-(Acrylamido)phenylboronic Acid Copolymer

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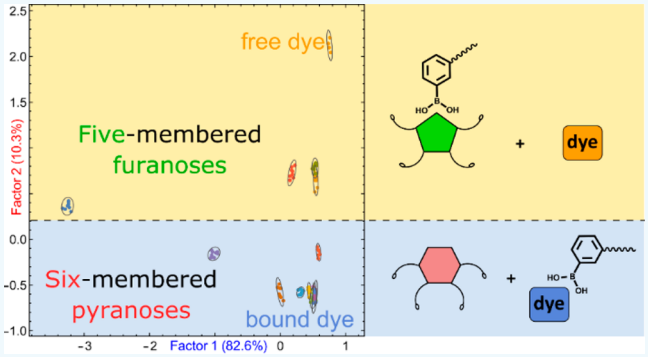
Supporting Information

ABSTRACT: Carbohydrate and saccharide sensing in water remains very challenging. Using the covalent reversible interaction between boronic acids and the diol groups in carbohydrates is still one of the most common approaches. However, the binding affinity and solubility of isolated boronic acids are poor in water, which significantly limits their application in physiological conditions. In this work, we improved the solubility and affinity of this interaction by connecting phenylboronic acid moieties to a hydrophilic poly(methacrylic acid) (PMAA) polymer to generate a water-soluble poly(methacrylic acid)-*co*-3-(acrylamido)phenylboronic acid (PMAA-*co*-AAPBA) copolymer as a supramolecular receptor. An indicator displacement assay including oxidized hematoxylin dye was used to study the interaction of the PMAA-*co*-AAPBA receptor with monosaccharides (e.g., fructose, glucose, galactose, and ribose) and disaccharides (e.g., lactose, maltose, sucrose, and trehalose). Data interpretation using linear discriminant analysis (LDA) confirmed that the [PMAA-*co*-AAPBA·dye] complex successfully differentiated both monosaccharides and disaccharides in neutral aqueous solution. The observed binding affinity trend provided further evidence in support of the fact that boronic acids bind more efficiently to vicinal *cis*-diols on five-membered rings than on six-membered rings. This system demonstrates the enhanced affinity and water solubility attainable through grafting a supramolecular sensing motif to an appropriate polymeric support, with an eye to future applications in biological and environmental contexts.



KEYWORDS: *carbohydrates, boronic acids, acrylates, array sensing, chemical fingerprinting, optical spectroscopy, absorbance, fluorescence*

INTRODUCTION

The quest for stable artificial receptors with high affinities has been very active in sensing science in the past decades, with particular emphasis on supramolecular analytical contributions.[1−4] Understanding the supramolecular interactions most suited to the analyte of interest is fundamental to design effective receptors and generate recognition systems. There has long been great interest in the interaction between boronic acids and molecules containing vicinal *cis*-diols.[5−14] In fact, boronic acids are known to covalently and reversibly bind to *cis*-diol functional groups, spurring their use to recognize diol-containing compounds such as small sugar molecules and glycoproteins.[15−22]

The binding affinity of boronic acids to carbohydrates is solvent- and pH-dependent.[23−25] Usually, boronic acids bind much more efficiently to sugars in organic solvents than in water, aided also by the lower desolvation cost in organic solvents. The binding affinity of boronic acids to sugars was also found to be higher in basic aqueous solution.[25] Most of the previous studies in this field therefore were performed in mixed aqueous solutions with a high percentage of organic solvent or at high pH.[26−32] Sensors for sugars that work in neutral water have been recently reported,[33−39] including some that use pattern-based recognition for carbohydrate discrimination,[40,41] indicating the importance and urgency to develop viable sensors in physiological conditions.

The Bonizzoni group previously reported on a differential array based on poly(amidoamine) dendrimers (PAMAM) modified with boronic acids, and two dyes (4-methylesculetin and alizarin red S), to differentiate common monosaccharides including fructose, glucose, galactose, and ribose in neutral buffered water.[15] PAMAM dendrimers, however, are costly and not amenable to surface immobilization. To move our detection paradigm further, we report here on the design,

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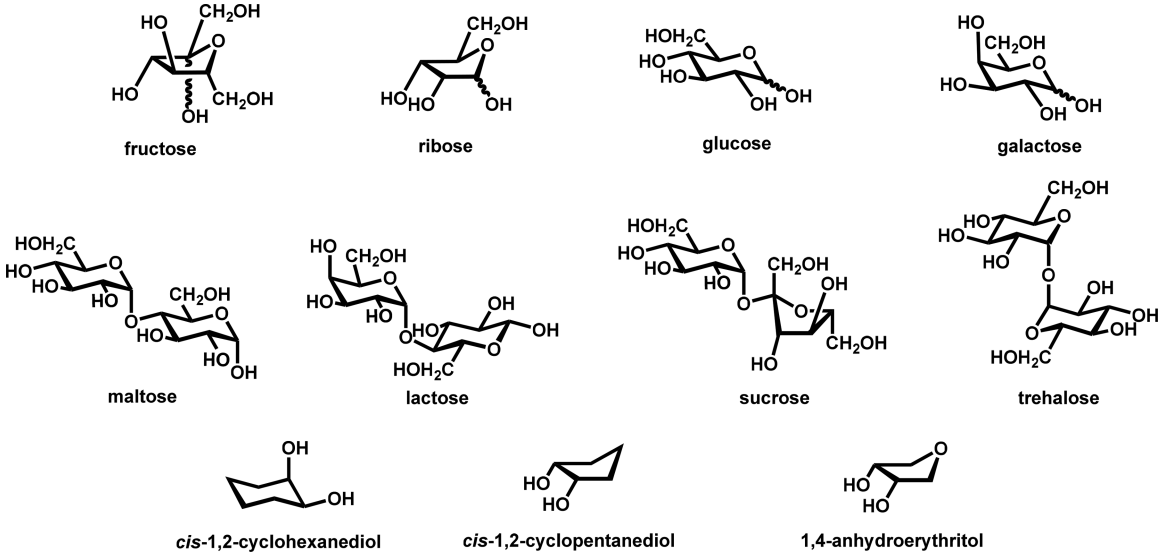
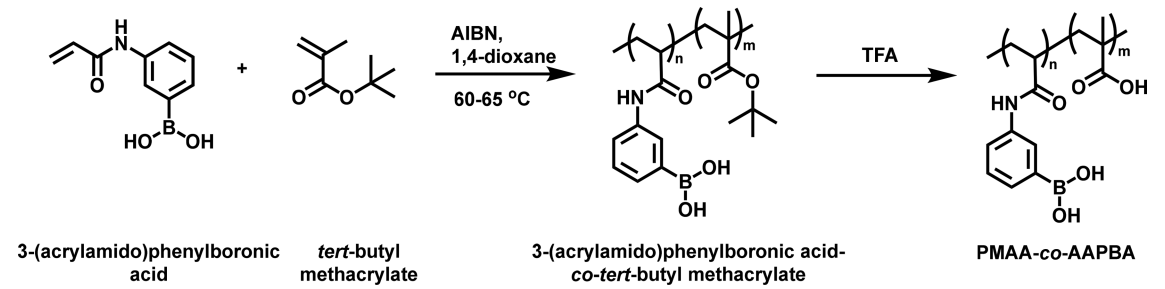
Scheme 1. Chemical Structures of Fructose, Ribose, Glucose, Galactose, Maltose, Lactose, Sucrose, Trehalose, *cis*-1,2-Cyclohexanediol, *cis*-1,2-Cyclopentanediol, and 1,4-Anhydroerythritol



Scheme 2. A New Water-Soluble Copolymer for Carbohydrate Sensing Obtained by Copolymerizing a Boronic Acid-Modified Acrylamide with a Methacrylate Ester Monomer via Free Radical Polymerization[a]



[a]The monomer ratio directly controls the percentage of phenylboronic acid in the polymer. Synthesis of the 3-(acrylamido)phenylboronic acid-*co*-*tert*-butyl methacrylate polymer was followed by removal of the *t*Bu ester to produce poly(methacrylic acid)-*co*-3-(acrylamido)phenylboronic acid (PMAA-*co*-AAPBA).

characterization, and analytical performance of a multivariable detection method relying on optical signals (UV−vis absorption) that uses a water-soluble poly(methacrylic acid)-*co*-3-(acrylamido)phenylboronic acid copolymer (PMAA-*co*-AAPBA) and oxidized hematoxylin dye to differentiate eight mono- and disaccharides in neat H_2O buffered at neutral pH.

Previous NMR and computational studies showed that boronic acids bind more efficiently to *cis*-diols on five-membered furanose rings than to those on six-membered pyranose ones; this is imputed to the much smaller dihedral angle between the two hydroxy groups on five- vs six-membered rings.[25,42−44] For example, *cis*-1,2-cyclopentanediol binds more strongly to phenylboronic acid than *cis*-1,2-cyclohexanediol;[44] it is also a common observation that boronic acid-based receptors bind to fructose more strongly than to, for example, glucose in water.[45]

To investigate whether this still holds true when boronic acids are conjugated to more complex multivalent receptors such as the one presented here, in this work we chose three cyclic molecules containing 1,2-*cis*-diol moieties as model probes—*cis*-1,2-cyclopentanediol, *cis*-1,2-cyclohexanediol, and 1,4-anhydroerythritol (Scheme 1)—in addition to a panel of analytically relevant small carbohydrates. We observed that *cis*-1,2-cyclopentanediol and 1,4-anhydroerythritol behaved very similarly to fructose and ribose (five-membered ring structures); on the other hand, *cis*-1,2-cyclohexanediol was more similar to glucose and galactose, which provided further evidence to support the trend above and gave an explanation of the binding affinity trend observed with small sugars as well.

EXPERIMENTAL SECTION

Synthesis of Poly(methacrylic acid)-*co*-3-(Acrylamido)-phenylboronic Acid (PMAA-*co*-AAPBA). The boronic acid-containing poly(methacrylic acid) copolymer PMAA-*co*-AAPBA was synthesized via free radical polymerization. For the synthesis of PMAA-*co*-AAPBA containing 6% boronic acid monomer units, 2.0 mL of *tert*-butyl methacrylate (*t*BMA), 0.25 g of (3-acrylamidophenyl)boronic acid (AAPBA), and 0.02 g of 2,2′-azobis(isobutyronitrile) (AIBN) were dissolved in 10 mL of dioxane. Solutions were degassed by N_2 bubbling, followed by three freeze−thaw cycles, to remove dissolved O_2. The mixture was then heated to 67 °C for 3 h under nitrogen, with continuous stirring. After the reaction was terminated, the *tert*-butyl methacrylate-*co*-3-(acrylamido)phenylboronic acid (*t*BMA-*co*-AAPBA) was precipitated in a 4:1 MeOH:H_2O mixture. The precipitated copolymer was dissolved in dioxane, precipitated in the methanol/water mixture twice, and dried under vacuum. The *t*BMA protecting group was removed by adding trifluoroacetic acid to a CH_2Cl_2 solution of the material; the solution was then left to stir overnight. The resulting free acid copolymer was dialyzed against DI water using a Spectra/Por

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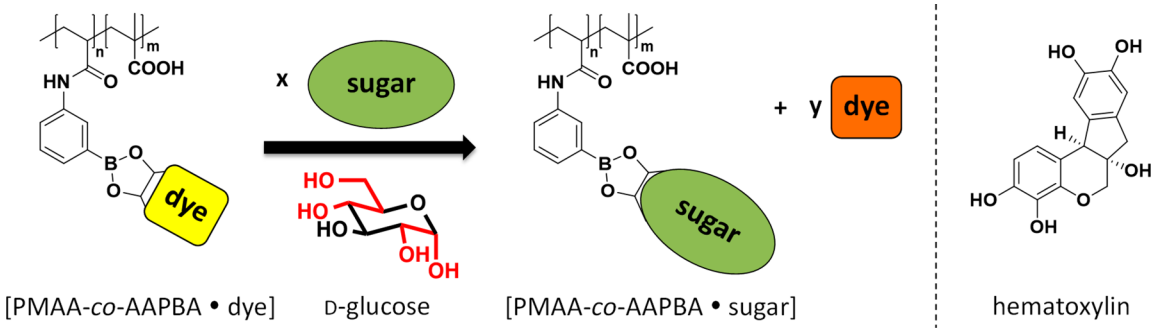
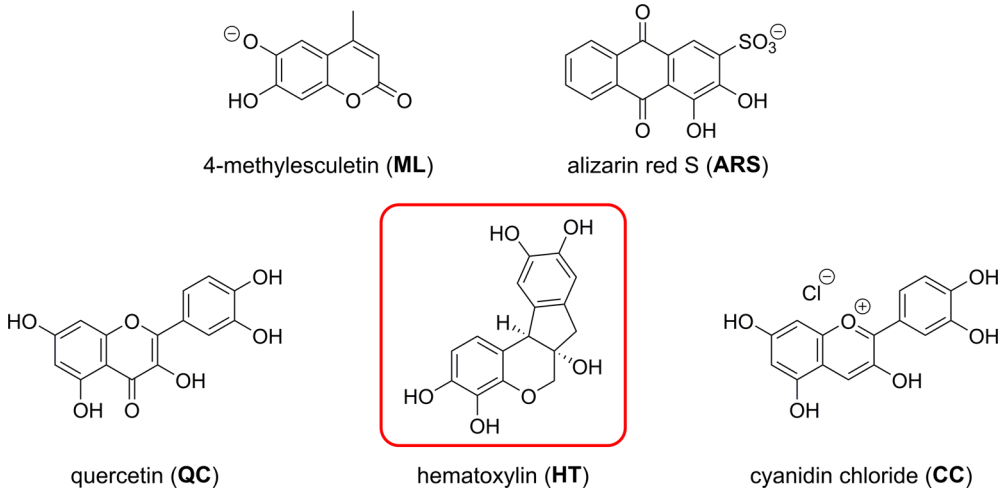


Figure 1. (left) After the addition of a sugar to the [PMAA-*co*-AAPBA·dye] complex, the sugar displaces the dye. The characteristic signal of the free dye is then detected by UV/vis absorbance and fluorescence emission, thereby indicating the complexation of the sugar molecule. (right) Chemical structure of hematoxylin dye (HT) in its reduced form.

Scheme 3. Structures of 4-Methylesculetin (ML), Alizarin Red S (ARS), Quercetin (QC), Hematoxylin (HT), and Cyanidin Chloride (CC) Dyes



Float-A-Lyzer with a MWCO of 10 000 Da and lyophilized. The composition of the *t*BMA-*co*-AAPBA copolymer before (5 mg/mL in $CDCl_3$) and after hydrolysis (10 mg/mL in D_2O) was determined using ^1H NMR recorded on a Bruker 500 MHz NMR spectrometer (see the Supporting Information). The molecular weight of PMAA-*co*-AAPBA was determined using a gel permeation chromatograph (GPC, Waters) calibrated using linear polystyrene standards (the weight-average molecular weight of PMAA-*co*-AAPBA-6 was 40 500 g/mol, $Đ$ = 1.2). Infrared spectroscopy performed on a Bruker (Alpha) attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrometer indicated that both components (methacrylic acid and phenylboronic acid moieties) were present in the copolymer (see the Supporting Information).

Titration Conditions and Multivariate Analysis. See the Supporting Information.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Polymeric Receptors. Inspired also by previous work on polymeric glucose receptors,[46] we built a multivalent boronic acid-modified copolymer containing a controlled percentage of boronic acid moieties with excellent water solubility across a wide pH range due to the addition of PMAA hydrophilic segments (Scheme 2).[47,48] PMAA was chosen as the polymer component because it affords excellent hydrophilic properties to enhance the boronic acids' poor solubility in water.[49] We were able to directly control the molecular weight and composition of our PMAA-*co*-AAPBA copolymer by varying the ratio of monomers during synthesis. In particular, we found that the copolymer with 6% of the molar ratio of 3-(acrylamido)phenylboronic acid units retained water solubility over a very wide range of pH, so we used this material as a water-soluble polymeric receptor to detect and differentiate sugars.

Constructing a Sensing System Based on PMAA-*co*-AAPBA. Optical measurements can be performed rapidly, with inexpensive and widely available high-throughput instrumentation (e.g., microwell plate readers), while maintaining high specificity and good sensitivity. Therefore, we used an indicator displacement assay (IDA) approach (Figure 1) to turn the PMAA-*co*-AAPBA copolymer into a sugar sensor,[50,51] so this material would be able to report on the presence and identity of small carbohydrates through macroscopic changes in the optical properties (e.g., absorbance and fluorescence emission) of its solutions.

The first step was to choose a suitable dye to bind to the PMAA-*co*-AAPBA polymer and form [polymer·dye] complexes that would serve as sensing units in our IDA approach. The dyes considered for this study are shown in Scheme 3. Our previous results showed that 4-methylesculetin (ML) and alizarin red S (ARS) bind to boronic acid moieties grafted onto the surface of cationic poly(amidoamine) (PAMAM) dendrimers in neutral aqueous buffer.[15] In those conditions, binding of the dye to the modified boronate polymer caused significant changes in the absorbance and emission properties

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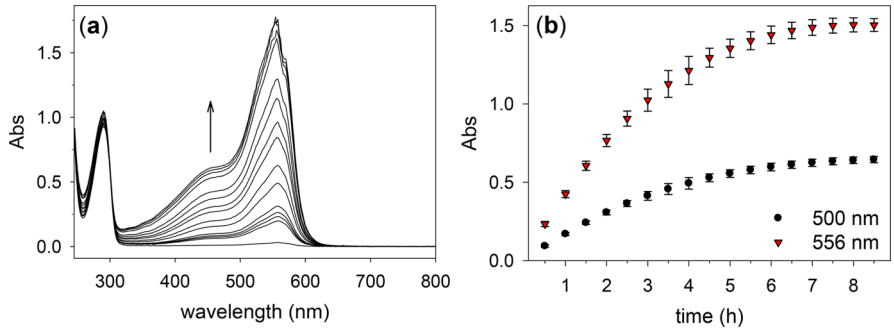


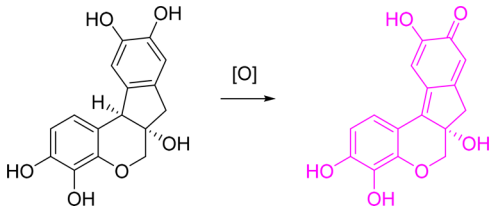
Figure 2. Time evolution of the oxidation of hematoxylin (HT) by atmospheric O_2 in buffered water (50 mM HEPES buffer) at pH 7.4 and room temperature: (a) family of absorbance spectra recorded as a function of time; (b) time evolution profile of the absorption at 556 nm.

of these dyes. However, when ML or ARS was added to PMAA-*co*-AAPBA at pH 7.4 in aqueous buffer, no optical signal changes could be observed, indicating poor binding between these dyes and PMAA-*co*-AAPBA. This was likely caused by charge repulsion between these anionic dyes and the similarly anionic PMAA-*co*-AAPBA, which could not be overcome by diol binding at the boronic acid moieties. To bind efficiently to the anionic PMAA-*co*-AAPBA, a neutral or positively charged dye was required.

We tested two neutral dyes, hematoxylin (HT) and quercetin (QC), and one positively charged dye, cyanidin chloride (CC), for their binding to PMAA-*co*-AAPBA. As the copolymer was added to HT or CC, both dyes were shown to bind to PMAA-*co*-AAPBA efficiently (see below for HT and the Supporting Information, Figure S8, for CC), but QC did not (see Figure S9), so the latter dye was not considered further. Furthermore, later studies showed that although CC could bind to PMAA-*co*-AAPBA with high affinity, nevertheless the [PMAA-*co*-AAPBA·CC] complex made no significant contribution to sugar differentiation (see Table S2). Therefore, we focus here on hematoxylin (HT).

Stabilization of Hematoxylin Solutions. When ML and ARS dyes interact with boronic acid moieties, significant changes are observed in their absorbance spectrum, and their fluorescence emission is enhanced, so we expected to see similar changes as the HT dye bound to the boronic acid groups on PMAA-*co*-AAPBA. However, when HT was dissolved in pH 7.4 buffered water, the absorption of this solution changed slowly over time due to oxidation of the dye by atmospheric oxygen (a representative time evolution profile for this process is shown in Figure 2). Indeed, HT is known to be easily oxidized to hematein in aqueous solution (Scheme 4).[52,53] In this process, the absorption at 556 nm increases from close to 0 to 1.8 au within 90 min (Figure 2), making it nearly impossible to monitor the binding of HT to PMAA-*co*-AAPBA in these conditions.

Scheme 4. Hematoxylin (HT, Left) Is Easily Oxidized to Hematein in Air (Right)



The oxidation of hematoxylin to hematein is well-known (hematein is a common cell-staining dye); in fact, the hematein product is a much stronger chromophore than HT itself. Because the oxidized hematein form still retains one 1,2-*cis*-diol moiety and can react with boronic acids, we realized that using the oxidized form could improve our signal/noise ratio and increase the sensitivity of our measurements.

However, solutions of hematoxylin are known to "ripen" on standing through continuous slow oxidation. To achieve repeatable results in our studies, we needed to control this oxidation process, so we developed a protocol for controlled oxidation of hematoxylin to hematein. Once acceptable color had developed on standing, we stopped the oxidation process by adding a suitable antioxidant to the solution. We selected ascorbate for this for two reasons: on the one hand, it acts as an effective and colorless antioxidant in neutral aqueous solution; additionally, it contains a vicinal diol functionality as well, so it itself may be able to interact with the boronate polymer and perturb the dye−polymer−carbohydrate binding system, thereby causing further differential behavior, useful for analytical differentiation. We found conditions to oxidize hematoxylin in air until the absorption of the solution reached an optimal level for our experiments (between 0.3 and 0.5 au) and then added 2 equiv of ascorbate to prevent further oxidation and stabilize the solution's optical properties. Partially oxidized HT solutions at different concentrations could all be stabilized for up to 10 h (see Scheme S1).

Figure S6 shows that more concentrated HT solutions were quicker to develop optimal color (between 0.3 and 0.5 au), reducing preparation time. On the other hand, however, too high a concentration of oxidized HT may reduce the sensitivity of PMAA-*co*-AAPBA toward sugars because the excess HT would compete effectively with sugars for the boronic acid binding sites and only allow their binding at high sugar concentration. To balance these two factors, we settled on a 0.1 mM dye concentration and a preoxidation time of 30 min to prepare the oxidized HT solution used in this study. From here on, we will refer to this preoxidized and stabilized preparations simply as HT. These partially oxidized dye preparations proved remarkably stable over multiple hours and very repeatable: controlling incubation time and temperature, we could routinely reproduce the same starting conditions. Time evolution spectra demonstrating this stability are included in the Supporting Information (Figure S7). Only ~12% of the total dye is in its oxidized, highly colored hematein form in our working solution, the rest of the hematoxylin being in its reduced form (see the Supporting Information for calculations).

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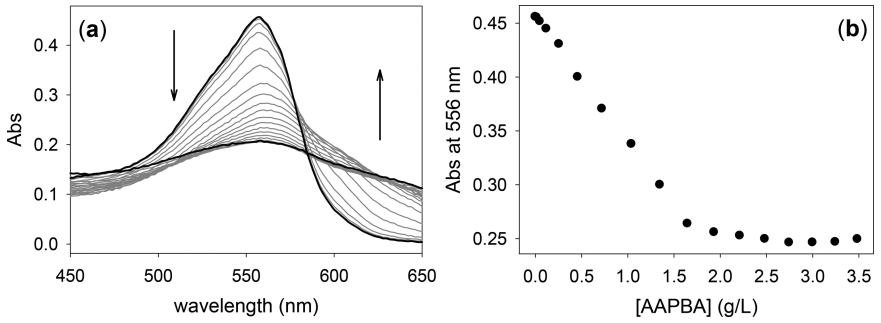

Figure 3. Binding titration of with PMAA-*co*-AAPBA into a solution of preoxidized, stabilized HT dye. T = 25 °C; pH = 7.4 in buffered water (50 mM HEPES); [HT] = 0.10 mM; [ascorbate] = 0.20 mM. (a) Family of absorbance spectra recorded as a function of copolymer concentration. (b) Titration isotherm at 556 nm.

Sugar Binding Studies. As aliquots of PMAA-*co*-AAPBA were added to a stabilized HT solution, absorption at 556 nm decreased (Figure 3) to a plateau, at which point dye binding was complete.

Neither the sugars of interest nor the PMAA-*co*-AAPBA copolymers have any color or fluorescence, so sugar binding would be optically silent, and it could not be monitored using optical spectroscopy. We therefore combined the PMAA-*co*-AAPBA (receptor) with the HT dye (indicator) to build an indicator displacement assay (IDA) capable of reporting on the binding of sugars to this copolymer through a change in optical signal.

Our past experience indicates that an optimal balance between sensitivity and responsiveness of an IDA assay can be achieved when ~80% of the dye is bound to its receptor.[54,55] Appropriate conditions were identified on the basis of the HT binding data obtained above (Figure 3). D-(−)-Ribose was studied first: upon addition of aliquots of a ribose solution to an aqueous solution containing [PMAA-*co*-AAPBA·HT] at pH 7.4, the absorption at 560 nm gradually increased (Figure 4).

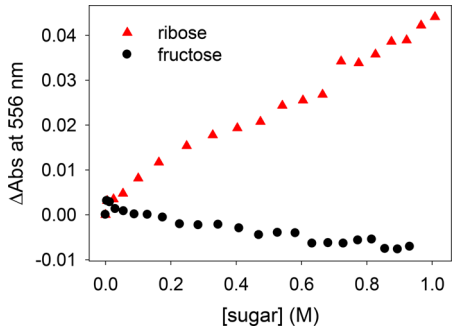

Figure 4. Changes in absorption associated with binding experiments with D-ribose (triangles) and D-fructose (squares) using [PMAA-*co*-AAPBA·HT]. T = 25 °C; pH = 7.4 in water (50 mM HEPES); [HT] = 0.10 mM; [ascorbate] = 0.20 mM; [PMAA-*co*-AAPBA] = 1.50 g/L.

This trend is the opposite of that observed upon dye binding (Figure 3): the dye is being displaced from its complex with PMAA-*co*-AAPBA by ribose, indicating that the sugar is binding to the polymer.

Moving from ribose to D-fructose, we were surprised to find that in this case the absorption at 560 nm decreased as the sugar was added to [PMAA-*co*-AAPBA·HT] (Figure 4), in contrast with the ribose results shown above. This indicated that fructose was not simply displacing HT dyes from the [PMAA-*co*-AAPBA·HT] complex. Instead, a more complex three-component interaction between the sugar, the HT dye, and the PMAA-*co*-AAPBA receptor is more likely, whose nature has not been fully elucidated.

The different nature of the interaction notwithstanding, this still represents an acceptable analytical signal: in fact, the [PMAA-*co*-AAPBA·HT] complex responds to the presence of carbohydrates through an easily measurable change in its optical properties. Furthermore, significant differences in the behavior of these sugars (Figure 4) provide a valuable differential response that can be harnessed to accomplish analytical sugar discrimination.

Differentiation of Sugars through Multivariable Detection. On the basis of the results presented above, we set out to construct a multivariable detection system for simple sugars using the PMAA-*co*-AAPBA copolymer as chemical receptor. We first attempted the discrimination of the simple carbohydrates shown in Scheme 1. Our first sensing array included all dyes showing a significant interaction with the PMAA-*co*-AAPBA polymer, resulting in the inclusion of the following sensing species: oxidized and stabilized HT dye, cyanidin chloride (CC) dye, [PMAA-*co*-AAPBA·HT] complex, and [PMAA-*co*-AAPBA·CC] complex. We collected absorption and fluorescence intensity of solutions containing these sensors upon interaction with each simple carbohydrates from Scheme 1. Ten replicate samples were measured for each analyte. A total of 44 instrumental variables (i.e., absorbance and fluorescence emission at varying wavelengths for each sensor) were acquired for each replicate. Samples were laid out on black polystyrene 384 well plates with transparent bottom, together with relevant blanks and standards. Absorbance and fluorescence emission measurements were then acquired on a BioTek Synergy II microwell plate reader. Further experimental details are described in the Supporting Information and in our previous work.[15]

We analyzed the raw multidimensional data obtained from the optical measurements using linear discriminant analysis (LDA) to identify linear combinations of those instrumental measurements that produced the highest discrimination among different analytes.[56] For each sample, we retained LDA scores along the first two factors, so sample properties could be summarized by two numerical values; each sample was then plotted using these values as coordinates on a scatter plot (the LDA score plot).

The result of LDA processing and reduction for the data obtained from the sensing array described above are shown in the Supporting Information (LDA score plot: Figure S3; LDA loadings: Table S2). Perusal of the LDA loadings from this analysis, which indicate which among the sensors in the array

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are most important for differentiation, led us to conclude that the contribution of species containing the CC dye was almost negligible and could be ignored, thereby significantly simplifying the sensing array and attendant data collection. We therefore reran the sensing experiment after eliminating the CC dye and its complex from the array. This reduced the system to a single sensors, the [PMAA-*co*-AAPBA·HT] complex. We used the same analytes as in the previous attempt. The result of LDA processing and reduction on this data are shown in Figure 5. We were glad to still find a large

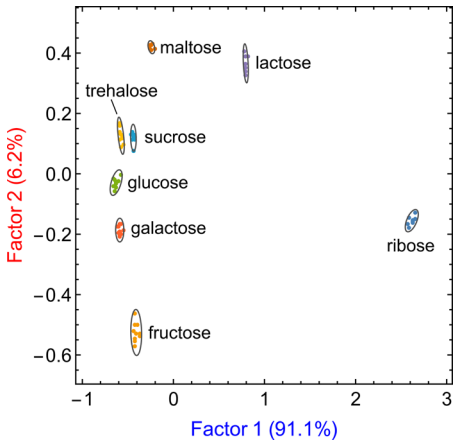

Figure 5. LDA score plot of the data resulting from the interaction between a [PMAA-*co*-AAPBA·HT] complex and eight among mono- and disaccharides in neutral buffered water (50 mM HEPES at pH 7.4). Scores for each sample along the first two LDA factors are plotted here. For each factor, the fraction of the total information explained by that factor is also indicated, as a percentage in each axis label.

separation among these clusters, indicating that the system's high discriminatory power was retained even after removal of the underperforming CC dye. Reducing the data representation to two dimensions for plotting still captured almost all the information available (97.3%), as indicated in the score plot shown in Figure 5.

Structural Features Responsible for Sugar Discrimination. Previous studies[42,44] showed that 1,2-*cis*-diol groups on five-membered rings usually bind to boronic acids more efficiently than those on six-membered rings. Therefore, we expected that fructose and ribose, for which a higher percentage of five-membered furanose form is present in aqueous solution, would bind more strongly to the boronic acid-modified polymer than other sugars that prefer a pyranose conformation in water.[44] Indeed, the LDA plot in Figure 5 confirmed this hypothesis: fructose and ribose were found far from other sugars in the scatter plot. On the other hand, glucose, galactose, maltose, and lactose have 1,2-*cis*-diols in pyranose rings, so they were able to bind to boronic acid moieties on the polymers, but not as efficiently as furanose-based fructose and ribose. Furthermore, disaccharides like sucrose and trehalose, which do not contain vicinal *cis*-diols, were found close to each other on the LDA plot; indeed, sucrose is known not to bind effectively to boronic acid moieties.[44]

We hypothesized that a major factor for differentiation in our system was the structure of the boronate ester resulting upon binding to boronic acid moieties, in accordance with previous results obtained on small-molecule isolated boronic acid ligands.[44] To confirm this hypothesis, we expanded the sample set in our patterning experiment to include binding probes with a single 1,2-*cis*-diol moiety, either on a five-membered ring (i.e., *cis*-1,2-cyclopentanediol and 1,4-anhydroerythritol, its heterocyclic equivalent) or on a six-membered one (*cis*-1,2-cyclohexanediol) (Scheme 1), in addition to the previously considered small sugars. We also added samples of free as well as bound dye (i.e., the [PMAA-*co*-AAPBA·HT] complex) to the set to act as a yardstick by which to measure relative binding affinities of the PMAA-*co*-AAPBA polymer for each analyte through the LDA score plot. In fact, strongly interacting analytes would displace most of the HT dye from the complex, so in their presence the solution would have properties similar to those of the free dye itself; on the other hand, poor binding would result in little to no dye displacement, and the solution would be nearly indistinguishable from the [PMAA-*co*-AAPBA·HT] sensor complex itself. In other words, analytes clustering close to the free dye cluster would be interpreted to display high binding affinity, whereas analyte clusters close to the sensor complex indicate low binding affinity.

We expected samples of the five-membered *cis*-1,2-cyclopentanediol and 1,4-anhydroerythritol to cluster close to fructose and ribose on the resulting expanded LDA score plot because these analytes contain *cis*-diols on five-membered rings and they should bind more strongly to PMAA-*co*-AAPBA. On the other hand, *cis*-1,2-cyclohexanediol should be more like those sugars that prefer to form six-membered pyranose rings.

The results of this experiment, after processing with LDA and data reduction, are shown in Figure 6. *cis*-1,2-Cyclo-

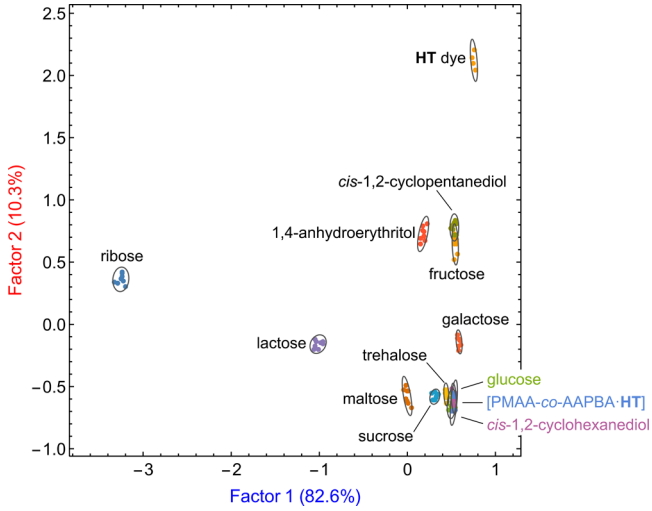

Figure 6. LDA score plot of the data resulting from the interaction between a [PMAA-*co*-AAPBA·HT] complex and *cis*-1,2-cyclopentanediol, *cis*-1,2-cyclohexanediol, 1,4-anhydroerythritol, and four mono- and four disaccharides. Scores for each sample along the first two LDA factors are plotted here. For each factor, the fraction of the total information explained by that factor is also indicated as a percentage in each axis label.

pentanediol, 1,4-anhydroerythritol, fructose, and ribose were found closer to the free HT dye cluster than other sugars, which confirmed our assumption that these four analytes bind more strongly to boronic acids. Indeed, the *cis*-1,2-cyclopentanediol cluster had significant overlap with the fructose cluster, showing that these two structures established very

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similar interactions with the PMAA-*co*-AAPBA receptor. On the other hand, maltose, sucrose, trehalose, glucose, and *cis*-1,2-cyclohexanediol were found very close or even overlapping with the [PMAA-*co*-AAPBA·HT] cluster (i.e., similar to bound, nondisplaced dye), confirming that these analytes did not bind as well to PMAA-*co*-AAPBA because they either had their *cis*-diol group on a six-membered ring or they did not have any *cis*-diols at all. Finally, lactose and galactose had an intermediate behavior. Although they each have *two* sets of *cis*-diols, these are located on six-membered rings: as a result, these sugars bind better than those with only one *cis*-diol group on a six-membered ring, but still worse than those containing a single *cis*-diols on a *five*-membered ring. In other words, we argue that preorganization beats multivalency in this system: one five-membered binding site, which forms a geometrically more advantageous boronate ester upon binding, bestows higher affinity on the containing structure than two less-advantageous six-membered binding sites. We hypothesize that the energetic cost of organizing the analyte's and polymer's binding sites for multivalent binding may overwhelm the energetic gain from a second, inferior binding interaction stemming from a diol on a six-membered ring. Indeed, literature results indicate that the open-chain, flexible sorbitol (hexane-1,2,3,4,5,6-hexol, $HOCH_2(CHOH)_4CH_2OH$) binds much more strongly than any of the sugars considered here,[45] including fructose and other furanoses, presumably because its conformational flexibility allows its diol moieties to achieve an optimal binding geometry.

Furthermore, sugars containing glucopyranose subunits formed a "glucose supercluster" toward the bottom right corner in the score plot (Figure 6), well separated from fructose and ribose, which are present in water as furanose structures. Finally, it is noteworthy that most glucopyranose-containing carbohydrates were found in the vicinity of the "bound dye" cluster (i.e., the one corresponding to the [PMAA-*co*-AAPBA·HT] sensing complex), particularly as concerns their score along factor 2 in the LDA plot. This indicates lower binding affinity; in fact, their concentration being equal, these sugars bind to boronic acids to a lesser extent, thereby displacing less of the bound dye from its complex with the PMAA-*co*-AAPBA receptor, so the spectroscopic signature of their solutions closely resembles that of the plain receptor−dye sensing complex. In this respect, a guest's score along factor 2 in this analysis appears correlated to the affinity of that guest for the PMAA-*co*-AAPBA receptor. This is further supported by the observation that samples from high affinity guests such as fructose and ribose have similar scores along factor 2 and move comparatively closer to the free dye cluster. In fact, higher affinity is reflected in higher dye displacement, so their solutions are markedly different from the bound dye, instead veering close to the free dye cluster.

CONCLUSIONS

We reported on the synthesis, characterization, and analytical applications of PMAA-*co*-AAPBA, a new sugar-sensing water-soluble polymeric receptor containing boronic acid moieties. Receptor PMAA-*co*-AAPBA dissolved well in neutral water and bound efficiently to simple sugars, whose binding was successfully studied using an indicator displacement assay (IDA) paradigm in which partially oxidized hematoxylin dye acted as the indicator. Its displacement from the [PMAA-*co*-AAPBA·HT] complex by simple carbohydrates brought about a change in optical properties that was used to monitor the progress of these binding events.

Our studies highlighted small but informative differences in the binding behavior of a panel of simple carbohydrates that were reflected in their displacement of HT dye from [PMAA-*co*-AAPBA·HT]. Harnessing these differences through linear discriminant analysis (LDA) allowed us to successfully differentiate eight common sugars, including epimer pairs, differing only by the configuration of a single chiral center (e.g., glucose and galactose).

We also correlated the observed affinity with the chemical structures of the sugar guests by studying model molecules *cis*-1,2-cyclohexanediol (representing a diol on a *six*-membered ring) and *cis*-1,2-cyclopentanediol and 1,4-anhydroerythritol (representing a diol on a *five*-membered ring). We thereby confirmed that boronic acid moieties bind more efficiently to vicinal *cis*-diols on five-membered furanose ring (e.g., furan and ribose), whereas they do not bind efficiently to *cis*-diol on six-membered pyranose ring.

The [PMAA-*co*-AAPBA·HT] system not only provided a novel analytical tool for the discrimination of carbohydrates in neutral aqueous solution but also offered corroborating evidence to support and extend the understanding of the binding of boronic acids to sugars.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsapm.9b00141.

Materials and methods; characterization of copolymer; multivariate data analysis techniques and details; time-dependent spectroscopic data on the spontaneous oxidation of hematoxylin, and on the stability of ascorbate + dye solutions; copolymer binding data for quercetin and cyanidin chloride (PDF)

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Notes

The authors declare no competing financial interest.

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