Boronic Acid Homopolymers as Effective Polycations for Sugar-Responsive Layer-by-Layer Assemblies

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Abstract

Due to their reversible diol-binding ability, phenylboronic acid (BA) derivatives have gained considerable interest for the development of saccharide sensors and drug delivery systems. In particular, BA-containing polymers have been used for the realisation of sugar-responsive layer-by-layer (LbL) films. Herein, a LbL system based on cationic BA-homopolymers (PBA) is presented, and its subsequent sugar-induced disassembly is described. The BA-linear polymers (PBA) were intercalated with poly(vinylsulfonic acid, sodium salt) to form (PBA/PVS)_n film assemblies. Different orientations of the boronic acid substituent (*ortho*-, *meta*- and *para*-) in the PBA polymer were investigated for their influence on the LbL assembly. The rate of disassembly in the presence of glucose or fructose was analyzed by UV-vis spectroscopy. Additionally, the PVS component was replaced by an anionic fluorophore, pyranine (PYR), which allowed the assembly and disassembly to be monitored by fluorescence.

Introduction

Stimuli-responsive layer-by-layer (LbL) films have been widely investigated, in which the disassembly of the multilayer films can be stimulated by numerous triggers, such as pH,¹⁴ ionic strength,⁵ temperature,^{6, 7} light,^{8, 9} mechanical stimulation,^{10, 11} or in the presence of small biomolecules.¹²⁻¹⁶ Typically, these external stimuli weaken the electrostatic interactions between adjacent layers, leading to disassembly of the LbL film.^{14, 17-20} In this context, sugar-responsive phenylboronic acid polymers (PBA) have gained much attention in LbL systems, in particular for the development of insulin releasing structures.²⁰⁻³⁰ Lewis acidic boronic acids (BAs) have the ability to reversibly bind to *cis*-1,2-diol substituents of saccharides, such as glucose, fructose, or galactose, via the formation of cyclic boronate esters.³¹ Under the optimal pH conditions,

typically at values between the pK_a of the BA (~9) and the BA ester (~6), sugar introduction can significantly disrupt interactions in a LbL assembly through competitive binding.¹⁹

Watahiki, *et al.*³² produced multilayer films on glass slides composed of BA dendrimers and poly(vinyl alcohol) (PVA) that could stimulate disassembly in the presence of glucose. The layers were assembled under neutral or basic conditions by covalent interactions forming cyclic boronate ester bonds between the BA dendrimers and the diol groups in PVA. Disassembly of the layers was dependent on the competitive binding to glucose over the diol groups in PVA, where the layers could be completely disassembled within 30 min in the presence of 100 mM glucose in pH 7.4 buffer at 37 °C.³²

Similarly, Suwa, *et al.*³³ fabricated LbL films with a BA dendrimer modified with poly(amidoamine) and intercalated with PVA to form a sugar-responsive film. The film was found to be unstable in weakly acidic solutions of pH 4-6, similar to that of Watahiki, *et al.*,³² but could be assembled in more basic solutions of pH 7-9, through the formation of stable boronate ester bonds. The disassembly of the film was monitored in sugar solutions and could be disassembled by ~20% in the presence of 30 mM glucose, at pH 7.4 or pH 9.0. The same film disassembled by 60% in 10 mM fructose at pH 7.4, and by 90% in 10 mM fructose at pH 9.0.³³

Levy, *et al.*¹⁴ formed LbL assemblies by alternating poly(acrylic acid)phenylacrylamido-BA with the polysaccharide mannan, which were held together by boronate ester bonds between the BA polymer and polysaccharide, on the surface of a gold-coated quartz crystal or fluorescently labelled colloidal CaCO_3 templates.¹⁴ Upon introduction of common monosaccharides, such as glucose, fructose, galactose and mannose, disassembly of the LbL film occurred. Investigation using a quartz crystal microbalance in the case of the quartz crystal, and fluorescence for the templated capsules, showed that the film could be disassembled within 5 min in 25 mM of all

monosaccharides.¹⁴ The layers were assembled in aqueous solutions close to the pK_a of the BA at pH 9-11. Overall, the LbL film was most responsive to fructose, owed to a notably higher binding constant than that of glucose, galactose and mannose.¹⁴ Similarly, the LbL-coated capsules released their content within 15 min in the presence of 10 mM fructose or 100 mM of the other saccharides. Below the critical sugar concentration, the films took hours to disassemble. Since this system was pH sensitive, the film could also be disassembled in solutions of pH 1-8 after less than a minute, in the absence of any saccharides.¹⁴

In all these studies the LbL assembly principle relied on the reversible formation of cyclic boronate esters between two alternating polymeric layers, that could be dissassembled through competititve binding with a secondary diol containing compound: typically, a monosaccharide of interest. Herein we instead employ cationic PBA chains (Figure S1), namely poly[*N*-(*ortho*-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide] (PoBA), poly[*N*-(*meta*-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide] (PmBA) and poly[*N*-(*para*-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide] (PpBA), alternated with anionic poly(vinylsulfonic acid, sodium salt) (PVS) for the formation of LbL films. Subsequent disassembly, in the presence of monosacharrides, results from the formation of an anionic cyclic boronate ester form upon sugar binding and the generation of an induced conformational change around boron. This disrupts electrostatic interactions between the alternating layers, thereby inducing disassembly.

Results and Discussion

The linear BA polymers (PoBA, PmBA and PpBA) were synthesized from the monomers *o*BA, *m*BA and *p*BA, which have been previously reported by us for hydrogel fabrication with applications as non-invasive glucose sensors for diabetes monitoring.³⁴⁻³⁶ The monomers,

composed of cationic BA derivatives, were polymerized by photo-induced radical polymerization, as described in the experimental section. Precipitation of the polymers was completed through the addition of cold diethyl ether and the filtered polymer was collected as a white solid. The polymers were subsequently characterized by ^1H NMR, where successful polymerization was confirmed by the absence of the monomeric vinyl group (~ 6.1 ppm (1H, s, CH_2) and ~ 5.7 ppm (1H, s, CH_2)) in the polymer spectrum.³⁵

The zeta potential of the PBA polymers was investigated at different pH values to determine the average surface charge, thereby quantifying their ability to function as a polycation. Measurement of surface charge proves considerably important for the formation of LbL films which rely on electrostatic interactions, by ensuring comparable zeta potentials between the polycation and polyanion layers, necessary for optimal assembly.³⁷ The zeta potential for PpBA was determined over a pH range of 5.2 to 12.1. At higher pH, the zeta potential of the polymer decreased, due to the increased concentration of the boronate anion. Figure 1 shows the linear trend of the zeta potential for PpBA in aqueous solution with increasing pH. These results indicate that the PBA polymers can function as polycations under physiological pH conditions (pH 7.4), exhibiting a zeta potential of approximately +25 mV. This allowed for efficient assembly with PVS, where the zeta potential for PVS is -36.4 mV.

Figure 1. Zeta potential of PpBA (1 mg mL^{-1}) between pH 5.2 to 12.1 measured by dynamic light scattering (DLS). The trend illustrates a linear dependence of surface charge with pH.

The assembly of the LbL films on quartz substrates was monitored by UV-vis spectroscopy (Figure 2, Figure S2-3). The $(\text{PEI/PVS})_n$ and $(\text{PBA/PVS})_n$ layers were deposited by dip-coating the substrate in alternating solutions containing the polyelectrolytes. Two bilayers of poly(ethyleneimine) and PVS $(\text{PEI/PVS})_2$ polyelectrolytes were initially adsorbed on to the slides to reduce any influence of substrate morphology on the film growth and to enhance the assembly of subsequent bilayers.⁴ The efficiency of LbL assembly for PoBA, PmBA and PpBA polymers was tested in a film of 5 bilayers intercalated with PVS. Two absorbance bands were observed, centered at 230 and 265 nm, respectively, corresponding to π - π^* transitions.³⁸ The bilayer deposition was followed at $\lambda_{\text{max}} = 230 \text{ nm}$.

From Figure S3, the trend in the slopes for the assembly of each film can be related to assembly efficiency and therefore, to the orientation of the BA group in the PBA polymer. The increase in the absorbance band at $\lambda_{\text{max}} = 230 \text{ nm}$ versus the number of bilayers for $(\text{PEI/PVS})_2(\text{PoBA/PVS})_5$ showed a linear trend with a slope of 0.032, while a slope of 0.051 and

0.064 was obtained in the case of (PEI/PVS)₅(P*m*BA/PVS)₅ and (PEI/PVS)₅(P*p*BA/PVS)₅, respectively (Figure S3), for the same number of bilayers. This increase in slope can be attributed to the orientation of the BA group as it is positioned further away from the N⁺ moiety. In the case of the *ortho* derivative, the BA group is next to the N⁺ charge, which would allow for an N⁺-B⁻ interaction above the p*K*_a of the molecule. Although further away, in the case of the *meta* derivative, N⁺-B⁻ interaction is still possible through mediation of solvent molecules.^{39, 40} This intramolecular interaction competes with the electrostatic interactions between the ammonium (in PBA) and sulfonate (in PVS) groups required for LbL assembly with PVS. Consequently, the assembly of the (PEI/PVS)₅(P*p*BA/PVS)₅ film is optimum since the BA group is positioned the furthest away from the N⁺ moiety, where this through-space intramolecular N⁺-B⁻ interaction is not possible, leading to enhanced deposition of the polyions for film assembly. For this reason, the P*p*BA was the polymer chosen for further studies.

A film comprising 15 bilayers of P*p*BA intercalated with PVS was assembled to further check the tendency of layer deposition (Figure 2). The linear increase of absorbance with the number of bilayers indicates that the same amount of material has been deposited in each step evidencing the efficiency of LbL assembly and the right choice parameters (concentration, pH).

Figure 2. UV-vis spectra of the quartz substrate after each bilayer deposition, during the fabrication of $(\text{PEI/PVS})_2(\text{PpBA/PVS})_{15}$ LbL film. The inset shows a plot of the absorbance at $\lambda_{\max}^{\text{abs}} = 230 \text{ nm}$ with increased number of (PpBA/PVS) bilayers. Error bars represent the standard deviation error for $n = 3$ films.

On exposing the LbL films to a solution containing glucose or fructose, a release assay could be performed. At pH 7.4, below the determined $\text{p}K_{\text{a}}$ of the BA derivatives ($\text{p}K_{\text{a}} \sim 8.7$), the conformation of the BA in the deposited PBA layers is predominantly trigonal planar and the boron atom is neutral. On introducing the monosaccharide, sugar binding can result in the formation of an anionic cyclic boronate ester, rendering boron negatively charged. Consequently, the electrostatic interactions between the PBA and PVS can become weakened to initiate disassembly in the LbL film. In order to study sugar-induced disassembly of the $(\text{PEI/PVS})_2(\text{PoBA/PVS})_5$, $(\text{PEI/PVS})_2(\text{PmBA/PVS})_5$ and $(\text{PEI/PVS})_2(\text{PpBA/PVS})_5$ films, the quartz substrates containing 5 bilayers of each film were placed into sugar containing solutions at pH 7.4 at 36 °C with gentle stirring.

When the LbL films were placed in a glucose solution of 100 mM, the absorbance at λ_{max} for the (PEI/PVS)₂(PpBA/PVS)₅ film decreased by 39% in approximately 1h, while for the (PEI/PVS)₂(PoBA/PVS)₅ film a decrease in absorbance by ~13% was recorded after the same amount of time (Figure S4). In both cases, no significant changes in absorbance were recorded after the first hour in 100 mM glucose. The better disassembly of the PpBA film over the PoBA film can be attributed to the orientation of the BA group in the *ortho* versus the *para* position. In the *ortho* position, glucose binding can be limited by steric hindrance of the methyl groups attached to the adjacent N⁺ moiety. The close proximity of the N⁺ moiety also enhances stabilization in the PBA by forming N⁺-B⁻ intramolecular interactions, to neutralize the induced negative charge on boron upon glucose binding. As a result of this enhanced stabilization upon glucose binding, the disassembly process is impeded. In the *para* isopolymer this is not the case. The BA group is positioned opposite the N⁺ moiety, where a through-space N⁺-B⁻ intramolecular interaction is not permitted. Consequently, upon glucose binding to the PpBA polymer there is no stabilization effect and hence, disassembly of the LbL film can occur. Nevertheless, the disassembly in each case is still limited and less successful compared to previously reported studies.^{14, 32, 33}

Instead, an accelerated disassembly profile was observed when the (PEI/PVS)₂(PBA/PVS)₅ LbL films were immersed in a 10 mM fructose solution. In this case, the PoBA, PmBA and PpBA containing LbL films showed a total decrease in absorbance at $\lambda_{\text{max}}^{\text{abs}} = 230 \text{ nm}$ by 65%, 71% and by 84%, respectively, after approximately 12 minutes (Figure 3-4). Comparing these films after 2.5 min exposure time to 10 mM fructose at pH 7.4 the films showed a decrease in absorbance by 58%, 67% and 84%, respectively (Figures 3-4, S5-6). The first order disassembly constant (k) for the fructose-induced disassembly of (PEI/PVS)₂(PBA/PVS)₅ in the case of the

PoBA, PmBA and PpBA containing LbL films was estimated by fitting the values of the absorbance at 230 nm using Microsoft Excel Solver and Equation 1 (Figure 4). The rate constant (k) was found to be 1.64 min^{-1} for the PoBA-containing film, 2.10 min^{-1} for the PmBA-containing film and 2.81 min^{-1} for the PpBA-containing film. This rapid response to fructose over glucose, can be explained by the higher binding constant of fructose with phenylBA, in comparison to other sugars such as glucose.^{32, 41, 42} James, *et al.*⁴³ have reported the binding constant of phenylBAs as 110 M^{-1} with glucose and 4370 M^{-1} with fructose. The furanose ring form of fructose binds with 40 times greater affinity to phenylBAs in contrast to the pyranose form of glucose. Since 99% of glucose exists in the pyranose form and less than 1% of glucose exists in the furanose form,⁴³ this low concentration of the furanose form of glucose accounts for the discrepancies between these binding constants. To monitor the disassembly with fructose more closely, the release assay profile was monitored in 5 s intervals (Figure 4). This figure illustrates that the PpBA film was disassembled in the presence of 10 mM fructose by 27% after 5 s, by 71% after 30 s and by 84% after 150 s.

Figure 3. Absorbance spectra for the disassembly of the $(\text{PEI/PVS})_2(\text{PpBA/PVS})_5$ film in 10 mM fructose (left) and the average absorbance at $\lambda_{\text{max}}^{\text{abs}} = 230 \text{ nm}$ ($n = 3$) over time, illustrating the

disassembly of the LbL film. The points on the curve represent the mean $\pm$ the standard deviation.

Figure 4. Normalized absorbance (A/A_0) at 230 nm following the disassembly of the PBA films; PoBA ($\blacklozenge$), PmBA ($\blacksquare$) and PpBA ($\blacktriangle$) with 10 mM fructose at pH 7.4, where the red square represents the stabilization of the films in buffer for 60 min before the addition of fructose; A_0 is the stable measurement in buffer and A is the measured absorbance after the addition of fructose. The experimental values were fitted using a single exponential model (Equation 1) to determine the disassembly constant (k) for the sugar-induced disassembly.

Previous work has shown that incorporation of drug molecules between adjacent layers can offer a means of producing drug-delivery systems.^{13, 43-47} In this regard, the fluorescent dye pyranine (PYR) was employed as a model for anionic drugs, in an attempt to understand more about the interaction between the PBA polymer and small anionic molecules, and also as a means of monitoring the sugar-induced disassembly process by fluorescence spectroscopy. It has been previously demonstrated that small molecules (*e.g.* dyes) can be used for the fabrication of LbL assemblies in combination with oppositely-charged polyions.⁴⁶

Figure 5 shows the assembly of the $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$ film tracked by UV-vis spectroscopy. The PpBA polymer was used since it showed the optimum results in the previous film assembly and disassembly study. The absorbance bands observed in the LbL film are characteristic for those of the PpBA polymer at 230 and 250 nm and for pyranine at 290, 373, 405 and 465 nm. The inset of Figure 5 confirms the linear deposition of the bilayers onto the quartz slide with a slope of 0.050 at $\lambda_{\text{max}}^{\text{abs}} = 405$ nm, demonstrating an assembly similar to that of the $(\text{PEI/PVS})_2(\text{PpBA/PVS})_5$ film.

Figure 5. UV-vis spectra of the quartz substrate before (in black) and after each bilayer deposition, during the fabrication of the $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$ LbL film. The inset shows a plot of the absorbance at $\lambda_{\text{max}}^{\text{abs}} = 405$ nm with increased number of (PpBA/PYR) bilayers. Error bars represent the standard deviation error for $n = 3$ films.

To determine the performance of the $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$ film in response to saccharides, the film was placed in a fructose solution and monitored in tandem by UV-vis and fluorescence spectroscopy. The $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$ film was initially stabilized in pH 7.4 buffer for 60 min at 36 °C under gentle stirring, which resulted in a slight decrease in the absorbance at $\lambda_{\text{max}}^{\text{abs}} =$

230 nm, attributed to dissolution of some weakly bound material. Subsequent immersion of the film in a 30 mM fructose solution showed a decrease in absorbance by 63% within approximately 4.5 min (Figure 6, black). In comparison with the (PEI/PVS)₂(PpBA/PVS)₃ film which disassembled by 84% in 2.5 min, the disassembly of the fluorescent (PEI/PVS)₂(PpBA/PYR)₃ film occurred to a smaller extent, indicating that the intermolecular interactions within the film were stronger than in the PpBA/PVS case. Similarly, the first order disassembly constant (k) for the fructose-induced disassembly of (PEI/PVS)₂(PpBA/PYR)₃ was found to be 1.05 min⁻¹ (Figure S7), smaller than that obtained for the (PEI/PVS)₂(PpBA/PVS)₃ film under similar experimental conditions. The disassembly of the (PEI/PVS)₂(PpBA/PYR)₃ film was examined by simultaneously monitoring the absorbance of the film and the fluorescence of the solution, following the specific pyranine bands (Figure S8-9). The film was placed in a pH 7.4 phosphate buffer solution at 36 °C under gentle stirring to stabilize the film before fructose was introduced. From Figure S8 the absorbance spectra of the (PEI/PVS)₂(PpBA/PYR)₃ film can be seen, showing the characteristic absorbance bands for pyranine. These reveal a decrease of absorbance intensity at 375 and 410 nm and an increase in the absorbance band at 460 nm, when the dry film is exposed to the 7.4 buffer solution. These absorbance differences can be attributed to changes in hydrogen bonding interactions between the -OH group of pyranine with the aqueous solvent.⁴⁸ After stabilization of the LbL film in the pH buffer, introduction of the film into the fructose solution (30 mM) caused a decrease by 63% (relative to buffer) within 4.5 min in the characteristic absorbance bands of pyranine (Figure 6, black), indicating film disassembly. Simultaneously, the fluorescence of the solution was monitored at 500 nm ($\lambda_{\text{ex}} = 405$ nm) to examine the dissolution of pyranine into the fructose solution due to LbL disassembly (Figure 6, blue). A decrease in the emission band centered at $\lambda_{\text{em}} = 500$ nm increased by 48% after ~4.5 min

in the fructose solution. As shown in Figure 6, the curve representing the decreasing in the absorbance of the film (black) and the curve showing fluorescence increase of the solution (blue) are complementary, indicating that the two processes occur simultaneously.

Figure 6. Graph showing the disassembly of the fluorescent $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$ film in fructose solution (30 mM) at pH 7.4, 36 °C, where the film was first stabilized in PBS buffer for 60 min. The decrease in absorbance of the film was monitored at $\lambda_{\text{max}}^{\text{ex}} = 405$ nm (black points). The fluorescence of the sugar solution simultaneously increases due to dissolution of pyranine, where the excitation wavelength was 405 nm and the fluorescence in solution was tracked at $\lambda_{\text{max}}^{\text{em}} = 500$ nm (blue points). Error bars represent the standard deviation error for $n = 3$ (corresponding to the disassembly of 3 different LbL films).

Conclusions

In summary, two types of LbL films were assembled, based on $(\text{PEI/PVS})_2(\text{PBA/PVS})_5$ and $(\text{PEI/PVS})_2(\text{PpBA/PYR})_5$, that could disassemble in response to monosaccharides. The films

disassembled to a greater extent in the presence of fructose over glucose, where the (PEI/PVS)₂(PpBA/PVS)₂ showed the optimum response to both sugars by disassembling by 39% in glucose (100 mM) and by 93% in fructose (10 mM). In this regard, the PpBA polymer was employed to construct a fluorescent film with the anionic fluorophore pyranine to mimic a drug delivery model. Overall, the PpBA films showed optimum responses and would be recommended for implementation into sugar monitoring or drug delivery systems. Future work could involve the fabrication of these LbL films upon microcapsules or microtubes encapsulated with insulin for sustained drug release in a drug delivery system.

Experimental

Hydrogen peroxide (30 wt% in H₂O), poly(ethyleneimine) (PEI, branched; 50% w/v in water), poly(vinylsulfonic acid, sodium salt) solution (PVS; 25 wt.% in H₂O), 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine; PYR, >97%), phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (PBPO), acetic acid, sodium hydroxide, D-glucose, D-fructose were purchased from Sigma Aldrich, Ireland and used as received. 2-(Bromomethyl)phenylboronic acid, 3-(bromomethyl)phenylboronic acid and 4-(bromomethyl)phenylboronic acid were purchased from Fluorochem, UK and used as received. All fluorescence measurements were recorded using a JASCO FP-8300 spectrofluorometer (JASCO Applied Science, Southampton, UK) at 20 °C and UV-vis absorption measurements were carried out on a Varian Cary 50 Probe spectrophotometer (Agilent, CA, USA), in a precision cell made from Quartz Suprasil that had a path length of 10 mm and a volume of 3 mL. Dynamic light scattering (DLS) measurements for determination of the zeta potentials of PpBA at various pH were measured using a Malvern Zetasizer Nano ZS (Malvern Panalytical, Almelo, the Netherlands) with a specialized Malvern cell, DTS1070 folded capillary cell. Solutions of

PpBA in pH 5.4-12.1 were measured at a concentration of 1 mg mL⁻¹. All pH measurements were carried out using a VWR SympHony SP70P pH meter (VWR, PA, USA). Deionized water (18.2 MΩ cm⁻¹) was purified using a Merck Millipore Milli-Q Water Purification System (Merck Millipore, Darmstadt, Germany). The phosphate buffer solution at pH 7.4 was prepared from 0.1 M potassium dihydrogen phosphate (KH₂PO₄; 100 mL) and 0.1 M sodium hydroxide (NaOH; 78 mL) salts and was made up to 200 mL using deionized water.

The BA monomers were synthesized according to a literature procedure.^{34, 35} Briefly, a one-step synthesis was used to produce a series of polymerisable BA monomers. 2-(Dimethylamino)ethyl methacrylate underwent a quaternization reaction with a bromomethyl substituted phenylboronic acid to form the BA-ammonium bromide monomeric salts, namely *N*-(2-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide (*oBA*), *N*-(3-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide (*mBA*) and *N*-(4-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide (*pBA*), where the boronic acid is positioned in the *ortho*-, *meta*-, or *para*-position to the ammonium chain, respectively. Upon quaternization of the nitrogen atom, the BA monomers are rendered water-soluble, which is an advantage for subsequent polymerization strategies.

The BA monomers were polymerized by a radical polymerization procedure. The BA monomer (260 mg; 0.7 mmol) was dissolved in DMSO (500 μL) in a 1 mL glass vial. The photoinitiator PBPO (2 mg; 0.005 mmol) dissolved in THF (100 μL) was added last. The vial was placed under high intensity white light irradiation for 1h to polymerize the monomer. Cold diethyl ether was added to the polymerized mixture to precipitate the polymer. The polymer was filtered and washed with diethyl ether and collected as a white solid.

The LbL films were assembled on to quartz slides (40 x 10 x 1 mm). The slide was primed using $\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{H}_2\text{O}$ (1:1:5, v:v:v) and $\text{HCl}/\text{H}_2\text{O}_2/\text{H}_2\text{O}$ (1:1:6, v:v:v) hydrophilization solutions at 75 °C for 10 min and washed with deionized water. PEI and PVS solutions were prepared at concentrations of 1 mg mL⁻¹ and 4 μL mL⁻¹, respectively. The PYR solution was prepared at 1 mM. The PBAs were dissolved in DI H₂O at a concentration of 1 mg mL⁻¹ and the pH was altered to pH 7.4 using acetic acid and sodium hydroxide and measured using a SympHony pH meter. All LbL films were assembled with 2 initial (PEI/PVS)₂ bilayers to minimize any interference with the quartz or glass substrate,⁴ in a process as follows: (1) the substrate was immerse in PEI (1 mg mL⁻¹) for 3 min, (2) in deionized water for 30 s, (3) in PVS (4 μL mL⁻¹) for 3 min and (4) in deionized water for 30 s. Subsequently, the substrate was immersed into the respective PBA and PVS polymer solutions for 3 min to deposit the linear chains. The polymer solutions during LbL assembly for all films were kept at room temperature. A washing step using deionized water was performed for approximately 30 s to avoid contamination between the polyelectrolyte solutions. This cyclic process was repeated until all desired 5 or 15 bilayers were afforded. A UV-vis spectrum of the slide after air drying was recorded after each bilayer deposition.

To disassemble the LbL films, the slide was simply dipped in the pH 7.4 buffer solution at 36 °C with gentle stirring. This equilibrated and stabilized the layers in the LbL film by washing away any weakly bound material. Once stabilized, the slide was placed in glucose (up to 100 mM) or fructose (up to 30 mM) solutions at pH 7.4 at 36 °C under gentle stirring for approximately 5 min. The slide was air dried and this disassembly process was monitored at specific time intervals by UV-Vis spectroscopy or fluorescence spectroscopy. A single exponential model (Equation 1) was used to determine the disassembly constant for the sugar-induced disassembly of (PEI/PVS)₂(PBA/PVS)₅ and (PEI/PVS)₂(PpBA/PYR)₅ LbL films,

$$y = a * e^{-kt} + b \quad (1)$$

where y is the absorbance at 230 nm ((PEI/PVS)₃(PBA/PVS)₃) or 405 nm ((PEI/PVS)₃(PpBA/PYR)₃), respectively, a is the scaling factor, k is the first order disassembly rate constant (min⁻¹), b is the baseline offset, and t is time (min).

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge. UV-Vis and fluorescence data for the LbL assembly and disassembly studies (PDF).

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ABBREVIATIONS

BA, phenylboronic acid/bornic acid; LbL, layer-by-layer; PBA, BA-homopolymers/BA-linear polymers/phenylbornic acid polymers; PVS, poly(vinylsulfonic acid, sodium salt); *o*-, *ortho*; *m*-, *meta*; *p*-, *para*; PYR, pyranine; PVA, poly(vinyl alcohol); PoBA, poly[*N*-(*ortho*-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide]; PmBA, poly[*N*-(*meta*-

boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide]; PpBA, poly[*N*-(*para*-boronobenzyl)-2-(methacryloyloxy)-*N,N*-dimethylethane-1-ammonium bromide]; NMR, nuclear magnetic resonance; *s*, singlet; PEI, poly(ethyleneimine); *A*, absorbance; *A*₀, initial absorbance; *k*, disassembly constant.

REFERENCES

1. Sukhishvili, S. A; Granick, S. Layered, Erasable Polymer Multilayers Formed by Hydrogen-Bonded Sequential Self-Assembly, *Macromolecules* **2002**, *35*, 301-310.
2. Shiratori, S. S.; Rubner, M. F. pH-Dependent Thickness Behavior of Sequentially Adsorbed Layers of Weak Polyelectrolytes, *Macromolecules* **2000**, *33*, 4213-4219.
3. Yoo, D.; Shiratori, S. S.; Rubner, M. F. Controlling Bilayer Composition and Surface Wettability of Sequentially Adsorbed Multilayers of Weak Polyelectrolytes, *Macromolecules* **1998**, *31*, 4309-4318.
4. Campos, P. P.; Fernandes Fraceto, L.; Ferreira, M. Layer-by-Layer Films Containing Emodin or Emodin Encapsulated in Liposomes for Transdermal Applications, *Colloids Surf., B* **2018**, *162*, 69-75.
5. Schuler, C.; Caruso, F. Decomposable Hollow Biopolymer-Based Capsules, *Biomacromolecules* **2001**, *2*, 921-926.
6. Lu, C. H.; Wei, F.; Wu, N. Z.; Zhao, X. S.; Luo, C. Q.; Cao, W. X. Micropatterned Self-Assembled Film Based on Temperature-Responsive Poly(*N*-isopropylacrylamide-co-acrylic acid), *J. Colloid Interface Sci.* **2004**, *277*, 172-175.

7. Sun, Q. Q.; Deng, Y. L. Encapsulation of Polystyrene Latex with Temperature-Responsive Poly(N-isopropylacrylamide) via a Self-Assembling Approach and the Adsorption Behaviors Therein, *Langmuir* **2005**, *21*, 5812-5816.
8. Tao, X.; Li, J. B.; Mohwald, H. Self-Assembly, Optical Behavior, and Permeability of a Novel Capsule Based on an Azo Dye and Polyelectrolytes, *Chem.–Eur. J.* **2004**, *10*, 3397-3403.
9. Campos, P. P.; Dunne, A.; Delaney, C.; Moloney, C.; Moulton, S. E.; Benito-Lopez, F.; Ferreira, M.; Diamond, D.; Florea, L. Photoswitchable Layer-by-Layer Coatings Based on Photochromic Polynorbornenes Bearing Spiropyran Side Groups, *Langmuir* **2018**, *34*, 4210–4216.
10. Gao, C.; Donath, E.; Moya, S.; Dudnik, V.; Mohwald, H. Elasticity of Hollow Polyelectrolyte Capsules Prepared by the Layer-by-Layer Technique, *Eur. Phys. J., E* **2001**, *5*, 21-27.
11. Gao, C. Y.; Leporatti, S.; Moya, S.; Donath, E.; Mohwald, H. Stability and Mechanical Properties of Polyelectrolyte Capsules Obtained by Stepwise Assembly of Poly(styrenesulfonate sodium salt) and Poly(diallyldimethyl ammonium) Chloride onto Melamine Resin Particles, *Langmuir* **2001**, *17*, 3491.
12. Sato, K.; Imoto, Y.; Sugama, J.; Seki, S.; Inoue, H.; Odagiri, T.; Hoshi, T.; Anzai, J. Sugar-Induced Disintegration of Layer-by-Layer Assemblies Composed of Concanavalin A and Glycogen, *Langmuir* **2005**, *21*, 797-799.
13. Inoue, H.; Sato, K.; Anzai, J. Disintegration of Layer-by-Layer Assemblies Composed of 2-Iminobiotin-Labeled Poly(ethyleneimine) and Avidin, *Biomacromolecules* **2005**, *6*, 27-29.

14. Levy, T.; Déjugnat, C.; Sukhorukov, G. B. Polymer Microcapsules with Carbohydrate-Sensitive Properties, *Adv. Funct. Mater.* **2008**, *18*, 1586-1594.
15. Joseph, V. S.; Hong, J.-D. Phenylboronic Acid-Modified Oligoamine Sensitive to Monosaccharides and Carbon Dioxide under Physiological Conditions, *Colloids Surf. Physicochem. Eng. Asp.* **2018**, *553*, 312–316.
16. Campos, P. P.; Moraes, M. L.; Volpati, D.; Miranda, P. B.; Oliveira Jr., O. N.; Ferreira, M. Amperometric Detection of Lactose Using β -Galactosidase Immobilized in Layer-by-Layer Films, *ACS Appl. Mater. Interfaces* **2014**, *6*, 11657–11664.
17. Choi, D.; Hong, J. Layer-by-Layer Assembly of Multilayer Films for Controlled Drug Release, *Arch. Pharm. Res.* **2014**, *37*, 79-87.
18. Guan, Y.; Zhang, Y. Dynamically Bonded Layer-by-Layer Films: Dynamic Properties and Applications, *J. Appl. Polym. Sci.* **2014**, *131*, 40918-40929.
19. Wang, B.; Yoshida, K.; Sato, K.; Anzai, J. Phenylboronic Acid-Functionalized Layer-by-Layer Assemblies for Biomedical Applications, *Polymers* **2017**, *9*, 202-218.
20. De Geest, B. G.; Sanders, N. N.; Sukhorukov, G. B.; Demeestera, J.; De Smedt, S. C. Release Mechanisms for Polyelectrolyte Capsules, *Chem. Soc. Rev.* **2007**, *36*, 636-649.
21. Kost, J.; Wolfrum, J.; Langer, R. Magnetically Enhanced Insulin Release in Diabetic Rats, *J. Biomed. Mater. Res.* **1987**, *21*, 1367-1373.
22. Edelman, E. R.; Kost, J.; Bobeck, H.; Langer, R. Regulation of Drug Release from Polymer Matrices by Oscillating Magnetic Fields, *J. Biomed. Mater. Res.* **1985**, *19*, 67-83.

23. Saslawski, O.; Weingarten, C.; Benoit, J. P.; Couvreur, P. Magnetically Responsive Microspheres for the Pulsed Delivery of Insulin, *Life Sci.* **1988**, *42*, 1521-1528.
24. Zhu, Y.; Zheng, L. Y. Preparation and Characterization of Glucose-Sensitive Hydrogel Submicron Particles Using Inverse Microemulsions, *J. Drug Delivery Sci. Technol.* **2006**, *16*, 55-58.
25. Wu, J.-Z.; Williams, G. R.; Li, H. Y.; Wang, D. X.; Li, S. D.; Zhu, L. M. Insulin-Loaded PLGA Microspheres for Glucose-Responsive Release, *Drug Deliv.* **2017**, *24*, 1513–1525.
26. Zhang, X.; Guan, Y.; Zhang, Y. Dynamically Bonded Layer-by-Layer Films for Self-Regulated Insulin Release, *J. Mater. Chem.* **2012**, *22*, 16299-16305.
27. Qi, W.; Yan, X.; Fei, J.; Wang, A.; Cui, Y.; Li, J. Triggered Release of Insulin from Glucose-Sensitive Enzyme Multilayer Shells, *Biomaterials* **2009**, *30*, 2799-2806.
28. Qi, W.; Duan, L.; Li, J. Fabrication of Glucose-Sensitive Protein Microcapsules and Their Applications, *Soft Matter* **2011**, *7*, 1571-1576.
29. Chen, X.; Luo, J.; Wu, W.; Tan, H.; Xu, F.; Li, J. The Influence of Arrangement Sequence on the Glucose-Responsive Controlled Release Profiles of Insulin-Incorporated LbL Films, *Acta Biomater.* **2012**, *8*, 4380-4388.
30. Ding, Z.; Guan, Y.; Zhang, Y.; Zhuh, X. X. Layer-by-Layer Multilayer Films Linked with Reversible Boronate Ester Bonds with Glucose-Sensitivity Under Physiological Conditions, *Soft Matter* **2009**, *5*, 2302-2309.

31. James, T. D.; Phillips, M. D.; Shinkai, S. Boronic Acids in Saccharide Recognition, RSC Publishing, Ed. J. F. Stoddart, 2006, pp. 3-176.
32. Watahiki, R.; Sato, K.; Suwa, K.; Niina, S.; Egawa, Y.; Seki, T.; Anzai, J. Multilayer Films Composed of Phenylboronic Acid Modified Dendrimers Sensitive to Glucose Under Physiological Conditions, *J. Mater. Chem., B* **2014**, 2, 5809-5817.
33. Suwa, K.; Nagasaka, M.; Niina, S.; Egawa, Y.; Seki, T.; Anzai, J-I. Sugar Response of Layer-by-Layer Films Composed of Poly(vinyl alcohol) and Poly(amidoamine) Dendrimer Bearing 4-Carboxyphenylboronic Acid, *Colloid Polym. Sci.* **2015**, 293, 1043–1048.
34. Delaney, C.; Florea, L.; Bruen, D.; Diamond, D. Boronic Acid Derivatives for Sugar-Sensing Hydrogels, GB Patent, GB1805226.6, 29 March 2018.
35. Bruen, D.; Delaney, C.; Diamond, D.; Florea, L. Fluorescent Probes for Sugar Detection, *ACS Appl. Mater. Interfaces* **2018**, 10, 38431-38437.
36. Bruen, D.; Delaney, C.; Florea, L.; Diamond, D. Glucose Sensing for Diabetes Monitoring: Recent Developments, *Sensors* **2017**, 17, 1866-1887.
37. 36. Decher, G. Fuzzy Nanoassemblies: Towards Layered Polymeric Multicomposites, *Science* **1997**, 277, 1232-1237.
38. Karabacak, M.; Kose, E.; Atac, A.; Asiri, A. M.; Kurt, M. Monomeric and Dimeric Structures Analysis and Spectroscopic Characterization of 3,5-Difluorophenylboronic Acid with Experimental (FT-IR, FT-Raman, ¹H and ¹³C NMR, UV) Techniques and Quantum Chemical Calculations, *J. Mol. Struct.* **2014**, 1058, 79–96.

39. Sun, X.; James, T. D. Glucose Sensing in Supramolecular Chemistry, *Chem. Rev.* **2015**, *115*, 8001-8037.
40. Hansen, J. S.; Christensen, J. B.; Petersen, J. F.; Hoeg-Jensen, T.; Norrild, J. C. Arylboronic Acids: A Diabetic Eye on Glucose Sensing, *Sens. Actuators, B* **2012**, *161*, 45-79.
41. Egawa, Y.; Gotoh, R.; Seki, T.; Anzai, J. Sugar Response of Boronic Acid-Substituted Azobenzene Dye-Modified Polymer, *Mater. Sci. Eng. C* **2009**, *29*, 115–118.
42. Camara, J. N.; Suri, J. T.; Cappuccio, F. E.; Wessling, R. A.; Singaram, B. Boronic Acid Substituted Viologen Based Optical Sugar Sensors: Modulated Quenching with Viologen as a Method for Monosaccharide Detection, *Tetrahedron Lett.* **2002**, *43*, 1139–1141.
43. Wu, X.; Li, Z.; Chen, X.-X.; Fossey, J. S.; James, T. D.; Yun-Bao Jiang, Y.-B. Selective Sensing of Saccharides Using Simple Boronic Acids and Their Aggregates, *Chem. Soc. Rev.* **2013**, *42*, 8032-8048.
44. Zhuk, I.; Jariwala, F.; Attygalle, A. B.; Wu, Y.; Libera, M. R.; Sukhishvili, S. A. Self-Defensive Layer-by-Layer Films with Bacteria-Triggered Antibiotic Release, *ACS Nano* **2014**, *8*, 7733-7745.
45. Schmidt, D. J.; Moskowitz, J. S.; Hammond, P. T. Electrically Triggered Release of a Small Molecule Drug from a Polyelectrolyte Multilayer Coating, *Chem. Mater.* **2010**, *22*, 6416-6425.
46. Ariga, K.; Lvov, Y.; Kunitake, T. Assembling Alternate Dye-Polyion Molecular Films by Electrostatic Layer-by-Layer Adsorption, *J. Am. Chem. Soc.* **1997**, *119*, 2224-2231.

47. Costa, R. R.; Alatorre-Meda, M.; Mano, J. F. Drug Nano-Reservoirs Synthesized Using Layer-by-Layer Technologies, *Biotechnol. Adv.* **2015**, *33*, 1310-1326.
48. Heo, W.; Uddin, N.; Park, J. W.; Rhee, Y. M.; Choi, C. H.; Joo, T. Coherent Intermolecular Proton Transfer in the Acid-Base Reaction of Excited State Pyranine, *Phys. Chem. Chem. Phys.* **2017**, *19*, 18243-18251.

A series of linear cationic boronic acid homopolymers are synthesized and assembled into layer-by-layer films. Their response to common saccharides, such as glucose and fructose is probed in solution to induce disassembly in the films. Fluorescent films using the anionic fluorophore pyranine were also assembled to mimic a drug delivery model. Facile polymer synthesis, combined with UV-vis and fluorescence analysis detailing comprehensive solution studies, has allowed for the generation of layer-by-layer films with a tailored saccharide response.