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Protein complexation and pH dependent release using boronic acid containing PEG-polypeptide copolymers^a

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Abstract

New poly(L-lysine)-*b*-poly(ethylene glycol) copolypeptides have been prepared, where the side-chain amine groups of lysine residues are modified to contain ortho-amine substituted phenylboronic acid, i.e. Wulff-type phenylboronic acid (WBA), groups to improve their pH responsive, carbohydrate binding properties. These block copolymers form nanoscale complexes with glycosylated proteins that are stable at physiological pH, yet dissociate and release the glycoproteins under acidic conditions, similar to those found in endosomal and lysosomal compartments within cells. These results suggest that WBA modified polypeptide copolymers are promising for further development as degradable carriers for intracellular protein delivery.

^a **Supporting Information** is available online from the Wiley Online Library or from the author.

FIGURE FOR ToC_ABSTRACT

Block copolymers containing Wulff-type phenylboronic acid groups form nanoscale complexes with glycosylated proteins that are stable at physiological pH, yet dissociate and release the glycoproteins under acidic conditions, similar to those found in endosomal and lysosomal compartments within cells. These results suggest that these polypeptide copolymers are promising for further development as degradable carriers for intracellular protein delivery.

1. Introduction

Many biological therapeutics, such as for enzyme-based therapies, require a carrier to reduce their clearance by the immune system as well as for delivery into cells or cellular compartments where they are needed [1]. Stimulus responsive carriers, such as those that respond to pH or redox, are especially desirable as they can be a means to effect release of therapeutic cargos upon cellular uptake [2]. For these reasons, the reversible and pH responsive diol-binding characteristics of phenylboronic acid (PBA) groups have been used in many carriers proposed for delivery of glycosylated therapeutics [3]. Recent efforts in this area have focused on PBA groups that have been modified with tethered OH and NR₂ groups [4], which adjust the properties of diol-PBA complexes so that they have increased stability at neutral pH, and can yet release their cargos at more acidic pH as found in endosomal and/or lysosomal compartments.

Although some PBA-containing polypeptides have been reported for development as therapeutic carriers, these have primarily utilized simple PBA groups, which form only weak complexes with carbohydrate diol groups at physiological pH [5]. Here, we have prepared new poly(L-lysine)-*b*-poly(ethylene glycol), K₈PEG₄₄, copolypeptides, where the side-chain amine groups of lysine residues have been modified to contain ortho-substituted amine PBA, i.e. Wulff-type phenylboronic acid (WBA) [6], groups (K^{WBA}₈PEG₄₄, **1**, **Equation 1**) to improve their pH responsive diol binding properties [4a,7]. These block copolymers were found to form nanoscale complexes with glycosylated proteins that were stable at physiological pH, yet were found to dissociate and release the glycoproteins at acidic conditions (pH 5.0), similar to those found in endosomal and lysosomal compartments.

Previously, polypeptides modified with PBA groups have been prepared mainly via amide linkages between para- and meta-substituted PBAs and the side-chain functional groups of lysine, glutamic acid, and aspartic acid [5]. These functional polypeptides, pioneered by Shinkai [5a,b], were studied for their carbohydrate binding abilities and nanoparticle formation upon complexation with diol-containing, as well as thermoresponsive or oppositely charged, polymers. A drawback of these systems is the low stability of the PBA-diol complexes at physiological pH, due to the high pK_a of PBA groups, which prohibits stable complex formation using PBA-diol binding alone [7]. It has been shown that substitution of the phenyl ring of PBAs can be used to reduce the pK_a of these groups [7], which increases the stability of the their diol-complexes at physiological pH, and allows for complex dissociation at pH 5 to 6 that is desirable for endosomal or lysosomal release within cells.

Examples of such polymer side-chain modifications include meta-nitro-PBAs [8], benzoboroxoles [4b-d], and WBAs (ortho-amino-PBAs) [4a,7], which have been studied in a number of carrier designs. A potential limitation of some of these systems is their use of fully non-degradable polymers [4a-c], which may limit any future development for human applications. Here, we sought to incorporate WBA functional groups into degradable polypeptide segments to improve their characteristics for development as carriers for endosomal/lysosomal delivery of protein therapeutics. Since WBA functionalization of polypeptides had not been previously reported, we developed methods to incorporate this functionality in a straightforward manner (Equation 1), and also sought to compare the

properties of the resulting polymers to the more widely studied PBA analog. To encourage robust protein complexation, high densities of WBA or PBA groups on the polypeptide segments were desired.

2. Experimental Section

2.1. Materials and Methods.

Unless otherwise stated, copolymer functionalization reactions were performed in glass vials, under ambient pressure. Room temperature reactions were performed at *ca.* 22 °C. Hexanes and THF were deoxygenated by sparging with N₂, then dried by passing through alumina columns. Commercial anhydrous DMF was used as received. All other solvents were used as received from Fisher Scientific. Alizarin Red S, horseradish peroxidase, bovine serum albumin, and ABTS were purchased from Sigma Aldrich. Alpha-L-iduronidase was provided by the laboratory of Dr. Patricia Dickson (LA Biomed). 2-formylphenylboronic acid and 4-carboxyphenylboronic acid were purchased from Combi-Blocks Inc. (San Diego, CA). *N*-Carboxybenzyloxy-L-lysine-*N*-carboxyanhydride (Cbz-Lys NCA) was prepared by a previously reported procedure [9]. Isocyanate-terminated, 2000 kDa poly(ethylene glycol) (PEG₄₄-NCO) was prepared by a previously reported procedure [10]. Dialysis was performed using deionized water (18.2 MΩ-cm) prepared by passing in-house deionized water through a Millipore Milli-Q Biocel A10 unit. Regenerated cellulose dialysis tubing was from Spectrum Labs (Rancho Dominguez, CA). In all other experiments, in-house deionized water was used. NMR spectra were recorded on a Bruker AV400 instrument with chemical shifts reported relative to the solvent signal. Dynamic light scattering was conducted with a Malvern Zetasizer Nano ZS model Zen 3600 (Malvern Instruments Inc., Westborough, MA). Fluorimetry was conducted on a QuantaMaster 40 Spectrofluorometer (Photon Technology International Inc., Birmingham, NJ). UV/Vis spectroscopy was conducted using an Agilent/HP diode-array 8453 UV-Visible spectrophotometer. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a SSI Accuflow Series III liquid

chromatograph pump equipped with a Wyatt DAWN EOS light scattering (LS) and Optilab rEX refractive index (RI) detectors. Separations were achieved using xStream H₂O 500 Å Jordi Labs 5 µm mixed bed column with 0.5 wt% potassium trifluoroacetate in HFiP as the eluent at 60 °C. All GPC/LS samples were prepared at concentrations of 10 mg/mL.

2.2. Experimental Procedures

Poly(L-lysine hydrochloride)₈-*b*-poly(ethylene glycol)₄₄, K₈PEG₄₄. This copolymer was prepared by a procedure similar to methods previously reported [9]. In a N₂ filled glove box, Cbz-Lys NCA (570 mg, 1.9 mmol) was dissolved in anhydrous DMF (50 mg/mL). To the stirred solution was added (PMe₃)₄Co (67 µL of a 50 mg/mL solution in 1:1 DMF/THF) via syringe. After 2 h an aliquot (20 µL) was removed for analysis by FTIR, which confirmed that all of the NCA had been consumed. To the reaction mixture was then added PEG₄₄-NCO (480 mg, 2.6 eq. per Co). The mixture was stirred for an additional 16 h and then removed from the glove box. The majority of the solvent was removed under reduced pressure, and the polymer was then precipitated by addition of water (15 mL) to the concentrated solution (*ca.* 4 mL) followed by additional washing with water (3 x 15 mL), then was lyophilized to dryness. The crude polymer product (*ca.* 1.9 mmol lysine residues) was dissolved in TFA (25 mL), and the mixture cooled in an ice bath. To this solution was added 33% HBr/AcOH (1.7 mL, 9.2 mmol) and the reaction was stirred for 5 h, followed by solvent removal using rotary evaporation. The product was transferred into 2000 Da MWCO dialysis tubing and dialyzed against 3 mM HCl_(aq) for 24 h, followed by Milli-Q water for 48 h with changes of dialysate (4 times daily). The product was isolated by lyophilization to yield a white powder (380 mg, 85% yield). Poly(L-lysine) segment length was determined by comparing ¹H NMR integrations of the 3.03 ppm resonance from lysine residues (-CH₂NH₂) to the 3.73-3.80 ppm resonances from PEG repeats (-CH₂CH₂O-). ¹H NMR (400 MHz, D₂O, 25 °C): δ 4.34 (t, 1H), 3.73-3.80 (m, 176H), 3.40 (s, 3H), 3.03 (t, 2H), 1.40-1.81 (m, 6H). GPC/LS analysis of K₈PEG₄₄ gave M_w/M_n = 1.13 (see SI, **Figure S1**).

Poly(*N*ε-(2-boronobenzyl)-L-lysine)₈-*b*-poly(ethylene glycol)₄₄, K^{WBA}₈PEG₄₄ (1). K₈PEG₄₄

(15 mg, 0.030 mmol lysine residues) and 2-formylphenylboronic acid (6.0 mg, 0.036 mmol) were mixed in a round bottom flask and dissolved in MeOH (0.5 mL). To this solution was added triethylamine (42 μL, 0.30 mmol) and MgSO₄ (*ca.* 3-5 mg). The reaction was stirred for 16 h at room temperature under N₂, sodium borohydride (2 mg, 0.061 mmol) was then added, and the reaction was stirred for another 24 h. The solution was transferred into 2000 Da MWCO dialysis tubing and dialyzed against 3 mM HCl_(aq) for 24 h, followed by Milli-Q water for 48 h with changes of dialysate (4 times daily). The product was isolated by lyophilization to yield a white powder (19 mg, 92% yield). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ 8.33 (d, 1H), 8.25 (d, 1H), 8.12 (d, 1H), 7.89 (d, 1H), 2.97 (s, 2H), 2.90 (s, 2H).

4-Carboxyphenylboronic acid succinimidyl ester. Prepared using a previously reported procedure [11]. 4-Carboxyphenylboronic acid (250 mg, 1.5 mmol) and *N*-hydroxysuccinimide (210 mg, 1.8 mmol) were mixed in a round bottom flask and dissolved in DMF (15 mL). To the resulting solution was added EDC-HCl (410 mg, 2.1 mmol). The reaction was stirred for 24 h at room temperature under N₂. The solution was diluted with water (30 mL) and the product was extracted with EtOAc (3 times 20 mL). The organic layer was washed with water, then brine, and then dried over MgSO₄. The filtered solution was then concentrated and the product crystallized under vacuum overnight (330 mg, 83% yield). The product was used without further purification. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ 7.38 (s, 1H), 6.75-7.17 (m, 3H), 3.92 (t, 1H), 3.26-3.68 (m, 181H), 2.44 (t, 2H), 1.10-1.78 (m, 6H).

Poly(*N*ε-(4-boronobenzoyl)-L-lysine)₈-*b*-poly(ethylene glycol)₄₄, K^{PBA}₈PEG₄₄ (2).

K₈PEG₄₄ (8.0 mg, 0.019 mmol lysine residues) was dispersed in MeOH in a round bottom flask and 4-carboxyphenylboronic acid succinimidyl ester (26 mg, 5 eq. per lysine residue) was added. To this mixture was added triethylamine (14 μL) and the reaction was stirred for 16 h at room temperature. The solution was transferred into 2000 Da MWCO dialysis tubing and dialyzed against 3 mM HCl_(aq) for 24 h, followed by Milli-Q water for 24 h with changes

of dialysate (4 times daily). The product was isolated by lyophilization to yield a white powder (7.4 mg, 73%). ^1H NMR (400 MHz, D_2O w/ NaOD, 25 °C): δ 7.29-7.51 (br s, 4H), 4.00 (t, 1H), 3.26-3.60 (m, 179H), 3.13 (t, 2H), 1.10-1.78 (m, 6H).

Alizarin Red S (ARS) Fluorescence Assay. ARS displays a significant shift in wavelength and intensity of visible light absorption upon complexation with aromatic boronic acids [12]. Copolymer **1** was combined with ARS in 100 mM pH 7.4 HEPES buffer at final concentrations of 1.0 mM (**1**) and 0.1 mM (ARS). These solutions were prepared in the presence of varying concentrations of catechol (0 to 100 mM) to compete with ARS for complexation of boronic acid groups in **1**. Fluorescence emission spectra for the different mixtures were collected upon excitation at 480 nm.

Characterization of Copolymer-Protein Assembly. Complexation between boronic acid-containing copolymers (**1** or **2**) and proteins (HRP, BSA, or IDUA) was detected using dynamic light scattering. All precursor solutions, buffers and Milli Q water were first passed through 0.2 μm pore size Whatman Nucleopore Track-etched polycarbonate membranes to remove extraneous dust prior to mixing. Samples were prepared in disposable microcuvettes at final volumes of 450 μL . Microcuvettes were rinsed three times with filtered Milli Q water, then ethanol, and dried upside down to prevent contamination by dust. Copolymer and protein solutions were initially analyzed separately to confirm minimal self-aggregation, as demonstrated by total scattering intensities below 100 kcps, in 125 mM pH 7.4 HEPES, pH 6.5 HEPES, and pH 5.0 citrate buffers. Initial analyses were carried out at the following concentrations: **1**, HRP and IDUA at 0.10 mg/mL, and BSA at 0.050 mg/mL and **2** at 0.033 mg/mL due to aggregation seen in these samples at higher concentrations. For preparation of sample mixtures, stock solutions of polymers and proteins were prepared at 0.2 mg/mL then combined at a *ca.* 10 to 1 ratio of boronic acid to monosaccharide groups, to yield samples with final polymer concentrations of 0.025 mg/mL. Due to the tendency of BSA to aggregate in buffer, the 10 to 1 mixtures were performed to yield samples for this protein with final

polymer concentrations of 0.0125 mg/mL. Since BSA is non-glycosylated, a mass ratio of copolymer to protein similar to that for HRP analysis was used. Complexation was confirmed by observation of scattering intensities above 150 kcps and a shift in the number distribution to larger diameters. Number distributions were used to enable better visualization of data for non-aggregating molecules.

HRP Activity Assay using ABTS. 2-2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was used as a colorimetric probe to assay peroxidase activity of HRP [13]. The activity of free HRP was analyzed by preparing the enzyme-ABTS solution with final concentrations of 0.033 units/mL (HRP) and 8.7 mM (ABTS), in 125 mM pH 7.4 HEPES buffer. Upon addition of 0.30% w/w hydrogen peroxide in water (0.10 mL, final conc. 0.01% w/w), UV-vis spectra were collected every 10 s for a total time of 120 s. To test the activity of HRP when complexed with **1**, the copolymer solution was added to the HRP-ABTS solution as described above for HRP (final copolymer concentration: 0.0022 mg/mL). ABTS incubated with H₂O₂ final conc. (0.01% w/w) but without HRP was found to show no significant changes in absorbance over time. The absorbance data at 405 nm were normalized by setting absorbance to zero at the beginning of each experiment. All data were collected at 25 °C.

3. Results and Discussion

In initial studies, we attempted to prepare conjugates of either meta-nitro-PBAs or WBAs to the side-chain amine groups in poly(L-lysine). At high degrees of functionalization, the meta-nitro-PBA modified poly(L-lysine) samples possessed minimal water solubility, and the WBA modified poly(L-lysine) samples only possessed reasonable water solubility at short chain lengths. Based on these results, we designed and prepared block copolymer **1**, which contains a 2 kDa PEG segment attached to a short, WBA functionalized, oligo(L-lysine) segment (Equation 1). The PEG segments were used as biocompatible, non-interacting, water

solubilizing chains to limit aggregation of WBA-diol complexes [2]. To prepare the WBA conjugates, ortho-formyl-PBA was attached to lysine side-chains via reductive amination [5c,7c,14], which directly introduced the ancillary ortho-aminomethyl functionality, characteristic of WBAs, into the PBA groups. Previous studies have shown that the secondary aminomethyl-WBA groups, as used here, behave similarly to tertiary aminomethyl-WBA groups that are also of interest [7]. The resulting WBA-containing copolymer **1** was found to be water soluble over a range in pH from 3.0 to 9.0, and was thus suitable for diol-binding studies. We also prepared the para-amide linked PBA analog of **1**, (K^{PBA}_8 PEG₄₄, **2**, see SI, **Scheme S1**) for use as a control polymer in complexation experiments.

To verify that copolymer **1** is capable of binding specifically to diol groups, we performed initial complexation studies using Alizarin Red S (ARS). Since its optical properties change upon complexation of its catechol group to aryl boronic acids, ARS has been used to detect binding of PBAs to diols via UV/Vis or fluorescence spectroscopy [12]. When **1** was added to ARS in aqueous solution, a color change from red to yellow-orange was observed. Fluorimetry was used to further probe the specificity of the interaction between ARS and **1** (**Figure 1**). To an aqueous solution of ARS (0.1 mM) and **1** (1.0 mM) at pH 7.4 was added increasing amounts of catechol (0 to 100 mM, see SI, **Figure S2**), which was used to compete with ARS for complexation with **1**. The fluorescence of the ARS-**1** complex decreased with increasing catechol concentration, as would be expected for conversion of ARS-**1** into catechol-**1** complexes in the presence of excess catechol (**Figure 1**, **Figure S2**). These studies confirmed that copolymer **1** was capable of reversibly binding diol-containing molecules.

To evaluate the ability of copolymer **1** to form complexes with a glycosylated protein, our initial studies utilized horseradish peroxidase (HRP), a readily available, glycosylated enzyme with a total carbohydrate content of 18 to 22% [15]. Complex formation between HRP and **1** was studied by dynamic light scattering (DLS) analysis of mixtures of **1** and HRP

(*ca.* 10 boronic acid groups per monosaccharide) in aqueous buffers ranging from pH 7.4 to pH 5.0 (**Figure 2**). DLS analysis showed that **1** and HRP, separately in solution, did not show any aggregation behavior in the range of pH studied. When solutions of **1** and HRP were mixed, their aggregation into multimeric complexes of *ca.* 25 to 65 nm average diameter was found to occur at both pH 7.4 and pH 6.5. At pH 5.0, no aggregation was seen in mixtures of **1** and HRP, showing that complexation between **1** and HRP is pH-dependent and that no complexation occurs at this pH (Figure 2). The exact ratio of **1** to HRP in the complexes was not be determined due to the unknown and variable degree and type of glycosylation in this protein [15].

The complexes between **1** and HRP at pH 6.5 and 7.4 were stable over time in buffer (48 hours, 22 °C), and possessed average diameters that would be desirable for bloodstream circulation and passive targeting via the enhanced permeation and retention (EPR) effect [16]. To determine if the HRP complexed with **1** remained in active form, an assay using the chromogenic HRP substrate 2-2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was conducted and showed that complexed HRP did not lose activity relative to free HRP (see SI, **Figure S3**) [13]. As an additional control to confirm that aggregation of **1** with HRP involved glycosylation sites on HRP, we also studied the ability of copolymer **1** to form complexes with a non-glycosylated protein, bovine serum albumin (BSA). At pH 7.4, no complex aggregates were observed by DLS to form between **1** and BSA (**Figure 3a**), which suggests that the presence of surface carbohydrates on a protein are necessary for strong complexation with **1** under these conditions. Further, we also observed that addition of catechol to complexes of **1** and HRP resulted in dissociation of the complexes, due to the preferential binding of catechol to WBA groups in **1** (see SI, **Figure S4**) [12]. To confirm that pH responsive complexation of HRP with **1** is due to its WBA groups, we also studied the ability of PBA containing copolymer **2** to form complexes with HRP. Copolymer **2** was found by DLS analysis to be unable to form complex aggregates with HRP even at pH

7.4, which is likely due to the high pK_a of the PBA groups (Figure 3b) [7]. These control experiments show that complexes of **1** and HRP do not denature the enzyme, are likely due to complex formation between WBA and surface carbohydrate groups, and that the structure of the WBA group greatly enhances complex stability at physiological pH, and allows for HRP release at pH 5.0.

To show the results obtained above for **1** and HRP are not specific to that protein, and also show an example with therapeutic potential, we studied the ability of copolymer **1** to form complexes with alpha-L-iduronidase (IDUA), a glycosylated enzyme used for enzyme replacement therapy for the treatment of mucopolysaccharidosis I [17]. Complex formation between IDUA and **1** was studied using DLS analysis of mixtures of **1** and IDUA (*ca.* 10 boronic acid groups per monosaccharide) in aqueous buffers ranging from pH 7.4 to pH 5.0 (**Figure 4**). Similar to results seen with HRP, DLS analysis showed that **1** and IDUA, separately in solution, did not show any aggregation behavior in the range of pH studied. When solutions of **1** and IDUA were mixed, their aggregation into complexes of *ca.* 50 to 125 nm average diameter was found to occur at both pH 7.4 and pH 6.5. At pH 5.0, no aggregation was seen in mixtures of **1** and IDUA, showing that complexation between **1** and IDUA is pH-dependent and that no complexation occurs at this pH (Figure 4). These results are similar to those obtained using HRP, and suggest that copolymer **1** has potential for pH dependent complex assembly with a variety of different glycosylated proteins.

4. Conclusions

We have described the synthesis of a new WBA functionalized polypeptide-PEG copolymer, **1**. While traditional PBA functionalized polypeptides and copolymers have been prepared in the past [4,5], **1** displays the ability to bind strongly to catechols and glycosylated proteins at physiological pH. The structural features of the WBA groups also allow for pH-dependent complexation of glycosylated enzymes. The nanoscale dimensions of the complexes, and the physiological relevance of the pH transition between complexed and non-complexed proteins,

suggest **1** is suitable for further development as a carrier for intracellular delivery of glycosylated enzymes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Abbreviations α -amino acid *N*-carboxyanhydride (NCA), poly(ethylene glycol) (PEG), trifluoroacetic acid (TFA), horseradish peroxidase (HRP), bovine serum albumin (BSA), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), molecular weight cutoff (MWCO), Alizarin Red S (ARS), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 2-(*N*-morpholino)ethanesulfonic acid (MES), 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC-HCl), α -L-iduronidase (IDUA).

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Figure 1. Fluorescence intensity of ARS (0.1 mM, solid line), ARS (0.1 mM) complexed with **1** (1.0 mM, dashed line), and the ARS + **1** complex mixed with catechol (Cat, 100 mM, dotted line) in 100 mM pH 7.4 HEPES buffer. Excitation at 480 nm.

Figure 2. DLS analysis of **1** (0.1 mg/mL, ◆), horseradish peroxidase (HRP, 0.1 mg/mL, □), and mixtures of **1** and HRP ([**1**] = 0.025 mg/mL, 10 eq boronic acid per monosaccharide, ●) in buffers (125 mM) of different pH. A) pH 7.4 HEPES. B) pH 6.5 MES. C) pH 5.0 citrate.

Figure 3. DLS analysis of control complexation experiments using a non-glycosylated protein, bovine serum albumin (BSA), or the PBA based copolymer **2**. A) **1** (0.1 mg/mL, $\blacklozenge$), BSA (0.05 mg/mL, $\square$) and mixture of **1** and BSA at the same mass ratio used for HRP ($[1] = 0.0125$ mg/mL, $\bullet$). B) **2** (0.033 mg/mL) ($\blacklozenge$), HRP (0.1 mg/mL) ($\square$) and mixture of **2** and HRP ($[2] = 0.025$ mg/mL, 10 eq boronic acid per monosaccharide, $\bullet$).

Figure 4. DLS analysis of **1** (0.1 mg/mL, $\blacklozenge$), alpha-L-iduronidase (IDUA, 0.1 mg/mL, $\square$), and mixtures of **1** and IDUA ($[\mathbf{1}] = 0.025$ mg/mL, 10 eq boronic acid per monosaccharide, $\bullet$) in buffers (125 mM) of different pH. A) pH 7.4 HEPES. B) pH 6.5 MES. C) pH 5.0 citrate.

ToC Caption:

Wulff-type phenylboronic acid functionalized polypeptide block copolymers form nanoscale complexes with glycosylated proteins that are stable at physiological pH, yet dissociate and release the glycoproteins at acidic conditions, such as found in endosomal and lysosomal compartments within cells. These new functional polypeptide copolymers are promising for further development as carriers for intracellular protein delivery.

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Protein complexation and pH dependent release using boronic acid containing PEG-polypeptide copolymers^b

ToC figure (110 mm wide × 20 mm high)

^b Supporting Information is available online from the Wiley Online Library or from the author.