ACS Macro Letters

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Temperature, pH, and Glucose Responsive Gels via Simple Mixing of Boroxole- and Glyco-Based Polymers

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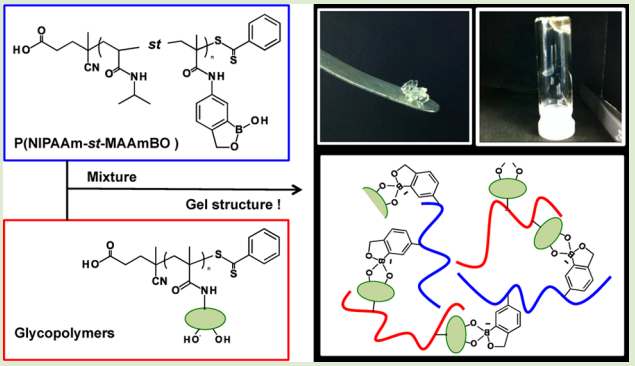
Supporting Information

ABSTRACT: Statistical copolymers of *N*-isopropylacrylamide (NIPAAm) and 5-methacrylamido-1,2-benzoxaborole (MAAmBo) have been synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization. The solution properties of the NIPAAm homopolymers and statistical copolymers were investigated and it was found that, besides temperature and pH, the statistical copolymers were also responsive to the presence of free glucose in solution. Furthermore, responsive hydrogels and nanogels were formed spontaneously by simply mixing the statistical copolymers of P(NIPAAm-*st*-MAAmBO)s and well-defined glycopolymers. These gels were found to have temperature, pH, and glucose responsive properties.



Boronic acids and their esters have been applied to a wide range of fields such as catalysis for stereocontrolled synthesis, diagnosis, and medical treatment for human immunodeficiency virus (HIV), obesity, diabetes, cancer, and many others applications resulting from their unique properties.[1] The reversible boronic-diol interaction between phenylboronic acid (PBA) and glucose was specially utilized as a trigger for insulin release in the designed structure with copolymer, micelle, gel, and so on.[2] The extent of insulin release was found to be dependent on the concentration of free glucose in solution. Recently, Hall and co-workers reported a benzoxaborole group, which has a higher affinity than PBA toward saccharides in a buffer solution at a pH of 7.4.[3] The benzoxaborole groups were also used for the selective binding of Thomsen−Friedenreich (TF)-antigen disaccharide.[4] Recently, Ellis et al. reported that the enhanced delivery of a protein toxin in the cytosol of cells using benzoxaborole.[5] Moreover, the polymeric benzoxaborole is known to neutralize HIV by combining with mannose on the gp120.[6]

Narain and co-workers have synthesized glyco-based polymers and copolymers with linear and hyperbranched structures for several applications.[7] These low toxicity materials were conjugated with metal nanoparticles, carbon nanotube, and nucleic acid.[8] In this communication, well-defined poly(*N*-isopropylacrylamide-*st*-5-methacrylamido-1,2-benzoxaborole) (P(NIPAAm-*st*-MAAmBO)) and glycopolymers have been synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization. Hydrogels and nanogels were formed by simply mixing the P(NIPAAm-*st*-MAAmBO)s and glycopolymers. The interactions of the MAAmBO with hydroxyl groups of the sugars allowed such cross-linking of the polymer chains and this process was found to be fully reversible as a function of temperature and the presence of free glucose (Figure 1).

The polymers, P(NIPAAm-*st*-MAAmBO)s were polymerized at different MAAmBO contents. Glycopolymers, poly(3-gluconamidopropyl methacrylamide) (PGAPMA) and poly(2-lactobionamidoethyl methacrylamide) (PLAEMA), were synthesized using the same conditions as in previous reports.[7c,9] The properties of these polymers are shown in Table 1. For the P(NIPAAm-*st*-MAAmBO) polymers, the MAAmBO contents were kept at 3, 6, and 12 mol %, respectively, and the molecular weights were determined by gel permeation chromatography (GPC) as 12, 9, 13, and 4 kg/mol, respectively, with relatively low molecular weight distributions. On the other hand, the molecular weights of two PGAPMAs and PLAEMA were determined to be 10, 43, and 10 kg/mol. PNIPAAm is a well-known temperature responsive polymer, which shows reversible hydrophilic/hydrophobic properties at about 32 °C which is the lower critical solution temperature (LCST).[10,11] Copolymerization of NIPAAm with MAAmBO leads to the formation of a new copolymer with temperature, pH, and glucose responsive properties. Figure 2A shows the transmittance change as a function of temperature for $PNIPAAm_{178}$, $P(NIPAAm_{101}\text{-}st\text{-}MAAmBO_3)$, $P(NIPAAm_{72}\text{-}st\text{-}MAAmBO_5)$, and $P(NIPAAm_{90}\text{-}st\text{-}MAAmBO_{12})$. LCSTs were determined

Received: February 16, 2013
Accepted: March 5, 2013
Published: March 7, 2013

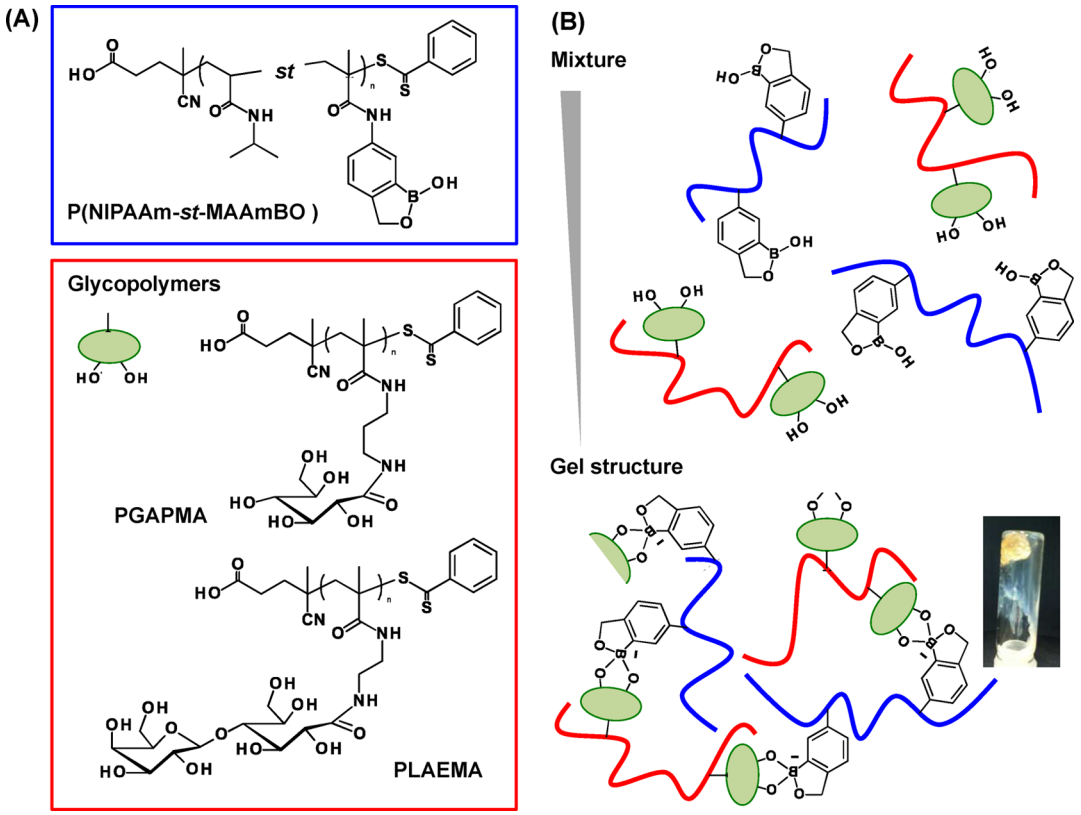


Figure 1. (A) Chemical structure of polymers; poly(*N*-isopropylacrylamide-*st*-5-methacrylamido-1,2-benzoxaborole) (P(NIPAAm-*st*-MAAmBO)), poly(3-gluconamidopropyl methacrylamide) (PGAPMA), and poly(2-lactobionamidoethyl methacrylamide) (PLAEMA). (B) Gel formation via boronic-diol interaction between P(NIPAAm-*st*-MAAmBO) and glycopolymers.

Table 1. Characterization of PNIPAAm, P(NIPAAm-*st*-MAAmBO)s, and Glycopolymers

polymer compositions[a]	MAAmBO content in feed (mol %)	MAAmBO content in copolymer[b] (mol %)	M_n[c] (kg/mol)	M_w/M_n[c] (−)	LCST[e] (°C)	LCST with glucose[f] (°C)
$PNIPAAm_{178}$			21	1.59	31.4	31.3
$P(NIPAAm_{101}$-*st*-$MAAmBO_3)$	3	3	12	1.48	29.6	30.1
$P(NIPAAm_{72}$-*st*-$MAAmBO_5)$	5	6	9	1.24	26.9	29.6
$P(NIPAAm_{90}$-*st*-$MAAmBO_{12})$	10	12	13	1.27	21.1	28.2
$P(NIPAAm_{26}$-*st*-$MAAmBO_4)$	10	12	4	1.38	21.8	26.1
$PGAPMA_{30}$			10	1.10		
$PGAPMA_{132}$			43[d]	1.58[d]		
$PLAEMA_{21}$			10	1.10		

[a]The polymer compositions were calculated by GPC and ^{1}H NMR. [b]Determined by ^{1}H NMR. [c]Determined by GPC (PNIPAAm and P(NIPAAm-*st*-MAAmBO)s: 10 mM LiBr DMF; glycopolymers: 0.5 M CH_3COONa/CH_3COOH water solution). [d]Determined by GPC using 0.1 M $NaNO_3$ water solution. [e]LCSTs were determined at the temperature with 50% of transmittance. [f]The glucose added was 10× higher as compared to MAAmBO units (same amount of glucose was added to $PNIPAAm_{178}$ as with $P(NIPAAm_{90}$-*st*-$MAAmBO_{12})$).

at the temperature with 50% of transmittance. All the polymers were dissolved in pH 7.4 Dulbecco's phosphate buffered saline solution (PBS) at 0.1 wt %. The LCSTs of P(NIPAAm-*st*-MAAmBO)s decreased to 29.6, 26.9, and 21.1 °C, respectively, with increasing MAAmBO contents of 3, 6, and 12 mol %, respectively, as compared to 31.4 °C in $PNIPAAm_{178}$ due to the hydrophobic nature of the MAAmBO. Interestingly, the addition of glucose to the solution causes an increase in the LCSTs due to the strong interaction of MAAmBO units with the hydrophilic glucose molecules. Figure 2B shows the transmittance change of P(NIPAAm-*st*-MAAmBO)s as function of temperature in the presence of glucose that are added in the polymer solution with 10 times higher concentration (mol-content) as compared to MAAmBO contents in the copolymer, that is, 3, 6, and 12 mol %. The LCSTs were increased by 30.1 (MAAmBO, 3 mol %), 29.6 (MAAmBO, 6 mol %), and 28.2 °C (MAAmBO, 12 mol %), respectively, while the LCST of $PNIPAAm_{178}$ did not change in the presence of glucose. The difference of LCSTs in $P(NIPAAm_{90}$-*st*-$MAAmBO_{12})$ between presence and absence of glucose was recorded to be 7.1 °C and this difference was found to increase with increasing MAAmBO contents. Moreover, the LCSTs were found to be sensitive to

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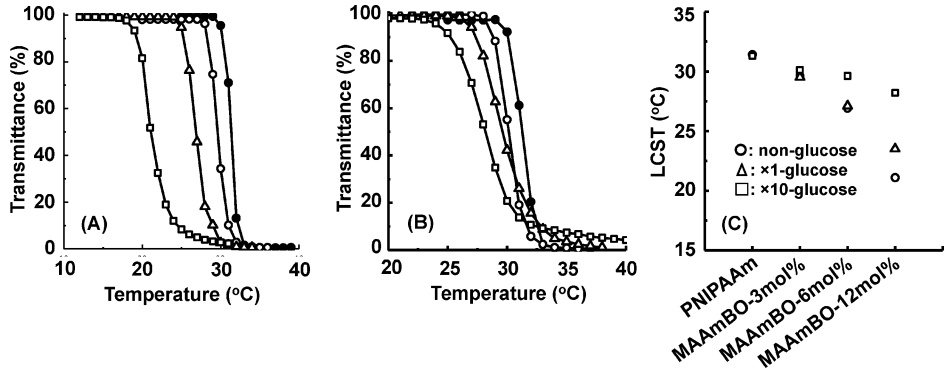

Figure 2. Transmittance change of 0.1 w/v% aqueous solution (A) in absence or (B) in presence (10× of mol content as compared to MAAmBO unites) of glucose, (●) PNIPAAm$_{178}$, (○) P(NIPAAm$_{101}$-*st*-MAAmBO$_3$), (Δ) P(NIPAAm$_{72}$-*st*-MAAmBO$_5$), and (□) P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) as a function of temperature. (C) LCST of PNIPAAm and P(NIPAAm-*st*-MAAmBO)s as a function of the glucose concentration.

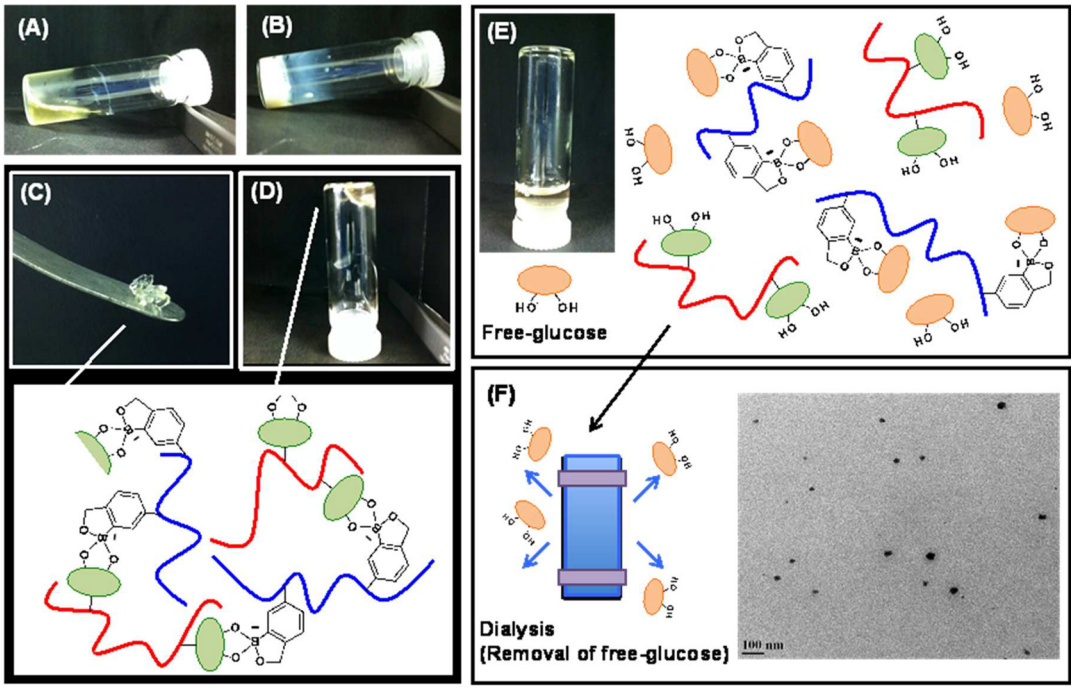

Figure 3. P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) in pH 7.4 PBS (400 μL, 10 wt %) at (A) 4 and (B) 30 °C. Gel formation between P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) (400 μL, 10 wt %) and (C) PGAPMA$_{30}$ (200 μL, 20 wt %) or (D) PLAEMA$_{21}$ (200 μL, 20 wt %) in pH 13 at 4 °C. (E) Highlight gel formation by adding excess free glucose solution (600 μL, 10 wt %) into P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$)-PGAPMA$_{30}$ gel sample in pH 13 at 4 °C. (F) TEM image of nanogel consisting of P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) and PGAPMA$_{30}$ by dialysis in a large amount of pH 12 solution at 4 °C.

the glucose concentrations (Figure 2C). These results suggest that the P(NIPAAm-*st*-MAAmBO)s have both temperature and glucose responsive properties and their LCSTs can be controlled by varying the amount of MAAmBO as well as the glucose concentrations.

Interestingly, we found that these P(NIPAAm-*st*-MAAmBO)s can spontaneously form cross-linked structures by simply mixing with glycopolymers such as PGAPMA and PLAEMA. Kikuchi et al. reported that the simple mixing of a copolymer of PBA and poly(vinyl alcohol) (PVA) can form a hydrogel instantly.[12] Li et al. used the PBA groups on telodendrimer as cross-linker with another telodendrimer having dihydroxybenzene group to prepare cross-linked micelles.[13] As compared to vinyl alcohol, which can also interact with boroxole, the glycomonomers are much easier to copolymerize with other functional monomers which make them more attractive.[7,8,14] Due to the interesting biological properties of glycopolymers, the glucose responsive gels formed to mixing glycopolymers with the P(NIPAAm-*st*-MAAmBO) could find drug delivery applications. For instance, the galactose based hydrogels formed by simple mixing of PLAEMA and P(NIPAAm-*st*-MAAmBO)s could be exploited for the targeted delivery of drugs and genes to the liver.[7d,8e,15] Figure 3 shows the formation of the responsive gels by simple mixing of the solution of P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) and glycopolymers. P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) was completely dissolved in 400 μL of PBS (pH 7.4, 10 wt %) at 4 °C. The addition of 200 μL of PGAPMA$_{30}$ solution (pH 7.4 PBS, 20 wt %) to the P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) solution resulted in an increase in viscosity, but no gel was observed. As shown in Figure 3C,D, the gel formation was formed only by changing the solution pH to 13. Boronic acids are known to interact with a range of sugars such as glucose, galactose, fructose, mannose, and so on.[1,3,16] When a glucose solution (600 μL, 10 wt %) was added

dx.doi.org/10.1021/mz400076p | *ACS Macro Lett.* 2013, 2, 260−264

to the gels at 4 °C, the cross-linked structure was completely destroyed and a clear solution was obtained. The addition of an excess of glucose caused the dissociation of the glycopolymer from the P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) and the instant association of the P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) to the added free glucose molecules (Figure 3E). When the clear solution (see Figure 3E) was dialyzed against an alkaline solution (pH 12) at 4 °C for 2 days, spherical nanogels could be obtained. As the small glucose molecules passed out through the dialysis membrane, the P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) chains could again gradually interact with PGAPMA$_{30}$ chains. Due to the strong association of those two polymer chains, nanogels with spherical morphology were formed over time. After dialysis, the nanogel samples were recovered after centrifugation (17,000 rpm, 10 min, 4 °C) and analyzed by TEM (see Figure 3F).

Due to the high pH for the formation of the gel that can limit their applications, we investigated the LCSTs of P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) at different glucose concentrations as shown in Figure 4A. The LCSTs were found to increase with increasing glucose concentrations. The addition of glucose (3 wt %) did not increase the LCST as compared to that of 1 wt % of glucose, and the transmittance change was slow (Figure S2). Moreover, LCSTs of P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) were also measured at pH 12. In the absence of glucose, the LCST of P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) was found to be 50.6 °C due to the formation of charged MAAmBO units at that pH. The LCSTs were notably increased to 65.7, and 76.1 °C in the presence of 0.1 and 0.3 wt % of glucose, respectively (Figure S2). MAAmBO units can strongly interact with glucose under alkaline condition, which is in agreement with the result for gel formation in Figure 3. On the other hand, LCSTs of PNIPAAm$_{119}$ were independent of glucose at both pH 7.4 and 12 (Figure S2). These results suggested that the formation of gel at pH 7.4 is possible if we use higher amount of glycopolymers as compared to P(NIPAAm-*st*-MAAmBO)s. Indeed, the addition of P(NIPAAm$_{26}$-*st*-MAAmBO$_4$) (22 μL, 10 wt % in pH 7.4 PBS) to PGAPMA$_{132}$ (200 μL, 20 wt % in pH 7.4 PBS) at 4 °C resulted in the formation of the gel. As a result, gel was successfully constructed in pH 7.4 PBS (Figure 4B). Interestingly, lower molecular weight glycopolymers such as PGAPMA$_{30}$ and PLAEMA$_{21}$ did not produce the gel under similar conditions. Therefore, the chain lengths of the polymers also play an important in the formation of the gel structures.

Stabilities of the gel structures were also investigated in different solution conditions. The gel composed of P(NIPAAm$_{26}$-*st*-MAAmBO$_4$) (400 μL, 10 wt %) and PGAPMA$_{132}$ (200 μL, 20 wt %) prepared at pH 12 was immersed in solutions at pH 7.4 and at two different temperatures (Figure 5a). At 40 °C, no degradation of the gel structures was observed after 3 days (Figure 5b). Interestingly, the gel structure did not collapse completely at 4 °C due to their strong interactions (Figure 5c). These gels were also immersed under different conditions of temperature, pH, and glucose for 1 day (Figure 5d–g). The gel structures were completely destroyed at both temperatures (4 and 40 °C) in a weakly

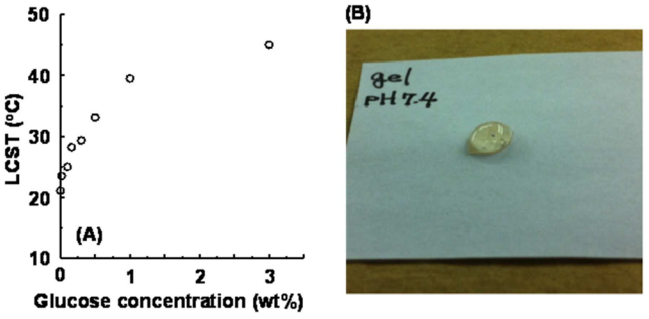

Figure 4. (A) LCSTs of 0.1 wt % P(NIPAAm$_{90}$-*st*-MAAmBO$_{12}$) as a function of the glucose concentration in pH 7.4 PBS. (B) Gel formation between P(NIPAAm$_{26}$-*st*-MAAmBO$_4$) (22 μL, 10 wt %) and PGAPMA$_{132}$ (200 μL, 20 wt %) in pH 7.4 PBS at 4 °C.

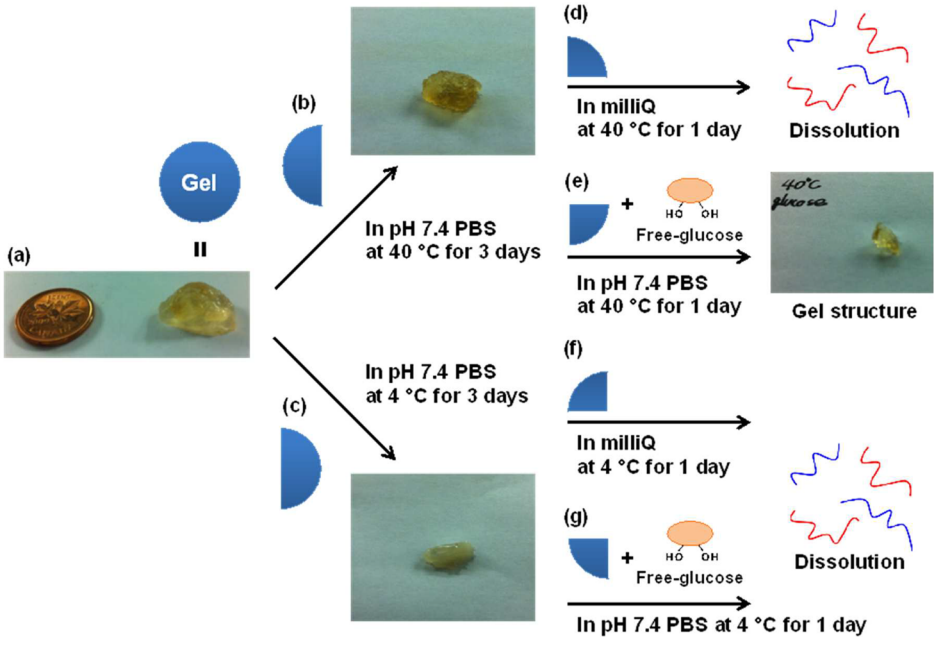

Figure 5. Stability test of gel structures composed of P(NIPAAm$_{26}$-*st*-MAAmBO$_4$) (400 μL, 10 wt %) and PGAPMA$_{132}$ (200 μL, 20 wt %) immersed (a) in pH 12 at 4 °C, (b) in pH 7.4 PBS at 40 °C, (c) in pH 7.4 PBS at 4 °C, (d) in Milli-Q at 40 °C, (e) in pH 7.4 PBS at 40 °C with 2.5 wt % glucose, (f) in Milli-Q at 4 °C, and (g) in pH 7.4 PBS at 4 °C with 2.5 wt % glucose.

dx.doi.org/10.1021/mz400076p | *ACS Macro Lett.* 2013, 2, 260–264

acidic solution. Gels immersed in pH 7.4 PBS buffer solution at 4 °C containing glucose (2.5 wt %) was also completely dissolved due to the interaction of P(NIPAAm$_{26}$-*st*-MAAm-BO$_4$) with free glucose rather than the PGAPMA$_{132}$ glycopolymer. On the other hand, at 40 °C, the gel structures remained intact in the presence of excess glucose and neutral pH. These results suggested that the prepared gels possess temperature, pH, and glucose responsive properties. This responsive gelation system could be very useful for the encapsulation of therapeutic drugs and their slow release could be triggered by temperature, pH, and glucose concentration.

In conclusion, P(NIPAAm-*st*-MAAmBO)s were synthesized by RAFT polymerization at different MAAmBO contents to control their stimuli-responsive properties. Moreover, we found that the P(NIPAAm-*st*-MAAmBO)s interact strongly with linear glycopolymers to form responsive gels, and these structures are also dependent on temperature, pH, and the presence of free glucose. This novel gelation method is promising for the incorporation/conjugation of various materials for several applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis method of MAAmBO and P(NIPAAm-*st*-MAAmBO) and their characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was partially supported by the Natural Sciences Engineering Research Council of Canada (NSERC) and Government of Canada Post-Doctoral Research Fellowship.

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