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Glycosylation of Polyphosphazenes by Thiol-Yne Click Chemistry for Lectin Recognition

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Abstract

Strong carbohydrate–lectin binding interactions in biological systems can be mimicked through the synthesis of glucose containing macromolecules, particularly the glycosylated polymers. Herein, an amphiphilic glycosylated polyphosphazene was synthesized by mixed substitution of poly(dichlorophosphazene) with *n*-butylamine and propargylamine, and a subsequent "thiol-yne" click reaction between poly[(propargylamine/*n*-butylamine)phosphazene] (PPBP) with 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-glucopyranose. Polyphosphazenes with different glycosyl densities, P-37% and P-58%, were obtained and investigated. It was found that these amphiphilic glycopolymers could self-assemble to form global micelles in aqueous solution. An increase in size occurred when the polyphosphazenes interacted with concanavalin A (Con-A). Such interaction was further confirmed by variations in absorbance demonstrated by the ultraviolet–visible spectrum. Disaggregation of the polyphosphazene-Con-A complex was observed in presence of free glucose, which was possibly due to variable ratios of glycosyl-to-alkyl moieties in the glycosylated PPBP. The glucosylated polyphosphazene bound specifically to Con-A but not to peanut agglutinin, as determined by fluorescence microscopy.

Keywords: polyphosphazene, carbohydrate-lectin interactions, self-assemble, Con-A

1. Introduction

Biological saccharides, in the form of glycoprotein, glycolipids and proteoglycans, undertake versatile physiological functions, including cell adhesion, protection against pathogen invasion, immune response, fecundation and blood coagulation, [1-6] through ligand–lectin recognition. Monovalent carbohydrate–protein interactions are generally thought to be associated with weak binding affinities, including hydrophobic interactions, Van der Waals' forces and hydrogen bonding.[7] By contrast, polymers containing multiple copies of sugar ligand can undergo high binding affinities to lectins, which was known to be “cluster glycoside effect.[8,9] Over the past few decades, various glycosylation methods have been used to synthesize oligosaccharides and glycopolymers to optimize binding strength.[10,11] In this regard, several factors may influence the affinity and specificity of multivalent binding as follows. First, the structure of the individual saccharide residues and the corresponding structure of the binding protein play a critical role in signaling process.[12,13] For instance, mannose residues bind specifically to concanavalin A (Con-A) and gatactose residues bind specifically to ricinus communis agglutinin (RCA). Second, the binding epitope density of saccharides along the glycopolymer chains should be taken into consideration, i.e. an increase in the degree of glycosylation normally gives rise to enhanced saccharide–lectin interactions.[6,14-18] Finally, the relative spatial orientation of the saccharide recognition elements and the structural features of the substrates upon which the saccharide residues are displayed also have great influence.[19,20] In this case, hyperbranched, α-helix glycopolymers could be designed to adjust biological status when exposed to carbohydrate receptors. Based on these factors, a large number of different glycopolymers have been designed to interfere with lectins, and thus facilitate a deeper investigation on the "cluster glycoside effect” occurring during biological events.[21]

Polyphosphazenes have inorganic main chains containing alternating nitrogen and phosphorus atoms, and two organic side groups bound to each phosphorus atom. Great interest on

polyphosphazenes stems out primarily because of their main chain flexibility,[22-24] structural tailorability,[25-27] nontoxic biodegradability and biocompatibility.[28-31] Such properties make polyphosphazene derivatives desirable for synthesizing glycopolymers with moderate binding sites and for applications in clinical events involving carbohydrate–protein affinity.[32-35] For instance, Heyde et al.[32,33] introduced galactose pendants to polyphosphazene and demonstrated that a large proportion of galactose was favorable for multivalent carbohydrate–protein interactions. In another case, Yang et al.[34] synthesized galactosylated polyphosphazene and found that the galactose pendants could be used as targeting ligands and showed specific affinity for the asialoglycoprotein receptor. Based on these results, it becomes quite apparent that the designing of novel protein-based glycopolymers, in which both the backbone flexibility and the number of saccharides on the backbone can be controlled, is of critical importance.[16]

Traditional work on multivalency between lectins and glycopolymers mainly focused on hydrophilic hydrogen bonding interactions. However, hydrophobic interactions also have a considerable effect on lectin binding.[36-39] The hydrophobic parts of the saccharide molecules can undergo strong hydrophobic interactions with the side chains in the aromatic amino acids of the lectins. The natural glycolipids also contain hydrophobic parts and form glycolated surface with high density. Additionally, hydrophobic moieties can also be introduced to glycopolymers to construct vesicles, micelles or nanoparticles through self-assembly and to enhance binding affinities in presence of lectins.[40-43]

Previously, we have synthesized polyphosphazenes with controllable glucosyl densities through “thiol-yne/ene” click chemistry that could lead to highly functionalized polymers.[44-49] In this work, we describe the synthesis of amphiphilic polyphosphazenes, derived from poly[(propargylamine/*n*-butylamine)phosphazene] (PPBP), with different saccharide densities: poly[(*β*-D-glucose/*n*-butylamine)-phosphazene]-37% (P-37%) and

poly[(*β*-D-glucose/*n*-butylamine)-phosphazene]-58% (P-58%). The self-assembly behavior and the binding affinities of these polymers toward lectin were further studied. Dynamic light scattering (DLS) and ultraviolet–visible (UV-Vis) spectroscopy showed that P-37% and P-58% had quite different tertiary structures in the presence of concanavalin A (Con-A) and free glucose, respectively.

-- Scheme 1 --

2. Experimental Section

2.1. Materials

Hexachlorocyclotriphosphazene (Bo Yuan New Materials & Technique, Ningbo, China) was purified by recrystallization from heptane and subsequent vacuum sublimation at 60 ºC. Poly(dichlorophosphazene) was synthesized via the thermal ring-opening polymerization of the purified hexachlorocyclotriphosphazene in an evacuated Pyrex tubes at 250 ºC. The polymer was dissolved in light petroleum (Sinopharm Chemical Reagent, China 60-90 degree b.p. fraction) to remove any unpolymerized hexachlorocyclotriphosphazene under a dry nitrogen atmosphere. Poly[(propargylamine/n-butylamine)phosphazene] (PPBP) was synthesized as previously reported.[50] Glycosylated PPBP was synthesized in-house (see supporting information). With a purpose to differentiate the density of glycosylation, the polymers were designated as poly[(*β*-D-glucose/*n*-butylamine)phosphazene]-37% (P-37%) and poly[(*β*-D-glucose/*n*-butylamine)phosphazene]-58% (P-58%). The average molecular weights (M_n) of P-37% and P-58% were 1.56×10^4 and 1.89×10^4 g/mol, respectively. Con-A tagged by fluorescein isothiocyanate (FITC-Con-A) as well as PNA tagged by fluorescein isothiocyanate (FITC-PNA) were purchased from Vector Laboratories, America. Bovine serum albumin (BSA) was supplied by Sino-American Biotechnology Co, LDT. 4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer solution (10 mM) was

prepared according to the standard method.[50] Water used in all the experiments was deionized and ultra-filtered to 18 MV·cm.

2.2. Preparation of the Self-assembled Polyphosphazene and Interaction with Lectins

Self-assembly of the amphipathic glycosylated polyphosphazenes was achieved using the dialysis method. P-37% and P-58% (200 mg each) were dissolved separately in N,N-Dimethylformamide (DMF) (20 mL). Then, the mixture was dialyzed against pure water for 3 days together with water exchange at a constant time interval using MWCO 3500 as the dialysis membrane. The solution was diluted to either 0.5 or 1 mg/mL for characterizations. Con-A or BSA was dissolved in HEPES buffer solution (1 mg/mL). Aqueous solutions of P-37% and P-58% (0.2 and 0.5 mg/mL) were added to the Con-A or BSA solution (1 mg/mL), respectively. The resulting precipitate was ultra-centrifuged and glucose solutions, in a range of 0.5–10 mg/mL, were added to the mixture to investigate whether disaggregation occured between Con-A and P-37% or P-58%.

2.3. Characterizations

P-37% and P-58% (0.2 mg/mL) were self-assembled in aqueous solution. Transmission electron microscopy (TEM) of the microstructure of the self-assembled polyphosphazenes was carried out on a JEM-1200EX (NEC, Japan) microscope at an accelerating voltage of 120 kV. The amphipathic polyphosphazene was dissolved in HEPES buffer solution (0.2 mg/mL), then filtered through a 0.22 μm pore diameter membrane. A 90-plus DLS instrument (Brookhaven, Germany) was employed to measure the hydrodynamic diameter and the dispersity. Pyrene was dissolved in acetone (solubility 6.0×10^{-7} mol/L) and the mixture was placed in a volumetric flask followed by removal of the acetone by evaporation. Subsequently, polyphosphazene solutions with different concentrations (0.5, 0.1, 0.02, 0.004, 0.0008, 0.00016, 0.000032, 0.0000064, and 0.00000128 mg/mL) were mixed with the pyrene. The

resultant solutions were kept at 40 ºC for 4 h and then at 25 ºC for 6 h. The critical micelle concentration was determined from fluorescence spectrum (FLS) using a RF-3510PC (Shimadzu, Japan) spectrometer equipped with a pyrene probe.

FITC-Con-A and FITC-PNA were used to investigate the specific interactions between the polyphosphazene and lectin. The polymer sample was blended either with FITC-Con-A or FITC-PNA and subsequently stirred at room temperature (RT) for 4 h in absence of light. The resultant precipitate was separated through centrifugation at 12000 rpm and rinsed repeatedly with HEPES buffer solution to remove any free lectin. The product was placed on a slide glass and dried at RT under vacuum. Finally, images from fluorescence microscopy (FLM) were visualized through a computer using a Nikon ECLIPES Ti-U (Shimadzu Nikon) microscope. Aqueous solutions of P-37% and P-58% (0.5 mg/mL) were mixed with Con-A or BSA (1 mg/mL) and subjected to UV-Vis spectroscopy. Absorbance versus time curves of the samples were recorded using a UV-2450 spectrophotometer (Shimadzu, Japan) at 360 nm.

3 Results and Discussions

3.1. Synthesis of Poly[(*β*-D-glucose/n-butylamine)phosphazene]

The proportion of glucose/butyl moieties of poly[(*β*-D-glucose/n-butylamine)phosphazene] was calculated from the relative intensity of 3.3-3.4 ppm (multiple broad signals of hydroxyl groups) with 0.86 ppm (signal of the methyl groups) in reference ^{1}H NMR (Figure S2 and Figure S3). Poly[(*β*-D-glucose/*n*-butylamine)-phosphazene]-37% (P-37%) and poly[(*β*-D-glucose/*n*-butylamine)-phosphazene]-58% (P-58%) were successfully synthesized, thereby proving that the number of functional groups along the inorganic chain can be controlled by variation ratios of different nucleophilic reagents in the primaty step. Owing to steric hindrance from the SH-GlcAc$_4$ moieties, a maximum saccharide density was as much as

80% after a click reaction of 4 h. Overall, using the thiol-yne click reaction for glycosylation achieved high efficiency of glycosylation and controllable glucose density.

3.2. Self-assembly of the Amphipathic Polyphosphazenes

As shown in Fig. 1, P-37% and P-58% were assembled into micelles with diameters of approximately 55 and 92 nm, respectively. The TEM micrographs of Global P-37% and P-58% micelles (Fig. 2) showed that the diameters of P-37% and P-58% were 30 nm and 75 nm, respectively. The micrographs also exhibited the tendency of the micelles to aggregate, which is possibly due to strong hydrophobic interactions, as well as hydrogen bonding among the glucose moieties. It should be noted that the diameters observed via TEM are smaller than those obtained from DLS. This is because DLS records the hydrodynamic diameter, i.e. the solvated particle diameter, whereas in TEM, any solvent is evaporated within the TEM chamber. This evaporation might have also caused deformation to our micelles.

-- Figure 1 --, -- Figure 2 --

To further study the self-assembly of the amphipathic polyphosphazenes, pyrene fluorescent spectroscopy was used to measure the critical micelle concentration (CMC) in aqueous solution. The intensity ratios (I_{333}/I_{338}) obtained from the pyrene excitation spectra were plotted against log concentrations (log C) of the amphiphilic polyphosphazenes (Fig 3). At low concentrations, the change in the intensity ratio (I_{333}/I_{338}) was negligible implying that no aggregates existed. However, at the critical concentration, the intensity ratios began to show a substantial decrease, which signified a shift of the pyrene probe from an aqueous phase to a hydrophobic one. Self-assembly could be observed when the concentrations of P-37% and P-58% were 0.079 and 0.10 mg/mL, respectively, which also supported the view that higher hydrophobicity renders a lower CMC.[51,52]

-- Figure 3 --

3.3. The Specific Interaction between Polyphosphazene and Con-A

Con-A, one of the typical plant lectins, can continue to form an oligomer until the oligomer's molecular weight reaches 26 kDa. Moreover, Con-A binds specifically to mannose and glucose in the presence of Ca^{2+} and Mn^{2+}.[53] Similar to a number of animal and bacterial lectins, Con-A can also activate cellular signaling on the cell surface. Hence, a glycopolymer bound to Con-A has the potential to regulate physiological processes involving cell signal transduction, such as cell adhesion, proliferation and survival.[54-56] To understand the interaction between a polyphosphazene and Con-A, P-37% and P-58% (0.5 mg/mL) were added to two separate solutions of Con-A (1 mg/mL). Both the solutions turned opaque indicating that Con-A played a key role in the agglutination of the self-assembled micelles. Interestingly, on further addition of pure glucose to both the turbid solutions of P-58% and P-37%, the former became transparent again, whereas, the latter showed no change (Fig. 4).

In all cases, the agglutination and anti-agglutination between Con-A and P-37% or P-58% micelles affected the turbidity of the solution. Based on this observation, UV–Vis spectra were recorded to study the interaction between the polyphosphazene and the lectin. As shown in Fig. 4 (a), the absorbance at 360 nm increased after the addition of Con-A to P-37%, indicating a strong interaction between the glycosylated polyphosphazene and Con-A. However, no change in the absorbance was observed on further addition of excess free D-glucose to the above mixture and the complex of Con-A and P-37% remained stable when exposed to free D-glucose. A similar increase in absorbance at 360 nm was observed when Con-A was added to P-58% [Fig. 4 (b)]. However, in this case, the absorbance decreased on addition of free D-glucose to the mixture, which is because the cross-linked P-58%-Con-A structure through protein-carbohydrate interaction were disaggregated when exposed to free D-glucose.

-- Figure 4 --, -- Scheme 2 --

These results prompted for further investigation into the variation of size during agglutination and anti-agglutination. An increase in the size of the micelles was observed when the glycosylated polyphosphazenes were exposed to Con-A. DLS results (Fig. 5a) showed that, the diameter of P-37% increased dramatically from 55.2 to 305.0 nm, indicating that agglutination occurred between the micelles and the Con-A through carbohydrate-lectin bonding interactions. The diameter stayed almost constant on further addition of α-D-glucose (Fig. 5b). BSA, which showed no binding affinities to α-D-glucose, was used for comparison with Con-A. With BSA, the diameter was 6.6 nm, which can be ascribed to the fact that the amount of BSA particles was far more than micelles formed by P-37% (Fig. 5c).

-- Figure 5 --

The interaction of P-58% with Con-A was similar to that of P-37%. The diameter increased from 92.6 to 482.9 nm (Fig. 5d) due to bonding of the two species. Further addition of free D-glucose caused a reduction in diameter to 70 nm (Fig. 5e), which provided evidence that free D-glucose impaired the previous binding between P-58% and Con-A. Similar to the results of P-37%, in BSA-P-58% mixed solution, the diameter was 7.4 nm owing to the predominance of BSA particles in solution (Fig. 5f).

From these different behaviors of P-37% and P-58%, it can be inferred that hydrophobic interactions have a stabilizing effect on the Con-A–polyphosphazene interaction. In the case of P-37%, the high ratio of hydrophobic alkyl groups to β-D-glucose residues considerably enhanced the hydrophobic interactions with Con-A. Therefore, any disaggregation of P-37%-Con-A complex was not observed. For P-58%, the high proportion of glycose moieties allowed a strong interaction with Con-A at the cost of hydrophobic forces between the micelles. As a result, the unbalanced Con-A–micelle and micelle–micelle interactions played a key role in the stability of polymer-lectin complex. In addition, the amount of the free glucose molecules

that disrupted the aggregation, far outnumbered the glycosyl residues of P-58% (16–32 times), and the glycosidic clustering effect dramatically enhanced the stability of the conjugation between Con-A and the highly glycosylated polymer. When a high concentration of glucose (10 mg/mL) was added to the system, the interaction of the glucose with Con-A became dominant and P-58% redissolved in aqueous solution.

To determine whether such interactions were specific to Con-A, fluorescence measurements were performed. Peanut agglutinin (PNA), a type of lectin that binds galactose but not D-glucose, was used for comparison. As shown in Fig. 6(a, c), fluorescence signals were detected owing to the specific interaction between the polyphosphazenes (P-37%, P-58%) and FL-Con-A. By contrast, almost no signals were observed when FL-PNA was mixed with P-37% or P-58% [Fig. 6(b, d)], which indicated that there was no specific interaction between PNA and the polyphosphazenes. Such specific binding affinity could be used to target substances containing Con-A, and enable their separation and release from a mixture.

-- Figure 6--

4. Conclusions

The amphiphilic polyphosphazenes, P-37% and P-58%, with different densities of saccharide residues, were successfully synthesized using the “thiol-yne” click reaction. Self-assemblies of P-37% and P-58% were observed in aqueous solution by TEM images. The hydrodynamic diameter of P-37% and P-58% were 55 nm and 75 nm, respectively. Further studies using UV-visible and fluorescence spectroscopy indicated that the interaction between the polyphosphazenes and Con-A were specific. It should be noted that the interaction was reversible in the case of P-58% owing to weak hydrophobic interactions. Such glycopolymers have promising applications in protein separation, as biosensors and lectin adsorption materials.

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References

[1] Q. Yang, C. Kaul and M. Ulbricht, *Langmuir*, 2010, **26**, 5746.

[2] M. Toyoshima and Y. J. Miura, *Polym. Sci. Part A: Polym. Chem.*, 2009, **47**, 1412.

[3] M. X. Hu, L. S. Wan, Z. S. Fu, Z. Q. Fan, Z. K. Xu, *Macromol. Rapid Commun.*, 2007, **28**, 2325.

[4] K. Zhang, D. Peschel, T. Klinger, K. Gebauer, T. Groth, S. Fischer, *Carbohyd. Polym.*, 2010, **82**, 92.

[5] K. Zhang, D. Peschel, E. Bäucker, T. Groth and S. Fischer, *Carbohyd. Polym.*, 2011, **83**,1659.

[6] G. S. Chen and N. L. Pohl, *Org. Lett.*, 2008,**10**, 785.

[7] N. Sharon and H. J. Lis, *Agric. Food Chem.*, 2002, **50**, 6586.

[8] R. T. Lee and Y. C. Lee, *Glycoconjugate*, 2000, **17**, 543.

[9] A. W. Kawaguchi, H. Okawa and K. J. Hashimoto, *Polym. Sci. Part A: Polym. Chem.*, 2009, **47**, 2032.

[10] X. P. Chen and N. Ayres, *Macromolecules*, 2010, **43**, 1341.

[11] L. Taylor, X. P. Chen and N. Ayres, *Polym. Int.*, 2014, **63**, 127.

[12] W. I. Weis and K. Drickamer, *Annu. Rev. Biochem.*, 1996, **65**, 441.

[13] S. Elgavish and B. Shaanan, *Trends Biochem. Sci.*, 1997, **22**, 462.

[14] C. W. Cairo, J. E. Gestwicki, M. Kanai and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 1615.

[15] N. Horan, L. Yan, H. Isobe, G. M. Whitesides and D. Kahne, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 11782.

[16] C. Wang, J. Wu and Z. K. Xu, *Macromol. Rapid Commun.*, 2010, **31**, 1078.

[17] H. Charville, J. Y. Jin, C. W. Evans, M. A. Brimble and D. E. Williams, *RSC Advances*, 2013, **3**,15435.

[18] C. C. Lin, Y. C. Yeh, C. Y. Yang, G. F. Chen, Y. C. Chen, Y. C. Wu and C. C. Chen, *Chem. Commun.*, 2003, 2920.

[19] Y. C. Lee and R. T. Lee, *Acc. Chem. Res.*, 1995, **28**, 321.

[20] V. T. Marchesi, T. W. Tillack, R. L. Jackson, J. P. Segrest and R. E. Scott, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 1445.

[21] J. Li, X. J. Huang, J. Vienken, Z. K. Xu and T. Groth, *Langmuir*, 2013, **29**, 8363.

[22] M. Deng, S. G. Kumbar, Y. Wan, U. S. Toti, H. R. Allcock and C. T. Laurencin, *Soft Matter*, 2010, **6**, 3119.

[23] E. W. Barrett, M. V. B. Phelps, R. J. Silva, R. P. Gaumond and H. R. Allcock, *Biomacromolecules*, 2005, **6**, 1689.

[24] N. R. Krogman, A. L. Weikel, K. A. Kristhart, S. P. Nukavarapu, M. Deng, L. S. Nair, C. T. Laurencin and H. R. Allcock, *Biomaterials*, 2009, **30**, 3035.

[25] S. P. Nukavarapu, S. G. Kumbar, J. L. Brown, N. R. Krogman, A. L. Weikel, M. D. Hindenlang, L. S. Nair, H. R. Allcock and C. T. Laurencin, *Biomacromolecules*, 2008, **9**, 1818.

[26] H. R. Allcock and R. L. Kugel, *J. Am. Chem. Soc.*, 1965, **87**, 4216.

[27] H. R. Allcock, D. B. Patterson and T. L. Evans, *J. Am. Chem. Soc.*,1977, **99**, 6095.

[28] H. R. Allcock, L. B. Steely and A. Singh, *Polym. Int.*, 2006, **55**, 621.

[29] N. R. Krogman, A. Singh, L. S. Nair, C. T. Laurencin, H. R. Allcock, *Biomacromolecules*, 2007, **8**, 1306.

[30] H. R. Allcock and A. M. A. Ambrosio, *Biomaterials*, 1996, **17**, 2295.

[31] A. M. A. Ambrosio, H. R. Allcock, D. S. Katti and C. T. Laurencin, *Biomaterials*, 2002, **23**, 1667.

[32] M. Heyde, M. Claeyssens and E. H. Schacht, *Biomacromolecules*, 2008, **9**, 672.

[33] M. Heyde, M. Moens, L. Van. Vaeck, K. M. Shakesheff, M. C. Davies and E. H. Schacht, *Biomacromolecules*, 2007, **8**, 1436.

[34] Y. X. Yang, Z. W. Zhang, L. L. Chen, W. W. Gu and Y. P. Li, *Biomacromolecules*, 2010, **11**, 927.

[35] Y. Hu, L. J. Meng, L. Niu and Q. H. Lu, *Langmiur*, 2013, **29**, 9156

[36] B. N. Murthy, S. Sinha, A. Surolia, S. S. Indi and N. Jayaraman, *Glycoconj. J.*, 2008, **25**, 313.

[37] N. Jayaraman, *Chem.Soc.Rev.*, 2009, **38**, 3463.

[38] J. Voskuhl, M. C. A. Stuart and B. J. Ravoo, *Chem. Eur. J.*, 2010, **16**, 2790.

[39] Y. Miura, H. Sato, T. Ikeda, H. Sugimura, O. Takai and K. Kobayashi, *Biomacromolecules*, 2004, **5**, 1708.

[40] X. H. Dai and C. M. Dong, *J. Polym. Sci. Part A: Polym. Chem.*, 2008, **46**, 817.

[41] S. R. S. Ting, E. H. Min, P. Escale, M. Save, L. Billon and M. H. Stenzel, *Macromolecules*, 2009, **42**, 9422.

[42] C. M. Dong and E. L. Chaikof, *Colloid Polym. Sci.*, 2005, **283**, 1366.

[43] C. M. Dong, K. M. Faucher and E. L. Chaikof, *J. Polym. Sci. Part A: Polym. Chem.*, 2004, **42**, 5754.

[44] X. Huang, X. J. Huang, A. G. Yu, C. Wang, Z. W. Dai and Z. K. Xu, *Macromol. Chem. Phys.*, 2011, **212**, 272.

[45] N. Ren, X. J. Huang, X. Huang, Y. C. Qian, C. Wang, Z. K. Xu, *J. Polym. Sci. Part A: Polym. Chem.*, 2012, **50**, 3149.

[46] C. Chen, X. J. Huang, Y. Liu, Y. C. Qian, Z. K. Xu, *Polymer*, 2014, **55**, 833.

[47] A. B. Lowe, C. E. Hoyleb, C. N. Bowman, *J. Mater. Chem.*, 2010, **20**, 4745.

[48] B. D. Fairbanks, T. F. Scott, C. J. Kloxin, K. S. Anseth, C. N. Bowman, *Macromolecules*, 2009, **42**, 211.

[49] B. D. Fairbanks, E. A. Sims, K. S. Anseth, C. N. Bowman, *Macromolecules*, 2010, **43**, 4113.

[50] J. S. Zigler, J. L. Lepe-Zuniga, B. Vistica and I. Gery, *In Vitro Cell. Dev. Biol.*, 1985, **21**, 282.

[51] S. C. Lee, Y. Chang, J. Yoon, C. Kim, I. C. Kwon, Y. H. Kim and S. Y. Jeong, *Macromolecules*, 1999, **32**, 1847.

[52] K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, 1977, **99**, 2039.

[53] H. Kogelberg and T. Feizi, *Curr. Opin. Struct. Biol.*, 2001, **11**, 635.

[54] J. Bouckaert, T. Hamelryck, L. Wyns and R. Loris, *Curr. Opin. Struct. Biol.*, 1999, **9**, 572.

[55] S. H. Barondes, V. Castronovo, D. N. W. Cooper, R. D. Cummings, K. Drickamer, T. Feizi, M. A. Gitt, J. Hirabayashi, C. Hughes, K. Kasai, H. Leffler, F. T. Liu, R. Lotan, A. M. Mercurio, M. Monsigny, S. Pillai, F. Poirer, A. Raz, P. W. J. Rigby, J. M. Rini and J. L. Wang, *Cell*, 1994, **76**, 597.

[56] N. L. Perillo, M. E. Marcus and L. G. J. Baum, *Mol. Med.*, 1998, **76**, 402.

Figure Captions

Scheme 1 Synthesis and self-assembly of PPBP

Figure 1 The mean diameter of P-37% and P-58% in aqueous solution

Figure 2 TEM micrographs of (a) P-37%, and (b) P-58%

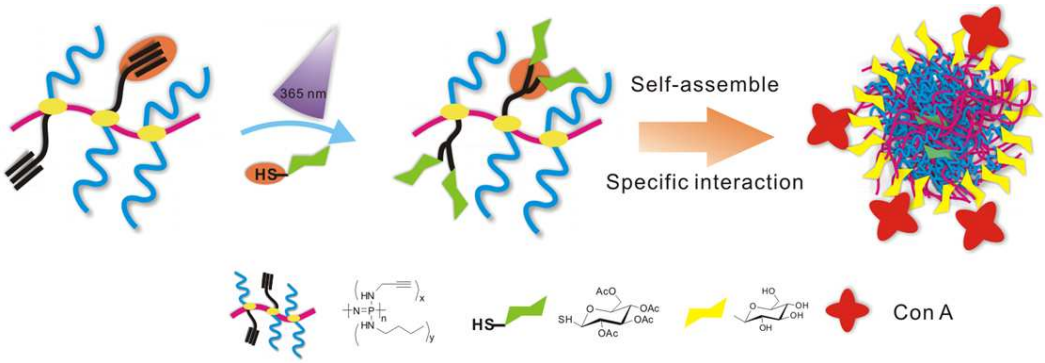
Figure 3 Plot of I_{333}/I_{338} (from excitation spectra of pyrene) versus the logarithm of the concentration for aqueous solutions of (a) P-37%, and (b) P-58%

Figure 4 The interactions of (a) Con-A and BSA (1.0 mg/mL) with P-37% (0.5 mg/mL), and (b) Con-A and BSA (1.0 mg/mL) with P-58% (0.5 mg/mL)

Scheme 2 The adhesion/de-adhesion behavior of PPBP (a) P-37%, (b) P-58%

Figure 5 The mean diameter of (a) P-37% + Con-A, (b) P-37% + Con-A + glucose, and (c) P-37% + BSA (d) P-58% + Con-A, (e) P-58% + Con-A + glucose, and (f) P-58% + BSA

Figure 6 Fluorescent microscope image after lectin recognition: (a) P-37% interacted with Con-A, (b) P-37% interacted with PNA, (c) P-58% interacted with Con-A, and (d) P-58% interacted with PNA



SCHEME 1

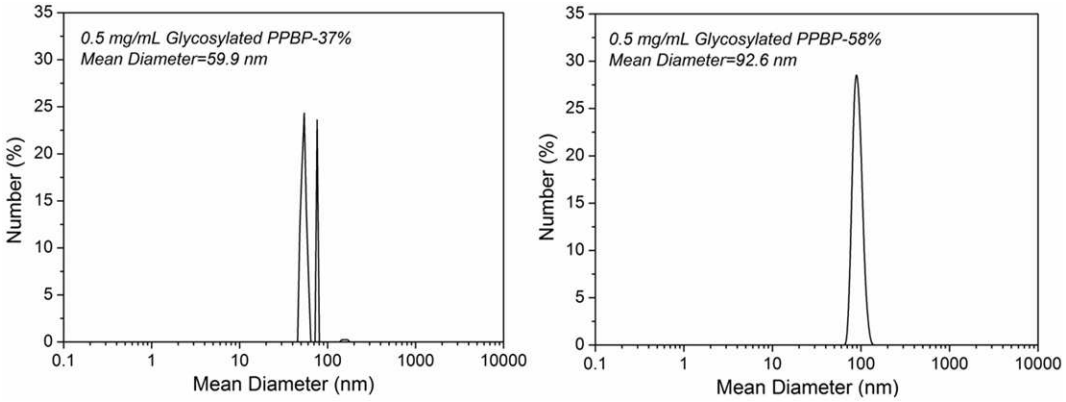

0.5 mg/mL Glycosylated PPBP-37%
Mean Diameter=59.9 nm
Number (%)
Mean Diameter (nm)
0.5 mg/mL Glycosylated PPBP-58%
Mean Diameter=92.6 nm
Number (%)
Mean Diameter (nm)

Fig. 1

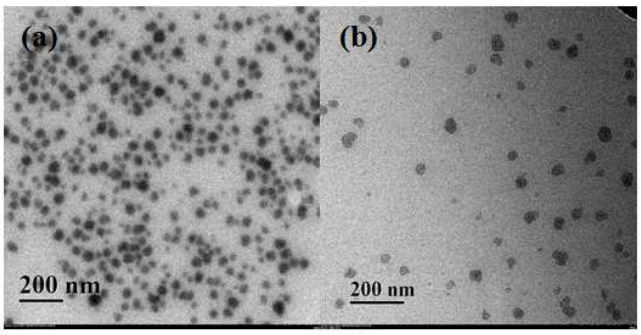


Fig. 2

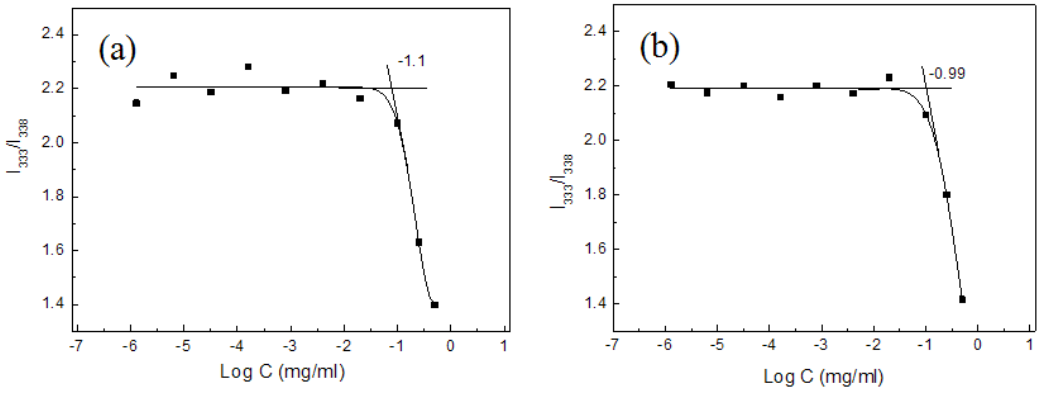


Fig. 3

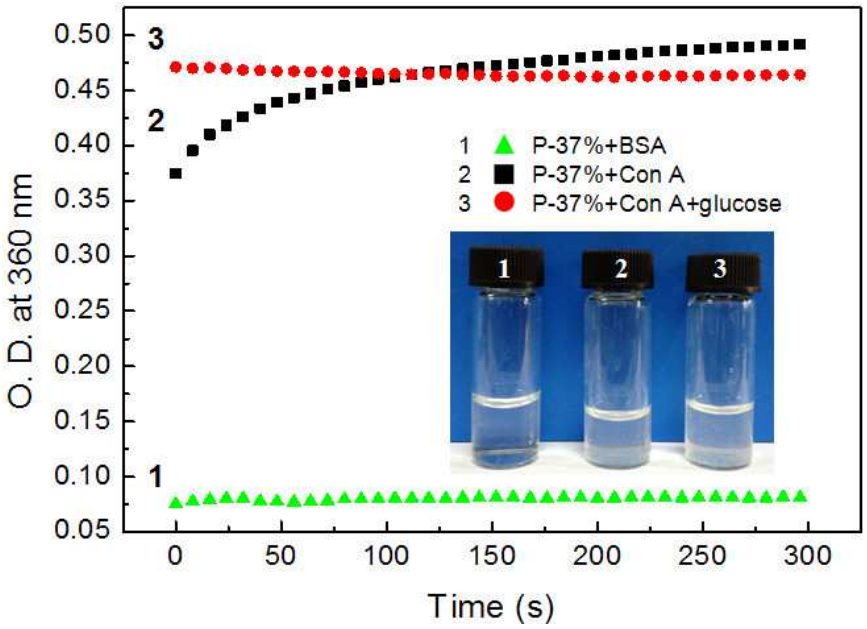

O. D. at 360 nm
Time (s)
1 P-37%+BSA
2 P-37%+Con A
3 P-37%+Con A+glucose
1
2
3

Fig. 4 (a)

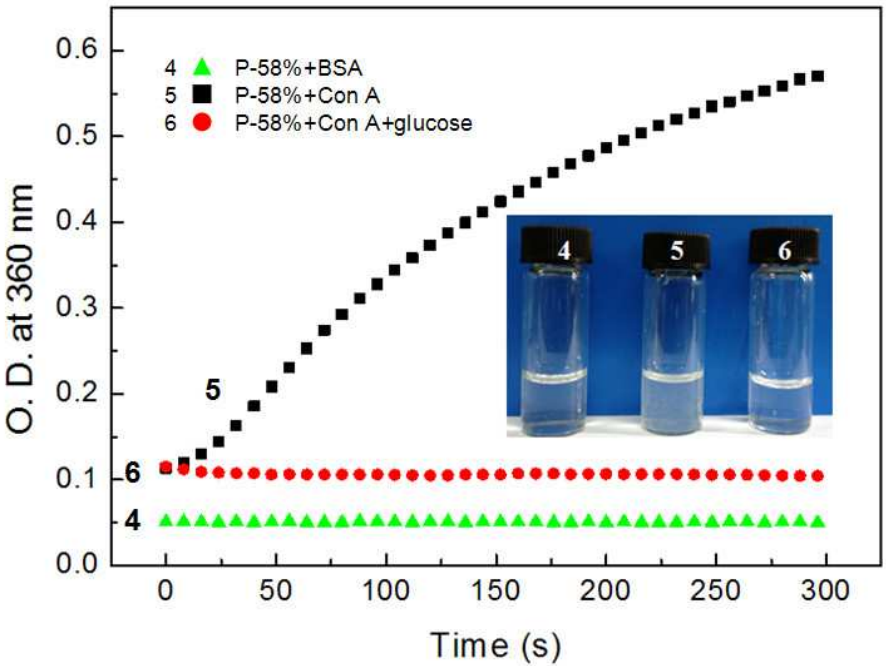
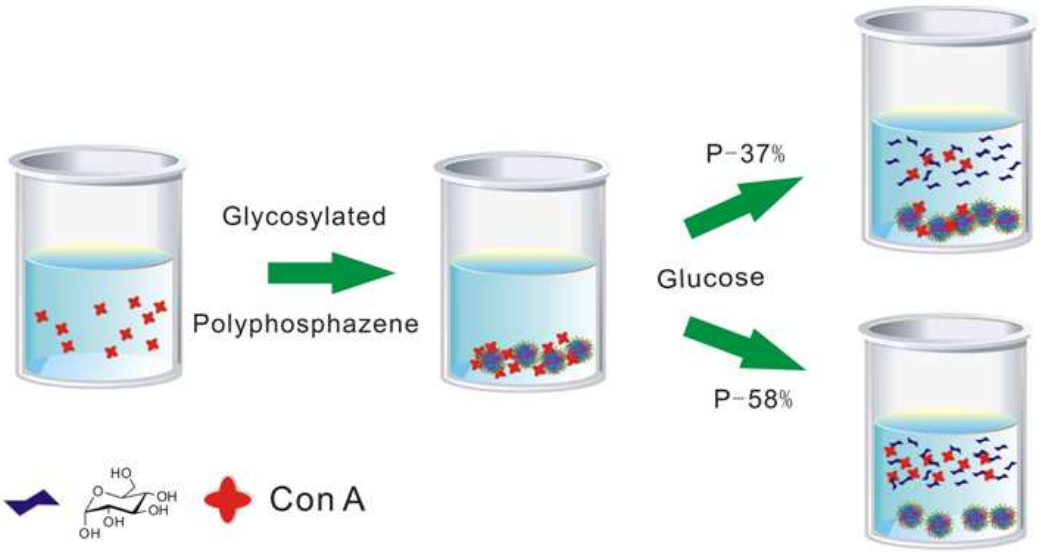

O. D. at 360 nm
Time (s)
4 P-58%+BSA
5 P-58%+Con A
6 P-58%+Con A+glucose
4
5
6

Fig. 4 (b)


Glycosylated
Polyphosphazene
P-37%
Glucose
P-58%
HO
OH
OH
OH
Con A

SCHEME 2

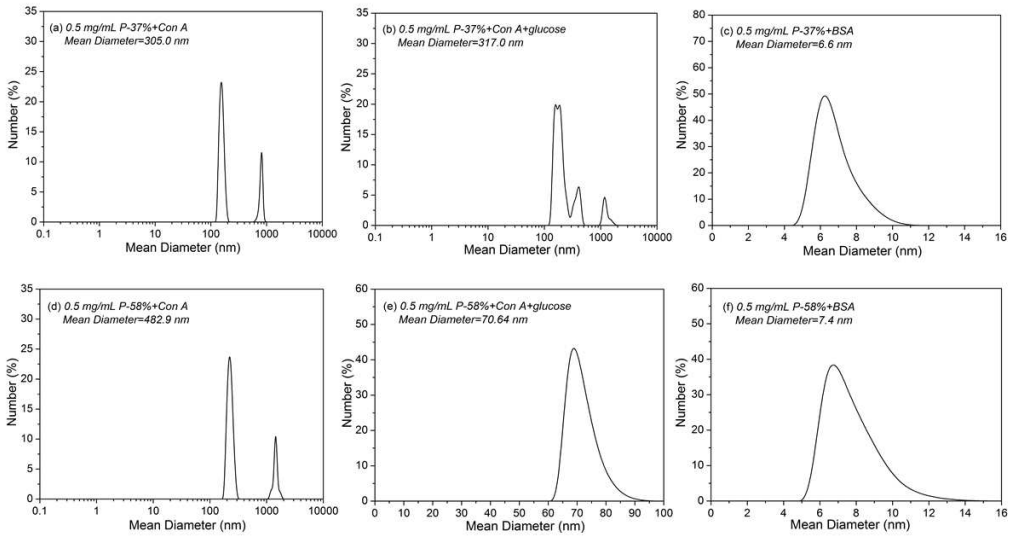

(a) 0.5 mg/mL P-37%+Con A
Mean Diameter=305.0 nm
(b) 0.5 mg/mL P-37%+Con A+glucose
Mean Diameter=317.0 nm
(c) 0.5 mg/mL P-37%+BSA
Mean Diameter=6.6 nm
(d) 0.5 mg/mL P-58%+Con A
Mean Diameter=482.9 nm
(e) 0.5 mg/mL P-58%+Con A+glucose
Mean Diameter=70.64 nm
(f) 0.5 mg/mL P-58%+BSA
Mean Diameter=7.4 nm
Number (%)
Mean Diameter (nm)

Fig. 5

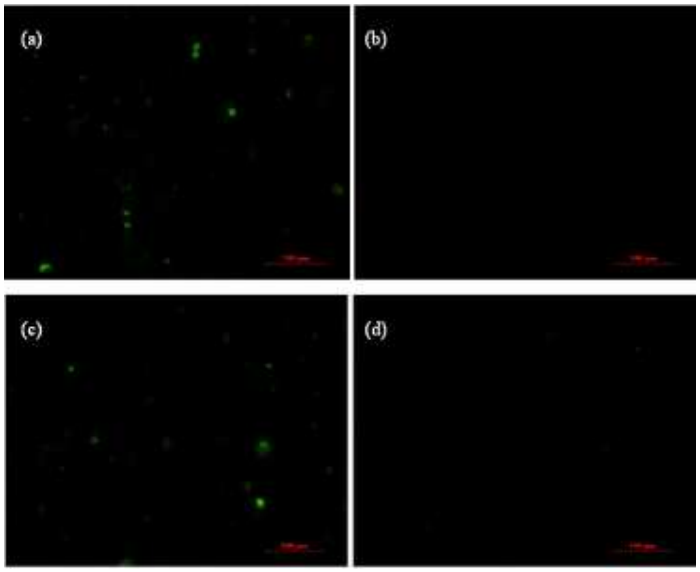


Fig. 6