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Non-covalent interactions in controlling pH-responsive behaviors of self-assembled nanosystems†

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Self-assembly and associated dynamic and reversible non-covalent interactions are the basis of protein biochemistry (*e.g.*, protein folding) and the development of sophisticated nanomaterial systems that can respond to and amplify biological signals. In this study, we report a systematic investigation on non-covalent interactions that affect the pH responsive behaviors and the resulting supramolecular self-assembly of a series of ultra-pH sensitive (UPS) block copolymers. An increase of hydrophobic and $\pi-\pi$ stacking interactions led to a decrease of pK_a values. In contrast, enhancement of direct ionic binding between cationic ammonium groups and anionic counter ions gave rise to increased pK_a. Moreover, hydration of hydrophobic surfaces and hydrogen bonding interactions may also play a role in the self-assembly process. The key parameters capable of controlling the subtle interplay of different non-covalent bonds in the pH-triggered self-assembly of UPS copolymers are likely to offer molecular insights to understand other stimuli-responsive nanosystems. Selective and precise implementation of non-covalent interactions in stimuli-responsive self-assembly processes will provide powerful and versatile tools for the development of dynamic, complex nanostructures with predictable and tunable transitions.

Introduction

Multifunctional nanomaterials are of growing interest and importance in a variety of optical, electrical, thermal and mechanical systems in a wide range of applications such as sensing, self-healing, adaptable surface adhesion and drug delivery.[1–3] Self-assembly has emerged as the most practical strategy in the synthesis of well-organized and stable nanostructures.[4] Such strategies have been exploited in a number of stimuli-responsive nanosystems such as thermo-sensitive hydrogels (*e.g.* poly(*N*-isopropylacrylamide),[5] elastin like proteins[6]), pH responsive nanoprobes[7,8] and oligonucleotide-functionalized gold nanoparticles.[9,10] Despite the widespread interest, molecular understanding of the underlying supramolecular chemistry remains elusive. Part of the challenge is

the lack of identification and evaluation of key structural and environmental parameters that affect their stimuli-response and accompanied supramolecular self-assembly, which hampers our capability in the rational design of responsive nanomaterials in a predictable fashion.[11]

Upon external physical or chemical stimuli, responsive nanomaterials can respond and adapt to the surrounding environment through conformational or chemical changes. In many cases, the conformational changes come in the form of external signal-triggered supramolecular self-assembly. At the molecular level, supramolecular self-assembly is the spontaneous association of molecules under equilibrium conditions into thermodynamically stable and structurally well-defined nanostructures joined by non-covalent bonds.[12,13] Supramolecular chemistry offers a toolbox of multiple non-covalent interactions for the formation of well-defined nanostructures.[14] These interactions range from relatively weak forces such as hydrophobic interactions, hydrogen bonding, or $\pi-\pi$ stacking interactions to ionic bonds. The keys to develop functional nanomaterials *via* external signal-induced self-assembly are to understand and control the non-covalent connections between building blocks or molecules, and to understand and overcome the intrinsically unfavorable thermodynamic barrier involved in bringing many molecules together in a single aggregate of a nanoparticle.[4] A major

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challenge in studying self-assembly in natural and synthetic systems is the interdependence of many non-covalent interactions and their compensating effects on the composite behaviors of the nanosystem.[15] To pinpoint specific contribution from individual non-covalent interactions, we need to establish a model system in which key parameters can be changed easily and independently.

Recently, we reported a library of methacrylate-based ultra-pH sensitive (UPS) nanoprobes with sharp pH transitions that are finely tunable in a broad range of physiological pH (4.0–7.4).[16,17] Besides the introduction of acid labile moieties like acetal groups,[18,19] the incorporation of ionizable groups such as amines or carboxylic acids serves as the general strategy in the development of pH sensitive nanomaterials.[20–24] The nanoprobes consist of a block copolymer, PEO-*b*-PR, where PEO is poly(ethylene oxide) and PR is an ionizable tertiary amine block. The UPS nanoprobes achieved over 100 fold fluorescence intensity increase within 0.3 pH units, which is critical for broad tumor imaging and endosome maturation studies.[25,26] The hydrophobic and π–π stacking interactions can be intensified by increasing the hydrophobic chain length and incorporating aromatic moieties in the copolymer, respectively. The hydrogen bonding network and hydration of macromolecular solutes in aqueous solution are also dependent on the presence of salt and its concentration. The strength of ionic bonds between charged polymers and counter ions is known to be ion species dependent. By selectively tailoring structural (*e.g.*, substituents of amines) and solution parameters (ion species and concentrations), we are able to strengthen or weaken ion pair interactions in the pH-triggered supramolecular self-assembly of UPS block copolymers.

Herein we report a systematic investigation of key factors that impact the pH responsive behavior and the resulting self-assembly of UPS block copolymers. This study aims to provide fundamental understanding of the effect of multiple non-covalent interactions (*e.g.*, hydrophobic interactions, π–π stacking, hydrogen and ionic bonding) on the pH-triggered supramolecular self-assembly. It also offers useful insights for further development of polymeric pH sensors with predictable and tunable transition pH.

Results and discussion

UPS block copolymer synthesis by ATRP method

We used the atom transfer radical polymerization (ATRP) method with CuBr as a catalyst and the *N,N,N′,N′,N″*-pentamethyl-diethylenetriamine (PMDETA) ligand for the copolymer syntheses (Scheme 1). PEO-*b*-PR copolymers with a homopolymeric PR block were synthesized using a single methacrylate monomer (Fig. S1 and S2†) as previously described.[16] At low pH, micelles dissociate into cationic unimers with protonated ammonium groups. When pH increases, neutralized PR segments become hydrophobic and the block copolymers self-assemble into core–shell micelles (Fig. 1, S2 and S3†). The delicate balance between the hydrophobic and hydrophilic seg-
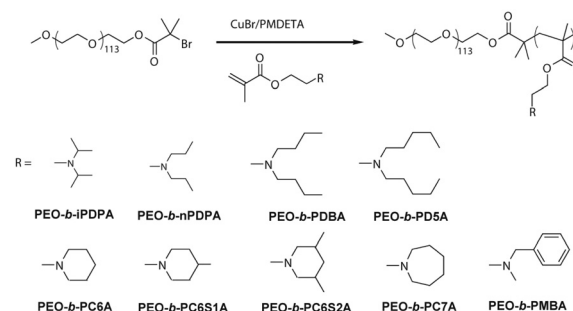
Scheme 1 Schematic syntheses and structures of PEO-*b*-PR diblock copolymers. PR is the hydrophobic segment with ionizable tertiary amines.
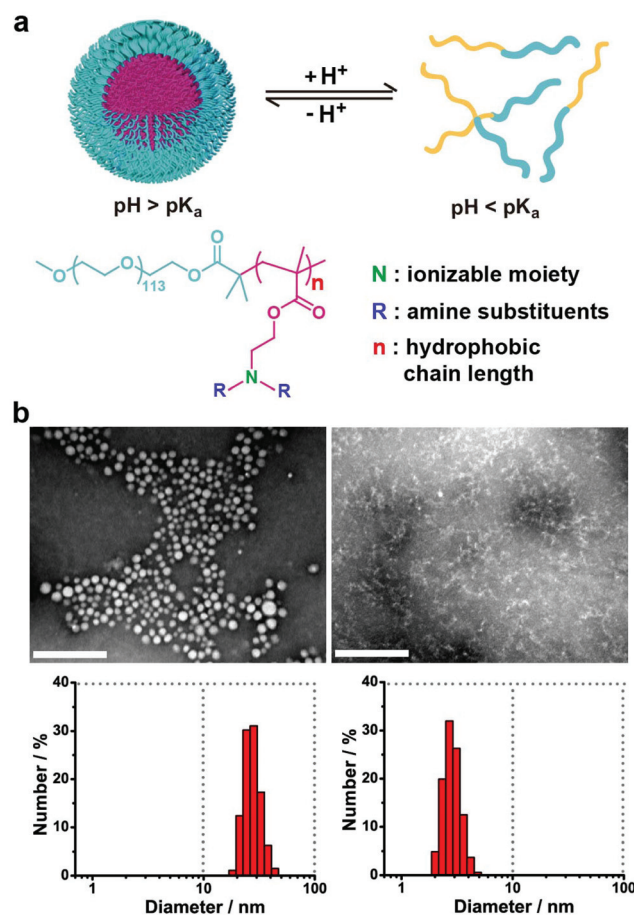
Fig. 1 pH activation of ultra-pH sensitive (UPS) micellar nanoparticles. (a) At pH above pK_a, the neutralized PR segment (purple in color) drove the formation of core–shell micelles. Upon pH decrease (<pK_a), positively charged ammonium groups in the PR segment (yellow) resulted in micelle dissociation into cationic unimers. (b) TEM and DLS characterization of PEO-*b*-nPDPA block copolymers in unimer (pH = 5.0) and micelle states (pH 7.4) (scale bar: 200 nm).

ments as a result of external pH changes drives the formation of thermodynamically stable micelles. The phase transition pH, where reversible supramolecular self-assembly occurs,

depends on the pK_a values of UPS block copolymers around which tertiary amines are reversibly protonated.

pH titration offers critical information on how pH sensors respond to external pH changes along the titration coordinate. We performed pH titration experiments by adding HCl solution into UPS block copolymers' micelle solution prepared following a solvent evaporation procedure.[16] Without specific indication, pH titration experiments were performed at a weight concentration of 2.0 mg ml^{-1} of copolymers in the presence of 150 mM NaCl to mimic the physiological level of salt concentration. We treated the initial micelle solution as having a protonation degree of 0% when no HCl was added. We considered the tertiary amines as 100% protonated when the addition of HCl yielded the sharpest change of pH (Fig. S4†). The apparent pK_a value was determined as the pH at which 50% of all the tertiary amines were protonated.[27–29]

Effect of hydrophobic interactions on pH responsive behavior

Naturally existing self-assembly systems like proteins and synthetic assemblies designed for biomedical applications such as UPS nanoprobes are primarily used in aqueous media. In aqueous solution, the hydrophobic interactions constitute the predominant driving force for the polymeric molecules to self-organize into nanostructures. We began the investigation of the structure–property relationship by determining how changing hydrophobic interactions affected the pH responsive behavior and self-assembly of UPS block copolymers. Early studies on thermo-responsive polymers have shown that the hydrophobicity of polymers has substantial impact on the transition temperature of nanomaterials.[6,30,31]

An obvious strategy in increasing hydrophobic interactions of amphiphilic PEO-*b*-PR block copolymers is to increase the hydrophobic chain length. For proof of concept studies, we synthesized a series of PEO$_{114}$-*b*-nPDPA$_x$ block copolymers with fixed hydrophilic poly(ethylene oxide) chains but systematic changes in the hydrophobic PR chain length (x = 5, 10, 20, 60 and 100). pH titrations of these copolymers were performed at the same molar concentration of tertiary amines at 6.75 mM. The transition pH of PEO$_{114}$-*b*-nPDPA$_{100}$ block copolymers, with the longest hydrophobic segment, yielded the lowest pK_a at 6.2 (Fig. 2a). In contrast, PEO$_{114}$-*b*-nPDPA$_5$, with the shortest hydrophobic chain length, displayed the highest transition pH around 6.7. The plot of pK_a values as a function of the hydrophobic chain length of PEO-*b*-nPDPA copolymers showed a dramatic hydrophobic chain length-dependent transition pH shift (Fig. 2b). It is also interesting to note that a longer hydrophobic chain length also resulted in a sharper pH transition, as measured by $\Delta pH_{10-90\%}$ (the pH range where the protonation degree of all tertiary amines increases from 10% to 90%) (Fig. 2a and S5†).

Strengthening the hydrophobic interactions can also be achieved by increasing the hydrophobicity of the amine substituents of UPS block copolymers. To accomplish this goal, we synthesized a series of ultra-pH sensitive PEO$_{114}$-*b*-PR$_{80}$ block copolymers with an identical poly(methacrylate) backbone and similar chain length but different linear terminal alkyl groups
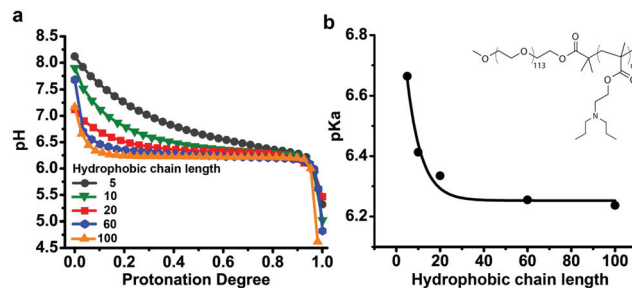

Fig. 2 The effect of hydrophobic chain length on the transition pH of UPS block copolymers. pH titration curves (a) and pK_a values (b) of PEO-*b*-nPDPA block copolymers with the number of PDPA repeating units at 5, 10, 20, 60 and 100. An increase of hydrophobic chain length not only lowers the pK_a but also sharpens the pH transition.

on the side chain. All polymers showed a strong buffer effect as proven by the plateau along the majority of the pH titration coordinates (Fig. 3a). The PEO-*b*-PD5A with the most hydrophobic pentyl group yielded the lowest pK_a at 4.4. Meanwhile, the PEO-*b*-iPDPA with the least hydrophobic isopropyl group as an amine substituent showed the highest pK_a, close to 6.6. PEO-*b*-nPDPA and PEO-*b*-PDBA had pH transitions at 6.2 and 5.3, respectively. These results demonstrated that PEO-*b*-PR block copolymers with more hydrophobic amine substituents have a lower pK_a. We calculated the octanol–water partition coefficients ($\log P$) of the repeating unit of the PR segment (neutral form) and used them as a quantitative measure of
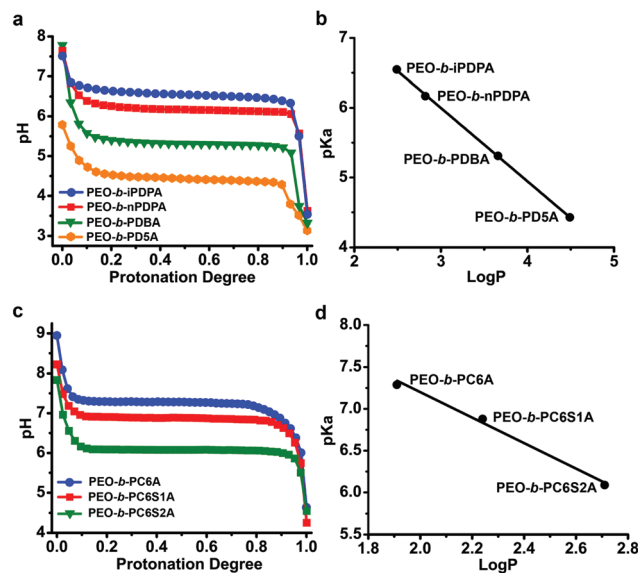

Fig. 3 The effect of hydrophobicity of tertiary amine substituents on the pK_a of UPS block copolymers. pH titration curves (a) and pK_a values (b) of a series of PEO-*b*-PR block copolymers with the same poly(methacrylate) backbone but different acyclic amine substituents. pH titration curves (c) and pK_a values (d) of a series of PEO-*b*-PR block copolymers with the same poly(methacrylate) backbone but different cyclic amine substituents. The pK_a values were correlated with the hydrophobicity parameter ($\log P$) of the repeating unit of the PR segment (b, d).

molecular hydrophobicity and the strength of hydrophobic interactions. The plot of pK_a values as a function of $\log P$ (Fig. 3b) showed a linear correlation.

To confirm the effect of enhancing hydrophobic interactions on the transition pH of UPS block copolymers, we synthesized another series of PEO_{114}-b-PR_{80} block copolymers with the same backbone and similar chain length, but cyclic terminal alkyl groups. For PEO-b-PC6A, we observed the highest pH transition at 7.3. Incorporation of one or two extra methyl groups on the piperidine ring resulted in lower transition pH values of 6.8 and 6.1, respectively (Fig. 3c). A plot of pK_a values as a function of $\log P$ also showed a linear correlation (Fig. 3d). Besides six-membered rings as cyclic substituents, we also synthesized another block copolymer, PEO-b-PC7A, with seven-membered rings as cyclic substituents. C7A repeating units had similar hydrophobicity ($\log P = 2.33$) to that of C6S1A (2.25) and they indeed showed a very close pK_a value around 6.9 (Fig. S6†).

Based on the above experiments, we concluded that stronger hydrophobic interactions (both in amine substituents and hydrophobic chain length) generally lead to a decrease of the transition pH of UPS block copolymers. An increase of hydrophobic interactions will stabilize the micelles and shift the equilibrium to the direction of neutralization of protonated tertiary amines, corresponding to the decrease of pK_a. In this case, the pH-triggered supramolecular self-assembly occurred at lower pH. This is in accordance with early reports that the lower critical solution temperature (LCST) of poly(N-isopropyl-acrylamide)(PNIPAM) based copolymers can be controlled by changing the hydrophobic chain length.[32–34]

Effect of π–π stacking on pH responsive behavior

π–π stacking has been reported to direct the formation of structured ensembles *via* the self-assembly of individual magnetic particles.[35,36] We then investigated whether the incorporation of aromatic rings also affected the pK_a values of UPS block copolymers. PEO-b-PMBA block copolymers were synthesized *via* polymerization of (methylbenzylamino)ethyl methacrylate (MBA, $\log P = 2.91$) monomers. The hydrophobicity of the amine substituents of PEO-b-PMBA was similar to that of PEO-b-nPDPA, but the pK_a value (4.86) was significantly lower (Fig. S7†). This suggested that the introduction of aromatic rings and the resulting π–π stacking may further decrease the pK_a of UPS block copolymers, in addition to hydrophobic interactions.

To further investigate the π–π stacking effect on the pH response of UPS block copolymers, we synthesized a series of PEO-b-P(MBA-r-C7A) copolymers. The molar fraction of the two monomers can be precisely controlled prior to polymerization, leading to a random copolymerized P(MBA-r-C7A) block with a predesigned MBA molar ratio. The pH titration experiments showed that incorporation of more hydrophobic MBA monomers into PEO-b-P(MBA-r-C7A) copolymers resulted in a decrease of pK_a (Fig. 4a). Further quantification by plotting the pK_a values of PEO-b-P(MBA-r-C7A) copolymers as a function of MBA molar ratio showed a linear correlation (Fig. 4b).
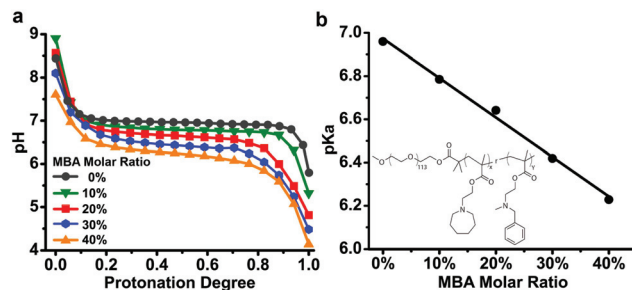

Fig. 4 The effect of incorporation of the (methylbenzylamino)ethyl methacrylate (MBA) monomer in the PEO-b-P(C7A-r-MBA) copolymers on the transition pH of the copolymers. pH titration curves (a) and dependence of pK_a on the MBA molar ratio (b) are shown.

Summary of the effect of tertiary amine substituents in changing pH responsive behavior

Upon external physical or chemical stimuli, responsive nanomaterials can respond and adapt to the surrounding environment through conformation or chemical changes such as self-assembly. The pH-triggered reversible micellization of UPS block copolymers represents a supramolecular self-assembly process, which employs a multitude of non-covalent interactions (*e.g.*, electrostatic and hydrophobic interactions, hydrogen bonding, π–π stacking, *etc.*) to achieve thermodynamically stable nanostructures. Non-covalent interactions besides hydrophobicity may also impact the pH responsive behavior and shift the pK_a of UPS block copolymers in aqueous solution. Fig. 5 summarized the pK_a values of all the UPS block copolymers used in this study as a function of the hydrophobicity of PR segment repeating units. The hydrophobicity of PR segment repeating units in PEO-b-P(C7A-r-MBA) copolymers was calculated as the statistical average of C7A and MBA repeating units. All PEO-b-PR block copolymers with aliphatic alkyl groups as amine substituents, either linear or cyclic, followed a linear pK_a–$\log P$ correlation. The pK_a of PEO-b-PR
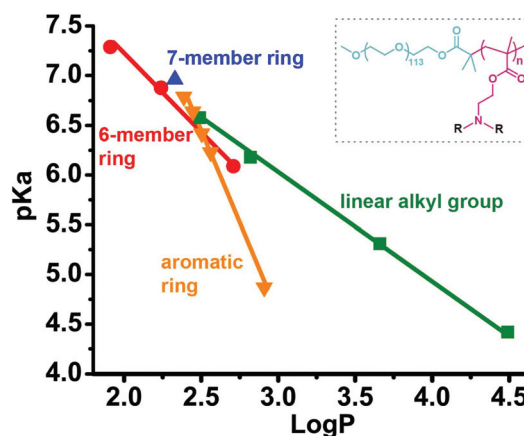

Fig. 5 Correlation of the pK_a values of PEO-b-PR block copolymers with the hydrophobicity parameter ($\log P$) of PR segment repeating units.

block copolymers did not change much by replacing the six-membered ring cyclic substituents (C6S1A) with seven-membered rings (C7A) as long as the hydrophobicity of repeating units stayed the same. The geometry of the alkyl substituents, namely PEO-*b*-iPDPA *vs.* PEO-*b*-nPDPA, appeared to affect the pK_a of the copolymers, but fit in the same linear curve in the pK_a–log P plot.

Interestingly, the incorporation of π–π stacking *via* an introduction of aromatic rings also affect the pH responsive behavior of UPS block copolymers, as proved by the fact that the pK_a values of PEO-*b*-P(C7A-*r*-MBA) copolymers did not fit in the linear correlation of pK_a–log P that existed in PEO-*b*-PR block copolymers with only aliphatic alkyl substituents. The observed drastic decrease in the pK_a of PEO-*b*-P(MBA-*r*-C7A) copolymers as a function of the hydrophobicity of PR segment repeating units suggested that π–π stacking further lowered the pK_a of UPS block copolymers. In the micelle state, the aromatic rings on the PR segment were close to each other to form aromatic stacking. In the unimer state, the π–π stacking effect was significantly minimized because the aromatic rings were far from each other as a result of electrostatic repulsion between cationic ammonium groups. Incorporation of π–π stacking stabilized the micelles and shifted the equilibrium to the direction of neutralization of protonated tertiary amines, corresponding to the decrease of pK_a. These data also suggested π–π stacking as an additional strategy in fine-tuning the transition pH of the UPS block copolymers.

Effect of hydrogen bonding and ionic bonds on pH responsive behavior

Self-organization of molecules *via* multiple intra- and inter-molecular hydrogen bonds has served as an important strategy in the development of self-assembled structures.[12,37] Ions have been known to greatly affect multiple chemical and biological processes in aqueous solution because of their ability to interfere with the hydrogen bonding networks and solvent polarity of water.[38,39] Early reports indicated that the increase of NaCl concentration resulted in a decrease of the LCST of thermo-responsive PNIPAM.[38] We first investigated whether a change of ion concentration could impact the pH response of UPS block copolymers. Here we used the PEO-*b*-nPDPA block copolymer and NaCl as a model system. A series of pH titration experiments of PEO-*b*-nPDPA block copolymers in aqueous solution were performed in the presence of various NaCl concentrations (Fig. 6a). As the NaCl concentration increased from 1 to 150 mM while keeping the polymer concentration the same, the apparent pK_a values of PEO-*b*-nPDPA block copolymers increased from 5.1 to 6.2 (Fig. 6a). Quantitative correlation indicated an exponential increase of pK_a as a function of NaCl concentration (Fig. 6d).

Hofmeister anions have been well known for their effects on the solubility of proteins in aqueous solution, though the underlying mechanism remains elusive.[40,41] The Hofmeister anion series has been divided into water structure makers (kosmotropes) and breakers (chaotropes) with a distinct effect on the protein solubility. SO_4^{2-} and ClO_4^- anions are classical
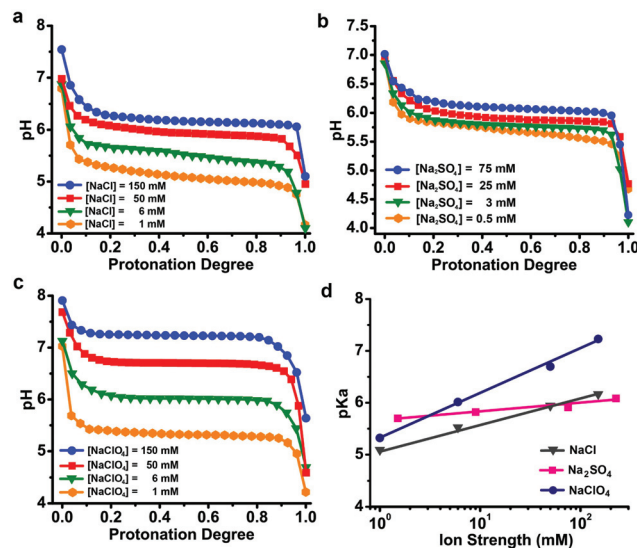
Fig. 6 The effect of anion species and concentration on the transition pH of UPS block copolymers. pH titration curves of PEO-*b*-nPDPA polymers in the presence of NaCl (a), Na_2SO_4 (b) and $NaClO_4$ (c) at different salt concentrations. (d) Quantitative analysis of pK_a values of PEO-*b*-nPDPA block copolymers as a function of ionic strength of aqueous solution. An increase of salt concentrations generally leads to an increase in pK_a. ClO_4^- has the most impact whereas SO_4^{2-} has the least effect on pK_a increase.

kosmotropes and chaotropes, respectively. Cl^- is considered as a neutral anion. We investigated whether ion species can have a different effect on the transition pH of UPS block copolymers. A series of pH titration experiments of PEO-*b*-nPDPA micelle solutions were performed using H_2SO_4 and $HClO_4$ in the presence of Na_2SO_4 and $NaClO_4$ salts, respectively. As shown in Fig. 6b, an increase of SO_4^{2-} concentration also resulted in an increase of pK_a values, although not as notable as Cl^-. Most notably, an increase of ClO_4^- concentration resulted in the most drastic pK_a increase (Fig. 6c). Quantitative analysis showed that the pK_a values of PEO-*b*-nPDPA were directly proportional to the logarithmic of ionic strength. The slopes of pK_a values as a function of ion strength (dpK_a/dlog[I]) for SO_4^{2-}, Cl^- and ClO_4^- were 0.16, 0.49 and 0.85, respectively (Fig. 6d).

These data demonstrate that both the anion species and concentration have significant impact on the transition pH of PEO-*b*-PR block copolymers. An increase of salt concentration generally leads to an increase of the transition pH of UPS block copolymers. The salt effect on the transition temperature of thermo-responsive nanostructures has been studied in multiple systems.[6,42] However, such an effect on the responsive behavior of pH-sensitive nanomaterials is less investigated. As addressed by previous reports,[38] interactions among anions, macromolecules and hydration water molecules all have potential impact on the stimuli-triggered supramolecular self-assembly behaviors of responsive nanomaterials.

We attempt to rationalize the effect of Hofmeister anions on the pH responsive behaviors of PEO-*b*-nPDPA by three

plausible mechanisms (Fig. S8†). First, the hydrated anions are capable of polarizing adjacent water molecules which may form hydrogen bonds with the nitrogen atoms on the tertiary amines. The polarization is likely to weaken the hydrogen bond and make the lone pair electrons of amine nitrogen more accessible to protons. In this case, an increase of salt concentration will favor the protonation of tertiary amines and lead to an increase of pK_a values. Second, anions may also interfere with the hydration of hydrophobic surfaces by water molecules by increasing the surface tension at the water/hydrophobic interface. This dehydration effect will lead to a decrease of pK_a because of the decreased solubility of hydrocarbons. The decrease of pK_a as a result of a salt-induced increase of surface tension can partly offset the H bond-induced pK_a increase. The order of anions' ability in decreasing H-bond interactions between water and tertiary amines and strengthening surface tension is $SO_4^{2-} > Cl^- > ClO_4^-$. Third, direct bonding between anions and cationic ammonium groups can neutralize the positive charges of protonated tertiary amines through the formation of ion-pairs. The neutralization will shift the equilibrium to the direction of protonation of amines, corresponding to the increase of pK_a. The ability to form stable ion pair interactions is much stronger with chaotropic anions (e.g., ClO_4^-, I^-) than kosmotropic anions (SO_4^{2-}). As we reported previously,[39] the order of ion pair strength between ammonium groups and specific anions is $ClO_4^- > Cl^- > SO_4^{2-}$, consistent with prior reports on the binding of anions to amides of PNIPAM.[38] We attribute that the ion pair interactions play a more dominant role than the other two factors for the observed influence of ClO_4^- anions on the pK_a shift of the PEO-b-nPDPA copolymer. PEO-b-PR block copolymers may serve as a good model system for the further delineation of the solvation effect, hydrogen bonding and ionic interactions between the Hofmeister anion series and synthetic macromolecules.

Effect of polymer concentration on pH responsive behavior

The effect of ion species and concentration on the pK_a values of UPS block copolymers suggests that the solution parameters also play a critical role in the pH-triggered supramolecular self-assembly process. Functional nanomaterials for biomedical applications are usually designed to transport therapeutic or diagnostic modalities from the point of administration to the site of action. One potential challenge is to ensure that dose dilution in the journey from injected sites to the action sites, such as in blood, will not compromise the performance of the nanomaterials. To address this question, we investigated whether the polymer concentration, a key solution parameter of polymers in water, may affect the transition pH of UPS block copolymers. We used PEO-b-nPDPA as a representative system in this study. The critical micelle concentration (CMC) of PEO-b-nPDPA, measured in 0.2 M sodium phosphate buffer at pH = 7.4, was around 1 μg ml⁻¹ (Fig. S9†). We performed all the experiments in the concentration range above the CMC.

In the presence of low NaCl concentrations (e.g., 1 mM), the pK_a values of PEO-b-nPDPA increased significantly with the increase of polymer concentration (Fig. 7a and c). More specifically, when the polymer concentration was increased from 0.2 to 10 mg ml⁻¹, pK_a values jumped from 4.7 to 5.3 (Fig. 7a and c). In the presence of 150 mM NaCl to mimic the physiological conditions, however, the pK_a values of PEO-b-nPDPA copolymers stayed the same at 6.2 (Fig. 7b and c). High salt concentrations normalized the variations in pH transition from different polymer concentrations.

To further validate the observation, we conjugated a fluorescent dye, tetramethylrhodamine (TMR), to the hydrophobic PDPA segment to evaluate the fluorescent transition pH as a function of polymer concentration in the presence of 150 mM NaCl. At high pH, neutralization of ammonium groups leads to the formation of micelles and resulting quenching of fluorescence. At low pH, protonation of tertiary amines resulted in the dissociation of micelles into unimers, accompanied by resurrection of fluorescence. The fluorescence intensity of PEO-b-nPDPA-TMR copolymers was measured in a series of sodium phosphate buffers with different buffering pH. A plot of normalized fluorescence intensity as a function of pH suggested that the fluorescence on/off transition pH stay almost the same (Fig. 7d and S10†) over 100-fold change in the polymer concentration (0.02 to 2 mg ml⁻¹), consistent with the above pH titration data. It is worth noting that the administrated concentration of UPS nanoprobes for in vivo tumor imaging studies varies from 0.5 to 2.0 mg ml⁻¹. The nanoprobe concen-
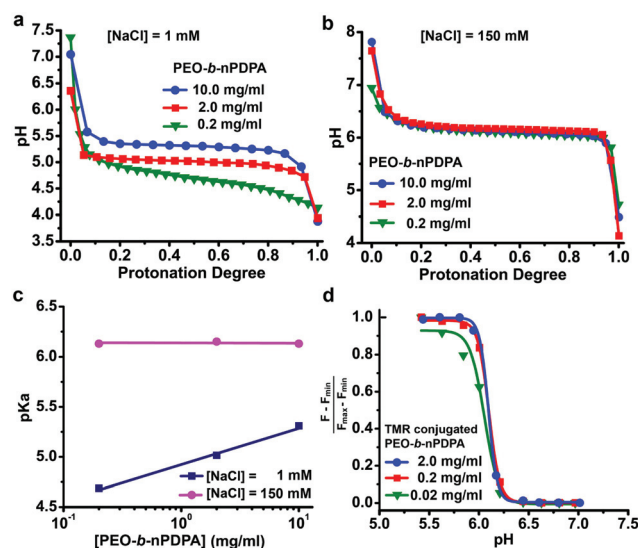
Fig. 7 The effect of polymer concentration on the transition pH of UPS block copolymers. pH titration curves of PEO-b-nPDPA block copolymers in the presence of 1 mM NaCl (a) and physiological level of 150 mM NaCl (b). (c) Quantification of pK_a values as a function of PEO-b-nPDPA concentration. (d) Normalized fluorescence intensity of TMR-conjugated PEO-b-nPDPA block copolymers at different polymer concentrations. In the presence of high NaCl concentrations, the pK_a and fluorescent transition pH stayed approximately the same over 100-fold change in polymer concentration.

tration in plasma 24 h after intravenous injection was approximately 0.02 to 0.1 mg ml^{-1}.[25] These data suggest that the physiological level of salt concentration (*i.e.*, 150 mM NaCl) may be critical to reduce the variability from probe dilution and maintain the nanoprobe integrity in biological studies. Besides tumor imaging, the administered concentration of responsive polymers may vary significantly for different applications such as drug delivery or biosensing. Elucidation of polymer concentration–transition pH relationships will help predict and improve the performance of pH responsive nanomaterials.

Conclusions

In this study, we systematically investigated a variety of non-covalent interactions that impacted the pH responsive behavior and the resulting supramolecular self-assembly of amphiphilic UPS block copolymers. An increase in the strength of both hydrophobic interactions and the π–π stacking effect stabilized the micelles, which favored the neutralization of protonated tertiary amines and resulted in a decrease of pK_a values. Formation of ion pairs between protonated ammonium groups and chaotropic anions drove the protonation of tertiary amines and led to an increase of the pK_a values of UPS block copolymers. A series of key parameters that affected the responsive behaviors of UPS block copolymers such as chain length, hydrophobicity of the substituents of tertiary amines and salt concentration were identified and evaluated, which help establish useful guidelines for rational development of nanomaterial based pH sensors with predictable and tunable transition pH. Without NaCl, the transition pH of UPS block copolymers was polymer concentration-dependent. In the presence of 150 mM NaCl, the transition pH of UPS block copolymers remains unchanged over a 100-fold range in polymer concentration. Ensuring the performance of pH sensors across a wide dose range is crucial for biomedical applications such as molecular imaging and drug delivery.

Introduction of stimuli-responsive moieties has become a general strategy in the design of responsive, functional nano-materials. Temperature-sensitive materials usually contain amide bonds surrounded by hydrophobic groups (*e.g.*, PNIPAM,[5] elastin-like peptides[6]). pH responsive materials are composed of amines,[43] carboxylic acids,[44] or pH-sensitive labile bonds.[24] Disulfide bonds are used to synthesize redox-responsive systems.[45] These stimuli-triggered supramolecular self-assembly sensors arise from a multitude of molecular interactions that exist ubiquitously in various natural and synthetic macromolecular systems. Self-assembly based on selective and precise control of non-covalent interactions provides powerful and versatile tools for the development of complicated nanostructures at the molecular level. The development of functional nanomaterials has a growing emphasis on the identification and optimization of specific design parameters crucial to performance. The key parameters that affect the pH-triggered self-assembly from this study may also serve as a useful guideline for tailoring the structure of other stimuli-responsive systems. Moreover, different responsive groups can be introduced in the same polymeric structures for the development of multi-responsive nanomaterials.[46]

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