

# Hydrophobic Monomer Incorporation Tunes the pH Responsivity of Polycationic Brushes

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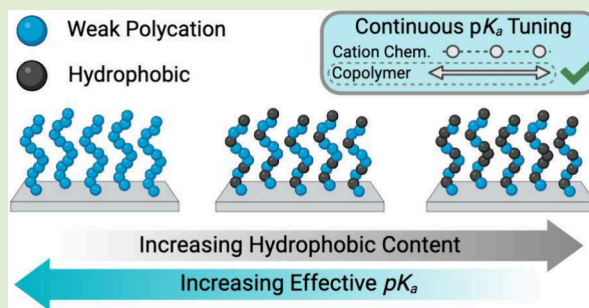


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**ABSTRACT:** We tune the pH-responsive swelling response of polycationic brushes via the incorporation of hydrophobic repeat units. Careful compositional tuning realizes apparent brush  $pK_a$  values, which we term  $pK_a^*$ , between 5.0 and 5.7. Further, we observed monotonic decreases in  $pK_a^*$  with an increase in hydrophobic monomer incorporation. Although brush  $pK_a^*$  was readily tuned with composition, the cooperativity of deprotonation was influenced only by polycation identity and not hydrophobic monomer incorporation. Interestingly, hydrophobicity-driven  $pK_a^*$  decreases were observed exclusively for brushes and not untethered, compositionally matched polymers, suggesting that confining polymers in the form of nanoparticle-tethered brushes might grant more fine-tuned control over pH-responsivity than the tunability offered by free polymers alone. We readily reproduced  $pK_a^*$  trends from planar substrates to nanoparticle-tethered brushes, potentiating applications in intracellular pH sensing and nucleic acid delivery that demand precise  $pK_a$  tuning. We will apply  $pK_a^*$ -tuned brush-grafted nanoparticles to overcome intracellular barriers that have previously hindered free polymers.



Polymer brushes are ultrathin coatings tens to hundreds of nanometers thick that are formed by grafting polymer chains at sufficiently high grafting densities.<sup>1</sup> Owing to their high grafting densities, polymer brushes adopt extended conformations, driving unique physical behavior that imparts brushes with interfacial properties that are distinct from free polymers in solution.<sup>2</sup> Chemists have synthesized brushes of diverse compositions, densities, and architectures, investigated complex polymer brush physics, and engineered stimuli-responsivity (e.g., pH-, temperature-, or solvent-responsive brushes).<sup>3–6</sup> These fundamental investigations unveiled useful properties that promote applications as sensors,<sup>7</sup> microfluidic gates,<sup>8</sup> drug delivery systems,<sup>9</sup> and cell-interfacing substrates.<sup>10</sup> An overarching goal is to predict how polymer characteristics (i.e., composition, molecular weight, and grafting density) and the aqueous environment (i.e., pH, ionic strength, counterion identity) jointly modulate brush swelling.<sup>11–22</sup> However, a complete picture remains elusive, hindering applications such as intracellular pH sensing and polycation-mediated gene delivery that demand precise  $pK_a$  regulation. Here, brushes must swell or deswell in response to changes in intracellular pH as small as 0.5–1 pH units.<sup>23</sup> Recognizing the urgent need to uncover chemical design principles for tuning the pH-responsive swelling of polycationic brushes, we present a hydrophobicity-centric approach to tailoring the polymer brush  $pK_a$  and the cooperativity of deprotonation.

Polycationic brushes do not exhibit the same  $pK_a$  as their constituent monomers. For some tertiary amine methacrylate

polymers, the  $pK_a$  reduces by 1–3 units upon polymerization, with larger decreases noted for bulkier alkyl substituents.<sup>24</sup> By tuning the  $pK_a$ , we can control the pH values at which polymer brushes bind or release DNA payloads<sup>25</sup> or undergo reversible hydrophobic-to-hydrophilic phase transitions.<sup>26</sup> Unlike small molecules where pH-driven deprotonation happens independently across each molecule, the distribution of protonation states in polymers is sensitive to molecular weight,<sup>27</sup> the spatial arrangement of repeat units,<sup>28,29</sup> and monomer chemical identity.<sup>30</sup> Similarly, Marciel, Conrad and co-workers modified the swelling response via copolymerization with water-soluble neutral monomers.<sup>21,22</sup> Other methods of  $pK_a$  modulation have been explored. For example, replacing methyl groups with ethyl or isopropyl substituents in tertiary amines decreases the  $pK_a$ .<sup>24,31</sup> Changing polymer backbones from acrylate to methacrylate decreases the  $pK_a$ .<sup>32</sup> Both exemplify hydrophobicity-driven phenomena; however, changes to the ionizable monomer  $pK_a$  or polymer backbone realized discrete jumps in  $pK_a$ , not a continuum of  $pK_a$  values demanded by intracellular applications. Hydrophobicity has been employed

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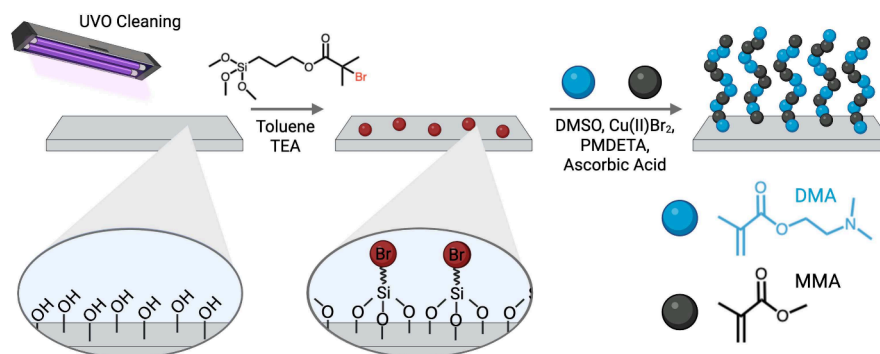


**Figure 1.** Synthetic scheme for surface-initiated atom transfer radical polymerization of hydrophobicity- and  $pK_a$ -tunable copolymer brushes incorporating ionizable cationic monomers (DMA) and neutral hydrophobic monomers (MMA).

as a design handle in pH-responsive polymeric micelles, achieving remarkably sharp and controllable transitions, but polymer brushes have yet to demonstrate the same capacity.<sup>33,34</sup> We hypothesized that introducing hydrophobicity via copolymerization with neutral hydrophobic monomers will tune polymer brush  $pK_a$  to a finer degree than previous approaches.

Unlike in previous work where hydrophobicity reduced the  $pK_a$  in both free polymers and brushes,<sup>31,32</sup> we engineered  $pK_a$  reductions solely in brushes, reductions that we did not observe in composition-matched free polymers. Although simulations predicted that hydrophobic monomer incorporation must reduce the  $pK_a$  for free polymers,<sup>35</sup> we observed mild increases instead. Further, we extended our hydrophobicity-mediated  $pK_a$  tuning approach from planar substrates to nanoparticles. Polycationic brushes grafted from nanoparticles are being applied for nucleic acid delivery due to their loading capacity and stimuli-responsivity.<sup>25,36</sup> Our work will guide the design of  $pK_a$ -tunable nanoparticle-grafted polycationic brushes for intracellular pH sensing and delivery.

We synthesized five copolymer brushes from ionizable 2-(dimethylamino)ethyl methacrylate (DMA) and neutral hydrophobic methyl methacrylate (MMA): DMA100-MMA0, DMA95-MMA05, DMA84-MMA16, DMA73-MMA27, and DMA69-MMA31. Activators regenerated by electron transfer (ARGET) surface-initiated atom transfer radical polymerization (SI-ATRP) yielded well-defined brushes of uniform brush thicknesses and targeted MMA incorporation (Figure 1). Spectroscopic ellipsometry revealed brushes with dry thicknesses varying from 30–34 nm (Table 1). Within this narrow

In situ liquid cell ellipsometry compared brush swelling across a range of pH values (Figure 2A). We use  $pK_a^*$  as a stand-in for  $pK_a$ <sup>20</sup> because swelling measurements capture observable brush thickness-averaged protonation and not local  $pK_a$  variations.<sup>38</sup> At a pH of 3, well below the reported  $pK_a$  of free DMA polymers ( $pK_a = 7$ ), DMA brushes are completely protonated.<sup>39</sup> DMA100-MMA0 brushes swell considerably (Figure 2A), reaching a swollen brush thickness that is 80% greater than the dry thickness. As the pH increases to 4–5, brush deprotonation sets in, decreasing swelling. Brush collapse is evident at pH values above 7, where most amine groups are deprotonated. In the pH range of 4–7, where we observe the transition from swollen to collapsed brushes, we extracted the effective  $pK_a^*$  by identifying the inflection point (eq S3). We detected hysteresis<sup>15,40</sup> in pH-dependent swelling curves between the forward and backward directions and quantified this hysteresis as the difference between the forward and backward  $pK_a^*$  values (Figure 2B,D). Importantly, as brushes grow enriched in the hydrophobic MMA (Figure 2A,D), brush swelling is suppressed, resulting in decreasing  $pK_a^*$ . Even with a 5% increase in MMA incorporation (DMA100-MMA0 to DMA95-MMA05), the  $pK_a^*$  dropped sharply by 0.32 units. Even at dopant levels, MMA repeat units will self-associate and promote hydrophobic interactions, frustrating the infiltration of water.<sup>35,41,42</sup> Further MMA incorporation decreases  $pK_a^*$  hysteresis (Figure 2B); DMA69-MMA31 displays almost no hysteretic behavior ( $\Delta pK_a^*$  of 0.03).

The  $pK_a$  values of polymeric gene carriers and intracellular pH sensors must be calibrated to the endosomal pH of cells, which may vary by as much as 1 pH unit between cell types. For instance, cancer cells have an endosomal pH of 5–5.5 whereas T-cells have a higher endosomal pH of 6.2.<sup>43</sup> In Figure 2D,  $pK_a^*$  values are separated by 0.75–0.94 pH units, a range well-suited for intracellular applications.

Next, we tested whether our strategy of increasing the level of hydrophobic incorporation to tune  $pK_a$  is generalizable to other copolymer pairings. We synthesized copolymers from 2-(diethylamino)ethyl methacrylate (DEA) and *n*-butyl methacrylate (BMA) (Figure 2C). Because of the single methylene group difference between DEA and DMA, DEA polymers, whether brushes or free polymers, exhibit a lower  $pK_a$  than DMA polymers.<sup>24,31</sup> Further, BMA (clogP of 2.9) is far more hydrophobic than MMA (clogP of 1.4). Unlike DMA, fully deprotonated DEA is insoluble in water, and brush swelling drops to nearly zero above the  $pK_a$ . Similar to DMA-MMA, we observed hydrophobicity-dependent decreases in  $pK_a^*$  and

**Table 1. Summary of Brush Properties**

| sample ID   | dry thickness (nm) | mol % DMA (%) | $\sigma$ (chains/nm <sup>2</sup> ) |
|-------------|--------------------|---------------|------------------------------------|
| DMA100-MMA0 | 30.0 ± 0.3         | 100 ± 1       | 0.9 ± 0.1                          |
| DMA95-MMA05 | 33.7 ± 0.1         | 95 ± 2        | 1.1 ± 0.1                          |
| DMA84-MMA16 | 30.4 ± 0.3         | 84 ± 2        | 1.1 ± 0.1                          |
| DMA73-MMA27 | 31.4 ± 0.4         | 73 ± 2        | 1.2 ± 0.1                          |
| DMA69-MMA31 | 31.6 ± 0.4         | 69 ± 3        | 1.0 ± 0.1                          |

range, brush thickness will not confound  $pK_a$  comparisons.<sup>37</sup> Brush composition spanned 0–31% MMA, which is close to our target incorporation (0–40% MMA). Reactivity ratio measurements ( $r_{\text{DMA}} = 1.28$  and  $r_{\text{MMA}} = 0.62$ ) and simulated copolymer sequences indicate that DMA-MMA copolymers do not differ significantly from perfectly random ( $r_{\text{DMA}} = 1.00$  and  $r_{\text{MMA}} = 1.00$ ) copolymers (Figure S11).

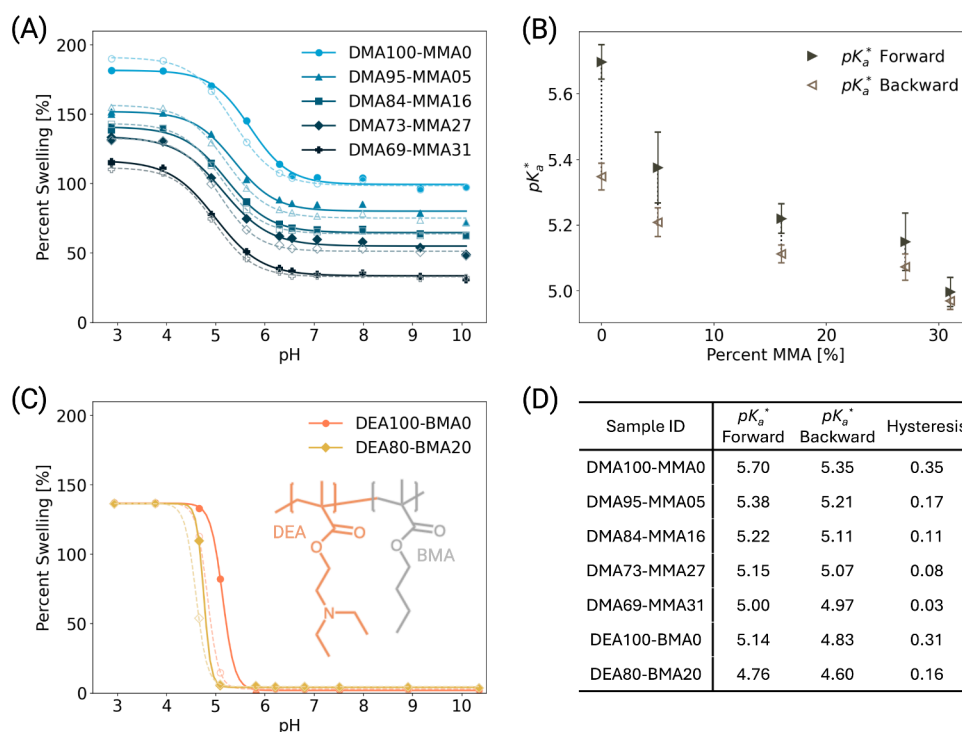


**Figure 2.** Brush hydrophobicity tunes the apparent  $pK_a$  or  $pK_a^*$  and hysteretic behavior. (A) Brush  $pK_a^*$  decreases monotonically with MMA incorporation, as measured by in situ liquid cell ellipsometry. (B) MMA incorporation also minimizes hysteresis. (C) Hydrophobicity-driven  $pK_a^*$  tuning was also observed with DEA-BMA copolymers, a more hydrophobic system than DMA-MMA. We also observed sharper changes in brush swelling with pH for DEA-BMA than with DMA-MMA. (D) Summary of pH-responsive swelling properties of DMA-MMA and DEA-BMA copolymers.

hysteresis (Figure 2D), indicating that our strategy is robust across cationic and hydrophobic repeat units.

While protonated and deprotonated states in DMA-MMA copolymer brushes spanned 2 pH units, transitions between swollen and collapsed brushes were far sharper for DEA-BMA, spanning only 0.5 pH units (Table S7). We attribute this sharper pH response of DEA-BMA copolymers to greater cooperativity in deprotonation.<sup>23</sup> Unlike DMA-MMA polymer brushes, deprotonation events in DEA-BMA brushes are not independent. Rather, each deprotonation event drives neighboring polymer segments to deprotonate and expel water, which, in turn, raises the hydration penalty for neighboring protonated amines and promotes their deprotonation. This phase separation establishes a positive feedback loop that compresses the pH range within which brushes transition from swollen to collapsed. Unlike DEA-BMA, deprotonated DMA-MMA polymer brushes still retain some water and do not phase separate (Figure 2A vs C). Hence, the hydrophilic-to-hydrophobic transition spans a broader pH range for DMA-MMA than DEA-BMA.

To quantify cooperativity, we measured the slope of the swelling curve at the inflection point and the width of the titration region in pH units (Figure S13). Both metrics indicated a sharper hydrophilic-to-hydrophobic transition for DEA-BMA than for compositionally similar DMA-MMA analogs (Table S7). For instance, the slope at the inflection point was 0.4–0.5 for DMA84-MMA16 as opposed to 2.9 for DEA80-BMA20, a 5–6-fold increase. The width was 1.75–1.9 pH units and 0.5 pH units for DMA84-MMA16 and DEA80-BMA20, suggesting a sharper transition between protonated and deprotonated states for the latter. The slope and width

were indifferent to the MMA/BMA molar content, suggesting that cation identity determines cooperativity.

Varying MMA incorporation in Figure 2A tuned the  $pK_a^*$  (forward direction) between 5.00–5.70, with successive compositions separated by as little as 0.07 units (DMA73-MMA27 to DMA84-MMA16) and as much as 0.32 (DMA100-MMA0 to DMA95-MMA05). In contrast, switching alkyl substituents in tertiary amines or modifying polymer backbone chemistry moved  $pK_a^*$  in larger jumps. In Figure 2D, replacing DMA with DEA (compare DMA100-MMA0 and DEA100-BMA0) lowered the  $pK_a^*$  by as much as 0.56 units with no access to intermediate values between 5.14 and 5.70. Copolymer compositional tuning therefore realizes a continuum of  $pK_a^*$  values and pH-responsive swelling behaviors that are inaccessible via monomer substitution alone.

Hydrophobic comonomer enrichment imposes an energetic penalty for brush hydration, destabilizing protonated states, promoting DMA deprotonation, and lowering the brush  $pK_a$  (Figure 3). If this mechanism holds, replacing MMA with the more hydrophobic BMA (clogP 2.9 vs 1.4) must further lower the  $pK_a^*$ . We observed a 0.08 reduction from 5.17 (DMA84-MMA16) to 5.09 (DMA83-BMA17, Figure S14). This decrease is comparable to the error in  $pK_a^*$  measurements. However, we must consider that DMA-BMA brushes were grafted at roughly half the density ( $\sigma$  of 0.6 vs 1.1 chains/nm<sup>2</sup>) of DMA-MMA (Figure S14). This reduced grafting density for DMA-BMA brushes should raise  $pK_a^*$  toward values approaching free polymers.<sup>37</sup> Replacing MMA with BMA lowered the  $pK_a^*$  despite diluted grafting density. Sparsely grafted brushes, which resemble untethered free polymers, should exhibit  $pK_a$  values closer to free polymer equivalents.<sup>37</sup> We estimate our grafting densities to be 0.9–1.2 chains/nm<sup>2</sup> (Tables 1 and S6),

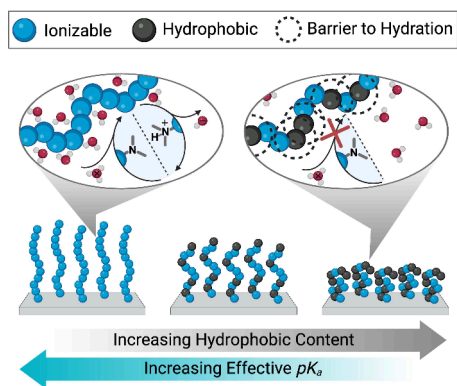


**Figure 3.** Schematic depicting a possible mechanism by which hydrophobicity lowers the  $pK_a$  of weak polycationic brushes. We propose that hydrophobic monomers impose unfavorable interactions with water creating a barrier to hydration that prevents the further influx of water into the brush layer. More acidic conditions are required to overcome this barrier, lowering the  $pK_a$ .

indicating that all five brushes are in the dense brush regime, and supporting the conclusion that observed  $pK_a^*$  trends are primarily associated with hydrophobicity rather than grafting density variations.

Surprisingly, hydrophobicity-driven reductions in  $pK_a$  were not observed for untethered, “free”, polymers (Figure 4). Free

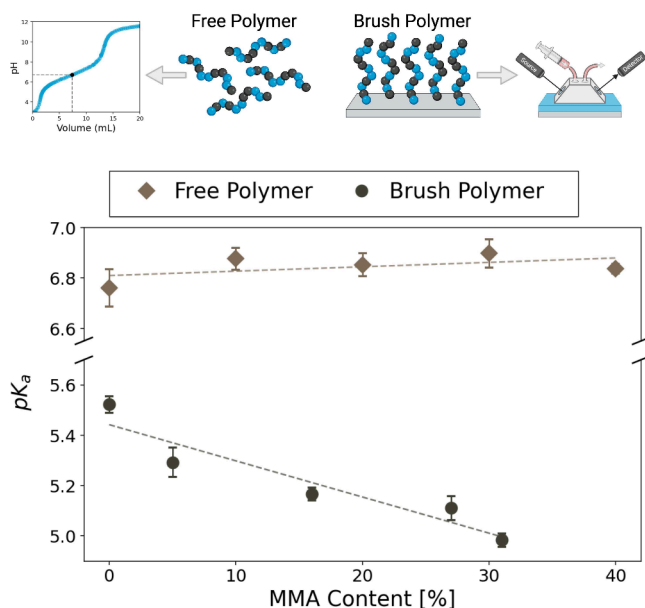


**Figure 4.** Free polymers do not experience hydrophobicity-dependent reductions in  $pK_a$ , unlike brushes. Note the break in the y-axis as untethered polymers display higher values of  $pK_a$ . Free polymer  $pK_a$  was measured via acid–base titration, while brush  $pK_a^*$  was assessed via in situ liquid cell ellipsometry.

polymers were synthesized with the same compositions as polymer brushes with molecular weights comparable to the estimated molecular weights of brushes (Tables S9 and S10). Free polymers exhibit negligible increases in  $pK_a$  with increasing MMA incorporation, contrary to simulations.<sup>35</sup> This is because MMA spacers isolate DMA repeat units from each other and realize higher  $pK_a$  values approaching monomeric DMA.<sup>39</sup> Unlike in polymer brushes, hydrophobic incorporation failed to realize  $pK_a$  decreases in equivalent free

polymers. Therefore, hydrophobicity needs to operate within the highly concentrated environment of densely (chain spacing  $\ll 2R_g$ ) grafted brushes to modulate  $pK_a^*$ . Robinson and Kegel demonstrated that hydrophobic reservoirs stabilize deprotonated segments and induce shifts in  $pK_a$ .<sup>44</sup> Such reservoirs are abundant in brushes due to their high grafting density but absent in free polymers. Introducing hydrophobic motifs within free polymers did not elicit  $pK_a$  reductions; these polymers must be presented in the form of polymer brushes to transform hydrophobicity into a knob for  $pK_a$  tuning.

When DMA repeat units become protonated, the resulting charged moieties must be hydrated, and their negatively charged counterions must penetrate the brush. Further, tethered polymer chains cannot swell to solvate charged DMA repeat units because swollen brushes would promote increased water contact with MMA. Unable to lower this energetic penalty conformationally, protonated states become destabilized, effectively decreasing the  $pK_a$  value. In contrast to polymer brushes, free polymers enjoy greater conformational freedom to rearrange polymer segments such that charged repeat units remain hydrated, while still shielding MMA from water. Consequently, hydrophobicity exerts a negligible influence on the free polymer  $pK_a$ .

We translated hydrophobicity-tuned  $pK_a^*$  reduction from planar brushes to 300 nm silica nanoparticles. After grafting equivalent DMA-MMA brushes from silica nanoparticles, we tracked changes in the hydrodynamic diameter of brush-grafted nanoparticles across pH values to monitor brush swelling or collapse. Comparing DMA69-MMA31 and DMA homopolymer (DMA100-MMA0) brushes, the onset of brush collapse and the inflection points are located at a lower pH for DMA69-MMA31 (dotted lines in Figure 5). Thus, hydro-

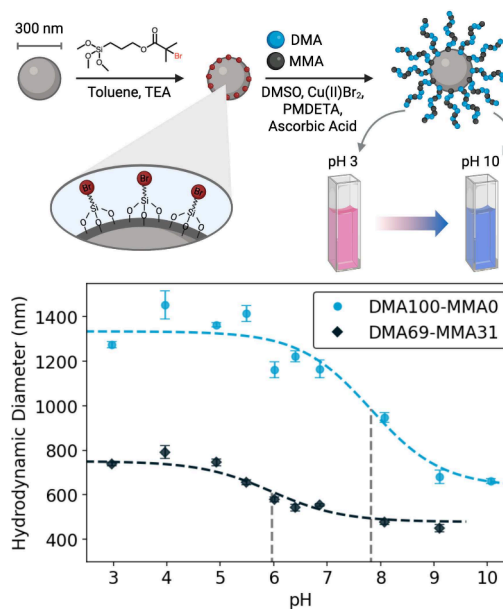


**Figure 5.** MMA incorporation lowers the  $pK_a^*$  of polymer brush grafted silica nanoparticles. Brushes were grafted from silica nanoparticles after an initiator immobilization step. We measured the gradual decrease of hydrodynamic diameter triggered by pH-dependent brush collapse via dynamic light scattering. Data points from DMA100-MMA0 and DMA69-MMA31 were fit to a sigmoidal function, and the inflection point of that curve is denoted by a dotted line. DMA69-MMA31 at pH 10 was excluded due to particle aggregation.

phobic monomer incorporation is an equally effective strategy to tune the  $pK_a^*$  of nanoparticle-tethered brushes as it is for planar brushes. Given that DMA-grafted silica nanoparticles have been applied in nucleic acid delivery,<sup>45</sup> our  $pK_a$  tuning strategy may augment their capacity to navigate endosomal barriers.<sup>46</sup>

While we apply MMA incorporation to increase brush hydrophobicity, MMA repeat units also serve as spacers that separate ionizable DMA units and promote ionization. To decouple these contributions, we prepared copolymer brushes incorporating hydrophilic (rather than hydrophobic) comonomers at matched compositions and checked for increases in  $pK_a^*$  with increasing hydrophilic spacer incorporation. Noting that comonomer hydrophilicity must exceed that of ionized DMA, we selected hydrophilic comonomers that steered clear of confounding effects such as association with DMA (e.g., anionic monomers) or counterion competition with DMA (e.g., cationic monomers) and settled upon zwitterionic comonomers ([2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide or DMAPS). Unfortunately, we ran into synthetic challenges while attempting to make DMA-DMAPS copolymer brushes. Although our proposed mechanism of how hydrophobicity modulates  $pK_a$  is plausible, our study would benefit from computational modeling to clearly illustrate this effect. Both in situ liquid cell ellipsometry and acid–base titration report thickness- and polymer-averaged ionization behavior of polymer brushes and free polymers and not their local  $pK_a$  distribution. Coarse-grained simulations would have deepened our mechanistic understanding by furnishing spatially resolved  $pK_a$  values. Anticipating that the addition of salt would elevate the  $pK_a^*$ , increase hysteresis,<sup>22</sup> and obscure hydrophobicity-dependent trends, we performed swelling ratio measurements and  $pK_a$  titrations in Milli-Q water instead of physiological (150 mM) ionic strength. However, we confirmed that hydrophobicity-driven decreases in  $pK_a^*$  are retained at physiological ionic strength (Figure S22). Future work should determine whether presenting DMA polymers in the form of brushes would trigger comparable levels of toxicity and nonspecific protein adsorption as their free polymer and micellar analogues.

Hydrophobic monomer incorporation is a powerful design handle for tuning the  $pK_a^*$  of weakly polycationic brushes; we achieved a  $pK_a^*$  reduction of 0.32 units with dopant-level (5%) hydrophobic monomer incorporation. Increasing the cationic monomer lipophilicity and augmenting the hydrophobicity of neutral monomers leverage cooperative deprotonation to sharpen the pH-triggered deswelling response and eliminate hysteretic swelling. Relying on surface confinement and crowding, we achieved brush-specific hydrophobicity-dependent  $pK_a^*$  modulation effects. In contrast to polymer brushes that exhibited monotonic decreases in  $pK_a^*$  with hydrophobic monomer incorporation, the  $pK_a$  of compositionally equivalent free polymers increased slightly. We translated our  $pK_a^*$  tuning strategy from planar brushes to brushes grafted from nanoparticles, potentially opening up applications in intracellular pH sensing and nucleic acid delivery. Subtle changes to local hydrophobicity can dramatically reshape the pH responsiveness of polycationic brushes. By engineering pH-sensitive brushes that instantaneously and cooperatively swell or deswell in response to small changes in pH, we could potentially realize pH-targeted delivery to biological environments characterized by dysregulated pH (e.g., hypoxic tumor environments and lysosomal storage disorders).<sup>34,47,48</sup>

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmacrolett.6c00398>.

Experimental procedures and additional polymer characterization data, including XPS, NMR, grafting density estimates, supplemental brush swelling data, and supplemental  $pK_a$  titration data (PDF)

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### Author Contributions

All authors were involved in data acquisition, curation, analysis, and manuscript writing.

### Notes

The authors declare no competing financial interest.

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