UV and NIR-Responsive Layer-by-Layer Films Containing 6-Bromo-7-hydroxycoumarin Photolabile Groups

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INTRODUCTION

Layer-by-layer (LbL) self-assembly is a versatile technique for the preparation of functional materials capable of responding to various stimuli. Forces driving the assembly of materials, notably ion-pairing interactions between polycations and polyanions, allow for the fabrication of films with thicknesses in the nanometers–micrometers range using aqueous polymer solutions for deposition. Disruption of these attractive forces in response to specific stimuli can yield triggered changes in film properties, such as swelling or dissolution. Additionally, the applicability of the LbL technique to a diversity of substrate shapes and sizes allows for the coating of micro- and nanoparticles, comprising gold, polystyrene, or quantum dot cores, among others, provide new opportunities for miniaturized composite, functional materials. Materials assembled using the LbL approach find practical use in a variety of fields, such as drug delivery, antibacterial coatings, and nanofiltration devices.

A host of physical, chemical, and biological stimuli have been utilized to disrupt ion pairing interactions in LbL films. The use of light as a stimulus for responsive materials, including for LbL films, has unique advantages, including enhanced spatiotemporal resolution and potential for wavelength selectivity. Our group has previously reported the use of UV and visible light with wavelengths less than ~450 nm to induce solubility of LbL films fabricated on macroscopic planar substrates and silica microparticles. The design for light-responsiveness of these films is a photolysis-driven charge shift in one of the polyelectrolyte materials. Generally, the photolysis of neutral photolabile esters attached to cationic polymers reveals carboxylate anions at neutral or basic pH, creating an amphoteric polymer in which the positive charges are balanced by the photoinduced negative charges.

Materials that dissolve upon exposure to near-infrared light are important for biological applications, as tissues are maximally transparent to wavelengths between 650 and 950 nm, a “biological transparency window” with minimal absorbance of both water and hemoglobin. LbL films capable of harnessing and responding to NIR light have been reported, notably gold nanoparticle-containing films that undergo photothermal dissolution. Additionally, upconverting nanoparticles (UCNPs) have been reported to degrade LbL films in which they are incorporated through energy transfer to labile nitrobenzyl groups. As an alternative, several photolabile groups are capable of photolysis upon efficient two-photon absorbance (TPA) making them especially important for study and integration into functional materials. The 6-bromo-7-hydroxycoumarin (BHC) group has an appreciable two-photon cross section of photolysis δ_u ≈ 1−2 GM (Goeppert-Meyer Unit, for which 1 GM = 10−50 cm4 s/photons) at 740 nm. As a comparison, the highest two-photon uncaging cross sections for o-nitrobenzyl ester derivatives are on the order of δ_u ≈ 0.1 GM at 750 nm. Given the importance of the BHC group in photoresponsive...
materials, our objectives in this study were to prepare LbL films comprising the 6-bromo-7-hydroxycoumarin moiety and to determine the stability of such films depends on both pH and exposure to either UV or NIR light.

**EXPERIMENTAL SECTION**

**Materials.** All synthetic procedures were performed under an argon atmosphere with magnetic stirring. Silica gel (230–400 mesh) was used as the stationary phase for purification via flash chromatography. Quartz slides were purchased from Advalabs Technologies. Silicon dioxide microparticles, 1 μm in diameter, were purchased from Sigma-Aldrich. Glass bottom dishes for multiphoton experiments were purchased from ibidi GmbH (μ-Dish 35 mm, high, Glass Bottom: Ø 35 mm, high wall (2 mL volume), # 1.5H (170 μm ± 5 μm) D 263 M Schott glass, sterilized). All commercial chemicals were used without further purification with the following exceptions: anhydrous toluene was obtained from an Innovative Technologies PureSolv 400 solvent purifier, and pyridine was distilled immediately prior to use.

**Characterization.** NMR characterization of synthesized organic compounds was performed on a Bruker Avance III 500 MHz NMR spectrometer. Molecular weight distributions of polymers were determined by gel permeation chromatography (GPC) using a Shimpadz chromatograph and Tosoh polystyrene standards. GPC chromatograms of the polymers derived in this paper were obtained using a Tosoh Bioscience TSKgel GMHHR column (7.8 mm ID x 300 cm, 5 μm) equipped with a TSKgel Guard Hi, 6 (6 mm ID x 4.0 cm, 5 μm). Polymer samples were eluted with a mobile phase of 2% triethylamine in THF at a flow rate of 0.75 mL/min, and monitored by UV−vis and refractive index detectors. Absorbance spectra of solid and liquid samples were obtained using a Varian Cary 100 Spectrophotometer in double beam mode. Fluorescence spectra were obtained using a PTI Quantum Master 4 equipped with a 75 W Xe lamp. Zeta potential measurements were made using a Malvern Zetasizer Nano Series with disposable capillary cells (DTS10070). AFM images were obtained using a Veeco D31005-1 in tapping mode. Gwyddion 2.4 software was used to process results.

**Synthetic Procedures.** Synthesis of 2. Previously reported condensation of 4-bromoresorcinol with ethyl 4-chloroacetocetate afforded halogenated coumarin 1.

\[ \text{(5 mL) was added acetic anhydride (0.30 mL, 3.2 mmol). The reaction mixture was stirred at room temperature. After 16 h, the reaction mixture was concentrated under vacuum, dissolved in CH}_2\text{Cl}_2, \text{chloroform, and purified by flash chromatography (20% ethyl acetate/hexanes) to afford 3 as an off-white solid.} \]

Yield: 0.246 g, 80%. \( \text{H NMR (500 MHz, CDCl}_3\text{)}; \delta (ppm) 7.82 (s, 1 H), 7.24 (s, 1 H), 6.54 (s, 1 H), 6.29 (s, 1 H), 5.76 (s, 1 H), 5.35 (s, 2 H), 2.43 (s, 3 H), 2.05 (s, 3 H). \text{13C NMR (125 MHz, CDCl}_3\text{)}; \delta (ppm) 168.0, 166.3, 159.5, 153.4, 150.8, 148.0, 135.3, 127.9, 127.6, 116.8, 113.9, 113.2, 112.3, 61.22, 20.9, 18.4. HRMS calculated mass [M + H]+: 338.9866. Experimentally determined [M + H]+: 338.9840.

**Preparation of Alexa 488-labeled Poly(ethylene imine).** Alexa-488-labeled poly(ethylene imine) (PEI) was prepared as described in a previous publication by conjugating the N-hydroxysuccinimimidyl (NHS) ester of Alexa Fluor 488 to PEI. We estimate a 0.3% loading of the dye based on the measured absorbance and the extinction coefficient of Alexa-488.

**Polymeric Solutions Used for LbL Assembly Experiments.** Four different polyelectrolyte solutions were prepared to fabricate the various LbL films studied in this manuscript. All polyelectrolyte solutions were prepared to have a concentration of 0.2% (w/v). Three polycationic solutions were prepared: (i) solutions of P1 were adjusted to pH 3.3 with 0.1 M HCl and 0.1 M NaOH, (ii) Alexa-488-labeled PEI (PEI-488), as synthesized above, was prepared in 0.1 M NaCl with pH adjusted to 3.3 with 0.1 M HCl and 0.1 M NaOH, and (iii) photoinert poly(diallyldimethylammonium chloride) (PDADMAC) solutions were prepared to adjust pH 3.3 with 0.1 M HCl and 0.1 M NaOH. The single polyanionic material used was polystyrenesulfonate (PSS), prepared in a solution of 0.1 M NaCl.

**LbL Assembly of Planar Thin Films.** Slides of quartz were cleaned with dust-free cloth to remove debris, and then air plasma cleaned using a Harrick Plasma cleaner (PDC-32G) for 1 min immediately prior to use. Substrates were first submerged into a 0.2% (w/v) polycation solution for 9 min, followed by a 1 min rinse in deionized water. The films were then submerged into a polyanion solution for 9 min, rinsed with 1 min, and the cycle repeated for the desired number of bilayers. After the final washing step of the deposition, the completed films were gently dried over a stream of compressed air. Films comprising eight bilayers were prepared for absorbance and pH dependence studies. The single polyanionic material used was polystyrenesulfonate (PSS), prepared in a solution of 0.1 M NaCl.
providing a light power density of approximately 15 mW/cm² between 295 and 435 nm. Alexa Fluor 488 fluorescence was monitored with an excitation wavelength of 470 nm, emission window of 485–685 nm and slit widths corresponding to a bandpass of 5 nm for both excitation and emission monochromators.

**Determining Extent of Coumarin Photolysis.** To determine percent photocleavage of coumarin side chains necessary to promote film dissolution, the rinse solution of an irradiation experiment was analyzed. Specifically, a (P1/PSS)₃ film was rinsed with water for 1 min and irradiated for 15 min as above (11 mW/cm²). The irradiated film was then rinsed in 2 mL of 0.1 M NaHCO₃ for 15 min. A 0.5 mL aliquot of the rinse solution was dialyzed against 0.1 M NaHCO₃ for 3 days, and diluted to 1 mL with 0.1 M NaHCO₃. Another 0.5 mL aliquot of the rinse solution was stored in the dark for 3 days and then diluted to 1 mL with 0.1 M NaHCO₃. The two solutions were comparatively analyzed by absorbance and fluorescence (365 nm excitation wavelength, emission window of 400–600 nm, slit widths corresponding to a bandpass of 5 nm for both excitation and emission monochromators).

**pH Dependent Irradiation Experiments.** For pH-dependent irradiation studies, both 295 and 435 nm long pass filters were used, providing a total power density of 20 mW/cm². To demonstrate pH-dependent dissolution, a (P1/PSS)₃ film was soaked in 0.1 M HCl for 5 min, rinsed with water, dried, and measured by absorbance. The film was then rinsed with water again and irradiated for 15 min. After irradiation, the film was soaked in 0.1 M NaHCO₃ for 5 min, rinsed with water, dried, and measured by absorbance. The film was then rinsed with 0.1 M NaHCO₃ and irradiated in its batchetomically shifted state. Absorbance measurements again followed 0.1 M NaHCO₃ soaking for 5 min, rinsing with water, and drying.

**LBL Assembly of Films on Silica Microparticles.** A 0.1 mL aliquot of a 5% aqueous suspension of silica microparticles (1 μm diameter) was washed with deionized (DI) H₂O prior to use. A typical wash step proceeded as follows: the particles were dispersed in 1 mL of DIH₂O, shaking vigorously for 1 min. The particles were then collected by centrifugation (2 min), transferred to a cuvette, and held in the dark for 15 min. The spheres were then dispersed in 1.5 mL of polycation solution, and the supernatant was measured. After washing, the particles were then dispersed in 1.5 mL of polycation solution, and mixed by vigorous shaking for 1 min. The suspensions were then mixed on a VWR waver for 60–120 min, until a charge shift in zeta potential was observed by dynamic light scattering (DLS) (see Supporting Information). After centrifugation, the spheres were washed with deionized water three times as detailed above. Repeated alternating dispersal steps in the two polyelectrolytes, each time followed by three washes in deionized water, was utilized to build up films comprising a total of four bilayers. For the third polycation layer, PEI-488 replaced P1. (P1/PSS/PEI-488/PSS)₂[P1/PSS] film was then rinsed with 0.1 M NaHCO₃ and irradiated in its batchetomically shifted state. Absorbance measurements again followed 0.1 M NaHCO₃ soaking for 5 min, rinsing with water, and drying.

**UV Light Irradiation Experiments for Film Release from Microparticle Substrates.** A 0.1 mL aliquot of LbL-coated microparticles was washed with 10 mL phosphate buffer (pH 7.8) two times before use. As a control experiment, the particles were dispersed in 1 mL of buffer, transferred to a cuvette, and held in the dark for 15 min. The spheres were collected by centrifugation (2 min, 2000×g), and the filtered supernatant (0.2 μm filter pore size) was measured by fluorescence. Alexa Fluor 488 fluorescence was monitored, with an excitation wavelength of 470 nm, emission window of 490–690 nm, and slit widths corresponding to a bandpass of 5 nm. The spheres were dispersed in buffer, transferred to a cuvette, and irradiated for 15 min with a 200 W Hg/Xe arc lamp with deep UV light filtered out using a 295 nm long pass filter, providing a total power density of 30 mW/cm². The irradiated suspensions were again collected by centrifugation, and the fluorescence of the filtered supernatant was measured.

**Single-side LBL Assembly for NIR Experiments.** To be suitable for analysis by multiphoton microscopy, LBL films were deposited onto ibidi μ-dishes. The borosilicate glass bottoms were plasma cleaned for 1 min immediately prior to use. The top surface of the coverglass bottom was completely submerged in polycation solution for 9 min, which was then drawn out of the dish via pipet. The film was then washed twice with ~1 mL of deionized water to ensure removal of all excess solution, first for approximately 5 s, and then again for 1 min. After the two washing steps, the polyanion solution was added to the μ-dish and rinsed as above. This procedure was repeated multiple times to accumulate bilayers of film on the glass substrate. All films coated onto μ-dishes for NIR experiments comprised 14 bilayers. Three different types of films were prepared: (i) (P1/PSS)₄ for basic multiphoton experiments, (ii) [(P1/PSS)₄(PEI-488/PSS)₂](P1/PSS), to monitor dye release, and (iii) films in which PDADMAC replaced P1 [(PDADMAC/PSS)₂(PEI-488/PSS)₂](PDADMAC/PSS), as negative controls.

**Two-Photon Imaging and Irradiation Experiments.** Two-photon excited fluorescence (TPEF) imaging and photolysis experiments were performed using a Leica TCS SP8 confocal microscope. Excitation was performed at 720 nm with a mode-locked Ti:sapphire laser (Insight DS+, Spectra-Physics, Inc.), focused with a dry 10×/0.40 HC PL APO objective lens. TPEF images were collected over a 1.55 × 1.55 mm field of view with 0.75×z zoom and 512 × 512 binning, using a hybrid GaAsP avalanche photodiode detector (Leica HyD) and a S25 ± 25 nm bandpass filter. Sixteen frames were accumulated for each TPEF image at a frame rate of 1.3 Hz and using low laser power in order for the photoinduced dissolution to proceed slowly during imaging. A 488 nm diode laser was used to perform single photon fluorescence imaging of PEI-488-containing films with detection by a Leica HyD detector.

First, a (P1/PSS)₃ film (deposited on an ibidi microdish) was immersed in water before imaging. The top face of the coverslip (and therefore the film) was brought into focus by finding the peak local reflectance of a 488 nm laser. For scans intended for photolytic film dissolution, a square zoomed-in region (232.5 × 232.5 μm) was irradiated continuously at higher laser power for a duration of 5 min. The power required to induce dissolution was determined empirically and could vary significantly between experiments executed on different days due to the nonlinear nature of the photochemical reaction and variations in laser focus on the sample. The film was then imaged by TPEF after returning to the original zoom (1.55 × 1.55 mm) (Figure 7, top).

Second, to monitor this process by single-photon excitation fluorescence, scans were performed on a [(P1/PSS)₄(PEI-488/PSS)₂](P1/PSS) film which had been submerged in 0.1 M NaHCO₃. Again, a zoomed-in (116.25 × 116.25 μm) region was irradiated continuously for 2 min with 720 nm light at 53 mW laser power, while simultaneously collecting TPEF images at a 1.3 Hz sampling rate (see Supporting Information for TPEF intensity traces). This irradiation was repeated three additional times. After the four irradiation experiments, a zoomed out (1.55 × 1.55 mm) fluorescence image was captured via 488 nm excitation over a spectral bandwidth of 500–600 nm at 0.1 mW laser power and averaged over 20 frames. An analogous negative control experiment was performed on a [(PDADMAC/PSS)₂(PEI-488/PSS)₂](PDADMAC/PSS) film. Four zoomed in (116.25 × 116.25 μm) areas were irradiated at 53 mW laser power for 2 min each. A zoomed out (1.55 × 1.55 mm) fluorescence image was captured via 488 nm excitation over a spectral bandwidth of 510–600 nm at 0.1 mW laser power and signal averaged over 20 frames (Figure 7, bottom).

Finally, dependence of film dissolution on laser power was also determined. A [(P1/PSS)₄(PEI-488/PSS)₂](P1/PSS), film was submerged in 0.1 M NaHCO₃ and a zoomed-in (116.25 × 116.25 μm) area was irradiated continuously for 2 min with 720 nm light. Four separate irradiation experiments were performed, at 20%, 10%, 5%, and 2% (52, 26, 13, and 5 mW, respectively) laser power (Figure 8).

After fluorescence imaging, films were rinsed with 0.1 M NaHCO₃ for 5 min, water for 1 min, and allowed to dry under ambient conditions. Atomic force microscopy (AFM) was used to measure film height of irradiated films.
RESULTS AND DISCUSSION

Experimental Design. We prepared a random copolymer of coumarin-substituted methacrylate 3 and DMAEMA as the polycation for a PEM material. DMAEMA was chosen as a light-inert comonomer to bear positive charge in neutral or acidic solution. The coumarin-functionalized methacrylate monomer 3 was utilized as the light-responsive unit, capable of exposing a negative charge upon photolysis. As depicted in Figure 1, photolysis of an LbL film containing this polycation results in photocleavage of the coumarin groups and consequent generation of negative charge and increasing hydrophilicity through carboxylate deprotection. The use of 6-bromo-7-hydroxycoumarin, a chromophore capable of undergoing single- and multiphoton uncaging processes, allowed for this change in overall net charge and increase in polymer hydrophilicity to be possible with both UV and IR light.

Polymer Synthesis. As shown in Scheme 1, preparation of coumarin-derivatized methacrylate 3 required a three-step process in 40% overall yield. Alkylation of methacrylic acid with 1 provided a facile approach for preparation of the photoresponsive coumarinyl ester. Protection of the phenol in the 7-position with acetic anhydride, to prevent the phenol moiety from completely inhibiting subsequent radical polymerizations, afforded the target monomer 3. Radical chain random copolymerization of 3 with DMAEMA yielded the polymeric precursor to coumarin-containing P1. The molecular weight distribution of polymer samples prepared in this manner had number-average and weight-average molecular weights of approximately 4–5 kDa and 12–14 kDa as determined by gel permeation chromatography relative to polystyrene standards. Increasing overall monomer concentration or decreasing molar ratio of the radical initiator did not increase these molecular weights. We suspect that the small concentrations of phenol groups, revealed by adventitious acetate hydrolysis, may have been responsible for the limited degrees of polymerization. In agreement with the feed ratio in the reaction, the molar ratio of the monomers within the isolated polymer was 1:1 (coumarin/DMAEMA), as determined by $^1$H NMR spectroscopy. Complete hydrolysis of the acetyl protecting group after polymerization was possible by stirring the polymer in water at room temperature for 1 week, yielding target polymer P1. As shown in Figure 2, the changes in proton chemical shifts of P1 after this reaction are consistent with phenol deprotection.

Figure 1. Design of photoactive polycation that changes in net charge and hydrophobicity upon photolysis and subsequent film dissolution.

UV-Induced Dissolution of P1/PSS Films. Before demonstration of susceptibility to NIR irradiation, we first showed that traditional photolysis of the coumarin pendants, using UV light, promoted PEM film degradation. Initially, absorbance of the film (P1/PSS) before and after a 15 min irradiation, but before rinsing, showed only a decrease in absorbance of 0.02 OD at 334 nm (Figure 4, left). In addition, there is no decrease at 220 nm, the absorbance band primarily due to the arylsulfonate rings of PSS. We used 10 mM phosphate buffer (pH 7.8) to rinse the films for 15 min after irradiation to ensure that any carboxylic acids would be deprotonated and negatively charged, reducing the overall net positive charge on the photosensitive polymer and encouraging film dissolution. After a single irradiation and buffer rinse, film absorbance decreased by approximately 60% at both 220 and 335 nm. Repeated irradiation and rinsing steps resulted in near complete disappearance of absorbance. On the basis of UV/vis and fluorescence spectrophotometric analysis of the rinsing solution of a dissolved film upon dialysis to separate small molecule coumarin photolysis products from coumarin bound to polymer chains, we estimate that full dissolution of such P1/PSS films occurs upon photolysis of ~60–80% of the coumarin groups (see Supporting Information).

Moreover, as shown in Figure 4, we also monitored release of a photoinert, dye-conjugated polycation from these PEM films. Specifically, a solution of poly(ethylenimine) (PEI) functionalized with dye Alexa Fluor 488 replaced the photolabile polycation during the assembly process for a single layer on PEM-coated microparticles, and two layers in the films on planar quartz. For the planar [(P1/PSS)$_2$]/(PEI-488/PSS)$_2$/(P1/PSS)$_2$] films (Figure 4, center), we measured the intensity of
fluorescence from the dye-conjugated polymer in the solution used for rinsing the films after irradiation, to monitor release of the fluorescent dye during photoinduced film degradation. Release of Alexa 488 dye occurs as shown by a 10-fold increase in fluorescence signal of the rinsing solution after irradiation. This result suggests that the decrease in film absorbance upon irradiation and rinsing is in fact indicative of degradation of the film.

Release of dye-functionalized PEI was also demonstrated for \((\text{P1/PSS})_2(\text{PEI-488/PSS})_1(\text{P1/PSS})_1\) films deposited on SiO\(_2\) microparticles. Figure 4 (right) shows fluorescence measurements demonstrating the selective release of Alexa Fluor 488 upon irradiation with light \(\lambda > 295\) nm. First, the spheres were dispersed in 10 mM phosphate buffer (pH 7.8) and held in the dark for 15 min as a control experiment. Fluorescence measurement of the filtered supernatant solution upon centrifugation showed negligible fluorescence. Subsequent irradiation of the suspended spheres for 15 min, however, resulted in a 30-fold increase in fluorescence intensity from the filtered supernatant.

**Response of Unirradiated P1/PSS to pH.** As a control experiment, \((\text{P1/PSS})_8\) films were immersed in 0.1 M NaHCO\(_3\) without irradiation, to ensure that photolysis was necessary for film dissolution. While the films were resistant to dissolution, the absorbance spectra of these films displayed a bathochromic shift at high pH. As shown in Figure 5, this shift was reversible, with initial rinsing in 0.1 M HCl (pH 1.0) for 5 min showing a spectrum comparable to the films as prepared with a \(\lambda_{\text{max}}\) of 336 nm. After 5 min of immersion in 0.1 M NaHCO\(_3\), the \(\lambda_{\text{max}}\) of the spectrum shifted to 349 nm with a shoulder at approximately 400 nm. Subsequent rinsing in 0.1 M HCl yielded a nearly identical absorbance spectrum to that initially observed, suggesting no degradation of the film.

We attribute this shift in absorbance to the Brønsted-Lowry acidity of the phenol group of the coumarinyl pendants of P1; deprotonation of the phenol increases donor–acceptor character of the coumarin chromophore and results in a bathochromic shift of absorbance.\(^4\) Consistent with this explanation, increasing pH of an aqueous solution containing only P1 results in a reversible bathochromic shift of the coumarin chromophore (Figure 5). Through spectrophotometric titration of such aqueous solutions of P1, we estimate the \(pK_a\) of this phenol to be 5.7, consistent with a previously reported value of 5.9.\(^5\)

This stability of unirradiated \((\text{P1/PSS})_8\) films at basic pH is particularly noteworthy due to the insight it provides on the nature of the forces that dictate film disruption upon photolysis. Deprotonation of the phenol presents the same shift in the formal charge of the side chain of P1 (neutral to negative) as photolysis, yet only photolysis promotes film dissolution. We therefore conclude that it is not only the decrease in net positive charge and change in ion pairing of P1 upon photolysis that results in film dissolution, but also some combination of reducing interchain dispersion interactions and increasing P1

![Figure 2. 1H NMR spectra (in DMSO-\(d_6\)) of the precursor to polymer P1, before hydrolysis, and P1, showing successful postpolymerization acetate hydrolysis. 1H NMR spectra of corresponding protected and deprotected coumarinyl methacrylates in DMSO-\(d_6\) provide comparisons that show closely matched chemical shifts of the aromatic protons (a, b, and c).](image)

![Figure 3. Film absorbance showed a linear dependence on the number of bilayers deposited. Top: Spectra of \((\text{P1/PSS})\) film deposited on planar quartz, dried with compressed air. In order of increasing absorbance: 0, 1, 2, 4, 6, and 8 bilayers. Bottom: Plot of increasing film absorbance at 334 nm as a function of deposited bilayers.](image)
Figure 4. Left: Decrease of solid-state absorbance signal before (green) and after (red) 15 min UV photolysis, as well as after a 15 min buffer rinse (blue). The result of a second irradiation and rinsing step is also shown (black). Center: Release of Alexa Fluor 488-functionalized PEI from planar [(P1/PSS)2(PEI-488/PSS)2(P1/PSS)] film after 5 min buffer rinses before and after 15 min UV irradiation. Right: Release of Alexa Fluor 488 from silica microparticles coated with a [(P1/PSS)2(PEI-488/PSS)2(P1/PSS)] film before and after 15 min UV irradiation.

Figure 5. Top: Dependence of (P1/PSS)n film absorbance on pH. Film manipulations in order: Black, 5 min rinse in 0.1 M HCl. Red, 5 min rinse in 0.1 M NaHCO3. Blue dots, 5 min rinse in 0.1 M HCl. Bottom: Solution-state spectra of P1 (30 μg/mL) at different protonation states. Red: pH 2.6. Violet: pH 7.1. Blue: pH 9.8.

The observed acidochromism of the coumarin side chains also enables longer wavelengths of light in the visible range of the spectrum to yield coumarin photolysis and film dissolution. As shown in Figure 6, (P1/PSS) films first immersed in 0.1 M HCl, to fully protonate all phenol groups of P1, did not dissolve upon irradiation with light from a 200 W Hg/Xe lamp passed through a 435 nm long pass filter, followed by a rinse in 0.1 M NaHCO3. This is consistent with the poor extinction of P1 at these wavelengths at low pH. Subsequent irradiation at λ > 435 nm and rinsing under identical conditions, after the previous exposure to base, led to film dissolution, as the phenoxide-substituted chromophore absorbs these wavelengths of light. This feature takes advantage of the known effect of the bromine substituent on the coumarin chromophore increasing the acidity of the phenol and increasing the extinction coefficient at longer wavelengths.

Two-Photon Induced Imaging and Dissolution of Films. Two-photon excitation processes offer the potential for using optimal wavelengths of light for biological efficacy but require high laser power density in order to increase the local photon flux sufficiently. To demonstrate the use of NIR light for disrupting BHC-containing LbL films, we performed two-photon excitation experiments on planar LbL films comprising 14 bilayers of P1 and PSS. In these experiments, we used 720 nm as the excitation wavelength, as BHC has a measurable absorbance at 360 nm, using a mode locked Ti-sapphire laser. We executed NIR irradiation experiments while films were submerged in water or 0.1 M NaHCO3 within microdishes (Figure 7). Laser power used to collect fluorescence images were lower than those used for inducing photolysis, which was determined empirically for experiments executed on different days.

As seen in the fluorescence image in Figure 7 (top), the area of the (P1/PSS)14 film that was irradiated with higher laser power was no longer fluorescent. The fluorescence microscopy image suggests dissolution occurred, as the fluorescence of the NIR-irradiated area is comparable to that of the nearby area that had been removed by scoring with a scalpel. In a control experiment to establish the necessity of the BHC group to observe this magnitude of photoinduced decrease in fluorescence, we compared the effect of 720 nm irradiation on the fluorescence images of two films (Figure 7, bottom). One film comprised the following pattern of deposition [(P1/PSS)2(PEI-488/PSS)2]14(P1/PSS)2 totaling 14 bilayers. Four irradiated squares in those fluorescence images showed >80% decrease in fluorescence intensity. Alternatively, films in which P1 was replaced with the photochemically inert PDADMAC showed only a 10−15% decrease in fluorescence intensity upon identical exposure to 720 nm, which we attribute to photobleaching of the fluorescent dye.

To further demonstrate NIR-induced disruption of P1/PSS films, we measured the topology of irradiated films by atomic force microscopy (AFM). When scored with a razor blade, this (P1/PSS)14 film had an average thickness of approximately 70 nm (see Supporting Information for line scan of scored film). Figure 8 shows an AFM image of a corner of the NIR-photolyzed area, which shows dissolution of the irradiated film. Line scans of AFM images reveal an average change in height of...
77 ± 5 nm between the irradiated and unirradiated areas with a small RMS roughness of the irradiated area of 1.8 nm (see the Supporting Information for the line scans). Together, these experiments demonstrate PEM film dissolution upon irradiation at 720 nm. In a separate experiment we also showed qualitatively that increased laser power correlated with increased diminution of fluorescence and decreased film thickness as measured with AFM (Figure 8, bottom). Specifically, four areas were irradiated at specific laser powers of 20%, 10%, 5%, and 2% laser power (52, 26, 13, and 5 mW,
respectively) and then imaged by fluorescence. The squares were then imaged by AFM, showing a correlation in film height comparable to that of the change in fluorescence decrease as a result of irradiation.

■ CONCLUSION

This work has several important implications for stimuli-responsive layer-by-layer films from both fundamental and applied perspectives. First, it broadens the suite of photo-cleavable groups that can be included into polyelectrolyte multilayer films by establishing the successful integration of the BHC group into the assembly and photoinduced disassembly of films. Second, it demonstrates the potential for using near-infrared light to promote photochemical disruption of layer-by-layer films through two-photon absorbance and resulting photocleavage of the BHC group. This feature expands the outlook of these materials in applications, such as triggered delivery of cargo, which would require penetration into tissues. Third, the observation that pH-induced charge shifting of phenol to phenolate did not dissolve LBL films highlights that hydrophobic and/or dispersion interactions can play a critical role in film stability, and that such factors should be considered in the design of related stimuli-responsive materials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b01469.

NMR spectra of all new compounds and polymers, zeta potential measurements, AFM and TPEF trace measurements (PDF)

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Notes
The authors declare no competing financial interest.

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