Supporting Information

Increased Protein Sorption in Poly(acrylic acid)-Containing Films Through Incorporation of Comb-like Polymers and Film Adsorption at Low pH and High Ionic Strength

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[†]Professor Baker passed away unexpectedly on October 17, 2012. We dedicate this work as a memorial to him.

Figure S11. Ellipsometric thicknesses of $(PAH/PAA)_5$ multilayers deposited from polyelectrolyte solutions at various pH values in the presence and absence of 0.5 M NaCl.

Figure S12. Lysozyme sorption in $(PAH/PAA)_5$ multilayers as a function of pH.

Figure S13. Reflectance FTIR spectra of $(PAH/PAA)_5$ and $(PAH/PAA)_5PAH$ films before and after sorption of lysozyme.

Figure S14. Lysozyme sorption in $(PAH/PAA)_5$ multilayers as a function of the concentration of NaCl in the 1 mg/mL lysozyme sorption solution.

Figure S15. Time-evolution of lysozyme sorption in $(PAH/PAA)_5$ multilayers immersed in a 1 mg/mL lysozyme solution in 20 mM phosphate buffer (pH 7.4).

Figure S16. AFM images of $(PAH/PAA)_5$ films (a) before and (b) after sorption of lysozyme. The films were adsorbed from pH 3 solutions containing 0.5 M NaCl.

Figure S17. AFM images of $(PAH/PAA)_5$ films (a) before and (b) after adsorption of lysozyme. The films were adsorbed from pH 9 solutions containing 0.5 M NaCl.

Figure S18. Reflectance FTIR spectra of (a) $(PAH/PAA)_5$ films and (b) $(PAH/PHEMA\text{-}g\text{-}PAA)_5$ films after immersion in 20 mM phosphate buffers adjusted to different pH values.

Figure S19. Repetitive lysozyme sorption on $(PAH/PAA)_2$ films.

Figure S20. Reflectance FTIR spectra of $(PAH/PAA)_2$ films before and after immersion in a 20 mM phosphate solution (pH 7.4).

Figure S21. Reflectance FTIR spectra of $(PAH/PAA)_5$ films before and after immersion in a 20 mM phosphate solution adjusted to different pH values. $(PAH/PAA)_5$ films were deposited from pH 9.0 polyelectrolyte solutions containing 0.5 M NaCl.

Figure S22. Lysozyme binding capacities of (a) (PAH/PHEMA-g-PAA)n and (b) (PAH/PAA)n multilayers (n=1~5) deposited from polyelectrolyte solutions at pH=3 both in the presence and absence of 0.5 M NaCl.

Preparation of PHEMA-*g*-PAA

ATRP of HEMA

PHEMA was prepared by ATRP using a modified literature procedure.[1] Ethyl 2-bromoisobutyrate (EB*i*B) (97 mg, 0.50 mmol), bpy (156 mg, 1.00 mmol), MEK (2 mL), isopropanol (1 mL), and HEMA (6.44 g, 6.0 mL, 50 mmol) were added to a 25-mL Schlenk flask and degassed by three freeze-pump-thaw cycles. CuCl (50 mg, 0.50 mmol) was added under a flow of N_2, and the mixture was stirred at room temperature for 16 h. The polymer solution was then exposed to air and diluted in acetone/isopropanol (volume ratio 2:1). The polymer solution was passed through a basic alumina column, and the purified polymer was recovered by evaporating the solvent. ^{1}H NMR ($CDCl_3$, 10% d^6-DMSO): δ = 3.87 (-CH_2-OCO, s, 2H), 3.59 (-CH_2-OH, s, 2H), 2.05-1.60 (-CH_2-C, br, 2H), 0.89 (-CH_3, s, 1H), 0.73 (-CH_3, s, 2H) ppm.

Preparation of the macroinitiator, poly(2-(2-bromoisobutyryloxy)ethyl methacrylate) (PBIEM)[2]

Over 60 min, α-bromoisobutyryl bromide (7.4 g, 32 mmol) was added dropwise to a 0 °C solution of PHEMA (2.0 g, 15 mmol of OH groups) in anhydrous pyridine (30 mL). The mixture was stirred for 3 h at 0 °C and then for 12 h at room temperature. The insoluble pyridinium salt was removed by filtration, and the solvent was removed by rotary evaporation. The crude polymer was dissolved in 10 mL THF and purified by precipitation in 250 mL methanol. Yield: 31 %. ^{1}H NMR ($CDCl_3$, 10% d^6-DMSO): δ = 4.17 (-CH_2-OCO, s, 2H), 4.00 (-CH_2-OCO, s, 2H), 1.77 (-C(Br)(CH_3)$_2$, s, 6H), 1.65 (-CH_2-C, s, 2H), 0.87 (-CH_3, s, 1H), 0.72 (-CH_3, s, 2H) ppm.

Preparation of PHEMA-*g*-poly(*tert*-butyl acrylate) (PHEMA-*g*-P*t*BA)[2]

In a 25 mL Schlenk flask, PBIEM (0.23 g, 0.82 mmol 2-bromoisobutyryloxy groups), $CuBr_2$ (9 mg, 40 mmol), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA) (150 g, 86 mmol), *t*BA (10.5 g, 12 mL, 82 mmol) and acetone (3 mL) were combined and degassed by three freeze-pump-thaw cycles. CuBr (0.118 g, 0.82 mmol) was added under a flow of N_2, and the mixture was stirred at 60 °C for 23 h. The

resulting polymer was dissolved in acetone and purified by passage through a basic alumina column (to remove catalyst) and precipitation in water. ^{1}H NMR ($CDCl_3$): δ = 2.36-2.07 (-CH_2-C, br, 2H), 1.90-1.68 (-C*H*-COO*t*Bu, br, 1H), 1.47-1.20 (-OC(CH_3)$_3$, br, 9H) ppm.

Hydrolysis of PHEMA-*g*-P*t*BA to PHEMA-*g*-PAA[3]

In a 10-mL round bottom flask equipped with a condenser, a solution of PHEMA-*g*-P*t*BA (3.0 g, 23 mmol *tert*-butyl ester groups), dioxane (45 mL) and concentrated HCl (15 mL, 0.48 mol) was heated to reflux. After about 2 h, the solution was cooled, and the excess reagents were removed by evaporation under vacuum. ^{1}H NMR (D_2O, pH>10 adjusted by NaOD): δ = 2.37-2.03 (-C*H*-COO^-, br, 1H), 1.90-1.35 (-CH_2-C, br, 2H) ppm.

Table S1. Efficiency of lysozyme elution from $(PAH/PAA)_5$ films as a function of the eluent KSCN concentration and elution time. Sorption solutions contained 1 mg/mL of lysozyme in 20 mM phosphate buffer (pH 7.4), and binding occurred for 16 h. The eluent was prepared in the same buffer. The elution efficiency was calculated from the amount of lysozyme in the film before and after elution as determined from FTIR spectra. The $(PAH/PAA)_5$ film was adsorbed from a pH 3 solution containing 0.5 M NaCl.

	ratio of eluted – to – bound lysozyme		
elution time	0.10 M KSCN	0.25 M KSCN	1.00 M KSCN
1 min	0.56	0.88	0.96
10 min	0.81	0.93	-
30 min	0.84	0.93	-
1 hour	0.85	0.94	-

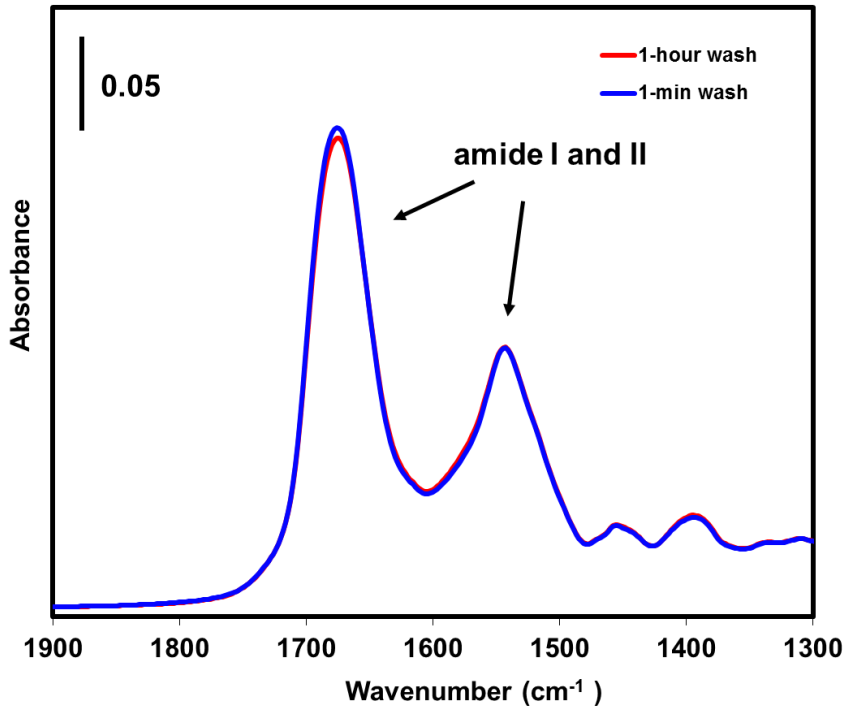


Figure S1. Reflectance FTIR spectra of $(PAH/PAA)_5$ films immersed for 16 h in 20 mM, pH 7.4 phosphate buffer containing 1 mg/mL lysozyme. Prior to obtaining the spectra, the films were rinsed for either 1 min or 1 h in washing buffer, and then rinsed with 10 mL water and dried with N_2 in both cases before FTIR measurements. For the 1-h rinse, the sample was immersed in washing buffer in a vial and shaken for 1 h. The 1-min rinse occurred from a Pasteur pipette.

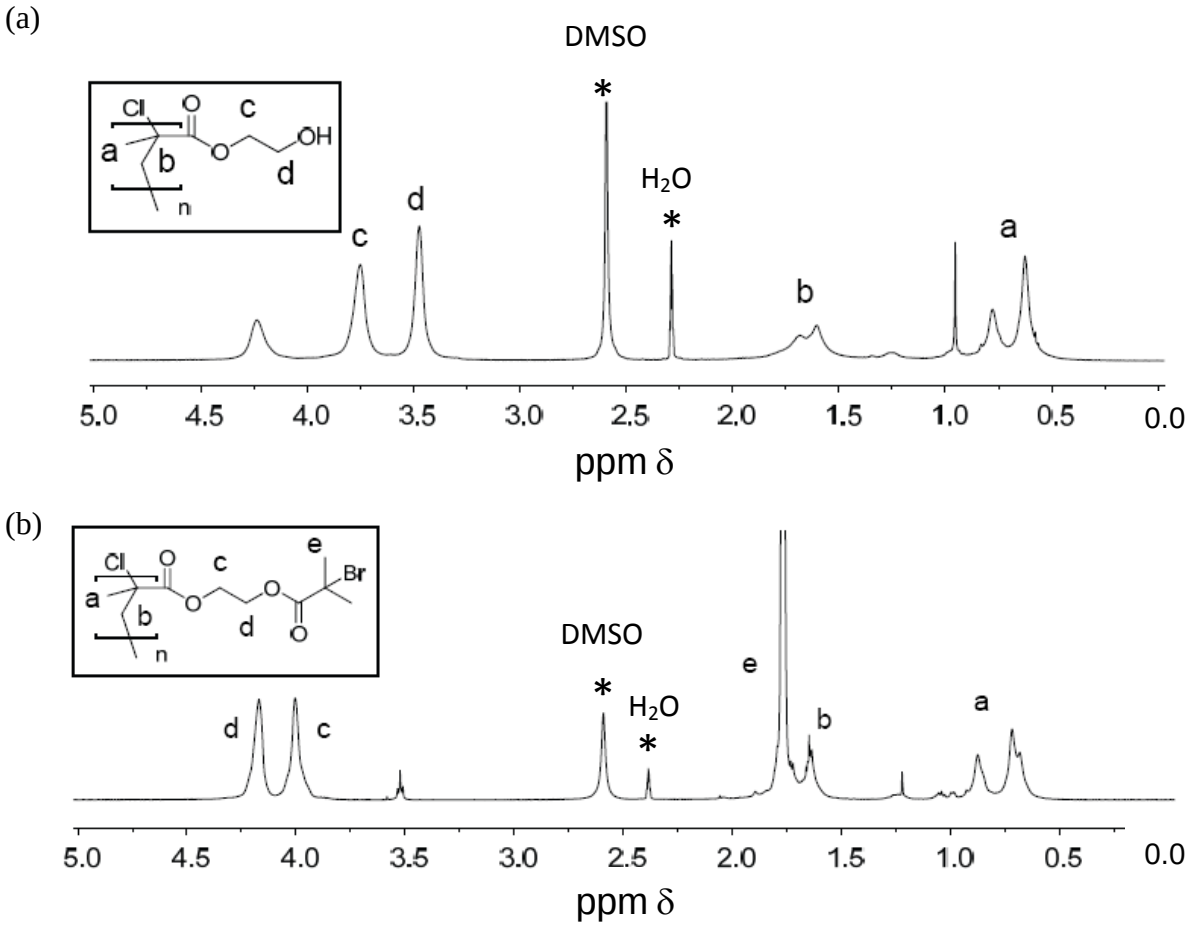


Figure S2. ^{1}H NMR spectra of (a) PHEMA and (b) PBIEM. For both analyses, the solvent was 90% $CDCl_3$/10% d^6-DMSO.

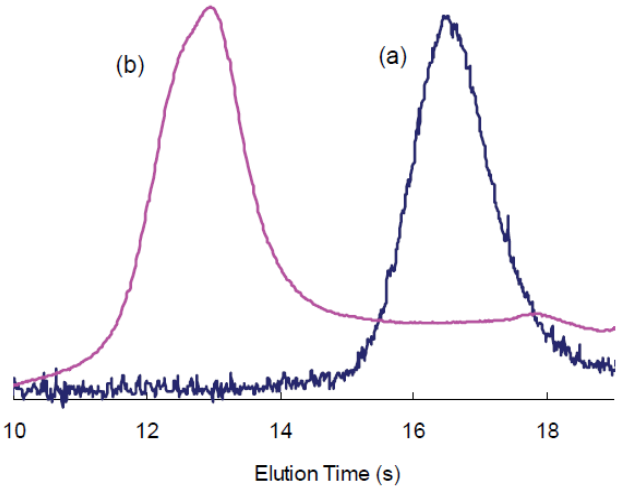


Figure S3. Gel-permeation chromatograms of (a) PBIEM and (b) PHEMA-g-P*t*BA. The molecular weights were determined by gel permeation chromatography at 35 °C, using two PLgel 10µ mixed-B columns in series (manufacturer-stated linear molecular weight range of 500 to 10×10^6 g/mol), and THF as the eluting solvent at a flow rate of 1 mL/min. An Optilab rEX (Wyatt Technology) refractive index detector and a DAWN EOS 18-angle light scattering detector (Wyatt Technology) with a laser wavelength of 684 nm were used to calculate absolute molecular weights.

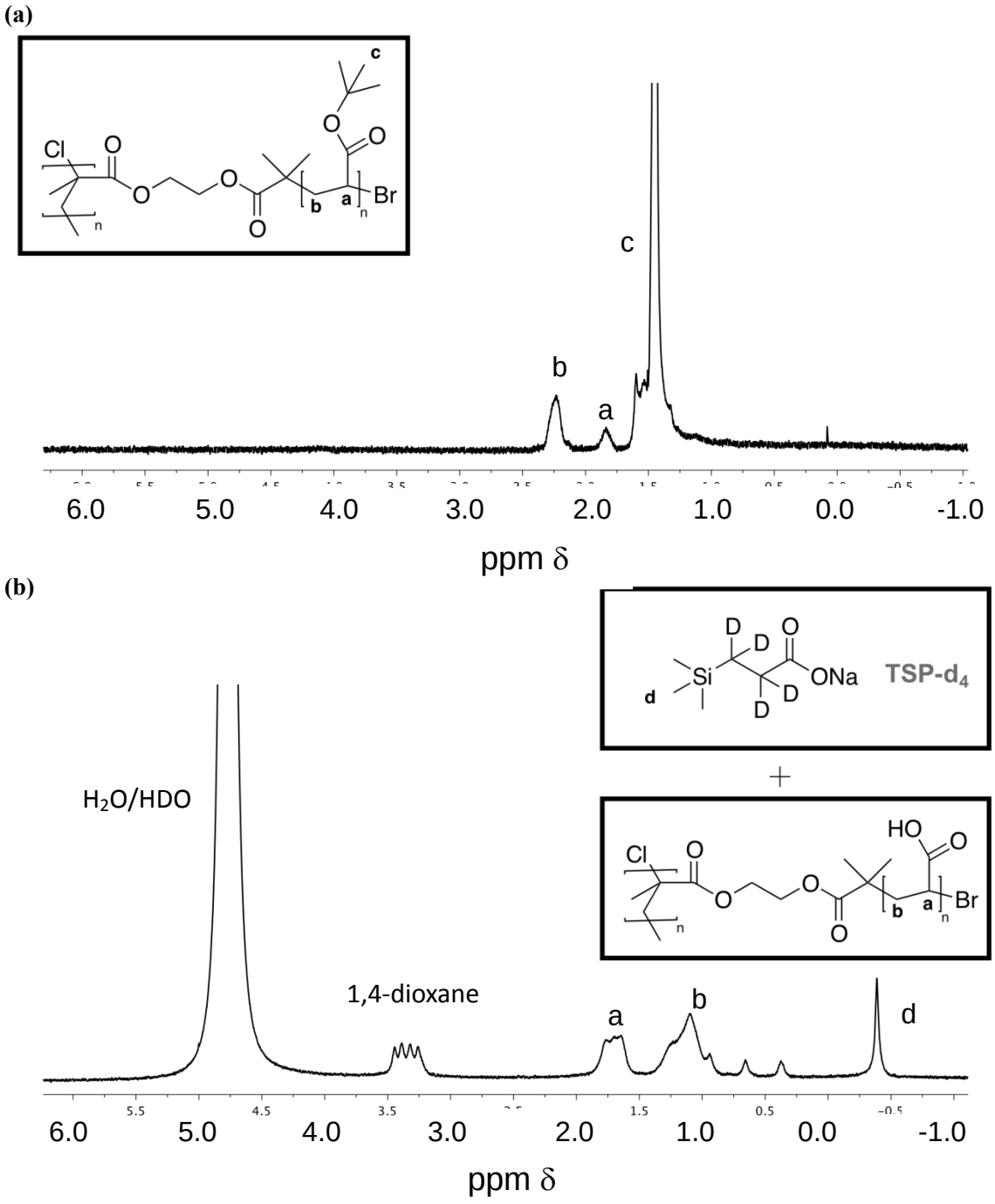


Figure S4. ^{1}H NMR spectra of (a) PHEMA-*g*-P*t*BA in $CDCl_3$ and (b) PHEMA-*g*-PAA in D_2O (pH>10 adjusted by NaOD) with 1mM 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4) internal standard.

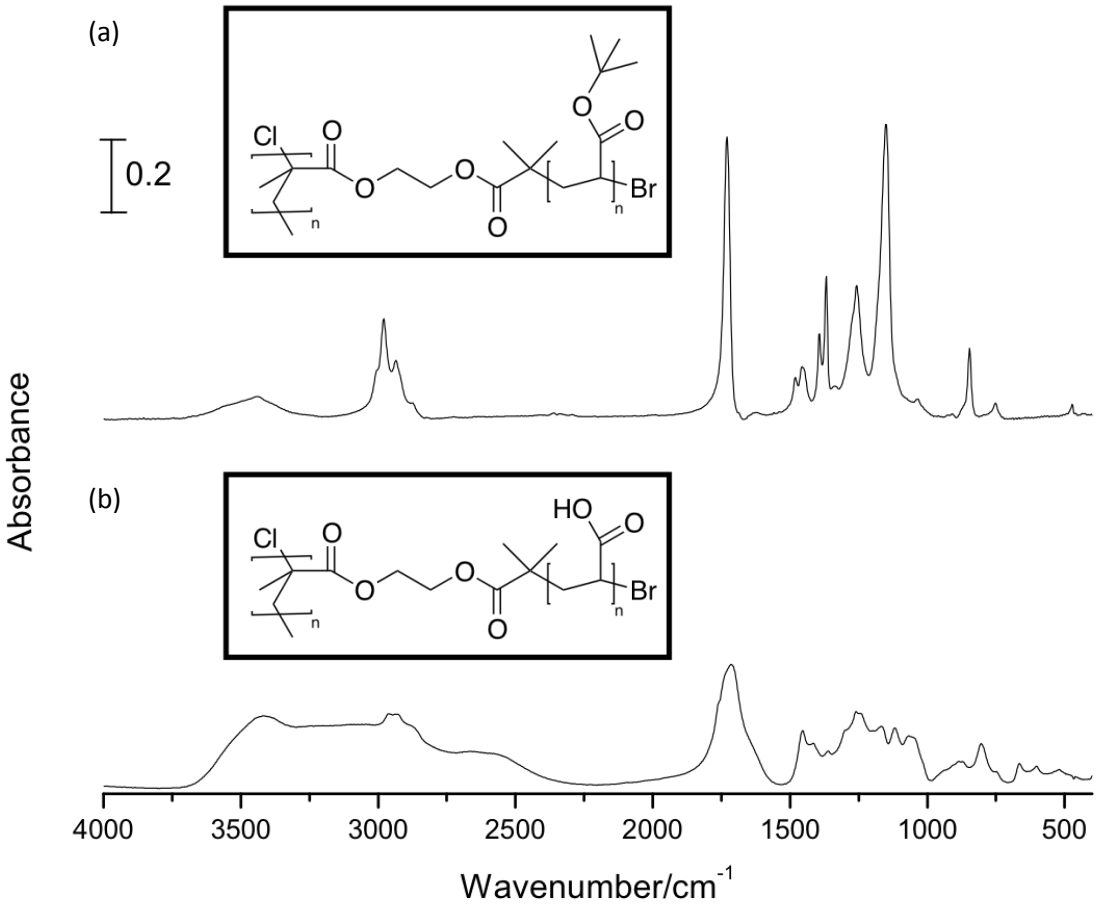


Figure S5. FTIR spectra of (a) PHEMA-*g*-P*t*BA in KBr (the *t*-butyl group shows two C-H bending bands at 1340-1400 cm^{-1}) and (b) PHEMA-*g*-PAA in KBr (the absence of C-H bending bands at 1340-1400 cm^{-1}, a broad O-H stretch at 2500-3500 cm^{-1}, and a strong, broad C=O stretching band near 1700 cm^{-1} confirm hydrolysis of the *t*-butyl group).

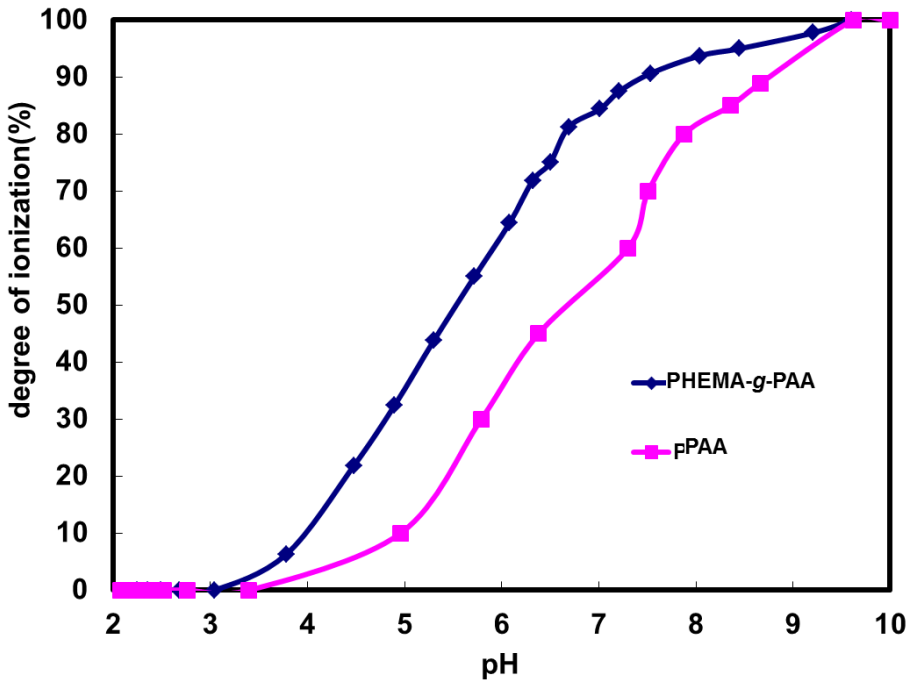


Figure S6. Titration curves for aqueous PAA and PHEMA-*g*-PAA solutions. We calculated the degree of ionization with the following equation, assuming that all the –COOH groups were deprotonated at pH 10.

$H^+ \, added = COOH + OH^- neutralized + increase \; in \; \; free \; H^+ \; in \; solution.$ The titration curve for PAA is similar to that in the literature.[4]

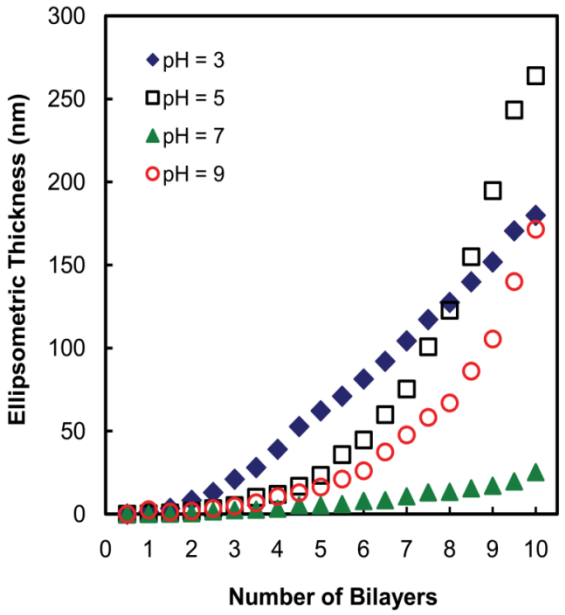


Figure S7. Ellipsometric thicknesses of (PAH/PHEMA-*g*-PAA)$_n$ films deposited from polyelectrolyte solutions with various pH values and no supporting electrolyte. Integer numbers of bilayers indicate films terminated with PHEMA-*g*-PAA, and films with an extra half bilayer end in PAH.

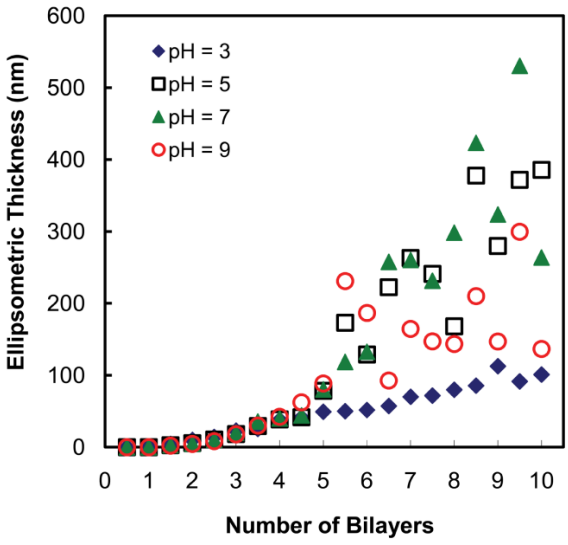


Figure S8. Ellipsometric thicknesses of (PAH/PHEMA-*g*-PAA)$_n$ films deposited from polyelectrolyte solutions containing 0.5 M NaCl at various pH values. Integer numbers of bilayers indicate films terminated with PHEMA-*g*-PAA, and films with an extra half bilayer end in PAH. Films with more than 5 bilayers layers are relatively rough, so there is significant scatter in the ellipsometric results.

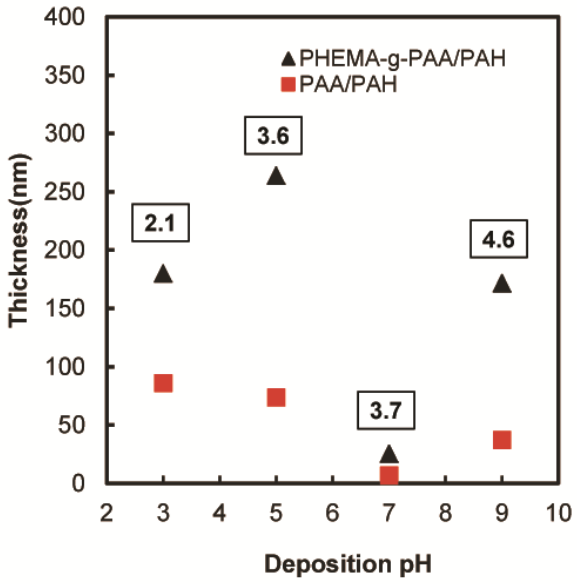


Figure S9. Ellipsometric thicknesses of (PAH/PHEMA-*g*-PAA)$_{10}$ and (PAH/PAA)$_{10}$ films deposited from polyelectrolyte solutions with various pH values and no supporting electrolyte. Numbers in the figure represent the ratios of the (PAH/PHEMA-*g*-PAA)$_{10}$ and (PAH/PAA)$_{10}$ thicknesses.

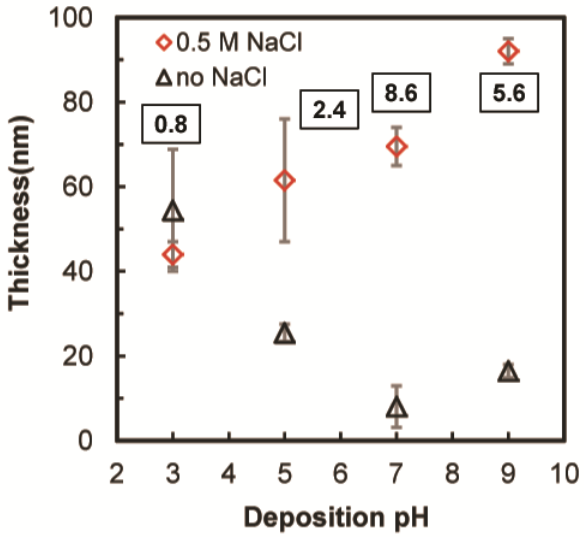


Figure S10. Ellipsometric thicknesses of (PAH/PHEMA-*g*-PAA)$_5$ multilayers deposited from polyelectrolyte solutions at various pH values in the presence and absence of 0.5 M NaCl. The boxed numbers show the ratios of thicknesses for films prepared with and without NaCl.

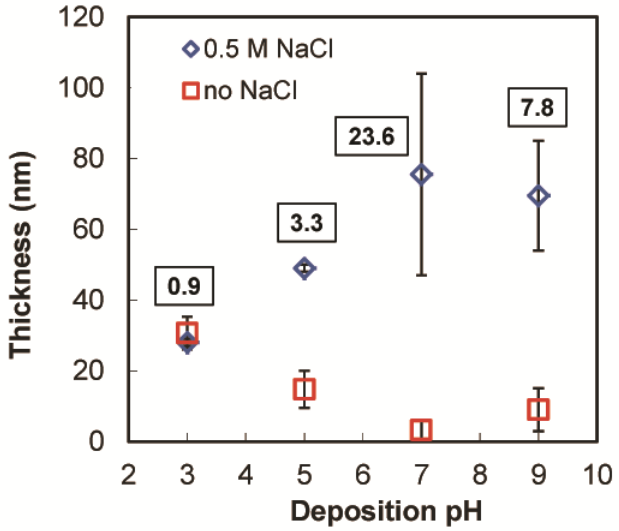


Figure S11. Ellipsometric thicknesses of $(PAH/PAA)_5$ multilayers deposited from polyelectrolyte solutions at various pH values in the presence and absence of 0.5 M NaCl. The boxed numbers show the ratios of thicknesses for films prepared with and without NaCl.

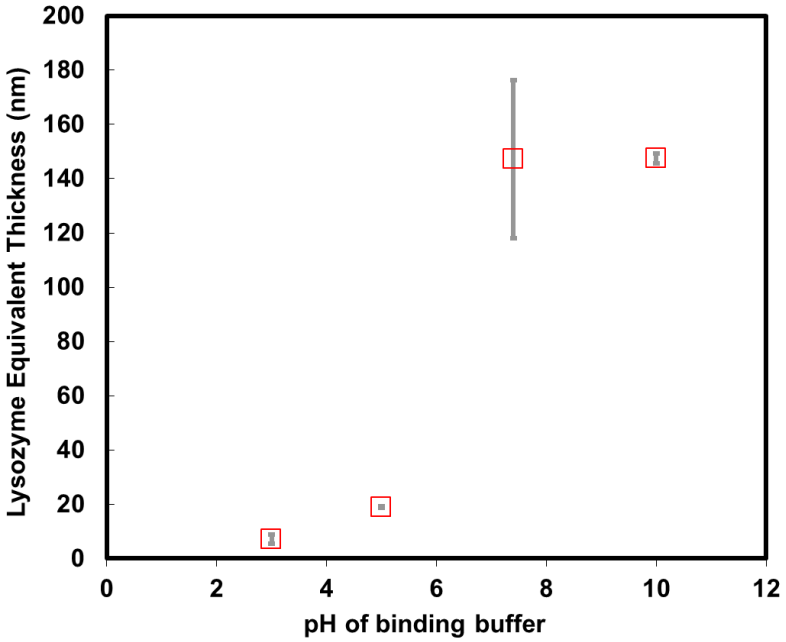


Figure S12. Lysozyme sorption in $(PAH/PAA)_5$ films as a function of the pH of the 1 mg/mL lysozyme sorption solution. $(PAH/PAA)_5$ coatings were deposited from solutions containing 0.5 M NaCl at pH 3. Lysozyme sorption solutions contained 20 mM phosphate adjusted to different pH values and no supporting salt. The equivalent thickness is the thickness of spin-coated lysozyme that would give an FTIR absorbance equivalent to that of the sorbed lysozyme.

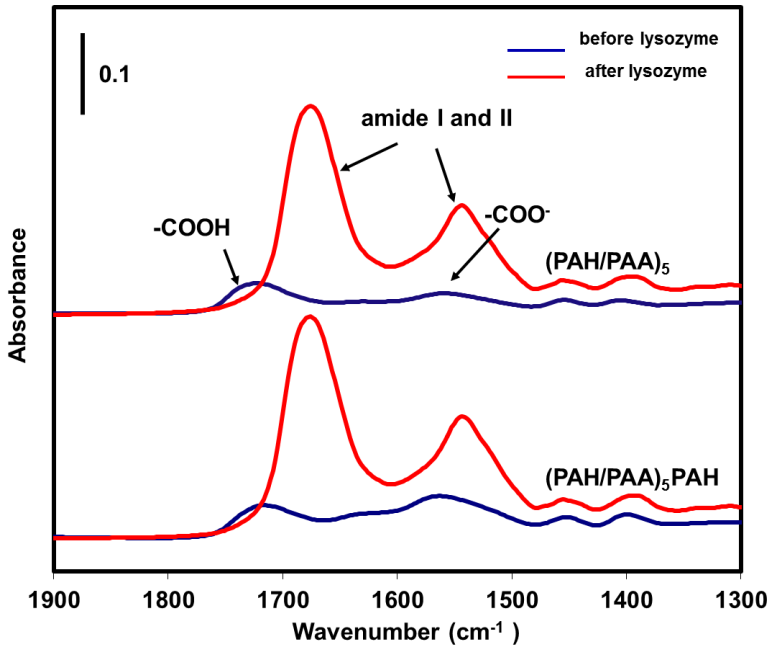


Figure S13. Reflectance FTIR spectra of $(PAH/PAA)_5$ and $(PAH/PAA)_5PAH$ films before and after sorption of lysozyme. Polyelectrolyte films were deposited from solutions containing 0.5 M NaCl at pH 3.0. Sorption of lysozyme occurred for 16 h from 20 mM phosphate buffer (pH 7.4) containing 1 mg/mL of lysozyme.

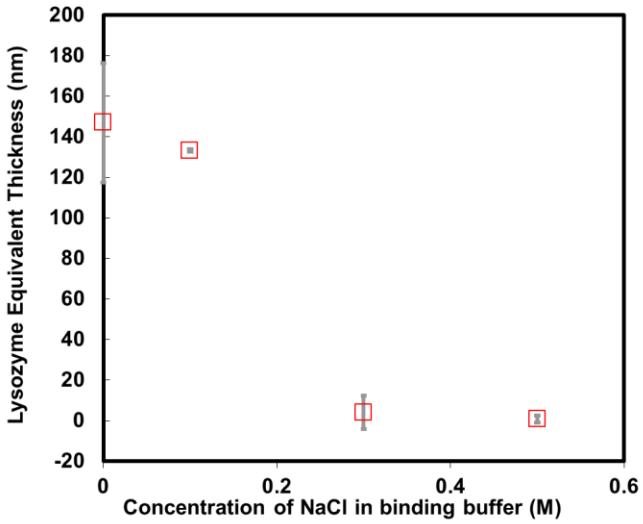


Figure S14. Lysozyme sorption in $(PAH/PAA)_5$ multilayers as a function of the concentration of NaCl in the 1 mg/mL lysozyme sorption solution. $(PAH/PAA)_5$ films were deposited from solutions containing 0.5 M NaCl at pH 3. The sorption solution also contained 20 mM phosphate buffer at pH 7.4. The equivalent thickness is the thickness of spin-coated lysozyme that would give an FTIR absorbance equivalent to that of the sorbed lysozyme. The error bars represent the range of values in measurements on two different films.

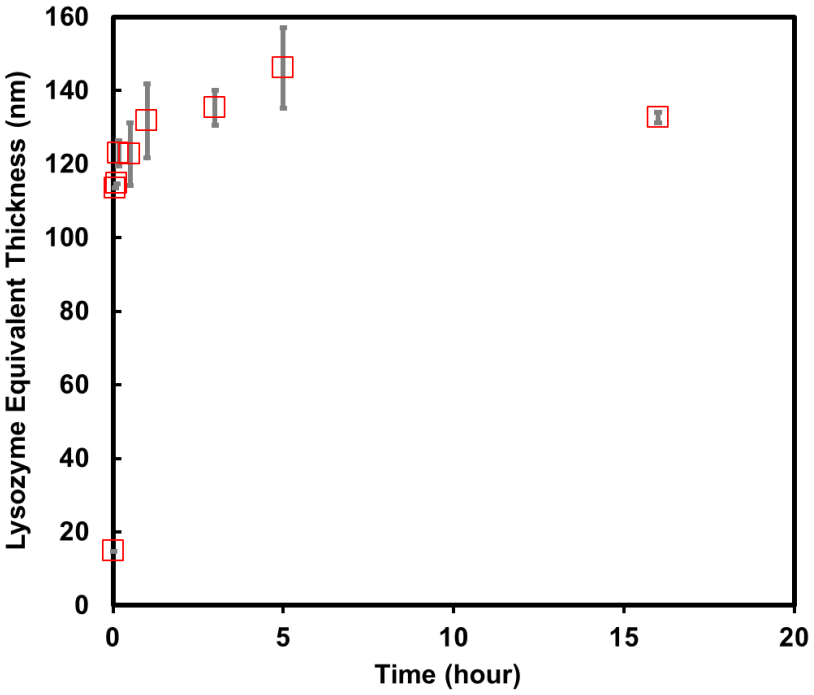


Figure S15. Time-evolution of lysozyme sorption in $(PAH/PAA)_5$ multilayers immersed in 1 mg/mL lysozyme solutions in 20 mM phosphate buffer (pH 7.4). The $(PAH/PAA)_5$ films were deposited from pH 3 solutions containing 0.5 M NaCl. The equivalent thickness is the thickness of spin-coated lysozyme that gives an FTIR absorbance equivalent to that of the sorbed lysozyme. The error bars represent standard deviations of measurements on 3 different films.

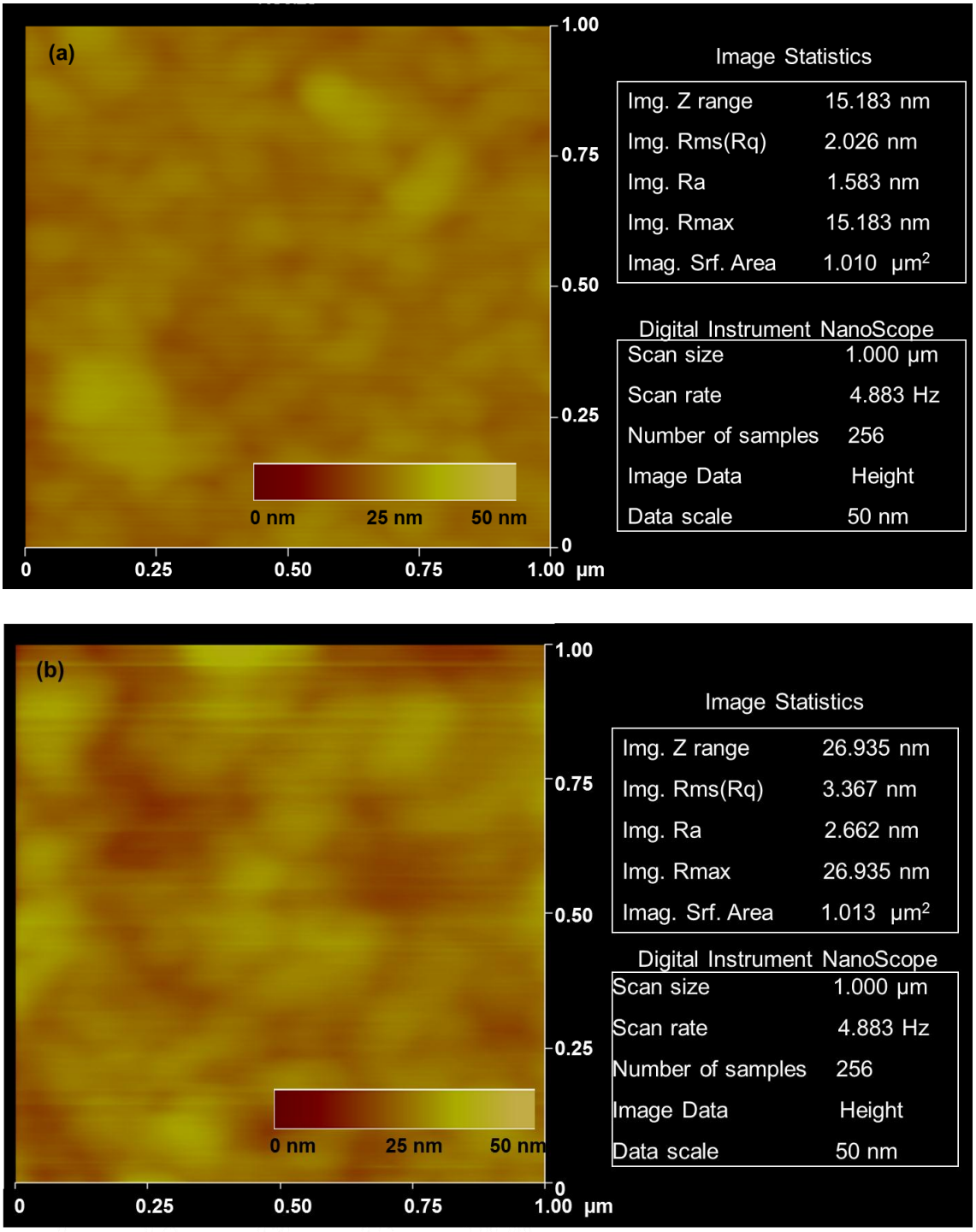


Figure S16. AFM images of $(PAH/PAA)_5$ films (a) before and (b) after sorption of lysozyme. The films were adsorbed from pH 3 solutions containing 0.5 M NaCl. Lysozyme sorption occurred from a 1 mg/mL lysozyme solution in phosphate buffer (pH = 7.4) for 16 h.

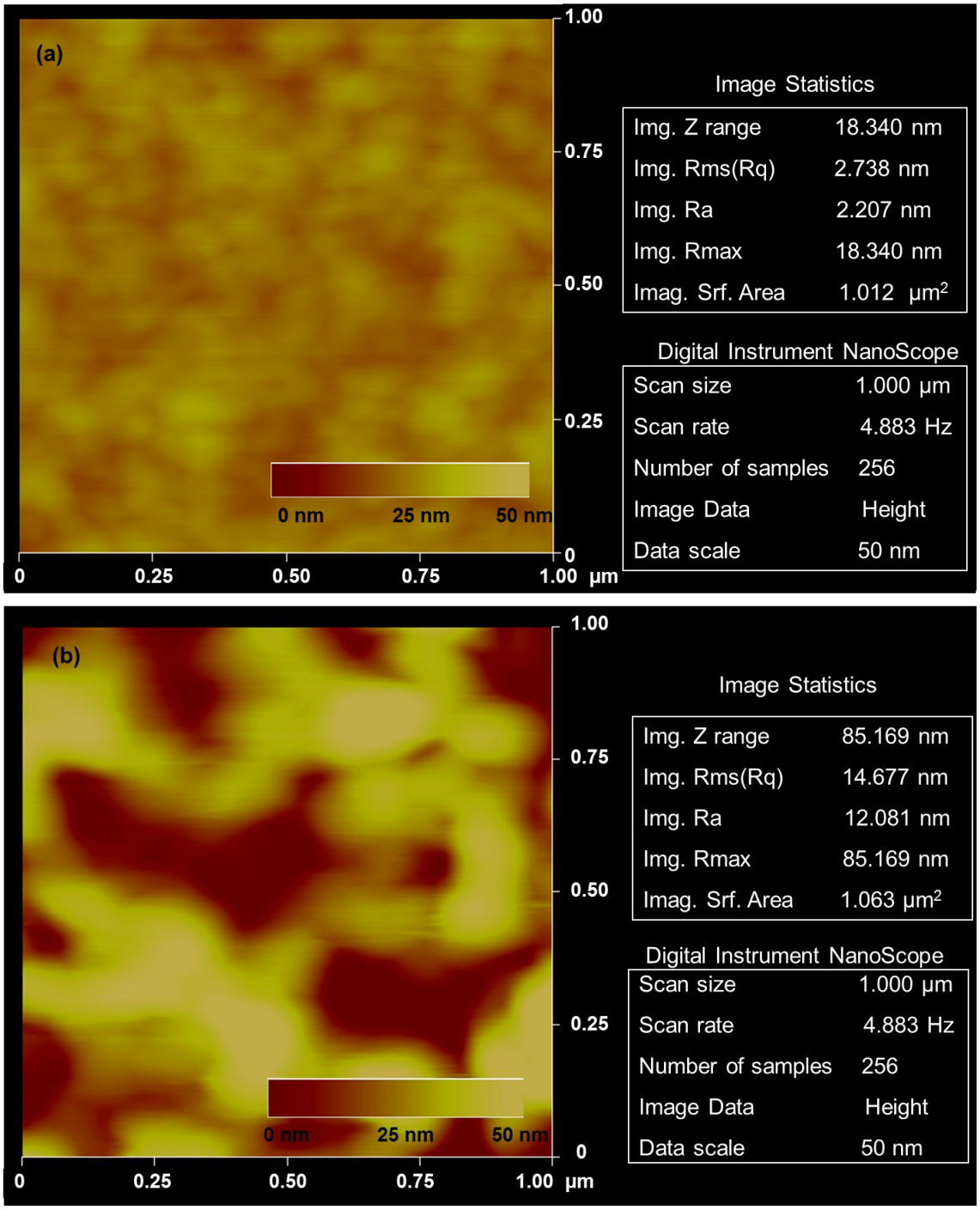


Figure S17. AFM images of $(PAH/PAA)_5$ films (a) before and (b) after adsorption of lysozyme. The films were adsorbed from pH 9 solutions containing 0.5 M NaCl. Lysozyme sorption occurred from a 1 mg/mL lysozyme solution in phosphate buffer (pH = 7.4) for 16 h.

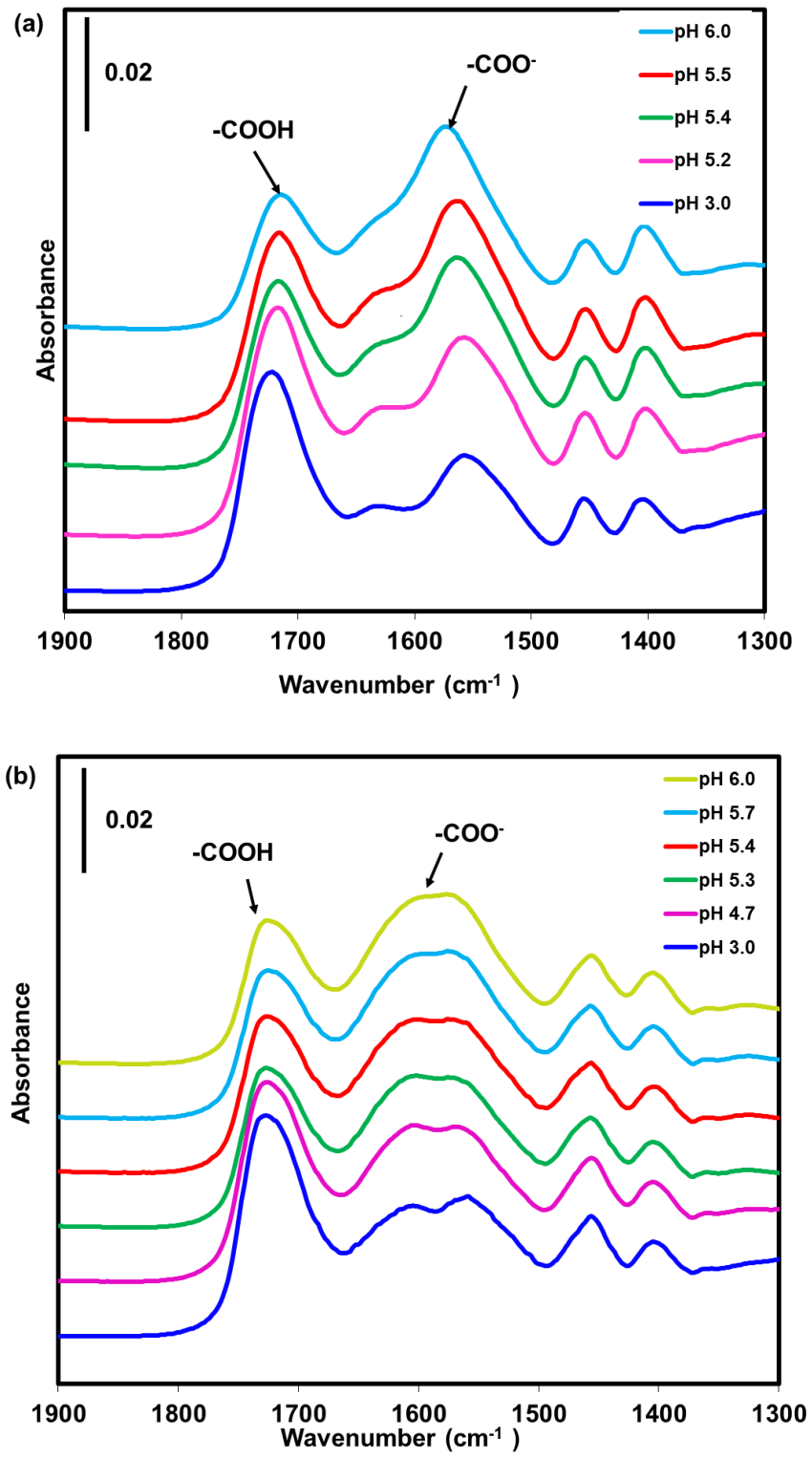


Figure S18. Reflectance FTIR spectra of (a) $(PAH/PAA)_5$ films and (b) $(PAH/PHEMA\text{-}g\text{-}PAA)_5$ films after immersions in 20 mM phosphate solutions adjusted to different pH values. Prior to obtaining the IR spectra the films were rinsed only in deionized water. The films were prepared

from pH 3 polyelectrolyte solutions containing 0.5 M NaCl. Assuming the same extinction coefficients for acid carbonyl and carboxylate absorption bands,[5] the degree of ionization, α, of PAA is estimated from the equation: $\alpha = \frac{A_{COO^-}}{A_{COO^-}+A_{COOH}}$ x 100%. In this equation, A_{COO^-} is the maximum absorbance of the carboxylate band and A_{COOH} is the maximum absorbance of the acid carbonyl band. Based on this equation, the estimated pKa value of the -COOH groups is 5.3 for PAA and 5.4 for PHEMA-g-PAA.

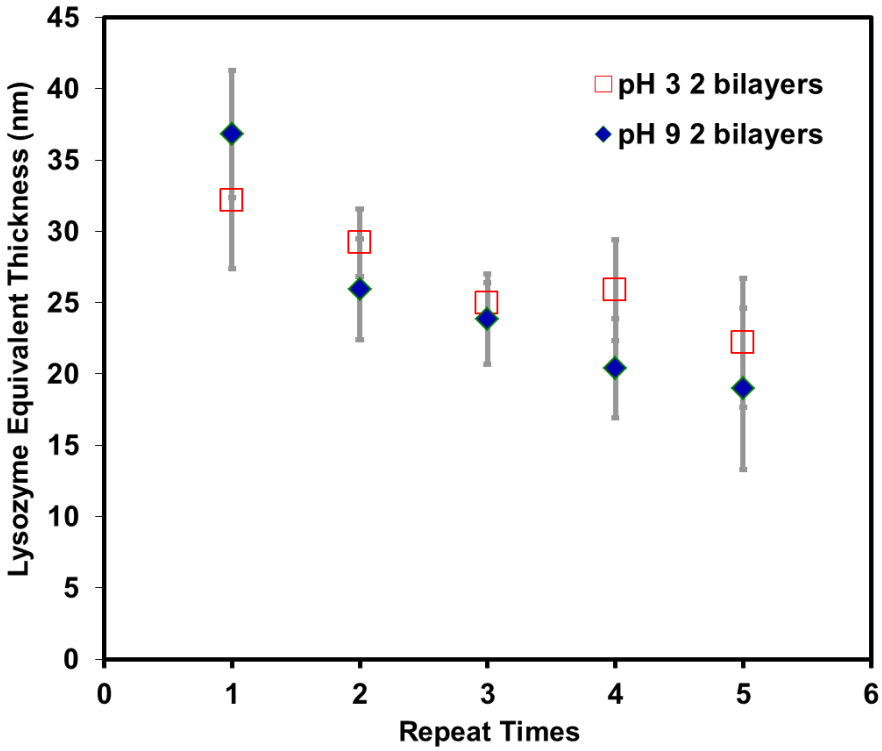


Figure S19. Repetitive lysozyme sorption on $(PAH/PAA)_2$ films in a 1 mg/mL lysozyme sorption solution. $(PAH/PAA)_2$ films were deposited from polyelectrolyte solutions containing 0.5 M NaCl at pH 3.0 or pH 9.0. Between each binding, the sorbed lysozyme was eluted with 20 mM phosphate buffer containing 1 M KSCN. The sorption solution also contained 20 mM phosphate buffer at pH 7.4. The equivalent thickness is the thickness of spin-coated lysozyme that would give an FTIR absorbance equivalent to that of the sorbed lysozyme. The error bars represent the standard deviations of measurements on 3 films.

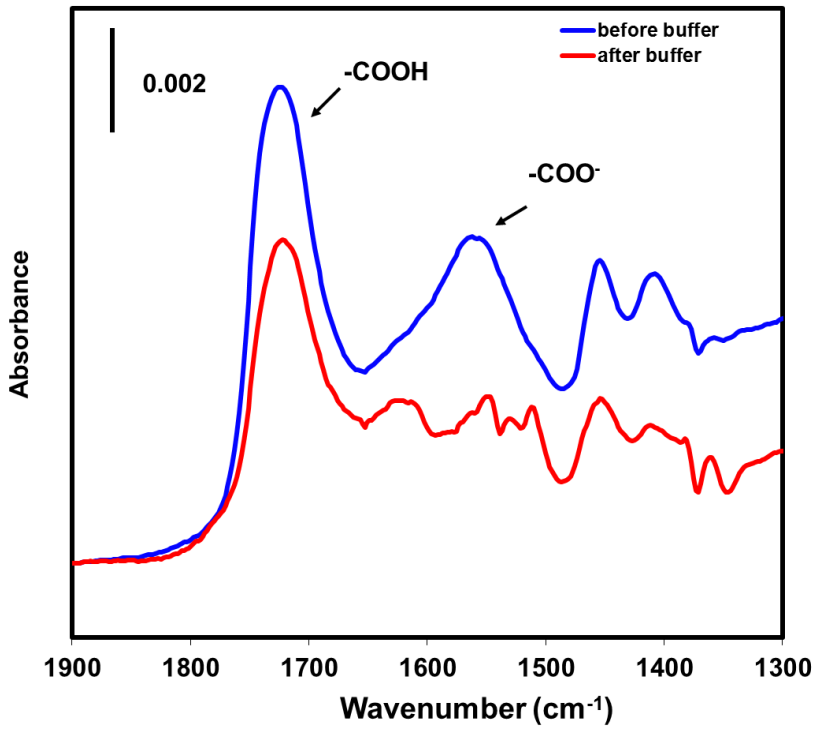


Figure S20. Reflectance FTIR spectra of $(PAH/PAA)_2$ films before and after immersion in a 20 mM phosphate solution (pH 7.4). $(PAH/PAA)_2$ films were deposited from pH 3.0 polyelectrolyte solutions containing 0.5 M NaCl. The film was immersed in the buffer overnight and washed with pH 3.0 phosphate buffer and water before obtaining FTIR spectra.

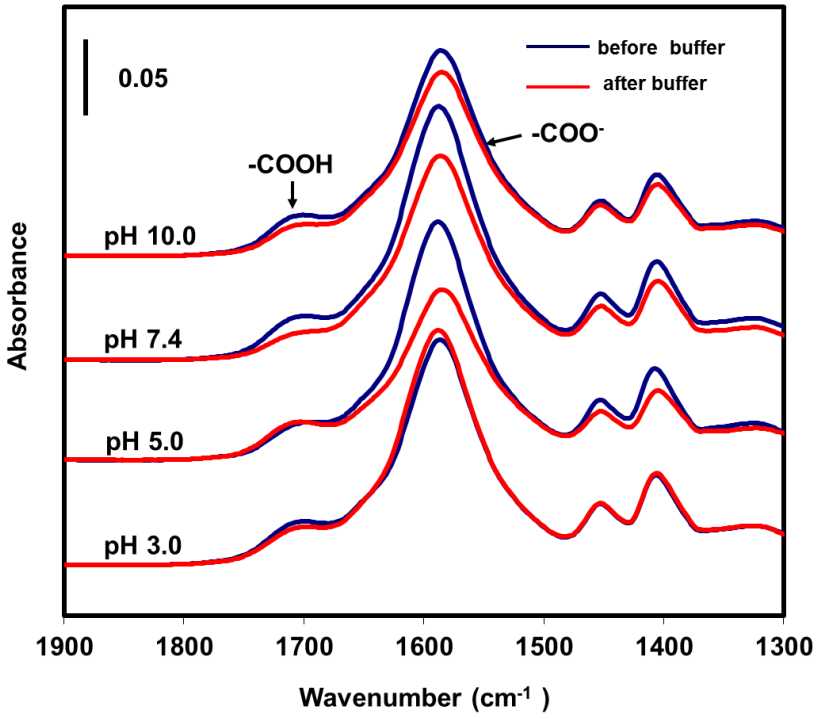
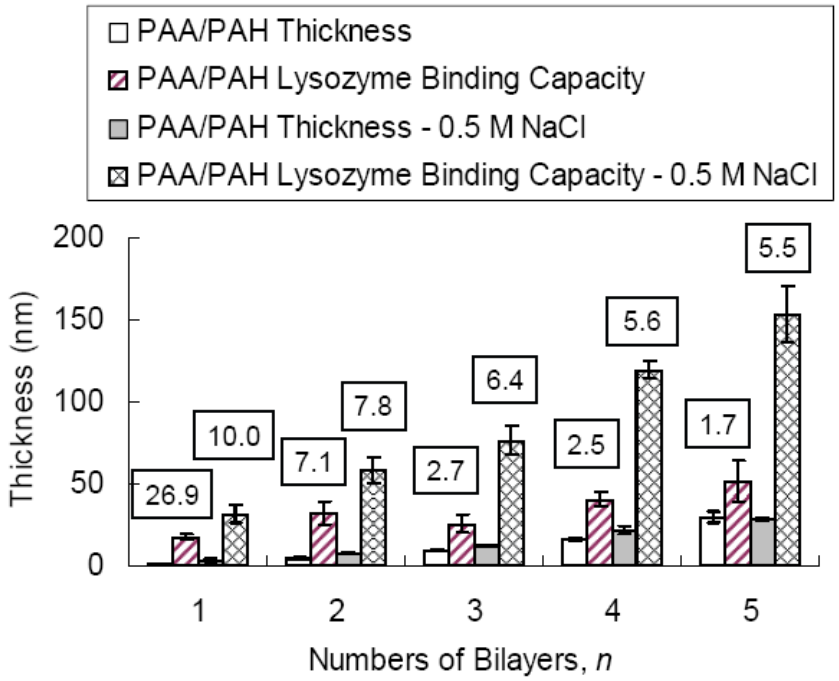


Figure S21. Reflectance FTIR spectra of $(PAH/PAA)_5$ films before and after immersion in a 20 mM phosphate solution adjusted to different pH values. $(PAH/PAA)_5$ films were deposited from pH 9.0 polyelectrolyte solutions containing 0.5 M NaCl. The films were immersed in the buffers overnight and washed with pH 9.0 buffer and water before obtaining FTIR spectra.

(a)



(b)

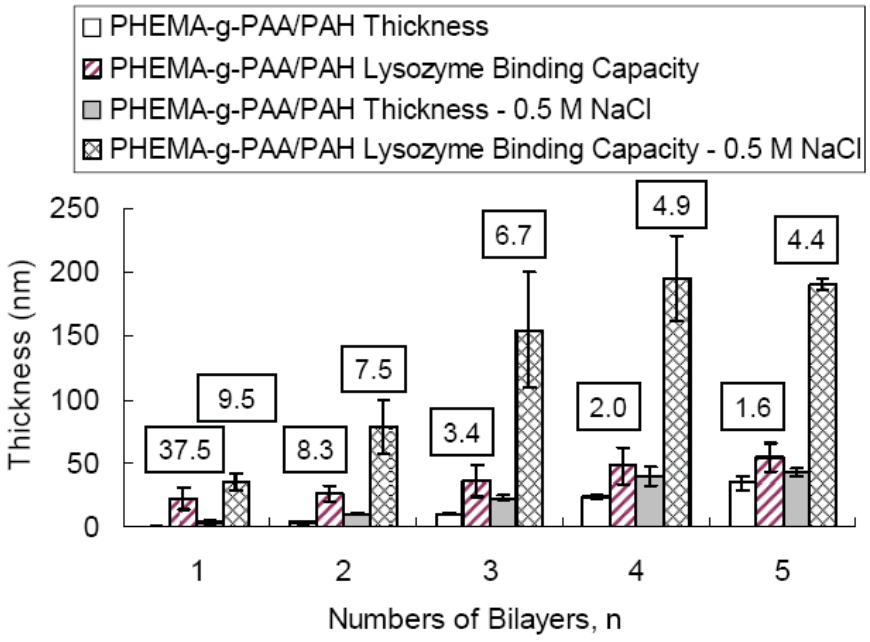


Figure S22. Lysozyme binding capacities of (a) $(PAH/PAA)_n$ and (b) $(PAH/PHEMA\text{-}g\text{-}PAA)_n$ multilayers (n=1~5) deposited from polyelectrolyte solutions at pH=3 both in the presence and absence of 0.5 M NaCl. The numbers above the bars represent the ratios of the lysozyme equivalent thickness to the film thickness. The equivalent thickness is the thickness of spin-coated lysozyme that would give an FTIR absorbance equivalent to that of the sorbed lysozyme.

References

(1) Beers, K. L.; Boo, S.; Gaynor, S. G.; Matyjaszewski, K. Atom Transfer Radical Polymerization of 2-Hydroxyethyl Methacrylate. *Macromolecules* **1999**, *32*, 5772-5776.
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(4) Choi, J.; Rubner, M. F. Influence of the Degree of Ionization on Weak Polyelectrolyte Multilayer Assembly. *Macromolecules* **2005**, *38*, 116-124.
(5) Mendelsohn, J. D.; Barrett, C. J.; Chan, V. V.; Pal, A. J.; Mayes, A. M.; Rubner, M. F. Fabrication of Microporous Thin Films from Polyelectrolyte Multilayers. *Langmuir* **2000**, *16*, 5017-5023.