Electronic Supplementory Information

Reversibly switchable polymer with cationic/zwitterionic/anionic behavior through synergistic protonation and deprotonation

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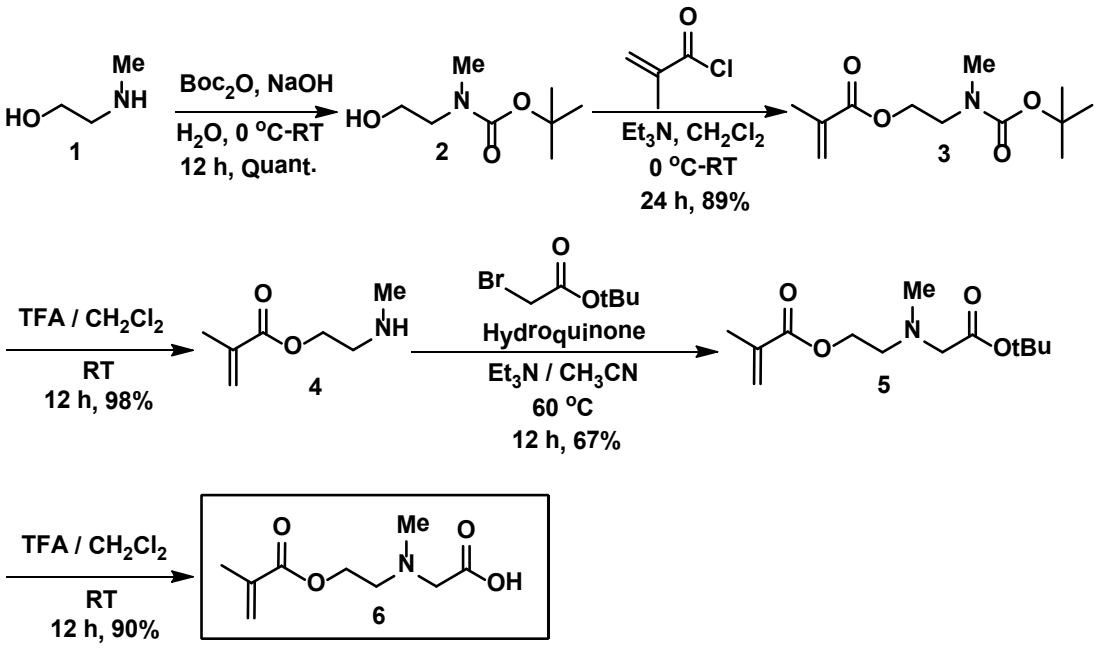
Materials and Methods

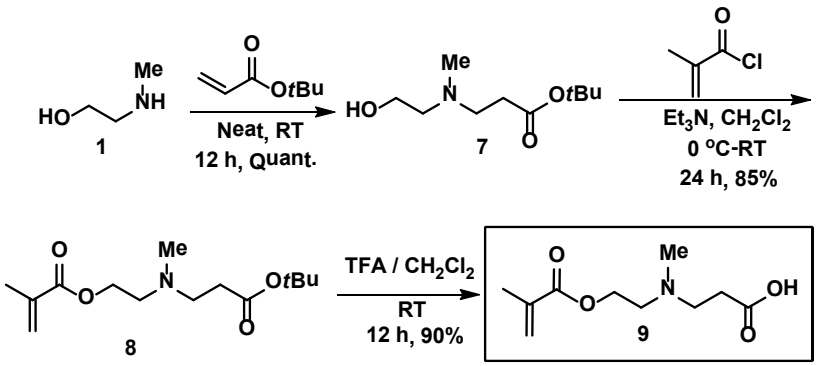
Chemicals: Tetrahydrofuran (THF) was purchased from OmniSolv (Salisbury, NC). Ethanol (200 proof) was purchased from Decon Laboratories, Inc. (King of Prussia, PA). Phosphate-buffered saline (PBS, 0.01M phosphate, 0.138M sodium chloride, 0.0027M potassium chloride, pH 7.4), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 11-Mercaptoundecan-1-ol**,** 4-(chloromethyl)phenyl isocyanate, dibutyltin dilaurate, diethylammonium salt of diethyldithiocarbamic acid, 2-(*N*,*N*'-dimethylamino) ethyl methacrylate (DMAEMA, 98%), triethylamine, anhydrous acetone, methanol, and tributylphosphine were purchased from Sigma-Aldrich (St. Louis, MO). Sodium carbonate anhydrous was purchased from EMD Chemicals (Darmstadt, Germany). Sodium chloride (NaCl) and ether were purchased from J.T. Baker (Phillipsburg, NJ). Sodium acetate anhydrous was purchased from Fluka (subsidiary of Sigma Aldrich, St. Louis, MO). Pepsin (from porcine gastric mucosa, M_W = 35 kDa), lysozyme (from chicken egg white, M_W = 15 kDa) and albumin from bovine serum (BSA, M_w = 66 kDa) were purchased from Sigma-Aldrich. Pooled human blood plasma was purchased from BioChemed Services (Winchester, VA). The water used in these experiments was purified using a Millipore water purification system with a minimum resistivity of 18.2 MΩ cm.

Synthetic Scheme And Procedures for the CBMA-1 Tertiary Amine (CBMA-1-TAM)

(A)



(B)



Scheme S1. (A) Synthesis of CBMA-1 tertiary amine and (B) synthesis of CBMA-2-TAM tertiary Amine monomers.

N-tert-Butoxycarbonyl-N-methyl-ethanolamine (2): The synthetic schemes for the monomers are given in schemes S1 and S2 (supporting information). *N*-Methyl-ethanolamine (13.9 g, 185 mmol) was dissolved in 1 N sodium hydroxide (250 mL) and the solution was cooled to 0 ºC. Di-*tert*-butyl dicarbonate (45.0 g, 206 mmol) was added to the mixture and the reaction was stirred and allowed to warm to room temperature overnight. The product was then extracted with ethyl acetate (4 x 125 mL) and the combined organic phase was dried over anhydrous sodium sulfate. After removal of the ethyl acetate under reduced pressure, the pure product was obtained as a colorless oil (31.8 g, 181 mmol). Yield: 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.71 (t, 2H, J = 6.0 Hz), 3.36 (t, 2H, J = 6.0 Hz), 2.90 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 156.7, 79.4, 60.6, 50.9, 35.2, 28.1.

N-tert-Butoxycarbonyl-N-methyl-2-aminoethyl-methacrylate (3): N-tert-Butoxycarbonyl-N-methyl-ethanolamine (14.5 g, 82.7 mmol) and triethylamine (34.7 mL, 249 mmol) were dissolved in anhydrous dichloromethane (150 mL) and the solution was cooled to 0 ºC. Methacryloyl chloride (12.3 mL, 125 mmol) was added dropwise and the solution was stirred at room temperature for 24 h. The reaction was quenched at 0 ºC by slow addition of ice-water then diluted with dichloromethane (100 mL). The solvent phases were separated and the organic phase was washed with H_2O (4 x 25 mL) and brine (25 mL). After drying over sodium sulfate, the solvent was removed under reduced pressure and the crude mixture was purified by silica gel chromatography using a gradient of pure hexane to hexane:ethyl acetate 10:1. The pure product was obtained as a yellow oil (17.8 g, 73.2 mmol). Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.15 (s, 1H), 5.56 (s, 1H), 4.28 (t, 2H, J = 5.9 Hz), 3.27 (s, 3H), 2.90 (t, 2H, J = 5.9 Hz),

1.96 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 166.6, 155.1, 135.7, 125.4, 79.2, 62.3, 47.5, 34.8, 27.9, 17.9.

N-methyl-2-aminoethyl-methacrylate (4): N-tert-Butoxycarbonyl-N-methyl-2-aminoethyl-methacrylate (15.0 g, 61.7 mmol) was dissolved in anhydrous dichloromethane (100 mL). Trifluoroacetic acid (24.0 mL, 313 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the crude residue was purified by silica gel chromatography using a gradient of pure dichloromethane to dichloromethane:methanol 2:1. The pure product was obtained as a light yellow oil (8.65 g, 60.4 mmol). Yield: 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.11 (s, 1H), 5.55 (s, 1H), 4.17 (t, 2H, J = 5.9 Hz), 3.10 (s, 3H), 2.70 (t, 2H, J = 5.9 Hz), 1.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 166.5, 134.9, 126.5, 59.4, 47.6, 32.9, 17.5.

2-((2-(tert-Butoxy)-2-oxoethyl)(methyl)amino)ethyl methacrylate (5): N-methyl-aminoethyl-methacrylate (12.1 g, 84.5 mmol) and triethylamine (35.3 mL, 253 mmol) were dissolved in anhydrous acetonitrile (150 mL). *t*-Butylbromoacetate (19.0 mL, 129 mmol) was added dropwise and the mixture was stirred at 60 ºC for 12 h. The reaction was cooled to room temperature and the solvent was removed under reduced pressure. The crude residue was dissolved in ethyl acetate (150 mL) and the organic phase was washed with H_2O (4 x 25 mL). After drying over sodium sulfate and evaporation of the solvent, the crude mixture was purified by silica gel chromatography using a gradient of pure hexane to hexane:ethyl acetate 1:1. The pure product was obtained as a light yellow oil (14.5 g, 56.3 mmol). Yield: 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.13 (s, 1H), 5.58 (s, 1H), 4.28 (t, 2H, J = 5.9 Hz), 3.27 (s, 2H), 2.90 (t, 2H, J = 5.9 Hz), 2.48 (s, 3H), 1.96 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 169.7, 166.7, 135.8, 125.0, 80.3, 62.5, 58.8, 54.3, 42.0, 27.7, 17.9.

2-((2-(methacryloyloxy)ethyl)(methyl)amino)acetic acid (6): Compound **5** (8.50 g, 33.0 mmol) was dissolved in anhydrous dichloromethane (100 mL). Trifluoroacetic acid (12.6 mL, 165 mmol) was added and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure and the product was crystallized using a 2:1 mixture of ether and methanol and the obtained white solid was dried under high vacuum (5.99 g, 29.8 mmol). Yield: 90%. ^{1}H NMR (300 MHz, D_2O) δ (ppm): 6.10 (s, 1H), 5.63 (s, 1H), 4.40 (t, 2H, J = 4.9 Hz), 3.67 (s, 2H), 3.48 (t, 2H, J = 4.9 Hz), 2.86 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, D_2O) δ (ppm): 169.9, 168.6, 135.1, 127.7, 59.2, 58.7, 55.0, 41.8, 17.3.

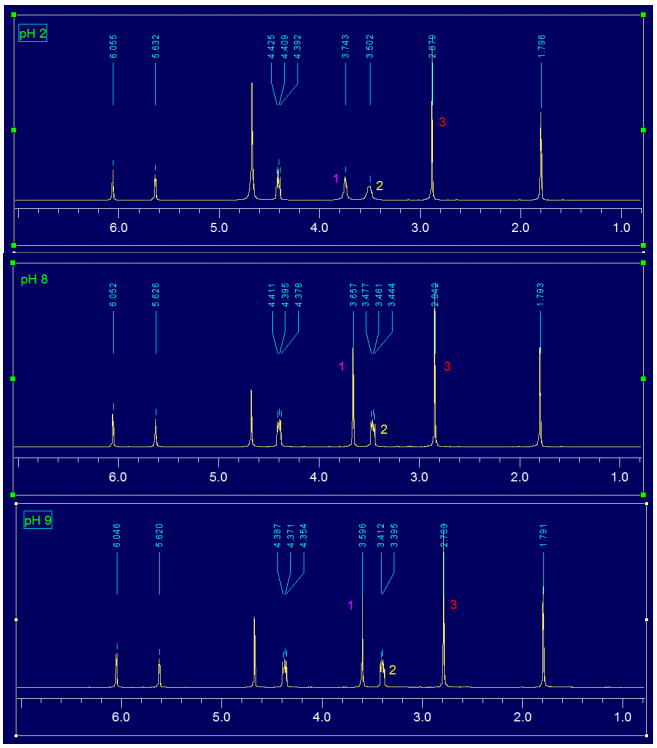
tert-Butyl 3-((2-hydroxyethyl)(methyl)amino)propanoate (7): N-Methyl-ethanolamine (15.6 g, 208 mmol) and *tert*-butyl acrylate (45.6 mL, 312 mmol) were added to a 500 mL round bottom flask and the mixture was stirred at room temperature for 12 h. The reaction was concentrated on the rotovap to remove unreacted *tert*-butyl acrylate and further dried under high vacuum to afford the product as a yellow oil (42.1 g, 207 mmol). Yield: 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.49 (t, 2H, J = 5.3 Hz), 3.10 (s, 1H), 2.61 (t, 2H, J = 6.9 Hz), 2.44 (t, 2H, J = 5.3 Hz), 2.29 (t, 2H, J = 6.9 Hz), 2.17 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.8, 80.3, 58.6, 52.5, 41.5, 33.6, 27.8.

2-((3-(tert-butoxy)-3-oxopropyl)(methyl)amino)ethyl methacrylate (8): Compound **7** (17.5 g, 85.9 mmol) was dissolved in dichloromethane (200 mL) in a 500 mL round bottom flask and the mixture was cooled to 0 ºC. Triethylamine (36.0 mL, 258 mmol) and methacryloyl chloride (12.7 mL, 129 mmol) were added successively and the reaction was stirred at room temperature for 24 h. After the reaction was quenched by addition of ice, the phases were separated and the aqueous phase was extracted with more dichloromethane (3 x 25 mL). The combined organic phases were dried over sodium sulfate, concentrated on the rotovap and the crude mixture was purified by silica gel chromatography using a gradient of hexane:ethyl acetate (10:1 to 5:1). The pure product was obtained as a light yellow oil (19.9 g, 73.3 mmol). Yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.05 (s, 1H), 5.50 (s, 1H), 4.18 (t, 2H, J = 5.9 Hz), 2.68 (t, 2H, J = 7.3 Hz), 2.65 (t, 2H, J = 5.9 Hz), 2.32 (t, 2H, J = 7.3 Hz), 2.25 (s, 3H), 1.88 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 171.4, 166.9, 136.0, 125.2, 79.9, 62.5, 55.2, 53.1, 42.1, 33.7, 27.8, 18.0.

3-((2-(methacryloyloxy)ethyl)(methyl)amino)propanoic acid (9): Compound 8 (10.3 g, 37.9 mmol) was dissolved in dichloromethane (45.0 mL) in a 500 mL round bottom flask. Trifluoroacetic acid (15.0 mL, 196 mmol) was added to the mixture and the reaction was stirred at room temperature for 12 h. The solvent and trifluoroacetic acid were removed under vacuum on the rotovap and the resulting crude residue was purified by silica gel chromatography using a gradient of dichloromethane:methanol (20:1 to 5:1) to afford the pure product as a light brown oil (7.35 g, 34.1 mmol). Yield: 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.07 (s, 1H), 5.59 (s, 1H), 4.47 (t, 2H, J = 5.9 Hz), 3.46 (t, 2H, J = 7.3 Hz), 3.45(t, 2H, J = 5.9 Hz), 2.88 (s, 3H), 2.80 (t, 2H, J = 7.3 Hz), 1.86 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 173.7, 166.4, 134.9, 126.7, 58.5, 54.1, 52.3, 49.4, 39.9, 17.6.

Synthesis of Dithiocarbamate Photoiniferter: Synthesis of photoiniferter, 11-mercaptorundecane-1-[4({[(diethylamino) carbonothioyl] thioethyl} phenyl) carbamate] was performed as reported in the literature.[1]

^{1}H NMR studies of the CBMA-1-TAM at different pH values: To study the pH-dependent changes of the monomer (Fig. S1), samples of the compound were dissolved in deuterated water and the pH values were adjusted by using hydrochloric acid and sodium hydroxide. The chemical shifts for three different sets of protons (1, 2 and 3) were observed via ^{1}H-NMR. NMR spectra of the monomer at key pH values as well as the corresponding proposed structures of the monomer are shown in Figure S1.



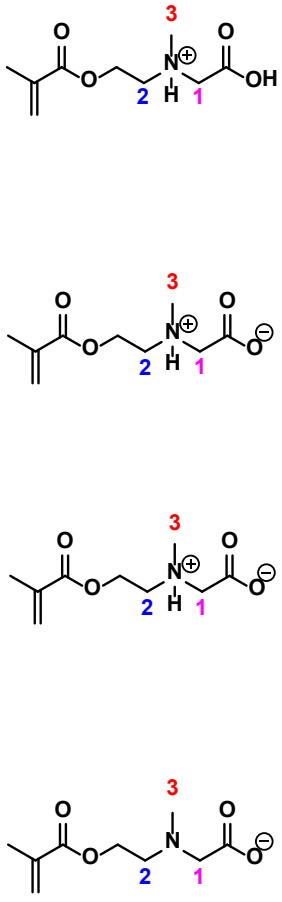


Figure S1. ^{1}H-NMR spectra of the monomer in different pH solutions and corresponding proposed structures.

Titration of monomer solution: A monomer solution of 5 mL with a concentration of 1 mg/mL was made using DI water. This was titrated against 0.1 N NaOH solution by adding 100 µL of NaOH solution each time. The change in pH of the solution was noted using a calibrated potentiometer.

SPR Sensor, Chips, and Calibration of the Surface Sensitivity: A laboratory SPR sensor developed at the Institute of Photonics and Electronics, Prague, Czech Republic was used as described previously.[2,3] This custom built SPR is based on the attenuated total reflection method and wavelength modulation. It is equipped with a four-channel flow-cell, temperature control, and uses a peristaltic pump for delivering samples. SPR sensor chips were made of a glass slide coated with an adhesion-promoting titanium film (~2 nm) followed by a gold film (~48 nm) using an electron beam evaporator. Since the SPR sensitivity depends on the distance of the binding event from the SPR active surface, the sensor response due to the polymer films had to be calibrated.[4] This was done using previously described methods.[1,4] For example a ~29 nm resulted in a calibration factor of 1.35. Hence, a 1 nm shift in the resonant wavelength corresponded to a change in protein surface coverage of ~ 23.0 ng/cm^2.[1,5]

Preparation of photoiniferter-coated SAMs on SPR chips: Prior to the formation of photoiniferter SAMs on gold coated SPR chips, the bare substrates were washed with water and ethanol, cleaned with a UV-ozone cleaner for 20 min, washed with water and ethanol, and then dried under a stream of filtered air. The photoiniferter SAMs were then formed by soaking the cleaned gold coated substrates in THF containing 0.2 mM photoiniferter at room temperature for 24 h. Before polymerization, the substrates were rinsed with THF, placed in pure THF for 1-2 min, and then dried with a stream of filtered air.

Surface Initiated Photoiniferter-Mediated Polymerization (SI-PIMP): The monomer solution was first prepared by weighing out the appropriate monomer and adding solvents to achieve the desired monomer concentration and solvent composition (methanol:water = 90:10) all under nitrogen protection. The substrate prepared with the photoiniferter SAM was placed in a quartz reaction tube manufactured from rectangular quartz tubing. The test tube was sealed with a rubber septum and placed under nitrogen protection. The degassed monomer solution was then transferred to the reaction tube using a syringe under nitrogen protection. A stream of nitrogen was allowed to pass through the monomer solution in the reaction tube for 1-2 min before being removed and wrapped with parafilm. In order to prevent the cleavage of the thiol-

gold bond of the photoiniferter SAM, a 280 nm cutoff filter was mounted to the outside of the reaction tube. Samples were then irradiated with 302 nm-centered UV light (UVP, model UVM-57) for the desired reaction time. Following the polymerization, the substrates were removed from the reactor, washed with PBS, and then stored in PBS overnight.

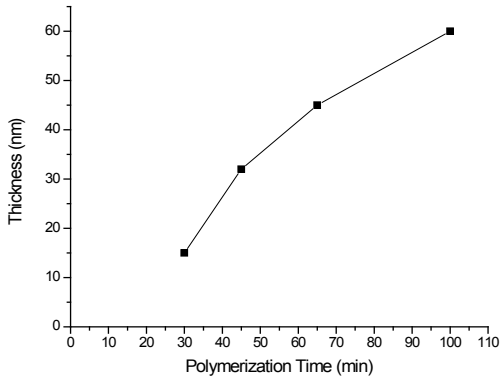


Figure S2. The photopolymerization kinetics for CBMA-1-TAM.

Ellipsometry: The thickness of the polymer films were determined using an ellipsometer (Model alpha-SE, J.A. Woollam, Lincoln, NE) using the 380 – 900 nm wavelength range at an incidence angle of 70°. The results were fitted to a Cauchy module.

Measurements of Non-specific Protein Adsorption by SPR: The non-specific protein adsorption of the polymer films was determined with a SPR biosensor using a flow rate of 50 μL/min at 25 °C. After first establishing a baseline using PBS, the protein solution (single proteins in PBS, undiluted human plasma and serum) was flowed for 10 minutes, followed by buffer to reestablish the baseline. Protein adsorption was quantified as the difference between buffer baselines and converted to a surface coverage.

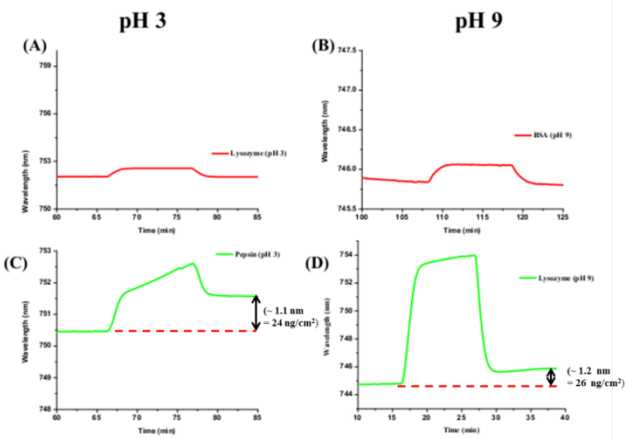


Figure S3. SPR sensorgrams for lysozyme at pH 3 (A); BSA at pH 9 (B); Pepsin at pH 3 (C) and lysozyme at pH 9 (D). At pH 3 the adsorption of pepsin and repulsion of lysozyme indicates the surface is positive at pH 3. At pH 9 adsorption of lysozyme and repulsion of BSA indicates the surface is negatively charged.

Simulation Details. Quantum calculations were carried out using the Gaussian 2009 at the B3LYP/6-311++G (d, p) level.[6] At the first step, molecular modeling with a classical UFF force field[7] was carried out in the aim to generate the inputs of geometric structure for quantum calculations. For CB-1-TAM, we rotated the N–C–C–O dihedral. Seven different structures with relatively low energies were selected for the zwitterionic form. For CB-2-TAM, we rotated the N–C–C–C dihedral and selected ten different structures for the zwitterionic form. All of the selected structures were then optimized in an implicit water solvent at the B3LYP/6-311++G (d, p) level. For each molecule, the structure with the lowest potential energy was chosen for single point energy calculations to generate the electrostatic potential surfaces.

For the radial distribution function calculation, all atom models were used to describe the CB-1-TAM, CB-2-TAM and water molecules. The SPC/E water model[8] was used to describe water molecules. The potential energy of intermolecular interactions was calculated as a combination of a Lennard-Jones (L-J) 12-6 potential and a Columbic potential. The force field parameters for CB-1-TAM were from our previous work.[9] For CB-2-TAM, its L-J interaction parameters were from OPLS-AA force field[10] and its partial charges were calculated as previously descirbed.[11]

The simulation system was a periodic water box containing 1100 water molecules and 80 zwitterionic molecules. The concentration of each zwitterion was 4 M. The molecular dynamics (MD) simulations were performed using Gromacs[12] (version 4.5.4) in an NPT ensemble. After

energy minimization and a 2.0 ns MD run for equilibrium, another 2.0 ns run was carried out for data collection. Long-range electrostatic interactions were computed with the particle mesh Ewald method with periodic boundary conditions in all three dimensions. The short-range van der Waals interactions were calculated with a cutoff distance of 1.1 nm. The system was maintained at 298 K and 100.0 KPa with the Berendsen algorithm.[13] The radial distribution function for the intermolecular interation of nitrogen proton with that of carboxylate is plotted in Fig. S4.

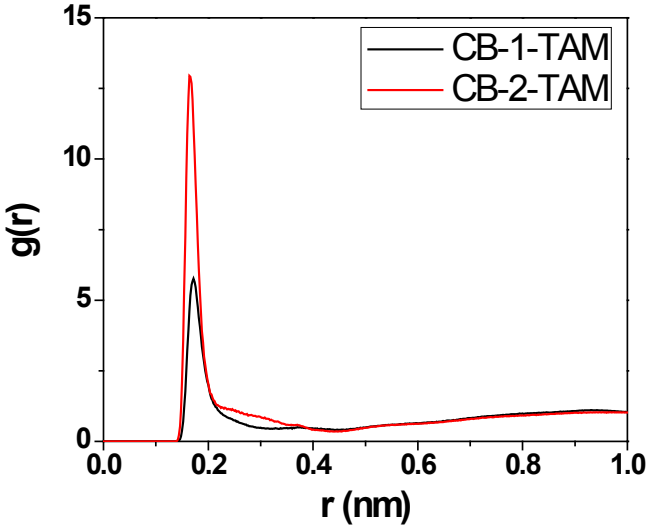


Figure S4. The radial distribution function for the intermolecular interactions of the nitrogen proton with the oxygen atoms of the carboxylate for CB-1-TAM ($^{-}OOCCH_2N^{+}H(CH_3)_2$) and CB-2-TAM ($^{-}OOCCH_2CH_2N^{+}H(CH_3)_2$). The data shows significantly higher intermoleular interactions for the two-carbon spacer which can lead to aggregation within a polymer brush, thereby resulting in higher fouling.

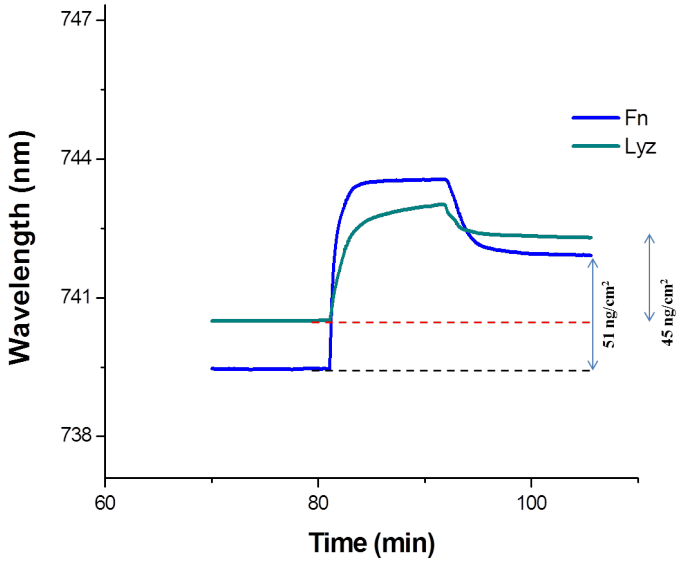


Figure S5. SPR sensorgrams for fouling experiments using lysozyme and fibrinogen at pH 7.4 (PBS) for the two carbon spacer polymer (CBMA-2-TAM) surface.

References

1. J. E. Krause, N. D. Brault, Y. Li, H. Xue, Y. Zhou, S. Jiang, *Macromolecules*, 2011, **44**, 9213-9220.

2 H. Vaisocherova, W. Yang, Z. Zhang, Z. Cao, G. Cheng, M. Piliarik, J. Homola, S. Jiang, *Anal. Chem.*, 2008, **80**, 7894-7901.

3 J. Homola, *Chem. Rev.*, 2008, **108**, 462-493.

4 W. Yang, H. Xue, W. Li, J. Zhang, S. Jiang, *Langmuir*, 2009, **25**, 11911-11916.

5. J. Homola, J. Dostalek, Editors. Surface Plasmon Resonance Based Sensors, Berlin: Springer, 2006.

6. M. J. Frisch, G. W. Trucks, H. W. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, Gaussian 09, revision A.1, Gaussian, Inc., Wallingford CT 2009.

7 A. K. Rappe, C. J. Casewit, K. S. Colwell, W. A. Goddard, W. M. Skiff, UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations. *J. Am. Chem. Soc.*, 1992, **114**, 10024–10035.

8. H. J. C. Berendsen, J. R. Grigera, T. P. Straatsma, *J. Phys. Chem.*, 1987, **91**, 6269-6271.

9. A. White, S. Jiang, *J. Phys. Chem. B*, 2011, **115**, 660-667.

10. W. L. Jorgensen, D. S. Maxwell, J. TiradoRives, *J. Am. Chem. Soc.*, 1996, **118**, 11225-11236.

11. Q. Shao, Y. He, A. D.White, S. Jiang, *J. Phys. Chem. B*, 2010, **114**, 16625-16631.

12. D. Van der Spoel, E. Lindahl, B. Hess, G. Groenhof, A. E. Mark, H. J. C. Berendsen, *J. Comp. Chem.*, 2005, **26**, 1701-1718.

13. H. J. C. Berendsen, J. P. M. Postma, W. F. Vangunsteren, A. Dinola, J. R. Haak, *J. Chem. Phys.*, 1984, **81**, 3684-3690.