

Formation of surface-grafted polymeric amphiphilic coatings comprising ethylene glycol and fluorinated groups and their response to protein adsorption

Shafi Arifuzzaman, Ali E. Özçam, and Kirill Efimenko

Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905

Daniel A. Fischer

Ceramics Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

Jan Genzer^{a)}

Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905

(Received 19 February 2009; accepted 13 March 2009; published 21 May 2009)

Amphiphilic polymer coatings were prepared by first generating surface-anchored polymer layers of poly(2-hydroxyethyl methacrylate) (PHEMA) on top of flat solid substrates followed by postpolymerization reaction on the hydroxyl terminus of HEMA's pendent group using three classes of fluorinating agents, including organosilanes, acylchlorides, and trifluoroacetic anhydride (TFAA). The distribution of the fluorinated groups inside the polymer brushes was assessed by means of a suite of analytical probes, including contact angle, ellipsometry, infrared spectroscopy, atomic force microscopy, and near-edge x-ray absorption fine structure spectroscopy. While organosilane modifiers were found to reside primarily close to the tip of the brush, acylchlorides penetrated deep inside PHEMA thus forming random copolymers P(HEMA-*co*-fHEMA). The reaction of TFAA with the PHEMA brush led to the formation of amphiphilic diblocks, PHEMA-*b*-P(HEMA-*co*-fHEMA), whose bottom block comprised unmodified PHEMA and the top block was made of P(HEMA-*co*-fHEMA) rich in the fluorinated segments. This distribution of the fluorinated groups endowed PHEMA-*b*-P(HEMA-*co*-fHEMA) with responsive properties; while in hydrophobic environment P(HEMA-*co*-fHEMA) segregated to the surface, when in contact with a hydrophilic medium, PHEMA partitioned at the brush surface. The surface activity of the amphiphilic coatings was tested by studying the adsorption of fibrinogen (FIB). While some FIB adsorption occurred on most coatings, the ones made by TFAA modification of PHEMA remained relatively free of FIB. © 2009 American Vacuum Society. [DOI: 10.1116/1.3114502]

I. INTRODUCTION

One of the outstanding issues of modern materials science involves the development of coatings that resist the deposition of biomaterials.¹ While early strategies have relied primarily on tuning the coating's chemical composition, recent developments in the field have revealed that combating adsorption of biomass onto man-made surface requires not only control over the coating's surface chemistry but also tailoring its topography, charge, mobility of the surface groups, and mechanical properties.^{2,3} Polymeric materials are particularly well suited for preparing antifouling surfaces because they enable facile control over the chemical composition, shape, charge, mobility, and modulus. In this contribution we describe the formation and characterization of coatings whose chemistry and responsive nature have been tailored in order to achieve effective protection against marine biofouling.

Materials designed to resist protein adsorption typically contain ethylene glycol (EG) (Refs. 1 and 4), phosphazene,⁵ or zwitterionic surface groups.^{6,7} However, numerous studies carried out over the past decade have demonstrated that these

types of coatings are not always very effective in preventing adsorption of marine organisms.³ Because of the amphiphilic nature of bio-organisms, designing an effective coating that is capable of resisting bioadhesion is a very challenging task. While some species, such as *Ulva*, settle heavily on hydrophobic surfaces, others, such as *Navicula*, prefer hydrophilic surfaces.⁸ It thus appears that single substrate chemistry cannot be utilized single-handedly in designing multipurpose marine antifouling coatings. To this end, several groups designed amphiphilic coatings comprising EG and fluorinated chemistries that both act in accord in order to minimize adhesion of numerous marine organisms. For instance, Wooley and co-workers synthesized a series of polymeric networks comprising hyperbranched fluoropolymers and poly(EG) chains and studied their resistance against a variety of proteins and zoospores of green fouling alga.^{9–11} The group reported that the coating's performance depended on the chemical composition and the topographical heterogeneity of the surface. In another strategy, Krishnan *et al.* used amphiphilic diblock copolymers comprising EG and fluorinate blocks.¹² Their study demonstrated that these amphiphilic coatings facilitated the removal of both *Ulva* and *Navicula*

^{a)}Electronic mail: jan_genzer@ncsu.edu

by applying a simple water jet cleaning. These two studies represent excellent examples illustrating the potential for developing amphiphilic coatings that contain both hydrophobic as well as hydrophilic components that act in concert to prevent (or at least minimize) marine adhesion.

The formation of functional fluorocarbon-based antifouling coatings typically requires sophisticated chemical routes that are further complicated by rather strong chemical immiscibility among the various components (i.e., those between hydrocarbons and fluorocarbons). Here we propose a new strategy that could circumvent the aforementioned synthetic drawbacks and suggest that functional coatings can be successfully prepared by postpolymerization chemical modification of surface-grafted macromolecules. Specifically, we demonstrate that amphiphilic polymer coatings can be prepared by first decorating substrates with end-grafted EG moieties based on poly(2-hydroxyethyl methacrylate) (PHEMA) polymers followed by attaching commercially available fluorinated units to the hydroxyl terminus present in HEMA. Specifically, we discuss three different chemical routes leading to the formation of EG-fluorinated amphiphiles by attaching (1) organosilanes, (2) acylchlorides, and (3) trifluoroacetic anhydride to the PHEMA brush backbone. The distribution of the fluorinated groups inside the PHEMA brushes is governed both by the head-group chemistry present in the fluorinated modifier that reacts with the $-OH$ groups in PHEMA and by the “bulkiness” of the fluorinated mesogen. In order to address the interplay between the structural nature of the fluorinated modifiers and its distribution inside the PHEMA brush, we use modifiers with various types of mesogens. We employ a suite of surface-sensitive analytical probes to assess the spatial distribution of the fluorinated units inside the brush. We also test the response of such-prepared amphiphilic coatings to fibrinogen, which was shown to exhibit the adsorption characteristics that are similar to those of *Ulva*.¹³

II. MATERIALS AND METHODS

A. Preparation of PHEMA brushes on silicon wafers

After chilling 30 ml of anhydrous toluene (dried over $MgSO_4$) in a glass vial to $-20\text{ }^{\circ}C$, $2.5\text{ }\mu l$ of (11-(2-bromo-2-methyl)propionyloxy)undecyltrichlorosilane (BMPUS) was added, which was synthesized by following previous reports.¹⁴ Silicon wafers (Silicon Valley Microelectronics) were cut into $1 \times 7\text{ cm}^2$ pieces and exposed for 10 min to ultraviolet/ozone treatment in order to generate a large number of surface hydroxyl groups that served as attachment points for BMPUS. Each wafer was then added to the toluene solution of BMPUS and allowed to sit at $-20\text{ }^{\circ}C$ for 18 h, after which the wafer was removed, rinsed copiously with toluene, and sonicated in neat toluene twice for 10 min. BMPUS thus deposited forms an organized self-assembled monolayer (SAM) on the silica-covered substrate. Measurements using variable angle spectroscopic ellipsometry (VASE) (J. A. Woollam, Co.) confirmed that only a monolayer of BMPUS was formed and that BMPUS molecules

were homogeneously distributed on the substrate. The bromoisobutyric terminus in BMPUS acted as an initiation point for polymerization of HEMA using atom transfer radical polymerization.

The polymerization reaction solution was prepared in a round-bottom, single-neck Schlenk flask equipped with an all-Teflon® Airfree® valve (Chemglass) under nitrogen purge in order to maintain an oxygen-free environment. In order to generate linear (uncross-linked) PHEMA brushes, we followed the procedure suggested in Ref. 15. The flask was first flushed with nitrogen and the chamber was charged with solution comprising 75.65 g of 2-hydroxyethyl methacrylate (HEMA) (98%, ACROS), 51.5 g of methanol [high performance liquid chromatography (HPLC) grade, Fisher Scientific], 14.2 g of de-ionized water (DIW), 8.41 g of bipyridine (99%, ACROS), 2.71 g of $CuCl$ (99.99%, Sigma-Aldrich), and 0.2 g of $CuCl_2$ (99.99%, Sigma-Aldrich). Methanol and DIW were used as cosolvents. The solvents and monomer were purged with nitrogen prior to polymerization in order to remove any traces of oxygen. The reaction mixture was stirred for 2 h to dissolve and homogenize all the salts, monomer, and solvents. A positive pressure of nitrogen was maintained in the flask to avoid any ambient oxygen contamination. Two silicon wafers covered with BMPUS were placed back to back in a 30 ml nitrogen purged empty vial. The polymerization of HEMA was carried out at $25\text{ }^{\circ}C$. The polymerization time was chosen to form PHEMA brushes of desired thickness (polymerization rate was $\approx 1\text{ }\text{\AA}/\text{min}$). After a predetermined period of time the substrate was removed from the reaction mixture, exposed to air, and thoroughly washed with MeOH and DIW and blow-dried with nitrogen. The substrates were further extracted with methanol in a Soxhlet extraction chamber to remove trace amount of copper salts and unreacted monomers. Two types of substrates containing homogeneous PHEMA polymer brush of 10 and 50 nm dry thicknesses were prepared in this method.

B. Fluorination of PHEMA brushes

Three different chemical routes were employed to prepare P(HEMA-*co*-fHEMA) copolymers, where fHEMA denotes the fluorinated HEMA segment. Specifically, we used commercial fluorine-containing agents based on (1) organosilane, (2) acylchloride, and (3) trifluoroacetic anhydride chemistries to couple with the hydroxyl terminus of the pendent group in HEMA. All reactions were carried out on previously polymerized PHEMA brushes anchored to solid substrates.

Numerous research groups reported on the reaction of organosilanes either in solution or in vapor, with hydroxyl groups present on the surfaces of metallic (e.g., CuO , AgO) or inorganic oxides (e.g., SiO_2) with subsequent formation of SAMs.^{16,17} Generally, the reactive silane groups first undergo a hydrolysis step to form silanols, followed by the condensation reaction with the surface-anchored hydroxyl groups resulting in the formation of covalent bonds with the surface. The end-functional group of formed SAM dictates the

chemical composition of the surface. Typically, chlorosilanes are employed in these coupling reactions because of their high reactivity relative to those of alkoxy-silanes. Two research groups reported on successful introduction of alkyl side chains to PHEMA in the bulk using organosilane coupling agents.^{18,19} Our goal is to extend the previous work and introduce short fluorocarbon chain to the surface-grafted PHEMA macromolecules. Silica substrates containing PHEMA brush were cut into 1×1 cm² pieces. We used two fluorine-containing organosilane coupling agents (both obtained from Alfa-Aesar): 1*H*,1*H*,2*H*,2*H*-perfluorodecyl dimethylchlorosilane (mF8H2) and 1*H*,1*H*,2*H*,2*H*-perfluorodecyl trichlorosilane (tF8H2). Because of differences in solubilities of PHEMA and the organosilane, we explored a large variety of solvents that would facilitate successful coupling of the organosilane precursors to PHEMA. Details of our experiments are provided in Appendix B. Optimal modification was obtained when the PHEMA substrate was treated with 10 mM silane solution in anhydrous cyclohexane with *n*-butyl-dilauryltin as a catalyst (concentration of 4 μ l per 30 ml of solution). The coupling reaction was carried out for 12 h at room temperature. One concern when working with organosilane modifiers involves the stability of the Si–O–C bond, which is known to be susceptible to large variations in solution pH.²⁰ In Appendix C we provide details of stability experiments, which indicate that both mF8H2-PHEMA and tF8H2-PHEMA remained stable for many hours when exposed to solutions whose pH ranged from 4 to 9.

Attachment of fluorocarbon modifiers bearing an acid chloride head group was performed according to the procedure described by Jennings and co-workers.^{21–25} Three different acylchlorides were employed (all obtained from Sigma-Aldrich): heptafluorobutyl chloride (C₃F₇COCl, F3), pentadecafluoro-octanoyl chloride (C₇F₁₅COCl, F7), and pentafluorobenzoyl chloride (C₆F₅COCl, PFA). Substrates with PHEMA brushes were exposed to 80 mM solutions of a given acylchloride with 100 mM pyridine in dichloromethane for 24 h at room temperature to modify PHEMA films with fluorinated side chains (see Fig. 1). After the coupling reaction, the films were rinsed with dichloromethane and dried by dry nitrogen gas.

Finally, we modified the –OH groups in PHEMA brushes with trifluoroacetic anhydride (TFAA), obtained from Sigma-Aldrich, according to the procedure suggested by Valdes *et al.*²⁶ PHEMA substrates were incubated in TFAA at 50 °C for 0.5 h, then washed and sonicated with DI water. Finally the sample was blow-dried with nitrogen gas.

C. Protein solution preparation and protein deposition on surfaces

Dry fibrinogen (FIB) from human plasma was obtained from Sigma-Aldrich. The dry powder contained $\approx 60\%$ protein, 15% sodium citrate, and $\approx 25\%$ sodium chloride. A stock solution of 0.1 mg/ml solution was prepared at a desired pH level by dissolving it in 1X-PBS buffer solution obtained from Fisher Scientific (composition: 0.137M NaCl,

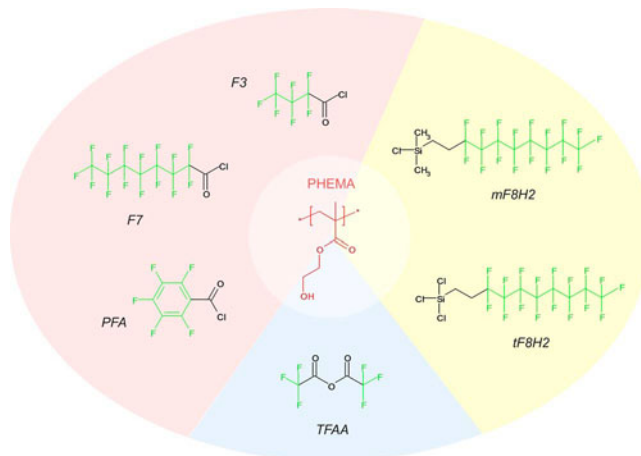


FIG. 1. (Color online) Schematic representing the structure of building blocks leading to the formation of P(HEMA-*co*-fHEMA) random copolymers. Three families of fluorinated modifiers have been employed that include (1) organosilanes (mF8H2 and tF8H2), (2) TFAA, and (3) acylchlorides (F3, F7, and TFA).

0.0027M KCl, and 0.0119M phosphates). The solution pH was adjusted to the pH levels of 4.4, 5.4, 6.4, and 7.4 by adding an appropriate amount of 0.1N of HCl/NaOH before addition of any protein. NaN₃ was added to the buffer to reach a final concentration of 0.2%, which was sufficient to inhibit any bacterial growth during the experiment. Finally, the solution was filtered through a 0.2 μ m filter and further diluted to make a 0.01 mg/ml solution. Previous experiments performed by Guicai *et al.* indicated that a full FIB monolayer was deposited on the surface when protein adsorption was administered at this concentration level.²⁷

Adsorption of FIB was accomplished by incubating silica substrates coating with the P(HEMA-*co*-fHEMA) brushes in protein solution for 16 h at room temperature. After incubation the wafers were sonicated in DI water for 5 min and washed thoroughly with DI water and blow-dried with nitrogen gas. The samples were subsequently stored in a plastic Petri dish for further characterization.

D. Characterization methods

The Nicolet 6700 infrared spectrometer in an attenuated total reflection (ATR) mode [Fourier transform infrared (FTIR)] equipped with Ge crystal and purged with dry air was utilized to verify bonding of the fluorinated groups to PHEMA and assess relative concentration (and in the case of tF8H2 structure) in the P(HEMA-*co*-fHEMA) layers. All measurements were carried out for at least 2048 scans with the resolution of 4 cm^{–1} with the simultaneous background and ATR corrections in order to enhance the signal-to-noise ratio due to a small thickness of the organic coating.

We used ellipsometry to measure the thickness of PHEMA before and after fluorination in order to establish the degree of modification. Additionally, ellipsometry was utilized to measure the extent to protein adsorption on top of P(HEMA-*co*-fHEMA) films. Ellipsometry measurements were performed using a J. A. Woollam Co. VASE with

WVASE32 software for modeling. Measurements were taken with the light incident at a 70° angle with respect to the surface normal using wavelengths ranging from 400 to 1100 nm with a step of 10 nm. A three-layer Cauchy model comprising Si/SiO_x/P(HEMA-*co*-fHEMA) was employed to evaluate the index of refraction of the P(HEMA-*co*-fHEMA) films, whose refractive index varied between that of pure PHEMA and pure fluorinated component. The thickness of FIB on top of P(HEMA-*co*-fHEMA) layer was evaluated using a four-layer Cauchy model for Si/SiO_x/P(HEMA-*co*-fHEMA)/FIB. We used the index of refraction of P(HEMA-*co*-fHEMA) obtained by fitting the ellipsometry data from sample after fluorination (see above) and assumed the refractive index of the protein layer to be 1.54.²⁸

Contact angles by DIW were measured on each sample surface using a Ramé–Hart contact angle goniometer and analyzed with the RAMÉ–HART software. The advancing contact angles were recorded by injecting 8 μ l of probing liquid; the receding contact angles were determined by removing 4 μ l of probing liquid from the droplet. At least three measurements were performed on each substrate. The contact angle hysteresis (CAH), defined here as the difference between the advancing and receding contact angles, provides information about topographical and/or chemical heterogeneity of the substrates. CAH $\leq 10^\circ$ is typically considered a signature of a molecularly uniform surface.²⁹

The surface chemical composition of P(HEMA-*co*-fHEMA) specimens was determined with a Kratos Axis Ultra DLD x-ray photoelectron spectroscopy (XPS) instrument using monochromated Al *K* α radiation with charge neutralization. Survey and high-resolution spectra were collected with pass energies of 80 and 20 eV, respectively, by using both electrostatic and magnetic lenses for single angle spectra collection. Angle-resolved XPS was used to gain information about depth-dependent distribution of the fHEMA units in the sample. The angle-resolved XPS measurements were conducted at different take-off angles, defined here as the angle between the sample surface and the detector; only electrostatic lens was utilized in order to achieve better angular resolution. Small take-off angles probe deeper areas in the sample, while larger take-off angles probe layers close to the surface; the estimated probing depths are ≈ 9 and ≈ 4.5 nm for take-off angles of 90° and 30° , respectively.³⁰ Elemental chemical compositions from XPS measurements were quantified from spectral regression using VISION and CASAXPS software packages.

Near-edge x-ray absorption fine structure (NEXAFS) spectroscopy was performed on each sample at NIST/Dow Soft X-ray Materials Characterization Facility at the National Synchrotron Light Source at Brookhaven National Laboratory. NEXAFS spectroscopy involves the resonant soft x-ray excitation of a *K*- or *L*-shell electron to an unoccupied low-lying antibonding molecular orbital of σ symmetry, σ^* , or π symmetry, π^* .³¹ The initial state *K*-shell excitation gives NEXAFS its element specificity, while the final-state unoccupied molecular orbital provides NEXAFS with its bonding

or chemical selectivity. A measurement of the partial electron yield (PEY) intensity of NEXAFS spectral features thus allows for the identification of chemical bonds and determination of their relative population density and orientation within the sample.³² The NEXAFS data were collected at various angles, $\theta = 20^\circ$, 50° , and 90° , where θ denotes the angle between the sample normal and the direction of the electric vector of the x-ray beam. The pre-edge and postedge in the NEXAFS spectra were normalized to 0 and 1, respectively. Several characteristic peaks can be identified in each set of the PEY NEXAFS spectra. These correspond to the $1s \rightarrow \sigma^*$ transitions associated with the C–H ($E = 287.5$ eV), C–F ($E = 292.0$ eV), and C–C ($E = 295.5$ eV) bonds. The orientation of the fluorinated moieties on the surfaces of the samples was determined by comparing the spectra collected in the glancing ($\theta = 20^\circ$) and normal ($\theta = 90^\circ$) geometries.

The changes in the surface morphology of PHEMA after fluorination and subsequent deposition of fibrinogen were recorded using scanning probe microscopy (SPM) (Quesant, Ambios Technology Inc.) on dry samples. The $5 \times 5 \mu\text{m}^2$ lateral scans were performed in the SPM “BB wavemode” for at least three different areas in each sample. All observed features were consistent within each sample. Final images were mathematically corrected using second order polynomial function to remove artificial tilt recorded during the scanning.

III. RESULTS AND DISCUSSION

As discussed in Sec. I, amphiphilic fluorine-containing polymer brushes were prepared by first synthesizing PHEMA via “grafting from” polymerization of HEMA from surface-bound initiators followed by postpolymerization reaction using various fluorination agents directly on the PHEMA brushes. Such postpolymerization reaction routes commonly lead to the formation of random copolymers (RCPs), as reported earlier by several groups.^{24,33,34} The amount of modifying agent that produces fHEMA units as well as the distribution of the two comonomers, HEMA and fHEMA, will depend on several system parameters, including (1) grafting density (σ_{PHEMA}) and (2) molecular weight (M_{PHEMA}) of the parent PHEMA homopolymer brush, (3) the size of the fluorinating agent, and (4) the reactivity between the function group on the parent homopolymer brush (–OH in the case of PHEMA) and the head group present in the modifying agent. Previous theoretical and experimental studies on polymer/nanoparticle hybrids prepared by diffusing nanoparticles inside swollen homopolymer brushes have provided clear evidence that the penetration depth of the particles depends on the interplay between the size of the particle and the grafting density and molecular weight of the brush.^{35–41} Similar effects are expected to control the distribution of the fluorinating moieties in the P(HEMA-*co*-fHEMA) RCP brushes. In order to address the interplay between the size and reactivity of the modifying agent, we employed a variety of commercially available fluorinated compounds. Figure 1 summarizes pictorially the chemical modification routes employed in this work; the procedures

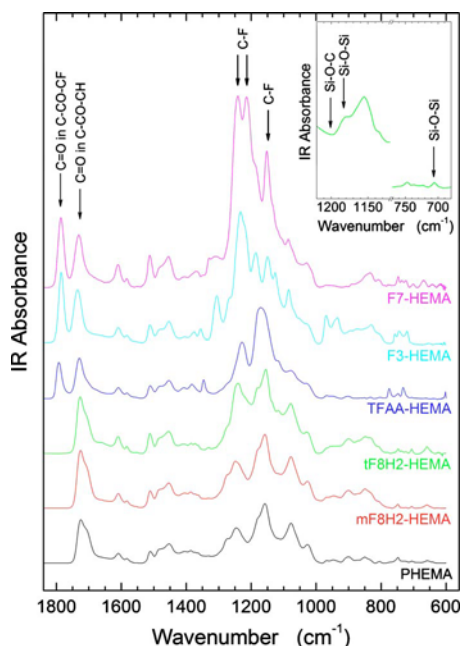


FIG. 2. (Color online) FTIR spectra collected in the ATR mode from P(HEMA-*co*-fHEMA) random copolymer brushes made by modifying PHEMA brushes (dry thickness of ≈ 50 nm) with mF8H2, tF8H2, TFAA, F3, and F7 fluorinating modifiers. The inset depicts details of the IR spectrum of PHEMA modified with tF8H2. Characteristic IR stretches are marked with arrows.

leading to P(HEMA-*co*-fHEMA) RCP brushes are detailed in Sec. II. In order to explore how the properties of the parent PHEMA affect the concentration and distribution of fHEMA inside the brush, we kept the σ_{PHEMA} constant at 0.45 nm^{-2} (Ref. 42) and prepared PHEMA brushes having two different dry thicknesses: 10 and 50 nm, which corresponded to the molecular weights of PHEMA of ≈ 12 and ≈ 60 kDa, respectively.⁴³ Details pertaining to the formation of PHEMA brushes have been given in Sec. II. We employed a suite of surface analytical tools in order to assess the concentration and distribution of the fluorinated species inside P(HEMA-*co*-fHEMA) RCP brushes and to monitor the response of such surface to FIB solutions.

The chemical coupling of the fluorinated agents and their relative population inside the sample were accessed via FTIR-ATR. In Fig. 2 we present IR absorbance curves for P(HEMA-*co*-fHEMA) specimens for the various fluorinating agents. The following conclusions can be deduced from the spectra. Fluorination of PHEMA brushes with mF8H2 does not lead to large increases in the fluorine signal inside the sample; the mF8H2-PHEMA spectrum is very similar to that of pure PHEMA. Later in the article we provide evidence that mF8H2 attaches to PHEMA, albeit only in small amounts. In contrast, addition of tF8H2 to PHEMA causes substantial increases in the fluorine signal. The strong increase in the ATR-FTIR signals around 1150, 1214, and 1240 cm^{-1} can be attributed to the presence of semifluorinated alkyl. Moreover, the existence of C–O–Si bond is evident by the vibrational stretch at 1200 cm^{-1} (see inset to Fig. 2) demonstrating coupling between tF8H2 and PHEMA.

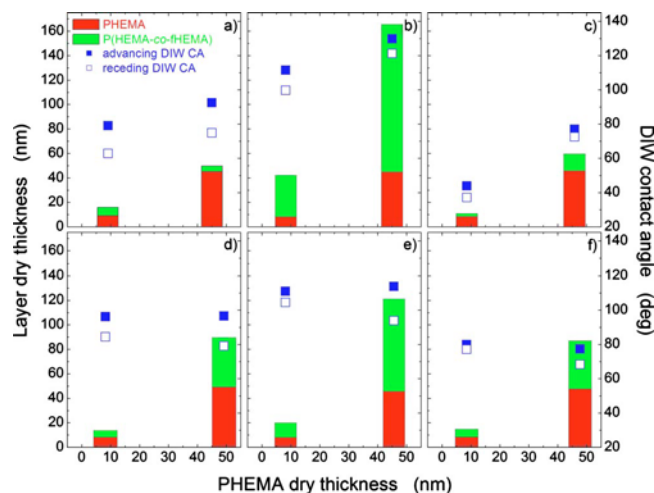


FIG. 3. (Color online) (Left ordinate) Dry thickness of PHEMA (red/darker column) and P(HEMA-*co*-fHEMA) (green/lighter column) and (right ordinate) DIW contact angle (advancing: solid symbols; receding: open symbols) as a function of PHEMA dry thickness for fHEMA prepared by modifying PHEMA brushes with (a) mF8H2, (b) tF8H2, (c) TFAA, (d) F3, (e) F7, and (f) PFA. Error in thickness and DIW contact angle is less than ± 0.5 nm and $\pm 1.5^\circ$, respectively.

Moreover, we also detect a signal corresponding to Si–O–Si bonds (stretches at 700 and 1187 cm^{-1} , see inset of Fig. 2) that suggests the formation of silane network between neighboring trifunctionalized tF8H2. The attachment of TFAA and all acylchlorides to PHEMA is clearly demonstrated by the presence of the stretch at 1786 cm^{-1} corresponding to formation of the carbonyl group CH–COO–CF. By exploring the intensity of this stretch in combination with –CF₂–vibrational stretches at 1150, 1214, and 1240 cm^{-1} one can conclude that the loading of the fluorinated agent inside PHEMA decreases in the following fashion: $F7 > F3 > TFAA$. While useful in providing proof of coupling and information about relative loading of the PHEMA brush with the fluorinating agent, FTIR alone cannot be used to quantify the amount of fluorination and the spatial distribution of the fluorinated moieties inside the sample. We therefore used additional complementary analytical tools, i.e., ellipsometry, contact angle, and angle-resolved XPS, to supplement the FTIR data.

In Fig. 3 we plot the thickness (left ordinate) of PHEMA (red/darker columns) and P(HEMA-*co*-fHEMA) (green/lighter column) brushes and the corresponding contact angles (right ordinate) using DIW as a function of the thickness of the original PHEMA brush. From the data, the loading of fHEMA inside P(HEMA-*co*-fHEMA) increases with increasing length of the PHEMA brush; one exception to this trend represents PHEMA modified with mF8H2, which exhibits a small decrease in the amount of fHEMA with increasing PHEMA thickness. The analysis of our data revealed that ellipsometry was not sensitive enough to determine the variation of the fHEMA concentration inside the brush; in all cases data modeling indicated that fHEMA is a “graded” layer, whose composition resides between that of pure HEMA and pure fHEMA. The only exception was the

sample prepared by modifying PHEMA with tF8H2, whose analysis detected that a pure layer of tF8H2 may be present on top of P(HEMA-*co*-fHEMA). Combined with the IR data, the latter observation strongly suggests that the surfaces of tF8H2-modified PHEMA brushes are decorated with dense tF8H2 SAM networks stabilized by in-plane linkages among neighboring molecules. We will return to the discussion of this sample in detail later in this section.

The ellipsometric thickness data can be quantified by invoking a simple model, which relates the variations in thickness to changes in the overall chemical composition of P(HEMA-*co*-fHEMA). To this end, the grafting density of a PHEMA brush can be expressed as

$$\sigma_{\text{PHEMA}} = \frac{h_{\text{PHEMA}} \rho_{\text{PHEMA}} N_A}{M_{\text{PHEMA}}}, \quad (1)$$

where h_{PHEMA} is dry PHEMA thickness, ρ_{PHEMA} is PHEMA density, M_{PHEMA} is PHEMA molecular weight, and N_A is Avogadro's number. One can write a similar equation for the grafting density of the P(HEMA-*co*-fHEMA) RCP:

$$\begin{aligned} \sigma_{\text{P(HEMA-co-fHEMA)}} &= \frac{h_{\text{P(HEMA-co-fHEMA)}} \rho_{\text{P(HEMA-co-fHEMA)}} N_A}{M_{\text{P(HEMA-co-fHEMA)}}} \\ &= \frac{h_{\text{P(HEMA-co-fHEMA)}} \rho_{\text{PHEMA}}}{\rho_{\text{P(HEMA-co-fHEMA)}}} \frac{M_{\text{P(HEMA-co-fHEMA)}}}{M_{\text{PHEMA}}}. \end{aligned} \quad (2)$$

Assuming that no chain cleavage occurs during the fluorination reaction, the grafting density of the polymer remains the same after fluorination, i.e., $\sigma_{\text{PHEMA}} = \sigma_{\text{P(HEMA-co-fHEMA)}}$. By combining Eqs. (1) and (2) and after some algebra, one arrives at

$$\frac{h_{\text{P(HEMA-co-fHEMA)}}}{h_{\text{PHEMA}}} = \frac{\rho_{\text{PHEMA}}}{\rho_{\text{P(HEMA-co-fHEMA)}}} \frac{M_{\text{P(HEMA-co-fHEMA)}}}{M_{\text{PHEMA}}}. \quad (3)$$

Equation (3) can be further simplified by considering that the degree of polymerization of the polymer brush does not change after fluorination. Hence:

$$\frac{h_{\text{P(HEMA-co-fHEMA)}}}{h_{\text{PHEMA}}} = \frac{\rho_{\text{PHEMA}}}{\rho_{\text{P(HEMA-co-fHEMA)}}} \frac{M_{o,\text{HEMA-co-fHEMA}}}{M_{o,\text{HEMA}}}, \quad (4)$$

where $M_{o,\text{HEMA-co-fHEMA}}$ is an “effective” molecular weight of the copolymer unit and $M_{o,\text{HEMA}}$ is the molecular weight of HEMA.

The density and molecular weight of HEMA-*co*-fHEMA are given by Eqs. (5) and (6), respectively. They read

$$\frac{1}{\rho_{\text{HEMA-co-fHEMA}}} = \frac{w_{\text{fHEMA}}}{\rho_{\text{fHEMA}}} + \frac{1 - w_{\text{fHEMA}}}{\rho_{\text{HEMA}}}, \quad (5)$$

$$\frac{1}{M_{o,\text{HEMA-co-fHEMA}}} = \frac{w_{\text{fHEMA}}}{M_{o,\text{fHEMA}}} + \frac{1 - w_{\text{fHEMA}}}{M_{o,\text{HEMA}}}. \quad (6)$$

In Eqs. (5) and (6), ρ_{fHEMA} and $M_{o,\text{fHEMA}}$ stand for the density and molecular weight of fHEMA, respectively, ρ_{HEMA} is the

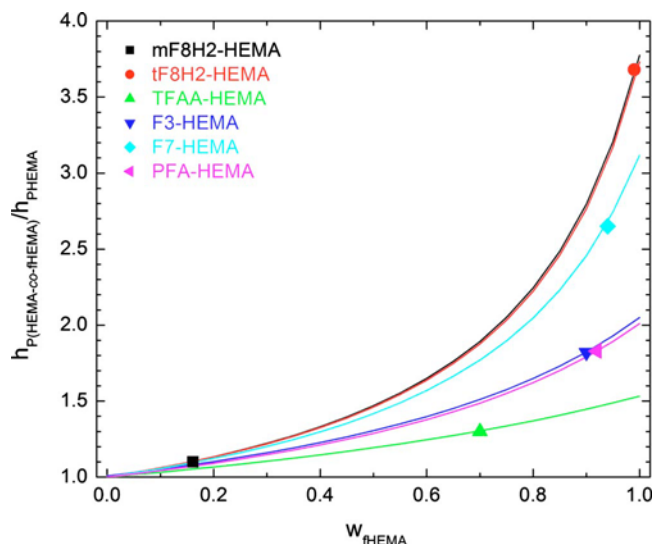


FIG. 4. (Color online) Ratio of a dry thickness of P(HEMA-*co*-fHEMA) and PHEMA as a function of the weight fraction of fHEMA in P(HEMA-*co*-fHEMA) for various fluorinated modifiers. The thickness of the parent PHEMA brush was ≈ 50 nm. The solid lines represent predictions using the model described in the text. The symbols depict the experimental data.

density of HEMA (assumed for simplicity to be equal to that of PHEMA), and w_{fHEMA} represents the weight fraction of fHEMA in P(HEMA-*co*-fHEMA). The method for determining ρ_{fHEMA} and $M_{o,\text{HEMA-F}}$ is outlined in Appendix A.

In Fig. 4 we plot $h_{\text{P(HEMA-co-fHEMA)}}/h_{\text{PHEMA}}$ as a function of w_{fHEMA} . The curves were generated by combining Eqs. (4)–(6) and using the corresponding values of unit molecular weight and density given in Table I. In the same figure we also plot (using solid symbols) the experimentally measured values of $h_{\text{P(HEMA-co-fHEMA)}}/h_{\text{PHEMA}}$ for each fHEMA system prepared on PHEMA with the initial dry thickness of ≈ 50 nm. The weight fractions of fHEMA can be converted into mole fractions, x_{fHEMA} ; the latter values are given in the last column in Table I. The data in Fig. 4 indicate that the loading of the fluorinated modifier inside ≈ 50 nm thick PHEMA brushes depends significantly on the type of the chemical modifier. As will be discussed later in the article, this is likely due to both the size of the modifier as well as the reactivity of the modifier's head group with the hydroxyl terminus in PHEMA's pendent group. The data can be split roughly into four groups. mF8H2 displays the smallest uptake ($\approx 4\%$, see Table I) inside the PHEMA brushes. In contrast, the amount of tF8H2 is the highest ($\approx 100\%$). However, because some tF8H2 is likely present on top of the sample in the form of tF8H2 networks, as mentioned previously, the actual uptake of tF8H2 inside PHEMA brushes is difficult to estimate. The concentration of fHEMA after reaction with TFAA is halfway between that of tF8H2 and tF8H2, $\approx 57\%$. Finally, the concentration of the acylchloride-based species is $\approx 80(\pm 2\%)$. We next complement the IR and ellipsometry measurements with contact angle, angle-resolved XPS, and NEXAFS data and use those to provide a complete picture of the distribution of the fHEMA units inside the sample.

TABLE I. Molecular properties of individual components and fHEMA-based species. M_o is the component molecular weight, ρ is the component density, w_f is the weight fraction of the fluorinated modifier in fHEMA [$w_f = M_{o,f} / (M_{o,f} + M_{o,\text{HEMA}})$], $M_{o,\text{fHEMA}}$ is the molecular weight of fHEMA, ρ_{fHEMA} is the density of fHEMA, w_{fHEMA} is the weight fraction of fHEMA in P(HEMA-co-fHEMA), and x_{fHEMA} is the mole fraction of fHEMA in P(HEMA-co-fHEMA).

Component	Individual components		w_f	fHEMA		P(HEMA-co-fHEMA)	
	M_o (Da)	ρ (g/cm ³)		$M_{o,\text{fHEMA}}$ (Da)	ρ_{fHEMA} (g/cm ³)	w_{fHEMA}	x_{fHEMA}
PHEMA	129.0	1.07	0.00	129.0	1.07	0.00	0.00
mF8H2	540.5	1.51	0.80	634.3	1.39	0.16	0.04
tF8H2	581.5	1.54	0.80	636.2	1.41	1.00	1.00
TFAA	97.0	1.51	0.43	226.0	1.22	0.70	0.57
F3	232.5	1.56	0.60	326.1	1.32	0.90	0.78
F7	432.5	1.56	0.75	526.1	1.40	0.94	0.78
PFA	230.5	1.60	0.60	324.1	1.34	0.92	0.82

In spite of their simplicity, contact angle measurements often reveal valuable information about the chemical composition of the uppermost regions of substrates. As a reference, the DIW contact angle (θ_{DIW}) of pure PHEMA brush is $\approx 45^\circ$,⁴⁴ and a SAM made of densely packed fluorinated material (tF8H2) on a flat silica-coated substrate exhibits $\theta_{\text{DIW}} \approx 115^\circ$.² For simplicity we split the data into three categories depending on the value of θ_{DIW} . The first category involves TFAA- and PFA-modified samples, which remain partially hydrophilic after fluorination ($\theta_{\text{DIW}} < 90^\circ$); presumably both fHEMA and HEMA components are present close to the sample surface. The second category involves specimens prepared by fluorination of HEMA using mF8H2 and F3. Their θ_{DIW} are still below but close to the values expected for a uniform fluorinated SAM surface. Interestingly, the CAH in these samples is relatively high indicating possible structural and chemical heterogeneity of the surface. One may hypothesize that the surfaces are made of heterogeneous regions rich in the fluorinated component. The last category includes samples formed by fluorinating PHEMA brushes with tF8H2 and F7. They both exhibit the highest measured θ_{DIW} values. In fact θ_{DIW} is much higher than that corresponding to a uniform fluorinated SAM surface. This can only be explained by the presence of a rough hydrophobic surface.^{2,45,46}

We employed angle-resolved XPS to gain information about the spatial distribution of fHEMA in the samples. In Fig. 5 we plot the elemental concentration of carbon, oxygen, fluorine, and silicon as a function of the take-off angle for samples prepared by fluorinating parent PHEMA brushes having initial dry thickness of ≈ 50 nm. The horizontal lines in the figure correspond to the expected elemental concentration in fully fluorinated HEMA. For instance, after quantitatively coupling F7-acylchloride to PHEMA, the overall chemical composition of F7-HEMA should be $\text{C}_{10}\text{H}_9\text{O}_4\text{F}_7$. The solid symbols represent the elemental compositions measured experimentally with XPS. The data from HEMA modified with TFAA and acylchlorides reveal that the surfaces of the samples are made of completely fluorinated HEMA units. Given the maximum penetration depth of ≈ 9

nm, one can conclude that the chemical composition in the topmost part of the samples corresponds to completely modified HEMA samples. It is imperative to stress that XPS measurements are performed under high vacuum, a medium that promotes the segregation of the hydrophobic fHEMA moieties. The distribution of fHEMA deduced from XPS may thus not correspond to the “true” concentration that would be encountered in aqueous solutions. The data collected from organosilanes exhibit large deviations between the expected and measured elemental compositions. For instance, the concentration of fluorine in tF8H2-modified sample is much higher than the value expected for tF8H2-HEMA. Concurrently, the concentrations of carbon and oxygen are much lower. No dependence on the XPS take-off angle is detected. These results thus unquestionably lead to the conclusion that tF8H2 forms dense multilayers on top of PHEMA brushes.

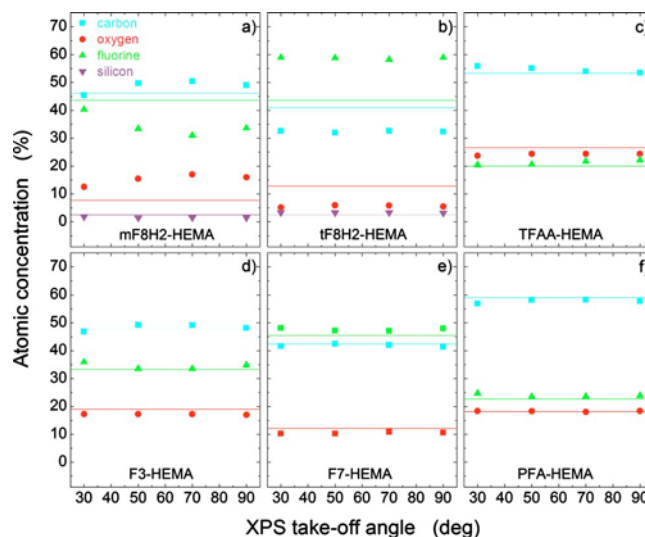


FIG. 5. (Color online) Atomic concentration of elements present in P(HEMA-co-fHEMA) samples as a function of the XPS take-off angle for P(HEMA-co-fHEMA) prepared modifying PHEMA brushes with (a) mF8H2, (b) tF8H2, (c) TFAA, (d) F3, (e) F7, and (f) PFA. The thickness of the parent PHEMA brush was ≈ 50 nm. The lines denote atomic concentration of completely fluorinated PHEMA samples.

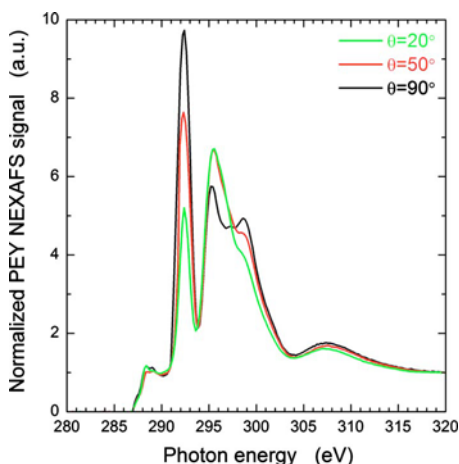


FIG. 6. (Color online) PEY NEXAFS spectra from a P(HEMA-*co*-tF8H2-HEMA) sample collected at the carbon *K* edge at three different sample orientations, θ , where θ denotes the angle between the sample normal and the electric vector of the x-ray beam. The thickness of the parent PHEMA brush was ≈ 50 nm.

While some tF8H2 may still penetrate into the PHEMA brush, it is hard to resolve. This result confirms our earlier observation with IR, ellipsometry, and contact angle measurement. In contrast to tF8H2, attaching mF8H2 to PHEMA does not lead to complete fluorination of PHEMA. Earlier in the article we discussed the results from ellipsometry, which revealed that the concentration of mF8H2 in the sample is very low. This finding is supported by the XPS data, which show a much smaller than expected concentration of fluorine, accompanied with an excess of carbon and oxygen in mF8H2-HEMA. Interestingly, at low take-off angles, where the measurement becomes very sensitive to the concentration at the sample surface, the predicted and measured elemental concentrations in mF8H2-HEMA agree very well. This result thus points to a nearly quantitative attachment of mF8H2 to HEMA at the surface of the sample, which was earlier suggested by the contact angle data. The combination of the results obtained from all analytical methods thus points to a depth-dependent distribution of mF8H2 inside PHEMA. While the bottom parts of the sample remain nearly free of mF8H2, there is a strong segregation of mF8H2 close to the tip of the brush.

As a final installment of our structural characterization we present and discuss the NEXAFS data collected from P(HEMA-*co*-fHEMA) specimens. NEXAFS signal was collected in the partial electron yield mode that is sensitive to the topmost ≈ 2 nm of the sample.^{47,48} All PEY NEXAFS spectra collected at various sample geometries (see Sec. II) were identical indicating no orientation of the fluorinated moieties on the surface of the P(HEMA-*co*-fHEMA) samples. The only exception was the specimen prepared by modifying PHEMA brushes with tF8H2. Here orientation of tF8H2 roughly perpendicular to the sample surface can be seen from the NEXAFS spectra (see Fig. 6). Recall that we have previously suggested that tF8H2 forms multilayers on top of PHEMA brushes. The observation from NEXAFS

only reinforces the fact the tF8H2 forms organized SAM layers that reside on top of PHEMA brushes.

Before we discuss the response of P(HEMA-*co*-fHEMA) substrates to FIB solutions, let us recall the major structural differences among the samples as revealed by the different analytical probes. Those would be important when discussing the FIB adsorption results. The concentration of mF8H2 in the RCPs is rather small and the fluorinated moieties are present predominantly close to the sample surface. It is likely that the two bulky methyl groups attached to the silicon head group cause steric hindrance not allowing mF8H2 to penetrate deeper into the brush. In contrast to mF8H2, the amount tF8H2 grafted to PHEMA is much higher but the molecule resides primarily on the sample surface, forming a thick overlayer of F8H2. Having three reactive groups attached to the silicon atom makes tF8H2 much more reactive than mF8H2 but the tendency of the silanols to condense and form networks in solution leads to large molecular aggregates that cannot penetrate deep into the brush. As a consequence, the substrate surface is made of nearly homogeneous fluorinated layer, which exhibits a large degree of molecular orientation of F8H2, as revealed by NEXAFS. The uptake of all acylchlorides into PHEMA brush is quite high, $\approx 80(\pm 2\%)$. This is because the head group in this class of modifiers is not as bulky as those present in the organosilane moieties and also because of rather high reactivity between $-\text{COCl}$ and $-\text{OH}$. While the two shorter molecules (F3 and PFA) distribute more or less evenly within the brush, there is likely some segregation of F7 at the surface, as suggested by the contact angle experiments. By combining the IR, ellipsometry, contact angle, XPS, and NEXAFS measurements one can conclude that the modification of PHEMA with TFAA leads to amphiphilic copolymers that have an approximate structure of diblocks; the bottom part of the copolymer consists of unmodified PHEMA and the top comprises P(HEMA-*co*-fHEMA) with enhanced segregation of fHEMA close to the sample surface. Among all materials studied the structures prepared by “chemically coloring” PHEMA brushes with TFAA exhibit the best promise in designing functional substrate resisting biomaterial adhesion, as will be demonstrated below.

All P(HEMA-*co*-fHEMA) specimens were tested as substrate for adsorption of FIB. Prior to the FIB adsorption experiments, we exposed P(HEMA-*co*-fHEMA) samples to aqueous solutions of various pH (ranging from 4.4 to 7.4) and after drying remeasured the brush thicknesses. In all cases we recovered the original dry thickness value (error $< 3\%$), which indicated that P(HEMA-*co*-fHEMA) did not undergo degradation due to chain scission or detachment of the fluorinated modifier from the brush. Protein adsorption was conducted in solutions having four different values of pH ranging from 4.4 to 7.4. The highest protein adsorption was expected to occur close to the isoelectric point of FIB ($pI \approx 5.5$),⁴⁹ where FIB appears nearly neutral and thus experiences minimal repulsion from neighboring proteins arriving from solution to the substrate. While very hydrophobic samples were expected to lead to substantial amount of ad-

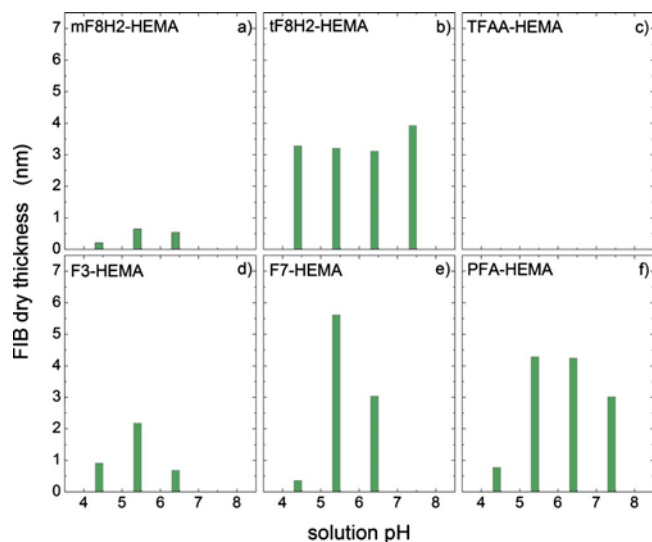


FIG. 7. (Color online) Dry thickness of FIB as a function of solution pH for P(HEMA-*co*-fHEMA) samples prepared by modifying PHEMA brushes with (a) mF8H2, (b) tF8H2, (c) TFAA, (d) F3, (e) F7, and (f) PFA. The error in thickness is less than ± 0.5 nm.

sorbed FIB, increasing the degree of hydrophilicity should lead to decreased amount of FIB on the surface. As a reference point, we also measured FIB adsorption of a parent PHEMA. No FIB could be detected on those samples with ellipsometry. In Fig. 7 we plot the thickness of FIB on substrates prepared by fluorination of parent PHEMA using the studied modifying agents (the dry thickness of the parent PHEMA brush was in all cases ≈ 50 nm). With the exception of tF8H2-modified PHEMA brushes, which exhibit no pH dependence in FIB, we detect the highest amount of FIB at pH close to the *pI* of FIB. The highest amounts of FIB were detected on the samples prepared by reacting PHEMA brushes with tF8H2 and F7. Those amounts indicated that nearly a full monolayer of FIB covered the surface of the sample given the cross section of dried FIB of $\approx 5\text{--}7$ nm.⁵⁰ This behavior is fully consistent with the previously discussed results that indicated that those two specimens exhibited the highest concentration of the fluorinated groups at the surface and thus the highest degree of hydrophobicity. Interestingly, also PFA-modified sample showed substantial amounts of FIB on the surface. Likely the concentration of the PFA groups in the subsurface region was high enough that it led to enhanced fouling on the substrate by the protein. While protein coverage on the aforementioned sample was also close to a full FIB monolayer, FIB deposited onto mF8H2- and F3-modified PHEMA brushes in submonolayer amounts. Based on the contact angle data the surfaces of these two specimens were not made of completely fluorinated groups. The hydrophilic groups and presumably the surface heterogeneity (islands of fluorine-rich and HEMA-rich regions, as deduced from the contact angle hysteresis) lead to smaller amount of FIB adsorbed. TFAA-modified samples performed the best in the FIB adsorption tests. No FIB was detected with ellipsometry in any of the specimens studied.

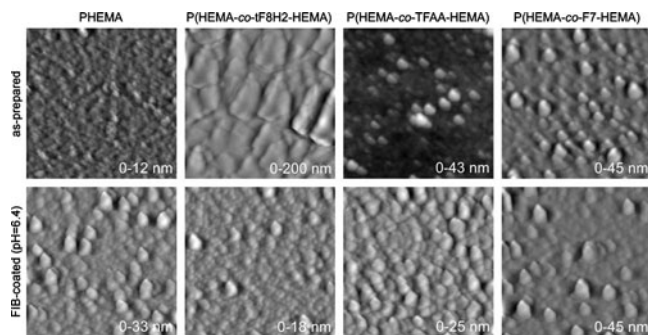


FIG. 8. Atomic force micrographs collected from dry P(HEMA-*co*-fHEMA) samples after fluorination (top row) and after subsequent FIB adsorption at pH=6.4 (bottom row). The height variation indicated in each image corresponds to the color change from black to white. The size of each image is $5 \times 5 \mu\text{m}^2$.

The surface topography of samples before and after FIB adsorption was visualized by means of atomic force microscopy (AFM). In Fig. 8 we present AFM micrographs of selected samples before (top row) and after (bottom row) exposure to FIB solutions at their isoelectric point. Based on the previous discussion these specimens exhibited the lowest (PHEMA- and TFAA-modified PHEMA) and highest (tF8H2-modified and F7-modified PHEMA) FIB adsorption. The surface of PHEMA is relatively smooth. After exposure to the FIB solution, a small amount of FIB traces can be detected, however. Note that those were not “visible” in ellipsometry measurements. TFAA-modified PHEMA surfaces are considerably rougher than those of PHEMA. Presumably some TFAA molecules formed clusters close to the surface. Although ellipsometry did not detect any FIB on top of TFAA-modified PHEMA surfaces, similar to the case of the PHEMA brushes small amounts of FIB aggregates can be detected on top of TFAA-modified PHEMA. Previously in the article we discussed that tF8H2 formed thick surface layers comprising well-organized tF8H2 SAMs resting on top of the polymeric supports. Those aggregates, seen in the AFM scan, are $\approx 1 \mu\text{m}^2$ in size. After exposure to FIB solution, the surface gets filled uniformly with FIB molecules that fill in any gaps on the surface in between tF8H2 clusters. As we discussed previously, the FIB adsorption was the highest on this very hydrophobic substrate. The surfaces of F7-HEMA samples also exhibit in-plane structural features; F7 molecules are long enough to induce strong intermolecular ordering among F7 mesogens on the surface. Those give rise to the aforementioned hydrophobicities and serve as attachment points for FIB molecules adsorbing from solution. Overall, the AFM micrographs are consistent with the findings reported earlier, although they also point out to minute contamination of the PHEMA- and TFAA-modified PHEMA surfaces with FIB.

As suggested earlier from the structural analysis, TFAA modification of PHEMA brushes likely leads to the formation of amphiphilic copolymers comprising an unmodified PHEMA bottom block and heavily fluorinated P(HEMA-*co*-fHEMA) top block, PHEMA-*b*-P(HEMA-*co*-fHEMA). The existence of substrate-anchored amphiphiles is likely respon-

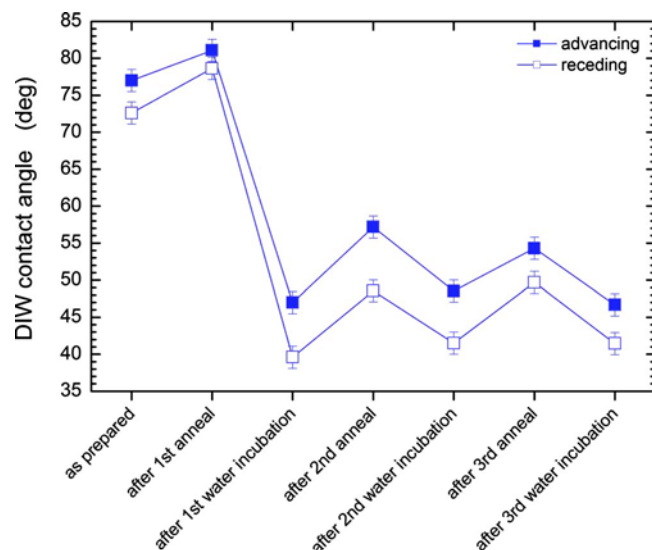


FIG. 9. (Color online) DIW contact angles for TFAA-modified PHEMA brushes after several cycles of anneal and water incubation.

sible for the ability of such macromolecules to resist heavy FIB adsorption. Recall that while FIB adsorbs strongly to hydrophobic substrates, only minute adsorption of FIB was detected on top of TFAA-modified PHEMA brushes with AFM. One plausible explanation for this behavior can be offered by considering the comonomer distribution in the PHEMA-*b*-P(HEMA-*co*-fHEMA) specimen. When in contact with air, the P(HEMA-*co*-fHEMA) block segregates to the free surface. In contrast, in hydrophilic environments, such as water, the PHEMA block tends to partition close to the free surface. We have designed a simple experiment to test this hypothesis. Specifically, we let the PHEMA-*b*-P(HEMA-*co*-fHEMA) sample undergo temperature annealing/water swelling cycles by drying the sample in a vacuum oven at 80 °C for 12 h (annealing) and exposing them to water at room temperature for 12 h and measured the DIW contact angles immediately after removing the sample for the respective environment (followed by briefly drying the sample with nitrogen gas). The contact angles for each cycle are plotted in Fig. 9. After first anneal, the hydrophobicity of the sample increases indicating segregation of the fluorinated component to the surface. After exposing the specimen to water, the contact angle decreases dramatically, even below the original contact angle measured in the as-prepared sample. Additional annealing and water solvation cycles result in contact angle oscillation between 54° and 47°, respectively. We attribute the dramatic changes in the contact angle in the first annealing/water solvation cycle to either relaxation of the chain or to the fact that some unreacted HEMA monomer could be trapped inside the HEMA brush after polymerization and some of it may have even gotten modified with TFAA. Cycling between annealing and water swelling removed these HEMA/TFAA-HEMA complexes. Regardless of the actual cause for these initial changes in the DIW contact angle, the TFAA-modified PHEMA brushes sustained their amphiphilic behavior.

IV. SUMMARY AND OUTLOOK

We formed amphiphilic copolymers comprising EG and fluorinated groups by fluorinating PHEMA brushes chemically anchored to flat solid substrates. A total of six fluorinating agents were employed in PHEMA modification that included three different types of chemical attachment of the fluorinated moieties to the hydroxyl terminus in the pendent group of HEMA, involving the condensation of organosilanes, acylchlorides, and TFAA. For two classes of fluorinated modifiers (organosilanes and acylchlorides) we explored compounds with various degree of bulkiness of the fluorinated mesogen. In all cases studied we observed attachment of the fluorinated groups to the HEMA monomer. A general conclusion that can be drawn from this study is that the spatial distribution of the fluorinated moieties inside the brush depends on a delicate interplay between the size of the group, its reactivity, and the spatial confinement imposed by the brush. A set of experimental probes was utilized that provided complementary information about the distribution of the fluorinating agents in the sample; none of the techniques alone would have allowed an unambiguous determination of the comonomer distribution inside the sample. Because of their bulky head groups (mF8H2) and tendency to form large molecular aggregates in solution (tF8H2) semifluorinated organosilanes remained attached close to the outer periphery of the brush. Acylchlorides were capable of penetrating deeper into the brush and forming P(HEMA-*co*-fHEMA) copolymers with a relatively high loading of the fluorinated groups (~80%).

Chemical modification of PHEMA brushes with TFAA resulted in amphiphilic grafts that had a character of a diblock copolymer with the bottom block being made of unmodified HEMA units and the top block comprising P(HEMA-*co*-fHEMA) with a high concentration of fHEMA. All specimens were tested as substrates for the adsorption of FIB, a protein, whose adsorption properties resemble closely those of *Ulva*. The strongest adsorption of FIB was detected in solutions whose pH was close to the pI of FIB (~5.5). Among all amphiphilic samples the one prepared by the reaction between TFAA and PHEMA performed the best. The very same sample exhibited an interesting reversible behavior in response to cycling the outside environment between hydrophobic and hydrophilic characters.

Protein adsorption experiments described in this work involved solutions with relatively low FIB concentrations (0.01 mg/ml). While at these conditions FIB forms a full monolayer on the substrate,²⁷ in real situations, the protein concentration may be much higher. For instance, FIB concentration in blood plasma is ~3 mg/ml;⁵¹ at those concentrations FIB is expected to form multilayers. Future studies should thus concentrate on performing FIB adsorption tests on the present substrates with solutions having higher FIB concentrations.

In this work we only restricted ourselves to studying the fluorination on brushes having a fixed grafting density of the PHEMA brushes. More work clearly has to be done on carrying out similar postpolymerization reactions on systems

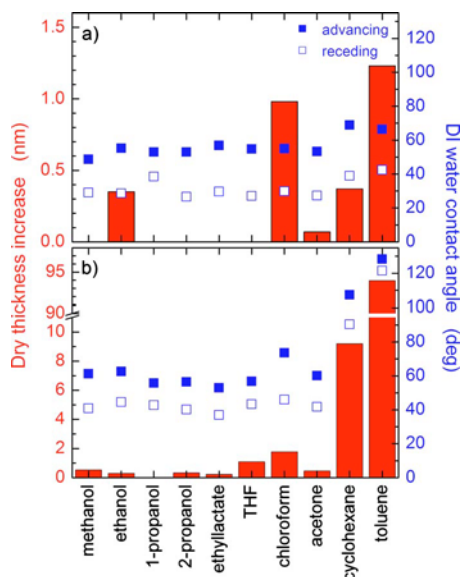


FIG. 10. (Color online) Thickness change (left ordinate, columns) and the corresponding DI water contact angles (right ordinate, symbols) associated with attaching (a) mF8H2 and (b) tF8H2 to PHEMA brush in different solvents. The average advancing and receding contact angles of pure PHEMA brushes were 60.2° and 50°, respectively.

with various grafting densities of PHEMA. Tailoring the density of PHEMA brushes will provide additional control parameter that will both govern the spatial distribution of the “coloring” species along the copolymer as well as lead to tailored responsive behavior of such amphiphilic grafts. Testing the performance against a variety of other biological moieties is also needed in order to fully understand the role of amphiphilic groups along the copolymer in governing bioadhesion. To this end, adsorption of biological moieties that prefer to settle on hydrophilic surfaces, such as *Avicula*, is required. While we have not performed experiments along those lines as of the time of writing of this article, we plan to carry them out shortly. We expect that the TFAA-based copolymer would perform well, given their responsive nature detected and reported in this article.

ACKNOWLEDGMENTS

This work was supported by the Office of Naval Research (Grant Nos. N00014-05-1-0613 and N00014-07-1-0258). Partial support from the National Science Foundation (Grant No. CBET-04-03535) is also greatly appreciated. NEXAFS spectroscopy experiments were carried out at the National Synchrotron Light Source, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Division of Materials Sciences and Division of Chemical Sciences.

APPENDIX A: DETERMINING THE DENSITY AND MOLECULAR WEIGHT OF THE PHEMA UNIT

The density and molecular weight of the fHEMA component are obtained from the densities and molecular weights of the fluorinated and HEMA units by means of Eqs. (A1) and (A2), respectively:

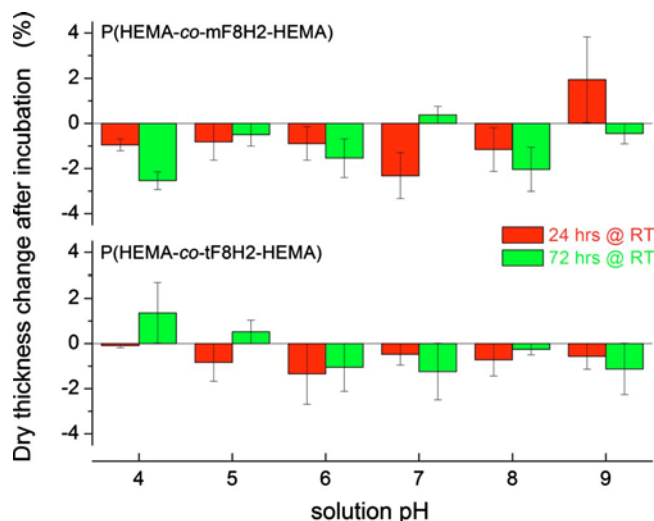


FIG. 11. (Color online) Dry thickness change in mF8H2-(top) and tF8H2-(bottom) modified PHEMA brushes after incubation in aqueous solutions of various pH. The original PHEMA thickness was ≈ 80 nm.

$$\frac{1}{\rho_{\text{HEMA}}} = \frac{w_f}{\rho_f} + \frac{1 - w_f}{\rho_{\text{HEMA}}}, \quad (\text{A1})$$

$$\frac{1}{M_{o,\text{HEMA}}} = \frac{w_f}{M_{o,f}} + \frac{1 - w_f}{M_{o,\text{HEMA}}}, \quad (\text{A2})$$

where w_f is the weight fraction of the fluorinated modifier, given by

$$w_f = \frac{M_{o,f}}{M_{o,f} + M_{o,\text{HEMA}}}. \quad (\text{A3})$$

Note that $M_{o,f}$ represents the molecular weight of the fluorinated modifier in the bonded form rather than its native form. For instance, for mF8H2 we calculate the molecular weight of $\text{F}(\text{CF}_2)_7(\text{CH}_2)_2\text{Si}(\text{CH}_3)_2$ rather than $\text{F}(\text{CF}_2)_7(\text{CH}_2)_2\text{Si}(\text{CH}_3)_2\text{Cl}$, etc. We also assume that the density of the bonded form of the fluorinated modifier can be closely represented by the density of the component in its native form.

APPENDIX B: ATTACHMENT OF ORGANOSILANES TO PHEMA BRUSHES FROM VARIOUS SOLVENTS

Because of the large differences in solubilities between semifluorinated organosilanes and PHEMA, we tested the deposition of mF8H2 and tF8H2 from various solvents. In Fig. 10 we plot the thicknesses of the P(HEMA-co-fHEMA) films (left ordinate) and the corresponding contact angles (right ordinate) for each solvent tested. While most polar solvents did not perform well in these tests, chloroform, cyclohexane, and toluene produced P(HEMA-co-fHEMA) layers. The large increases in the thickness for cyclohexane- and toluene-based samples, however, indicated that the organosilane molecules may have formed aggregates in solution that got subsequently deposited onto (and perhaps even into) the PHEMA layers. The samples for our studies were prepared

by using anhydrous cyclohexane with *n*-butyl-dilauryltin as a catalyst (concentration of 4 μ l per 30 ml of solution). The catalyst was added in order to promote the coupling of the organosilane to PHEMA and to minimize the tendency of organosilane to form large molecular aggregates in solution. The coupling reaction was carried out for 12 h at room temperature.

APPENDIX C: STABILITY OF ORGANOSILANE-MODIFIED PHEMA

As we pointed out earlier, one of the concerns when working with organosilane modifiers is the stability of the Si–O–C bond, which is known to be susceptible to large variations in solution *pH*.⁵² In order to test the stability of the organosilane-modified PHEMA, we exposed mF8H2- and tF8H2-based PHEMA samples to solutions of various *pH* ranging from 4 to 9. We measured the thickness of the layer before and after the deposition for extended periods of time. The data in Fig. 11 indicate that there is no substantial cleavage of the organosilane material from the specimen.

- ¹M. Mrksich and G. M. Whitesides, *Annu. Rev. Biophys. Biomol. Struct.* **25**, 55 (1996).
- ²J. Genzer and K. Efimenko, *Biofouling* **22**, 339 (2006) and references therein.
- ³S. Krishnan, C. J. Weinman, and C. K. Ober, *J. Mater. Chem.* **18**, 3405 (2008).
- ⁴S. Herrwerth, W. Eck, S. Reinhardt, and M. Grunze, *J. Am. Chem. Soc.* **125**, 9359 (2003).
- ⁵S. Satz, C. Henn, P. Christoph, P. Kurz, U. Stampfl, S. Stampfl, F. Thomas, B. Radeleff, I. Berger, M. Grunze, and G. M. Richter, *Invest. Radiol.* **42**, 303 (2007).
- ⁶S. Chen, J. Zheng, L. Li, and S. Jiang, *J. Am. Chem. Soc.* **127**, 14473 (2005).
- ⁷G. Cheng, Z. Zhang, S. Chen, J. D. Bryers, and S. Jiang, *Biomaterials* **28**, 4192 (2007).
- ⁸L. K. Ista, M. E. Callow, J. A. Finlay, S. E. Coleman, A. C. Bolasco, R. H. Simons, J. A. Callow, and G. P. López, *Appl. Environ. Microbiol.* **70**, 4151 (2004).
- ⁹D. Gan, A. Mueller, K. L. Wooley, *J. Polym. Sci., Part A: Polym. Chem.* **41**, 3531 (2003).
- ¹⁰C. S. Gudipati, J. A. Finlay, J. A. Callow, M. E. Callow, and K. L. Wooley, *Langmuir* **21**, 3044 (2005).
- ¹¹C. S. Gudipati, C. M. Greenleaf, J. A. Johnson, P. Pornpimol, K. L. Wooley, *J. Polym. Sci., Part A: Polym. Chem.* **42**, 6193 (2004).
- ¹²S. Krishnan, R. Ayothi, A. Hexemer, J. A. Finlay, K. E. Sohn, R. Perry, C. K. Ober, E. J. Kramer, M. E. Callow, J. A. Callow, and D. A. Fischer, *Langmuir* **22**, 5075 (2006).
- ¹³S. Schilp, A. Kueller, A. Rosenhahn, M. Grunze, M. E. Pettitt, M. E. Callow, and J. A. Callow, *BioInterphases* **2**, 143 (2007).
- ¹⁴K. Matyjaszewski *et al.*, *Macromolecules*, **32**, 8716 (1999).
- ¹⁵K. L. Robinson, M. A. Khan, M. V. de Paz Banez, X. S. Wang, and S. P. Ames, *Macromolecules* **34**, 3155 (2001).
- ¹⁶A. Ulman, *Chem. Rev. (Washington, D.C.)* **96**, 1533 (1996).
- ¹⁷F. Schreiber, *Prog. Surf. Sci.* **65**, 151 (2000).
- ¹⁸C. G. L. Khoo, J. B. Lando, and H. Ishida, *J. Polym. Sci., Part B: Polym. Phys.* **28**, 213 (1990).
- ¹⁹X. M. Deng, E. J. Castillo, and J. M. Anderson, *Biomaterials* **7**, 247 (1986).
- ²⁰S. Spange, U. Eismann, S. Hohne, and E. Langhammer, *Macromol. Symp.* **126**, 223 (1998).
- ²¹E. L. Brantley and G. K. Jennings, *Macromolecules* **37**, 1476 (2004).
- ²²M. R. Bantz, E. L. Brantley, R. D. Weinstein, J. Moriarty, and G. K. Jennings, *J. Phys. Chem. B* **108**, 9787 (2004).
- ²³E. L. Brantley, T. C. Holmes, and G. K. Jennings, *J. Phys. Chem. B* **108**, 16077 (2004).
- ²⁴G. K. Jennings and E. L. Brantley, *Adv. Mater. (Weinheim, Ger.)* **16**, 1983 (2004).
- ²⁵E. L. Brantley, T. C. Holmes, and G. K. Jennings, *Macromolecules* **38**, 9730 (2005).
- ²⁶T. I. Valdes, W. Ciridon, B. D. Ratner, and J. D. Bryers, *Biomaterials* **29**, 1356 (2008).
- ²⁷L. Guicai, S. Xiaoli, Y. Ping, Z. Ansha, and H. Nan, *Solid State Ionics* **179**, 932 (2008).
- ²⁸J. Brandrup, E. H. Immergut, E. A. Grulke, and D. Bloch, *Polymer Handbook*, 4th ed. (Wiley, New York, 2005), pp. VI/576.
- ²⁹A. Ulman, *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly* (Academic, Boston, 1991), p. 442.
- ³⁰The probing depth of XPS, *d*, is defined as $3\lambda \sin \alpha$, where λ is the electron mean free path and α is the take-off angle. Electron mean free paths for the compounds studied are all ≈ 3 nm, as estimated from the contributions of individual electron mean free paths of C (≈ 3.3 nm), O (≈ 3.0 nm), F (≈ 2.7 nm), and Si (≈ 3.6 nm) [J. C. Vickerman, *Surface Analysis: The Principle Techniques* (Wiley, Chichester, 1997); D. J. O'Connor, B. A. Sexton, and R. St. C. Smart, *Surface Analysis Methods in Materials Science*, 2nd ed. (Springer, Berlin, 2003)] and the atomic percentage of each element in the sample determined at $\alpha = 90^\circ$.
- ³¹J. Stöhr, *NEXAFS Spectroscopy* (Springer-Verlag, Berlin, 1992).
- ³²J. Genzer, K. Efimenko, and D. A. Fischer, *Langmuir* **18**, 9307 (2002).
- ³³Y. K. Jhon, J. J. Semler, and J. Genzer, *Macromolecules* **41**, 6719 (2008).
- ³⁴S. Diamanti, S. Arifuzzaman, A. Elsen, J. Genzer, and R. A. Vaia, *Polymer* **49**, 3770 (2008).
- ³⁵E. P. K. Currie, J. van der Gucht, O. V. Borisov, and M. A. Cohen Stuart, *Langmuir* **14**, 5740 (1998).
- ³⁶E. P. K. Currie, G. J. Fleer, M. A. Cohen Stuart, and O. V. Borisov, *Eur. Phys. J. E* **1**, 27 (2000).
- ³⁷R. A. Gage, E. P. K. Currie, and M. A. Cohen Stuart, *Macromolecules* **34**, 5078 (2001).
- ³⁸Z. Liu, K. Pappacena, J. Cerise, J. Kim, C. J. Durning, B. O'Shaughnessy, and R. Levicky, *Nano Lett.* **2**, 219 (2002).
- ³⁹J. U. Kim and B. O'Shaughnessy, *Phys. Rev. Lett.* **89**, 238301 (2002).
- ⁴⁰R. R. Bhat and J. Genzer, *Appl. Surf. Sci.* **252**, 2549 (2006).
- ⁴¹S. Diamanti, S. Arifuzzaman, J. Genzer, and R. A. Vaia, *ACS Nano* (in press).
- ⁴²We estimate that $\sigma_{\text{PHEMA}} \approx 0.45 \text{ nm}^{-2}$, based on previous report, in which the grafting density was measured directly. In both cases the procedure leading to the formation of the initiator SAM was identical.
- ⁴³A rough correlation between the brush dry thickness (*h*) and molecular weight of the brush can be established ($M = 1200h$, where *h* is in nanometers), as detailed in M. R. Tomlinson and J. Genzer, *Langmuir* **21**, 11552 (2005).
- ⁴⁴Note that the contact angle of PHEMA brushes may vary by as much as 15° depending on the humidity present. "Bone dry" PHEMA layers typically exhibit contact angles of $\approx 55^\circ$. When moisture is present in the air, PHEMA absorbs and becomes more hydrophilic.
- ⁴⁵M. Callies and D. Quéré, *Soft Matter* **1**, 55 (2005).
- ⁴⁶A. Marmur, *Langmuir* **22**, 1400 (2006).
- ⁴⁷K. E. Sohn, J. Dimitriou, J. Genzer, D. A. Fischer, C. J. Hawker, and E. J. Kramer, *Langmuir* (in press).
- ⁴⁸S. Turgman-Cohen, D. A. Fischer, P. K. Kilpatrick, and J. Genzer (submitted).
- ⁴⁹E. Triantaphyllopoulos and D. C. Triantaphyllopoulos, *Biochem. J.* **105**, 393 (1967).
- ⁵⁰C. E. Halland H. S. Slayter, *J. Biophys. Biochem. Cytol.* **5**, 11 (1959).
- ⁵¹J. M. Grunkemeier, W. B. Tsai, C. D. McFarland, and T. A. Horbett, *Biomaterials* **21**, 2243 (2000).
- ⁵²S. Spange, U. Eismann, S. Hohne, and E. Langhammer, *Macromol. Symp.* **126**, 223 (1998).