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Simple, Benign, Aqueous-Based Amination of Polycarbonate Surfaces

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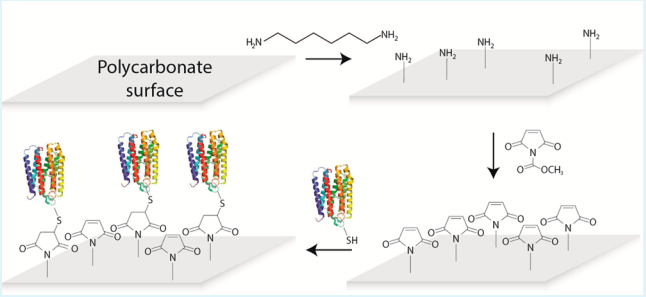
Supporting Information

ABSTRACT: Polycarbonate is a desirable material for many applications due to its favorable mechanical and optical properties. Here, we report a simple, safe, environmentally friendly aqueous method that uses diamines to functionalize a polycarbonate surface with amino groups. The use of water as the solvent for the functionalization ensures that solvent induced swelling does not affect the optical or mechanical properties of the polycarbonate. We characterize the efficacy of the surface amination using X-ray photo spectroscopy, Fourier transform infrared spectroscopy (FT-IR), atomic force microscopy (AFM), and contact angle measurements. Furthermore, we demonstrate the ability of this facile method to serve as a foundation upon which other functionalities may be attached, including antifouling coatings and oriented membrane proteins.



KEYWORDS: *polycarbonate, aminated surface, surface chemistry, bacteriorhodopsin, antibiofouling, functionalized surface*

INTRODUCTION

Polycarbonate (PC) is a desirable material in many applications due to its high impact strength, toughness, heat resistance, and optical transparency.[1] PC has widespread industrial applications ranging from lab-on-a-chip diagnostic devices to compact discs. In many of these applications, particularly those related to biomedical devices, the ability to functionalize PC is central to its utility.[2] To that end, a number of methods have been developed to modify the surface chemistry of PC for specific applications. All previous methods, however, either require expensive, specialized equipment or create extremely toxic products. Even worse, the harsh chemicals employed in these methods can damage microscale surface features or create macroscale inhomogeneities.[3] We propose an aqueous treatment of PC that provides a simple route to aminated surfaces.

One of the earliest techniques developed to functionalize PC involved exposing the surface to an ammonia plasma to create ammonium groups.[2] This method, however, requires expensive vacuum equipment, and the mean free path of the plasma limits the ability of the reaction to proceed inside tortuous microchannels. Another common method is the use of UV and ozone to form carboxyl groups, which have been used for the development of DNA biochips.[1] Here, specialty UV−ozone equipment is required, and reactions into small dimensions are diffusion limited. Alternatively, 1-fluoro-2-nitro-4-azidobenzene (FNAB) may be evaporated onto the surface and then irradiated with UV, which activates the surface to bind proteins via reaction with amino groups.[4−6] Again, expensive vacuum equipment and high voltage sources are required, while deposition is limited to the line-of-site, as is typical in physical vapor deposition techniques.[7] In another approach, concentrated nitric acid (30%) at elevated temperature provides an electrophilic aromatic substitution to generate nitro groups, which can then be reduced to primary amines with $NaBH_4$. This nitric acid technique has been used to generate PC-based systems for the detection of single nucleotide polymorphisms on compact discs,[8] hapten-linked immoassays,[9] ELISA detection of *Samonella typhi*,[10] and capture of circulating biomarkers.[11] This approach is both potentially dangerous and environmentally destructive, as it generates the toxic green-

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house gas NO_2. Further, the reactions are reported to have rather low efficiency.[8,12] Another technique modifies terminal residues on the surface of the PC in compact discs via a complicated multistep reaction to attach ligands for biomolecule screening.[3] Instead of covalently modifying the PC surface, other researchers have taken the approach of depositing a thin coating on top of the PC that can then be reacted with their ligand of choice.[7,12] This approach is suboptimal, and the coating material may drastically alter the optical, mechanical, and electrical properties of the materials system and may not necessarily be amenable for microsystems with close tolerances. For many applications, such as compact discs, it is important that the functionalization procedure not modify the optomechanical properties of the PC.[9]

A method to attach amine functionalized multiwalled carbon nanotubes to PC in bulk was recently developed.[13] However, this approach involved melt blending at 260 °C, which is typically not suitable for surface modification.

Considering the substantial drawbacks of current methods to safely and simply provide a fundamental surface functionalization for a widely used material, we developed a benign, straightforward, aqueous technique that employs a single commercially available chemical to aminate PC surfaces. We validate the efficacy of this amination method to provide PC surfaces with convenient chemical handles by subsequently modifying the PC surface for practical applications including resistance to biofouling and targeted protein binding. In particular, we show the covalent attachment of PEG to create an antifouling surface and the facile conversion of the amine to a maleimide used to direct the oriented attachment of the protein bacteriorhodopsin, a light activated, directional proton pump.

EXPERIMENTAL SECTION

Chemical and Reagents. Polycarbonate thin films were made from 99% pure polycarbonate beads (Aldrich) by dissolving 1 g of PC beads in 10 mL of chloroform (Fisher, 99.9% HPLC grade) and spin-coating onto piranha-cleaned single crystal (100) silicon at 2000 rpm for 60 s. *(CAUTION: "Piranha" solution reacts violently with organic materials; it must be handled with extreme care.)* The PC thin films were dried overnight at 60 °C under vacuum (<1 mTorr). The resultant film thickness was 2290 + 50 nm (N = 5), as measured with a Dektak IIA profilometer. Commercial polycarbonate sheets (1 mm thick) were obtained from McMaster-Carr (8574K19). Hexamethylene diamine (HMDA), methoxycarbonyl maleimide, acetic anhydride, methoxy-terminated PEG (5k), and bovine serum albumin conjugated to biotin (BSA-biotin) were obtained from Sigma-Aldrich (NY). AlexaFluor 488-Streptavidin conjugate, AlexaFluor 594 NHS ester conjugate, and AlexaFluor 488 maleimide were obtained from Life Technologies (Carlsbad, CA). mPEG-NHS ester (5k) was obtained from Nanocs Inc. (NY).

Surface Amination and Maleimide Functionalization Procedures. PC was aminated by immersion in a 1 wt % aqueous solution of HMDA for a time ranging from 24 to 72 h at room temperature with the solution sealed from exposure to the atmosphere. After treatment, samples were rinsed with copious amounts of DI water, dried with nitrogen, and then either used immediately or stored under inert atmosphere. For some applications, amines were converted to maleimides via reaction with *N*-methoxycarbonyl maleimide under basic conditions (fresh solution of 5 wt %/vol in saturated $NaHCO_3$ for 1 h at room temperature).

X-ray Photoelectron Spectroscopy Measurements. X-ray photoelectron spectroscopy (XPS) was performed using a Kratos Axis Ultra DLD instrument with a monochromatic Al Kα (1486.6 eV) source. Full survey spectra were collected with an analyzer pass energy of 160 eV and a step size of 1 eV. High resolution spectra were collected with an analyzer pass energy of 20 eV and step sizes of 0.1 eV. The analyzer was used in hybrid mode with a large spot size of 300 μm by 700 μm, elliptically. To minimize the deleterious effects of sample charging, charge neutralization was employed. Due to differential charging considerations, a 90° take off angle was used, which results in an estimated inelastic mean free path of 3 nm.[14] C 1s, N 1s, and O 1s XPS spectra were recorded for at least three unique locations on each sample. Data from unfunctionalized PC were fitted to Gaussian−Lorentzian peaks after Shirley background subtraction. Subsequently, spectra from aminated and maleimide-functionalized surfaces were fitted, constraining both the location of a given peak and its position relative to the other peaks to within ±0.2 eV. The peak width (fwhm) and the Gaussian−Lorentzian ratio for a given peak were held constant throughout all spectra.

Contact Angle Measurements. Static contact angle measurements on spin-coated PC samples were performed on a Kruss goniometer after a 5 μL droplet of deionized water had equilibrated for 2 min. This was repeated for 5−7 unique test sites per sample.

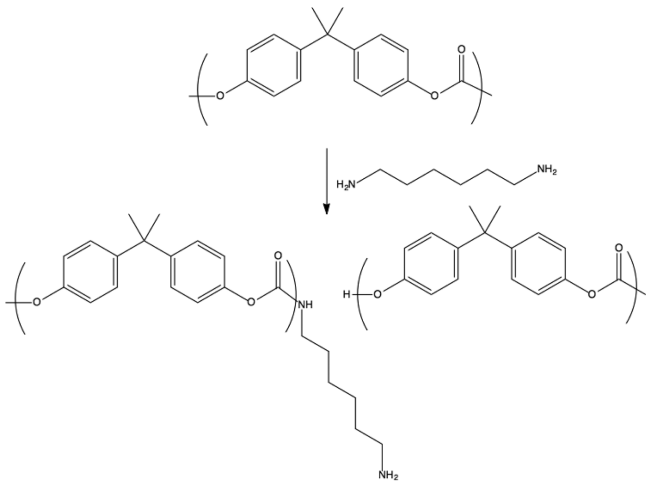
Fourier Transform Infrared Spectroscopy Measurements. Grazing angle Fourier transform infrared spectroscopy (FT-IR) measurements were performed on spin-cast PC thin films with a Thermo Scientific Nicolet 6700 spectrometer. The sampling area was 16 mm in diameter. 256 scans were performed at 1 cm^{-1} resolution with a background measurement directly before each sample and a 15 min purge before each measurement.

Atomic Force Microscopy Measurements. Atomic force microscopy (AFM) measurements on spin-coated PC were performed on a Park Scientific Instruments Autoprobe Cp. Topographs were acquired at 2 Hz collection speed, 512 × 512 pixels over a 5 μm × 5 μm area, and 50 pN force. Five unique test sites were measured per sample. AFM on commercial PC samples was performed using an MFP-3D-SA system, equipped with a closed loop *XY* scanner and all-digital ARC2 Controller (Asylum Research, Santa Barbara, CA). Imaging was performed in AC mode, in air, using Olympus AC240TS Si probes ($k \sim 2$ N/m). Topographs were generally acquired at 512 × 512 pixels, 1 Hz scan speed, with other parameters optimized while scanning to impart minimal forces. Images were processed, and height profiles were generated using the manufacturer's provided software.

RESULTS AND DISCUSSION

Amination of Surface of Polycarbonate. The postulated reaction used to functionalize PC is shown in Scheme 1. Upon addition of the HMDA solution to the PC surface, the carbonyl group undergoes nucleophilic substitution by the diamine. This reaction results in scission of the polymer chain and the formation of a terminal hexylaminocarbamate. Addition of the diamine results in a surface decorated with amine groups that

Scheme 1. Amination of Polycarbonate



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are available for subsequent functionalization reactions. Alternatively, other functionalization groups conjugated to amines could be attached to the PC surface using the same chemistry. We chose to use the readily available hexamethylene diamine because of its water solubility and the value of an amine substituted surface. One could easily imagine using glycine or something similar to generate a carboxylated surface. Use of water (a nonsolvent for PC) as the solvent for the functionalization instead of an organic solvent (e.g., ethanol, dichloromethane) confines the reaction to the surface and prevents swelling or dissolution that could compromise the integrity of the substrate. Such deleterious effects not only are simply limited to deformation of microstructural features (e.g., microchannels) but also may create thickness variations which cannot be mechanically smoothed without physical removal of the surface and its functionalization. PC surfaces aminated with the process presented here, however, appeared unchanged under examination by AFM (see Figure S1, Supporting Information), with samples having similar morphology and surface roughness before (average roughness/Å of 6.6 ± 2.0) and after 72 h of amination treatment (average roughness/Å of 4.1 ± 2.2).

X-ray Photoelectron Spectroscopy. PC thin films immersed in 1 wt % aqueous HMDA for 0 h (as-cast PC) to 24 h were evaluated via X-ray photoelectron spectroscopy (XPS). Cross correlation of chemical shifts in the C 1s, N 1s, and O 1s spectra confirm the scission of the polymer chain and the formation of a terminal hexylaminocarbamate as outlined in Scheme 1. Characteristic XPS spectra are presented in Figure 1. Survey spectra and detailed quantification of all XPS data are provided in Figure S2 and Tables S1 and S2, Supporting Information.

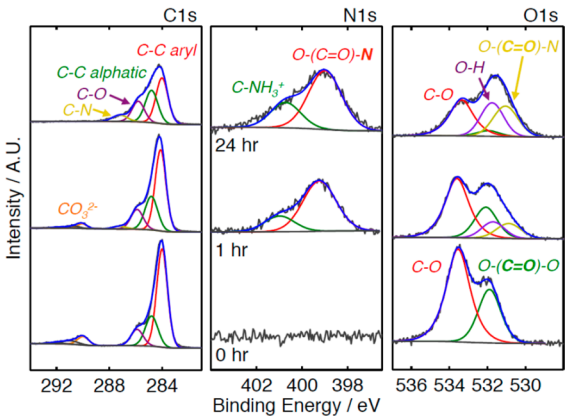

Figure 1. XPS data of PC thin films aminated for 0 h (as-cast), 1 h, or 24 h (bottom, middle, and top, respectively). Left, middle, and right columns correspond to carbon 1s, nitrogen 1s, and oxygen 1s spectra, respectively.

In the C 1s spectra, four peaks consistent with pure polycarbonate are observed:[15,16] (1) carbon in the phenyl ring not bonded to oxygen (C−C aryl, 284.0 eV), (2) carbon in the methyl groups and bridging carbon (C−C alphatic, 284.8 eV), (3) carbon in the phenyl ring bonded to oxygen (C−O, 285.8 eV), and (4) carbon in the carbonate group (CO_3^{2-}, 290.0 eV). Adjacent to CO_3^{2-} are two $\pi-\pi^*$ shakeup peaks, consistent with literature reports and readily seen at higher magnification in Figure 3.[15] The relative fraction of each peak is consistent with the molecular formula of PC. Upon immersion in the amination solution, a peak attributed to carbon bonded in the carbamate appears (C−N, 287.1 eV) at the expense of the CO_3^{2-} peak.[17,18] Alkyl carbons bonded to nitrogen are at binding energies similar to those of C−O, resulting in an increased relative intensity of the C−O peak.[18] As the amination time increases, the relative intensity of C−C alphatic, C−N, and C−O grows, while the C−C aryl decreases, consistent with the formation of a alkylaminocarbamate on the PC surface. By 24 h, CO_3^{2-} disappears, indicating that nearly all surface CO_3^{2-} has been consumed as the carbamate is formed.

In the O 1s spectra, two peaks consistent with literature reports of pure PC are observed:[15,17,19,20] (1) oxygen singly bonded to both a phenyl ring and the carbon in CO_3^{2-} (C−O, 533.4 eV) and (2) oxygen double bonded to carbon in CO_3^{2-} (O−(C═O)−O, 532.0 eV). As predicted by the PC molecular formula, the ratio of the experimentally recorded areas C−O/−(C═O)−O is 2:1. Once the PC is immersed in the amination solution, the O 1s spectrum becomes broader and shifts to lower binding energies. This shift is most simply explained by the addition of peaks consistent with the phenol[20,21] and carbamate[22] of Scheme 1: (1) oxygen bonded in the terminal phenol (O−H, 531.7 eV), and (2) oxygen double bonded in the carbamate (O−(C═O)−N, 530.9 eV). As the amination time increases, the fraction of oxygen bonded as O−(C═O)−O decreases from 33% to 4.4%, while the O−H and O−(C═O)−N increase to each occupy 29% of the O 1s spectrum. These results are consistent with those of the C 1s spectra, indicating nearly all carbonate has reacted to form a carbamate and a phenol.

The N 1s spectra is featureless for pure PC, though immersion in the amination solution produces two peaks: (1) nitrogen in a protonated amine ($C-NH_3^+$, 400.9 eV),[18,23] and (2) nitrogen bonded in a carbamate (O−(C═O)−N, 399.1 eV).[18] While deprotonated primary amines may appear near 399 eV, all primary amines in this study are protonated; copious rinsing and sonicating in DI water (pH ∼ 6) is sufficient to protonate HMDA, with a pK_a ∼ 11. As time increases, the total surface composition (C, N, O only) increases from 0 at% nitrogen to 6.4 at% nitrogen.

From the C 1s, O 1s, and N 1s spectra, it is repeatedly observed that the carbonate group is eliminated and an alkylaminocarbamate is formed. Simple adsorption or electrostatic bonding of HMDA onto the PC surface is insufficient to explain this behavior; the XPS data can only be explained by a new chemical species. Specifically, C 1s, O 1s, and N 1s each identify formation of a carbamate species, while C 1s and O 1s reveal elimination of the carbonate species. Additionally, C 1s and N 1s show that alkyl and amino groups are added to the surface. All trends are consistent over time and imply a nearly fully functionalized surface (all surface CO_3^{2-} reacted) after 24 h.

During the reaction illustrated in Scheme 1, it is possible that both amino groups of a single HMDA molecule react at nearby sites on the PC surface to form carbamates. The fraction of HMDA molecules which bridge the surface, versus those with the desired free amine tail, may be calculated from the N 1s spectra. Only the free amine tails are represented by the $C-NH_3^+$ peak, while all fixed ends are contained in the O−(C═O)−N peak. After 1 h in the amination solution, only 44% of HMDA is present on the surface with a free amine tail, increasing to 57% after 24 h in solution. This trend is explained by the depletion of adjacent pairs of CO_3^{2-} sites as the surface

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reaction progresses. As the availability of these paired sites decreases, an increasing number of HMDA molecules are limited to bonding only one amino group, increasing the fraction of HMDA with a free amine tail. The increased concentration of these free amine tails should noticeably alter the surface wettability, as polycarbonate is relatively hydrophobic and amines are relatively hydrophilic.

Contact Angle. The static contact angle of the PC surface was examined as a function of amination treatment time. The contact angle, as shown in Figure 2, exhibits a drop from 84 ± 1° for the untreated PC down to 61 ± 2° over the course of 24 h, after which the contact angle remained constant. Droplet size was 5 μL. Measurements were made at six unique sites on each sample, with the uncertainty representing one standard deviation of the data. This decrease in contact angle closely follows the increase in surface concentration of free amine tails, plotted in Figure 2 and represented by the nitrogen bonded as $C-NH_3^+$ as a percentage of the overall surface composition (C, N, O only).

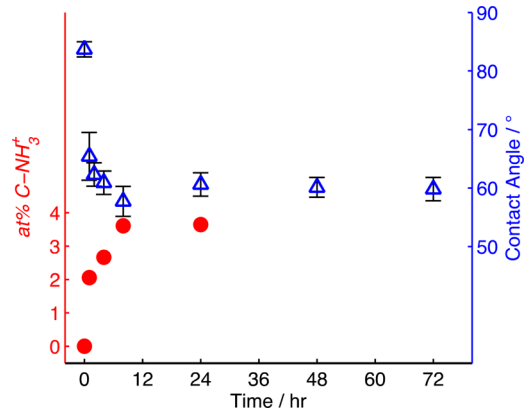

Figure 2. at% of surface present nitrogen bonded as $C-NH_3^+$ (solid circles) and static contact angle (open triangles) of PC surface as a function of amination time.

FT-IR. The confinement of the amination reaction to the surface of the polycarbonate was confirmed with grazing angle FT-IR measurements, as shown in Figure 3. All the peaks match the Sigma-Aldrich reference spectrum except for the broad peak near 2100 cm^{-1}, which is an interference peak twice the thickness of the PC film. After 72 h in amination solution, there are no signs of peaks corresponding to the N–H stretch of a primary amine, C=O stretch from carbamate, or C–N stretch from the carbamate, which would appear near 3350, 1710, and 1630 cm^{-1}, respectively. For reference, HMDA pressed in a KBr pellet is provided in Figure 3. This indicates that the HMDA did not significantly penetrate the PC thin film, either as a free molecule or as a reactant. Spectra of samples conditioned for less than 72 h appear the same as the 72 h spectra shown in Figure 3.

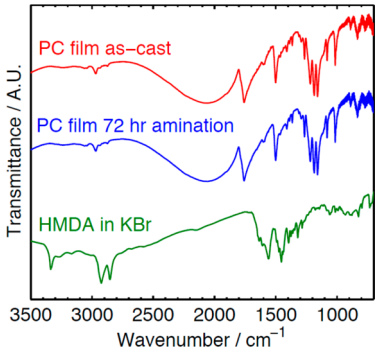

Figure 3. Grazing angle FT-IR spectra of polycarbonate as-cast (upper red line) and after 72 h of amination treatment (middle blue line), compared to a reference spectra of HMDA in KBr pellet (lower green line).

The detailed characterization of the PC surface via XPS, FT-IR, contact angle, and AFM was performed on polycarbonate thin films that were cast from 99% pure polycarbonate beads, which supports our assertion that the proposed chemistry is not a side effect of reactions with unknown plasticizers or other contaminants that might be present in sheets of commercial PC. However, similar characterization experiments show that the amination chemistry is just as effective on commercial PC (Figures 4 and S1 and S4, Supporting Information).

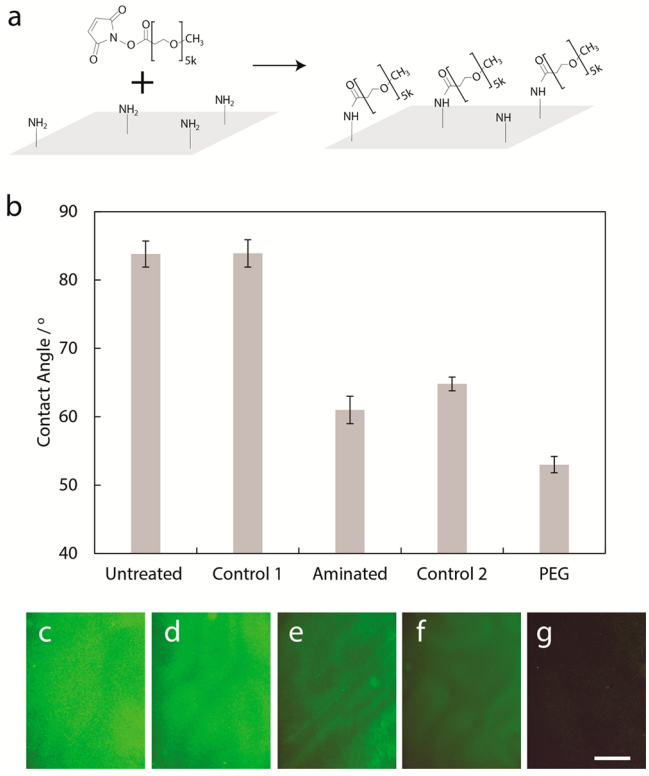

Figure 4. (a) Cartoon of the attachment of 5k PEG to the PC surface. (b) Static contact angle measurements of PC surfaces. (c–g) Fluorescence micrographs indicating adsorption of biotinylated BSA labeled with Alexa Fluor 488-Streptavidin onto PC for untreated PC (c), control 1 (NHS-PEG on untreated PC) (d), aminated PC (e), control 2 (aminated PC treated with methoxy terminated PEG) (f), and aminated PC treated with NHS ester terminated PEG (g). Scale bar is 50 μm.

PEGylation for Antifouling. Although the amination of the PC surface alone provides a chemically useful, positively charged surface that can be utilized to bind a variety of targets including proteins, macromolecules, and eukaryotic cells, we further show that the aminated surface can be used to covalently tether secondary functional molecules to the PC surface. As a first demonstration, we bound polyethylene glycol (PEG) to introduce antifouling properties to the PC, as

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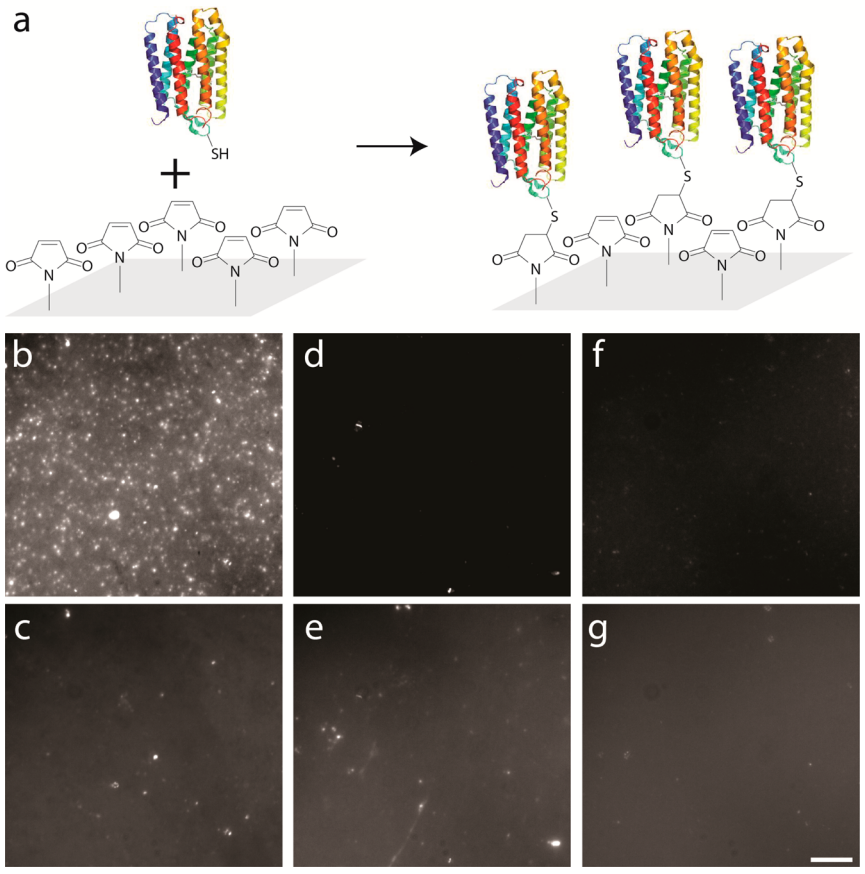

Figure 5. (a) Cartoon of attachment scheme of cysteine mutant BR to maleimide functionalized PC for selective orientation of purple membrane patches. Purple membrane patches consist of an array of BR proteins with associated lipids in a hexagonal lattice. (b) All purple membrane patches are labeled with an NHS ester conjugated dye. (c) Maleimide functionalized dye shows purple membrane patches that are in the wrong (cysteines-up) orientation. (d) and (e) show the cysteine mutant BR patches on unfunctionalized polycarbonate labeled with an NHS ester dye and a maleimide functionalized dye, respectively. (f) and (g) show wild-type BR patches on maleimide functionalized polycarbonate labeled with an NHS ester dye and a maleimide functionalized dye, respectively. Scale bar is 10 μm.

one might need for applications such as filtration. Specifically, the aminated PC surface was reacted with 50 mg/mL mPEG-NHS ester in 100 mM sodium bicarbonate, pH 8.3, for 1 h at RT. The PC surface was then rinsed with DI water. Additionally, control experiments were performed by applying: (1) mPEG-NHS ester to plain polycarbonate and (2) methoxy-terminated PEG (5k) to aminated PC to show that the attachment of NHS ester-PEG to the PC surface was not due to nonspecific adsorption. Successful attachment of NHS ester-PEG was demonstrated by contact angle measurements (droplet size 5 μL), as shown in Figure 4. The methoxy-terminated PEG on the aminated PC control displayed the same contact angle as the aminated PEG, showing that PEG does not adsorb nonspecifically onto the aminated PC surface. With the NHS ester-PEG, the PEG is attached covalently to the surface and the long chain length of the PEG effectively covers the surface and produces a more hydrophilic contact angle.

To demonstrate that the PEG functionalization effectively prevented fouling of the PC surface, a solution of fluorescently labeled BSA-biotin, a protein that has a strong tendency to adhere to surfaces,[24] was applied to the untreated, aminated, control, and PEGylated PC surfaces for 1 h at RT (details of protocol in the Supporting Information). Figure 4 clearly shows that the PEGylated PC had much less adsorption of fluorescent protein than the aminated, untreated, or control surfaces. These qualitative observations were quantified by fluorescence microscopy and the fluorescence intensities for the untreated, aminated, control 1, control 2, and PEGylated surfaces were 1167 ± 62, 828 ± 39, 1082 ± 54, 748 ± 30, and 475 ± 34 au (standard deviation designates variability within each image), respectively, confirming the effective antifouling character of the amine-mediated PEGylation.

Oriented Protein Attachment. As a second demonstration of the value of this amine-tethering concept, terminal amines were converted to maleimides using the protocol detailed in the Experimenal Section. Upon converting the surface from amine to maleimide, no new peaks appeared in the XPS spectra (Figure S4, Supporting Information), as expected, but the relative peak intensities changed in a manner consistent with maleimide termination. The concentration of oxygen bonded as O−(C═O)−N or O−(C═O)−O (at both 530.9 and 532 eV) increased consistently with the increased oxygen content of a maleimide-terminated surface. Also, the relative intensity of the C 1s carbamate/amide (O−(C═O)−N) increased from 5.6% to 7%. In the N 1s spectra, the concentration of nitrogen bonded as $C-NH_3^+$ decreased from 9% to 3% as the free tails of the diamines are consumed to form maleimides.

The maleimides were then used to mediate the oriented attachment of a mutated bacteriorhodopsin (BR) proton pump on the PC surface. BR is a 7 α-helix transmembrane protein that functions as a directional, light-driven proton pump from a

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H. salinarum and is purified from the bacteria in large (~1 μm) membrane patches commonly referred to as purple membranes due to their characteristic bright purple color. Directing the oriented attachment of these pumps is central to their characterization and potential utility *in vitro*. These particular BR proteins were modified by site-directed mutagenesis (see the Supporting Information) to contain a single cysteine residue on the cytoplasmic side of the protein.[25] When purple membrane composed of the cysteine mutant was exposed to the maleimide-functionalized PC surface, membrane patches oriented with cysteines against the surface (cytoplasmic side down) covalently attach to the surface. Patches with the opposite orientation, on the other hand, were not bound and could be washed off. Attached cysteine mutant purple membrane patches are shown in Figure 5b, where a NHS ester dye is used to label all the patches, regardless of orientation. Protein orientation was confirmed using a maleimide functionalized dye, which only labels purple membrane patches oriented the wrong-way (cysteine-up), as shown in Figure 5c, indicating that very few of the patches are oriented in the wrong orientation. As a negative control, we show the cysteine mutant does not adhere to an unfunctionalized PC surface (Figure 5d,e). Wild-type protein, which has no cysteines, did not adhere to the maleimide functionalized PC (Figure 5f,g).

CONCLUSION

In conclusion, here we presented a novel yet simple method to aminate PC surfaces that can be used to facilitate secondary functionalization, such as PEGylation or oriented, covalent protein attachment. Because the modification is near the monolayer in functionality, bulk properties are not changed and optical effects are minimized, unlike what is common with thicker modification layers or exposure to harsh solvents. Given the desirable material properties of PC, this new method will facilitate diverse polycarbonate device applications.

ASSOCIATED CONTENT

Supporting Information

Experimental details, methods, AFM images, survey XPS spectra and quantification of XPS data for PC films, and XPS spectra for commercial PC. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare the following competing financial interest(s): D.R.W., V.V., and L.J.S. have applied for intellectual property protection for the reported work. There are, however, no concrete financial interests.

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ABBREVIATIONS

PC, polycarbonate
XPS, X-ray photoelectron spectroscopy
PEG, polyethylene glycol
NHS, *N*-hydroxysuccinimide
BR, bacteriorhodopsin

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