pH-responsive aqueous/LC interfaces using SGLCP-b-polyacrylic acid block copolymers†

Dong-Yul Lee, a Jung-Min Seo, a Waliullah Khan, a Julia A Kornfield, b Zuleikha Kurji b and Soo-Young Park ab

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Block copolymers that combine a side-group liquid crystalline polymer (SGLCP) block and a pH-responsive hydrophilic block, poly(acrylic acid) (PAA), are shown to confer pH-dependent anchoring of the director orientation at the aqueous/LC interface. The SGLCP block, poly-(4-cyanobiphenyl-4-oxynundecylacrylate), was chosen based on its ability to influence the director field of the 5CB (4-cyano-4'-pentylibiphenyl). At low pH the PAA block collapses and the inherent, planar alignment tendency of 5CB at a water interface prevails. As pH increases, the polyelectrolyte block becomes increasingly charged and expands, producing a change to homeotropic anchoring. The change in anchoring occurs as quickly as the buffer can be changed (within ~2 s) and is reversible, with a response that is repeatable over as many cycles as were tested (approximately 20 cycles). The polymer-mediated anchoring persists for 6 days, indicating that the SGLCP block secures the self-assembled layer on the 5CB, even under conditions that cause repulsive interactions among the PAA blocks. Thus, SGLCP blocks can translate conformational changes of a responsive hydrophilic block into rapid, reversible changes in the director field.

Introduction

Liquid crystals (LCs) are responsive to small changes in temperature, shear, electric (or magnetic) fields, and the structure of solid surfaces with which they are in contact.1–3 Subtle changes in interfacial structure result in long-range consequences in the director field of the LC; the resulting amplification was used by Gupta et al. to enable facile detection of antigen binding at solid/LC interfaces.4 More recently, environmentally-responsive aqueous/LC interfaces5–15 have been shown to control the orientational order of the LC, responding actively and/or reversibly to a broad range of stimuli. Examples of such systems are the assembly of amphiphiles, such as phospholipids7,9,15 and surfactants,6,8,11,12 at an aqueous/LC interface leading to changes in the alignment of the LC that can be observed in real time using the birefringence of the LC.14,16 These changes in orientational order result from coupling between the aliphatic tails of the adsorbed amphiphiles and the mesogens of the LC. Chemical or physical events in the aqueous phase that perturb the organization of the monolayer, such as the binding or enzymatic action of a protein, alter the anchoring condition in a manner that depends on the structure of the amphiphiles (e.g., tail length or head group structure).6–8,11,15,17 Building upon knowledge of surfactants at the aqueous/LC interface, a diblock copolymer functionalized with hydrophobic (N-decyl acrylamide) and hydrophilic (N-[3-(dimethylamino) propyl] acrylamide) side groups (polymer 1) was examined by Abbott and co-workers and shown to assemble at the aqueous/LC interface.18 This adsorbed diblock copolymer responds reversibly to changes in pH of the aqueous phase, coupling the order of the LC to changes in the physicochemical properties of the aqueous environment.

Here we examine block copolymers that combine a hydrophilic block and a LC-philic, side-group liquid crystalline polymer (SGLCP) block as responsive interfacial agents. The self-assembly of block copolymers in the bulk,19 in solution,20 and at interfaces21 can be systematically tuned by choice of the blocks and their length. In the context of block copolymers for use at aqueous/LC interfaces, the structure of the LC-philic block can be used to dictate the strength and direction of director anchoring. The length of the LC-philic block can be used to stabilize the interfacial layer. The structure of the hydrophilic block can be chosen to confer responsiveness to changes in temperature, pH, and ionic strength; reorganization of the polymers in response to environmental stimuli in the aqueous phase can then be translated into changes in anchoring of the LC.

To facilitate examination of the effects of diverse stimuli, a weak polyelectrolyte, in this case, poly(acrylic acid) (PAA), was chosen for the hydrophilic block. One of the most extensively characterized small molecule liquid crystals, 4-cyano-4'-pentylbiphenyl (5CB), was selected as the LC; therefore, the LC-philic block was chosen based on its solubility in 5CB and to switch the anchoring direction from that observed at a bare aqueous/5CB interface (planar) to homeotropic. The SGLCP, poly(4-cyano-biphenyl-4-oxynundecylacrylate) (denoted LCP), meets these criteria. The polyelectrolyte PAA block changes from almost uncharged to fully charged over the pH range explored (from pH = 2 to 12). The LCP block is completely miscible with 5CB so...
that there are extensive interactions between 5CB and the LCP block. Therefore, PAA-\(b\)-LCP diblocks assembled at the aqueous/5CB interface were chosen to illustrate general principles for the design of block copolymers to translate changing conditions in the aqueous phase into readily detectable changes in director anchoring. Specifically, aqueous/5CB interfaces functionalized with PAA-\(b\)-LCP were immobilized using a transmission electron microscopy (TEM) grid mounted in a cell that permitted rapid exchange of the aqueous medium, providing a versatile system for examining a variety of environmental stimuli: pH, ionic strength and small amounts of proteins. The changes in director anchoring that occur in response to the pH in the aqueous phase were used to determine the response time, reversibility and repeatability of the restructuring of the block copolymer monolayer. To illustrate transduction of other stimuli, the effects of ionic strength and of small concentrations of protein (e.g. lysozyme) were also examined.

Experimental

Materials

2,2'-Azobisisobutyronitrile (AIBN, Junsei) was purified by recrystallization from methanol. THF (Aldrich) was refluxed with sodium and distilled under nitrogen. DMF (Aldrich) was refluxed over CaH2, and then distilled. Tert-butyl acrylate (tBA, Aldrich, 98%) was purified by passing over alumina. All other chemicals, for example, trifluoroacetic acid (TFA, Aldrich, 99%), 4-cyano-4'-pentylbiphenyl (5CB, TCI, 100%), methanol (Aldrich), buffer solutions (pH = 2–12, Samchun®), dichloromethane (DCM, Aldrich), and lysozyme (MP biomedicals) were used without further purification. Copper specimen grids (G75, with grid hole width 285 μm, pitch 340 μm, bar width 55 μm, size 3.05 mm and thickness 18 μm) were bought from Ted Pella, Inc. The LC monomer, 4-cyano-4'-oxyundecylacrylate (LC11), was prepared according to literature protocols.22 (The NMR spectrum of LC11 can be found in the ESI, Fig. S1†). A chain transfer agent, s-methoxycarbonylphenylmethyl dithiobenzoate (MCPDB) was synthesized according to the reported procedure.23 (The NMR spectrum of MCPDB can be found in Fig. S2†.) The polymerization for the synthesis of the block copolymer has been illustrated in Scheme 1.

Polymerization of PrBA homopolymer as a macromolecular chain transfer agent

MCPDB (19.380 mg, 0.0641 mmol) and AIBN (5.469 mg, 0.0192 mmol) were combined in a Schlenk flask. The flask was degassed by three freeze–pump–thaw cycles. tBA (1.456 mL, 10 mmol) and previously-degassed (by bubbling argon through it for 60 min) DMF (1.463 mL) were then introduced to the flask using a syringe that was purged with dry nitrogen. The flask was then placed in a 70 °C oil bath and stirred for 18.5 h. The resulting polymer solution was poured into a large volume of methanol–water mixture (1/1, v/v). The precipitated polymer, poly(tBA)-CTA (PrBA-CTA) was purified 3 times by dissolution in small volumes of THF, followed by precipitation into a large volume of methanol–water mixture (1/1, v/v). The polymer was then dried under vacuum at room temperature for 12 h, yielding 0.90 g (52%). The polymer had an \(M_n = 2.65 \times 10^4\) with an \(M_w/M_n\) of 1.21, which were measured by gel permeation chromatography (GPC, Younglin instrument co., Acme 9000) calibrated with polystyrene standards (PSs) with THF as eluent.

Polymerization of PrBA-\(b\)-LCP diblock copolymer

AIBN (0.688 mg, 0.0042 mmol), LC11 (83.85 mg, 0.20 mmol), and PrBA-CTA (110.9 mg, 0.0042 mmol) were added into a Schlenk flask. The flask was degassed by three freeze–pump–thaw cycles. DMF (0.32 mL), that was previously degassed by bubbling dry nitrogen through it for 60 min, was then introduced to the flask using a syringe purged with nitrogen. The flask was then placed in an oil bath thermostated at 70 °C and reacted for 39.5 h. The resulting polymer solution was poured into a large volume of methanol–water mixture (1/1, v/v). The precipitated polymer, PrBA-\(b\)-LCP was purified 3 times by first dissolution in small volumes of THF, followed by precipitation into a large volume of methanol–water mixture (1/1, v/v). The polymer was then dried under vacuum at room temperature for 12 h yielding 125 mg (52%). The polymer had an \(M_n = 3.27 \times 10^4\) with an \(M_w/M_n\) of 1.27.

Preparation of PAA-\(b\)-LCP

PrBA-\(b\)-LCP (0.1 g) and TFA (1.0 mL) were dissolved in DCM (5.0 mL), and after stirring at room temperature for 24 h, the
solution was concentrated under reduced pressure with a rot-vap. PAA-b-LCP was precipitated from ethyl ether and dried under vacuum at 40 °C for 24 h. The NMR spectrum of PAA-b-LCP can be found in Fig. S3;\textsuperscript{†} \textsuperscript{1}H-NMR (d-DMSO): 1.24–1.35 (s, 14H, –(CH\textsubscript{2})\textsubscript{7}), 1.35–1.58 (m, main chain, –CH\textsubscript{2}), 1.59 (m, 2H, –CH\textsubscript{2}), 1.72 (m, 2H, –CH\textsubscript{2}), 2.11–2.31 (s, main chain, –CH), 4.02 (t, 2H, –CH\textsubscript{2}), 4.09 (t, 2H, –CH\textsubscript{2}), 7.05 (d, 2H, aromatic), 7.17 (d, 2H, aromatic), 7.70 (d, 2H, aromatic), 7.83–7.89 (q, 4H, aromatic), 12.4 (s, 1H, –OH) ppm. The IR spectra of PAA-b-LCP can be found in Fig. S4.\textsuperscript{†}

Preparation of the TEM grid cell and the flow chamber

A series of experiments were conducted in a home-made polydimethylsiloxane (PDMS) flow chamber designed to permit rapid and controlled exchange of the aqueous solutions (and hence, rapid and controlled changes in pH) at the PAA-b-LCP functionalized aqueous/5CB interface, and to facilitate repeated displacement and introduction of fresh buffer solutions to these interfaces without requiring repeated exposure of the interface to air. The TEM grid cells and the flow chambers were prepared according to the procedures in the literature with small modifications.\textsuperscript{6,9,24} Fig. 1 shows the schematic of the flow chamber used in this study and a picture of the TEM grid cell in the flow chamber. The glass microscope slides were cleaned according to published procedures\textsuperscript{25} and coated with octadecyltrichlorosilane (OTS).\textsuperscript{26} Copper specimen grids (that were cleaned sequentially in methylene chloride, ethanol, and finally, in methanol) were placed onto the surface of the small OTS-coated glass slide that was then glued onto another common slide glass with epoxy (see Fig. 1a). 1 \mu L of 5CB was dispensed onto each grid by dropping, and the excess LC was removed by contact with a capillary tube. The thickness of the 5CB layer on the TEM grid was approximately 20 \mu m. The PAA-b-LCP solution was prepared by the following method: PAA-b-LCP was first dissolved for 2 days at 60 °C in dioxane, after which toluene was added to obtain a 0.1 wt% solution in a dioxane/toluene (6/4, v/v) mixture. (A dioxane/toluene (6/4, v/v) mixture was used for a PAA-b-LCP solvent because PAA-b-LCP did not dissolve in the typical organic spreading solvents, (such as pure chloroform or toluene) due to its polarity.) The PAA-b-LCP solution spread on the water in the bath (see Fig. 1a) and the solvent was evaporated completely for 1 h at room temperature, creating a Langmuir film. The effective surface area of one block copolymer was \approx 1271 \AA\textsuperscript{2}. The density of the dioxane/toluene (6/4, v/v) mixture is lower than that of water, which facilitates deposition and the solvent mixture is sufficiently hydrophobic to prevent loss during spreading. Langmuir–Schaefer transfer of the film was achieved in the following manner: The TEM grid on the OTS-coated glass (glued on the glass slide) was slowly inserted (grid side down) into the bath, coming to rest on silicon spacers attached to a glass slide, previously placed in the bath (see Fig. 1b). The two glass slides spaced with silicon rubber were clipped with binding clips. Inlet and outlet ports for exchanging buffer solutions were made by needles that were punched through silicon rubber. The entire TEM grid cell and flow chamber were then flipped over and observed through a polarized optical microscope (Samwon, LSP-13) equipped with crossed polarizers (see Fig. 1c). The aqueous subphase consisted of the buffer solution with different pHs to which 1 M NaCl salts were added; all aqueous phases in this study contained 1 M NaCl salts if not mentioned otherwise. Fig. 1d shows a picture of the flow chamber and the TEM grid cell.

The dioxane/methanol (6/4, v/v) mixture was a good solvent for PAA-b-LCP and this solution spread well on water. One drop (30 \mu L) of the solution formed a monolayer on the water where the hydrophilic PAA and the hydrophobic LCP blocks were in and above the water surface, respectively, as shown in Fig. 1b. This spread monolayer on the water was deposited on the 5CB in the TEM grid cell where the PAA and LCP blocks were above...
and inside the aqueous/5CB interface in the TEM grid, respectively. The buffer solution was used with addition of 1 M NaCl salts to increase ionic strength, which will be discussed later. The LCP block has a cyanobiphenyl mesogenic group that is completely miscible with the 5CB due to the similar chemical structure. This LCP block might penetrate easily into the 5CB in the interfacial region and anchored them tightly due to a good interaction between the 5CB and the LCP block.

Results and discussion

Fig. 2 shows the optical micrographs of the TEM grid cells with a PAA-b-LCP monolayer on the 5CB observed under a polarized optical microscope with a cross-polar state at the different pHs. The image at pH = 12 appears dark, indicating homeotropic anchoring at both the aqueous/LC interface and at the OTS coated substrate; in contrast, without the PAA-b-LCP, similarly prepared TEM grid cells with 5CB alone show characteristic four-brush textures that indicate planar anchoring at the aqueous/LC interface (see Fig. S5†). In the homeotropic case, each square of the copper grid possesses a small bright edge corresponding to the region of the LC in which interactions with the surface of the grid perturb the orientation of the LC. The pH was changed by injecting different buffer solutions into the cell without changing the LC-loaded TEM grid, eliminating possible variability in sample preparation. The optical micrograph of the PAA-b-LCP treated LC at pH = 2 reveals a four-brush texture indicating planar orientation; in the absence of the block copolymer, no switching occurs, since planar orientation is maintained across the entire range of pH examined (from 2 to 12 in increments of 1 pH unit) (see Fig. S5†). Starting from low pH, LC treated with PAA-b-LCP maintained planar orientation up to pH = 9: the planar-to-homeotropic orientational change occurred upon increasing the pH from 9 to 10 and the homeotropic orientation persisted to the highest pH examined (pH = 12). With increasing pH, a conformational change in the PAA chain from a random coil in acidic solution to an extended conformation at high pH occurs (Fig. 3). As the PAA coils expand and repel each other, the block junction points become localized and their Boltzmann-weighted separation increases. This regular separation may allow the SGLCP blocks to adopt configurations that are oblate, such that the backbone no longer extends away from the interface. Such an oblate configuration of the backbone preferentially orients the director normal to the interface.

Abbott and co-workers have studied the effects of the local density of phospholipids on the LC orientation. They demonstrated that high areal densities of phospholipids were required to promote homeotropic alignment of 5CB at the aqueous/5CB interface. A compact coil of the PAA block (e.g., at pH = 2) would cover a small area above the aqueous/5CB interface, and the connected LCP block might also cover a small area on the 5CB (below the aqueous/5CB interface) which would allow the main chain of the SGLCP block to penetrate more deeply into the 5CB straight along a direction perpendicular to the interface (see Fig. 3, left). The mesogenic groups of the LCP block (perpendicular to the main chain) would therefore be parallel to the interface, reinforcing the usual anchoring condition at an aqueous/5CB interface. However, the expanded coil of the PAA block (e.g., at pH = 12) would cover a larger area on the interface, and the connected SGLCP block might relax and adopt an oblate configuration (Fig. 3, right), with backbone segments...
preferentially parallel to the interface and the pendant mesogens preferentially perpendicular to it. Such a change in conformation would be consistent with the observed homeotropic anchoring at high pH. The homeotropic-to-planar orientational change occurred at a greater pH than the pKₐ of the PAA. This indicates that, for this PAA block length, a high extent of charge is required to induce the conformational change of the SGLCP block. The PAA chains continuously expand above the pKₐ of PAA and the expansion of PAA may reach a critical value, pH*, at which the homeotropic orientation is produced. Ongoing studies are examining the dependence of pH* on the ionic strength, the block copolymer composition, and the density of PAA-b-LCP on the interface (which can be controlled using a Langmuir–Blodgett film fabrication method), etc.

The charge density of PAA depends not only on the pH, but also on the salt concentration. Fig. 4 shows optical micrographs of the TEM grid cells with a PAA-b-LCP monolayer on the 5CB observed under a polarized optical microscope in a cross-polar state at pH = 12 with different amounts of added NaCl salts. When the coating of PAA-b-LCP on the 5CB in the TEM grid cell was prepared at pH = 12 without NaCl salts in the aqueous phase, the homeotropic orientation was observed immediately. However, it changed to a planar orientation within 10 min as shown in Fig. 4a while its color was not as bright as that of the typical planar orientation as shown in Fig. 2. This orientational change might be supposed to indicate desorption of the PAA-b-LCP from the aqueous/5CB interface. However, as soon as the 1 M NaCl buffer solution at pH = 12 was replaced, the color returned to black indicating that desorption did not occur as shown in Fig. 4d. The image of the homeotropic orientation became darker and clearer as the NaCl concentration increased. All experiments in this article were done with the addition of 1 M NaCl salts in the aqueous phase, because it was determined that the 1 M NaCl solution provided the best image of the homeotropic orientation. The increase of the ionic strength by the addition of NaCl salts in the aqueous phase might decrease the electrostatic cost of dissociation in the carboxylic groups of the PAA block.

The orientational change of the 5CB in the TEM grid cell (by environmental stimuli in the aqueous phase or interactions with other foreign materials) can be used for sensing pH or detecting small amounts of foreign materials (such as proteins), particularly cationic ones that can form complexes with the COO⁻ in the PAA block. For these purposes, the speed, reversibility, and durability of the response of the TEM grid cell are important characteristics. In order to study the speed of the homeotropic to planar orientational change, a video was recorded during the change of the pH in the buffer solution (see ESI†). Fig. 5a shows the change of the average grayscale intensity in each frame in the video during the altering of the pH from 12 to 2, 2 to 12, 12 to 2, and 2 to 12. These frames of the video were analyzed in 0.5 s increments. The grayscale intensities at pH = 12 and 2 were low and high, respectively, due to the darkened image of the homeotropic orientation and the brightened image of the planar orientation. The homeotropic (at pH = 12) to planar (at pH = 2) (or vice versa) orientational change occurred within ~2 s. This fast response is a good indicator of the suitability of this TEM grid cell for sensor applications. In order to study the reversibility of the TEM grid cells, the pH of the buffer solutions was alternated from pH = 12 to 2 and back repeatedly. Fig. 5b shows the average grayscale intensity of the image of the TEM grid cell up to 20 cycles (see the original images in Fig. S7†). The TEM grid cell changed reversibly from a homeotropic to a planar orientation in each cycle. The grayscale intensity of the image of
the TEM grid cell at the 20th cycle was almost same as that at the 1st cycle. This indicates that PAA-b-LCP was not desorbed from the aqueous/5CB interface, and the LCP block penetrated deeply into the 5CB anchored in the interfacial region by the strong interactions between the mesogens of the LCP block and the 5CB. We did not perform experiments above 20 cycles, but we believe that the reversibility of the TEM grid cell can extend higher than 20 cycles. In order to study the durability of the TEM grid cell, the images of the TEM grid cell at pH 12 were taken over time and Fig. 5c shows their average grayscale intensity (see the original images in Fig. S8). The average grayscale intensity did not appear to change until the 6th day, and slightly increased at the 7th day. The reversible nature of the change in optical appearance of the 5CB with change in the pH of the aqueous phase, the short time scale over which these changes occur, and the durability of the homeotropic orientation at pH = 12 demonstrate that the orientational changes of the 5CB in the interfacial region are not the result of desorption or re-adsorption of PAA-b-LCP at the aqueous/5CB interface. Instead, our results suggest that the response of the PAA-b-LCP-laden interfaces to changes in the pH of the aqueous phase arises from the fast pH-dependent conformational changes of the PAA block and the complete miscibility of the LCP block.

PAA brushes represent an exceptional surface modification useful for unspecific protein immobilization and can bind proteins regardless of the sign of the protein’s net charge. In order to test the sensing capability of the TEM grid cell for detecting proteins, small amounts of the lysozyme protein were dissolved in the aqueous phase at pH = 12. The complex formation between COO\(^{-}\) of the PAA block and lysozyme. Repeated tests were performed for varying lysozyme concentrations, sequentially decreasing the concentration in the aqueous phase by half until the planar orientation was no longer observed. The homeotropic to planar orientational change was observed at as little as 0.0022 µmol ml\(^{-1}\) (Fig. 6f) and did not occur again until the lysozyme concentration was at 0.000068 µmol ml\(^{-1}\) (Fig. 6g). Thus, the aqueous/5CB interface functionalized with PAA-b-LCP in the TEM grid cell can be utilized as a sensor for detecting extremely small amounts of the proteins without the use of sophisticated devices. Further studies on the effects of the composition of the block copolymer, the density of the monolayer (on the water during preparation of the TEM grid cell by using a Langmuir–Blodgett film fabrication method), and the type of protein on the performance of the TEM grid cell for use as a sensor, are currently under way.

**Conclusions**

Fast, reversible, and durable switching between homeotropic and planar anchoring at the aqueous/LC interface in response to environmental stimuli is mediated by an appropriately designed diblock copolymer. The switching time between homeotropic and planar anchoring appeared to be mass-transfer limited (changing as quickly as the buffer could be exchanged, within ~2 s). Reversible, and repeatable switching was sustained for as many cycles as were tested (~20 cycles). The homeotropic orientation at pH = 12 remained unchanged for 6 days, indicating that the LCP block secures the self-assembled layer on the LC interface and maintains its conformation over time. What distinguishes the present approach from previous responsive aqueous/LC interfaces is the orientational coupling between the backbone and the mesogenic side groups of the SGLCP block. Thus, the change in conformation of the polyelectrolyte “responsive” block exerts its influence through a single covalent connection to the SGLCP “transducer” block, rather than through the local density of the hydrophobes in the 5CB. Using an SGLCP as the “transducer” block opens the way to systematic optimization by choice of the mesogen, the mode of attachment (side-on vs. end-on) and the spacer. The SGLCP can be
connected to a wide variety of “responsive” blocks, such as single-stranded DNA,31,32 aptamer-binding RNA,33,34 peptides35 or proteins.36,37

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