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Design of Biomolecular Interfaces using Liquid Crystals Containing Oligomeric Ethylene Glycol

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Abstract

We report an investigation of nematic LCs formed from miscible mixtures of 4-cyano-4'-pentylbiphenyl (5CB) and 2-(2-[2-{2-(2,3-difluoro-4-{4-(4-*trans*-pentylcyclohexyl)-phenyl-phenoxy}ethoxy)ethoxy]ethoxy)ethanol (EG4-LC), the latter being a mesogen with a tetra(ethylene glycol) tail. Quantitative characterization of the ordering of this LC mixture at biologically-relevant aqueous interfaces revealed that addition of EG4-LC (1–5% by weight) to 5CB causes a continuous transition in the ordering of the LC from a planar (pure 5CB) to a perpendicular (homeotropic) orientation. The homeotropic ordering is also seen in aqueous dispersions of micrometer-sized droplets of the LC mixture, which exhibit enhanced stability against coalescence. These observations and others, all of which suggest partitioning of the EG4-LC from the bulk of the LC to its aqueous interface, were complemented by measurements of the adsorption of bovine serum albumin (BSA) to the aqueous-LC interface. Whereas adsorption of BSA to the interface of a LC mixture containing 1% wt/wt of EG4-LC triggered an ordering transition, higher concentrations of EG4-LC (>2% wt/wt) prevented this ordering transition, consistent with a decrease in adsorption of BSA. This conclusion is supported by epifluorescence measurements using fluorescently labeled BSA and comparisons to LC interfaces at which EG4-containing lipids are adsorbed. Overall, these results demonstrate a general and facile approach to the design of LCs with interfaces that present biologically relevant chemical functional groups, assume well-defined orientations at aqueous interfaces, and lower non-specific protein adsorption.

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The bulk of the LC serves as a reservoir of EG4-LC, thus permitting easy preparation of these interfaces and the potential for spontaneous repair of the EG4-decorated interfaces during contact with biological systems.

Keywords

functionalized liquid crystals; biomolecular interfaces; surface-induced ordering; oligoethylene glycol

1. Introduction

Interfaces between thermotropic liquid crystals (LCs) and immiscible aqueous phases have received substantial recent attention owing to the strong coupling that can occur between the ordering of the LCs and organization of assemblies of molecules that can be formed at these interfaces.^[1–28] For example, it has been shown that the self-assembly of surfactants,^[2, 11–16] lipids^[3, 17–20] and polymers^[4, 5, 21–24] at these interfaces is strongly coupled to the ordering of the LCs, and that the presence and organization of these molecules can be reported through changes in the optical appearance of the LCs. It has also been reported that more complex interfacial phenomena, such as specific binding events involving proteins,^[3, 6] bacteria,^[7] viruses,^[7] enzymatic reactions,^[8, 25–28] hybridization of DNA,^[9] and the culture of human embryonic stem cells (hESCs)^[10] at aqueous-LC interfaces can trigger dynamic orientational transitions in the LCs. Those studies suggest that aqueous-LC interfaces define a promising class of biomolecular interfaces for reporting interfacial phenomena; herein we report a new approach to design of such interfaces for biological studies.

Surfaces modified with oligoethylene glycol (OEG) and polyethylene glycol (PEG) are well-known for their tendency to minimize protein adsorption, although the precise mechanism by which this occurs is not fully understood (and it likely differs substantially depending on whether oligomers or polymers are employed).^[29, 30] Past studies have reported immobilization of OEG and PEG on solid substrates, such as gold, silicon and polydimethylsiloxane (PDMS), to form self-assembly monolayers (SAMs) and polymeric brushes, respectively.^[31, 32] For example, Prime and Whitesides first reported OEG-terminated SAMs by self-assembling alkanethiols onto gold.^[33, 34] They demonstrated that OEG-terminated SAMs were effective in minimizing non-specific protein adsorption, and it was suggested that this property of the system has an entropic origin.^[35] Later studies revealed the conformations of EG units and the extent of hydration of the SAMs to be important in determining the tendency of these systems to minimize protein adsorption.^[35, 36] The grafting of PEG to solid surfaces has also been widely explored to reduce or eliminate protein adsorption.^[37] High molecular weights and high grafting densities of the PEG chains generally decrease protein adsorption,^[38, 39] presumably through steric interactions that prevent direct protein interactions with the surface underlying the PEG brush.^[40, 41] Inspired by these prior studies of PEGylated surfaces, we sought to establish a methodology to engineer interfaces of LCs that would minimize non-specific adsorption of proteins. In particular, we sought to determine how mesogens containing ethylene glycol moieties would impact the orientational ordering of LCs at aqueous interfaces, and also modify the interactions of proteins with these LC interfaces.

This report describes the functionalization of aqueous-LC interfaces with OEG moieties by using a mesogen with a tetra(ethylene glycol) tail (EG4-LC) (Figure 1). We report on surface-induced orientational ordering transitions in nematic mixtures of 4-cyano-4'-pentylbiphenyl (5CB) containing EG4-LC that are placed into contact with aqueous phases,

using both film (planar) and droplet (non-planar) geometries. We also describe ordering transitions induced at these LC interfaces by adsorption of bovine serum albumin (BSA) from bulk aqueous solution. These results are interpreted by using epifluorescence microscopy to quantitatively measure the amount of fluorescently-labeled BSA adsorbed at the interfaces, and the measurements are compared to BSA adsorption at LC interfaces decorated with a lipid containing a tetraethylene glycol head group.

2. Results and Discussion

Our first set of experiments employed differential scanning calorimetry (DSC) to determine if the EG4-LC was miscible with 5CB. Figure 1 shows the molecular structure of 5CB and the EG4-LC mesogen used in this study. The EG4-LC mesogen is comprised of two parts: a hydrophobic mesogenic core and hydrophilic oligo(ethylene glycol) moiety with a terminal hydroxyl group. Fluorine groups are attached to the mesogenic core to lower the temperatures at which LC phases are obtained.^[42] Past studies have reported that when the hydroxyl group is functionalized with methacrylate, the monomer can be polymerized to form ion-conductive films.^[43] In our experiments, 5CB was mixed with various amounts of EG4-LC, reported below as % by weight of EG4-LC in 5CB.

The DSC measurements revealed that upon heating, EG4-LC exhibited the following phase behavior: Cr 25.6 °C S_x 97.2 °C S_A 116 °C I (abbreviations are as follows: Cr, crystalline phase; N, nematic phase; S_x , undefined ordered smectic phase; S_A , smectic A phase; I, isotropic phase). The DSC of the 5CB containing 5% and 10% EG4-LC was found to be qualitatively similar to pure 5CB (nematic to isotropic transition temperature (T_{NI}) of 35.8 °C), with the presence of the EG4-LC causing a small downward shift of the nematic-to-isotropic transition temperature to 34.1 °C (for 5% EG4-LC) and 33.9 °C (for 10% EG4-LC) as shown in Figure S1. Significantly, for the mixtures of 5CB and EG4-LC, there was no evidence of the S_x -to- S_A transition at 97.2 °C nor the S_A -to-I transition of pure EG4-LC at 116 °C. The principal conclusion extracted from these results is that 5CB and EG4-LC appear well-mixed in the bulk.

Next, we sought to determine the influence of the EG4-LC on the orientational ordering of mixtures of 5CB and EG4-LC at interfaces to air and aqueous solutions. In a typical experiment, a thin film of nematic 5CB mixed with EG4-LC was wicked into the pores (283 $\mu\text{m} \times 283 \mu\text{m}$) of a 20 μm -thick gold electron microscopy grid. The LC-filled grids were supported on a glass microscope slide treated with octadecyltrichlorosilane (OTS). The OTS treatment of the glass anchors 5CB and a variety of other LCs in an orientation that is perpendicular (homeotropic) to the LC-glass interface.^[44, 45] The results reported below indicate that mixtures of 5CB and EG4-LC also assume a homeotropic orientation at the surface of OTS-treated glass. To promote mixing of 5CB and EG4-LC, the mixture was heated into its isotropic phase (~120 °C) and vortexed for 3 min prior to placement into the gold grids. The LC-filled grids were equilibrated in air for at least 10 min, and then immersed under an aqueous phase, resulting in the formation of a stable approximately planar interface between the aqueous phase and LCs.

Figure 2A shows optical micrographs (crossed polars) of nematic 5CB confined within a gold grid exposed to air. As reported previously, nematic 5CB aligns perpendicular to the interface with air (so-called homeotropic anchoring).^[2] When combined with the influence of the OTS-treated glass (which also causes homeotropic anchoring), the LC film assumes a uniform orientation that is perpendicular to both surfaces thus leading to a dark appearance between cross-polars. The director profile is shown in Figure 2C. Note that regions of the LCs within ~10 μm of each grid bar assume a bright optical appearance, due to interactions of the LCs with the vertical surfaces of the gold grid. When the 5CB-filled grid was

submerged under an aqueous solution of phosphate buffer (pH 7.4, see the Experimental section for composition), the optical appearance of the LCs became bright, consistent with an orientational ordering transition induced by contact with water (Figure 2B). Past studies have established that the easy axis of nematic 5CB is parallel to an interface with water (so-called planar anchoring).^[2] In order to accommodate the planar anchoring at the LC-aqueous interface and the perpendicular anchoring at the OTS-glass interface, the LC undergoes a continuous splay and bend distortion between the two confining interfaces (Figure 2D).

Similar to pure 5CB, we observed 5CB containing 5% EG4-LC in contact with air to possess a dark appearance caused by a homeotropic orientation of the LC (Figure 2E). Immediately after immersion under the aqueous phosphate buffer, the 5CB containing 5% EG4-LC exhibited a bright optical appearance (Figure 2F). The interference colors, which were bright greens and pinks, indicate that the LCs had assumed a tilted state (that is, an orientational state that is distinct from that of pure 5CB shown in Figure 2B). Inspection of Figure 2F also reveals that some regions of the LCs contained in the grid had transformed to a homeotropic orientation, as evidenced by the black regions; in some squares of the grid, both bright and dark regions coexist. Figure 2G and 2H show the optical appearance of the same film of 5CB containing 5% EG4-LC approximately 1 min and 30 mins, respectively, after contact with the aqueous phosphate buffer. These images reveal that the 5CB containing 5% EG4-LC undergoes a dynamic ordering transition towards a homeotropic orderings over this period of time, although we observe both dark and bright regions simultaneously (see discussion below). The tilted orientation of the LCs at short times, when combined with the observation of a slow dynamic ordering transition towards the homeotropic orientation over a period of minutes, is suggestive of a physical mechanism whereby the EG4-LC adsorbs and organizes itself at the aqueous-LC interface following contact with the aqueous phase. We note here that pure 5CB remains in a parallel orientation for days following contact with the aqueous phase. Thus, the ordering transition observed in Figure 2E-H is caused by the presence of the EG4-LC in the 5CB. Similar results were obtained when pure water was used instead of the aqueous phosphate buffer, indicating that the phosphate buffer was not the cause of the dynamic ordering transition described above.

We also investigated the influence of the concentration of EG4-LC on the orientational ordering of mixtures of 5CB and EG4-LC. These measurements revealed that 5CB containing either 1% or 2% EG4-LC, upon contact with phosphate buffer, also underwent an ordering transition to a homeotropic orientation over a time-scale comparable to that shown in Figure 2E-H (~ 30 mins). It is noteworthy, however, that in all cases where 5CB was mixed with EG4-LC and subsequently contacted with aqueous phosphate buffer, the transition to the homeotropic orientation was typically incomplete (see Figure 2H). The grid squares containing LCs that did not transition to the homeotropic orientation within 10–20 mins (the bright regions in Figure 2H) possessed a bright optical appearance for weeks (>2 weeks).

To provide insight into the unexpected variation in orientation of the 5CB containing 5% EG4-LC that is evident in Figure 2H, we sought to determine if these patterned orientations corresponded to equilibrium states or were kinetically trapped states of the system. To this end, we sequentially heated and cooled the mixture of 5CB and EG4-LC through T_{NI} while in contact with the aqueous phase. Figure 3A shows the initial state of a mixture of 5CB and 5% EG4-LC that was incubated in phosphate buffer at room temperature (rt) for 2 hrs. Consistent with the image shown in Figure 2H, some LC regions contained within the grid have assumed a homeotropic orientation whereas other regions are clearly tilted from the normal of the interface. Figure 3B shows the optical appearance of the 5CB containing 5% EG4-LC after heating the system into its isotropic phase at 40 °C (above T_{NI} ~ 34.6 °C).

The dark optical appearance of the 5CB (crossed polars) is caused by the formation of the isotropic phase. Figure 3C shows the system upon cooling back into the nematic phase ($\sim 33^\circ\text{C}$) – the interference colors indicate that the initial orientation of the nematic phase is not along the normal of the interface. In contrast to the experiment shown in Figure 2, however, almost the entire film of LC transitioned to the homeotropic orientation within a few seconds ($< 10\text{ s}$). We note here that the time-scale of this transition (10 s) as well as the uniformity of the final orientational state (almost entirely homeotropic) differ from the orientational transition shown in Figure 2. The former observation (dynamics) suggest that the EG4-LC is likely adsorbed and pre-organized at the interface to the aqueous phase when the 5CB containing the EG4-LC was heated into the isotropic state, thus allowing a rapid orientation transition upon cooling of the mixture into its nematic state. The latter observation suggests that the non-homeotropic regions of LCs evident in Figure 2H and 3A are likely kinetically trapped states of the system (note that the mixture of LCs shown in Figure 2H was contacted with the aqueous solution in the nematic state). Although our results support the proposition that the bright regions in Figure 2H and 3A correspond to non-equilibrium states, we note that a number of aspects of this phenomenon remain to be fully understood. For example, inspection of Figure 3A reveals that the boundaries between the homeotropic and non-homeotropic regions are defined by the grid – it is not obvious to us what role the grid may be playing in this phenomenon. We note that previous studies of lipids adsorbed at the aqueous-LC interface have revealed that lateral diffusion of lipid at the interface of the LCs cannot take place between the grid squares.^[3] We speculate that compartmentalization of the interface by the grid may be playing a role in the patterned orientation of the LCs shown in Figure 3A.

In order to further test the proposition that EG4-LC adsorbed and organized at the aqueous-LC interface underlies the orientational behaviors shown in Figure 2 and 3, we measured the orientations of 5CB doped with different concentrations of EG4-LC. By quantifying the retardance of the LCs using a birefringence mapping system (see Supporting Information), we determined the tilt angle of the LCs at the aqueous-LC interface (Figure 4A). We used mixtures containing 0%, 0.05% or 0.1% EG4-LC in these experiments. Each of these mixtures assumed a homeotropic orientation at the air-LC interface prior to immersion into the aqueous phase. After immersion into aqueous phosphate buffer, all the samples were subjected to a temperature excursion into the isotropic phase and then cooled to the nematic phase at room temperature, as shown in Figure S3A-C, Supporting Information. Measurements of the retardance of these LCs was used to determine that the tilt angle of the LCs changed continuously from 90° to 0° , as measured from the interface normal, as the weight fraction of EG4-LC increased from 0% to 1% (Figure 4B). Mixtures of 5CB containing higher concentrations of EG4-LC (between 1% and 5%) exhibited an orientational behavior that was similar to that shown for the 5% EG4-LC sample in Figure 2 and 3 (largely homeotropic orientation). After a temperature excursion into the isotropic phase, these LCs also assumed uniform homeotropic orientations (Figure S3D and E). Finally, we examined mixtures of LCs comprised of 5CB and 10% EG4-LC. Those mixtures exhibited an inhomogeneous appearance (Figure S3F) which persisted after a temperature excursion into the isotropic phase, suggesting that 5CB and 10% EG4-LC may not be completely miscible at the aqueous interface in this composition range. We note that our DSC measurements showed no evidence of bulk immiscibility of 10% EG4-LC in 5CB and thus it appears likely that this phenomenon observed at the interface of samples containing 10% EG4-LC is induced by the contact with the water. In summary, the continuous decrease in tilt angle from 90° to 0° with the increase of EG4-LC content from 0% to 1% is consistent with our proposition that the EG4-LC partitions to the aqueous-LC interface and influences the orientation ordering of the LCs at this interface.

Although the orientation of the LC shown in Figure 3F corresponds to a homeotropic orientation, it is not possible to conclude from the results reported in Figure 3F that the easy axis of the 5CB containing 5% EG4-LC is homeotropic when the lower interface confining the LCs (OTS-treated glass) also promotes a homeotropic orientation. For example, the orientation shown in Figure 3F is also consistent with the proposition that the EG4-LC causes weak planar anchoring energy of the LC mixture. To determine if EG4-LC does, in fact, cause the 5CB containing EG4-LC to possess an easy axis that is perpendicular to the interface, we prepared emulsion droplets of 5CB and 5% EG4-LC in aqueous solutions. We examined the ordering of the LCs within the emulsion droplets as well as the stability of the LC emulsions. We prepared the emulsions by sonication of 1 vol% LC (5CB or 5CB mixed with 5% EG4-LC) in aqueous phosphate buffer. The emulsion droplets possessed sizes in the range of 1-20 μm . As observed in Figure 5A, pure 5CB within the droplets exhibited optical characteristics indicative of a bipolar configuration (Figure 5C), where two point defects (“Boojums”) are located at the poles of the droplet. This configuration of the LC is consistent with planar anchoring at the aqueous interface of the droplets.^[46] However, droplets containing 5% EG4-LC in 5CB (Figure 5B), exhibited an optical appearance consistent with a radial configuration of the LC (Figure 5D). For droplets of the size indicated in Figure 5C, the configuration is indicative of homeotropic anchoring at the aqueous-LC interface.^[47] We note that the variation of the optical appearances of the droplets in Figure 5 is caused by variation in the location of the focal plane relative to the droplet centers. Interestingly, the radial configuration of the droplets comprised of 5CB mixed with 5% EG4-LC was observed immediately after the formation of droplets. The appearance of the radial ordering of the LCs within the droplets is thus faster than the appearance of the homeotropic orientation seen within the grids shown in Figure 2F-H. We speculate that sonication of the LC droplets promotes mixing and transport of the EG4-LC to the interface of the droplet with the aqueous phase, thus leading to rapid homeotropic anchoring. The emulsion of pure 5CB was observed to be stable for ~ 1 day, with coalescence evident over longer storage times. However, the emulsion prepared from 5CB mixed with 5% EG4-LC was found to be stable for at least a week. We note that nonionic surfactants containing PEG have been widely used to stabilize oil-in-water emulsions,^[48] and we speculate that the EG4-LC influences the emulsion stability through a similar physical mechanism, involving hydration of the EG4 chains located at the interface.^[49] In the context of the current paper, the results reported here regarding stability of the emulsion droplets are significant because they provide further evidence that EG4-LC partitions to the aqueous-LC interface to modify properties of the interface. We also conclude that the level of solubility of EG4-LC in water is low because after 12hrs incubation of 5% EG4-LC in phosphate buffer, the LCs maintains its homeotropic alignment (see Figure S4).

As discussed in the Introduction, OEG-decorated interfaces have been widely investigated in biomolecular systems because of their tendency to minimize non-specific adsorption of proteins.^[29, 30] Building from these past studies, we sought to determine if the incorporation of EG4-LC into 5CB would also reduce non-specific adsorption of proteins onto interfaces of the LCs in contact with aqueous phases. In a typical experiment, an aqueous film of 5CB containing a predetermined concentration of EG4-LC was immersed under aqueous phosphate buffer, and the optical appearance of the LC between crossed polars was recorded after thermal annealing. Subsequently, the samples were transferred into aqueous buffer containing 10 μM Alexa Fluor[®] 488-labeled bovine serum albumin (BSA) for 2 hrs. When immersed in the buffer, the pure 5CB assumed a planar orientation, and the mixtures of 5CB and EG4-LC (not 10%, see above) exhibited a homeotropic alignment. For the pure 5CB sample, after incubation in the BSA solution for 2 hrs, the orientation of the LCs was indistinguishable from the 5CB in contact with buffer (planar). This observation is consistent with prior reports that have shown adsorbed proteins to promote planar orientations of LCs.^[3] In contrast, when the 5CB containing 1% EG4-LC, the presence of

the BSA in the phosphate buffer caused an ordering transition from a homeotropic state to a tilted state, resulting in a bright optical appearance of the LCs (see Figure S5). Upon increasing the concentration of EG4-LC dissolved into the 5CB to either 2% or 5% by weight, we observed the orientation of the 5CB containing the EG4-LC to remain homeotropic following contact with the BSA solution. Similarly, the optical appearance of the LC sample containing 10% EG4-LC was not changed by exposure to BSA (remained homeotropic). The observation that the LC mixture containing 1% EG4-LC underwent an ordering transition upon exposure to BSA whereas mixtures containing the high weight percents of EG4-LC did not suggests that either (i) a higher interfacial concentration of EG4-LC lowered the extent of the BSA adsorption and, as a result, the 5CB containing EG4-LC did not undergo an orientational ordering transition in the presence of the low amount of adsorbed BSA or (ii) the amount of BSA adsorbed at the aqueous-LC interface is not necessarily lowered by the EG4-LC but the high interfacial concentrations of EG4-LC at the aqueous-LC interface achieved by using high bulk concentrations of EG4-LC prevent the homeotropic orientation of LCs being perturbed by the adsorbed BSA.

To distinguish between the two physical scenarios described above, we quantified the amount of BSA adsorbed at the aqueous-LC interface as a function of the concentration of EG4-LC dissolved in 5CB. After a 2 hr incubation in aqueous buffer containing 10 μ M fluorescently labeled BSA, the LCs were contacted five times with 100 mL phosphate buffer to remove BSA from the bulk solution as well as to remove reversibly adsorbed BSA from the aqueous-LC interface. The interface of the LCs was then imaged by using fluorescent microscopy (see Supporting Information for additional detail). The fluorescence intensity of each interface was quantified and used to determine the relative amounts of BSA adsorbed at the aqueous-LC interface. As references against which to compare BSA adsorption at the aqueous-LC interfaces, OTS-treated glass (hydrophobic surface) and PEG-treated glass (see below) were used. The PEG-treated glass has been the subject of prior studies and is known to exhibit good resistance to protein adsorption.^[32, 50, 51]

Inspection of Figure 6 reveals that after incubation with labeled BSA, the fluorescence intensity of the aqueous interface of 5CB (no EG4-LC) is comparable to that of the aqueous interface of OTS-treated glass. We used ellipsometry to determine the optical thickness of BSA adsorbed onto an OTS-treated silicon wafer (see Supporting Information for details regarding the experimental procedure). The value was determined to be 3.1 ± 0.2 nm, corresponding approximately to monolayer coverage of BSA (the size of a BSA molecule is $4 \text{ nm} \times 4 \text{ nm} \times 14 \text{ nm}$.^[52]). This suggests that the loading of BSA at the aqueous interface of 5CB is close to monolayer coverage. We also prepared a PEG-treated glass surface, using Si-PEG₍₄₅₎ to graft PEG onto the glass slide through a silanization reaction. This surface was used as we expected it to yield low levels of protein adsorption. Inspection of Figure 6 reveals that the amount of BSA adsorbed to the aqueous-LC interface was decreased substantially (by a factor of almost 4) by incorporation of the EG4-LC into the 5CB. The effect is particularly pronounced between 1% and 5% EG4-LC, with the incorporation of 10% EG4-LC not revealing a significant decrease in BSA adsorption relative to the sample containing 5% EG4-LC. These results demonstrate that incorporation of EG4-LC into 5CB is an effective strategy to lower protein adsorption at the LC interface, although the levels of protein adsorption are not lowered to values comparable to PEG-treated glass (Figure 6). This latter difference in level of non-specific adsorption may be because the PEG-treated glass is decorated with a longer ethylene glycol chain (45 repeat units) than the interface of the LC (4 repeat units). We also note that the fluidity of the interface of the LCs will allow the LC interface to deform and reorganize upon BSA adsorption in ways that are not possible on flat solid surfaces.

In order to provide an additional perspective on the effectiveness of the EG4-LC as a means to lower non-specific protein adsorption at LC interfaces, we also prepared nematic 5CB interfaces decorated with the lipid, $C_{14}E_4$, which has the same number of ethylene glycol groups as EG4-LC (Figure 7A). We decorated the aqueous-5CB interface with $C_{14}E_4$ by incubating aqueous solutions containing 1 mM, 2 mM, and 3 mM of $C_{14}E_4$ against 5CB for 1 hr. As shown in Figure 7B, D and F, a change in optical appearance of 5CB from bright (planar alignment) to uniformly dark (homeotropic alignment) was observed to accompany the adsorption of $C_{14}E_4$ to the interface for the three different concentrations. This observation is consistent with our past studies that have reported that non-ionic amphiphiles can cause homeotropic orientations of 5CB via interactions between the aliphatic tails of the amphiphiles and the LC.^[26] Figure 7B corresponds to an experiment in which 5CB was incubated with 1 mM $C_{14}E_4$. After 1 hr, the excess $C_{14}E_4$ was rinsed away and the aqueous-5CB interface was exposed to 10 μ M fluorescently labeled BSA solution for 2 hrs. The optical appearance after contact with the BSA solution is shown in Figure 7C, revealing that an ordering transition was induced by adsorption of the BSA at the aqueous-LC interface. However, when 5CB was incubated with a higher concentration of $C_{14}E_4$, e.g., 2 mM or 3 mM, the orientational ordering of the LCs did not change after exposure to the BSA solution (Figure 7E and G). To determine the amount of BSA adsorbed at the $C_{14}E_4$ -decorated interfaces giving rise to the above described ordering transitions in the LCs, we used fluorescence methods identical to those used above for quantitation of BSA adsorption to the interface of mixtures of 5CB and EG4-LC. Inspection of Figure 8 reveals that the $C_{14}E_4$ has an influence on BSA adsorption that is remarkably similar to the EG4-LC. In particular, with an increase in the $C_{14}E_4$ concentration from 1 mM to 3 mM, the amount of BSA adsorbed onto the $C_{14}E_4$ -decorated aqueous-5CB interface decreased linearly to a value that is very similar to that obtained with 5% EG4-LC. These observations also show that a higher interfacial concentration of $C_{14}E_4$ at the aqueous-5CB interface leads to a greater resistance to protein adsorption.

We make one final observation regarding BSA adsorption to the LC interface decorated with OEG moieties. We note that the fluorescence intensity of aqueous-5CB interfaces incubated in 1 mM $C_{14}E_4$ is similar to the intensity measured using 2% EG4-LC in Figure 6. This result suggests that similar amounts of BSA adsorbed onto both interfaces. However, we point out that the former sample ($C_{14}E_4$) underwent an ordering transition from homeotropic to planar alignment under these conditions whereas the latter one (5CB + EG4-LC) remained in a homeotropic orientation. This result indicates that the aqueous/EG4-LC interface differs from the $C_{14}E_4$ -decorated interface of 5CB in terms of the orientational response to non-specifically adsorbed protein. The aqueous/EG4-LC interface is apparently more tolerant, possibly due to strong homeotropic anchoring caused by EG4-LC relative to $C_{14}E_4$, or the presence of EG4-LC within the bulk of the LC (EG4-LC can adsorb from the bulk onto the interface during adsorption of the BSA).

3. Conclusions

In summary, this paper describes a general strategy for preparing interfaces of LCs with chemical functionality that is relevant to the design of biotic-abiotic interfaces based on LCs. We illustrate the approach by introducing a mesogen that contains tetra(ethylene glycol) group (EG4-LC) into a nematic phase of 5CB. A series of results presented in this manuscript (obtained using both film and droplets geometries) support our conclusion that the mesogen EG4-LC partitions to the aqueous interface of the nematic mixture to change the orientational ordering of the LC as well as the interactions of the interface of the LC with proteins. In particular, the interfacial partitioning of the EG4-LC lowers the level of non-specific adsorption of BSA to the interface of the LC. Minimization of non-specific adsorption of proteins to the interfaces of synthetic materials represents a key challenge in

the design of biotic-abiotic interfaces. A significant merit of this approach is that the bulk of the LC serves as a reservoir of the EG4-LC, thus permitting easy preparation of these interfaces as well as spontaneous repair of the EG4-decorated interfaces during contact of these materials with biological systems. Whereas the study reported in this paper employs EG4-functionalized mesogens, the approach is a generalizable one that can be extended to the design of mesogens that are functionalized with specific binding groups for biological targets.

4. Experimental

Materials

Sodium hydroxide (NaOH) and potassium dihydrogen phosphate (KH_2PO_4) were obtained from Sigma-Aldrich (Milwaukee, WI). Tetraethylene glycol monotetradecyl ether (C_{14}E_4) was obtained from Sigma-Aldrich (St. Louis, MO). Deionization of a distilled water source was performed using a Milli-Q system (Millipore, Bedford, MA) to give water with a resistivity of 18.2 M Ω cm. Octadecyltrichlorosilane (OTS), hydrogen chloride, hydrogen peroxide (30% w/v) and sulfuric acid were all obtained from Fisher Scientific (Pittsburgh, PA). The glass microscope slides were Fisher's Finest Premium Grade obtained from Fisher and silicon wafers were obtained from Silicon Sense (Nashua, NH). Gold specimen grids (20 μm thickness, 333 μm pitch, and 55 μm bar width) were obtained from Electron Microscopy Sciences (Fort Washington, PA). 4-cyano-4'-pentylbiphenyl (5CB) was obtained from EM Sciences (New York, NY). The mesogen 2-(2-[2-{2-(2,3-difluoro-4-{4-(4-*trans*-pentylcyclohexyl)-phenyl-phenoxy}ethoxy)ethoxy}ethoxy)ethanol (EG4-LC) was synthesized, as reported previously by Kato and coworkers [43]. Poly(ethylene glycol) tri(ethoxy)silane (Si-PEG₍₄₅₎, $M_w \sim 2000$) was obtained from ProChimia (Gdansk, Poland). Alexa Fluor^R-488 bovine serum albumin (BSA) was obtained from Invitrogen (Carlsbad, CA).

Preparation of Optical Cells Containing LCs

Details regarding the preparation and examination of the optical cells filled with LCs that were used in our study can be found in one of our past publications [2]. Briefly, glass microscope slides were piranha cleaned and coated with octadecyltrichlorosilane (OTS). The quality of the OTS layer was assessed by checking for perpendicular alignment of nematic 5CB confined between two OTS-treated glass slides. Gold specimen grids (rinsed three times each in ethanol, methanol and dichloromethane and dried) were then placed onto the surface of a piece of OTS-treated glass (5 mm $\times$ 5 mm). 1 μL of 5CB was dispensed onto each grid and the excess 5CB was removed by using a 10 μL syringe (Fisher Scientific) in order to obtain a uniformly filled grid. The OTS-treated glass supporting the gold specimen grid was placed into 8 mL glass wells containing 5 mL phosphate buffer (pH 7.4, prepared using 39.5 mM NaOH and 50 mM KH_2PO_4). Six mixtures of EG4-LC and 5CB were prepared by mixing (0.05, 0.1, 1, 2, 5, 10 % wt/wt of EG4-LC). Each mixture was heated into the isotropic phase of the EG4-LC ($\sim 120^\circ\text{C}$) and mixed for 3 min using a vortex mixer. This mixing procedure was performed before filling each grid with LC. Each filled grid was equilibrated in air for at least 10 min prior to immersion in an aqueous phase.

Preparation of PEG-Treated Substrates

Glass slides and silicon wafers were piranha cleaned (using a procedure described in past studies) [2] and immediately immersed in a silane solution [53]. The silane solution contained 3 mM Si-PEG₍₄₅₎ dissolved in water and acidified with 0.8 mL of concentrated HCl per liter. After incubation for 2 hrs at room temperature, each substrate was thoroughly washed with water and dried under a stream of N_2 .

Adsorption of C₁₄E₄ at the Aqueous-LC Interface

C₁₄E₄ (11.7 mg, 0.03 mmol) was dissolved in 5 mL phosphate buffer (pH 7.4, see above for details) to obtain a 6 mM solution of C₁₄E₄. The cloudy solution of C₁₄E₄ was sonicated using a tip sonicator (15 cycles of 1 min at 10 W, below ~ 60 °C) to obtain a clear solution. C₁₄E₄ was adsorbed onto the aqueous-5CB interface by immersing gold grids hosting 5CB under phosphate buffer and adding an aliquot of 6 mM C₁₄E₄ to obtain the desired concentration of C₁₄E₄. The grids were incubated at room temperature for 1 hr. The aqueous solution containing C₁₄E₄ was subsequently exchanged with phosphate buffer five times, keeping the aqueous-5CB interface submerged under the aqueous phase at all times.

Protein Adsorption at Aqueous-LC Interfaces

Naked aqueous-LC interfaces and interfaces of LC decorated with C₁₄E₄ were contacted with 10 μ M Alexa Fluor^R-488 bovine serum albumin (BSA) in phosphate buffer (pH 7.4). After incubation at room temperature in the dark for 2 hrs, the aqueous solution was contacted five times with 100 mL phosphate buffer, keeping the aqueous-5CB interface under the aqueous phase at all times. The same experimental procedure was used to investigate protein adsorption onto OTS-treated glass slides and PEG-treated glass slides.

Preparation of LC Emulsions

The LC-in-water emulsions (1 vol.%) were formed by mixing 10 μ L of LCs (5CB or mixture of 5CB and EG4-LC) with 1 mL of the phosphate buffer with a tip sonicator at a power of 10 W for 60 s.

Differential Scanning Calorimetry (DSC)

To prepare a sample containing 5% EG4-LC for DSC, 24.98 mg EG4-LC was dissolved in CHCl₃ (5 mL) in a mess-flask, and then 500 μ L of the resulting CHCl₃ solution was added to 47.51 mg 5CB. The sample was heated at 100 °C for 1 hr to remove CHCl₃, and then mixed at 120 °C. DSC (Netzsch, DSC204) was performed using an aluminum pan containing 2–5 mg of LC and a reference pan. The sample was sequentially heated to 140 °C at 10 °C/min, equilibrated for 10 min, cooled to –20 °C at 10 °C/min, and equilibrated for 10 min. The sample was then heated from –20 °C to 140 °C at 10 °C/min, equilibrated for 10 min and then cooled to –20 °C at 10 °C/min [54]. The temperatures at which the nematic-to-isotropic phase transition occurred (T_{NI}) were determined from the heating curves.

Optical Characterization of LC Ordering

The orientational ordering of 5CB within each specimen grid was determined by using plane-polarized light in transmission mode on an Olympus BX60 microscope with crossed polarizers. The optical cells were placed on a rotating stage located between the polarizers. Orthoscopic examinations were performed with the source light intensity set to 50% of full illumination and the aperture set to 10% in order to collimate the incident light. Homeotropic alignments of the LCs were determined by first observing the absence of light transmitted during a 360° rotation of the sample. Insertion of a condenser below the stage and a Bertrand lens above the stage allowed conoscopic examination of the optical cell. An interference pattern consisting of two crossed isogyres indicated homeotropic alignment. In-plane birefringence was indicated by the presence of brush textures, typically four-brush textures emanating from a line defect, when the sample was viewed between crossed polarizers. All images were captured using a digital camera (Olympus C-2040 Zoom) mounted on the microscope and set to an f-stop of 2.6 and a shutter speed of 1/320 s. All images shown in this paper correspond to the equilibrium alignment of the LCs except specially stated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Lockwood NA, Gupta JK, Abbott NL. *Surf Sci Rep.* 2008; 63:255.
2. Brake JM, Abbott NL. *Langmuir.* 2002; 18:6101.
3. Brake JM, Daschner MK, Luk YY, Abbott NL. *Science.* 2003; 302:2094. [PubMed: 14684814]
4. Kinsinger MI, Sun B, Abbott NL, Lynn DM. *Adv Mater.* 2007; 19:4208.
5. Zou J, Fang J. *Langmuir.* 2009 Article ASAP.
6. Hartono D, Xue CY, Yang KL, Yung LYL. *Adv Funct Mater.* 2009; 19:3574.
7. Sivakumar S, Wark KL, Gupta JK, Abbott NL, Caruso F. *Adv Funct Mater.* 2009; 19:2260.
8. Bi XY, Hartono D, Yang KL. *Adv Funct Mater.* 2009; 19:3760.
9. Price AD, Schwartz DK. *J Am Chem Soc.* 2008; 130:8188. [PubMed: 18528984]
10. Lockwood NA, Mohr JC, Ji L, Murphy CJ, Palecek SR, de Pablo JJ, Abbott NL. *Adv Funct Mater.* 2006; 16:618.
11. Brake JM, Mezera AD, Abbott NL. *Langmuir.* 2003; 19:8629.
12. Lockwood NA, de Pablo JJ, Abbott NL. *Langmuir.* 2005; 21:6805. [PubMed: 16008390]
13. Gupta JK, Abbott NL. *Langmuir.* 2009; 25:2026. [PubMed: 19140731]
14. Price AD, Ignés-Mullol J, Vallve MA, Furtak TE, Lo YA, Malone SM, Schwartz DK. *Soft Matter.* 2009; 5:2252.
15. Bahr C. *Phys Rev E.* 2006; 73:030702.
16. McCamley MK, Ravník M, Arntsen AW, Opal SM, Zumer S, Crawford GP. *J Appl Phys.* 2009; 105:123504.
17. Brake JM, Daschner MK, Abbott NL. *Langmuir.* 2005; 21:2218. [PubMed: 15752009]
18. Brake JM, Abbott NL. *Langmuir.* 2007; 23:8497. [PubMed: 17595119]
19. Gupta JK, Meli MV, Teren S, Abbott NL. *Phys Rev Lett.* 2008; 100:048301. [PubMed: 18352339]
20. Meli MV, Lin IH, Abbott NL. *J Am Chem Soc.* 2008; 130:4326. [PubMed: 18335929]
21. Tjijto E, Cadwell KD, Quinn JF, Johnston APR, Abbott NL, Caruso F. *Nano Lett.* 2006; 6:2243. [PubMed: 17034091]
22. Lockwood NA, Cadwell KD, Caruso F, Abbott NL. *Adv Mater.* 2006; 18:850.
23. Gupta JK, Tjijto E, Zelikin AN, Caruso F, Abbott NL. *Langmuir.* 2008; 24:5534. [PubMed: 18419143]
24. Sivakumar S, Gupta JK, Abbott NL, Caruso F. *Chem Mater.* 2008; 20:2063.
25. Hartono D, Bi XY, Yang KL, Yung LYL. *Adv Funct Mater.* 2008; 18:2938.
26. Park JS, Abbott NL. *Adv Mater.* 2008; 20:1185.
27. Birchall LS, Ulijn RV, Webb SJ. *Chem Commun.* 2008:2861.
28. Hartono D, Lai SL, Yang KL, Yung LYL. *Biosens Bioelectron.* 2009; 24:2289. [PubMed: 19162466]
29. Prime KL, Whitesides GM. *J Am Chem Soc.* 1993; 115:10714.
30. Kingshott P, Griesser HJ. *Curr Opin Solid State Mat Sci.* 1999; 4:403.
31. Szleifer I. *Curr Opin Solid State Mat Sci.* 1997; 2:337.
32. Papra A, Bernard A, Juncker D, Larsen NB, Michel B, Delamarche E. *Langmuir.* 2001; 17:4090.
33. Palegrosdemange C, Simon ES, Prime KL, Whitesides GM. *J Am Chem Soc.* 1991; 113:12.

34. Prime KL, Whitesides GM. *Science*. 1991; 252:1164.
35. Wang RLC, Kreuzer HJ, Grunze M. *J Phys Chem B*. 1997; 101:9767.
36. Wang RLC, Kreuzer HJ, Grunze M. *Phys Chem Chem Phys*. 2000; 2:3613.
37. Harris, JM.; Zalipsky, S. *Poly(ethylene glycol): Chemistry and Biological Applications*. American Chemical Society; Washington DC: 1997.
38. Jeon SI, Lee JH, Andrade JD, Degennes PG. *J Colloid Interface Sci*. 1991; 142:149.
39. McPherson T, Kidane A, Szleifer I, Park K. *Langmuir*. 1998; 14:176.
40. Szleifer I. *Biophys J*. 1997; 72:595. [PubMed: 9017189]
41. Halperin A. *Langmuir*. 1999; 15:2525.
42. Hird M. *Chem Soc Rev*. 2007; 36:2070. [PubMed: 17982522]
43. Kishimoto K, Yoshio M, Mukai T, Yoshizawa M, Ohno H, Kato T. *J Am Chem Soc*. 2003; 125:3196. [PubMed: 12630858]
44. Tingey ML, Wilyana S, Snodgrass EJ, Abbott NL. *Langmuir*. 2004; 20:6818. [PubMed: 15274590]
45. Tercero Espinoza LA, Schumann KR, Luk YY, Israel BA, Abbott NL. *Langmuir*. 2004; 20:2375. [PubMed: 15835699]
46. Lavrentovich OD. *Liq Cryst*. 1998; 24:117.
47. Gupta JK, Sivakumar S, Caruso F, Abbott NL. *Angew Chem Int Ed*. 2009; 48:1652.
48. Tadros, T. *Novel Surfactants: Preparation Applications and Biodegradability*. CRC; New York: 2003.
49. Tadros T. *Adv Colloid Interface Sci*. 2009; 147–148:281.
50. Delamarche E, Geissler M, Bernard A, Wolf H, Michel B, Hilborn J, Donzel C. *Adv Mater*. 2001; 13:1164.
51. Delamarche E, Donzel C, Kamounah FS, Wolf H, Geissler M, Stutz R, Schmidt-Winkel P, Michel B, Mathieu HJ, Schaumburg K. *Langmuir*. 2003; 19:8749.
52. Peters, TJ. *All About Albumin: Biochemistry, Genetics and Medical Applications*. Academic Press; San Diego, CA: 1996.
53. Hellmich W, Regtmeier J, Duong TT, Ros R, Anselmetti D, Ros A. *Langmuir*. 2005; 21:7551. [PubMed: 16042494]
54. Sharma D, MacDonald JC, Iannacchione GS. *J Phys Chem B*. 2006; 110:16679. [PubMed: 16913806]

Figure 1.
Molecular structures of 5CB and EG4-LC.

Figure 2.

Optical micrographs (crossed polars) of 5CB hosted in gold grids supported on OTS-treated glass slides in contact with (A) air and (B) aqueous solutions of phosphate buffer (pH 7.4). Schematic illustrations of the director profiles of 5CB exposed to air (C) and water (D) leading to homeotropic and planar anchoring, respectively. Optical images (crossed polars) of 5CB containing 5% EG4-LC hosted in gold grids supported on OTS-treated glass slides in contact with (E) air and aqueous solutions of phosphate buffer (pH 7.4) at (F) 0 s; (G) 1 min and (H) 30 mins respectively. Scale bar, 300 μm .

Figure 3.

Optical images (crossed polars) of 5% EG4-LC in 5CB hosted in gold grids supported on OTS-treated glass slides and placed into contact with an aqueous solution of phosphate buffer (pH 7.4) in (A) the nematic phase, and (B) heated into the isotropic phase, and the cooled back to the nematic phase for (C) 0 s; (D) 5 s; (E) 10 s and (F) 30 s. Scale bar, 300 μm . (See Figure S2 for color images)

Figure 4. Influence of EG4-LC concentration on the anchoring of 5CB at aqueous-LC interfaces. (A) Schematic illustration of LC director profile (tilt angle was defined relative to the interface normal). (B) Quantification of the tilt angle of LCs comprised of EG4-LC in 5CB at the aqueous-LC interface. The error bars on the values of the tilt angles are smaller than the symbols.

Figure 5. Optical images (crossed polars) of (A) 5CB droplets and (B) 5% EG4-LC in 5CB droplets, both dispersed in phosphate buffer (pH 7.4). Scale bar, 10 μm . Cartoons in (C) and (D) show the bipolar and radial configurations of the LC droplets, respectively.

Figure 6.

Fluorescence intensities of fluorescently labeled BSA adsorbed at aqueous interfaces of either 5CB, OTS, 1% EG4-LC in 5CB, 2% EG4-LC in 5CB, 5% EG4-LC in 5CB, 10% EG4-LC in 5CB or PEG-treated glass (see text for details). Each interface was contacted with 10 μ M fluorescently labeled BSA (in phosphate buffer pH 7.4) for 2 hrs.

Figure 7.

(A) Molecular structure of $C_{14}E_4$. Optical images (crossed polars) of 5CB contained in gold grids supported on OTS-treated glass slides and placed into contact with aqueous $C_{14}E_4$ at concentrations of (B) 1 mM; (D) 2 mM and (F) 3 mM for 1 hr, and corresponding images (C, E, G) after contacting the interfaces with 10 μ M fluorescently labeled BSA solution for 2 hrs. Scale bar, 300 μ m. (See Figure S6 for color images)

Figure 8.

Fluorescence intensities of labeled BSA adsorbed at interfaces of either 5CB, OTS, 5CB decorated with C₁₄E₄ by incubation with 1 mM, 2 mM and 3 mM C₁₄E₄, or PEG-treated glass (see text for details). The intensities were measured after contact of each interface with 10 μ M fluorescently labeled BSA solution for 2 hrs.