Sensors

DOI: 10.1002/ange.201306630

Liquid Crystal Chemical Sensors That Cells Can Wear**

Uttam Manna, Yashira M. Zayas-Gonzalez, Rebecca J. Carlton, Frank Caruso, Nicholas L. Abbott, and David M. Lynn**

Micrometer-scale droplets of thermotropic liquid crystals (LCs) suspended in aqueous media can act as exquisitely sensitive reporters of environmental analytes.[1–8] Aqueous emulsions of the nematic LC 4-cyano-4′-pentylbiphenyl (5CB), for example, can quantify exposure to bacterial endotoxin (a key component of the outer membranes of Gram-negative bacteria and a major cause of disease and contamination) at pg mL^{-1} concentrations.[4] The interaction of amphiphilic species with LC droplets promotes changes in orientational order that can be detected as changes in optical appearance[2,8] that reflect both the concentration and structure of the analyte.[2,4] The speed and sensitivity with which these changes occur, combined with the ease with which they can be detected using optical methods (e.g., polarized light microscopy), provide new principles for the design of dispersed, droplet-based sensors that can report on the presence of chemical and biological agents in aqueous solutions.[1–8]

Here, we report the design of droplet-based LC sensors that can be immobilized directly on the surfaces of cells. We demonstrate that cells decorated with encapsulated LC droplets can report—in real-time and at the level of single droplets and individual cells—on the presence of toxic agents in surrounding media. This approach provides principles for the design of droplet-based LC sensors and methods for the local (μm scale) detection of agents in cellular environments in ways that are difficult to achieve in situ using free-floating LC droplets or other analytical methods. Our approach is based on the confinement of small droplets of nematic LC within covalently crosslinked, cell-adhesive polymer microcapsules.

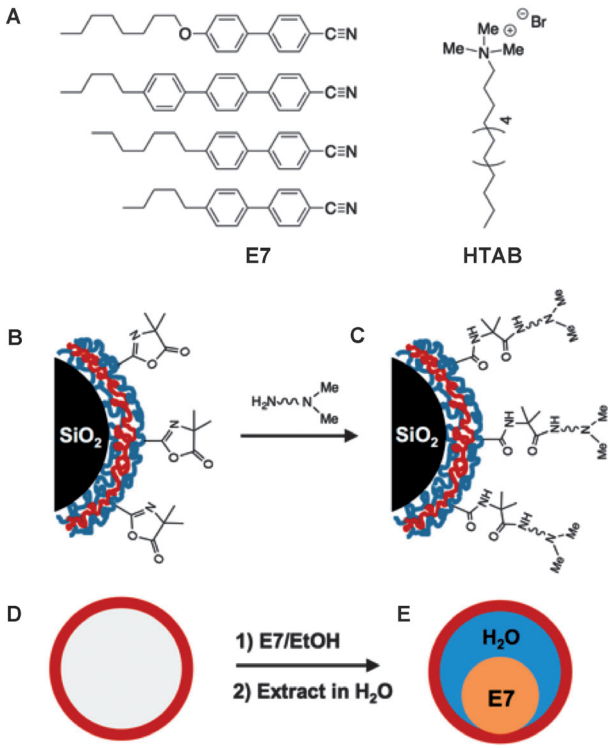

Figure 1. A) Structures of E7 and HTAB. B, C) Schematic showing a SiO_2 particle coated with PEI/PVDMA multilayers (B) and reaction of azlactones to yield amine-functionalized coatings (C). Etching of SiO_2 yields microcapsules (D). D, E) Infusion of an isotropic solution of E7 yields capsules (red) containing droplets of E7 (yellow) surrounded by water (blue; E).

Our design incorporates several features important for the manipulation and immobilization of LC droplets in cellular environments: 1) encapsulation of LCs in polymeric microcapsules provides means to control LC droplet size, 2) sequestration of LC in a protective membrane prevents LC droplets from coalescing or wetting other surfaces (e.g., culture dishes) and can insulate cells from direct contact with the LC, 3) polymer capsules can be decorated with functionality that can interact with cell membranes to anchor droplets in specific locations, and 4) the use of capsules with semipermeable walls can protect the LC from contact with macromolecular components of culture media, while allowing smaller analytes to pass through unhindered. The work reported here demonstrates proof of concept and underscores the utility of these design features in the context of cell-based sensing using several different well-defined model systems.

We selected the thermotropic LC known as E7 (Figure 1 A) here because it exhibits a nematic/isotropic transition temperature (ca. 60 °C) well above that used for mammalian

[*] U. Manna,[+] Y. M. Zayas-Gonzalez,[+] R. J. Carlton, Prof. N. L. Abbott, Prof. D. M. Lynn
Department of Chemical and Biological Engineering
University of Wisconsin—Madison
1415 Engineering Drive, Madison, WI 53706 (USA)
E-mail: abbott@engr.wisc.edu
dlynn@engr.wisc.edu
Prof. F. Caruso
Department of Chemical and Biomolecular Engineering
The University of Melbourne
Melbourne, Victoria 3010 (Australia)
[+] These authors contributed equally to this work.

[**] Financial support was provided by the NSF through a grant to the MRSEC at UW-Madison (DMR-1121288) and the Office of Naval Research (N00014-07-1-0255), and made use of NSF-supported shared facilities.

Supporting information (including a detailed description of experimental procedures) for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201306630.

cell culture (37 °C). We fabricated polymer-encapsulated droplets of LC by infusing E7 into covalently crosslinked microcapsules[9] fabricated by reactive layer-by-layer assembly of poly(ethyleneimine) (PEI) and the azlactone-containing polymer poly(4,4-dimethylazlactone) (PVDMA).[10] We used this approach, as opposed to capsules based on polyelectrolyte multilayers (PEMs)[1–3] because of the ease with which these reactive capsules can be functionalized after assembly.[9] We fabricated films composed of four PEI/PVDMA bilayers on monodisperse 5 μm SiO_2 spheres (Figure 1B; see Figure S1A in the Supporting Information for details). We estimate these films to be approximately 30 nm thick based on analysis of films fabricated on planar silicon.[11]

Past studies demonstrate that residual azlactone functionality in PEI/PVDMA films (Figure 1B) can be reacted with primary amine-containing compounds to install additional surface functionality after fabrication.[9–12] We treated our reactive film-coated SiO_2 particles with 3-dimethylaminopropylamine to install tertiary amine functionality (Figure 1C) to enhance colloidal stability and promote ionic interactions with the negatively charged surfaces of cell membranes. Subsequent etching of SiO_2 cores using HF (see Figure S1A)[1,3] yielded PEI/PVDMA microcapsules approximately 6.5 ± 0.2 μm in diameter (depicted in Figure 1D; see Figure S2 for additional characterization of coated particles and hollow capsules). The average diameter of these capsules was approximately 1.5 μm larger than that of the particle templates on which they were fabricated, suggesting that they swell in aqueous environments.

Infusion of an isotropic solution of E7 and EtOH (5%, v/v) into these PEI/PVDMA capsules, followed by evaporation of EtOH and extraction of capsules into water, yielded aqueous suspensions of capsules containing droplets of LC (Figure 1E; see also Figure S1). The image in Figure 2A shows LC-filled capsules suspended in cell culture media (DMEM, pH 7.4). Interestingly, these images reveal the capsules to be only partially filled with LC (also depicted in Figure 1E). This morphology differs from that reported in past studies on the encapsulation of the LC in PEM-based capsules (in those studies, capsules were completely filled with LC).[1–3] The reasons for this difference are not completely understood, but the morphology observed here appears to arise, at least in part, from the swelling of these PEI/PVDMA capsules on extraction into water rather than from underfilling during infusion or the loss of E7 upon extraction into water. The ζ potential of these LC-loaded capsules was 8.4 ± 1.7 mV (in PBS buffer; pH 7).

This procedure yields freely suspended polymer capsules that contain "caged" droplets of LC surrounded substantially by aqueous media. We note, however, that the LC droplets were always observed to be situated in the capsules asymmetrically, such that part of the droplets maintained physical contact with the walls of the capsules (Figures 1E and 2A), presumably a result of ionic interactions between the negatively charged droplets (ζ potential $= -35.5 \pm 1.7$ mV in PBS) and the walls of these amine-functionalized capsules. This configuration maintains, to a significant extent, the aqueous/LC interface characteristic of emulsions of bare (uncoated) LC droplets investigated in past studies,[3,4,8] while

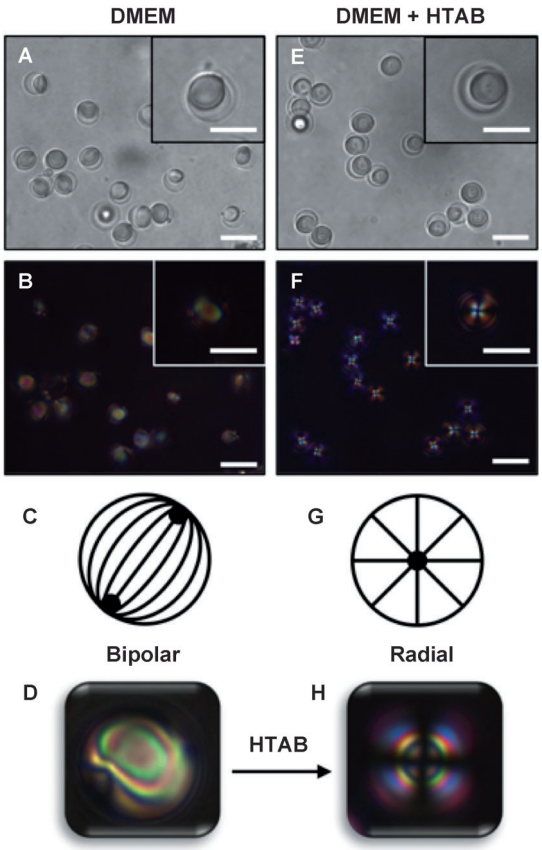

Figure 2. A, B; E, F) Bright-field and polarized light microscopy images of PEI/PVDMA capsules containing droplets of E7. A, B) Capsules suspended in DMEM. E, F) Capsules after addition of HTAB (10 μM). Images in (B) and (F) were imaged through crossed polarizers. Scale bars are 10 μm (5 μm for insets); polarizers were oriented parallel/perpendicular to scale bars. C, G) Director profiles for spherical LC droplets in bipolar (C) and radial (G) states. D, H) Polarized light microscopy images of bare (uncoated) droplets of E7 (approximately 5 μm) suspended in buffer (pH 7) before (D) and after (H) addition of HTAB. The droplet in (D) is in the bipolar state; the droplet in (H) is in the radial state.

preventing the physical contact of droplets with other droplets or surfaces. We demonstrate below that contact of the LC with capsule walls does not prevent the droplets from responding to the presence of analytes in ways that are similar to those of bare, free-floating droplets.

Characterization of the partially filled capsules shown in Figure 2A using polarized light microscopy revealed the LC droplets to exist in the so-called bipolar configuration (Figure 2B),[2,13–16] in which the director of the LC connects two point defects at opposite poles of a droplet (we note that the droplets in these capsules are slightly nonspherical in shape (Figure 2A); a schematic showing the director profile for a model spherical droplet is shown in Figure 2C, and additional interpretation of the optical appearances of these droplets is shown in Figure S3).[1,2,4,13–16] The characteristic optical appearance of these encapsulated bipolar droplets is similar to that observed for bare droplets of E7 (4–6 μm) suspended in aqueous media (Figure 2D).

Addition of the cationic amphiphile HTAB (Figure 1A; 10 μM) to these capsules resulted in an apparent decrease in

the area of contact between the LC droplets and the walls of the capsules (Figure 2E). Inspection of Figure 2F reveals that the addition of HTAB also resulted in a rapid change from the bipolar state to the so-called radial configuration[2,13–16] containing a defect at the core of the droplet (see Figure 2G). This change in orientation results in a change in the optical appearance of the droplets to the characteristic cross-like pattern[2,13–16] shown in Figure 2F when viewed between crossed polarizers. This bipolar-to-radial transformation is similar to that observed when HTAB is added to bare droplets of E7 (Figure 2H), and occurred in approximately 100% of encapsulated droplets at concentrations of HTAB at or above 10 μM. Below this concentration, droplets exhibited a mixture of bipolar and radial configurations, with some also exhibiting preradial configurations discussed in detail below. We conclude that HTAB is able to permeate rapidly through the walls of the capsules and trigger diagnostic bipolar-to-radial transformations in encapsulated LC droplets similar to those observed upon adsorption of HTAB to bare LC droplets.

We performed a series of experiments to determine whether these cationic LC-filled microcapsules could be immobilized on the surfaces of mammalian cells, and whether capsules fixed to cells could respond to and report on the presence of cytotoxic agents (illustrations of these concepts are shown in Figure 3A–C). We selected COS-7 cells for these proof-of-concept experiments because this cell type does not readily internalize particles of the size used in this study. LC-filled capsules placed into culture media sedimented onto cells over a period of 1 h (Figure 3D,E; additional fields of view are included in Figure S4). A live/dead assay indicated that LC-filled capsules were not substantially cytotoxic at levels up to 10^5 capsules per mL over this period (Figure S5)

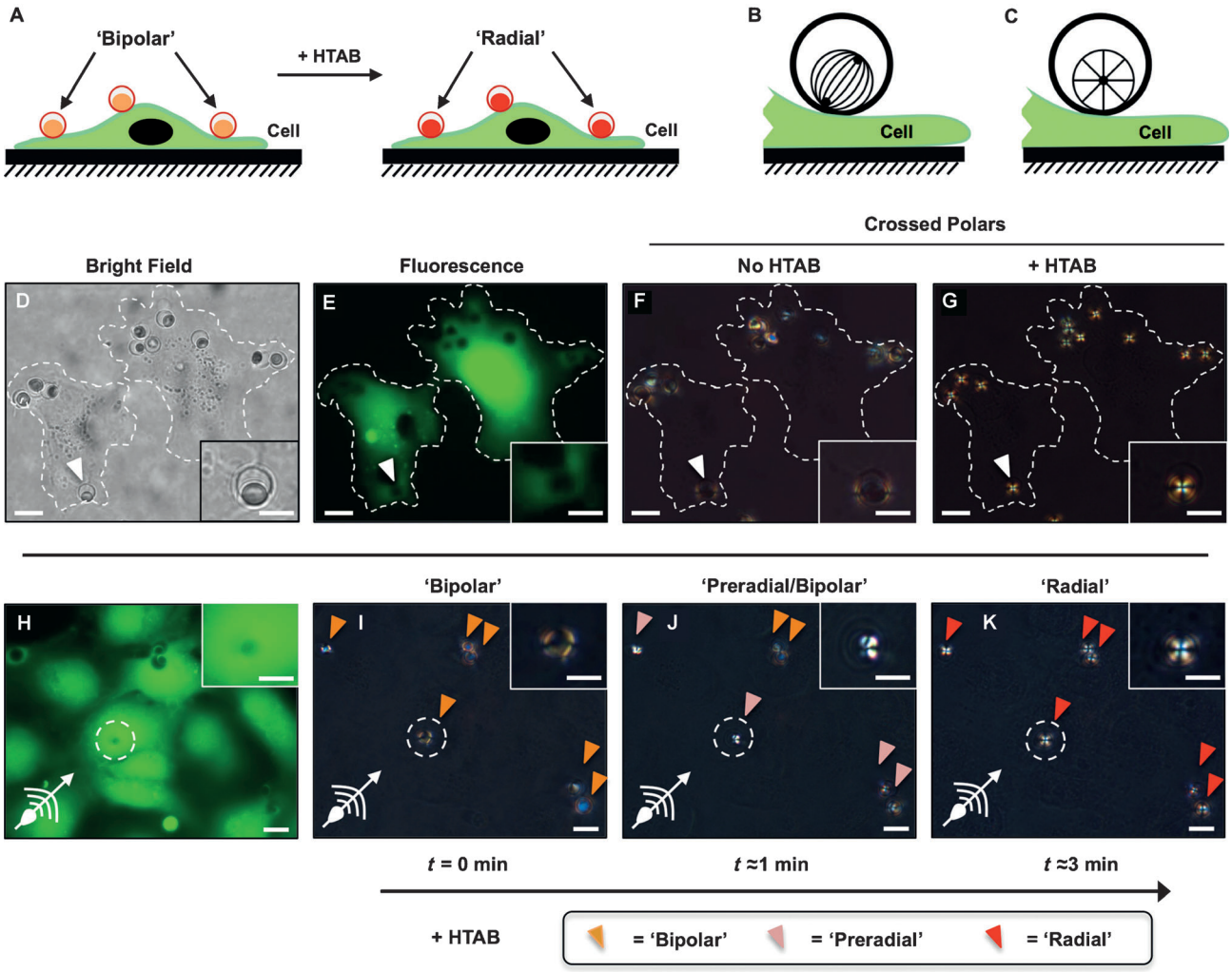

Figure 3. A) General concept: LC-filled capsules are anchored to cells; LC droplets are in bipolar states (left) until HTAB is added to promote a transition to radial states (right). B,C) Hypothetical director profiles of LC droplets in cell-associated capsules: (B) bipolar, (C) radial. D–G) Bright-field (D), fluorescence (E), and polarized light (F,G) microscopy images of cells decorated with LC-filled capsules. Cell-associated droplets in insets are indicated by white arrowheads; dotted lines indicate cell borders. Droplets were bipolar (F) before but transitioned to radial (G) after addition of HTAB. See Figure S4 for additional fields of view. H–K) Experiment in which HTAB was allowed to diffuse slowly across the well (direction indicated by arrows in lower left). I–K) Capsule-decorated cells 0, 1, and 3 min after addition of HTAB; the droplet in the inset is indicated by a dotted circle. Scale bars are 10 μm (5 μm for insets). For images in (F,G) and (I–K), polarizers were oriented parallel and perpendicular to scale bars.

or for up to 24 h (Figure S6). The majority of cells were associated with LC-filled capsules, with many associated with (or "wearing") multiple capsules (as many as 5–10 capsules/cell; e.g., Figure 3D,E). In experiments using bare (uncoated) droplets of E7, droplets were readily swept away by removing media and manually rinsing with buffer, thus indicating that they were not strongly bound to cells (Figure S7; bare droplets were also observed to wet and spread on the culture plates). In contrast, LC-filled capsules associated with cells were not readily removed from cell surfaces by manual rinsing, thus demonstrating that they were strongly attached (presumably through ionic interactions with negatively charged cell membranes).

Characterization of droplet-decorated cells using polarized light microscopy revealed LC droplets within cell-associated capsules to remain bipolar (Figure 3F,B and Figure S3). However, addition of HTAB (to a final concentration of 50 μM) 1 h after immobilization resulted in uniform and almost instantaneous (in approximately 1 s) transitions of cell-attached droplets from bipolar to radial states (Figure 3G,C) similar to those observed above for free-floating LC-filled capsules characterized above (Figure 2F). These results establish 1) that contact of the capsules with cell surfaces does not promote changes in the orientation of encapsulated LC, and 2) that droplet-decorated cells can be used to report, in real time, on the addition of a potentially harmful agent. Additional experiments demonstrated that LC droplets in both suspended and cell-attached capsules were bipolar in culture media containing serum (2%, v/v) and remained able to transition to radial states upon addition of HTAB (Figures S4 and S8). Bipolar-to-radial transitions were rapid under these conditions (< 1 min), but were slower than those observed in serum-free medium and required higher concentrations of analyte (e.g., 100 μM) to promote radial configurations in 100% of the droplets.

HTAB is a small-molecule surfactant that is toxic to cells. At high concentrations, HTAB disrupts cell membranes, and live/dead assays revealed the onset of substantial cytotoxicity after 1 h of exposure at concentrations as low as 100 μM (Figure S9). The results above demonstrate that cells decorated with LC-filled capsules can report on concentrations of HTAB at levels significantly lower than those that are cytotoxic under these conditions. The sensitivity of these cell-attached capsules and the speed with which they respond to the presence of added HTAB suggest potential utility as beacons that can rapidly identify and report on the presence of harmful amphiphilic agents in the immediate vicinity of cells before they reach toxic concentrations, or as a means to map or report in real time (and at the single-cell level) on the local concentrations of chemical agents in ways that are otherwise difficult to achieve in cell-culture-based experiments.

To demonstrate proof of the latter concept and investigate the potential of cell-immobilized LC droplets to provide real-time information on changes in the concentration of a toxic agent, we performed the set of experiments shown in Figure 3H–K. In the experiments described above (Figure 3D–G), a concentrated solution of HTAB was added to culture media and then rapidly mixed to achieve a desired concentration that was uniform across the culture well. For experiments shown in Figure 3H–K, a small aliquot of concentrated HTAB (10 mM) was carefully added at one edge of a culture well, and HTAB was allowed to diffuse slowly across it; LC-decorated cells far removed from the location at which HTAB was added were then monitored to characterize time-dependent changes in the orientational order of the LC. The images in Figure 3H,I show cells immediately after addition of HTAB, and reveal all droplets in the field of view (Figure 3I) to exist in bipolar configurations (see also Figure S3). The image in Figure 3J shows the same cells approximately 1 min after the addition of HTAB. This image reveals a change in the configuration of several (but not all) cell-associated LC droplets in this region from bipolar to the so-called preradial configuration.[2,15,16]

The preradial configuration has a characteristic appearance arising from a director that radiates outward from a single point defect displaced from the center of the droplet (in contrast to the profile for the radial configuration in Figure 2G).[2,15,16] This shift from bipolar to preradial is observed at concentrations of adsorbate below those needed to promote transitions to a radial configuration; as a result, addition of higher concentrations of adsorbate can push preradial droplets into radial states.[2] The presence and relative locations of the preradial droplets in Figure 3J are consistent with a time-dependent increase in the concentration of HTAB as it diffuses across the culture well. This view is further supported by results shown in Figure 3K (acquired approximately 3 min after the addition of HTAB), which reveal all droplets to transform to radial states at longer times. These results demonstrate that LC droplets immobilized on cells can be used to report changes in chemical gradients in cell culture environments (on μm scales and at the level of individual droplets and cells). This feature could prove useful, with further development, as a tool for the real-time correlation of changes in local cell behavior to the presence, arrival, or increase in concentration of bioactive substances that cannot be readily detected, visualized, imaged, or mapped using other analytical methods in cell culture environments.

The experiments above demonstrate basic principles and highlight important features of this encapsulated/immobilized LC droplet system using a model capsule design and a model analyte. We anticipate, however, that these cell-adhesive capsules can be adapted to detect the presence of more complex analytes of commercial and health-related importance. To this end, we note that initial experiments reveal these capsules to transform rapidly to optically distinguishable states upon exposure to bacterial endotoxin (lipopolysaccharide; Figure S10). We also anticipate that the principles reported here, demonstrated in the context of cell-based sensing, could also be useful for immobilization of LC-based droplets on other organic and inorganic surfaces. Finally, we note that microscale objects that can be attached to living cells are also useful in other contexts.[17,18] Studies to exploit the properties of these LC-filled capsules in these and other applied contexts are currently underway.

Received: July 29, 2013
Revised: September 30, 2013
Published online: November 29, 2013

Keywords: azlactone · cells · liquid crystals · multilayers · polymers

[1] J. K. Gupta, S. Sivakumar, F. Caruso, N. L. Abbott, *Angew. Chem.* **2009**, *121*, 1680 – 1683; *Angew. Chem. Int. Ed.* **2009**, *48*, 1652 – 1655.
[2] J. K. Gupta, J. S. Zimmerman, J. J. de Pablo, F. Caruso, N. L. Abbott, *Langmuir* **2009**, *25*, 9016 – 9024.
[3] S. Sivakumar, K. L. Wark, J. K. Gupta, N. L. Abbott, F. Caruso, *Adv. Funct. Mater.* **2009**, *19*, 2260 – 2265.
[4] I. H. Lin, D. S. Miller, P. J. Bertics, C. J. Murphy, J. J. de Pablo, N. L. Abbott, *Science* **2011**, *332*, 1297 – 1300.
[5] V. J. Aliño, J. Pang, K. L. Yang, *Langmuir* **2011**, *27*, 11784 – 11789.
[6] W. Khan, J. H. Choi, G. M. Kim, S. Y. Park, *Lab Chip* **2011**, *11*, 3493.
[7] T. Bera, J. Y. Fang, *J. Mater. Chem.* **2012**, *22*, 6807 – 6812.
[8] D. S. Miller, N. L. Abbott, *Soft Matter* **2013**, *9*, 374 – 382.
[9] E. M. Saurer, R. M. Flessner, M. E. Buck, D. M. Lynn, *J. Mater. Chem.* **2011**, *21*, 1736 – 1745.
[10] M. E. Buck, D. M. Lynn, *ACS Appl. Mater. Interfaces* **2010**, *2*, 1421.
[11] M. E. Buck, J. Zhang, D. M. Lynn, *Adv. Mater.* **2007**, *19*, 3951 – 3955.
[12] M. E. Buck, A. S. Breitbach, S. K. Belgrade, H. E. Blackwell, D. M. Lynn, *Biomacromolecules* **2009**, *10*, 1564 – 1574.
[13] R. Ondris-Crawford, E. P. Boyko, B. G. Wagner, J. H. Erdmann, S. Zumer, J. W. Doane, *J. Appl. Phys.* **1991**, *69*, 6380 – 6386.
[14] P. S. Drzaic, *Liquid Crystal Dispersions*, World Scientific Publishing, Singapore, **1995**.
[15] O. O. Prishchepa, A. V. Shabanov, V. Y. Zyryanov, *Phys. Rev. E* **2005**, *72*, 031712.
[16] D. S. Miller, R. J. Carlton, P. C. Mushenheim, N. L. Abbott, *Langmuir* **2013**, *29*, 3154 – 3169.
[17] D. B. Weibel, P. Garstecki, D. Ryan, W. R. Diluzio, M. Mayer, J. E. Seto, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11963 – 11967.
[18] A. J. Swiston, C. Cheng, S. H. Um, D. J. Irvine, R. E. Cohen, M. F. Rubner, *Nano Lett.* **2008**, *8*, 4446 – 4453.

