

Supplementary Information

Interfacing Biodegradable Molecular Hydrogels with Liquid Crystals

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S1: Materials and Methods

1,2-Dilauroyl-sn-glycero-3-phosphocholine (12:0 PC; DLPC) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Sulfuric acid, methanol, ethanol, chloroform, dichloromethane, and sodium chloride were obtained from Fischer Scientific (Loughborough, Leicestershire, England). Hydrogen peroxide (30 % w/v), heptane, 4'-pentyl-4-biphenylcarbonitrile (5CB, nematic, 95%), silicone isolators (press-to-seal; 1 well, 2.5 mm x 20 mm; silicone/adhesive), subtilisin (protease from *Bacillus licheniformis*, product code P4860), chymotrypsin (α -chymotrypsin from bovine pancreas; ≥ 40 units/mg protein), potassium chloride (99.0 %), and glucose ($C_6H_{12}O_6$; α -D-glucose waterfree) were from Sigma-Aldrich (Gillingham Dorset, England). Fmoc-Thr-OH monohydrate, L-leucine methyl ester hydrochloride (L-OMe-HCl, ≥ 99 %) and thermolysin from *Bacillus thermoproteolyticus rokko* were obtained from Fluka Chemicals through Sigma-Aldrich (Gillingham Dorset, England). n-Octadecyltrichlorosilane (OTS; 95 %) was obtained from Alfa Aesar (Heysham, Lancashire, United Kingdom). Potassium dihydrogen phosphate was obtained from Lancaster Synthesis Ward Hill, MA, USA). Monosodium phosphate (sodium phosphate monobasic) was obtained from Fisons Laboratory Reagent (Leicestershire, England). Disodium phosphate (sodium phosphate, dibasic) was obtained from BDH Laboratory Reagent (Poole, England). Elastase (97 % protein) was obtained from Worthington Biochemical Corporation (Lakewood, NJ, USA). All chemicals were used as obtained. Coverslip glass slide with dimension 22 x 50 mm was obtained from Menzel-Glaser, Gerhard Menzel, Glasbearbeitungswerk GmbH & Co. KG (Braunschweig, Germany). Copper TEM grids (G100-Cu; 18 μ m thickness, 45 μ m wide bars, and 205 μ m grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA, USA). Glass based dish (35 mm petri dish with 12 mm glass base; sterile) was obtained from IWAKI, Asahi glass Co. Ltd (Tokyo, Japan). A distilled water source was used for all experiments.

S1.1 Preparation of Aqueous Dispersions of DLPC.

Dispersions of DLPC were prepared following published procedures with minor modifications.¹ Briefly, DLPC powder was dissolved in chloroform and dispensed into glass vials. The solvent chloroform was evaporated under a stream of N_2 , and the vial containing the lipids was then placed under vacuum for at least 2 hours. The dried lipid was resuspended in a buffer solution of interest overnight. This procedure resulted in a slightly cloudy solution. Subsequent sonication (Tip sonicator from BandelinSonopuls HD3200; 40 % power, 15 second sonication and 15 second pulse for 5 minute total, sample was kept in

water bath to prevent overheating) resulted in a clear solution. The vesicle solution was typically used within 1 week after its preparation. The final concentration of DLPC vesicles was 500 μM .

S1.2 Preparation of Fmoc-TL-OMe hydrogel with or without DLPC incorporation.

Fmoc-TL-OMe hydrogels were prepared according to a previously published method.² In brief, Fmoc-T-OH monohydrate (6.83 mg, 0.02 mmol), L-OMe•HCl (14.44 mg, 0.08 mmol) and thermolysin (0.5 mg, 36.5 units/mg) were mixed in 1 mL 100 mM pH 8 phosphate buffer (0.964 g monosodium phosphate and 2.497 g disodium phosphate in 100 mL distilled water, adjusted pH to 8). The mixture was vortex mixed for 30 seconds at maximum strength (vortex mixer (Whirlimixer) from Fisherbrand) and then sonicated for 1 minute in a water bath (Sonicator from CAMLAB Serving Science, Transsonic T460). Gelation occurred at room temperature overnight. The resulting Fmoc-TL-OMe hydrogel was semi-transparent in appearance. For Fmoc-TL-OMe hydrogel with DLPC incorporated, the same preparation procedure was followed as for Fmoc-TL-OMe hydrogel preparation. However, 1 mL 500 μM DLPC vesicular dispersion (in 100 mM pH 8 phosphate buffer) was substituted for 1 mL pH 8 phosphate buffer.

S1.3 Preparation of Optical Cells and LC observations

Detailed descriptions of procedures used to prepare LC-filled grids can be found in past publication from Brake and Abbott.³ Briefly, coverslip slides were cleaned by piranha solution and coated with OTS. The quality of the OTS layer was assessed by checking the alignment of 5CB confined between two OTS-coated coverslip slides. Any surface not causing homeotropic anchoring (perpendicular alignment of 5CB) of 5CB was discarded. Copper specimen grids that were used as obtained and were placed onto the surface of OTS-coated coverslip slides. Approximately 1 μL of 5CB was dispensed onto each grid and then excess LC was removed by contacting a capillary tube with the droplet of 5CB. Each LC-filled grid was equilibrated at ambient temperature and subsequently contact with the hydrogel solution or hydrogel solid block of interest at ambient temperature.

Optical cells were prepared as follows (**Figure S1**). A press-to-seal orange silicone isolator was attached to the center of a glass-based plastic petri dish. The center coverslip within the Petri dish allowed further microscopic measurement as the coverslip did not possess birefringence property. The silicone isolator was used to confine the hydrogel/solution total volume up to maximum ~ 1 mL. Subsequently, the LC sample supported on an OTS-coated glass slide was placed in the center of the optical cell. Hydrogel solution (125 μL) was then added onto the top of the LC interface and gelation was allowed to occur overnight at room temperature. Second hydrogel solution (125 μL ; with DLPC incorporation) was

further added onto the first gelled hydrogel; the gelation was also allowed to occur overnight. During the overnight gelation, the optical cell was covered by a lid and sealed by Parafilm to prevent solution evaporation.

Over the top of these layered hydrogel/LC chambers was added the appropriate solution (750 μL): subtilisin (0.15 μM , 0.03 units/mL in 100 mM pH 8 phosphate buffer, diluted 1 in 100 from 15 μM stock solution); elastase (1 μM in 100 mM pH 6 phosphate buffer, 0.1 mg in 4 mL buffer); glucose (10 mM in 100 mM pH 8 phosphate buffer); 100 mM pH 8 phosphate buffer; 100 mM pH 6 phosphate buffer; pH 7.4 PBS buffer. Each solution was held within the silicone gasket.

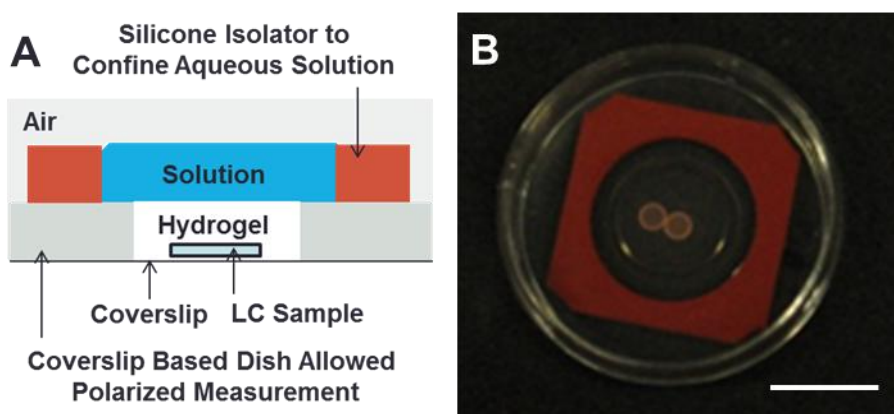


Figure S1. Preparation of the optical cells. (A) Schematic illustration and (B) photograph of the optical cell used in this study. The center-hollowed glass based Petri dish is used to facilitate the polarized microscope measurement. LC sample supported on an OTS-coated coverslip was placed in the center of the Petri dish. The diameter of the center glass coverslip is 12 mm. The small well can hold up to $\sim 280 \mu\text{L}$ solution with a convex meniscus. The silicone gasket is used to confine the total hydrogel/aqueous solution volume up to 1 mL used in the system. Scale bar is 1 cm.

The alignment of 5CB was observed by using a Zeiss Imager A1 microscope with crossed polarizers (transmission mode). Optical cell with LC specimens were placed on a stage located between the polarizers and imaged with LC interface facing up. Orthoscopic examination is performed with the source light intensity set to 3200k, the 2-position filter wheels set to 6 and 50, and the iris set to 0.2 for 10 \times objective so as to collimate the incident light. Homeotropic alignments of the LC are determined by first observing the absence of transmitted light during a manual 360° rotation of the sample at the specimen stage.³

To differentiate homeotropic-aligned LC anchoring and LC-dewetting phenomena (loss of some LC to give a e.g. half-full or empty grid) within the TEM grid wells without a Bertrand lens, a brightfield condenser is swiveled in to increase the image contrast, the LC thin film with homeotropic anchoring therefore reveals gray hue against the background, where the LC absence area appears pure black hue.

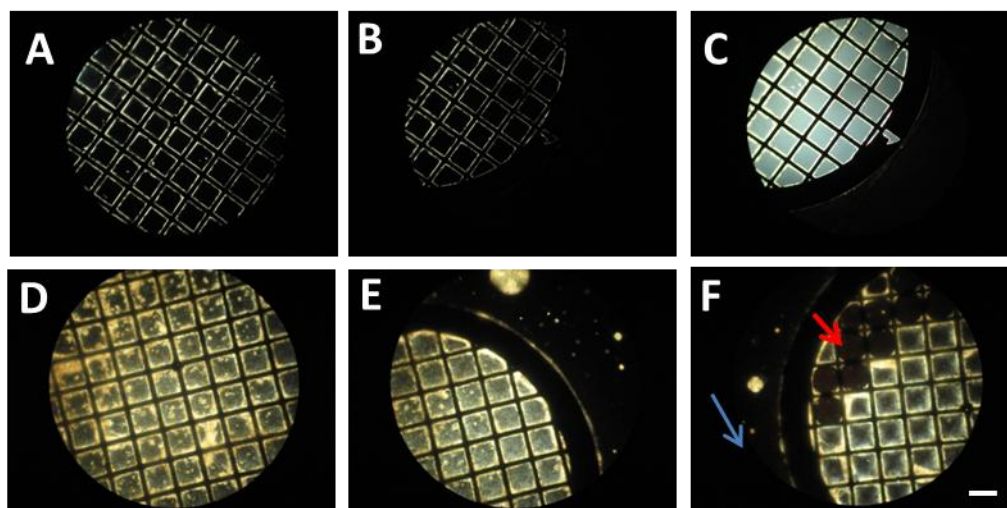


Figure S2. Optical examination of liquid crystal with homeotropic alignment. (A-C) 5CB-filled specimen supported on an OTS-coated glass slide exposed to air; a LC homeotropic anchoring is adopted. (A and B) Optical images taken without a brightfield condenser swiveled in; (C) image taken with a condenser swiveled in. (D-F) DLPC-adsorbed LC-aqueous interface. (D and E) optical images taken without a brightfield condenser swiveled in. (F) Image taken with a condenser swiveled in; this image demonstrates the optical appearance of LC with homeotropic anchoring, LC dewetted phenomena (red arrow), and LC absence area (blue arrow) under the polars. Scale bar is 200 μm .

Figure S2A shows an optical image of a 5CB-filled TEM grid supported on an OTS-coated glass slide and exposed to air under the orthoscopic examination. The OTS monolayer and air boundary conditions dictate the LC to adopt homeotropic alignment through the $\sim 20 \mu\text{m}$ thin film and reveals uniform black hue. **Figure S2B** reveals an optical image of the same sample near the TEM grid edge. Without a brightfield condenser to enhance the light intensity, uniform black hue is both observed within and outside the TEM grid wells. (**Figure S2C**) Optical image of the same sample with a brightfield condenser swiveled in to enhance the image contrast, the LC with homeotropic alignment therefore reveals gray hue against the black background, where non-birefringent materials exist. (**Figure S2D to F**) Optical images of LC homeotropic anchoring at the DLPC-adsorbed LC-aqueous interface with a brightfield condenser swiveled in. (**Figure S2F**) Optical image of 5CB anchoring under the same experimental

condition as mentioned in **Figure S2D**; a brightfield condenser is used here to enhance image contrast. This image demonstrates the optical appearance of LC with homeotropic anchoring, LC dewetted phenomena (red arrow), and LC absence area (blue arrow) under the polars. These images, when combined, confirm the uniform black hue shown in **Figures S2A to S2F** results from homeotropic anchoring and not an artifact. Images were captured with a microscope-mounted digital camera (Canon Power Shot G6) set to an f-stop of 3.5, ISO 200 and a shutter speed of 1/30 s or 1/25s.

S1.4 Fluorescence emission spectroscopy

Fluorescence emission spectra were collected on a Perkin-Elmer LS55 fluorimeter. The hydrogels (1 mL) were prepared in cuvettes (Fisher Scientific, CX1-110-005J) and the emission spectra were recorded between 300 and 600 nm after excitation at 295 nm. Enzyme solutions (3 mL) were added, the mixtures incubated at 20 °C and changes in emission recorded after 24 hours.

S1.5 HPLC analysis

Aliquots (30 µL) of pre-gelation solutions were added to Eppendorf tubes, and left to gel overnight. The enzyme solutions (90 µL, as described previously) were added to the gel samples and the digests were incubated at 20 °C before quenching at the desired time points by the addition of HPLC solution (1 mL (1:1 v:v water:acetonitrile with 0.1 % TFA)). The sample was sonicated to ensure complete dissolution then analysed by HPLC, which was carried out on a Dionex P680 HPLC system fitted with a UVD170U detector. An aliquot of sample (10 µL) was injected into a Macherey-Nagel C18 column of the following dimensions: length 250 mm; internal diameter 4.6 mm; particle size 5 µm; flow rate, 1 mL min⁻¹. The gradient used was a linear exchange between 20 % acetonitrile in water at 5 min to 80 % acetonitrile/water at 35 min. Fmoc-peptides were identified by comparison with known retention times.

S1.6 AFM analysis

Trimmed mica sheets were adhered to AFM support stubs using clear nail varnish. Clean mica surfaces were prepared by removing the top layer of mica with adhesive tape immediately before sample deposition. The Fmoc-peptide containing gelation solutions (50 µL) were deposited onto the mica covered slides, the slides were kept in a wet chamber until gelation had taken place. Next, either phosphate buffer (50 µL, 100 mM, pH 8) or enzyme solution (50 µL) was added to the top of the gel and

allowed to digest for four hours, the slides were kept in a wet chamber during this period to prevent drying out. The samples were then washed with water (10 x 100 μL) and allowed to air dry overnight.

Samples were imaged by intermittent contact mode in air using a Veeco Multimode atomic force microscope with a Nanoscope IIIa controller and an 'E' scanner. Imaging was performed using high-aspect-ratio etched silicon probes (OTESPA -Veeco Instruments S.A.S.) with nominal spring constant of 42 N m⁻¹. Cantilever oscillation varied between 300 and 350 kHz while the drive amplitude was determined by the Nanoscope software. The amplitude setpoint was adjusted to just below the point at which tip-sample interaction was lost. Height and phase images with scan sizes of 5 μm \times 5 μm were captured at a scan rate of 1.97 Hz and at a relative humidity of < 40 %. Data were first-order flattened using the Nanoscope software before image export and the instrument was periodically calibrated using a grating with 180 nm deep, 10 mm² depressions.

S2: LC anchoring during Fmoc-TL-OMe hydrogel formation

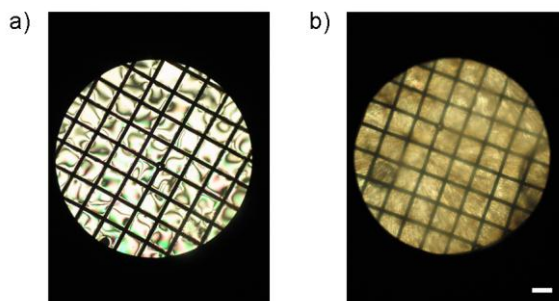


Figure S3: Optical images of 5CB anchoring at 5CB-aqueous interfaces (a) immediately after Fmoc-T-OH/L-OMe/thermolysin mixture addition and (b) after gelation (image take after 24 h at room temperature). The 5CB reveals planar alignment is maintained both before and after gelation. Scale bar is 200 μm .

S3: LC anchoring during Fmoc-TL-OMe/DLPC hydrogel formation

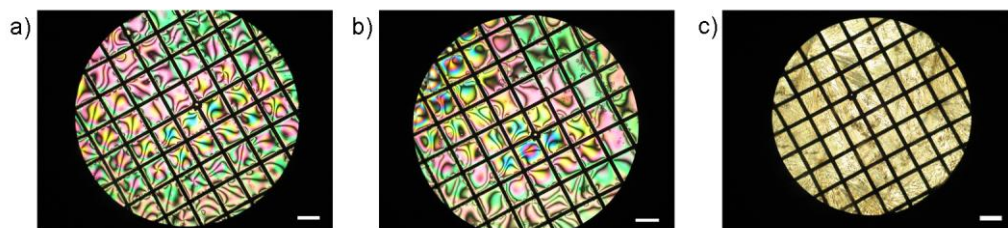


Figure S4. Optical polarized images of 5CB LCs in contact with Fmoc-T-OH/L-OMe/thermolysin/DLPC solution at the 5CB interface. After (a) 2 minutes contact with Fmoc-T-OH/L-OMe/thermolysin/DLPC solution; (b) 20 minutes contact with Fmoc-T-OH/L-OMe/thermolysin/DLPC; (c) after overnight (~24 h) incubation, 5CB now in contact with Fmoc-TL-OMe/DLPC hydrogel formed *in situ*. Scale bar is 200 μm .

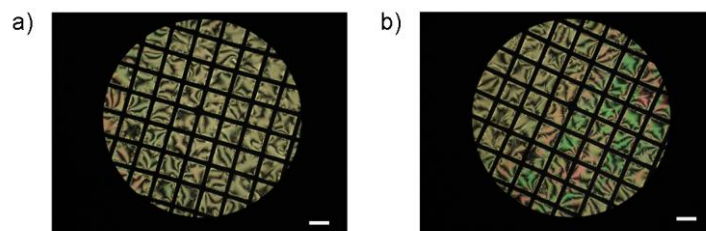


Figure S5. DLPC liberation from Fmoc-TL-OMe/DLPC hydrogel. The Fmoc-TL-OMe or Fmoc-TL-OMe/DLPC hydrogel was incubated in pH 8 phosphate buffer for 24 h at room temperature. Supernatant of the mixture was removed and LC samples were immersed into the supernatant. (a) Optical image of 5CB anchoring upon contact the supernatant of Fmoc-TL-OMe gel for 48 h at room temperature. (b) Optical image of 5CB anchoring upon contact the supernatant of DLPC-containing Fmoc-TL-OMe gel for 48 h at room temperature. Scale bar is 200 μm

DLPC liberation from Fmoc-TL-OMe/DLPC hydrogels: LC images in Figure S5(a) and (b) both reveal 4th to 5th interference colors after 48 hour incubation, suggesting planar anchoring of the LCs. These results suggest the trace DLPC-leakage does not lead to LC planar-to-homeotropic anchoring transition; the LC planar anchoring preserves as the DLPC is mainly held within the Fmoc-TL-OMe structure. Combined with experimental results discussed in the main text, these results imply the 5CB planar-to-homeotropic anchoring transition upon specific enzyme-induced DLPC-containing-Fmoc-peptide-gel-digestion is not resulted from the spontaneous DLPC leakage.

S4: Reproducibility of optical observations on LCs

To the Fmoc-TL-OMe dual hydrogel/LC system (prepared as described in Sections S1.1 to 1.3) were added and optical observations made during 24 h:

Phosphate buffer (pH 6): 5 of 5 TEM grids preserved planar LC anchoring

Subtilisin: 5 of 6 TEM grids revealed an LC planar anchoring transition

Elastase: 5 of 5 TEM grids preserved planar LC anchoring

Elastase + subtilisin: 8 of 10 TEM grids revealed an LC planar anchoring transition

Glucose: 5 of 5 TEM grids preserved planar LC anchoring

Glucose + subtilisin: 5 of 5 TEM grids revealed an LC planar anchoring transition

PBS buffer: 5 of 5 TEM grids preserved planar LC anchoring

PBS buffer + subtilisin: 5 of 5 TEM grids revealed an LC planar anchoring transition

S5: Fluorescence spectroscopy and HPLC data for Fmoc-TL-OMe incubated with pH 8 buffer

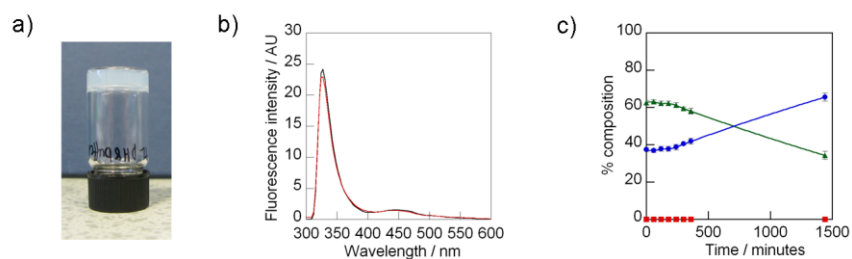


Figure S6. (a) Images of a vial inversion test showing the Fmoc-TL-OMe hydrogel remains intact after incubation with pH 8 buffer. (b) Changes in the fluorescence spectrum of Fmoc-TL-OMe hydrogels both before (—) and after the addition of pH 8 buffer (---). (c) HPLC analysis of the products from the incubation of Fmoc-TL-OMe hydrogel with pH 8 buffer: Fmoc-TL-OMe (■); Fmoc-TL-OH (●); Fmoc-T-OH (▲). Curve fits are to guide the eye.

S6: LC observations for optical cells incubated with pH 8 phosphate buffer, Fmoc-TL-OMe, subtilisin, Fmoc-T and L-OMe.

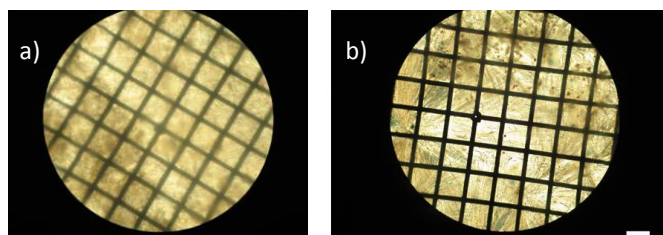


Figure S7. Optical polarized images of 5CB LCs in contact with Fmoc-TL-OMe hydrogel after incubation in pH 8 phosphate buffer for (a) 0 h and (b) 24 h at room temperature. Each grid is 205 μm wide.

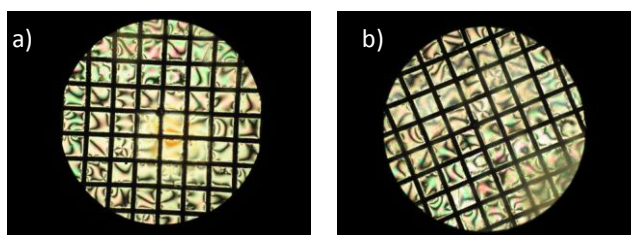


Figure S8. Optical polarized images of 5CB LCs in contact with subtilisin (0.15 μM) after incubation in pH 8 phosphate buffer for (a) 0 h and (b) 24 h at room temperature. Each grid is 205 μm wide.

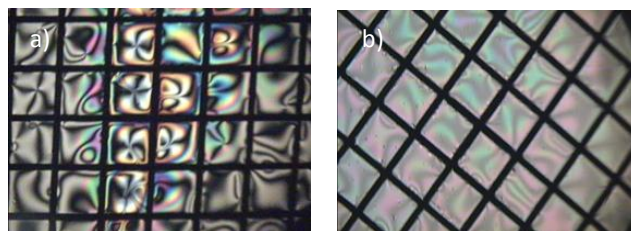


Figure S9. Optical polarized images of 5CB LCs in contact with Fmoc-T (2 mM) after incubation in pH 12 phosphate buffer for (a) 0 h and (b) 8 days at room temperature. Sample in pH 12 buffer due to low solubility at pH 8. Each grid is 205 μm wide.

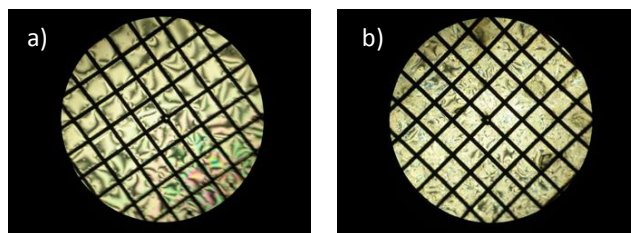


Figure S10. Optical polarized images of 5CB LCs in contact with L-OMe (80 mM) after incubation in pH 8 phosphate buffer for (a) 0 h and (b) 8 days at room temperature. Each grid is 205 μm wide.

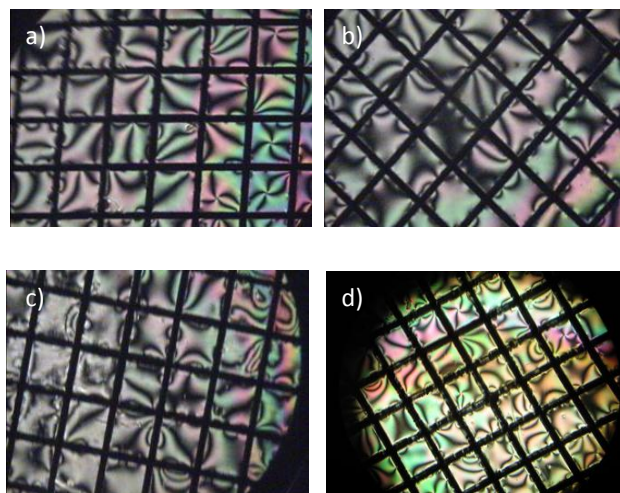


Figure S11. Optical polarized images of 5CB LCs in contact with (a) 2 mM Fmoc-F, (b) 2 mM Fmoc-A, (c) 2 mM Fmoc-AA, and (d) Fmoc-K after 24 h incubation at room temperature. Each grid is 205 μm wide.

S7: Fluorescence spectroscopy/vial inversion tests for Fmoc-TL-OMe hydrogel incubated with pH 6 buffer and HPLC data for Fmoc-TL-OMe hydrogel incubated with subtilisin at pH 6.

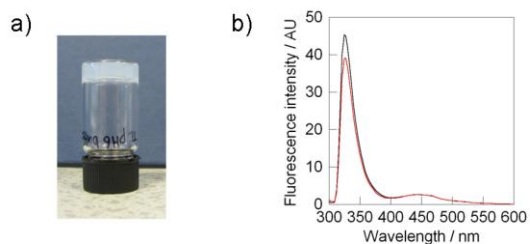


Figure S12. (a) Images of a vial inversion test showing the Fmoc-TL-OMe hydrogel remains intact after incubation with pH 6 buffer. (b) Changes in the fluorescence spectrum of Fmoc-TL-OMe hydrogels both before (—) and after the addition of pH 6 buffer (—).

After incubation of the Fmoc-TL-OMe hydrogel with subtilisin at pH 6, HPLC analysis showed the following product ratios: Fmoc-T = 46.4%, Fmoc-TL-OH = 53.6%, Fmoc-TL-OMe = 0% after 6 h (subtilisin at pH 8 gave Fmoc-T = 64.7%, Fmoc-TL-OH = 35.4%, Fmoc-TL-OMe = 0%). After incubation of the Fmoc-TL-OMe hydrogel with subtilisin for 24 h, the product ratios were Fmoc-T = 93.8%, Fmoc-TL-OH = 6.2%, Fmoc-TL-OMe = 0% (subtilisin at pH 8 gave Fmoc-T = 93.3%, Fmoc-TL-OH = 6.7%, Fmoc-TL-OMe = 0%).

S8: References

¹ J. M. Brake, M. K. Daschner and N. L. Abbott, *Langmuir*, **2005**, *21*, 2218–2228.

² M. Hughes, P. W. J. M. Frederix, J. Raeburn, L. S. Birchall, J. Sadownik, F. C. Coomer, I-H. Lin, E. J. Cussen, N. T. Hunt, T. Tuttle, S. J. Webb, D. J. Adams and R. V. Ulijn, *Soft Matter* **2012**, *8*, 5595-5602.

³ J. M. Brake and N. L. Abbott, *Langmuir*, **2002**, *18*, 6101–6109.