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Hierarchical Assemblies of Soft Matters From Polymers and Liquid Crystals on Structured Surfaces

Apiradee Honglawan
University of Pennsylvania, apih@seas.upenn.edu

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Hierarchical Assemblies of Soft Matters From Polymers and Liquid Crystals on Structured Surfaces

Abstract
Hierarchical, multifunctional materials hold important keys to numerous advanced technologies, including electronics, optics, and medicine. This thesis encompasses generation of hierarchical structures with novel morphologies and functions through self-assembly directed by lithographically fabricated templates. Here, two soft materials, amphiphilic random copolymers of photopolymerized acryloyl chloride (ranPAC) and smectic-A liquid crystal (SmA-LC) molecule, 4′(5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heptadecafluorododecyloxy)-biphenyl-4-carboxylic acid ethyl ester, are synthesized as model systems to investigate the governing principles at the topographic surface/interface.

The ranPAC can self-organize into nanomicelles with high regularity and stability, typically not possible in random copolymer systems. The morphology can be controlled by the photopolymerization conditions and solvent; the crosslinked shell makes the micelles robust against drying and storage. Using SU-8 micropillar arrays with spatially controlled surface chemistry as templates, we construct hierarchical microporous structures with tunable pore size and symmetry (e.g. square array), and uncover a new evaporative assembly method. By functionalizing the ranPAC nanovesicles with cationic poly(ethyleneimines), we encapsulate the anticancer drug, doxorubicin hydrochloride, and mRNA at a high payload, which are delivered to HEK 293T cells in vitro at a low cytotoxicity level.

SmA-LC are characterized by arrangement of molecules into thin layers with the long molecular axis parallel to the layer normal, forming a close-packed hexagonal array of topological defects known as focal conic domains (FCDs) in a thin film. Using a series of SU-8 micropillar arrays with different size, shape, height, and symmetry as topological templates, we investigate the epitaxial and hierarchical assemblies of FCDs; whether the system favors confinement or "pillar edge-pinning" depends on balance of the elastic energy of LCs and the surface energy imposed by the template. The conservation of toric FCD (TFCD) textures over large LC thickness manifests a remarkably unique outcome of the epitaxial growth of TFCDs. On shorter pillars, however, the system favors the "pinning" of FCD centers near pillar edges while avoiding the opposing effect of confinement, leading to the break of the underlying symmetry of the pillar lattice, exhibiting tunable eccentricity, and a nontrivial yet organized array of defects balancing the elastic energy of LCs and the surface energy imposed by the template.

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HIERARCHICAL ASSEMBLIES OF SOFT MATTERS FROM POLYMERS AND LIQUID CRYSTALS ON STRUCTURED SURFACES

Apiradee Honglawan

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Supervisor of Dissertation

__________________________

Shu Yang, Ph.D.
 Associated Professor, Materials Science and Engineering (MSE), Chemical and Biomolecular Engineering (CBE)

Graduate Group Chairperson

__________________________

Raymond J. Gorte, Russell Pearce and Elizabeth Crimian Heuer Professor, CBE

Dissertation Committee

Russell J. Composto, Ph.D. Daeyeon Lee, Ph.D.
Professor, MSE Assistant Professor, CBE

Kathleen J. Stebe, Ph.D., Richer and Elizabeth Goodwin Professor of Engineering and Applied Science, CBE; Deputy Dean for Research, SEAS
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arrays with different size, shape, height, and symmetry as topological templates, we investigate
the epitaxial and hierarchical assemblies of FCDs; whether the system favors confinement or
“pillar edge-pinning” depends on balance of the elastic energy of LCs and the surface energy
imposed by the template. The conservation of toric FCD (TFCD) textures over large LC thickness
manifests a remarkably unique outcome of the epitaxial growth of TFCDs. On shorter pillars,
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lattice, exhibiting tunable eccentricity, and a nontrivial yet organized array of defects balancing
the elastic energy of LCs and the surface energy imposed by the template.
# TABLE OF CONTENTS

ACKNOWLEDGMENT .............................................................................................................................II

ABSTRACT ............................................................................................................................................... III

LIST OF TABLES ................................................................................................................................... VII

LIST OF ILLUSTRATIONS ................................................................................................................ VIII

PREFACE ................................................................................................................................................. XX

CHAPTER 1 ................................................................................................................................................ 1

FACILE SYNTHESIS OF NANOPARTICLES VIA ASSEMBLY OF PHOTOPOLYMERIZED
AND SELF-CROSSLINKED RANDOM COPOLYMERS IN SELECTIVE ORGANIC MEDIA 1

Supporting Information ...........................................................................................................................25

CHAPTER 2 ............................................................................................................................................. 31

EVAPORATIVE ASSEMBLY OF ORDERED MICROPOROUS FILMS AND THEIR
HIERARCHICAL STRUCTURES FROM AMPHIPHILIC RANDOM COPOLYMERS ........ 31

Supporting Information ...........................................................................................................................56
CHAPTER 3 ............................................................................................................................................. 58

DESIGN FOR MULTIFUNCTIONAL NANOPARTICLES AS DRUG AND MRNA CARRIERS FOR CANCER THERAPEUTICS............................................................................................................................................. 58

CHAPTER 4 ............................................................................................................................................. 91

PILLAR ASSISTED EPITAXIAL ASSEMBLY OF TORIC FOCAL CONIC DOMAINS OF SMECTIC-A LIQUID CRYSTALS ............................................................................................................................................. 91

Supporting Information............................................................................................................................................. 106

CHAPTER 5 ........................................................................................................................................... 131

TOPOGRAPHICALLY-INDUCED HIERARCHICAL ASSEMBLY AND GEOMETRICAL TRANSFORMATION OF FOCAL CONIC DOMAIN ARRAYS IN SMECTIC LIQUID CRYSTALS ............................................................................................................................................. 131

Supporting Information............................................................................................................................................. 152

CONCLUSIONS AND PROSPECTIVE ......................................................................................................................... 165
LIST OF TABLES

Table 1.1 Summary of the effects of UV dosage to the synthesized polymers and their assemblies based on light scattering measurements and SEM analysis. *The particle size from SEM was determined by ImageJ analysis averaged over 200 particles. ........................................ 6

Table 1.2 Summary of Hansen solubility parameters (\(\delta_D\): dispersion, \(\delta_P\): polarity, \(\delta_H\): hydrogen interaction) of various solvents and acryloyl chloride and Flory-Huggins interaction parameters (\(\chi\)) for various organic solvents against acryloyl chloride. Note that the solubility was determined by observation of the polymer solution while the morphology was based on the results of SLS and DLS. The reported particle size was hydrodynamic diameter. The polymers were synthesized at the UV dosage of 2,000 mJ/cm\(^2\), followed by centrifugation and redispersion in various solvents. ........................................................................................................................................................................ 18

Table S1.1 Summary of dynamic and static light scattering results of the polymer assemblies in various organic solvents. Radius of gyration, \(R_g\) is determined by Guinier approximation and morphology of the aggregates is based on the value of \(\rho (= R_g / R_H)\)............ 29

Table 2.1 Summary of Flory-Huggins interaction parameters (\(\chi\)) for various solvents and acryloyl chloride based on Hansen solubility parameters,(Hansen 2007) their corresponding vapor pressures at 20°C and boiling points.(Afeefy, Liebman et al.) .......................................................... 36

Table 3.1: Summary of drug loading efficiencies and zeta potentials of Dox/nanoparticle complexes with and without PEI grafting by two drug packaging strategies. The experiments were performed in aqueous solution........................................................................................................................................................................ 74
LIST OF ILLUSTRATIONS

Figure 0.1: Illustration of block copolymer morphologies assembled in melt (or bulk as you used here) and in solution.(Bucknall and Anderson 2003) .................................................. xxv

Figure 0.2: (a) Schematics of block copolymer fractions with respective cryogenic transmission electron microscopy images showing vesicles or worm micelles and spherical micelles. (b) Schematic scaling of polymersome membrane thickness with copolymer molecular weight (MW). PEG, polyethylene glycol.(Discher and Ahmed 2006) ...................................................... xxvi

Figure 0.3: (a,b) Phase images from TappingMode™ scanning force microscopy of thin SBS films on Si substrates after annealing in chloroform vapor at partial pressure of 0.62. The surface is everywhere covered with an 10-nm-thick PB layer. Bright (dark) corresponds to PS (PB) microdomains below this top PB layer. Contour lines calculated from the corresponding height images are superimposed. (c) Schematic height profile of the phase images shown in (a,b). (d) Simulation of an A₃B₁₂A₃ block copolymer film in one large simulation box of 352 × 32 × H(x)grid points with increasing film thickness H.(Knoll, Horvat et al. 2002) ......................... xxvii

Figure 0.4: Templating and modeling 3D self-assembled structures of poly(styrene-b-dimethylsiloxane) (PS-b-PDMS) (A to K) SEMs of the oxidized PDMS (ox-PDMS) microdomains templated by post arrays. White and light gray areas represent hydrogen silsequioxane (HSQ) posts and ox-PDMS, respectively. Ox-PDMS microdomains were commensurate in the same direction [(A) and (B)] or formed perpendicular and angled mesh-shaped structures [(C) to (F)], cylinders on top of ellipsoids (G), cylinders on top of spheres (H), cylinders on top of perforated lamellae (I), and periodic superstructures [(J) and (K)]. (L) Summary of the experimentally determined morphologies. Circles denote cylinders oriented along x or y; triangles, stars, and squares denote cylinders oriented in a diagonal direction. (M to Q) SCFT simulation results for
representative post periods. Top images, isometric views; bottom images, top-down views. Surface contours of constant minority-block (PDMS) density $f$ are plotted; $f = 0.5$ represents the boundary between the PS and PDMS blocks. (Tavakkoli K. G., Gotrik et al. 2012) .................. xxviii

Figure 0.5: A schematic illustrating the formation of the structure in polymer films. The images are color-coded, with blue and orange denoting low and high temperatures, respectively, relative to room temperature. (A) The conditions under which the experiment is performed. (B) Evaporation of the solvent cools the solution surface, thus initiating the nucleation and growth of moisture. (C) Because of the convective currents arising from the evaporation as well as from the airflow across the surface, the water droplets pack into a hexagonal array. (D to F) The ordered array sinks into the solution, thus leaving the surface of the solution free for the nucleation and growth of moisture to form another ordered array of water droplets. (G) When all of the solvent has evaporated, the film must return to room temperature, thus allowing the water droplets to evaporate as well while leaving behind the scaffold. (Srinivasarao, Collings et al. 2001) ............ xxxiv

Figure 1.1 Schematic illustration of the synthesis and assembly of random copolymers of PAC/X in acetone. ............................................................................. 5

Figure 1.2 (a) SEM image of non-collapsed nanoparticles dispersed in acetone and captured on a Si wafer. The polymers were synthesized at a UV dosage of 2,000 mJ/cm$^2$. The polymer concentration is 0.13 mg/mL. Scale bar is 500 nm for the inset. (b) TEM image of the particles synthesized in (a). (c) TEM image of the polymer assembly as in (a) but stained with TOPO- stabilized CdSe (2 nm in diameter) in toluene (5 wt. %). Inset: higher magnification, highlighting the thin membrane of the vesicle.......................................................... 5

Figure 1.3 FT-IR spectra of random copolymers from photopolymerized AC with addition of [AA]$_0$ at (a) 0, (b) 5, (c) 10 and (d) 20 mol%. The total volume is 3 mL. The total UV dosage is 2,000 mJ/cm$^2$. ........................................................................................................ 8
Figure 1.4 (a) Schematic of possible chemical structure of photopolymerized AC containing PAC chains and crosslinked X groups. (b) Schematic of the cross-sectional view of the random copolymer vesicle synthesized at UV dosage of 1,000 mJ/cm² or greater. ........................................ 9

Figure 1.5 SEM images of particles formed from photopolymers with [AA]₀ at (a) 0, (b) 5, (c) 10, and (d) 20 mol%. The UV dosage was 2,000 mJ/cm². The polymers were dispersed in acetone at 0.13 mg/mL. Scale bar: 200 nm, applicable to all images. ........................................ 10

Figure 1.6 FT-IR spectra of copolymers from photopolymerized AC at 2,000 mJ/cm² UV dosage for a total volume of 3 mL with variable processing environment. (a) The polymers were photopolymerized at ambient environment using non-anhydrous solvents. (b-c) The polymers were photopolymerized in a glovebox (H₂O ~ 2 ppm) using anhydrous solvents (b) and non-anhydrous solvents (c). The polymer solutions prepared in (b) were exposed to air for 2 h (d) and 24 h (e) prior characterization. ........................................................................ 11

Figure 1.7 Hydrodynamic diameters from DLS measurement of the assemblies in acetone as a function of UV exposure dosage. The polymer concentration was at 0.03 g/mL. SEM images of nanoparticles photopolymerized at a UV dosage of 500 (a), 2,000 (b) and 10,000 (c) mJ/cm², followed by dispersing in acetone at 0.13 g/mL. The particles were captured on Si wafers and air-dried. Scale bar: 500 nm, applicable to all images. ................................. 14

Figure 1.8 FT-IR spectra of random copolymers polymerized at UV dosage of (a) 200, (b) 500, (c) 1,000, (d) 2,000, (e) 4,000, and (f) 8,000 mJ/cm². .................................................. 15

Figure 1.9 The distribution and hydrodynamic diameter of particles of nanoparticles photopolymerized at UV dosage of 2,000 mJ/cm², followed by centrifugation and dispersion in various solvents: (a) acetonitrile, (b) acetone, (c) THF and (d) 1,4-dioxane. The polymer concentration is 0.13 g/mL. ......................................................................................... 17

x
Figure S1.1 Photographs of (a) CdSe nanocrystal solution in toluene at 10 wt. % and (b) a mixture of random copolymer solution in acetone (400 mg/mL) and nanocrystal solution in (a) after vigorous mixing (b). ............................................................ 26

Figure S1.2 Photographs of the photopolymerized products of AC with an initial AA composition of 0, 5, 10 and 20 mol% at a total volume of 3 mL. The UV dosage is 2,000 mJ/cm2 and the loading of photoinitiator, Darocur® 1173, is 2 v/v %. ........................................ ................ 26

Figure S1.3 SEM images of photopolymers synthesized at 2000 mJ/cm2 in a glovebox, followed by dispersion in anhydrous acetone at a concentration of 0.13 mg/mL. ...................... 27

Figure 2.1 (a) Chemical structure of the photopolymerized random copolymer. (b) SEM image of a typical ordered porous film prepared at a polymer concentration of 75 mg/mL in acetone (1mL) and toluene (80 µL). (c) Schematic illustration of the synthesis and assembly of microporous film via solvent induced phase separation after dip coating the polymer solution on a Si wafer. Step (i): condensation of toluene droplets (blue) on evaporating polymer film. Step (ii): polymer imprint of the droplet arrays after complete evaporation. ........................................... 35

Figure 2.2 SEM images of ranPAC films dip coated on clean Si wafers from 1 mL polymer/acetone solution (100 mg/mL) mixed with various amounts of toluene: (a) 30 µL, (b) 60 µL, (c) 90 µL, (d) 200 µL, (e) 300 µL and (f) 500 µL. Scale bar of 10 µm is applicable to (a) to (e). ............................................................................................................................................. 36

Figure 2.3 SEM images of ranPAC films dip coated on clean Si wafers from 1 mL polymer/acetone solution (100 mg/mL) mixed with 80 µL different nonsolvents. (a) Chloroform. (b) Toluene. (c) Benzene. (d) Xylene. (e) Cyclohexane. ................................................................................................................................................ 40

Figure 2.4 SEM images of ranPAC films formed on various substrates. (a) Si wafer after O₂ plasma. (b) Clean Si wafer with native oxide. (c) SU-8. (d) HF etched Si wafer. (e)
Fluorosilane treated Si wafer. (f) SU-8 pillar array \((D = 600 \text{ nm}, AR = 4 \text{ and } P = 1.5 \mu\text{m})\). The polymer concentration is 75 mg/mL (1 mL acetone and 80 \(\mu\text{L} \) toluene).................

Figure 2.5 SEM images of porous ranPAC films generated on clean Si wafers from different initial polymer concentrations. (a) 30 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, (d) 100 mg/mL, (e) 150 mg/mL, and (f) 200 mg/mL in 1 mL acetone and 80 \(\mu\text{L} \) toluene. .................

Figure 2.6 SEM images of ranPAC assembled on set I SU-8 pillar array \((D = 600 \text{ nm}, AR = 4 \text{ and } P = 1.5 \mu\text{m})\) at different initial polymer concentrations. (a) 10 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, and (d) 100 mg/mL in 1 mL acetone and 80 \(\mu\text{L} \) toluene. (d), Inset: schematic of directed assembly of polymer-rich and polymer-poor phases on SU-8 pillar array. ..................

Figure 2.7 SEM images of ranPAC assembled on set II SU-8 pillar array \((D = 4 \mu\text{m}, AR = 0.62 \text{ and } P = 15 \mu\text{m})\) at different polymer concentrations. (a) 20 mg/mL, (b) 30 mg/mL, (c) 50 mg/mL, and (d) 75 mg/mL in 1 mL acetone and 80 \(\mu\text{L} \) toluene..........................

Figure 2.8 SEM and fluorescent images of CdSe functionalized porous ranPAC films on a Si wafer (a and b), a hexagonal SU-8 pillar array with \(D = 1 \mu\text{m}, AR = 2 \text{ and } P = 1.5 \mu\text{m}\) (c and d), square SU-8 pillar arrays with \(D = 600 \text{ nm}, AR = 4 \text{ and } P = 1.5 \mu\text{m}\) (e and f), and \(D = 4 \mu\text{m}, AR = 0.62 \text{ and } P = 15 \mu\text{m}\) (g and h). The initial polymer concentrations in acetone were 100 mg/mL (a-f) and 50 mg/mL (g-h) in 1 mL acetone and 80 \(\mu\text{L} \) toluene. ............................................

Figure S2.1 SEM images of porous structures of ranPAC formed on SU-8 micropillar arrays with hydroxyl groups activated at different locations. The polymer concentrations are 75 mg/mL (a and c of set I pillars) and 50 mg/mL (b and d of set II pillars), respectively, in a mixture of 1 mL acetone and 80 \(\mu\text{L} \) toluene. (a-b) Hydroxyl groups were activated only on pillar tops by sitting water droplets for 5 min. (c-d) Hydroxyl groups were activated everywhere on pillars by complete immersing the pillars into water for 15 min...........................................................
Figure S2.2 SEM image of a porous film of ranPAC formed on a flat SU-8 substrate. The polymer concentration is 150 mg/mL in a mixture of 1 mL acetone and 80 µL toluene. 

Figure 3.1: (a) Schematic illustration of the synthesis and assembly of PAA and PEI nanoparticles. (b) Chemical structure of photopolymerized random copolymer.

Figure 3.2. FT-IR spectra of different nanoparticles: PAC NPs, PAA NPs and PEI NPs.

Figure 3.3. Zeta-potentials of PAA and PEI nanoparticles in water and 10 mM NaCl solution. PEI has molecular weights of 1,800 and 10,000. The particle concentration is 1 mg/mL.

Figure 3.4. Size (a) and surface charge (b) of PEI NPs synthesized at various PEI concentrations in water measured by dynamic light scattering. Note that there was no readable data for the sample at 5 mg/mL due to gross aggregation and large polydispersity of NPs.

Figure 3.5. SEM (a,c,e) and TEM (b,d,f) images of particles modified at different states: PAC NPs (a and b), PAA NPs (c and d), and PEI NPs (e and f).

Figure 3.6. Optical (left) and fluorescent (right) images of FITC tagged PEI NPs drop-cast onto a clean Si-wafer at the particle concentration of ~ 1 mg/mL in aqueous solution. Large particles were synthesized at high UV dosage of 4,000 mJ/cm² prior surface modification with FITC tagged PEI (MW 10,000) at ratio of FITC to PEI = 3:1 (molar).

Figure 3.7. The hydrodynamic diameters of functional particles in different pH buffer solutions (b) and over a 15-day period in water at neutral pH and with 10 mM NaCl (b) determined by DLS. In all samples, particle concentration was kept at 1 mg/mL.

Figure 3.8. Schematic illustration of two different drug packaging strategies. The names of NPs are denoted by their synthesis process sequentially.
Figure 3.9. (a) UV-Vis spectra of initial and unloaded Dox solutions after separated from various samples of Dox loaded NPs at initial Dox loading concentration of 100 µg/mL. (b) A photograph of the samples of PEI NPs loaded with Dox by encapsulation method (1) and physisorption method (2) after centrifugation for separation. (c) TEM image of a single Dox/PEI NP. (d and e) Fluorescent (d) and optical (e) images of Dox/PEI NP aggregates. ....................... 75

Figure 3.10. Release of Dox from different particles loaded at 100 µg/mL in 10 mL buffer (10 mM NaCl) solution. Accumulative percentages of loaded Dox released at pH 7 for the first 120 min and pH 4 for the following 3 h are based on UV-Vis measurement of Dox released in the solution. The names of NPs are denoted by their synthesis process sequentially......................... 76

Figure 3.11. Relative fluorescent intensity of free Dox and Dox loaded PAA and PEI NPs at different normalized Dox concentrations based on flow cytometry analysis of HEK 293T cells after 4 h incubation........................................................................................................................................ 78

Figure 3.12. Delivery efficiency of luciferase encoding mRNA to HEK 293T cells by various gene carriers, including PEI particles of different sizes (105, 165 and 220 nm in diameter), PEI complexed mRNA, TransIT complexed mRNA and substrate as a signal baseline at different N/P ratios (1 – 81), which were determined by the detected luciferase activity. .............................. 79

Figure 3.13. HEK 293T cell viability after treated with increasing concentrations of PEI NPs and incubated at 37°C for 24 h. ........................................................................................................................................ 80

Figure 4.1 Epitaxial assembly of TFCDs on SU-8 square pillar arrays with diagonal separation $S \leq S_c$. a) Schematic illustration of SmA LCs confined by a SU-8 square pillar array with $S < S_c$. b) SEM image of the SU-8 square pillar array with diameter $D = 1$ µm, diagonal separation $S = 3$ µm, and height $H = 1.5$ µm. c-d) SEM images of the corresponding TFCDs assembled on the SU-8 pillar array (b) at various LC thicknesses, $h = 1.5$- 2.5 µm (c) and 3.5 µm (d). d) inset: polarized optical image at high magnification. e-f) SEM images of TFCDs assembled xiv
on the SU-8 pillar array with $D = 5 \mu m$, $S (\sim S_c) = 5 \mu m$, $H = 2.5 \mu m$ at various LC thickness, $h = 2.5 - 4 \mu m$ (e) and $4.0 \mu m$ (f). f) inset: high magnification. The blue arrows indicate satellite TFCDs formed between the neighboring pillars. .................................................................95

Figure 4.2 Epitaxial assembly of TFCDs on SU-8 square pillar arrays with diagonal separation $S > S_c$ and diameter $D > D_c$. a) Schematic illustration of SmA LCs confined by a SU-8 square pillar array with $S \geq S_c$ and $D > D_c$. b) SEM image of a SU-8 square pillar array with diameter $D = 10 \mu m$, diagonal separation $S = 15 \mu m$, and height $H = 7.5 \mu m$. c-f) The corresponding TFCDs assembled on the SU-8 pillar array (b) at various LC thicknesses: $h = 2 \mu m$ (c), $7.5 \mu m$ (d), $8 \mu m$ (e), and $9 \mu m$ (f). .........................................................................................................................99

Figure 4.3 Calculated TFCD energy relative to the flat-layer state vs. $\rho = a/h$ using $h = H = 3.0 \mu m$, $D = 1 \mu m$, and varying pillar spacing with diagonal separation $S$. A sharp energy penalty is evident for TFCDs whose diameter exceeds $S$, while smaller sized TFCDs have the same energy as on a flat substrate. Materials constants for calculation(Kim, Yoon et al. 2009) include the splay elastic constant $K = 5 \times 10^{-11} N$, the defect core size $\xi = 3 \times 10^{-9} m$, and the energy per unit area for molecules oriented normal to the LC/air interface $\sigma_{\perp}^{air} = 20 \times 10^{-3} N/m$. The energy per unit area for molecules oriented parallel vs. normal to the LC/SU-8 interface is determined. $\Delta\sigma_{\perp}^{subr} = -1.1 \times 10^{-3} N/m$. This is found by requiring that the calculated TFCD energy allow energetically stable TFCDs only for values of $h \geq h_c = 1.5 \mu m$. ........................................................................................................................................100

Figure 4.4 Epitaxial assembly of TFCDs on SU-8 hexagonal pillar arrays. a) SEM images of the SU-8 hexagonal pillar array with diameter $D = 1 \mu m$, diagonal separation $S = 1 \mu m$, height $H = 2 \mu m$. b-c) The corresponding TFCDs on the hexagonal pillar array (a) at various LC thickness vs. pillar height, $h-H= 0-8 \mu m$ (b) and $8 \mu m$ (c). .........................................................................................................................103
Figure S4.1 Schematics of the synthesis of LC molecules.........................................................107

Figure S4.2 $^1$HNMR (a) and $^{19}$FNMR (b) spectra of LC, compound D. .........................108

Figure S4.3 (A) Chemical structure of the liquid crystal molecule and (B) its phase transition determined by X-ray scattering, which is consistent with report by Yoon et al (Yoon, Choi et al. 2007). (C) SEM images (a, c) and a polarized optical microscopy (POM) image (b) of toric focal conic domains (TFCDs) formed on a flat Si wafer. ..................................................................................................................112

Figure S4.4 SEM image of SmA LCs assembled on the SU-8 square pillar array ($D = 5 \, \mu m$, $S (~ S_c) = 5 \, \mu m$, $H = 2.5 \, \mu m$) at a LC film thickness $h = 40 \, \mu m$, illustrating 3D confinement of LCs by the pillars..............................................................................................................................113

Figure S4.5 SEM images of SmA LCs assembled between the SU-8 square pillar array ($D = 10 \, \mu m$, $S = 15 \, \mu m$, $H = 7.5 \, \mu m$) at LC film thickness $h = 2 \, \mu m$ (a), $6 \, \mu m$ (b), and $7.5 - 8 \, \mu m$ (c) at a tilting angle of $30^\circ$, $45^\circ$ and $52^\circ$, respectively. Scale bar in (c) applies to all the images. ........................................................................................................................................113

Figure S4.6 SEM images of SmA LCs assembled between the Au coated (sputtered for 40 sec) SU-8 square pillar arrays. (a) $D = 5 \, \mu m$, $S = 5 \, \mu m$, $H = 2.5 \, \mu m$, and LC film thickness $h = 2 \, \mu m$. (b) $D = 10 \, \mu m$, $S = 15 \, \mu m$, $H = 7.5 \, \mu m$, $h = 7.5 - 9 \, \mu m$ .................................................................................................................................114

Figure S4.7 TFCD energy relative to the flat-layer state versus domain radius ($a$) for a flat substrate, with LC thickness $h$ increasing from uppermost to lowermost curves: $h = 2 \, \mu m$, $2.5 \, \mu m$, $3 \, \mu m$, $3.5 \, \mu m$, $4 \, \mu m$, $5 \, \mu m$. The value of $a$ where $F$ changes from positive to negative, which determines $S_c$ and $D_c$, is approximately $0.5 \, \mu m$ for all LC thicknesses $h \geq 2 \, \mu m$..............126
Figure S4.8 (a) Geometry of a cross-section of a TFCD. Dashed lines divide the two regions of integration and the region external to the domain where the layers are flat. (b) Geometry of a cross-section of a TFCD confined by pillars, drawn as gray rectangles. .......................... 127

Figure S4.9 Geometry of the unit cell for a TFCD confined by pillars (drawn as gray circles). The circular defect bounding the TFCD is the dotted curve; the location of the central line defect is given by the thick black dot. Dashed lines mark the boundaries of the fundamental unit cell, with area one-eighth that of the original unit cell. ........................................................................... 128

Figure S4.10 TFCD energy relative to the flat-layer state versus domain radius (a) for defects on top of the pillar at varying pillar diameters, $D$ using $h - H = 3\, \mu m$. Thermodynamically stable domains form only for $D > D_c \approx 1.0\, \mu m$. For $1.0\, \mu m < D < 3.0\, \mu m$, the domain radius equals $D/2$. For $D > 3.0\, \mu m$, the domain radius is the same as on the flat substrate, $a \approx 1.5\, \mu m$. Each curve agrees with the $D > 2(h - H)$ curve for values of $a < D/2$. ......................................................................................................................... 129

Figure 5.1 (a-e) Formation of FCD arrays on 1 \( \mu m \) tall SU-8 pillars with variable sizes and shapes. Optical images of top view of SU-8 pillars (1) and LC defect textures on pillars without (2) and with crossed polarizers (3). Scale bars: 20 \( \mu m \). (a) Circular pillars with diameter $D = 5.5\, \mu m$, the center-to-center spacing of the nearest pillars $W = 8.5\, \mu m$ and the diagonal center-to-center distance of the next-nearest pillars $S = 12.0\, \mu m$. (b) Elliptically shaped pillars with major axis length $2A = 6.2\, \mu m$, minor axis length $2B = 5.2\, \mu m$, $W = 7.4\, \mu m$ (along the shorter lattice vector) and $S = 12.2\, \mu m$. (c) Elliptically shaped pillars with $2A = 7.0\, \mu m$, $2B = 3.4\, \mu m$, $W = 6.3\, \mu m$ (along the shorter lattice vector) and $S = 11.8\, \mu m$. (d) Y-shaped post with equal peripheral dimension of 30 \( \mu m \) at all sides. (e) Triangularly shaped pillars with each side of length 10 \( \mu m \). The LC
thickness \( h \) is \( \sim 7 \mu m \) (a – c) and \( \sim 10 \mu m \) (d and e). (d4) AFM height profile of LC defects assembled on a Y-shaped post with equal lateral dimensions of 30 \( \mu m \).

Figure 5.2 A plot of the numerically calculated free energy \( \Delta F \), relative to the reference state of planar layers, as a function of the relative position of the circular pillar center along the line connecting the two TFCD centers for different pillar heights \( (H = 0.5 – 4 \mu m) \). The TFCD radius is set to 5.2 \( \mu m \) at LC thickness \( h = 10 \mu m \) on the pillar array with radius of 5.72 \( \mu m \). Schematics illustrate the TFCD arrangements on the pillar with edge-pinning and confinement effects.

Figure 5.3 Surface characterization of FCD formation on the circular (a-d) and elliptical pillar arrays (e-h). The latter corresponds to pillars seen in Fig. 5.1c. Optical images (a and e) reveal a defect texture with the polarizer and analyzer at a relative angle of 45°, and the corresponding surface topography of FCDs arrays obtained from SEM (b and f). (c and g) 3D maps of the surface of the LC films extracted from AFM measurements based on their height profiles with color representation of relative thickness of the film. (d and h) Plots of the height profiles along the dashed white lines in (c) and (g).

Figure 5.4 Schematic illustration of internal structures of FCDs with zero (a) and nonzero (0.2) (b and c) eccentricity in regions bounded by a cylinder (a and b) or a cone configuration (c). (d) Representation of a possible arrangement of FCDs with nonzero eccentricity on circular pillars with the edge-pinning effect.

Figure 5.5 Schematic illustrations of (a) a single FCD bounded by a cone, (b) a smectic layer construction bridging between two bounding cones with circular arcs concentric about \( l_u \) in the plane containing the cone generator through \( E_u \) and the cone normal direction along this generator, and (c) a complete layer construction of four FCDs surrounding a pillar for all \( u \in [0,2\pi] \) based on (b). (d) A 2-D map of topmost surface of (c) with a color representation of surface
Figure S5.1 Substrate interface (a) and elastic (b) energy contributions to the free energy $\Delta F$ plotted in Figure 2 of two TFCDs sharing one cylindrical pillar. The free energy is given relative to the reference state of planar layers as a function of the relative position of the circular pillar center along the line connecting the two TFCD centers for different pillar heights (0.5 – 4 µm). The TFCD radius is set to 5.2 µm at LC thickness of 10 µm on the pillar array with radius of 5.72 µm. The air interface energy is independent of pillar position.

Figure S5.2 Schematic diagram of the FCD arrangement assumed for the pillar topography of Figure 1a, viewed from above. The circular pillar, of diameter $D$, is represented in gray. The elliptical focal curves of four FCDs, assumed to lie in the plane of the substrate, are each tangent at four points to squares comprising one fourth of the unit cell. The solid black circles are the ellipse foci as well as the intersections of the hyperbolic focal curves with the plane; these lie at the pillar edge. The open circle is the center of one ellipse. The x- and y-axes of the coordinates used in Equation (1) are as shown in the diagram.
PREFACE

Nature offers numerous remarkable examples of complex hierarchical architectures via spontaneous self-assembly of molecular building blocks known as the bottom-up approach, exhibiting fascinating functions and properties, such as the dual-scale roughness on lotus leaves for self-cleaning and superhydrophobicity, combination of regular and irregular structures on butterfly wings for angle-independent iridescence, the sophisticated aquiferous system of sea sponges, the interconnected fibrous network of wood and bone structures for excellent mechanical property, and cellular membranes that regulate a variety of cellular activities from protein production to transportation of ions and molecules. Bottom-up assembly relies on the chemical nature of building blocks and intermolecular interactions between them, such as van der Waal forces and hydrogen bonding, to assemble smaller building blocks into more complex structures with desired shapes and functions. Most of today’s technologies, however, have relied on top-down processes that cut, mill and shape materials, such as photolithography and etching techniques, to create structures by reducing lateral dimensions of bulk materials into desired shape and order. Top-down approaches are highly effective in manufacturing materials with small footprints of rather simple structures and homogenous composition with high precision. Nevertheless, the top-down processes could be soon overwhelmed by the ever-increasing demand for more complex, multileveled structures with heterogeneous compositions at a smaller lengthscale, as well as the soon-approaching fundamental limits in scalability. In comparison, driven by thermodynamics to form stable structures, self-assembly offers a promising route to mass produce hierarchical and functional materials in parallel and at a much lower cost. Thus, self-assembly has rapidly gained momentum in research and manufacturing.

Tremendous efforts have been invested in two different but interrelated directions to control self-assembly. One is to control environmental parameters (e.g. temperature, (Alexandridis, Zhou et al. 1996), solvent quality (Jung and Ross 2009), concentration, (Khougaz, Gao et al. 1994) and
interface (Oslanec, Costa et al. 2000; Green and Limary 2001), which impact the assembly process of building blocks both in solution and in bulk. For example, Eisenberg and colleagues thoroughly investigated the effect of solvent on micelle formation of block copolymers, poly(styrene-b-acrylic acid). By tuning the strength of polymer-solvent interaction parameter, they varied the dimensions of both the core and corona of the polymer micelles in solution, leading to the transition from spherical micelles to cylindrical micelles and vesicles. (Yu, Zhang et al. 1998)

The other path exploits innovative design of the building blocks to customize molecular interactions (e.g. H-bonding and hydrophobic interaction), enabling supramolecular structures. (Percec, Ahn et al. 1998; Lehn 2002) For example, Percec and colleagues have synthesized a rich library of dendrons as self-assembly building blocks, leading to the formation of complex structures such as chiral supramolecular dendrimers. (Percec, Ahn et al. 1998; Percec, Mitchell et al. 2004; Percec, Won et al. 2007; Percec, Imam et al. 2008) Selective dendritic crowns, for instance, self-assemble into helical pyramidal columns that form 2D columnar hexagonal lattices with intracolumnar order while others self-assemble into spherical supramolecular dendrimers that self-organize into cubic and tetragonal lattices. (Percec, Imam et al. 2008) Self-assembly approaches have undoubtedly remarkable prospects to create complex and multifunctional architectures. Yet, the poor controllability of the individual components and assemble them into an arbitrary size and shape, and lack of reproducibility due to kinetic trapping of intermediate structures have limited the practical applications of self-assembly processes.

To overcome the disadvantages of top-down and bottom-up approaches, one way is to marry the two together via template-assisted self-assembly. Typically, a top-down method is employed to fabricate templates with precise geometry and surface chemistry to direct the assembly of building blocks by exerting directional forces such as capillary, covalent and noncovalent interactions. This approach offers greater control of self-assembly to fabricate 2D and 3D hierarchical structures, which could potentially result in novel morphologies and functions. So far, there have been considerable efforts to direct the assemblies of block copolymers (Wang and
Composto 2002; Li, Coenjarts et al. 2005; Stoykovich, Müller et al. 2005; Cheng, Ross et al. 2006) and colloids(Kitaev and Ozin 2003; Dziomkina and Vancso 2005) on both flat and chemically and topographically patterned substrates in 2D. However, there are comparatively few studies to direct self-assembly of other soft materials in 3D with spatial control of surface chemistry and interface. For that, it has potential to be a model study/design for both theoretical and technological aspects.

The most fascinating aspect of soft matter lies in its ability to form higher order structures at macroscopic length scale with its spatial extent ranging from nanometer to micrometer scale through bottom-up approach. Some of the most important and well-studied examples of soft matter are amphiphilic molecules such as block copolymers and surfactants. Amphiphilic molecules, which comprise of at least two chemically dissimilar segments, are known to spontaneously self-organize into various nano- and microstructures, for instance, spherical micelles, cylindrical micelles, vesicles, compound micelles, and inverted micelles in solution and lamellae, gyroids and hexagonal structures in bulk by the repelling and coordinating action between the two segments in respond to the surrounding environment (see Fig. 0.1). These structures are of great importance in a number of research fields ranging from lithography, surface patterning, to fabrication of innovative functional materials such as nanotubes, nanosheets and nanoribbons for electrical and medical devices as the assembled structures are recognized to exhibit intriguing and useful physical properties that are desired in today technologies. (Alexandridis, Zhou et al. 1996; Kim, Solak et al. 2003; Bita, Yang et al. 2008; Kim, Yoon et al. 2010)

In solution, the feature size and morphology of the assemblies from block copolymers at equilibrium state can be regulated by various parameters such as the interaction parameter,(Svensson, Olsson et al. 2000) molecular architecture, polymer molecular weight,(Bermudez, Brannan et al. 2002; Choucair, Lavigueur et al. 2004) composition,(Choucair,
Lavigueur et al. 2004; Choi, Koo et al. 2005; Adams, Butler et al. 2006; Yu, Azzam et al. 2009) polymer concentration, and solvent quality.(Choucair, Lavigueur et al. 2004; Sanson, Schatz et al. 2010) Discher and Ahmed et al. described assembled morphology dependent on the relative mass or volume fraction of each block in aqueous solution where the time average molecular shape can be considered in the form of a cone, wedge or cylinder for hydrophilic fraction, $f > 50\%$, $\sim 40 - 50\%$, $\sim 25 - 40\%$, respectively (see Fig. 0.2).(Ahmed and Discher 2004; Discher and Ahmed 2006) Based on the geometric force, block copolymers with $f > 50\%$ will readily self-assemble into small micelles where hydrophobic blocks are removed from the aqueous environment to achieve a state of minimum free energy. On the other hand, when $f \sim 25 - 40\%$, the polymer forms a cylindrical shape due to the balance of geometric volume between hydrated hydrophilic fraction and large hydrophobic fraction. This results in assembly of vesicles, a closed bilayer structure with hydrophobic core and hydrophilic corona. The molecular weight of the polymer dictates the thickness of the vesicle membrane as the thickness of the vesicle ($d$) increases with the molecular weight copolymer. For instance, $d \approx 8 - 21\ nm$ for the molecular weights ranging from 2,000 – 20,000 Da.(Bermudez, Brannan et al. 2002; Discher and Ahmed 2006) The adjustable membrane thickness from block copolymer is typically thicker than that from lipid ($d \approx 3 - 5\ nm$), therefore, providing greater robustness to the vesicle structure and in turn, addressing the key limitation of lipid based delivery system in medical applications.(Discher and Ahmed 2006)

In bulk, the amphiphilic block copolymers with highly incompatible blocks microphase separate into ordered microstructures, depending on xxx. In thin film, the role of surface/interfacial energetics is significantly enhanced and becomes predominant for structure formation.(Fasolka and Mayes 2001; Smith, Douglas et al. 2001) by tuning film thickness, the surface chemistry or a choice of annealing solvents, we can modulate the orientation and alignment of the ordered domains such that the polymers form interesting microstructures that
deviate from the bulk structures through minimization of surface/interfacial energy (Bita, Yang et al. 2008; Jung and Ross 2009).

In addition to pattern chemical groups, topographical substrates or templates are utilized to achieve a higher level of control of the self-assembly block copolymer thin films. A variety of well-aligned and complex line patterns (Kim, Solak et al. 2003) defect-free dot patterns (Bita, Yang et al. 2008) and aperiodic patterns (Stoykovich, Kang et al. 2007) are demonstrated. The key to various patterns of micro-phase separated block copolymers lies in the minimization of the entropic penalty of the system due to the commensurability/incommensurability between the localized polymer domain period and the periodicity of the templates. The template-assisted self-assembly approach offers an effective route not only to scale-down the patterns smaller than those of assisting templates, but also to expand the fine level of control to complex 3D structures with variable geometry for numerous applications, particularly in an incorporation of block copolymer lithography to nanodevice fabrication as illustrated in Fig. 0.4 (Ruiz, Kang et al. 2008; Tavakkoli K. G., Gotrik et al. 2012). Over decades, the well-defined systems of block copolymer have been exploited for a wide range of applications, for example, micelles of different morphologies for storage and delivery of food, dyes, drugs and other active molecules (Ahmed and Discher 2004; Champion, Katare et al. 2007; Chen, Meng et al. 2010), and nanostructures of block copolymer thin films as templates in nanofabrication of devices, including patterned magnetic recording media, transistors, flash memory and gas sensors (Stoykovich, Müller et al. 2005; Sakatani, Boissière et al. 2007; Bita, Yang et al. 2008; Tang, Lennon et al. 2008) However, the rapid revolution of technology today demands even more innovative, adaptive and smarter materials than those from simple block copolymer systems. The synthetic processes to prepare well-defined responsive block copolymers, however, are often tedious and complicated. Amphiphilic random copolymers,
Figure 0.1: Illustration of block copolymer morphologies assembled in melt (or bulk as you used here) and in solution. (Bucknall and Anderson 2003)
Figure 0.2: (a) Schematics of block copolymer fractions with respective cryogenic transmission electron microscopy images showing vesicles or worm micelles and spherical micelles. (b) Schematic scaling of polymersome membrane thickness with copolymer molecular weight (MW). PEG, polyethylene glycol. (Discher and Ahmed 2006)
Figure 0.3: (a,b) Phase images from TappingMode™ scanning force microscopy of thin SBS films on Si substrates after annealing in chloroform vapor at partial pressure of 0.62. The surface is everywhere covered with an 10-nm-thick PB layer. Bright (dark) corresponds to PS (PB) microdomains below this top PB layer. Contour lines calculated from the corresponding height images are superimposed. (c) Schematic height profile of the phase images shown in (a,b). (d) Simulation of an A₃B₁₂A₃ block copolymer film in one large simulation box of |352 × 32 × H(Campo)| grid points with increasing film thickness H.(Knoll, Horvat et al. 2002)
Figure 0.4: Templating and modeling 3D self-assembled structures of poly(styrene-b-dimethylsiloxane) (PS-b-PDMS) (A to K) SEMs of the oxidized PDMS (ox-PDMS) microdomains templated by post arrays. White and light gray areas represent hydrogen silsequioxane (HSQ) posts and ox-PDMS, respectively. Ox-PDMS microdomains were commensurate in the same direction [(A) and (B)] or formed perpendicular and angled mesh-shaped structures [(C) to (F)], cylinders on top of ellipsoids (G), cylinders on top of spheres (H), cylinders on top of perforated lamellae (I), and periodic superstructures [(J) and (K)]. (L) Summary of the experimentally determined morphologies. Circles denote cylinders oriented along x or y; triangles, stars, and squares denote cylinders oriented in a diagonal direction. (M to Q) SCFT simulation results for representative post periods. Top images, isometric views; bottom images, top-down views. Surface contours of constant minority-block (PDMS) density \( f \) are plotted; \( f = 0.5 \) represents the boundary between the PS and PDMS blocks. (Tavakkoli K. G., Gotrik et al. 2012)
therefore, potentially could offer promising alternatives due to its simplicity in synthesis and ability to access a wide range of functionality. A number of organized structures from random copolymer have been reported. For example, robust giant vesicles are formed by crosslinking the membrane via specific interactions such as H-bonding in random copolymers of poly(styrene-ran-methacrylic acid), and complementary random copolymers functionalized with complementary diaminopyridine and thymine moieties. (Ilhan, Galow et al. 2000; Liu, Kim et al. 2005; Thibault, Uzun et al. 2006) Zhu and Liu et al. investigated self-assembly of designed amphiphilic random copolymers containing hydrophobic dodecyl chain and hydrophilic L-glutamic acid and learned similar behavior as in block copolymer system: the assembled structures are highly dependent on the balance of hydrophobic and hydrophilic portions along polymer chain. (Zhu and Liu 2011) In this case, when $f \approx 10\%$, formation of giant vesicles with diameters ranging from 1 – 20 µm and the average membrane thickness of 18 nm was observed in a mixed solution of ethanol and water. Increasing $f$ to approximately 24%, the copolymers formed smaller vesicles (a few hundred nm in diameter) since a large water soluble portion of L-glutamic acid could stabilize a larger surface area of smaller spherical assemblies. Nevertheless, lack of control in synthesis of amphiphilic random copolymers will result in polymers with ill-defined architecture, heterogeneous chemistry along the chains and large polydispersity index.

The first half of the thesis presents the synthesis and assembly of a novel amphiphilic random copolymer system, denoted as ranPAC. The random copolymers, comprising of poly(acryloyl chloride) (PAC) as a solvophilic portion and solvophobic self-crosslinked acid anhydride are synthesized through bulk photopolymerization of acryloyl chloride (AC). Relying on partial hydrolysis of highly reactive AC with environmental humidity, the photosynthesis readily and reliably yields amphiphilic polymer rather than homopolymer of PAC, owing to the reaction between AC and its hydrolyzed compound (acrylic acid), which contributes to the formation of crosslinked acid anhydride groups in the copolymer. In a selective organic media, ranPAC
polymer chains self-assemble into ordered structures with various morphologies (e.g. spherical micelles, vesicles, compound aggregates and ordered porous films) and sizes (from nanometers to micrometers) in a controlled manner much like those typically found in block copolymers. In Chapter 1, we discuss the detailed study of the polymer synthesis, the underlying principle of its assembly, the effects of UV dosage applied during polymerization, polymer concentration and solvents to polymer composition, molecular weight and micelle formation are investigated comprehensively. The study reveals that the molecular weight (MW) and degree of crosslinking increase with UV dosage applied to the monomers. Consequently, the UV dosage becomes a convenient parameter to control the feature size and morphology of the assembled ranPAC, an important tool in polymer synthesis to fully assess the potential of the assemblies toward various applications such as encapsulation and controlled release systems, nanoreactors, responsive templates for functional materials, and building blocks for fabrication of hierarchical structures. (Sakatani, Boissière et al. 2007; Chen, Schönherr et al. 2009; Li, Zhong et al. 2009; Lu, Proch et al. 2009; Min, Kim et al. 2010)

The effect of solvent is utmost important for the self-assembly of amphiphiles in solution. While manipulating the hydrophobic and hydrophilic portions of block copolymers as a means to control association forces such as hydrophobic interaction or hydrogen bonding in aqueous solution is well established in literature, the analogy of the assembly closely applies to water incompatible copolymers. In nonaqueous system, the balance between the solvent-liking (solvophilic) and solvent-hating (solvophobic) portions of copolymers determines the interactions of the polymer to external solvent, elasticity along polymer chains and thus their packing in the solution. The polymers in solution form equilibrium structures in order to minimize the totally energy of the system which directly relates to polymer solubility. Among approaches developed to determine polymer solubility, a 2D lattice based model developed by Flory and Huggins is commonly used to describe polymer solutions in which polymer segments and solvent molecules are assumed to have the same unit size, and each segment can occupy a single lattice
space. (Flory 1941; Huggins 1942) In this model, the Gibbs free energy of mixing, $\Delta G_m$, can be calculated as a function of the number of solvent, $n_1$, and polymer molecules, $n_2$, the volume fraction, $\varphi$, and the Flory-Huggins interaction parameter, $\chi$ between the polymer and solvent as shown in Eq. 1:

$$\Delta G_m = k_BT(n_1 \ln(\varphi_1) + n_2 \ln(\varphi_2) + \chi n_1 \varphi_2)$$  \hspace{1cm} (1)

where $k_B$ is Boltzmann’s constant, $T$ is the absolute temperature.

The value of $\chi$ can be approximated from solubility parameter, $\delta$, a numerical estimate of the degree of interaction between materials such as polymer and solvent. In general, two materials with similar value of $\delta$ are miscible whereas materials with very different values of $\delta$ do not mix. For solvent, is directly related to the molar energy of vaporization, $\Delta E^v$ representing the energy change after isothermal vaporization of the saturated liquid to the ideal gas state at infinite dilution and molar volume, $V$ of a pure liquid as the following:

$$\delta = \sqrt {\frac {\Delta E^v} {V}}$$  \hspace{1cm} (2)

where $\Delta E^v = \Delta H^v - RT$, $\Delta H^v$ is the enthalpy of vaporization.

For polymer solubility, $\chi$ is typically estimated with Hansen solubility parameters (HSP) which account for dispersion forces ($\delta_D$), permanent dipole-permanent dipole forces ($\delta_P$) and hydrogen bonding ($\delta_H$). (Hansen 2007) The interaction parameter can be calculated as the following:

$$\chi = \frac {VR_a^2} {4RT}$$  \hspace{1cm} (3)

$$\left(R_a\right)^2 = 4(\delta_D^2 - \delta_D^2) + (\delta_P^2 - \delta_P^2) + (\delta_H^2 - \delta_H^2)$$  \hspace{1cm} (4)
where $R_a$ is the solubility parameter distance between two materials based on their respective partial solubility parameter components. $V$ is the molar volume of the solvent. $R$ is the gas constant, and $T$ is the absolute temperature.

The Flory-Huggins interaction parameter is used to determine a degree of solubility of random copolymer in solvent in Chapter 1 and 2. $\chi < 0.5$ represents high affinity between the polymer and the solvent while $\chi > 0.5$ indicates that the polymer has poor solubility in a given solvent.

Like amphiphilic block copolymers and surfactants, ranPAC can form ordered microporous films via evaporative assembly (see Chapter 2). While a typical fabrication of 2D and 3D ordered porous films with honeycomb structures, known as breath figure (BF), relies on generation of condensing water droplets on the cold surface of the evaporating polymeric solution in a high humidity environment, organization of the droplets, driven by the surface convection and capillary force and solidification of the polymer film molded around the sacrificial templates of the droplets (see Fig. 0.5), the amphiphilicity of ranPAC enables similar organization into ordered porous films based on a specific compatibility and ratio between an organic solvent pair such as acetone and toluene so that a moderately poor solvent for ranPAC behaves like water in BF by condensing into spherical droplets and templating the polymer film. In the same chapter, self-assembly of ranPAC on 2D lithographic templates with spatial chemistry and physical confinement is studied, in which the periodicity and arrangement of the patterned templates determine the resulting symmetry and size of the pore structures in thin films through selective wetting on the surface. The study does not only demonstrate the versatility of ranPAC system, but also provides a simple route that may be applicable for other polymer systems to create and control a variety of hierarchical porous structures through evaporative, template-assisted assembly.

Further, we exploit the design of "smart" and pH-responsive nanocarriers from ranPAC vesicles for encapsulation and controlled delivery of drug and mRNA (see Chapter 3) as a new
means of cancer therapeutics. Toward development of safe and effective delivery systems, most vehicles are engineered to respond to different external stimuli. Among others, pH stimuli remain one of the most important functions for clinical drug delivery due to the different pH conditions in normal organs and tumors. The average pH value in tumor tissues is relatively low, approximately pH 6.5 compared to pH 7.4 in the normal extracellular physiological environment. (Ganta, Devalapally et al. 2008) In primary and secondary lysosomes, pH value can drop to as low as pH 4.5. (Ganta, Devalapally et al. 2008; Min, Kim et al. 2010) Thus, this significance change in pH value within the body offers great opportunities to develop smart medicines with pH responsive carriers.

The particle synthesis from ranPAC described in Chapter 1 manifests its merit in which the size (10 nm to 500 nm) and morphology (spherical core-shell micelles, vesicles with extremely thin membrane and large spherical aggregated compounds) are tunable over a wide range encompassing the specific criteria for effective delivery system. The synthetic formulation involves simple yet highly effective routes to functionalize the shell of the vesicles with poly(ethylene imines) (PEI), a well-studied polycation for pH responsiveness and improved stability, package active agents onto the carriers with high payload and finally deliver medicine to sites of therapeutics based on the responsiveness of the carriers to external environment. Our in vitro studies in HEK 293T cells suggests that the formulation of PEI modified particles is a potent platform in delivery of both mRNA and anticancer drug. Moreover, the proposed packaging approach by coassembly of polymeric vesicles together with active agents in organic media can be applied for many other active molecules particularly hydrophobic drugs and vitamins, whose encapsulation by current technologies remain inefficient. (Rösler, Vandermeulen et al. 2001; Letchford and Burt 2007; Fleige, Quadir et al. 2012)
**Figure 0.5:** A schematic illustrating the formation of the structure in polymer films. The images are color-coded, with blue and orange denoting low and high temperatures, respectively, relative to room temperature. (A) The conditions under which the experiment is performed. (B) Evaporation of the solvent cools the solution surface, thus initiating the nucleation and growth of moisture. (C) Because of the convective currents arising from the evaporation as well as from the airflow across the surface, the water droplets pack into a hexagonal array. (D to F) The ordered array sinks into the solution, thus leaving the surface of the solution free for the nucleation and growth of moisture to form another ordered array of water droplets. (G) When all of the solvent has evaporated, the film must return to room temperature, thus allowing the water droplets to evaporate as well while leaving behind the scaffold. (Srinivasarao, Collings et al. 2001)
Liquid crystals (LCs) are another fascinating class of soft matters, which exhibit unique physical properties between liquid and crystalline phases. Comprised of rod-like or disk-like molecules, LC manifests its anisotropy through a variety of remarkable optical, electrical and magnetic properties, such as birefringence, polarization, dielectric and diamagnetic phenomena and formation of distinguish patterns and structures (viscous fingering) based on their molecular alignments that are highly sensitive to external stimuli (i.e. electric and magnetic fields, surface chemistry and confined geometry). (Bragg 1934; Kang, Maclennan et al. 2001; Lee and Clark 2001; Choi, Pfohl et al. 2004; Choudhury, Rao et al. 2010; Bisoyi and Kumar 2011; Miyajima, Araoka et al. 2012) Because of these unique characteristics of LCs, they have played important roles in a number of technologies today, ranging from liquid crystal displays, highly efficient and low cost sensors, fast switchable opto-electronic devices to energy-absorbing-bullet proof materials.(Gardiner and Coles 2006; Bisoyi and Kumar 2011)

LC molecules also offer excellent prototypes to study self-assembly of soft materials as they resemble a number of biological compounds (proteins, viruses, lipids, carbohydrates and nucleic acids) and demonstrate the general principle of self-organization and structure formation in living systems by embracing nearly all kinds of supramolecular interactions such as van der Waals interaction, dipolar and quadrupolar interactions, charge transfer and π-π interaction, metal coordination and hydrogen bonding etc.(Paleos and Tsiourvas 2001; Kato, Yasuda et al. 2009) Depending on the level of ordering of the LC molecules, there are various LC phases: 1) nematic phases having only orientational order where molecules self-align along their common long axis known as director, 2) smectic phases showing a degree of both orientational and translational long range orders and thus resulting in molecular alignments into layers or planes, and 3) more complex LC phases, including cholesteric or chiral nematic phases exhibiting chirality, blue phases having a regular 3-D cubic structure of defects and possessing icosahedral symmetry similar to quasicrystals, discotic phases assembled from disc-like molecules, etc.
Over the past decades, controlling LCs into complex geometries has been extensively studied not only for scientific curiosity but also to develop novel display and many other advanced technologies. Topological defects has long been a topic of great interest to physicists as they play a vital role in a number of intriguing phenomena in the field ranging from early universe cosmology to condensed matter. LCs with long range order are often employed as models in studying those phenomena because they can either intrinsically or extrinsically generate textures and patterns in various geometries under different conditions. From a technological point of view, the manufacturing of advanced materials has progressively transformed from defect-free systems (i.e. the first geometries of displays) to control defects as a means to create innovative materials. (Drzaic 1995; Crawford 1996) Therefore, the ability to tailor LCs with complex, topological defect arrays will offer new clues for the next generation of switchable optic/electric technologies and beyond.

Tremendous effort has been devoted to discovering and establishing fundamental properties of topological defects present in nematic phases, for instance, by controlling the stability of emulsions and interaction between colloidal particles in the elastic ocean of nematic LCs. (Poulin, Cabuil et al. 1997; Poulin, Stark et al. 1997; Smalyukh, Lavrentovich et al. 2005) In comparison, much less attention has been paid to smectic systems because the formation of topological defects known as focal conic domains (FCDs) is often irregular in bulk. Not until recently that FCDs have significant interests because the defects can be arranged in a controlled manner in the micronscale in the smectic-A (SmA) LC phase on a flat and topographic substrates. The smectic layers in each FCD form concentric sections of Dupin cyclides, generalizations of tori, with two linear focal sets (centers of curvature), an ellipse and a confocal hyperbola. (Alexander, Chen et al. 2010) One interesting property of FCD is that the family of curved surfaces of smectic layers can be wrapped around these two focal lines preserving their equidistance everywhere except at the very defect cores. The 3D configuration of the directors inside the domain is rather
complex: any line that connects a point on the ellipse to a point on the hyperbola, is the local optic axis.

Whereas FCDs arise as the prototypical, kinetically-trapped texture in bulk, a 2D hexagonal lattice of axially symmetric toric FCDs (TFCDs) with negative Gaussian curvature can be robustly produced in thin smectic films with antagonistic boundary conditions of tangential anchoring at the surface of substrate and homeotropic anchoring toward the air (see Fig. 0.6a). Thus, a TFCD array is simply a result of director deformation in thin film. On surface, a defect domain appears as a circular, cone-shaped dimple at the LC/air interface (Fig. 0.6b), forming a characteristic Maltese cross pattern under cross polarizers (Fig. 0.6c). The polarized texture indicates that the director field with respect to the plane of the substrate is radial bounded by concentric basis of a domain ellipse. The dark background between each domain corresponds to zero birefringence indicating that the smectic planes are parallel to the substrate and air interfaces. In the standard smectic ground state, the smectic layers are flat and parallel to the substrate and thus the molecular orientation points normal to both the LC/air and LC/substrate interfaces. The TFCDs form spontaneously when the decrease in surface energy obtained by tangential anchoring on the substrate outweighs the elastic energy cost of bending the layers and the increase in surface energy due to the dimple-like deformation of the LC/air interface. These TFCD arrays have been used to fabricate functional surfaces,(Kim, Yoon et al. 2009; Kim, Jeong et al. 2010) to direct the self-assembly of soft Microsystems,(Yoon, Choi et al. 2007; Pratibha, Park et al. 2010; Milette, Relaix et al. 2012) to template new nanoscale patterns,(Kim, Yoon et al. 2010) and to enhance charge in photovoltaics and transistors.(O'Neill and Kelly 2003) To expand the applications of FCDs, the prerequisite would be the control of the type, feature size, and spatial distribution of the defects in 3D, which have not been well addressed in previous studies.
Figure 0.6: (a) Schematic illustrating smectic layer (red plane) construction of a tori focal conic domain on randomly planar anchoring induced surface where green rods represent liquid crystal molecules. (b) SEM image of array of tori focal conic domains and (c) its corresponding cross polarized image.
In thin film, the relation between film thickness \((h)\) and the size of TFCD, specifically its lateral diameter \((2r)\) is well established by Fournier et al. in which the derived expression (Eqs. (5 and 6)) for the energy of a FCD, \(F\) describes quantitatively all experimental observations for 10CB (4-n-decyl-4'-cyano-biphenyl). (Fournier, Dozov et al. 1990) The total energy of FCD simply results from a balance between the elastic energy of LC, \(\Delta F_{el}\) (the first two terms in Eq. (5)) and the surface energy at both the air and substrate interfaces, \(\Delta F_{air}, \Delta F_{subs}\) (the last two terms in Eq. (5)). By minimizing \(F\) with respect to \(r\), \(h\) is found to be linearly proportionate to \(r\) as shown in Eq (7), which is in agreement with most smectic systems. (Guo, Herminghaus et al. 2008; Kim, Yoon et al. 2009)

\[
\Delta F = \Delta F_{el} + \Delta F_{air} + \Delta F_{subs}
\]

\[
F = 2\pi \beta K h + 2\pi \alpha Kr - \pi r^2 \Delta \sigma_{sub} + \frac{\pi \sigma_{air} r^4}{12h^2}
\]

\[
h = \left[\frac{\sigma_{air} r^3}{6\sigma_{sub} (r - \frac{2\alpha K}{\sigma_{sub}})}\right]^{1/2}
\]

\(K\) is the mean elastic constant, \(\sigma_{air}\) is the surface tension of air, \(\Delta \sigma_{sub}\) the surface tension difference between hometotropic and planar anchoring on the substrate, \(\alpha\) and \(\beta\) are dimensionless unknown constants.

The growth and arrangement of TFCD arrays can be effectively manipulated by confinement of SmA LCs utilizing either chemically or topologically patterned substrates. (Ki Yoon, Deb et al. 2010; Yoon, Yoon et al. 2010) For example, Guo et al. presented a simple method to pattern Si-wafers by thermal evaporation of gold through standard TEM grids as lithographic masks to obtain alternating molecular anchoring surfaces such that the gold coated surface promoted homeotropic alignment of LC molecules. (Guo, Herminghaus et al. 2008) FCDs of 8CB (4-n-octyl-4-cyanobiphenyl) were found above the uncoated regions, of which the width restricted the maximum size of the defect. Controlling the size and spatial patterning of defect domains by
geometric confinement was first demonstrated in Choi *et al.* (Choi, Pfohl *et al.* 2004). The study shows that the defects behaved like colloidal objects: confining 8CBs in the surface-modified microchannels yielded uniform FCDs that arranged themselves in quasi-2D ordered patterns with triangular symmetry. Kim *et al.* also studied assembly of high density TFCDs in 1D microchannels, and reported an important finding that domain formation was strongly influenced by both the channel width ($W$) and, even more dramatically, by the channel depth ($H$). (Kim, Yoon *et al.* 2009) They found that in order to form an energetically stable, hexagonal array of TFCDs, $W > W_c \sim 4 \mu m$ and $H > H_c \sim 2 \mu m$. (Kim, Yoon *et al.* 2009) In parallel, Shojaei-Zadeh *et al.* investigated the effect of 3D confinement with mixed surface anchoring to defect formation, its size, size distribution and packing structure of smectic 8CB. (Shojaei-Zadeh and Anna 2006) In the study, the microchannels with closed tops (rather than a free surface) were fabricated by soft lithography with polydimethylsiloxane (PDMS) elastomer which intrinsically induced homeotropic anchoring of 8CBs and tangential anchoring when oxidized in air plasma. The optical transparency of PDMS enabling observation of LC textures in all directions became the main advantage of this system. The importance of confined geometry was highlighted by Kim *et al.* where different shapes of 1D channels (rectangular, V-shaped, and isosceles trapezoidal) with random planar anchoring surfaces ensued distinct FCD textures (alternating fan shape or half FCD, no FCD, complete circular TFCD, respectively) due to variation in surface energy from the side walls and spatial spaces available for LC to form a complete toroidal structure. (Kim, Kim *et al.* 2011) Further, Jeong *et al.* reported the *in situ* transition of the topological defects from a point to a line defect when the aspect ratio of PDMS prolate spheroids in which smectic LC was confined increased due to mechanical stretching of the elastomer film. (Jeong and Kim 2012) To understand the transition, the authors derived a simple energy model to describe defect structures in terms of the misorientation and undulation instability of the smectic layers within various confined geometries.
Since a FCD is a 3D topological defect whose textures correspond to the molecular configuration of the domain and its placement with respect to the confinement, energetically complied a given boundary condition of molecular anchoring, it is possible to harvest and control defect location and orientation rather than the typical hexagonal lattice of TFCDs simply by tuning film thickness and anchoring conditions. While unidirectional planar anchoring on crystalline surfaces such as polybdenite and mica is known to form 1D arrays of parallel linear cylindrical defects in thin film,(Michel, Lacaze et al. 2006; Zappone, Lacaze et al. 2011) Zappone et al. reported a 2D hexagonal arrays of non-toroidal FCDs with high eccentricity, tilted away from the normal of the substrate with identical anchoring condition when $H < a$ minor axis of the ellipse or ~ 10 smectic layers.(Zappone, Meyer et al. 2012) Thus, the array of non-zero FCDs resulted in a unique texture on the free surface, resembling biological skin patterns such as fish, reptiles and pineapples. In a separate study, a topographically curved surface such as microwrinkle grooves were employed to confine smectic LC for a uniform linear array of staggered non-zero FCDs along the groove direction.(Ohzono, Takenaka et al. 2012) This work created yet another surface profile of FCDs, expanding the range of shapes and symmetries accessible for applications based on self-ordered defects in smectic with non-zero eccentricity.

So far, most attention has been devoted to the precise manipulation of the size and arrangement of FCDs in 2D lattices by confining defect domains within small regions through patterning of the substrate. Little is known, however, about a higher level of control of FCDs in 3D. Thus, the second half of the thesis highlights systematic studies of topological defect structures from controlled assembly of a rod-like SmA LC molecules, 4’(5,5,6,6,7,7,8,8,9,9,10,10,11,-11,12,12,12-heptadecaflu-orododecyloxy)-biphenyl-4-carboxylic acid ethyl ester using SU-8 micropillars as topographic confinement in 3D.(Honglawan, Beller et al. 2011) SU-8 is a multifunctional epoxy photoresist, which naturally promotes tangential anchoring to the SmA LC molecules. By design, LC casted on the SU-8 pillar array is confined by a 3D hybrid cell of tangential anchoring at the substrate and homeotropic anchoring at the LC/air...
interface, suitable for formation of TFCDs. The SU-8 pillar arrays with variable size, shape, and symmetry (e.g. square and hexagonal lattices) are fabricated by photolithography and soft lithography techniques to study directed SmA LC assembly. The study reported in Chapter 4 reveals that the most important requirements for the directed assembly and determination of TFCD formation is the design of the pillar array, specifically its dimension including height, diameter, center-to-center spacing of the nearest two diagonal pillars (for a square lattice). From a series of experiments, a critical value for each parameter defining the pillar array is found, above which the system energetically favors the formation of stable TFCDs. Within the designed 3D cell, each TFCD can be grown either on the top of each pillar or between neighboring pillars or both depending on the LC thickness relative to the pillar height. As a result, a variety of new TFCD arrays beyond the close-packed hexagonal arrangement are formed where both dimension and symmetry of the pillar arrays propagate through the TFCDs for LC thicknesses up to 40 µm above the pillar top, demonstrating the unique long-range ordering into the bulk from surface epitaxy. The epitaxial approach offers an entirely new and promising organizational principle for smectic LC systems using simple topographic substrates.

By decreasing the pillar height below its critical value, a transition from confinement of isolated domains to the “pillar edge-pinning” effect of multiple FCDs yielding hierarchical growth of FCDs, tangent to their neighbors, with their hyperbolic focal lines pinned near the pillar edges is observed as a result of minimization of the global free energy of FCDs. The mechanism of this phenomenon is investigated in detail in Chapter 5. Along the line, the key strategy for constructing the specific form of hierarchically organized FCD arrays is discovered that the center-to-center spacing of the nearest two diagonal pillars (for a square array of circular pillars) must be approximately 4 times the domain radius such that the nonpatterned region surrounded by 4 neighboring pillars can accommodate multiple domains with tangential ellipses as opposed to just one domain as in the previous case in Chapter 4. The size and shape of the pillars (circular, elliptical, triangular, and Y shapes) can be used to control the type of hierarchical FCD
arrangement; the anisotropy of the pillar shape enables a precise prediction of the locations of FCDs relative to the substrate patterning due to the competing effect between the effective attraction of FCDs to pillar corners and the steric repulsion between domains. Furthermore, these organized FCDs within the edge-pinning regime are found to have non-zero eccentricity, yielding a variety of unique surface profiles of the LC structure. The nontrivial, but apparently smooth, matching of smectic layers between neighboring FCDs on a non-uniform substrate presents an intriguing theoretical problem for which a geometric ansatz is proposed and is found in good agreement with the experimental data. Chapters 4 and 5 in this thesis establish the fundamental design of the pillar arrays with variable dimensions such that they impose both molecular anchoring and geometric restrictions on the 3D structure of FCDs with an exceptionally fine level of control from feature size, spatial distribution, symmetry to intrinsic topology of FCDs. Last but not least, these findings through template-assisted self-assembly approach should expand a wide range of current and potential applications of the identified assembled structures from soft materials as well as open a new avenue toward assembly of more complex 3D structures and geometries, essential for revolutionizing the future technologies from photonics, phononics, chemical and biological sensing, data storage to electronic devices.
References


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CHAPTER 1

FACILE SYNTHESIS OF NANOPARTICLES VIA ASSEMBLY OF PHOTOPOLYMERIZED AND SELF-CROSSLINKED RANDOM COPOLYMERS IN SELECTIVE ORGANIC MEDIA


Introduction

Nanocarriers and nanoreactors are of interest for potential applications, including catalysis, (Joly, Kane et al. 2000; Chen, Schönherr et al. 2009; Lu, Proch et al. 2009; Kim, Meeuwissen et al. 2010) encapsulation and controlled delivery of drugs, proteins, DNAs, RNAs, cosmetics, nutrients and food, (Discher and Ahmed 2006; Li and Szoka 2007; van Dongen, van Hoog et al. 2009; Kim, Meeuwissen et al. 2010) Amphiphilic molecules, such as phospholipids and block copolymers, which comprise of at least two chemically dissimilar segments, are known to spontaneously self-organize into various nanostructures, including spherical micelles, cylindrical micelles, vesicles, compound micelles, and inverted micelles. In the case of assemblies from block copolymers, the size and shape of the assemblies are regulated by the interaction parameter, (Svensson, Olsson et al. 2000) molecular architecture, polymer molecular weight, (Bermudez, Brannan et al. 2002; Choucair, Lavigueur et al. 2004) composition, (Choucair, Lavigueur et al. 2004; Choi, Koo et al. 2005; Adams, Butler et al. 2006; Yu, Azzam et al. 2009) polymer concentration, and solvent quality, (Choucair, Lavigueur et al. 2004; Sanson, Schatz et al. 2010) Among them, vesicles are specifically attractive because of potential high loading efficiency and the ability to encapsulate both hydrophobic and hydrophilic molecules in different portions of the structures, (Rösler, Vandermeulen et al. 2001)
Compared to well-defined block copolymers, random copolymers are readily available with a wide range of chemistry and compositions. However, their assemblies in solutions are often ill-defined and unstable, and the size is rather larger (a few hundreds of nm’s to a few microns) due to the chemical heterogeneity in the polymer chains. Recently, several groups have shown elegant designs of amphiphilic random copolymers to assemble well-defined nano- and microparticles. (Ilhan, Galow et al. 2000; Pollino, Stubbs et al. 2003; Liu, Kim et al. 2005; Lutz, Pfeifer et al. 2006; Thibault, Uzun et al. 2006; Gao, Li et al. 2008; Tian, Yu et al. 2008) Robust vesicles are obtained by crosslinking the membrane via specific interactions, for example, hydrogen bonding in random copolymers of poly(styrene-co-methacrylic acid), (Liu, Kim et al. 2005) and complementary random copolymers functionalized with complementary diaminopyridine and thymine moieties. (Ilhan, Galow et al. 2000; Thibault, Uzun et al. 2006)

While many of the studies are reported from polymers self-assembled in aqueous solutions, (Discher, Won et al. 1999; Burke and Eisenberg 2001; Discher and Eisenberg 2002; Choucair, Lavigneur et al. 2004; Discher and Ahmed 2006) assemblies in non-aqueous media via solvophobic effect (Zhang, Yu et al. 1996; Zhang, Shen et al. 1997; Burke and Eisenberg 2001; LaRue, Adam et al. 2004; Bang, Jain et al. 2006; Korczagin, Hempenius et al. 2006; Wang, Liu et al. 2009) are also of interest to create new morphologies, and to improve the loading of hydrophobic and large molecular weight encapsulants by utilizing the cavity and outer shell of the vesicles. Therefore, it will be attractive to develop a simple method to prepare stable vesicular nanoparticles in a non-aqueous medium, which could be transferred and utilized in an aqueous media. Acryloyl chloride (AC) is one such interesting monomer. It is highly reactive and sensitive to moisture. Its homopolymerization is known to be rather difficult due to side reactions, which could lead to crosslinking. (Serenson and Campbell 1961) If crosslinked, the polymer chains will precipitate from the good solvent of poly(acryloyl chloride) (PAC). Meanwhile, the AC groups can be hydrolyzed to carboxylic acid groups before and after the polymerization for further functionalization.
Here, we report synthesis of vesicular nanoparticles (~ 60 - 100 nm in diameters) assembled from amphiphilic and partially self-crosslinked random copolymers from PAC, consisting a small amount of anhydride groups, in various organic solvents. The random copolymers were photopolymerized in bulk from AC with partially hydrolyzed byproduct, acrylic acid (AA) (< 10 mol%) due to reaction with the moist air at ambient environment. FT-IR spectra and electron micrographs suggested that AA was responsible for the formation of anhydride, leading to the change of solubility from the majority PAC chains in a selective solvent, such as acetone. Transmission electron microscopy (TEM) images and light scattering results implied that the nanoparticles were vesicular in nature with rather thin membranes (~ 2.3 nm). The particles were highly robust and maintained the spherical shape after air-drying. When AA was deliberately introduced to the monomer solutions during photopolymerization, or varying the UV dosage (from 200 mJ/cm\(^2\) to 10,000 mJ/cm\(^2\)), we observed changes in ratio of acid anhydride groups to PAC from FT-IR analysis, particle size (from a few nanometers to a few microns), and morphology (from small spherical micelles to vesicles and large aggregates) from polymers assembled in acetone. In contrast, polymers photopolymerized in a dry glovebox and then dispersed in anhydrous acetone failed to produce particles. These results confirmed that hydrolysis of AC monomers and the formation of acid anhydride groups during photopolymerization played important roles to the amphiphilic nature of the random copolymers, and thus the morphology and stability of the assemblies. The amphiphilic property of the polymers was further investigated from their assemblies in a wide range of organic solvents of different solubility, including acetonitrile, tetrahydrofuran (THF), 1,4 dioxane, isopropanol (IPA), ethanol, toluene, xylene, and hexane.

Results and Discussion Section

Polymer synthesis and formation of nanoparticles. As seen in Fig. 1.1, AC monomers were first photopolymerized in bulk in a glass vial under UV irradiation (\(\lambda = 365 \text{ nm}\)). After purified by centrifugation in toluene, the photopolymerized product was dispersed in acetone and
spontaneously formed spherical nanoparticles with high reproducibility, suggesting the photopolymers might be amphiphilic. At UV dosage of 2,000 mJ/cm$^2$, the particles have an average size of 43.1 ± 4.9 nm in radius (over 200 particle counts) according to SEM images (see Fig. 1.2). The hydrodynamic radius ($R_H$) of the polymer assembly in acetone was measured by DLS as 45.3 ± 3.5 nm with relatively low polydispersity index, 0.27. The radius of gyration, $R_G$, of the structure was estimated 46.2 ± 1.9 nm by SLS using Guinier approximation model. The morphological ratio of the aggregate, $\rho = R_G / R_H$, was 1.02, close to 1, characteristic of vesicular geometry (see Table 1.1).

The vesicular morphology of the particles were confirmed by TEM with the appearance of a thin dark ring due to the higher density in the membrane region (Fig. 1.2b). (Hickey, Haynes et al. 2011) The TEM contrast was further enhanced by staining the particles with a trace amount of TOPO stabilized CdSe nanocrystals (~ 2 nm in diameter), which selectively associated to the solvophobic chains of the polymers without disrupting the assembly process (see Supplementary Information and Fig. S1.1). The stained nanoparticles revealed a membrane thickness of ~ 2.3 nm (Fig. 1.2c inset). The thin membrane is typical for assemblies from random copolymers since the interfacial energy between the core and shell molecules is rather small. (Du and O'Reilly 2009; Zhu and Liu 2011) Surprisingly, the obtained nanoparticles were highly robust against drying. When the nanoparticle solution was deposited on the Si wafer, followed by air-drying and vacuuming in the SEM and TEM chambers, the nanoparticles maintained the spherical shape and hollowness without collapse (see the inset of Fig. 1.2a) until the particle size was greater than half micron obtained at a much higher UV dosage (details in the following section).

**Amphiphilicity of the photopolymer.** Typically, to prepare homopolymers of PAC, the initial reactants need to be carefully purified and handled in a dry environment to prevent hydrolysis. In our experiments, the photopolymerization was carried out in an ambient indoor...
Figure 1.1 Schematic illustration of the synthesis and assembly of random copolymers of PAC/X in acetone.

Figure 1.2 (a) SEM image of non-collapsed nanoparticles dispersed in acetone and captured on a Si wafer. The polymers were synthesized at a UV dosage of 2,000 mJ/cm². The polymer concentration is 0.13 mg/mL. Scale bar is 500 nm for the inset. (b) TEM image of the particles synthesized in (a). (c) TEM image of the polymer assembly as in (a) but stained with TOPO-stabilized CdSe (2 nm in diameter) in toluene (5 wt. %). Inset: higher magnification, highlighting the thin membrane of the vesicle.
**Table 1.1** Summary of the effects of UV dosage to the synthesized polymers and their assemblies based on light scattering measurements and SEM analysis. *The particle size from SEM was determined by ImageJ analysis averaged over 200 particles.*

<table>
<thead>
<tr>
<th>UV dosage (mJ/cm²)</th>
<th>500</th>
<th>1,000</th>
<th>2,000</th>
<th>4,000</th>
<th>8,000</th>
</tr>
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<tbody>
<tr>
<td>DLS $R_h$ (nm)</td>
<td>17.7±1.6</td>
<td>30.1±1.1</td>
<td>45.3±3.5</td>
<td>72.2±30.3</td>
<td>320.1±102.2</td>
</tr>
<tr>
<td>PDI</td>
<td>0.15</td>
<td>0.22</td>
<td>0.27</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>SLS $R_G$ (nm)</td>
<td>14.5±2.1</td>
<td>31.7±1.5</td>
<td>46.2±1.9</td>
<td>70.9±27.2</td>
<td>N/A</td>
</tr>
<tr>
<td>$\rho$</td>
<td>0.82</td>
<td>1.05</td>
<td>1.02</td>
<td>0.98</td>
<td>N/A</td>
</tr>
<tr>
<td>Morphology</td>
<td>Micelle</td>
<td>Vesicle</td>
<td>Vesicle</td>
<td>Vesicle</td>
<td>Vesicle*</td>
</tr>
<tr>
<td>SEM $R_{SEM}$ (nm)</td>
<td>15.9±1.2</td>
<td>28.4±1.7</td>
<td>43.0±4.9</td>
<td>77.3±14.8</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* Determined by SEM image. $R_{SEM}$ = an average of measured particle radius of over 200 particles from SEM by ImageJ analysis. N/A for the samples that were too polydispersed for a measurement or contained multiple size distributions.
environment (20 – 40% humidity; 20 – 30°C). Therefore, it is likely that certain percentage of AC was hydrolyzed to acrylic acid (AA). However, no hydroxyl peaks appeared in the FT-IR spectrum of the synthesized polymers (see Fig. 1.3a). Instead, we observed an asymmetric carbonyl C=O stretch peak at 1778 cm\(^{-1}\), attributed to PAC or acid anhydride, an ester C-O stretch peak at 1039 cm\(^{-1}\) from the acid anhydride, and a characteristic peak of alkyl halide, C-Cl stretch at 731 cm\(^{-1}\).

It has been reported that when photopolymerized in bulk by radical initiator, 2,2'-azobisisobutyronitrile (AIBN), PAC could be partially crosslinked due to the photolysis of AC to ketene (R'R''C=C=O) through elimination of an HCl from AC. (Egorova, Migunova et al. 2002) Ketenes could undergo [2+2] cycloaddition reactions with carbonyl groups (C=O), forming \(\beta\)-lactones. They could also react with hydroxyl groups (R-OH) and another ketene to yield ester groups (-O-R-CO-R'-CO-) or anhydride groups (R'OC-O-CO-R''). Therefore, it is likely that the detected anhydride groups were the side products of the bulk photopolymerization of AC and/or through intra- or intermolecular chain reactions between AC and AA groups. We have attempted to characterize the chemical composition via \(^1H\) NMR using CDCl\(_3\), CD\(_2\)Cl\(_2\) and THF-d8 as solvents, respectively. However, the acid or acid anhydride peak was not well detected and the peak assignment was not always consistent, suggesting that the polymers did not completely dissolve in these solvents, possibly due to crosslinking and micelle formation in the solvent. If the photopolymerized chains are partially crosslinked, they become amphiphilic in an organic media, such as acetone: the PAC chains are highly solvophilic, whereas the crosslinked anhydride ones are solvophobic. Further confirmation of chain amphiphilicity and the micelle formation in various selective solvents will be presented later. Here, for simplicity, we refer to the photopolymer product as a random copolymer (see Fig. 1.4a for representation of the chemical structure) consisting of PAC and a small amount of self-crosslinking component (X) comprising of acid anhydride groups.
Figure 1.3 FT-IR spectra of random copolymers from photopolymerized AC with addition of [AA]₅ at (a) 0, (b) 5, (c) 10 and (d) 20 mol%. The total volume is 3 mL. The total UV dosage is 2,000 mJ/cm².
Figure 1.4 (a) Schematic of possible chemical structure of photopolymerized AC containing PAC chains and crosslinked X groups. (b) Schematic of the cross-sectional view of the random copolymer vesicle synthesized at UV dosage of 1,000 mJ/cm² or greater.

To verify the role of AA in photopolymerization and nanoparticle formation, we fixed the UV dosage (2,000 mJ/cm²) and photoinitiator concentration (2% v/v), but added variable amount of AA ([AA]₀/[AC + AA]₀ = 5 – 20 mol%) for photopolymerization. The total monomer volume (3 mL) was kept constant. As expected, the addition of AA increased the C-O stretch peak intensity at ~ 1060 cm⁻¹, and broadened the C=O stretch peak at ~ 1770 cm⁻¹ due to overlapping carbonyl signals of acid anhydride with alkyl halide of PAC (see Fig. 1.3). The results clearly indicated that AA participated in the formation of acid anhydride. However, no acid or hydroxyl peaks were observed from the copolymer when the initial AA concentration, [AA]₀ was less than 20 mol%, suggesting that the acid groups were nearly consumed and contributed to crosslinking reactions. When [AA]₀ was increased to ≥ 20 mol%, the photopolymer immediately precipitated out of the monomer solution (see Fig. S2) during photopolymerization and a broad peak at 2533 – 3512 cm⁻¹, representing O-H stretch of carboxylic acid, appeared in the FT-IR spectrum (see Fig. 3d). Since the reactivity of AA was higher than that of AC, the observed large aggregates in the solution should be mainly composed of poly(acrylic acid) (PAA) due to its immiscibility with the monomer.(Palit and Ghosh 1962; Jenkins and Jenkins 1996) These results unequivocally confirmed the role of AA in the chain crosslinking, which rendered the photopolymers amphiphilic, leading to vesicle formation with crosslinked X core and PAC corona as shown in Fig. 1.4b.

The size and morphology of polymer assemblies also changed with the addition of AA. As seen from SEM images in Fig. 1.5, when [AA]₀ was ≤10 mol%, the copolymers assembled in acetone (0.13 mg/mL) appeared as spherical particles with the size (82 ± 3 nm in diameter) comparable to those without addition of AA, 90.6 ± 6 nm in diameter (Fig. 1.5 a and b). At [AA]₀ = 10 mol%, a mixture of spherical particles (75 ± 2 nm in diameter) (Fig. 1.5c) and small aggregates (~ 5 nm in diameter) was observed. When [AA]₀ = 20 mol%, only irregular aggregates appeared
throughout the sample (Fig. 1.5d), in agreement with the observed cloudiness and precipitation of polymers seen in Fig. S1.2. Therefore, we estimated that $[AA]_0$ in the monomer solution should be no greater than 10 mol% of $[AC]_0$ to form well-defined nanoparticles.

**Figure 1.5** SEM images of particles formed from photopolymers with $[AA]_0$ at (a) 0, (b) 5, (c) 10, and (d) 20 mol%. The UV dosage was 2,000 mJ/cm$^2$. The polymers were dispersed in acetone at 0.13 mg/mL. Scale bar: 200 nm, applicable to all images.
Figure 1.6 FT-IR spectra of copolymers from photopolymerized AC at 2,000 mJ/cm² UV dosage for a total volume of 3 mL with variable processing environment. (a) The polymers were photopolymerized at ambient environment using non-anhydrous solvents. (b-c) The polymers were photopolymerized in a glovebox (H₂O ~ 2 ppm) using anhydrous solvents (b) and non-anhydrous solvents (c). The polymer solutions prepared in (b) were exposed to air for 2 h (d) and 24 h (e) prior characterization.
To further confirm the importance of hydrolysis of AC to AA to the resulting polymer amphiphilicity, we investigated contributions from two factors, including environmental humidity and trace of water in solvents. When the synthesis of PAC was carried out following the same procedure described earlier (UV dosage of 2000 mJ/cm²), but using a fresh bottle of AC in a glovebox purged with argon (H₂O ~ 2 ppm), no C-O peak of acid anhydride was observed in the FT-IR spectrum (Fig. 1.6b). No vesicles were observed from DLS and SEM (see Fix. S3) when the polymers were dispersed in anhydrous acetone. Instead, small aggregates (Dₜ = 18.2 ± 1 nm) and a layer of polymer film were deposited on Si-wafer, suggesting formation of homopolymer of PAC in the glovebox.

We then examined whether trace water in the dispersing organic solvents contributed to the hydrolysis of PAC, and subsequent formation of anhydride. PAC prepared in the glovebox was purified and dissolved in non-anhydrous toluene and acetone, respectively, outside the glovebox. FT-IR spectrum of the polymer revealed a small ester C-O peak at 1039 cm⁻¹, indicating occurrence of hydrolysis and formation of anhydride (see Fig. 1.6c). However, no vesicles were obtained from these polymers. This may be explained by the rather small portion of crosslinked anhydride in relative to PAC in the polymer chains; therefore, no phase separation occurred between anhydride chains and PAC. When the anhydrous polymer solution prepared in the glovebox was left in air (31% humidity, 16°C) for 2 h, a small C-O peak from acid anhydride appeared, however, the relative intensity ratio of the C-O peak at 1042 cm⁻¹ to the alkyl halide C-Cl peak at 735 cm⁻¹ was much less than that of the control amphiphilic photopolymer (see Fig. 1.6a and d). Therefore, only small aggregates with Dₜ of 13.54 ± 1.2 nm were detected by DLS. These results suggest that hydrolysis of an appreciable amount of AC is necessary for vesicle formation. When the above prepared polymer solution was left in air overnight, the polymers became partially soluble in acetone, and exhibited a relatively large C-O peak in FT-IR from the solution casted on the NaCl plate (see Fig. 1.6e). Accordingly, aggregates with multimodal size distribution were observed from DLS. Based on the above results, we conclude that
environmental humidity contributes to the hydrolysis of AC monomers, and the degree of hydrolysis determines the relative composition of solvophobic crosslinked anhydride vs. solvophilic PAC, which in turn dictates the morphology of polymer assemblies in the organic solvent.

**Effects of UV dosage to assembly morphology.** The size and packing morphology could be tuned by UV dosage applied to the monomer solution. As seen from static and dynamic light scattering results and SEM images shown in Fig. 1.7 and Table 1.1, the particle diameter increased from $35.5 \pm 4 \text{ nm} (500 \text{ mJ/cm}^2$, Fig. 1.7a) to $90.6 \pm 6 \text{ nm} (2000 \text{ mJ/cm}^2$, Fig. 1.7b), and to $882.2 \pm 75 \text{ nm} (10,000 \text{ mJ/cm}^2$, Fig. 1.7c). Polymers synthesized at UV dosage of 500 mJ/cm$^2$ formed small spherical micelles with PDI of 0.15 (from DLS) and $\rho = 0.82$ (from SLS), whereas polymers synthesized at 1,000 mJ/cm$^2$ or higher dosages self-organized to stable vesicles in acetone with PDI $\geq 0.22$ and $\rho$ close to 1. The results implied that molecular weight (MW) and degree of crosslinking increased with UV dosage. We attempted to characterize the MW of polymers polymerized at different UV dosages using size exclusion chromatography (SEC). In eluent, THF, SEC gave the number-average of molecular weight ($\bar{M}_n$) of 429 and 29,150 g/mol and weight-average of molecular weight ($\bar{M}_w$) of 437 and 45,600 g/mol for polymers exposed at UV dosage of 500 and 2,000 mJ/cm$^2$, respectively, followed by esterification of acryloyl chloride groups for SEC. However, as we show later, the polymers would form spherical micelles in THF. At higher UV dosage, large aggregates were obtained (see Table 1.1) and most likely filtered before entering SEC columns. Thus, the MW characterized by SEC was not sufficient to study UV dosage effect.

FT-IR spectra of polymers obtained at different UV dosages (see Fig. 1.8) showed that the peak signal of the anhydride $\text{C}=\text{O}$ stretch at $1774 \text{ cm}^{-1}$ became stronger and broader with
Figure 1.7 Hydrodynamic diameters from DLS measurement of the assemblies in acetone as a function of UV exposure dosage. The polymer concentration was at 0.03 g/mL. SEM images of nanoparticles photopolymerized at a UV dosage of 500 (a), 2,000 (b) and 10,000 (c) mJ/cm², followed by dispersing in acetone at 0.13 g/mL. The particles were captured on Si wafers and air-dried. Scale bar: 500 nm, applicable to all images.
Figure 1.8 FT-IR spectra of random copolymers polymerized at UV dosage of (a) 200, (b) 500, (c) 1,000, (d) 2,000, (e) 4,000, and (f) 8,000 mJ/cm².
increase of UV dosage in relative to the C-O stretch peak at 1056 cm$^{-1}$, confirming the increase of the MW and relative ratio of solvophobic to solvophilic portions, thus, leading to the change of polymer morphology (from spherical micelle to vesicle as determined by light scattering measurements) in solution. In short, the lower UV dosages (100 – 500 mJ/cm$^2$) afforded low MW polymers or oligomers with a much higher content of X, leading to aggregates at any concentration. Conversely, when the size of the flexible PAC chains in solution becomes sufficiently large and comparable to that of the anhydride when UV dosages is greater than 500 mJ/cm$^2$, vesicles are formed with X core and PAC corona. Based on the uniformity of particle size and shape, we conclude that UV dosage of 2,000 mJ/cm$^2$ is optimal for photopolymerization to assemble vesicular nanoparticles.

**Self-assembly mechanism and solvency effect on particle formation.** To further elucidate the amphiphilic nature of the photopolymers and their assembly, we investigated the solvency effect by dispersing the photopolymers obtained at 2,000 mJ/cm$^2$ in a series of organic solvents. The solubility of PAC in different solvents was evaluated by calculating the Flory-Huggins interaction parameters ($\chi$) between AC and solvents using Hansen solubility parameters (see details in Supporting Information and summary in Table 1.2). (Hansen 2007) Due to the similar chemical and solubility nature, we used AC monomer to approximate PAC. We then compared the $\chi$ values with the morphologies of polymer assembly in different solvents determined by light scattering measurements (see Fig. 1.9, Table 1.2 and Table S1.1). As a result, vesicular nanoparticles (70-530 nm in diameter) were formed in relatively good solvents with $\chi \leq 1.12$, including acetonitrile, acetone, THF and 1,4-dioxane: larger vesicles were obtained in the solvent with higher affinity of the PAC chains due to relaxation of the surface tension at the membrane shell, allowing more polymer chains to pack in the membrane. It is shown that the particle distribution also became more dispersed with large particles such as ones assembled in acetonitrile when the particle diameter was over half a micron with standard deviation of over 200 nm. In contrast,
Figure 1.9 The distribution and hydrodynamic diameter of particles of nanoparticles photopolymerized at UV dosage of 2,000 mJ/cm², followed by centrifugation and dispersion in various solvents: (a) acetonitrile, (b) acetone, (c) THF and (d) 1,4-dioxane. The polymer concentration is 0.13 g/mL.
Table 1.2 Summary of Hansen solubility parameters (δ_D: dispersion, δ_P: polarity, δ_H: hydrogen interaction) of various solvents and acryloyl chloride and Flory-Huggins interaction parameters (χ) for various organic solvents against acryloyl chloride. Note that the solubility was determined by observation of the polymer solution while the morphology was based on the results of SLS and DLS. The reported particle size was hydrodynamic diameter. The polymers were synthesized at the UV dosage of 2,000 mJ/cm², followed by centrifugation and redispersion in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>δ_D</th>
<th>δ_P</th>
<th>δ_H</th>
<th>χ</th>
<th>Morphology and size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acryloyl chloride</td>
<td>16.2</td>
<td>11.6</td>
<td>5.4</td>
<td></td>
<td>Vesicle ~ 530 ± 212 nm in diameter</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>16.0</td>
<td>12.8</td>
<td>6.8</td>
<td>0.02</td>
<td>Vesicle ~ 90.6 ± 4.9 nm in diameter</td>
</tr>
<tr>
<td>Acetone</td>
<td>15.5</td>
<td>10.4</td>
<td>7.0</td>
<td>0.04</td>
<td>Vesicle ~ 90.9 ± 24 nm in diameter</td>
</tr>
<tr>
<td>THF</td>
<td>16.8</td>
<td>5.7</td>
<td>8.0</td>
<td>0.35</td>
<td>Vesicle ~ 67.7 ± 19 nm in diameter</td>
</tr>
<tr>
<td>Dioxane</td>
<td>19.0</td>
<td>1.8</td>
<td>7.4</td>
<td>1.12</td>
<td>Insoluble</td>
</tr>
<tr>
<td>IPA</td>
<td>15.8</td>
<td>6.1</td>
<td>16.4</td>
<td>1.16</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Ethanol</td>
<td>15.8</td>
<td>8.8</td>
<td>19.4</td>
<td>1.18</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Toluene</td>
<td>18.0</td>
<td>1.4</td>
<td>2.0</td>
<td>1.36</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Xylene</td>
<td>17.6</td>
<td>1.0</td>
<td>3.1</td>
<td>1.53</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Hexane</td>
<td>14.9</td>
<td>0.0</td>
<td>0.0</td>
<td>2.22</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>
polymers precipitated out in relatively poor solvents of PAC, including IPA, ethanol, toluene, xylene, and hexane. The solvency study confirms the amphiphilic nature of the photopolymers and their resulting assembly morphologies in various solvents.

Conclusions

We presented a facile route to synthesize highly robust hollow nanoparticles with variable size from the assemblies of amphiphilic random copolymers of PAC in selective organic solvents. The copolymers were synthesized through bulk photopolymerization of acryloyl chloride at ambient condition. FT-IR analysis verified that AA, the hydrolyzed AC monomer, played an important role to the formation of acid anhydride in the polymer chain, leading to solubility change from the solvophilic PAC chains in an organic medium. Solubility study, TEM images and light scattering measurements suggested that the polymers were amphiphilic and the particles assembled from polymers synthesized with UV dosage of 2000 mJ/cm\(^2\) were vesicular. The particle size and structure could be further tuned by AA concentration, UV dosage applied to the monomer solution, and the choice of solvent for assembly. Since the shell of the nanoparticles is PAC, they can be converted to PAA, making the nanoparticles compatible with water. Their chemical and physical properties can be further tuned by surface functionalization of the carboxyl groups for a wide range of potential applications. We currently exploit the nanoparticles as carriers in drug and gene delivery.
Experimental Section

Materials. Monomers, AC (> 97.0%) and AA (99.5%) were purchased from Sigma-Aldrich and used as received. Photo-initiator, 2-hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur® 1173) was obtained from Ciba Specialty Chemicals. Solvents including acetonitrile (HPLC grade), tetrahydrofuran (THF, HPLC grade), acetone (ACS grade), acetone (99.8%, H₂O < 50 ppm, ACS grade), 1,4 dioxane (ACS grade), 2-isopropanol (IPA, HPLC and ACS grade), ethanol (99.5%, ACS grade), toluene (HPLC grade), toluene (99.8%, H₂O < 30 ppm, ACS grade), xylene (ACS grade), and hexane (HPLC grade) and all other reagents were purchased from Fisher Scientific and used as received unless noted.

Synthesis of polymer nanoparticles. The polymers were synthesized by photopolymerization in bulk. In a typical procedure, AC (3 mL) was mixed with the photoinitiator, Darocur® 1173 (2% v/v) in a 5 mL glass vial in open air. The glass vial was then capped and placed horizontally under a UV light source (97435 Oriel Flood Exposure Source from Newport, intensity of 54 mW/cm² at λ = 365 nm) for irradiation at different set dosages. After irradiation, polymer/AC mixture (200 µL) was mixed with an excess amount of poor solvent, toluene (HPLC grade), and purified by centrifugation (10,000 rpm, 30 min), followed by alternating rinsing with toluene and acetone three times. The purified polymer was then redispersed in acetone to form nanoparticles. Photopolymerization was also carried out in a glovebox filled with argon (MBRAUN, 2 ppm H₂O) for comparison.

Characterization. The morphologies of nanoparticles were characterized by scanning electron microscopy (SEM) on FEI Strata DB235 focused ion beam (FIB) system at 5 kV and transmission electron microscopy (TEM) on the high resolution TEM JEOL 2010 at 80 kV. The nanoparticles were cast on silicon wafers (2 cm x 2 cm), which were cleaned by soaking in 2% v/v of Detergent 8 (Alconox) on a hot plate at 60 °C for 1 h, followed by rubbing with a cotton swab and rinsing with DI water several times. The wafers were then dried under compressed air and cleaned...
additionally by oxygen plasma cleaner (PDC-100, Harrick Scientific Products, 30 W) for 1 h. To capture the nanoparticles, the freshly cleaned Si wafers were submerged in the polymer solutions described above for 10 s and dried with compressed air. The size of the assemblies and their distributions were measured from SEM images using ImageJ software. Each data point was averaged over 200 particles.

For TEM, the particles (0.13 g/mL in acetone) were first purified by centrifugation 3 times using excess toluene and redispersed in acetone, followed by drop-cast on a holy copper grid (200 meshes from Electron Microscopy Sciences). To enhance contrast of the particle shell for structural analysis, a trace amount (10 µL) of CdSe nanocrystals (2 nm in diameter) stabilized with trioctylphosphine oxide (TOPO) in toluene (5 wt. %) was added to the prepared polymer/acetone solution. After shaking for a few seconds, the solution was drop-casted on the TEM grid.

Fourier transform infrared (FT-IR) spectra were obtained from Nicolet 450 FT-IR equipped with an MCT/B detector at a resolution of 1.93 cm^{-1}. To prepare the sample, purified random copolymers (60 mg) were dispersed in acetone (2 mL), followed by drop casting on a NaCl disk. The sample was dried in vacuo for 3 h before taking FT-IR spectra. Characteristic peaks of FT-IR (film). Random copolymers: 2935 and 2863 (C-H anhydride and C-H alkane, stretch), 1778 (C=O anhydride, stretch and Cl-C=O, acyl halide, stretch), 1039 (C-O anhydride, stretch), and 731 cm^{-1} (C-Cl, stretch). Random copolymers with initial [AA] of 5 and 10 mol% of the total monomers: 2929 (C-H anhydride and C-H alkane, stretch), 1767 (C=O anhydride, stretch and Cl-C=O, acyl halide, stretch), 1049 (C-O anhydride, stretch), 735 cm^{-1} (C-Cl, stretch). Random copolymers from initial [AA] of 20 mol%: 3528 - 2541 (O-H carboxyl, stretch), 2911 and 2850 (C-H anhydride and C-H alkane, stretch), 1749 (C=O anhydride, stretch and Cl-C=O, acyl halide, stretch), 1048 (C-O anhydride, stretch), 740 cm^{-1} (C-Cl, stretch).
Dynamic light scattering (DLS) was carried out on a Malvern Nano-S zetasizer ($\lambda = 633$ nm) to obtain hydrodynamic diameter ($D_H$) and polydispersity index (PDI) of the assemblies in acetone solutions prepared from different UV dosages. For each measurement, the polymer solution (0.03 g/mL) was filtered through a Millipore filter of nominal 5 µm pore size. The solutions were stabilized at 20 °C for 15 min before DLS measurements.

Static light scattering (SLS) was performed at 20 °C on a BI-APD detector and ALV laser goniometer ($\lambda = 632.8$ nm, Brookhaven Instruments) with 200 µm pinhole. Before each measurement, the sample/acetone solution (65 mg/mL) was filtered through a Millipore filter of nominal pore size of 0.45 µm. The accessible scattering angle range is from 10° to 150°.

Acknowledgments

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METAL NANOPARTICLES

SHELLS

POLYSTYRENE

BLOCK COPOLYMERS

UTZ

VENSSON

RANDOM VACROMOLECULES

GLUTAMIC ACID

NUCLEOBASES

L. NUCLEOBASES


SANSON, C., C. SCHATZ, ET AL. (2010). "BIOCOMPATIBLE AND BIODEGRADABLE POLY(TRIMETHYLENE CARBONATE)-B-POLY(L-


6274.


Supporting Information


Proof of binding between CdSe nanocrystals to the photopolymerized random copolymers

When CdSe nanocrystal/toluene solution (red, 10 wt. %, 3.75 mL, Fig. S1.1a) was first mixed with concentrated solution of random copolymer (opaque in acetone, 400 mg/mL, 1 mL), they phase separated. After vigorous mixing for a few seconds, the opaque polymer layer became light yellowish while the red color in toluene phase turned to yellow (Fig. S1.1b), indicating selective sequestering of the nanocrystals to the crosslinked acid anhydride groups in the copolymers due to solvophobic interaction.
**Figure S1.1** Photographs of (a) CdSe nanocrystal solution in toluene at 10 wt. % and (b) a mixture of random copolymer solution in acetone (400 mg/mL) and nanocrystal solution in (a) after vigorous mixing (b).

**Figure S1.2** Photographs of the photopolymerized products of AC with an initial AA composition of 0, 5, 10 and 20 mol% at a total volume of 3 mL. The UV dosage is 2,000 mJ/cm² and the loading of photoinitiator, Darocur® 1173, is 2 v/v %. 
Figure S1.3 SEM images of photopolymers synthesized at 2000 mJ/cm² in a glovebox, followed by dispersion in anhydrous acetone at a concentration of 0.13 mg/mL.
Esterification of the photopolymerized random copolymers and MW analysis.

Because PAC is highly polar, for size exclusion chromatography (SEC) analysis, the as-synthesized copolymers were esterified by methanol. Approximately 1.4 mg of purified copolymers was obtained after centrifugation of 500 µL photo-polymerized product in 1.5 mL toluene at 11,000 rpm for 30 min, followed by rinsing with toluene and acetone, respectively, for three cycles and drying in vacuo at room temperature for 3 h. Excess anhydrous methanol (2 mL) was then added to the polymers under nitrogen atmosphere. The solution was kept at room temperature overnight allowing for a complete conversion of alkyl halide and acrylic acid to methyl ester. The resulting polymers were then dried in vacuo overnight before dissolving in 1 mL acetone for IR analysis, in CD$_2$Cl$_2$ for $^1$H NMR measurement and in THF for molecular weight analysis on SEC. The results confirmed a complete reaction and removal of monomers. FT-IR: 3007 -2853 (C-H alkane, stretch), 1731 (C=O carbonyl, ester, stretch), 1534 (C=O carbonyl, stretch), 1456 (C-H methyl, bending) and 1260 - 969 cm$^{-1}$ (C-O ester, stretch). $^1$H NMR: PMA: δ 3.6, 3.4 (t, 3H, CH$_3$, 2.3-2.1 (m, 1H, CH), 1.9-1.6, 1.2 (m, 2H, CH$_2$).

The molecular weight and distribution of the esterified photo-polymers were characterized by SEC equipped with an Applied Biosystems 785A UV-Vis detector, a Viscotek dual refractive index and viscometric detector Model 250, Waters M510 pump, and Waters U6K injector. The esterified polymers (PMA) were dissolved in THF for 48 h and filtered before being sampled against 20 polystyrene standards (PSS, Warwick, USA) in three 5-µm PL gel columns having pore sizes of 50 Å, 500 Å and a mixed C (Polymer Laboratories, Amherst) at 40 °C. The eluent flow rate was 1 mL/min. The data were processed by Omni-SEC (Viscotek) Software package, version 3.1.
Table S1.1 Summary of dynamic and static light scattering results of the polymer assemblies in various organic solvents. Radius of gyration, $R_g$, is determined by Guinier approximation and morphology of the aggregates is based on the value of $\rho (= \frac{R_g}{R_H})$.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Acetonitrile</th>
<th>Acetone</th>
<th>THF</th>
<th>Dioxane</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_g$ (nm)</td>
<td>N/A</td>
<td>46.2</td>
<td>45.0</td>
<td>30.8</td>
</tr>
<tr>
<td>$\rho$</td>
<td>N/A</td>
<td>1.02</td>
<td>0.99</td>
<td>0.91</td>
</tr>
<tr>
<td>Morphology</td>
<td>Vesicle*</td>
<td>Vesicle</td>
<td>Vesicle</td>
<td>Vesicle</td>
</tr>
</tbody>
</table>

*Morphology observed from SEM.*
Calculation of polymer-solvent interaction parameter.

The Flory-Huggins interaction parameter between polymer and solvent ($\chi$) was estimated with Hansen solubility parameters (HSP) which account for dispersion forces ($\delta_D$), permanent dipole-permanent dipole forces ($\delta_P$) and hydrogen bonding ($\delta_H$). (Hansen 2007) The interaction parameter can be calculated as the following:

$$\chi = \frac{VR_a}{4RT}$$

$$\left(R_a\right)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2$$

where $R_a$ is the solubility parameter distance between two materials based on their respective partial solubility parameter components. $V$ is the molar volume of the solvent. $R$ is the gas constant, and $T$ is the absolute temperature.

The Flory-Huggins interaction parameter was used to determine a degree of polymer solubility in solvent. $\chi < 0.5$ represents high affinity between the polymer and the solvent while $\chi > 0.5$ indicates that the polymer has poor solubility in a given solvent. Since the Hansen solubility parameters of the random copolymers and PAC homopolymers were not available, the interaction parameters between the monomer acryloyl chloride and solvents were calculated and used analogously to determine the solubility and interaction values of the solvent with the synthesized random copolymers under an assumption that the majority of synthesized polymer was PAC having the same Hansen solubility parameters as the monomer, AC. The assumption was justified by the agreement between the calculated results and the experimental results as shown in Table 1.2.

Reference

CHAPTER 2

EVAPORATIVE ASSEMBLY OF ORDERED MICROPOROUS FILMS AND THEIR HIERARCHICAL STRUCTURES FROM AMPHIPHILIC RANDOM
COPOLYMERS

Honglawan et al. Soft Matter 2012, 8 (47), 11897-11904.

Introduction

Because of their large surface area, light weight and well-defined porosity, ordered microporous polymeric films are of great interests for potential applications, including separation, filtration, catalysis, tissue engineering, photonics, and electronics. (Pieracci, Crivello et al. 2001; Srinivasarao, Collings et al. 2001; Davis 2002; Yamamoto, Tanaka et al. 2007; Xu, Wang et al. 2008; Li, Zhong et al. 2009; Tsai, Xu et al. 2010; Wong, Stenzel et al. 2010; Innocenzi, Malfatti et al. 2011) A number of methods have been developed to fabricate porous films in two dimensions (2D) and three dimensions (3D), including templating from arrays of colloid assembly, amphiphiles (e.g. surfactants and block copolymers) and emulsions, reaction-induced phase separation, and solvent-induced phase separation. (Yu, Meiser et al. 2003; Lu and Schüth 2006; Zhao and Collinson 2010; Guillen, Pan et al. 2011; Wu, Xu et al. 2012) Among them, breath figures (BFs) formed from polymers have been widely studied due to the simplicity and low cost in fabrication of microporous films with honeycomb structures over a large area. (Widawski, Rawiso et al. 1994; Srinivasarao, Collings et al. 2001; Cui, Peng et al. 2005; Cui, Xuan et al. 2005; Madej, Budkowski et al. 2008; Li, Zhao et al. 2010; Ferrari, Fabbri et al. 2011; Maniglio, Ding et al. 2011; Chen, Yan et al. 2012; Escalé, Rubatet et al. 2012)

The formation of BF relies on the generation of condensing water droplets on the cold surface of the evaporating polymeric solution and the subsequent organization of the droplets,
driven by the surface convection and capillary force. With continuing solvent evaporation, the polymer molds around the droplets as sacrificial templates prior complete solidification, leading to honeycomb structure. Hence, the morphology and periodicity of BF is dependent on the solvency of the organic solvent, airflow rate, temperature, and more specifically, the environmental humidity (typically > 50%).(Karthaus, Maruyama et al. 2000; Srinivasarao, Collings et al. 2001; Cui, Peng et al. 2005; Connal, Vestberg et al. 2008) Recently, a few groups have reported BF formation in a dry environment by introducing water in the polymer solution via mixing during polymer preparation prior film casting and solvent evaporation.(Li, Zhao et al. 2010; Chen, Yan et al. 2012) It will be intriguing to create highly ordered microporous structures in a water-free or low humidity environment through evaporative assembly.

Meanwhile, there has been increasing demand for creating hierarchical porous structures to emulate outstanding optical, wetting and mechanical properties manifested in nature by diatom,(Fuhrmann, Landwehr et al. 2004) butterfly wing scales,(Gu, Uetsuka et al. 2003) bone,(Weiner and Wagner 1998) wood,(Fratzl and Weinkamer 2007) and the aquiferous system of sea sponges.(Yao, Fang et al. 2011) Most existing fabrication techniques, however, are limited to the construction of porous structures on a flat surface via self-assembly approaches, which always yield hexagonal lattices, which may not always be favorable in device fabrication. By combining “top-down” and “bottom-up” approaches, a few groups have demonstrated hierarchical structures with arbitrary symmetries from colloids,(Yin, Lu et al. 2001) block copolymers(Kim, Solak et al. 2003; Hong, Wang et al. 2009; Munoz-Bonilla, Ibarboure et al. 2009; Arora, Du et al. 2010; Byun, Bowden et al. 2010; Byun, Han et al. 2010; Han, Byun et al. 2011; Han and Lin 2012) and liquid crystals(Honglawan, Beller et al. 2011) by controlling the position and ordering of superlattices using well-defined templates with desired surface chemistry.

Herein, we report fabrication of microporous films and their hierarchical structures through evaporative assembly of amphiphilic random copolymers (ranPAC), consisting of
poly(acryloyl chloride) (PAC) chains and self-crosslinked acid anhydride groups. The structures were obtained via dip coating a substrate in the copolymer solution, followed by air drying under ambient condition. The copolymers were dissolved in acetone with a trace amount of nonsolvent, toluene. It was found that the final morphology and dimensions of the assembled structures were highly dependent on the initial polymer concentration and the choice of solvent in a typically low relative humidity (20% – 40%) environment. Honeycomb structures were observed with an average pore diameter decreased from 2.5 µm to 600 nm when the copolymer concentration was increased from 75 to 200 mg/mL. It was hypothesized that toluene droplets were condensed at the polymer-solvent-air interface during evaporation of acetone, which then directed formation of the microporous film. To confirm this, we studied solvent vapor pressure and solvency of the minority solvent, and surface chemistry of the substrate. It was found that to obtain an ordered porous film, the minority solvent should be a moderately poor solvent of PAC with polymer-solvent interaction parameter value, \( \chi \) of 1.36 -1.53 and have low vapor pressure relative to acetone; the substrate should be relatively hydrophilic (e.g. Si wafer and SU-8) but not having strong interactions with the PAC chains. We then utilized SU-8 micropillar arrays with variable dimensions (diameter, \( D = 600 \text{ nm} - 4 \mu \text{m} \), aspect ratio, \( AR = 0.62 - 4 \), and pitch, \( P = 1.5 - 15 \mu \text{m} \))symmetries (square and hexagonal lattices), and chemistry (with and without activated hydroxyl groups) to create hierarchical porous films with tailored pore size, position, and arrangement. Finally, we demonstrated a highly fluorescent microporous film by selectively incorporating CdSe nanocrystals into the hydrophobic core of the amphiphilic copolymers in acetone/toluene solution, followed by solvent evaporation.
Results and Discussion

Evaporative assembly of microporous films from ranPAC on Si wafers. Random copolymers of acryloyl chloride were photopolymerized in bulk at ambient condition. Our earlier studies suggest that the copolymers are amphiphilic, comprising of solvophilic PAC chains and solvophobic self-crosslinked acid anhydride groups (see Fig 2.1a). (Honglawan, Xu et al. 2012) In a dilute solution (0.13 mg/mL) of acetone, a selectively good solvent of PAC, the ranPAC formed vesicle-like hollow nanoparticles, which maintained the shape when cast on a Si wafer due to the crosslinked anhydride core. When the copolymer solution was dip coated on a Si wafer at a much higher polymer concentration (≥ 75 mg/mL in acetone) with trace amount of toluene (a nonsolvent of PAC), highly ordered honeycomb microporous films were obtained (see Fig. 2.1b) over a large area (1 cm x 1 cm) within a few seconds, which is usually observed from well-defined polymers with amphiphilicity or complex architecture such as star.

Phase separation in a three-component system, consisting of a polymer, a solvent and a nonsolvent, has been a key process to create porous membranes. (Wang, Liu et al. 2006; Guillen, Pan et al. 2011; Samuel, Umapathy et al. 2011) The nonsolvent is a prerequisite for phase separation, thus, pore generation. When the polymer solution is in contact with a vapor or liquid of a nonsolvent, there is diffusion-induced change of the local composition of the polymer film, leading to demixing. When the polymer-rich phase is subject to a nonsolvent vapor, the nonsolvent vapor coagulates and diffuses into the polymer film. At equilibrium, membranes with spherical pores arranged in periodic structures are often generated. Such evaporation induced phase separation to create ordered structures requires a volatile solvent and a less volatile nonsolvent. In the case of BF formation, the less volatile solvent is water. Although the process is straightforward to create porous structures, the experimental conditions need to be carefully controlled since any undesired loss of solvent/nonsolvent will generate meta- or unstable compositions, leading to the loss of ordering during phase separation.
Figure 2.1 (a) Chemical structure of the photopolymerized random copolymer. (b) SEM image of a typical ordered porous film prepared at a polymer concentration of 75 mg/mL in acetone (1mL) and toluene (80 µL). (c) Schematic illustration of the synthesis and assembly of microporous film via solvent induced phase separation after dip coating the polymer solution on a Si wafer. Step (i): condensation of toluene droplets (blue) on evaporating polymer film. Step (ii): polymer imprint of the droplet arrays after complete evaporation.
Figure 2.2 SEM images of ranPAC films dip coated on clean Si wafers from 1 mL polymer/acetone solution (100 mg/mL) mixed with various amounts of toluene: (a) 30 µL, (b) 60 µL, (c) 90 µL, (d) 200 µL, (e) 300 µL and (f) 500 µL. Scale bar of 10 µm is applicable to (a) to (e).

Table 2.1 Summary of Flory-Huggins interaction parameters ($\chi$) for various solvents and acryloyl chloride based on Hansen solubility parameters, (Hansen 2007) their corresponding vapor pressures at 20°C and boiling points. (Afeefy, Liebman et al.)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\chi$</th>
<th>Vapor pressure (mmHg) at 20°C</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>0.04</td>
<td>184.0</td>
<td>56.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.36</td>
<td>22.0</td>
<td>110.6</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.47</td>
<td>75.0</td>
<td>80.1</td>
</tr>
<tr>
<td>Xylene</td>
<td>1.53</td>
<td>7.0</td>
<td>138.5</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>1.74</td>
<td>95.0</td>
<td>80.7</td>
</tr>
<tr>
<td>Hexane</td>
<td>2.22</td>
<td>132.0</td>
<td>69.0</td>
</tr>
</tbody>
</table>
In our system, we believe that toluene acts as the nonsolvent, which has a higher boiling point (b.p. 110.6°C) and lower vapor pressure (22.0 mmHg at 20°C) than acetone (b.p., 56°C and vapor pressure, 184.0 mmHg at 20°C). Acetone as a good solvent of polymer should evaporate first while toluene is condensed into droplets and phase-separated from the polymer film. Similar to surfactants in emulsions, the amphiphilicity of the copolymer functions to stabilize the nonpolar toluene droplets in thin films to minimize the interfacial tension while the droplets organize in close-packed fashion to minimize the entropic energy (see Fig. 2.1c). After toluene is completely evaporated, the polymer film is vitrified into honeycomb structures.

One of the major advantages of our system is that the ordered porous films are formed in an ambient environment at a low relative humidity (20 – 40%). In comparison, BF formation requires high relative humidity (> 50%) or addition of water in the solution to induce water condensation and control of solvent evaporation rate.

To verify our hypothesis, we added various volumes of toluene (30 - 500 µL) to 1 mL polymer /acetone solution (kept at 100 mg/mL) and investigated evolution of the porous structures (see Fig. 2.2). With increasing toluene volume, pores gradually appeared in the polymer films with increasing porosity and uniformity in pore size. In comparison, no porous film was generated in absence of toluene at any composition. At addition of 90 µL toluene, a highly uniform porous film with average pore diameter of ~ 1.8 µm was observed (Fig. 2.2c). Further increase of toluene led to dramatic increase of pore size (e.g. 2.7 µm with 200 µL toluene seen in Fig. 2.2d) and reduced pore regularity. When 300 µL toluene was introduced into the solution, nanoparticles together with microporous network were observed (see Fig. 2.2e), inferring the lack of control of toluene condensation at the polymer-solvent-air interface. The nanoparticles are similar to those cast from polymer/acetone solution, however, at a much lower concentration (1-25 mg/mL), where acetone is a relatively good solvent of PAC chains but a relatively poor solvent of anhydride groups.(Honglawan, Xu et al. 2012) It was likely that some polymers began to form
micelles when increasing toluene amount; supporting this was the appearance of giant vesicles with a complete loss of the porous network when 500 µL toluene was added to the solution (see Fig. 2.2f). Similar morphological transition influenced by nonsolvent has been reported in solvent evaporation induced phase separation systems. (Zhu and Liu 2011; Chen, Yan et al. 2012; de León, Muñoz-Bonilla et al. 2012) These results clearly confirm that toluene is responsible for pore generation in the film.

**Effect of nonsolvent.** Previously, we show that the polymer-solvent interaction parameter, $\chi$, plays a crucial role in micelle formation in acetone solution. (Honglawan, Xu et al. 2012) In order to further elucidate the pore formation mechanism and possibly broaden applications of ranPAC system, several organic solvents (each 80 µL) besides toluene were introduced as a nonsolvent to the polymer/acetone solution. The $\chi$ value was calculated using AC to approximate ranPAC, which was shown in reasonably good agreement with experimental observations. (Honglawan, Xu et al. 2012) As seen in Table 2.1 and Fig. 2.3, porous membranes with high regularity were produced from moderately poor solvents, including toluene, benzene and xylene, with $\chi$ value ranging from 1.36 to 1.53. When cyclohexane ($\chi = 1.74$) was mixed with acetone solution, an irregular polymeric film was obtained (see Fig. 2.2e), while polymers aggregated immediately when hexane ($\chi = 2.22$) was added to the acetone solution.

**Effect of surface chemistry of the substrates.** It is known that the surface characteristics of the supporting substrate will strongly influence the organization of polymer thin films (< 5 µm thick). Thus, we prepared substrates with different surface chemistry, wettability and topographies, including flat Si wafers and SU-8 films, and SU-8 micropillar arrays, to investigate the influence of substrates to the membrane formation. As seen in Fig. 2.4, periodic pores were formed on Si wafers with native oxide, SU-8 flat films, and SU-8 pillar arrays, which had apparent static water contact angles (WCA) of 34°, 75°, 141°, respectively. On SU-8 pillars, the high WCA is attributed to surface topography while the local WCA is 75°. Because of ring opening reaction during
photocuring, there are always a small amount of hydroxyl groups on as-prepared SU-8 films. However, irregular membranes with sparse pores were obtained on oxygen plasma treated Si wafers (WCA of ~ 0°). This may be explained by the preferential interaction between the polar PAC and the hydroxyl groups on the Si wafers, leading to strong segregation of toluene droplets from the polymer-rich. On the other hand, on HF etched, hydrogen-terminated and fluorosilane treated Si wafers, which had WCA of 82° and 98°, respectively, the polymer solution completely de-wetted. These results substantiate that surface chemistry of the substrate is also important to dictate the porous film formation at the micro-scale. On Si wafers with native oxide and SU-8 films (both flat and pillar arrays), the presence of a moderate density of hydroxyl groups facilitated the local wetting and spreading of the polymer solution on the substrates. Although the HF etched Si wafer has similar WCA as SU-8, there was no hydroxyl groups on surface, therefore, polymer failed to wet on such surface.

Control of pore size. It has been suggested that pore size decreases with increasing polymer concentration as denser polymers impede the growth of the nonsolvent droplets, thus, energetically confine smaller droplets. (Karthaus, Maruyama et al. 2000; Chen, Yan et al. 2012) Here, we prepared polymer solutions in 1 mL acetone and 80 µL toluene at different concentrations and dip coated them on Si wafers to investigate the pore size and its distribution. As seen in Fig. 2.5a, at a low polymer concentration, 30 mg/mL, only nanoparticles were observed on the Si wafer. A film with connected pores began to form at the polymer concentration of 50 mg/mL but with poor control of pore size and regularity (Fig. 2.5b). Ordered pore networks were obtained when increasing the polymer concentration to 75 – 150 mg/mL with gradual decrease of the average pore diameter from 2.5 µm to 600 nm (Fig. 2.5c-e). At 200 mg/mL, small pores (~ 300 nm in diameter) with a few giant pores (> 100 µm in diameter) were observed (Fig. 2.5f), suggesting the upper limit to form ordered and uniform membranes due to lack of control of toluene condensation.
Figure. 2.3 SEM images of ranPAC films dip coated on clean Si wafers from 1 mL polymer/acetone solution (100 mg/mL) mixed with 80 μL different nonsolvents. (a) Chloroform. (b) Toluene. (c) Benzene. (d) Xylene. (e) Cyclohexane.
Figure 2.4 SEM images of ranPAC films formed on various substrates. (a) Si wafer after O₂ plasma. (b) Clean Si wafer with native oxide. (c) SU-8. (d) HF etched Si wafer. (e) Fluorosilane treated Si wafer. (f) SU-8 pillar array ($D = 600$ nm, $AR = 4$ and $P = 1.5$ µm). The polymer concentration is 75 mg/mL (1 mL acetone and 80 µL toluene).
Generation of hierarchical porous structures on micropillar arrays. The ability to create highly ordered honeycomb structures with (sub)micron pores on a substrate with desired surface chemistry leads us to question whether it is possible to create a porous membrane with an arbitrary symmetry using a topographic template. Symmetry plays an important role in photonic and phononic bandgap materials. (Srinivasarao, Collings et al. 2001) Previously, we have demonstrated epitaxial assembly of smectic-A liquid crystals into arrays of arbitrary symmetries (e.g. a square lattice) directed by SU-8 pillar arrays in contrast to the naturally formed hexagonal arrays on a flat surface. (Honglawan, Beller et al. 2011) SU-8 has been widely used to create micropillar arrays by photolithography, replica molding and capillary force lithography. (Zhang, Lin et al. 2010) As discussed earlier, the polymer/acetone solution wets well on SU-8. By varying the pillar dimension (diameter, height and spacing) and symmetry together with the polymer concentration, we expect to create hierarchical structures with tunable pore size, symmetry and architecture.

As seen in Fig. 2.4f, a square array of pores was obtained from the corresponding SU-8 pillar template; each pore centered around the pillar, suggesting that the toluene droplets were pinned on the as-prepared SU-8 pillar surface. Meanwhile, a tall SU-8 pillar array offers us a topographic substrate to manipulate the surface chemistry at different locations, for example, using water to render the amount of hydroxyl groups on pillar tops vs. everywhere, leading to spatial control of the pore generation and formation of hierarchical structures.

To better understand the template directed formation of porous membranes, we fabricated three sets of SU-8 pillar arrays: I) a square lattice with pillar diameter, $D = 600$ nm, aspect ratio, $AR$ (= height/diameter) = 4, and pitch, $P = 1.5$ µm (see Fig. 2.6); II) a square lattice with $D = 4$ µm, $AR = 0.62$ and $P = 15$ µm (see Fig. 2.7); and III) a hexagonal lattice with $D = 1$ µm, $AR = 2$, and $P = 1.5$ µm (see Fig. 2.8 c-d). In all cases, toluene droplets were pinned at the pillars during solvent evaporation while polymer-rich phase filled between the pillars due to capillary
effect as illustrated in the inset in Fig. 2.6d, thus, enabling templating the symmetry of the pore array.

In the case of set I SU-8 pillars, since the surface was highly hydrophobic (WCA of 141°) and had high AR, water did not wet into the grooves. By placing the water on top of pillars for a short period of time (5 min), the pillar tops became rather hydrophilic due to surface reconstruction of SU-8, exposing hydroxyl groups on pillar top or near top regions. Pores were found in-between the activated pillars (see Fig. S1a and b), possibly due to dewetting of nonpolar toluene from the hydrophilic pillar tops. This differs from the previous observation of pores pinned on the as-prepared SU-8 pillars. When the pillars were immersed in water for 15 min, the entire pillar surface became more hydrophilic. For the tall pillars (set I, AR = 4), a thin polymer film with no pores was formed on top of the pillars, which were collapsed during drying due to capillary force (see Fig. S2.1c). For the much shorter set II pillars (AR = 0.62), pores were formed randomly on top of and in-between the pillars (see Fig. S2.1d). These results were similar to the observation on a flat Si wafer treated with O₂ plasma. As discussed earlier, the strong interactions between PAC polymers and hydroxyl groups on treated surface interfered with toluene condensation, resulting in loss of ordering.

Since the SU-8 pillars directs the condensation of toluene droplets, both the pore size and symmetry of the porous membrane can be determined by the diameter and symmetry of the pillars. Larger pore size with high uniformity was obtained from pillar arrays with larger diameters ($D = 4 \, \mu m$ in Fig. 2.7, set II pillars vs. $D = 600 \, nm$ in Fig. 2.6, set I pillars), and at the appropriate concentration, pores formed on pillars ($\sim 4 \, \mu m$ in Fig. 2.7c) could be tuned larger than those formed on a flat Si wafer ($2.5 \, \mu m$ in Fig. 2.5b). Likewise, the smallest pillar arrays could reduce the effective pore size to $\sim 600 \, nm$ (Fig. 2.6d), smaller than the minimum size ($\sim 1 \, \mu m$) formed on a flat SU-8 substrate (Fig. S2.2). These results clearly demonstrated the topographic supports as
Figure 2.5 SEM images of porous ranPAC films generated on clean Si wafers from different initial polymer concentrations. (a) 30 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, (d) 100 mg/mL, (e) 150 mg/mL, and (f) 200 mg/mL in 1 mL acetone and 80 µL toluene.
**Figure 2.6** SEM images of ranPAC assembled on set I SU-8 pillar array ($D = 600$ nm, $AR = 4$ and $P = 1.5$ µm) at different initial polymer concentrations. (a) 10 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, and (d) 100 mg/mL in 1 mL acetone and 80 µL toluene. (d), Inset: schematic of directed assembly of polymer-rich and polymer-poor phases on SU-8 pillar array.
Figure 2.7 SEM images of ranPAC assembled on set II SU-8 pillar array (D = 4 µm, AR = 0.62 and P = 15 µm) at different polymer concentrations. (a) 20 mg/mL, (b) 30 mg/mL, (c) 50 mg/mL, and (d) 75 mg/mL in 1 mL acetone and 80 µL toluene.
a powerful tool to direct the phase separation, and thus the control of film pore size and symmetry.

Further, by simply dip coating SU-8 pillars with different polymer concentrations, we created a variety of polymer architectures. For instance, at a low polymer concentration (10 – 20 mg/mL) in 12.5:1 v/v acetone/toluene mixture, ranPAC nanoparticles were found decorating pillar surface (Fig. 2.6a and 2.7a) while ordered pores arranged in square arrays as of their supporting pillars were obtained at higher polymer concentrations (50 - 100 mg/mL for smaller pillars, set I, and 30 - 75 mg/mL for larger pillars, set II) (Fig. 2.6 and 2.7 b-d). In general, the pore size decreased as polymer concentration increased on SU-8 pillars, much like those on a flat substrate except that the pore size was approximately 20% smaller on the tall pillar supports (set I) than that on a flat surface at the same polymer concentration (see Fig. 2.4c and f), which could be attributed to physical confinement imposed by the pillars. Such topographic effect diminished when the pillars became very short and when the pillar diameter ($D = 4 \mu m$) was much larger than the natural pore size (1.2 $\mu m$ at 75 mg/mL polymer, see pillars set II, Fig. 2.7d, $AR = 0.62$). Then the pore size on pillars became comparable to that formed on a flat SU-8 substrate at the particular concentration. In this case, a hierarchical pore structure was obtained: the porous membrane contoured around the pillars to accommodate the physical barrier while each pillar top housed several pores.

**Functionalization of hierarchical structures with quantum dots.** Nanoparticles are promising building blocks due to their remarkable electronic, magnetic, and optical properties, which are determined by their size, shape, composition, and surface chemistry. Honeycomb films have been utilized as scaffolds to organize nanoparticles by means to synergistically integrate additional functionality to the porous structure.(Boker, Lin et al. 2004; Sakatani, Boissière et al. 2007; Sun, Ji et al. 2008)
Previously, we show that the TOPO-CdSe nanocrystals preferentially bind to the crosslinked anhydride groups of ranPAC via solvophobic interaction in an organic media.\textsuperscript{33} Here, by mixing the same TOPO-CdSe nanocrystals (2 wt% in toluene) with the ranPAC /acetone solution, followed by dip-coating on Si wafers and SU-8 pillars, we created fluorescent porous films (see Fig. 2.8). The fluorescent intensity throughout the porous films was highly uniform, suggesting that CdSe nanocrystals were well-dispersed in the polymer solutions as the pore size and symmetry remained unchanged from the comparison of fluorescent images and optical images. The approach to incorporate nanocrystals to the amphiphilic ranPAC in solution prior to membrane formation offers several advantages compared to post-treatment of the porous membranes: 1) only a small amount of CdSe nanocrystals is needed and they did not interfere with the porous film formation, thus, the pore size and symmetry are maintained after addition of nanocrystals (see Fig. 2.7c vs. Fig. 2.8h); 2) no chemical reactions are needed between the nanocrystals and polymer films; and 3) the method is applicable to any colloidal system, and thus potentially enabling generation of a variety of advanced materials with tunable structure and properties.

Conclusions

We presented a simple yet versatile route to fabricate ordered microporous films with variable pore size (600 nm to 4 \( \mu \)m) and arbitrary symmetry (square vs. hexagonal lattices) by evaporative assembly from a ternary system, consisting of 1) amphiphilic random copolymers of poly(acryloyl chloride), 2) acetone (solvent), and 3) trace of toluene (nonsolvent). We hypothesized that fast evaporation of acetone led to organized droplets of toluene, which was subsequently stabilized by polymer-rich phase. The highly ordered porous films were all formed at a relative low humidity (20 - 40\%) in an ambient environment. The morphology and pore size of the porous films were found dependent on the relative composition of toluene vs. acetone and the initial polymer concentration. At a low polymer concentration (1 mg/mL), hollow, spherical vesicles were formed,
and honeycomb structures were observed when the polymer concentration was increased to 50 mg/mL and greater. Further study of solvency of the minority solvent suggested that highly ordered films were obtained only from moderately poor solvents ($\chi$, 1.36 -1.53) and low vapor pressure (relative to acetone), including toluene, benzene, and xylene. Using SU-8 pillar arrays of variable dimension, symmetry and surface chemistry (thus, wetting location), we created highly ordered hierarchical porous structures with well-defined pore size, symmetry and spatial resolution. The evaporative assembly from an amphiphilic polymer solution with a trace amount of nonsolvent does not require high environmental humidity, therefore, will offer an attractive alternative of BF to create highly ordered and/or hierarchical porous structures over a large area. The method is versatile such that functional nanocrystals (e.g. TOPO stabilized CdSe) could be incorporated into the porous structures through selective binding nanocrystals with the hydrophobic chains of the amphiphilic copolymers, followed by evaporative assembly. The insights of generating porous films using an organic nonsolvent and a template could shed light to create arbitrary structures in 2D and 3D for a wide range of potential applications, including sensors, optics, microelectronics, controlled delivery, and tissue engineering.
**Figure 2.8** SEM and fluorescent images of CdSe functionalized porous ranPAC films on a Si wafer (a and b), a hexagonal SU-8 pillar array with $D = 1 \, \mu\text{m}$, $AR = 2$ and $P = 1.5 \, \mu\text{m}$ (c and d), square SU-8 pillar arrays with $D = 600 \, \text{nm}$, $AR = 4$ and $P = 1.5 \, \mu\text{m}$ (e and f), and $D = 4 \, \mu\text{m}$, $AR = 0.62$ and $P = 15 \, \mu\text{m}$ (g and h). The initial polymer concentrations in acetone were 100 mg/mL (a-f) and 50 mg/mL (g-h) in 1 mL acetone and 80 µL toluene.
Experimental Section

**Materials.** Acryloyl chloride (AC) (> 97.0%), cyclohexane (anhydrous, 99.5%), benzene (anhydrous, 99.8%) and xylene (certified A.C.S.) were purchased from Sigma Aldrich. Photoinitiator, 2-hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur® 1173) was obtained from Ciba Specialty Chemicals while all other chemicals and solvents were purchased from Fisher Scientific and used as received unless noted.

**Synthesis of random copolymers, ranPAC, and their evaporative assembly on a substrate.**

The polymers were synthesized by photopolymerization in bulk following the procedure reported in literature.(Honglawan, Xu et al. 2012) In a typical procedure, 3 mL AC was mixed with 2% v/v Darocur® 1173 in a 5 mL glass vial in an ambience indoor environment (20 – 40% relative humidity at 20 – 30°C). The vial was then placed horizontally under a UV light source (97435 Oriel Flood Exposure Source from Newport, intensity of 54 mW/cm² at λ = 365 nm) for irradiation at a dosage of 4,000 mJ/cm². To prepare porous films, the irradiated product (0.75 g/mL) was washed with an excess amount of toluene, a good solvent of monomer AC but a poor solvent of the polymer, and centrifugated at 11,000 rpm for 30 min. The obtained polymers were further rinsed with toluene for three times and acetone once, respectively, before re-dispersed in a mixture of acetone (1 mL) and various amounts of nonsolvent (e.g. toluene). A clean substrate was dip-coated in the polymer solution for 10 s and air-dried to obtain porous films.

**Substrate Preparations.** Si wafers and photocrosslinked SU-8, a bisphenol A epoxy derivative, were used as substrates for casting random copolymer solutions. The Si wafers were diced to 1 x 1 cm and cleaned by soaking in 2% v/v of Detergent 8 (Alconox, Inc.) on a hot plate at 60 °C for 1 h, followed by rubbing with a cotton swab and rinsing with deionized (DI) water multiple times. The wafers were then dried with compressed air and cleaned additionally by oxygen plasma cleaner (PDC-100, Harrick Scientific Products, 30 W) for 1 h. To create hydrophobic surfaces, the freshly oxidized Si wafers were functionalized with (heptadecafluoro-1, 1, 2, 2-tetrahydrodecyl)
trichlorosilane (Gelest, Inc.) by vapor deposition in a vacuum desiccator for 1 h. The silanized substrate was cleaned by sonication in ethanol and acetone for 30 min, respectively, followed by drying with compressed air. To remove native oxide on Si wafers, the wafers were submerged in a mixed solution of 50% NH$_4$F and 50% HF (2:1 v/v) for 10 s, followed by rinsing with DI water and drying by the compressed air. SU-8 pillar arrays (diameter, $D = 600$ nm – 4 µm, aspect ratio, $AR = 0.62 – 4$, and pitch, $P = 1.5 – 15$ µm) in square and hexagonal lattices were fabricated by capillary force lithography (CFL), followed by UV curing (250 mJ/cm$^2$) following the reported procedure. (Zhang, Lin et al. 2010)

**Incorporation of CdSe nanocrystals in the microporous films.** In a typical process, 1 mL polymer/acetone solutions at variable concentrations were mixed with 80 µL TOPO stabilized CdSe nanocrystals (3 nm in diameter) in toluene (2 wt%), followed by the same evaporative assembly procedure described above.

**Characterization.** The morphologies of polymers deposited on various substrates were characterized by scanning electron microscopy (SEM) on the FEI Strata DB235 focused ion beam (FIB) system at 5kV. The fluorescent images of nanocrystal hybrid films were taken from optical microscopy (Olympus BX61). Static water contact angles (WCA) were measured by Ramé-Hart standard automated goniometer (model 290) using a 5.0 µL water droplet and averaged over three different spots.

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References


Figure S2.1 SEM images of porous structures of ranPAC formed on SU-8 micropillar arrays with hydroxyl groups activated at different locations. The polymer concentrations are 75 mg/mL (a and c of set I pillars) and 50 mg/mL (b and d of set II pillars), respectively, in a mixture of 1 mL acetone and 80 µL toluene. (a-b) Hydroxyl groups were activated only on pillar tops by sitting water droplets for 5 min. (c-d) Hydroxyl groups were activated everywhere on pillars by complete immersing the pillars into water for 15 min.
Figure S2.2 SEM image of a porous film of ranPAC formed on a flat SU-8 substrate. The polymer concentration is 150 mg/mL in a mixture of 1 mL acetone and 80 µL toluene.
CHAPTER 3
DESIGN FOR MULTIFUNCTIONAL NANOPARTICLES AS DRUG AND mRNA CARRIERS FOR CANCER THERAPEUTICS

Introduction
Recent advances in molecular biology and polymer science have opened up new exciting possibilities in innovative medicine, particularly in cancer therapeutics, where polymeric nanoparticles (NPs) are used as drug and/or gene carriers for highly efficient and safe anticancer therapies. (Liu, Miyoshi et al. 2007; Lai, Wang et al. 2009) In carrier design, not only the carrier should deliver active agents to the therapeutic site, it is also important to address potential side effects due to nonspecific delivery, poor physiochemical properties and bioavailability of active molecules to improve the overall therapeutic efficacy. While different formulations of DNA nanoparticles have been prepared with various carriers, for example, cationic lipids and, polymers, so far they show relatively modest efficiencies. (Peer, Karp et al. 2007) In contrast, messenger RNA (mRNA) delivery to primary cells is extremely potent, approaching 100% efficiency and amenable to chemical modifications, allowing us to adjust the duration of translation of the delivered mRNA. Modified mRNA can be utilized as an immunogen delivery vehicle to stimulate the immune system and to mobilize it to use its cellular and molecular tools to fight against cancer. (Rittig, Haentschel et al. 2011; Fotin-Mleczek, Zanzinger et al. 2012) Thus, it is of great interest to develop a suitable delivery system for mRNA as a cancer vaccine that delivers immune adjuvant and tumor cell active proteins that will lead to tumor reduction.

Despite the tremendous efforts in the design and synthesis of various types of NPs, site-specificity, efficacy, stability and toxicity of the gene/drug carriers remain the major hurdles for the clinical application of NP based carriers in cancer therapeutics. Compared to lipid-based NPs, polymeric NPs are structurally more robust and versatile due to their higher molecular weight and
wide range of functionalities. (Ahmed and Discher 2004) Well-defined block copolymers have been widely used to prepare NPs by self-assembly process, especially vesicles, which have been shown to offer high loading efficiency and ability to encapsulate both hydrophobic and hydrophilic molecules in different portions of the structure. (Rösler, Vandermeulen et al. 2001; Sanson, Schatz et al. 2010) In comparison, random copolymers are much simpler to synthesize, scalable, and available with a wide range of chemistry and composition. However, particle size and morphology are often not well-defined due to the random nature of chemistry and composition of random copolymer. From a large scale manufacturing standpoint, a random copolymer system that can form nano-vesicles with modest uniformity offers a great promise as a delivery vehicle for both drug and gene in advanced cancer therapy.

Besides the choice of materials and morphologies of the carriers, there are a number of parameters in NP design, including size, (Decuzzi, Godin et al. 2010; He, Hu et al. 2010) shape, (Champion, Katare et al. 2007; Decuzzi, Godin et al. 2010) surface charge, (Gratton, Ropp et al. 2008) and sensitivity to external stimuli (Fleige, Quadir et al. 2012) (e.g. pH, (Min, Kim et al. 2010; Qiao, Zhang et al. 2011) redox potential, (Koo, Min et al. 2012) temperature (Qiao, Zhang et al. 2011)), as they influence the biodistribution, clearance of the NPs from the body and mode of delivery. (Davis, Chen et al. 2008; Gratton, Ropp et al. 2008) in systemic therapy, entities with a size greater than a few hundred nanometers are most likely cleared by reticuloendothelial system (RES). Smaller particles (< 6 nm), on the other hand, will be eliminated from the bloodstream through glomerular filtration in the kidney. The fate of NPs is also highly dependent on surface charge of NPs such that if not optimized, NP aggregation, nonspecific cell adhesion and clearance by scavenger endothelial cells will occur.

To engineer targeted delivery for cancer therapy, a passive targeting approach is typically utilized. The strategy exploits various distinct features of disease pathology or cellular compartments, including leaky vasculatures providing an easy access to the tumors known as
enhanced permeability and retention effect (EPR), as well as the acidic microenvironment of the cancerous cells (pH < 7.2), endosome and lysosome (4.5 < pH < 6.5, for gene therapy as one intracellular uptake mechanism), enabling controlled release of active agents at the specific site. Hence, it is desirable to develop NPs whose physical properties (e.g. swelling/deswelling, particle degradation, aggregation) are highly receptive to the change of environmental pH. (Chen, Meng et al. 2010)

Here, we present a versatile platform of multifunctional NPs from assembly of random copolymers as a novel drug and mRNA delivery system. Our previous study has shown that amphiphilic polymers, synthesized through a single step photopolymerization of acrylyol chloride (AC) are comprised of poly(acrylyol chloride) (PAC) as a solvophilic portion and self-crosslinked acid anhydride as a solvophobic component, enabling self-assembly in selective organic solvents into particles with variable morphologies (micelles and vesicles) and sizes (20 nm – half a micron) depending on the UV-dosage applied to the monomers during polymerization. (Honglawan, Xu et al. 2012) Polymers synthesized at a UV dosage of 2,000 mJ/cm$^2$ were selected in this study owing to the formation of rather uniform vesicles, potentially high loading efficiency of active agents, and the particle size (90.6 ± 7 nm in diameter) within the optimal range for drug/gene delivery. First, the particle shell, mainly consisting of PAC, was converted to polyacrylic acid-rich shell (denoted as PAA NPs) via hydrolysis in aqueous solution. The PAA NPs were then reacted to branched poly(ethyleneimine) (PEI), a cationic polymer that is commonly used in drug and gene delivery, of different molecular weights through both electrostatic interaction and amide bond formation between amine groups on PEI and activated ester groups on PAA NPs. Each step of particle modification was thoroughly characterized with Fourier transform infrared (FT-IR) spectroscopy, scanning electronic microscopy (SEM) and transmission electron microscopy (TEM), zeta-potential and dynamic light scattering (DLS). The modified NPs (both PAA and PEI NPs) demonstrated high pH sensitivity determined by a large change of particle size at different pHs. Further, it was shown that PEI grafting enhanced stability of the NPs.
For delivery, two packaging strategies of the anticancer drug, doxorubicin (Dox), with PEI NPs were investigated: 1) encapsulation of Dox during particle assembly in acetone prior to surface modification, and 2) conjugation of Dox onto modified PEI NPs in aqueous solution. The results from UV-Vis measurements of Dox release and in vitro delivery to HEK 293T cells suggested that packaging Strategy 1 (Dox/PEI NPs) yielded the best samples in terms of drug loading and delivery efficiency. We then tested in vitro delivery of mRNA to HEK 293T cells, where mRNA was conjugated to the surface of PEI NPs (Strategy 2) based on electrostatic interaction. It was shown that the conjugated mRNA/PEI NPs were highly effective in vitro at a low cytotoxicity level in comparison to naked PEI and standard complexing agents, such as TransIT-mRNA. Cell viability was maintained at the optimal concentration of PEI NPs for effective delivery of both drug and mRNA.
Results and Discussion

Nanoparticle synthesis. Based on our previous study, uniform NPs with different size and morphology can be produced via self-assembly of amphiphilic random copolymers (ranPAC) comprised of solvophilic poly(acryloyl chloride) (PAC) and solvophobic acid anhydride as a lightly crosslinked portion in selective organic solvent such as acetone (Figure 3.1). The characteristics of the NPs can solely be regulated by the UV dosage applied to the mixed monomer (acryloyl chloride, AC and its hydrolyzed product), which determines the degree of polymerization and composition of the polymer. Thus, the versatility of the system offers a unique modality of delivery system of cancer therapeutics.

To achieve optimal systemic delivery and cellular uptake using NP based carriers, it is important control the particle size and size distribution, as well as a high loading efficiency for active molecules. Vesicular NPs with an average hydrodynamic diameter ($D_h$) of 90.6 ± 7 nm in acetone were produced at UV dosage of 2,000 mJ/cm$^2$. Upon transferring the NPs to an aqueous solution, carboxylic acid terminal decorated NPs (denoted as PAA NPs) were obtained through hydrolysis of the PAC shells. The carboxylic acid groups are negatively charged, thus, can be utilized as a reacting or grafting site to incorporate additional functionalities to the NPs. In this work, branched poly(ethylene imines) (PEI), a well-studied pH-responsive cationic polymer with a sponge effect that facilitates controlled release of active molecules in intracellular matrix for cancer therapy was selected to functionalize the NPs though a combination of electrostatic interaction and amide formation from active ester and amine groups.(Boussif, Lezoualch et al. 1995; Akinc, Thomas et al. 2005)

Characterizations of functional NPs. Successful surface modifications of the PAA and PEI NPs from PAC NPs were verified with FT-IR (Fig. 3.2) and zeta potential measurements (see Fig. 3.3). As shown in Fig. 3.2, the appearance of characteristic peaks of hydroxyl groups at 2994-3470 and 961 cm$^{-1}$ and broadening of the C=O, carbonyl peak at 1757 cm$^{-1}$ of PAA NPs indicated that
Figure 3.1: (a) Schematic illustration of the synthesis and assembly of PAA and PEI nanoparticles. (b) Chemical structure of photopolymerized random copolymer.
Figure 3.2. FT-IR spectra of different nanoparticles: PAC NPs, PAA NPs and PEI NPs.
Figure 3.3. Zeta-potentials of PAA and PEI nanoparticles in water and 10 mM NaCl solution. PEI has molecular weights of 1,800 and 10,000. The particle concentration is 1 mg/mL.
Figure 3.4. Size (a) and surface charge (b) of PEI NPs synthesized at various PEI concentrations in water measured by dynamic light scattering. Note that there was no readable data for the sample at 5 mg/mL due to gross aggregation and large polydispersity of NPs.
the shell of PAC NPs was converted to carboxylic acid termini after hydrolysis in aqueous solution for 24 h. Supporting this was a significant shift of zeta-potential from near 0 to \(-8.6 \pm 2.0\) mV after reaction in aqueous solution for 24 h due to the presence of negatively charged carboxylic acid groups.

Branched PEI contains approximately 25% primary amine groups, 50% secondary amine groups, and 25% tertiary amine groups with pKa values of 4.5, 6.7 and 11.6, respectively. Depending on the molecular weight of branched PEIs (MW: 10,000 and 1,800 Da), PEI NPs in aqueous solution at pH 7 exhibited positive surface charges of 58.2 ± 17.1 mV and 18.71 ± 13.5 mV, respectively, at the optimal PEI grafting concentration, 2 mg/mL. The optimal concentration of PEI was chosen based on the following parameters: 1) the lowest \(D_H\) and polydispersity index of NPs, 2) no aggregation of NPs and PEI after purification, and 3) the high value of zeta-potential with smallest standard deviation (see Fig. 3.4). The FT-IR spectrum of PEI NPs also confirmed the chemical transformation of the particle shell with several characteristic peaks of amine groups at 3570-3188 cm\(^{-1}\) (stretch) and 1640-1554 cm\(^{-1}\) (bend and scissor).

PEI can bind to the surface of hydrolyzed NPs via two routes: 1) electrostatic interaction and 2) chemical reaction between the reactive carboxyl groups and amine groups. It was shown earlier that hydrolysis of poly(acrylate) shell yields negatively charged NPs, which should complex with polycations such as branched PEI electrostatically. However, the peaks representing amide bond were also observed at 3680 - 3500 cm\(^{-1}\) for amide N-H stretch and 1654 cm\(^{-1}\) for amide C=O stretch in the FT-IR spectrum of PEI NPs. Although the reactivity between carboxylic acid and amine groups is effectively low without a coupling agent, such as EDC and/or DCC to activate carboxyl groups, amide bond can be formed if there are active ester groups that are highly reactive to amino groups. Since hydrolysis and functionalization of PAC NPs occurred concomitantly, it was possible that amide bonds were formed through reactions between the active ester groups of acyl halide on the NPs and the amine groups of PEI. Therefore, PEI can be
introduced to the NPs via both electrostatic attraction and amine bond formation. (Boussif, Lezoualch et al. 1995; Akinc, Thomas et al. 2005)

Morphologies of NPs at various stages of synthesis are shown in Fig. 3.5, all illustrating spherical vesicular structures. The results suggested that the surface modification process did not alter the morphology of the NPs, which could be attributed to the cross-linked core of the vesicles, as evidenced from our previous study. (Honglawan, Xu et al. 2012) Based on the contrast of the membrane in TEM images (Fig. 5 b, d and f), the shell of the NPs in the aqueous solution expanded from a few nm (PAC NPs) to ~ 25 nm (PAA NPs) and ~ 40 nm (PEI NPs), confirming the swelling of the hydrolyzed polymeric shell and successful grafting of PEI. Additional support for PEI grafting to the NPs can be seen in the optical and fluorescent images in Fig. 3.6, where PEI (MW 10,000) was tagged by fluorescent FITC at a molar ratio of FITC/PEI=3:1. The grafting density of PEI on the NPs was estimated to be 2.1 wt% of PAC NPs, functionalized at the optimal PEI concentration (2 mg/mL) by measuring UV-Vis absorbance at 484 nm, which was calibrated from aqueous solutions of FITC tagged pure PEI at different FITC concentration.

**pH responsiveness of the functional NPs.** It is known that both PEI and PAA are sensitive to pH changes. We monitored the pH responsiveness of the particles using DLS by measuring $D_h$ in different pH buffer solutions. As shown in Fig. 7a, the average $D_h$ of PAA NPs increased over 2 fold from pH 7 to pH 10 due to the deprotonation of carboxylic acid groups, but de-swelled to 38.21 ± 5.57 nm at pH 4, since PAA had a pKa of ~ 4.5. When the particles were immersed in aqueous solution without NaCl salt at pH 7 for 24 h, the particle size increased by nearly 5 folds (see Fig. 7b), and become unstable due to large surface charge present on NPs. This behavior is consistent with observations of swelling of electrolyzed polymeric NPs reported in the literature. (Guo, Weiss et al. 1999; Jabbari and Nozari 2000)

The opposite trend of pH responsiveness was observed from PEIs NPs and the magnitude of change was dependent on MW of PEI. When the solution pH was reduced from pH
Figure 3.5. SEM (a,c,e) and TEM (b,d,f) images of particles modified at different states: PAC NPs (a and b), PAA NPs (c and d), and PEI NPs (e and f).
Figure 3.6. Optical (left) and fluorescent (right) images of FITC tagged PEI NPs drop-cast onto a clean Si-wafer at the particle concentration of ~ 1 mg/mL in aqueous solution. Large particles were synthesized at high UV dosage of 4,000 mJ/cm² prior surface modification with FITC tagged PEI (MW 10,000) at ratio of FITC to PEI = 3:1 (molar).
Figure 3.7. The hydrodynamic diameters of functional particles in different pH buffer solutions (b) and over a 15-day period in water at neutral pH and with 10 mM NaCl (b) determined by DLS. In all samples, particle concentration was kept at 1 mg/mL.
7 to pH 4, the NPs grafted with large MW PEI (10,000 MW) revealed a significant increase of the particle size by approximately 50%, whereas those grafted with lower MW PEI (1,800 MW) did not change much due to lower positive charge density. Importantly, all PEI grafted NPs remained stable and did not change size over a 15-day period in aqueous solution with and without NaCl salt (Fig. 3.7b). The tunability of swollen membrane by solution pH or MW of grafted PEI, which determines the permeability of the particle shell, is of great interest for encapsulation and controlled release. (Ganta, Devalapally et al. 2008) Based on the above results, the NPs grafted with 10,000 MW PEI with high sensitivity to pH change and stability in aqueous solution are investigated here as smart delivery systems for anticancer drugs, doxorubicin, and mRNA for cancer therapeutics.

**Drug loading and releasing efficiency of functional NPs.** In order to engineer an effective drug delivery system with high drug loading and encapsulating efficiencies, two strategies of packing doxorubicin (Dox), an anticancer drug model were first investigated, including 1) loading Dox during PAC NP assembly in an organic solvent prior to particle hydrolysis and functionalization, and 2) complexing Dox on the surface of PEI functionalized NPs (see Fig. 3.8).

Using UV-Vis (see Fig. 3.9a and Table 3.1), we measured the amount of free Dox left in the solution at 484 nm in each sample. It is revealed that the drug packaging strategy 1, where Dox was encapsulated during NP assembly was ~ 10 times more efficient than the second approach which relied on physisorption of Dox onto the surface of preformed NPs. The efficacy of two strategies to package Dox can be readily observed from the centrifugated samples (see Fig. 3.9b), where the sample solution prepared by method 2 appeared orange and slightly cloudy, suggesting majority of Dox remained in solution, in agreement with a low encapsulating efficiency (4.8%) calculated from UV-Vis results. In contrast, when Dox was encapsulated during particle assembly, followed by PEI grafting, here, termed as Dox/PEI NPs, after centrifugation, the red color was nearly disappeared in aqueous solution, indicating that the majority of Dox was
Figure 3.8. Schematic illustration of two different drug packaging strategies. The names of NPs are denoted by their synthesis process sequentially.
Table 3.1: Summary of drug loading efficiencies and zeta potentials of Dox/nanoparticle complexes with and without PEI grafting by two drug packaging strategies. The experiments were performed in aqueous solution.

<table>
<thead>
<tr>
<th></th>
<th>Total Dox (mg in 10 mL)</th>
<th>Loading Efficiency (wt. %)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Dox</td>
<td>1.72</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>PAA NP/Dox</td>
<td>0.04</td>
<td>0.93</td>
<td>-2.11</td>
</tr>
<tr>
<td>PEI NP/Dox</td>
<td>0.15</td>
<td>3.11</td>
<td>+6.73</td>
</tr>
<tr>
<td>Dox/PAA NP</td>
<td>0.51</td>
<td>11.85</td>
<td>-57.25</td>
</tr>
<tr>
<td>Dox/PEI NP</td>
<td>1.36</td>
<td>27.28</td>
<td>+42.6</td>
</tr>
</tbody>
</table>
Figure 3.9. (a) UV-Vis spectra of initial and unloaded Dox solutions after separated from various samples of Dox loaded NPs at initial Dox loading concentration of 100 µg/mL. (b) A photograph of the samples of PEI NPs loaded with Dox by encapsulation method (1) and physisorption method (2) after centrifugation for separation. (c) TEM image of a single Dox/PEI NP. (d and e) Fluorescent (d) and optical (e) images of Dox/PEI NP aggregates.
Figure 3.10. Release of Dox from different particles loaded at 100 µg/mL in 10 mL buffer (10 mM NaCl) solution. Accumulative percentages of loaded Dox released at pH 7 for the first 120 min and pH 4 for the following 3 h are based on UV-Vis measurement of Dox released in the solution. The names of NPs are denoted by their synthesis process sequentially.
effectively bound to the NPs prepared by method 1 (with an encapsulation efficiency of nearly 80%). In Fig. 3.9c-e, the TEM image of individual Dox/PEI NPs and fluorescent and optical images of NP aggregates substantiated the loading of fluorescent Dox inside the cavity of hollow PEI NPs. we note that in method 1, the loaded drug did not significantly alter the physicochemical properties of the NPs, including surface charge and particle size, unlike method 2. Therefore, it is possible to independently functionalize drug carriers and drugs, a vital prerequisite of a truly versatile platform of a multitargeted delivery system. Dox/PEI NPs had the highest drug loading efficiency (27.28 wt%, or polymer to Dox ratio = 4:1 mol/mol), which was ~2 - 4 fold of a typical value using liposomes and polymersomes as carriers, and comparable to some of the best encapsulating vehicles reported in literature.(Shuai, Ai et al. 2004; Sanson, Schatz et al. 2010; Upadhyay, Bhatt et al. 2010) The unique morphology of the assemblies may explain the high loading efficiency. Compared to vesicles assembled from block copolymers, the vesicular structures assembled from random copolymers, such as ranPAC, typically have an extremely thin membrane due to the small interfacial energy between the core and shell portions of the membrane, which enables every portion of the structure accessible for the drug molecules.(Du and O'Reilly 2009; Zhu and Liu 2011) The fact that Dox/PEI NPs had more than twice the loading efficiency than in PAA NPs (Dox/PAA NP) suggested that PEI grafting on the particle surface enhanced particle stability and potentially reduced drug leakage out of the particles.

Drug release of each Dox/particle sample and their sensitivity to solution pH were monitored over a 5 h period (Fig. 3.10). Dox/PEI NPs (blue line) exhibited a minimal burst effect with accumulative release of less than 30% within the first two hours in buffer solution at pH 7, whereas more than half of loaded drug was released from other particle formulations. This again supported the high stability of the Dox/PEI NPs prepared by method 1. When solution pH was changed to pH 4, Dox/PEI NPs released additional loaded drugs by over 30% within 30 min and nearly 60% within 2 hrs. Thus, the results above suggest that Dox/PEI NPs is a promising
Figure 3.11. Relative fluorescent intensity of free Dox and Dox loaded PAA and PEI NPs at different normalized Dox concentrations based on flow cytometry analysis of HEK 293T cells after 4 h incubation.
Figure 3.12. Delivery efficiency of luciferase encoding mRNA to HEK 293T cells by various gene carriers, including PEI particles of different sizes (105, 165 and 220 nm in diameter), PEI complexed mRNA, TransIT complexed mRNA and substrate as a signal baseline at different N/P ratios (1 – 81), which were determined by the detected luciferase activity.
Figure 3.13. HEK 293T cell viability after treated with increasing concentrations of PEI NPs and incubated at 37°C for 24 h.
candidate for a targeted delivery system with a controlled fast release profile triggered by change in solution pH, which is a commonly exploited in cancer therapeutics.

**In vitro Delivery of Doxorubicin.** To further corroborate the effective packaging strategy of drug encapsulated NPs, Dox/PEI NPs were delivered to human embryonic kidney (HEK) 293T cells and the efficacy was determined *in vitro* by flow cytometry analysis of cells after a 4 h incubation with drug/particles in comparison to free Dox and Dox/PAA NPs. Dox loaded PEI particles increased delivery efficiency of Dox to 293T cells by 10 fold, relative to the free Dox. In contrast, Dox/PAA NPs failed to deliver Dox due to the burst release effect shown in Fig. 3.10. The results from *in vitro* drug delivery were consistent with the results reported earlier, that is, Dox encapsulated by PEI NPs by method 1 was highly stable with minimal drug leakage, therefore, offering the highest cellular uptake, while the use of PEI reduced effective dosage.

**Delivery of mRNA in vitro.** Owing to the incompatibility of mRNA with acetone, the nucleic acid cannot be loaded into NPs during PAC assembly (packaging strategy 1) unless a different organic solvent such as DMSO was used for polymer assembly. Therefore, in the initial trials, we coupled to the PEI NP surface through electrostatic interaction between positively charged PEI and negatively charged mRNA (packaging strategy 2). We then studied delivery efficiency as a function of PEI particle size (105, 165, 220 nm in diameters) and particle/RNA ratio (N/P = 1 to 81). According to the detected luciferase activity, which was encoded by the mRNA, all PEI particles investigated here demonstrated high delivery efficiency to HEK 293T cells, whereas the particle size did not show much difference (Fig. 3.12). The results were comparable to those obtained from highly potent delivery agents, including TransIT mRNA and pure PEI, suggesting that the particle sizes were within the optimal range of *in vitro* cellular uptake and the PEI NPs efficiently delivered mRNA to HEK 293T cells.

**Cytotoxicity of PEI NPs.** Lastly, the cytotoxicity of PEI NPs at various concentrations was evaluated on HEK 293T cells using flow cytometry analysis. Using Aqua dead cell or propidium...
iodide staining as a marker for dead cells, the cells remained viable at PEI NP concentrations up to 9 µg/mL (>90%). When PEI NP concentration was increased to 27 µg/mL (Fig. 3.13), cell viability was reduced dramatically to ~50%. The result confirmed the known toxicity of PEI to cells at high concentrations. Nevertheless, appreciable efficiency of the drug and gene delivery could be achieved at a relatively low PEI content (9 µg/mL) for safe drug and gene therapy.

Conclusions

pH responsive nanoparticles were synthesized via self-assembly of amphiphilic random copolymers of PAC and engineered as a drug and gene delivery system for cancer therapies. In acetone, the PAC copolymers were assembled into nanovesicles of a mean hydrodynamic diameter of ~90 nm, a thin membrane (a few nm). The small phase separation within ranPAC membrane and the proposed packaging formulation through coassembly between the polymer with drug molecules contributed to high loading efficiency for doxorubicin. The particle shell was hydrolyzed to negatively charge PAA NP with carboxyl termini, which could be used for binding to PEI, leading to the formation of positively charged NP. The size of functionalized NPs varied in response to change of solution pH from 4 to 10: PAA NPs (~40 – 160 nm) and PEI NPs (~60 – 180 nm), depending on the molecular weight of PEI. Unlike PAA NPs, PEI NPs were stable in aqueous solution with and without NaCl salt (10 mM) for over a 15-day period. We then tested the use of PEI (MW 10,000) NPs as cancer drug (Dox) and gene (mRNA) carriers for in vitro delivery to HEK 293 T cells. First, we investigated two approaches to package Dox with PEI NPs, including coassembly during NP formation in acetone, followed by PEI grafting and physisorption of Dox on PEI NPs. The data from controlled release experiments monitoring the amounts of drug loaded and released revealed that coassembly of Dox and polymer was an extremely effective packaging approach with high Dox loading efficiency (~30 %) and encapsulation efficiency (nearly 80%). In vitro, the particles modified with PEI demonstrated improved drug delivery efficiency by 10 fold compared to free Dox. Separately, the PEI particles coupled with mRNA
through electrostatic interaction mediated high cellular uptake of mRNA with little cytotoxicity to cells. Encouraged by the in vitro drug and mRNA delivery studies, we have begun to investigate in vivo delivery of mRNA in murine models. It is important to note that this particle model is extremely versatile, such that it is possible to attach multiple functional molecules (e.g. poly(ethylene glycol) for enhancement of cell viability and reduced protein binding and folic acid, cholesterol, monoclonal antibodies, receptor ligands for specific cell targeting) to the carboxyl terminated NPs in a single step process. In addition, the assembly of vesicles in organic media could potentially improve loading efficiency of many other active molecules particularly hydrophobic drugs, which are difficult to be encapsulated inside nanoparticles.
Experimental

Materials. Acryloyl chloride (AC) monomers (97.0%) and doxorubicin hydrochloride (Dox) (98.0-102.0%, HPLC) were purchased from Sigma-Aldrich. Photo-initiator, 2-hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur® 1173) was obtained from Ciba Specialty Chemicals. Branched poly(ethylene imines) (PEI) (99%, molecular weight (MW) of 1,800 and 10,000 Da) were from Polysciences, Inc. HEK 293T cells (ATCC, Rockville, MD) in Dulbecco's modified Eagle's medium supplemented with glutamine (Invitrogen, Rockville, MD) and 10% FCS (Gemini Bio-Product, Foundation FBS) (complete medium) were utilized in in vitro experiments.

Posttranscriptionally capped-TEV-firefly luciferase-A101 mRNA was synthesized as described in Karikó et al. (Kariko, Muramatsu et al. 2008). Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. (Kariko, Muramatsu et al. 2012) All other reagents were purchased from Fisher Scientific and used as received unless noted.

Synthesis of PAC nanoparticles. The polymers were synthesized by photopolymerization in bulk. In a typical procedure, AC (3 mL) was mixed with the photoinitiator, Darocur® 1173 (2% v/v) in a 5 mL glass vial in open air. The glass vial was then capped and placed horizontally under a UV light source (97435 Oriel Flood Exposure Source from Newport, intensity of 54 mW/cm² at λ = 365 nm) for irradiation at 2,000 mJ/cm². After irradiation, polymer/AC mixture (200 µL) was mixed with an excess amount of poor solvent, toluene (HPLC grade), and purified by centrifugation (10,000 rpm, 30 min), followed by alternating rinsing with toluene and acetone several times. The purified polymer was then redispersed in acetone to form NPs.

Functionalization of nanoparticles. 300 µL particle /acetone solution (40 mg/mL) was added drop-wise into an aqueous solution for PAA NP synthesis and 10 mL PEI solution (2 mg/mL PEI in water) for PEI NP synthesis with magnetic bar stirring for over 3 h to ensure complete
evaporation of acetone. The NP solutions were purified 3 times with water by centrifugation at 3,000 rpm for 10 min. The purified functionalized nanoparticles were then re-dispersed and kept in water or freshly prepared aqueous buffer solution (10 mM of NaCl) for usage.

**Characterizations.** SEM images were taken on a FEI Strata DB235 focused ion beam (FIB) system at 5kV. For imaging, Si wafers were diced to 2 x 2 cm and soaked in 2% v/v of Detergent 8 (Alconox, NY) on a hot plate at 60°C for 1 h, followed by rubbing with a cotton swab and rinsing with DI-water multiple times. The wafers were then dried with compressed air and cleaned by oxygen plasma cleaner (PDC-100, Harrick Scientific Products, 30 W) for 1 h. To capture the particles, the freshly cleaned Si wafers were submerged in the NP solutions prepared as described above for 10 sec, followed by rinsing with IPA and dried with compressed air. TEM images were taken with the high resolution TEM JEOL 2010 at 200 kV, where the NPs were drop-cast on a holy copper grid (200 mesh from Electron Microscopy Sciences) without further treatment.

The hydrodynamic diameter of particles ($D_{hv}$) and their size distribution in different pH buffer solutions were measured by dynamic light scattering (DLS) using a Zetasizer Nano Particle Analyzer Series S ZEN1600. For the study of pH responsiveness of the particles, 10 mM NaCl was used as a buffer solution while the pH value was adjusted with 0.2 M HCl and NaOH. Before any measurement, the particle solution was sonicated for 30 min and stabilized in the buffer for 5 min. Zeta potential values of the particles were obtained with DelsaTM Nano Submicron Particle Size and Zeta Potential Particle Analyzer (Beckman Coulter, Inc. CA).

FT-IR spectra were obtained on a Nicolet 450 FT-IR equipped with an MCT/B detector at a resolution of 1.93 cm$^{-1}$. To prepare the samples, ~ 4 mg of random copolymers dispersed in 2 mL acetone, PAA NPs and PEI NPs in aqueous solution were drop-cast on NaCl and AgCl disks. The samples were dried in vacuo for 3 h before taking FT-IR spectra. FT-IR. Random copolymers (PAC NPs): 2902 and 2839 (=C-H anhydride and C-H alkane, stretch), 1804 - 1757 (C=O,
anhydride, stretch and Cl-C=O, acyl halide, stretch), 1251 and 1025 (C-O, carbonyl, stretch), and 730 cm\(^{-1}\) (C-Cl, stretch). PAA NPs: 3470 – 2994 (O-H, carboxyl, stretch), 2920 and 2845 (=C-H, anhydride and C-H, alkane, stretch), 1838 – 1698 (C=O anhydride, stretch, Cl-C=O, acyl halide, stretch and HO-C=O, carboxylic acid, stretch), 1195 (C-O, carbonyl, stretch), and 961 cm\(^{-1}\) (O-H, bending). PEI NPs: 3570 – 3188 (N-H, amine, stretch), 2968 and 2877 (=C-H, anhydride and C-H, alkane, stretch), 1654 (C=O, amide, stretch), 1640 – 1554 (N-H, amine, bending and N-H\(_2\), amine, scissoring), 1248 – 1010 (C-O, carbonyl, stretch and C-N, amine, stretch), and 930 – 860 cm\(^{-1}\) (N-H, amine, wag).

**Drug loaded nanoparticles and delivery.** Method 1: Dox was loaded in the nanoparticles during assembly process by mixing 20 µL of Dox solution prepared in DMSO (5 µg/µL) with the NP solution in acetone (5 mg/mL in the total volume of 1.5 mL) for 30 min prior to transferring to aqueous solution for purification and surface modification with PEI as described earlier. Method 2: 20 µL of Dox solution in DMSO was added to the preformed PEI grafted NPs in aqueous solution (2 mg/mL of the particles in the total volume of 10 mL) and incubated overnight before centrifugation for separation at 3000 rpm for 15 min 3 times. The eluents from the samples were collected to determine drug loading efficiency (LE) and drug encapsulated efficiency (EE). LE and EE are defined by the following:

\[
LE(\%) = \frac{\text{wt. of encapsulated Dox}}{\text{total wt. of the sample}} \times 100,
\]

\[
EE(\%) = \frac{\text{wt. of encapsulated Dox}}{\text{wt. of initial Dox}} \times 100,
\]

\[
\text{(wt. of encapsulated Dox)} = \text{(wt. of initial Dox)} - \text{(wt. of free Dox)},
\]

which were estimated from UV-Vis measurements of the eluents (Cary 5000 spectrophotometer) using water as reference.

Release profiles of Dox from each prepared sample were obtained from UV-Vis spectra (Varian UV-Vis-NIR Cary 5000 spectrophotometer) of the collected solutions containing Dox.
released out of the particles over a 5 h period. 2 mL aqueous solution (10 mM NaCl) of Dox loaded particles in dialysis membrane bags with MW cutoff at 3,000 was placed in a 10 mL buffer solution (10 mM NaCl) at pH 7 for 2 h, followed by pH 4 for 3 h. The solutions containing released Dox were sampled how often?. The released Dox were quantified with the absorbance at 490 nm, characteristics of Dox. All measurements were repeated 3 times.

For in vitro delivery experiments, Dox loaded NPs were incubated with HEK 293T cells (ATCC, Rockville, MD) in complete medium composed of Dulbecco's modified Eagle’s medium supplemented with glutamine (Invitrogen, Rockville, MD) and 10 vol% fetal calf serum (FCS) (Gemini Bio-Products) for 4 h at 37°C. The uptake of Dox was determined by flow cytometric analysis utilizing a FACSCalibur flow cytometer with a 585/42 filter (BD Bioscience).

RNA loaded nanoparticles and delivery. mRNAs encoding firefly luciferase were coupled to the as-prepared PEI particles at various N/P ratios (1-81) prior to delivery to 293T cells. The cells were incubated for 4 h before flow cytometric analysis. TransIT-mRNA from Mirus Bio containing non-liposomal cationic polymer/lipid formulation was utilized as a control delivery agent.

Cytotoxicity of PEI NPs. Aqueous solutions of PEI NPs at various concentrations (0 – 81 µg/mL) were incubated with 293T cells for 24 h before detaching the cells with phosphate buffered saline (PBS) solution and 5 mM ethylenediaminetetraacetic acid (EDTA). The collected cells were washed with complete medium to get rid of excess EDTA and resuspended in PBS with 2 vol% FCS. Flow cytometer with Propidium Iodide or Live/Dead Fixable Aqua Dead Cell Stain (Invitrogen) on a BD LSR II was used to measure cell death.

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References


CHAPTER 4

PILLAR ASSISTED EPITAXIAL ASSEMBLY OF TORIC FOCAL CONIC
DOMAINS OF SMECTIC-A LIQUID CRYSTALS


Introduction

Self-assembly, self-processing, and bottom-up design are ever more important tools for the development of new materials of both fundamental and technological interest owing to their robust capability for generating complex, hierarchical structures. In general, self-assembling materials, including colloids, block copolymers, and supramolecules/DNA form thermodynamically stable structures over a broad range of length scales, from the micro- to nano-scales. Structure formation in these long-range ordered phases is often governed by entropic and geometric considerations, leading frequently to a limited variety of optimal, close-packed structures. However, close-packed structures are not always appropriate in device applications. Some control has been gained through so-called graphoepitaxy, which exploits substrates with topological( Segalman, Yokoyama et al. 2001; Kim, Solak et al. 2003; Stoykovich, Müller et al. 2005; Bita, Yang et al. 2008; Arora, Du et al. 2010) or chemical(Edwards, Montague et al. 2004) surface relief patterns that nearly match the domain structures of block copolymers, for instance, and direct their epitaxial assembly into nanostructures with long-range positional order and orientation in thin films. However, epitaxial assembly of highly ordered square arrays has only been recently achieved in both triblock copolymers( Tang, Bang et al. 2008; Chuang, Gwyther et al. 2009) and supermolecular assemblies of hydrogen-bonding diblock copolymer in thin films.(Tang, Lennon et al. 2008)

Because of their geometrical, mechanical, and electronic anisotropy, liquid crystals (LCs) are not only highly sensitive to external aligning fields but can also exquisitely control the
propagation of electromagnetic phenomena. Consequently, the patterning of LC molecules has
long been of interest for scientific discovery and technological advancement.

Smectic-A (SmA) LCs are characterized by arrangement of molecules into layers with the
long molecular axis parallel to the layer normal. When the surface chemistry promotes planar
alignment of LC molecules, smectics-A spontaneously form highly ordered hexagonal arrays of
toric focal conic domains (TFCDs)(De Gennes and Prost 1993) in which smectic layers wrap
around a pair of disclination lines formed by a circle and a straight line passing through the circle
center. In surface measurements, a defect domain appears as a circular, cone-shaped dimple at
the LC/air interface. The bending of the LC layers away from the flat equilibrium SmA to form
TFCDs results from the competing effects of planar anchoring at the LC/substrate interface and
homeotropic anchoring at the LC/air interface. In the standard smectic ground state, the smectic
layers are flat and parallel to the substrate and thus the molecular orientation points normal to
both the LC/air and LC/substrate interfaces. The TFCDs form spontaneously when the decrease
in surface energy obtained by planar anchoring on the substrate outweighs the elastic energy
cost of bending the layers and the increase in surface energy due to the dimple-like deformation
of the LC/air interface. Regular hexagonal lattices of TFCDs have been used to create microlens
arrays(Kim, Jeong et al.), matrices for the self-assembly of soft microsystems(Yoon, Choi et al.
2007; Martinez-Miranda and Lynn 2009; Pratibha, Park et al.), lithographic templates(Choi, Pfohl
et al.), two-dimensional (2D) charge transport models(Choudhury, Rao et al. 2010), and
patterned, functional surfaces. The ability to control the size and arrangement of TFCDs is
currently under investigation; for instance, studies have employed substrates presenting different
surface chemistries,(Zappone and Lacaze 2008; Choudhury, Rao et al. 2010; Simon, Burton et
al.; Zappone, Lacaze et al.) confinement within 1D microchannels,(Choi, Pfohl et al. 2004; Mitya,
and randomly patterned planar and depressed substrates.(Guo, Herminghaus et al. 2008) Little is
known, however, about a higher level of control of TFCDs into three dimensions (3D). (DiDonna
Controlling topological defects and smectic LC phases in 3D is of particular interest to the generation of blue phases and other topologically structured materials which will lead to possibly disruptive display technologies.

Here, we demonstrate, for the first time, epitaxial assembly of SmA LCs into arrays of TFCDs with variable sizes and arbitrary symmetries (e.g. a square lattice) directed by pillar arrays. We utilize materials that induce planar anchoring of LC molecules, such as SU-8, a bisphenol A epoxy derivative. By varying the pillar dimensions (size, height and spacing) and thickness of the LC film, we can confine and direct the growth of each TFCD. As a result, we promote a new variety of TFCD arrays beyond the close-packed hexagonal arrangement formed spontaneously on a flat surface by controlling the size and symmetry of the underlying pillar pattern. We hope that this template-directed assembly method will benefit a number of engineering applications and advanced device concepts.
Results and Discussion

The liquid crystals used in this communication are rigid biphenyl molecules with semi-fluorinated chains (see chemical structure in Figure S4.1). They have a smectic-A LC phase at ~ 114 °C, and retain TFCD structure when quenched to room temperature. The LCs were synthesized by a 2-step reaction following the literature,(Johansson, Percec et al. 1996; Percec, Johansson et al. 1996) and their structure was confirmed by Fourier transform infrared (FT-IR) spectroscopy and ¹HNMR (see Supporting Information). Highly ordered hexagonal arrays of TFCDs were observed on bare Si wafers and SU-8 coated Si wafers via scanning electron microscopy (SEM) with corresponding Maltese cross patterns in polarized optical microscopy (POM) (see Figure S4.3a-c). The same morphology has been reported by Kim et al. (Kim, Yoon et al. 2009) on Teflon-AF coated Si and glass substrates, suggesting high planar anchoring strength of the LCs on these substrates. It has been suggested that the TFCD radius a, measured as half of the center-to-center distance between neighboring TFCDs, is roughly equal to half of the LC film thickness (h) in these regions.(Guo, Herminghaus et al. 2008; Kim, Yoon et al. 2009) In our system, the average TFCD radius <a> on the flat Si and SU-8 surface is ~ 2.5 µm for h = 5 µm, in agreement with the literature.

Using 1D microchannels, Kim et al. studied confined assembly of high density TFCDs and reported that domain formation was strongly influenced by both the channel width (W) and, even more dramatically, by the channel depth (H).(Kim, Yoon et al. 2009) They found that in order to form an energetically stable, hexagonal array of TFCDs, \( W > W_c \sim 4 \mu m \) and \( H > H_c \sim 2 \mu m \).(Kim, Yoon et al. 2009) Here, we use SU-8 pillar arrays with varying pillar diameter, height, spacing, and symmetry as a 3D confinement system for SmA LCs.

Figures 4.1 and 4.2 demonstrate different TFCD morphologies from the assembly of SmA LCs directed by confinement and interaction with the underlying square pillar arrays at variable length scales. The geometry created by a square array of pillars is described by three
Figure 4.1 Epitaxial assembly of TFCDs on SU-8 square pillar arrays with diagonal separation $S \leq S_c$. a) Schematic illustration of SmA LCs confined by a SU-8 square pillar array with $S < S_c$. b) SEM image of the SU-8 square pillar array with diameter $D = 1 \mu m$, diagonal separation $S = 3 \mu m$, and height $H = 1.5 \mu m$. c-d) SEM images of the corresponding TFCDs assembled on the SU-8 pillar array (b) at various LC thicknesses, $h = 1.5-2.5 \mu m$ (c) and $3.5 \mu m$ (d). d) inset: polarized optical image at high magnification. e-f) SEM images of TFCDs assembled on the SU-8 pillar array with $D = 5 \mu m$, $S (\sim S_c) = 5 \mu m$, $H = 2.5 \mu m$ at various LC thickness, $h = 2.5 - 4 \mu m$ (e) and $4.0 \mu m$ (f). f) inset: high magnification. The blue arrows indicate satellite TFCDs formed between the neighboring pillars.
parameters: $S$, the smallest distance between the edges of two diagonally neighboring pillars; $D$, the pillar diameter; and $H$, the pillar height. Depending on the values of these parameters and of the LC thickness $h$, the pillars define anchoring points for TFCDs at the centers of the pillars’ top surfaces, or on the substrate positioned symmetrically between four neighboring pillars, or both.

When the LC thickness $h$ exceeds the pillar height $H$ by at least 1.5 µm, a single TFCD forms on the circular top of each pillar (see Figure 4.1a). Figure 4.1c and Figure S4.5c show an area of unlevelled LC thickness on pillar arrays, clearly revealing that the top surface of each pillar defines an anchoring point of one TFCD (see a whole pattern and its polarized image in Figure 4.1d). No TFCDs were observed on the top of pillars, however, when $D < 1$ µm. This is due to inadequate anchoring area for a circular defect. For such small TFCD diameters, the negative energy contribution from the surface term at the LC-substrate boundary is outweighed by the elastic energy cost and the increased surface area at the air-LC interface. The experimental value of this critical diameter, $D_c \approx 1$ µm, agrees well with our calculations of TFCD energies in 3D pillar arrays (see details in Supporting Information), which predict the minimum domain diameter of an energetically stable TFCD to be 1.0 µm. Notably, this critical diameter does not change over a wide range of $h-H$. Thus, with directing pillars of $D \geq D_c$, a square array of TFCDs could be generated that grow into the bulk. Indeed, we find that both the square symmetry and dimension of the pillar array was maintained in the TFCDs for LC thicknesses up to 40 µm (see Figure S4.4), demonstrating the long range ordering into the bulk from surface epitaxy. Since the main aim of our study is to control the arrangements of TFCDs other than the natural close-packed structures using geometric confinement by pillars, in all experiments we avoided making both $D$ and $h-H$ so large as to generate multiple close-packed TFCDs on the top surface of a single pillar.

Our experimental data also show that the pillar array defines TFCD anchoring points on the substrate between pillars: in the center of each unit cell defined by four neighboring pillars, a single TFCD is observed when the diagonal pillar spacing $S$ exceeds a critical value, $S_c \sim 5$ µm.
and the LC thickness $h$ exceeds the critical height, $h_c \sim 1.5 \mu m$. The pillars cause this transition in two ways: they limit the surface area on the substrate available to the TFCD for planar anchoring, and they impose additional planar anchoring conditions along the vertical pillar sides, requiring the smectic layers to orient horizontally as they approach the pillars. As a result, when $S < S_c$, LC layers align parallel to the bottom of the substrate, filling up the spaces between pillars without forming a defect domain at any LC thickness, as shown in Figure 4.1c (region marked with an arrow), where $S = 3 \mu m < S_c$ and $h = 1.5 \mu m \sim h_c$. Conversely, TFCDs formed between the pillars when $S = 15 \mu m > S_c$ for $h > h_c$, as shown in Figure 4.2c and d. When $S > S_c$, $D > D_c$, and $h$ surpasses the pillar height $H$ by at least 1.5 $\mu m$ (i.e. $h - H > h_c$), TFCDs are observed to develop from both anchoring sites, the surface between pillars and the top surface of the pillars, as shown in Figure 4.2f. Square lattices of TFCDs between pillars were observed for $H$ as low as 0.5 $\mu m$; therefore, the critical pillar height $H_c$, which marks the transition from a hexagonal close-packed lattice of TFCDs to a square lattice, must be at a smaller length scale but, presumably, much greater than the layer spacing (3.2 nm).

It is noted that the formation of the TFCDs was not only influenced by the geometry of the pillar array alone, but also the surface chemistry of the confinement. From an energetic standpoint, the formation of TFCDs in a thin film smectic LC requires surface chemistry that promotes planar alignment of LCs, as provided by materials such as SU-8, a widely used photoresist to fabricate high aspect ratio pillar arrays. Otherwise, there would be no TFCD developed within the confinement at any dimension. The importance of surface chemistry is further strikingly demonstrated when the SU-8 pillar arrays are coated with Au, as a result of which no TFCDs form (see Figure S4.6). To this point, our results clearly demonstrate that this geometric 3D confinement of SU-8 pillars essentially permits the controlled growth of the multi-scale TFCD array which is not achievable through a simple 1D patterning approach.
At the critical spacing, \( S \sim S_c (5 \, \mu\text{m}) \), Figures 4.1e and 4.1f reveal evidence of coexistence between TFCDs on the substrate between pillars (the small dimples indicated by blue arrows in Figure 4.1f) and the flat-layer state, where no dimple forms in the surface between pillars. This coexistence may be due to kinetics preventing complete equilibration. The inconsistency with which TFCDs form between pillars in Figures 4.1e and 4.1f is to be contrasted with the nearly perfect square array of TFCDs that form on top of the pillars in the same images. The TFCDs on top of the pillars exhibit a larger dimple in the surface because they are centered at a lesser depth below the surface. Indeed, our calculations (Figure 4.3) of smectic bending and surface energies predict that when the LC is confined within a space smaller than a critical spacing, homeotropic alignment of molecules at the substrate surface becomes energetically preferable to the formation of a TFCD between pillars. The predicted \( S_c \) is, however, \( \sim 1 \, \mu\text{m} \). The discrepancy between experiment and theory may arise from our assumption that the smectic layers distort only within the TFCD (see detailed discussion in Supplemental Information, Figures S4.7-S4.10), so that there is an energy penalty only when the TFCD comes into contact with the pillar. Through this assumption, we have ignored more complicated layer organization that may occur at the sharp corner where the pillar meets the substrate and the boundary conditions change rapidly. Such structures could allow the pillars to effectively “repel” TFCDs at a distance. Further experimentation will be pursued to test the influence of sharp corners of pillars.

It is interesting that only single domains appear in the space between four neighboring pillars at any LC thickness even though multiple domains having equilibrium diameter roughly equivalent to \( h \) were expected for sufficiently large \( S \) (\( \sim 15 \, \mu\text{m} \)) and low LC thickness as, for instance, \( h = 2 \, \mu\text{m} \) (Figure 4.2c). Our experimental results suggest that the topographical confinement effect of pillars remains influential for LC alignment at this length scale, although it is also possible that the variation in both surface chemistry and curvature of each pillar could lead to a varying anchoring strength of LC on the substrate, which would result in larger domains.
Figure 4.2 Epitaxial assembly of TFCDs on SU-8 square pillar arrays with diagonal separation $S > S_c$ and diameter $D > D_c$. a) Schematic illustration of SmA LCs confined by a SU-8 square pillar array with $S \geq S_c$ and $D > D_c$. b) SEM image of a SU-8 square pillar array with diameter $D = 10$ µm, diagonal separation $S = 15$ µm, and height $H = 7.5$ µm. c-f) The corresponding TFCDs assembled on the SU-8 pillar array (b) at various LC thicknesses: $h = 2$ µm (c), 7.5 µm (d), 8 µm (e), and 9 µm (f).
Figure 4.3 Calculated TFCD energy relative to the flat-layer state vs. \( \rho = a/h \) using \( h = H = 3.0 \ \mu m \), \( D = 1 \ \mu m \), and varying pillar spacing with diagonal separation \( S \). A sharp energy penalty is evident for TFCDs whose diameter exceeds \( S \), while smaller sized TFCDs have the same energy as on a flat substrate. Materials constants for calculation (Kim, Yoon et al. 2009) include the splay elastic constant \( K = 5 \times 10^{-11} \ \text{N} \), the defect core size \( \xi = 3 \times 10^{-9} \ \text{m} \), and the energy per unit area for molecules oriented normal to the LC/air interface \( \sigma_{\perp}^{\text{air}} = 20 \times 10^{-3} \ \text{N/m} \). The energy per unit area for molecules oriented parallel vs. normal to the LC/SU-8 interface is determined. \( \Delta \sigma_{\text{ubs}} = -1.1 \times 10^{-3} \ \text{N/m} \). This is found by requiring that the calculated TFCD energy allow energetically stable TFCDs only for values of \( h \geq h_c = 1.5 \ \mu m \).
Our results demonstrate three important features with regard to epitaxial assembly of LC molecules confined and directed by an SU-8 pillar array. First, it is possible to alter the naturally-occurring close-packed lattice of the TFCD arrays via anchoring. To further confirm the control of TFCD arrangement by the supporting pillars, we confined the LCs on a hexagonal pillar array with $D = 1 \, \mu m$, $S = 1 \, \mu m$, $H = 2 \, \mu m$ (Figure 4.4a). As anticipated, the top surface of the underlying pillars served as anchoring points of the TFCDs, resulting in a hexagonal array of the defect domains with a domain diameter of $\sim 1 \, \mu m$, equal to $S$ (see Figures 4.4 b-c).

Second, defect size and spacing can be controlled simply by varying the dimensions of the directing pillars, which enables generation of TFCD arrays with defect size and spacing smaller than previously observed in the same material at any given LC thickness ($\sim 5 \, \mu m$ on treated and untreated flat Si surfaces and $\sim 2.6 \, \mu m$ in a 1D microchannel for the smallest tested $h = 5 \, \mu m$). The possibility of down-scaling the spacing between defects will be beneficial especially for LC-based device fabrication.

The third and most important implication of pillar directed epitaxial assembly of LCs is the conservation of the symmetry and dimension of TFCD arrays at high LC thickness, experimentally observed up to 40 $\mu m$ despite the fact that on a flat non-patterned substrate the domain size scales as the film thickness.(Guo, Herminghaus et al. 2008; Guo and Bahr 2009) This behavior is a direct result of LC confinement and epitaxial growth of individual TFCDs, in which the geometry of the pillar sets the upper limit for the size of a domain by imposing a sharp energy barrier to further domain growth. Furthermore, the minimum allowable domain size is also independent of LC thickness, as predicted by the energy model: in Figure S4.7, we plot the calculated energy of a TFCD relative to the flat-layer state as a function of domain radius; the energy curve crosses zero at approximately the same domain radius ($0.5 \, \mu m$) at any $h \geq 2 \, \mu m$, setting $S_c$ and $D_c$ independently of $h$. Our results are further supported by the observation of smectic 8CBs assembled on a circularly patterned flat substrate,(Guo, Herminghaus et al. 2008)
where the diameter of the TFCDs is determined solely by the diameter of the circular units regardless of the film thickness. As a result, the simple proof-of-principle experiments presented here together with modeling provide a viable technique to generate a uniform array with arbitrary symmetry of equal sized TFCDs that extend into the bulk.

**Conclusion**

We have demonstrated epitaxial assembly of smectic-A LCs using top-down fabricated polymer pillar arrays. The 3D nature of the pillar array is crucial to confine and direct the formation of toric focal conic domains on the top of each pillar as well as between neighboring pillars. Independent of LC thickness (above a critical thickness $h_c$), the pattern of SU-8 pillar arrays determined the final crystal habit of the TFCD array: both highly ordered square and hexagonal array TFCDs were obtained. The epitaxial approach presented here offers an entirely new and promising organizational principle for smectic LC systems using simple topographic substrates. In turn, it may lead to the formation of more complex LC phases in 3D that are critical to the advancement of LC based electronic and optical devices,(DiDonna and Kamien 2002; DiDonna and Kamien 2003) and perhaps generation of novel materials when incorporating functional units such as nanoparticles, nanocrystals and carbon nanotubes into the LC layers.
Figure 4.4 Epitaxial assembly of TFCDs on SU-8 hexagonal pillar arrays. a) SEM images of the SU-8 hexagonal pillar array with diameter $D = 1 \, \mu m$, diagonal separation $S = 1 \, \mu m$, height $H = 2 \, \mu m$. b-c) The corresponding TFCDs on the hexagonal pillar array (a) at various LC thickness vs. pillar height, $h-H = 0-8 \, \mu m$ (b) and $8 \, \mu m$ (c).
References


Supporting Information

Experimental

Unless specifically noted, all chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as received.

Synthesis of liquid crystal. The semi-fluorinated smectic liquid crystal, (4’–(5,5,6,6,7,7,8,8,9,9,10,10,11,-11,12,12,12-heptadecafluoro-ododecyloxy)-biphenyl-4-carboxylic acid ethyl ester) was synthesized by a two-step reaction following the literature (Johansson, Percec et al. 1996; Percec, Johansson et al. 1996) (see Figure S1). In brief, a mixture of 1H, 1H, 2H, 2H, 3H, 3H, 4H, 4H-perfluorododecan-1-ol (A) (1.63 g, 3.15 mmol) (Synquest Laboratories, Inc., Alachua, FL, USA), phase-transfer catalyst, Aliquat™ 336 (60 µL), and 48% HBr aqueous solution (ACS grade) (4 mL) was heated in an oil bath to 100 °C. After 20 h reaction, the mixture was cooled to room temperature and extracted with ethanol. The organic layer was washed several times with H₂O, dried over MgSO₄ powder, and filtered. The solvent was removed with a rotatory evaporator at room temperature for 30 min. 1.34 g (82.2% yield) of 1H, 1H, 2H, 2H, 3H, 3H, 4H, 4H-perfluorododecyl bromide (B) as a product was obtained as orange oil. Next, a mixture of B (1.34 g, 2.34 mmol), ethyl 4’-hydroxy-4-biphenyl carboxylate (0.584 g, 2.34 mmol) (C), K₂CO₃ (0.96 g) and dimethylformide (DMF) (13 mL) was heated in an oil bath under N₂ atmosphere at 65 °C for 20 h. The mixture was quenched by cooling to room temperature and mixed with 100 mL ice water. The raw product was acidified with concentrated aqueous HCl solution (20 vol%), followed by vacuum filtration and drying in air. Finally, the product (D) was recrystallized with acetone to yield light yellow crystals (75 % yield).
Figure S4.1 Schematics of the synthesis of LC molecules.
Figure S4.2 $^1$HNMR (a) and $^{19}$FNMR (b) spectra of LC, compound D.
Compounds B and D were confirmed by $^1$H and $^{19}$F NMR shown in Figure 4.2S, and consistent with the literature. (Johansson, Percec et al. 1996; Percec, Johansson et al. 1996) NMR spectra were recorded on a Bruker Advance DMX 360 (360 MHz) at 25°C and analyzed using TOPSPIN software. $^1$HNMR (CDCl$_3$, $\delta$, ppm). B: 1.75-2.18 (overlapped peaks, 6H, CF$_2$CH$_2$CH$_2$CH$_2$), 3.44 (t, 2H, CH$_2$Br). D: $^1$H NMR: 1.26 (m, 2H, CF$_2$CH$_2$CH$_2$), 1.39 (t, 3H, OCH$_2$CH$_3$), 1.6 - 1.91 (m, 4H, CF$_2$CH$_2$CH$_2$CH$_2$), 4.04 (m, 2H, OCH$_2$(CH$_2$)$_3$), 4.46 (m, 2H, OCH$_2$CH$_3$), 6.97 (d, 2H, ortho to O), 7.60 (t, 4H, ortho to ortho), 8.07 (d, 2H, ortho to CO$_2$CH$_3$). $^{19}$FNMR (CDCl$_3$, $\delta$, ppm). B: -80.7 (t, 3F, CF$_3$), -114.2 (m, 2F, CF$_2$CH$_2$), -121.9 (m, 6F, ((CF$_3$)$_2$CF$_2$CH$_2$), -122.8 (m, 2F, CF$_2$CF$_2$CF$_2$CF$_3$), -123.5 (m, 2F, CF$_2$CF$_2$CF$_2$CF$_3$), and -126.0 (m, 2F, CF$_2$CF$_3$). D: -80.7 (t, 3F, CF$_3$), -114.3 (m, 2F, CF$_2$CH$_2$), -121.8 (m, 6F, (CF$_3$)$_2$CF$_2$CH$_2$), -122.8 (m, 2F, CF$_2$CF$_2$CF$_2$CF$_3$), -123.4 (m, 2F, CF$_2$CF$_2$CF$_3$), -126.0 (m, 2F, CF$_2$CF$_3$).

**Fabrication of SU-8 pillar arrays.** Silicon wafers were used as substrates for SU-8 pillar fabrication. The wafers were cleaned in a solution of Detergent 8 (Alconox, NY, USA) (2 vol%) at 60 °C for 1 h, followed by rubbing with a cotton swab and rinsing with DI water multiple times. The wafers were then dried with compressed air and cleaned additionally by oxygen plasma cleaner (PDC-100, Harrick Scientific Products, 30 W) for 1 h before use.

SU-8 square pillar arrays were fabricated on cleaned Si wafers by contact lithography using the OAI Model 200 mask aligner, following the guideline from MicroChem Corp. for SU-8 2010 solution. Hexagonal arrays of pillars ($D = 1 \mu$m, $S = 1 \mu$m, $H = 2 \mu$m) were fabricated via capillary force lithography (CFL) from the SU-8 pillar masters following a procedure reported previously. (Zhang, Lin et al. 2010) Briefly, PDMS molds were prepared by pouring the mixture of PDMS prepolymer and crosslinker (RTV615 from GE Silicones, 10:1 wt/wt) on top of the pillar masters, followed by curing at 65 °C for 4 h. The cured PDMS molds were peeled off for use in CFL. SU-8 2 solution (from MicroChem Corp., Newton, MA) was spin-coated onto a clean Si wafer at 3,000 rpm for 30 s to obtain a film thickness of 1.5 µm. The SU-8 thin film was soft-
baked at 65 °C for 2 min and 95 °C for 2 min before placing the PDMS mold on the molten SU-8 film for an additional 2 min at 95 °C for CFL. A slight pressure was applied to ensure conformal contact between the mold and SU-8 film. The films were then cooled down to room temperature. To enhance the pillar mechanical strength, we exposed the pillars together with the PDMS mold to UV light (Newport 97435 Oriel Flood Exposure Source, 54 mW cm⁻² at λ = 365 nm) with an overall dosage of 100 mJ cm⁻². Finally, the films were post-exposure baked at 65 °C for 2 min and 95 °C for 2 min. After cooling to room temperature, the SU-8 pillar array was peeled off from the PDMS mold. The SU-8 pillars were rinsed with isopropanol (IPA) and dried with compressed air.

**Assembly of LC molecules on substrates.** The crystalline powder of the LC molecules was dissolved in a fluorinated solvent (Fluorinert FC-770, 3M) at a concentration of 500 mg mL⁻¹. Different amounts of LC solution (10 µL – 120 µL) were drop-cast onto the flat and pillar substrates to vary the thickness of LC films. The LC films and substrates were heated on a Mettler FP82 and FP90 thermo-system hot stage to an isotropic phase at 200 °C, cooled to 114 °C at 5 °C min⁻¹ to form a smectic-A phase, and subsequently quenched to room temperature.

**Characterization.** The quenched structures of LCs confined on various substrates were characterized by scanning electron microscopy (SEM) on FEI Strata DB235 focused ion beam (FIB) system at 5 kV. The LC phase transition from the isotropic phase, through the smectic-A phase, to the crystal phase were monitored with Zeiss AxioImager M1m upright microscope with crossed polarizers (on a Minus K Vibration Isolation Platform with monochrome camera: AxioCam HSm, and color camera: AxioCam HRc) through transmittance mode after the LC crystals on glass substrate were heated to 200 °C, followed by cooling down at 5 °C min⁻¹ to room temperature.

The LC phases were confirmed by X-ray diffraction using a Bruker-Nonius Fr591 generator with a 0.2x2 mm² focus, operated at 3.7 kW. CuKα photons were selected and focused using Osmic confocal optics and three sets of pinholes. Measurements were made at
fixed sample-detector distances of 54 cm and 11 cm, respectively. The scattered photons were collected using a Bruker HiStar area detector. An integral vacuum was maintained from the focusing optics to the detector. The samples were loaded in glass capillaries, and thermally regulated using a Linkam temperature controller. Primary data analysis was accomplished using Datasqueeze software.
Figure S4.3 (A) Chemical structure of the liquid crystal molecule and (B) its phase transition determined by X-ray scattering, which is consistent with report by Yoon et al. (Yoon, Choi et al. 2007). (C) SEM images (a, c) and a polarized optical microscopy (POM) image (b) of toric focal conic domains (TFCDs) formed on a flat Si wafer.
Figure S4.4 SEM image of SmA LCs assembled on the SU-8 square pillar array (D = 5 µm, S (~ S₈) = 5 µm, H = 2.5 µm) at a LC film thickness h = 40 µm, illustrating 3D confinement of LCs by the pillars.

Figure S4.5 SEM images of SmA LCs assembled between the SU-8 square pillar array (D = 10 µm, S = 15 µm, H = 7.5 µm) at LC film thickness h = 2 µm (a), 6 µm (b), and 7.5 – 8 µm (c) at a tilting angle of 30°, 45° and 52°, respectively. Scale bar in (c) applies to all the images.
Figure S4.6 SEM images of SmA LCs assembled between the Au coated (sputtered for 40 sec) SU-8 square pillar arrays. (a) $D = 5 \, \mu m$, $S = 5 \, \mu m$, $H = 2.5 \, \mu m$, and LC film thickness $h = 2 \, \mu m$. (b) $D = 10 \, \mu m$, $S = 15 \, \mu m$, $H = 7.5 \, \mu m$, $h = 7.5 - 9 \, \mu m$. 
Calculation of the energy of a TFCD (by Daniel A. Beller)

We first calculate the energy of a TFCD centered on the substrate between neighboring pillars. In the presence of a square array of pillars, the energy of a TFCD relative to the flat-layer state is the sum of three components:

\[ F = F_s + F_{el} + F_{pillar} \]

The first term is the surface energy,

\[ F_s = \sigma_{\perp \text{air}} A' + \sigma_{\parallel \text{subs}} A - \sigma_{\perp \text{air}} A + \sigma_{\perp \text{subs}} A = \sigma_{\perp \text{air}} A' + (\Delta \sigma_{\text{subs}} - \sigma_{\perp \text{air}}) A, \]

where \( A \) is the "undistorted" area taken up by the TFCD on the substrate; \( A' \) is the surface area of the distorted LC exposed to the air; \( \sigma_{\perp \text{air}} \) is the energy per unit area for molecules at the air-LC interface with orientation perpendicular to the interface; and \( \Delta \sigma_{\text{subs}} = \sigma_{\parallel \text{subs}} - \sigma_{\perp \text{subs}} \) is the difference in energy per unit area for molecules at the LC-substrate boundary with orientation parallel vs. perpendicular to the boundary.

The second term is the elastic energy,

\[ F_{el} = \int dV f_{el}, \]

where the integral is over the volume of the TFCD, and the elastic energy density is

\[ f_{el} = K \left( \frac{1}{R_1} + \frac{1}{R_2} \right)^2 + \frac{K}{2} \frac{1}{R_1 R_2} + \frac{1}{2} B \delta^2, \]

where \( R_1, R_2 \) are the layers' principal radii of curvature. Here, the first term is proportional to the splay elastic constant multiplied by the mean curvature, the second term is the saddle-splay
elastic constant multiplied by the Gaussian curvature, and the third term is a compression modulus $B$ multiplied by a change $\delta$ in the layer spacing. Because TFCDs are equally spaced, we will assume that the costly compression term vanishes and that $\delta = 0$ throughout. As in Kim et al. (Kim, Yoon et al. 2009), we take $K = 0$. Thus we take

$$f_{el} = \frac{K}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)^2.$$  

The elastic energy is always positive, whereas the surface energy may be negative. In the absence of pillars, the balance of the elastic energy and surface energy determines whether a TFCD of a given radius is energetically favorable compared to the flat-layer ground state.

The third term in $F$ is the "pillar energy", an extra surface energy that arises when a pillar occupies part of the cylindrical region that the TFCD would take up on a flat substrate, i.e. when $a > S/2$ (where $a$ is the radius of the domain and $S$ is the spacing between the closest points on two pillars that are next-nearest neighbors). In this case, the tilt of the layers as determined by the TFCD geometry requires the molecules at the liquid crystal-pillar boundary to lie in a direction not completely parallel to the side of the pillar, inducing an extra surface energy cost $F_{\text{pillar, side}}$. When the pillar height $H$ is less than the LC thickness $h$, there is also a small negative energy contribution, $F_{\text{pillar, top}}$, from the top surface of the pillar: Whereas molecules in the flat-layer state are oriented uniformly perpendicular to this surface, molecules in a TFCD are slightly tilted away from the perpendicular wherever the layers are not completely horizontal. The sum of these two energies gives $F_{\text{pillar}}$.

**Case 1: $a < S/2$**

First consider the case where the TFCD radius, $a$, is smaller than $S/2$. This calculation is described in the Supporting Information to Kim et al. (Kim, Yoon et al. 2009). The smectic layers
are distorted from the horizontal ground state inside a cylindrical volume of radius \(a\). With respect to the origin at the center of the unit cell, we first use the cylindrical coordinates \(z\), the height above the substrate; \(s\), the radial distance from the origin in a plane parallel to the substrate; and \(\phi\), an azimuthal angle. We assume that the layers are distorted into portions of tori as follows: If a layer's height above the substrate is \(\eta\) for \(s > a\) \(\left(0 \leq \eta \leq h\right)\), then \[z(s) = \sqrt{\eta^2 - (s-a)^2}\] for \(s \leq a\). Fixing \(\phi\) chooses a half-plane, in which we define new planar polar coordinates \((r, \theta)\) relative to an origin at the point \((z = 0, s = a, \varphi)\). \(\theta\) lies in the interval \([0, \pi / 2]\), and \(r\) lies in the interval \([0, h]\). The two principal radii of curvature (of opposite sign) are

\[
R_1 = r \\
R_2 = -\left(\frac{a - r \sin \theta}{\sin \theta}\right) = r - \frac{a}{\sin \theta}
\]

where \(R_1\) corresponds to curvature about the circular defect, and \(R_2\) corresponds to curvature around the central line defect. Therefore,

\[
f_{el} = \frac{K}{2} \left(1 + \frac{1}{r-a/\sin \theta}\right)^2 = \frac{K}{2r^2} \left(\frac{2r-a/\sin \theta}{r-a/\sin \theta}\right)^2
\]

and the area measure is

\[
dS = r(a/\sin \theta - r) \sin \theta d\theta d\phi = r(a - r \sin \theta)d\theta d\phi.
\]

We divide the cylindrical region occupied by the TFCD into two: Region I, a cone of hypotenuse \(h\), defined by:

\[
\xi \leq r \leq a/\sin \theta - \xi, \quad \arcsin\left(\frac{a}{h}\right) \leq \theta \leq \pi/2,
\]

and Region II:

\[
\xi \leq r \leq h, \quad 0 \leq \theta \leq \arcsin\left(\frac{a}{h}\right).
\]
(See Figure S8a) Here, $\xi$ defines the core size of the line defect in the center of the TFCD; its value of 3 nm is comparable to the layer spacing. Let $\rho = a / h$ and $\theta^* = \arcsin(\rho)$. The total elastic energy is

$$F_{el} = \int_0^{2\pi} d\phi \left( \int_0^{\pi/2} \frac{d\theta}{\sin \theta} \int_{\xi}^{\pi/2} dr a \cos \theta dr + \int_0^{\theta^*} d\theta \int_{\xi}^{\rho - \xi} dr r(a - r \sin \theta) \frac{K}{2} \left( \frac{2r - a \sin \theta}{r - a \sin \theta} \right)^2 \right)$$

$$= 2\pi Kh \rho \left( \ln \left( \frac{h \rho}{\xi} \right) - 2 \left( \frac{\pi}{2} - \theta^* \right) \right) - 2\pi Kh \rho \int_{\theta^*}^{\pi/2} d\theta \ln(\sin \theta) + 4\pi Kh \left( \sqrt{1 - \rho^2} - 1 \right)$$

$$+ \pi Kh \rho^* \ln(h \rho^* \xi) - \pi Kh \rho \int_0^{\theta^*} d\theta \ln(\rho - \sin \theta)$$

To calculate the surface energy, we need the undistorted area $A = \pi r^2$ and the distorted area of the air-liquid crystal interface

$$A' = \int_0^{2\pi} d\phi \int_0^r \frac{sh}{\sqrt{h^2 - (s-a)^2}} ds d\phi = \int_0^r \int_0^{2\pi} d\phi \int_0^r \frac{sh ds}{\sqrt{h^2 - (s-a)^2}}$$

$$= 2\pi h^2 \left( \sqrt{1 - \rho^2} - 1 + \rho \theta^* \right)$$

In this case, $F_{pillar} = 0$. The total energy of the TFCD relative to the flat-layer state is

$$F = 2\pi Kh \rho \left( \ln \left( \frac{h \rho}{\xi} \right) - 2 \left( \frac{\pi}{2} - \theta^* \right) \right) - 2\pi Kh \rho \int_{\theta^*}^{\pi/2} d\theta \ln(\sin \theta) + 4\pi Kh \left( \sqrt{1 - \rho^2} - 1 \right)$$

$$+ \pi Kh \rho^* \ln(h \rho^* \xi) - \pi Kh \rho \int_0^{\theta^*} d\theta \ln(\rho - \sin \theta) + 2\sigma^a_{\perp} h^2 \left( \sqrt{1 - \rho^2} - 1 + \rho \arcsin \rho \right)$$

$$+ (\Delta \sigma^s - \sigma^a_{\perp}) h^2 \rho^2.$$
This energy as a function of the domain radius $a$ is plotted in Figure S4.7 for various values of $h$.

**Case 2: $a > S/2$**

When the TFCD is sufficiently large that the layers are not completely horizontal when they come into contact with the side of the pillar, the calculations of $F_s$ and $F_{el}$ become somewhat more complicated, and the additional surface energy $F_{pillar}$ must be added to the TFCD energy. (When TFCDs form both on the substrate and on top of the pillar, it is assumed that there is no overlap of their volumes.)

In order to calculate $F_{pillar}$, we need to determine the planar distance $d(\phi)$ from the TFCD center to the edge of a pillar at angle $\phi$. Call the diameter of the pillars $D$, and let $w = W + D$ be the lattice spacing of the pillar centers (where $W$ is the nearest distance between pillar edges). Then

$$d(\phi) = \frac{1}{2} \left[ w(\sin \phi + \cos \phi) - \sqrt{w^2(\sin 2\phi - 1) + D^2} \right]$$

Suppose that, at a given point on the side of the pillar, a molecule is oriented at an angle $\omega$ out of the local tangent plane to the pillar. Then the energy relative to the flat-layer state is $\sigma_{||} \cos \omega - 1 + \sigma_{\perp} \sin \omega$. Define $\phi_p$ as the minimum value of $\phi$ for which the TFCD is in contact with the pillar:

$$\begin{align*}
  d(\phi_p) &= a & \text{if } a \leq \sqrt{(w/2)^2 + (W/2)^2} \\
  \phi_p &= \arctan(1 - D/w) & \text{otherwise}.
\end{align*}$$
Now define an azimuthal angle \( \gamma \) relative to an origin at the center of a pillar, such that 
\[
\phi = \pi / 4 \iff \gamma = \pi / 4 \quad \text{and} \quad \phi = \arctan(1 - D / w \iff \gamma = 0),
\]
as shown in Figure S4.9. In general,
\[
d(\phi)\cos \phi = x(\gamma) = (w / 2) - (D / 2)\sin \gamma \\
d(\phi)\sin \phi = y(\gamma) = (w / 2) - (D / 2)\cos \gamma
\]
which give
\[
d(\gamma) = \sqrt{\left(\frac{w^2}{2}\right) + \left(\frac{D^2}{4}\right) - \left(\frac{wD}{2}\right)\left(\sin \gamma + \cos \gamma\right)}.
\]
Define
\[
\gamma^* = \begin{cases} 
- \arcsin\left(\left[a \cos(\phi_p) - w / 2\right] / (D / 2)\right) & \text{if } a \leq \sqrt{\frac{w^2}{2} + \left(\frac{W}{2}\right)^2} \\
0 & \text{if } a \geq \sqrt{\frac{w^2}{2} + \left(\frac{W}{2}\right)^2}.
\end{cases}
\]
Then we have \( \phi = \phi_p \iff \gamma = \gamma^* \). The pillar energy cost due to the side of the pillar can then be written
\[
F_{pillar, side} / 8 = \int_0^\pi / 4 d\gamma_p \int_{\gamma_p}^{\pi / 4} \frac{D}{2} d\gamma \left(\sigma_{\parallel} \sin \omega + \sigma_{\perp} \cos \omega\right)
\]
(By symmetry we may consider only \( 0 \leq \phi < \pi / 4 \) and then multiply by 8.) The quantity \( \cos\omega \) is given by the dot product \( \hat{n} \cdot \hat{n}' \) of the layer unit normal \( \hat{n} \) with the projection \( \hat{n}' \) of the layer normal onto the pillar side's local tangent plane (normalized to unit length). The layer surface equation
\[
z - \sqrt{\eta^2 - (s - a)^2} = 0
\]
gives the normal direction
\[
\hat{n} = \hat{z} + \hat{s} \frac{s - a}{\sqrt{\eta^2 - (s - a)^2}}.
\]
This
expression is then converted to Cartesian coordinates using \( s = \sqrt{x^2 + y^2} \) and

\[
\hat{s} = \hat{x}(x/s) + \hat{y}(y/s).
\]

Here,

\[
\eta(z_{\text{pillar}}, \gamma) = \sqrt{z_{\text{pillar}}^2 + (d(\gamma) - a)^2}.
\]

The projected normal vector is in the direction

\[
\hat{n}' = (\hat{n} \cdot \hat{z})\hat{z} + (\hat{n} \cdot \hat{\gamma})\hat{\gamma}
\]

where \( \hat{\gamma} = \hat{x}\cos \gamma - \hat{y}\sin \gamma \). Computing the dot product \( \hat{n} \cdot \hat{n}' \) in Cartesian coordinates gives \( \cos \omega \) and hence \( \sin \omega \) as functions of \( z_{\text{pillar}} \) and \( \gamma \), which can then be integrated over to determine the energy cost \( F_{\text{pillar, side}} \).

The slight energy benefit due to the top surface of the pillar is given by

\[
F_{\text{pillar, top}} / 8 = \int_{\phi_p}^{\pi/4} d\phi \int_{d(\phi)}^{a} ds \left( \sigma_{\text{sub}} \sin \alpha + \sigma_{\phi} \left( \cos \alpha - 1 \right) \right),
\]

where \( \alpha \) is the angle that a layer makes with the horizontal, in the radial \( \hat{s} \) direction, at cylindrical coordinates \( (z = H, s, \phi) \):

\[
\tan \alpha = \left. \frac{dz}{ds} \right|_{z=H} = \left. \frac{d}{ds} \sqrt{\eta^2 - (s-a)^2} \right|_{z=H} = \frac{a-s}{z} = \frac{a-s}{H}.
\]

The surface energy \( F_s \) is found using the undistorted surface area given by

\[
A / 8 = \int_{\phi_p}^{\pi/4} d\phi \left[ \int_{0}^{a} sd\nu + \int_{0}^{\pi/4} d\phi \int_{d(\phi)}^{a} s ds \right] = \frac{1}{2} a^2 \phi_p + \frac{1}{2} \int_{\phi_p}^{\pi/4} d\nu \left[ d(\phi) \right]^2
\]

and the distorted surface area given by
\[
A'/8 = \int_{\phi}^{\phi_{p}} d\phi \int_{s_{b}}^{a} ds \frac{sh}{\sqrt{h^2 - (s - a)^2}} + \int_{\phi_{p}}^{\phi} d\phi \int_{s_{b}}^{a} ds \frac{sh}{\sqrt{h^2 - (s - a)^2}} \\
= \phi_{p} h^2 \left[ \sqrt{1 - \rho^2} - 1 + \rho \theta^* \right] \\
+ h^2 \int_{\phi_{p}}^{\phi} d\phi \left[ \sqrt{1 - \rho^2} - \sqrt{1 - (d - a)^2} \right] h^2 + \rho \left( \arcsin \left( \frac{d - a}{h} \right) + \theta^* \right) 
\]

In order to calculate the elastic energy, \( F_{el} \), if the pillar height is less than the liquid crystal thickness, it is necessary to determine when a given value of \( \theta \) corresponds to a ray through the top of the pillar or through the side of the pillar. Specifically, does the value of \( \theta \) corresponding to the edge of the top of the pillar,

\[
\theta_{c}(\phi) \equiv \arctan \left[ \frac{a - d(\phi)}{H} \right],
\]

reside in Region I (\( \theta_{c}(\phi) < \theta^* \)) or Region II (\( \theta_{c}(\phi) > \theta^* \)) (see Figure S4.8 b) There are three possibilities. Subcase A: If \( \theta_{c}(\phi = \pi / 4) < \theta^* \), then \( \theta_{c}(\phi) < \theta^* \) for all \( \phi \). Subcase B: If \( \theta_{c}(\phi_{p}) > \theta^* \), then \( \theta_{c}(\phi) > \theta^* \) for all \( \phi \). Subcase C: If neither of these extremes holds, then define an angle \( \phi_{c} \) such that \( \theta_{c}(\phi) < \theta^* \) for \( \phi < \phi_{c} \) and \( \theta_{c}(\phi) > \theta^* \) for \( \phi > \phi_{c} \). The value of \( \phi_{c} \) is determined numerically by finding the root of the expression \( \theta_{c}(\phi_{c}) - \theta^* \).

Let \( J \) stand for the integrand in the elastic energy integral:

\[
J = r \left( a - r \sin \theta \right) \frac{K}{2} \left( \frac{2r - a / \sin \theta}{r - a / \sin \theta} \right)^2.
\]

First consider Subcase C. The elastic energy is a sum of eight integrals
\[ F_{el} / 8 = I_{1a} + I_{1b} + I_{2a} + I_{2b} + I_{2c} + I_{3a} + I_{3b} + I_{3c} \]

where

\[ I_{1a} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{H/\cos \theta}^{h} dr J \]

\[ I_{1b} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{H/\cos \theta}^{h} dr J \]

\[ I_{2a} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{a/(\sin \theta - \xi)}^{\pi/2} dr J \]

\[ I_{2b} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{H/\cos \theta}^{h} dr J \]

\[ I_{2c} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{a/(\sin \theta - \xi)}^{\pi/2} dr J \]

\[ I_{3a} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{H/\cos \theta}^{h} dr J \]

\[ I_{3b} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{a/(\sin \theta - \xi)}^{\pi/2} dr J \]

\[ I_{3c} = \int_{0}^{\phi_p} d\phi \int_{0}^{\pi/2} d\theta \int_{H/\cos \theta}^{h} dr J. \]

The calculation of \( F_{el} \) for Subcase A is similar to that for Subcase C, with the following changes:

\( I_{3a} = I_{3b} = I_{3c} = 0 \), and \( \phi_p \) is replaced with \( \pi/4 \) wherever it appears in the upper limits of integrals \( I_{2a}, I_{2b}, I_{2c} \). Likewise, \( F_{el} \) for Subcase B can be obtained from the above results for Subcase C by taking \( I_{2a} = I_{2b} = I_{2c} = 0 \) and replacing \( \phi_p \) by \( \phi_p \) in the lower limits of integrals \( I_{3a}, I_{3b}, I_{3c} \).

As shown in Figure 4.3 in the main text, for any \( S > 2h \) ("Case 1"), the TFCD energy as a function of its radius is the same as in the case of a flat substrate. For smaller values of \( S \) ("Case 2"), the pillars make the domain more energetically costly for \( a > S/2 \). Each curve agrees with the
$S > 2a$ curve for values of $\rho$ below $\rho h = S/2$. Energetically stable domains are expected to form only when $S \geq S_c$, where the critical pillar spacing is in this case $S_c \approx 0.98 \mu m$.

If the domain radius $a$ is greater than half of the pillar lattice spacing, $w/2$, then neighboring domains meet at a tilt wall, inducing an extra energy cost $F_{wall}$. As given by de Gennes and Prost,(De Gennes and Prost 1993) the free energy per unit area of the wall is

$$f_{wall} = \frac{2}{3} \frac{K}{\lambda} \psi^3.$$ Here, $\lambda$ is a length comparable to the layer spacing of 3.2 nm; we assume that $\lambda$ in fact equals the layer spacing. $\psi$ is the angle that the layers make with the horizontal at a given point on the tilt wall, at which

$$r = \sqrt{(W/2)^2 + (W \tan(\phi)/2)^2} = W/(2 \cos \phi).$$

$\psi$ is a function of both $\phi$ and $z$:

$$\psi = \arctan \left[ \frac{d}{dr} \left( \eta^2 - (r - a)^2 \right) \right]_{r=W/(2 \cos \phi)} = \arcsin \left[ a - W/(2 \cos \phi) / \eta \right]$$

where

$$\eta = \sqrt{z^2 + (W/(2 \cos \phi) - a)^2}.$$ The tilt wall on the right side of the unit cell will extend from $-\phi_{wall}$ to $\phi_{wall}$, where $\phi_{wall} = \arctan[a/(W/2)].$ (In the case that $\phi_{wall} > \phi_p$ as defined above, $\phi_p$ is used instead of
$\phi_{wall}$ as the limit of integration.) Since there are two complete tilt walls per domain, the total tilt wall energy is

$$F_{wall} = 2 \int_{-\phi_{wall}}^{\phi_{wall}} d\phi \int_{0}^{h^2-(W/(2\cos\phi)-a)^2} dz \left[ \frac{2K}{\lambda} \psi(z, \phi) \right].$$

**Calculation of critical pillar diameter.** On a flat substrate, TFCDs form only when the LC thickness $h$ exceeds the critical thickness, $h_c$. Similarly, TFCDs may also form on the top surfaces of pillars if $h - H > h_c$ (where $H$ is the pillar height) and the pillar diameter $D$ exceeds a critical diameter $D_c$. For smaller diameters, the negative energy contribution from the surface term at the LC-substrate boundary is outweighed by the elastic energy cost and the increased surface area at the air-LC interface. The energy of a TFCD of radius $a$ on the top surface of the pillar is calculated in the same manner as for Case 1 above, with the following change to the surface energy when $a > D/2$:

$$F_s = \sigma_{air} (A' - A) + \Delta \sigma_{sub} \pi (D/2)^2.$$

The critical diameter is the value of $D$ below which no domain size is energetically stable relative to the flat-layer state. The value of $D_c$ is predicted to be 1 $\mu$m (independent of $h-H$) as shown in Figure S4.10, in good agreement with the experimentally determined $D_c \approx 1 \mu$m. Since we neglected the Gaussian curvature this good agreement indicates that this effect is governed by surface anchoring and should apply to other materials as well.
Figure S4.7 TFCD energy relative to the flat-layer state versus domain radius (a) for a flat substrate, with LC thickness h increasing from uppermost to lowermost curves: h = 2 µm, 2.5 µm, 3 µm, 3.5 µm, 4 µm, 5 µm. The value of a where F changes from positive to negative, which determines $S_c$ and $D_c$, is approximately 0.5 µm for all LC thicknesses $h \geq 2$ µm.
Figure S4.8 (a) Geometry of a cross-section of a TFCD. Dashed lines divide the two regions of integration and the region external to the domain where the layers are flat. (b) Geometry of a cross-section of a TFCD confined by pillars, drawn as gray rectangles.
Figure S4.9 Geometry of the unit cell for a TFCD confined by pillars (drawn as gray circles). The circular defect bounding the TFCD is the dotted curve; the location of the central line defect is given by the thick black dot. Dashed lines mark the boundaries of the fundamental unit cell, with area one-eighth that of the original unit cell.
Figure S4.10 TFCD energy relative to the flat-layer state versus domain radius (a) for defects on top of the pillar at varying pillar diameters, $D$ using $h - H = 3 \, \mu m$. Thermodynamically stable domains form only for $D > D_c \approx 1.0 \, \mu m$. For $1.0 \, \mu m < D < 3.0 \, \mu m$, the domain radius equals $D/2$. For $D > 3.0 \, \mu m$, the domain radius is the same as on the flat substrate, $a \approx 1.5 \, \mu m$. Each curve agrees with the $D > 2(h - H)$ curve for values of $a < D/2$. 
References


CHAPTER 5

TOPOGRAPHICALLY-INDUCED HIERARCHICAL ASSEMBLY AND

GEOMETRICAL TRANSFORMATION OF FOCAL CONIC DOMAIN ARRAYS

IN SMECTIC LIQUID CRYSTALS

Honglawan and Beller et al. PNAS, 2012.

Introduction

Liquid crystals (LCs) are anisotropic materials with physical properties that depend sensitively on both global and local molecular alignment. In LCs, average local molecular orientations assume geometries that can be controlled by boundary conditions (Lee and Clark 2001; Brake, Daschner et al. 2003) and external fields (Kang, Maclennan et al. 2001; Miyajima, Aroaka et al. 2012) while the resulting mechanical and electric anisotropies of LCs provide powerful tools in controlling the propagation of light and the assembly of soft materials (Poulin, Stark et al. 1997; Muševič, Škarabot et al. 2006; Koenig, Lin et al. 2010; Lavrentovich, Lazo et al. 2010; Smalyukh, Lanksa et al. 2010; Moreno-Razo, Sambriski et al. 2012) A quintessential example is the blue phase LC organized around a three-dimensional (3D) disclination network (Coles and Pivnenko 2005; Ravnik, Alexander et al. 2011) As a display component, it offers rapid response time without surface alignment (Gardiner and Coles 2006) The ability to tailor LCs with complex, topologically-structured geometries will be necessary for the next generation of display technologies and beyond.

Under appropriate boundary conditions, the smectic-A (SmA) LC phase develops a regular array of micron-scale defect structures known as focal conic domains (FCDs), which have gone from mere geometric curiosities to the focus of much attention in recent years as an enabling technological tool (Yoon, Choi et al. 2007; Guo, Herminghaus et al. 2008; Zappone and Lacaze 2008; Yoon, Deb et al. 2010) The smectic layers in each FCD form concentric sections of
Dupin cyclides, generalizations of tori, with two linear focal sets (centers of curvature), an ellipse and a confocal hyperbola. (Alexander, Chen et al. 2010) Whereas FCDs arise as the prototypical, kinetically-trapped texture in bulk, a two-dimensional (2D) lattice of axially symmetric toric FCDs (TFCDs) can be robustly produced in thin smectic films with antagonistic boundary conditions at the substrate and air interfaces. These TFCD arrays have been used to fabricate functional surfaces, (Kim, Yoon et al. 2009; Kim, Jeong et al. 2010) to direct the self-assembly of soft microsystems, (Yoon, Choi et al. 2007; Pratibha, Park et al. 2010; Milette, Relaix et al. 2012) to template lithographic patterns, (Kim, Yoon et al. 2010) and to enhance charge transport in photovoltaics and transistors. (O’Neill and Kelly 2003) So far, most attention has been devoted to the precise manipulation of the locations of FCDs in 2D lattices by confining individual domains within small regions through both chemical and topographical patterning of the substrate. (Guo, Herminghaus et al. 2008; Kim, Yoon et al. 2009; Yoon, Deb et al. 2010) For device applications, it is desirable to produce FCDs with prescribed arrangements in 2D and 3D over large regions and to scale down the LC patterning. Recently, we have demonstrated epitaxial assembly of a TFCD lattice with tailored domain size and symmetry using polymer-based micropillar arrays. (Honglawan, Beller et al. 2011)

Here, we present a new level of control to direct the growth of FCD arrays by inducing hierarchical assembly of multiple FCDs centered at the edges of micropillars with non-overlapping elliptical focal curves. Below a critical pillar height, the confining effects produced by anchoring conditions on the pillar sides are diminished, but the LC elastic and surface energies remain sensitive to the positions of the FCDs on the patterned substrate. Consequently, multiple FCDs “share” a single pillar and self-assemble in a hierarchical manner; changing the shape of the pillars promotes a variety of new FCD arrangements. We employ a simple energetic model for the smectic LC that predicts the transition between this hierarchical assembly and topographic confinement of FCDs as the pillar height varies. Additionally, we exploit the size and spacing of the pillar array to tune the eccentricity, e, of the FCDs (see description in Supplementary
Information). In the case of TFCDs with circular focal curves, $e = 0$, while parabolic FCDs have $e = 1$. The ability to tune the eccentricity allows the creation of a versatile assortment of asymmetric FCD arrays, the first step towards the formation of 3D networks and more complex geometries. Finally, we present a geometric ansatz for the layer configurations that allows us to numerically investigate the energetic effects of nonzero eccentricity. These calculations are consistent with the observed nonzero eccentricity in the samples.
Results and Discussion

In a thin film geometry, smectic layers spontaneously assemble into FCDs in response to antagonistic boundary conditions, with homeotropic anchoring at the air interface and degenerate planar anchoring at substrate, in our system composed of the polymer SU-8. The total free energy of the system becomes a sum of three terms, the elastic energy of the LC and surface energies at both the air and substrate interfaces: \( \Delta F = \Delta F_{\text{el}} + \Delta F_{\text{air}} + \Delta F_{\text{subs}} \) where \( \Delta F_{\text{subs}} \) is highly dependent on topography of the substrate and we measure the free energy with respect to horizontal flat layers. Previously, we used circular micropillar arrays of SU-8 to confine SmA LCs both by limiting the surface area on the substrate available to each FCD with degenerate planar anchoring and by imposing frustrating degenerate planar anchoring conditions along the vertical pillar sides.\(^{(Honglawan, Beller et al. 2011)}\) It is natural to ask 1) how the FCD arrangement changes when the pillars become short enough so that their vertical sides do not present an insurmountable barrier to local FCD anchoring, and 2) how the arrangement of FCDs and the smectic layer structure depend on pillar shapes and lateral dimensions. Previous research on nematic LCs in micropillar arrays has highlighted the importance of pillar shape in determining the texture and controlling the placement of defects, demonstrating an inherently bistable LC display.\(^{(Kitson and Geisow 2002; Kitson and Geisow 2004)}\)

Here, we fabricated three sets of short \((H = 1 \, \mu m)\) SU-8 micropillar arrays of differing cross-section (circular vs. elliptical) (see Fig. 5.1a-c). The SmA LC film thickness cast on pillars was kept constant, \(h \approx 7 \, \mu m\), so that \(h-H > h_c \approx 1.5 \, \mu m\), the minimum film thickness at which FCDs form. In the case of circular pillars, the center-to-center spacing between pillars along a diagonal of the square lattice is \(S = 12 \, \mu m\), roughly twice the TFCD diameter (7.2 \, \mu m) that minimizes the free energy of a single TFCD on a substrate with degenerate planar anchoring at the same \(h\). As seen in Fig. 5.1a, four FCDs formed on each pillar with their centers lying on the lattice diagonal and near the pillar edges. On elliptical pillars, the number of FCDs on each
Figure 5.1 (a-e) Formation of FCD arrays on 1 µm tall SU-8 pillars with variable sizes and shapes. Optical images of top view of SU-8 pillars (1) and LC defect textures on pillars without (2) and with crossed polarizers (3). Scale bars: 20 µm. (a) Circular pillars with diameter $D = 5.5$ µm, the center-to-center spacing of the nearest pillars $W = 8.5$ µm and the diagonal center-to-center distance of the next-nearest pillars $S = 12.0$ µm. (b) Elliptically shaped pillars with major axis length $2A = 6.2$ µm, minor axis length $2B = 5.2$ µm, $W = 7.4$ µm (along the shorter lattice vector) and $S = 12.2$ µm. (c) Elliptically shaped pillars with $2A = 7.0$ µm, $2B = 3.4$ µm, $W = 6.3$ µm (along the shorter lattice vector) and $S = 11.8$ µm. (d) Y-shaped post with equal peripheral dimension of 30 µm at all sides. (e) Triangularly shaped pillars with each side of length 10 µm. The LC thickness $h$ is ~ 7 µm (a – c) and ~ 10 µm (d and e). (d4) AFM height profile of LC defects assembled on a Y-shaped post with equal lateral dimensions of 30 µm.
Figure 5.2 A plot of the numerically calculated free energy $\Delta F$, relative to the reference state of planar layers, as a function of the relative position of the circular pillar center along the line connecting the two TFCD centers for different pillar heights ($H = 0.5 – 4 \mu m$). The TFCD radius is set to $5.2 \mu m$ at LC thickness $h = 10 \mu m$ on the pillar array with radius of $5.72 \mu m$. Schematics illustrate the TFCD arrangements on the pillar with edge-pinning and confinement effects.
pillar decreased from four, to three \((A/B = 1.2, \text{Fig. 5.1b})\), to two \((A/B = 2.5, \text{Fig. 5.1c})\). This is in sharp contrast to our previously reported confinement effect with taller pillars \((H \geq 1.5 \mu m)\) (Honglawan, Beller et al. 2011) where FCD centers were positioned only in the centers of pillars or evenly spaced between neighboring pillars (see schematics in Fig. 5.2). Simply by reducing the height of pillars, we have effectively changed the interaction between pillar sides and FCD centers from repulsive to attractive, thereby promoting “edge-pinning” of FCD centers to the boundaries of short pillars. As the pillar’s minor axis length decreases, fewer FCDs are packed with their centers on the pillar edge.

To further elucidate the edge-pinning effect, we prepared an array of 1 \(\mu m\) tall, Y-shaped pillars with each side of length 30 \(\mu m\), a much larger lateral scale than the cylindrical pillars. Under crossed polarizers, the Maltese cross patterns of each FCD were clearly distorted at the edges of the Y-shaped pillar (Fig. 5.1d). The distortion was even more apparent in the 3D topography of the top surface imaged by AFM (Fig. 5.1d): the surface was depressed at the periphery of the Y pattern but relaxed in the middle to the height of the surrounding flat region, confirming that the attraction of FCD centers to pillar edges is strong enough to disrupt the assembly of close-packed hexagonal lattices of TFCDs. The hierarchical nature of the epitaxial assembly leads to geometric relations among the orientations of FCD groups from one pillar to the next over regions spanning the whole pillar array. For example, in Fig. 5.1a, the centers of the four FCDs surrounding each circular pillar form a square aligned with the substrate patterning’s lattice directions consistently from pillar to pillar. Different pillar shapes yield distinct hierarchical arrangements of FCDs. An especially interesting case is presented by the most eccentric pillars in Fig. 5.1c, where the two FCDs on each pillar are connected by a line slightly rotated off the major axis of the ellipse. This pattern breaks mirror symmetry along the pillar’s major axis, and the choice of ground state is consistent over regions spanning tens of pillars, even though the substrate patterning does not break this symmetry. In this way, simply by varying the pillar height
and shapes, we are able to transform the arrangements of FCDs into anisotropic patterns, exhibiting order over large regions.

The importance of pillar shape to hierarchical assembly of FCDs is further evidenced by the attraction of domains to the more highly curved regions of convex pillar edges shown in Fig. 5.1c. We attribute this effect to the strong steric repulsion between the neighboring FCDs. Two FCDs will prefer to position themselves as far apart as possible while remaining tangent and keeping their centers pinned to the pillar edge. The effective attraction of FCD centers to pillar “corners” is especially evident in pillars with triangular cross-sections (see Fig. 5.1e).

The transition from surface confinement to edge-pinning results from a delicate balance of the elastic (layer curvature) energy in the bulk and the surface energy of both the LC-air and the LC-substrate interfaces (see Supplemental Information). Degenerate planar anchoring along the pillar’s vertical surface imposes an energy penalty for rod-like molecules tilted out of the vertical direction unless the molecule happens to tilt in the tangent plane to the pillar edge. For tall pillars (empirically, $H \geq 1.5 \mu m$), the substrate surface energy favors smectic layers horizontal at the pillar edge, a condition which is not satisfied in the interior of an FCD. Thus, the surface energy promotes confinement, with the FCD centers as far as possible from the pillar edges. In contrast, the elastic energy is concentrated most strongly near the elliptic and hyperbolic defect curves of the FCD, the focal set of the Dupin cyclides. This effect would be further enhanced by including a core energy for the defect curves; in this analysis, we have omitted this core energy because its form is uncertain and a transition from confinement to edge-pinning occurs by considering only the bulk elastic energy. If the dimension of the pillar and the LC thickness are chosen so that two or more FCDs form for each pillar, then the elastic energy often favors “hiding” the lower portion of the hyperbolic defect curve inside of the pillar, removing a significant fraction of the elastic energy (Supplemental Information). The role of the pillar’s top surface is more subtle, but the degenerate planar anchoring conditions on this surface generally favor the edge-
pinning configuration for short pillars. The balance of these energies promotes edge-pinning as the confining effects of the pillar diminish with decreasing pillar height.

To understand the transition from confinement to edge-pinning with decreasing pillar height, we use numerical energy calculations to investigate a simplified scenario: Two TFCDs and one pillar in the shape of a circular cylinder. Fig. 5.2 presents the calculated free energy $\Delta F$ relative to the reference state of equally-spaced, horizontal planar layers, as a function of the relative position of the pillar center along the line connecting the two TFCD centers (see calculation in Supplemental Information). We set the pillar radius to 1.1 times the TFCD radius, so that it is possible to “hide” portions of both straight-line focal curves within the pillar. The LC thickness is 10 $\mu$m and the TFCD radius is 5.2 $\mu$m, chosen to minimize the analytic expression for $\Delta F$ on a flat substrate. (Kim, Yoon et al. 2009) For all values of $H$, local minima in $\Delta F$ are seen when the pillar is centered directly at the center of either TFCD and when the pillar is positioned symmetrically between the two TFCDs. The results reveal that the global minimum changes as $H$ decreases: for $H > 2 \mu$m, the energy is minimized by centering the pillar at the center of either TFCD, corresponding to a confinement effect. For shorter pillars, the global minimum switches to a symmetric configuration of two TFCDs “sharing” a pillar equally, with their centers near the pillar edges. This calculation correctly captures the transition of FCDs from confinement to edge-pinning with decreasing pillar height.

The edge-pinning regime provides a novel geometric means to tune the eccentricity of FCDs. By comparing the AFM height profile of FCD arrays formed on circular and elliptical pillar arrays to LC textures observed in optical and SEM images (Fig. 5.3), we find two surprising features. First, the LC thickness is typically smaller over the pillar, where the substrate is raised, than over the lower regions between pillars. Along a line that passes through successive cusp-like indentations marking the terminations of hyperbolic defects at the air interface, we measure an alternating set of large and small arcs on the topmost layer. Moreover, there is a lack of axial
Figure 5.3 Surface characterization of FCD formation on the circular (a-d) and elliptical pillar arrays (e-h). The latter corresponds to pillars seen in Fig. 5.1c. Optical images (a and e) reveal a defect texture with the polarizer and analyzer at a relative angle of 45°, and the corresponding surface topography of FCDs arrays obtained from SEM (b and f). (c and g) 3D maps of the surface of the LC films extracted from AFM measurements based on their height profiles with color representation of relative thickness of the film. (d and h) Plots of the height profiles along the dashed white lines in (c) and (g).
Figure 5.4 Schematic illustration of internal structures of FCDs with zero (a) and nonzero (0.2) (b and c) eccentricity in regions bounded by a cylinder (a and b) or a cone configuration (c). (d) Representation of a possible arrangement of FCDs with nonzero eccentricity on circular pillars with the edge-pinning effect.
symmetry about the cusp. The absence of axial symmetry clearly implies that the edge-pinned FCDs are not toric; their eccentricity is nonzero.

FCDs with zero and nonzero eccentricity are illustrated in Fig. 5.4a-c. Non-toric FCDs select a particular direction in the plane given by the direction in which the hyperbola points. In the topmost layer profile, the periodic alternation of small and large arcs suggests the following model: the hyperbolæ face inward toward the pillar centers, and thus face away from each other over the space between the pillars (Fig. 5.4d).

Why should nonzero eccentricity be favored in the edge-pinning regime? We propose an answer based on geometry. Consider the square array of circular pillars (Fig. 5.1a) where \( S \) slightly exceeds four times the pillar radius of 2.75 \( \mu \text{m} \). Consequently, TFCDs in a symmetrical arrangement with their centers pinned to the edges of one pillar could not possibly have their ellipses tangent to those of the corresponding TFCDs of the neighboring pillars. A small gap would be left in between, creating extra area on the substrate with unfavorable, homeotropic anchoring rather than the preferred degenerate planar anchoring.

However, the FCD array can close the gap by shifting to small but nonzero eccentricity. Like the TFCD, an FCD of any eccentricity enjoys degenerate planar anchoring on a level surface in the area enclosed by its ellipse. Because the hyperbolic defect passes through the focus of the ellipse rather than through its center, nonzero eccentricity can shift the FCD center so that the ellipse is tangent to the ellipse of a neighboring pillar’s FCD, while maintaining edge-pinning of the hyperbolic defect. Based on the dimensions presented for Fig. 5.1a, this geometric model predicts an eccentricity, \( e = 0.12 \), which agrees reasonably well with a separate estimate \( e = 0.08 \), based on the AFM data in Fig. 5.3c-d (Supplemental Information). Furthermore, nonzero eccentricity can decrease the elastic energy by bending the hyperbolic focal curve toward the pillar center, thus “hiding” more of the FCD’s high-curvature central region inside the pillar.
The regular assembly of FCDs in groups of two to four poses an interesting problem as to how the LC molecules fill the interstices between the domains. For TFCDs, the interstices are filled by horizontal layers, onto which the layers of the TFCD match with continuous layer normals on a right cylinder, intersecting the circular focal curve. FCDs of nonzero eccentricity oriented in different directions cannot be joined by planar layers. Instead, elliptic-hyperbolic FCDs typically assemble in groups with their hyperbolic focal curves, or their extensions, all intersecting at a single point \( P \), and with the elliptical focal curves tangent to their neighbors. Friedel showed that FCDs associated in this way can be joined, with continuous layer position and normal direction, across bounding surfaces in the form of right circular cones with apices at \( P \) and which include the elliptical focal curves. (Friedel 1922)

It is geometrically possible for smectic layers to fill the space outside of these “corresponding cones” with portions of spheres concentric about \( P \), again without discontinuity in layer position or normal direction, as demonstrated by Sethna and Kléman. (Sethna and Kléman 1982) In a model originally proposed by Bragg (Bragg 1934) and confirmed in experiments by Lavrentovich, (Lavrentovich 1986) the sample is divided into quasi-pyramidal regions, each filled by FCDs and spheres organized around a given point \( P \), as well as wedges between the pyramids which are filled by portions of still more FCDs. This construction fills a region entirely with layer configurations possessing only zero- and one-dimensional focal sets.

In thin film smectics, there is no energetic prohibition of more general layer configurations with two-dimensional focal sets outside the FCDs, provided that the focal sets of these interstitial regions lie below or above the sample as “image cusps” that are not physically realized in the smectic. In this sense, confinement dramatically expands the range of possible layer geometries even in an ideal system.

To quantitatively estimate the effect of nonzero eccentricity on the free energy, we propose an ansatz configuration for the layers in the case of four FCDs around a circular pillar as...
in Fig. 5.1a. (We focus on this case for modeling because it enjoys the highest symmetry.) Our ansatz employs the conical bounding surfaces of Friedel but not the concentric spheres of Sethna and Kléman or Bragg’s pyramids. First, we choose a point on the hyperbolic focal curve to serve as the apex of a right circular cone $C$ that passes through all points on the elliptical focal curve. $C$ provides a boundary separating the FCD on the inside from some other layer configuration on the outside (Fig. 5.5a).

But how will we bridge the gaps between the cones? Because $C$ consists of generators for the Dupin cyclides, the layers meet the cone at right angles. Generators are straight lines consisting of surface normals to parallel layers, which remain constant from layer to layer in the normal direction, and which point toward the center of curvature. Consider a point $E_u$ on the elliptical focal curve parameterized by $u \in [0, 2\pi)$ (see Supplemental Information), and the subset of the FCD with one center of curvature at $E_u$, the other center lying on a variable point on the hyperbola (Fig. 5.5b). This subset consists of circular arcs concentric about $E_u$. Continuity of the layer normal across the bounding cone requires that the cone generator through $E_u$ is also the generator of the layers just outside the cone. We thus choose a new center of curvature along the same generator. A natural choice is the intersection $I_u$ of the cone generator with the corresponding generator of the neighboring FCD’s bounding cone. This intersection lies somewhere below the sample. The simplest reasonable construction is to fill in the regions outside the bounding cones with circular arcs concentric about $I_u$, in the plane containing the cone generator through $E_u$ and the cone normal direction along this generator.

As the construction is repeated for all $u \in [0, 2\pi)$, we obtain a set of parallel surfaces that matches the FCD layers along the boundary cone and bridges the space between FCDs in a manner compatible with the observed four-fold symmetry (Fig. 5.5c). Using this construction, we can produce simulated AFM data for the topmost layer that agrees reasonably well with the experimental data (Fig. 5.5d vs. Fig. 5.3c). In combination with the structure of the FCD itself, this
Figure 5.5 Schematic illustrations of (a) a single FCD bounded by a cone, (b) a smectic layer construction bridging between two bounding cones with circular arcs concentric about \( I_u \) in the plane containing the cone generator through \( E_u \) and the cone normal direction along this generator, and (c) a complete layer construction of four FCDs surrounding a pillar for all \( u \in [0, 2\pi] \) based on (b). (d) A 2-D map of topmost surface of (c) with a color representation of surface height. (e) A plot of total free energy of LC geometry in (c) as a function of eccentricity of FCDs.
ansatz provides a family of space-filling smectic layer geometries parameterized by the eccentricity. We numerically evaluate the elastic- and surface- energy integrals for these geometries and plot the total $\Delta F$ as a function of $e$ in Fig. 5.5e. This plot shows that $\Delta F$ for eccentricity $e \leq 0.04$ is comparable to that at $e = 0$, whereas $\Delta F$ increases nearly monotonically for larger $e$. This result is consistent with the experimental observation of stability of nonzero eccentricity on the order of 0.1. Adding a core defect energy for the hyperbolic focal curve would decrease the free energy at $e \approx 0.1$ relative to that at $e = 0$, due to a portion of the hyperbola disappearing inside the pillar as discussed above.

Could this system alternatively be modeled by the pyramids and wedges construction of Bragg? In the case of four FCDs around a circular pillar, we could imagine constructing an indented square pyramid around each pillar, containing four FCDs whose hyperbolae intersect at the pyramid’s apex, along with portions of concentric spheres. A roughly tetrahedral wedge containing a portion of an FCD is inserted between every pair of neighboring pyramids. Finally, each four-corner meeting point of the pyramids' bases on the substrate also serves as the apex of an inverted square pyramid filled only with concave-down portions of spherical layers, forming the purple regions in Fig. 5.3c. This model predicts a concave-up region above the center of every pillar, where the layers would form portions of spheres concentric about a point above the sample. It is possible that the slight depression in the middle of the smaller arc of Fig. 5.3d is evidence of such a concave-up region. However, the concave-up region in the AFM data is no more than one micron in width, implying that the ellipse eccentricity exceeds 0.7, far greater than our estimate $e \approx 0.080$ based on calculations independent of our model for the interstices (see Supplemental Information). Furthermore, similar slight depressions are arguably visible in Fig. 35.h, for the case of two FCDs around elliptical pillars, but are not expected in a model using Sethna-Kléman filling with concentric spheres: If the bounding cones of FCDs are tangent to those of their neighbors, then the white dashed line in Fig. 5.3g passes from one FCD to the next without going through a region of spherical layers over the pillars. Future studies will probe the
theoretical and experimental differences between these models. With maximum parsimony in mind we propose our model which fits the geometry and eccentricity more readily.

In conclusion, we have shown that using SU-8 micropillar arrays of variable dimension and geometry (height, shape and spacing) as topographical templates, we can introduce hierarchical assembly of FCDs and tune their eccentricity in a SmA LC assembly. By decreasing the micropillar height, we observe a transition from confinement of isolated domains to the hierarchical growth of FCDs, tangent to their neighbors, with their hyperbolic focal lines pinned near the pillar edges. The size and shape of the pillars can be used to control the type of hierarchical FCD arrangement; the anisotropy of the pillar shape allows us to reliably predict the locations of FCDs relative to the substrate patterning due to the effective attraction of FCDs to pillar corners. The nontrivial, but apparently smooth, matching of smectic layers between neighboring FCDs on a non-uniform substrate presents an intriguing theoretical problem for which we have suggested a geometric ansatz. These topographic tools significantly enrich the library of possible FCD arrays, and open a new avenue to create more complex 3D structured soft systems beyond trivial assembly.
Materials and Methods

**LC Synthesis.** The SmA LC used in this study, \( (4' - (5,5,6,6,7,7,8,8,9,9,10,10,11,-11,12,12,12-
heptadecafluoro)dodecyloxy)-biphenyl-4-carboxylic acid ethyl ester) \), was synthesized by a two-step reaction following the literature.(Percec, Johansson et al. 1996)

**Fabrication of patterned substrates.** Arrays of triangular and Y-shaped pillars with equilateral dimensions of 10 and 30 µm, respectively, were fabricated from SU-8 2010 (MicroChem Corp.) on clean silicon (Si) wafers by contact lithography using an OAI Model 200 mask aligner. SU-8 micropillars in square arrays were fabricated on Si wafers by capillary force lithography (CFL)(Zhang, Lin et al. 2010) (see details in Supplemental Information). We use three sets of pillar arrays (see Fig. 5.1): 1) a square lattice of circular pillars of diameter \( D = 5.5 \) µm, with pitch or center-to-center distance \( W = 8.5 \) µm, and corresponding diagonal pillar spacing \( S = 12 \) µm; 2) a rectangular lattice of pillars of elliptical cross section with major axis \( 2A = 6.2 \) µm, minor axis \( 2B = 5.2 \) µm, pitch \( W = 8.0 \) µm, and diagonal pillar spacing \( S = 12.2 \) µm; and 3) a rectangular lattice of elliptical pillars with major axis \( 2A = 8.5 \) µm, minor axis \( 2B = 3.4 \) µm, pitch \( W = 6.3 \) µm, and diagonal pillar spacing \( S = 13 \) µm. All pillars have the same height, \( H = 1.0 \) µm.

**Assembly of LC molecules on substrates.** Crystalline powders of the LC molecules were dissolved in a fluorinated solvent, Fluorinert FC-770 (3M), at a concentration of 500 mg mL\(^{-1}\). 30 µL LC solution was drop-cast onto the SU-8 pillar array and heated on a Mettler FP82 hot stage with FP 90 controller to form an isotropic phase at 200 °C for 5 min. It is subsequently cooled down to 114 °C at 5 °C min\(^{-1}\) to form the SmA phase, which was quenched to room temperature.

**Characterizations of LC films.** We imaged LC structures formed on various substrates by scanning electron microscope (SEM) on a FEI Strata DB235 focused ion beam (FIB) system at 5 kV and an Olympus BX61 motorized optical microscope with crossed polarizers using CellSens software. The surface topography of the LC was characterized by a Dimension 3000 Atomic
Force Microscope (AFM, Digital Instruments) in tapping mode using open source software, Gwyddion, for image-processing.

**Calculation of Free Energy.** Numerical integrations are conducted using Mathematica 7.0 and 8.0. Details of the calculations can be found in the Supplemental Information.

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References


150


Supporting Information

Capillary force lithography of SU-8 pillar arrays.
Briefly, a mixture of PDMS prepolymer and crosslinker (RTV615 from GE Silicones, 10:1 wt/wt) was poured on top of the pillar master, followed by curing at 65 °C for 4 h to prepare the PDMS mold. SU-8 2 solution (MicroChem Corp.) was spin-coated onto a clean Si wafer at 3,000 rpm for 30 s to obtain a film thickness of 1.5 µm. The SU-8 thin film was soft-baked at 65 °C for 2 min and 95 °C for 2 min before placing the PDMS mold on the molten SU-8 film for another 2 min at 95 °C for capillary force lithography (CFL). A slight pressure was applied to ensure conformal contact between the mold and the SU-8 film. The film was then cooled down to room temperature. To enhance the pillar mechanical strength, we exposed the pillars together with the PDMS mold to UV light (Newport 97435 Oriel Flood Exposure Source, 100 mJ cm\(^{-2}\) at 365 nm), followed by post-exposure baking at 65 °C for 2 min and 95 °C for 2 min to crosslink the SU-8. Finally, the SU-8 pillar array was peeled off from the PDMS mold after cooling to room temperature. The SU-8 pillars were rinsed with isopropanol (IPA) and dried with compressed air. To fabricate pillars with elliptical cross-sections, a 1.2 mm thick rectangular (1 x 3 cm) PDMS mold with a circular hole array was stretched uniaxially at 5% and 25% strain levels, followed by CFL described above.

Free energy calculations (by Daniel A. Beller)
In this chapter, all calculations for the free energy of a smectic layer configuration are taken relative to a reference state where all of the layers are horizontal planes with equal spacing. The free energy is a sum of three terms,

\[ \Delta F = \Delta F_{\text{el}} + \Delta F_{\text{air}} + \Delta F_{\text{subs}}. \]  

(1)

The first term is the elastic energy,

\[ \Delta F_{\text{el}} = \int dV \left[ 2k_r \lambda z^2 + \frac{1}{2}R_g \right]. \]
where $H$ is the layer mean curvature, $G$ is the layer Gaussian curvature, and $K_1$ and $K_2$ are associated elastic moduli. The second term is the surface tension energy associated with the dimple-like deformation in the topmost layer,

$$\Delta F_{\text{air}} = \sigma_{\text{air}} \int dA \left[ \sqrt{1 + (\nabla h)^2} - 1 \right],$$

where $\sigma_{\text{air}}$ is the surface tension associated with homeotropic anchoring at the air interface and $h(x, y)$ is the height function describing the topmost layer. The third term represents the preference for degenerate planar anchoring on the substrate. For the horizontal substrate surfaces on pillar tops and between pillars, it has the form

$$\Delta F_{\text{subs}} = \Delta \sigma_{\text{subs}} \int dA \left[ 1 - (\hat{\rho} \cdot \hat{z})^2 \right]$$

where $\hat{\rho}$ is the smectic layer’s unit normal vector at the substrate, $\hat{z}$ is the unit vector in the vertical direction, and $\Delta \sigma_{\text{subs}} = \sigma_{\text{subs}} - \sigma_{\text{air}} < 0$ is the relative energy per unit area of degenerate planar versus homeotropic anchoring. The anchoring potential is of the commonly used Rapini-Papoular form. For the pillar side surfaces,

$$\Delta F_{\text{subs}} = \Delta \sigma_{\text{subs}} \int dA \left[ - (\hat{\rho} \cdot \hat{\nu})^2 \right],$$

where $\hat{\nu}$ is the unit normal vector of the pillar side. Numerical integration is performed using Mathematica 7.0 and 8.0. Numerical values of constants are given below.

We now describe the calculation of each energy component in more detail. To calculate the elastic free energy, we use expressions for the curvature energy adapted from Kléman and Lavrentovich. (Kleman and Lavrentovich 2000)

$$\Delta F_{\text{el}} = W_1 + W_2$$

$$W_i = W_{i,1} + W_{i,2}, \quad i = 1, 2$$

$$W_{i,1} = \int_0^{2\pi} du \int_0^{\arccos \left( \frac{\pi}{\pi} \right)} dt \int_{e \cos u + \rho \hat{\nu}}^{\sec t - \rho \hat{\nu}} d\rho \Theta(u, t, \rho) w_i(u, t, \rho)$$

$$W_{i,2} = \int_0^{2\pi} du \int_0^{\arccos \left( \frac{\pi}{\pi} \right)} dt \int_{e \cos u + \rho \hat{\nu}}^{\phi - \rho \hat{\nu}} d\rho \Theta(u, t, \rho) w_i(u, t, \rho)$$
Here, $a$ is the FCD semi-major axis length; $e$ is the FCD eccentricity; $K_1$ is the splay (mean-curvature) elastic modulus; $\Lambda \equiv \overline{K} + 2K_1$ where $\overline{K}$ is the Gaussian curvature modulus; $u$ parameterizes the elliptical focal curve; $t$ parameterizes the hyperbolic focal curve; $\rho$ parameterizes the layers, measuring distance in the layer normal direction divided by $a$; $\Phi$ measures the maximum value of $\rho \times a$ at the air interface and is comparable to the LC thickness; $T$ determines which point on the hyperbola serves as the apex of the bounding cone as described in the text; Region I corresponds to generators that terminate on the hyperbola; Region II corresponds to generators that terminate at the air interface; and $\theta(u,t,\rho)$ is a unit step function whose value is zero inside of the pillar (where there is no LC) and one outside of the pillar.

Whereas Kléman and Lavrentovich obtain an elegant result for the integrated elastic free energy by assuming that the hyperbolic focal curve extends to infinity, here the nontrivial boundaries at the pillars and the air interface require us to compute $\Delta F_{el}$ numerically.

Because the boundary of the pillar is more naturally expressed in Cartesian coordinates than in the FCD coordinates $(u,t,\rho)$, we employ the following transformation to calculate $\theta(u,t,\rho)$ for an FCD with its hyperbola in the $xz$ plane:

$$
\begin{pmatrix}
X \\
Y \\
Z
\end{pmatrix} = \frac{1}{1 - e \cos t \cos u} \begin{pmatrix}
\rho a (e - \cos u \cos t) + a(1 - e^2) \cos u \\
a \sqrt{1 - e^2} \sin u (1 - \rho \cos t) \\
a \sqrt{1 - e^2} \sin t (\rho - e \cos u)
\end{pmatrix}.
$$

To calculate $\Delta F_{air}$, we use the area measure on the smectic layer at $\rho = \Phi/a$, again adapted from (Kleman and Lavrentovich 2000):

$$
\Delta F_{air} = a^3 h a^2 (1 - e^2) \int_0^{2\pi} du \int_{\arccos(\frac{\Phi}{a})}^{T} dt \left| \frac{1 - \left( \frac{\Phi}{a} \cos t \right) \left( e \cos u - \left( \frac{\Phi}{a} \right) \right)}{(1 - e \cos u \cos t)^2} \right|.
$$

154
To calculate the substrate interface energy $\Delta F_{\text{subs}}$, we need the smectic layers’ normal direction $\hat{\rho}$ as a function of $x, y, z$. In the region of the substrate that is enclosed by the elliptical focal curve and outside of the pillar, $\hat{\rho}$ has no $z$-component, so the energy per unit area is simply $\Delta \sigma_{\text{subs}}$. However, for the pillar top and side surfaces, we are required to compute $\hat{\rho}$. In the FCD coordinates, we have the analytic expression

$$\hat{\rho}(u, t) = \left( \frac{\hat{\rho}_x}{\hat{\rho}_y} \right) = \frac{1}{1 - e \cos u \cos t} \begin{pmatrix} e - \cos u \cos t \\ -\sqrt{1 - e^2 \sin u \cos t} \\ \sqrt{1 - e^2 \sin t} \end{pmatrix}. \quad (2)$$

We then need $(u, t)$ as functions of $(x, y, z)$, i.e. the inverse of transformation (1). While no analytic relation for this transformation is available for arbitrary eccentricity $e$, at $e = 0$ (TFCD case) we can readily write

$$u = \arctan \left( \frac{y}{x} \right) \quad (3a)$$

$$t = \arctan \left( \frac{z}{b - \sqrt{x^2 + y^2}} \right) \quad (3b)$$

$$\phi \equiv \beta \rho = \sqrt{(b - \sqrt{x^2 + y^2})^2 + z^2} \quad (3c)$$

where $b$ is the domain radius. Remarkably, it has been shown that the coordinates at arbitrary $e$ are related to those of a TFCD by a Lorentz transformation: \cite{Alexander, Chen et al. 2010}

$$x' = \gamma (x - \beta \phi), \quad \phi' = \gamma (\phi - \beta x), \quad y' = y, \quad z' = z. \quad (4)$$

This transformation maps each point on a TFCD in the unprimed system to a corresponding point on an FCD of arbitrary eccentricity having the same values of $u$ and $t$ in the primed system. Here we make the identifications $\beta \equiv -e, \gamma \equiv (1 - \beta^2)^{-\frac{1}{2}}$, and $\phi' \equiv \rho' a$, where $a$ is the semi-major axis length in the primed system. The semi-minor axis length $b = a \sqrt{1 - e^2}$ in
the primed system equals the TFCD radius in the unprimed system, so $\phi = \rho b$ as defined above.

By substituting Equation (3c) into the transformation (4), we obtain a quartic equation in $x$ whose
coefficients depend on $(x', y', z')$ and $e$:

$$
0 = \left[ y^{-4} \right] x^4 - \left[ 4x'y'^{-3} \right] x^3 + \left[ 4x'^2 y'^{-2} + 2(x'^2 y'^{-2} - \beta^2 (b^2 + y'^2 + z'^2)) y'^{-2} - 4\beta^4 b^2 \right] x^2
$$

$$
- \left[ 4x'y'^{-1} (x'^2 y'^{-2} - \beta^2 (b^2 + y'^2 + z'^2)) \right] x
$$

$$
+ \left[ (x'^2 y'^{-2} - \beta^2 (b^2 + y'^2 + z'^2)) y'^{-2} - 4\beta^4 b^2 y'^2 \right].
$$

Equation (5) is solved numerically for $x$, from which $u$ and $t$ are obtained via Equations
(3a) and (3b). Finally, we can compute $\tilde{\rho}(u, t)$ as in Equation (2) at the Cartesian coordinates
$(x', y', z')$ corresponding to the pillar boundaries.

For the geometric ansatz connecting the smectic layers between neighboring FCDs, the
ergetic contribution of the regions outside the FCDs is calculated from an explicit expression
for the height of the topmost layer $h(x, y)$ constructed from a union of circular arcs as described in
the text. The height function gives the principal curvatures $k_1^0$ and $k_2^0$, reciprocal to radii of
curvature $R_1^0$ and $R_2^0$, as well as the unit normal direction $\tilde{n}$. From each point $(x, y)$ on the
topmost layer, a generator is constructed by traveling downward along $\tilde{n}$ until reaching the
substrate, and the generator is parameterized by $\phi$, which has units of length and equals zero at
the topmost layer. Along the generator, the assumption of parallel layers implies that the principal
radii of curvature are $R_i(\phi) = R_i^0 + \phi$ for $i = 1, 2$, and thus the area measure on a layer varies as

$$
da(\phi) = dx dy \sqrt{1 + (\nabla h)^2} \left( \frac{R_1^2 + \phi}{R_1^0} \right) \left( \frac{R_2^2 + \phi}{R_2^0} \right).
$$

These expressions allow us to integrate the mean and Gaussian curvatures over $\phi$ along
each generator, and then integrate over all generators parameterized by their values $(x, y)$ at the
air interface, weighted by the appropriate area measure. Implicit in these calculations is the
assumption that no focal sets exist outside the FCDs. The contribution to $\Delta F_{\text{air}}$ is similarly
calculated from $h(x, y)$ and the area measure. Additionally, the contribution to $\Delta F_{\text{subs}}$ on the
horizontal substrate surfaces is calculated from $h(x, y)$ by finding the value $\phi^*$ of $\phi$ where a given
generator intersects the substrate, setting \( \hat{\rho} \cdot \hat{z} = \left[ 1 + (\nabla h)^2 \right]^{-\frac{1}{2}} \), and integrating over generators using the area measure \( dA(\phi') \). For the contribution to \( \Delta F_{\text{subs}} \) on the pillar sides, we approximate the layer normal direction at \((x, y, z)\) to be the same as the normal direction of the topmost layer at the same \((x, y)\), which is valid in the limit of slowly varying \( h(x, y) \).

The ansatz requires us to choose a value of \( T \), which marks the point on the hyperbola that serves as the apex of the FCD’s bounding cone. Because the bounding cone apex is expected to be located above the LC/air interface, a lower bound for \( T \) is provided by \( T_{\min} = \arccos \left( \frac{a}{\Phi} \right) \), corresponding to the termination of the hyperbolic focal curve at the air interface. An upper bound for \( T \) is provided by \( T_{\max} = \arccos \left( \frac{c}{a} \right) \), which corresponds to the point on the hypothetically extended hyperbola positioned directly over the point on the ellipse at \( u = 0 \). If \( T > T_{\max} \), then the layer normal would be vertical at some point within the FCD, which is not observed in the AFM data. Generally, we found that the choice of \( T \) in the interval \([T_{\min}, T_{\max}]\) had little effect on the calculation result or on agreement with the AFM data. We therefore chose \( T = \frac{1}{2}(T_{\min} + T_{\max}) \) for the results presented here.

**Estimation of FCD eccentricity**

The FCD eccentricity is estimated from the AFM data by the following procedure. First we recall a standard parameterization for the FCD layers:(Kleman and Lavrentovich 2000)

\[
\vec{x} = (x, y, z)
\]

\[
x(u, t, \phi) = \frac{\phi(c - a \cos u \cos t) + b^2 \cos u}{a - c \cos u \cos t}
\]

\[
y(u, t, \phi) = \frac{b \sin u (a - \phi \cos t)}{a - c \cos u \cos t}
\]

\[
z(u, t, \phi) = \frac{b \sin t (\phi - c \cos u)}{a - c \cos u \cos t}
\]

Here \( a \) is the ellipse semi-major axis, \( b \) the semi-minor axis, and \( c = \sqrt{a^2 - b^2} \). The variable \( u \in [0, 2\pi) \) marks the point on the ellipse through which the generator containing \( \vec{x} \).
passes. The variable \( t \in [0, \pi] \) marks the point on the hyperbola through which the generator containing \( x \) passes. The variable \( \phi \) parameterizes the parallel layers. At the topmost layer, we say \( \phi = \Phi \). Let \( t_* < \frac{\pi}{2} \) be the value of \( t \) where the hyperbola terminates at the air interface. By setting \( y = 0 \) for all \( u \), we find \( t_* = \arccos(a/\Phi) \). The plane of the hyperbolic focal curve is chosen to be the \( xz \) plane. The hyperbola is given by \( \tilde{H}(t) = (c \sec t, 0, b \tan t) \). It follows that, for two FCDs with hyperbolic focal curves oriented toward each other, the distance between their ellipse foci (where the hyperbolae meet the \( xy \) plane at \( t = 0 \)) is

\[
d_f = \delta x + 2c(\sec t_* - 1) = \delta x + 2c(\Phi/a - 1),
\]

where \( \delta x \) is the horizontal distance between the two cusps in the AFM data.

Now consider the geometry observed in Figure 5.1a, with four FCDs per pillar on a square array of pillars with circular cross-section. We assume that each FCD is tangent at four points to a square with one vertex at the center of the pillar, with side length equal to half of the center-to-center spacing \( W \) of nearest-neighbor pillars, and whose sides are aligned with the substrate patterning lattice directions (see Figure S5.2). This geometry requires that

\[
\sqrt{\frac{a^2 + b^2}{2}} = W/4.
\]

Replacing \( b^2 \) with \( a^2 - c^2 \), we obtain the relation

\[
a = \sqrt{\frac{1}{2} \left( \frac{W^2}{8} + c^2 \right)}.
\]

The distance from the ellipse center to the ellipse focus is simply \( c \). Furthermore, the distance from the pillar center to the ellipse center is \( \sqrt{a^2 + b^2} = \sqrt{2}W/4 \). We thus obtain another expression for the distance between the ellipse foci of two FCDs facing each other,

\[
d_f = 2\left( \frac{\sqrt{2}W}{4} - c \right)
\]

Equating the two expressions (6) and (8) for \( d_f \) yields
To obtain an expression for the cusp angle \( \alpha_c \), as measured along the ellipse's major axis in the AFM data (Figures 3d, h), we compute the two layer normal vectors

\[
\partial_{\Phi} \hat{X} \big|_{(u, t, \Phi)} = (0, \arccos(a/\Phi), \Phi), \quad \partial_{\Phi} \hat{X} \big|_{(u, t, \Phi)} = (\pi, \arccos(a/\Phi), \Phi),
\]

normalize them, and equate their dot product to \( \cos(\pi - \alpha_c) = -\cos \alpha_c \). The general form of the unit normal vector is

\[
\hat{N} = \frac{\partial_{\Phi} \hat{X}}{||\partial_{\Phi} \hat{X}||} = \frac{(c - a \cos u \cos t, -b \sin u \cos t, b \sin t)}{a - c \cos u \cos t}.
\]

After some algebraic simplification, we find

\[
-\cos \alpha_c = \frac{\Phi^2 - a^2 - b^2}{\Phi^2 - c^2} = \frac{\Phi^2 - 2a^2 + c^2}{\Phi^2 - c^2},
\]

(10)

We substitute for \( a \) and \( \Phi \) the functions of \( c \) given by Equations (7) and (9). The result is an equation relating \( c, \alpha_c, \delta x \), and \( W \) (implicitly through \( a \)). The latter three are measured quantities from the AFM data. For the samples in Figure 5.1a, we measure \( \alpha_c \approx 147^\circ, \delta x \approx 4.8 \mu m, \) and \( W \approx 8.5 \mu m. \) Using these values in Equation (10) gives \( c \approx 0.17 \mu m, a \approx 2.1 \mu m, \) and \( \Phi \approx 7.5 \mu m. \) Dividing \( c \) by \( a \) gives the eccentricity, \( e \approx 0.080. \) The value of \( \Phi \) obtained by this method is in good agreement with the liquid crystal thickness, \( h \approx 7 \mu m. \)

We can follow a similar calculation for the geometry in Figure 5.1c. Here, we assume that each FCD is tangent to two neighbors, with both points of tangency lying along the major axis. The FCD semi-major axis is then related to the diagonal center-to-center separation \( S \) of next-nearest-neighbor pillars by

\[
a = S/4
\]

(11)
in place of Equation (7). Equation (8) is replaced with \( d_f = 2(a - c). \) Equating this to the right-hand side of Equation (6) gives
\[ \Phi = \frac{a}{2c} (2a - \delta x) \]  

(12)

in place of Equation (9). As before, Equations (11) and (12) are substituted into Equation (10), allowing us to solve for \( c \). For the samples in Figure 5.1c, we measure \( \alpha_c \approx 163^\circ, \delta x \approx 4.7 \text{ \mu m}, \) and \( S \approx 13.0 \text{ \mu m} \). Using these values in Equation (10) gives \( c \approx 0.13 \text{ \mu m}, a \approx 3.3 \text{ \mu m}, \) and \( \Phi \approx 22.0 \text{ \mu m} \). Dividing \( c \) by \( a \) gives the eccentricity, \( e \approx 0.041 \).

Returning to the geometry of Figure 5.1a, we consider the predictions of an alternative model for the interstices between FCDs, in which the spherical layers of Sethna and Kléman fill the space between FCDs over the pillars (see text). The cones bounding the FCDs around a given pillar all have a common apex above the pillar center, where the extensions of the four hyperbolic focal curves meet. Therefore, each bounding cone has in its interior a vertical generator line connecting the point on the ellipse at \( u = 0 \) to the point on the hyperbola directly above. Where this generator meets the topmost layer, the height profile in Figure 5.3d would form a local maximum, with tangent passing through the horizontal. The distance \( \delta m \) between the two local maxima in Figure 5.3d, which is at least as great as the width of the concave-up region, equals the minimum distance between ellipses on opposite sides of the pillar. Referring to the geometry of Figure S5.2, this means

\[ \frac{1}{2} \delta m = \frac{\sqrt{2}}{4} W - a = \frac{\sqrt{2}}{4} W - \frac{1}{2} \left( \frac{w^2}{a} + c^2 \right). \]

Solving for \( e = c/a \),

\[ e = \sqrt{\frac{1}{\pi} \left( \frac{\delta m}{W} \frac{\sqrt{2}}{4} \right)^2}. \]

Substituting \( \delta m = 1 \text{ \mu m} \) and \( W = 8.5 \text{ \mu m} \) gives \( e \approx 0.75 \).
Numerical values of constants

The numerical values of all constants are the same as in Honglawan and Beller et al. (Honglawan, Beller et al. 2011): $K_1 = 5 \times 10^{-11}$ N, $\rho \times a = 3 \times 10^{-9}$ m, $\sigma^\text{air} = 20 \times 10^{-3}$ N m$^{-1}$, $\Delta \sigma^{\text{subs}} = -1.1 \times 10^{-3}$ N m$^{-1}$. We also set $R = K_1$. 
Figure S5.1 Substrate interface (a) and elastic (b) energy contributions to the free energy $\Delta F$ plotted in Figure 2 of two TFCDs sharing one cylindrical pillar. The free energy is given relative to the reference state of planar layers as a function of the relative position of the circular pillar center along the line connecting the two TFCD centers for different pillar heights (0.5 – 4 µm). The TFCD radius is set to 5.2 µm at LC thickness of 10 µm on the pillar array with radius of 5.72 µm. The air interface energy is independent of pillar position.
Figure S5.2 Schematic diagram of the FCD arrangement assumed for the pillar topography of Figure 1a, viewed from above. The circular pillar, of diameter $D$, is represented in gray. The elliptical focal curves of four FCDs, assumed to lie in the plane of the substrate, are each tangent at four points to squares comprising one fourth of the unit cell. The solid black circles are the ellipse foci as well as the intersections of the hyperbolic focal curves with the plane; these lie at the pillar edge. The open circle is the center of one ellipse. The $x$- and $y$-axes of the coordinates used in Equation (1) are as shown in the diagram.
References


CONCLUSIONS AND PROSPECTIVE

In this thesis, we presented stepping stone studies toward innovation of multifunctional and multidimensional structured materials by means of \textit{template-assisted self-assembly}. Specifically, we studied two interesting soft materials, amphiphilic random copolymer (ranPAC) and smectic A liquid crystals (SmA LCs), and their assemblies on SU-8 micropillar arrays fabricated by photolithography and soft lithography techniques. In each system, we investigated the self-assembly of individual components in a solvent or on a flat film in comparison to their assemblies on lithographically defined micropillar arrays. We studied the governing forces at the surface/interface to uncover the self-assembly principles from which the most important implication is how to design templates to direct self-assembling materials by regulating the incommensurability between the dimensions of the guiding templates and the equilibrium dimension of the assembling system. These studies should offer important geometric cues toward assembly of more complex structures and advanced materials.

The ranPAC self-organized into nanomicelles with high regularity and stability that was typically not possible in random copolymer systems. The size and morphology could be controlled by the photopolymerization conditions, and choice of solvent; the crosslinked shell made the micelles robust against drying and storage. We then exploited the amphiphilic ranPAC to 1) create ordered microporous films via evaporative assembly and 2) synthesize cationic nanocarriers for drug and gene delivery. Using SU-8 micropillar arrays with spatially controlled surface chemistry to direct the evaporative assembly, we constructed hierarchical microporous structures with tunable pore size and symmetry (e.g. square array vs. hexagonal array), and uncovered a new evaporative assembly method using volatile organic solvents, which was different from conventional humidity sensitive breath figure formation. By functionalizing the ranPAC nanovesicles with branched poly(ethyleneimines) (PEI), we attempted encapsulation of model anticancer drug, doxorubicin hydrochloride, and mRNA at a relatively high payload, which were then delivered to HEK 293T cells \textit{in vitro} at a low cytotoxicity level. The simplicity in particle
synthesis, tunable chemistry, high sensitivity of solution pH and effective delivery of both drug and gene shown in our particle system offer an attractive modality to develop smart nanocarriers in biomedical applications.

For a system as unique as ranPAC, there are a number of untapped aspects and unresolved problems associated with the studies that are vital to complete the fundamental understanding of the system as well as to expand its applications. Below is a list of some of the important areas that could be further investigated in the future. Note that the direct potential applications for the resultant structures will be excluded here as they are explicitly discussed in the previous chapters.

Characterization of random copolymer in a solution – It is generally difficult to characterize random copolymers because of their heterogeneous composition and ill-defined architecture. In chapter 1, we have performed a series of FT-IR analysis to verify the two major components of the copolymers, the solvophilic poly(acryloyl chloride) and the solvophobic crosslinked acid anhydride group which were responsible for micelle assembly in selective organic solvents. The exact composition of the two components was not quantified in the study due to the limitation of the available instrument. However, the composition could be better characterized with 2D FT-IR spectroscopy which is based on a statistical correlation of dynamic spectral variations induced by an external perturbation. This approach allows for deciphering the environment of a particular chemical group, intermolecular and intramolecular interactions thus, giving more quantitative information of the resolution and chemical nature of a complex molecule such as ranPAC.

Besides polymer composition, we could not accurately measure the average molecular weight (MW) of the synthesized polymers by SEC due to formation of micelles from the amphiphilic copolymers in the eluent solvent (THF) and partially crosslinked nature of the shell. The results from SEC analysis merely suggested a correlation between the molecular weight and
UV dosage applied to the monomer mixture. The MW of the polymers is a key to create a phase diagram of the copolymers both in bulk and in solution, which potentially could lead to realization of new morphologies. Alternatively, mass spectroscopy can be utilized to analyze MW of the copolymer based on measurement of mass-to-charge ratio of analyte ions. Although mass spectroscopy offers the advantage of high mass resolution, high mass accuracy and high sensitivity for analyzing polymers with narrow polydispersity, it is challenging to characterize complex systems such as random copolymers with broad polydispersity. For example, differences in charge state distributions for different chain length oligomers can complicate the determination of MW. Other possible issues, including sample preparation, sample discrimination, effect of solvent, multiple charging, laser fluence, and instrument dynamic range, may also complicate the data collection and interpretation. Therefore, it is important to analyze the complex random copolymer system using different techniques.

**Study of random copolymer assemblies in bulk vs. in a thin film** – Chapter 2 describes a new evaporative assembly strategy for preparation of ordered porous thin films on solid substrates. The study demonstrated the importance of the interfacial interaction between the polymer and the substrate. As interesting as ranPAC itself, it would also be intriguing to thoroughly investigate the interplay between the strength of the surface and the modulated phases of the copolymers, including wettability, spinodal decomposition of the polymers in a multiphase system, and surface-induced ordering and orientation. Comparison between the equilibrium phases in bulk and modulated phases in a thin film of ranPAC will provide the means to understand general mechanisms governing phase behaviors, and enrich a wider class of ordered polymeric structures beyond the self-assembly from the block copolymers.

**Mechanism for the formation of ordered porous structures from ranPAC** – Although the control of porous films from evaporative assembly of ranPAC (Chapter 2) can be generalized based on several regulating factors, including polymer concentration, choice of solvent, polymer-
solvent interaction parameters, and surface chemistry of substrates, the exact mechanism of this system remains unresolved as its analog, breath figure. While breath figure method relies on both condensation of moist air to function as the sacrificial spherical template of water droplets and evaporative cooling of polymer solution, our process does not involve the former. Proven experimentally, the spherical pore template in the system is formed by toluene, the volatile, nonsolvent of the ranPAC, which is initially introduced into the polymer/acetone mixture. The collective experimental data in Chapter 2 led us believe that the mechanism is most likely driven by nonsolvent induced phase separation or demixing of the 3 - 4 component mixtures: polymer (two chemically dissimilar components of amphiphilic molecules), good solvent, and nonsolvent. In this case, building up a triangular or a more complex phase diagram of the polymers will offer a quantitative view of polymer phase behaviors and predict the stable, metastable and unstable regions for a given mixture.

It is clear that the formation of ranPAC porous films involves complex and multiple mechanistic steps from growth of toluene droplets to organization of the droplet array. The formation of droplets in this process differs fundamentally from that in breath figure since it does not involve cooling of water, but the organization of the droplets for both methods may share similarity. There are two widely accepted theories in the studies of breath figure formation that may be relevant to our system explaining why the polymer/water droplets do not coalesce; the first is the thermal gradient effect (Marangoni convection and the classic thermal capillary effect), the other is the minimization of the interfacial tension facilitated by the thin polymer layers. Unfortunately, it remains challenging to prove experimentally which of these two mechanisms is dominant in formation of porous films in our system. More mechanistic experiments are needed to elucidate the details of the solvent evaporation process and droplet formation. Several important questions remain to be answered: what is the driving force for nucleation of toluene droplets? What is the kinetics of the toluene droplet formation? What is the effect of temperature to the demixing process in this specific system? Can the pore shape and distribution be regulated? To
address these questions, it requires precise control of the kinetics and thermodynamics of the spinodal decomposition of the polymer solution, from the nucleation of the toluene droplets to the growth of the stabilized phase of the suppressed coalescence. The fundamental studies will guide the design of experiments to possibly capture new morphologies of porous structures in a hidden phase.

The studies in Chapters 4 and 5 shed light on topographic design of 2-D templates for controlling epitaxial growth and hierarchical arrangement of FCDs of SmA LC. The former is based on confinement of FCDs by SU8 pillar arrays which serve as 3-D hybrid cells of mixed boundary conditions for local FCDs: tangential anchoring at the flat bottom of the arrays and vertical side walls of the pillars and homeotropic anchoring at air interface. As a result, an individual FCD could be directly grown either in between 4 neighboring pillars or on top of each pillar, yielding highly ordered arrays of FCDs that were in registry with the guiding templates. The conservation of toric FCD (TFCD) textures over large LC thickness above the guiding pillars manifested a remarkably unique outcome of the epitaxial growth of TFCDs. In this case, one of the most important requirements is the dimensions of the pillar arrays including height, diameter, center-to-center spacing of the nearest two diagonal pillars (for a square lattice), whose values must be equal to or greater than their critical values for energetically favorable formation of FCDs. The other scenario with remarkable results for hierarchical arrangements of multiple FCDs on a single pillar detailed in Chapter 5 can be observed when the pillar height is smaller than its critical value for confinement and when the center-to-center spacing of the nearest two diagonal pillars must be approximately 4 times the domain radius. The system favored the “pinning” of FCD centers near pillar edges while avoiding the opposing effect of confinement, leading to the break of the underlying symmetry of the pillar lattice, exhibiting tunable eccentricity, and a nontrivial yet organized array of defects balancing the elastic energy of LCs and the surface energy imposed by the template. As far as this, we established the fundamental design of the pillar arrays with variable dimensions such that they imposed both molecular anchoring and geometric restrictions.
on the 3-D structure of FCDs with an exceptionally fine level of control from feature size, spatial
distribution, symmetry to intrinsic topology of FCDs. These findings should expand current
applications of FCD arrays to a great extent; however, they encompass the very first step toward
the formation of 3D networks and more complex geometries as a top leveled agenda for
revolutionizing technology such as blue phase liquid crystal displays. To proceed toward the
ultimate goal, here are some of the most relevant future directions for research.

Complex templates – Most studies so far focus on confining liquid crystals with uniform
boundary conditions at different interfaces in order to produce predictable pattern or topology of
liquid crystals with high regularity. It is known that boundary conditions regulate molecular
anchoring of liquid crystals at interfaces and in turn influence macroscopic structures. Hence, a
direct and simple route to exploit new forms of liquid crystal structures is to study liquid crystals in
more complex templates with curved surfaces, gradient boundary conditions, for example,
surfaces/templates with varying anchoring strengths, varying physical geometries such as
nonuniform pillar height or pillar shape and responsive kinds in which circular post arrays or
patterns on the templates could even be dynamically altered with heat or an electric field, for
example, making the posts in a certain region elliptical. This microscopic geometric cue would
travel up the layers of liquid crystal and produce micrometer-scale changes on the surface. It
would be profoundly interesting to investigate how LC molecules respond to these complex
confinements while satisfying heterogeneous boundary conditions by balancing competition
between elastic inner force imposed by LC molecules and surface anchoring forces imposed by
the template.

Directed assemblies from other liquid crystal phases – While we demonstrate the
unprecedented control of FCDs from SmA LCs, it will be interesting to broaden the scope of the
study and investigate assemblies on variable 1-D to 3-D templates from other LC phases. For
example, confinement of smectic C* phase, which is constructed with rotating directors from one
layer to the next forming a helix, should yield new 3-D orders and chirality due to energetic frustration of SmC* LCs from their equilibrium forms. Cholesteric phase, known as chiral nematic, is another LC phase that deserves much attention. The chiral nematic exhibits a number of unique structural related properties, such as high temperature sensitivity and selective reflection of light, which have already been extensively utilized in today technologies, ranging from LC based thermometers, sensors, advanced displays, clothing, inks to paints, etc. Therefore, the ability to create new chiral nematic structures with a geometric means should immediately benefit its current applications and beyond.

Templat ing nanomaterials and other applications of SmA LC – Over decades, a number of novel functional nanomaterials ranging from semiconductor and metal nanoparticles, quantum dots, fullerences, supramacromolecular aggregates, graphenes to carbon nanotubes have been discovered and characterized for their exceptional properties. These materials are the key components from which emerging and future nanotechnologies will be fabricated. However, an assimilation of these materials into larger configurations with engineered order remains the major challenge for the preparation of useful materials and functional devices. The ability to control the assembly of SmA LCs in 3-D with variable degree of orientational and translational orders and to efficiently produce a variety of long-range periodic and anisotropic structures on designed surfaces such as wedge cells, microgroove wrinkle surfaces, 1-D microchannels, and 2-D pillar arrays as shown in our studies hence prompts a potential path to organize these functional materials.

Because the layers of LCs transmit elastic energy, they can be used to do mechanical work such as assembling nanomaterials and inducing topological defects in LC. To some extent, the feasibility of this scheme has been illustrated in the lab scale, for instance, linear and hexagonal arrays of nanoparticles elastically trapped at various sites of smectic defects ranging from grain boundary, curvature wall, dislocation to disclination and aligned parallel sheets of single wall carbon nanotubes organized between smectic layers based on specific interactions.
between π-π interactions between the hexagonal rings of the carbon nanotubes and the aromatic moieties of LC molecules. (Jeong, Ko et al. 2010; Coursault, Grand et al. 2012; Milette, Relaix et al. 2012) The researchers are essentially in the process of laying the foundation of a directed assembly technique that can be used with any SmA LC. What is anticipated to become clearer in the near future is that the potential applications of controlled LC structures will extend beyond sensors and displays and forward to nanomanufacturing and many more.

An emerging application of SmA is found in the fabrication of lithographic parts and smart surfaces such as a surface with selective wetting or with superhydrophobicity where the properties depend on complex physical and chemical features on the surface as self-assembly of SmA LC can provide a simple and inexpensive route to produce a variety of unique and interesting topographic surfaces or patterns over a large area in a short time period. The possibility of patterns generated from this process is not limited to ordered arrays of micro-dimples, (Kim, Yoon et al. 2010) fish skin texture, (Zappone, Meyer et al. 2012) circular ring (Kim, Jeong et al. 2010) and alternating hill and valley surfaces as shown in Chapter 5. While creation of a superhydrophobic surface from an array of dimple like SmA defects in thin film has been demonstrated through simple surface modification, (Kim, Yoon et al. 2009) the inverse of the texture which features an array of sharp points can function as a stamp to print nanometer patterns in other materials. The dimples now become sharp points after printing, which in turn can be used as lenses and localized surface plasmon resonance hot spots for chemical and biological sensing.

Without doubt, template-assisted self-assembly is a powerful tool to create functional materials at various length scales. As demonstrated in this thesis, the technique combines merits of both bottom-up and top-down approaches and at the same time addresses the fundamental limitations of each approach, for example, poor structural controllability in bottom-up approaches, and the high cost in scaling down dimensions to nanoscale and formation of hierarchical structures using top-down approaches. We believe that in the near future, template-assisted self-
assembly will become a main fabrication process for structural materials that are of vital importance for the development of advanced technologies, including photonics, nanophotonics, chemical and biological sensing, data storage, and electronic devices.
References


