### **Supporting Information**

### Chiral textures inside 2D achiral domains

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#### S1) Experimental procedures.

*Materials.* Hemicyanine dye, 4-[4-hydroxi)styryl]-1-docosylpyridinium bromide (SP) and dimyristoyl-phosphatidic acid (DMPA) were purchased from Sigma-Aldrich and used as received. Their molecular structures are depicted in Scheme 1. A mixture of chloroform:methanol, ratio 3:1 (v/v), was used as spreading solvent for solving both components. The pure solvents were obtained without purification from Aldrich (Germany). Ultrapure water, produced by a Millipore Milli-Q unit, pre-treated by a Millipore reverse osmosis system (> 18.2 M $\Omega$  cm), was used as a subphase. The subphase temperature was 21°C with pH 5.7.

*Methods*. Two different models of Nima troughs (Nima Technology, Coventry, England) were used in this work, both provided with a Wilhelmy type dynamometric system using a strip of filter paper: a NIMA 611D with one moving barrier for the measurement of the reflection spectra, and a NIMA 601, equipped with two symmetrical barriers to record BAM images. The monolayers were compressed at a speed of ~ 0.1nm nm<sup>2</sup> min<sup>-1</sup> molecule<sup>-1</sup>.

UV–visible reflection spectra at normal incidence as the difference in reflectivity ( $\Delta R$ ) of the dye film-covered water surface and the bare surface<sup>1</sup> were obtained with a Nanofilm Surface Analysis Spectrometer (Ref SPEC<sup>2</sup>, supplied by Accurion GmbH, Göttingen, Germany).

Images of the film morphology were obtained by Brewster angle microscopy (BAM) with a I-Elli2000 (Accurion GmbH) using a Nd:YAG diode laser with wavelength 532 nm and 50mW, which can be recorded with a lateral resolution of 2  $\mu$ m. The image processing procedure included a geometrical correction of the image, as well as a filtering operation to reduce interference fringes and noise. The microscope and the film balance were located on a table with vibration isolation (antivibration system MOD-2 S, Accurion, Göttingen, Germany) in a large class 100 clean room.

Circular Dichroism measurements were recorded in a Jasco J-715 CD spectrometer at room temperature. Spectra were taken in the interval between 280 and 600 nm with a step resolution of 0.5 nm and a bandwidth of 1 nm. The scanning rate was 20 nm/min with a response time of 1 s.

S2) Additional BAM images: comparison with the *L*-DMPA:HSP mixed monolayer.



**Figure S1**: Surface pressure-area ( $\pi$ -A) isotherms of the mixed HSP:DMPA monolayer in a molar ratio 1:1 at T = 21 °C (top-left). A)-H) BAM images of the mixed HSP:DMPA = 1:1 monolayer under different surface pressures (PCA =0°, 0°, 0°). I) BAM images with PCA = 2°, 2°, 8°). J) BAM images of the mixed SP:DMPA = 1:1 monolayer (Gonzalez-Delgado et al., *J. Phys. Chem. C*, 114, **2010**, 16685). Image size: 215 µm width.

S3) BAM pictures of the SP(x):HSP(y):DMPA(1) mixed monolayer at the air/water interface, where x+y = 1.



Figure S2

BAM images obtained for the mixed films: Left) SP(0.5):HSP(0.5):DMPA(1) and Right) SP(0.1):HSP(0.9):DMPA(1). Both images were obtained at a surface pressure of 10 mN/m.

S3) BAM pictures of the *rac*-DMPG:SP = 1:1 mixed monolayer at the air/water interface. Pictures acquired after a second compression run of the monolayer.



For the mixed monolayer *rac*-DMPG:SP, circular domains without inner chiral textures have been observed for the first cycle of compression. However, after a decompression of the monolayer and a subsequent second compression, a slow growth process of the domains around the already formed nuclei takes place. In this scenario, although most of the domains show no chiral texture, some domains show chiral textures with both handednesses *i. e.*, S-shaped and reverse S-shaped. The chiral domains are highlighted within orange circles.

S5) BAM pictures of the HSP (prochiral):DMPA (L-enantiomer) = 1:1 mixed monolayer at the air/solid interface. Solid support: quartz. Surface pressure for transfer: 30 mN/m. 1 monolayer was transferred.



The HSP:DMPA mixed monolayer was transferred from the air/water interface to a quartz solid support by the Langmuir-Blodgett technique. Transfer ratio was close to unity. As observed in Figure S3, the shape of the domains was conserved after the transfer process. The significant roughness of the support, as well as the weaker contrast prevents for observing by BAM the chiral inner textures. However, the chirality in the transferred samples is assured by Circular Dichroism measurements (see Figure 3 of the manuscript). Therefore, we conclude that the supramolecular structure is similar for the HSP:DMPA monolayer at both air/water and air/solid interfaces.

# S6) BAM experiments on the mixed monolayer DMPA:HSP: Discarding of linear birefringence

The following BAM experiment has been conducted: the polarization angles of the incident light (noted as P and C, polarizer and compensator, respectively), as well as the polarization angle of the camera (noted as A, analyzer) have been systematically varied during the recording of the BAM picture of the mixed monolayer DMPA:HSP.

The Figure S5 shows BAM pictures recorded using values of PCA = $\gamma$ ,0,0, with the value of  $\gamma$  ranging from -90° to 90°. The Figure S6 shows BAM pictures recorded using values of PCA = 0,0, $\eta$ , with the value of  $\eta$  ranging from -90° to 90°.

The helical textures inside the domains, which we relate with the chiral supramolecular structures, seem to rotate with the variation of  $\gamma$  or  $\eta$ . For the cases of  $\gamma = \eta$  (Fig. S5 and S6), the inner textures of the domains show approximately the same helical rotation. Therefore, the existence of linear birefringence effects is non likely to be present for these S-shaped structures, at least for the wavelength used in the BAM experiments (532 nm).



Figure S5

Figure S6

S7) UV-vis reflection spectra of HSP:SA= 1:1 mixed monolayers at the air/water interface. The UV-vis normalized reflection spectra,  $\Delta R_{\text{norm}}$ , of HSP:SA= 1:1 monolayers on a ultrapure water subphase at different surface areas (see isotherm of Figure 1) are shown in Figure S4. For comparison, the Figure S4 (dotted line) shows the solution spectrum of the HSP:DMPA = 1:1 mixed in Chloroform:methanol 3:1 (v/v).



For low values of absorption, the reflection  $\Delta R$  has been shown to be proportional to the surface concentration of the dye.<sup>1-4</sup> In this case, the corresponding product  $\Delta R \times A = \Delta R_{\text{norm}}$  could be expressed by means<sup>5</sup>

$$\Delta R_{norm} = \Delta R \times A = 5.407 \times 10^{-8} f_{orient} \varepsilon$$
<sup>(1)</sup>

where the extinction coefficient  $\varepsilon$  is given as L mol<sup>-1</sup> cm<sup>-1</sup>,  $f_{orient}$  is a numerical factor that takes into account the different average orientation of the square transition moment of the dye in solution as compared to the monolayer at the air–water interface, A is the surface area per dye molecule and  $\Delta R_{norm}$  is expressed in nm<sup>2</sup>.

At low surface pressures, the spectra presented a low-energy band at ~390 nm, corresponding to the  $\pi$ - $\pi$ \* transition of the chromophore in trans configuration.<sup>6-7</sup> (In solution the maximum wavelength appears at 414 nm As the surface pressure increased, two phenomena were observed in the reflection band:

1) The maximum wavelength of the band was shifted to longer wavelengths, from 390 nm to 403 nm, although the band shows a shoulder at 423 nm. In the case of the SP:DMPA system, the band shifted from shorted wavelength, to 475 nm to 459 nm.<sup>5</sup>

2) The width of the band increases

3)  $\Delta R_{\text{norm}}$  decreased under compression, which could be related to the decreasing of the polar tilt angle of the chromophores. The polar angle  $\theta$  is defined as the angle between the HSP transition moment and the normal to the air–water interface.

#### S8) Calculation of the polar tilt angle of the H and J component of the HSP headgroup

For very thin films, i.e., for weak absorption values (< 1%), and according to Vandevyver et al.,<sup>8</sup> the dichroic ratio DR can be expressed as:

$$DR = \frac{Ab_s}{Ab_p} = \left[\frac{n_1 \cos(r) + n_3 \cos(i)}{n_1 \cos(i) + n_3 \cos(r)}\right] \left[\frac{n_2^4 \left\langle\sin(\theta)^2\right\rangle}{2n_1^3 n_3 \sin(i)^2 \left\langle\cos(\theta)^2\right\rangle + n_2^4 \left\langle\sin(\theta)^2\right\rangle \cos(i)\cos(r)}\right]$$
(2)

where  $n_1$  (= 1),  $n_2$  and  $n_3$  (= 1.43) are the refractive index of air, film and quartz, respectively, *i* is the incidence angle of the light,  $r = \arcsin[n_1 \sin(i)/n_3]$  is obtained from Snell's ratio,  $\theta$  is the angle between the transition dipole and the normal to the support, and brackets denote average values.

From equation (2)  $(n_2 = 1.6^5 \text{ and } i = 45^\circ \text{ or } i = 60^\circ)$ , the tilt angle from the *DR* values has been calculated, obtaining  $\theta = 23^\circ \pm 2^\circ$  for  $\lambda \approx 335$  nm (H component), and  $\theta \approx 70^\circ \pm 4^\circ$  for  $\lambda \approx 500$  nm (J component). In any case, these values should be carefully considered, given that the H and J components of the absorption band could not be completely resolved.

# S9) Reflection of an anisotropic thin film on isotropic substrate and simulation of circular domains

The anisotropic thin film is assumed to be a biaxial material with complex refractive indices  $(N_{1x}, N_{1y}, N_{1z})$ . The reflection incidence angle,  $\alpha_0$ , and transmission angle,  $\alpha_2$ , of multiply reflected waves (Figure S5) are constants and related by  $n_0 \sin(\alpha_0) = n_2 \sin(\alpha_2)$ , where  $n_0 = 1$  (air) and  $n_2 = 1.333$  (water) show the real refractive indices of the ambient and substrate, respectively.



In BAM microscopy the incident light is p-polarized and when the analyzer is set to  $0^{\circ}$ , only the p component of the reflection is observed. In this case, the light reflection at the ambient/film interface can be expressed as follows:<sup>9</sup>

$$r_{01pp} = \frac{N_x N_z \cos(\alpha_0) - n_0 \left(N_z^2 - n_0^2 \sin(\alpha_0)^2\right)^{1/2}}{N_x N_z \cos(\alpha_0) + n_0 \left(N_z^2 - n_0^2 \sin(\alpha_0)^2\right)^{1/2}}$$
$$r_{12pp} = \frac{n_2 \left(N_z^2 - n_2^2 \sin(\alpha_2)^2\right)^{1/2} - N_x N_z \cos(\alpha_2)}{n_2 \left(N_z^2 - n_2^2 \sin(\alpha_2)^2\right)^{1/2} + N_x N_z \cos(\alpha_2)}$$

The total reflection coefficients for p-polarizations

$$r_{012pp} = \frac{r_{01pp} + r_{12pp} \exp(-i2\beta_p)}{1 + r_{01pp} r_{12pp} \exp(-i2\beta_p)}$$

where

$$\beta_{\rm p} = \frac{2\pi d}{\lambda} \frac{N_{\rm x}}{N_{\rm z}} \left(N_{\rm z}^2 - n_0^2 \sin\left(\alpha_0\right)^2\right)^{1/2}$$

being  $\lambda = 532$  nm the wavelength of the laser beam and d the thickness of the biaxial thin film. The reflectances for p-polarized waves are obtained by

$$\mathbf{R}_{\mathrm{p}} = \left| \mathbf{r}_{\mathrm{012pp}} \right|^2$$

The complex refractive indices of anisotropic materials along the x, y, and z axes, and are given by  $N_x = n_x - ik_x$ ,  $N_y = n_y - ik_y$ , and  $N_z = n_z - ik_z$ , respectively. As only the p-component of the reflection is analyzed, the N<sub>y</sub> component does not affect to the final result. On the other hand, the absorption coefficients can be expressed by means of

$$k_{x} = k_{0} \sin(\theta)^{2} \cos(\phi)^{2}$$

$$\mathbf{k}_{z} = \mathbf{k}_{0} \cos(\theta)^{2}$$

where the angles  $\theta = 70^{\circ}$  (J component) and  $\phi$  are defined in Scheme 1 (see the manuscript) and  $\alpha_0 = 53.15^{\circ}$  (Brewster angle). To simulate the reflectivity, we used the same parameters used previously for the HP:DMPA system:  $n_x = n_z = 1.6$ , d = 3.7 nm (thickness of the film including DMPA and HSP), and  $k_0 = 0.15$ , because the absorption is lower than that of SP. In such way, the reflectivity depends exclusively on  $\phi$ .

The reflectivity for any radius of the circular domain, can be determined by modifying  $\phi$  between  $\phi_0$  and  $\phi_0 + 2\pi$ . Figure S6 displays the reflectivity obtained for  $\phi_0 = -30^\circ$ . Also, this figure displays the polar representation of the reflectivity.



Furthermore, we assume that  $\phi$  varies linearly with distance r, as

$$\phi(\mathbf{r}) = \phi_0 + \frac{\mathbf{r}}{\mathbf{R}}\gamma$$

where R is the maximum radius of the domain,  $\phi_0$  the value of  $\phi$  for r = 0 and  $\gamma$  is the rotation of the J component along the entire domain. Thus, assuming that  $\gamma = 75^{\circ}$ , and for the example of the previous figure which corresponds to a situation with r = 0 and  $\phi_0 = -30^{\circ}$ , we have that for r = R,  $\phi = -30^{\circ} + 75^{\circ} = 45^{\circ}$ , which in this case the reflectivity curve is shown in Figure S7.



With all the reflectivity curves ranging from r = 0 ( $\phi = -30^{\circ}$ ), to r = R ( $\phi = 45^{\circ}$ ), we construct a data matrix, which is represented in the xy plane, although, for the simulation of BAM domains, the reflectivity is transformed in gray levels from 255 (white) to 0 (black). The simulated domain in Scheme 2D correspond to  $\phi_0 = -30^{\circ}$  y  $\gamma = +75^{\circ}$  (domains II and III) and  $\phi_0 = -60^{\circ}$  y  $\gamma = +75^{\circ}$  (domains V and VI).

The contrast and clarity of the image can be improved by means of the instrumental option of the BAM, the auto set gray level. When the auto set option is clicked, the gray level is rescaled by increasing the exposure time to the light in such a way that only the reflectivity under the threshold value can be discerned, being this threshold the new maximum reflectivy, that is, 255. Figure S8, shows an example of the above, where the reflectivity is truncated to 50% of the maximum reflectivity. Reflectivity greater than this value are taken as a target (255).

The brightness depends on the maximum and minimum reflection, which, in turn, depends on the time of exposure of the monolayer at BAM, an increase of exposure time can increase the contrast of the image as is illustrated in Figure 5D from the manuscript, which shows two experimental domains, designated I and IV. Images II and III represent two simulations of domain I, which have used the same optical parameters, modifying only the exposure time. The same applies to the images V and VI, which correspond to the simulation of domain IV.



SI-11

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