SUPPORTING INFORMATION

AGGREGATION AND STRUCTURAL STUDY OF THE MONOLAYERS FORMED BY AN AMPHIPHILIC THIAPENTACARBOCYANINE.

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1. ANISOTROPIC THIN FILM ON ISOTROPIC SUBSTRATE

The anisotropic thin film is described with the complex refractive indices of N_x , N_y , N_z . As shown in Scheme S1, the reflection incidence angle α_0 and transmission angle α_2 of multiply reflected waves are constants and are related by $n_0 \sin(\alpha_0) = n_2 \sin(\alpha_2)$. $n_0 = 1$ (air) and $n_2 = 1.333$ (water) represent the real refractive indices of the ambient and substrate, respectively.



Scheme S1: Schematic illustration of reflection at a three-layer system in which layer 0, 1 and 2 represent is air, the thin film, and the subphase (water in this case). Left: Black arrows represent the incident light. Right: Red arrow represents the transition dipole direction, and x-y represent the interface plane.

The Fresnel expressions for the reflection of s- and p- polarized radiation under different α_0 originated for a anisotropy biaxial film between two isotropic media, e g., air and water are provided by:^[1]

$$r_{01pp} = \frac{N_{x}N_{z}\cos(\alpha_{0}) - n_{0}\left(N_{z}^{2} - n_{0}^{2}\sin(\alpha_{0})^{2}\right)^{1/2}}{N_{x}N_{z}\cos(\alpha_{0}) + n_{0}\left(N_{z}^{2} - n_{0}^{2}\sin(\alpha_{0})^{2}\right)^{1/2}}$$

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

The total reflection coefficients

$$r_{012xx} = \frac{r_{01xx} + r_{12xx} \exp(-2 \cdot i \cdot \beta_x)}{1 + r_{01xx} r_{12xx} \exp(-2 \cdot i \beta_x)}$$

Where x = s or p and

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

Where λ is the wavelength and *d* is the thickness of the thin film. We obtain the reflected amount of light for x = s or p-polarized waves as follows:

$$R_{\rm x} = \left| r_{\rm 012\,xx} \right|^2$$

The complex refractive indices of anisotropic materials along the x, y, and z axes are given $N_q = n_q -i \times k_q$, where, $q \equiv x$, y, z, being n_q the real part of the reflective index and k_q the absorption constant along each axes. The variations in reflection are assumed to be caused mainly by absorption, and $n_x = n_y = n_z = n_1$ can be assumed. Furthermore, the absorption constants can write as:

$$k_{x} = 3k_{iso} \left\langle \sin(\theta)^{2} \right\rangle \left\langle \cos(\phi)^{2} \right\rangle$$

$$k_{y} = 3k_{iso} \left\langle \sin(\theta)^{2} \right\rangle \left\langle \sin(\phi)^{2} \right\rangle$$

$$k_{z} = 3k_{iso} \left\langle \cos(\theta)^{2} \right\rangle$$
(1)

Where the brackets indicate average values, k_{iso} is the absorption constant in an isotropic media, θ is the angle between the normal to the air-water interface (z axis) and the direction of the transition dipole, and Π is the angle between the projection of the transition dipole on the xy plane and the x axis, see Scheme 1(right).

1.1. Polarization study of the absorption bands by using UV-Vis Reflection Spectroscopy under variable angle incidence.

The Fresnel expressions for the reflection of s- and p- polarized radiation under different α_0 can be used for obtaining the variation between the reflection of incoming radiation in presence and in absence of a Langmuir monolayer, ΔR .^[2] For uniaxial materials, and assuming that the film is isotropic in the plane as an average, it is possible to rewrite the equations (1) as.

$$k_{x} = k_{y} = \frac{3}{2} k_{iso} \left\langle \sin(\theta)^{2} \right\rangle = \frac{3}{2} k_{iso} P(\theta)$$

$$k_{z} = 3k_{iso} \left\langle \cos(\theta)^{2} \right\rangle = 3k_{iso} \left[1 - \left\langle \sin(\theta)^{2} \right\rangle \right] = 3k_{iso} \left[1 - P(\theta) \right]$$
(2)

Where $P(\theta)$ is the order parameter. In the region of spectral absorption, the dispersion of the real part of the refractive index can be determined via the Kramers-Kronig relation^[3]

$$n_{1}(w) = n_{\infty} + \frac{2}{\pi} \int_{0}^{\infty} \frac{x \cdot k(x)}{x^{2} - w^{2}} dx$$
(3)

Using this model the reflection of the film at a given incidence angle, α_0 , and wavelength, λ , depends of four experimental parameters; film thickness (*d*), reflective index, n_1 , k_{iso} and $P(\theta)$. From theory, a wide range of experimental measurements of ΔR_p and ΔR_s as a function of α_0 would allow the determination of the four parameters of the described model, i. e., k_{iso} , $P(\theta)$, *d* and n_1 by numerical fitting. However, this information cannot be accessed due to experimental limitations. These limitations can be overcome by separately measuring or estimation of n_{∞} and *d*. Thus, a value, $n_{\infty} = 1.6$ has been assumed, and the film thickness was estimated from CPK models, taking into account the molecular area given by the compression state of each Langmuir monolayer, obtaining $d \approx 2$ nm.

Under normal incidence the reflection spectrum depend on three parameters, k_x , n_1 and d, exclusively, but not depending on k_{iso} and $P(\theta)$ in a separate way.^[2] This behavior can be used to determine the dependence of n_1 with λ . This procedure has been applied as follows; first, an approximate k_x value from the reflection spectrum under normal incidence and high surface pressure (ΔR_n , unpolarized radiation) has been determined from the following expression:

$$k_x^0 \approx \frac{3}{2} \frac{\lambda \cdot \Delta R_n}{8\pi \cdot d \cdot \sqrt{R_s}}$$
(4)

Where, $R_S \approx 0.02$ is the reflection of in the absence of the Langmuir monolayer. From this set of k_x^0 values, a first set of n_1^0 values was obtained solving numerically the integral (3).^[3] With these sets of k_x^0 and n_1^0 values the reflection spectrum under normal incidence ΔR^0 , is simulated and compared with the experimental one, ΔR_n . Next, a new set of k_x values could be obtained from the relationship $k_x^1 = k_{iso}^0 \times (\Delta R_n / \Delta R^0)$, and next n_1^1 could be obtained applied again equation (3). These corrected k_x^1 and n_1^1 values are used for a new correction, and the process being repeated until experimental and theoretical reflections match. Four iterations are enough to obtain calculated results showing a quite good agreement with the experimental values of reflection under normal incidence. k_x and n_1 values obtained for $\pi = 38$ mN/m are shown in Figure S1.



λ, nm

Figure S1: k_x and n_1 values obtained from the reflection spectrum under normal incidence. $\pi = 38$ mN/m.

In the next step, the wavelengths corresponding to each absorption maxima are selected, and the values of n_1 according to the results shown in the figure S1 are fixed. Thus, for $\lambda = 600$ nm, 760 nm and 822 nm, the n_1 values were 1.5, 1.75 and 2.01, respectively. Then, once fixed *d* and n_1 , the k_{iso} and $P(\theta)$ values are obtained by fitting the experimental data to the theoretical model, following the procedure described previously.^[2] The ΔR values for different angles of incidence and s-and p radiation are used as input experimental data.

The experimental ΔR_s and ΔR_p values at the absorption maxima, $\lambda = 600$ nm, $\lambda = 760$ nm and $\lambda = 822$ nm *at* $\pi = 38$ mN/m, are shown in Figures S2, S3 and S4, respectively (circles). The solid lines in Figure S2, S3 and S4 correspond to the theoretical predictions for the values of the obtained parameters after the numerical

fitting. Thus, $k_{iso} = 0.38 \pm 0.04$ and $P(\theta) = 0.95 \pm 0.09$, for $\lambda = 600$ nm, $k_{iso} = 0.58 \pm 0.07$ and $P(\theta) = 1.0 \pm 0.1$, for $\lambda = 760$ nm and $k_{iso} = 0.62 \pm 0.07$ and $P(\theta) = 0.9 \pm 0.1$, for $\lambda = 822$ nm are obtained.



Figure S2: Circles: Experimental ΔR_s and ΔR_p of the OTCC monolayer, correspond at λ = 600 nm for π = 28 mN/m The solid lines correspond to the theoretical variation of ΔR_s (s) and ΔR_p (p) vs α_0 for n_1 = 1.5, d = 2 nm, k_{iso} = 0.38 and $P(\theta)$ = 0.95.



Figure S3: Circles: Experimental ΔR_s and ΔR_p of the OTCC monolayer, correspond at λ = 760 nm for π = 28 mN/m The solid lines correspond to the theoretical variation of ΔR_s (s) and ΔR_p (p) vs α_0 for n_1 = 1.75, d = 2 nm, k_{iso} = 0.58 and $P(\theta)$ = 1.0.





Figure S4: Circles: Experimental ΔR_s and ΔR_p of the OTCC monolayer, correspond at λ = 822 nm for π = 28 mN/m The solid lines correspond to the theoretical variation of ΔR_s (s) and ΔR_p (p) vs α_0 for n_1 = 2.01, d = 2 nm, k_{iso} = 0.62 and $P(\theta)$ = 0.9.

1.2 The physical origin of the in-plane anisotropy: relationship between reflectivity and ∏angle

As obtained in the previous section, $P(\theta) \approx 1$, and therefore $\theta \approx 90^{\circ}$. In these circumstances equations (1) can be expressed as:

$$k_{x} = 3k_{iso} \left\langle \cos(\phi)^{2} \right\rangle$$
$$k_{y} = 3k_{iso} \left\langle \sin(\phi)^{2} \right\rangle$$
$$k_{z} = 0$$

Figure S5 (continuous line) shows the reflection under p-polarized radiation, according to Fresnel's equations, for a typical film in which $\theta \approx 90^{\circ}$, $n_1 = 1.6$, d = 2 nm, $k_{\rm iso} = 0.5$ and $\lambda = 600$ nm, being $\alpha_0 = 53.15^{\circ}$ (Brewster angle).



Figure S5: Plot of R_p , *vs* Π angle (Continuous black line), according to Fresnel's equations, for $\theta = 90^\circ$, $n_1 = 1.6$, d = 2 nm, $k_{iso} = 0.5$, $\lambda = 600$ nm, and $\alpha_0 = 53.15^\circ$ (Brewster angle). Circles: Plot of equation (5) *vs* Π angle.

In Figure 5, is also represented (circles) the equation:

$$R_{p} = A \times \cos(\phi)^{4} + B = \left[R_{p}(\phi = 0^{\circ}) - R_{p}(\phi = 90^{\circ})\right] \times \cos(\phi)^{4} + R_{p}(\phi = 90^{\circ})$$
(5)

Where A and B, are constants related to the reflections at 0 and 90 degrees, as shown in the Equation (5). A quite good agreement between the calculated values from Fresnel equations and equation (5) is obtained. Using equation (5) to replace the Fresnel equations at the Brewster angle is correct provided that $\theta = 90^{\circ}$ and $k_{iso} \le 2$.

Equation (5) allows us to establish a simple relationship between the transition dipole orientation (ϕ angle) and the intensity of each pixel of BAM image (0-255 grayscale) as described in manuscript.

2. Dichroic ratio of the Langmuir-Schaefer films

The OTCC monolayers could be transferred onto solid substrates by horizontal lifting or Langmuir-Schaefer method. The monolayers were transferred at $\pi = 12$ mN/m, with the transfer ratio being close to unity in all the cases. To estimate the average tilt of the transition dipoles with respect to the surface, absorption spectra under s-(blue line) and p-(red line) polarized light at 45° of incidence angles were recorded (Figure S6).



Figure S6. Polarized absorption spectra, s (blue line) and p (red line), under 45° incidence angle of the OTCC LS film (one monolayer). Inset: Variation of DR.

The spectra obtained are slightly different from those obtained at the same surface pressure at the air-water interface (see DR yellow line in Figure 4, manuscript), presumably due to a partial disaggregation, though all absorption bands are clearly recognizable. The results can be interpreted by using the dichroic ratio, $DR = Ab_s/Ab_p$ (see Inset in Figure S6). Thus, for an incidence angle of 45°, $DR \approx 1.6 \pm 0.1$ in the range 550-820 nm. The measurement was repeated four times, showing good reproducibility.

For very thin films containing molecules with non-degenerated transition dipole components, and according Vandevyver et al. we can writte:^[4]

$$DR = \frac{Ab_s}{Ab_p} \approx \frac{n_0 \cos(\alpha_2) + n_2 \cos(\alpha_0)}{n_0 \cos(\alpha_0) + n_2 \cos(\alpha_2)} \left[\frac{n_0^3 n_2 \sin(\alpha_0)^2}{n_1^4} \left(\frac{2\left\langle \cos(\theta)^2 \right\rangle}{\left\langle \sin(\theta)^2 \right\rangle} \right) + \cos(\alpha_0) \cos(\alpha_2) \right]^{-1}$$

Where, θ represents the angle between the single transition dipole and the normal to the plane support (and the rest of parameters are defined in Scheme S1). This equation is obtained under the consideration that the films are isotropic in the support plane, which has been experimentally verified by recording different absorption spectra of the LS films under normal incidence and rotating the linear polarized light, and similar spectra in all the experiments were obtained.

On quartz the previous equation predicts DR = 1.57 for $\theta = 90^{\circ}$ independently of the n_1 value, in very good agreement with the obtain result. This result confirm that all the absorption component are polarized in the interface plane, even the disaggregate OTCC.

3. Semi empirical Study of the OTCC tetramer system.

OTCC (cationic) and CBS (anionic) were building by using HyperChem software. The distribution of electronic density and other structural parameters were optimized in a separate way by using PM3 semi-empirical method (RMS gradient 0.001kcal/Å×mol).A set of four OTCC molecules were positioned parallel with an intermolecular distance of ca. 0.4 nm, and 4 CBS molecules were located below each OTCC polar group for keeping the electroneutrality. The complete set of molecules was place in a periodic box of size $5.6 \times 2.7 \times 1.3$ nm³ including 248 water molecules located in the region below the OTCC molecules, see Figure S7.

The geometry was optimized by PM3 semi-empirical methods (RMS gradient 0.01kcal/Å×mol). The final structure obtained is shown in Figure S7. Other structures with lower energy were checked by performing three runs of molecular dynamics simulations under the molecular mechanics method MM+. Each run consisted of heating the structure to 400 K, running a molecular dynamics simulation for 5 ps, and then annealing to 0 K. Finally, the resulting structure was again optimized by using PM3 method. No significant differences in the final structures were observed.



Figure S7: Final structure after the PM3 geometry optimization corresponding to $(OTCC/CBS)_4$ complex located in a periodic box including 248 water molecules. The four OTCC molecules are represented by different color for a better visualization of the final structure.

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