

Supporting Information

Self-assembled Solvato-morphologically Controlled Photochromic Crystals**

Larisa Florea, Silvia Scarmagnani, Fernando Benito-Lopez, Dermot Diamond**

*The INSIGHT Centre for Data Analytics, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland

Fax: (+) 353 1 700 7995

E-mail: fernando.lopez@dcu.ie and dermot.diamond@dcu.ie

*CIC microGUNE, Arrasate-Mondragón, Spain

Fax.: +34 943739805

Fax: (+) 34 943739805

E-mail: fbenito@cicmicrogune.es

Table of Contents

1. Methods
2. Synthesis of SP-COOH
3. Protocol for the formation of SP-COOH microstructures and equilibrium of SP-COOH derivative in EtOH/water solution
4. FT-IR spectroscopy of SP-COOH
5. Micro-elemental analysis of SP-COOH microstructures
6. Interfacial self-assembly
7. Scanning Electron Microscopy
8. Absorbance spectra of SP-COOH microstructures
9. Microscopy imaging of SP-COOH 25% microstructures under cross polarizers.
10. Guided interfacial aggregation
11. References

1. Methods and procedures

¹H-NMR Spectroscopy

¹H-NMR Spectroscopy was performed using a Bruker Avance® spectrometer (400 MHz.). Deuterated chloroform (CDCl₃) was employed as the solvent and was used as purchased from Sigma-Aldrich® Ireland Ltd. Approximately 10 mg of sample per 0.7 mL of deuterated solvent was used. Spectra were run at 30 °C and were calibrated with residual protonated solvent peak (*i.e.* CHCl₃ in CDCl₃).

UV-Vis Spectroscopy

UV-Vis Spectroscopy was employed to characterize SP-COOH solution in EtOH (10⁻³ M, Figure S3A) and EtOH/water solution (Figure S3B). The UV/Vis absorption spectra were obtained using a Perkin Elmer Lambda 900 UV/Vis/NIR spectrometer. All spectra were obtained in the wavelength region spanning from 300 to 800 nm, using a 1 nm data interval. For the switching of the spiropyran to the merocyanine form, the solution was irradiated for 30 s with UV light. The UV irradiation source was an array of 9 UV LEDs placed at a distance of 1 cm from the spiropyran solution. The LEDs have an emission wavelength peak at 365 nm and an optical output power of 1.2 mW, supplied by Roithner Lasertechnik Austria. The white light irradiation source used for the switching of MC back to SP form was a LMI-6000 LED 211 Fiber Optic Illuminator obtained from Dolan-Jenner Industries. The irradiation time was also in this case fixed to 30 s.

Changes in the absorbance spectra of the spiropyran microstructures under different illumination conditions were recorded in reflectance mode using two fiber-optic light guides connected to a Miniature Fiber Optic Spectrometer (USB4000 - Ocean Optics) and aligned using an in-house made holder (Figure S1). The in-house-designed holder was fabricated using a 3D printer (Dimension SST 768) in black acrylonitrile butadiene styrene co-polymer (ABS) plastic in order to minimise interferences from ambient light. The two parts of the holder were designed using ProEngineer CAD/CAM software package and fixed together to ensure no interferences from ambient light (Figure S1). The light source was a LS-1 tungsten halogen lamp (white light) obtained from Ocean Optics, Inc. Data from the spectrometer was processed using Spectrasuite software provided by Ocean Optics Inc. For clarity, the absorbance spectra recorded were smoothed using Origin software using Savitzky–Golay algorithm.

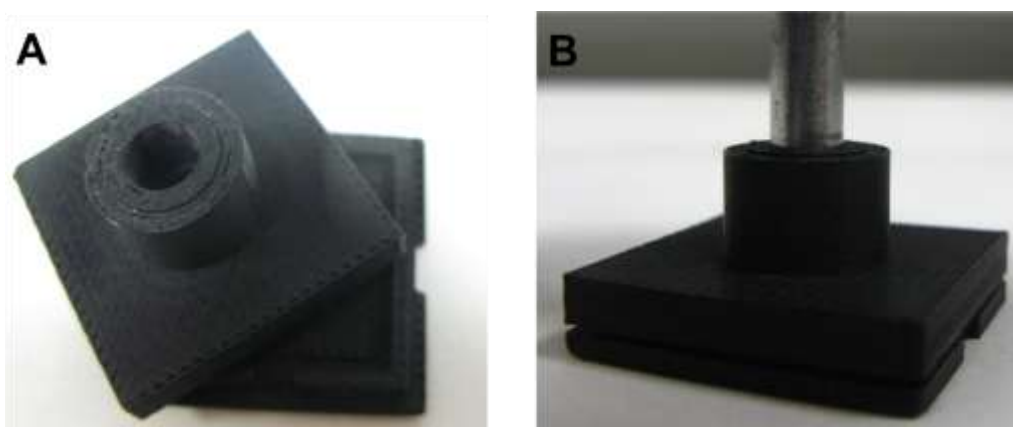


Figure S1. In house designed holder used for absorbance measurements of the SP-COOH microstructures.

FT-IR ATR Spectroscopy

IR spectroscopy was employed to study the chemical structure of SP-COOH microstructures in comparison with SP-COOH obtained throughout the evaporation of a homogeneous EtOH solution (Figure S4). IR spectra were recorded on a Perkin Elmer Spectrum GX FT-IR System® using an ATR unit. 64 scans were performed in the spectral range from 4000 cm⁻¹ to 600 cm⁻¹ with a resolution of 1.00 cm⁻¹.

Microscopy

Microscopy images of the microstructures were performed on an Aigo digital microscope (The Dolomite Centre Ltd) equipped with auxiliary objective to give 60X, 180X or 540X total magnification.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) was performed using a Carl Zeiss EVOLS 15 system at an accelerating voltage of 9 to 11 kV. For the sample preparation, about 10 ml of the SP-COOH EtOH/water dispersion (15%, 20% and 25% EtOH, respectively, prepared as described in the ESI, section 3) were poured in a 5.5 cm OD Petri dish. After exposure to daylight for 2 minutes, microstructures were transferred from the EtOH/water interface to clean silicon wafers, and the residual solvent was left to evaporate at room temperature. Prior to imaging, the microstructures were sputter-coated with a 10 nm Au layer.

Elemental microanalysis

Elemental microanalyses were performed on an Exeter Analytical CE 440 elemental analyser. Three samples of each of the microstructures, SP-COOH 15%, SP-COOH 20% and SP-COOH 25% were analysed. Prior to elemental analysis, the microstructures were filtrated from the rest of the solution through a nylon membrane (pore size = 0.2 μm , Millipore), washed with water and dried under high vacuum for 24 hours to insure they are dried.

2. Synthesis of SP-COOH

The spiroopyran derivative used in this study, namely 11'-(3-carboxypropyl)-3,3'-dimethyl-6-nitrospiro-[2H-1]-benzopyran-2,2'-indoline (SP-COOH), was produced in a three-step sequence as described elsewhere[1]. ^1H NMR (300 MHz, CDCl_3): 1.19 (3H, s, 3'- CH_3), 1.29 (3H, s, 3'- CH_3), 1.95 (2H, m, N- $\text{CH}_2\text{CH}_2\text{CH}_2$ -), 2.41 (2H, t, N- $\text{CH}_2\text{CH}_2\text{CH}_2$ -), 3.22 (2H, m, N- $\text{CH}_2\text{CH}_2\text{CH}_2$ -), 5.88 (1H, d, H-3), 6.62 (1H, d, H-7'), 6.74 (1H, d, H-8), 6.88 (1H, m, H-5'), 6.89 (1H, d, H-4), 7.09 (1H, d, H-4'), 7.19 (1H, t, H-6'), 7.99 (2H, m, H-5 and H-7). Identical ^1H NMR spectra were obtained in the case of all the microstructures.

3. Protocol for the formation of SP-COOH microstructures and equilibrium of SP-COOH derivative in EtOH/water solution

To prepare the microstructures, SP-COOH was dissolved in ethanol (EtOH), which is a good solvent for most benzospiroopyrans, at a concentration of 10^{-3} M. The resulting solution had a strong purple color due to the absorbance of MC centered at 550 nm (Figure S2-A).

To this solution, DI water was added so that the final EtOH volume percent (V%) in the water/EtOH solution was 15% (SP-COOH 15%), 20% (SP-COOH 20%) and 25% (SP-COOH 25%), respectively. After this, the solution was left to rest in the dark for 12 h. Immediately after mixing the two solvents, and following the exothermic reaction (due to mixing of water with EtOH), the solution is clear and has a pale red color. However, over time the solution becomes turbid (Figure S2-B). This phenomenon is due to the formation of MC-COOH individual micro-size structures caused by the poor solubility of MC-COOH in water. The only water-soluble species is $\text{MCH}^+\text{-COO}^-$ formed throughout the partial dissociation of carboxylic acid ($\text{pK}_a \sim 4.9^{[2]}$) in DI water (pH ~ 6) and successive protonation of the SP-COO $^-$ form (typical pK_a for MCH^+ is $\sim 6-7^{[3]}$), see Scheme 1.

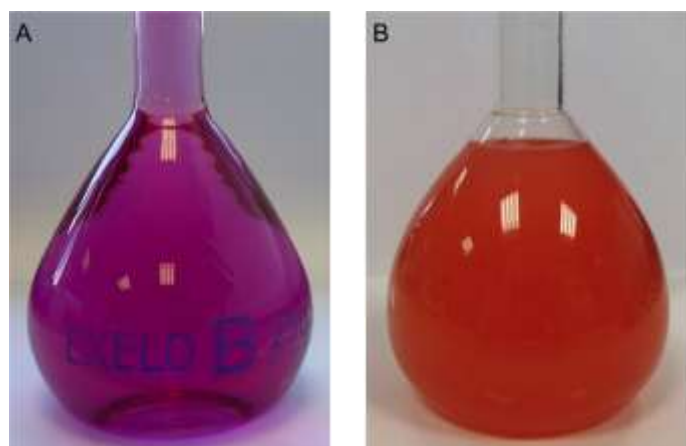


Figure S2. Photos of (A) SP-COOH solution in EtOH (10^{-3} M) in comparison with (B) the same solution after the addition of 75% deionised water, corresponding to SP-COOH 25%. Both solutions were photographed immediately after they were taken from dark.

Figure S2 shows photos of the SP-COOH solution in EtOH (10^{-3} M) in comparison with the same solution after the addition of 75% deionised water (SP-COOH 25%) in the dark. One can observe that while SP-COOH solution in EtOH (10^{-3} M) shows good transparency indicating the full solubilization of SP-COOH in EtOH at 10^{-3} M concentration. The addition of 75% (V%) deionized water causes a change in color due to changes in polarity and increased turbidity due to the formation MC-COOH individual micro-scaled structures caused by the poor solubility of the MC-COOH in water.

UV-Vis Spectroscopy was employed to characterize SP-COOH solution in EtOH (10^{-3} M) and EtOH/water after the removal of the insoluble microstructures in order to indicate the particular species dissolved in the solvent mixture (Figure S3).

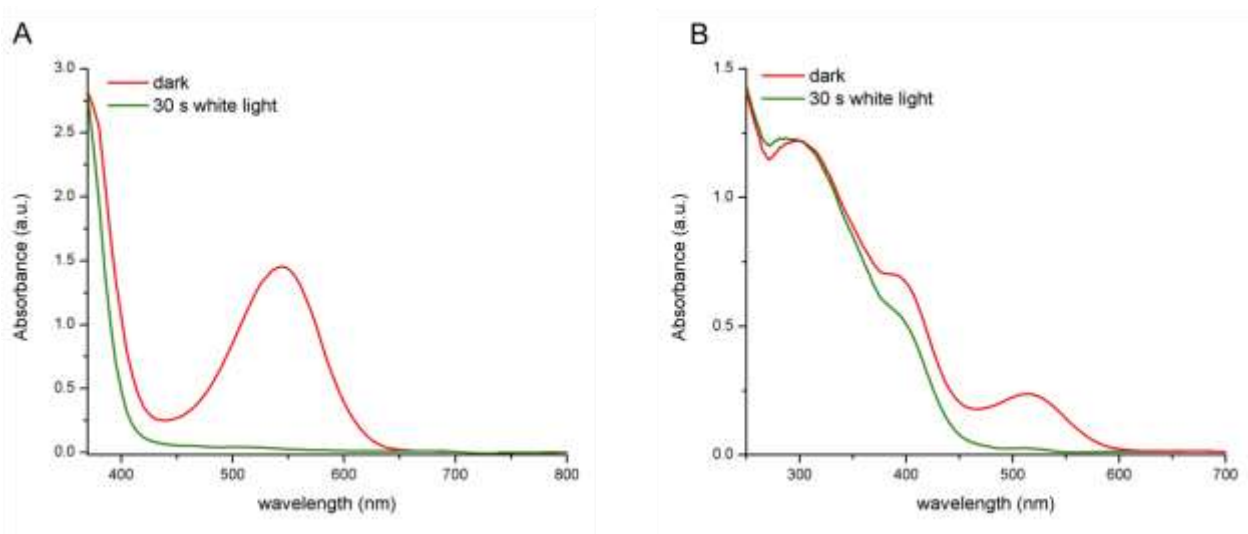
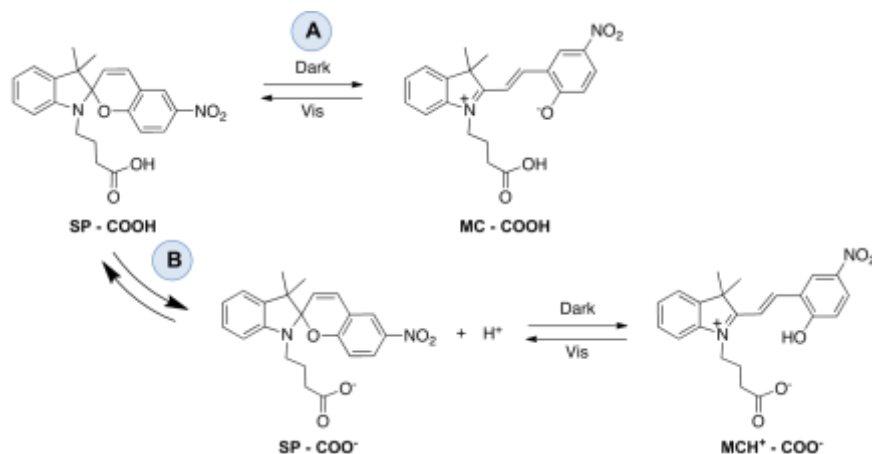


Figure S3. Absorbance spectra of SP-COOH derivative in EtOH (10^{-3} M) (A) and EtOH/water after the removal of the insoluble microstructures (B) under different illumination conditions (dark and 30 s white light irradiation, respectively).

As it can be seen from Figure S3, the MC-COOH form in EtOH shows an absorbance band in the visible region centered around 550 nm. In contrast, the SP-COOH form in EtOH does not show any absorbance band in the visible region.

Mixing of SP-COOH 10^{-3} M solution in EtOH with water (V% of water – 85%, 80% and 75%, respectively) causes the formation of individual MC-COOH microstructures due to decreased solubility of the SP-COOH derivative in the EtOH/water mixture compared to pure EtOH. However, a substantial amount of the spiropyran derivative still remains soluble in the solvent mixture (about 0.11 ± 0.01 mM, $n = 3$, in the case of the 3:1 (V:V) water:EtOH

solution). Scheme S1 shows the possible reversible structural transformations of SP-COOH in EtOH (A) - water (B) solution in response to light. The corresponding absorbance spectra of SP-COOH in EtOH/water solution are shown in Figure S3B. Similar spectra were obtained for all of the solutions studied (filtrates obtained from SP-COOH 15%, SP-COOH 20% and SP-COOH 25%, respectively) with the intuitive observation that as the V% of water increases, the absorbance band corresponding to the MC-COOH form decreases. Figure S3B shows a representative example of SP-COOH 25% after the removal of the microstructures by filtration.



Scheme S1. Reversible structural transformations of SP-COOH EtOH (A) / water (B) solution in response to light.

As seen from Figure S3B, the solution of SP-COOH in 25% EtOH/75% water taken from the dark shows three main absorbance bands. The first absorption band (300 nm) is characteristic of the SP-COOH form (Scheme S1). The band centered on 515 nm is indicative of the MC-COOH form. This absorption decreases upon irradiation with white light due to switching of the MC-COOH back to the SP-COOH form (Scheme S1A). The absorbance band centered around 490 nm is associated with MCH⁺-COO⁻, which is formed through the protonation of SP-COO⁻ by the H⁺ coming from the partial dissociation of the carboxylic acid group in water (Scheme 1B). This band also decreases upon irradiation with white light due to the formation of SP-COO⁻.

Comparable microstructures, dimensions, shape, and behavior (*e.g.* aggregation at the liquid-air interface), were obtained when other solvents with similar characteristics to ethanol, *e.g.* methanol or isopropanol, were used.

4. FT-IR spectroscopy of SP-COOH

IR spectroscopy was employed to compare the chemical structure of these microstructures with MC-COOH obtained through the evaporation of a homogeneous EtOH solution (Figure S4). IR spectra were recorded on a Perkin Elmer Spectrum GX FT-IR System® using an ATR unit. 64 scans were performed in the spectral range from 4000 cm⁻¹ to 600 cm⁻¹ with a resolution of 1.00 cm⁻¹. The typical FT-IR bands and their assignments are shown in Table S1[4, 5]. As can be seen, identical spectral features are apparent between the FT-IR spectrum of the solid SP-COOH obtained after evaporation under UV light of a homogeneous EtOH solution, and that of the solid SP-COOH (15%) microstructures under similar illumination conditions, which supports the interpretation that the microstructures are produced in their merocyanine form.

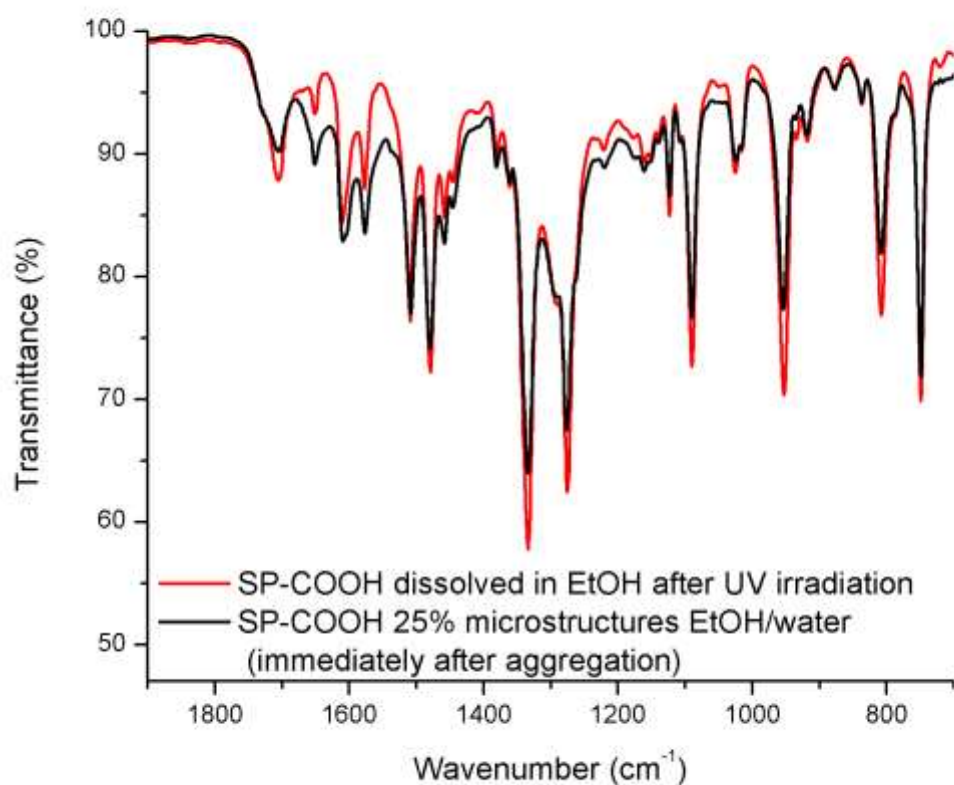


Figure S4. FT-IR spectra of MC-COOH obtained from an EtOH solution under UV irradiation and MC-COOH self-assembled microstructures obtained from EtOH/water mixture – immediately after aggregation.

Table S1. Important FT-IR frequencies for SP-COOH from solution and SP-COOH self-assembled microstructures and their assignments.

Assignment	MC-COOH from EtOH	Self-assembled Microstructures
	Wavenumber (cm ⁻¹)	Wavenumber (cm ⁻¹)
C=O	1705	1705
C=C stretch	1609	1609
C=N ⁺	1577	1576
NO ₂ sym stretch	1509	1508
C-C aromatic ring stretch	1479, 1458	1479, 1457
C-O ⁻	1445	1445
NO ₂ asym stretch	1334	1334
C-O-C ether sym stretch	1275	1276
C-O ester stretch	1161	1161
C-O ester stretch	1090	1090
C-C-N bend	1025	1024
O-C-N stretch, C=CH; CH out of plane deformation (<i>cis</i>)	953	954
C-H bending	809	808
C-H bending	749	749
C-H bending	688	688

5. Micro-elemental analysis of SP-COOH microstructures

Microanalysis of the dried microstructures have show that all the microstructures – SP-COOH 15%, SP-COOH 20% and SP-COOH 25%, respectively, have very similar % content of C, H and N, indicating no significant composition differences between the different structures.

Microstructures	C %	H %	N %
SP-COOH 15%	65.875 ± 0.055 , n=3	5.475 ± 0.055 , n=3	6.785 ± 0.045 , n=3
SP-COOH 20%	66.15 ± 0.073 , n=3	5.50 ± 0.070 , n=3	6.850 ± 0.070 , n=3
SP-COOH 25%	66.14 ± 0.02 , n=3	5.545 ± 0.045 , n=3	6.79 ± 0.05 , n=3

6. Interfacial self-assembly

Interfacial self-assembly at the liquid/air interface was followed using an Aigo digital microscope (The Dolomite Centre Ltd.) at a 60X magnification. A representative example is given in Figure S5 for SP-COOH 25%.

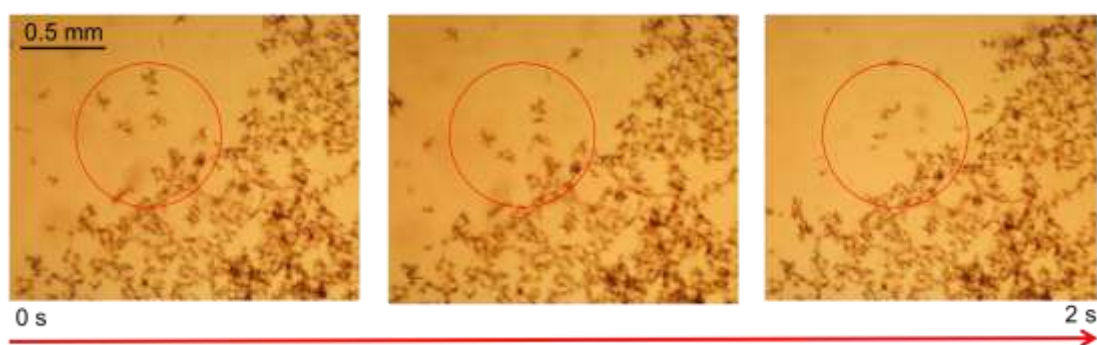


Figure S5. Sequence of microscopy images showing the self-assembly of SP-COOH 25% microstructures into a single film at the liquid/air interface. For the full video please refer to Video S1.

Pictures of the interfacial self-assembly of SP-COOH 15% and 25% are shown in Figure S6 and S7.

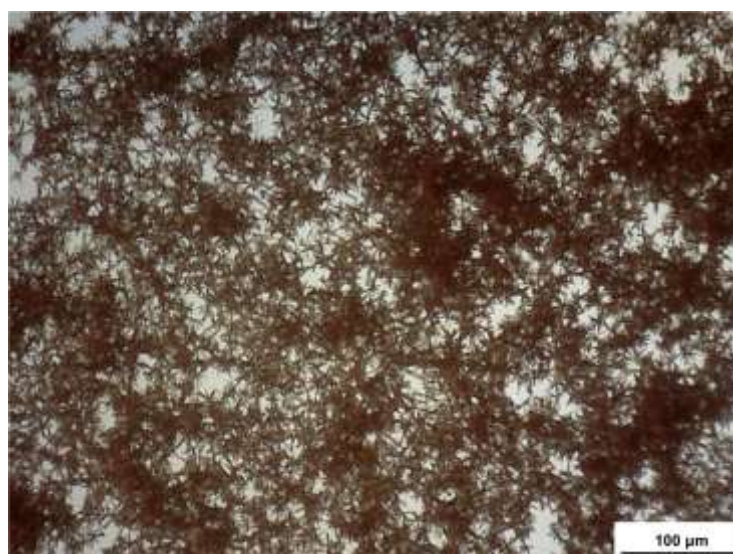


Figure S6. Interfacial agglomeration of SP-COOH 15%.



Figure S7. Interfacial agglomeration of SP-COOH25%.

7. Scanning Electron Microscopy

Scanning Electron Microscopy was used to image the self-assembled microstructures corresponding for SP-COOH 25% (Figure S8), SP-COOH 20% (Figure S9) and SP-COOH 15% (Figure S10). The imaging was performed on a Carl Zeiss EVOLS 15 system at an accelerating voltage of ~ 10 kV. Prior to imaging the samples were coated with a 10 nm layer gold.

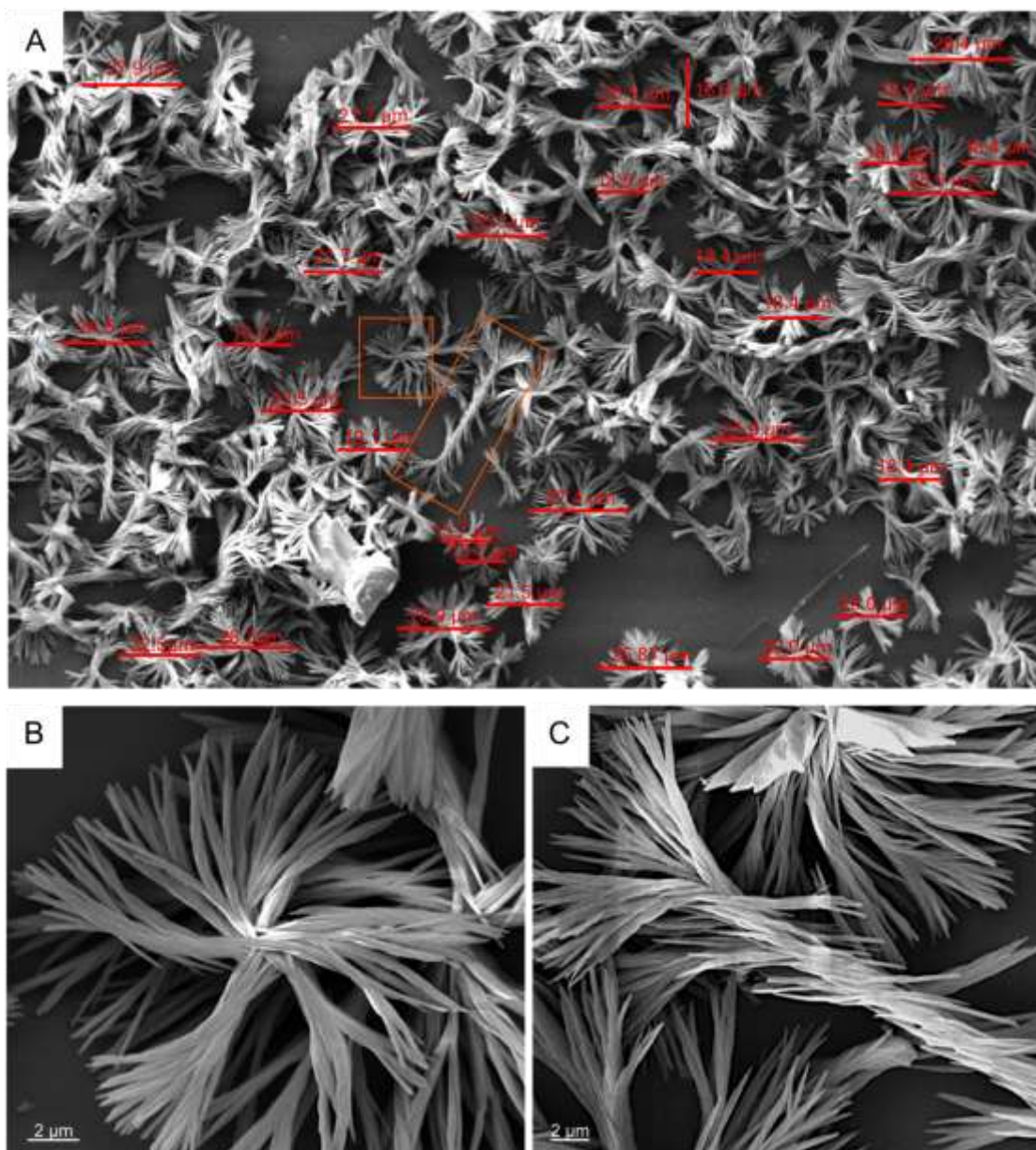


Figure S8. Self-assembled micro-structures of SP-COOH 15%.

As seen in Figure S8, the assembled microstructures presented diameters ranging from 14 μm to 27 μm . The majority of the structures showed a ribbon-like appearance assembled in a daisy-like structure (Figure S8, B) although few elongated structures (Figure S8, C) were also observed.

Daisy-like structures obtained from SP-COOH 20% had diameters ranging from 34 μm to 53 μm (Figure S9).

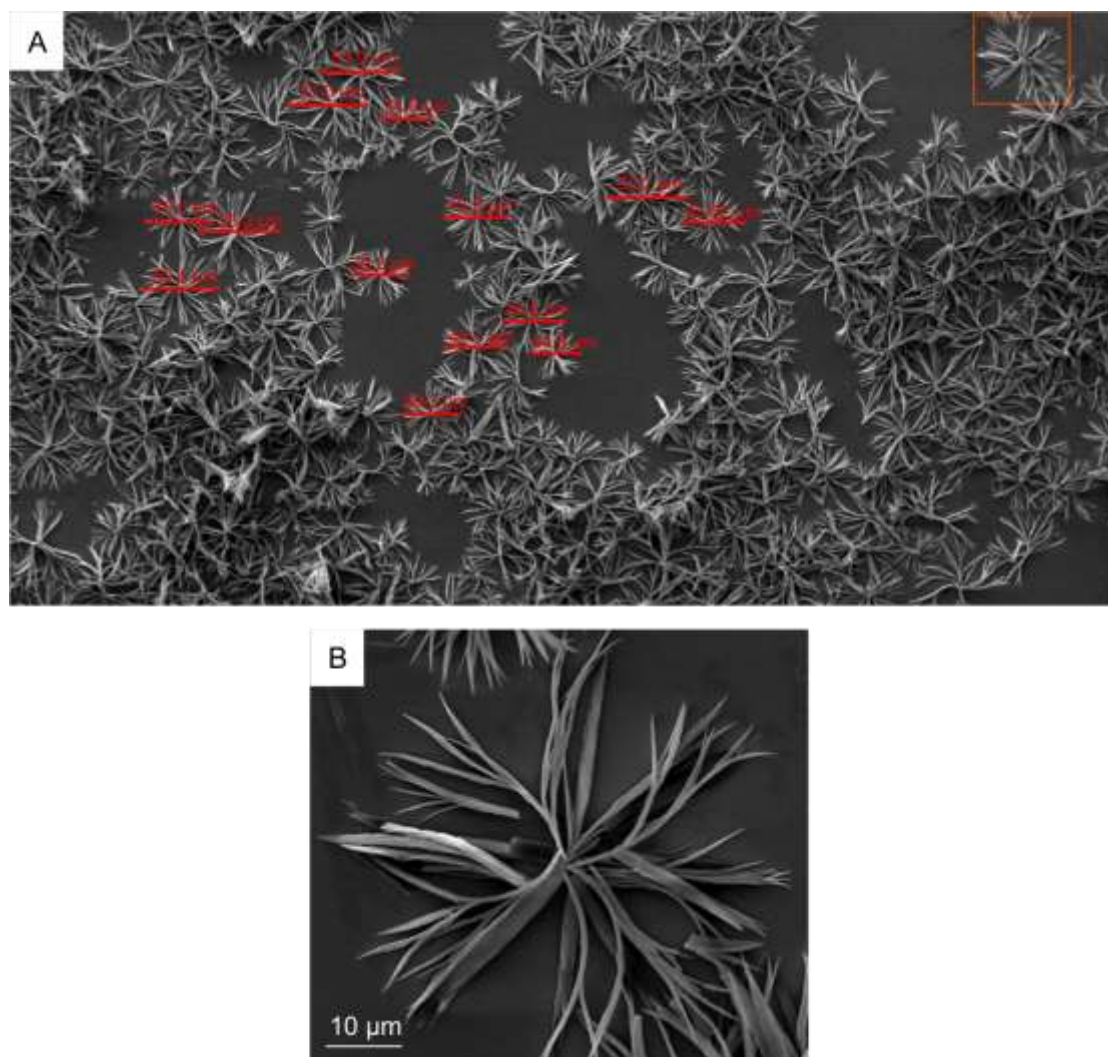


Figure S9. Self-assembled microstructures of SP-COOH 20%.

Daisy-like structures formed from flat crystals obtained using SP-COOH 25%. The diameter of these structures varied from 65 μm to 78 μm , although smaller structures or larger single unit crystals were also observed (Figure S10).

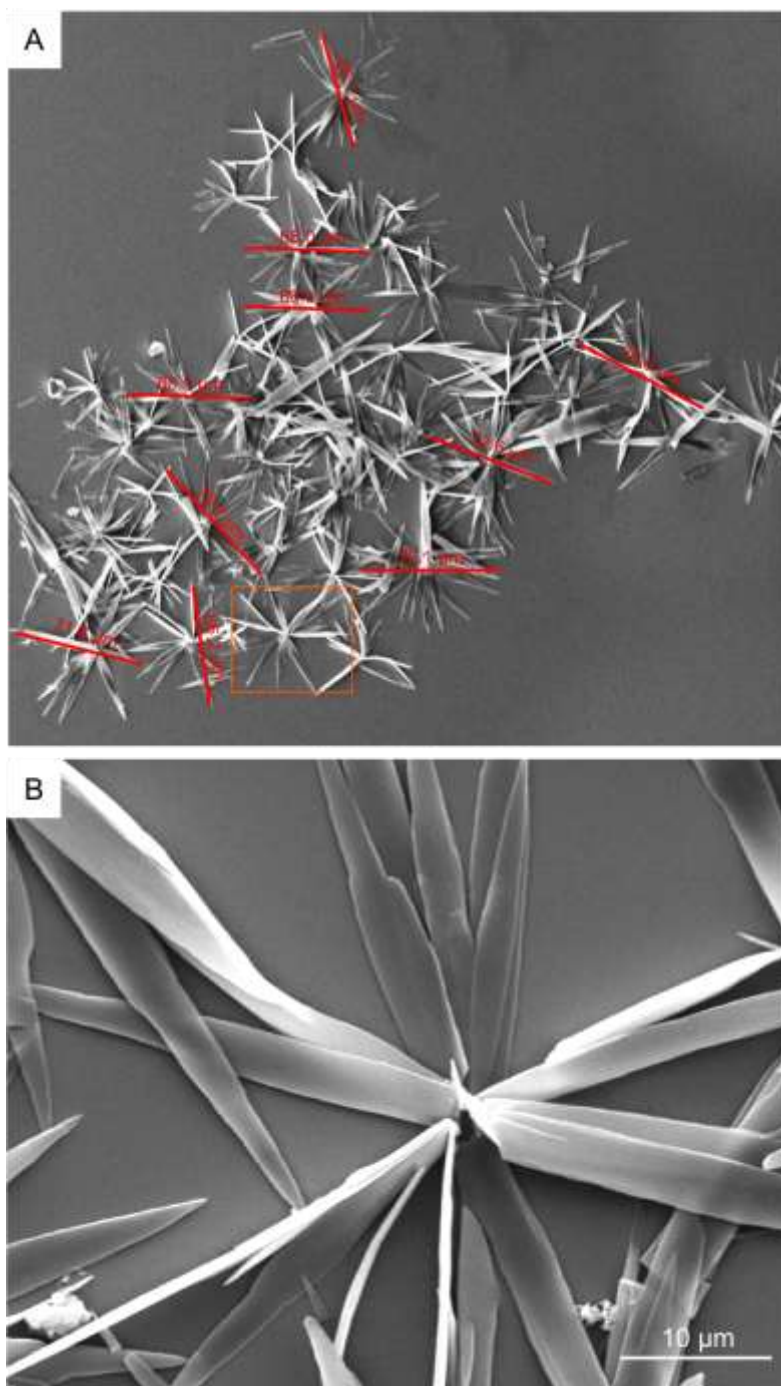


Figure S10. Self-assembled microstructures of SP-COOH 25%.

8. Absorbance spectra of SP-COOH microstructures

UV-Vis spectroscopy was used to study the photochromic behavior of spiropyran microstructures (SP-COOH 15%, SP-COOH 20% and SP-COOH 25%). The absorbance spectra were recorded in reflectance mode using a fiber-optic light guide connected to a Miniature Fiber Optic Spectrometer (USB4000 – Ocean Optics) and a specially designed probe holder. The light source was a LS-1 tungsten halogen lamp (white light) obtained from Ocean Optics, Inc. Data from the spectrometer was processed using Spectrasuite software provided by Ocean Optics Inc. For clarity, the absorbance spectra recorded were smoothed using Origin software using Savitzky-Golay algorithm. Photoconversion of the microstructures from SP to MC form was achieved using an in-house fabricated UV light source consisting of three UV LEDs (Roithner LaserTechnik GmbH, emission $\lambda_{\text{max}} = 375 \text{ nm}$). The microstructures were placed 2 cm from the source and irradiated at a power of 0.4 mW cm^{-2} for 1 min. The white light irradiation was provided via a LMI-6000 LED Fiber Optic Illuminator obtained from Dolan-Jenner Industries and was used to switch the MC form back to the closed SP. The photo-conversion was observed both at the liquid/air interface (Figure S11), and in the solid state (Figure S12).



Figure S11. Sequence of photos showing the formation of the self-assembled aggregates at the liquid air/interface and their switching between the SP and MC form.

As can be seen from Figure S12, immediately after aggregation at the liquid air interface, reddish microstructures form, which are composed of mixture of protonated merocyanine and merocyanine, as indicated by the absorbance bands centered at 430 nm and 550 nm, respectively. The higher the water content in the water-ethanol mixture, the more protonated merocyanine is presented, due to the equilibrium of SP-COOH in water, Scheme S1-A. Aggregate of the microstructures occurs when they are in the merocyanine forms (MC and MC-H⁺), supporting the interpretation that the aggregation mechanism is driven by the polar nature of merocyanine, and its well-known ability to form aggregates. However, once formed, the microstructures can be switched reversibly between spiropyran predominant and (Figure S12) and merocyanine predominant (Figure S12) compositions, when irradiated with white light, and UV light, respectively.

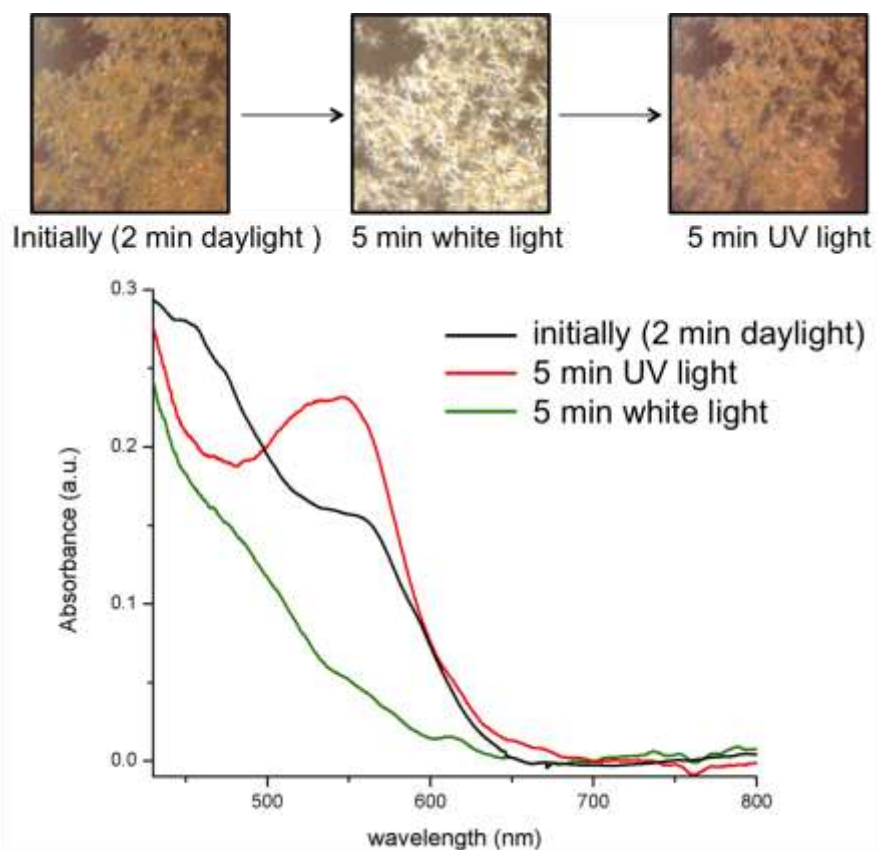


Figure S12. Absorbance spectra and photos of SP-COOH 25% self-assembled microstructures under different illumination conditions.

9. Microscopy imaging of SP-COOH 25% microstructures under cross polarizers.

Spontaneously forming SP-COOH 25% assemblies showed a bright image under the microscope with crossed polarizers (Figure S13).

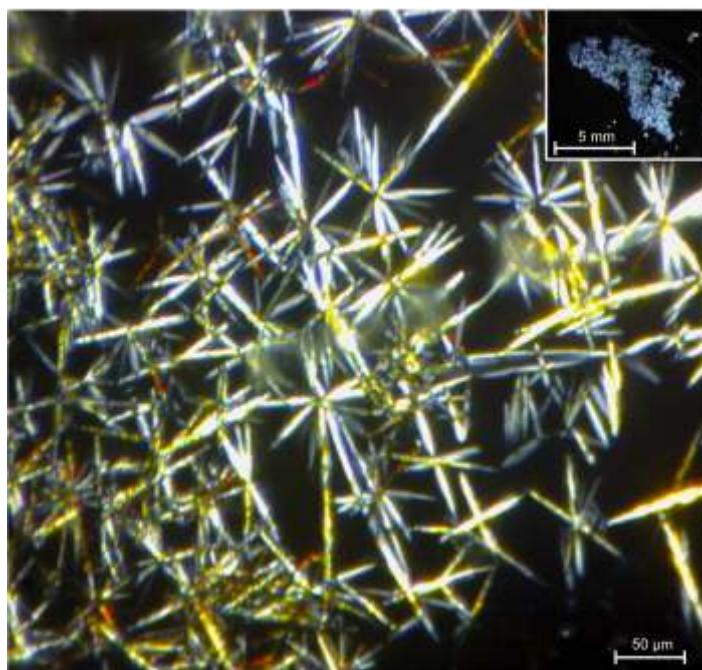


Figure S13. Microscopy image of SP-COOH 25% under crossed polarizers.

10. Guided interfacial aggregation

As the assembly mechanism of the microstructures towards the liquid/air interface is driven by the evaporation of EtOH, guided evaporation was achieved throughout the use of a 150 W DDL Halogen lamp obtained from Polytec GmbH Waldbronn, Germany. The lamp was placed at a distance of 4 cm from one side of the Petri dish. The process is shown in Video S2. The microstructures form immediately at the interface and they are moving towards the illuminated area. In time, new assemblies of various sizes are formed that will start to migrate in the same direction. The video is presented in real time, showing the fast movement of the structures towards the light. Because of this behavior of the SP-COOH 25% crystals, we call them “crystal moths”.

Temperature influences the rate of aggregation since it has an effect on the rate of ethanol evaporation. Furthermore, elevated temperatures (< 50 °C) dramatically change the equilibrium of the solution in favor of merocyanine formation, which greatly improves the stability of the dispersion, and inhibits microstructure aggregation.

11. References

1. Rosario, R., et al., *Photon-modulated wettability changes on spiropyran-coated surfaces*. Langmuir, 2002. **18**(21): p. 8062-8069.
2. Bacarella, A.L., et al., *The potentiometric measurement of acid dissociation constants and pH in the system methanol-water - pKa values for carboxylic acids and anilinium ions*. Journal of Organic Chemistry, 1955. **20**(6): p. 747-762.
3. Sumaru, K., et al., *Characteristic phase transition of aqueous solution of poly(N-isopropylacrylamide) functionalized with spirobenzopyran*. Macromolecules, 2004. **37**(13): p. 4949-4955.
4. Delgado-Macuil, R., et al., *ATR spectroscopy applied to photochromic polymer analysis*. Materials Characterization, 2007. **58**(8-9): p. 771-775.
5. Fries, K.H., et al., *Spectroscopic Analysis of Metal Ion Binding in Spiropyran Containing Copolymer Thin Films*. Analytical Chemistry, 2010. **82**(8): p. 3306-3314.