Supplementary Information (SI)

From Lyotropic to Thermotropic Behavior: Solvent-Free Liquid Crystalline Phases in Polymer-Surfactant-Conjugated Rod-shaped Colloidal Viruses

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1. Materials and Methods

1.1 Materials

Glycolic acid lauryl ether ethoxylate (Average Mn~ 690 g mol^{-1}, Polymer Surfactant: PS, 463256), 1-Ethyl-3-(3-dimethyl aminopropyl carbodiimide), ≥ 97 % (EDC) were all purchased from Sigma-Aldrich (India) and used as received. N-Hydroxysuccinimide ≥ 97% (NHS), anhydrous Dichloromethane (DCM), anhydrous Methanol, anhydrous (DMSO), Potassium Di -Hydrogen Phosphate (KH_2PO_4), Di-Potassium Hydrogen phosphate (K_2HPO_4) all with purity ≥ 97% were purchased from the SRL chemicals (Mumbai, India) and used as received. MWCO (10 KDa) cut-off Snakeskin dialysis tube brought from Thermo-fisher Scientific (India), and for all the experiments, we used the Milli-Q water.

1.2 Synthesis of NHS functionalized Polymer Surfactants (PS-NHS)

In a dry Schlenk flask, 1 g of Polymer surfactant (PS) was dissolved in 9.5 mL of anhydrous dichloromethane (DCM) containing 0.5 mL of anhydrous methanol (co-solvent). The solution was cooled in an ice-water bath under a nitrogen environment for 15 minutes with constant stirring (300 rpm). Later, 0.234 g of NHS (0.2 M) was added to the above solution, followed by the addition of 0.389 g of EDC (0.2 M), and the reaction was carried out under constant stirring in an inert environment. The crude product was filtered to remove solid impurities. The crude sample was purified by recrystallisation. The sample with a minimal amount of DCM was poured into 50 ml of cold diethyl ether, stirred for 10 minutes, and kept at -20 °C for 6 hours. The residue was collected by decantation. This procedure was repeated twice to remove unreacted reactants. Then the product was vacuum dried and stored at -20 °C under N_2 environment. The NHS functionalization characterized by ^{1}H and ^{13}C NMR **(Figures S1 and S2)**, ATR-FTIR **(Figure S3)** and UV absorption[1] **(Figure S4)** methods.

1.3 Determination of PS-NHS functionalization

For the characterization of the functionalization of NHS with polymers, the UV measurements were carried out at 260 nm.[1] For the blank analysis, PS and PS-NHS were dissolved in methanol (5 mg mL^{-1}). Each 100 µL of the above solutions was thoroughly mixed with 900 µL of 0.1 M NH_4OH separately and kept reaction aside for 20 minutes, followed by the UV measurement. PS with NHS functionalization showed the absorbance peak at 260 nm (ε = 9700 cm^{-1} M^{-1}).

1.4 Bioconjugation of *fd* bacteriophages

The *fd* virus was dispersed in phosphate buffer (100 mM, pH 7.8) to form a suspension with a final concentration of 1 mg mL^{-1}. 10 mL of the above suspension was taken in an ice-water bath, and then PS-NHS dissolved in 0.2 mL of anhydrous DMSO was added slowly to the above *fd* virus suspension. The molar ratio 1:10 (virus to polymer) was maintained to obtain the maximum grafting density. The reaction was allowed to continue for 4 hours at 4 °C. The excess of free polymers was removed by dialysis against the phosphate buffer (10 mM, pH 7.8) in a snakeskin dialysis tubing of MWCO (10K). The dialysis was done for three days with repeated buffer changes every 12 hours. The bio-conjugated samples were lyophilized for 48 hours to get waterless bio-conjugates (PS-*fd*). The PS-*fd* samples were annealed at 60 °C for 15 minutes, resulting in a highly viscous liquid-like material.

1.5 Estimation of grafting density of polymers on the surface of the *fd* bacteriophage

The different n-*fd* and PS-*fd* sample concentrations were determined by measuring absorbance at 269 nm (*fd* gives an absorbance peak at 269 nm; however, the polymer does not absorb at this wavelength). The polymer concentrations were determined by weighing the samples. The refractive index of the different concentrations of n-*fd*, PS-*fd* and polymer samples was measured at a fixed wavelength (λ) of 589.3 nm using an Anton Paar refractometer Abbemat 350. The *dn/dc* values were obtained from the concentration slope versus the increase

in the refractive index plot. The number of polymers conjugated per virus particle calculated using the formula:[2,3]

$$N = \frac{(dn/dc)_{PS-fd} - (dn/dc)_{fd}}{(dn/dc)_{PS-NHS}} \times \frac{Mw_{fd}}{Mw_{PS-NHS}} \quad (1)$$

2. Characterization

2.1 UV-visible spectroscopy

The UV-visible measurement was performed on a Cary 100 (Agilent, USA). The absorption was measured at 269 nm for native *fd* (n-*fd*) and bio-conjugated *fd* (PS-*fd*) samples (molar absorption coefficient 3.84 mg mL^{-1}) for concentration measurements. The blank measurement was carried out using the phosphate buffer (100 mM, pH 7.8). The 10 µL of centrifuged samples (n-*fd* or PS-*fd*) was mixed with 990 µL of phosphate buffer taken in the 1 mL quartz cuvettes and UV measured at 269 nm, and the concentration was determined using Beer's Lambert's law.

2.2 Nuclear Magnetic Resonance (NMR) spectroscopy

The characterization of end functionalization of polymer surfactant was carried out using ^{1}H and ^{13}C NMR spectroscopy. For this purpose, the Bruker 500 MHz NMR instrument was used. Approximately 5-10 mg of samples dissolved in a small amount of deuterated chloroform taken in the NMR tube. After the measurements for ^{1}H and ^{13}C NMR studies, data was processed using Bruker Topspin software.

2.3 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (Bruker Alpha) was used for the functional group analysis of PS-NHS and to confirm the α-helicity of the n-*fd* and PS-*fd*. For the measurements, around 4-5 mg of the lyophilized samples (PS, NHS, PS-NHS, n-*fd*, and PS-*fd*) were placed in the sample chamber and measured in the range of 500 cm^{-1} to 4000 cm^{-1}.

2.4 Differential Refractive Index (*dn/dc*) experiments

The differential refractive index increments experiments were carried out on an Anton Par Abbe 350 refractometer, with a refractive index resolution of ($\pm 10^{-5}$) and an accuracy of ($\pm 10^{-4}$). The measurements were done at a wavelength of 589.3 nm. For performing the experiments, all the samples were prepared in Milli-Q water.

2.5 Zeta potential measurements

The zeta potential measurements were done in the Omega quartz cuvettes. 350 µL of n-*fd* and PS-*fd* sample each in phosphate buffer (100 mM, pH = 7.8) was taken in the Omega cuvettes and were measured at 25 °C.

2.6 Differential Scanning Calorimetry (DSC)

The DSC experiments were carried out to obtain the phase transition temperatures in the PS-*fd*, PS-NHS and PS samples. The Perkin Elmer DSC 6 instrument was used to perform the experiments. About 4-5 mg of the lyophilized PS-*fd* samples were sealed in a standard aluminium pan for the measurements, and the sealed empty aluminium pan was used as a reference; the DSC experiments were done in the range of -30 °C to 40 °C at a rate of 10 °C min^{-1} under N_2 environment. The measurements were carried out over three cycles to avoid the hysteresis effects.

2.7 Rheology

All the rheology experiments on PS-*fd* were carried out using the MCR 102 rheometer (Anton Paar) with a cone plate geometry (CP 25 mm). The temperature-dependent viscosity measurements were performed in the range of 18–80 ℃ at a constant shear rate of 15 s^{-1}. The amplitude sweep experiment was performed in the range of 0.01 to 5 shear strain (%) at a constant temperature of 25 ℃ and angular frequency of 10 rad s^{-1}. The linear visco-elastic region was found to be around 0.01 to 0.1 shear strain (%). The frequency sweep experiment was performed in the range of 0.1 to 100 rad s^{-1} in the linear visco-elastic region.

2.8 Small Angle X-ray Scattering (SAXS)

The small-angle X-ray scattering experiments were implemented to obtain the changes in the microstructure of the samples with respect to the thermal transition changes. The Xenocs Xeuss 2.0 instrument was used for the measurements, and the multi-temperature experiments were performed using Linkam TST250V (-30 °C to +150 °C). The sample to detector distance was maintained at 1500 mm for the SAXS measurements and 350 mm for the WAXS measurements. The Copper anode is used as an X-ray source (Cu-Kα wavelength 1.54 Å). The silver behenate used to calibrate the sample to detector distances. For the temperature-dependent SAXS/WAXS measurements, a small mass of lyophilized samples of PS, PS-NHS and PS-*fd* were sealed in a gel sample holder using Kapton sheets. The temperature-dependent measurements were carried in the range of 0 °C to 50 °C (equilibration time of 10 minutes at each temperature) with the exposure time of 15 minutes. The background subtraction was done using the empty Kapton sheets. All the experiments were carried out under a vacuum and measured with the absolute intensity parameters. The 2D data collected in the Eiger R 1M detector was Fourier transformed to 1D data using Foxtrot software provided by Xenocs.

2.9 Temperature-Dependent Polarized Optical Microscopy (POM)

Olympus polarizing microscope (BX-51) equipped with a Linkam heating stage (LTS 350 or THM 600) and a color camera (JAI, CV-M7) was used for optical characterizations. For the POM studies, a small drop of PS-*fd* sample was taken on a glass slide and covered with a coverslip. The temperature-dependent optical microscopy studies were carried out with repeated heating and cooling from 0 °C to 40 °C at a rate of 10 °C per minute (**Figure S7**), and all the samples were equilibrated for 10 minutes each before taking the images. The cooling experiments below room temperature were carried out using liquid nitrogen as a coolant.

Figures:

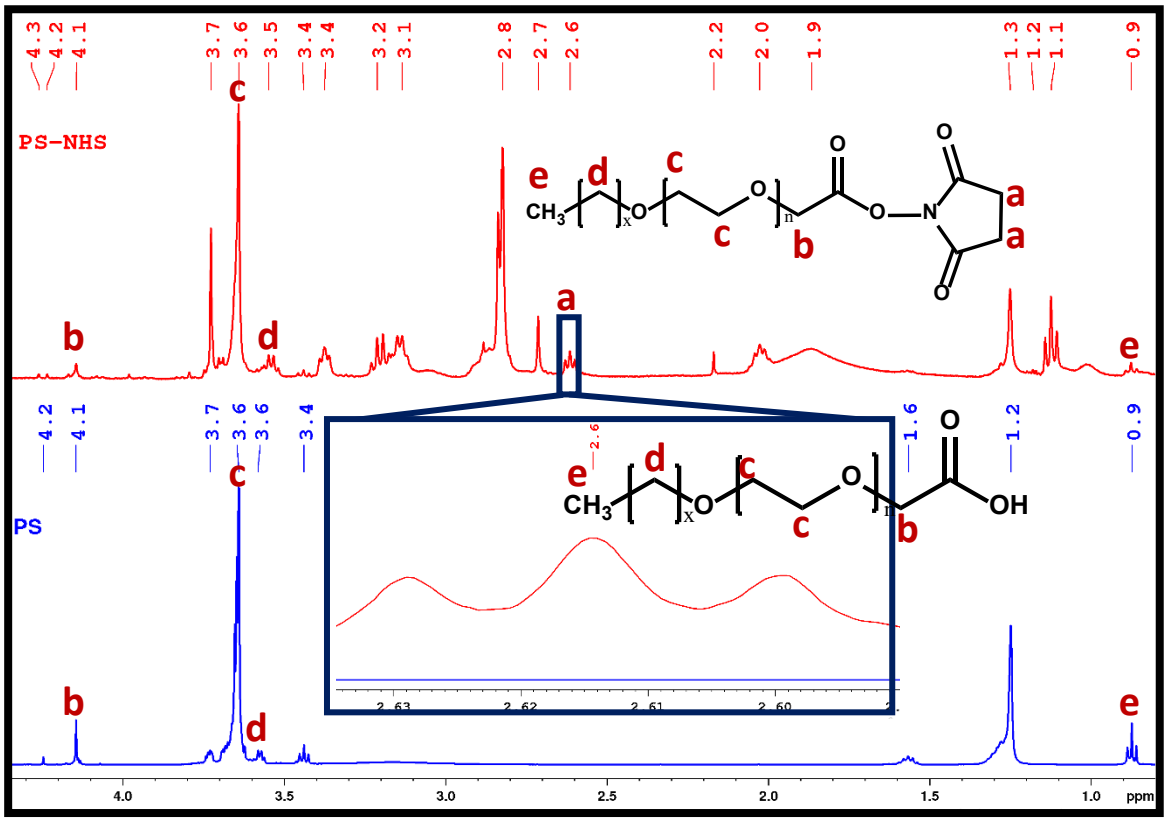

Figure S1: Comparison of ^{1}H NMR of PS and PS-NHS. The PS-NHS shows the characteristic chemical shift value of 2.6 ppm (triplet) for the -CH_2 group of the NHS.

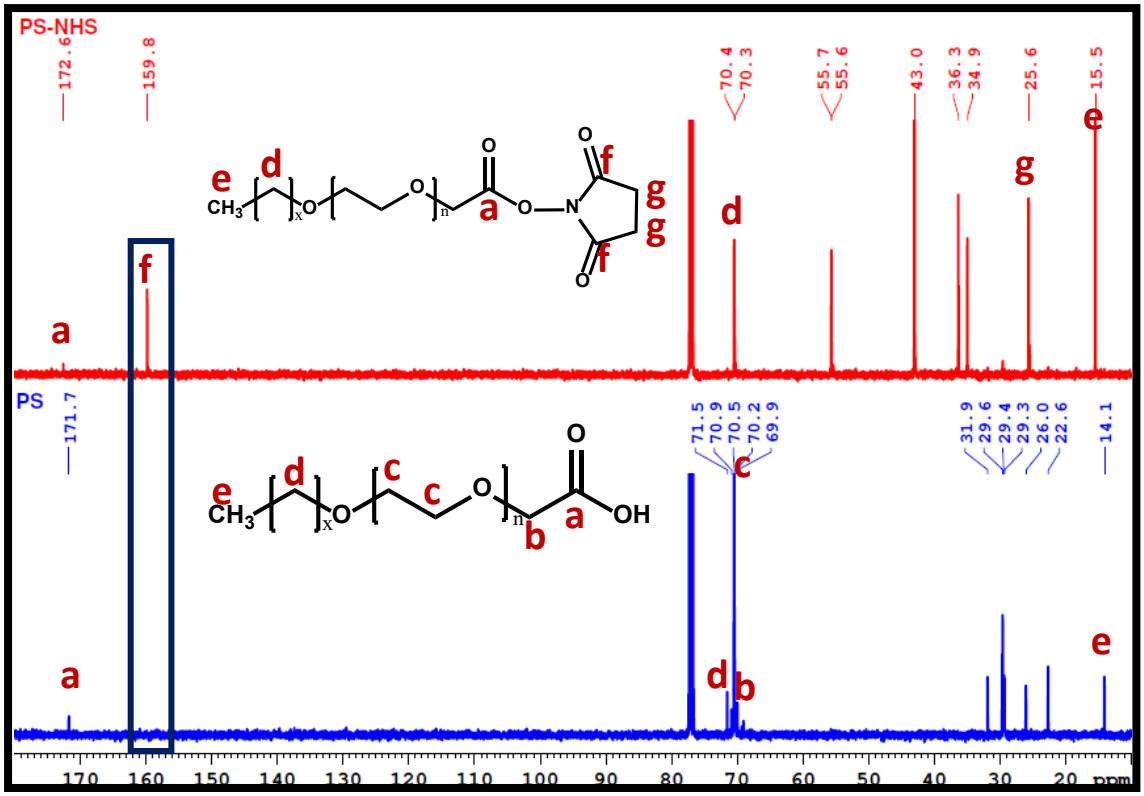

Figure S2: ^{13}C NMR Data of PS-NHS showing the chemical shift values for carbonyl (C=O) groups at 171.8 ppm and 159.9 ppm, chemical shift value at 25.6 ppm for the -CH_2 group of NHS and chemical shift value at the 172 ppm for the carbonyl group of the PS.

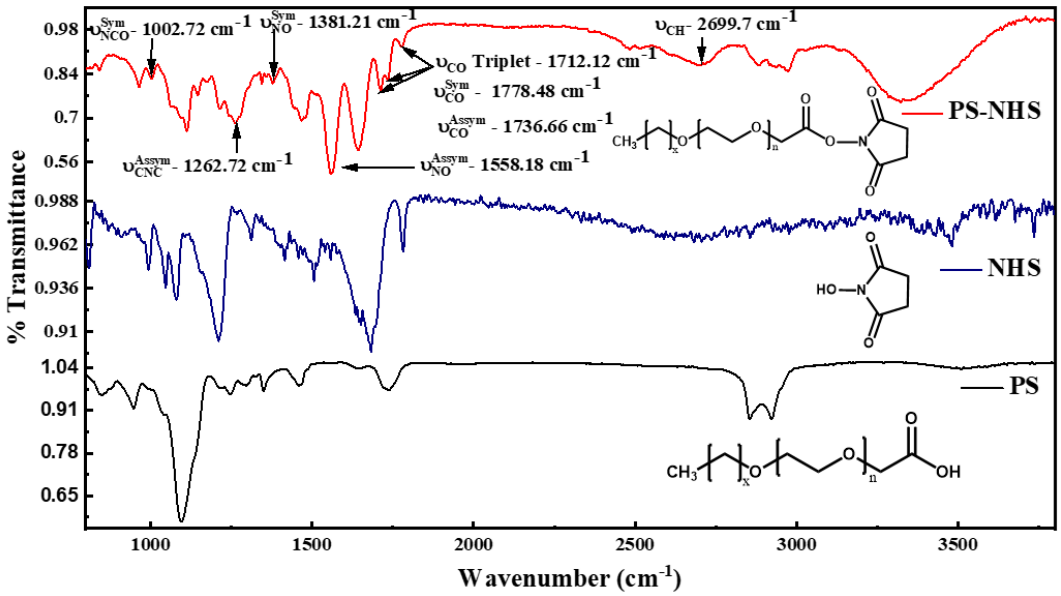

Figure S3: ATR-FTIR data of PS, NHS and PS-NHS showing IR frequencies of different functional groups. The PS-NHS shows the N-O stretching frequencies at 1558 cm^{-1} and 1381 cm^{-1} and C=O stretching frequencies at 1778 cm^{-1} and 1736 cm^{-1}.

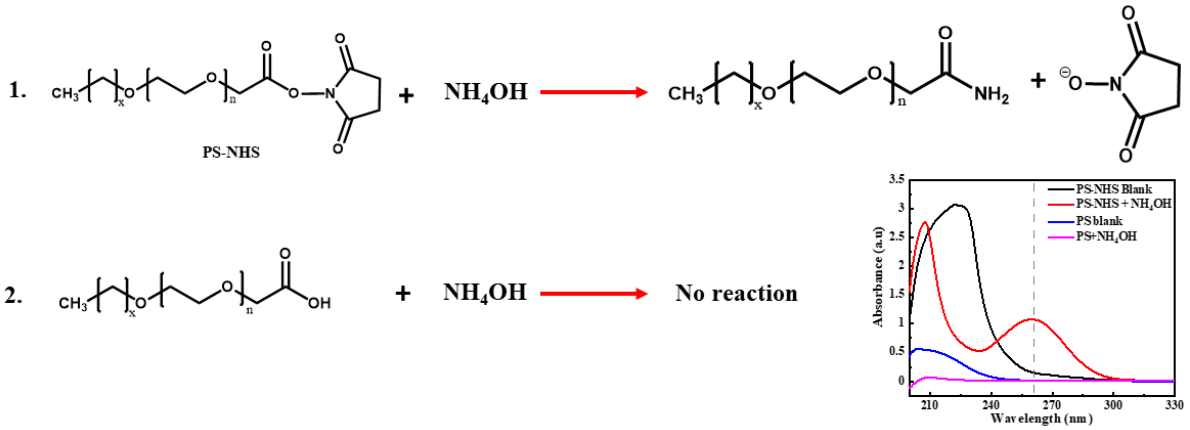

Figure S4: Determination of the functionalisation of NHS onto the PS by esterification reaction. The UV-visible spectra show the absorbance peak at 260 nm for the product formed after reaction with 0.1 M ammonia for the PS-NHS, whereas the 260 nm peak was absent for the control experiment (i.e., for the PS reacting with the ammonia).

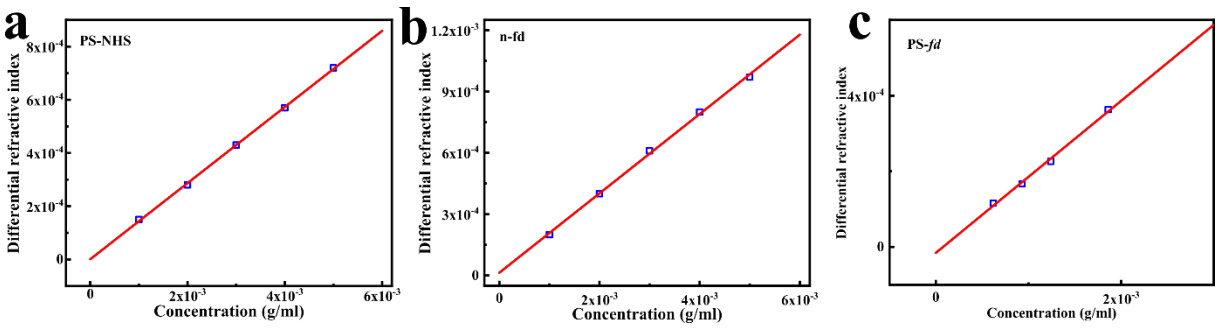

Figure S5: (a) Increment in refractive index plots showing *dn/dc* values for **(a)** PS-NHS - 0.143 **(b)** n-*fd*- 0.194, **(c)** PS-*fd* - 0.201.

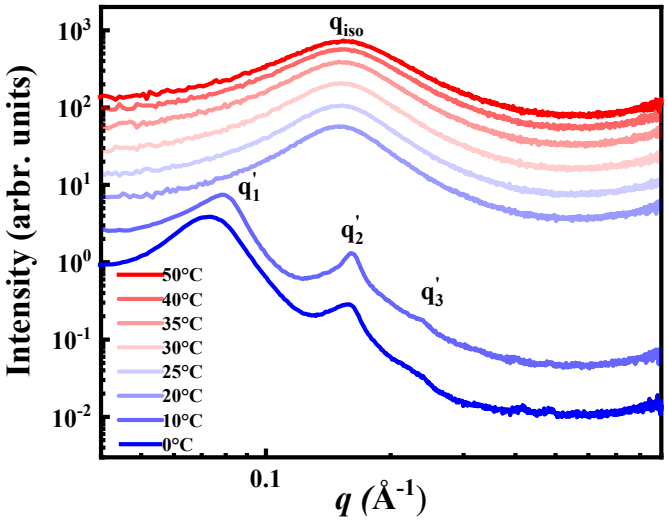

Figure S6: SAXS patterns of pure PS (melting transition temperature, T_m = 16.4 °C). During cooling, the SAXS profiles exhibit a broad peak at $q \approx 0.15$ Å^{-1} above the melting transition, indicative of a disordered or isotropic state. Below $T < T_m$, multiple sharp peaks appear at $q \approx$ 0.079, 0.160, 0.236 Å^{-1}, corresponding to a main d-spacing of 7.9 nm. The relative positions of these peaks exhibit a 1:2:3 ratio with respect to the first peak at q ≈ 0.079 Å^{-1}, which is characteristic of a lamellar structure.

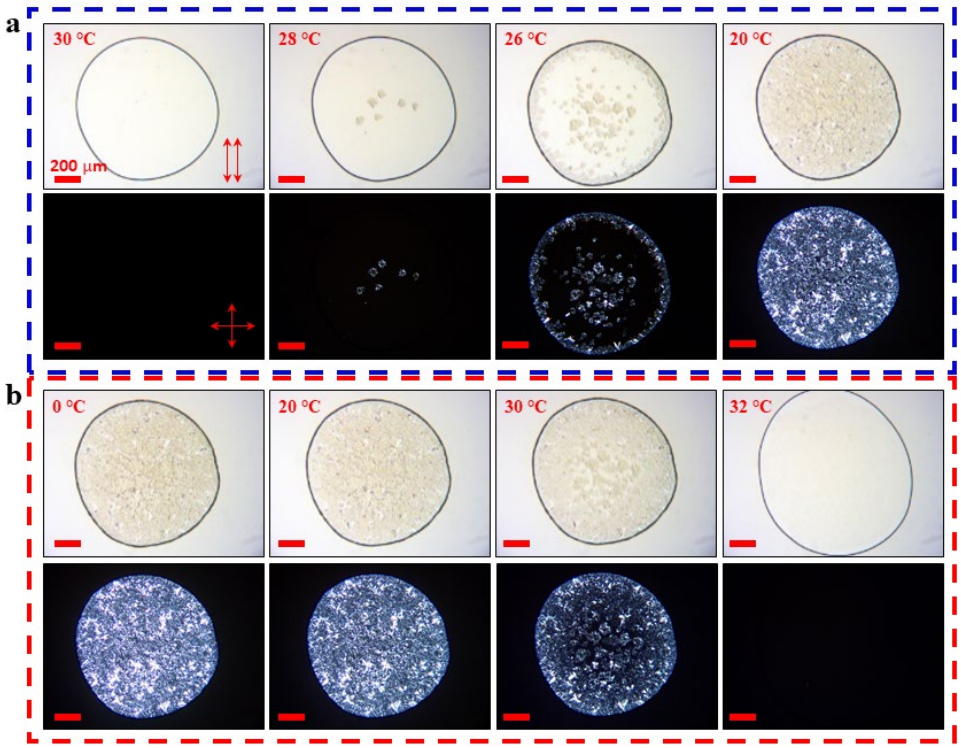

Figure S7: Parallel and cross-polarized optical micrographs of solvent-free PS-*fd* during **(a)** cooling and **(b)** heating cycles (10°C min^{-1}). POM images reveal birefringence arising from the formation of fan-shaped textures below the melting transition temperature T_m (25-30 °C). During cooling, nucleation and growth of the ordered structures begin around 28 °C. Upon heating, the disappearance of birefringence and therefore of the ordered structure on heating above 30 °C, indicates a transition to the isotropic phase (Magnification 5 ×, scale bar = 200 µm).

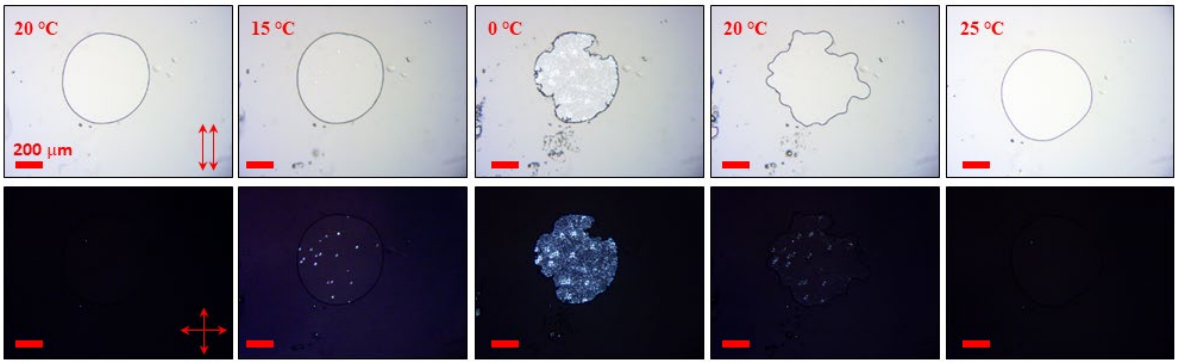

Figure S8: Polarized optical microscopy (POM) images of pure PS during thermal cooling and heating cycles (10°C min^{-1}). The birefringence associated with the formation of a lamellar phase (see **Figure S6**) appears upon cooling around 15 °C and disappears upon heating above 20 °C.

References:

(1) Miron, T.; Wilchek, M. A Spectrophotometric Assay for Soluble and Immobilized N-Hydroxysuccinimide Esters. *Anal. Biochem.* **1982**, *126* (2), 433–435. DOI: 10.1016/0003-2697(82)90540-1.

(2) Grelet, E.; Fraden, S. What Is the Origin of Chirality in the Cholesteric Phase of Virus Suspensions? *Phys. Rev. Lett.* **2003**, *90* (19), 198302. DOI: 10.1103/PhysRevLett.90.198302.

(3) Grelet, E.; Rana, R. From Soft to Hard Rod Behavior in Liquid Crystalline Suspensions of Sterically Stabilized Colloidal Filamentous Particles. *Soft Matter* **2016**, *12* (20), 4621–4627. DOI: 10.1039/C6SM00527F.