Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

"Cross-linked Fibrous" Spherulites from a Low Molecular Weight Compound, Fmoc-functionalized phenolic amino acid

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Materials and Methods

Fmoc-L-Tyrosine (ASF 2521) was purchased from Avra chemicals (India). TFE (T63002), HFIP (105228) and Rhodamine B (83689) were obtained from Sigma Aldrich.

Sample preparation

FmocY was dissolved in HFIP (2 mg/ml) at room temperature while FmocY was dissolved in TFE (2 mg/ml) at 55 °C due to low solubility at room temperature.

Optical and Polarized Microscopy

About 60 μL of FmocY in HFIP (2 mg/ml) solution was added drop wise on a glass cover slip (9 mm diameter). Spherulite was formed after complete evaporation of HFIP solvent at 27 °C. Before coating sample, glass cover slip was washed with 50 mM acetic acid solution and dried at room temperature. Similar procedure was adopted for the preparation of fascia-like structure of FmocY from TFE solvent. The experiments were repeated several times to ascertain the formation of spherulite and fascia-like structures under selected conditions. Multiple experiments suggest that the formation of these structures were reproducible under the experimental conditions studied here. The optical images, with and

without polarizer, of spherulite and fascia-like structures were captured using Olympus microscope (Model BX50F4).

Scanning Electron Microscopy

The sample used in the optical microscopic analysis was used for SEM analysis except that the dried FmocY was coated with a thin layer of gold under vacuum before SEM measurements. SEM images of spherulite and fascia-like structures were captured using FEI Quanta 200 SEM operating at10 kV.

Fluorescence Microscopy

The sample preparation procedure was same as optical microscopy measurements except 10 µl of HFIP and TFE containing Rhodamine B (RB) were added to the corresponding FmocY solution (2 mg/ml) before drying on a glass cover slip. The final concentration of RB was 0.34 mM. Fluorescence microscopic images were taken with blue filter (495 nm excitation and 523 nm emission) using Leica DM IRB fluorescent microscope.

Fluorescence Spectroscopy

All fluorescence spectra were measured using PerkinElmer LS45 Luminescence spectrometer at room temperature. For solution measurements, a stock solution of FmocY in HFIP and TFE was diluted with the respective solvents to get the final concentration of 0.2 µg/ml. For fluorescence spectra of spherulite and fascia-like structures, a similar procedure as that of optical microscopy was followed except that FmocY in HFIP and TFE solution (60 µl) was casted on a quartz slide instead of glass. The fluorescence emission spectra were measured between 300 and 500 nm with an excitation wavelength of 285 nm. The excitation spectra were measured between 250 and 330 nm by exciting at two different wavelengths

(440 and 420 nm). In all experiments, the emission slit width and scan speed was 10 nm and 200 nm/min, respectively.

Electronic Circular Dichroism

All CD spectra were measured on JASCO-715 Spectropolarimeter. The concentration of FmocY in TFE and HFIP was 0.5 mg/ml. A rectangular quartz cell with path length of 1 mm was used. The CD spectra were measured between 350 and 200 (spherulite and fascia-like structures) or 218 (monomer in HFIP and TFE) nm with a scan speed of 100 nm/min. The parameters such as band width, data pitch and response time of 1 nm, 0.5 nm, 1 sec was used, respectively. Each spectrum was an average of three individual scans. For base line correction, the CD spectrum of corresponding solvent was subtracted from the sample CD. For the CD spectrum of spherulites and fascia-like structures, the samples were prepared on a quartz slide. The CD was measured at three different angles (0˚, 90˚ and 180˚) with respect to light beam axis, to check linear dichroism and birefringence, if any.

Wide Angle x-ray Scattering (WAXS)

As mentioned earlier, a fixed volume of FmocY in TFE and HFIP solution was placed on glass plate and kept for complete solvent evaporation at room temperature. The dried FmocY self-assembled structures (spherulites and fascia-like structures) scraped subsequently from the glass plate. The formation of spherulites and fascia-like structures were confirmed by optical microscopy. WAXS measurements were carried out on XEUSS WAXS system using a Genix microsource from Xenocs operated at 50 kV and 0.6 mA. The Cu Kα radiation (l = 1.54 Å) was collimated with FOX2D mirror and two pairs of scatter less slits from Xenocs.

Solid State NMR Measurements

The ^{13}C MAS NMR spectra were recorded after scraping of FmocY in the form of spherulites and fascia-like structure from glass surface (confirmed by optical microscope). The solid-state NMR experiments were performed on a Bruker Avance-III HD 400 WB NMR spectrometer (9.4 T). The carbon resonance frequencies were 400.07 and 100.61 MHz respectively. Samples for ^{13}C analysis were prepared as mentioned in WAXS. ^{13}C MAS spectra of FmocY spherulites and fascia-like structures were recorded at room temperature using a double resonance 4 mm MAS probe. ^{13}C cross polarization/total sideband suppression (CP/TOSS)[1] experiment were performed at a spinning speed of 7 kHz to get sideband free spectrum. A contact time of 3 ms and a ^{1}H 90° pulse length of 4 μs were used. Typically, 1024 scans were acquired with a relaxation delay of 5s. Cross polarization combined with polarization inversion (CPPI)[2] was used to provide spectral editing of the ^{13}C spectrum. Here a short, polarization-inverting spin-lock period of 50 μs is applied on ^{13}C and ^{1}H channels following the initial contact time of 3 ms, to achieve spectral editing. This experiment nulls methine, inverts methylene and leaves methyl and quaternary carbon signals without much change and thus leads to spectral discrimination. MAS rate of 7 kHz were employed for CPPI experiment and 2048 scans were acquired with a relaxation delay of 5s. SPINAL-64[3] decoupling sequence was used to decouple protons during the acquisition by employing radiofrequency field strength of 83 kHz. All FIDs were subjected to an exponential multiplication function with a line broadening value of 30 Hz prior to Fourier transform. ^{13}C chemical shifts are referenced to the carbonyl signal of glycine at 176.2 ppm as an external reference standard.

Fourier Transform Infrared Spectroscopy

FTIR spectra were collected using an ABB MB3000 spectrometer. Typical KBr methodology was adapted to measure FTIR spectra of spherulite and fascia-like structures. Part of the FmocY sample prepared for solid state measurement were mixed with KBr (1:10) and FTIR spectra were collected at room temperature. For solution measurement, FmocY was dissolved in HFIP (30 mg/ml). The solution was transferred to a 50 μm fixed path length containing CaF_2 windows. For baseline correction, solvent alone spectrum was measured separately and subtracted from the sample spectrum. All FTIR spectra were collected at 8 cm^{-1} resolution and by averaging 32 scans, expect 1024 scans for solution measurement.

Molecular Packing Model: The monomer of FmocY was energy minimized by the Gaussian program using quantum mechanical calculations (b3lyp/3-21g level). The molecular packing model of FmocY was built manually based on the WAXS results.

References:

[1] W. T. Dixon. *J. Chem. Phys*. **1982**, *77*, 1800.

[2] X. Wu, K. W. Zilm. *J. Magn. Reson*. **1993**, *102*, 205.

[3] B. M. Fung, A. K. Khitrin, K. Ermolaev. *J. Magn. Reson*. **2000**, *142*, 97.

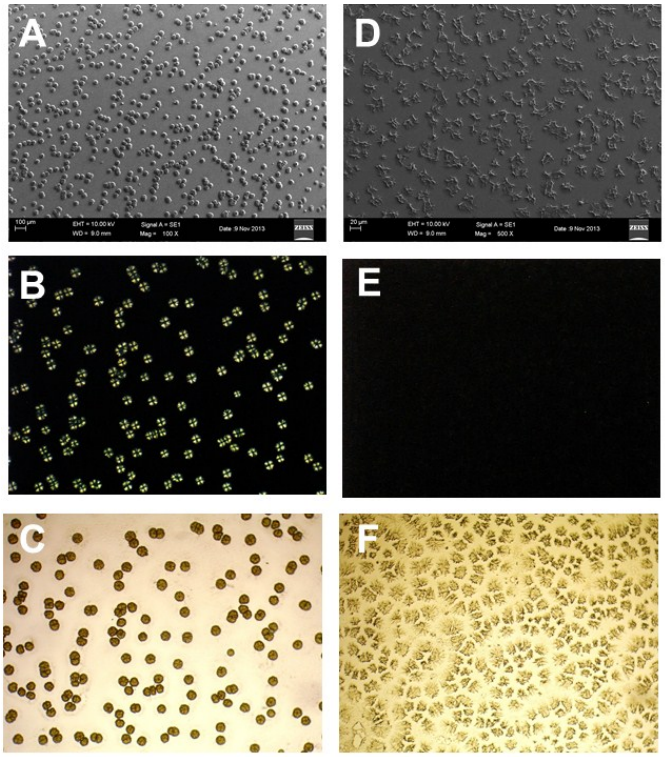

Figure S1 Representative SEM (A), polarized (B) and normal (C) optical microscopic images of spherulites under lower magnification and representative SEM (D), polarized (E) and normal (F) optical microscopic images of fascia-like structures under lower magnification.

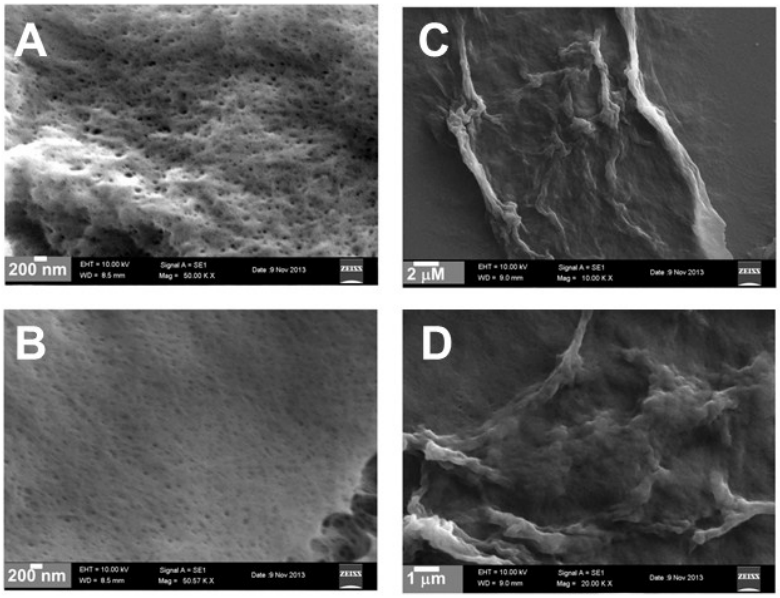

Figure S2 Representative SEM images of spherulites (A and B) and fascia-like (C and D) structure at different magnifications.

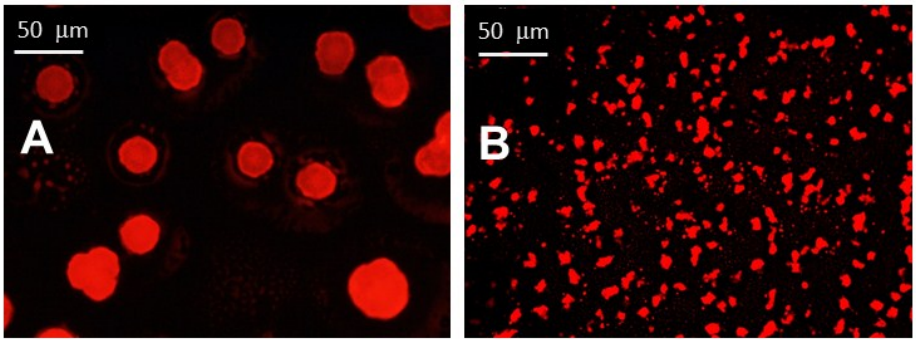

Figure S3 Representative fluorescence microscopic images of spherulites (A) and fascia-like (B) structures.

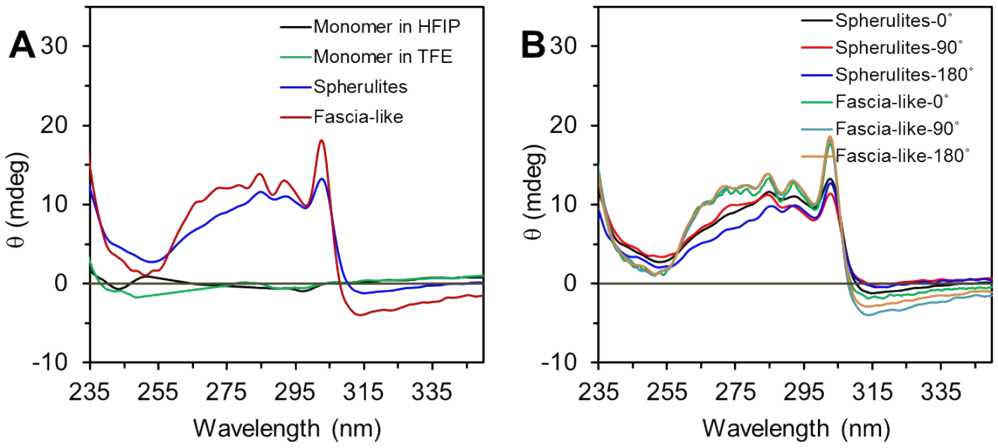

Figure S4 (A) CD spectra of FmocY in HFIP and TFE solvents and (B) CD spectra of spherulites and fascia-like structures formed on quartz surface at different angles with respect to light beam axis. The CD of spherulites and fascia-like structure measured at different angles displays a similar shape and intensity, indicating an absence of linear dichroism in the solid state CD spectra. Base line of the CD spectra in the region of 310-350 nm were shifted from zero line, which could be attributed to reduced transparency of the solid film.

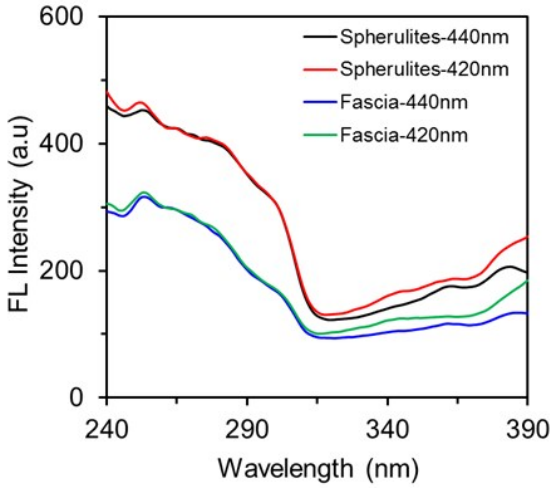

Figure S5: Excitation spectra of spherulites and fascia-like structures with the different excitation wavelengths.

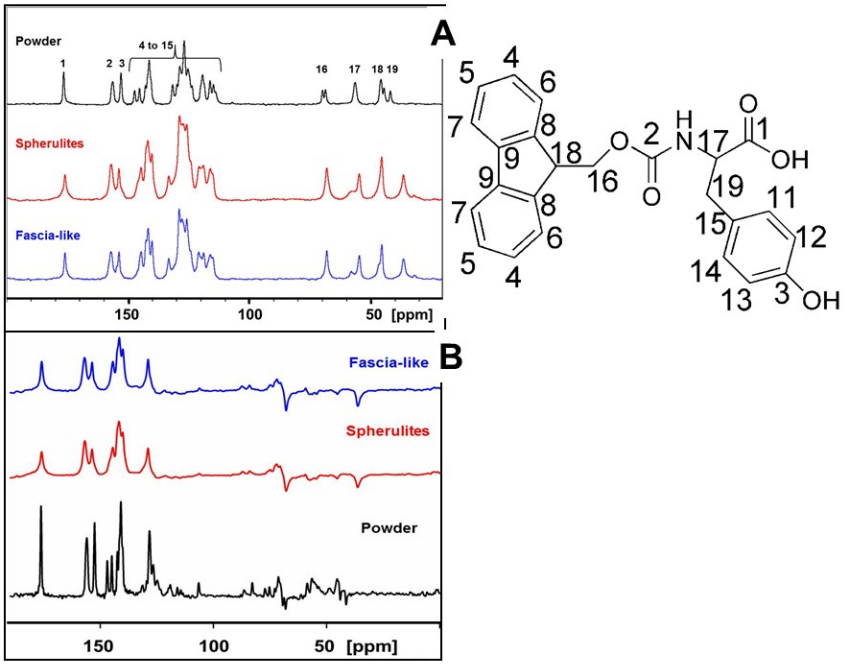

Figure S6: **(A)** ^{13}C MAS NMR spectra and (B) ^{13}C Cross polarization combined with polarization inversion (CPPI) NMR spectra of FmocY in spherulite, Fascia-like, and powder form, respectively. CPPI method was used to assign the carbon resonances tentatively.