SUPPORTING INFORMATION

Supramolecular Block Copolymers under Thermodynamic Control

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1. Methods

All solvents were obtained from Biosolve, Acros or Aldrich and used as received. 6,6',6"-Nitrilotris(N-(3,7-dimethyloctyl)nicotinamide) (S-TPyA, S-1), 6,6',6"-Nitrilotris(N-dodecynicotinamide) (a-TPyA, a-1), 4,4',4"-nitrilotris(N-dodecylbenzamide) (a-TPA, a-2) and 4,4',4"-nitrilotris(N-((S)-3,7-dimethyloctyl)benzamide) (S-TPA, S-2) were synthesized as previously reported1 and vacuum-oven dried before use. Cage-dyes were obtained from Abberior®2 and used as received.

1H-NMR measurements were conducted on a 500 MHz Bruker five-channel liquid-state spectrometer equipped with a high sensitivity QXI cryoprobe with cooled proton channel. Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS).

UV/Vis and circular dichroism (CD) measurements were performed on a Jasco J-815 spectropolarimeter, for which the sensitivity, time constants and scan rates were chosen appropriately. Corresponding temperature-dependent measurements were performed with a Jasco PFD-425S/15 Peltier- type temperature controller with a temperature range of 263–393 K and adjustable temperature slope. In all experiments the linear dichroism was also measured and in all cases no linear dichroism was observed. Fluorescence spectra were measured with Jasco FMO-427S/15 fluorimeter implemented in the CD spectrometer. For spectroscopic measurements spectroscopic grade solvents were employed and different cells were employed. For CD, UV/Vis and fluorescence measurements a sealable quartz cuvette with optic path of 1 cm × 1 cm was used. Fluorescence was tested also with a fluorescence cuvette with 1 cm x 1 mm path, the results obtained with the two cuvettes are comparable. Solutions (50 μM) were prepared by weighing the necessary amount of compound for the given concentration and dissolved with a weighted amount of solvent based on its density. The stock solutions were heated up, sonicated till complete dissolution and slowly cooled down to room temperature every time before use unless otherwise specified. All the spectroscopic and microscopy measurement were performed with freshly prepared solutions (max. 1 week after the preparation of the stock solution).

iPAINT images were acquired using a Nikon N-STORM system equipped with ~158.3 mW/cm² (λ = 405 nm), ~488 mW/cm² (λ = 561 nm) and ~1335 mW/cm² (λ = 647 nm) laser lines configured for quasi-total internal reflection fluorescence (TIRF) imaging. The angle at which the inclined excitation was performed was finely tuned to maximize the signal-to-noise ratio. The fluorescence emission was collected by means of an oil immersion objective (Nikon 100x, 1.49 NA) A quad-band pass dichroic filter (97335 Nikon) was used to separate the excitation light from the fluorescence emission. Timelapses of 15 × 10³ frames were recorded onto a 256 × 256 pixel region (pixel size 170 nm) of an EMCCD camera (ixon3, Andor) at a rate of 47 f/s. Two different photo-activated dyes were used to perform two-color iPAINT measurements: Cage-552 and Cage-635 (Abberior®). These caged rhodamines reside in a dark, non-fluorescent state; upon UV irradiation (λ = 405 nm) the photolysis of the photo-activated moiety is carried out, leading to an open-fluorescent form through the Wolff rearrangement.2 Upon excitation with the proper wavelength (λ = 561 nm for Cage-552, λ = 647 nm for
Cage-635), the fluorescence of the dyes was collected. To perform single-molecule experiments, a low UV laser light power (< 1% power) was used. In this way a small amount of dyes per frame is uncaged, ensuring statistically a spatial separation greater than the diffraction limit of light \( \frac{\lambda}{2NA} \sim 200 \, \text{nm} \), for \( \lambda = 500 \, \text{nm} \). Subsequently, the sample was irradiated with a 100% power laser at the proper wavelength to achieve both excitation and bleaching of the single molecules previously photo-activated. In such a way the excited and bleached molecules are no longer contributing to the reconstruction of the super-resolved image. The iPAINT image was analyzed with NIS-element Nikon software. The sample chamber used to perform iPAINT imaging was made from a coverslide and a coverslip held together by double-sided tape, creating a chamber volume of \( \sim 30 \, \mu\text{L} \). Prior assembly of the sample chamber, the coverslip was cleaned by piranha etching, followed by rinsing and sonication in acetone, isopropanol, MQ-water (18.2 MΩ) and finally blown-dry with nitrogen. To perform super-resolution imaging in organic solvents, a protocol based on adding a small amount of an alcohol to the sample solution was followed, to achieve the uncaging of the caged dyes. Hence, iPAINT polymers \( (c = 200 \, \mu\text{M}) \) was carried out in methylcyclohexane (MCH), adding 1% v/v of isopropanol (i-PrOH) to the solution, and 0.5% v/v of the cage-dyes \( (c = 10 \, \text{mM in DMSO}) \).

To obtain images representative of the samples, the measurements were performed after brief incubation of the solution in the chamber. Then, a check of the overlying solution (by proper variation of the quasi-Total internal reflection fluorescence (TIRF) angle) was performed to verify the absence of diffusing objects. The imaging was performed when no objects were found in the solution and the majority of the fibers were hence physisorbed on the glass coverslip. Further insight on the techniques can be found in literature.
2. Sample preparation

The supramolecular copolymerizations were performed with tri-\(p\)-carboxamide triphenylamine (TPA) and tri-5-carboxamide tri(pyrid-2-yl)amine tricarboxyamide (TPyA). Specifically: 6,6’,6’’-nitrilotris(\(N\)-(\(S\))-3,7-dimethyloctyl)nicotinamide) (\(S\)-TPyA, \(S\)-1), 6,6’,6’’-nitrilotris(\(N\)-dodecyl nicotinamide) (\(a\)-TPyA, \(a\)-1), 4,4’,4’’-nitrilotris(\(N\)-dodecyl benzamide) (\(a\)-TPA, \(a\)-2) and 4,4’,4’’-nitrilotris(\(N\)-((\(S\))-3,7-dimethyloctyl)benzamide) (\(S\)-TPA, \(S\)-2) (Scheme S1). 1

Scheme S1. Chemical structures. Tri-5-carboxamide tri(pyrid-2-yl)amines (with \((S\))-dimethyloctyl chain (\(S\)-TPyA, \(S\)-1) and achiral C12 chain (\(a\)-TPyA, \(a\)-1)), tri-\(p\)-carboxamide triphenylamines (with \((S\))-dimethyloctyl chain (\(S\)-TPA, \(S\)-2) and achiral C12 chain (\(a\)-TPA, \(a\)-2))

The techniques used to supramolecularly copolymerize TPyA and TPA were developed taking inspiration from classical copolymerization techniques as stepwise addition and random polymerization. The two techniques used in this work are: “addition of self-assembled homopolymers” and “slow cooling of monomers”.

We investigated the supramolecular copolymerization of the following couples of monomers, \(S\)-2: \(a\)-1 (poly[\((S\)-2\)]\(_x\)co-(\(a\)-1)\(_{1-x}\))), \(S\)-1: \(a\)-2 (poly[\((S\)-1\)]\(_x\)co-(\(a\)-2)\(_{1-x}\))), and \(S\)-1:S-2 (poly[\((S\)-1\)]\(_x\)co-(\(S\)-2)\(_{1-x}\))), where \(x\) and \((1-x)\) are the respective feed ratios.
2.1. Copolymerization in state I, under thermodynamic control

As previously reported, both the homopolymers are subject to pathway complexity. In MCH both poly(S-1) and poly(S-2) show two different assembled states with opposite handedness.\textsuperscript{1} State I, with positive CD signal, is formed at high temperatures from a molecularly dissolved solution via a nucleation-elongation mechanism. State II, is formed below room temperature through a sharp transition from the first assembled state I. We reported that, applying a slow cooling-heating cycle on the systems, the CD curves display hysteresis in the temperature window below room temperature. Besides, out of the hysteresis region, the CD reports the same value while heating and cooling, meaning that above room temperature the system is under thermodynamic equilibrium in the state previously identified as state I.

Recent discovery indicates that the temperature where the transition I $\rightarrow$ II occurs is dominated by the concentration of water dissolved in aliphatic solvents.\textsuperscript{5}

In the previous work the necessity of analyzing the polymerization of S-1 in decalin isomer mixture for increasing the solubility was addressed. Poly(S-1) shows the same assembling path in decalin and MCH: in ambient condition in decalin the transition I$\rightarrow$ II occurs at 0 °C.\textsuperscript{1}

For this reason, we chose to perform the supramolecular copolymerization in decalin isomer mixture and for completeness we investigated the assembling behavior of S-2 in decalin isomers mixture. We confirmed the analogous behavior observed in MCH (Figure S1). Thus, in order to perform all the copolymerization under thermodynamic control in state I, all the measurements were performed at temperatures higher that 20 °C. The solutions were prepared in ambient conditions and stored in sealable vials or cuvettes.

![Figure S1. Cooling curves till -10 °C. CD cooling curve of (a) poly(S-1) ($\lambda$ = 333 nm) and (b) poly(S-2) ($\lambda$ = 350 nm) in decalin isomers mixture $c_{S-1} = 45$ μM, $c_{S-2} = 50$ μM, cooling/heating rate: 15 °C h$^{-1}$.](image-url)
2.2. **Stepwise addition of self-assembled homopolymers**

Poly(S-1) and poly(S-2) were assembled in decalin isomer mixture ($c = 50 \mu M$) from the respective monomerically dissolved state (100 °C) via slow cooling (cooling rate = 15 °C h$^{-1}$). Poly(S-1), was placed in a cuvette and poly(S-2) was stepwisely added at a fixed temperature of 40 °C. The system was equilibrated for 5-10 minutes after every addition in order to stabilize the signal. Spectra were recorded after the equilibration time. Based on the stage of the stepwise addition the ratio between S-1 and S-2 was variable (in the text expressed as poly[(S-1)$_{x}$-co-(S-2)$_{1-x}$]) while the total concentration of the mixed solution was kept at 50 μM.

The same procedure was performed at 20 °C to kinetically trap the self-sorted state. The self-sorted solution eventually evolves into the supramolecular copolymer via equilibration or heating.

2.3. **Slow cooling of monomerically dissolved state**

The supramolecular copolymerization via slow cooling was performed in decalin isomers mixture combining monomers S-1 and S-2 in a 1:1 ratio to obtain poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$]. Slow cooling of monomers was performed at $c_{\text{tot}} = 50 \mu M$, meaning a concentration per each monomer of 25 μM. The solution was heated to 100 °C to reach the molecularly dissolved state and slowly cooled down (cooling rate of 15 °C h$^{-1}$) leading to the formation of the copolymer under thermodynamic control.

2.4. **Mixed sergeant and soldiers experiment**

Mixed sergeant and soldiers experiments were performed to investigate the possibility of interaction between different supramolecular monomers or polymers and were measured for the couples S-1:a-2, poly[(S-1)$_{x}$-co-(a-2)$_{1-x}$], and S-2:a-1, poly[(S-2)$_{x}$-co-(a-1)$_{1-x}$], (Figures S14, S15).

Mixed sergeant and soldiers experiments were performed via “stepwise addition at 40 °C” and “slow cooling of monomers” and prepared as described before for S-2:S-1 couple.

The achiral molecules show I $\rightarrow$ II transition at higher temperatures compared to the chiral analogues. Therefore, all the measurements were performed at $T > 30 ^\circ C$, preventing in this way, the transition to state II.
2.5. iPAINT solution

The solutions for iPAINT were prepared in MCH since previous work showed the possibility to do super resolution imaging in MCH. As previously reported, the self-assembling properties of triarylamines in MCH and in decalin are comparable. Poly(S-1) and poly(S-2) were polymerized individually under thermodynamic control (cooling rate 15 °C h⁻¹) in MCH (c = 200 μM). Then, at 20 °C poly(S-1) was stained with 0.5% v/v of Cage-635 (c = 10 mM in DMSO) and 1% of i-PrOH. Poly(S-2) was stained with 0.5% v/v Cage-552 (c = 10 mM in DMSO) and 1% of i-PrOH. Both solutions were vortexed and brought to the temperature required for the mixing.

**Addition at 40 °C:**

The stained solutions of poly(S-1) and poly(S-2) were brought to 40 °C in a water bath, equilibrated for 5 minutes, then mixed in a 1:1 ratio. The mixed solution was injected in the sample chamber and consequently imaged.

**Addition at 20 °C:**

Stained poly(S-1) and poly(S-2) were mixed in a 1:1 ratio at 20 °C, one aliquot was injected in the sample chamber to be imaged directly after the mixing. Another aliquot was kept at 20 °C and then imaged one hour after the mixing.

**Additional note:**

The background observed in the images (Figures 6, 8 main text) is due to the physisorption of dye molecules on the coverslip. The low solubility of the dyes in MCH makes the dye deposition on the coverslip much higher compared to analogous techniques in water.
3. Stepwise addition of homopolymers

UV–vis spectra show a linear transition from poly(S-1) to poly(S-2) with an isosbestic points at λ = 319 nm (Figure S2a) similarly to what observed for the CD (Figure 2b, main text). The spectra were compared with the linear combination (Figure S3) of the two homopolymers, and they are remarkably similar (Figure S4). The fluorescence excitation (Figure S2b) and emission (Figure 2c, main text) spectra recorded do not follow a linear trend. The early stages of the supramolecular copolymerization show a sharp increase a red shift comparable with the spectra of poly(S-2). A drastic change is observed between poly(S-1) and poly[(S-1)_{0.80-co-(S-2)_{0.20}}] while the following additions do not significantly modify the emission spectra (Figure 2c main text). The sharp enhancement is caused by the dilution effect of poly(S-2) by mixing it with poly(S-1). For this reason, it is not possible to compare the fluorescence intensity with the theoretical linear combination. However, it is possible to compare the shape of the fluorescence spectra with the theoretical linear combination. Interestingly, the mixed solution does not present the emission shoulder of S-1 at λ = 360 nm (main text Figure 4c, Figure S4b).

Figure S2. Supramolecular copolymerization via stepwise addition at 40 °C. (a) UV–vis, (b) excitation spectra of the copolymer mixture for poly[(S-1)_{x-co-(S-2)_{1-x}}]. Red line: poly(S-1), green line: poly(S-2), light blue line poly[(S-1)_{0.53-co-(S-2)_{0.47}}]. Measurements performed in decalin isomer mixture at 40 °C with c_{S-1} = c_{S-2} = 50 μM.
Figure S3. Linear combination of homopolymers spectra at 40 °C. (a) CD, (b) emission, (c) UV-vis, (d) excitation spectra obtained by linear combination of poly(S-1) and poly(S-2) spectra at different ratio. Linear combination calculated as: \[ x \times \text{poly(S-1)} + (1-x) \times \text{poly(S-2)} \]. Red line: poly(S-1), green line: poly(S-2), black dotted line: linear combination \[0.53 \times \text{poly(S-1)} + 0.47 \times \text{poly(S-2)}\]. Homopolymer measurements performed in decalin isomer mixture at 40 °C with \(c_{S-1} = c_{S-2} = 50 \mu\text{M}\).
Figure S4. Comparison of experimental spectra of addition at 40 °C and the relative linear combination. (a) CD, (b) emission, (c) UV-vis, (d) excitation spectra. Blue line: poly[(S-1)$_{0.53}$-co-(S-2)$_{0.47}$], black dotted line: linear combination of homopolymers [0.53 × poly(S-1) + 0.47 × poly(S-2)].
4. $^1$H NMR Analysis

4.1. $^1$H NMR of homopolymers

Proton NMR experiments were performed with the aim of verifying potential shifts in the aromatic and amide signals upon supramolecular (co)-polymerization. Due to the limited solubility at high concentration of triarylamines in deuterated MCH, the samples were prepared in CDCl$_3$ ($c = 5$ mM). The solutions were cooled to -40 °C to favor supramolecular interactions in chloroform. Both $S$-1 (Figure S6) and $S$-2 (Figure S5) show aggregation in CDCl$_3$ at -40 °C visible in the -NH peak that is subject to deshielding and it loses resolutions in its multiplicity. Aromatic peaks display a smaller shift: $S$-1 shows partially an increase in the shielding and loss of peaks’ resolution, while $S$-2 displays just small broadening of the AB system.

![Figure S5. $^1$H NMR of S-2. CDCl$_3$ c$_S$-2 = 5 mM. Red line: molecularly dissolved at 25 °C, purple line: small aggregates at-25 °C, blue line: aggregated state at -40 °C.](image)
Figure S6. $^1$H NMR of S-1. CDCl$_3$ cS-1 = 5 mM. Red line: molecularly dissolved state at 25 °C, purple line: small aggregates at-25 °C, blue line: aggregated state at -40 °C.
4.2. $^1$H NMR analysis of the supramolecular copolymer

The 1:1 mixing of poly(S-1) and poly(S-2) was performed at room temperature, then the mixture ($c_{\text{tot}} = 5$ mM, $c_{S-1} = c_{S-2} = 2.5$ mM) was cooled to $-40 ^\circ$C in order to favor aggregation in CDCl$_3$. Similar to what was observed for the homopolymers, the mixture shows aggregation in CDCl$_3$ at $-40 ^\circ$C as observed from the shifting and broadening of the peaks (Figure S7).

![Figure S7. $^1$H NMR of the supramolecular copolymer poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$]. CDCl$_3$ $c_{\text{tot}}= 5$ mM. Red line: molecularly dissolved at 25 $^\circ$C, blue line: (r), aggregated state at $-40 ^\circ$C (blue line).](image)

The NMR spectrum of poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$] was compared with the NMR spectra of the aggregated homopolymers at the same temperature. Interestingly significant shifts can be noticed between the peaks belonging to the homopolymers and the ones of the copolymer solution (Figure S8). Remarkably, the –NH peak of poly(S-1) shows a small shift to upfield in the copolymer compared to the homopolymer (6.62 vs 6.68 ppm), while poly(S-2) shows a downfield shift in the copolymer compared to the homopolymer (6.29 vs 6.17 ppm).
Figure S8. $^1$H NMR comparison of the supramolecular copolymer with supramolecular homopolymers. Poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$] Blue line; poly(S-1) red line and poly(S-2) green line. Measurements performed in CDCl$_3$ $c = 5$ mM at $-40 \, ^{\circ}$C.

$^1$H NMR experiment confirms the presence of S-1/S-2 interactions showing shifts in core protons although it is not possible to draw a direct relation of what observed in CDCl$_3$ at $-40 \, ^{\circ}$C and in decalin at $20 \, ^{\circ}$C.
4.3. **NOE analysis on supramolecular copolymers**

In order to verify the presence of interactions between the two monomers, 1D NOE experiments were performed on the mixed solution poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$]. Measurements were carried out in CDCl$_3$ at −40 °C at $c_{\text{tot}} = 5$ mM meaning $c_{\text{S-1}} = c_{\text{S-2}} = 2.5$ mM.

NOE measurements were executed irradiating different signals belonging to both the monomers and good NOE effects are detected both from the molecule irradiated and from the other not directly excited. All the measurements present negative NOE signals. This is assigned to the Overhauser effect occurring intermolecularly.$^7$

The NOE was explored irradiating the aromatic protons of $\text{S-1}$ (Figures S9, S10) and $\text{S-2}$ (Figures S11, S12) and the amide proton of $\text{S-2}$ (Figure S13). The irradiation of the peak at 7.0 ppm captures both the signal of $\text{S-2}$ and $\text{S-1}$ so is less diagnostic of hetero-interactions (Figure S12). Upon irradiation of the pyridine protons, we observed responses from both tripyridylamine, $\text{S-1}$, and triphenylamine, $\text{S-2}$, protons (Figures S9, S10). Analogously, irradiating $\text{S-2}$ we recorded $\text{S-1}$ response (Figure S11).

These results show the presence of hetero-interactions between $\text{S-1}$ and $\text{S-2}$ although it is not possible to quantify the ratio of homo-homo contacts vs hetero-hetero contacts.

![Figure S9. NOE spectrum of poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$].](image)

Figure S9. NOE spectrum of poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$]. Irradiation on $\text{S-1}$ aromatic proton at 8.7 ppm. Blue arrow irradiated proton. Orange, red dots symbols for $\text{S-1}$ protons, Green stars symbols for $\text{S-2}$ protons. All the aromatic protons belonging to the two monomers are visible, while the −NH signal of $\text{S-2}$ is not detected because of the distance (zoom panel section 6.5-6.1 ppm).
Figure S10. NOE spectrum of \(|(S-1)_{0.5} - co - (S-2)_{0.5}|\). Irradiation on \(S-1\) aromatic proton at 8.05 ppm. Blue arrow irradiated proton. Orange, red dots symbols for \(S-1\) protons, Green stars symbols for \(S-2\) protons. All the aromatic protons belonging to the two monomers are visible, while the –NH signal of \(S-2\) is not detected because of the distance (zoom panel section 6.5-6.1 ppm).

Figure S11. NOE spectrum of \(|(S-1)_{0.5} - co - (S-2)_{0.5}|\). Irradiation on \(S-2\) aromatic proton at 7.67 ppm. Blue arrow irradiated proton. Orange, red dots symbols for \(S-1\) protons, Green stars symbols for \(S-2\) protons. All the aromatic protons belonging to the two monomers are visible even if the one belongings to \(S-1\) show low intensity (zoom in panel 8.8-7.9 ppm). While the –NH signal of \(S-1\) is not detected because of the distance (zoom panel section 6.9-6.4 ppm).
Figure S12. NOE spectrum of [(S-1)s-c-o-(S-2)s.s]. Irradiation on S-2 aromatic proton at 7.1 ppm. Blue arrow irradiated proton. The aromatic proton of S-1 residing at 7.1 ppm is partially irradiated as well. Orange, red dots symbols for S-1 protons, Green stars symbols for S-2 protons. All the aromatic protons belonging to the two monomers are visible even if the one belongings to S-1 show low intensity (zoom in panel 8.8-7.9 ppm). While the –NH signal of S-1 is not detected because of the distance (zoom panel section 6.8-6.1 ppm).

Figure S13. NOE spectrum of [(S-1)s-c-o-(S-2)s.s]. Irradiation on S-2 -NHc proton at 6.3 ppm. Blue arrow irradiated proton. Orange, red dots symbols for S-1 protons, Green stars symbols for S-2 protons. The aromatic protons belonging to S-2 are visible while S-1 results silent in all its protons (zoom in panel 8.8-7.9 ppm) and (7.1-6.5 ppm)
The irradiation of the amide did not give any NOE effect for hetero interactions, we address this absence to the fact that the amide lies further away from other protons and just homo-NOE effect can be still observed (Figure S13).

5. Mixed sergeant and soldiers experiments

In standard8 and mixed sergeant and soldier (S&S) the chiral monomer is CD active while the achiral one is CD silent. However, in the mixed sergeant and soldier the achiral species (i.e.: a-2) has a different CD feature than the chiral one (i.e.: S-1). This results that just a mixed S&S interaction (i.e.: a-2 with S-1) can induce a CD signal of the achiral species present.

The couples S-2:a-1 and S-1:a-2 were analyzed applying both the supramolecular copolymerization techniques developed.

The couple S-2:a-1 does not show any increase of CD signal originating from a-1, hence no transfer of chirality was observed (Figure S14). Therefore, no strong interaction among S-2 and a-1 occurs. We hypothesize that this is due to the scarce solubility of a-14 and to the difference in the thermodynamic properties of the two supramolecular polymers.1 As previously reported,1 poly(S-2), shows less cooperativity and a lower \( T_e \) than poly(S-1). This probably makes S-2 not suitable for imposing chirality on a-1 aggregates in significant extent.

By contrast, the system S-1:a-2 shows transfer of chirality from S-1 to a-2 and the CD signal of a-2 arises upon addition of S-1 (Figure S15). Differently from the S-1:S-2 couple (Figure 2 main text, Figure S2) that show fast copolymerization kinetics at 40 °C, the addition of poly(a-2) to poly(S-1) shows slow kinetic. Directly after the addition, a drop in CD intensity of poly(S-1) (\( \lambda_{\text{CD max}} = 333 \) nm) is recorded, then a red shift is observed overtime. After 5 hours the CD shows a maximum at \( \lambda = 341.5 \) nm, indicating that the copolymerization between poly(S-1) and poly(a-2) occurred (Figures S15a, S15b). Hence, the interaction among S-1 and a-2 monomers takes place.

The cooling curve measured at \( \lambda = 341 \) nm for poly[(S-1)$_{0.50}$-co-(a-2)$_{0.50}$] (Figure S15d) displays \( T_e \) at 85 °C (the same of poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$] (Figure 3b Main text)). Differently from poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$], poly[(S-1)$_{0.5}$-co-(a-2)$_{0.5}$] displays another smooth transition at 60 °C. We hypothesize that between 85 °C and 65 °C S-1 polymerizes mostly with itself. Then, when the temperature is close to the \( T_e \) of a-2, the second monomer starts to polymerize on the preformed poly(S-1) nuclei. Therefore, the second transition occurs when this second process ensues. This hypothesis is supported by the progressive red shift of \( \lambda_{\text{CD max}} \) as a function of temperature (Figures S15c, S15e). At high temperature \( \lambda_{\text{CD max}} = 333 \) nm (poly(S-1) CD maximum) while at 30 °C it lies at \( \lambda = 341 \) nm, indicating a cointeraction between S-1 and a-2.

According to the theoretical model (Figure S22b), the presence of a double transition in the cooling curve is an evidence of a small enthalpy gain (\( \Delta H_{AB} \)) upon mixing. This effect would drive to longer
blocks (Figure S24), compared to systems with higher $\Delta H_{AB}$. This hypothesis is in agreement with the low chiral amplification, which can be due to the partial segregation of the two species.

Figure S14. Mixed sergeant and soldier experiment between $S$-2 and $a$-1. Attempt of supramolecular copolymerization via stepwise addition of homopolymers, (a) CD and (b) UV-vis spectra recorded at 40 °C. Attempt of supramolecular copolymerization via slow cooling of monomers for poly{(S-2)$_{0.5}$-co-(a-1)$_{0.5}$}: (c) CD Spectra and (d) CD cooling curve monitored at $\lambda = 341$ nm. (decalin isomers mixture, $c_{tot} = 50 \mu$M, cooling rate 15 °C h$^{-1}$)
Figure S15. Mixed sergeant and soldier experiment between S-1 and a-2. (a, b) Copolymerization via addition of a-2 to S-1 at 40 °C. (a) CD spectra as a function of time. (b) Analysis of the $\lambda_{CD\ max}$ and the relative CD intensity (inset) as a function of time. (c, d, e) Copolymerization via slow cooling. (c) CD spectra, (d) CD cooling curve ($\lambda = 341$ nm, 15 °C h⁻¹). (e) Analysis of the $\lambda_{CD\ max}$ and the relative CD intensity as a function of temperature. Measurement for poly[(S-1)$_{0.5}$-co-(a-2)$_{0.5}$], decalin isomers mixture, $c_{tot} = 50$ μM.
6. Slow cooling of monomers

The supramolecular copolymerization via slow cooling of monomers was monitored while cooling from 100 °C to 20 °C (cooling rate 15 °C h⁻¹). UV-vis, CD and fluorescence spectra were recorded every 5 °C (Figure 3 main text, Figures S17, S18). The results were compared with the spectra of the poly(S-1) and poly(S-2) recorded under the same conditions (Figure S16). UV-vis spectra show a trend comparable to the one observed for the homopolymers (Figures S16a, S16b vs Figure S17a). The CD spectra show an incremental growth of the CD intensity and a small red shift while cooling (Figure 3c, main text). Plotting the λ\text{CD max} as a function of temperature, we followed the red-shift of the CD while cooling and polymerizing. The analysis confirmed that at the beginning λ\text{CD max} = 334 nm, close to poly(S-1) maximum, while at high degree of supramolecular polymerization λ\text{CD max} = 341.5 nm, namely the 1:1 combination of λ\text{CD poly(S-1)} and λ\text{CD poly(S-2)} (Figure S17c).

The fluorescence spectra upon cooling and copolymerization display a red shifted emission and a small decrease in intensity (Figure S18c). At 20 °C the maximum of fluorescence resides at λ = 382 nm. The results are compared with the trend of fluorescence of the two homopolymers (Figures S18a, S18c). Both poly(S-1) and poly(S-2) show a red shift upon aggregation, however poly(S-2) displays a quenching in intensity of about 50% upon aggregation while poly(S-1) shows an increment. Both the systems show higher emission at lower concentration (Figure S18b, S18d). This diverse behavior complicates the comparison of the behavior of poly[(S-1)₀.₅₀-co-(S-2)₀.₅₀] with poly(S-1) and poly(S-2). However, we compared the general trend of the intensity at λ = 382 nm of poly[(S-1)₀.₅₀-co-(S-2)₀.₅₀] (c\text{S-1} = c\text{S-2} = 25 μM, c\text{tot} = 50 μM) with the sum of fluorescence of the homopolymers ([poly(S-1)] + poly(S-2)) with c\text{S-1} = c\text{S-2} = 25 μM or with the linear combination of the homopolymers ([0.5 × poly(S-1) + 0.5 × poly(S-2)] with c\text{S-1} = c\text{S-2} = 50 μM). It results that the copolymer trend is closer to the linear combination obtained from the homopolymers at 50 μM (Figure S18f). This hints that the interaction between the two homopolymers is present showing lower intensity compared to the sum of fluorescence recorded with c = 25 μM.
Figure S16. Supramolecular homopolymerization via slow cooling of monomers. (a, c, e) Poly(S-1) and (b, d, f) poly(S-2). (a, b) UV-vis, (c, d) CD spectra, and (e, f) cooling curves (CD at λ = 341 nm, cooling rate: 15 °C h⁻¹). Measurements performed in decalin isomers mixture, (a, b, c, d) c = 50 μM; (e, f) c = 25 μM (red and green lines) and c = 50 μM (dark red line, dark green line).
Figure S17. Supramolecular copolymerization of poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$] via slow cooling of monomers. (a) UV-vis spectra as a function of temperature (increment every 5 °C). (b) Normalized CD cooling curves (λ = 341 nm, concentrations in the legend). (c) Analysis of λ$_{CD}$ max and the relative CD intensity of poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$] as a function of temperature. Measurements performed in decalin isomers mixture, cooling rate 15 °C h$^{-1}$, $c_{tot}$ = 50 μM, $c_{S-1}$ = $c_{S-2}$ = 25 μM.
Figure S18. Fluorescence spectra of supramolecular homo- and copolymerization via slow cooling. (a, c, e) Emission spectra as a function of temperature: (a) poly(S-1), (c) poly(S-2), (e) poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$]. (b, d, f) Fluorescence cooling curves (λ = 382 nm, cooling rate: 15 °C h$^{-1}$): (b) poly(S-1), (d) poly(S-2), (f) poly[(S-1)$_{0.50}$-co-(S-2)$_{0.50}$]. (f) The copolymer intensity is compared with the sum of homopolymers’ intensities: [poly(S-1) + poly(S-2)] with $c_{S-1} = c_{S-2} = 25$ μM (dotted black curve) and with the linear combination of homopolymers intensities: [0.5 × poly(S-1) + 0.5 × poly(S-2)] with $c_{S-1} = c_{S-2} = 50$ μM (dash-dotted black curve). Measurements performed in decalin isomer mixture, $\lambda_{exc} = 320$ nm, sealed cuvette 1 cm × 1 cm, $c_{exc} = 50$ μM, $c_{S-1} = c_{S-2} = 25$ μM.
7. Techniques comparison: slow cooling of monomers vs stepwise addition of homopolymers

We compared the CD and fluorescence spectra obtained via “slow cooling of monomers” with the ones recorded via “addition of homopolymers at 40 °C” (main text Figures 4b, 4c, Figures S19). Strikingly the CD and the fluorescence show the same features, meaning that the two techniques bring to the same copolymer structure poly[(S-1)-co-(S-2)].

Figure S19. Techniques comparison: Slow cooling vs Addition at 40 °C. (a) CD and (b) normalized emission spectra of poly[(S-1)-co-(S-2)] obtained via slow cooling (blue line for poly[(S-1)0.50-co-(S-2)0.50]) or via stepwise addition at 40 °C (light blue line for poly[(S-1)0.53-co-(S-2)0.47]). Measurements performed in decalin isomers mixture at 40 °C, λexc = 320 nm. ctot = 50 μM.
8. Decomposition of the variable temperature copolymer CD spectra

The homopolymers poly(S-1) and poly(S-2) do not present shift in the wavelength of CD spectra during the polymerization: the maximum of the CD stays constant during the polymerization process. Besides, the copolymer CD spectra (Figure 3c, main text), and the relative wavelengths were the CD maximum falls (Figure S17c), are dependent from the temperature. This effect is due to the change in the effective composition of the copolymer while cooling the system. In order to estimate the effective composition of poly[(S-1)0.5-co-(S-2)0.5] while cooling and polymerizing, we analyzed the changes in the CD spectrum as a function of temperature.

From the variable temperature CD spectra of poly(S-1), poly(S-2) and poly[(S-1)0.5-co-(S-2)0.5] (Figures S16c, S16d, 3c main text, respectively), we extracted the values of CD at three wavelengths (\(\lambda = 333\) nm, 338 nm, and 350 nm). \(\lambda = 350\) nm corresponds to poly(S-2) CD maximum, \(\lambda = 333\) nm to poly(S-1) CD maximum and \(\lambda = 338\) nm to the wavelength where the intensities of poly(S-1) and poly(S-2) are similar (main text, Figure 5a). The cooling curves obtained were normalized such that the area under the curves for all three wavelengths is the same, and the CD signal at \(\lambda = 338\) nm at the lowest temperature equals 1 (Figure S20a, S20c, S20e).

In standard one-component system (assuming the formation of a single supramolecular species in solution) the CD spectrum of the supramolecular polymer should vary just in intensity while cooling and polymerizing. Thus, their normalized cooling curves followed at multiple wavelengths should overlaps. As expected, the normalized cooling curves for the three wavelengths reasonably overlap both for poly(S-1) and poly(S-2) (Figures S20a, S20c). Nevertheless, for poly[(S-1)0.5-co-(S-2)0.5] this does not happen; between 85 °C and 50 °C the CD at \(\lambda = 333\) nm is more intense, while below 50 °C the CD at \(\lambda = 350\) nm is more intense (Figure S20e). This effect can be attributed to the composition (ratio S-1 to S-2) of the copolymer that is temperature dependent.

We then used the non-normalized cooling curves at the two wavelengths where the CD intensities of poly(S-1) and poly(S-2) are maximal, i.e., \(\lambda = 333\) nm and 350 nm respectively, (Figure S20b, S20d) to analyze this temperature dependence of the copolymer composition based on the assumption that the measured CD signal of poly[(S-1)0.5-co-(S-2)0.5] is the linear combination of the signals of the two monomer types:

\[
CD^\lambda_{poly[(S-1)0.5-co-(S-2)0.5]} = \alpha CD^\lambda_{poly(S-2)} + \beta CD^\lambda_{poly(S-1)}. \tag{S8.1}
\]

That is, the CD signal of poly[(S-1)0.5-co-(S-2)0.5] at a certain temperature T and wavelength \(\lambda\) \((CD^\lambda_{poly[(S-1)0.5-co-(S-2)0.5]})\) is the amount (in \(\mu\)M) of polymerized S-2 (\(\alpha\)) at that temperature multiplied by the CD signal per \(\mu\)M poly(S-2) at that wavelength \((CD^\lambda_{poly(S-2)})\) plus the amount (in \(\mu\)M) of polymerized S-1 (\(\beta\)) at the same temperature multiplied by the CD signal per \(\mu\)M poly(S-1) at that wavelength \((CD^\lambda_{poly(S-1)})\). Note that \(\alpha\) and \(\beta\) may depend on the temperature, but are independent of \(\lambda\).

The CD signals per \(\mu\)M polymerized S-1 and S-2 \((CD^\lambda_{poly(S-1)}\) and \(CD^\lambda_{poly(S-2)}\)) we extract from the
homopolymer CD spectra at the lowest temperature (293K) assuming that all material (50 μM) is polymerized at that temperature.

For the wavelengths $\lambda = 333$ nm and $\lambda = 350$ nm we thus have:

$$CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{333} = \alpha CD_{\text{poly}(S-2)}^{333} + \beta CD_{\text{poly}(S-1)}^{333}$$  \hspace{1cm} (S8.2)

and

$$CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{350} = \alpha CD_{\text{poly}(S-2)}^{350} + \beta CD_{\text{poly}(S-1)}^{350}$$  \hspace{1cm} (S8.3)

Together, these Equations (S8.2) and (S8.3) constitute two equations with two unknown, i.e., $\alpha$ and $\beta$, which can be rewritten as:

$$\alpha = \frac{(CD_{\text{poly}(S-2)}^{350} \cdot CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{333}) - (CD_{\text{poly}(S-1)}^{333} \cdot CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{350})}{Q}$$  \hspace{1cm} (S8.4)

$$\beta = \frac{-(CD_{\text{poly}(S-2)}^{350} \cdot CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{333}) + (CD_{\text{poly}(S-1)}^{333} \cdot CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{350})}{Q}$$  \hspace{1cm} (S8.5)

with:

$$Q = CD_{\text{poly}(S-2)}^{333} \cdot CD_{\text{poly}(S-1)}^{350} - CD_{\text{poly}(S-2)}^{350} \cdot CD_{\text{poly}(S-1)}^{333}$$  \hspace{1cm} (S8.6)

The temperature dependence of $\alpha$ (i.e. equivalent concentration $S\text{-2}$ in aggregates) and $\beta$ (i.e. equivalent concentration $S\text{-1}$ in aggregates) can be determined via above equations from $CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{333}$ and $CD_{\text{poly}(S-1)\text{-co-}(S-2)}^{350}$ at different temperatures. This yields to the Figures S22d and 3b of the main text. Given $\alpha$ and $\beta$, the CD signal can be recalculated via Equation (S8.1). At $\lambda = 333$ nm as well as at $\lambda = 350$ nm (Figures S20b, S20d blue and black scattered-line curve), the so calculated CD signal exactly matches the experimental curve for the copolymer. This is expected since these wavelengths were used to determine $\alpha$ and $\beta$. At other wavelengths, e.g. $\lambda = 338$ nm (Figure S20f), the match is not necessarily perfect. Deviations can be explained by the three assumptions made on the model:

1) $CD_{\text{poly}(S-1)}^{\lambda}$ and $CD_{\text{poly}(S-2)}^{\lambda}$ are estimates based on the assumption that at 20 °C all material is polymerized,

2) the shape of the CD spectra of $S\text{-1}$ and $S\text{-2}$ are completely temperature independent,

3) the CD signal of the copolymer is the exact linear combination of the signals of the two homopolymers.

However, the differences are acceptable and the resulting concentrations of copolymerized material (main text Figure 5b, Figure S22d) seem reasonable. These results clearly show that at high temperature $S\text{-1}$ is the majority copolymer constituent, while at 20 °C the copolymers balance at 50:50 ratio.

Given the full spectra of the pure poly($S\text{-1}$) and pure poly($S\text{-2}$) at 20 °C (Figures S16c, S16d), we can also use the calculated values for $\alpha$ and $\beta$ at the different temperatures to predict using Equation (S8.1) the full CD spectra of the copolymer solution at those temperatures (Figure S21a). We then analyzed the variation of $\lambda_{\text{CD max}}$ as a function of temperature for the predicted spectra (Figure S21b) and we
compared these with the experimental results (Figures 3c main text, S17c). Although the real system does not show a perfect linear combination of the CD spectra of the homopolymers (as visible in the band at $\lambda = 297$ nm) the model shows a similar trend in the shift of $\lambda_{\text{CD} \text{ max}}$ while cooling.

Figure S20. Cooling curve analysis and prediction by linear algorithm. Normalized experimental cooling curve of (a) poly(S-1), (c) poly(S-2), (e) poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$] followed at $\lambda = 333$ nm (red scatter-line), $\lambda = 338$ nm (blue scatter-line), $\lambda = 350$ nm (green scatter-line). Experimental cooling curve for poly(S-1) (red scatter line), poly(S-2) (green scatter line) and poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$] (blue scatter line) followed at (b) $\lambda = 333$, (d) $\lambda = 350$ and (f) $\lambda = 338$ nm. Calculated results for poly[(S-1)$_{0.5}$-co-(S-2)$_{0.5}$] (black scatter line).
Figure S21. Prediction of CD spectra and cooling curve via linear algorithm. (a) Predicted CD spectra based on calculated \( \alpha \) and \( \beta \) (in Figure S22d) and (b) Analysis of \( \lambda_{\text{CD max}} \) and the relative CD intensity of the predicted spectra of poly\([S-1]_{0.50-}
\text{co-}[S-2]_{0.50}\) as a function of temperature.
9. Copolymerization model

A) Introduction
Here we describe a model to investigate the supramolecular copolymerization of two monomers that individually polymerize with a distinct cooperativity. In section B) the reactions describing the copolymerization are given as well as the relations on the equilibrium constants that are required to obey detailed balance.

The thermodynamic analysis can be performed using mass-balance equations. In this work we extend the derivation reported by Das et al.6 to allow the copolymerization of two monomers that individually polymerize with a distinct cooperativity.

Additionally, the kinetics of the copolymerization can be followed using stochastic simulations. In such simulations, individual copolymers (including the exact order of monomers inside them) are tracked, allowing to visualize the supramolecular polymers and to extract the fraction of bonds that involve two different monomers.

The model and the simulations were performed on two general monomers called $A$ and $B$ that homopolymerize via a nucleation elongation pathway into poly($A$) and poly($B$) respectively.

B) Model description: The reactions
Denoting one monomer by $A$ and a polymer poly($A$) consisting of $i$ monomers by $P_i$, the corresponding reactions are:

$$P_iA + A \xrightleftharpoons{K_{PA,A}}{\sigma_A} P_{i+1}A$$

with $i \geq 1$ for the elongation phase, and for the dimer formation (i.e. the nucleation phase):

$$A + A \xrightleftharpoons{\sigma_A K_{PA,A}}{\sigma_A} AA$$

where $\sigma_A$ is the cooperativity.

Analogously, denoting the other monomer by $B$, the polymerization of poly($B$) can be described by the reactions:

$$P_iB + B \xrightleftharpoons{K_{PB,B}}{\sigma_B} P_{i+1}B$$

with $i \geq 1$ for the elongation phase, and for the dimer formation:

$$B + B \xrightleftharpoons{\sigma_B K_{PB,B}}{\sigma_B} BB$$

where $\sigma_B$ is the cooperativity for this second monomer type.

Assuming that the equilibrium constants $K_{PA,A}$ and $K_{PB,B}$ are not only independent on the length $i$ but also on the exact composition (i.e., the ratio of $A$ and $B$ monomers) of polymer $P_i$, the above elongation
reactions also describe elongation of copolymer $P$ via $AA$ and $BB$ contacts. The co-assembly of $A$ and $B$ into copolymer $P$ is then fully described with the additional reactions:

$$P_i A + B \overset{K_{PA-B}}{\rightleftharpoons} P_i AB$$
$$P_i B + A \overset{K_{PB-A}}{\rightleftharpoons} P_i BA$$

and:

$$A + B \overset{K_{A-B}}{\rightleftharpoons} AB$$
$$B + A \overset{K_{B-A}}{\rightleftharpoons} BA$$

These additional reactions contain 4 new equilibrium constants. However, if we assume that the polymers can grow from both sides and that above reactions describe both growth mechanisms, the equilibrium constants cannot all be chosen independently. Once $K_{PA,B}$ is chosen, detailed balance yields the remaining parameters, i.e., (Scheme 2):

$$K_{PB-A} = \frac{\sigma_A}{\sigma_B} K_{PA-B}$$

and (Scheme 3):

$$K_{A-B} = K_{B-A} = \sigma_A K_{PA-B} = \sigma_B K_{PB-A}$$.

Scheme S2: Detailed balance yields a relation between equilibrium constants $K_{PB-A}$ and $K_{PA-B}$. 

- $\frac{\sigma_A K_{PA-A} K_{PB-B}}{\sigma_B K_{PB-A} K_{PA-B}} = 1 \Rightarrow K_{PB-A} = \frac{\sigma_B}{\sigma_A} K_{PA-B}$
Scheme S3: Detailed balance yields a relation between equilibrium constants $K_{A-B}$ and $K_{PA-B}$.

Thus, once we characterized the homopolymerization of the two individual components, copolymerization is described using a single new equilibrium constant $K_{PA-B}$. This yields the final reaction scheme, with dimerization steps:

\[
\begin{align*}
A + A & \rightleftharpoons \sigma_{A}K_{PA-A} \rightarrow AA \\
A + B & \rightleftharpoons \sigma_{A}K_{PA-B} \rightarrow AB \\
B + A & \rightleftharpoons \sigma_{B}K_{PB-A} \rightarrow BA \\
B + B & \rightleftharpoons \sigma_{B}K_{PB-B} \rightarrow BB
\end{align*}
\]

and elongation steps:

\[
\begin{align*}
P_i A + A & \rightleftharpoons \frac{K_{PL-A}}{} \rightarrow P_i AA \\
P_i A + B & \rightleftharpoons \frac{K_{PL-B}}{} \rightarrow P_i AB \\
P_i B + A & \rightleftharpoons \frac{K_{PB-A}}{} \rightarrow P_i BA \\
P_i B + B & \rightleftharpoons \frac{K_{PB-B}}{} \rightarrow P_i BB
\end{align*}
\]

C) The mass balance model

C.1. Algebraic relations for copolymers

The goal of this section is to compute, in an equilibrium situation with given total concentrations of $A$ and $B$ monomers, the concentrations of the various possible copolymers and their composition. Since the binding of a monomer at the top of an existing copolymer depends on the monomer at the top, concentrations of copolymers with top $A$ and top $B$ are treated separately. Let $c_k^A$ be the concentration of copolymers with length $k$ and top $A$ and let $c_k^B$ be the concentration of copolymers with length $k$ and top $B$. 
Moreover, let \( a = [A] \) and \( b = [B] \) be the concentrations of free \( A \) and free \( B \) monomers, respectively. In equilibrium the concentrations of copolymers with top \( AA \) are given by:

\[
[P_{k-1}AA] = K_{PA-A}a[P_{k-1}A] = K_{PA-A}ac_k^A \quad \text{for } k \geq 2. \tag{S9.1}
\]

Similarly, the concentrations of copolymers with top \( BA \) are given by:

\[
[P_{k-1}BA] = K_{PB-A}a[P_{k-1}B] = K_{PB-A}ac_k^B \quad \text{for } k \geq 2. \tag{S9.2}
\]

Since each copolymer with top \( A \) is either of the form \( P_{k-1}AA \) or of the form \( P_{k-1}BA \), this implies that:

\[
c_{k+1}^A = K_{PA-A}ac_k^A + K_{PB-A}ac_k^B
\]

In the same way the relation:

\[
c_{k+1}^B = K_{PA-B}bc_k^A + K_{PB-B}bc_k^B
\]

can be derived. In both relations \( k \geq 2 \) since the equilibrium constants of the elongation phase are used.

For the nucleation phase these relations become:

\[
c_2^A = \sigma_A K_{PA-A}a^2 + \sigma_B K_{PB-A}ab \quad \text{and} \quad c_2^B = \sigma_A K_{PA-B}ab + \sigma_B K_{PB-B}b^2
\]

These recurrence relations for the copolymer concentrations can be written in one system as

\[
\begin{pmatrix} c_k^A \\ c_k^B \end{pmatrix} = M_2^{k-1} \cdot \begin{pmatrix} \sigma_A a \\ \sigma_B b \end{pmatrix} \quad \text{for } k = 2, 3, ...
\] \tag{S9.3}

where the 2 x 2 matrix \( M_2 \) is given by:

\[
M_2 = \begin{pmatrix} K_{PA-A} a & K_{PB-A} a \\ K_{PA-B} b & K_{PB-B} b \end{pmatrix} \tag{S9.4}
\]

For chemically realistic situations all the eigenvalues of \( M_2 \) must have an absolute value smaller than 1. Otherwise the concentrations given in Equation (S9.3) will not tend to zero if \( k \to \infty \), which is necessary for a system with a finite total concentration. If the free monomer concentrations \( a \) and \( b \) are known, the system in Equation (S9.3) allows to compute the concentration of copolymers of all lengths.

However, it does not give information on the composition of these copolymers, which is needed for the mass-balance approach.

To obtain information on the amount of \( A \) and \( B \) occurring in copolymers of a given length, two additional notions are introduced:

\[
n_k^A = \text{average number of } A \text{ monomers occurring in a copolymer of length } k \text{ with top } A,
\]

\[
n_k^B = \text{average number of } A \text{ monomers occurring in a copolymer of length } k \text{ with top } B.
\]

Note that, by definition, \( n_k^A \geq 1 \), whereas \( n_k^B \geq 0 \).

To find an expression for \( n_{k+1}^A \), note that copolymers of length \( k + 1 \) (for \( k \geq 2 \)) that have a top \( A \) are either of the form \( P_{k-1}AA \) or of the form \( P_{k-1}BA \), where \( P_{k-1} \) is an arbitrary copolymer of length \( k - 1 \). The average number of monomers \( A \) in copolymers of the form \( P_{k-1}AA \) is given by \( n_k^A + 1 \) and the average number of monomers \( A \) in copolymers of the form \( P_{k-1}BA \) is given by \( n_k^B + 1 \). Hence \( n_{k+1}^A \) is given by the weighted average:

\[
n_{k+1}^A = (n_k^A + 1)f_{k+1}^A + (n_k^B + 1)f_{k+1}^B \tag{S9.5}
\]
where $f_{k+1}^A$ is the fraction of all copolymers of length $k+1$ and top $A$ that are of the form $P_{k-1}AA$ and $f_{k+1}^B$ is the fraction of all copolymers of length $k+1$ and top $A$ that are of the form $P_{k-1}BA$. These fractions are easily computed using Equations (S9.1) and (S9.2) as:

\[
f_{k+1}^A = \frac{[P_{k-1}AA]}{c_{k+1}} = \frac{K_{PA-A}c_k^A}{c_{k+1}} \quad \text{and} \quad f_{k+1}^B = \frac{[P_{k-1}BA]}{c_{k+1}} = \frac{K_{PB-A}c_k^B}{c_{k+1}}.
\]

Substitution of these relations in Equation (S9.5) leads to:

\[
n_{k+1}^A = (n_k^A + 1) \frac{K_{PA-A}c_k^A}{c_{k+1}} + (n_k^B + 1) \frac{K_{PB-A}c_k^B}{c_{k+1}}
\]

Now define $g_k^A = c_k^A n_k^A$. Then $g_k^A$ is the equivalent concentration\(^1\) of $A$ monomers that occur in a copolymer of length $k$ with top $A$. Similarly $g_k^B = c_k^B n_k^B$ is the equivalent concentration of $A$ monomers that occur in a copolymer of length $k$ with top $B$. Rewriting Equation (S9.6) with these notions leads to:

\[
g_{k+1}^A = K_{PA-A}a g_k^A + K_{PB-A}a g_k^B + K_{PA-A}ac_k^A + K_{PB-A}ac_k^B.
\]

In a similar way the recurrence relation for $n_k^B$ and $g_k^B$ can be derived. That results in:

\[
n_{k+1}^B = n_k^B K_{PA-B}b c_k^A + n_k^B K_{PB-B}b c_k^B
\]

and finally in:

\[
g_{k+1}^B = K_{PA-B}b g_k^A + K_{PB-B}b g_k^B
\]

Note that the “+1” terms in Equations (S9.5) and (S9.6) are missing in these expressions, since the number of $A$ monomers does not change if the copolymer is elongated by the addition of an $B$ monomer at the top.

The Equations (S9.3), (S9.7) and (S9.8) can be written in matrix form as:

\[
\begin{pmatrix}
c_{k+1}^A \\
c_{k+1}^B \\
g_{k+1}^A \\
g_{k+1}^B
\end{pmatrix} = M \cdot \begin{pmatrix}
c_k^A \\
c_k^B \\
g_k^A \\
g_k^B
\end{pmatrix}
\]

for $k = 2, 3, \ldots$, with:

\[
M = \begin{pmatrix}
K_{PA-A}a & K_{PB-A}a & 0 & 0 \\
K_{PA-B}b & K_{PB-B}b & 0 & 0 \\
0 & K_{PA-A}a & K_{PB-A}a & K_{PB-A}a \\
0 & 0 & K_{PA-B}b & K_{PB-B}b
\end{pmatrix}
\]

The iteration process can even be started from $k = 1$ by:

\[
\begin{pmatrix}
c_k^A \\
c_k^B \\
g_k^A \\
g_k^B
\end{pmatrix} = M^{k-1} \cdot \begin{pmatrix}
\sigma_A a \\
\sigma_B b \\
\sigma_B a \\
0
\end{pmatrix} \quad \text{for} \quad k = 2, 3, \ldots
\]

\(^{1}\)The equivalent concentration of monomers in (co)polymers is defined as the monomer concentration that results if all those (co)polymers are broken down. Thus, $g_k^B$ is the concentration of monomers $A$ that results if all copolymers of length $k$ with top $A$ are broken down.
Also the concentrations in Equation (S9.11) will only tend to zero for \( k \to \infty \) if all the eigenvalues of \( M \) have an absolute value smaller than 1. Since the eigenvalues of \( M \) are the same as the eigenvalues of \( M_2 \), this condition is the same as the condition for a finite system described by Equation (S9.3).

### C.2. The mass-balance equations

The total equivalent concentration of \( A \) monomers that is present in copolymers of length at least two is given by:

\[
G(a, b) = \sum_{k=2}^{\infty} (g_k^A + g_k^B) = \sum_{k=1}^{\infty} \left( (M^k \cdot z)_3 + (M^k \cdot z)_4 \right) \quad \text{where} \quad z = \begin{pmatrix} \sigma_A a \\ \sigma_B b \\ \sigma_A a \\ 0 \end{pmatrix} \quad (S9.12)
\]

Using the matrix identity \( \sum_{k=1}^{\infty} M^k = (I - M)^{-1} \cdot M \), where \( I \) is the 4 x 4 identity matrix, the total equivalent concentration of \( A \) monomers in copolymers can be written as:

\[
G(a, b) = (u_3 + u_4), \quad (S9.13)
\]

where the 4 dimensional vector \( u \) is defined as:

\[
u = (I - M)^{-1} \cdot M \cdot z. \quad (S9.14)
\]

For the mass-balance equations, also the total equivalent concentration of \( B \) monomers that occur in copolymers is needed. In analogy with \( g_k^A \) the equivalent concentration of \( B \) monomers that occur in a copolymer of length \( k \) with top \( A \) will be denoted by \( h_k^A \) and the equivalent concentration of \( B \) monomers that occur in a copolymer of length \( k \) with top \( B \) will be denoted by \( h_k^B \). Since each copolymer of length \( k \) contains \( k \) monomers, either \( A \) or \( B \), the following two relations hold:

\[
\begin{align*}
g^A_k + h^A_k &= k c^A_k \\
g^B_k + h^B_k &= k c^B_k.
\end{align*} \quad (S9.15)
\]

Using Equations (S9.11) and (S9.15) we can write the equivalent \( B \) concentration in polymers of length at least two as:

\[
H(a, b) = \sum_{k=2}^{\infty} (h^A_k + h^B_k) = \sum_{k=2}^{\infty} \left( k c^A_k - g^A_k + k c^B_k - g^B_k \right) \\
= \sum_{k=2}^{\infty} \left( (k M^{k-1} \cdot z)_1 + (k M^{k-1} \cdot z)_2 \right) - \sum_{k=2}^{\infty} \left( (M^{k-1} \cdot z)_3 + (M^{k-1} \cdot z)_4 \right) \quad (S9.16)
\]

The first term in the resulting formula can be computed using the matrix identity \( \sum_{k=2}^{\infty} k M^{k-1} = (I - M)^{-2} - I \). Using the vector:

\[
\nu = ((I - M)^{-2} - I) \cdot z \quad (S9.17)
\]

and the vector \( u \) defined above (Equation (S9.14)), the amount of \( B \) monomers present in copolymers can now be written as:

\[
H(a, b) = (v_1 + v_2 - u_3 - u_4). \quad (S9.18)
\]

The mass-balance equations for a system that only contains monomers \( A \) and \( B \), and corresponding copolymers are given by

\[
\begin{align*}
a + G(a, b) &= a_{tot} \\
b + H(a, b) &= b_{tot}
\end{align*} \quad (S9.19)
\]
where \( a_{\text{tot}} \) and \( b_{\text{tot}} \) are the total A and B concentrations respectively, \( a \) and \( b \) are the free monomer concentrations of A and B, respectively, and \( G(a, b) \) and \( H(a, b) \) are the total equivalent concentrations of A and B in the copolymers, as given by Equations (S9.13) and (S9.18) respectively. The mass-balance equations (Equation (S9.19)) simply state that each A monomer is either present as a free monomer or it occurs in a copolymer, and also each B monomer is either a free monomer or it occurs in a copolymer. The total equivalent concentrations of monomers in aggregates \( p_{\text{tot}} \) is finally given by \( p_{\text{tot}} = G(a, b) + H(a, b) \).

D) Calculating melting curves

The free monomer concentrations (and the equivalent concentrations of monomers in aggregates) can be calculated for a given set of equilibrium constants and total concentrations of the copolymerizing monomers \( (a_{\text{tot}} \) and \( b_{\text{tot}} \)) by (numerically) solving the mass balance equations (Equation (S9.19)). To create cooling curves, we calculate these free monomer concentrations (and the equivalent concentrations of monomers in aggregates) for fixed overall concentrations \( (a_{\text{tot}} \) and \( b_{\text{tot}} \)) at various temperatures, where the temperature dependence of the equilibrium constants is given \( K = \exp(-\Delta G^0/RT) \), with \( \Delta G^0 \) the standard free energy differences of the corresponding reaction. The homopolymerization reactions are described with a single entropy term and two enthalpy terms per system, i.e., \( \Delta S_{\text{NN}}, \Delta H_{\text{NN}}, \) and \( N_{\text{PNN}} \) for \( A \) and \( \Delta S_{\text{BB}}, \Delta H_{\text{BB}}, \) and \( N_{\text{PBB}} \) for \( B \). Thus:

\[
K_{P_{\text{A}}-\text{A}} = e^{-\Delta H_{\text{NN}}/RT} \quad \text{and} \quad \sigma_{\text{A}} = e^{N_{\text{PNN}}/RT}
\]

for \( A \), and

\[
K_{P_{\text{B}}-\text{B}} = e^{-\Delta H_{\text{BB}}/RT} \quad \text{and} \quad \sigma_{\text{B}} = e^{N_{\text{PBB}}/RT}
\]

for \( B \).

For the extra equilibrium constant for the copolymerization, we write:

\[
K_{P_{\text{B}}-\text{A}} = e^{-\Delta H_{\text{AB}}+T\Delta S_{\text{AB}}}/RT
\]

The copolymerization is thus described using the two additional energy terms \( \Delta H_{\text{AB}} \) and \( \Delta S_{\text{AB}} \).

In the current manuscript we took all entropy terms equal, i.e., \( \Delta S_{\text{AA}} = \Delta S_{\text{AB}} = \Delta S_{\text{BB}} = \Delta S \).

E) Mass-balance analysis results and discussion

In order to simulate systems similar to poly(\( S-1 \)) and poly(\( S-2 \)) (Figures S16e, S16f), we used for the hypothetical poly(\( A \)) and poly(\( B \)) as simple parameters as possible, applying different parameters for the two homopolymers just where needed. To do so, we selected the same entropic term for the two homopolymerizations and varied just the enthalpy of elongation \( (\Delta H) \), to account for the difference in elongation temperature between poly(\( S-1 \)) and poly(\( S-2 \)), and the nucleation penalties \( (\text{NP}) \), to account for the difference in shape of the poly(\( S-1 \)) and poly(\( S-2 \)) cool curves around their respective elongation temperatures. The values selected are the following:

poly(\( A \)): \( \Delta H_{\text{AA}} = -53 \text{ kJ mol}^{-1}, \Delta S_{\text{AA}} = -0.06 \text{ kJ mol}^{-1}, N_{\text{PNN}} = -40 \text{ kJ mol}^{-1} \)

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poly(B): $\Delta H_{BB} = -50 \text{ kJ mol}^{-1}, \Delta S_{BB} = -0.06 \text{ kJ mol}^{-1}, N_{P_B} = -20 \text{ kJ mol}^{-1}$

The resulting homopolymer cooling curves for $c = 25 \mu\text{M}$ (Figure S22a) reasonably match the cooling curves of respectively poly(S-1) and poly(S-2) (Figures S16e, S16f) at that same concentration. Varying the value of $\Delta H_{AB}$ between $-53 \text{ kJ mol}^{-1} \leq \Delta H_{AB} \leq -25 \text{ kJ mol}^{-1}$ (while keeping $\Delta S_{AB}$ equal to $\Delta S_{BB}$ and $\Delta S_{AB}$) we could simulate how a copolymerization cooling curve would look like (Figure S22b). Low negative $\Delta H_{AB}$ corresponds to low tendency of mixing while high negative $\Delta H_{AB}$ values correspond to an enthalpic gain in the cointeraction. We observed that with $\Delta H_{AB} \geq -30 \text{ kJ mol}^{-1}$ the cooling curves show low $T_e$ and an additional transition (Figure S22b, black curve), similarly to what is predicted by the linear combination of cooling curves (Figures 3b main text, S22e, black curve). With $-40 \text{ kJ mol}^{-1} \leq \Delta H_{AB} \leq -31 \text{ kJ mol}^{-1}$ the systems show only one transition, $T_e$, and the cooling curves display an almost linear behavior (Figure S22b, light blue curve). Increasing further the tendency to co-interact (more negative $\Delta H_{AB}$ values) the $T_e$ shifts to higher temperatures and the cooling curves display a more cooperative behavior (Figure S22b, pink, orange curves).

The model can also predict how the two monomers are incorporated in the copolymer chain as a function of the temperature (Figure S22c). We selected 4 different copolymers characterized by the following enthalpic values: $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$ (low gain to co-interact); $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$ (modest gain to co-interact); $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ (moderate gain to co-interact) and $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$ (high gain to co-interact). We noticed that for $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$ the monomer B begins to polymerize where the second transition occurs, suggesting indeed a self-sorting of the system. Increasing the enthalpic gain, the two individual monomers cooling curves get closer and closer, at high negative $\Delta H_{AB}$ the two curves overlaps.

Based on the linear shape of the experimental cooling curve and on its $T_e$ (that coincides with the $T_e$ of the hypothetical self-sorting (main text Figure 3b, Figure S22e)) we hypothesize that our system is similar to the simulated copolymer corresponding to $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$ (main text, Figure 5c). Additionally, the comparison of the incorporation of the two monomers in the copolymer chain obtained by the decomposition of the CD cooling curve (main text Figure 5b, Figure S22d) with the simulation predicted for $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$ supports our speculation.

Additionally, we calculated for the four cases of interest ($\Delta H_{AB} = -25 \text{ kJ mol}^{-1}, -37 \text{ kJ mol}^{-1}, -42 \text{ kJ mol}^{-1}, -47 \text{ kJ mol}^{-1}$) the polydispersity of the system (Figure S23). We simulated the number distribution as ‘equivalent concentration’ per degree of polymerization (number of monomeric units in the supramolecular copolymer) for the copolymers at 50 °C and 20 °C. The model predicts the bimodal distribution for the case of self-sorted polymers with $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$ (Figure S23a) and single distribution for the other cases (Figure S23b-d). All the systems, as expected, are polydisperse but the dispersity varies with $\Delta H_{AB}$ displaying larger distribution for more negative values of $\Delta H_{AB}$ (Figure S23e, S23f).
Figure S22. Comparison between theoretical model and experimental curves. Theoretical cooling curves of (a) homopolymers poly(A) (orange lines) and poly(B) (green lines). Simulation for $c_{S^{-2}} = c_{S^{-1}} = 25$ μM (light green and light orange lines) and for $c_{S^{-2}} = c_{S^{-1}} = 50$ μM (dark green and dark orange lines). Theoretical cooling curves of (b) the relative copolymers poly[(A)$_{0.5}$-co-(B)$_{0.5}$] ($c_{tot} = 50$ μM) while varying $\Delta H_{AB}$. (c) Analysis of the concentration of polymerized monomers A and B in poly[(A)$_{0.5}$-co-(B)$_{0.5}$] ($c_{tot} = 50$ μM) as a function of temperature for different values of $\Delta H_{AB}$. (d) Concentration of polymerized monomer $S^{-1}$ and $S^{-2}$ in poly[(S$^{-1}$)$_{0.5}$-co-(S$^{-2}$)$_{0.5}$] as a function of temperature. Values for S-1 and S-2 calculated on the experimental cooling curve via decomposition of the CD cooling curve. (e) Experimental cooling curve (blue line) for poly[(S$^{-1}$)$_{0.5}$-co-(S$^{-2}$)$_{0.5}$] $c_{tot} = 50$ μM and the linear combination (black dotted curve) calculated as [poly(S-1) + poly(S-2)] $c_{tot} = 50$ μM, $c_{S^{-1}} = c_{S^{-2}} = 25$ μM.
Figure S23. Number distribution of supramolecular copolymers. Number distribution of poly[(A)$_{0.5}$-co-(B)$_{0.5}$] at 50 °C (red lines) and 20 °C (black lines) for different $\Delta H_{AB}$: (a) $\Delta H_{AB} = -25$ kJ mol$^{-1}$, (b) $\Delta H_{AB} = -37$ kJ mol$^{-1}$, (c) $\Delta H_{AB} = -42$ kJ mol$^{-1}$, (d) $\Delta H_{AB} = -47$ kJ mol$^{-1}$. Comparison of the number distribution at (e) 50 °C and (f) 20 °C.
For 4 copolymers poly[(A)_{0.5}-co-(B)_{0.5}] corresponding to: $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$; $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$; $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$, we run stochastic simulations in order to visualize the copolymer microstructures. The simulations were performed from two different starting points, free monomer, and pre-assembled homopolymers, where we followed 10,000 monomers (5000 A and 5000 B) during $10^{11} - 10^{12}$ reaction steps. We assumed diffusion-controlled association rate constants ($10^{10} \text{ M}^{-1}\text{s}^{-1}$) for all monomer association and dimer formation reactions. The simulations give distributions of lengths of copolymers with different microstructures based on $\Delta H_{AB}$ (Figures S24a, S24c, S25a, S25c). The evolution of the system was followed monitoring the fraction of A-B contacts in the copolymers as a function of time (e.g., for a copolymer $\text{AABBBBBA}$ the fraction of A-B contact is 0.25, i.e., 2 out of 8 contacts are A-B, while 1 is AA and 5 are BB). It is important to note that the fraction of A-B contacts as a function of time displays that both starting from monomers and from assembled homopolymers the system reaches the same copolymer microstructure rapidly (Figures S24b, S25b, S25d, main text Figure 5d), indicating that the equilibrium is reached well within the time scale of the simulations.

The simulations were performed for $c = 50 \mu\text{M}$ at 50 °C. The simulations were performed at 50 °C to avoid working with longer polymers at lower temperature. However, the ratio of A-B contact is not highly influenced by the temperature of these simulations.

As theorized, the system with the smallest enthalpy gain upon A-B contact formation ($\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$) shows self-sorting of poly(A) and poly(B) with few di-block copolymers (Figures S24a, S24b). For $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$ the system shows a multi-block-structure (Figures S24c, main text Figure 5e) supporting our hypothesis. For larger enthalpy gains, the system shows a random distribution (Figures S25a, S25b) with the fraction A-B contacts around 0.5 for $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and a predominantly alternated organization (Figures S25c, S25d) with the fraction A-B contacts close to 1 for $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$.
Figure S24. Stochastic simulation. Polymer distributions and microstructures for (a) $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$ and (c) $\Delta H_{AB} = -37 \text{ kJ mol}^{-1}$. Evolution on time of the fraction of $AB$ contacts in the copolymer starting from free monomers (blue x) or from assembled homopolymers (light blue cross) for (b) $\Delta H_{AB} = -25 \text{ kJ mol}^{-1}$. 
Figure S25. Stochastic simulation. Polymer distribution and microstructure for (a) $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and (c) $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$. Evolution on time of the fraction of $AB$ contacts in the copolymer starting from free monomers (blue x) or from assembled homopolymers (light blue cross) for (b) $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and (d) $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$. 

$\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$

$\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$

Figure S25. Stochastic simulation. Polymer distribution and microstructure for (a) $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and (c) $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$. Evolution on time of the fraction of $AB$ contacts in the copolymer starting from free monomers (blue x) or from assembled homopolymers (light blue cross) for (b) $\Delta H_{AB} = -42 \text{ kJ mol}^{-1}$ and (d) $\Delta H_{AB} = -47 \text{ kJ mol}^{-1}$. 

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10. CD spectroscopy on iPAIN environment

Since iPAIN microscopy (main text Figures 6, 8) is performed on concentrated solutions (poly[(S-1)_{0.5-co-(S-2)_{0.5}}, c = 200 μM in MCH) and in presence of small percentage of polar solvents (0.5% DMSO and 1% i-PrOH),\(^4\) we aimed to verify that these conditions are representative for the dilute solutions (c = 50 μM in decalin) used for the spectroscopic studies (main text Figures 2-5, 7, supporting information Figures S2, S4, S19, S27, S28).

We hence measured the CD spectra of poly(S-1) and poly(S-2) in MCH (c = 200 μM) at 20 °C confirming the same features reported in dilute conditions (Figure S26a vs. Figure 2b main text). 0.5 % of DMSO and 1% of i-PrOH were then added to the solutions, which were then shook and transferred to the cuvette. Due to the high absorbance, we performed the CD analysis in 1 mm cuvette. The presence of protic solvents partially denatures the assembly of the homopolymers, which display lower CD values but unaltered CD features (Figure S26a vs. Figure S26b, green and red lines). We then combined poly(S-1) and poly(S-2) in a 1:1 ratio at 40 °C, shook and measured the CD spectrum at 20 °C. The CD spectrum recorded for poly[(S-1)_{0.5-co-(S-2)_{0.5}}] (c = 200 μM) in pure MCH confirmed the formation of the copolymer displaying the expected CD spectrum (Figure S26a, blue line vs. Figure 2b main text). The CD spectrum recorded for poly[(S-1)_{0.5-co-(S-2)_{0.5}}] (c = 200 μM) in MCH mixed with 0.5% DMSO and 1% i-PrOH in volume verified that the different environment does not affect the formation of the copolymer significantly, showing a spectrum comparable with the one reported in absence of protic solvents (Figure S26c).
Figure S26. CD spectroscopy in iPINT environment. CD spectra of poly(S-1) (red lines), poly(S-2) (blue lines) and poly[(S-1)_{0.5-co-(S-2)_{0.5}}] (blue and black line, respectively) in (a) MCH (c = 200 μM) and in (b) MCH with 0.5% DMSO, 1% i-PrOH (v/v) (c = 200 μM). Comparison of the CD spectra of poly[(S-1)_{0.5-co-(S-2)_{0.5}}] obtained in MCH (c = 200 μM, blue line) and in MCH with 0.5% DMSO, 1% i-PrOH (v/v) (c = 200 μM, black line). Measurements performed at 20 °C in 1 mm cuvette.
11. Self-sorting through stepwise addition of homopolymers at low temperature and supramolecular copolymerization through heating

In order to kinetically trap the self-sorted supramolecular homopolymers, we performed the stepwise addition of poly(S-2) to poly(S-1) at 20 °C in 1:1 ratio.

At 20 °C the CD spectrum overlaps perfectly with the linear combination (Figure S27a) and the emission spectrum does not show the amplified emission observed at 40 °C (Figure S27b).

The self-sorted solution \([0.5 \times \text{poly}(S-1) + 0.5 \times \text{poly}(S-2)]\) evolves into the supramolecular copolymer poly[(S-1)\(_{0.50}\)-co-(S-2)\(_{0.50}\)] via heating or equilibration. The self-sorted solution obtained at 20 °C was slowly heated (heating rate: 15 °C h\(^{-1}\)) and monitored through CD and fluorescence spectroscopy. The heating curve obtained following the CD maximum between 20 °C and 100 °C shows a transition at 25 °C (main text Figure 7c). The transition corresponds to a small but net decrease in CD intensity and a sharp increase in fluorescence (Figures S28a, S28b, respectively). At temperatures higher than 30 °C the CD heating curve, CD and fluorescence measurements match with the same experiments performed with the supramolecular copolymers solution obtained by slow-cooling (Figures S28a, S28b).

Alternatively, the copolymerization was performed allowing the self-sorted solution to equilibrate at 20 °C. We monitored the evolution following the fluorescence emission over time. The transition from \([0.5 \times \text{poly}(S-1) + 0.5 \times \text{poly}(S-2)]\) to poly[(S-1)\(_{0.50}\)-co-(S-2)\(_{0.50}\)] is characterized by a progressive enhancement of fluorescence intensity and the vanishing of the shoulder of poly(S-1) emission at \(\lambda = 360\) nm (Figures S28c, S28d).
Figure S27. Comparison of the spectra obtained by addition at 20 °C with the copolymer spectra obtained via slow cooling. (a) CD and (b) emission spectra recorded at 20 °C. (c) CD and (d) emission spectra recorded at 40 °C. Measurements performed in decalin isomer mixture, microcuvette 1 cm × 1 mm, $c_{\text{tot}} = 50 \ \mu\text{M}$, $c_{S1} = c_{S2} = 25 \ \mu\text{M}$, $\lambda_{\text{exc}} = 320 \ \text{nm}$
Figure S28. Self-sorting by addition of homopolymers at 20 °C and subsequent copolymerization. (a) CD and (b) emission spectra as a function of temperature while heating (measurements performed in decalin isomer mixture, microcuvette 1 cm × 1 mm, heating rate 15 °C h⁻¹, \(c_{tot} = 50 \mu\text{M}, c_{S-1} = c_{S-2} = 25 \mu\text{M}\)). (c) Emission spectra and (d) emission intensity at \(\lambda = 382\) nm at 20 °C as a function of time. Measurements performed in decalin isomer mixture, microcuvette 1 cm × 1 mm, \(c_{tot} = 50 \mu\text{M}, c_{S-1} = c_{S-2} = 25 \mu\text{M}, \lambda_{exc} = 320\) nm).
12. References

(1) Adelizzi, B.; Filot, I. A. W.; Palmans, A. R. A.; Meijer, E. W. Chem. A Eur. J. 2017, 23 (25), 6103–6110.

(2) Belov, V. N.; Mitronova, G. Y.; Bossi, M. L.; Boyarskiy, V. P.; Hebisch, E.; Geisler, C.; Kolmakov, K.; Wurm, C. A.; Willig, K.; Hell, S. W. Chem. A Eur. J. 2014, 20 (41), 13044.

(3) Alo, A.; Vargas Jentzsch, A.; Vilanova, N.; Albertazzi, L.; Meijer, E. W.; Voets, I. K. J. Am. Chem. Soc. 2016, 138 (9), 2953–2956.

(4) Adelizzi, B., Alo, A., Van Zee, N. J., Palmans, A.R.A., Meijer, E.W., Voets, I. K. ACS Nano 2018, DOI: 10.1021/acsnano.8b00396

(5) Unpublished results

(6) Das, A.; Vantomme, G.; Markvoort, A. J.; ten Eikelder, H. M. M.; Garcia-Iglesias, M.; Palmans, A. R. A.; Meijer, E. W. J. Am. Chem. Soc. 2017, 139 (20), 7036–7044.

(7) Claridge, T. D. W.; High-Resolution NMR Techniques in Organic Chemistry; 2016; 315–380.

(8) Smulders, M. M. J.; Schenning, A. P. H. J.; Meijer, E. W. J. Am. Chem. Soc. 2008, 130 (2), 606–611.

(9) ten Eikelder, H. M. M.; Markvoort, A. J.; de Greef, T. F. A.; Hilbers, P. A. J. J. Phys. Chem. B 2012, 116 (17), 5291–5301.

(10) Markvoort, A. J.; Eikelder, H. M. M. ten; Hilbers, P. A. J.; de Greef, T. F. A. ACS Cent. Sci. 2016, 2 (4), 232–241.

(11) De Greef, T. F. A.; Smulders, M. M. J.; Wolffs, M.; Schenning, A. P. H. J.; Sijbesma, R. P.; Meijer, E. W. Chem. Rev. 2009, 109 (11), 5687–5754.