

Supporting Information for

Co-crystal Formation Between Poly (ethylene glycol) and Small Molecular Drug Griseofulvin

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Materials and Sample Preparation

Griseofulvin (Gris) was purchased from Alfa Aesar (Lot #G8629A, 97% purity). Poly (ethylene glycol) (PEG), with number-average weight of 400, 2000, 6000 and 20000 g/mol was obtained from Alfa Aesar. All the materials were used without further purification.

Griseofulvin and PEG were dried in a desiccator over phosphorus pentoxide powder for at least one week before use. Physical mixture of griseofulvin/PEG (except for PEG 400 which is in the liquid state at room temperature) was prepared by the solvent evaporation method. Griseofulvin and PEG with different weight ratios (total weight 1.0 g) were dissolved in 20 ml methylene dichloride, and stirred for 2 hours at room temperature to ensure complete dissolution. The solvent was then removed using a rotary evaporator. The solid dispersions obtained were collected and further dried under vacuum at room temperature for 24 h to remove residual solvent. The resulted materials were milled gently with a pestle and mortar, and stored again in a desiccator over phosphorus pentoxide powder at room temperature before use.

Samples for hot stage microscopy (HSM) observation were prepared by melting 20-30 mg of Gris/PEG solid dispersion powder at 220 °C for 1 min on a clean microscope cover slip (18 mm×18 mm). Gris/PEG 400 sample was prepared by directly mixing a proper amount of Gris and PEG 400 on a cover slip and heating to melt. The melt was then covered with another cover slip to obtain a sandwiched sample and was quenched to predesigned temperatures. The sandwiched samples were then annealed at these predesigned temperatures to observe the crystallization behavior.

Gris/PEG solid dispersion powders were directly used for differential scanning calorimetry (DSC) analysis. 3-5 mg of solid dispersion powder was sealed in an aluminum pan and heated to 230 °C to generate homogeneous melt, followed by quenching and annealing at specific temperatures.

To prepare samples for powder X-ray diffraction (PXRD) and nuclear magnetic resonance spectroscopy (NMR) study, proper amount of Gris/PEG solid dispersion powder was spread out on a clean cover slip and then covered with a piece of aluminum foil before melt. After melting and being held at about 230 °C for 1-2 min to generate a homogeneous melt, these samples were allowed to quench to 0 °C by contact with an aluminum plate as described above, and then to solidify at room temperature before the aluminum foil was gently removed to expose a free surface. The samples were annealed at pre-designed temperatures and etched with de-ionized water to completely remove excess crystalline PEG. Co-crystals obtained on cover slips can be directly observed by HSM or collected for PXRD analysis and NMR characterization.

Samples for X-ray fiber diffraction were prepared by melting a proper amount of Gris/PEG solid dispersion powder between a cover slip and a polyimide (PI) film, followed by annealing on a hot-stage of 50-60 °C for 8-12 h until large spherulites formed. The PI film was gently removed and the sample was etched with de-ionized water to completely remove excess crystalline PEG.

Characterization

Polarized optical microscopy (XS-402, Nikon) equipped with a digital camera (WV-GP420, Panasonic) and a hot stage (KEL-XMT-3100, WEITU, China), was used to observe the crystallization behavior of Gris/PEG system.

Thermal analysis was conducted with differential scanning calorimeter (DSC-60, SHIMADZU). Temperature calibration was carried out using an indium metal standard supplied with the instrument. Gris/PEG solid dispersion powders were weighed out (2-2.5 mg) in aluminum pans and analyzed from 25 °C to 240 °C at heating and cooling rates both of 10 °C/min using a similar empty pan as a reference. An inert atmosphere was maintained in the calorimeter by purging nitrogen gas at a flow rate of 50 mL/min.

Powder X-ray diffraction measurement was carried out at room temperature on a diffractometer (D/max-2500, Rigaku), with scans taking at a speed of 2° 2 θ /min and 0.02° 2 θ

per step under Cu K α radiation.

X-ray fiber diffraction measurement was carried out at room temperature on a single-crystal diffractometer (R-Axis-Spider, Rigaku) under Mo K α radiation.

^1H NMR analysis was performed on a nuclear magnetic resonance spectrometer (JNM-ECA300, JOEL). Deuterated DMSO was used as solvent.

All solid-state NMR spectra were acquired using a Bruker AV 300 spectrometer operating at 75.4MHz for ^{13}C (7T static magnetic field). Samples were packed into a 4mm ZrO₂ rotor and sealed with Kel-F caps. Experiments were performed using 4mm double resonance MAS probe. All ^{13}C spectra were acquired under magic angle spinning (MAS) at 5 kHz, using ramped-amplitude CP and tppm decoupling. A 3 ms (100 μs for PEG) contact time was used in experiments. Glycine was used as an external standard, with carbonyl resonance at 176.03ppm. All experiments were conducted at room temperature. ^1H T_1 relaxation times were measured using the saturation recovery experiment through ^{13}C observation. A 90 degree pulse width of 2.5 μs was used in the experiments. ^1H $T_{1\rho}$ relaxation times were measured by varying the spin-lock duration time following a 90 degree pulse. A recycle delay of about 2 times the measured T_1 was used to maximize the signal-to-noise ration.

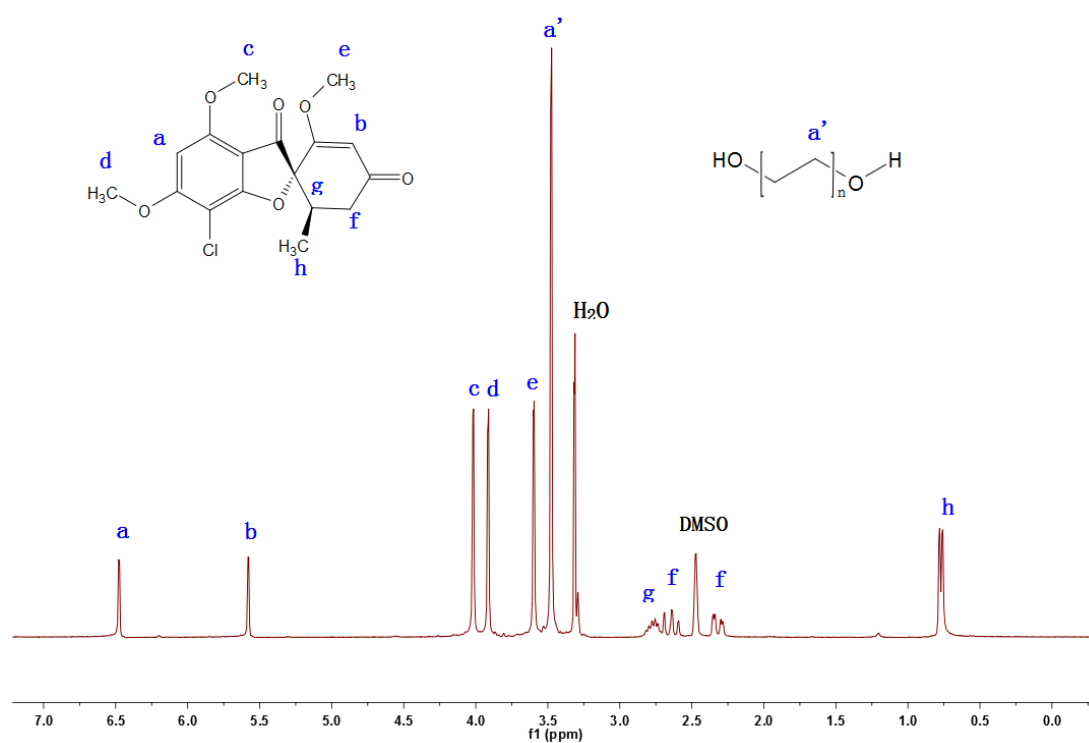


Figure S1. ¹H-NMR characterization of etched samples (50/50, w/w). Characteristic peaks corresponding to different protons in Gris and PEG molecules are marked with corresponding letters, indicating the presence of PEG molecules in crystal lattice.

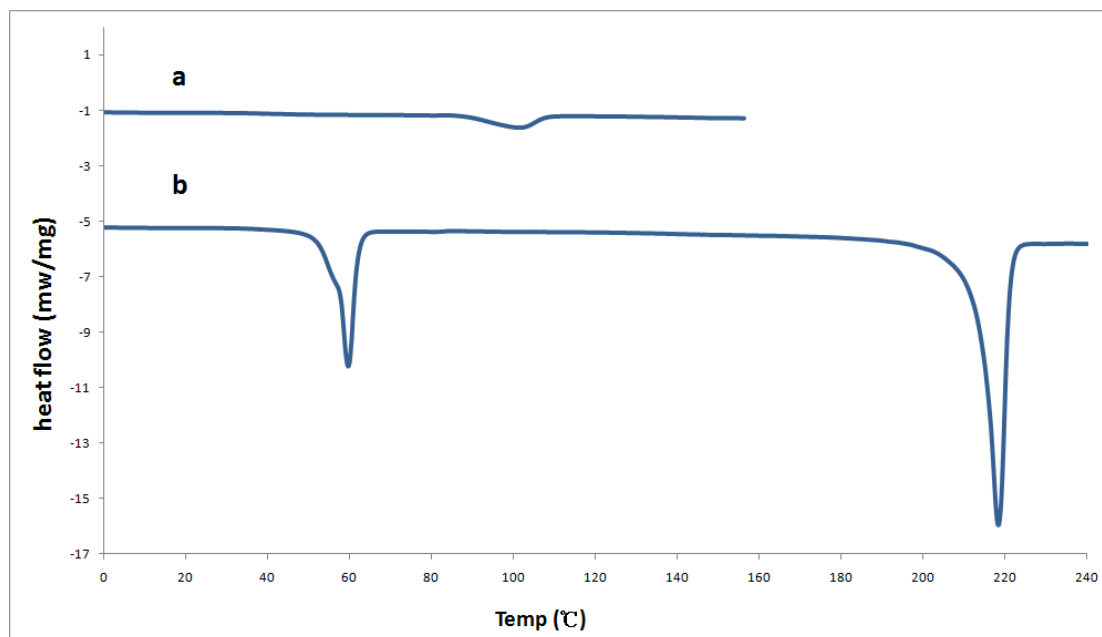
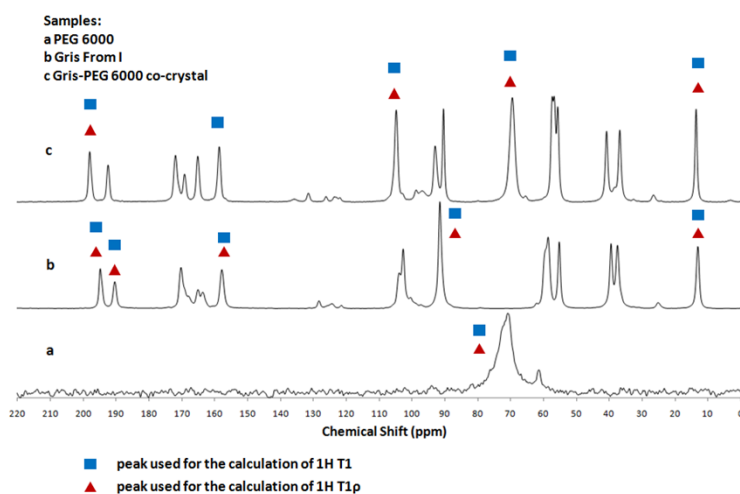


Figure S2. DSC thermograms of the etched sample. **a** first heating, a weak endothermal event corresponding to the dissociation of the co-crystal occurred around 110 °C. **b** second heating after storage at room temperature for 12 hours, melting peaks corresponding to PEG and Gris known polymorph can be seen clearly. Heating rate of 10 °C/min was used for these scans.

Table S1. The ^1H T_1 and ^1H $T_{1\rho}$ values of Gris Form I, PEG and Gris-PEG co-crystal



Sample	Relaxation Type	Integral region (ppm)	Relaxation Time ^a
Gris-PEG 6000 co-crystal	^1H T_1 (s)	201.744-195.442	0.899 (0.028)
		161.946-156.032	0.893 (0.028)
		109.545-101.660	0.965 (0.021)
		74.482-64.625	0.958 (0.019)
		16.959-10.641	0.902 (0.019)
	^1H $T_{1\rho}$ (ms)	202.704-195.745	9.47 (0.07)
		110.135-100.986	9.90 (0.18)
		75.746-63.665	8.42 (0.13)
		18.661-8.787	10.65 (0.17)
Gris (Form I)	^1H T_1 (s)	200.598-192.965	0.837 (0.012)
		192.965-186.461	0.729 (0.007)
		161.255-152.847	0.722 (0.012)
		95.914-85.973	0.711 (0.005)
		17.970-9.562	0.684 (0.010)
	^1H $T_{1\rho}$ (ms)	198.862-192.830	307 (2.4)
		192.830-186.411	530 (4.3)
		161.137-153.589	497 (4.1)
		96.234-85.670	487 (4.5)
		18.122-10.573	443 (2.9)
PEG 6000	^1H T_1 (s)	103.530-32.646	4.91 (0.03)
	^1H $T_{1\rho}$ (ms)	113.825-41.980	0.365 (0.007)

^aThe numbers in parentheses indicate the standard error associated with the fit.

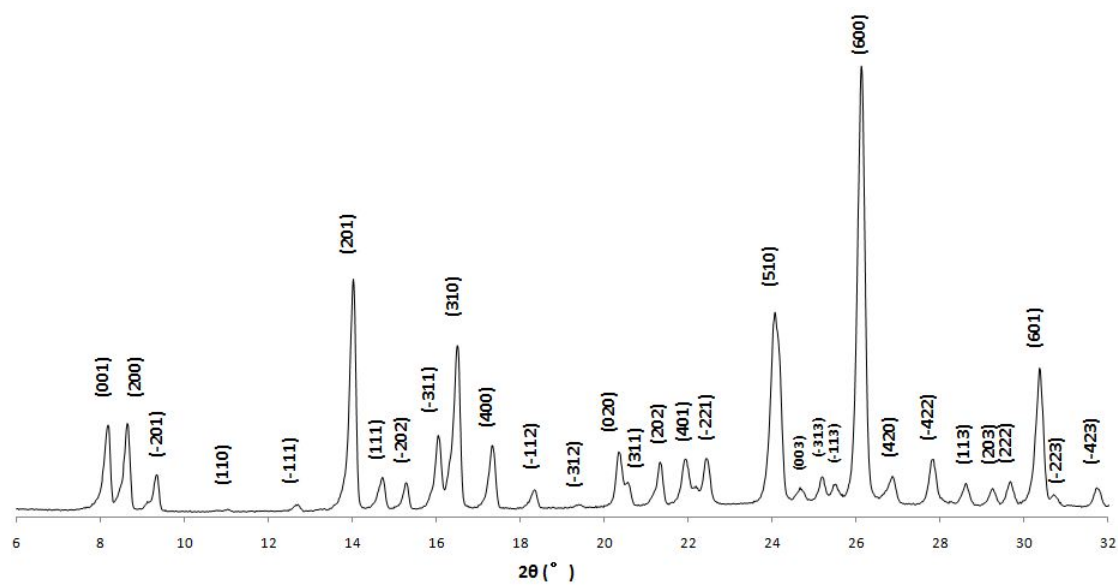


Figure S3. The indexing results of the griseofulvin-PEG co-crystal diffractogram by using parameters: monoclinic crystal, space group C2, $a = 22.169 \text{ \AA}$, $b = 8.716 \text{ \AA}$, $c = 11.730 \text{ \AA}$, $\beta = 112.73^\circ$. Computer program: *Jade 6.0*, Materials Data, Inc.

Table S2. Comparison between the experimental data and the indexing results

$d(\text{exp})/\text{\AA}$	$d(\text{cal})/\text{\AA}$	$(h\ k\ l)$	$ d(\text{cal})-d(\text{exp}) /\text{\AA}$
10.8026	10.8194	(0 0 1)	0.0160
10.2277	10.2237	(2 0 0)	0.0040
9.4794	9.4811	(-2 0 1)	0.0017
8.0086	8.0180	(1 1 0)	0.0095
6.9853	6.9665	(-1 1 1)	0.0187
6.3123	6.3125	(2 0 1)	0.0002
6.0209	6.0204	(1 1 1)	0.0005
5.8005	5.7960	(-2 0 2)	0.0045
5.5211	5.5254	(-3 1 1)	0.0043
5.3741	5.3691	(3 1 0)	0.0050
5.1156	5.1118	(4 0 0)	0.0038
4.8343	4.8371	(-1 1 2)	0.0028
4.5714	4.5689	(-3 1 2)	0.0025
4.3626	4.3581	(0 2 0)	0.0045
4.3205	4.3149	(3 1 1)	0.0056
4.1642	4.1627	(2 0 2)	0.0015
4.0548	4.0561	(4 0 1)	0.0013
3.9623	3.9598	(-2 2 1)	0.0025
3.7015	3.7022	(5 1 0)	0.0008
3.6070	3.6065	(0 0 3)	0.0006
3.5341	3.5325	(-3 1 3)	0.0016
3.4951	3.4930	(-1 1 3)	0.0021
3.4090	3.4079	(6 0 0)	0.0011
3.3167	3.3164	(4 2 0)	0.0003
3.2064	3.2083	(-4 2 2)	0.0019
3.1186	3.1171	(1 1 3)	0.0014
3.0521	3.0513	(2 0 3)	0.0008
3.0097	3.0102	(2 2 2)	0.0005
2.9417	2.9411	(6 0 1)	0.0006
2.9100	2.9093	(-2 2 3)	0.0007
2.8186	2.8184	(-4 2 3)	0.0002

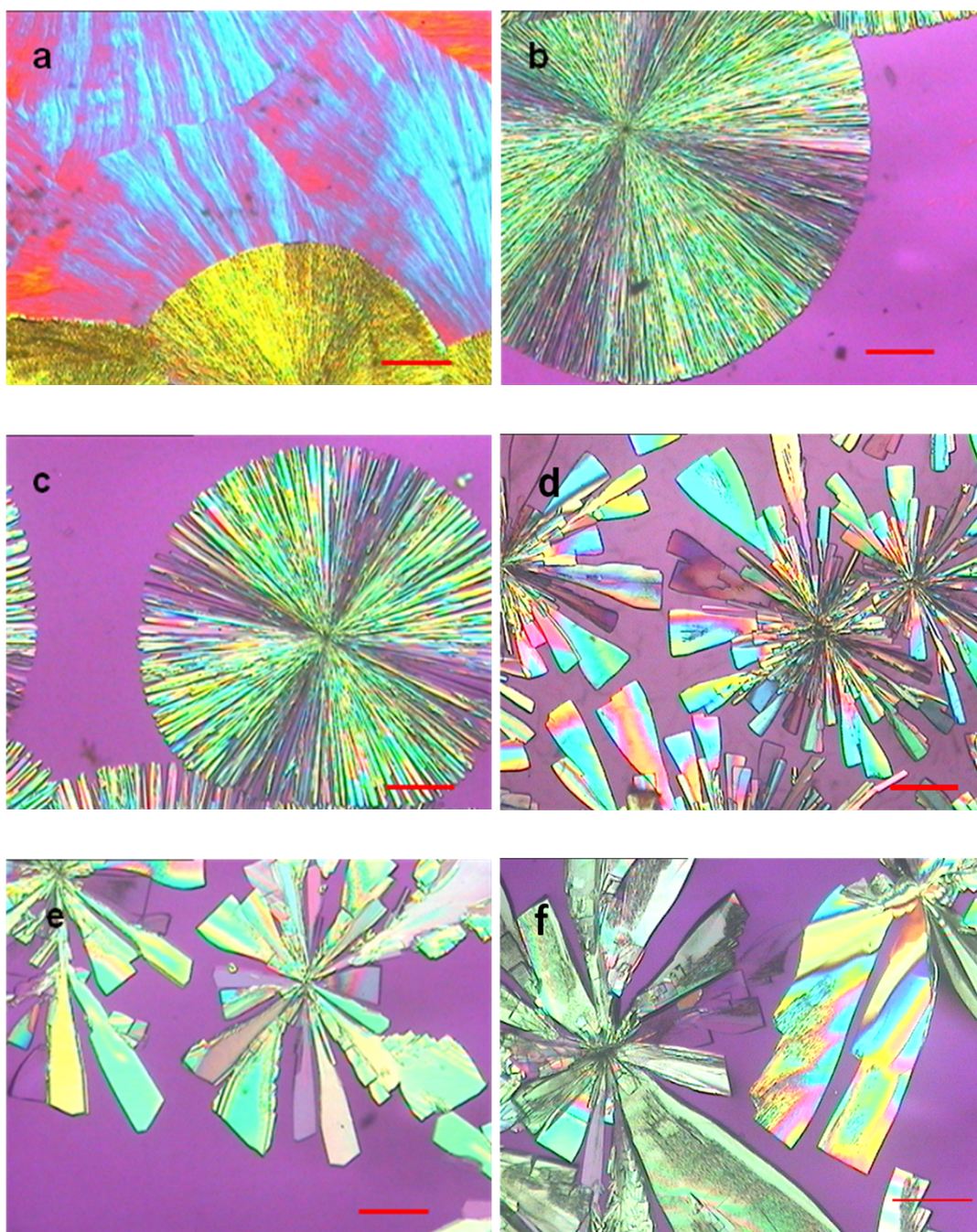


Figure S4. Gris/PEG 6000 co-crystals can be obtained in a wide range of annealing temperature with gradually changed morphologies (from **a** to **f**, 45 °C, 50 °C, 60 °C, 70 °C, 80 °C and 90 °C). At low annealing temperature (45 °C), it can be seen that, spherulites of co-crystals grew in the crystalline matrix of PEG, while at higher temperatures co-crystals grew in PEG melt. The identity of these co-crystals was confirmed by the melting behavior on heating. The bars in these pictures represent 200 μm .

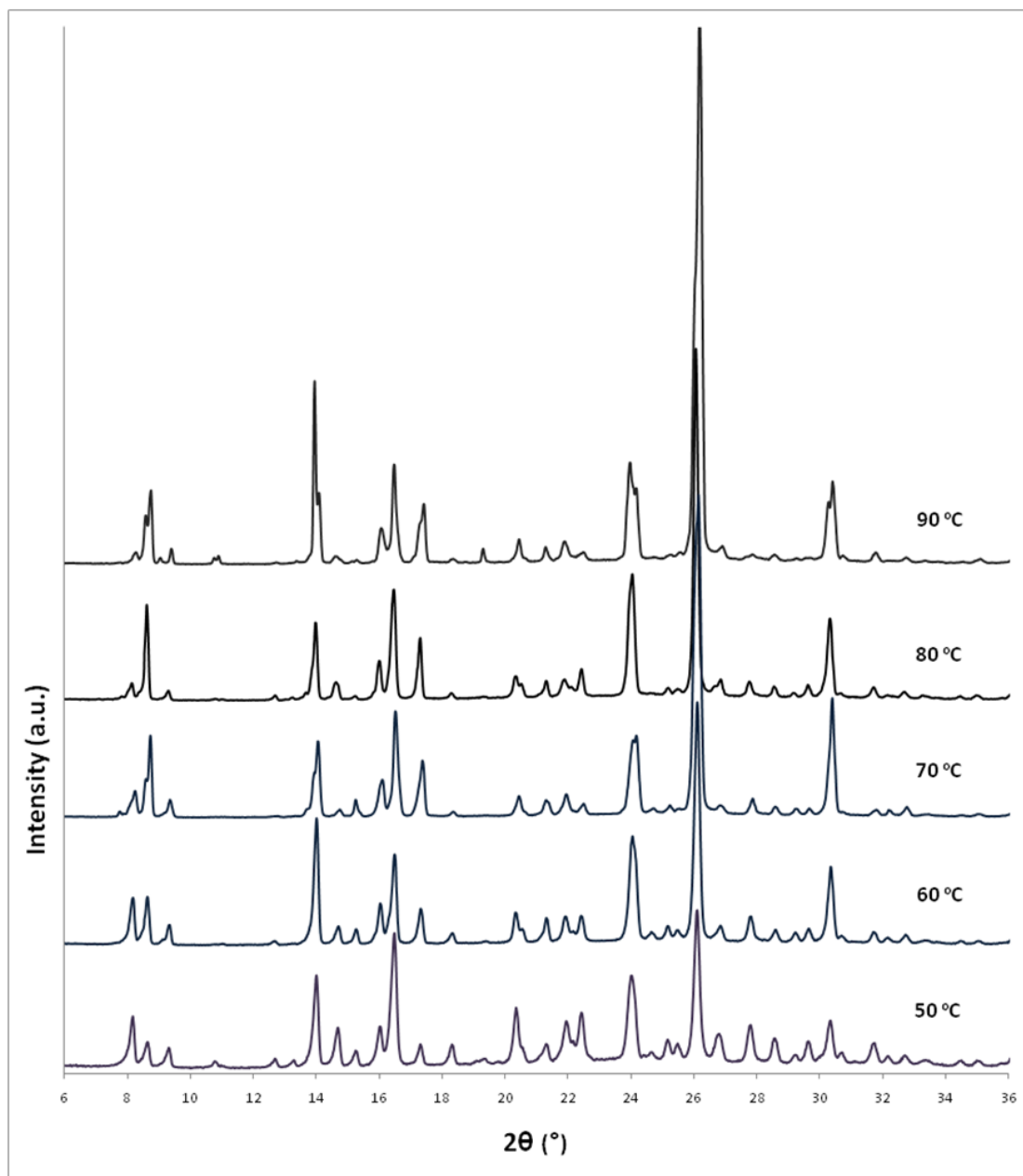


Figure S5. PXRD patterns of Gris/PEG 6000 co-crystals formed at different annealing temperatures (numbers on each curve indicates the annealing temperature of the sample).

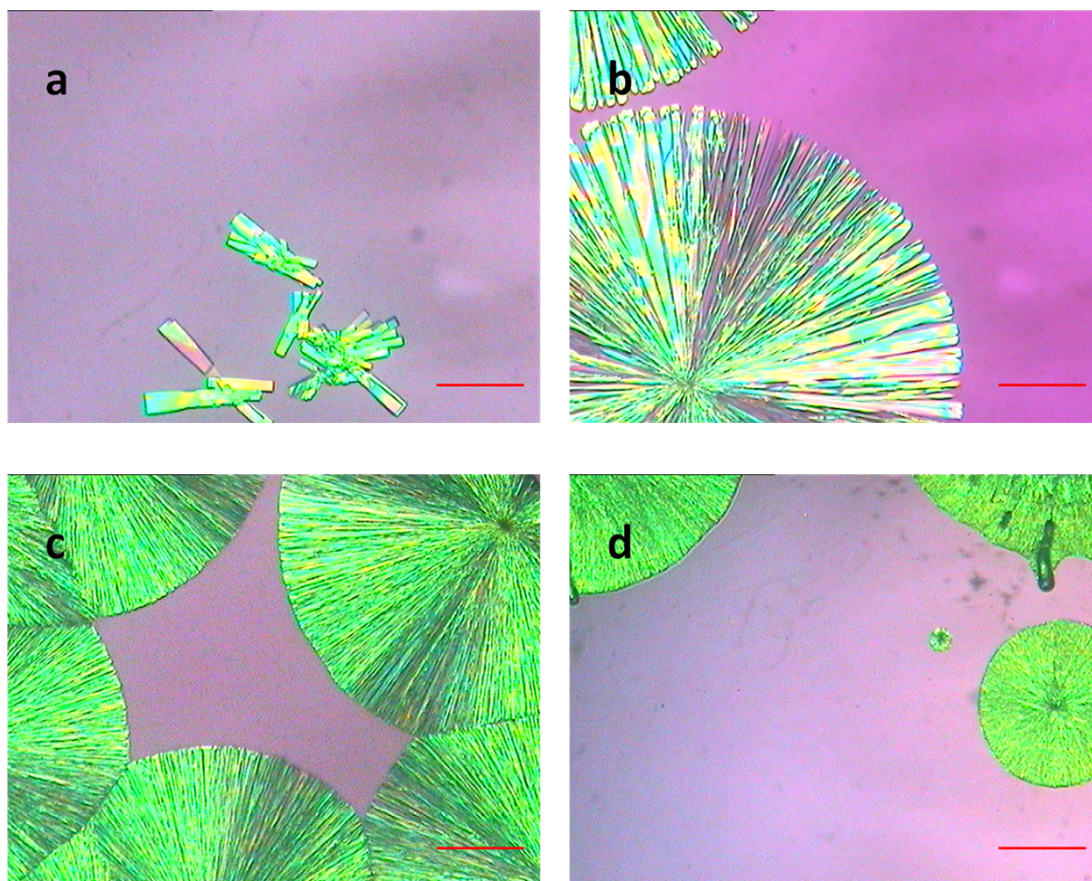


Figure S6. Formation of Gris/PEG 6000 co-crystals in Gris/PEG melt of different compositions.

a. 20/80 (w/w), 65 °C; **b.** 40/60 (w/w), 65 °C; **c.** 60/40 (w/w), 65 °C; **d.** 80/20 (w/w), 85 °C. The identity of these co-crystals was confirmed by the melting behavior on heating. The bars in these pictures represent 200 μm .

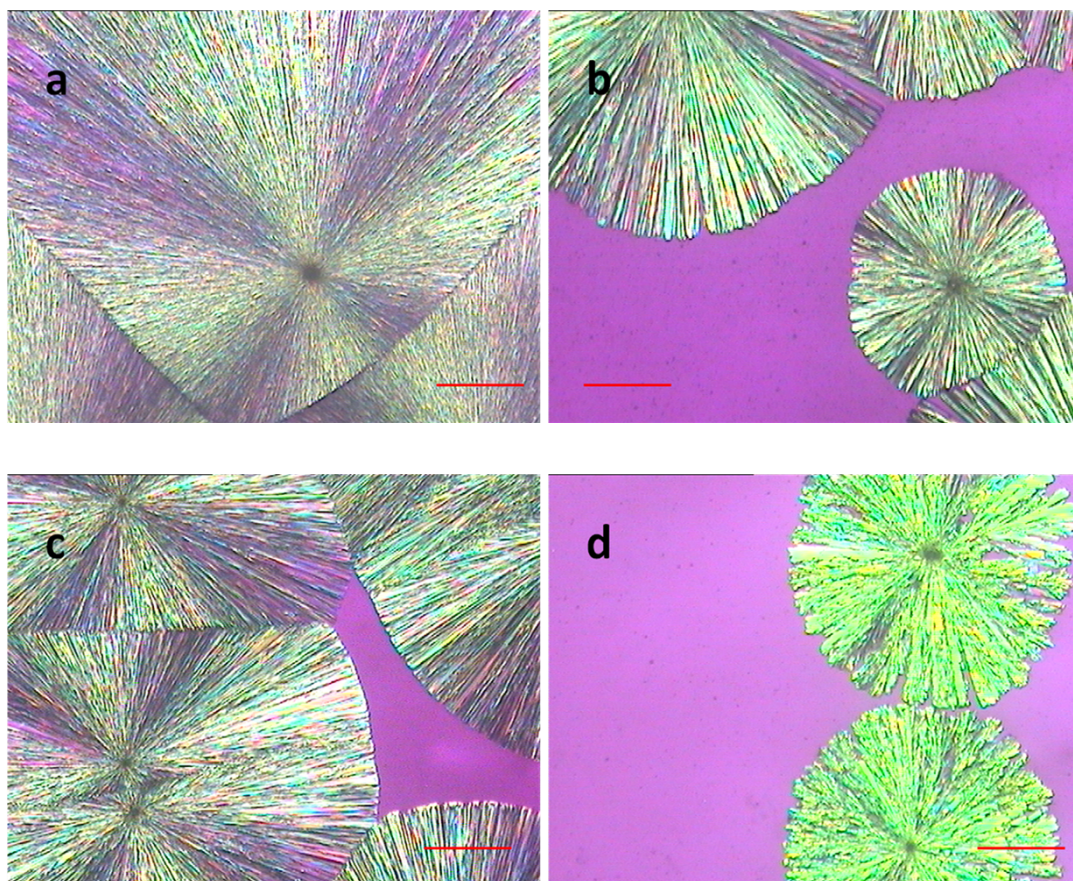


Figure S7. Co-crystals formed by Gris with PEG of different number-averaged molecular weight. **a.** PEG 400, 50/50 (w/w), r.t.; **b.** PEG 2000, 50/50 (w/w), 65 °C; **c.** PEG 6000, 50/50 (w/w), 65 °C; **d.** PEG 20000, 50/50 (w/w), 65 °C. The identity of these co-crystals was confirmed by the melting behavior on heating. The bars in these pictures represent 200 μm .

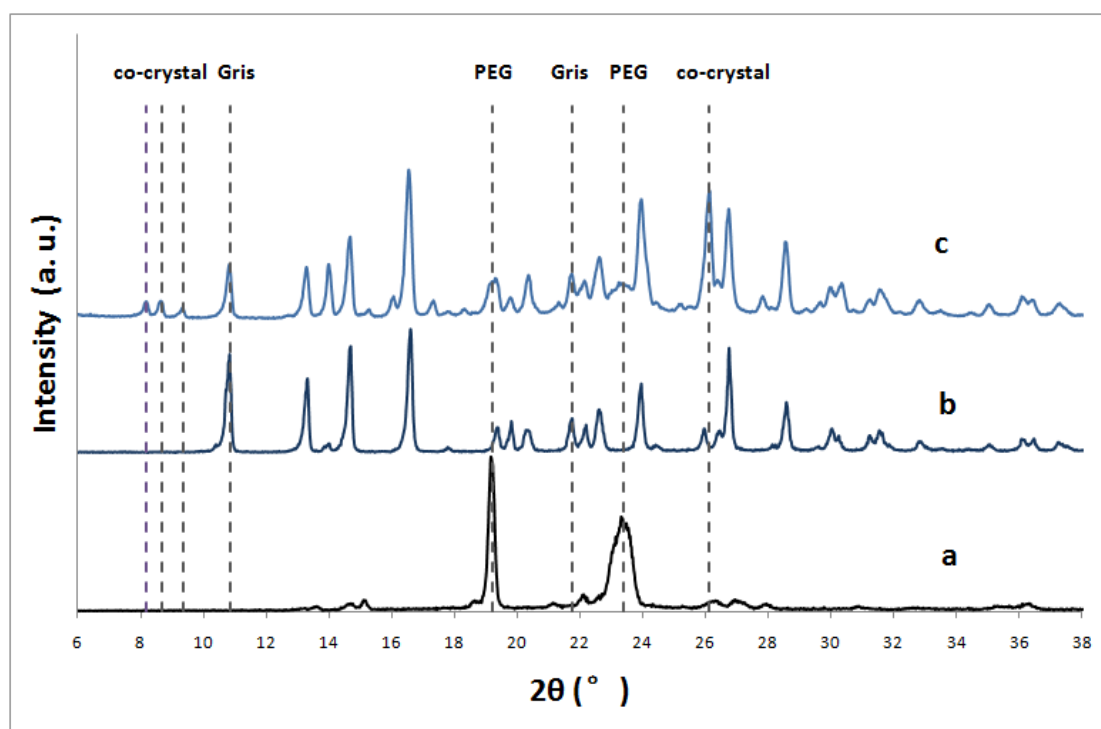


Figure S8. PXRD patterns of **a.** PEG 6000; **b.** Gris known polymorph; **c.** Gris/PEG 6000 co-crystal after storage at room temperature for 10 months. Characteristic peaks corresponding to each material are indicated by the dashed lines. The combination of reflections of PEG, Gris and Gris/PEG co-crystal in curve c clearly evidences a slow transformation from Gris/PEG co-crystal to the separated phases on long-time storage. All measurements were performed at room temperature.