

SUPPLEMENTARY MATERIALS

Co-crystallization Kinetics of 2:1 Benzoic Acid – Sodium Benzoate Co-crystal: The Effect of Templating Molecules in a Solution

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MATERIALS AND METHODS

Materials. Benzoic acid (HBz, > 99.5% purity, m.p.: 121–125 °C, MW: 122.12 g/mol, Lot: SZBE3380V) and sodium benzoate (NaBz, > 99% purity, m.p. > 300 °C, MW: 144.1 g/mol, Lot: SZBF2150V) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH, ≥ 98% purity, Lot: MA-7708-69) was purchased from Macron Fine Chemicals TM. All of the chemical reagents were used without any further purification.

Solvents. Ethanol (C₂H₅OH, 99.5% purity, b.p.: 78.37 °C, MW: 46.07 g/mol, Lot: 262611) was purchased from Echo Chemical Co. Ltd. (Taipei, Taiwan). Water was clarified by reverse osmosis (RO) using a water purification system, model Mili-RO plus, bought from Millipore (Billerica, MA, USA). All solvents were used without any further purification.

Reaction co-crystallization of 2:1 HBz-NaBz co-crystal without template. The co-crystallization of 2:1 HBz-NaBz co-crystals was conducted using the concept of direct assembly from the reaction of benzoic acid and sodium hydroxide [22].

Solution A was prepared by dissolving HBz in 50 mL of 4:1 (v/v) ethanol–water co-solvent at 25 °C (Table S1). Separately, Solution B was prepared by dissolving NaOH in 6 mL of 2:1 (v/v) ethanol–water co-solvent (Table S1). The higher amount of water in the co-solvent used for Solution B was necessary to completely dissolve NaOH pellets. Afterwards, solutions A and B were mixed together in a 100 mL round-bottom flask at 25 °C using a spin bar at 175 rpm for 1 h to dissolve all solids completely and eliminate all seeds. After 1 h of mixing, 2:1 HBz-NaBz co-crystals were not produced until the saturated solution was cooled from 25° to 16 °C along with the 5 L water bath, which gave a slow temperature gradient of 0.8 °C/min to avoid supercooling. The solution was maintained at 16 °C for up to 150 min at an agitation rate of 175 rpm, during which, the system underwent nucleation and crystal growth.

To understand the effect of supersaturation, co-crystallization experiments of 2:1 HBz-NaBz co-crystal were carried out at different initial degrees of supersaturation (S_0). S_0 was defined as the ratio between the initial concentration of the mother liquor, C_0 , at 16 °C and the solubility value of the co-crystal, C^* , at 16 °C, as shown in Equation (S1):

$$S_0 = \frac{C_0}{C^*} \quad (\text{S1})$$

The solubility value, C^* , of 2:1 HBz-NaBz co-crystal in 4:1 (v/v) ethanol–water co-solvent was determined to be 0.196 kg/L at 16 °C. Different S_0 values of 1.66, 1.54, 1.48, and 1.43 in 56 mL of 4:1 (v/v) ethanol–water co-solvent were used by adjusting the amounts HBz and NaOH dissolved in their respective Solutions A and B before mixing based on the corresponding formulations listed in Table S1.

Table S1. List of the theoretical amounts of produced 2:1 HBz-NaBz co-crystals and the amounts of HBz in Solution A and NaOH in Solution B required at different S_0 values.

S_0 at 16 °C	$C_0 \times 10^3$ (kg/L) at 25 °C	2:1 HBz-NaBz co-crystal product based on stoichiometry		HBz in solution A at 25 °C		NaOH in solution B at 25 °C	
		Mass (g)	Mol (mol)	Mol (mol)	Mass (g)	Mol (mol)	Mass (g)
1.66	326.007	18.256	0.047	0.141	17.223	0.047	1.880
1.54	302.441	16.937	0.044	0.131	15.978	0.044	1.744
1.48	290.657	16.277	0.042	0.126	15.355	0.042	1.677

1.43	282.802	15.837	0.041	0.122	14.940	0.041	1.631
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$C^* = 0.196 \text{ kg/L}$ at 16 °C in 4:1 (v/v) ethanol-water co-solvent

Template effect on the co-crystallization of 2:1 HBz-NaBz co-crystal. Three different dissolved templating molecules were studied: pure NaBz crystals, 2:1 HBz-NaBz co-crystals, and 1:1 HBz-NaBz co-crystals. These templating powders, with the amount of 4.2 mol% (1–5 wt%) with respect to the theoretical number of moles of 2:1 HBz-NaBz co-crystals, were completely dissolved into Solution A at 25 °C prior to mixing with Solution B. After all of the templating powders were dissolved, the solution was well mixed with Solution B. From this point on, the rest of the procedure was the same as explained in the previous section. The exact amounts of templating molecules which were added at different S_0 s are listed in Table S2.

Table S2. Amounts of templating molecules added into Solution A for the co-crystallization of 2:1 HBz-NaBz co-crystals.

S_0 at 16 °C	Mol of 2:1 HBz-NaBz co-crystal product based on stoichiometry (mol)	Templating molecules in Solution A at 25 °C					
		Purchased NaBz		Synthesized 2:1 HBz-NaBz co-crystal		Synthesized 1:1 HBz-NaBz co-crystal	
		Mol (mol)	Mass (g)	Mol (mol)	Mass (g)	Mol (mol)	Mass (g)
1.66	0.047	0.0020	0.288	0.0020	0.776	0.0020	0.532
1.54	0.044	0.0019	0.267	0.0019	0.720	0.0019	0.493
1.48	0.042	0.0018	0.257	0.0018	0.692	0.0018	0.474
1.43	0.041	0.0017	0.250	0.0017	0.673	0.0017	0.461

The templating powders of 2:1 HBz-NaBz co-crystal were prepared by dissolving 17.22 g (0.141 mol) of benzoic acid (HBz) in 50 mL of 4:1 (v/v) ethanol–water co-solvent and introducing a solution containing 1.88 g (0.047 mol) of NaOH dissolved in 6 mL of 2:1 v/v (v/v) ethanol–water co-solvent at 25 °C, based on the formulation at $S_0 = 1.66$ at 16 °C in Table S1. Then, the solution was cooled to 16 °C to crystallize out the pure 2:1 HBz-NaBz co-crystals [12]. The preparation of the templating powders of 1:1 HBz-NaBz co-crystal was performed by dissolving 5 g (0.0347 mol) of sodium benzoate in 10 mL of water at 25 °C. Then, 0.1 mL of 1 M HCl_(aq) was added into the sodium benzoate aqueous solution by a pipette every 30 s until the volume of HCl reached 2.8 mL [12]. All the templating powders were filtered, and oven dried at 40 °C overnight, and characterized.

Determination of Induction Time, Crystal Growth, and Characterizations of 2:1 HBz-NaBz Co-crystals. UV–Vis was employed to measure and monitor the concentrations of the 2:1 HBz-NaBz co-crystal solution at different time points during co-crystallization, starting at 16 °C. About 1 mL of a supersaturated solution or a slurry was withdrawn from the flask at every designated time point, quickly ultra-filtered by using a 0.22 μm PVDF syringe filter, and diluted with 4:1 v/v ethanol–water co-solvent for UV–Vis spectrophotometric analysis. Ultra-filtration separated out the solids from the mother liquor, while the dilution of the mother liquor prevented unwanted nucleation in the UV–Vis cuvette. The concentration measurement was based on the change of the absorbance intensity at 200 nm of the absorption peak. At the end

of the UV–Vis spectrophotometric measurement, a desupersaturation curve, as in Figure 3, was obtained. The end of the induction period was indicated by a concentration plummet between two consecutive time points. Crystal growth rate was determined by monitoring the concentration decrease from right at the end of induction period until the system reached the solubility concentration, C^* , of 0.196 kg/L at 16°C. Besides UV–Vis spectrophotometry, slurry samples were also examined by optical microscopy (OM) throughout the experiment. At the end of co-crystallization, 2:1 HBz-NaBz co-crystals were filtered and oven dried at 40 °C for 24 h for further characterization.

Instruments. Optical micrographs were taken by an Olympus BX-51 (Tokyo, Japan) equipped with a Moticam 2000 (Hongkong, China) digital camera. Fourier transform infrared (FTIR) spectroscopy was performed using the Perkin Elmer Spectrum One (Norwalk, CT, USA) by the KBr pellet method. TGA analysis was performed by the Perkin Elmer TGA 7 (Norwalk, CT, USA), with the samples placed on a platinum pan. The heating rate of 10 °C/min was used for TGA analysis. Powder X-ray diffraction (PXRD) patterns were collected by the Bruker AXS D8 Advance (Karlsruhe, Germany) using an X-ray source of $\text{CuK}\alpha_1$ ($\lambda = 1.54 \text{ \AA}$) and a scanning rate of 1°/min over an angular 2θ range of 5° to 35°. UV–Vis spectrophotometry was conducted on the Perkin Elmer Lambda 25 (Norwalk, CT, USA) to measure 2:1 HBz-NaBz co-crystal concentration in the mother liquor. The UV calibration curve was prepared by different concentrations of 2:1 HBz-NaBz co-crystal in 4:1 (v/v) ethanol–water co-solvent. A linear calibration curve was obtained within the concentration range of 0.001 to 0.006 mg/mL and was used throughout this study.

Software. Mercury 3.9 by the Cambridge Crystallography Data Centre (CCDC) and Diamond v3.2k by CrystalImpact GbR were used to visualize the crystal structure of the 2:1 HBz-NaBz co-crystal. KrystalShaper v1.5.0 by Steffen Weber, Ph.D. was used to visualize the theoretical morphology of the 2:1 HBz-NaBz co-crystal.

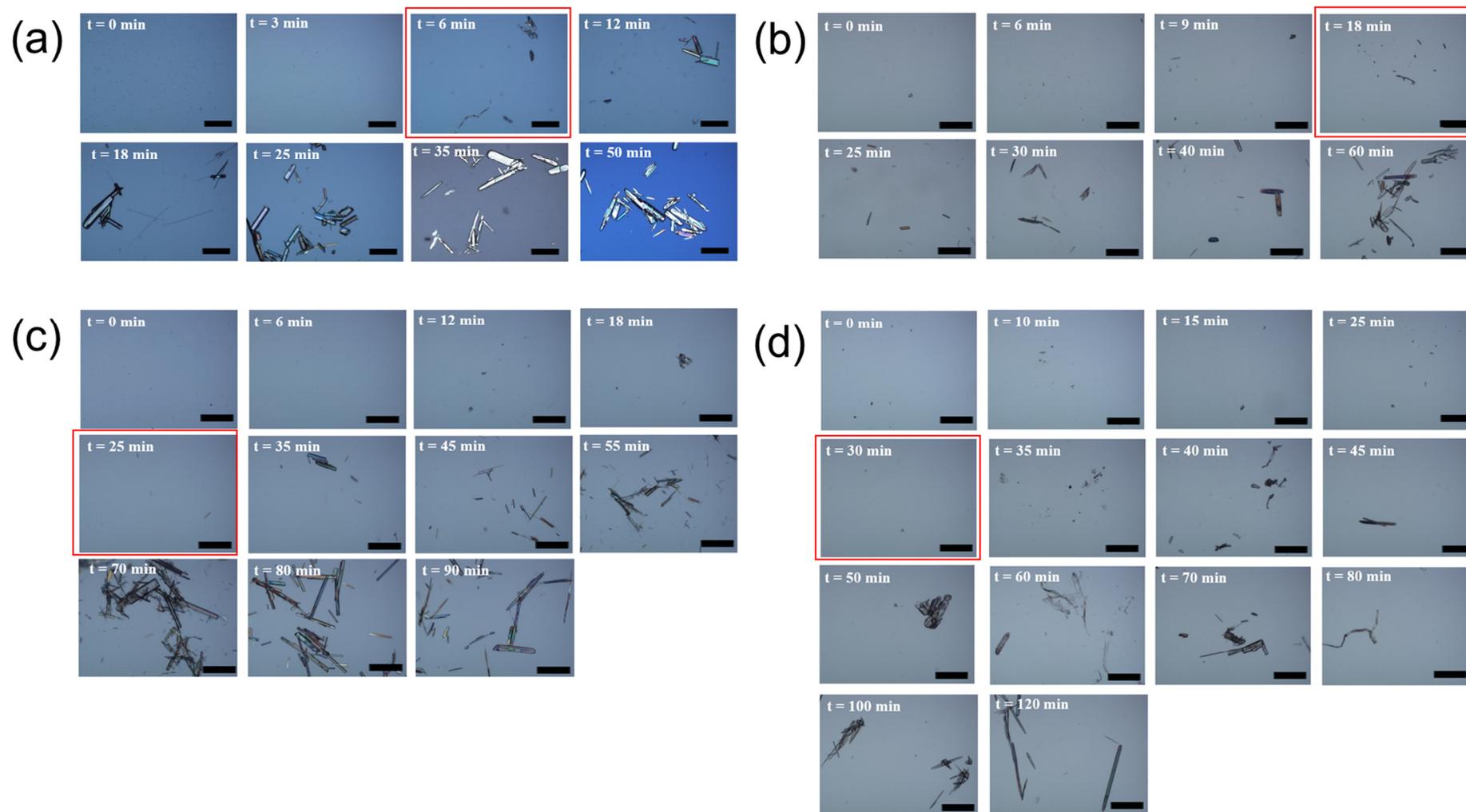


Figure S1. OM images of crystallization of 2:1 HBz-NaBz co-crystals without templating molecules at the initial degree of supersaturation (S_0) of: (a) 1.66, (b) 1.54, (c) 1.48, and (d) 1.43. Red boxes indicate the induction time as tabulated in Table 1. (Scale bar: 100 μm).

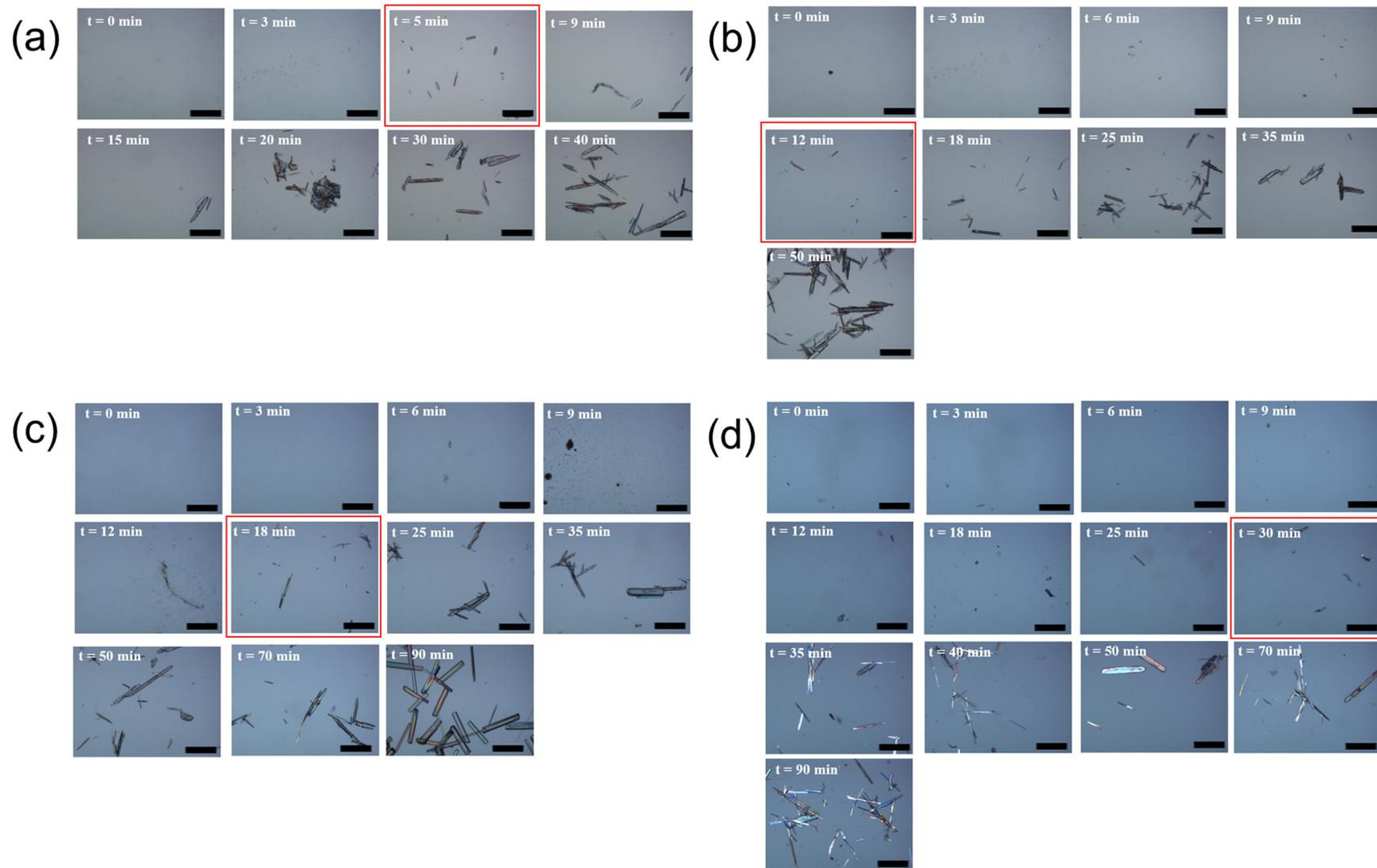


Figure S2. OM images of crystallization of 2:1 HBz-NaBz co-crystals with NaBz templating molecules at the initial degree of supersaturation (S_0) of: (a) 1.66, (b) 1.54, (c) 1.48, and (d) 1.43. Red boxes indicate the induction time as tabulated in Table 1. (Scale bar: 100 μm).

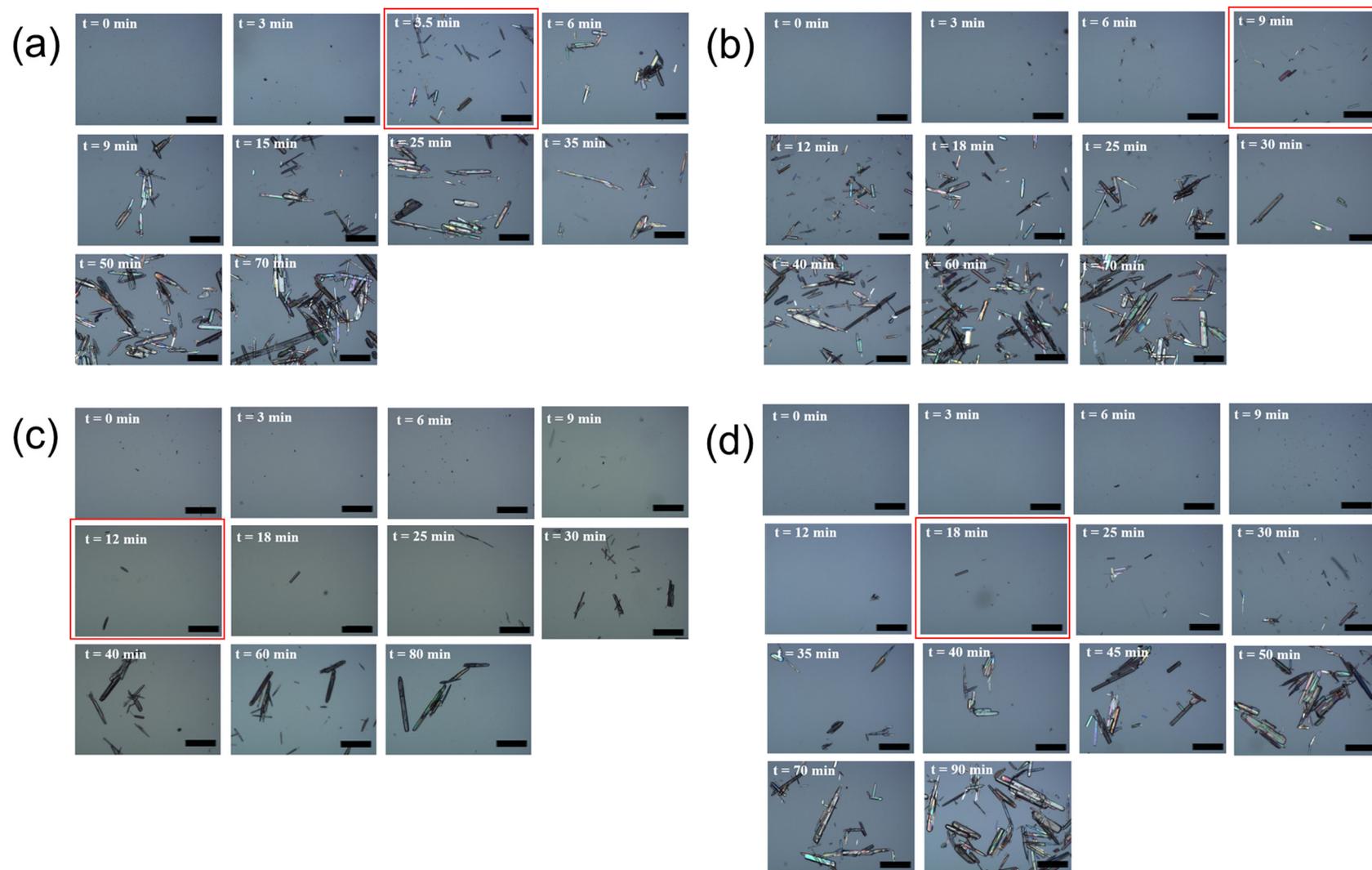


Figure S3. OM images of crystallization of 2:1 HBz-NaBz co-crystals with 2:1 HBz-NaBz co-crystal templating molecules at the initial degree of supersaturation (S_0) of: (a) 1.66, (b) 1.54, (c) 1.48, and (d) 1.43. Red boxes indicate the induction time as tabulated in Table 1. (Scale bar: 100 μm).

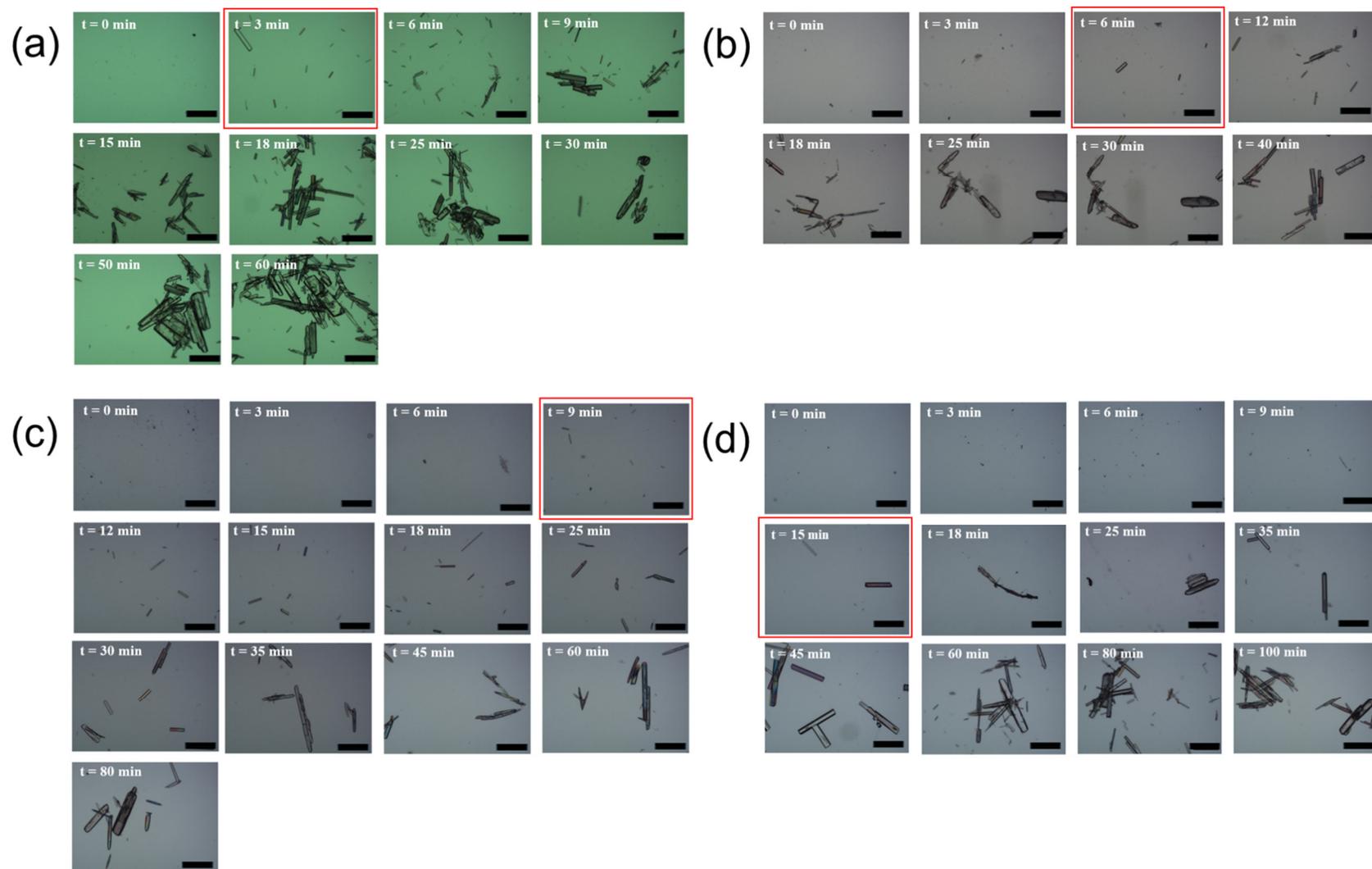


Figure S4. OM images of crystallization of 2:1 HBz-NaBz co-crystals with 1:1 HBz-NaBz co-crystal templating molecules at the initial degree of supersaturation (S_0) of: (a) 1.66, (b) 1.54, (c) 1.48, and (d) 1.43. Red boxes indicate the induction time as tabulated in Table 1. (Scale bar: 100 μm).

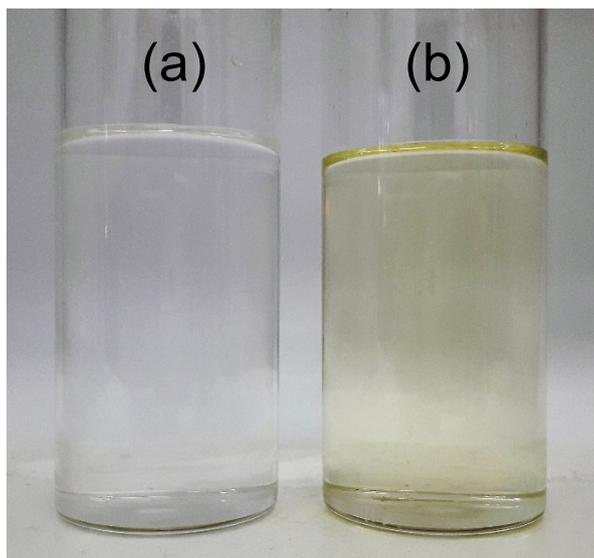


Figure S5. Photo image of (a) clear solution obtained by dissolving 2:1 HBz-NaBz co-crystals in 4:1 (v/v) ethanol–water co-solvent, and (b) yellow solution derived by reaction co-crystallization of HBz and NaOH in 4:1 (v/v) ethanol–water co-solvent.

In a 2:1 HBz-NaBz co-crystal, the weight percentage of HBz is calculated as:

$$\begin{aligned} \text{wt\% of HBz} &= \frac{2 \times \text{Molar mass of HBz}}{(2 \times \text{Molar mass of HBz}) + (1 \times \text{Molar mass of NaBz})} \times 100\% \\ &= \frac{2 \times 122.12 \frac{\text{g}}{\text{mol}}}{\left(2 \times 122.12 \frac{\text{g}}{\text{mol}}\right) + \left(1 \times 144.11 \frac{\text{g}}{\text{mol}}\right)} \times 100\% = 62.89 \text{ wt\%} \end{aligned} \quad (\text{S2})$$

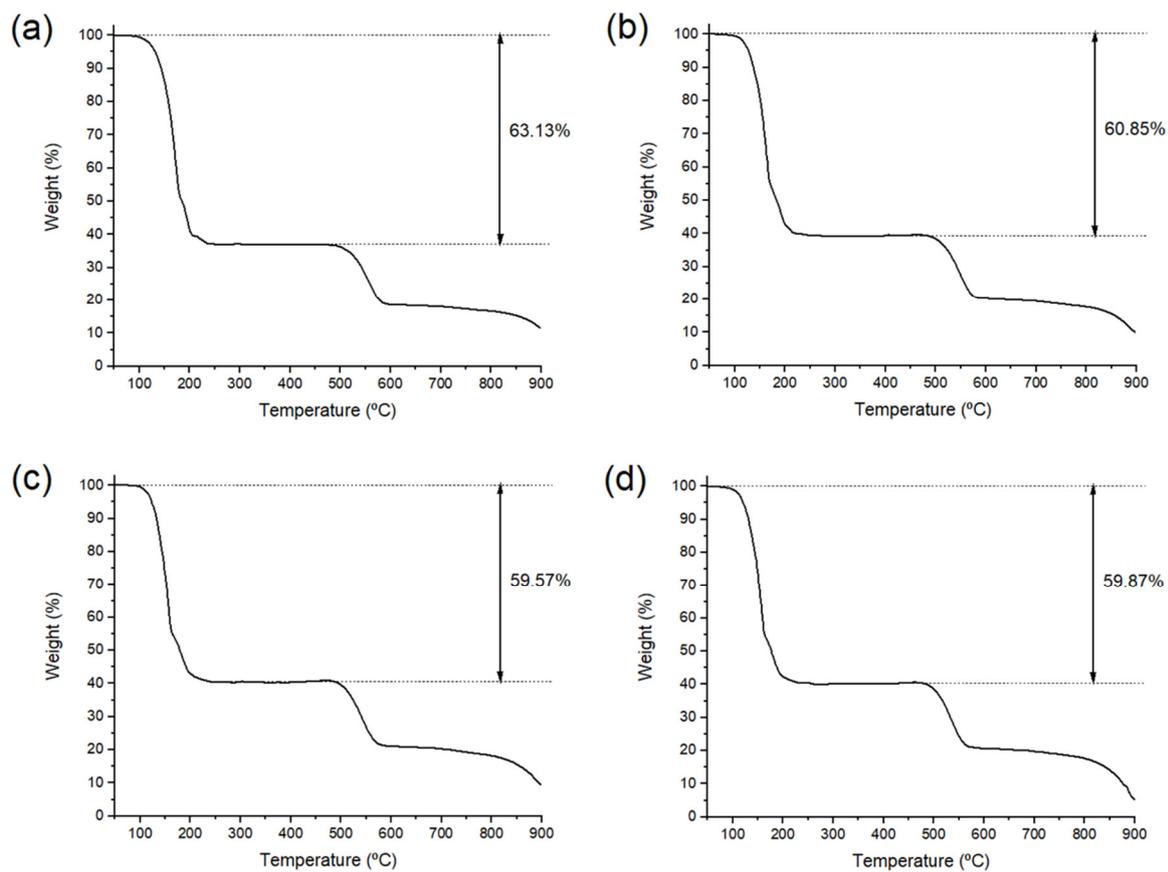


Figure S6. TGA scans of 2:1 HBz-NaBz co-crystals generated without templating molecules at different initial degrees of supersaturation: (a) $S_0 = 1.66$; (b) $S_0 = 1.54$; (c) $S_0 = 1.48$; and (d) $S_0 = 1.43$.

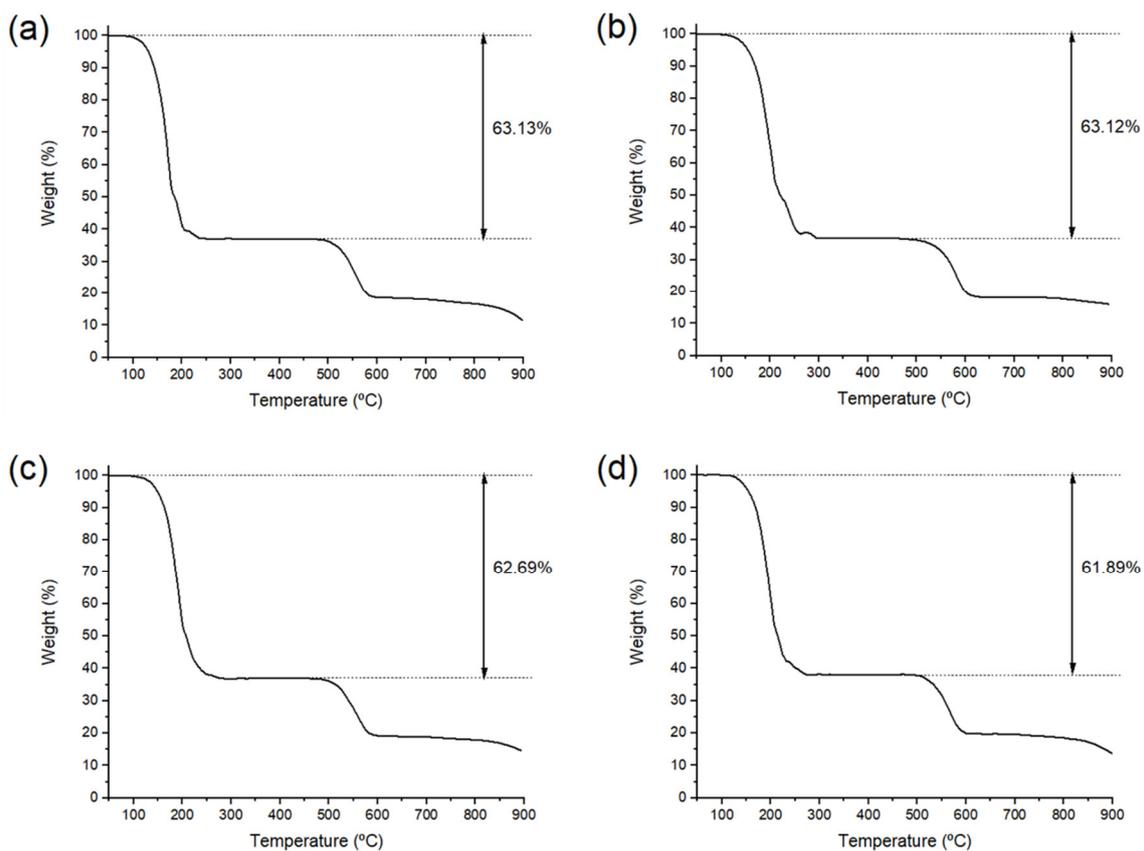


Figure S7. TGA scans of 2:1 HBz-NaBz co-crystals generated at $S_0 = 1.66$: (a) without templating molecules, (b) with dissolved NaBz templating molecules, (c) with dissolved 2:1 HBz-NaBz co-crystal templating molecules, (d) with dissolved 1:1 HBz-NaBz co-crystal templating molecules.

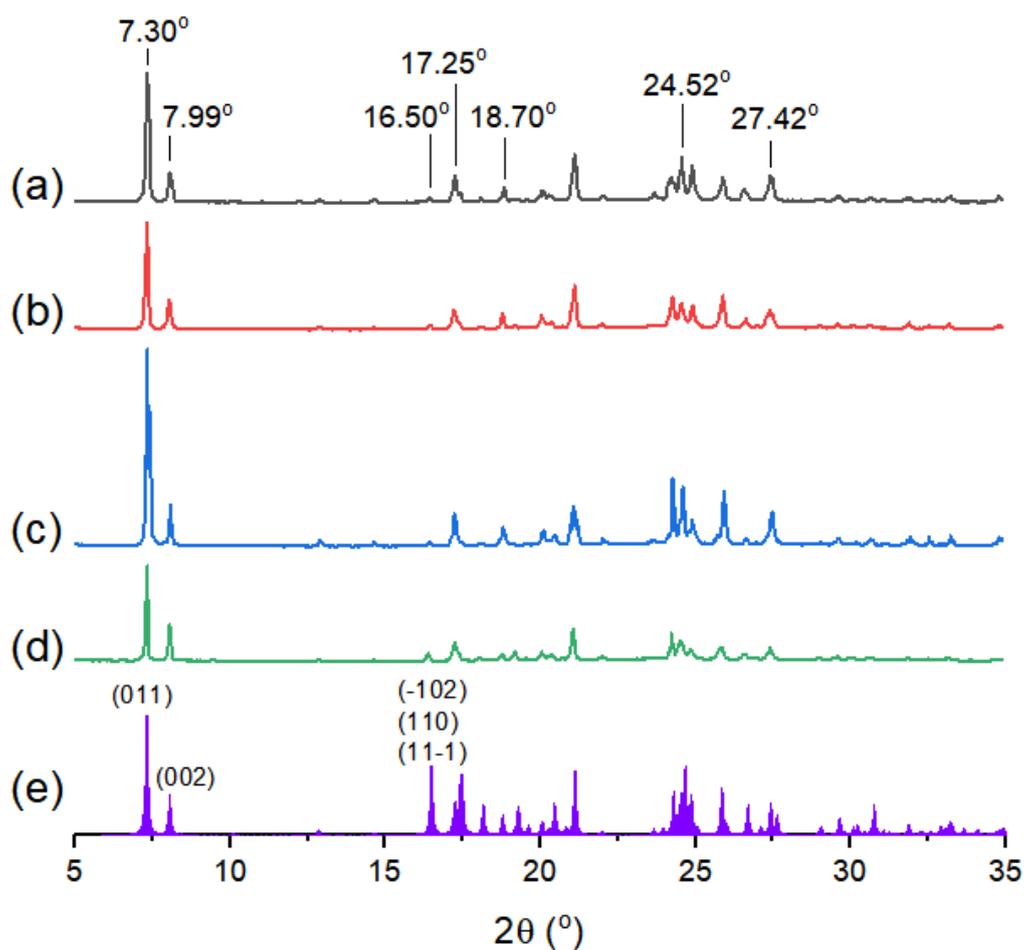


Figure S8. PXRD patterns of 2:1 HBz-NaBz co-crystals generated without templating molecules from (a) $S_0 = 1.66$, (b) $S_0 = 1.54$, (c) $S_0 = 1.48$, and (d) $S_0 = 1.43$, and (e) simulated PXRD patterns of 2:1 HBz-NaBz Form A co-crystal (CCDC No. 875040).

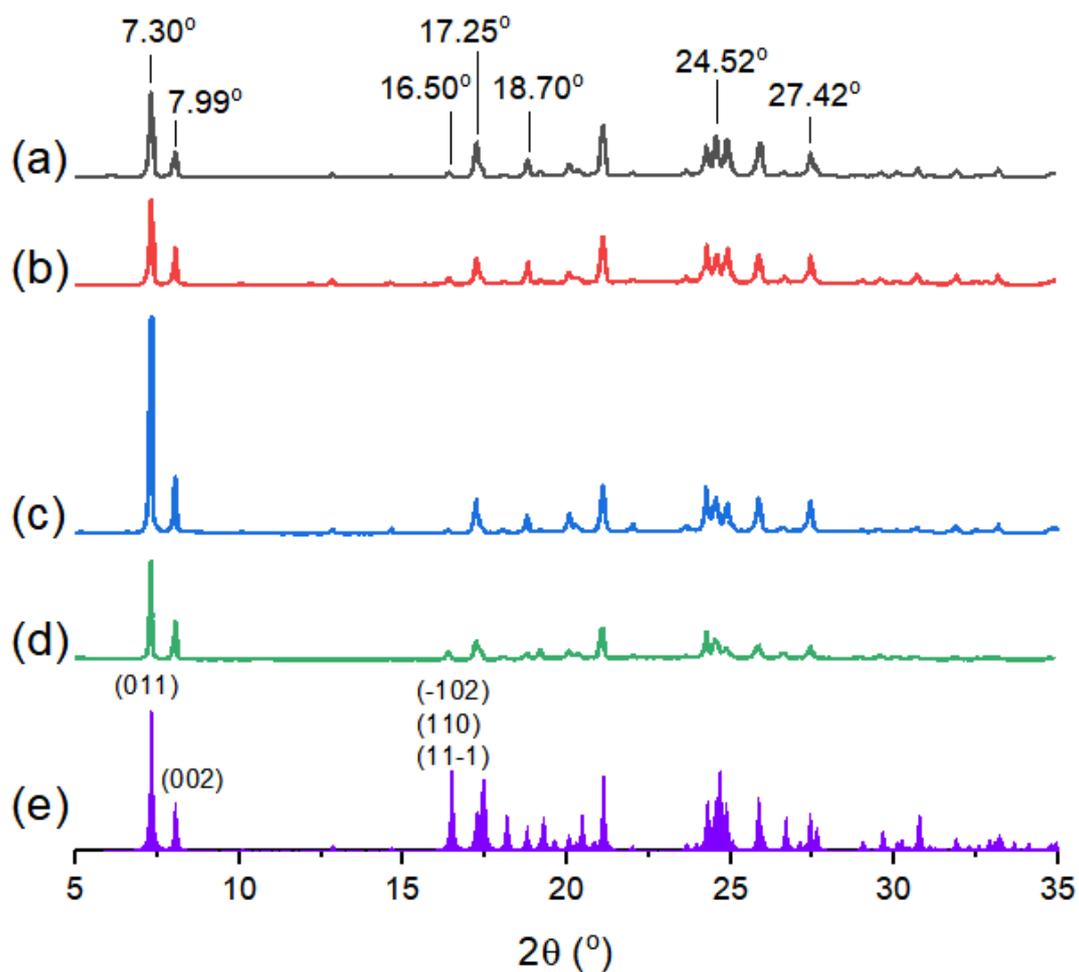


Figure S9. PXRD patterns of 2:1 HBz-NaBz co-crystals generated at $S_0 = 1.66$: (a) without templating molecules, (b) with dissolved NaBz templating molecules, (c) with dissolved 2:1 HBz-NaBz co-crystal templating molecules, and (d) with dissolved 1:1 HBz-NaBz co-crystal templating molecules, and (e) simulated PXRD pattern of 2:1 HBz-NaBz Form A co-crystal (CCDC No. 875040).