Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Integrated plug flow synthesis and crystallisation of pyrazinamide

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Supporting Information

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1. Experimental Parameters

Apparatus:

Pumping and temperature control unit for flow synthesis – Vapourtec R2+ and R4

Air and carrier fluid pumps – Ismatec gear pump REGLO-Z

Circulating baths for tubing jackets – Grant TC-120-R1

Table S1 Flow rates for all integrated plug flow chemistry and crystallisation experiments

	Solution	Air	Carrier Fluid	Total
Flow rate (ml/min)	1	6.3	2.1	9.4

1.1 Uncontrolled nucleation

A 100 ml, 0.25 M aqueous solution of pyrazinecarbonitrile was prepared at room temperature and kept stirring throughout the experiment to ensure a homogenous feed. An adjustable omnitfit column (100 mm, 6.6 mm ID) of variable volume (0.1 to 2.4 ml) was packed with 0.98 g of MnO_2 powder mixed with 0.2 g of celite to prevent over-backpressure and capped with celite to prevent egress of MnO_2 powder from the column. The column was heated to 80 °C, a 12 bar back pressure regulator (BPR) was connected to the outlet of the omnifit column, the outlet tubing (1.5 mm internal diameter, ID, fluoroethylenepropylene, FEP) of which was encased in a tubing jacket (75 °C, regulated by a circulating bath). The jacketed tubing was then connected to a PEEK (polyether ether ketone) cross-piece mixer with a thru-hole of 1.25 mm. The carrier fluid and air feeds were transferred to the cross-piece *via* 1 mm, ID PTFE tubing. The segmentation unit was temperature controlled *via* a custom water bath (70 °C, Figure sx). The outlet temperature of the solution slugs was 63.8 °C (measured internally). The outlet of the segmentation unit connected to the 15 m 3.2 mm ID FEP crystalliser tubing *via* a 10 cm long portion of 3.2 mm ID marprene tubing. The 15 m crystalliser tubing was coiled into three distinct coils, the first housed within a glass holder and the latter two held in aluminium coil holders. The outlet of the crystalliser is designed to separate the solution and carrier fluid prior to online suction filtration as described previously.[1]

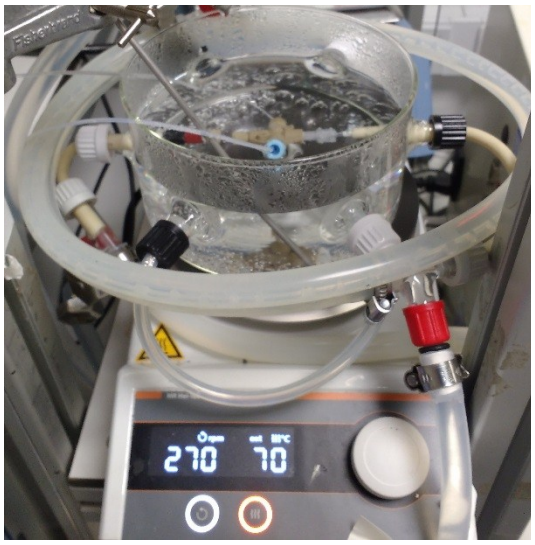

Figure S1 Custom water bath used for the segmentation unit

During periods of blocking a heat gun was used to partially dissolve the material blocking the tubing.

1.2 Controlled nucleation

These experiments were identical to the uncontrolled nucleation experiments excepting a solution concentration of 0.28M and the inclusion of a 1.2 m length of 3.2 mm ID FEP jacketed tubing between the outlet of the water bath and the main crystalliser tubing. A second circulating bath was used to control the temperature within the tubing jacket (set to 10 °C). A zero volume union was used to connect this tubing to the main crystalliser tubing. No blocking occurred within this set-up.

1.3 Synthesis only experiments

In order to ascertain the % conversion a flow synthesis only run was performed with identical parameters to the integrated runs but collecting the product immediately after the BPR. In three runs with 20 ml of 0.28 M aqueous pyrazinamide solution, the solvent was evaporated giving an average isolated yield of 0.6871 g (91.7 %) measured. NMR and DSC (see below) confirmed the purity of the product.

2. Analysis

2.1 Microscope Images

Figure S2 Images from an uncontrolled nucleation run. N.B. major gridlines are 1 mm, minor gridlines 0.1 mm

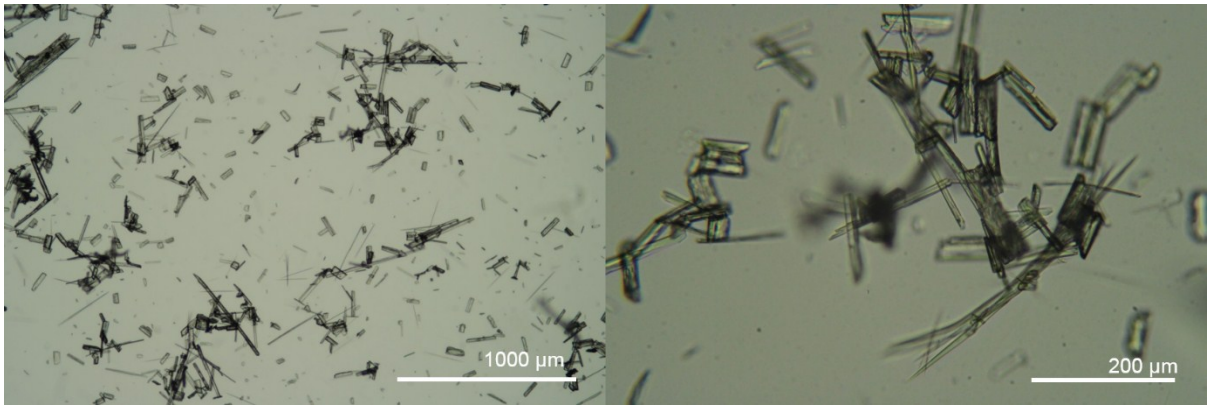

Figure S3 Microscope images of crystals from a controlled nucleation run

2.2 Slug size analysis

Slug volume was measured through 11 collections of 10-28 slugs.

Table S2 slug volumes of captured solvent slugs for average slug volume calculations

No. of Slugs	Starting Volume (ml)	End Volume (ml)	Total Volume (ml)	Average Slug size (ml)
28	0	2	2	0.07
12	4.4	5.4	1	0.08
10	5.6	6.4	0.8	0.08
10	6.6	7.4	0.8	0.08
13	7.4	8.6	0.8	0.06
12	8.8	9.6	0.8	0.06
27	0	2	2	0.07
13	2.4	3.6	1.2	0.09
12	3.8	4.8	1	0.08
10	5	5.8	0.8	0.08
13	6	7	1	0.08
Total average slug Volume				0.075

Slug lengths were calculated through measuring images of the slugs within coils. The pixel number to the tube size was normalised for every slug to account for perspective.

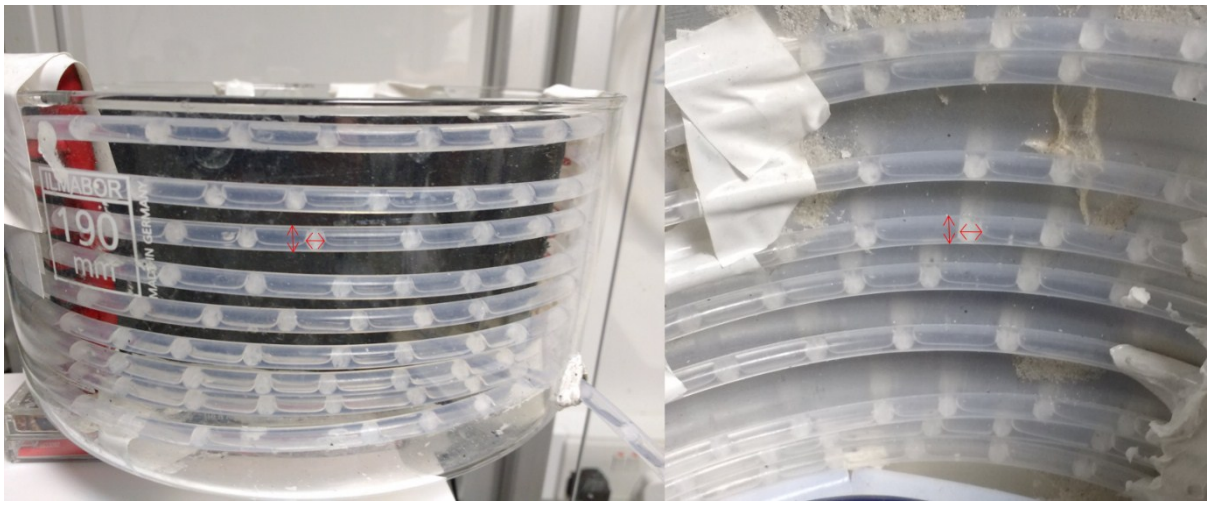

Figure S4 Images of slugs in coils 1 and 2 used for average slug size analysis, red arrows indicate the lengths measured

Table S3 slug length calculations from pixel widths

	Tubing	Slug

	height	width
mm	4.76	2.97
pixels	53	33.00
		2.97
	53	33.00
		3.41
	53	38.00
		3.02
	52	33.00
		2.61
	51	28.00
		2.58
	53	28.40
		2.86
	54	32.40
		3.04
	61	39.00
		2.78
	60	35.00
		2.92
	57	35.00
		3.15
	59	39.00

Table S4 Slug size calculations from pixel data

	Max	Average	Min	St dev	Aspect ratio	Volume of cylinder (ml)
Width (mm)	3.41	2.94	2.58	0.23	1.08	0.08
% change	16.25		12.29			
ml change	0.03		0.02			

N.B. The actual volume of the slugs will be slightly lower than the calculated volume of a cylinder due to wetting effects at the front and rear of the slug. The measured average volume of 0.075 ml is therefore in very good accordance with the calculated 0.08 ml.

2.3 Particle size analysis

Due to the large size, undesirable particle attributes and low yield of the uncontrolled nucleation runs, the particle sizing was performed through image analysis similar to the slug size analysis. Table S1 details the average length and breadth of 30 crystals analysed from four collections using a microscope grid slide with minor divisions of 0.1 mm. The surface area was calculated assuming rectangular shapes.

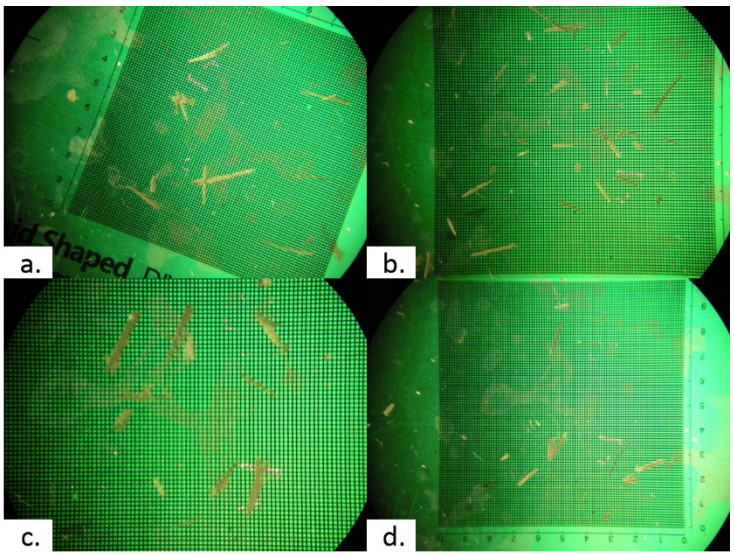

Figure S5 Images used for particle size analysis of pyrazinamide crystals resulting from uncontrolled nucleation runs

Table S5 Particle size analysis from pixel width calculations from images with microscope grid slide

	Length	Width	Surface Area (mm^2)
Average (mm)	1.02	0.12	0.13
St.Dev	0.546741	0.04825	0.133443

Particle size analysis of the controlled nucleation runs was performed using laser diffraction on a Malvern Mastersizer 3000. The particles were suspended in a saturated solution of pyrazinamide in isopropanol to avoid particle dissolution. A manual Malvern Hydro SM dispersion cell (stirring rate 2000 rpm) was used to disperse the sample as the needle like particles have a tendency to aggregate. After 30 mins, the majority of these aggregates had dispersed (Figure S6) without damage to the particles. This methodology was successful for disaggregation of the larger aggregates (>1000 µm) but smaller aggregates remained. All measurements discussed further were taken once sample stability was achieved (numbers 20 to 50).

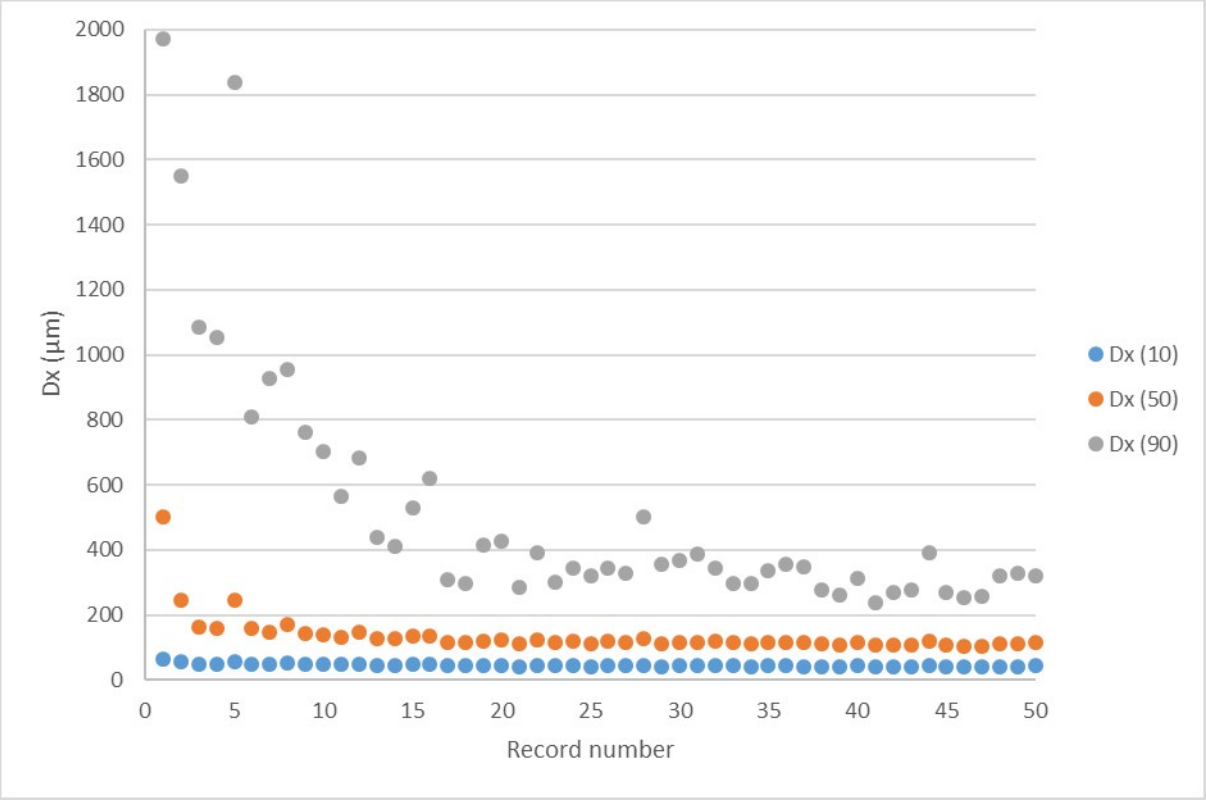

Figure S6 D10, D50 and D90 in microns as a function of the experiment number. Each record takes around 20 seconds to acquire. Large aggregate dispersal and thus sample stability is achieved at 30 min (record 20).

The Malvern Mastersizer 3000 software version 3.36 fits the diffraction data to an irregular sphere. As the particles here are needle shaped, it can be presumed that a combination of all orientations of the needle crystals with respect to the laser beam will be represented in the data. A higher proportion of the long axis parallel to the flow (perpendicular to the laser) can be expected for needle crystals in flow.

The lower size range shown in Figure 7 corresponds to particles presenting parallel to the laser, hence projecting only their width, measured between 3-20µm. All other orientations of the particles are detected in the range of 20-300µm, maximum population expression at 100µm. The maxima at 100 µm most likely corresponds to the particles aligned with the flow in the cell, showing their actual length, this is in accordance with optical microscopy observations (Figure S3).

The sizes detected over 300µm correspond in part to occasional long particles, but mainly to aggregates breaking and reforming over the 30 measurements. One can indeed observe that the regions of the size distributions corresponding to the particles themselves are superimposed, showing their stability, while this region shows a very high variability between records, which is explained by the fact these aggregates are not stable and will break down then reform randomly over time.

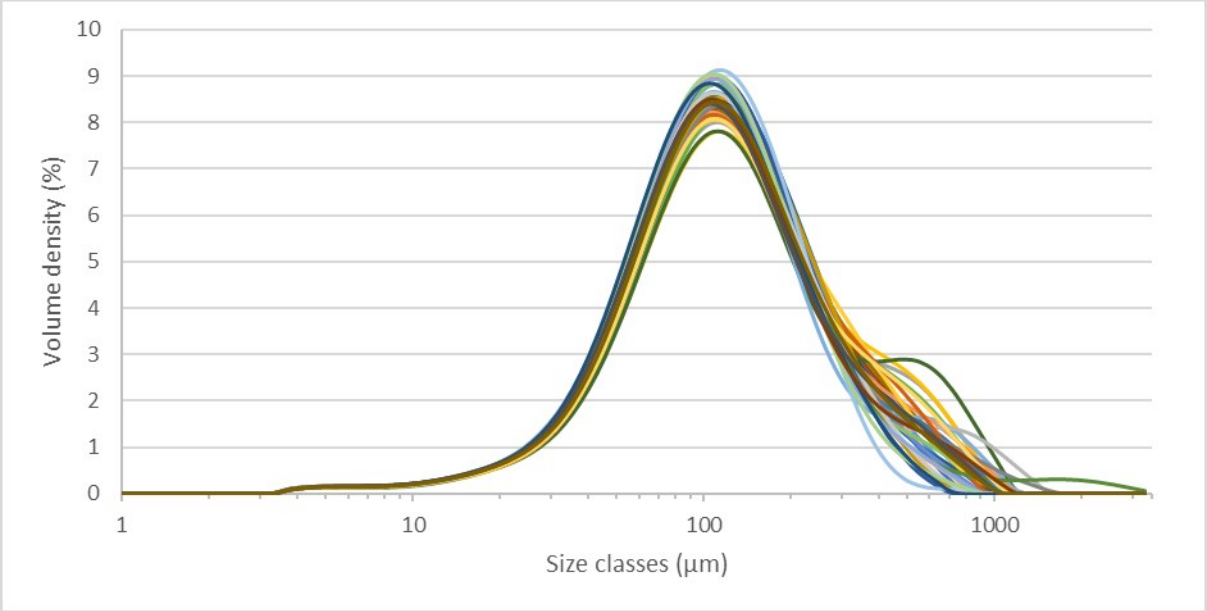

Figure S7 Volume density measurements as a function of size class, superimposing records 20 to 50.

These results are confirmed by SEM analysis from two separate controlled nucleation experiments using a Jeol JSM-6610LV instrument. Figure S8 below shows an average range of particles between 100-300 µm with some larger particles present.

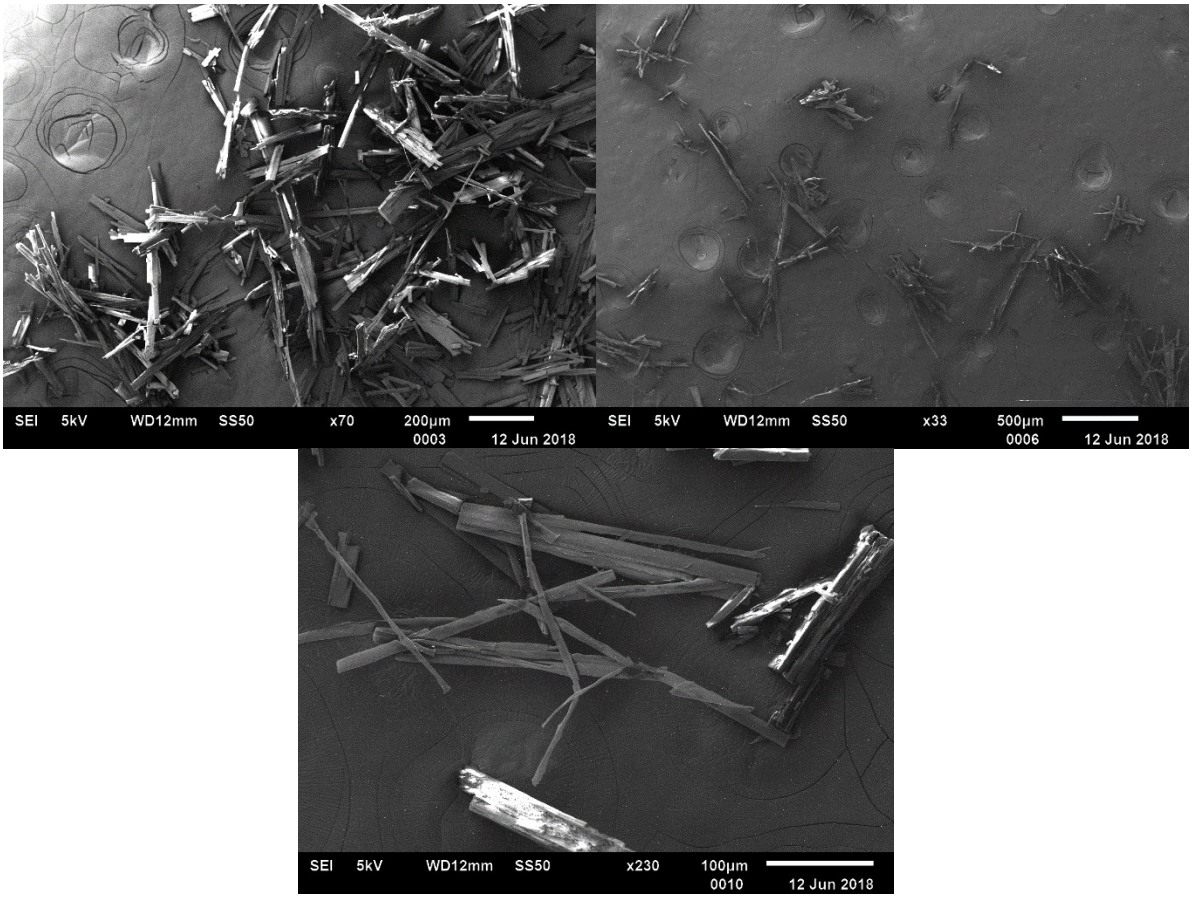

Figure S8 SEM analysis on pyrazinamide crystals produced from controlled nucleation experiments

2.4 Powder X-Ray Diffraction (PXRD)

PXRD of samples from uncontrolled nucleation runs with and without major blockages and a controlled nucleation run. To confirm whether the reaction mixture or crystallisation conditions determined the resultant polymorph, the filtrate of a controlled nucleation run was evacuated to dryness and the resultant precipitate analysed. Simulated patterns calculated from single-crystal X-Ray Diffraction data obtained from the Cambridge Structural Database ver. 2018, refcodes: PYRZIN (α) and PYRZIN04 (γ)

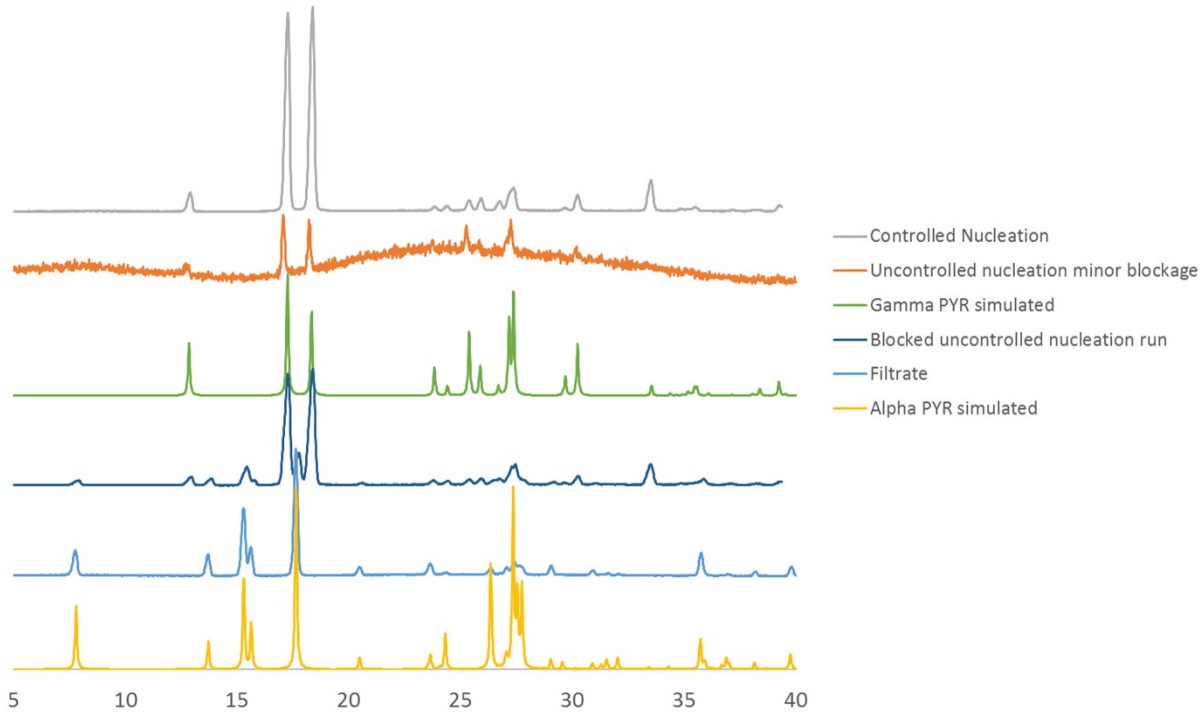

Figure S9 PXRD pattern of different integrated synthesis and crystallisation runs. N.B. the low quality of the pattern of the uncontrolled minor blockage is due to the poor yield

2.5 Differential Scanning Calorimetry (DSC)

Polymorphic identity was further confirmed by DSC with reference to[2]

Endothermic events between 145-160 °C in filtrate and blocked run (20x magnification) correspond to α- to γ- transition. The endothermic event at 185-195 in filtrate and controlled nucleation run corresponds to melting of the γ phase.

20x magnification of the blocked run DSC trace is necessary due to the low concentration of α-pyrazinamide in the sample.

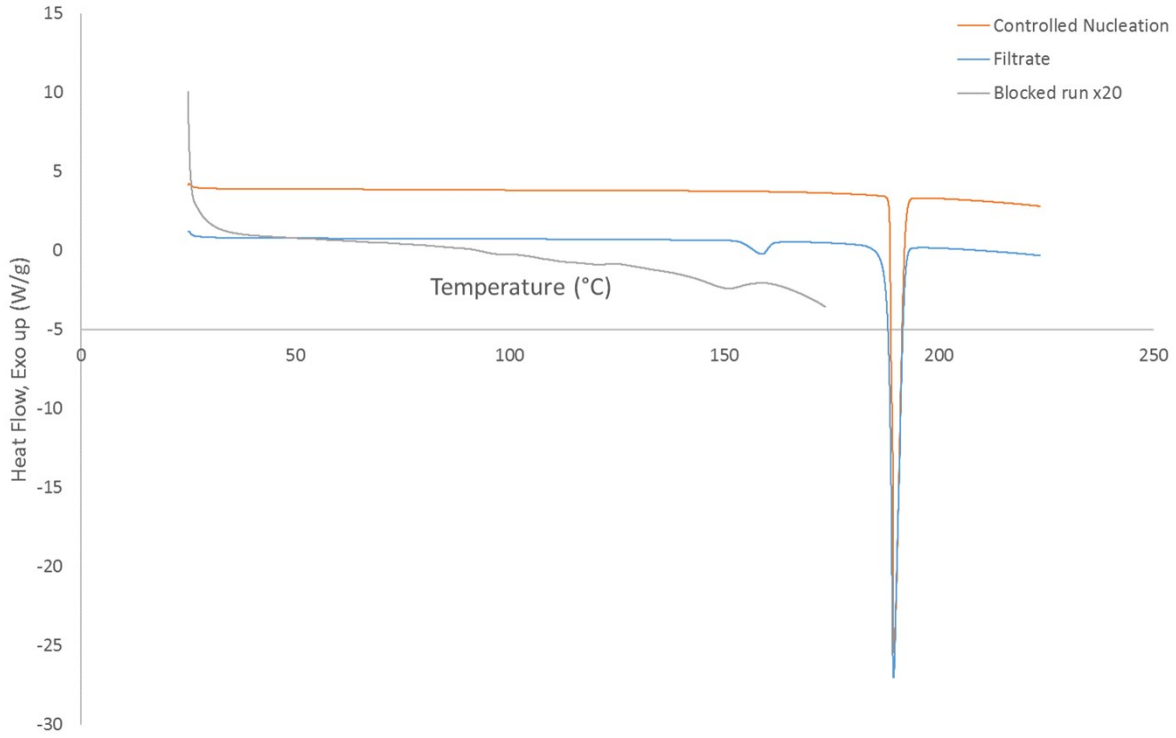

Figure S10 DSC analysis of products obtained from synthesis only ('filtrate'), controlled nucleation and uncontrolled nucleation ('blocked run') experiments. N.B. due to the small percentage of α-pyrazinimide present in the 'blocked run' sample the heat flux has been magnified 10x to show the endothermic recrystallisation event)

2.6 ^{1}H NMR

All NMR analysis was carried out using a 400 MHz Bruker Advance

2.6.1 Controlled nucleation product

Prior to analysis, the recovered solid product from a controlled nucleation run was dried overnight.

δH(400 MHz; d6-DMSO; 25 °C) 7.87 (1 H, br. s), 8.27 (1 H, br. s), 8.71 (1 H, dd, J 2.5 Hz, J 1.5 Hz), 8.84 (1 H, d, J 2.5 Hz), 9.17 (1 H, d, J 1.5);

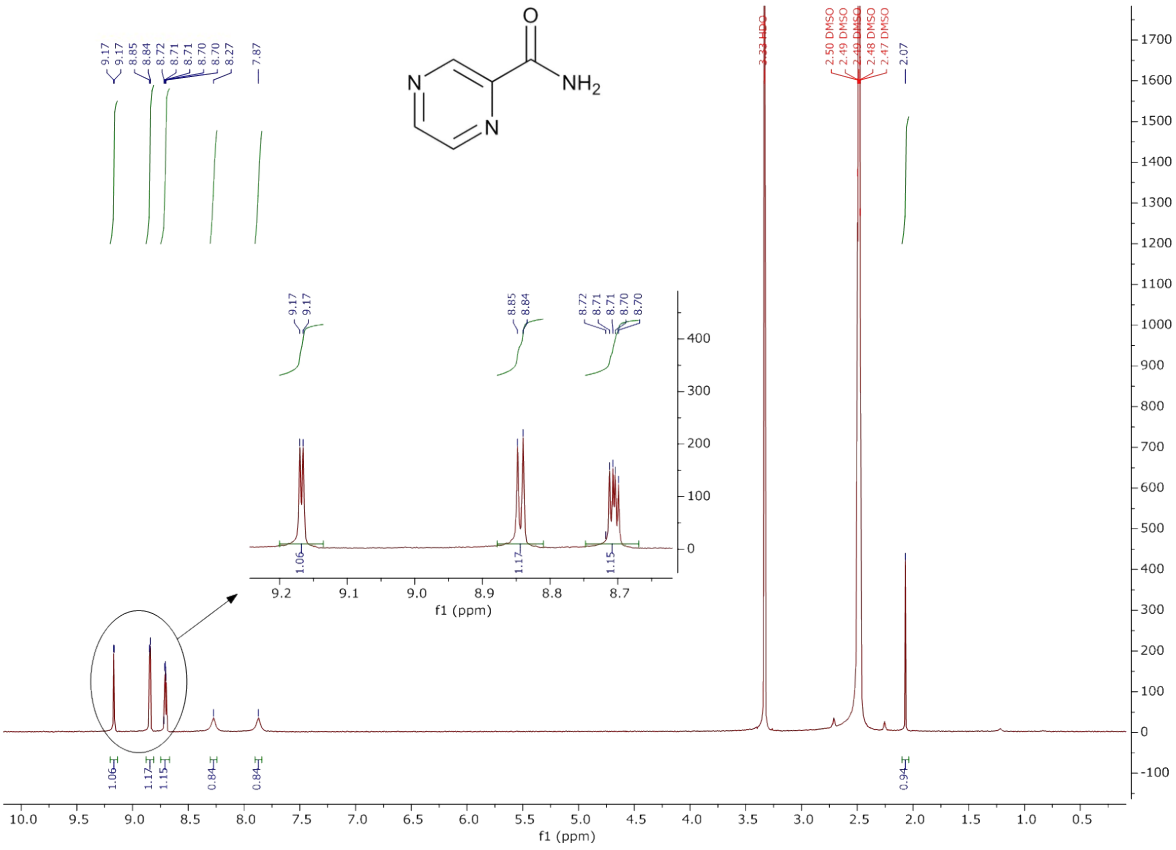

Figure S11 NMR spectrum of the product from a controlled nucleation crystallisation run

2.6.2 Synthesis only product

The product obtained after evaporation of solvent was oven dried before being prepared for NMR analysis without purification. N.B. the excess water in the sample is due to wet d6-DMSO.

δH(400 MHz; d6-DMSO; 25 °C) 7.87 (1 H, br. s), 8.27 (1 H, br. s), 8.71 (1 H, dd, J 2.5 Hz, J 1.5 Hz), 8.84 (1 H, d, J 2.5 Hz), 9.17 (1 H, d, J 1.5);

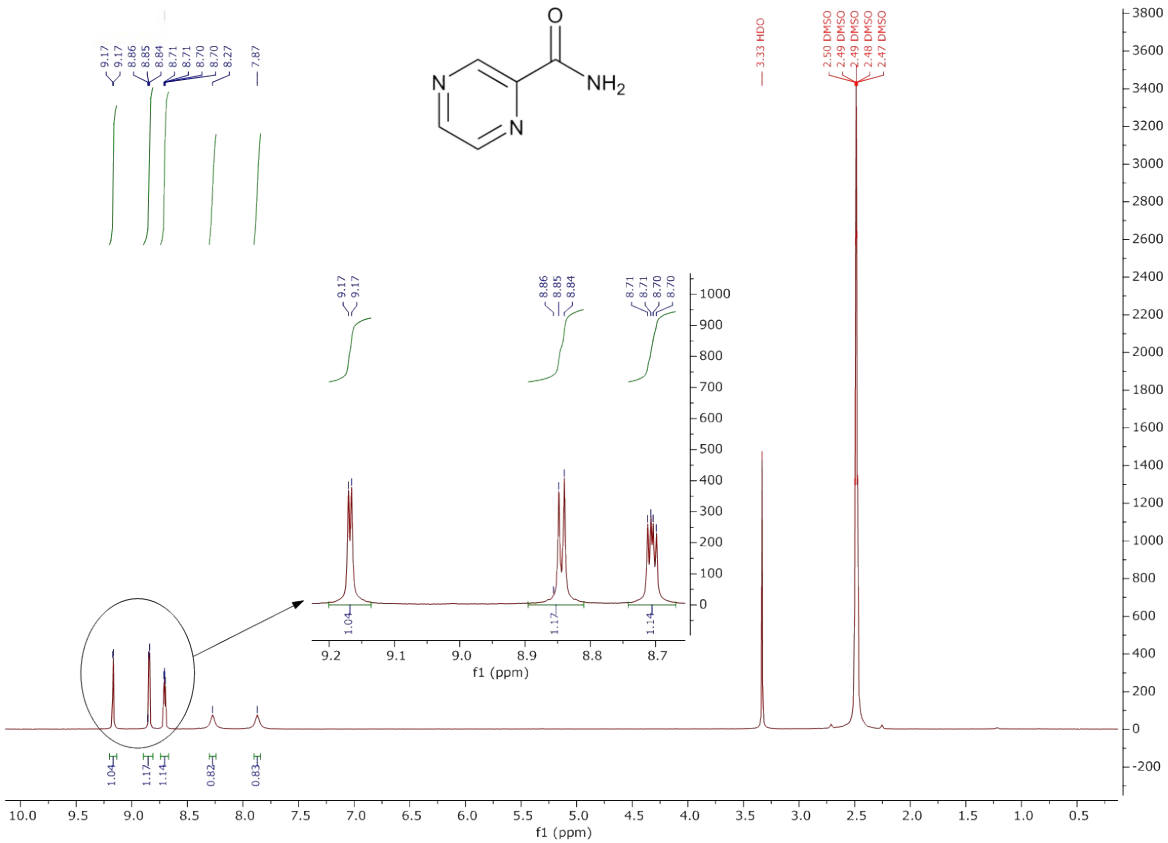

Figure S12 NMR spectrum from a synthesis only experiment

3. References

1. K. Robertson, P.-B. Flandrin, A. R. Klapwijk and C. C. Wilson, *Crystal Growth and Design*, 2016, **16**, 4759-4764.
2. S. Cherukuvada, R. Thakuria and A. Nangia, *Crystal Growth & Design*, 2010, **10**, 3931-3941.