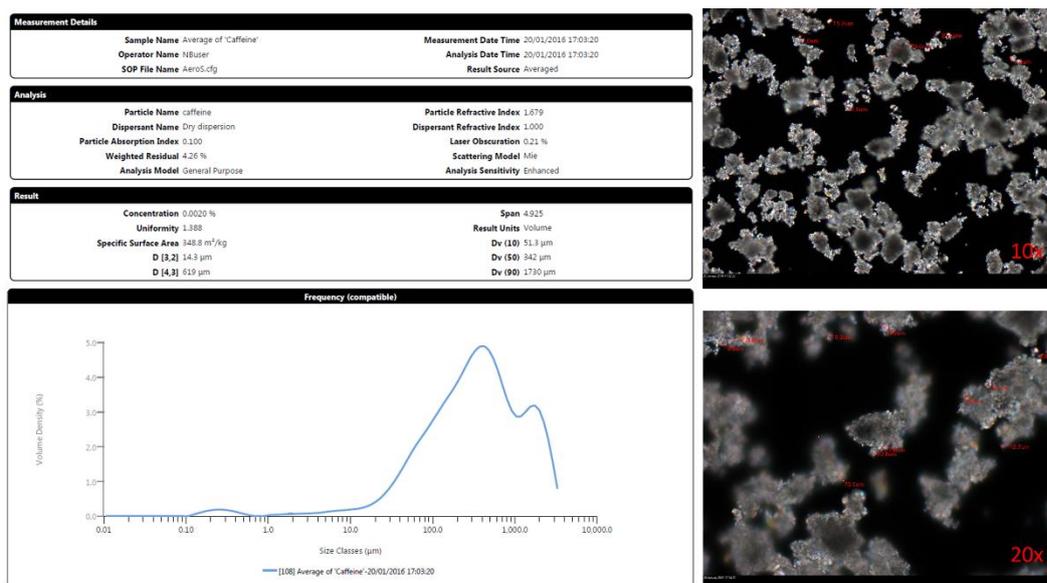


# **Tuning the 'spontaneous' formation kinetics of caffeine:malonic acid co-crystals**

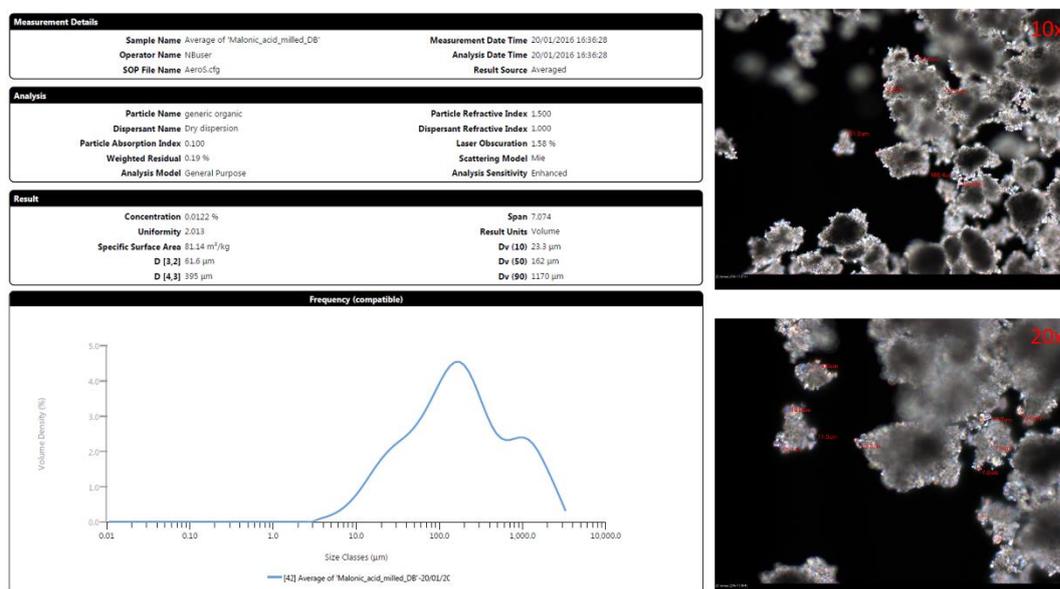
**Karol Nartowski, Yaroslav Khimyak and David Berry**

**Supporting information**

## 1. Particle size measurement

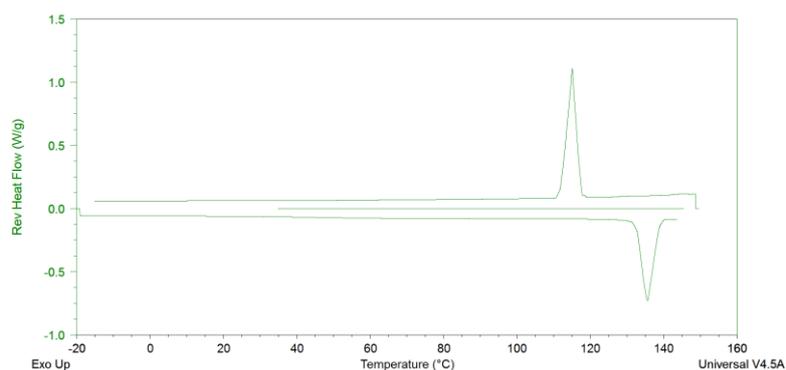


**Figure S1.** Displaying particle size of anhydrous caffeine post ball-milling. Laser diffraction was not able to determine the size of primary particles in this instance due to the cohesiveness of the milled material (AeroS). It was not possible to separate these agglomerates with higher pressure as the maximum of 4 Bar was used. Solution approaches (HydroEV) led to dissolution of the material so were abandoned. Optical microscopy shows the primary particle size to be in the order of 10 micrometres.

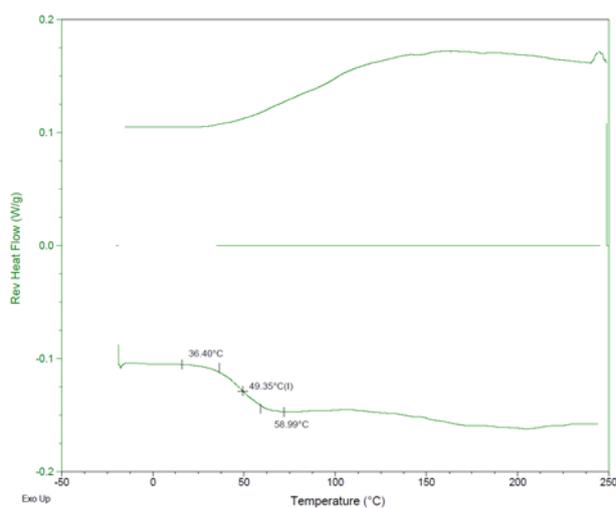


**Figure S2.** Displaying particle size of anhydrous caffeine post ball-milling. Laser diffraction was not able to determine the size of primary particles in this instance due to the cohesiveness of the milled material (AeroS). It was not possible to separate these agglomerates with higher pressure as the maximum of 4 Bar was used. Solution approaches (HydroEV) led to dissolution of the material so were abandoned. Optical microscopy shows the primary particle size to be in the order of 10 micrometres.

## 2. Amorphous property determination

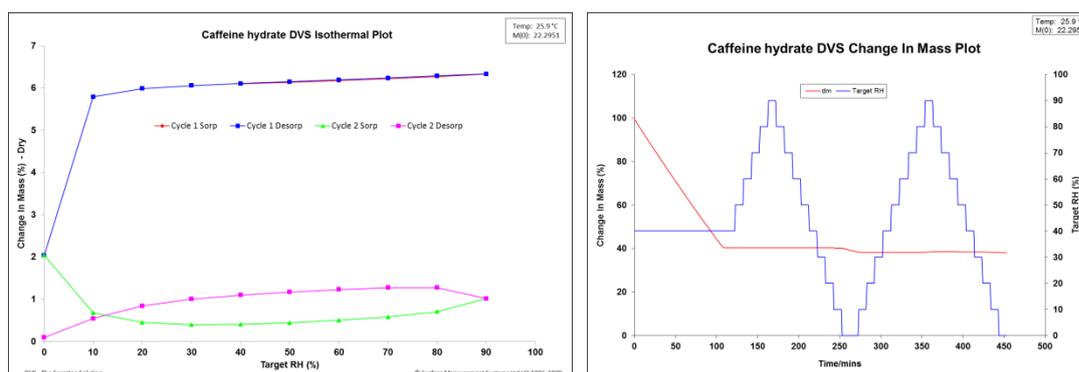


**Figure S3.** Displaying the reversing heat flow vs. temperature for a heat cool heat cycle for malonic acid and recrystallization on the cooling cycle. The first heating cycle was run as standard DSC, further cycles were run with modulated DSC with an underlying heating and cooling rate of 3°C/min and a modulation period of +/- 1°C/min.

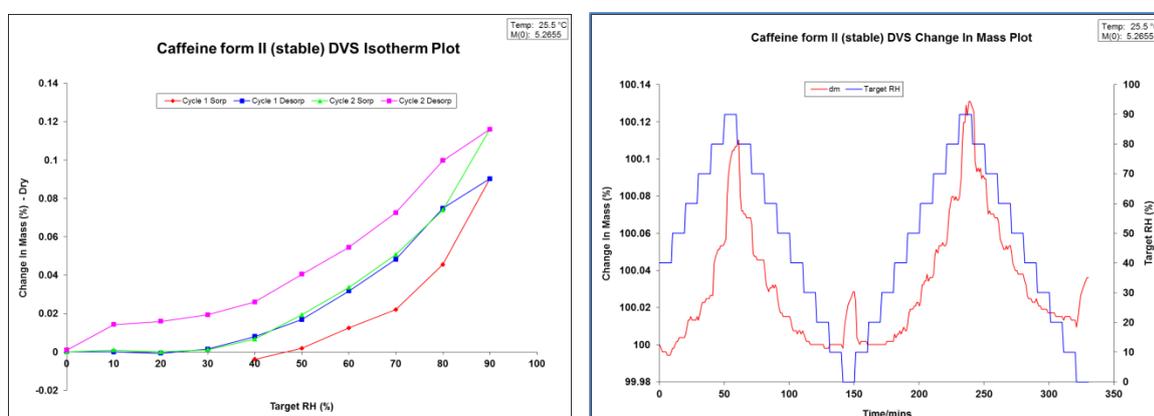


**Figure S4.** Displaying the reversing heat flow vs. temperature for a heat cool heat cycle for caffeine, showing no re-crystallisation and a glass transition at around 49°C. The first heating cycle was run as standard DSC, further cycles were run with modulated DSC with an underlying heating and cooling rate of 3°C/min and a modulation period of +/- 1°C/min.

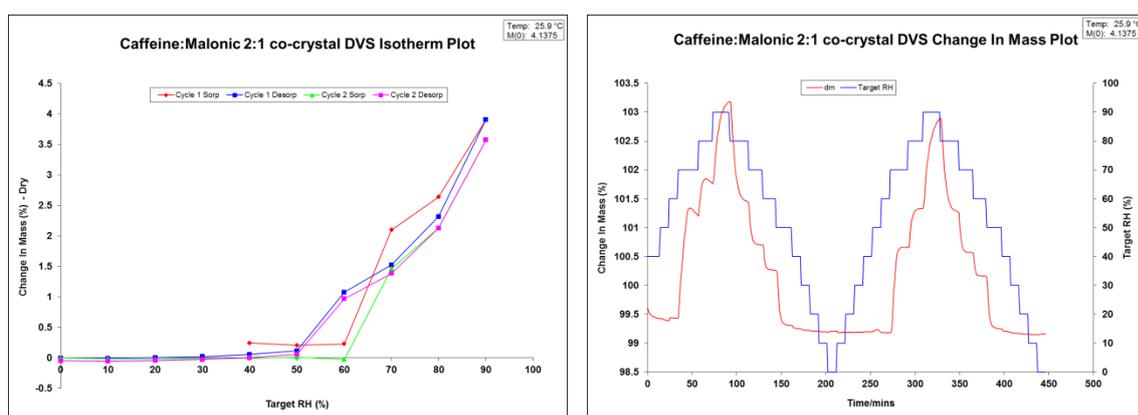
### 3. Dynamic Vapour Sorption (DVS)



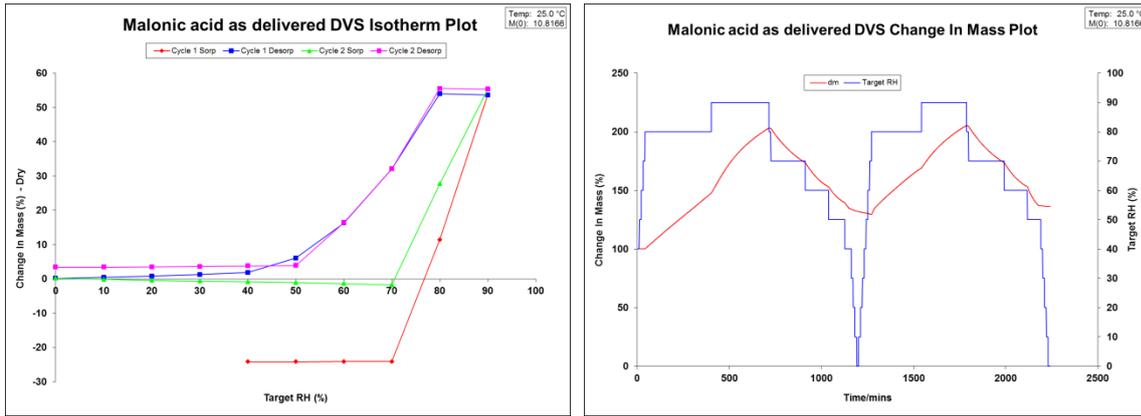
**Figure S5.** Caffeine hydrate sorption and desorption isotherms. With the hydrate displaying conversion back to the anhydrous counterpart after the first cycle of dehydration.



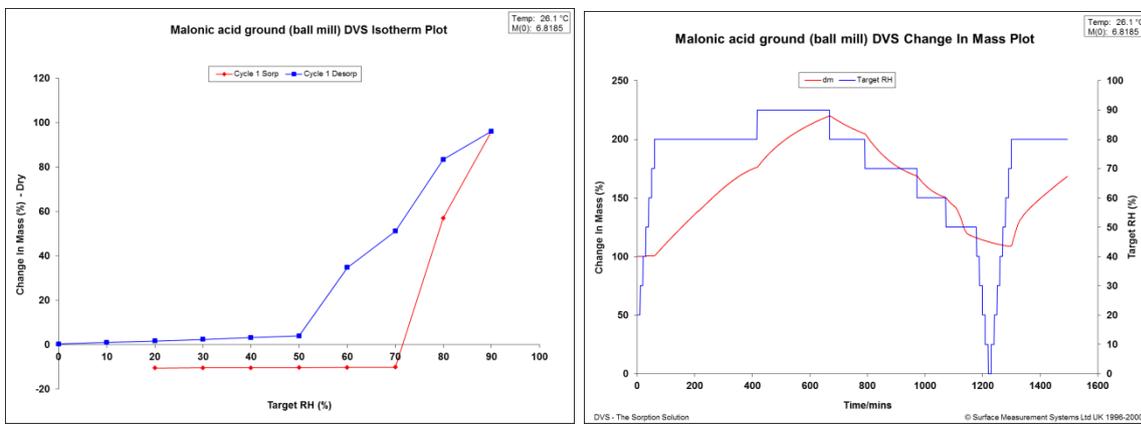
**Figure S6.**  $\beta$ -anhydrous caffeine. Displaying little change against humidity. N.B. see scale.



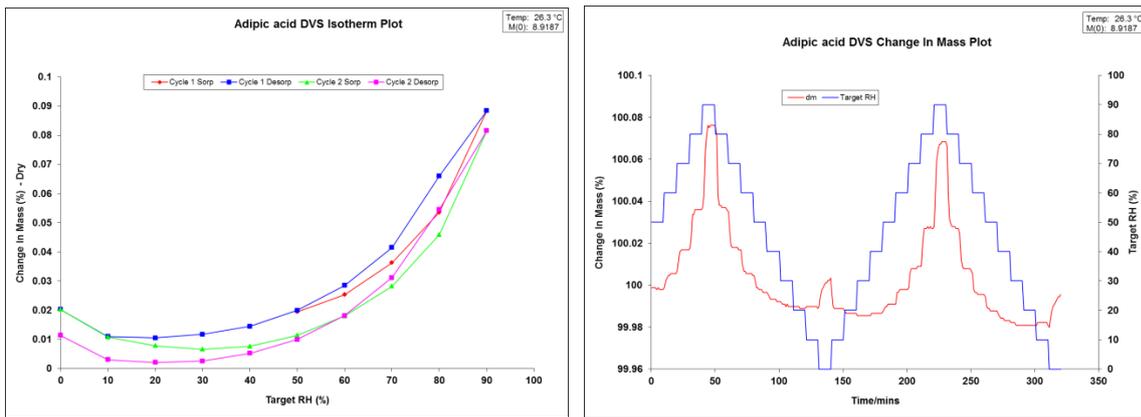
**Figure S7.** Caffeine: malonic acid co-crystal. Displaying moisture sorption above 60% RH. There is a degree of hysteresis in the isotherm plots, displaying the potential of a previously unreported hydrated form. This has not been seen elsewhere during (i.e. within in-situ X-ray and SSNMR transition monitoring), or been further investigated by this study.



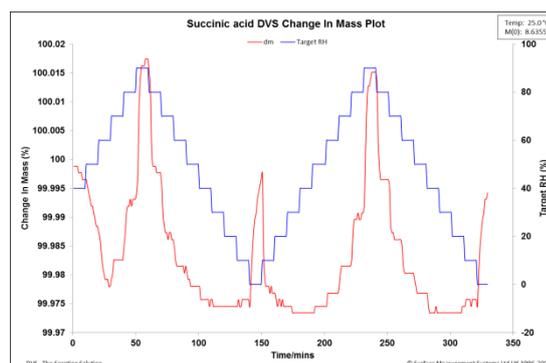
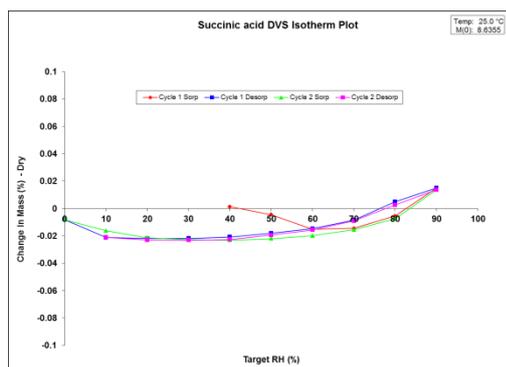
**Figure S8.** Malonic acid as delivered. Displaying significant hygroscopicity beyond 70% relative humidity.



**Figure S9.** Malonic acid once ground. Displaying greater hygroscopicity than the larger particle size form (Figure 8) beyond 70% relative humidity.



**Figure S10.** Adipic acid, showing no hygroscopicity. N.B. see scale.



**Figure S11.** Succinic acid, showing no hygroscopicity. N.B. see scale.

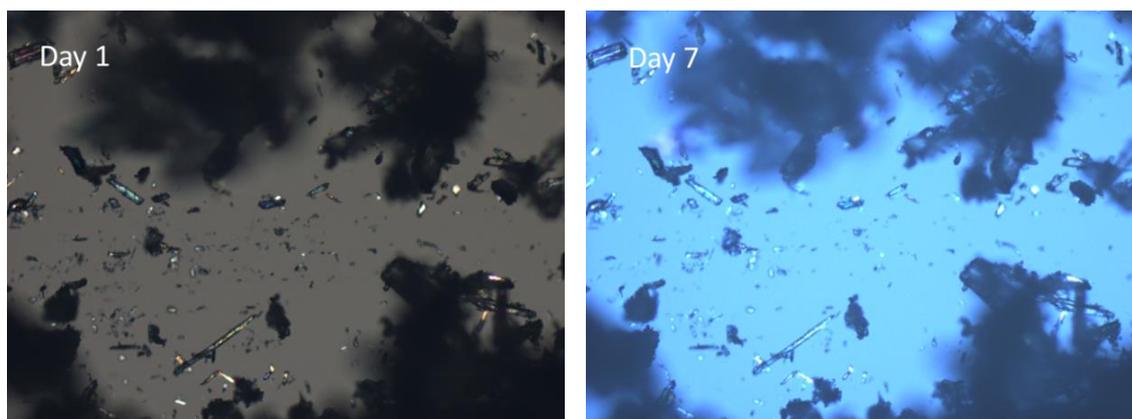
#### 4. Polarised optical microscopy



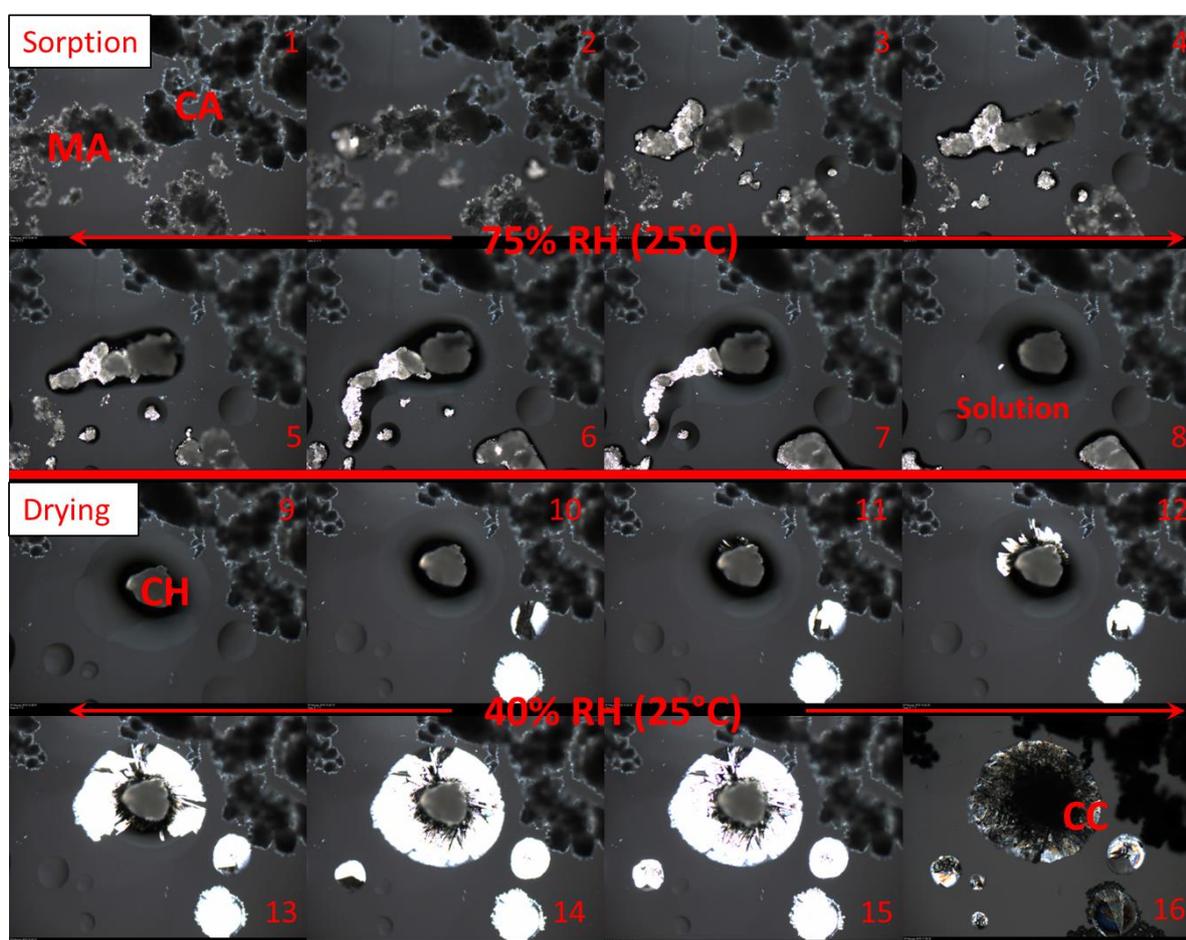
**Figure S12.** Humidity chamber setup, utilising inverted glass petri dishes. The material under investigation was suspended in a chamber above a saturated salt solution (salt + pure water). For the production of RH please see ESI Table S1 (below). All humidity's were checked with a Rotronic A/H hygrometer (temperature and RH).

**Table S1.** Salt solutions to prepare increased humidity environments.<sup>1</sup>

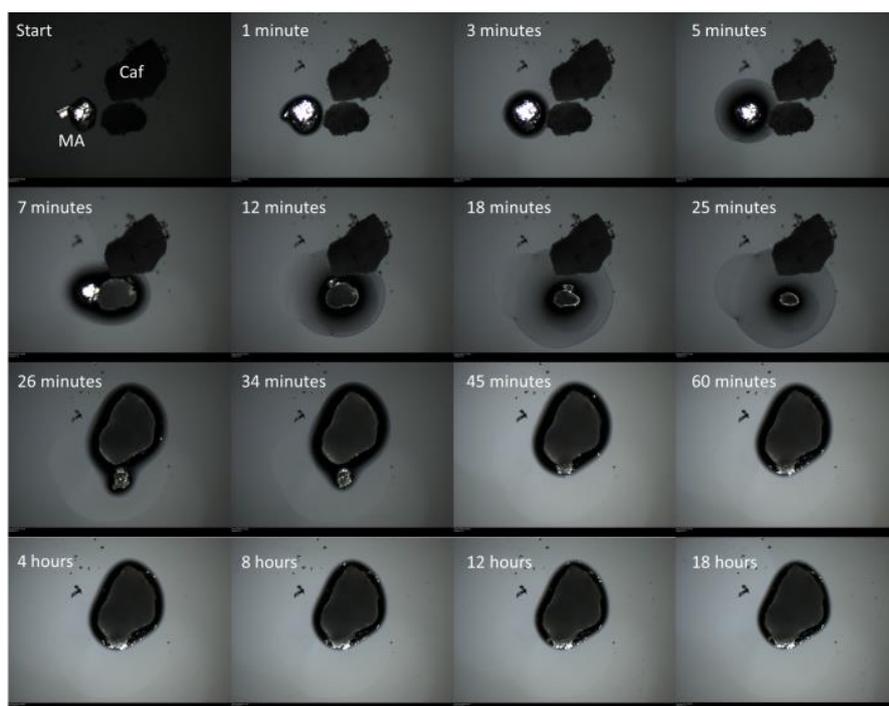
Desired Relative humidity at 25°C	Salt
75% RH	Sodium chloride
57% RH (labelled as 60%RH)	Sodium bromide
52% RH (labelled as 50% RH)	Magnesium nitrate
11% RH	Lithium chloride



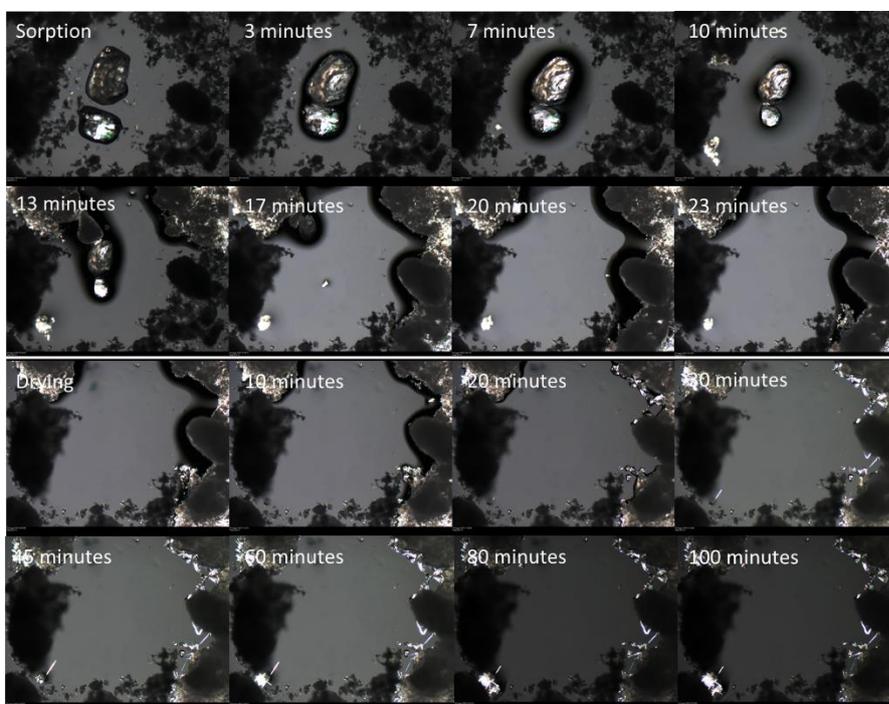
**Figure S13.** Caffeine: malonic acid ground samples at 40% RH (25°C) – ambient. No change was evident after 7 days.



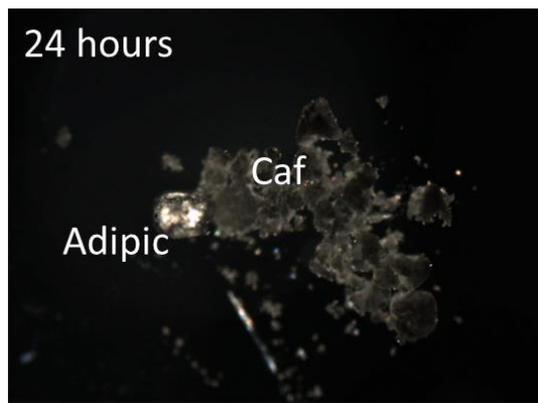
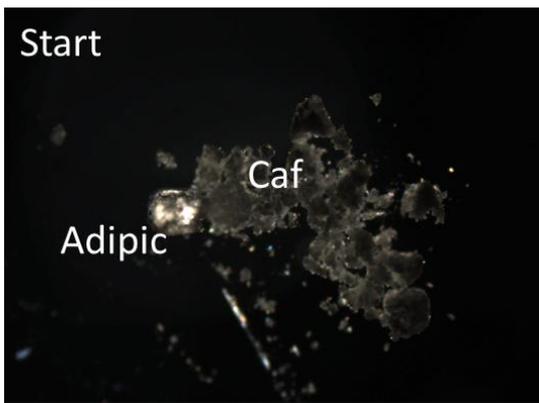
**Figure S14.** Caffeine and malonic acid micronized samples at 75% RH (25°C). Showing hygroscopicity of the malonic acid and conversion of caffeine to the hydrate (image 3) followed by complete dissolution of the malonic acid and growth of the co-crystal on the surface of the caffeine hydrate. Images are 2 minutes apart for the sorption section (1-8) and at 0, 89, 94, 96, 101, 120, 140 seconds and 1 hour respectively (9-16).



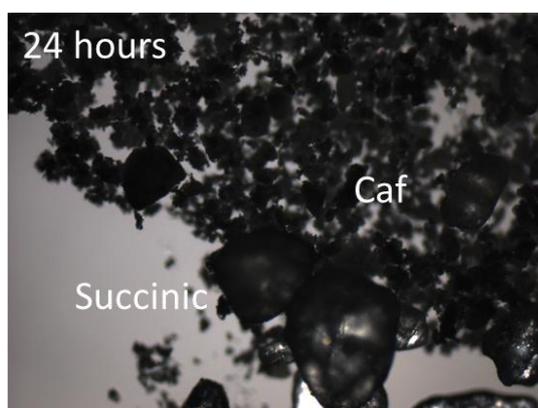
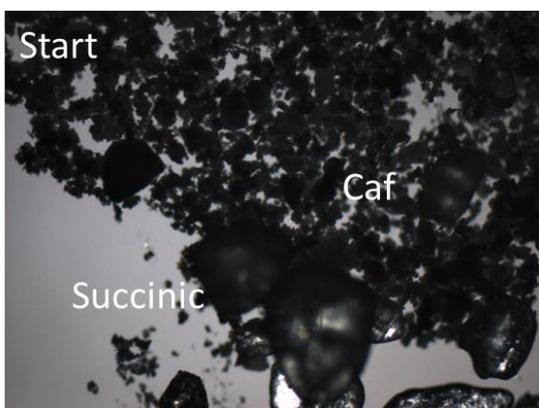
**Figure S15.** Caffeine: malonic acid ground samples at 50% RH (25°C). Showing deliquescence of the malonic acid followed by surface crystal growth on the caffeine.



**Figure S16.** Caffeine: malonic acid ground samples at 60% RH (25°C). Showing deliquescence of the malonic acid followed by co-crystal growth (most obvious bottom left of drying section).



**Figure S17.** Caffeine: Adipic acid, displaying no conversion to the co-crystal after 24 hours.

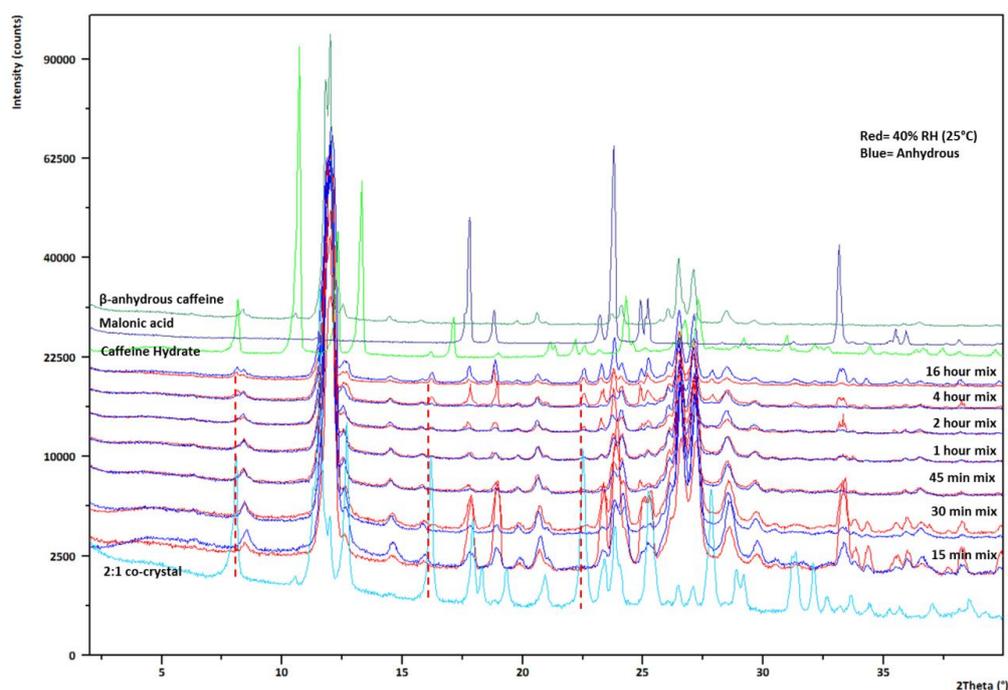


**Figure S18.** Caffeine: Succinic acid, displaying no conversion to the co-crystal after 24 hours.

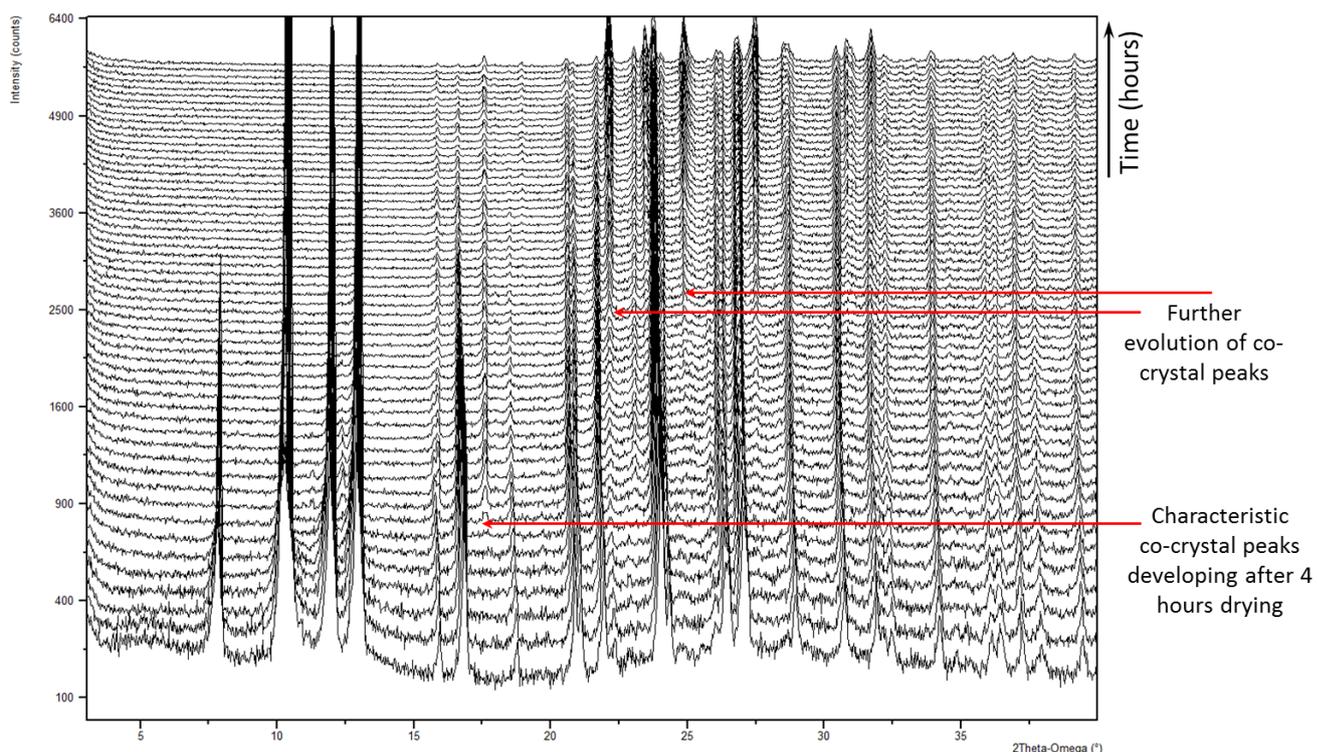
## 5. Roller Grinding and X-ray mapping of conversion



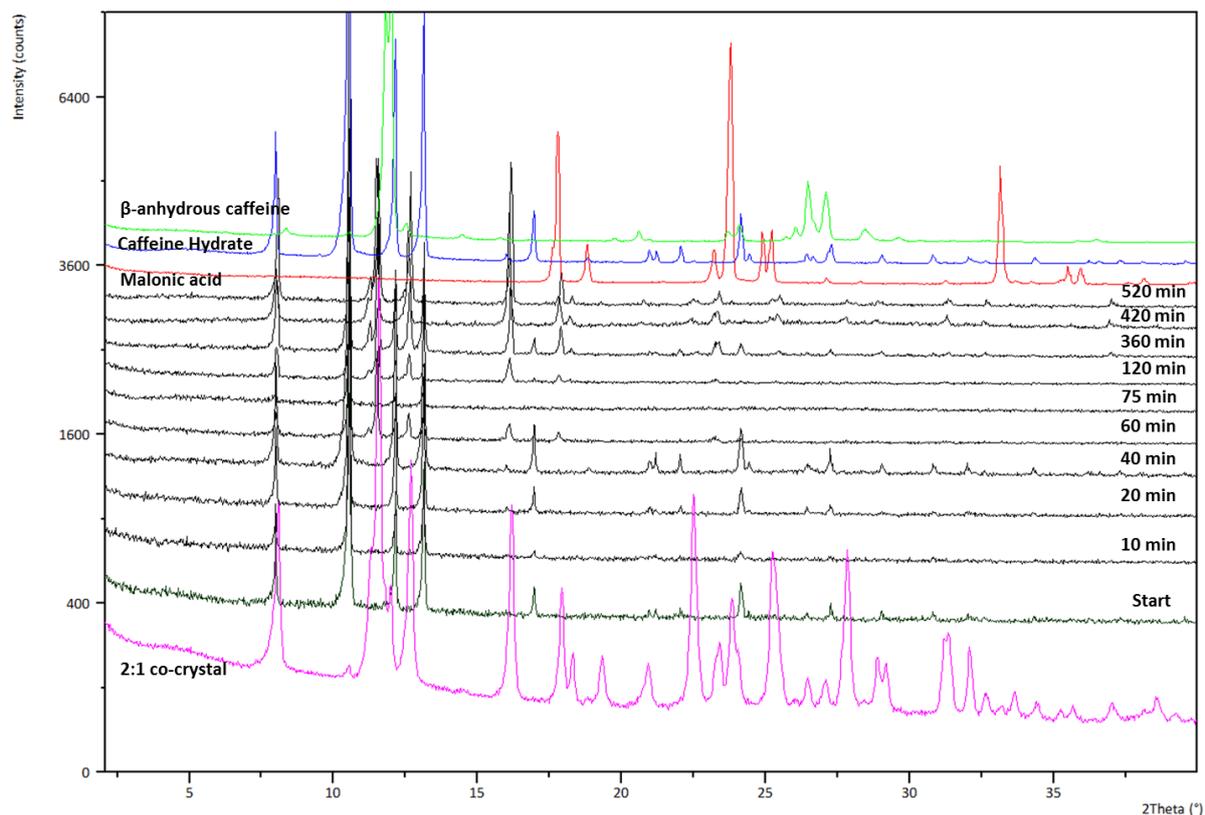
**Figure S19.** Grinding in roller mixer- no detectable difference in turnover to the co-crystal was seen in 24 hours i.e. Rate of co-crystal production is similar with or without water when levels were at ambient conditions (as per previous reports of co-crystal conversion through grinding).



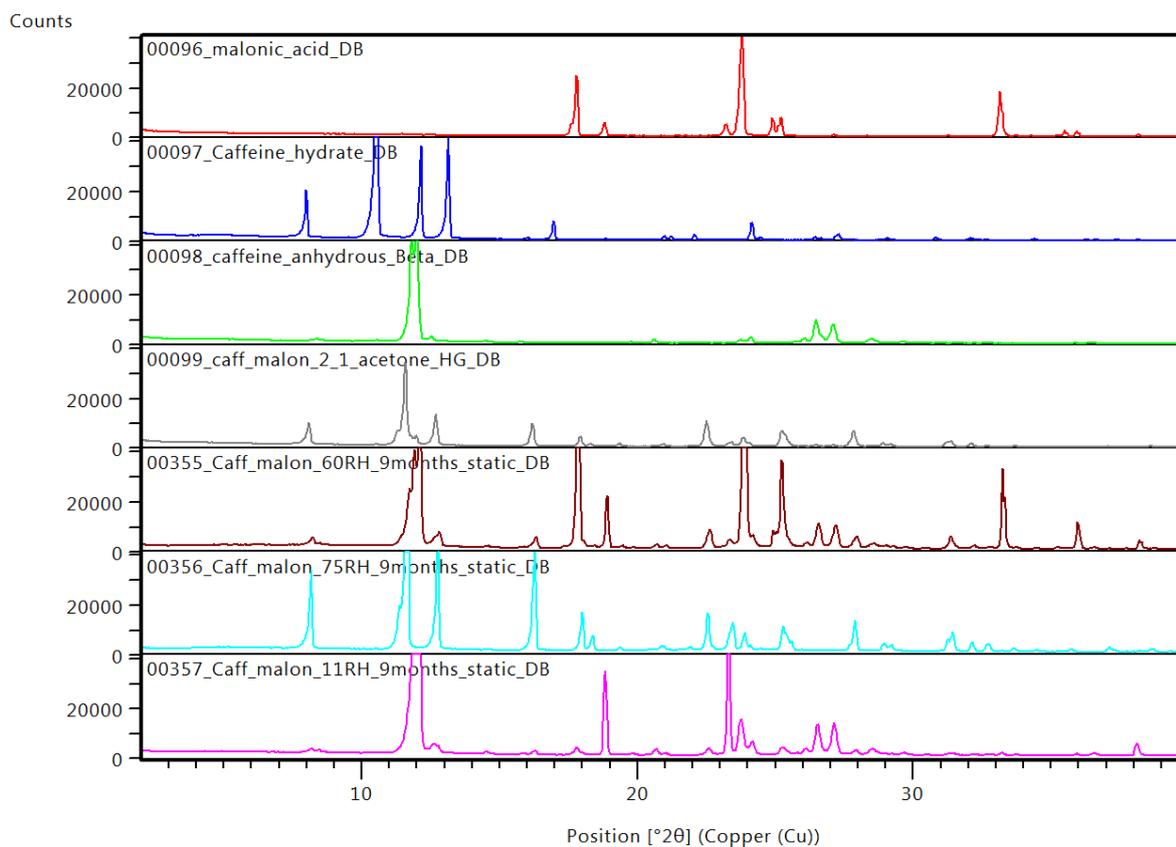
**Figure S20.** Showing Powder X-ray diffraction (full scale) for the mechanical grinding experiment. Conversion of the mixture to the co-crystal is not accelerated by moisture at ambient levels. Quantitative peak analysis has not been attempted due to uncertainty over the mixing process and subsequent sample selection. Simply the first time point at which characteristic peaks appear is noted, which is equivalent in both samples.



**Figure S21.** Showing Powder X-ray diffraction for the  $\beta$ -anhydrous caffeine drying experiment. Diffractograms were taken at 15 minute intervals. In this experiment 1.24g of MA and 2.31g of Caf water were used to produce a mixture, with the solids mixed together in an agate mortar and pestle by hand before the addition of molar quantities of water comparable to that seen on DVS deliquescence of MA (3.95g of pure water). In this instance the sample was allowed to dry until the first appearance of solids, then subsequent drying was undertaken in a deep fill sample holder on the diffractometer. The hydrate is the first phase seen followed by appearance of characteristics peaks of the co-crystal at 4 hours. Slight peak shifts associated with zero point error arising from the reducing sample height on drying can be seen.

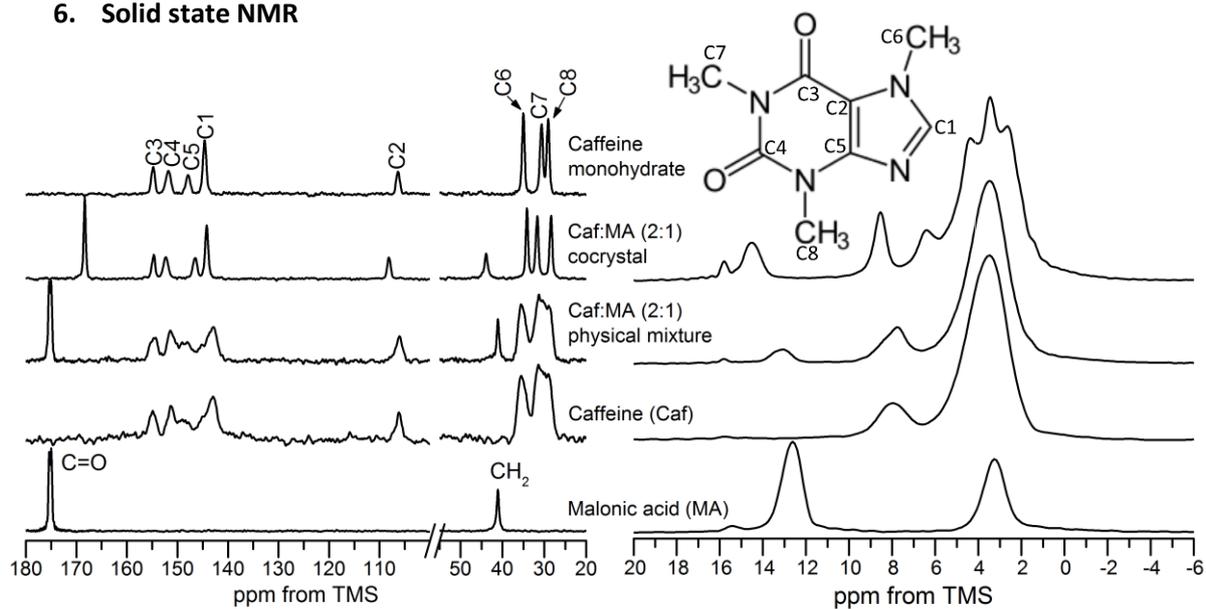


**Figure S22.** Showing Powder X-ray diffraction for the caffeine hydrate drying experiment. In this instance samples were taken at regular intervals and individually loaded onto the diffractometer cf. ESI Figure S20. In this experiment 1.24g of MA and 2.31g of Caf hyd were used to produce a mixture, with the solids mixed together in an agate mortar and pestle by hand before the addition of molar quantities of water comparable to that seen on DVS deliquescence of MA (3.95g of pure water). The hydrate failed to fully dissolve under these conditions and was seen to convert fully to the co-crystal after 420 minutes.



**Figure S23.** Displaying X-ray diffraction patterns for 2:1 mixtures of caffeine and malonic acid which were stored for 9 months under 3 humidity conditions. In this instance samples of Caf and MA were gently shaken together at ambient RH before being left under the humidity condition. Full conversion to the co-crystal can be seen in the 75% RH condition with the 60% RH condition displaying partial conversion. The 11% RH environment shows no evidence of conversion and appears to be simply a mixture of caffeine and malonic acid.

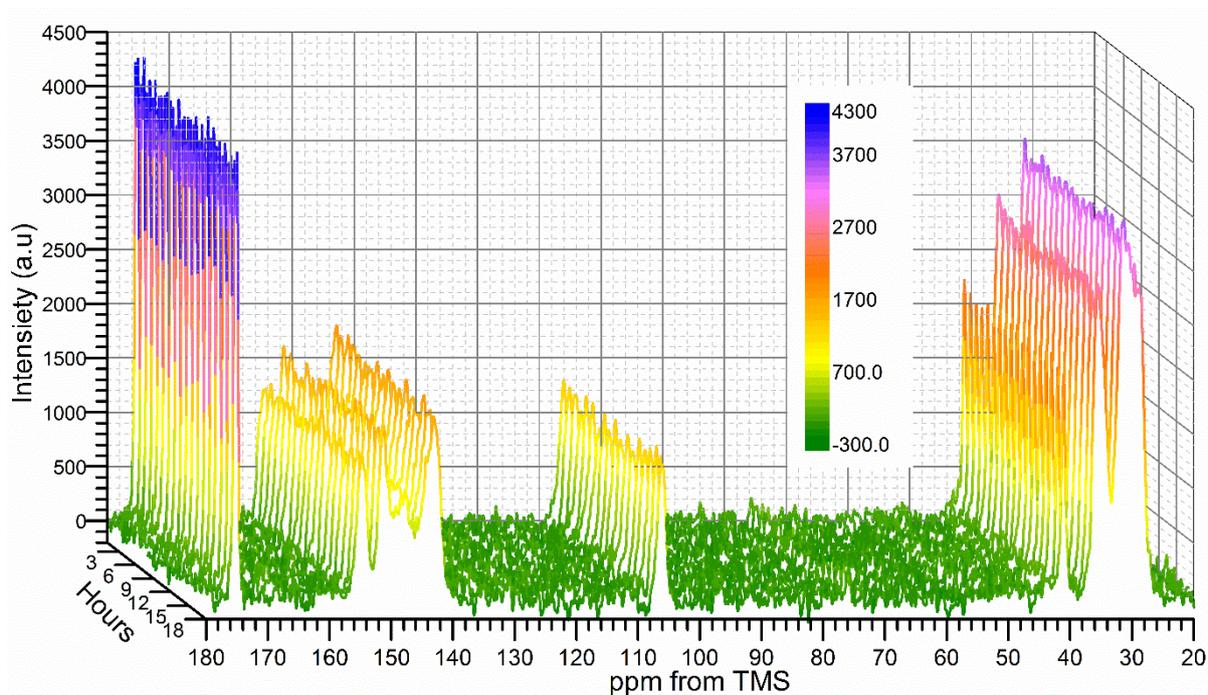
## 6. Solid state NMR



**Figure S24.**  $^1\text{H}$ - $^{13}\text{C}$  solid-state NMR spectra (left) and  $^1\text{H}$  CRAMPS spectra (right) of malonic acid, caffeine anhydrous, caffeine monohydrate and Caf:MA physical mixture and Caf:MA co-crystal. Assignment of the caffeine monohydrate peaks is in agreement with previously published data.<sup>2,3</sup>

### Co-crystallisation kinetics of dry materials:

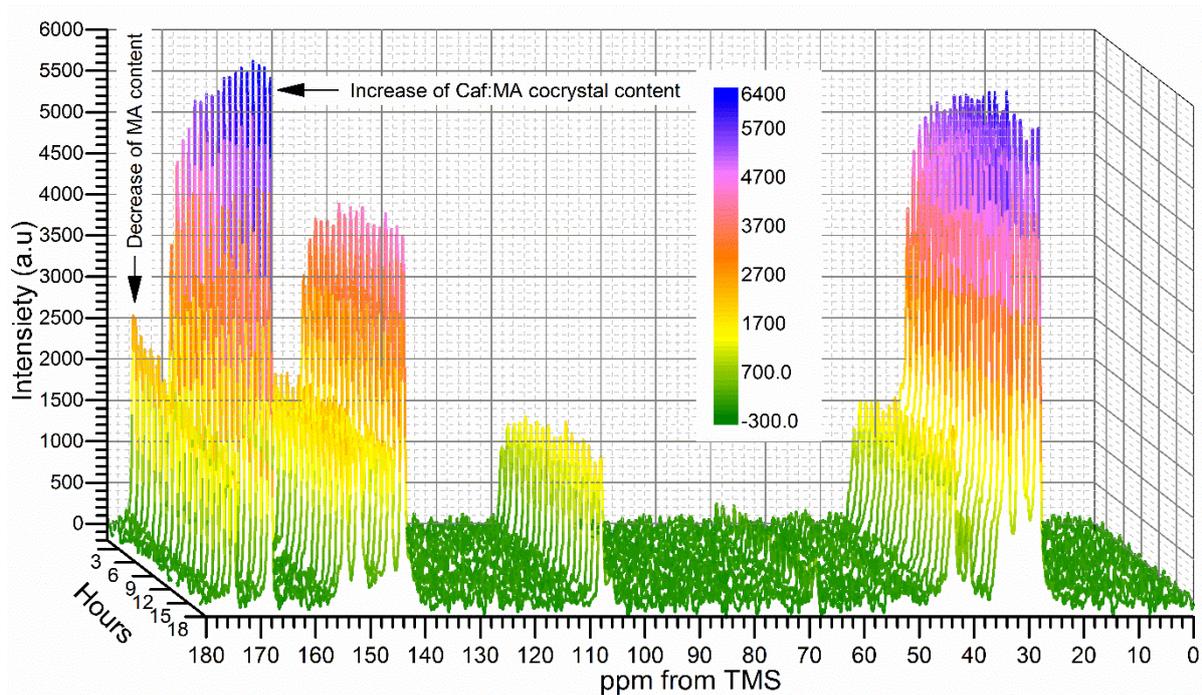
Dry mixture of Caf and MA shows a very slow transition to Caf:MA co-crystal over a period of 24 h during spinning at an MAS rate of 10 kHz (and further storage over P<sub>2</sub>O<sub>5</sub> for another 72 hours) (ESI Figure S24, ESI Figure S26).



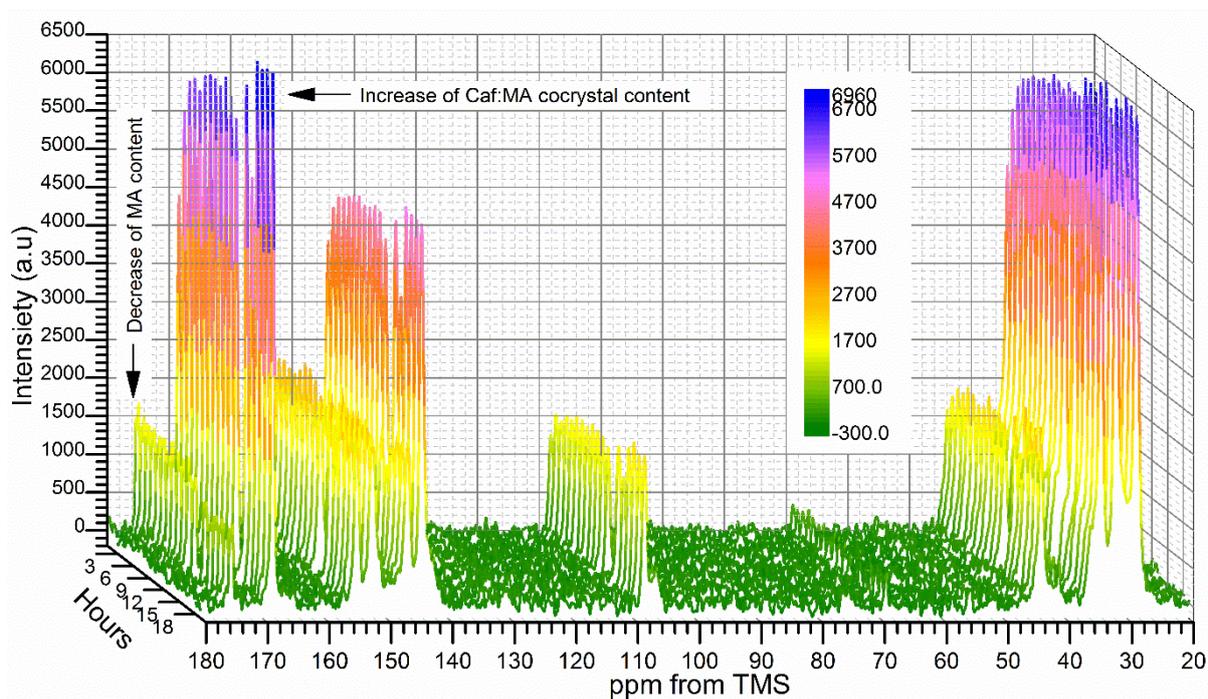
**Figure S25.** Series of <sup>1</sup>H-<sup>13</sup>C solid-state NMR spectra of dry Caf and MA mixture recorded within first 18 hours of kinetic experiment. Very slow transition to Caf:MA co-crystal is observed.

### Co-crystallisation kinetics of Caf:Caf hydr (25:1 and 20:1 molar ratio) with MA:

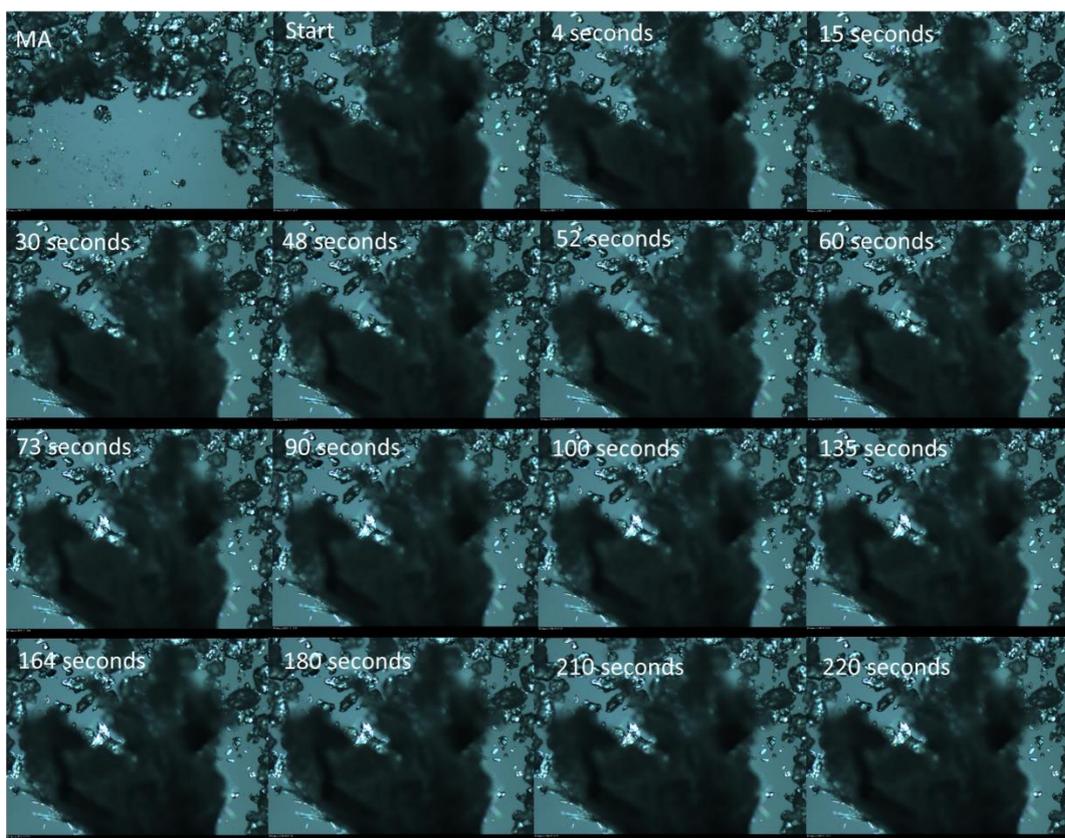
Co-crystallisation of MA and Caf:Cad hydrate mixtures has been monitored using solid-state NMR. During the first 18 hours of the process the content of the pure MA decreased while a gradual increase of the Caf:Ma co-crystal was observed (ESI S26, S27).



**Figure S26.** Series of  $^1\text{H}$ - $^{13}\text{C}$  solid-state NMR spectra of Caf:Caf hydr (25:1) and MA mixture recorded within first 18 hours of kinetic experiment.



**Figure S27.** Series of  $^1\text{H}$ - $^{13}\text{C}$  solid-state NMR spectra of Caf:Caf hydr (20:1) and MA mixture recorded within first 18 hours of kinetic experiment.



**Figure S28.** Caffeine hydrate contact with MA. Immediate co-crystal formation can be seen in the centre of the images. A better representation can be seen in the video of these data Figure S29 ESI.



caff\_hydrate\_malonic  
c\_contact\_3.avi

**Figure S29.** Embedded video of the movement of water on the contact of the caffeine hydrate with malonic acid.

## Materials

All materials were purchased from Sigma Aldrich at the highest available purity. Malonic acid was used as the delivered  $\beta$ -form when both delivered and after subsequent grinding. Caffeine was delivered as the anhydrous  $\beta$ -form and subsequent to grinding was still in this form. To create the caffeine hydrate a slurry of caffeine and pure water was created and magnetically stirred for a 48 hour period. This was filtered to dryness.

## Methods

**Grinding.** This was achieved using a Retsch ball mill, each chamber was filled with 2 g of the respective material and run at 20Hz 10 minutes in 50mL steel chamber with a 1.5cm steel ball. Post grinding annealing was conducted in a 50°C oven for a 48 hour period.

**Particle size analysis.** A Mastersizer 3000 was used with the dry dispersion AeroS stage. A pressure of 4 bar was used with the micro-sample plate used for powder delivery.

**Microscopy.** For microscopy a Leica DM2700P microscope was used with a QIcam Fast-1594 camera, with images recorded through the Linksys software ((Linkam Scientific Instruments Ltd., Tadworth, UK). For particle size analysis a stage graticule was used for lens special calibration with a 10x and 20x Hiplan polarising lens used. All other microscopy used a 4x Hiplan polarising lens.

**X-ray Powder Diffraction (XRPD).** X-ray powder diffraction patterns were recorded on a PANalytical Empyrean diffractometer using Cu K $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ), tube voltage of 40kV and 40mA current. Intensities were measured from 2° to 40° 2 $\theta$  with 0.04 rad. Soller slits and an incident beam divergent slit of 1/8°, anti-scatter slit of 1/4° and diffracted beam anti-scatter slit of 7.5mm (PIXcel).

**Differential Scanning Calorimetry (DSC).** Differential scanning calorimetry scans were recorded on a TA Q2000 using standard aluminium pans, calibration was undertaken before experimentation with indium and sapphire standards for heat of fusion and heat capacity respectively. A modulated method was used with an underlying 3°C/minute heating rate and a modulation of +/- 1°C/minute. A heat, cool, heat cycle was used with a 'standard' ramp used to initially melt the material, before checking for reversing heat capacity changes on the cooling and subsequent heating stages.

**Dynamic Vapour Sorption (DVS).** DVS was undertaken using an SMS DVS-1 with a 10% RH step between humidity values with equilibrium achieved at 0.01% weight change before moving to the next step. Methods were started at the humidity of the room at ambient (measured by a Rotronic A/H hygrometer) with subsequent increase to 90%RH before cycling to 0%RH, to 90%RH, to 0%RH. Sample weights of between 5-20mg were used for all samples (M(0) displayed in ESI Figures).

**Mixing.** Mixing was conducted on samples of milled  $\beta$ -anhydrous caffeine and  $\beta$ -malonic acid at the 250mg scale using a 2:1 blend of caffeine to malonic acid. One set of samples were dried in the oven at 50 °C for 24 hours prior to analysis, these were then sealed into vials in-situ and left sealed until analysis. The other set of samples were left at room temperature and humidity. Mixing was undertaken on a 'tube roller'. This ensured a gentle rolling motion in the powder bed which was consistent in all samples due to uniform size of sample and container. Analysis was conducted by powder X-ray diffraction.

**Solid-state NMR.** All NMR experiments were performed using a Bruker 400 MHz Avance III solid-state NMR spectrometer equipped with a triple resonance probe operating at frequencies of 400.23 MHz ( $^1\text{H}$ ) and 100.64 MHz ( $^{13}\text{C}$ ). Materials were packed in the 4 mm zirconia rotors and rotated at an MAS rate of 10 kHz. All materials were characterised using  $^1\text{H}$  single pulse,  $^1\text{H}$ - $^{13}\text{C}$  cross-polarization magic angle spinning (CP/MAS) techniques ( $^1\text{H}$   $\pi/2$  pulse length 3.5  $\mu\text{s}$ ,  $^{13}\text{C}$   $\pi/2$  pulse length 3.5  $\mu\text{s}$ ,  $^1\text{H}$ - $^{13}\text{C}$  CP contact time 2 ms, SPINAL64 decoupling was used during signal acquisition). Recycle delay of 40 s was used and total number of 90 scans was acquired in all kinetics experiments in agreement with previously published data.<sup>4</sup> The Hartmann-Hahn conditions for  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS NMR experiment were set with hexamethylbenzene (HMB). The  $^{13}\text{C}$  chemical shifts were recorded with respect to TMS. All spectra were acquired at 20 °C using dry nitrogen as a spinning gas.

High resolution  $^1\text{H}$  spectra were recorded using the FSLG homonuclear decoupling pulse sequence at an MAS spinning rate of 6.8 kHz.<sup>5</sup> In  $^1\text{H}$  CRAMPS experiments (Combined Rotation and Multiple Pulse Spectroscopy) 32  $\mu\text{s}$  wplmg1 cycle was applied with effective *rf* decoupling power of 100 kHz. A short acquisition window (10-15 ms) was used in order to acquire a complete data set and to avoid probe overheating.

#### **Kinetic experiment in dry conditions**

Both  $\beta$ -anhydrous caffeine and  $\beta$ -malonic acid were dried in the oven at 50 °C for two hours prior to analysis. Materials in 2:1 (Caf:MA) molar ratio were mixed for 30 seconds using vortex at maximum speed according to the procedure described previously by Mandala *et al.* and divided into two batches.<sup>4</sup> First batch was packed in the 4 mm NMR Zirconia rotor, analysed using solid-state NMR in series of one hour  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS experiments for 24 hours and further stored in dry conditions (sealed in the Zirconia rotor over  $\text{P}_2\text{O}_5$ ) for another 72 hours. The second batch was left in an open vial on the bench at *ca.* 45% RH and analysed after 96 hours. The time from vortex mixing to start of NMR experiment was shorter than 5 minutes. It is important to note that even a gentle mixing of dry compounds using mortar and pestle results in fast transformation to the co-crystal.

#### **Kinetic experiments with caffeine monohydrate**

B-Anhydrous caffeine and malonic acid in 2:1 molar ratio were weighted accurately to two separate vials and stored in the oven at 50 °C for 2 hours prior to each experiment to avoid presence of water adsorbed at the material surface. In order to investigate effect of controlled amount of water on the formation of Caf:MA acid co-crystal caffeine monohydrate was mixed together with dried anhydrous caffeine. Both materials were mixed thoroughly using vortex, then malonic acid was added and materials were further vortexed for another 30 seconds at maximum speed. The samples were packed in a 4 mm NMR Zirconia rotor and analysed using solid-state NMR. The time from vortex mixing to start of NMR experiment was shorter than 5 minutes.

## References

- 1 LB. Rockland LB., *Analytical Chemistry*, 1960, **32**(10), 1375-6.
- 2 GD. Enright GD, et al., *Crystal Growth & Design*, 2007, **7**(8), 1406-10.
- 3 FG. Vogt, et al., *Crystal Growth & Design*, 2009, **9**(2), 921-37.
- 4 VS. Mandala, et al., *The Journal of Physical Chemistry Letters*, 2014, **5**(19): 3340-4.
- 5 E Vinogradov, et al., *Chemical Physics Letters*, 1999, **314**(5-6), 443-50.