Highly Unusual Triangular Crystals of Theophylline: The Influence of Solvent on the Growth Rates of Polar Crystal Faces

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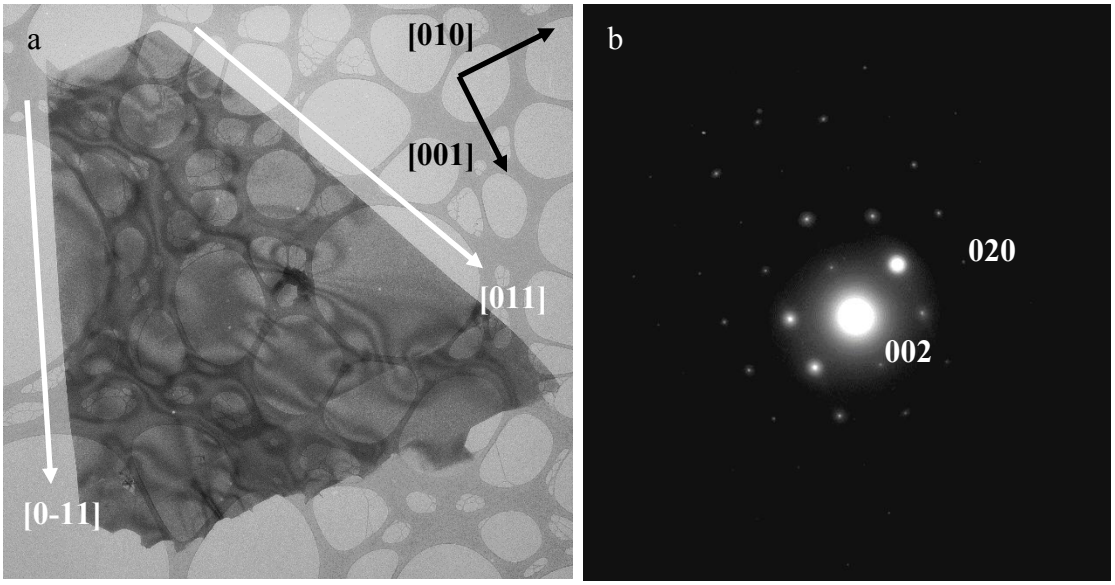

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Introduction

The large diversity observed in the habits of crystals,[1,2] ranging from simple cubic blocks to the intricate hexagonal structures of snowflakes,[3,4] results from the complex interplay of the many different processes involved in crystal growth.[5-11] Understanding these phenomena is not only of academic importance, but also critical in many industrial sectors since the behavior of crystalline materials can be strongly influenced by crystal habit.[12-14] For example, in many applications the shape and size of particles must be carefully controlled to ensure that product performance is consistent from batch to batch.[12, 14-16] Furthermore, while the process of generating a desired particle size and morphology profile in a batch of material has traditionally been performed post crystallization through techniques such as milling and micronisation,[17, 18] it is more desirable to gain sufficient control of the crystallization step such that crystals with optimum characteristics are grown directly.[18-20] For these reasons, focus has recently been directed to gaining further control and understanding of crystallization processes.[21-25]

In addition to morphology, crystallization conditions can also be used to control which crystal form (polymorph) of a compound is generated.[26-29] This is important because the various crystal forms a compound may adopt can have very different properties.[17, 30, 31] While it is still usually impossible to say 'a priori' which crystal form will result from a given set of crystallization conditions,[32] notable advances have been made in recent years in the prediction of aspects of polymorphic behaviour.[33-36] For example, using crystal structure prediction it is now routinely possible to computationally generate plausible crystal structures for a simple organic compound and determine which is likely to be the most stable form on the basis of lattice energy calculations.[33, 37-39] An important question, however, is why crystal structure prediction often generates many more low energy crystal forms for a compound than are found to exist in practice[32, 40] One possibility is that these forms do exist experimentally, but only in trace amounts as minor components of mixtures with other crystal forms, and so are rarely detected.[40] An example of such behavior is presented in this study.

Theophylline is a pharmaceutically active compound used to treat asthma and cardiopulmonary disease for which there are six previously reported crystal forms (crystallographic data for those forms for which crystal structure determination has been performed are given in the Supporting Information, Table S1).[40, 41-46] Form II (orthorhombic, space group $Pna2_1$), is the most commonly encountered polymorph, and was long believed to be the thermodynamic form of theophylline under ambient conditions, though it has recently been shown that Form IV is in fact more stable.[47] The crystal structure of Form II was determined by Ebisuzaki *et al* using crystals grown by sublimation that were reported to have a thin-plate morphology. Notably, Form II has subsequently been reported to crystallize with a highly

unusual triangular habit, during both supercritical CHF_3 crystallization and solution growth from nitromethane, though no discussion of the growth mechanism was given.[45, 46]

In this study, the factors leading to growth of Form II theophylline crystals with a triangular habit are elucidated. It is demonstrated that there is a strong influence of solvent on the resulting crystal habit, with both solvent polarity and the ability of a solvent to form hydrogen bonding interactions contributing to whether or not triangular crystals are obtained. Transmission electron microscopy is used to map crystallographic directions to crystal habit allowing the growth of triangular crystals to be rationalized on the basis of the arrangement of molecules in the polar crystal structure of Form II . The model of triangular crystal growth thus derived is then used to interpret interesting growth features on the surfaces of theophylline crystals identified by atomic force microscopy. We also present a second type of triangular theophylline crystal which has a shape that does not fit this model. It is suggested that these crystals correspond to a new polymorph of theophylline, the crystal structure of which is proposed from an analysis of electron diffraction data and comparison with a set of putative structures generated computationally by global lattice energy searching.

Experimental Section

All chemicals were purchased from Sigma-Aldrich (purity ≥ 99%) and used without further purification.

Triangular crystals of Form II of theophylline were grown by solution crystallization from a variety of solvents including nitromethane, ethyl acetate, dioxane and toluene using either

evaporation or cooling to induce supersaturation and crystal growth. The following conditions were used to generate samples for analysis by transmission electron microscopy and atomic force microscopy and are given as an example: 10.0 mg of theophylline was dissolved in 2.0 ml of nitromethane with heating. The resulting solution was then stored at -20 °C to induce supersaturation and precipitation. No stirring or solvent evaporation was performed.

Optical microscopy was performed on a Leica DM1000 instrument with a polarizing filter.

Transmission electron microscopy (TEM) characterization was performed at room temperature on a Philips CM30 instrument operating at 300 kV and data were collected on photographic films which were scanned in order to generate digital images. Samples were supported on holey-carbon films on 300 mesh copper grids held within a double tilt sample holder. The diffraction patterns were used for crystal form identification and indexed by comparison with the reported crystal structure of Form II of theophylline (CSD ref BAPLOT01)[42]. The positions of reflections in experimental diffraction patterns were measured, converted to d-spacings and matched to calculated values for the Form II structure. The experimental diffraction patterns were then compared with simulated diffraction patterns of the appropriate zone axes to ensure a match using CrystalMaker SingleCrystal software. The relative orientations of images and diffraction patterns were determined through analysis of crystals of molybdenum trioxide (where the relationship between crystal structure and crystal habit is known)[48].

Atomic force microscopy (AFM) images were recorded using a Veeco Instruments Multimode atomic force microscope, operated by a Nanoscope IIIa controller, interfaced with a Quadrex extender module. Samples were prepared on a mica coverslip attached to a stainless steel AFM sample disc using a sticky tab and imaged using a J-scanner (150 μm maximum scan size) in

contact mode. Data were analyzed using NanoscopeTM software version 6 and images were flattened prior to analysis.

Crystal structure prediction (CSP): Putative crystal structures of theophylline were generated with the CrystalPredictor program,[49] using rigid molecular geometries derived from density functional theory calculations on the isolated molecules. Trial crystal structures were created with quasi-random unit cell dimensions, molecular positions and orientations with $Z' = 1$ in 25 common space groups. The resulting crystal structures were then re-optimized using the programs DMACRYS[50] and CrystalOptimizer,[51] with intermolecular interactions described by an empirically parameterized exp-6 repulsion-dispersion potential (the FIT potential described by Coombes *et al*)[52] and an atomic multipole electrostatic model, with multipoles derived from a distributed multipole analysis[53] of the calculated molecular charge density. Full details of the CSP methodology have been given elsewhere.[54]

The electrostatic potential surface for theophylline was calculated using the DFT BLYP functional[55-57] and 6-31G(d) basis set as implemented in Tonto[58] within the software CrystalExplorer.[59]

Results and Discussion

(i) Analysis of the growth of triangular crystals of theophylline

The growth of triangular crystals of Form II of theophylline by evaporative crystallization from a drop of nitromethane solution placed onto a glass slide was followed by optical microscopy (Figure 1). The habit adopted by these crystals can be described more accurately as

plate-like isosceles triangles which have an acute angle at the vertex. The vertex of the triangle is located at the point of nucleation and crystal growth occurs in a direction perpendicular to the base of the triangle. It is common for multiple crystals to nucleate at the same nidus and to grow out radially. Nucleation can also occur on the faces of existing crystals as highlighted with an asterisk in Figure 1c (see also Supporting Information Figure S1), particularly under the high supersaturation conditions that are induced by the fast rate of solvent evaporation occurring in the glass slide experiment, with the daughter crystals being aligned with the parent suggesting an epitaxial relationship. The polymorphic form of the triangular crystals was confirmed to be Form II by powder X-ray diffraction (see Supporting Information Figure S2).

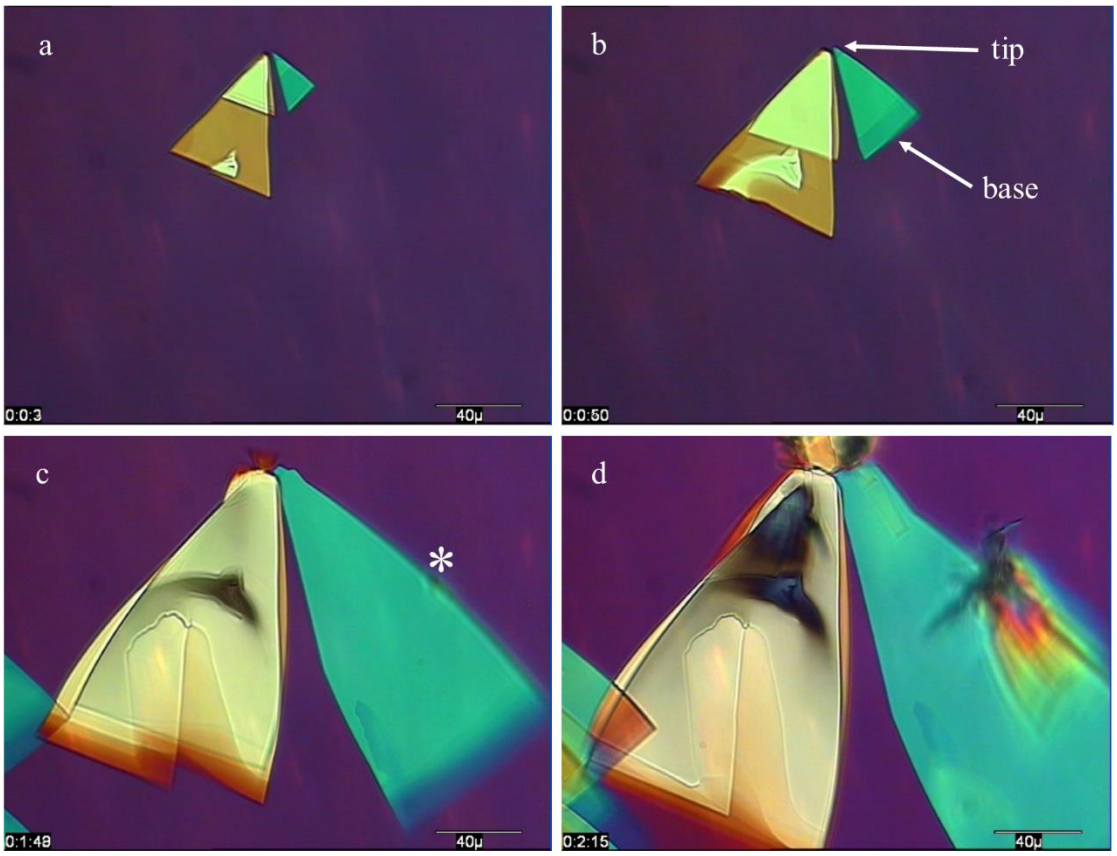

Figure 1. Optical microscope images showing the growth of triangular crystals of Form II of theophylline during evaporation of a small drop of a solution of theophylline in nitromethane (placed on a glass slide) over a period of 2 minutes and 15 seconds. The crystals grow in a direction from tip to base. The asterisk in image (c) marks a site on the triangular crystal surface where nucleation of further crystals of theophylline occurs.

The orientation of the theophylline Form II crystal structure was related to the observed triangular crystal habit using transmission electron microscopy (ordinarily this mapping could be performed using single crystal X-ray diffraction, but as the theophylline crystals prepared in this

study were too small to enable such an analysis, having sub-micron thicknesses, TEM was used for its combination of high magnification imaging and diffraction capability). A representative TEM image of a theophylline crystal is shown in Figure 2 along with a corresponding electron diffraction pattern. The b-axis of the crystal runs parallel to the base of the triangular crystals, meaning that growth occurs in the [001] direction within a growth sector bounded by the [011] and [0-11] directions. The angle between the two edges of the triangle is measured to be approximately 47°, which is consistent with the calculated interplanar angle for (011) and (0-11) of 48.5°.

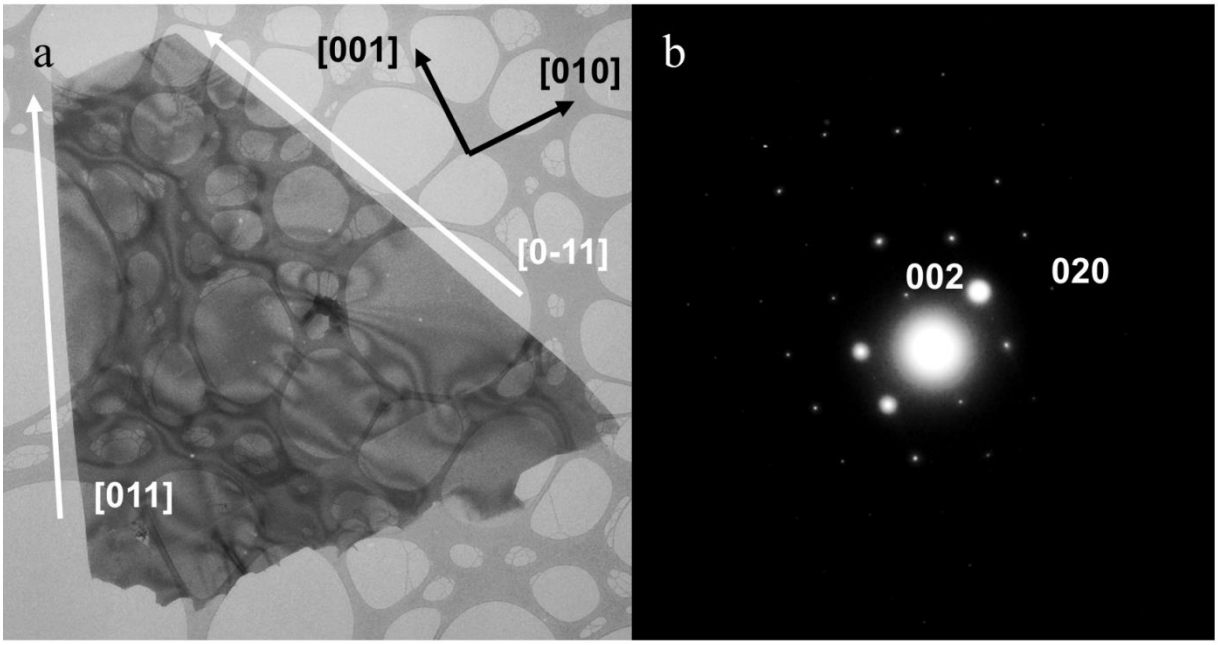

Figure 2. (a) TEM image showing a triangular plate crystal of Form II of theophylline. The tip of the triangle has fractured off prior to analysis,[46] and there is evidence of uneven growth at the rapidly growing crystal face which forms the base of the triangle. (b) Corresponding rotation corrected <100> zone axis electron diffraction pattern (signifying that the electron beam was

aligned with the a-axis of the crystal when the electron diffraction pattern was recorded). The reflections in the diffraction pattern were indexed on the basis of d-spacings and interplanar angles, allowing crystallographic directions to be assigned to the TEM image (further details of the indexing process are given elsewhere)[46].

(ii) Solvent dependence of the crystal morphology of theophylline Form II

The growth of triangular plate-like crystals of theophylline is not limited to crystallization from nitromethane. For example, such crystals are also obtained on crystallizing theophylline from the solvents ethyl acetate and dioxane (Figures 3a-b). The common feature of these solvents is that they possess hydrogen bond acceptor groups, but no donor groups. In addition, triangular theophylline crystals can be prepared from highly non-polar solvents such as toluene (Figure 3c). Importantly, however, triangular crystals do not always form. For example, solvents with both hydrogen bond donor and acceptor groups, such as methanol, ethanol and isopropyl alcohol, yield lath shaped crystals (Figures 3d-f). It is notable that crystal growth in these alcohols is relatively slow. Furthermore, crystals obtained from chloroform, a molecule with an intermediate polarity and no hydrogen bonding capability, are laths with tapered ends (Figure 3g). Crystallization by sublimation, i.e. in the absence of solvent, leads to the formation of rectangular plates (Figure 3h).

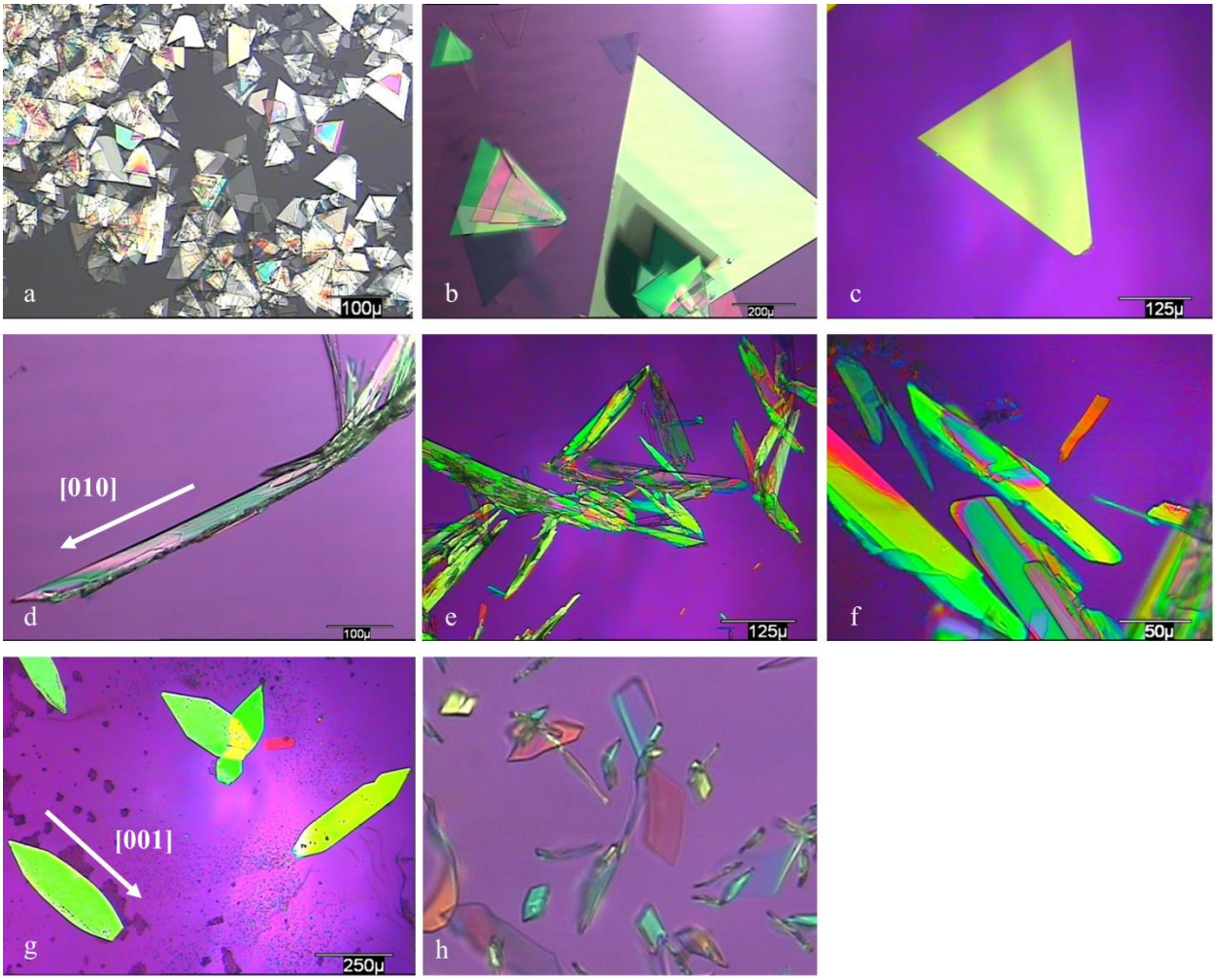

Figure 3. Polarized light microscopy (PLM) images of crystals of Form II of theophylline prepared by solution crystallization from (a) ethyl acetate, (b) dioxane, (c) toluene, (d) methanol, (e) ethanol, (f) isopropyl alcohol, (g) chloroform. The crystals were grown by cooling saturated solutions of theophylline in the respective solvents and were removed from the crystallization solvent prior to analysis. (h) Form II crystals prepared by sublimation. Crystallographic directions have been included in images (d) and (g).

(iii) Understanding the growth of triangular theophylline crystals

The observed solvent dependence of crystal habit was rationalized by considering the crystal packing in Form II of theophylline.

In the Form II structure theophylline molecules assemble through hydrogen bonding interactions to form 1D chains (Figure 4a). The chains stack (with parallel rather than antiparallel alignment) to give 2D layers as shown in Figures 4b-c. Importantly, hydrogen bonded chains in one layer are oriented at 48.5° to those in the next layer, resulting in an ABAB type packing arrangement (Figures 4d-e). Furthermore, the polar nature of the Form II structure leads to (001) and (00-1) crystal faces being inequivalent. Hydrogen bond donating NH groups are exposed at the (100) face (in both A-layers and B-layers) whereas hydrogen bond accepting nitrogen atoms possessing a lone pair of electrons (N:) are exposed at (00-1). In contrast, the (010) and (0-10) faces are equivalent.

In the Figures I wonder if the double headed arrows would be better replaced with single-headed to emphasis the polarity and maybe add A and B to the figure as well as (00-1) and (100)? See attachment.

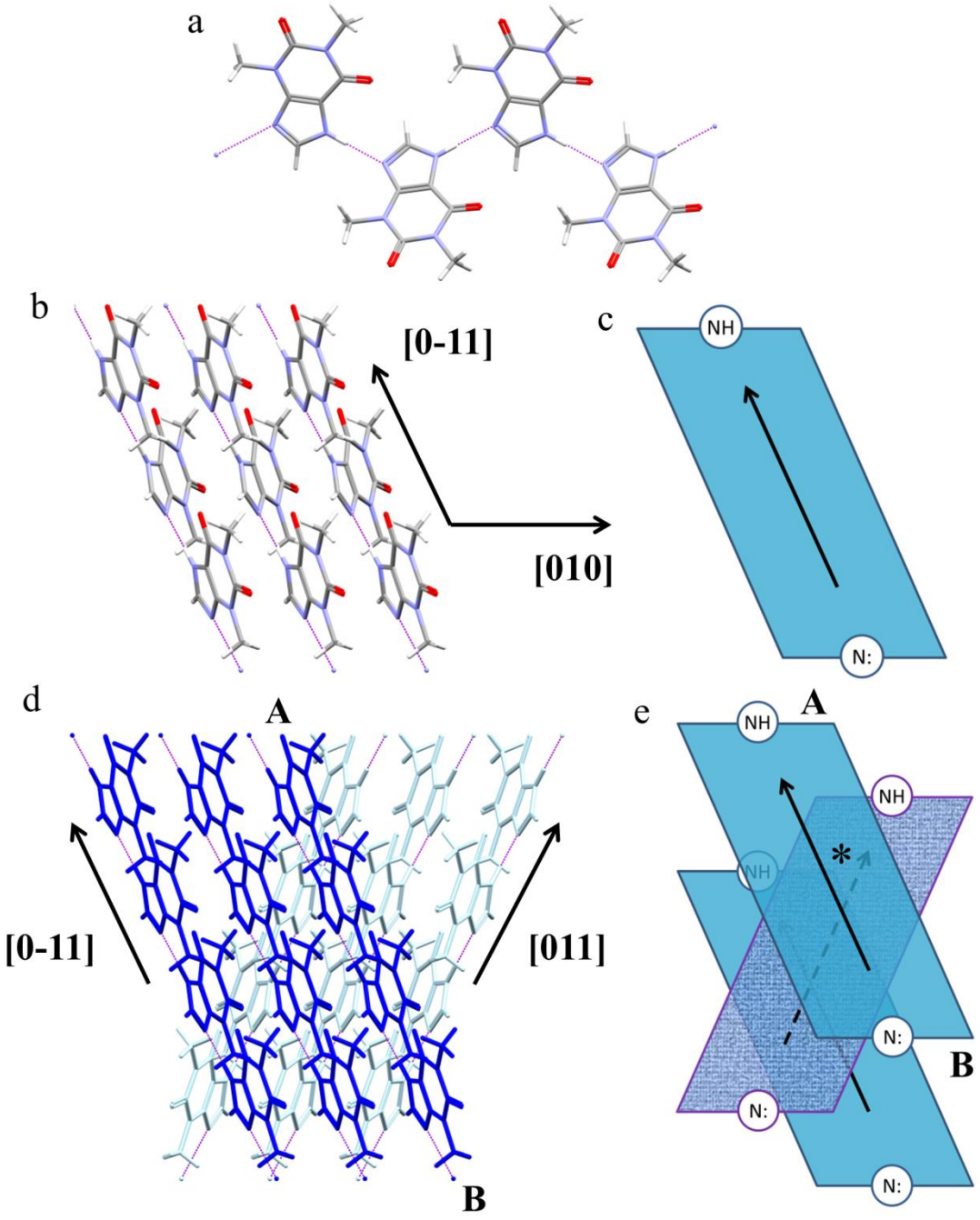

Figure 4. (a) An image showing hydrogen bonding between theophylline molecules in the crystal structure of Form II of theophylline. The imidazole rings in adjacent theophylline molecules are linked through NH···N hydrogen bonds to give a linear chain. (b) A single layer of theophylline molecules viewed down the a-axis (i.e. the layer is parallel with the plane of the

page). Hydrogen bonded chains of theophylline molecules are shown running in the [0-11] direction and stacking (through weaker dispersive forces) in the [010] direction in a parallel manner. (c) A schematic representation of the layer of molecules shown in (b). The circled NH signifies that at the top edge of the layer the theophylline chains are terminated by NH groups whereas the circled N: signifies that at the bottom edge chains are terminated by N: groups. (d) Image of the full crystal structure of Form II of theophylline viewed down the a-axis. Molecules in odd and even numbered layers have been shaded light and dark respectively to highlight the difference in the orientations of chains of theophylline molecules in adjacent layers. The chains in A-layers run parallel to the <011> axis whereas those in B-layers are parallel to the <0-11> axis. (e) A schematic representation of the Form II structure showing the ABAB type stacking arrangement of layers (A-layers have been given a textured appearance whereas B-layers have been given a smooth appearance). The arrows show the orientations of the chains of molecules in each layer. The angle between chains in adjacent layers, as marked with an asterisk, is 48.5°.

The growth rates of different theophylline crystal faces can be related to the Form II structure. Firstly, interactions between layers in the Form II structure are weak, leading to a slow growth rate at the {100} crystal faces. These faces form the dominant surfaces of all Form II crystals, whether triangular plates or rectangular laths. For crystals prepared by sublimation, growth would be expected to be to be quickest in the direction parallel the chains of theophylline molecules, due to the favorable hydrogen bonding interactions. The fact that this direction is different in A and B layers of the Form II structure is a complicating factor, but it is assumed that the rate of maximum crystal growth would be in the <001> axis as this lies directly between the hydrogen bonding directions in A and B layers.

During crystallization from solution, solvent molecules attach to Form II crystal faces and influence their growth rates. For example, a solvent capable of forming hydrogen bonds can interact with the ends of the growing chains of theophylline molecules, slowing the addition of further theophylline molecules and inhibiting the elongation of the chains. Critically, if the solvent possesses a hydrogen bond acceptor group, but no donor group (as is the case for nitromethane, ethyl acetate and dioxane) this inhibition will occur only at the NH terminated end of each theophylline chain. In such a situation, the growth of chains of theophylline molecules in A-layers would be inhibited in the [011] direction and occur primarily in the [01-1] direction, whereas chains in B-layers would grow primarily in the [0-1-1] direction (Figure 5a). Four growth sectors can be defined for theophylline crystals, as shown in Figure 5b, and it follows that during crystallization from a hydrogen bond acceptor solvent, the growth of A-layers would be inhibited in sectors i and iii, while the growth of B-layers would be inhibited in sectors i and ii. It is only within sector iv that the growth of both A-layers and B-layers is favored. Growth will be limited to this triangular shaped sector, and this explains why theophylline crystals with a triangular habit are observed experimentally.

Evidence to support this hypothesis for the growth mechanism of triangular crystals of theophylline comes from the observation that the crystallographic orientation of sector iv matches the crystallographic orientation of experimentally prepared triangular theophylline crystals, as determined by TEM (see Figures 2a and 5b).

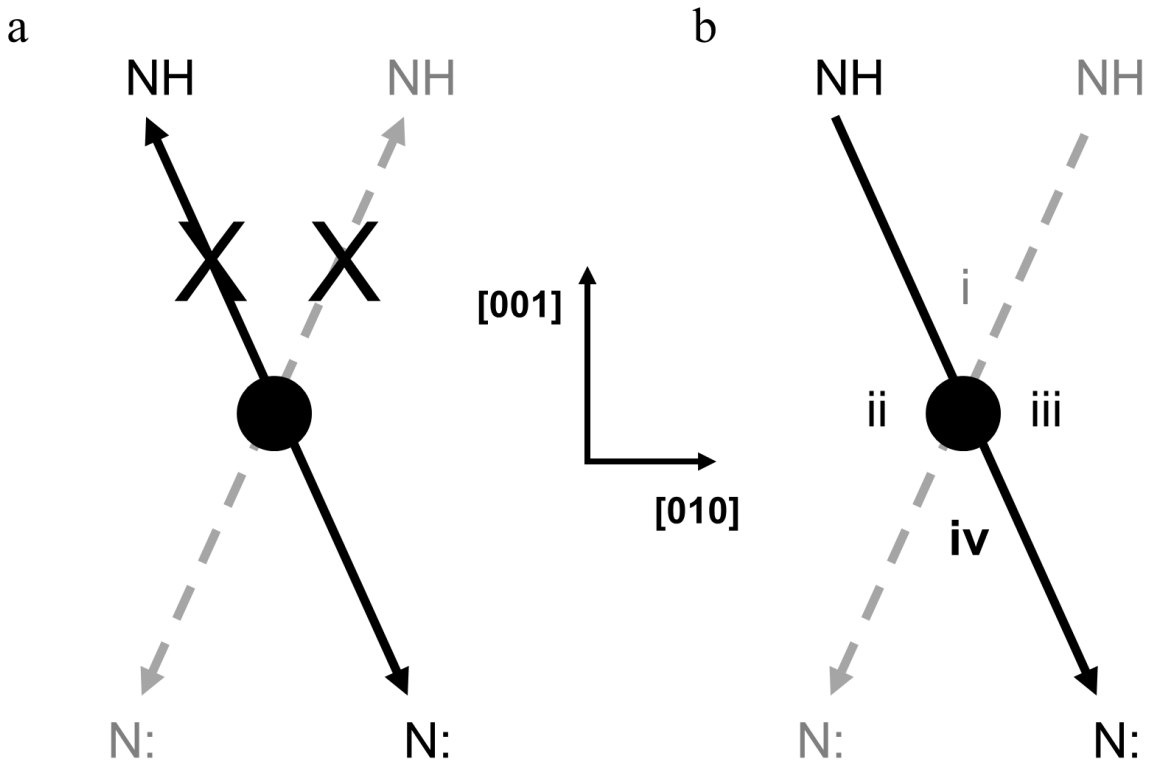

Figure 5. (a) Schematic showing the orientation of hydrogen bonded chains of theophylline molecules in A-layers (dashed arrow) and B-layers (solid arrow) in the Form II structure. The crosses signify that during crystallization from a solvent with a hydrogen bond acceptor group (and no potential hydrogen bond donating group), the growth of chains is inhibited in one direction due to interaction of the solvent with the exposed theophylline NH groups. (b) Schematic defining four growth sectors for Form II crystals. During crystallization from a solvent with a hydrogen bond acceptor group (and no potential hydrogen bond donating group) crystal growth is limited to the triangular shaped sector labelled iv.

The observation that triangular crystals are also obtained from toluene, despite this solvent having no hydrogen bond donor or acceptor groups, indicates that hydrogen bonding is not the only factor influencing crystal habit. In this case, the polarity of the solvent is important. As shown in Figure 6, the electrostatic potential around theophylline molecules in the Form II structure differs strongly in the direction of the polar <001> axis. A strongly lipophilic solvent such as toluene will interact more strongly at the (00-1) side of the theophylline molecule (the bottom side as displayed in Figure 6) than at the (001) side, leading to inhibition of the growth of chains of theophylline molecules at the N: terminated ends. If the previously described model of theophylline growth sectors is applied to crystal growth from toluene it can be determined that crystal growth will be favored in sector i. The triangular shape of this growth sector matches the triangular habit of crystals grown from toluene.

On crystallizing theophylline from alcohols, solvents which are both hydrogen bond donors and acceptors, it would follow that the growth of chains of theophylline molecules would be inhibited at both the N: and NH terminated ends, limiting crystal growth in the <001> axis. This hypothesis is supported by TEM analysis of lath shaped crystals obtained from methanol, which revealed that crystal growth occurred primarily in the <010> axis (Figure 7). This direction is associated with the stacking of hydrogen bonded chains of theophylline molecules in both A and B layers. The observed slow relative crystal growth rates from alcohol solvents results from molecular addition occurring primarily through this stacking, rather than through stronger hydrogen bonding interactions. In contrast, chloroform does not interact strongly with either end of the hydrogen bonded chains of theophylline molecules because it has no hydrogen bond donors or acceptors and is not highly lipophilic. In this case, growth occurs predominantly along the <001> axis, as was seen for crystals grown by sublimation.

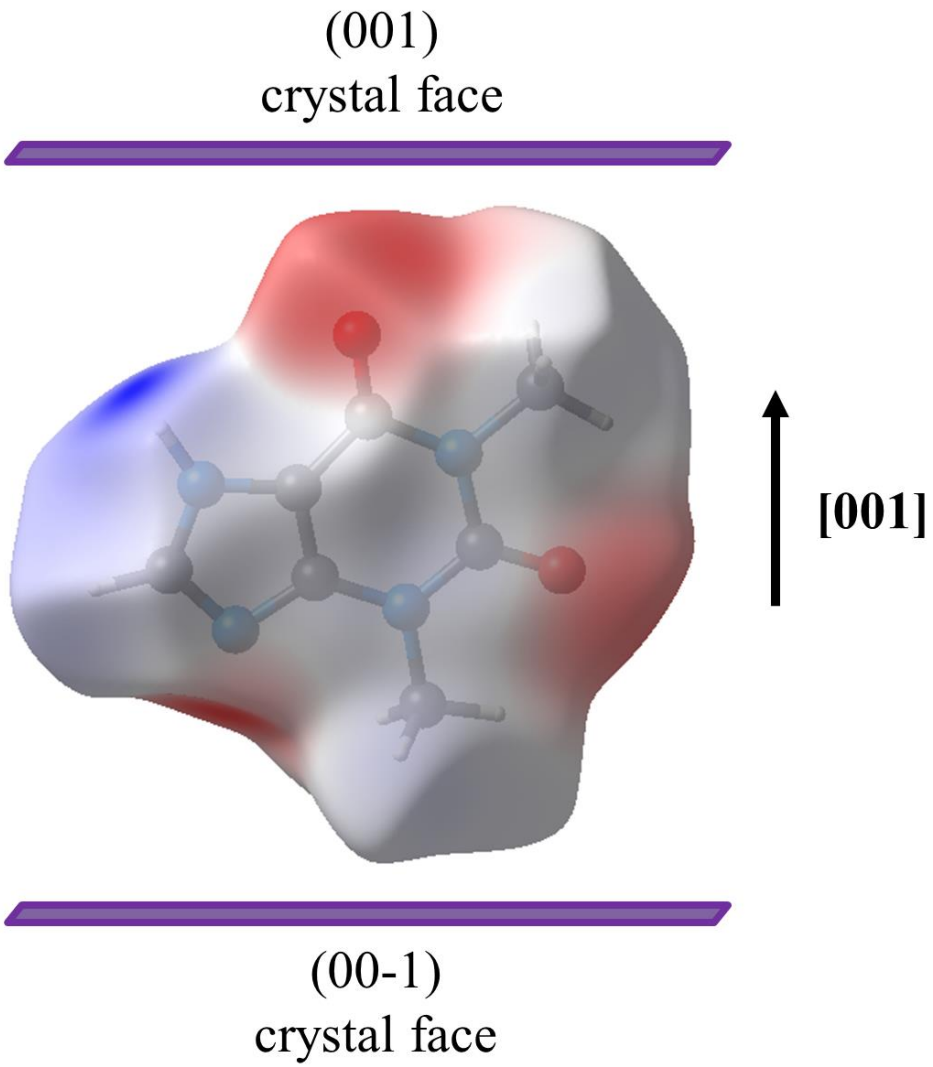

Figure 6. Electrostatic potential surface around a molecule of theophylline in the Form II crystal structure (calculated from CSD structure BAPLOT01)[42]. The inequivalent (001) and (00-1) crystal faces are also shown in the image (edge on). It is evident that the electrostatic potential surface of theophylline molecules is significantly more polar at the (001) crystal face than at the (00-1) face.

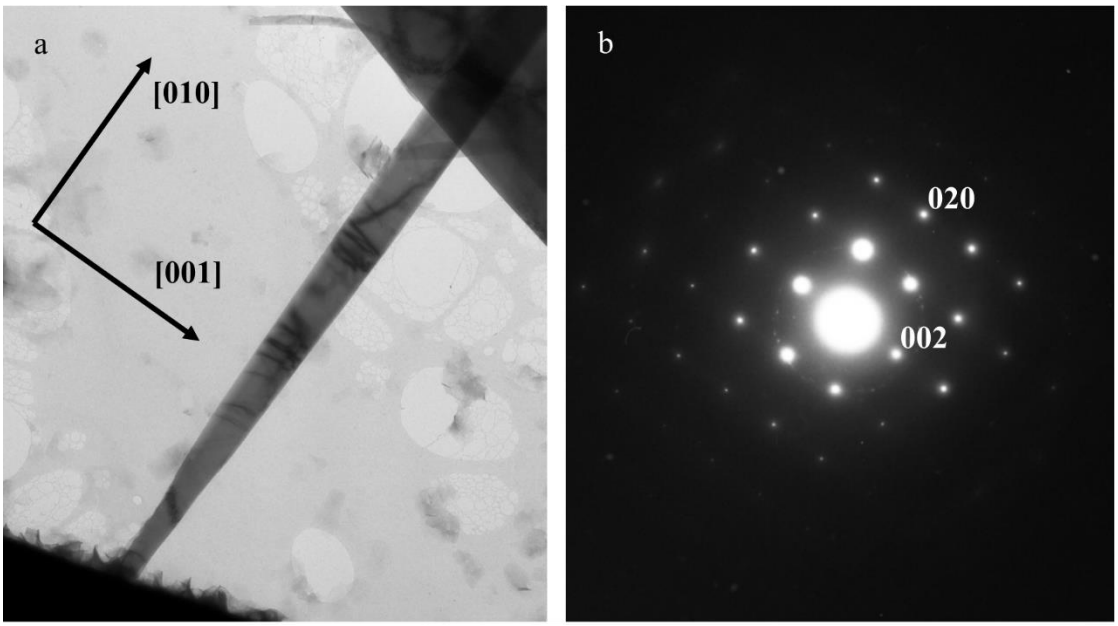

Figure 7. (a) TEM image showing a thin lath shaped crystal of Form II of theophylline crystallized from methanol. (b) Corresponding rotation corrected <100> zone axis electron diffraction pattern.

(iv) Further observations relating to theophylline crystal growth

It is clear from the above observations that the habit of theophylline Form II crystals is strongly influenced by solvent. Interestingly, there is evidence that habit can also be influenced in the solid state. In a previous study, a 1:1 caffeine:theophylline cocrystal was identified and, on heating, found to undergo a solid state dissociation wherein the caffeine and theophylline molecules separated and recrystallized separately.[60] The crystal habit of the resulting Form II theophylline crystals was plate-like, with shapes ranging between triangles and rectangles. As described above, crystallization of theophylline by sublimation yields rectangular plates and it might have been expected that the theophylline crystals obtained from the cocrystal dissociation

experiment would also have had this habit. The observation that many of the theophylline crystals forming after cocrystal dissociation had a triangular character is postulated to be due to the interaction of caffeine molecules with the growing theophylline crystal faces. Caffeine is a molecule with hydrogen bond acceptor groups, but no donor groups, which means it could potentially inhibit elongation at the NH terminated ends of chains of theophylline molecules during crystal growth, and thereby influence crystal habit, in much the same way as described above for solvents such as nitromethane and ethyl acetate during solution crystallizations.

Atomic force microscopy analysis of the dominant (100) surface of triangular Form II crystals revealed interesting growth features as shown in Figure 8a. The triangular shaped 'hillocks' evident in the image are regions where the growth of several layers of theophylline molecules has initiated at screw dislocations emergent at the crystal surface, and their shape and orientation mirror that of the bulk crystal (see Figure 8b). The spiral pattern formed by layers generated at the screw dislocations can be seen more clearly in Figure 8c. The triangular appearance of the layer growth at these screw dislocations is unusual (emergent screw dislocations typically give rise to a symmetrical pattern of layers around the dislocation)[61-65] and can again be explained by the action of the solvent in inhibiting the elongation of chains of theophylline molecules at the NH terminated ends. Furthermore, the ABAB type arrangement of layers of molecules in the Form II structure is evident in the surface topology. All surface layers originating at the screw dislocations have expanded in the direction of the base of the triangle, within a triangular shaped growth sector, but growth towards the edge that runs parallel to <011> is slightly faster for A-layers than B-layers (in fact growth of A-layers in this direction seems to be limited by the extent of growth of the B-layers below) and growth towards the edge that runs parallel to <0-11>

is slightly faster for B-layers than A-layers. This leads to an alternating pattern in the appearance of layers at theophylline surfaces as shown in the inset of Figure 8a.

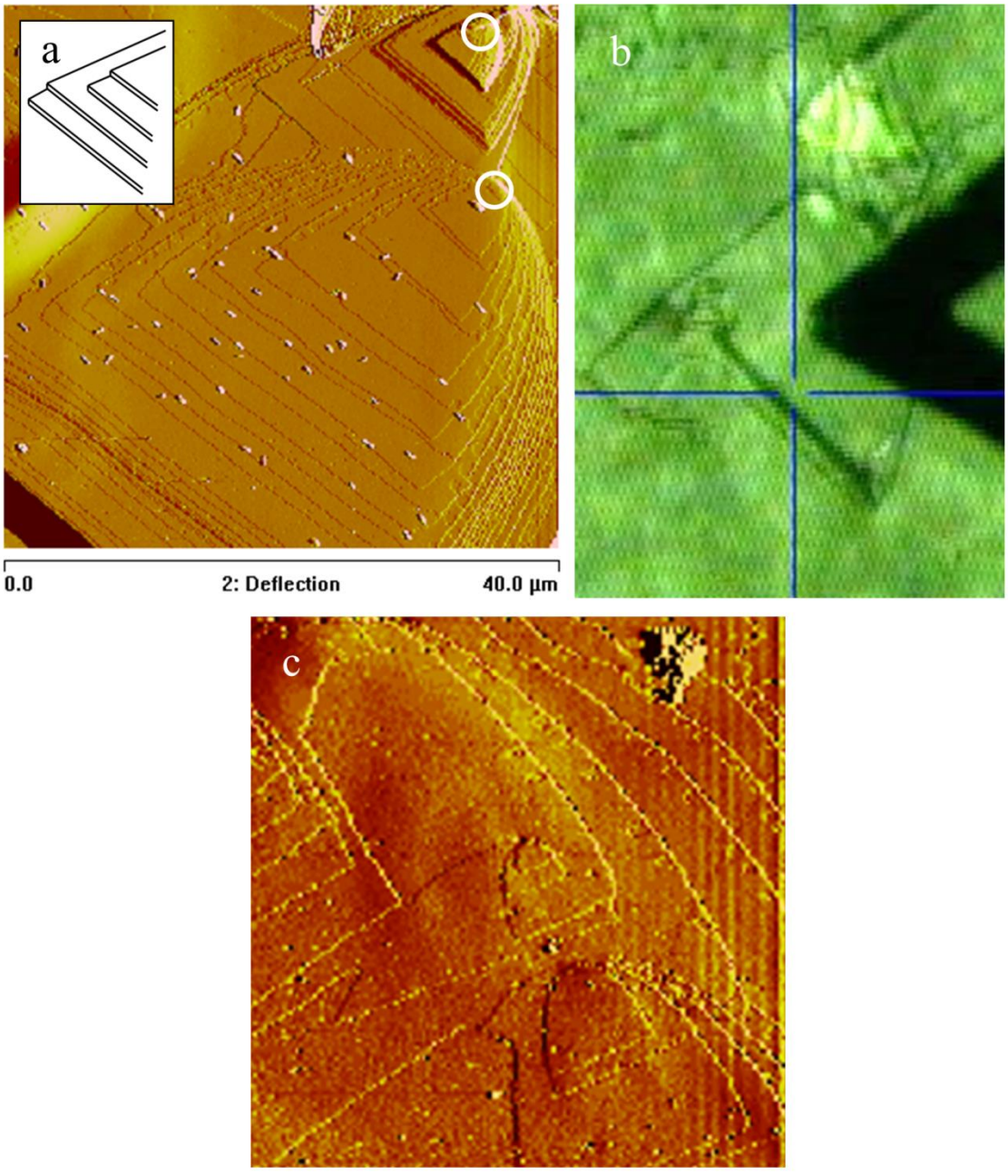

Figure 8. (a) AFM deflection image of the (100) surface of a crystal of Form II of theophylline crystallized from nitromethane (recorded in contact mode). The image shows a region where two screw dislocations are emergent at the crystal surface (circled). Several layers of theophylline molecules have grown at each of the screw dislocations, the edges of which can be seen as lines on the image, resulting in triangular shaped screw dislocation hillocks. The layers have heights of approximately 1.2 nm, consistent with the spacing between molecules in the [100] direction (A figure showing the layers in the crystal structure of theophylline is included in the Supporting Information (Figure S3)). It is evident that there is a slight difference in the way that adjacent layers of molecules grow from the screw dislocations. In general, all layers can be seen to grow in the direction of the base of the triangle, but the A-layers have grown preferentially to one side of the triangle (in the direction of the top left of the image) and the B-layers have grown preferentially to the other side (in the direction of the bottom right of the image), as shown schematically in the inset. (b) A corresponding optical image showing the whole of the crystal (the tip of the crystal has fractured off prior to analysis)[46]. It is apparent that the triangular shaped screw dislocation hillocks are aligned with the triangular morphology of the crystal under analysis. The dark feature on the right hand side of the image is the AFM cantilever. (c) AFM deflection image of the (100) surface of a second theophylline crystal showing the spiral nature of layer growth at a screw dislocation.

(v) Evidence for a new polymorphic form of theophylline

During this study, theophylline has been observed to crystallize with a second type of triangular morphology clearly distinguishable from the triangular habit described above. This

new variety of triangular crystal possesses a wider growth angle, as shown in Figures 9a and 9b, and can be categorized as an obtuse isosceles form. The wide triangles have been obtained from both nitromethane and ethyl acetate, but are observed infrequently, approximately once in every 20 crystallizations, and always occur concomitantly with the conventional triangular crystals of Form II, never forming more than a small percentage (< 1 %) of any sample. As a result, crystallographic and spectroscopic information could not be obtained from these crystals using standard approaches, but it was possible to analyze individual crystals by TEM. The electron diffraction pattern in Figure 9c, recorded from one such crystal, is consistent with diffraction from the <1-20> zone axis of Form II of theophylline. Interestingly, however, when crystallographic directions are mapped onto the crystal it is evident that the [010] crystallographic direction runs from tip to base and the base of the triangle is approximately parallel with the [001] direction. This is opposite to what was found for the conventional triangular crystals described previously. Additionally, crystal defects (which are identified from the distortions to bend contours that occur in the vicinity of the defects) can be seen running from tip to base in this crystal. In contrast, defects in conventional triangular crystals of Form II have been reported to run parallel to the base of the triangles.[46] Furthermore, the model for crystallization of theophylline from a hydrogen bond acceptor solvent described above and shown in Figure 5 cannot explain the growth of obtuse triangular crystals. On the basis of these observations it is postulated that the obtuse triangular crystals are in fact a new crystal form of theophylline.

It is striking that the wide triangular crystal of theophylline shown in Figure 9b is similar in shape to the growth sectors labelled ii and iii in Figure 5b. As described above, the growth of Form II is not favored in these sectors during solution crystallization from a hydrogen bond

acceptor solvent such as nitromethane. If, however, each of the A-layers were to be rotated by 180°, so that N: groups are exposed at the (001) and NH groups exposed at the (00-1) face, this would give a hypothetical theophylline crystal form which would be expected to grow primarily in growth sector ii, and therefore to crystallize as wide triangles (Figure 9d).

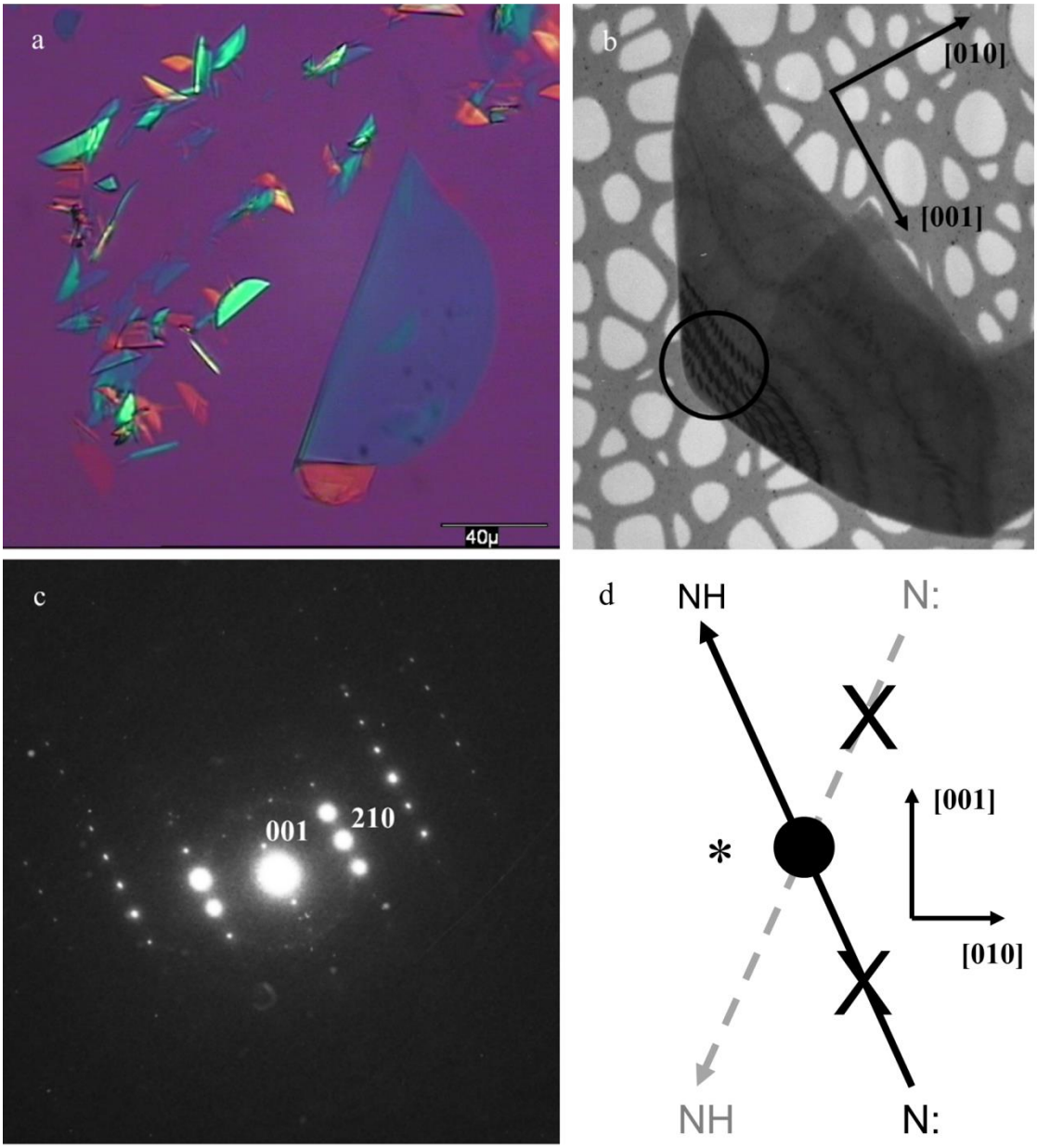

Figure 9. (a) PLM image of a small selection of theophylline crystals within a sample grown from ethyl acetate. Several of the crystals resemble obtuse isosceles triangles rather than the acute isosceles triangles described earlier. (b) TEM image of an obtuse triangular crystal of theophylline grown from nitromethane. The angle between the two equivalent edges of this triangular crystal is approximately 130°. (c) Corresponding rotation corrected electron diffraction pattern (recorded from the region highlighted with a circle in the TEM image). Reflections have been indexed on the basis that diffraction was from the <1-20> zone axis of Form II of theophylline (crystallographic directions were added to image (b) on this basis also). (d) A model of a hypothetical crystal form of theophylline which would be expected to crystallize as obtuse triangular crystals (within the growth sector marked with an asterisk) from a hydrogen bond acceptor solvent (note that the functional groups at the ends of the dashed arrow are reversed in comparison to the similar schematic for Form II in Figure 5a).

An ensemble of putative crystal structures of theophylline was generated using crystal structure prediction (CSP) calculations and analyzed in an attempt to identify a crystal structure which is consistent with the model for the growth of obtuse triangular crystals shown in Figure 9d. Such a structure was identified and was found to be that with the 14th lowest lattice energy within the ensemble (Figure 10a). This structure has a calculated energy 5.9 kJ.mol^{-1} greater than Form II, an energy difference which is consistent with systems where polymorphism has been observed.[66-69] It should be noted that the electron diffraction pattern in Figure 8c, which was recorded from an obtuse triangular crystal, is consistent with both CSP structure #14 and Form II (diffraction from the <10-2> zone axis or <1-20> zone axis respectively), but the positions of reflections are closer to that expected for diffraction from CSP structure #14. These

results provide support to the hypothesis that the wide triangles are a new crystal form of theophylline. This would correspond to the seventh polymorph to be isolated to date and will be referred to hereafter as Form VII.

The arrangement of molecules in the crystal structure of Form VII of theophylline is shown in Figure 10b, and is closely related to that in Form II. The primary difference between the two forms is the relative orientations of hydrogen bonded chains in the A and B layers (see also the schematic in Figure 10c). Form VII crystallizes in the same space group as Form II, $Pna2_1$, and is calculated to have similar lattice parameters (a = 26.597 Å, b = 8.707 Å, c = 3.876 Å for Form VII, *cf.* a = 24.612 Å, b = 3.830 Å, c = 8.501 Å for Form II). The higher energy of Form VII stems partly from less favorable electrostatic interactions between hydrogen bonded chains (accounting for 3.1 $kJ.mol^{-1}$ of the energy difference) and partly from poorer packing efficiency (resulting in a smaller stabilizing dispersion contribution to the lattice energy), and provides an explanation for why this phase is observed so infrequently in comparison to Form II.

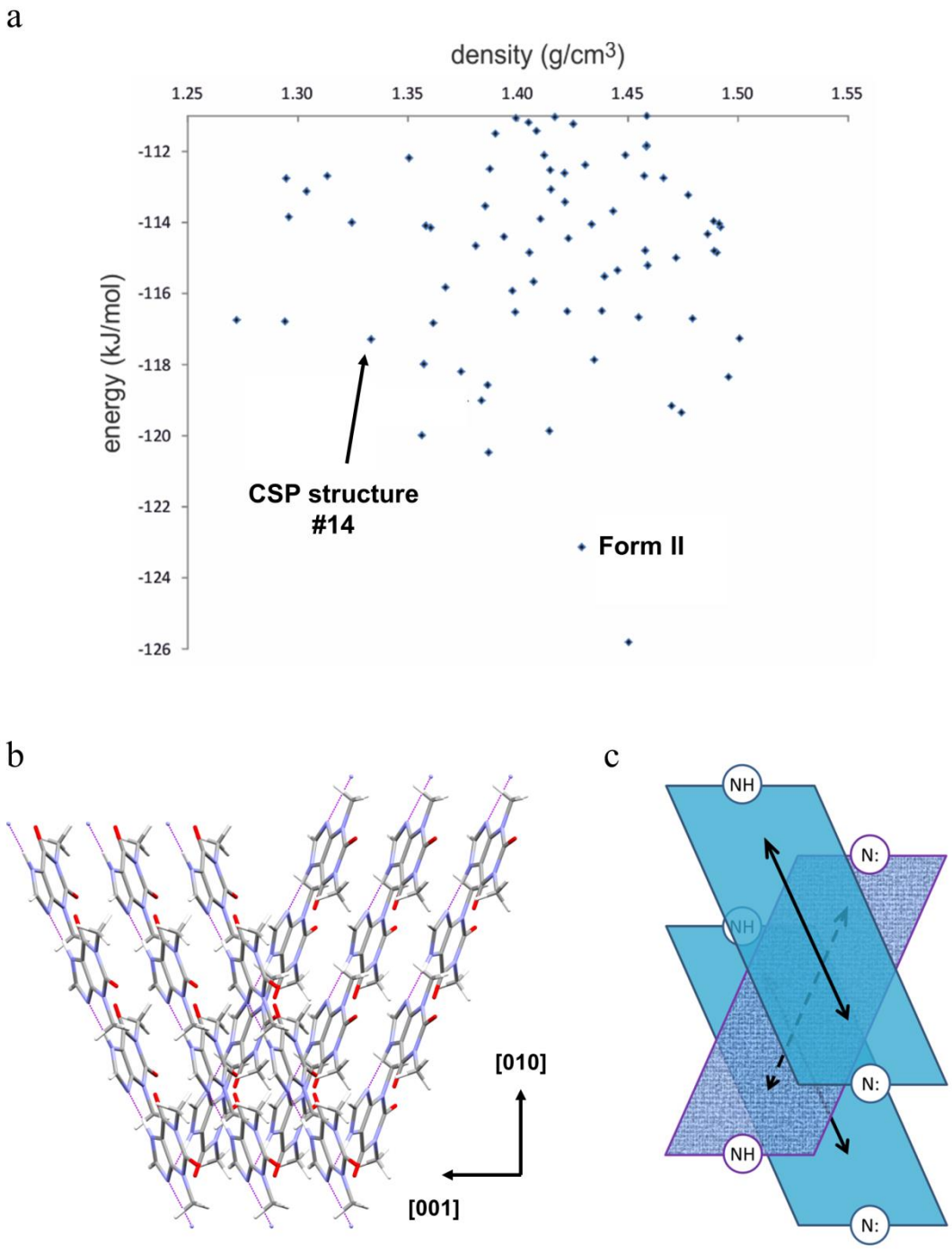

Figure 10. (a) The results of crystal structure prediction with theophylline. The 73 low energy putative structures within 15 kJ.mol^{-1} of the global minimum are plotted by lattice energy and density. The computationally derived crystal structure which corresponds to Form II, #2, and that which fits observations of wide triangular crystals of theophylline, #14, are highlighted. (b) The

crystal structure of CSP structure #14/Form VII of theophylline viewed down the a-axis. (c) A schematic representation of the Form VII structure showing the ABAB type stacking arrangement of layers (A-layers have been given a textured appearance, whereas B-layers have been given a smooth appearance). The arrows show the orientations of hydrogen bonded chains of theophylline molecules in each layer. Note that the chains in A-layers are rotated by 180° with respect to equivalent chains in the Form II structure.

Conclusions

Form II of theophylline has been shown to form a triangular crystal habit when crystallized from hydrogen bond accepter solvents and highly hydrophilic solvents due to solvent induced inhibition of crystal growth in all but one growth sector. Two key structural features which enable Form II to grow as triangular crystals have been identified: the presence of a polar axis and an ABAB type arrangement of layers wherein the A and B layers are neither parallel nor orthogonal. It is postulated that crystal forms of other compounds which share these characteristics may also form triangular crystals under the right conditions.

A second, obtuse triangular crystal morphology has been identified for theophylline, and the combination of TEM analysis and crystal structure prediction provided evidence that these wide triangular crystals are a new polymorphic form of theophylline. This new polymorph is observed rarely, and only as a minor phase in mixtures with Form II, which is likely to be why it has not been identified previously. The use of TEM was a critical part of this study, enabling the mapping of crystal structure to crystal habit in theophylline Form II crystallites and allowing individual crystals of the new theophylline polymorph to be characterized. This is the second

time that the combination of TEM and crystal structure prediction has been used to identify a crystal structure for a metastable polymorph of theophylline (present as a minor phase in a mixture with Form II).[40] This combination of techniques could prove to be a valuable tool for investigating the polymorphic behavior of other compounds, particularly for the analysis of trace polymorphic impurities whose presence may be undetected by more common analytical methods.

The observations reported here lend weight to the idea that more, if not most, of the computationally predicted low energy crystal structures for a compound can be observed experimentally under the right crystallization conditions and with the use of appropriate analytical tools.

ASSOCIATED CONTENT

Supporting Information. Additional optical micrographs of the growth of theophylline Form II crystals, a PXRD diffractogram of triangular theophylline crystals and a diagram showing the nature of height changes at the (100) theophylline crystal surface are supplied as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Groth, P. H. R., *Chemische Kristallographie. Erster Teile*; W. Englelemann: Leipzig, **1906**.

(2) Sunagawa, I., *Morphology of Crystals, Parts A and B*; Terra Science Publishers: Tokyo, **1989**.

(3) Bentley, W. A.; Humphreys, W. J., *Snow Crystals*; Dover Publications: USA, **1962**.

(4) Bentley, W. A., Conical Snow. *Science* **1932**, 75, 383.

(5) Eddleston, M. D.; Jones, W. *Cryst. Growth Des.* **2010**, *10*, 365-370.

(6) Nanev, C. N. *Crystallogr. Rev.* **1994**, 4, 3-71.

(7) Myerson, A. S. *Handbook of Industrial Crystallization*. Second edition; Butterworth-Heinemann: United States, **2002**.

(8) Bailey, M.; Hallett, J. *J. Atmos. Sci.* **2004**, 61, 514–544.

(9) Dhanaraj, G.; Byrappa, K.; Prasad, V.; Dudley, M., *Springer Handbook of Crystal Growth*; Springer-Verlag: Germany, **2010**.

(10) Wells, A. F. *Philos. Mag.* **1946**, 37, 184-99.

(11) Hartman, I. P.; Perdok, W. G. *Acta Crystallogr.* **1955**, 8, 49-52.

(12) Tiwari, A. K., Crystal Habit Changes and Dosage Form Performance. In *Encyclopedia of Pharmaceutical Technology*, Third edition; Swarbrick, J., Ed.; Informa Healthcare: New York, USA, **2006**; pp 820-833.

(13) Bandyopadhyay, R.; Grant, D. J. W. *Pharm. Dev. Technol.* **2000**, 5, 27-37.

(14) Calderon De Anda, J.; Wang, X. Z.; Lai, X.; Roberts, K. J. *J. Process Control* **2005**, 15, 785-797.

(15) Sun, Z.; Ya, N.; Adams, R. C.; Fang, F. S. *Am. Pharm. Rev.* **2010**, 13, (4).

(16) Mirza, S.; Miroshnyk, I.; Heinamaki, J.; Antikainen, O.; Rantanen, J.; Vuorela, P.; Vuorela, H.; Yliruusi, J. *AAPS PharmSciTech* **2009**, 10, 113-119.

(17) Lee, A. Y.; Myerson, A. S. *MRS Bull.* **2006**, 31, 881-886.

(18) Rasenack, N.; Steckel, H.; Mueller, B. W. *Powder Technol.* **2004**, 143-144, 291-296.

(19) Chow, A. H. L.; Tong, H. H. Y.; Chattopadhyay, P.; Shekunov, B. Y. *Pharm. Res.* **2007**, 24, 411-437.

(20) Kawashima, Y. *Arch. Pharmacal Res.* **1984**, 7, 145-51.

(21) For example, the UK funding body EPSRC launched a Grand Challenge in 2009 titled 'Directed Assembly of Extended Structures with Targeted Properties'.

(22) Thomas, L. H.; Wales, C.; Zhao, L.; Wilson, C. C. *Cryst. Growth Des.* **2011**, 11, 1450-1452.

(23) Brown, C. J.; Ni, X.-W. *CrystEngComm* **2012**, 14, 2944-2949.

(24) Zhao, L.; Raval, V.; Briggs, N. E. B.; Bhardwaj, R. M.; McGlone, T.; Oswald, I. D. H.; Florence, A. J. *CrystEngComm* **2014**, 16, 5769-5780.

(25) Wittering, K.; King, J.; Thomas, L. H.; Wilson, C. C. *Crystals* **2014**, 4, 123-140.

(26) Miller, J. M.; Rodriguez-Hornedo, N.; Blackburn, A. C.; Macikenas, D.; Collman, B. M. *Biotech. Pharm. Aspect.* **2007**, 6, 53-109.

(27) Threlfall, T. *Org. Process Res. Dev.* **2000**, 4, 384-390.

(28) Bernstein, J., Crystal Polymorphism. In *Engineering of Crystalline Materials Properties*, Novoa, J. J.; Braga, D.; Addadi, L., Eds.; Springer: Dordrecht, The Netherlands, **2008**; pp 87-109.

(29) Byrn, S.; Pfeiffer, R.; Ganey, M.; Hoiberg, C.; Poochikian, G. *Pharm. Res.* **1995**, 12, 945-54.

(30) Singhal, D.; Curatolo, W. *Adv. Drug Delivery Rev.* **2004**, 56, 335-347.

(31) Blagden, N.; de Matas, M.; Gavan, P. T.; York, P. *Adv. Drug Delivery Rev.* **2007**, 59, 617-630.

(32) Price, S. L. *Acta Crystallogr., Sect. B Struct. Sci.* **2013**, 69, 313-328.

(33) Day, G. M. *Crystallogr. Rev.* **2011**, 17, 3-52.

(34) Galek, P. T. A.; Fábián, L.; Motherwell, W. D. S.; Allen, F. H.; Feeder, N. *Acta Crystallogr., Sect. B Struct. Sci.* **2007**, B63, 768-782.

(35) Galek, P. T. A.; Allen, F. H.; Fábián, L.; Feeder, N. *CrystEngComm* **2009**, 11, 2634-2639.

(36) Vippagunta, S. R.; Brittain, H. G.; Grant, D. J. *Adv. Drug Delivery Rev.* **2001**, 48, 3-26.

(37) Day, G. M.; Cooper, T. G.; Cruz-Cabeza, A. J.; Hejczyk, K. E.; Ammon, H. L.; Boerrigter, S. X. M.; Tan, J. S.; Della Valle, R. G.; Venuti, E.; Jose, J.; Gadre, S. R.; Desiraju, G. R.; Thakur, T. S.; van Eijck, B. P.; Facelli, J. C.; Bazterra, V. E.; Ferraro, M. B.; Hofmann, D. W. M.; Neumann, M. A.; Leusen, F. J. J.; Kendrick, J.; Price, S. L.; Misquitta, A. J.; Karamertzanis, P. G.; Welch, G. W. A.; Scheraga, H. A.; Arnautova, Y. A.; Schmidt, M. U.; van de Streek, J.; Wolf, A. K.; Schweizer, B. *Acta Cryst., Sect. B: Struct. Sci.* **2009**, B65, 107-125.

(38) Price, S. L. *Accounts Chem. Res.* **2009**, 42, 117-126.

(39) Neumann, M. A.; Leusen, F. J. J.; Kendrick, J. *Angew. Chem., Int. Ed.* **2008**, 47, 2427-2430.

(40) Eddleston, M. D.; Hejczyk, K. E.; Bithell, E. G.; Day, G. M.; Jones, W. *Chem. Eur. J.* **2013**, 19, 7883-7888.

(41) Fucke, K.; McIntyre, G. J.; Wilkinson, C.; Henry, M.; Howard, J. A. K.; Steed, J. W. *Cryst. Growth Des.* **2012**, 12, 1395-1401.

(42) Ebisuzaki, Y.; Boyle, P. D.; Smith, J. A. *Acta Crystallogr., Sect. C Cryst. Struct. Commun.* **1997**, C53, 777-779.

(43) Phadnis, N. V.; Suryanarayanan, R. *J. Pharm. Sci.* **1997**, 86, 1256-63.

(44) Khamar, D.; Pritchard, R. G.; Bradshaw, I. J.; Hutcheon, G. A.; Seton, L. *Acta Crystallogr., Sect. C Cryst. Struct. Commun.* **2011**, C67, 496-499.

(45) Roy, C.; Vega-Gonzalez, A.; Subra-Paternault, P. *Int. J. Pharm.* **2007**, 343, 79-89.

(46) Eddleston, M. D.; Bithell, E. G.; Jones, W. *J. Pharm. Sci.* **2010**, 99, 4072-4083.

(47) Seton, L.; Khamar, D.; Bradshaw, I. J.; Hutcheon, G. A. *Crystal Growth & Design* **2010**, 10, 3879-3886.

(48) Fultz, B.; Howe, J. M., *Transmission Electron Microscopy and Diffractometry of Materials*. Fourth edition; Springer: Berlin Heidelberg, 2013; p 784.

(49) Karamertzanis, P. G.; Pantelides, C. C. *J. Comput. Chem.* **2004**, 26, 304-324.

(50) Price, S. L.; Leslie, M.; Welch, G. W. A.; Habgood, M.; Price, L. S.; Karamertzanis, P. G.; Day, G. M. *Phys. Chem. Chem. Phys.* **2010**, 12, 8478-8490.

(51) Kazantsev, A. V.; Karamertzanis, P. G.; Adjiman, C. S.; Pantelides, C. C. *J. Chem. Theory Comput.* **2011**, 7, 1998-2016.

(52) Coombes, D. S.; Price, S. L.; Willock, D. J.; Leslie, M. *J. Phys. Chem.* **1996**, 100, 7352-60.

(53) Stone, A. J.; Alderton, M. *Mol. Phys.* **1985**, 56, 1047-64.

(54) Eddleston, M. D.; Hejczyk, K. E.; Bithell, E. G.; Day, G. M.; Jones, W. *Chem. Eur. J.* **2013**, 19, 7874-7882.

(55) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B Condens. Matter* **1988**, 37, 785-9.

(56) Becke, A. D. *Phys. Rev. A Gen. Phys.* **1988**, 38, 3098-100.

(57) Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, 157, 200-6.

(58) Jayatilaka, D.; Grimwood, D. J., Tonto: A Fortran Based Object-Oriented System for Quantum Chemistry and Crystallography. In *Computational Science - ICCS 2003*, Sloot, P. M. A.; Abramson, D.; Bogdanov, A. V.; Gorbachev, Y. E.; Dongarra, J. J.; Zomaya, A. Y., Eds. Springer: Berlin Heidelberg, 2003; pp 142-151.

(59) Spackman, M. A.; McKinnon, J. J.; Jayatilaka, D. *CrystEngComm* **2008**, 10, 377-388.

(60) Eddleston, M. D.; Lloyd, G. O.; Jones, W. *Chem. Commun.* **2012**, 48, 8075-8077.

(61) Burton, W. K.; Cabrera, N.; Frank, F. C. *Nature* **1949**, 163, 398-9.

(62) Verma, A. R. *Nature* **1951**, 167, 939.

(63) Amelinckx, S. *Nature* **1951**, 167, 939-40.

(64) Ward, M. D. *Chem. Rev.* **2001**, 101, 1697-1725.

(65) Rimer, J. D.; An, Z.; Zhu, Z.; Lee, M. H.; Goldfarb, D. S.; Wesson, J. A.; Ward, M. D. *Science* **2010**, 330, 337-341.

(66) Beyer, T.; Day, G. M.; Price, S. L. *J. Am. Chem. Soc.* **2001**, 123, 5086-5094.

(67) Bernstein, J., *Polymorphism in Molecular Crystals*; Oxford University Press: United States, **2002**; p 410.

(68) Day, G. M.; Motherwell, W. D. S.; Jones, W. *Cryst. Growth Des.* **2005**, 5, 1023-1033.

(69) Gavezzotti, A.; Filippini, G. *J. Am. Chem. Soc.* **1995**, 117, 12299-305.

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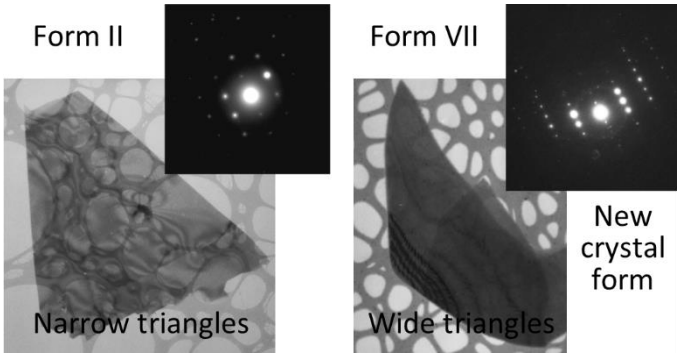

The polar nature of the theophylline Form II crystal structure leads to the growth of triangular crystals from hydrophobic solvents and solvents that act as hydrogen bond acceptors, but not donors. A second, wider, triangular crystal habit is also occasionally observed and interpreted as a new polymorphic form of theophylline, Form VII. This study utilized transmission electron microscopy and crystal structure prediction.