Expanding High Throughput Nanolitre Solution-Phase Crystallisation for Polymorph Screening

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Introduction

Polymorphism is a solid-state phenomenon where a chemical species may adopt different conformational or packing arrangements, therefore allowing for the formation of more than one distinct crystal structure.

Crystal polymorphs can exhibit varying physical and chemical properties. These properties such as solubility, hardness, colour, and melting point can all be important in the design of a compound in the crystal engineering and pharmaceutical industries.[1, 2]

Typically, conducting polymorph screens requires a combination of classical crystallisation techniques which demand both large quantities of material and time to complete.

Herein, we describe how encapsulated nanodroplet crystallisation (ENaCt) can be applied for low-quantity rapid polymorph screening using highly polymorphic 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY) and nicotinamide as test compounds.

ENaCt polymorph screening resulted in the growth, and analysis, of single crystals of a new polymorph of ROY 'O22'.

Crystal forms of both ROY and nicotinamide previously thought to be inaccessible to solution-phase crystallisation were also successfully grown, highlighting the capabilities of ENaCt for screening a diverse polymorphic landscape.

Figure 1: ROY

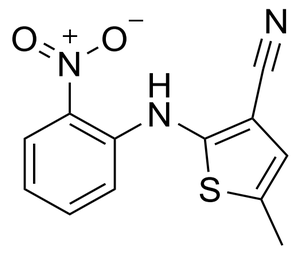
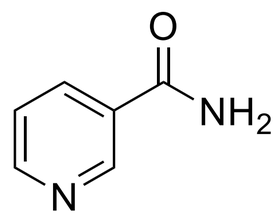


Figure 2: Nicotinamide



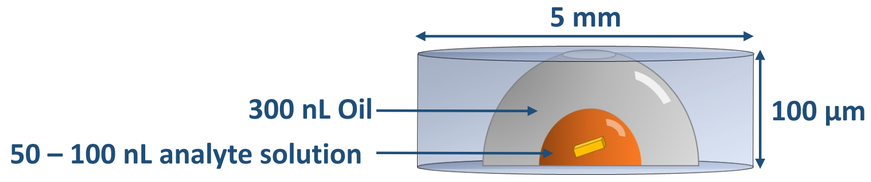
Encapsulated Nanodrop Crystallisation

Encapsulated nanodroplet crystallisation (ENaCt) is a high-throughput, robot-assisted crystallisation technique aimed at the crystallisation of organic-soluble molecules on a small scale.[3]

Nano-litre droplets of analyte solution are encapsulated within droplets of inert oils in 96-well crystallisation plates (Figure 3).

Encapsulated solution droplets reach high-degrees of supersaturation where crystal nucleation and growth occurs.

Figure 3: Side-on view of an ENaCt experiment

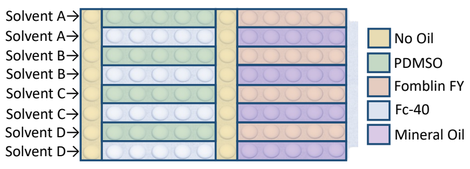


Key Advantages:

- **Low sample quantities:** Typically only 0.25-5µg per experiment.
- **Rapid:** Up to 96 unique crystallisation experiments can be dispensed in ~1 min.
- **High quality crystals:** Crystals suitable for analysisby SC-XRD are observed from conditions that would be challenging classically.

ENaCt Screening Method

01 Design

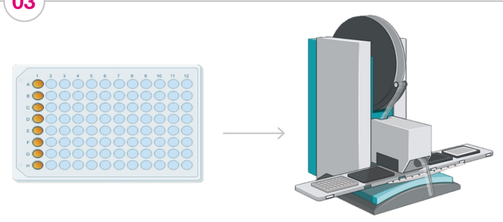


- Preliminary solubility testing was used to identify classes of suitable solvents/ antisolvents for ROY and nicotinamide.
- Plates were designed to examine 4 solvent conditions in conjunction with 4 different inert oils creating 20 unique crystallisation conditions per plate. (Allowing for 4/5 replicants per plate)

02 Dissolve

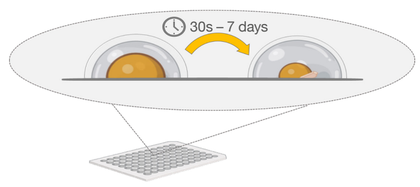
- ~1mg of ROY/nicotinamide was weighed for each solvent condition to be examined.
- Each sample was dissolved in the minimum solvent (or prepared to the desired concentration).
- Optical microscopy was used to confirm complete dissolution.

03 Dispense



- 100 nL of analyte solution was pipetted into 96-well crystallisation plates using an SPT Labtech mosquito® liquid handling robot.
- Plates were sealed with a glass cover-slip and crystallisation was monitored for 7 days.

04 Detect



- Crystals suitable for analysis by SC-XRD were identified by cross-polarised optical microscopy and harvested from wells. Their polymorphic form was identified by a unit cell determination.
- Single crystal X-ray diffraction data of each form were collected using a Bruker D8 Venture with Cu–Kα X-radiation.

Results

ROY

ENaCt polymorph screen of 280 solvent/antisolvent + oil conditions resulted in the successful growth of single crystals suitable for analysis by SC-XRD of 8 of the 13 previously known ROY polymorphs as well as a new polymorph 'O22' (Figure 5).

Generally, polymorphs well-known to solution-phase crystallisation Y, ON, YN, and R, were most common throughout the screen, making up the majority of the single crystals observed (Figure 4).

Conditions to reliably grow crystal forms Y, R18, and Y19 were determined while selectivity among other forms was low, and appeared more statistical in nature, perhaps owing to the congested lattice energy landscape, with 12 of the 13 forms that have calculated lattice energies existing within 5 kJ/mol of one another.[6]

Two of the polymorphs grown, Y04 and Y19, were previously thought to be inaccessible to solution-phase crystallisation techniques having only been reported from melt-based seeding methods to date.[4, 5]

Y19 is the highest energy polymorph known while Y04 is a metastable crystal form prone to polymorphic transformations and nucleation of more thermodynamically stable forms such as R.[6, 7]

Figure 4: Distribution of ROY polymorphs observed.

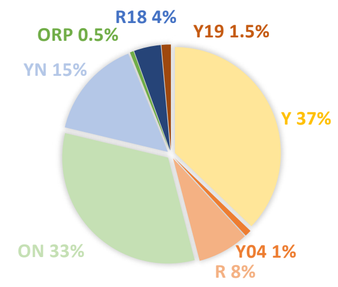


Figure 5: ROY polymorphs observed in ENaCt screen, crystal structure and their lattice energies, relative to Y.

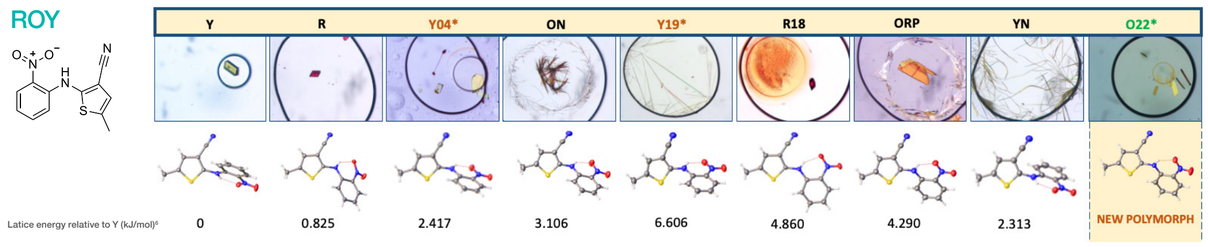
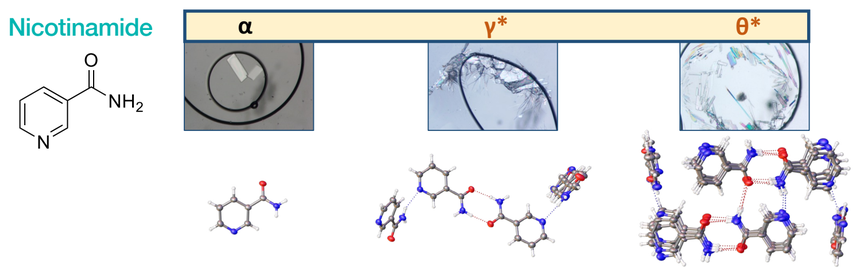


Figure 6: Nicotinamide polymorphs observed in ENaCt screen and their crystal structures.



Nicotinamide

- ENaCt polymorph screen of 80 solvent/antisolvent + oil conditions resulted in the successful growth of single crystals suitable for analysis by SC-XRD of 3 of the 9 currently known polymorphs (Figure 6).
- Two of the polymorphs grown, γ and θ, were previously thought to be inaccessible to solution-phase crystallisation techniques having only been reported from melt-based methods to date.[8]

Conclusions

- Encapsulated nanodroplet crystallisation was successfully applied as a viable small-scale, rapid polymorph screening technique for both ROY and nicotinamide.
- ENaCt polymorph screen of ROY resulted in the discovery of new ROY polymorph O22.
- Both Roy and nicotinamide screens yielded crystal forms previously thought to be inaccessible to solution phase crystallisation techniques.
- Results suggest the ENaCt technique may trap kinetically favourable, metastable crystal forms, therefore giving rise to greater polymorphic diversity than classical solution-phase crystallisation techniques.

References

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