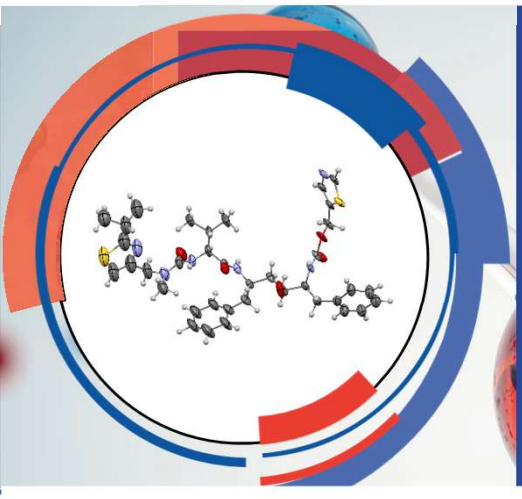


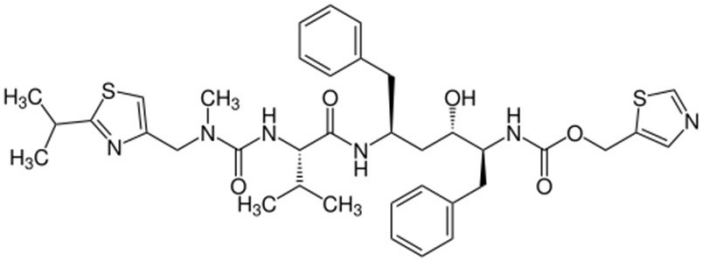
3D ED and CSP:

Derisking Drug Development

Identifying all relevant polymorphs of an active pharmaceutical ingredient (API) is crucial to finding a safe formulation for any new drug. Crystal Structure Prediction (CSP) has become an integral part of pharmaceutical solid form development to complement experimental polymorph screening and make sure no form is missed. However, confirming all relevant predictions by crystallographic structure determinations remains challenging. 3D electron diffraction (3D ED) offers both the high throughput and the sensitivity to find even minor forms in phase mixtures, making 3D ED and CSP the perfect combination to establish a full understanding of the polymorph landscape of a new API.

The Ritonavir Polymorph Incident

The HIV-protease inhibitor ritonavir represents one of the most prominent cases of a late appearing polymorph, meaning a polymorph that was not known at the time the product was released. Only after ritonavir was marketed in 1996 as polymorph 1, the more stable polymorph 2 unexpectedly appeared. Due to its lower solubility, polymorph 2 did not have a sufficient bioavailability in the chosen formulation. At the same time, seed crystals of form 2 had already contaminated the production line, which made it impossible to produce form 1. Ultimately, the drug had to be withdrawn from the market for reformulation, which left patients without treatment and caused heavy financial and reputational losses to the producer. Therefore, exhaustive solid form screening is now standard practice to mitigate polymorphism-related risks in pharmaceutical development. For ritonavir, two more polymorphs (plus many more solvates) have been reported to date, but form 4 has eluded structural characterisation so far, so the picture is still not complete.



Chemical formula of ritonavir

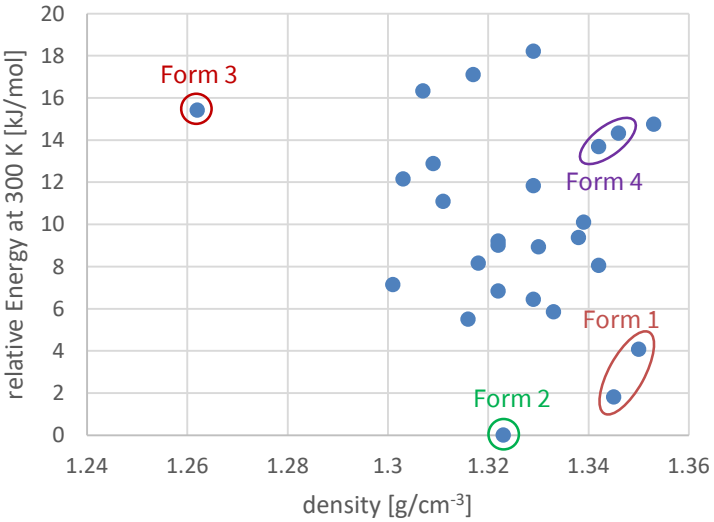
3D ED and CSP

As comprehensive experimental polymorph screening is a very time-consuming process, CSP is a very helpful addition to estimate the number of expected polymorphs and point towards missing ones if there are low energy predictions without an experimental match. On the other hand, experimental verification of predictions is crucial as not all thermodynamic minima are kinetically accessible. Finding all relevant predictions by crystallographic structure determinations can be challenging, because often not all can be prepared phase pure or crystallised in suitable size for single crystal X-ray diffraction (SC-XRD). Both challenges can be handled very well by 3D ED, making it the perfect tool to experimentally confirm elusive polymorphs. Furthermore, the ability to measure with high throughput and use powder samples without the need for growing large single crystals can significantly speed up the screening. The combination of both methods can boost the efficiency of solid form screening and effectively derisk drug development.

Considering that neither CSP nor 3D ED were established technologies in the 1990s, scientists from MSD and ELDICO Scientific decided to revisit and complete the ritonavir polymorph landscape and see if the incident could have been predicted and averted with today's methods.

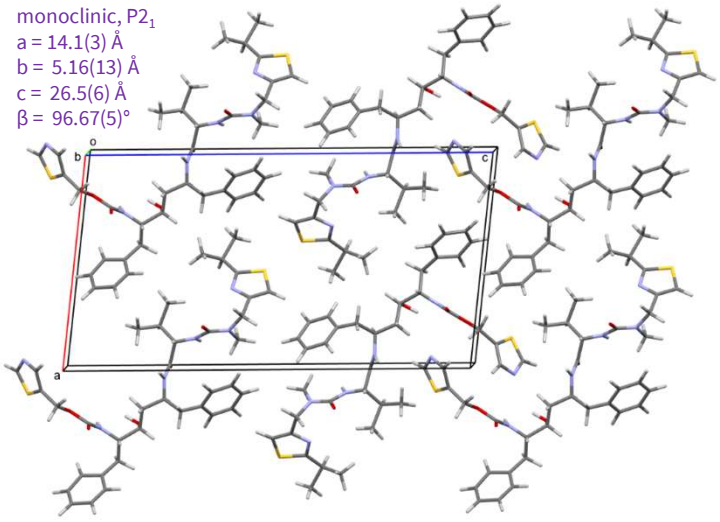
read the paper:
L. Iuzzolino, A. W. Kelly, M. T. Chaudhry, C. Jandl, D. Stam, A. Y. Lee, *Commun. Chem.* **2025**, *8*, 404.
https://doi.org/10.1038/s42004-025-01814-6.

Case Study: Ritonavir



26 best predictions in the crystal energy landscape of ritonavir (Form 1 and 4 appear twice due to isopropyl group disorder)

Ritonavir Form 4
monoclinic, $P2_1$
a = 14.1(3) Å
b = 5.16(13) Å
c = 26.5(6) Å
β = 96.67(5)°



packing view of ritonavir form 4 determined by 3D ED (disorder omitted)

Completing the Polymorph Landscape

The CSP study was performed using the GRACE software package and was limited to one molecule in the asymmetric unit (Z' = 1).[1] It indeed predicted form 2 as the most stable polymorph of ritonavir (see Figure above) and also contains all other forms of ritonavir reported to date. Form 3 was not generated by CSP due its high Z' and instead was optimised from the literature structure. The CSP landscape confirms that the late appearing form 2 could actually have been predicted with the computational techniques available today. Such a prediction would likely have prompted further screening until the missing polymorph was found and with this knowledge a suitable formulation could have been chosen from the start.

Form 4 is reported to be metastable and proved challenging to prepare in pure form, so in the end it could only be obtained as a powder mixture with form 1, which makes it a perfect case for electron diffraction. 135 crystallites were measured on an ELDICO *ED-1* and clustered by unit cell. The majority belonging to form 1 allowed straightforward structure solution. The minor phase form 4 was intentified only in 17 crystallites, 14 of which had suitable quality to be used further. As the data were also affected by beam damage, *ab initio* structure solution was challenging, but by using a predicted model selected due to its similarity with the experimental PXRD of form 4 or by simulated annealing the structure could be obtained and refined. This highlights another synergy between 3D ED and CSP: sometimes the number of high quality crystals is limited and additional complications like beam damage (especially if structure determination is intended at room temperature) or preferred orientation (like for the needle-shaped crystals of ritonavir forms 1 and 4) can further reduce the amount of useful reflection data. In such cases it can be tough to reach the completeness required for *ab initio* structure solution, but CSP can help with this by directly providing the initial model for refinement as was shown in the present case.

Form 4 is closely related to form 1 in unit cell, molecular conformation, and even the disorder of one isopropyl group, but siginificantly higher in energy, explaining its instability. The crystal structure of form 4 completes the characterisation of ritonavir polymorphs reported so far, showcasing the potential of 3D ED and CSP working together to explore API polymorphism.

Conclusion

The combination of CSP and high throughput 3D ED is a powerful tool to make solid form development of APIs as efficient as possible, safeguarding against polymorphism-related risks. Revisiting the polymorphism of ritonavir indeed proved that the polymorph incident could have been prevented with the techniques that are available today.

read the paper:
L. Iuzzolino, A. W. Kelly, M. T. Chaudhry, C. Jandl, D. Stam, A. Y. Lee, *Commun. Chem.* **2025**, *8*, 404. https://doi.org/10.1038/s42004-025-01814-6.

[1] M. A. Neumann, F. J. J. Leusen, J. A. Kendrick, *Angew. Chem. Int. Ed*. **2008**, *47*, 2427–2430.