Cultivating crystal forms

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All the means of action – the shapeless masses – the materials – lie everywhere about us. What we need is the celestial fire to change the flint into the transparent crystal, bright and clear. That fire is genius.
Henry Wadsworth Longfellow

It was less than a generation ago that the chemical literature contained many papers whose titles began with "The Crystal and Molecular Structure of..." (This is not meant to be a criticism; I authored some of them!) Papers of this type were justified then by the considerable effort involved in solving a single crystal structure and in searching the literature for related compounds as a basis for comparison of structural and chemical characteristics. The evolution of X-ray diffraction methods for structure solution by both single crystal and powder methods, along with rapid and increasingly comprehensive access to the chemical literature in general and to the structural literature in particular (mainly through the Cambridge Structural Database (CSD)), has changed that situation. X-ray crystal structure determination has become a (nearly) routine analytical tool so that structure determination is now an integral (and often required) component of synthetic chemistry. The resulting proliferation of crystal structures has led to a new awareness of the existence of multiple crystal forms (Fig. 1) and their importance in a wide variety of applications.

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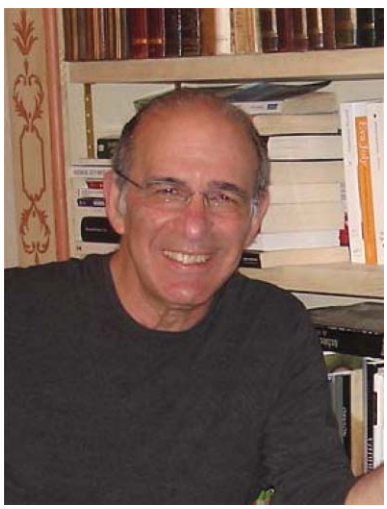

Joel Bernstein was born in Cleveland, Ohio in 1941. After obtaining a BA degree at Cornell University, he earned a Ph.D. degree in physical chemistry at Yale University for research on the solid-state spectroscopy of organic compounds. Following two-year postdoctoral stints in X-ray crystallography with Ken Trueblood at UCLA and in organic solid-state chemistry with Gerhardt Schmidt at the Weizmann Institute of Science in Rehovoth, Israel, he joined the faculty of the newly established Ben-Gurion University of the Negev, where he is now Professor of Chemistry. His research interests center on the organic solid state, with particular emphasis on understanding and utilizing polymorphism, structure–property relationships, hydrogen-bonding patterns and graph sets and organic conducting materials. He has published over 130 research and review articles on these subjects and is the sole author of a recent (2002) book entitled "Polymorphism in Molecular Crystals", published by Oxford University Press. He has also served as a consultant to a number of pharmaceutical companies and as a testifying witness in litigations on the solid-state chemistry of drugs. His career has been punctuated by visiting professorships at the University of Illinois, Cornell University, and the University of Minnesota, more recently at the Universities of Bologna, Barcelona and Johannesburg and as a visiting scientist at the Cambridge Crystallographic Data Centre.

Among those crystal forms polymorphism was first recognized in 1823[1] and co-crystals (albeit often with some alternate designation) have been studied for nearly a century.[2] However, the development of new technology,[3] the attempts to design and control crystal structure,[4] combined with some spectacular encounters with new (and undesired) crystal forms[5] and some high profile pharmaceutical patent litigations[6] have resulted in a recent spate of chemical crystallographic papers with considerably more enticing titles. A sampling of the titles reveals the breadth of current interest in obtaining, characterizing and controlling crystal forms:

- the awareness of the richness of the older literature, including some long-standing challenges ("Polymorphism in Benzamide – Solving a 173-Year Old Riddle");[7]
- the need to update and correct some of the earlier classic experiments ("Polymorphism of Cinnamic and α-Truxillic Acids: New Additions to an Old Story");[8]
- the application of crystal engineering principles based on hydrogen-bonding patterns to the preparation of new multi-component solids ("Crystal Engineering of the Composition of Pharmaceutical Phases. Do Pharmaceutical Co-crystals Represent a New Path to Improved Medicines?"[9a] or "Total Synthesis Supramolecular style: Design and Hydrogen-bond Directed Assembly of Ternary Supramolecules"[9b]);
- the induction of new crystal forms by incorporating a variety of functional groups onto a polymer backbone ("Crystalline Polymorph Selection and Discovery with Polymer Nuclei");[10]
- the development of high-throughput crystallization techniques ("Elucidation of Crystal Form Diversity

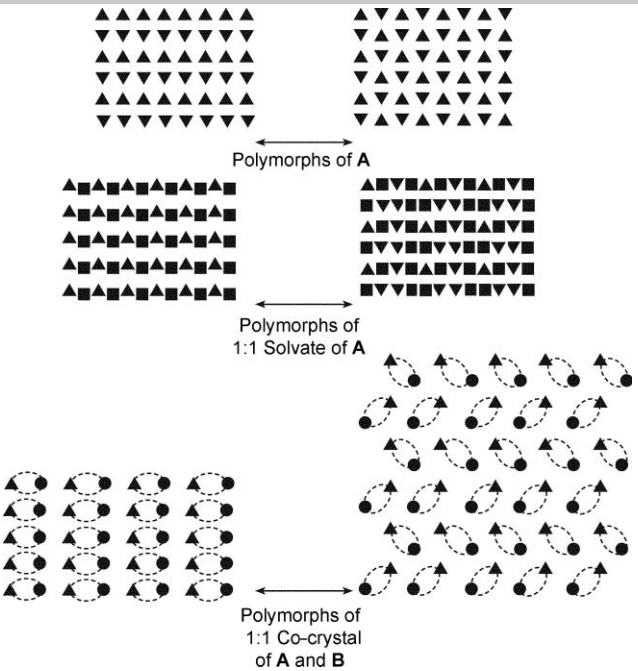

Fig. 1 Schematic representation of the crystal forms discussed. Upper: polymorphs of compound A, represented by ▲. Middle: polymorphs of the 1 : 1 solvate of A, where ■ represents a solvent molecule. If ■ were water this would be defined as a monohydrate. Lower: polymorphs of a co-crystal between A and another compound B, represented by ●. The broken lines connecting A and B suggest the presence of hydrogen bonding, although other intermolecular interactions and relative orientations are possible. As discussed in the text these diagrams represent idealized situations. As in much of chemistry there are real situations that will not fit neatly into one of these idealized schemes, which nevertheless provide a useful framework for the understanding, discussion and comparison of various crystal forms.

of HIV Protease Inhibitor Ritonavir by High-throughput Crystallization'');[3]

● the utilization of solid–solid reactions (''Making Crystals from Crystals: a Green Route to Crystal Engineering and Polymorphism'',[11] ''Selective Polymorph Transformation *via* Solvent-drop Grinding''[12]);

● the setting of records for the discovery and characterization of new polymorphs (''New Polymorphs of ROY and New Record for Coexisting Polymorphs of Solved Structures'')[13] or solvates (''Over One Hundred Solvates of Sulfathiazole'',[14] ''Five New Pseudopolymorphs of *sym*-Trinitrobenzene''[15]).

All of these papers (and an exponentially increasing number on similar topics) deal in one way or another with attempts to obtain new crystal forms, or to control the production of those already known – qualities we associate with the term 'cultivation'. Hence the title of this contribution.

The proliferation of crystal forms has spawned debate about the definition of at least two of the phenomena illustrated schematically in Fig. 1,[16]† solvates and co-crystals. Definitions are indeed important in chemical thinking. They serve to provide a conceptual framework which assist us to understand, categorize, make generalizations, predict, seek outliers, and design new experiments for the verification of unusual phenomena or the development of new models. Our definitions need not be all-inclusive – in fact they are often more valuable as narrow rather than broad, allowing us to recognize the exceptions to the rules. In fact it is the exceptions that drive our curiosity to test models and develop new theories. Chemists are also very comfortable mixing narrowly defined concepts – witness the ionic character of the covalent bond or the ease with which we move between and mix concepts of the molecular orbital and valence bond models. So it is with the schematic definitions in Fig. 1; they represent the simplest way for me to conceptualize these structural phenomena in an idealized fashion, while recognizing that many real cases will fall somewhere in between these idealized ones. For instance, one might legitimately argue that the difference between a solvate and a co-crystal depends in many cases on the intent of the experimenter. Indeed many solvates will have the structural features of co-crystals and *vice-versa*. However, both the seminal references on the current incarnation of co-crystals (*e.g.* Ref. 17) and those reflecting the current wave of interest in the subject (*e.g.*[3,9,11]) all do include an *element of design* in the preparation of the crystalline product. More often than not that design involves the utilization of specific interactions, such as hydrogen bonds, to create predetermined *synthons* or building blocks that are the basis for the three-dimensional crystal structure.[18] On the other hand, while the search for solvates, or the attempts to modify known solvates, may involve some quite sophisticated chemistry, the element of design is rarely, if ever, present in that effort. The absence of the design element regarding the formation of solvates reflects our limited knowledge and understanding about solute/solvent interactions and the mechanism of solvate formation. In the sense of cultivating crystal forms we are at a stage where we can suggest molecular pairs that have a reasonable chance of forming co-crystals, and we can make an educated guess as to the structural nature of the synthons that will be present in the eventual structure. On the other hand we have virtually no idea which solvent will form solvates for a particular solute, or what would be the eventual structural relationship between solute and solvent should a solvate be obtained.

I will readily concede that neither definition of solvates and co-crystals is perfect (how many definitions are perfect?), but I will also contend that in many cases the chemical community would like to distinguish between solvates and co-crystals. Just as McCrone[19] suggested a number of tests to verify that

† I have consciously avoided the use of the term '*pseudo*polymorph', which I believe is an unnecessary misnomer. See reference 16*b* for part of the current debate.

two (or more) substances are polymorphic (even those tests are not perfect [Ref. 6 pp. 148–149]), in principle it is possible to prescribe a number of tests to clarify the distinction. As Merton and Barber demonstrated so elegantly and eloquently in their recent book on the history and use of the single word "serendipity",[20] definitions are dynamic and may even vary from one discipline to the next, but that should not cause us to desist from making them, and from making them as succinct and distinct as possible.

In many ways the cultivation of crystal forms runs contrary to classical chemical thinking. As many undergraduate textbooks testify, crystallization or recrystallization is the traditional method for purifying a solid material. Yet polymorphs by definition have different structures, and solvates and co-crystals have different chemical composition from the principal component, in addition to the variation in structure. When any of these crystallize concomitantly, we may obtain chemical purity (in the case of strict polymorphism) but the product contains solids with potentially different properties. In cultivating crystal forms we are attempting to mediate, or in some cases, overcome what might be a natural tendency for a compound to crystallize in a particular way. For example, under certain conditions a particular crystal form may have only an ephemeral existence, and quite sophisticated methods may be required to isolate it for sufficient time to characterize it.[7]

High-throughput crystallization technology[3] was developed to provide a tool for rapidly and economically surveying crystal space. In general it involves the use of automated methods such as robotics for the preparation of hundreds or even thousands of simultaneous or sequential crystallization experiments. The crystallization experiments are monitored by the utilization of sophisticated analytical and statistical techniques (again usually automated) such as image analysis, pattern recognition and comparison, data mining, *etc.* to detect and characterize crystal forms and the conditions that lead to their production.

Such information is particularly important in the pharmaceutical industry quite early in the drug development process for the appropriate choice of crystal form for formulation, for the protection of intellectual property, and for the anticipation and prevention of the (possibly disastrous) appearance of undesired crystal forms in later stages of the drug life cycle.[5] The concept of high-throughput methods conjures up a 'shotgun' approach – the possibility of an unlimited number of experiments. In practice, the amount of sample available is often severely limited requiring automated preparation, monitoring, identification and characterization on a micro scale, with corresponding technological challenges. Moreover in pharmaceutical applications the choice of solvents is limited to those listed as GRAS (generally regarded as safe) by the FDA. Hence, considerable chemical understanding, knowledge and insight as well as considerable technological sophistication are required for successfully conducting high-throughput crystallization experiments.

Chemists have long employed additives to influence the outcome of a crystallization.[21] In a new variation on that theme, Matzger *et al.*[10] have recently combined the concept of 'tailor-made additives' with high-throughput methods by using a variety of highly cross-linked polymers with a combination of functional groups as nucleating agents. In addition to identifying known crystal forms for four substances (a *sine qua non* for any proof-of-concept crystallization experiment for generating multiple crystal forms), they discovered a new

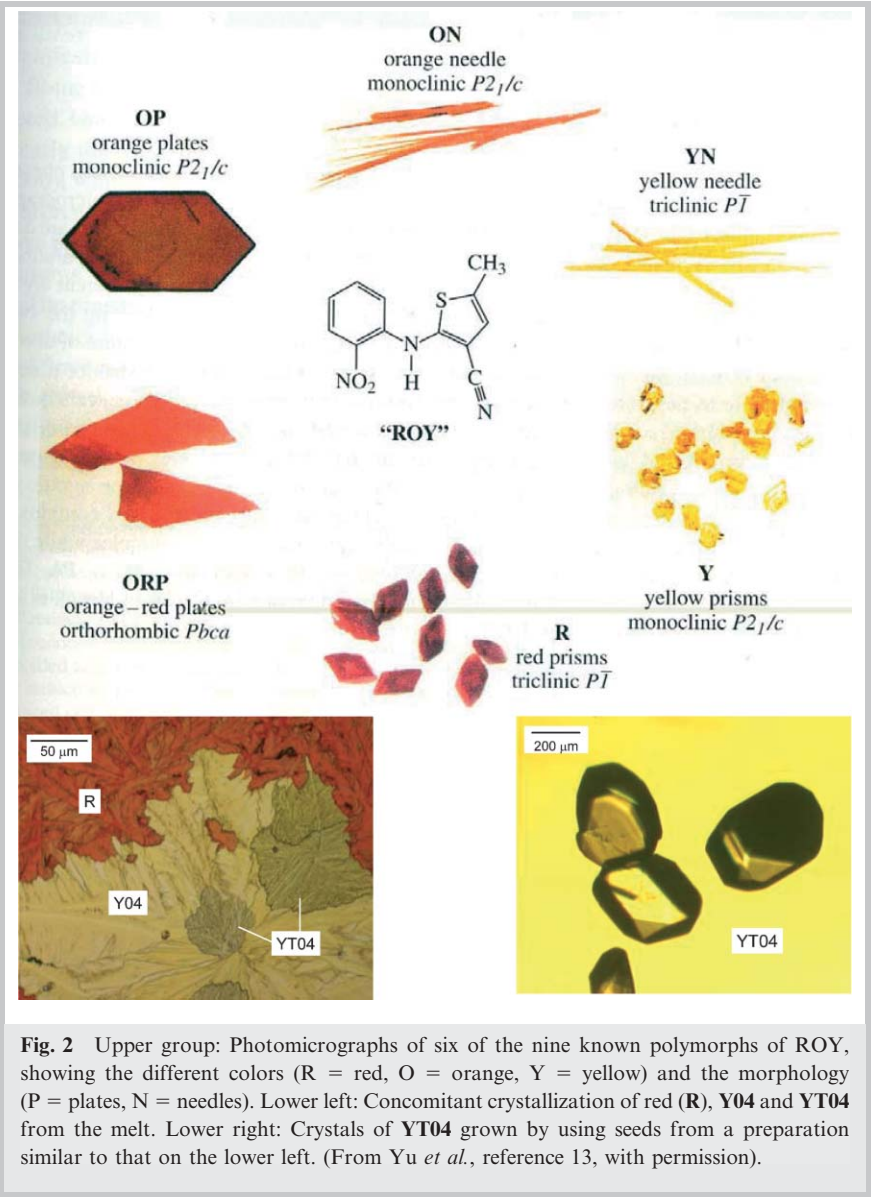

Fig. 2 Upper group: Photomicrographs of six of the nine known polymorphs of ROY, showing the different colors (R = red, O = orange, Y = yellow) and the morphology (P = plates, N = needles). Lower left: Concomitant crystallization of red (**R**), **Y04** and **YT04** from the melt. Lower right: Crystals of **YT04** grown by using seeds from a preparation similar to that on the lower left. (From Yu *et al.*, reference 13, with permission).

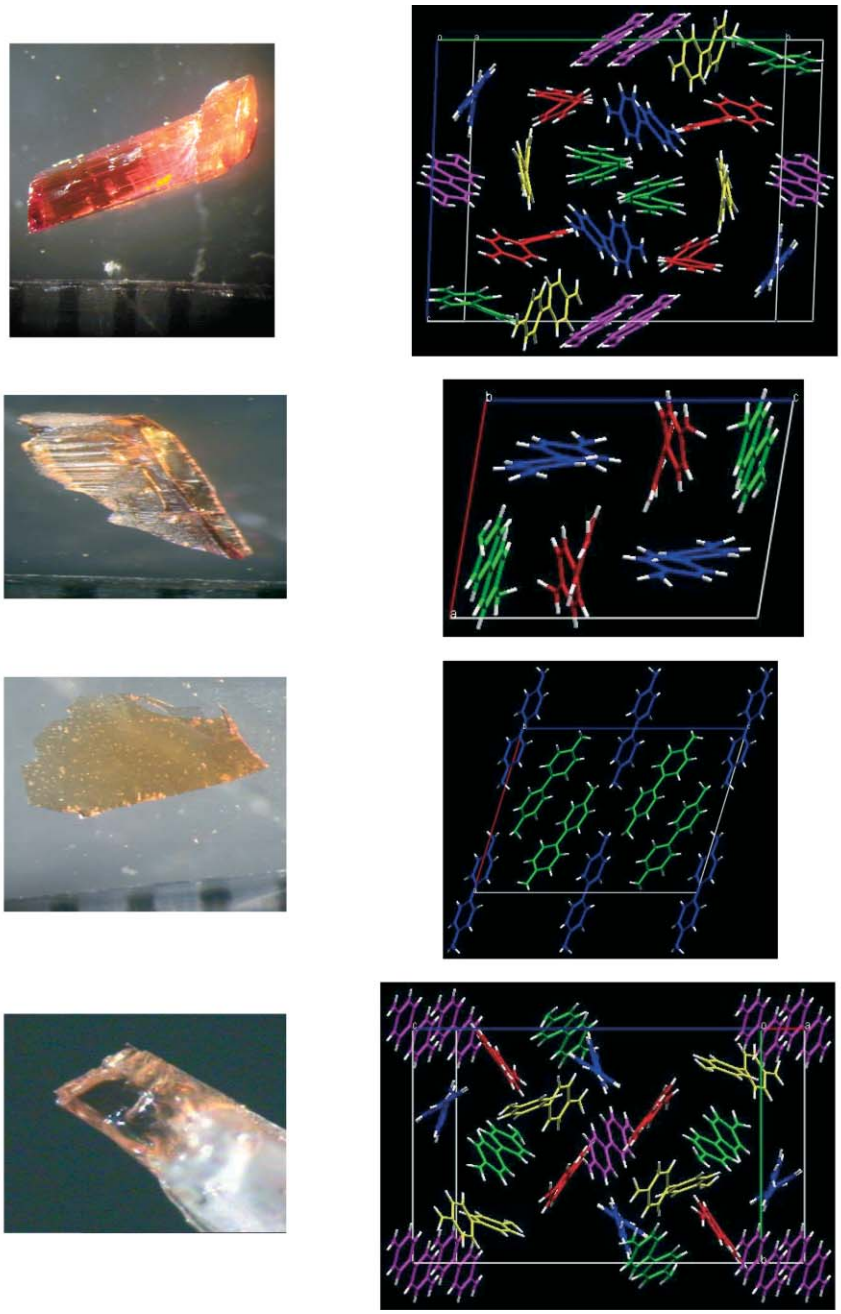

Fig. 3 Photographs of crystals and packing diagrams of the four polymorphic forms of benzidine. It is worthy of note that the crystals of Forms 2–4 are not particularly well formed and are often difficult to distinguish from one another. In pre-CCD diffractometer days it was likely that such crystals would have been rejected for measurement either as unsuitable (too thin, poorly shaped) or as resembling one another. Hence the existence of polymorphism might have been overlooked. The ease and rapidity of screening such crystals now readily facilitates such investigations. In each packing diagram the different colored molecules designate crystallographically independent molecules in the asymmetric unit. From top to bottom, Form 1 (monoclinic, $P2_1/n$, $Z' = 4.5$); Form 2 (triclinic, $P\bar{1}$, $Z' = 3$); Form 3 (monoclinic, $P2_1/n$, $Z' = 1.5$); Form 4 (monoclinic, $P2_1/n$, $Z' = 4.5$) (Michal Rafilovich, personal communication).

form of widely studied carbamazepine and two new forms of sulfamethoxazole.

They also worked on a system that has become the latter-day quintessential model for a polymorphic system, 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile, commonly known as ROY for the red, orange and yellow colors of its various polymorphs (Fig. 2).[13]‡ Significantly, the experiments of Matzger *et al.* did yield the six polymorphs originally reported by Yu *et al.*, but failed to yield three additional forms subsequently reported. The reasons for this are instructive. The seventh form of ROY was obtained by

‡ Among previous claims for the record number of identified polymorphs – but not crystal structures – were phenobarbital (12)[22] and *p*′-methylchalcone (13).[23]

a crystallization from the vapor phase on a succinic acid substrate, while the eighth and ninth forms were obtained, respectively, from a melt crystallization and a solid-state transformation, three crystallization methods which are not part of the standard high-throughput protocols. This example points out the necessity of using a number of crystallization *methods* in the cultivation of crystal forms.

It is of interest that the ninth form of ROY discovered is apparently the second most stable of all the known forms. This is essentially consistent with Ostwald's assertion[24] that later forming or appearing crystal forms will tend to be more stable than their predecessors. In spite of their now holding the record (7) for solved crystal structures of a polymorphic system Yu *et al.* specifically raise doubts about whether they have found all the polymorphs, or even the most stable polymorph. As they correctly point out, the utilization of the historically well developed but generally forgotten methods of chemical microscopy,[25] combined with modern spectroscopic and analytical techniques often leads to the discovery of additional crystal forms. Additional developing methods for cultivating new crystal forms include reactions between crystals and crystals or crystals and gases,[11] solvent-free synthesis,[26] the desolvation of solvated crystals,[27] and crystallization in supercritical solvents.[28]

As noted above, an old[2]/new[9,17,29] approach to new crystal forms is the preparation of co-crystals (Fig. 1). The general strategy is to utilize the complementarity in intermolecular interactions, primarily those of hydrogen bonds, to generate new crystals of two or more components. Since the number of candidate co-crystal formers is much greater than the current list of GRAS materials for solvents or solvates, this approach has great potential in the pharmaceutical field, both for the modification of solid state properties of an active pharmaceutical ingredient, and for the development of new intellectual property.

Even with some initial successes in the rational preparation of co-crystals (but not in the design of their *crystal structure*) there is still much to be learned in this area of cultivating crystals, as in those previously described. For instance, since the desired or attempted preparation of a co-crystal of a particular substance involves the addition of a second complementary substance, the second substance may act to enhance or inhibit the nucleation and/or growth of a particular form rather than act to form a co-crystal. This can lead to new polymorphs of either or both of the substances. For instance, in our own laboratory, attempts to prepare co-crystals of benzidine with triphenylphosphine oxide have led to the preparation of four polymorphs of benzidine, for which no crystal structure has been previously reported (Fig. 3), while attempts to prepare co-crystals of glutamine and aspartic acid have yielded respectively, the previously reported trihydrate and sesquihydrate of oxalic acid. In both of these cases, though, we did succeed also in preparing a co-crystal with the desired components.

Along with serendipity, the abovementioned evolution of crystallography has played an important role in the discovery and apparent proliferation of new crystal forms. With the advent of single crystal X-ray diffractometers with CCD detectors any strange or unusual crystal (see *e.g.* Fig. 3) is a candidate for a rapid (*i.e.* a few hours) crystal structure determination to be identified as a possible new crystal form. Many of our own recently reported new forms have been discovered in this way.

The revolution in X-ray single crystal methods has not relegated other analytical methods to lesser roles in the discovery and characterization of crystal forms. On the contrary, the crystal farmer has an increasingly sophisticated armory in his analytical tool shed. As noted earlier, hot stage microscopy is enjoying a well-deserved renaissance, and the developments in IR, Raman SSNMR spectroscopies, DSC/TGA thermal methods, X-ray powder diffraction techniques, and the 'hyphenated' combination of many of them, especially reduction to microscale technology, all bode well for the discovery and characterization of an increasing number of crystal forms.

For chemists, crystallization has always been an art: inducing on the order of 10^{15} or more molecules to line up in perfect order to produce well shaped (and often colored) crystals has given satisfaction to generations of practitioners. We heat it, filter it, cool it, scratch it, freeze it, bang on it, seed it; the gestation period often taxes our patience or seems to approach infinity, but opening the cupboard or the refrigerator to find a glittering harvest of well-shaped crystals never fails to provide a moment of fulfillment, much like that of the farmer who has labored to cultivate his yearly harvest. Cultivating both new and old crystal forms is providing both new challenges and vast opportunities for creating new materials.

Acknowledgements

I wish to acknowledge a community of outstanding chemical crystallographers who keep making this field ever more exciting and challenging. Many have gone unmentioned here, due to lack of space, but by no means lack of high regard for their contributions. I am grateful to Dario Braga, Fabrizia Grepioni (Bologna) and Juan Novoa (Barcelona) for gracious hospitality during a sabbatical leave in the course of which this manuscript was prepared. My thanks to two of my students, Michal Rafilovich and Mazal Wenger, who allowed me to cite their work prior to publication.

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