

INTERNATIONAL UNION OF CRYSTALLOGRAPHY

IUCr Newsletter

Volume 28 · Issue 4

Collected archival edition

Original article text and available illustrations

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LETTER FROM THE PRESIDENT

The extraordinary has become ordinary

Sven Lidin



I'm afraid this is another bleak one, and I must be honest about this, things are truly bleak. There are those who will tell you that Newton did his best work when the universities were closed down because of the plague and that getting all this time without travel and physical meetings is a blessing in disguise, allowing us to work away on our own. Well, I'm afraid they are wrong. Science is a social endeavor, and scientists are social beings. We do not work well in splendid isolation.

Still, there is hope. There are ways to make things work and, as we all experience in our daily work life we can still get interaction of sorts with students, business partners and colleagues. It is a very healthy sign of our vibrant community that the list of virtual meetings for November 2020 in the [IUCr calendar of events](#) has been full and exciting.

In our response to the pandemic, I'm reminded of the plight of one my early PhD students. During his studies, he suffered an attack of labyrinthitis, a sudden deterioration of his hearing and a grave disruption of his sense of balance, leaving him disoriented and incapacitated from vertigo and nausea, making simple everyday tasks difficult. He told me how he slowly got used to this condition and how his body adjusted. Then he was hit by a new bout of dizziness as the infection receded and his physician explained to him that this was most likely caused by his body having to relearn how to get his bearings all over again.

As the pandemic ebbs and flows, our societies and our professional and social circles are disrupted over and over again. We try to adapt, but things change quickly and with the time-lag of incubation periods, it

is difficult to respond appropriately. We all get disoriented and we will continue to suffer this social vertigo for still some time to come.

The extraordinary has become ordinary.

We adapt surprisingly quickly to something that was inconceivable just 10 months ago.

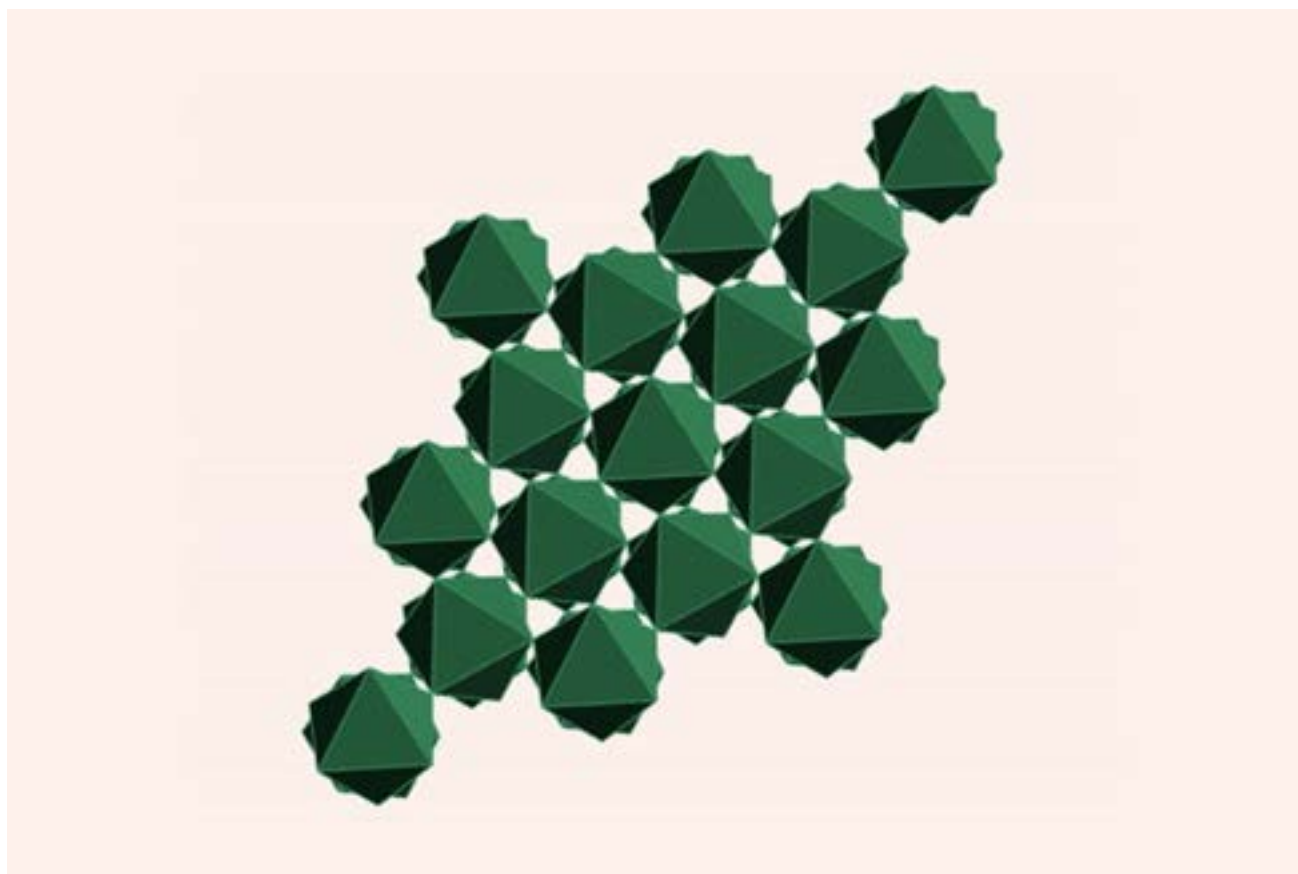
This ability to adapt to change is an enormous advantage and is helping us now, but we must also be prepared for the fact that going back to what was once our normality may take time and that it may also be a challenge.

Thank you everyone for your patience in these difficult and strange times!!

EDITORIAL

Editorial

Mike Glazer



As I write this, the news of the COVID-19 pandemic is finally starting to lighten, with the development of new vaccines that seem to be highly effective. So far, two in Russia have been approved, but without the usual Phase 3 trials needed for international use. Two others (Pfizer and Moderna) seem to have something like a 90–95% efficacy. There are many more in various stages of development and testing. The remarkable speed with which this has happened is a real triumph of science, in which crystallography has played a significant role. Here at Oxford University, working with AstraZeneca, a third vaccine is showing promise. So, with a bit of luck, within the next few months, we shall be able to resume some normal life. It has been a long haul, hasn't it? But we are not finished yet. I do seem to remember that I used to have an office back in the lab; I look forward to some time soon being able to get back to the lab and meet face to face (rather than *Zoom*) with my colleagues over morning coffee. And as I write, the UK has approved the Pfizer vaccine!

Recently, the [Gregori-Aminoff Prize for 2021](#) was awarded to Henry Chapman (University of Hamburg, Germany), Janos Hajdu (Uppsala University, Sweden) and John Spence (Arizona State University, Tempe, AZ, USA). I am pleased that John Spence agreed to write a [special article](#) for us in this issue of the *IUCr Newsletter* about serial crystallography at X-ray free-electron lasers and synchrotrons. The enormous speed with which vast amounts of data can be collected using modern techniques like this is just breathtaking, especially for someone like me, who grew up taking X-ray crystallographic data using the old-fashioned film cameras. Today, X-ray data can be collected in seconds, where it used to take several weeks. Crystallographers of today have it much easier!

When I first began research in crystallography, we were all told that crystals were defined in terms of periodic arrangements of molecules and atoms, maybe with a little disorder thrown in to make them more real. However, nature has a habit, now and then, of throwing a curveball, with the historic discovery of quasicrystals by Dan Shechtman in 1984. Years before, crystallographers like Alan Mackay at Birkbeck College London already had the foresight to question the doctrine of strict periodicity. In this issue, István and Balazs Hargittai tell us about Alan Mackay and the latest Nobel Laureate, Roger Penrose. Intriguingly, the story involves a legal dispute over the Penrose pattern and ... wait for it ... toilet paper! Read about it [here](#).

The Seventh Crystal Structure Prediction (CSP) Blind Test has been [announced](#). The ability to predict a crystal structure, especially one containing organic molecules, is of great importance to the pharmaceutical industries in their bid to design new drugs. Most new pharmaceutical compounds are patented using crystallographic data, and one of the areas of interest is polymorphism, where a molecule can form crystals with different structures. Each polymorph may have different properties, such as solubility differences that affect the drug uptake, and can be patented separately. The Cambridge Crystallographic Data Centre invites applications to participate.

Clearly, structure prediction is gaining in importance. By coincidence, just the other day, another type of structure prediction has hit the news. This is the announcement that has excited the media headlines from the recent Critical Assessment of Protein Structure Prediction (CASP) experiments. These have shown that it is possible, with apparently staggering success, to start with an amino acid sequence and predict the way it folds up into a protein molecule. For crystallographers, this raises a question. Do we still need to do diffraction measurements in structural biology? John Helliwell [discusses this important topic](#) in this issue of the Newsletter.

One of the most disagreeable aspects of getting older is that you hear ever more often of the passing of old friends. So, it was with regret that I heard about the [recent death](#), after a long illness, of Sir John Meurig Thomas. We both go back a long way, having first encountered each other when he sent in a manuscript for publication, and I was asked by the journal editor of the time to be a reviewer. It turned out, by chance, that I happened to be the only other person in the UK who had inside knowledge about the topic. I won't bore you with the details, but suffice it to say that we got into a friendly discussion on the subject of his paper, which then led to a long-lasting friendship. John was a superb scientist, working both in the fields of crystallography and materials science. He was also an excellent speaker and science presenter. In 1987, when he was Director of the famous Royal Institution in London, he gave that year's Christmas Lectures on Crystals and Lasers. I recommend that you watch these yourself [here](#). I was lucky enough to be in the audience during his first lecture.

But it doesn't stop there. I have just heard that another old friend, Patience Thomson, has also passed on this week. Patience was the younger daughter of W. L. Bragg and granddaughter of W. H. Bragg. I got to know her when we were planning the [Two Braggs Exhibition](#) at the University of Warwick in 2013 (and see <https://www.diamond.ac.uk/Home/News/LatestNews/02-09-14.html>). Some years earlier, I was invited to give a lecture on the Braggs, and I was able to meet up with Lady Margaret Heath, Patience's elder sister. She very kindly (and I thought rashly) let me take home the Bragg family photograph album (now in the archives of the Royal Institution). Margaret then introduced me to Patience. She, and other members of the Bragg family, joined in the planning, giving access to their homes and material, much of which has never been published. Patience was such a lovely person and was well known to the crystallography community. I used to enjoy visiting her at her home south of Oxford and seeing all the great paintings and drawings made by her father and grandfather, and chatting about all the famous people she had met. That resulted in a joint book on the autobiographies of Sir Lawrence and Lady Bragg. Patience was married to David, son of G. P. Thomson and grandson of J. J. Thomson (that's four Nobel Prize winners!) – quite a record.



Patience and David Thomson at the Two Braggs Exhibition in 2013.

Have you seen the stunning [new website for the IUCr](#)? If you have not done so already, do have a look. I should also like to mention that the early back issues of the *Newsletter* are now online and available [here](#).

In the meantime, I hope you will stay safe and wish you well for the New Year.

IUCr ACTIVITIES

Family-friendly meetings

Alex Ashcroft



Those of you who saw [my article](#) in the last edition of the *IUCr Newsletter* will have seen that the organisers of ECM32 (Vienna, Austria) in 2019 had arranged for awards to be presented by some of the children of participants, which I thought was a lovely idea. Three photos were used to illustrate my previous article, but you can see more of them above and below. The parents of the children kindly gave their permission for us to reproduce the photographs, and then we found that the three photos that were most relevant to last month's article all featured the same young girl. We thought it was important to include the other photos as we wanted all of the parents who were kind enough to grant their permission to get the opportunity to see their child in the *Newsletter*.



Reading about the family-friendly nature of ECM32 reminded me that one of the highlights, for me, of the excellent ECM31 in Oviedo, Spain, in 2018 was to see a young father playing with his infant daughter at the welcome buffet while his wife was busy networking with other academics. That really brought the family-friendly policy to life for me and helped me to understand why ECM31 Chair Santiago García-Granda had been so keen on this idea when it had been discussed at the IUCr Congress in Hyderabad, India, in 2017. Some of you may know that my wife, Nicola, has been editing *Acta Cryst. A* and *International Tables* for more years than either of us want to count, so I have (mostly) fond memories of being at home looking after our son, Tom, while Nicola went to the IUCr Congresses in Florence, Osaka, Madrid and Montreal. Enjoyable though it (usually) was to have more time with Tom, it would have been lovely for us all to have been able to attend the Congresses together. I'm sure we are all hoping that the [Prague Congress](#) can go ahead as a physical meeting in August 2021, and I know Radomír Kužel, the Conference Chair, is planning a special "Children's Zone" with supervision available from experienced teachers for the children of delegates who register for this. More family-friendly features are currently being planned, so I hope that more families can enjoy Congresses together in future.

FEATURE ARTICLE

Serial crystallography at XFELs and synchrotrons

J. C. H. Spence

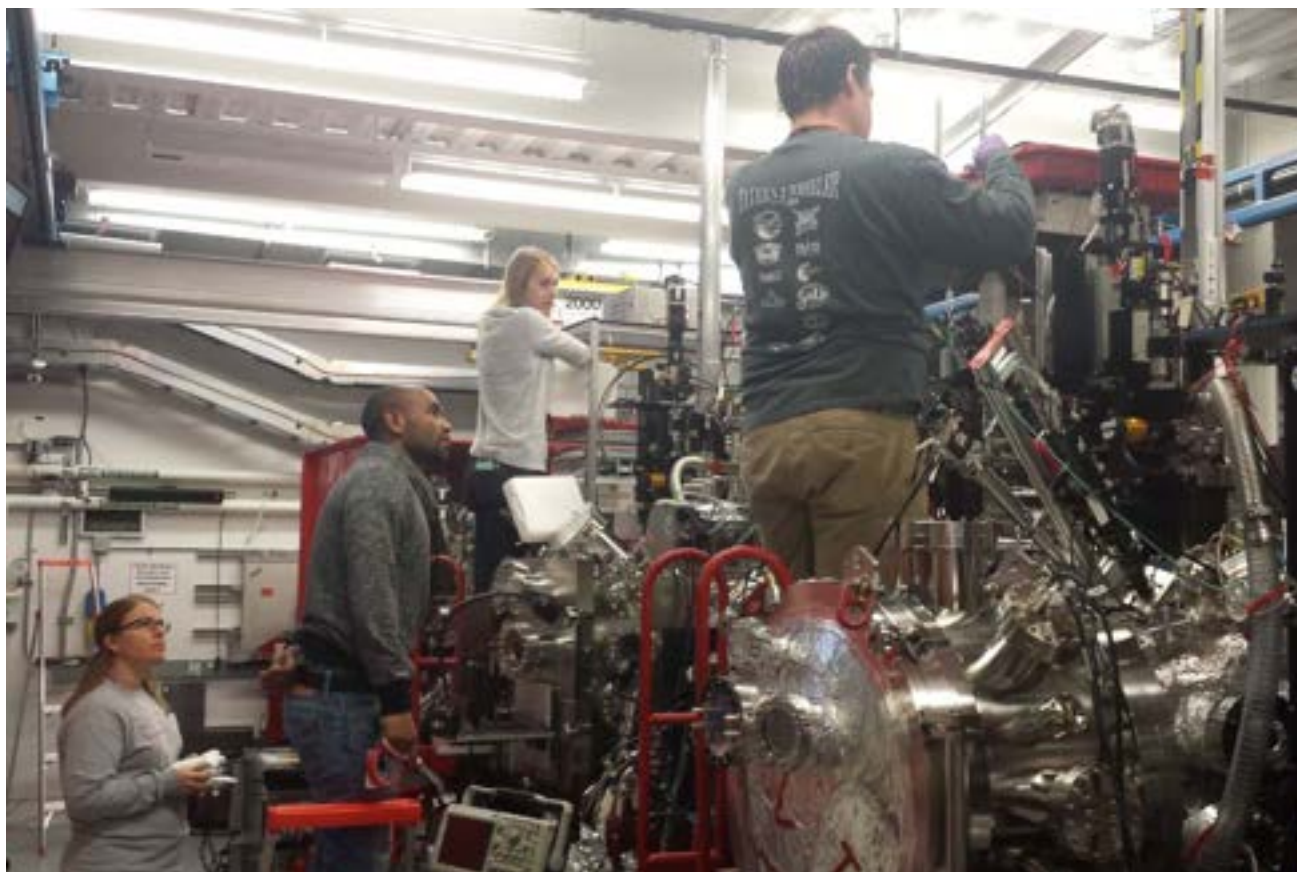


Figure 1. Graduate students prepare a crystallography beamtime at the Linac Coherent Light Source (LCLS) at SLAC in 2015.

It is now about a decade since the first results of crystallography at an X-ray free-electron laser (XFEL) were published [1], so perhaps the dust has settled a little. For summaries of the considerable literature, and at the risk of offending contributors who may be overlooked, I refer readers to reviews of structural biology at XFELs generally [2,3] and to the following summaries of crystallography at the major hard X-ray FELs in California (the LCLS [4]), South Korea (PAL [5]), Switzerland (SwissFEL [6]), Germany (European XFEL – EuXFEL [7]) and Japan (SACLA [8]). All have now turned their attention to the COVID-19 threat, as briefly discussed below. Additional references can be found through keyword searches in, for example, the open Google Scholar search engine, which include the most recent contributions to the rapidly growing COVID literature.

A brief history of serial crystallography

XFELs provide intense pulses of soft or hard X-rays with pulse durations between about one femtosecond (fs) and perhaps 100 fs. The repetition rate of most is about 100 Hz (often limited by the readout rate of the detector) but will soon increase to the kilohertz region and may be varied. A special case concerns the megahertz rate of the EuXFEL, which is discussed below. The unsuitability of the XFEL for standard crystallography owing to instabilities was immediately evident when work started at the LCLS in 2009,

with up to 15% variation in beam intensity between shots, 0.1% bandwidth and a spikey time-dependence to each pulse. In addition, one needed to record and save 120 diffraction patterns per second using a fully coherent beam, a few micrometres in diameter, which drilled holes instantly in stainless steel. (A typical shot contains about 10^{12} incident photons, about the same as provided by many synchrotrons in one second.) New area detectors with a much larger dynamic range were needed. The aforementioned issues were dealt with by developing the method of serial crystallography (SC, or SFX for serial femtosecond crystallography), in which thousands of hydrated randomly oriented micrometre-sized crystals are exposed successively. Each exposed microcrystal is destroyed by the beam after providing a useful Bragg diffraction pattern before the femtosecond X-ray pulse ends. The diffraction conditions for each microcrystal are determined later by computer analysis, and the serious scaling problem which arises owing to differences in crystal size and orientation must then be addressed. Given these fluctuations in both beam and sample conditions, it is remarkable that sufficiently accurate structure-factor measurement is now possible to allow both native phasing and time-resolved crystallography at XFELs. Preliminary results from the even more difficult time-resolved small-angle X-ray scattering (SAXS) technique look promising. A large effort from many collaborating research groups and beamline scientists around the world has contributed to the current state of affairs, where, after a few days of beam time, small groups of users can now work with local staff to obtain and analyse high-quality diffraction data from protein microcrystals at room temperature with negligible radiation damage, as seen in Fig. 1.

The early excitement over the ability of this 'diffract-then-destroy' femtosecond snapshot crystallography to outrun damage has led to a deeper understanding of damage processes (distinguishing primary initial ionization from later irreversible nuclear motion) and the principle has held up provided that sufficiently brief pulses (perhaps 20 fs in duration) are used. In brief, both Bragg diffraction and a limited amount of ionization occur before the termination of a sufficiently brief X-ray pulse, after which the photoelectron cascade destroys the crystal. Resolution degrades with increased pulse duration [1]. Meanwhile, the strengths of the method – the ability to solve room-temperature structures without significant damage and hence to record protein dynamics (without immobilizing the structure by cooling) under near-physiological conditions – have been much more fully developed. This has opened the way to structural studies under controlled environmental conditions, such as temperature changes, photon exposure of light-sensitive proteins in pump-probe studies and diffusion of ligands into protein microcrystals using time-resolved snapshot diffraction. The subsequent transfer of this SC technique to synchrotrons since 2009 for similar studies of protein dynamics, enabled by new fast detectors and diffraction-limit upgrades, is one of the most important impacts that XFEL research has had on the field of protein crystallography.

The beginnings of SC are almost as old as crystallography itself. As soon as the early crystallographers in the 1920s attempted to merge the data from more than one crystal, or from different areas of the same large crystal, many of the scaling issues now dealt with in modern SC arose. This merging was carried out in order to share the damaging radiation dose over a larger volume; generally, these scaling issues were addressed by the standard statistical methods of maximum likelihood optimization. When using film recording with the oscillation method, the problem later arose of merging partial reflections (those not integrated fully in angle across the rocking curve) from one crystal with those from another. This problem was addressed by defining a 'degree of partiality' in a 'post-refinement' approach pioneered by Michael Rossmann and his group (Purdue University, West Lafayette, IN, USA). A new form of fast SC for microcrystals was developed around 2004 in preparation for use at the first XFELs, as discussed below.

For SFX, the solution to the data-analysis problem with multiple crystals was to adopt a Monte Carlo integration approach [9], summing the same indexed reflections from different microcrystals and thereby averaging over all the large statistical fluctuations from several independent sources. These include size variation among the crystals, shot-to-shot beam-intensity variations, the wavelength distribution in the beam and the orientation variation of crystals at each reflection. With enough diffraction patterns from

different microcrystals in random orientations, sampling would occur at a sufficient number of points across the rocking curve to perform the required angular integration. The error (R split) can then be seen to fall as $k/\sqrt{N}$ for N patterns, with k a constant. We recall that the height of a Bragg peak is proportional to the square of the number of molecules in the crystal, while the angle-integrated intensity is proportional to the number of molecules, and inversely proportional to the size of the unit cell. Since that time, many improvements over this global averaging approach have been published with the effect of reducing k through some form of iterative modelling of the rocking curve or spectrum. Many of these improvements are now incorporated as options in several software packages, including the *DIALS* and *CrystFEL* suites, to which the reader is referred for details of data analysis in SC. This includes methods to resolve the indexing ambiguity which arises when merging data from different crystals [10]. Prior to indexing and merging, useful data must be isolated ('hit finding') from the total dataset, which will include blank shots where the beam misses the crystals, the background must be treated and detector corrections must be applied. Merging depends on autoindexing methods, which may involve sparse datasets, either in the sense of showing too few Bragg spots to allow indexing by conventional macromolecular crystallography (MX) software or by showing very weak reflections or both. There have recently been dramatically effective approaches to both of these problems [11, 12]. The use of the expectation maximization and compression (EMC) algorithm employs a similar approach to that used for single-particle XFEL data merging: it does not assign crystallographic indices but treats the entire nanocrystal as one molecule. This adds the parameter of crystal size to the three angular-orientation unknowns in single-particle analysis, resulting in a powerful approach for data from weaker storage-ring sources for the very smallest crystals of small proteins (which give stronger Bragg spots than larger molecules), but it comes with a very large computational cost. A second method of indexing sparse patterns, for somewhat larger crystals producing sparse patterns, collects pairs of reflections and the angle between them and then searches a look-up table for the most probable orientation.

As at synchrotrons, most phasing for SFX is done using molecular replacement; however, native sulfur anomalous diffraction (SAD) phasing has been achieved using an XFEL, as has isomorphous replacement. New possibilities for phasing arise [3] as the coherence width of the beam typically exceeds the dimensions of the crystals, producing interference effects between Bragg reflections. (Conventional Bragg crystallography requires only that the coherence width across the beam be larger than the unit cell, not the crystal.)

Closely linked to the data-analysis problem has been the sample-delivery problem, and it has been fascinating to see how the SC methods first developed for XFELs before 2009 have now been taken over successfully and often improved on at most synchrotrons. The term 'serial crystallography' has come to encompass methods as varied as those which run tens of thousands of hydrated micrometre-sized crystals in single file in a liquid water jet across the beam to those at synchrotrons where multiple exposures are recorded using different beam positions on a single large crystal mounted on a goniometer. The term also includes the increasingly popular methods where hundreds of small crystals lie on a membrane spanning a semiconductor chip, which is raster scanned across the beam. The success of the synchrotron work reflects the fact that most processes in structural biology occur on a time scale of microseconds or longer, so that *the much higher time resolution of XFELs is usually more valuable for reducing radiation damage, allowing the study of reactions at room temperature, than for its speed.* (Study of the early stages of reactions in light-sensitive proteins, however, does benefit from femtosecond time resolution.) The experimental origins of fast SC can be traced to an early proposal [13] to inject bioparticles in a continuous single-file stream across a beam for diffraction analysis using a Rayleigh jet. The first tests of such a system with protein microcrystals were undertaken at the Advanced Light Source [14]. A liquid-injection system was developed for the first SFX experiments in 2009 [1] by incorporating a gas dynamic virtual nozzle (GDVN) into the vacuum chamber of the world's first XFEL, the LCLS at SLAC (see Fig. 2). That system was immediately modified to allow optical laser pump-probe measurements suitable for studies of photosynthesis. Mixing jets were also developed soon after [15] to allow time-resolved X-ray snapshots to be recorded during a chemical reaction, using the mix-and-inject

serial crystallography or 'MISC' method [16, 17]. This allows the XFEL to outrun both radiation damage and diffusion, for sufficiently small micrometre-sized crystals. It is understood that these studies are limited to molecules and enzymes that remain chemically active in crystalline form (but with slower kinetics [16, 17]), a result established as long ago as 1969.

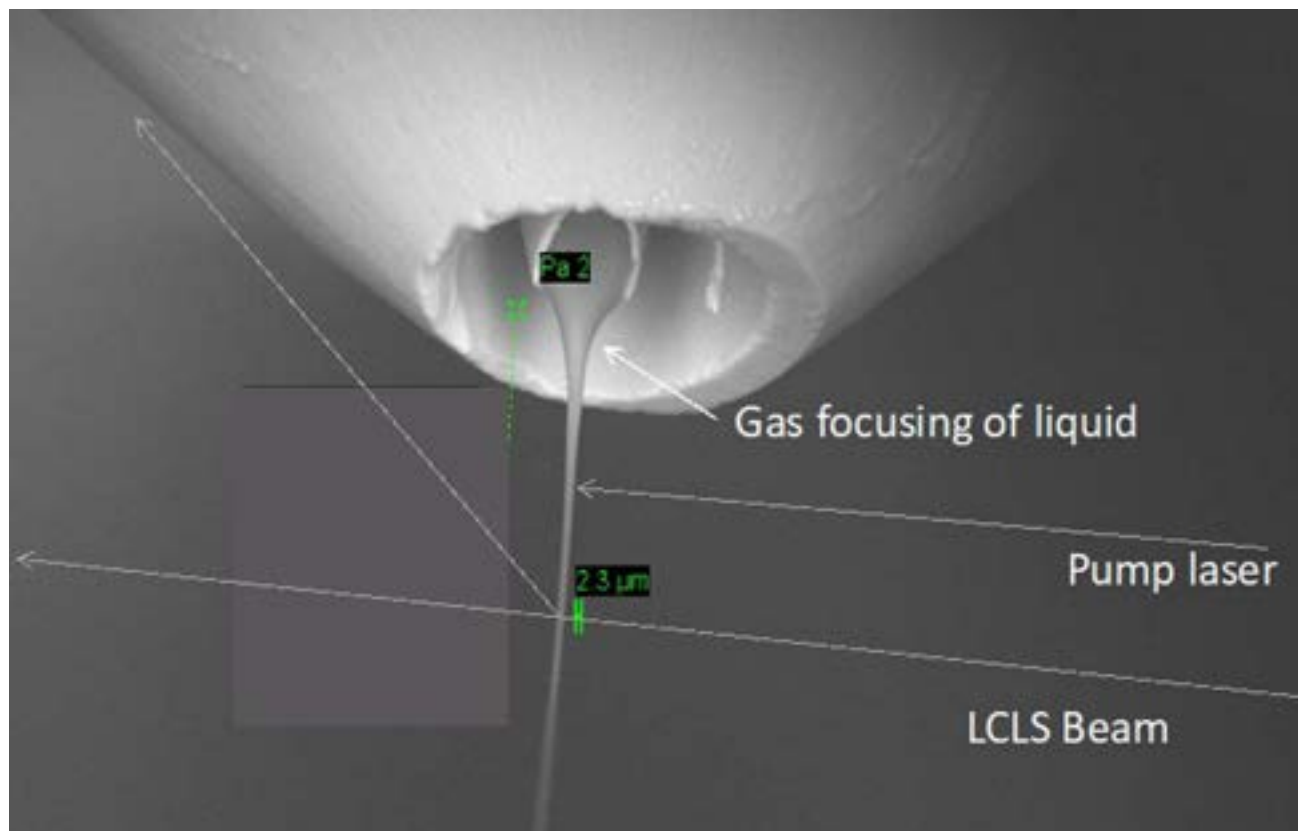


Figure 2. GDVN jet. Showing how the high-speed gas jacket focuses the liquid stream (containing protein microcrystals in single file) by speeding it up, while avoiding the need for a smaller nozzle which may clog [1]. The jet is a few micrometres wide. A Bragg beam runs up to the top left corner.

Sample-delivery methods

Since that time, a plethora of different sample-delivery schemes have been developed for the three main modes of SC operation: static structure determination for protein microcrystals, such as the G-protein coupled receptor (GPCR) drug targets; pump-probe studies on light-sensitive protein microcrystals, as shown in Figs. 3 and 4 (movie) [18]; and mixing-jet experiments on microcrystals for the study of chemical dynamics, such as time-resolved imaging of enzymes [16, 17]. In addition, similar sample-delivery methods were required for closely related XFEL techniques such as single-particle analysis (with one virus per shot), fast spectroscopy and time-resolved solution scattering. In each case, one must consider the need for a hydrated sample environment, possibly in vacuum (or helium gas) together with possible laser pumping or solution-mixing facilities, while delivering over a thousand samples per second across the beam. These are demanding constraints, with the ultimate goal of minimizing sample waste between shots and obtaining a high hit rate. Sample-delivery methods include scanned stages ('fixed target' systems), liquid jets, double-focusing jets, viscous jets, conveyor-belt systems, electrospray, acoustic levitation, mix-and-inject systems and electrokinetic injectors. For some modes (such as pump-probe work on light-sensitive proteins), a completely reliable system for XFELs that wastes little protein has yet to be developed. SFX at the EuXFEL (and at LCLS II) is complicated by very high pulse repetition rates of 1 MHz or more (in separated pulse trains). For static structure determination, the simplest and most reliable system for protein microcrystals that can be grown in lipid cubic phase (LCP) appears to be the slow-moving LCP jet [19], which does not suffer from clogging and wastes little protein. Scanned stages, with their very high hit rate, have been highly successful at lower repetition rates and have

involved a variety of robotic or flow-based loading systems. A system for mixing droplets onto chip-mounted microcrystals can also be found [20], in addition to a simple mixing jet which is easy to assemble and does not require lithography or 3D printing [21]. A conveyor-belt (tape drive) system has been described, which provides high hit rates. Conveyor belts, with droplets dispersed in single file along them, allow the belt speed across the beam to be adjusted to match the XFEL repetition rate. Work toward synchronized droplet jets (triggered by the XFEL photocathode signal) continues, including a system that inserts long slugs of oil between droplets of buffer containing microcrystals. The oil travels across the beam between X-ray pulses, conserving protein [22] (see Fig. 5). Very recently, this system has succeeded in producing diffraction patterns at the LCLS synchronized with droplets containing protein microcrystals. For the future, with ever-increasing repetition rates it may be that only the liquid jets are fast enough. (For the GDVN, the free-running droplet generation rate is about 1 MHz.) Current XFEL upgrades to 1 kHz or more are common, with data acquisition limited by detector readout speed. These microfluidic systems and mixing nozzles are increasingly fabricated using two-photon 3D laser printers with sub-micrometre resolution, as shown in Fig. 6. It is remarkable that the same 3D CAD drawing can be used both to 3D print the device and for fluid-dynamics computer simulations, with good agreement between them. The sub-micrometre resolution of two-photon 3D printers has greatly contributed to making jet delivery highly reproducible for monodispersed particles. Alternatively, XFELs can be intentionally run at reduced repetition rates to allow scanned-sample systems to be used with their very high hit rate. Viscous jets, which avoid clogging and are perhaps the simplest and most reliable system, have recently been used for time-resolved pump-probe work [23] with low background and protein waste, and should be usable up to about 1 kHz. The segmented flow system holds promise for high-repetition-rate sample delivery while conserving protein. References to articles on all these systems, including the advanced 'Roadrunner' scanned-stage system, can be found in two recent comprehensive reviews (with over 200 references) of XFEL sample delivery for biology [24, 25]. Examples of well-developed systems and results for time-resolved SC at synchrotrons are described in the following references [20, 23]. Systems for static SC are now becoming available at most synchrotrons worldwide.

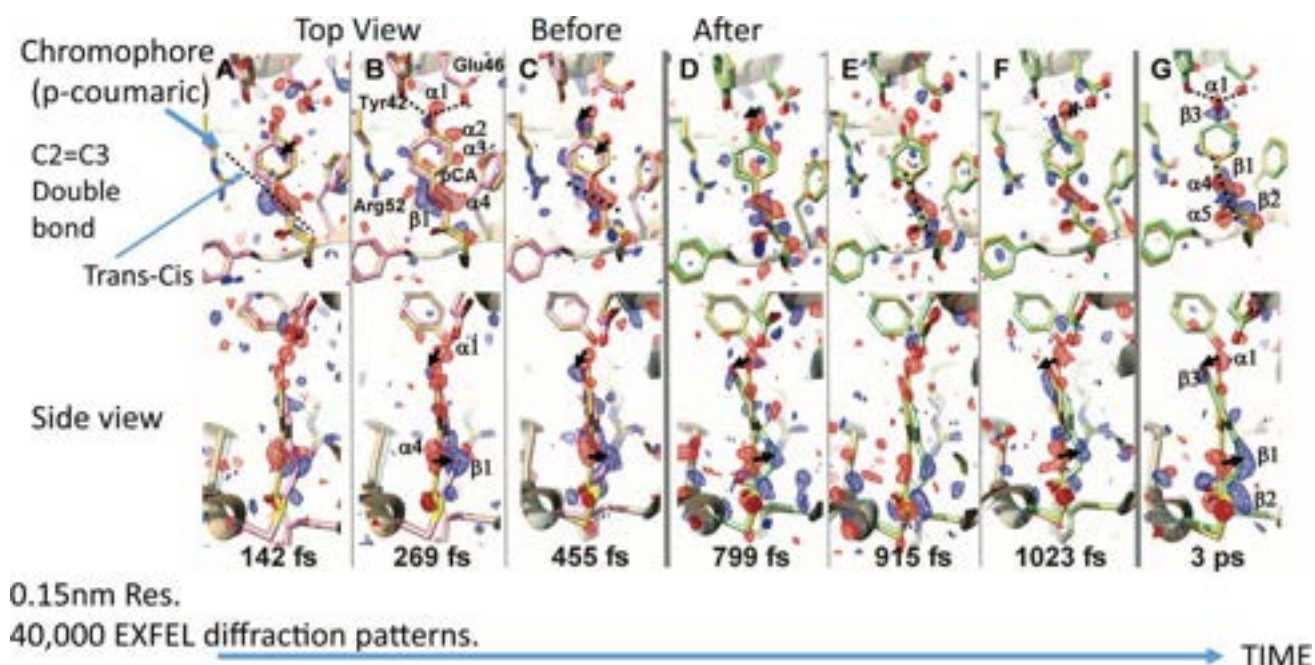


Figure 3. The motion of the *p*-coumaric acid chromophore in photoactive yellow protein (PYP) undergoing a *cis-trans* isomerization reaction owing to absorption of light. The torsion angle rotation about the double bond shown can be measured against time from these difference density (DED) snapshot frames (across the page) taken about 150 fs apart. This is a conical intersection, which must pass through a degeneracy in nuclear coordinates. Yellow: reference dark structure. Pink: DED before *cis-trans* rotation. Green: after. Obtained by pump-probe SC at LCLS [18].

Time-resolved structural analysis

As a result of all these developments in data analysis, XFEL sources and sample-delivery methods, we have seen very rapid progress over the last 10 years in the SC method, both at XFELs (for SFX) and at synchrotrons (SC). For XFELs, this has allowed for static structural determination of crystals grown *in vivo*, perhaps of submicrometre size, and from challenging targets such as membrane proteins, grown in the viscous LCP sample-delivery medium. Many radiation-sensitive GPCR drug targets have been solved (13 new XFEL structures since 2013), in addition to supramolecular assemblies such as ribosomes. For time-resolved XFEL studies, the small crystal size allows for rapid diffusive saturation in mix-and-inject analysis of biochemical reactions, with microsecond diffusion times, shorter than reaction times, for sufficiently small crystals. Here the MISC technique is proving powerful for structural analysis of intermediates, allowing a structure to be associated with each time point in the catalytic cycle of an enzyme, for example, as identified from spectroscopic or electron paramagnetic resonance (EPR) studies of the kinetics. A recent MISC movie of the progress of the RNA riboswitch through its four intermediate states while regulating gene expression provides a good example [26]. The method has also shown us the mechanism by which the β -lactamase enzyme gains resistance to an antibiotic drug [16]. The heavily studied light-sensitive macromolecules rhodopsin, photosystem I and II, and PYP have all produced spectacular multi-frame 'movies' of conformational change owing to light absorption. Here a small fraction of molecules in the crystal respond to the sharp optical trigger to follow one or more reaction paths, producing changes in the Bragg intensity with delay between the trigger and the XFEL snapshot. The trigger-delay-snapshot process is repeated until all orientations of microcrystals have been sampled, and for many delays, each delay providing one frame of a movie. Based on the accurately known structure of the unexcited crystal, a combination of single-value decomposition analysis [16] and modelling can extract a time-resolved image of the molecular motion. We note, however, that these 'movies' of kinetics, recovered from the time-dependence of Bragg spots, show a spatial periodic average of the amounts of the various photoactivated species present at each time point and not a true molecular movie of, for example, bond formation and breakage, which occurs on the much faster time scale of dynamics. Nevertheless, XFEL movies of molecular machines have now been published with time resolutions between 150 fs and seconds. In these studies of light-sensitive proteins it has often proven difficult to accurately calibrate and provide the correct pump-light fluence. For studies of microcrystals into which small molecules are diffused, similar considerations apply regarding the interpretation of crystallographic 'movies'. To obtain a sharp trigger to initiate a reaction in these systems, it has been suggested that optogenetics or caged compounds may be used to render them light sensitive.

Figure 4. A movie of the ultrafast early phase of the photoreaction in PYP shown in Fig. 2 is shown on a time scale spanning from 100 fs to 10 ps. This time scale is covered by ten time-resolved X-ray datasets obtained after reaction initiation by ultrafast blue laser pulses from a powerful femtosecond laser. The pump-probe TR-SFX data were all collected on needle-like PYP microcrystals grown with malonate as the precipitant, and injected into vacuum by a liquid injector to interact with the laser and the X-ray pulses. Datasets for the first nine time delays were collected at the LCLS with a 120 Hz X-ray pulse repetition rate, the last time point (10 ps) was obtained at the EuXFEL using 564 kHz X-ray pulses. Structural models of PYP were determined at each time delay by interpreting so-called extrapolated electron-density maps where the extent of reaction initiation was expanded to 100%. The movie was produced by 'UCSF Chimera' by morphing between the ten resulting structural models at consecutive time delays [18].

2020 COVID research

Since March 2020, the five worldwide XFELs have begun to offer special beam times for COVID-19 research. For crystallography, this has mainly meant solving the crystallized component proteins of the virus; however, more ambitious studies are also planned, including time-resolved diffraction. XFELs can offer room-temperature structures without significant damage, the ability to study nanocrystals and snapshot imaging of kinetics. Several projects have focused on the possibility of developing protease inhibitors, as for the virus responsible for AIDS. Many proposals for inhibiting viral enzymes have been described in the literature, as outlined in a [recent review](#) in the *IUCr Newsletter*. On 22 July, there were 47 COVID structural models contributed to the PDB by cryo-EM and 250 by MX. At least five beam times

had been awarded at LCLS alone for COVID work, some now completed successfully. (The National Science Foundation Science and Technology Center 'BioXFEL', devoted to the application of XFELs to structural biology, is a significant contributor.) Publications can be found using the keywords COVID and XFEL in the open Google Scholar search engine, in addition to the various pre-publication archives.

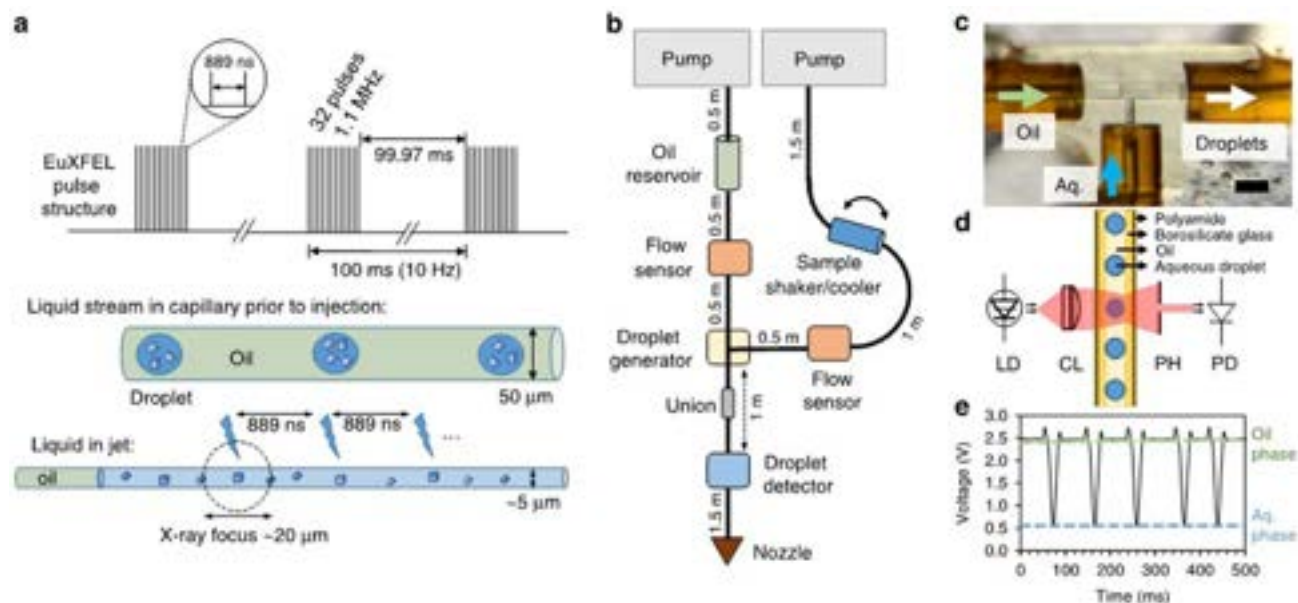


Figure 5. Synchronized delivery for high repetition-rate and readout-rate XFELs. The droplets of buffer containing protein microcrystals are interspersed with oil, which runs across the beam between X-ray pulses. An applied electric pulse triggered by the XFEL photocathode may be used to synchronize the arrival of the droplets with the beam [22].

The future of serial crystallography

This new field – imaging protein kinetics by SC at XFELs or synchrotrons – is now entering its most exciting phase. As sample-delivery methods mature we are bound to see many more time-resolved studies of molecular chemistry with atomic spatial and femtosecond time resolution, recorded under near-physiological conditions. From these it should be possible to extract both kinetics and (by modelling) dynamic mechanisms, energy landscapes, rate coefficients and activation barriers for reactions, and intermediate structures for both reversible and irreversible (catalyzed) reactions. In addition to associating a structure with each stage of the reaction kinetics, time-resolved serial crystallography (TR-SC) is unique in providing the sequence of events in a reaction – it can tell us which species came first in a multi-step reaction when times are short. New reaction pathways might be discovered, intermediate states suitable as drug targets identified and the images used to refine the force fields used for modelling over a larger range of times. The MHz repetition rate of the EuXFEL allows one to collect in one hour data which would take days to collect at the other XFELs worldwide. This raises the exciting possibility of multi-parameter data collection, in which a wide variety of chemical conditions are varied in real time during data collection, such as temperature, pH, applied potentials, ligand diffusion, terahertz pumping and the use of light-sensitive caged compounds to trigger reactions. Two very recent intriguing new ideas have also been investigated: that XFEL diffraction data report the correct oxidation state of heavy atoms in proteins (without the chemical reduction owing to photoelectrons in synchrotron studies) and that damage may also be outrun using sufficiently brief pulses for hard X-ray inner-shell spectroscopy. Time-resolved density maps have also been obtained during application of an electric field to proteins [27].

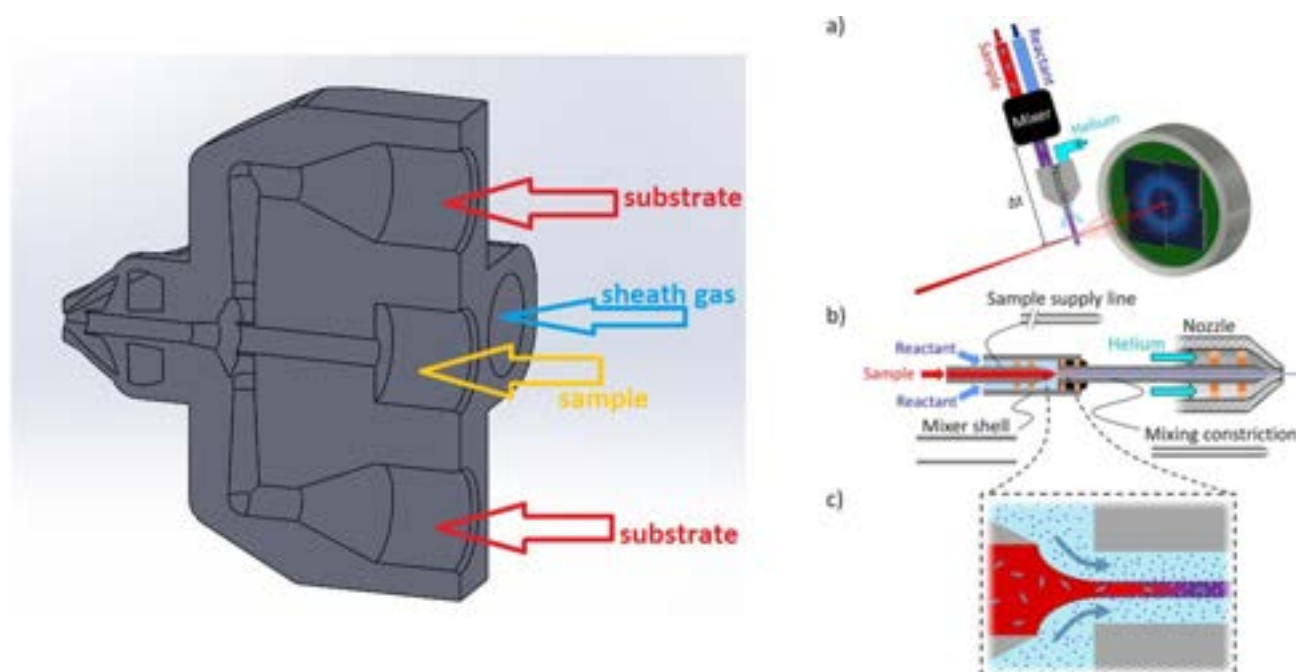


Figure 6. Mixing jets. 3D-printed at left [22] and modular rapid-assembly Lego mixer at right [21].

Instrumentation developments are also occurring rapidly in the field of accelerator physics, where new ideas for X-ray lasers abound in the literature. A compact XFEL, which fills a large room, is already under construction at Arizona State University [28]. This is based on spatial modulation of the MeV electron beam and use of a counter-propagating laser as a wiggler. New designs are also under consideration for a possible large machine in the UK. The companion field of XFEL SAXS is progressing rapidly, to expand studies of protein dynamics to motions unrestricted by crystal constraints. These experiments with very low signals and large backgrounds (lacking the 'Bragg boost' of crystallography) are very difficult, despite recent success in producing the desirable 'sheet jet' delivery systems. The highly stable conditions of the SAXS sample cell and beam available at a synchrotron are hard to replicate but early results from pump-probe studies of the reaction center and myoglobin are encouraging.

In summary, the development of SC at both XFELs and synchrotrons has opened up many new opportunities in structural biology, especially in the exciting new field of atomic-resolution imaging of protein kinetics, including enzyme catalysis, under controlled chemical conditions.

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John Spence (Department of Physics, Arizona State University, Tempe, AZ, USA) is Main Editor (Physics and FEL Science and Technology) of IUCr, and has recently been awarded the Gregori Aminoff Prize 2021 along with Henry Chapman and Janos Hajdu “for their fundamental contributions to the development of X-ray free electron laser based structural biology”.

FEATURE ARTICLE

DeepMind and CASP14

John R. Helliwell



Structure of lysozyme, PDB ref 1LYZ.

What do I as a protein crystallographer think of the use of AI by DeepMind to predict protein folds?

The Critical Assessment of Techniques for Protein Structure Prediction (CASP) challenge is a serious and very well done effort these last decades carried out by global organisers: Dr John Moult (chair), University of Maryland, USA; Dr Krzysztof Fidelis, UC Davis, USA; Dr Andriy Kryshchuk, UC Davis, USA; Dr Torsten Schwede, University of Basel and SIB Swiss Institute of Bioinformatics, Switzerland; and Dr Maya Topf, Birkbeck, University of London, UK and CSSB (HPI and UKE) Hamburg, Germany. The CASP14 Press release of 30th November 2020 https://predictioncenter.org/casp14/doc/CASP14_press_release.html stated that:

'Today (Monday), researchers at the 14th Community Wide Experiment on the CASP14 will announce that an artificial intelligence (AI) solution to the challenge has been found.' And 'During the latest round of the challenge, DeepMind's AlphaFold program has determined the shape of around two-thirds of the proteins with accuracy comparable to laboratory experiments*. AlphaFold's accuracy with most of the other proteins was also high, though not quite at that level.'

The asterisk above is all-important to exploring precisely what has been achieved in this undoubted breakthrough:

'Notes to editors: *AlphaFold produced models for about two-thirds of the CASP14 target proteins (JRH: 84 targets; full details are here: <https://predictioncenter.org/casp14/index.cgi>) with global distance test scores above 90 out of 100. Above the 90-score threshold, remaining differences between the models and the experimental structures are small and of the size expected for experimental artefacts and errors and alternative low energy local conformations. Note that these CASP targets are single proteins or domains, not protein complexes, which are a next frontier. The global distance test is a measure of how closely the shape of the protein model matches the shape from lab experiments [1, 2]."

A dramatic advance has happened then, but what, exactly?

This is clearly, i.e. all the expert judges of the CASP series of challenges agree, a breakthrough in the field of prediction of protein folds. To understand this better, we need to clarify what is a fold, and what is a structure? The polypeptide chain fold is described by simply linking the alpha carbon in each peptide in the protein's amino acid sequence, one after the other. This leads to the well known ribbon representation of a protein. Then, where the individual atoms are in the structure is a critical aspect, and as W. L. Bragg most famously said: with crystallography, we can see atoms.

The experimental probes of X-rays, electrons and neutrons in crystallography, and electrons in microscopy, as well as NMR, then strive for precise protein models derived as the best fit to their measurements. The term accuracy, which means as close as we can get to the truth, requires more than one method and, in this context, is termed integrated structural biology. Our experimental methods have weaknesses though. There is radiation damage to the protein structure, if not usually to the fold, by X-rays or electrons according to the dose absorbed by the crystal sample. Then there are changes to the details of the structure and its structural dynamics according to whether cryo-temperatures are used. In recent years experimentalists have explored more and more of these changes, termed 'artefacts', and strive for damage-free structures at physiologically relevant temperatures. Cryo-structures can be substantiated by accompanying the structure with a functional assay and/or undertaking one of a group of structures at room temperature, including comparing structures in solution with those in the crystal such as by solution X-ray or neutron scattering. The characterisation of what precision of a protein structure has been achieved is a big topic, but in 1999 Durward Cruickshank formulated the Diffraction Precision Index [3] to assist with this assessment. The CASP14 press release's sentence, "Above the 90-score threshold, remaining differences between the models and the experimental structures are small and of the size expected for experimental artefacts and errors, and alternative low energy local conformations", has I think to be assessed carefully, case by case, and the precision of placement atom by atom. I suspect that a new terminology has arrived: allowing for prediction artefacts to add to our own list of experimental artefacts.

Some history of the protein folding problem: key steps and how the folding problem has held great scientific minds over the last 60 years

Referred to as Levinthal's paradox, in 1969 Cyrus Levinthal [4] noted that, because of the vast number of degrees of freedom in an unfolded polypeptide chain, the molecule has an astronomical number of possible conformations. Based upon the observation that proteins fold much faster than this, Levinthal then proposed that a random conformational search does not occur, and the protein must, therefore, fold through a series of metastable intermediate states.

A famous article in this field is by Christian Anfinsen [5], which stated in conclusion that 'empirical considerations of the large amount of data now available on correlations between sequence and three-dimensional structure, together with an increasing sophistication in the theoretical treatment of the energetics of polypeptide chain folding are beginning to make more realistic the idea of the a priori prediction of protein conformation. It is certain that major advances in the understanding of cellular

organization, and of the causes and control of abnormalities in such organization, will occur when we can predict, in advance, the three-dimensional phenotypic consequences of a genetic message.'

Indeed the Nobel Prize in Chemistry in 1972 (<https://www.nobelprize.org/prizes/chemistry/1972/press-release/>) was awarded 'one half to Christian B. Anfinsen for his work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation, the other half jointly to Stanford Moore and William H. Stein for their contribution to the understanding of the connection between chemical structure and catalytic activity of the active centre of the ribonuclease molecule.'



Here David Phillips unveils the lysozyme's molecular model (on the table in front of him) to the public during a historic lecture at the Royal Institution in London in 1965. Hanging from the ceiling was a 32-foot-long string of the enzyme's 129 amino acids. And on the counter next to Phillips was the same string, this time folded into a globular shape a few feet across. The folding, Phillips explained, locked the amino acids together in a way that gave the enzyme its catalytic activity. Reproduced with the permission of the Royal Institution of Great Britain (RIGB). This photo and caption were located via Google at the C&EN website, who we also thank.

The great protein crystallographer David Phillips, who with his team determined the first enzyme crystal structure [6], and later was Sir David Phillips and then Lord Phillips of Ellesmere, wrote already in 1966 in *Scientific American* [7] of the folding of lysozyme: 'It seems a reasonable assumption that, as the synthesis proceeds, the amino end of the chain becomes separated by an increasing distance from the point of attachment to the ribosome, and that the folding of the protein chain to its native conformation begins at this end even before the synthesis is complete. According to our present ideas, parts of the polypeptide chain, particularly those near the terminal amino end, may fold into stable conformations that can still be recognized in the finished molecule and that act as 'internal templates', or centres, around which the rest of the chain is folded.'

At this point in the history, one should note that [8] '(while) Anfinsen (1973) showed that all information needed for a polypeptide to fold into basic secondary structure elements such as α -helices and β -strands and their subsequent collapse into a compact structure is entirely contained within the amino acid sequence. (and) Although small single-domain proteins can fold spontaneously and in a reversible way, most multi-domain proteins need the help of chaperones to adopt the ultimate native and active conformation.... These proteins thus violate Anfinsen's rule.'

How did DeepMind make this advance [9, 10]?

From the DeepMind blog <https://deepmind.com/blog/article/AlphaFold-Using-AI-for-scientific-discovery> the public at large can learn that DeepMind's methods 'relied on deep neural networks that are trained to predict properties of the protein from its genetic sequence. The properties our networks predict are: (a) the distances between pairs of amino acids and (b) the angles between chemical bonds that connect those amino acids. The first development is an advance on commonly used techniques that estimate whether pairs of amino acids are near each other.'

They had already entered the CASP13 competition in 2018, which they described in [10]. In that, they make clear [9] that:

'Source code for the distogram, reference distogram, and torsion prediction neural networks, together with the neural network weights and input data for the CASP13 targets, are available for research and non-commercial use at https://github.com/deepmind/deepmind-research/tree/master/alphafold_casp13. We make use of several open-source libraries to conduct our experiments, particularly HHblits36, PSI-BLAST37, and the machine-learning framework TensorFlow (<https://github.com/tensorflow/tensorflow>) along with the TensorFlow library Sonnet (<https://github.com/deepmind/sonnet>), which provides implementations of individual model components⁵⁰. We also used Rosetta9 under license.

Data availability: Our training, validation, and test data splits (CATH domain codes) are also available from https://github.com/deepmind/deepmind-research/tree/master/alphafold_casp13.

The following versions of public datasets were used in this study: PDB 2018-03-15; CATH 2018-03-16; Uniclust30 2017-10; and PSI-BLAST nr dataset (as of 15 December 2017).'

The CASP14 work involved rewriting their software such that there were accuracy improvements in AlphaFold in CASP14 over that used by them in CASP13 which 'lead to more accurate interpretations of function; better interface prediction for protein-protein interactions; better binding pocket prediction and improved molecular replacement in crystallography.'

Most importantly for us experimentalists, we read: 'All the neural network models are trained on structures extracted from the PDB. We extract non-redundant domains by utilizing the CATH7 35% sequence similarity cluster representatives (CATH version: 2018-03-16). This gives 31 247 domains, which are split into train and test sets (29 427 and 1820 proteins, respectively), keeping all domains from the same homologous superfamily (H-level in the CATH classification) in the same partition.'

Getting into the mind of DeepMind: its other projects

A major strength of DeepMind is the breadth of its other areas of research involving AI. Further details of these are available at their website and include:

[Traffic prediction with advanced graph neural networks](#)

[Using AI to predict retinal disease progression](#)

[Using WaveNet technology to reunite speech-impaired users with their original voices](#)

[Advanced machine learning helps Play Store users discover personalised apps](#)

[AlphaStar: Grandmaster level in StarCraft II using multi-agent reinforcement learning](#)

[Using machine learning to accelerate ecological research](#)

[Using AI to give doctors a 48-hour head start on life-threatening illness](#)

[DeepMind AI reduces Google data centre cooling bill by 40%](#)

Some competing interests: an AI company and its use of an open-access data archive of experimental structures, the PDB

In their Nature article [9] Acknowledgements, the authors state, 'We thank ...the CASP13 organisers and the experimentalists whose structures enabled the assessment.' So, that is good, but in the blog of DeepMind's view of experimental methods (<https://deepmind.com/blog/article/AlphaFold-Using-AI-for-scientific-discovery>) I find wide of the mark their use of the words 'trial and error':

'Over the past five decades, researchers have been able to determine shapes of proteins in labs using experimental techniques like cryo-electron microscopy, nuclear magnetic resonance, and X-ray crystallography, but each method depends on a lot of trial and error, which can take years of work, and cost tens or hundreds of thousands of dollars per protein structure. This is why biologists are turning to AI methods as an alternative to this long and laborious process for difficult proteins. The ability to predict a protein's shape computationally from its genetic code alone - rather than determining it through costly experimentation - could help accelerate research.'

This also brings us back to being clear about fold versus structure and the all-important precision of atom placements, whether by experiment [3] or by AI prediction, as yet not parameterised.

There is also the question that while the reproducibility of the DeepMind Nature paper is feasible via the authors placing the workflows at github, the competing interests statement in their Nature paper states: 'A.W.S., J.K., T.G., J.J., L.S., R.E., H.P., C.Q., K.S., A.Ž. and A.B. have filed provisional patent applications relating to machine learning for predicting protein structures.'

And re. reproducibility, (https://github.com/deepmind/deepmind-research/tree/master/alphafold_casp13), 'This code can't be used to predict the structure of an arbitrary protein sequence. It can be used to predict structure only on the CASP13 dataset (links below). The feature generation code is tightly coupled to our internal infrastructure as well as external tools. Hence we are unable to open-source it. We give guide as to the features used for those accustomed to computing them below.....'

This, to me, seems like a culture clash of the fully open PDB versus the private enterprise and profit of DeepMind.

Future outlook

As I have explored in this article, it is the detailed protein structure that determines function, not the protein fold per se, but DeepMind are clearly entering the area of positioning atoms in detail, although not really with a clear level of precision at the atom-by-atom detailed level. Secondly, multi-domain proteins are currently outside of this achievement. Thirdly, where a structure is known, I note that structural dynamics studies start, i.e. in effect, while there may be one fold, there isn't one structure in function terms! In our experimental arena, kinetic crystallography is set to thrive with an expansion of X-ray lasers, the ESRF EBS, Diamond II etc. building on many earlier developments [11]. Diffuse scattering is all there for the measuring and interpreting, i.e. structural dynamics again.

Onto more specific topics: a third of all proteins are metalloproteins. Predicting where a metal might bind and which metal element it is, is not solved. And not just metal ions but all sorts of other ligands too will still have to be done experimentally, although see DeepMind's Nature paper Extended Data Fig. 8 and caption statement on ligands, and thereby drug design/discovery:

'Ligand pocket visualizations for T1011. T1011 (PDB 6M9T) is the EP3 receptor bound to misoprostol-FA55. a, The native structure showing the ligand in a pocket. b, c, Submission 5 (78.0 GDT TS) by AlphaFold (b), made without knowledge of the ligand, shows a pocket more similar to the true pocket than that of the best other submission (322, model 3, 68.7 GDT TS) (c). Both submissions are aligned to the native protein using the same subset of residues from the helices close to the ligand pocket and visualized with the interior pocket together with the native ligand position.'

The impact on the AI field as a whole in its efforts with predicting protein structure from amino acid sequence has already been major. Since the DeepMind publication [9] it has been cited 248 times since January 2020. A wide-ranging very recent review of the whole field, with 252 references, is in press in the journal Patterns [12].

I am keen to get started with DeepMind, who are owned by Google, which I imagine will proceed as follows:

JRH: Google, I am starting research on protein 427 in human chromosome 14. What is DeepMind's prediction of the 3D structure?

Google: I will submit the job to our computer cluster...(2 minutes later)...The nearest 3D structure is PDB entry 8XYZ but has a GDT score of only 55%. So, DeepMind offers a fold with an 89% GDT score. NB. it is likely a single-domain protein, so DeepMind is very confident in this GDT score estimate. This 3D structure prediction is in your mailbox.

JRH: Thankyou Google.

Google: You're welcome. Good luck with your research.

[NB For DeepMind to provide a GDT estimate without an experimental structure available is not yet solved!]

Envoi

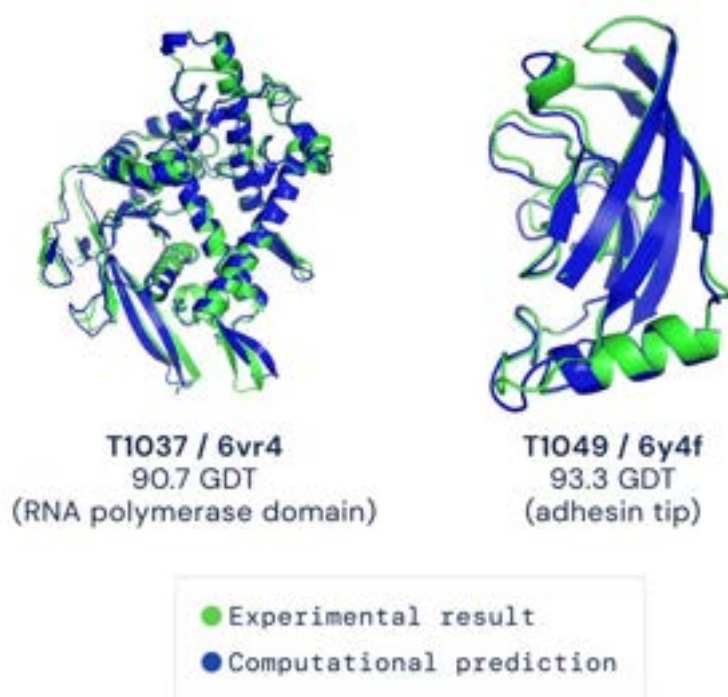
I am going to leave the last words to DeepMind out of respect for their achievement:

'Some rare diseases involve mutations in a single gene, resulting in a malformed protein which can have profound effects on the health of an entire organism. A tool like AlphaFold might help rare disease researchers predict the shape of a protein of interest rapidly and economically.'



The DeepMind AlphaFold team, in Zoom gallery view mode, at the moment that they got the email from Dr John Moult, Chair of CASP14, read out to them by their Team Leader John Jumper. Image credit: DeepMind.

Also, here is their figure animation, kindly provided by the DeepMind Press Office.



Two examples of protein targets in the free modelling category. AlphaFold predicts highly accurate structures measured against experimental result. image credit: DeepMind.

The more complete 8-minute video from DeepMind on their work is to be found here, which I found also highly interesting: <https://www.youtube.com/watch?v=gg7WjuFs8F4&feature=youtu.be>

Acknowledgements

I am very grateful to Mike Glazer for helpful discussions during the preparation of this article, as well as to Sarah Froggatt and Brian McMahon of IUCr Chester for expert technical help. I thank the DeepMind Press Office and the Royal Institution of Great Britain for prompt responses to my requests for figure, gif and movie materials.

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Announcement of two new Main Editors for Journal of Synchrotron Radiation



Dibyendu Bhattacharyya (left) and Kristina Kvashnina.

The entire *Journal of Synchrotron Radiation* (JSR) editorial team would like to take this opportunity to thank Professor Dr Ilme Schlichting (Max Planck Institute, Heidelberg, Germany) for her many years of service as a Main Editor of JSR, and wish her well as she steps down at this time.

Following our advertisement for a new JSR Main Editor earlier in the summer, together with other discussions, we are pleased to announce the appointment of two new JSR Main Editors:

- Dr Dibyendu Bhattacharyya – Head, Synchrotron Science and Multilayer Physics Section, Atomic and Molecular Physics Division, Bhabha Atomic Research Centre, Mumbai, India, and also Professor at Homi Bhabha National Institute, Mumbai, India.
- Professor Dr Kristina Kvashnina – ERC group Head at the European Synchrotron – ESRF, Grenoble, France, at the beamline supported by Helmholtz-Zentrum Dresden-Rossendorf, Germany; also Professor of Chemistry, Moscow State University, Russia.

While not previously associated with IUCr journals as such, Dibyendu is actively involved in the IUCr Commission on XAFS, and has published several papers in JSR. He will bring in a new regional perspective, and we hope more papers from India as India continues to develop its own major facilities.

Kristina is an existing Co-editor of JSR. By being an active committee member of the review panels at several synchrotron and free-electron laser facilities across different countries, she is comfortable with

the open-access publishing environment, and she will bring valuable insights for guiding *JSR* into the fully open-access regime.

Dibyendu and Kristina will work with *JSR*'s other Main Editors, Professor Yoshiyuki Amemiya (University of Tokyo, Japan) and Professor Ingolf Lindau (SLAC/Stanford University, USA), and of course with the entire *JSR* Editorial Board.

This article was originally published in *J. Synchrotron Rad.* (2020). **27**, 1459.

IUCR JOURNALS NEWS

Announcement of conversion of Journal of Synchrotron Radiation to 100% open access from January 2022



The International Union of Crystallography (IUCr) has concluded that the time for converting *Journal of Synchrotron Radiation* (JSR) to become fully open access has arrived. With this November 2020 issue we are pleased to announce that JSR will become a fully open-access journal from the beginning of 2022. More specifically, and with the approval of the IUCr Executive Committee, the decision has been made to convert JSR to fully open access from the January 2022 issue.

Full open access will require the contact author of each accepted paper (or their institution) to pay an article processing charge (APC). The APC will apply to all accepted papers (after reviews are completed), unless a waiver or discount has been agreed between the contact author and the Editorial Office. Authors from developing countries will be able to apply for a full or 50% waiver of the APC. All papers in new JSR issues from January 2022 will be made open access and freely available to all researchers (*i.e.* they will be gold open access). The costs of peer review, of journal production, and of online hosting and archiving will be met from the APC.

Further details will be made available as we get nearer to the conversion date, and the following transitional timetable will apply:

1. Submission for publication from now and through all of 2021 – this will be the open-access transition period, in which publication of conventional behind-pay-wall articles is still possible but open access will be increasingly encouraged. Discounts in the open-access APC will be available

where appropriate. Each 2021 *JSR* issue will reprise the announcement of full open access coming to *JSR* for papers published from January 2022.

2. For papers submitted from 1 October 2021, the contact author will be asked to agree to payment of the open-access APC if their article is accepted, unless a waiver or discount has been agreed between the contact author and the Editorial Office.
3. *JSR* becomes fully open access from the January 2022 issue. Conventional behind-pay-wall articles will no longer be possible, although APC discounts (and sometimes waivers) will still be available.

While this is a major change for *JSR*, presenting both challenges and opportunities, ultimately it is made in the spirit of expansion rather than contraction.

In this connection, we much appreciate the help of *JSR*'s supporting institutions, and we hope other major facilities will also consider supporting *JSR* as the journal of choice for papers focused on synchrotron or FEL-based X-ray structural science by taking up an open-access facility arrangement.

This article was originally published in *J. Synchrotron Rad.* (2020). **27**, 1460.

COMMENTARY

In and out: crystal engineering for reversible iodine uptake

Guillermo Minguez Espallargasa

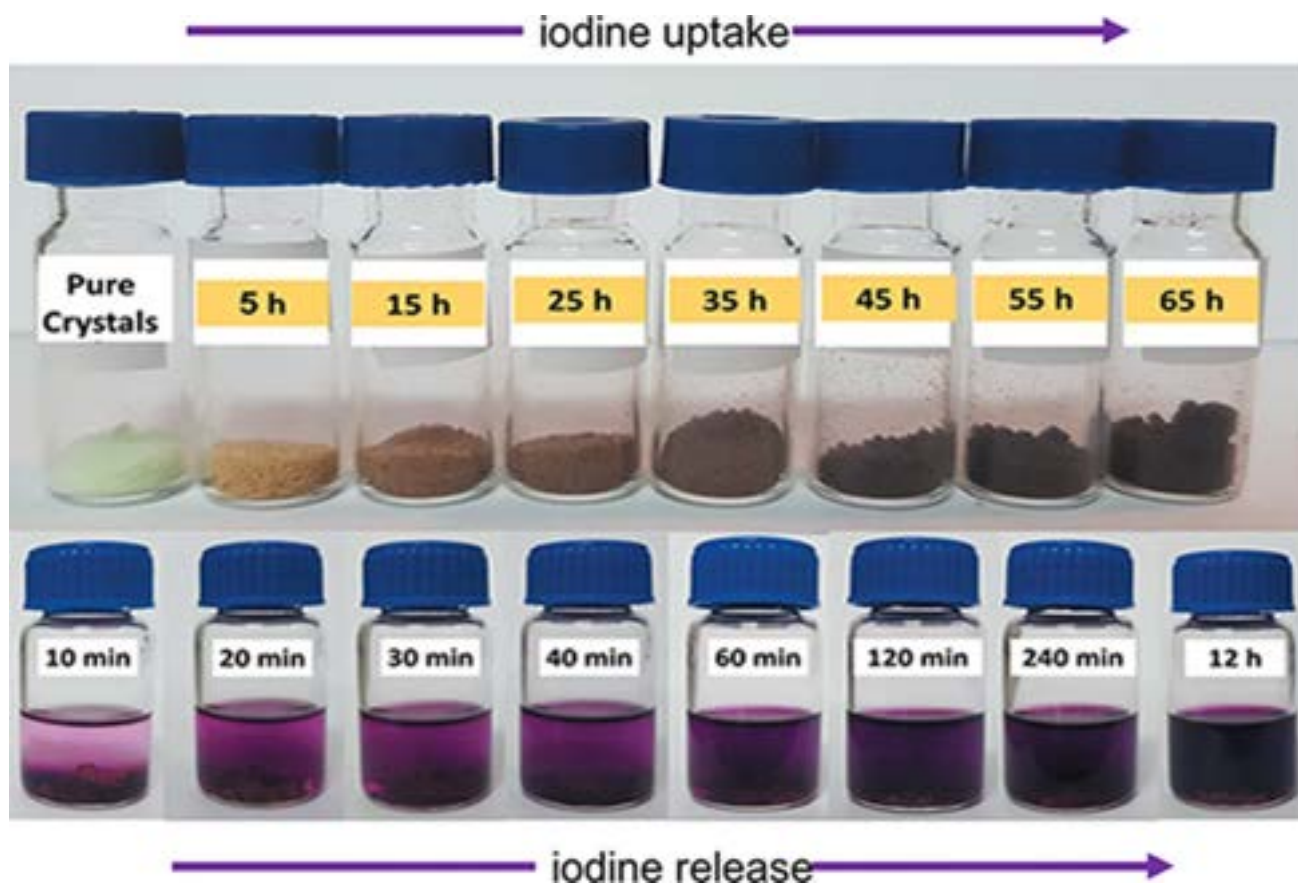


Figure 1. Combined photographic images taken from two figures in the paper by Moghzi *et al.* (2020) showing the colour change on iodine uptake and iodine release.

In the October 2020 issue of *Acta Crystallographica Section B*, Moghzi *et al.* (2020) report the chemical design of a metal-organic compound with an outstanding iodine (I_2) sorption capability. Sequestration of I_2 , an extremely volatile gas, has important applications in terms of controlling radioactive gases, as various radionuclides of I_2 are released during the fission of nuclear fuels. Thus, the preparation of porous materials that can efficiently capture and store I_2 is of importance not only from an academic point of view, but also for real-life applications.

In their report, the authors nicely illustrate the process that has been followed to prepare the final material, demonstrating the importance of careful evaluation of the different crystal structures obtained. In other words, Moghzi and co-workers show an example of the crystal engineering process, *i.e.* the understanding of intermolecular interactions in the context of crystal packing and the utilization of such understanding in the design of new solids with desired physical and chemical properties. In this sense, they have fine-tuned the synthetic conditions in order to control the dimensionality of the product, from discrete units (0D), to chains (1D) and layers (2D). More specifically, the authors show that the dimensionality of the final coordination polymer is dependent on the coordination mode of the *N*-heterocyclic polycarboxylic acid used (pyridine-2,6-dicarboxylic acid or pyrazine-2,3-dicarboxylic acid).

This, in turn, depends on the pH of the initial solution, which is varied with the addition of piperazine, a base that also acts as a second ligand. These findings, key for the successful chemical design, have been made possible thanks to a detailed analysis of the crystal structures.

In order to further expand the dimensionality of the coordination polymers to form a 3D metal-organic framework (or MOF), Moghzi and co-workers replaced the *N*-heterocyclic polycarboxylic acid with a Zn-based metalloligand, with the aim of connecting the 2D layers in the third direction thus forming a 3D structure. The combination of the metalloligand with a praseodymium salt results in a bimetallic crystalline material which expands in the three dimensions, as was anticipated by the authors. In addition, this structure is thermally stable (up to 400°C), an important aspect for applications.

This MOF, upon exposure to iodine vapours, is capable of incorporating I₂ molecules, as revealed by the gradual colour change of the solid, which turns dark brown over time (see Fig. 1, top). In addition, gravimetric measurements reveal a mass increase over time, with a remarkable maximum amount of sorbed I₂ (490 mg g⁻¹) after 45 h. This value places this novel material among the best MOFs in terms of I₂ sorption.

The reverse process, *i.e.* the release of I₂, has also been investigated by this team. In particular, they used cyclohexane, a common organic solvent, to extract the incorporated I₂, and used spectroscopic techniques to follow the process. However, as iodine solutions are strongly coloured, the process can also be followed visually, as can be seen in Fig. 1 (bottom). Still, UV-vis spectroscopy was used to confirm that release is completed after 4 h.

Once the reversibility of the process was established, Moghzi and co-workers studied its cyclability, revealing that the capacity of this compound remains almost intact after four cycles of adsorption-desorption. Although clearly this is not a massive number of cycles, it is a first step that reveals the potential of the material for iodine sequestration.

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This article was originally published in *Acta Cryst.* (2020). **B76**, 722–723.

COMMENTARY

Looking at local classical and quantum forces in stable crystals using multipole-model refined electron densities and orbital-free DFT approximations

Carlo Gatti

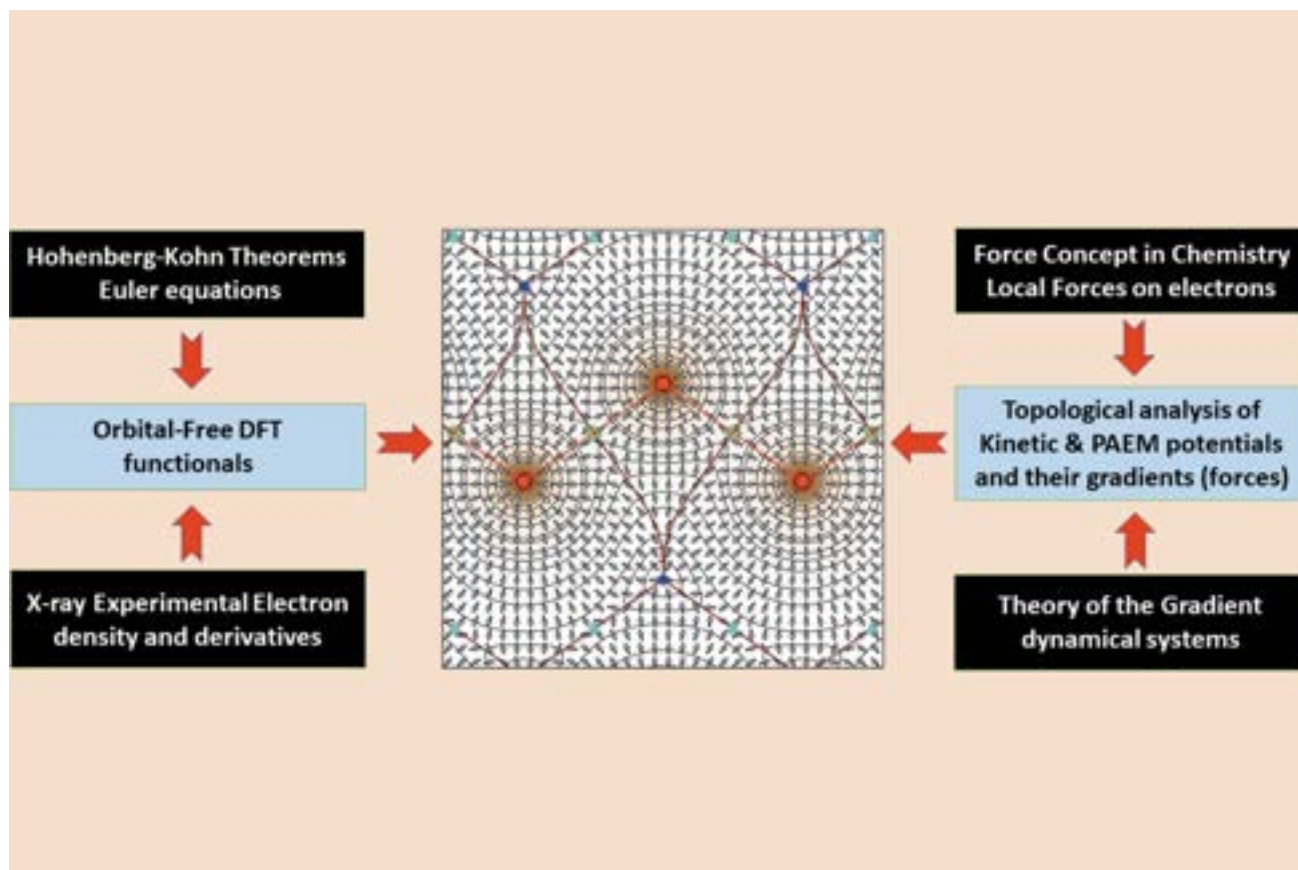


Figure 1. Ingredients and concepts from several disciplines are ingeniously combined in a *Quantum Crystallography* approach to analyse the balance of local forces acting on electrons in stable crystals (see text). Forces are derived as gradients of their associated classical and orbital-free DFT quantum mechanical potentials, evaluated using experimental electron densities and derivatives from high-resolution X-ray diffraction experiments. The central plot [taken from Fig. 5(b) in Tsirelson & Stash (2020)] shows the kinetic potential iso-contour lines and the kinetic force field arrows in the (110) plane of diamond. The space of the crystal turns out to be partitioned in atomic-like basins.

Two distinct approaches, that of *energy* and that of *force*, are adopted in quantum mechanics to gain insights on chemical processes. In the second, the net forces acting on the electrons and nuclei in a system (Ehrnefest and Hellmann–Feynman forces, respectively) are determined and a *local* version of the approach, in terms of force density fields rather than forces, has also been proposed for electrons. This is the path followed by Tsirelson & Stash (2020) in the October 2020 issue of *Acta Crystallographica Section B*, to study for the first time the spatial distribution of the electronic forces of different nature acting in stable crystals. Interestingly, by relying on approximations taken from orbital-free DFT, all components of the inner-crystal force can be easily retrieved from multipole-model refined experimental electron

densities and their derivatives. No less important is that these calculations are becoming easily doable for any X-ray density crystallographer thanks to a new version of the computer program *WinXPRO*, purposely developed in the study which is discussed in this commentary.

In their article, Tsirelson and Stash (T&S) describe a method to obtain local mapping of electrostatic and quantum forces acting on electrons in stable crystals. To achieve their goal, T&S have carefully selected and ingeniously combined several ingredients that might not be immediately familiar to crystallographers. The ingredients include the quantum mechanical *force approach* in chemistry (Deb, 1973), the theory of *gradient dynamical systems* (Abraham & Shaw, 1992), *Orbital-Free Density Functional Theory* (Wesolowski & Wang, 2013) and the old, but recently broadened and revitalized field of *Quantum Crystallography* (Genoni *et al.*, 2018). Each of these bits and pieces of science deserves a brief introduction on its own, before analysing the role they play when combined together (Fig. 1).

In quantum chemistry, rather than using the more customary energy approach, chemical bonding and molecular processes may be studied using the force approach, that is in terms of the forces of various nature acting on the electrons and nuclei in a system. In the *local force* form adopted by T&S, forces acting on electrons are expressed in terms of force density fields (Deb, 1979; Bader *et al.*, 2007; Pendás & Hernández-Trujillo, 2012; Pendás *et al.*, 2016). The latter may be obtained either from the expectation values of the gradients of the corresponding potential operators or, as performed by T&S, as gradients of the associated scalar potential fields.

These scalar potentials and their gradient fields (the forces) are analysed by T&S in terms of their topology via the theory of *gradient dynamical systems*, using its typical entities (attractors, repellers, basins, gradient paths, critical points) and concepts (homeomorphism). It is a tool common to most of the quantum chemical topology methods, such as Bader's quantum theory of atoms in molecules (QTAIM) (Bader, 1990). *Attractors and repellers* are those local potential maxima and local potential minima where gradient paths (the force lines) converge to and are expelled from, leading to a partitioning of chemical systems into space-exhaustive pieces, named *basins*. Each basin is traversed by all ascending gradient paths (GPs) terminating at its own 3D attractor (an alternative partitioning in terms of descending GPs and 3D repellers is equally possible). In most cases the basins bear a chemical meaning. Those with 3D attractors are often atom-like in shape and in QTAIM they are identified as the (quantum) atoms in molecules. The *critical points* (CPs) are those points where the gradient of the potential, the force, vanishes and are characterized by their signature. The signature is given by the dimensionality of the space traversed by the ascending GPs expelled from the CP minus that of the space traversed by the ascending GPs with terminus at the CP. Signatures -3 and $+3$ correspond to attractors (local maxima) and repellers (local minima), while the -1 and $+1$ signatures denote first- and second-order saddle points, respectively. If two potential fields exhibit the same number of CPs for each signature, the fields are said to be *homeomorphic* within the system where this occurs. Basins sharing a portion of their bounding surface imply a CP of -1 signature lying on their common surface and being the potential maximum for it. Two unique ascending GPs originate at this CP. One GP ends up at the attractor of one of the two basins sharing the surface and the other at the attractor of the remaining basin. The union of such paths is a line joining these two attractors and with maximum potential value relative to any lateral displacement from the line. In QTAIM it is known as a *bond path*, a line of maximum electron density ρ linking the nuclei of a pair of atoms. Accumulation of ρ along the bond path has been associated with chemical bonding (Bader, 1990). T&S discuss the analogy and dissimilarity of the topology of the scalar potential and force fields they analyse with that typical of QTAIM (where ρ is taken as the potential and its gradient $\nabla\rho$ as the corresponding force).

Orbital-free density functional theory (OF-DFT) and the methods derived thereof represent a fascinating challenge to go beyond the established routes in computational DFT. In principle, Hohenberg-Kohn theorems make it possible to describe the ground state of a multi-electron system exactly without

constructing the wavefunction explicitly. Yet, the extreme difficulty of building sufficiently accurate approximations to the electron density (ED) functional for the kinetic energy of a many-electron system progressively led to the adoption of orbital-based formal frameworks in practical DFT (the Kohn-Sham DFT method being the most well known solution). For this reason and despite its conceptual superiority, OF-DFT was put aside for a long time since in its early Thomas-Fermi implementation all stable molecules were found to dissociate. However, in the last two decades the situation has dramatically changed and research on OF-DFT has been revitalized. Since their computational cost grows quasi-linearly with the size of the system, OF-DFT methods have a great appeal for treating extremely large systems, while the conventional orbital-based methods have a well known highly non-linear dependence. This pleasing OF-DFT feature has fuelled studies on the exact properties of the kinetic energy density functional and the hunt for its increasingly better approximations, making current OF-DFT computations more reliable by far (Wesolowski & Wang, 2013). Besides, the kinetic energy and the electrostatic, exchange and correlation energy densities, all entering with their corresponding functionals in OF-DFT approaches, were demonstrated to be of great importance for interpreting key chemical concepts (Bader, 1990; Gatti, 2005; Wesolowski & Wang, 2013; Pendás *et al.*, 2016). Using information from experimental EDs and their derivatives, Tsirelson (2007) has in the past pioneered the study of bonding in crystals in terms of these various local OF-DFT energies. T&S now take a significant step forward. Rather than the energies, they study the OF-DFT potentials and forces derived thereof, using again experimentally derived densities and examining which forces behave as *heterotropic* and which as *homotropic* (Revici, 1961). The former tend to preserve homogenous disorganization of matter, while the latter favour its local organization, such as the electronic shell structure around the nuclei.

Next to 'orbital-free', the little known discipline of Quantum Crystallography (QCr) appears in the title of T&S's paper before the aim of 'viewing forces in crystals' is declared. QCr was originally defined as the operation of using 'crystallographic information to enhance quantum mechanical calculations and the information derived from them' (Massa *et al.*, 1995) or, conversely, of that of performing '*ad hoc* quantum mechanical calculations to enhance the accuracy of the crystallographic refinement' (Huang *et al.*, 1999). None of these definitions strictly applies to the work performed by T&S, yet it surely does to the more recent, highly broadened QCr view (Genoni *et al.*, 2018), embracing many other active and emerging research areas involving quantum mechanics and scattering experiments. Significantly, in 2017 the IUCr Commission on Charge and Spin Momentum Densities was renamed as the Commission on Quantum Crystallography. Orbital-free QCr can then be seen as a successful attempt of using precious information obtained from crystallographic methods for evaluating potential and forces in crystals within a fundamental quantum mechanical approach.

After illustrating their multi-faceted scientific framework, the results obtained by T&S deserve a brief overview and concluding comments. Within DFT, and considering a number of simplifications (Tsirelson & Stash, 2020), the local one-electron potential $\mu(\mathbf{r})$ is given by the sum of a kinetic potential, $\nu_{\text{kin}}(\mathbf{r})$, and of the so-called potential acting on an electron in a molecule (PAEM; Zhao & Yang, 2014), $\nu_{\text{PAEM}}(\mathbf{r})$. For a stable system, $\mu(\mathbf{r})$ vanishes everywhere. Thereby, the two electron potentials balance themselves everywhere, $\nu_{\text{kin}}(\mathbf{r}) = -\nu_{\text{PAEM}}(\mathbf{r})$, and so do their corresponding forces $-f_{\text{kin}}(\mathbf{r}) = f_{\text{PAEM}}(\mathbf{r})$ [with $\nabla \nu_{\text{kin}}(\mathbf{r}) = -f_{\text{kin}}(\mathbf{r})$ and $-\nabla \nu_{\text{PAEM}}(\mathbf{r}) = f_{\text{PAEM}}(\mathbf{r})$]. Since ν_{kin} and ν_{PAEM} differ in sign, they have the same total number of CPs and are placed at the same locations, but each with reversed signature. For instance, the local maxima of one potential become the local minima of the other and vice versa. The kinetic potential includes two terms, one due to the electron fluctuations arising from the uncertainty principle and the other to the corrections arising from the wavefunction antisymmetry requirements. Both terms are clearly quantum mechanical in nature. Instead, PAEM is composed of the electrostatic, the exchange and the correlation potential contributions, the first being purely classical.

T&S have analysed all ν_{kin} and ν_{PAEM} components for two simple stable crystals, diamond and rock salt, using ED and its derivatives obtained from high-resolution X-ray diffraction experiments, followed by an

accurate multipole-model refinement of the electronic structure factors. Many are the intriguing results of their study. First, it was found that the forces of kinetic origin draw electrons from the nuclei and are heterotropic in nature, whereas all f_{PAEM} components behave as homotropic. The latter push electrons to the nuclei that therefore act as sinks for the PAEM field, rather than as local maxima for v_{kin} . Hence, only the kinetic potential and its associated forces are able to create the electronic shells yielding atomic-like structures and basins resembling those of ρ in the QTAIM, yet with noticeable differences (Tsirelson & Stash, 2020). Both v_{kin} and v_{PAEM} fields concur to the accumulation of electronic charge along curved lines (GPs) joining nearest atoms. However, not all atomic pairs linked by bond paths in the ρ field (QTAIM) are found to be joined by the corresponding GPs in the v_{kin} field. This fact has an impact on the topologies of the two fields that are therefore not always homeomorphic. Even more importantly, it opens the question on the physical meaning of both the lack of a shared portion of bounding surface and of a common CP of signature -1 in some circumstances (in the present case, the $\text{Cl}\cdots\text{Cl}$ interaction in the NaCl crystal, which is missing in the v_{kin} field and, instead, present in the ρ field). Interestingly, the missing CPs in the v_{kin} field topologically correspond to the QTAIM bond critical points (bcps), whose meaning is and has been widely debated in the literature [see for instance, Pendás et al. (2007)]. Understanding the reasons behind the possible dissimilarities of the ρ and the v_{kin} fields could shed further light on the physical interpretation of bcps. It will certainly be a challenge for forthcoming contributions in the area pioneered by T&S in their paper.

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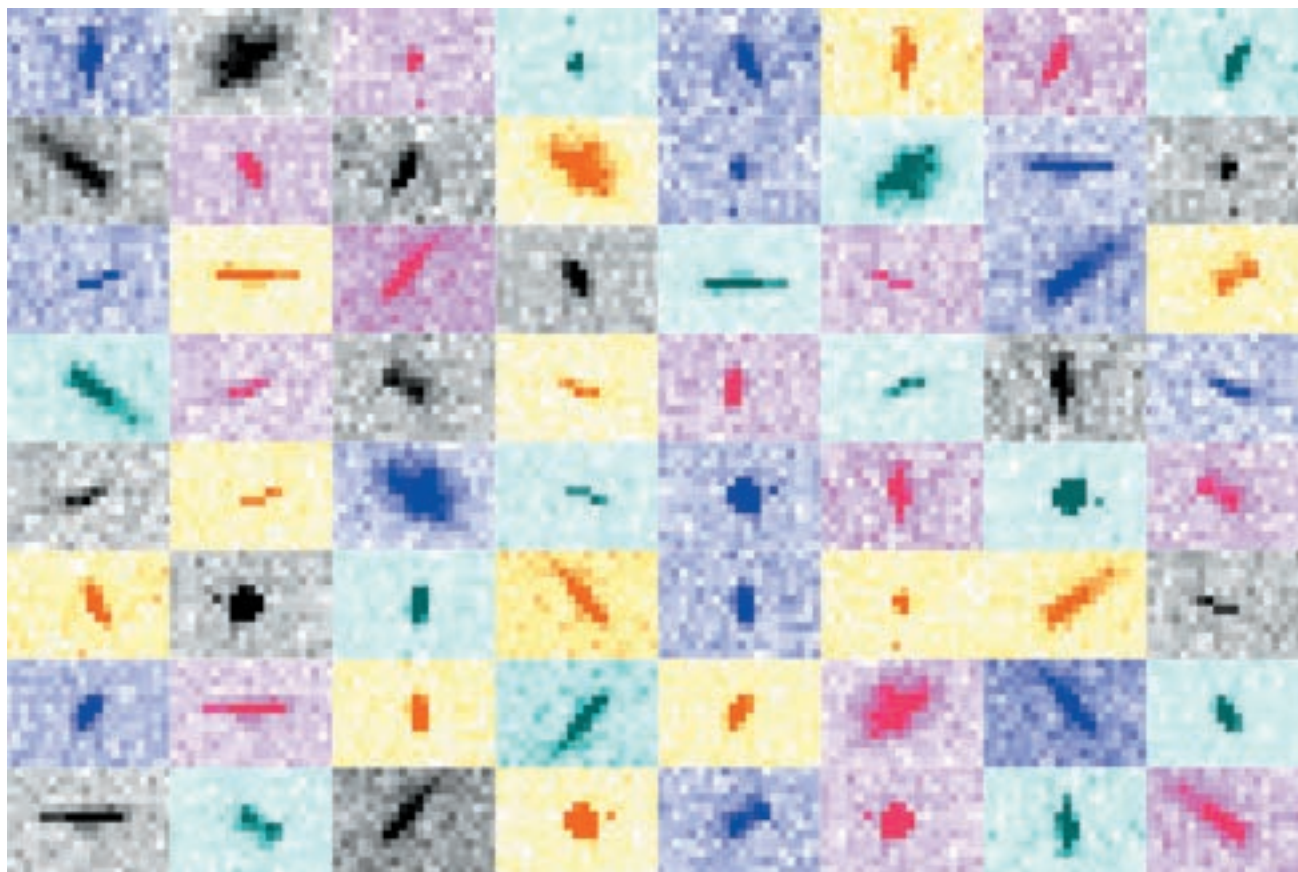
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This article was originally published in *Acta Cryst.* (2020). **B76**, 724–726.

COMMENTARY

Pixel modelling - a new age in SFX data analysis

Karol Nass



Collage of partial Bragg spots recorded during SFX experiments. For each pixel in the 'shoe-box' of a given Bragg spot, *diffBragg* applies precise modelling of the various parameters affecting their shape and intensity, such as lattice orientation, unit-cell dimensions, mosaic structure, incident photon spectra and partiality.

Over a decade ago, the first proof-of-principle serial femtosecond crystallography (SFX) experiment started the era of nano- and micro-crystallography at X-ray free-electron lasers (XFELs) (Chapman *et al.*, 2011). This opened up unmatched opportunities for structural studies of radiation-sensitive metalloproteins (Kern *et al.*, 2015; Suga *et al.*, 2020) and small, weakly diffracting protein crystals, including membrane proteins (Johansson *et al.*, 2017).

Moreover, the femtosecond XFEL pulse duration allows light to be shed on the structure-function relationship of photo-activated proteins by enabling sub-picosecond time-resolution in optical-pump X-ray-probe SFX studies (Orville, 2020). In this case, small crystal sizes are a necessity due to the limited penetration depth of the pump laser in protein crystals. By combining radiation-damage-free data collection from tiny crystals at room temperature with the sub-picosecond time-resolution in pump-probe experiments, SFX is a very powerful tool in the hands of structural biologists (Spence, 2017).

However, despite continuous efforts to improve state-of-the-art SFX, it still suffers from major technical

challenges that limit the throughput and accessibility of this relatively new technique, especially for non-expert user groups. These challenges occur in all aspects of the experiment, starting with radically different sample requirements (Beale *et al.*, 2019), non-standardized methods and complex sample delivery equipment (Grünbein & Nass Kovacs, 2019), and finishing with demanding and heavily supervised data collection and analysis that requires significant computational resources.

In particular, analysis of the SFX data is complex. A fundamental limitation is the femtosecond pulse duration precluding rotation of the crystal during exposure, which results in measuring diffraction patterns from still crystals in random orientations that contain only partially integrated reflections. Moreover, it suffers from many sources of errors due to the stochastic nature of XFEL pulses and morphological differences between measured crystals. The XFEL pulse instability affects shot-to-shot intensity, width and centre of the wavelength spread, and the exact shape of the energy spectrum. On the sample side, the distribution of crystal sizes and unit-cell dimensions and the degree of non-isomorphism and mosaicity contribute to the uncertainty in SFX data analysis. All this makes assembly of a complete set of full and accurate structure factor amplitudes for molecular replacement strategies challenging, and even more so for *de novo* structure determination (Nass *et al.*, 2016).

To date, the most popular SFX data processing programs use the so-called 'Monte Carlo' method, which is based on averaging partial intensity measurements of equivalent reflections from many different crystals. This method can decrease the contribution of random sources of errors described above to the structure factor amplitudes, but it is inefficient in terms of sample and beam time usage, as it requires tens of thousands of indexed diffraction patterns to converge (Kirian *et al.*, 2010). Extension of this 'Monte Carlo' method with post-refinement and partiality corrections recently provided tangible improvements to the determination of accurate anomalous structure factor differences required for *de novo* phasing by needing significantly fewer diffraction images than before (Nass *et al.*, 2020). However, these methods still rely on integration of the Bragg spot intensities located under globally defined, fixed-width areas predicted by incomplete diffraction models without taking into account the shape and size of the spots (Fig. 1).

In this issue of *IUCr*, Mendez and co-workers (Mendez *et al.*, 2020) present a new data analysis approach, *diffBragg*, that proposes to increase the accuracy of structure factor amplitudes attainable in SFX experiments. In contrast to the Monte Carlo approach defined in Kirian *et al.*, *diffBragg* employs an elaborated physical model and maximum-likelihood estimation to describe the intensity of all observed pixels in Bragg spots and in their vicinity across all images. As opposed to other SFX data processing programs, the model presented by the authors takes into account most of the important factors that determine the intricate shapes, sizes and intensity profiles of Bragg spots to improve accuracy of final structure factor amplitudes. The parameters optimized by the pixel modelling include crystal orientation, unit-cell parameters, intensity scale factor, mosaic parameters, incident photon spectra and a starting list of structure factor amplitudes provided by an initial round of conventional data processing.

This work paves the way for next-generation SFX data analysis by enabling us to decouple contributions of these various experimental sources of error from the measured Bragg spot intensities, which otherwise obscure structure factor amplitude determination. The authors achieve an order of magnitude reduction in the number of required diffraction images yielding the same accuracy of the anomalous structure factor amplitudes, compared with the conventional Monte Carlo approach. Although these improvements were demonstrated with simulated diffraction data of ytterbium-derivative lysozyme crystals, the method was shown to be robust against many experimental sources of error (e.g. background scattering, measurement noise, typical detector panel displacement). This indicates that *diffBragg* could have a remarkable impact on future SFX experiments by addressing one of the main bottlenecks of SFX – the need for high amounts of data, translating into large sample quantities. Furthermore, the high accuracy achieved with pixel-level refinement can provide a clear view on

extremely sensitive details such as two differently oxidized metal atoms (Sauter *et al.*, 2020). The ability to observe such level of detail expands opportunities for new and exciting experiments to consider for the upcoming years of SFX science.

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- This article was originally published in *IUCrJ*. (2020). **7**, 949–950.

COMMENTARY

Twinning and intertwined microcrystals in an intriguing, yet elusive, mineral

Sven Hovmöller

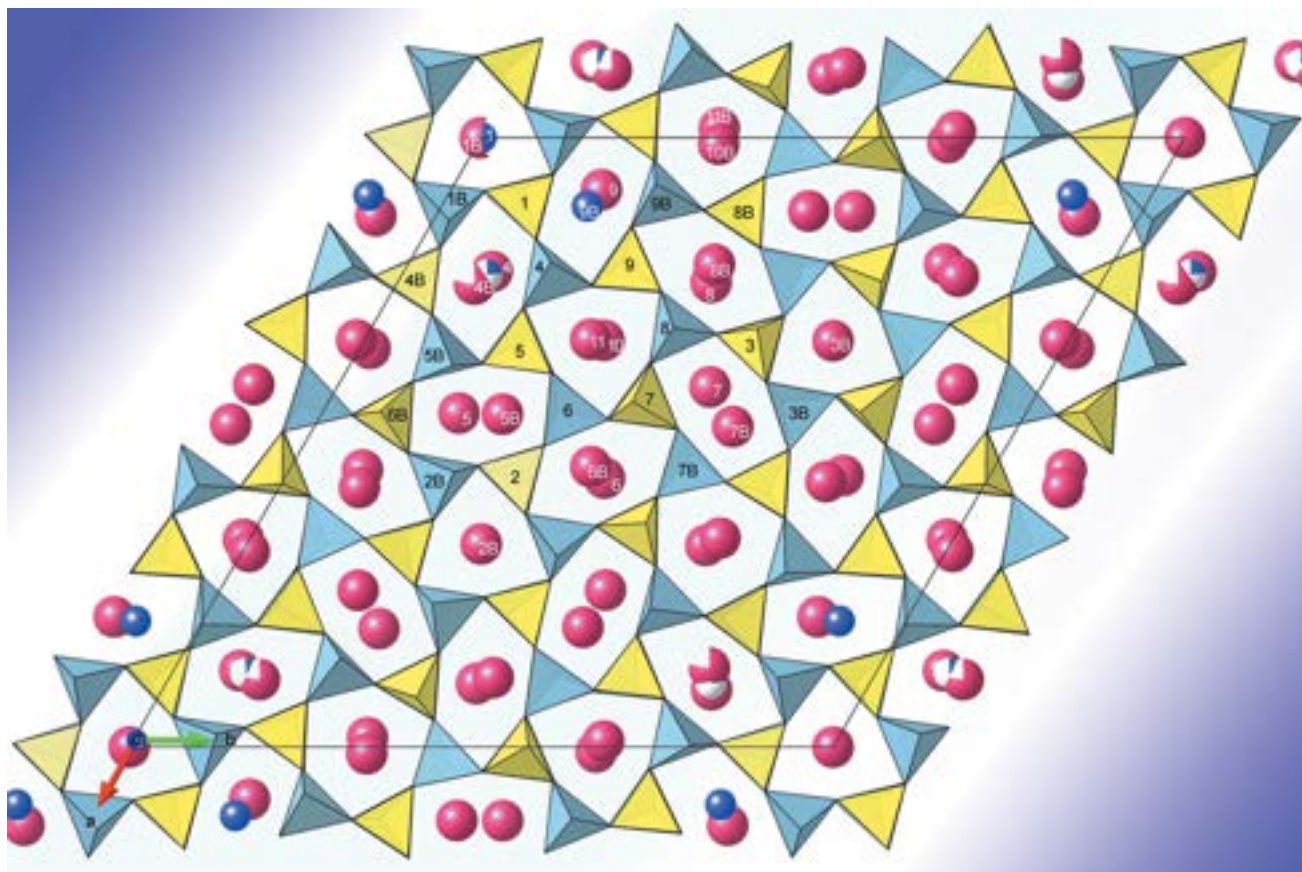


Figure 1. A unit cell of kaliophilite in space group $P3$, $a = 27.06$ and $c = 8.56$ Å. Si tetrahedra are shown in yellow, Al tetrahedra are shown in sky blue, Na cations are shown in blue and K cations are shown in red. Reproduced from Mugnaioli *et al.* (2020).

Like Sleeping Beauty in the traditional story, after one hundred years of sleep, a number of enigmatic crystal structures are now coming to life. In the November 2020 issue of **IUCr**, the group of Mauro Gemmi in Pisa report the structure of KAlSiO_4 (Mugnaioli *et al.*, 2020). This is at least the third complicated crystal structure that has been studied by X-ray crystallography for a century without being solved, waiting for electron crystallography to reach its present level of sophistication. Recently, Ute Kolb's group in Mainz solved the mineral vaterite (Mugnaioli *et al.*, 2012) and Ken Inge *et al.* in Xiaodong Zou's group in Stockholm solved the structure of the much used medicine bismuth subgallate (Wang *et al.*, 2017). The reason for this surge in solving hard problems in crystallography is the rapid development of instrumentation, software and theory in electron crystallography.

X-ray crystallography is a (set of) scientific methods for determining atomic structure. Its results are so profound and spectacular, from the first ever correct description of a chemical structure [NaCl by Bragg (1913)], that one feels obliged to apologize when saying there are cases where other techniques are better. Yet, it is clear from the name of the method, X-ray 'crystallography', that it should be applied to crystals. Although very many things around us are crystalline, from NaCl to almost any mineral and including many organic and biological compounds if pure and concentrated, not everything is crystalline.

This holds not only for gases and liquids, but also for very many solid objects, from some minerals to biological tissues. Notably, (to the general public) the most spectacular and well known result of X-ray crystallography, the double helix structure of DNA, is strictly speaking not a crystal but a rather well ordered fibre.

Having thus duly given our apologies, we may consider where we may apply variations of the tremendously powerful technique of X-ray crystallography, in order to depict atomic structures in cases where X-ray crystallography fails or at least is limited. The first consideration may be how to overcome the need for (large) crystals. Even a grain of sand, invisible to the human eye, is almost 10 micrometres in each direction and contains some 10^{12} – 10^{15} atoms. Many minerals are made up of crystals much smaller than this. They may form twins of random or preferred relative orientations or even sets of related but nonidentical structures. Luckily there is an alternative to X-rays for such cases; the use of electrons rather than X-rays.

Electrons react orders of magnitude more strongly with matter than do X-rays, and this opens the door to studying smaller crystals and even non-crystalline objects. Unfortunately, this extremely strong interaction has a major drawback: if the sample is more than a few nanometres thick, the electrons may scatter more than once on their way through the sample. This multiple scattering soon leads to uninterpretable results. For many decades, it was believed that this would make electron crystallography unsuitable for solving atomic structures in a fashion similar to X-ray crystallography. Only a very small number of groups pursued structure determination by electron diffraction and electron microscopy. However, in the last decade, their endurance has proven to be fruitful. A spectacular development in instrumentation, with super-fast and accurate electronic cameras and computer-controlled electron microscopes, has made it possible to collect complete 3D electron diffraction data on a crystal a million times smaller than needed for X-ray crystallography, in but a few seconds. It has turned out that the most severe problem for electron crystallography was not the multiple scattering, but the lack of complete 3D data.

The group led by Mauro Gemmi in Pisa is one of those who can take full advantage of the spectacularly improved situation. In collaboration with excellent mineralogists, they are now in a position where many enigmatic mineral structures can be accurately determined by electron crystallography.

Twinning and superstructures, with not only two but numerous intergrowing crystals, are notoriously hard to solve. In a very informative electron micrograph [see Fig. 2 of Mugnaioli *et al.* (2012)], a typical part of the mineral is shown. Although the sample is less than 100×200 nm, it is obvious that there are several small crystals intergrowing. Even without any knowledge of crystallography, it should be clear that it must be very difficult to solve the atomic structure of such a compound. And, for those who know the pros and cons of single-crystal and powder X-ray crystallography, it should be evident why this mineral has resisted being solved, in spite of numerous attempts, some even before W. H. Bragg and W. L. Bragg invented X-ray crystallography in 1912. The crystal size is smaller than what is needed even for X-ray powder diffraction.

Now, Gemmi and his group and several experienced mineralogists have at last solved the atomic structure of this mineral. It was possible by electron crystallography. For one thing, the very much stronger interaction of electrons than of X-rays with matter, makes it possible to use extremely small samples for the crystallographic analysis. It is clear from the above-mentioned figure that although the mineral is heavily twinned, small areas of some tens of unit cells (*i.e.* a few tens of nanometres) may in fact be single crystals.

Electron crystallography has the unique advantage over X-ray and neutron crystallography that both real and reciprocal space can be directly observed. Electron microscopy data, unlike any type of diffraction data, contain the crystallographic structure factor phases.

In addition to the contribution to the development of electron crystallography, the mineral kaliophilite (KAlSiO_4), studied here, is of general interest for several reasons. It is one of a dozen minerals found with almost the same stoichiometric composition, but with varying degrees of superstructure. The present is by far the most complicated of them all, with a unit cell 27 times larger than the smallest in the series. These compounds are built up by chains and rings of AlO_4 and SiO_4 tetrahedra. The rings are mainly 6-rings, but they can vary in how the tetrahedra point upwards (U) or downwards (D). A most intriguing pattern in kaliophilite, making up just one unit cell, is shown in Fig. 1. The present mineral is the first with three different such schemes. Is this a portal into a world of infinite variations of these (and other!) minerals? It will certainly prompt X-ray crystallographers to keep an eye on what goes on in electron crystallography. In a wide number of fields, also outside mineralogy and pharmaceuticals, the many structures being quickly and accurately solved by electron crystallography should encourage the use of not only single-crystal and powder X-ray and neutron diffraction, but also electron diffraction.

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This article was originally published in *IUCrj* (2020). **7**, 951–952.

COMMENTARY

Structural details of the enzymatic catalysis of carbonic anhydrase II via a mutation of valine to isoleucine

Daumantas Matulis

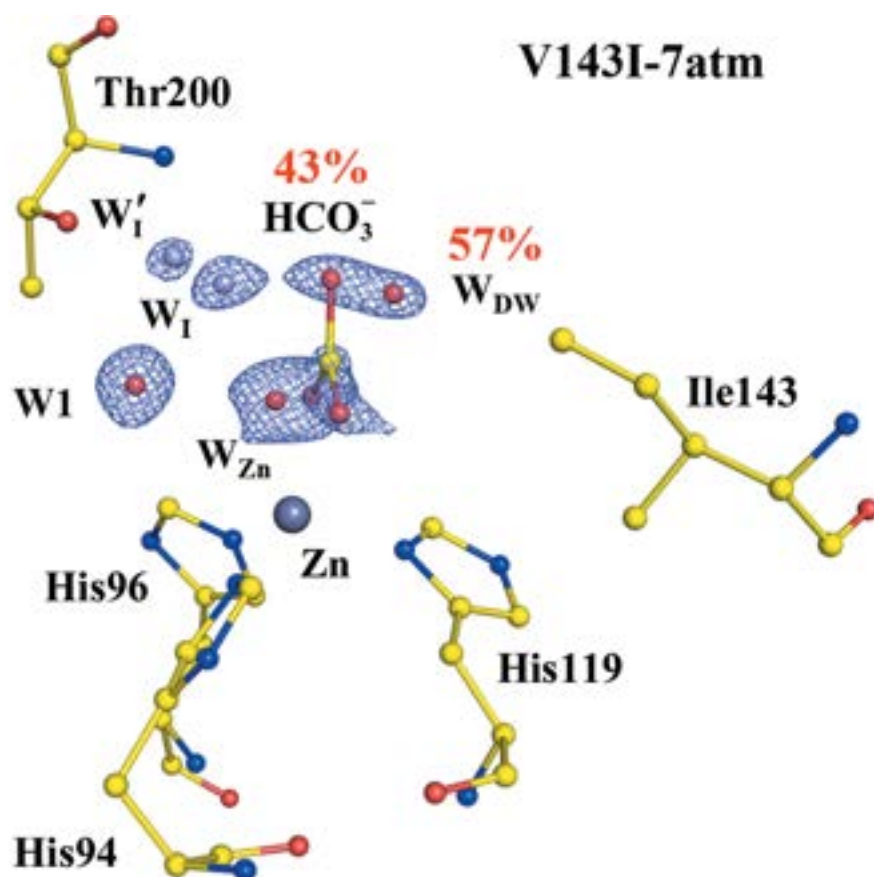


Figure 1. $\text{CO}_2/\text{HCO}_3^-$ -binding site of V143I CA II. The intermediate waters (W_1 and W_1') are coloured steel blue for clarity. The electron density ($2F_o - F_c$) is contoured at 1.5σ . Partial occupancies of HCO_3^- and W_{DW} are shown. Reproduced from Kim *et al.* (2020).

Carbonic anhydrase (CA) is one of the most studied proteins in molecular life sciences. It has been especially widely used as a biophysical model for protein stability and binding studies (Krishnamurthy *et al.*, 2008). CA is also one of the fastest known enzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate anion and acid protons. Humans contain 15 highly homologous CA isoforms. Three of them do not perform the catalytic function of CA owing to the absence of Zn(II) in the active center as at least one of the three His residues holding the metal cation has changed to another amino acid. The remaining 12 isoforms have variable expression in tissues: some of them are localized intracellularly in the cytosol or mitochondria, whereas others are located outside of and attached to the cell membrane via a lipid anchor or a transmembrane helix. Several of these isoforms have been implicated and are over-expressed in numerous cancers. Thus research in the CA field is quite active and has a significant impact on all sectors of protein science (Dodgson *et al.*, 1991; Chegwiddden *et al.*, 2000; Frost & McKenna, 2014; Supuran & Simone, 2015; Matulis, 2019).

Since the discovery of sulfanilamide and acetazolamide in the 1940s, it has been established that primary sulfonamides are highly specific binders and inhibitors of CAs. Numerous sulfonamides bearing thiazide, furosemide and other moieties have become clinical drugs used to treat hypertension and edema, as well as being used as diuretics. Dorzolamide and brinzolamide have been used as antiglaucoma agents. However, there is also a discussion on the applicability of some recently designed CA inhibitors (Jonsson & Liljas, 2020).

Since the first X-ray crystal structure of a CA (Liljas *et al.*, 1972), the atomic structures of ten human CA isoforms have been determined. The missing structures are isoforms expressed in mitochondria, CA VA and CA VB. However, isoform CA II is by far the most abundant in the Protein Data Bank (Linkuvienė *et al.*, 2018). Most efforts to crystallize CA isoforms have been in the field of drug design for understanding how low-molecular-weight compounds recognize and distinguish CA isoforms. A good drug is supposed to selectively inhibit only one CA isoform, the target of a disease.

Despite these numerous studies, a truly detailed atomic level understanding of CA enzymatic activity, interaction of the enzyme with its substrate CO_2 and especially the behavior of water molecules are not fully understood. The situation is further complicated by the substrate being gaseous, making the methodology more complex.

Writing in the November 2020 issue of **IUCrJ**, Kim *et al.* (2020) advance our understanding of the catalytic mechanism of CA II by producing a mutation and comparing it with the native protein. The mutation (V143I) has previously been introduced (Fierke *et al.*, 1991; West *et al.*, 2012). The change of valine to isoleucine is one of the smallest possible perturbations of a protein structure (where one hydrophobic amino acid is changed to another hydrophobic amino acid that differs by a single CH_2 group) that can be introduced into a protein with the hope that such a change would cause almost no deviation in the catalytic properties of the enzyme.

The mutation actually changed the catalytic properties of the enzyme quite substantially. The k_{cat} value was not significantly affected, but the affinity of the substrate CO_2 for CA II decreased tenfold, from 10 mM K_{m} for CA II to 100 mM K_{m} in the mutant. The study by Kim *et al.* (2020) demonstrates the structural arrangement of the CO_2 in the active site and shows its diminished mobility in the mutant as a result of the decrease of available space in the substrate binding pocket.

As in a previous study where pressurized CO_2 was applied (Kim *et al.*, 2016), this study determined the crystal structures not only at ambient pressure in air, but also at 7, 13 and 15 atm pressure of CO_2 gas (the 7 atm binding site is illustrated in Fig. 1). The concentration of CO_2 in the experiment increased from approximately 300 ppm in ambient air to more than 10 atm in the pressurized setting. Thus, the increase was approximately 30-thousand-fold, enabling visualization of the CO_2 molecule bound in the active site of the protein. The mutation caused a shift of its position and partial conversion to an HCO_3^- bicarbonate anion.

Furthermore, the study demonstrated that the proton transfer pathway around His64 has been largely unaffected, but there were subtle changes in the structure of the entrance conduit (EC) water molecules. It would be very interesting to see the positions of each proton in the conduit, but this would require neutron crystallography (Fisher *et al.*, 2012). Still, this remarkable study shows the effect of a mutation on the structure of the catalytic mechanism of CA II in the greatest detail so far and will be used in the design of compounds for pharmaceutical purposes.

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This article was originally published in *IUCrJ* (2020). **7**, 953–954.

COMMENTARY

Screening cells for crystals: a synergistic approach

Terese BergforsSoneya Majumdar

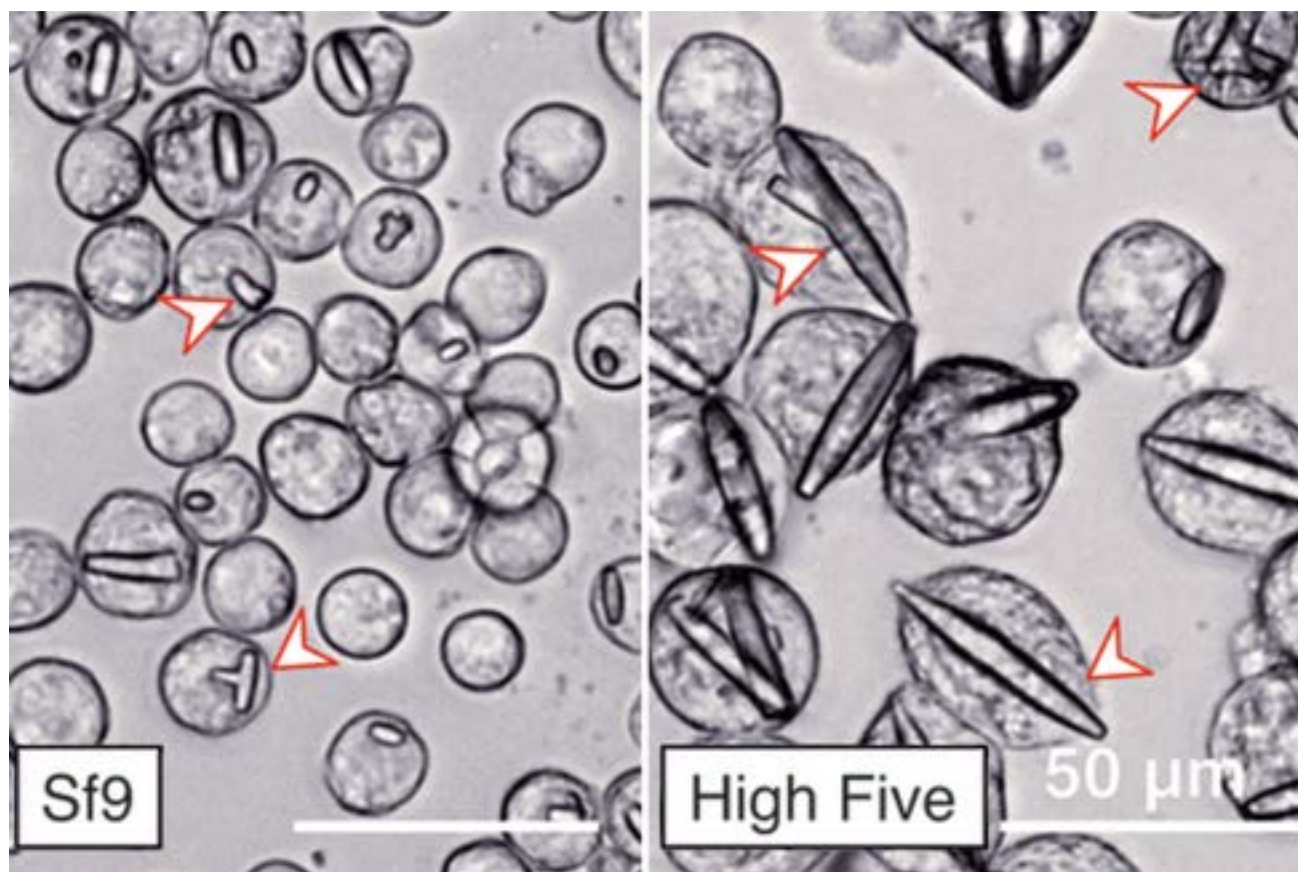


Figure 1. Differential interference contrast light microscopy of intracellular *Neurospora crassa* HEX-1 crystals grown in (a) Sf9 and (b) High Five insect cells. Red arrowheads highlight selected intracellular crystals. Images courtesy of Lahey-Rudolph *et al.* (2020).

In cellulo crystallization – the formation of crystals inside living cells – is an intriguing phenomenon. How does nature manage to produce *in vivo* crystals while crystallization in the laboratory is so difficult? An understanding of nature's own crystallogenesis would expand our possibilities to use this process. However, one difficulty hampering the study of *in vivo* crystals is detecting them inside their host cells. Spatial constraints limit the crystal size and the chaotic background of the host cell gives a high signal-to-noise ratio. In the October 2020 issue of *Journal of Applied Crystallography*, Lahey-Rudolph *et al.* (2020) tackled these problems by combining small-angle X-ray scattering (SAXS) with X-ray powder diffraction (XRPD). Their work has resulted in a rapid and sensitive method to screen for crystals *in cellulo*.

In cellulo crystallization occurs in all kingdoms of life. Plants, viruses, insects, yeast, fungi and mammals use it as a way to store proteins, to protect them from proteolysis and to make solid-state catalysts (Schönherr *et al.*, 2018). *In vivo* crystals can also result from non-native conditions, for example during heterologous overexpression or some pathologies. XRPD was used early on to characterize the unit cells of *in vivo* crystals, for example from *Bacillus thuringiensis* (Li *et al.*, 1988). But it is thanks to increased availability of free-electron lasers and fourth-generation synchrotrons that *in vivo* and other nano- and

microcrystals can now be used for diffraction studies with serial femtosecond X-ray crystallography or microfocus beams.

In vivo crystals can be recovered for diffraction by lysing the host cells, but this often affects the crystal quality (Gallat *et al.*, 2014). It would be better to diffract the crystals while still *in cellulo*, and in a proof-of-concept experiment, Axford *et al.* (2014) did this with cypovirus polyhedrin crystals. The resulting structure showed the potential of *in cellulo* crystallization and diffraction.

However, to implement *in cellulo* data collection into a structure pipeline, a method needs to be in place to screen the cells for possible crystals. The screen should require minimum sample preparation and be rapid enough that the cells remain viable. It must be sensitive enough to detect the crystals, even when few in number. It should give direct proof of crystallinity. Available microscopies – bright field, transmission electron and second-harmonic generation – are unsatisfactory in one way or another in meeting all these requirements. Neither are UV fluorescence and micro-electron diffraction completely adequate for detection purposes, owing to the background from high cellular protein concentration in the former and the need for ultra-thin samples in the latter.

With these challenges in mind, Lahey-Rudolph *et al.* overexpressed four test proteins known to crystallize *in cellulo* (Fig. 1). The crystal-bearing host cells were placed into quartz X-ray capillaries for small-angle X-ray scattering (SAXS), a method designed to work with dilute solutions of macromolecules. The X-ray scattering curves showed distinct peaks (in comparison to the controls), indicating crystalline elements within the scattering volume. The sensitivity was established by recording the scattering curves from a dilution series of the crystal-containing cells. Peak intensities systematically decreased, but the overall trend of the scattering curve remained unaltered. Eight data sets were collected in under 30 min, during which time the cells remained viable.

SAXS can detect weak scattering signals even from noisy backgrounds, like those from living cells. However, it does not allow extraction of unit-cell parameters. To overcome this problem, the authors converted the SAXS data from $I(s)$ to $I(2\theta)$ for analysis by XRPD software packages. SAXS data indicate that the Bragg peaks are due to crystallinity, and XRPD analysis allows refinement of unit-cell parameters. Every crystal form has a characteristic diffraction pattern; hence, XRPD is extremely sensitive to crystal polymorphism. By combining the SAXS and XRPD data, Lahey-Rudolph *et al.* harnessed the strengths of both methods for successful identification of crystals *in cellulo*. However, only a few, low-resolution Bragg reflections could be identified, preventing *ab initio* indexing. Initial cell constants and space-group symmetry were, therefore, estimated from reported structures in the Protein Data Bank and then refined from the XRPD data sets (Pawley, 1981).

It would be beneficial to solve the indexing problem. However, even without knowing the cell constants, the method can still be used to study environmental parameters that affect formation of *in cellulo* crystals. This opens the possibility to repurpose overexpression systems for production of crystals rather than proteins. Another positive aspect of *in cellulo* crystallization is that the proteins are in their physiological environment – the living cell. Here they are presumably surrounded by their genuine cofactors and protein partners. Post-translational modifications are also enabled, as seen in the structure solved with *in vivo* crystals of cathepsin B (Redecke *et al.*, 2013). Furthermore, *in cellulo* data collections are done at room temperature, eliminating structural artifacts induced by cryotemperatures and reducing mosaicity.

The *in cellulo* screening method is a welcome addition to the structural biology toolbox. Ultimately, a better understanding of crystal biogenesis might allow us to transform its occurrence as an incidental or accidental event into an intentional one. *In vivo* crystals may be more common than we realize – under both native and non-native conditions – because we do not screen for them. Without reliable detection methods, they could be hiding in plain sight.

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This article was originally published in *J. Appl. Cryst.* (2020). **53**, 1414–1415.

COMMISSION NEWS

Growing pressure for progress: activities of the IUCr Commission on High Pressure

Kamil Dziubek



Incumbent CHP members and consultants, and the *local workshop chairs* at the 2018 CHP Workshop in Honolulu. From left to right: *Przemysław Dera* (2018 CHP Workshop chair), Kamil Dziubek, *Ronald Miletich* (2019 CHP Workshop Chair), Andrzej Katrusiak, Amy Lazicki, Guoyin Shen and Haozhe Liu.

Since the 17th IUCr Congress in Seattle in 1996, the IUCr Commission on High Pressure (CHP) has been working on the promotion of extreme conditions crystallography – a vibrant multidisciplinary field with relevance to different disciplines including Earth and planetary science, chemistry, physics, material research, nanotechnology and biology. Whereas it is not possible to present here a detailed account of the Commission's activity, it is worth noting some major initiatives that have taken place.

The CHP has been organizing annual workshops each year since its inception, except for the years of IUCr Congresses. In 2015 and 2016 the workshops were held at the sites of synchrotron light sources: LLNS in Campinas, Brazil (local organizer **Narcizo Souza-Neto**) and PAL in Pohang, South Korea (local organizer Yongjae Lee), respectively. Remarkably, the meeting in Brazil was most probably the first international high-pressure crystallographic event in the Southern Hemisphere. The last two workshops took place in venues known for establishing top high-pressure labs. In 2018, it was hosted in University of Hawai'i at Mānoa (USA) by **Przemysław Dera** and accompanied by a full day of high-pressure single-crystal data analysis training. In 2019 the workshop was organized in Vienna, Austria, by Ronald Miletich and for the first time it was held jointly under the auspices of both CHP and European Crystallographic

Association SIG-11 (Crystallography under Extreme Conditions) as a satellite event to the 32nd European Crystallographic Meeting.

Furthermore, the CHP members have been active in the organization of other meetings, schools and workshops. One of the most prestigious and important of these is the International School of Crystallography held on an annual basis in Erice, Italy. So far, three courses were devoted to high-pressure studies: in 2003 (directors **Andrzej Katrusiak** and Paul McMillan), 2009 (directors **Elena Boldyreva** and **Przemysław Dera**) and 2016 (directors **Francesca Fabbiani**, **John Parise** and Malcolm Guthrie). The next high-pressure Erice school is planned for 2022, with **Kamil Dziubek**, Shanti Deemyad and Helen Maynard-Casely serving as the directors. Another remarkable event was the 26th International Conference on High Pressure Science and Technology (AIRAPT) co-organized in 2017 in Beijing, China, by the incumbent CHP Chair, **Haozhe Liu**. And this summer saw the IUCr/DKG High-Pressure School take place in Bayreuth, Germany - see article in this issue [here](#).

The promotion of high-pressure crystallography was also achieved through publication of a [special issue](#) of *Acta Cryst. B* on Non-ambient Crystallography in 2014 (Guest Editors: David Billing and **Andrzej Katrusiak**). Recently, the informative CHP website has been completely redesigned and launched on the IUCr servers with the invaluable support of the IUCr Research and Development Officer, Brian McMahon. The [web resource](#) provides the CHP terms of reference, public documents including annual reports, information on past events and the calendar of forthcoming meetings. Moreover, a public mailing list has been set up for discussing ideas on high-pressure research and as a platform to announce forthcoming meetings or events and disseminate information about open job positions and involvement opportunities. It is fitting here to express sincere appreciation and gratitude to two former CHP members: Ingo Loa who served for many years as the previous CHP home page webmaster and John Loveday who maintained the old mailing list. Another ongoing task of CHP is proposing high-pressure data and metadata descriptors and guidelines for metadata depositions, in line with the IUCr Committee on Data recommendations.

As a final note, it is with great pleasure to announce that the next CHP workshop, the IUCr High-Pressure Workshop 2021, will be held from 1 to 6 February 2021 in Novosibirsk, Russia, and organized by **Elena Boldyreva**, one of the world's leading high-pressure researchers, former CHP member and former IUCr Executive Committee member. Given the COVID-19 pandemic situation, the workshop will be held online using Zoom software. Its purpose is to bring together scientists working in the field of high-pressure crystallography to discuss the latest achievements in fundamental research, techniques and practical applications. Special attention is being given to educational aspects: a number of tutorials are planned, as well as a special session for young scientists. Participation is free for all registered participants. The deadline for submissions is 15 January for posters, 31 January for participation without a contribution, and 31 December for applications for oral contributions, the number of which is limited. More detailed information about the event is available on the [workshop website](#).

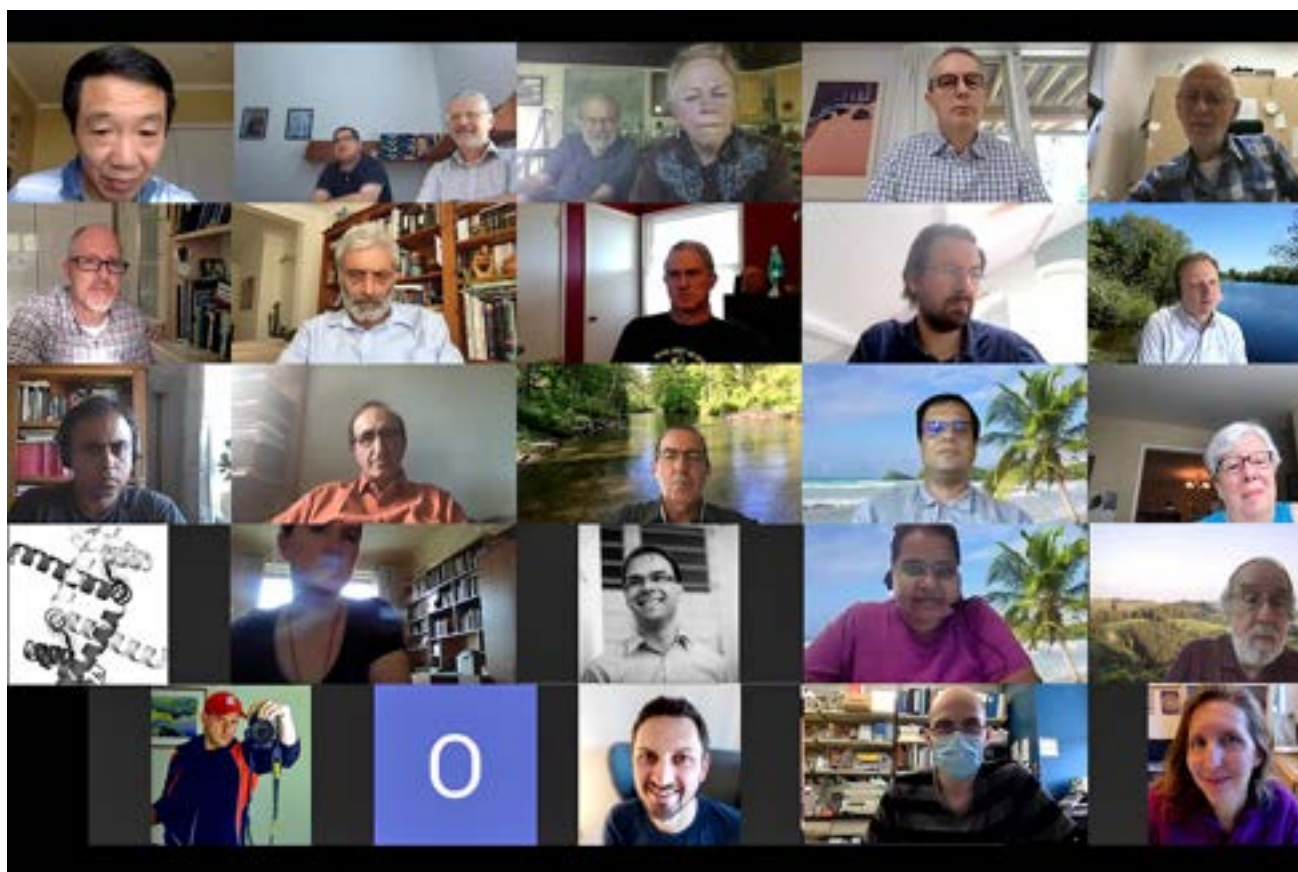
Note: the names of former and current CHP members participating in numerous activities are highlighted in boldface.

Kamil Filip Dziubek is Secretary of the IUCr Commission on High Pressure.

SPECIAL REPORT

Nucleic Acid Valence Geometry Working Group

Bohdan Schneiderlan Bruno, Stephen K. Burley, David A. Case, Jiří Černý, Rhiju Das, Martin Egli, Paul Emsley, Zukang Feng, Mariusz Jaskolski, Robbie P. Joosten, Catherine L. Lawson, Nigel W. Moriarty, Abhik Mukhopadhyay, Garib Murshudov, Gary Parkinson, Jane S. Richardson, Oleg V. Sobolev, Brinda Vallat, Sameer Velankar, Evan Worden, Ivan Nikolay ZheludevHelen M. Berman



Participants in the virtual workshop to establish the Nucleic Acid Valence Geometry Working Group.

This communication reports on an initiative to establish the Nucleic Acid Valence Geometry Working Group, whose task will be to define and implement a uniform dictionary for nucleic acid valence geometry parameters for use in modeling, refinement and validation systems.

Biomolecular structure refinement depends both on experimental data and on chemical restraints that encode prior knowledge regarding acceptable geometries. Chemical restraints used for nucleic acid (NA) valence geometries (bond lengths and angles) in the major refinement and validation software packages are divergent, especially in the standard deviations used and in whether they are dependent on sugar pucker, RNA versus DNA, etc. This impacts many aspects of NA-containing structure studies including model building, refinement and validation. We propose to review those dictionaries and protocols, first seeking agreement on a common compromise.

This effort is an initiative of the ELIXIR Community on Structural Bioinformatics, 3D-Bioinfo (Orengo *et al.*, 2020) "to encourage development and use of software tools to describe, analyze, annotate, and predict nucleic acid (NA) structures. In particular, standards are needed for initial model building and refinement

of nucleic acid molecular structures.” This task has become urgent as techniques such as cryo-EM (Afonine *et al.*, 2018; Lawson *et al.*, 2020) and high-resolution neutron diffraction (Liebschner *et al.*, 2019) are generating experimental data on 3D structures containing RNA and DNA molecules.

To initiate the process, we held a virtual meeting on 24–25 August 2020. More than 20 subject-matter experts in the field discussed the status of standards for valence geometry in nucleic acids. We heard 15 talks on the topic from various perspectives. We learned that the most commonly used software tools for refinement and validation use different sets of values for the valence geometries. Of particular concern is the fact that the refinement programs *REFMAC* (Murshudov *et al.*, 2011) and *Phenix* (Liebschner *et al.*, 2019), which includes X-ray, neutron and cryo-EM, use somewhat different values. The wwPDB (Berman *et al.*, 2003) validation pipeline (Gore *et al.*, 2017) uses the values published on the current NDB site (Berman *et al.*, 1992) while *Phenix* uses the Molprobity (Williams *et al.*, 2018) values. Lack of consistency between refinement and validation dictionaries leads to validation reports that may identify unforeseen or even spurious outliers.

Valence geometry standards have traditionally been determined separately for the nitrogenous bases and the sugar-phosphate backbone. The first set of values was determined by the Kennard/CSD group in 1982 (Taylor & Kennard, 1982a) (Taylor & Kennard, 1982b). Using a larger number of crystal structures, somewhat more detailed parameters were determined for the bases (Clowney *et al.*, 1996) and the sugar backbone (Gelbin *et al.*, 1996; Parkinson *et al.*, 1996). Current refinement and validation programs use some combination of those 1996 values. Recently an updated set of CSD-based values was developed again separately for the bases (Gilski *et al.*, 2019), phosphates (Kowiel *et al.*, 2016) and sugars (Kowiel *et al.*, 2020). The latter two analyses show the correlation between the valence geometry and conformation. These values have been tested for a limited number of refinements. The complicated geometry of the phosphodiester linkage was also investigated as a function of charge (Schneider *et al.*, 1996).

Extensive discussion at the workshop resulted in two conclusions. One is that the valence geometry standards cannot be isolated from the other geometric and conformational descriptors of nucleic acids, including sugar puckers, phosphate conformations, base pair geometry and hydrogen bonding. These all need updates of standards using state-of-the-art statistical tools and well-curated sets of experimental data before adoption, especially by the rightly conservative wwPDB validation. Ways to find correlated geometry values for larger building blocks such as the dinucleotide unit were suggested and need to be further discussed. A second conclusion is that in the short term, while that research is being done and evaluated by the community, it is very important for all stakeholders to agree on a uniform, optimal compromise for the simpler restraint values currently being used.

Outcomes and recommendations of this Workshop are the following:

- To establish the Nucleic Acid Valence Geometry Working Group, whose task will be to make these changes happen.
- The short-term goal of the Working Group will be to define, obtain agreement on, and implement a conservative and uniform compromise on simple parameters for current use in all the major modeling, refinement and validation systems.
- More complex definitions that take into account local identities and conformations should be compared, tested and tried out by the community, to reach a more accurate, next-generation set of valence parameters.
- Further research and testing should eventually be expanded to conformationally (primarily dihedral-angle) dependent restraints.

Acknowledgements

This project has been supported by the implementation plan of the ELIXIR Community on Structural Bioinformatics, 3D-Bioinfo.

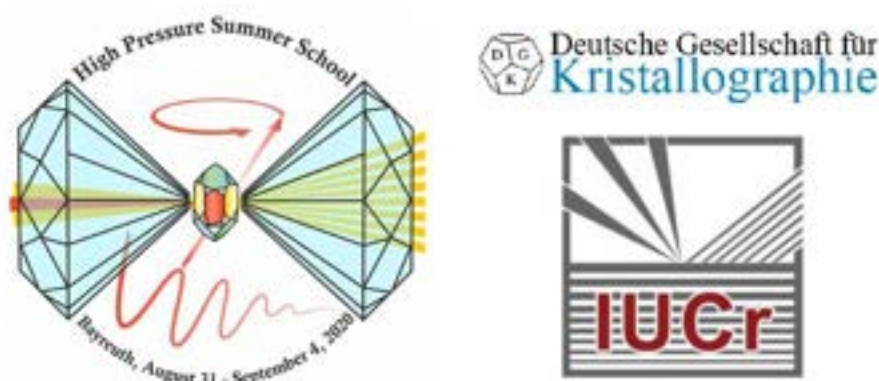
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MEETING REPORT (IUCR SUPPORTED)

The IUCr/DGK International High-Pressure Summer School 2020, Bayreuth, Germany

Natalia Dubrovinskaia, Dominique Laniel, Thomas Meier, Leonid Dubrovinsky



Introduction to Novel Methods of Atomic and Electronic Structure Studies at High Pressures

Natalia Dubrovinskaia, Dominique Laniel, Thomas Meier and Leonid Dubrovinsky

The International Summer School on Novel Methods of Atomic and Electronic Structure Studies at High Pressures was held in Bayreuth, Germany, from 31 August to 4 September 2020 (<https://www.dubrovinskaia.uni-bayreuth.de/en/High-Pressure-Summer-School-2020/index.html>). It was approved by the IUCr (through the IUCr Commission on High Pressure, CHP) and by the German Society for Crystallography (DGK). We expected to host our students from all over the world at the University of Bayreuth. However, the COVID-19 pandemic prevented the participants from gathering in the heart of Bavaria, but they enthusiastically supported the idea put forward by the organizers to attend via Zoom. The School was a great success, and we truly believe that it will become an annual event.

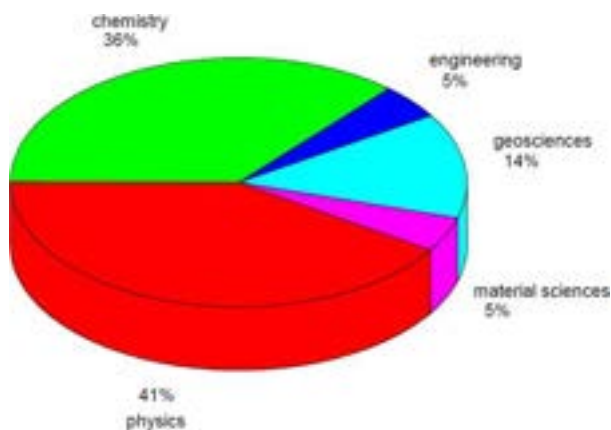


Bayreuth Team of lecturers and students (from the left to the right and from the last row to the front row): Timofey Fedotenko, Saiana Khandarkhaeva, Dominique Laniel, Thomas Meier, Kirill Vlasov, Sergey Ovsyannikov, Alena Krupp, Andrey Aslandukov, Alena Aslandukova, Natalia Dubrovinskaia, and Leonid Dubrovinsky (Achim Schaller is not in the photo).

The idea of the School emerged from a wish to share the experience collected within the Dubrovinsky-Dubrovinskaia group (<https://www.dubrovinskaia.uni-bayreuth.de>), whose research during the last decade has been mainly focused on the development of high-pressure single-crystal X-ray diffraction (sc-XRD) and nuclear magnetic resonance (NMR) in a diamond anvil cell (DAC). Our efforts enabled sc-XRD and NMR experiments at simultaneous high pressures of over 180 GPa and temperatures of several thousand degrees. This has led to remarkable findings in solid-state physics, chemistry and materials science at extreme conditions.

The School, whose organization was pushed by Dominique Laniel and Thomas Meier, aimed to provide an introduction to novel methods of atomic and electronic structure studies at high pressures in a DAC to early-career stage scientists (Masters and PhD students, post-docs), as well as scientists working in the field of high-pressure research. The focus was set on the new developments pioneered in high-pressure sc-XRD and NMR.

An overview of the necessary fundamental theoretical and methodological principles, including DAC preparation and strategy of experiments, was covered through lectures accompanied by multiple demonstrations, hands-on sessions and step-by-step tutorials. At the end of the School, participants were expected to be able to perform basic high-pressure sc-XRD and NMR experiments, as well as data processing and interpretation.



The academic background of the participants.

The School was attended online by 25 participants from 7 countries: Germany (8), India (6), France (3), China (3), Italy (2), UK (2), and Slovenia (1). Among them were 11 PhD students, seven post-docs, 2 Masters students, and 5 staff scientists. They were taught by 12 lecturers and instructors, ten of whom are either present or former members of our Bayreuth group.

The curriculum of the School comprised 30 teaching hours (6 hours per day) distributed as follows: The sc-XRD part of 23 hours included crystallography lectures (10 hours), covering topics ranging from the basics of crystallography and X-ray diffraction to advanced themes on data handling and software, lectures on the technical aspects of working with DACs, including a historical insight (3 hours), and lectures on synchrotron setups (2 hours); 2 hours were dedicated to application cases aimed at giving an insight into a wide range of scientific problems in physics, chemistry and materials sciences, which could be addressed using the methods taught at the School. The sc-XRD hands-on sessions included 6 hours of exercises (6 different hands-on sessions were led by four instructors for 4 groups of students in parallel). The NMR part of 7 hours included NMR lectures (4 hours) and hands-on sessions (3 hours) for three groups at different times.



Left: 28.08.20 - The Bayreuth team is ready to start! Right: 31.08.2020 - The lecture has just finished!

Undoubtedly, a School via Zoom differs a lot from an on-site workshop. As pointed out by many lecturers, the absence of immediate audience feedback, especially during the 'teaching' lectures, created an essential difficulty. Interestingly, the majority of lecturers attended the lectures of the other lecturers as well. For those who joined us online from the east coast of the USA, the School started in the middle of the night (at 2:30 a.m.) but, as one of the participants wrote, they 'enjoyed the School very much despite the time-zone differences with Germany.'

Taking into account the large variety of backgrounds and prior knowledge of the participants, the programme of the School was very diverse. The lectures spanned topics from the basics of symmetry and NMR to very complex material for advanced crystallographers and interesting cases encountered in real experiments. The majority of the participants evaluated this broadness very positively: on the one hand, everyone could follow the material corresponding to their own knowledge, and hence could

improve it. On the other hand, one could see the material beyond one's own level and consider it as the next goal for studying.

We do believe that our experience would be useful for other colleagues who intend to run online schools and workshops in the future. We have summarized a few technical recommendations which could make online events even more efficient:

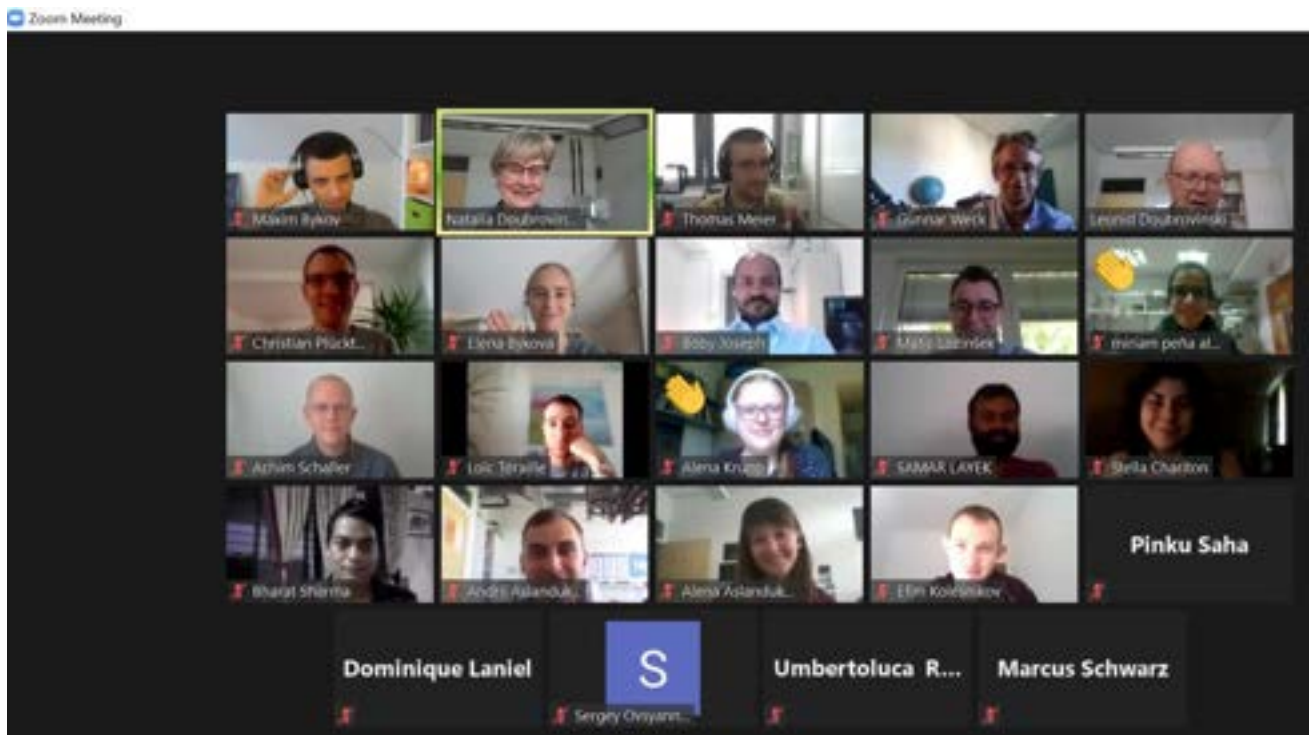
- It would be very helpful if the participants could download all materials and software at least one week before the course starts with no rush or stress;
- During the week prior to the course, we would suggest organizing one or two online sessions for testing the software: it would help to eliminate possible problems in advance and save valuable time at the beginning of the course;
- It should be specified in advance that the use of a large monitor or two separate monitors during hands-on sessions is an important technical prerequisite in order to realize the practical exercises efficiently. As several programs have to run in parallel and one has to quickly switch back and forth between the shared screen of the instructor and one's own running programs, just a single small monitor is insufficient.
- Time should be more generously allocated for the hands-on sessions; a common suggestion for our sc-XRD part was to increase it by a factor of 1.5, i.e. to plan 9 hours of hands-on instead of 6. This means that almost 40% of the teaching time should be allocated for hands-on.
- One should take care of the breaks between the lectures. According to the participants, a reasonable time (up to 15 min) for breaks would allow one to think about the lecture that had just finished and to get prepared for the following one.



Our teachers: ND - Natalia Dubrovinskaia (Bayreuth), DL - Dominique Laniel, TM - Thomas Meier, KG - Konstantin Glazyrin, SK - Saiana Khandarkhaeva, AS - Achim Schaller, VP - Vitali Prakapenko, TF - Timofey Fedotenko, SC - Stella Chariton, SO - Sergey Ovsyannikov, EB - Elena Bykova, MB - Maxim Bykov, KF - Karen Frieze, LSD - Leonid Dubrovinsky.

Student's feedback: 'All speakers did their talks in a 'not boring way', for that I would like to say special thanks to them.'

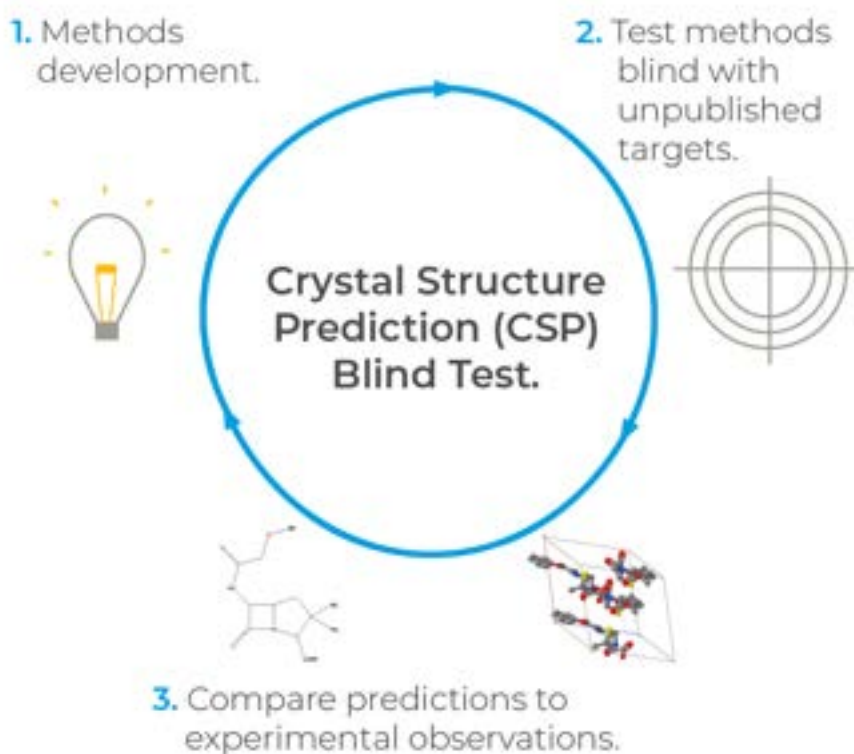
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The School is over. Thanks to all and goodbye!

NOTICE

Seventh Crystal Structure Prediction Blind Test brings new challenges for computational chemists



How the CSP Blind Test initiative works to help develop computational techniques to predict crystal structures. This field has the potential to revolutionise drug and agrochemical design and development methods.

Announcing the new challenge

The Seventh Crystal Structure Prediction (CSP) Blind Test – a major challenge in computational chemistry – began at the end of October 2020. It will see the world-leading methods in CSP put to the test on real targets – with a high bar set by previous work. The seven test systems have been sourced from leading experimentalists in industry and academia for prediction.

The importance of CSP

The 3D structure and packing of crystalline materials have a big impact on their properties – this is most relevant in drug discovery and development.

Small-molecule drugs are usually administered in crystalline, solid form. This form undergoes extensive testing to ensure it is stable, effective and safe. However, it is possible for a small molecule to have multiple crystal forms – polymorphs – where the molecular structure is the same, but the packing is different. This phenomenon is widely observed – for example cocoa butter has six polymorphs, resulting in different melting temperatures that impact the texture of chocolate.

Predicting how and when an alternative polymorph will form is not simple. In the context of medicines, the impact can be huge – with different polymorphs being unstable in storage, ineffective or at worst unsafe. Ritonavir, an antiretroviral drug for HIV/AIDS, had to be pulled from the market in 1998 when a second polymorph suddenly appeared that was less soluble and hence less effective (Bauer *et al.*, 2001).

The ability to predict *in silico* if another polymorph is possible and probable, would allow pharmaceutical companies to identify such risks earlier.

CSP is already a key part of most pharma companies' discovery and development process, but the science is still developing, with initiatives like the CSP Blind Test playing a key role.

How does the CSP Blind Test work?

The Cambridge Crystallographic Data Centre (CCDC) has administered and run the CSP Blind Test since the first iteration in 1999. The team selects a set of solved but unpublished structures to use as the test targets. The 2D structures are provided to participants, who have around one year to predict the 3D structure and crystalline properties using their best methods. Once predictions are in, the solved structures are revealed and comparing the two allows further method refinement and learnings.

Leading scientists enter the test from academia and industry, to see how their novel methods perform. The [results of each test](#) are published in the literature, with great leaps of progress seen every time.

"The CSP Blind Test is a fantastic example of how scientific methods can be advanced through careful and organised initiatives," said Anthony Nicholls, President and CEO of OpenEye Scientific Software. "The very principle of blind testing is at the core of the scientific method, and the CCDC acting as facilitators allows CSP methods to be put to the test in a realistic way that helps them to be progressed and improved upon."

How does CSP compare to other blind challenges?

Blind tests in evaluation of software methods have a long and illustrious history. Along with historical CSP challenges, various groups have held other blind challenges such as protein folding, docking challenges and property prediction challenges.

Protein folding has risen through computational chemistry circles into the popular science spotlight, with tech companies like Google's DeepMind entering the Critical Assessment of protein Structure Prediction (CASP) challenge (see John Helliwell's [article](#) in this issue), and games like FoldIt raising awareness more broadly. But CSP presents an even bigger challenge; there's more chemical diversity, a huge number of possible structures to be evaluated and often no single correct result. Further, for CSP to be a useful method the structural models generated need to be correct at a finer scale than protein predictions.

"Reliable and computationally tractable CSP would revolutionise aspects of drug & agrochemical development and would lead to new organic material design methods in other fields." Jason Cole, Senior Research Fellow at CCDC explains. "Being able to accurately predict the properties of crystalline materials before they are synthesised would have huge impacts in drug safety, material stability and more. But the myriad of factors that affect small molecule structure, packing and stability make this a difficult problem to tackle - the blind test aims to support further development in this important field."

Find out more about the CSP Blind Test, see previous results and apply to participate on the [CCDC website](#).

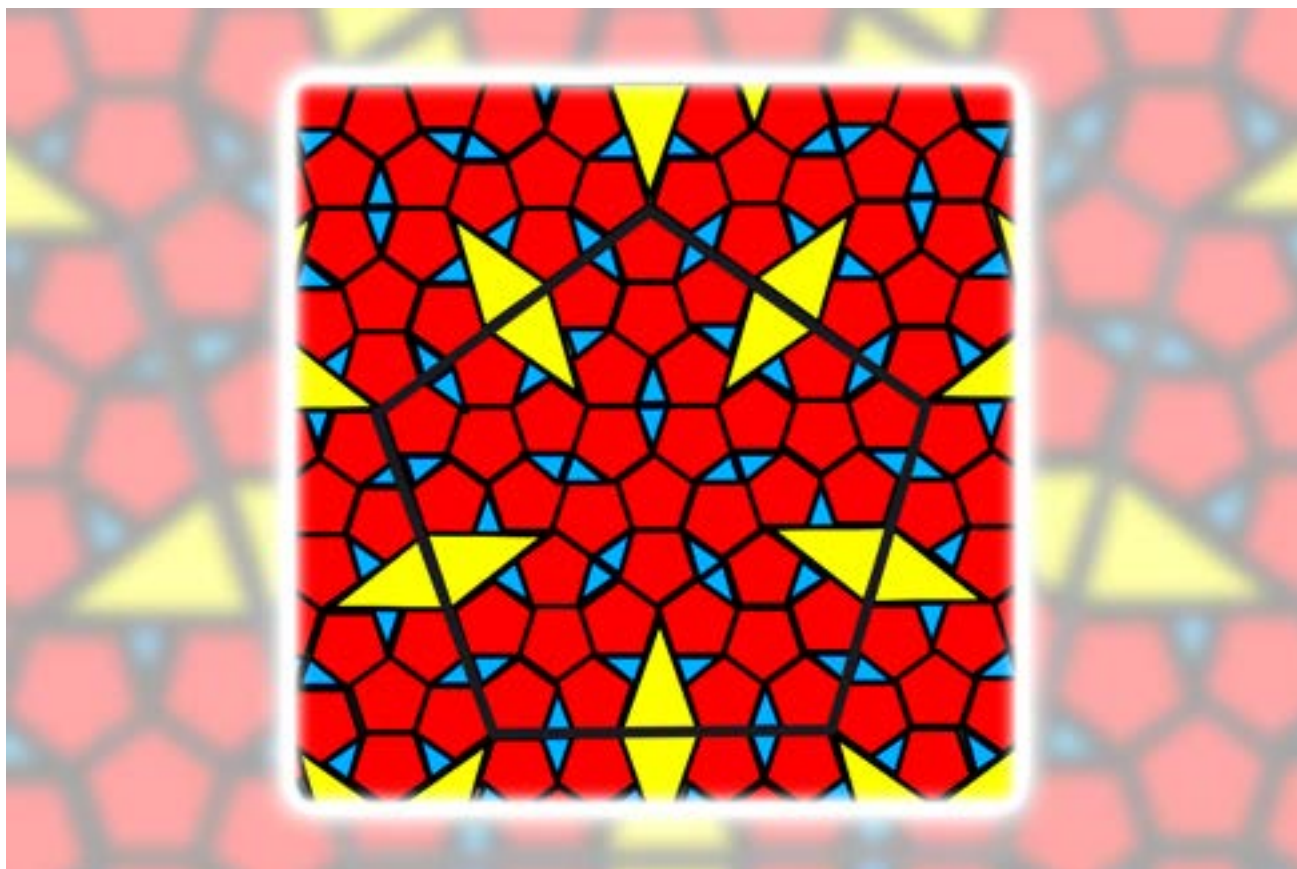
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Bauer, J., Spanton, S., Henry, R., Quick, J., Dziki, W., Porter, W. & Morris, J. (2001). *Pharm. Res.* **18**, 859–866.

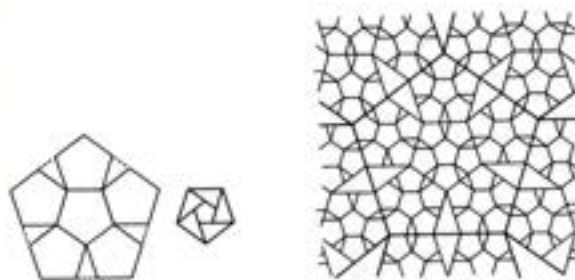
HISTORY OF CRYSTALLOGRAPHY

Penrose-Mackay-Shechtman and bringing down the dogma about fivefold symmetry

Istvan HargittaiBalazs Hargittai



Crystallographers have known Roger Penrose for a long time for the Penrose patterns. For the fresh (2020) Physics Nobel laureate, serious science and playful doodling have never been rigorously separated. Once, around 1972, he noticed a pentagonal logo on a letterhead: a regular pentagon at its center, surrounded by five same-size pentagons, making a larger pentagon. The contour of this scheme left five triangles uncovered. Penrose cut up a seventh same-size pentagon, which yielded five triangles to fill the gaps and left a smaller-size pentagon unused. The continuation of this scheme resulted in the simplest Penrose pattern. He built more sophisticated patterns, all involving fivefold symmetry, and published a paper about these patterns in an obscure mathematical journal [1].



Left: Roger Penrose in 2000 in his office at Oxford University, UK (photograph by IH), and right: generating the simplest Penrose pattern (courtesy of Alan L. Mackay).

When Martin Gardner wrote about the Penrose patterns in *Scientific American* [2] it garnered a great deal of attention, but was still considered to be hardly more than a mathematical curiosity.

At some point in time there was an unrelated reason that added to the fame of the Penrose pattern. It was a lawsuit because of an unauthorized use of the Penrose pattern on a toilet paper. A company, called Pentaplex, had been operating, making things based on Penrose's designs, and another company encroached on it without agreement. Not much has transpired about the lawsuit because there was an out-of-court settlement. The Penrose pattern still appeared on the toilet paper following the settlement, but, by then, it did so with an agreement. The issue was not whether the pattern on the toilet paper was produced by some mathematical formula. The question was whether a certain pattern had been directly copied. This is an important distinction. A piece of mathematics could not be judged as being private property or public property, because it could only be judged as public property.



Dan Shechtman (left) and Alan L. Mackay (right) in 1995 in the Hargittais' home in Budapest, Hungary (photograph by IH).

Enter Alan L. Mackay who had been a champion of questioning dogmas in crystallography (and elsewhere). He was a J. Desmond Bernal disciple at Birkbeck College and icosahedral structures used to be one of his major interests. He had long been intrigued by the rule of classical crystallography according to which fivefold symmetry was impossible in extended structures. The Penrose pattern made him initiate a simulation experiment: to shine light onto such a pattern and see whether a diffraction pattern might result. It did, and it indicated tenfold symmetry, being as forbidden in classical crystallography as fivefold symmetry. He published his simulated diffraction pattern in 1982 and issued a warning that we might ignore fivefold symmetries even if we experience them if we blindly believe the dogma about their impossibility [3]. In September 1982 Mackay gave two lectures on fivefold symmetry in Budapest and repeated his warning. One of us (IH) was present at the lectures (actually, IH had initiated Mackay's visit). The reason IH was not more impressed than he was was because his primary interest was in molecular structures where no such rule of restriction ever existed. Unbeknownst to Mackay and his audience, earlier in the same year, in April 1982, the Israeli scientist Dan Shechtman, visiting the US National Bureau of Standards (as it was then), did observe tenfold symmetry. It was displayed in the electron diffraction patterns of an aluminium/manganese alloy. Shechtman was aware of the dogma – as a student once he had to prove it mathematically as part of an exam. Nonetheless, he believed what he observed and recognized its significance, but it then took a long time for him to convince others and to have his observation published [4]. What he observed were quasicrystals – a name coined by the theoretical physicists Paul Steinhardt and Dov Levine, at the time at the University of Pennsylvania. They constructed a model to describe such structures but alas, they kept their model in a drawer and published it only after Shechtman's observation had finally appeared in print [5]. This should be considered an effect of the dogma about fivefold symmetry in extended structures. This further points to Mackay's courage and integrity in this story. When Shechtman's well-deserved Nobel Prize for the quasicrystal discovery was announced in October 2011, Penrose wrote a letter to Mackay in which he stressed: 'If anyone had been clear in the prediction that quasi-symmetric 5-fold/10-fold patterns might underlie a completely new area of crystallography – where the very way that such materials might indeed be identified through their characteristic diffraction patterns – it was clearly you.' [6]



Computer-automated drawing of a “Pentagonal snow crystal” by Robert H. Mackay in 1975. This was drawn originally by hand by Roger Penrose, and he autographed it in 2005 (courtesy of Robert H. Mackay).

Back in 1975, Alan Mackay informed Roger Penrose about the simulated light diffraction experiment on the Penrose pattern. At the time they met, Penrose was experimenting with an extended pentagonal network that could be considered to be a pentagonal snow crystal as compared with the usual hexagonal snowflakes. Mackay's son, Robert, was present and Penrose gave him a copy of his hand-drawn pattern. Robert was a student at York, and when he returned to his computer, he automated Penrose's drawing. The computer ran out of time at some point, hence the pattern of the pentagonal snow crystal was left incomplete. This has the advantage of showing many different parts of its generation, and it is more informative of how it came about than a completed pattern would have been. This computer-generated pattern is shown above. At the time, Penrose and the two Mackays thought this to be a theoretical exercise and years later they were astonished when Shechtman turned up the real thing. Thirty years later, Robert asked Penrose to autograph the drawing.

For further reading on the background and historical details, see also references [7] and [8].

Literature

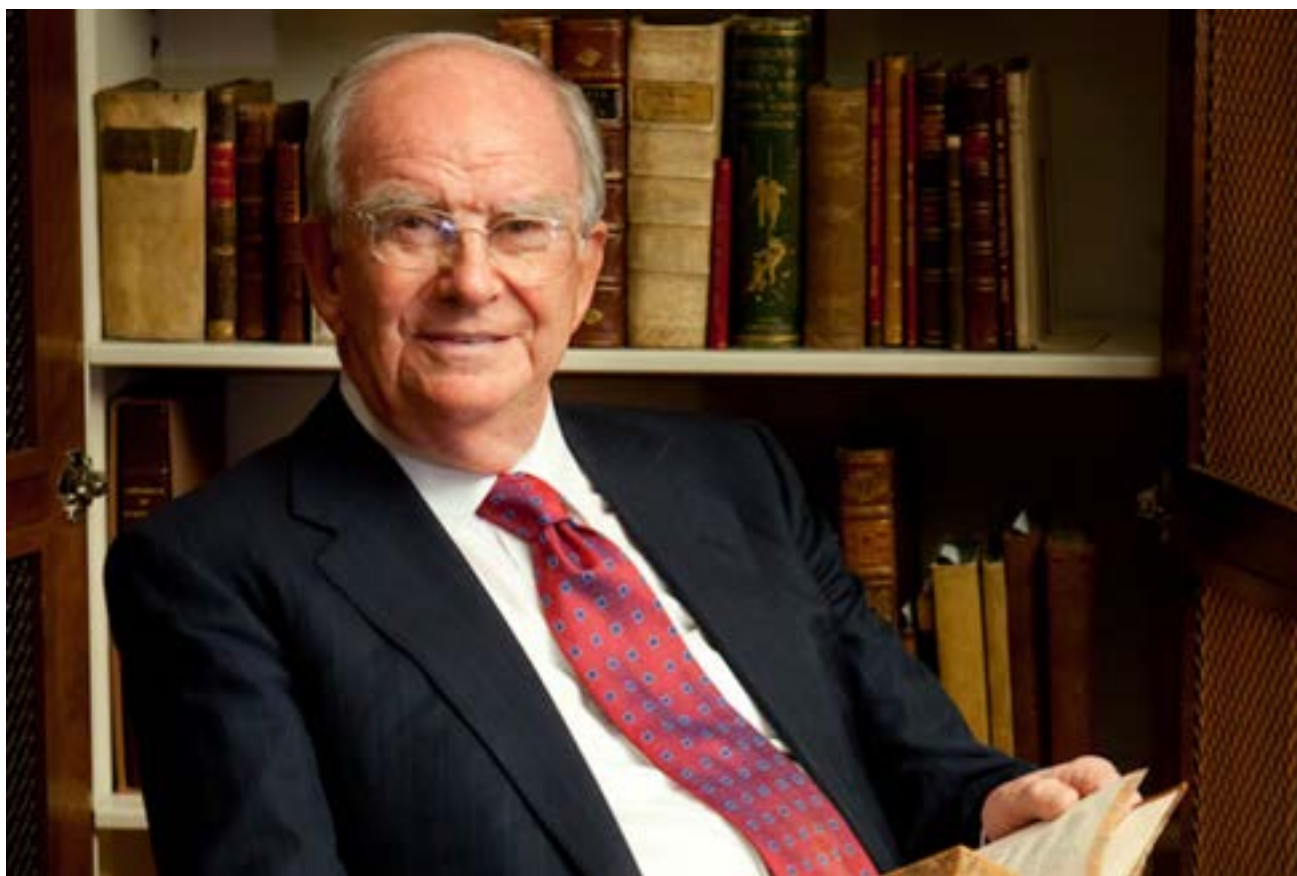
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OBITUARY

Sir John Meurig Thomas (1932–2020)

Richard Catlow



Sir John Meurig Thomas. Science History Institute, [CC BY-SA 3.0](#), via Wikimedia Commons.

John Meurig Thomas, who was a world-renowned solid-state scientist, sadly died on 13 November 2020 at the age of 87.

Thomas was born the son of a coal miner in the Gwendraeth Valley in South Wales. He graduated with BSc and PhD degrees in Chemistry from the University College of Swansea. From 1958, he held academic positions at the University of Wales, initially as Lecturer at Bangor and then (from 1969) as Professor at Aberystwyth. In 1978, he was appointed as Professor and Head of the Department of Physical Chemistry at the University of Cambridge, before taking up the prestigious role of Director of the Royal Institution (RI) in 1986. Subsequently, he was Master of Peterhouse while continuing his research programme at the RI, and later took up honorary appointments in the Department of Materials Science at the University of Cambridge and the School of Chemistry at Cardiff University.

Thomas made major contributions to many key areas of chemical and materials sciences: his early work focused on the developing field of the physical chemistry of solids and included key contributions to mineralogy but his most significant scientific legacy will be in catalytic science, where he pioneered new techniques and systems, focusing on the development of fundamental knowledge that has allowed the optimisation and development of new catalytic technologies. His science in the 1990s and the earlier part of this century focused on microporous catalytic solids – zeolites and microporous aluminophosphates – where he and his team developed new materials with novel structural and catalytic

properties. Much of his work in this field was accomplished in the Davy Faraday Laboratory of the RI, where Thomas continued the long history of world-leading scientific innovation.

Thomas published prolifically with over 1100 papers reviews and books during his long career. He also received recognition worldwide for his achievement, including the Royal Medal of the Royal Society, the Willard Gibbs Award of the American Chemical Society and the Bragg Prize Lectureship of the British Crystallographic Association.

Thomas's contributions to science were, however, much broader than his wide-ranging research achievements: he mentored and encouraged scientists, especially younger colleagues, at key stages in their career; he showed great prescience in appreciating the significance of new developments, for example, solid-state NMR and computation in materials chemistry; and he was a passionate and eloquent advocate of the wonder and elegance of scientific knowledge and never happier than when lecturing to audiences of all ages, giving several discourse and schools lectures at the RI, perhaps most notably the Christmas lectures in 1987, which he gave jointly with David Phillips. He was also fascinated by the history of science and wrote lucidly and with great insight about some of the great figures of science. As Director of the RI in the 1980s, he was able to combine his passions for research, advocacy and the history of science.



Photo by Nathan Pitt.

Thomas was also a patriotic Welshman who loved his native land, language and literature and indeed is celebrated in Wales not only as a scientist but as a poet. His work will be a lasting contribution to materials, structural and catalytic science and he will be missed, remembered and honoured worldwide.

For a list of papers by Thomas appearing in IUCr journals click [here](#).

OBITUARY

Nicolae C. Popa (1945-2020)

George-Adrian Lungu



On behalf of the National Institute of Materials Physics at Bucharest-Măgurele, Romania, and as a last collaborator of Dr Nicolae C. Popa, a Romanian physicist with many contributions to IUCr journals (and, lastly, to Volume H of *International Tables for Crystallography*) as both author and reviewer, I am charged with announcing his passing away on 15 September 2020, after a very long physical illness and a very short time after his wife's decease (on 23 August 2020).

Dr Nicolae C. Popa was born in Deduleşti (Argeş), Romania, on 17 December 1945. He graduated from the Faculty of Physics within the University of Bucharest in 1968, and in that very year was employed as a researcher at the Institute of Atomic Physics at Bucharest-Măgurele, defending his doctoral dissertation entitled *Extinction processes in diffraction of neutrons and X-rays in quasi-ideal crystals* on 3 February 1989, under supervision of Dr Dorel Bally.

He was an employee of several Romanian research centers during his career, including the Institute of Atomic Physics at Bucharest-Măgurele (1968–1977), Institute of Nuclear Power Reactors at Piteşti (1977–1981), Institute of Nuclear Physics and Engineering, Bucharest-Măgurele (1989–1990), and National Institute of Materials Physics, Bucharest-Măgurele, formerly the Institute of Physics and Technology of Materials (1987–1989, 1990–2000, 2001–2002, 2007–2016), and fellow of three foreign centers for longer or shorter periods: University of Rio de Janeiro (December 1973–December 1974) as IAEA fellow, Joint Institute for Nuclear Research, Dubna, USSR (November 1981–April 1987) as researcher, National Institute of Standards and Technology, Boulder, CO, USA (June 2000–December 2001) as guest

researcher, and again Joint Institute for Nuclear Research, Dubna, Russia (October 2002–May 2007), this time as Neutron Physics Laboratory Deputy Director.

During his career, Dr Nicolae C. Popa published both theoretical and experimental studies in X-ray and neutron diffraction, 18 original and highly visible papers [see e.g. Popa (1998)] being published in IUCr journals since 1976 (Popa, 1976). As a recognition of his contributions to the applied crystallography field, he was invited to join the author contributors for the new volume of the prestigious *International Tables for Crystallography* on the topic of powder diffraction, in which he wrote on determination of elastic stress and strain in polycrystalline samples via powder diffraction (Popa, 2019).

With sincere regrets for this loss, Dr George-Adrian Lungu.

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This article was originally published in *J. Appl. Cryst.* (2020). **53**, 1628.

OBITUARY

Antonio Ricardo D. Rodrigues (1951–2020)

Aldo F. Craievich



Photograph courtesy of Liu Lin (LNLS).

Antonio Ricardo Droher Rodrigues passed away in Campinas, Brazil, on 3 January 2020, at the age of 68. Known to his friends and colleagues as Ricardo, he was the Project Leader responsible for constructing the synchrotron light sources at the Brazilian Synchrotron Light Laboratory (LNLS), now considered the greatest Brazilian achievement of experimental science and technology.

Ricardo began his research activities in 1974 at the X-ray Optics Laboratory of the Physics Department at the Federal University of Paraná, Curitiba, Brazil. He obtained his PhD in Physics at King's College, University of London, UK, under the supervision of Michael Hart, from 1976 to 1979. In his thesis, titled 'X-ray optics for synchrotron radiation', Ricardo proposed and characterized two-crystal monochromators with nearly parallel crystallographic planes for efficient harmonic rejection of X-rays, which are currently used in many synchrotron beamlines. He carried out the experimental part of his thesis at HASYLAB in Hamburg, Germany, by 1978, thus becoming the first Brazilian user of a synchrotron light facility.

Soon after his return from the UK to Brazil in 1981, Ricardo participated in the Synchrotron Radiation Project, the precursor organization that led, in 1986, to the creation of LNLS. Ricardo joined LNLS from the very beginning and became the Project Leader responsible for the construction of the first LNLS synchrotron light source, based on a 1.37 GeV electron storage ring named UVX. This second-generation synchrotron source was commissioned in 1997. Since then, it was in successful operation until 2019. The UVX synchrotron light source was the first in operation in the southern hemisphere. In addition to his

decisive role in the construction of the UVX source, Ricardo collaborated actively on the design of new instrumentation for several beamlines and experimental stations.

In recognition of Ricardo's great scientific and technological accomplishments, he received the title of 'Comendador da Ordem Nacional do Mérito Científico' awarded by the President of Brazil.

In 2009, Ricardo became Project Leader of the team responsible for the design and construction of the second LNLS synchrotron light source, Sirius, a 3.0 GeV fourth-generation source, which started its commissioning by mid-2019. This new state-of-the-art project presented great challenges related to engineering issues that were unprecedented not only in the Brazilian context but also worldwide. Ricardo and his team overcame these challenges by applying, in many cases, original ideas and using predominantly Brazilian technology.

The contribution of Ricardo's achievements to the development of Brazilian science and technology was of paramount importance. This is evident after 22 years of operation of the UVX synchrotron source and associated beamlines. Between 1997 and 2019 the LNLS UVX source was used by more than 6000 researchers from many fields of science and their results have been explored in hundreds of Master and PhD theses. The LNLS user community, including many crystallographers from Brazil and other Latin American countries, benefited greatly from the fruits of Ricardo's work.

I met Ricardo for the first time in Warsaw during the Eleventh Congress of the International Union of Crystallography (IUCr) in 1978. At that time, he told me about his pioneering investigations on harmonic-free two-crystal monochromators for X-rays. During my initial engagement with the early Brazilian Synchrotron Radiation Project in Rio de Janeiro (1982–1986), later on as Adjoint Director of LNLS (1986–1997), and finally as a frequent LNLS user, I have always appreciated Ricardo's outstanding competence, extreme dedication and excellent personal qualities. In my last contact with him in late December 2019, he told me with serenity and restrained satisfaction that the fourth-generation 3.0 GeV Sirius source had just reached its nominal electron energy and first preliminary experiments of X-ray transmission imaging had been carried out. Sadly, Ricardo passed away a few days later.

People who were lucky enough to meet and know Ricardo consider that, besides being an exceptional professional, he was also a human being with unique qualities. Cylon Gonçalves da Silva, Director of LNLS from 1986 until 1998, summed up everyone's feelings about his departure from this world as follows: "Ricardo left a vacuum, not only in Brazilian science and technology, but also in the hearts of his wife, his daughter, his two sons, his family and his innumerable friends. His death reminds us of the ancient Greeks reflections that only after death can you tell if a man was happy. Looking at his legacy, Ricardo certainly could say he was happy. And all of us, family and friends, scientists and technicians, old and young students, who benefited from his immense contributions to our personal and professional lives, can, in a small measure, feel happy for having been part of his happiness. The vacuum in our hearts becomes an equally immense space full of light. And this is the great legacy of Ricardo to Brazil and to all of us: the intense brightness of synchrotron light. Without him, it is hard to imagine that it would exist today."

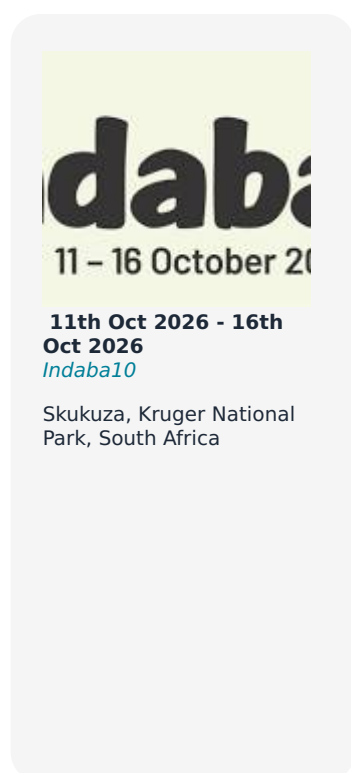
This article was originally published in *J. Synchrotron Rad.* (2020). **27**, 1753–1754.

IUCr SUPPORTED EVENTS

IUCr supported meetings

During this period of meeting cancellations and postponements because of international health concerns, the IUCr will make best efforts to indicate the status of advertised meetings whenever we receive updates. Please refer in all cases to any website maintained by the meeting organizers for definitive information.

These forthcoming meetings are supported by grants from the IUCr Calendar Committee.





5th Dec 2026 - 10th Dec 2026

African Materials Research Society (AMRS 2026)

Nairobi, Kenya

MEETINGS CALENDAR

Meetings calendar

During this period of meeting cancellations and postponements because of international health concerns, the IUCr will make best efforts to indicate the status of advertised meetings whenever we receive updates. Please refer in all cases to any website maintained by the meeting organizers for definitive information.

This is a list of meetings of interest to crystallographers occurring within the next twelve months, as recorded in the IUCr Calendar of Events. Please let us know of any that are missing by [completing this form](#) or sending an email to forthcoming.meetings@iucr.org.

	Indaba10 South Africa 11th Oct 2026 - 16th Oct 2026
	Synchrotron Radiation: Science, Knowledge, Innovations, Future (SKIF-2026) Russian Federation 18th Oct 2026 - 23rd Oct 2026
	CCP-EM/Diamond Icknield Workshop United Kingdom 9th Nov 2026 - 13th Nov 2026
	Instruct-ERIC hands-on workshop on Advanced cryo-FIB-SEM methods Germany 9th Nov 2026 - 13th Nov 2026
	Crystal36-AXAA 2026 Meeting Australia 26th Nov 2026 - 2nd Dec 2026
	African Materials Research Society (AMRS 2026) Kenya 5th Dec 2026 - 10th Dec 2026
	Joint Polish-German Crystallographic Meeting 2027 Germany 15th Feb 2027 - 18th Feb 2027