Time-resolved crystallography

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Dynamic crystal structure of a molecular framework

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X-ray diffraction analysis typically affords the static 3D structures of given compounds or materials, but to understand chemical processes, the visualization of fast structural changes is desirable. Time-resolved femtosecond crystallography has now been used to monitor the structural dynamics of a photoactive metal–organic framework.

Single-crystal X-ray crystallography has been considered the 'gold standard' for determining the molecular and crystal structures of molecules and materials in the solid state since the pioneering work of the Braggs more than a century ago[1]. However, until recently, the use of this technique has often been limited to determining either the structures of the starting materials or of the reaction products, without providing insight on the structural changes occurring along the reaction pathway.

Indeed, studying the reactivity of a given compound in crystallo is challenging. Since the early 1990s, attempts have been made to develop time-resolved crystallographic techniques that would enable the visualization of the structural changes taking place in a given crystal in response to external stimuli, along with the determination of the reaction dynamics[2]. The majority of the experiments have involved the interaction of crystalline solids with light, changes in temperature or pressure, or the application of electric fields. The development of the methodology has been aided by technological advances such as the increase in the intensity and brightness of X-ray sources (synchrotrons and X-ray free-electron lasers (XFELs)), the rapidity and accuracy of X-ray detectors, improvements in cryogenics and lasers, and increases in computing power and data storage capacity. Now, writing in *Nature Chemistry*, Ihee and co-workers present the use of time-resolved serial femtosecond crystallography to visualize the structural changes in a metal–organic framework and elucidate its structural dynamics (Fig. 1)[3].

Early work on nanosecond time-resolved crystallography reported[4] back in 1996 focused on the photodissociation of CO in a CO complex of myoglobin (MbCO). The experiment was carried out at the European Synchrotron Radiation Facility where a pump–probe methodology was used. This involved the use of an initial laser 'pump' pulse that initiates the photodissociation, followed by a Laue (white beam) X-ray 'probe' pulse timed to arrive after a specific time delay that monitors changes in the structure. Datasets recorded at 4 ns and 1 µs showed regions of negative electron density at the expected initial position of the coordinated CO molecule, confirming that Fe–CO bond cleavage had occurred. The development of XFELs later on enabled even faster studies, with pump–probe time-resolved serial femtosecond crystallography (TR–SFX) being used to investigate the dynamics of structural transitions in proteins[5,6]. In these macromolecular structures, compared with smaller molecular structures, the diffraction patterns are densely packed with Bragg peaks making indexing of the patterns easier. In addition, given that many of the components of proteins are known, often there is no need to resolve the structure to atomic resolution, and entities such as amino acid residues can be modelled. Consequently, large quantities of data could be collected relatively quickly before the crystal deteriorated, making it possible to solve the structure of the short-lived intermediates.

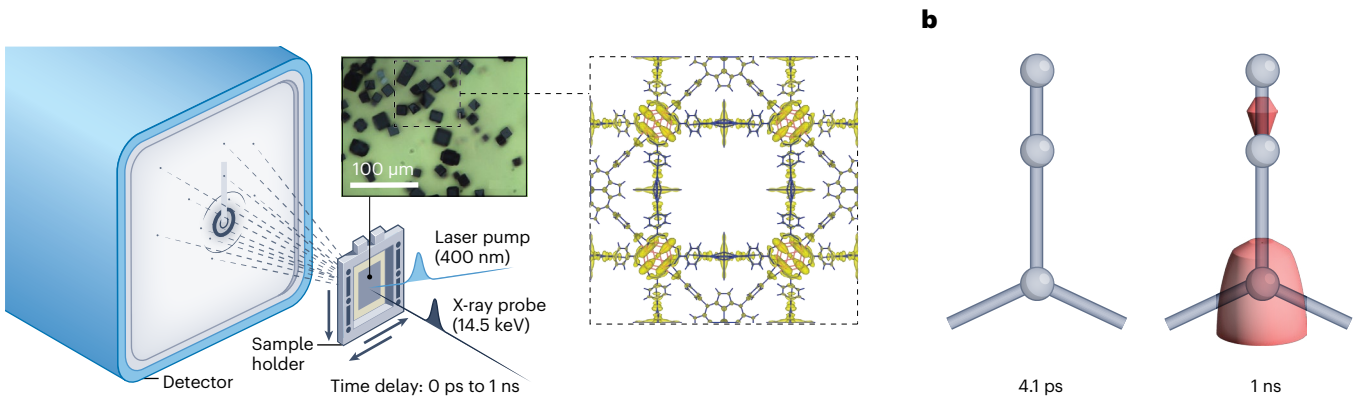

Fig. 1 | Femtosecond serial crystallographic study of a metal organic framework. a, The data collection set-up for the TR–SFX experiment with the fixed-target sample holder, along with a depiction of the crystal structure of PCN-224(Fe)–CO before photoactivation. **b**, A part of the time-resolved difference electron density map showing the region around the Fe–CO unit after 4.1 ps and 1 ns showing the changes in electron density. Figure adapted from ref. 3 under a Creative Commons licence CC BY 4.0.

In contrast, time-resolved crystallography studies on molecular systems have lagged behind — for these systems, crystallography has largely focused on the study of metastable and longer-lifetime excited-state complexes[7] with the structures being determined to atomic resolution using monochromatic radiation or Laue (white beam) sources at synchrotrons[8]. The work by Ihee and co-workers goes one step further and applies time-resolved serial femtosecond crystallography to study the structural transformations in a metal–organic framework (MOF), and the elucidation of its structural dynamics, during the dissociation of a ligand[3].

Specifically, the team has focused on PCN-224(Fe), which consists of a porous coordination network of iron porphyrin units that act as photoantennae, linked with hexa-zirconium nodes, Zr_6. The iron centres can bind CO to yield a MOF designated PCN-224(Fe)–CO. On photoexcitation, the CO ligand is dissociated from the iron porphyrin, which causes significant perturbation to the MOF structure. In a way, conceptually these experiments are reminiscent of those performed on the macromolecular monoxy-myoglobin (MbCO) in the initial nanosecond time-resolved experiment (see earlier)[4].

Ihee and colleagues collected data at negative time delays and the resultant electron density (ED) maps confirmed the structure of PCN-224(Fe)–CO. They calculated the difference ED (DED) maps of the positive time delays by subtracting the averaged structure factor amplitudes at negative time delays from each set of structure factor amplitudes for each positive time delay. The most significant features of these DED maps were that at time zero the CO position showed a negative electron density, consistent with dissociation of this ligand. The Fe atom also showed a negative density, indicating a displacement from its original position, and the Zr_6 node showed increased disordering. After 1 ps these features became more evident, while at late time delays the DED maps are consistent with the presence of vibrationally hot metal atoms. Overall, the complete DED features show periodic strengthening and weakening in the early time delays consistent with a coherent oscillation.

In the work by Ihee and co-workers, the X-ray energy used was 14.5 keV, higher than the energy of 5–12 keV typically used for macromolecular studies, because with the higher energy more Bragg peaks are accessible by the detector in a single shot, partially overcoming the issue of the sparse diffraction pattern associated with the smaller crystallographic unit cells in molecular systems. The crystals of PCN-224(Fe)–CO were typically 10–20 μm and Ihee and co-workers used a translatable fixed-target sample holder, unlike the injector-style crystal delivery systems usually used for macromolecular systems. The advantages of this for molecular systems is that with a fixed-target sample holder the amount of sample consumed is greatly reduced, and the issue of chemical compatibility between crystals and the injector carrier fluid is removed. The resolution of the diffraction patterns obtained was better than 1 Å.

Overall, Ihee and colleagues have shown that it is possible to carry out successful TR–SFX studies on molecular systems, and through dynamic and kinetic studies on the picosecond timescale have identified three structural pathways that occur within the PCN-224(Fe)–CO MOF. They have observed coherent oscillatory motion of the Fe and Zr centres, a transient structure in which levels of disorder and framework structures occur, and a vibrationally hot structure at longer time delays.

This study will act as a benchmark for future TR–SFX investigations of molecular systems, leading to a better understanding of short-lifetime dynamics in molecular materials that cannot be investigated fully by other analytical techniques. At the same time, the study complements fast spectroscopic methods that have been used very successfully in solution. The methodology development in the investigation also shows what changes are necessary to successfully study molecular species compared with macromolecular species, where the use of XFELs in short-lifetime dynamic studies is more established.

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Competing interests

The authors declare no competing interests.