MicroED

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Microcrystal Electron Diffraction (MicroED) for Small-Molecule Structure Determination

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absolute configuration · crystalline sponge · electron diffraction · structure elucidation · X-ray diffraction

Abstract: *The development of new methods to analyze and determine molecular structures parallels the ability to accelerate synthetic research. For many decades, single-crystal analysis by X-ray diffraction (SXRD) has been the definitive tool for structural analysis at the atomic level; the drawback, however, is that a suitable single crystal of the analyte needs to be grown. The recent innovation of the crystalline sponge (CS) method allows the microanalysis of compounds simply soaked in a readily prepared CS crystal, thus circumventing the need to screen crystallization conditions while also using only a trace amount of the sample. In this context, electron diffraction for the structure determination of small molecules is discussed as potentially the next big development in this field.*

Single-crystal X-ray crystallography (SXRD) has been developed steadily over the last 100 years. It has become an indispensable tool to obtain structural information on organic, inorganic, organometallic, and macromolecular systems, as it provides information about the connectivity and placement of individual atoms within a given molecule. Additionally, SXRD is the only method that can determine the absolute configuration of chiral molecules on the basis of anomalous scattering effects of heavy atoms; NMR and other spectroscopic methods, in principle, only determine the relative stereochemistry.[1] For SXRD, however, arduous sample preparation is necessary, as a crystal of suitable size (approximately $100 \times 100 \times 100\ \mu m^3$ for standard in-house measurements) and sufficient quality needs to be grown. The quality needs to be very high if the absolute configuration is to be determined accurately in the absence of heavy atoms. Depending on the nature of the sample, this can even be close to impossible because either the morphology of the obtained crystals is not appropriate or the analyte is simply not available in necessary quantities to simultaneously test a sufficient number of conditions for crystallization. Special effort is required if the analyte is a gas or liquid near room temperature, further hampering routine high-throughput measurements or automatization attempts.

Fujita and co-workers succeeded in improving the SXRD method and addressed these disadvantages in a series of publications;[3] subsequent studies were carried out by other groups.[4] The crystalline sponge (CS) method allows structural analysis of a target compound by SXRD without the need to grow a single crystal of the analyte. In this general method, a porous crystalline coordination network is soaked in a solution of the compound and together subsequently subjected to X-ray diffraction (Figure 1). The compound is found in a more or less ordered fashion within the pores, bound by weak interactions. Although the CS method only needs minute amounts of the analyte and circumvents the crystallization procedure, the soaking of the sponge needs to be optimized and not all classes of compounds are compatible with the commonly used coordination networks.[3a,b]

In two recent publications, both Grüne et al.[5] as well as Gonen and co-workers[6] convincingly demonstrated the potential application of microcrystal electron diffraction (MicroED) to elucidate the structures of small organic molecules. MicroED was originally developed to study large biological macromolecules, specifically proteins.[7] In contrast to SXRD, an electron beam is used to observe the scattering of crystals 1/6000th the volume of a crystal typically used for SXRD. In these two new studies, the researchers show that the technique can be applied to obtain the molecular structure of nanocrystals present in finely graded powders, as readily obtained from chemical suppliers and even from seemingly amorphous films of chromatography fractions in a scintillation vial. The Grüne group were able to elucidate the structure of acetaminophen (**1**) from a commercial flu relief medicine in the presence of several other pharmaceutically active and non-active ingredients. In addition, a single needle (approximately $1 \times 2\ \mu m^2$ and hence too small for standard SXRD measurement) of a novel methylene blue derivative provided a dataset of 0.9 Å resolution from its mother liquor within four hours.[5] Similar impressive results were independently reported by Gonen and co-workers, who analyzed a total of 11 different, biologically active, organic compounds. From a commercial sample of progesterone (**2**), estimated to be 20 years old, approximately 1 mg was ground between two glass slides to produce a fine powder. After depositing the sample on a holey carbon-copper grid, it was cooled to liquid

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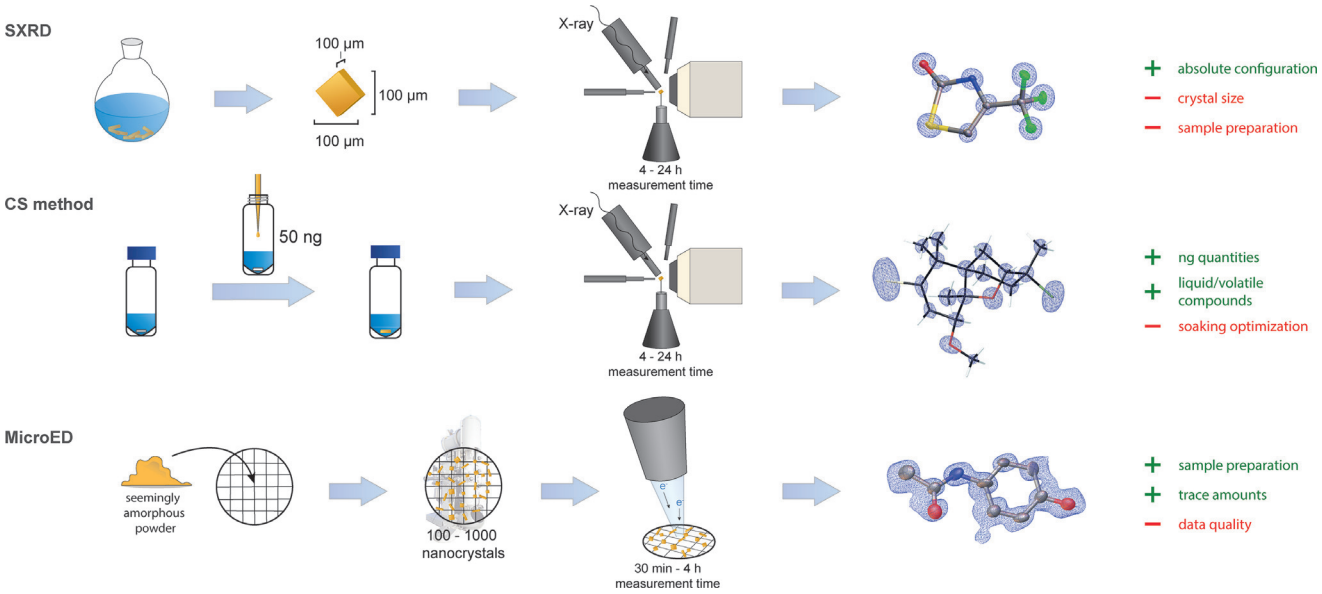

Figure 1. Different types of small molecules solved by SXRD (top),[2] the CS method (middle),[3e] and MicroED (bottom),[6] including key specifications and average measurement times. Arbitrary electron density maps suggest the different qualities of the obtained data, which significantly vary for each analyte.

nitrogen temperature and transferred to a Thermo Fisher Talos Arctica cryoelectron microscope. Upon imaging, several hundreds of nanocrystals suitable for diffraction and with sufficient resolution to determine the connectivity could be identified. The diffraction of one of these nanocrystals was good enough to collect a dataset of 1.0 Å resolution of steroid **2**, with the molecular structure obtained at atomic resolution within 30 minutes.[6] However, the absolute configuration of small molecules cannot be determined by MicroED at the current state of development. [7a] Conveniently, MicroED can be processed using broadly available standard software for SXRD, as stated by the authors.[6]

Additionally, ten structures including of brucine (**3**), (+)-limaspermidine (**4**), and thiostrepton (**5**) could be derived from milligram quantities (Figure 2). Amazingly, the structures of acetaminophen **1** and ibuprofen (**6**) were obtained after grinding tablets bought at a local pharmacy. Astonishingly, both groups were able to determine novel and known molecular structures from mixtures of crystalline and non-crystalline crude samples.[5,6] Furthermore, the Gonen group collected four datasets of four different compounds from a heterogeneous mixture.

The interaction of the electrons with the electrostatic potential of both protons and electrons in the crystal yields more accurate hydrogen bond lengths compared to SXRD datasets, where they appear shortened because of the predominant interaction with the electrons in the bond.[5,8] In both recent reports on MicroED, analyte hydrogen atoms were readily observed during the refinement, although not for all the compounds presented. A significant drawback of the MicroED method—the inability to determine the absolute configuration—might be overcome in the future and will further fuel electron crystallography.

Further developments and the next series of breakthroughs for this impressive technique are eagerly awaited,

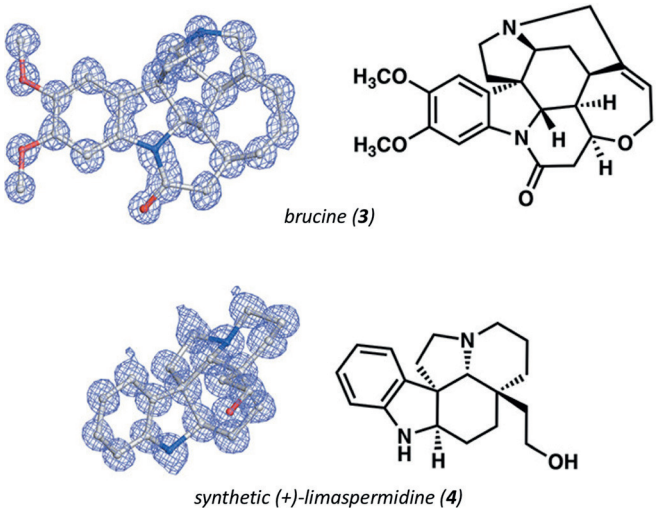

Figure 2. The molecular structures of brucine (**3**) and synthetic (+)-limaspermidine (**4**), including superimposition of the F_o electron density maps obtained by MicroED.[6]

which will determine whether MicroED will become more practically applied than SXRD. Natural product and pharmaceutical chemistry, in particular, rely on techniques that allow the analysis of compounds that are only available in minute amounts. The ability of synthetic chemists to quickly gain structural information about the molecules in hand is crucial for the acceleration of innovations across many fields.

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Conflict of interest

The authors declare no conflict of interest.

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