ANALYTICAL CHEMISTRY

Tiny crystals, big potential

A method called microcrystal electron diffraction can rapidly image the structures of small molecules, including those found in mixtures. Will it usurp X-ray crystallography for determining small-molecule structures?

ALAN BROWN & JON CLARDY

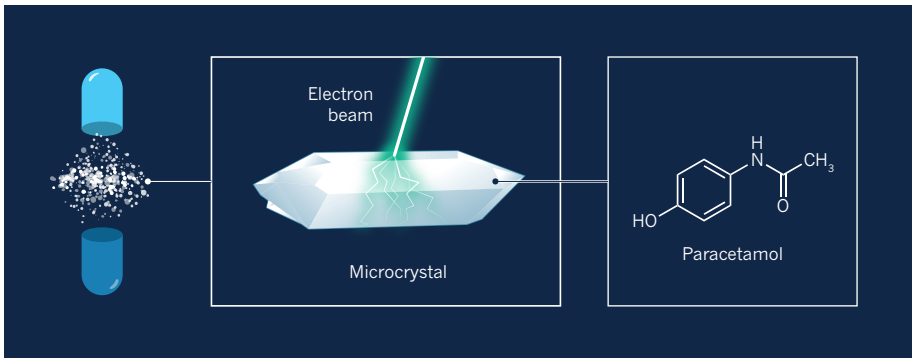

Figure 1 | Microcrystal electron diffraction (MicroED) applied to small molecules. Gruene *et al.*[1] and Jones *et al.*[2] have demonstrated that MicroED can determine the structures of small molecules in impure samples — a feat that could not be accomplished using X-ray crystallography. For example, Gruene and colleagues solved the structure of paracetamol from tablets of a cold medication that contained several biologically active and inactive ingredients. They focused an electron beam precisely at microcrystals of paracetamol in the medication, and then recorded and analysed the diffraction patterns to visualize the structure.

For decades, X-ray crystallography has been the gold-standard method for determining the structures of small molecules. But two papers, one by Gruene *et al.*[1] in *Angewandte Chemie* and the other by Jones *et al.*[2] in *ACS Central Science*, illustrate the potential of using an electron cryomicroscopy (cryo-EM) method called microcrystal electron diffraction (MicroED) for this purpose. Using different laboratory set-ups, the two groups have produced strikingly concordant sets of results: high-resolution structures of a suite of small molecules, some of which were even solved from impure samples.

In X-ray crystallography, molecules are crystallized before being bombarded with X-rays. This is because the scattering of X-rays from single molecules is too weak to be used for structure determinations, whereas the regularly repeating molecules in a crystal lattice focus the scattering into stronger patterns that can be analysed to generate a 3D atomic model of the molecule. However, X-ray crystallography requires samples to be relatively pure. Moreover, growing crystals large enough to diffract X-rays that will produce a measurable signal is an artisanal skill, and a bottleneck for structure determination.

In MicroED, crystals are exposed to a focused electron beam in an electron microscope, rather than to X-rays. The technique was originally used to solve protein structures from microcrystals[3], but the new papers demonstrate its potential for determining the structures of small organic molecules. Electrons interact with matter much more strongly than X-rays do, which makes it possible to use crystals just one-millionth of the size of the crystals used in X-ray diffraction. The crystals are rotated in the electron beam by tilting the microscope stage to create a series of diffraction patterns. Because the stage has a limited tilt range, the method often requires data from multiple crystals to be merged.

The stronger interaction of the irradiating electrons with the molecular target comes at a price: more damage is done to the sample than in X-ray crystallography. To reduce damage, MicroED uses a highly attenuated electron beam, and the crystals are frozen and imaged under cryogenic conditions. Freezing prolongs the life of the crystals, and thereby extends the range of samples that can be studied, compared with related electron-diffraction techniques that work at room temperature[4,5] and are suitable only for highly stable crystals.

Jones and colleagues report that they were able to use MicroED to solve the structure of paracetamol from over-the-counter painkillers ground up using a mortar and pestle. Gruene and co-workers independently solved the same structure from tablets of a cold medication that contained several biologically active and inactive ingredients (Fig. 1). Both groups also solved other structures to illustrate the broad applicability of MicroED. One particularly impressive example reported by Jones *et al.* was the structure of thiostrepton, a complex peptide antibiotic that has a globular structure ten times larger than paracetamol's. In all cases, both groups used crystals that were orders of magnitude smaller than those required for X-ray crystallography.

The possibilities of MicroED are tantalizing. Using this technique, it should be possible to obtain the structures of compounds that cannot be coaxed to form large crystals. Additionally, imperfect or intergrown crystals could also be studied by either breaking them into smaller, regular pieces or directing the electron beam at small parts of the crystal.

However, the most exciting application would be to obtain structures of compounds in tiny samples of mixtures. This should be possible because electron beams can be finely controlled and directed only at crystals, ignoring non-crystalline contaminants. For example, one could imagine using MicroED to study compounds extracted from natural sources, which are often obtained in extremely small quantities. Genome-based approaches are uncovering naturally occurring molecules at an ever-increasing rate[6], the structural characterization of which has lagged behind their discovery.

It remains to be seen whether MicroED will have the same revolutionary impact on chemistry that other cryo-EM methods have had on other fields (particularly structural biology). The excitement that has been generated around MicroED should be tempered by the fact that several other methods — such as atomic-force microscopy[7] and crystalline sponges[8] — have previously been feted as replacements for X-ray crystallography, but have failed to live up to expectations for one reason or another.

One factor that might allow MicroED to succeed is that the electron microscopes and detectors needed for it are already found in most modern electron-microscopy facilities. The software used for data acquisition will also be familiar to many electron microscopists, and electron-diffraction data can be processed using the same software as that used

to process X-ray-diffraction data. The entry barrier for any structural biologist or chemist wanting to use MicroED is therefore low. But chemists might need to compete for time on electron microscopes with their colleagues in biology departments.

Ultimately, the adoption of MicroED might depend on what percentage of small molecules are amenable to the technique. Previous work[3] in which MicroED was used to solve protein structures from microcrystals suggests that there is no limitation on molecular size — it should work for everything from small organic molecules to large, multiprotein complexes. Nevertheless, MicroED does not remove the need for crystals, and not every molecule will crystallize. The technique is also unable to distinguish between mirror-image isomers of molecules, which is a drawback because such isomers can have very different biological properties.

Should the technique take off, the next step will be to develop electron microscopes specifically for small-molecule analysis. These microscopes would be the same as those currently used in structural biology, but would have detectors that are optimized for electron diffraction, and stages that have a greater tilt range and that can be more finely controlled. The development of systems for automated data collection and structure determination would allow the rapid, routine determination of structures from complex mixtures. Given that data collection is fast (a whole data set can be collected from one microcrystal in just three minutes), many thousands of crystals could be imaged from a single sample of material.

Small-molecule MicroED might also teach us a lot about how electrons interact with matter. Unlike X-rays, which interact only with electron clouds in molecules, electrons interact with both protons and electrons. Finally, knowledge gained from small-molecule structures solved at atomic resolution should help to improve the quality of all structures solved by cryo-EM methods, from small-molecule drugs to multiprotein biological machines. ■

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1. Gruene, T. *et al. Angew. Chemie Int. Edn* **57**, 16313–16317 (2018).
2. Jones, C. G. *et al. ACS Central Sci.* **4**, 1587–1592 (2018).
3. Shi, D., Nannenga, B. L., Iadanza, M. G. & Gonen, T. *eLife* **2**, e01345 (2013).
4. Mugnaioli, E., Gorelik, T. & Kolb, U. *Ultramicroscopy* **109**, 758–765 (2009).
5. Su, J. *et al. Microporous Mesoporous Matter* **189**, 115–125 (2014).
6. Zerikly, M. & Challis, G. L. *ChemBioChem* **10**, 625–633 (2009).
7. Gross, L., Mohn, F., Moll, N., Liljeroth, P. & Meyer, G. *Science* **325**, 1110–1114 (2009).
8. Inokuma, Y. *et al. Nature* **495**, 461–466 (2013).

NEUROSCIENCE

Brain circuits of compulsive addiction

A study in mice identifies a brain adaptation that underlies the compulsive behaviour associated with drug addiction, and which might explain why some drug users behave compulsively whereas others do not. SEE ARTICLE P.366

PATRICIA JANAK

Drugs of abuse have complex pharmacological effects that trigger many changes in brain function. One of these effects, the direct or indirect activation of neurons that release the neurotransmitter dopamine, is common to all drugs of abuse and has long been assumed to contribute to the development of addiction. On page 366, Pascoli *et al.*[1] report on the neurobiological mechanisms induced by the repeated activation of dopamine neurons that might explain why some drug users seek reward despite facing negative consequences — a type of compulsive behaviour that is a defining feature of human addiction[2].

The authors took an optogenetics approach to mimic the activation of the brain's dopamine systems by drugs of abuse: they used laser light delivered through an optical fibre to activate dopamine neurons in the ventral tegmental area (VTA) of the brains of genetically engineered mice. The mice could directly stimulate these neurons themselves by pressing a lever, and performed this action avidly during a test period of 40 minutes a day for almost 2 weeks.

On subsequent days, the mice received a brief electric shock to their feet on one-third of the lever-pressing occasions, at random. Their behaviour under this condition revealed an intriguing variability: 40% of the mice (termed renouncers) greatly reduced the frequency of lever-pressing when given foot shocks (Fig. 1a), whereas the remaining 60% (perseverers) were willing to receive painful punishment for the opportunity to self-stimulate their dopamine neurons (Fig. 1b). As some of these authors have previously shown[3], the persevering mice provide a model for persistent drug use despite negative consequences, and parallel the subset of human drug users whose drug use becomes compulsive.

The authors next tried to determine what was different between the brains of perseverers and renouncers. They measured the activity of neurons connecting different brain areas in real time to determine which networks were active when mice pressed the lever. Communication between the orbitofrontal cortex (OFC), an area involved in decision-making, and the dorsal striatum, which is engaged in voluntary

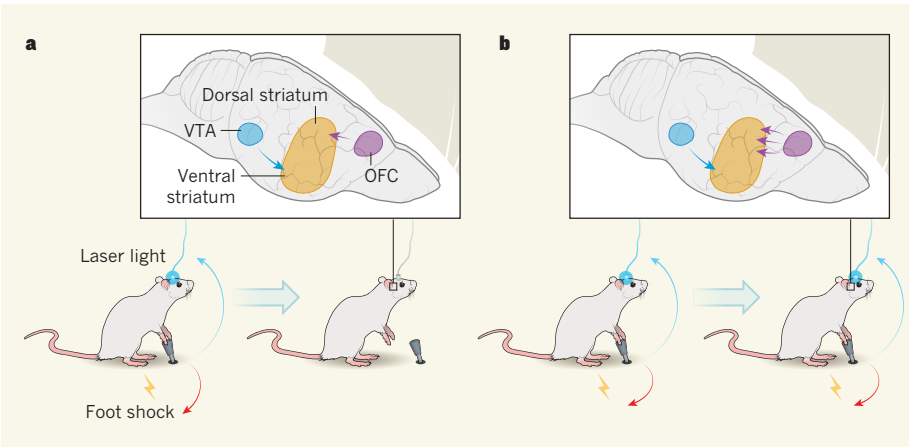

Figure 1 | Compulsive activation of dopamine neurons in the brain. In the study by Pascoli *et al.*[1], mice pressed a lever to activate dopamine-releasing neurons through the delivery of laser light conducted by an optical fibre. These neurons, which project from the ventral tegmental area (VTA) to the ventral striatum in the brain, are associated with reward. **a**, Some mice, termed renouncers, reduced the lever-pressing behaviour when it was associated with a painful electric shock to their feet. The strength of the connections between neurons of the orbitofrontal cortex (OFC) projecting to the dorsal striatum was low in these mice. **b**, Other mice, termed perseverers, continued to press the lever despite the punishment — a hallmark of compulsive behaviour. The neural connections between the OFC and the dorsal striatum were stronger in these mice than in renouncers. When the authors weakened these connections in persevering mice, the animals' compulsive behaviour decreased (not shown).