Revolutionary method for probing molecular structure unravels

Authors concede problems with X-ray technique for hard-to-crystallize molecules.

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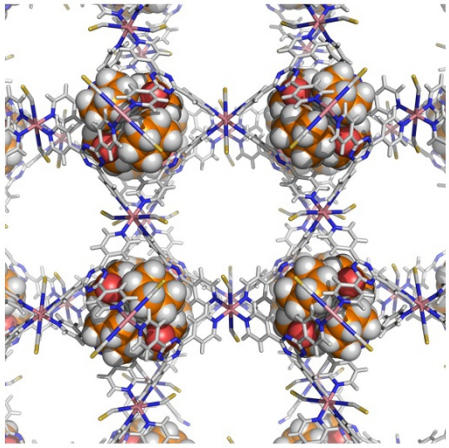
24 September 2013

Coaxing molecules to form crystals is a dark art, equal parts luck and labour. So, many chemists were in awe when a team reported an apparently simple way of getting otherwise intractable small molecules to adopt orderly arrangements — the first step towards determining their structure using X-rays.

The method uses 'crystalline sponges' to hold molecules in the regular order needed to perform X-ray crystallography. After it was presented in a *Nature* paper[1], researchers rushed to bring the technique to their own labs but, after six months, many have struggled to get it to work. Furthermore, crystallography experts are now raising questions about the most impressive aspects of the paper.

"It really would have been transformational," says Jon Clardy, a biological chemist at Harvard Medical School in Boston, Massachusetts, who wrote a glowing News and Views to accompany the paper when it was first published. "Nobel prizes have been given for less," wrote drug-discovery chemist Derek Lowe on his widely read blog *In the Pipeline* in March.



Y. Inokuma and M. Fujita

Some experts have questioned whether embedding molecules into a sponge-like cage can help to determine their structures using X-rays.

Sponge-worthy molecules

X-ray crystallography was the technique that revealed DNA's double helix. It has become one of the workhorses of structural biology, uncovering the shapes of countless proteins and other complexes. Chemists also use the method to determine the structures of molecules that may be used as drugs, many of which are derived from plants and marine organisms, and are isolated in vanishingly small amounts. Determining their structure is key to learning how to make them in the lab, and how to tweak their chemical properties.

For the technique to work, the molecules must hold still in regular crystalline structures, which enable researchers to reconstruct the shape of the individual molecule from the pattern of X-rays diffracted by the crystal. But whereas some molecules form crystals easily, others never do, says Clardy. The crystalline-sponge method offered the tantalizing possibility of overcoming that bottleneck by absorbing molecules of interest into structures called metal–organic frameworks, which contain regularly spaced pockets that hold the molecules in place. The research was led by Makoto Fujita, a chemist at the University of Tokyo.

Fujita's team tested the approach on number of simple chemicals and several more complex ones, including some that are found in the peel of citrus fruit. Most impressively, says Clardy, the technique revealed the shape of an elongated molecule called miyakosyne A, which is made by a marine sponge. The precise structure had been unknown, because the molecule's elongated shape caused it to flop around, making crystallization all but unthinkable.

Ambiguous centres

But on 12 September, Fujita's team published a correction to its paper, conceding that important aspects of the miyakosyne A structure were incorrect or ambiguous and could not be revealed using the crystalline-sponge technique. The errors involve places in the molecule called chiral centres, at which the arrangement of atoms can cause otherwise identical molecules to have different chemical properties.

Curtis Moore, an X-ray crystallographer at the University of California, San Diego, was the first person to raise concerns to manuscript editors at *Nature*, according to himself and Fujita. He obtained the raw X-ray diffraction data from Fujita, and says that he found irregularities in the way they were analysed.

To infer the structure of a molecule, scientists must sift through diffraction patterns created by other chemicals, such as solvents — and in this case, by the crystalline sponge. As a work-around, crystallographers often predict what the diffraction patterns for the

molecule of interest ought to look like, and then determine whether their data fit with that model. Moore says that Fujita and his team introduced too many assumptions about the shape of miyakosyne A and other molecules during this process. “They went looking for their molecule and that’s what they found,” he says.

Fujita rejects the idea that his team based its miyakosyne A structure on pre-existing assumptions. “We have no intention of suppressing negative information,” he says. He adds that his team corrected the paper after a colleague determined in June that a key aspect of the structure was incorrect, by synthesizing the molecule from scratch. Chemical synthesis is a more reliable way of determining structure than X-ray diffraction, says Fujita.

Trial and error

Meanwhile, other researchers have struggled to get the technique to work as advertised. Clardy says that he and his lab members have succeeded only with very simple molecules, “but we have had no luck with any interesting, or even mildly interesting, structures”. Large molecules and those that contain alkaline chemical groups are particularly problematic, he says.

Fujita says that his team has helped industrial and academic groups to master the technique in one to two weeks, and hopes soon to publish a more detailed paper in *Nature Protocols*. “I believe that the problem of the difficulty in replicating our method will be mostly solved when the protocol paper is published,” he says.

Data deposition

One further issue with the original *Nature* paper is that it does not contain important raw X-ray diffraction data that could have helped crystallographers to determine whether the miyakosyne A structure was correct, says Anthony Spek, an X-ray crystallographer at Utrecht University in the Netherlands. Journals published by the International Union of Crystallography in Chester, UK, require authors to deposit raw data online, Spek points out, but *Nature* currently has no such conditions. “My hope is that this type of high-profile paper may catalyse the implementation of a deposition requirement,” says Spek. “It would never be accepted in our crystallography journals.”

Fujita gave the raw data to Moore, and is open to making them public. “We don’t mean to hide our data,” he says. “However, it is impossible to convince all the crystallographers in the world. The crystal-sponge method is a newborn method and, unlike the crystallography of small molecules, the criteria for the data validation need to be discussed.”

Nature will consider requiring data deposition for future manuscripts, says Karl Ziemelis, chief physical-sciences editor for the journal, which is based in London. “When questions about the crystallographic aspect of this paper were first raised, they immediately prompted us to explore the issue and we will indeed review our own policies as they apply to the provision and deposition of crystallographic data,” he says**.**

Problems with Fujita's technique and data take the sheen off the initial excitement, says Clardy, but his lab has not yet given up. “I’m still optimistic enough that we’re plugging away and trying some things. It’s certainly not going to be a panacea for all structural problems.”

That attitude is much more measured than early opinions. If the technique had stood up to initial promise, “it would have been revolutionary”, says Clardy.

Nature | doi:10.1038/nature.2013.13798

References

1. Inokuma, Y. *et al*. *Nature* **495**, 461–466 (2013).

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Nature (and other journals) have different standards for different types of data. For example, it would be unthinkable for a genomics or genetics paper to be published, even in Nature, without the data being deposited in a public repository beforehand. While multi-disciplinary journals may not be able to list the data sharing requirements for all experiments in detail, editors should ask reviewers specifically to evaluate whether the necessary data for review have been released. After all, how

could they endorse (or reject) the validity of a paper without having adequate access to the results?