Structures solved by locking molecules in place

Covalently linking compounds to a chiral MOF steadies them for structural studies via X-ray diffraction

*By **Bethany Halford***

SCIENCE & TECHNOLOGY CONCENTRATES

Chemists have long relied on X-ray crystallography to unambiguously determine a molecule's structure. But in some cases, it can be devilishly difficult to grow the crystals required for this technique. A few years ago, chemists introduced the

Covalently linking (+)-jasmonate to a chiral MOF (yellow) allowed researchers to determine its absolute stereochemistry.
Credit: Omar Yaghi/UC Berkeley

crystalline sponge method, in which a metal-organic framework, or MOF, is soaked like a sponge with a solution of a compound. Weak interactions between the MOF and the compound hold it inside the MOF, allowing the compound's structure to be determined by X-ray diffraction. But the method is not perfect and is often used as a last resort. **Omar M. Yaghi <http://yaghi.berkeley.edu/>** , Seungkyu Lee, and Eugene A. Kapustin of the University of California, Berkeley, have now devised a way to make this kind of structure determination more reliable. The UC Berkeley chemists lock the molecules they are studying in place by covalently linking them to the aluminum atoms of a chiral framework known as MOF-520 (*Science* 2016, DOI: **10.1126/science.aaf9135 <http://dx.doi.org/10.1126/science.aaf9135>**). This
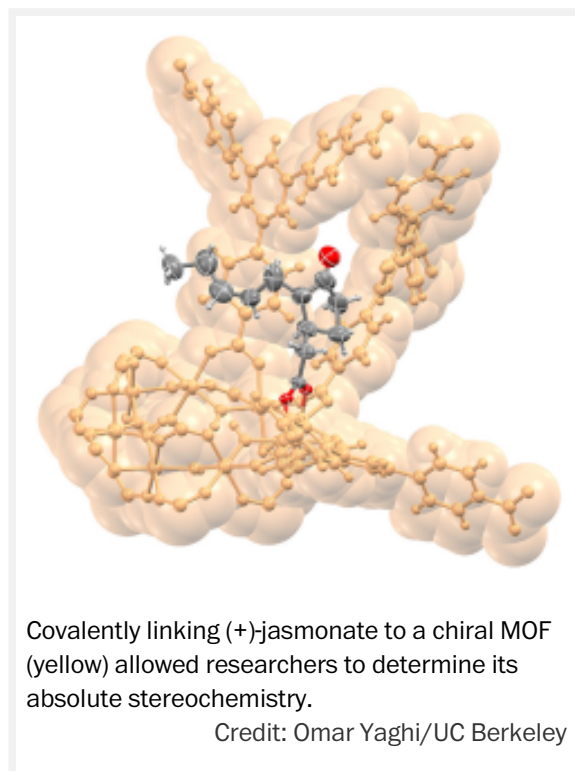
ensures the molecules align within the MOF, making it easier to solve structures via X-ray analysis. The team demonstrated the technique works with primary alcohols, phenols, vicinal diols, and carboxylic acids. Using the chiral MOF makes it possible to determine absolute stereochemistry, as the researchers demonstrate with jasmonate, a compound for which no crystal structure had been reported previously.

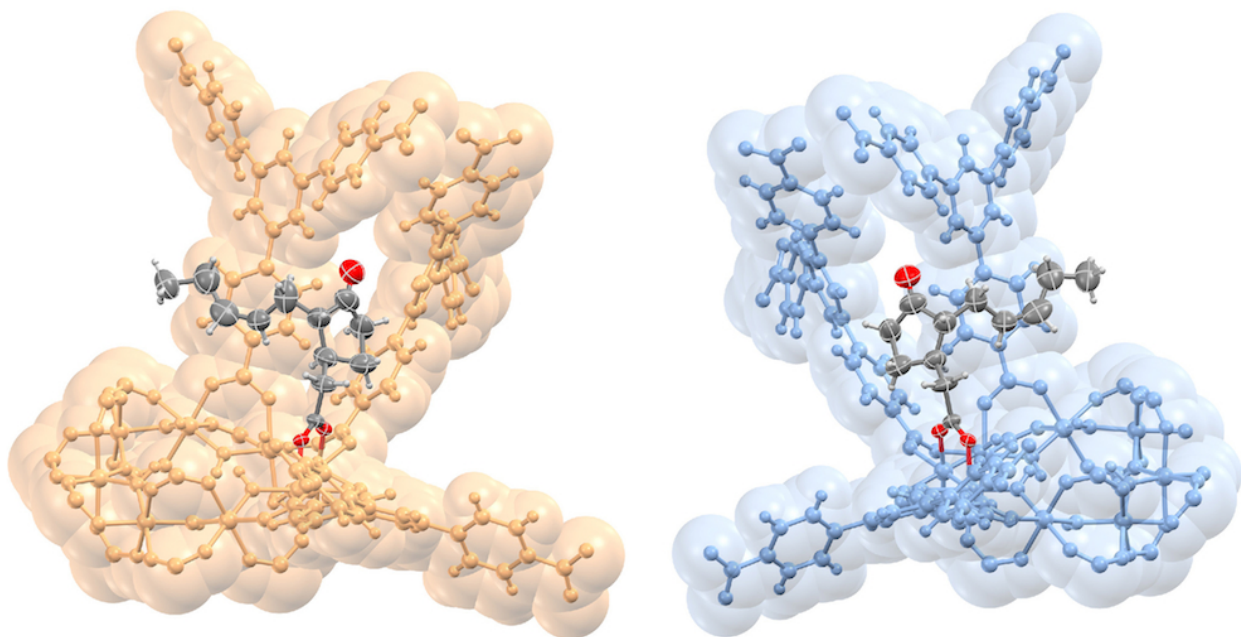Chemical & Engineering News
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A New Way to Display the 3-D Structure of Molecules

Metal-organic frameworks provide a new platform for solving the structure of hard-to-study samples

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Mirror, mirror: *This rendering shows opposite configurations in the molecular structure of a plant hormone called jasmonic acid (gray and red) that are bound to nanostructures (gold and blue) called MOFs, or metal-organic frameworks. (Credit: S. Lee, E. Kapustin, O. Yaghi/Berkeley Lab and UC Berkeley)*

Researchers at the Department of Energy's Lawrence Berkeley National Laboratory (Berkeley Lab) and UC Berkeley have created a sort of nanoscale display case that enables new atomic-scale views of hard-to-study chemical and biological samples.

Their work, published online Aug. 18 in the journal *Science*, could help to reveal new structural details for a range of challenging molecules—including complex chemical compounds and potentially new drugs—by stabilizing them inside sturdy structures known as metal-organic frameworks (MOFs).

The researchers introduced a series of different molecules that were chemically bound inside these porous MOFs, each measuring about 100 millionths of a meter across, and then used X-ray techniques to determine the precise molecular structure of the samples inside the MOFs.

The samples ranged from a simple alcohol to a complex plant hormone, and the new method, dubbed "CAL" for covalent alignment (the molecules form a type of chemical bond known as a covalent bond in the MOFs), enables researchers to determine the complete structure of a molecule from a single MOF crystal that contains the sample molecules in its pores.

The MOFs in the study, which are identical and are easy to manufacture in large numbers, provided a sort of backbone for the sample molecules that held them still for the X-ray studies—the molecules otherwise can be wobbly and difficult to stabilize. The researchers prepared the samples by dipping the MOFs into solutions containing different molecular mixes and then heating them until they crystallized.

"We wanted to demonstrate that any of these molecules, no matter how complex, can be incorporated and their structure determined inside the MOFs," said Omar Yaghi, a materials scientist at Berkeley Lab and chemistry professor at UC Berkeley who led the research.

The MOFs also possess a particular handedness known as "chirality"—like a left-handed person vs. a right-handed person—that selectively binds with molecular samples that also possess this handedness. The difference in a molecule's handedness is particularly important for pharmaceuticals, as it can mean the difference between a medicine and a poison.

"This is one of the holy grails: how to crystallize complex molecules, and to determine their chirality," Yaghi said.

Seungkyu Lee and Eugene A. Kapustin, Berkeley Lab researchers and UC Berkeley graduate students who participated in the latest work, said hard-to-study proteins, such as those important for drug development, are high-priority targets for the new technique.

"We are aiming for those molecules that have never been crystallized before," Kapustin said. "That's our next step. So we cannot only show the arrangement of atoms, but also the handedness of molecules, in which pharmaceutical companies are interested."
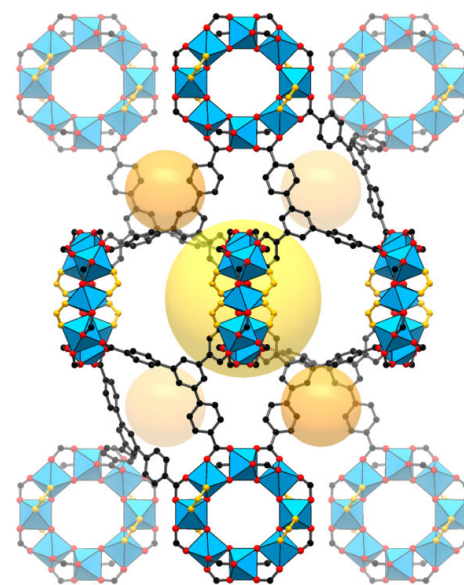
This illustration shows the structure of a nanostructure known as a metal-organic framework or MOF. The structure possesses a handedness (like a right-handed vs. left-handed person), known as "chirality," that enables researchers to identify the same kind of handedness in molecules that bind within it. (Credit: S. Lee, E. Kapustin, O. Yaghi/Berkeley Lab and UC Berkeley)

One of the best methods for studying any molecule's 3-D structure in atomic detail is to form it into a crystal. Then, researchers point intense X-ray light at the crystal, which produces a pattern of spots—like light off of a disco ball. Such patterns serve as a fingerprint for fully mapping the molecule's 3-D structure.

Some molecules are difficult to form into crystals, though, and the process of crystallizing a single molecule can in some cases involve years of effort and expense.

"To crystallize a molecule typically involves a trial-and-error method," Yaghi said. "Every chemist and biologist has to submit to this process. But in this MOF material you don't need all that—it traps the molecule and orders it. It's a way to bypass that trial-and-error approach to crystallography."

Different types of MOFs, with different pore sizes, could be tested to find out which ones work best with different types of samples, Lee said.

Importantly, the MOFs in the latest study did not appear to distort the natural, intact structure of the molecules. Researchers say it's possible to determine the complete 3-D structure of a molecule even if the samples only fill about 30 percent of a MOF's pores.

Researchers determined the atomic structure of the MOFs and the bound molecules with X-rays at Berkeley Lab's Advanced Light Source (ALS), and they also studied the MOFs using a technique called nuclear magnetic resonance (NMR) at Berkeley Lab's Molecular Foundry.

In all, the researchers studied 16 different molecules bound inside the MOF pores, including a plant hormone called jasmonic acid whose chiral structure had never been directly determined before, other plant hormones known as gibberellins, methanol, and other acids and alcohols.

The metals in the MOF framework itself can actually serve to enhance the quality of the X-ray images, Kapustin said, adding that in one case the technique allowed researchers to distinguish between two nearly identical plant hormones based on the difference in a single atomic bond.

Researchers could see structural details down to hundredths of a nanometer—less than the diameter of some atoms. "You can see with such precision whether it is a double bond or a single bond, or if this is a carbon atom or some other atom," Lee said. "Once you bind a molecule in the MOF, you can learn the absolute structure very precisely since the chirality of the MOF serves as a reference during the structure refinement."

For more
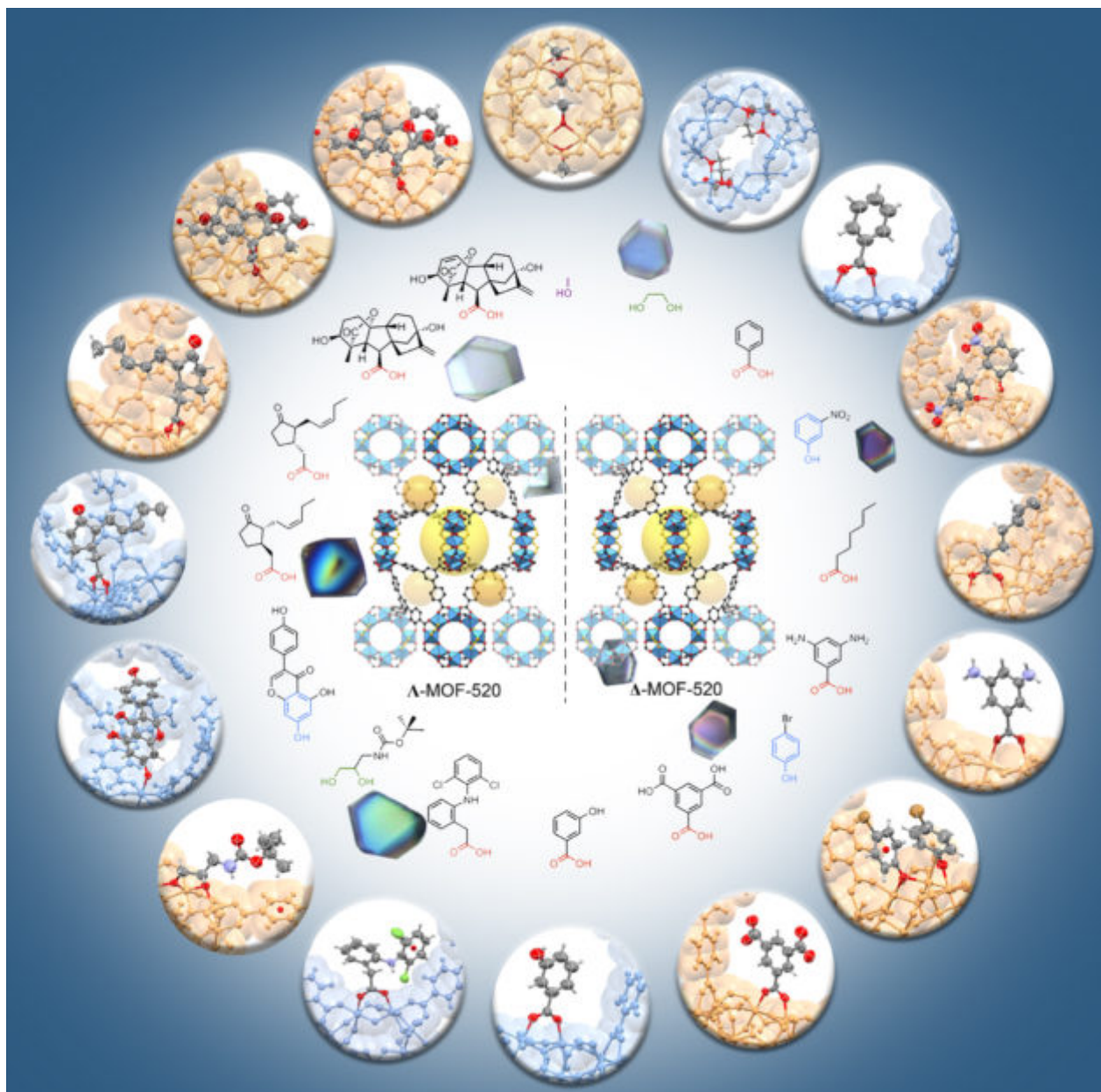
This illustration shows the structure of 16 molecules that were studied while bound to metal-organic frameworks (MOFs) that exhibit handedness. The frameworks stabilized the molecules for study with X-rays. (Credit: S. Lee, E. Kapustin, O. Yaghi/Berkeley Lab and UC Berkeley)

information about Omar Yaghi's research, visit http://yaghi.berkeley.edu/.

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excel at generalizing those patterns to new data. For instance, search engines use this technology to automatically label the contents of billions of internet photos.

The authors use a convolutional neural network to learn the relationship between millions of daytime satellite images (which are rich in detail) and nighttime images (where light areas are assumed to be wealthy). In this way, the network learns which features in the daytime imagery are indicative of economic activity (see the figure). Knowledge of those features enabled the authors to accurately reconstruct survey-based indicators of regional poverty, improving on results from simpler models that relied solely on nightlights or mobile phone data.

How might these results change the way that we measure and target poverty? Perhaps the most immediate application is as a source of inexpensive, interim national statistics. Jean *et al*.'s results indicate that a model trained in one country can be used in another, creating options for countries where no recent survey data exist. For social welfare programs, some of which already use satellite imagery to identify eligible recipients (*14*), higher-fidelity estimates of poverty can help to ensure that resources get to those with the greatest need.

Other applications are on the horizon. Remotely sourced satellite and mobile phone data are updated frequently and can be used to generate nearly real-time estimates of regional vulnerability. Once it is possible to estimate short-term changes in wealth and poverty, new approaches to program monitoring and impact evaluation will follow.

Considerable validation and calibration are required before proof-of-concept studies such as that of Jean *et al*. can be used in practice. However, as their study illustrates, there is exciting potential for adapting machine learning to fight poverty. As the economist Sendhil Mullainathan has asked, "Why should the financial services industry, where mere dollars are at stake, be using more advanced technologies than the aid industry, where human life is at stake" (*15*)? ∎

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10.1126/science.aah5217

Predicting poverty
Satellite images can be used to estimate wealth in remote regions.

Neural network learns features in satellite images that correlate with economic activity

Daytime satellite photos capture details of the landscape

Convolutional Neural Network (CNN) associates features from daytime photos with nightlight intensity

Road
Metal roof
Water
Concrete

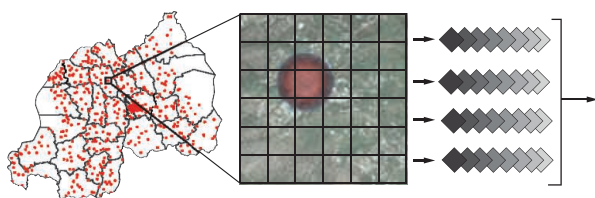**Satellite nightlights** are a proxy for economic activity
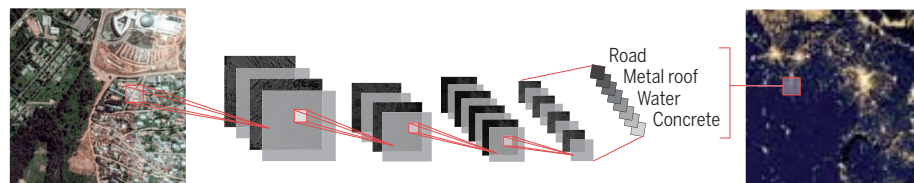
Daytime satellite images can be used to predict regional wealth

Household survey locations

CNN processes satellite photos of each survey site

Features from multiple photos are averaged

Ridge regression model reconstructs ground truth estimates of poverty

Now you see me too
Attaching chiral molecules to a chiral framework allows their molecular structures to be determined

By **Lars Öhrström**

Knowledge of three-dimensional (3D) molecular structures is crucial for scientific advances in fields ranging from materials chemistry to medicine. For solar cell materials, human proteins, or new drugs, the revelation of the exact arrangement of atoms and bonds vastly advances understanding of their properties. On page 808 of this issue, Lee *et al*. (*1*) report an approach that allows better structural data to be obtained for large, complex organic molecules that are difficult to crystallize on their own.

The method of choice to obtain structure information is single-crystal x-ray diffraction, a method so important that UNESCO declared 2014 the International Year of Crystallography. However, this method requires not only a pure substance, but also the ability to grow crystals of it—no crystals, no crystal structure data. The main complementary method, nuclear magnetic resonance, mainly provides structures of compounds in solution, often at great detail, but sometimes with inherent uncertainty, especially for chiral (handed) molecules with complicated stereochemistry.

Although long hours in the lab may produce crystals, some substances are notoriously difficult to crystallize or yield crystals with defects and disorder that prevent a complete structure determination. On the other hand, the molecular structures of small solvent molecules, trapped between the larger molecules that are the principal constituents of a specific crystal, are determined over and over again; for example, 1989 molecular structures of pyridine, C_5H_5N, are reported in the Cambridge Crystallographic Database (*2*). This occurs because the form and intermolecular interactions of the larger molecules sometimes generate voids in the crystal. Scientists

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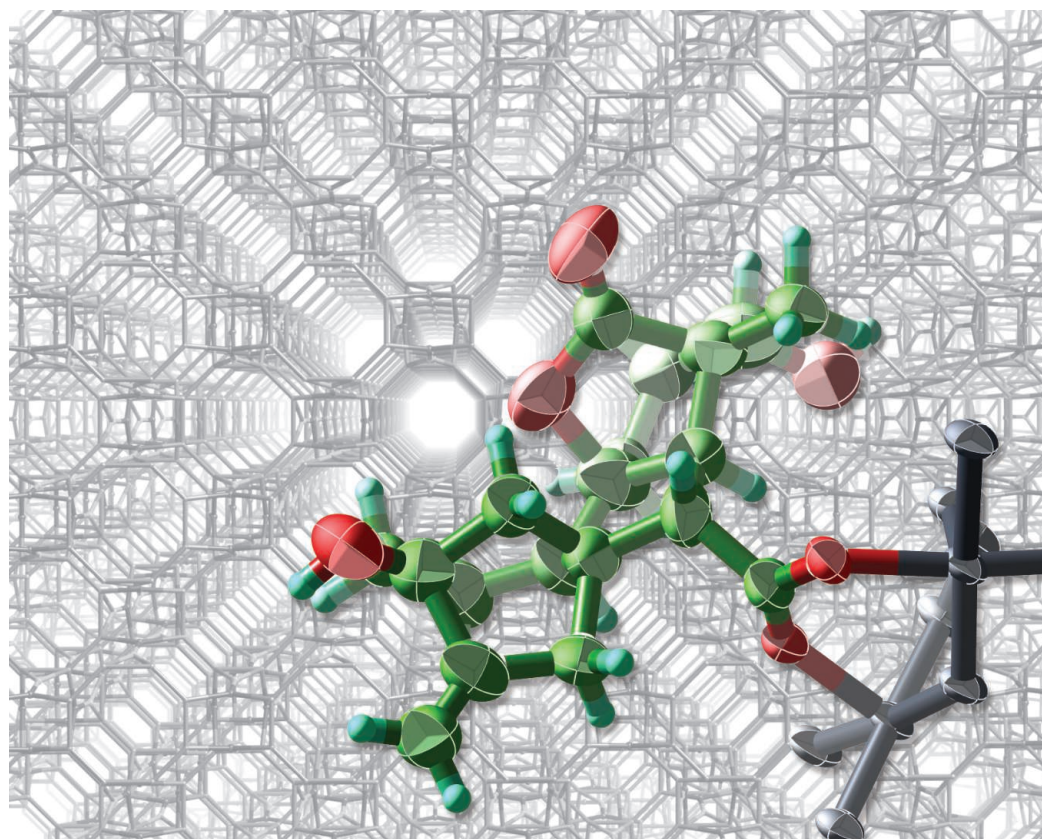
have therefore been exploring the idea that difficult-to-crystallize molecules could benefit from a similar approach if large enough voids could be deliberately engineered to trap the target molecules in.

An early example of such void engineering is the use of resorcinarenes and related substances (bowl-shaped molecules that assemble into hollow dimers) to encapsulate compounds and determine their structures (*3*). However, it was not until the discovery of coordination polymers and metal-organic frameworks (MOFs) (*4*) that a general protocol could be developed for the inclusion and structure determination of difficult-to-crystallize molecules.

MOFs consist of metal ions or clusters bridged by organic molecules (ligands) to form crystalline 3D networks with large potential voids and channels. First-generation MOF-based structure determination matrices were based solely on the void properties and are known as crystalline sponges (*5*). They work by soaking up the desired molecules from a

Lee *et al*. determined the structure of the plant hormone gibberellin A1 [carbon (green), hydrogen (light green), oxygen (red)] by trapping it inside MOF-520 (gray). The hormone is attached to aluminum ions (slate blue) in the MOF. The shapes of the atoms reflect data quality, with smaller and more spherical atoms indicating better precision (closer to attachment points to the MOF).

solution. Information on molecular chirality has been obtained from molecules trapped in crystalline sponges (*6*), but the crystalline sponges themselves are nonchiral. They therefore do not provide a frame of reference (like a system of left hands could easily distinguish between right- and left-handed gloves) for absolute chirality assignment. Also, the probed molecules are only weakly attached to the framework. This can result in large thermal motions in the crystal and thus less precise data.

Lee *et al*. present a substantial improvement in data quality by using MOF-520. The bridging ligand in this MOF, 1,3,5-benzenetribenzoate, has a propeller-like handedness. The MOF forms separate crystals of either chirality, even though the 3D network in itself, assigned the topology symbol "sum," is not intrinsically chiral (*7*, *8*) (a well-known achiral topology is that of diamond; a chiral topology is that of quartz). The chiral framework makes it much easier to determine the stereochemistry of the trapped molecule; this information is crucial for understanding its potential biological activity.

To create the crystals, the authors first impregnated MOF-520 with fresh solvent and then soaked it in a saturated solution of the target molecule. The latter substitutes the small formate ions (HCOO⁻) that are part of the original framework. The target molecule is thus firmly attached to the framework, reducing thermal motion and improving the precision of the data. However, it requires the probed substances to have a functional group that can be coordinated to a metal site. Fortunately, such functional groups are common in the molecules of interest.

> "*The approach...is an important advance, especially for difficult-to-crystallize natural products.*"

The approach reported by Lee *et al*. is an important advance, especially for difficult-to-crystallize natural products. However, having the molecular structure does not solve all crystallographic problems associated with a potential drug molecule. For legislation and patent reasons, a crystal structure of the pure compound or any of its pharmaceutically acceptable salts or co-crystals are also needed.

Beyond structure determination, Lee *et al*. show that MOFs could be used to exactly position molecular components. Such crystal engineering is, for example, of interest for solar energy applications (*9*), where it may reduce the need for costly covalent organic synthesis (*10*). Indeed, an early idea was that MOFs could be used to hang molecules on. Lee *et al*. show that MOF-520 provides both good hangers and a chiral wardrobe for complex molecular structures. ∎

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Now you see me too
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