A new method for clicking molecules together

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Scientists at EPFL have developed a quick and simple method for connecting and assembling new molecules together, paving a new road for synthetic chemistry, material science, chemical biology, and even drug discovery.

Thiols are sulfur-containing molecules found in most proteins of the human body. Characterized by their 'garlicky' smell, they also give coffee, sweat and the spray of skunks their unique odor. Because they are so widespread in biology, medicine and materials science, thiols are ideal targets for connecting molecules like drugs or polymers together, except that they must first be fitted with a chemical group that acts like an adaptor to other molecules. One of the most potentially useful of these 'adaptors' are the alkynes, which can be extremely powerful in bioconjugation. The problem is that adding alkynes to thiols ('alkynylation') has been too difficult to be of any use outside the lab. But now, in a patent-pending publication in the *Journal of the American Chemical Society*, EPFL scientists have found a way to make the connection in five minutes and at room temperature, paving the way to commercial, medical and perhaps industrial applications.

Thiol alkynylation is part of 'modular' or 'click' chemistry, a field that has far-reaching applications in drug discovery, nanotechnology and bioengineering. The idea is to synthesize large molecules by quickly "clicking together" smaller ones, allowing us to change previously-inaccessible complex molecules. Although relatively new, click chemistry is actually inspired by natural processes. For example, after being synthesized in the cell, proteins are further modified with

carbohydrates and lipids to change their properties. This process, known as post-translational modification, involves a whole host of complex enzymes that can be sidestepped in the lab by using click chemistry methods.

In order to achieve alkynylation, researchers from the group of Jerôme Waser at EPFL used an alkyne-transfer reagent called TIPS-ethynyl-benziodoxolone (TIPS-EBX), which they have made commercially available by collaborating with Sigma-Aldrich. The EPFL researchers had found that previous alkynylation reactions were limited by slow reaction rates and also needed a relatively unstable gold catalyst. By using TIPS-EBX, the researchers found that they were able to alkynylate thiols with very short reaction times and without having to use any metal catalyst.

TIPS-EBX is a highly reactive microcrystalline powder (like sugar) that is stirred in a liquid to make a solution. When the solution was mixed in with alkynes and thiols, it proved to be excellent in 'clicking' them together. Because of its structure, TIPS-EBX is also considerably more stable than previous alkynylation reagents. But the real benefit for Waser's group was that TIPS-EBX allowed them to carry out the thiol alkynylation reactions within five minutes, at room temperature (23oC) and in open-flask conditions, meaning that this previously challenging transformation can now be performed quickly, safely and under normal conditions.

Thiols are important building blocks in the synthesis of natural, pharmaceutical and medicinal products, but they also contain chemical components which could obstruct the alkynylation reaction. To confirm their new alkynylation method, Waser's group tested it on a range of thiols that contained the most common and reactive chemical groups present in bioactive molecules. Of the 35 thiols tested, 29 produced yield above 92%, confirming that the TIPS-EBX reaction can be applied

across a wide range of thiols – even those in human proteins, which is expected to impact biotechnology as we know it. Waser says: "Introducing an alkyne into thiol-containing molecules allows to further modify them in order to make future bioconjugates".

The breakthrough has enormous implications for chemical biology, drug design and materials science, and the researchers have even filed a patent on the method. Following their success with alkynylating a cysteine amino acid, the group is now testing their method on actual proteins, attempting to change their properties by modifying associated molecules like lipids, glucose derivatives and even synthetic polymers.

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