New cyclization reactions for synthesizing macrocyclic drug leads

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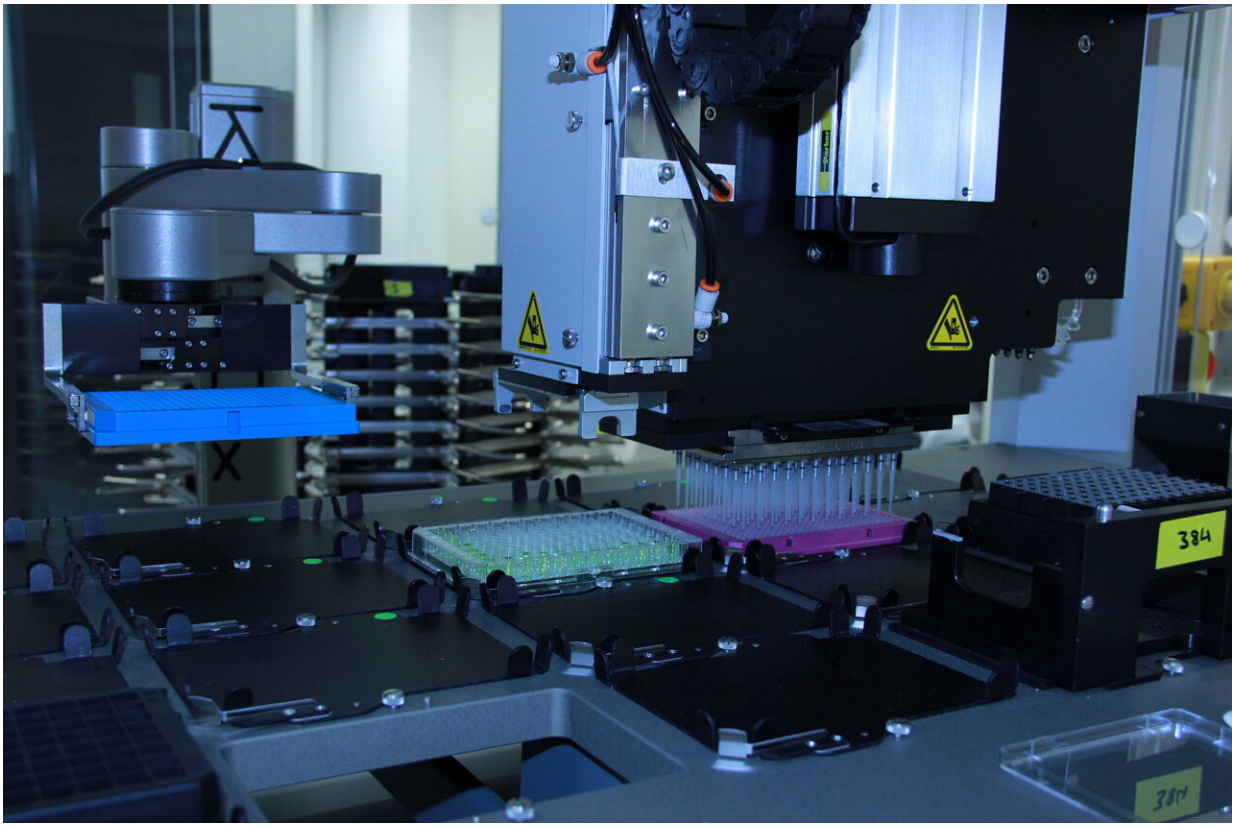


Integrated robotic workstation used at EPFL's Biomolecular Screening Facility for performing combinatorial synthesis of macrocycles and subsequent high-throughput target-based screening assays. Credit: Antoine Gibelin (EPFL)

Scientists at EPFL have developed a new method to synthesize and

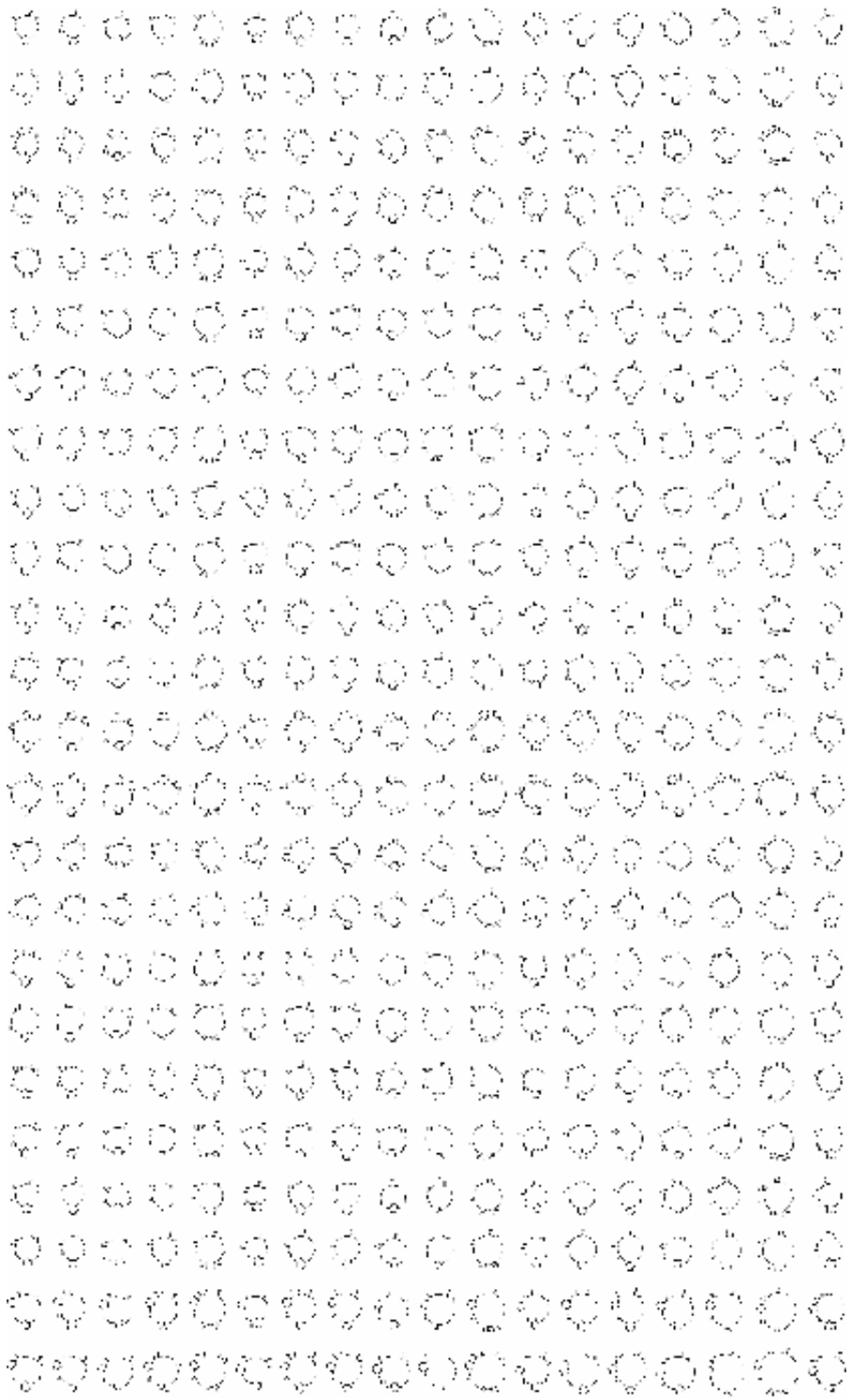
screen thousands of macrocyclic compounds, a family of chemicals that are of great interest in the pharmaceutical industry. The study is published in *Science Advances*.

Macrocyclic compounds are ring-shaped molecules made by connecting two ends of linear molecules. One of their unique and exciting properties is that their cyclical configuration reduces their flexibility, which means that macrocycles need less energy to bind targets than conventional small molecules.

In fact, macrocycles show a great ability to bind difficult targets that have flat, featureless surfaces. This has raised tremendous interest in the pharmaceutical industry, which is particularly interested in macrocyclic compounds with a molecular weight below 1 KDa, which would be small enough to cross the cell membrane and reach intracellular disease targets, e.g. proteins or even genes in the cell.

Still, there is a hurdle: there aren't enough suitable macrocycle libraries or methods to generate such small macrocycles. The compound libraries that pharmaceutical companies use today in high-throughput screens do contain 1-2 million different molecules, but those are mostly classical small molecules and only a handful are actual macrocyclics—at most, only a few hundred. This is too small a number for the screens to yield good hits when searching for possible drug candidates against challenging disease targets.

Now, scientists at EPFL have found a way to generate libraries of more than 9,000 of macrocyclic molecules below 1 KDa, all with high structural diversity. "Initially, what we wanted to do is generate orally available or cell-permeable macrocyclic drugs," says Professor Christian Heinis, whose lab led the study.



Chemical structures of macrocycle backbones illustrating the large structural diversity of the newly generated macrocyclic compound libraries. Credit: Dr. Sangram Kale (Heinis lab, EPFL)

The libraries were generated by "cyclizing" short linear peptides in combination with diverse linker reagents, which promote chemical bonding. The yields of the macrocyclization reactions turned out to be so efficient that there was no need for purification. And in a key breakthrough, the new method also led to the discovery of surprisingly efficient macrocyclization reactions based on the ligation of thiol and amino groups of short peptides.

The work was supported by EPFL's Biomolecular Screening Facility (BSF), headed by Gerardo Turcatti. "EPFL has already developed the liquid handling processes to perform the combinatorial synthesis and to screen the macrocyclic compound libraries," he says. Screening identified binders of different disease targets, including inhibitors of thrombin, an important target of coagulation disorders. X-ray structure analysis of a thrombin inhibitor by partners in Italy showed a snug fit of the macrocycle to its target.

Heinis's lab is now further developing the macrocycle synthesis approach in order to screen even larger combinatorial libraries. Working closely with the BSF with the support of NCCR Chemical Biology, the next step is to generate macrocyclic inhibitors of intracellular protein-protein interactions, for which we currently have no good inhibitors.

More information: "Thiol-to-amine cyclization reaction enables screening of large libraries of macrocyclic compounds and the generation of sub-kDa ligands" *Science Advances* (2019). DOI: 10.1126/sciadv.aaw2851 , advances.sciencemag.org/content/5/8/eaaw2851

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