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Single chip tests thousands of enzyme mutations at once

The technique vastly speeds up understanding of how the proteins function and how to target drugs.

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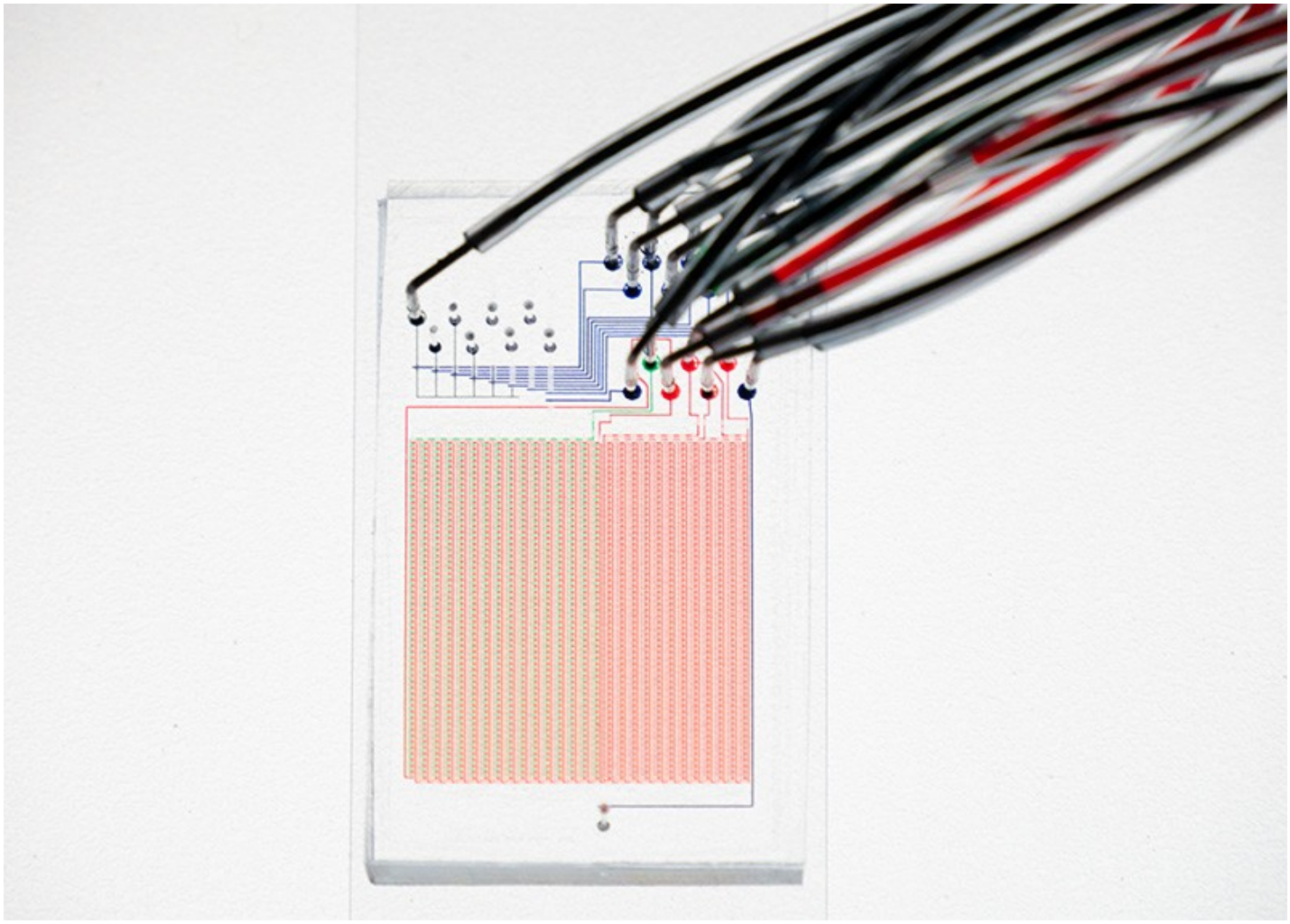

The silicone microfluidic chip has an array of 1,568 reaction chambers. Channels etched in the chip connect the chambers to control pipes attached to the top. Credit: Daniel Mokhtari

Figuring out how a protein or enzyme works, and understanding how genetic mutations affect these molecules that are fundamental to life, can often take years. Researchers must alter hundreds of the molecule's amino acid building blocks one by one, produce each mutated enzyme in the lab and test how each mutation affects the enzyme's ability to carry out its job.

Now, a glass chip etched with tiny channels could reduce that time to mere hours by allowing researchers to test more than 1,000 mutations at a time. A 22 July paper[1] in *Science* describes how the new system, called High-Throughput Microfluidic Enzyme Kinetics (HT-MEK), could provide a faster way for scientists to study disease-causing proteins, develop enzymes that break down environmental toxins and understand the evolutionary relationships between different species.

To develop HT-MEK, bioengineer Polly Fordyce and biochemist Daniel Herschlag at Stanford University in California and their colleagues worked for six years, ending up with a US$10 chip about 7 cm^2 in size. The chip contains 1,568 tiny wells that can each contain a mutated version of the enzyme, and a microfluidic system that delivers reagents to all the mutants at the same time.

To test the system, Fordyce and Herschlag chose a bacterial enzyme called PafA that is involved in modifying other proteins. They created a 'library' of different mutant enzymes by designing DNA sequences in which each of PafA's 526 amino acids was swapped for a different amino acid. A robot put these DNA sequences into individual wells on the chip, then added reagents that allowed the proteins to be produced. The chip then added a chemical that gives off light when it is processed by PafA. A scanner measured the amount of light given off by the chemical: mutations that made PafA less effective made the enzyme produce less light.

Rather than simply telling the researchers whether the experiment worked or not, the platform allowed them to examine the speed at which each mutant enzyme carried out the reaction and determine how chemicals or pH changes affected the way the enzyme folds and functions. "It was like being able to take off the cover of the protein and kind of look inside and see an architectural drawing," Fordyce says.

Meet the neighbours

Because it can screen so many mutants at a time, the system could allow researchers to look beyond mutations in the active site — the part of an enzyme that actually carries out its main function and usually attracts the most research attention. Mutations in other regions might still affect an enzyme's function by changing the way it folds or binds to other proteins, for instance. HT-MEK identified 161 such sites on PafA. The extent of the mutations' impact was surprising, says Herschlag, who has spent many years studying the enzyme. "It's like you really know your neighbourhood where you live, but you never leave," he says. "You realize that there are all these different neighbourhoods and all these different effects."

He and Fordyce say that being able to identify the functions of these distant mutations might allow researchers to target enzymes that are considered 'undruggable' because their active sites are structurally similar to those of other, healthy enzymes. Figuring out which additional regions help a cancer-causing enzyme function, for instance, might lead to the development of drugs that target those regions instead.

"It's a really impressively large amount of work," says Douglas Fowler, a protein scientist at the University of Washington in Seattle. "It'll be exciting to see where this technology goes, the scale-up here is pretty impressive." He expects that HT-MEK will make many tasks easier and faster, but that it remains to be seen whether the system will work as well for every type of enzyme as it did for PafA.

Although the instructions for building a HT-MEK system have been published online[2], Fordyce and Herschlag hope to create a centre where researchers can come to test the enzymes they're interested in. Herschlag says he is especially excited about the potential to understand how certain genetic mutations lead to diseases and how these mutant enzymes can be targeted with drugs. If researchers can analyse the functions of these mutations more quickly, he says, "we'll be creating the knowledge necessary for going from the molecular changes to ultimately predicting disease outcomes".

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References

1. Markin, C. J. *et al. Science* **373**, eabf8761 (2021).

2. Markin, C. J. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2020.11.24.383182 (2021).