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Tiny Laboratory Enables Huge Leaps in Mapping Protein Function

Howard Hughes Medical Institute researchers have designed a laboratory about the size of a quarter that is capable of conducting thousands of experiments simultaneously to measure how specialized proteins bind their DNA targets. This tool provides a new way of measuring the activity and function of proteins. Having those measurements may help scientists predict the behavior of individual proteins in biological systems without making any direct measurements on model organisms.

Howard Hughes Medical Institute investigator Stephen Quake, whose lab is at Stanford University, and co-author Sebastian Maerkl published a research article describing their new approach in the January 12, 2007, issue of the journal *Science*.

"To test theories of systems biology, we should now be able to predict biology without making any measurements on the organism itself."

— **Stephen R. Quake**

The goal of systems biology is to understand how biological networks act in concert to enable an organism to function, said Quake. Very powerful high-throughput methods have been developed to map the network topologies, revealing what interacts with what. But if you point to a particular node on the network and ask how it is functioning, frequently all you can say is that there is some interaction going on. You rarely have the biochemical depth of knowledge to properly understand network function from the bottom up.

We ought to be able to make biophysical characterizations of all these protein machines, and if we understand how these machines work, then, in principle, the only other information we need to predict how the organism works is a blueprint from the genome, he said. The microlaboratory that Quake and Maerkl have developed could help bring researchers closer to this goal.

To understand complex biological systems and predict their behavior under particular circumstances, it is essential to characterize molecular interactions in a quantitative way, Quake said. Binding energy—the energy with which one

protein bind to another or to DNA—is one important quantitative measurement researchers would like to know. But these interactions are highly transient and often involve extremely low binding affinities, so they are difficult to measure on a large scale. To overcome this hurdle, Quake and Maerkl set out to develop a microlaboratory that could trap a type of protein known as a transcription factor. Once the transcription factor was trapped, the scientists hoped to measure the binding energy of the transcription factor bound to specific DNA sequences.

But simply measuring the binding energy between a transcription factor and a single DNA sequence is not enough, Quake said. He said it would be more meaningful to know the energy involved in a transcription factor binding to many different DNA sequences. This would give researchers a more complete picture of the DNA binding energy landscape of each transcription factor.

To determine the binding energy landscape, Quake and Maerkl's microlaboratory needed to conduct thousands of binding-energy experiments at once. The apparatus they created, which they called mechanically induced trapping of molecular interactions (MITOMI), consists of 2,400 individual reaction chambers, each controlled by two valves and including a button membrane. Each of the chambers is less than a nanoliter in volume. That's one-billionth of a liter—enough to hold a snippet of human hair only as long as the hair's diameter. The MITOMI apparatus fits over a 2,400-unit DNA microarray, or gene chip, onto which the researchers can dab minute amounts of DNA sequences. Each spot of DNA is then enclosed in its own reaction chamber.

The researchers constructed the MITOMI apparatus by first producing silicon molds using the same photolithography process used to make microelectronic circuits. They then cast the elements of the MITOMI apparatus in rubber, before bonding them together to create the finished device.

The MITOMI process begins by pumping a transcription factor into the chambers containing the thousands of slightly varied DNA sequences anchored to the microarray chip. There, the transcription factor interacts with the DNA. The scientists then lower the button membrane in each chamber onto the microarray surface, physically trapping the transcription factors that have bound to the DNA. The researchers can then conduct measurements that reveal the binding energy of the trapped transcription factor. Quake emphasized that MITOMI can be used to measure the interactions between any two proteins, as well as between a protein and DNA.

To demonstrate the technique, Quake and Maerkl characterized the binding energy landscape of two versions of a human transcription factor called MAX, as well as two yeast transcription factors, called Pho4p and Cbf1p. They chose the transcription factors because they represent a large family of proteins that have a characteristic structure called a basic helix-loop-helix. This family regulates genes involved in an array of cellular processes ranging from cell proliferation and development to metabolism. The MAX

transcription factors also play a role in some cancers.

Quake said the MITOMI technique enabled them to predict the biological function of these transcription factors purely from the physical measurements of the binding energies. We discovered a wealth of interesting things—to me the most important being that using the binding energy data for different sequences, we could predict which genes the yeast transcription factors would regulate, he said.

Quake and Maerkl also used MITOMI to test the widely held assumption that each nucleotide unit of a DNA sequence functions independently in contributing to the binding of a transcription factor. Their measurements of how the transcription factors bound to a multitude of slightly different DNA sequences revealed a flaw in that assumption: They showed that the nucleotides act cooperatively in establishing the binding energy of the transcription factor.

As a rapid, high-throughput technique for measuring binding between proteins, MITOMI is a powerful new approach to mapping biological networks, said Quake. We would like to use this technique to map all the protein-protein binding energies in an organism. For even a small bacterium, this means millions of interactions, but with MITOMI, we can hope to accomplish such measurements, he said. The next effort in his laboratory will be to use measurements of the binding energy of unknown transcription factors as clues to identify the genes they regulate.

According to Quake, MITOMI brings scientists closer to an important goal. To test theories of systems biology, we should now be able to predict biology without making any measurements on the organism itself, he said.