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A New Way to Count

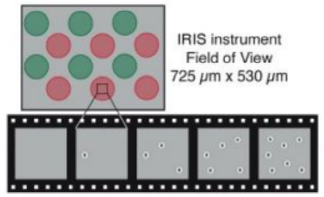
important yet diverse molecules, such as micro-RNAs.

"Our new method allows us to step past the trade-off of sensitivity versus specificity," says Derin Sevenler, a postdoctoral fellow in Ünlü's lab. "By recording the duration of the individual binding events on video, we can better determine which were the target molecule and eliminate the signal from other non-target molecules."

The work described in the *PNAS* paper builds on Sevenler's years-long thesis work published in *ACS Nano* last year. That paper describes a new digital microarray technique that can detect single molecules using gold nanorods; it's comparable to other state-of-the-art, single-molecule detection systems called digital counting. This new work uses that technique plus an algorithm to process video of the reaction.

The algorithm sorts each binding event and determines if it was the right molecule that bound. And like all other assays, this one must be calibrated. By documenting the number of binding events with solutions where the concentrations of the target molecules are already known, the researchers can build a dataset. Then, when the algorithm determines the number of binding events per hour, it can relate that back to the data and give a readout of the concentration of target molecules.

In the *PNAS* paper, the researchers added this video-processing algorithm to dynamically track single-binding events, improving the technique from the *ACS Nano* paper. When the new results were compared to the results from using only the previous method without the video-processing algorithm, they showed that their technique can detect a much smaller concentration—36 times smaller—than it could before.



Individual molecules bind to an atomically flat surface and are detected with Interference Reflectance Imaging Sensing (IRIS). Each molecule is labeled with a gold nanoparticle, which is visible as a faint dark spot when it contacts the surface. Above is a graphical representation of how the IRIS software tracks how long these binding events last before the bond between the molecule and the surface breaks: each event is circled in a different color.

Traditional endpoint assays like ELISA can't compare to this dynamic-tracking technique because they can't detect single molecules. And dynamic tracking not only outperforms previous digital counting methods because of its 36-times better sensitivity, it also has a wide field of view and can see 12 different reactions at once.

This level of detection could open the door to accurately see micro- and messenger-RNA, and single-strand biomarkers in ultra-low concentrations, applications that could be used for cancer diagnostics, pre-symptomatic viral infection detection and determining how susceptible an infection is to an antibiotic. ■

Innovation through Bio-Inspired Metamaterials

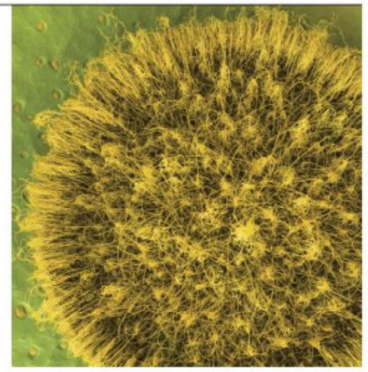
Professor Xin Zhang (ME, ECE, BME, MSE) is known for her pioneering work in building metamaterials, artificially designed materials that have properties not found in nature. Now, Aobo Li, a doctoral student in Zhang's lab, has flipped that concept around to study the properties and functions of a natural material to improve on metamaterial designs.

Li chose to study a type of unicellular algae called diatoms. Their cell walls, or frustules, are made of silica and are hard, like a shell; depending on the species, these shells vary in shape, size and other physical characteristics. In work published in *Small* and *Advanced Functional Materials*, both as cover images, Zhang, Li and collaborators studied a particular diatom's structure, used it as a scaffold to grow nanowires and, most recently, mimicked its structural pattern when making their own metamaterials.

"A lot of researchers find inspiration in their daily lives. Sometimes it's a random occurrence or thought that connects two ideas together, or sometimes it's more purposeful and they choose to study a phenomenon they become curious about," Zhang notes. "In my field, that typically isn't the case, but Aobo has shown how we can expand our inspiration and draw from natural elements and perhaps innovate in ways we couldn't before."

As an engineer, Zhang is accustomed to building for a purpose, maybe to fill a gap in current technology. Li has brought science into the lab, seeking answers for the sake of curiosity and then turning those answers into specific purposes.

After understanding the diatom's properties and its unique structure, they chose to mimic those properties in making the completely artificial metamaterial. The frustule they used as inspiration has a hierarchical pattern of hexagonally spread pores; each pore is inside a group of hexagonally arranged pores as the pores get smaller and smaller. The original frustule looks like a honeycomb, an intricate, repeating pattern of tiny holes arranged in a hexagonal pattern even as they get smaller.



Gold nanowires grow on the frustule of the diatom.

Other researchers have attempted to make metamaterials with hexagonally arranged pores, but instead of arranging those hexagons in hexagons, they arranged them in lines. Preserving the hierarchical pattern in a metamaterial gave Zhang and Li a metamaterial with new properties and the potential to create broadband infrared absorbers. Although this offers a practical application, the biggest takeaway from their research investigating is why this new metamaterial is such a good infrared absorber—and that comes back to the bio-inspired hierarchical patterns of the pores in the metamaterial. ■

IMAGES COURTESY OF *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES* & *SMALL*

Strength in Numbers

SYNTHETIC BIOLOGISTS ADD COOPERATIVE MOLECULES TO THEIR TOOLKIT

Elaborate molecular networks inside living cells enable them to sense and process many signals from the environment in order to perform desired cellular functions. Synthetic biologists have been able to reconstruct and mimic simpler forms of this cellular signal processing, but now, a new toolset powered by self-assembling molecules and predictive modeling will allow researchers to construct the complex computation and signal processing found in eukaryotic organisms, including human cells.

This work by **Associate Professor Ahmad 'Mo' Khalil** (BME), Assistant Professor Caleb Bashor of Rice University, BME graduate student Nikit Patel, and other colleagues at MIT, Harvard, the Broad Institute and Brandeis University has been published in *Science*.

The type of combinatorial signal processing that they've been able to engineer synthetically is what cells naturally and elegantly do to enable intricate tasks, like those in embryonic development and differentiation.

"By taking a common principle that we know exists in nature—the ability for regulatory molecules to collaborate and form higher-order assemblies—you can program cells to execute very difficult computational and combinatorial problems," Khalil explains. "This represents a very different way of engineering genetic circuits than is traditionally done, and it can open up a new class of cellular functions that we can mimic and control."

First, the researchers built a library of simple, synthetic molecular components that can interact with one another—each of these components has its own unique chemical and kinetic makeup, which can be used to understand its behavior. Using these known properties, they constructed a quantitative model that can predict how different combinations of those molecules come together to build higher-order assemblies. They could then use that predictive model as a guide to design genetic circuits that take advantage of combinatorial assembly to execute desired signal processing functions.

The complex itself is made up of three components: a synthetic transcription factor that controls gene activation; the sequence of DNA where that transcription factor binds; and a synthetic "clamp" molecule that secures the three pieces together. That complex can allow them to tune the strength of the cell's response to an input signal, and shut the response on and off at desired times.

But it's also much more than that.

"What we are harnessing and trying to build is one of the most powerful and universal features of biology: cooperativity," Khalil points out. "One way to think about cooperativity is that it allows for the whole to be greater than the sum of its parts."

"You can think about cooperativity as the same type of signal-processing feature that gives you an analog-to-digital converter," Bashor adds. "An analog-to-digital converter takes something that's basically linear and turns it into something switch-like."

Using their system, engineered cells are made to produce assembly components in response to a desired chemical or environmental input. In one experiment, they programmed yeast cells to respond to two different chemicals, and specifically to respond to varying concentrations of the two chemicals in either analog or digital fashion.

In the analog circuits, the response is continuous; if a small concentration of either or both chemicals is present, there is a graded response. But in the digital circuits, there is an all-or-nothing, discrete response—like a signal conversion to binary code, which is only comprised of 0s and 1s, off and on.

By taking advantage of their new ability to adjust the cooperativity between assembly components, they showed that they could convert the cell's response from dull to sharp—the more cooperative the complex, the sharper the response. The sharpness of a response—how acutely and intensely a system responds when a signal reaches a critical threshold—is key for digital signal processing.

"Engineering this type of response into transcription factors was central for allowing us to program cells to perform a diverse array of complex functions, such as Boolean logic, time-dependent filtering and even frequency decoding," Khalil notes.

Associate Professor Ahmad 'Mo' Khalil

That upgrade from analog to digital is the culmination of years of research. The analog-to-digital converter and their other synthetic gene circuits can be used to explore and manipulate the regulatory programs that guide immune and stem cell functions, with the ultimate goal of developing transformational cell-based therapeutics from engineered human cells.

"It's well known that nature has perfected very powerful information processing with only a small number of parts, but deconvoluting precisely how this works is virtually impossible in human cells due to their complexity," Dr. Tom Ellis (who was not part of the research) says. "By re-creating the way human cells process information at the DNA level, but in a simple yeast cell model with synthetic parts, they have been able to re-create complex signaling from first principles. This is an excellent example of how thinking like an engineer can unlock a new way to answer major biology questions." ■