

Engineers build DNA 'nanotowers' with enzyme tools

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Duke engineers have added a new construction tool to their bio-nanofabrication toolbox. Using an enzyme called TdTase, engineers can vertically extend short DNA chains attached to nanometer-sized gold plates. This advance adds new capability to the field of bio-nanomanufacturing.

"The process works like stacking Legos to make a tower and is an important step toward creating functional nanostructures out of biological materials," said Ashutosh Chilkoti, associate professor of biomedical engineering at Duke's Pratt School of Engineering.

The prefix nano means a billionth and refers to the billionth-of-a-meter scale of such structures.

Last year, Chilkoti and his team demonstrated an enzyme-driven process to "carve" nanoscale troughs into a field of DNA strands. By combining this technique with the new method of adding vertical length to the DNA strands, they can now create surfaces with three-dimensional topography.

"The development of bio-nanotechnological tools and fabrication strategies, as demonstrated here, will ultimately allow the automated study of biology at the molecular scale and will drive our discovery and understanding of the basic molecular machinery that defines life," said Stefan Zauscher, assistant professor of mechanical engineering and materials science.

This research was published online on Sept. 27, 2005, and will be published in the print Journal of the American Chemical Society (JACS). The authors include Chilkoti, Zauscher, postdoctoral fellow Dominic Chow and graduate student Woo-Kyung Lee.

"Compared with semi-conductor fabrication, bio-nanomanufacturing is in the stone age. There are few tools for working with bio building blocks that work well in water, the natural milieu of biomolecules," Chilkoti said. "And it makes little sense to blindly copy the semi-conductor industry because their techniques don't work with water-based materials," he said. "So Duke is creating the tools that will make bio-manufacturing possible at an industrial scale."

The team starts with a forest of short DNA strands that cover nanoscale patches of gold, lithographed onto a silicon substrate. The researchers then submerge the substrate in a solution that contains the TdTase (terminal deoxynucleotidyl transferase) enzyme, a cobalt catalyst and the molecular building blocks, called nucleotides, of DNA chains.

Over an hour, the TdTase enzyme grabs the free-floating nucleotides and builds nanoscale "towers" above the surface by extending each DNA strand, increasing its height a hundredfold. In addition, the process works at room temperature in an incubator that maintains humidity, Chilkoti said.

"Working with water-based biological materials requires a humidity-controlled environment, but it is a plus for industry that this surface-initiated polymerization works at room temperature. No special heating or cooling is needed," he said.

"The process is like a surface-initiated polymerization reaction in polymer chemistry, with the important difference that it uses biological materials and is enzymatically catalyzed," adds Zauscher. "Developing

the tools to harness biological reactions on the molecular scale opens a whole new arena for materials syntheses."

Biologists have known about the TdTase enzyme for decades, but it has only been used for a few specialized tasks in molecular biology, Chilkoti said. His group was interested in the enzyme because it doesn't just copy DNA, it builds DNA.

"Biologists call the TdTase enzyme promiscuous because it just builds and builds using whatever is available. We now recognize the enzyme offers us fabulous flexibility for bioengineering. We can use it with any sequence of DNA we need," Chilkoti said.

The Duke team sees enzymes as a rich source of tools for bio-nanomanufacturing. "Enzymes are the body's production factories, so it makes sense to copy nature's tools and use them in much the same way. We are trying to bring as many different enzymes as possible to bear on the biomanufacturing problem," Chilkoti said. "The new fabrication strategy allows exquisite control over the structure and composition of the DNA nanostructures, a prospect that offers interesting possibilities for bionanofabrication as it allows specific molecular adapters to be encoded along the vertical direction of the DNA chains," said Zauscher.

Chilkoti said the next step towards bio-nanofabrication is to create a little crane to pick up, move and place biological molecules in precise locations on three-dimensional DNA surfaces.

"When we can place molecules in the right configuration, then we can get them to function. At that point, we can design and create biological machines that accomplish something," he said.

The article is available at: [pubs3.acs.org/acs/journals/doi ...
oi=10.1021/ja052491z](https://pubs3.acs.org/acs/journals/doi/10.1021/ja052491z)

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