

Engineers building 'erasable' detectors, 'nanobrushes' and DNA 'highrises'

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A Duke University engineering group is doing pioneering work at very diminutive dimensions. Their basic studies could lead to genetically engineered proteins that can form erasable chemical detectors; self-grown forests of molecular "bottlebrushes" that keep themselves contamination-free; and auto-assembled DNA "towers" that could become anchors for the tiniest of devices.

Professor of biomedical engineering Ashutosh Chilkoti of Duke's Pratt School of Engineering will describe such advances in designing bio-detectors and structures scaled in the millionths and billionths of a meter in a Wednesday, March 29, 2006, talk at the American Chemical Society's 231st national meeting in Atlanta.

The proposed erasable detectors are made of artificial elastin-like polypeptides (ELPs), which are short segments of proteins normally soluble in water. Crafted through genetic engineering with the aid of bacteria, such ELPs have the useful property of coming out of a solution to form a solid whenever a slight temperature increase or other alterations to the water induces a phase change.

Chilkoti's group reported in the November 1999 issue of the journal *Nature Biotechnology* that an ELP could also be chemically linked with another protein so that both "fusion proteins" leave solution together after such phase changes.

Following that discovery, for which Duke has applied for a patent,

Chilkoti's team reported in the February 2003 issue of *Analytical Chemistry* that this method could be used to create a "reversible" protein sensor on a glass slide.

After dotting such a slide with microscopic amounts of surface-bound ELPs, the researchers discovered that dissolved fusion proteins would selectively attach to those microdots upon leaving the solution.

They also found the "captured" fusion proteins could pull other select proteins from solution so those could be chemically identified. Finally, they confirmed that microdot array could then be wiped clean of all attached proteins simply by "reversing the phase transition," Chilkoti said in an interview.

In this case, the researchers added salt to the solution to induce the same kind of phase changes as does raising the water temperature.

"It's a way of creating what I would call a cleanable surface for sensing," Chilkoti said. "We can create a surface for a sensor, do a binding reaction, detect a signal, then release everything. Then we could repeat the same process with the same fusion protein, or a different one."

But the dots used in that experiment were "microns" wide -- at the millionths of a meter scale. Chilkoti's team wondered if the process would also work at the thousand-times-smaller "nanometer" scale (billionths of a meter) to capture a few hundred individual molecules.

So they collaborated with Stefan Zauscher, a Duke assistant professor of mechanical engineering and materials science whose group has an Atomic Force Microscope that can deposit nanoscale amounts of material through a process called "dip pen nanolithography" (DPN).

Instead of using a glass slide, that collaboration fabricated a gold surface

on which to bind ELP nanodots because "DPN really works well on gold," Chilkoti said. Repeating the reversible phase change experiments to draw proteins from solution for detection, "we found it worked even better at the nanoscale," he added.

A major reason for their improved success is that the gold surface was specially modified to prevent stray proteins from attaching to the experimental array, he said. "There was nothing binding in the background, so we could get extraordinary reversibility. We would have a clean surface, and we could do it over and over."

The goal of keeping away stray proteins also motivated Chilkoti's group to grow forests of special 15-nanometer-high polymer brushes with fuzzy branches that could act as raised platforms on which to locate ELP protein sensors or other molecular sized devices.

In a paper in the February 2004 issue of the journal *Advanced Materials*, Chilkoti and colleagues described building such a "non-fouling" platform by inducing methyl methacrylate molecules to grow into tall stalks from a gold surface through a self-assembly process known as "atom transfer radical polymerization."

In the same process, molecules of polyethylene glycol (PEG) were also induced to form fuzzy branches extending from those stalks, creating the overall look of bottle brushes.

In this case, the PEG branches formed a protective barrier that kept unwanted proteins from coming out of solution and sticking to the platform. "PEG is the gold standard for making a film or coating that is protein resistant," Chilkoti said. "But it has been difficult to get it to work on a range of materials."

In an attempt to use nature's method to grow chain-like polymers,

Chilkoti's and Zauscher's laboratories are now exploring a method to build nanotowers of DNA -- the master molecule that makes up genes -- block by block from the surface.

In a paper published online on Sept. 27, 2005, in the *Journal of the American Chemical Society*, the Duke researchers described how the enzyme terminal deoxynucleotidyl transferase (TdTase) could be used to induce short DNA strands to form extensive chains. Those "polymerizing" chains, growing vertically from nanodots of gold patterned onto silicon, [assembled into tower-like structures](#).

The process worked in a solution of enzyme and DNA building blocks -- called nucleotides -- with the TdTase grabbing floating nucleotides and pulling those into the extending structure.

"We believe that TdTase-catalyzed surface-initiated polymerization of DNA will be a useful tool for the fabrication of complex biomolecular structures with nanoscale resolution," the researchers wrote.

Source: Duke University

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