

## Building a Better — But Still Tiny — Sensor

*DNA aptamers improve sensing ability of atomic force microscopy*

**F**or an atomic force microscope, familiarity can breed better images — in more ways than one. When the microscope's tip has a sensing molecule tethered to it, the molecule enables the device to recognize targets in the sample being scanned. That chemical familiarity also enables the microscope to perform biological imaging of complex samples in water, something it could not accomplish otherwise.

The standard choice for a sensing molecule has been an antibody, which helps pick out proteins for which it has an affinity. Now a group from Arizona State University in Tempe has used DNA aptamers — manufactured single-stranded DNA molecules. Stuart Lindsay, a professor of physics and chemistry and leader of the research team, noted that aptamers offer a significant advantage over the typical antibody approach because their chemistry is simpler. "The simpler chemistry seems to make everything work better," he said. "Better signal to noise, for example."

In atomic force microscopy, a stylus with a very sharp point is brought close to a sample surface. The tip is mounted on the end of a cantilever, and the interaction between the tip and the sample deflects the cantilever. That deflection is amplified by an optical lever in which a laser is fired at the cantilever and bounces off it; the reflected beam is detected by a photodiode. Because the lever arms are long, they magnify the deflection by a factor of thousands or more, making it possible to spot small movements and achieve molecular-scale imaging.

For biomolecular imaging, one of the biggest problems with this technique is that it is nearly impossible to distinguish between proteins, even if their molecular weight is very different, based on a topographical image alone. So researchers have attached sensor molecules, such as antibodies, to the tip of the stylus and bounced the probe up and down slightly. As the tip moves across the surface, the oscillation changes when the sensor binds to a target on the surface. This information



*Figure 1. A schematic, not to scale, shows an atomic force microscope probe with attached aptamer scanning a surface. Because of its structure and chemistry, the aptamer alters the probe's response near particular molecules, enabling the location of that type to be mapped. Image courtesy of Stuart Lindsay, Arizona State University.*

is used to locate the target proteins.

The scheme is promising, but the investigators note that one issue is the high surface concentration under the probe. Consequently, the chemical recognition signal may appear even if the affinity between the antibody and a molecule is small. The result is noise that can mask the true signal.

In contrast to antibodies, which are natural, aptamers are man-made. Starting with a random sequence of DNA or RNA, they are created through a process called systematic evolution of ligands by exponential enrichment. Aptamers can recognize specific ligands and bind to nucleic acids, proteins or small organic compounds. Because they consist of a single DNA strand, they are easy to synthe-

size and store, and they are easily attached to the microscope's probe tip.

Aptamers also have a high affinity for some small molecules, which might allow recognition imaging of minor chemical changes, such as elements of an epigenetic code, information in addition to that encoded in the DNA. "My own goal is to map epigenetic markings at the molecular level," Lindsay said.

For a demonstration of their approach, which was published in the June issue of *Biophysical Journal*, the scientists chose the aptamer that binds to human immunoglobulin E (IgE). They did so primarily because this aptamer produces significant adhesion in atomic force microscopy force curves.

They functionalized the probes with an ethylene-glycol oligomer, leaving a thiol-reactive maleimide at the end of the molecule to act as a tether for the aptamer. Because of the tether length, the resulting resolution was ~5 nm. They used an atomic force microscope from Tempe-based Molecular Imaging Corp., now part of Agilent Technologies Inc.

The researchers coated a mica substrate with a solution containing IgE. After some additional processing, they imaged the substrate at a scanning speed of about 2  $\mu\text{m/s}$  and with an oscillation amplitude of ~5 nm. They found that nine out of 10 features on the surface of the right size to be IgE molecules were picked up by the aptamer sensor, a recognition efficiency of 90 percent. Moreover, there was a clear difference between the background signal and that of a legitimate spot, indicating a higher signal-to-noise ratio than is typically achieved by antibody-based sensors.

The investigators demonstrated that the effect was specific. When IgE molecules were injected into the solution, the

recognition signal disappeared because it was blocked. When they imaged surfaces coated with another protein, thrombin, there were no recognition events. Also, when they imaged a surface coated with a mixture of thrombin and IgE, the recognition events tracked to a degree the molar ratio of the mixture. The actual ratio of molecular recognition was higher than the molar ratio, a difference that could have resulted from preferential surface adsorption of the IgE.

The improved signal-to-noise ratio of the aptamer approach as compared with one based on antibodies was not due to stronger binding. Tests showed that the force required to break the aptamer bonds was actually somewhat smaller than the force required to break the antibody bonds. However, other tests have shown a similarly small difference, but with the aptamer bond slightly stronger than the antibody one.

In applying the technique, it would be necessary to have the appropriate aptamers, with the molecules designed to bind to a given target, Lindsey noted. Therefore, work must be done developing and producing the aptamers. That can be a challenge, but the researchers are working on it.

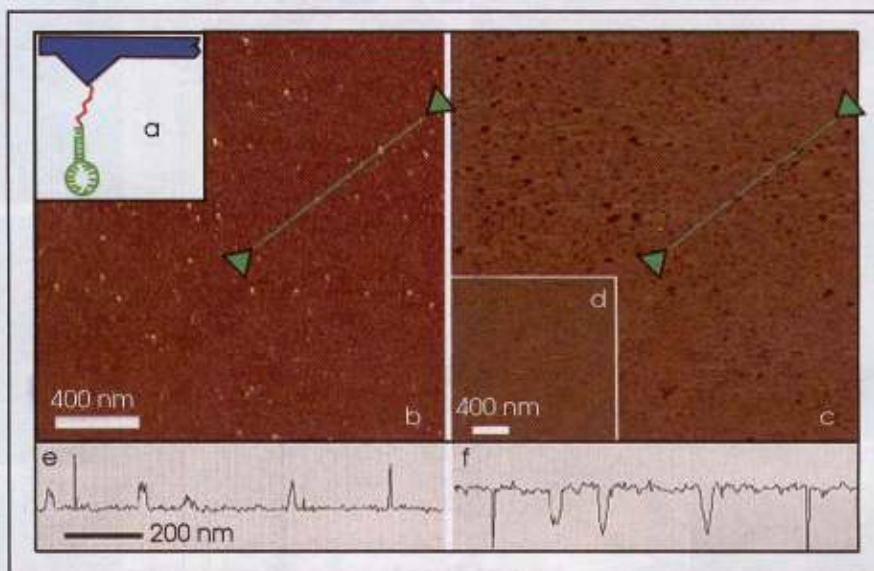


Figure 2. Immunoglobulin E (IgE) was imaged with a DNA aptamer, a recognition molecule shown in green and tethered to an atomic force microscope probe via a polyethylene glycol linker, shown in red (a). The technique enables the depiction of the topography of the IgE molecules on a mica substrate (b) as well as simultaneous acquisition of recognition signals derived from the aptamer's interaction with IgE (c). Adding a solution of IgE blocked further signals (d). Traces (between the green arrows) are shown for the topography (e) and recognition (f) steps.

"We are building an aptamer factory, but it's not easy. On the other hand, if we get things working, we'll avoid the batch-to-

batch variation that plagues natural products like antibodies," Lindsay said. □

Hank Hogan

## Lighting up nitric oxide with a little bit of copper

*Technique provides a way to see the molecule itself — not just its effects*

Nitric oxide, an antioxidant primarily known for its role as a messenger in cellular signaling events, also is generated by cells to accomplish a variety of other tasks. Macrophages, for example, create it to eliminate bacteria and other foreign particles that they encounter.

Biologists who study the effects of nitric oxide on living tissues are interested in clearly identifying the presence of the molecule within cells. However, current imaging methods, including chemiluminescence, electron paramagnetic resonance spectrometry and amperometry, have low spatial resolution and often are costly. Fluorescence microscopy can image

nitric oxide in biological contexts with suitable spatiotemporal resolution; however, commonly used fluorescent nitric oxide sensors are unable to monitor the molecule directly.

For several years, Stephen J. Lippard and his colleagues at MIT in Cambridge, Mass., have attempted to find a way to trigger fluorescence directly in the presence of nitric oxide. Using a variety of metal ions — such as iron, ruthenium, cobalt and rhodium — in combination with a metal-bound fluorophore, they found that some metal-based compounds could elicit fluorescence in the presence of nitric oxide, but also in the presence of other substances, even water.

Now the group has developed a fluorescent probe — made with copper — that directly and rapidly reacts with nitric oxide, enabling imaging of the molecule in living cells. The researchers — Lippard, Mi Hee Lim and Dong Xu — report their findings in the July issue of *Nature Chemical Biology*.

They created the probe by combining a fluorescein-based ligand with  $\text{CuCl}_2$ . In the resulting complex, the fluorescence of the modified fluorescein was quenched. But when nitric oxide was introduced, the compound immediately exhibited an approximately elevenfold increase in fluorescence. The researchers have not measured the precise speed of the reaction yet