When proteins, antibodies and other biological molecules kiss, a new kind of biosensor can tell

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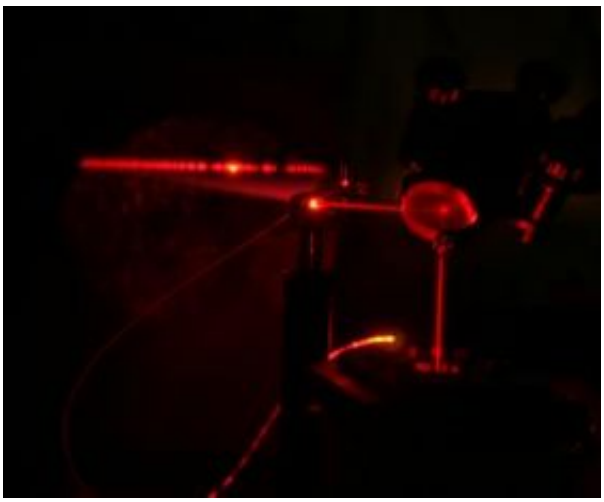

The back-scattering interferometer uses a red-light laser identical to those used in grocery store scanners. Credit: Daniel Dubois

When biological molecules kiss, a new kind of biosensor can tell. A new and deceptively simple technique has been developed by chemists at Vanderbilt University that can measure the interactions between free-floating, unlabeled biological molecules including proteins, sugars, antibodies, DNA and RNA.

That is precisely the kind of capability needed to capitalize on the new avenues of research that have been opened up by the 15-year-plus effort

to sequence the human genome. DNA is the blueprint of all living creatures. But, just as the blueprint of a building is much simpler than the actual structure, so too DNA is far simpler than the myriad of molecules that make up living bodies. As a result, scientists need powerful new methods to study the actual behavior of all these molecules, particularly how they work together.

The new method is called back-scattering interferometry (BSI). By shining a red laser like those used in barcode scanners into a microscopic, liquid-filled chamber where two kinds of molecules are mixed, the instrument can measure the strength with which they react, even when the interactions are extremely weak. In fact, the researchers have demonstrated that it is sensitive enough to detect the process of protein folding, they report in the Sept. 21, 2007 issue of the journal *Science*.

“Molecular interactions are the very heart of biology,” says Professor of Chemistry Darryl J. Bornhop, who headed the 12-year development process.

“Pharmaceuticals depend on reactions between proteins and small molecules or between pairs of proteins or between interactions between RNA and DNA or pairs of DNA molecules. So the ability to measure how that happens is very advantageous.”

The members of the Bornhop research team are post doctoral students Joey Latham and Dmitry Markov; graduate student Amanda Kussrow; Henrik Sorenson, who is now at the Risø National Laboratory in Denmark; and Senior Research Associate Richard Jones.

The method represents an entirely new application of interferometry, a powerful technique that combines light from multiple sources to make precise measurements. Interferometry is used in everything from

astronomy to holography to geodetic surveys to inertial navigation.

The equipment required for the new biosensor is surprisingly modest: a helium-neon laser like those used in grocery store scanners, a mirror, a CCD detector like those used in digital cameras and a special glass microfluidic chip. The chip contains a channel about one fiftieth the size of a human hair. There is a “Y” at one end that allows the researchers to inject two solutions simultaneously, each containing a different kind of molecule. It is followed by a serpentine section that mixes the two.

Finally, there is a straight observation section where the interactions are measured. An unfocused laser beam is directed through the channel at this point. The beam is reflected back and forth inside the channel about 100 times. Each time the light beam strikes the channel some of the light is transmitted back up to the mirror where it is directed to the detector. There it forms a line of alternating light and dark spots called an interference pattern.

It turns out that the interference pattern is very sensitive to what the molecules are doing. If the molecules begin sticking together, for example, the pattern begins to shift. The stronger the binding force between the molecules, the larger the shift. This allows the system to measure interaction forces that vary a million-fold. That includes the entire range of binding forces found in living systems.

The reason the system works so well is still something of a mystery. The researchers know that it responds to minute changes in the index of refraction, which is a measure of how fast the light travels through the liquid in the chamber compared to its speed in a vacuum. They suspect that it has to do with the rearrangement in the water molecules that cover the surface of the proteins: When two proteins react they squeeze the water molecules out of the area where they bind together. This displacement changes the density of the liquid slightly which, in turn,

alters its index of refraction.

BSI has some potential cost advantages compared to current techniques. “The price of the equipment required for BSI is modest and the entire system could easily be miniaturized and integrated with ‘lab on a chip’ systems,” says Bornhop. It is also easy to adapt for high-throughput operation: processing hundreds or thousands of different samples at the same time, he says.

Vanderbilt has applied for and received two patents on the process and has several other patents pending. The university has issued an exclusive license to develop the technology to Molecular Sensing, Inc. Bornhop is one of the founders of the start-up and serves as its chief scientist. The company plans on completing a prototype system this fall.

Source: Vanderbilt University