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Researchers Create Molecule-Sized Test Tubes

In a Howard Hughes Medical Institute laboratory in Illinois, a new kind of test tube, one-thousandth the diameter of a human hair and small enough to hold only a few molecules of DNA, is revolutionizing the way researchers observe the behavior of single molecules of DNA, RNA, or proteins. The test tubes are actually bubble-like nanocontainers that are porous to small molecules. Researchers can easily feed needed ions and other chemicals into the ultra-tiny reaction chambers.

Many scientists say that more can be learned about the dynamics of chemical reactions that power biological processes by studying the behavior of individual molecules rather than observing the collective behavior of many molecules, as scientists do now. But techniques for single-molecule studies are limited and often highly specialized. The new nanocontainers, however, will make single-molecule techniques both more accessible and more powerful, said Taekjip Ha, a Howard Hughes Medical Institute investigator at the University of Illinois at Urbana-Champaign. Ha and his Illinois colleagues are the creative force behind the new technology.

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> — **Taekjip Ha**

This technique enables study at the single-molecule level of any complex interactions between DNA, RNA and proteins that can be modulated by small molecules, Ha explained. These include how cancer drugs interfere with reactions central to the growth of tumors, and the mechanism by which motor proteins enable movement of molecules within the cell. It may even be possible to use this approach for ultrasensitive high-throughput screening of candidate drugs that can inhibit specific interactions between proteins.

The researchers say their technique can be easily applied in other laboratories, to enable scientists to study individual molecular reactions free of the complications of analyzing reactions in bulk solution. The new approach also improves on other methods used for observing the behavior of single molecules. One of the most common methods required that single molecules be tethered to a surface. With nanocontainers, however, the vesicles themselves are attached to a surface, meaning the molecules inside do not have to be. This simplifies analysis, because the effects of the surface on the reaction do not have to be taken into account, the researchers said.

The researchers published their advance during the week of May 21, 2007, in the online Early Edition of the *Proceedings of the National Academy of Sciences*. The Ha laboratory team includes lead author Ibrahim Cisse, a graduate student in the department of physics, and recent graduates Burak Okumus and Chirlmin Joo.

In earlier work, Ha and his colleagues created the nanocontainers, which are roughly one-thousandth the diameter of a human hair, from lipid films. They used the nanocontainers, called vesicles, to entrap and study the behavior of large molecules like proteins and DNA. However, the earlier work hit an impasse when researchers realized that there was a limit to the kinds of reactions they could study inside the nanocontainers. The main limiting factor was the inability to introduce chemicals through the impermeable lipid membrane.

Over a period of months, the researchers perfected their technology, ultimately solving the problem and adapting the nanocontainers so that reactants can pass through. To do so, they formed the nanocontainers from a lipid that was transitioning between a liquid and a gel. This instability caused defects in the lipid membrane, which produced pores.

We could not control the pore size, Cisse said, but we were lucky that the lipids we used to form the nanocontainers had pores just big enough to let molecules such as ATP and ions pass, yet small enough to keep large molecules like proteins and DNA inside. ATP is an energy-containing molecule that powers biochemical reactions.

As a demonstration of the technique, Ha, Cisse, and their colleagues analyzed how a protein called RecA attaches to DNA to form filaments. RecA is a major component of a mechanism for repairing abnormal DNA, and filament formation is central to the repair process. The researchers trapped RecA and a DNA labeled with fluorescent dye molecules inside porous nanocontainers. They then infused ATP into the nanocontainers, and observed in detail how the ATP triggered RecA proteins to interact with DNA to uncoil the DNA to form filaments.

An expert in single-molecule study, Ha is particularly excited about this advance because of its broad applicability and ease of use. I think this technique will go a long way toward my goal of commoditization of *in singulo* techniques, getting them out of specialists' labs to the general research community, he noted.

The convenient thing about this technique is that it is a self-assembly process, Cisse explained. A researcher need only select the protein, DNA or other biomolecule they want to study, adjust the conditions for lipid encapsulation, and the vesicles will self-assemble, trapping the number of molecules they wish. We have provided information on how to design those conditions. According to Cisse, the major drawback to the technique is that the pore size cannot be precisely controlled, but he and his colleagues are experimenting with bacterial toxin that introduces pores of a precise size into the nanocontainer membrane.