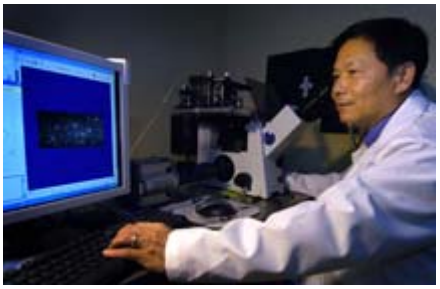
January 30, 2007

New single-molecule imaging system ends pRNA debate over phi29 motor

WEST LAFAYETTE, Ind. - Scientists are able to view active molecules within a biological motor of the nanometer scale with the help of a new imaging system far more sensitive and powerful than existing optical microscopes.

A Purdue University researcher has created a single-molecule imaging system to view deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and other tiny biological molecules. This ability helps settle a seven-year debate within the virology and nanomedicine fields over the shape and structure of a tiny biological motor that has potential use in nanotechnology and nanomedicine, including the diagnosis and treatment of diseases such as cancer, AIDS and influenza.

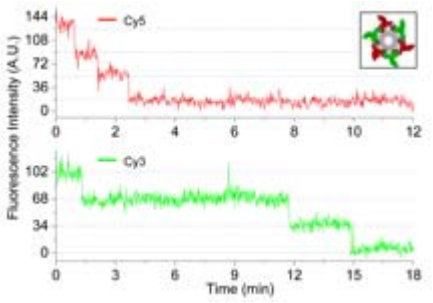


Peixuan Guo
Download photo
caption below

Scientists had disputed the number of packaging ribonucleic acid (pRNA) molecules contained in the DNA-packaging motor of the phi29 virus. The number of these molecules present determines the shape of the motor and expands understanding of the way it works. The new imaging system definitively concludes that six pRNA molecules were present. The research, funded by the National Institutes of Health, will be published in the upcoming issue of the European Molecular Biology Organization Journal, EMBOJ.

"The microscopes commercially available were not sensitive enough to distinguish individual molecules of this scale," said Peixuan Guo, professor of molecular virology and biomedical engineering and creator of the imaging system. "Our system, through its highly sensitive detection system and dual color viewing ability, is capable of distinguishing

and counting individual molecules within a nanodevice."



dual view graph
Download image
caption below

Nanotechnology deals with materials within the nanometer scale. A nanometer is one-billionth of a meter, or 100,000 times smaller than the diameter of a human hair. The most powerful optical microscopes available offer a resolution of 200 nanometers, meaning anything closer together than 200 nanometers would blur together and appear as a single unit.

"Many of the molecules involved in the cellular processes of the human body are much smaller than this 200-nanometer diffraction limit," Guo said. "For example, the phi29 motor is approximately 20 nanometers in size and a strand of DNA is only 2 nanometers wide."

Electron microscopes, which use a beam of electrons instead of light to detect a specimen, can reach a resolution of tenths of a nanometer. However, materials that have a versatile or flexible structure but are not electron-dense, such as RNA, are not easily imaged using electron microscopes, he said.

Active molecules performing their functions can be observed in real time through Guo's system, which can detect the motion of the molecules and also count individual molecules within a few tens of nanometers.

"This was greatly needed for nanotechnology research," he said. "Researchers need a clear view of their subjects to fully understand them. Direct observation of these molecules will answer many of the questions facing this field and will help in the design of novel nanodevices. The strength of our system is the sensitivity of detection."

Guo, who leads Purdue's NIH-funded National Nanomedicine Development Center and also is a member of Purdue's Cancer Center, used readily available parts to construct the innovative imaging system.

The system, called a single-molecule dual viewing total internal reflection fluorescence imaging system (SMDV-TIRF) is an improvement of total internal reflection microscopy. Its improved sensitivity comes from the use of a newly assembled laser combiner to control the release of two or more lasers as the light source and the direct delivery of this laser light through optical fibers to the detector.

Other modifications, such as an advanced cooling system and the placement of the quartz prism on the top of the chamber, minimize background noise and leakage of light that could confuse the detector. In addition, the application of binomial distribution for dual view technology has led to new findings in imaging.

The molecules of interest are marked with red and green fluorescent labels that emit signals. These signals are divided into different paths according to their colors before they reach the detector and a computer analyzes the information and converts the signals to images.

The modifications to the system assure that the signal from the labels retain their strength and are clearly detected, Guo said.

The system recognizes when different labels overlap, a sign of interaction between the different types of molecules, and presents a yellow color to differentiate it from the red and green markers. As the system analyzes the sample, the computer displays an image and a graph.

The image displays dots of red, green and yellow, showing the location of the DNA and RNA and where and when the two overlap.

The graph displays lines similar to stairsteps, which are used to count the individual molecules. As the light shines on the fluorescent markers to illuminate them, it also degrades and, eventually, destroys the marker. As this destruction, called photobleaching, occurs, less light is sent to the detector.

"The fluorescent molecules naturally depart one by one when exposed to intensive illumination," Guo said. "The graph drops as each molecule's marker fades until none are left and the graph reaches the baseline. Each stairstep on the final graph represents one single molecule with one marker."

Guo and his team, including co-authors Hui Zhang and Dan Shu, and postdoctoral researcher Wulf-Dieter Moll were able to count the number of pRNA molecules present and observed the motion of the phi29 motor as it packaged DNA. By observing the interaction of the DNA and RNA they were able to determine the structure and mechanics of the nanomotor.

"The pRNA molecules are unstable and quickly degrade," Guo said. "This caused confusion over the number of pRNA molecules present. Researchers were unable to directly count the molecules when the motor is in motion and had to estimate the number of molecules present based on the chemical composition of large samples. The degradation of the RNA affected the estimates, and no one could solve the problem."

Guo said to construct this novel system, he received

assistance and advice of many single molecule biophysicists, including Nils Walter, David Rueda, Peter Stockley, Taekjip Ha, Eckhard Jankowsky, Toshio Yanagida, Chris Meiners, Meredith Lambert, Faqing Huang and Mark Browne from Ando Technology.

Guo's team next plans to count the individual protein components within the phi29 DNA packaging motor and will further characterize the real-time motion of the motor.

Writer: Elizabeth K. Gardner, (765) 494-2081, ekgardner@purdue.edu

Source: Peixuan Guo, (765) 494-7561, guop@purdue.edu

Purdue News Service: (765) 494-2096; purduenews@purdue.edu

PHOTO CAPTION:
Peixuan Guo, director of the Purdue Nanomedicine Development Center and professor of molecular virology and biomedical engineering, observes biological processes at the nanometer scale using the single-molecule imaging system he created. (Purdue News Service photo/David Umberger)

A publication-quality photo is available at http://news.uns.purdue.edu/images/+2007/guo-imaging.jpg

IMAGE CAPTION:
A dual view graph generated by the imaging system shows fluorescence intensity vs. time for two types of pRNA. Each step down represents the photobleaching, or loss, of one marker. Each step on the final graph represents one single molecule. The insert illustrates the phi29 motor ring of six pRNA. (Purdue graphic/Guo Laboratories)

A publication-quality image is available at http://news.uns.purdue.edu/images/+2007/guo-graphs.jpg

---

ABSTRACT

Counting of six pRNAs of phi29 DNA-packaging motor with customized single molecule dual-view system

Dan Shu, Hui Zhang, Jiashun Jin, and Peixuan Guo

Direct imaging or counting of RNA molecules has been difficult due to its relatively low electron density for EM and insufficient resolution in AFM. Bacteriophage phi29 DNA-packaging motor is geared by a pRNA ring. Currently whether the ring is a pentagon or hexagon is under fervent debate. We report here the assembly of a highly sensitive

imaging system for direct counting of the copy number of pRNA within this 20-nm motor. Single fluorophore imaging clearly identified the quantized photobleaching steps from pRNA labeled with a single-fluorophore and concluded its stoichiometry within the motor. Almost all of the motors contained six copies of pRNA before and during DNA translocation, identified by dual-color detection of the stalled intermediates of motors containing Cy3-pRNA and Cy5-DNA. The stalled motors were restarted to observe the motion of DNA packaging in real time. Heat-denaturation analysis confirmed that the stoichiometry of pRNA is the common multiple of 2 and 3. EM imaging of procapsid/pRNA complexes clearly revealed six ferritin particles that were conjugated to each pRNA ring.

To the News Service home page