

Polarized microscopy technique shows new details of how proteins are arranged

April 17 2011

Whether you're talking about genes, or neurons, or the workings of a virus, at the most fundamental level, biology is a matter of proteins. So understanding what protein complexes look like and how they operate is the key to figuring out what makes cells tick. By harnessing the unique properties of polarized light, Rockefeller scientists have now developed a new technique that can help deduce the orientation of specific proteins within the cell. By turning their instruments toward the nuclear pore complex, a huge cluster of proteins that serves as a gateway to a cell's nucleus, the scientists say they have filled in the gaps left by other techniques and made important new discoveries about how the complex works.

"Our new technique allows us to measure how components of large protein complexes are arranged in relation to one another," says Sandy Simon, head of the Laboratory of [Cellular Biophysics](#). "This has the potential to give us important new information about how the [nuclear pore complex](#) functions, but we believe it can also be applied to other multi-protein complexes such as those involved in DNA transcription, [protein synthesis](#) or [viral replication](#)."

Although researchers have spent years studying the workings of the nuclear pore complex, there is still much that has remained mysterious. One problem is that there is a "resolution gap" between the two techniques primarily used to visualize protein complexes. [Electron microscopy](#) can reveal the broad outlines of a large protein complex, but it can't show details. X-ray crystallography, meanwhile, can show minute

detail but only of a small piece of the complex; it can't say how the individual pieces fit together. To further complicate matters, both techniques require fixed samples – while they can give you an idea of what something looks like at a moment in time, they can't tell you how its pieces might move.

The new technique was developed by Simon along with postdoc Alexa Mattheyses, graduate student Claire Atkinson and Martin Kampmann, a former a member of Günter Blobel's Laboratory of Cell Biology who is currently at the University of California, San Francisco. It takes advantage of the properties of polarized light to show how specific proteins are aligned in relation to one another. After genetically attaching fluorescent markers to individual components of the nuclear pore complex, the scientists replaced the cell's own copy of the gene that encodes the protein with the new form that has the fluorescent tag. Then, they used customized microscopes to measure the orientation of the waves of light the fluorescently tagged proteins emitted. By combining these measurements with known data about the structure of the complex, the scientists can confirm or deny the accuracy of previously suggested models.

"Our experimental approach to the structure is synergistic with other studies being conducted at Rockefeller, including analysis with X-ray crystallography in Günter's lab and electron microscopy and computer analysis in Mike Rout's lab," says Simon. "By utilizing multiple techniques, we are able to get a more precise picture of these complexes than has ever been possible before."

The scientists used the technique to study nuclear pore complexes in both budding yeast and human cells. In the case of the human cells, their new data shows that multiple copies of a key building block of the nuclear pore complex, the Y-shaped subcomplex, are arranged head-to-tail, rather than like fence posts, confirming a model proposed by Blobel

in 2007.

"As a graduate student with Günter Blobel, I determined the three-dimensional structure of the Y-shaped subcomplex using electron microscopy," says Kampmann. "However, it was still a mystery how these 'Y's are arranged. The new technique we have developed reveals the orientation of building blocks in the cell, and we hope that it will eventually enable us to assemble individual crystal structures into a high-resolution map of the entire nuclear pore complex."

Eventually, the scientists say their technique could go even further. Because the proteins' fluorescence can be measured while the cells are still alive, it could give scientists new insights into how [protein](#) complexes react to varying environmental conditions, and how their configurations change over time.

"What happens when other proteins pass through the nuclear pore? Does the orientation of the nucleoporins change? With this technique, can find out not only what the pore looks like when it's sitting still, but what happens to it when it's active," Simon says. Their first characterization of the dynamics of the nuclear pore proteins was published recently in *The Biophysical Journal*.

Provided by Rockefeller University

Citation: Polarized microscopy technique shows new details of how proteins are arranged (2011, April 17) retrieved 20 June 2024 from <https://phys.org/news/2011-04-polarized-microscopy-technique-proteins.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.