Fluorescence Measures Tiny Cell Movements

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Our cells contain parts that are constantly moving. For instance, pores on the cell membrane open and close in response to their environment, some organelles move along microtubules to areas of the cell where they are needed, and DNA moves into position for cell division.

Understanding how small cellular parts move and why they sometimes don't work properly could lead to a better understanding of certain diseases and to better drug therapies for those diseases. Meanwhile, these small movements have already allowed researchers to make their own machines from DNA.

A technique called fluorescence resonance energy transfer makes it possible to measure the tiny movements of cellular parts, which can be as small as 1 Å. Researchers attach pairs of molecules that fluoresce when excited by a laser or other light source. When excited, one molecule acts as a donor, transferring energy to the other molecule, the acceptor.

Because the efficiency of energy transfer strongly depends on the distance between a pair, it can be used to calculate that distance. The efficiency is measurable because the transfer of energy usually causes the acceptor to emit more light than it would if no donor were present, and it causes the donor to emit less light. The researchers visualize movement by measuring how the donor-acceptor distance changes when the structure is in different states.

Kinesin motors

The kinesin protein motor uses the adenosine triphosphate (ATP) molecule as fuel to move along microtubules in the cell. It sometimes carries organelles and also plays roles in meiosis and mitosis, two forms of cell division.

Kinesin consists of two heads connected by a neck. The heads bind to ATP, converting it to energy by releasing a phosphate and leaving adenosine diphosphate (ADP). The heads also alternately bind to the microtubule, producing kinesin's movement.

To understand how specific sites on kinesin move and what part of the energy cycle induces movement, researchers studied heads in simulated states of the cycle. For each stage, they attached a donor of green fluorescent protein to a site on the neck. They fused an acceptor of tetramethylrhodamine on the head, which binds with ATP and the microtubules. The donors were excited with a 300-W mercury lamp at 475 nm, and acceptor emission measured at 575 nm with a K2 spectrofluorometer from ISS Inc. of Champaign, Ill.

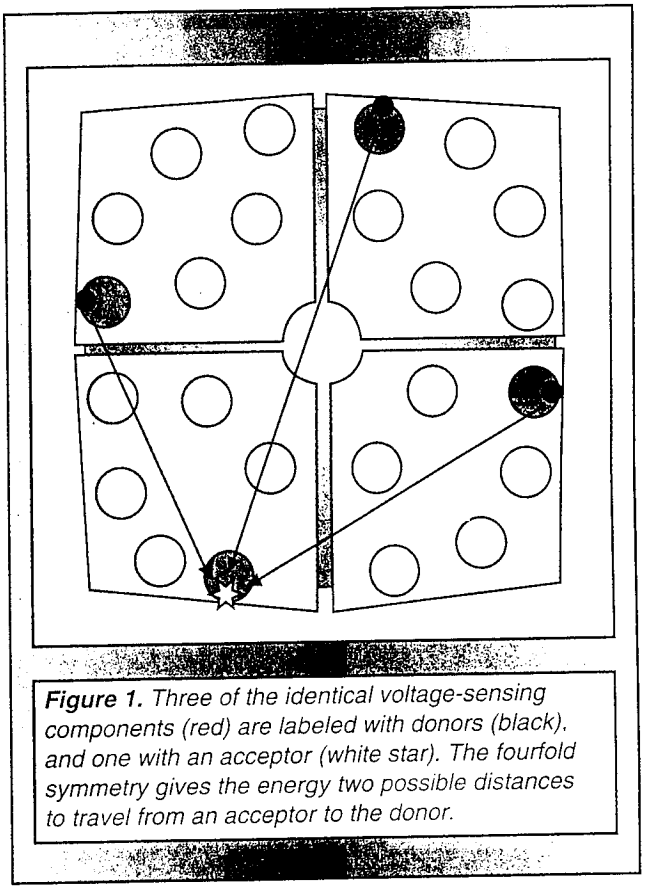

Figure 1. *Three of the identical voltage-sensing components (red) are labeled with donors (black), and one with an acceptor (white star). The fourfold symmetry gives the energy two possible distances to travel from an acceptor to the donor.*

Efficient energy transfer between the donor and acceptor occurred when kinesin was bound to the microtubule and ATP. However, in other states, transfer of energy was not efficient. This told the researchers that the neck region did not move when bound to the microtubule and ATP but became mobile after ATP converted to ADP. When not bound to the microtubule, the neck region moved whether or not ATP or ADP was present.

It is more difficult to study structures inside cells. Here, researchers use a modification of the fluorescence technique, luminescence resonance energy transfer, which employs rare-earth metals instead of organic molecules as donors. The metal donors give off light only when both a donor and acceptor are present. This method has helped researchers at the University of California in Los Angeles School of Medicine and the University of Illinois in Urbana-Champaign to understand how a voltage-dependent potassium pore opens and closes by studying it in the cell.

The Shaker pore, which helps form signals in neurons, consists of four subunits, each made of six components (Figure 1). The pore's fourfold symmetry allows the use of three donors and one acceptor, instead of the usual pairs. The distances between donors and the acceptor differ. The pores may get any combination

of acceptors and donors, but only those with at least one pair give off light.

The research group used terbium-chelate maleimide as the donor and fluorescein maleimide as the acceptor on the four identical voltage-sensing components in the Shaker pore. This component probably controls when the pore opens and closes by responding to the surrounding charges.

The researchers induced the pore to open by applying voltage to the cell culture. They viewed the molecules using an Axiovert 10 microscope from Carl Zeiss after exciting them with a 337-nm nitrogen laser. The light was measured with a gallium-arsenide R943-02 photomultiplier tube from Hamamatsu. This was repeated for several sites on the voltage-sensing component.

As the pore opened, they found that some sites on the component moved farther from the pore, some moved closer and others remained at the same distance. This suggested that the voltage-sensing component ratates instead of moving up and down, as previously thought. By rotating, charged parts of the subunit can reach extracellular fluid when the pore opens.

DNA motors

Fluorescence resonance energy transfer can also confirm movement in man-made motors. Researchers at Lucent Technologies' Bell Labs recently used the technique to construct and measure movement in a DNA motor. Cellular motors inspired the team to develop the motor, which uses DNA as the machine, the fuel and the waste.

The motor, which acts like tweezers, consists of three single strands of DNA when open (Figure 2). Half of strand A is complementary to strand B and the other half complementary to strand C. In the open position, half of strands B and C are free. To close the tweezers, the fuel, strand F, is introduced and binds to the free parts of B and C, bringing the unpaired arms together. Another strand complementary to strand F can remove it, leaving the tweezers open and strand F as a double-stranded waste product.

To determine how many of strands B and C to add, the researchers labeled the 5' end of strand A with tetrachlorofluorescein phosphoramidite and the 3' end with carboxy-tetramethylrhodamine. They measured the fluorescence using silicon photodiodes after exciting the molecules with a 514.5-nm argon-ion laser.

When A is unbound, the labels are close together and transfer energy efficiently, causing the 5' label's fluorescence to drop by a factor of six. When the energy was no longer transferred efficiently, the researchers knew they had added a sufficient number of strands B and C to bind with the A strands in the solution.

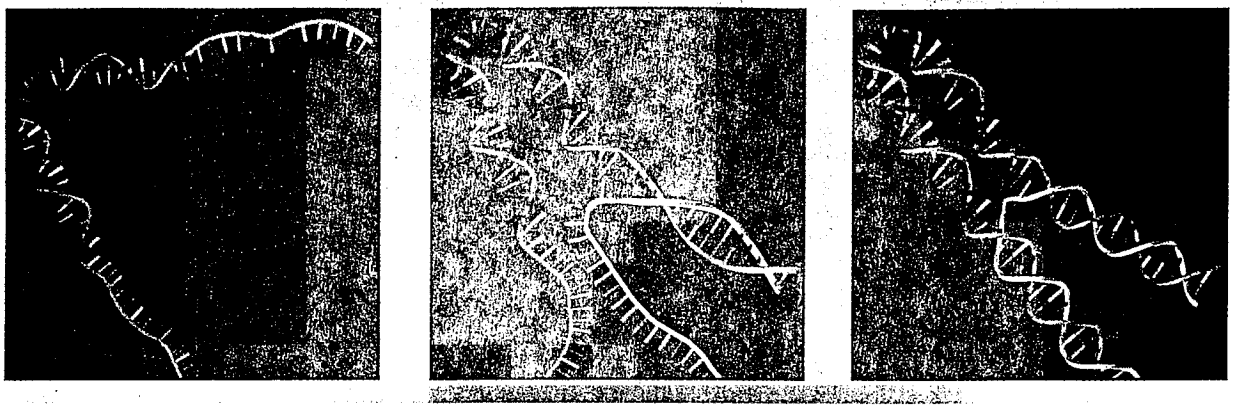

Figure 2. *At left, the DNA tweezers are in an open position, with the blue strand (A) bound to half of the red strand (B) and half of the green strand (C). Fluorescent molecules at each end of the blue strand tell the researchers whether the tweezers are open or closed. In the center, the silver strand (F) is binding to the free parts of the red and green strands of DNA, bringing the strands together. At right, the tweezers are in a closed position. A strand complementary to the silver strand can remove it and return the tweezers to an open position.*

They also used energy transfer to figure out that the machine takes 13 seconds to open or close. To do this, they measured how long it took fluorescence to drop by a factor of six, corresponding to a closed position, while adding strand F.

The order of bases in the DNA fuel could possibly be used to carry information from one machine to another or to coordinate individual machines in complex jobs. According to Bell Labs, the ability to make small movements could prove important in constructing powerful computer chips containing billions of transistors, instead of the millions in today's computers. □