

How Plants Avoid Feeling the Burn

Photoprotective effect measured for the first time at single biomolecule level

Too much sun – for plants as well as people – can be harmful to long-term health. But to avoid the botanical equivalent of “lobster tans,” plants have developed an intricate internal defense mechanism, called photoprotection, which acts like sunscreen to ward off the sun’s harmful rays.

“We knew that biomolecules called carotenoids participate in this process of photoprotection, but the question has been, how does this work?” said Iris Visoly-Fisher, a postdoctoral research associate in the Biodesign Institute at Arizona State University.

Carotenoids act as ‘wires’ to carry away the extra sunlight energy in the form of unwanted electrons, somehow wicking away the extra electrons across long distances from locations that could damage plant tissues and photosynthesis. During photoprotection, the consensus school of thought was that carotenoids—the source of the orange pigments in carrots and Vitamin A — become oxidized, or charged, losing an electron in the process.

Now, Fisher and other ASU scientists have found a way to measure for the first time the electrical conductance within such an important biomolecule. And in doing so, the team has produced a new discovery which shatters the prevailing view. The research team found that oxidation is not required for photoprotection, but rather, carotenoids in a neutral, or uncharged state, can readily handle the electron overload from the sun.

Their findings have been published in the prestigious journal Proceedings of the National Academy of Sciences (PNAS) under the title “Conductance of a Biomolecular Wire” (<http://www.pnas.org/cgi/content/abstract/0600593103v1>).

“This is a remarkable experimental tour-de-force and the result is quite unexpected,” said Lindsay, who directs Fisher’s work in the Biodesign Institute’s Center for Single Molecule Biophysics. “Carotene was regarded as the poster child for this molecular mechanism, but it turns out that a much simpler mechanism works just fine.”

The innovative work was a collaboration between several ASU departments and the Universidad Nacional de Rio Cuarto in Argentina. In addition to Fisher, who was lead author on the paper, contributions from chemistry and biochemistry professors Devens Gust, Tom Moore and Ana Moore of ASU’s Center for the Study of Early Events in Photosynthesis were instrumental in the project.

“The initial interest was to more fully understand how photosynthesis works,” said Fisher. Because our center focuses on electron transport in a single molecule, Devens Gust and Tom and Ana Moore suggested that we look at single molecule transport in carotene.”

To get at the heart of the problem, Fisher had to attempt an experiment that had never been done before for any biomolecule: to control the charge of the biomolecule while measuring its ability to hold a current.

By holding a carotenoid under potential control, Fisher could control whether the biomolecule was in a neutral state or in the charged state (the oxidized state), while simultaneously measuring the electron transport through a single molecule.

“The importance of this result is not only for understanding natural systems and photosynthesis, but also for the fact that technically, for the first time, we could hold a molecule in a state pretty close to the natural conditions found in the plant,” said Fisher.

To make the experimental measurements, Fisher first needed to work out several technically challenging variations to a method first pioneered by electrical engineering professor Nongjian Tao of ASU’s Fulton School of Engineering. In concept, it’s much like trying to measure the current of a wire found in an everyday household appliance, only in this case, the “wiring” is a miniscule 2.8 nanometers long and less than a single nanometer thick, or about 10,000 times smaller than the width of a human hair.

One measurement problem is that carotenoids are highly prone to react with water and oxygen, so all measurements had to be performed in an environment that would both protect the molecule and immerse it in an environment mimicking a biological cell membrane, where the carotenoids are found in nature.

Other innovations included developing a new insulating coat of polyethylene for the probe tip of a Scanning Tunneling Microscope (STM), which is used to measure the electron flow across single molecules. Also, the chemical ends of the carotenoids had to be modified so they could chemically stick to the STM probe’s gold tipped electrodes.

To make a single measurement, the carotenoid molecules, which lie flat on the surface of a tiny reaction chamber, are first picked up by the STM probe’s gold tip and chemically bound between these two electrodes, forming a kind of nanoscale bridge. “Gold is a soft metal, and when you pull it apart, eventually, you can measure the conduction of a single carotenoid molecule between the gold electrodes,” said Fisher.

The research team found that, especially when compared to metals, carotenoids are not very conductive, even when measuring the most oxidized form. However, the electrical conduction was two orders of magnitude higher when compared to what is needed for the photoprotective effect to work.

The group also measured how fast the electrons traveled across the carotenoid bridge between the electrodes. By measuring carotenoids of different chemical lengths, the team showed that the travel rate was fast enough to match or exceed measurements performed in the plant system.

One of the greatest challenges of the experiment came down to the human endurance of taking thousands of measurements over an intense, six month period. “We needed to keep this finicky molecule away from the light, so sometimes, the microscope room became like a cave, where I was sitting for hours and hours in the dark,” said Fisher.

For Fisher and the rest of the team, however, the main satisfaction was being able to break down a complex process to understand its simplest components and produce a groundbreaking discovery.