Cleared for Takeoff

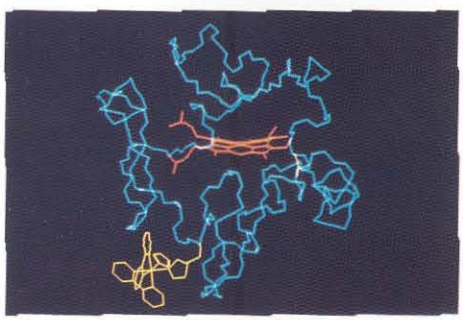
Some members of the collaboration: (back row, from left) Morten Bjerrum, Winkler, Gray, David Beratan, DiBilio, Juri Germanas; (front row) Jorge Colón, Gary Mines, Chang, Debbie Wuttke, Danny Casimiro, and Zhong Huang.

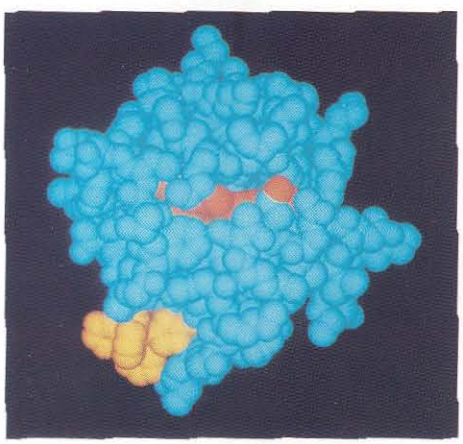
Electrons do more frequent flying than most corporate executives. Electrons commuting from molecule to molecule power life's basic processes. And many important metalloproteins, such as the cytochromes that help power our cells, keep the metabolic economy humming by dispatching electrons from a metal atom at the heart of the molecule to various sites on its periphery. Harry Gray, Beckman Professor of Chemistry and Director of the Beckman Institute, and Jay Winkler, a Member of the Beckman Institute, are studying these intramolecular electron transfers in hopes of discovering how the rate of electron transfer varies with the distance to the destination and with the molecular terrain along the way. Proteins are made of smaller molecules called amino acids, and the physical contours and electrical properties of the particular amino acids on the route traveled by the electron can greatly affect its passage. The researchers' eventual goal is to make another transfer—to apply the rules behind nature's exquisitely engineered metabolic machinery to the design of similar chemical machinery that would turn out made-to-order substances (pharmaceuticals, plastics, or what have you) on an industrial scale.

The researchers act as molecular travel agents. First, the electron's reservation is confirmed by replacing the amino acid at the electron's destination with another amino acid—histidine—to which a ruthenium atom can be attached. The ruthenium atom provides a landing site for the electron and undergoes a spectroscopically detectable change upon its arrival, allowing its time of flight to be measured. These new electron-transfer proteins are built to spec in collaboration with members of Professor of Organic Chemistry John Richards's group.

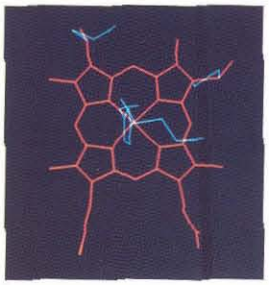
Electrons in living cells take wing in response to processes that are hard to duplicate in the lab, so the researchers use a laser pulse to excite the central metal atom, causing it to emit an electron. But most of the biologically important metals stay excited for mere trillionths of a second—not long enough to emit an electron. Several researchers at other institutions have successfully substituted zinc—whose excited state lasts for several thousandths of a second—for iron, around which hemoglobin, the cytochrome family of proteins, and a slew of other molecules are built, but this technique doesn't work for other metals. An iron-containing protein has its metal atom mounted in an elaborate bit of scaffolding called a heme complex, around which the protein is assembled. When researchers popped the iron atom out of the framework and slipped a zinc atom in, the protein obligingly reassembled itself around the modified heme complex. But atoms of other metals are directly bound to the amino acids that

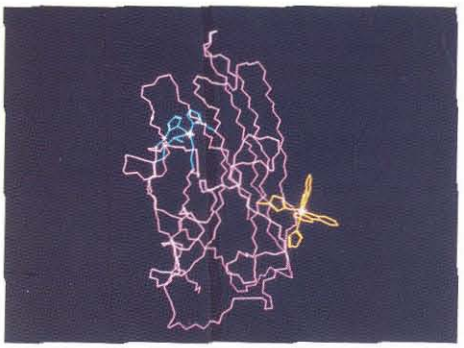
Left: Winkler measuring fluorescence from a sample in the Beckman Institute's laser center. Below: Two views of a cytochrome molecule. The heme complex is red, the amino acids are blue, and the histidine–ruthenium assembly is orange. The upper view shows only the "backbone" of the structure. The lower view shows all the atoms. Note how the heme complex, which lies perpendicular to the page from this perspective, is buried in the middle of the protein.





Above: The heme complex. The iron atom lies at the intersection of the two diagonal lines in its center. The light blue lines show points of attachment to the surrounding protein, including two directly above and directly below the iron atom, relative to the plane of the page. Electrons can also travel to the protein via the two "legs" projecting toward the bottom of the image, which are more loosely connected to the protein. Below: There are only four connections (light blue) to the copper atom (purple cross) in an azurin molecule.





make up the protein. The metal's identity determines the shape of the protein around it to such an extent that replacing the metal with zinc distorts the protein to the point of altering its behavior.

This past year, postdoc I-Jy Chang figured out how to do the experiment with the original metal left in place, by turning the laser on the ruthenium atom instead. When the right substituents are added to it, ruthenium's excited state lasts about 50 billionths of a second, just long enough to clear an electron for takeoff. So instead of prodding the zinc atom, the researchers excite the ruthenium atom, sending electrons from the outskirts in toward the center. And if the experiment demands that the electrons be outbound, the researchers can add a chemical reagent that makes the ruthenium atom electron-deficient, causing it to steal electrons from the central atom when excited.

Now that electrons can be booked onto any itinerary that the researchers want to study, postdoc Angelo DiBilio and grad student Ralf Langen are applying the technique to azurin, an intensely blue, copper-containing protein found in bacteria. Azurin is a particularly nice protein to study, because the amino acids enfolding the copper atom attach to it at only four specific points. Thus an electron has only four possible routes to or from the copper atom. The ruthenium atom's placement determines which path the electron follows, allowing each one of the four to be studied unambiguously. Heme, by contrast, has an elaborate honeycomb structure, and the metal-heme complex resembles a golf ball pushed halfway through a chicken-wire fence. There are many possible journeys an electron could make through this complex, and it's almost impossible to chart with certainty the course the electron actually traverses.

The group has just discovered that an electron's speed depends on its route, and the specific amino acid attached to the copper atom appears to make the difference. Two of the attachment points are histidines, one is a cysteine, the fourth is a methionine. Cysteine is the express route; the methionine route appears to be a puddle-jumper, taking several hundred times longer. The measurements were made with the ruthenium runway sited some distance from the copper center, so the proof isn't ironclad yet. The scientists plan to move the runway closer to the center, but they know from experiments with cytochrome that the electrons start traveling too fast for the current spectroscopic system as the distance between takeoff and landing shrinks. Up to 15 atomic diameters as the electron flies can be covered in less than ten billionths of a second. A new system that will enable the group to follow electron transfers in trillionths of a second is being built. □ —*DS*