nanometers before the opposite charges recombine and release energy either as another photon or as heat. To get around this problem, researchers have long created two-layer devices: One organic layer contains electron-deficient molecules known as "acceptors" that attract electrons; the other contains electron-rich "donor" molecules that attract holes. If, during its random wanderings, an exiton encounters the junction between the two layers, it should be pulled apart by the donor and acceptor molecules, allowing the charges to be delivered to electrodes attached to each layer. But only about 10% of the exitons encounter the donor-acceptor junction before they recombine.

To improve these odds, the two groups—one led by Alan Heeger, Fred Wudl, and Gang Yu at UCSB, the other by Friend and J. J. M. Halls at Cambridge—essentially scrambled their organic donor and acceptor materials together, which effectively created junctions throughout the heart of the device. But while this strategy makes it easier to separate the charges, it creates another potential problem: how to get the separated charges to the correct electrode. For electrons to move to an electrode, they must be able to jump from one acceptor molecule to another in an unbroken chain. Similarly, holes must be able to jump from one donor molecule to the next. But scrambling the two organic materials together can create isolated pockets of donor and acceptor material.

To minimize this drawback, both teams used one polymer—known as MEH-PPV—as the donor and another called CN-PPV as the acceptor. The result: Electrons and holes can travel along the polymer chains and jump from one chain to another to find a route to an electrode. At the European Materials Research Society conference in Strasbourg, France, in May, both teams reported making devices in which between 1% and 2% of the absorbed photons was converted into stored energy. And last week, the Cambridge group published its results in the journal *Nature*.

In addition to these dual polymer devices, the UCSB researchers also reported making similar devices using the spherical carbon molecule C_{60} as their acceptors instead of CN-PPV. Although C_{60} isn't a polymer, it's more efficient at grabbing electrons than its polymer counterpart, explains Heeger. As a result, the C_{60} devices achieved a slightly higher efficiency than did the dual polymer ones. And Yu suspects that in the end, the best device may use a combination of both C_{60} and CN-PPV, so that the C_{60} can efficiently grab electrons and pass them to the CN-PPV for transport to the electrode. If so, and if researchers can find a way to lengthen the lives of their polymers, plastics may yet brighten the prospects for solar electricity.

–Robert F. Service

BIOCHEMISTRY

New Angle for Classic Tale of Respiratory Protein and Oxygen

The angle between cellular life and death, according to biochemistry textbooks, is acute. Life-giving oxygen is stored within muscle cells by a protein called myoglobin, and that storage is made possible by the crooked angle at which oxygen binds to the protein. The angle is crucial, for oxygen has competition for space in this molecular shuttle: poisonous carbon monoxide (CO), which prefers to sit upright. When myoglobin's structure forces CO over on its side, however, the poisonous interloper falls by the wayside. Or so the textbooks say.

Now some recent studies suggest that "the textbook is wrong," says George Phillips, a professor of biochemistry and cell biology at Rice University in Houston. One such study appears on page 962 of this issue, where a team led by Philip Anfinrud from Harvard University provides evidence that a nearly perpendicular CO fits quite comfortably in myoglobin, and that forced bending has little to do with CO exclusion.

"It puts another nail in the coffin of the [textbook] idea" of how myoglobin suppresses CO binding.

—Joel Berendzen

Anfinrud's evidence is proving extremely persuasive because, using an infrared (IR) light spectroscopy technique, his group was able to pin down the binding angle to within just a few degrees. "It's a beautiful piece of work," enthuses chemist Robin Hochstrasser at the University of Pennsylvania, Philadelphia. "It puts another nail in the coffin of the idea that the binding angle controls the ability of CO to bind," says Joel Berendzen, who does both x-ray crystallography and spectroscopy at the Los Alamos National Laboratory in New Mexico.

Researchers are now offering a variety of other ideas for myoglobin's oxygen (O_2) favoritism. One is that the protein may restrain unbound CO from binding; another is that particular amino acids in the protein may help attract O_2. Although there is no unanimity as to the nature of this new mechanism—"It's a very contentious issue," says Stanford University chemist Jim Collman—and there are still holdouts who favor the angle theory, there is an emerging consensus that the new studies are helping researchers gain insights into ever finer details of protein structure.

Both CO and oxygen bind to an iron atom in the middle of a ring-shaped portion of myoglobin known as the heme group. But scientists have long been puzzled by the fact that heme, when isolated in experiments, binds to CO 10,000 times as strongly as it does to O_2. Yet, when embedded in the myoglobin protein, it binds only 20 to 30 times as strongly as O_2. (Organisms can live with this bias because there is far more O_2 in their system than CO, which is produced by the breakdown of heme in the body.) The inescapable conclusion, says Anfinrud, is that "the protein must be doing something to suppress carbon monoxide relative to O_2."

To solve this riddle, researchers originally turned to x-ray diffraction. X-rays fired through a crystal of a molecule are scattered in different directions by different types of atoms. By analyzing the scatter patterns, researchers are able to identify the location of the hundreds or thousands of these atoms, and thus the structure of the molecule.

In the 1970s, these studies of CO bound to isolated heme showed that CO stuck straight up. But similar studies in the 1980s of CO bound to the complete myoglobin protein showed it was bent over—most commonly from 20 to 40 degrees. The textbook conclusion: CO's preferred binding position is perpendicular, but myoglobin forces it to bend. And that departure from its natural state was the reason CO binding is suppressed. "Everyone was feeling comfortable" with this notion, says Steven Boxer, a professor of chemistry at Stanford University. "Then a couple of chinks appeared in the armor."

One crack opened up last year when a team of researchers, led by Paul Champion and Tim Sage at Northeastern University in Boston, did a structural study of a myoglobin crystal using not x-ray diffraction but another method: infrared spectroscopy. To get structural information using spectroscopy, researchers direct light at a target—in this case myoglobin molecules ordered in a regular crystalline array—and determine the orientation of various parts of the molecule, such as the CO bound to the heme, by measuring how much light is absorbed along different axes of the crystal.

The Northeastern scientists found that

bound CO was nearly upright—with at most a 10-degree bend—and reported it the 4 May 1994 issue of the *Journal of the American Chemical Society* (*JACS*). Another *JACS* article in the 30 November issue, by Tom Spiro and his colleagues at Princeton, reached a similar conclusion.

But the preponderance of evidence—both from x-ray diffraction and other spectroscopy studies—still favored a bent CO bond. At best, however, these results had a margin of error of about ±8 degrees. So Anfinrud and his colleagues jumped into the fray in order to see if they could whittle down this error margin, in part by increasing the computer control of the experiment. This improved the precision and accuracy of their measurements "head and shoulders above the others," says Berendzen.

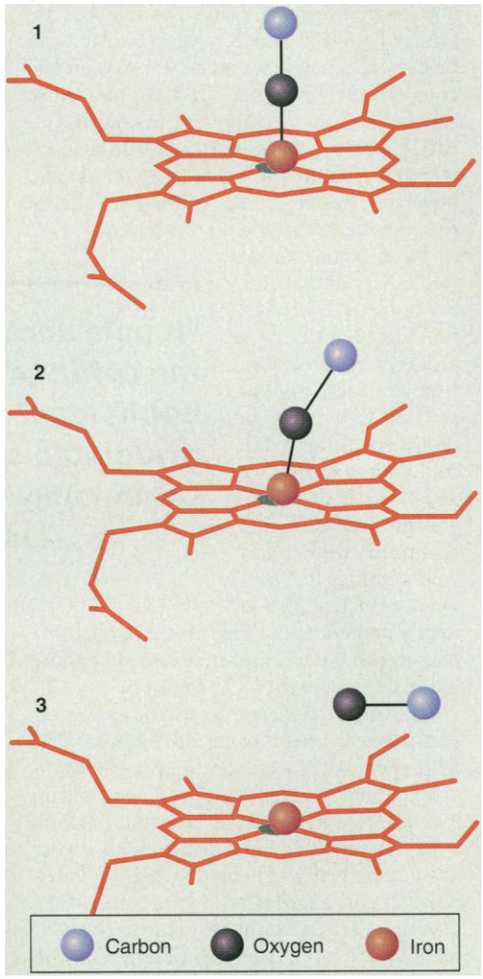
The Harvard researchers began with a thin cell filled with myoglobin in solution. Their goal was to determine the orientation of the hemes and their bound COs in this solution; the researchers accomplished this with laser light pulses, in which the light's electromagnetic waves were polarized, or oscillated, either vertically or horizontally.

They first sent a pulse of horizontal IR light into the cell, which is absorbed most strongly by horizontally oriented COs. This gave them a background figure on how much IR light is absorbed when COs are randomly oriented in solution. Next, they fired a single pulse of green light, polarized vertically. Green light is absorbed by heme groups, and in this case most efficiently by those that are also vertical. Photons from this pulse energize the hemes, causing them to shake off their bound COs. That left the solution with fewer COs pointing in one particular orientation.

A second pulse, again of horizontally polarized IR light, followed a fraction of a second later, and they recorded how much light was absorbed. Any decline in absorption of this pulse—compared to the initial IR pulse—was related to the orientation of the COs before they were knocked free: If the absorption dropped sharply, it meant the COs started out nearly horizontal—and therefore perpendicular to the vertical hemes. If only weakly, then the COs started out bent more closely to the vertical.

When repeated thousands of times, with the polarization of the pulse of green light changed back and forth between vertical and horizontal, the researchers could knock COs off hemes with particular orientations, and—by comparing the relative IR absorption—home in on the characteristic orientation of the CO-heme bond to within a few degrees. And that orientation, Anfinrud's team concluded, was nearly perpendicular.

Although many, such as Hochstrasser, support this conclusion, agreement isn't universal. Gregory Petsko at Brandeis University in Waltham, Massachusetts, for one, says he's more inclined to believe the crystallography data, the bulk of which shows a bent CO. "[Spectroscopy] is an indirect measure of the position of atoms. But the crystallography is looking right at it," he says. Berendzen and others counter that x-ray measurements are imprecise, as they are strongly diffracted by the heavy iron atom in the heme, making it harder to see the weaker signals nearby, such as the diffraction from the CO. They are also prone to artifacts, adds Berendzen. It's possible, he explains, that the x-ray studies measure a bent CO angle accurately, but this bent CO is created by the formation of the crystal itself. "I think they got the angle right," he says. "It's just bent in the crystal." The way in which the proteins pack into a crystal, he explains, may force the CO to bend.



A new twist. Carbon monoxide (CO) prefers to bind perpendicularly to heme (1). But when heme is embedded in myoglobin, little CO binding takes place. Researchers long believed that was because the protein forces CO to bind at an angle (2), but new work suggests it may be because unbound CO near the binding site is pinned on its side (3).

If the new spectroscopy results are to be believed, it creates a new problem. "It's not enough just to tear down the old paradigm for how myoglobin discriminates against CO. You have to offer something in its place," says Boxer.

Anfinrud does have something to offer: He believes researchers have had the CO binding story backwards. Rather than allowing the unbound CO to roam free and then forcing it to bind in a bent orientation, additional spectroscopy results from his study indicate that unbound CO, floating near the binding site, is constrained by the shape of the protein and forced to lie flat on its side. In order to bind, the CO must break free of this restraint and bind straight up and down. Because this requires more energy than what is needed for the molecule to simply diffuse out of the protein, most of the CO is prevented from binding. "He's saying that there's a barrier to getting upright to bind," says John Olson, a professor of chemistry and cell biology at Rice University. "And I think that's probably true."

But while Anfinrud explores CO barriers, Olson has been investigating another idea: O_2 boosters. In 1988, he and his colleagues analyzed x-ray diffraction data to show that in the absence of CO and O_2 the pocket in which they dock doesn't remain empty: A water molecule drops into the space. So, in order to bind to the iron, CO or O_2 must elbow this water out of the way. And O_2 may be better at this than its competitor, Olson says, because it is pulled in by a neighboring amino acid in the protein. When O_2 binds to iron, it forms a weak hydrogen bond to a histidine, which helps hold the O_2 in place. "The oxygen is actually attracted by the hydrogen bonds, whereas CO is not," says Princeton's Spiro. "I think that's a big part of the story.

Phillips and Olson and a raft of colleagues gave this theory a boost in a 1993 paper in the *Journal of Molecular Biology* when they used genetically engineered mutant myoglobins—made by Barry Springer and Stephen Sligar at the University of Illinois, Urbana—that replaced this histidine with other amino acid groups, such as leucine, which don't bond to oxygen. The amino acid substitution drastically lowered the ability of O_2 to bind to the iron when compared to the CO.

There are other possible mechanisms for how the protein favors O_2 binding as well. Regardless of which mechanism researchers finally settle on, many believe that Anfinrud's work has shown that spectroscopic techniques have the ability to home in on fine details of protein structure that more global techniques such as x-ray diffraction can only guess at. "Protein crystallography has been in a phase of measuring the shapes of coastlines," says Berendzen. "Now we need to look at the rivers. The spectroscopic techniques are beginning to give us that level of detail. The different techniques complement each other well."

–Robert F. Service