What looks like a mountain on top of a huge worm is actually a fluorescent quantum dot attached to a DNA repair protein as the protein scans a segment of DNA. Attaching the relatively huge quantum dot (about 20 nanometers) to the protein (about 3 nanometers) is like appending a boulder to a pebble.

DNA REPAIR LIVE AND IN REAL TIME

BY JOE MIKSCH

HOW DOES IT DO THAT?

If our DNA cannot repair itself, we are well and truly sunk. Dead, actually.

With each lesion, whether caused by ultraviolet light, other radiation, additional insults, or even normal metabolic doings, the damage mounts. Without a mechanism by which cells can identify the harm and call in a repair crew to remedy the disruption, cells can die or reproduce with rabbit-like rapidity, causing tumors. Inheritable diseases can be passed on.

Scientists know there are proteins that monitor the health of our DNA, locating errors and fixing them. One big question, though, is "How can a small team of proteins find and undo damage efficiently enough?" Human DNA contains 3 billion base pairs. An organism whose DNA we know more about, *Escherichia coli*, has a few million base pairs. For every 188,000 or so *E. coli* base pairs, there's likely to be just one DNA repair protein.

On the surface, that ratio is as absurd as having only a handful of highway workers to repair every pothole in Pittsburgh. There's just no way they can get the job done quickly enough to be effective. Right? There's no way one pothole can be filled before a dozen others crop up.

Enter Bennett Van Houten.

COURTESY BENNETT VAN HOUTEN

Not only has he solved the mystery of the apparent labor shortage, he's also managed to find a way to watch the DNA repair process happen live and in person and to identify the tools by which these molecular road crews get the job done.

His work may one day allow scientists to rescue the DNA repair process when it fails and open up new treatments for DNA-damage-related disease.

Van Houten, a PhD, leads the University of Pittsburgh Cancer Institute molecular and cell biology program and is the Richard M. Cyert Professor of Molecular Oncology and a professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh. He came to Pitt from the National Institute of Environmental Health Sciences (NIEHS, where he held a precedent-setting joint appointment in both its intramural and extramural research divisions).

At his PC in his office at the Hillman Cancer Center, Van Houten sets up a running slideshow on DNA repair, specifically nucleotide-excision repair (NER), a DNA-mending pathway that employs proteins to identify and cut out damage and to fill in gaps. Van Houten works with *E. coli*, but the process is similar, though more complex, in mammalian cells. In fact, NER is universal among all biological organisms. It's kind of a catch-all repair mechanism in that it addresses a wide range of insults to DNA. In humans, NER is the major mechanism used to protect DNA from damage from ultraviolet light.

Van Houten is one of those guys who seems larger than he is. It's probably his ebullient personality that creates the illusion of physical hugeness. Van Houten, who arrived at Pitt in September, is "there."

His gestures are sweeping. His enthusiasm is engaging. He is a rhetorical question machine. He is very willing to spend nearly two hours explaining the minutiae of DNA repair proteins, their function, and his novel method for watching them in action in real time—an exercise that involves frequently interjecting terms such as "cool," "really cool," "very cool," and "super impressive." And that's just what half of his lab works on. (Van Houten is also pursuing studies that demonstrate how mitochondrial DNA become damaged from oxidative stress.)

Caroline Kisker, a PhD and chair of structural biology at the Rudolf Virchow Center for Experimental Biomedicine in Wuerzburg, Germany, has collaborated with Van Houten on this DNA repair work. She says that Van Houten has an outsized passion for his work, a passion so strong that a spirited discussion can sound like a confrontation.

"It is wonderful to talk to him about science even if we disagree," says Kisker. "It may even sound sometimes like a fight when we try to convince each other about our ideas, but at the end, even if we disagree, we are always best friends and have learned from each other a lot.

"The way he talks about science clearly reveals how deeply he thinks about it but also how much he is excited about it."

With his floppy mop of blondish hair, Van Houten swings in his swivel chair, turns to this reporter, and—after saying, "I'm really big into visualization"—begins to paint a picture in PowerPoint of a professional obsession: the machinery of the system that prevents injuries to DNA from killing us.

"Imagine being on a road crew, and your job is to fix potholes," he says as images click by. "Unfortunately, there aren't many of you, and you have to drive from California to New York and fix every road in between. That's what these repair proteins [in an enzyme group called Uvr] are doing."

"They're basically looking for any perturbation in the structure of the DNA. I don't know how many miles of roads there are in the United States, but if you think about this problem, you've got to ask, 'How do you even attempt to do that?'"

The road crew consists of UvrA, UvrB, and UvrC. UvrA is the dummy of the bunch. It sees a lesion in the DNA first, but then it's stumped.

"I call it big and stupid," Van Houten says. "It says, 'You know, there's something wrong with the DNA, but I can't tell you what it is.'"

UvrA is not a particularly discerning worker, so it calls upon UvrB, the road crew supervisor, kind of. UvrB's job is to confirm that, yes, there is something wrong here, a pothole in the DNA, if you will.

Here's where UvrA, that dim bulb, lends a hand. It helps B bind to the DNA so smarty-pants can confirm A's suspicion. Such DNA damage can thoroughly bollix transcription and replication and cause irreparable damage—fatal damage—to a cell.

So in comes UvrC. C cleans crumbling asphalt from the DNA pothole by snipping out the lesion with the aid of a DNA helicase (a master at pulling apart double-helix strands). Then two colleagues, other proteins, visit the work site to fill and seal the gap.

But in an *E. coli* cell, there are between 25 and 200 UvrA molecules to 4.7 million base pairs of DNA. The ratio is similar in human cells. And the generation time of *E. coli*, even under the best of circumstances, is no more than 20 minutes. That's a lot of road to monitor in very little time. So how does the Uvr crew get the job done?

"Think about how you would go about searching every road in the United States," Van Houten says. "One way would be to fly around in a helicopter and just randomly come down on the road in certain places. But that's a three-dimensional search, and that would take a long time," particularly with all that surveying from the sky, landing, checking, and launching again.

How about a surface search? Like an "intelligent" car that could travel the DNA highways. It could trundle along each road and, when a flaw is found, notify the repair system. That might be faster, but not by a huge margin, Van Houten says.

That's not fast enough if you'd like to see all of your DNA

Or, what if the smart car could hop along like a hovercraft, bounding over good DNA? Maybe it would be more like the Tarzan—"I can't come up with a good road metaphor here," Van Houten says—of the DNA highway repair crew, swinging from strand to strand, never still or in the "air" for long?

You could guess and hypothesize all day long and, with the aid of the scientific method, come up with a rational, falsifiable idea of what's going on. Or, you could just take a look.

How? Call on the quantum dot.

Quantum dots may sound like something used to help the Starship Enterprise rend the fabric of the space-time continuum, but in actuality they are little beacons of light. Consisting of semiconductors, they absorb light of any wavelength and then emit a bright band of a discrete color. The biggest quantum dots—about 20 nanometers—emit red. The smallest ones—around 12 nanometers—give off blue light. "And they're super bright," Van Houten says.

Attaching quantum dots, as small as they are, to much smaller individual proteins is kind of like appending a boulder to a pebble. But Van Houten and his colleagues found a way to mate the dot with the protein. At least they thought they did. Van Houten's lab colleague Hong Wang, PhD and master of atomic force microscopy, was able to confirm this.

Atomic force microscopy uses a tiny probe with a sharp tip (only 50 nanometers) attached to a cantilevered arm. Like a stylus on a record player, the arm sweeps across the mica surface. As it bumps over molecules, it causes a laser to deflect off the arm. The instrument captures this deflection and converts it into an image.

Van Houten pulls up a movie on his computer that's part of the PowerPoint show. Neil Kad, a lecturer in biophysics at the University of Essex, made the movies with Hong's help. "Neil worked out a way to stretch the DNA," says Van Houten, "which usually resembles a hopeless mess of spaghetti, into a tightrope, [allowing us] to watch the proteins do their stuff.

"So now we can watch proteins move," he says.

checked for damage and repaired before you're singing with the choir invisible.

Stretched out strands of DNA resemble a green spider web. A bunch of little red blobs, which are the UvrA-affixed quantum dots fluorescing, engage in a spastic dance. Some pause along the DNA web while others flash and move. No one had witnessed this life-saving dance until Van Houten's team came along. "This is a huge breakthrough," Van Houten says.

But something didn't make sense.

"The UvrA is just doing a three-dimensional (helicopter) search," Van Houten says. "But jumping from strand to strand is going to take a while. Over some pints in a pub, Neil and I calculated that with the amount of protein and the amount of DNA, it would take the proteins about four generations of *E. coli* to search all the DNA."

That's not fast enough, obviously, if you'd like to see all of your DNA checked for damage and repaired—what's supposed to be a never-ending process—before you're singing with the choir invisible. Van Houten and colleagues then suspected that UvrB might contribute to the search. So, B gets added to the mix with A, and …

"What UvrB has done here, and I don't know of any examples of this [described] in the literature, is take a protein that moves in three dimensions and turn it into a one-dimensional search," Van Houten says. That's A and B together, kind of sliding randomly on a strand of DNA. How about B on its own?

Van Houten says he would have bet a lot of money that UvrB wouldn't move at all without being told to by chemical signals from its buddies. That's a bet he would have lost. After confirming the damage first recognized by UvrA, sometimes UvrB seems to get tired of waiting for UvrC. At that point, with C already having been signaled for, B strikes out on its own, moving along one axis but somehow directed.

It's not just one protein moving in one way trying to identify DNA damage, Van Houten realized. A, by itself, does what Van Houten calls a three-dimensional search, swinging around strands of DNA. A and B together randomly walk around. And B occasionally heads out on its own to what seems to be a predetermined destination.

"Together, these three types of motion allow Uvr to clearly see all the roads in the United States," says a satisfied Van Houten.

There is, of course, a next step. And, Kisker says, Van Houten wouldn't be Van Houten if he weren't eager to take it. "He is always driven to understand different topics and will not hesitate to analyze them from all different angles to obtain a more complete picture," she says.

At the moment, as Van Houten notes, he and his colleagues can only see the Uvr proteins with the aid of quantum dots. What interests Van Houten is unraveling the structure of these proteins. Knowing how they're composed is key to knowing how they work, how they find damaged DNA. Function follows form.

Interpreting a molecular dance is key to damage assessment. "The DNA is wiggling and dancing and the repair enzymes are looking at that wiggling and dancing," Van Houten says. "If you put a lesion in the DNA, it affects the dynamics, and that's a big part of the recognition process."

Okay, but how do the proteins themselves figure out when the wiggling has gone awry? Structural biologists have found that UvrA has "zinc fingers," structures that look and act like fingers and bind to zinc. What they do, says Van Houten, is function like chemical probes. The fingers insert themselves into the DNA, poking around for chemical signals that indicate damage.

UvrB then gets into the picture. It has a structure called a helicase fold, which Van Houten says acts like the "jaws of life" emergency responders use to extricate crash victims from their cars. The fold spreads open the DNA, allowing another structure called a beta hairpin to go into the DNA and probe to confirm UvrA's suspicion of damage.

"The UvrABC system had long resisted structural analysis until Ben teamed up with Caroline Kisker to achieve a sophisticated breakthrough," says former National Institutes of Health colleague Jan Drake, a PhD who is chief of the NIEHS laboratory of molecular genetics in North Carolina.

This breakthrough, says Kisker, may have wide-ranging benefits for human health. "Maintenance of the correct genetic information is crucial for all living organisms," she says. "Mutations are the primary cause of hereditary diseases, as well as cancer, and may also be involved in aging. Eighty to 90 percent of all human cancers are ultimately due to DNA damage."

The bacterial Uvr proteins have human analogues, Van Houten says: "I think these are fundamental findings that are going to be true for the same system in human cells."

Sorting this out, says Drake, could open a window through which scientists can manipulate the DNA repair process for the better.

"Understanding key cellular systems allows [us to] recognize when mutations or environmental impacts have disabled them," Drake says. "This work provides the foundation for a new generation of studies, which have the potential to manipulate or fix repair systems to our advantage."

"I tell people that before I retire or expire, I want to change these little blobs [which represent proteins on a PowerPoint slide] into structures," says Van Houten. "My goal, and I'm really serious about this, is to make a molecular movie of how all these proteins work."

And he says this with a big smile. ■