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The Power of the LDL Receptor is All in the Fold

For Stephen Blacklow, striking a balance between helping people and doing research hasn't always been easy, and he didn't want to choose between them. So in 1993, Blacklow, armed with a recent MD and a PhD in chemistry, searched for a postdoctoral position that would fulfill both objectives.

"Stephen Blacklow, a former HHMI postdoctoral fellow in the laboratory of Hughes investigator Peter Kim, has made a key discovery that may explain why some people have high cholesterol levels."

He found it with HHMI investigator Peter Kim, a biologist at MIT's Whitehead Institute for Biomedical Research. Blacklow realized during his interview that Kim's lab could be the perfect place to focus his biochemistry expertise on serious medical problems.

Score one for instinct. In the September issue of *Nature Structural Biology*, Blacklow—who has just finished his HHMI postdoctoral fellowship at MIT—and Kim reported a finding that is changing the way researchers understand familial hypercholesterolemia (FH), an inherited disease marked by high cholesterol levels that can lead to coronary heart problems.

Following a hunch, Blacklow and Kim discovered how gene mutations can wreck the low-density lipoprotein (LDL) cell receptor. The receptor's job is to pluck cholesterol-carrying lipoproteins from the bloodstream, shuttling cholesterol inside the cell for use. Blacklow and Kim suggest that mutations in the LDL receptor cause it to fold incorrectly by interfering with the receptor's ability to bind calcium, which is needed by the receptor to function properly.

When the LDL receptor works normally, cholesterol levels stay somewhat in balance. If the LDL receptor malfunctions, however, and fails to bind or sweep cholesterol-laden proteins into cells, then cholesterol levels skyrocket. One result is FH, which affects about one in 500 people.

Blacklow decided several years ago to pursue the LDL receptor project in Kim's lab because the protein's cell biology was well understood. "Michael Brown and Joseph Goldstein had already taught us so much about LDL uptake processing and receptor recycling," Blacklow said, "that it was an ideal system to take a step further and look at the molecular details of structural biology."

To study the LDL receptor, Blacklow zeroed in on one particular fragment, LDL ligand-binding repeat 5 (LDLR5), previously identified as crucial to receptor function, as well as being mutated in people with FH. He set out to characterize LDLR5, which contains a conserved gene sequence with several negative charges. Many researchers think LDLR5's negatively charged cluster binds to positive ones on cholesterol-carrying proteins. Until then, researchers thought that any major gene mutation in the LDL receptor must directly affect this receptor-ligand link. As Kim put it, "That was the dogma of the field."

Blacklow's results would soon contradict that dogma, however. In studying LDLR5, he first inspected how the protein fragment folds normally. Every protein is a linear sequence of amino acids that subsequently folds into a three-dimensional shape. This unique shape determines, in part, with which other proteins it can bind and interact to cause a variety of reactions between and within cells.

So Blacklow began to isolate samples of LDLR5. He placed the linear protein fragments into test tubes with a buffer containing calcium (to keep the environmental pH constant). He included calcium simply because it is standard in such buffers; and, as most researchers knew, the receptor needed a jolt of calcium to bind LDL.

After the LDLR5 sequences curled into their expected three-dimensional shape, Blacklow used nuclear magnetic resonance spectroscopy to chart their structure. For well-folded proteins, Blacklow expected to see spectra filled with peaks, and he did. When he repeated the experiment on LDLR5 sequences in a buffer without calcium, however, he noticed something odd: The perfectly normal LDLR5 fragments produced sloppy spectra, the kind

one might expect from a misfolded or denatured protein.

Blacklow then realized that calcium was far more crucial to basic LDL receptor folding than had previously been recognized. "It was like, 'Hey! I have a project!'" he recalls. Kim, too, knew something was up. "There come certain moments when you get a result that is not expected," Kim said. "And that's when you know you're on to something."

Further experiments by Blacklow confirmed calcium's importance. He showed that mutant LDLR5 fragments have a low affinity for calcium, that is, they don't bind calcium efficiently. This suggested that the crucial role for LDLR5's negative charge is not to bind the positive part of cholesterol-carrying proteins, as previously thought, but instead to bind calcium, which is also positively charged. During folding, the LDL receptor apparently needs calcium to help guide its final shape.

"The evidence we have more strongly suggests that the likely effect of [LDL receptor point] mutations is to interfere with the binding of calcium, which is coupled to formation of [the receptor's] proper shape," Blacklow says. "The result is a misshapen LDLR5 repeat. Because the whole repeat is misshapen, binding fails, uptake fails, and the whole pathway malfunctions."

LDL's newfound structural defects could shed light on other diseases partly blamed on poor protein folding, including cystic fibrosis, Alzheimer's disease, Marfan syndrome, retinitis pigmentosa and α -1-antitrypsin deficiency, which causes emphysema.

"It's yet another testimony to the importance of doing basic science research," said Kim. "We study protein folding, and HHMI has supported protein folding research for a long time. That's about as basic as you can get. Yet we now know that several human diseases are in fact caused by defects in this very fundamental process."

Blacklow also knows now that he really can do basic research and help people at the same time. "I'm still interested in the general relationship between protein structure and function," said Blacklow, now an assistant professor of pathology at Stanford University. "And, of course, how it goes awry in human disease."