Antibody Replacements Just a 'Click' Away

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Chemists at the California Institute of Technology (Caltech) and The Scripps Research Institute (SRI) have developed an innovative technique to create cheap but highly stable chemicals that have the potential to take the place of the antibodies used in many standard medical diagnostic tests. James R. Heath, Ph.D., principal investigator of the Nanosystems Biology Cancer Center at Caltech, one of eight Centers of Cancer Nanotechnology Excellence, and K. Barry Sharpless, Ph.D., SRI, and their colleagues describe the new technique in the journal *Angewandte Chemie International Edition*.

Last year, Dr. Heath and his colleagues announced the development of the integrated blood-barcode chip, a diagnostic medical device about the size of a microscope slide that can separate and analyze dozens of proteins using just a pinprick of blood. The barcode chip employed antibodies, which are proteins utilized by the immune system to identify, bind to, and remove particular foreign compounds such as bacteria, viruses, and other proteins.

“The thing that limits us in being able to go to, say, 200 proteins in the barcode chip is that the antibodies used to detect the proteins are unstable and expensive,” said Dr. Heath. “We have been frustrated with antibodies for a long time, so we wanted to be able to develop antibody equivalents—what we call protein capture agents—that can bind to a particular protein with very high affinity and selectivity and that pass the following test: You put a powder of them in your car trunk in August in Pasadena, and you come back a year later and they still work.”

In the new work, Dr. Heath and his colleagues have developed a protocol to quickly and cheaply make such highly stable compounds, which are composed of short chains of amino acids, or peptides. The technique makes use of the "in situ click chemistry" method introduced by Dr. Sharpless in 2001, in which chemicals are created by joining—or "clicking"—smaller subunits together.

To create a capture agent for a particular protein, the scientists devised a stepwise approach in which the first subunit of the capture agent is identified, and then that unit, plus the protein, is used to identify the second subunit, and so on. For the first subunit, a fluorescent label is added to the protein, which then is incubated with a bead-based library of tens of millions of short-chain peptides, representing all the potential building blocks for the capture agent. When one of those peptides binds to the protein of interest, the fluorescent label is visualized on the bead (red, blue, or green, depending on the type of label), allowing the linked protein-peptide complex to be identified.

That first peptide, which is about one-third of the length of the final capture agent the scientists are trying to make, then is isolated, purified, and modified on one end by the addition of a chemical group called an alkyne. This is the anchor peptide, which then is incubated, together with the same protein, with the bead-based library. The bead-based library now contains peptides that have been chemically modified to contain an azide group at one end. The alkyne group on the added peptide can potentially chemically react with the azide group of the library's peptides to create a new peptide that is now two segments long.

However, the reaction can occur only when the second peptide comes into close contact with the first on the surface of the target protein, which means that both must have affinity for that protein; essentially, the protein itself builds an appropriate capture agent. The two-segment-long peptide then is isolated and purified, "and then we modify the end of

that with an alkyne and add it back to the library to produce a three-segment peptide, which is long enough to be both selective for and specific to the target protein," Dr. Heath said.

"What Dr. Heath has shown is that in several iterations, a high-affinity ligand for a protein can be created from blocks that do not bind to the protein all that well; the trick is to repeat the in situ screen several times, and the binding improves with every iteration," noted Dr. Sharpless.

"This is about as simple a type of chemistry as you can imagine," said Dr. Heath. The process, he said, makes "trivial" the "Herculean task of finding molecules that bind selectively and with high affinity to particular proteins. I see no technical reason it couldn't replace any antibody."

This work, which is detailed in the paper "Iterative in situ click chemistry creates antibody-like protein-capture agents," was supported by the NCI Alliance for Nanotechnology in Cancer, a comprehensive initiative designed to accelerate the application of nanotechnology to the prevention, diagnosis, and treatment of cancer. An abstract is available at the journal's Web site.

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