Staying ahead of antibiotic resistance: Protein structure research paves the way for more efficient treatment

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The recent coronavirus pandemic shows just how quickly a deadly pathogen can sweep across the globe, killing tens of thousands in the

U.S. and disrupting daily life for millions more in the span of a few months. A lack of medicines to treat the disease is one reason coronavirus is so deadly. COVID-19 is caused by a virus—this means that antibiotics, which kill bacteria, can't be used to treat the virus.

However, antibiotics give us a false confidence about our ability to stamp out bacterial and other microbial pathogens. Penicillin, the first antibiotic, has been saving lives since 1928, but because pathogens have acquired antibiotic resistance—due partly to antibiotic overuse—penicillin is now harmless to many pathogens it used to quickly kill. This is because most antibiotics are derived from other bacteria, which means many pathogens have genes that can quickly respond to what are seen as natural threats. Because they are prepared for such threats, the pathogens that cause diseases like tuberculosis or staph infections often quickly evolve antibiotic resistance that evades or defeats antibiotic treatments.

"Efforts to produce new antibiotics have not been sufficient to cope with the emergence of these new antibiotic-resistant strains," said Andrzej Joachimiak of the Center for Structural Genomics of Infectious Diseases. "There is an urgent need to advance new strategies for antimicrobial drug development to tackle the rising global threats of antimicrobial resistance."

The Structure Study

One way researchers are looking for new antibiotics is by directly studying proteins inside pathogens that could be deactivated in new ways. They are especially interested in the essential proteins that allow pathogens to survive, grow, and reproduce inside a human host.

"We look at protein structure to see how the protein works and how to maybe break them down in a way that bacteria can't readily fix," says

PNNL researcher Garry Buchko. "If the pathogen doesn't recognize the type of antibiotic attack, it won't have a natural resistance at the ready, so it could take longer to develop an arsenal to fight against it."

Buchko's work on the structure of pathogen proteins bookends a special issue of *Protein Science* devoted to antibiotic resistance. In one paper, he presents research on a protein family whose function is unknown, a DUF, or "domain of unknown function." DUF proteins represent 20 percent of all protein families that aren't linked to a specific role.

Thanks to advances in DNA sequencing, researchers know the amino acid sequence of all the proteins encoded in a pathogen's genomes. But even when they know the amino acid sequences of these proteins, they often may not know the protein's real role, or primary function, inside the cell.

Unlike viruses, whose genomes only encode for tens of proteins, microbial pathogens have genomes that can code for thousands of proteins. Furthermore, the scientists don't know what the function of many of these proteins are. This means that proteins may be lurking inside a microbe to counterattack a new antibiotic.

Bacteria are good at having backup plans. While a family of proteins may be the primary enzyme used to catalyze one specific chemical reaction, they often are promiscuous and catalyze other reactions but at a lower efficiency. That's why even if an antibiotic knocks out the main protein that performs an important reaction, there might be two or three others with a similar enough structure that lets the organism still perform those vital reactions well enough to survive.

"Protein structure is a way to understand the biochemistry inside microorganisms," says Buchko, explaining that different protein structures encourage different chemical reactions. "We have an idea of

which proteins are important for disease but as we learn the biochemistry, we can be more intelligent about turning them off."

All in the Protein Family

Researchers have a few different tools to study protein structure, but Buchko's specialty is nuclear magnetic resonance (NMR) spectroscopy, a method of observation so detailed he can measure the motion of individual atoms in a protein. Atoms are arranged not only in the amino acid strings that form the backbone of a protein, but also in the three-dimensional organization of atoms in unique molecular side chains that enable proteins to perform their specialized chemical reactions.

One of the powers of NMR spectroscopy is that it allows researchers to monitor each proton in the nuclei of atoms in a large protein and see the effects of adding a chemical, such as an antibiotic, to know the specific location the antibiotic binds to. Being so exact can give researchers an idea of which amino acids are most important, provide clues toward the function of proteins, and enable a molecular understanding of protein changes as they carry out chemical reactions.

"NMR has long been used to study protein structure changes," says Nancy Washton, the NMR capability lead at EMSL, the Environmental Molecular Sciences Laboratory, a U.S. Department of Energy, Office of Science, user facility located at PNNL, which provides Buchko's NMR resources. "One important advantage of NMR over other techniques is that the protein can be investigated in live conditions. This provides us with results more relevant to biological systems."

The first of Buchko's papers studied proteins from the bacteria that cause tuberculosis. By solving structures using NMR spectroscopy, he discovered that proteins previously thought to be in one big DUF family were actually composed of two sub-families with structural differences

that make them behave very differently. The second paper is about a structure for a fungal pathogen with a very unusual cellular organization. The protein contains an iron-sulfur cluster, and the NMR data suggests the structure of this protein changes with the redox state of the cluster.

These findings contribute to a larger picture that enhances our understanding of how proteins work. Having detailed structures for proteins creates blueprints that researchers can use to design new antibiotics to outsmart pathogens before they become overwhelming problems.

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