Computer program predicts MRSA's next move

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This image is a visualization of the 3-D crystal structure of the top-scoring DHFR protein mutant, determined from experimental data. DHFR is a likely molecule for attack by a drug to stop MRSA bacteria infections. The new K* Algorithm predicted both the structures of the mutants and affinity, how well the mutants would interact both with the inhibitor drug molecule and the native substrate. Scientists' next step would be to redesign the inhibitor or design a new inhibitor that better binds to the mutant DHFR protein, as predicted by Algorithm K*, as well as to the wild-type MRSA bacterium DHFR. Alternately, they may try to design a new inhibitor that would bind better to the mutant DHFR proteins predicted by K*, and use a combination therapy of the Old inhibitor (which binds well to the wild-type MSRA DHFR) plus the New inhibitor, to block mutants that may come along over time. These mutations (shown as stick residues) cause an 18-fold loss in affinity, and crystal structures show a conformation with significantly reduced protein:inhibitor interactions. The mutations are the colored (green and red) sticks that stick out of the rainbow-colored ribbon drawing of the DHFR protein. The mutations are in two places because the mutant has two amino acid mutations. Credit: Image Courtesy of Bruce R. Donald, Duke Univ. Medical Center

Researchers at Duke University Medical Center are using computers to identify how one strain of dangerous bacteria might mutate in the same way a champion chess player tries to anticipate an opponent's strategies.

The predictive software could result in better drug design to beat antibiotic-resistant mutations.

"This work shows a way to predict bacterial resistance to antibiotics under development, before research progresses and tests of the antibiotics begin in people, and even before doing laboratory procedures to explore potential resistance," said Bruce Donald, Ph.D., Duke's William and Sue Gross Professor of Computer Science and Biochemistry. "The protein-design algorithms that predict mutations could be used in a drug-design strategy against any pathogen target that could gain resistance through mutation. It's very expensive and labor-intensive to go back to square one and redesign a drug when a bacterium gains resistance to a drug's existing structure."

A paper describing the work will appear in the Proceedings of the National Academy of Sciences the week of Monday, July 19.

Certain bacteria, like MRSA (methicillin-resistant Staphylococcus aureus) are dangerous because they mutate swiftly and cleverly to evade drugs designed to block the pathogen's essential biological pathways. In this study, the researchers examined mutations in a MRSA enzyme called dihydrofolate reductase (DHFR), which is targeted by several drugs. Almost every living organism has a version of DHFR, because it is an enzyme needed at a critical step in a pathway that takes folic acid and turns it into thymidine, one of the four building blocks of DNA—the "T" in the A-C-G-T nucleotides.

"We are excited about the prediction power we have, in this case with MRSA, because we used a sophisticated algorithm that models protein and drug flexibility while searching for mutants," Donald said. "We used our algorithm to find mutation candidates that satisfy both a positive design - structures that still allow the bacterial enzyme to do its work - and also negative design - they block the ability of a brand new antibiotic drug to do its job. The algorithm found candidates that would be able to block the antibiotic while at the same time allowing the native reaction of the bacterial enzyme to occur."

"We're basically trying to do a pre-emptive strike, and this study is a step toward identifying antibiotics that can pre-emptively deal with possible resistance in nature," said lead author Ivelin Georgiev, Ph.D., who did the work while he was a graduate student in the Donald lab and has since moved to the National Institutes of Health.

Donald said that some bacteria, such as MRSA, escape antibiotics by evolving mutations to change the shape of the active site of their enzymes. "Our algorithm tries to predict that process," he said.

The authors will provide their algorithm freely and publish the open-source software they devised.

Donald added: "My kids are now 9 and 11, and when I ask about the antibiotics they took 10 years ago, I'm now told these are not strong enough to treat the same illnesses, which is a real-life example of drug resistance," Donald said.

Working with longtime collaborators from the University of Connecticut under Amy Anderson, the Duke team built on its computer program for designing enzyme structures to uncover the possible "chess moves" that MRSA might make to evade a drug that binds to DHFR to slow or stop its actions.

That algorithm features a "dead-end elimination" feature that can process all of the possible chemical interactions to sort through outcomes that would not work well for the bacterium. Last year, Donald, Georgiev, Duke student Cheng-Yu Chen and Anderson published a paper in PNAS about this K* (K Star) algorithm, which can search through all the possible shapes and changes of a desired enzyme.

For the MRSA study, the Connecticut team tested its new inhibitor molecule with four promising potential enzyme mutants predicted by the Donald lab using K*. Three of the mutants maintained activity but also displayed lower affinity for the drug molecule, a propargyl-linked antifolate inhibitor. When team members determined the crystal structure of the top-ranked mutant enzyme bound to the inhibitor drug, the structure showed that the drug clearly had significantly fewer interactions with the protein, providing insight into the reasons why this mutant DHFR evades the drug.

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