STRUCTURE_OF_PROTEINS_AND_DNA

NEW WEAPONS FOR THE GERM WARS

INEXPENSIVE POLYMERS CAN EXTEND THE RANGE OF NATURE'S GERM-FIGHTER ARSENAL.

2

This story begins with frogs. A lily pad is a fine place to perch in the moonlight and broadcast mating calls if you're a frog, but the picturesque lily pond of a Monet painting is a cesspool of microorganisms. If you or I swam in similar water, we'd risk our lives.

How do frogs manage to live happily in filthy water? It's a simple question that no one asked until the 1980s when it intrigued a scientist named Michael Zasloff. In 1986 he found the answer — the skin of a frog harbors armies of protein-like germ fighters. Zasloff isolated a molecule he named magainin — Hebrew for "shield." Structurally like a protein only smaller — just a few amino acids on a peptide chain — magainin has the instincts of a secret service agent. Squads of these agents patrol a frog's skin, where they attack and destroy the cells of bacteria that threaten infection.

The discovery of magainin launched a worldwide wave of research that's still going. Since 1986 scientists have identified nearly 500 other anti-microbial peptides. They've learned that plants and animals, including some that lack the immune system of mammals, harbor a diverse collection of defensive peptides, and they've learned that each species has its own unique peptide arsenal, targeted to the bacteria, viruses and other pathogens of that species' environment.

The prospects to create new germ fighters from the model of mother nature's peptides are potentially boundless. Topping the list is the need for new antibiotics that can defeat the protean ability of bacteria to resist conventional penicillin-like antibiotics. That promise is especially tantalizing in that a range of studies show that bacteria have little or no ability to resist antimicrobial peptides.

But the practical obstacles are huge. "The big catch," says Michael Klein, Hepburn Professor of Physical Science at the University of Pennsylvania, "is you need 20 or 30 steps of organic synthesis to make these molecules, and you end up with such high cost that it's equivalent to grinding up diamonds."

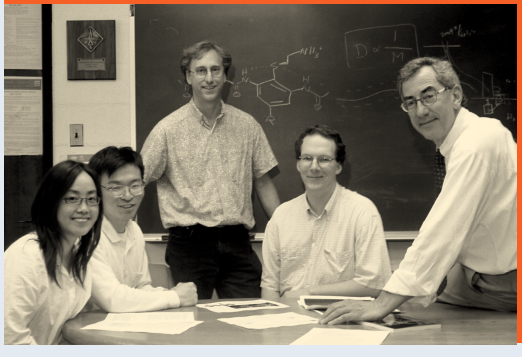
Still other possibilities involve using molecules modeled on magainin and its cousins to create germ-resistant materials such as bandages that kill bacteria or toilet seats that sanitize themselves. With recent studies citing hospital-acquired infection as the fourth-leading cause of death in the United States, such possibilities and others — antiseptic operating tables, surgical gowns, pillows and sheets — provide a strong impetus for research.

PLAYING COPYCAT WITH MOTHER NATURE

Initial efforts to mimic nature's molecular germ fighters led to several laboratory-synthesized peptides, one of which came from Klein's University of Pennsylvania colleague William DeGrado. While these synthetic peptides represent a step forward, they also come with built-in obstacles to practical use. "These natural peptides as well as their synthetic analogues are expensive to prepare and difficult to produce on a large scale," says DeGrado, George W. Raiziss Professor of Biochemistry and Biophysics, "which limits their potential use."

Klein, who directs Penn's Center for Molecular Modeling, collaborated with DeGrado, providing theory-based molecular simulations to help guide his laboratory work. In 2001 these two scientists joined forces again to explore in a different, potentially more practical direction. "We posed a question," says Klein. "Can we mimic the peptide with something that's cheap to make?" Based on initial results, the answer appears to be yes.

Klein and his co-workers Bin Chen and Robert Doerksen used LeMieux, PSC's terascale system, to test the possibilities of creating a polymer — an organic molecule easier to make than peptides — structurally similar to magainin and with similar germ-fighting ability.



University of Pennsylvania research team (left to right): Dahui Liu, Bin Chen, William DeGrado, Robert Doerksen and Michael Klein. Not in photograph: Gregory Tew, now at University of Massachusetts, Amherst.

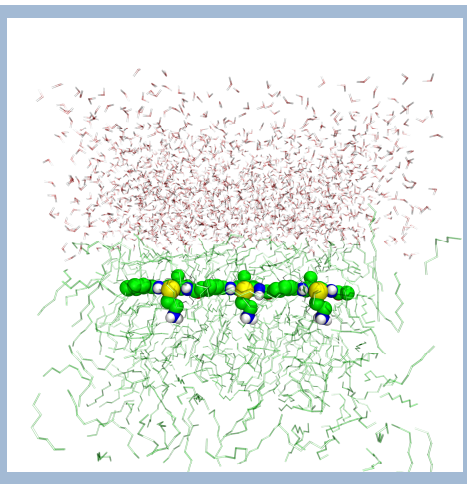
With a series of computations, they developed an accurate computational model to forecast how the polymer would behave in the cellular environment.

With design guided by these computations, DeGrado's team — Greg Tew, Dahui Lihui and Justin Kaplan — synthesized this relatively simple polymer, called an arylamide. Lab tests show the arylamide has antibacterial action similar to magainin and other peptides. The good news of this work — reported in the *Proceedings of the National Academy of Sciences* (April 2002) — is that a feasible new pathway is now open to extend the range of mother nature's anti-microbial arsenal.

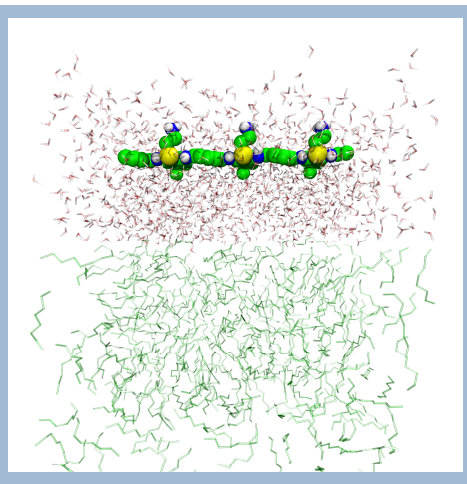
CONT'D →

MOLECULAR DYNAMICS OF ARYLAMIDE

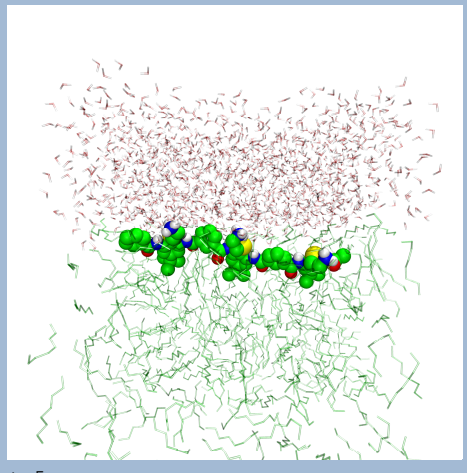
These snapshots show the arylamide polymer in two sets of simulations, both in an oil-water solution. In one set (right), the polymer starts out in the water (red & white); in the other (left), it's inside the oil (green). In both simulations, the polymer moves to the oil-water interface and stays there. Time is in picoseconds (trillionths of a second). The arylamide polymer is composed of carbon (green), oxygen (red), nitrogen (blue), hydrogen (white) and sulfur (yellow).



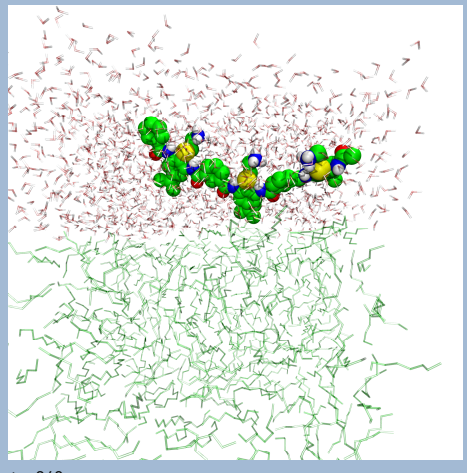
t = 0



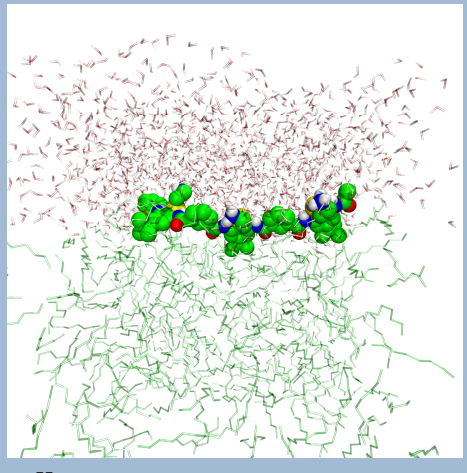
t = 0



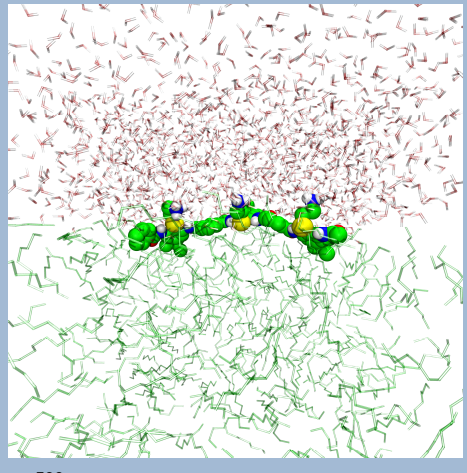
t = 5



t = 240



t = 55



t = 500

WITH HOSPITAL-ACQUIRED INFECTION AS THE FOURTH-LEADING CAUSE OF DEATH IN THE UNITED STATES, APPLICATIONS SUCH AS ANTISEPTIC OPERATING TABLES AND SURGICAL GOWNS PROVIDE A STRONG IMPETUS FOR RESEARCH.

TWO-FACED MOLECULES

What do nearly 500 different natural germ-killer peptides have in common? Like Janus, the Roman god of gates and doors, they face in and out at the same time. All these small anti-microbes can, when circumstances dictate, assume a shape in which, in chemical terminology, they're amphiphilic — one side of the molecule avoids water (hydrophobic) while the other side likes it (hydrophilic).

This two-faced structure — scientists believe — is an essential part of how nature equips peptides to destroy bacterial cells. While the hydrophilic face turns out to the watery environs of the cell exterior, the hydrophobic face can attach to lipids, the oil-like molecules that form cell membranes, to pierce the membrane and create holes that eventually kill the cell.

To create a polymer with similar properties, DeGrado and Tew approached Klein and asked him to model a class of polymers with a fairly simple structure, called arylamides. "We were taking a further step away from the protein backbone as the structural model," says DeGrado, "which should give us good stability at reduced expense. We had a shape that we thought should be compatible with the biological activity we want. But this molecule could adopt many shapes, and the question was whether it's really happy in this shape, whether it's energetically favorable."

To answer this question, Klein, Chen and Doerksen set out to simulate the arylamide polymers in solutions that represent the cellular membrane and its surrounding water, to see if they maintain structure and behave similarly to magainin. The modeling tool for this job is molecular dynamics, a method that tracks the shape and movement of a molecule and its interaction with surrounding atoms. Most often used to model proteins, molecular dynamics relies on "force fields" to represent the forces acting between the atoms of the molecules. Initial attempts to model the arylamide polymer showed that standard force fields gave inaccurate results.

The problem had to do with the uniqueness of the arylamide backbone structure, chosen so that it wouldn't freely rotate, keeping hydrophobic side chains on one side and hydrophilic on the other. To solve this problem, Klein's team carried out a series of demanding quantum computations, an approach called density functional theory, to systematically derive accurate readings of the rotational resistance of the arylamide backbone. With about 60,000 hours of computing using 128 LeMieux processors at a time, they derived the force fields they needed.

The researchers confirmed the accuracy of their revised molecular dynamics model by simulating an arylamide structure and comparing it to the actual structure from experiment. They then ran molecular dynamics with several different versions of the arylamide polymer in an oil-water solution. These simulations (facing page) show the polymer moving toward the oil-water interface and lodging there, mimicking the behavior of the natural anti-microbial peptides.

Taking their cue from these results, DeGrado's team synthesized the polymer and tested its antibacterial properties. Based on the success of this work, the University of Pennsylvania filed for several patents and created a company, POLYMEDIX, to exploit the possibilities for useful applications.

"We've identified a class of compounds," says Klein, "that the drug industry would refer to as a possible lead compound. Some of these short polymers are effective, but it will require systematic studies to develop this further." While it's only a first step, it's a big one, demonstrating not only the possibilities of using polymers to mimic nature's peptide germ fighters, but also how computational simulations and laboratory experiment can work together to custom design molecules for particular purposes.

MORE INFORMATION: http://www.psc.edu/science/klein2002.html

The structure of magainin (left) compared to the arylamide polymer mimic (right). Amphiphilic structure is apparent, with hydrophobic side chains (green) on one side and hydrophilic (blue) on the other.