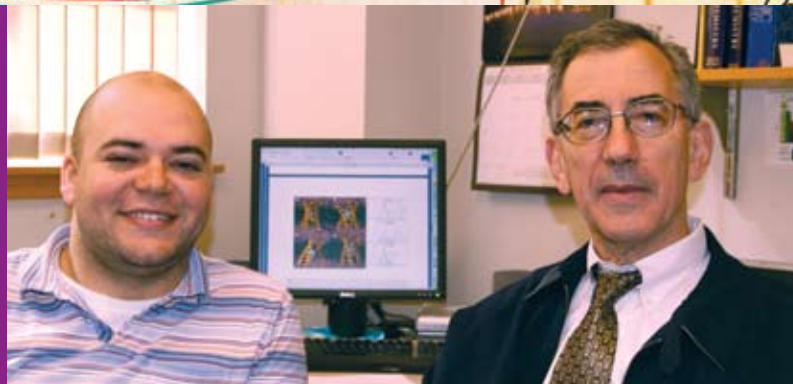


HEAVY METAL INVASION

Matteo Dal Peraro
(left) and Michael
Klein, University of
Pennsylvania



So much for miracles. Penicillin and its derivatives have saved uncountable lives, but evolution, in its robustly creative way, has improvised potent countermeasures to Alexander Fleming's miracle drug. Infectious bacteria have shown protean ability to adapt, survive and reassert themselves as a threat to human health.

Over the last two decades, bacterial resistance to antibiotics has become widely recognized as a serious problem. The National Health Council estimates that 14,000 Americans die annually in U.S. hospitals because available antibiotics don't work. Like *Nosferatu* the undead, tuberculosis has resurrected itself as a worldwide killer because of the TB bacterium's antibiotic resistance. Many other bacteria have also developed resistance. In response, molecular biologists, as if in a weapons race, have devised ever-newer drugs to overcome bacterial defenses. Various multi-drug regimens have proven to be effective, but current research, nevertheless, finds more and more bacteria capable of resisting even these drugs.

The most widely used antibiotics are penicillin and its derivatives, a family called "beta-lactam" antibiotics — so-named for their chemical structure. As you might expect, because of wide use over more than half a century, bacteria have evolved a defense to these drugs — an enzyme that attacks and inactivates the beta-lactam structure. "The clinical use of beta-lactams, the most widespread antibiotics on the market today," says University of Pennsylvania biophysicist Matteo Dal Peraro, "has exerted a large evolutionary pressure on bacteria, triggering several resistance mechanisms. The most effective of these mechanisms is biosynthesis of beta-lactamases, a group of enzymes that inactivate the drugs."

Dal Peraro, a post-doctoral researcher, collaborates with Michael Klein, who directs the University of Pennsylvania's Center for Molecular Modeling and who leads a range of studies on enzymes and how they catalyze reactions. For a number of years, Klein's research group has used PSC systems to good effect, and they were among the first research groups to have success with BigBen, PSC's Cray XT3. "For the kind of calculations we do," says Klein, "the XT3 is the most productive resource available to our group. It is allowing us to undertake projects we couldn't do before in a reasonable time."

Klein's focus on enzymes and his development of powerful computational methods to study them, led him and Dal Peraro along with Alejandro Vila and Paolo Carloni to look at a particularly nefarious class of beta-lactamases, a group that includes zinc, a heavy metal, in its structure. Known as metallo-beta-lactamase (MBL), this class of enzymes is newer than other beta-lactamases and so far not as widespread. Existing multi-drug regimens, however, are ineffective against MBLs, and they pose a potentially serious threat.

In a series of simulations starting with LeMieux, PSC's first terascale system, and finishing this year with BigBen, the Klein team uncovered previously unknown atomic-level information about how MBL works and the reactions that occur as it attacks beta-lactam antibiotics — vital information for pharmaceutical researchers as they work to develop drugs that can inhibit MBLs before the bacteria that produce them become more dangerous.

*QUANTUM SIMULATIONS WITH THE XT3 TAKE
A STEP TOWARD DEFEATING A DANGEROUS
STRAIN OF ANTIBIOTIC-RESISTANT BACTERIA*

BREAKING THE RING

Although beta-lactams have been used since the 1940s, scientists still lack a complete, exact picture of how they work. What's clear is they disrupt bacterial ability to synthesize cell walls, and the disruption is lethal.

The distinctive feature of beta-lactams is the beta-lactam ring — a four-sided structure, three carbon and one nitrogen atoms. The antibiotic effect depends on the integrity of this ring. Beta-lactamase enzymes attack the ring and “hydrolyze” it, cutting it and rendering the drug harmless as an antibiotic.

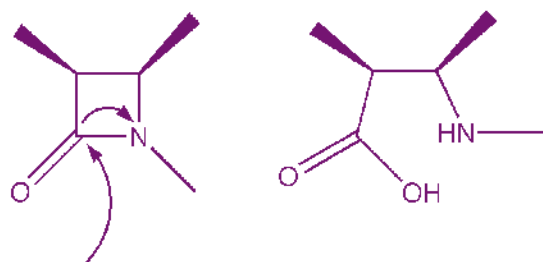
The ability of beta-lactamase enzymes to break the beta-lactam ring depends on the presence of a “nucleophile” — a chemical group that can donate electrons — in the active site of the enzyme. In the older, better known beta-lactamases the nucleophile is attached to the amino-acid serine.

“These normal beta-lactamases,” says Dal Peraro, “which are sometimes called serine beta-lactamases, have been around since the 1970s, and inhibitors have been designed to limit their effectiveness.” A widely used multi-drug formulation is augmentin, which teams the antibiotic amoxicillin in a one-two punch with clavulanic acid, an inhibitor that binds with and occupies the beta-lactamases, allowing amoxicillin to have its lethal effect.

In alarming ways, however, MBLs differ from their serine beta-lactamase cousins. They wield the same chemical tactic, a nucleophile in the active site, but they rely on zinc ions, either one or two — mono or bizinc MBLs — rather than serine, and because of this difference, they are immune to existing inhibitors.

“This is a new class of beta-lactamases,” says Dal Peraro, “first discovered more than 10 years ago. There are no useful inhibitors in the market to inactivate these enzymes, so all beta-lactam antibiotics are ineffective against MBL bacteria. Fortunately, MBLs aren't as widespread as the older beta-lactamases, but microbiologists who study this problem find they are continuously spreading in the clinical setting. They are already dangerous and have led to deaths from post-operative infection, but they will be more dangerous in the future if they continue to spread.”

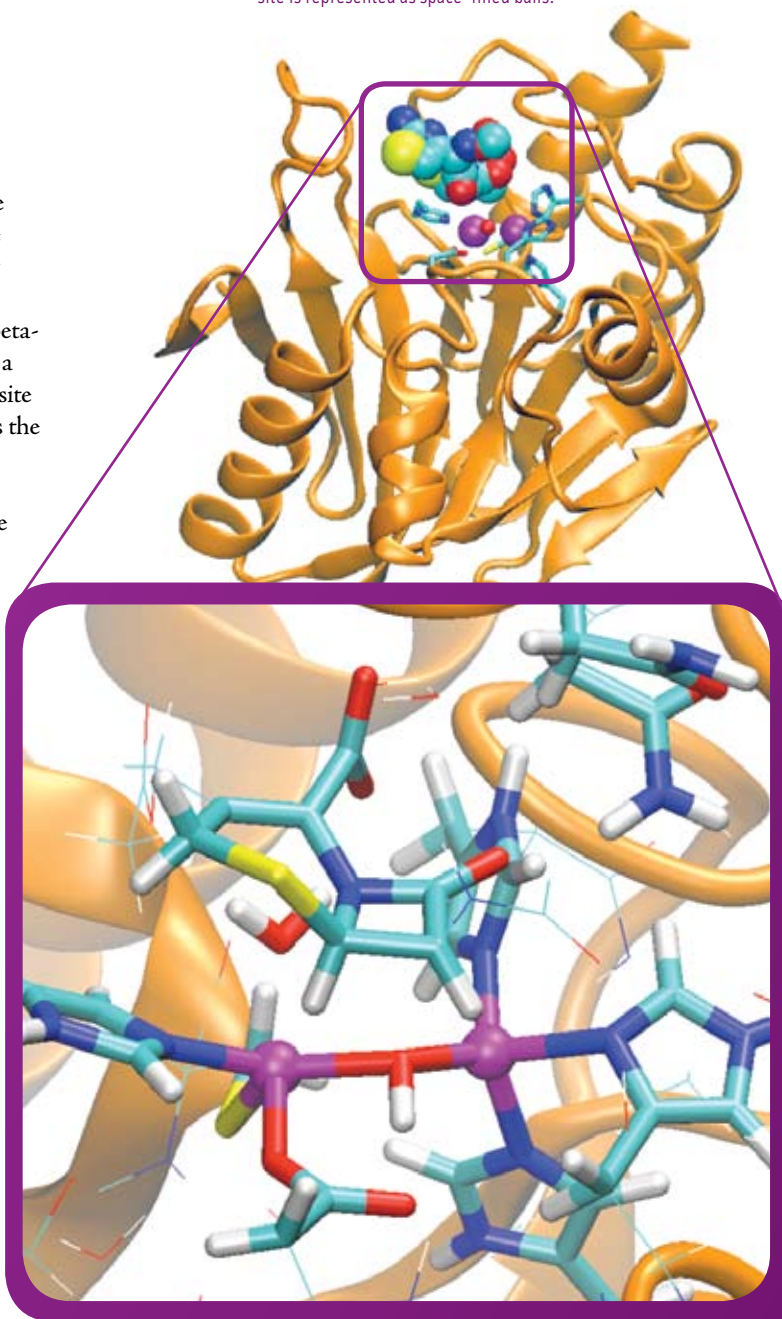
“There is an urgent need,” says Klein, “to understand the function of these enzymes at the molecular level.”



nucleophile(metallo β -lactamase)+water

Metallo beta-lactamase enzymes wield a nucleophile to break the beta-lactam ring.

This ribbon structure depicts the metallo-beta-lactamase enzyme simulated by the researchers. Two zinc ions (purple spheres) are in the enzyme's active site with amino acids coordinating the metals (represented as sticks). A beta-lactam antibiotic bound at the active site is represented as space-filled balls.



Closeup on the Action

This closeup on the bizinc MBL active site shows the conformation of the reactants (in stick representation) from the QM/MM simulation. The beta-lactam ring of the antibiotic is in the center of the picture. Other parts of the enzyme, not included in the quantum-mechanical part of the simulation, are shown as thin lines. The QM treatment of the active site reveals that a hydroxide group (red & white, OH⁻) bridging the two zinc ions (purple spheres) interacts with a water molecule (red with two white wings, above the left zinc atom) between one of the metals and the antibiotic. This water shuttles a proton (H⁺) to break the beta-lactam ring.

GETTING A FIX ON ZINC

For the pharmaceutical industry to identify and gain approval of a new antibiotic is a major undertaking, requiring as much as eight years and \$500 million. As a big step in the direction of an MBL inhibitor, the Klein team set out to precisely characterize the reactions that occur when an MBL enzyme interacts with a beta-lactam antibiotic.

“Computational studies of enzyme catalysis,” says Klein, “go back a long way — to the pioneering work of Arieh Warshel and Michael Levitt, now at USC and Stanford respectively, who introduced the notion of QM/MM [quantum mechanics/molecular mechanics], which treat the active site with quantum mechanics and the rest by classical molecular dynamics. More rigorous treatments are possible now in both regions. Our modest contribution is to employ Car-Parinello density-functional based methodology for the QM region. To implement this, our group uses code developed by one of our former post-doctoral researchers, Ursula Roethlisberger of EPFL (Ecole Polytechnique Fédérale de Lausanne) Switzerland and her group.”

Traditional classical “molecular dynamics” tracks the movement of atoms and, therefore, changes in molecular structure with time, but doesn't track the making and breaking of bonds that occur during reactions. The powerful Car-Parinello approach makes it feasible to obtain this quantum-level information with a practical amount of computing.

Reactions involve transition states that occur almost instantaneously as a reaction moves from reactant to product states. “With this kind of study,” says Dal Peraro, “we want to characterize the transition state of the reaction, because what we know from experiment is only the resting state of the enzyme, not the pathway that leads to the products. With this information you have a better chance to design an efficient inhibitor.”

The role of zinc in the MBL enzyme reaction hasn't been well defined, despite a large amount of experimental study. Klein and Dal Peraro designed a simulation that tracked a particular bizinc MBL with their QM/MM approach as it reacts with three different beta-lactam antibiotics.

“FOR THE KIND OF CALCULATIONS WE DO THE XT3 IS THE MOST PRODUCTIVE RESOURCE AVAILABLE TO OUR GROUP.”

For initial phases of the project, the researchers relied on LeMieux, PSC's terascale system, but when BigBen became available in late 2005, they turned to the new system and it became a major advantage to their work. With the QM/MM code, Dal Peraro was able to use up to 128 BigBen processors at a time, twice as many as he could efficiently use on LeMieux. He submitted ensemble jobs using up to 1,280 BigBen processors for up to 10 different parallel, simultaneous 128-processor simulations that scanned the reaction pathway in several windows over a total reaction time of more than 50 picoseconds (trillionths of a second).

After a series of these simulations, they arrived at several new findings. They found that a water molecule forms a bridge between the MBL active site and the antibiotic, and this water shuttles a proton (H⁺) that is instrumental in cleaving the carbon and nitrogen of the beta-lactam ring. “This is important,” says Dal Peraro, “because you can model inhibitors based on the position of that water molecule and its importance in the enzyme reaction.”

What also emerged from the simulations is that the bizinc form is more efficient. “The second zinc,” says Dal Peraro, “helps to make the reaction faster.” With bizinc MBL, the reaction happens in one step, as opposed to two steps with the monozinc form. This suggests that the bizinc form is more of a threat, a finding that aligns with experimental evidence that bizinc MBL “is the most evolutionarily advanced form of the enzyme and ultimately more dangerous.”

Another surprising finding has to do with the “coordination sphere” of the zinc ions, which refers to the number and geometry of bonds associated with the ions. The zincs shift their coordination during the reaction, between being bound to either four or five other molecular groups. This shift and the flexibility it shows, say the researchers, is of paramount importance in appreciating how the enzyme works, and it is revealed only by computation. “Experimentally you can't see these movements,” says Dal Peraro, “because it's really fast and also because zinc and other metals are silent, not visible to spectroscopic techniques.”

“All these ingredients,” the researchers conclude, “are important and need to be considered for the design of new inhibitors.” (MS)

MORE INFORMATION:

<http://www.psc.edu/science/2006/enzyme.html>