The Microchip Microbe Hunters

Their weapons are computers rather than microscopes, and they're hot on the trail of new antiviral drugs—including a cure for the common cold

AS THE CURRENT INFLUENZA EPIDEMIC, the worst in several years, sweeps through North America, it provides a painful reminder that viruses still have the upper hand in their battle with humanity. And influenza is just the tip of the iceberg. For all the deadly viral diseases that afflict humanity—including AIDS, viral pneumonia, and hepatitis, among many others—there are almost no effective therapeutic agents.

The few viral diseases that have been tamed (polio and smallpox, for example) have been vanquished by vaccines. But for many viral diseases vaccines are no solution. Because of the number of different strains (as in the case of influenza) or the virus's great genetic variability and complexity (as in the case of HIV), it may be immensely difficult to come up with a single effective vaccine. Therapies are clearly needed.

Yet compared with the remarkable success biochemistry has achieved against bacterial diseases, the search for antiviral agents has been painfully slow. In contrast to the hundreds of effective antibiotics now available, there are only a handful of approved antiviral agents—and most of them are only partially effective. Furthermore, because viruses (unlike bacteria) rely heavily on the human host cell in order to reproduce, agents that kill viruses are often quite toxic for human tissues; a good example is the anti-AIDS agent AZT which is highly toxic for bone marrow cells.

But researchers have not given up on finding the viral equivalent of penicillin. In fact, many of them believe computer-aided drug design—the 10-year-old marriage of high-powered computational techniques, theoretical chemistry, x-ray crystallography, and molecular biology—is dramatically improving the odds in the battle against viruses. That trend, they think, will become readily apparent in the next few years. Indeed, the first new drugs resulting from this process are already in the pipeline.

The most tangible success resulting from this computer-assisted process—a single anticold drug in clinical trials—has come from a large collaboration among workers at universities and in industry. That collaboration includes Michael G. Rossman and his colleagues at Purdue University, Mark McKinlay's group at the Sterling-Winthrop Research Institute, Roland Rueckert and his co-workers at the University of Wisconsin at Madison, and Terry Lybrand at the University of Minnesota.

Like drug designers at pharmaceutical firms and universities everywhere, one of their chief dicta is to know the enemy. Hence the first step in the process of computer-aided design is to produce models of the virus in atomic detail on the graphics monitors of supercomputers and high-end workstations. Rossman and his collaborators have been going after the picornaviruses, a large family whose members cause colds, polio, heptatitis A, foot-and-mouth disease, and encephalomyocarditis, but other investigators are applying the same computational tools to many other viruses.

Once the virus has been put up on the screen, the investigators begin to probe it for its molecular Achilles heel. In real time, drug designers test one drug after another for their ability to bind to vulnerable spots in the virus—without resorting to time-consuming and expensive in vitro assays. Out of hundreds of compounds imaginable, only a few pass this "in calculo" assay and then move to the lab for chemical synthesis and actual assaying.

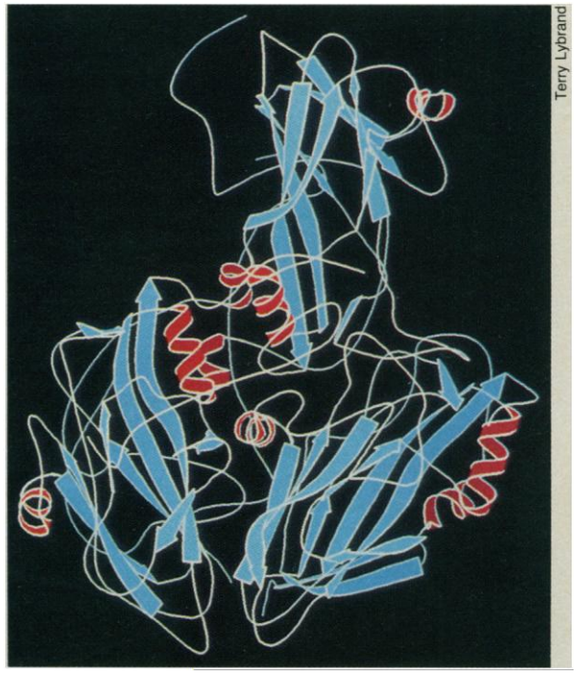
"What [computer-aided drug design] represents is a faster way of suggesting changes that might improve the activity of molecules you already have in hand," says Rossman.

Some in the field believe the new computational approaches offer much more than this ability to tinker with compounds already known to have antiviral activity. According to Scott Dickson of SmithKline Beecham, who has been perfecting some of the software involved in computational methods, "computer-aided design also gives us a new tool for suggesting completely different approaches to drug development."

"So much of medicinal chemistry today," Dickson adds, "is interpretive. You determine the biological activity of several closely related compounds and then make small structural changes and test again. The idea with computer-aided drug design is to take the wealth of biological and chemical data available and take a much bigger step to entirely new structures, not just minor derivatives."

Why haven't these methods already revolutionized the search for new drugs? Well, one reason is that computer-aided drug design is far from cheap. The basic supercomputer—a Cray X-MP 14SE—costs $2.5 million; renting time on such a machine ranges from $500 to $1000 an hour. Such costs are obviously enough to deter companies and universities—unless there is a clear prospect of a substantial economic payoff.

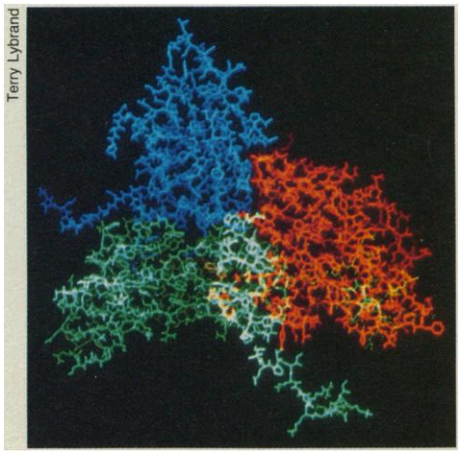
And in several cases there is. In the United States viruses account for an estimated 60% of all illnesses, compared to the 15% that are caused by bacteria. Considering that $2 billion a year is spent on medications that merely mask cold symptoms, a true cold



Code in the nose. *Three proteins—VP1, VP2 (a typical cold virus), and VP3—that form the outer shell of rhinovirus 14 are shown in a schematic surface view.*

medication might earn a drug company many billions over its lifetime.

It's not surprising, then, to find that much of the computer-aided antiviral effort is aimed at finding drugs to kill cold viruses. Indeed, that's what Rossman and his collaborators have been aiming at. And their most tangible success—the drug that Sterling-Winthrop has in clinical trials—is an anti-cold medication. (Although its development is fairly far advanced, the new compound does not yet have a name because Sterling-Winthrop's policy is to simply number drugs until they are ready for marketing.)



Tight squeeze. *Close-up of a putative antiviral drug in the binding pocket formed by the surface proteins of rhinovirus 14.*

The work leading to the formulation of this still anonymous drug provides a good example of the strengths—and the current limits—of computer-aided drug design. In 1986 Rossman and his colleagues, working with virus particles grown at Wisconsin, solved the x-ray crystallographic structure of rhinovirus 14, a typical cold virus belonging to the picornavirus group. Picornaviruses share two traits: Their genetic material is RNA rather than DNA and their outer shells, or capsids, are made of four proteins that assemble into an icosahedron.

While Rossman was working on the virus's structure, the Sterling-Winthrop researchers developed an initial group of antiviral agents that inhibited the ability of the picornaviruses in general to infect cells as measured by test-tube assays. They then turned back to Rossman to determine how the drugs worked.

Thomas J. Smith, then a postdoctoral fellow working for Rossman and now an assistant professor at Purdue, began by determining how nine of the new compounds interact with the proteins that make up the capsid of human rhinovirus 14. Three capsid proteins, designated VP1, VP2, and VP3, wrap themselves into barrel-shaped structures that have cavities at their centers. Smith solved the crystal structures of the drug-virus complex and found that the antiviral drugs diffuse into the cavity of VP1, which is found at the center of a structure called a beta-barrel. By altering the chemistry of the viral protein, the binding of drug and capsid protein can stop the spread of the viral infection. Naturally, the next step in the process of computer-aided drug design was to find out how this happens.

"The capsid of a picornavirus is in a tenuous state," Smith explains. "It is stable enough to get into a cell, but it is unstable enough that once inside the cell it falls apart, releasing its RNA so that it can make more copies of itself. These drugs get inside the beta-barrels and stabilize them, preventing them from collapsing and keeping the capsid from falling apart."

It turns out that both the new drugs and the cavity of the beta-barrel are hydrophobic. Hence the cavity is a perfect haven for the drugs, which are trying to get out of the aqueous environment of the body. Once inside VP1, the drugs act like girders that reinforce the protein's three-dimensional structure against changes in *p*H.

"Normally, the *p*H outside a cell is different than inside the cell," said Smith, "and it is this *p*H change that makes the capsid fall apart once it enters the cell. But with the drug in place, the *p*H change has no effect on VP1's structure, and the capsid remains in one piece." As a result, the viral RNA is not released into the cell, and the process of infection is interrupted.

Having found such an interesting compound and having understood something about how it works, the investigators were able—using their computational tools—to fine tune the drug to enhance its desirable properties. Using high-powered computer graphics, the Purdue and Sterling-Winthrop researchers modified the drug molecules to make compounds that bind more effectively in the beta-barrel—at least on the computer screen. For a better estimate of actual binding properties in the body, the data were sent to Lybrand, who has access to the Crays at the Minnesota Supercomputer Center in Minneapolis.

Lybrand goes beyond a simply look-and-see, static approach to binding and attempts to understand the dynamic processes involved in drug binding. To do so, he uses the tools of molecular mechanics to calculate the approximate behavior of all atoms in a molecule. This process does have practical limits, which are imposed by the state of the art in computation. "In the ideal situation we would use quantum mechanics to determine intramolecular motions," said Lybrand, "but a virus is just too large for this to be feasible. Instead, we have to make the approximation that the bonds between atoms are like Newtonian springs, that atoms have the size of their Van der Waals radii, and so forth."

Within these limits the process of dynamic simulation is quite powerful. Its starting point is provided by the atomic positions given by the x-ray crystal structure. Rather than use the entire 240-protein virus structure, Lybrand simplified the calculations by working with one 4-protein unit.

Random thermal vibrations were added and the molecule was allowed to settle into its lowest energy states. The next step was to see how the molecule moves between those states—which is the dynamic part of the simulation. Atomic positions were calculated at intervals of about 1 femtosecond for about 100 picoseconds. The results of this 100,000-step calculation, which takes 24 to 26 hours on even the fastest supercomputer, were assembled into a film loop by a graphics program running on a workstation.

Once Lybrand had accomplished this task with the four-protein viral unit, he added a drug molecule to the simulation. But he didn't use only one drug. By repeating the simulation with several different drugs, he could compare the motions of the various drug-protein combinations with those of the viral protein complex alone. That work enabled him to pinpoint some of the intermolecular forces between the drug and the protein that tend to stabilize the viral coat and render it harmless.

In addition, the data generated in these simulations make it possible to calculate differences in free energy of binding for various drugs and correlate them with their in vitro activity. "With this data, we can suggest whole new avenues to explore in terms of what parts of the drug molecule might increase the binding energy and what parts aren't as important," said Lybrand.

Medicinal chemists want to understand the binding process for at least two reasons. First, they would like to know how to increase the affinity of the drug for its target. Second, if they know which parts of a drug are not critical for binding, those regions can be modified to enhance properties such as solubility and transportability that are needed in a pharmaceutical compound.

Although this process represents a great advance over older, trial-and-error methods of large-scale screening, it isn't exactly equivalent to designing a drug from scratch on the computer. "True drug design, where you come up with a new active compound de novo—that's still down the road a way," says William C. Ripka of E. I. Du Pont's Medical Products Department.

Part of the reason de novo design is still a

ways down the road is the complexity of the information needed to characterize the desired molecule. One way to simplify the problem is to think of it as one of geometry: find molecules that are complementary to the shape of the target site. The rules of chemistry—bond lengths, atomic sizes, Van der Waals forces, and so on—then limit the available choices. "You would think there would be something in graph theory that would apply to this problem, but we haven't found it yet," says Ripka.

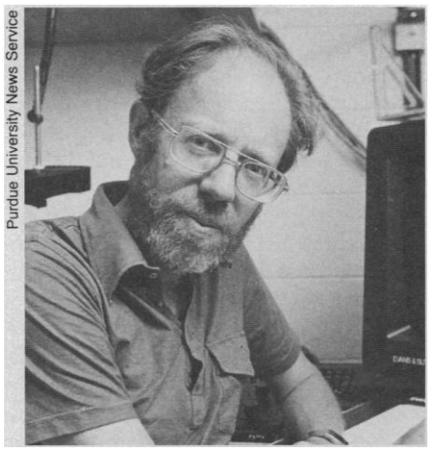
What is available, however, is a simpler approximation method developed by I. D. Kuntz at the University of California at San Francisco and Dickson at SmithKline Beecham. The program that has been developed by Kuntz and Dickson roughs out the shape of the target site and then attempts to fill it with drugs of corresponding shape. "Having the three-dimensional crystal structure of your target provides a lot of information about atomic positions and so forth," says Dickson, "and part of that information describes the empty spaces within that target, the places where your drug is going to interact with the target. So since we can determine the shape of those spaces from crystallographic data, we decided to look for molecules that would best fill that space."

To find those molecules, Kuntz developed a neat trick for calculating the shape of the target space. The space was first treated as if it were completely filled with balls of various radii. Kuntz then wrote a computer program that matches the "ballspace" with the "ballshapes" corresponding to small drug molecules. Meanwhile Dickson, relying heavily on structural information in the Cambridge Crystallographic Data Base, developed a library of such molecular shapes for use with the program.

According to Dickson, both the SmithKline and UCSF groups have used this approach to generate molecules that "look quite reasonable," and some that even inhibit target enzymes in vitro. They are now studying some of those molecules in molecular dynamics simulations to see if they can be further refined.

Others are also searching for a route to de novo drug design. Yvonne C. Martin of Abbott Laboratories has developed an approach that makes it possible to match shapes when the crystal structure of a virus has not yet been fully resolved. According to Martin, "like all companies, we have these smart medicinal chemists who have made 300 or so compounds of varying activity, and we can use these data to infer something about the target site and the binding points for these drugs, and develop an approximate shape for that site."

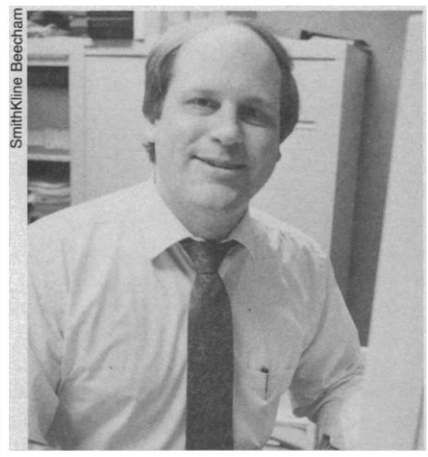
Shape-matching programs, such as the ones developed at Abbott or by Kuntz and Dickson, can then sift through a molecular library and identify compounds for further testing. Though not as accurate as shapes determined by crystallographic data, the approach has generated compounds showing in vitro antiviral activity.



Structure buff. *Michael G. Rossman of Purdue worked out the arrangement of proteins in rhinovirus 14.*

With supercomputers becoming more widely available, workstations increasing in power, and commercial molecular graphics programs proliferating, computer-aided design is fast becoming a standard part of the drug screening routine. Although company officials are reluctant to discuss their efforts for publication, scientists at several drug houses said they were quite pleased with the results produced by their in-house computer efforts.

The search for compounds effective against HIV and polio virus are perhaps benefiting the most from computer-aided design methods, though hepatitis B and herpes and influenza viruses are also under study. The main obstacle to a more important role for computer methods in antiviral research is the lack of virus crystal structures. As more of these structures are solved, this research will no doubt pick up steam.



Match game. *Scott Dixon of SmithKline Beecham, who developed a computer program for matching drugs and viral targets.*

Computer-aided drug design represents a powerful tool for the medicinal chemist and, as such, its future is bright. "What we've done, really, is develop tools for identifying possible drug candidates," said Ripka. "We might identify 100 or so molecules with the right shape. You could then use molecular dynamics calculations to reduce the list further and identify those compounds you want to try first in your in vitro assay.

"It's really a question of narrowing the odds for success, of making the most intelligent choices you can from the wealth of biological data that is available."

At least one drug in clinical trials—the anonymous Sterling-Winthrop agent—and possibly two others were developed with computer-aided design programs. But many observers feel it will take at least a decade for these techniques to have their full impact on the search for new antiviral agents. As the potential benefits of computer-aided methods become apparent, more companies and universities are willing to pony up the money needed to buy time on the supercomputers that drive the work, and so some of the financial limitations on use of computer methods have begun to fade away.

As the financial obstacle is decreased, however, another obstacle comes into increasing prominence: a shortage of scientists who have the broad combination of skills—biochemical, theoretical, and computational—that are required to make further gains. That obstacle, too, will certainly be overcome, but only as academics such as Lybrand begin to teach the next generation of computational chemists.

Once that generation is in place, the field may take off with great speed, Lybrand and others say. One reason is that the interdisciplinary nature of the field, which may slow it at the beginning, will actually be a source of rapid advances in the future—as developments in different fields begin to feed off one another. According to Lybrand, "we're not only gaining a better idea of how a drug binds to a particular protein, but we're also learning more about the various interactions that occur between molecules. This enables us to better refine our models and in the long run, make better predictions.

"You have to remember, though, that this is really a new field, and we're just getting to the point where we can study large systems with any confidence. Computer-aided drug design is not a near-term thing," he adds, "but it is a tool that will become an important part of the pharmaceutical chemist's armament." ■ JOSEPH ALPER