Viral Shell Game

A surprising view of how a virus shell assembles itself

By IVARS PETERSON

Like a microscopic, heavily armored spacecraft, a virus cruises intercellular space in search of a cell to invade and colonize. A tough, rigid shell protects its cargo of nucleic acids — strands of DNA or RNA — until contact. Then, clinging to its target, the shell opens up to inject its contents into the hapless cell.

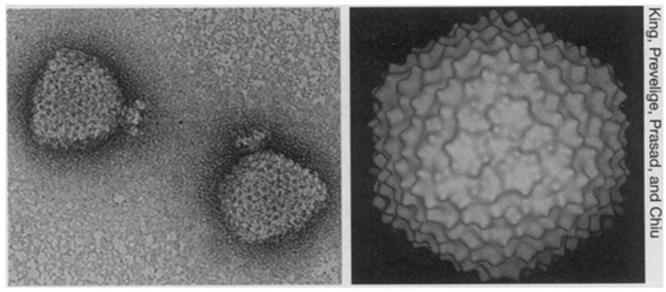
The success of a virus in infecting its host depends largely on how well its outer coat functions. Made up of protein building blocks, this tightly closed shell usually has a geometric structure.

As the virus reproduces inside the cell it has infected, new shells must assemble themselves out of a broth of identical protein subunits. Researchers have long sought to understand how they do it. "This is an unsolved problem in modern biochemistry and structural biology," says Jonathan A. King of the biology department at the Massachusetts Institute of Technology.

Now, biologists have a new perspective from which to tackle the question of virus self-assembly. Mathematical research suggests that sets of simple rules, which define the way an individual protein can stick to another, will automatically lead to the kinds of virus structures that biologists observe.

"The rules completely guarantee the final shell shape," says applied mathematician Bonnie A. Berger of MIT, who worked with mathematician Peter W. Shor of AT&T Bell Laboratories in Murray Hill, N.J., and others to devise this new, mathematical model of virus shell construction.

Such research may offer a novel and potentially lifesaving strategy for short-circuiting the infection process. Instead of looking for ways to prevent a fully formed shell from binding to a cell, researchers can now think about interfering with the growth of a virus shell to render it ineffectual.



King, Prevelige, Prasad, and Chiu

Electron micrograph showing a pair of P22 bacterial viruses (left). A computer reconstruction based on electron micrographs of the mature P22 virus shell reveals its icosahedral shape (right).

"It's a very important piece of work," says Peter E. Prevelige Jr. of the Boston Biomedical Research Institute. "It allows you to think about what happens when things don't work correctly. You can try to poison the growing [virus shell] complex to make it go down a dead end."

Of the myriad viruses that cause a variety of ailments in plants and animals, most have highly regular structures. Many display the 20 triangular faces of an icosahedron. This large group includes the adenoviruses, which can cause respiratory problems in humans, the herpesvirus, the poliovirus, and HIV, the AIDS virus.

The shells of these viruses typically consist of a hundred or more copies of a single protein, though in some cases the shell contains two or three different kinds of proteins. Assembly occurs quickly and spontaneously — often, in a matter of seconds.

To explain how these shells assemble themselves, biologists initially focused on their regularity and symmetry. They postulated a two-step process. First, five or six protein molecules link to form pentagonal or hexagonal rings. Then these rings connect to make icosahedrons (see diagram). In this way, construction would proceed in an orderly and direct manner to the final structure.

But this model didn't address the issue of how individual proteins join to create larger units, the rings and the shells. "How does one little protein know where to go to form such a highly structured shape?" Berger asks.

Moreover, experimental studies of the growth process revealed the presence of single proteins and completed shells, but not the pentagonal and hexagonal rings of the postulated intermediate steps. These results challenged the existing model.

"We had strong experimental data demonstrating that almost certainly the shells were built from monomers [single protein units]," King says. "The high symmetry of the end product did not account for how [a shell] got built."

In some instances, researchers found that certain virus shells even require a protein scaffolding to aid assembly. This disposable framework serves the same stabilizing function as the scaffolding needed to construct a cathedral's dome or bridge's arch, and it disappears once the structure is completed.

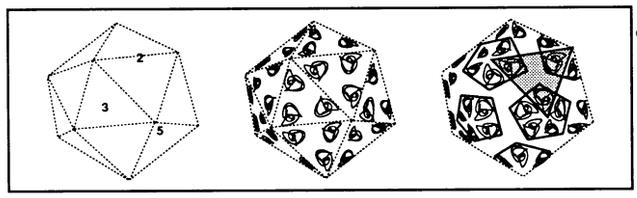
Several years ago, King became interested in using computers to visualize the ways in which virus shells might grow. He and his colleagues had spent more than 15 years studying virus assembly experimentally, and they wanted a better understanding of how the process works in three dimensions.

King suggested the problem to Berger, who has a background in both computer science and mathematics. He hoped she could find some mathematical recipes, or algorithms, that would make it possible to depict protein polymerization — how proteins join together to form larger units — on the computer screen.

"I did not know that a much deeper insight would necessarily come out of this," he says.

Berger shared the problem with Peter Shor, and they began exploring the kinds of rules that might specify how one protein links to another. Instead of concentrating on the actual geometry of virus shells, they looked at the web of possible connections between individual proteins.

Berger and Shor discovered that they could formulate a set of simple rules specifying how one protein links with another to directly create the desired final structure — *if* they assumed that the protein molecules, each identical in its chemical composition, could fold



Berger

In this example of a virus shell with icosahedral symmetry, each triangular face of an icosahedron (left) has three protein molecules (middle). This protein arrangement can also be viewed as an array of pentagonal rings (right).

themselves into a specific number of different shapes, or conformations.

"It seemed a reasonable assumption to make," Berger says. For the P22 bacterial virus (or bacteriophage), which had long been the target of King's investigations, the mathematics led to the desired structure if the protein could assume seven different conformations, or perhaps only four.

When Berger checked with King, she was delighted to find that he and his colleagues had recently obtained experimental evidence strongly suggesting just such a possibility. His micrographs showed that the protein molecule that makes up the P22 shell apparently has seven different shapes, of which four look particularly distinctive.

"These structural studies had shown that the same chemical subunits are in different, stable conformations in different positions in the shell," King says. "Until very recently, that was considered a heretical notion."

With this encouragement, Berger and Shor, assisted by graduate student Lisa Tucker-Kellogg of MIT's Laboratory for Computer Science, went on to develop a more complete mathematical theory of virus shell assembly.

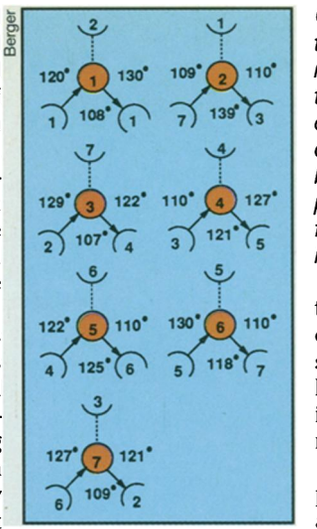
"If the protein subunits assume different conformations during the assembly process depending on their relative positions, a protein binding to the structure has enough 'local' information to 'know' where to bind," the researchers reported in the Aug. 2, 1994, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

They also predicted the existence of shell geometries that the old biological model of virus shell construction appeared to rule out. Alerted to this possibility, researchers began searching for and are now starting to find examples of these "anomalous" forms.

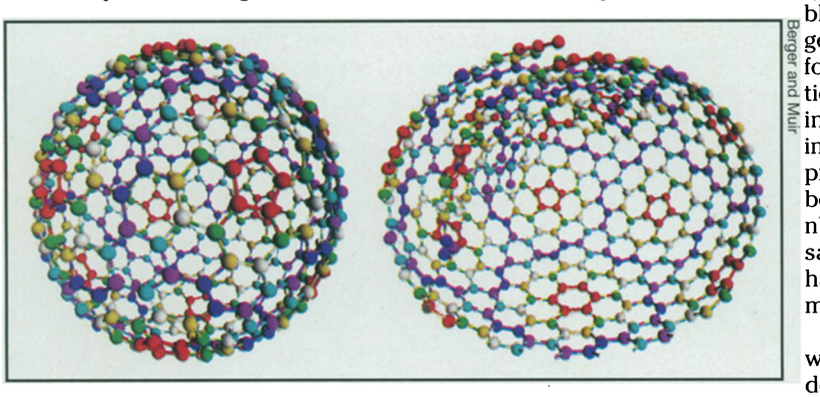
Computer simulations verified that the rules worked to create closed shells. They also demonstrated that changing the assumed interaction angles and distances by small amounts didn't necessarily disrupt shell growth.

But with larger deviations, the shells often failed to close, and deformed structures appeared. For example, with one false step, a polymerizing shell could start growing into a spiral, mimicking a malformation occasionally observed in nature (see color illustration).

Berger is now interested in modeling the rates at which different types of shells grow. "Before in our models, when a protein came along and bonded on according to the rules, it stayed there," she says. "Now, we're looking at a protein soup and allowing things to come and go, sometimes bonding and sometimes not."



One possible set of rules for the binding of protein molecules in three dimensions to form a P22 virus shell. Each of the seven protein conformations is represented by a numbered circle. These proteins link in specific ways, fitting together like keys going into locks.



Computer-generated image of the shell resulting from the rules given above (left). A single misstep in the building process can lead to a spiraling defect, which keeps the virus shell from closing (right).

To keep things as simple as possible, Berger and her coworkers initially assumed that virus shells contain a single kind of protein. These proteins, however, could take on a certain number of different conformations.

For each conformation, the rules specified which other molecular shapes it could bind with. They also designated the approximate angles and distances between the two interacting proteins.

For one model of the P22 bacteriophage, the researchers came up with seven protein conformations and seven rules (see diagram). Interacting only with their immediate neighbors according to the given rules, the proteins could assemble themselves into the required structure of P22's exterior. As soon as one link was made, the others followed automatically to create the complete shell.

Berger and Shor tried various sets of rules for different types of virus structures. In a number of cases, they discovered alternative strategies for achieving the same result, sometimes using fewer different protein conformations.

The mathematical models proposed by Berger and her colleagues have already had some impact on biological studies.

In one instance involving the P22 bacteriophage, Berger's rules suggested that scaffolding proteins would have to have a particular structure to fit with the shell proteins. This insight enabled Peter Prevelige to make sense of several puzzling experimental findings.

"We had worked for a number of years trying to discern what sorts of [combinations] of scaffolding proteins exist in solution," Prevelige says, but the combinations they had found didn't appear to fit into any known shell-building model.

By suggesting what types of protein units might be present and how they might interact, Berger's rules provided a possible explanation. "Having a theory allowed us to say that our results were plausible," Prevelige says.

"The theory tells us what to pay attention to," he adds. "It's like looking for something that's lost. If you know what you're looking for, it's much easier to find."

Understanding how virus shells assemble themselves also suggests new opportunities for therapeutic intervention. "In general, people in the pharmaceutical industry haven't seen this process as a target, partly because this process hadn't been sorted out," King says. Now, researchers have a picture of what may occur.

Prevelige and his coworkers have already demonstrated that a dose of small dye molecules can block virus shell formation in vitro by binding with the protein subunits at critical positions. "That's quite surprising because these proteins have large surface areas and the dye molecules are comparatively small," King says.

Apparently, the dye molecules zero in on the same molecular sites that, according to the mathematical model, dictate the binding of proteins to each other. "You can stop these reactions with small, weakly bound molecules," King argues.

Prevelige is now trying to refine this strategy. The preferred course is not to go after individual proteins, but to rechannel the growth into misshapen viral forms that can no longer function properly.

"We're going to think about and use Berger's rules in our work," Prevelige says.

"There are many problems we face as biologists where we need the tools of the mathematician to come to our aid," King notes. "The intersection of biology and mathematics can be extremely productive when both sides tune in to the actual problem, the actual need." □