Scientists unveil structure of adenovirus, the largest high-resolution complex ever found

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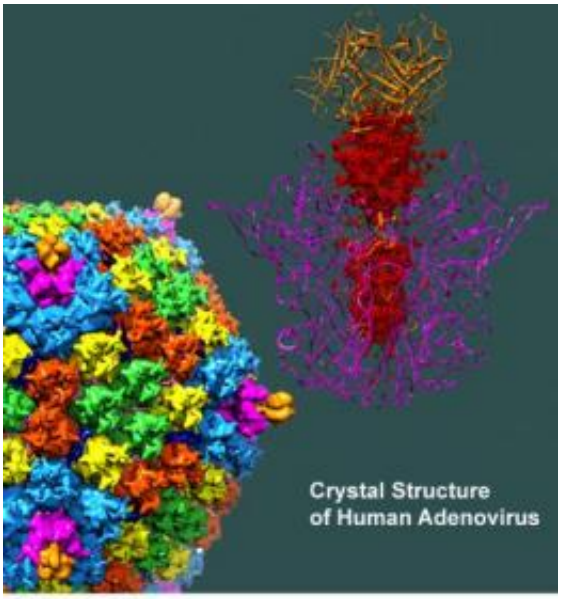

Scripps Research scientists have pieced together the structure of a human adenovirus (two views illustrated here). Image courtesy of the Nemerow and Reddy labs at the Scripps Research Institute.

Scripps Research scientists have pieced together the structure of a human adenovirus (two views illustrated here). Credit: Courtesy of the Scripps Research Institute

After more than a decade of research, Scripps Research Institute scientists have pieced together the structure of a human adenovirus—the largest complex ever determined at atomic resolution. The new findings about the virus, which causes respiratory, eye, and gastrointestinal

infections, may lead to more effective gene therapy and to new anti-viral drugs.

The study was published in the prestigious journal *Science* on August 27, 2010.

"We learned a number of important things about the virus from the structure, including how its key contacts are involved in its assembly," said Scripps Research Professor Glen Nemerow, who, together with Scripps Research colleague Associate Professor Vijay Reddy, led the study. "That's very important if you want to reengineer the virus for gene therapy."

"Even though a number of viral structures have been solved by x-ray crystallography, this is the biggest to date," said Reddy. "The adenovirus is 150 megadaltons, which contains roughly 1 million amino acids—twice as big as PRD1, previously the largest virus ever solved to atomic resolution."

The Promise of Gene Therapy

First discovered in the 1950s, adenovirus is a major class of disease-causing agents that include viruses that causes the common cold. While the body is usually able to eventually fight off infection, infants and people with compromised immune systems can be susceptible to severe complications. No medications are currently available against adenoviruses, so current treatment focuses on managing symptoms.

While adenovirus has plagued humankind for millennia, more recently scientists have sought to exploit some of its properties—such as its stability and ability to infect many different types of cells—to engineer cures for other diseases. The hope is that modified adenovirus could play a role in gene therapy, used as a vector (carrier) for delivering

therapeutic genes to the interior of cells.

"Adenovirus was used early on in pioneering gene therapy trials for the treatment of cystic fibrosis," said Nemerow. "Those trial failed because scientists didn't understand the biology and virus-host cell interactions to be able to use the virus properly."

Despite early setbacks, adenovirus is still being used in about 25 percent of human gene therapy trials, mostly for cancer and cardiovascular disease, according to Nemerow. A better understanding of the virus could help advance those efforts, which, if successful, could have a major impact on a host of conditions.

The Marathon Begins

So, in 1998 Nemerow and Reddy set out to determine the molecular structure of adenovirus—with no idea it would take them 12 years to succeed.

The scientists turned to a technique known as x-ray crystallography, the gold standard of molecular structure determination for large complexes. In this method, scientists produce large quantities of a protein or virus then turn it into crystal form. The crystal is then placed in front of a beam of x-rays, which diffract when they strike the atoms in the crystal. Based on the pattern of diffraction, scientists can reconstruct the shape of the original molecule.

Several steps in this process, however, can be problematic.

The first challenge is producing crystals. Other scientists' attempts at crystallizing adenovirus had failed due to long, spoke-like fibers that stick out of the vertices of the complex. These fibers interfere with crystallization, which requires close packing of the particles.

The Nemerow lab, however, produced an adenovirus lacking these long spindles, sparking hope that these particles could be crystallized. But the scientists found that the new virus was somewhat unstable. In another attempt, the Nemerow lab produced a version of the virus that had short fibers rather than long ones.

"This was a 'best of both' situation," said Reddy. "We had a stable virus and short fibers so we could pack the particles to produce crystals."

However, the production of crystals also required high concentrations of protein in solution. This turned out to be a problem because at high concentrations the adenovirus clumped together, again becoming unstable. After trial and error with various chemical additives and buffers, eventually Nemerow and Reddy arrived at conditions that kept the virus solublized.

The scientists were able to produce crystals at last.

But when Nemerow and Reddy took the crystals to the Advanced Light Source synchrotron facility at Lawrence Berkeley National Laboratory, the crystals did not diffract.

Persevering in the Face of Adversity

What the scientists needed was higher quality crystals.

Nemerow and Reddy persevered, continuing to vary the conditions under which the crystals were formed in an attempt to produce diffraction-quality crystals. At this point, Reddy and Nemerow had the idea of turning to robotic crystallization—at that time (around 2002) still somewhat of a novelty and a relatively expensive fee-for-service proposition.

"In the lab, we were limited by the dispensing of small volumes of sample," Reddy explained. "We could not pipette less than one microliter at a time. These robotic trials used very small—50 nanoliter—trials. With these small drops, we could explore a large number of conditions. Crystals also grow faster in these small drops."

After a series of robotic trials, the scientists were able to identify conditions that would produce high-quality crystals. They then reproduced these conditions in the lab and manufactured enough crystals to make another trip to the Berkeley Lab synchrotron. There, Nemerow and Reddy found that the crystals diffracted—but only to 10 angstroms, not enough to solve the structure.

Luckily, around that time (now 2005) a cutting-edge new synchrotron, the 23 ID-D beamline at Advanced Photon Source at Argonne National Laboratory, was coming online in Chicago. Reddy's mentor, Scripps Research Professor Jack Johnson, suggested they give that facility a try.

After traveling to Chicago with their samples, Nemerow and Reddy discovered that, using the new beamline, their adenovirus crystals indeed diffracted to 3-4Ĺ resolution.

Toward the Finish Line

But the team's challenges were not over. The scientists found that the crystals deteriorated under the force of the x-rays, limiting amount of data that could be obtained from a single crystal.

"This is such a big assembly that there are people who think that in solution viruses in general are breathing entities," said Reddy. "In crystals you need to have perfect packing. Every molecule has to be in lock step. That's why any disruption can affect the packing of the virus—and then you don't have diffraction."

Nemerow and Reddy continued to vary the conditions under which the crystals were grown and prepared to produce more robust crystals. The pair also made the realization that they had the most success with fresh crystals that had just completed their two-month growth cycle. The scientists started timing their trips to Chicago to coincide with the period when the crystals were freshest.

Nemerow and Reddy began travelling to Chicago at least three times a year, grateful that their hosts in Chicago were willing to provide generous use of the beamline. In total, the scientists collected data from nearly 1,000 crystals, about 10 percent of which diffracted.

"We used about 100 crystals to solve the structure," said Reddy. "That's nearly 20 million reflections. So we started calling ourselves 'millionaires'…"

As the data was collected from crystal after crystal, Reddy took on the task of merging the massive amounts of information to finally piece together a picture of the virus.

"Normally, more than one person does that work," Nemerow noted. "In this situation, Vijay was the one who did it all pretty much by himself."

A New Understanding

The picture of the virus that emerged from this work comprises the majority of the adenovirus. Small, elusive portions resisted placement, probably because these regions of the virus are dynamic, essentially moving too much to assign a definitive location.

The new structure provides information on where the weakest links in the viral assembly are, as well as where the strength of the virus lies.

"The virus has to be stable to survive in the environment, but also has to be able to disassemble when it enters the cell," said Nemerow. "So the structure revealed places in the virus that are loosely bound so they can come apart upon cell entry."

Nemerow notes that it might be possible to use this information to develop drugs, for example compounds that prevent the virus from disassembling and thus from infecting cells. He is also excited about the potential of this new information for use in gene therapy.

"Like an electrical engineer who wants to go into a house and put in new wiring, [a genetic engineer] doesn't want to put something where there is another important contact," he said. "Knowing where these important regions are in the virus is crucial."

The scientists note that the adenovirus structure also revealed unanticipated changes in some of the key proteins involved in receptor interactions, highlighting the plasticity of these parts of the assembly.

In future research, the team plans to focus on better understanding the portion of the virus that resisted characterization in this study, as well as on comparing different types of adenovirus structures.

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