





By Design

SDSC supercomputer helps identify promising new target for bird flu vaccine

by Warren Froelich

In motion on the computer screen, the green-and-pink protein structure bobbed and weaved, wiggled and gyrated, like a bowl of jello on a slinky. Somewhere in this quivering mass, however, may lie a potential solution for stopping a feared virus—bird flu.

Extending her hand toward the highly animated structure, Rommie Amaro—a chemist at UC San Diego—points toward a small region in green and what appears to be an open cavity adjacent to it. That opening—already being dubbed a “hot pocket”—is being seen as a potential target for new drugs to combat what some worry may one day cause a world-wide pandemic.

Fighting Bird Flu

New targets are desperately needed since the only drugs now available—Tamiflu and Relenza—are in limited supply, and some strains of the virus already have developed resistance to these agents.

“If those resistant strains begin to propagate, then that’s when we’re going to be in trouble, because we don’t have any anti-virals active against them,” said Amaro. “So, we should have something as a backup, and that’s exactly why we’re working on this.”

The structure under study by Amaro, a postdoctoral fellow at UCSD, is neuraminidase (NA), an enzyme located on the surface of the avian influenza virus, needed to spread infection to new cells. Essentially, influenza comes in nine different varieties or subtypes of this enzyme, N1-N9. For avian flu,

technically known as H5N1 avian influenza, the neuraminidase of interest is N1.

Last October, a team of scientists from the United Kingdom identified the structure of several members of this family of enzymes, including N1, using a technique known as X-ray crystallography. The procedure represents a snapshot of the structure in question.

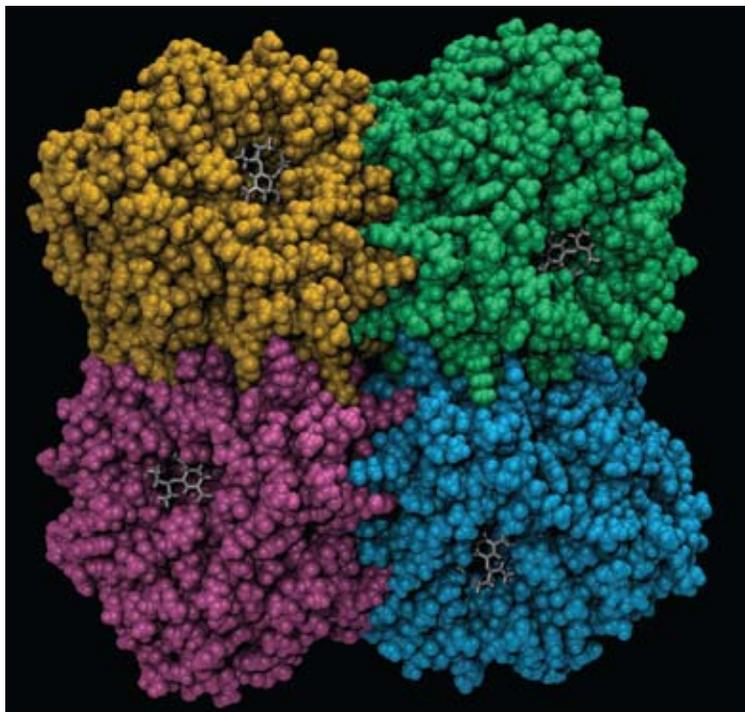
As reported in the journal *Nature*, the image revealed a loop-like area, called the “150 loop,” adjacent to an open cavity. The group said the newly revealed loop and cavity “might be exploited by drug designers” with new inhibitors capable of docking into these targets, thus blocking the enzyme’s activity—in effect, shutting down its ability to infect other cells.

Considered a potential breakthrough, the newly identified image nevertheless was limited since it captured just a single brief moment in time.

“Crystal structures are very important,” said Amaro, who works in the laboratory of J. Andrew McCammon, holder of the Joseph Mayer Chair of Theoretical Chemistry at UCSD. “There’s no doubt about it. They give us a real picture of the enzyme. But it’s just one picture.”

Proteins in Motion

Over the past decade or so, scientists have come to realize that proteins are far more dynamic than the sometimes colorful structures gleaned from standard crystallography studies. Instead of a still-life painting, these molecules act more like a



Fighting Bird Flu

This image shows a key enzyme on the surface of avian influenza, N1 neuraminidase, needed to spread infection to new cells. The anti-viral drug Tamiflu is shown bound in the active sites (yellow). Molecular dynamics simulations of this enzyme-drug complex are being run on SDSC supercomputers to help develop improved drugs to fight this disease. Image: R. Amaro, UCSD.

moving picture, constantly twitching and jiggling, making the goal of finding a specific inhibitor daunting. It's somewhat like a baseball pitcher attempting to throw strikes to a catcher who's doing handspings behind home plate.

To help capture these sometimes spasmodic activities, scientists work with molecular dynamics codes that simulate the activities and motions of these molecules as they obey the fundamental laws of physics. Understanding these motions helps scientists build better drugs.

Molecular dynamics simulations already have proved their value for other drug designs, said McCammon, one of the pioneers in the field. For example, the route to a potential new drug manufactured by Merck to combat HIV—an anti-integrase inhibitor—was discovered in McCammon's lab; the drug recently passed Phase III clinical trials and will soon be reviewed by the FDA for approval. Some of the same principles used in the HIV anti-integrase studies are now being applied to this new avian flu target.

“The new structural understanding we obtained through molecular dynamics simulations was subsequently exploited to come up with this promising new drug to attack HIV,” said McCammon, a Howard Hughes Medical Investigator. “We anticipate that the insights we're getting with our current work could provide value for the rational design of inhibitors to avian flu.”

Such is the complexity of the mathematical calculations needed for these simulations that scientists require the use

of supercomputers housed at the San Diego Supercomputer Center (SDSC). Here, a molecular dynamics program called NAMD is put through its paces on SDSC's DataStar, scaled to run on 128 processors. The results were two, 40 nanosecond (a billionth of a second) simulations of the N1 neuraminidase requiring about a day of compute time for each five to eight nanoseconds of simulations.

“That's really fast,” said Amaro. “And it's fast because of this really great program and DataStar's balanced architecture.”

A New Bird Flu Target

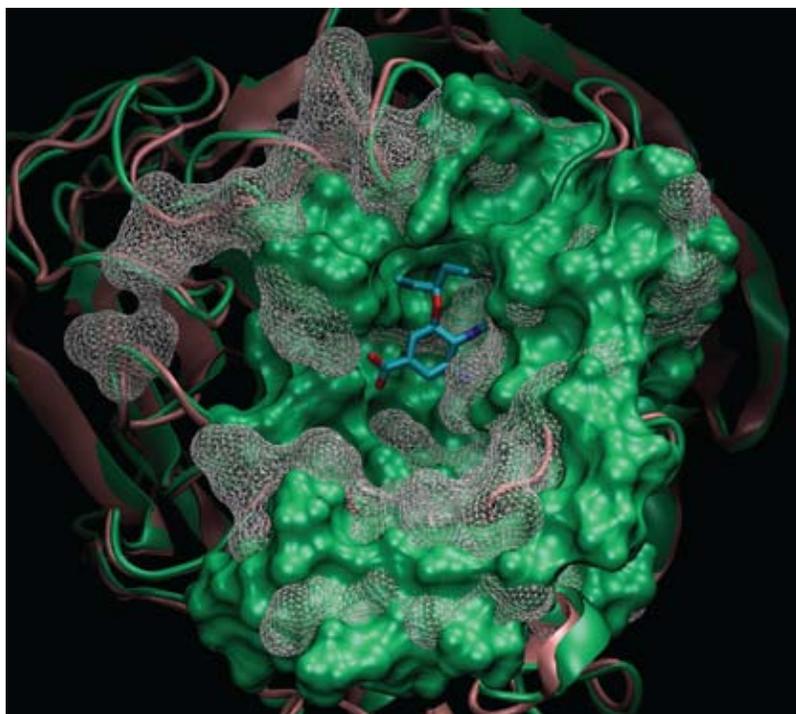
In a paper published earlier this year in the *Journal of the American Chemical Society*, the UCSD-led team described the nanosecond-by-nanosecond movements of the N1 molecule, structured like a four-leaf clover, as it was bathed in a water and salt solution—similar to a cellular environment. Some surprising new details of interest to drug designers emerged as the scientists watched the protein gyrate and wiggle over time.

First, the stretch of amino acids defined as the “150 loop” is far more flexible than speculated in the original paper. In the dynamic simulations, the loop not only opens—as seen in the static image—it also closes. What's more, it's capable of opening far wider than suggested by the crystal picture.

Second, the scientists identified another amino acid sequence near the 150-loop region, dubbed the 430-loop, which may be important. According to the simulation, these two

Virtual Molecules

Sophisticated supercomputer simulations create “virtual molecules” to explore new drug targets to combat bird flu. Alignment of the open crystal structure (pink) and a wide-open snapshot extracted from the simulation (green) shows the significant cavity expansion found in the simulation. The new structure can help guide development of novel antiviral therapeutics. Image: R.Amaro, McCammon Group, UCSD.



loops seem to act in concert to expand the “hot pocket” region well beyond what was seen in the crystal structure.

“The whole pocket appears to be very dynamic, very flexible,” said Amaro, who was the paper’s first author. “The topology of the pocket and the [amino acid] residues linking the pocket are significantly different than what we saw in the crystal structure.

“We were particularly interested in the wide open structure adjacent to the two loops, because in the wide open structure, there is an even larger area to target with inhibitors.”

Amaro said her group—which included investigators from The Scripps Research Institute and the National Taiwan University—already has identified several potential inhibitors that might represent potential new drugs against avian flu. Some of these might bind to the “hot pocket” region; others to the two amino acid loops; while another category might build onto the structures of existing anti-influenza drugs, such as Tamiflu. Indeed, a second simulation performed by Amaro and her team showed how Tamiflu binds to the “hot pocket” region, and its impact on the opening and closing of the cavity.

To help identify the best alternatives, the team is collaborating with researchers from the University of Malaysia, under the direction of Habibah Wahab, associate professor of pharmaceutical technology.

“We’re recommending a slew of compounds for her to test,” said Amaro. “And then based on what, if any, hits we get, then we begin putting these into the discovery pipeline.”

Added McCammon, the paper’s principal investigator: “In light of the urgency to find antiviral drugs against N5N1 bird flu, we’re hopeful that these simulations will assist in that effort.”

The work of the scientists is supported by the National Biomedical Computation Resource (an NIH Research Resource), the Center for Theoretical Biological Physics, the National Science Foundation, National Institutes of Health, and Accelrys, Inc., in addition to SDSC.

Project Participants

Rommie Amaro, David Minh, and J. Andrew McCammon, UCSD; William “Lindy” Lindstrom and Arthur Olson, TSRI; Lily Cheng and Wilfred Li, NBCR; Jung-Hsin Lin, National Taiwan U.; and Habibah Wahab, U. of Malaysia

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