Team charts new understanding of actin filament growth in cells

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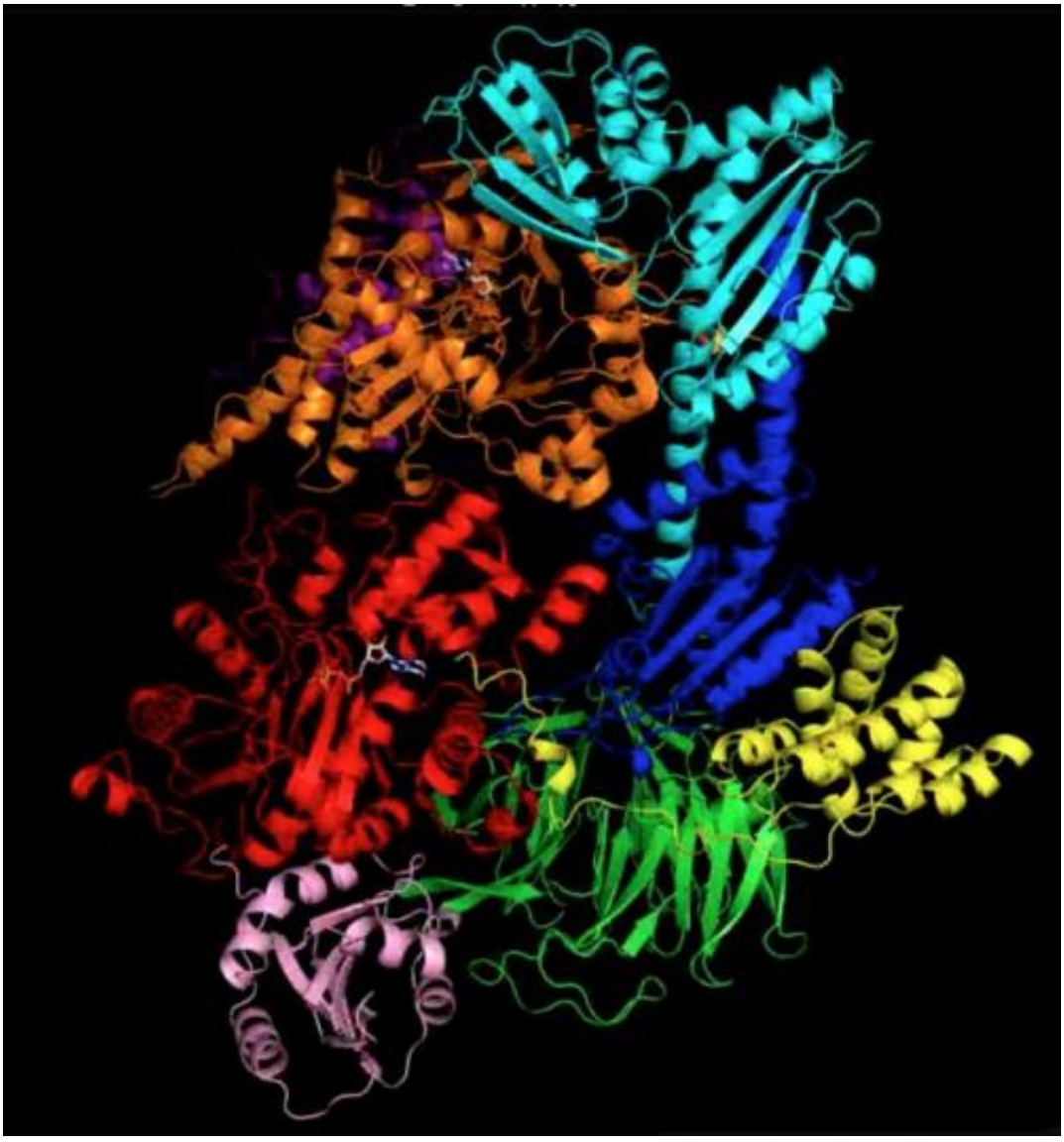

University of Oregon biochemists have determined how tiny synthetic molecules disrupt an important actin-related molecular machine in cells in one study and, in a second one, the crystal structure of that machine when bound to a natural inhibitor.

The accomplishments—done in the name of fundamental understanding, or basic science—provide new windows on the complexities of cellular structure and suggest a potential future route to therapeutic targeting, said Brad J. Nolen, a professor of chemistry and biochemistry at the UO, who was principal investigator on both studies.

The machinery is the actin-related protein 2/3 complex (Arp2/3), a large assembly of seven proteins that stick together. This complex is critical to cell motility—the ability to move and perform myriad duties—and for initializing the construction of a network of filaments known as the actin cytoskeleton that provides structural support for cells.

"In addition to cells having a lot of actin, they also have a lot of proteins that bind to actin to control its dynamics," said Nolen, who has just completed his second year as a Pew Scholar in the Biomedical Sciences. "And that's exactly what the complex does. It binds to the side of pre-existing actin filaments, where it nucleates the growth of new filaments. The Arp2/3 complex is very highly conserved, like actin. All of your cells are chock full of actin much like a yeast cell. There are very few differences between the molecule in yeast and in human cells."

The cell loses control of the actin cytoskeleton in various diseased states, including certain viral infections, such as HIV and cancer, he said.

Nolen, also a member of the UO's Institute of Molecular Biology, began studying the Arp2/3 complex's role in cytoskeletal network formation during postdoctoral research at Yale University, where he was part of a team that in 2009 identified two distinct classes of molecules that

inhibited normal activity of the machinery. The discovery, reported in the journal *Nature*, opened the way for exploring how the complex works.

Reporting in the May 23 issue of the journal *Chemistry & Biology*, Nolen and a team of UO researchers, in a series of biochemical, biophysical and X-ray crystallography experiments, exposed the complex to the two tiny synthetic molecules, which turned off actin-filament initiation as anticipated. More importantly, they were able to capture exactly where the molecules docked, or bound, with the much larger macromolecular Arp2/3 complex.

That binding activity, they found, was enough to block the ability of the machinery to align properly for activating filament production. "We found that these small molecules throw a monkey wrench in this macromolecular machine and lock it into the off state," Nolen said. "By locking it into the off state it prevents it from nucleating branched filaments."

What was seen in the Arp2/3 complex, Nolen said, will help to understand precisely how actin is controlled in cells. "Cell motility requires actin inside the cell to constantly be remodeled," he said. "A lot of studies are showing that the Arp2/3 complex is very important for cell motility. So if we can figure out the Arp2/3 complex works, we can better understand how it affects things like cellular motility and, therefore, how we might affect things like metastasis of tumors.

In the second paper, placed online July 28 in advance of regular publication in the journal *Nature Structural & Molecular Biology*, Nolen and Quing Luan, a research technician in the UO's Institute of Molecular Biology, report the first crystal structure of the Arp2/3 complex while bound with a natural occurring inhibitor, glial maturation factor, known as GMF.

"We have determined the three-dimensional structure of all of the atoms that make up each of the sub-units of the Arp2/3 complex, and we've created a 3-D picture of where this regulator binds to the complex by using X-ray crystallography," Nolen said. "What this tells us is the structural basis for how GMF regulates the Arp2/3 complex. It binds to the complex and blocks the initiation of Y-shaped branches that create new filaments. It also binds to the pre-existing branches and causes them to pop off, so it is involved in the disassembly of these networks."

There is a difference, he noted, in how inhibitor molecules in the two studies worked. The synthetic versions in the first study, while binding to specific locations, did not block separate filament-building activators from also binding to the complex but instead stopped activation by locking the complex into a non-productive position. GMF while bound to the complex, on the other hand, blocked activators from also locking on.

The different results, Nolen said, could guide future efforts therapeutic delivery of molecules, or drugs, to fight disease-related scenarios in damaged cells. His lab is now working with a computational chemist on the design of molecules that might drive desired alterations in the complex and related actin regulators without unintended consequences of toxicity.

"We are pursuing the potential clinical value," Nolen said. "It's basic research with a potential long-term payoff, or it may never happen. For now what we've provided is a basic-science tool."

"Researchers at the University of Oregon continue to further our understanding of the dynamic processes that inform multi-level health and well-being," said Kimberly Andrews Espy, vice president for research and innovation and dean of the UO graduate school. "By helping to elucidate the complexities of cellular structure, Dr. Nolen's

research may eventually lead to more effective targeting of tumors and other diseases."

Provided by University of Oregon