First sharp images reveal structure of key inflammatory protein

By STEPHANIE DUTCHEN | June 20, 2019 | Research

Protein engineering and cryo-electron microscopy revealed the long-awaited structure of NLRP3. Images: Wu and Mao labs

After decades of attempts by the scientific community, researchers at Harvard Medical School and Peking University have provided the first clear look at a protein implicated in a vast array of inflammatory conditions.
The finding, published June 12 in *Nature* lifts a blindfold that has hampered scientists' ability to intervene when the immune system overreacts to perceived threats.

**Get more HMS news here**

The protein, known as NLRP3, alerts immune cells when it senses invading viruses and bacteria and other dangers. The cells then marshal defenses, including assembly of molecules called inflammasomes that tell compromised cells to self-destruct to prevent the threat from spreading.

But sometimes NLRP3 sounds an unnecessary or disproportionate alarm. The proteins may call for assaults on arterial plaques or uric acid crystals or healthy tissues or fail to shut off after mobilizing an appropriate immune response.

The results: autoimmune diseases; chronic inflammation that contributes to cardiovascular disease, type 2 diabetes, gout and the most severe form of nonalcoholic fatty liver disease; neuroinflammation such as in Alzheimer's and Parkinson's diseases; and sepsis, a life-threatening systemic overreaction to an infection.

"Few proteins have been implicated in as many diseases as NLRP3," said study co-senior author Hao Wu, the Asa and Patricia Springer Professor of Structural Biology in the Blavatnik Institute at HMS and a senior investigator at Boston Children's Hospital.

By capturing the detailed, three-dimensional molecular structure of NLRP3 and revealing how another protein allows it to activate, Wu and colleagues have laid the foundation for designing drugs that bind to NLRP3 and muffle its alarm.
"There has been huge interest in developing anti-inflammatory drugs that stop NLRP3 activation," said Humayun Sharif, a postdoctoral fellow in the Wu lab and co-first author of the study. "Understanding its structure will help by providing a blueprint."

The discovery should also allow researchers to determine whether any existing drugs act on NLRP3 and tackle diseases that arise from mutations in the gene that produces NLRP3.

Not least, it will help biologists dig further into the basic mechanisms of inflammasomes.

Sticky situation
The main reason scientists had been stymied all those years was that the instruments that could reveal the structure of NLRP3 required clean, individual proteins—but NLRP3 insists on clumping together.

Former Wu lab postdoctoral researcher and co-first author Li Wang finally overcame this hurdle by conducting some clever protein engineering that convinced NLRP3 to stay single.

The team then used cryo-electron microscopy, or cryo-EM, to snap high-resolution pictures of NLRP3. Partnering with the lab of co-senior author Youdong Mao at Peking University, including co-first author Wei Li Wang, quintupled the number of images the researchers were able to obtain. In all, they took 2 million pictures and analyzed only the best 100,000.

The results delivered two surprises.

**Protein puzzle pieces**

At last, NLRP3's shape became clear. The researchers liken it to an earring, with a stud connected to a C-shaped hoop.

Next came the question of how NLRP3 goes from its dormant to its alarm-blaring state. Day after day, Sharif played a kind of protein Tetris on the computer to figure out whether the scientific community's top candidate, the protein NEK7, could lock on to NLRP3's unique shape and activate it.

"I couldn't sleep at night," said Sharif. "I would go home and see proteins move when I closed my eyes."

At last, NEK7 slotted into place.

Although it's not yet clear which other players may be involved, the work "settled some longstanding questions in the field about whether NEK7 is important in inflammasome activation," said Wu.
Simulations showed that NEK7 nestles into the cup of NLRP3's C-shaped hoop. Eleven NLRP3-NEK7 combos then form a ring, with the studs in the middle and the hoops out.

"NEK7 helps the components of the ring come together," Sharif said. "We never would have expected that. Seeing that was one of the coolest parts of this project."

From left: NLRP3 alone, bound to NEK7 (yellow) and linked with 10 others in an activated ring. Images: Wu lab

NEK7 was previously known to be involved in mitosis, or cell division. Since it can't bind to NLRP3 and to mitosis-related molecules at the same time, the researchers suspect that dividing cells can't sound the immune alarm and alarm-blaring cells can't divide.

**Long-term triumph**

The biggest surprise, though, said Wu, was that the team solved the structure at all.

The work was so difficult and painstaking that it took three generations of trainees in her lab to complete the project.

"There is a joke that the lawn outside our building is haunted by the ghosts of past postdocs who worked on this project," said Sharif.
"It's really satisfying that we got an answer," said Wu. "I feel like this means that it doesn't matter how difficult a problem is; if you put in enough effort, you'll get it."

**Funding, authorship and disclosures**

Wu lab members who co-authored the paper include Venkat Giri Magupalli, Liudmila Andreeva, Qi Qiao and Arthur Hauenstein. Other co-authors were Gabriel Núñez of the University of Michigan Medical School and Zhaolong Wu of Peking University. Mao and Wei Li Wang are also affiliated with the Department of Microbiology in the Blavatnik Institute at HMS and the Dana-Farber Cancer Institute.

Wu is co-founder of SMOC Therapeutics, a company developing drugs for diseases driven by the innate immune system. Hauenstein and Li Wang are employees.

This work was funded in part by the U.S. National Institutes of Health (grants DP1HD087988, R01AI124491 and R01AI06331), Intel Corporation, Thousand Talents Plan of China, National Natural Science Foundation of China (grant 11774012) and Natural Science Foundation of Beijing City (grant Z180016).