Profile of Hao Wu

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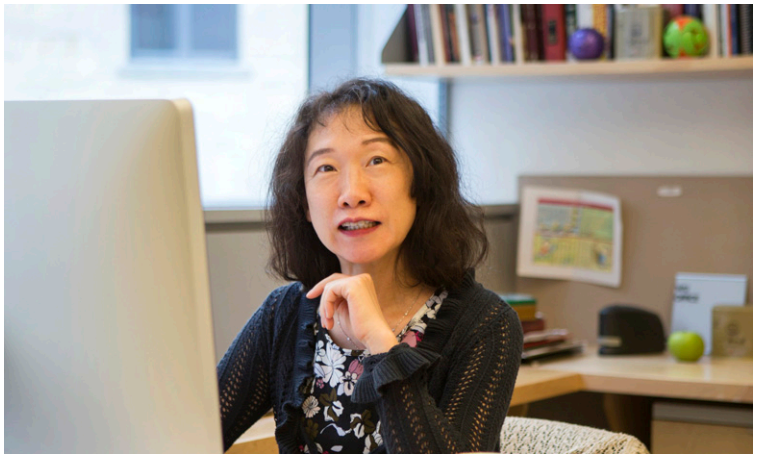
Researchers' understanding of cellular physiology has evolved over time with the identification of internal cell structures. Organelles, large and small, are key to cellular function and response to external stimuli. Harvard Medical School professor of structural biology Hao Wu, elected to the National Academy of Sciences in 2015, uses protein structure determination to gain insights into immune signaling pathways that cells use to combat infections and internal dangers. This work has led to the identification of protein-rich, intracellular signaling complexes called signalosomes or supramolecular organizing centers (SMOCs) (1). This discovery filled the conceptual gap between classic organelles and more transient, stimulus-dependent molecular complexes. Wu's Inaugural Article describes the assembly of a SMOC in the inflammatory pathway—an "inflammasome"—and provides insights into potential therapeutic targets (2).

Beijing Prologue

Wu comes from a long line of academics and scientists. She was born in Beijing to physicist parents. Her grandfather, Chengluo Wu, was a chemist known for unifying weights and measures in China. During the Chinese Cultural Revolution, however, these academic qualifications became liabilities. Wu's parents were remanded to the countryside to farm rice and raise pigs when she was 5 years old. Wu was sent to live with an aunt in northern China.

"They really tried to shelter my brother and me from the political situation," Wu says. After 2 years of exile, her parents were allowed to return to Beijing and the family reunited.

Influenced by a biology teacher with whom she made short films that explored subjects like marine zoology and convinced by her mother, Wu decided to attend Peking Union Medical College, initially established with the help of the Rockefeller Foundation. Wu picked up an interest in photography during her premed years at Peking University, which primed her career.



Hao Wu. Image credit: John Soares (photographer).

Fascinated by Crystallography

Wu researched the autoimmune disease lupus while at Peking Union Medical College. The work intrigued her but also prompted her to consider leaving medical school to pursue a doctorate. At an international meeting of biochemistry in Beijing in 1987, Wu attended a lecture by Purdue University professor of structural biology Michael Rossmann. Rossmann, a pioneer in X-ray crystallography, had helped British molecular biologist Max Perutz solve the structure of hemoglobin, work that led Perutz to receive the Nobel Prize for Chemistry in 1962. "Michael," Wu says, "came up with the idea to use internal symmetries to solve larger structures."

Wu explains that "X-ray crystallography has components of mathematics and physics that I have always been interested in but can also be used to solve biological questions. I was fascinated by the method's power."

Wu approached Rossmann after his talk and asked him about a possible position as a graduate student in his laboratory. Rossmann encouraged her to apply, and Wu left China for Purdue University in West Lafayette, Indiana.

Under Rossmann's tutelage, Wu read voraciously about crystallography and virology. Simultaneously, she taught herself Fortran and worked on computational methods to solve structures. "At the time crystallography had very little automation, computers were very primitive, and so we had to learn from the fundamental theories. I really enjoyed that part. In

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terms of problem-solving, it was good training," she remarks.

Wu believes that advancements in software have expanded the field of crystallography so that other biologists can use the technique without having to become experts. "Nowadays, the software is a black box," she says. "If your data is good, you can get your structure without fully understanding the underlying algorithm."

CD4 Structure

After completing her doctorate in 1992, Wu joined Wayne Hendrickson's laboratory at Columbia University in New York as a postdoc and continued working in crystallography. Hendrickson had developed a method called multiwavelength anomalous diffraction that transformed the field as it allowed researchers to more easily derive phases or structures.

When Wu joined Hendrickson's laboratory, the HIV/AIDS crisis was decimating populations across the world. She received a fellowship to solve the structure of the CD4 molecule (3), which is the receptor to which the retrovirus binds on T cells.

"The project really attracted me because it had important consequences. But those crystals were very finicky to produce. I spent a lot of time in the cold room fishing out crystals, then taking them to the synchrotron. But they would not diffract well."

As luck would have it, one crystal did diffract to a high enough resolution. "Then immediately after I collected the synchrotron data, the cooling mechanism failed." Wu recalls that she "did a huge amount of molecular replacement calculations to look for how the molecules sit in that crystal."

Wu's work revealed that CD4 formed a dimer during T cell signaling, which helped form a higher-order cluster at the immunological synapse. "Looking back," she said, "this was the first case where I encountered an oligomer, which has now become a central theme of my research."

"However, it took another 10 years before we discovered that intracellular signaling proteins can assemble into very large oligomeric complexes. Most of the time we threw them away because we thought they were just misaggregated or misfolded."

Innate Immunity

Wu accepted a tenure-track position at Weill Medical College of Cornell University in New York in 1997. Before embarking on her next project, she spent weeks in the library developing an independent research plan.

"What caught my eye was the tumor necrosis factor (TNF) signaling pathway, and my interest in TNF set the tone for the rest of my career in innate immunity."

In the 1990s, scientists had begun identifying signaling factors in the TNF pathway. These proteins play a role in a variety of immune responses from cellular proliferation and differentiation to inflammation and cell death. An emerging concept was that the composition of the elicited interactions in response to stimuli or stressors determined whether a cell lived or died.

"One pathway," Wu notes, "can go in many different directions."

Wu's laboratory at Cornell determined the structure of TNF receptor-associated factor 2 (TRAF2) and then soon after the structure of TRAF2 in complex with TNF receptor type 1-associated death domain protein (TRADD) (4, 5). The affinity between the proteins helped ensure that TRAF2 could put the brakes on TRADD's ability to initiate apoptosis, she says.

Death domains turn out to be extremely important in innate immunity. An evolutionarily conserved sequence consisting of six α-helices, death domains like the one found in TRADD recruit proteins involved in inflammation and apoptosis, often through the caspase family of cysteine proteases. Wu's laboratory demonstrated that to activate caspases, multiple death domains from different adaptor proteins, such as seven RAIDD (RIP-associated protein with a death domain) and five PIDD (p53-induced protein with a death domain) molecules, needed to assemble and form large, oligomeric complexes (6).

"Eventually, we solved the structure using the heavy-atom derivative method, and it turned out that there's no apparent symmetry in the complex," Wu notes.

Several years later when investigating a complex of proteins called the Myddosome, which is involved in Toll-like receptor signaling, another important component of innate immunity, Wu discovered that far from having no symmetry, death domain complexes have helical symmetry similar to the double helix (7). "It can be open-ended," Wu said, "so you can keep adding subunits from one side or the other."

Transforming Techniques

At this time, Wu began to integrate methods other than X-ray crystallography into her work. She says that, "these large complexes were much better pursued by a combination of technologies. Cryo-electron microscopy (cryo-EM), in particular, can solve these filament structures amazingly well. We had wonderful collaborators who helped us learn new tools and we were fortunate to meet the revolution in cryo-EM when we needed it."

Using a combination of techniques, Wu deduced the structure of a complex of proteins called the necrosome that is involved in inflammatory cell death or necroptosis. As opposed to apoptosis, in which an orderly, biochemical process terminates cellular activity and the cell dies, necrosis or necroptosis was previously viewed as a largely passive process until the discovery that a specific pathway was indeed involved.

Wu identified β-amyloid structures, which are a hallmark of many diseases, including Alzheimer's disease, in necrosome filaments as part of the signaling complex, revealing a role of amyloid structures as functional scaffolds (8). Recently, the addition of cryo-EM to her toolbox has allowed Wu to image many filament structures to near atomic resolution (9), which provided the structural basis to further establish the functional significance of these complexes.

"Whether it's necrosomes, inflammasomes, or other types of SMOCs, we're seeing that structural scaffolding through immunoprotein interaction leads to the crucial functional consequence," Wu observes.

Signalosomes

As her career progressed, Wu developed a metaphor for the intracellular interactions she has helped elucidate: "I started considering a cell as if it is a civilized society in which you have villages and cities. These are the sites where most reactions happen. Symmetry—including helical symmetry—and multivalent interactions bring molecules together into these sites to execute biochemical reactions locally, which drives the physiology of the cell."

These higher-order structures may also have an evolutionary advantage, she adds (10). "Multivalent interactions allow the complexes to assemble cooperatively, which act to modulate immune responses as a threshold, so that they don't overreact and cause apoptosis, necroptosis, or pyroptosis if they don't need to."

Targeted Therapeutics

After nearly two decades in New York City, Wu moved to Harvard Medical School and Boston Children's Hospital in 2012. She serves as the Asa and Patricia Springer Professor of Structural Biology. Although she continues to work on immunity, Wu has expanded her research scope to include examining the structures of proteins in the process that generates antibody diversity (11), the interplay between signal transduction and amplification in SMOCs such as inflammasomes (12), and downstream events that lead to pyroptotic cell death and cytokine release (13).

As in her Inaugural Article (2), Wu is pushing her work in a new direction, trying to aid the development of clinical applications for a variety of illnesses like cancers, autoimmune diseases, and inflammatory disorders.

"The molecules we study are involved in many different pathways. I'm really interested in how we may be able to contribute to targeted therapeutics using our knowledge of structural biology."

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