Protein recycling machine visualized

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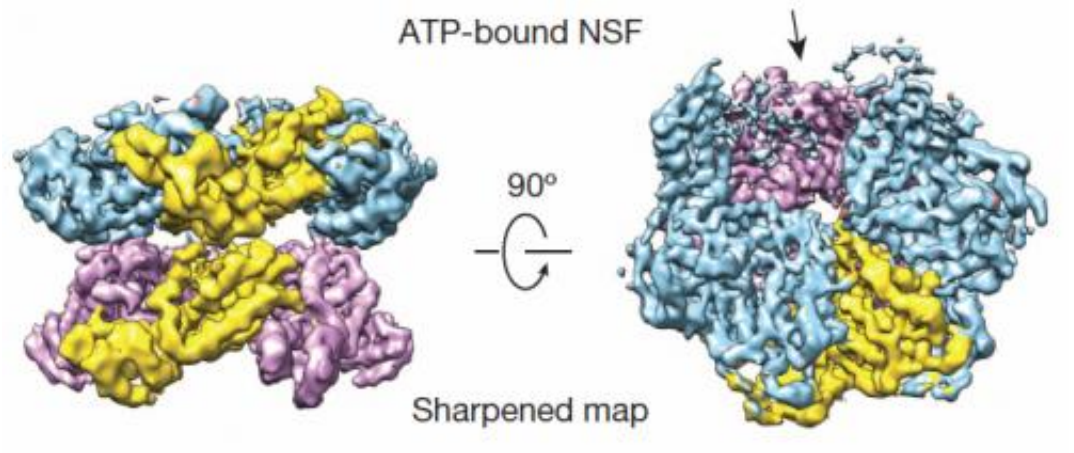

3D density map of ATP-bound NSF. Different views of the sharpened map of ATP-bound NSF filtered to a resolution of 4.2 angstroms with each domain colorcoded. A single chain of NSF (protomer) is colored in gold to help with visualization. The density of one D1 domain is not well resolved (see black arrow). Credit: Published in Zhao et. al., *Nature*, doi:10.1038/nature14148

Howard Hughes Medical Institute (HHMI) scientists have new structures of an essential cellular recycling machine that depict its structure with near atomic-level detail. The structures, which show a protein called NSF alone and interacting with its target, a protein complex called SNARE that is formed when membranes fuse together. Taken together, the structures offer new clues into how the machine does its work.

SNARE proteins on the surfaces of cells and cellular compartments called vesicles provide the energy necessary for those surfaces to fuse,

much as two soap bubbles come together to form one larger bubble. That fusion is essential for the transport and secretion of a variety of materials, including hormones and neurotransmitters. By dissassembling SNARE complexes and recovering their components after fusion has taken place, NSF ensures cells maintain the resources they need to carry out membrane fusion.

The new structures suggest that NSF may unwind SNARE complexes by gripping them tightly and exerting a strong torque to unwind their components, says Axel Brunger, an HHMI investigator who led the research at Stanford University. Brunger says this is the first time scientists have captured a detailed image of a disassembly machine as it prepares to pull apart its target. He and his colleagues, including Yifan Cheng, who is at the University of California, San Francisco (UCSF), and postdoctoral researchers Minglei Zhao at Stanford and Shenping Wu at UCSF, published their findings January 12, 2015, in the journal *Nature*.

During membrane fusion, SNARE proteins on the surface of each membrane zip together into a stable complex, creating a force that pulls the membranes together. "When this complex has done its job, it won't come apart on its own. It needs an active process to disassemble it," Brunger says. Membranes must fuse repeatedly inside cells, so rather than manufacturing new SNARE proteins for the next round, NSF steps in and salvages components of each SNARE complex so that they can be recycled.

Researchers have known for more than 10 years that cells need NSF to recover SNARE proteins after membrane fusion. They also know that NSF uses energy from the molecule ATP to drive SNARE disassembly. To better understand how that process works, Brunger and others have examined NSF's structure, striving to visualize the protein with as much detail as possible. In the late 1990s, they obtained structures that

depicted about two-thirds of the molecule. "But until recently, we have been unable to determine the full length NSF molecule, let alone its complex with SNARE complexes and the adapter protein, SNAP," Brunger says. "Believe me, we and many other people tried very hard."

As NSF latches onto a SNARE complex and takes it apart, molecules move and conformations shift. That makes obtaining structural information difficult. Electron microscopy, one technique for gleaning structural information, creates structures by averaging images from many different particles. When the particles in a sample are not identical, the resulting structure is blurred, Brunger explains. Heterogeneity was also problematic when researchers tried to solve the structure of NSF using x-ray crystallography, as were certain physical features that made the protein complex reluctant to form the crystals needed for that technique.

Improvements in electron microscopy technology—in particular, new direct electron detectors that collect higher-resolution micrographs—were essential for finally obtaining high-resolution structures that show NSF interacting with individual components of a SNARE complex, Brunger says. His team also had to develop biochemical methods to synchronize the complexes they used to capture the structure.

With these advances, Brunger and his colleagues captured several structures of NSF and its complex with SNAREs. The first shows NSF latching onto a SNARE complex, preparing to take it apart. Another shows NSF bound to the energy-storing molecule ATP, and another shows NSF after it has done its work, bound to the the nucleotide ADP. Two features of these structures were particularly striking, and hint at how NSF might work, Brunger says.

The SNARE complex resembles a rope with a left-handed twist, he

explains. In the team's structures, NSF sits beneath the complex, formed of two rings stacked atop one another, with upward extensions secured by adapter proteins called SNAPs. The SNAP adapters wrap around the SNARE complex. "When I first looked at that structure, what amazed me was that the SNAPs wrapped around the SNARE complex with the opposite twist – a right-handed twist," Brunger says. "That was entirely unexpected. It might suggest that to initiate disassembly, NSF essentially unwinds the SNARE complex."

Brunger says his team was also surprised to see that the SNAP adapter proteins connected to NSF at multiple points. "Usually you would expect just one interface between one part and the other part, but we discovered two interfaces," he says. Both interfaces are important: when the scientists mutated SNAP at those sites, the protein failed to disassemble SNARE complexes. "I think it's this interaction with the SNAPs at two interfaces that provides a much tighter connection, and maybe enables NSF to exert a torque on the SNAPs, which would eventually pull the complex apart," he says.

The structures of NSF bound to ATP and ADP were also important, because they revealed how the protein changes shape after it uses ATP's energy to disassemble the SNARE complex. The segments of the protein that connect with the SNAPs flip over, reaching down toward the rest of the NSF protein. "That suggests that upon hydrolysis [of ATP] they are able to exert a large force, which will be translated into the SNAPs with this very tight connection," Brunger says.

More work is necessary to fully understand how NSF does its work, Brunger says. So far, his team has snapshots of NSF at a few points in the process. "We don't see what happens in between these two states – but it's better than having nothing, like we did before," he says. His team plans further studies to determine how the disassembly process begins, what happens to the SNARE complex after binding and following the

hydrolysis of ATP, and how exactly the SNARE complex is torn apart.

More information: "Mechanistic insights into the recycling machine of the SNARE complex" *Nature* (2015) DOI: 10.1038/nature14148

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