How a molecular motor untangles protein

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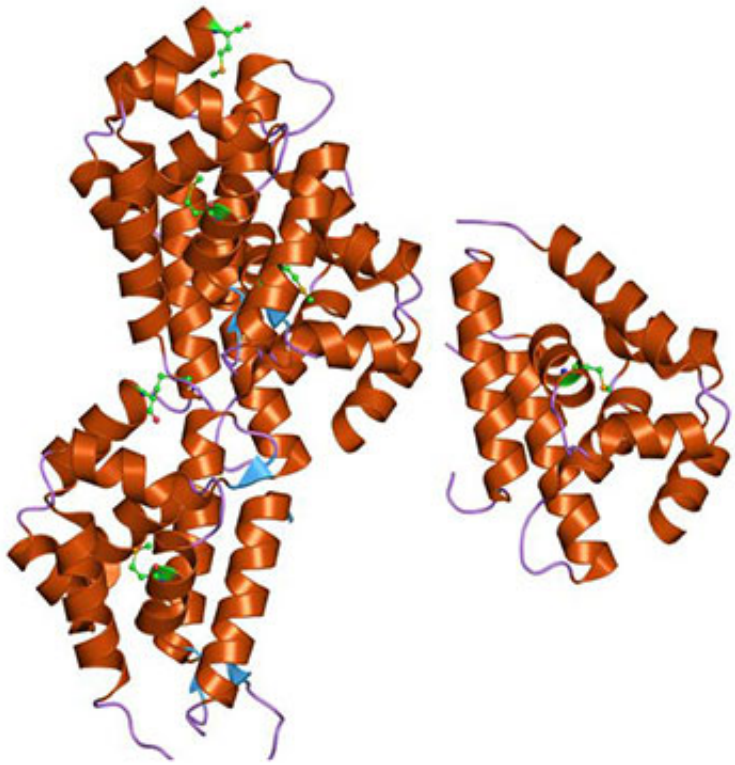

Molecular structure of the N-terminal domain of ClpB, a heat-shock protein. Credit: Jawahar Swaminathan and the European Bioinformatics Institute

A marvelous molecular motor that untangles protein in bacteria may sound interesting, yet perhaps not so important. Until you consider the hallmarks of several neurodegenerative diseases—Huntington's disease has tangled huntingtin protein, Parkinson's disease has tangled α-synuclein, and Alzheimer's disease has tangles of tau and β-amyloid. In

fact, a similar untangling motor from yeast has already shown effectiveness in mouse and nematode models of Huntington's disease.

So Aaron Lucius, Ph.D., professor in the University of Alabama at Birmingham Department of Chemistry, is studying the bacterial protein ClpB of E. coli, as a steppingstone to expanded research on medically significant models in coming years. The question is how does ClpB actually do its job to untangle proteins?

"We don't know how proteins get tangled, but if we can study how proteins get disaggregated, it may have clinical relevance," Lucius said.

ClpB is one of a vast assortment of similar molecular machines found in all living cells, known as hexameric AAA + enzymes. They have six subunits that form a hexagon with a hole in the middle, and they burn ATP for energy. While the machines are all similar, the kinds of work they do vary widely—examples include unwinding DNA, helping digest proteins, untangling proteins, cutting microtubules, helping shape plant cells and driving membrane fusion.

ClpB is closely related to the ClpA enzyme of E. coli. Unlike ClpB—which has the job of untangling a protein that has lost its proper shape—ClpA helps to digest unnecessary proteins into small peptide fragments. Proteins are chains of amino acids, linked together like beads on string, and then folded into a precise shape. ClpA is able to grab one end of a protein that has been marked for recycling, and pull it through the central hole of ClpA, like an anchor chain winched in through the hawse hole of a ship. ATP hydrolysis powers that processive pulling, and the unraveled protein chain is pushed into an attached ClpP enzyme, which cuts up the chain "like a molecular paper shredder," Lucius said.

A previous lab group had garnered evidence that ClpB is also a processive translocase, meaning that it pulls the protein chain all the way

through that central hole in a long series of stepwise tugs, but they were forced to introduce an artefact into the ClpB enzyme to do their experiments. Lucius and his fellow UAB researchers are now challenging that model. After finding a way to test ClpB without introducing the artefact, their experimental results show that the ClpB enzyme makes only one or two tugs on the tangled protein, and then lets go.

"Our results support a molecular mechanism where ClpB catalyzes protein disaggregation by tugging and releasing exposed tails or loops," they wrote in a paper recently published in the *Biochemical Journal*, similar to how someone would tug at the loose strands of a tangled ball of yarn.

This proposed new paradigm of how ClpB functions may apply to other untangling enzymes. "It will take time to see if it is accepted," Lucius said.

The study of hexameric AAA+ enzyme function requires sophisticated experimental approaches. "We can't see the proteins; we have to come up with clever ways to infer what they are doing," Lucius said.

His lab discovered such a clever technique in 2010 while working with ClpA. But when graduate student Tao Li tried to apply it to ClpB studies, and expected to find similar results of processive translocation that the earlier group had reported, "she did three years of every possible experiment to see if it translocates and found no evidence in support of translocation," Lucius said. So the UAB lab began to consider alternatives, which led to the finding that the ClpB enzyme made only one or two tugs before releasing the substrate protein. They also tested ClpB that had the artefact inserted and found evidence that what had appeared to be translocation to the previous researchers was only nonspecific protein degradation without translocation through the central

hole of ClpB.

The experimental approach

ClpB binds to the substrate protein in the presence of an ATP analog that promotes binding but cannot function to power the enzyme. The bound substrate has a fluorescent label attached to its far end, but that fluorescence is dampened by the binding to ClpB. The mixture is put into one syringe, and high concentration of ATP and unlabeled substrate is put into another syringe. With the press of a trigger, a piston powered by 120-pounds-per-square-inch nitrogen gas mixes the contents of the two syringes together within two-thousandths of a second, and now, in the presence of ATP, the ClpB machine can go to work. This technique is known as fluorescence stopped-flow.

The UAB researchers look for the increased fluorescence when ClpB releases the labeled substrate. If the enzyme is pulling the labeled substrate protein through the central hole of the hexamer, there will be a time lag before the fluorescence increases. That lag will increase as longer substrate proteins are tested. But if the ClpB only tugs once or twice, and then releases the substrate, there will be no lag. Conditions are set so that, after each single ClpB hexamer releases its fluorescently labeled substrate, the enzyme will not bind another because there is an excess of unlabeled substrate. Thus, this fluorescence stopped-flow method shows only a single turnover for each enzyme complex.

When this system was used with ClpA, there was a lag before fluorescence increase, and that lag increased with increased length of the substrate protein. Both those results are consistent with the ClpA enzyme, powered by ATP, pulling the substrate protein through its central hole. With a 127-amino-acid substrate, that lag lasted 10 seconds. When this system was used with ClpB, there was no lag, and the length of the substrate made no difference in how quickly the fluorescent signal

increased. Thus, ClpB was releasing the substrate quickly.

More information: "Escherichia coli ClpB is a non-processive polypeptide translocase." *Biochem J*. 2015 Aug 15;470(1):39-52. DOI: 10.1042/BJ20141457

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