UNIVERSITÉ DE GENÈVE

PRESS RELEASE

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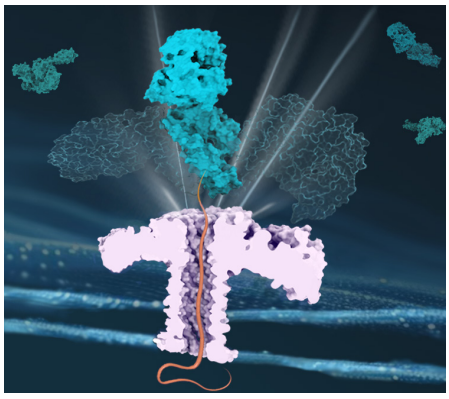
The secret strength of our cell guards

A team from UNIGE and EPFL has demonstrated how Hsp70 chaperone proteins help proteins move within cells.

Proteins control most of the body's functions, and their malfunction can have severe consequences, such as neurodegenerative diseases or cancer. Cells have therefore set up mechanisms to control the quality of the proteins. In animal and human cells, chaperones of the Hsp70 class are at the heart of this control system, overseeing a wide array of biological processes. Yet, despite their crucial role, the precise molecular mechanism of Hsp70 chaperones has remained elusive for decades. Using a cutting-edge nanopore single-molecule technique, a team from the University of Geneva (UNIGE), in collaboration with EPFL, has now made a significant breakthrough in determining how Hsp70 chaperones generate the force needed to manipulate the structure of their client proteins. These results, which put an end to a decade of debate, are published in *Nature Communications*.

Proteins need to fold into specific three-dimensional shapes to function correctly. Among their several roles, chaperone proteins like Hsp70s typically assist the correct folding of proteins. To successfully perform these tasks, Hsp70s need to forcefully manipulate the structure of the proteins, extracting them from aggregates that had formed spontaneously or by facilitating protein translocation into key cell compartments, such as mitochondria.



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A chaperone protein facilitates the passage of another protein through a nanopore, ensuring efficient transport.

High resolution pictures

In this context, during the 1990s and early 2000s, there was an intense debate about the mechanism allowing Hsp70 chaperones to drive protein translocation, with two main models proposed based on different sets of experiments, but with no definitive answer. In 2006, a new theory, called Entropic Pulling, was proposed by Prof. Paolo De Los Rios at EPFL and Prof. Pierre Goloubinoff at the University of Lausanne (UNIL) and their collaborators. Entropic Pulling could explain all existing observations for protein translocation into mitochondria and could also be applied to the other cellular functions of Hsp70s, such as protein disaggregation.

Experimental evidence

Over the years, this theory has allowed the interpretation of a growing number of results but had remained without a direct experimental confirmation. The group of Chan Cao, new assistant professor in the Department of inorganic and analytic chemistry at the UNIGE Faculty of science, specializes in single-molecule bioanalysis, particularly nanopore detection. This innovative approach involves reading

the ionic current response as a single molecule passing through a nanoscale pore, which can be either a biological protein assembly embedded in a lipid membrane or a fabricated solid-state material. The development of nanopore technology aims to create high-resolution sensors for detecting target molecules within complex matrices and for sequencing biopolymer.

In this recent work, the team leveraged nanopore technology to mimic the in vivo setup of protein translocation at the single-molecule level. Prof. Chan Cao explained: "our results provide clear evidence for the Entropic Pulling mechanism of Hsp70 chaperones, ruling out the other previously proposed two models, namely Power Stroke and Brownian Ratchet".

A strong force at the molecular level

In the Entropic Pulling mechanism, the chaperone, by pulling on the target protein, increases its range of movement, generating what is known as an entropic force. Verena Rukes, PhD student and the leading author of the study, explains "our analysis estimated the strength of Entropic Pulling to be approximately 46 pN over distances of 1 nm, indicating a remarkably strong force at the molecular level".

Prof. Paolo De Los Rios from the Institute of Physics and Institute of Bioengineering at EPFL explains: "our theory proposed in 2006 was accounting for most of the physics of the system comprising Hsp70, the translocating protein and the translocation pore, but in the end, it remained a theory, even if in indirect agreement with most observations. Thanks to the beautiful work of Prof. Chan Cao and her team, we have now a direct proof of it and, which is most important, a quantitative estimate of its strength, which turns out to be remarkably high, further explaining why Hsp70s are so effective at changing the structure of their target proteins".

This research also establishes nanopore approaches as a powerful single-molecule technique for exploring the molecular mechanisms of protein action.

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