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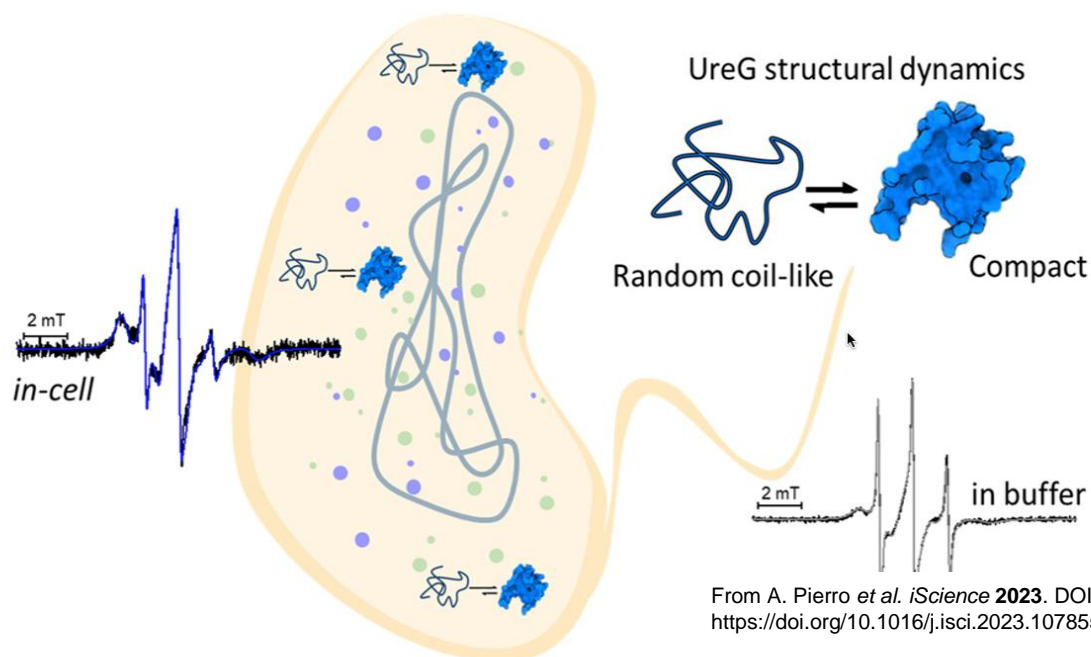
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Messages from cells: breaking new ground in deciphering protein behaviour

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UreG is molecular chaperone that functions in the activation of urease, a nickel-dependent enzyme required by many pathogenic bacteria and fungi to infect their host. UreG multitasks by also acting as a GTPase enzyme, collaborating with other chaperones to channel energy from GTP hydrolysis to deliver nickel ions essential for activating urease, thereby playing a key role in the cellular ecosystem and metal homeostasis pathway.

In a landmark study, Dr. Elisabetta Mileo from BIP Laboratory (Aix Marseille University and CNRS) and Professor Barbara Zambelli from the Dept of Pharmacy and

Biotechnology (University of Bologna), along with Professor Valérie Belle, Professor Bruno Guigliarelli, Dr. Emilien Etienne, Dr. Guillaume Gerbaud, Hugo Leguenno, Ketty Tamburrini, and Annalisa Pierro from Aix Marseille University, have uncovered the dynamic behavior of the GTPase UreG protein within live bacterial cells. This represents a major leap in our comprehension of protein functions in their natural habitat. Published in the esteemed journal *iScience*, their research elucidates how the UreG protein, indispensable for the activation of the urease enzyme in bacteria, exhibits remarkable flexibility in its physiological context that could be pivotal for its enzymatic activities.

Previously, UreG's role as a chaperone in aiding the activation of nickel-dependent enzymes like urease has been well documented through *in vitro* studies, which have evidenced the flexible behavior of this enzyme in solution. These data propel the understanding forward by deploying site-directed spin labeling combined with electron paramagnetic resonance (SDSL-EPR) spectroscopy to directly observe UreG's behavior inside living cells.

Dr. Zambelli highlighted the study's significance, stating, "The findings demonstrate that UreG maintains a diverse structural landscape *in-cell*, existing in a conformational ensemble of two major populations showcasing either random coil-like or compact properties. These insights affirm the physiological significance of UreG's inherently disordered nature and suggest a role of protein flexibility for this specific enzyme, possibly related to the regulation of broad protein interactions for metal ion delivery."

The researchers utilized innovative techniques to delve into the UreG protein's behavior within the cellular environment. "*In-cell* EPR experiments were conducted by first labeling the protein of interest produced by recombinant expression and then delivering it inside cells," Dr. Mileo explained. A delivery protocol involving calcium ions and a brief thermal shock (incubation at 42°C) was adapted for this study, facilitating optimal protein internalization that preserved cell viability.

This study not only highlights the intrinsic disorder and flexibility of the UreG protein but also marks a significant methodological advancement in the study of proteins within their native cellular context. "The application of nitroxide-based spin labels enabled EPR analysis at room temperature, conditions compatible with the life of the cells under study," Dr. Mileo added, underscoring the method's capability to observe proteins in their natural setting without compromising cell health.

By leveraging advanced SDSL-EPR spectroscopy, the research teams have provided valuable insights into the conformational landscape of UreG, setting the groundwork for future endeavors aimed at unraveling the intricate mechanisms underlying protein function in live cells. This research not only deepens our understanding of protein dynamics but also opens new pathways for the development of targeted therapies and biotechnological applications for the discovery of new drugs and anti-microbial molecules.

Reference: Annalisa Pierro et al., "In-cell investigation of the conformational landscape of the GTPase UreG by SDSL-EPR," *iScience*, 2023. DOI: <https://doi.org/10.1016/j.isci.2023.107855>.

About the Authors

Barbara Zambelli – Associate Professor from 2021, previously Assistant Professor at UniBO since 2008. Her research activity aims to understand, at the molecular and structural level, metal-driven protein interactions that, in biological systems, are responsible for regulating metabolic processes important for life. In particular, her main

research lines deal with the cellular metabolism of nickel and its effects on human health, with possible medical and pharmaceutical applications.

Elisabetta Mileo is a CNRS researcher at the laboratory “Bioénergétique et Ingénierie des Protéines” (BIP) in Marseille, FRANCE. She is a chemist and a spectroscopist with experience in the synthesis and use of nitroxide radicals as spin labels in structural biology. E. Mileo research activities are mainly focused on the investigation of protein structural dynamics, in particular in chaperones and other flexible proteins, with the objective of gaining information on how protein dynamics affects protein function. The originality of her work resides on the fact that protein investigation by SDSL-EPR is carried directly inside cells (in-cell EPR), in physiological conditions. Her studies are also aimed to the development of new tools and new spin labels to follow proteins “in action” by EPR directly into living cells.

