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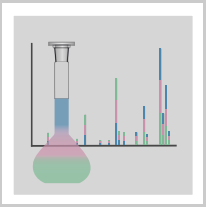

Highlights of Analytical Sciences in Switzerland

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Visualizing a Carbon-Fixing Nanowire Inside Bacteria

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Bacteria have evolved unusual ways to obtain energy in extreme environments with no oxygen. The hydrogen-dependent CO_2 reductase (HDCR) enzyme from acetogenic bacteria is one particularly ingenious example. HDCR performs the reversible conversion of molecular hydrogen and CO_2 into formic acid much more efficiently than any other known chemical catalyst. To understand how HDCR achieves such an impressive turnover rate, we sought to visualize its molecular structure.

Working in collaboration with the research groups of Volker Müller (University of Frankfurt) and Jan Schuller (University of Marburg), we used cryo-electron microscopy to obtain a high-resolution 3D structure of purified HDCR proteins. This advanced imaging technique, pioneered by Swiss Nobel laureate Jacques Dubochet, involves rapidly freezing the sample in vitreous ice to preserve its structure under the electron beam. The results revealed that HDCR is a novel type of biological nanowire, which is decorated by hydrogenase and formate dehydrogenase enzymes. The enzymes and nanowire proteins are all interconnected by a chain of iron-sulfur clusters, which efficiently shuttle electrons between the catalytic subunits, thereby coupling the hydrogen-splitting and carbon-fixation reactions.

Furthermore, we also used cryo-electron tomography to visualize the HDCR filaments in their native biological context inside *Thermoanaerobacter kivui* cells. These cells were preserved in vitreous ice and then thinned by focused ion beam (FIB) milling before tomographic imaging. The 3D reconstructions showed that around 100 HDCR nanowires bundle together to form ~200 nm circular superstructures attached to the cell membrane. This ordered bundling of HDCR filaments in the cell may be a concentration mechanism that further explains how these organisms are so efficient at carbon fixation. How the HDCR nanowires attach to the plasma membrane and interact with the other components in the acetogenic metabolic pathway are questions that remain to be explored.

Understanding the molecular architecture of HDCR paves the way towards its use in biotechnological applications. For example, it could be used to store hydrogen fuel in a more efficient and safer way as formate, or in carbon-capture technologies aimed at mitigating global warming.

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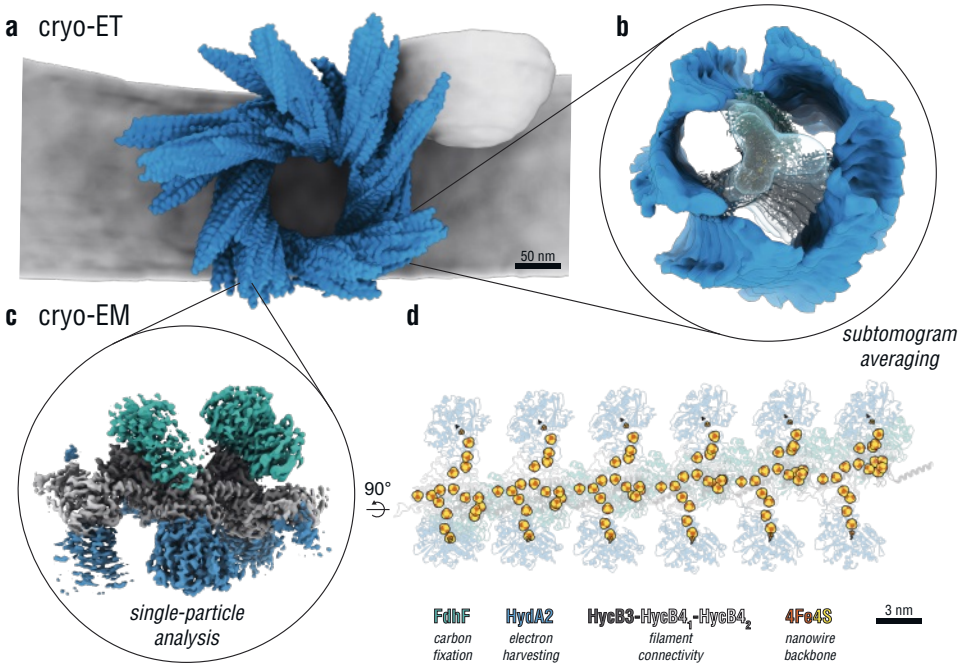
H. M. Dietrich, R. D. Righetto, A. Kumar, W. Wietrzynski, R. Trischler, S. K. Schuller, J. Wagner, F. M. Schwarz, B. D. Engel, V. Müller, J. M. Schuller, *Nature* **2022**, *607*, 823, https://doi.org/10.1038/s41586-022-04971-z.



Visualizing HDCR filaments using cryogenic electron microscopy techniques. a) Using cryo-electron tomography (cryo-ET) on FIB-milled *T. kivui* bacteria, we observed bundles of HDCR filaments (blue) attached to the cellular membrane (gray). b) These native HDCR filaments were resolved to 17 Å with subtomogram averaging. c) Single-particle cryo-electron microscopy (cryo-EM) of purified HDCR filaments resolved the structure to 3.4 Å. d) Combining the structural data from cryo-EM and cryo-ET at different scales, we built an atomic model for the HDCR filaments, showing that a nanowire of iron-sulfur clusters (orange and yellow) connects the catalytic centers of the hydrogen-splitting (HydA2) and carbon-fixing (FdhF) enzymes.

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