Fluorescent probes increase understanding of bacterium's electron transfer

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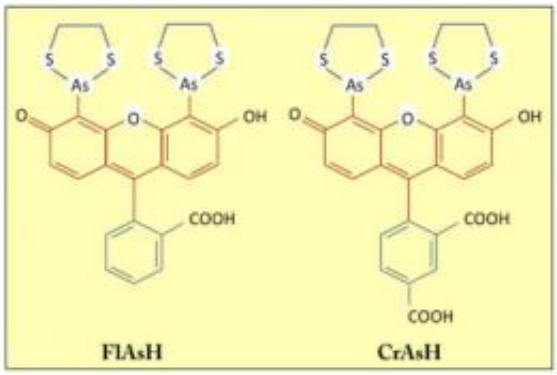

Structures of biarsenic fluorophores, CrAsH-EDT2 and FlAsH-EDT2. FlAsH-EDT2 represents a fluorescein scaffold derivatized with two As(III) groups (4′,5′-bis(1,3,2-dithioarsolan-2-yl)fluorescein). Each arsenic is capped with an ethanedithiol (EDT). Similarly, CrAsH-EDT2 represents 6-carboxy-FlAsH-EDT2.

(PhysOrg.com) -- When it comes to transporting a cell's valuable electrons, the metal-reducing microbe Shewanella oneidensis only trusts stable, mature proteins, according to scientists at Pacific Northwest National Laboratory. Immature proteins are degraded before they can take up the task, suggesting that protein trafficking to the outer membrane is tightly regulated.

These results mark another step toward understanding the cellular mechanisms that enable a bacterial protein-in this case, the cytochrome

MtrC-to transfer electrons to minerals in soil, sediment, and subsurface materials. This new information contributes to understanding protein stability and electron transfer between cells and minerals, which is important for applications in synthetic biology such as biofuel production. The results were published in the journal *Biochemistry*.

Electron transfer by MtrC, an outer membrane cytochrome on S. oneidensis, can stabilize contaminants, mitigating their impact on the population and environment. However, scientists believe that gaining insight into the electron transfer mechanisms could also play a role in directing the bacterium toward biofuel production.

"Our goal is to define the role of these cytochromes in the metabolic switching between different terminal electron acceptors," said Dr. Thomas Squier, a PNNL biochemist and senior author of the publication. "The long-term goal is to understand the stability and targeting mechanisms important to synthetic biology applications involving, for example, chemical sensing between living cells and electronic detectors as well as the development of biofuel cells."

These findings don't just relate to Shewanella, though it was in this microbe where MtrC was first seen. They also apply to many other bacteria, such as E. coli, notes Squier.

"This research ties very well into looking at and understanding microbial communities as a whole," he added.

Measuring MtrC's environmental stability requires the ability to differentiate an immature protein from a mature protein after it is secreted and assembled on Shewanella's outer membrane. To do this, the scientists constructed complementary fluorescent probes to label MtrC. The highly charged carboxy-FlAsH (CrAsH) probe selectively labels mature MtrC only on the outer cell membrane, while the cell-permeable

Fluorescein Arsenical Helix (FlAsH) probe labels all MtrC, including immature proteins within the cell.

More information: Xiong Y, et al. 2011. "Targeted Protein Degradation of Outer Membrane Decaheme Cytochrome MtrC Metal Reductase in Shewanella oneidensis MR-1 Measured Using Biarsenical Probe CrAsH-EDT2." *Biochemistry* 50(45):9738-9751 DOI: 10.1021/bi200602f

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