Seeing protein synthesis in the field

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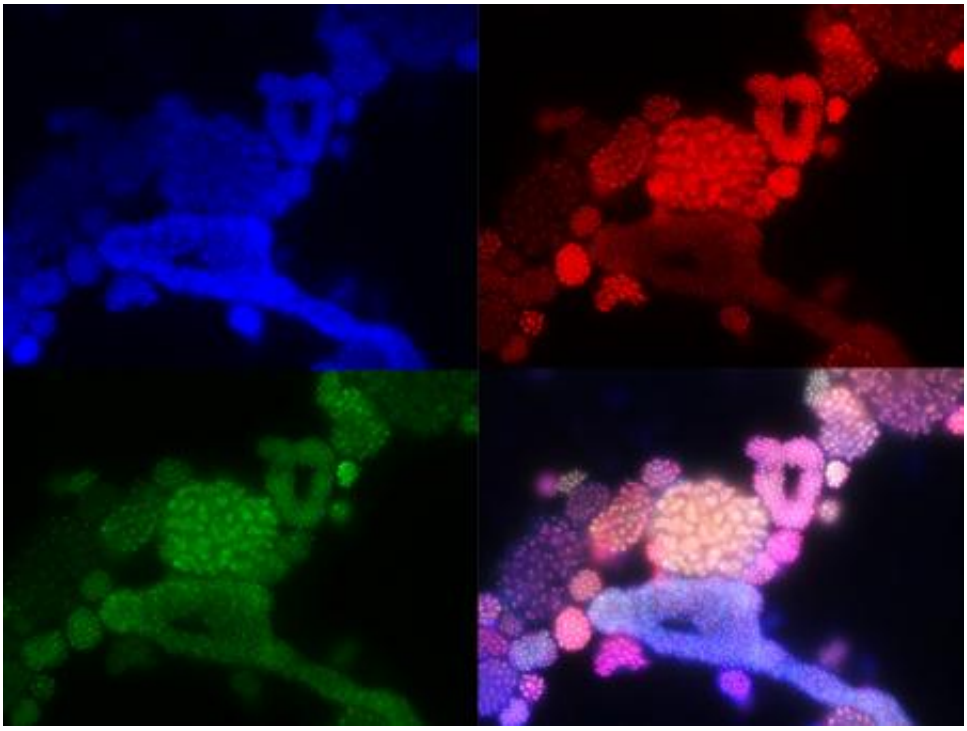

In this image, the entire microbial community in Caltech's lily-pond water is stained blue with a DNA dye; freshwater gammaproteobacteria are labeled with a fluorescently tagged short-chain ribosomal RNA probe, in red; and newly created proteins are dyed green by BONCAT. The green and orange cells in the composite image (lower right) show gammaproteobacteria and other rod–shaped cells that are actively making proteins. Credit: Roland Hatzenpichler and Victoria Orphan/Caltech

(Phys.org) —Caltech researchers have developed a novel way to visualize proteins generated by microorganisms in their natural

environment—including the murky waters of Caltech's lily pond, as in this image created by Professor of Geobiology Victoria Orphan and her colleagues. The method could give scientists insights to how uncultured microbes (organisms that may not easily be grown in the lab) react and adapt to environmental stimuli over space and time.

The visualization technique, dubbed BONCAT (for "bioorthogonal non-canonical amino-acid tagging"), was developed by David Tirrell, Caltech's Ross McCollum–William H. Corcoran Professor and professor of chemistry and chemical engineering. BONCAT uses "non-canonical" amino acids—synthetic molecules that do not normally occur in proteins found in nature and that carry particular chemical tags that can attach (or "click") onto a fluorescent dye. When these artificial amino acids are incubated with environmental samples, like lily-pond water, they are taken up by microorganisms and incorporated into newly formed proteins. Adding the fluorescent dye to the mix allows these proteins to be visualized within the cell.

For example, in the image, the entire microbial community in the pond water is stained blue with a DNA dye; freshwater gammaproteobacteria are labeled with a fluorescently tagged short-chain ribosomal RNA probe, in red; and newly created proteins are dyed green by BONCAT. The cells colored green and orange in the composite image, then, show those bacteria—gammaproteobacteria and other rod-shaped cells—that are actively making proteins.

"You could apply BONCAT to almost any type of sample," Orphan says. "When you have an environmental sample, you don't know which microorganisms are active. So, assume you're interested in looking at organisms that respond to methane. You could take a sample, provide methane, add the synthetic amino acid, and ask which cells over time showed activity—made new proteins—in the presence of methane relative to samples without methane. Then you can start to sort those

organisms out, and possibly use this to determine protein turnover times. These questions are not typically tractable with uncultured organisms in the environment." Orphan's lab is also now using BONCAT on samples of deep-sea sediment in which mixed groups of bacteria and archaea catalyze the anaerobic oxidation of methane.

Why sample the Caltech lily pond? Roland Hatzenpichler, a postdoctoral scholar in Orphan's lab, explains: "When I started applying BONCAT on environmental samples, I wanted to try this new approach on samples that are both interesting from a microbiological standpoint, as well as easily accessible. Samples from the lily pond fit those criteria." Hatzenpichler is lead author of a study describing BONCAT that appeared as the cover story of the August issue of the journal *Environmental Microbiology*.

More information: "In situ visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry." Hatzenpichler, Roland and Scheller, Silvan and Tavormina, Patricia L. and Babin, Brett M. and Tirrell, David A. and Orphan, Victoria J. (2014) In situ visualization of newly synthesized proteins in environmental microbes using amino acid tagging and click chemistry. *Environmental Microbiology*, 16 (8). pp. 2568-2590. ISSN 1462-2912. PMCID PMC4122687. resolver.caltech.edu/CaltechAU … S:20140303-103306009

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