Improving the robustness of engineered bacteria to nutrient stress

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Green fluorescent protein (shown in the middle) is used by engineered bacteria as a reserve of amino acids. When nutrients become scarce, the protein can be broken down to provide essential amino acids needed for survival. Credit: Klara Szydlo & Thomas Gorochowski

Researchers from the Universities of Bristol and Hamburg have engineered bacteria with internal nutrient reserves that can be accessed when needed to survive extreme environmental conditions. The findings, published in *ACS Synthetic Biology*, pave the way for more robust

biotechnologies based on engineered microbes.

Synthetic biology allows scientists to redesign organisms, harnessing their capabilities to lead to innovative solutions spanning the sustainable production of biomaterials to advanced sensing of pathogens and disease.

Dr. Thomas Gorochowski, joint senior author and a Royal Society University Research Fellow in the School of Biological Sciences at Bristol, said: "Many of the engineered biological systems we have created to date are fragile and break easily when removed from the carefully controlled conditions of the lab. This makes their deployment and scale-up difficult."

To tackle this problem, the team focused on the idea of building up reserves of protein within cells when times are good, and then breaking these down when conditions are difficult and additional nutrients are needed.

Klara Szydlo, first author and a Ph.D. student at the University of Hamburg, elaborated: "Cells require building blocks like amino acids to function and survive. We modified bacteria to have a protected reserve of these that could then be broken down and released when nutrients became scarce in the wider environment. This allowed the cells to continue functioning when times were tough and made them more robust to any unexpected challenges they faced."

To create such a system, the team engineered bacteria to produce proteins that could not be directly used by the cell, but which were recognized by molecular machines called proteases. When nutrients fluctuated in the environment, these proteases could then be called on to release the amino acids making up the protein reserve. The released amino acids allowed the cells to continue growing, even though the

environment lacked the nutrients required. The system acted similar to a biological battery that the cell could tap into when the mains power was cut.

Dr. Gorochowski added: "Developing such a system like this is difficult because there are many different aspects of the design to consider. How big should the protein reserve be? How quickly does this need to be broken down? What sorts of environmental fluctuation would this approach work for? We had lots of questions and no easy way to assess the different options."

To get around this problem, the team built a mathematical model that allowed them to simulate lots of different scenarios and better understand where the system worked well and where it broke. It turned out that a careful balance was required between the size of the protein reserve, the speed of its breakdown when required, and the length of time nutrients were scarce. Importantly though, the model also showed that if the right combination of these factors was present, the cell could be completely shielded from changes in the environment.

Professor Zoya Ignatova, joint senior author from the Institute of Biochemistry and Molecular Biology at the University of Hamburg, concluded: "We've been able to demonstrate how carefully managing reserves of key cellular resources is a valuable approach to engineering bacteria that need to operate in challenging environments. This capability will become increasingly important as we deploy our systems into complex real-world settings and our work helps pave the way for more robust engineered cells that can operate in a safe and predictable manner."

More information: Klara Szydlo et al, Improving the Robustness of Engineered Bacteria to Nutrient Stress Using Programmed Proteolysis, *ACS Synthetic Biology* (2022). DOI: 10.1021/acssynbio.1c00490

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