Non-invasive, ratiometric determination of intracellular pH in Pseudomonas species using a novel genetically encoded indicator

The ability of Pseudomonas species to thrive in all major natural environments (i.e. terrestrial, freshwater and marine) is based on its exceptional capability to adapt to physicochemical changes. Thus, environmental bacteria have to tightly control the maintenance of numerous physiological traits across different conditions. The intracellular pH (pHi) homoeostasis is a particularly important feature, since the pHi influences a large portion of the biochemical processes in the cell. Despite its importance, relatively few reliable, easy-to-implement tools have been designed for quantifying in vivo pHi changes in Gram-negative bacteria with minimal manipulations. Here we describe a convenient, non-invasive protocol for the quantification of the pHi in bacteria, which is based on the ratiometric fluorescent indicator protein PHP (pH indicator for Pseudomonas). The DNA sequence encoding PHP was thoroughly adapted to guarantee optimal transcription and translation of the indicator in Pseudomonas species. Our PHP-based quantification method demonstrated that pHi is tightly regulated over a narrow range of pH values not only in Pseudomonas, but also in other Gram-negative bacterial species such as Escherichia coli. The maintenance of the cytoplasmic pH homoeostasis in vivo could also be observed upon internal (e.g. redirection of glucose consumption pathways in P. putida) and external (e.g. antibiotic exposure in P. aeruginosa) perturbations, and the PHP indicator was also used to follow dynamic changes in the pHi upon external pH shifts. In summary, our work describes a reliable method for measuring pHi in Pseudomonas, allowing for the detailed investigation of bacterial pH homoeostasis and its regulation.
and productivity of engineered pathways and synthetic circuits. In order to circumvent this problem, we have designed a novel expression system based on the well-known XylS/Pm transcriptional regulator/promoter pair from the soil bacterium Pseudomonas putida mt-2, in which the key functional elements are physically decoupled. By integrating the xylS gene into the chromosome of the platform strain KT2440, while placing the Pm promoter into a set of standard plasmid vectors, the inducibility of the system (i.e. the output difference between the induced and uninduced state) improved up to 170-fold. We further combined this modular system with an extra layer of post-translational control by means of conditional proteolysis. In this setup, the target gene is tagged with a synthetic motif dictating protein degradation. When the system features were characterized using the monomeric superfolder GFP as a model protein, the basal levels of fluorescence were brought down to zero (i.e. below the limit of detection). In all, these novel expression systems constitute an alternative tool to altogether suppress leaky gene expression, and they can be easily adapted to other vector formats and plugged-in into different Gram-negative bacterial species at the user's will.

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Getting Bacteria in Shape: Synthetic Morphology Approaches for the Design of Efficient Microbial Cell Factories
Supported by the tools of contemporary synthetic biology, the field of metabolic engineering has advanced in its overarching purpose of contributing efficient bioprocesses for the synthesis of biochemicals by addressing a number of cell and process parameters. The morphology and spatial organization of bacterial biocatalysts has been somewhat overlooked in such endeavors. The shape, size, and surface features of bacteria are maintained over evolutionary timescales and, under tight control of complex genetic programs, are faithfully reproduced each generation—and offer a phenomenal target for manipulations. This review discusses how these structural traits of bacteria can be exploited for designing efficient biocatalysts based on specific morphologies of both single cells and natural and artificial communities (e.g., catalytic biofilms). Examples are presented on how morphologies and physical forms of bacterial cell factories can be programmed while engineering their biochemical activities. The concept of synthetic morphology opens up strategies for industrial purposes and holds the potential to improve the economic feasibility of some bioprocesses by endowing bacteria with emergent, useful spatial properties. By entertaining potential applications of synthetic morphology in the future, this review outlines how multicellular organization and bacterial biorobots can be programmed to fulfill complex tasks in several fields.

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