A Bio-electrical Interface for Biosensing Purposes

Coroiu Diana[1], Janardanan Amritha[1], and Pilizota Teuta[1]

Understanding bacterial gene expression and their physiology opens the possibility of engineering (and obtaining) biological machines which are often more effective and specific than their physical counterparts. Here, we exploit the bacterial motility machinery to design bacteria that can sense chemicals – specifically, rhamnose and zinc - in their environment, and respond to it by changing their flagellar rotation pattern, which can then be detected in various ways, including electrically. We also characterize the sensors in terms of response speed and sensitivity.

Key words: **biosensors, bacterial flagellar motor, CheY, chemotaxis, zinc sensor, rhamnose sensor, back-focal-plane interferometry, bacterial motility, bioelectrical chip**

I. INTRODUCTION

Bacterial species can be exploited for technological applications not only as chassis for synthesis of recombinant proteins and molecules of interest, but also as chemosensors, voltmeters, and mechanosensors [1]. Synthetic biology allows us to rewire native biochemical pathways to develop such sensing bacterial strains. Synthesis of Green Fluorescent Protein (GFP) is usually used as the output signal to indicate the presence of a particular chemical in the environment; however, this form of output often requires complicated equipment and access to mains power to operate. Alternative output signals are therefore of interest. Here, we develop strains which change the pattern of their flagellar motor rotation in response to chemical inputs. The approach opens the door for alternative methods of detecting biochemical outputs in response to environmental contents, including electrically.

The bacterial flagellar motor, which drives the rotation of the filament, is a complex made up of several protein units. The chemotactic network is responsible for regulating the direction of this rotation: by default, the motor rotates in a counterclockwise (CCW) direction; however, upon binding of an attractant molecule from the environment to its receptors, the CheY protein is phosphorylated by the ligand-bound receptor [2]. Phosphorylated CheY interacts with the flagellar motor and changes its rotational direction from counterclockwise to clockwise (CW). We use a mutant form of the CheY protein, CheY**, which mimics the phosphorylated form of CheY [3], and we engineer our bacterial strains in such a way that they only express CheY** when a chemical of interest is present in the environment. Thus, the number of CW rotations of the motor increases as the likelihood of the interaction between the flagellar motor and the CheY** protein increases in the presence of a chemical of interest. This change in flagellar behaviour upon addition of the chemical of interest is a mechanical form of output, rather than an optical one, so it can more easily be coupled to a simple and accessible physical transducer for subsequent detection of the biochemical response.

II. EXPERIMENTAL APPROACH

a) Development of the sensor strain

We designed an *E. coli* K-12-derived host strain lacking CheY that also carries a so-called *fliCsticky* gene insertion. The *fliCsticky* gene encodes for a form of flagellin (FliC) with a ~50 amino acid mutation, that results in a more hydrophobic form of the filament and, as such, it readily adheres to surfaces [4]. This is needed for attachment of polystyrene beads to filaments to assess the function of the sensors (see *Section b.* below). Lastly, we transform constructs with the *cheY*** gene downstream of rhamnose or zinc-inducible promoters – both of which are part of endogenous pathways in *E. coli* species – into our host strain, thus obtaining cells whose number of CW flagellar rotations increases in the presence of either rhamnose or zinc.

b) Assessment of the sensitivity range and response curve

We use back-focal-plane interferometry [5] – a heavily attenuated optical trap – to assess the behaviour of the flagellar motor in response to various concentrations zinc or rhamnose, by tracking the rotation of a polystyrene bead attached to the "sticky" filament. We build a response curve to rhamnose and zinc by tracking the so-called CW-Bias, a measure of the amount of time spent by the flagellum rotating clockwise relative to the total time spent rotating.

III. CONCLUSIONS

The project uses synthetic biology to develop novel outputs for whole-cell biosensors that have the potential to be used in today's medical, industrial, and environmental settings.

REFERENCES

[1] Krasnopeeva E, Barboza-Perez UE, Rosko J, Pilizota T, Lo C-J. Bacterial flagellar motor as a multimodal biosensor. Methods 2021;193:5–15. https://doi.org/10.1016/j.ymeth.2020.06.012.

[2] Bren A, Eisenbach M. The N terminus of the flagellar switch protein, FliM, is the binding domain for the chemotactic response regulator, CheY. Journal of Molecular Biology 1998;278:507–14. https://doi.org/10.1006/jmbi.1998.1730.

[3] Scharf BE, Fahrner KA, Turner L, Berg HC. Control of direction of flagellar rotation in bacterial chemotaxis. Proceedings of the National Academy of Sciences 1998;95:201–6. https://doi.org/10.1073/pnas.95.1.201.

[4] Kuwajima G. Construction of a minimum-size functional flagellin of Escherichia coli. Journal of Bacteriology 1988;170:3305–9. https://doi.org/10.1128/jb.170.7.3305-3309.1988.

[5] Denk W, Webb WW. Optical measurement of picometer displacements of transparent microscopic objects. Applied Optics 1990;29:2382. https://doi.org/10.1364/AO.29.002382.

[1] Institute of Cell Biology, School of Biological Sciences, The University of Edinburgh, Edinburgh, UK; e-mail: teuta.pilizota@ed.ac.uk