ABSTRACT

Halorhodopsin (HR), a light-driven ion pump originated from *Halobacterium* employs light to transport chloride ion (Cl⁻) into cells uni-directionally against osmolality. In our project, we integrated halorhodopsin into functional biobricks. Based on the characterized properties of halorhodopsin, we have furthermore developed and implemented two innovative applications: light-coupled computer-aided expression platform and entropy-mixing electrical power generation.

INTRODUCTION

ChloriColight implies our project aim, which involves the interaction between light and Cl⁻ through *E.Coli*. We achieve this by making use of a light-driven ion pump called Halorhodopsin. It is specific for Cl⁻. Our part comprises on extracellular concentration with extracellular light input. It can serve as a great plug-in tool that alters Cl⁻ concentration by light. We have done extensive characterization on our biobricks. The data suggest the possibility of turning our biobricks into applications.

CHARACTERIZATION

A. Biobrick Construction

We have successfully constructed 3 biobricks (BBa_K559010, BBa_K559000, BBa_K559001). The identity of each biobrick has been confirmed by BGI sequencing.

D. The effect of NaCl concentration on the bacterial growth

The growth of bacteria was unaffected in the range from 0 M to 0.4 M but it was inhibited above 0.4 M.

B. Expression of halorhodopsin in BL21 (DE3)

Western blotting was performed to confirm the expression of HR. Expression of halorhodopsin is induced by IPTG. Halorhodopsin was detected by anti-His antibody. 0.1 mM of IPTG was found to be the optimum.

C. The essential factors for Cl⁻ absorption

Intracellular Cl⁻ concentration was at maximum when light, IPTG and NaCl were present.

LIGHT INTRA-TUNABLE SYSTEM

Chloride-sensing cassette connects our biobrick (BBa_K559010) and responses to intracellular Cl⁻ concentration. *p*ₐₚ is one of the examples. *p*ₐₚ regulates gene transcription in Cl⁻ dependent manner. In our project, modified *p*ₐₚ operon, i.e. insert gene of *EGFP* (BBa_E0040), is coupled with our characterized HR system to construct a light intra-tunable system. The level of *EGFP* expression is regulated by the intracellular Cl⁻ concentration and thus by light. Hence the gene expression level can be tuned.

POWER GENERATION

The ocean is a gigantic energy reservoir. Its energy is stored as salinity potential. When seawater mixes with freshwater from river, massive amount of energy is released. Mixing-entropy battery with one pair of electrodes is designed to convert the salinity potential into electricity.

The halorhodopsin decreases Cl⁻ concentration of the electrolyte while Cl⁻ are absorbed into the bacteria. Those Na⁺ and Cl⁻ binding to the electrodes are released to the solution. When placed in dark, bacteria expressing halorhodopsin releases excess Cl⁻. The out flux of Cl⁻ causes the increase of the extracellular salinity. The cycle repeats when light is available again.

CONCLUSION

We have found the best condition for our biobrick (BBa_K559010). Using our biobrick, we can regulate any gene expression by controlling intracellular Cl⁻ concentration.

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REFERENCES