POTASSIUM, PHOSPHATE, AND NITRATE BIOSENSOR

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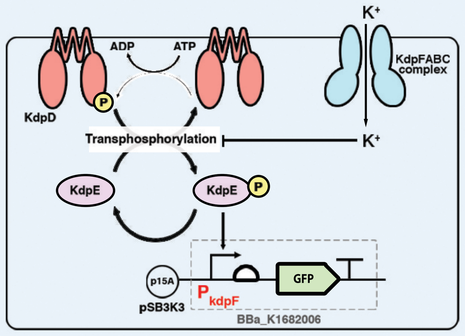
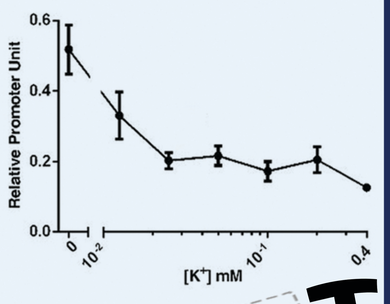
INTRODUCTION

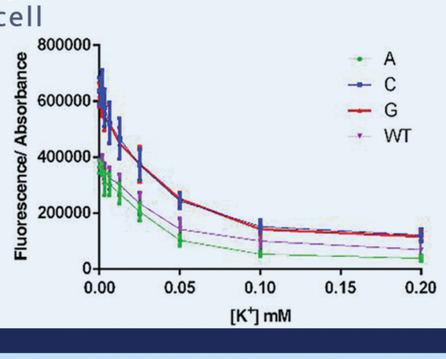
We want to engineer a biological sensor in *E. coli*, which can detect NITRATE, PHOSPHATE, and POTASSIUM levels in the surrounding environment and give responses in the form of colors. In addition, we are characterizing the effects of a dual output system, in contrast to a single output system, in order to anticipate the expression of multiple outputs in a single system.

POTASSIUM

We adopted the promoter kdpFp from kdpFABC operon. We designed another 3 mutants having change of one base-pair at -15 position from thymine to either cytosine , guanine or adenine. G mutant was chosen to be furtherly characterized with fluorescence-activated cell sorting (FACS).

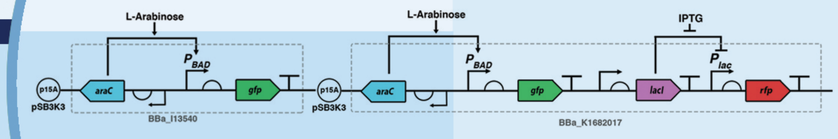


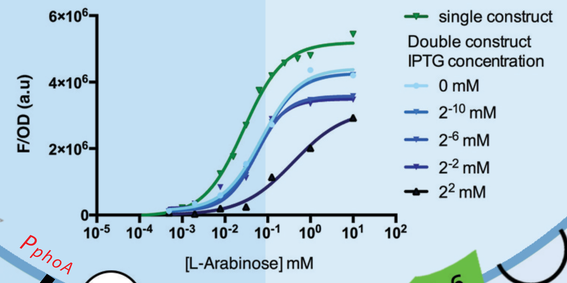




PARALLEL SENSOR

- Combined two constructs characterised by different inputs and outputs within one plasmid to see if there are differences in signal outputs for one construct in a single plasmid and multiple constructs in the same plasmid.
- NPK Simulation.



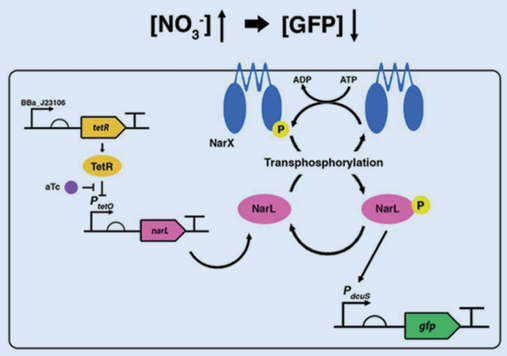


NITRATE

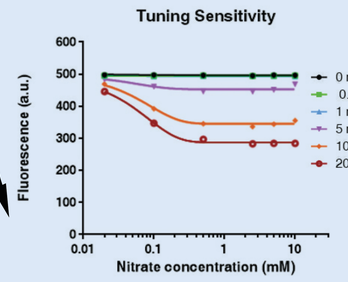
P_{dcuS}

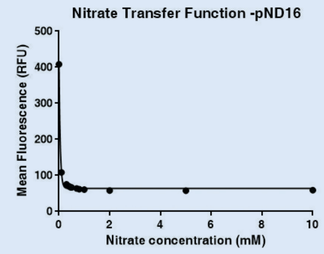
NarX-NarL two-component system

- NarL was put under aTc inducible control
  - Sensor behavior was characterized for several values of aTc
  - The sensor saturates in NarL before 20 ng/mL aTc
  - The higher the aTc concentration, the lower the fold response of the sensor



- For a saturating level of aTc, the sensor was further characterized to extract important parameters
- In the future, we would like to flip out the fluorescent reported for a chromophore, to give the sensor a colorimetric output

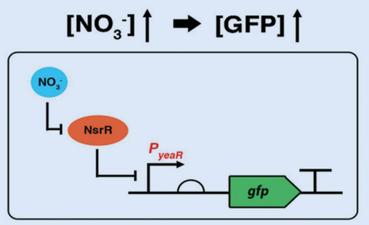


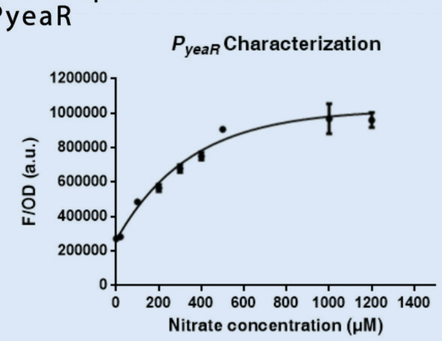


Parameters	Values
HIGH	408.3724
LOW	57.12321
K_1/2	0.020647
n	1.114473

P_{yeaR}

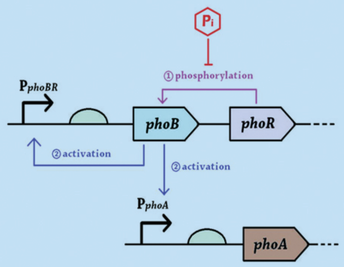
- Cross-regulated by NsrR and Nar system
- Presence of nitrate relieve repression from the two components→activate PyeaR

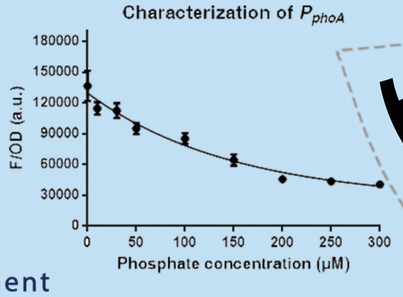




- Dynamic range: 0-500μM
- RFU increases by 3.12 folds in its dynamic range

PHOSPHATE



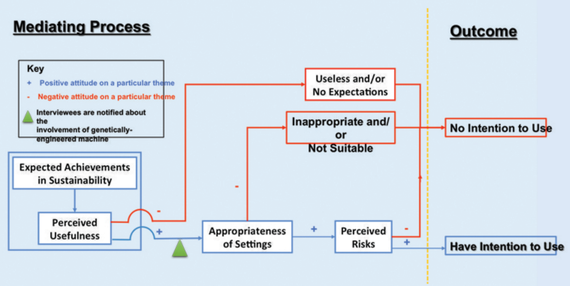


PhoR/PhoB two-component system
-P: PhoR will phosphorylate PhoB and PhoB-P will directly activate the expression of *PphoA*
+P: PhoR will repress PhoB phosphorylation which in turns inactivates *PphoA*

Results:
RFU decreases by 2.99 folds between 0μM - 200μM. Dynamic range is between 0μM - 200μM.

HUMAN PRACTICE

This research could help researchers decide if their design truly meets the need of different stakeholders and is considered to be appropriate in term of settings ; and if so, how to apply the device in order for achieving perceived benefits while minimising the risks.



APPLICATIONS

1. Paper-based cell lysate --> test nutrient level - success with preliminary experiment.
2. Controlled biofeltilizer system --> control nutrient's can theoretically (by transforming Potassium, Nitrate and Phosphate Biosensor to native soil species such as nitrogen-fixing bacteria)
3. Proof-of-concept experiment - positive result

CONCLUSION

- Characterized potassium, phosphate and nitrate bio sensors.
- Measured the effect of par allel sensing.
- Provide proof of concept experiment to prove the feasibility of biosensor.
- Collected public percep tion toward application of biosensors.

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