**ABSTRACT**

Cells can sense and respond to the presence of various gas molecules such as oxygen, nitrogen and carbon monoxide using gas sensor proteins.

CooA is a carbon monoxide (CO) sensing transcription factor. It is a member of the cAMP receptor protein (CRP)/fumarate nitrate reduction (FNR) family of transcriptional regulators. CooA switches on oxidation enzymes in *Rhodospirillum rubrum* which enables the bacterium to use CO as a carbon source.

CO is an odorless and colorless gas which can be extremely lethal. Our aim is to develop a cell sensor which can detect a wide range of CO concentration in the environment.

We are building CooA and CooA-responsive promoter biobricks which will be transformed into E.coli. Fluorescent proteins (GFP and RFP) will be utilized as dose-responsive signals of ambient CO.

**PROJECT DESCRIPTION**

**Objectives**
- To construct a carbon monoxide sensing cell sensor
- To increase the dynamic range of the sensor via strong/weak promoter coupling;

**Enhanced Dynamic Range (EDR)**

**How E-CO Sensor Works?**
- When CO is introduced into the medium, transcription from both strong and weak CooA responsive promoters will be initiated.
- Since affinity of CO bound transcription factor is higher for the strong promoter, GFP signal will dominate the RFP signal due to the higher transcription rate of the former.
- Increase in CO concentration will completely saturate strong promoter and after a point saturation of the second, weaker promoter will begin.
- As the concentration of the signal from weak promoter (RFP) increases, detected fluorescent signal will start to change from green to yellow.

**COOA EXPRESSION AND PURIFICATION**

We expressed and purified CooA using a combination of anion exchange and affinity chromatography.

**COOA-RESPONSE ELEMENT AFFINITY STUDIES**

We used pCooF and pCoom as positive controls to optimize Electrophoretic Mobility Shift Assay (EMSA). Retardation bands and decreased free DNA band intensity indicate CooA-response element binding.

**OPTIMIZATION OF CULTURING AND CO FEEDING**

We optimized three different setups for culturing and CO feeding; Fermentor, flask and controlled atmosphere chamber.

**CONCLUSIONS**

- We have built a carbon monoxide sensing cell sensor.
- 17 biobricks have submitted to iGEM library.
- Two binding characterization methods, EMSA and ITC, were optimized for affinity screening of mutated promoters.
- Three culturing and CO feeding setups for testing and performance evaluation of our gas sensing cell sensor were optimized.
- We showed by two independent experiments (EMSA and Cell sensor) that CooA has a low-level transcriptional activity even in the absence of CO.

**FUTURE WORK**

- Sensitivity and response time studies of the sensor will be conducted.
- Enhanced Dynamic Range (EDR) components will be implemented.
- Thermodynamic characterization of response element-COOA binding via Isothermal Titration Calorimetry (ITC) will be completed.

**FUTURE IMPLICATIONS**

- E-CD Sensor provides a technical framework for future gas sensing systems.
- EDR concept can be used in other contexts as a dose-responsive trigger component.
- We consider E-CD Sensor as the first step towards development of gas sensing and metabolizing cell factories which may take a role in future biohydrogen production and terraforming projects.