Introduction

Over the past decades, mankind has been looking for a new source of energy. It must be green, clean, and 100% renewable. Microbial fuel cells (MFC) can be considered as a plausible solution. However, MFC are insufficient and produce little electricity.

In order to improve the current design of MFC we aimed to introduce the following abilities through genetic modifications:

1. The ability to sense gold in the environment.
2. The production of nanoparticles via short peptide secretion.
3. Regulated electron, efflux through transmembrane protein complex
4. Overall system regulation in response to population viability and density.

Background

Salmonella typhimurium, a bacterium, within its genome contains a transcription activator called GoulS. It is responsible for expression of the gold detoxification system involving the transcription of goulB (gold ion binding protein) from the promoter PgoulB as well as goulI (transcription of itself gene) and goulT (gold ion transportation system) transcription from promoter PggulT (fig. 1A)[1].

In previous studies [2] the deletion of this regulon was shown to have an effect on bacterial mortality. Several attempts have been made to develop and characterise the gold sensing system of S. typhimurium. The activity of PggulTS and PggulB was analysed using fluorescent proteins GFP and GFP.

However, they faced some problems with basal expression levels of goulI, which was causing some inconsistencies within their results. We demonstrated that E. coli DH5α transformed with BBa_K112708 can respond to gold (fig. 2). The relative activity of β-galactosidase increased significantly in the presence of PggulTS and goulI (DH5α + alpha AB) but was reduced to the basal level when goulI was missing (DH5α alpha A).

The results of Knusal-Walliis were significant (X² = 20.75, df = 7, p-value < 0.01). This signifies the function of goulI as a gold-dependent transcriptional activator.

In all experiments, untransformed E. coli DH5α was used as our negative control and constructs were present within pBluescript plasmid.

2. Gold scavenging peptides

Background

Gold nanoparticles (AuNP) differ from solid gold on its own.

We decided to make different fusions with A3, Flg and MIDAS-2 tagging them with pelB signal modifications:

- PggulTS and PggulB fused with goulI (as goulI is not essential for the function of this operon).

Our design

We decided to make different fusions with A3, Flg and MIDAS-2, tagging them with pelB signal sequences to facilitate their expression. Also, due to small size of the peptides and potential degradation we also made fusions with M9F tag (=5.5kDa).

Four parts were submitted to iGEM. We cloned the parts under constitutive promoter and used media supplement for induction of AuNP formation and tried characterising them using Nanoparticle Tracking Analyzer (NanoSight LM10 ®).

Unfortunately, the results did not provide evidence for gold bio-mineralization because changes were subtle and not significant compared to controls.

Alternatives such as A3 or MIDAS-2 gold binding peptides were discovered [4-5]. These are short dodecamer peptides which have shown to coat AuNP and prevent them from aggregation. A3 derived peptides need and external gold reducing agent (HEPES buffer), whereas MIDAS-2 is able to reduce gold on its own.

3. Electricity production

The increasing interest in electricity production by microbes is reflected by the amount of research conducted and publications not only on scientific journals, but also in more public ones. For instance, Shewanella species bacteria are being extensively studied due to their unique cytochromes and ability to pump electrons through membrane, which are generated during respiration [5]. Specifically, cytochromes mtrCAB from its genome were previously used in iGEM and have shown functioning in E. coli.

So we tried cloning the mtrCAB complex into pBBR1CMV plasmid, unfortunately due to recurring nonsense mutations during assembly we did not have enough time to characterise the part. We did, however, submit a biobrick (BBa_K112706) which contains all three mtrCAB open reading frames and rbs for mtrC & mtrB.

4. The hybrid bistable switch

Bistability is commonly found in nature where resting is observed in either of two states (fig. 4). It usually involves a positive feedback loop accompanied by a sensitive regulatory step. These switches have capabilities to retain memories and make decisions, which has extreme biological importance [6].

We aimed to design a switch that is well regulated, tunable and robust. It must be bistable to synchronize current generation by the mtrCAB complex with gold mineralization by the peptides, as well as to manage resistance allocation with respect to fitness of the whole bacterial population (fig. 5). We ended up submitting part BBa_K112706 but without having the time to characterise it.

References

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Microbial fuel cell

These devices are basically batteries engineered to exploit living cells to produce current, from the electrons produced during cellular respiration. Although they have already been documented in 20th century, novel genetic engineering techniques have opened new ways of improving such systems.

However, traditional microbial fuel cells are bulky and inefficient. They are costly and less likely to be used on industrial scale. And so the need for miniaturized microbial fuel cells (mMFC) has grown extensively over the last decades. For example, Shogo Inoue and colleagues have studied due to their unique cytochromes and ability to pump electrons through membrane, which are generated during respiration [5]. Specifically, cytochromes mtrCAB from its genome were previously used in iGEM and have shown functioning in E. coli.

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Figure 1. Gold detoxification genes found in nature (A) and bacterial sensing of and resistance to gold salts. Mol. Microbiol. 66, 2681-2689.

Figure 2. A simple representation of a bistable switch.

Figure 3. Schematic representation of mtrCAB complex.

Figure 4. A simple representation of a bistable switch.

Figure 5. Illustration of a synthetic bistable switch for the expression on taR12. The genetic circuit is an extended version of quorum sensing from Vibrio Fischeri. AIA is induced to disrupt the feedback loop involving LuxR.

Figure 6. Miniaturized microbial fuel cell from team Bielefeld.

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