Magnetasense: a novel gene expression system induced by magnetic fields

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Overview: Magnetotactic bacterium, such as Magnetospirillum magnetum AMB-1 (M. magnetum), form magnetosomes in order to align themselves to the Earth’s magnetic field. However magnetosomes are complex prokaryotic organelles. Current attempts to form magnetosomes in other strains of bacterium have not been successful. However, many of these attempts have been focused on creating a structure that is membrane bound or reconstituting the complete magnetosome. Given the complex nature of the magnetosome, it is very difficult to fit into the structure of an iGEM project. Thus, we focused on a key aspect in the function of the magnetosome: biosynthesis of uniform magnetite crystals. Our project aims to provide a proof of concept for applications of biosynthesizing magnetite in E. coli.

Magnetasense is our proof of concept application for the formation of magnetite in E.coli. Magnetasense is a novel sensory pathway which is able to detect a magnetic field and express a gene accordingly. The induction of gene expression by a magnetic field offers a number of unique opportunities. Given that electrical currents create magnetic fields, an expression system utilizing magnetic field could play a role in interfacing the digital world with the biological. In addition to a proof of concept applications in vivo, uniformly shaped magnetite crystals have a number of nanotechnological applications as well.

A Background
Currently magnetotactic bacterium such as Magnetospirillum magnetum AMB-1 (M. magnetum) form a magnetosome, which can create magnetic nanoparticles. We have identified MmsE, MmsM, MamM, MamO and Mms6 as key genes in M. magnetum, which are involved in the production of the magnetic iron oxide (Fe₃O₄) magnetite. The Mam genes play an important accessory role in magnetosome formation. Mms6, on the other hand, has been demonstrated to directly interact with the magnetite crystal after in vitro synthesis. It is thought to be responsible for dictating the morphology of magnetite crystals (3). Based on this theory, we focused our efforts on expressing this protein in the periplasm. These newly formed crystals would in turn act as a ligand for our Mms6-ToxR receptor. Previous characterization of the Mms6 part by Team Lehtbridge in 2010 indicated that the protein may be toxic to the bacterium. We intend to use a inducible expression system which would allow us to select viable transformants.

B Magnetite Formation
• Once in the periplasm Mms6 will interact with the iron atoms.
• It has been shown that Mms6 helps to form an hydroxide intermediate in synthetic magnetite reactions
• Mms6 is then tightly bound to the newly formed magnetite crystal.
Removal of Mms6 can only be achieved by boiling the crystals in 1% SDS.

Construct A (Ex Vivo Assay) Construct A contains a constitutive promoter and ribosome binding site (BBa_B0034, BBa_B0001, BBa_K243002) allowing the expression of LacI (BBa_J23100). A Fur inducible tagged Mms6 (AT- Mms6) is under the control of a Fur repressible constitutive promoter with RBS (BBa_K243002).

Construct B (In Vivo Expression) • Construct B is similar to construct A.
• A DsbA periplasmic localization signal (BBa_K243002) has been fused to Mms6 (DsbA- Mms6).
• This signal peptide allows for the trafficking of ribosome-bound Mms6 to the periplasmic membrane where it will be co-translated into the periplasmic space.
• Active Mms6 protein in the periplasm is hypothesised to conduct formation of the magnetite crystals upon supplementation of ferrous iron followed by partial oxidations. The newly formed crystals would in turn act as ligand for our Mms6-ToxR receptor.

C Magneticosome
• When a magnet is brought close to the bacteria, the tethered magnetite crystals will aggregate.
• The aggregation of the ToxR cytoplasmic domain will lead to the activation of the cpx promoter and the expression of downstream genes.

D Periplasmic Localization

E Fusion Protein

F Future Directions
This year the Toronto iGEM team faced many challenges, the primary being a shortened competition year and a reorganization of the team’s operating structure. However we feel that our project has a strong theoretical background and will have a strong scientific impact. In the year to come we would like to:
1. Assay the effects of Mms6 in magnetite formation
2. Form proper magnetite-MMS6 complex used in signal propagation
3. Induce gene expression and characterize expression system
The continuation of this project will be through a voluntary process involving members of the UofT iGEM Club.

H Literature Cited
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