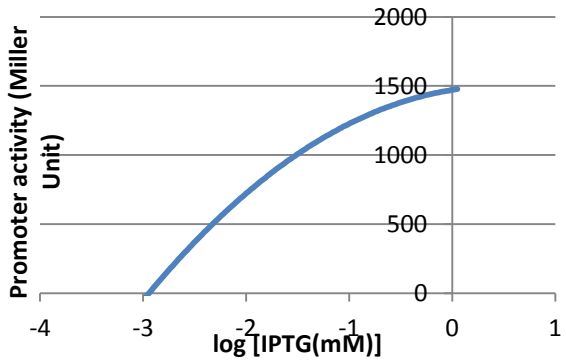
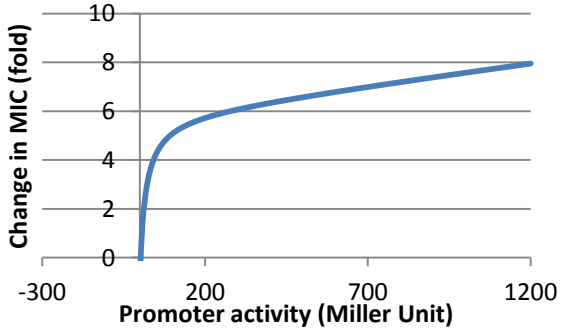
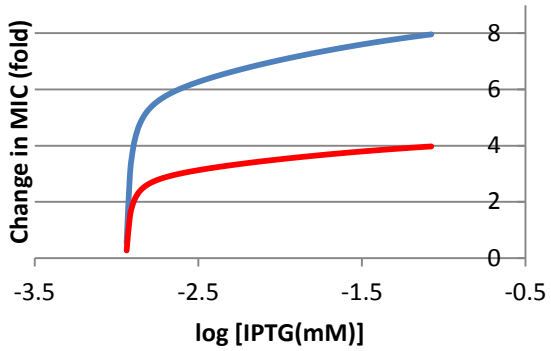
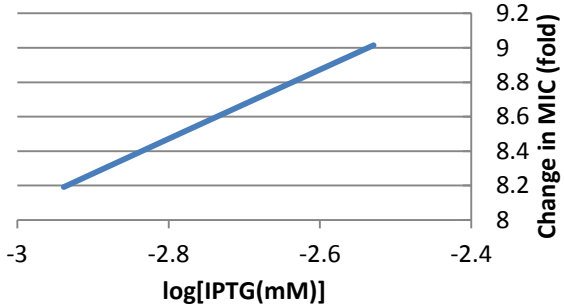
Collaboration

We are glad to cooperate with HKUST on modeling the hypothetical behavior of *bcr* gene if driven by a *lac* or T7 promoter. *bcr* is a membrane protein that actively pumps out a variety of substrates from the cell, notably being antibiotics such as tetracycline and kanamycin. Hence, expression of *bcr* leads to antibiotic resistance. The collaboration was made because of the similarity between halorhodopsin and *bcr* - both are active cell membrane transporters, and our familiarity with such scenario.

Modeling results

1. lac promoter activity vs. IPTG		Curve fitting using one-site total binding model from experimental data [4], [5].
2. Activity of native promoter [1] of *bcr* (acrAp+acrBp) vs. change in MIC (Blue - Tetracycline)		Curve fitting using one-site total binding model from experimental data [3], [6].

3. Change in MIC vs. IPTG (lac promoter construct) (Blue - Tetracycline; Red – Kanamycin)		By substituting the result of the 1st graph to the 2nd graph.
4. Change in MIC (effect of using other promoters)	BBa_K568003 (T7 promoter) 	By promoter activity data from [7] and feed into the 2nd graph.

Assumptions

- Steady-state approximation: bacteria were already transformed with the *plac+bcr* construct and are undergoing or have undergone the exponential phase, before exposing to the antibiotics.
- The inclusion of *plac* does not alter the metabolism in any other ways except expression of *bcr*.
- The addition of IPTG does not affect the bacteria in any other ways except inducing *plac*.

Major insights and highlights from modeling results

- Only a small amount of IPTG is required to induce the *bcr* to the extent where the bacteria shows antibiotic resistance similar to that seen in the wild type. However, further increase In IPTG has a much weaker effect on the strength of antibiotic resistance. This is probably due to the toxicity of antibiotics other than their main mode of action, at higher concentrations.

Reference

[1] RegulonDB
http://regulondb.ccg.unam.mx/operon?term=ECK120014773&format=jsp#myReferences
[2] J.H. Miller in "Experiments in Molecular Genetics" 1972 Cold Spring Harbor Laboratories p.352-355
[3] Increased expression of the multidrug efflux genes acrAB occurs during slow growth of Escherichia coli
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[4] http://www.expressys.com/main_applications.html
[5] http://partsregistry.org/Part:BBa_R0010:Experience
[6] Analysis of a Complete Library of Putative Drug Transporter Genes in Escherichia coli
J Bacteriol. 2001 October; 183(20): 5803–5812.
[7] http://2011.igem.org/Team:TU_Munich/lab/results