FATBUSTER

THE HONG KONG UNIVERSITY OF SCIENCE & TECHNOLOGY

Abstract

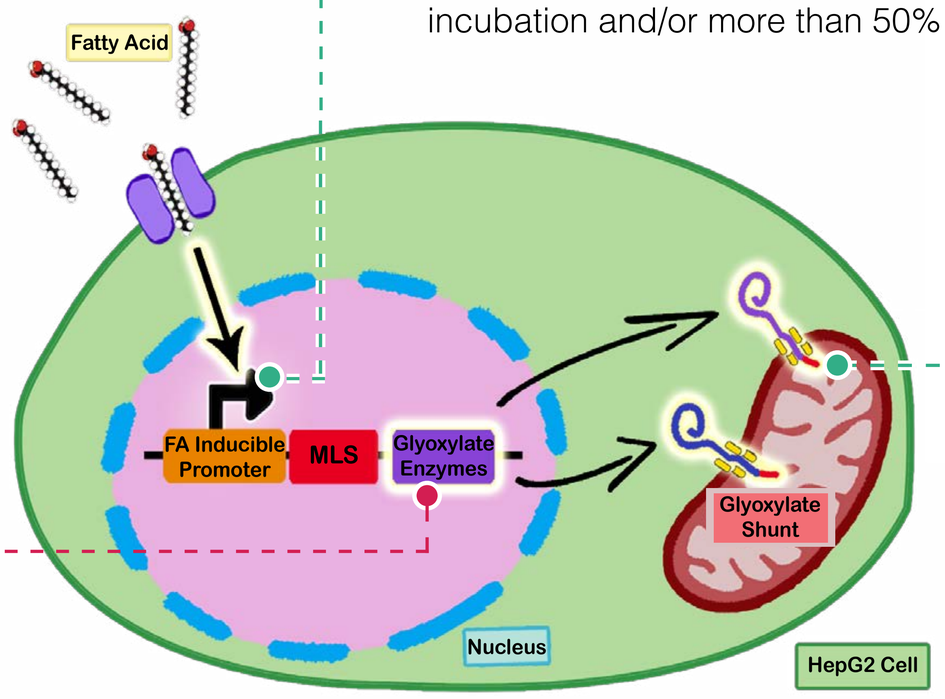
While a low-fat diet and regular exercise are popular approaches to combat obesity, one easy alternative is simply to increase energy metabolism. Employing a synthetic biology approach, we are working to create an artificial futile cycle in mammalian liver cells by introducing glyoxylate enzymes native to bacteria. Past research has shown that mice expressing enzymes facilitating an active glyoxylate shunt are resistant to diet-induced obesity. Our team plans to introduce an inducible system that allows us to couple the sensing of circulating fatty acid concentrations with a circuit of glyoxylate shunt. Our inducible system is intended to prevent the risk of fatty acid deficiency, while facilitating greater fatty acid uptake at higher fatty acid circulating concentrations. Such a system should increase the feasibility of a glyoxylate cycle engineered to function *in vivo.*

1. Fatty Acid Inducible system

In comparison with constitutive glyoxylate shunt, fatty acid induced circuit prevents fatty acid deficiency and triggers greater energy expenditure at high circulating fatty acid levels.

2. Cell Viability

We used MTT assay to test cell viabilities in different fatty acid concentrations. The objective was to determine a range of optimal concentrations of fatty acids to be introduced into HepG2 cell and achieve more than 60% viability after 24 hours incubation and/or more than 50% in 48 hours.



Inducible Glyoxylate Shunt

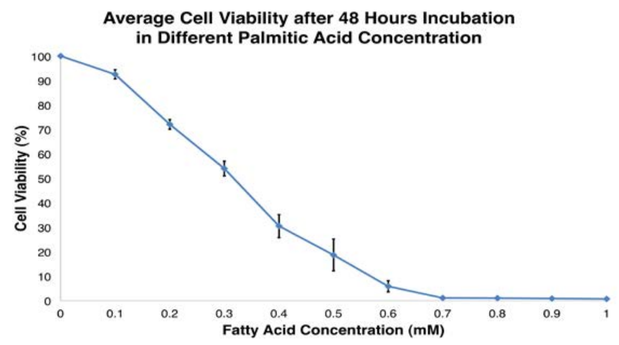


Figure 1 Average Cell Viability in Different Palmitic Acid Concentrations. HepG2 cells were incubated in gradient concentration of sodium palmitate from 0mM to 1.0mM and 2.0mM for 48 hours respectively. Cell viability was calculated using spectrophotometer measurement of formazan formation.

3. Glyoxylate Shunt

Research by Prof. James Liao's group at UCLA has shown that glyoxylate enzymes introduced into mammalian cells can generate an artificial futile cycle. This causes mice expressing these genes to be resistant to diet-induced obesity.

The expression of glyoxylate enzymes are driven by constitutive mammalian promoters, namely CMV and EF-1alpha promoters. We have characterized the CMV promoter.

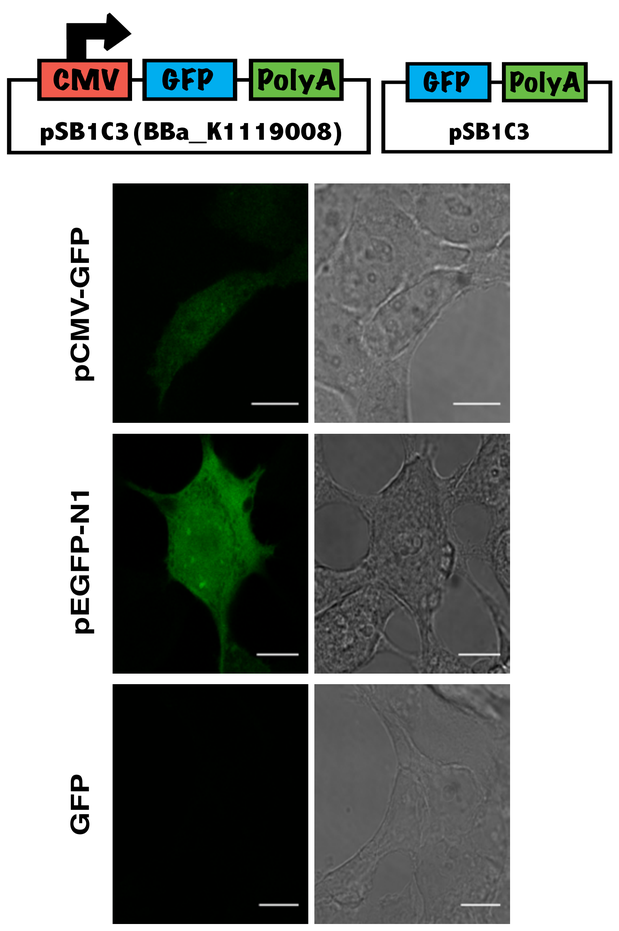
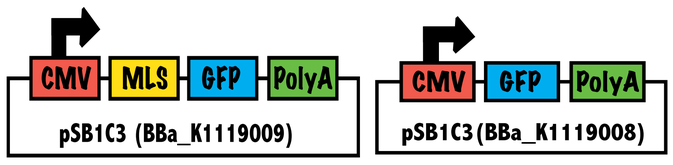


Figure 2. CMV promoter drives expression of GFP. HEK cells transfected with pCMV-GFP gave GFP signals. HEK cells transfected with the commercial pEGFP-N1 showed similar results, while the same construct without any promoter did not give any GFP signals.
Scale bar = 10 microns

4. Protein Trafficking

To guide the glyoxylate enzymes into the mitochondria, we attached Mitochondrial Leader Sequence (MLS) to their N-termini. We have constructed the MLS in its own into standard BioBricks (BBa_K1119000 & BBa_K1119001), and we quantitatively characterized their behavior using GFP reporter.



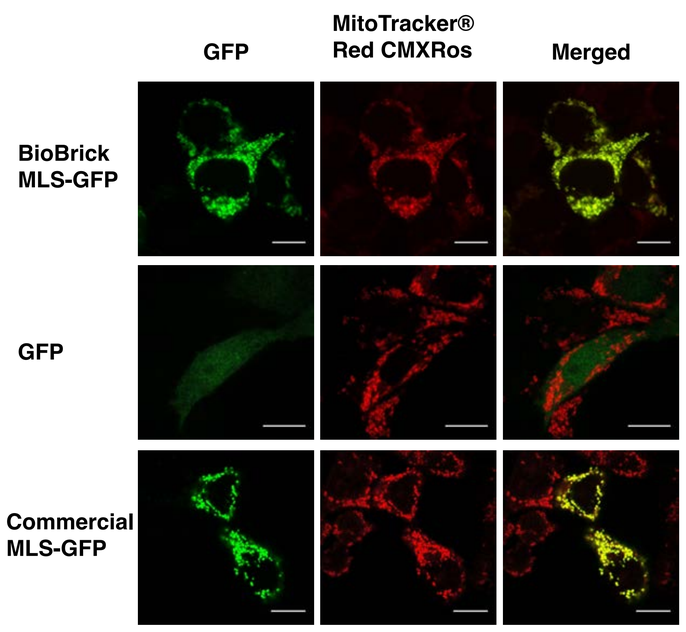


Figure 3. MLS (BBa_K1119000 and BBa_K1119001) directs GFP into mitochondria. When MLS is added to the N terminus of GFP, the GFP was directed to the mitochondria in the cells, giving patches of GFP signal that overlapped with the signals from MitoTracker®. When MLS is not added to the GFP, the GFP signal can be seen scattered all around in the cell. Scale bar = 10 microns

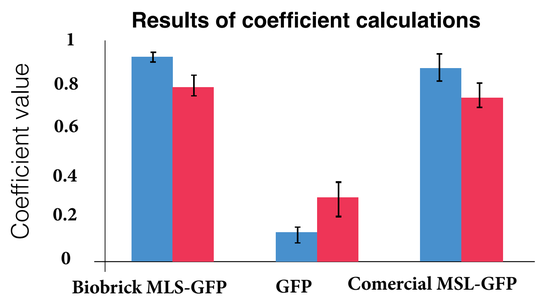


Figure 4. Mean Pearson correlation coefficient (rp) and mean Spearman correlation coefficient (rs) were shown in bar chart. Using ImageJ software and plugins, the Pearson correlation coefficient and Spearman correlation coefficient were generated. For every batch of transfected cells, four samples were used for quantification. Experimental BioBrick MLS-GFP and commercial MLS-GFP: Coefficients were close to 1, good colocalization; GFP: Coefficients were close to 0, poor colocalization. Error bars show standard deviation.

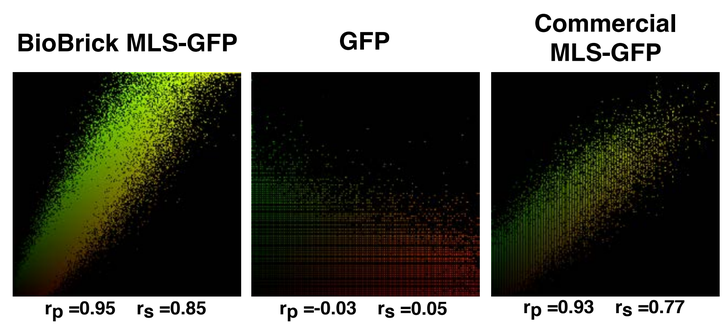


Figure 5. Scatter plots of fluorescence intensities of green (y-axis) and red (x-axis) from images shown in Figure 3. It showed that the BioBrick MLS-GFP and commercial GFP construct had linear relationship of green intensities and red intensities while the GFP generator had no relationship.

5. Human Practice

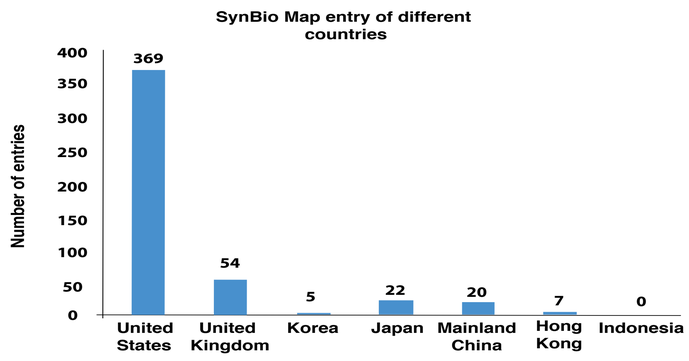
Country Profile of 5 East Asian Regions

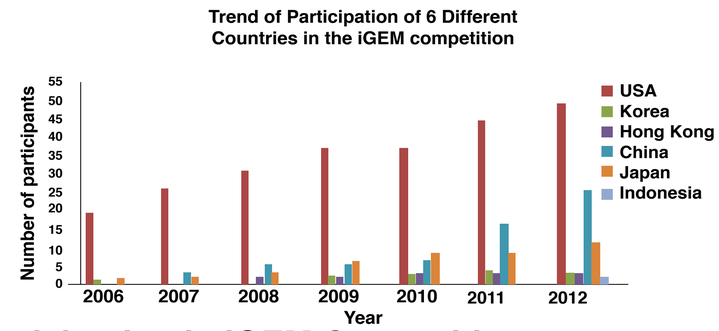
Our country profile provides information illustrating and comparing the development of synthetic biology in 5 Asian regions: Hong Kong, Korea, Indonesia, China and Japan. The following charts show two of the indicators we compiled that represent the development of synthetic biology.

Note: the SynBio map is a product of the Woodrow Wilson International Center for Scholars that shows the geographical distribution of institutions active in synthetic biology work.

SynBio Map Entries

We compared SynBio map entries in the 5 Asian regions of our study with those in the USA and UK, countries where synthetic biology is more established.





Participation in iGEM Competition

The number of participants in the iGEM competition has been continuously increasing every year. This trend holds true for some of the 5 Asian countries that we have investigated.

Acknowledgements

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