Oscillating BP reliever
Hust-iGEM Team, 2013

Abstract
Hypertension has become the leading risk factor for mortality worldwide. Human’s blood pressure (BP) has a basic daily rhythm with two peaks, 6:00 to 10:00 in the morning and 16:00 to 18:00 in the afternoon. The morning peak is also called “death time”, for it’s unlikely to take drugs before waking. Short chain fatty acid (SCFA), was recently shown to produce an acute hypotensive response. A GPCR called sfrp1 is expressed in smooth muscle cells of small blood vessel plays an important role. It could be activated by propionate and induce vasodilation and hypotension. 2013 HUST-China IGEM use a synthetic way to combine bio-oscillator with propionate-producing enzyme, trying to build a gut probiotic, which can release propionate periodically in accord with the rhythm of human BP. This could be a great substitute for chemical drugs by saving patients from drug dependence and the risk of sudden death at morning BP peak time.

Propionate Synthesis
A four-gene operon in E.coli K12 genome which includes sbmC,yfgC,yfgD and yfgE is significant in the metabolic pathway that converts succinate to propionate through Wood-Werkman reaction. We constructed an effective expression plasmid for SCFA to increase the expression of the four genes copies independently, hence the enzymes relating to propionate synthesis raising in the cytoplasm. We evaluated the output of each kind engineering E.coli by high performance liquid

Oscillator
The key part of the oscillator is araBAD lacZYA hybrid promoter. It is activated by the AraC protein in the presence of arabinose and repressed by the LacI protein in the absence of IPTG, constructing two feedback loops with opposite effects. And the differential activity of the two feedback loops can drive oscillatory behavior.

Future Work
There is a big challenge in our project that the recombination microbe has to be validated in the human intestine. Based on the pre-exisitng work, a plenty of works are coming to us in the near future.
1. Regulating the period of propionate utilizing the frequency divider with a ssaA tag analog attached to the end of enzyme.
2. Replace the report gene rfp encoding red fluorescence protein with key gene encoding enzymes in the synthetic pathway.
3. As we know that E.coli is the most popular chassis used in the synthetic biology. But according to the people we sent questionnaire to, they prefer eating food containing probiotics rather than eating bacteria. So we are going to transform the regulatory net into bifidobacterium, which enjoy a highly reputation among the dairy industry. We will measure the propionate outside of the human body.
4. Using mathematical modeling to imitate the environment in the intestine and the concentration decreasing of propionate in the blood circulation.

Human Practice
We have done a remarkable job to introduce biology and iGEM jamborees to high school students synthetic and motivated them towards future participation in the iGEM. We were not only work as a team but also collaborated with other two iGEM teams by sharing plasmids and characterizing their parts. Furthermore, we made a public speech in our school about what we achieved, sharing the feelings in the iGEM competition. To add more fun to this competition, we designed a simple crosswords to acquire you more knowledge on iGEM competition.

Results
1. Biobricks (we submit / Registry )

<table>
<thead>
<tr>
<th>Standard biobricks</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSB1C1-yfgE::OmpR (PstI)</td>
<td>According to Thomas Haller, the enzyme encoded by the second gene, yfgE, contains a common binding properties for XAS. They thought it might be a succinyl (or propionate-CoA ligase, or a novel (beta-ketone-independent) propionyl-CoA carboxylase).</td>
</tr>
<tr>
<td>pSB1C1-yfgC::OmpR (PstI)</td>
<td>This gene encodes propionate-CoA succinyl-CoA Transference that catalyzes a CoA transferase reaction from propionyl-CoA to succinyl generating propionate.</td>
</tr>
<tr>
<td>pSB1C1-yfgD::OmpR (PstI)</td>
<td>The third gene in the operon encoding methylmalonyl-CoA decarboxylase that catalyzes the decarboxylation of methylmalonyl-CoA to propionyl-CoA.</td>
</tr>
<tr>
<td>pSB1C1-sbmC::OmpR (PstI)</td>
<td>Sbm encodes methylmalonyl-CoA epimerase which catalyzes the reversible reaction of succinyl-CoA and methylmalonyl-CoA.</td>
</tr>
</tbody>
</table>

Team
HUST iGEM team comes from Huazhong University of Science and Technology, in which “Huazhong” means the location is in the central China as is displayed in the e-map. We regard our team as our little baby who is three years old now and will hopefully grow into a brilliant aspiring youth. This year our team is composed by eighteen undergraduates from the biological sciences, biomedical engineering, mathematics departments, as well as two faculty advisers and two project instructors in life school.