- ENABLE + Researchers

As an emerging field of discipline, Synthetic Biology actively interacts and collaborates with the classic fields of biology, expanding its development in this process. As active iGEMers, we have connected with professors from Clinical Medicine, Immunology, Histology and Embryology, and Neuroscience. Through this process, the enlightenment we gained can be classified into two aspects. On the one hand, they directed our attention to certain key aspects on our logic gate design. Questions like, 'can our design be applied universal?', 'Is our design the simplest?', 'Are all the parts necessary?', 'Compared to all the previous work on logic gates, what proves to be our innovation and significance point?', has been raised. Our considerations on these questions are concluded in our '*project introduction*'. In addition, to prove the universality our design, we also did many thought experiments, all of which are concluded in the supplementary part of '*introduction*'.

On the other hand, our connection with the professors inspired us to more fields of potential application for our ENABLE project. Considering the major fields of the clinical doctors we interviewed, our team brainstormed together during team meetings and put forward many suggestions. For more information on this part, please visit our project's "*future application*", opinions from the interviewed doctors were underlined.

As our project applies to foundational advance track, though it has more possibilities for practical application, we also face more difficulties in turning it from research into application compared to other track's projects. This calls for us to broaden our view, adopt a more open mindset, and make in-depth interactions with currently well-developed fields.

In this year's project, we not only established conversations between our team and researchers, but also took up some new methods, such as joint group meetings and joint project designing. We managed to form a deepened cooperation instead of the usual way of conversing with researchers in many fields, by means of developing possible applications of our project in joint efforts.

Through these of exchanges, we are delighted to find that our project holds huge application potential, and to find some of the limitations within our original design. In this way we also gained feedback on 'how to make our project more comprehensible'. All of the feedback further led the way of our project optimization.

- ENABLE + Cancer Therapy

  As a professional clinical thoracic surgeon, how do you think of the current clinical treatment of lung cancer?

Immunotherapy has already been used in treating lung cancer in clinics nowadays, and many clinicians are very concerned about its development. However, most people's vision is limited in the classic immuno-methods[经典的免疫治疗方式], which makes their ideas lack innovation. At the same time, although clinicians are looking forward to more efficient ways of immunotherapy, they lack the lab experience and background knowledge to change its current status, let alone making it better. Take PD-L1 as an example, less than 25% of patients has an actual (detectable?) high expression level of PD-L1 in clinical practice, which means the currently-used check point antibodies are unavailable to use on most patients. Fundamental research has gone very far, but we're still facing the biggest problem of transferring them to be usable in clinical practice, which makes translational medicine very crucial for the future.

Your view on our project:

Your project is very original. It's covering aspects which are very difficult for a clinical doctor to think about, but the big point is that you need to combine your work with that of the classic research, and try to transform it to application level. The core competency of your project is the ability to achieve more precise and orthogonal control of cells. Your originality lies in the logic circuits, which can identify membrane antigens and enable transmembrane signal transduction. Comparing to the traditional methods of treating cancer, your biggest advantage is that you can control the entire treatment process,

including the reaction intensity and time duration of treatment. In this way, the best therapeutic effect can be achieved while avoiding many side effects.

What are the prospects of application of our project in cancer research?

(There are mainly two prospects.) On the one hand, cancer is a disease which is always changing dynamically, and the surface antigens' expression level during different stages of cancer development varies. Current methods of antigen detection can only detect its level at a certain time point, which cannot reflect the dynamic changes. While your method of co-culturing cancer cells with cells carrying the assigned logic circuits can transform the cancer cells' antigen level into the signaling output of the 'tool cells' (cells with logic), so that the dynamics of cancer cell surface antigen can be reported in real time. On the other hand, tool cells can show the specific signal exchange condition[信息交流情况] between living cells, and can serve as a convenient and reliable tool for the study of cancer surface antigens.

Views on the use of our project on clinical cancer treatment

The research on extracellular antigens and the selection of output signals is already quite matured. Your project has also given these researches a good way of transferring their findings into application. If you want to use membrane antigens of cancer, for example, many classic antigens have already been found. Glycoprotein-type cancer marker, etc., is a good direction.

On the other hand, there are many clinical diseases which are caused by the body's loss of control of a once normal system. Since your system can enable precise control over a certain cell behavior, can we broaden our thinking and expand your system into the treatment of these diseases? For instance, SIRS-MODS, or other diseases caused by autoimmune. Right now, many intracellular signaling pathways have been well characterized, can your system treat diseases by exerting an additional control over these signaling pathways?

- ENABLE + Neurology [专业词汇对着谷歌瞎逼逼的，拜托校对更正]

Current status of neural tissue regeneration and repair

1. Repairing spinal cord using stem cell is a relatively mature method. But due to the existence of the blood-brain barrier and the difficulty of early diagnosis, treatment of cerebral injury, such as stem cells, is generally performed by stereotactic injection after injury. This method largely limits the stem cell repairment of deep brain tissue.
2. At present, the repair of nerve injury is mainly applied to Alzheimer's disease and spinal cord disconnection in clinical practice, while research on repairment of injury after stroke does not have much progress.
3. The major problems stem cell therapy has are tumorigenic ability, hard to trace the injected cells, cannot be used for disease prevention or ~~even~~ early recovery, and poor tissue localization.

4. The application of stem cells in brain tissue is not optimistic at present. Not only because the brain lacks the microenvironment required for functional neuronal differentiation, but also because it is difficult to solve the blood supply problem even after tissue repair, resulting in the failure of proper neuron growth even after cell differentiation.
5. When applied to clinical level, doctors hope that stem cells not only possess the ability of repairment which is already in the later stage, but also help to avoid initial damage. Because the neurons die in 6 hours while the operation usually takes place 16 hours after stroke.

What advantages would our project have when applied to cerebral injury repairment

1. If the stem cell can perform binary logic operation through transmembrane signaling, then it can be injected into the body before any damage takes place, and recognize the signal of cerebral injury without delay. The signal then serves to activate the downstream differentiation signals. Depending on the signal, our engineered cell can also identify specific tissue, thus enabling specificity in its performance.
2. Stem cells equipped with different logic circuits works in synergy, to achieve more complex repairing mechanisms. For example, the first-activated stem cells will create a microenvironment suitable for the neuronal differentiation, while another group of cells attaches

specifically near blood vessels and differentiates into glial cells to perform supporting and early hemostasis.

3. The output signal can also serve to trace the location of cells. In this way, we can directly demonstrate the effect and mechanism of stem cells to proof its function, no matter applied to treatment or scientific research.

How can our project be applied specifically in neurology research and clinical treatment?

1. AND Gate is recommended to use. The two input signals can be separately used to recognize brain nerve markers for localization and traumatic signals, thus achieving time and tissue specificity.
2. The output signal is suggested to express neurotrophic factor preferentially at early stages of activation, in order to create a microenvironment suitable for the differentiation and growth of neurons. Also, the early activation of SOX9 and Nfib is suggested, they can be used to induce astrocyte differentiation, which in turn leads to blood vessel repairment and serves in the role of matrix to support differentiation for later stages.
3. Use subarachnoid injection directly into brain tissue, to avoid crossing the blood-brain barrier.
4. Let the stem cells output fluorescent markers to help trace their position.
5. Do not limit your visage to after stroke tissue repairment, stem cells may have a better prospect of usage in diseases like Alzheimer's disease.

- ENABLE + Immunology

As a researcher in immunology, what do you think of our project?

Your project is similar to CAR-T to some extent. But comparing with the traditional CAR-T, designing logic circuits within cell allows the cell to recognize different patterns of cancer antigen, which enables more flexibility into your project as well as the ability to perform more complex tasks. One potential benefit of applying SynNotch is that it may be more suitable to target solid tumors compared to all the existing immunotherapy methods.

Do you have any suggestions for the application of our project in Immunology?

1. Use magnetic beads attached with tumor antigens to simulate antigen presentation in human. Or through in vitro MHC and antigenic epitope assembly, together with magnetic beads presentation, to simulate in vivo antigen presentation in a simple way.
2. Because SynNotch needs cell-cell contact for activation, it may be possible to let the output signal activate antibody-dependent cell-mediated cytotoxicity (ADCC), or express PD1 antibody through internal secretion to avoid the side effects caused by PD1 antibody-inhibited immune response.
3. Consider applying microenvironment control. For example, we can secrete cytokines to maintain T cell function when IL2 is low, to activate T

cell when IL2 is in middle level, and to activate the toxic effect of tumor cell death when IL2 level is high to enable more precise delivery of toxicities.

4. After recognizing an antigen with SynNotch, the downstream promoter expresses a T-cell receptor (TCR) and a restricted MHC molecule, thus solving the MHC restriction problem.

Do you have any improvements or suggestions to make towards our project?

1. Test whether SynNotch can achieve a strong anchor between cells. Consider how to anchor cells to the tumor to ensure more effective killing.
2. Using only one type of immune cells has only limited effect. Since 16 Boolean logic gates are completed, you can dwell upon the possibility of simultaneously injecting several immune cells into the body to cooperate with each other in tumor killing.

- ENABLE + Tissue Generation

What do you think of our project?

Tissue regeneration has great potential in both medical and scientific research. However, directed differentiation of tissue cells still needs induction on gene level, where irrelevant gene interference exists to some extent. Moreover, cell-cell communication cannot be established through current

methods, thus we're now unable to simulate the tissue differentiation in actual internal environment. Your project can enable the transmembrane communication of cell-to-cell signals, meaning that the information exchange between cells under natural condition can be realized through your project. And you only need to modify the stem cell to your needs to achieve it. At the same time, orthogonal signal transduction in your logic circuit can avoid the interference of some irrelevant gene during stem cell induction process.

If our project is to be used as a research tool in the field of tissue differentiation, where do you propose for us to begin?

The transmembrane signal of your system can be used as a tool to reflect on cell-cell interaction. The specific application may lie in stem cell research, where it can be used to reflect on the interaction of transmembrane signals among cells during stem cell differentiation.

Or it can be used as a tool to reflect the degree of differentiation. When a certain type of cell is differentiated, our 'engineered cell' could directly detect this cell by transforming the target antigen of this certain cell type into detectable output signals to show the differentiation status of the appointed tissue.

How can our project be better applied to in tissue regeneration?

First of all, your project application should be integrated with some fundamental research results in the field of tissue regeneration. Take tissue repair after skin burn as an example, Smad3 is a transcription factor which

localizes in the cytoplasm and enters the nucleus to perform functions. After skin burn, Smad3 enters the nucleus and initiates the expression of endogenous repair signals. You can use Smad3 as an ultimate output, inducing the 'tool cells' to start differentiating, which then leads to skin repair after burns. As an output signal, Smad3 has a unique advantage--it increases at the early stage of repairment and decreases when repair is nearing completion. This means that when its level is low, it serves as a termination signal instead. When Smad3 is missing, the biological behavior of the epidermis is enhanced, while enhancing the signal increases the fibroblast's behavior. In line with the knowledge that during the process of skin repair, the behavior between dermis and epidermis is mutually antagonistic. Your project can enable the full binary logic, so theoretically it is able to precisely complete the different stages of skin repair as well as repairing different layers of skin at the same stage, while outputting different intensity levels of Smad3 signals to better match the actual situation.

As for the signal to initiate repair, inflammatory signals such as some inflammatory mediators may be a good choice. TLR4 is a suitable candidate of the membrane ligand. TLR4 is a bacterial endotoxin that activates the TLR4 receptor, which in turn activates inflammatory mediators through its endogenous pathway. Inflammatory mediators may include some activated monocytes and immune cells (e.g. NK cells), which tend to infiltrate the area around the burned skin.

Moreover, your project has many potentials, including but not limited to post-burn repair. For example, when the burned area is too large and Smad3 signal gets out of control, the dermis will break through the epidermis and form scar tissue. In cases such as this, your logic circuits can serve to re-control the out-of-control signal. And this set of thinking is not limited to tissue regeneration as well. Many clinical diseases involve the out-of-control signal(s). The artificial manipulation of cell logic in your project means that you can broaden your horizon to different fields. And the project can be used in ~~more~~ any normal signals turning out-of-control situation to help regain control, such as SIRS whose clinical mortality is very high.

What needs to be improved in our project?

1. Although in theory, SynNotch can accomplish skin tissue repair by detecting skin burns and subsequently express Smad3; working in reality, it's likely that your system is not sensitive enough, that the level of activation signal is not enough to activate the repair signal. This calls for your team to considerate an amplifier in actual application.
2. Since you're dealing with research and application in tissue regeneration, your system must be capable of terminating the response to the repair signal at any time, which is the so-called braking mechanism.
3. If you want to target your project to a broader market, you should first try to make your project easier to understand. Try to present your project in a more attractive way.