HydroMagic

Novel Biological Approaches to solar-to-Chemical Conversion

Team Name: Nanjing-China 2016

Team Members:

Chen Chen, Miao Liu, Kuisong Song, Zhen Li, Jingwen Lin, Yang Sun, Shuangshuang Du, Bao Wang, Wenyin Su, Churong Xu, Wei Jin, Zijie Zhao, Rendong Yu

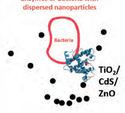
*All experiments were designed and performed by the iGEM team Nanjing-China 2016.

Solor-Driven Whole-Cell In Air !

Background

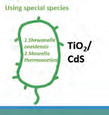
Method 1

Enzymes or Bacteria with dispersed nanoparticles



Method 2

Using spectal species



Solar energy is the most abundant form of energy but extremely hard to use. Scientists have been trying different methods to convert solar energy to chemical energy, all of which need to solve two problems: media for electron transduction and enzymes to capture the excited electrons. Current methods for electron transduction include photosynthesis process and semiconductor nanoparticles. But they all bear certain disadvantages. As for the enzymes, most of them are oxygen-intolerant, which makes it difficult to use them in air.

This year, our HydroMagic project successfully solved these two problems with our *in situ* fixed CdS nanoparticles on the surface of *E.coli*, and silicon encapsulation system. We have successfully proved the applicability of our systems through hydrogen production and propose that our systems can be universally applied in model organisms such as *B. subtilis* and yeast!

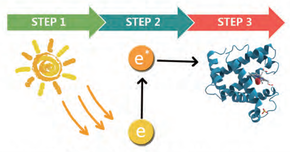


Figure 1. Basic steps to use solar energy

Design

To address the problem of electron transmitter and enzyme oxygen sensitivity, our design is devided into **two** parts: the construction of artificial PS system and the construction of in air hydrogen factory.

Induced precipitation of CdS nanoparticles on *E.coli* cell

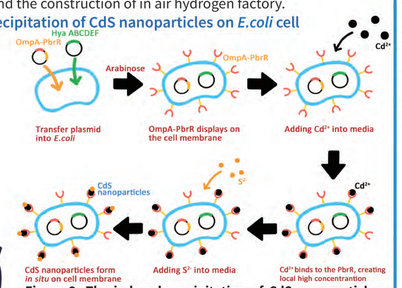


Figure 2. The induced precipitation of CdS nanoparticles using fused protein OmpA-PbrR

In this year's design our artificial PS system has no need for either chemically synthesized semiconductor nanoparticles or special organisms. The system is constructed on the outer cell membrane of *E.coli* using fused protein OmpA-PbrR.

PbrR based artificial PS system

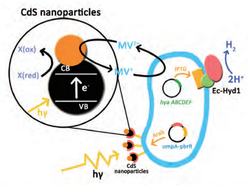


Figure 3. Light driven reaction of PbrR based artificial PS system

To address the problem of electron transmitter, CdS nanoparticles acts as semiconductors imitating photosynthetic system under illumination.

It provided excited electrons to a redox mediator methyl viologen (MV) which then penetrates into *E.coli* cells and transfer the electron to hydrogenase Ec-Hyd1, produced from induced expression under IPTG. Hydrogenase then produce one molecule of H2 out of 2 protons and 2 electrons. The semiconductor regains its lost electron from sacrificial electron donors.

Construction of in air hydrogen factory

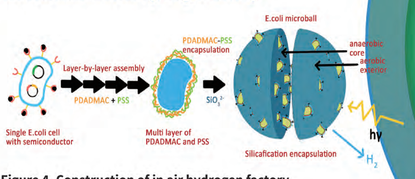


Figure 4. Construction of in air hydrogen factory

To overcome the oxygen sensitivity of hydrogenase, we use silicon encapsulation. Single *E.coli* cells with semiconductors are wrapped into multi layers of PDADMAC and PSS. Cells are then closed with silicification coats into microballs where the exterior consumes oxygen and creates an anaerobic core.

Test method: Hydrogen production

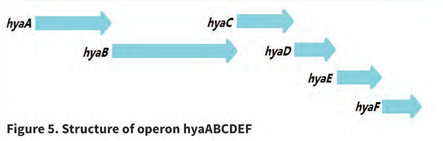
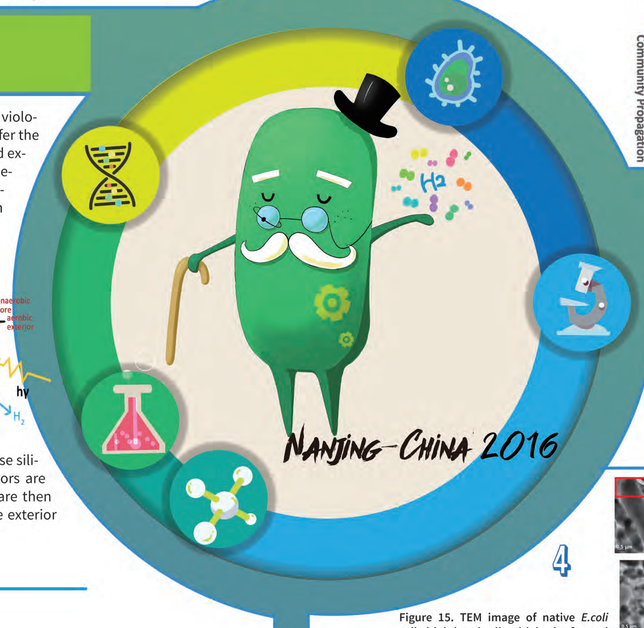


Figure 5. Structure of operon hyaABCDEF

We chose the hydrogenase I in *E.coli*, which is a [NiFe] core hydrogenase, to be our candidate since it is more stable and can easy to handle. EcHyd-1 in encoded by a six-gene operon hyaABCDEF on the genome of *E.coli*. The first two genes hyaA and hyaB encode the small and large subunit for the enzyme and hyaCDEF is of important function in the enzyme's maturation. All six genes will also be our Parts this year.



Modeling

Model 1: Simulation of hydrogen production system

In the first model, we have constructed a hydrogen production system through the diffusion equation of gas. In this system, we obtained the steady state solution of hydrogen-oxygen, and obtained the hydrogen-oxygen concentration, which is consistent with the experimental data.

● Single-bacteria model

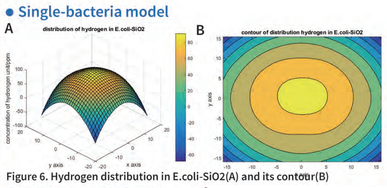


Figure 6. Hydrogen distribution in E.coli-SiO2(A) and its contour(B)

The concentration of hydrogen exponentially decreases by distance. It is easily known that microballs are spherically symmetric.

● Multi-bacteria model

$$\begin{cases} 0 = -D_H(r)\nabla^2 c_H(r) + g(r)q_H(c_O) \\ 0 = -D_O(r)\nabla^2 c_O(r) + g(r)q_O(c_O) \\ c_H(r_0) = 0 \\ c_O(r_0) = c_{O,total} \\ c_H'(r_0) = 0 \\ c_O'(r_0) = 0 \end{cases}$$

$$q_H(c_O) = \begin{cases} 1, c_O < c_{O,critical} \\ 0, otherwise \end{cases}$$

$$q_O(c_O) = -(1 - e^{-c_O})$$

$$g(r) = \begin{cases} \sin kr, \sin kr > 0 \\ 0, otherwise \end{cases}$$

$$D_{O,B}(r) = g(r) = \begin{cases} \sin(kr+\pi), \sin(kr+\pi) > D_{O,B} \\ D_{O,B}, otherwise \end{cases}$$

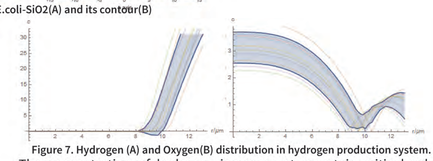


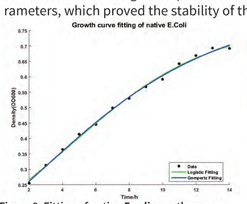
Figure 7. Hydrogen (A) and Oxygen(B) distribution in hydrogen production system.

The concentration of hydrogen increases at a certain critical value. Oxygen decease before the critical value and them increases slightly, which is basically consistent with the experimental results of sensor micro.

When we randomly change the ambient oxygen concentration, we can find that the trend of hydrogen concentration is stable. Therefore, the

Model 2: Growth Curve Fitting

In the second model, we used the Logistics equation and the Gompertz equation to fit the data of the growth phase of *E. coli*, and compared the correlation parameters, which proved the stability of the encapsulation.



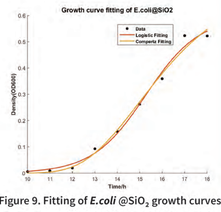
Fitted by Logistic equation:

$$D_L(t) = \frac{0.7728}{1 + e^{1.123 - 0.2422x}}$$

Fitted by Gompertz equation:

$$D_G(t) = 0.8334e^{-e^{-0.4699 - 0.1605x}}$$

Figure 8. Fitting of native *E.coli* growth curves



Fitted by Logistic equation:

$$D_L(t) = \frac{0.5942}{1 + e^{12.91 - 0.8453x}}$$

Fitted by Gompertz equation:

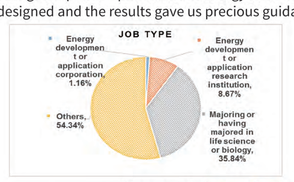
$$D_G(t) = 0.7271e^{-e^{-6.199 - 0.4142x}}$$

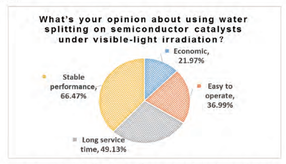
Figure 9. Fitting of *E.coli* @SiO_2 growth curves

The K value for encapsulated bacteria shrinks from 0.7728 in native E.coli to 0.5942. Only after 10h of culture do encapsulated group display exponential growth. This confirms the stability of our silicon shell which suppresses the multiply of bacteria.

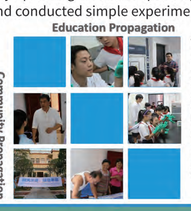
Human Practice

We investigated public opinion about energy issues by **questionnaires**. Questions closely related to our project have been designed and the results gave us precious guidance.





We promoted **education** by openning our labs to primary school students on June 1st. Eight children visited our lab and conducted simple experiments under our supervisions.

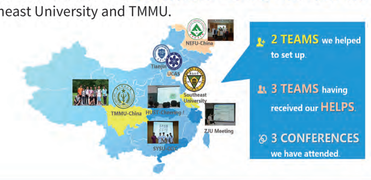


We conducted **counselling** with experts in hydrogen production who are experienced in developing hydrogen-based transportations for technical details, like Prof.Christopher Chang from UC-Berkeley and Mr.Zou (Zhigang Zou), a famous Academician of Chinese Academy of Sciences.

We are invited to CCiC, HUST-Cheering and ZJU **meeting**, which are three conferences held in China, which indeed helped us familiarize with our project as well as deepen the friendship between different teams in China.

Besides, we **helped** Team Tianjin, Team NEFU-China, Team UCAS with experimental guidance or materials.

We also made contributions to **the establishment of new iGEM teams**, which are from Southeast University and TMMU.



Results

1 Parts

We successfully constructed **SEVEN** parts this year and all tested their characterizations and proved their functions.

Gene	Register ID
hyaA	*BBa_K1958001*
hyaB	*BBa_K1958002*
hyaC	*BBa_K1958003*
hyaD	*BBa_K1958004*
hyaE	*BBa_K1958000*
hyaF	*BBa_K1958006*
pbrR	*BBa_K1958007*

Gene	Register ID
ompA	*BBa_J36836*

We improved the function of a previously existing part called OmpA and extended its application into the cell surface display system in *E.coli*.

2 Hydrogenase

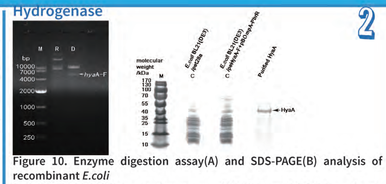


Figure 10. Enzyme digestion assay(A) and SDS-PAGE(B) analysis of recombinant *E.coli*

This indicates that we have amplified hyaABCDEF and expressed it successfully.

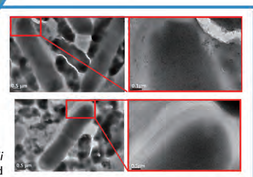


Figure 15. TEM image of native *E.coli* cells (right) and cells with in situ formed CdS nanoparticles (left)

The comparison of TEM image of native *E.coli* cells and cells with in situ formed CdS nanoparticles revealed success in CdS nanoparticles formation through PbrR.

PbrR-based artificial PS system

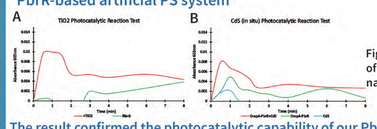


Figure 13. Photocatalytic reduction of MV by TiO2 and induced CdS nanoparticles

The result confirmed the photocatalytic capability of our PbrR-based precipitation of CdS nanoparticles.

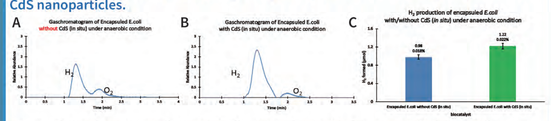


Figure 14. Gas chromatography test on hydrogen evolution of hydrogenase with CdS particles

This proved that the CdS system is compatible with hydrogenase as well.

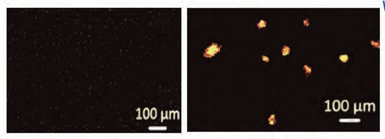


Figure 18. Fluorescent microscopic image of (A) native and (B) encapsulated *E.coli* cells

Whole cell-based hydrogen production

We determined the structure of encapsulated bacteria and found that encapsulated bacteria formed microballs, though with a slightly irregular shape.

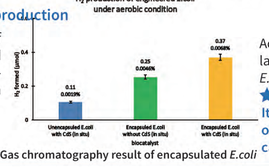


Figure 19. Gas chromatography result of encapsulated *E.coli*

According to the result, we found either unencapsulated *E.coli* or encapsulated E.coli without CdS produced less hydrogen than the encapsulated *E.coli* with CdS. This proved our success in "in air production" of hydrogen.

★ Biosafety

It is noteworthy that all our experiments are operated under supervisions of senior researchers and all our devices are standerdized. Besides, our cadmium sewage is strictly disposed according to national regulations.

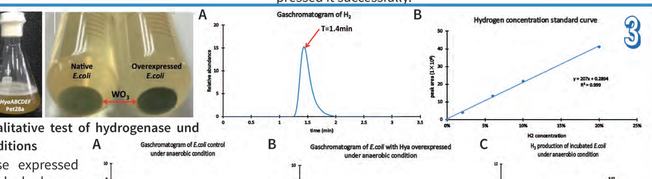


Figure 11. Qualitative test of hydrogenase und anaerobic conditions

The hydrogenase expressed above exhibited hydrogen production possibility, as is shown in the qualitative test. The gas produced has the ability to reduce WO3, which means that it might be hydrogen.

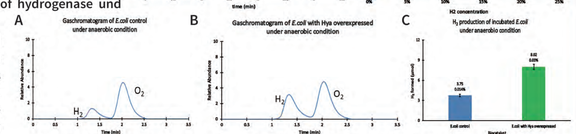


Figure 12. Gas chromatography quantitative test of hydrogenase under anaerobic condition

The gas chromatography test confirmed the qualitative test that the gas produced by hydrogenase proved to be hydrogen.

Silicon encapsulation

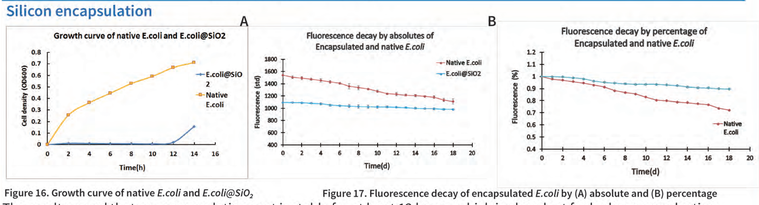


Figure 16. Growth curve of native *E.coli* and *E.coli@SiO*$_2$

Figure 17. Fluorescence decay of encapsulated *E.coli* by (A) absolute and (B) percentage

The result proved that our encapsulation coat is stable for at least 12 hours, which is abundant for hydrogen production.

This result demonstrated the viability of our encapsulated bacteria.

Expansion

Now that our two systems (the artificial photosynthetic system and encapsulation system) proved to be successful, unlike photosynthetic system constructed in special species, we can also expand the applications of our artificial photosynthetic system into other model organisms such as *B. subtilis* and yeast simply by replacing OmpA with TasA/CotC in *B. subtilis* and GCW21 in yeast, as all these proteins are cell surface display proteins. Likewise the downstream enzyme is not restricted to hydrogenase; the principle applies to other enzymes that produce different products.

Besides, we can also expand our encapsulation system into other oxygen-intolerant enzymes to make them work under aerobic conditions.

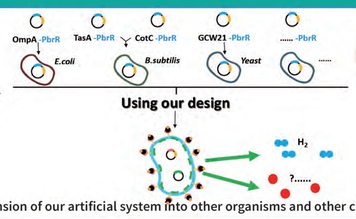


Figure 20. Expansion of our artificial system into other organisms and other circuits

Acknowledgements

Instructor&Advisor: Prof. Jing Zhao, Prof. Wei Wei, RA. Peiqing Sun
Prof. Zhigang Zou Nanjing University
Prof. Xinghua Xia Nanjing University
Prof. Peidong Yang UC.Berkeley
Prof. Chris Chang UC.Berkeley
Prof. Ruikang Tang Zhejiang University
Doc. Wei Xiong Zhejiang University
Prof. Qiwen Hu TMMU
Prof. Y. Honda Kyushu University
School of life science, Nanjing University

References

Peidong Y. et al. Science 351.6268(2016):74-77.
Tatsumi I. et al. Angewandte Chem. 2016
Parkin. Metal Ions in Life Sciences.14,99–124(2014).
Ruikang T, et al. Angewandte Chemie International Edition, 2015, 54(41): 11961-5.
Zhao. et al. Environmental Science & Technology 48.6(2014):3363-3371.
Sakimoto K K. et al. Science, 2016, 351(6268):74-77.
Honda Y. et al. Angewandte Chemie, 2016.
Xiong W. et al. Angewandte Chemie International Edition, 2015, 54(41):11961-5.
Wei W.et al. Environmental Science & Technology, 2014, 48(6):3363-71.