PIXCELL

Imperial College London iGEM

A.S.D.C Prunetto, D. Mujku, S. Chang, L. Chavez-Rodriguez, W. Beardall, Y. Yin, T. Caganek, L. Ripoll-Sanchez, J. Lawrence, S. Joshi, A. Singh, I. Mullor-Ruiz, R. Ledesma Amaro, and T. Ouldridge

Electronic control circuits have provided us with the complex technology we use everyday of our lives. **With PixCell we enable electronic control to synthetic biology,** and prove how it can provide the **spatiotemporal control** required for a key condition of biological complexity: **patterning**.

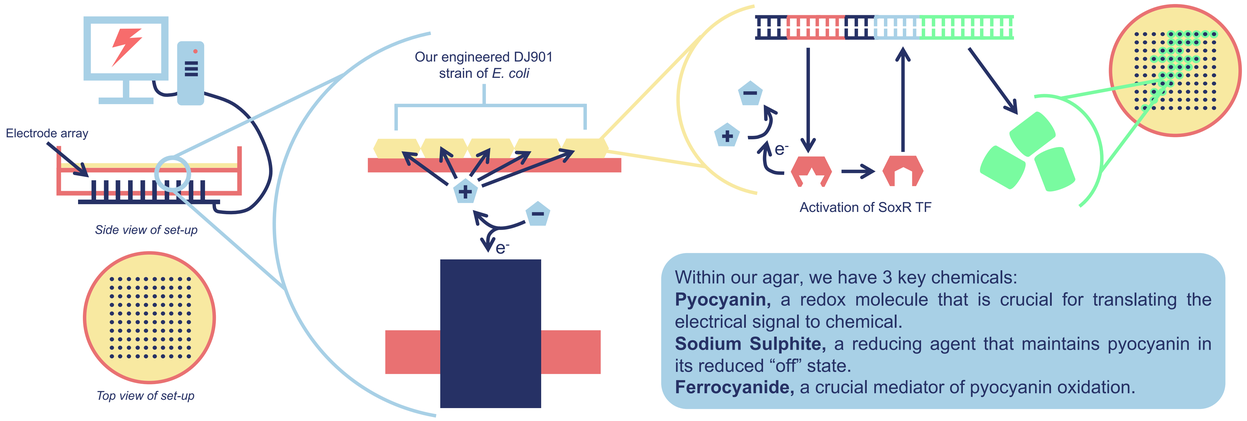
How do we control spatiotemporal gene activity using electricity?

1) We've designed our PixelDraw software to take input designs from the user

2) PixelDraw then communicates with our PCB electrode array. Electrodes at specific coordinates are activated and produce a local voltage of +0.5 V

3) Pyocyanin molecules in the local region are oxidised. These diffuse further in the agar and then into the monolayer of cells

4) Oxidised pyocyanin oxidises the transcription factor SoxR, switching it to its "on" state. SoxR goes onto bind the pSoxS promoter and recruits RNA polymerase to transcribe the GFP gene down stream.



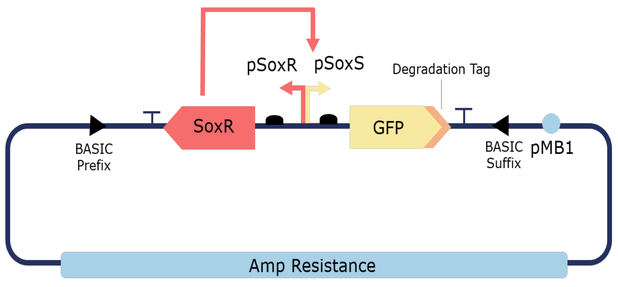
Within our agar, we have 3 key chemicals:
Pyocyanin, a redox molecule that is crucial for translating the electrical signal to chemical.
Sodium Sulphite, a reducing agent that maintains pyocyanin in its reduced "off" state.
Ferrocyanide, a crucial mediator of pyocyanin oxidation.

Our PixCell Construct

PixCell builds upon the research done by T. Tschirhart et al., where we have extended their work in two key ways:

- ✓ We have established electrogenetic control in an aerobic environment conditions.
- ✓ We have demonstrated control of gene activity in 2D space.

Furthermore we have created a SoxR and pSoxS parts library that can be assembled modularly, to further fine tune gene expression to our intent.



We would like to thank Imperial College London's Bioengineering and Life Sciences departments for all their supervision and support throughout the project

arbor biosciences · eppendorf · SnapGene® Software for molecular biology · Benchling · NEW ENGLAND BioLabs Inc. enabling technologies in the life sciences · clarafi · IDT Integrated DNA Technologies · BMG LABTECH The Microplate Reader Company · Biolegio

References
Tschirhart, T., Kim, E., McKay, R., Ueda, H., Wu, H., Pottash, A., Zargar, A., Negrete, A., Shiloach, J., Payne, G. and Bentley, W. (2017). Electronic control of gene expression and cell behaviour in Escherichia coli through redox signalling. *Nature Communications*, 8, p.14030.

Dry lab

Diffusion modelling

We first wanted to evaluate the concentration profile of pyocyanin in agar *in silico* to test the feasibility of spatially resolved oxidised and reduced populations. From our modelling, the **half-maximal width of the concentration profile is predicted to be 1.145 electrode diameters**, which confirms the feasibility of generating spatially resolved regions of oxidising or reducing environments.

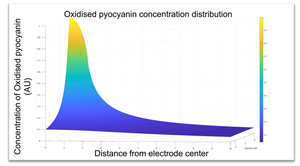


Figure 1. Oxidised pyocyanin concentration distributions throughout the volume of interest, extending out from electrode center

Curve fitting

We wished to fit a transcription/translation model to all of our data, in order **to estimate the relationships between growth condition and circuit activity. We** therefore constructed an ODE-based model of our genetic circuit. **We fitted our model to the entire time course of experimental data,** rather than merely the steady state GFP values for each pyocyanin concentration.

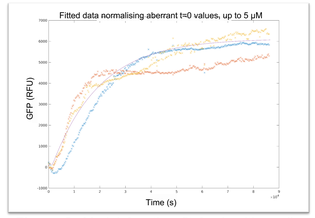


Figure 2. Model function fit for healthy cells, normalised for initial variable bias

Creating our programable electrode array

Once we experimentally demonstrated how our gene expression could be locally induced with our affordable 3-electrode setup (see wet lab), **we next built a higher-end electrode array able to induce genetic expression locally** at specific coordinates on a lawn of cells

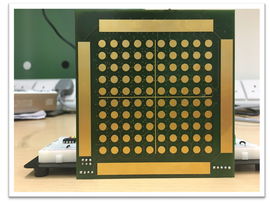


Figure 3. Our printed circuit board (PCB) 100-coordinate electrode array

Wet Lab

PixCell construct dose response to pyocyanin

Through a series of **plate reader experiments**, we tested LB media compositions of **varying pyocyanin, sodium sulphite and ferrocyanide concentrations** to identify the ideal conditions for induction of our PixCell construct. **We have identified 2.5µM Pyo, 0.02% sodium sulphite and 10mM ferrocyanide as the ideal values**, which works both in liquid and solid cultures.

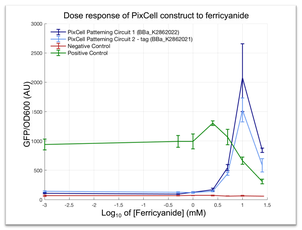


Figure 4. Oxidised ferrocyanide (ferricyanide) simulates an oxidising environment, which switches on our constructs

Stimulation of PixCell construct on agar

Cells with our PixCell construct were grown on agar with pyocyanin, ferrocyanide and sodium sulphite. They were **induced with an oxidising potential of +0.5V at the working carbon electrode.** Using a colony scanner, **significant expression of GFP was observed** at the working electrode, proving that **we can spatially induce GFP synthesis using electrical stimulation.**

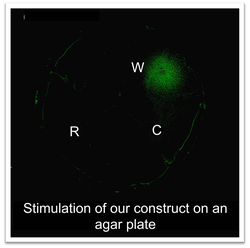


Figure 5. A +0.5V induction at the working (W) electrode shows significant GFP synthesis compared to the background, as well as the counter (C) and reference (R) electrodes.

Biocontainment application experiment

Biocontainment of GMOs is a major concern of both researchers and the public. We therefore, as a first step, proposed a system in which we control the spatial extent of bacterial populations through a "microbial electric fence". **We have shown that in an oxidising environment, our pSoxS-Gp2 circuit can inhibit cell growth by a factor of 2.**

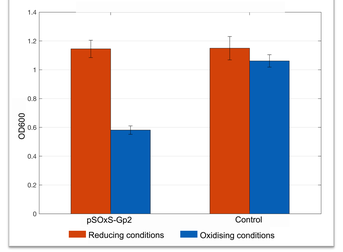


Figure 6. Our pSoxS-GP2 construct inhibits cells growth by a factor of 2 in oxidising conditions, which simulates induction by a oxidising potential.

Creating the PixCell parts library

The PixCell part library consists of **5 SoxR transcription factors and 8 pSoxS promoters** which were created for construction of electrogenetic circuits with variable activity. Using **BASIC assembly we constructed all 40 transcription factor and promoter combinations, and characterised them in DH5α** strain of *E. coli*. From our data, we are able to find combinations with varying expressions, which **allows for selection of a SoxR-pSoxS pairing with a specific induction strength.**

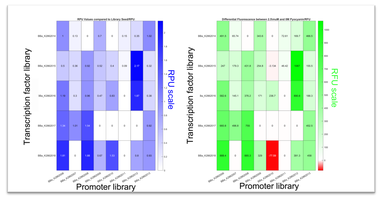


Figure 7. Left - relative promoter unit activity with respect to he library seed (top left corner). Right – comparison of RFU between pyocyanin induced and non-induced conditions

Conclusion

Through our project we have:

- ✓ Proven that we can have **spatiotemporal control of gene activity using electrical induction**, in aerobic conditions.
- ✓ **Simulated both the electrochemistry of pyocyanin** in 2D and 3D space, and created models that can predict cell interactions with pyocyanin.
- ✓ Created a **library of parts as a starter toolkit for the synthetic biology community** to use.
- ✓ Shown **proof of concept by creating a first generation biocontainment construct** to help alleviate the public and expert's concerns regarding GMOs.