A Synthetic Biology toolbox for electronic control of gene expression

Alicia Climent Catala[1,3], Dr. Rodrigo Ledesma Amaro[2,3], Dr. Danny O'Hare[2,3] and Dr. Thomas Ouldridge[2,3]
[1]Department of Chemistry, [2]Department of Bioengineering, and [3]Centre for Synthetic Biology at Imperial College London

Research motivation

Cells are naturally able to regulate metabolic processes in response to extrinsic and intrinsic signals (Fig. 1). The ability to process information and produce an output can be rationally engineered using **Synthetic biology.** SynBio is an emerging field that aims to provide a systematic framework for the engineering of biological systems.

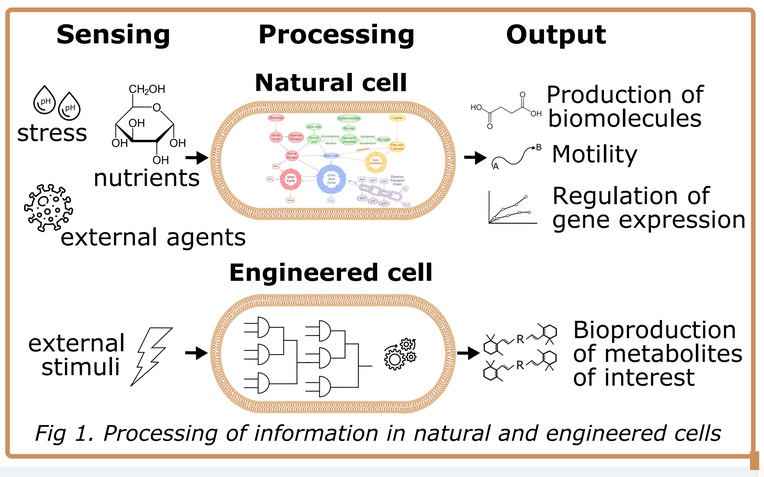


Fig 1. Processing of information in natural and engineered cells

Research Proposal

Electrogenetics is a novel field that aims to combine biology and electronics to directly control biological processes. Electrogenetics offers a framework to regulate bioproduction in both space and time as well as to use in large-scale bioreactors.

This **project** aims to develop a synthetic biology toolbox to control gene expression via electronic signals to broaden the possibilities for gene regulation in synthetic biology.

Objectives:

- Understand the performance of the synthetic circuit
- Characterization of the redox-sensitive circuit
- Production of high-value compounds
- Construction and test of the electro-chemical set-up

1) Electrogenetic synthetic circuit

SoxR is a **transcriptional regulator** that can undergo a reversible redox reaction to modulate its activity (Fig. 2). SoxR is **activated by oxidation** of its **[2Fe–2S] cluster**, which activates gene expression from PSoxR. **Redox-cycling drugs** can oxidise SoxR.

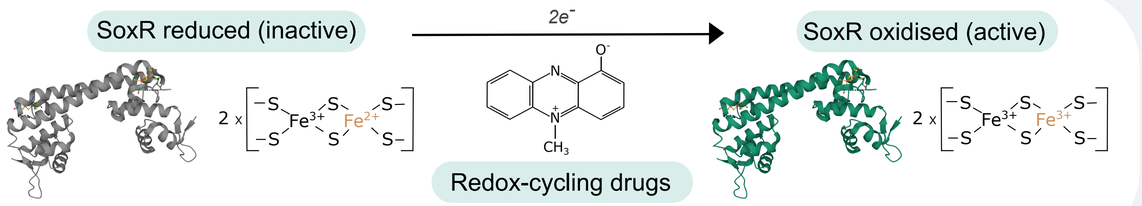


Fig 2. Activation mechanism of the transcriptional factor SoxR in E. coli by redox-cycling drugs

ELEMENTS OF THE ELECTROGENETIC DEVICE

Redox-cycling drugs

Redox-cycling drugs sequester electrons from metal centers of redox enzymes (e.g. phena-zines or viologens) (Fig. 3)

Redox-drugs are re-oxidised by the electron flow through the respiration machinery.

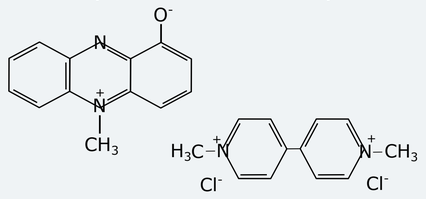
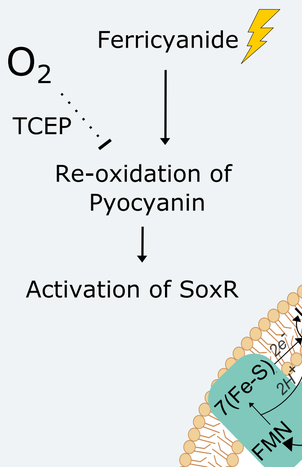


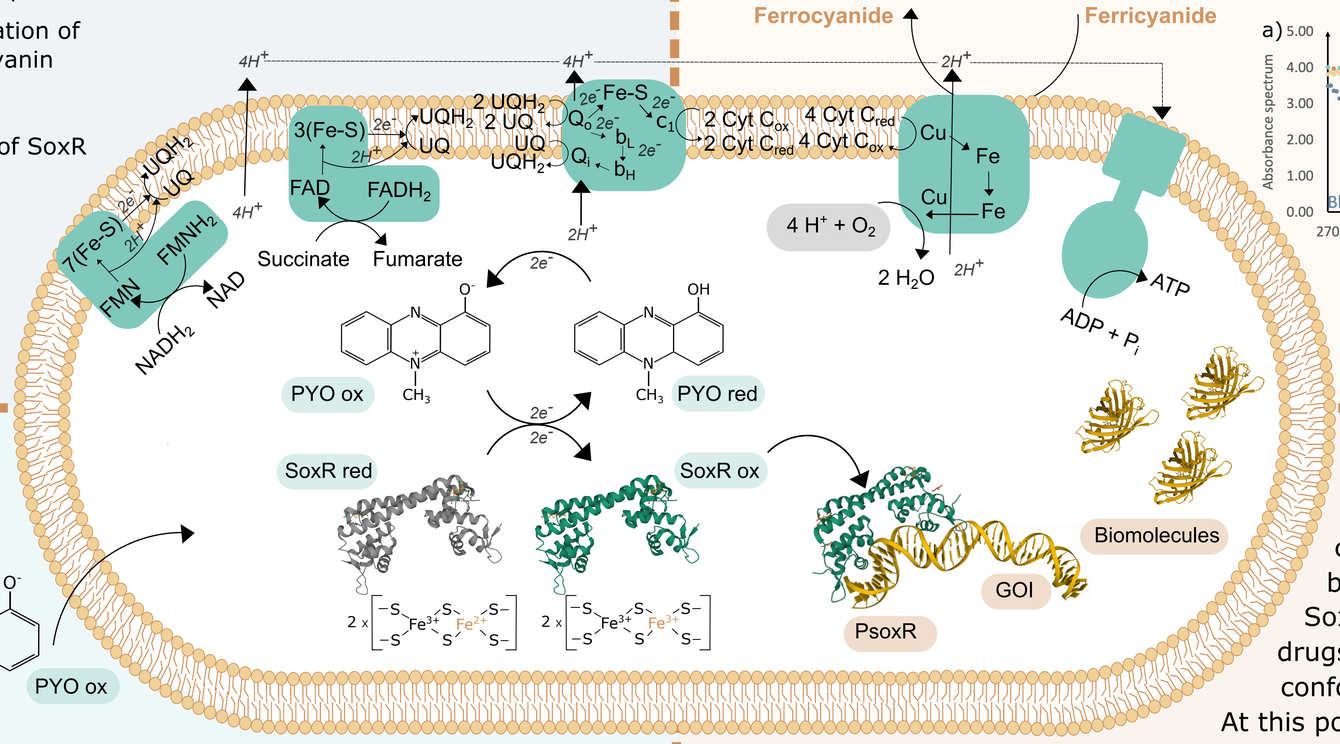
Fig. 3 Pyocyanin and paraquat chemical structures

Electrochemical control



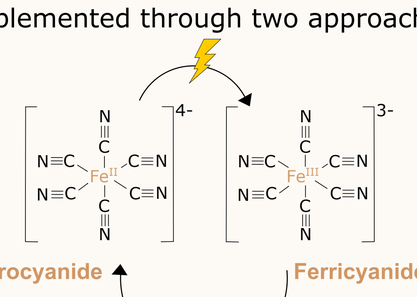
PsoxR promoter

PsoxR is activated when SoxR is oxidised.



2) Electrochemical device

Electrochemistry connects the flow of electrons to chemical changes which are often the oxidation or reduction of metals. This project implements electrochemical experiments to trigger the **oxidation of ferrocyanide** and thus, to reoxidise the redox-drug and activate SoxR in the system. The **three-electrode system** (Fig. 4 and 5) is implemented through two approaches:



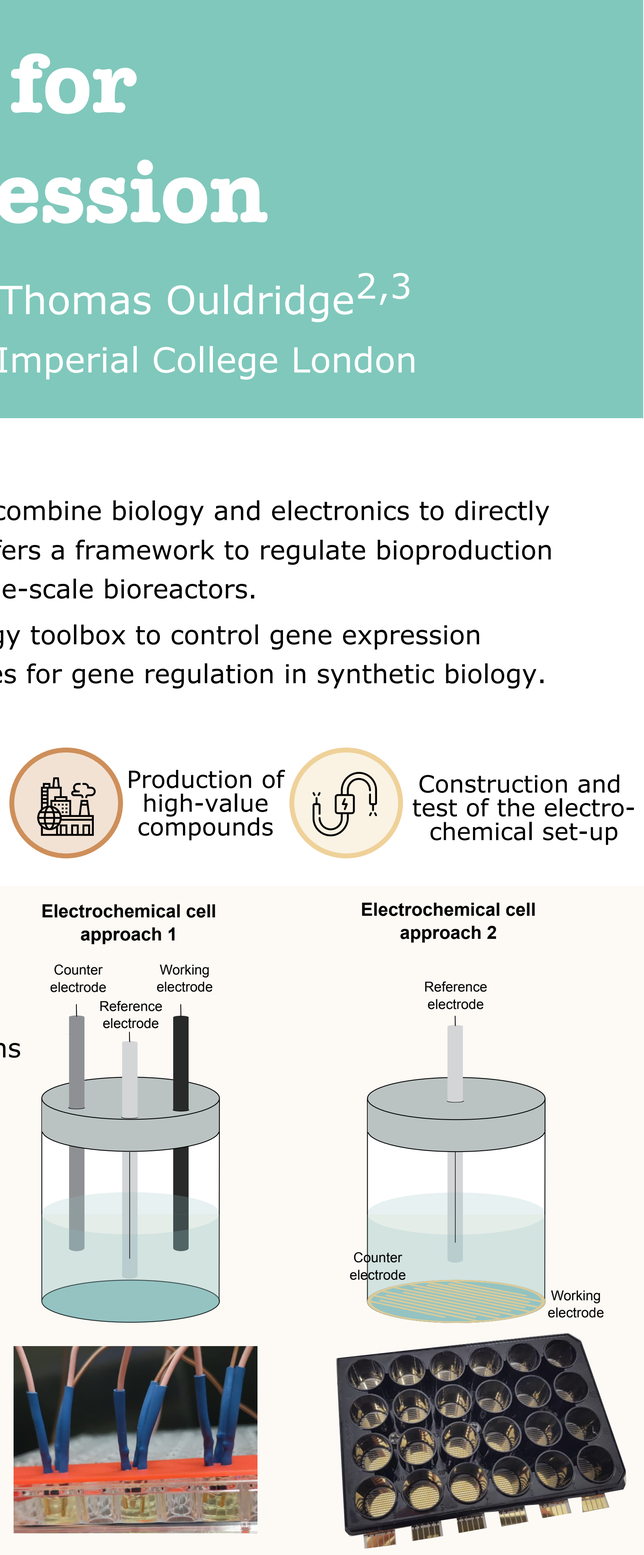


Fig 4. Schematic representation of both electrochemical cells proposed for this work for CV experiments.

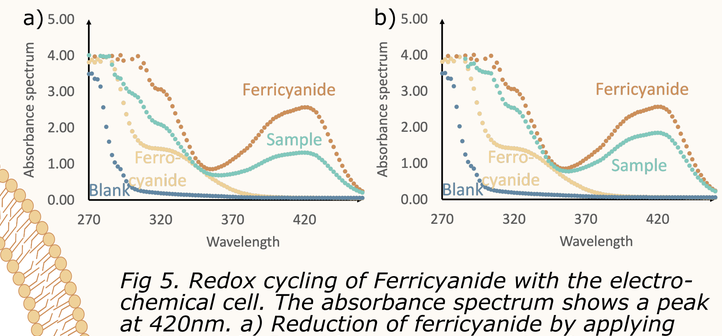
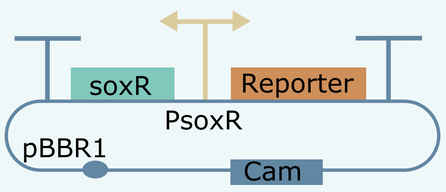


Fig 5. Redox cycling of Ferricyanide with the electro-chemical cell. The absorbance spectrum shows a peak at 420nm. a) Reduction of ferricyanide by applying -0.4V and b) Reoxidation reaction using 0.4V.

3) Redox-sensitive synthetic circuit

The **design of synthetic genetic circuit:**



3.1) Activation of SoxR with redox-drugs

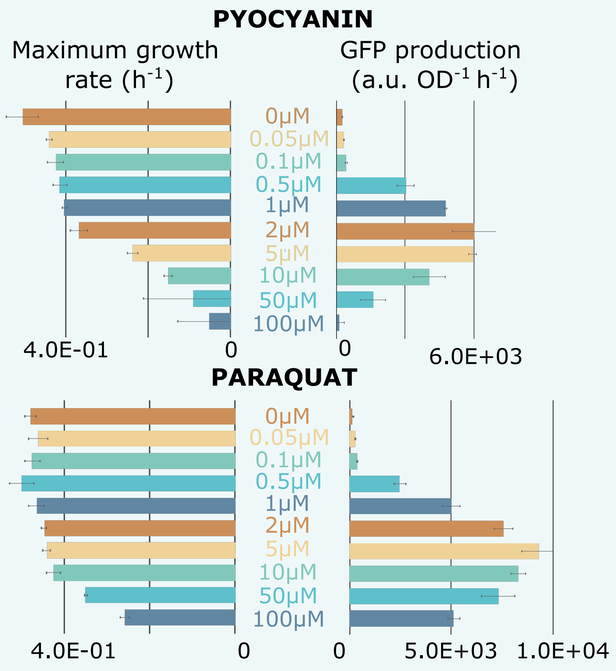
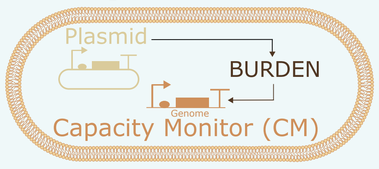


Fig. 6 Growth and production analysis with redox-drugs

3.2) Analysis of the genetic burden

The **capacity Monitor** (CM) is a constitutive fluorescent signal in the genome. CM production decreases if the synthetic plasmid imposes burden in the cell due changes in resource availability.



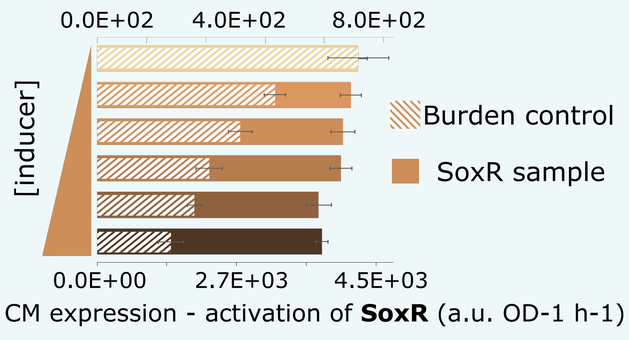
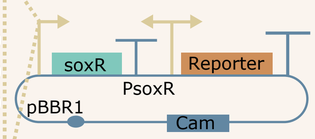


Fig. 7 Capacity monitor expression when SoxR is activate

4.1) Optimization of the system

Is the **concentration of SoxR** a limiting factor in the expression of biomolecules inside the cell? SoxR is expressed under constitutive promoters:

- J23113 (Rel.units:0.01)
- J23116 (Rel.units: 0.16)
- J23107 (Rel. units: 0.36)
- J23118 (Rel. units: 0.56)
- J23102 (Rel. units: 0.86)
- J23100 (Rel. units: 1)
- J23119 (Rel. units: 2)



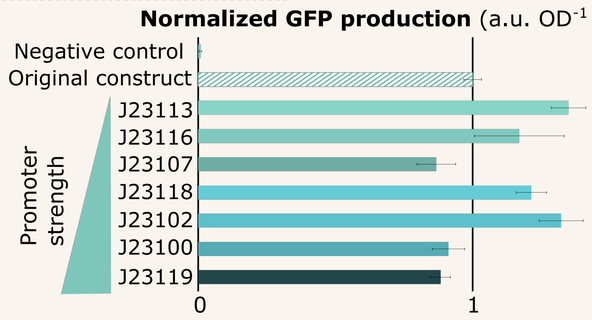


Fig. 8 GFP production levels when SoxR is expressed under the Anderson's promoters. Results are normalized with the original construct

4) Bioproduction

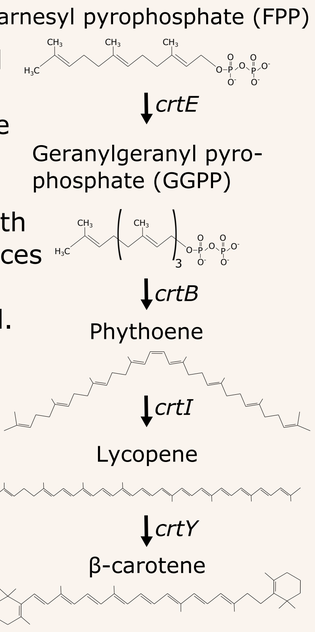
PsoxR is a bidirectional promoter that constitutively produces SoxR in one direction, but it is inducible in the other direction. SoxR is activated at the same time as the redox drugs are reduced. In this state, SoxR changes its conformation and recognises the PSoxR promoter. At this point, the transcription of the gene of interest / metabolic pathway to produce high-value compounds.

4.2) Metabolic pathways

1) β-carotene pathway

The enzymes involved in this pathway (crtE, crtB, crtI and crtY) are controlled by pSoxR.

Pyocyanin interferes with the pathway and reduces the concentration of β-carotenes in the cell.



2) Eumelanin

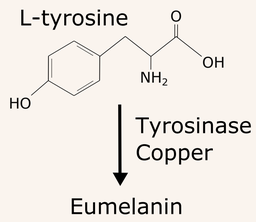


Fig. 9 Metabolic pathways of β-carotenes and eumelanin

Conclusions and Future steps

Preliminary results from the electrochemical device demonstrate that the oxidation state of ferrocyanide can be cycled. The next step is to activate the cellular response under low-oxygen conditions and to study how electricity can affect cell metabolism.

The electrogenetic device has been characterized across different conditions and redox-drugs in order to expand the use of this system in metabolic engineering. The next steps will be the implementation and monitoring reducing agents in cells.

Different architectures have been tested to try to improve the production, but results indicate that the concentration of SoxR is not a limiting factor for bioproduction. The next steps are to produce β-carotenes and eumelanin under this system with electricity.

References

[1] Tschirhart,T. et al., Electronic control of gene expression and cell behaviour in Escherichia coli through redox signalling. Nat. Commun., vol. 8, 2017.

[2] Ceroni, F. et al., Quantifying cellular capacity identifies gene expression designs with reduced burden. Nat Methods 12, 415–418, 2015.

[3] Sheplock, R. et al., Species-specific residues calibrate SoxR sensitivity to redox-active molecules. Mol. microbioly, 87(2), 368–381, 2013.

[4] Gu, M. & Imlay, J. A. The SoxRS response of Escherichia coli is directly activated by redox-cycling drugs rather than by superoxide. Mol. microbiology, 79(5), 1136–1150, 2011.