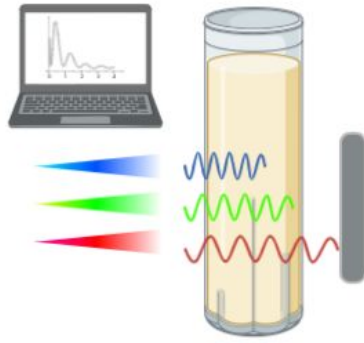
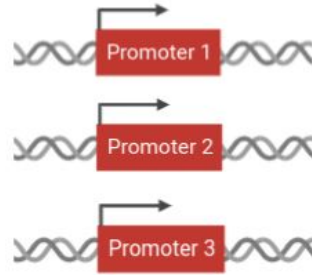


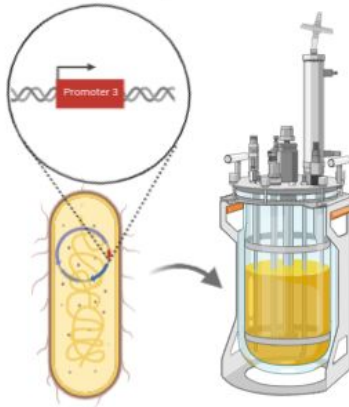
Project title		
Scaling up optogenetic control of recombinant protein expression		
Keywords⁽¹⁾		
Optogenetics, biomanufacturing, bioreactor, synthetic biology		
Type of project (experimental/computational/both)		
Both		
Academic supervisor		
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Co-supervisor(s) if applicable⁽²⁾		
Name	Affiliation	Email address
Project description		
Background (max 300 words; please include a diagram)		
<p>A core process in biotechnology is the production of recombinant proteins in microbes such as <i>Escherichia coli</i> bacteria. Typically, protein expression is induced once the microbial culture has reached a certain level of growth in order to optimise yield. Classical induction systems include the lac/T7 system, whereby expression of T7 RNA polymerase is induced by addition of the chemical IPTG to the media and this in turn activates expression of the target protein under a T7 promoter. Other induction systems require addition of antibiotics such as tetracycline or sugars such as arabinose. All of these are costly components when it comes to scaling up fermentation, with IPTG accounting for up to 40% of raw material costs.</p> <p>There are now a variety of genetic tools available to control the expression of proteins using light by integrating low-cost LEDs into bioreactors. Two outstanding questions concern the standardised characterisation of different light-driven promoters and their application to cultures of large scale (1 - 50 L). A small number of papers have applied optogenetic control systems at up to 2 L scale (e.g. Lalwani et al., 2018; Ohlendorf, 2012) but mostly they have been used in small cultures of < 500 ml. No papers have modelled the penetrance of light of different wavelengths through <i>E. coli</i> culture for this purpose or determined optimal light inducible genetic systems that could scale from prototyping to production. New systems are typically characterised in comparison to chemical induction by IPTG rather than in a cross-comparison to other light-inducible promoters so a standardised characterisation and model for optogenetic control are therefore needed to inform scale up strategies.</p>		



1 Model of light penetrance through *E. coli* culture and experimental validation



2 Characterise promoters with chosen wavelength in 96-well optogenetics rig



3 Test promoters in bioreactor at different culture scales



4 Design optogenetic module for protein production in Africa

Aims (max 200 words)

The overall aim of the project is to combine modelling and experimental work to deliver a combination of DNA modules and hardware that enables low cost, optogenetically controlled protein production in resource-constrained labs, specifically for production of recombinant proteins for research and diagnostics in Cameroon and Ghana.

This will include:

1. Constructing a model of light penetrance through *E. coli* culture at different wavelengths, scales and optical densities.
2. Validating the model through experimental work in *E. coli* cultures with LEDs and light sensors.
3. Identifying the wavelength with most potential for scale-up using a 96-well optogenetic test rig to characterise sets of promoters controlled by the selected wavelength and producing datasheets for those genetic parts.
4. Design of an optogenetic LED module for an open source bioreactor to be deployed in Cameroon and Ghana.
5. Testing the top candidate promoters in 2 L bioreactors for the production of DNA polymerases for diagnostics and molecular biology.

Scientific approach/methodology (max 300 words)

Constructing a model of light penetrance through *E. coli* culture and testing

A model of red, blue and green light penetrance at different intensities will be constructed using data on light scattering by *E. coli*, culture media absorbance values and other data obtained from the literature. This will be validated experimentally using a simple LED and sensor-based system in cell culture.

Selecting DNA parts for testing and cloning into standard plasmid backbone

Up to four DNA parts activated by the chosen wavelength will be selected from the literature and obtained from DNA repositories or *de novo* synthesis then cloned into a standard plasmid backbone using Type IIS assembly methods on the OpenTron PT2 pipetting robot.

96-well optogenetic test rig for standardisation of part characterisation

Plasmids containing the light inducible promoters will express a marker protein that can be measured by absorbance or fluorescence. These measurements will be carried out using an existing open source design for a 96-well optogenetic test rig. Datasheets will be constructed for each of the DNA modules.

Design of an optogenetic LED module for an open source bioreactor to be deployed in Cameroon and Ghana

Taking into account the optimal wavelengths and range of light intensities required, an LED-based module will be designed to enable illumination of a 2L culture in an existing open source, low-cost bioreactor designed by this research group.

Cloning and assembly of final constructs for production of OpenVent DNA polymerase

Two promoters will be selected for final testing in scaled up cell culture, cloned into a plasmid expressing a DNA polymerase and the level of induced expression will be compared to each other and to IPTG induction in cultures of 2 L using SDS-PAGE and fluorescent marker proteins at different light intensities.

Expected outcomes⁽⁴⁾

- 1) Model and experimental validation for red, blue and green light penetrance in *E.coli* cultures
- 2) Set of cloned plasmids encoding light-inducible promoters and basic characterisation dataset
- 3) Dataset investigating scalable induction of expression in 2 L cultures
- 4) Design for optogenetics module as add-on for existing low-cost, open-source bioreactor.

Provisional topic to be addressed in the expected mini-review⁽⁵⁾

Review topic: Optogenetic control of protein production for recombinant protein expression

This could be an extended review as there are currently only reviews of the variety of mechanisms for optogenetic control, not its application in biotechnology.

Further reading⁽⁶⁾

Miliadis-Argeitis, A. et al. Nature communications. <https://doi.org/10.1038/nature26141> (2016).

Ohlendorf, R. et al. Journal of molecular biology. <https://doi.org/10.1016/j.jmb.2012.01.001> (2012).

Lalwani, M. et al. bioRxiv. <https://doi.org/10.1101/845453> (2019).

Industrial collaboration (if any)⁽⁷⁾

N/A