Figure A: Healthy E. coli cells

Figure B: Unhealthy E. coli cells

Figure C: Unhealthy E. coli cells

Figure D: Unhealthy E. coli cells

Figure E: Unhealthy E. coli cells
“The Traffic Light Stress Sensor”
Aim of Our Project:

- Create biosensors to detect various cell stresses in bioreactors.
- Ligate different stress sensors together to build a traffic light system.
The Bio-processing World

Cells → Fermentation → Lysis → Primary recovery → Precipitation+ depth filtration → UF or TF → UF

- Buffer exchange
- Formulation
- Gel chromatography (Endotoxin removal)
- Size exclusion chromatography (RNase fragments and protein removal)
- Expanded bed ion-exchange chromatography (pDNA capture)
- Heat

Primary recovery

Polishing

Clarification
Stresses on *E.coli* cell

Oxygen deficient

Healthy *E.coli* cell

Over expression stressed

Shear stressed
Fermentation

Characterisation of stresses in a Fermenter

- Impeller damage - Shear stress
- Dead spots – lack of Oxygen
- General stress e.g. Misfolded protein
General shear stress sensor in *E. coli*

- Pilus
- β-barrels/OMPs
- Misfolded proteins and aggregations
- CpxR

OM

IM

P
General Oxygen Depletion Sensor

A $\text{O}_2$  

B $\text{O}_2$  

Cytoplasm
The Traffic Light Stress Sensor
Promoter from *E.coli* degP locus

-35 box - 10 box

BBa_K239000
Promoter from *E.coli spy* locus

-35 box - 10 box

BBa_K239001
Promoter from *E. coli* NarK locus

$\text{RNAP}^{\text{35 box}} \text{-} \text{10 box}^{+1}$

$\text{O}_2$

$\text{RNAP}^{\text{BBa_K239005 and BBa_K239006}}$
Low oxygen levels

Growth curve in a fermenter without stress

Normal growth curve, stressed cells.
Characterising the Spy & degP Sensor

• We used 3mM Cu$^{2+}$ as a proxy for shear stress. (Yamamoto & Ishihama, 2005)

• *E. coli* were grown in 96 well plates, 37°C, 300rpm in presence of 3mM Cu for 16 hours and time points taken. Fluorescence was measured with Tecan® spectrophotometer.
The degP sensor is shear responsive

- At the sensitive range, degP sensor respond to the shear stress. The overnight culture showed stronger fluorescence due to the high OD of the solution.
The Spy sensor is shear responsive

• At the sensitive range, spy sensor respond to the shear stress. The overnight culture showed stronger fluorescence due to the high OD of the solution.

* Y-axis: Relative Fluorescent Units
* GFP parts: excitation 501nm; emission 511nm
Summary of Additional Experiments

- Investigated challenge with more than 10 different chemicals with different concentrations for shear stress
- Currently investigating sensor activity during high cell density cultivation in bioreactors
- Currently investigating mechanical shear device mimics.
- Modifying Nark promoter sequence for increasing affinity (mNarK).
- Preliminary characterisations of oxygen sensor NarK and modified NarK promoters
- Investigated different ways of $O_2$ depletion
Future Work

- Further characterise activation of the degP and spy promoter.

- Modify the promoter sequences to build more sensitive and targeted biosensors, which can be applied to bio-processing.

- Continuing O\textsubscript{2} biosensor research

- Ligate different stress detectors together.

- Stress-actuated feedback control of heterologous protein expression.
Vision

Periplasmic space

OM

IM
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Questions ?