**Report 2: Synthetic, Genetic and Chronometric**

*The construction of genetic circuits that can exhibit robust oscillatory behaviour in the cellular environment is a major goal in the field of synthetic biology.*

*Several research groups have demonstrated working circuits, but cannot yet match the performance of corresponding systems that have evolved to operate within living organisms.*

In this report you should summarise the motivation, context and current status of this challenge. In particular you should discuss the important role played by techniques to analyse and model engineered, and detail how they can be used to help design, understand and improve the operation and performance of synthetic oscillators.

Throughout your report you should use results from computational modelling work to illustrate the topics discussed.

**Guidance:**

The breakdown below details some of the areas that could be included in your report. However you should adjust and rework this suggested structure and topics as you feel appropriate.

**1. Introduction.**

Here you can introduce the motivation and context for the research work. This might include.

**a) examples of natural oscillatory genetic systems, their roles, (proposed) mechanisms and properties (as currently understood).**

e.g. circadian oscillators, segmentation clock

Note: you can mention other biochemical oscillators here (e.g. cell-cycle, neural firing, metabolic oscillations), however I recommend you keep the majority of the discussion on oscillators that operate via gene regulation.

\*\* A tutorial is provided to code and simulate a simple circadian clock model, along with entrainment by night/day cycles.

**a) the motivation for investigating and developing synthetic versions of these systems;**

Note. This information can be found in review papers of the field, and also usually discussed in the introduction/outlook for papers describing experimental/theoretical work on genetic oscillators

**b) metrics and types of oscillatory behaviour, and the desirable qualities in engineered oscillatory systems;**

Describe the meaning of:

period/ frequency, amplitude, shape/waveform,

sustained, damped, limit-cycle oscillations

Describe how system behaviour can be shown as time evolution plots and phase diagrams

\*\*Note: in the first exercise in the assessed practical you generated example different types of oscillations, so can reuse your code here to produce suitable figures (adjusting parameters if necessary).

You might also briefly describe qualities that have particular relevance to the design and operation of genetic oscillatory circuits. (You can return to and discuss these qualities more fully in section 2.)

Keywords here could include:

robustness/resilience, uniformity/regularity/stability/fidelity, tunable/controlable, synchronicity, entrainment, metabolic load, temperature compensation, noise, stochasticity, interference

**2. A discussion of synthetic genetic oscillators.**

This should make use of computational models of one or more models of experimentally realised synthetic oscillators. Generate figures and write some short paragraphs that describe and demonstrate:

**a) key principles, features and qualities of genetic oscillators.**

e.g. discuss / demonstrate how negative feedback, delay and non-linearity can lead to limit cycle oscillations, and how these arise in genetic circuits.

\*\*Note: here you can reuse the code and work covered this is the week 9 computer room practical on the Goodwin oscillator.

**b) details of experimentally realised gene oscillators.**

Here you can discuss the workings of one or more engineered synthetic circuits (e.g. Repressilator, Dual Feedback oscillator) using figures created from your own simulations.

Referring to these figures you can describe and discuss the qualities of genetic oscillators as discussed in part (1).

\*\* Note: here you can reuse the simulation work carried out in the assessed practical on the Repressilator. You should be able to discuss:

* tuning. Demonstrate how properties of the oscillations are dependent on parameter values and mention if/how these can be achieved experimentally.
* synchronicity of single cell oscillations over long periods (use your plot of the averaged trajectory from stochastic simulation of the Repressilator)
* robustness of oscillations in the noisy cell environment (use your plots of amplitude and period distributions from stochastic simulation of the Repressilator).

\*\* A tutorial on coding and running the dual feedback oscillator (Hasty et al 2008) is provided.

\*\* Students taking the CELLM006 (Masters version of the module) should additionally show an example of a synthetic circuit that includes quorum sensing. A tutorial is provided to help code and run this.

**c) the ways in which simulation can aid the experimental and engineering process.**

e.g. discuss how computational modelling can aid the design, analysis and understanding of genetic systems.

This might cover the different types of analysis and simulation (ODE, DDE, stochastic) that can be carried out, along with their uses, advantages, disadvantages etc.

\*\* Example code for running the repressilator as a DDE (delayed differential equation model) is provided.

\*\* A tutorial is provided to show how to produce a bifurcation diagram for the dual feedback oscillator.

**3. Summary and outlook.**

Write a closing section that summarises the key points covered. An outlook might include details of up-to-date research results and a discussion of the future prospects for the field.

**Papers**

I have collected together some papers in the Dropbox folder that you can draw on while researching for and writing your report.

You are expected to use these selectively (rather than read everything) and are also encouraged to venture beyond this list as necessary.

**1 - Synthetic Biology:**

“A brief history of synthetic biology”

“Build to understand- synthetic approaches to biology”

“Engineered gene circuits”

“Five Hard Truths for Synthetic Biology”

“Foundations for the design and implementation of synthetic genetic circuits”

“Realizing the potential of synthetic biology”

“Recent advancements in synthetic biology- Current status and challenges”

**2 - Natural Cellular Oscillators:**

“Biological rhythms- Clocks for all times”

“Biological switches and clocks”

“Building a cell cycle oscillator- hysteresis and bistability in the activation of Cdc2”

“Combinatorial gene regulation by modulation of relative pulse timing”

“Computational approaches to cellular rhythms”

“Design Principles of Biochemical Oscillators”

“Frequency control of cell cycle oscillators”

“Functional Roles of Noise in Genetic Circuits”

“Functional Roles of Pulsing in Genetic Circuits”

“Mechanisms of noise-resistance in genetic oscillators”

“Modeling the Cell Cycle- Why Do Certain Circuits Oscillate”

“Polyphasic feedback enables tunable cellular timers”

“Sniffers, buzzers, toggles and blinkers- dynamics of regulatory and signaling pathways in the cell”

“Systems biology of cellular rhythms”

“The Ups and Downs of Modeling the Cell Cycle”

“Timing by Rhythms”

**3 - Circadian Clocks:**

“A Model for Circadian Rhythms in Drosophila”

“Biological clock began ticking 2.5 billion years ago “

“Circadian Clocks- A Tale of Two Feedback Loops”

“Circadian clocks limited by noise”

“Clocks, Metabolism, and the Epigenome”

“Keeping the Beat in the Rising Heat”

“Mechanics and resonance of the cyanobacterial circadian oscillator”

“Resilient circadian oscillator revealed in individual cyanobacteria”

“The daily rhythms of genes, cells and organs”

“Timing the day- what makes bacterial clocks tick”

**4 - Segmentation clock:**

“Autoinhibition with Transcriptional Delay- A Simple Mechanism for the Zebrafish Somitogenesis Oscillator”

“Intercellular Coupling Regulates the Period of the Segmentation Clock”

**5 - Synthetic Gene Oscillators:**

“A comparative analysis of synthetic genetic oscillators”

“A synthetic gene–metabolic oscillator”

“A tunable synthetic mammalian oscillator”

“How to make an oscillator”

“Robust network clocks- Design of genetic oscillators as a complex combinatorial optimization problem”

“Robustness and period sensitivity analysis of minimal models for biochemical oscillators”

“The pedestrian watchmaker- Genetic clocks from engineered oscillators”

**6 - Dual Feedback Oscillator:**

“A fast and tunable genetic oscillator”

“Entrainment of a Population of Synthetic Genetic Oscillators”

“Syncronised Quorum of Genetic Clocks”

**7 - Repressilator:**

“A synthetic oscillatory network of transcriptional regulators”

“Effects of temperature on the dynamics of the LacI-TetR-CI repressilator”

“Modeling a synthetic multicellular clock- Repressilators coupled by quorum sensing”

“Precision timing in a cell”

“Synchronous long-term oscillations in a synthetic gene circuit”