Engineering a molecular predation oscillator

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Abstract: The paper addresses the problem of designing and building a stable molecular based oscillator which can be controlled in terms of both amplitude and frequency. A study of previous oscillators of this type showed that they are inherently unstable. To overcome this problem a design was chosen which is based on Lotka–Voltera dynamics. An important aspect of the work was the use of what we term the Engineering Cycle; that is, the cycle of system specification, design, modelling, implementation, and testing and validation. The Lotka–Voltera dynamic, in the context of a predation oscillator, amounts to a predator–prey approach. This is the basis of the oscillator design. The oscillator was designed and detailed modelling undertaken to establish the modes of the dynamic; how it could be tuned for stability; and how to control its amplitude and frequency. The biological implementation of the design was undertaken using a number of BioBricks from the MIT registry (http://parts.mit.edu/registry/index.php/Main_Page), together with a number of parts which we designed and built.

1 Introduction

The paper describes the design and implementation of the molecular predation oscillator which formed the Imperial College submission to the iGEM competition in 2006. The team came second overall in the competition. The approach which was used was the traditional engineering approach for building a stable and flexible oscillator (in this case molecularly based). As will be shown in sub-sections of this paper, this approach involved a step-by-step development cycle comprising specifications, design, modelling, implementation and testing/validation. The full documentation for the project can be found on our OpenWetWare site: http://openwetware.org/wiki/IGEM:IMPERIAL/2006

2 Specifications

As with standard engineering oscillators (mechanical, electronic, fluidic etc.) it was necessary to define basic specifications. The key specifications are:

- stable oscillations for more than ten periods
- high signal-to-noise ratio
- controllable frequency and amplitude
- modular design for easy connectivity
- full documentation for quality control

Having defined the specifications, the next task was to study a range of approaches for implementing the oscillator as a biological system. This led to a decision to develop an oscillator design based on cell population dynamics. The mathematical basis for this was the Lotka–Voltera predation model. Its biological implementation was based on the quorum-sensing system found in Vibrio fischeri. Fig. 1, below, shows the basic dynamic of the oscillator design. This amounts to a predator–prey approach to the left-hand side of the diagram (i.e. the prey generator) representing the dynamic generation of the prey molecule population and the right-hand side of the diagram representing the dynamic generation of the predator molecules.

A key aim of the iGEM competition is to use, where possible, BioBricks which are available from the MIT Registry (parts.mit.edu). The central row in Fig. 1 shows the BioBricks which were used from the registry. These were added to new constructions which were developed during the project (and indeed in a number of cases became new BioBricks for the registry). Specifically, quorum sensing/quenching BioBricks from the registry were used in the design. The design of the oscillator was reduced to a system of two cell types in order to produce greater flexibility.

3 Modelling

A very important aspect of the design cycle was the development of a detailed computer model of the predator–prey system. This dynamical model was derived from a theoretical analysis. One of the key aspects of the modelling was to determine the conditions for stable oscillations which were controllable in terms of frequency, amplitude and profile. Fig. 2 shows details of the equations. Referring to the figure it can be seen that there are a number of parameters which can be controlled: a, b, c (population dependent); a0, b0, c0 (constants); and d1, d2, e (washout related).

Fig. 3 illustrates the functional and intermediate parts which were used in the oscillator. It can been seen from the figure that some of the parts were built, sequenced, tested, characterised and documented, while others were simply built and sequenced. It should also be noted from the diagram that parts were defined not only in
Fig. 1  Biological design of the predator generator

Fig. 2  Modelling of the full oscillator system, including limit cycle studies

Fig. 3  Contributions to the MIT Registry as a result of the project: functional and intermediate parts
4 Testing and validation

The initial step was to define testing protocols to satisfy component specifications. This was followed by the analysis of experimental data (both in terms of that acquired in the wet lab and from the model). This allowed the characterisation of different test constructs. The specific procedure for testing and validation comprised testing the part; predicting the model transfer function for the part; acquiring experimental data; fitting the model to the acquired data; and extracting the appropriate parameters. Fig. 4 illustrates this procedure. It should be noted from the figure that, for example, a plot of GFP against AHL shows wide variation for individual runs (but an expected characteristic, in terms of the model prediction). This is important because it shows that one of the features of biologically based oscillators is their high variability, high noise and so on. This is interesting because this was the situation when the original electronically based oscillators were built. Hence, it is anticipated that one of the key areas of development for biologically based oscillators will be (as with the original electronic versions) to significantly variability and noise.

5 Conclusion

Within the project a complete dynamical model was derived which describes the main biochemical reactions which characterise and drive the molecular oscillator. This was coupled to a full theoretical analysis and detailed computer simulation. On these bases, we were able to validate the design in relation to the original specifications. This resulted in the successful construction and characterisation of functional parts which are the building blocks for the planned oscillator.

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