ECE 6760: Modeling and Analysis of Biological Networks

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Lecture 8: Engineering Synthetic Gene Circuits

Introduction
- Can use electrical engineering principles to understand behavior of genetic circuits.
- Therefore, may be able to construct *synthetic genetic circuits* that behave like electrical circuits such as switches, oscillators, and communication networks.
- May even be possible to use these insights to design genetic circuits to correct faulty cellular mechanisms.
- Natural genetic circuits may not agree with our models because they are not designed well, so we should design better genetic circuits which agree with our models (Drew Endy).
- This lecture presents principles involved in engineering synthetic gene circuits as well as some examples.

Genetic AND Gate
- IPTG used to disable repression by *LacI*.
- aTc used to disable repression by *TetR*.

Genetic Logic Gates
Combinatorial Synthesis of Genetic Networks

- 'Combinatorial synthesis' uses subcloning and ligation with 15 promoter-gene units yielding a library of three-gene networks.
- Networks use $P_1$-lacI-$P_2$-cI-$P_3$-tetR where $P_1$, $P_2$, and $P_3$ are:
  1. $P_L^1$ (repressed by LacI)
  2. $P_L^2$ (repressed by LacI)
  3. $P_T$ (repressed by TetR)
  4. $P_\lambda$ (repressed by cI)
  5. $P_\lambda^2$ (activated by cI)

- For measurement, $P_\lambda$-gfp added to each plasmid.
- Inputs are IPTG and aTC, and output is GFP fluorescence.
- Grown in *E. coli* under four input conditions, output measured, and collection of logic gates found.

Modular Genetic Cloning Strategy

Two Example Genetic Circuits

Behavior of Selected Networks
Sequential Logic Circuits

- The output of sequential circuits depend not only on the current input, but also on the recent history of inputs.
- This history is recorded in the state of the circuit.
- State is maintained through the use of feedback.
- Feedback loops are important for stability in control systems.
- In autoregulation, protein modifies own rate of production.
- Feedback can be either positive or negative.
- Genes regulated by negative feedback should be more stable than those unregulated or regulated by positive feedback.
Positive Feedback and Bistability

Toggle Switch

AND Gate with Memory

Repressilator

- Oscillations used in control systems as central 'clocks' to synchronize behavior.
- Circadian rhythms manifest as periodic variations of concentrations of particular proteins in the cell.
- Though precise mechanism is unknown can generate a network that has a similar behavior.
- Note that not all parameter choices lead to oscillations.
- High protein synthesis and degradation rates, large cooperative binding effects, and efficient repression are all necessary.
- As result, strong and tightly repressible promoters selected, and proteins modified to make easy targets for proteases.
**Synthetic Transcriptional Oscillator**

Slow transcription and translation rates lead to internal noise.

- Internal noise can affect developmental pathway choice.
- Models of single gene predict that random variation in expression should scale linearly with translational rate and be independent of transcription rate.
- Experimental work found that size of fluctuations induced by translational step is inversely proportional to mRNA half-life.
- Therefore, fast mRNA turnover is means of mitigating noise.
- However, this increases energy requirements for protein production so evolution has a tradeoff here.
- Other experiments found that cascades of genes can act as attenuators for a noisy input signal.
- Circadian circuitry may actually exploit the noise.

**Intercell Signaling System**

- Signals necessary to coordinate behavior.
- Cellular membranes act to isolate cells, so communication must use specialized channels to pass through or activate receptors.
- Example network is quorum sensing in *Vibrio fischeri* in which behavior is regulated based on density of bacteria.
- In synthetic system, sender cells produce autoinducer while receiver cells produce GFP when autoinducer is detected.
- Theoretical analysis has shown that coupling cell’s production of autoinducer to oscillatory phase can synchronize population.

**Applications**

- Synthetic gene circuits can lead to new biological insights.
- May have biotechnology applications in the future.
- Using genetic logic blocks, integrated biological circuits can potentially be constructed in the future
- Wet nano-robots could be used for biosensing, synthesizing biomaterials, executing programmed cell death, and interfacing with microelectronics.
- Oncolytic adenovirus is capable of selectively killing tumour cells by detecting the state of the p53 protein.
- Inserting engineered networks into cells may be a way to probe gene expression to help identify the network topology.
Sources
- http://parts2.mit.edu/r/parts/index.html
Registry of Standard Biological Parts

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Registry of Standard Biological Parts

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Registry of Standard Biological Parts

**BBa_C0051** Basic Part

**Repressor, Lambda cI (RBS-LVA+)**

Coding region for the cI repressor based on repressor from lambda lysage lambda modified with an LVA site for rapid recognition of the site in cI repressor binds to the rBS sites (BBa_C0051).

**Usage and Biocompatibility**

*Please enter your experience with this part here.*

**Functional Properties**

**Sequence and Features**

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*View the actual sequence*