Feedback and Control in Biological Circuit Design

Richard M. Murray
Control and Dynamical Systems / Bioengineering
California Institute of Technology

Elisa Franco (CDS)    Jongmin Kim (BE)    Javad Lavaei (CDS)
Arjun Ravikumar (UG)    Somayeh Sojoudi (CDS)    Ophelia Venturelli (BMB)
Mary Dunlop (U. Vermont)    Arthur Prindle (UCSD)    Johan Ugander (Cornell)
Control Systems in Biology: Chemotaxis

“Classic” example of control system
• CDS 110a: Sensing, actuation and computation, in a feedback loop
• Key difference: overlapping functionality

Implements key principles of feedback
• Design of dynamics: search for regions with the highest nutrient concentration
• Management of uncertainty: perfect adaptation to mean concentration level
Analysis & Design of Biomolecular Feedback Systems

Overarching principles (v0.1)
- Data-driven, model-based approaches to rigorous modeling & analysis of phenotype
- Heterogeneous redundancy and crosstalk as mechanisms for robustness
- Systematic methods for design of dynamics and robust performance

Systems Biology
- Theory of phenotype
- Role of feedback
- System identification

In Vitro Testbeds
- RNA-based genelets
- Biomolecular wind tunnel
- Abstraction hierarchy

Biocircuit design
- Fast feedback
- Heterogeneous redundancy
- Systematic design
Analysis & Design of Biomolecular Feedback Systems

I. Motivation and Approach

II. Analysis of Feedback Circuits

• System identification using intrinsic noise
• Role of feedback in GAL pathway
• Joint work with M. Elowitz, H. El-Samad (UCSF)

III. Design of Feedback Circuits

• In vitro rate regulators (joint work with Erik Winfree)
• Design of oscillators using programmable delays
• Theory: delay-based control design

IV. Biomolecular “wind tunnel” (ongoing)

• Systematic approaches to biocircuit design
• Protein expression systems: preliminary results

V. Summary and Future Directions
System Identification Using Cell Noise

Traditional systems identification

- Engineering: forced response. Difficult to do in in vivo (eg, sinusoids are tricky)
- Biology: gene knockouts; steady state measurements using gene arrays

Idea: use noise as a forcing function

- Steady state distributions are not enough if extrinsic noise is present
- Need to use correlation data instead
System ID of a Synthetic Circuit (Dunlop, Elowitz & M)

E. coli Chromosome (4,600,000 base pairs) 1 copy per cell

Plasmid (7,500 base pairs) ~10 copies per cell

BNMC, 10 Nov 2010  Richard M. Murray, Caltech CDS
System ID of an in vivo circuit

Galactose regulation in *E. coli*
- GalE regulated by CRP via a feedforward loop
- GalR represses feedforward loop when fucose is present
- Promoter fusions measure GalS and GalE concentrations

**System ID shows FFL is not active**
- Addition of fucose shows no change in correlations => GalS is not actively regulating GalE

**Hypothesis: GalR repression dominant**
- If repression by GalR is large, GalS is always “off” => no connection
- Removal of GalR recovers expected correlations

**Implication:** useful for identifying *active* regulatory mechanisms
GAL Pathway in Yeast (Venturelli, Elowitz and M)

Motivation: copy number detection in cells
- How can we detect 2X different in copy number?
- Example: GAL pathway in yeast

Question: what are the relevant mechanisms?
- Multiple positive and negative feedback loops
  - GAL80 activates GAL2, GAL3, GAL80
  - Sequestration in cytoplasm, nucleus
- Promoter architecture may also be responsible
  - Cooperative interactions between GAL4/80
  - Number/location of binding sites

Approach
- Analysis: build model and study sensitivity of ultrasensitivity to various parameters (gains)
- Experiment: remove feedback pathways, mutate promoters; study resulting ultrasensitivity
- (Warning: not clear that these modifications make mathematical sense; could be shifting operating point of the system)
Analysis of GAL Ultrasensitivity

Build model that captures experimental data
• Use reaction rate equation model (ODEs)
• Best results w/ low order models (~10 states)
• Also exploring stochastic models (in progress)

Study parameter sensitivity in model
• High sensitivity in mechanism parameters => mechanism has strong effect on ultrasensitivity
• Can also use sensitivity analysis to understand the role of different feedback mechanisms

Sensitivity of slope of transfer curve

Infinitesimal parameter sensitivity analysis

• Note: difficult to do experimentally; requires analysis
**In Vitro Rate Regulator (Franco, Winfree & M)**

**Idea for a circuit:** produce two chemicals at same rates
- Common operation for metabolic networks - maintain stoichiometry
- Implemented using *in vitro* technology (test tubes instead of cells)

**Molecular programming for in vitro systems**
- Exploit Watson-Crick base pair binding (A-T, C-G)
- Can “compile” functional specifications into RNA and DNA sequences
- Circuits are biocompatible ⇒ some hope of embedding into cells
Rate Regulator Results

In vitro experiments
- Add templates + enzymes to test tube
- Use fluorophors to measure amount of repression

Rate regulator functions correctly
- When $T_1$ is high, get more repression of $T_1$ (to bring $R_1$, $R_2$ into balance)
- Can also use cross activation

Next steps
- Loading effects
- Sensing/actuation
- Integral feedback (Fei)
Improving the Performance of Oscillators

Toggelator

- Coupled oscillators
- Add additional “delay” (ACi)

Ugander, Dunlop & M, ACC 07
Improving Oscillator Performance by Adding Delay

Idea: add delay in dynamics by inserting “junk” DNA before coding region

Result: get much more “stable” oscillator

UG project (Ben Prindle)
- Added delay elements to repressilator circuit
- Results indicated that delay had effect, but not conclusive
Can we design control laws using (possibly variable) time delay?

- Easy to obtain in many bio systems
- Idea: combine signals with different amounts of delay to get control \( \hat{G}(s) \)
- Approach: given \( G(s) \), implement the impulse response using delay + 1-2 integrators (or lags)

\[
\hat{G}(s) : \quad \ddot{u} = \sum \alpha_i y(t - \tau_i)
\]

Preliminary results

*Theorem 3:* The approximation error \( \| G(j\omega) - \hat{G}(j\omega) \|_\infty \) satisfies the following inequality:

\[
\| G(s) - \hat{G}(s) \|_\infty \leq \sqrt{2} \int_0^{\tau_1} |g(t)| dt + \sqrt{2} \int_{\tau_k}^{\infty} |g(t)| dt + \sum_{i=1}^{k-1} \max_{\tau \in [\tau_i, \tau_{i+1}]} |g''(\tau)| \frac{\sqrt{2}(\tau_{i+1} - \tau_i)^3}{12}.
\]

- Can also get bounds on error when delays vary (possibly useful for jitter?)
Advances in Biomolecular System Design

Carlson, 2009

Synthesis productivity (bases/person/day)
Cost of gene synthesis ($/base)
Cost of oligo synthesis ($/base)

Winfree, 2008

Complexity (nt)

DNA 4-arm junctions (Seeley, 1959)

Purnick and Weiss, 2009

Systems

Year

Number of promoters

Max (18 months)
Moving average (18 months)

Year

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Biomolecular “Wind Tunnel” [Klavins]

Wind tunnel metaphor
- Use carefully controlled environment to gain understanding of the underlying physics
- Heavily instrumented with force balance, flow visualization, control of environmental conditions
- Design in a step-by-step fashion, checking understanding (and results) as you go
- Similar paradigm in most branches of engineering (eg, breadboards → circuit boards → IC chips)

Biomolecular equivalent
- Need a simple environment where we can test circuits and understand what they are doing
- One possibility: NEB pure express system
  - RNAP, NTPs, Ribosome, amino acids
  - ~20 additional enzymes (synthetases etc)
  - Double stranded DNA → protein(s) in 1 hour
- Development process for biomolecular circuit design
  - Sequence of PURExpress environments, designed to give step by step understanding

http://www.strongware.com
RNA-Based Antisense Regulation

Use antisense RNA to block translation
- 15-mer RNA strand, complementary to CATATGC GG GGGTTCT (blue = start)
- Multiple types of GFP DNA
  - Plasmid
  - Unpurified linear GFP (from PCR)
  - Purified linear GFP (QIAquick)

Results
- Plasmid GFP shows very high expression and repression
- Linear GFP shows decrease in expression level, more when unpurified
- Unpurified GFP still sufficient for testing and repression still effective

Conclusion
- Good option for combining genelet-based control with protein expression
Design of an event detector

Detect combination (and order of a set of inputs)
• Combines sensing circuit, with decision logic and protein output
• Requires some level of memory and timing
• Build logic out of RNA, inputs/outputs using proteins

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>No input</td>
<td>BFP, CFP, GFP, RFP</td>
</tr>
<tr>
<td>A</td>
<td>GFP, RFP</td>
</tr>
<tr>
<td>B</td>
<td>BFP, CFP</td>
</tr>
<tr>
<td>A then B</td>
<td>GFP</td>
</tr>
<tr>
<td>B then A</td>
<td>CFP</td>
</tr>
</tbody>
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Summary and Next Steps

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Some results to date
- System identification of active (feedback) pathways using intrinsic noise
- Sensitivity analysis as a first step in understanding the role of feedback mechanisms
- Design of RNA-based “genelet” circuits implementing feedback control concepts
- Time-delay as a mechanism for “design of dynamics” (mainly theory, so far)
- Biomolecular wind tunnel for systematic approach to design and understanding