Analysis and Optimization of Cellular Networks: a Systems Approach

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The success of human genome project has ushered a new era that emphasizes on a systemic or integrated approach known as systems biology, to ascertain the cellular behavior arising from complex cellular networks [1]. Systems biology lies at the interface of biology and systems theory including control systems engineering. Other than the familiar use of the word "systems" as a designation for physical systems, the term here also refers to the study of physical systems through: modeling, formulation of mathematical descriptions, analysis, and design [2]. Control systems approaches have been instrumental in this discipline, for example in the elucidation of chemotaxis adaptive mechanism [3], in the identification of control motifs in regulatory networks [4], and in the unraveling of design principles in circadian rhythm architecture [5]. In addition, several concepts from control engineering, in particular robustness, have diffused into systems biology to define many characteristics of cellular behavior. Robustness describes the ability of a system to maintain the desired performance/behavior under intrinsic and extraneous uncertainties [6]. In biological systems, these uncertainties can arise from inherent stochastic nature of gene expression (intrinsic) [7] or variations in the extracellular species concentration (extraneous). In fact, there appears to be an intimate link between the complexity and robustness in cellular functions [8].

Unraveling Design Principles using Sensitivity Analysis: Stochastic and Oscillatory Biological Systems

The size and complexity of cellular networks prevent the deduction of cellular behavior based solely on intuition. Systems analysis can help to unravel this complexity. One such method is sensitivity analysis [9], in which linear sensitivities quantify how much the system behavior changes as the parameters are varied. In cellular networks, high sensitivities point to the weakest links in the system which cellular behavior strongly depends on. By mapping these critical pathways back to the genotype, one can point to the set of genes and interactions that control the cellular behavior. Sensitivity analysis traditionally applies to continuous systems, i.e, differential equations, as these are the most common representation of engineered systems. However, the characteristics of cellular processes, such as nonlinear behavior (e.g., oscillations) and low concentration (nanomolar) of molecules, limit the application of classical sensitivity analysis and thus require the development of new methodologies for analysis.

One focus in my research is the development of non-traditional sensitivity analysis to investigate common systems in biology, in particular discrete stochastic and oscillatory systems. Sensitivity analysis has been formulated for discrete stochastic systems which prevail in cellular level modeling [10]. In these systems, the chemical reactions occur as

discrete events due to the low copy number of species involved, which accurately describe many cellular processes such as binding and unbinding of a transcription factor on a promoter [7]. Application of traditional sensitivity analysis to continuum representations of these systems can give incorrect results, in particular for systems with multiple steady states such as a bistable gene switch [10].

Another common dynamics of cellular networks is an oscillatory behavior of a limit cycle, for example in circadian rhythms, neuronal activities, and cell cycles. Here, past sensitivity analysis has mainly focused on the period and amplitude of the oscillations, with applications to models of circadian rhythm [5, 11, 12]. There exists very little work on the sensitivity analysis of the phase of oscillations, which describes the entrainment property of an oscillatory system such as circadian rhythm, to external forcing functions. The enabling concept for the phase analysis is the isochorns, collection of points that evolve to the same position in the limit cycle, which allows quantification of phase shifts between different limit cycles. Different measures of phase sensitivity analysis can be developed from this approach including the phase response curve (PRC) and the peak-to-peak sensitivity.

Optimizing Cellular Networks in Synthetic Biology: Stochastic Gene Switch

Advances in the recombinant DNA technology allow scientists to construct synthetic gene networks with specific functions such as a repressilator [13] and a gene switch [14]. These techniques set the foundation for building plug-and-play gene modules with predictable performance, which will make up a list of standardized parts [15]. From these parts, one can construct a functional module that can perform a specific task. The design effort in synthetic biology will become decoupled from the fabrication, analogous to the manufacture of integrated chip. Existing methodologies for designing the gene modules take on different approaches, such as combinatorial synthesis [16], design-then-mutate [17], and in silico evolution [18]. None of these approaches however considers the stochastic nature of cellular processes explicitly. As aforementioned, the inherent stochastic noise can induce distinguishing behavior that is not observable in continuum models [19].

Another focus in my research is in the development of methodology for the gene network optimization, using a bistable gene switch as an illustrative example. The optimization is formulated as a constrained nonlinear programming, where the stochastic effects are explicitly taken into account in the constraints. The goal is to produce plug-and-play gene modules that can robustly perform under the stochastic nature of cellular processes. The comparison between the proposed method and other design technique such as bifurcation analysis, highlighted the importance of stochastic effects in the design of a gene network.

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EDUCATION:

2003 **Ph.D. Chemical Engineering**, University of Illinois at Urbana-Champaign
Thesis title: *Modeling and Control of Transient Enhanced Diffusion of Boron in Silicon*
Thesis advisers: Richard D. Braatz and Edmund G. Seebauer

2000 **M.S. Chemical Engineering**, University of Illinois at Urbana-Champaign
Thesis title: *Dimensionality Reduction and Robustness Analysis of Large Scale Systems*
Thesis adviser: Richard D. Braatz

1998 **B.S. Chemical Engineering & Mathematics**, University of Wisconsin at Madison
Graduated with Highest Distinction,
Dean's Honor List 1994 – 1998
Hotaling Scholarship 1997

EXPERIENCE:

Postdoctoral Fellow, *University of California Santa Barbara* 9/2003 – present

- Performed research in the area of systems biology with interests in optimal experiment design, systems identification, dynamical sensitivity analysis, and discrete stochastic systems; with applications in circadian rhythm, frog egg cell cycle, genetic toggle switch, enzymatic futile cycle, and apoptosis.
- Developed iterative experiment design procedure and optimal measurement selection for iterative model development of biological systems, applied to the identification of caspase-activated apoptosis model.
- Developed dynamical sensitivity analysis for discrete stochastic systems, applied to gene regulatory models for circadian rhythm and genetic toggle switch.
- Developed dynamical phase sensitivity analysis for biological oscillatory systems, applied to circadian rhythm gene network.
- Developed analysis for robust photic entrainment of *Drosophila* circadian rhythm.
- Developed methodologies for the design of bistable genetic switch using bifurcation analysis and explicit stochastic approach, applied to enzymatic futile cycle and synthetic gene switch.
- Developed sensitivity analysis computation based on simultaneous stochastic perturbations.

- Actively involved in the development of sensitivity analysis tools BioSens, as part of open-source computational biology tools Bio-SPICE funded by DARPA BioComp program.
- Actively involved in the collaborative work of modeling staphylococcal enterotoxin B pathway in kidney cells (UCSB, UC Berkeley, UCLA, Walter Reed, Thomas Jefferson U, Indiana U, KGI, NYU, SRI) as part of Bio-SPICE (DARPA BioCOMP Project).
- Supervised four graduate and one undergraduate students in the area of systems biology.

Research Assistant, *University of Illinois at Urbana-Champaign* 8/1998 – 8/2003

- Performed research in the area of control systems with interests in robust control, optimal control, model reduction, experiment design, model identification, and hyperbolic systems; with applications in time delay systems, batch crystallization, microelectronics processing, and particulate systems.
- Developed model reduction and robustness analysis techniques for large-scale multivariable systems with uncertain time delays.
- Developed and applied optimal experiment design to determine kinetic parameters for nucleation and growth in batch crystallization of potassium dihydrogen-phosphate.
- Developed and analyzed a reaction-diffusion model for transient enhanced diffusion (TED) of boron in Si during the manufacture of ultrashallow p-n junctions for advanced CMOS.
- Employed maximum likelihood and Bayesian parameter estimation to identify the TED kinetic parameters from literature and experimental data provided by International Sematech.
- Designed the optimal annealing procedure that achieves the optimal p-n junction thickness.
- Developed a worst case analysis for the manufacture of ultrashallow p-n junctions to quantify the effects of model parameter uncertainties and control implementation errors.
- Developed high resolution methods for simulating population balance equations from batch crystallization modeling with size-independent and -dependent growth rates and aggregation.
- Developed a software package Particle Solver based on the finite volume method for simulating general particulate system dynamics described by population balance equations.

Lecturer, *University of Illinois at Urbana-Champaign* 8/2000 – 12/2000

- Responsible for lecture, exam preparation and grading, laboratory experiments and report grading of “Open-ended Experimental Design” course for M.S. students.
- The course is designed to teach scientists and engineers how to bring processes to production efficiently.
- Techniques covered in the course include data analysis, process modeling and simulation, design of experiments, parameter estimation, and process optimization.

- Crystallization processes were used as the primary examples to illustrate the ideas.

Teaching Assistant, *University of Illinois at Urbana-Champaign* 8/1999 – 5/2000

- Led a weekly discussion section, prepared solutions for homework and exams, and responsible for homework and exams grading of "Chemical Rate Processes and Reactor Design" course for senior undergraduates.
- Instructed laboratory sections and graded laboratory reports and exams of "Open-ended Experimental Design" course for senior undergraduates.

Undergraduate Researcher, *University of Wisconsin at Madison* 6/1997 – 5/1998

- Designed and implemented automated data acquisition for unit operations laboratory.
- Studied solubility of ethanol on a self-assembled monolayer of alkylsiloxanes using Monte Carlo simulations implemented on INSIGHT II.

GRANTS AND FELLOWSHIPS

UIUC Graduate Student Travel Grant 2002
(Finalists for the Burroughs-Wellcome Fund Career Awards – to be made in 11/2005)

PROFESSIONAL ACTIVITIES

Reviewer, Automatica (nominated as Automatica outstanding reviewer for 2004)
Member, American Institute of Chemical Engineers
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PUBLICATIONS

Patent:

1. "Methods for Controlling Dopant Concentration and Activation in Semiconductor Structures" with E. G. Seebauer, R. D. Braatz and M. Y. L. Jung, patent filed 8/2005.

Book Chapter:

1. R. Gunawan, K. Gadkar, and F. J. Doyle III. Methods to Identify Cellular Architecture and Dynamics from Experimental Data. In J. Stelling (Ed.), System Modeling in Cellular Biology, MIT Press, 2005. in press

Journal Articles:

1. R. Gunawan and F. J. Doyle III. Design of a bistable gene switch, 2005. in preparation
2. R. Gunawan and F. J. Doyle III. Entrainment of circadian rhythm: continuum vs. discrete models, 2005. in preparation
3. R. Gunawan and F. J. Doyle III. Isochron-based phase response analysis of circadian rhythms, Biophys. J., 2005. submitted.
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Peer-reviewed Conference Proceedings:

1. R. Gunawan and F. J. Doyle III. Phase Sensitivity Analysis of a Circadian Gene Network. In *Proc of 44th IEEE Conf. on Decision and Control and European Control Conference*, Seville, Spain, Dec. 2005.

2. R. Gunawan, M. Y. L. Jung, E. G. Seebauer, and R. D. Braatz. Optimal control of transient enhanced diffusion. In *Proc. of the IFAC Symp. on Advanced Control of Chemical Processes*, pp. 603-608, Hong Kong, China, 2003.
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