Multiphasic, Dynamic, High Throughput Measurements and Modeling for Postgenomic Cellular Biophysics

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Whence the Future for HTS and Biological Modeling?

• What will be required of future
  – High throughput screening (HTS) technologies?
  – Biological modeling?

• How best might they be coupled?
HTS Today

• Antibody/antigen binding
• Enzyme amplification
• Gene expression
• Ion channel modulation
  – Intracellular calcium
  – Transmembrane action potential
• Arrays
  – DNA
  – Proteins …
  – Cells…………
• Chemical libraries
• Fluorescent and magnetic tagging…

A variety of independent “slow” measurements of single-step operations on simple systems
Biological Modeling Today

• Electrical
  – Transmembrane action potential
  – Tissue-level action potential propagation
• Biochemical
  – Signaling and secondary messengers
  – Protein structure and function
  – Metabolic fluxes
• Biomechanical
  – Stress, strain
  – Hydrodynamics
  – Signal transduction
  – Molecular motors....

Frontal attack using ODE’s and PDE’s that involves nanomoles of equations and gigaflop years...
The Problem

- The “problem” is too big for measurement or models alone
- The models need data to drive and validate them
- The experiments need models for design and interpretation

- Advanced models and measurements will require new and coordinated technologies
Recent Progress in Biology

- Genomics
  - Structural genomics
- Proteomics
  - Structural proteomics
  - Functional proteomics
- What’s next?
Reductionism

- Thermodynamics
- Statistical mechanics
- Molecular/atomic dynamics
- Electrodynamics
- Quantum Chromodynamics
- Bulk solids
- Devices
- Continuum models
- Microscopic models
- Atomic physics
- Anatomy
- Physiology
- Organ
- Cell
- Protein
- Genome
Post-Reductionism

Thermodynamics
Statistical mechanics
Molecular/atomic dynamics
Electrodynamics
Quantum Chromodynamics

Bulk solids
Devices
Continuum models
Microscopic models
Atomic physics

Anatomy
Physiology
Organ
Cell
Protein
Genome
Spatial Resolution in Physiology

X-Ray / SEM / STM
Optical microscope
Magnifying glass
Unaided eye

Future = Cell
How do we study cellular-level responses to stimuli in both normal and patho-physiologic conditions?

Hypothesis: Great advances in physiology have been made by opening the feedback loop and taking control of the biological system.
Negative versus Positive Feedback

Opening the Feedback Loop

Hypothesis: Great advances in physiology have been made by opening the feedback loop and taking control

- Starling cardiac pressure/volume control
- Kao neuromuscular/humeral feedback
- Voltage clamp of the nerve axon

Simplified Hodgkin-Huxley

- For the resting cell, $E_{Na}$, $R_{Na}$ and inward $I_{Na}$ depolarize the cell with positive feedback
- $E_{K}$, $R_{K}$ and outward $I_{K}$ repolarize the cell and serve as negative feedback
- Ignore $Cl$

Hodgkin-Huxley: Closed-loop with positive and negative feedback

Adapted from Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259
Overriding Internal Control: Voltage Clamp

Adapted from Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259
Opening the Loop During External Control

Adapted from Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259
A Key to the Future: External Control of Cellular Feedback

✓ Electrical
  • Mechanical
  • Chemical
  • Cell-to-cell…
Signatures of Control

- Stability in the presence of variable input ($\Delta T = 50^\circ F$)
- Oscillations when excessive delay or too much gain
- Divergent behavior when internal range is exceeded or controls damaged

Control Stability

- Proportional control
- Proportional control with finite time delay
- Higher gain, same delay
- Same gain, longer delay
Intracellular Metabolic and Chemical Oscillations

- We know that oscillations and bursts exist
  - Voltage
  - Calcium
  - Glucose/insulin
  - Neurotransmitter

- **Prediction:** At higher bandwidths than provided by current instrumentation, we will see **chemical** bursts, oscillations, and chaotic behavior. **FIND THEM AND USE THEM!**

http://www.intracellular.com/app05.html
A Key to the Future
Probing and Controlling Cellular Metabolic and Signaling Pathways
Postgenomic Integrative/Systems Physiology/Biology

- Specify
  - Concentrations
  - Rate constants

- Add
  - Gene expression
  - Protein interactions
  - Signaling pathways

- Include
  - Intracellular spatial distributions, diffusion, and transport (ODE → PDE)

- ... Calculate how the cell behaves in response to a toxin or drug
The Catch

• Modeling of a single mammalian cell may require 100,000 variables and equations
• Cell-cell interactions are critical to system function
• $10^9$ interacting cells in some organs
• The data don’t yet exist to drive the models
• Micromoles of equations and teraflop years
• Hence we need to link models AND experiments to form hybrid digital/analog computers …
The Challenge

• Develop the tools and techniques for integrative, post-genomic **cellular** biology
  – Genes
  – Proteins
  – Metabolic and signaling pathways
  – Models
  – Instruments
  – **Wide-bandwidth dynamic control theory for cellular systems**

• **Needed**: Multiphasic, dynamical (fast) measurements and models of multi-step processes in complex cellular systems
Why Fast?

- Cellular-scale biochemistry can be very fast
- Wide measurement bandwidth, i.e., good response to high frequencies, is required to track cellular events
- Stable control requires a matching, high bandwidth
Physical and Biological Time Constants, Seconds

Mixing time to homogenize liquid in a large-scale bioreactor (10-100 m³) \(10^4 - 10^8\)
90% liquid volume exchange in in a continuous reactor \(10^5 - 10^6\)
Oxygen transfer (forced not free diffusion) \(10^2 - 10^3\)
Heat transfer (forced convection) \(10^3 - 10^4\)

Cell proliferation, DNA replication \(10^2 - 10^4\)
Response to environmental changes (temperature, oxygen) \(10^3 - 10^4\)
Messenger RNA synthesis \(10^3 - 10^4\)
Translocation of substances into cells (active transport) \(10^1 - 10^3\)
Protein synthesis \(10^1 - 10\)
Allosteric control of enzyme action 1

Glycolysis \(10^{-1} - 10^{-2}\)
Oxydative phosphorylation in mitochondria \(10^{-2}\)
Intracellular quiescent mass & heat transfer (dimension \(10^{-5}\) m) \(10^{-5} - 10^{-3}\)
Enzymatic reaction and turnover \(10^{-6} - 10^{-3}\)
Bonding between enzyme & substrate, inhibitor \(10^{-6}\)
Receptor-ligand interaction \(10^{-6}\)
Why Multiphasic?

Single measurements are woefully inadequate to study cellular-level responses to stimuli in both normal and patho-physiologic conditions!
The Problem

• Existing chemical and metabolic sensors and actuators are too slow to track biochemical events at the cellular level

• Many metabolic and signaling models are slow.

• Metabolic control is today possible only at the animal and organ level: metabolic clamp

• Post-genomic physiology needs cellular metabolic and signaling control
What do we need?

- Simultaneous, fast sensors (transducers) that detect a variety of changes within and outside the cell
- Actuators that control the microenvironment within and outside the cell
- Openers for the internal feedback loops
- System algorithms and models that allow you to close and stabilize the external feedback loop
- ...
Possible Approaches

- A biological cell or molecule inserted into a microinstrument, *e.g.*, a single-cell spectrophotometer or a whole-cell patch clamp
- A nanoinstrument inserted into the cell/molecule, *e.g.*, caged ATP

- Combine the cells, instruments, and software to form an integrated, closed-loop bio/nano/micro/info system
The Modeling Challenge

- Interpret, predict, and control the fast, dynamical response to interventions in closed-loop (internal or external) physiological control systems
The Experimental Challenge

- *Fast* requires small to beat diffusion time constants
- Small = < nL
- Massively-Parallel, Multi-Phasic Cellular Biological Activity Detector (MP²-CBAD)
- Chemical and Mechanical Clamp
Summary

- HTS today: A variety of “slow” measurements of single-step operations on simple systems
- Modeling today: Frontal attack using ODE’s and PDE’s that is heading towards micromoles of equations and gigaflop years…
- Molecular biology and physiology are converging on the cell, where the great questions reside
- Hypothesis: Great advances in physiology have been made by opening the feedback loop and taking control of the biological system
- Future: A hybrid approach using integrated, massively parallel, multiphasic, dynamic, high throughput measurements and modeling