VIIBRE – ICSE
VANDERBILT UNIVERSITY

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

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Instrumenting and Controlling the Single Cell (ICSC) Project

Physiome SAB, 10 November 2001

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

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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 <u>nanomoles</u> of equations and <u>gigaflop years</u>…

The Problem

- The “problem” is to 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

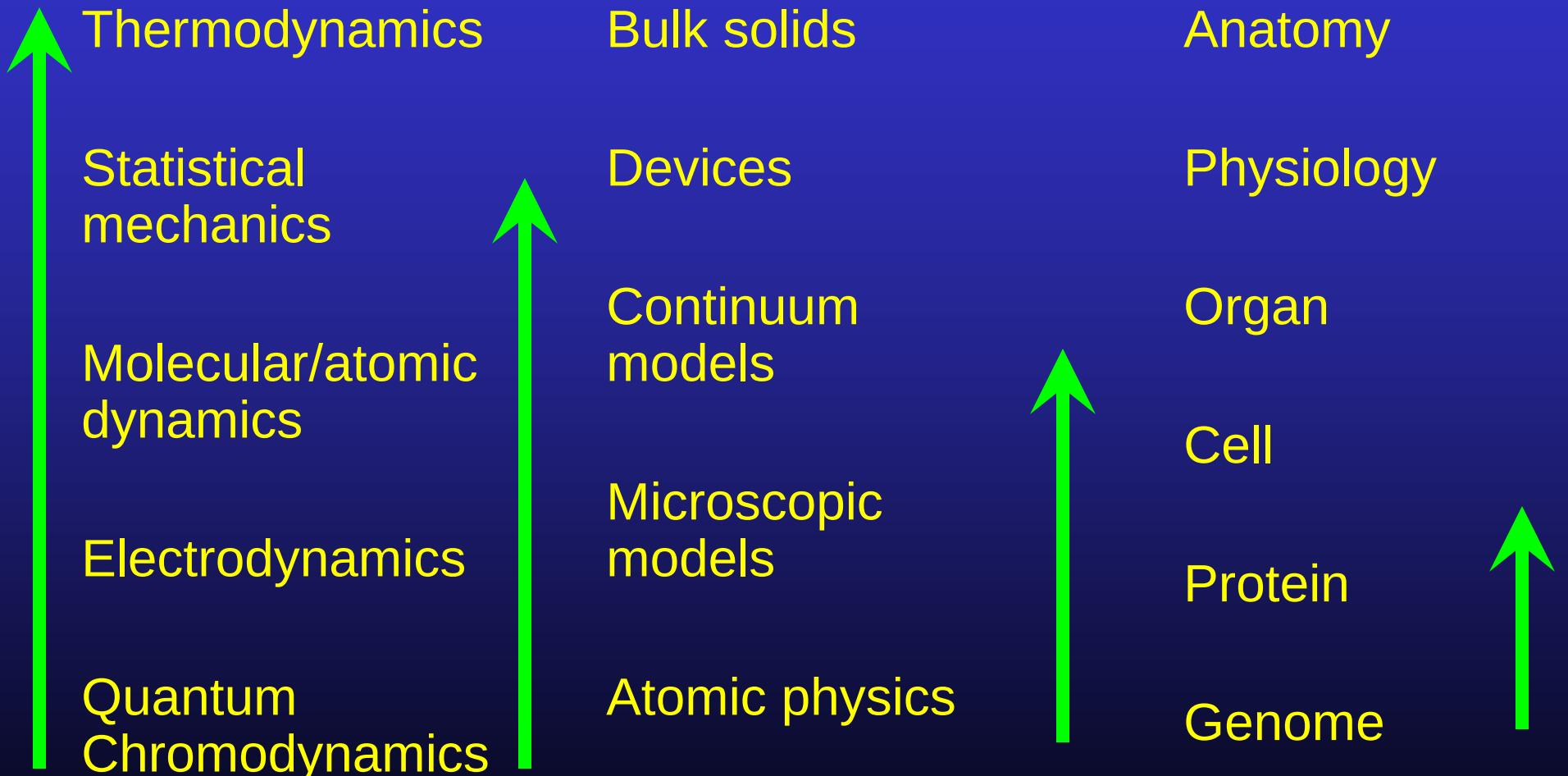
Organ

Cell

Protein

Genome

Post-Reductionism

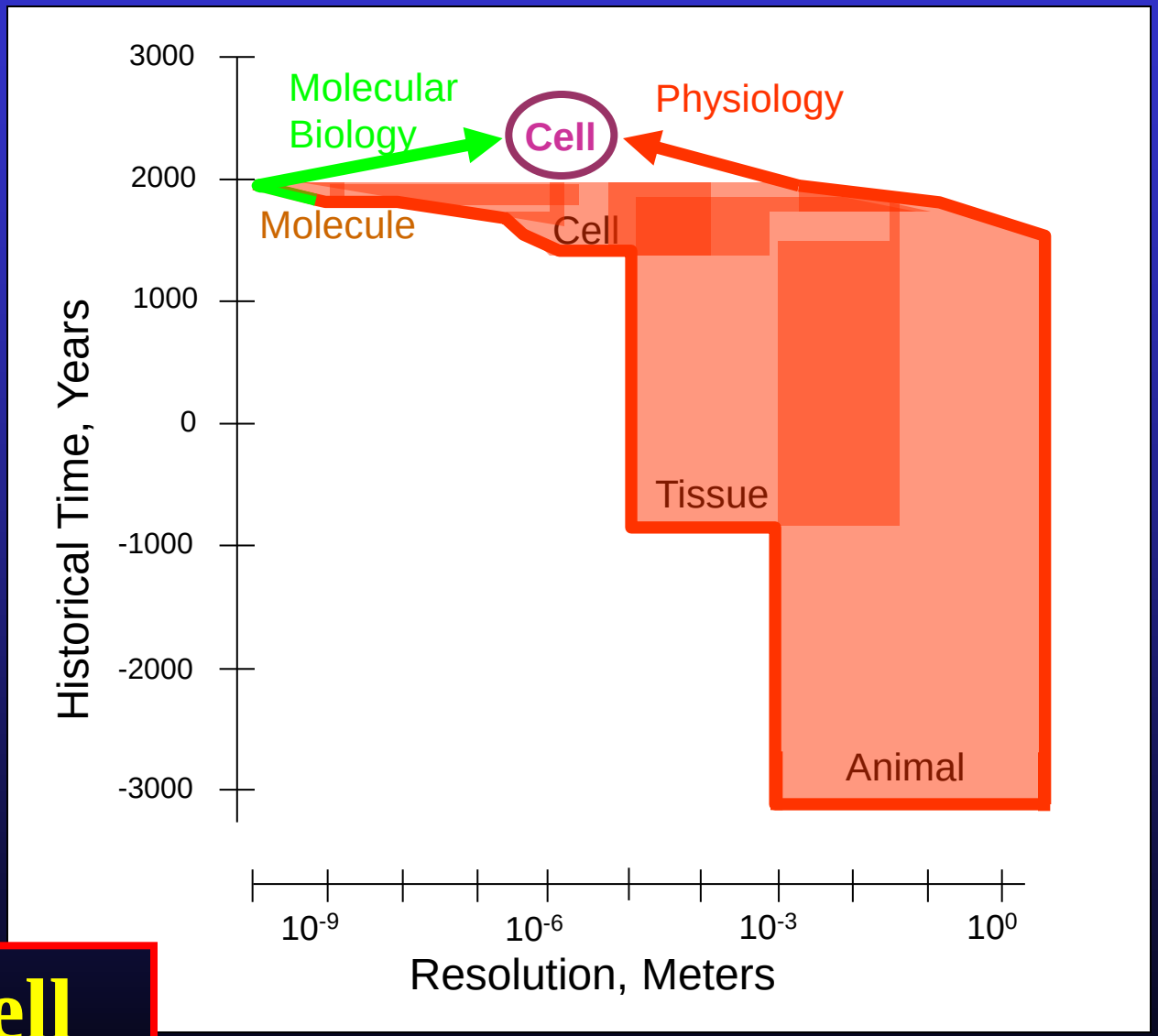


Spatial Resolution in Physiology

X-Ray / SEM / STM
Optical microscope

Magnifying glass

Unaided eye

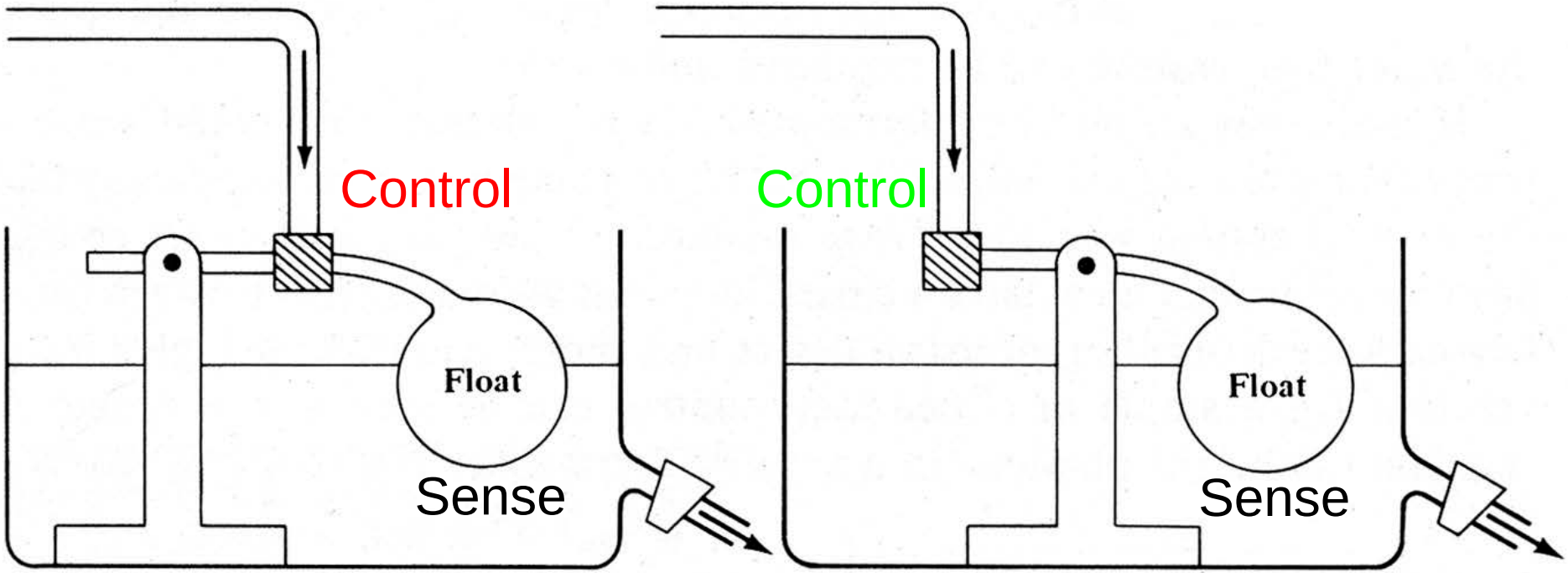


Future = Cell

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



Negative Feedback

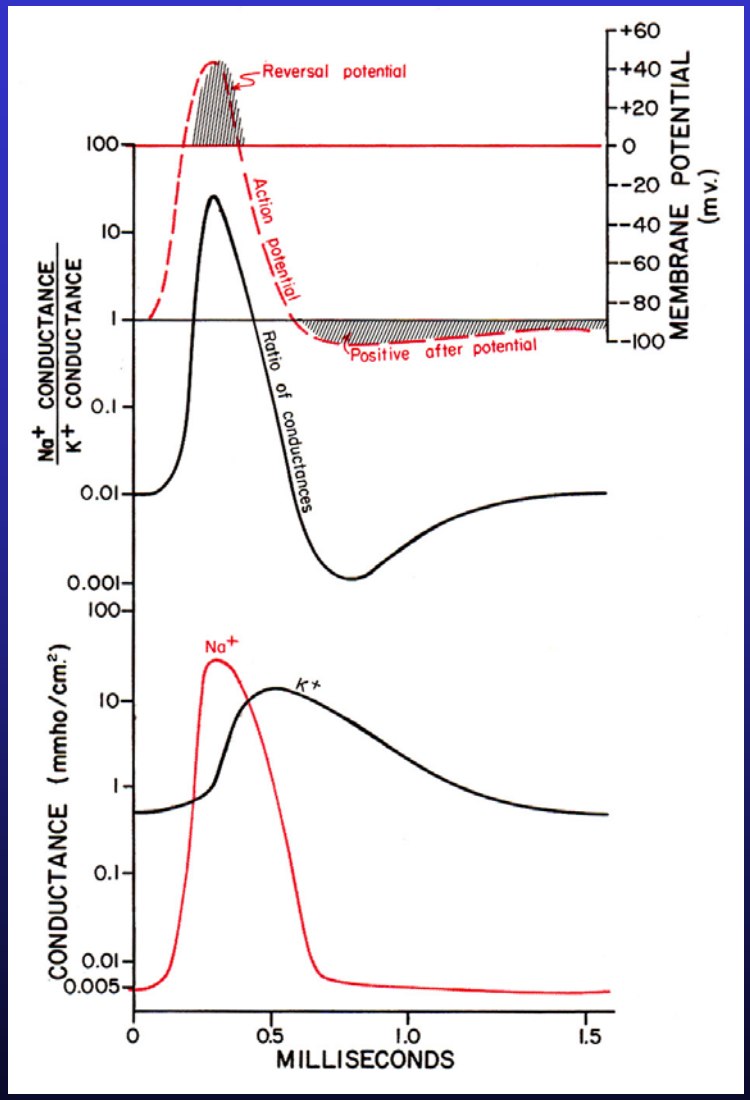
Positive Feedback

Metcalf, Harold J.; Topics in Classical Physics, 1981, Prentice-Hall, Inc., p.108

Opening the Feedback Loop

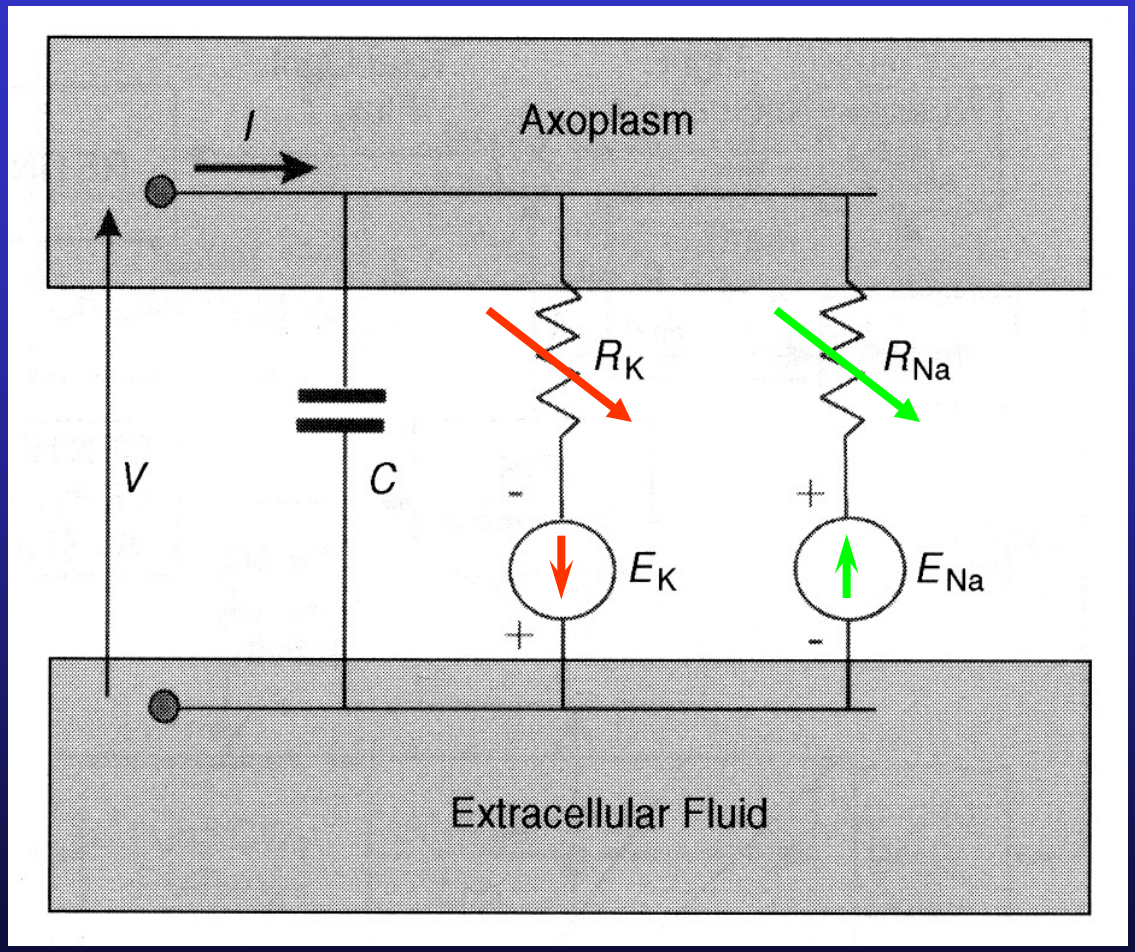
Hypothesis: Great advances in physiology have been made by opening the feedback loop and taking control

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



Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.110

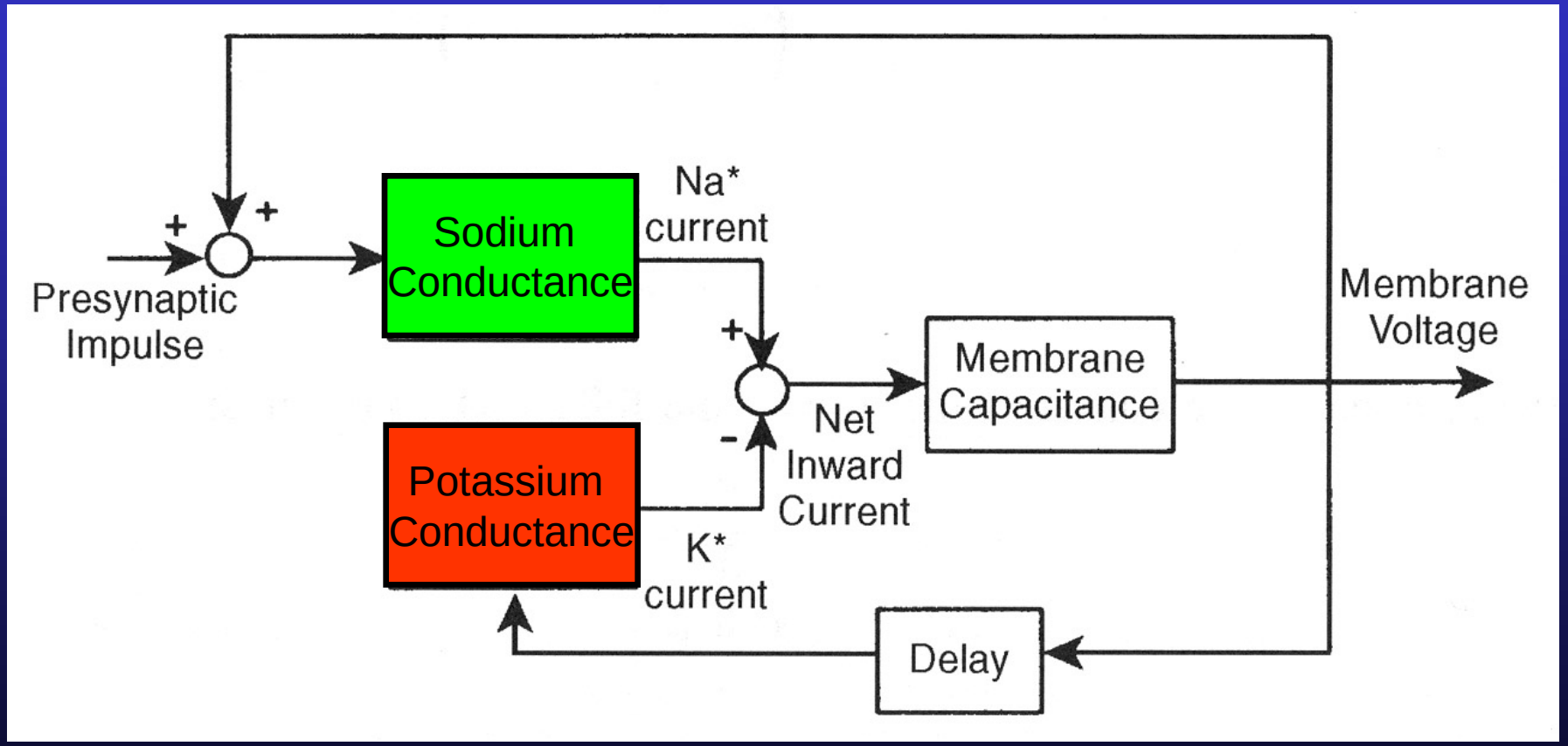
Simplified Hodgkin-Huxley



- 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

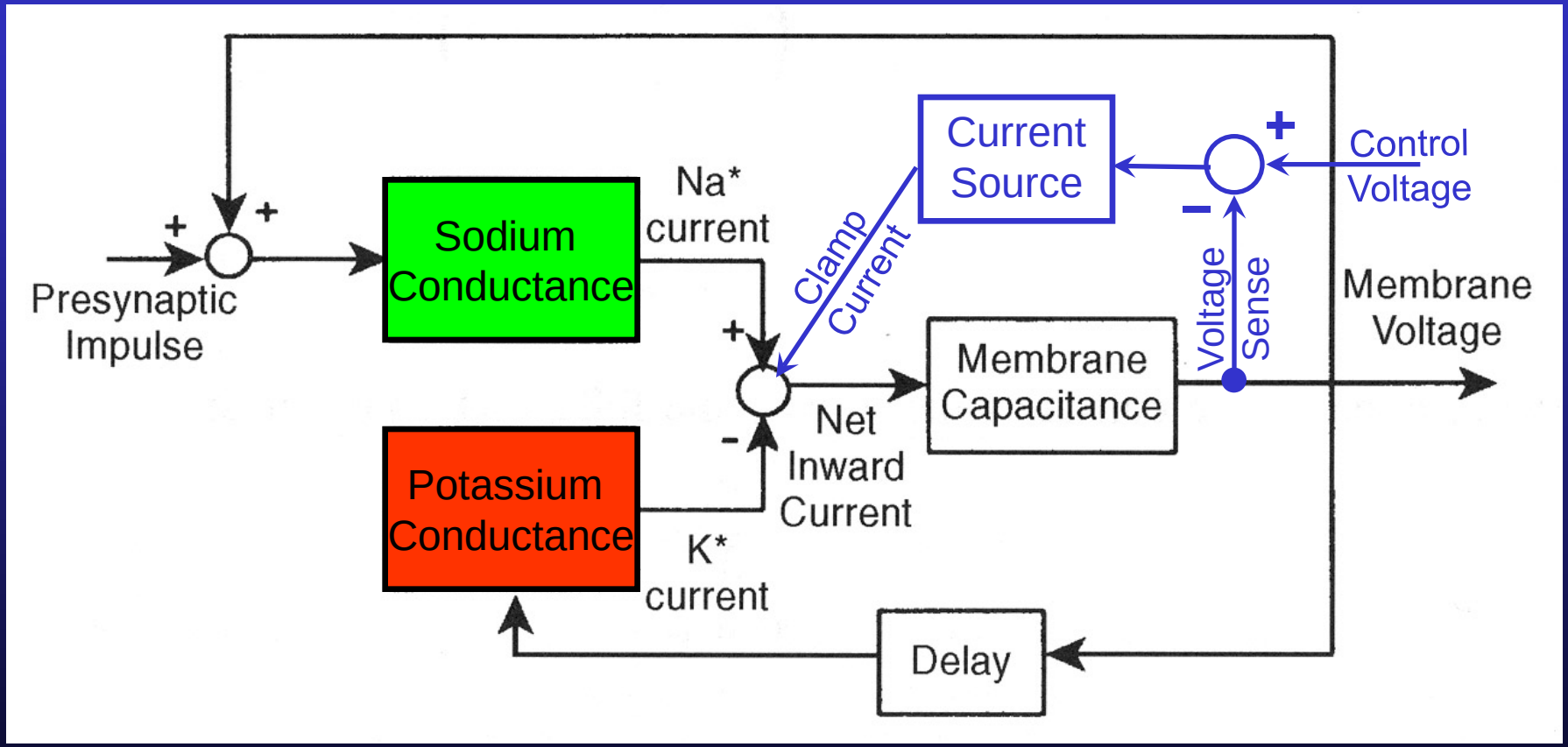
Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.187

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



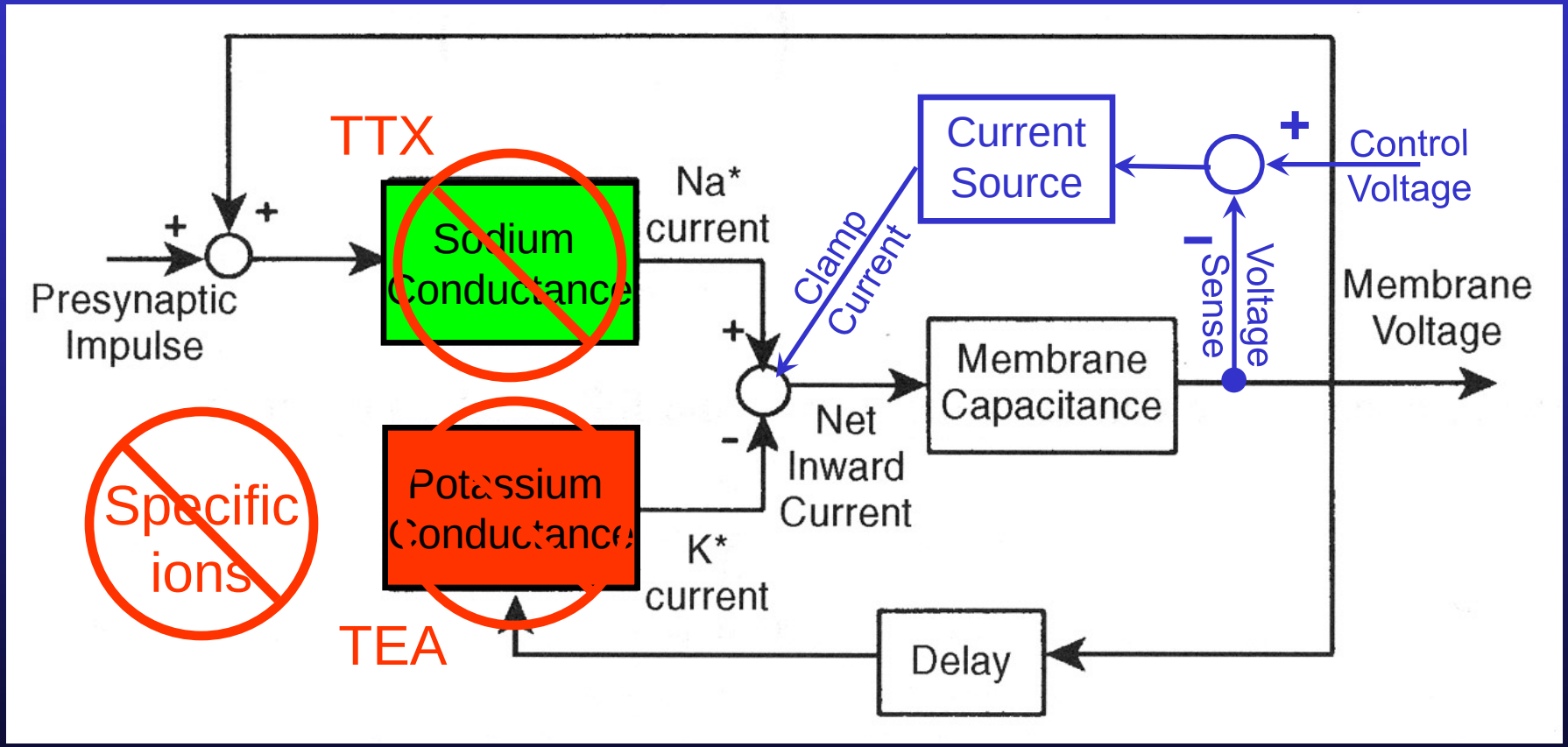
Adapted from Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259

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



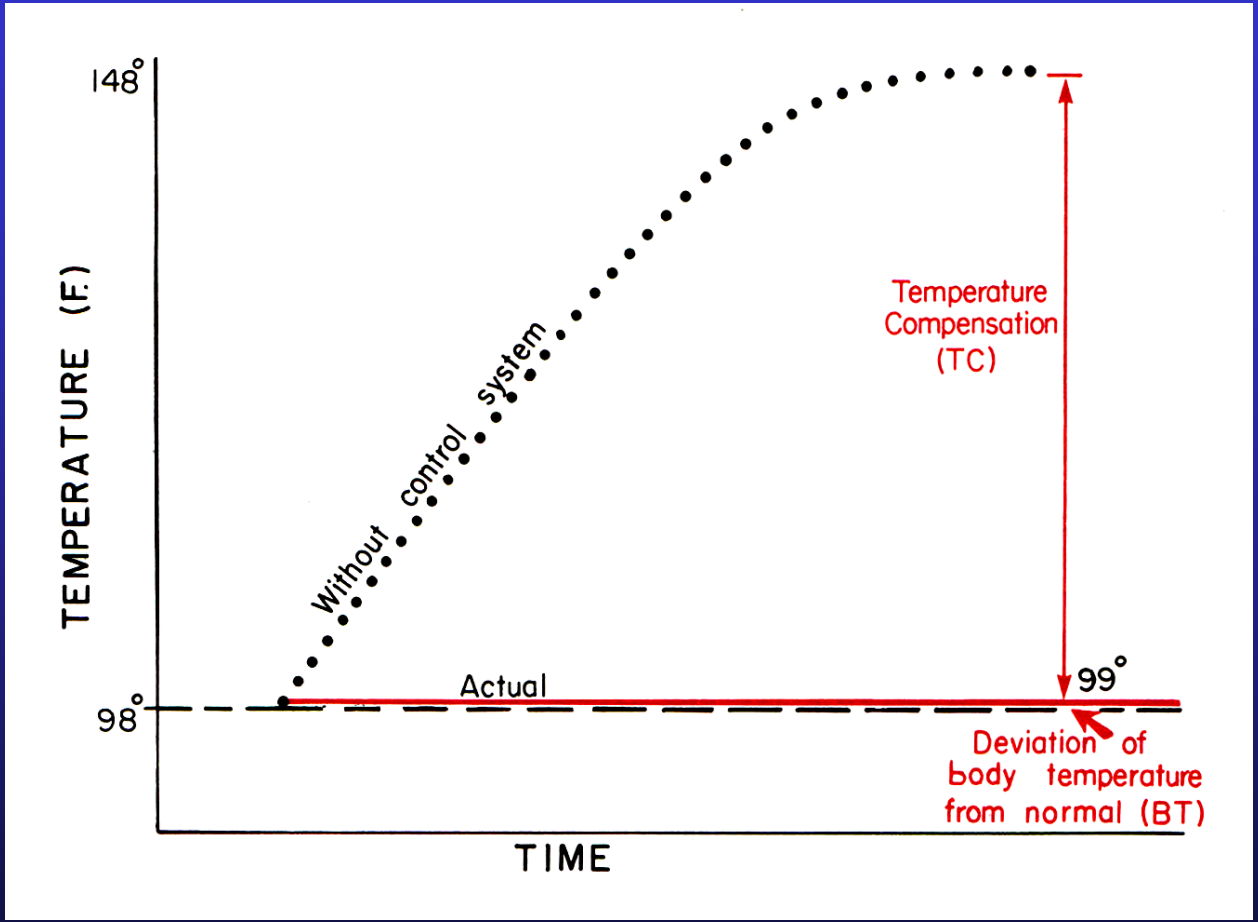
Adapted from Khoo, Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259

A Key to the Future: External Control of Cellular Feedback

- ✓ Electrical
- Mechanical
- Chemical
- Cell-to-cell…

Signatures of Control

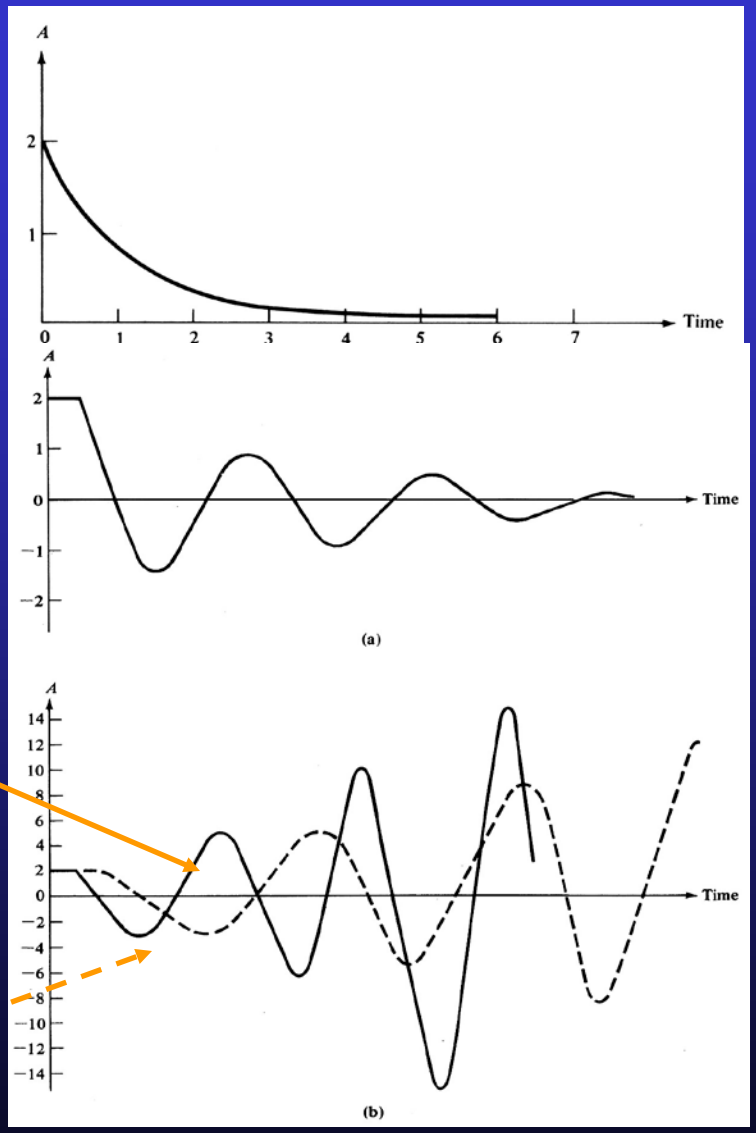
- Stability in the presence of variable input ($\Delta T = 50^{o}$ F)
- Oscillations when excessive delay or too much gain
- Divergent behavior when internal range is exceeded or controls damaged



Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.9

Control Stability

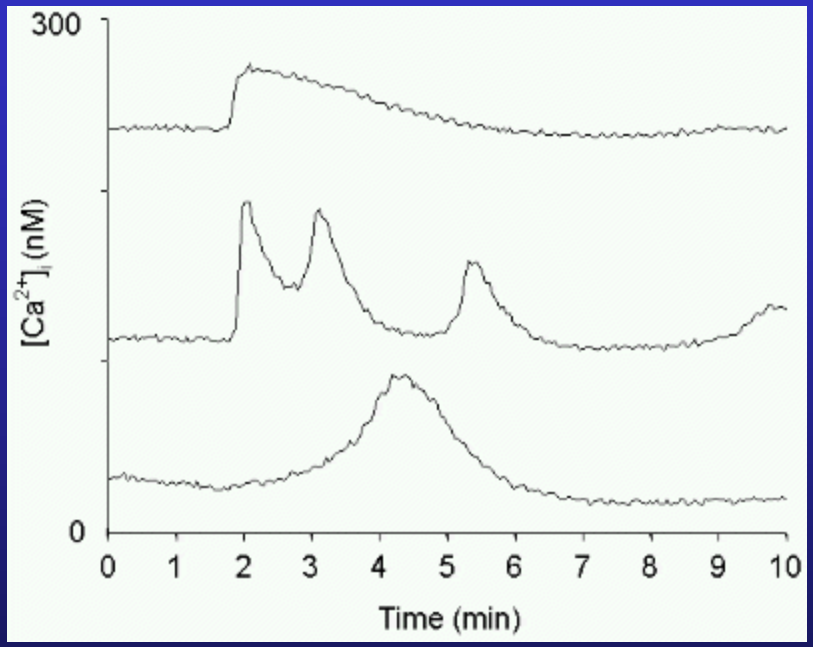
- Proportional control
- Proportional control with finite time delay
- Higher gain, same delay
- Same gain, longer delay



Metcalf, Harold J.; Topics in Classical Physics, 1981, Prentice-Hall, Inc., p.111, p.113

Intracellular Metabolic and Chemical Oscillations

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



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

http://www.intracellular.com/app05.html

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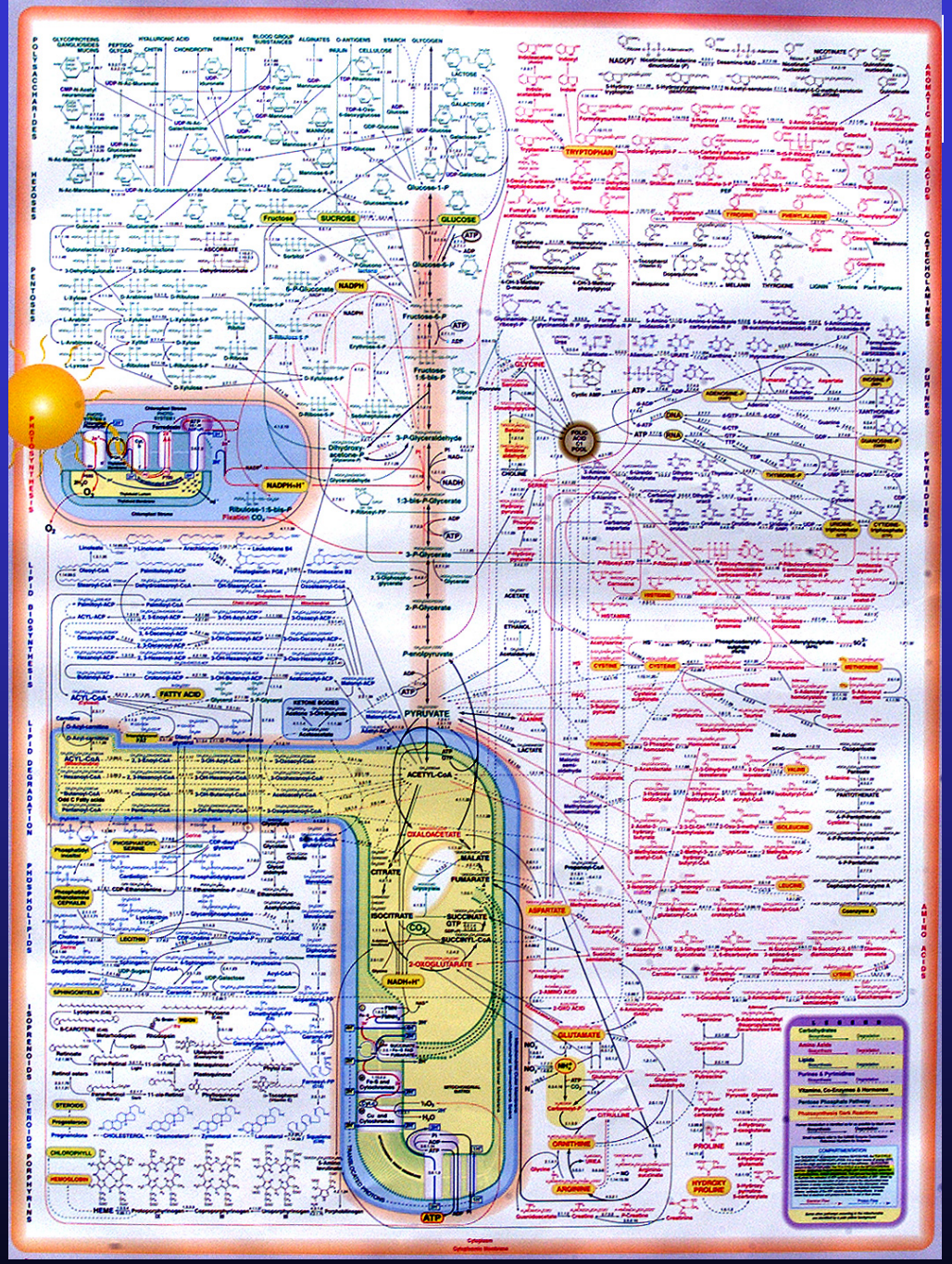
A Key to the Future

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 …

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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**
- **<u>Needed</u>: Multiphasic, dynamical (fast) measurements and models of multi-step processes in complex cellular systems**

Why Fast?

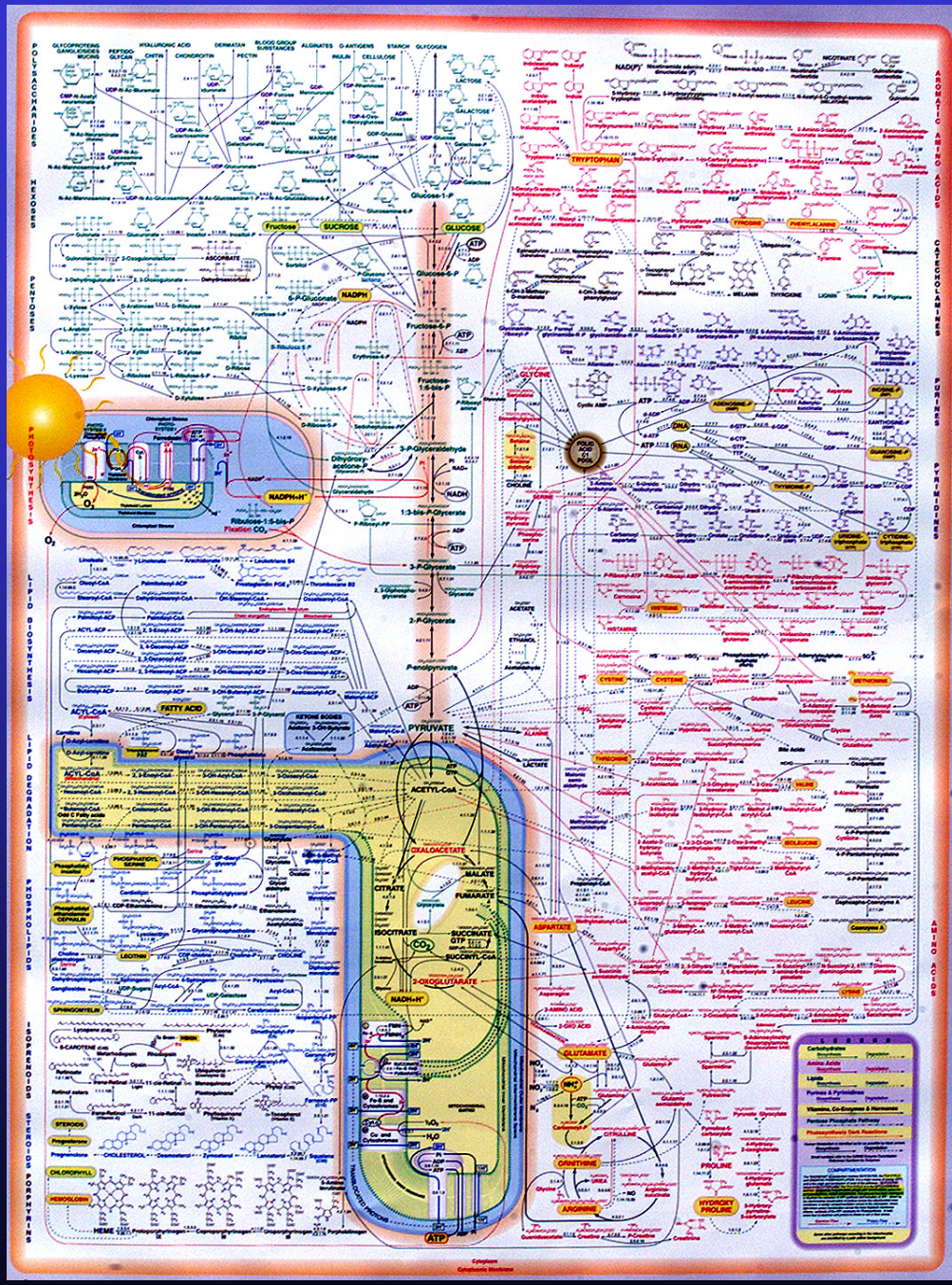
- Cellular-scale biochemistry can be very fast
- Wide measurement bandwidth, i.*e*., good response to high frequencies, is required to track cellular events
- Stable control requires a matching, high bandwidth

Physical and Biological Time Constants, Seconds

Mixing time to homogenize liquid in a large-scale bioreactor (10-100 m^3)	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!



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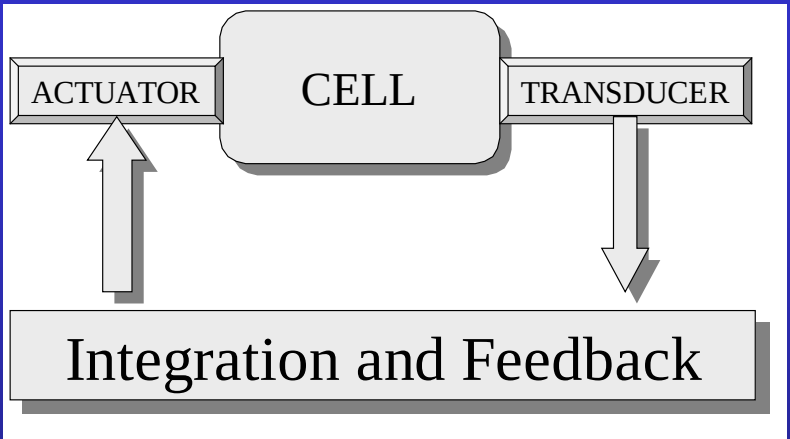
The Problem

- 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

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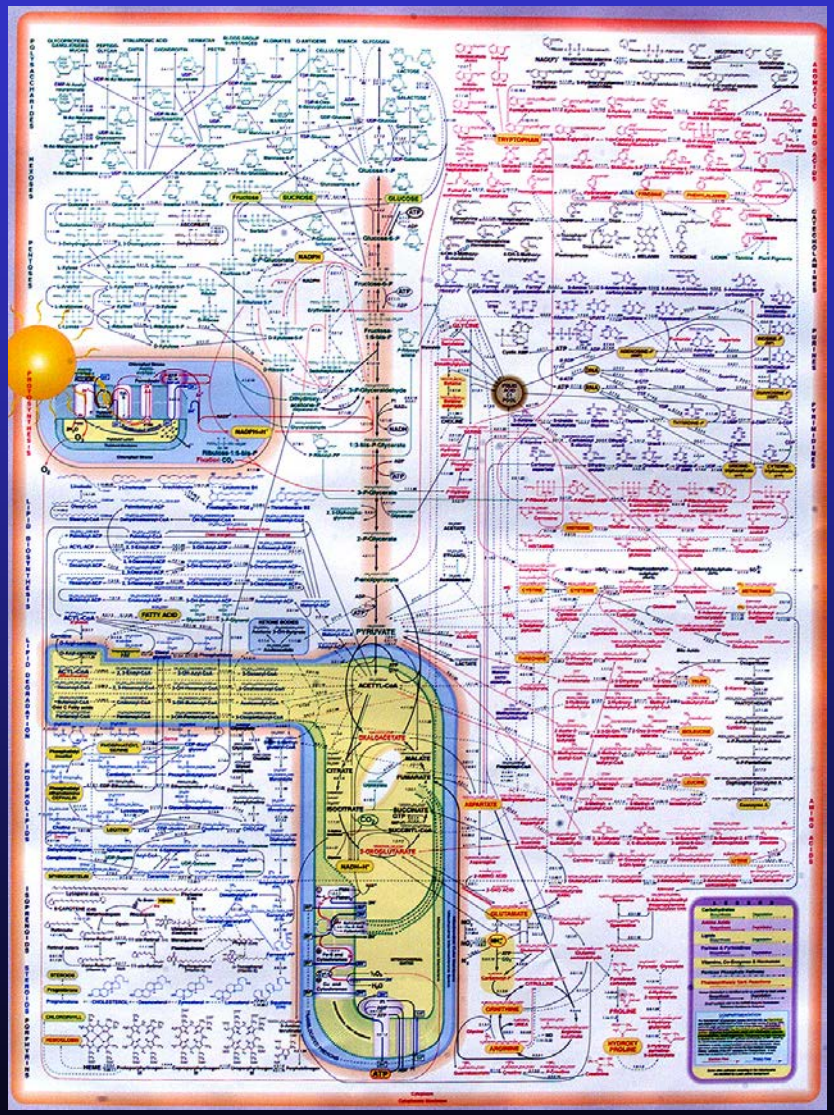
The Modeling Challenge

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



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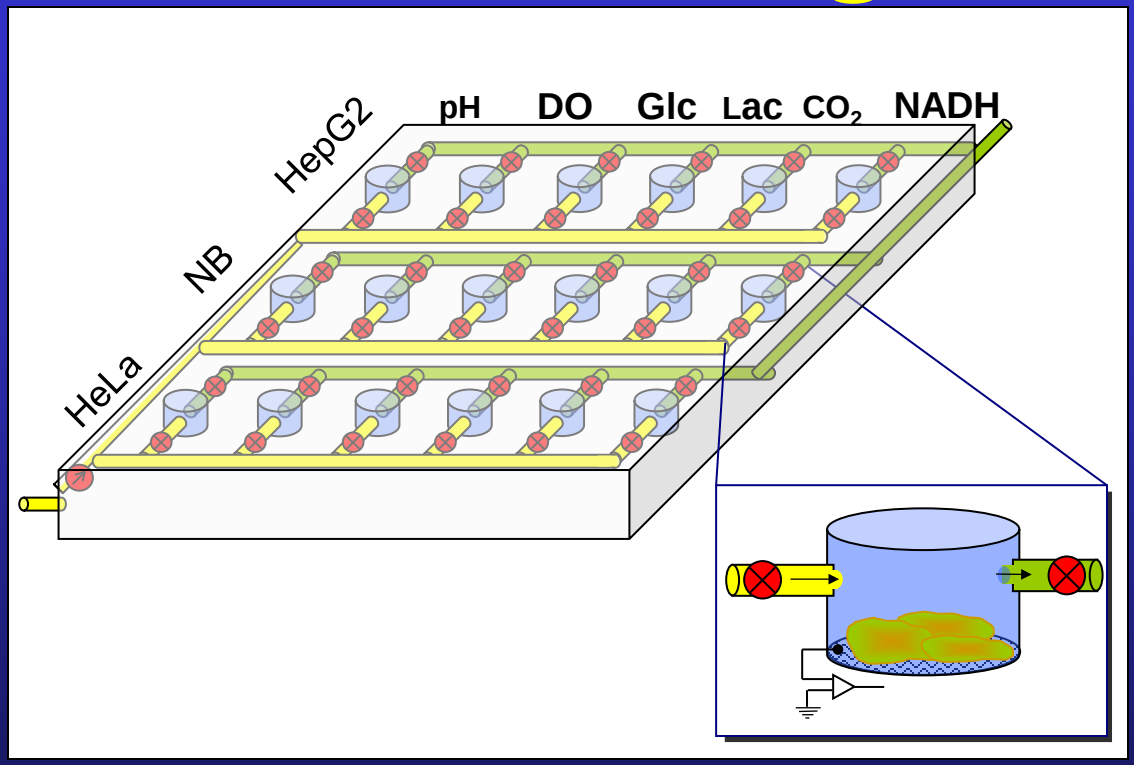
The Experimental Challenge

- ***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