The need for dynamic sensing and control of cells to specify and validate systems biology models

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Abstract

Burgeoning genomic and proteomic data are motivating the development of numerical models for systems biology. However, specification of the almost innumerable dynamic model parameters will require new measurement techniques. The problem is that cellular metabolic reactions and the early steps of intracellular signaling can occur in ms to s, but the 100 s to 100 ks temporal resolution of measurements on milliliter culture dishes and well plates is often limited by diffusion times set by the experimental chamber volume. Hence the instruments themselves must be of cellular dimension to achieve response times commensurate with key intracellular biochemical events, as is done with microelectrode recording of ion-channel conductance fluctuations and fluorescence detection of protein binding. More importantly, lessons from classical physiology suggest that control is as important as sensing, so that at the cellular level nanoactuators and closed-loop external control will be key to testing systems biology models. The engineering challenge is to develop BioMEMS and molecular-scale sensors and actuators to study the breadth of mechanisms involved in intracellular signaling, metabolism, and cell-cell communication.
Acknowledgements

- Mike Ackerman – Nanophysiometer fabrication
- Franz Baudenbacher, Ph.D. – Nanophysiometer and dynamic profiling
- Darryl Bornhop, Ph.D. – Optical detection of protein binding
- Richard Caprioli, Ph.D. – MALDI-TOF and mass spectrometry
- Eric Chancellor -- Picocolorimetry
- David Cliffel, Ph.D. – Cytosensor/electrochemical electrodes
- Elizabeth Dworska – Cell culture
- Sven Eklund – Microphysiometry
- Sebastian Eluvathingal - microfluidic modeling
- Shannon Faley – T-cell activation and signaling
- Todd Giorgio, Ph.D. – messenger recognition
- Igor Ges, Ph.D. – Nanophysiometer fabrication
- Frederick Haselton, Ph.D. – cell culture and protein capture
- Jacek Hawiger, M.D., Ph.D. – T cell activation/intracellular targeting
- Borislav Ivanov – pH sensors
- Duco Jansen, Ph.D. – T-cell activation
- Amanda Kussrow – Optical determination of protein binding
- Eduardo Andrade Lima – Multichannel potentiostats
- Jeremy Norris – MALDI-TOF
- David Piston, Ph.D. – Spectroscopy and fluorescent detection
- Sandra Rosenthal, Ph.D. – Q-Dots
- Phil Samson – Microscopy, microfluidics, and cell lysing
- David Schaffer – Nanophysiometer fabrication
- Mark Stremler – Microfluidic modeling
- Ian Thomlinson, Ph.D., – Q-Dots
- Roy Thompson, ECBC/Aberdeen – Class A toxin studies
- Momchil Velkovsky, Ph.D. – Statistical Analysis
- Mike Warnement – Glow in the dark
- Andreas Werdich – Cardiac nanophysiometer
- DARPA, AFOSR, NIH, Vanderbilt
Definition

Systems Biology is … quantitative, postgenomic, postproteomic, dynamic, multiscale physiology
Theme I

The complexity of postreductionist biology
Step 1 in Science: 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

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

Resolution, Meters:
- $10^{-9}$
- $10^{-6}$
- $10^{-3}$
- $10^0$

Historical Time, Years:
- $-3000$
- $-2000$
- $-1000$
- $0$
- $1000$
- $2000$
- $3000$

Levels of Analysis:
- Molecule
- Cell
- Tissue
- Animal
- Systems Biology
- Molecular Biology
The Problems

• Our understanding of biological phenomena is often based upon
  – experiments that measure the ensemble averages of populations of $10^6$ – $10^7$ cells, or
  – measurements of a single variable while all other variables are hopefully held constant, or
  – recordings of one variable on one cell, or
  – averages over minutes to hours, or
  – combinations of some of the above, as with a 10 liter bioreactor that measures 50 variables after a one-week reactor equilibration to steady state.

• Genomics is providing an exponential growth in biological information
The rate at which DNA sequences began accumulating was exponential.

- ~13 million sequence entries in GenBank
- Nearly 13 billion bases from ~50,000 species

Human Genome Project begun
Rapid DNA sequencing invented

Year

2002: 22,318,883


Courtesy of Mark Boguski
Moore’s Law vs. Growth of GenBank

Transistors/chip
DNA Sequences

Courtesy of Mark Boguski
Step 2 in Science: Post-Reductionism

Thermodynamics
Statistical mechanics
Molecular/atomic dynamics
Electrodynamics
Quantum Chromodynamics

Bulk solids
Devices
Continuum models
Microscopic models
Atomic physics

Behavior
Systems Biology
Physiology
Systems Biology
Organ
Systems Biology
Cell
Systems Biology
Protein
Structural Biology
Genome

P-P Cross-Section at low P
Si Step Edge Diffusion
Key Questions in Systems Biology

• Given the shockwave of genetic and proteomic data that is hitting us, **what are the possible limitations of computer models being developed for systems biology?**

• What are promising approaches?
  – Multiphasic, *dynamic* cellular instrumentation
  – Exhaustively realistic versus minimal models
  – Dynamic network analysis
Suppose you wanted to calculate how the cell responds to a toxin...
The complexity of eukaryotic gene transcription control mechanisms

Pol II-Mediated mRNA Gene Transcription is Controlled by the Coordinated Action of Multiple Co-Regulators Acting on Different "Targets"

Courtesy of Tony Weil, MPB, Vanderbilt
Molecular Interaction Map: Cell Cycle

Molecular Interaction Map: DNA Repair

Proteins as Intracellular Signals

A cell expresses between 10,000 to 15,000 proteins at any one time for four types of activities:

• Metabolic
• Maintaining integrity of subcellular structures
• Intracellular signaling
• Producing signals for other cells
MALDI-TOF: Cells express a lot of proteins…

Courtesy of Richard Caprioli,
Mass Spectrometry Research Center
Vanderbilt University
G-Protein Coupled Receptors

Light, Ca++, Odorants, Pheromones, Small molecules:
- amino-acids, amines
- nucleotides, nucleosides
- prostaglandins, PAF
- peptides...

Proteins:
- TSH
- LH
- FSH
- interleukins
- wingless
- chemokines
- α-latrotoxin

Effector:
- enzyme
- channels...

Intracellular messengers

G protein

Courtesy of Heidi Hamm
Pharmacology, Vanderbilt
The Time Scales of Systems Biology

- $10^9$ s Aging
- $10^8$ s Survival with CHF
- $10^7$ s Bone healing
- $10^6$ s Small wound healing
- $10^5$ s Atrial remodeling with AF
- $10^4$ s
- $10^3$ s Cell proliferation; DNA replication
- $10^2$ s Protein synthesis
- $10^1$ s Allosteric enzyme control; life with VF
- $10^0$ s Heartbeat
- $10^{-1}$ s Glycolosis
- $10^{-2}$ s Oxidative phosphorylation in mitochondria
- $10^{-3}$ s
- $10^{-4}$ s Intracellular diffusion, enzymatic reactions
- $10^{-5}$ s
- $10^{-6}$ s Receptor-ligand, enzyme-substrate reactions
- $10^{-7}$ s
- $10^{-8}$ s Ion channel gating
- $10^{-9}$ s
“A cell is a well-stirred bioreactor enclosed by a lipid envelope”....

Sure....
3.1 x 3.2 µm³

- ER, yellow;
- Membrane-bound ribosomes, blue;
- free ribosomes, orange;
- Microtubules, bright green;
- dense core vesicles, bright blue;
- Clathrin-negative vesicles, white;
- Clathrin-positive compartments and vesicles, bright red;
- Clathrin-negative compartments and vesicles, purple;
- Mitochondria, dark green.

“A cell is a well-stirred bioreactor enclosed by a lipid envelope”…

ODEs become PDEs …

Lots and lots and lots of PDEs
Suppose you wanted to **calculate** how the cell responds to a toxin...

- Specify concentrations and
- Rate constants
- Add gene expression,
- Protein\(^N\) interactions, and
- Signaling pathways
- Time dependencies
- Include intracellular spatial distributions, diffusion, and transport: ODE → PDE(t)

... and then you can **calculate** how the cell behaves in response to a toxin.
The Catch

• Modeling of a single mammalian cell may require >100,000 *dynamic* variables and equations

• Cell-cell interactions are critical to system function

• $10^9$ interacting cells in some organs

• Cell signaling is a highly *DYNAMIC*, multi-pathway process

• Many of the interactions are non-linear

• **The data don’t yet exist to drive the models**

• Hence we need to **experiment…**
The Grand Challenge

A cell expresses between 10,000 to 15,000 proteins at any one time for four types of activities:

• Metabolic
• Maintaining integrity of subcellular structures
• Intracellular signaling
• Producing signals for other cells.

There are no technologies that allow the measurement of a hundred, time dependent, intracellular variables in a single cell (and their correlation with cellular signaling and metabolic dynamics), or between groups of different cells.
Theme II

Instrumenting the Single Cell

**Goal:** Develop devices, algorithms, and measurement techniques that will allow us to instrument single cells and small populations of cells and thereby explore the complexities of quantitative, experimental systems biology.
## Sizes, Volumes, Diffusion Time Constants

<table>
<thead>
<tr>
<th>X</th>
<th>V, m³</th>
<th>V</th>
<th>Tau\text{Diff}</th>
<th>Example</th>
<th>N</th>
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<tr>
<td>1 m</td>
<td>1</td>
<td>1000 L</td>
<td>10⁹ s</td>
<td>Animal, bioreactor</td>
<td>100</td>
</tr>
<tr>
<td>10 cm</td>
<td>10⁻³</td>
<td>1 L</td>
<td>10⁷ s</td>
<td>Organ, bioreactor</td>
<td>100</td>
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<tr>
<td>1 cm</td>
<td>10⁻⁶</td>
<td>1 mL</td>
<td>10⁵ s = 1 day</td>
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<td>10</td>
</tr>
<tr>
<td>1 mm</td>
<td>10⁻⁹</td>
<td>1 uL</td>
<td>10³ s</td>
<td>µenviron, well plate</td>
<td>10</td>
</tr>
<tr>
<td>100 um</td>
<td>10⁻¹²</td>
<td>1 nL</td>
<td>10 s</td>
<td>Cell-cell signaling</td>
<td>5</td>
</tr>
<tr>
<td>10 um</td>
<td>10⁻¹⁵</td>
<td>1 pL</td>
<td>0.1 s</td>
<td>Cell</td>
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</tr>
<tr>
<td>1 um</td>
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<td>1 fL</td>
<td>1 ms</td>
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<tr>
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<td>10⁻²¹</td>
<td>1 aL</td>
<td>10 us</td>
<td>Organelle</td>
<td>2</td>
</tr>
<tr>
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<td>10⁻²⁴</td>
<td>1 zL</td>
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<tr>
<td>1 nm</td>
<td>10⁻²⁷</td>
<td>1 npL</td>
<td>1 ns</td>
<td>Ion channel</td>
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</tbody>
</table>
High-Content Toxicology Screening Using Massively Parallel, Multi-Phasic Cellular Biological Activity Detectors MP$^2$-CBAD

F Baudenbacher, R Balcarcel, D Cliffel, S Eklund, I Ges, O McGuinness, A Prokop, R Reiserer, D Schaffer, M Stremler, R Thompson, A Werdich, and JP Wikswo

Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE)
Edgewood Chemical and Biological Center (SBCCOM / ECBC)
MP²-CBAD Discrimination

Discrimination Matrix

Sensor Array

Output

Cell Types

HepG2

HeLa

NB

Toxin

pH

DO

Glc

Lac

CO₂

NADH

NanoPhysiometer
Simplified Metabolic Network

**Glucose**

- Glycolysis
- NADH
- Oxidative Phosphorylation
- Acidification
- Lactate
- CO₂
- NAD+
- TCA Cycle
- NADPH
- Oxidase
- FAD
- GDP
- 3 NADH
- 3 NADH + NAD(P)H
- 0.5 O₂ + 3 ADP + NADH
- 3 ATP + NAD⁺
- 0.5 O₂ + 2 ADP + FADH₂
- 2 ATP + FAD

**Oxygen**

- NADPH Oxidase

**Heat**

- Glucose + 2 ADP + 2 NAD⁺ → 2 Pyruvate + 2 ATP + 2 NADH
- Pyruvate + NADH → Lactate + NAD⁺
- Pyruvate + CoA + FAD + GDP + 3 NAD⁺ + NAD(P)⁺ → 3 CO₂ + FADH₂ + GTP + 3 NADH + NAD(P)H
- 0.5 O₂ + 3 ADP + NADH → 3 ATP + NAD⁺
The well size determines the bandwidth

- Microliter – 10-100 seconds
  Modified Cytosensor MicroPhysiometer

- SubNanoliter – 10-100 milliseconds
  Vanderbilt NanoPhysiometer
Multianalyte Microphysiometry

- The Multianalyte MicroPhysiometer (MMP) serves as a platform for studying large numbers of cells simultaneously.
- Upon activation, we can measure acidification rate, \(O_2\), lactate, glucose with \(\sim 1\) minute resolution.

**MicroPhysiometer: Modified sensor head**

Schematic drawing of modified sensor head for the microliter Molecular Devices Cytosensor microphysiometer.
Multianalyte Microphysiometry for Biotoxin Discrimination

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CHO cells with 720 s of 20 mM fluoride.

S.E. Eklund, D.E. Cliffel, et al.,

Automated Data Acquisition and Analysis
Eduardo Lima, Momchil Velkovsky, Ron Reiserer, et al.
3D Sensor Modeling
Mark Stremler and Sebastian Eluvathingal

- Production or consumption of analytes by cells in the microphysiometer chamber
- Convective-diffusive transport of analytes through the fluid
- Consumption of analyte by the electrochemical sensors
- Computational model built with CFD-ACE+ (CFD Research Corporation)

**Electrochemical Sensors:**
- Zero concentration at surface
- Sensor signal proportional to concentration gradient at surface
- Customizable location, geometry

**Flow Inlet:**
- Inlet velocity specified
- Specified analyte concentrations

**Channel Walls:**
- Impermeable

**Cells on Bottom Surface:**
- Uniform cell distribution
- Membrane fluxes specified

**Flow Outlet**

**O₂ Concentration Flush-Out**

**O₂ Concentration: Sensing Sequence**

- 10 seconds
- 30 seconds
The well size determines the bandwidth

- **Microliter** – 10-100 seconds
  Modified Cytosensor MicroPhysiometer

- **SubNanoliter** – 10-100 milliseconds
  Vanderbilt NanoPhysiometer
Lactate Diffusion Times

**Linear Dimension, microns**

Volume, liters

Diffusion Time, seconds

- Smaller = much faster
- mL = 3 x 10^4 sec
- µL = 300 sec
- nL = 3 sec
- pL = 30 msec
PDMS Soft Lithography
The Multianalyte NanoPhysiometer (MNP) will serve as a platform for studying, one at a time, large numbers of single cells.

Upon activation, we will measure pH, O, V_m, [Ca], lactate, glucose, Q-Dot binding.
Microelectrodes to measure extracellular potentials and stimulate cells

Optical fiber array to measure propagating calcium waves in a single cardiomyocyte

Microfabricated pH Electrodes

A) pH electrodes
B) pH calibration
C) Reference electrode
D) Calibration device
E) Temporal response to a 1 pH step change.
F) and G) Stop-flow acidification for A9L HD2 fibroblasts and M3 WT4 CHO cells

Ges et al., Biosensors and Bioelectronics, in press
First Generation Autoloading NanoPhysiometer

A. Prokop, et al.,

- Biological and Bioinspired Materials and Devices, MRS, 2004,

Goal: Instrumented bioreactors with individually addressable traps
Activated Primary T cells Labeled with anti-IL2Rα QDs in Traps
Shannon Faley, Mike Warnement, et al.
Optical Detection of Protein Binding: On-Chip Interferometric Backscatter Detector (OCIBD)  
Darryl Bornhop et al

- Optical interferometry to detect protein binding within the channel just downstream of the NanoPhysiometer

- 495 pL detection volume
- $7 \times 10^{-8}$ RIU detection limit
- 40 pM, $1.8 \cdot 10^{-20}$ mole; 10,800 molecules; 280 fg of IL-2
- This well within the anticipated range of IL-2 production by a single cell (10 pM).
Nanophysiometer Modeling
Mark Stremler et al.

- 3D computational model:

**Sensor:**
- 10 µm wide, 100 µm long
- Zero concentration at surface
- Sensor flux proportional to current

**Channel Walls:**
- No transport
- Zero velocity condition

**Inlet Flow:**
- Specified flowrate, velocity profile
- Specified concentrations
- Upstream diffusion allowed

- Possible device flow and sensing scenarios:

<table>
<thead>
<tr>
<th>Flow</th>
<th>Sensing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>Continuous</td>
</tr>
<tr>
<td>Intermittent</td>
<td>Intermittent</td>
</tr>
</tbody>
</table>
Inverse Sensor Model

• Model diffusion and reactions within the polymer matrix of the sensor.
• Enzyme concentration within the sensor assumed uniform.
• Production of $\text{H}_2\text{O}_2$ within sensor modeled with Michaelis-Menten kinetics.
• Sensor signal given by gradient of $\text{H}_2\text{O}_2$ at the surface.
• Model implemented analytically and with CFD-ACE

\[ \text{Substrate: } (\partial C_s / \partial n) = 0 \text{ on surface, } C_{\text{H}_2\text{O}_2} = 0 \text{ in bulk} \]

\[ \text{H}_2\text{O}_2 \rightarrow \text{Sensor matrix} \]

\[ \text{S} + E_1 \rightarrow \text{H}_2\text{O}_2 + E_2 \]

\[ \text{Fluid: analyte S carried to sensor by diffusion} \]
The Next Steps

- Inverse sensor model
- Inverse metabolic network model
- Additional metabolic parameters
- Apply experiments, models and analysis to examine the blocking or enhancing of metabolic pathways
OBJECTIVE: Use microfabricated picocalorimeters to measure the heat from single-cell metabolism, droplet evaporation, and protein denaturation.

METHODS: A custom-fabricated picocalorimeter has a sensitivity of 6 V/W, a 1 ms response time, 80 nV of noise, and a power sensitivity of 14 nW/Hz\(^{1/2}\). It can detect with \(~100:1\) S/N the evaporation of a 100 pL drop of water in < 1 sec. We will use a picoliter injector to add urea to a droplet of concentrated protein and measure the incremental heat of denaturation.

Theme III
Instrumenting and Controlling The Single Cell

**Goal:** Develop devices, algorithms, and measurement techniques that will allow us to instrument and control single cells and small populations of cells and thereby explore the complexities of quantitative, experimental systems biology.
How do we study cellular-level responses to stimuli in both normal and pathophysiologic 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

Hypoxia-Red Blood Cell Concentration

Variables
- Erythropoietin E
- Hypoxia A
- RBC

Glucose-Insulin Control

Opening the Feedback Loop

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

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

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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
Voltage clamp of the nerve axon

How do we study cellular-level responses to stimuli in both normal and pathophysiologic conditions?

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

Required: New devices to seize control of subsecond, submicron cellular processes.
A Key to the Future of Systems Biology: External Control of Cellular Feedback

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

- Stability in the presence of variable input (DT = 50° 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

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
  – Repair enzymes

• Prediction: At higher bandwidths than provided by present instrumentation, we will see in biosystems other chemical bursts, oscillations, and chaotic behavior. FIND THEM, USE THEM!

http://www.intracellular.com/app05.html
OBJECTIVE: Identify the mechanisms for intraislet signaling in glucose-stimulated insulin release

METHODS: Trap an intact pancreatic in a microfluidic channel that allows independent control of the glucose levels on opposite sides of the islet. Apply glucose to one side of the islet and look for calcium release on the opposite side.

Spatially Restricted Glucose Produces Spatially Restricted Calcium Oscillations

Mobility of Protozoa through Microchannels
W.Wang, L.Shor, E.LeBoeuf, D.Kosson, J. Wikswo

*Keronopsis* sp. squeezes through 20 mm constriction.

*MOVIE* *Euplotes vannus* entering a 20 x 20 mm channel (40 sec).
Ok, we’re convinced about feedback and control....

What do we need to study cellular dynamics?
What Do We Need to Study Cellular Dynamics?

- Multiple, fast sensors
- Intra- and extracellular actuators for controlled perturbations
- Openers (Mutations, siRNA, drugs) for the internal feedback loops
- System algorithms and models that allow you to close and stabilize the external feedback loop
- ...
Short-term goal: Measure ~ 10 dynamic variables from a single cell with sub-second response!
Quantum Dots to Report Protein Presence

- Quantum dots can be conjugated to an antibody that then binds to a membrane protein
QD Detection of Gene Upregulation
Activated Jurkat Cells Labeled with IL-2Ab Conjugated QDots
Shannon Faley, Mike Warnement, et al.

- **Red**: Anti-IL2 QDots
- **Green**: Yopro-1 nucleic acid stain (i.e. non-viable cells)
- Activated using PMA & Ionomycin for 72 hrs
- QDots label 50-70% of viable activated cells
Quantum Dot Quenching for Detection of Protein Binding and Enzyme Activity
Metal Nanoshells as Substrates for Surface-Enhanced Raman Spectroscopy

- $10^{12}$ Raman enhancement
  - optically-addressable intracellular nanothermometer?
- Molecular (vibrational) spectroscopy for protein identification and nanoparticle labeling, (Cullum at U. Maryland, Baltimore)
We need more cellular nanosensors!!
What about the cellular nanocontrollers/nanoactuators?
What should our cellular controllers look like?

They should be very, very small...

and very, very fast
Targeted Optical Delivery of Heat or Charge

- Metallic NanoShells (Halas at Rice, Cliffel at Vanderbilt, Tomchek at UES, ....)
- Infrared heating by bioconjugate nanoshells
  - Local control of enzymatic reactions
  - Selected destruction of tagged organalles
Magnetic Nanoparticles

- Translational and rotational forces
  - Viscosity -- Nanorheometry
  - Molecular motor characterization
- Magnetic separation
- Magnetic identification
  - Tagged cells
  - Tagged molecules
We need more cellular nanoactuators!!
What is the cellular sensor/actuator competition?

Proteins, proteins, proteins…
Bacterial Photosynthetic Reaction Center


(b)
Calcium Control of Conductance

Molecular Cell Biology, 2nd ed.
Darnell, J.; Lodish, H.; Baltimore,
W.H Freeman & Co. 1990, p.525
Gap Junctions

The Ultimate NanoMachine: 
The 1 nm pore in a gated ion channel
Cells have LOTS of different ion channels that serve as sensors and actuators!

Ion currents and ion channel clones

Current
- sodium current
- L-type calcium current
- T-type calcium current
- Na-Ca exchange
- \( I_{\text{to1}} \) (4-AP-sensitive)
- \( I_{\text{to2}} \) (Ca-activated)
- \( I_{\text{k2}} \)
- \( I_{\text{or l}} \)
- \( I_{\text{inward rectifier}} \)
- \( I_{\text{p}} \) (pacemaker current)

Probable clone
- H1, SCN5A*
- √*
- √
- Na-Ca exchanger
- Kv4.3 (?1.2, 1.4, 1.5, 2.1, 4.2)*
- --
- KvLQT1 + minK (IsK)
- HERG + MiRP1
- Kv1.5
- CFTR, TWIK (?others)
- Kir2.x
- Kir3.1/3.4; Kir6.x/SUR
- hCNG

*+sub-units
The Ultimate Instrumentation Question for Systems Biology

Can we develop nanodevices that allow *sensing and control* of cellular functions more effectively than natural or bioengineered proteins, but also provide *readout and external control*?
Sizes, Volumes, Time Constants

<table>
<thead>
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<th>X</th>
<th>V, m³</th>
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<td>10&lt;sup&gt;7&lt;/sup&gt; s</td>
<td>Organ, bioreactor</td>
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<td>1 mL</td>
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<td>10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>1 nL</td>
<td>10 s</td>
<td>Cell-cell signaling</td>
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<td>10&lt;sup&gt;-15&lt;/sup&gt;</td>
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<td>1 um</td>
<td>10&lt;sup&gt;-18&lt;/sup&gt;</td>
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<td>100 nm</td>
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</table>
Then…. Statistical Analysis of Activation Responses

- Correlations of protein expression and dynamical state
- Effective metabolic and signaling model
  - Metabolic Flux Analysis is primarily steady state
  - Dynamic measurements require dynamic network models
    - Accumulation and depletion of intracellular stores in short times
    - Enzyme concentrations fixed in the intermediate time period
  - Inverse analysis of exact models is intractable, so effective models are required
The Payoff

• The simultaneous measurement of the dynamics of a hundred intracellular variables will allow an unprecedented advance in our understanding of the response of living cells to pharmaceuticals, cellular or environmental toxins, CBW agents, and the drugs that are used for toxin prophylaxis and treatment.

• The general application of this technology will support the development of new drugs, the screening for unwanted drug side effects, and the assessment of yet-unknown effects of environmental toxins.
– Systems Biology –

The Ultimate Sensor and Actuator Challenge for the 21st Century