Keynote Lecture

Instrumentation Challenges for Systems Biology

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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 to 100k s 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. 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 -- picocalorimetry
- **David Cliffel, Ph.D. – Cytosensor/electrochemical electrodes**
- Elizabeth Dworska – Cell culture
- **Sven Eklund -- Microphysiometry**
- **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
- Phil Samson – Microscopy, microfluidics, and cell lysing
- David Piston, Ph.D. – Spectroscopy and fluorescent detection
- Sandra Rosenthal, Ph.D. – Q-Dots
- **David Schaffer – Nanophysiometer fabrication**
- 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

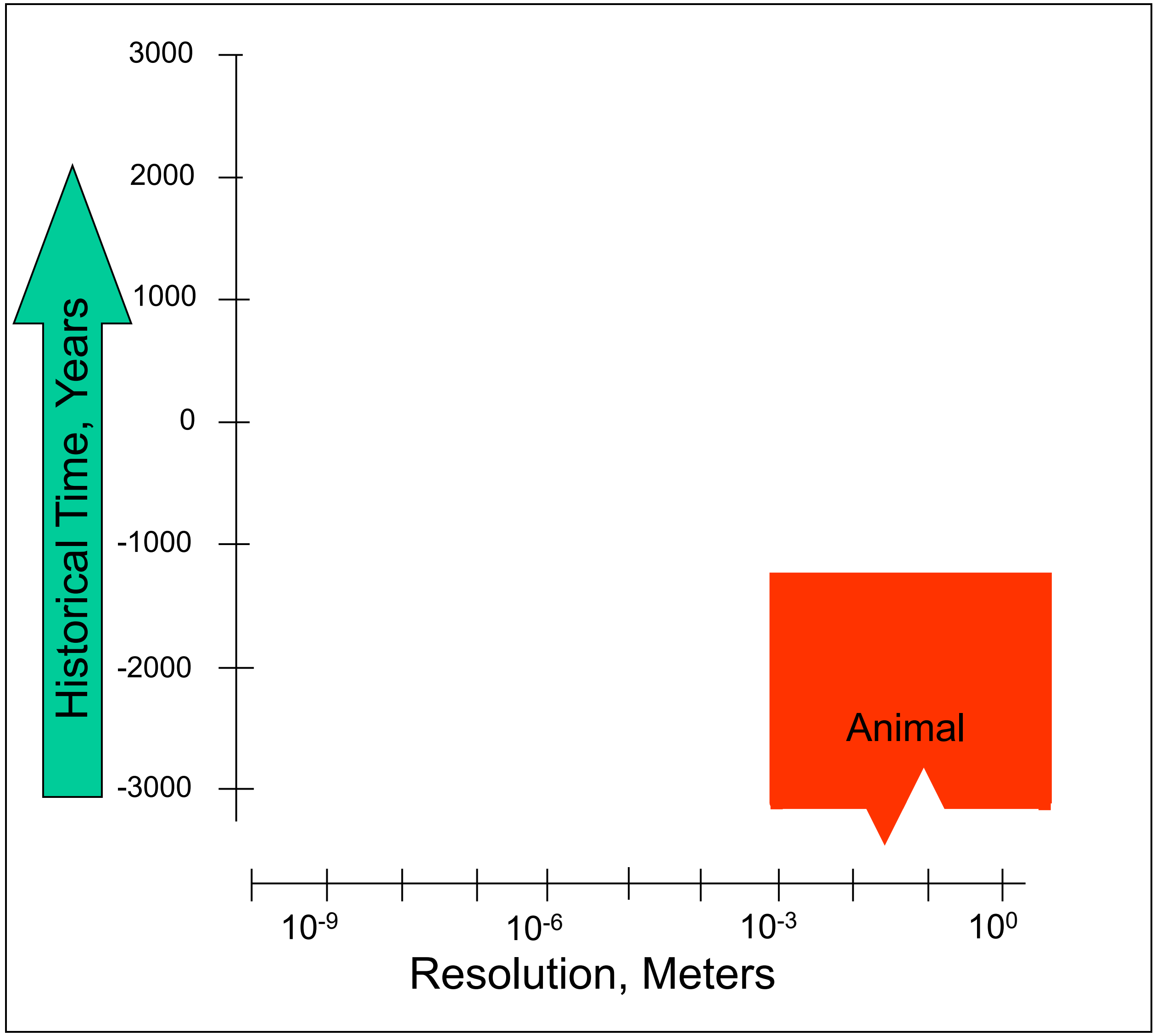
Computer

X-Ray / SEM / STM

Optical microscope

Magnifying glass

Unaided eye



The Problems

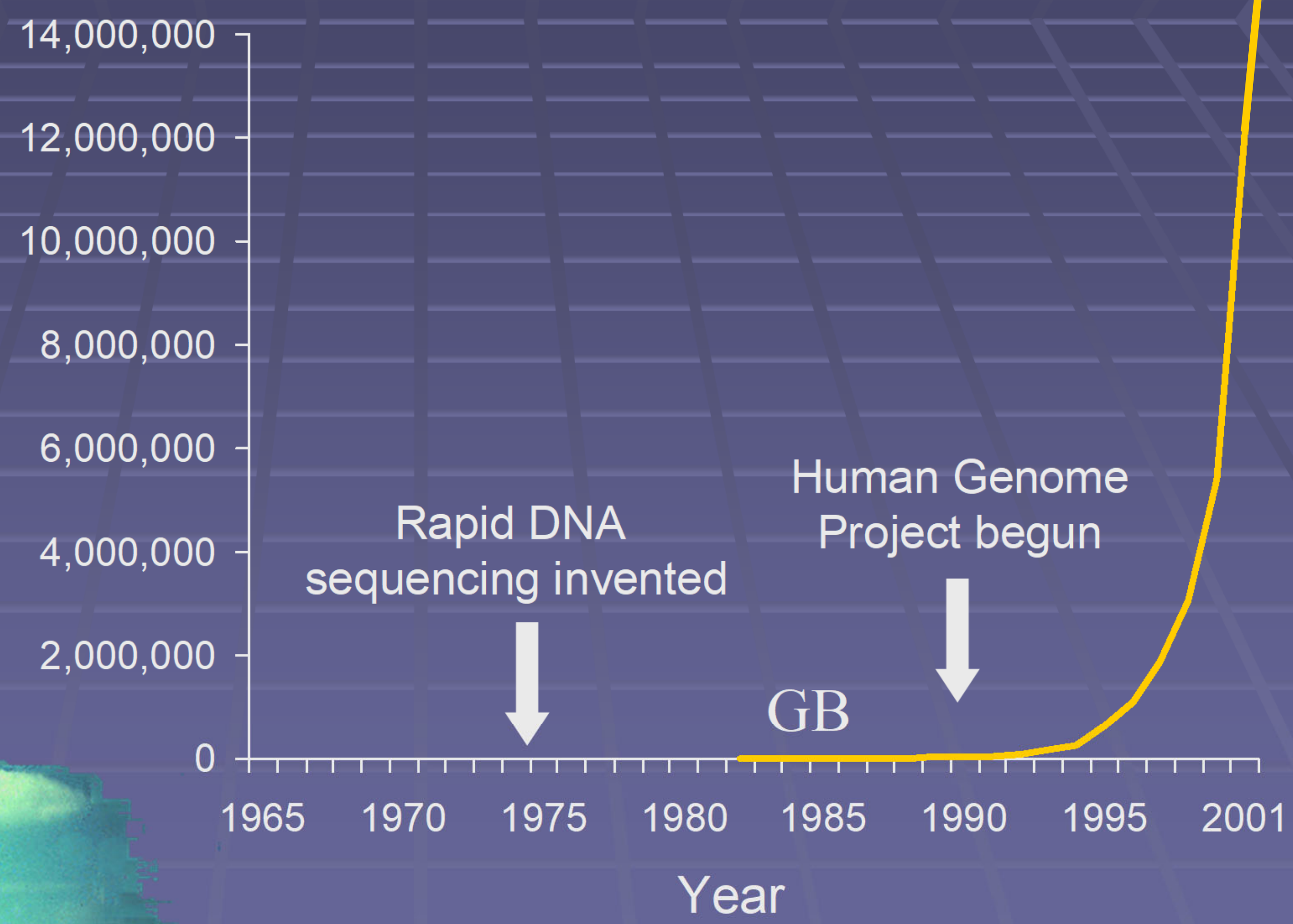
- Our understanding of biological phenomena is often based upon
  - experiments that measure the ensemble averages of populations of 10^6 – 10^7 cells, or
  - measurements of a single variable while all other variables are hopefully held constant, or
  - recordings of one variable on one cell, or
  - 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

2002: 22,318,883

~13 million sequence entries in GenBank

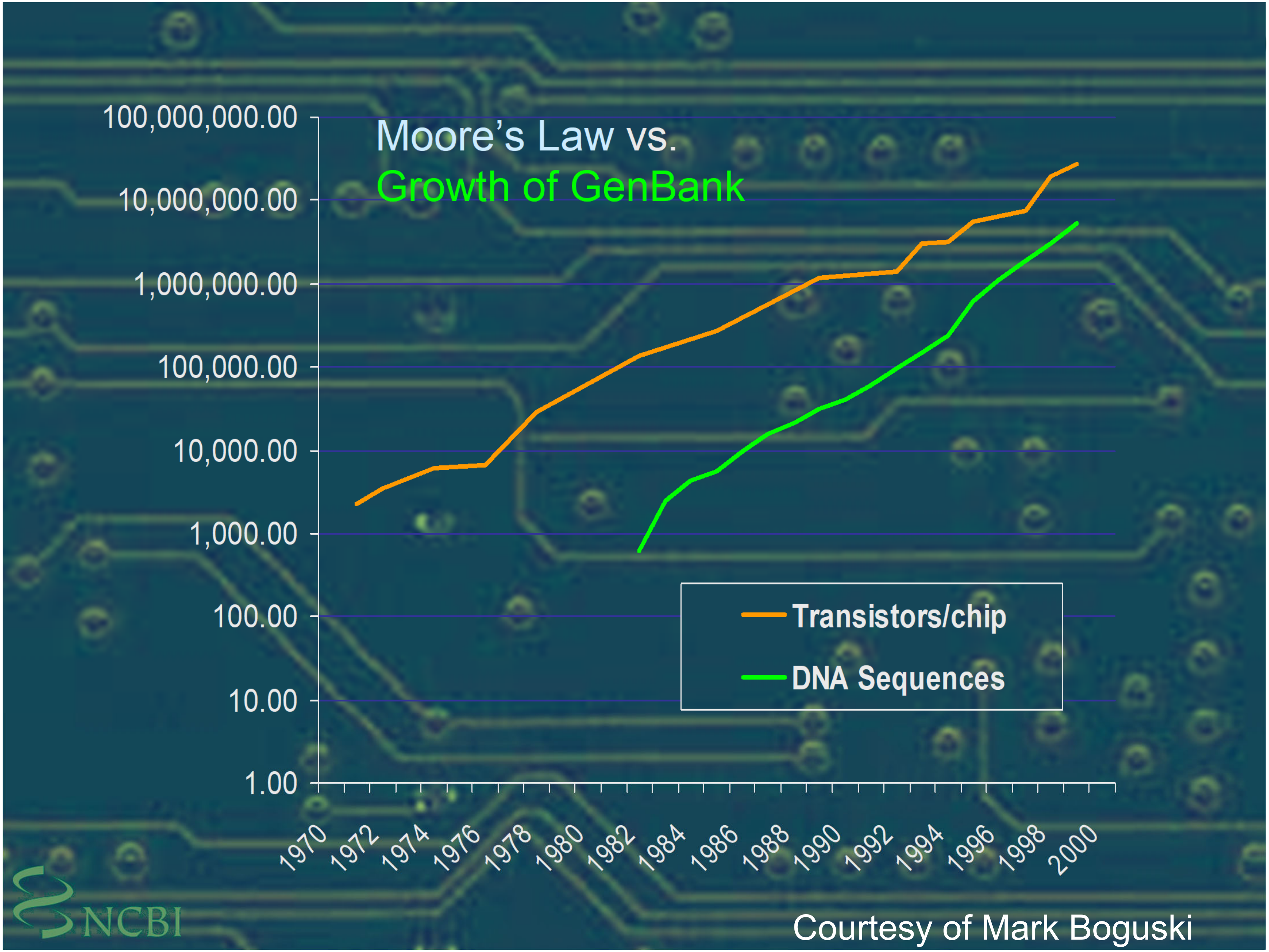
Nearly 13 billion bases from ~50,000 species



http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html

National Library of Medicine

Courtesy of Mark Boguski


Moore's Law vs.
Growth of GenBank
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1998
2000
Transistors/chip
DNA Sequences
NCBI
Courtesy of Mark Boguski

Step 2 in Science: Post-Reductionism

Thermodynamics

Statistical mechanics

Molecular/atomic dynamics

Electrodynamics

P-P Cross-Section at low P_t

Quantum Chromodynamics

Bulk solids

Devices

Continuum models

Si Step Edge Diffusion

Microscopic models

Atomic physics

Behavior

Systems Biology

Physiology

Systems Biology

Organ

Systems Biology

Cell

Systems Biology

Protein

Structural Biology

Genome

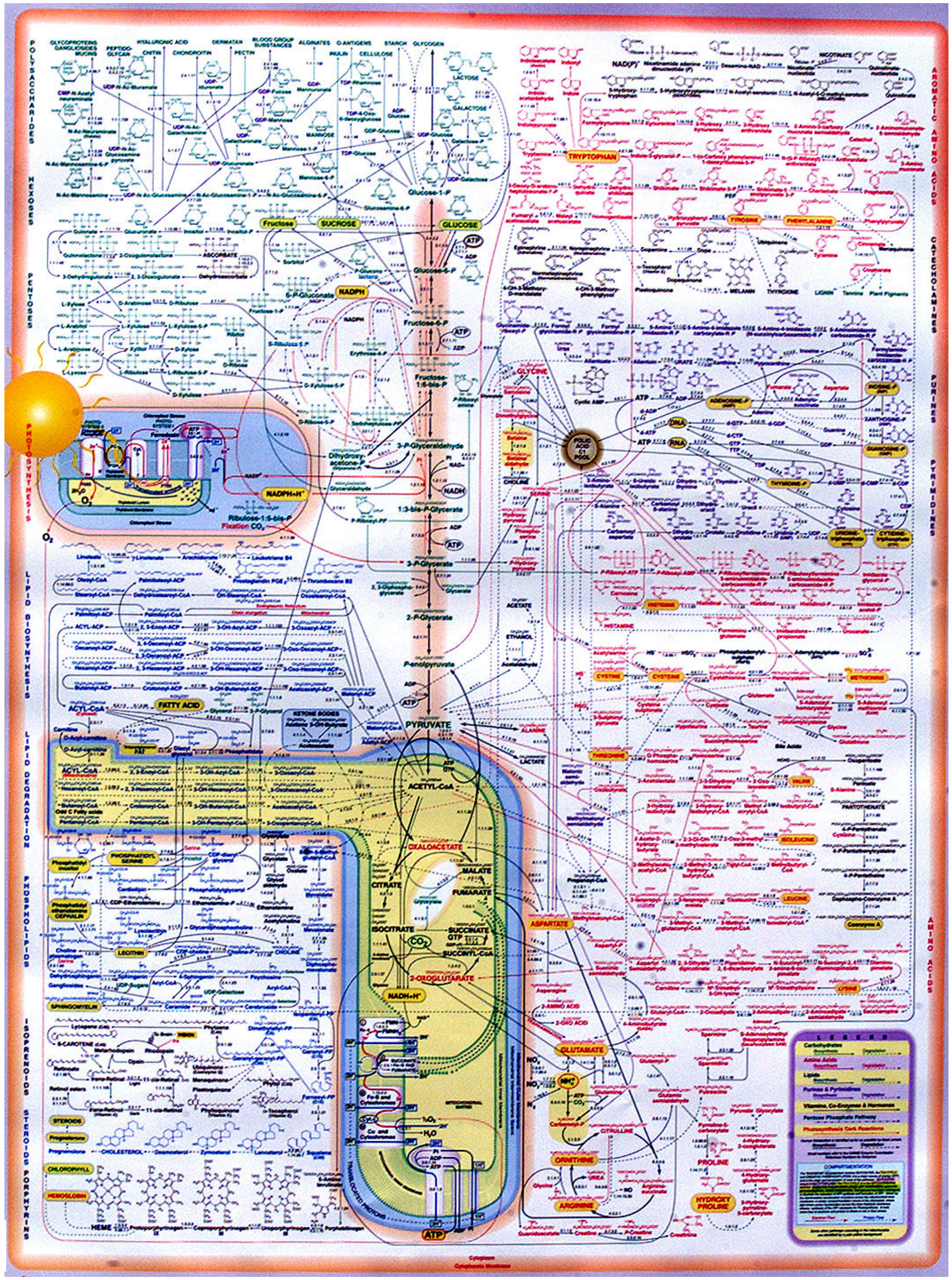
Key Questions in Systems Biology

- 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

VI∫BRE

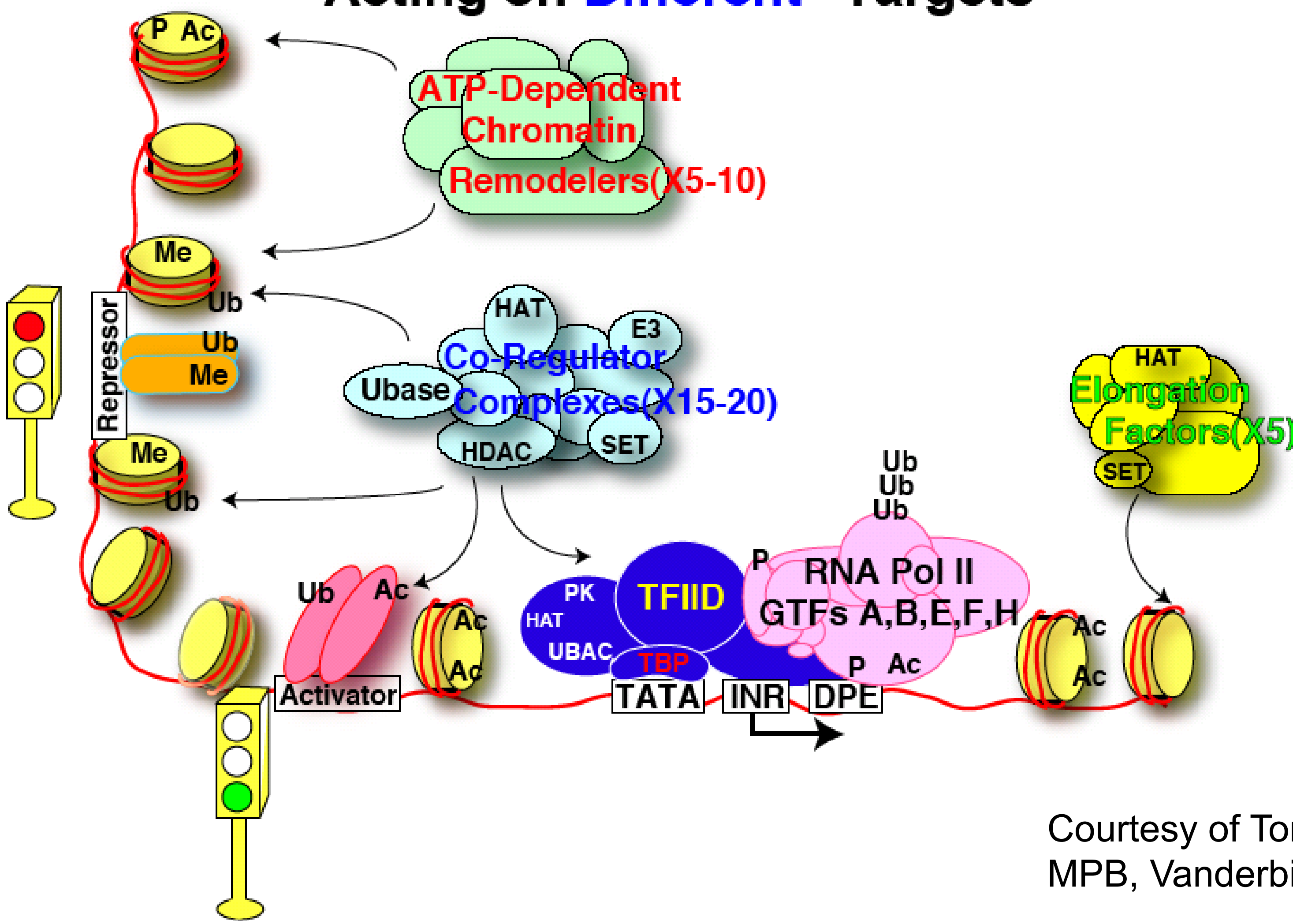
‘Postgenomic’ Integrative/Systems Physiology/Biology

- Suppose you wanted to **calculate** how the cell responds to a toxin…



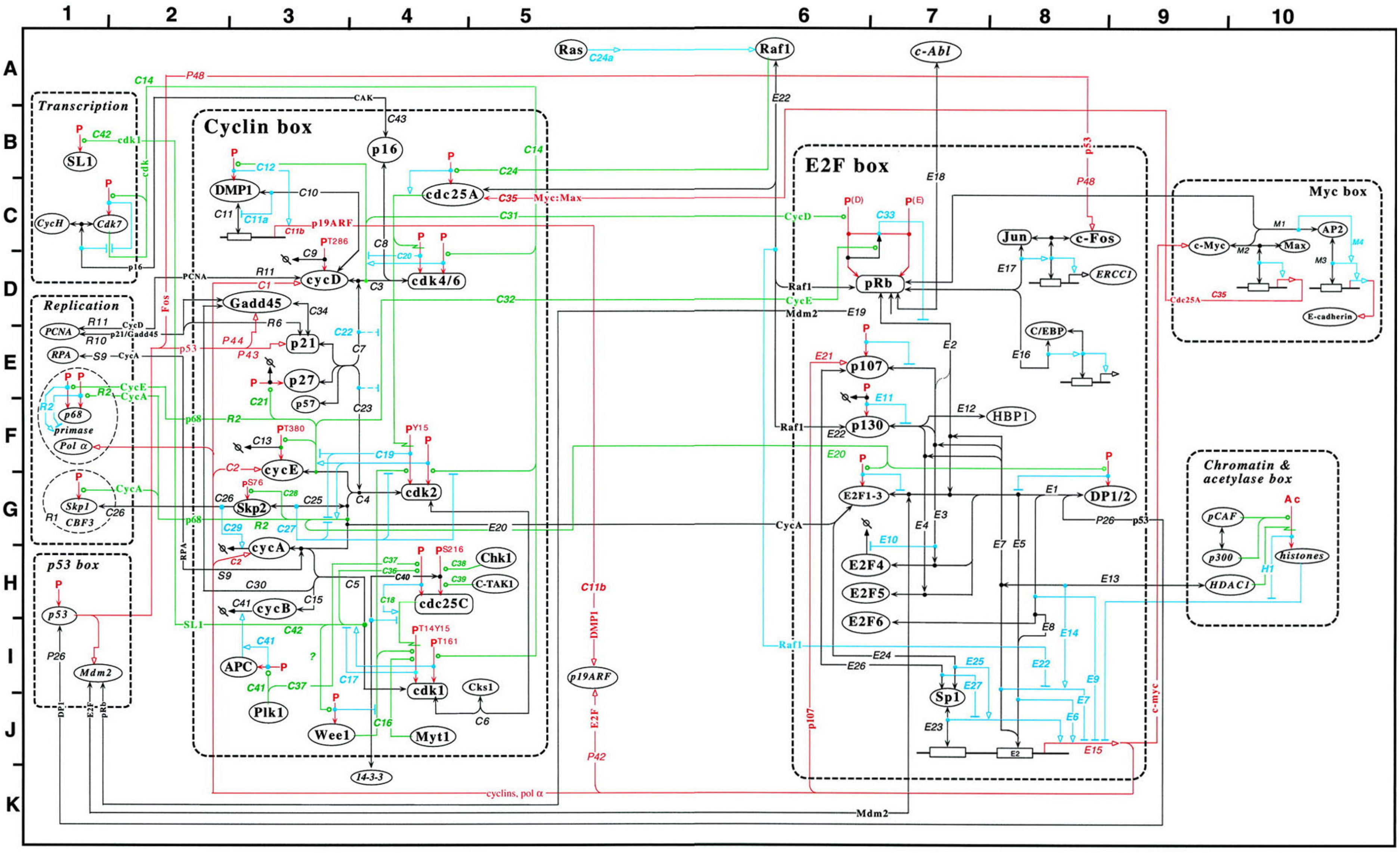
The complexity of eukaryotic gene transcription control mechanisms

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



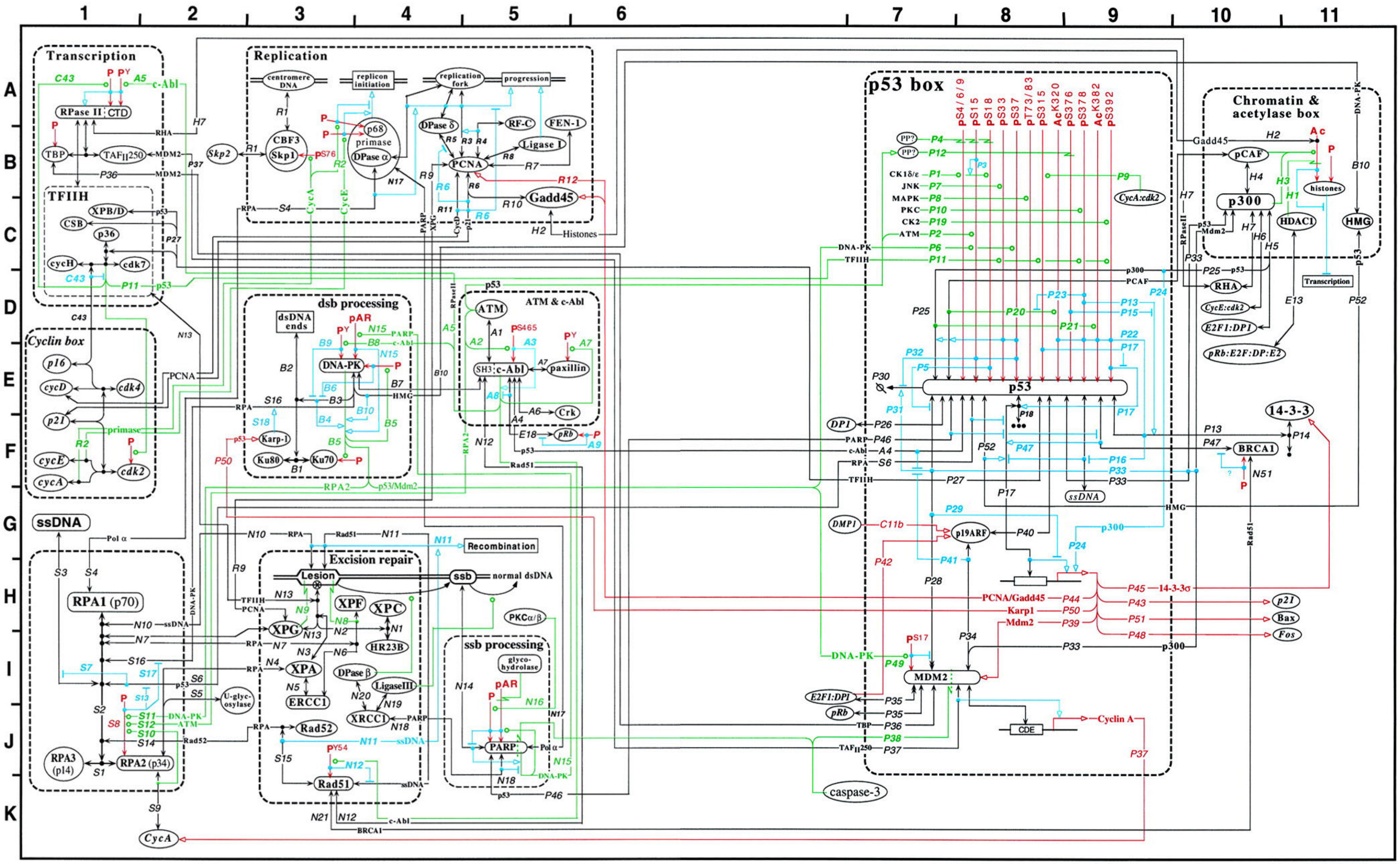
Courtesy of Tony Weil, MPB, Vanderbilt

Molecular Interaction Map: Cell Cycle



KW Kohn, “Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems,” *Mol. Biol. of the Cell*, 10: 2703-2734 (1999)

Molecular Interaction Map: DNA Repair



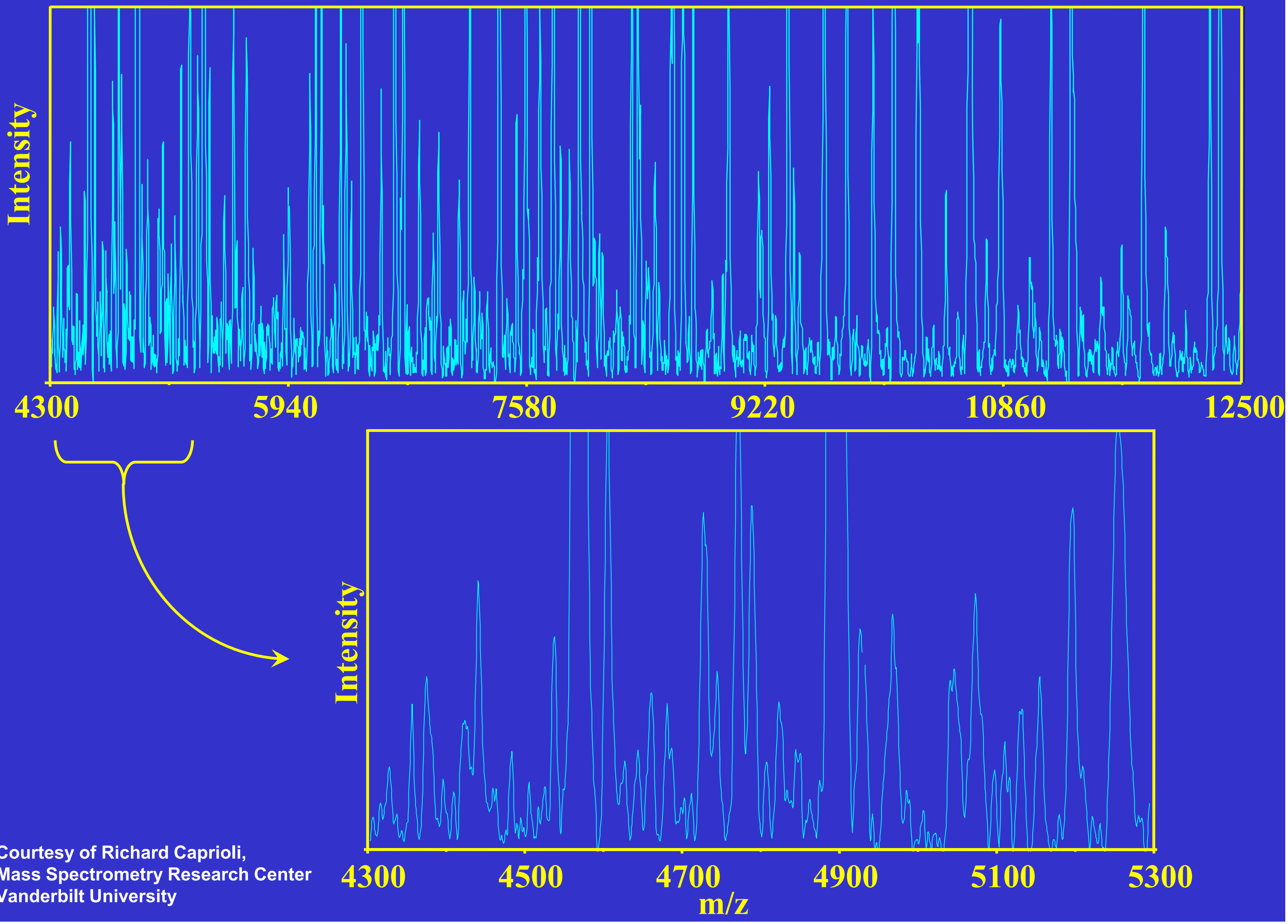
KW Kohn, “Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems,” *Mol. Biol. of the Cell*, 10: 2703-2734 (1999)

Proteins as Intracellular Signals

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

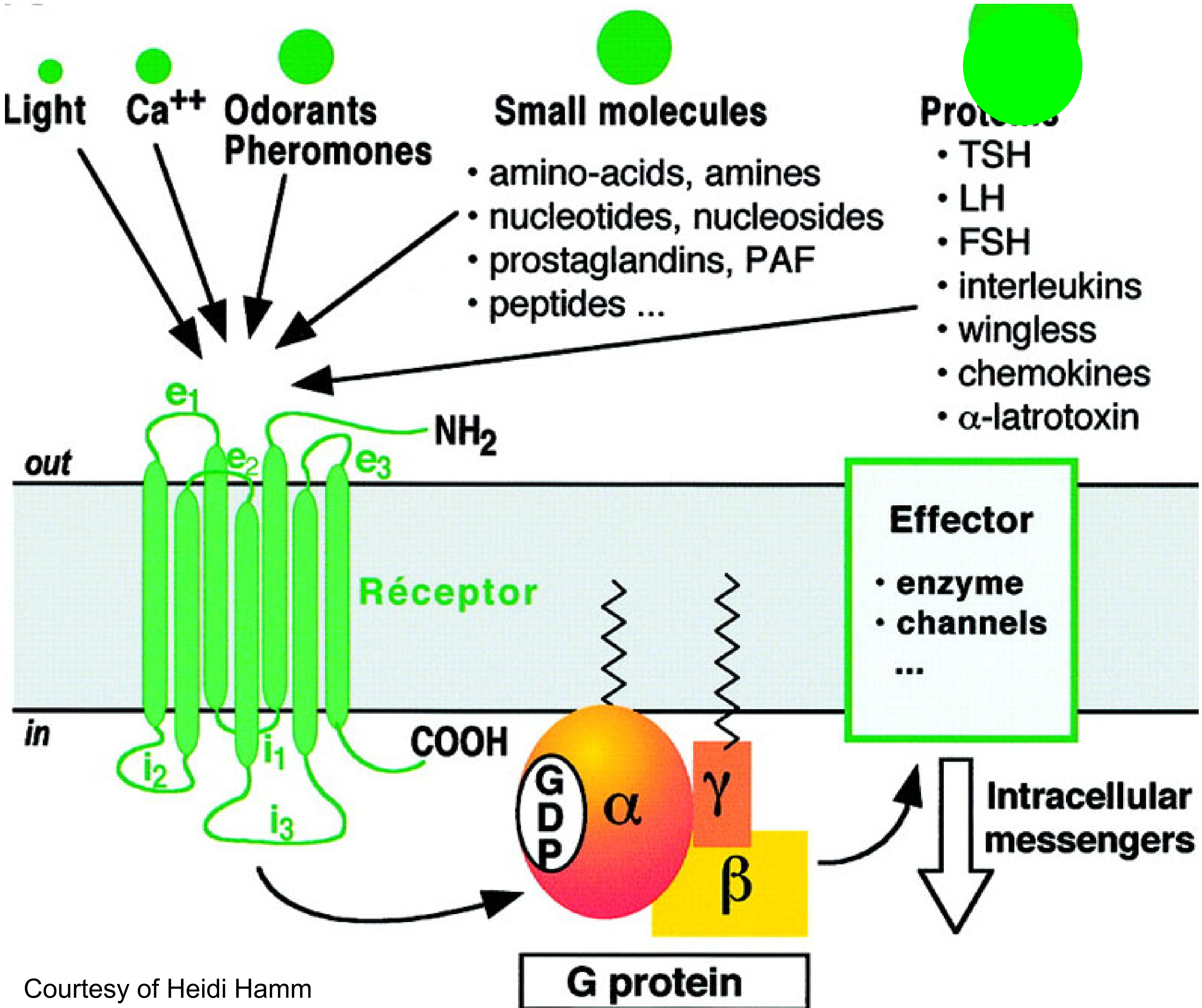
- Metabolic
- Maintaining integrity of subcellular structures
- Intracellular signaling
- Producing signals for other cells

MALDI-TOF: Cells express a lot of proteins…



Courtesy of Richard Caprioli,
Mass Spectrometry Research Center
Vanderbilt University

G-Protein Coupled Receptors



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

3.1 x 3.2 μm^3

- 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. .

6319movie6.mov

Marsh *et al.*, Organellar relationships in the Golgi region of the pancreatic beta cell line, HIT-T15, visualized by high resolution electron tomography. *PNAS* 98 (5):2399-2406, 2001.

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

ODEs become PDEs …

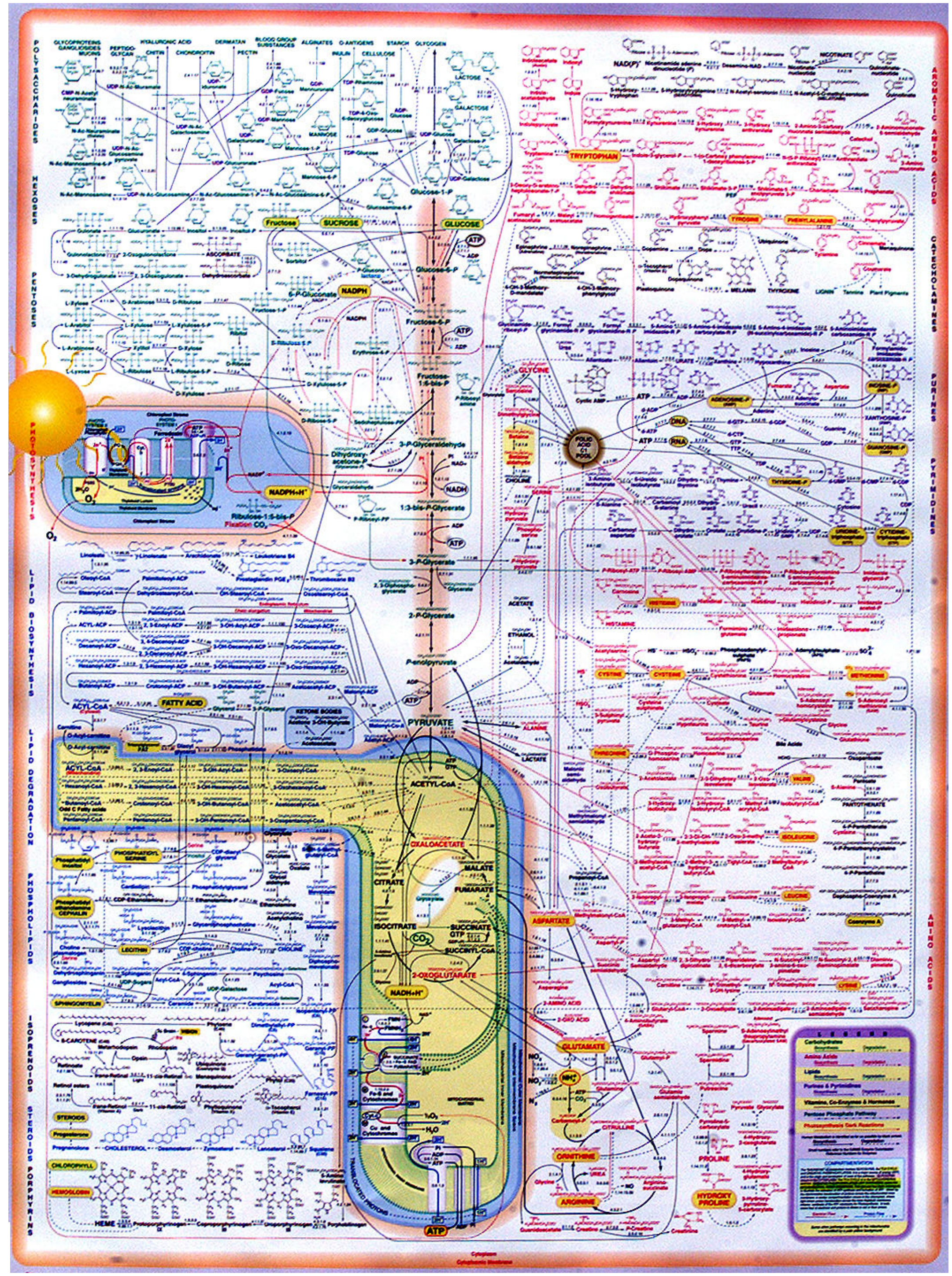
Lots and lots and lots of PDEs

VI∫BRE

'Postgenomic' Integrative/Systems Physiology/Biology

- 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:

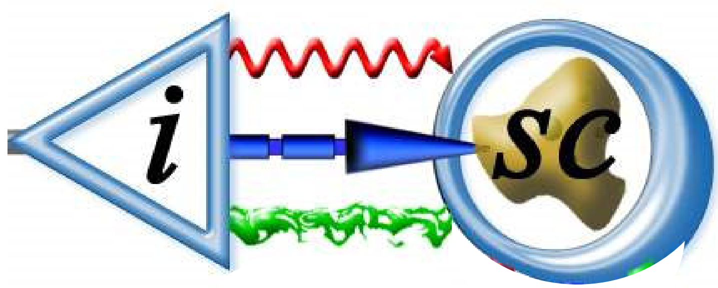
- Metabolic
- Maintaining integrity of subcellular structures
- Intracellular signaling
- Producing signals for other cells.

There are no technologies that allow the measurement of a **hundred,** time dependent, intracellular variables <u>in a single cell</u> (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, DiffusionTime Constants

X	V, m^3	V	Tau_{Diff}	Example	N
1 m	1	1000 L	10^9 s	Animal, bioreactor	100
10 cm	10^{-3}	1 L	10^7 s	Organ, bioreactor	100
1 cm	10^{-6}	1 mL	10^5 s = 1 day	Tissue, cell culture	10
1 mm	10^{-9}	1 uL	10^3 s	μenviron, well plate	10
100 um	10^{-12}	1 nL	10 s	Cell-cell signaling	5
10 um	10^{-15}	1 pL	0.1 s	Cell	10
1 um	10^{-18}	1 fL	1 ms	Subspace	2
100 nm	10^{-21}	1 aL	10 us	Organelle	2
10 nm	10^{-24}	1 zL	100 ns	Protein	1
1 nm	10^{-27}	1 npL	1 ns	Ion channel	1

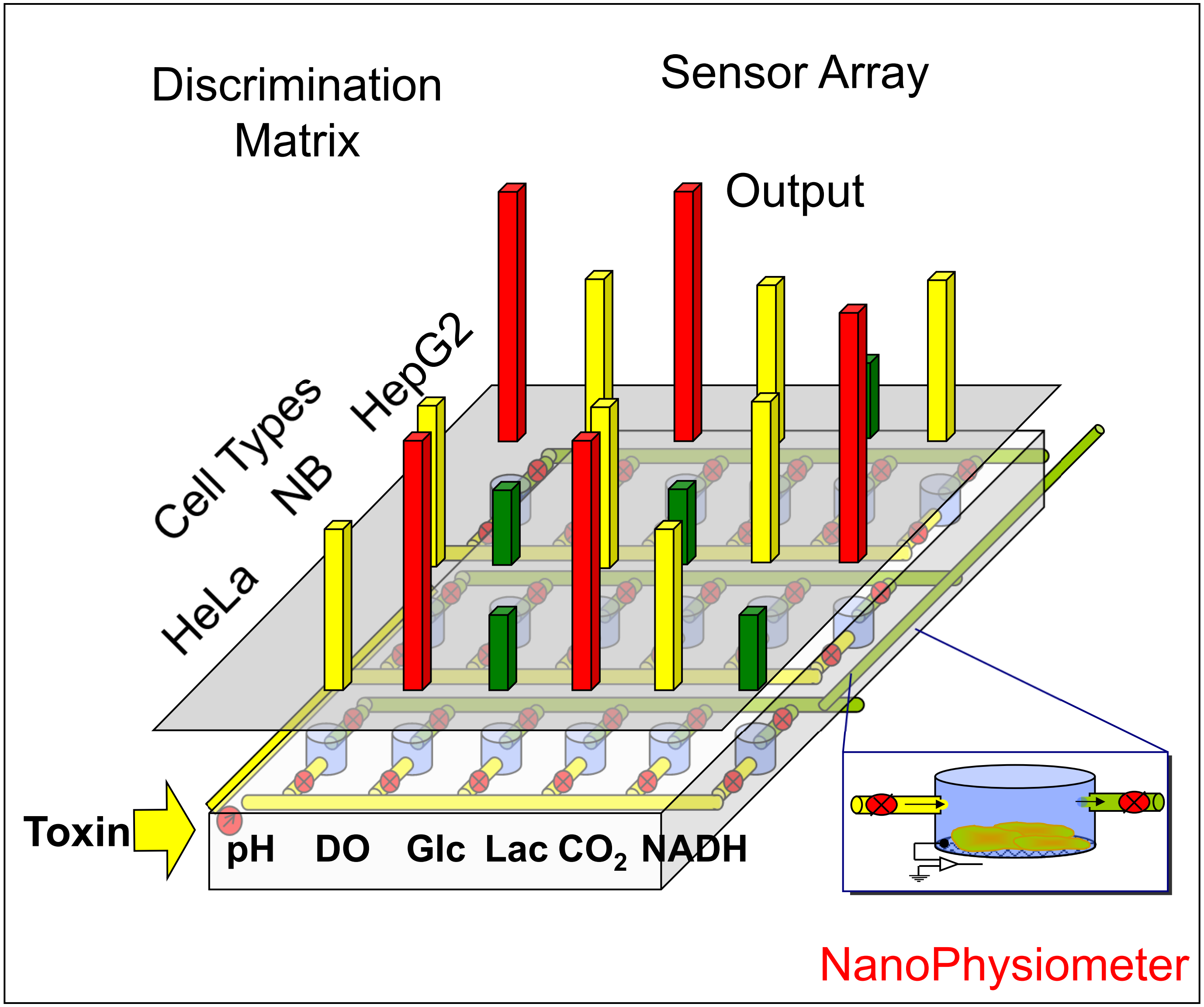
High-Content Toxicology Screening Using Massively Parallel, Multi-Phasic Cellular Biological Activity Detectors MP²-CBAD

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

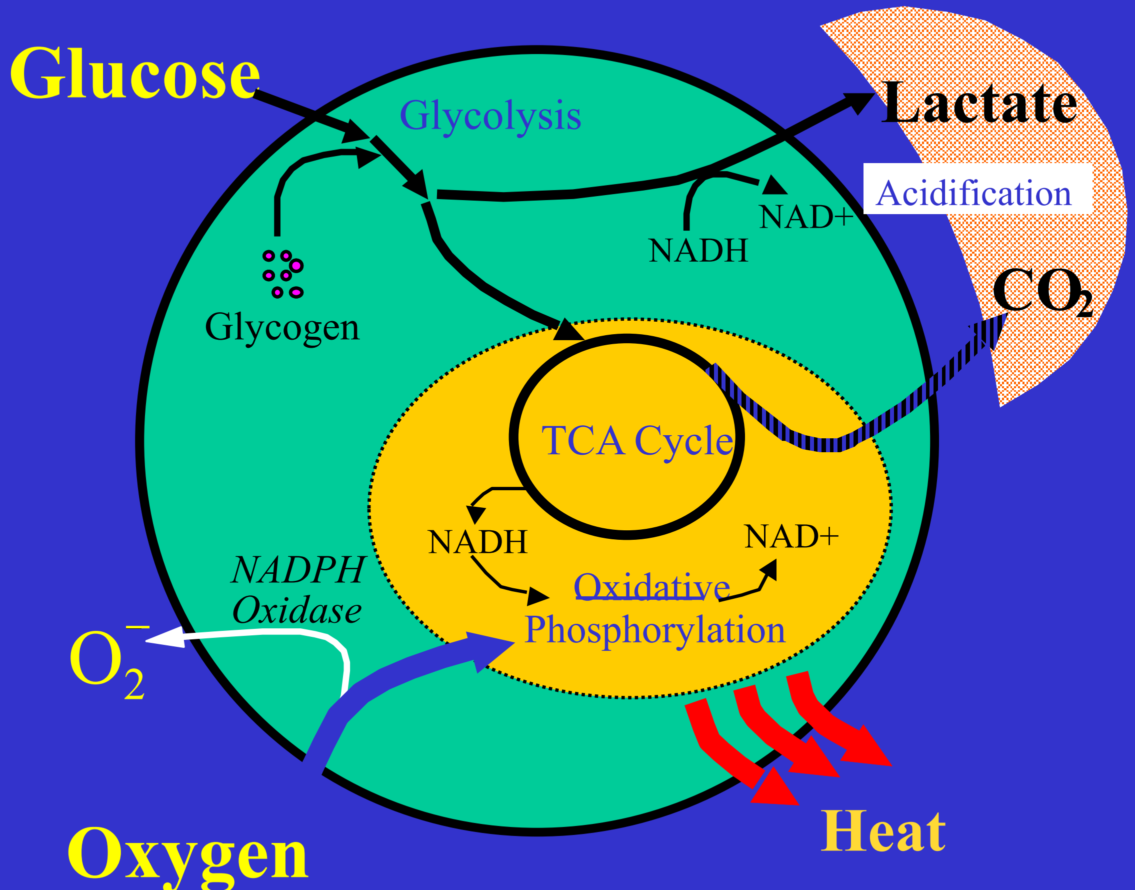
Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE)

Edgewood Chemical and Biological Center (SBCCOM / ECBC)

MP²-CBAD Discrimination



Simplified Metabolic Network



- Robert Balcarcel
- Franz Baudenbacher
- David Cliffel
- Ales Prokop
- Owen McGuinness
- John Wikswo

Glucose + 2 ADP + **2** $\mathbf{NAD^+}$	→	**2 Pyruvate** + 2 ATP + **2 NADH**
Pyruvate + **NADH**	→	**Lactate** + $\mathbf{NAD^+}$
Pyruvate + CoA + **FAD** + GDP + **3** $\mathbf{NAD^+}$ + $\mathbf{NAD(P)^+}$	→	**3** $\mathbf{CO_2}$ + **FADH2** + GTP + **3 NADH** + **NAD(P)H**
0.5 $\mathbf{O_2}$ + 3 ADP + **NADH**	→	3 ATP + $\mathbf{NAD^+}$
0.5 $\mathbf{O_2}$ + 2 ADP + $\mathbf{FADH_2}$	→	2 ATP + **FAD**

The well size determines the bandwidth

- Microliter – 10-100 seconds

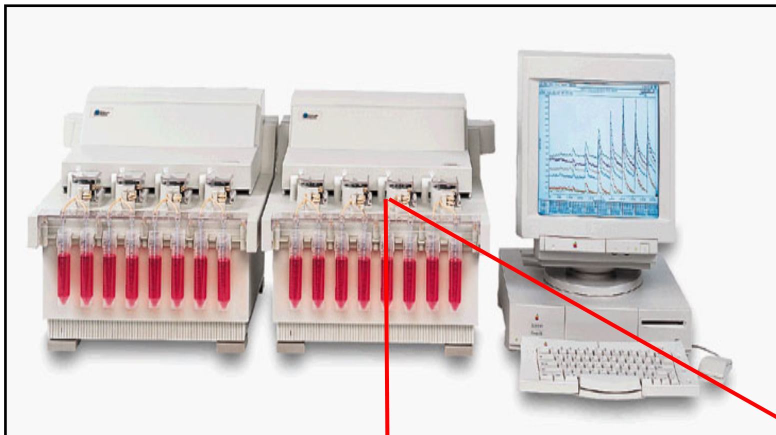
  Modified Cytosensor MicroPhysiometer

- SubNanoliter – 10-100 <u>milli</u>seconds

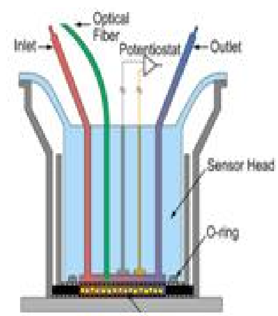
  Vanderbilt NanoPhysiometer

Multianalyte Microphysiometry

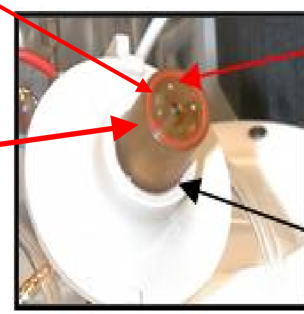
- The Multianalyate MicroPhysiometer (MMP) serves as a platform for studying large numbers of cells simultaneously
- Upon activation, we can measure acidification rate, O_2, lactate, glucose with ~1 minute resolution



MicroPhysiometer: Modified sensor head

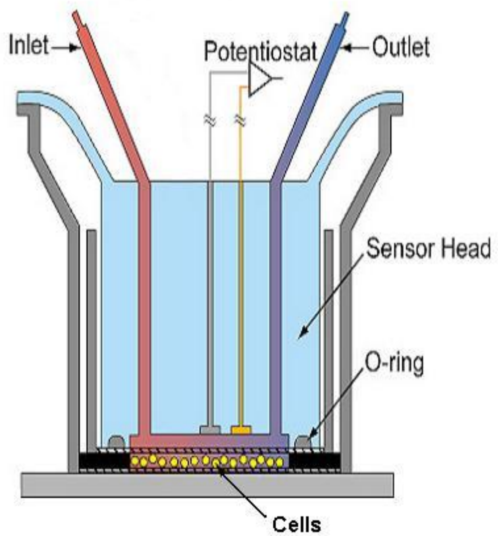


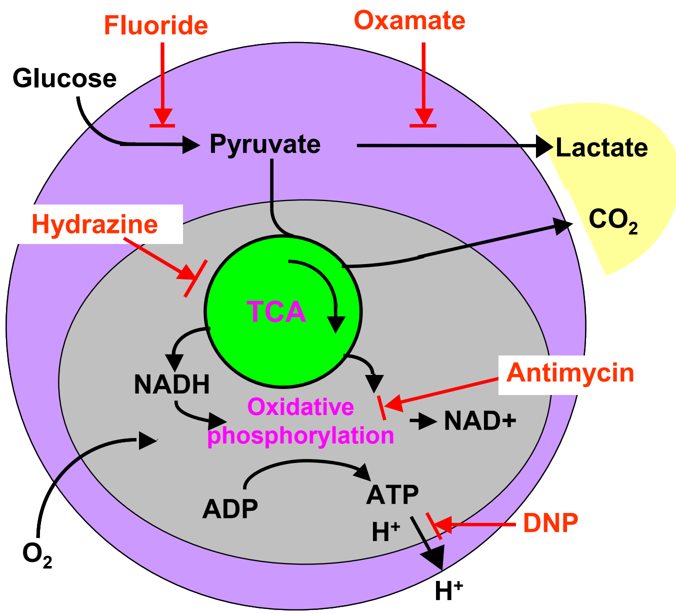
Schematic drawing of modified sensor head for the microliter Molecular Devices Cytosensor microphysiometer

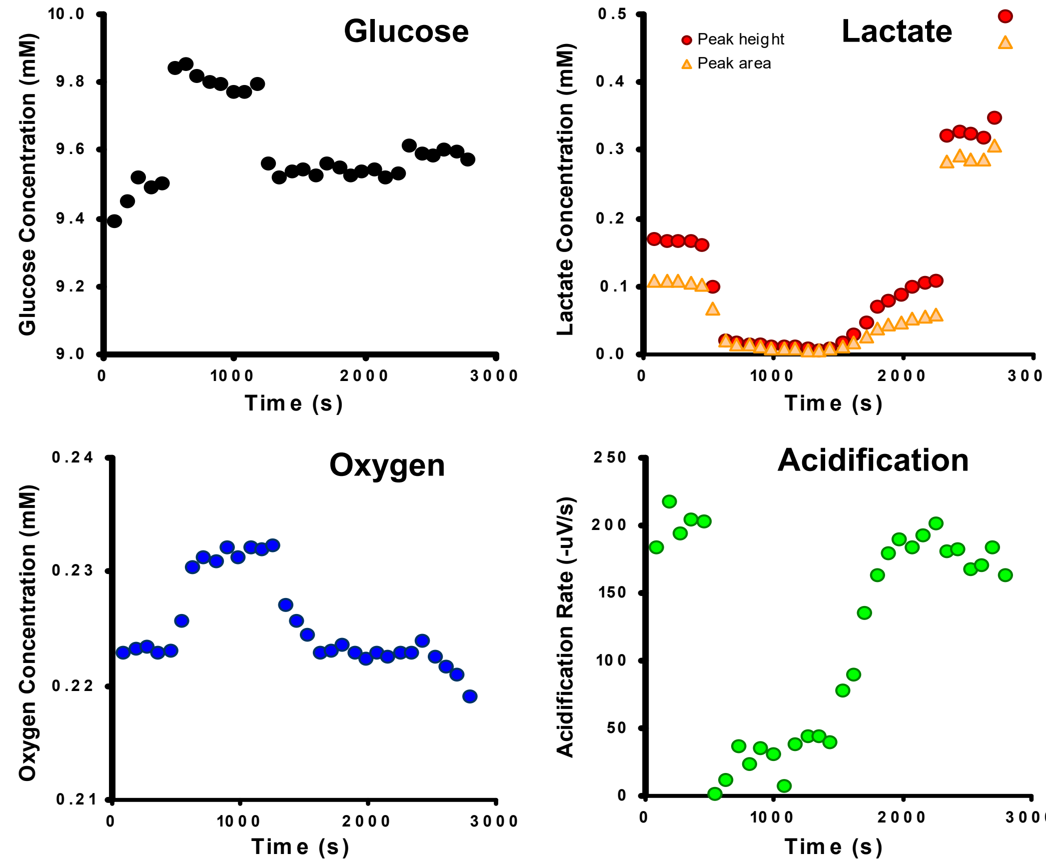


- Existing Cytosensor head

Multianalyte Microphysiometry for Biotoxin Discrimination





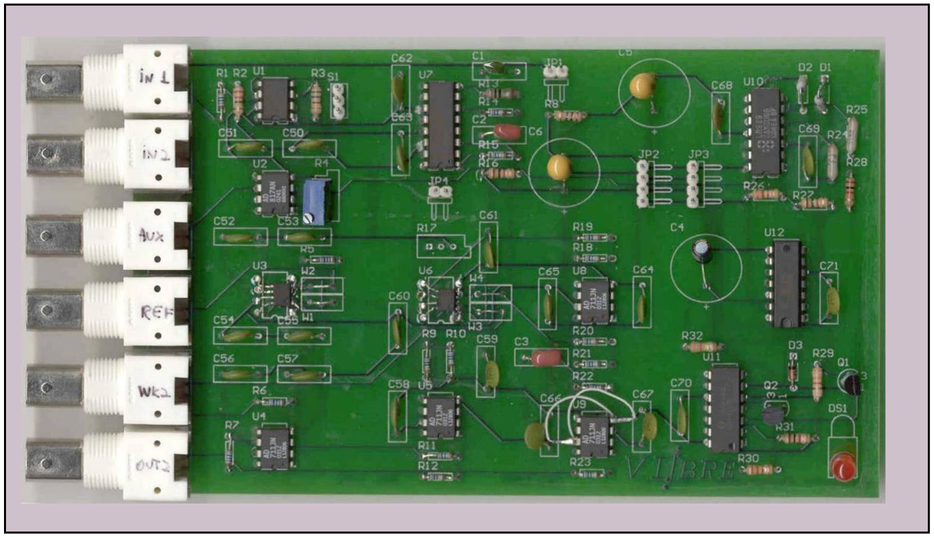


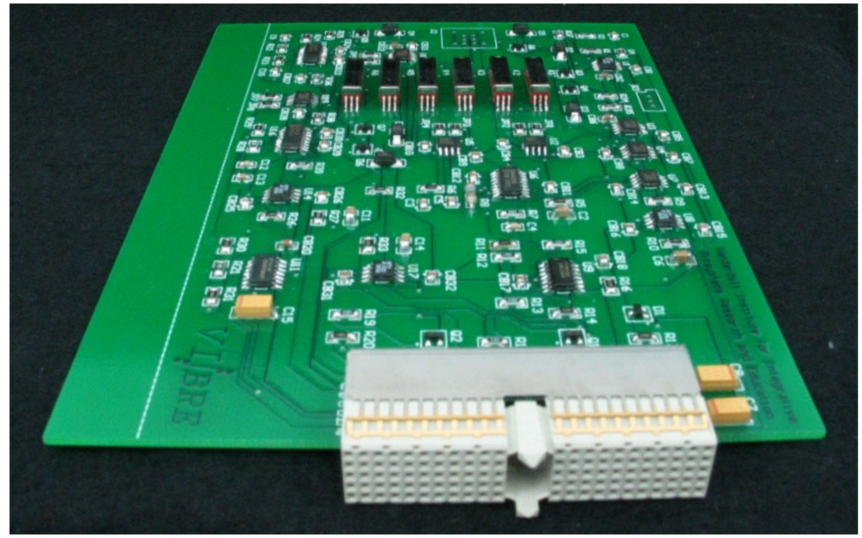
CHO cells with 720 s of 20 mM fluoride.

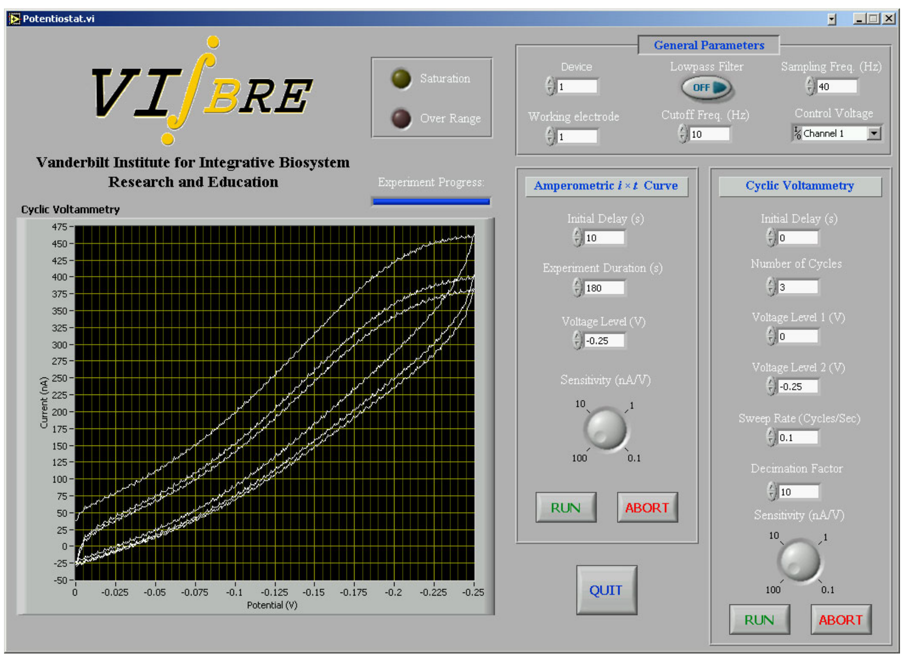
S.E. Eklund, D.E. Cliffel, et al.,

- *Anal.Chim.Acta* 496 (1-2):93-101, 2003;
- *Anal.Chem.* 76 (3):519-527, 2004;
- *Nanobiotechnology*, Humana Press, In Press.

Automated Data Acquisition and Analysis







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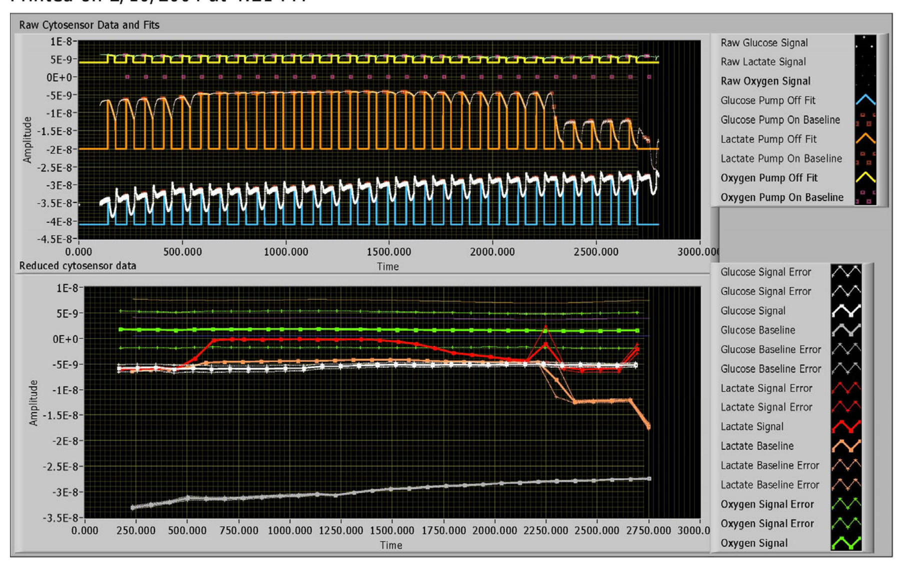


The well size determines the bandwidth

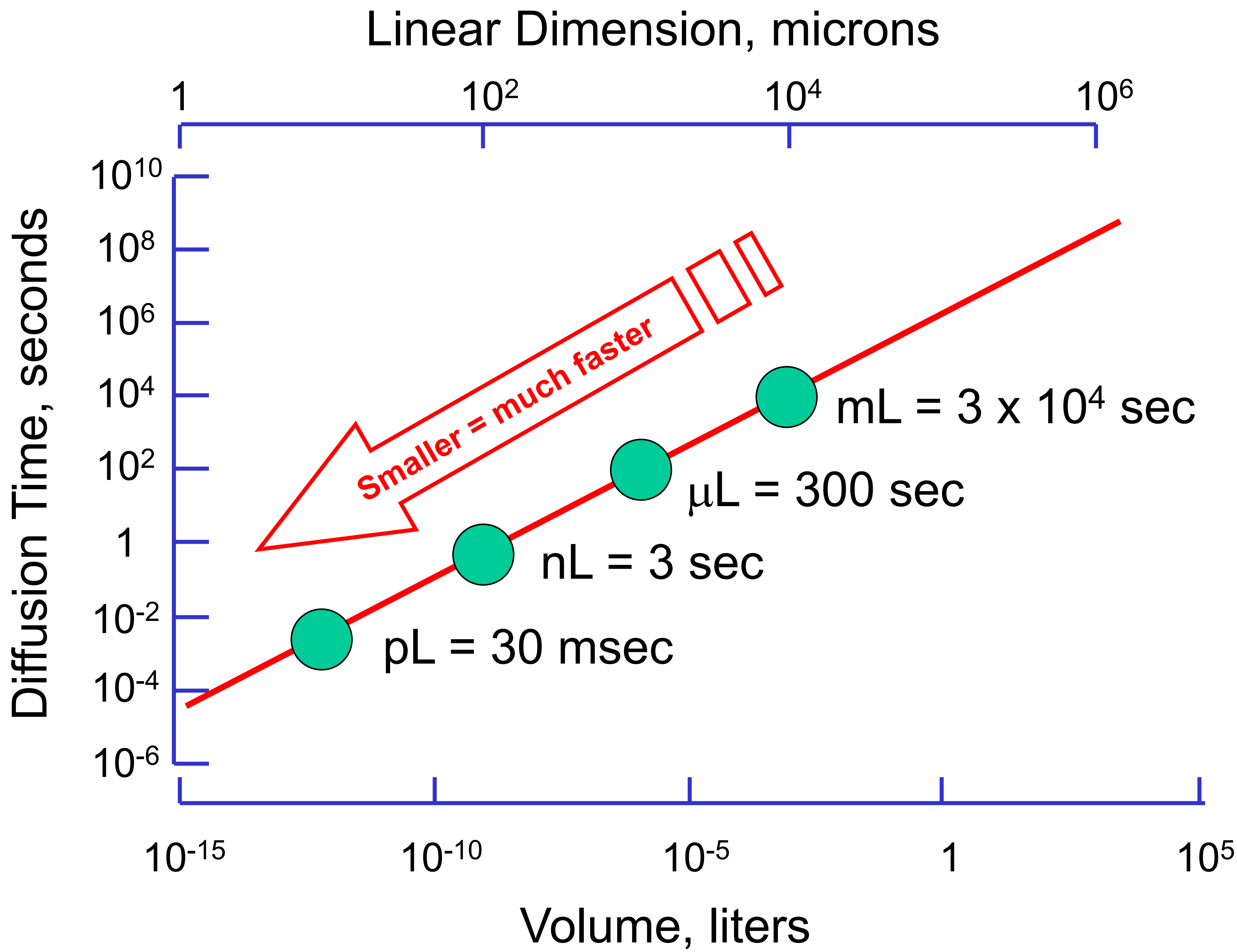
- Microliter – 10-100 seconds

  Modified Cytosensor MicroPhysiometer

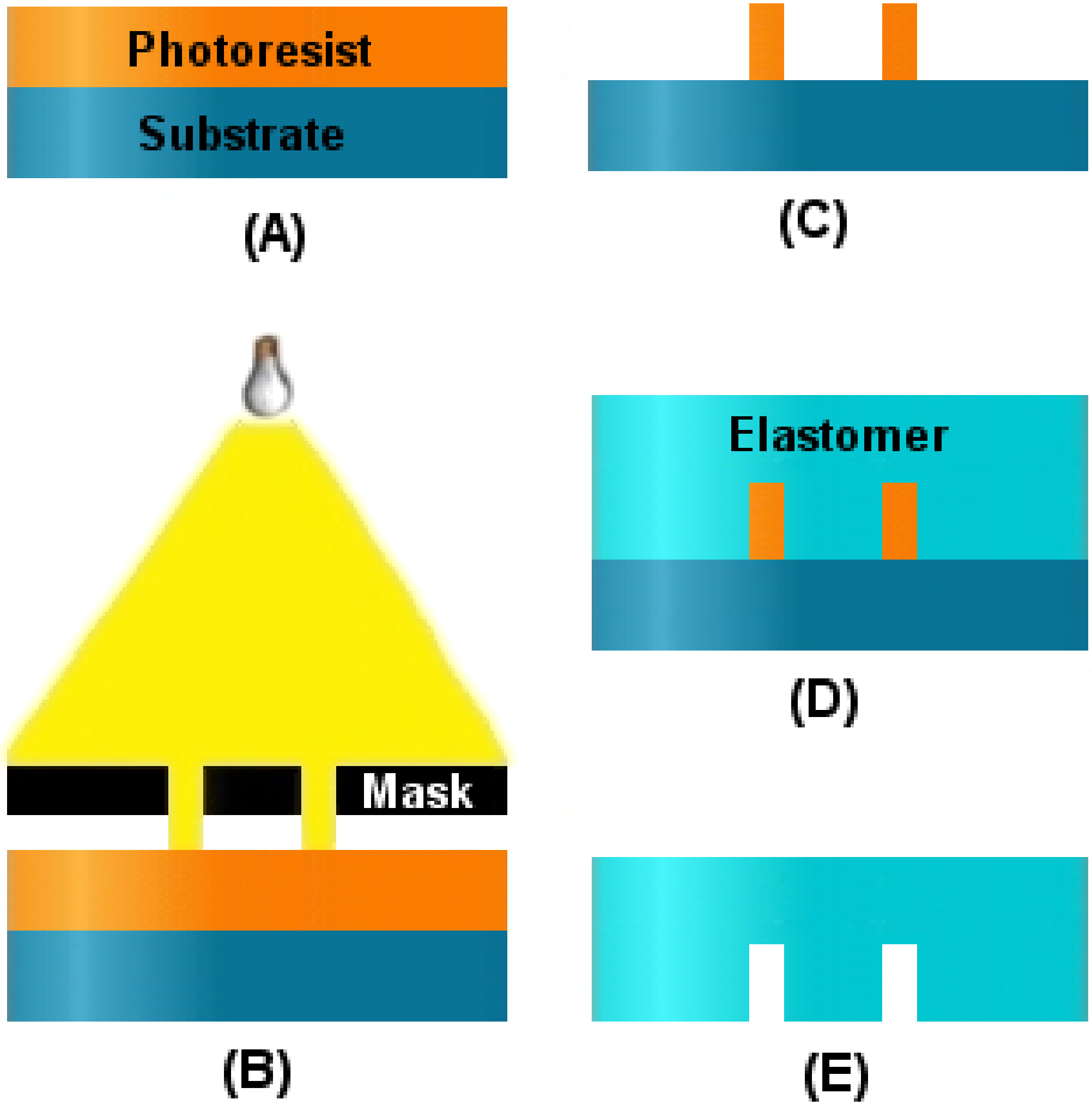
- SubNanoliter – 10-100 <u>milli</u>seconds

  Vanderbilt NanoPhysiometer

Lactate Diffusion Times

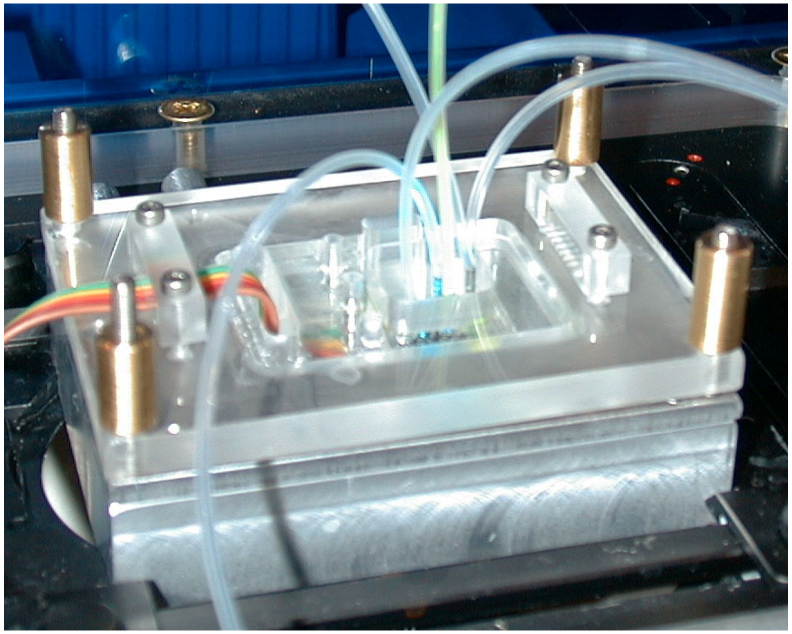


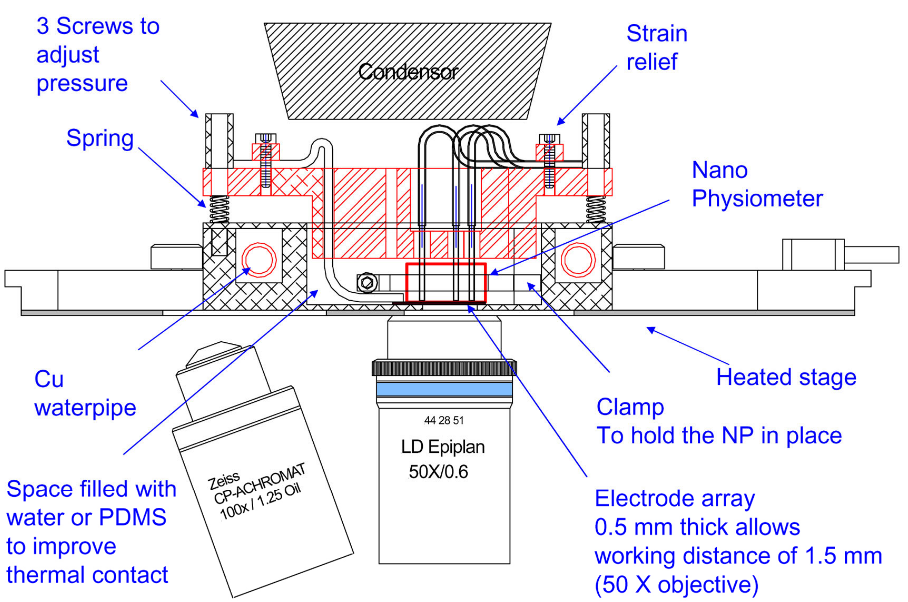
PDMS Soft Lithography

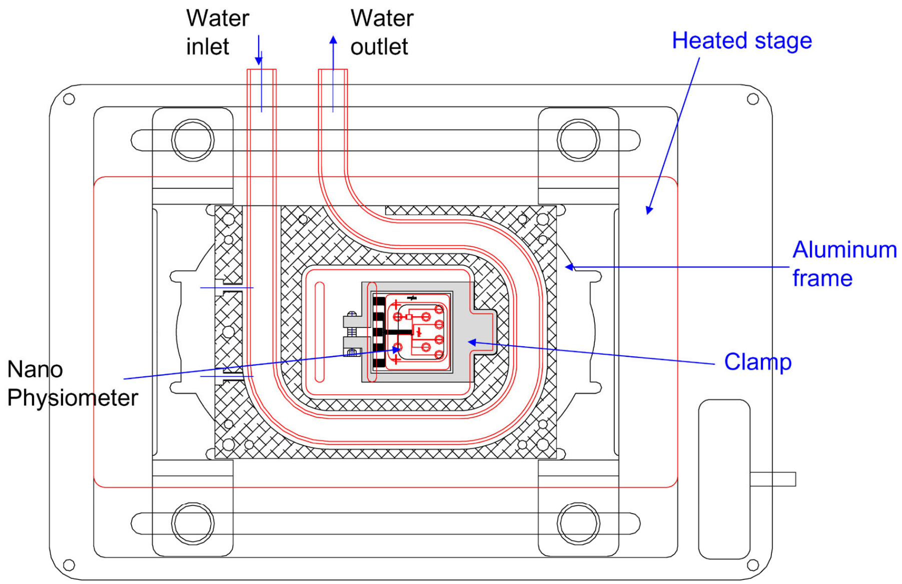


Nanophysiometer for Rapid Activation Dynamics (Baudenbacher)

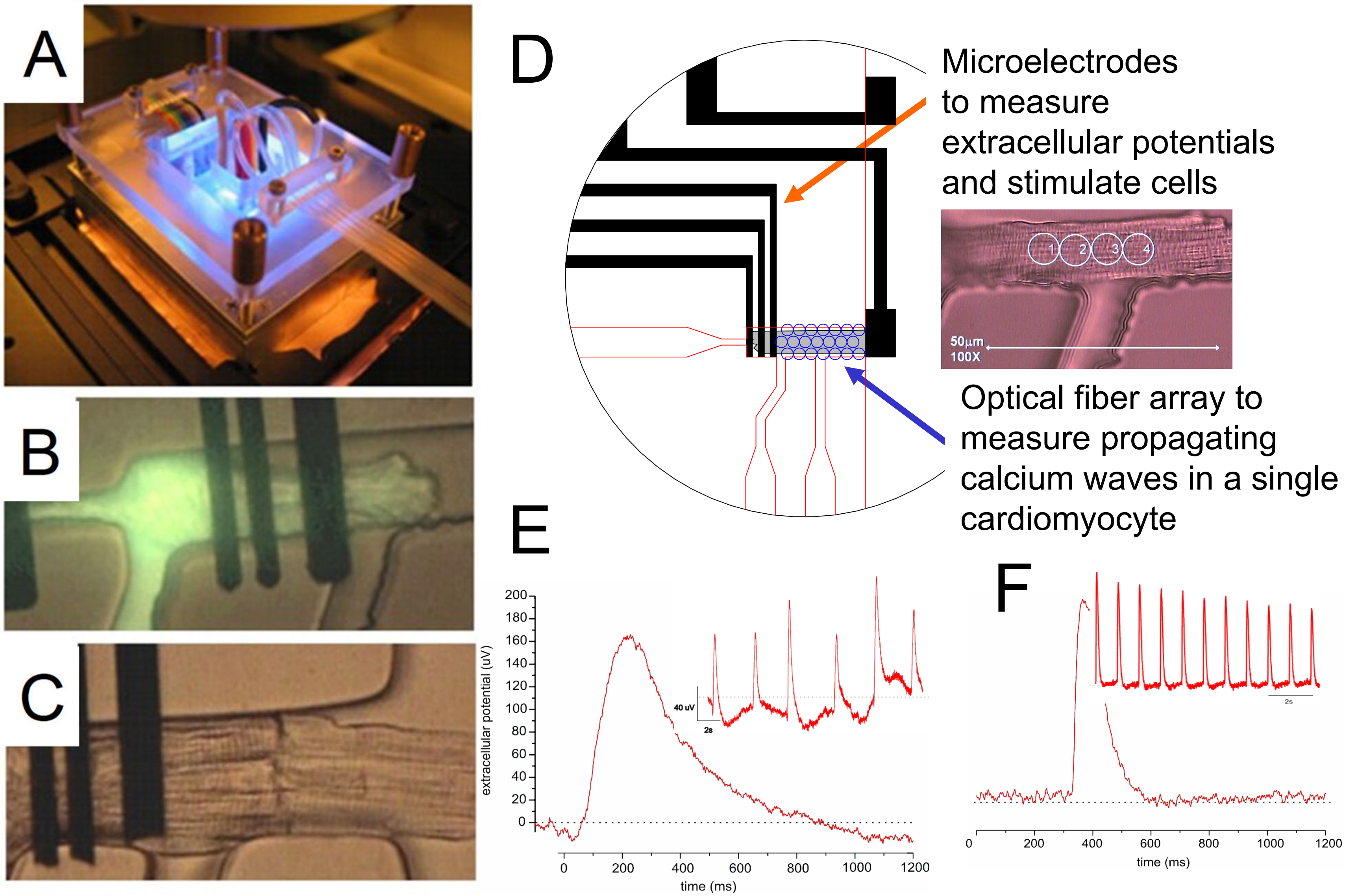
- The Multianalyte NanoPhysiometer (MNP) will serve as a platform for studying, one at a time, large numbers of single cells
- Upon activation, we will measure pH, O, V_m, [Ca], lactate, glucose, Q-Dot binding







Cardiomyocyte in the Nanophysiometer
F Baudenbacher and A Werdich



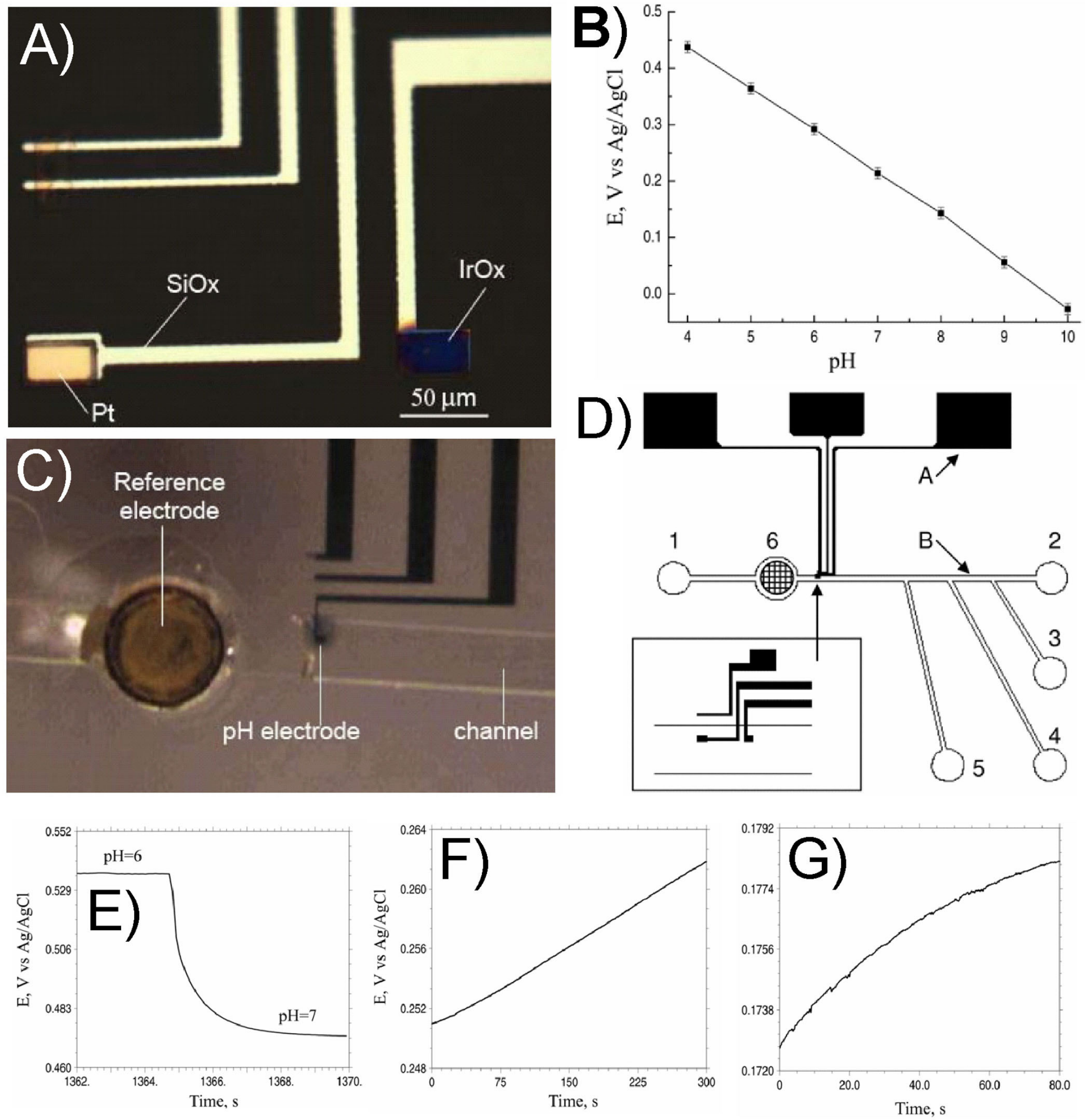
A. Werdich, et al *Lab on a Chip* 4 (4):357-362, 2004

Microfabricated pH Electrodes

I. Ges, B. Ivanov, F Baudenbacher

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

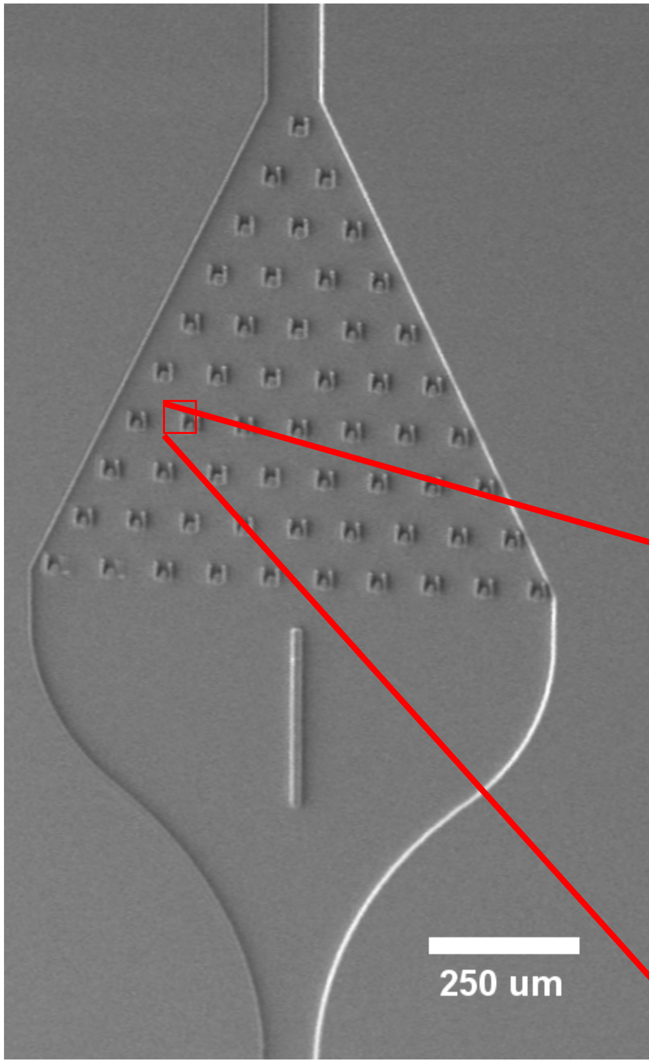
Ges et al., Submitted for publication

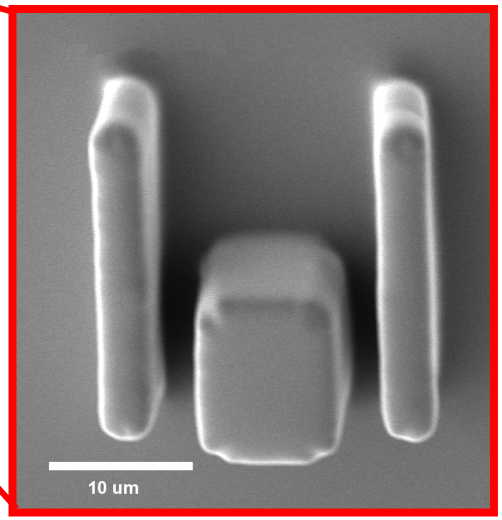


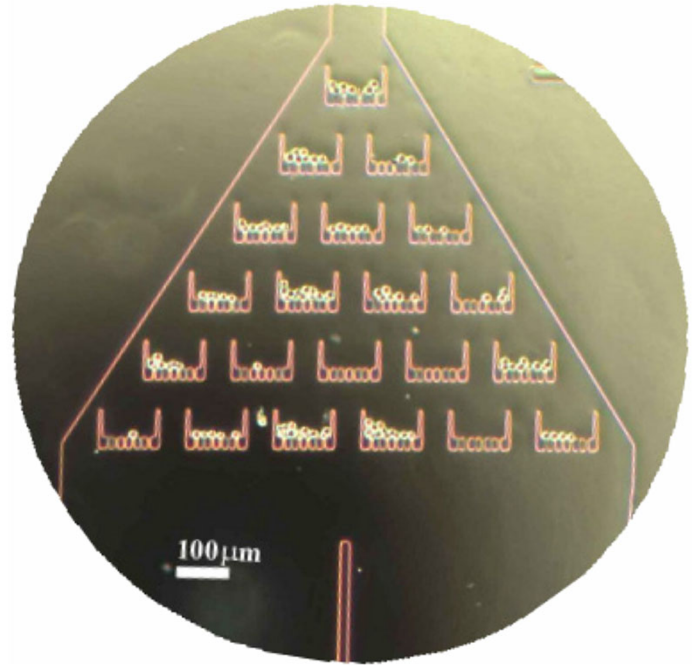
First Generation Autoloading NanoPhysiometer

A.Prokop, et al.,

- Biological and Bioinspired Materials and Devices, MRS, 2004,
- Biomedical Microdevices 6 (4):In Press, 2004.

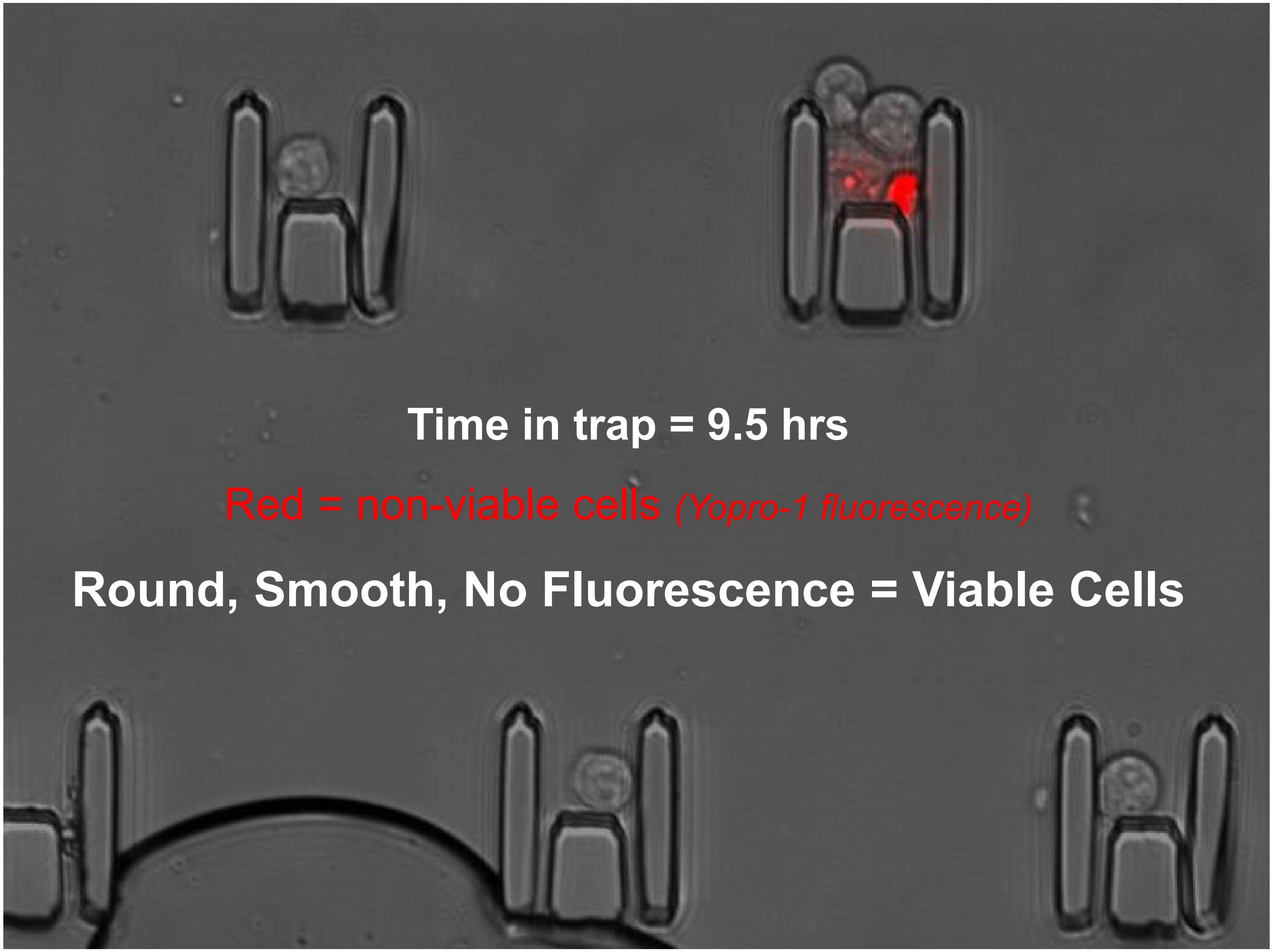






Goal: Instrumented bioreactors with individually addressable traps

Viability of Activated Jurkat cells in NanoPhysiometer Using CO_2-free media



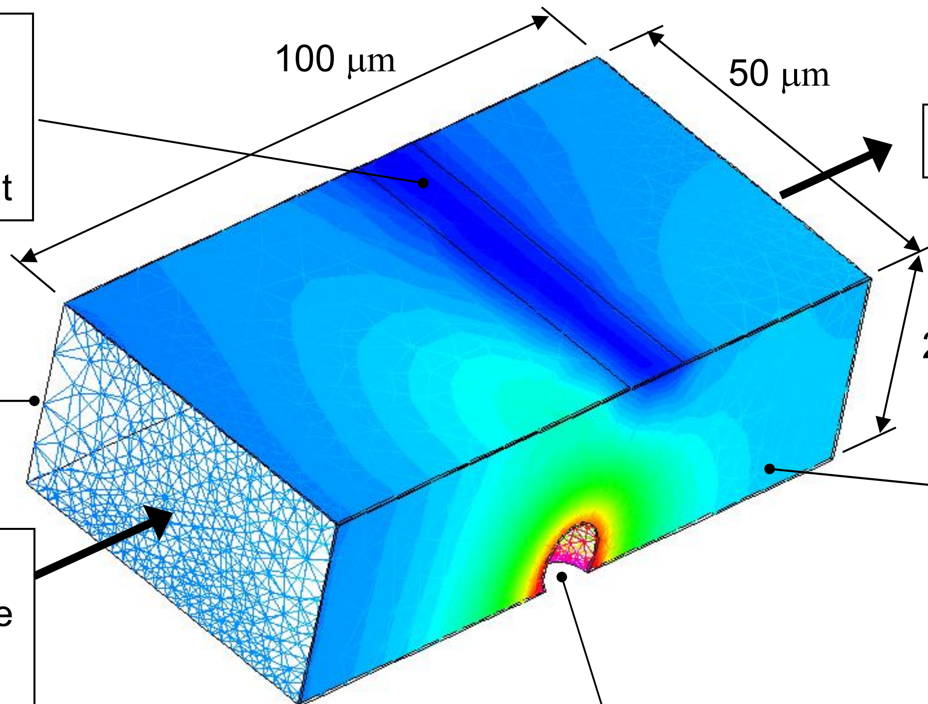
Nanophysiometer Modeling
Mark Stremler

- 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



Outlet

Symmetry plane

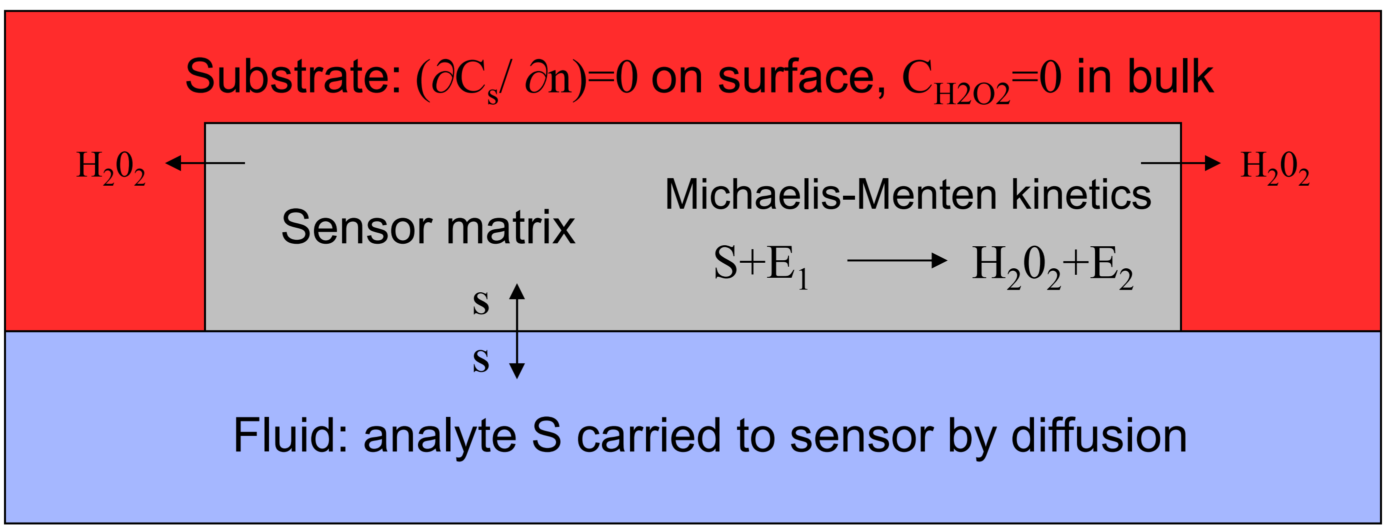
Single Cell:
- 10 μm diameter
- Specified membrane fluxes

- Possible device flow and sensing scenarios:

Flow	Sensing
Continuous	Continuous
Intermittent	Intermittent

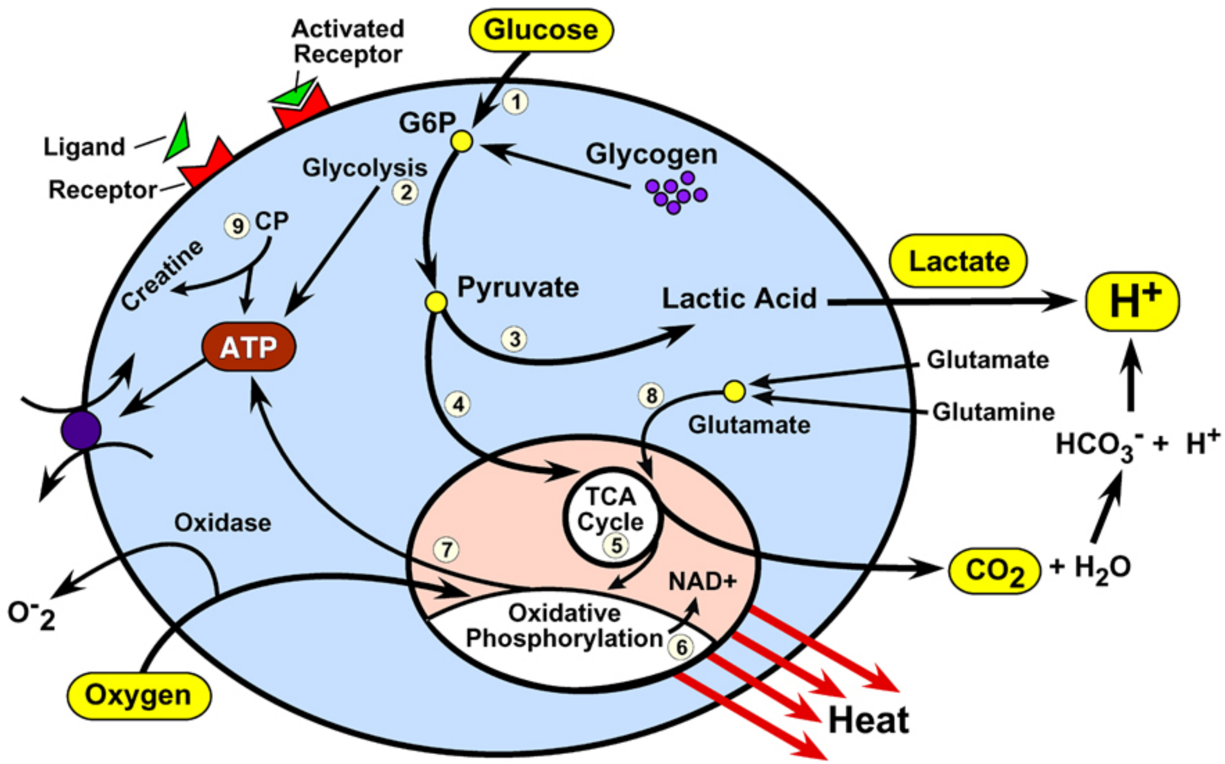
Inverse Sensor Model

- Model diffusion and reactions within the polymer matrix of the sensor.
- Enzyme concentration within the sensor assumed uniform.
- Production of H_2O_2 within sensor modeled with Michaelis-Menten kinetics.
- Sensor signal given by gradient of H_2O_2 at the surface.
- Model implemented analytically and with CFD-ACE



The Next Steps

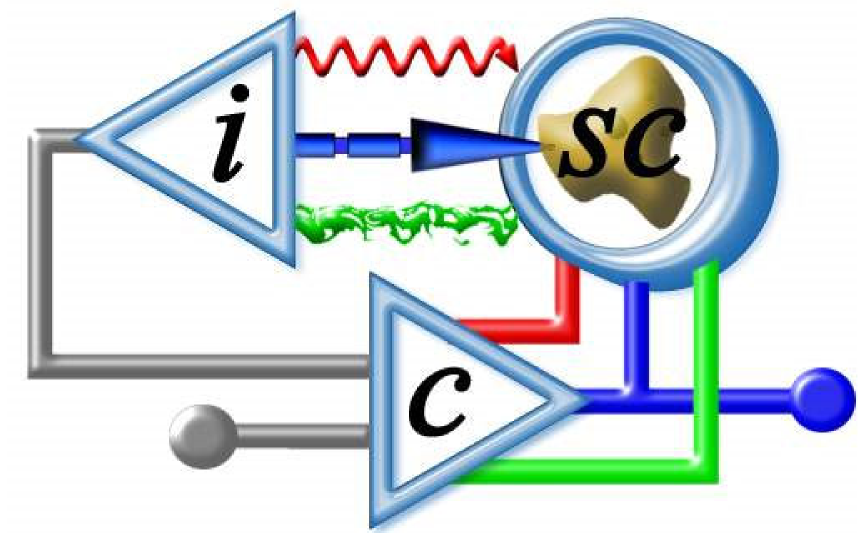
- Inverse sensor model
- Inverse metabolic network model
- Additional metabolic parameters
- Apply experiments, models and analysis to examine the blocking or enhancing of metabolic pathways



Theme III

Instrumenting and Controlling The Single Cell

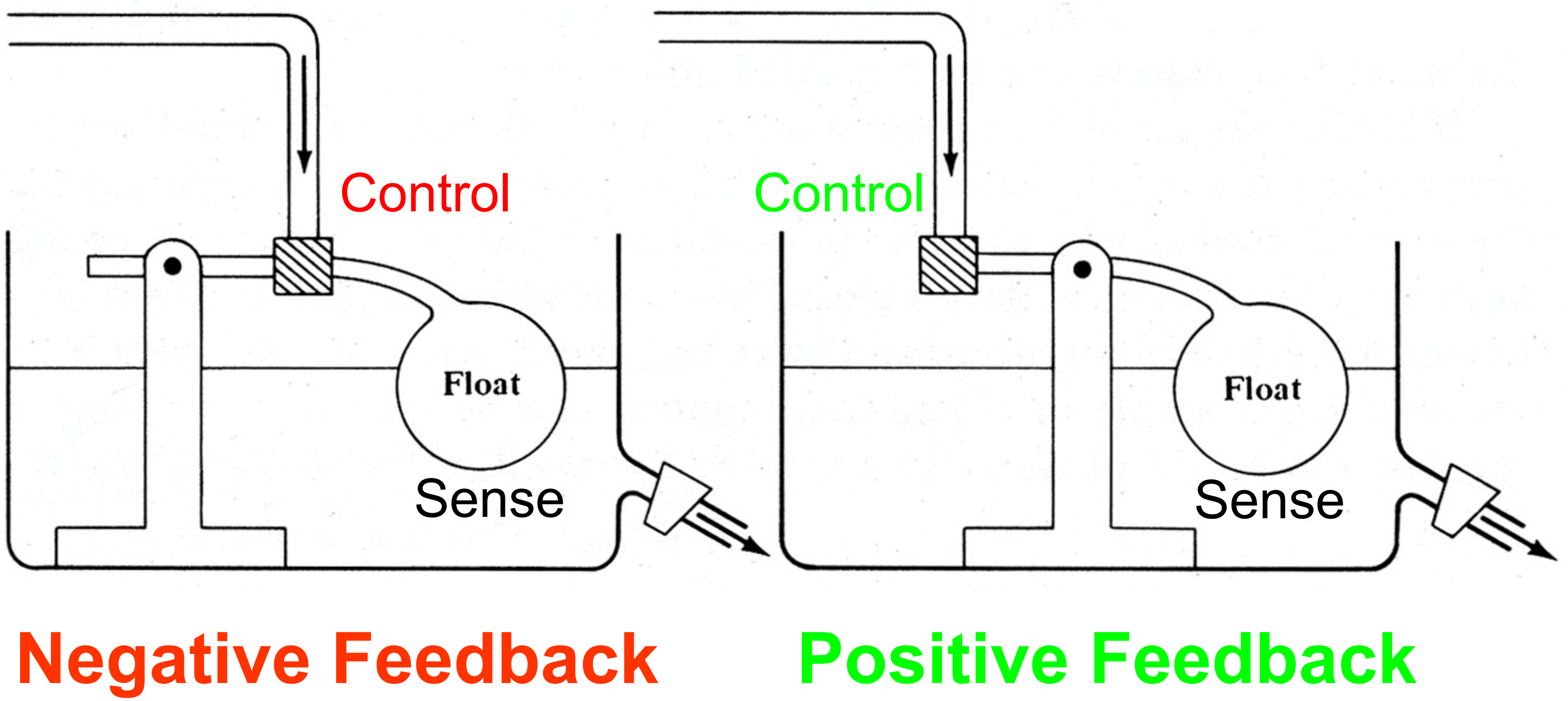
Goal: Develop devices, algorithms, and measurement techniques that will allow us to instrument and **control single cells** and small populations of cells and thereby explore the complexities of quantitative, experimental systems biology



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

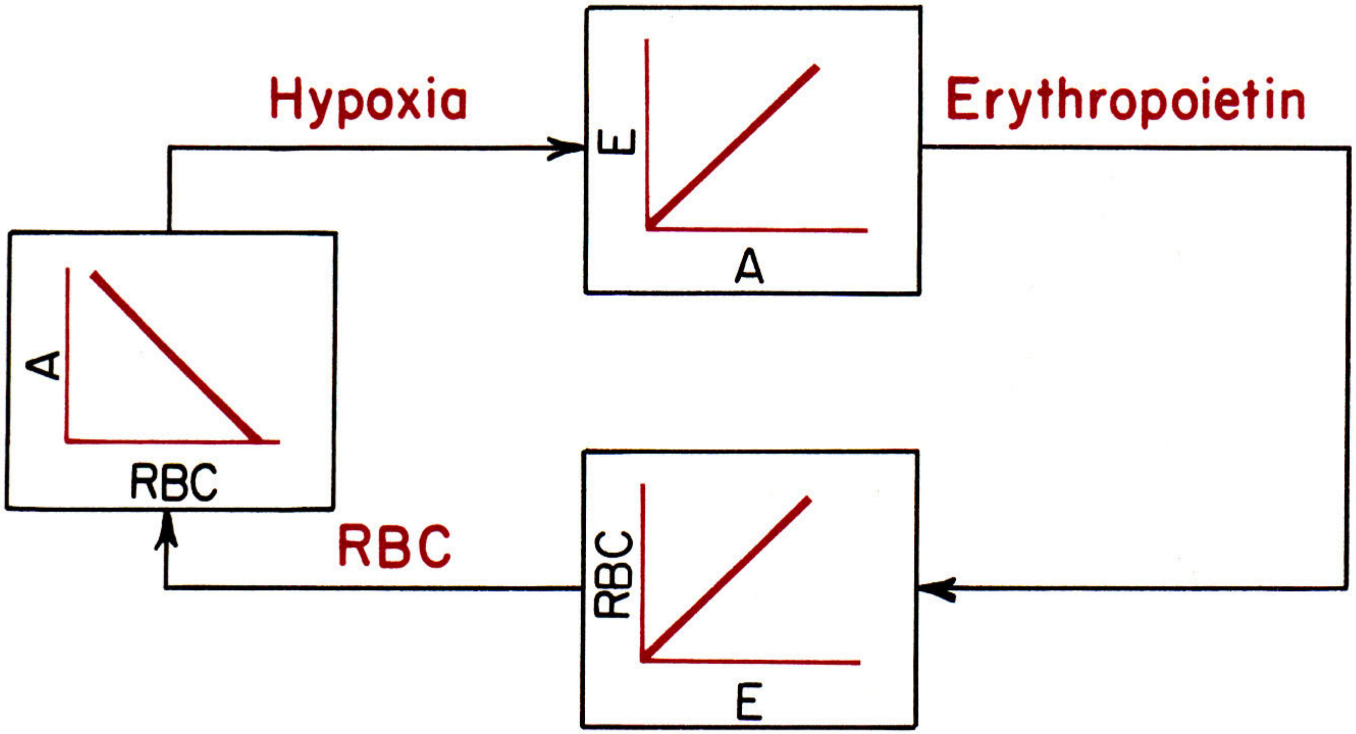
Hypothesis:	Great advances in physiology have been made by opening the feedback loop and taking control of the biological system

Negative versus Positive Feedback



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

Hypoxia-Red Blood Cell Concentration

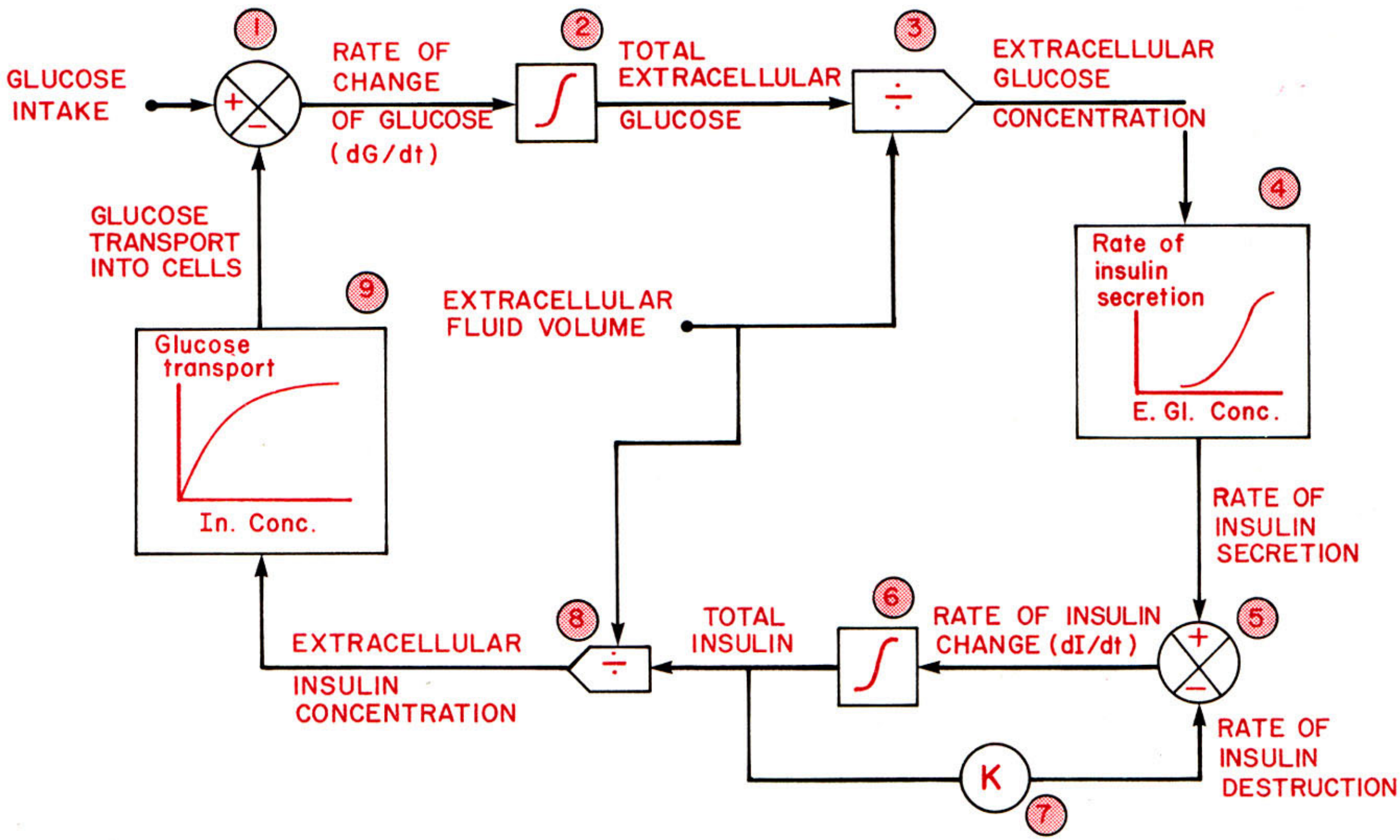


Variables

- Erythropoietin E
- Hypoxia A
- RBC

Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.59

Glucose-Insulin Control

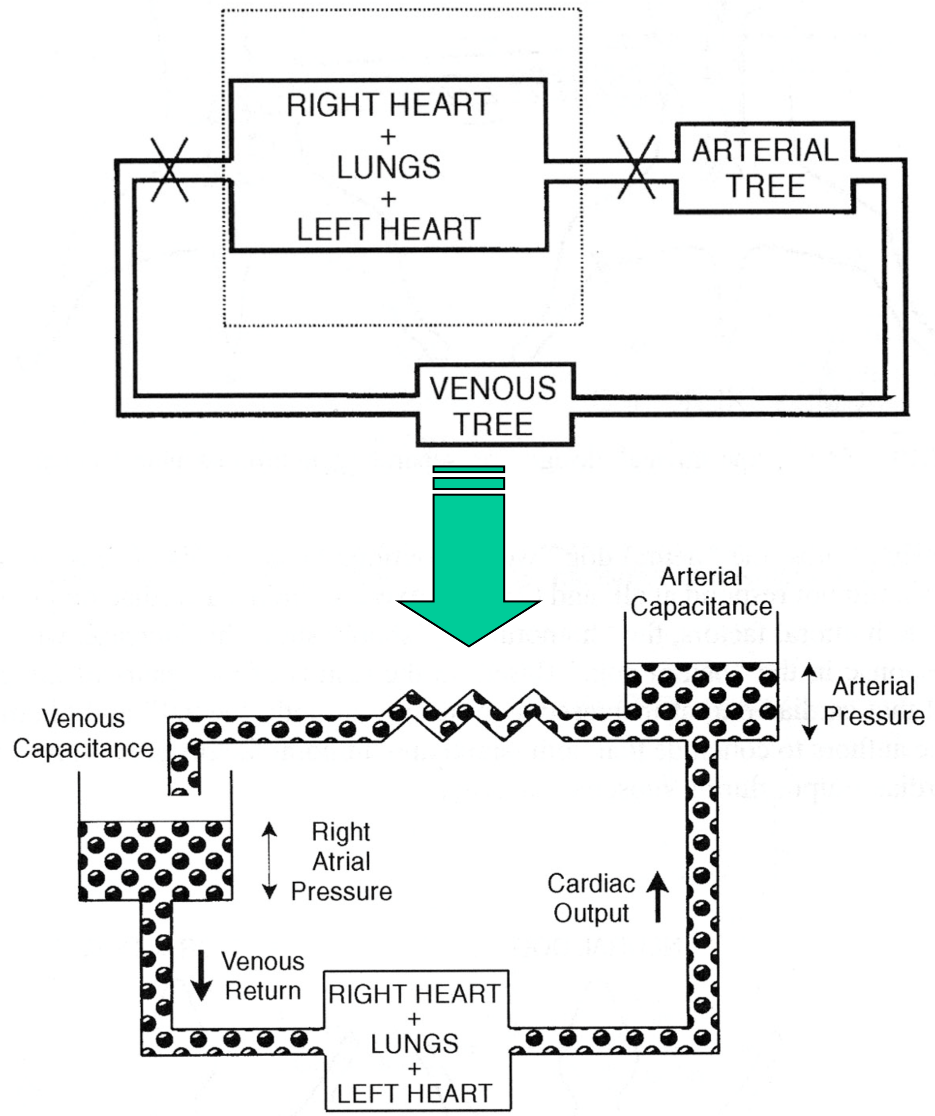


Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.9

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



Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.183

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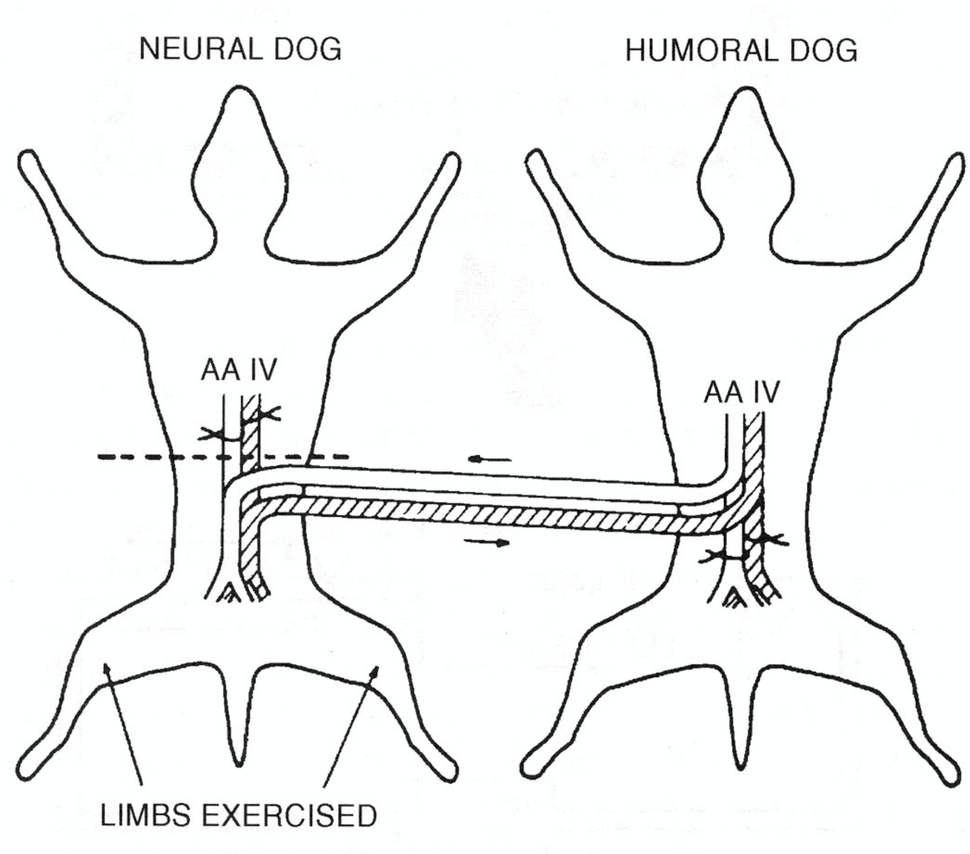

Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.184

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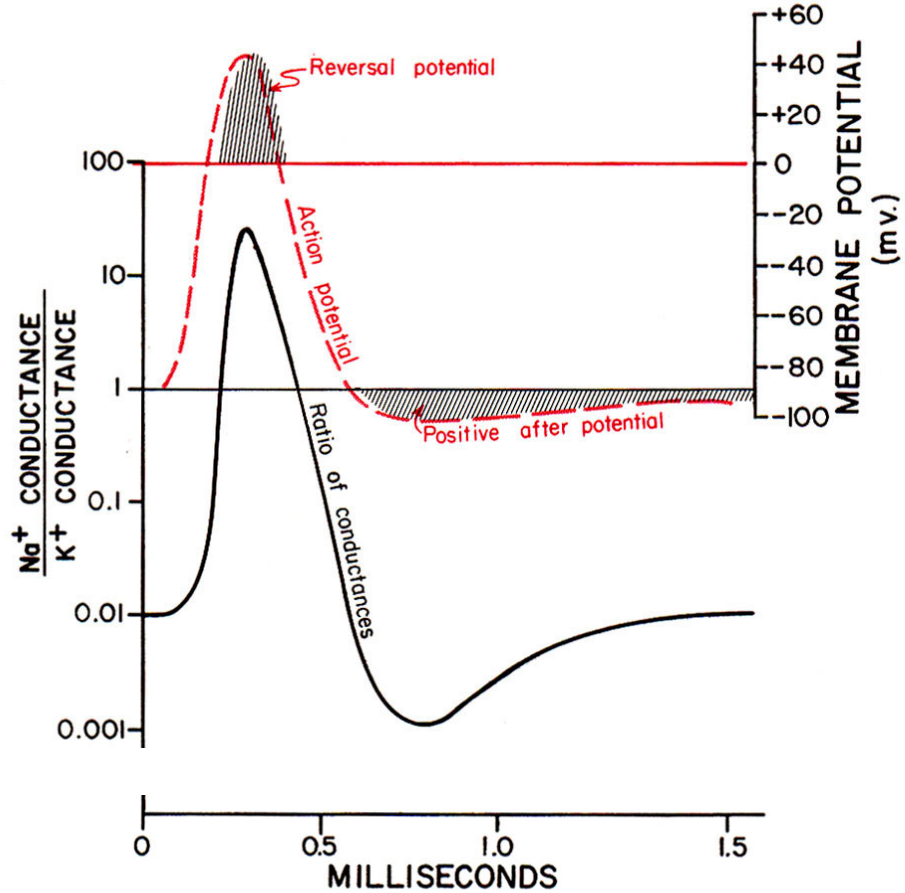
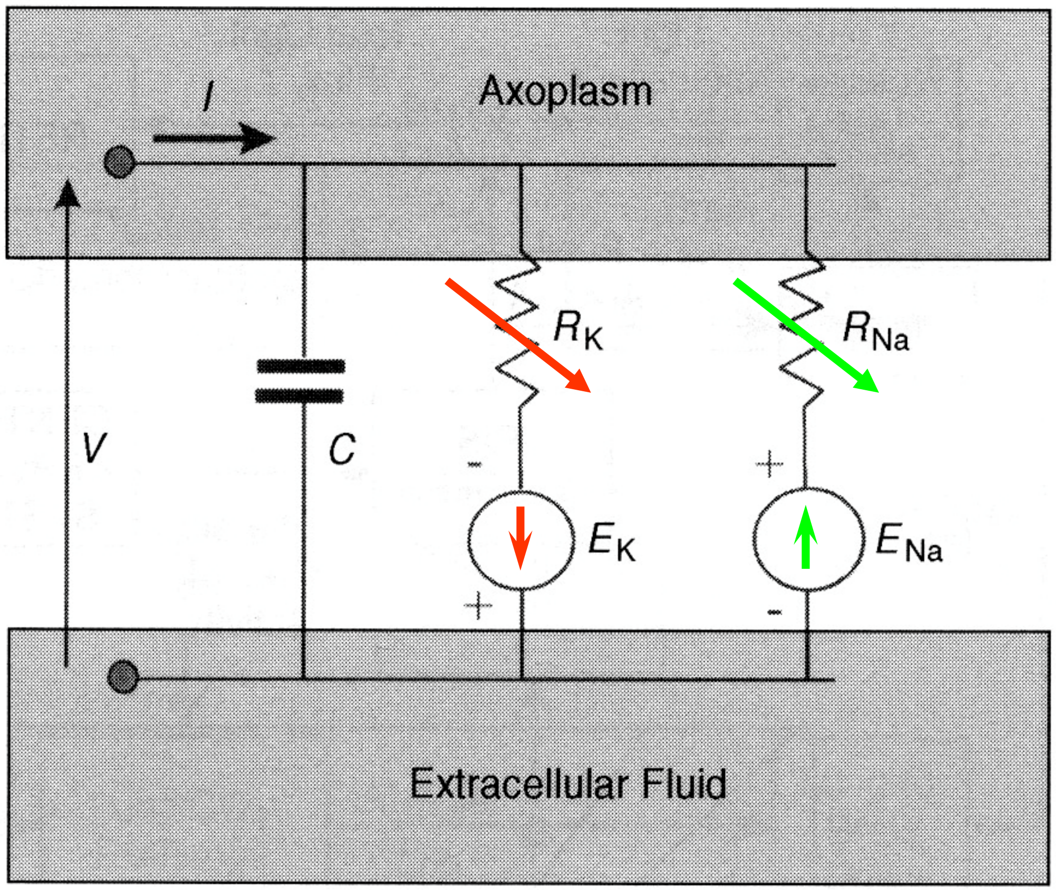


Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.110

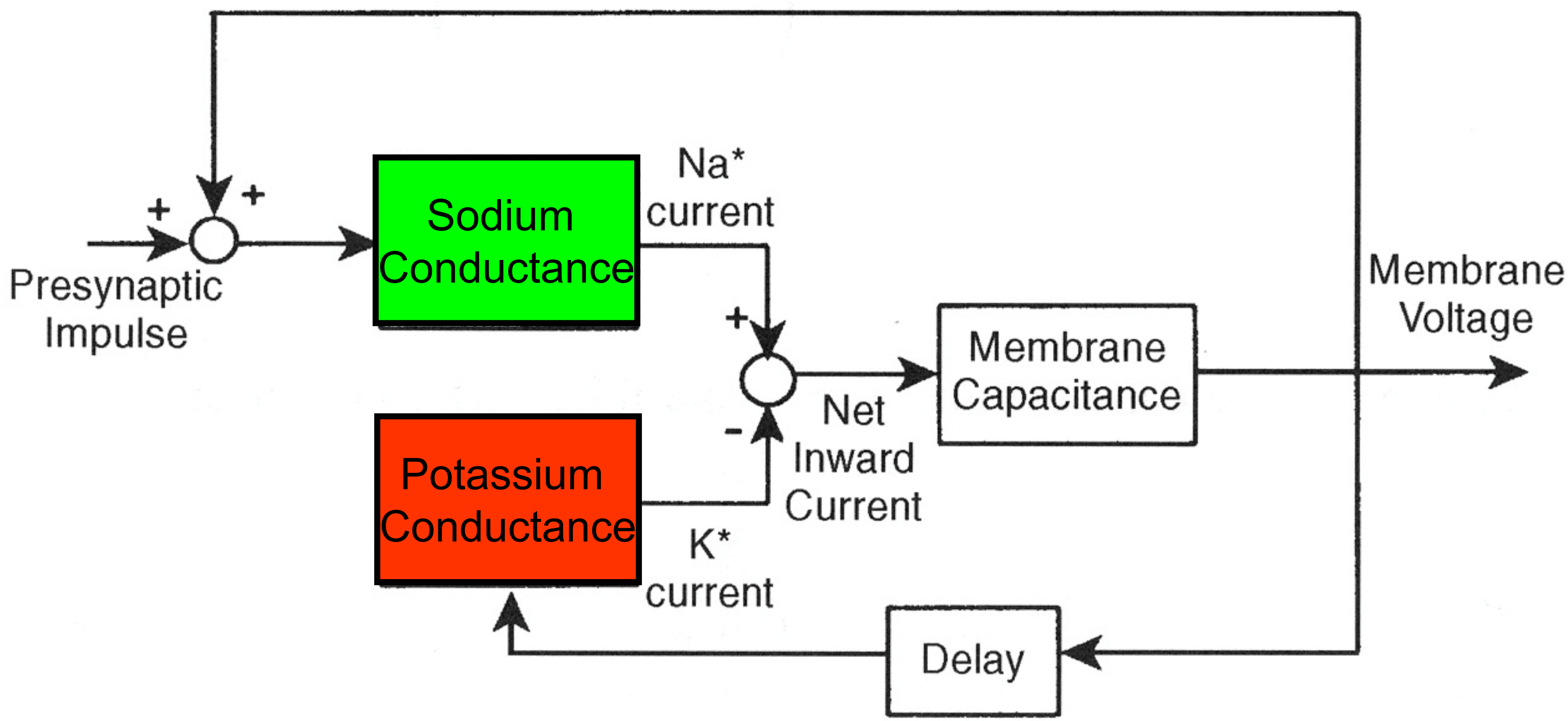
Simplified Hodgkin-Huxley



- 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

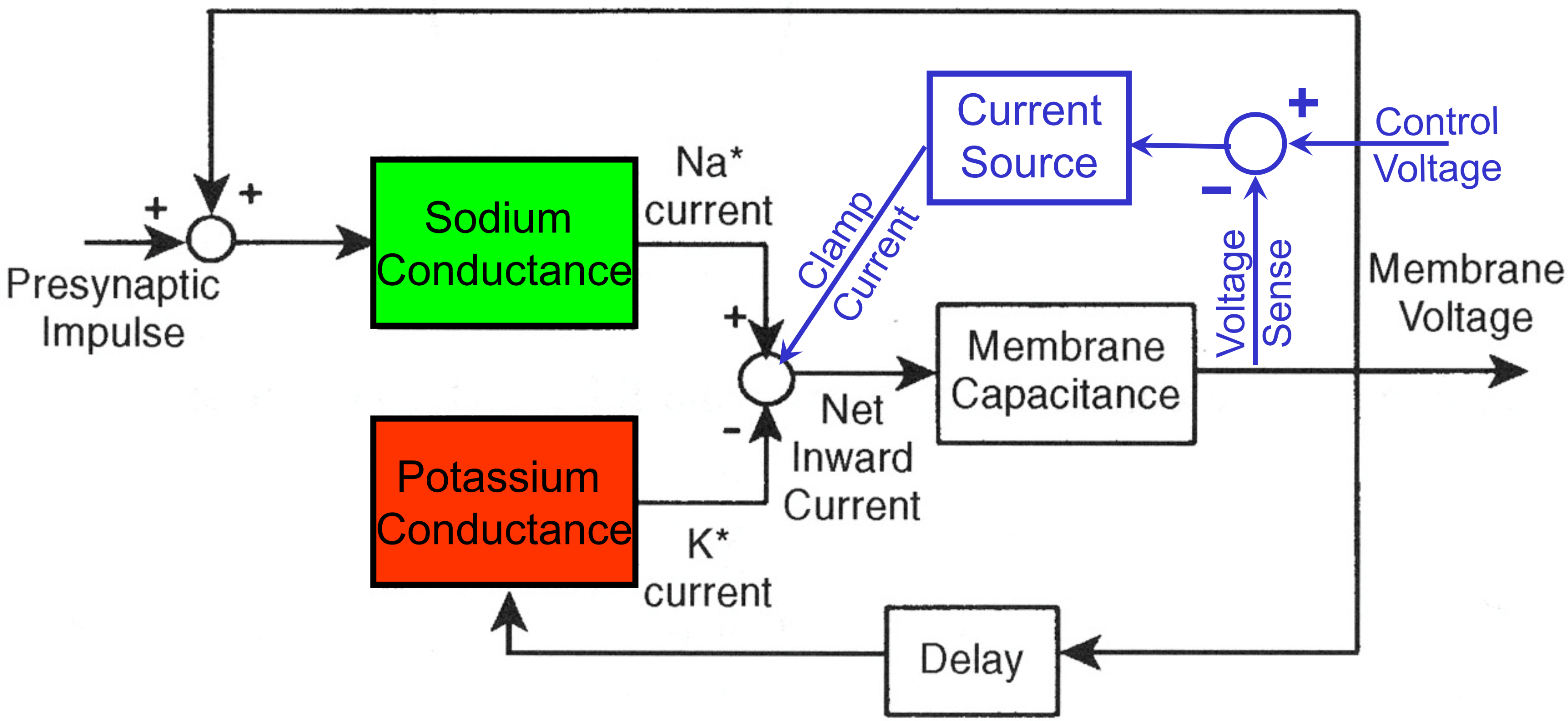
Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.187

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



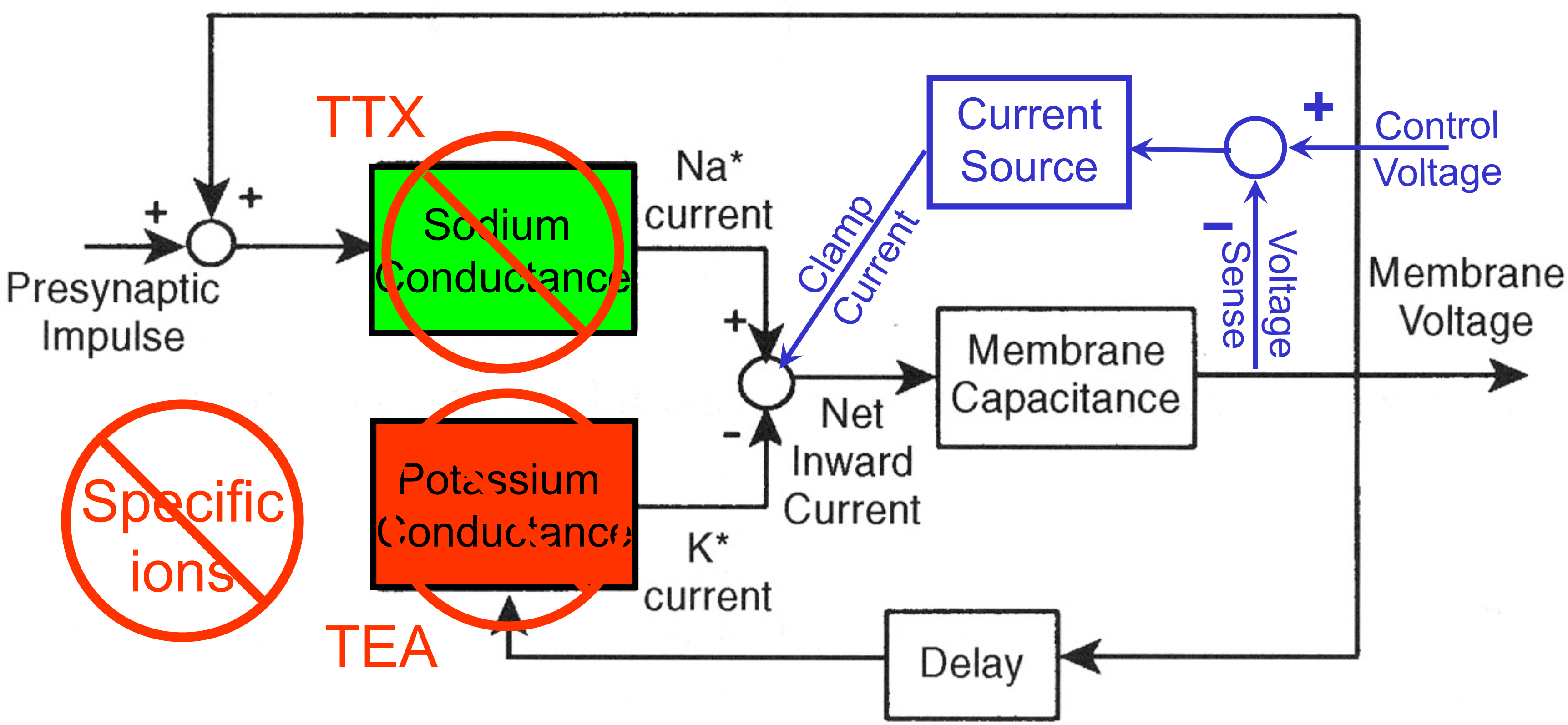
Adapted from Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259

Overriding Internal Control: Voltage Clamp



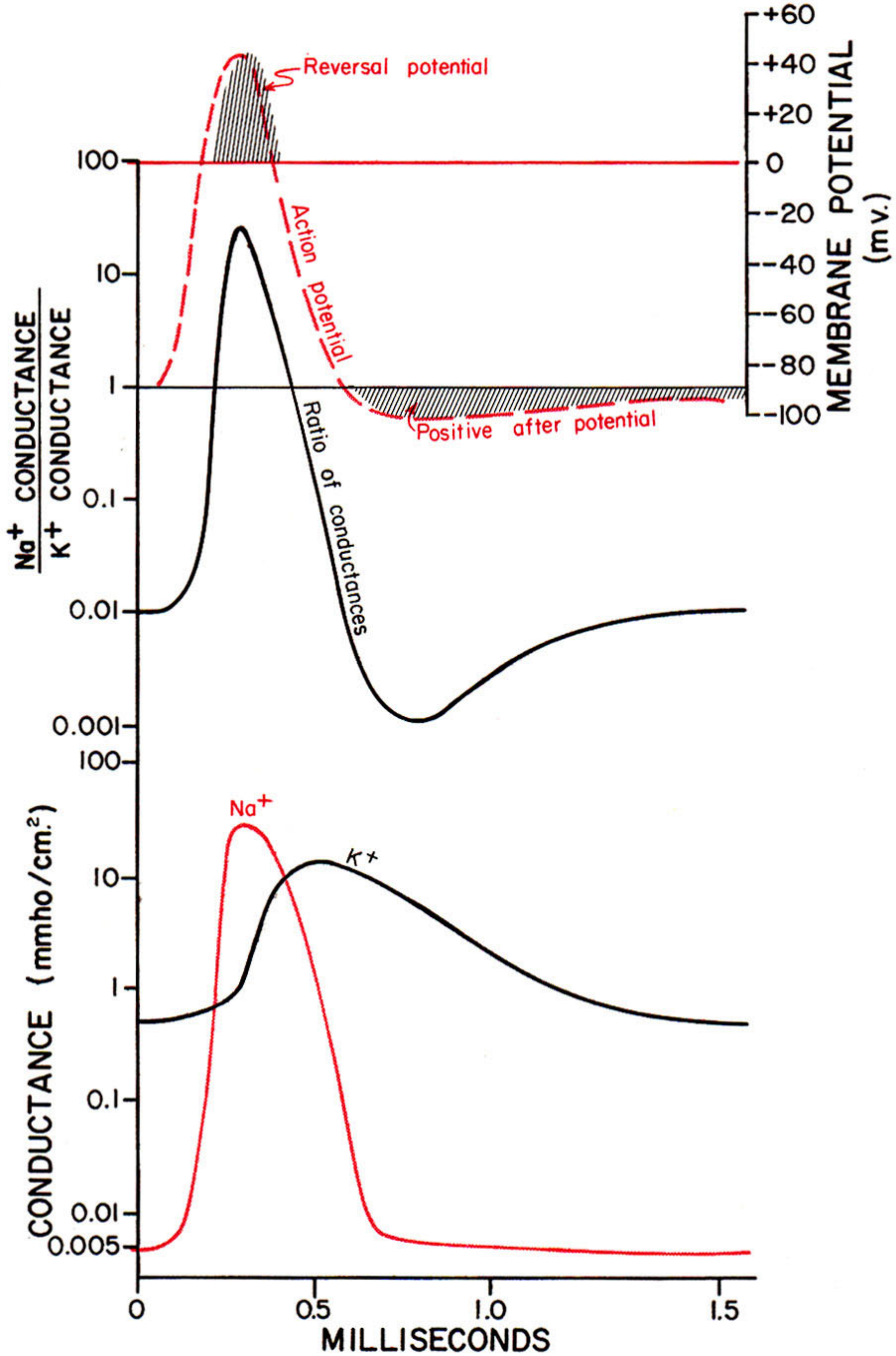
Adapted from Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.259

Opening the Loop During External Control



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

Voltage clamp of the nerve axon



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

VI∫BRE

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

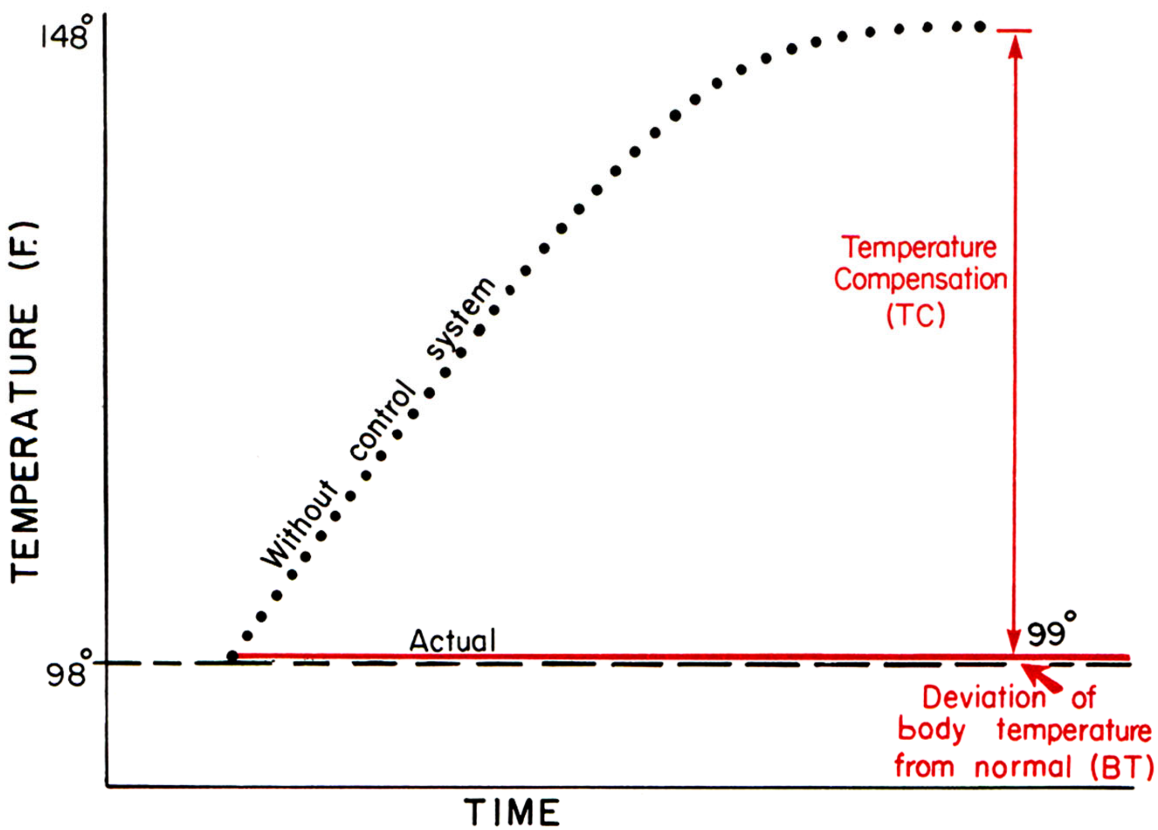
Required:	New devices to sieze control of subsecond, submicron <u>cellular</u> processes.

A Key to the Future of Systems Biology: External Control of Cellular Feedback

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

Signatures of Control

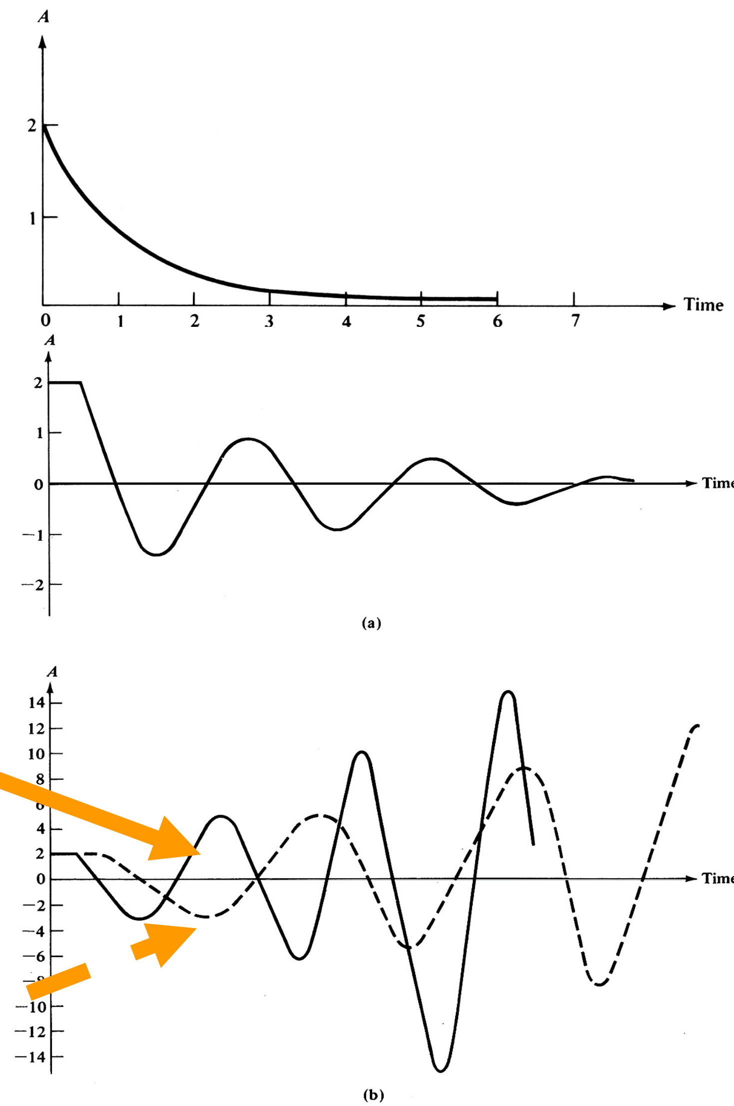
- Stability in the presence of variable input (DT= 50° F)
- Oscillations when excessive delay or too much gain
- Divergent behavior when internal range is exceeded or controls damaged



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

Control Stability

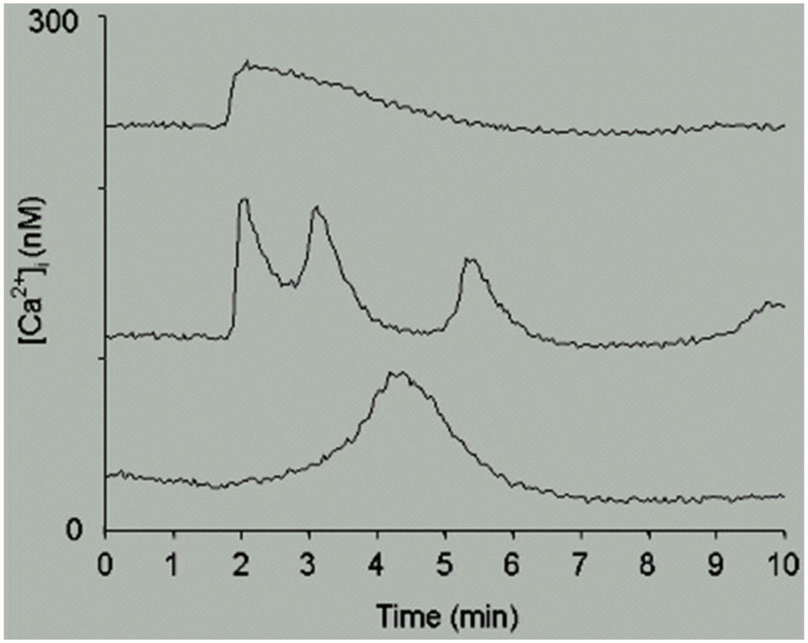
- Proportional control
- Proportional control with finite time delay
- Higher gain, same delay
- Same gain, longer delay



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
  - Repair enzymes



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

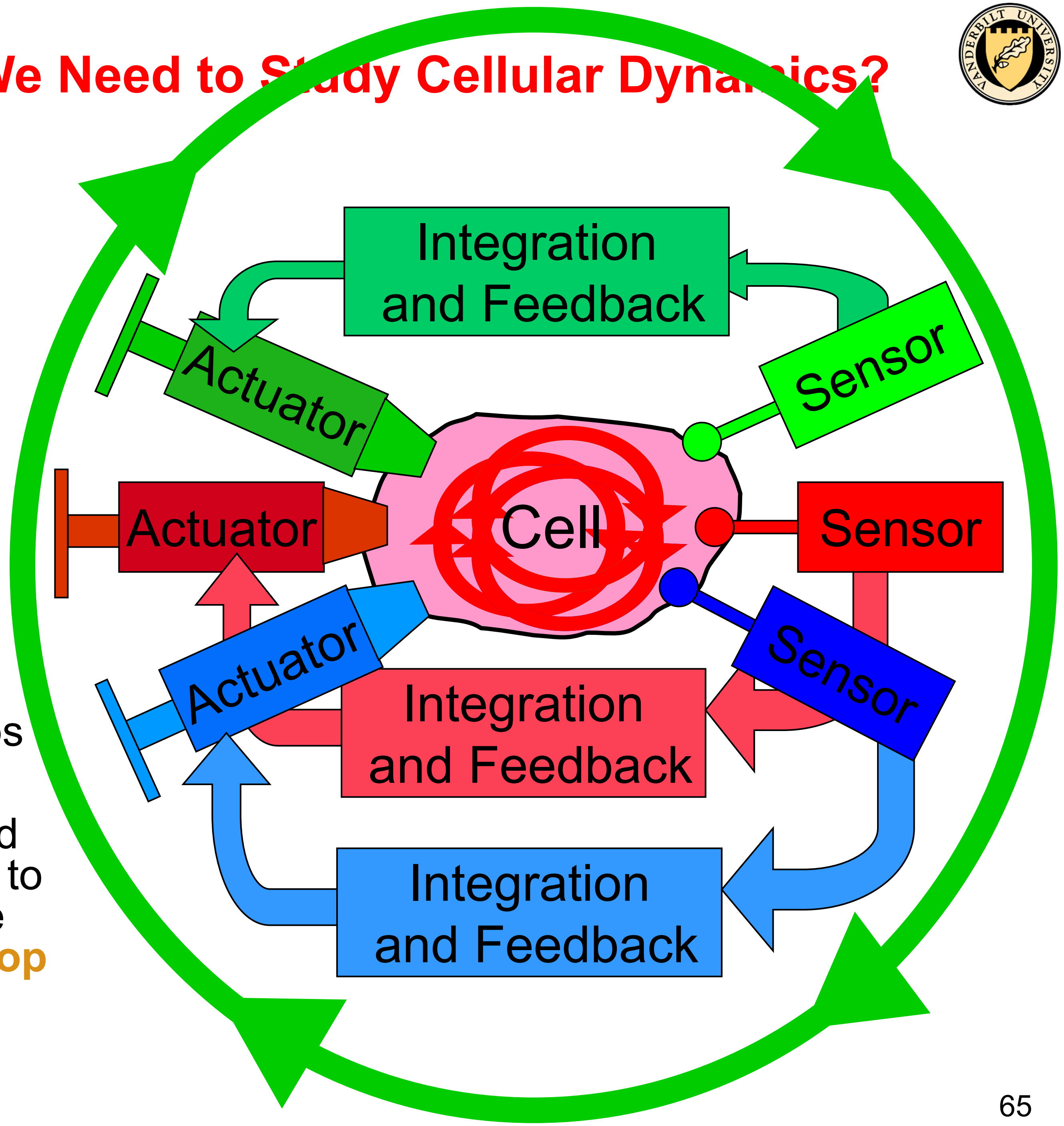
http://www.intracellular.com/app05.html

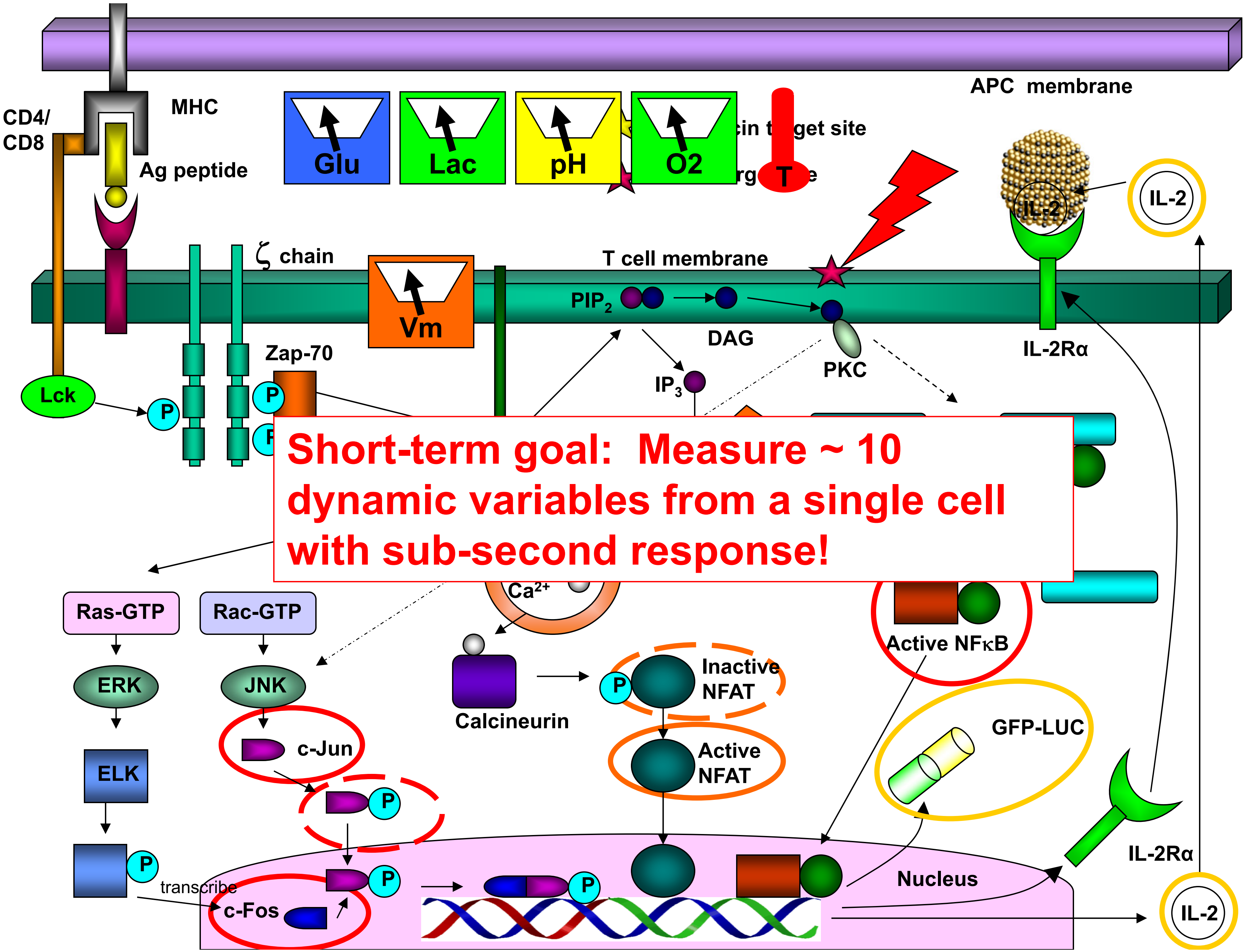
Ok, we're convinced about feedback and control….

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**
- …

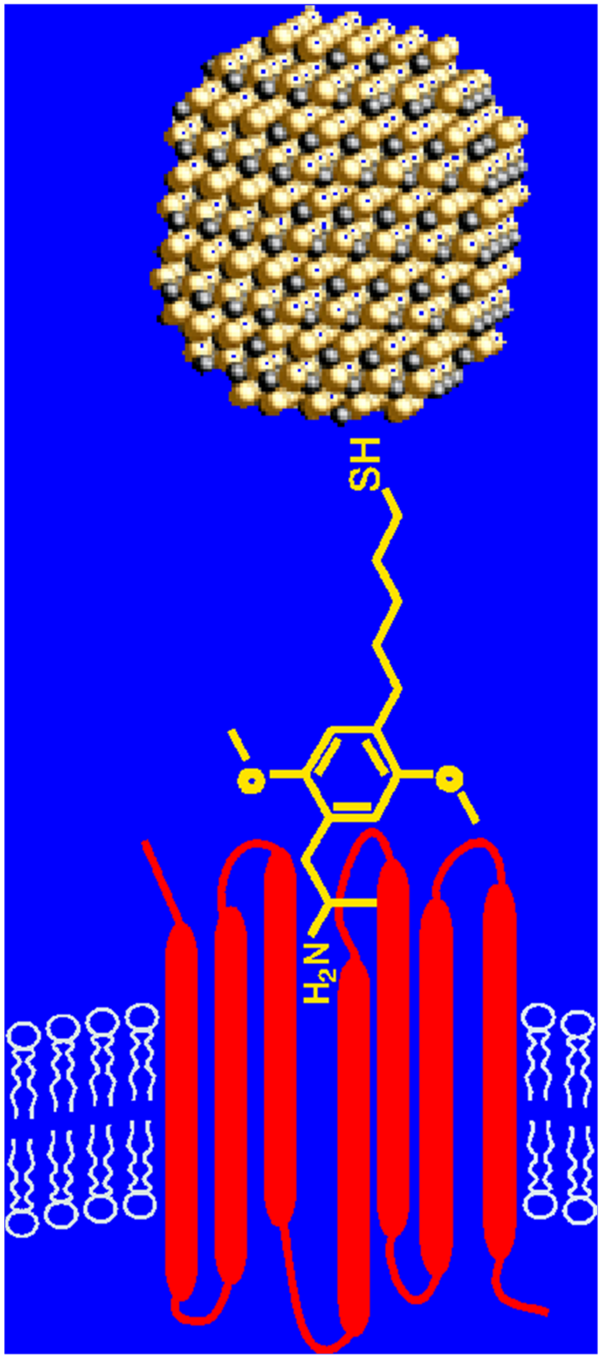




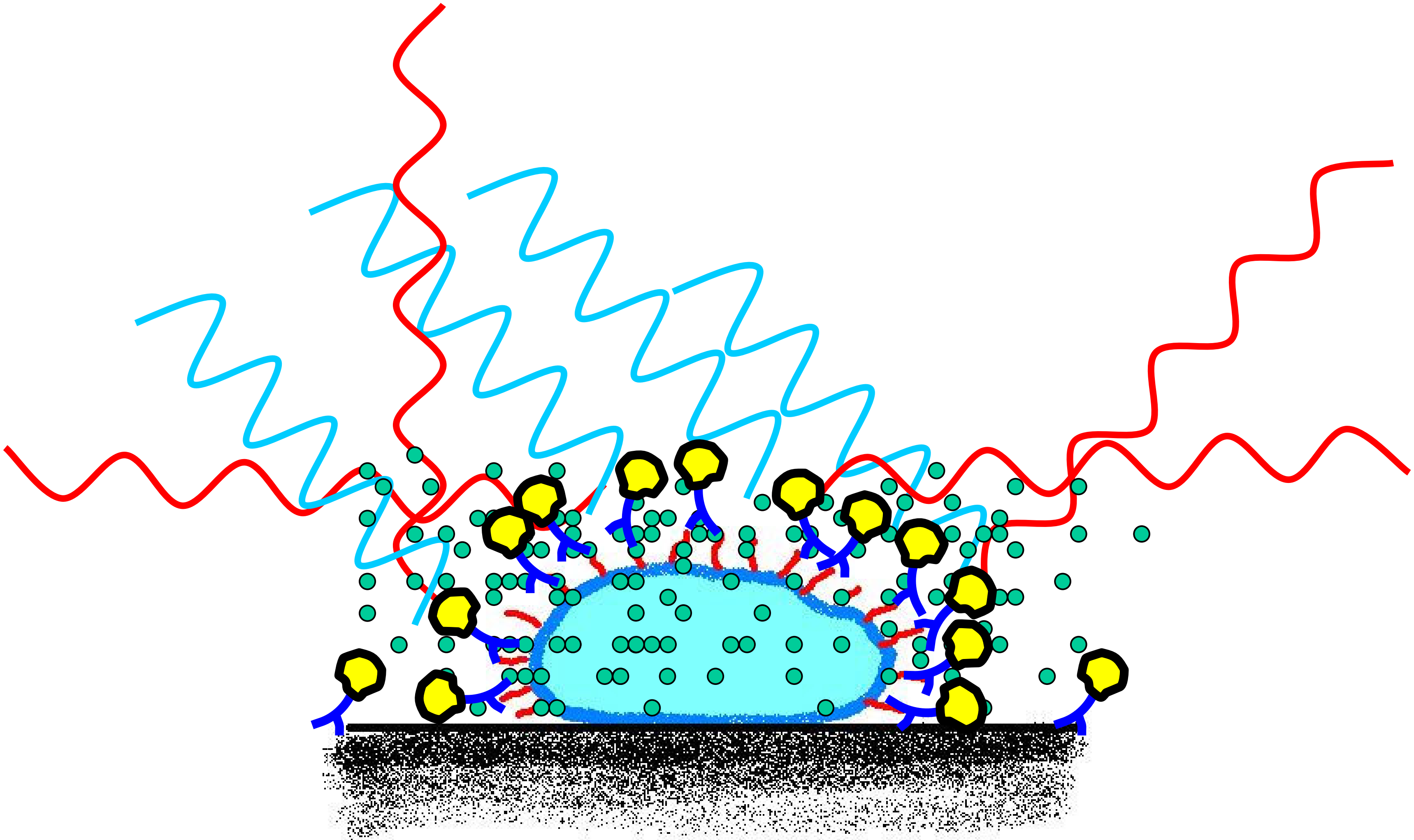
Short-term goal: Measure ~ 10 dynamic variables from a single cell with sub-second response!

Quantum Dots to Report Protein Presence

- Quantum dots can be congugated to an antibody that then binds to a membrane protein

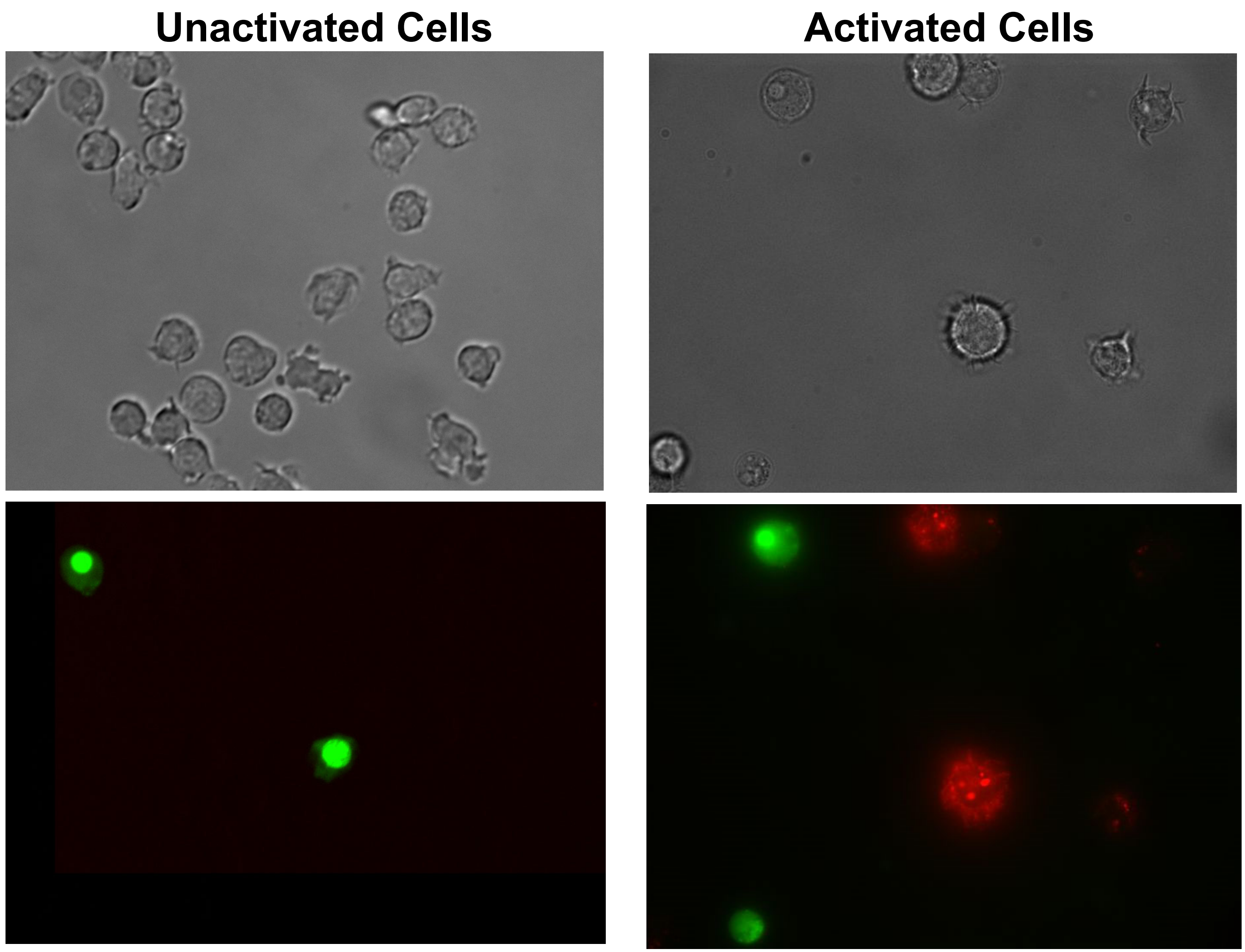


QD Detection of Gene Upregulation

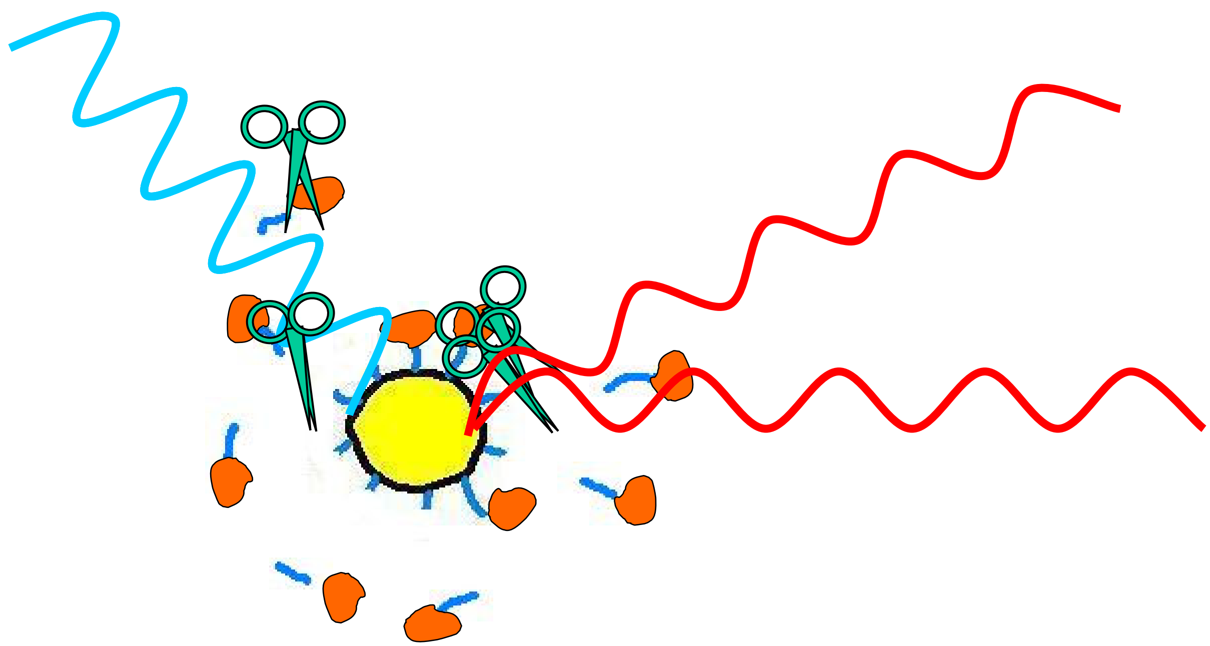


Activated Jurkat Cells Labeled with IL-2Ab Conjugated QDots

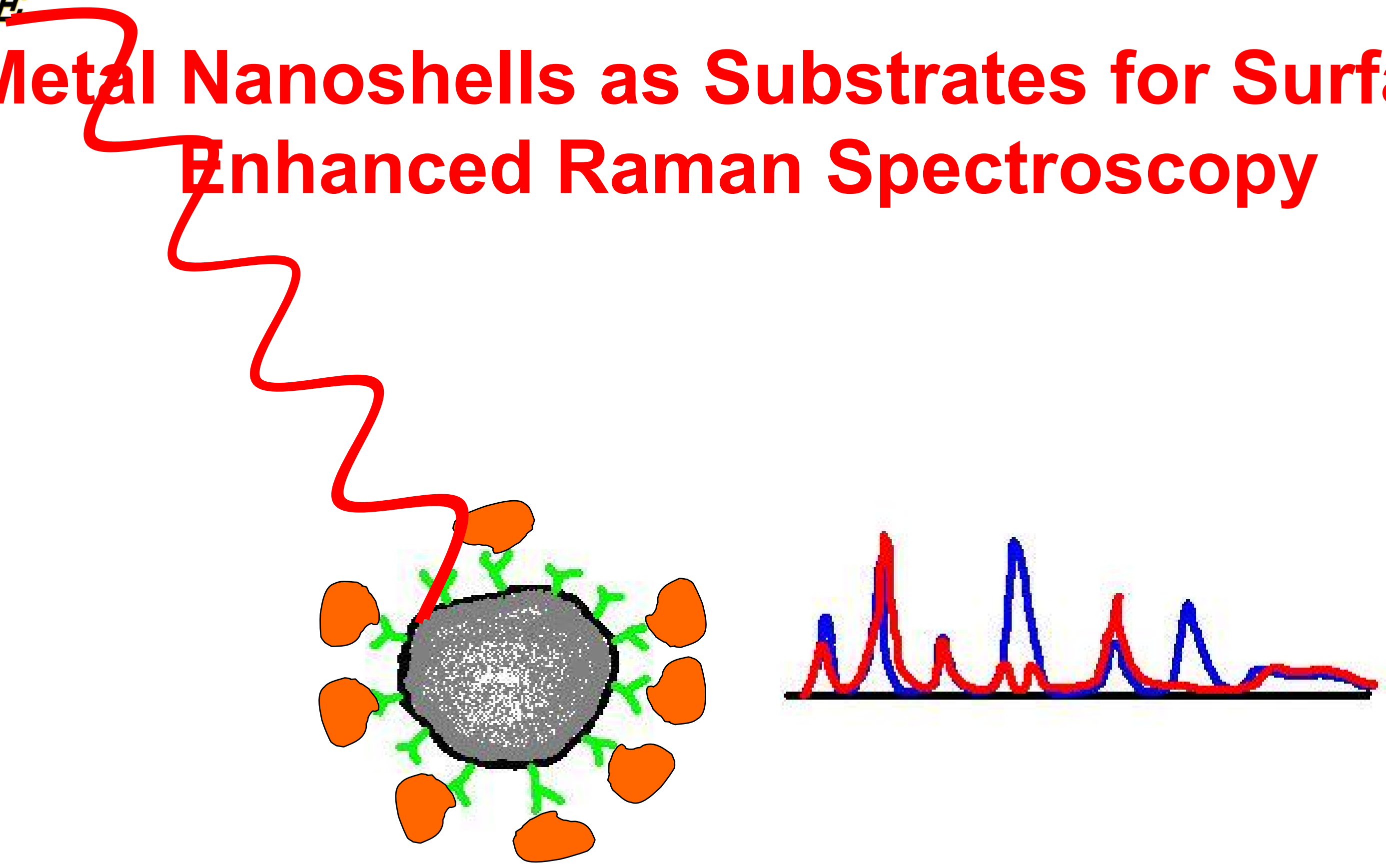
- Red: Anti-IL2 QDots
- Green: Yopro-1 nucleic acid stain (i.e. non-viable cells)
- Activated using PMA & Ionomycin for 72 hrs
- QDots label 50-70% of viable activated cells



Quantum Dot Quenching for Detection of Protein Binding and Enzyme Activity



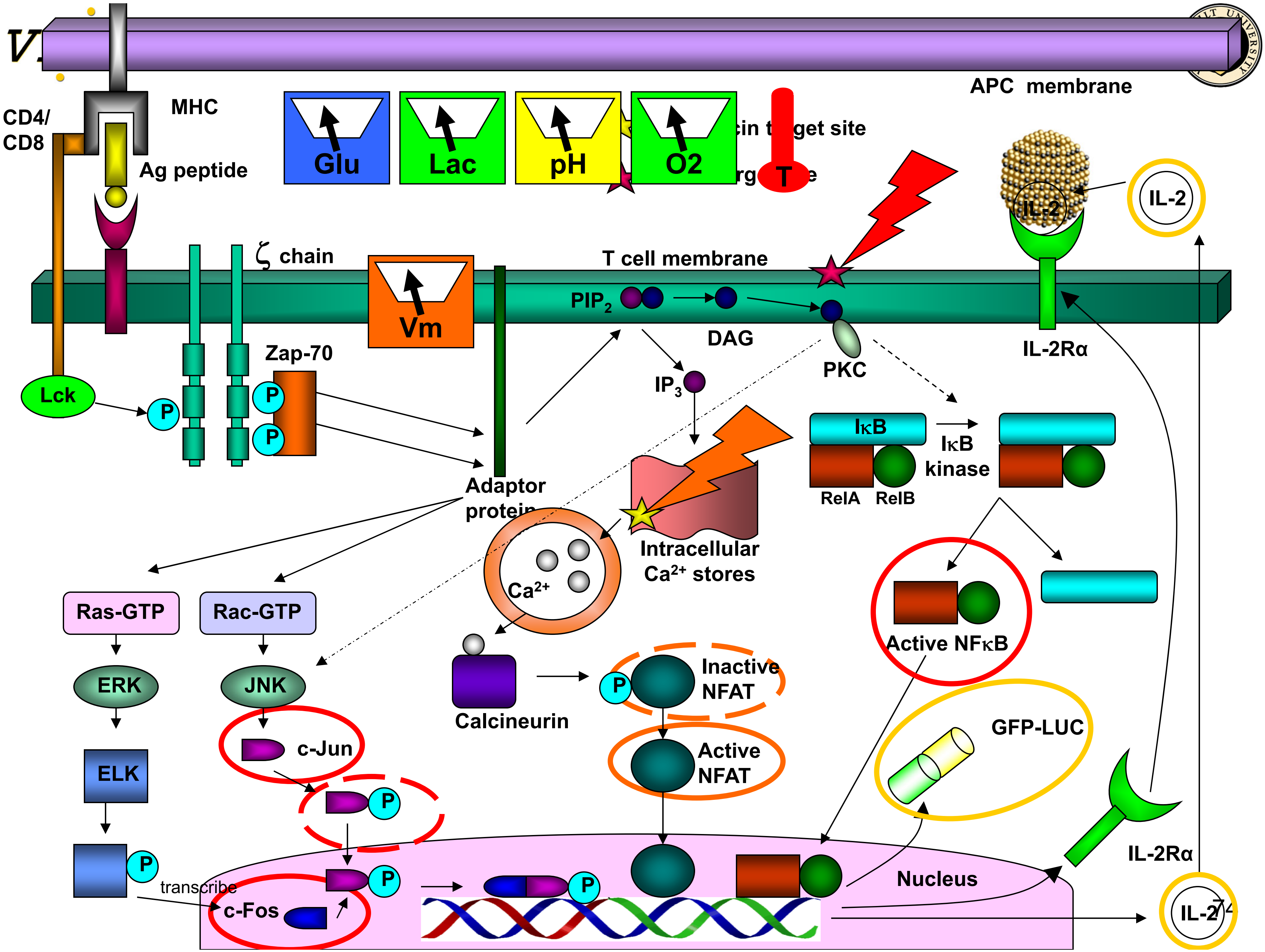
Metal Nanoshells as Substrates for Surface-Enhanced Raman Spectroscopy



- 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?


APC membrane
CD4/
CD8
MHC
Ag peptide
Glu
Lac
pH
O2
T
ζ chain
T cell membrane
Vm
PIP2
DAG
PKC
IL-2
IL-2
IL-2Rα
Zap-70
P
P
P
Lck
IP3
IκB
IκB
kinase
RelA
RelB
Adaptor
protein
Intracellular
Ca2+ stores
Ca2+
Active NFκB
Ras-GTP
Rac-GTP
ERK
JNK
Calcineurin
Inactive
NFAT
Active
NFAT
c-Jun
GFP-LUC
ELK
IL-2Rα
Nucleus
transcribe
c-Fos
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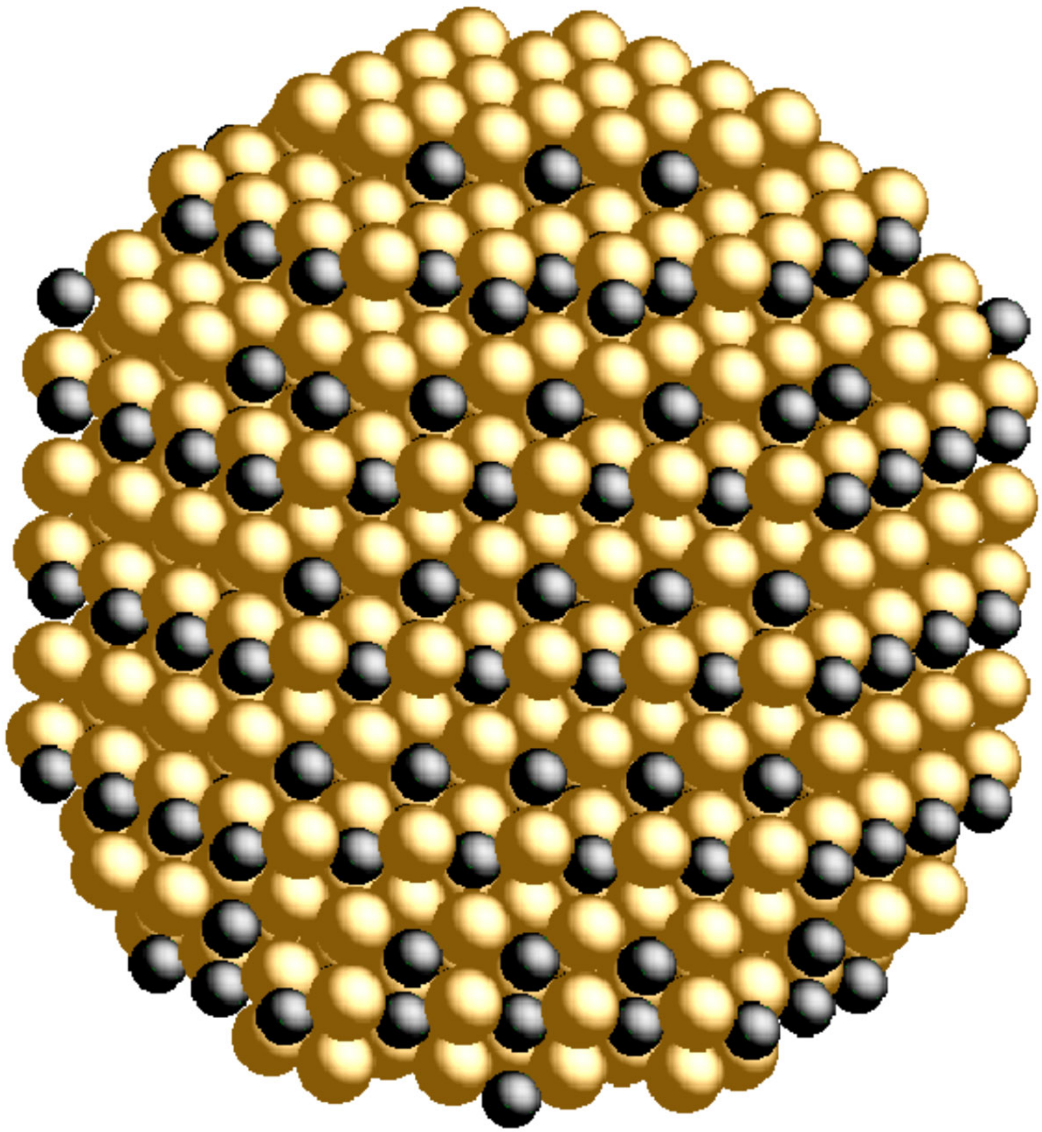
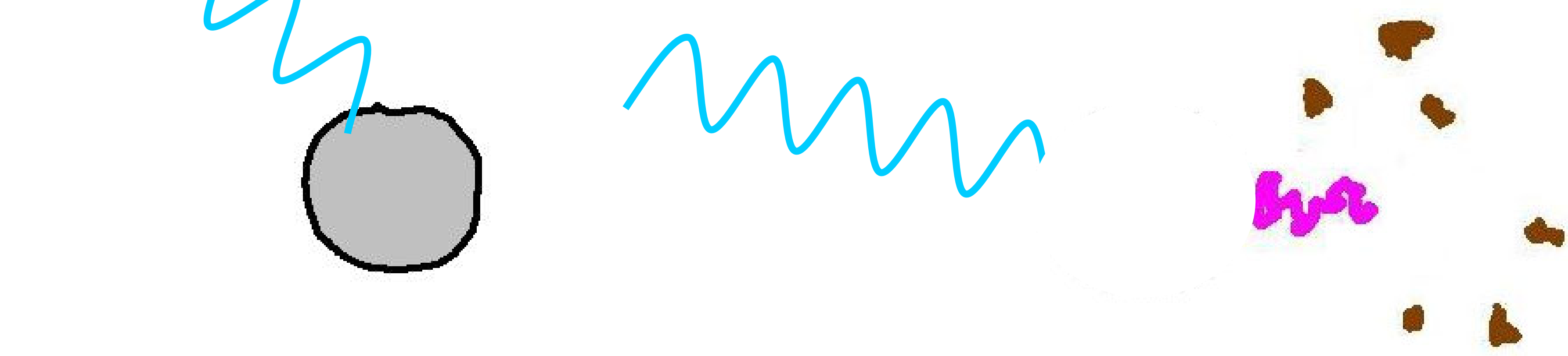


Th

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

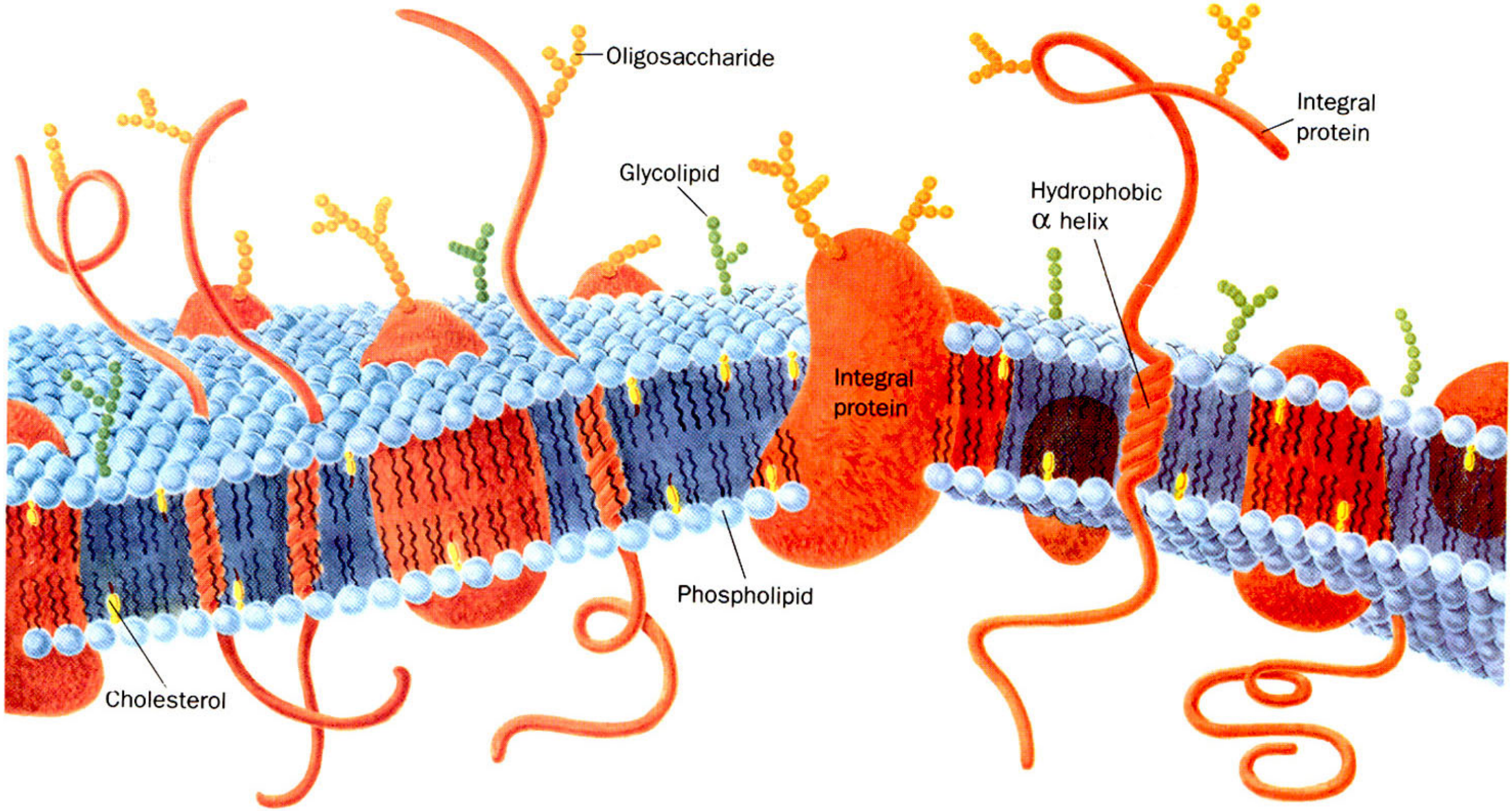
- Translational and rotational forces
  - Viscosity -- Nanorheometry
  - Molecular motor characterization
- Magnetic separation
- Magnetic identification
  - Tagged cells
  - Tagged molecules

We need more cellular nanoactuators!!

What is the cellular sensor/actuator competition?

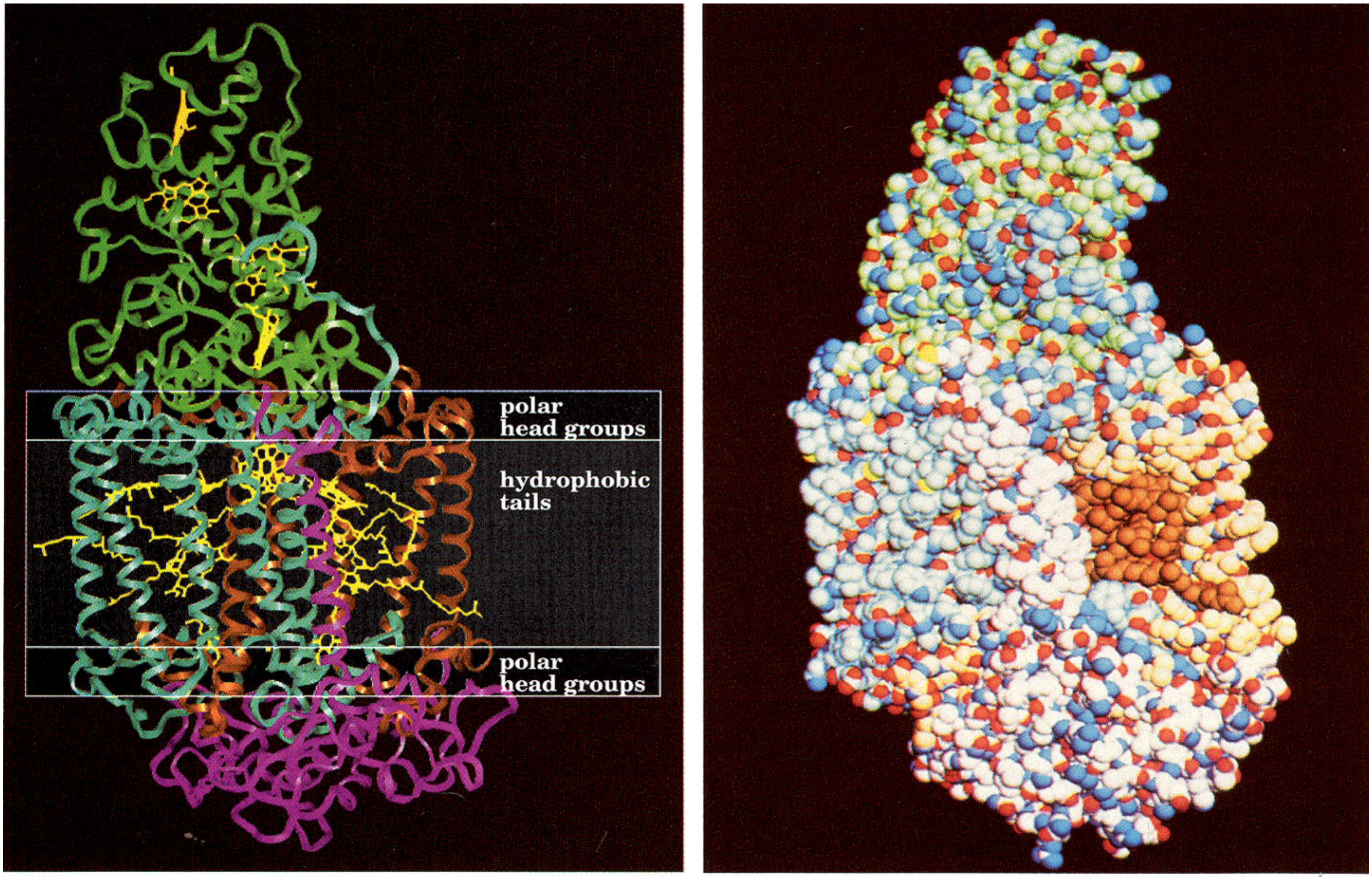
Proteins, proteins, proteins…

Plasma Membrane



Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 292

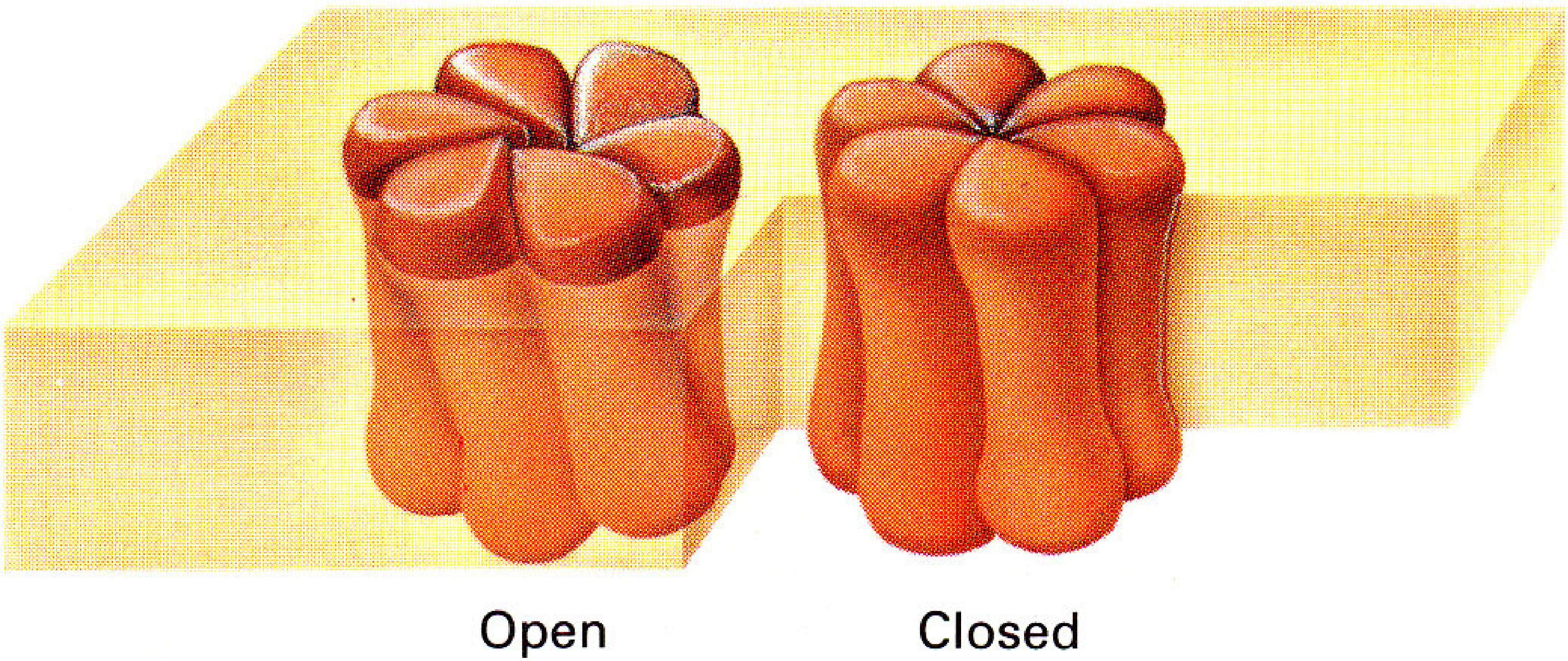
Bacterial Photosynthetic Reaction Center



(a) (b)

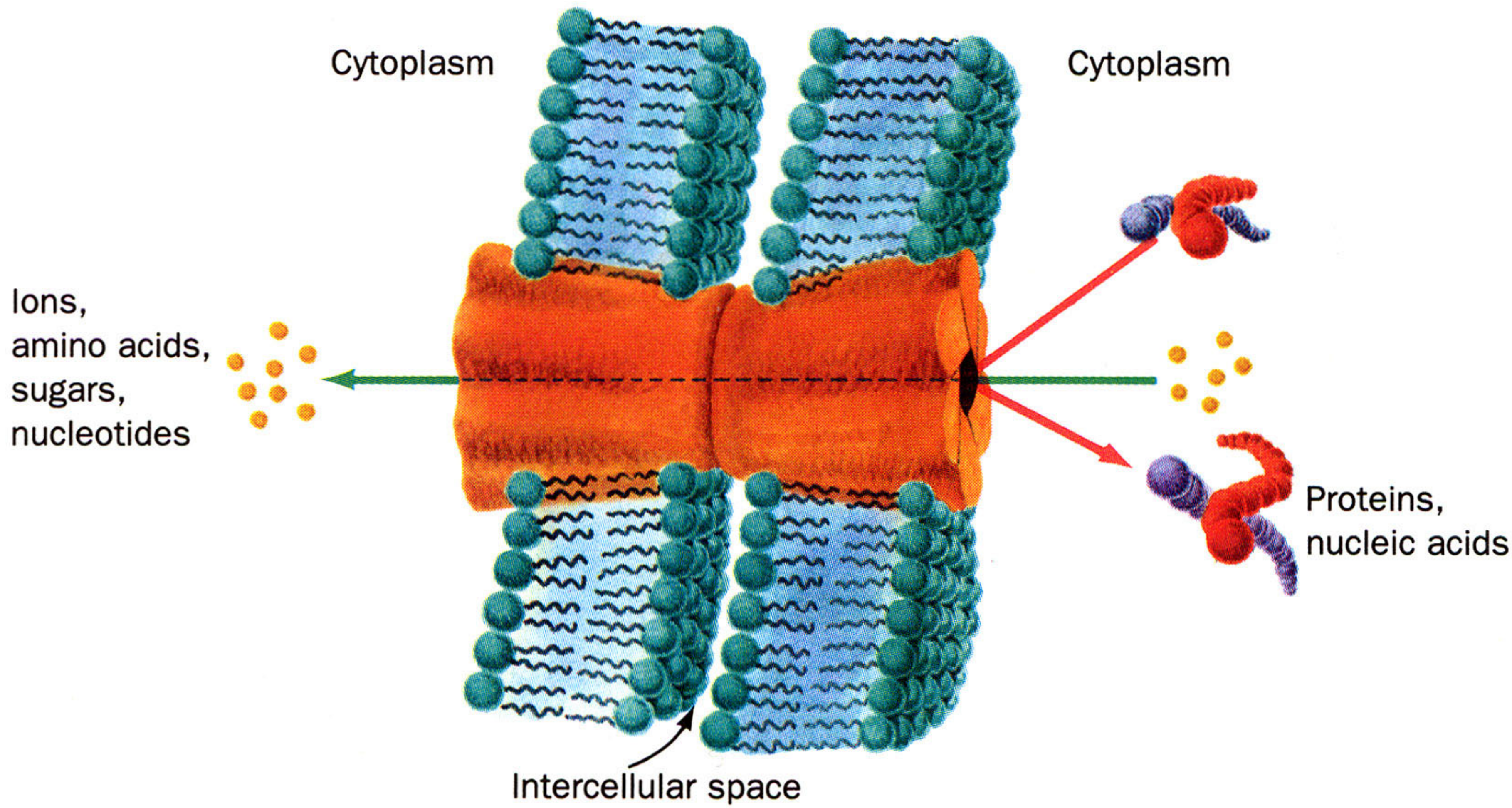
Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 296

Calcium Control of Conductance



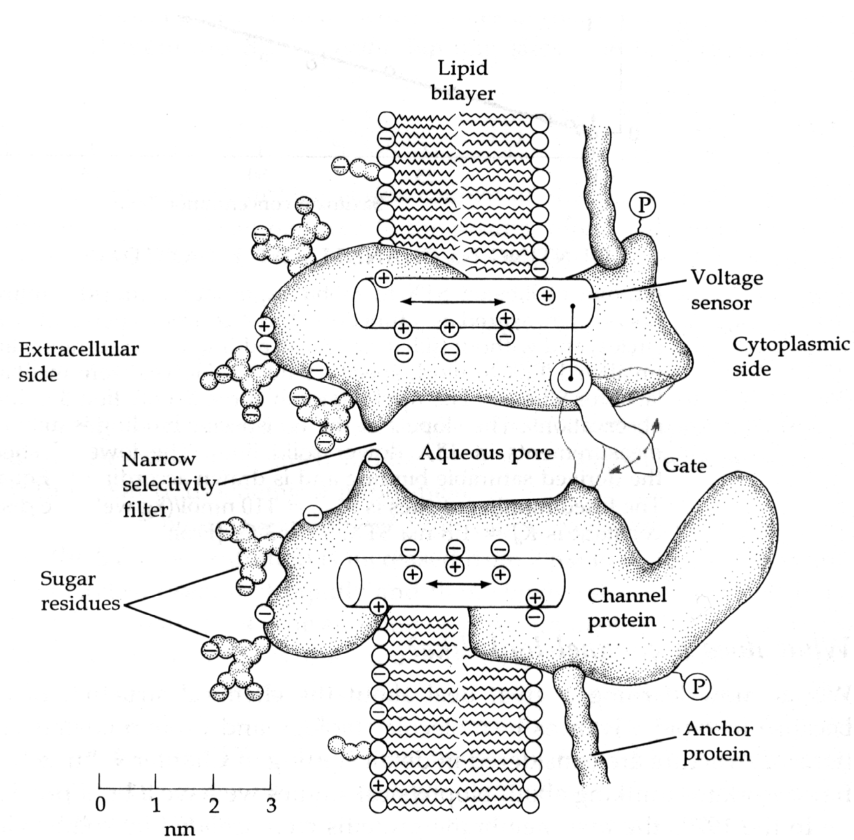
Molecular Cell Biology, 2nd ed.
Darnell, J.; Lodish, H.; Baltimore,
W.H Freeman & Co. 1990, p.525

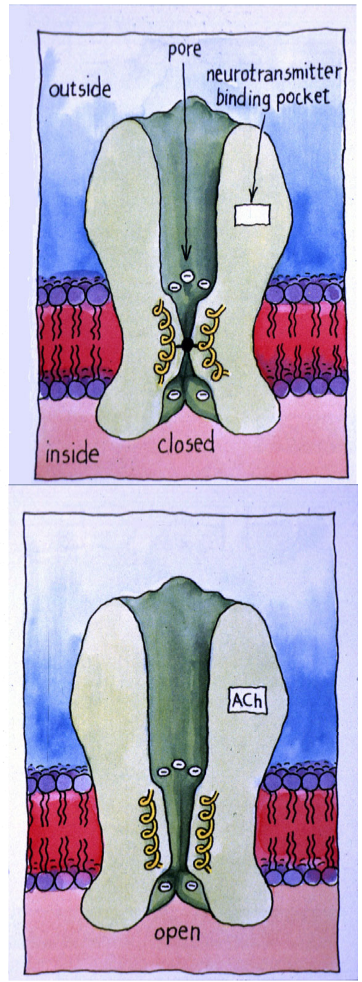
Gap Junctions



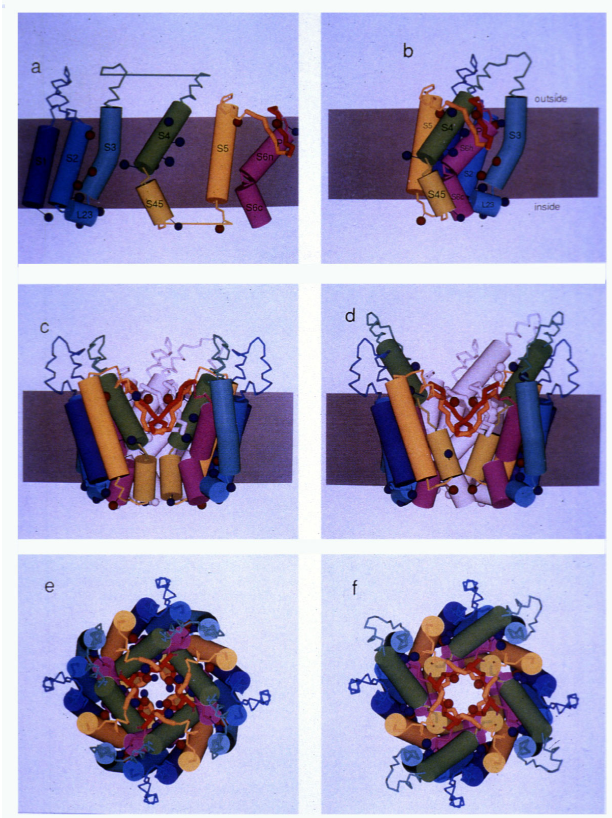
Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 304

The Ultimate NanoMachine: The 1 nm pore in a gated ion channel



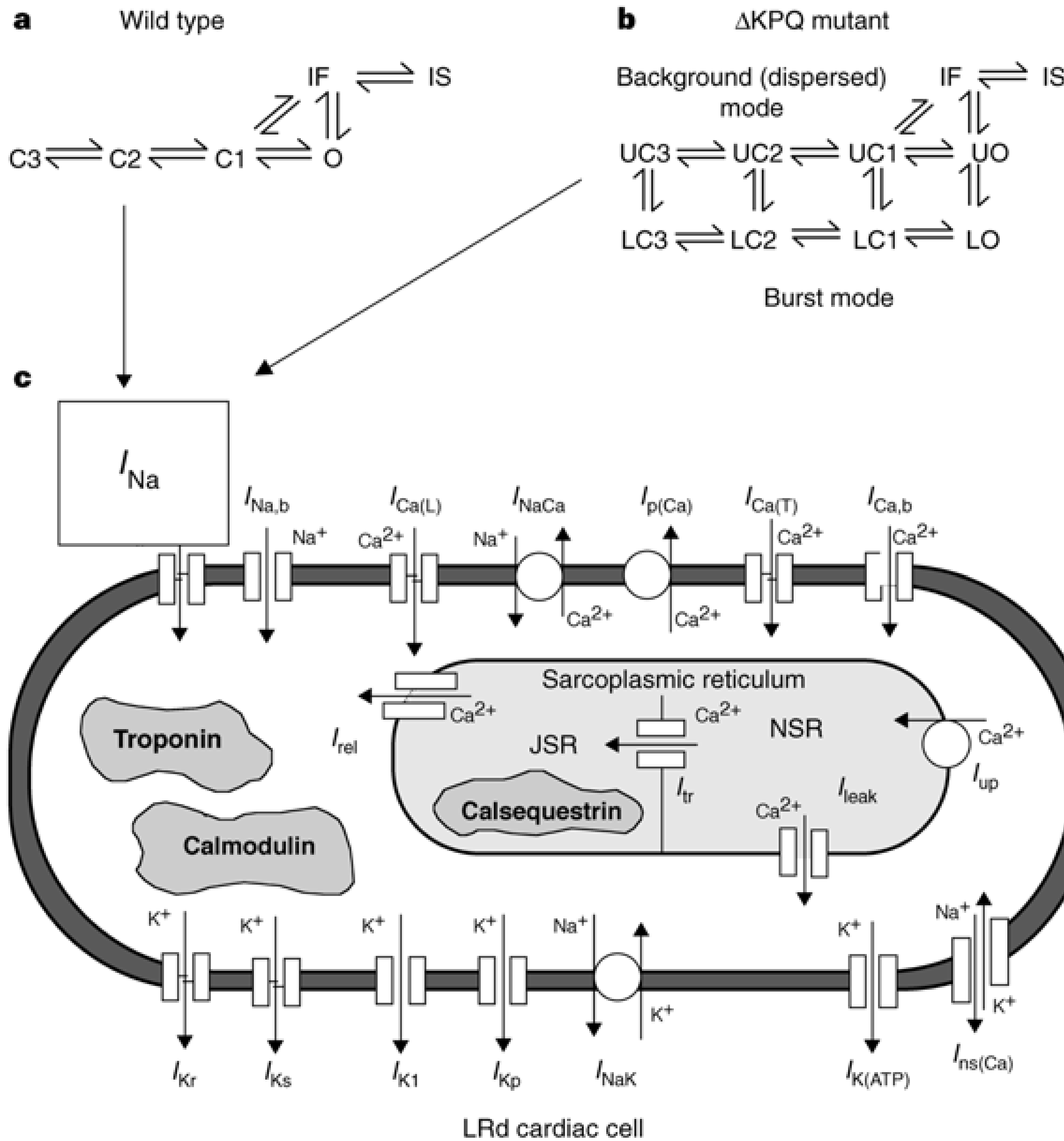


S.R. Durrell and H.R. Guy, *Biophysical Journal*, 62: Discussions 1992 238-250 (1992)

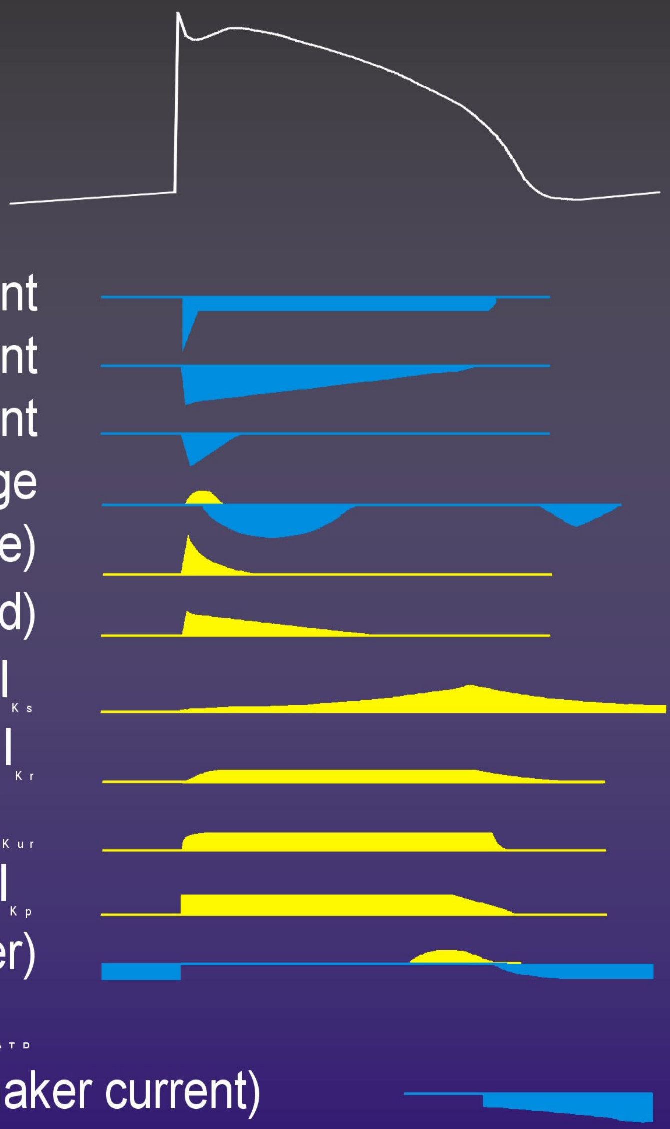


Cells have LOTS of different ion channels that serve as sensors and actuators!

Clancy, C. E. and Y. Rudy. Linking a genetic defect to its cellularphenotype in a cardiac arrhythmia. Nature 400 (6744) 566-569, 1999.



Ion currents and ion channel clones



Current	Probable clone
sodium current	H1, SCN5A*
L-type calcium current	✓*
T-type calcium current	✓
Na-Ca exchange	Na-Ca exchanger
I_{TO1} (4-AP-sensitive)	Kv4.3 (?1.2, 1.4, 1.5, 2.1, 4.2)*
I_{TO2} (Ca-activated)	--
I_{Ks}	KvLQT1 + minK (IsK)
I_{Kr}	HERG + MiRP1
I_{Kur}	Kv1.5
I_{Cl} or I_{Kp}	CFTR, TWIK (?others)
I_{K1} (inward rectifier)	Kir2.x
I_{K-ACh}; I_{K-ATP}	Kir3.1/3.4; Kir6.x/SUR
I_f (pacemaker current)	hCNG

*+sub-units

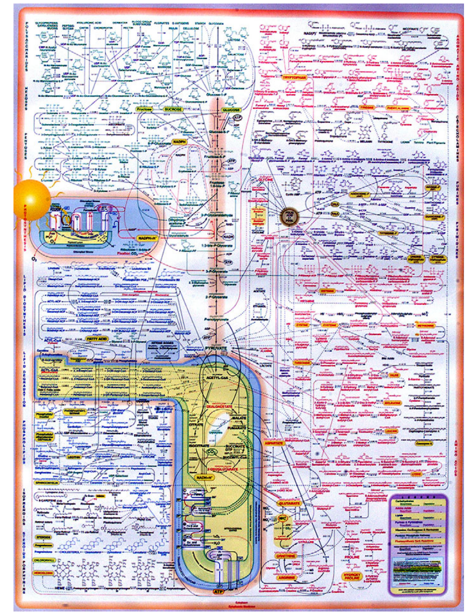
The Ultimate Instrumentation Question for Systems Biology

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***?

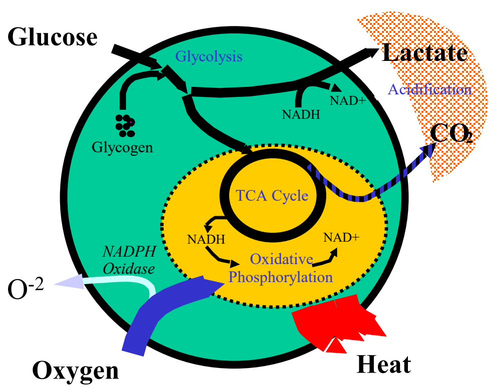
Sizes, Volumes, Time Constants

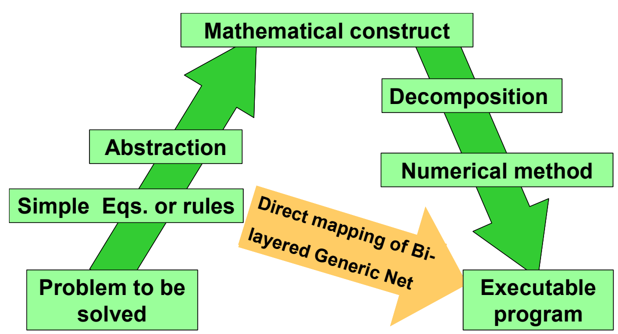
X	V, m^3	V	Tau_{Diff}	Example	N
1 m	1	1000 L	10^9 s	Animal, bioreactor	100
10 cm	10^{-3}	1 L	10^7 s	Organ, bioreactor	100
1 cm	10^{-6}	1 mL	10^5 s = 1 day	Tissue, cell culture	10
1 mm	10^{-9}	1 uL	10^3 s	µenviron, well plate	10
100 um	10^{-12}	1 nL	10 s	Cell-cell signaling	5
10 um	10^{-15}	1 pL	0.1 s	Cell	100
1 um	10^{-18}	1 fL	1 ms	Subspace	2 - ?
100 nm	10^{-21}	1 aL	10 us	Organelle	2 - ?
10 nm	10^{-24}	1 zL	100 ns	Protein	1
1 nm	10^{-27}	1 npL	1 ns	Ion channel	1

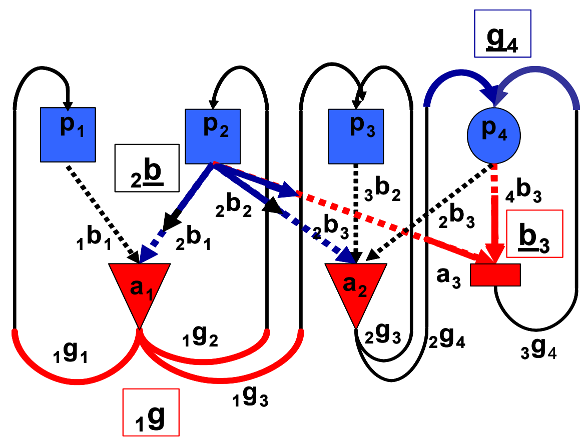
Then…. Statistical Analysis of Activation Responses



- 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 Challenge for the 21st Century