Need for Cellular and Molecular Sensors and Actuators

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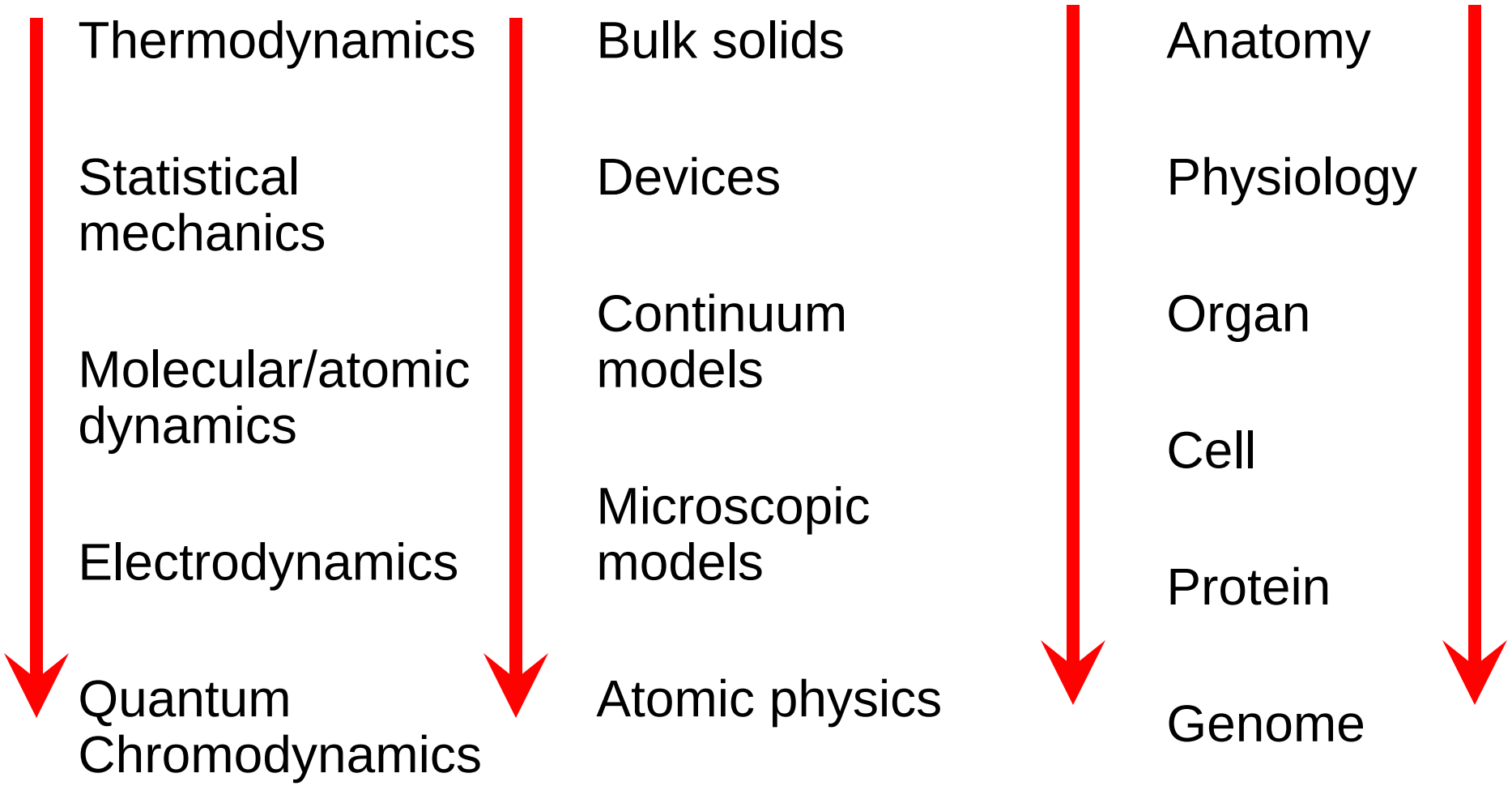
Mini-Symposium: Biomolecular Processors through Micro- and Nanotechnology The

Abstract

Systems biology may present the ultimate micro- and nanoengineering challenge: a single mammalian cell requires at least a hundred-thousand variables and equations to describe its dynamic state, cell-cell interactions are critical to system function, and some organs have a billion interacting cells. At present, biologists might record five dynamic intracellular variables simultaneously from a single cell – typically through fluorescent imaging. Historically, the assumption has been that it is sufficient to hold all but a few variables constant and make a limited number of measurements. In a realm of highly interconnected, distributed nonlinear networks, measurements made in this way cannot adequately capture system dynamics. The growing interest in nanobiology and nanomedicine is spawning extensive activity in artificial nanosensors that include ligand-gated ion channels, fluorescent nanocrystal reporters that bind to targeted sites, nanofibers that can deliver DNA, and metal nanoshells that can provide localized heating. The engineering challenges that must be met for nanoscience to make a broad impact in basic research in biology and medicine include techniques to record and control multiple dynamic variables in single cells; nanosensors that report the local environment rather than just position; and addressable nanoactuators that control more than just conductance or temperature.

Step 1 in Science: Reductionism



Spatial Resolution in Physiology

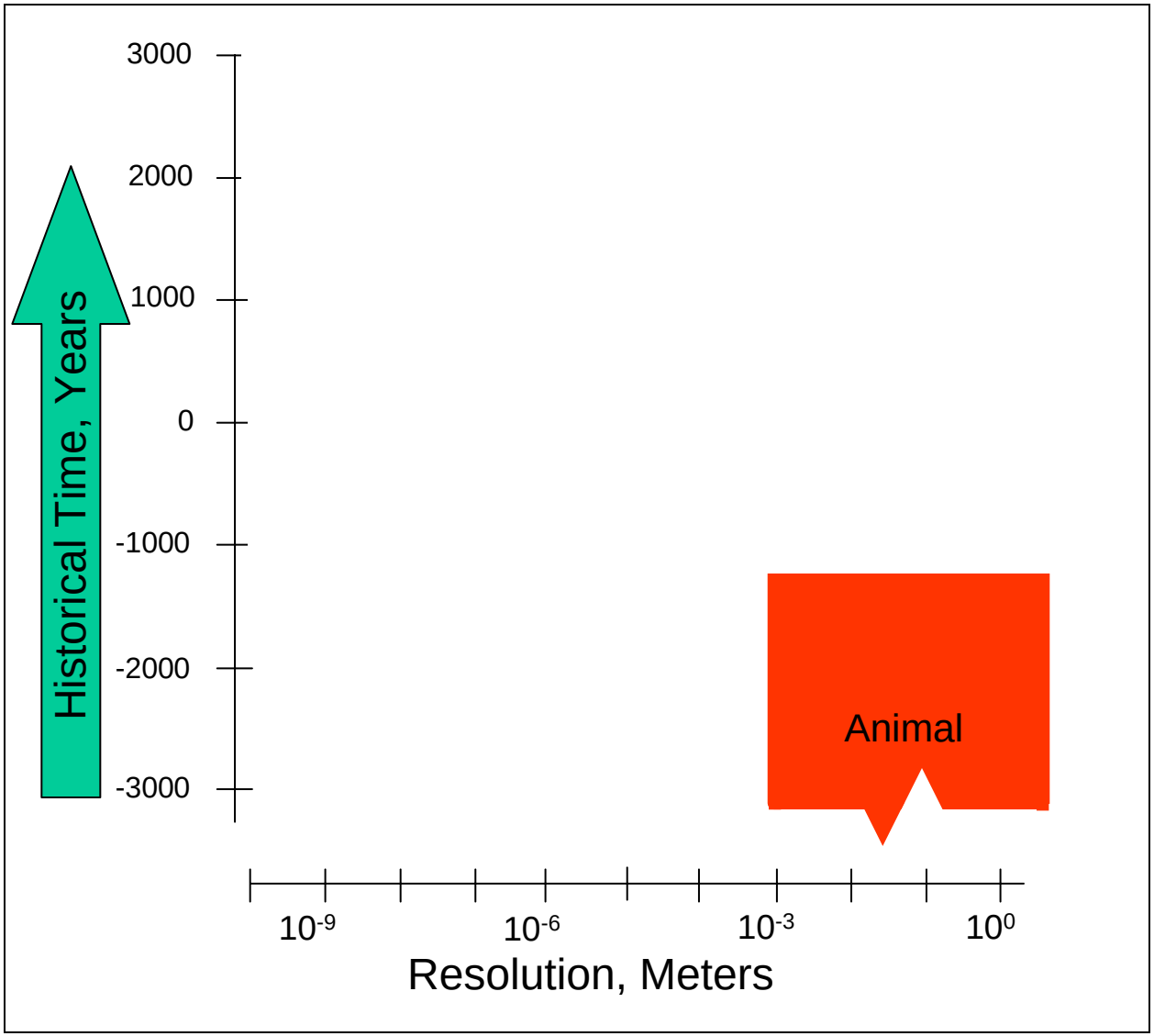
Computer

X-Ray / SEM / STM

Optical microscope

Magnifying glass

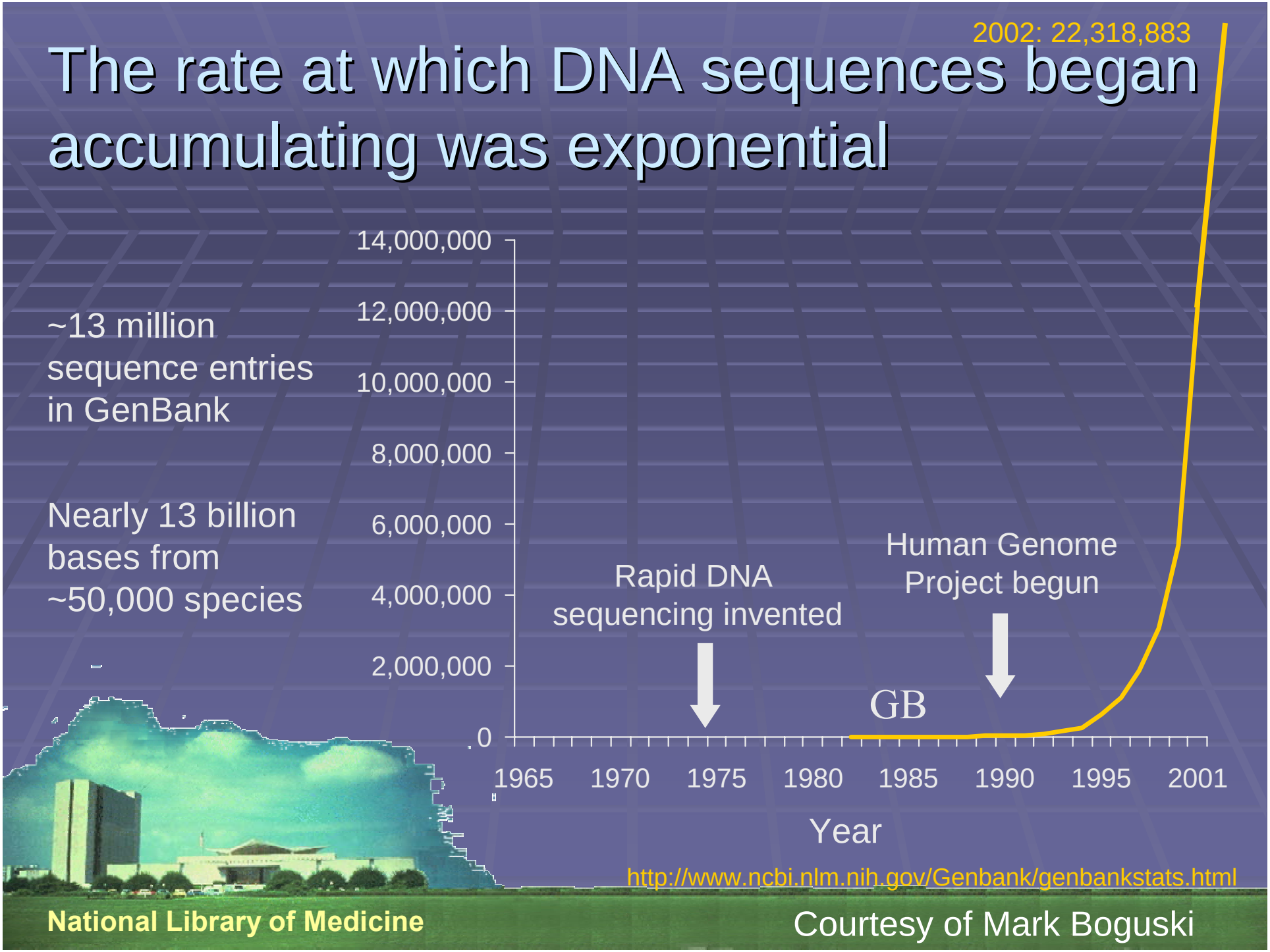
Unaided eye

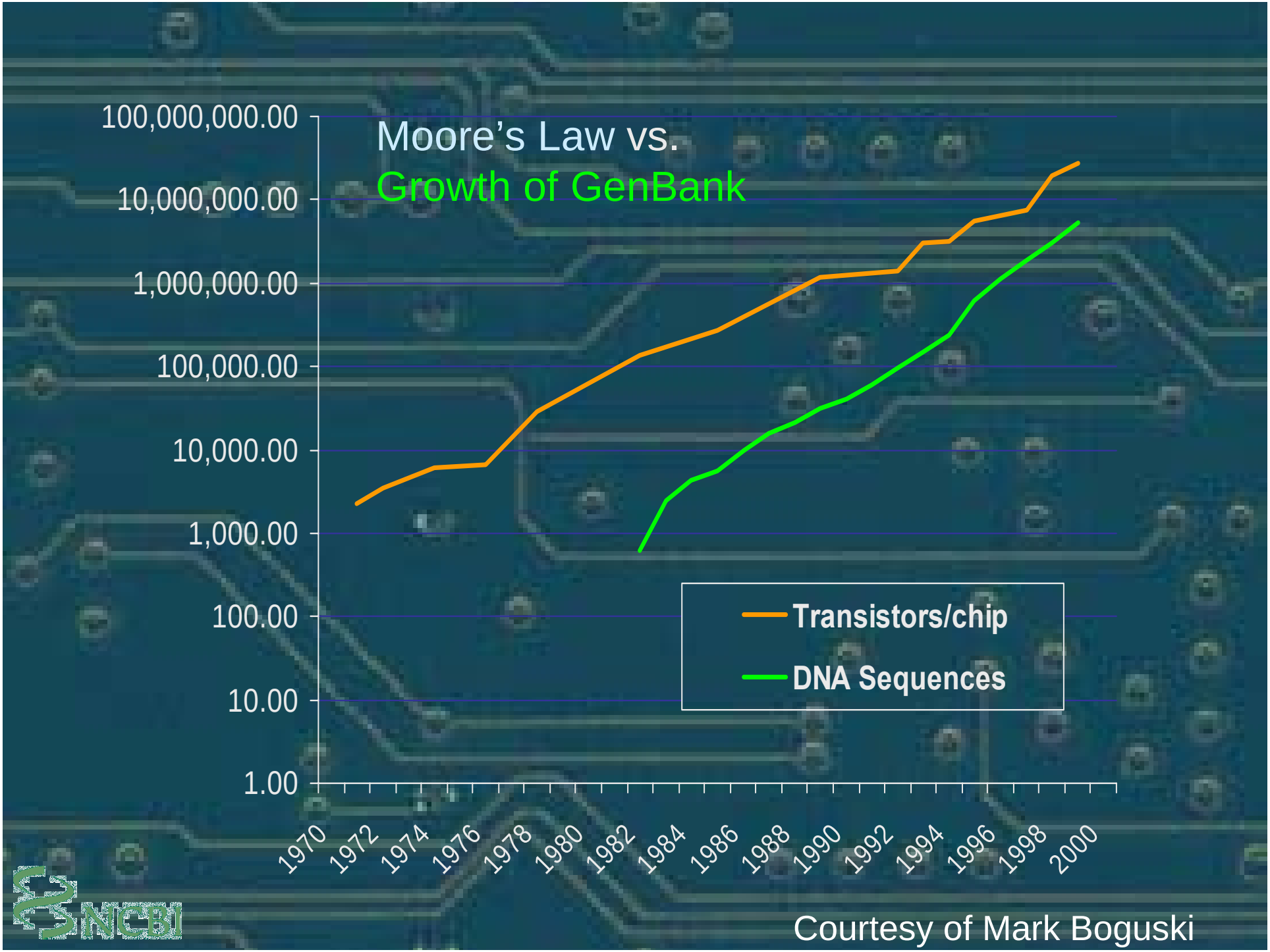


The Problems

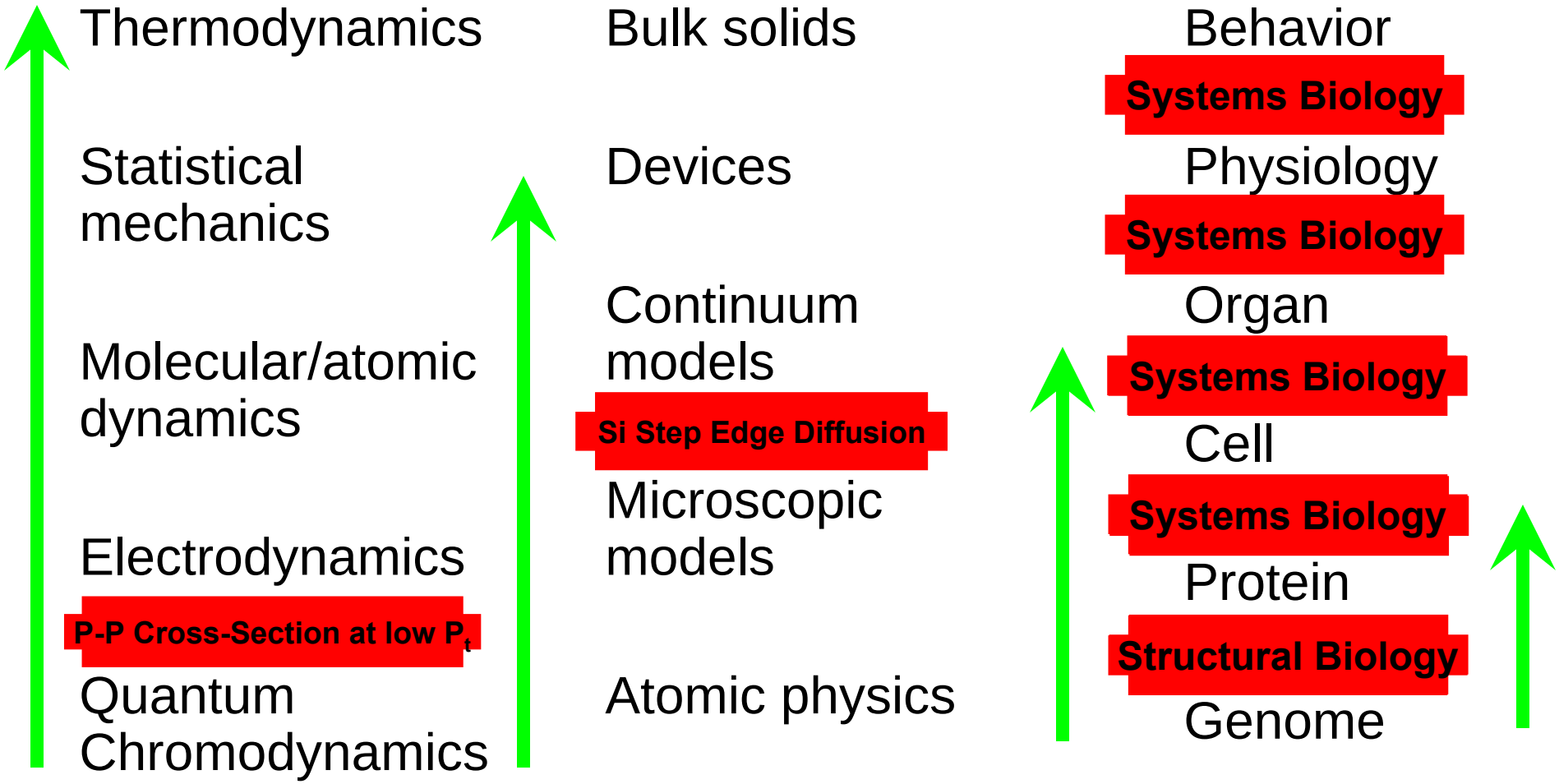
- Our understanding of biological phenomena is often based upon
  - experiments that measure the ensemble averages of populations of 10^6 – 10^7 cells, or
  - measurements of a single variable while all other variables are hopefully held constant, or
  - recordings of one variable on one cell, or
  -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




Moore's Law vs.
Growth of GenBank
100,000,000.00
10,000,000.00
1,000,000.00
100,000.00
10,000.00
1,000.00
100.00
10.00
1.00
1970
1972
1974
1976
1978
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
Transistors/chip
DNA Sequences
NCBI
Courtesy of Mark Boguski

Step 2 in Science:
Post-Reductionism



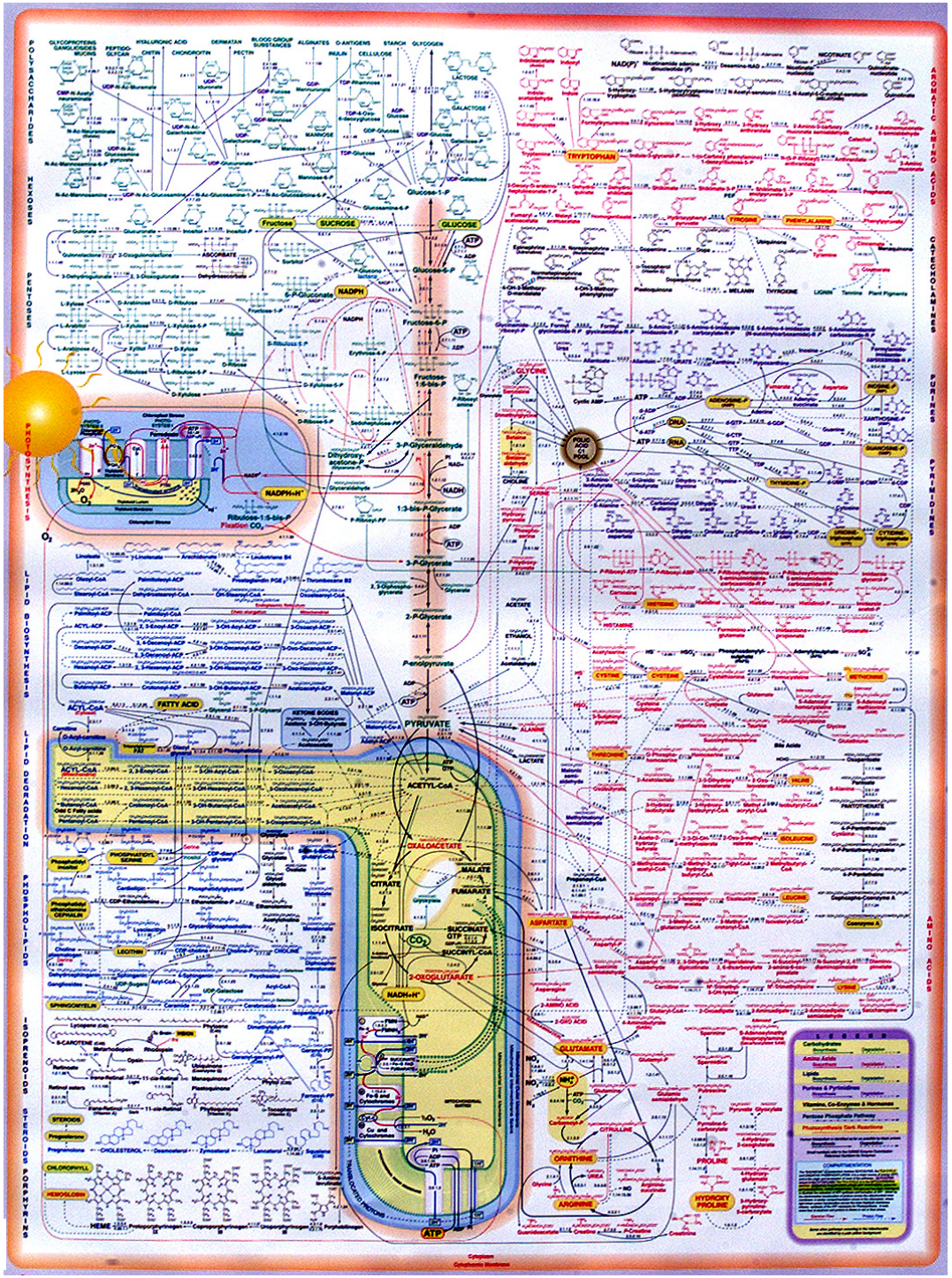
Key Questions in Systems Biology

- Are computer models the key to understanding quantitative, multiscale, postgenomic, postproteomic, *dynamic* physiology, i.e., systems biology?
- To what extent can we actually create, use and trust computer models of cell signaling networks to understand the shockwave of genetic and proteomic data that is hitting us?
- What are the potential problems, and their possible solutions?
  - Multiphasic, *dynamic* cellular instrumentation
  - Exhaustively realistic versus minimal models
  - Dynamic network analysis

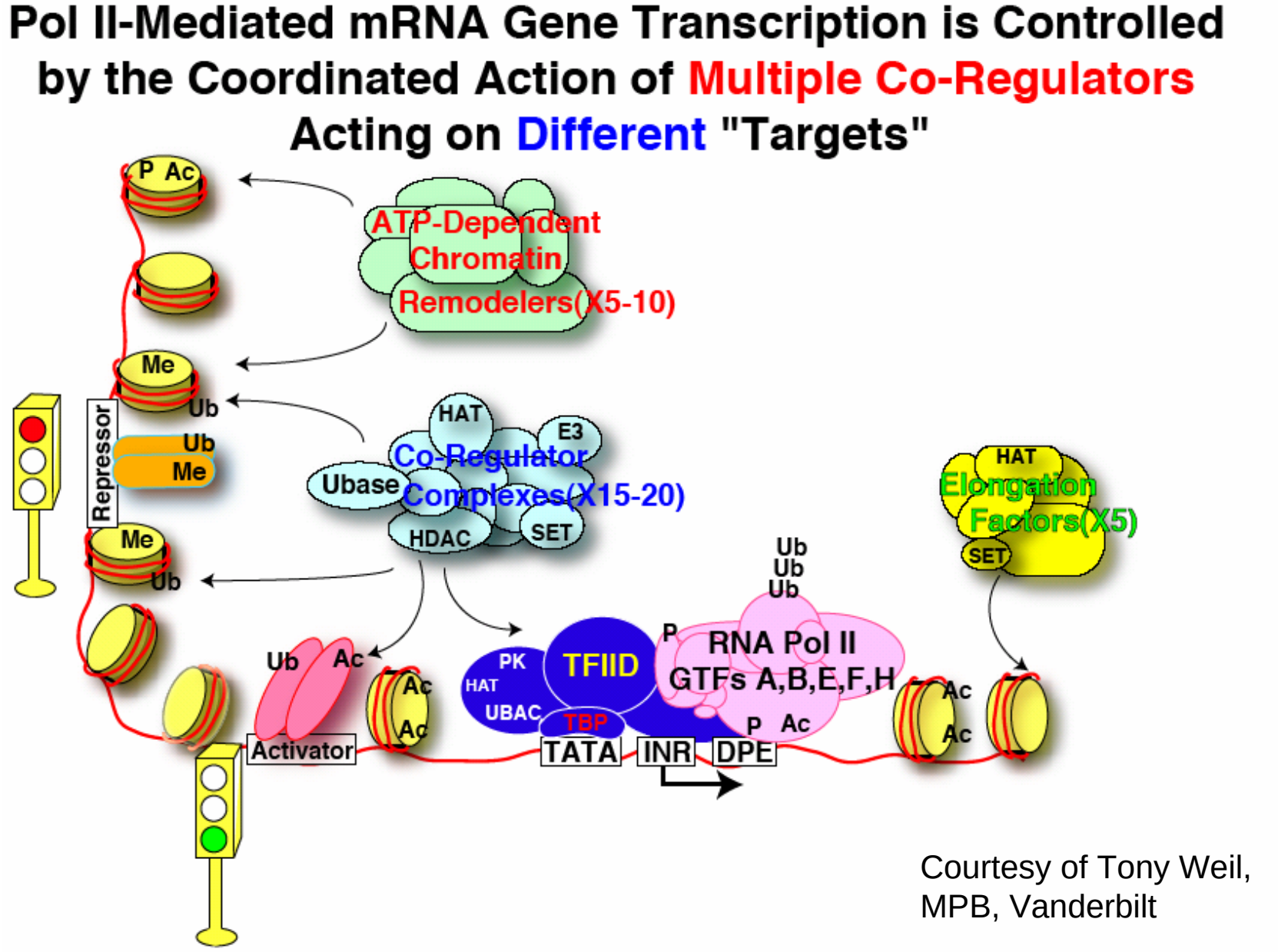
VIJBRE

'Postgenomic' Integrative/Systems Physiology/Biology

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

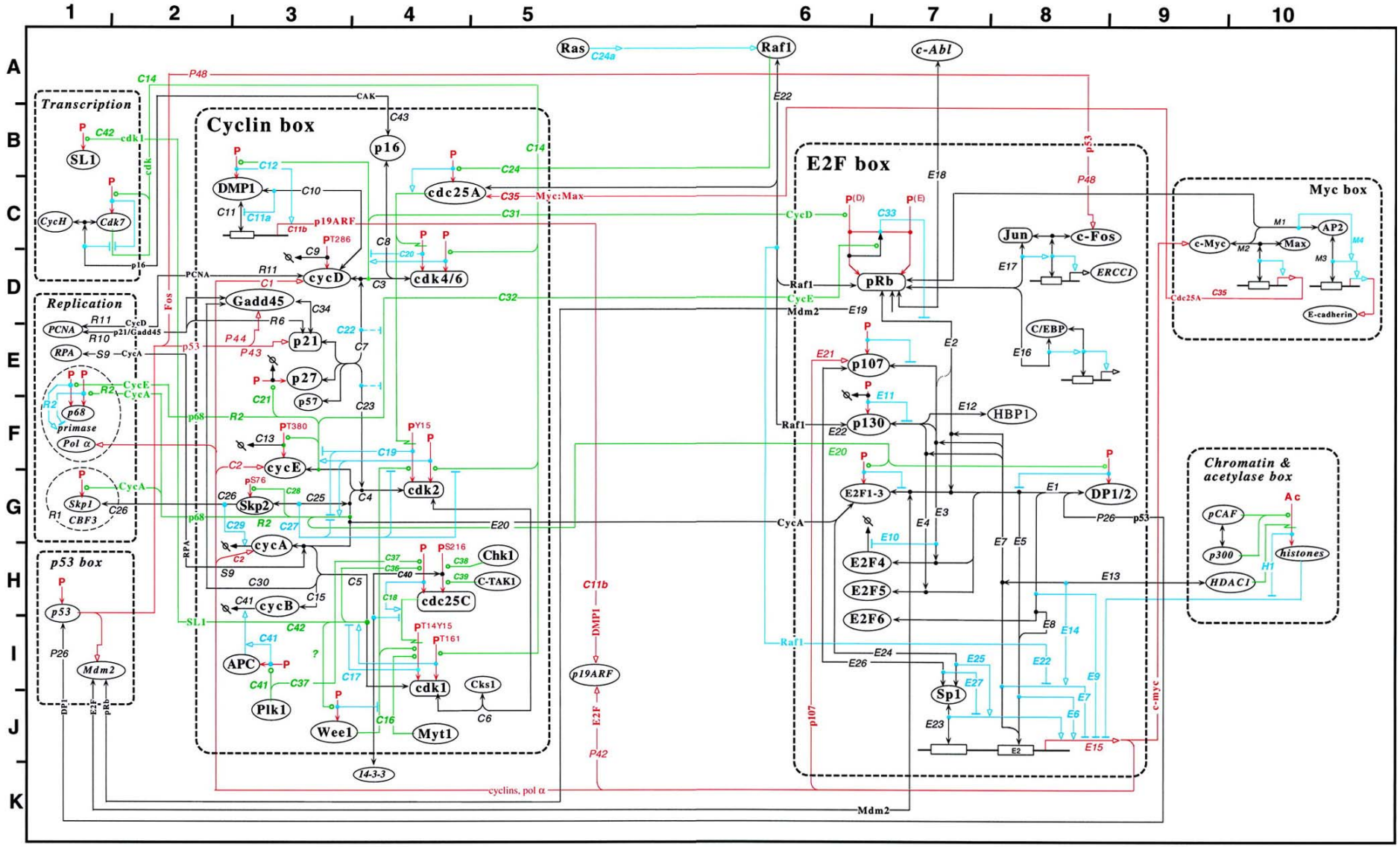


The complexity of eukaryotic gene transcription control mechanisms



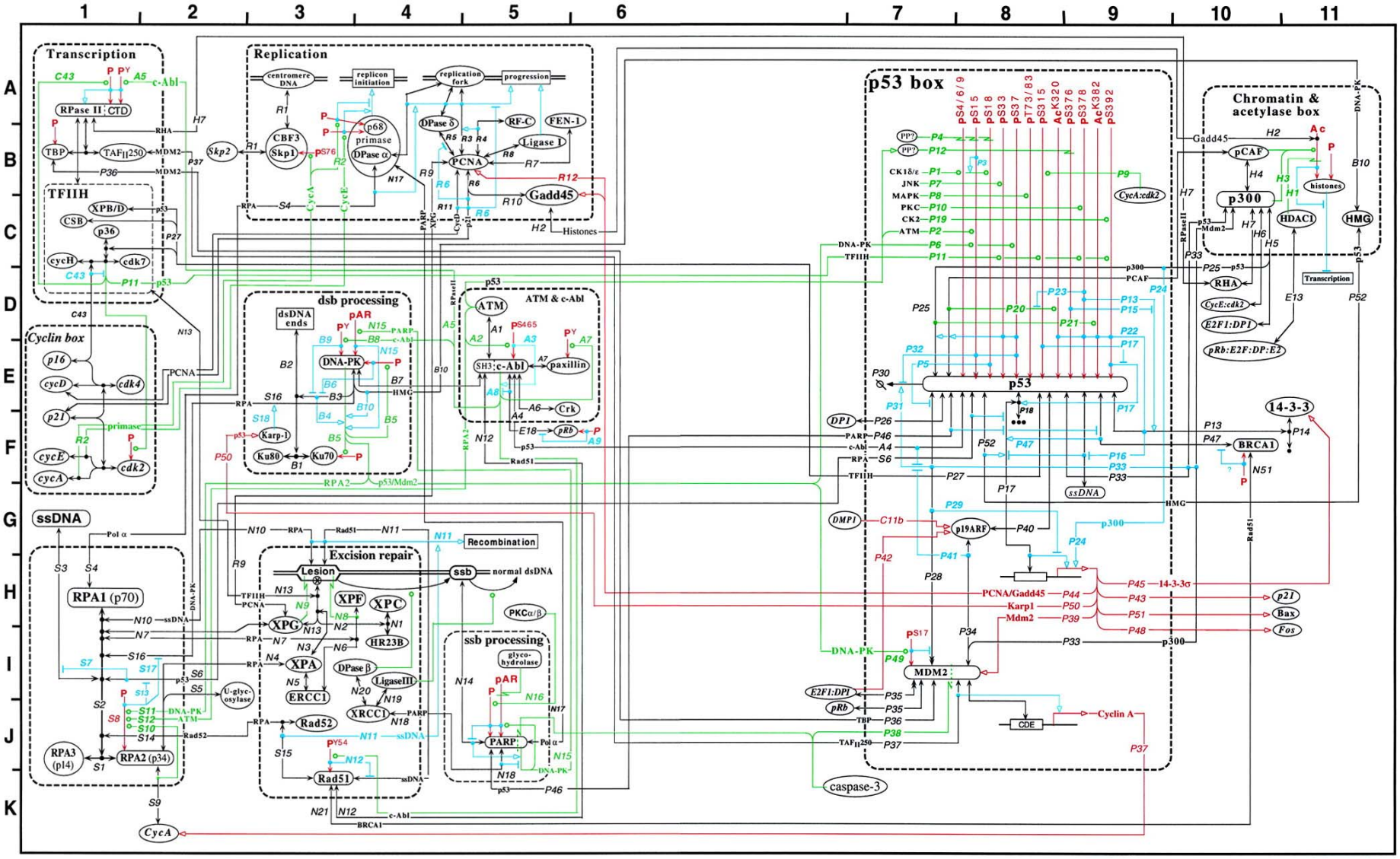
Courtesy of Tony Weil, MPB, Vanderbilt

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



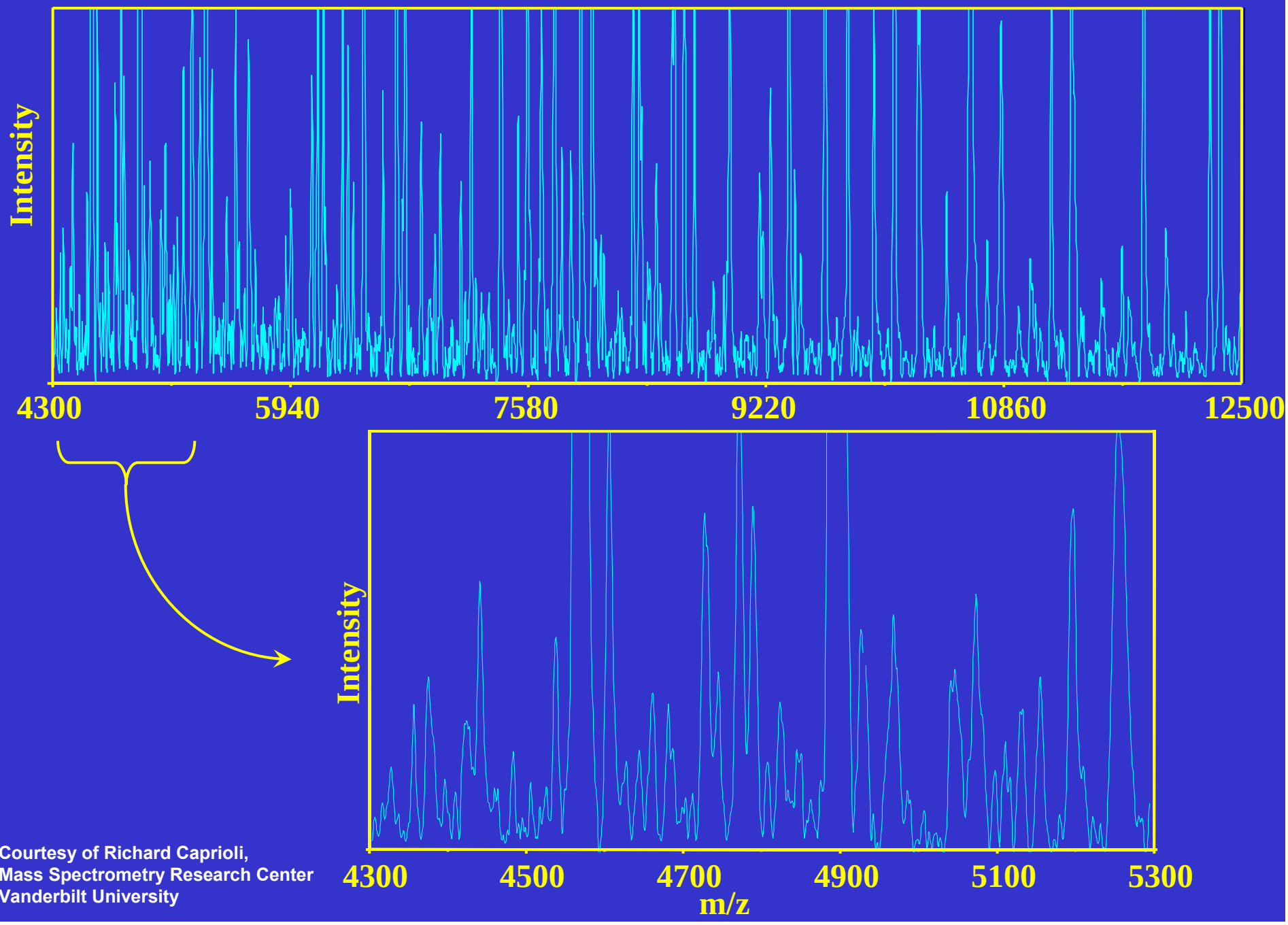
KW Kohn, "Molecular Interaction Map of the Mammalian Cell Cycle Control and DNA Repair Systems," *Mol. Biol. of the Cell*, 10: 2703-2734 (1999)

Proteins as Intracellular Signals

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

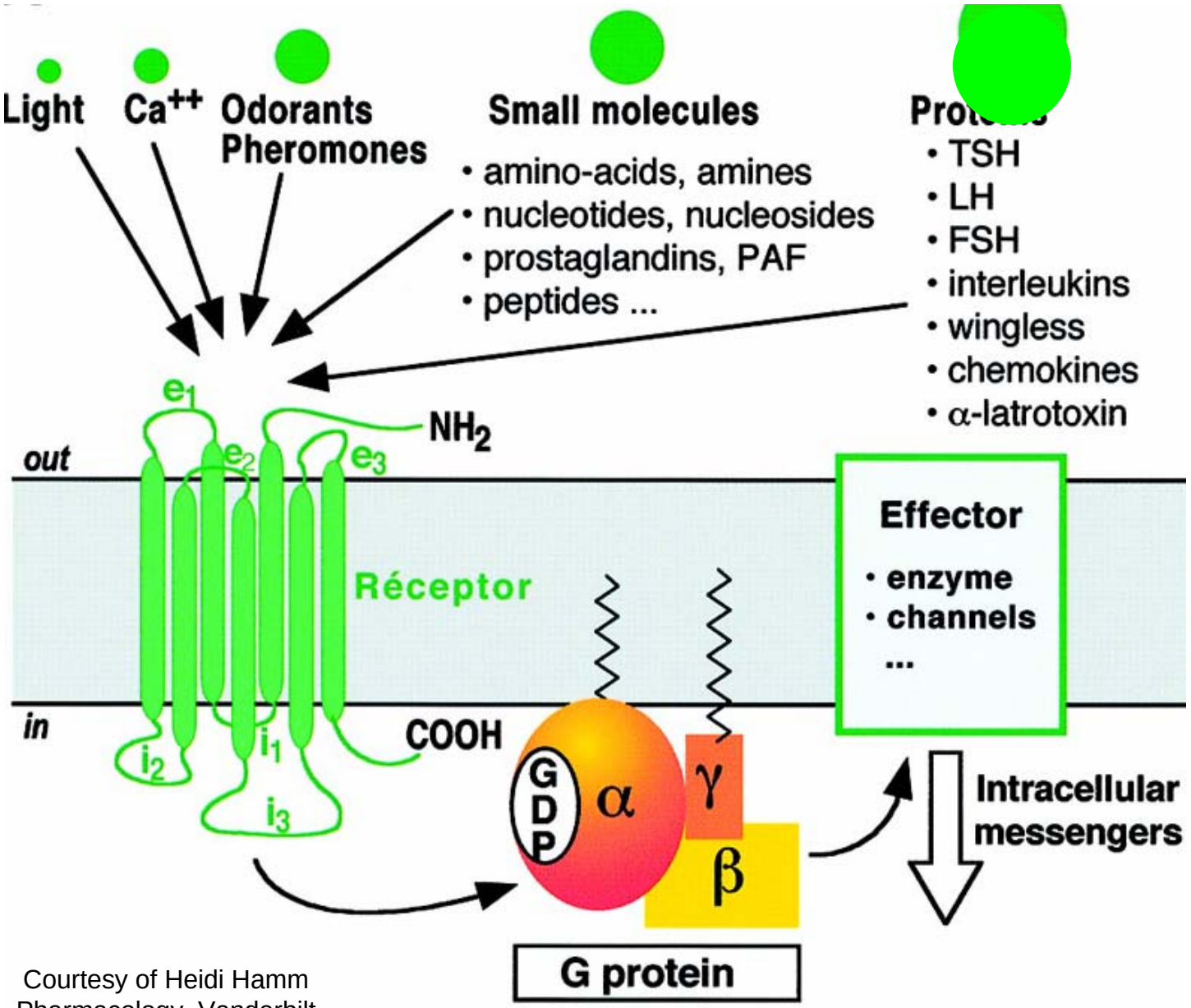
- Metabolic
- Maintaining integrity of subcellular structures
- Producing signals for other cells

MALDI-TOF: Cells express a lot of proteins…



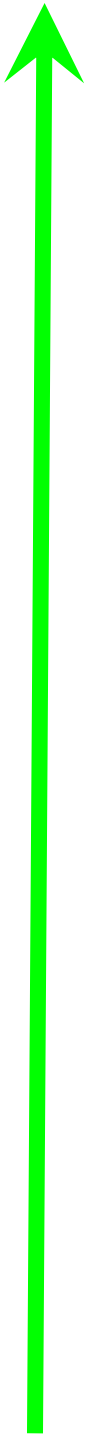
Courtesy of Richard Caprioli,
Mass Spectrometry Research Center
Vanderbilt University

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"....

Sure....

ODEs become PDEs ...

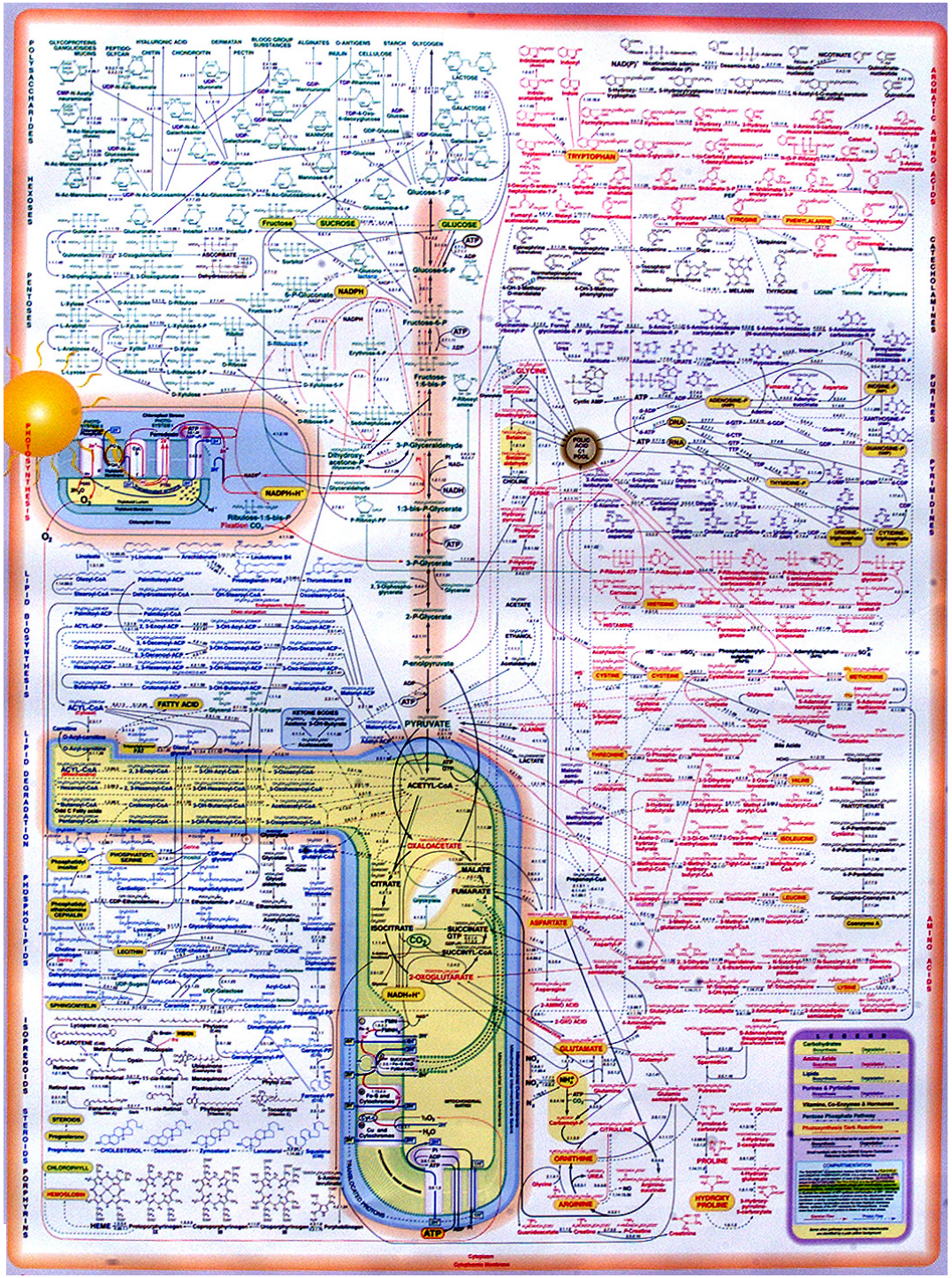
Lots and lots and lots of PDEs

VIBRE

'Postgenomic' Integrative/Systems Physiology/Biology

- Specify concentrations and
- Rate constants
- And gene expression,
- Protein interactions, and
- Signaling pathways
- Time responses
- Include intracellular spatial distributions, diffusion, and transport: ODE → PDE(t)
- … and then you can **calculate** how the cell behaves in response to a toxin

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



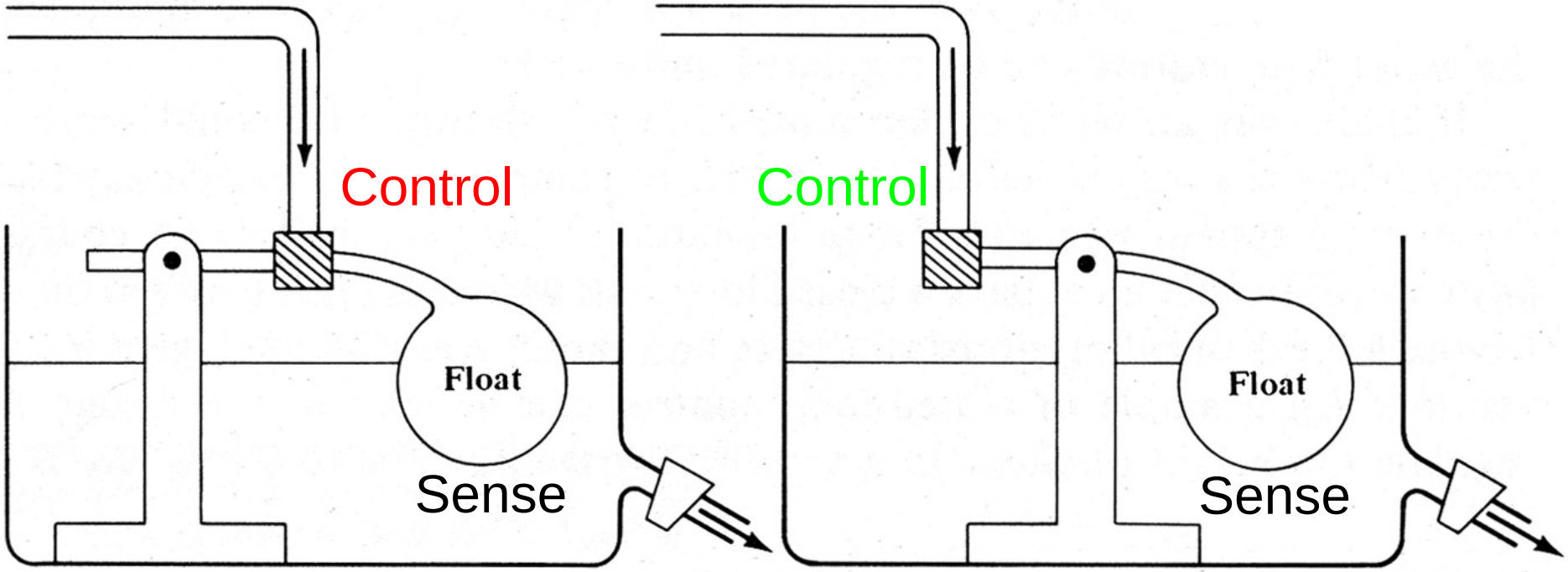
The Catch

- 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…**

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

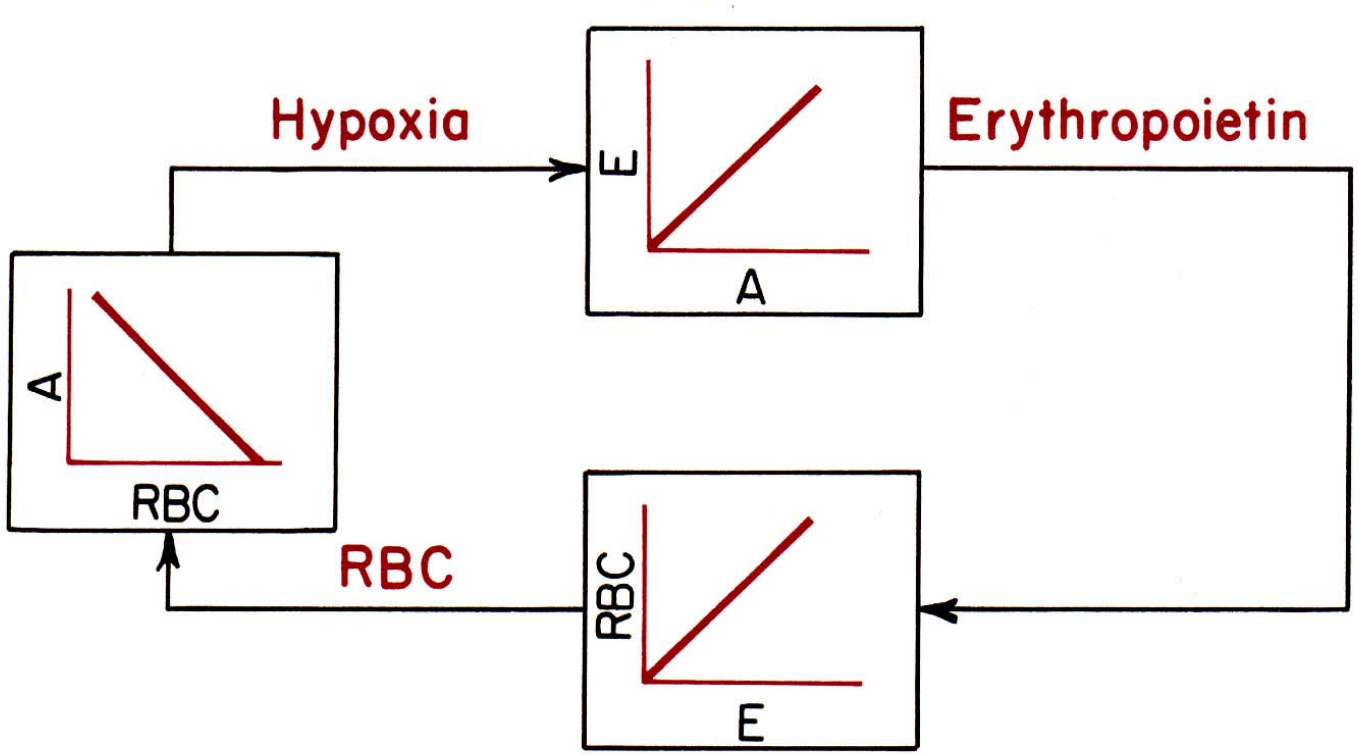
Negative versus Positive Feedback



Negative Feedback **Positive Feedback**

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

Hypoxia-Red Blood Cell Concentation

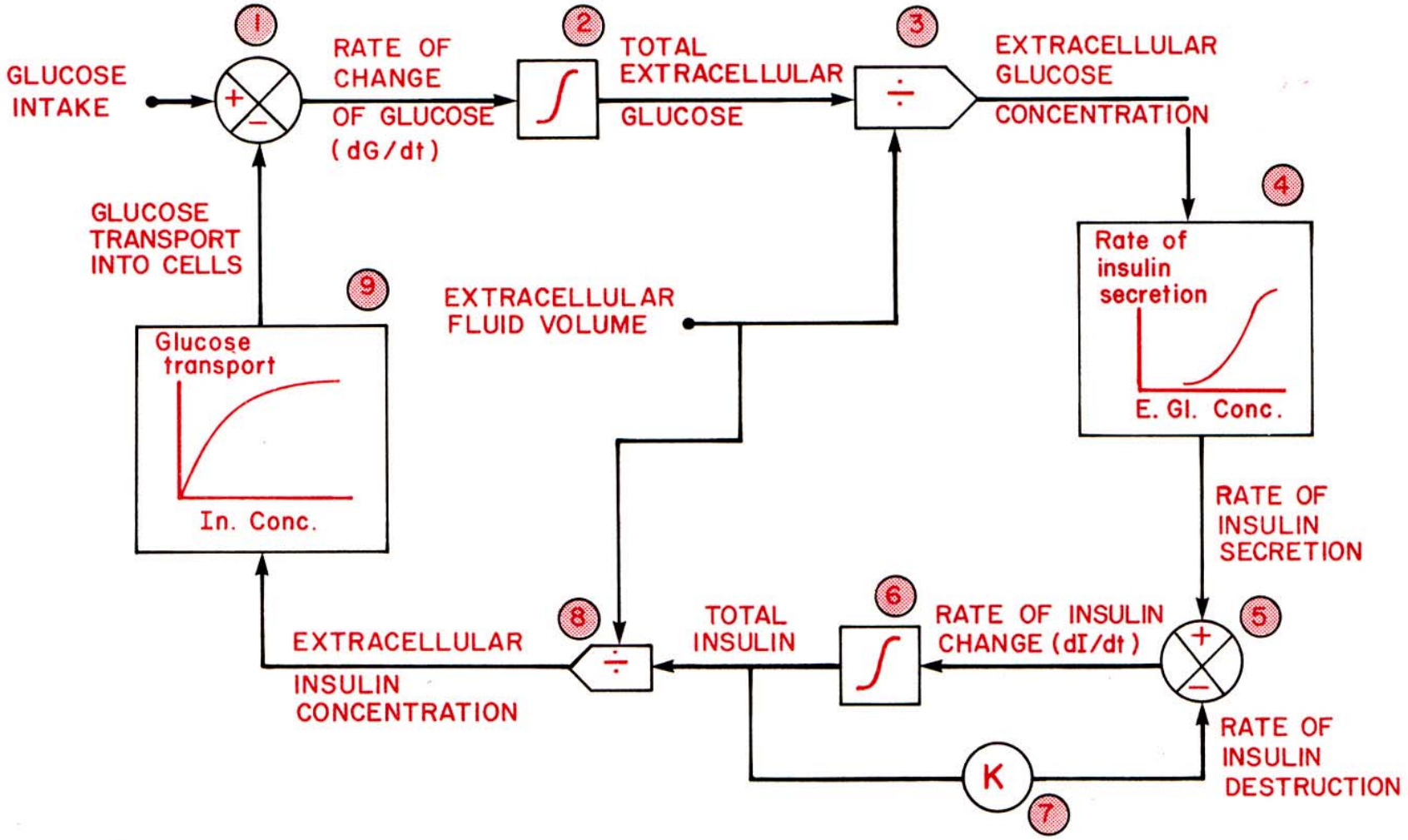


Variables

- Erythropoietin E
- Hypoxia A
- RBC

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

Glucose-Insulin Control

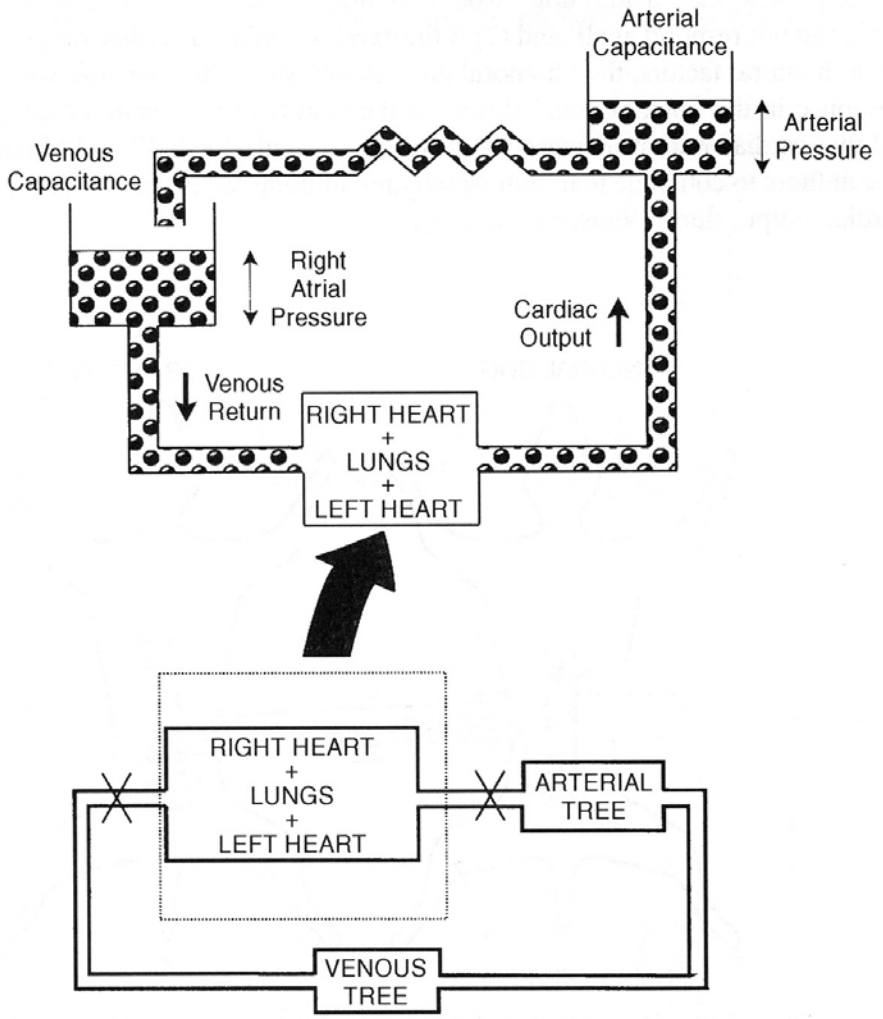


Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.9

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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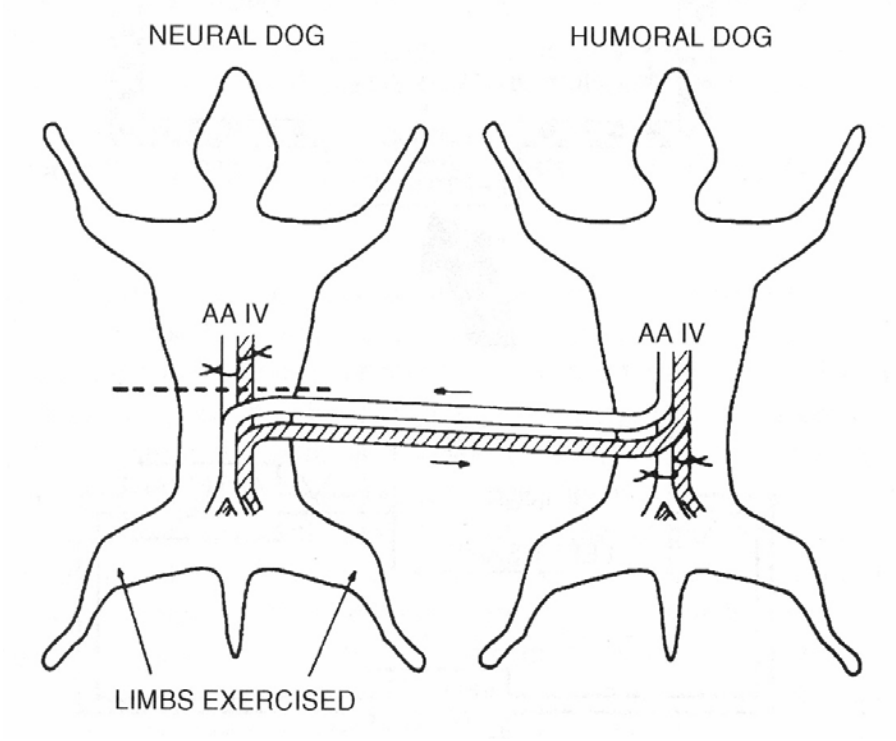

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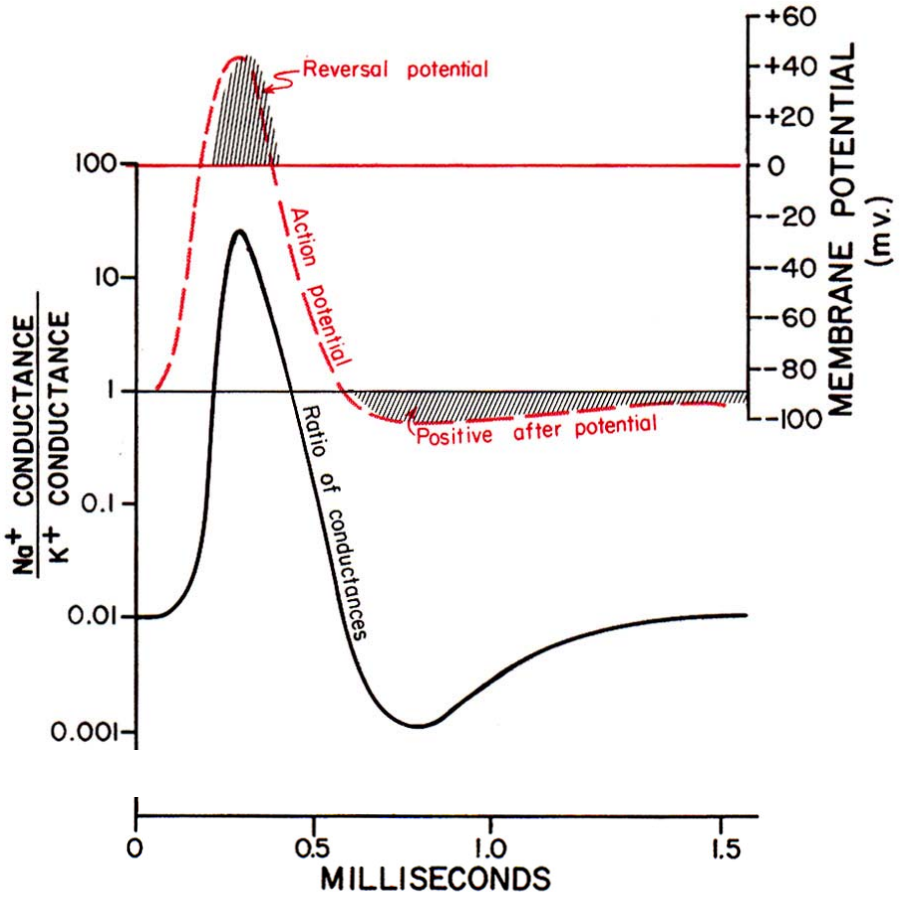
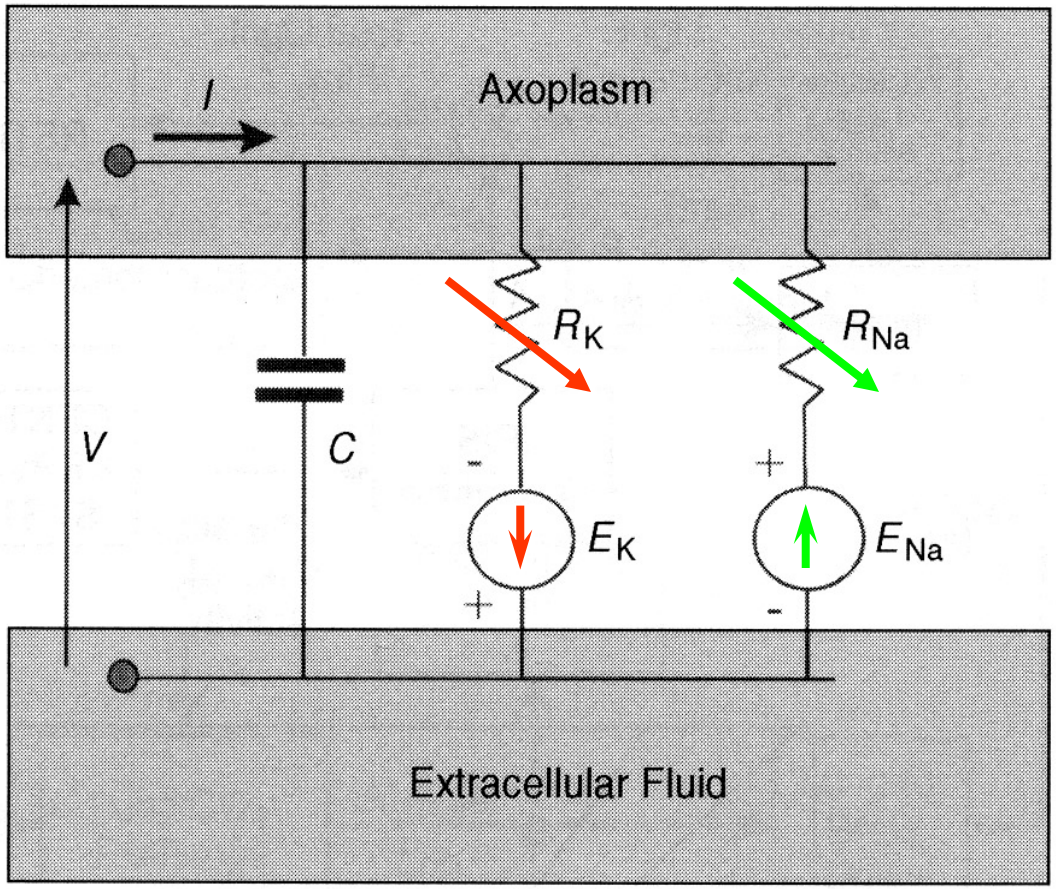


Guyton, Arthur C.; Textbook of Medical Physiology, 6rd ed.; 1981, W.B. Saunders, p.110

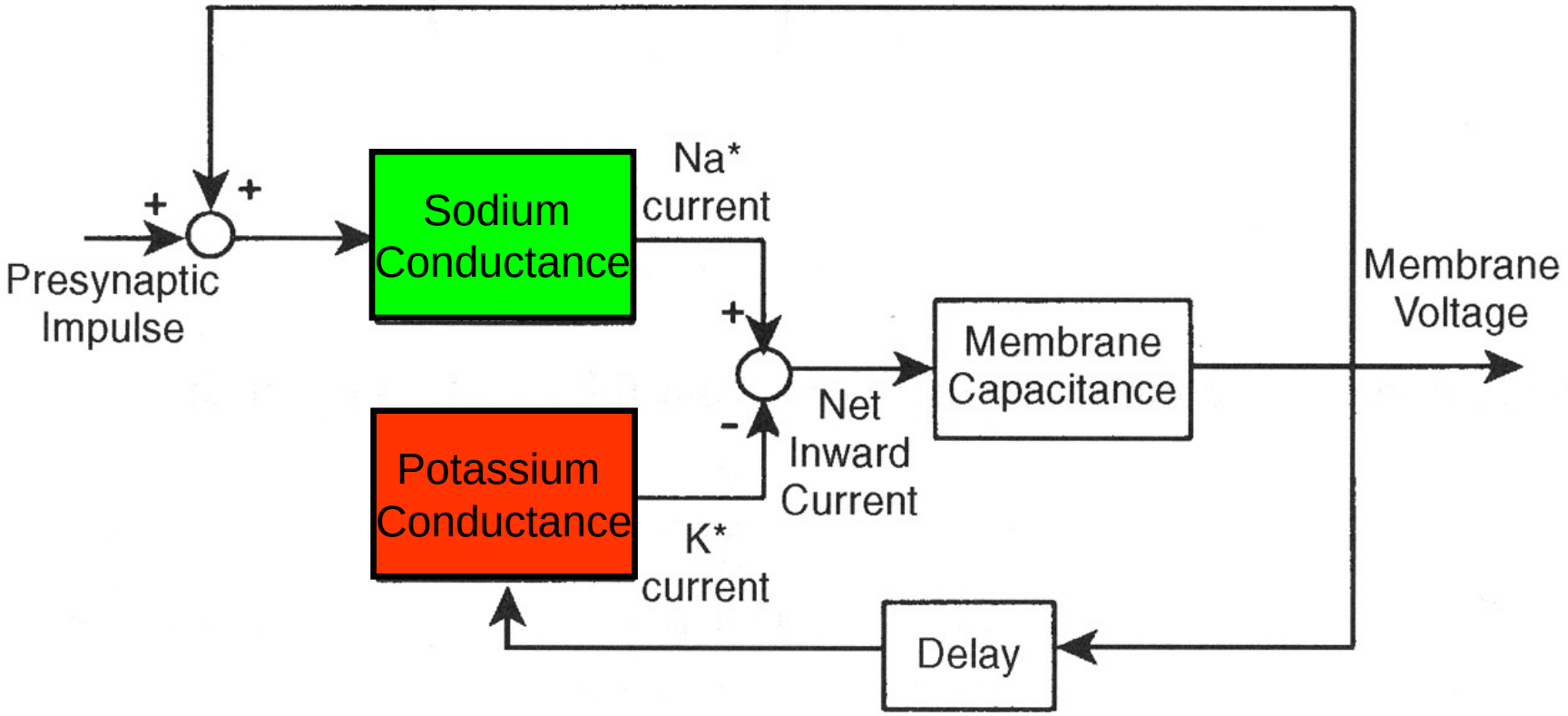
Simplified Hodgkin-Huxley



- 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

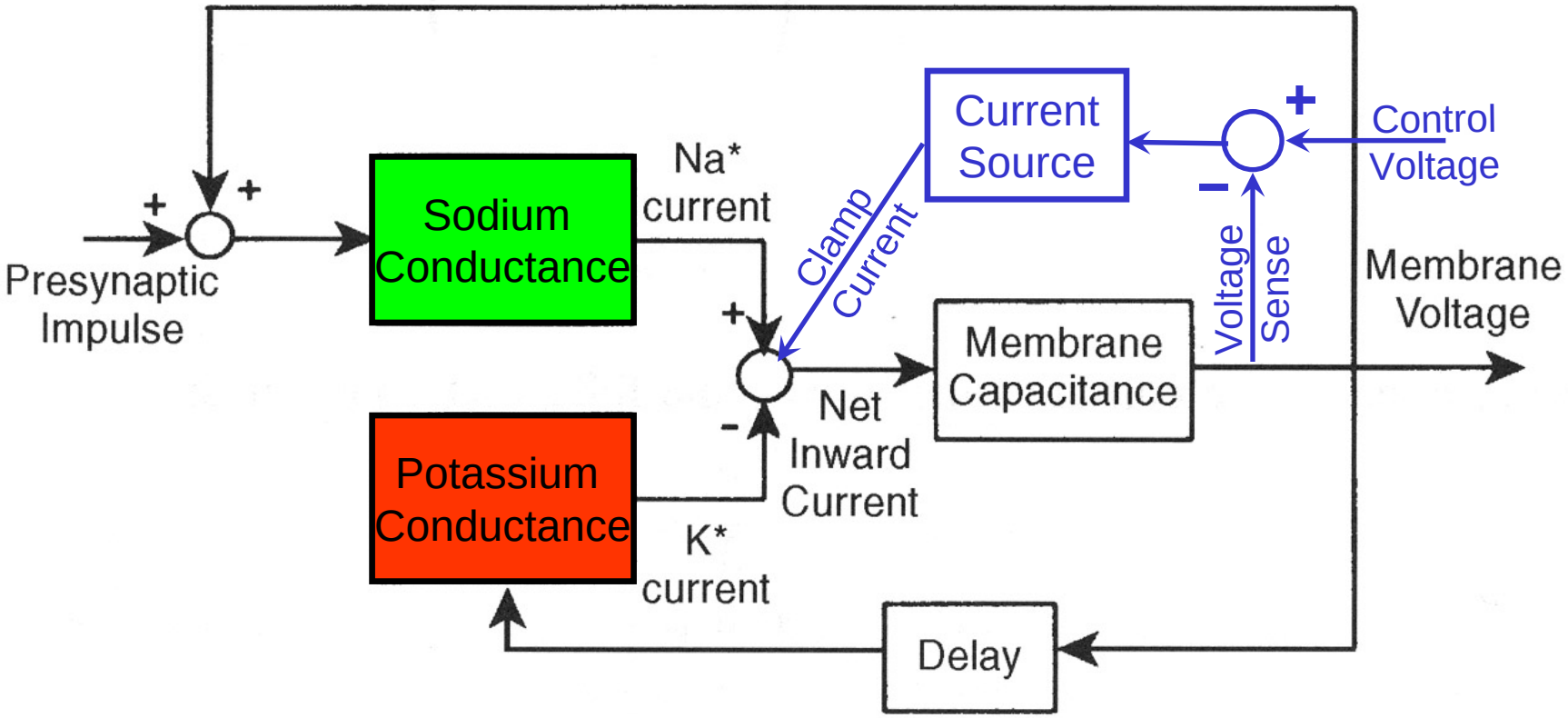
Khoo,Michael C.K.; Physiological Control Systems; 2000, IEEE Press, p.187

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



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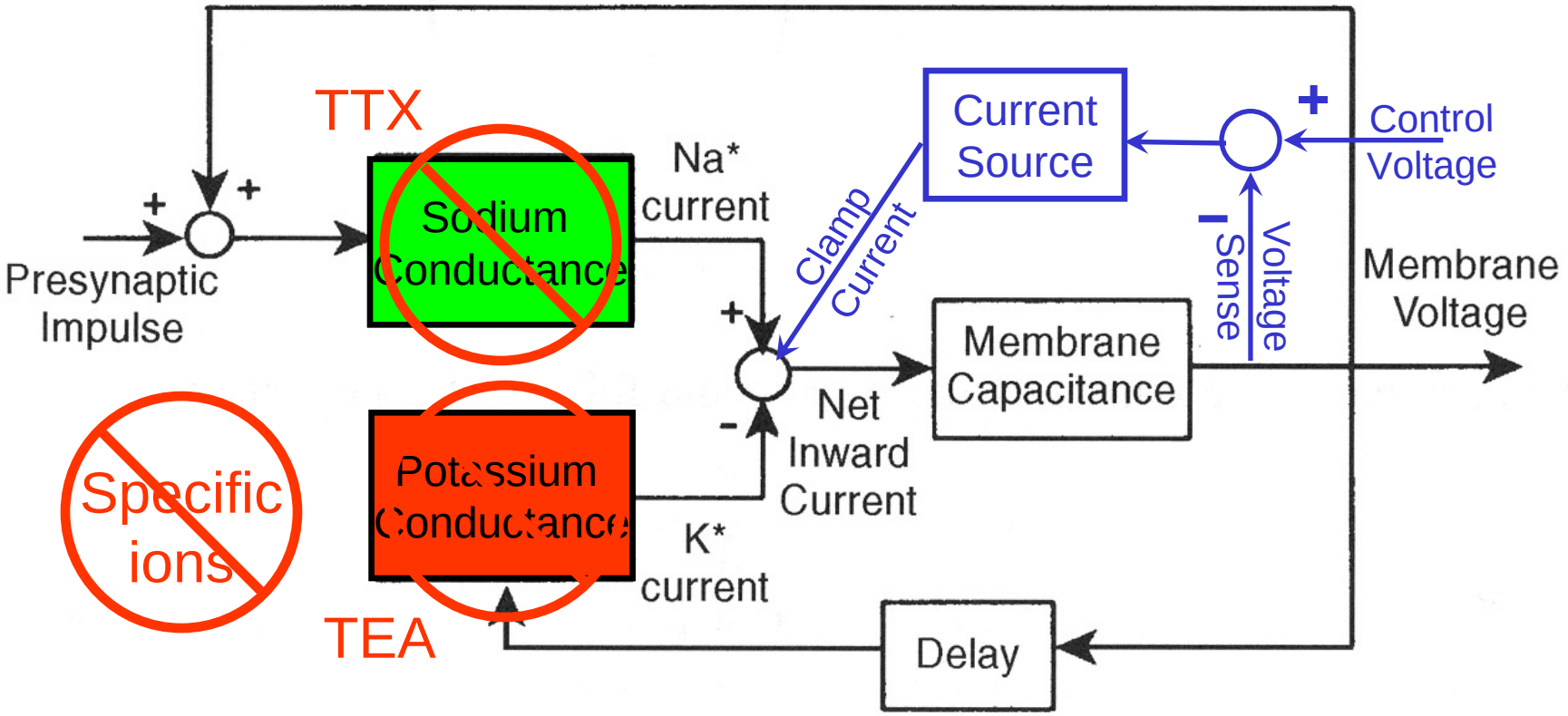
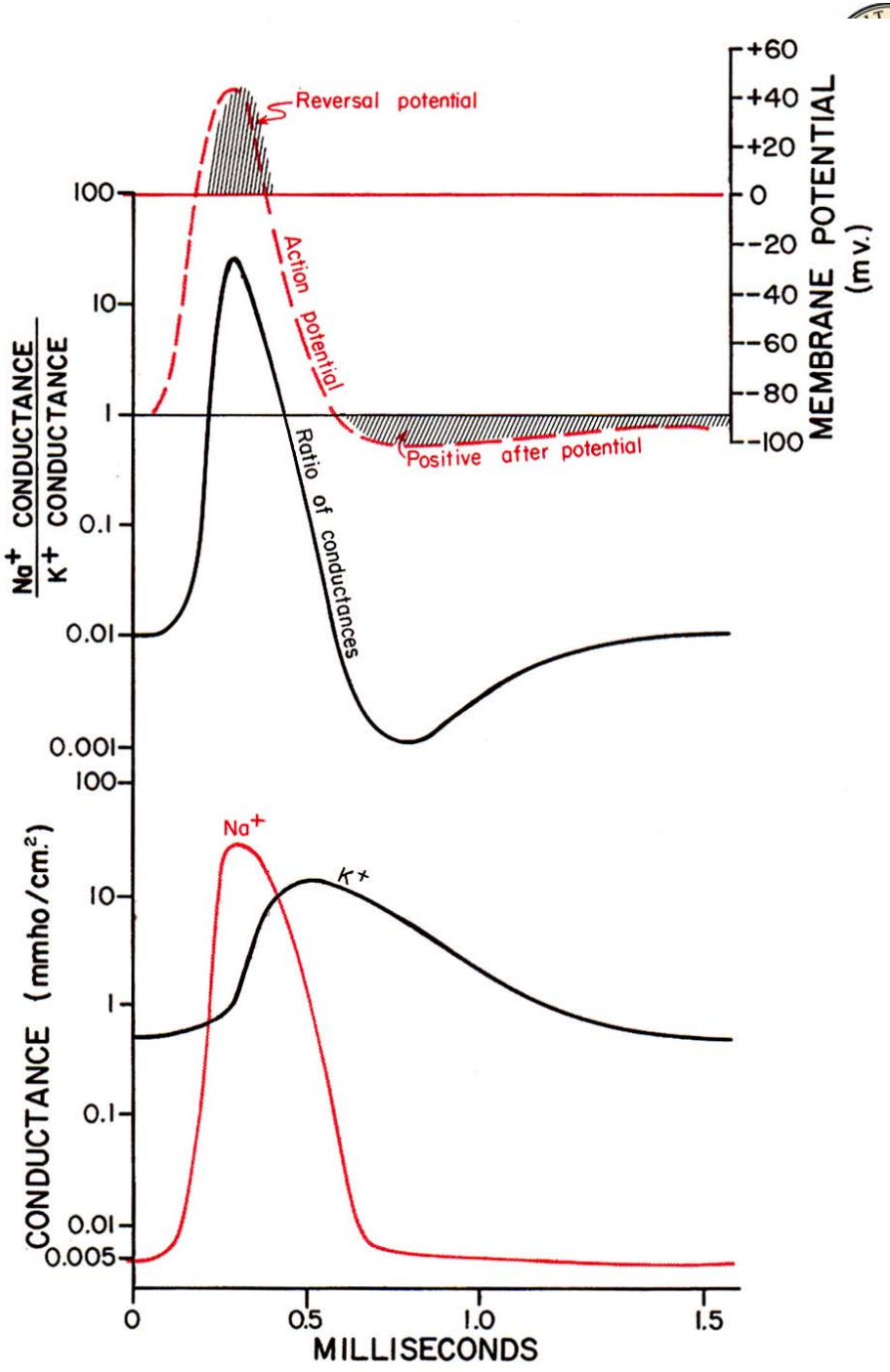
Opening the Loop During External Control



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

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

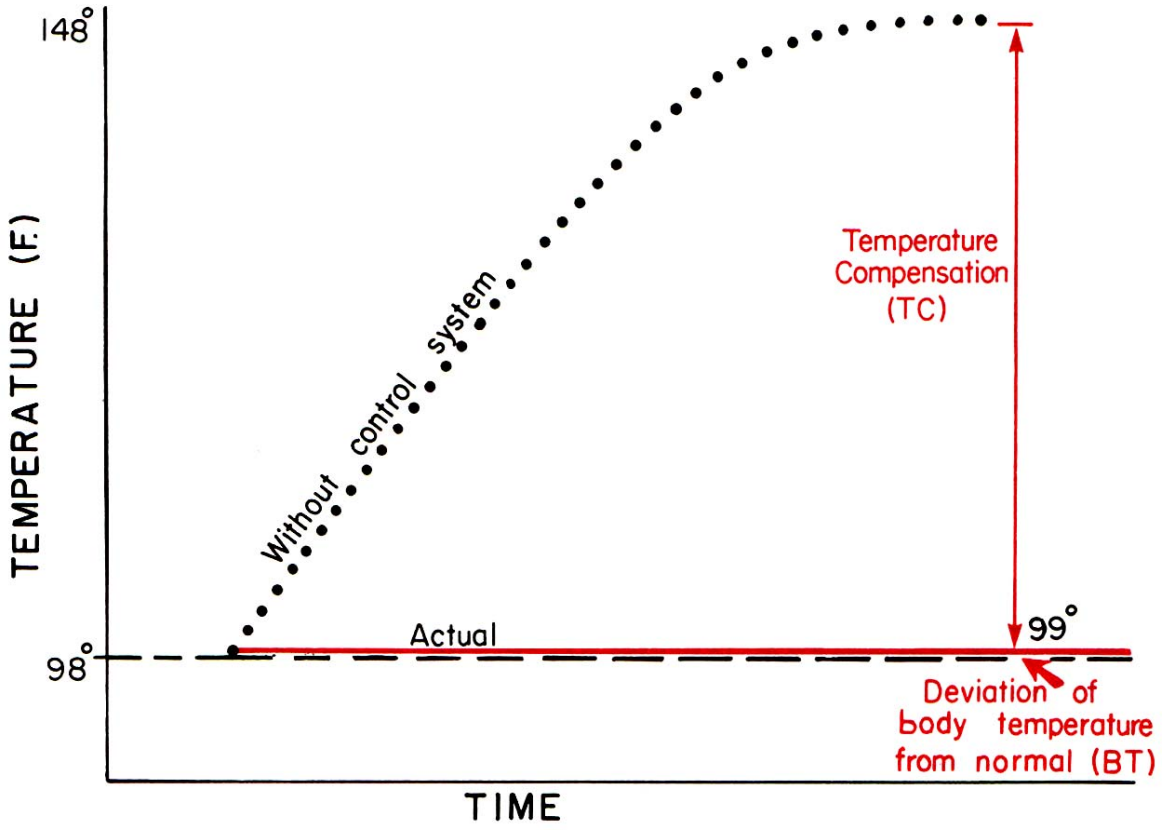
Required:	New devices to sieze control of subsecond, submicron cellular processes.

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

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

Signatures of Control

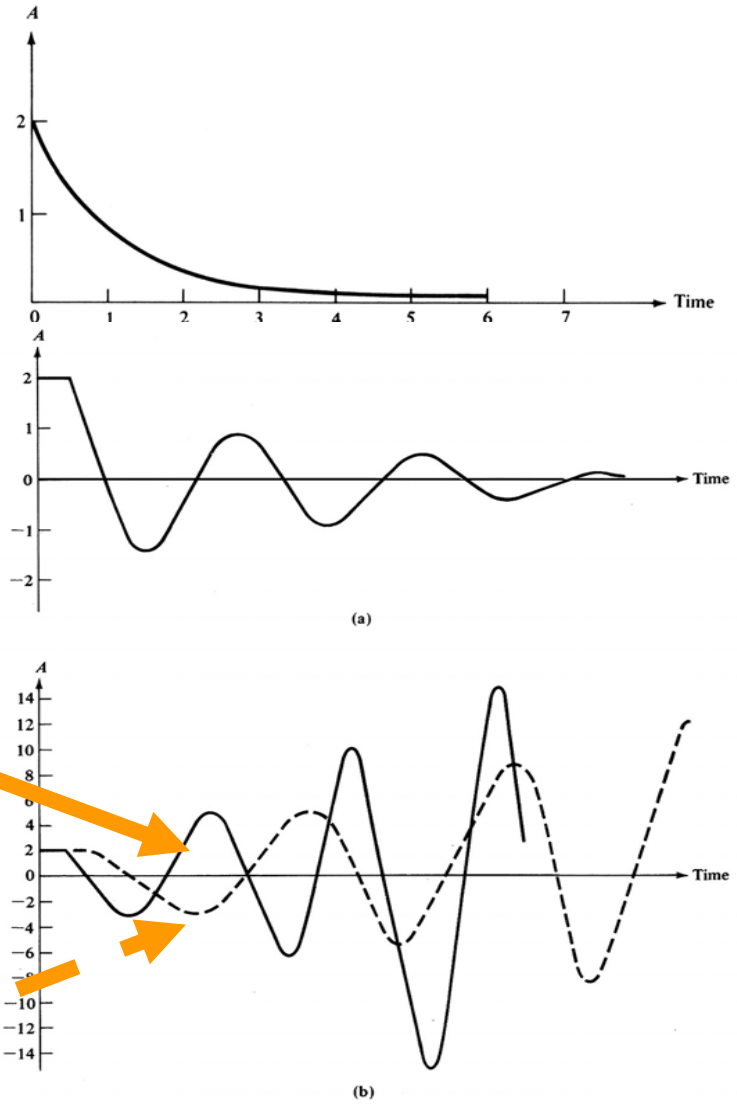
- Stability in the presence of variable input (DT= 50^{o} F)
- Oscillations when excessive delay or too much gain
- Divergent behavior when internal range is exceeded or controls damaged



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

Control Stability

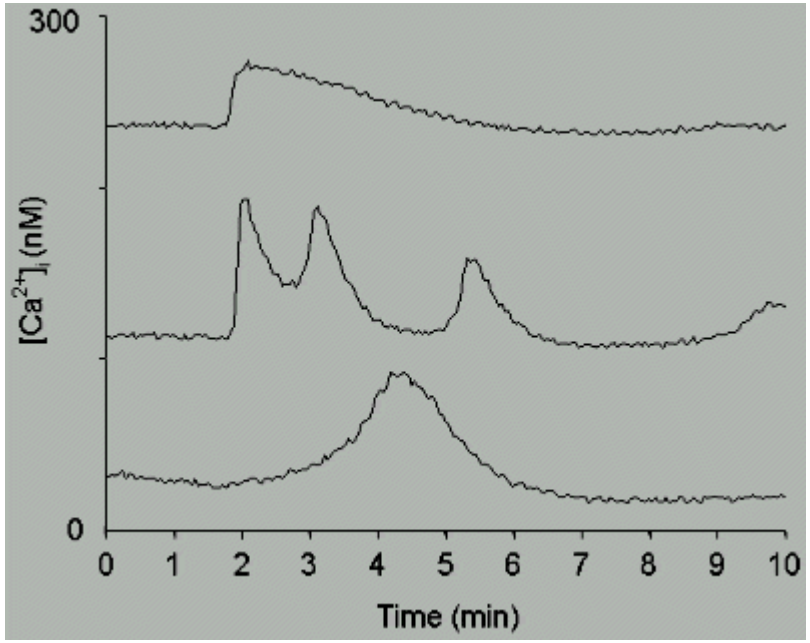
- Proportional control
- Proportional control with finite time delay
- Higher gain, same delay
- Same gain, longer delay



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

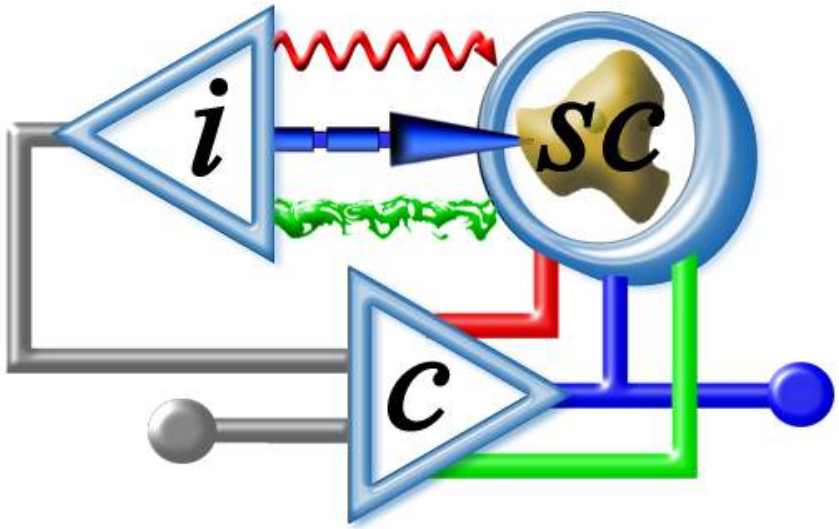


- **<u>Prediction</u>: At higher bandwidths than provided by present instrumentation, we will see <u>chemical</u> bursts, oscillations, and chaotic behavior. FIND THEM AND USE THEM!**

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

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



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

The Grand Challenge

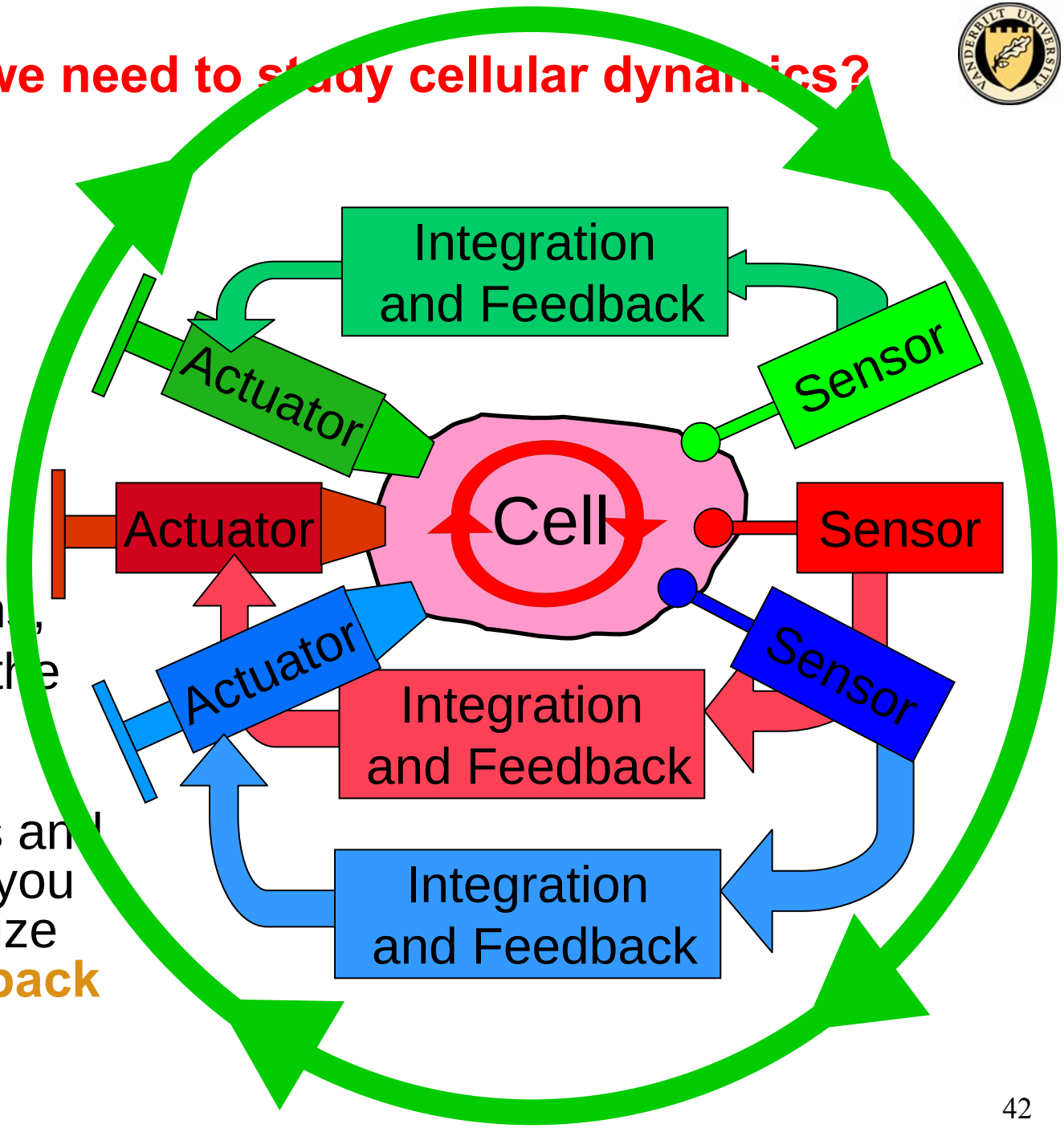
A cell expresses between 10,000 to 15,000 proteins at any one time for three types of activities:

- Metabolic
- Maintaining integrity of subcellular structures
- 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.

What do we need to study cellular dynamics?

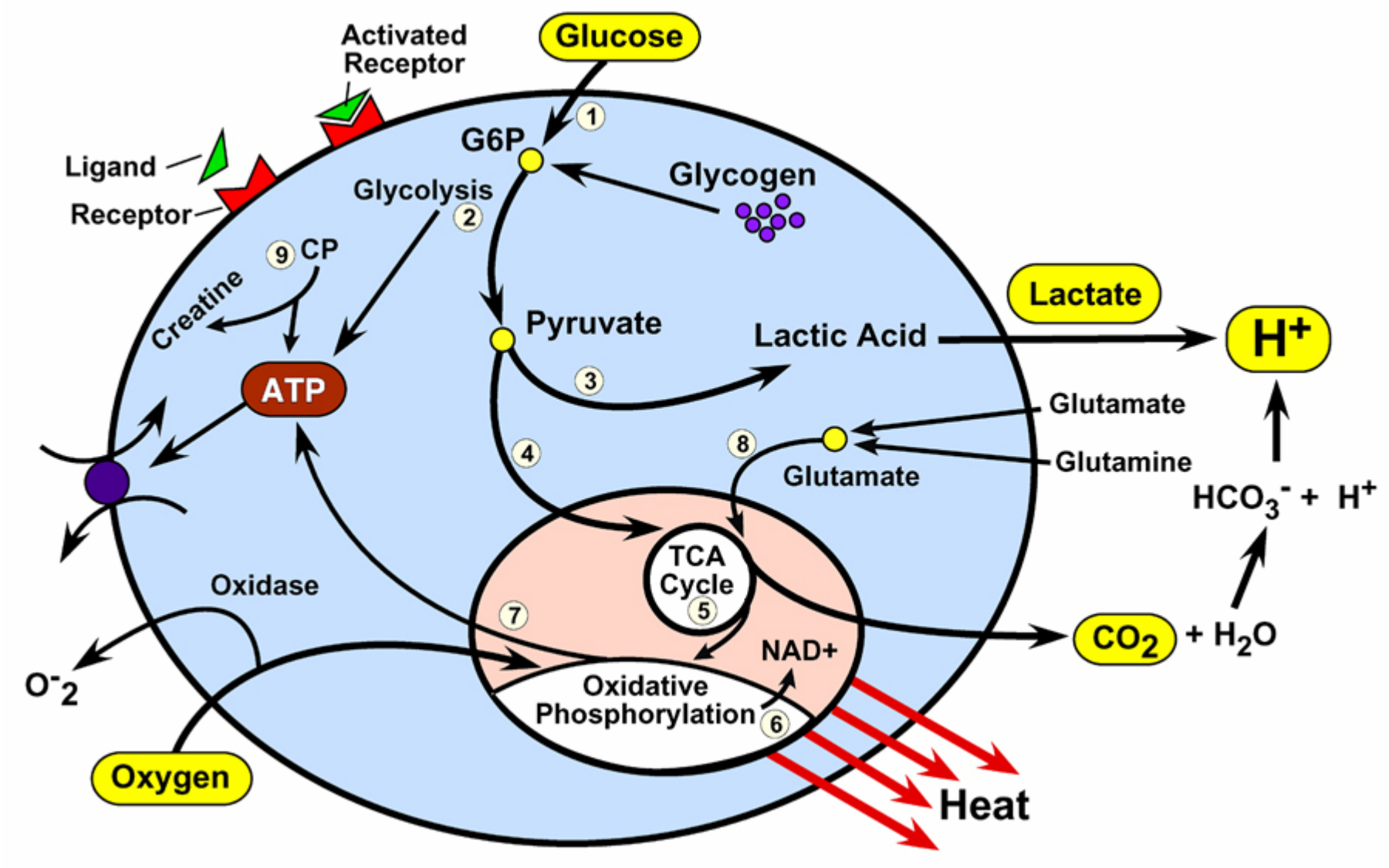
- Multiple, fast **sensors**
- Intra- and extracellular **actuators** **for controlled perturbations**
- Openers (Mutations, siRNA, drugs) for the internal feedback loops
- System algorithms and models that allow you to close and stabilize the **external feedback loop**
- …

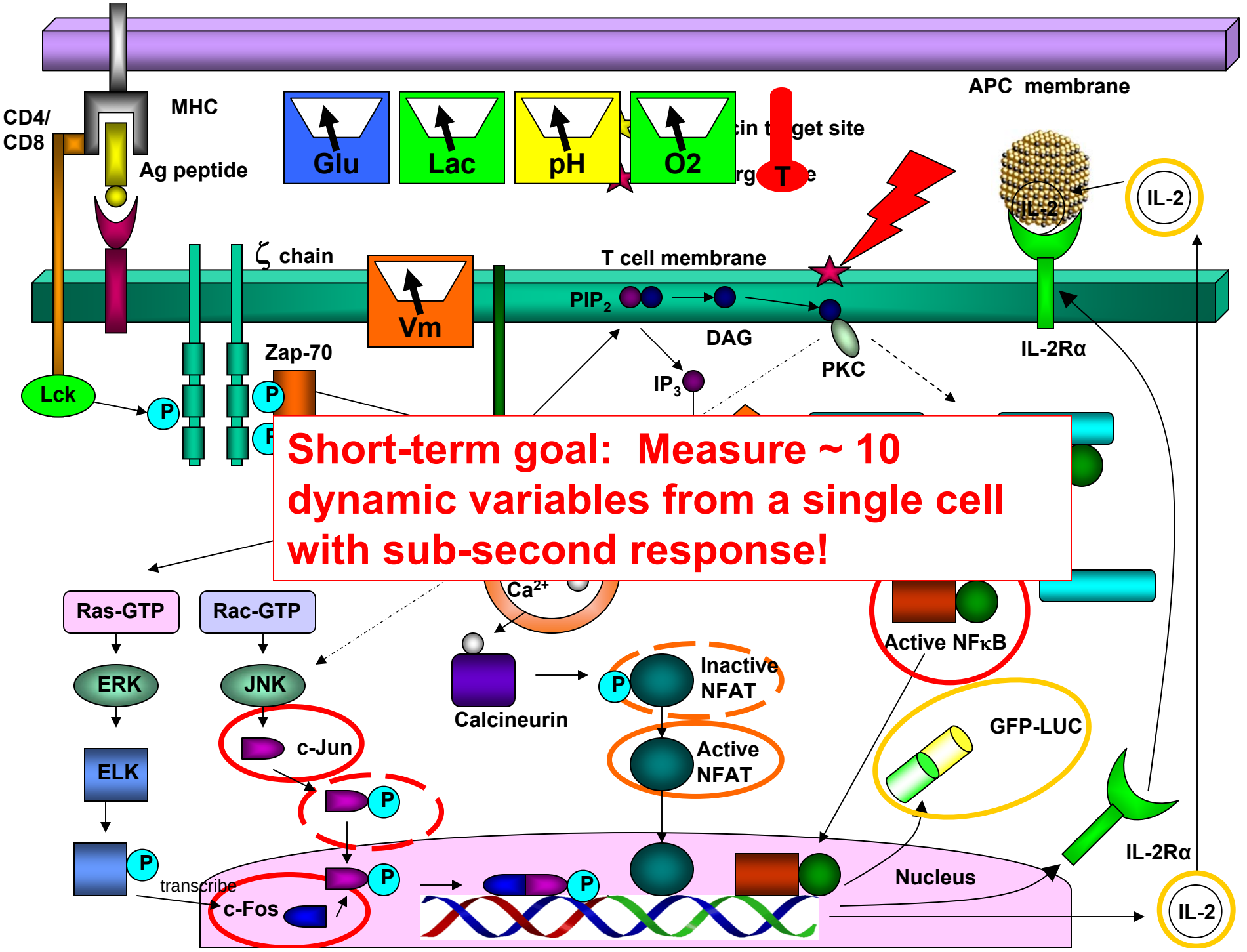


A Key to the Future

Probing and Controlling Cellular Metabolic and Signaling Pathways

Tools for Metabolomics: Experiments, Models and Analysis Blocking or Enhancing Metabolic Pathways




APC membrane
MHC
CD4/
CD8
Ag peptide
Glu
Lac
pH
O2
T
IL-2
IL-2
ζ chain
T cell membrane
Vm
PIP2
DAG
PKC
IL-2Rα
Zap-70
Lck
P
IP3
Short-term goal: Measure ~ 10 dynamic variables from a single cell with sub-second response!
Ca2+
Ras-GTP
Rac-GTP
Active NFκB
ERK
JNK
Inactive NFAT
Calcineurin
GFP-LUC
c-Jun
ELK
Active NFAT
transcribe
c-Fos
Nucleus
IL-2Rα
IL-2

What lers

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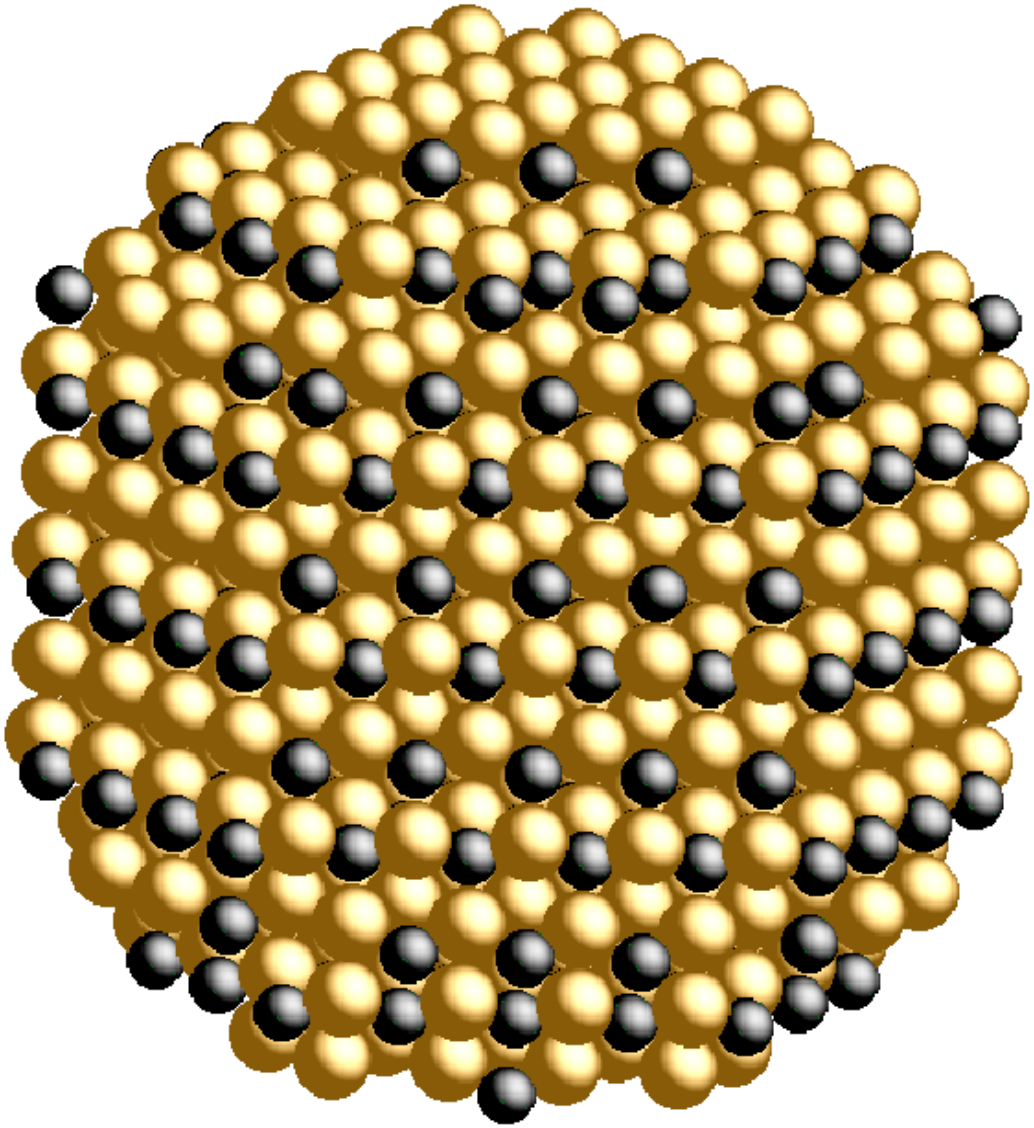
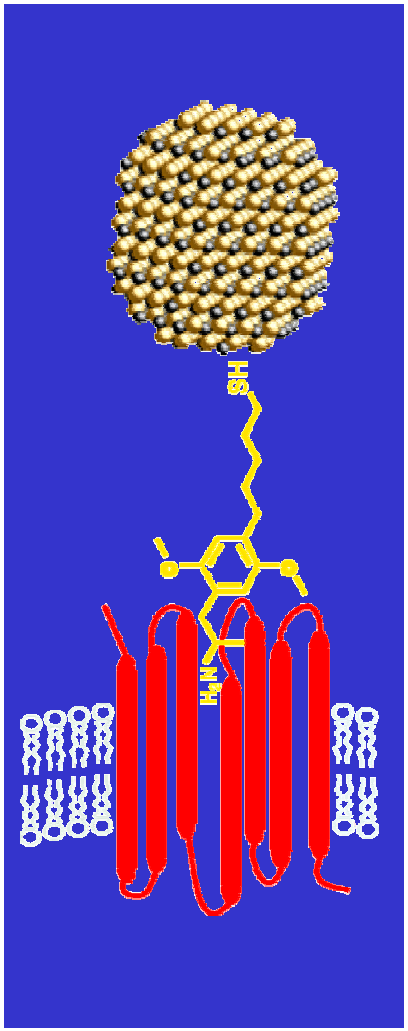
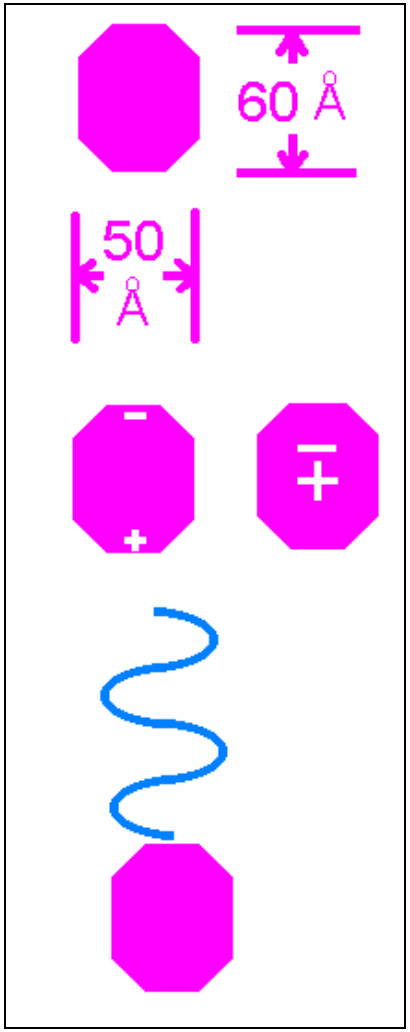


CdSe Nanocrystals

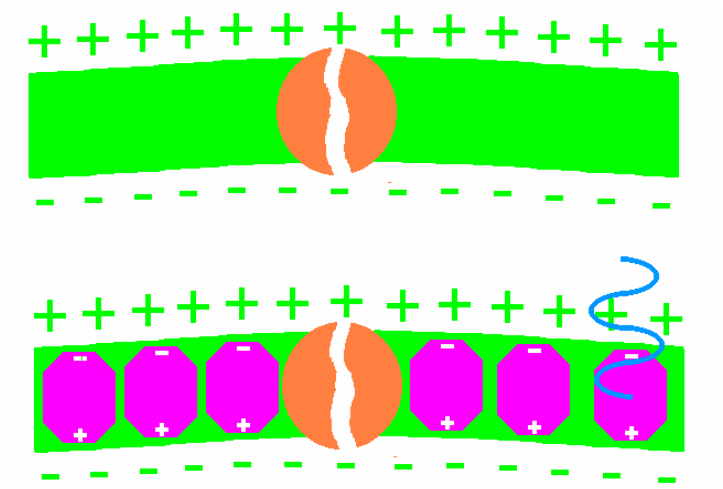
- Intrinsic shielded dipole moment of 70-80 debye for a 60 A nP
  - +/- 0.3 e at ends, or
  - +/- e separated by 17 A
  - 0.25 volt drop between ends of nP
  - 10^7-10^8 V/m internal field
  - nP dipole moment reduced by light

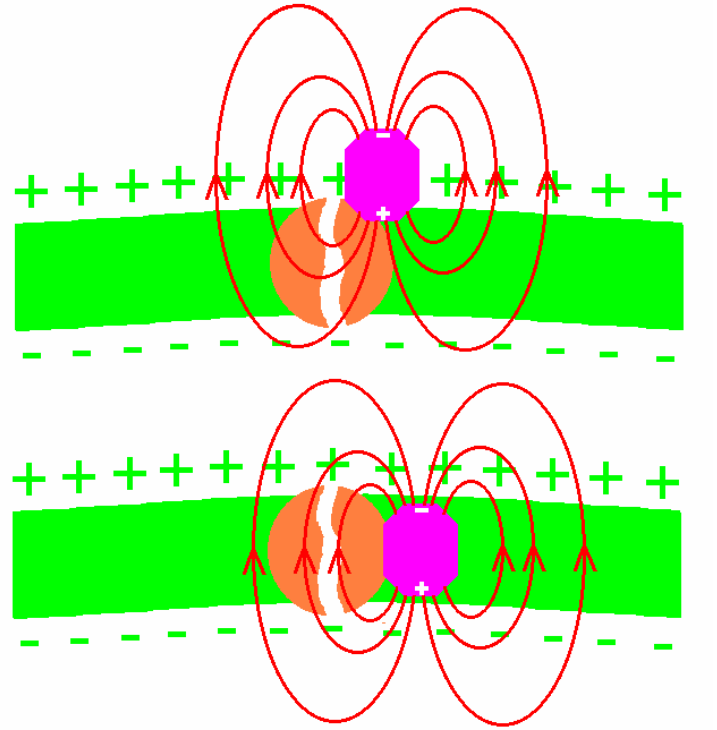




SemiConducting NanoCrystals as Optically-Controlled Dipole Moments

- Nanocrystals might be used to control cell membranes
  - In cell membrane
  - Bound to voltage-gated ion channel
- Shine light, no dipole





Metallic NanoShells (Halas@Rice)

- 10^{12} Raman enhancement
  - Is it possible to resolve the Stokes/AntiStokes lines or an adsorbed molecule and construct an optically-addressable intracellular nanothermometer?
- Infrared heating by bioconjugate nanoshells
  - Local control of enzymatic reactions
  - Selected destruction of tagged organalles

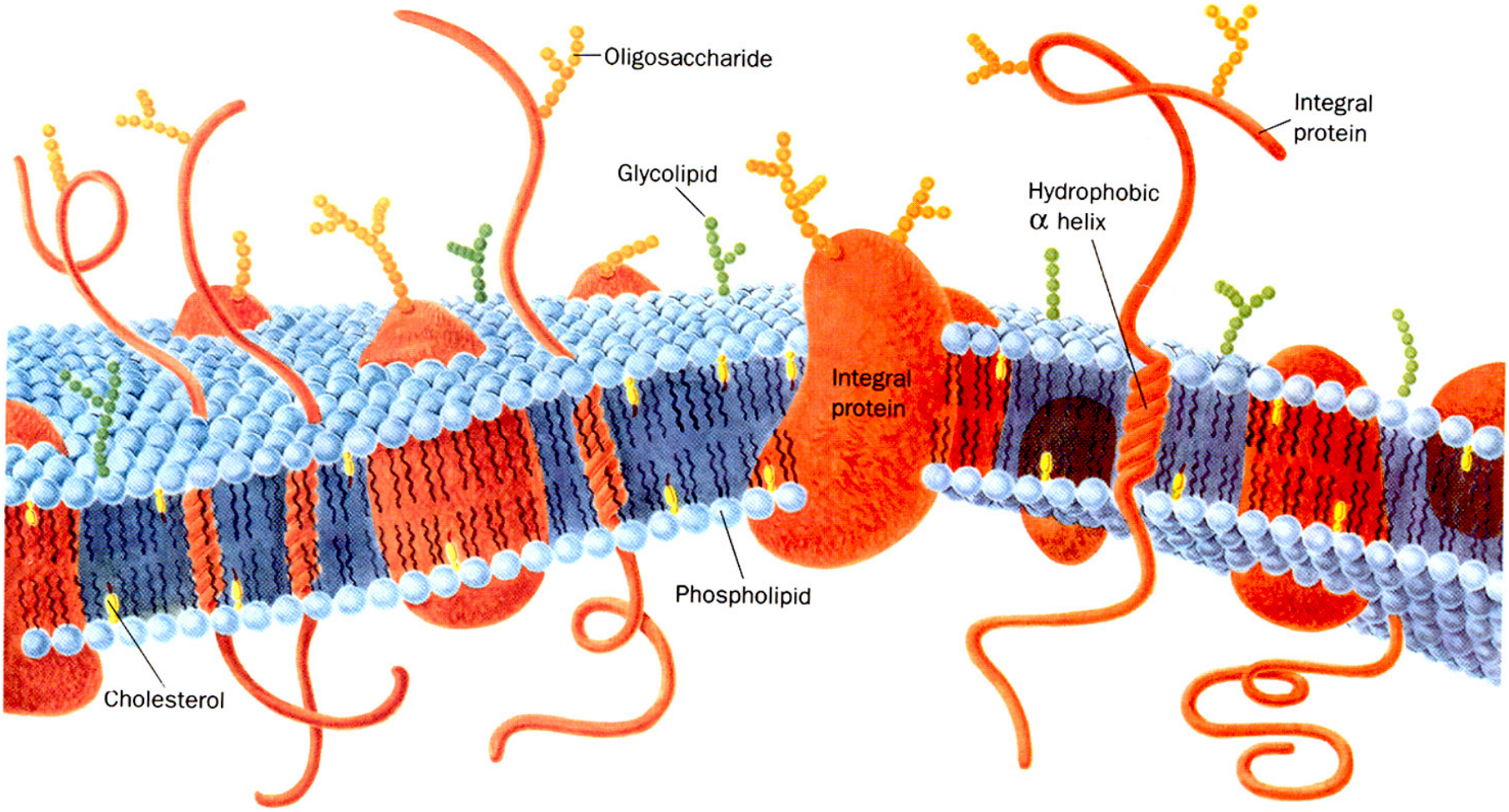
Magnetic Nanoparticles

- Translational and rotational forces
  - Viscosity -- Nanorheometry
  - Molecular motor characterization
- Magnetic separation
- Magnetic identification
  - Magnetobacteria
  - Determination of mechanisms of biomagnetic sensing
  - Tagged cells and molecules

What is the competition?

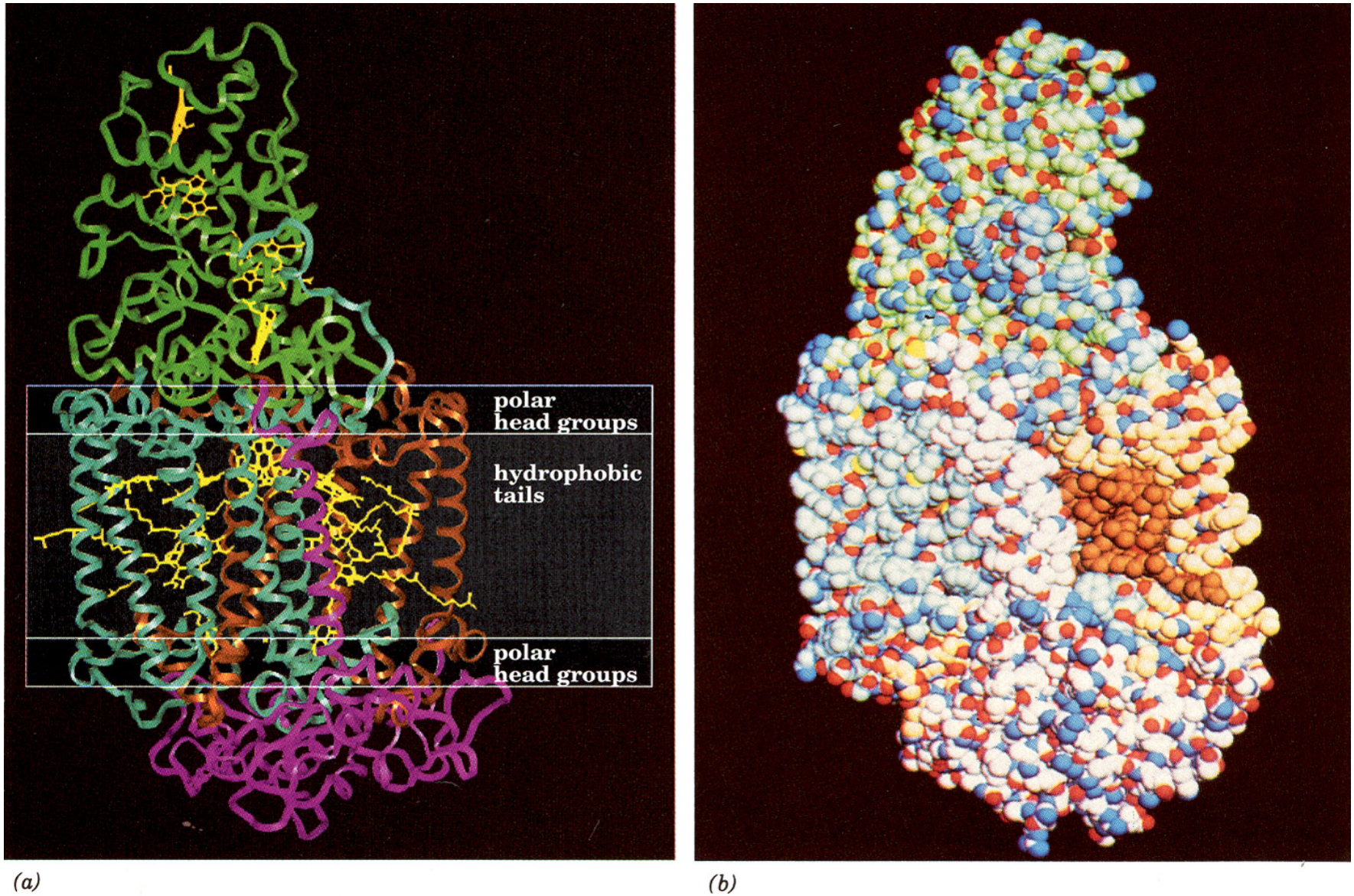
Proteins, proteins, proteins…

Plasma Membrane



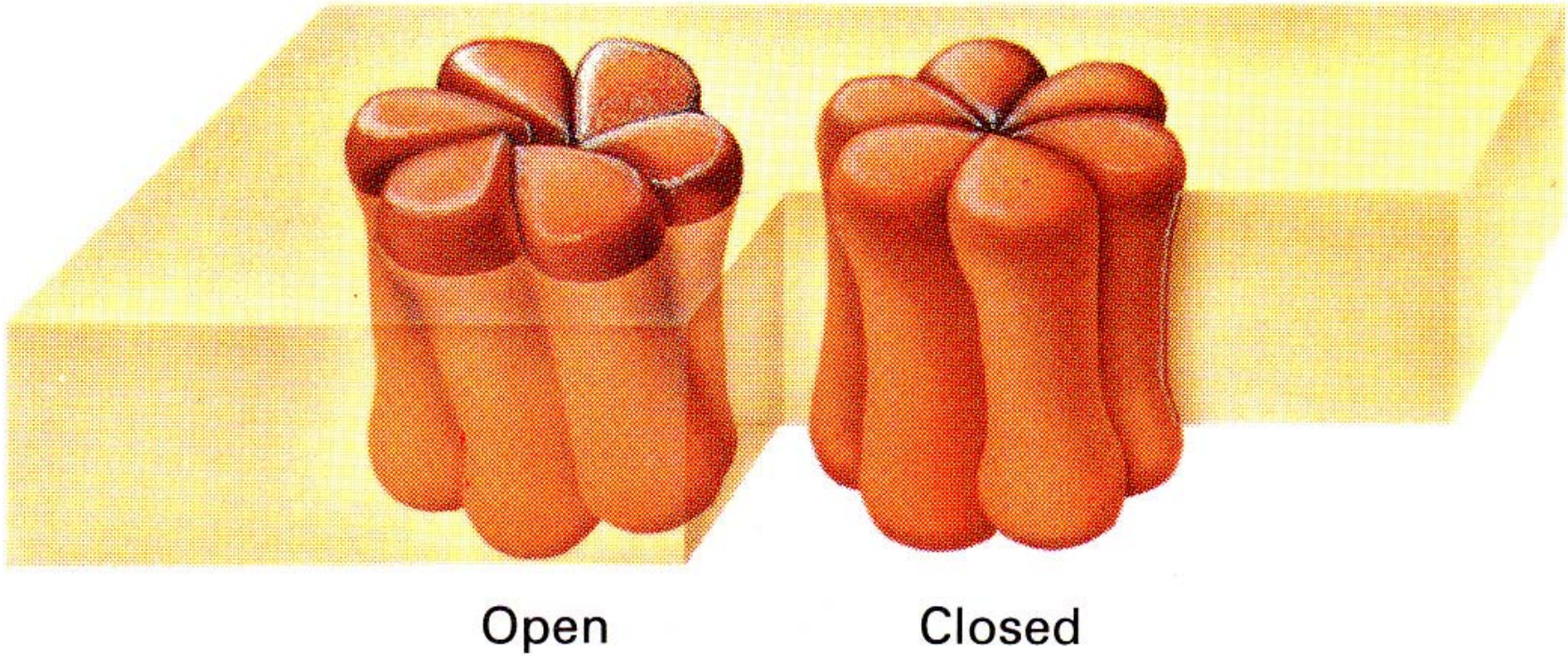
Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 292

Bacterial Photosynthetic Reaction Center



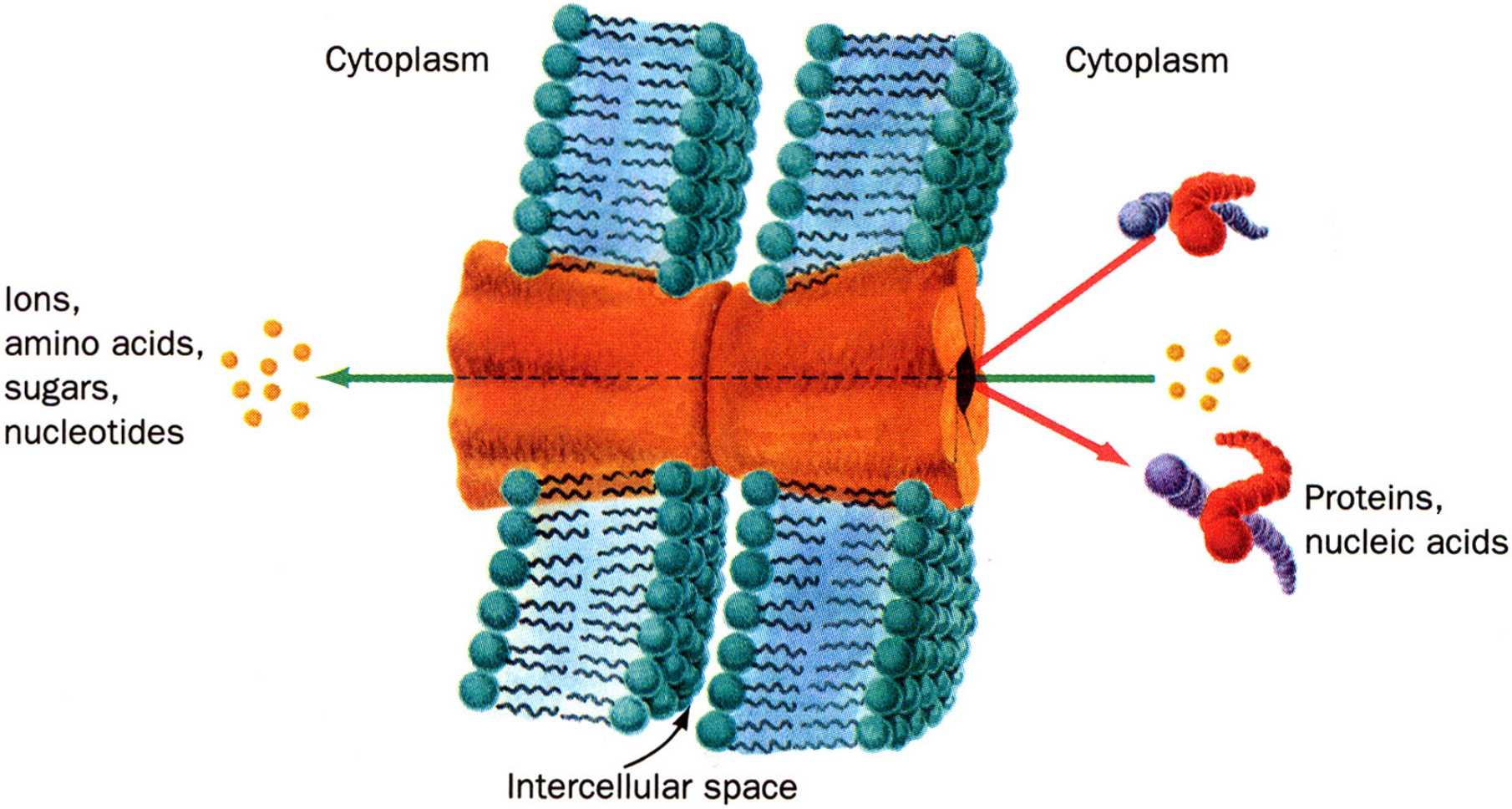
Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 296

Calcium Control of Conductance



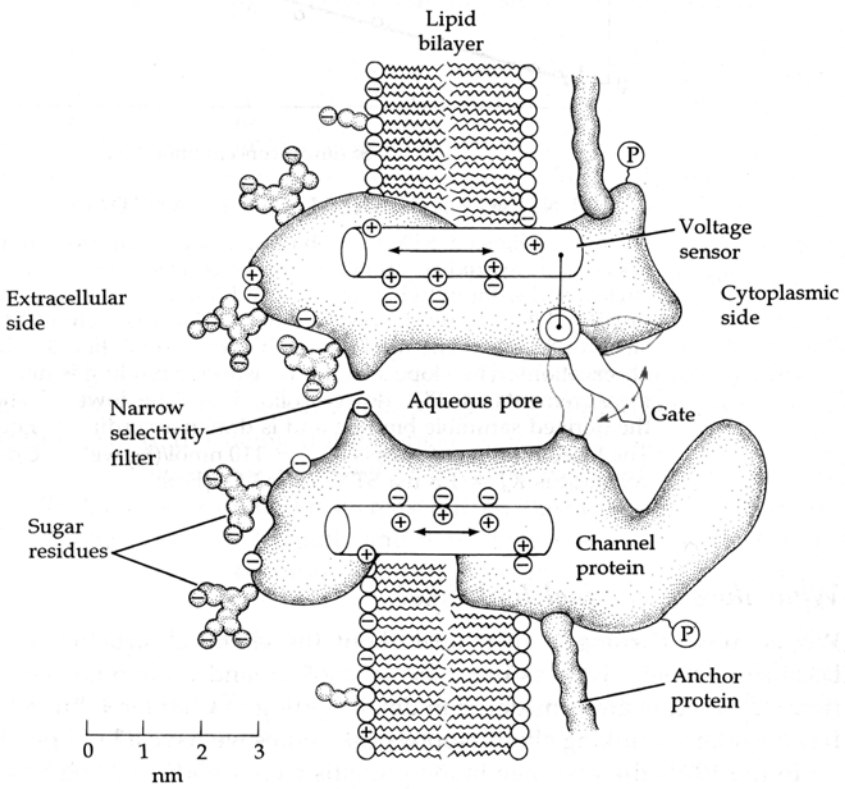
Molecular Cell Biology, 2nd ed.
Darnell, J.; Lodish, H.; Baltimore,
W.H Freeman & Co. 1990, p.525

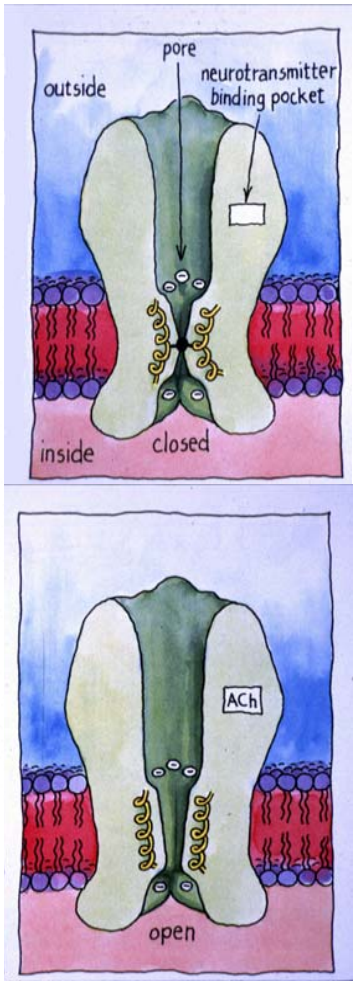
Gap Junctions

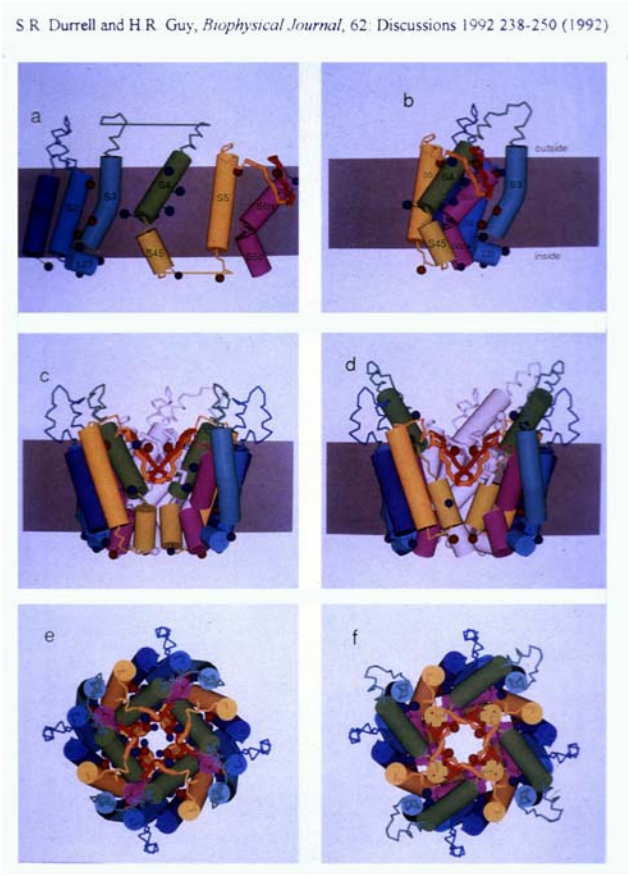


Biochemistry, 2nd ed. Voet, D.; Voet, J.G.; NY, John Wiley & Sons, 1995, p. 304

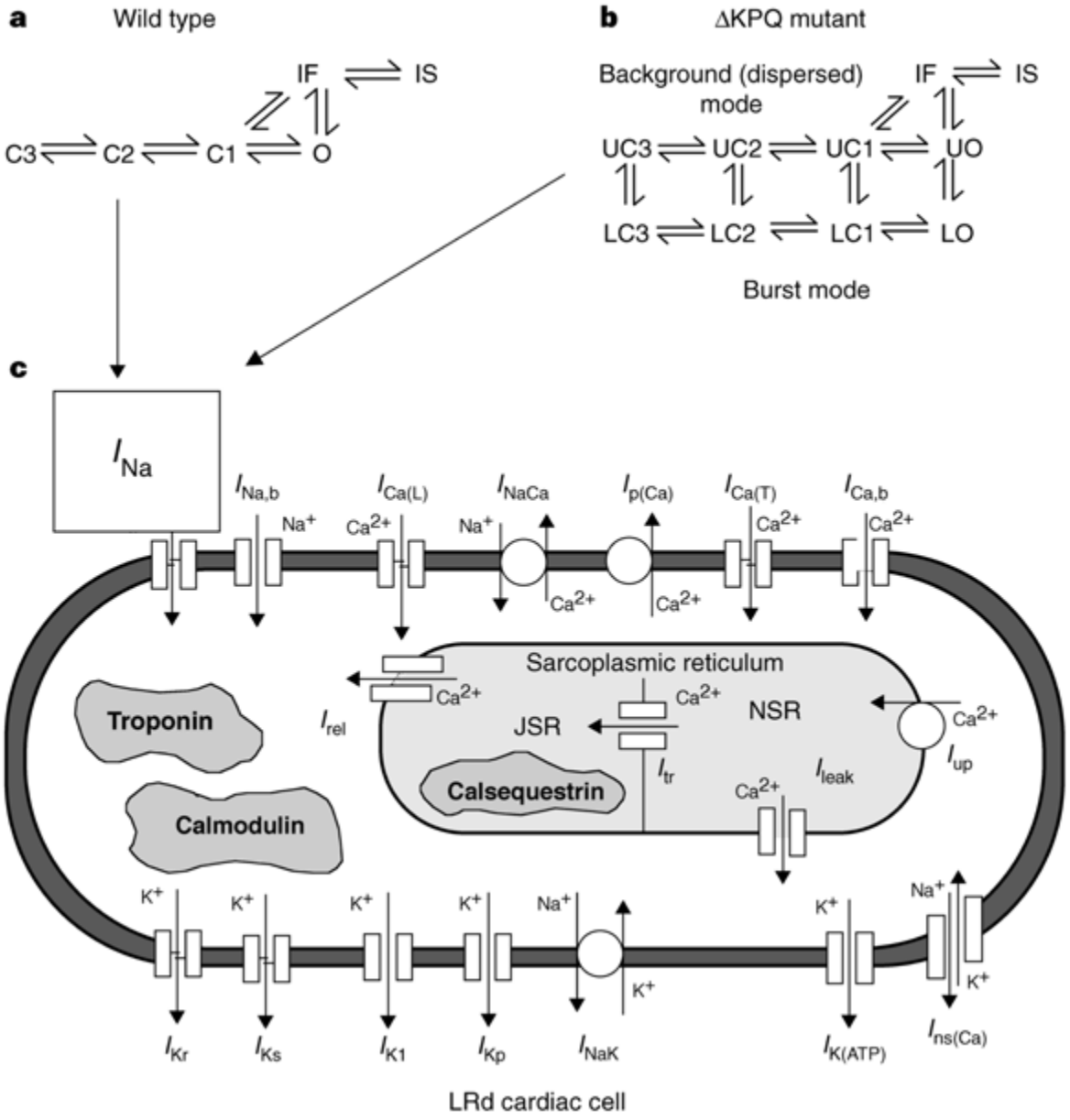
The Ultimate NanoMachine: The one-nanometer pore in a gated ion channel







Cells have LOTS of different ion channels!



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

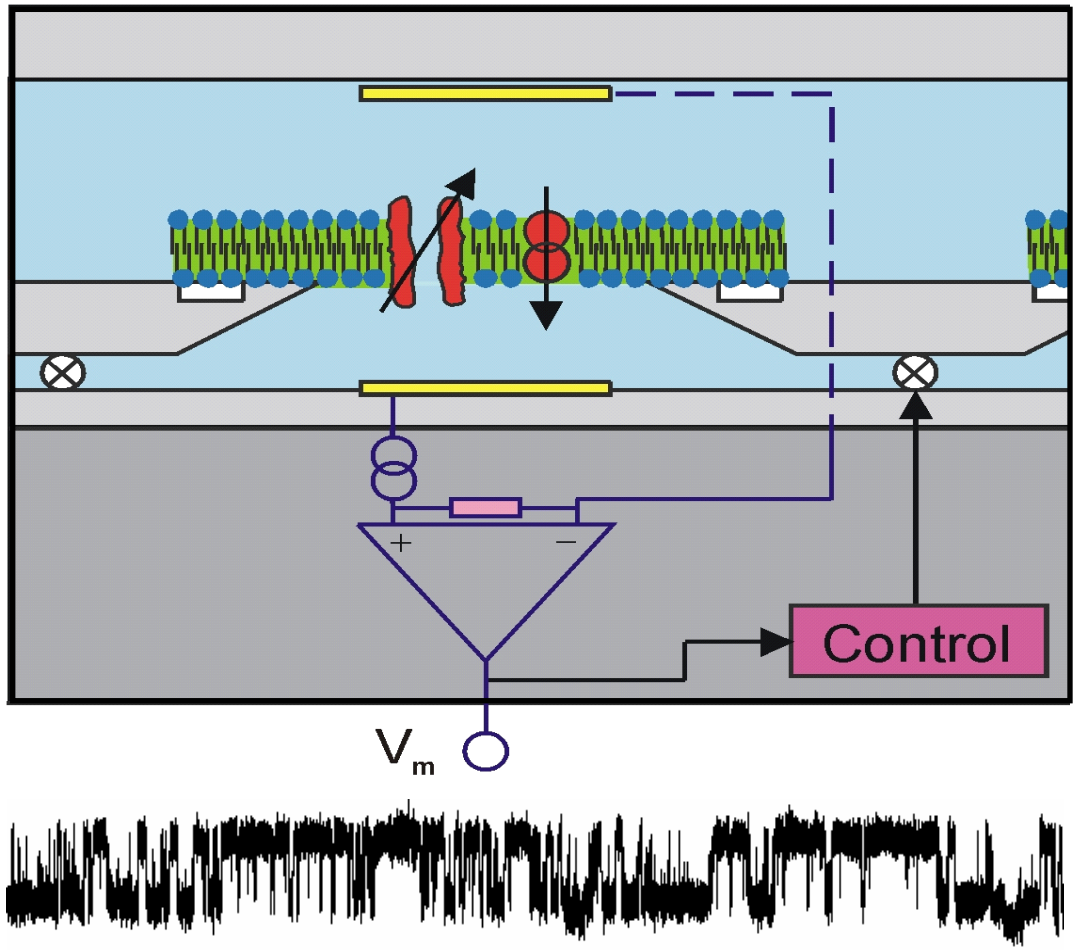
Ion currents and ion channel clones

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

How do you make an ion-channel biosensor?

- Make two small silicon bottles
- Connect with a small hole
- Cover the hole with a lipid membrane
- Put channel in the membrane
- Put different test solutions in one chamber
- Measure the current through the channel



What is the gain of a ligand-gated ion channel?

- Gain ≡ the number of ions that pass through the channel for one bound ligand

$$I = g \times V \qquad Q = I \times t \qquad N = \frac{Q}{z \times e} \qquad N = \frac{g \times V \times t_{bound}}{z \times e}$$

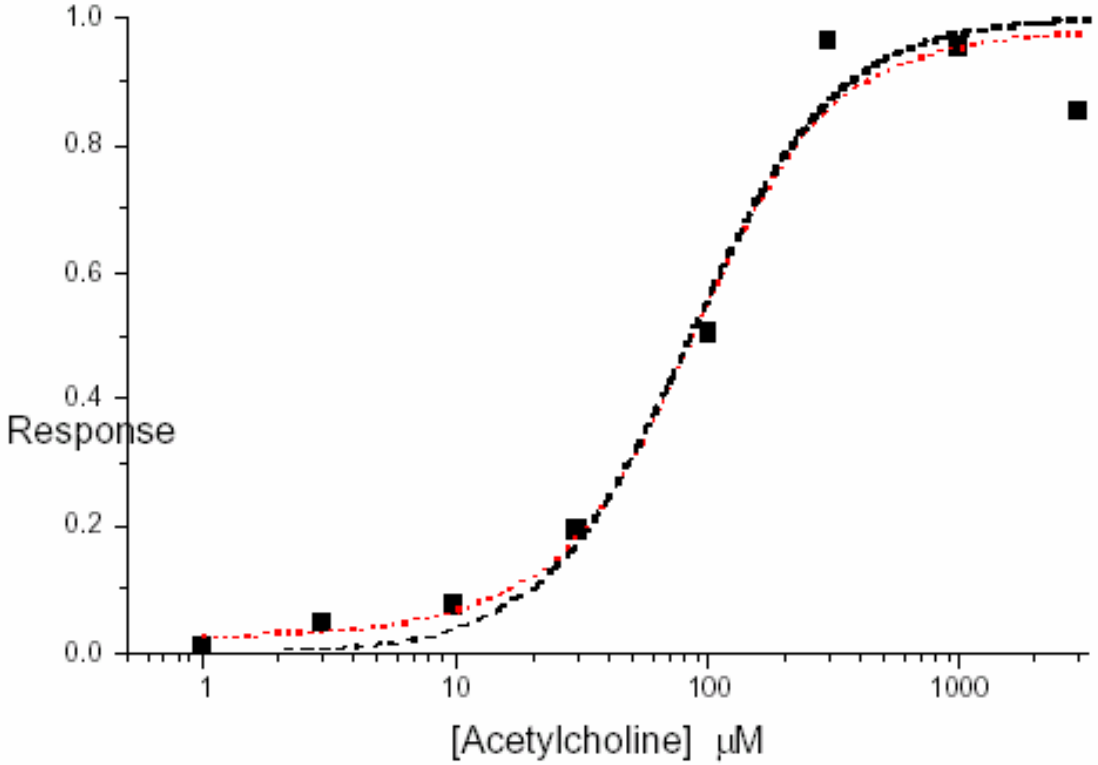
- 1 ms < t_{bound} < 10 ms
- 10^4 < flux < 10^7 ions per second
- 10 < Gain < 10^5.
- Large channels like gluR0 in normal [K^+] pass about 10^7 ions/s at a 100 mV driving force. In higher [K^+] or *Vm* they will pass more ions. The open time occurs in bursts that typically last for one second. **For these channels, the "gain", *i.e.*, the integrated ion flux/ligand binding, is >10^7**

What you need

- You want the ligand to stick well
- You don't want the ligand to stick so well that you can't reuse the channel
- You have to measure for a long time to get enough data to get a concentration
- You want the ligand to stick well enough to minimize blinking
- You need to measure a long time to get the channel kinetics

Other problems

- Binding is not a binary event
- Binding is not perfectly specific
- As we said, many channels have multiple binding sites and cooperative binding



http://www.biophysics.org/btol/img/Jackson.M.pdf

Solutions to get faster response and bigger signals

- A single ion channel is infinitely sensitive if you wait infinitely long, but you then couldn't measure the concentration…
- Put the ligand molecule in a $(0.1\ mm)^3$ box to get [L] = 1 mM. Detection straightforward thereafter
- Put multiple channels in the patch (~500)
  - Increase the current
  - Increase the probability of getting a binding event
  - Loose information about channel binding dynamics
- Use a massively parallel array of ion channels

Single Ion-Channel Conclusions

- Single channels have a very high internal gain as detectors where binding of one molecule can result in the transport of > 10^7 ions.
- A single-channel chemical detector is not a single molecule detector: it runs on a bimolecular reaction with [R][L].
- Single molecule sensors take time to respond that is dependent upon concentration in a diffusion-limited manner.
- To detect concentration, channel detectors must make repeated cycles of binding and unbinding since concentration is inferred from the time between binding events.
- While channels can be engineered to improve selectivity and responsiveness, diffusion places limits on the maximum speed of response.
- The use of channels as detectors requires the ability to distinguish different compounds in mixtures of different concentrations. This requires large parallel arrays.

The Ultimate Question for Systems Biology Instrumentation

Can we develop nanodevices that allow external control of cellular functions more effectively than natural or bioengineered proteins?

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

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