A Biophysics and Bioengineering Perspective:

What makes breast cancer a hard problem, and where are some keys to its prevention, control, and cure?

John Wikswo
Vanderbilt

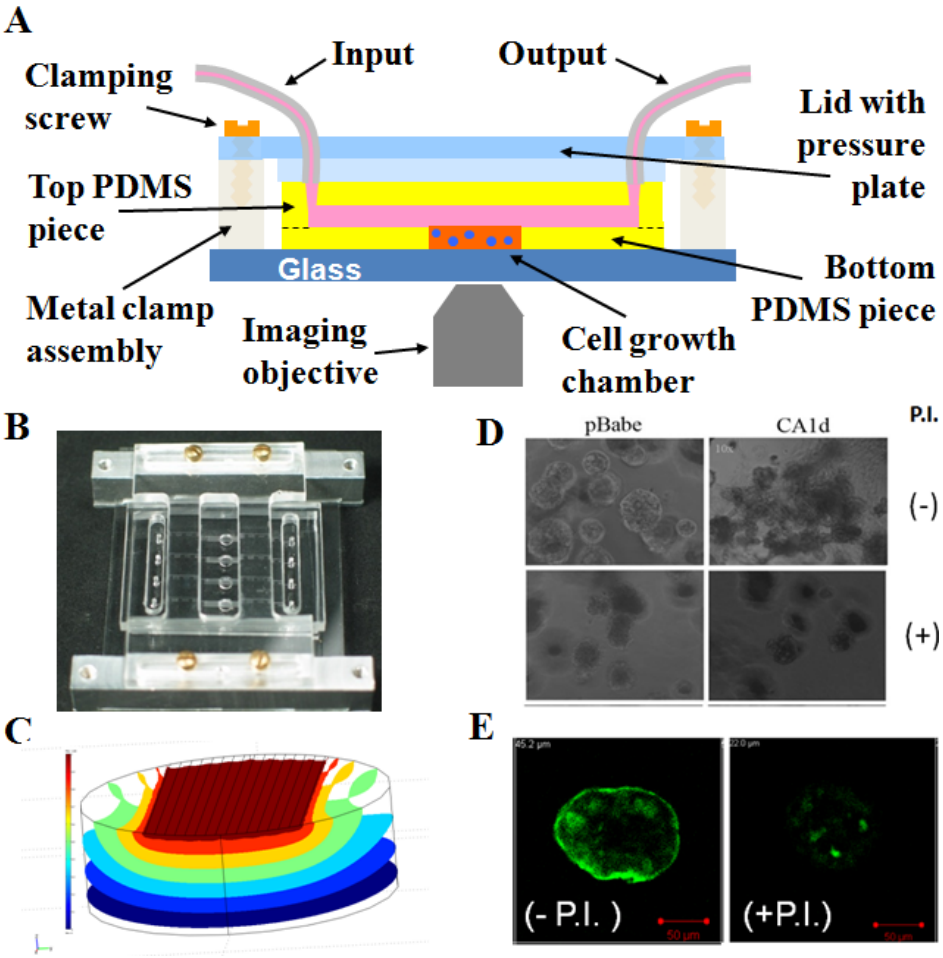
2010 NSF Advances in Breast Cancer Research Workshop
October 26-29, 2010
University of Arkansas

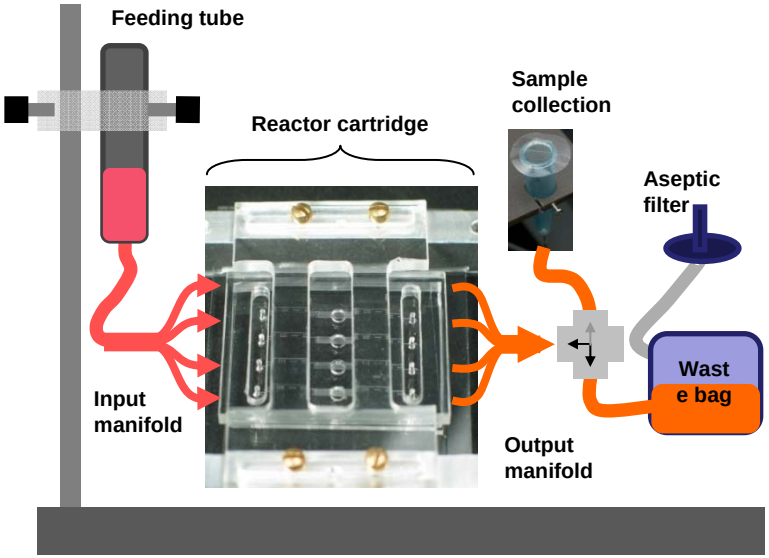
What I won't discuss

- Bioelectricity and biomagnetism in breast cancer
- Microfabricated devices for cancer research
  - Cell motility
  - Adhesion of rolling cells
  - Cell stiffness
  - Cell migration after patterning
  - Cellular haptotaxis on patterned substrates
  - Murine mammary fat pad windows
  - Three dimensional microbioreactors
  - Hollow fiber bioreactors
  - A perfused murine aorta model for angiogenesis
  - A microbioreactor for angiogenesis in the chick alantoic membrane

Thick Tissue Bioreactor:

Validated with organotypic cultures and testing of drug delivery of chemical inhibitors of mammosphere formation

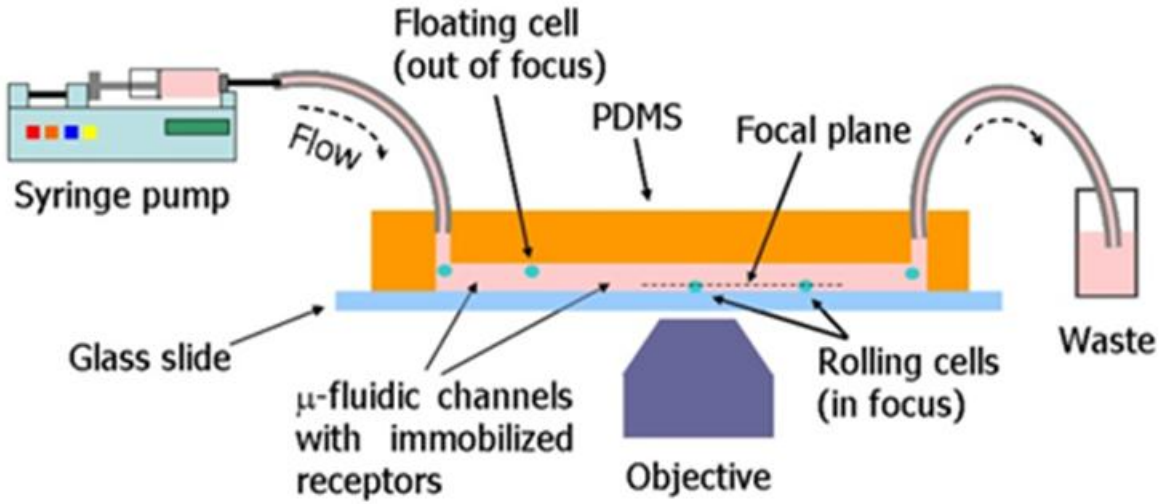


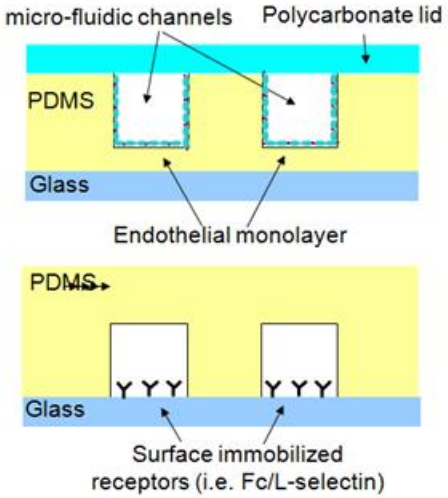


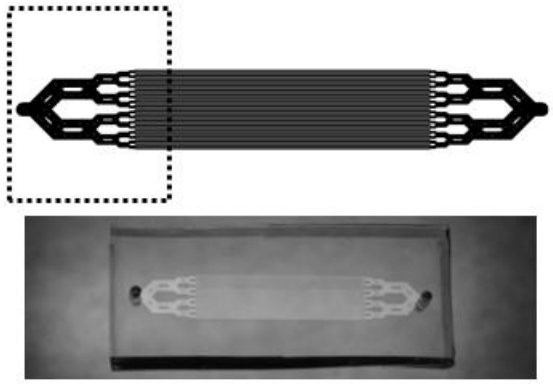
Lisa McCawley and Dmitry Markov, Vanderbilt

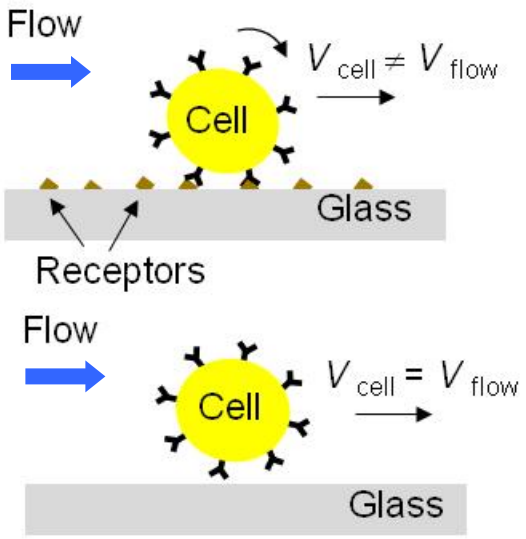
Planar PCPB (parallel capillary perfused bioreactors)

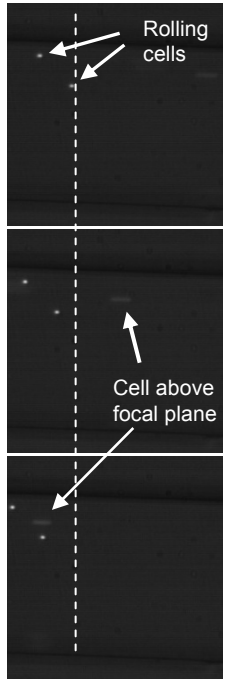
coating with various cell adhesion receptors and assaying neutrophil interaction.











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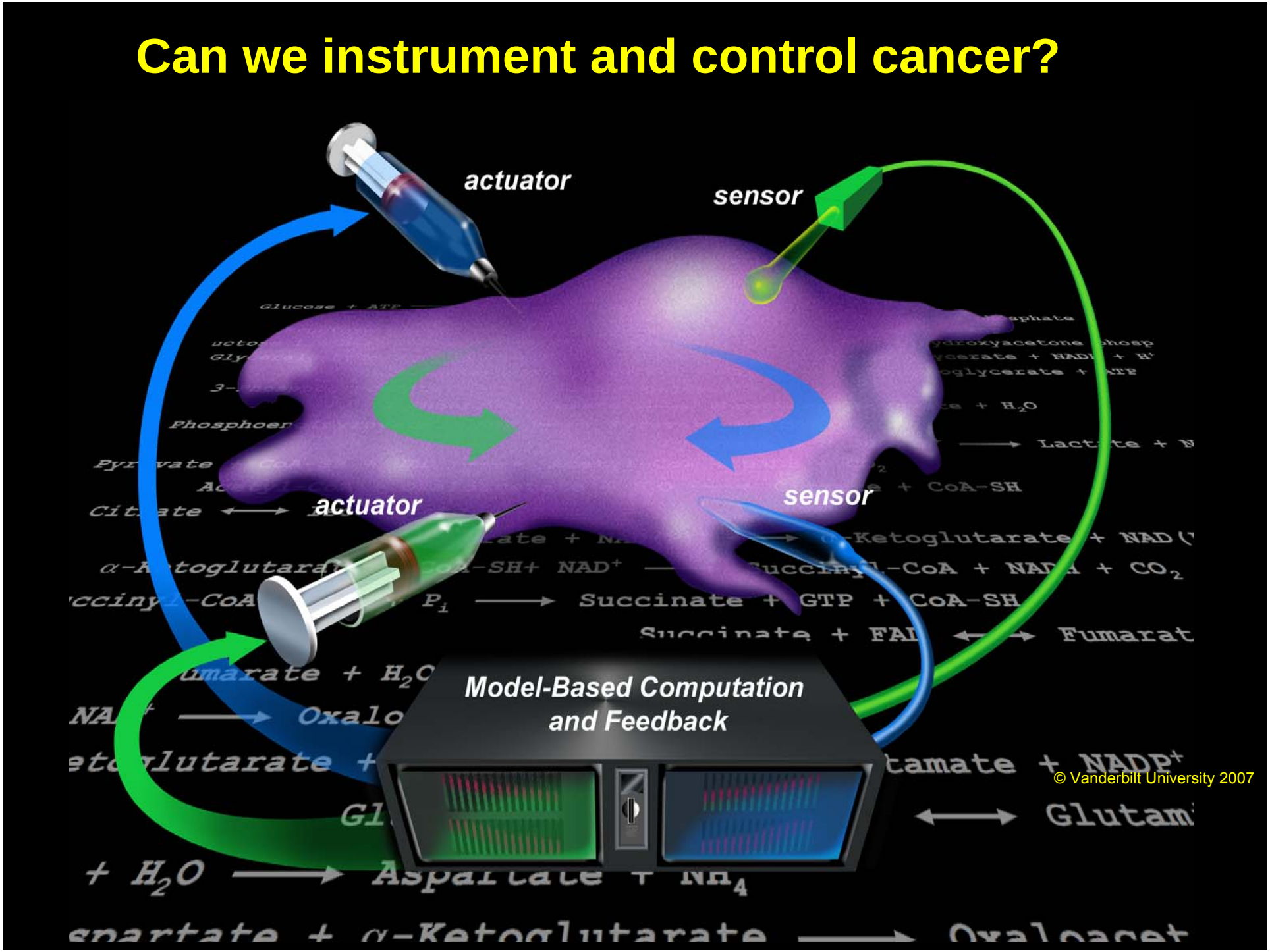
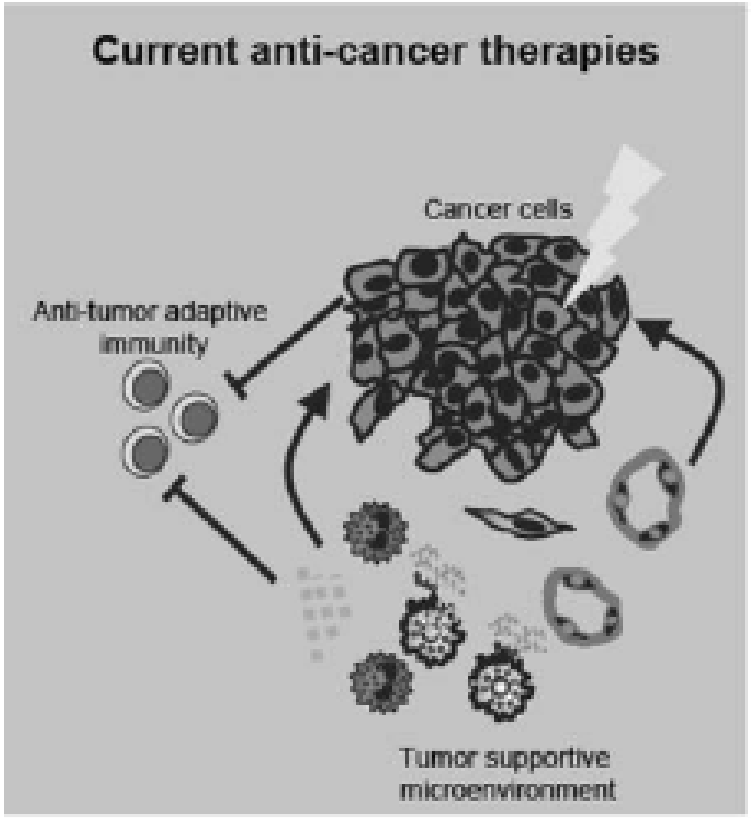
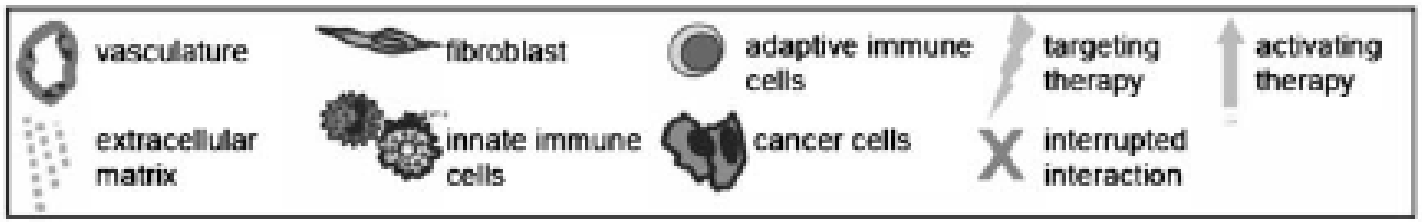
Let’s discuss control and the complexity of biology, starting with the end of the talk.

Can we instrument and control cancer?



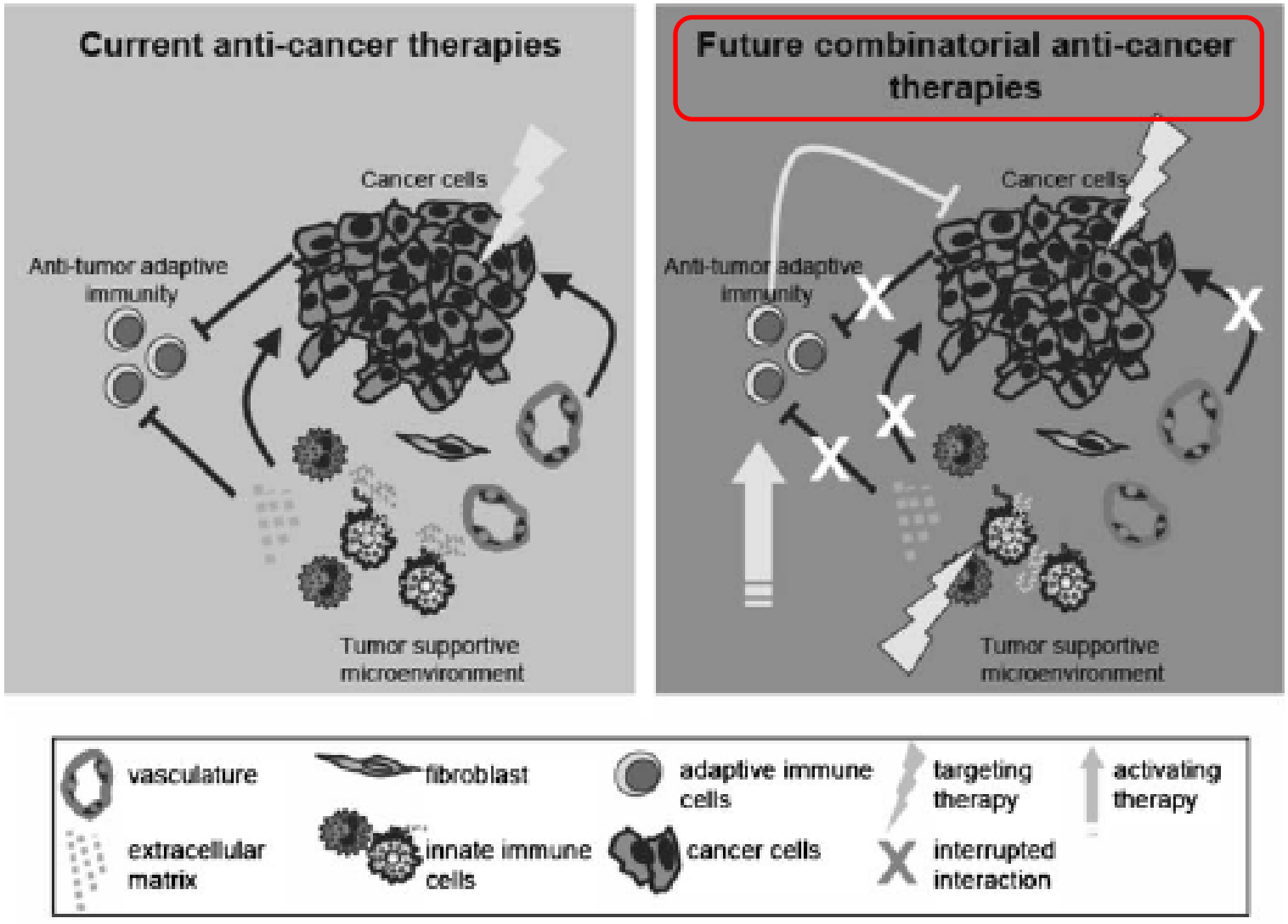
The future of biology and cancer medicine is distributed hybrid multiscale non-linear stochastic control





De Visser, Cancer Immunol Immunother (2008) 57: 1531-1539

The future of biology and cancer medicine is distributed hybrid multiscale non-linear stochastic control

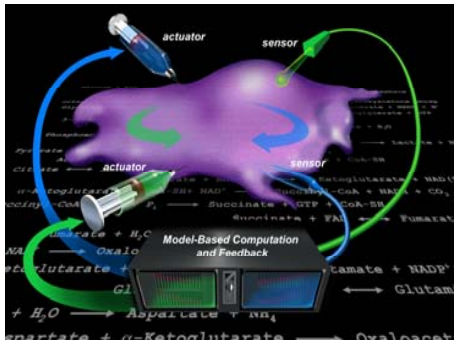


De Visser, Cancer Immunol Immunother (2008) 57: 1531-1539

INPUT ACTUATORS

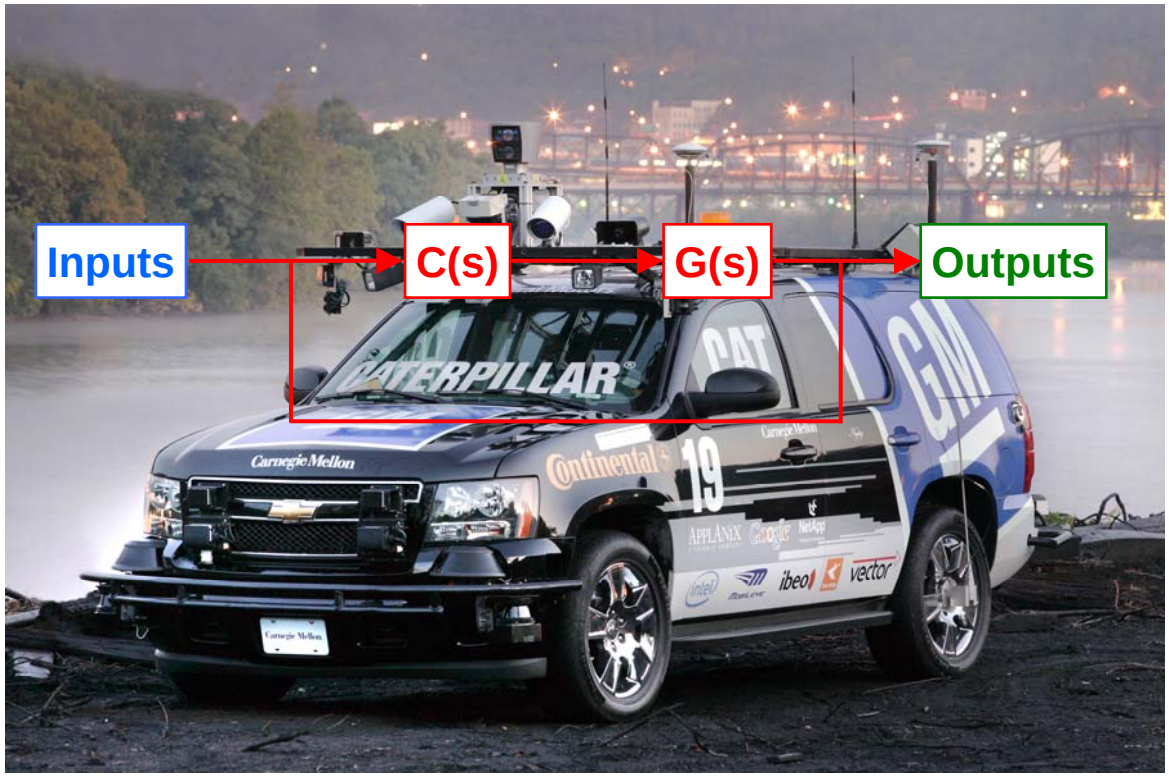
- Chemical
- Electrical
- Genetic
- Mechanical
- Optical
- Thermal
- Scaffolding

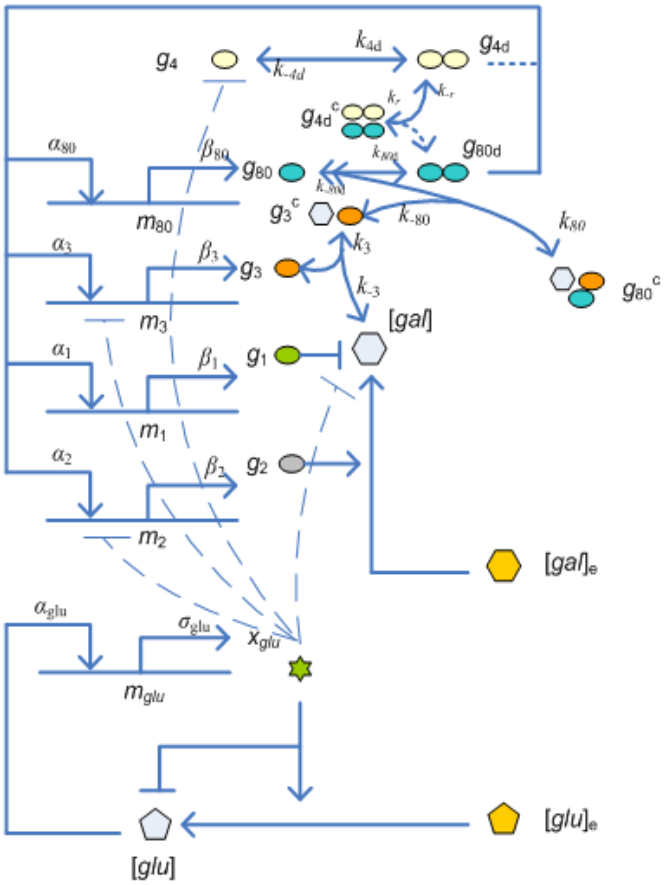
C(s) → G(s)



OUTPUT SENSORS

- Apoptosis
- Differentiation
- Gene / Protein Expression
- Growth
- Metabolism
- Motility
- Signal Transduction

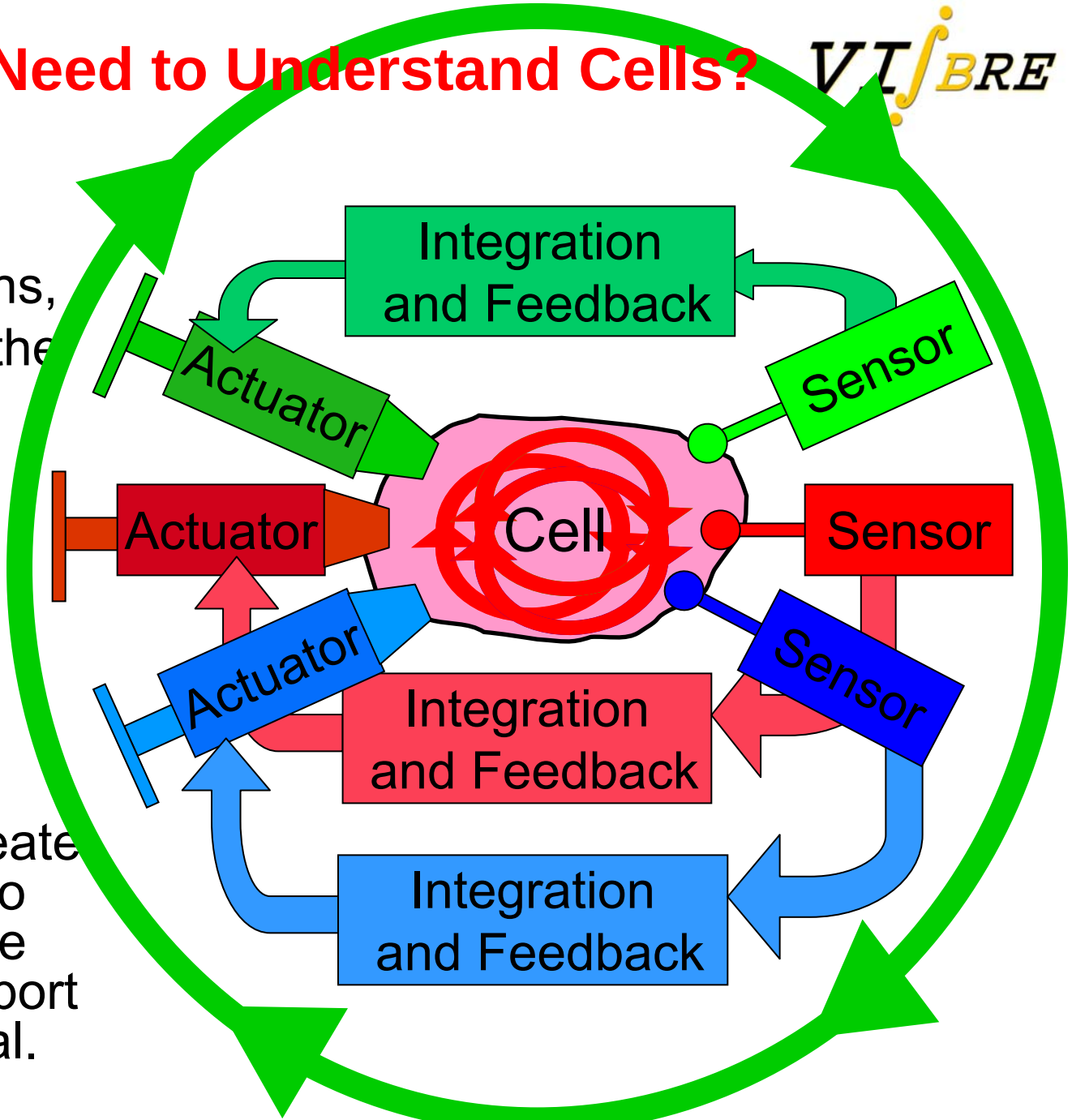




LeDuc, Messner, Wikswo. How do controls approaches enter into biology. *Submitted*, 2010.

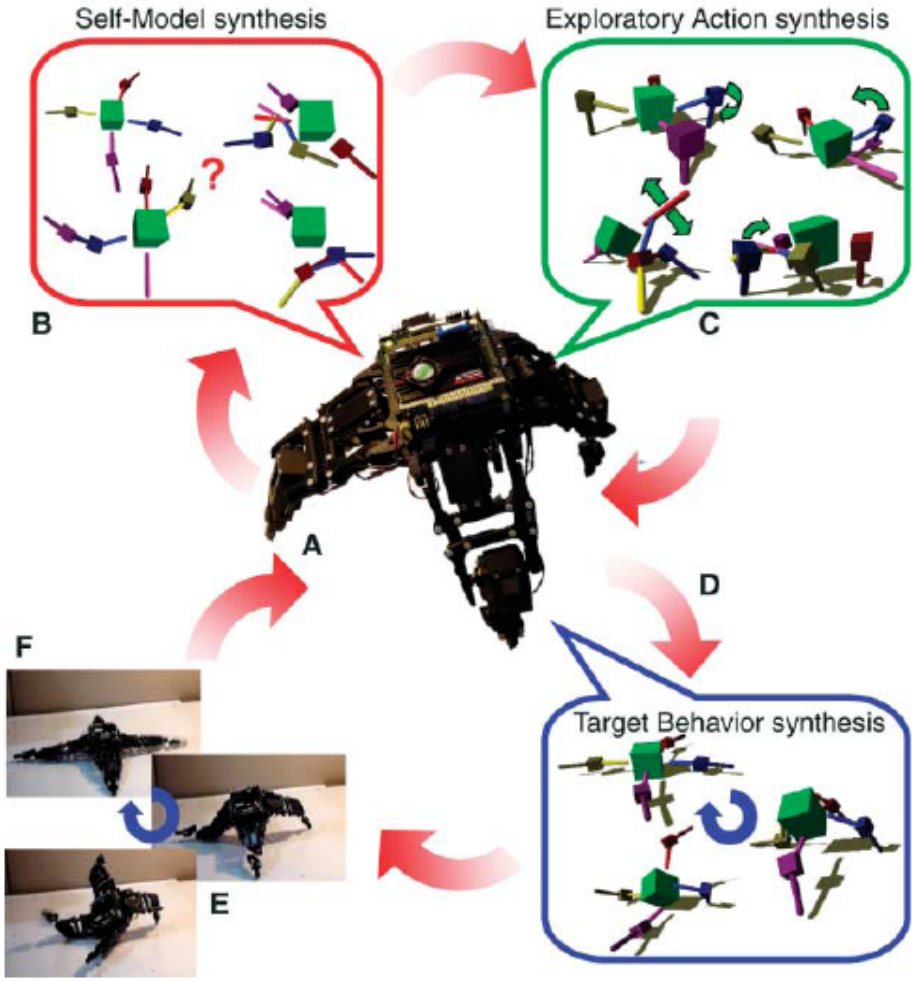
What Do We Need to Understand Cells?

- Multiple, fast **sensors**
- **Openers** (Mutations, siRNA, drugs) for the internal feedback loops
- Intra- and extracellular **actuators** **for controlled perturbations**
- Algorithms that create **feedback loops** to automatically probe the system and report the feedback signal.



Wikswo *et al*., IEE Proc Nanobiotechnol. 153: 81-101 (2006)

Machine Learning: A robot that can infer a model of "itself"

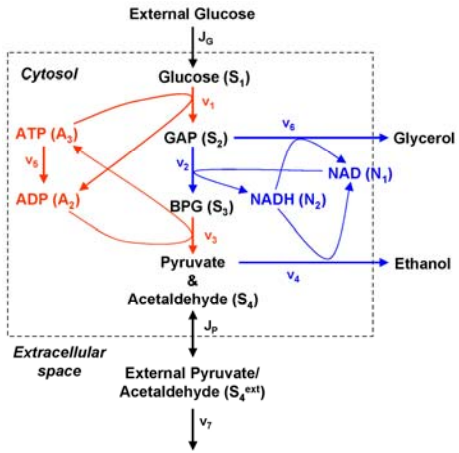


Hypothesis: Machine learning and model inference with automated experimentation can be extended from robots to bioreactors

J. Bongard, V. Zykov, and H. Lipson, Resilient Machines Through Continuous Self-Modeling, Science, 314, 1118-1121, 2006

Computational Synthesis

Inferring Metabolic Models



VIBRE

CFDRC

Target model placed in black box with 10% noise

$$\frac{dS_1}{dt} = 2.5 - \frac{100 * A_3 S_1}{1 + 13.68 * A_3^4}$$

$$\frac{dS_2}{dt} = \frac{200 * A_3 S_1}{1 + 13.68 * A_3^4} - 6 * S_2 \boxed{- 6 * S_2 N_2}$$

$$\frac{dS_3}{dt} = 6 * S_2 - 6 * N_2 S_2 - 64 * S_3 + 16 * A_3 S_3$$

$$\frac{dS_4}{dt} = 64 * S_3 - 16 * A_3 S_3 - 13 * S_4 - 100 * N_2 S_4 + 13 * S_5$$

$$\frac{dN_2}{dt} = 6 * S_2 - 18 * N_2 S_2 - 100 * N_2 S_4$$

$$\frac{dA_3}{dt} = -1.28 * A_3 - \frac{200 * A_3 S_1}{1 + 13.68 * A_3^4} + 128 * S_3 + 32 * A_3 S_3$$

$$\frac{dS_5}{dt} = 1.3 * S_4 - 3.1 * S_5$$

Model inferred without any a priori information

$$\frac{dS_1}{dt} = 2.53 - \frac{98.79 \cdot A_3 S_1}{1 + 12.66 \cdot A_3^4}$$ **Glucose**

$$\frac{dS_2}{dt} = \frac{200.23 \cdot A_3 S_1}{1 + 13.80 \cdot A_3^4} - 6.87 \cdot S_2 \boxed{- 6.87 \cdot N_2 + 0.95}$$ **G3P DP Pool**

$$\frac{dS_3}{dt} = 6.00 \cdot S_2 - 6.00 \cdot N_2 S_2 - 64.16 \cdot S_3 + 16.08 \cdot A_3 S_3$$ **BPG**

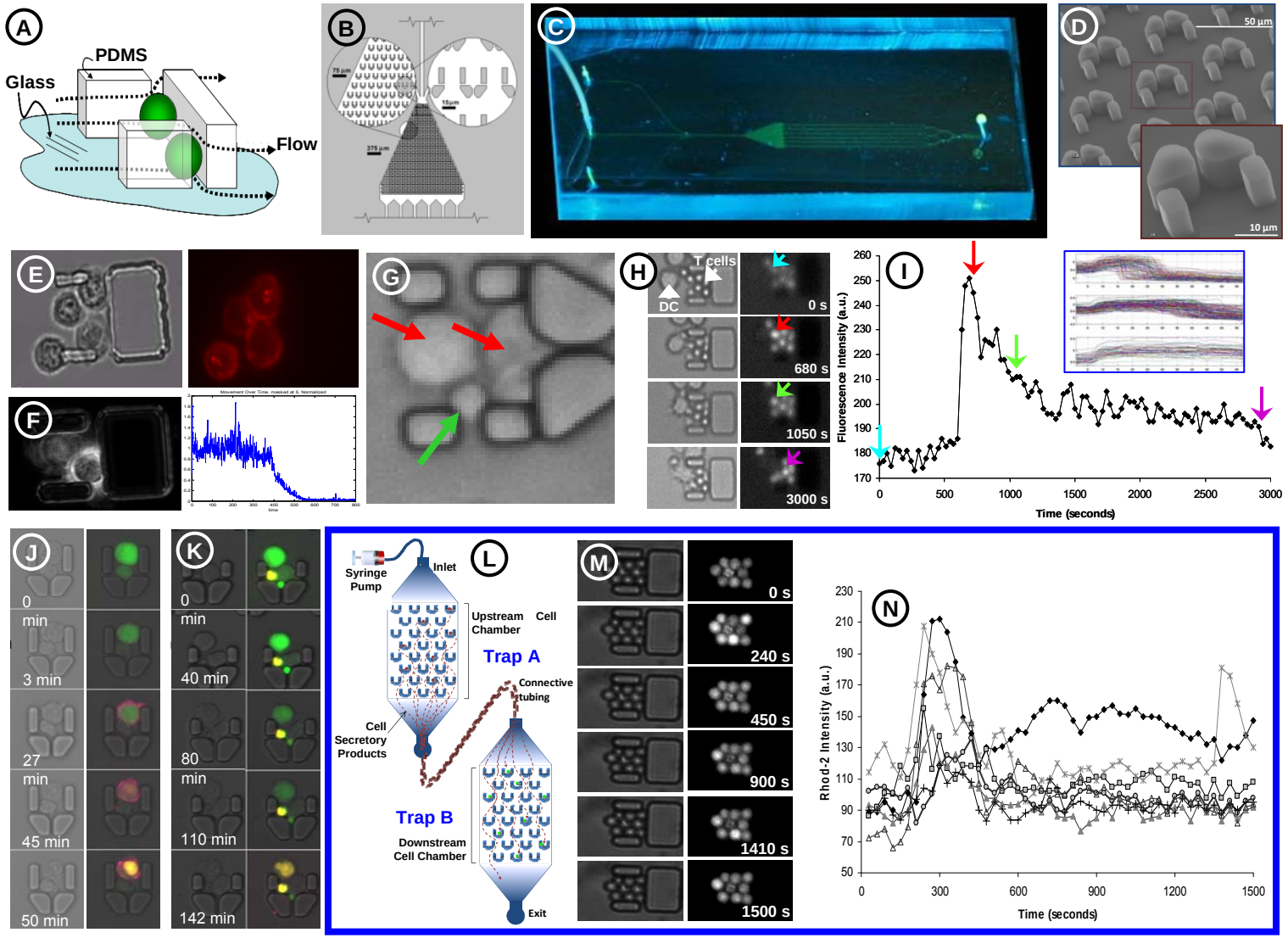
$$\frac{dS_4}{dt} = 64.04 \cdot S_3 - 16.03 \cdot A_3 S_3 - 13.03 \cdot S_4 - 100.11 \cdot N_2 S_4 + 13.21 \cdot S_5$$ **Pyr Act Pool**

$$\frac{dN_2}{dt} = \boxed{- 0.055} + 5.99 \cdot S_2 - 17.94 \cdot N_2 S_2 - 98.82 \cdot N_2 S_4$$ **NADH**

$$\frac{dA_3}{dt} = -1.12 \cdot A_3 - \frac{192.24 \cdot A_3 S_1}{1 + 12.50 \cdot A_3^4} + 124.92 \cdot S_3 + 31.69 \cdot A_3 S_3$$ **ATP**

$$\frac{dS_5}{dt} = 1.23 \cdot S_4 - 2.91 \cdot S_5$$ **$S4_{ext}$**

Microfabricated Multitrap Nanophysimeters (MTNPs) enable dynamic measurements on small populations of cells



Faley,S et al., Lab on a Chip, 8:1700-1712 (2008) & 9(18):2659-2664, 2009.

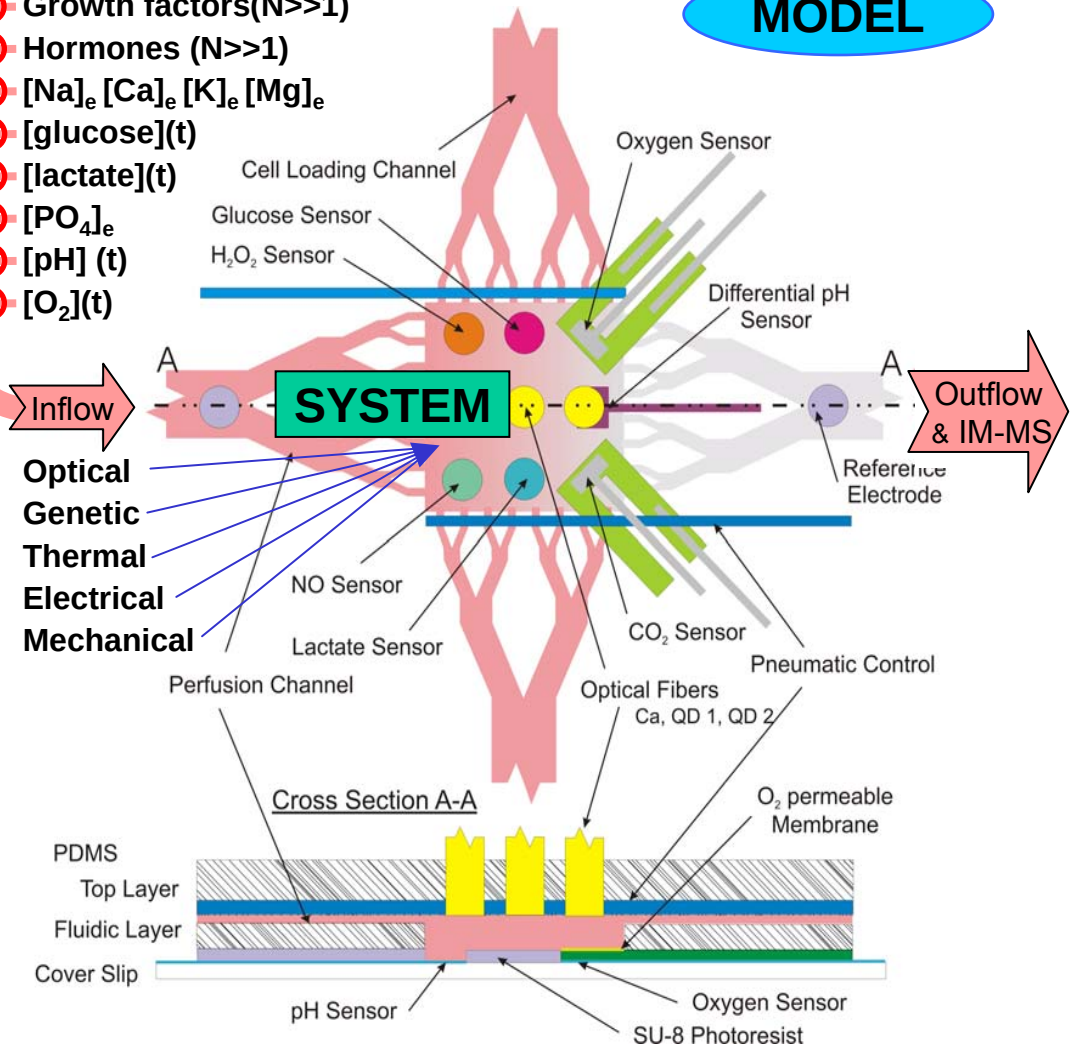
ACTUATORS (Inputs)

CONTROLS

SENSORS (Outputs)

MODEL

- **Base medium**
- **Amino acids (~15)**
- **Cytokines (N>>1)**
- **Growth factors(N>>1)**
- **Hormones (N>>1)**
- **$[Na]_e$ $[Ca]_e$ $[K]_e$ $[Mg]_e$**
- **[glucose](t)**
- **[lactate](t)**
- **$[PO_4]_e$**
- **[pH] (t)**
- **$[O_2](t)$**



Morphology
Size, shape, optical density, motility, division, organelle configuration

Force
Shear, tension, deformation

Intracellular Signaling (Optical)
GFP/luciferase reporters, $[Ca]_i$, pH_i, V_m, MMP, GFP FRET

Extracellular Electrolytes (Electrochemical)
$[Na]_e$, $[Ca]_e$, $[K]_e$, $[Mg]_e$, $[PO_4]_e$, $[Cl]_e$, $[HCO_3]_e$

Neurotransmitters (Electrochemical)
Seratonin, acetocholine, GABA, …

Extracellular Metabolites (Electrochemical)
[glucose](t), [lactate](t), [pH](t), $[O_2](t)$, NO(t), $H_2O_2(t)$ …

Extracellular Metabolites (GC IM-MS)
Amino acids, small metabolites, stable isotopic markers

Surface Expression
Specific affinity probes

Soluble Gene Expression (nESI IM-MS)
Cytokines, growth factors
hormones, enzymes

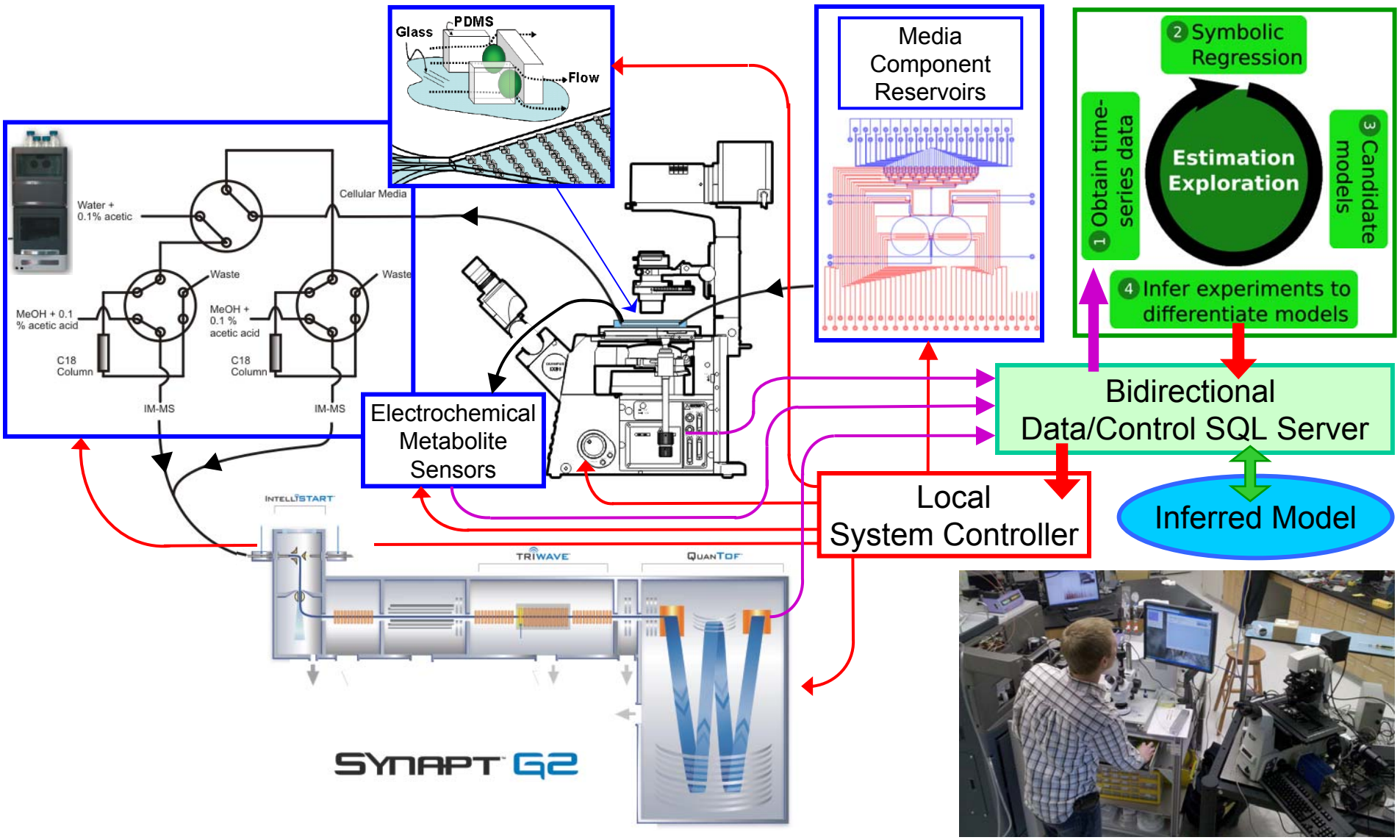
Cytosolic Proteins (MALDI IM-MS)

Lipids (Cell Lysate IM-MS)

Gene expression (mRNA Arrays)

. . .

Our Robot Scientist: VIIBRE Automated Omni-Omics



The End

How hard a problem is cancer?

Just how hard is a hard problem?

What is your favorite REALLY HARD problem?

- What is the nature of dark energy?
- What is the chemistry of interstellar space?
- What occurred at the origin of life?
- How does the brain work?
- Can we create life *de novo*?
- Can we save the planet from its human infestation?
- **...**
- How do describe fully the spatiotemporal multiscale complexity of a biological system?
- …
- How do you cure breast cancer?
- How do you control breast cancer?
- How do you prevent breast cancer?

A Really Hard Problem

If the human brain were so simple
that we could understand it,
we would be so simple
that we couldn't.

Emerson M. Pugh, 1938

A Really Hard Problem

The same observation applies to biology:

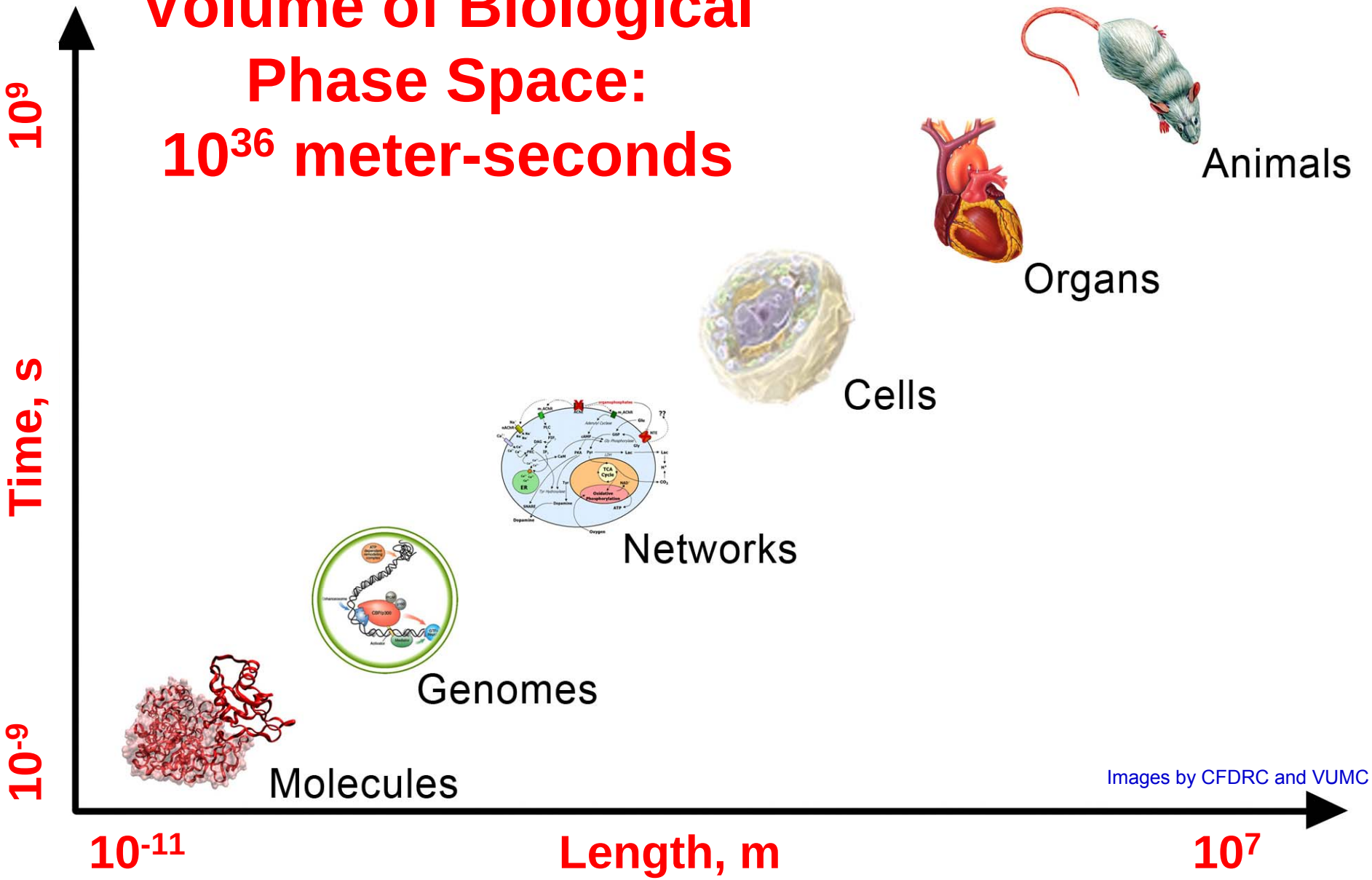
Human biology is too complicated for humans to fully comprehend.

John Wikswo

VIBRE

So just how hard are biological problems?

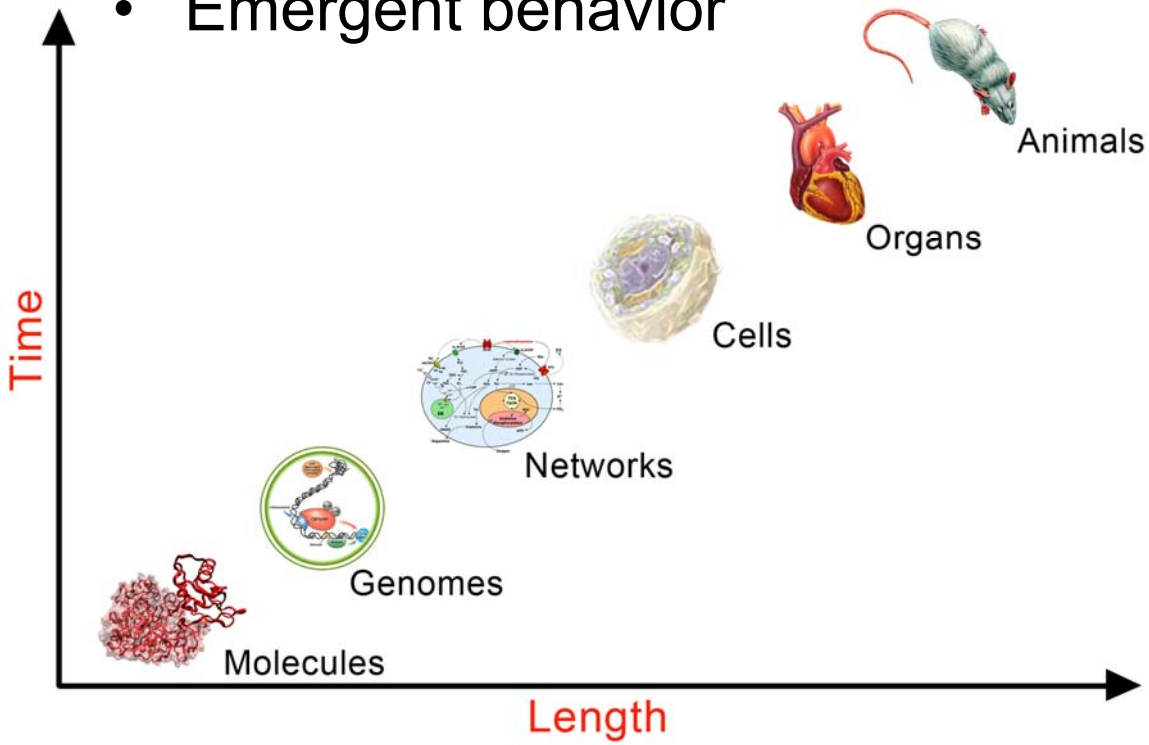
Volume of Biological Phase Space: 10^{36} meter-seconds

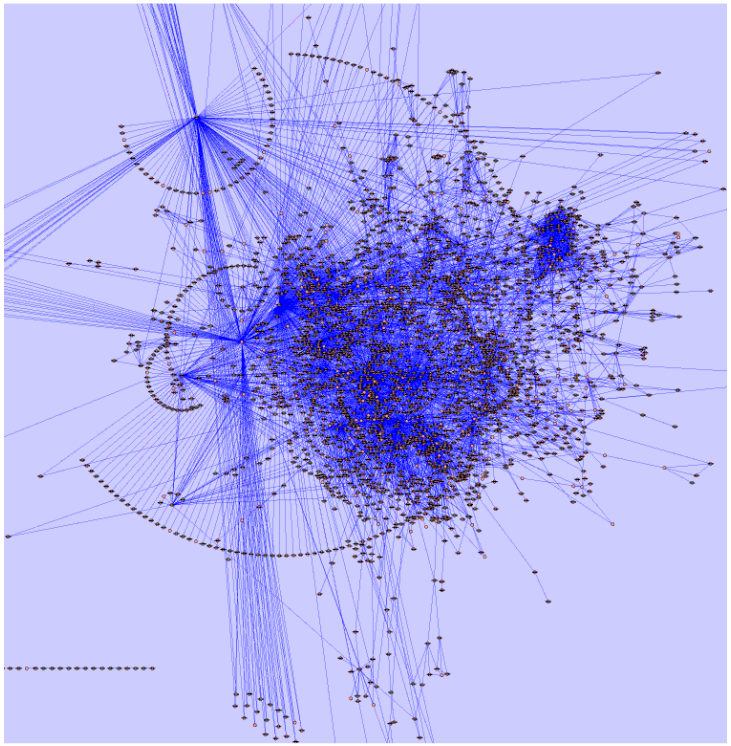


Images by CFDRC and VUMC

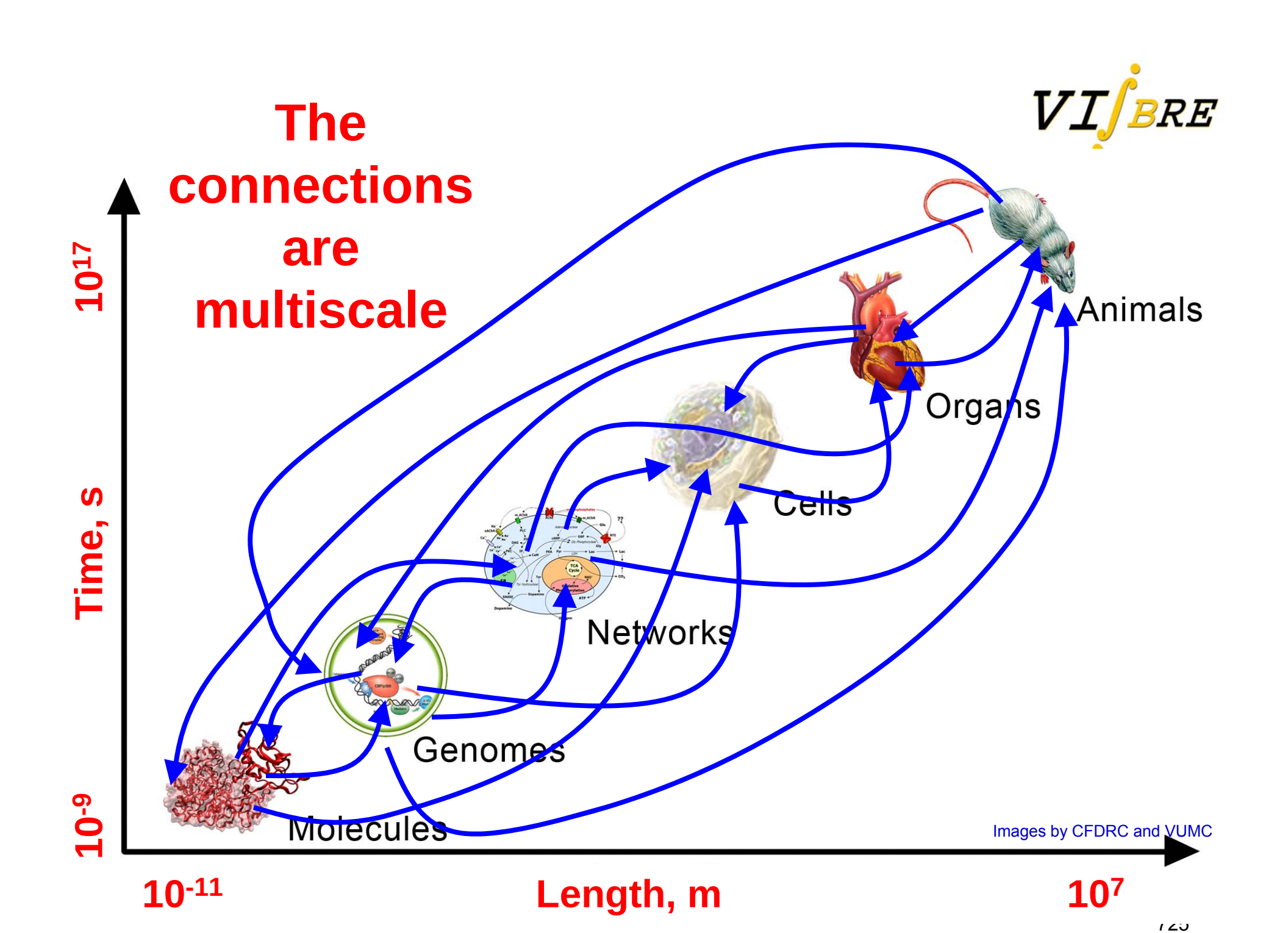
Multiscaling makes biological problems hard

- Spatial extent
  - Number of interfaces
- Temporal extent
- Number of molecular species
- Complexity of interactions
- Emergent behavior





Yeast Interactome


The connections are multiscale
VIBRE
Animals
Organs
Cells
Networks
Genomes
Molecules
Time, s
10^{17}
10^{-9}
10^{-11}
Length, m
10^{7}
Images by CFDRC and VUMC

Multiscaling makes cancer a very hard problem

Lymphatic metastasis is a major pathway for tumor dissemination

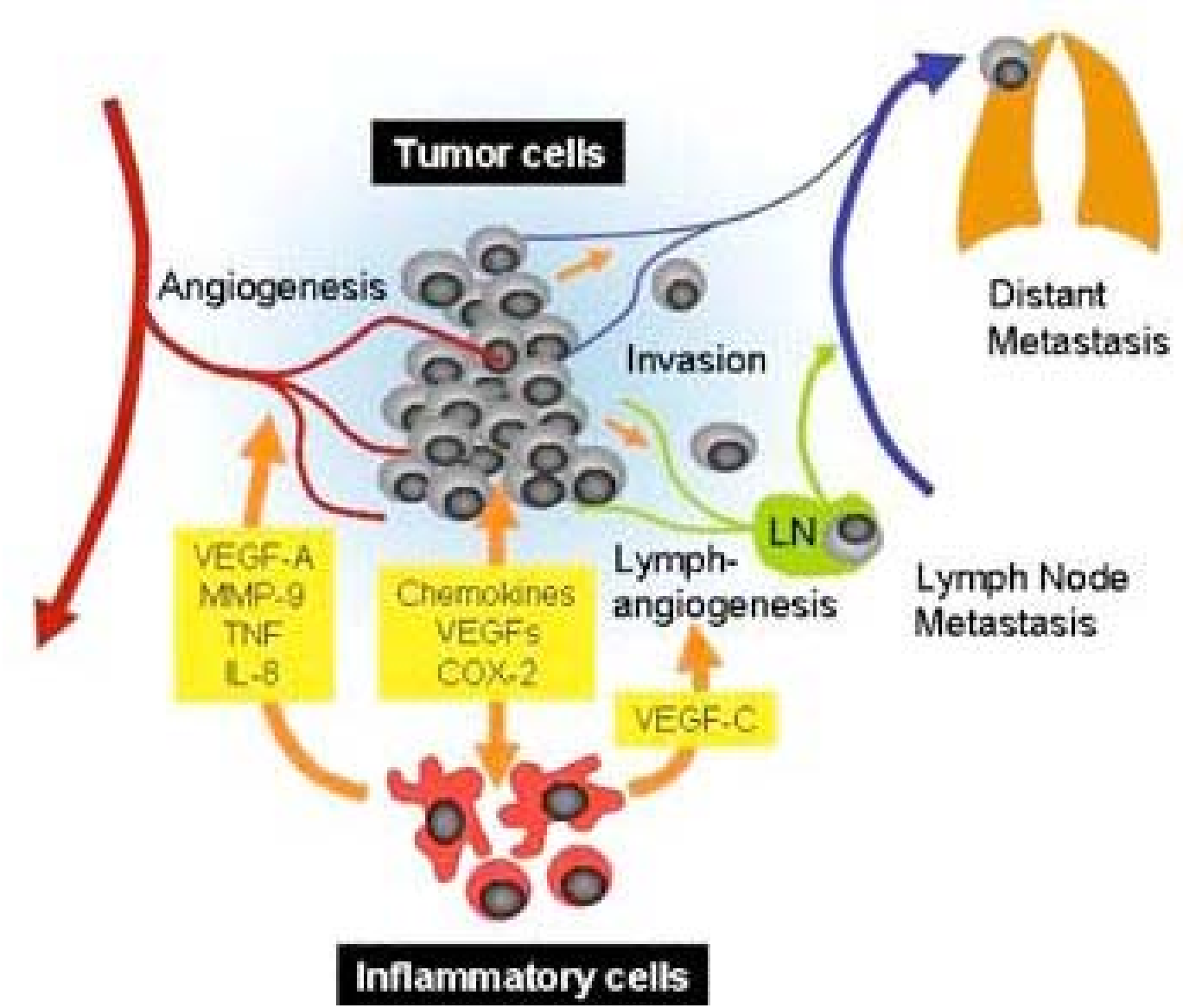


Image taken from: www.nccroncology.ch/scripts/index.aspx?idd=110.

3D microenvironment provides numerous biophysical cues

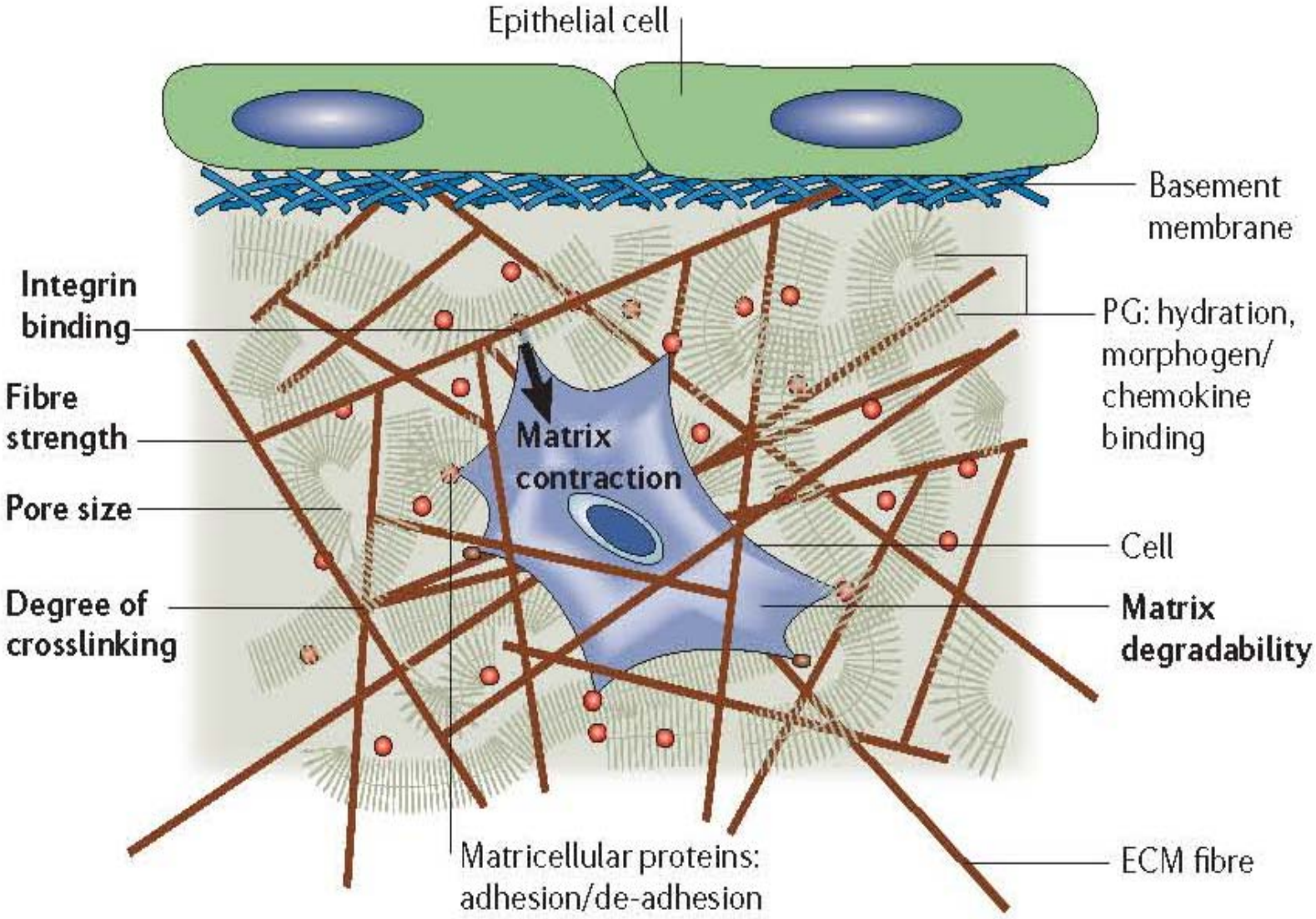
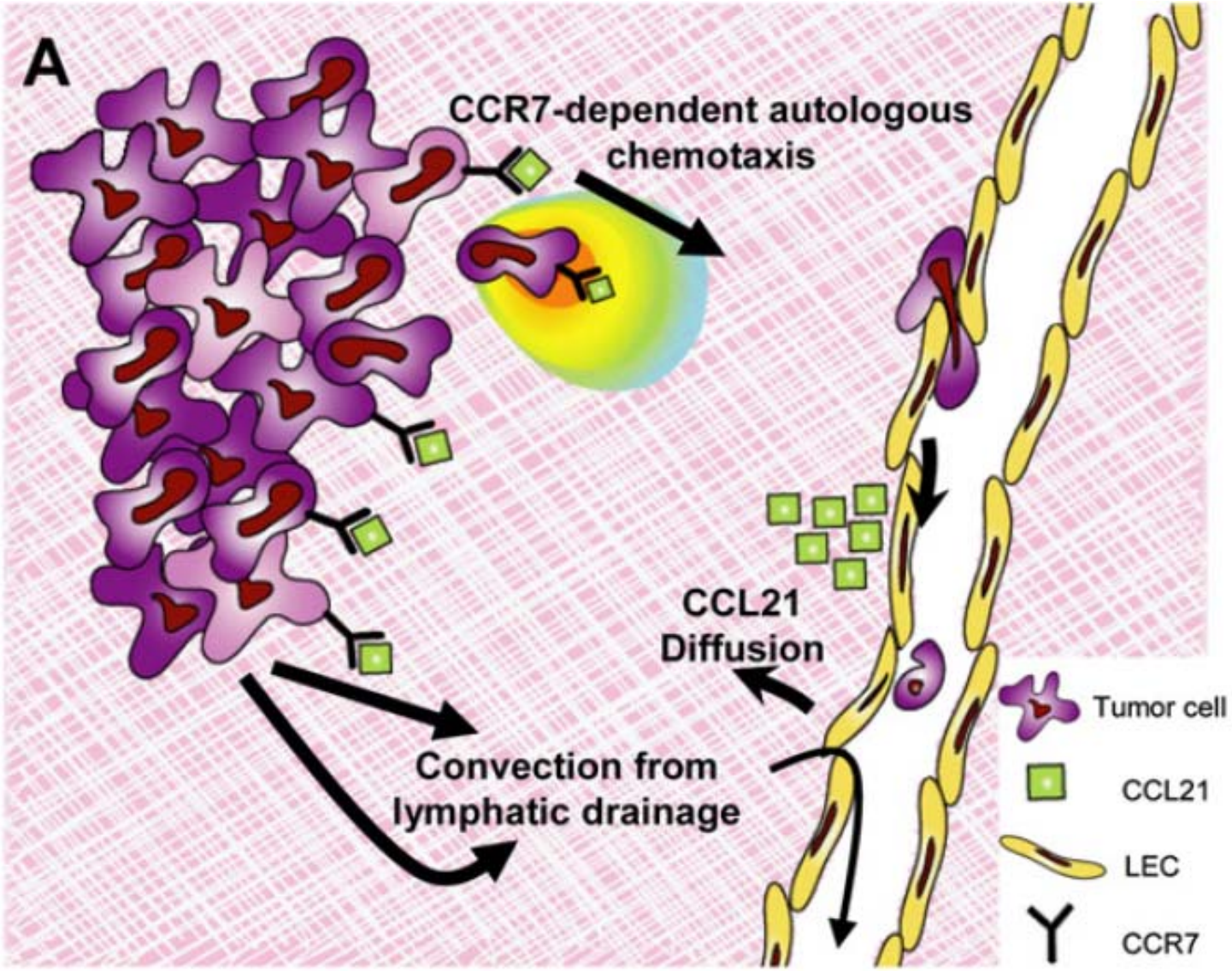


Image taken from: Griffith L.G. et al., *Nature Molecular cell Biology Review,* Vol.7 (2006)

Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics

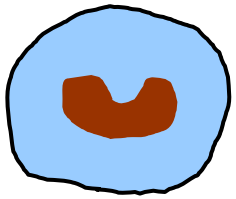


Shields, J.D., et al. *Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling.* Cancer Cell, 2007. **11**(6): p. 526-538

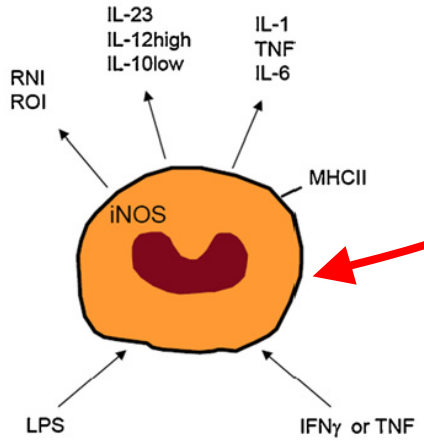
Monocytes into M_1 or M_2 Macrophages

In the presence of
- Interferon (IFN)
- Lipopolysaccharide (LPS)
- Other microbial products

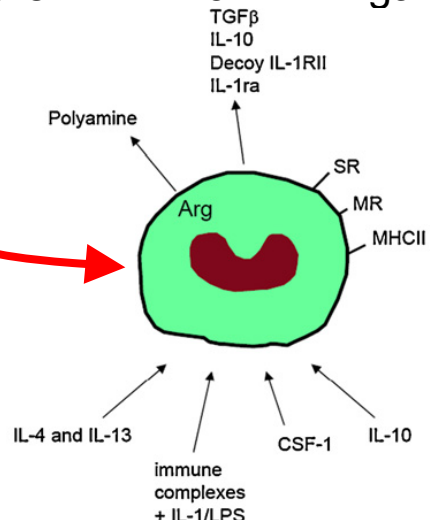
M



In the presence of
- Macrophage colony stimulating factor (CSF-1)
- Interleukin (IL)-4, IL-13, IL-10
- Immunocomplexes in association with either IL-1R- or TLR-ligands



M1



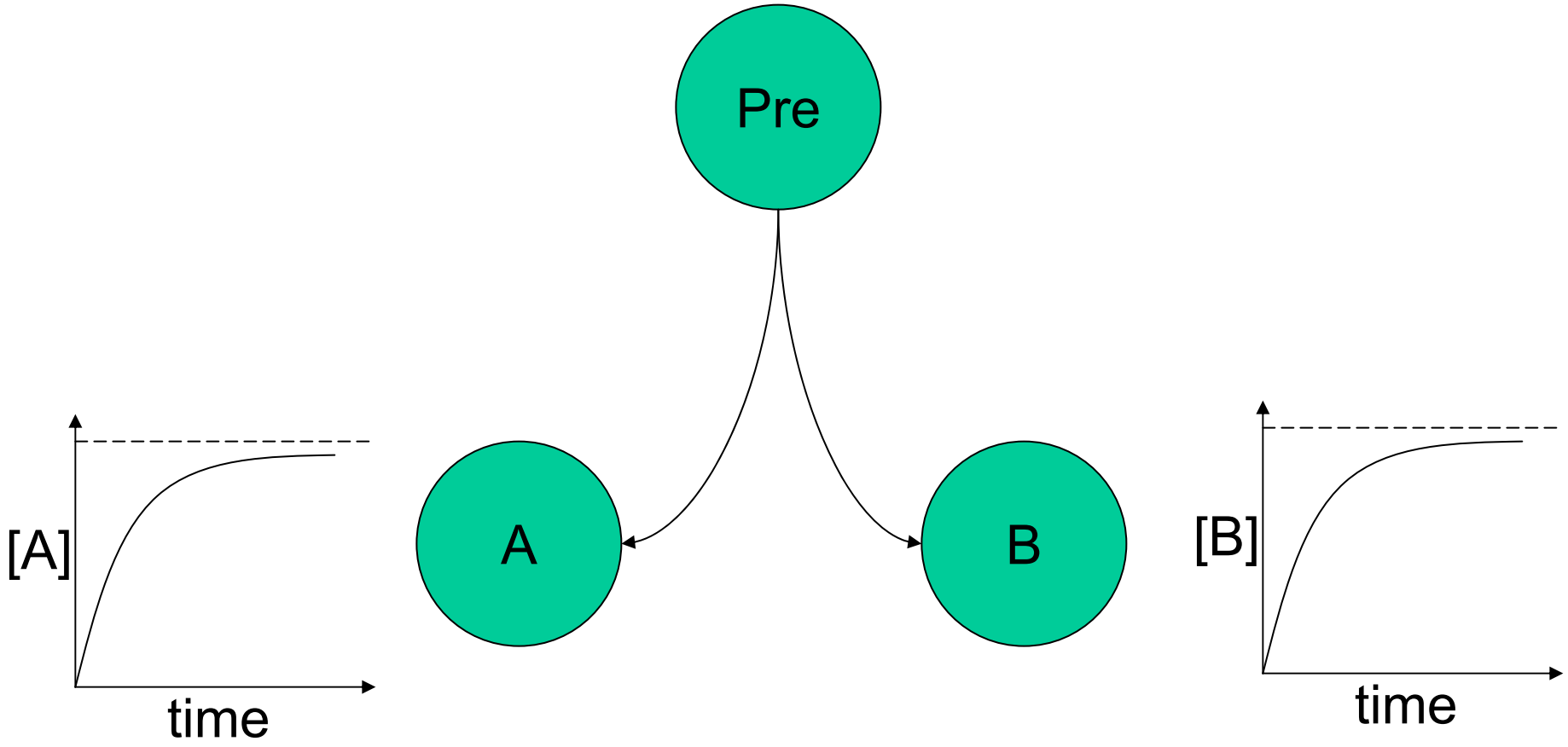
M2

- High microbicidal activity
- Kill intracellular parasites
- Immuno-stimulatory functions
- Tumor cytotoxicity

- High scavenging ability
- Kill and encapsulate parasites
- Promote tissue repair and angiogenesis by matrix repair and remodeling
- Favor tumor progression.

NAKFI Complexity 2008: Amy Bauer, Chen Hou, Wolfgang Losert, Roger Narayan, Leor Weinberger, John Wikswo, Lani Wu, Mingjun Zhang, Hadley Leggett

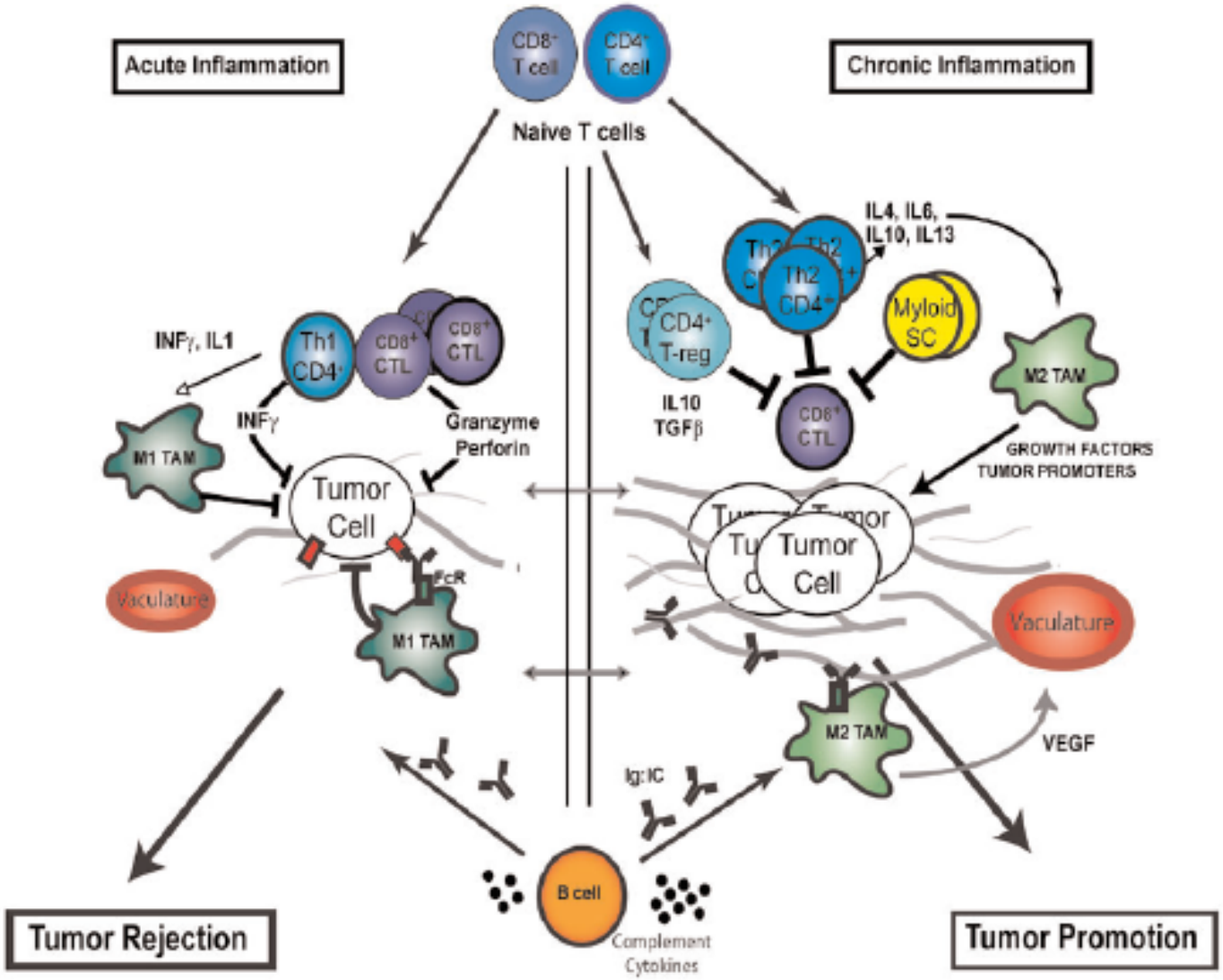
The Yogi Berra problem generalized: Control the A/B population



When a cell comes to a fork in the road, it takes it.

It is in fact a bit more complicated…

M1



M2

Breast Cancer Research Vol 9 No 4 DeNardo and Coussens

Cell differentiation
Rolling down the epigenetic landscape

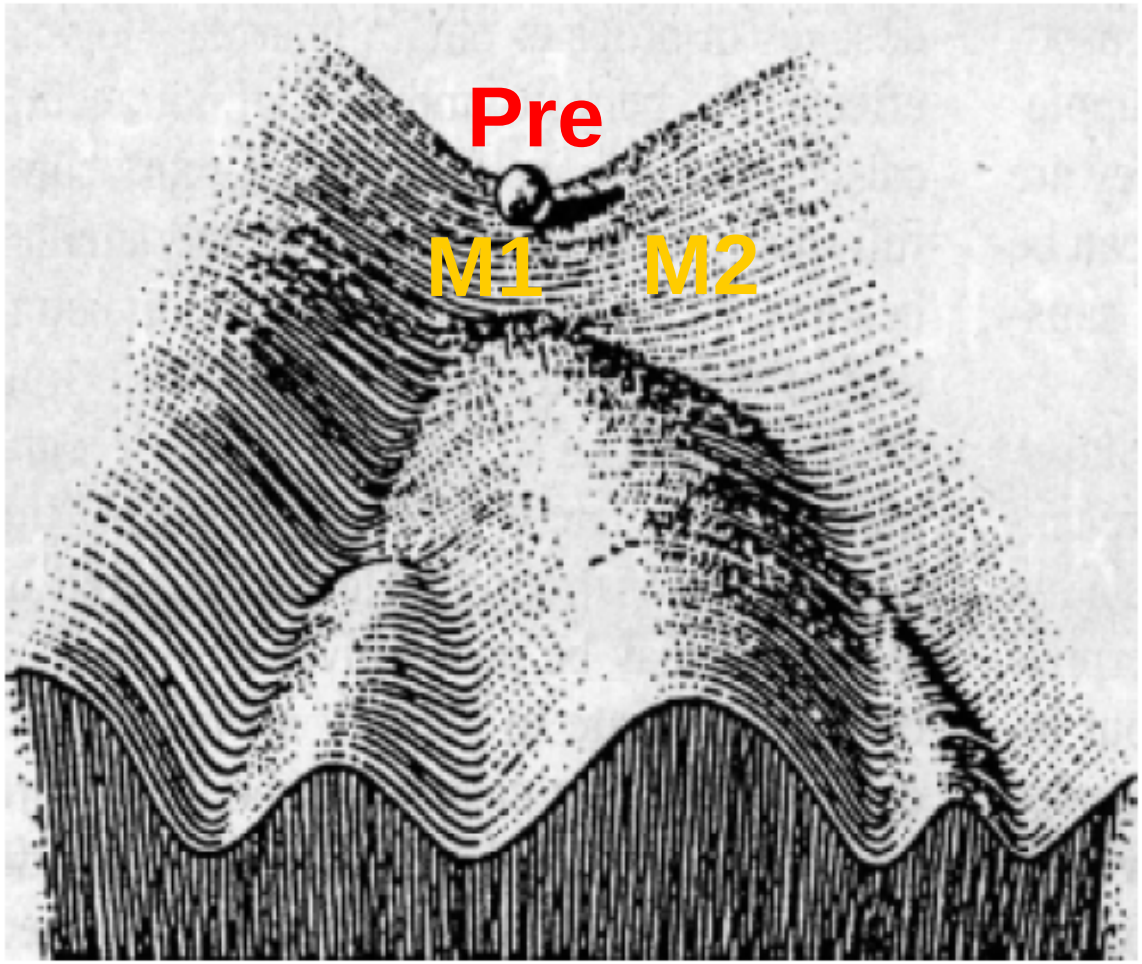
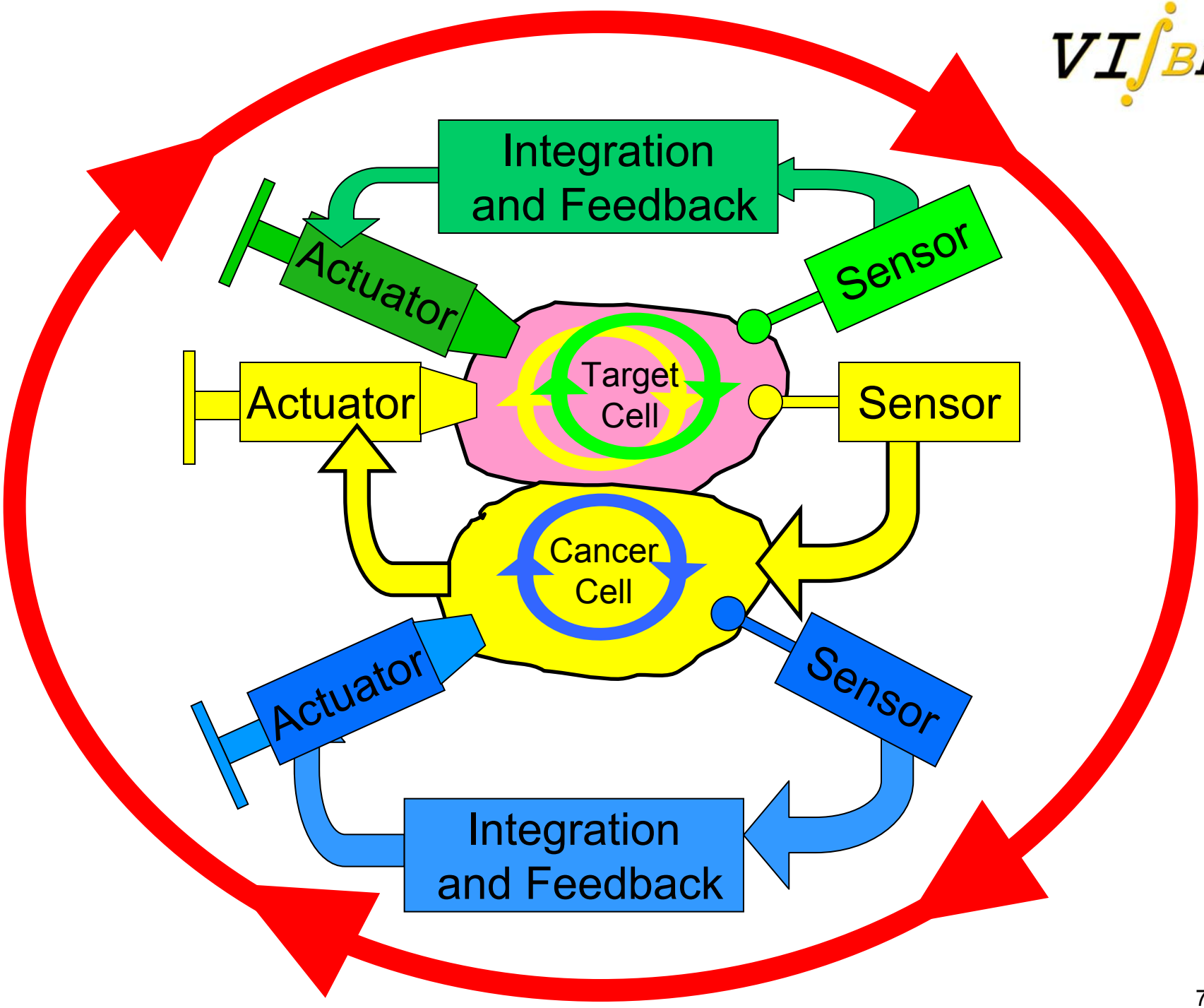


Fig. 3. Waddington's epigenetic landscape. Reproduced from C.F. Waddington, 1957 [64]. We postulate here that the metaphoric epigenetic landscape corresponds to the attractor landscape (Fig. 2) that can be reduced to the dynamics of a gene regulatory network.


Integration
and Feedback
Actuator
Sensor
Actuator
Target
Cell
Sensor
Cancer
Cell
Actuator
Sensor
Integration
and Feedback

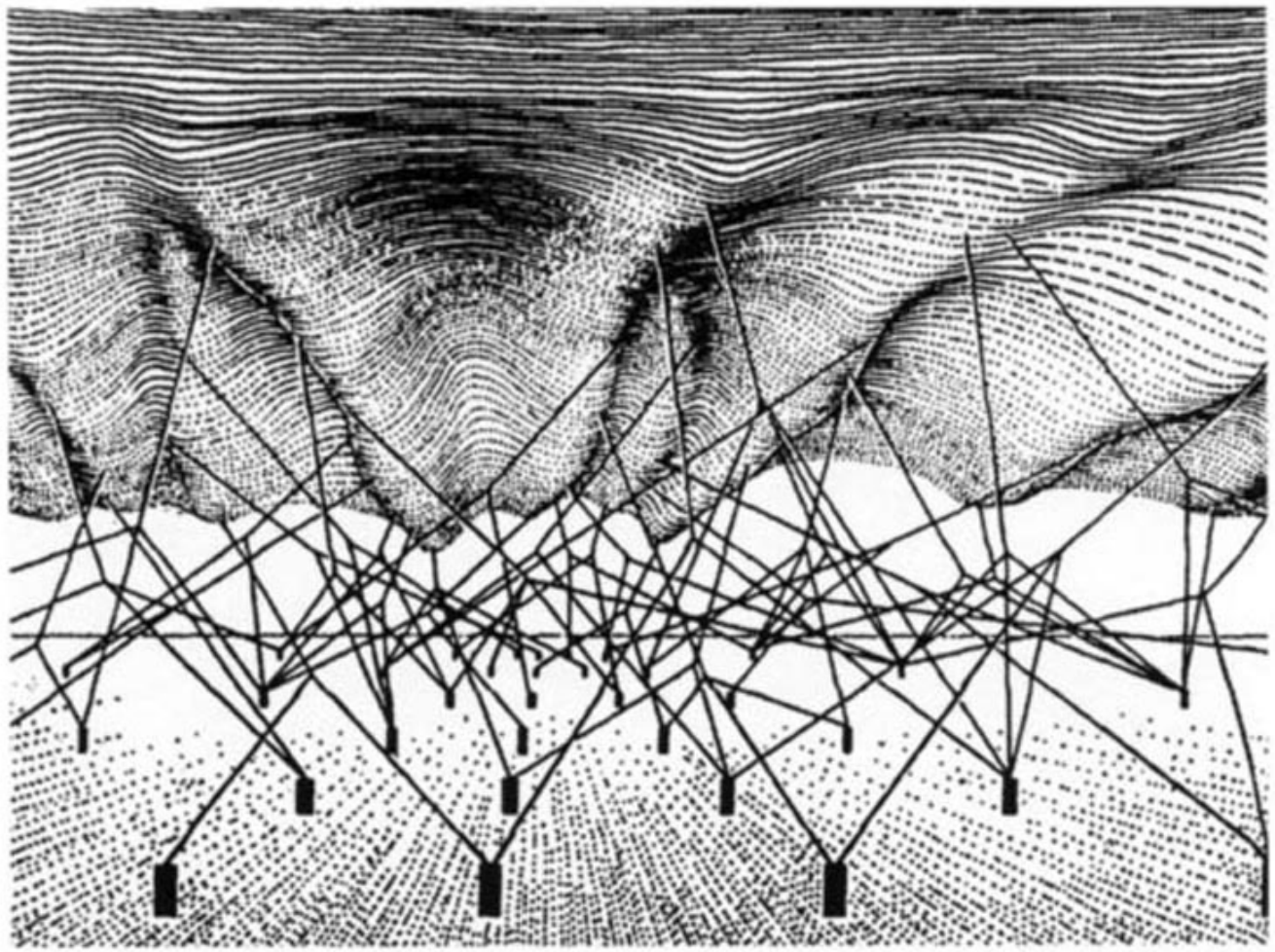
A Complex problem

- Suppose the disease is an emergent phenomenon…

  the collective effect of multiple mutations and extensive gene regulatory changes in an ensemble of cells leading to a new dynamic (dis)equilibrium state
  - Type II diabetes
  - Lupus
  - Schizophrenia
  - Most cancers

- And God said
  - Let it be cured by distributed hybrid multiscale non-linear stochastic control

The epigenetic landscape reflects complex and dynamic genetic control

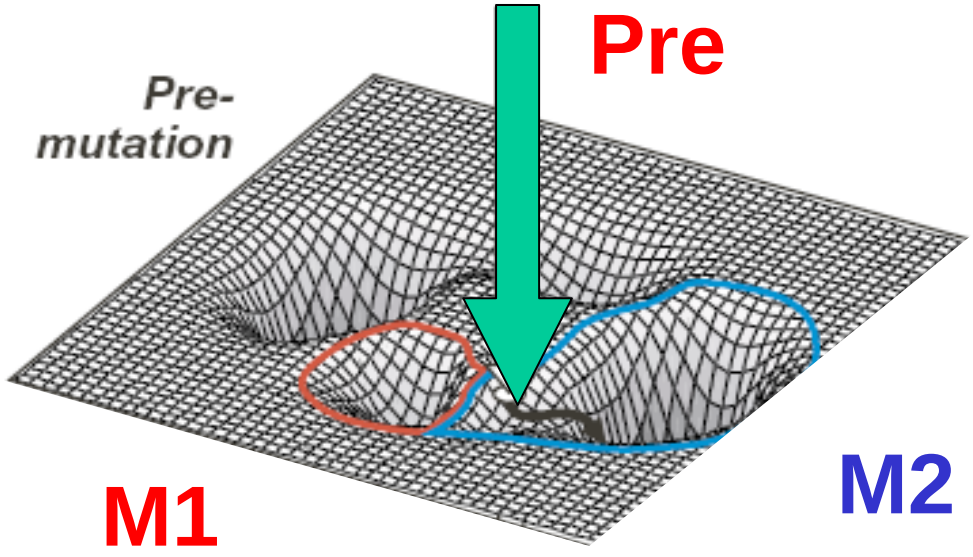


Nonequilibrium thermodynamics allows uphill motions

Waddington, 1957

Shift the Epigenetic Landscape to Control Cell Fate

S. Huang and D.E. Ingber / A Non-Genetic Basis for Cancer Progression and Metastasis



Shift the Epigenetic Landscape to Control Cell Fate

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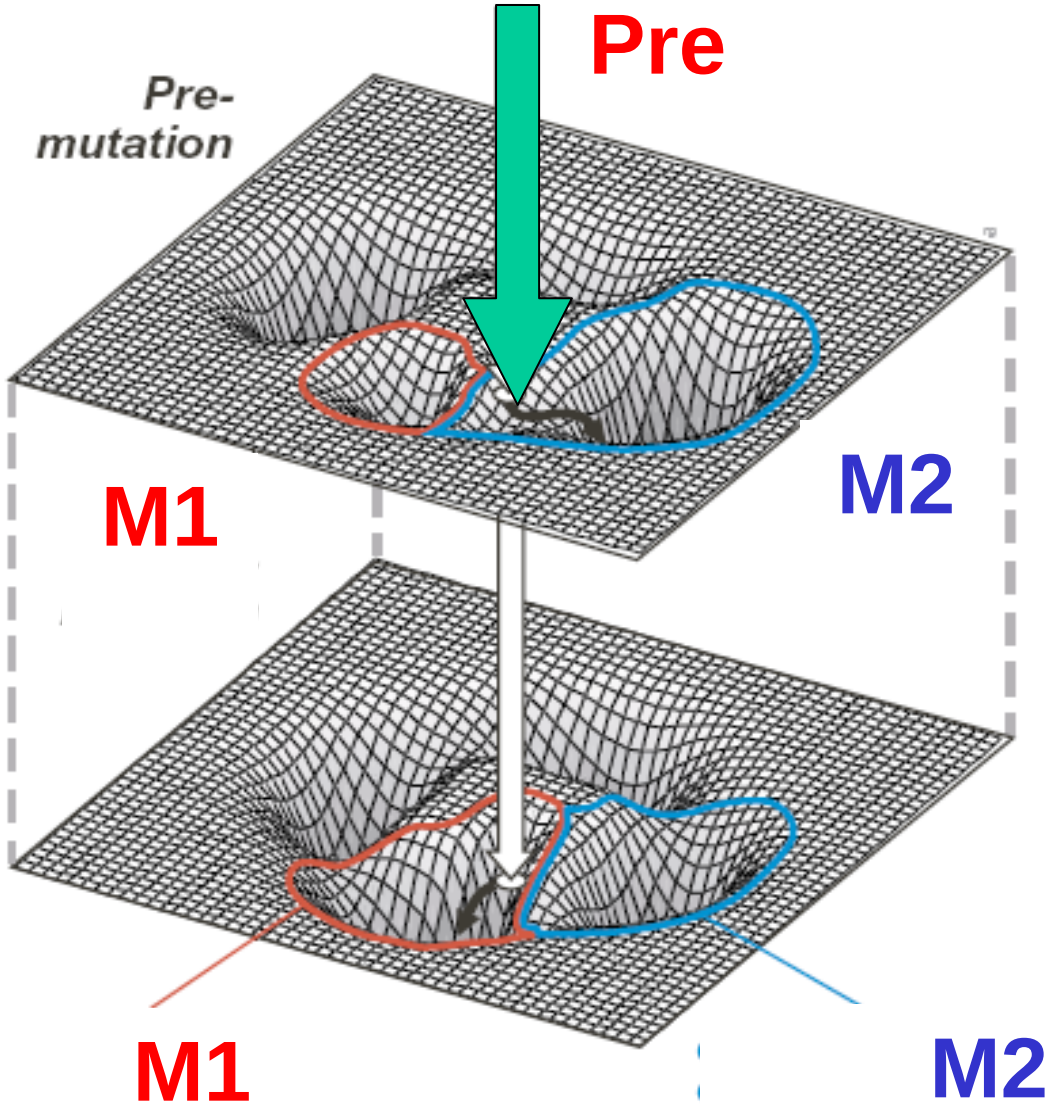
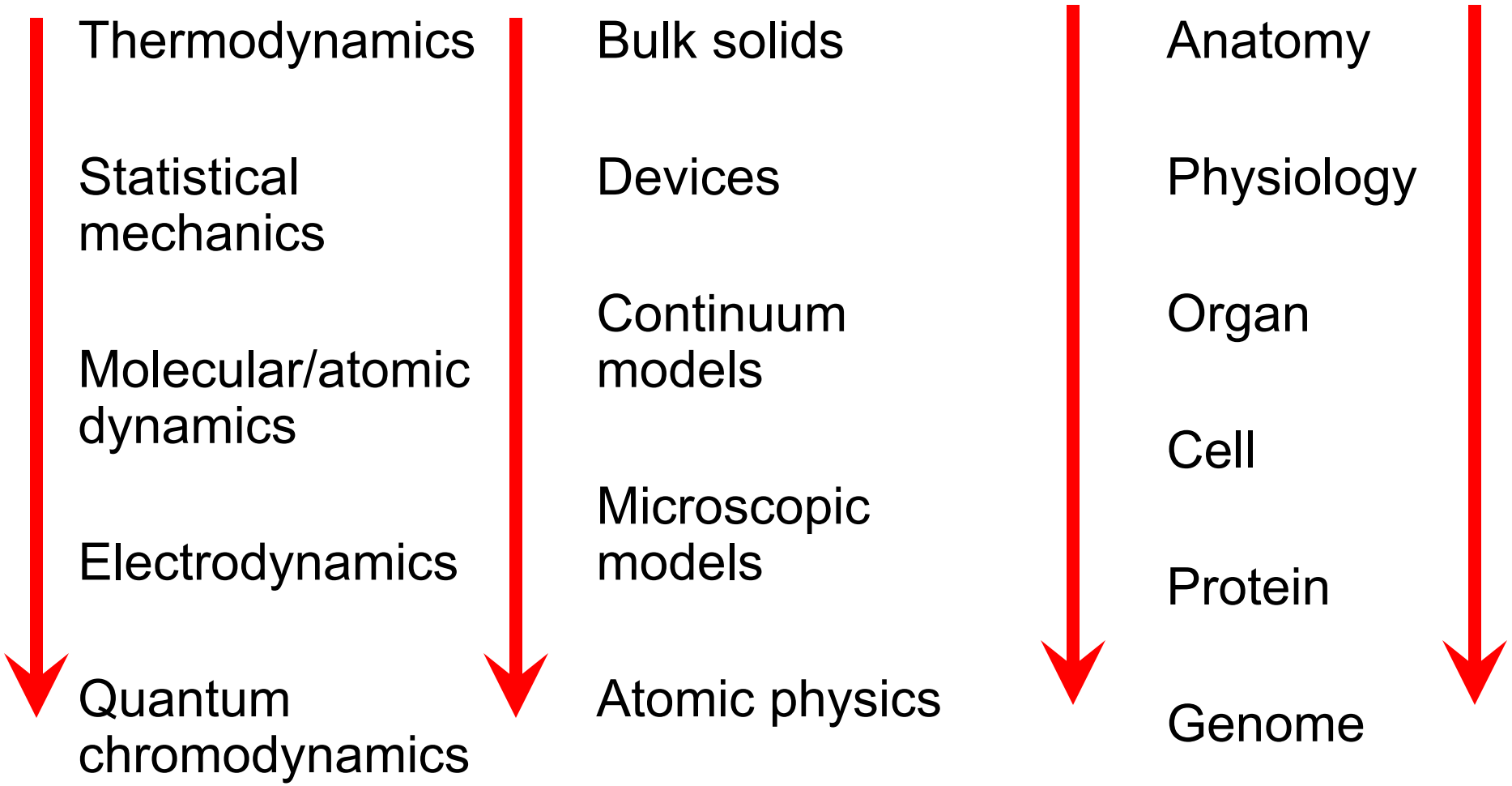
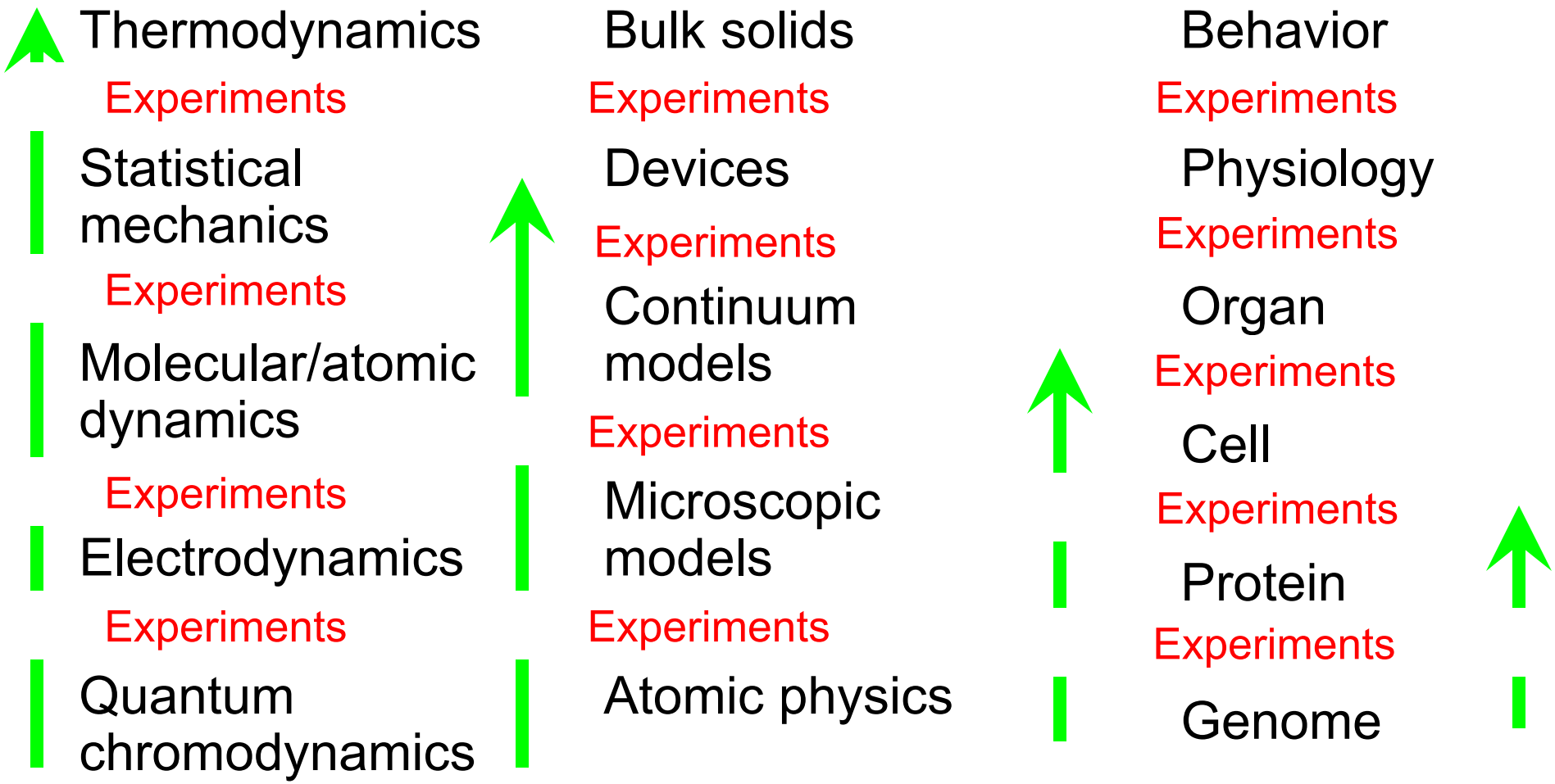


Can reductionist science solve the problem?

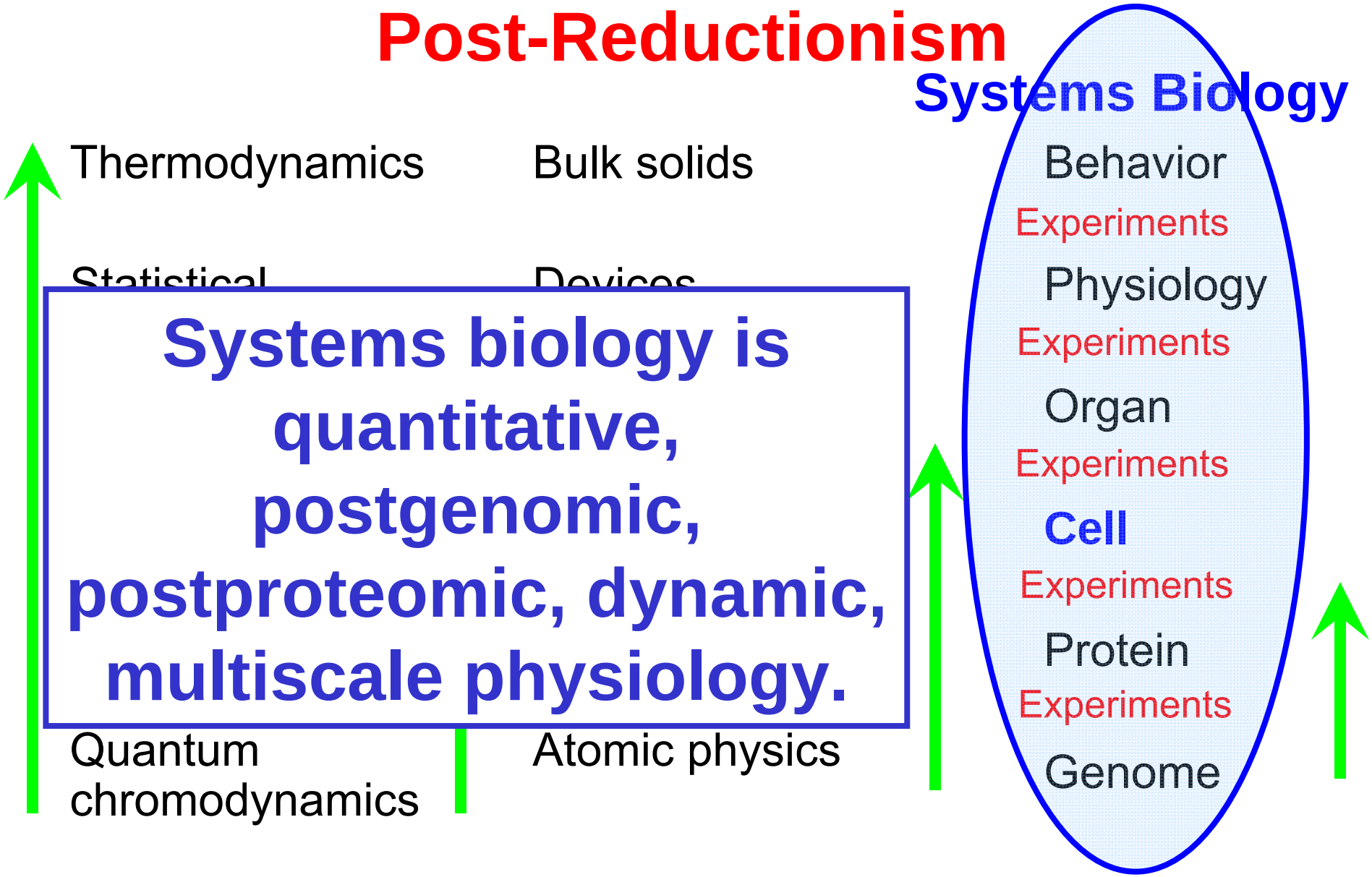
Step 1 in Science:
Reductionist Explanations



Step 2 in Science:
Post-Reductionist Theory



Step 2 in Science:
Post-Reductionism

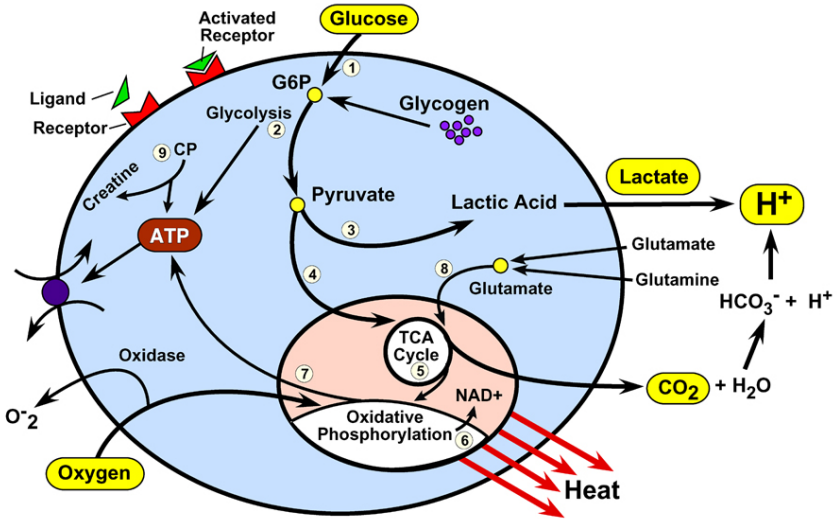


A Really Hard Problem: Metabolic and Signaling Kinetics in a Multiscale Environment

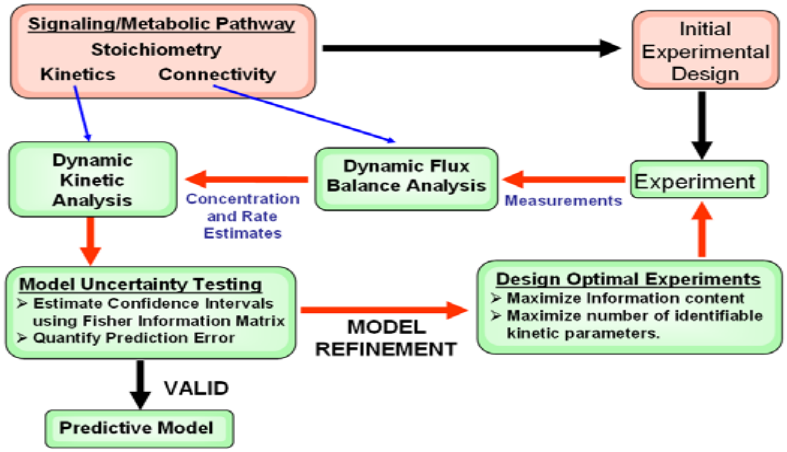
- Question:
  - How do we describe and interpret biological complexity over multiple spatiotemporal scales?
- The standard solution:
  - Genomics, proteomics, metabolomics arrays
  - Reductionist analysis of components
  - Mathematical modeling….

The Models

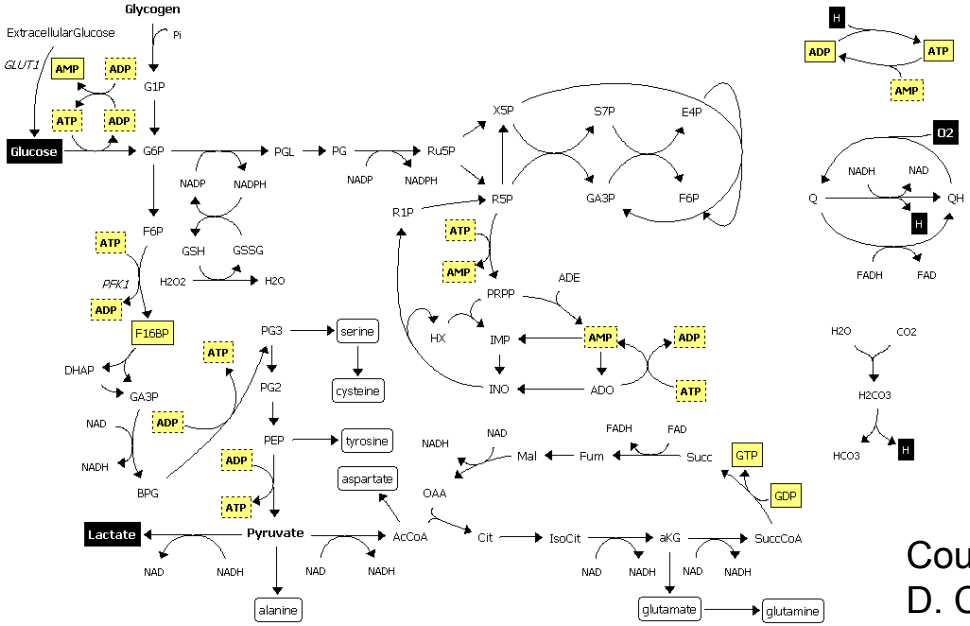
Effective Models



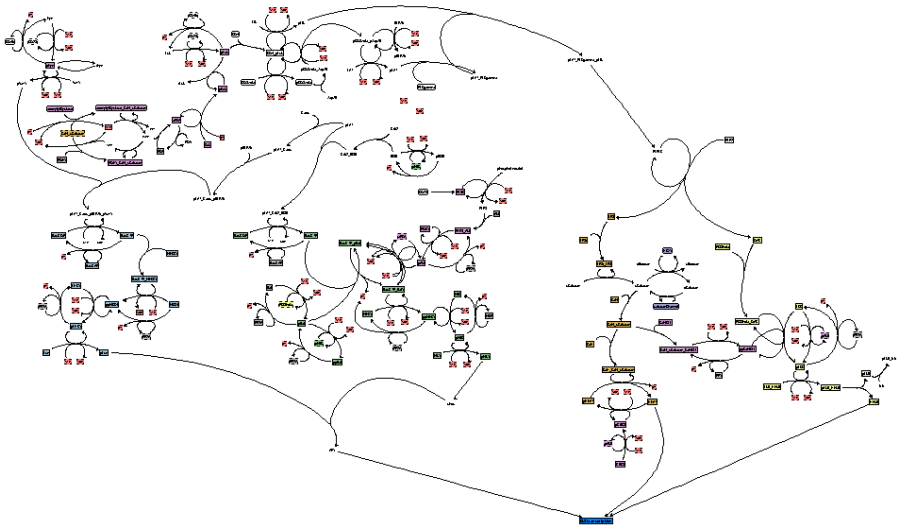
Algorithmic Framework



Central Carbon Metabolism

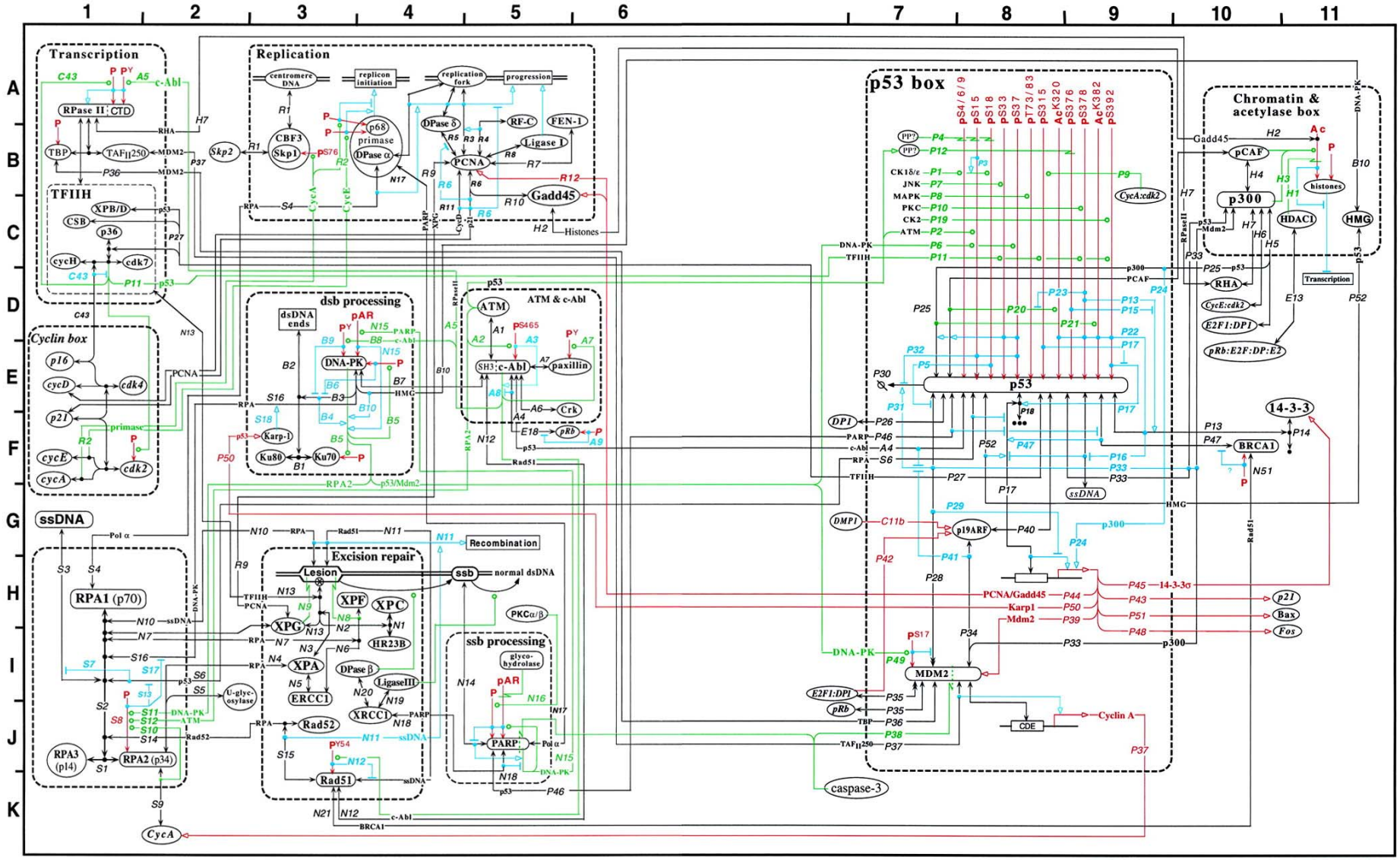


T-cell Signaling



Courtesy of S. Sundaram and Jerry Jenkins, CFDRC, D. Cliffel, Vanderbilt

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)

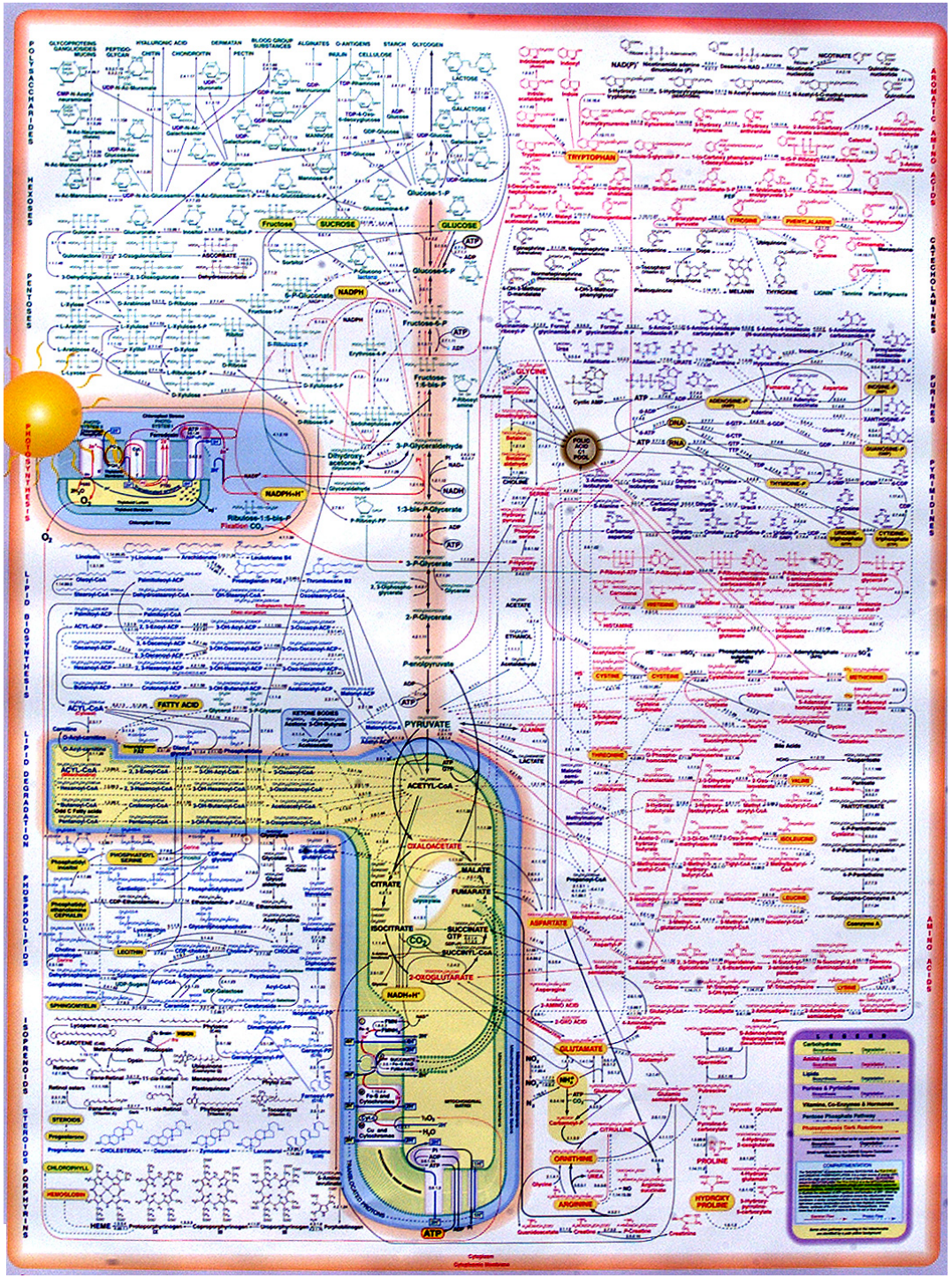
How Many Bits?

- 1 bit = Boolean Logic
- 2 bits = Bialekan Logic
- …
- N bits = Bio Logic

How big is N?

'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

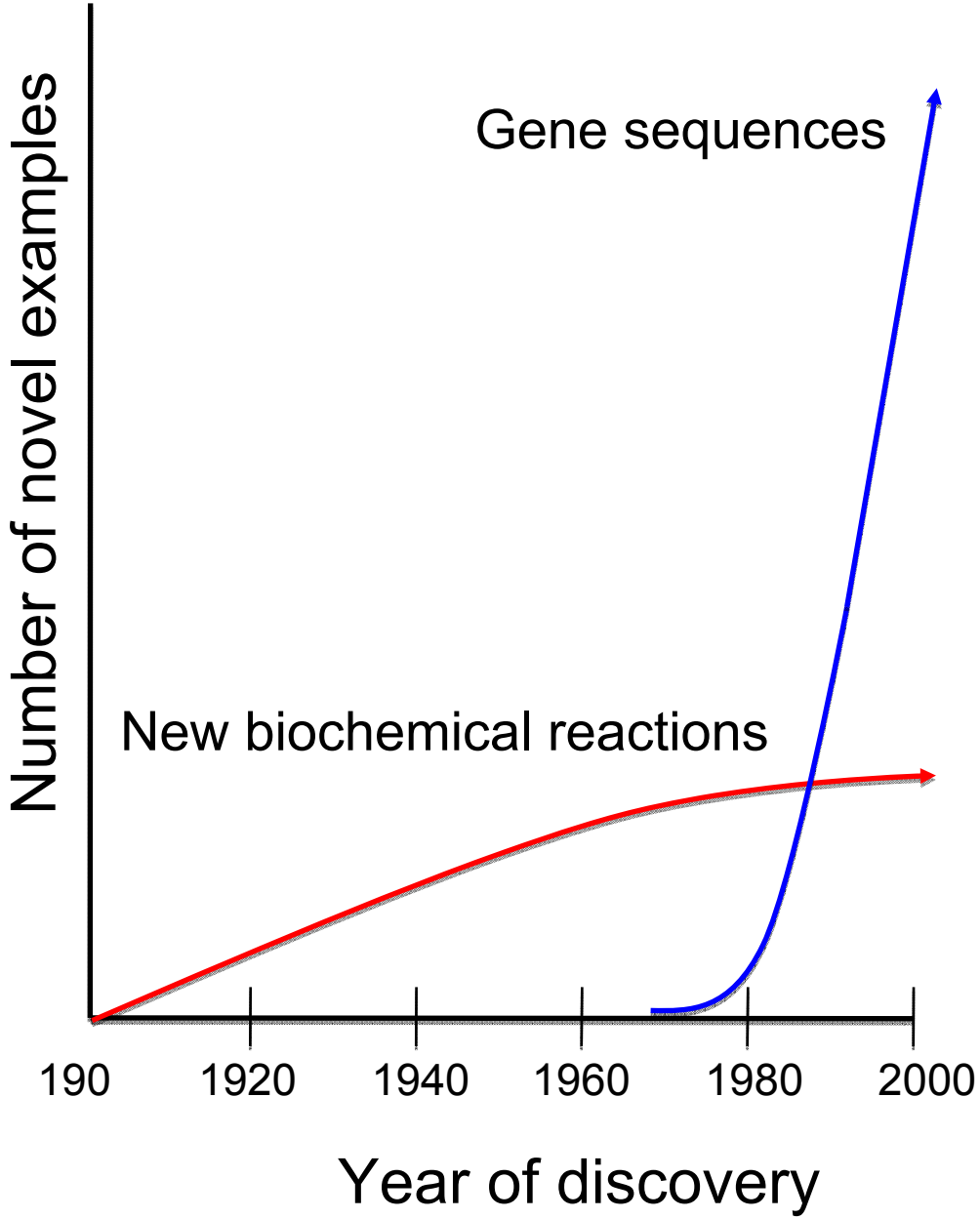
- Modeling of a single mammalian cell may require >100,000 *dynamic* variables and equations, maybe > 1,000,000
- Cell-cell interactions are critical to system function
- 10^9 - 10^{11} interacting cells in some organs
- Cell signaling involves highly *DYNAMIC* biochemical cascades with positive and negative feedback
- Multiple, overlapping regulatory mechanisms
- Many of the interactions are nonlinear
- Models might have a Leibnitz (1 L = N_a) of PDEs
- **The data don't yet exist to drive the models …**

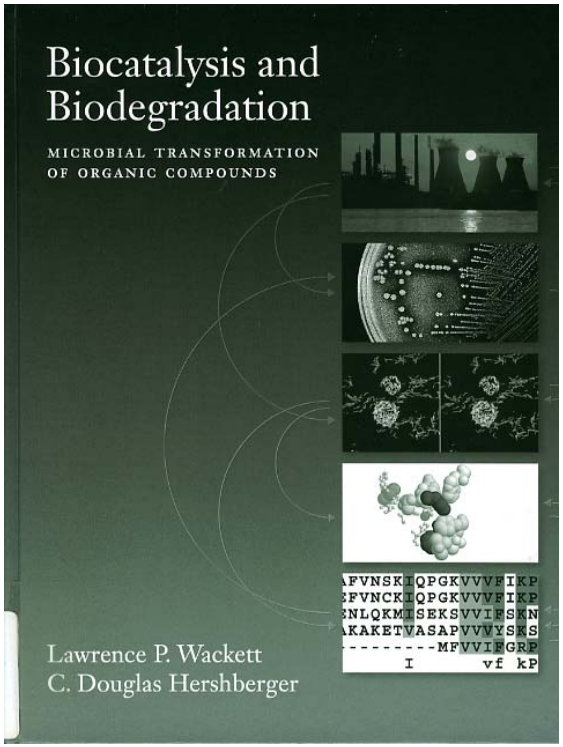
It’s the numbers….

Where do we get a mole of numbers?

The Practical Problems

- Our understanding of biological phenomena is <u>often</u> 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, one hopes, held constant, or
  - recordings of one *rapid* 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.
  - costly measurements of the expression of 12,600 genes (Can you afford to read mRNA every 30 minutes for 7 days from multiple cell cultures?)
- Even though we suffer from an explosion of qualitative genomic expression data, we don't have an adequate quantification of expressed protein concentrations or the underlying biochemical reactions they enable.





Functional annotation is not keeping pace with gene discovery

Slide courtesy of Jim Spain

Dennis Bray understands the problem….

- “The past few decades have seen such an explosion of knowledge about the contents of living cells that we now swim in an ocean of data.”

- “How can we come to terms intellectually with such an enormous number of interacting entities?”

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

A possible failure mode

Ontological failure: The phenomenon you are interested in requires elements or laws outside of the set you have been given.

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

The solution to ontological failure

Get more data…

The Catch

- Modeling of a single mammalian cell may require >100,000 *dynamic* variables and equations, maybe > 1,000,000
- Cell-cell interactions are critical to system function
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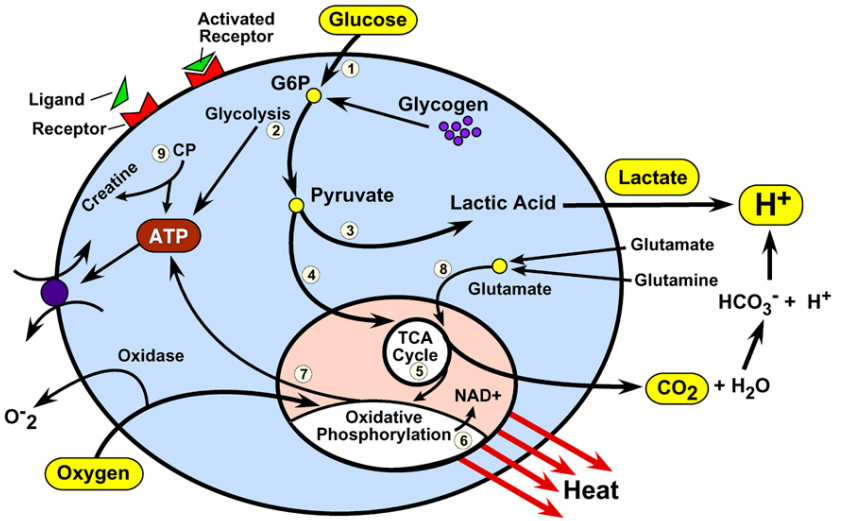
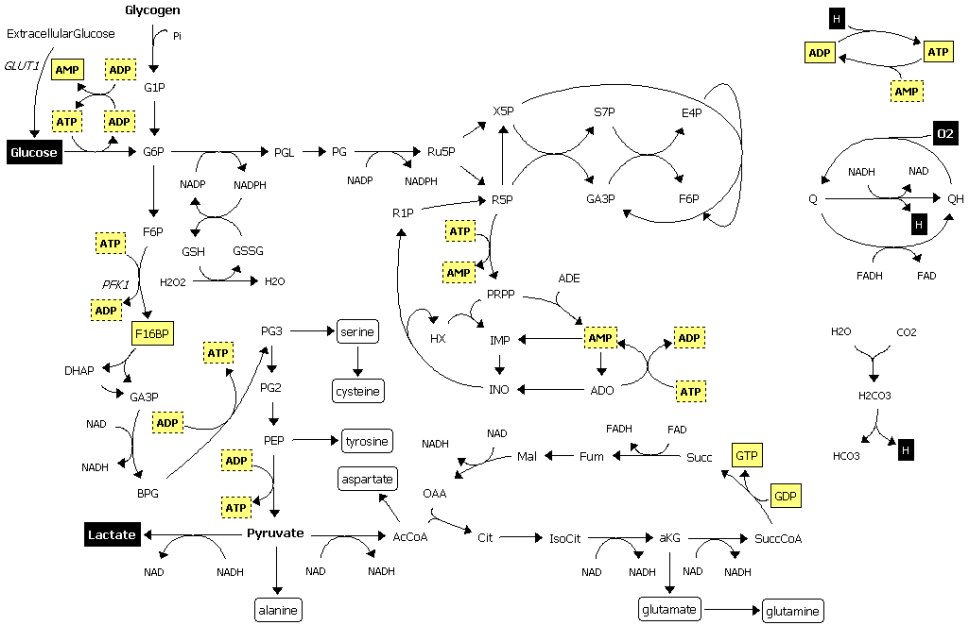
- Cell signaling involves highly *DYNAMIC* biochemical cascades with positive and negative feedback
- Multiple, overlapping regulatory mechanisms
- Many of the interactions are nonlinear
- Models might have a Leibnitz (1 L = N_a) of PDEs
- **The data don't yet exist to drive the models**
- Hence we need to **experiment…**

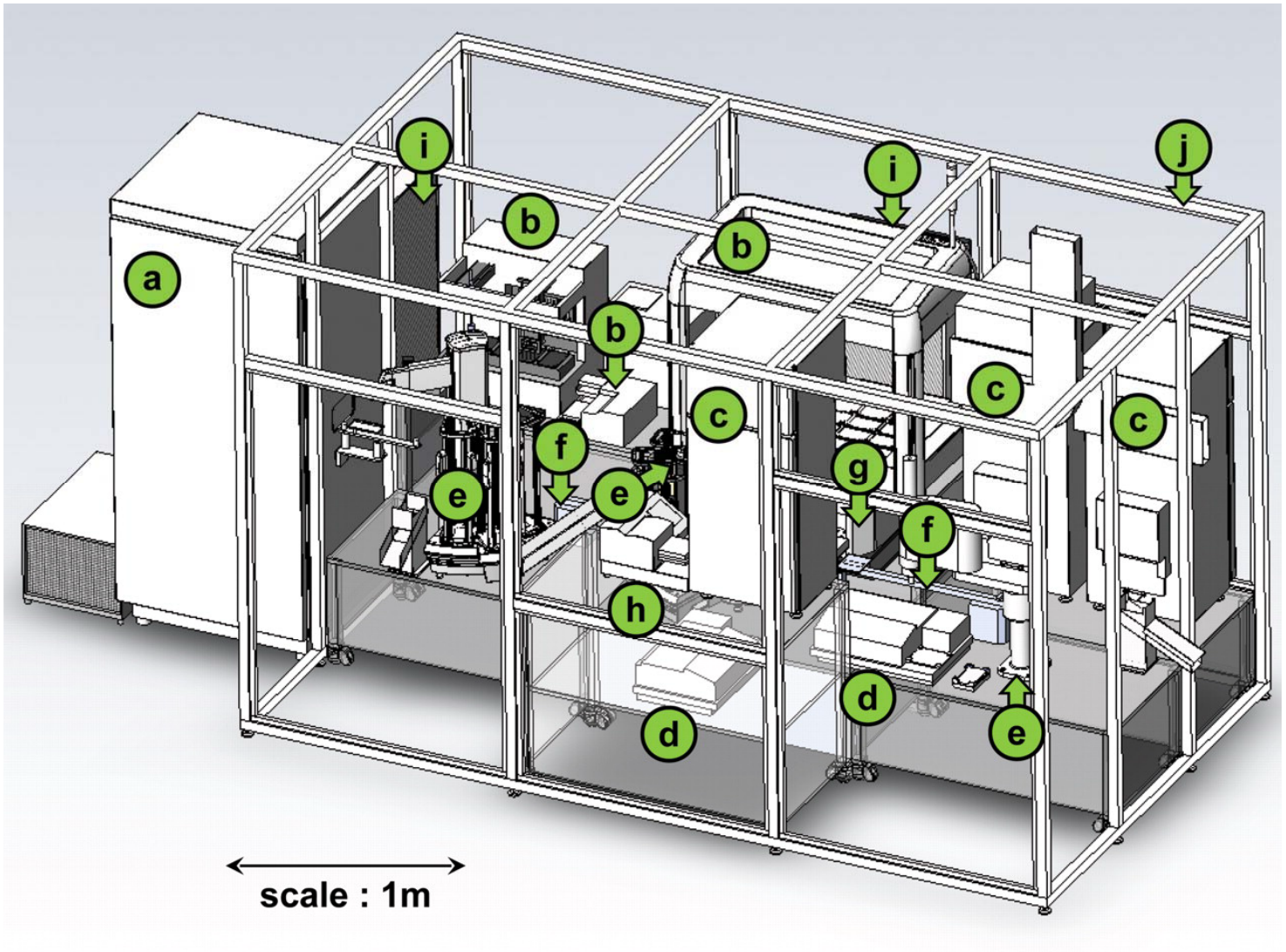
Grand Challenge

- Design and build a hybrid silicon/biological system that proposes and generates models and conducts experiments on itself to identify the underlying equations that govern the biology.
- Extracellular: $3 - 4 million and 3 - 5 years
- Intracellular: $15 - 20 million and 5 – 10 years





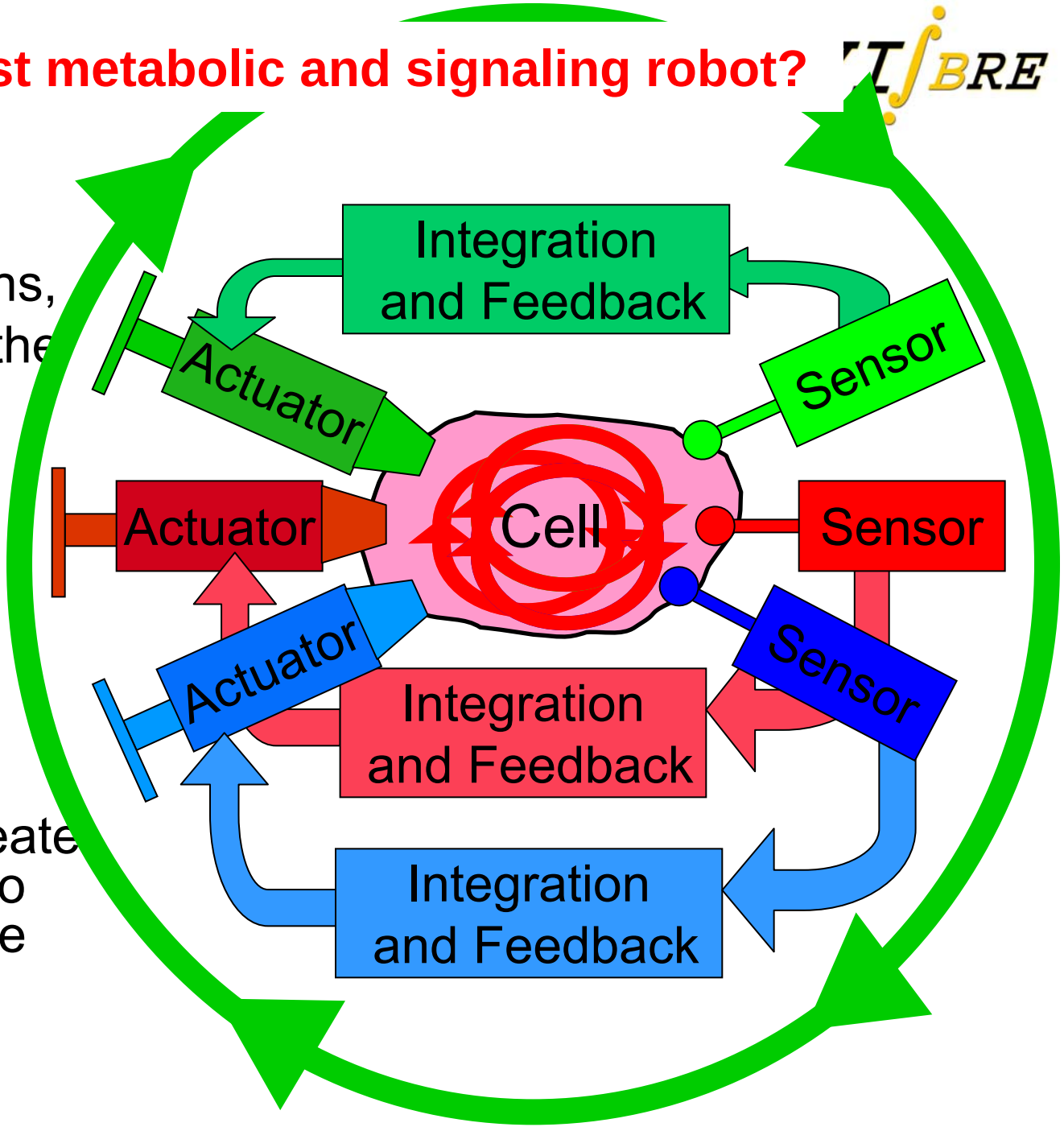
The Robot Scientist



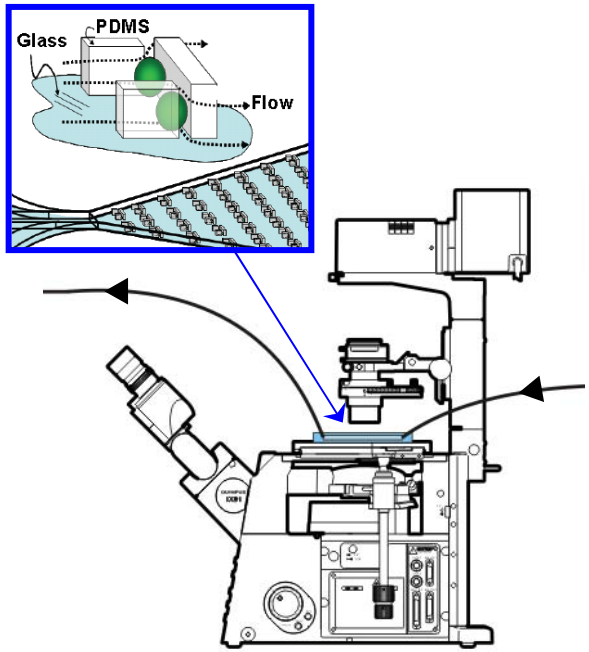
RD King et al. The Automation of Science. Science **324** (5923):85-89, 2009.

Can we build a fast metabolic and signaling robot?

- Multiple, fast **sensors**
- **Openers** (Mutations, siRNA, drugs) for the internal feedback loops
- Intra- and extracellular **actuators** **for controlled perturbations**
- Algorithms that create **feedback loops** to automatically probe the system
- …

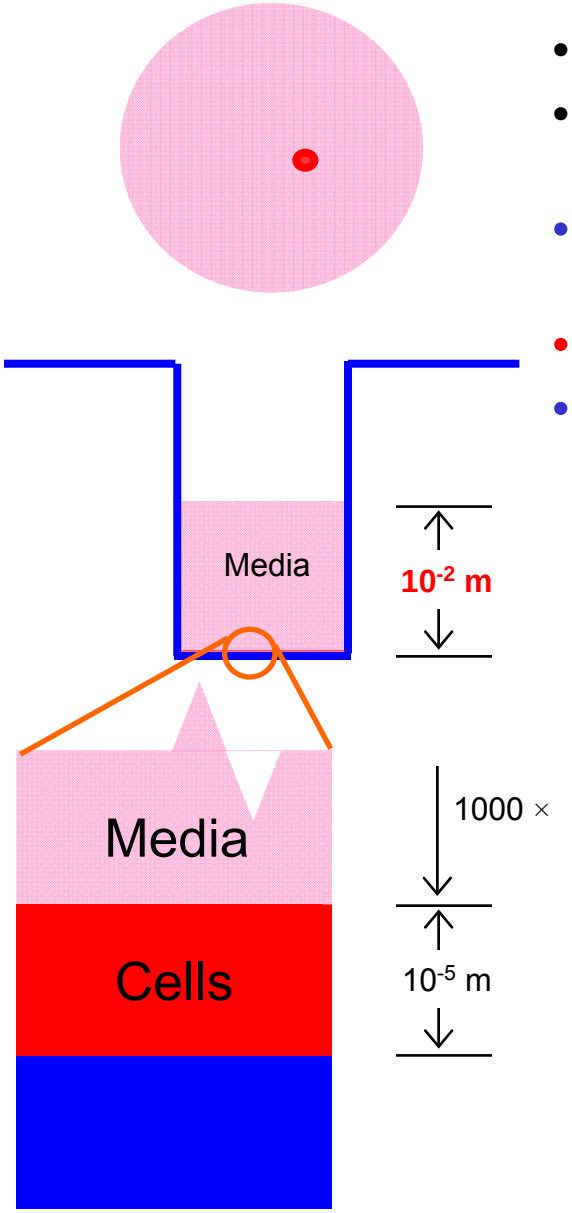


VIIBRE Automated Omni-Omics

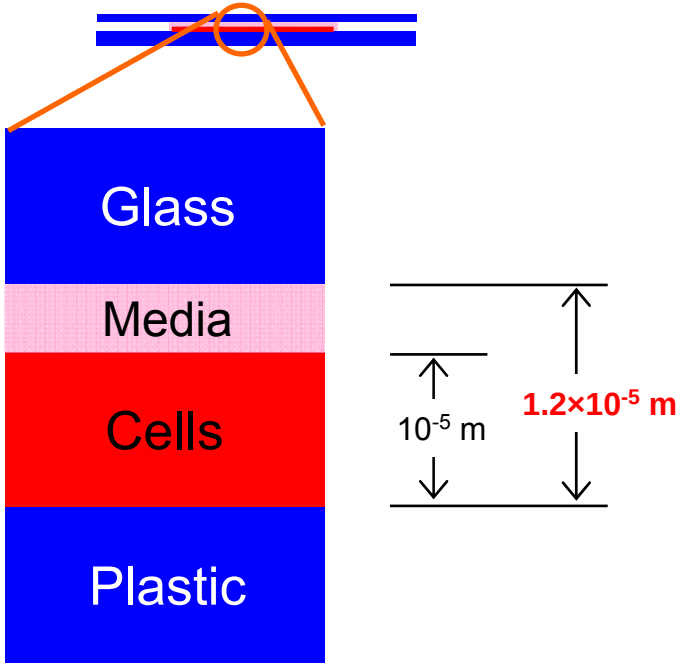


Cell Culture vs Microfluidics

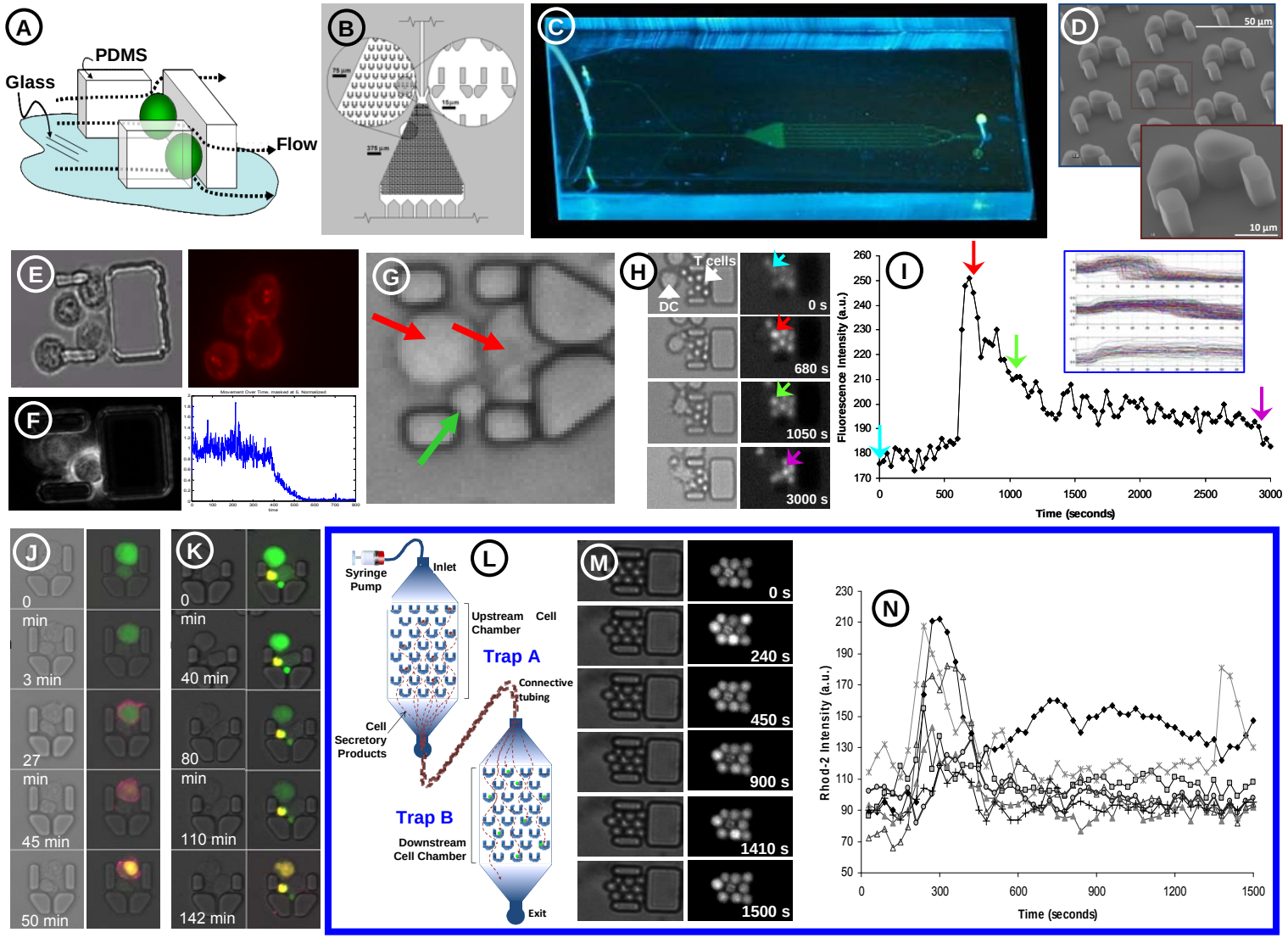
- A typical picoliter cell requires a nanoliter of media per day
- A 10 μm layer of cells is covered by a 10,000 μm layer of media
- 1 fluid change/day
- Metabolites, autocrine and paracrine factors are diluted 1000-fold



- BioMEMS
- A typical picoliter cell requires a nanoliter of media per day
- A 10 μm layer of cells is covered by a 2 μm layer of media
- 5000 fluid changes/day
- Metabolites, autocrine and paracrine factors are diluted by 1.2x

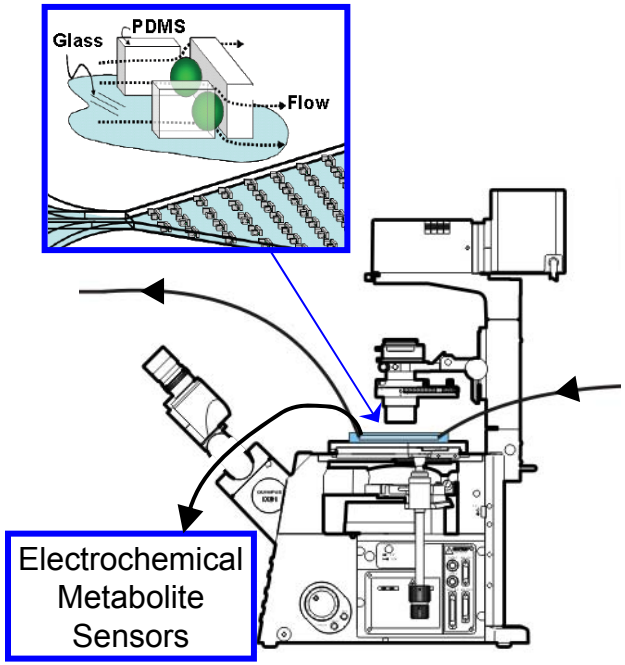


Microfabricated Multitrap Nanophysimeters (MTNPs) enable dynamic measurements on small populations of cells



Faley,S et al., Lab on a Chip, 8:1700-1712 (2008) & 9(18):2659-2664, 2009.

VIIBRE Automated Omni-Omics



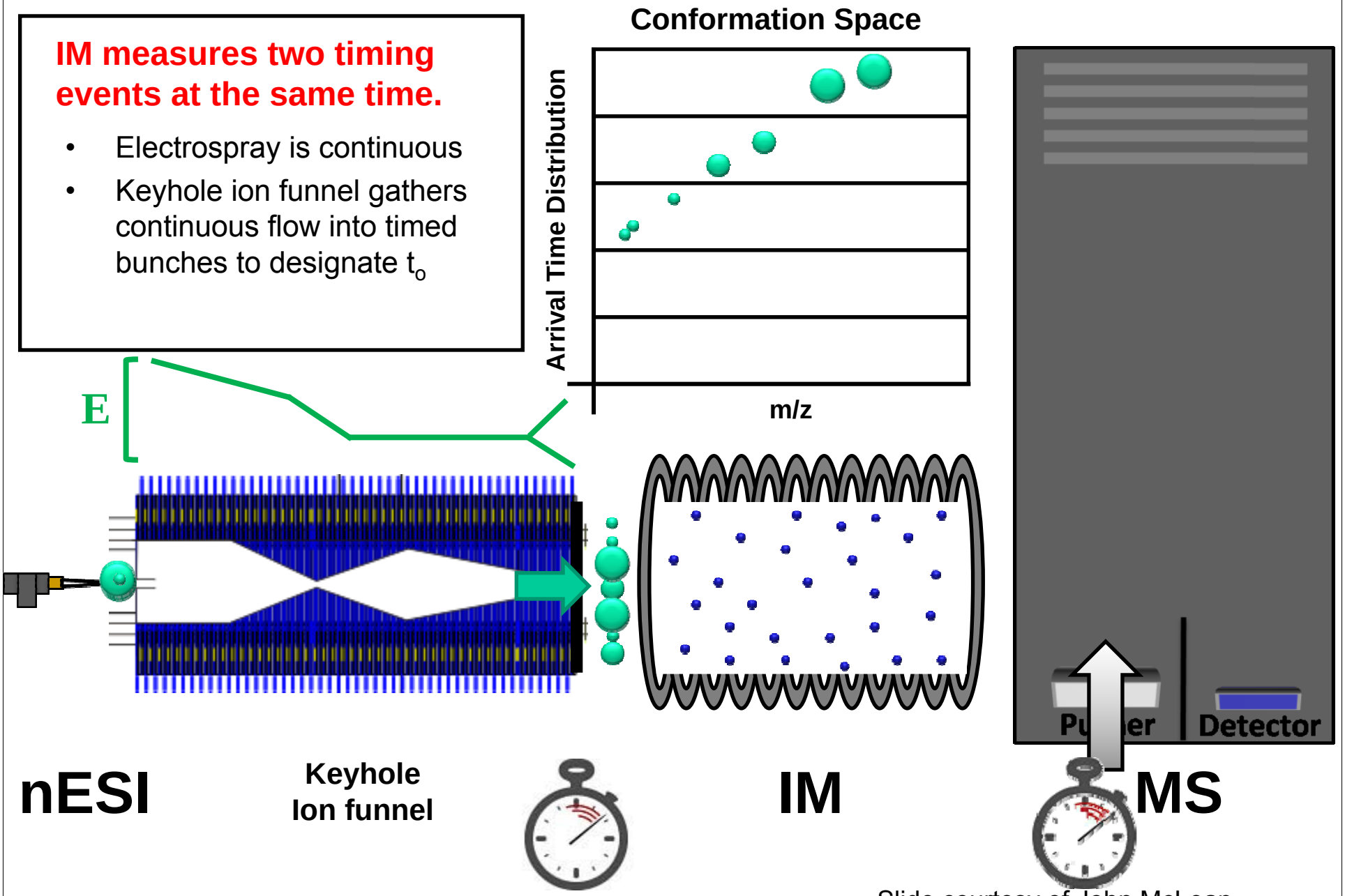
We need lots and lots and lots of numbers.

HPLC mass spectrometry?
An HPLC separation might require an hour!

Ion mobility mass spectrometry!

IM measures two timing events at the same time.

- Electrospray is continuous
- Keyhole ion funnel gathers continuous flow into timed bunches to designate t_o

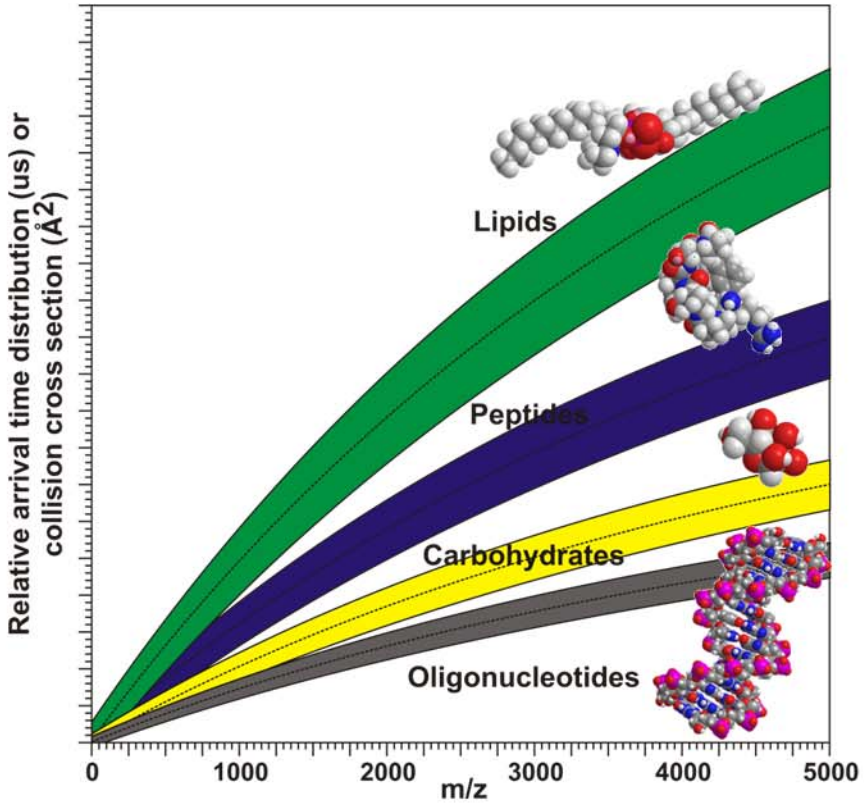


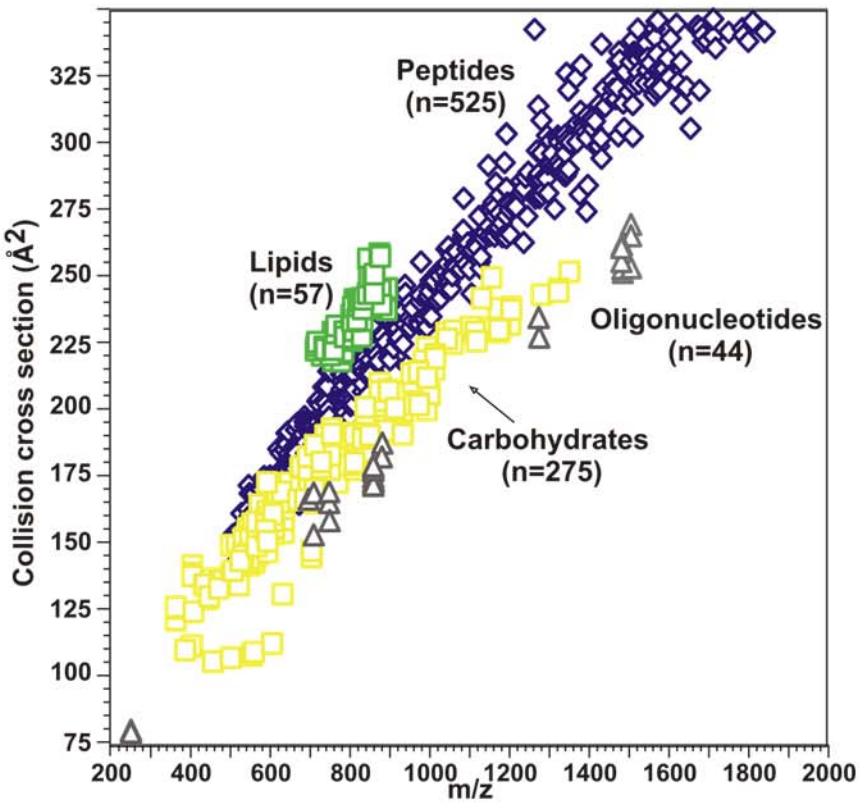
Slide courtesy of John McLean

MALDI/nESI-IM-TOFMS

Slide courtesy of John McLean

Real-time 2D identification of biomolecular signatures:
Integrated omics for dynamic systems biology





We will be adding GC for GC-IM-MS to improve detection of low mass metabolites

L. S. Fenn and J. A. McLean, *Anal. Bioanal. Chem*. 391, 905-9 (2008).

Slide courtesy of John McLean

Real-Time Structural Mass Spectrometry

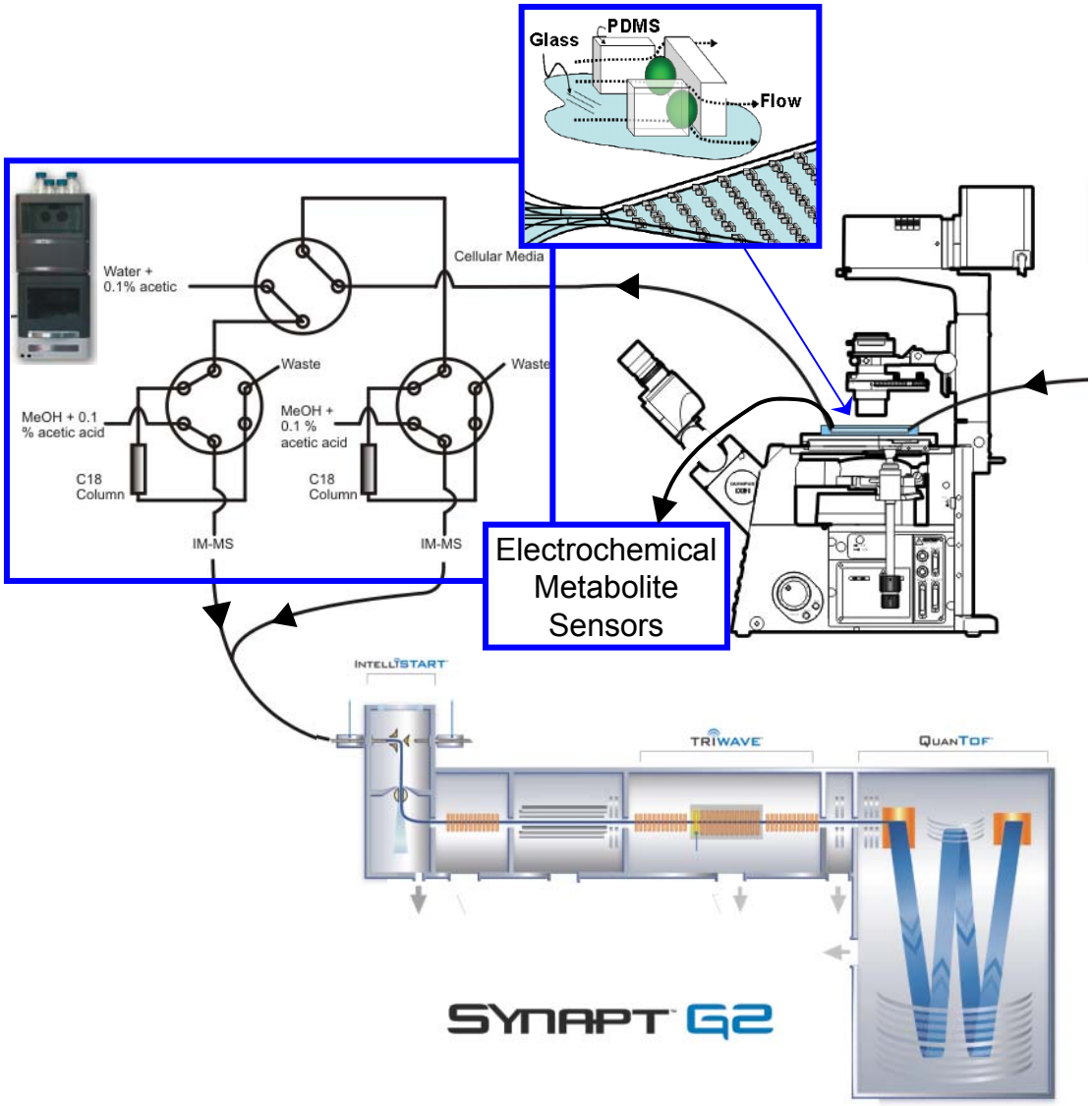
John A. McLean,[1-3] Jeffrey Enders,[1-3] Cody Goodwin,[1-3] Jody May,[1-3] Chrissy Marasco,[3-5] Sevu Sundarapandian,[1-3] Hod Lipson,[3,6] Michael Schmidt,[6] Kevin Seale,[3-5] and John P. Wikswo[3-5,7,8]

[1] Department of Chemistry, Vanderbilt University
[2] Vanderbilt Institute of Chemical Biology
[3] Vanderbilt Institute of Integrative Biosystems Research and Education
[4] Searle Systems Biology and Bioengineering Undergraduate Research Experience, Vanderbilt University
[5] Department of Biomedical Engineering, Vanderbilt University
[6] Department of Mechanical and Aerospace Engineering, Cornell University
[7] Department of Physics and Astronomy, Vanderbilt University
[8] Department of Molecular Physiology and Biophysics, Vanderbilt University

DTRA CB Basic Research Program (HTDTRAI-09-1-0013)

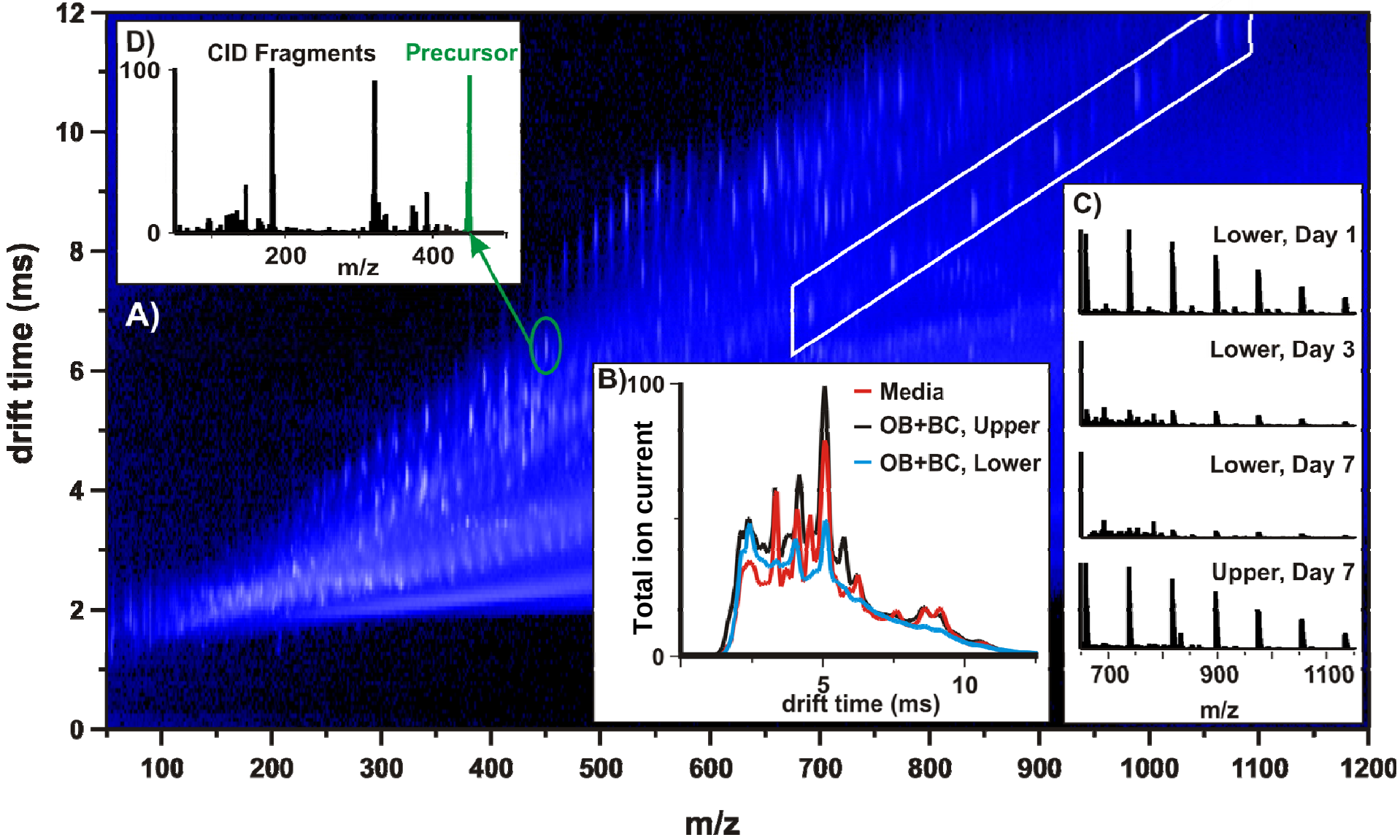
NIH-NIDA (RC2DA028981)

VIIBRE Automated Omni-Omics



Time-Lapse Bone Bioreactor Media Analysis

Nutrient consumption and metabolite production



Samples: Andrea Mastro and Erwin Vogler. Analysis: Jeff Enders and John McLean

OK – you now have enough data.

How do you deal with a Leibnitz of non-sparse PDEs involving 100,000 nonlinear variables?

Carefully, very carefully

A possible failure mode

Ontological failure: The phenomenon you are interested in requires elements or laws outside of the set you have been given.

There is a second possible failure mode

Epistemological failure: You have enough elements and the laws do apply, but you yourself cannot understand the explanation that they provide.

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

Houston, we have a problem.

- The human brain can process only seven pieces of data at a time.

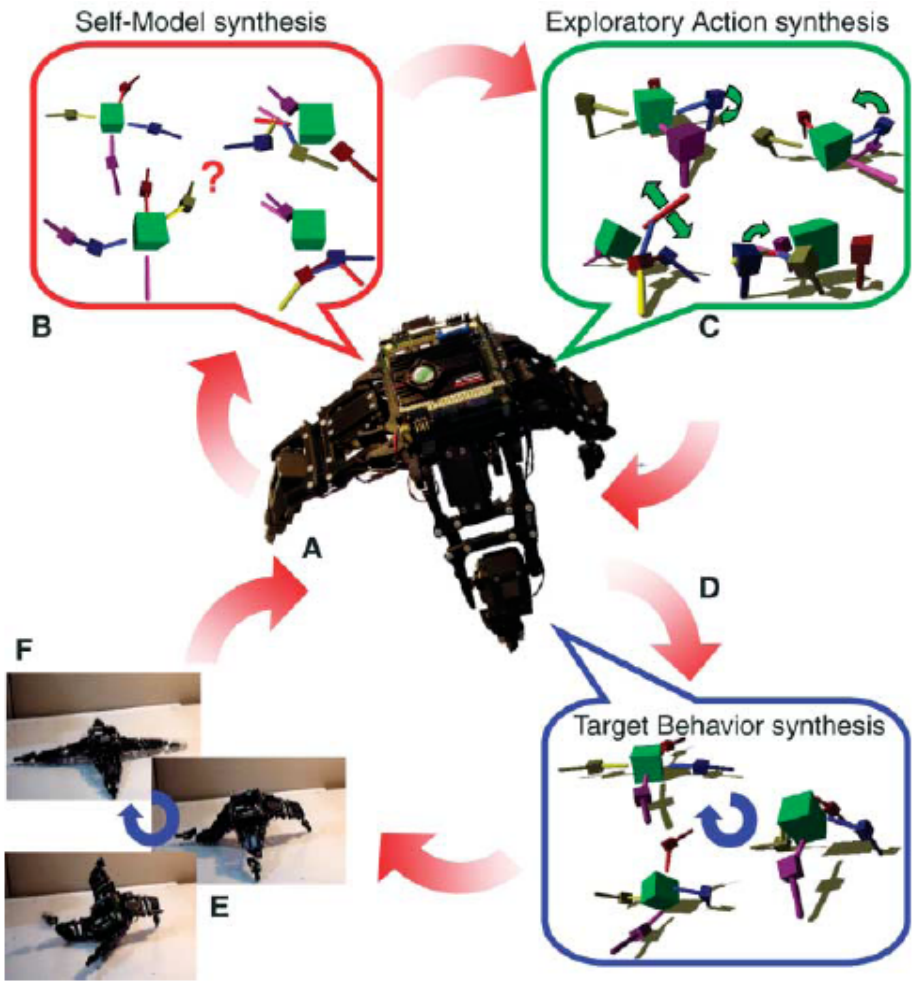
"…the seven-point rating scale, the seven categories for absolute judgment, the seven objects in the span of attention, and the seven digits in the span of immediate memory..."

G.A. Miller, "The Magical Number Seven, Plus or Minus Two: Some Limits on our Capacity for Processing Information," Psychological Review, 63, 81-97 (1956).

The solution to epistemological failure

Get a smarter, bigger brain…

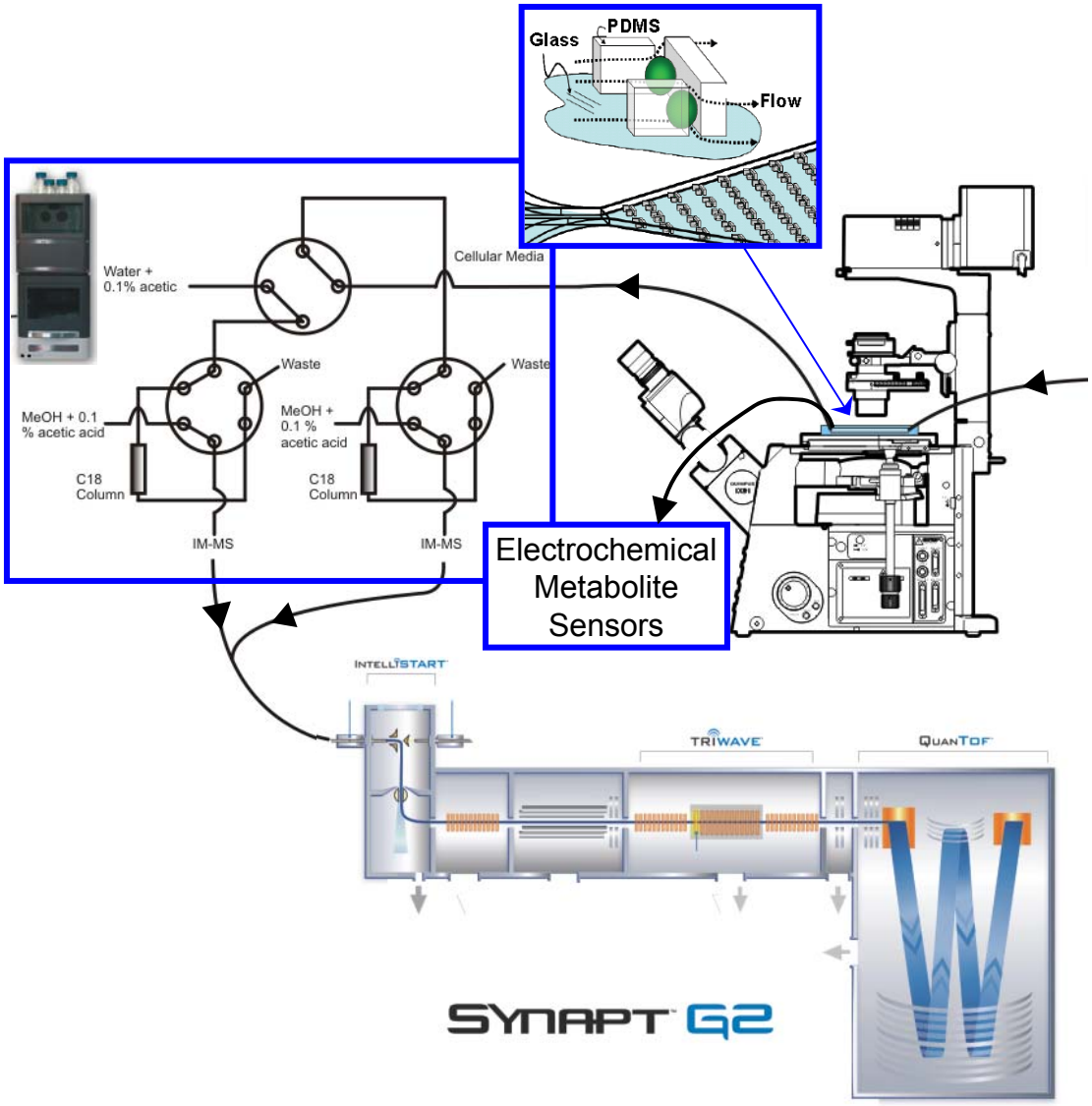
Machine Learning: A robot that can infer a model of "itself"

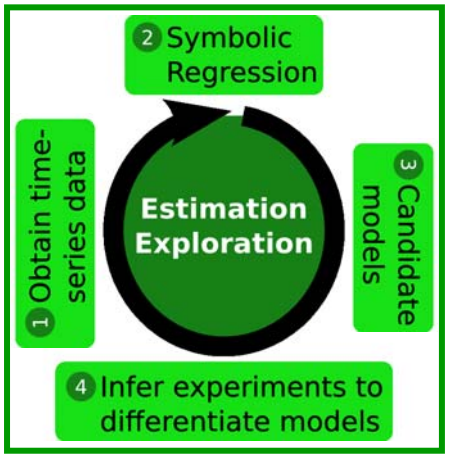


Hypothesis: Machine learning and model inference with automated experimentation can be extended from robots to bioreactors

J. Bongard, V. Zykov, and H. Lipson, Resilient Machines Through Continuous Self-Modeling, Science, 314, 1118-1121, 2006

VIIBRE Automated Omni-Omics





Automated Probing and Inference of Analytical Models for Metabolic Network Dynamics

Michael Schmidt,[1] Ravishankar Vallabhajosyula,[2] Jerry Jenkins,[2,3] Jonathan Hood,[2] Abhishek Soni,[2] John Wikswo,[4] Hod Lipson[1]

[1] Cornell University
[2] CFD Research Corporation
[3] Hudson Alpha Institute
[4] Vanderbilt University

Supported in part by DTRA, NIAID, NIDA, NSF and VIIBRE

Symbolic Regression
-- Hod Lipson, Cornell --

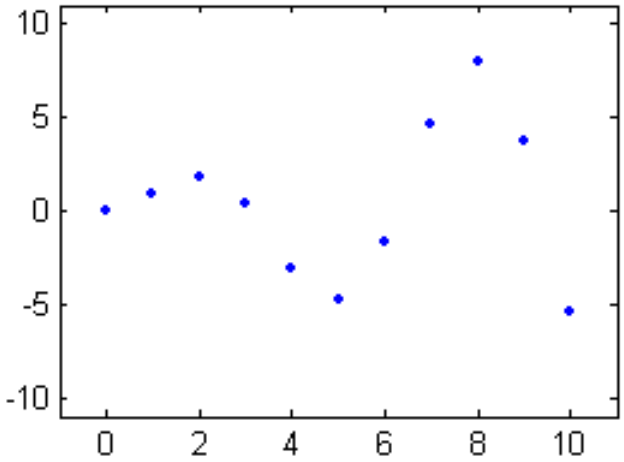
- Traditional regression
  - Model known, regress coefficients
  - Linear, nonlinear

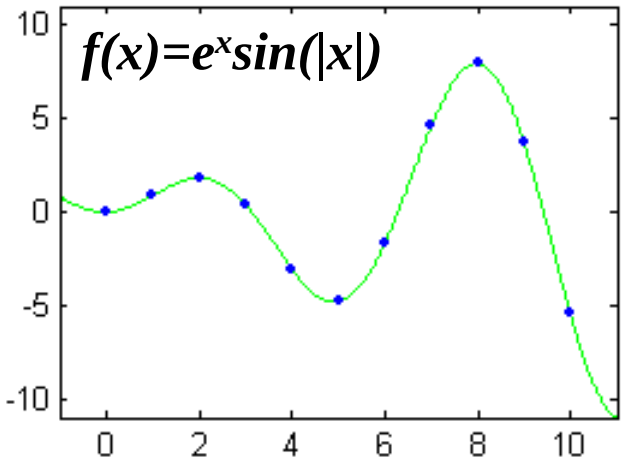
$$f(x)=ax^2+bx+c$$

- Symbolic regression
  - Model unknown
  - Model building blocks given
    - `{+,-,*,/,const,sin,cos,exp,log}`

$$f(x)=ae^{bx}+c$$

Eureqa: http://ccsl.mae.cornell.edu/eureqa





Estimation-Exploration algorithm designs experiments to select best symbolic model

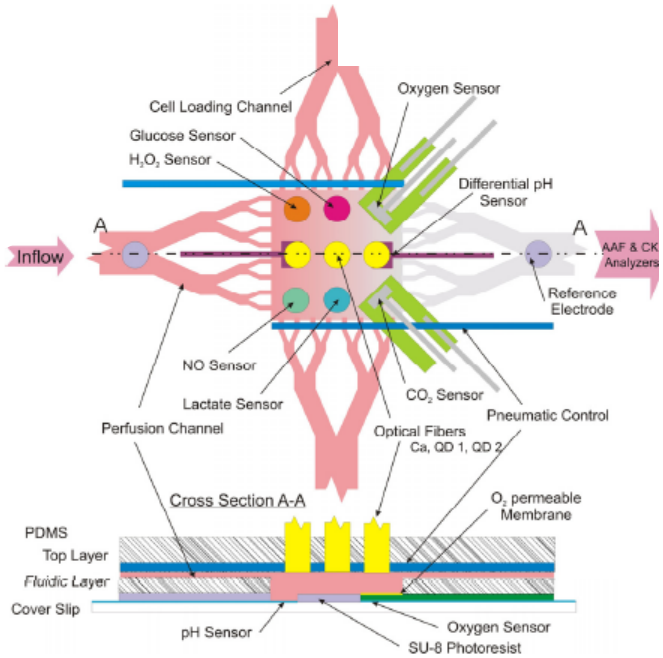
Computational Synthesis

CFDRC

VIBRE

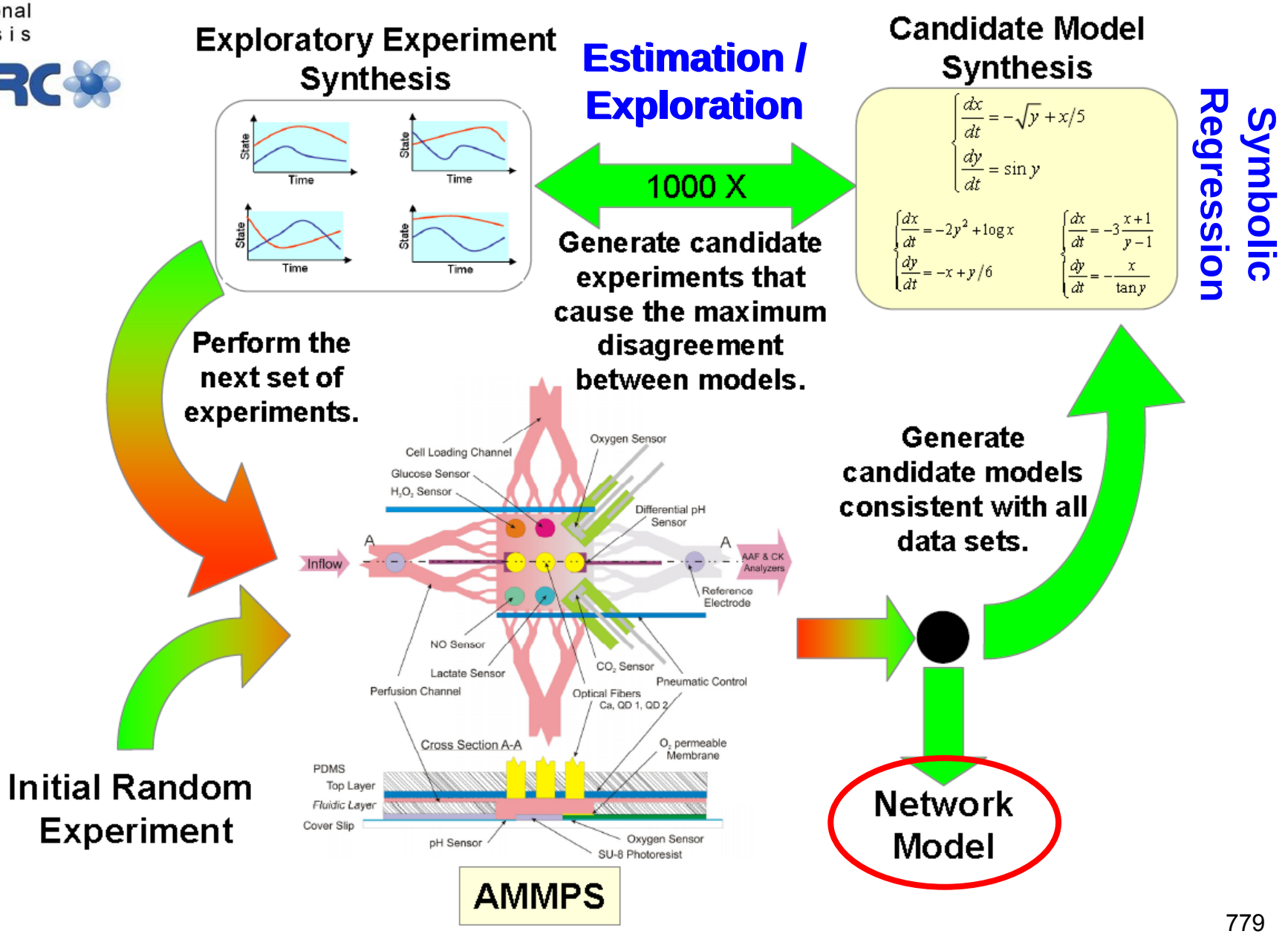
Estimation / Exploration

Symbolic Regression

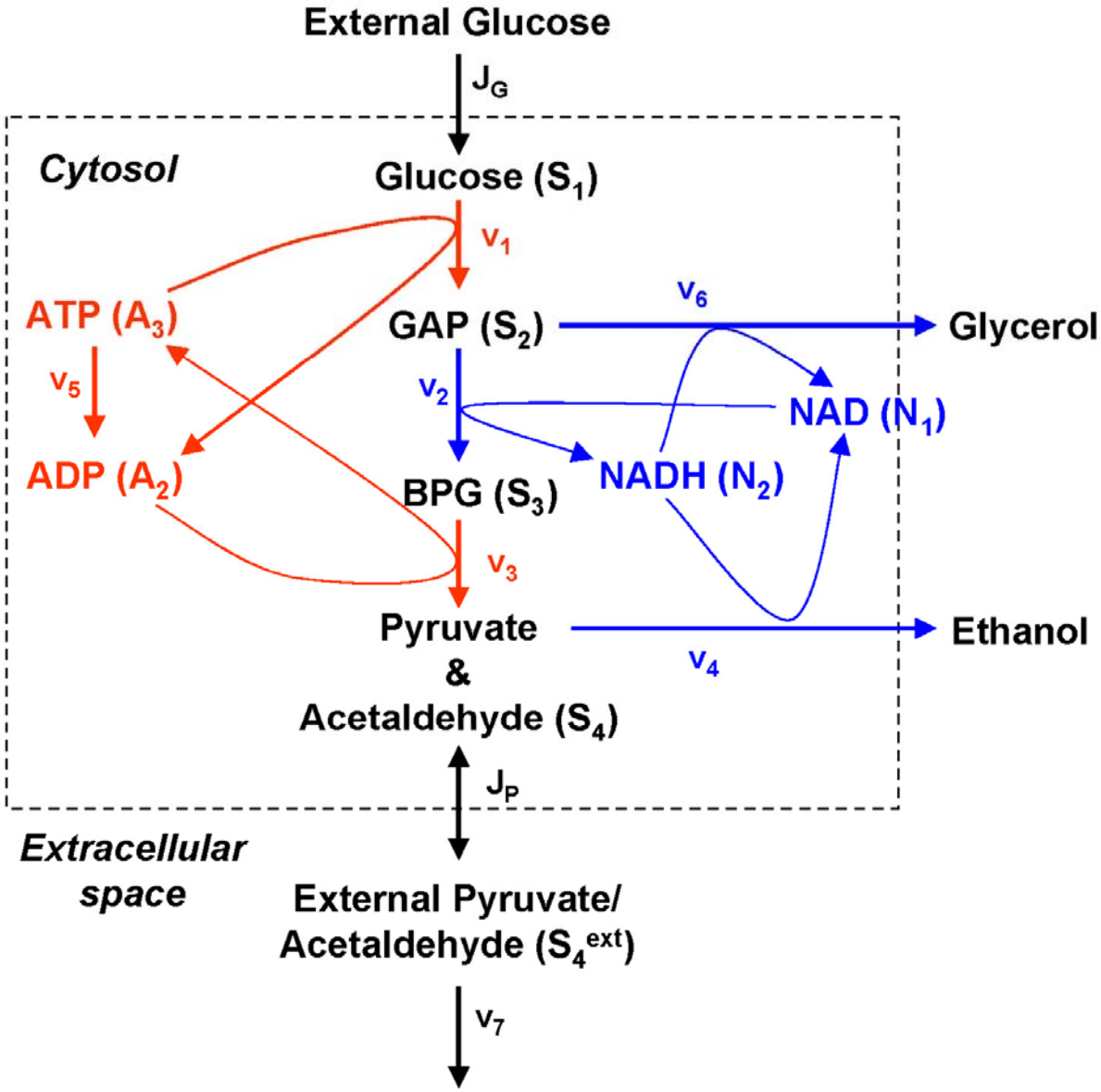


AMMPS

Estimation-Exploration algorithm designs experiments to select best symbolic model

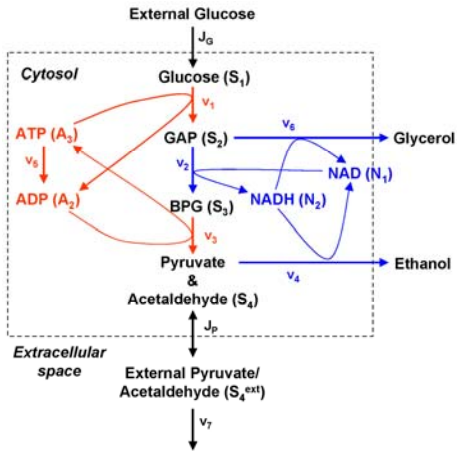


Into the Black Box: Yeast Glucose Oscillations



Computational Synthesis

Inferring Metabolic Models



VIBRE

CFDRC

Target model placed in black box with 10% noise

$$\frac{dS_1}{dt} = 2.5 - \frac{100 * A_3 S_1}{1 + 13.68 * A_3^4}$$

$$\frac{dS_2}{dt} = \frac{200 * A_3 S_1}{1 + 13.68 * A_3^4} - 6 * S_2 \boxed{- 6 * S_2 N_2}$$

$$\frac{dS_3}{dt} = 6 * S_2 - 6 * N_2 S_2 - 64 * S_3 + 16 * A_3 S_3$$

$$\frac{dS_4}{dt} = 64 * S_3 - 16 * A_3 S_3 - 13 * S_4 - 100 * N_2 S_4 + 13 * S_5$$

$$\frac{dN_2}{dt} = 6 * S_2 - 18 * N_2 S_2 - 100 * N_2 S_4$$

$$\frac{dA_3}{dt} = -1.28 * A_3 - \frac{200 * A_3 S_1}{1 + 13.68 * A_3^4} + 128 * S_3 + 32 * A_3 S_3$$

$$\frac{dS_5}{dt} = 1.3 * S_4 - 3.1 * S_5$$

Model inferred without any a priori information

$$\frac{dS_1}{dt} = 2.53 - \frac{98.79 \cdot A_3 S_1}{1 + 12.66 \cdot A_3^4}$$ **Glucose**

$$\frac{dS_2}{dt} = \frac{200.23 \cdot A_3 S_1}{1 + 13.80 \cdot A_3^4} - 6.87 \cdot S_2 \boxed{- 6.87 \cdot N_2 + 0.95}$$ **G3P DP Pool**

$$\frac{dS_3}{dt} = 6.00 \cdot S_2 - 6.00 \cdot N_2 S_2 - 64.16 \cdot S_3 + 16.08 \cdot A_3 S_3$$ **BPG**

$$\frac{dS_4}{dt} = 64.04 \cdot S_3 - 16.03 \cdot A_3 S_3 - 13.03 \cdot S_4 - 100.11 \cdot N_2 S_4 + 13.21 \cdot S_5$$ **Pyr Act Pool**

$$\frac{dN_2}{dt} = \boxed{- 0.055} + 5.99 \cdot S_2 - 17.94 \cdot N_2 S_2 - 98.82 \cdot N_2 S_4$$ **NADH**

$$\frac{dA_3}{dt} = -1.12 \cdot A_3 - \frac{192.24 \cdot A_3 S_1}{1 + 12.50 \cdot A_3^4} + 124.92 \cdot S_3 + 31.69 \cdot A_3 S_3$$ **ATP**

$$\frac{dS_5}{dt} = 1.23 \cdot S_4 - 2.91 \cdot S_5$$ **$S4_{ext}$**

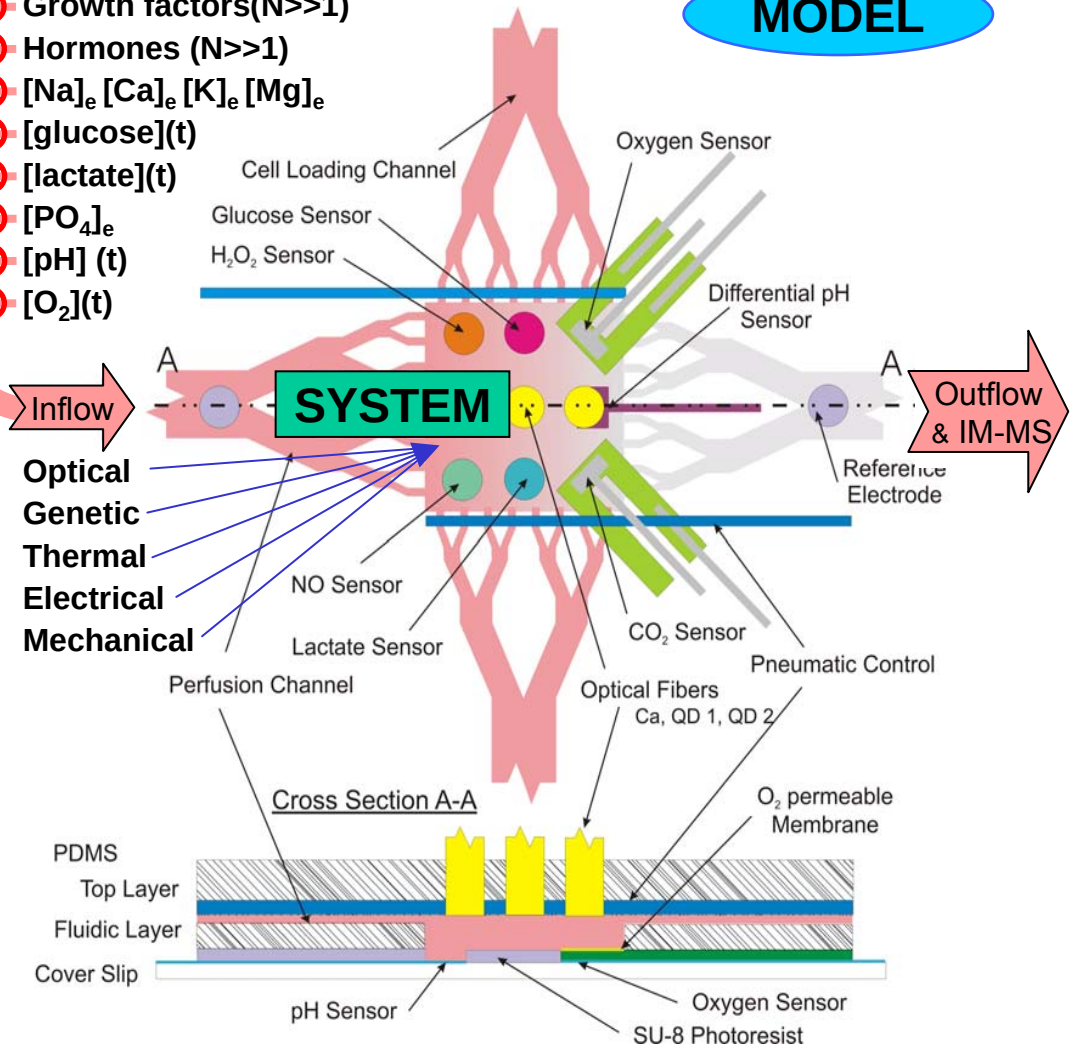
ACTUATORS (Inputs)

CONTROLS

SENSORS (Outputs)

MODEL

- **Base medium**
- **Amino acids (~15)**
- **Cytokines (N>>1)**
- **Growth factors(N>>1)**
- **Hormones (N>>1)**
- **$[Na]_e$ $[Ca]_e$ $[K]_e$ $[Mg]_e$**
- **[glucose](t)**
- **[lactate](t)**
- **$[PO_4]_e$**
- **[pH] (t)**
- **$[O_2](t)$**



Morphology
Size, shape, optical density, motility, division, organelle configuration

Force
Shear, tension, deformation

Intracellular Signaling (Optical)
GFP/luciferase reporters, $[Ca]_i$, pH_i, V_m, MMP, GFP FRET

Extracellular Electrolytes (Electrochemical)
$[Na]_e$, $[Ca]_e$, $[K]_e$, $[Mg]_e$, $[PO_4]_e$, $[Cl]_e$, $[HCO_3]_e$

Neurotransmitters (Electrochemical)
Seratonin, acetocholine, GABA, …

Extracellular Metabolites (Electrochemical)
[glucose](t), [lactate](t), [pH](t), $[O_2](t)$, NO(t), $H_2O_2(t)$ …

Extracellular Metabolites (GC IM-MS)
Amino acids, small metabolites, stable isotopic markers

Surface Expression
Specific affinity probes

Soluble Gene Expression (nESI IM-MS)
Cytokines, growth factors
hormones, enzymes

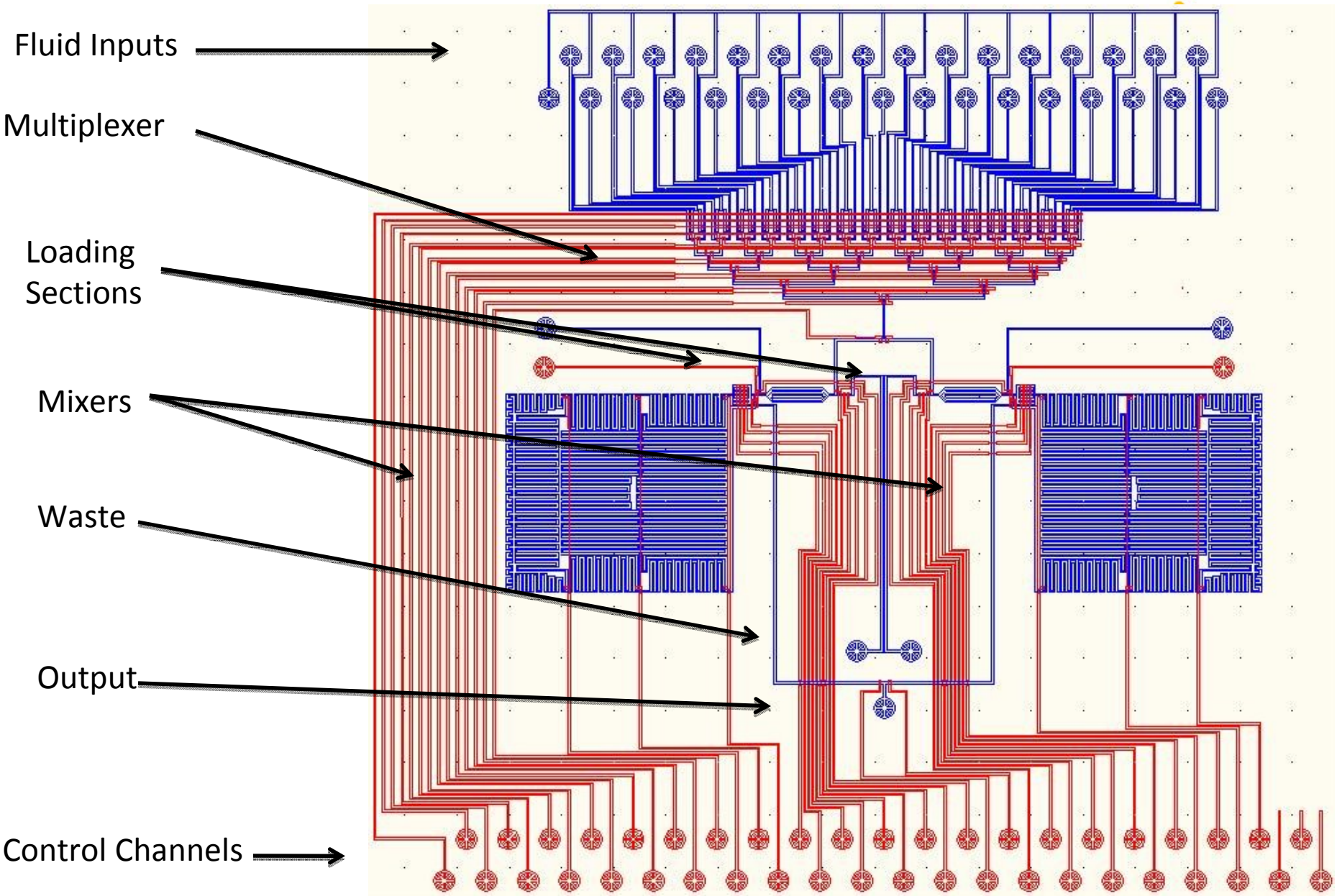
Cytosolic Proteins (MALDI IM-MS)

Lipids (Cell Lysate IM-MS)

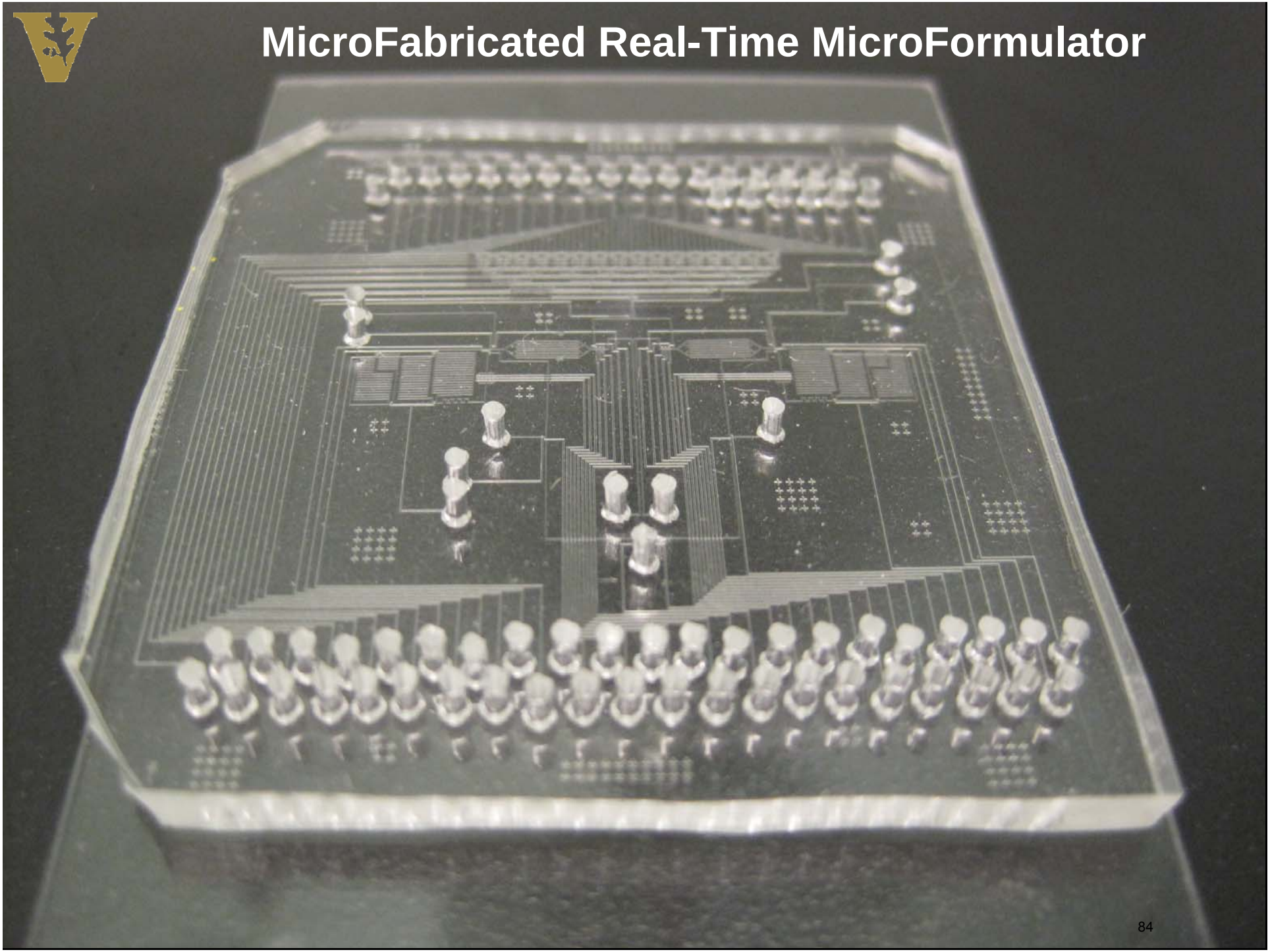
Gene expression (mRNA Arrays)

. . .

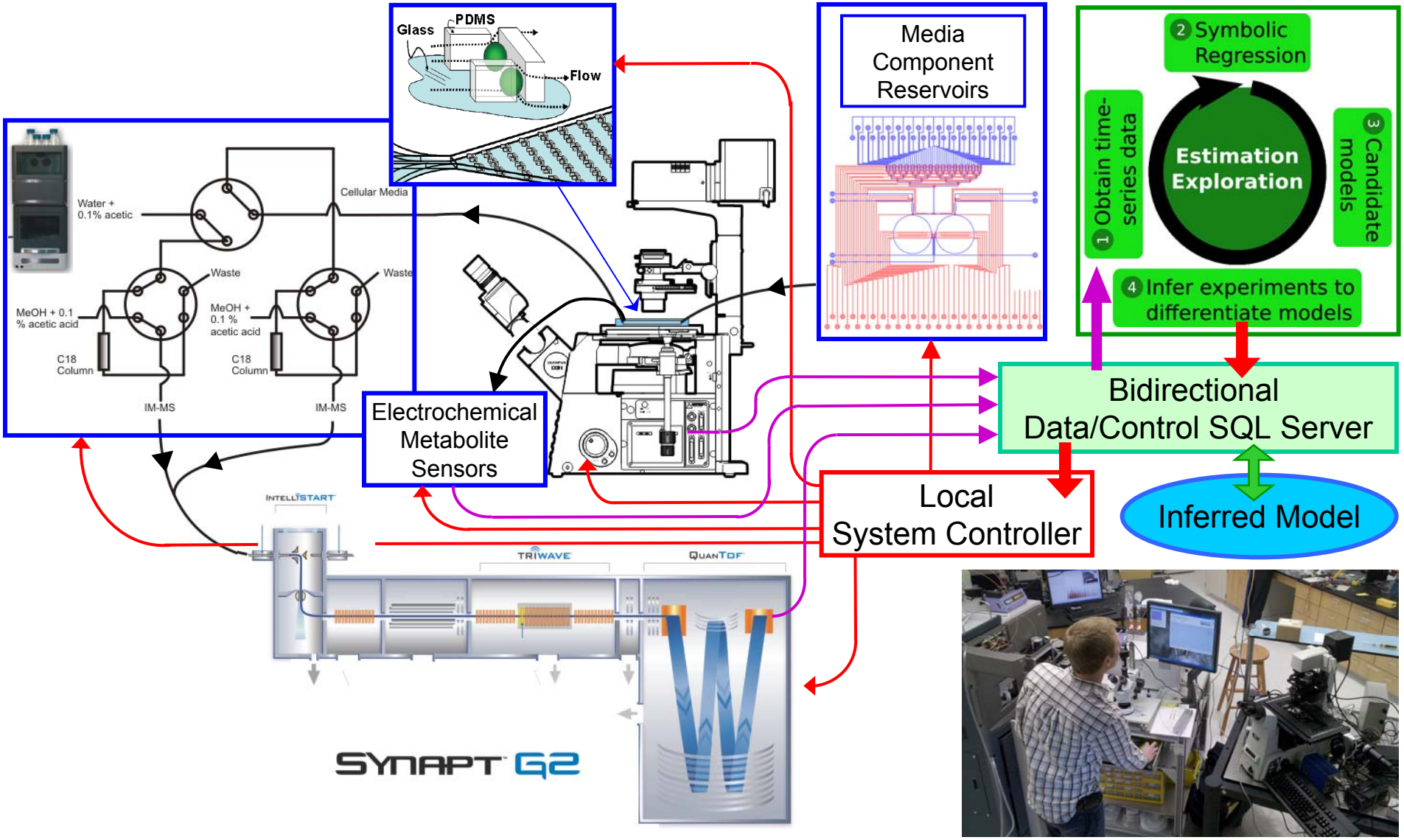
Will Matloff's MicroFormulator



MicroFabricated Real-Time MicroFormulator



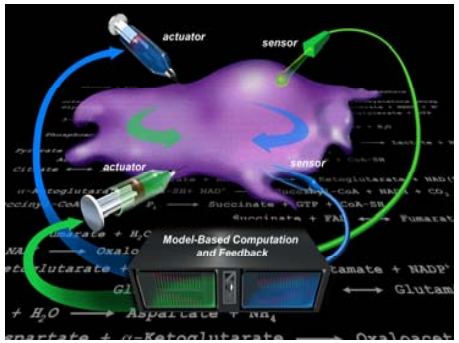
Our Robot Scientist: VIIBRE Automated Omni-Omics



INPUT ACTUATORS

- Chemical
- Electrical
- Genetic
- Mechanical
- Optical
- Thermal
- Scaffolding

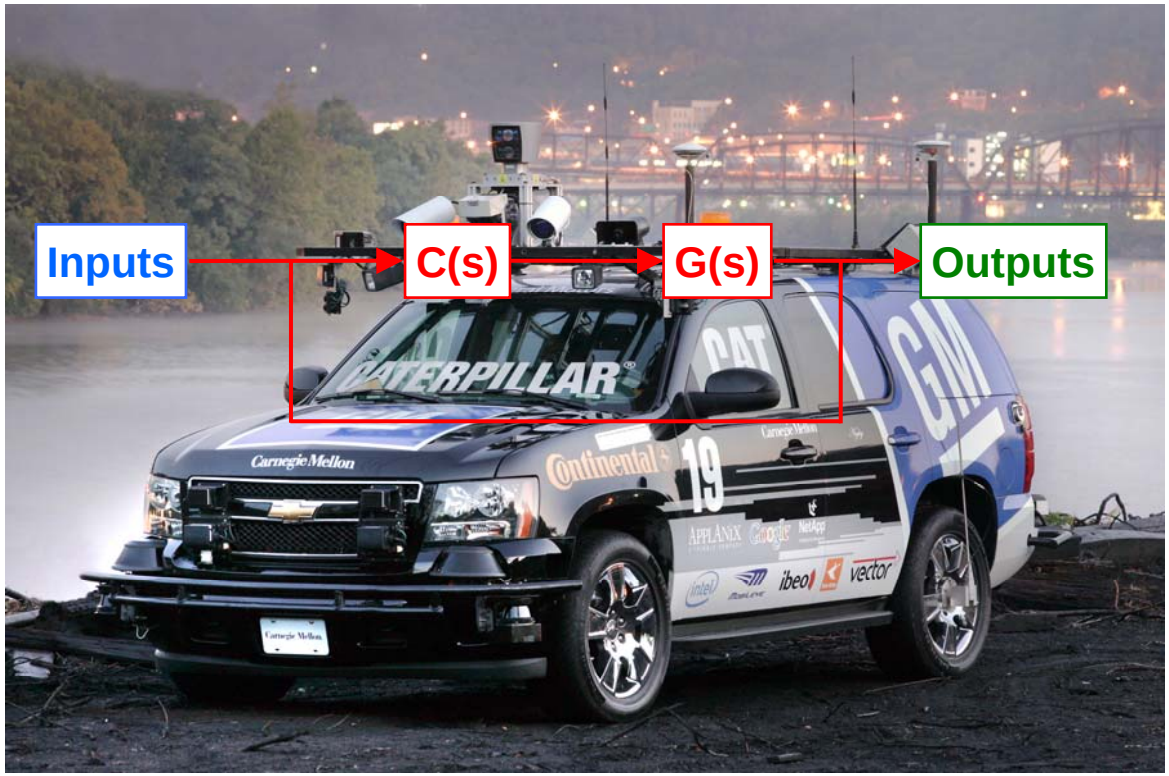
C(s) → G(s)

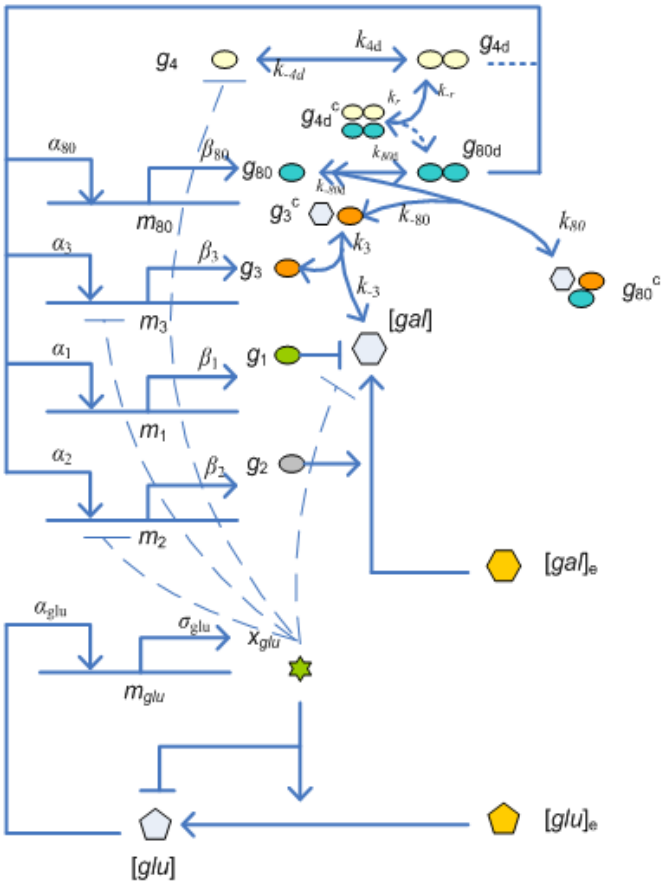


OUTPUT SENSORS

- Apoptosis
- Differentiation
- Gene / Protein Expression
- Growth
- Metabolism
- Motility
- Signal Transduction

VIBRE



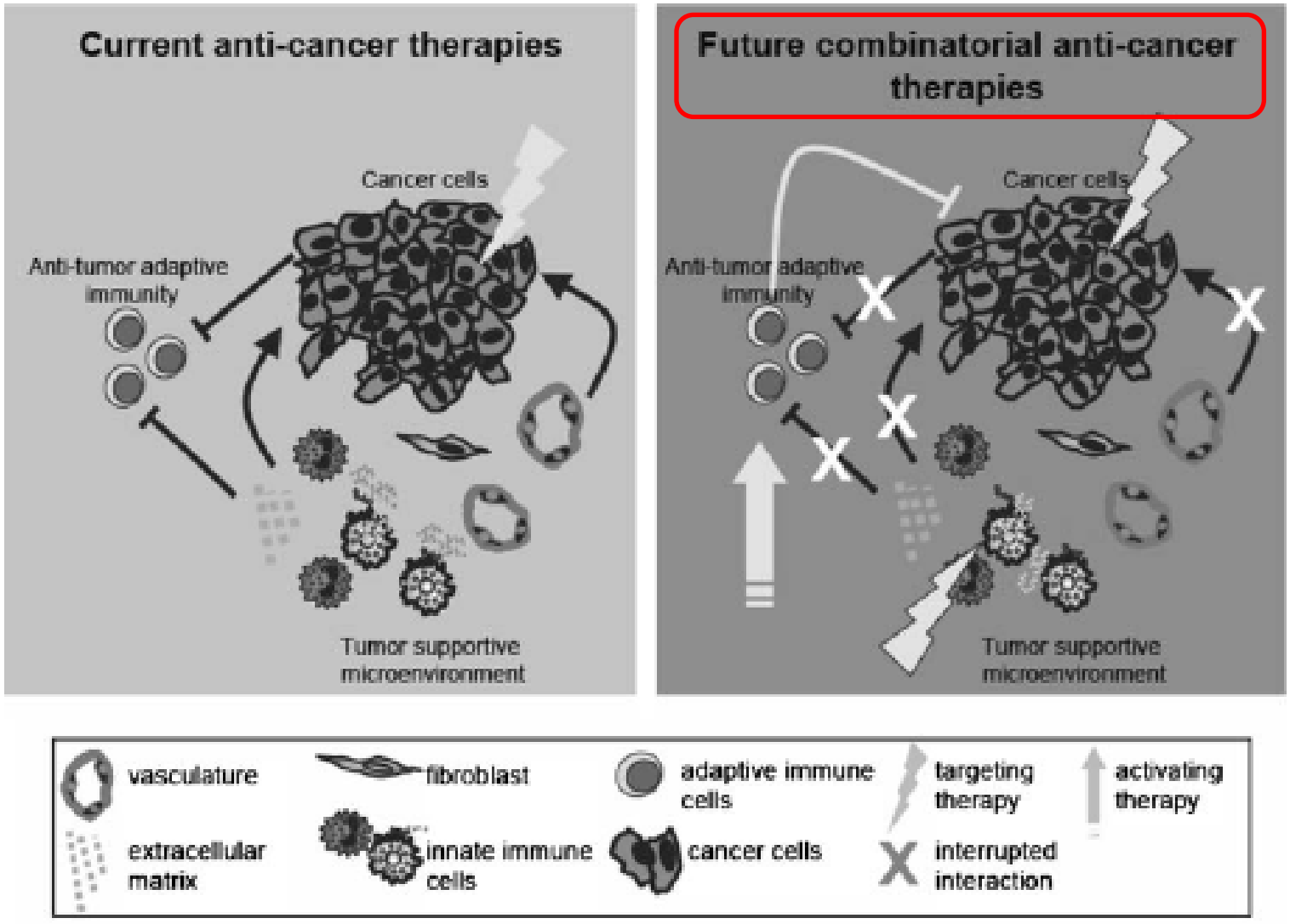


LeDuc, Messner, Wikswo. How do controls approaches enter into biology. *Submitted*, 2010.

Biophysics/Bioengineering & Cancer?

- The need for more realistic *in vitro* experiments
  - Massively parallel, cellular microenvironments for the study of cell-cell, cell-cell-drug, and cell-cell-drug-snp interactions
  - Real-time control of biological systems
- The need to control multiple parameters at the same time and measure multiple dynamic variables
  - Cell-scale sensors and actuators
  - Experiments that involve thousands of parameters
- The need to create complex, nonlinear models
  - Symbolic regression and exploration-estimation algorithms for machine learning in automated microbioreactors
  - Models to enable control of cellular responses and biomolecule production
- The need to raise research funds from more diverse sources
- The inability of the human mind (or at least those of the reviewers) to understand the complexity of what is being proposed and/or discovered

The future of biology and cancer medicine is distributed hybrid multiscale non-linear stochastic control



De Visser, Cancer Immunol Immunother (2008) 57: 1531-1539

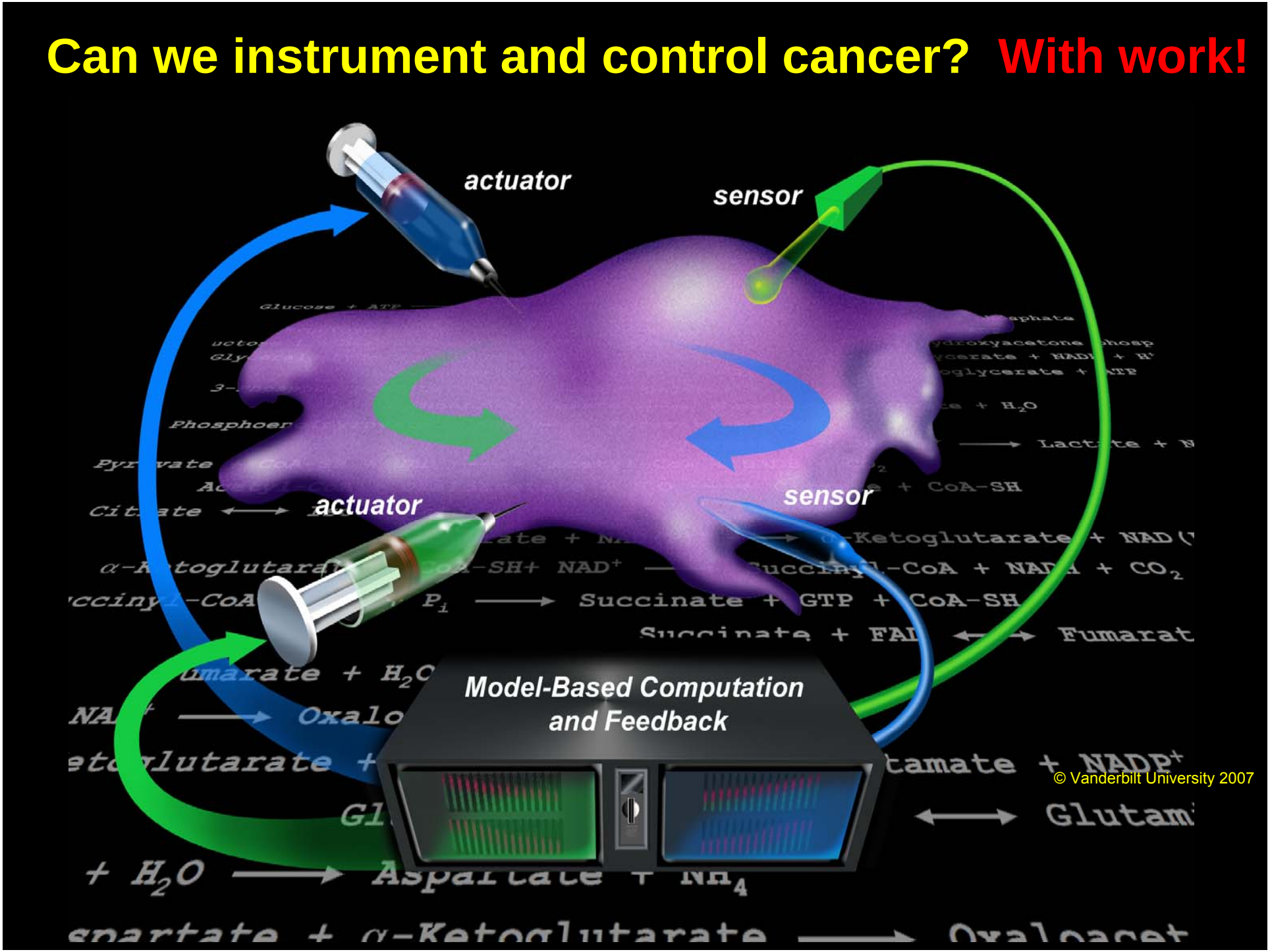
Really Hard Problems

However…

We do not have to fully understand a phenomenon to control or eliminate it.

John Wikswo

Can we instrument and control cancer? With work!



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Click to go past the end

There is yet one more potential problem…

- We may not be able to understand what the computer tells us about biology.
- The next challenge is to create computers that can explain their findings to us….

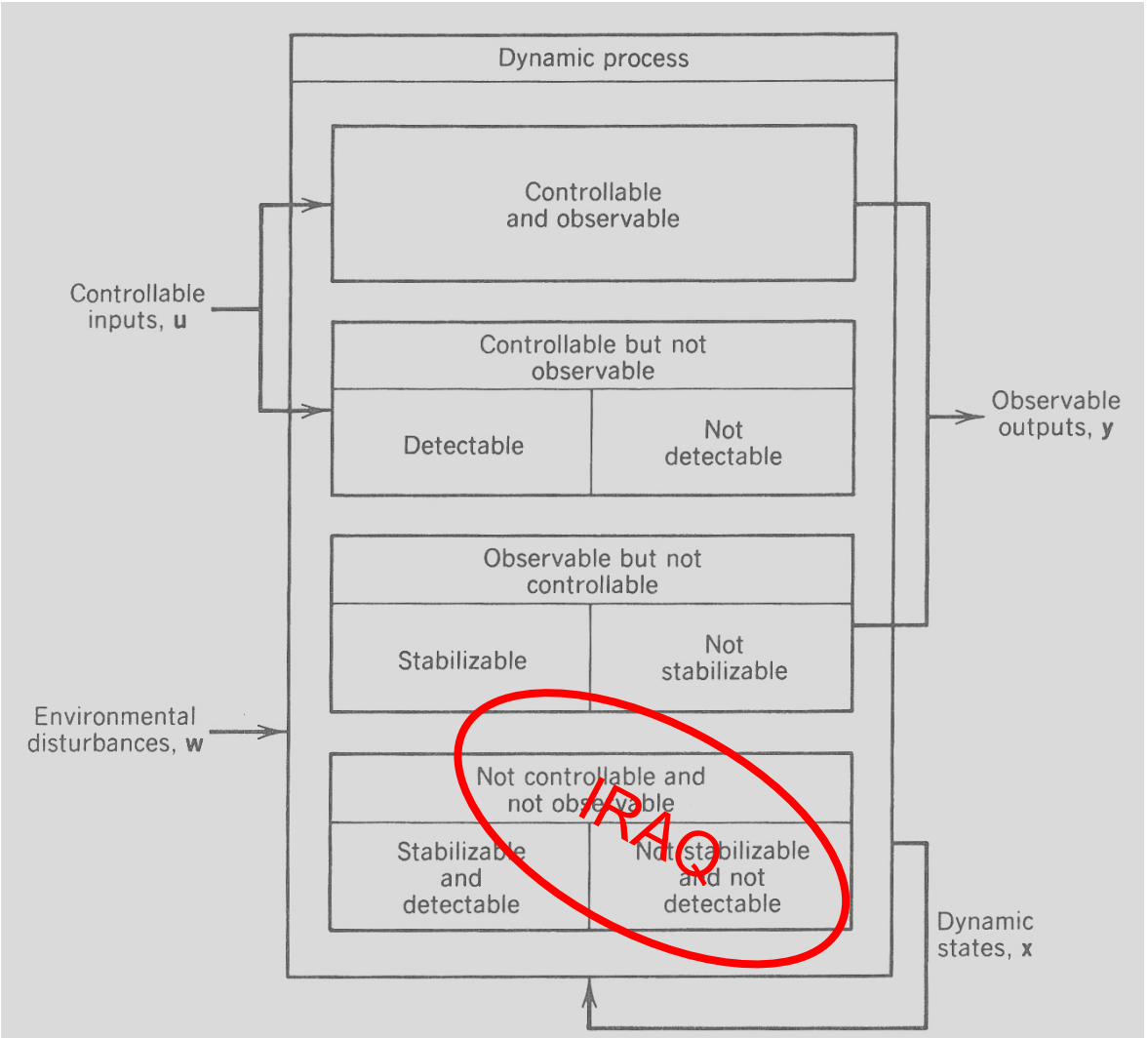
- It might be as hopeless as explaining Shakespeare to a dog.

Hod Lipson, 2009

Rumsfeld's Analysis of Complex Systems

- Observable?
- Controllable?
- Stabilizable?
- Detectable?

Iraq was neither observable, controllable, stabilizable, or detectable …



Stengel, Optimal Control and Estimation, Dover, 1994, p.7