Complexity, microphysiological systems, and closing the hermeneutic circle of biology

John Wikswo

International Organ-on-Chip Workshop:

From Systems Biology to Societal Issues

Milan, Italy, 14-15 February 2019

Disclosure

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Abstract

Deconvolving the multiscale, spatiotemporal complexity of biology requires not only understanding the governing laws of physics and chemistry, but also decoding billions of years of genetically encoded history. Simple, passive observations cannot expose the nested, redundant levels of regulation in the historical instruction set, and active interventions are needed to disable specific biological functions to expose others. The concept of the hermeneutic circle applies to biology – one cannot understand the whole until one understands the parts, and one cannot understand the parts without understanding the whole. In this context, coupled microphysiological systems meet the criteria for a successful toy model: complicated enough to recapitulate key regulatory processes but simple enough to understand. Revealing the functions of such coupled *in vitro* systems will require untargeted analysis of the genome, transcriptome, proteome, lipidome, interactome, and metabolome at the level of cells and tissues, which places demands on the accessibility and interconnection of each micro-organ and establishes lower limits on their sizes. The grand challenge is to devise and integrate the requisite cells, microfluidic bioreactors, sensors, analytical techniques, closed-loop controls, and mathematical models (which may be underspecified). Machine learning and automated design of experiments may be critical to closing the hermeneutic circle.

The central question for today

What can we do to accelerate our understanding of the complexities of biological systems?

Seven Themes

1. A brief history of biology
2. Just how complex is biology?
3. What is the role of physics in understanding the complexity of biology?
4. Why do we need to emphasize external control?
5. How might Organoids and Organs on Chips change the way we study biology?
6. What does Multi-Omics offer?
7. Closing the circle

Julia Wikswo

Seven Themes

1. **A brief history of biology**
2. Just how complex is biology?
3. What is the role of physics in understanding the complexity of biology?
4. Can sensors, actuators, controllers and robot scientists help address biological complexity?
5. How might Organoids and Organs on Chips change the way we study biology?
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Julia Wikswo

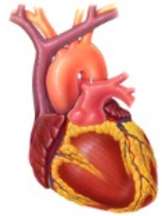
The history of biology…

The birth of physiology

Anatomy

Physiology

Animal



Organ

Galen of Pergamon
AD 129-216

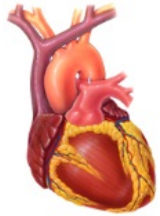
Isolated Organs

Anatomy

↓

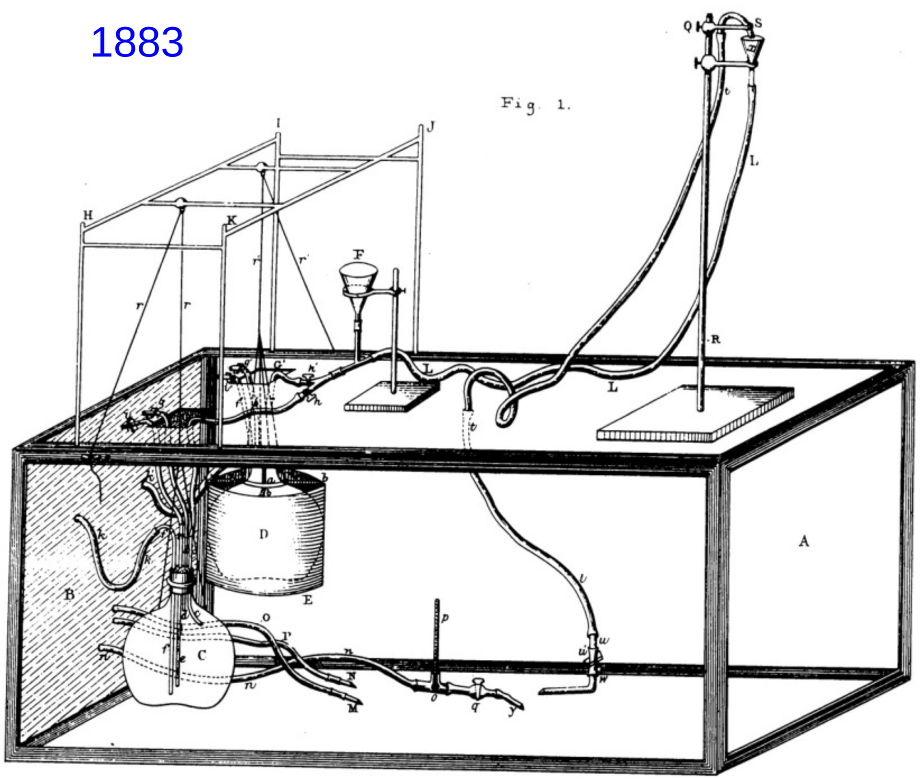
Physiology

Animal



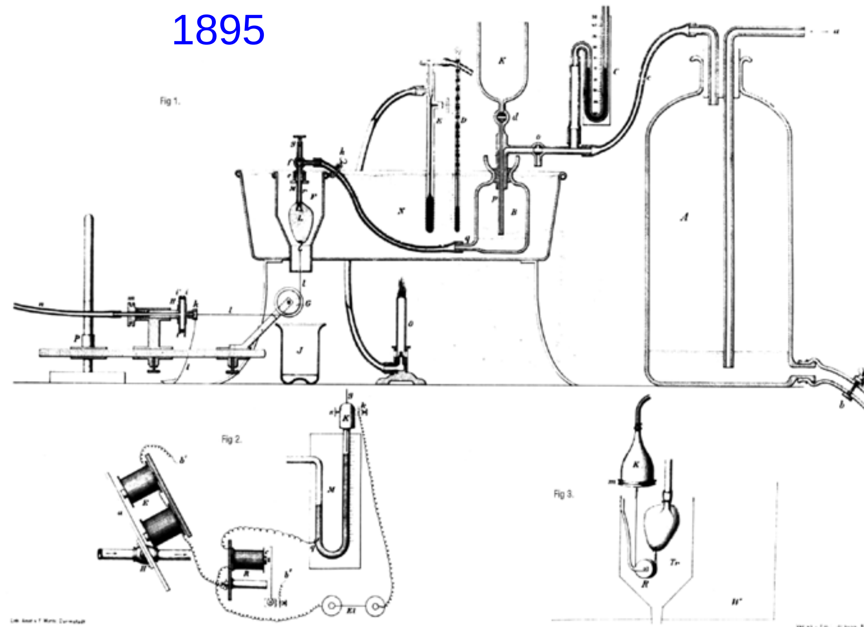
Organ

1883



Martin HN. The direct influence of gradual variations of temperature upon the rate of beat of the dog's heart. Philos Trans R Soc London 1883 Jan 1;174:663-88.

1895



O. Langendorff. Untersuchungen am Überlebenden Säugethierherzen. Pflug.Arch.Eur.J.Phy. 61 (6):291-332, 1895, as shown in H.G. Zimmer. The isolated perfused heart and its pioneers. News Physiol.Sci. 13:203-210, 1998.

Conclusion: Body temperature affects heart rate. Missing from the image: Dog with cannulated heart & lungs, cast iron, water-filled pan, and Bunsen burners…

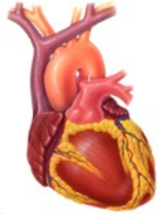
Isolated Organs

Anatomy

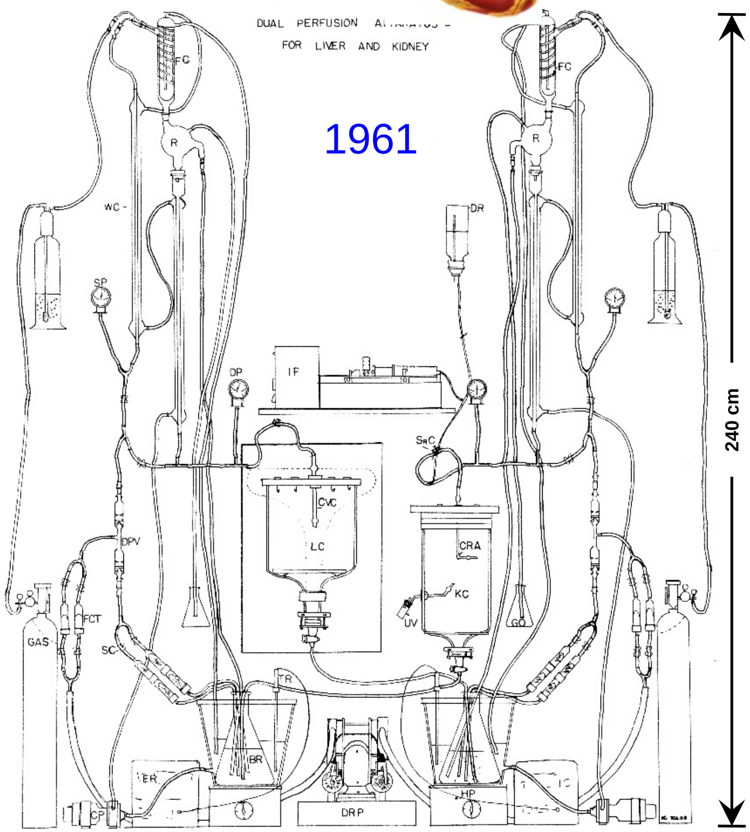
↓

Physiology

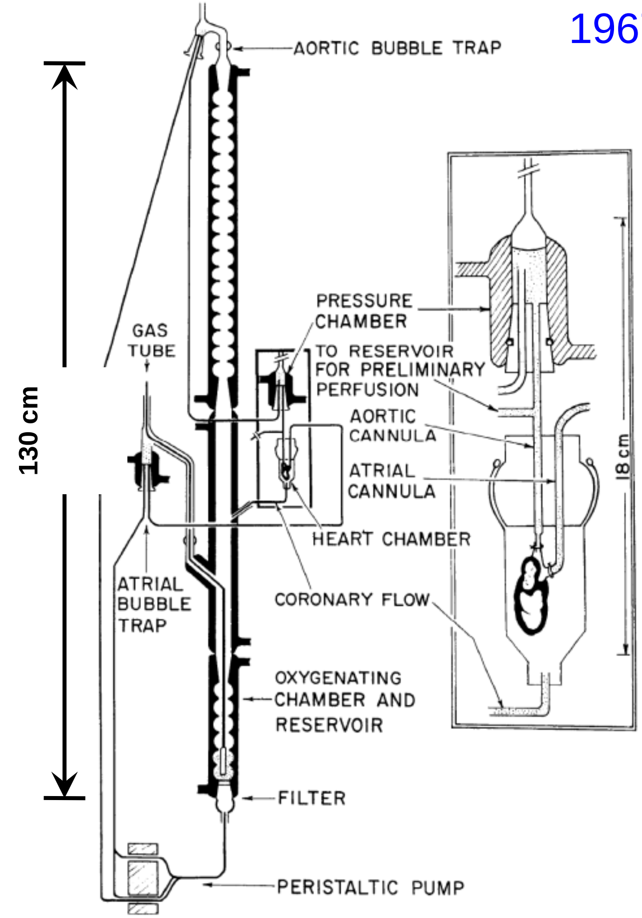
Animal



Organ



Avis FR. Investigations of liver and kidney. Design of a dual apparatus for research study. Science Teacher 1961;28(1):14-8.



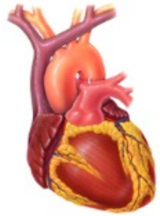
- Neely JR, Liebermeister H, Battersby EJ, Morgan HE. Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 1967 Apr 1;212(4):804-14.
- Neely JR, Liebermeister H, Morgan HE. Effect of pressure development on membrane transport of glucose in isolated rat heart. Am J Physiol 1967;212(4):815-22.

Isolated Organs

Anatomy

Physiology

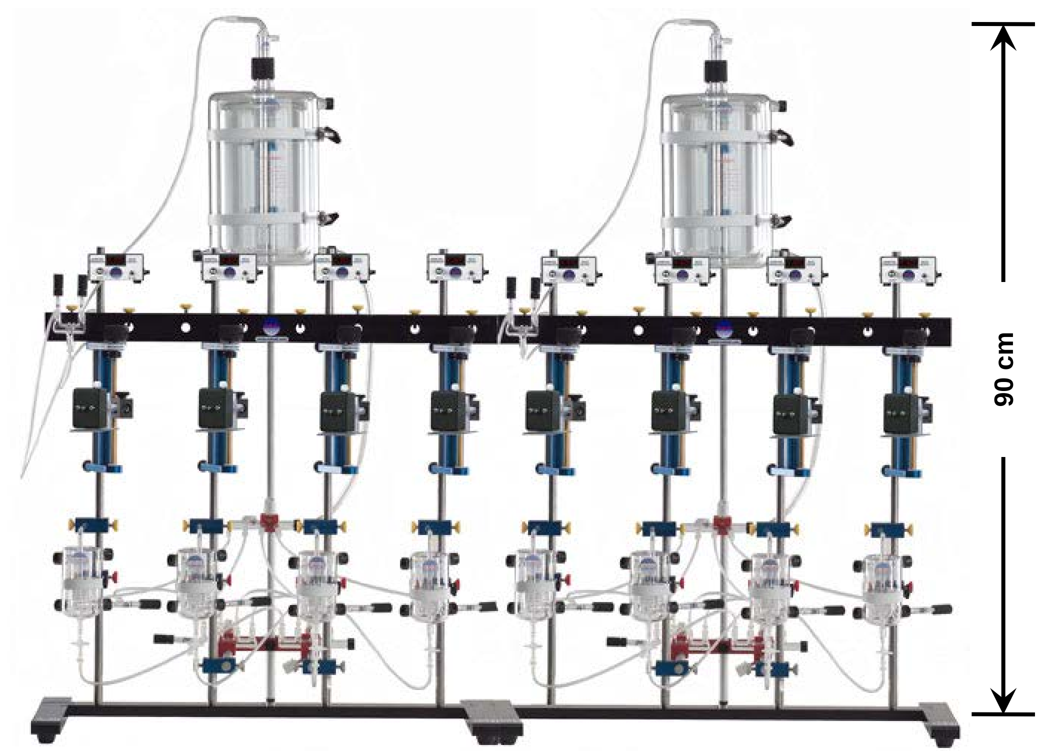
Animal



Organ

Today

Holzer JR, Fong LE, Sidorov VY, Wikswo JP and Baudenbacher FJ. High resolution magnetic images of planar wave fronts reveal bidomain properties of cardiac tissue. Biophys.J. 87 (6):4326-4332, 2004.



Courtesy of Desmond Radnoti of Radnoti LLC

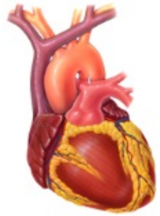
In regular use by academics and pharma

Cultured Cells

Anatomy

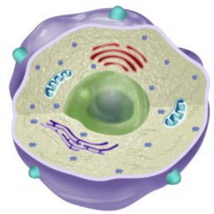
Animal

Physiology



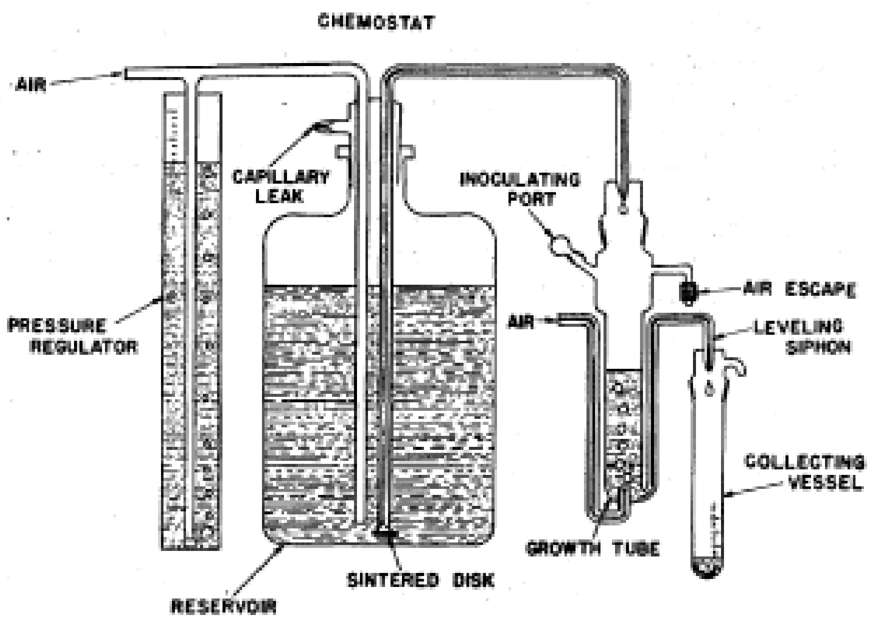
Organ

Cell Biology



Cell

1950's



Henrietta Lacks

http://www.lacksfamily.net/images/image359.jpg

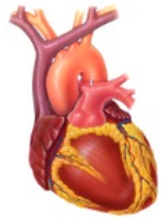
- Novick A and Szilard L. Experiments with the Chemostat on Spontaneous Mutations of Bacteria. PNAS 36 (12):708-719, 1950.
- Aaron. Novick and Leo Szilard. Description of the Chemostat. Science 112 (2920):715-716, 1950.

Anatomy

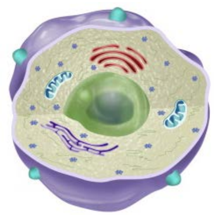
Physiology

Cell Biology

Animal



Organ



Cell

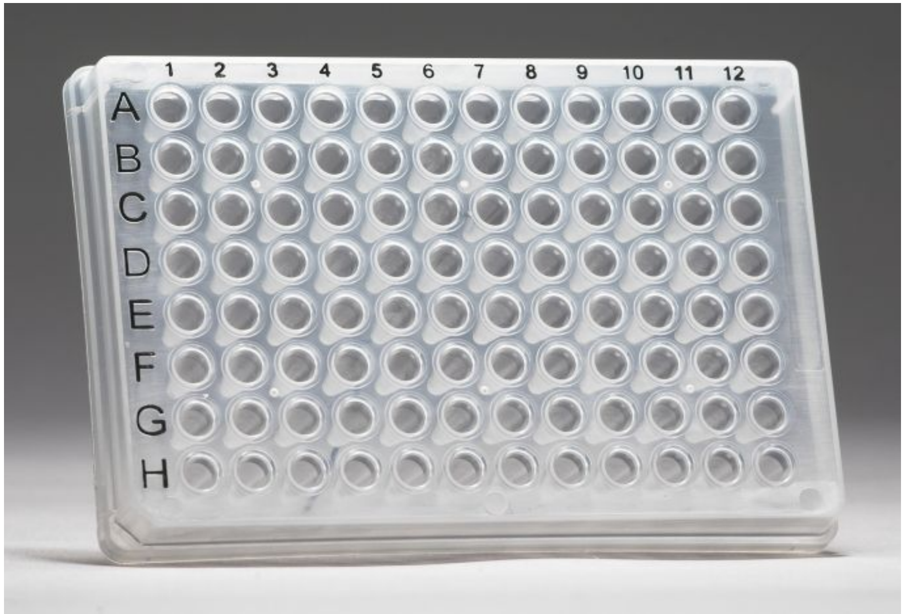
Cultured Cells

VI∫BRE

glasslaboratory.com/files/2245127/uploaded/GL-P100%20Petri%20Dish.jpg

Today

tpp.ch/page/bilder/Produkte/TC_flasks_standard/flasks_all2.jpg



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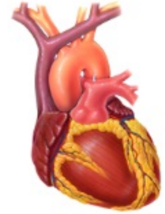
Anatomy

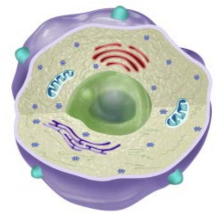
Animal

Physiology



Organ

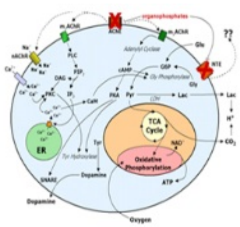
Cell Biology



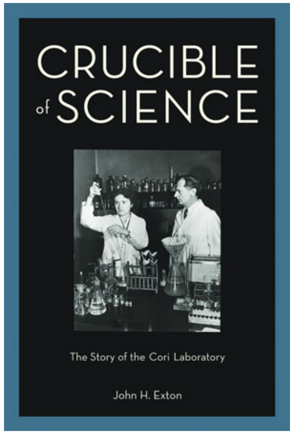
Cell

1930's to Today

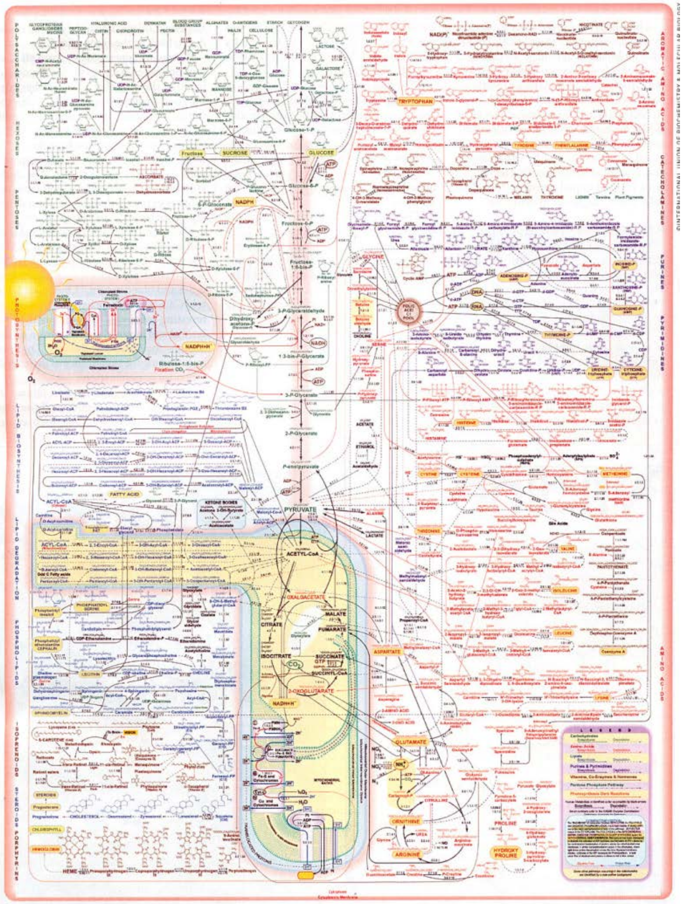
Molecular Biology



Network



Crucible of Science: The Story of the Cori Laboratory, John H. Exton, Oxford, 2013

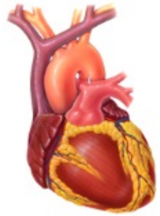


http://www.iubmb-nicholson.org/chart.html

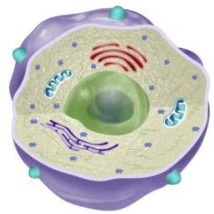
Anatomy

Animal

Physiology

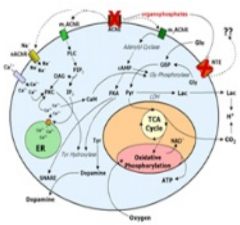


Organ



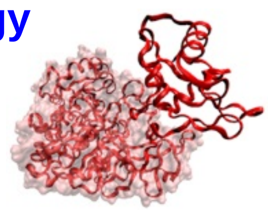
Cell

Cell Biology



Network

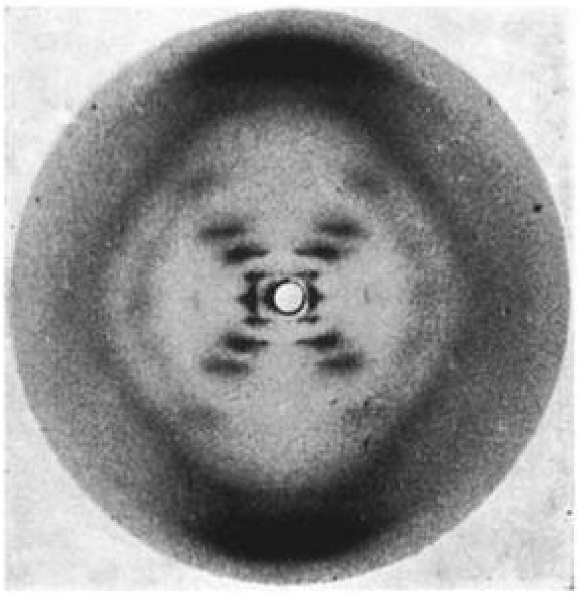
Molecular Biology



Molecule

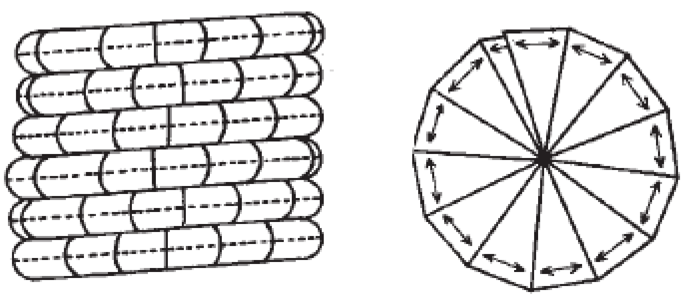
Structural Biology

1952



http://www.insight.mrc.ac.uk/2013/04/25/behind-the-picture-photo-51/

1955



Rosalind E. Franklin. Structure of Tobacco Mosaic Virus. Nature 175 (4452):379-381, 1955.

Anatomy

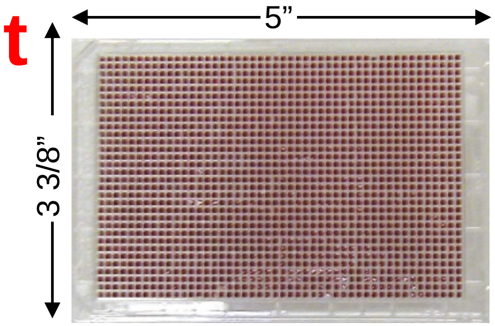
Animal

Automated high throughput screening

Physiology

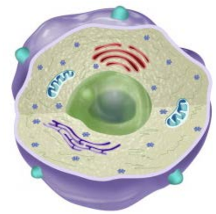
Organ

Today

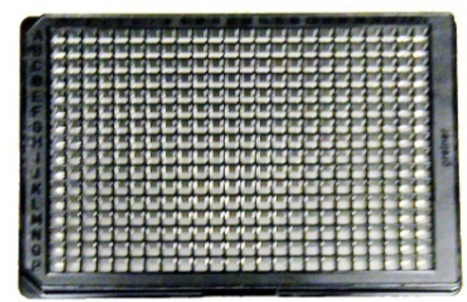


1536 Well ~8 μl

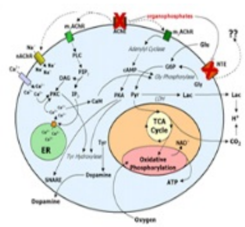
Cell Biology



Cell

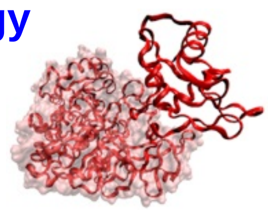


384 Well ~40 μl



Network

Molecular Biology



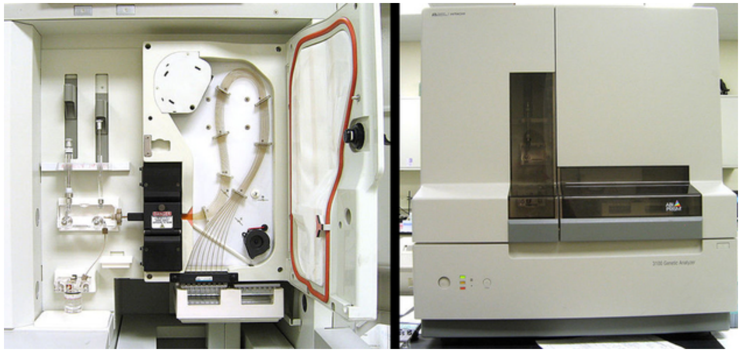
Molecule

Structural Biology

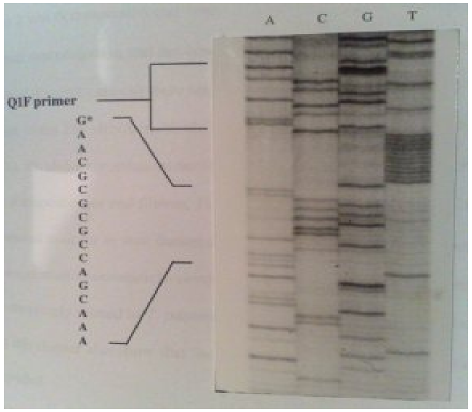
Images courtesy of
Dr. David Weaver, Vanderbilt VICB High-Throughput Screening Facility

The Human Genome Sequenced

2003



http://www.wired.com/2008/07/british-institu/

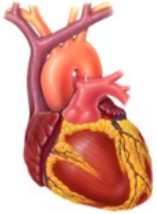


http://nsaunders.wordpress.com/2011/12/22/sequencing-for-relics-from-the-sanger-era-part-1-getting-the-raw-data/

Anatomy

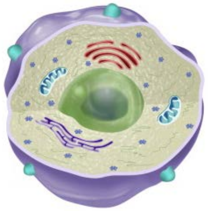
Animal

Physiology

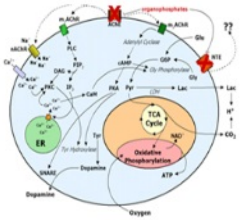


Organ

Cell Biology

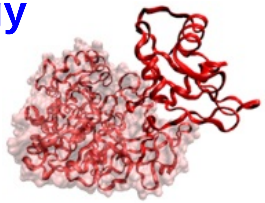


Cell



Network

Molecular Biology



Molecule

Structural Biology

Genome

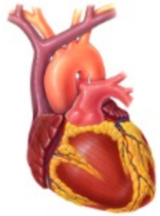
Genomics

The $1,000 Genome

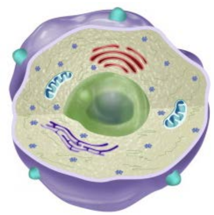
Anatomy

Animal

Physiology

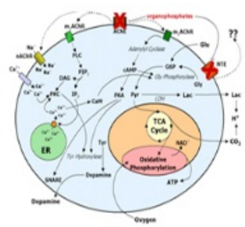


Organ



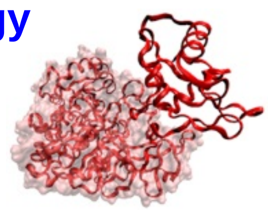
Cell

Cell Biology



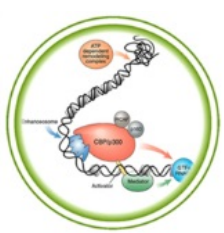
Network

Molecular Biology



Molecule

Structural Biology



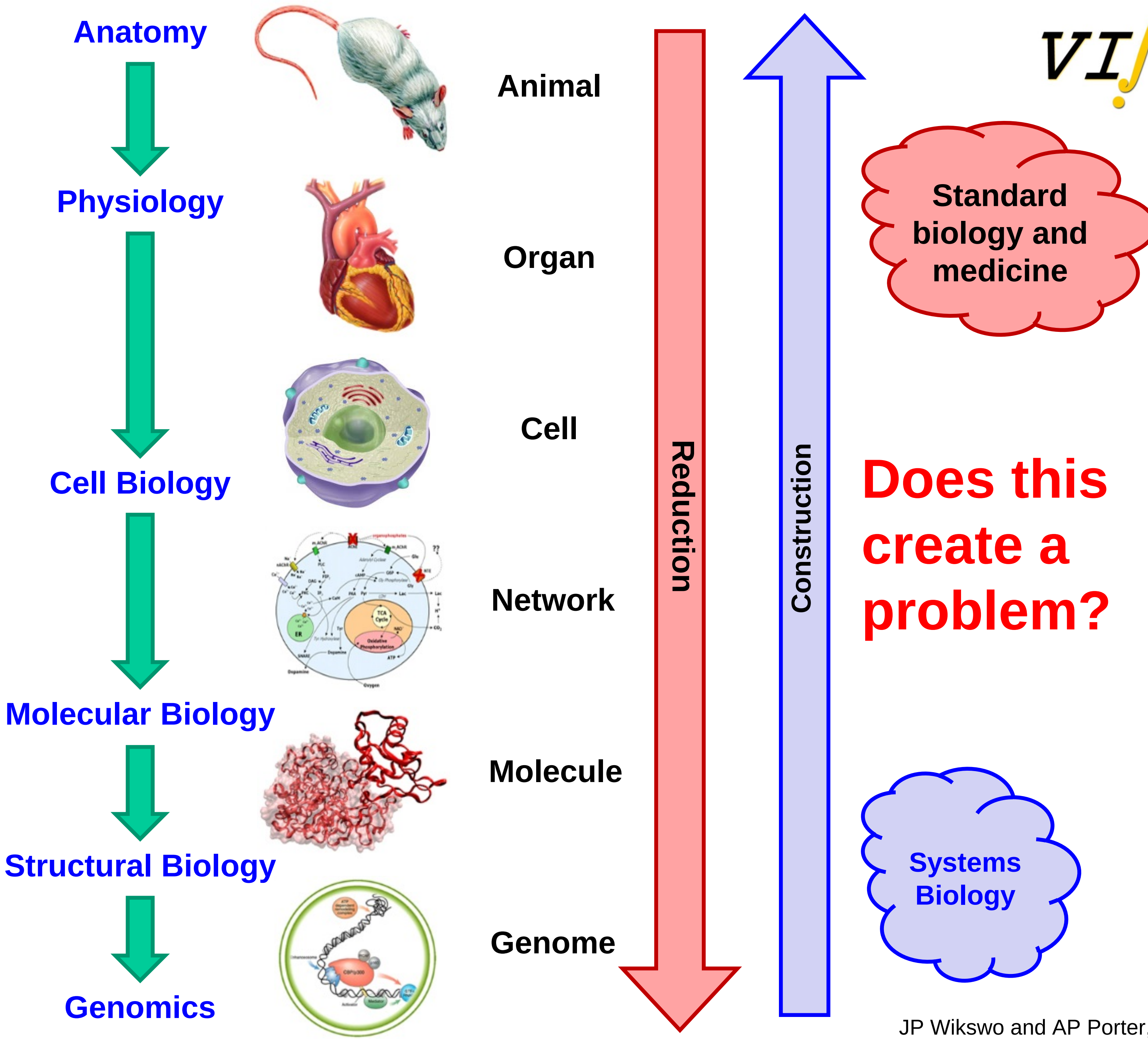
Genome

Genomics

Today

1000 Gb max output, 4000M max read number, 2x125bp max read length, 600 gigabases (Gb) per day per system
http://systems.illumina.com/systems/hiseq_2500_1500.ilmn

Conclusion: Technology drives major advances in biology!



JP Wikswo and AP Porter, *EBM*, 2015

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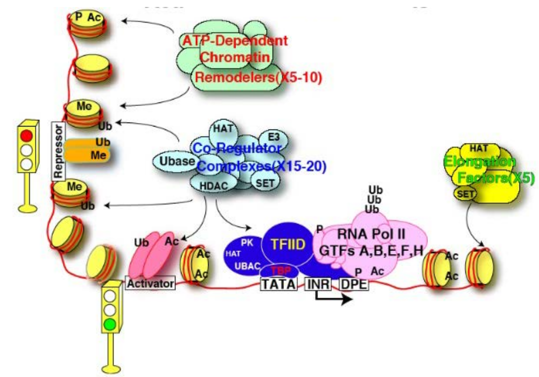
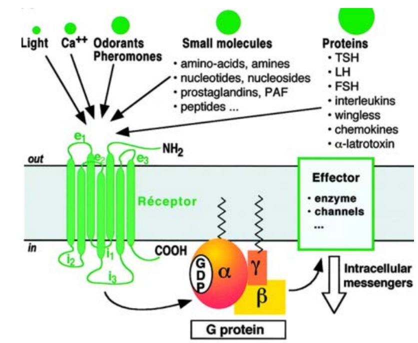
Julia Wikswo

Why is biology so complex?

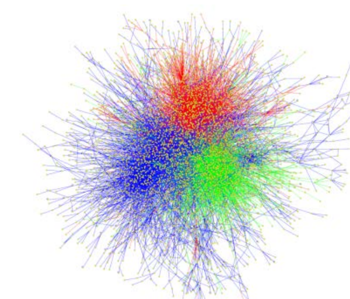
- The human genome codes for ~20,000 unique proteins
- RNA splicing and proteolysis may result in 50,000 to 500,000 unique proteins.
- Post-translational modifications may increase this to a million ….
- An individual cell expresses between 10,000 to 15,000 different proteins at any one time.
- Proteins interact with each other.



Transcription Regulation - Tony Weil



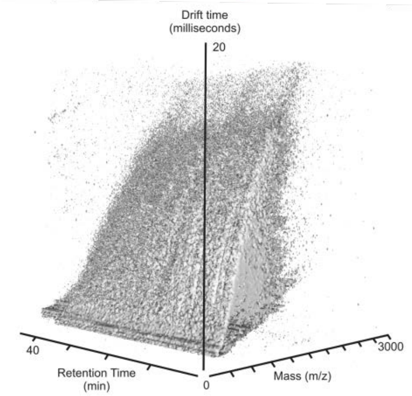
G-coupled protein receptors – Heidi Hamm



Human proteome, and its binding interactions - Simonis and Vidal.

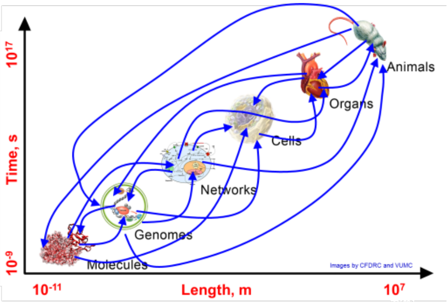
Why is biology so complex, con't?

- Today, one can easily detect 100,000 chemical species in 100 μL of rat serum.
- Cells are NOT well-stirred bioreactors but have anomalous diffusion and active transport.
- 10^9 - 10^{11} interacting cells in some organs.
- We must consider the microbiome.
- Cell signaling is dynamic, non-linear, multiscale, redundant, and has positive and negative feedback.



UPLC-nESI-IM-MS John McLean

3.1 x 3.2 x 1.2 μm^3 beta cell
Brad Marsh, PNAS, 2001



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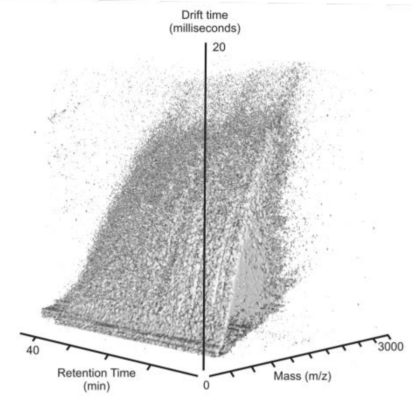
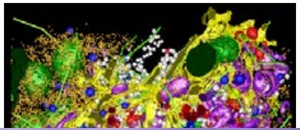
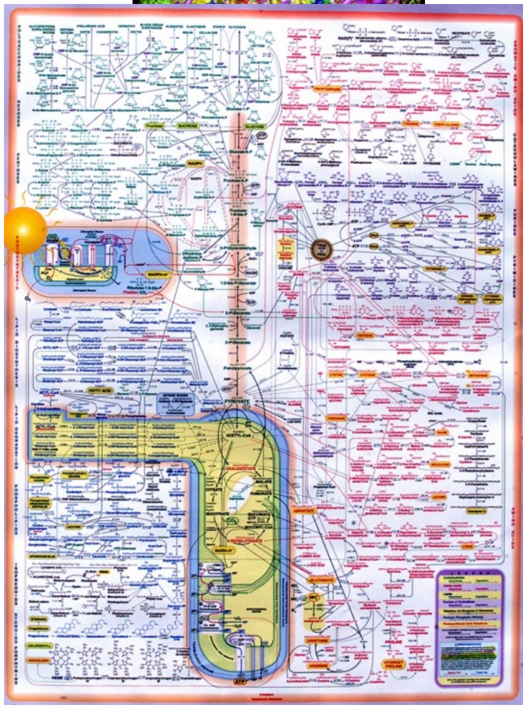
- 10^9 - 10^{11} interacting cells in some organs.
- We must consider the microbiome.
- Cell signaling is dynamic, non-linear, multiscale, redundant, and has positive and negative feedback.
- Metabolism has 8,000 reactions.
- Models might need Avogadro's number of PDEs, *i.e.*, a Leibniz of PDEs (1 L = N_a).
- We need new experimental approaches.



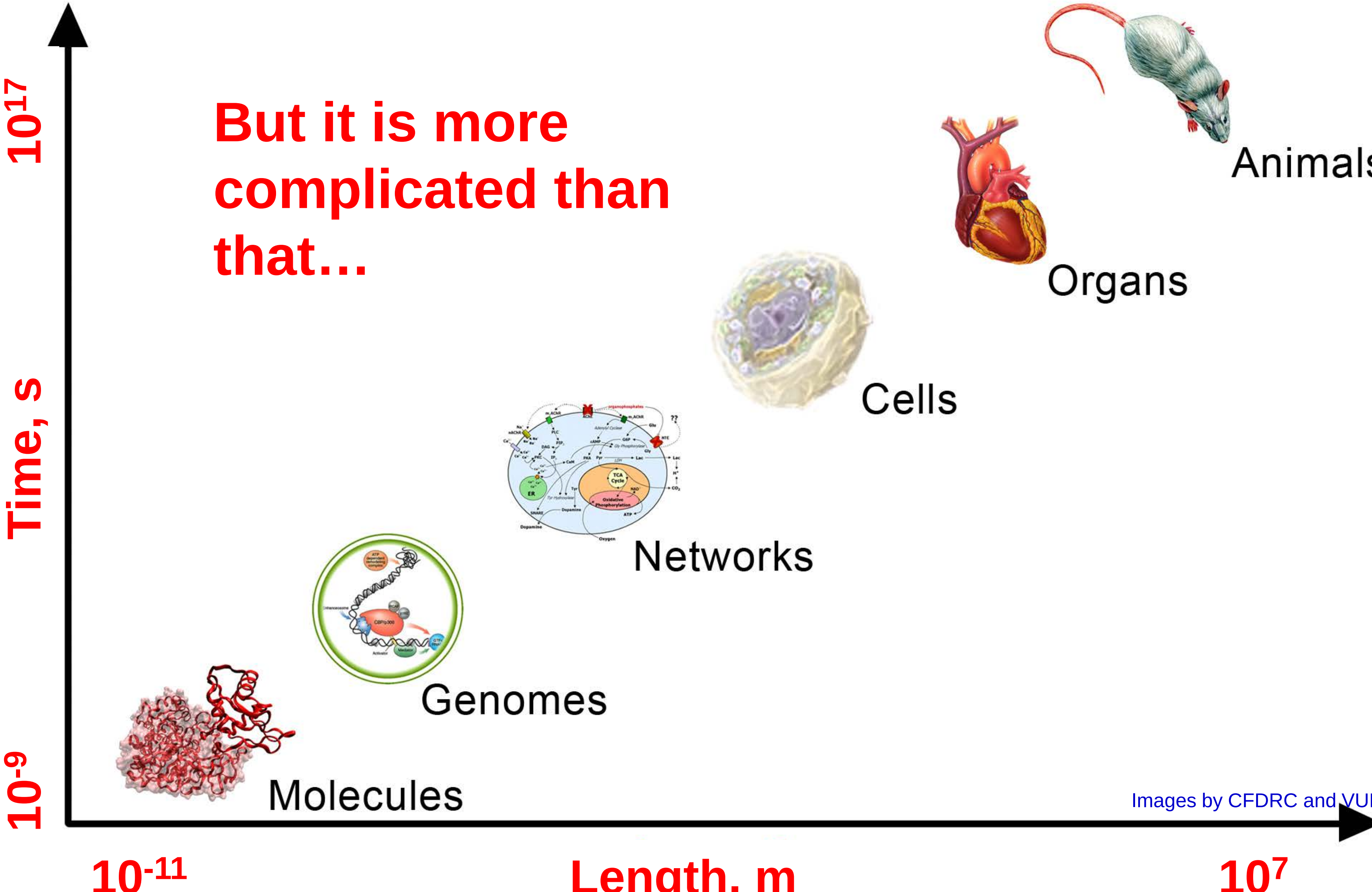
UPLC-nESI-IM-MS John McLean





Biology spans lots of space and time

But it is more complicated than that…



Part of the problem is that human biology is COMPLEX.

Organs talk to each other, but we seldom hear what they are saying.

Julia Wikswo

Organs, Organs, Organs

Cardiovascular
Heart
Blood
Blood vessels

Digestive
Salivary glands
Esophagus
Stomach
Liver
Gallbladder
Pancreas

Excretory
Kidneys
Ureters
Bladder
Urethra

Immune
Leukocytes
Tonsils
Adenoids
Thymus

Reproductive
Ovaries
Fallopian tubes
Uterus
Vagina
Mammary glands
Testes
Vas deferens
Seminal vesicles
Prostate

Julia Wikswo

Intestines
Colon
Rectum
Anus

Endocrine
Hypothalamus
Pituitary gland
Pineal gland
Thyroid
Parathyroids
Adrenals

Spleen
Appendix

Integumentary
Skin
Hair
Nails

Muscular
Muscles
Golgi tendon organ

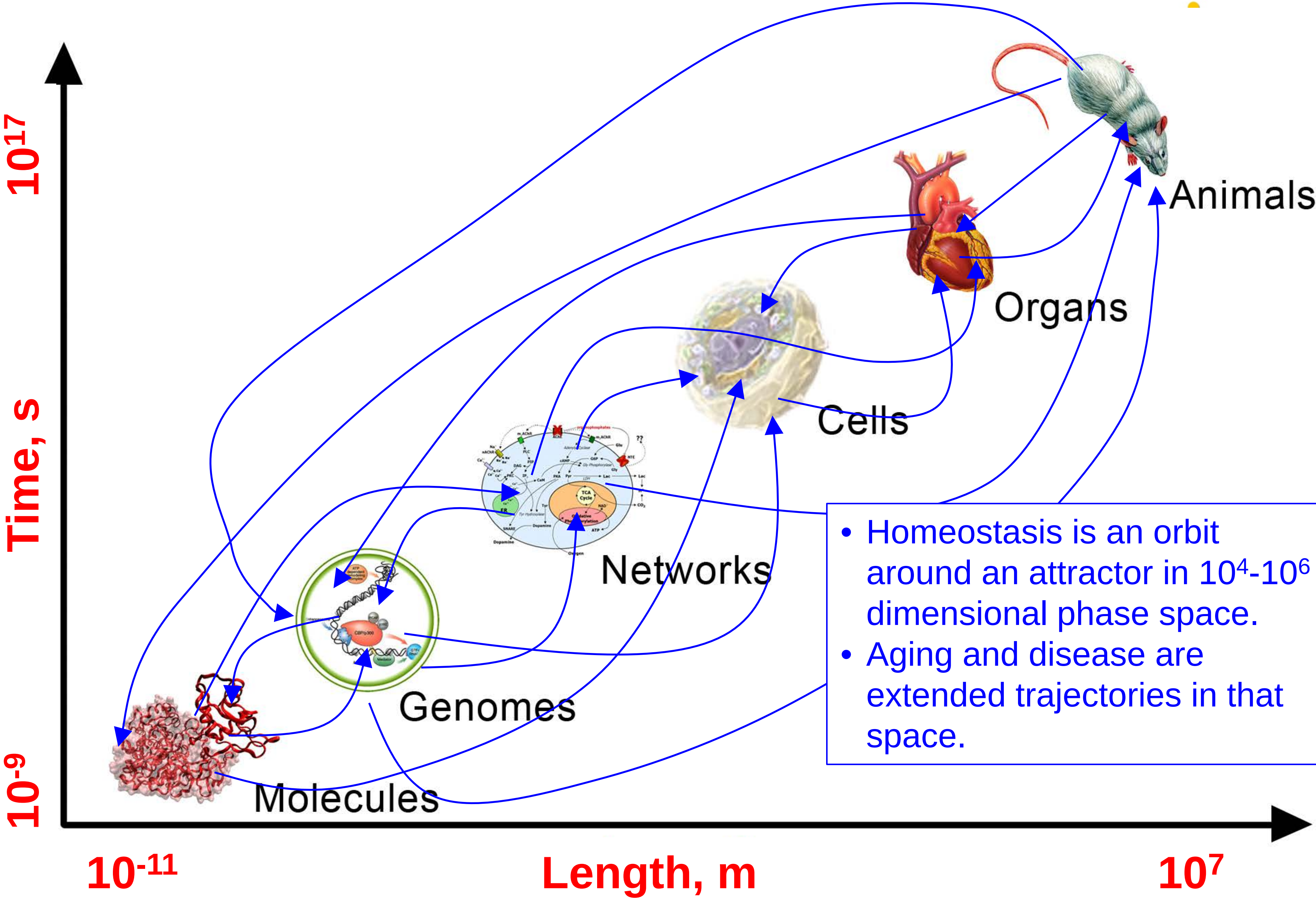
Nervous
Brain
Spinal cord
Nerves
Eyes

Penis

Respiratory
Pharynx
larynx
Trachea
Bronchi
Lungs
Diaphragm

Skeletal
Bones
Cartilage
Ligaments
Tendons

Complexity from multiscale interactions



- Homeostasis is an orbit around an attractor in 10^4-10^6 dimensional phase space.
- Aging and disease are extended trajectories in that space.

A possible failure mode

<u>Ontological failure</u>: 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…

It's the numbers….

Where do we get a mole of numbers?

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**
- Hence we need to **experiment…**

Seven Themes

1. A brief history of biology
2. Just how complex is biology?
3. **What is the role of physics in understanding the complexity of biology?**
4. Can sensors, actuators, controllers and robot scientists help address biological complexity
5. How might Organoids and Organs on Chips change the way we study biology?
6. What does Multi-Omics offer?
7. Closing the circle

Julia Wikswo

Helmholtz on Cells

"The behavior of living cells should be accountable in terms of motions of molecules acting under certain fixed force laws."

Herman von Helmholtz, 1870

Quoted in Max Delbruck, "A Physicist looks at biology" Trans. Conn. Acad. Arts Sci. 38:173-190, 1949.

Bohr on Complementarity

"… we should doubtless kill an animal if we tried to carry the investigation of its organs so far that we could describe the role played by single atoms in vital functions. In every experiment on living organisms, there must remain an uncertainty as regards the physical conditions to which they are subjected, and the idea suggests itself that the minimal freedom we must allow the organism in this respect is just large enough to permit it, so to say, to hide its ultimate secrets from us."

N. Bohr. "Light and Life,"
Nature 131 (3309):457-459, 1933.

Bohr on Complementarity

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N. Bohr. "Light and Life,"
Nature 131 (3309):457-459, 1933.

Schrödinger on Life

"Present day physics and chemistry could not possibly account for what happens in space and time within a living organism."

"… a living organism … can only keep … alive … by continually drawing from its environment negative entropy."

Schrödinger, *What is Life*, 1943

Perutz on Schrödinger

"When I was invited to review the influence of *What is Life?* I accepted with the intention of doing honor to Schrödinger's memory. To my disappointment, a close study of his book and of the related literature has shown me that what was true in his book was not original, and most of what was original was known not to be true even when it was written."

M. F. Perutz. Physics and the riddle of life. Nature 326 (6113):555-558, 1987.

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What was original was from Max Delbruck.

What makes biology so different from physics, chemistry, and engineering?

What makes biology different from physics or chemistry?

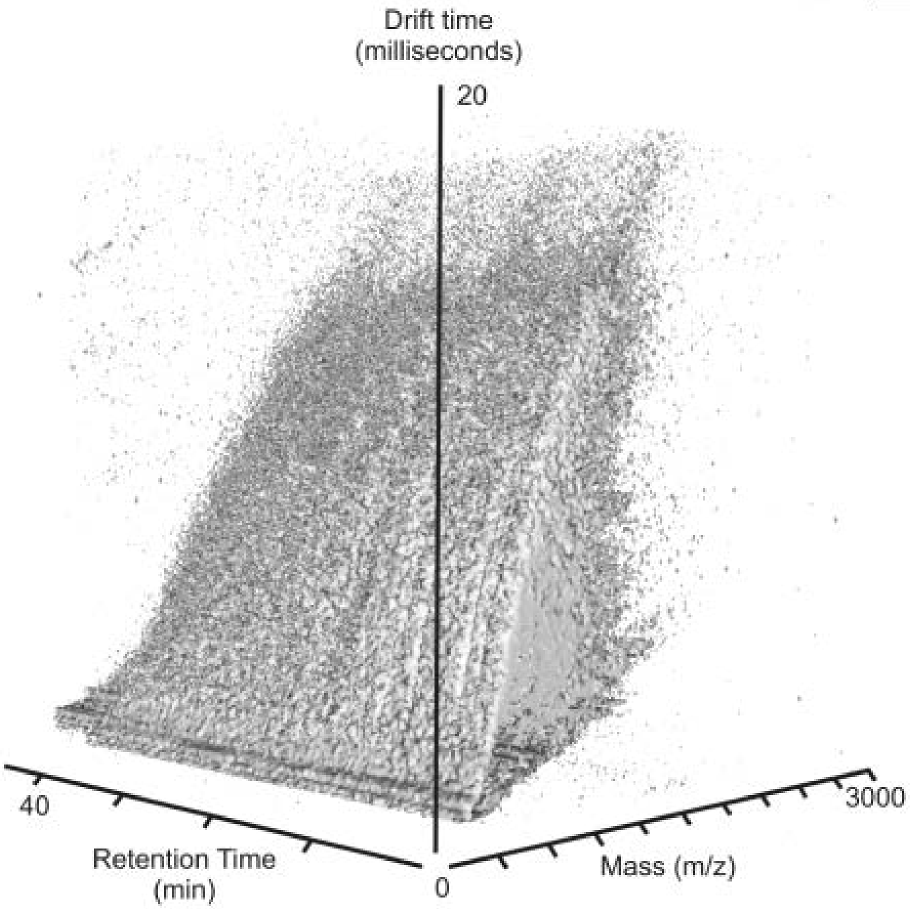
Physics and chemistry describe dynamic interactions in terms of fundamental or phenomenological laws that govern the state of the matter being studied.

*Ohm's law, Hooke's law, the Standard Model, … conservation of mass, Dalton's law, quantum mechanics …

Biology has laws, but the operation of every living organism is determined not only by the laws of biology, physics and chemistry, *but also by historic instructions that may be specific to each individual organism*.

". . . any living cell carries with it the experiences of a billion years of experimentation by its ancestors. You cannot expect to explain so wise an old bird in a few simple words."

Max Delbrück, "A Physicist Looks at Biology," 1949



Build untargeted mass spectrometers, and you will 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).

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

Yet one more Really Hard Problem

Pugh's observation applies to biology:

Human biology may be too complicated for humans to fully comprehend.

John Wikswo

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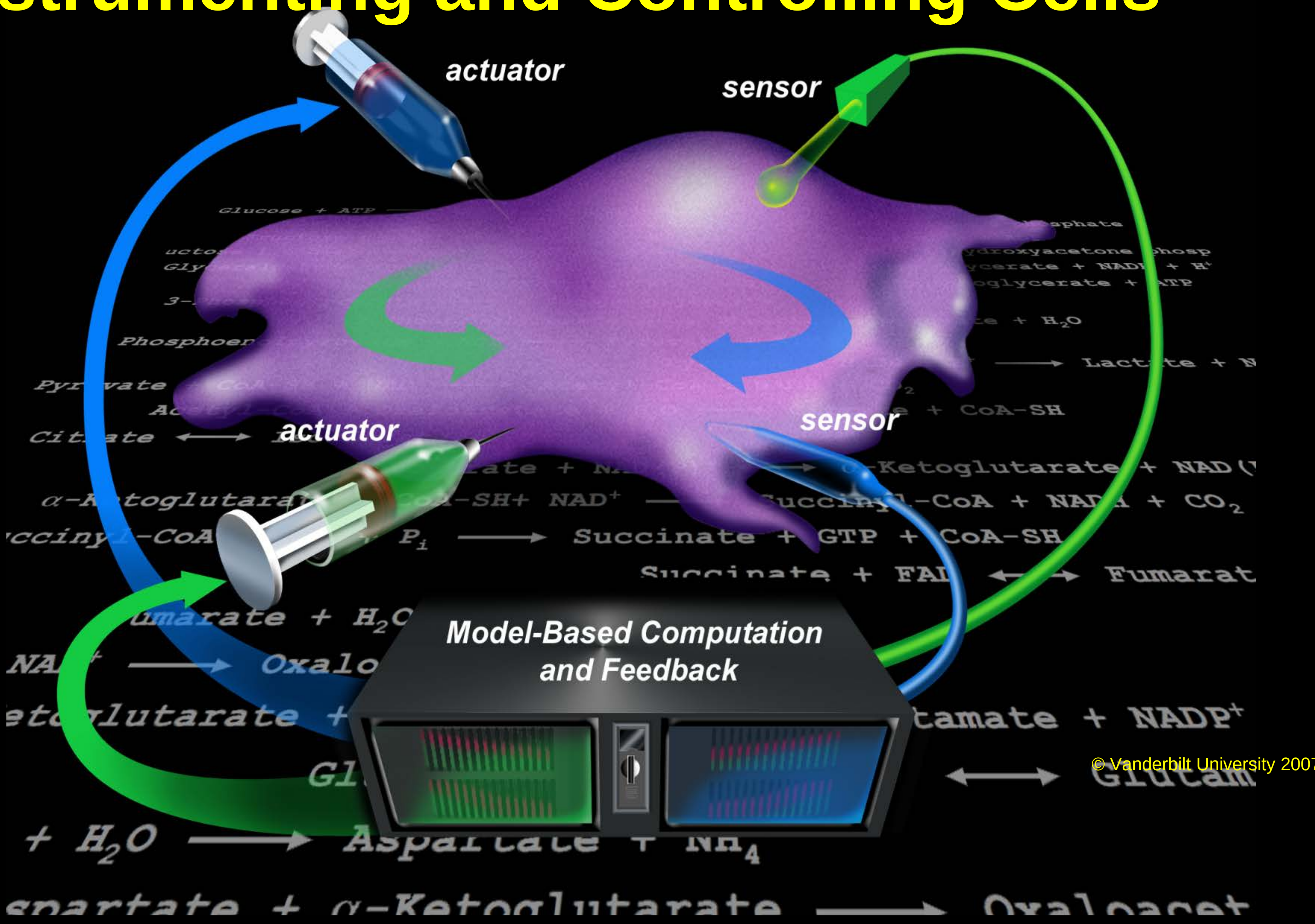
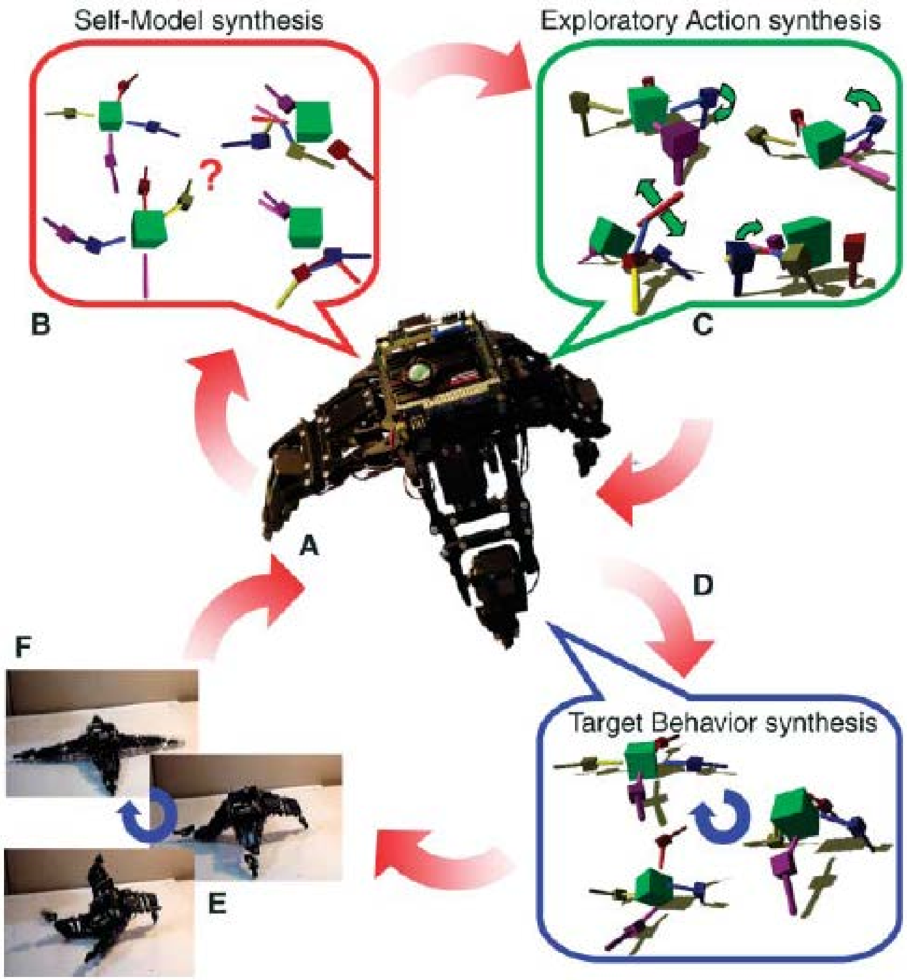
Julia Wikswo

VIIBRE 2001
Instrumenting and Controlling Cells



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



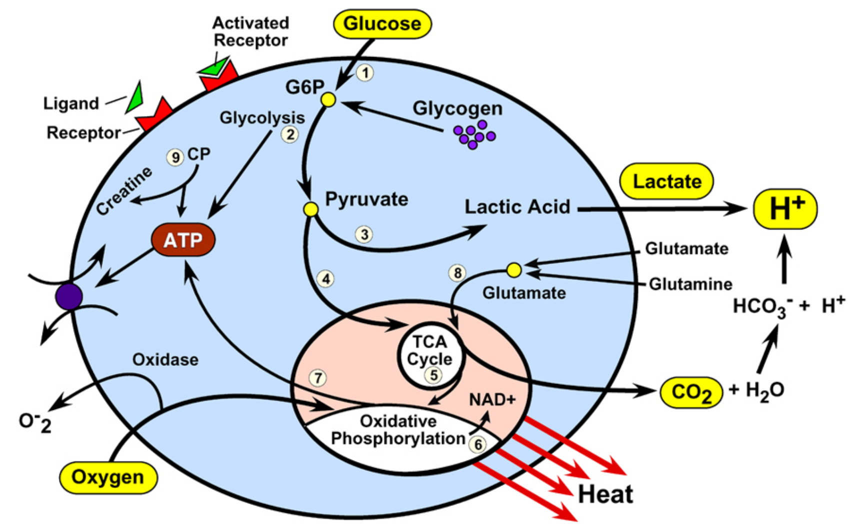
A robot can be programmed to conduct experiments to derive a model of itself.

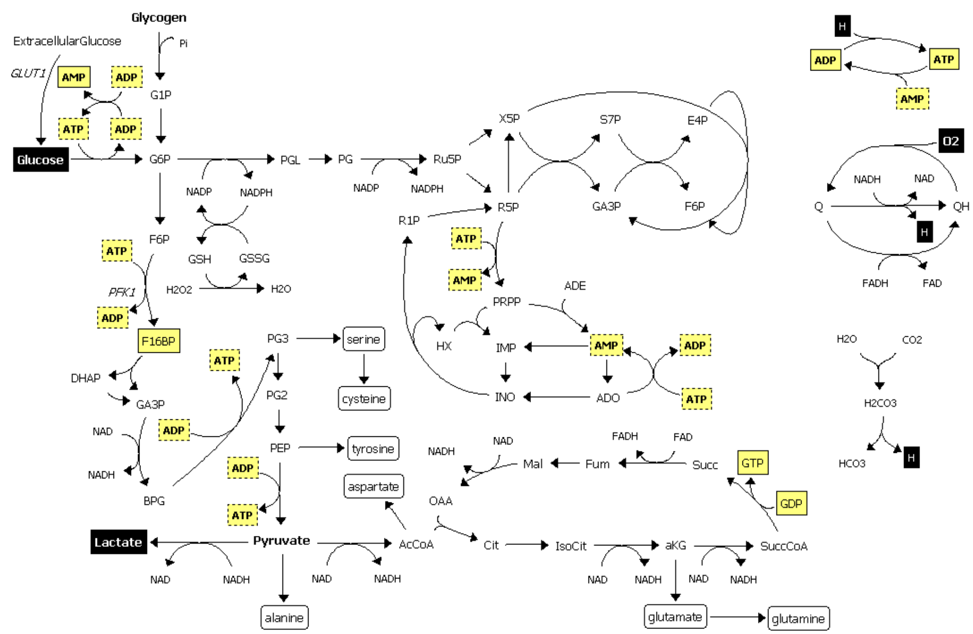
My hypothesis: Machine learning and model inference with automated experimentation can be extended from robots to bioreactors.

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

Note to self: 2006

- Can I 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 cellular dynamics?
- Extracellular: $3 - 4 million and 3 - 5 years
- Intracellular: $15 - 20 million and 5 – 10 years

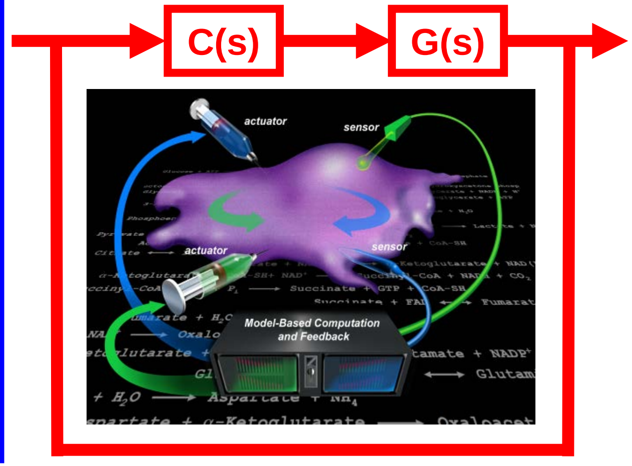




First step – control biology!

INPUT ACTUATORS

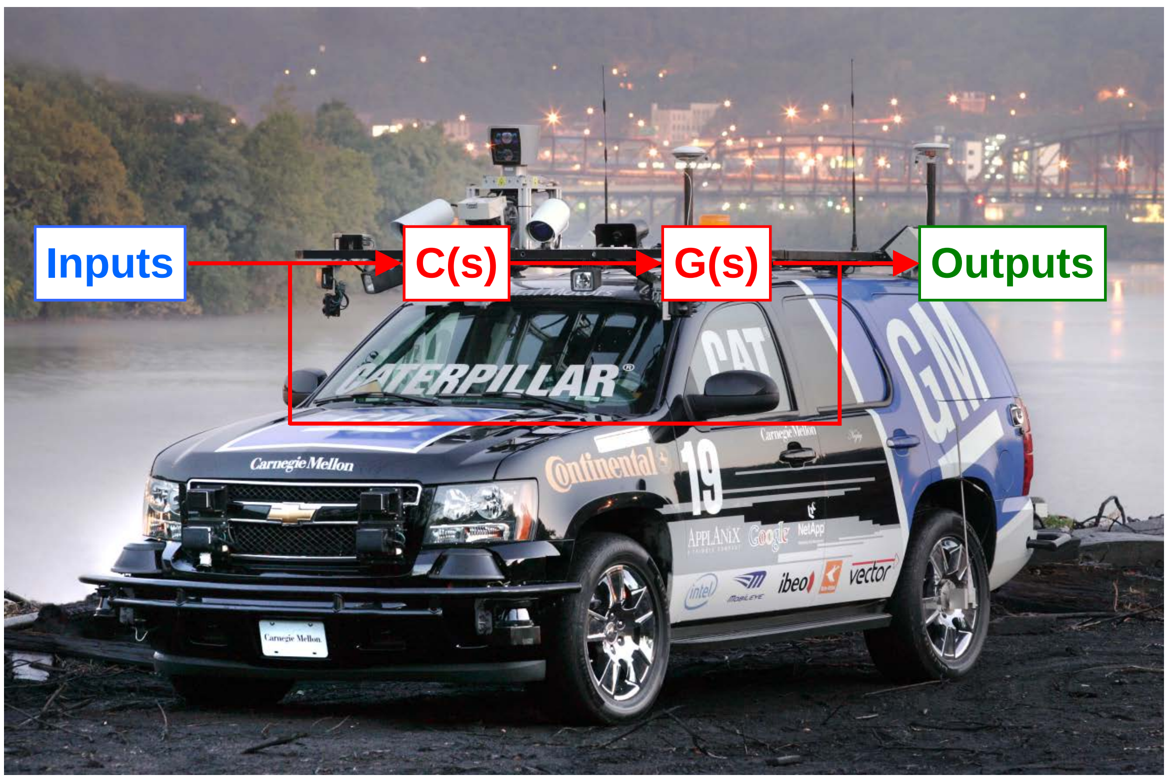
- Chemical
- Electrical
- Genetic
- Mechanical
- Optical
- Thermal
- Scaffolding



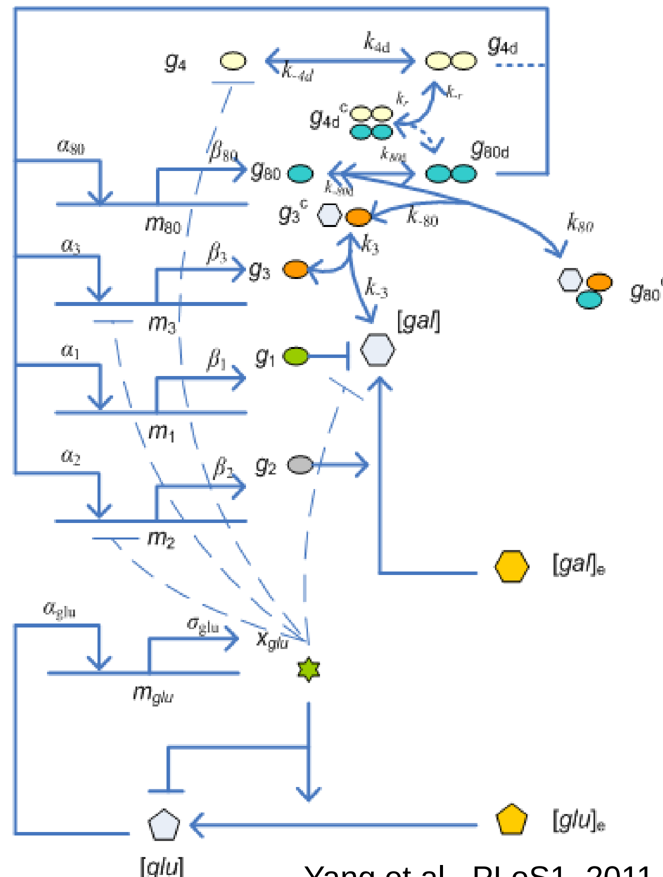
C(s) = Controller G(s) = System Dynamics

OUTPUT SENSORS

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



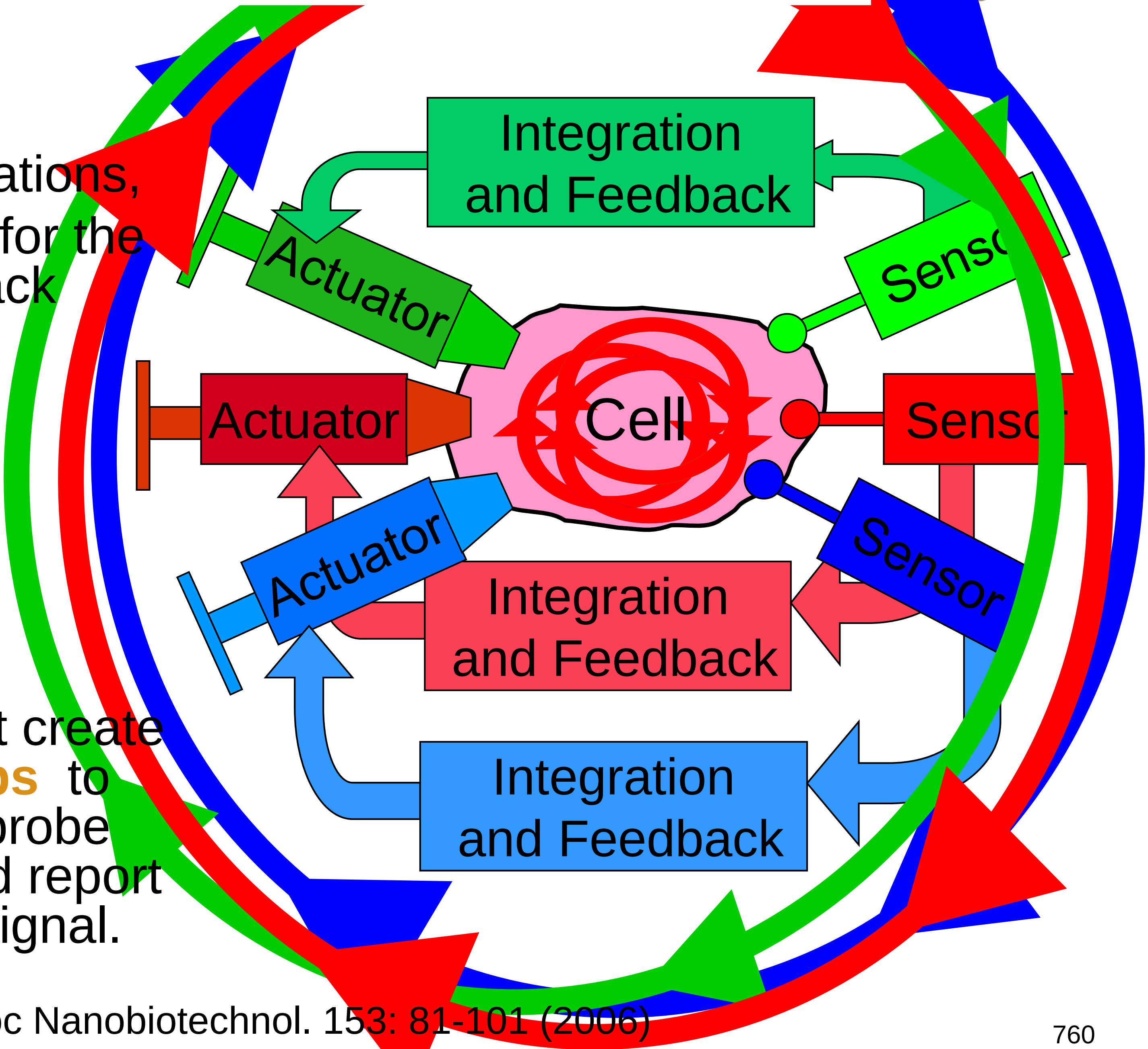
From: LeDuc, Messner, Wikswo. How do controls approaches enter into biology? *Annual Reviews of BME,* 2011.



Yang et al., PLoS1, 2011

Model-driven control of biological systems

- 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)

Artificial Intelligence, per Wikipedia

- **Narrow AI** (or "weak AI“) is the use of software to study or accomplish specific problem solving or reasoning tasks. Weak AI, in contrast to strong AI, does not attempt to simulate the full range of human cognitive abilities. (Siri, Alexa…)

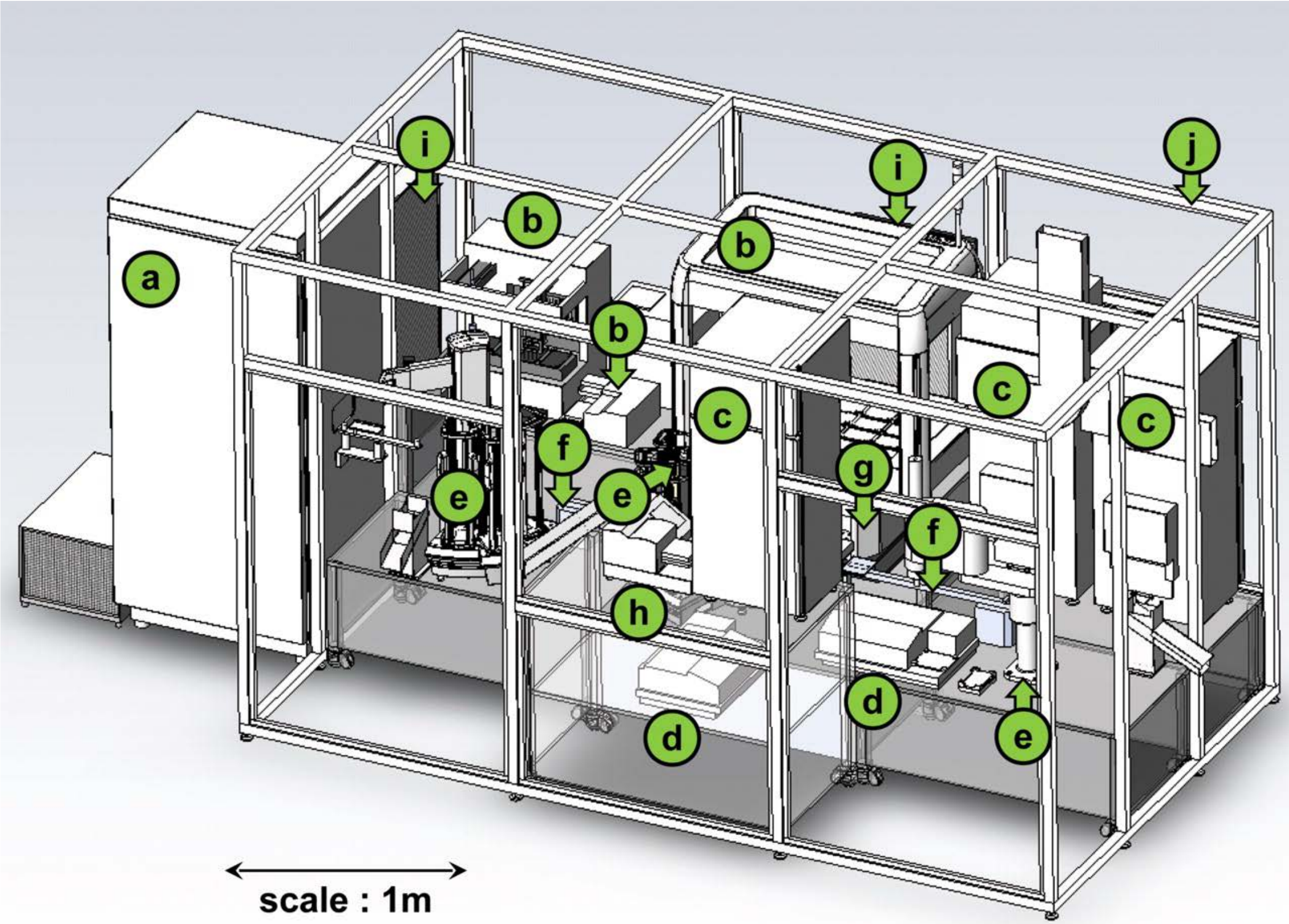
- **Strong AI** is artificial intelligence that matches or exceeds human intelligence — the intelligence of a machine that can successfully perform any intellectual task that a human being can. … Strong AI is associated with traits such as consciousness, sentience, sapience and self-awareness observed in living beings. (Turing test…)

Narrow versus Strong Automated Biology

- **Narrow AB:** the use of software to study or accomplish specific laboratory biology tasks
  - High throughput screening (HTS)
  - Automated microscopy (ImageJ)
  - Biomedical data mining (KEGG, MAGINE, SIMONE)
  - Computer-aided medical diagnosis

- **Strong AB:** automated biological experiments driven by autonomous, machine-learning systems
  - Identification of orphan gene function (King *et al.*)
  - Inference of metabolic networks (Schmidt *et al.*)
  - Optimal design of experiments
  - Development of iPSC differentiation control paradigms

Strong AB: King's Robot Scientist "Adam"

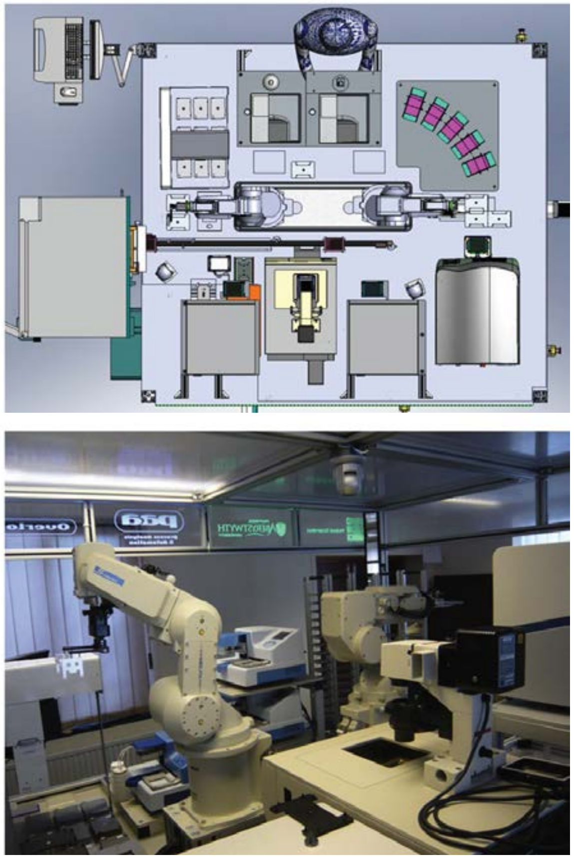


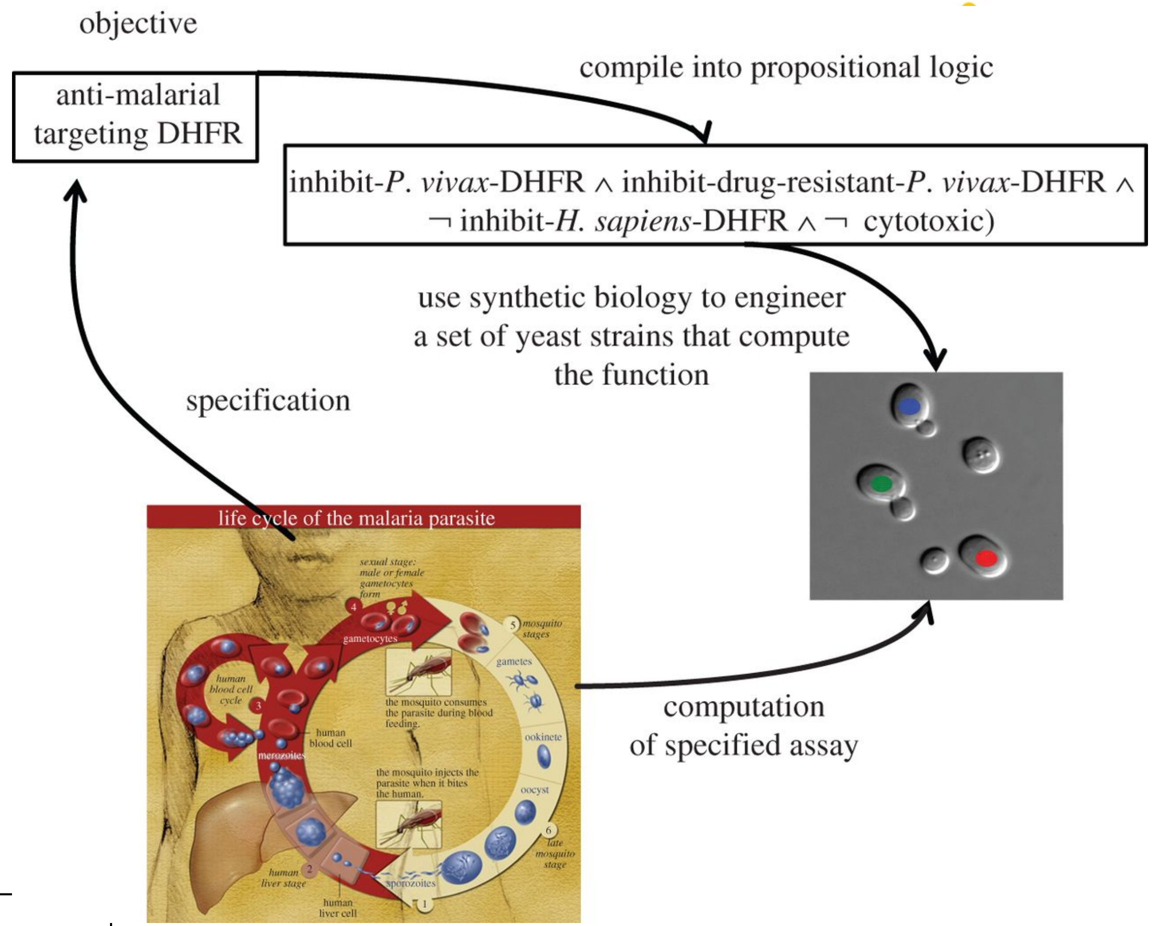
- 6,657,024 optical density measurements@595nm to form 26,495 growth curves
- Formulated and tested 20 hypotheses concerning genes encoding 13 orphan enzymes

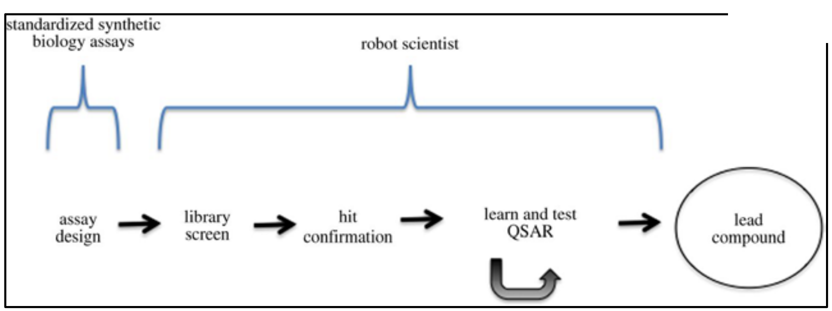
RD King et al. The Automation of Science. Science **324** (5923):85-89, 2009.

Strong AB: King's Robot Scientist "Eve"

(*a*)
(*b*)







Chimeric yeast can inform drug mechanism of action in mammalian cells.

Williams, et al., "Cheaper faster drug development validated by the repositioning of drugs against neglected tropical diseases," Royal Society Interface, 2015

What do we need for automated inference of biological models?

- Closed-loop control of biology
- Automated design of experiments
  - Cells
  - Matrix chemistry and architecture
  - Metabolic and signaling molecules and other clues
- Quantitative measurements
  - Fluorescent imaging
  - Electrochemical measurements of bioenergetics
  - Untargeted ion mobility-mass spectrometry
  - New types of data from new sensing modalities
- Automated data mining and interpretation
- Organotypic 3D tissue culture, including dynamic control of the extracellular matrix

Narrow versus Strong Automated Biology

- **Narrow AB:** the use of software to study or accomplish specific laboratory biology tasks
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Grand Challenge: 2006

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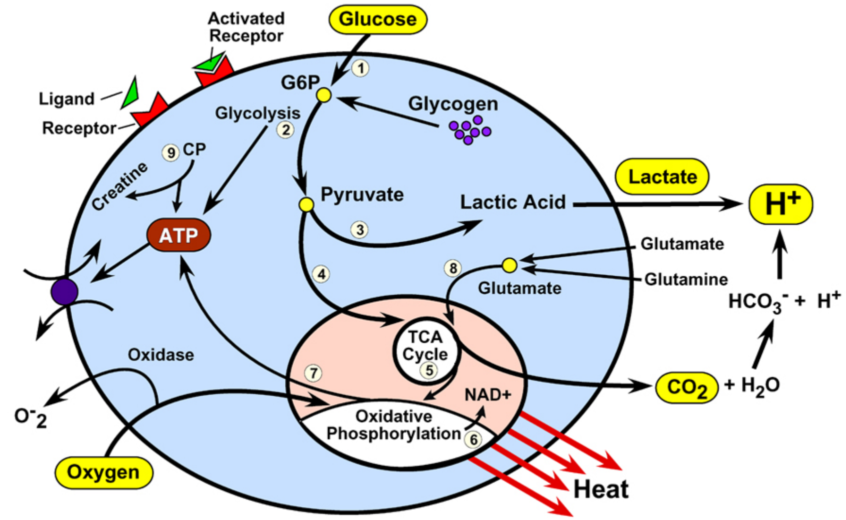
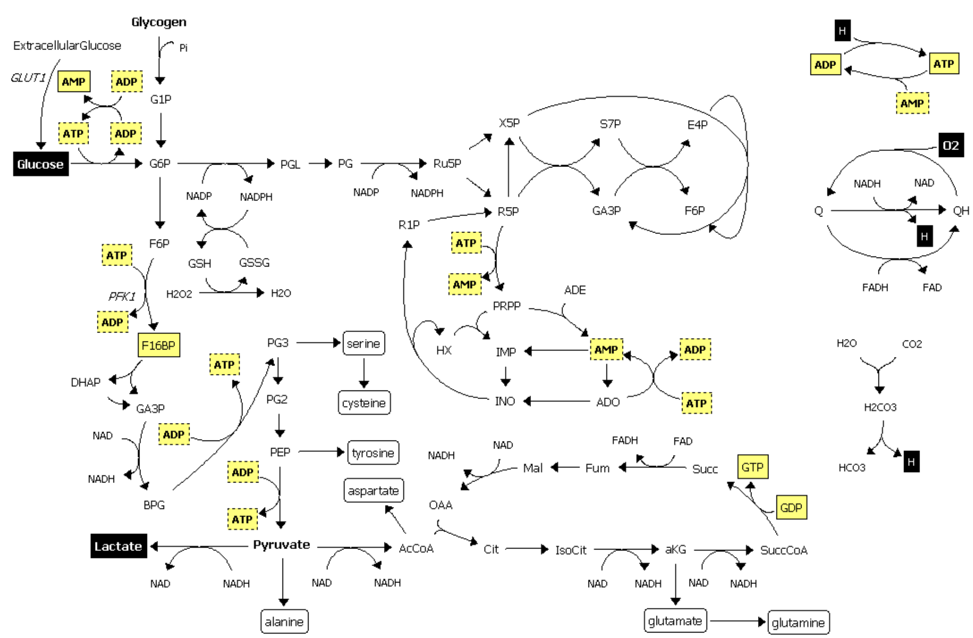
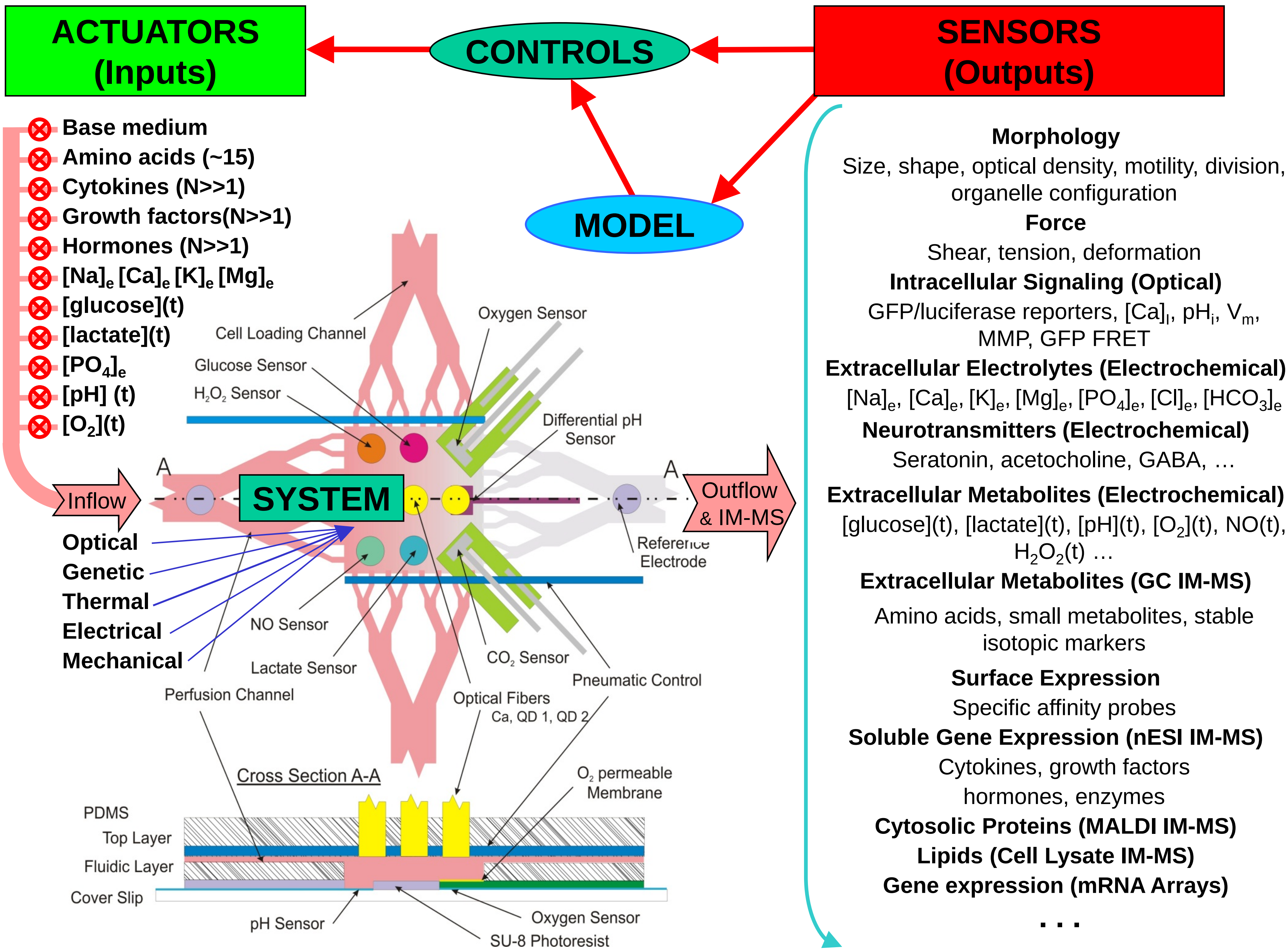






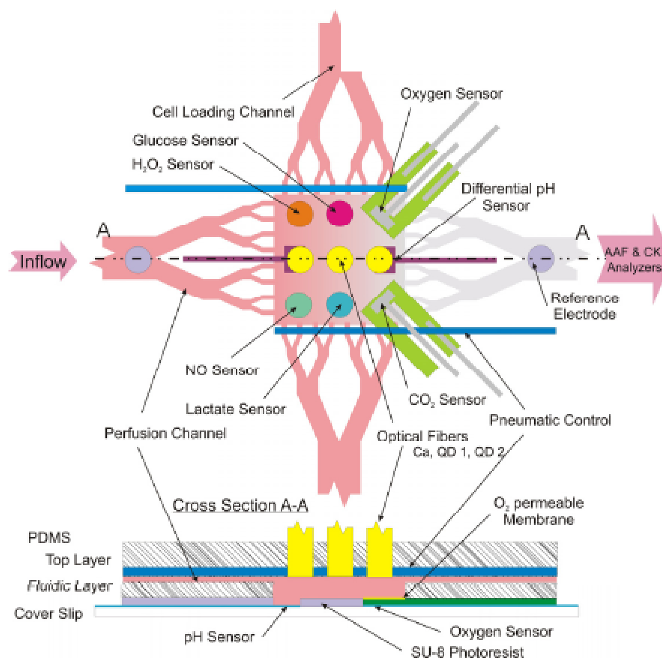
LeDuc, Messner, Wikswo, *Annual Reviews of BME,* 2011.

Computational Synthesis

Symbolic-Regression and Estimation-Exploration Algorithms can design experiments to select best symbolic model

Estimation / Exploration

Symbolic Regression



AMMPS

LeDuc, Messner, Wikswo, *Annual Reviews of BME,* 2011.

Inferring Metabolic Models using the SRA andEEA

Computational Synthesis

Target Model placed in black box with 10% noise

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

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

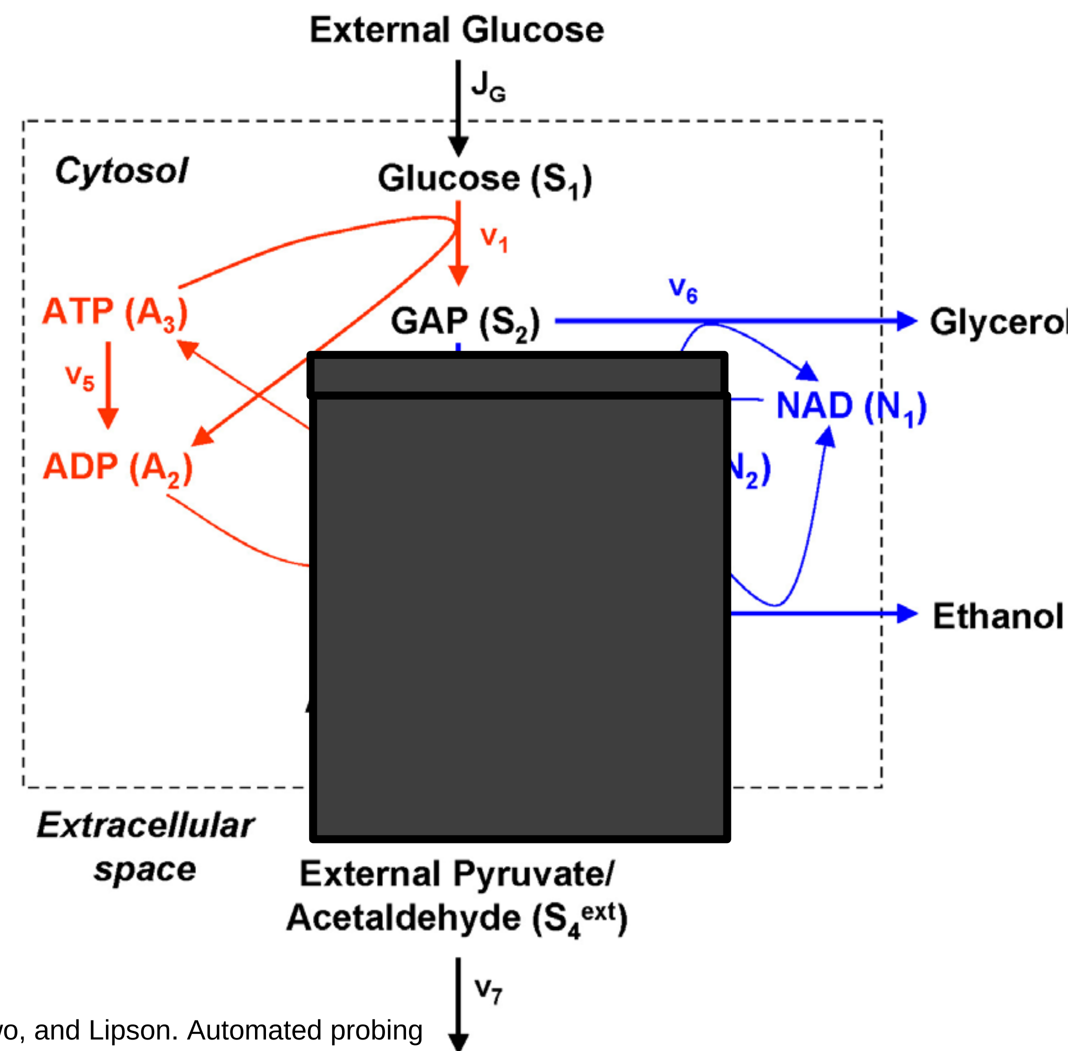
$$\frac{dS_3}{dt} = 6 * S_2 - 6 * N_2 S_2 - 64 * S_3 + 16 * A_3 S_3$$

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

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

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

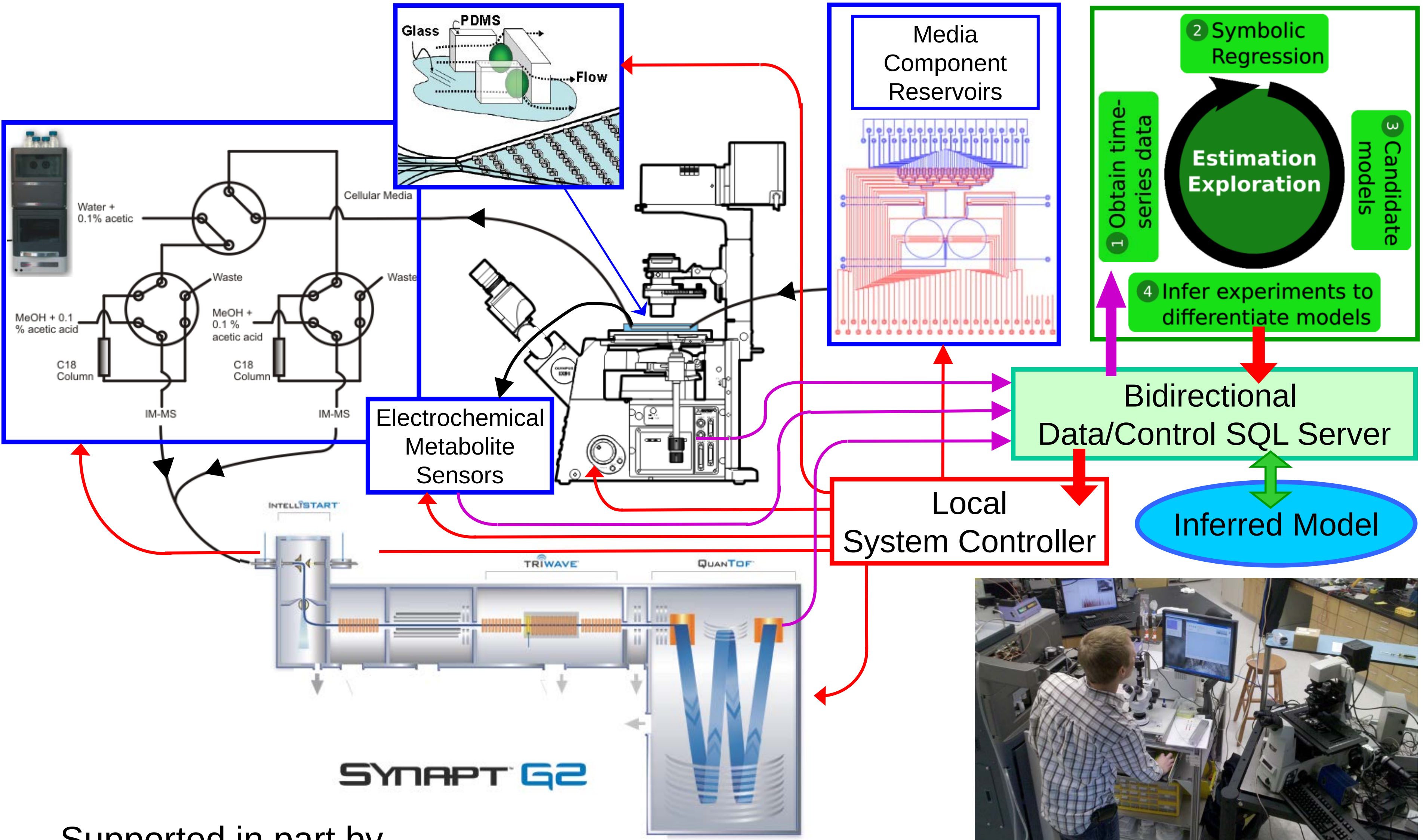
$$\frac{dS_5}{dt} = 1.3 * S_4 - 3.1 * S_5$$



Schmidt, Vallabhajosyula, Jenkins, Hood, Soni, Wikswo, and Lipson. Automated probing and inference of analytical models for metabolic networks. Phys.Biol. 8 (5):055011, 2011.

Model adapted from P. Ruoff et al. Biophysical Chemistry 106 (2003) 179-192

2014: VIIBRE's Robot Scientist for Automated Omni-Omics



Supported in part by DTRA and NIH/NIDA

Cornell's robot can control VIIBRE's robot microscope!

So how do we implement the biology for our Robot Scientist?

How have we been studying biology?

- <u>People</u>

  We are severely limited in isogenetic controls, interventions, and data when studying normal subjects and patients.

- <u>Animals</u>

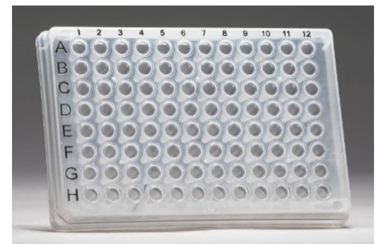
  Animals, including non-human primates, are not people and have significant genetic and physiological differences.

- <u>Cells *in vitro*</u>

  2D biology on plastic: Many biological experiments are conducted on cells that
  - have cancer,
  - are inbred,
  - are diabetic,
  - are potatoes on a <u>stiff</u> plastic couch without exercise
  - enjoy neither gender nor sex,
  - live almost entirely in the dark,
  - gorge themselves on sugar once a day,
  - may be slowly suffocating in an increasingly acidic environment,
  - live in their own excrement,
  - never bury their dead,
  - may take a complete or only partial bath every day or two,
  - and talk only to cells of like mind.

glasslaboratory.com/files/2245127/uploaded/GL-P100%20Petri%20Dish.jpg

tpp.ch/page/bilder/Produkte/TC_flasks_standard/flasks_all2.jpg



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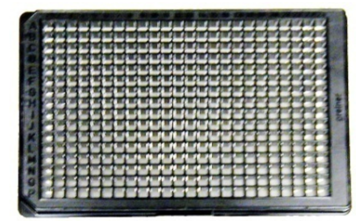
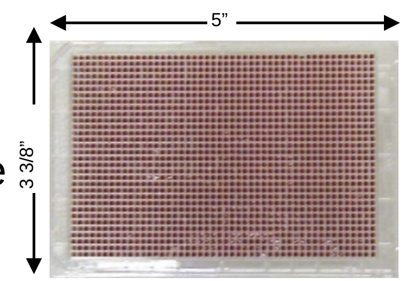


384 Well ~40 µl



1536 Well ~8 µl

384 and 1536 images courtesy of David Weaver

One might get reproducible, statistically significant results, but are they relevant to human biology and disease?

Watson, Hunziker, and Wikswo, Exper. Biol. and Med., 2017

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Julia Wikswo

Circa 2011, organoids and organs-on-chips break into the limelight

- Schmeichel and Bissell "Modeling tissue-specific signaling and organ function in three dimensions." J Cell Science (2003)
- https://www.ted.com/speakers/mina_bissell

A hot, new *in vitro* model for biology

Complex 3D biology is a better model than 2D biology.

- <u>3D Organoids</u>

  Are self-organizing models with tissue-level functions and disease phenotypes.

  Demonstrate development

  Can be transplanted

  Can be a medium-to-high throughput assay

  Hard to replicate an individual organoid

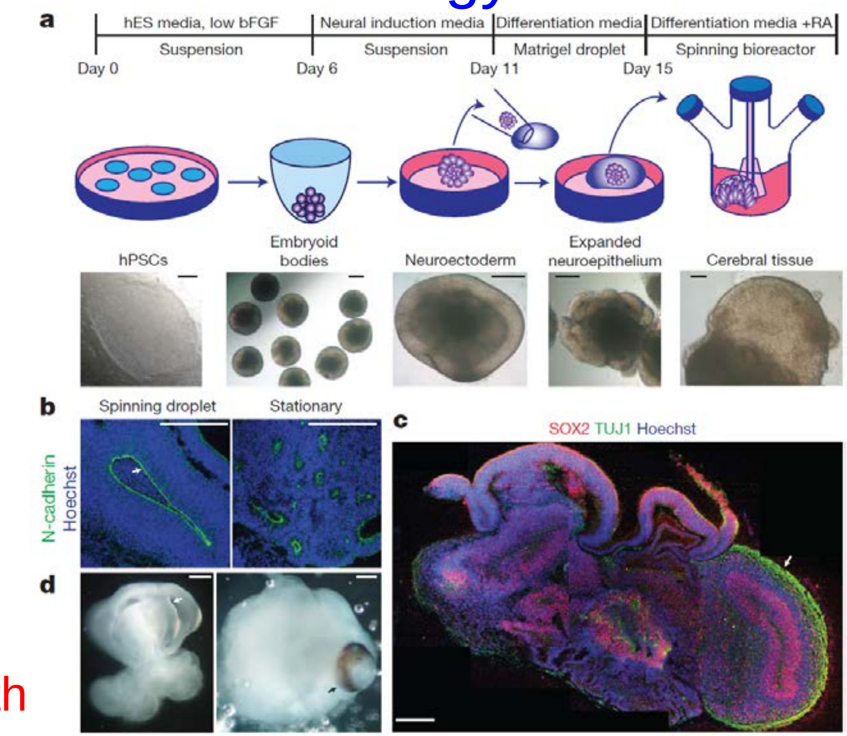
  May benefit from engineered hydrogels

  Hard to perfuse or apply uniform shear stress

  Hard to quantify barrier functions

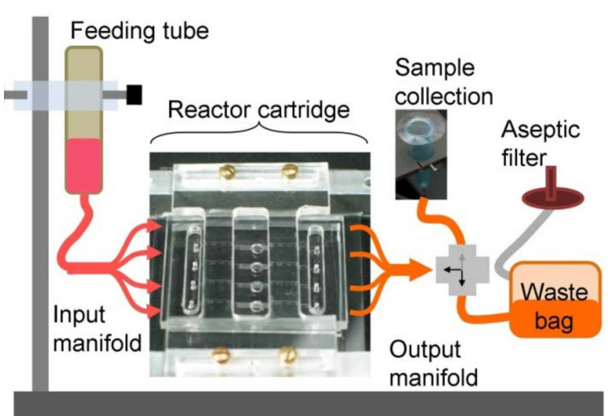
  Hard to visualize when living

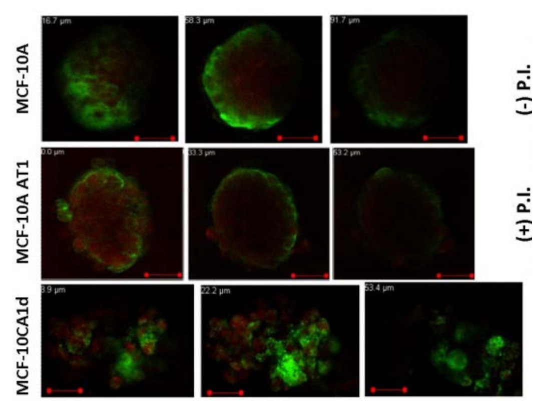
  Hard to integrate with other organ systems with proper volumes

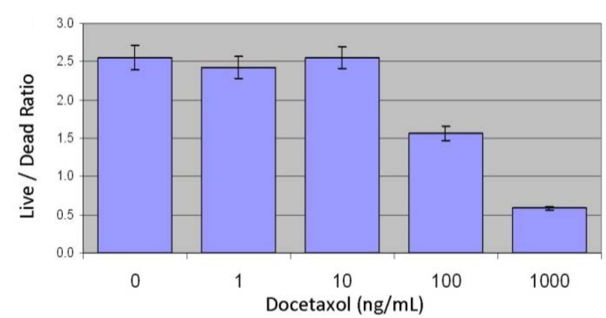


Lancaster, … , Knoblich. Cerebral organoids model human brain development and microcephaly. Nature, 2013.

Contributions from Kapil Bharti (NIH/NEI)





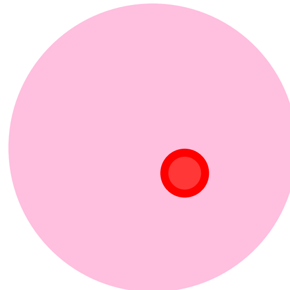


Markov, … , McCawley. Thick-tissue bioreactor as a platform for long-term organotypic culture and drug delivery. Lab Chip 12:4560-4568. 2012.

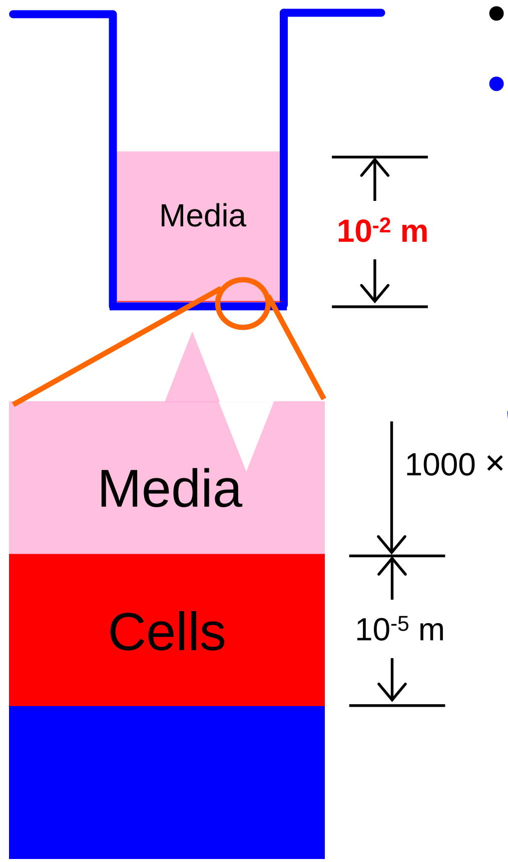
The "Media Volume" problem

Conventional culture

- A typical picoliter cell requires a nanoliter of media per day.
- A **10 µm layer of cells** covered by a **10,000 µm layer of media.**
- **A 5 nL spheroid in 5 µL of media**
- **1 or 2 days between fluid changes**
- Metabolites, endocrine, autocrine, and paracrine factors are **diluted 1000-fold.**

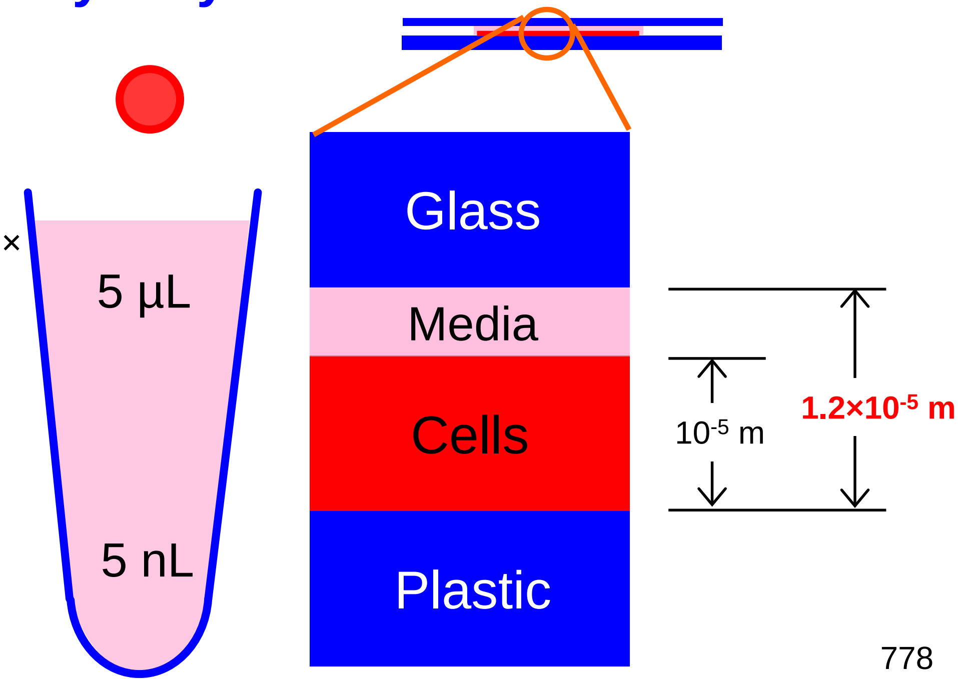


Relative sphere sizes: nL media vs pL cell



Microfluidic tissue culture

- 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, paracrine, and endocrine factors are **diluted by only 1.2x**



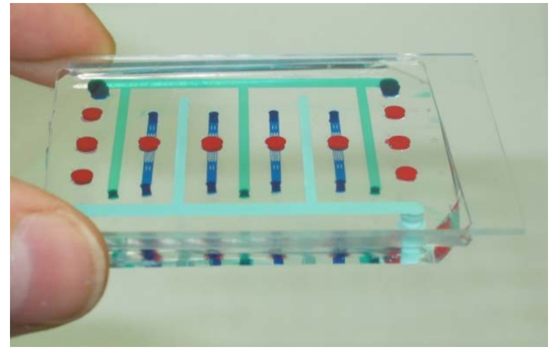
Another hot new *in vitro* model for biology

Complex 3D biology is a better model than 2D biology.

- <u>Organ Chips</u>

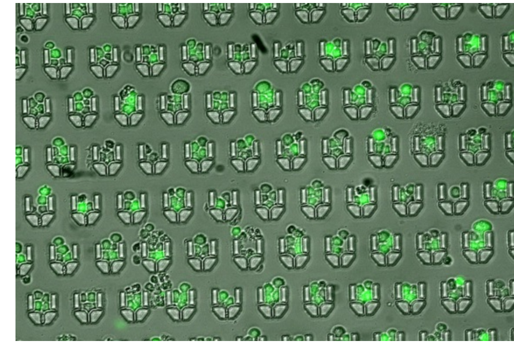
Better than 2D biology
Ideal for barrier functions
Can reproduce physiological flows
Provide a thick ECM for scaffolding and drug/factor binding
Support organ-organ interactions
Sufficient tissue for multi-omics of 10's to 1000's of variables
Can use minimal media volumes
Will be vascularized soon
May ultimately reduce drug costs
Possible to build a single-patient homunculus
Could build animals-on-chips
Can require microfluidics and control
Not yet high throughput
Are expensive today (hardware, effort, human cells, real estate)
Not fully validated vs *in vivo, e.g.,* no WGCNA yet
Can't be transplanted

Mammary gland on a chip



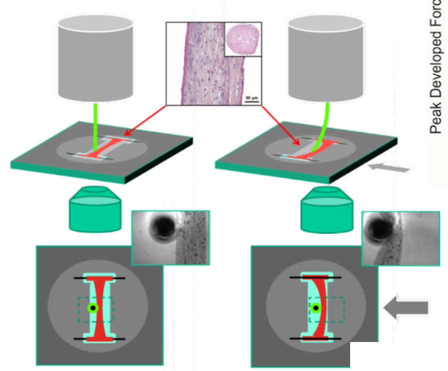
Lisa McCawley and Dmitry Markov, Vanderbilt

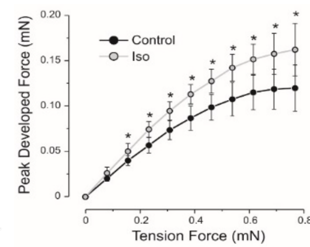
T cells in a lymph node on a chip

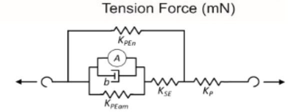


Shannon Faley, Kevin Seale and John Wikswo, Vanderbilt

Heart on a chip

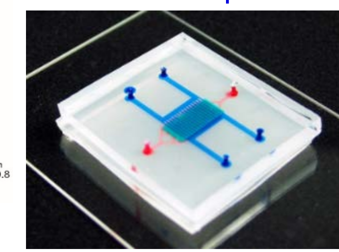




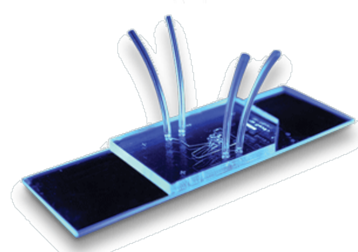


Veniamin Sidorov and John Wikswo.

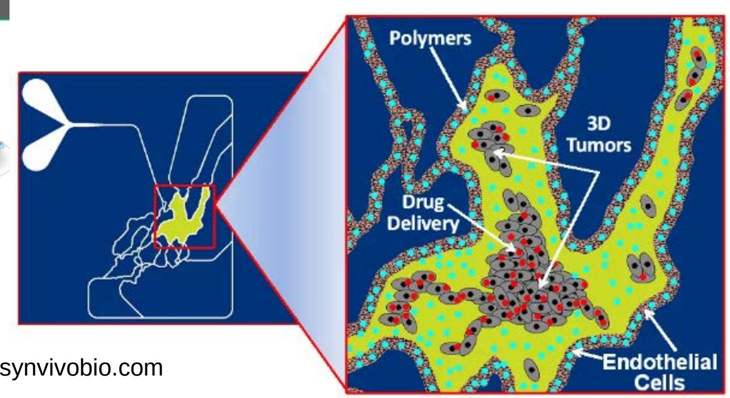
Brain on a chip



Jacquelyn Brown and John Wikswo, Vanderbilt

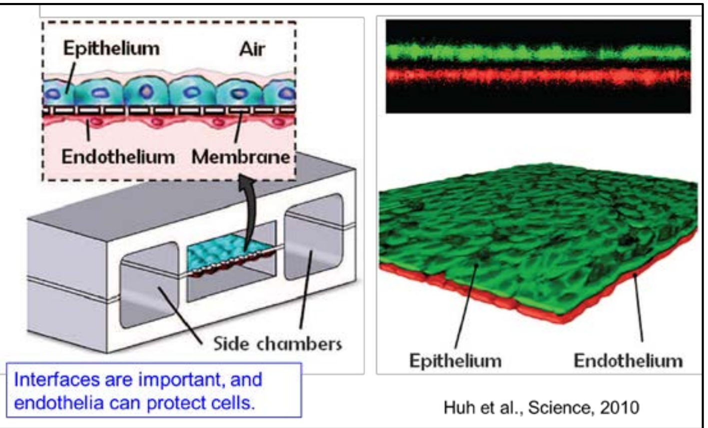


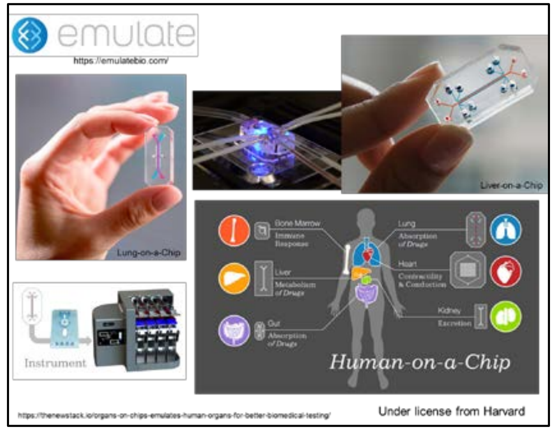
Prabhakar Pandian and Kapil Pant, SynVivo/CFDRC

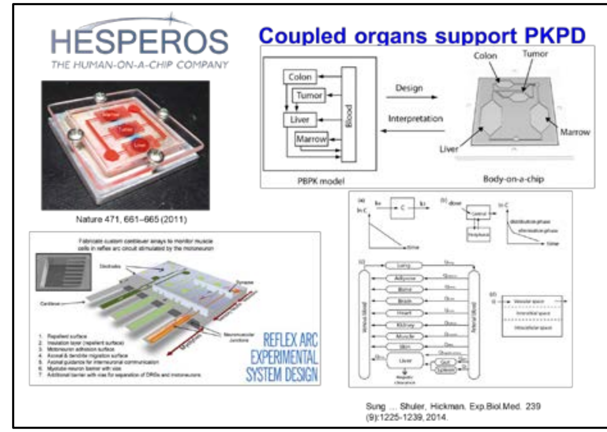


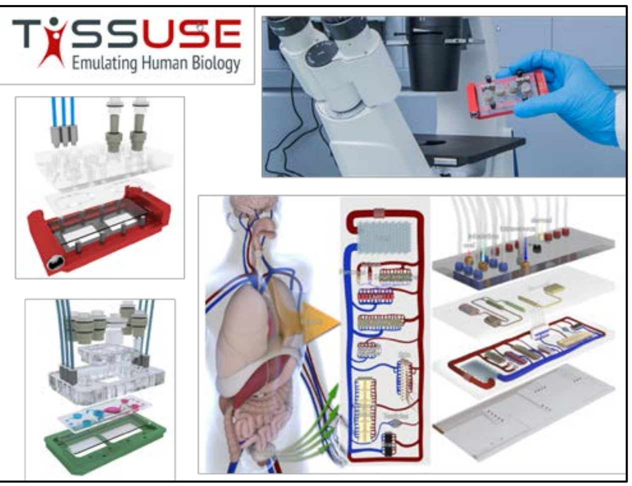
http://www.synvivobio.com

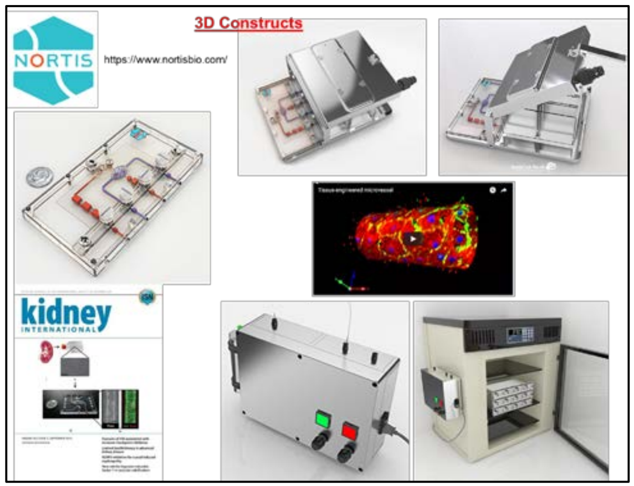
Organ-Chips, Organ Chips, Organ Chips…

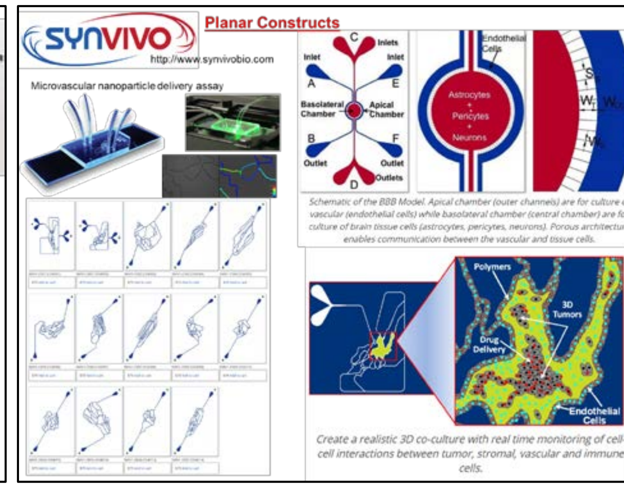


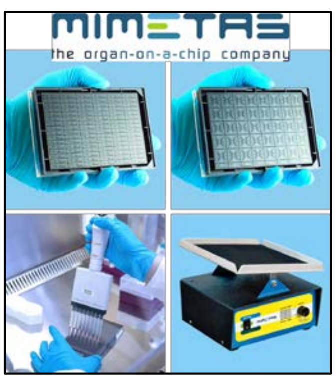






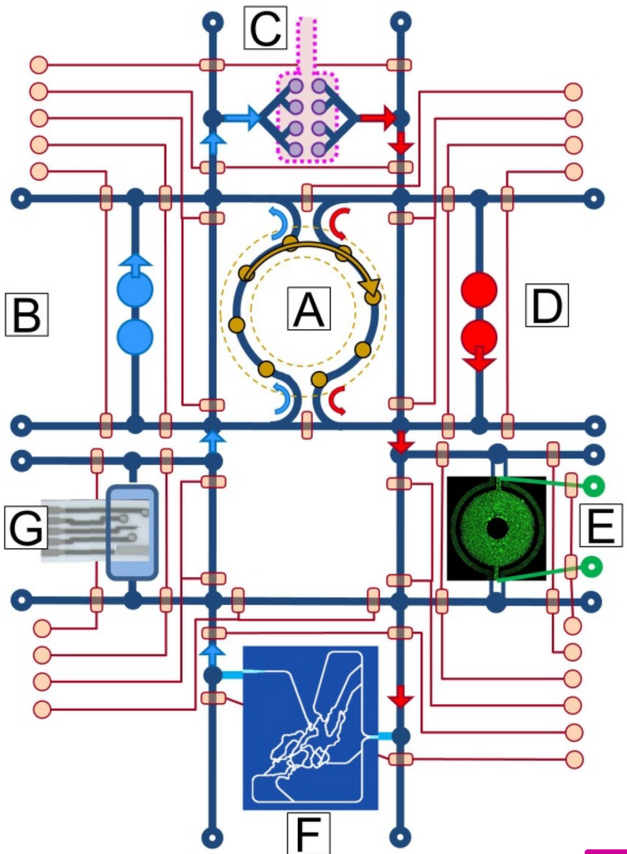




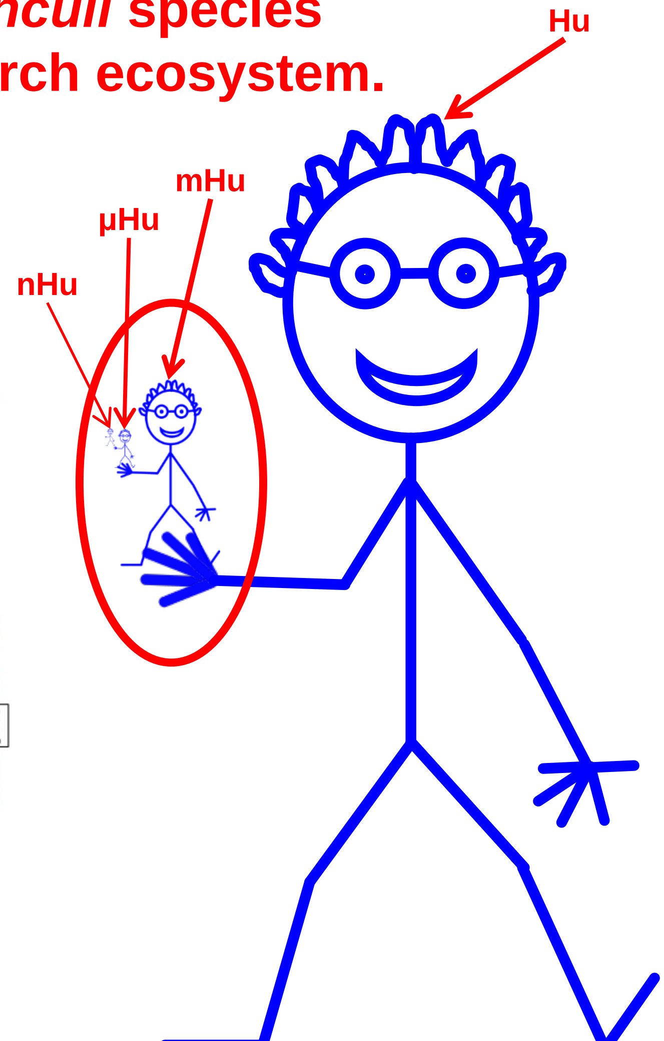


Others, and there's room to grow!

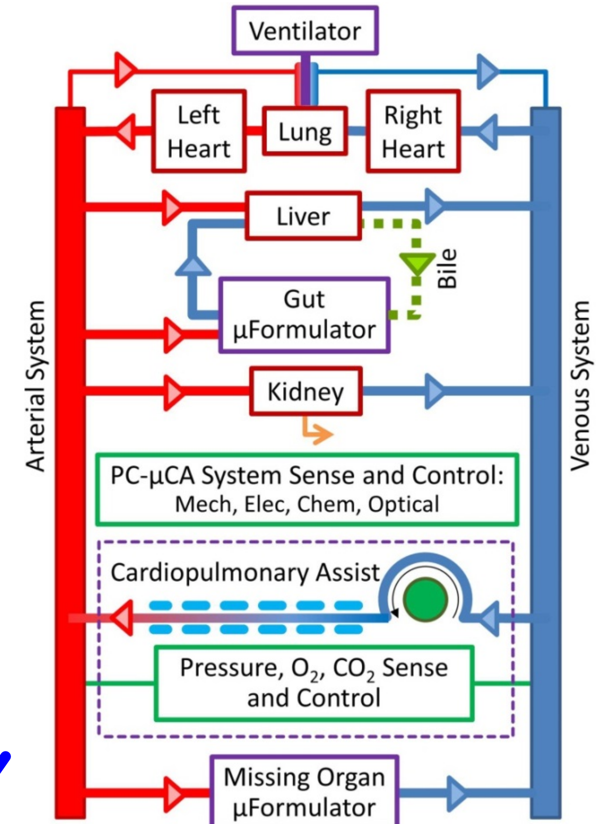
John Wikswo's goal – Determine how best to fit two new *Homunculi* species into the biomedical research ecosystem.



Homo chippiens
NanoHuman (nHu)



What can organs-on-chips do for basic research and tox-safety? Single organs and/or coupled-organ homunculi?



Homo minutus
MicroHuman (µHu)

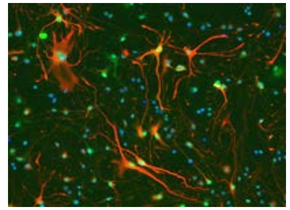
JP Wikswo, *et al.*, *Lab on Chip*, 2013.

Tissue Chips at Vanderbilt

NIH National Center for Advancing Translational Sciences

VIIBRE

- Human iPSC-derived neuronal cells



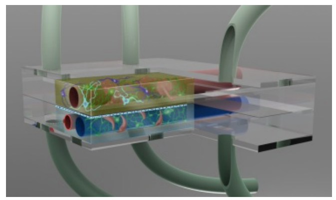
hiPSC glutamatergic neurons

Rare Diseases Clinical Research Network
Initiative of the National Center for Advancing Translational Sciences (NCATS)

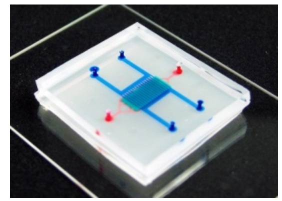
Tuberous Sclerosis Alliance

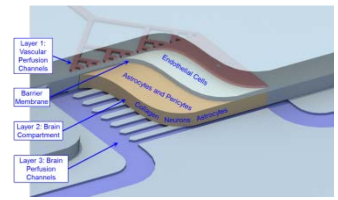
TSC-patient hiPSCs are being used to create brain microvascular endothelial cells, astrocytes, pericytes, and both excitatory and inhibitory neurons 2016

- Bioreactors

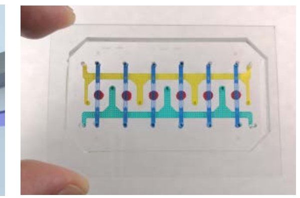


VIIBRE NVU concept 2012





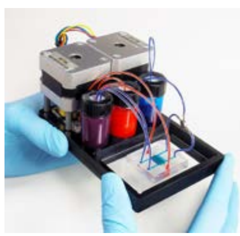
VIIBRE NVU as built 2014



Mammary gland-on-a-chip 2016

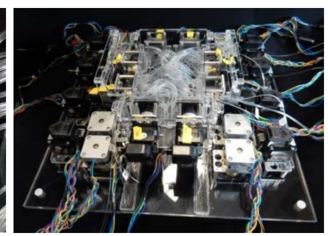
NIH National Cancer Institute

- Control hardware



VIIBRE NVU Perfusion Controller 2014

VIIBRE 24-port valve 2015

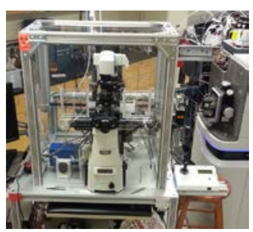


MicroFormulator 1.0, 2015

MicroFormulator 2.0, 2016

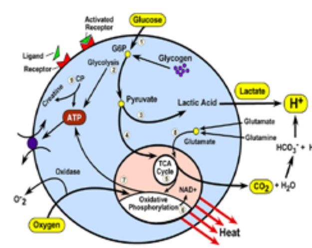
SmartMotor 2.0, 2016

- Analytical chemistry & metabolomics

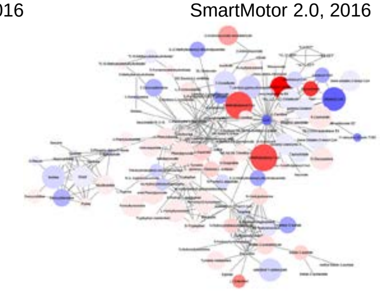


In-line MS of organ chip 2013

VIIBRE MicroClincial Analyzer 2014



Core Carbon Metabolism



MS metabolomics 2016

- Translation

United States Environmental Protection Agency

CFDRC

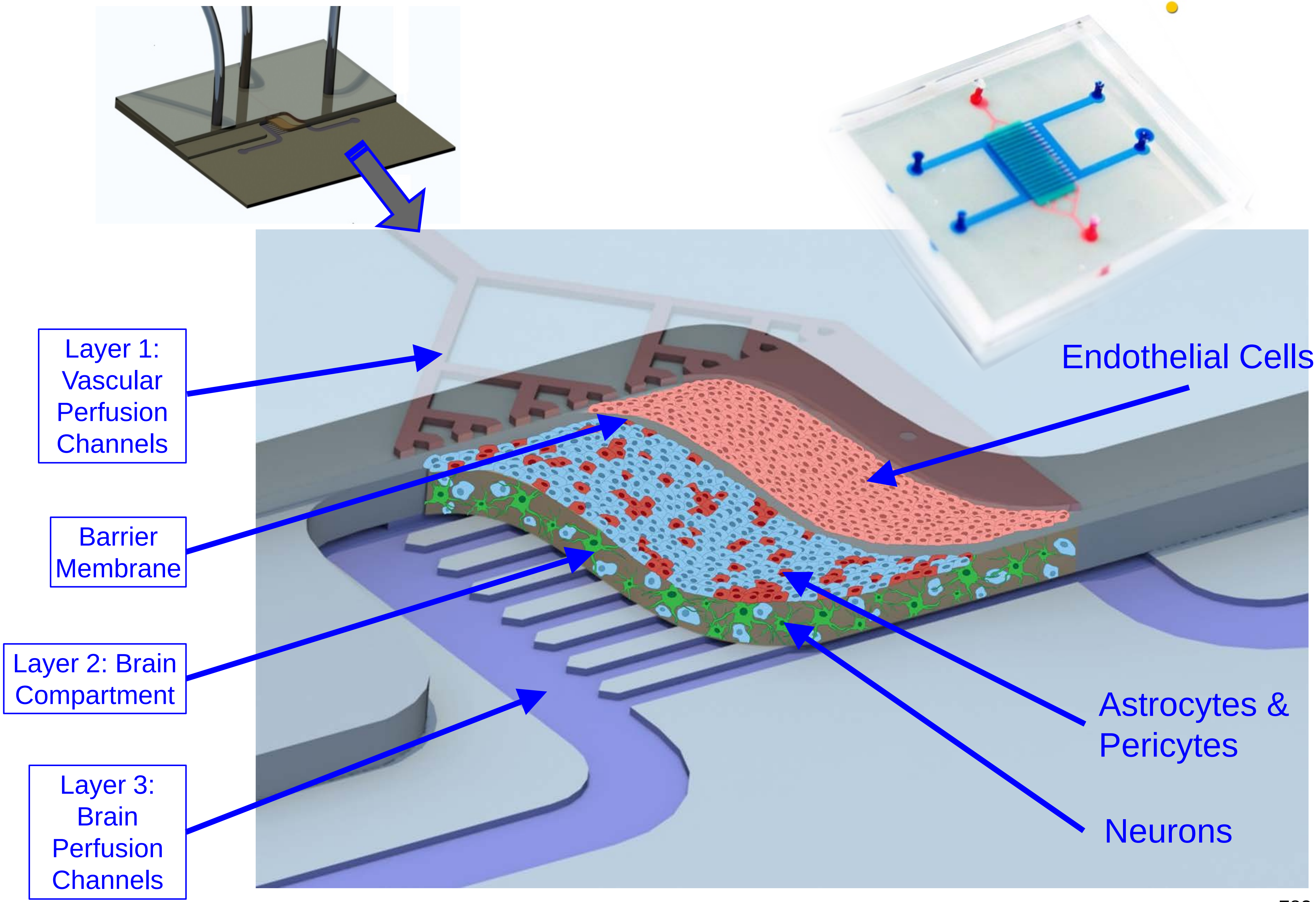
NIH National Center for Advancing Translational Sciences

KIYATEC Advanced Ex Vivo 3D Cell Culture Technologies

NORTIS

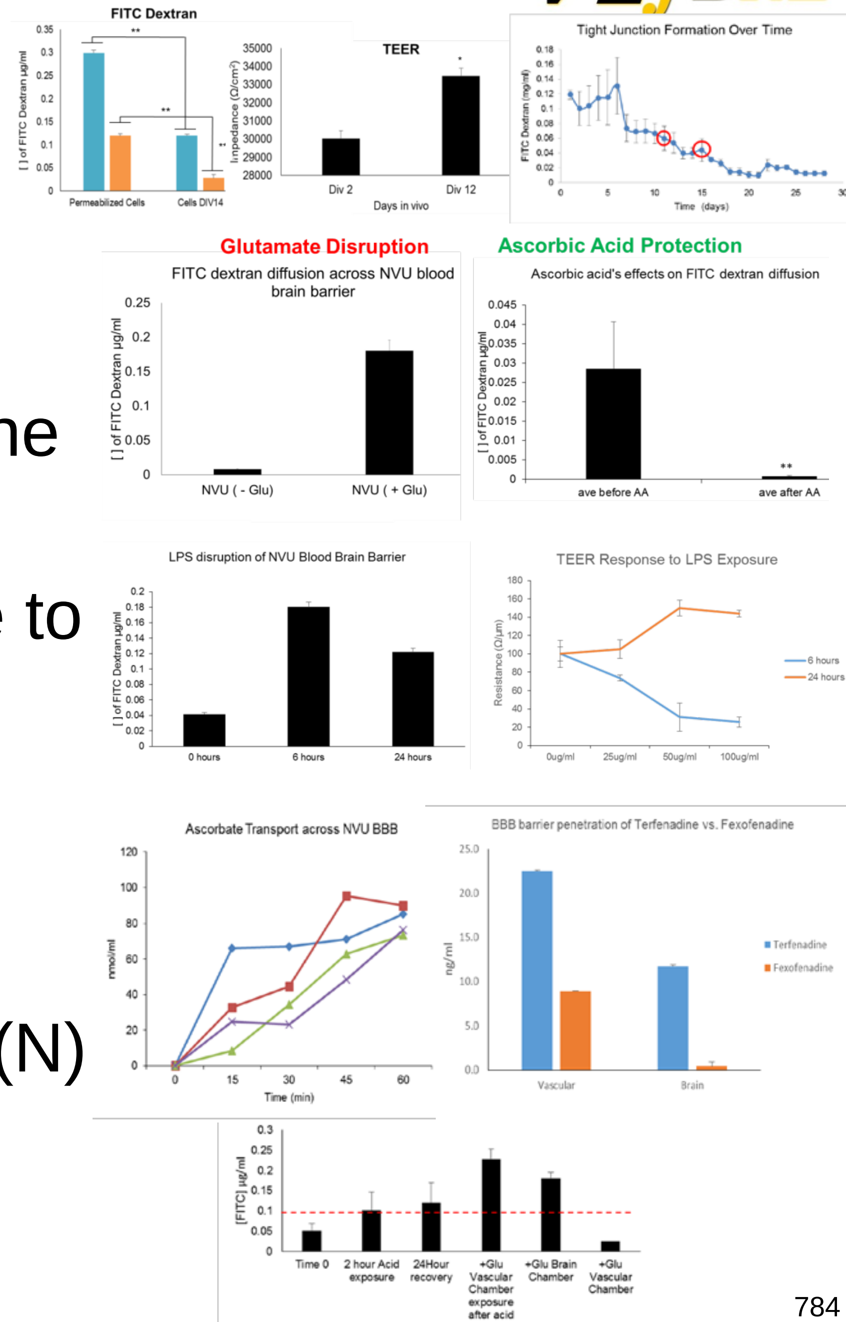
Issued U.S. patents: 7,435,578; 7,534,601; 7,704,745; 7,713,733; 7,790,443; 7,974,003; 7,977,089; 7,981,649; 8,129,179; 8,339,704

The VIIBRE NVU and BBB

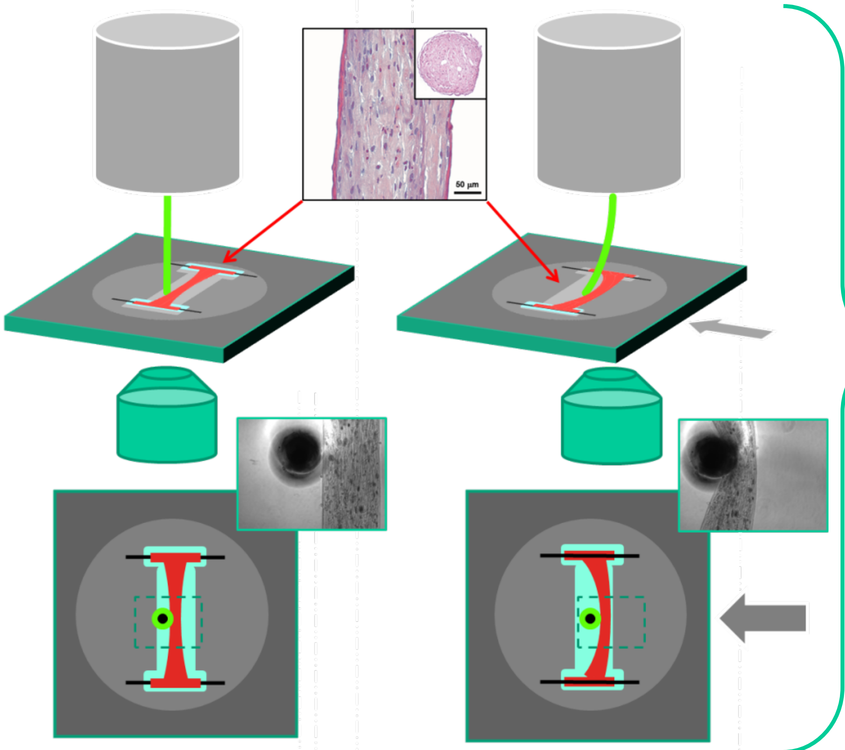


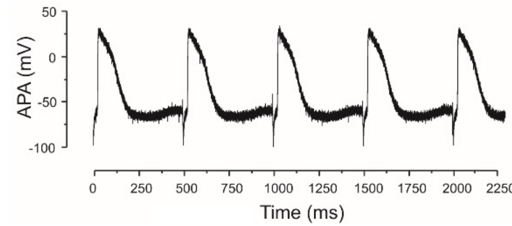
NVU/BBB measurements

- Tightening of the BBB with time after assembly
- Disruption by glutamate in the brain compartment
- Tightening by ascorbic acid in the vasculature
- Differential responses over time to inflammatory agents (LPS and cytokine cocktails)
- Differential transport across the BBB: ascorbic acid (Y), Terfenadine (Y), Fexofenadine (N)
- Response to combined insults (brain glutamate + acidification)

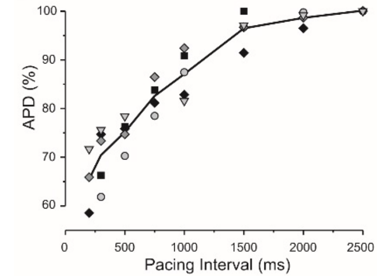


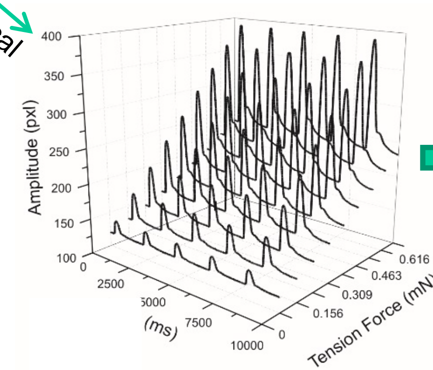
Cardiac I-Wire



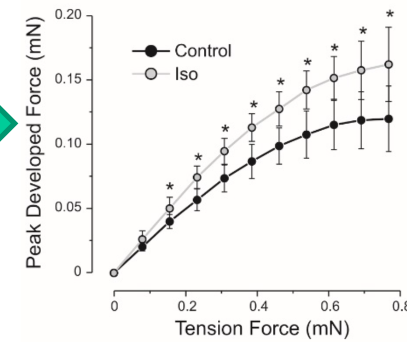


Restitution Curve

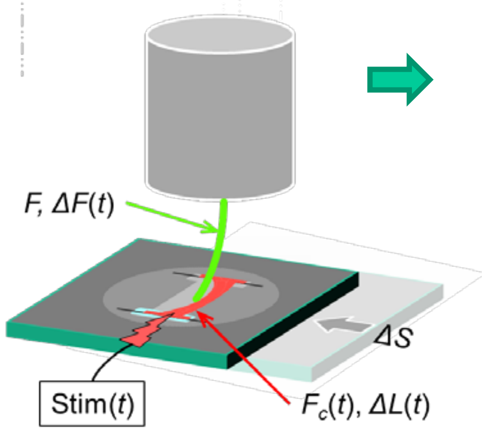




Frank-Starling Curve



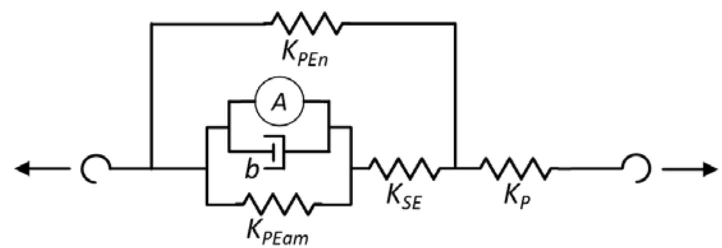
Siderov, *et al.*, Acta Biomateralia, 2016



$$F_P = K_P \Delta s_1$$
$$\Delta s_1 = \Delta s - \Delta s_2 = \Delta s - \sqrt{\Delta L(2L + \Delta L)}$$
$$\Delta s_2 = \sqrt{(L + \Delta L)^2 - L^2} = \sqrt{\Delta L(2L + \Delta L)}$$
$$\Delta L = \sqrt{L^2 + \Delta s_2{}^2} - L = \sqrt{L + \left(\Delta s - \frac{F_P}{K_P}\right)^2} - L$$
$$F_P = K_P\, \Delta s_1 = 2F_C \sin(\theta) = 2F_C\, \frac{\Delta s_2}{L+\Delta L}$$
$$F_C = \frac{1}{2} K_P \left(\frac{\Delta s}{\sqrt{\Delta L(2L+\Delta L)}} - 1\right)(L + \Delta L)$$
$$F_C = \frac{\left(K_{PEam} + K_{PEn} + \frac{K_{PEn}\, K_{PEam}}{K_{SE}}\right)}{1+\frac{K_{PEam}}{K_{SE}}}\left(\Delta L + \Delta L_{offset}\right)$$
$$\dot{\Delta L} = \frac{\left(1+\frac{K_{PEam}}{K_{SE}}\right) f_1(\Delta L) - K_M\left(\Delta L + \Delta L_{offset}\right) - F_A}{b\left(1 - \frac{f_2(\Delta L) - K_{PEn}}{K_{SE}}\right)}$$

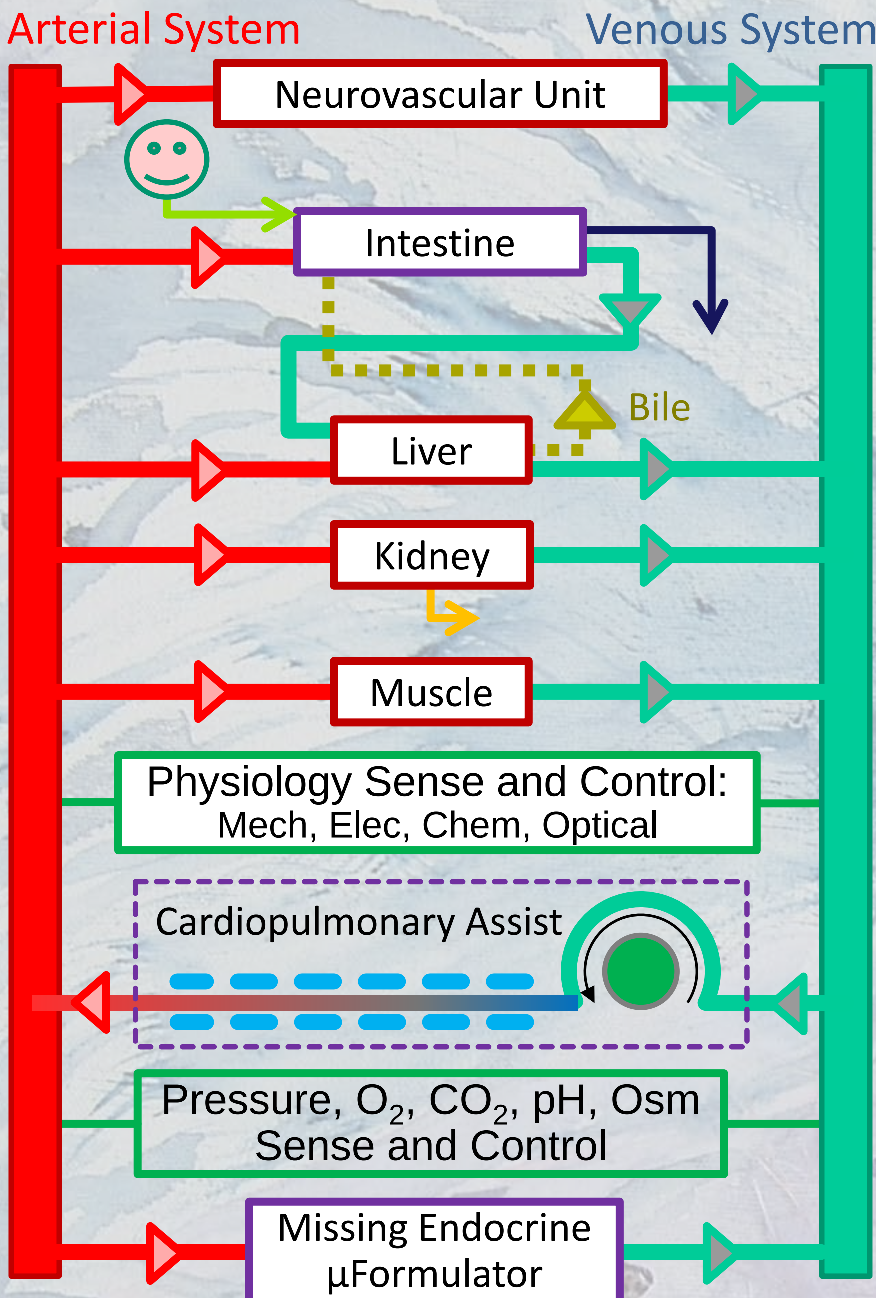
Nashville single-string guitar equation

Cardiac Hill model

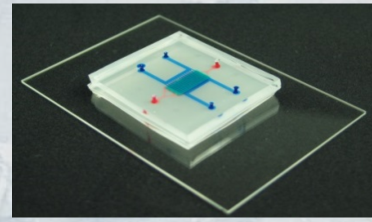


Schroer, *et al.*, Acta Biomateralia, 2016

NIH-NCATS MPS Integration

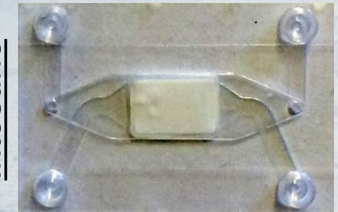


NVU



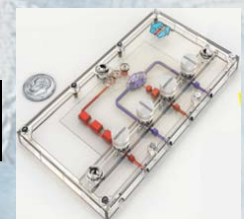
Vanderbilt

Intestine



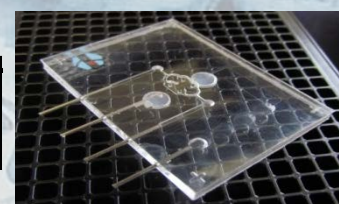
Hopkins, Baylor

Liver



Pittsburgh

Kidney



Washington

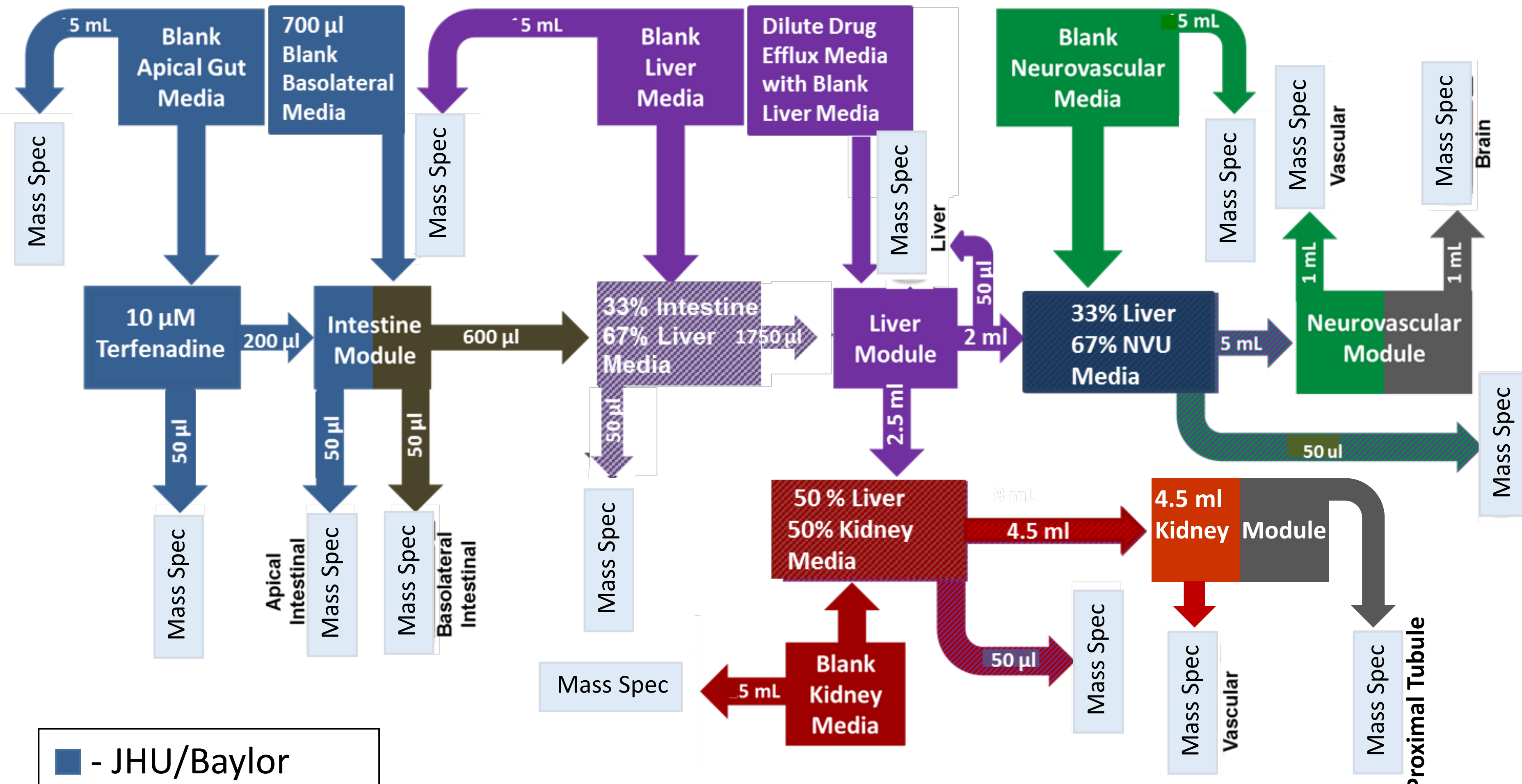
Muscle

Duke

ME-µF

Work Flow for Functional Coupling Experiment

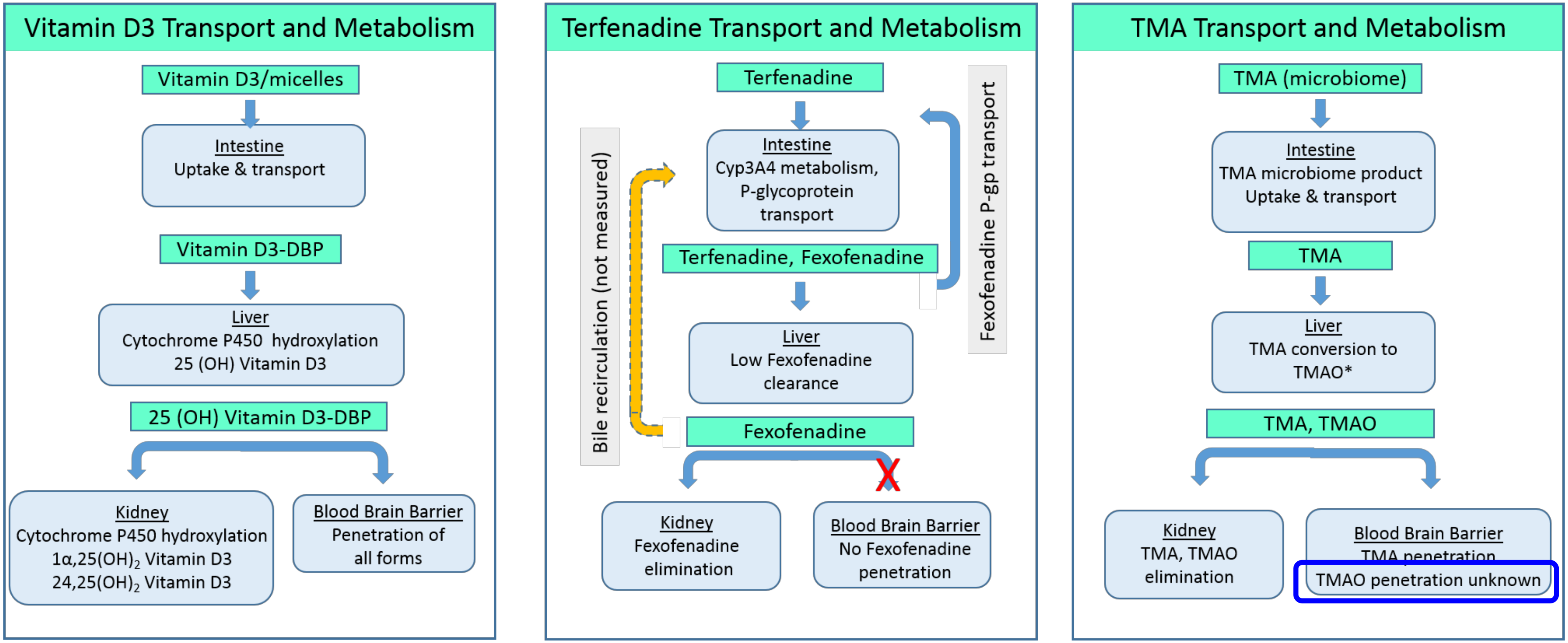
Goal: Couple Gut, Liver, Brain, and Kidney



- JHU/Baylor
- U Pittsburgh
- U Washington
- Vanderbilt

Vernetti, *et al.*, Scientific Reports, 2017

Work Flow for Functional Coupling Experiment



TMAO penetration into human CSF confirmed the NVU observation: Del Rio, et al., Nutrients, 2017

We found 26% TMAO penetration into the NVU brain chamber!

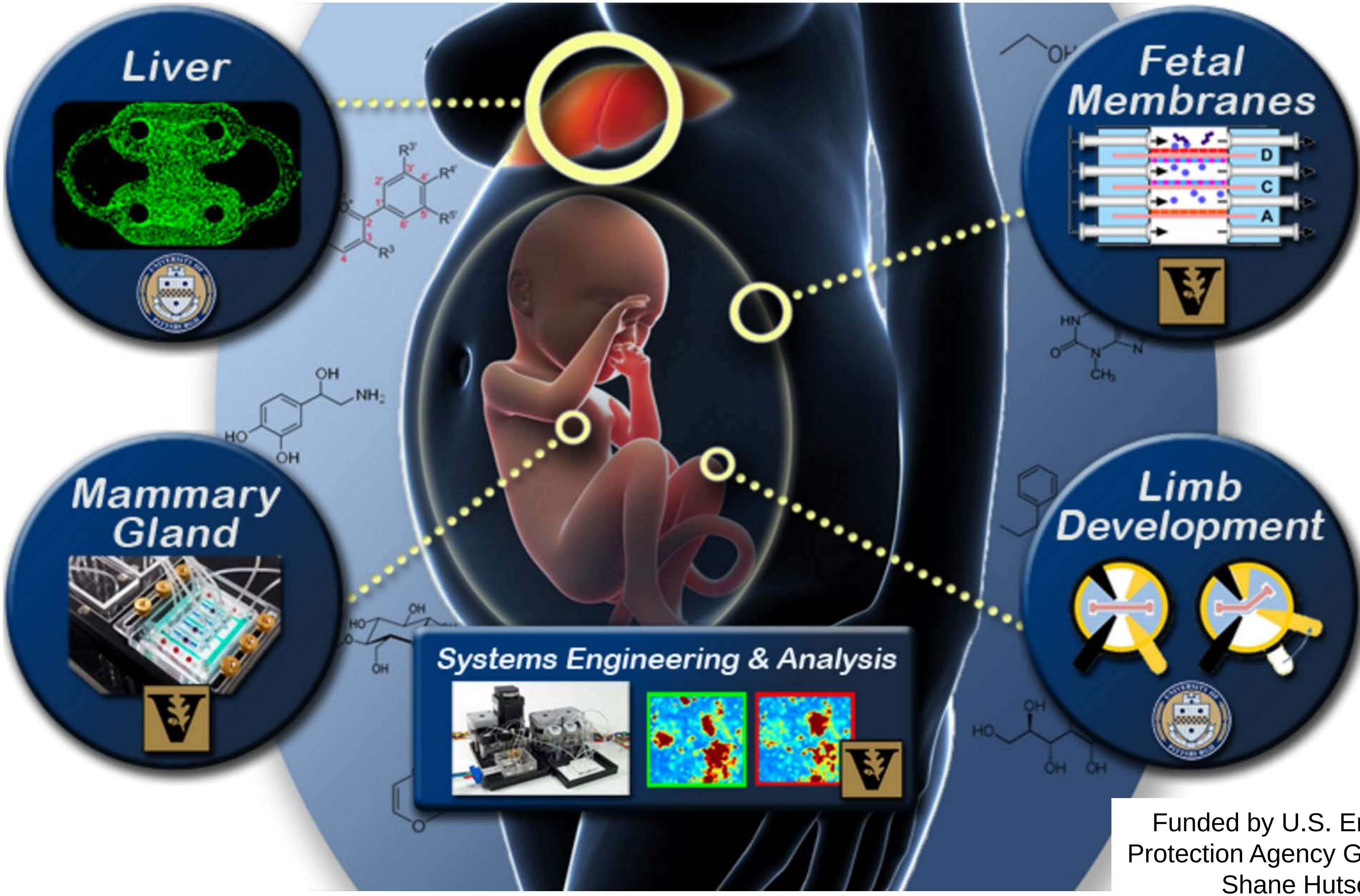
Test Agent/*Metabolites*	Clinical MPS Model	Intestine	Liver	Kidney	BBB
TMA *TMAO*	**Clinical**	Uptake & Transport	**TMA** → *TMAO* < 5% **TMA** Clearance	> 95% *TMAO* Excreted	*TMAO* Penetration: Unknown
	MPS	Uptake & Transport	**TMA** → *TMAO* < 1% TMA Clearance	~46% *TMAO* Excreted	26% *TMAO* Penetration
Terfenadine (Ter) *Fexofenadine (Fex)*	**Clinical**	**Ter** → *Fex*; *Fex* CounterTrans	< 1% Bio T < 95% *Fex* Clearance	11% *Fex* Excreted	~0% *Fex* Penetration
	MPS	**Ter** → *Fex*; *Fex* CounterTrans	< 1.4% Bio T (est.) < 80% *Fex* Clearance	~ 1% *Fex* Excreted	~ 0% *Fex* Penetration
Vitamin D3 (VD3) *25(OH)VD3; 1α,25(OH)$_2$VD3; 24,25(OH)$_2$VD3*	**Clinical**	Uptake & Transport No metabolism	**VD3** → *25(OH)VD3*	*25(OH) VD3 → 1α,25(OH)$_2$VD3 & 24,25(OH)$_2$VD3*	**VD3** & *25(OH)VD3* Penetration
	MPS	Uptake & Transport No metabolism	**VD3** → *25(OH)VD3 & 24,25(OH)$_2$VD3*	*1α,25(OH)$_2$VD3 & 24,25(OH)$_2$VD3* below LOQ	0.4% **VD3** & 6% *25(OH) VD3* Penetration

Key Concordances Between MPS and Clinical Fate for Three Test Agents. Key: Uptake - by jejunum endothelial cells ; Transport - from apical to basolateral media; → = Metabolism; CounterTrans = Transport from basolateral to apical media; est. = estimated. Excreted - into proximal tubule lumen; LOQ = limit of quantitation; Penetration - through blood-brain barrier.

Vernetti, *et al.*, Sci. Reports, 2017

VPROMPT

Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology



Funded by U.S. Environmental Protection Agency Grant 83573601
Shane Hutson, P.I.

Problems that are at the MPS cutting edge

VI∫BRE

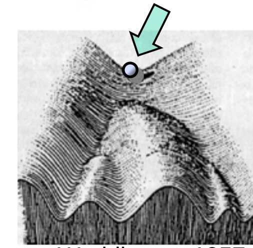
How accurately can we recreate micro-vasculature and the basement membrane?

- **The full metastatic cascade**
  - Localized formation of the primary tumor
  - Intravasation into vascular and lymph systems
  - Dissemination through vascular and lymph systems
  - Extravasation into a competent organ
  - Colonization and proliferation with seed-soil interactions

How accurately must we recreate adaptive immunity?

- **Testing immuno-oncology drugs**
  - Requires isogenetic innate and adaptive immune system, tumor, and metastatic niche to avoid host-versus-graft reactions and MHC-HLA incompatibilities.
  - May require organ-specific lymph nodes, immune-active spleen and bone marrow for proper programming of multiple types of immune cells.
  - CD34+ progenitor cells and B cells have yet to be derived from iPSCs.

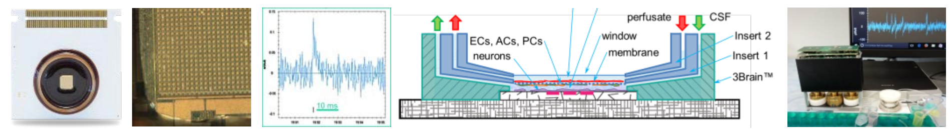
VIIBRE's Tissue Chips Challenges



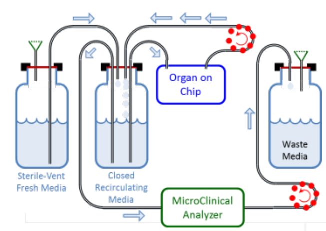
Waddington 1957

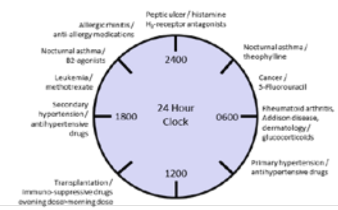
- Human iPSC-derived neuronal cells
  - Reduce costs
  - Shorten time from patient to iPSC to mature phenotypes
  - Develop genotype libraries
  - Learn how to control iPSC differentiation

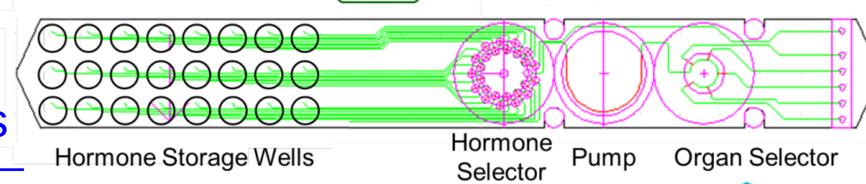
- Bioreactors
  - Reduce volumes
  - Vascularize
  - Eliminate PDMS
  - Add electrodes to the NVU



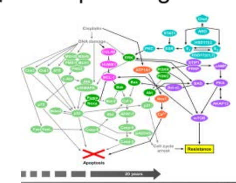
- Control hardware
  - Reduce volumes
  - Reduce size and cost
  - Recirculate
  - Add diurnal hormone and nutrient variations







- Analytical chemistry & metabolomics
  - Reduce volumes
  - Detect more analytes on-line at lower cost
  - Infer metabolic and signaling networks



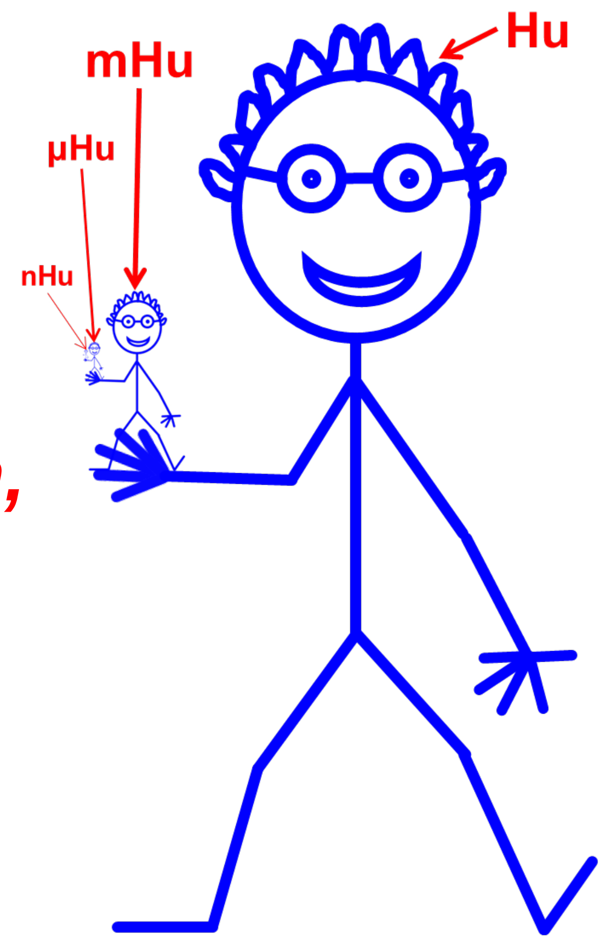
- Translation
  - Make it cost-effective and easy for conventional biologists, toxicologists, and pharmacologists to use organs on chips without a gigantic capital investment or an engineering degree
  - Start answering medical questions and solving medical problems

How good a model do we need?

- It depends upon the question you are asking

The best material model for a cat is another, or preferably the same cat.

Arturo Rosenblueth and Norbert Wiener. The Role of Models in Science. Philosophy of Science 12 (4):316-321, 1945.



Make your theories simple enough, but not too simple.

~Albert Einstein

Make your organs-on-chips systems simple enough, but not too simple.

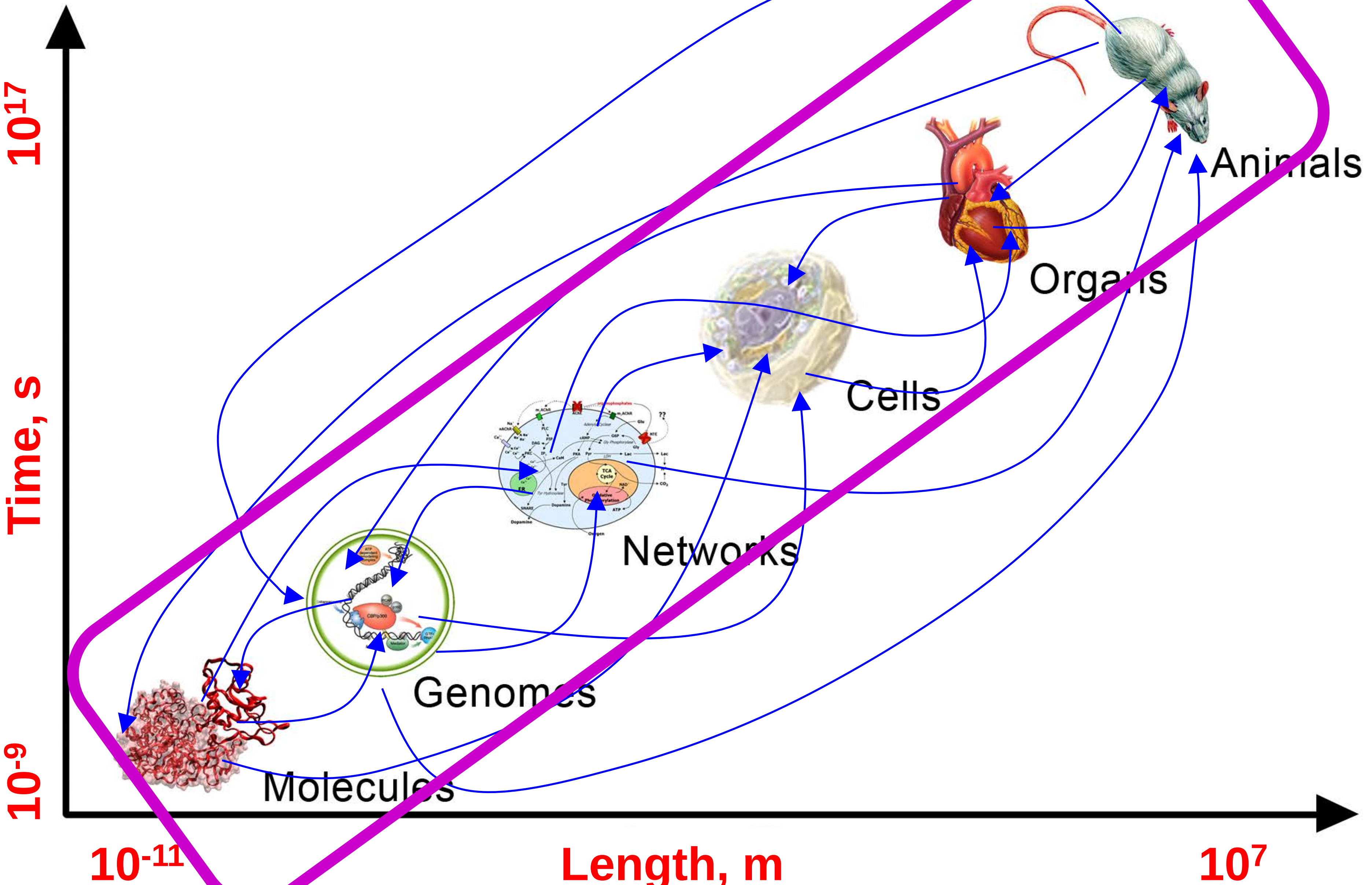
John Wikswo

Seven Themes

1. A brief history of biology
2. Just how complex is biology?
3. What is the role of physics in understanding the complexity of biology?
4. Can sensors, actuators, controllers and robot scientists help address biological complexity?
5. How might Organoids and Organs on Chips change the way we study biology?
6. **What does Multi-Omics offer?**
7. Closing the circle

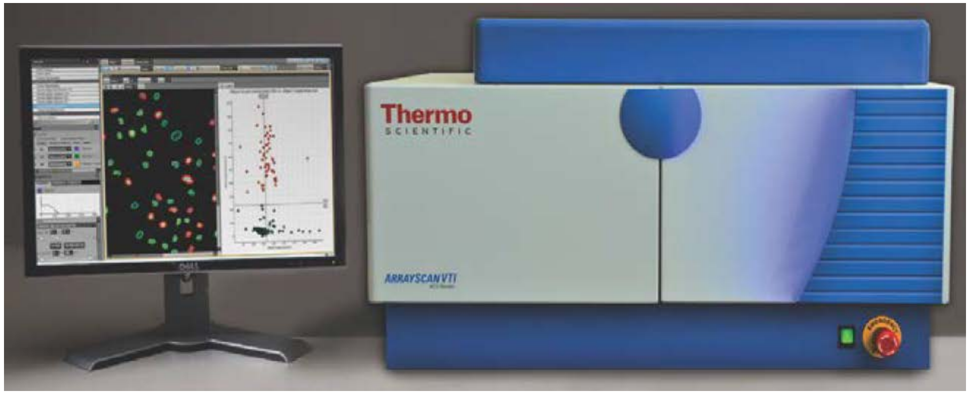
Julia Wikswo

Coupled Organ-Chips, Control, and Multi-Omics may hold the key!



Monitoring Organ Health/Toxin Response

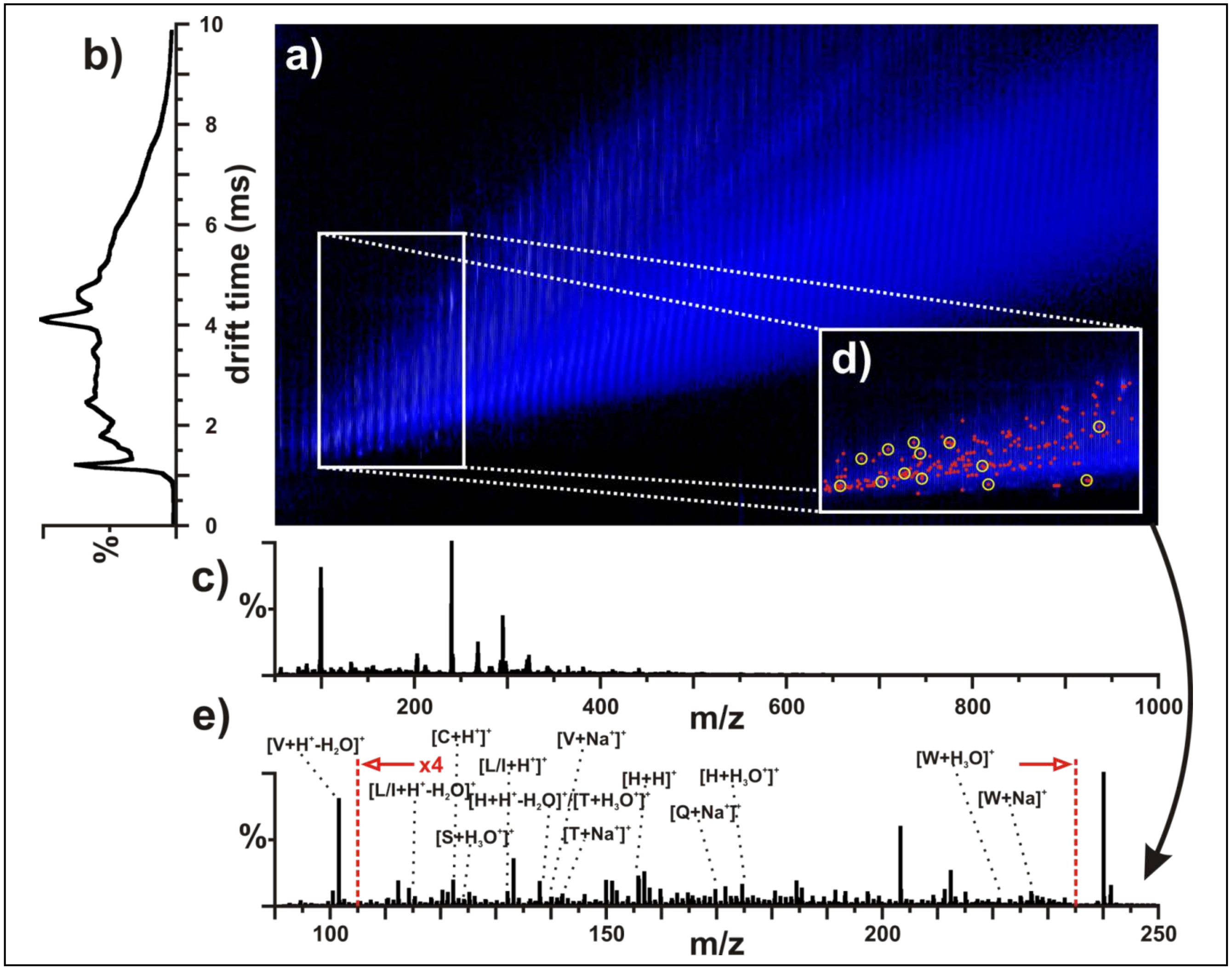
- Cellular morphology
  - Requires fluorescence microscopy
  - Organ-on-chip module should be HCS confocal compatible
- Genetically encoded fluorescent reporters
- TEER
- Bioenergetics
- Protein production
  - Albumin
  - Bile acids
  - Cytokines
  - Cyp activity
  - LDH release
- Transcriptomics
- Drug metabolism
- Untargeted metabolomics
- Metabolic activity (glucose, lactate, pH, oxygen)



The sensitivity of many assays is set by the ratio of cell volume to media volume!

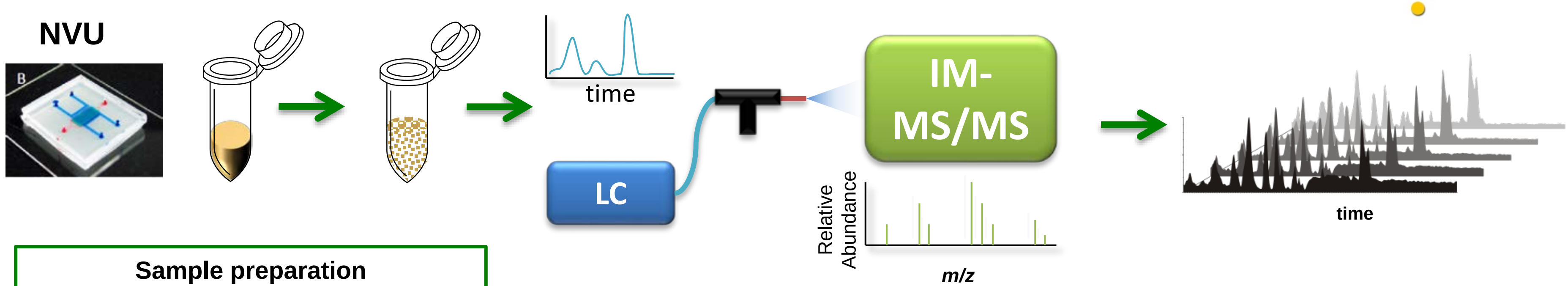
Tyrode's solution perfused through a normal beating mouse heart

Dynamic metabolomics



Sample from Franz Baudenbacher, slide courtesy of John McLean

NVU/BBB UPLC-IM-MS workflow



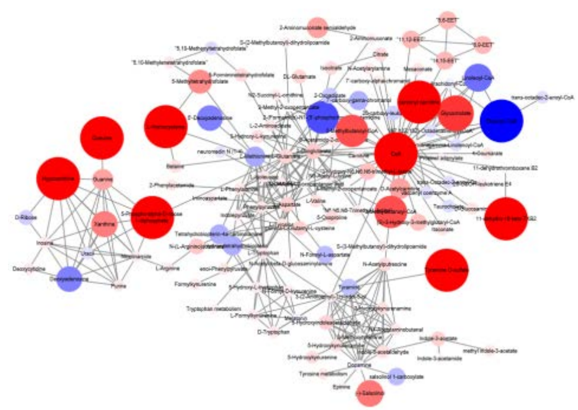
Sample preparation
metabolites extracted using ice cooled methanol:H_2O (80:20),
incubated -80ºC overnight,
spun down at 15,000 rpm, 15 min
dried down *in vacuo*

Sample Acquisition
LC IM-MS/MS of metabolite extracts

LPS or Cytokine treated samples

Data Alignment and Biostatistical Analysis
Progenesis QI

Pathways Analysis	p-value
Vitamin E metabolism	8.00E-05
Glutathione Metabolism	1.13E-03
Prostaglandin formation from arachidonate	6.48E-03
Aspartate and asparagine metabolism	9.95E-03
Drug metabolism - cytochrome P450	9.97E-03



Network & Pathway Module Output

Network and Pathway analysis
Mummichog

Metabolomic pathway analysis with high mass-accuracy UPLC-IM-MS is accelerating the incorporation of untargeted metabolomics into mechanism of action studies.

Brown *et al.*, J. Neuroinflammation, 2016

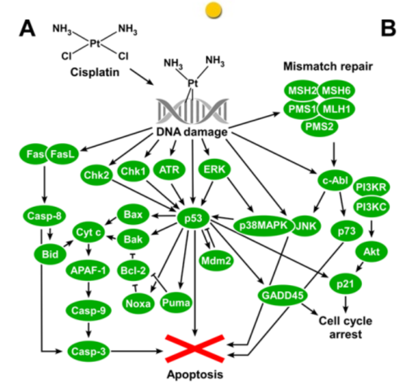
Rapid Threat Assessment (RTA) of MoA

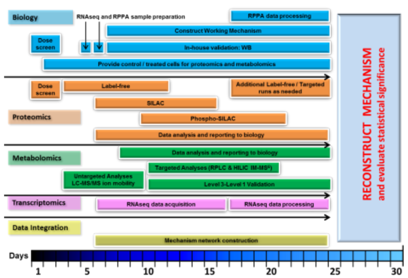
Richard Caprioli, PI, DARPA W911 NF-14-2-0022.

Objective: Use multiomics to characterize drug and toxin Mechanism of Action (MoA) in 30 days or less.

Challenge 1: A549 cells treated with 50 µM cisplatin for 1, 6, 24 and 48 h. MS proteomics (mudpit, SILAC, phospho-proteomics), IM-MS metabolomics, RNAseq, etc.

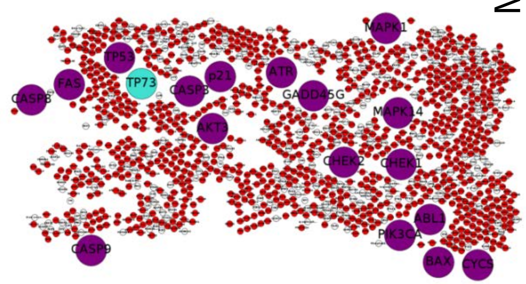
Canonical Cisplatin MoA Siddik 2003 plus literature





30-day workflow

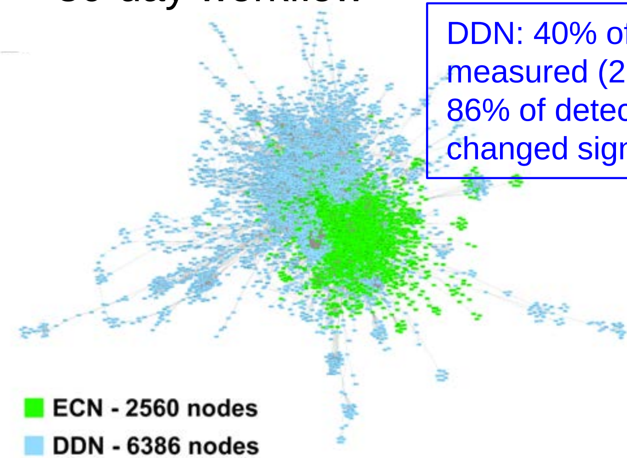
- 254,296 total features
- 55,898 unique species
- 13,483 (24%) species significantly changed



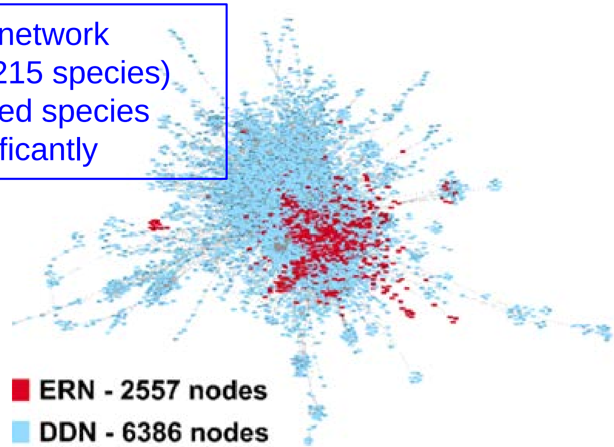
- Canonical Species, Detected
- Canonical Species, Not detected
- Expanded Canonical Species, Detected
- Expanded Canonical Species, Not detected

Expanded Canonical, Pino *et al.,* in preparation

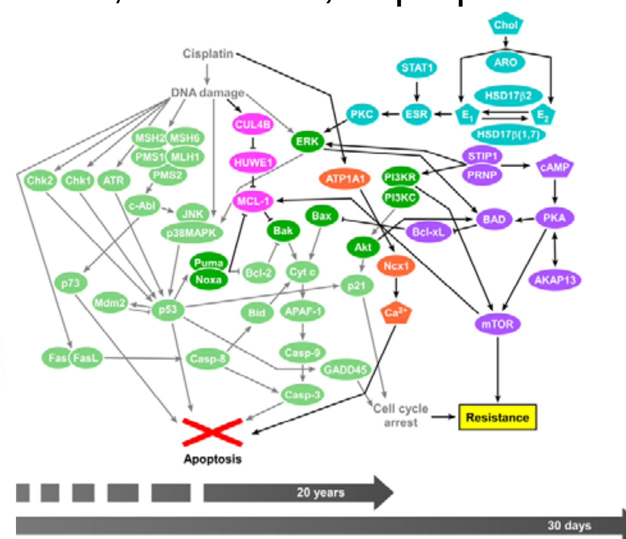
DDN: 40% of network measured (2,215 species)
86% of detected species changed significantly



Expanded Canonical vs Data Driven Network



Expanded Resistance vs Data Driven Networks



New Canonical Cisplatin MoA

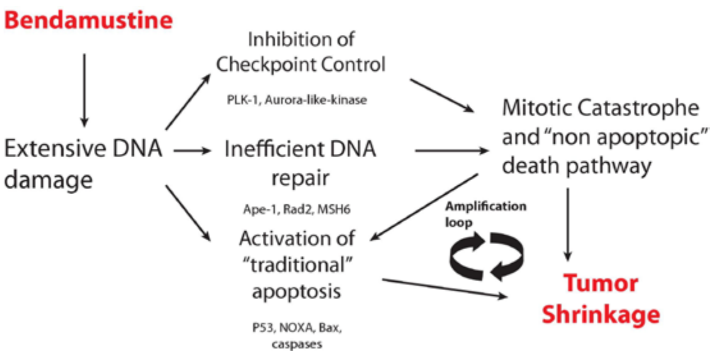
Norris *et al.,* J. Proteome Res. 2017

Time-resolved omni-omics has great potential!

RTA – Bendamustine MoA

VI∫BRE

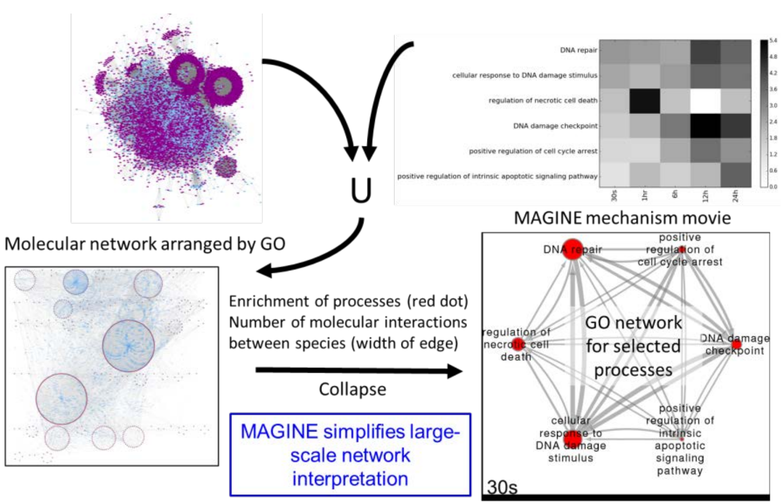
Known Mechanism of Action of Bendamustine



Leoni and Hartley, Seminars in Hematology (2011)

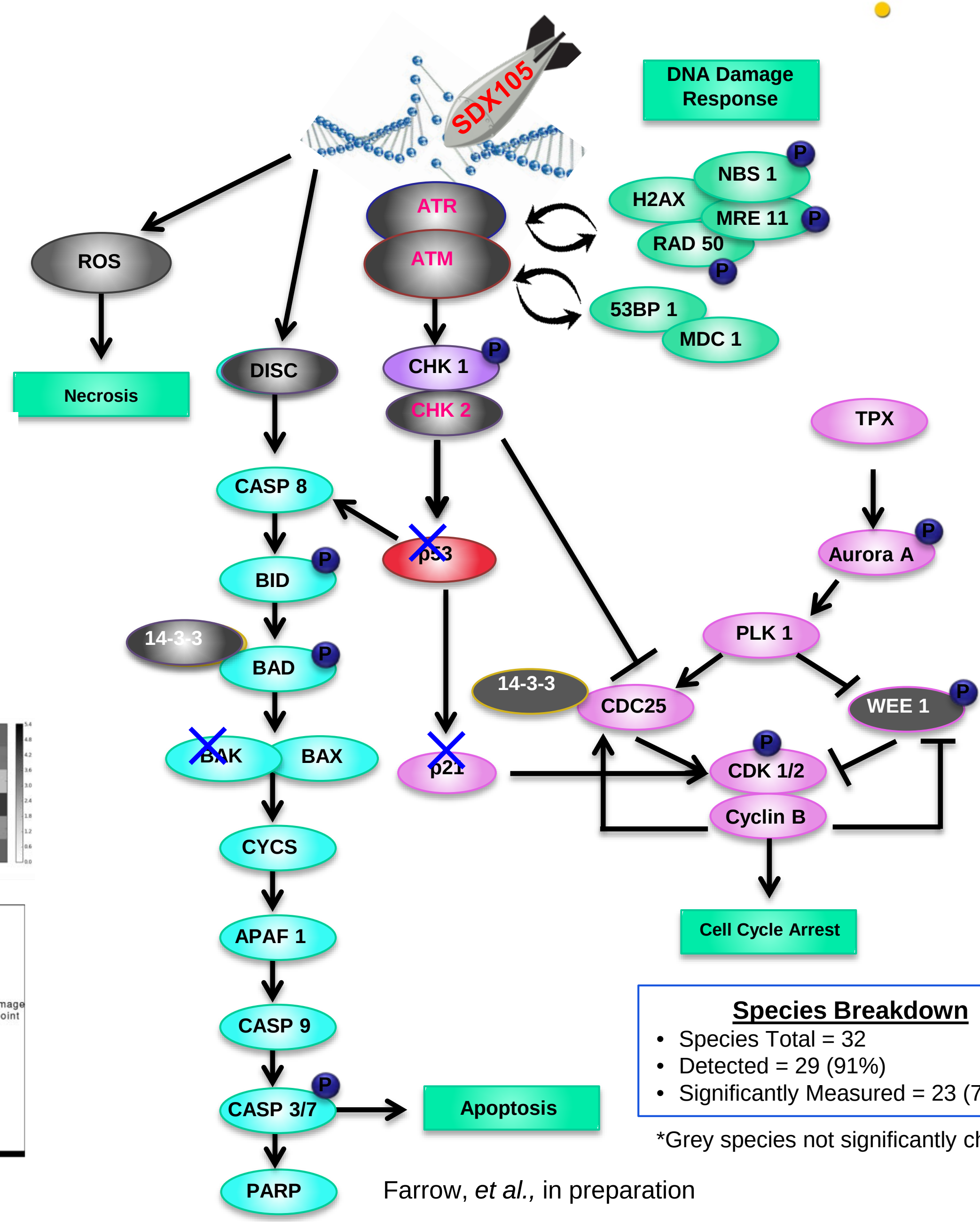
Vanderbilt RTA 2nd 30-day Challenge

- Acquire 781,072 data points spanning 12 time points and 7 platforms
- How do we extract and integrate knowledge from these data?



Pino, *et al.*, in preparation

Vanderbilt-RTA Postulated Mechanism of Action



Species Breakdown

- Species Total = 32
- Detected = 29 (91%)
- Significantly Measured = 23 (72%)

*Grey species not significantly changing

Farrow, *et al.*, in preparation

Seven Themes

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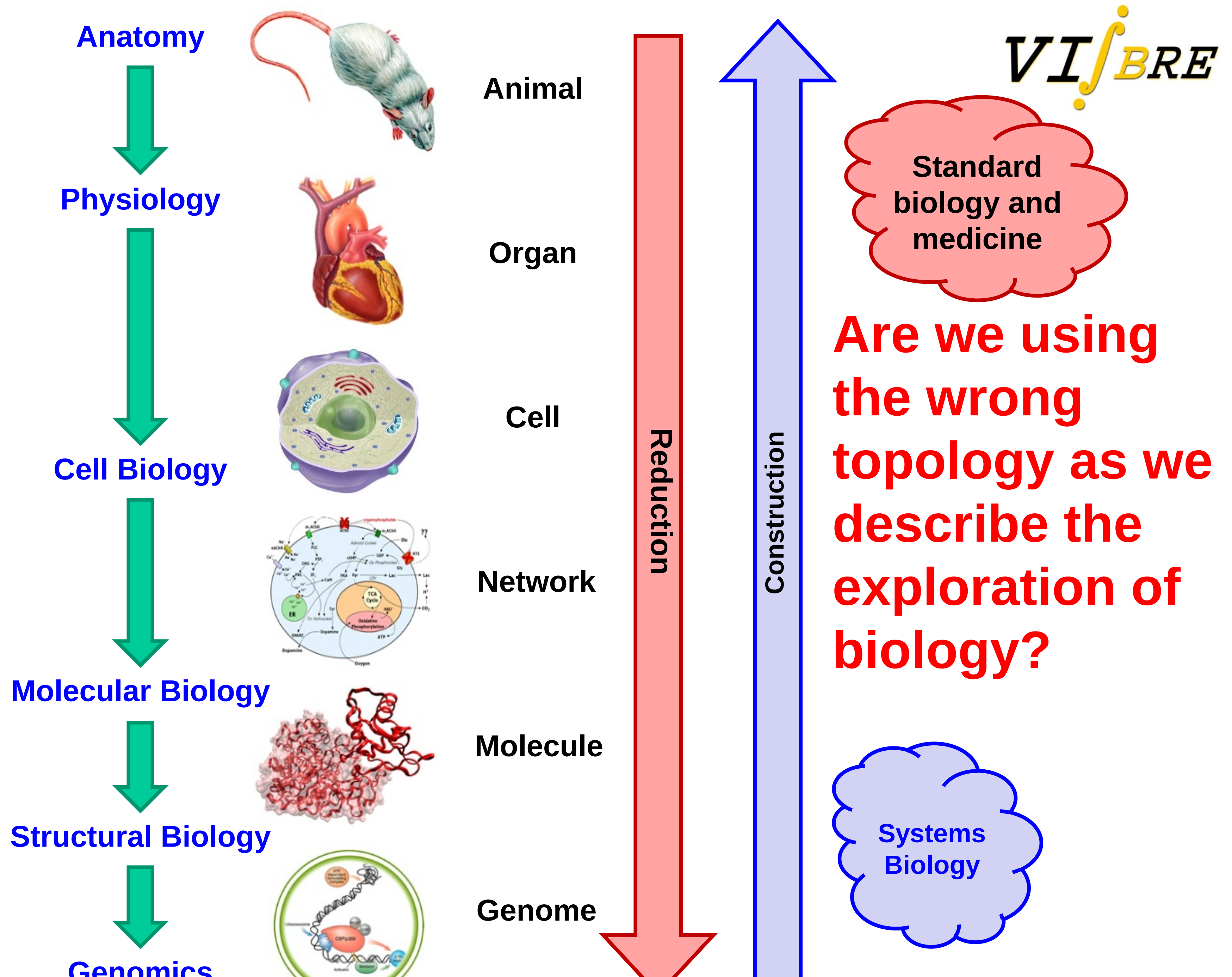
Julia Wikswo



JP Wikswo and AP Porter, *EBM*, 2015

Hermeneutics, *noun*

[hərməˈn(y)o͞odiks/]

The study of the methodological principles of interpretation (as of the Bible).

http://www.merriam-webster.com/dictionary/hermeneutic

The first order art and the second order theory of understanding and interpretation of linguistic and non-linguistic expressions.

http://plato.stanford.edu/entries/hermeneutics/

Julia Wikswo

Hermeneutic Circle, *noun*

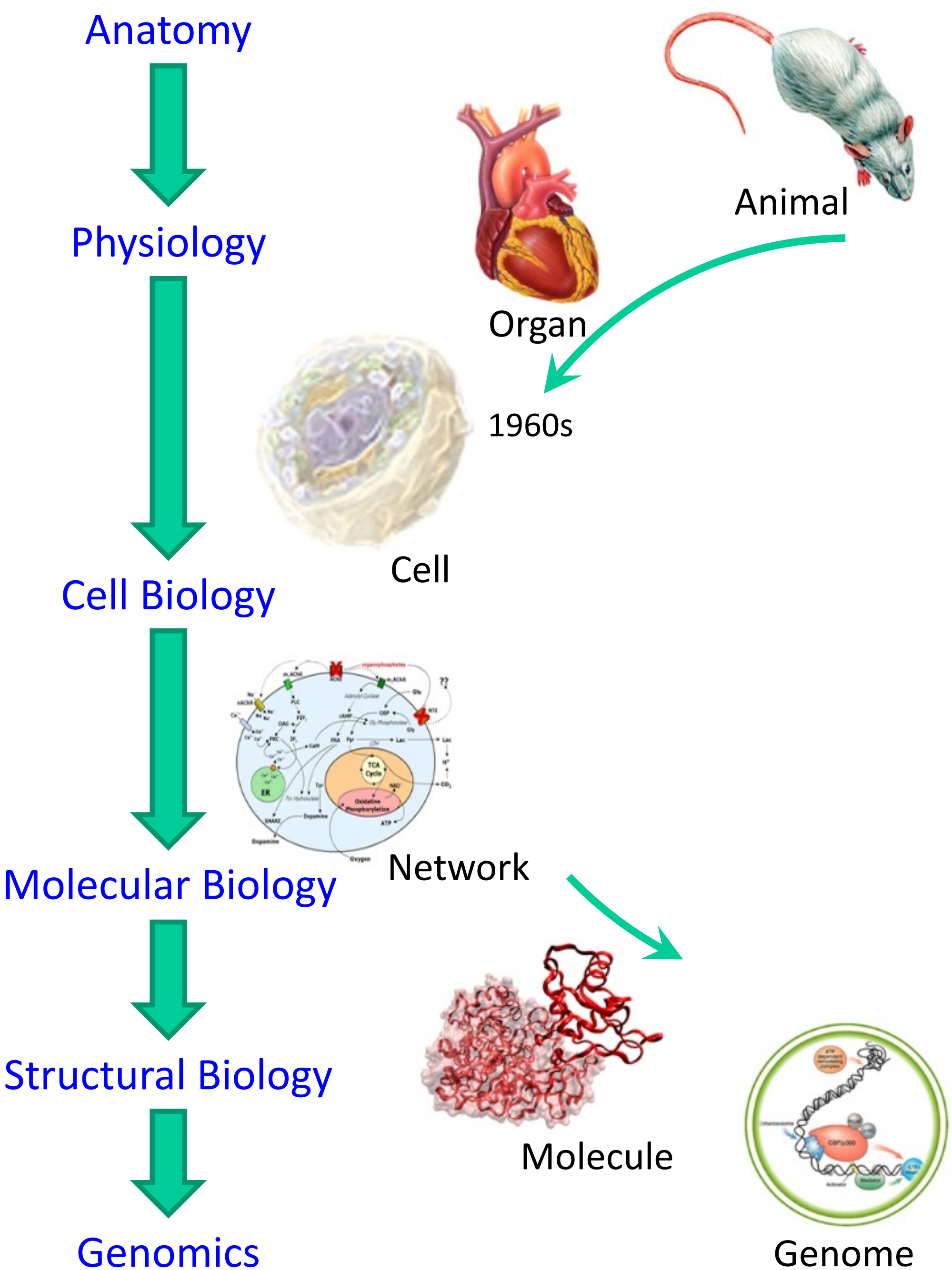
[hərməˈn(y)o͞odik ˈsərk(ə)l]

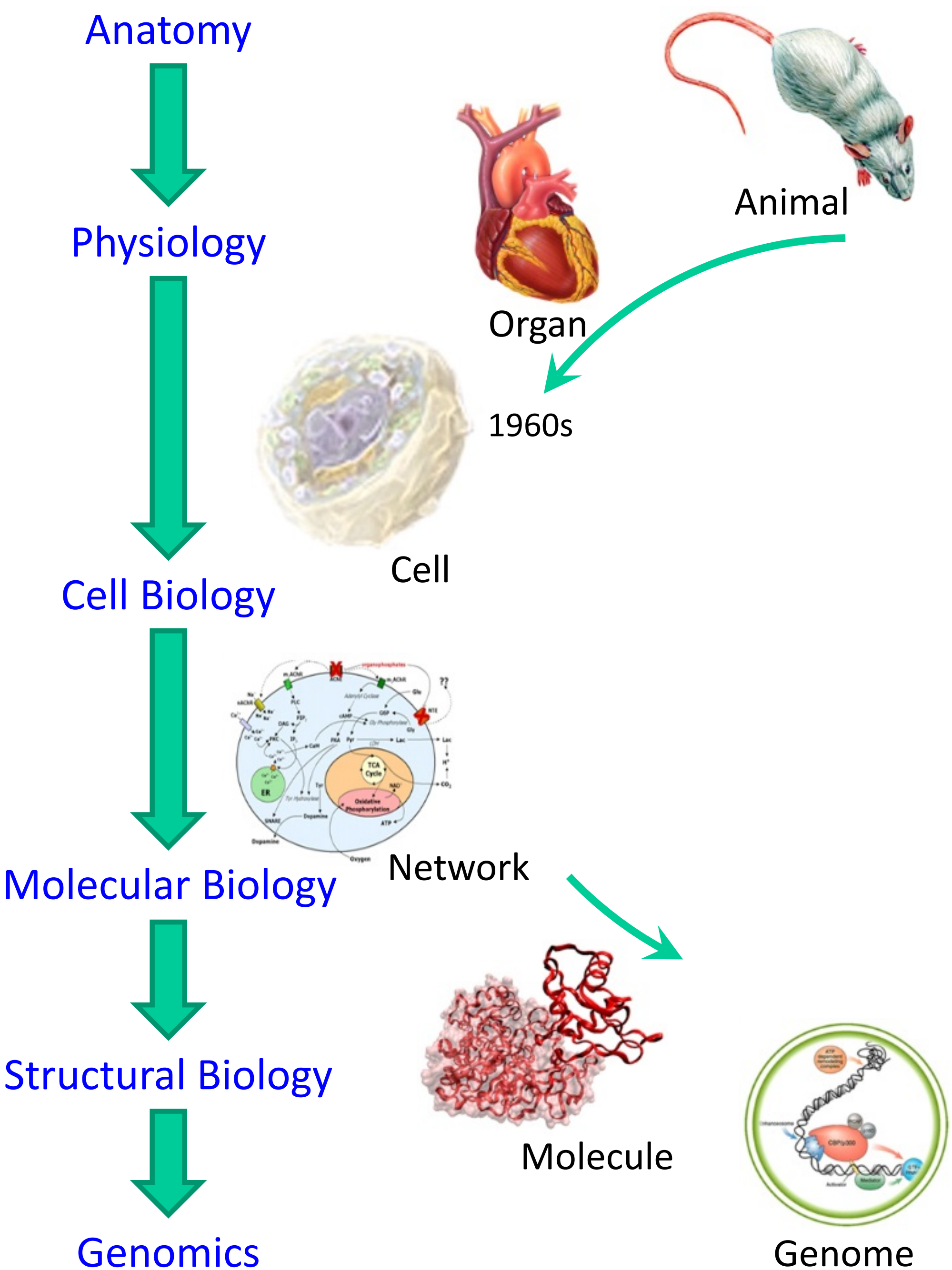
Whole

One cannot understand the whole until one understands the parts, and one cannot understand the parts until one understands the whole.

Parts

Gadamer,HG. Truth and Method. 2nd ed., Continuum, New York, 2000


Anatomy
Physiology
Cell Biology
Molecular Biology
Structural Biology
Genomics
Animal
Organ
1960s
Cell
Network
Molecule
Genome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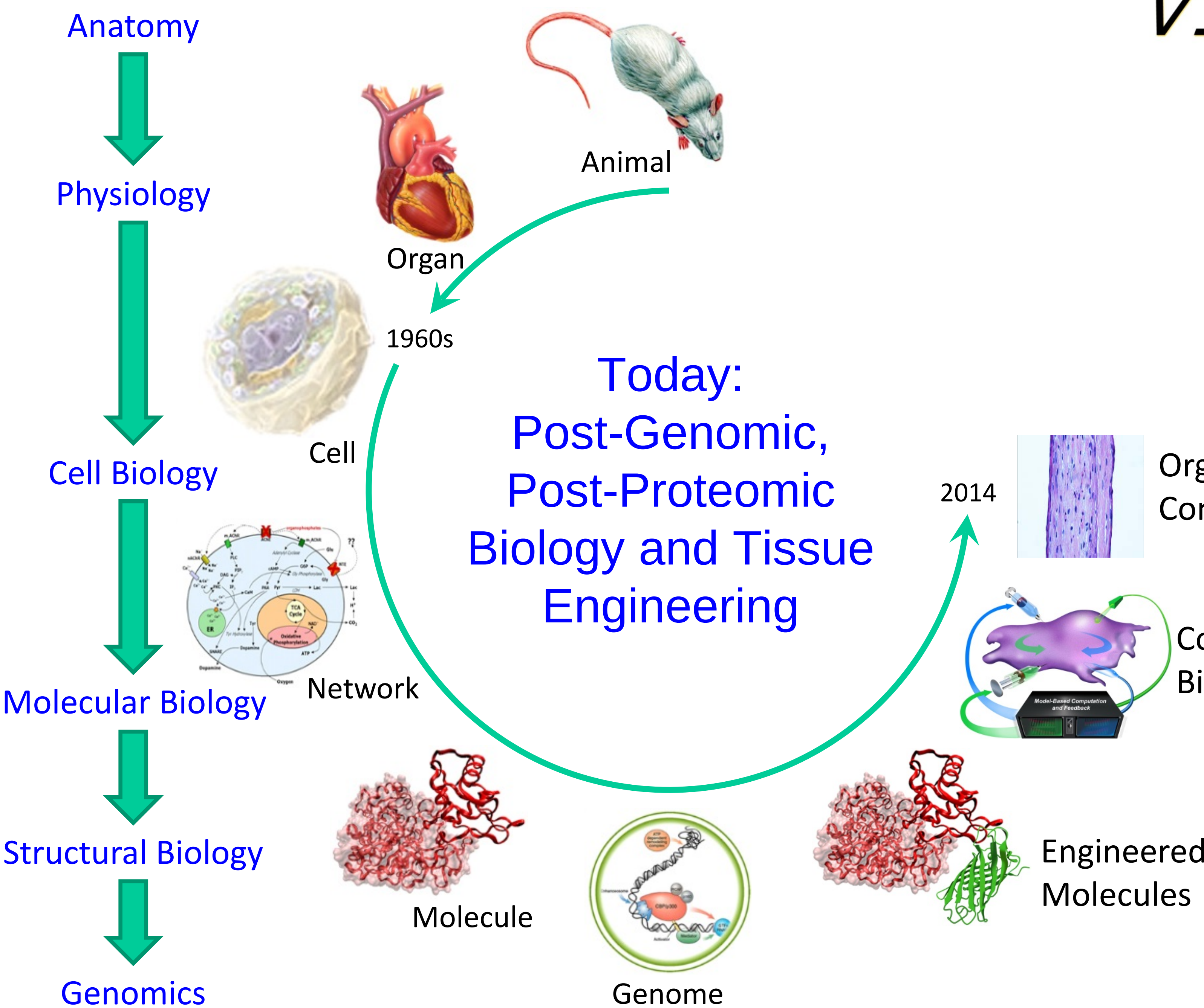
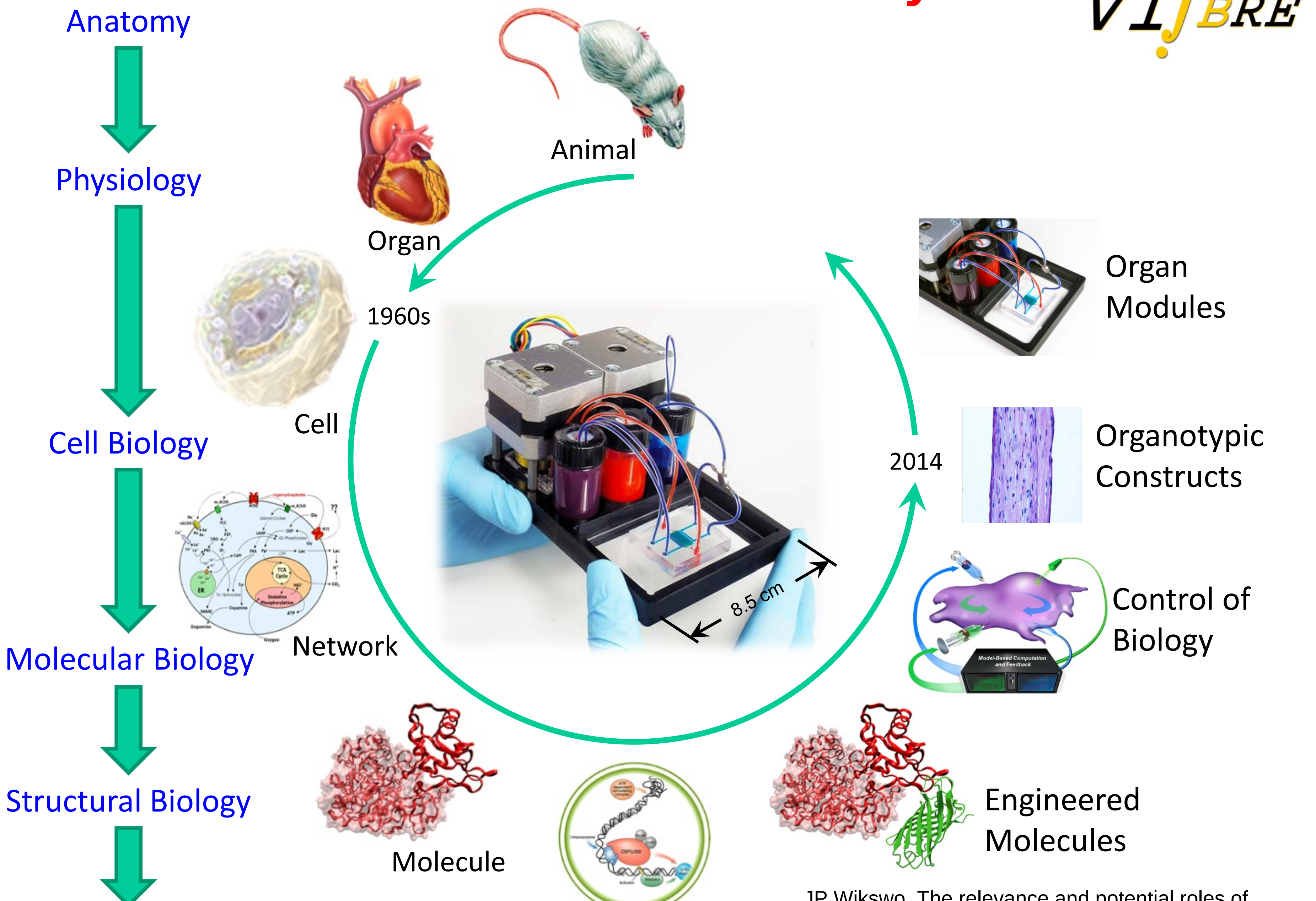


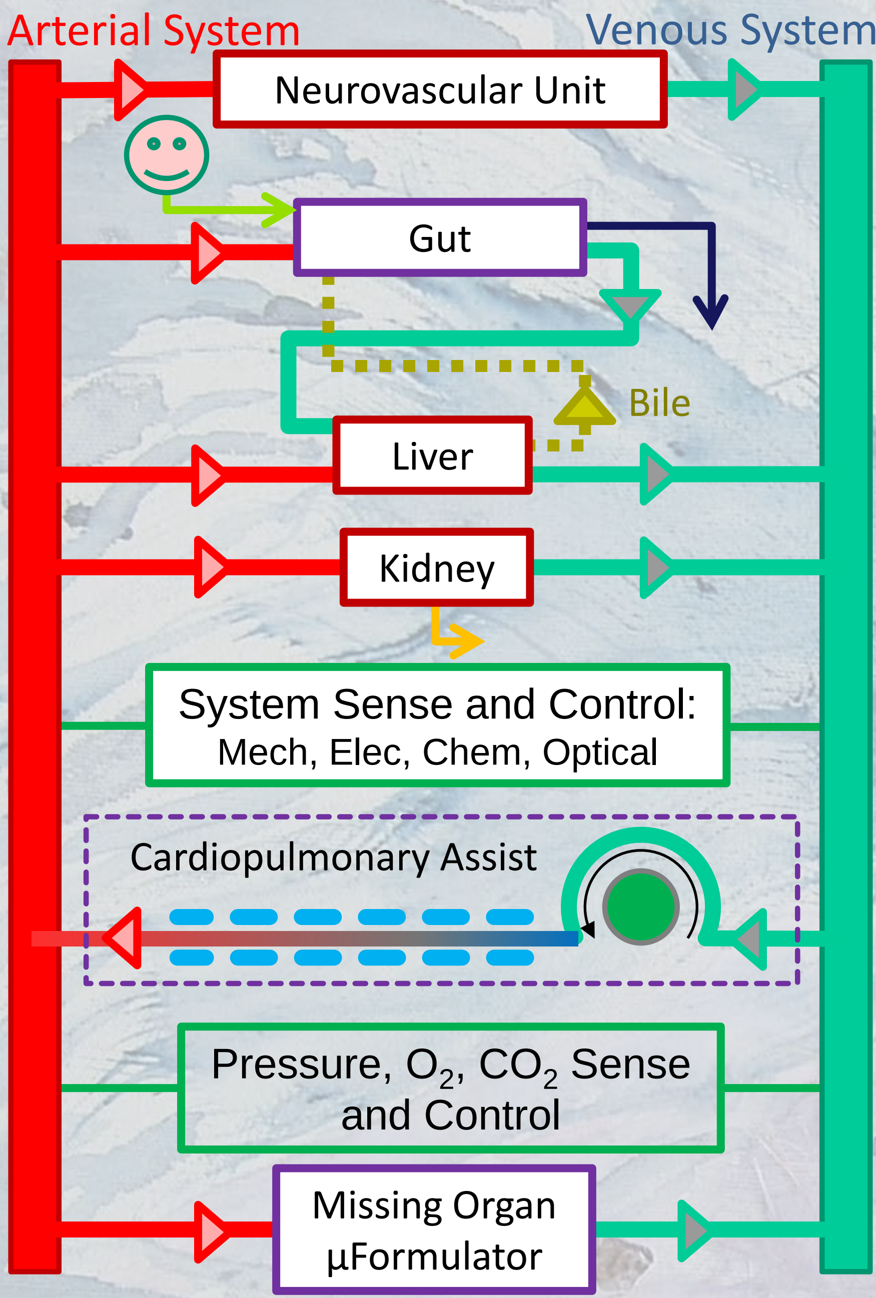
Anatomy
Physiology
Cell Biology
Molecular Biology
Structural Biology
Genomics
Animal
Organ
1960s
Cell
Network
Molecule
Genome
Today:
Post-Genomic,
Post-Proteomic
Biology and Tissue
Engineering
2014
Organotypic
Constructs
Control of
Biology
Model-Based Computation and Feedback
Engineered
Molecules

The next five years

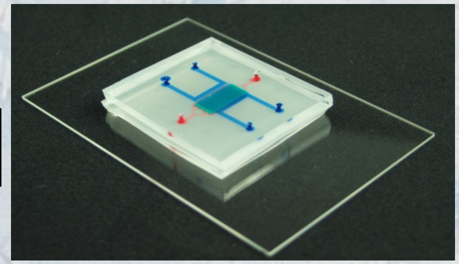


JP Wikswo. The relevance and potential roles of microphysiological systems in biology and medicine. Exp.Biol.Med. 239:1061-1072, 2014.

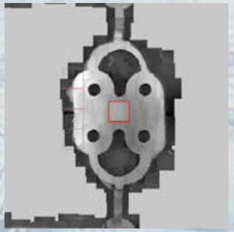
NIH NCATS MPS Integration:
Baylor, Johns Hopkins, MGH, Pittsburgh, Vanderbilt, Washington



NVU

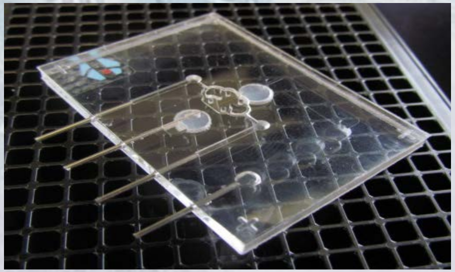


Gut



Liver

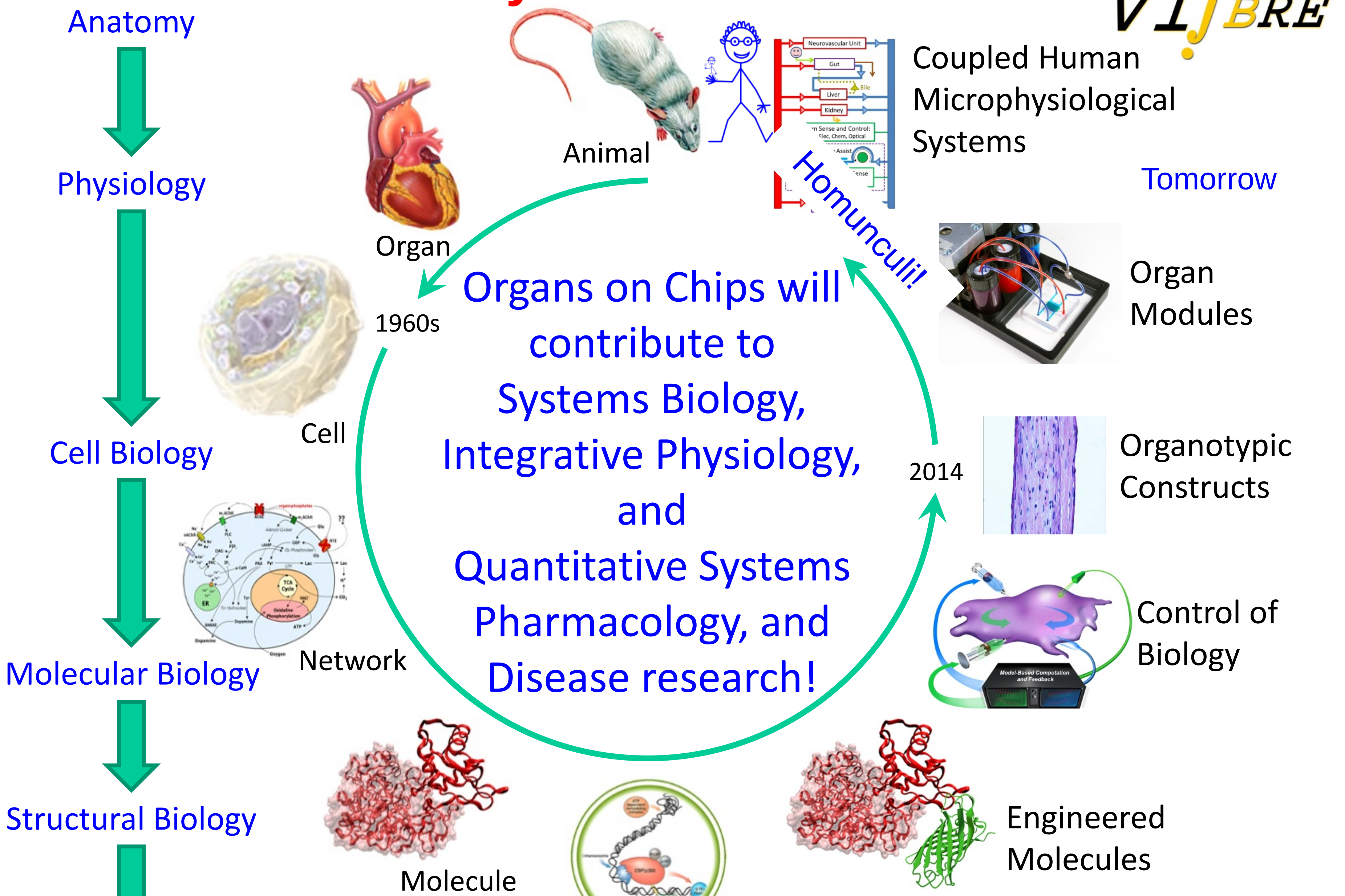
Kidney



NVU-µCA

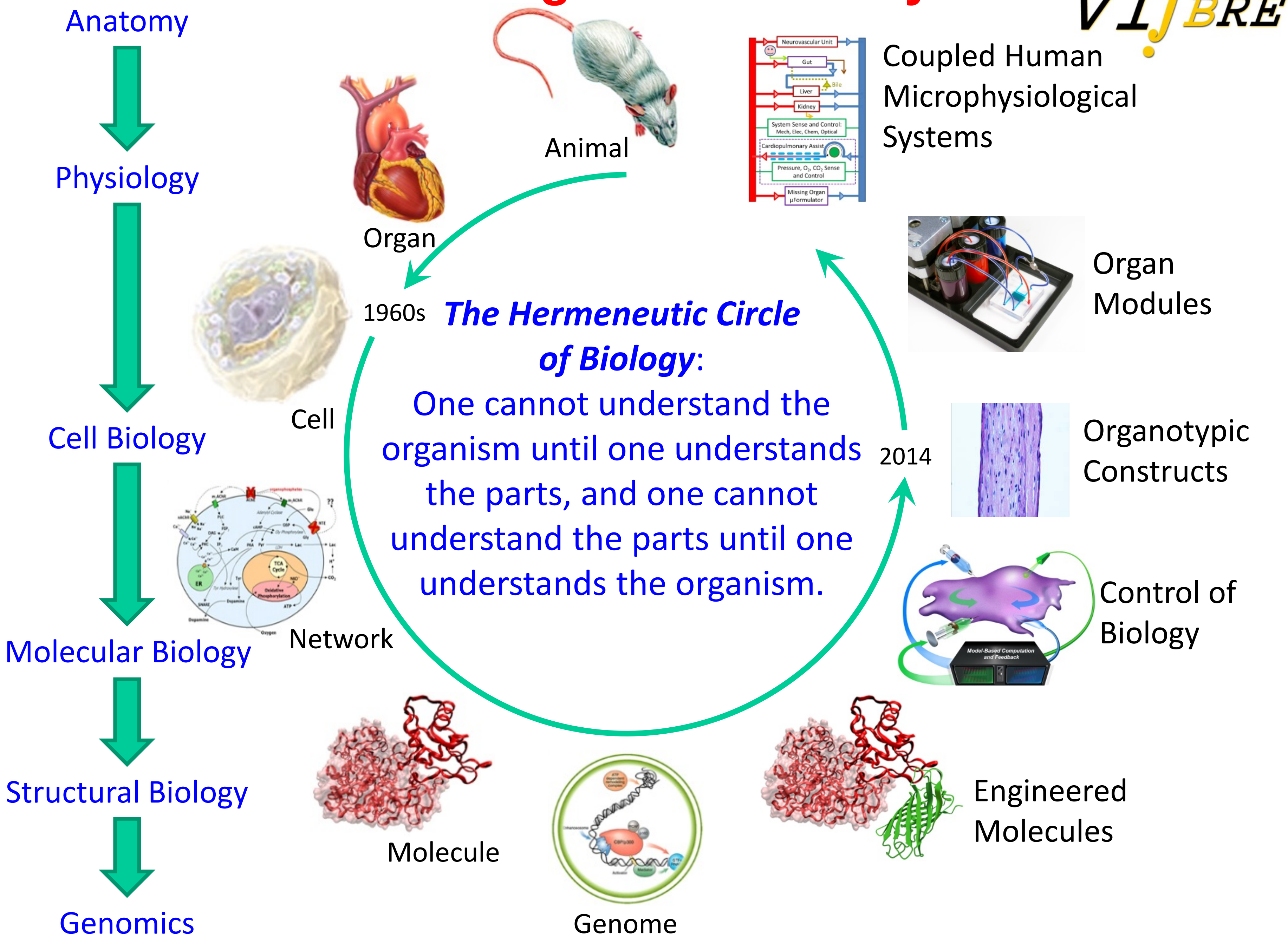
Julia Wikswo

The next five years: Disease Models

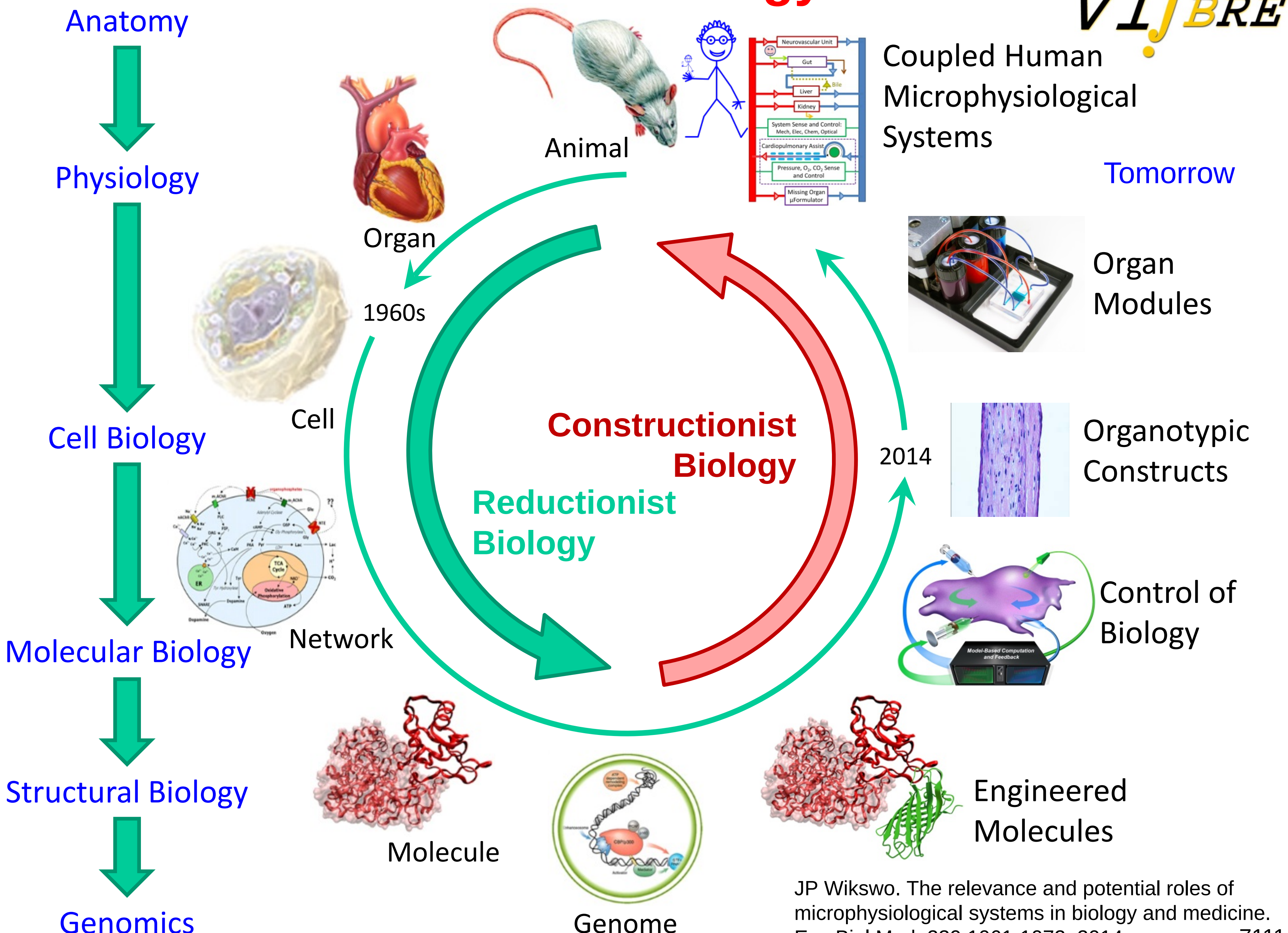


JP Wikswo. The relevance and potential roles of microphysiological systems in biology and medicine. Exp.Biol.Med. 239:1061-1072, 2014.

Almost time to begin a second cycle…



The Hermeneutic Circle of Biology



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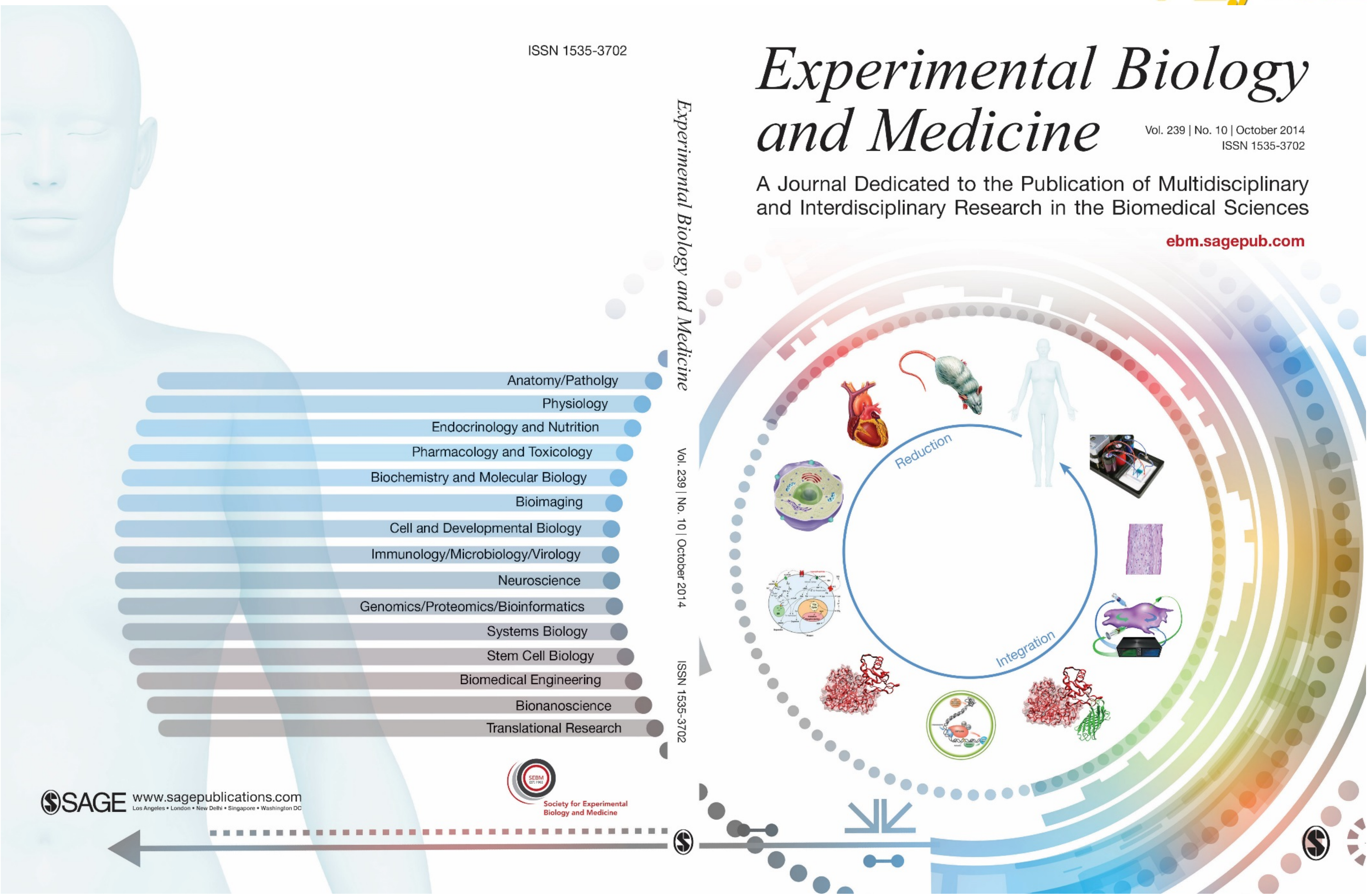
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Experimental Biology and Medicine
Vol. 239 | No. 10 | October 2014
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Systems Biology
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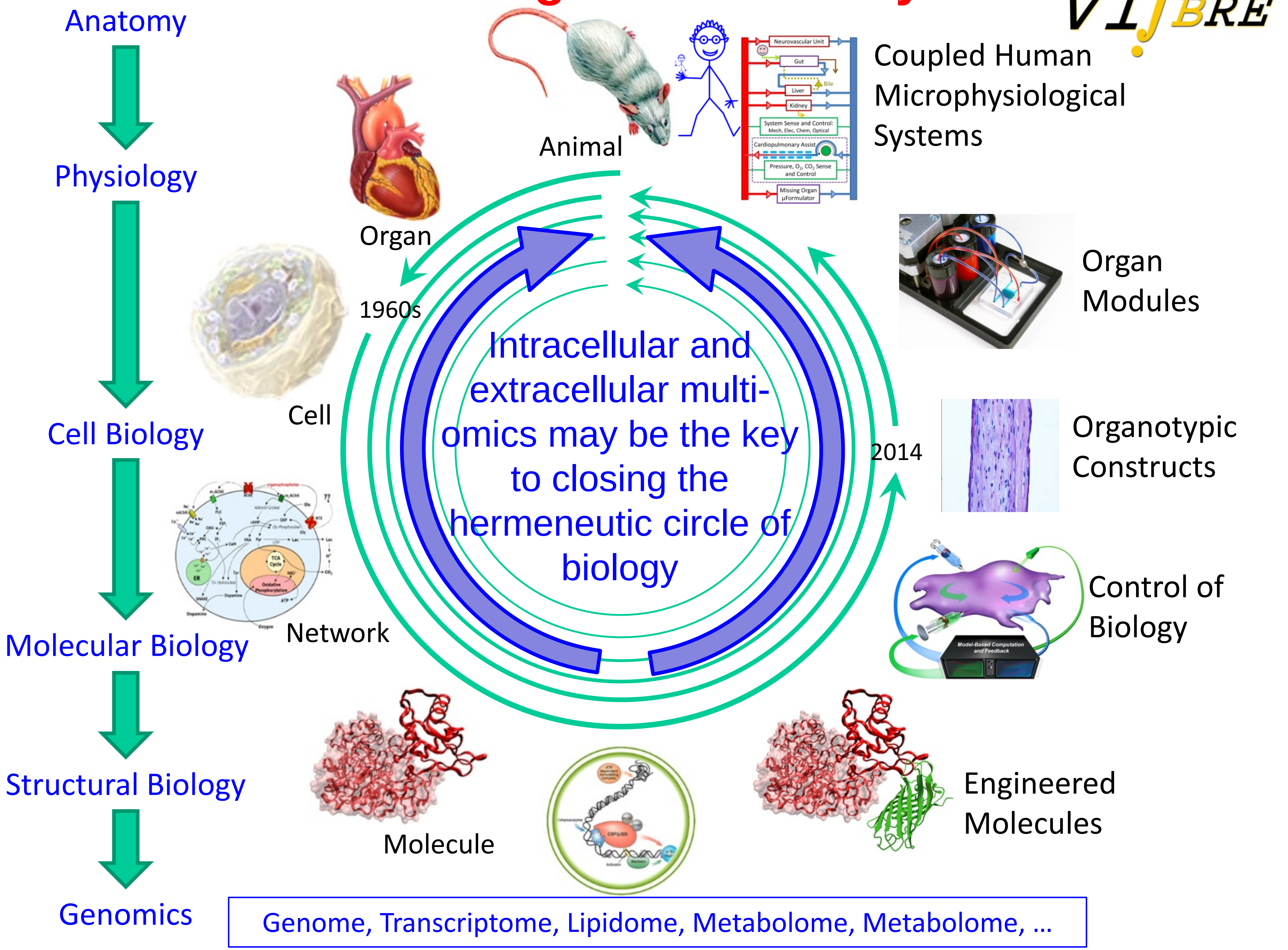
Almost time to begin a second cycle…



What's next for me?

- Missing organs
- Exploring pharmacokinetics (PK) without changing the medicinal chemistry
- Controlling iPSC differentiation

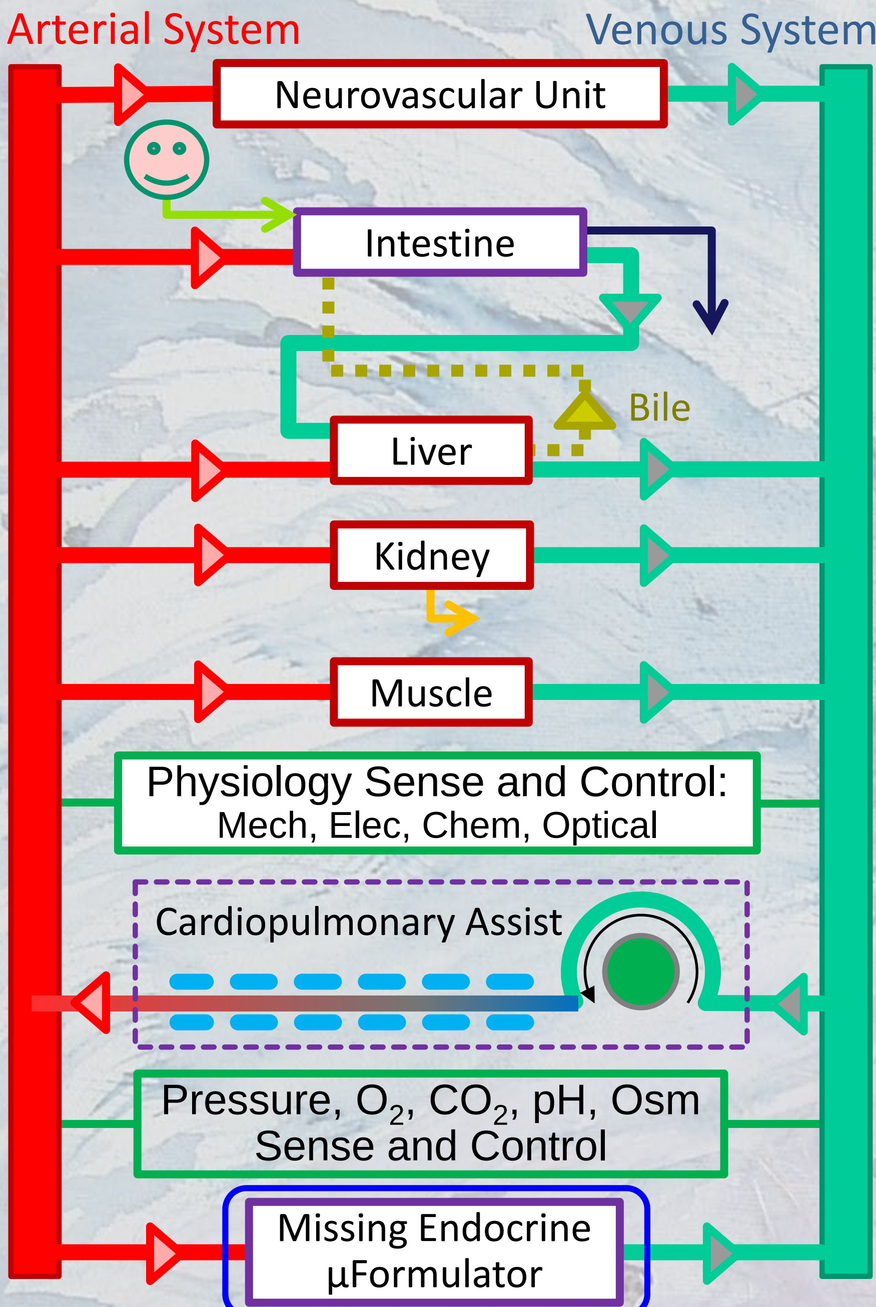
TAF

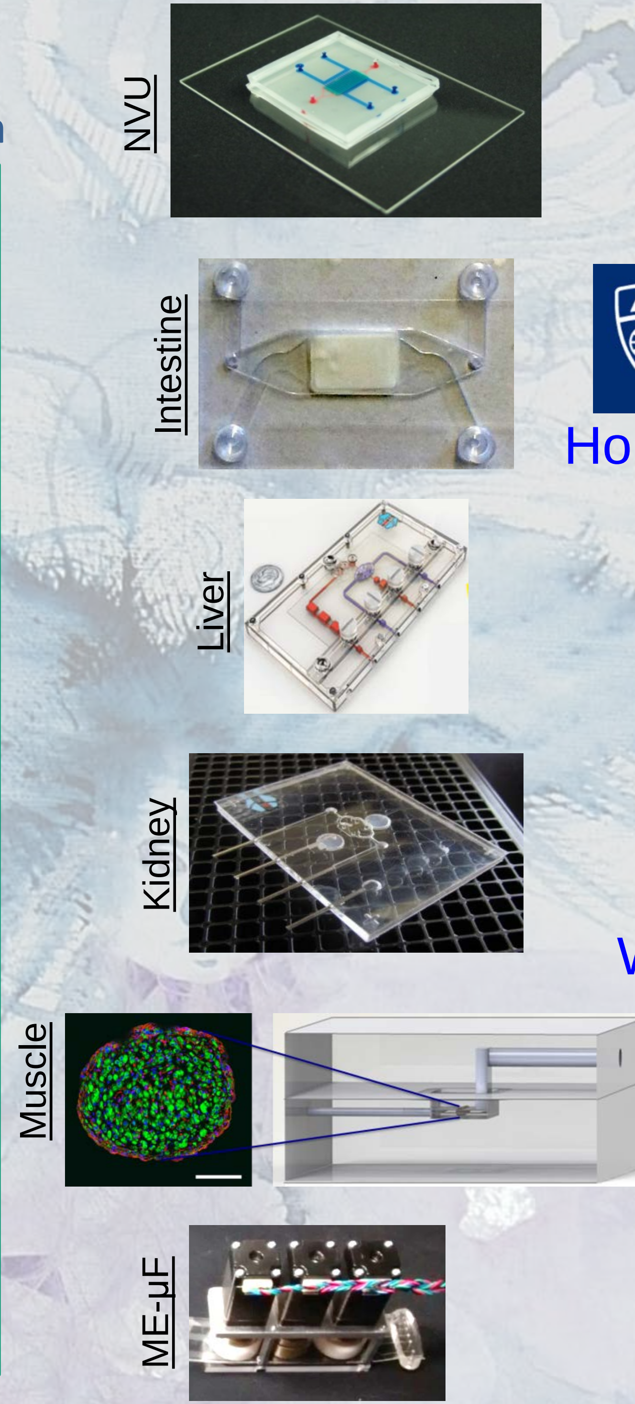
The "Missing Organ" Problem

- The human body has over a hundred organs.
- The Tissue Chips community is building "toy models" of humans, *i.e.,* Homunculi.
  - We cannot include every organ.
  - We should not include every organ.
- For a coupled organ system, there may always be a key organ that has been omitted.
- Missing <u>secretory</u> organs can be replaced with a ***Missing Organ Microformulator***.
  - Hormones, hormones, hormones

TAF

NIH-NCATS MPS Integration





Vanderbilt

Hopkins, Baylor

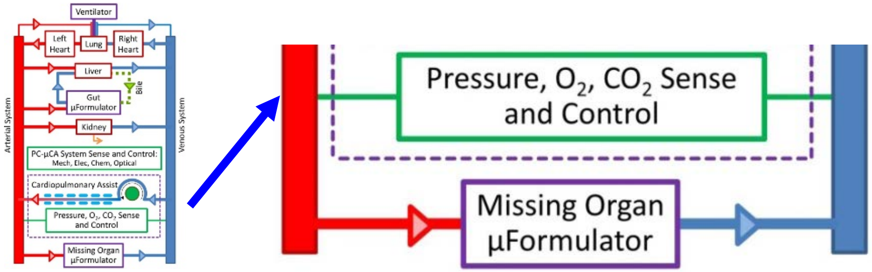
Pittsburgh

Washington

Duke

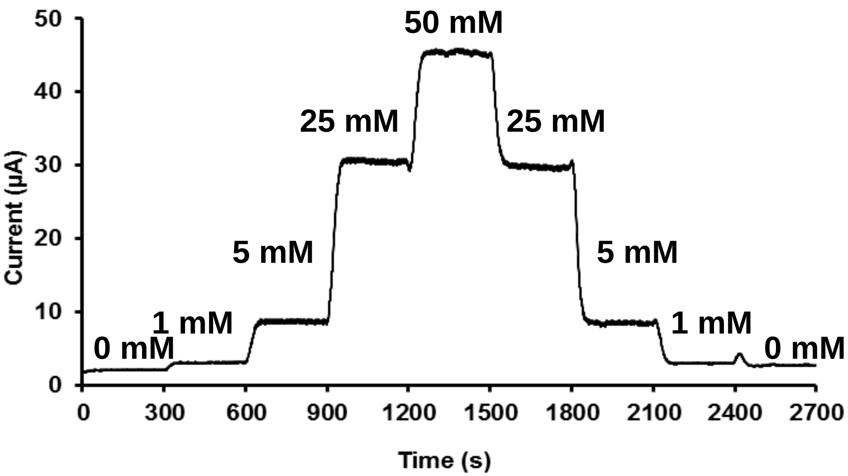
TAF

Missing Organ MicroFormulator (µF)

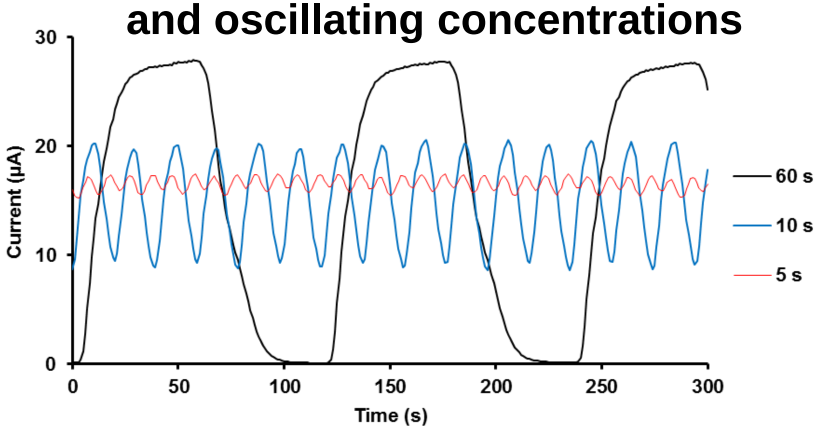


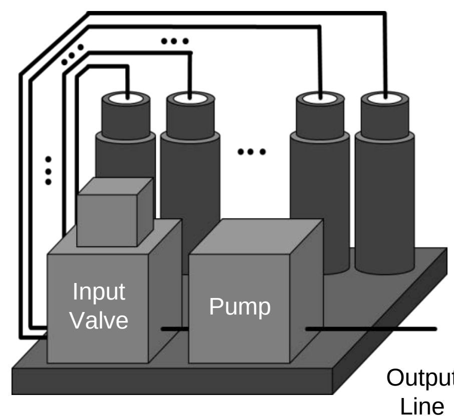
- Cliffel Group:
  - Testing performance with e-chem
  - Reduction of ferricyanide at -0.16V *vs.* Ag quasi-reference.
- Low leakage between ports
- Programming allows rapid switching between ports for dilution, gradients, and calibration of electrochemical sensors

Delivery of desired concentration

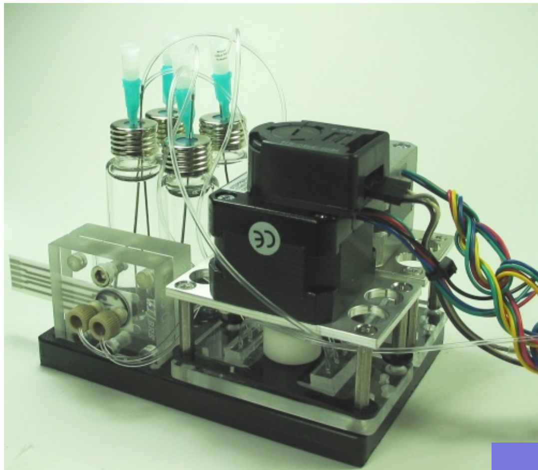


Time-division multiplexing and oscillating concentrations





United States Patent, 9,618,129 B2

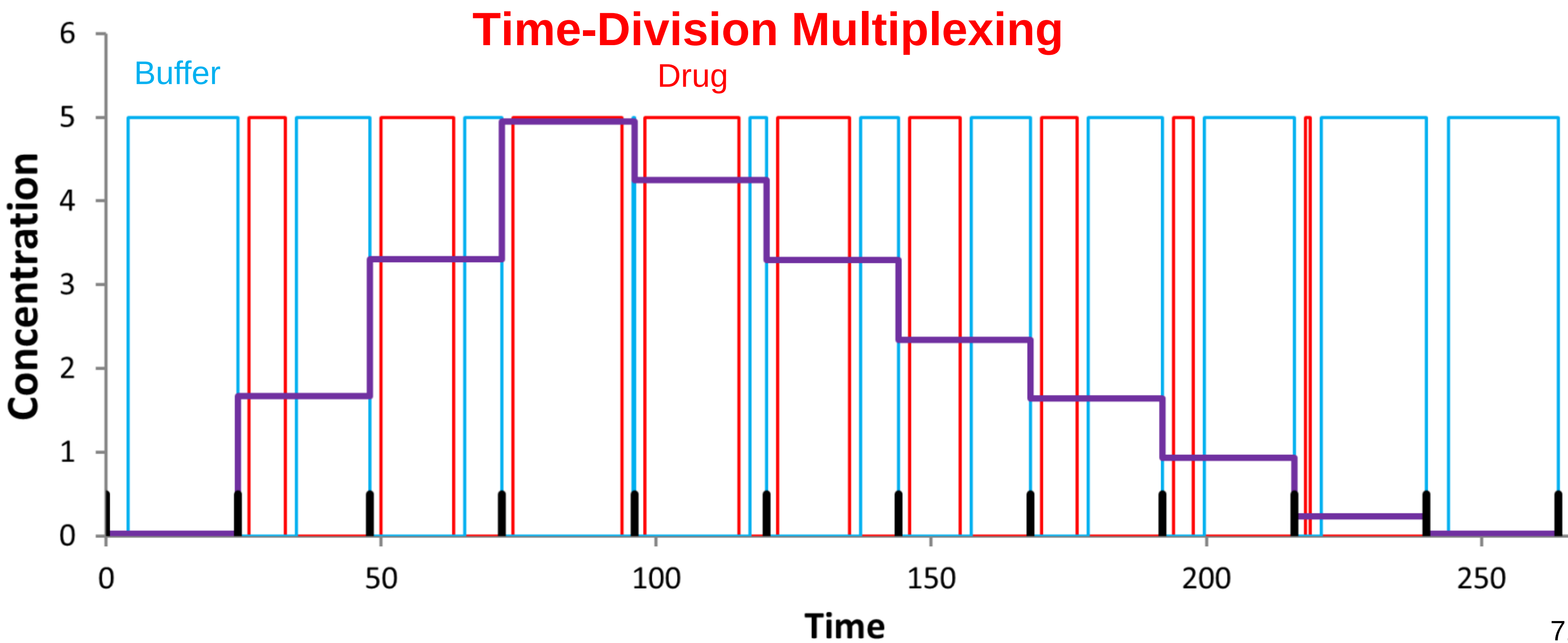


A normally closed rotary planar valve for microfluidic applications, F. E. Block III, J.R. McKenzie, P. C. Samson, D. A. Markov, and J. P. Wikswo, *In Preparation*.

TAF

What can you do with a μF-96?

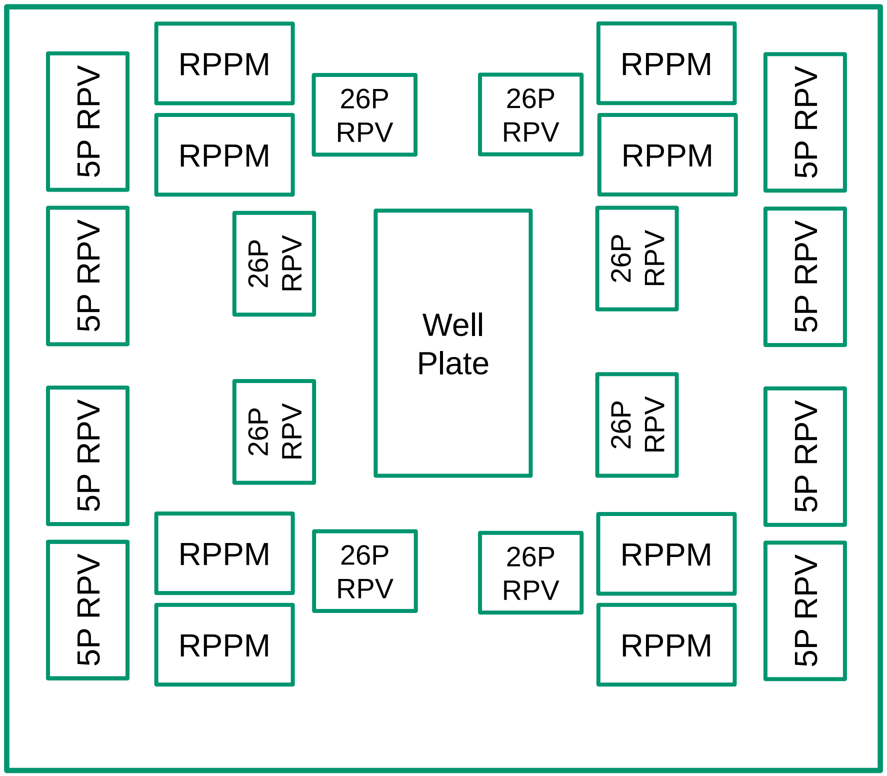
- Matt Wagoner – AstraZeneca: “Make me 96 of them! Use time-division multiplexing to create realistic PK drug-exposure profiles individualized for each and every well in a 96-well-plate HTS assay!”
  - Conventional cell culture
  - Massively parallel organs on chips
  - Organoid HTS arrays
    - Hanging drop
    - Transwells

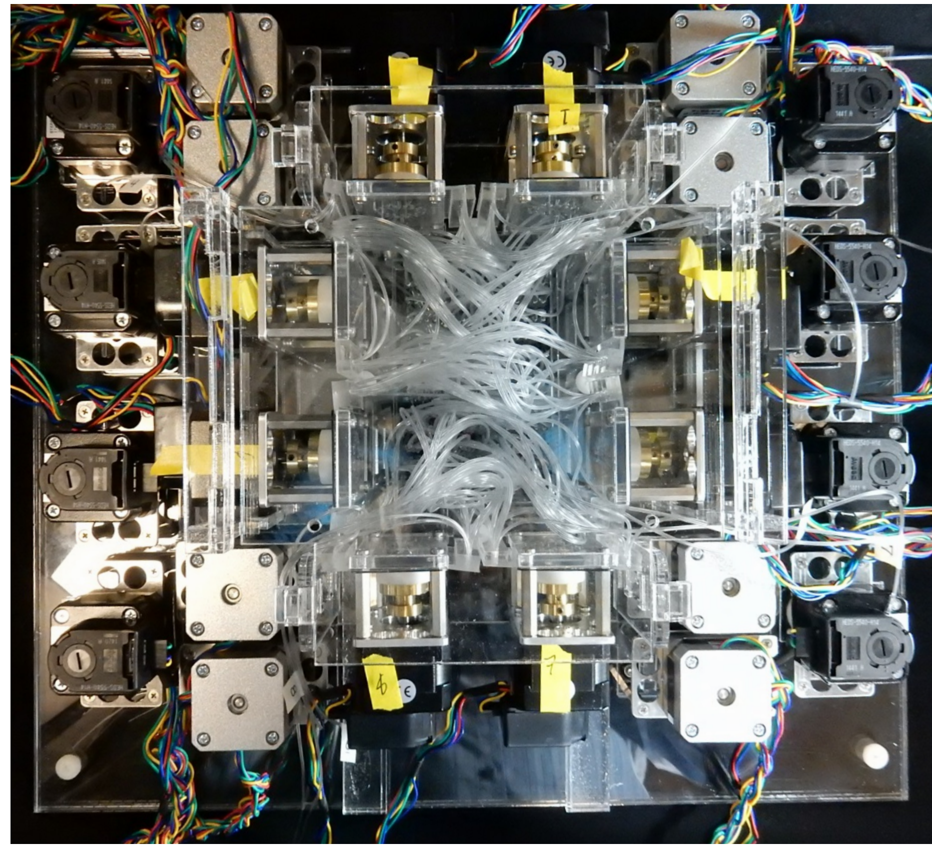


TAF

μF-96 v1.0: January 2016

Funded in part by AstraZeneca as a collaborative effort initiated by Matt Wagoner, with Jay Mettetal and postdoc Aditya Kolli. Now involving Kristin Fabre and Clay Scott, and postdocs Sudhir Deosarkar and Jingwen Zhang.







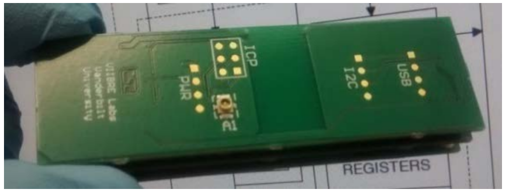


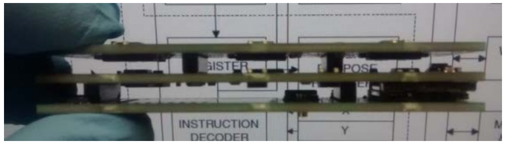

Can individually formulate, deliver, and remove custom media cocktails to each well of a 96-well plate to simulate PK profiles.

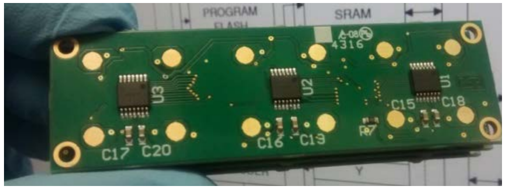
TAF

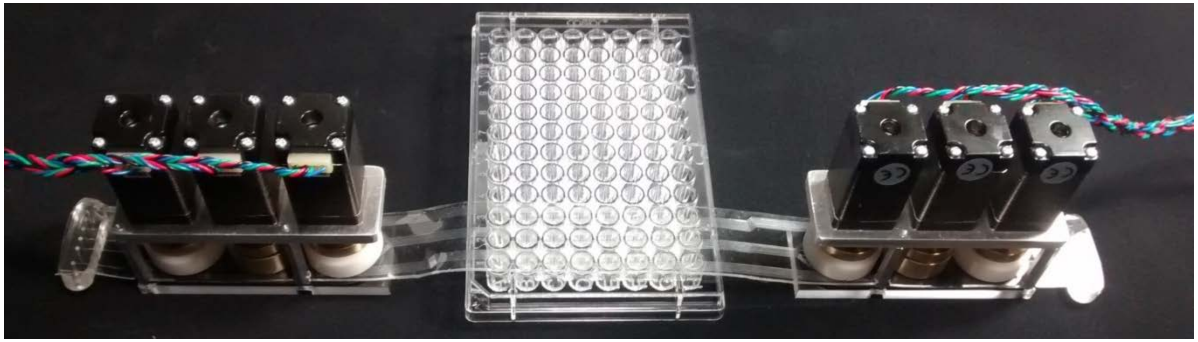
96-Channel MicroFormulator (μF-96), v2.0

- For each well, formulate a custom media/drug mixture in real time.
- Change 10% of the fluid in each well 40x/day.







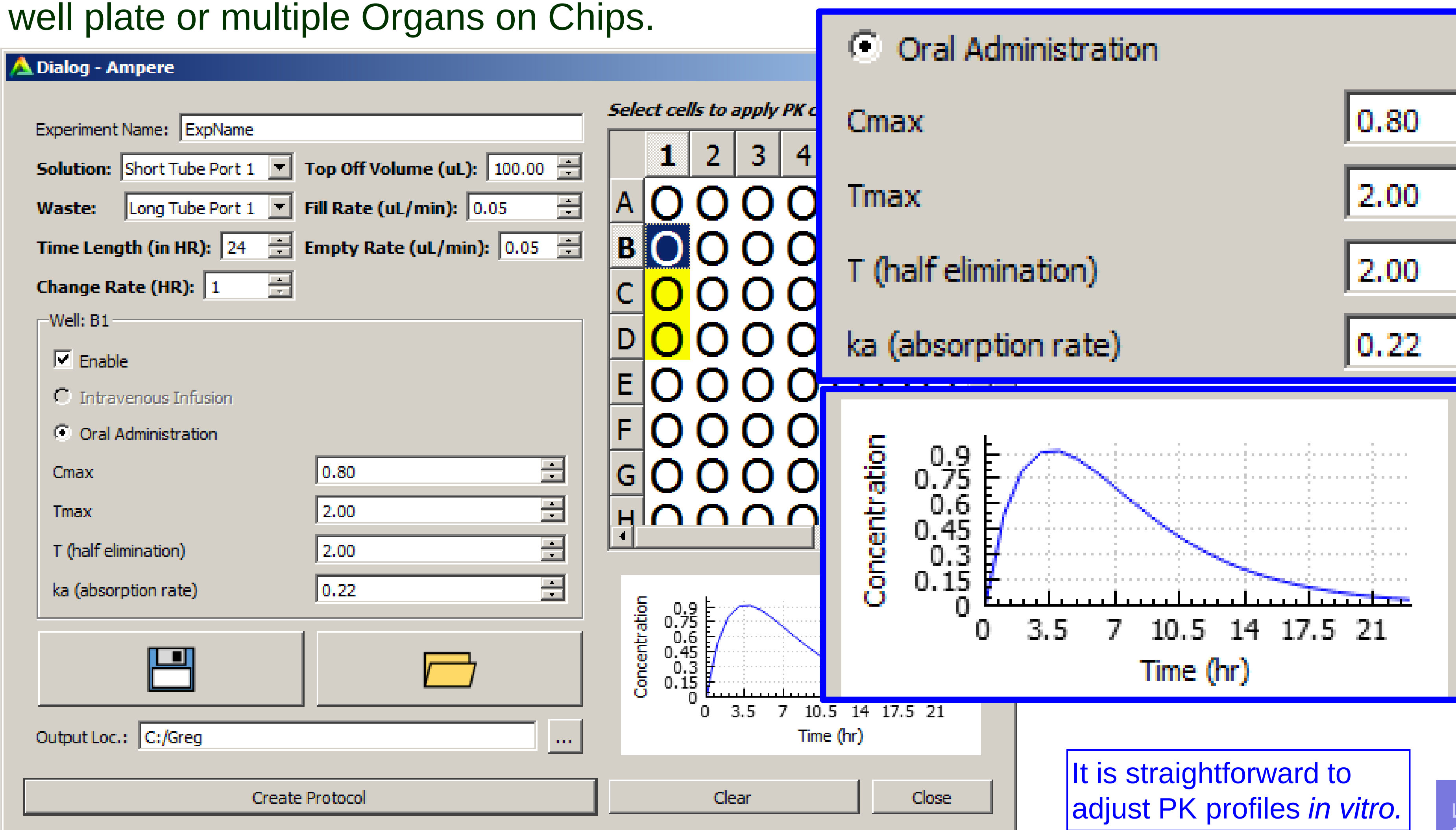


TAF

Funded in part by AstraZeneca and an NIH/NCATS SBIR to CFD Research Corporation. Licensed to CN Bio Innovations

Well Plate Tool

Challenge: Develop a tool for configuring and tracking fluid delivery (including PK exposure profiles) to individual wells in a 96-well plate or multiple Organs on Chips.



It is straightforward to adjust PK profiles *in vitro.*

TAF

Funded primarily by an NCATS SBIR to CFD Research Corp. Developed by Greg Gerken, VIIBRE

2019: CN Bio Innovations' PharmacoMimix™

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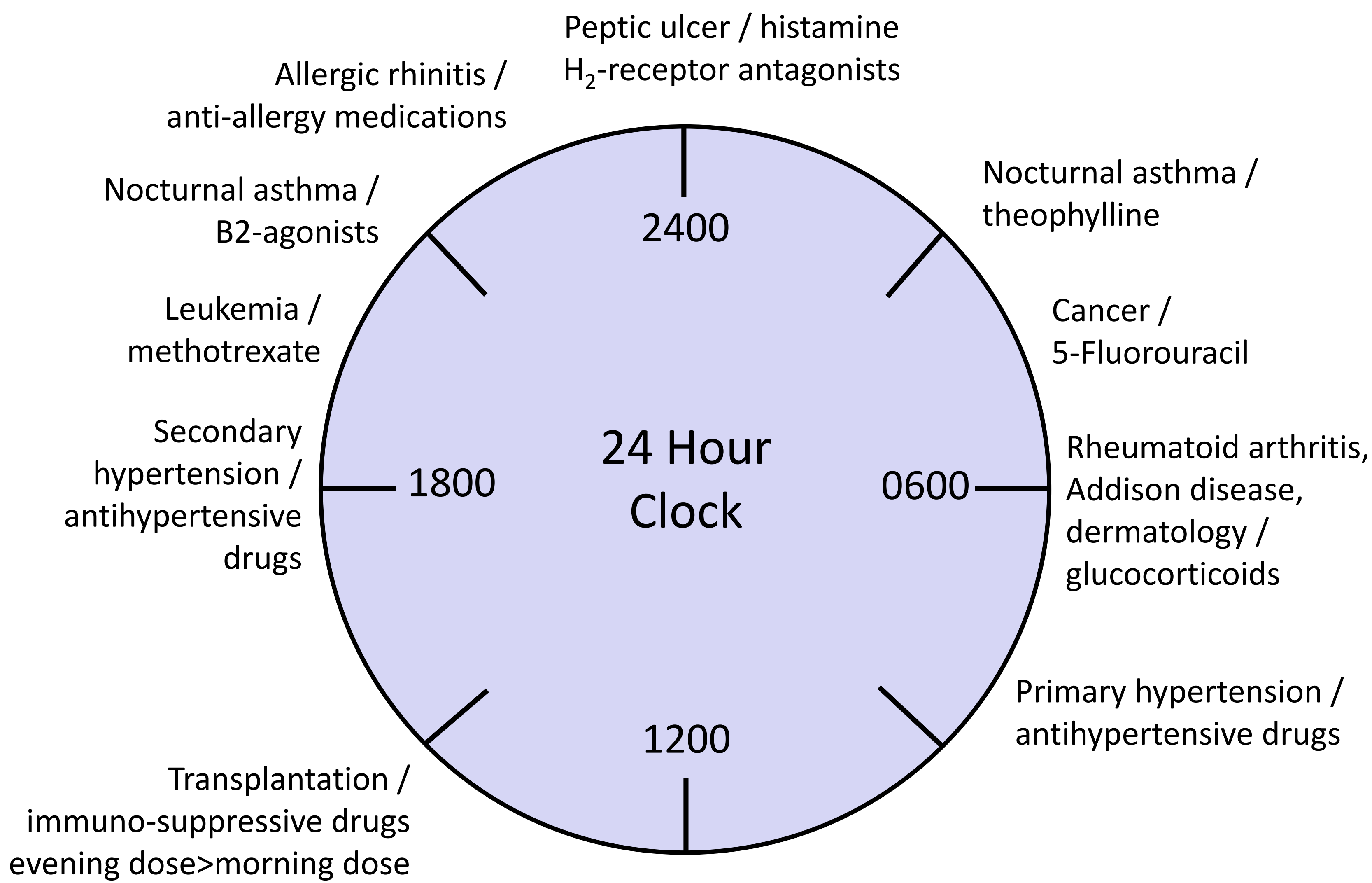
Diseases and Optimal Drug Dosing are Circadian



Adapted from Baraldo MD (2008) The influence of circadian rhythms on the kinetics of drugs in humans, Expert Opinion on Drug Metabolism & Toxicology, 4:2, 175-192,

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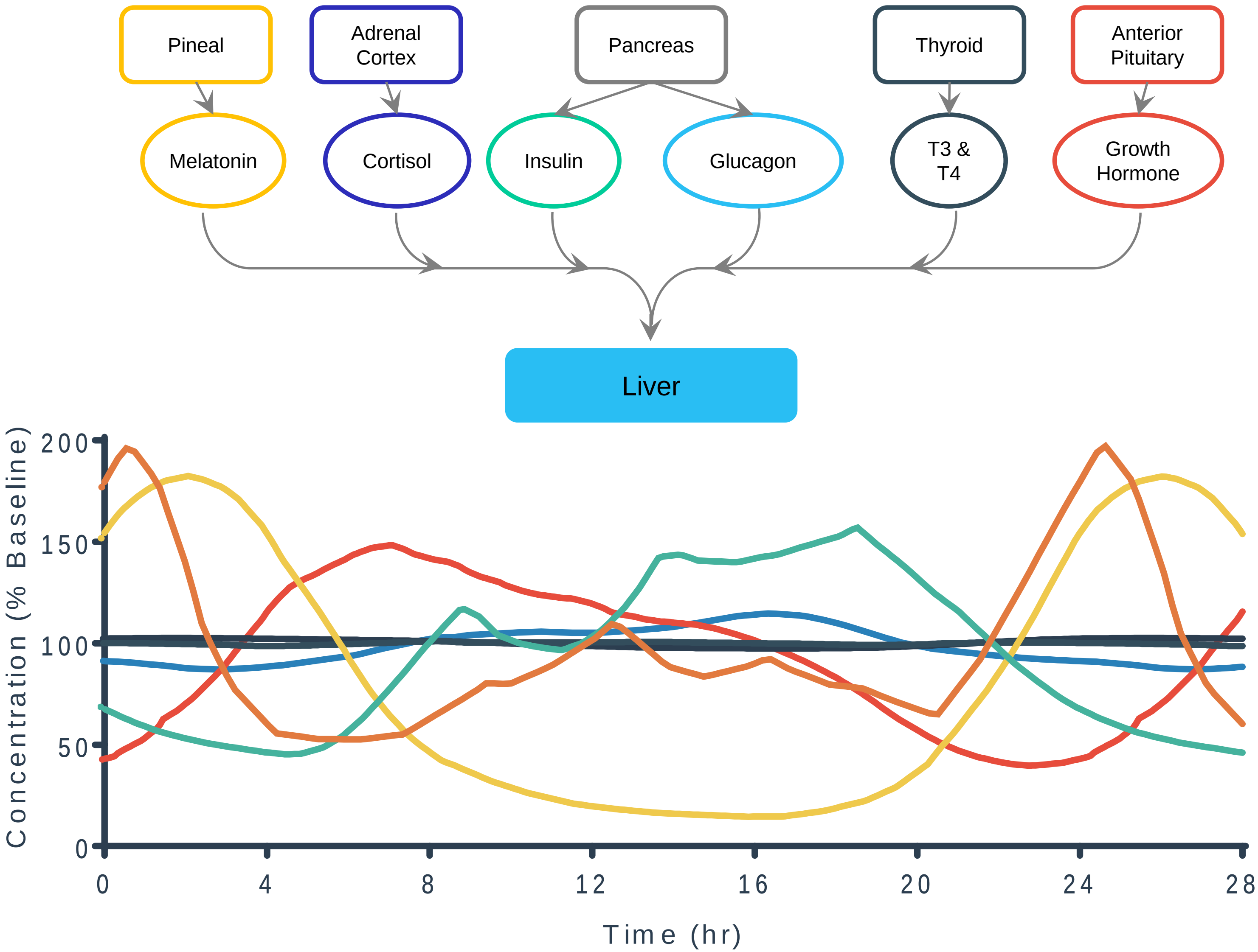
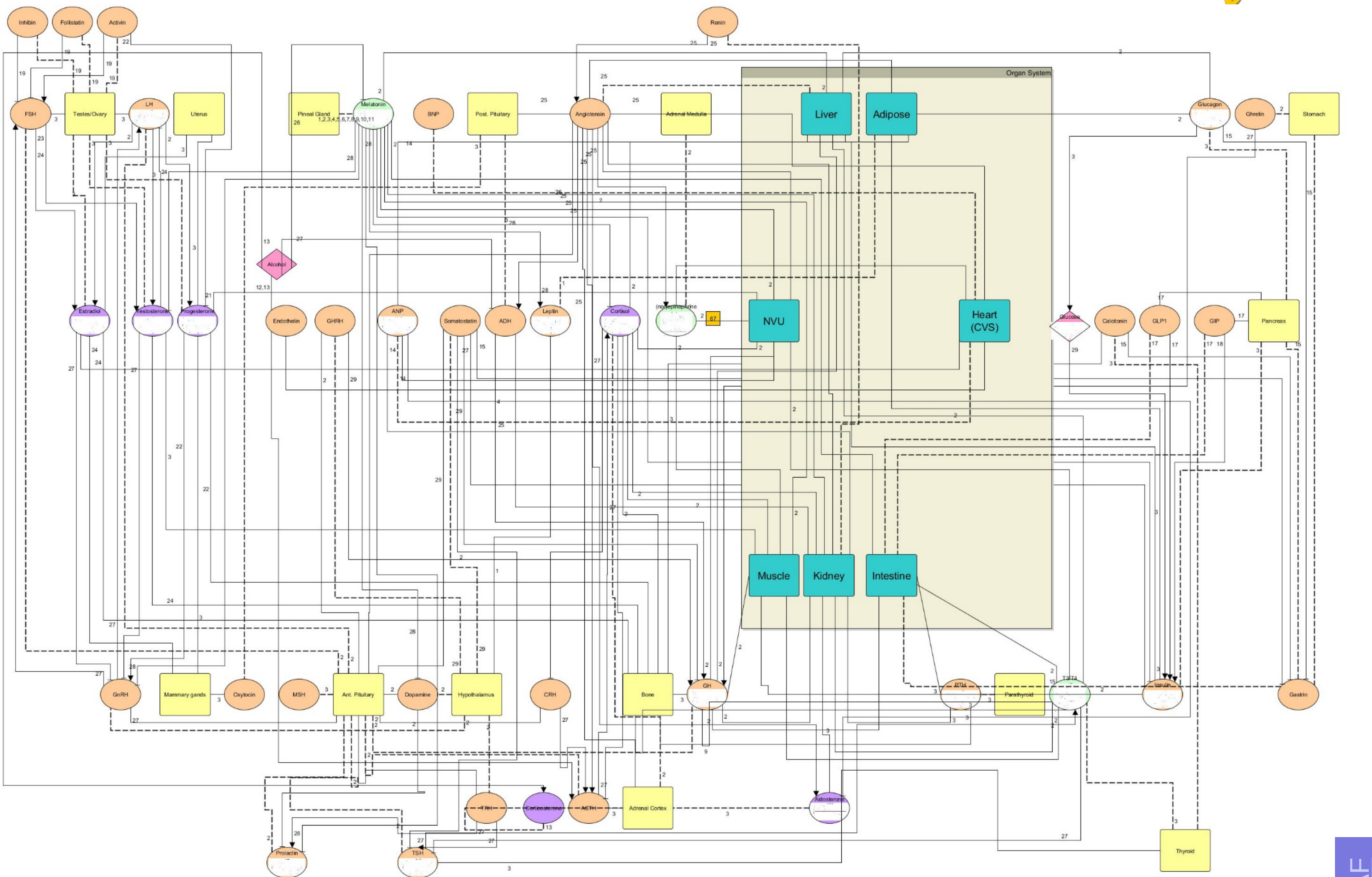
Diurnal Variations of Liver-Regulating Hormones



Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

Which endocrine organs / hormones do we need?



Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

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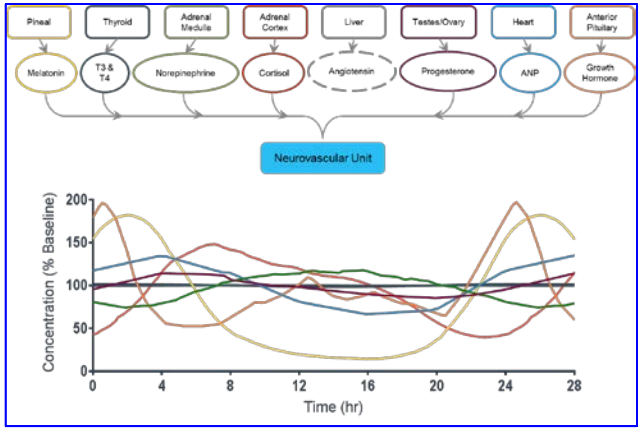
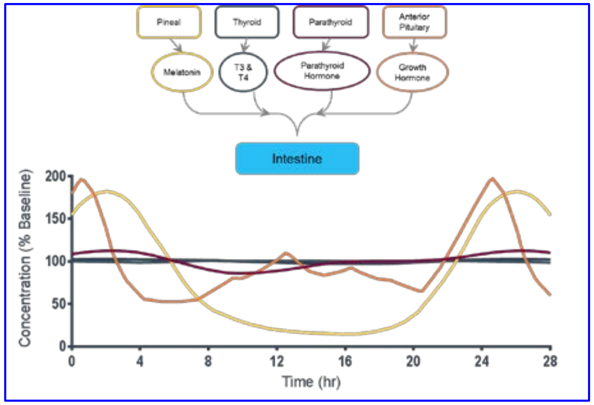
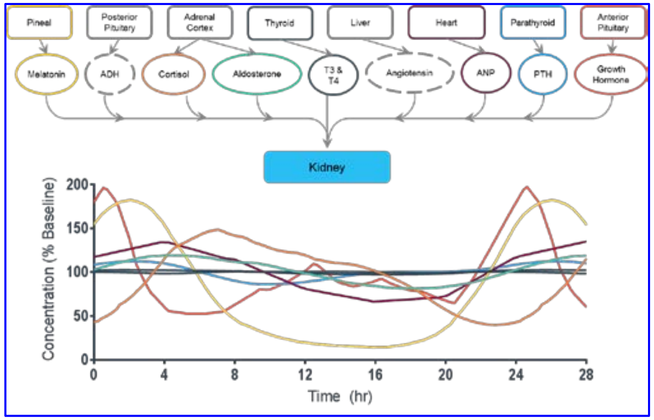
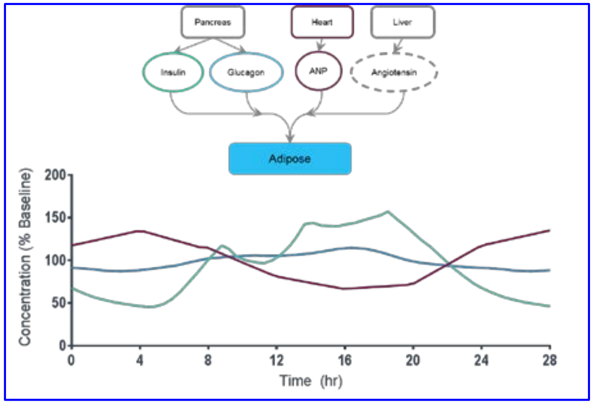
Diurnal Variations of Organ-Regulating Hormones

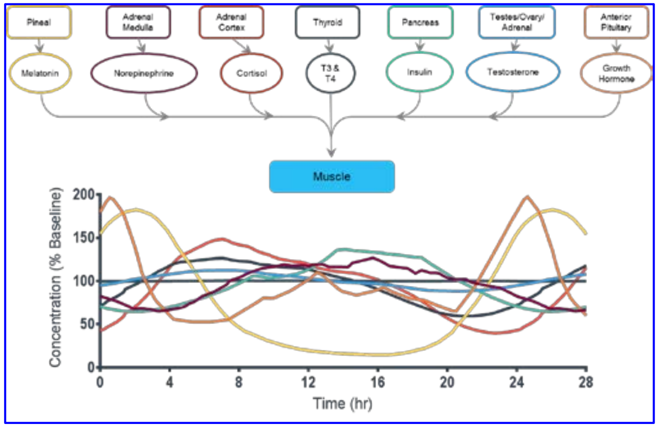


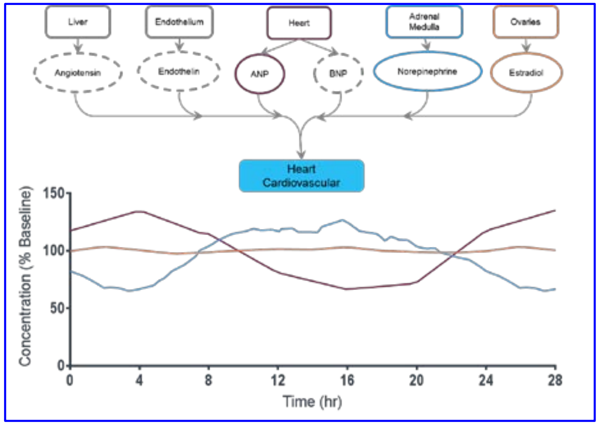


- Neurovascular Unit
- Kidney
- Muscle
- Adipose
- Heart / Cardiovascular









Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

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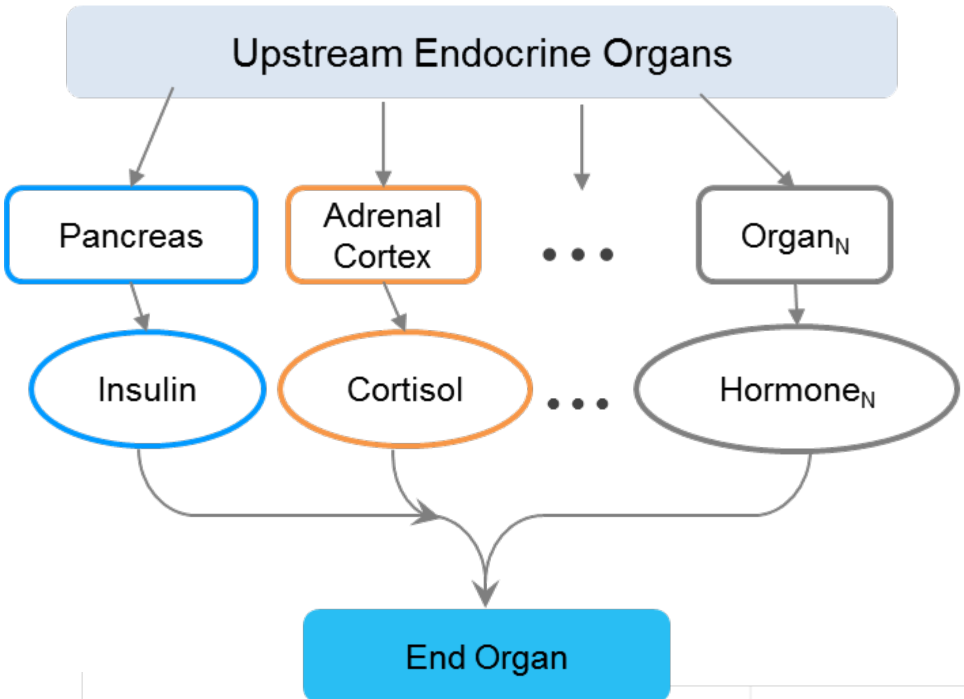
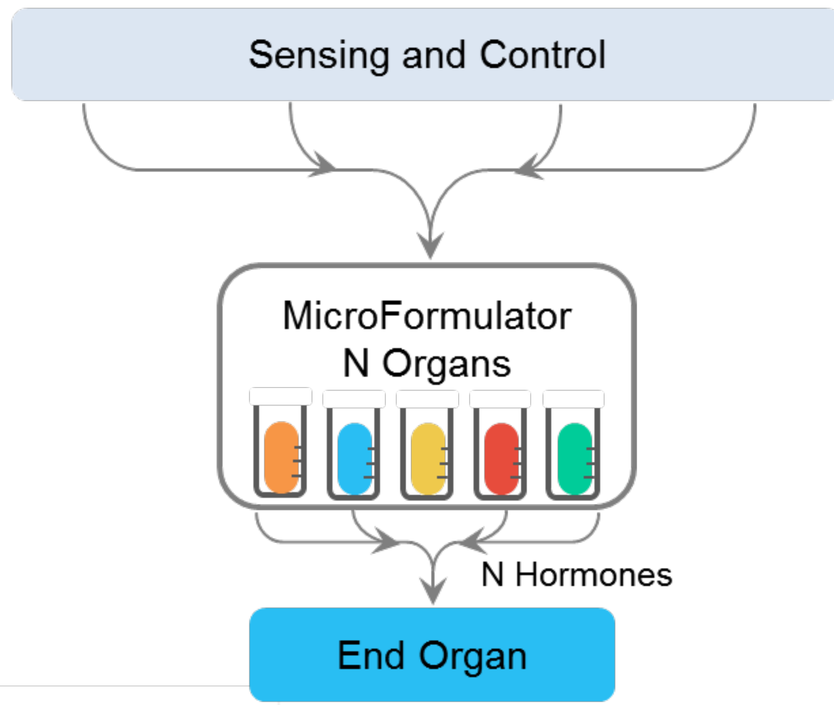
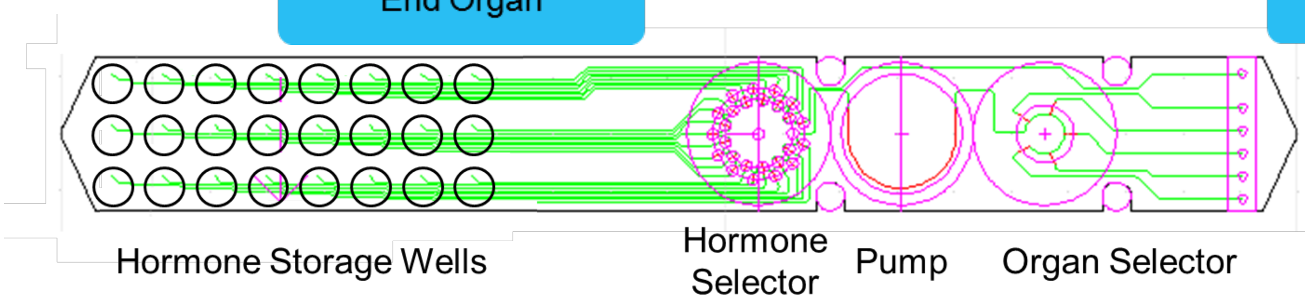
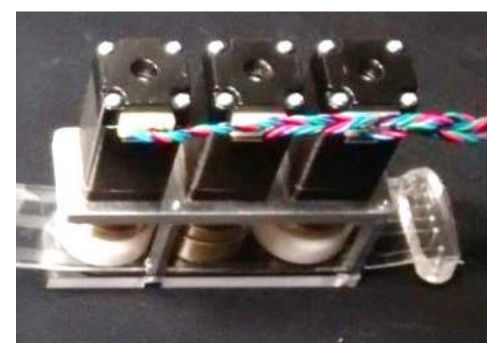
Diurnal Variations of Organ-Regulating Hormones







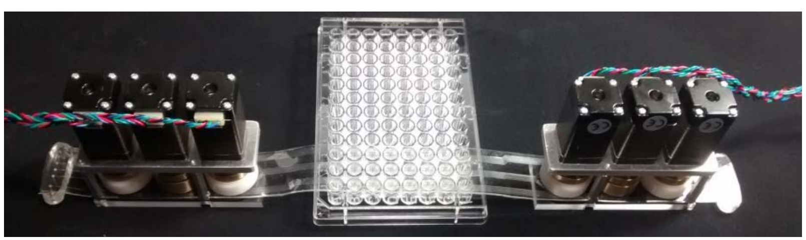


The MicroFormulator can bring diurnal rhythms to biology on plastic.

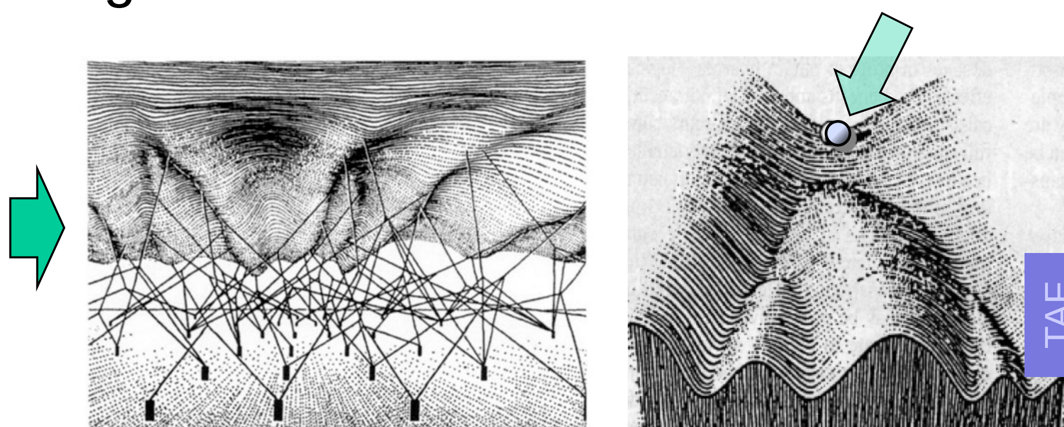
Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

What can you do with a µF-96?

- Use time-division multiplexing to create realistic PK drug-exposure profiles individualized for each and every well in a 96-well-plate HTS assay.
  - Conventional cell culture
  - Massively parallel organs on chips
  - Organoid HTS arrays
- Explore in a massively parallel manner the multitude of combinations of growth factors and other compounds that are needed to guide iPSC differentiation to specific cellular phenotypes.
  - Readily applicable to organoid developmental biology
  - Suitable for machine learning and automated model inference.



Add and remove growth factors, etc., at will



Waddington 1957

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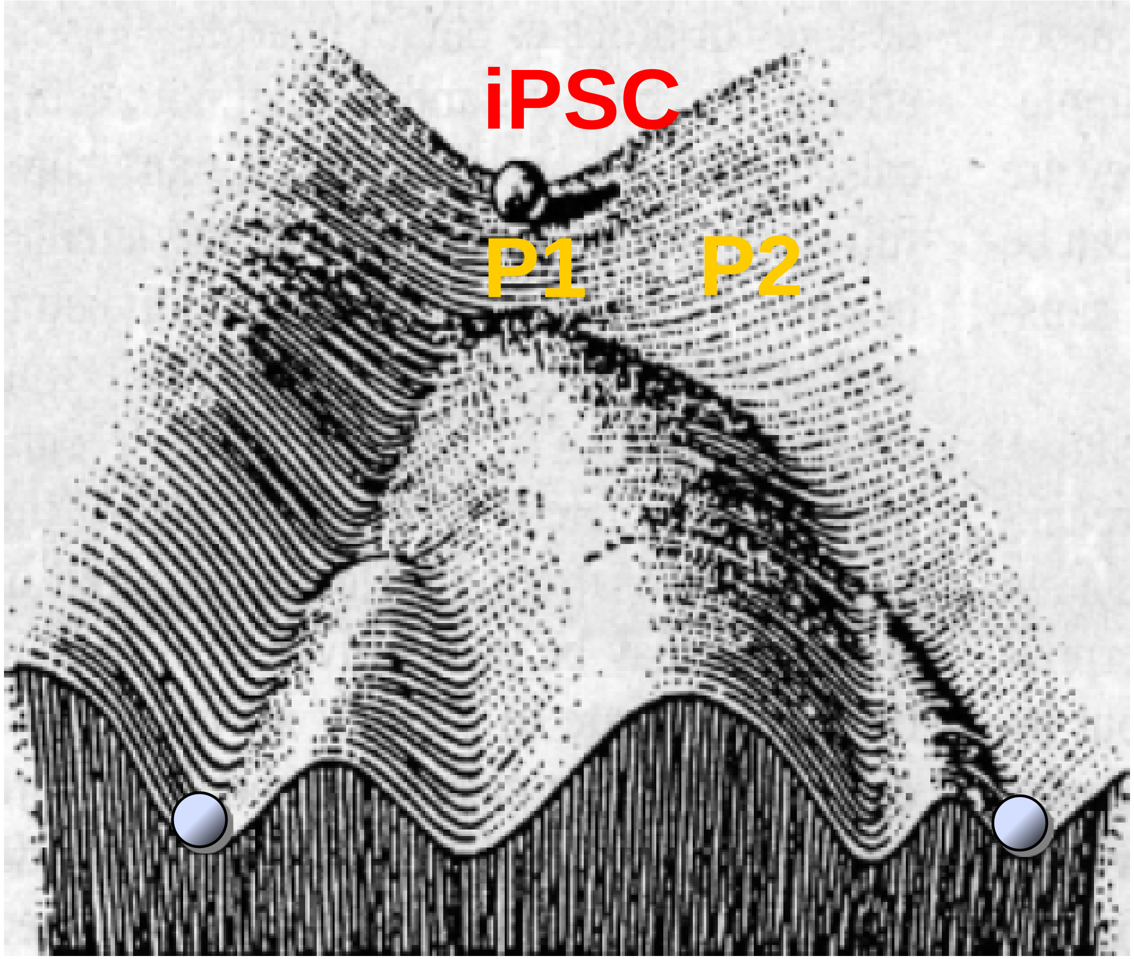
Stem Cell Differentiation: Cell phenotype rolling down the epigenetic landscape



This is a 10^2 to 10^5 dimensional surface

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.

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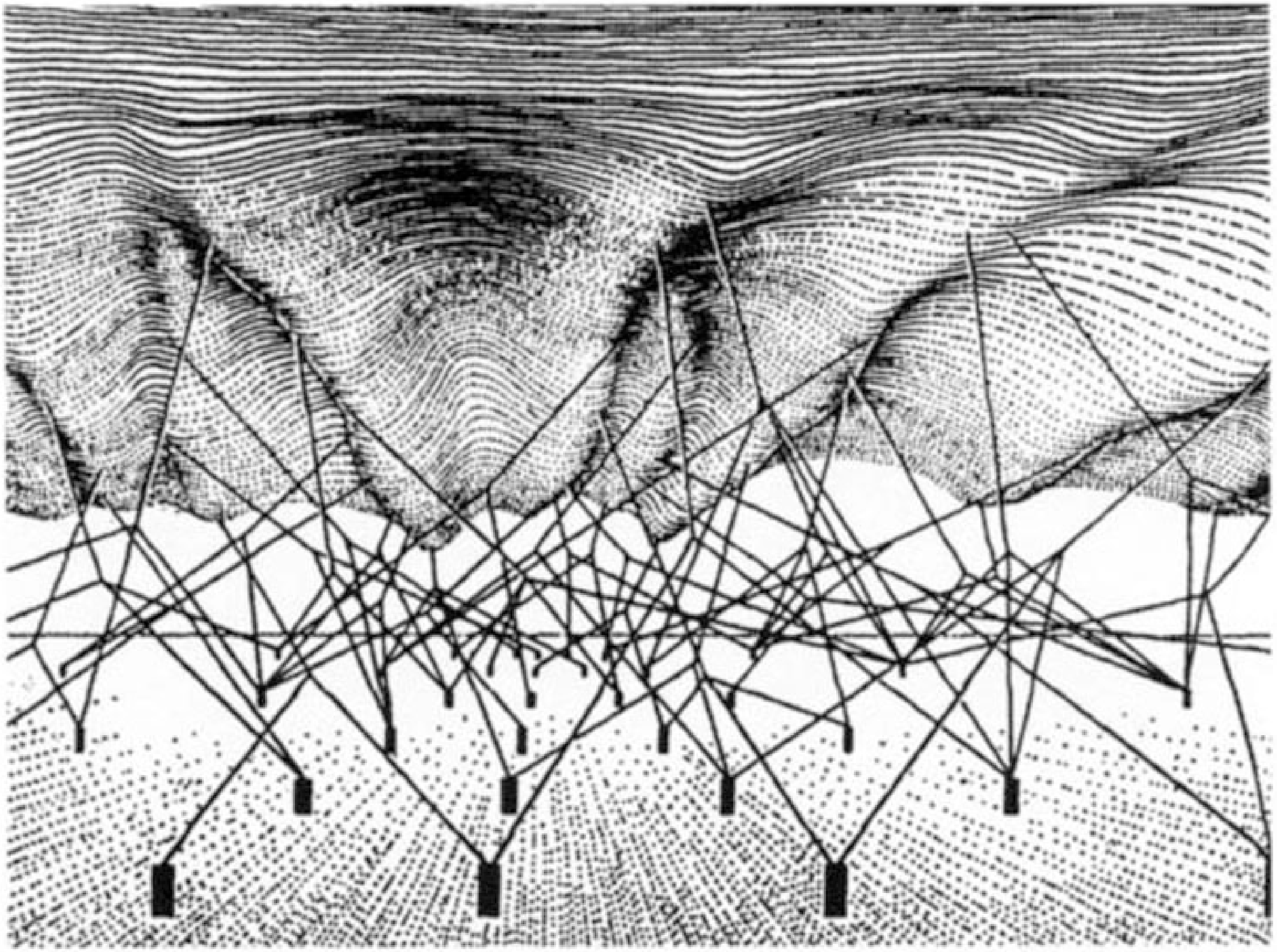
The epigenetic landscape reflects complex and dynamic genetic control.



- iPSCs
- ESCs
- SCNT-ESCs
- Epigenetic control

Nonequilibrium thermodynamics allows uphill motion.

We need to control the sticks!

Waddington, 1957

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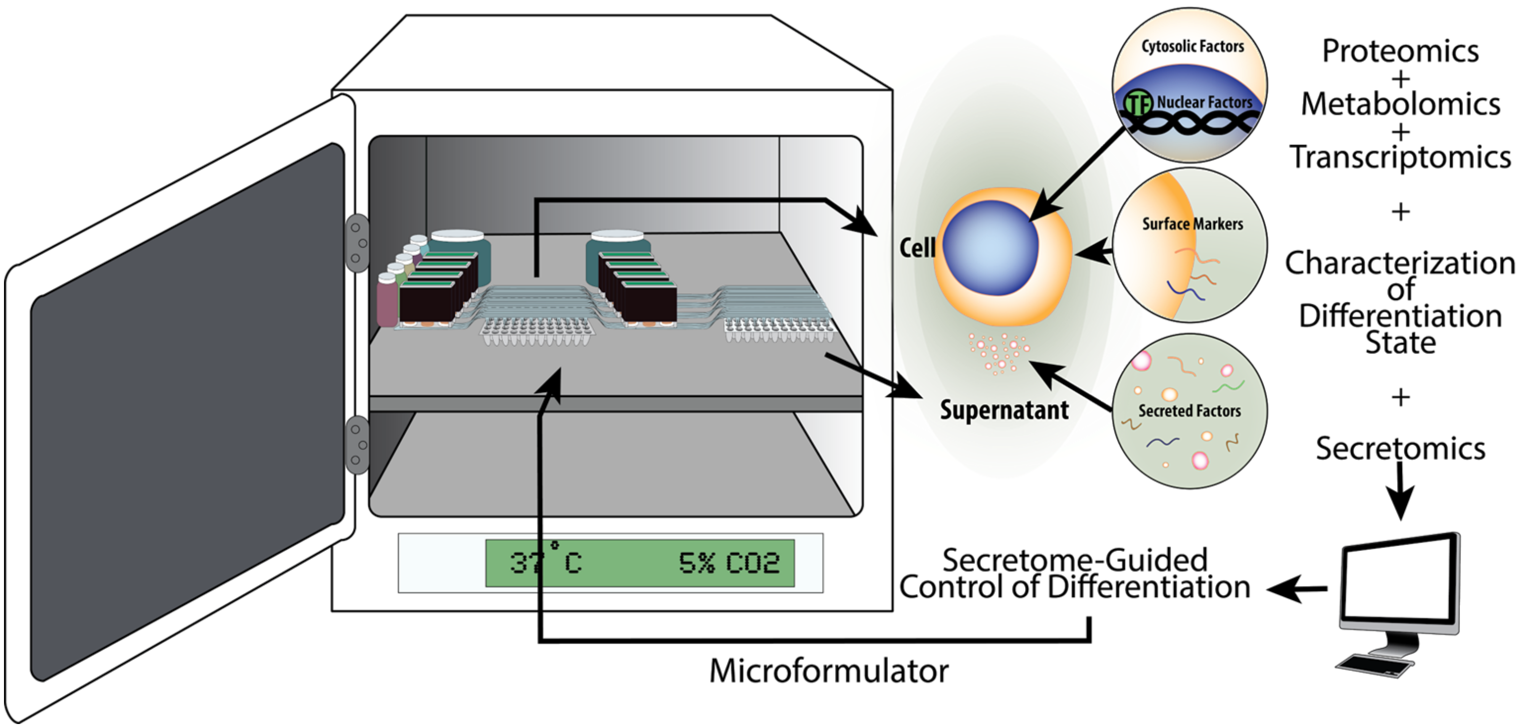
Can the secretome be used to control iPSC differentiation?

ATLAS
- Automated
- Quantitative guidance
- Non-destructive cell monitoring
- Combinatorics through multi-well plates



John Wikswo's 2017 Automated Biology Class: Kylie Balotin, Lauren Boller, Allison Bosworth, Caleb Casolaro, Natalie Hawken, Kyle Hawkins, Greg Lowen, Michael Raddatz, Joey Simmons, Tyler Taplin, John Vastola

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We need a non-destructive signal to control iPSC differentiation!

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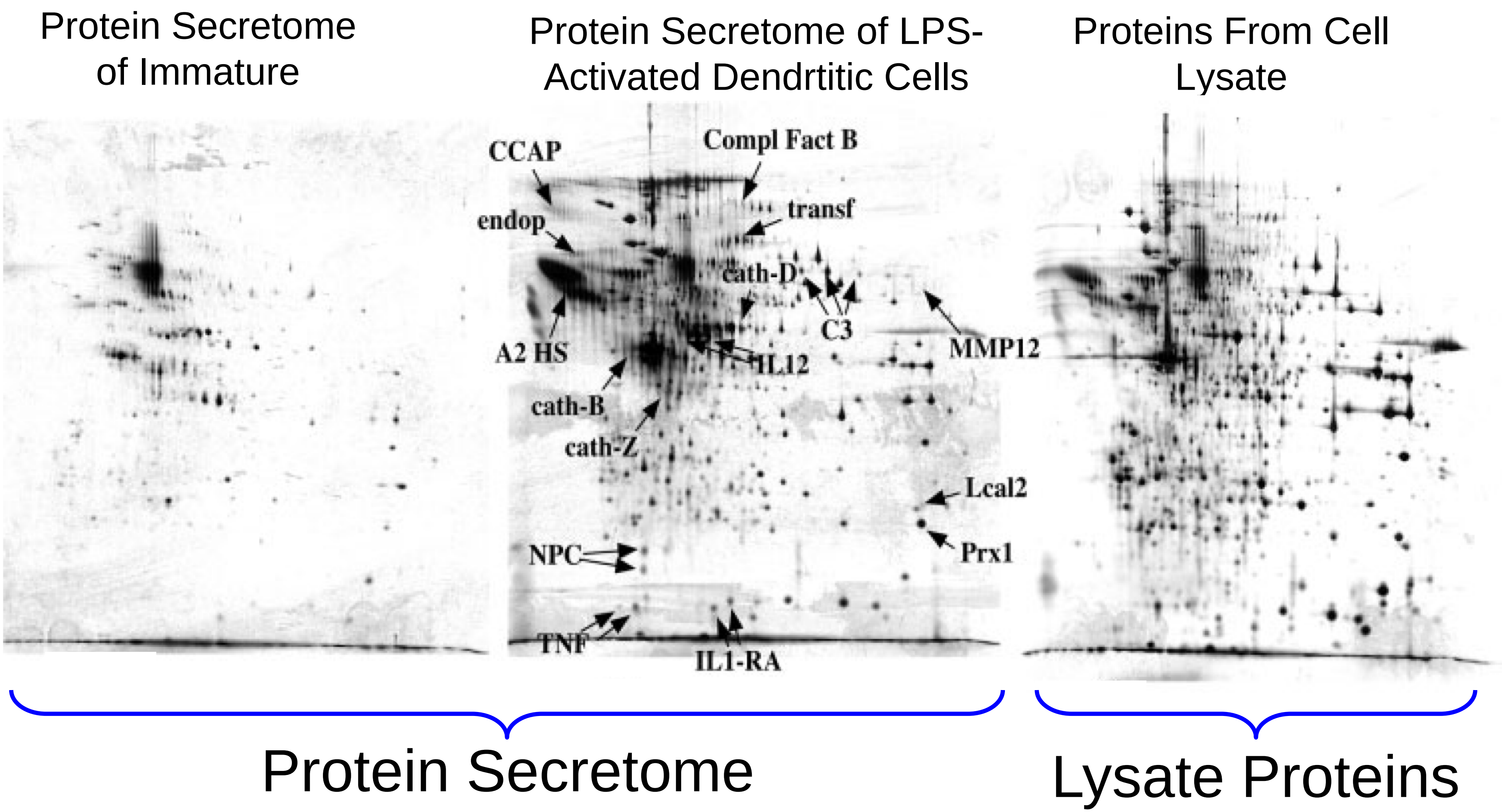
Proteins in Secretome vs Cytosol



What is the small-molecule, metabolite secretome?

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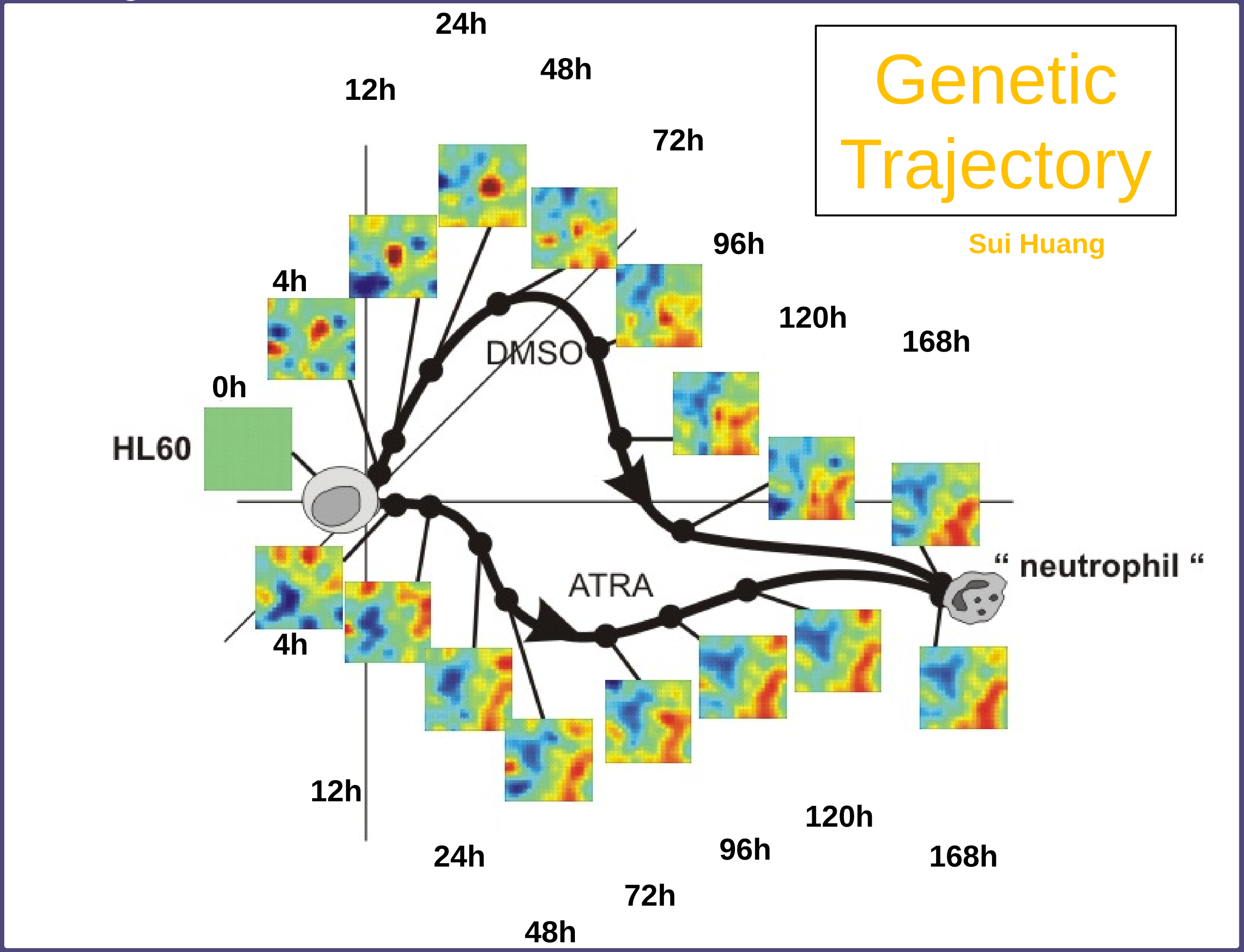
Chevallet, et al. *Proteomics.* 2007.

Cell fates as high-dimensional attractor states of complex gene regulatory network

Genome-wide gene regulatory networks govern the behavior of cells (*i.e.*, differentiation, death, etc.). Gene expression profiling can be used to show that two trajectories of neutrophil differentiation converge to a common state from different directions.



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Data from Huang S, Eichler G, Bar-Yam Y, et al. Cell fates as high-dimensional attractor states of a complex gene regulatory network. Phys Rev Lett. 2005 Apr 1;94(12):128701.

Cell fates as high-dimensional attractor states of complex gene regulatory network

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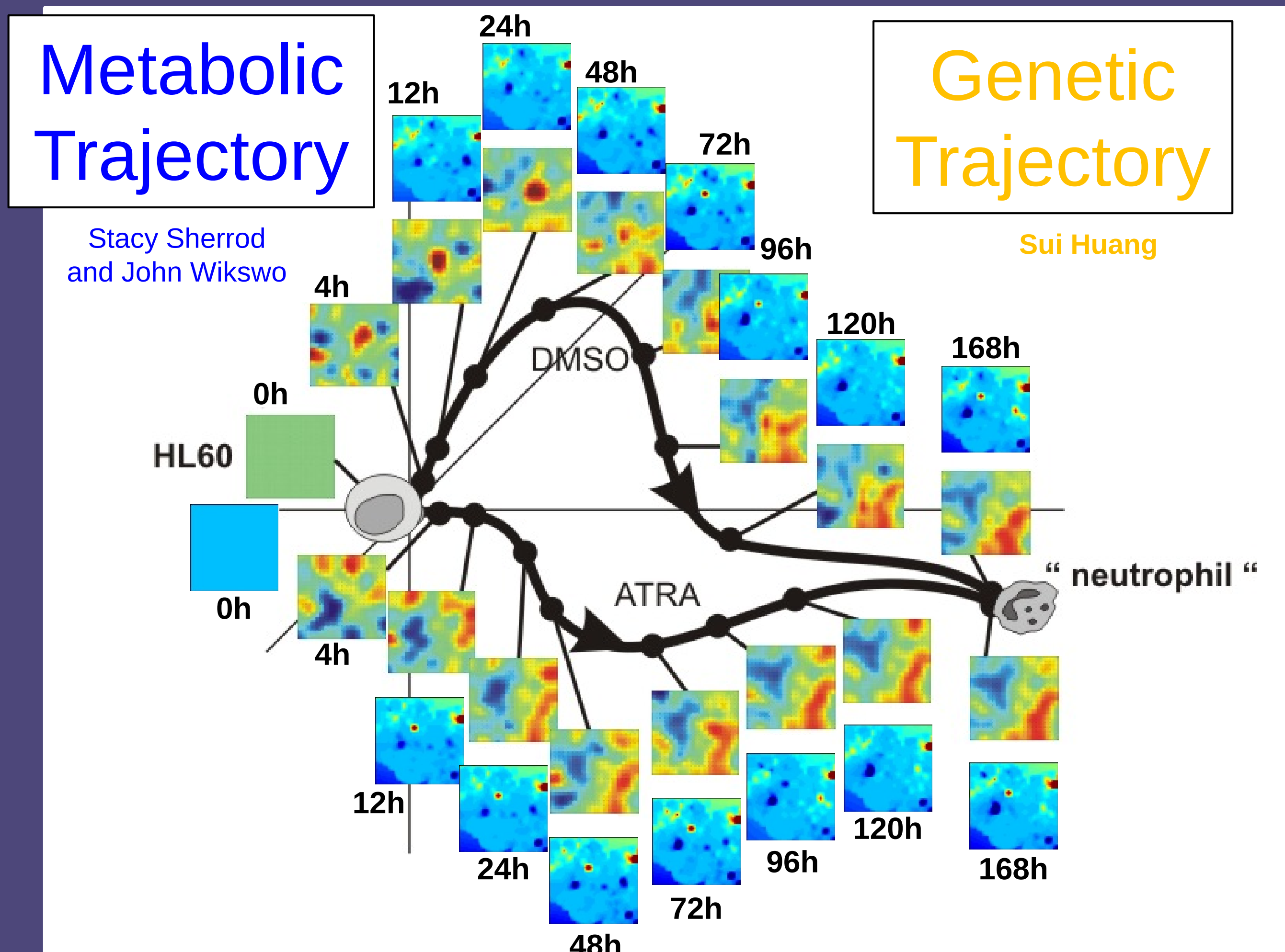

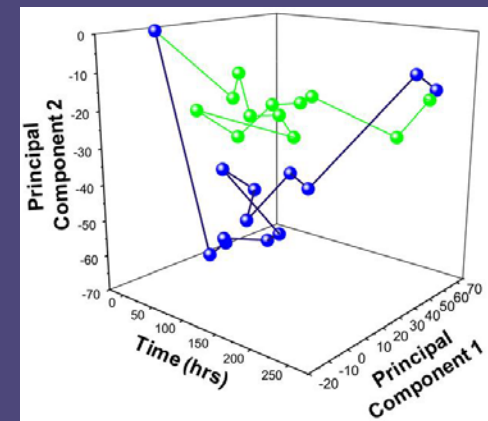

Secretome metabolomics can distinguish transitions in intracellular state

Stacy Sherrod and John Wikswo with the support of the Millipore Corporation

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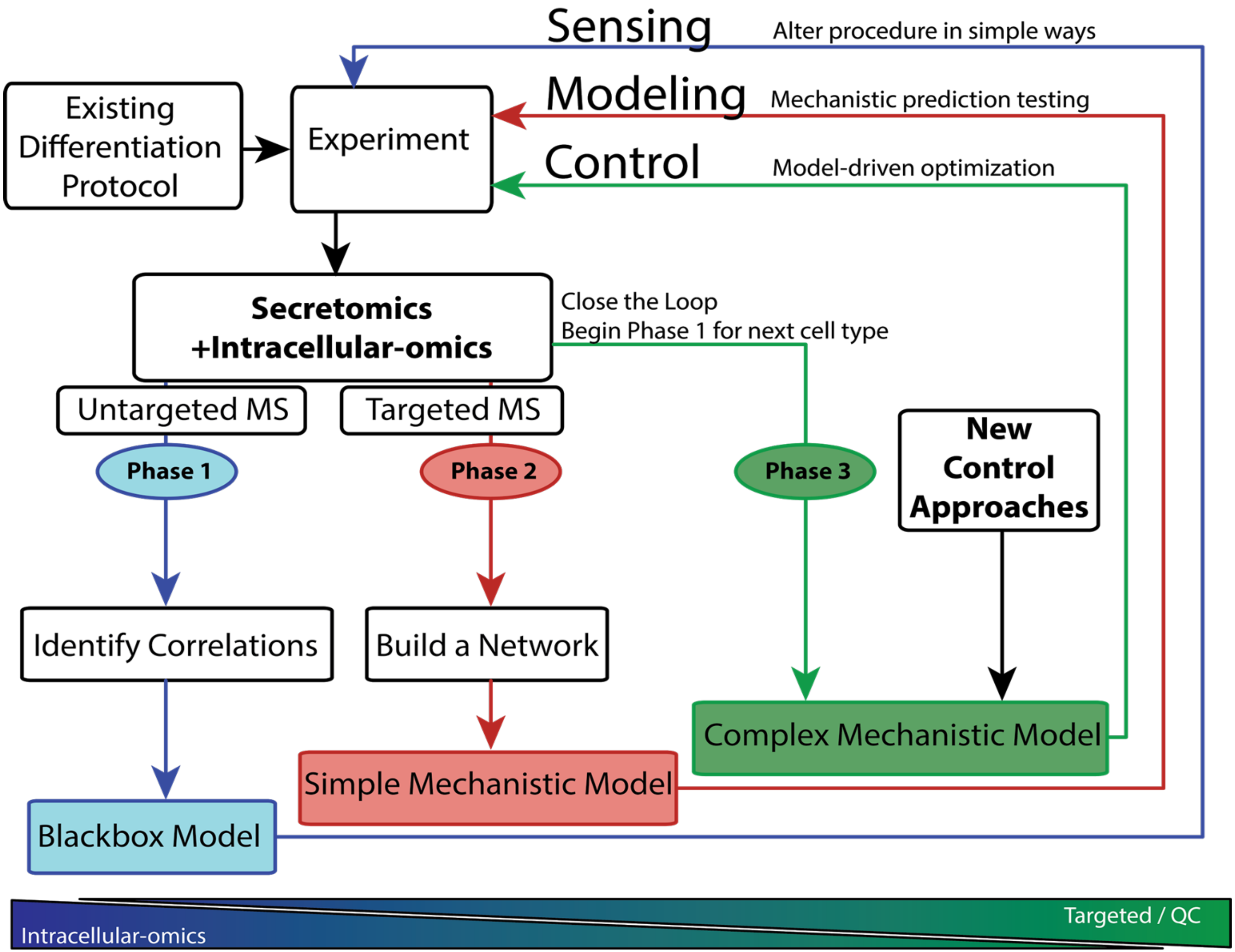
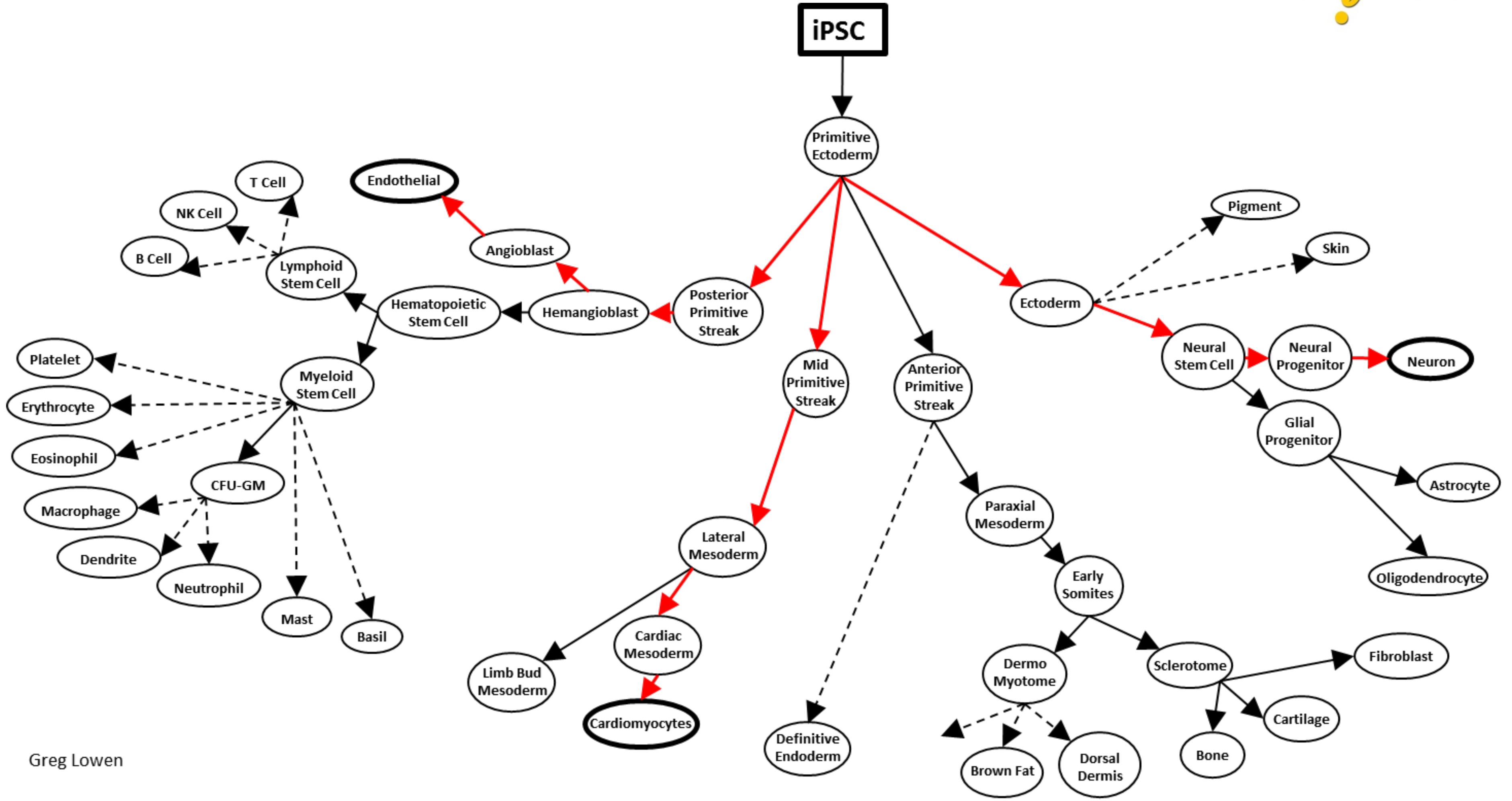
Transcriptomic data from Huang S, Eichler G, Bar-Yam Y, et al. Cell fates as high-dimensional attractor states of a complex gene regulatory network. Phys Rev Lett. 2005 Apr 1;94(12):128701.

Can the secretome be used to control iPSC differentiation?



John Wikswo's 2017 Automated Biology Class: Kylie Balotin, Lauren Boller, Allison Bosworth, Caleb Casolaro, Natalie Hawken, Kyle Hawkins, Greg Lowen, Michael Raddatz, Joey Simmons, Tyler Taplin, John Vastola

Controlling Cellular Differentiation



Where is Vanderbilt's tactical advantage for MicroFormulator and multi-omic control of cellular differentiation?

- Neurons?
- GI epithelium?

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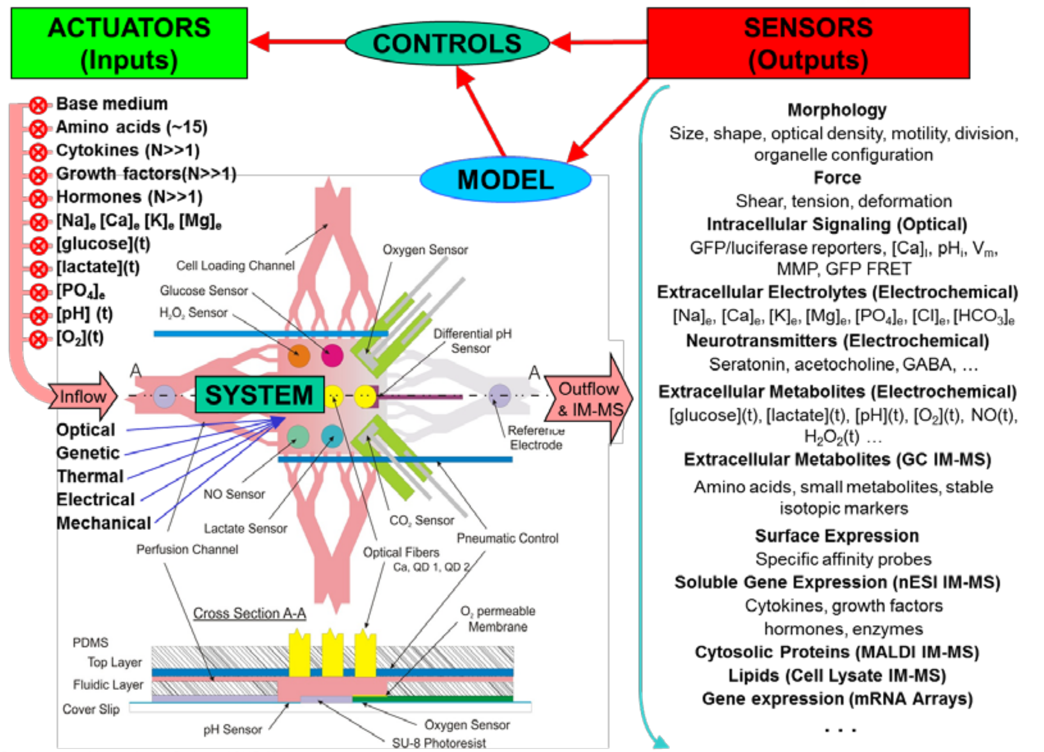
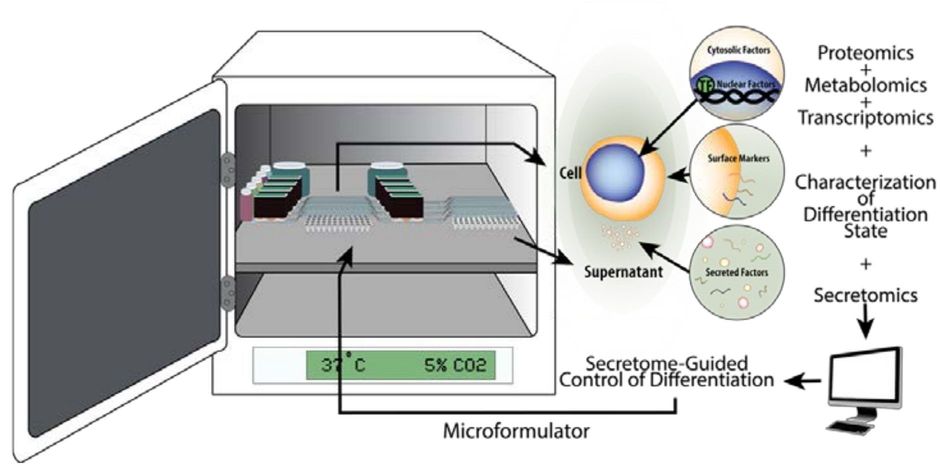
What else might we need?

- Advances in mathematics
- Genetically coded fluorescent reporters
- Optogenetics
- Addressing cellular heterogeneity
  - Single-cell FISH
  - CRISPR-CAS with Single-cell RNAseq
  - Single-cell mass spectrometry
  - …



LeDuc, Messner, Wikswo, *Annual Reviews of BME*, 2011.



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Almost done!

2012: Driving forces for the future of biology

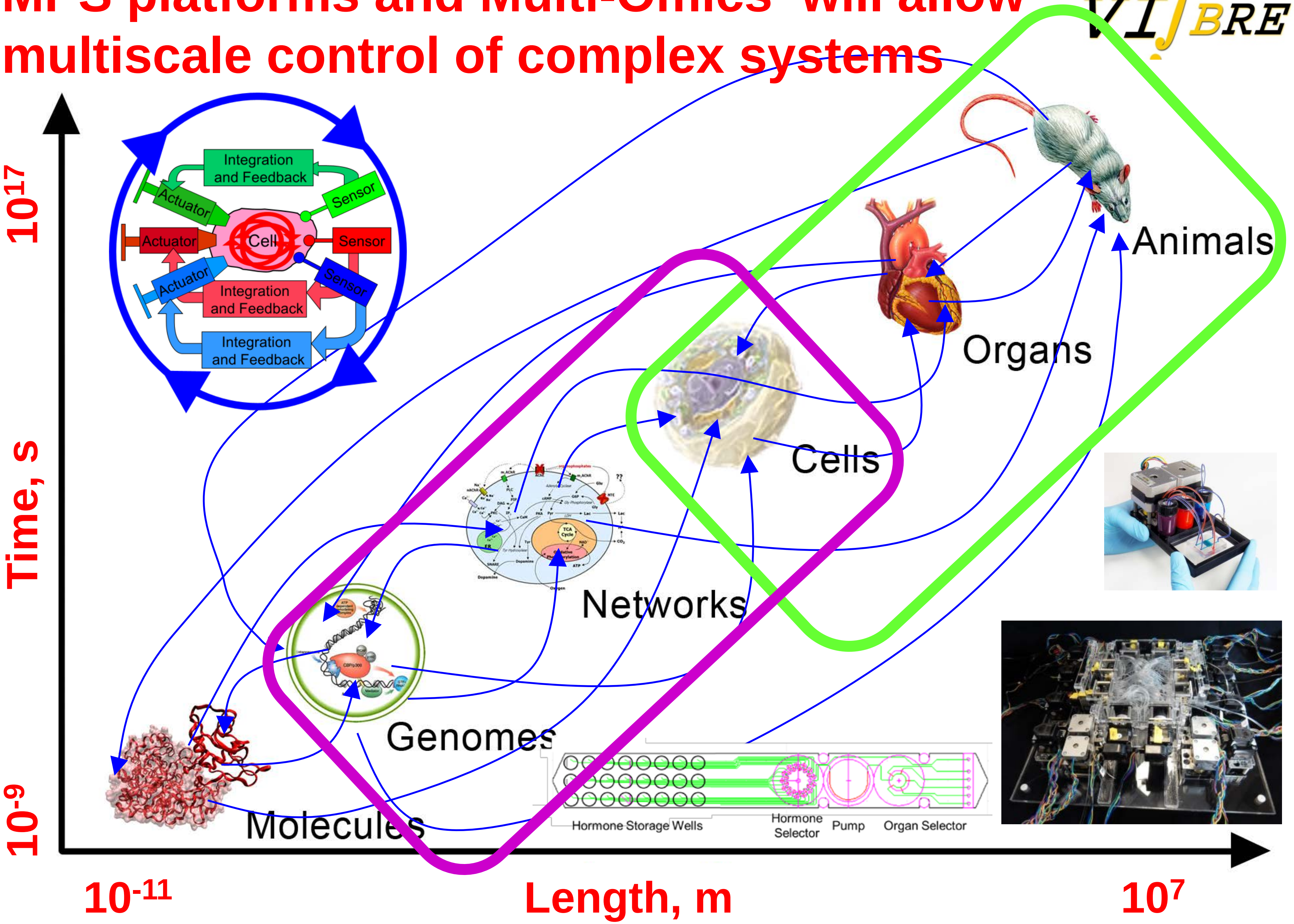
- **The need for more realistic *in vitro* experiments**
  - Massively parallel, cellular microenvironments for the study of cell-cell, cell-cell-drug, and cell-cell-drug-snp interactions
  - Real-time control of biological systems
- **The need to control multiple parameters at the same time and measure multiple dynamic variables**
  - Cell-scale sensors and actuators
  - Experiments that involve thousands of parameters
- **The need to create complex, nonlinear models**
  - New mathematics
  - 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**

Organs on Chips

MicroFormulator

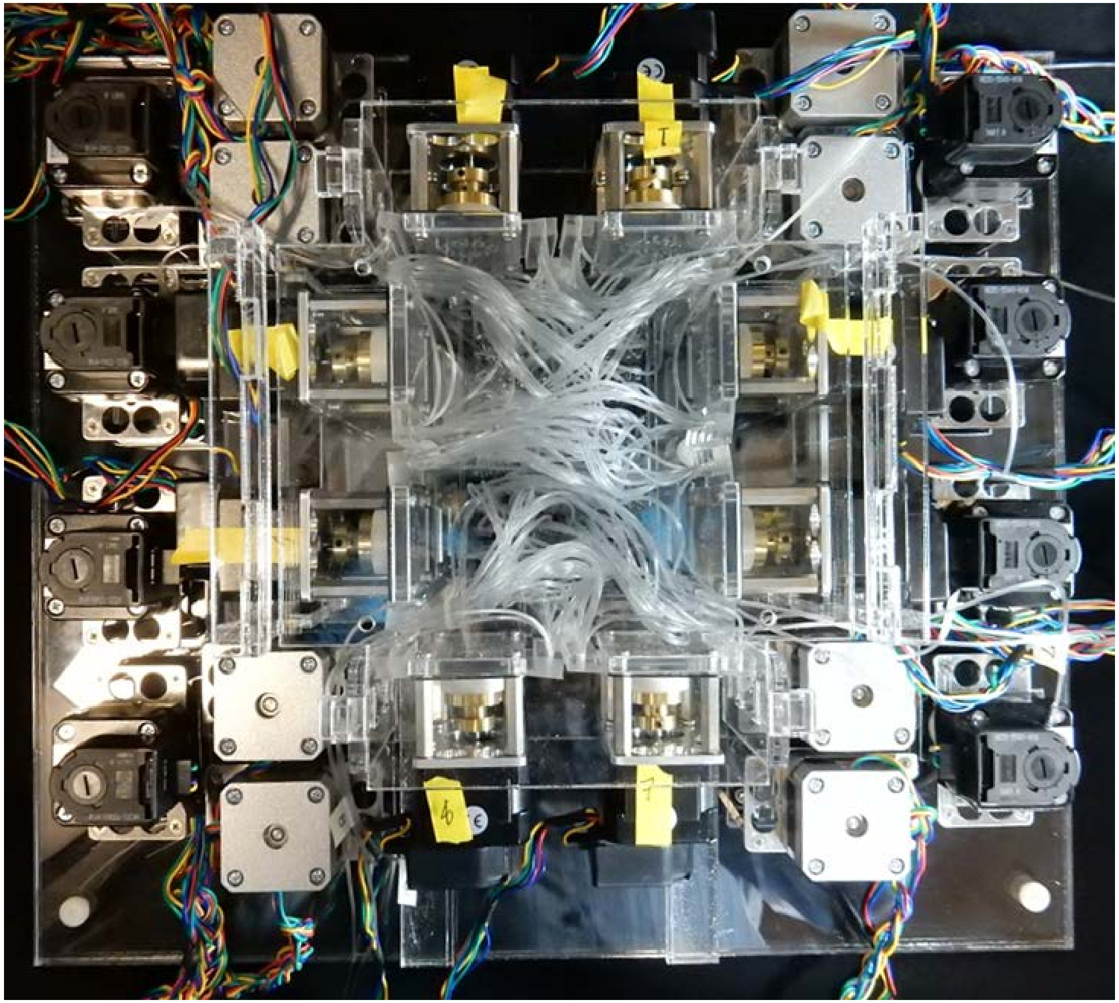
Graph Databases

MPS platforms and Multi-Omics will allow multiscale control of complex systems



JPW 2012: The bottom line…

- Ultimately, biology experiments may resemble particle physics experiments.
- Physicists have the requisite training and mindset compatible with large scale, automated biology, but are often bioignorant.
- Computational geometry and topology may be the new mathematics for biology.
- Should we teach Physics 101 and Topology 101 before Biology 101?



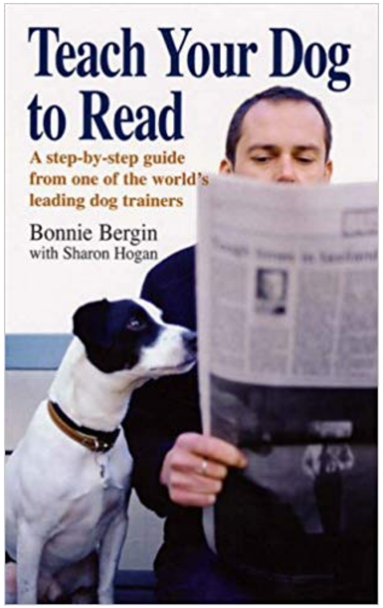
JPW January 2016: "Oh, shit. I've done it."

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



2006

See Spot Read: Willow the Dog understands written commands

"….the dog can now sit up when a card says 'Sit Up,' plays dead when a card reads 'Bang,' and wave a paw when a sign says 'Wave.' "

http://www.peoplepets.com/news/amazing/see-spot-read-willow-the-dog-understands-written-commands/1

Really Hard Problems

However…

We do not have to fully understand a phenomenon to control or eliminate it. An effective model can accomplish a lot.

John Wikswo

Organs on Chips are highly controlled, interconnected *in vitro* human organ preparations that support intensive data acquisition and control not possible in humans.

Use them accordingly.

VIIBRE Organ-on-a-Chip Collaborators

Vanderbilt

- Vanessa Allwardt
- **Frank Block III**
- Frank Block Jr.
- **Aaron Bowman**
- **Clayton Britt**
- **Jacquelyn Brown**
- Young Chun
- **David Cliffel**
- Erica Curtis
- John Scott Daniels
- **Jeffrey Davidson**
- Anna Davis
- **Mona Everheart**
- William Fissell
- **Greg Gerken**
- Lucas Hofmeister
- William Hofmeister
- Orlando Hoilett
- Chaz Hong
- Shane Hutson
- Deyu Li
- Chee Lim
- **Ethan Lippmann**
- Dmitry Markov
- **William Matloff**
- **Lisa McCawley**
- **Jennifer McKenzie**
- **BethAnn McLaughlin**
- **John McLean**
- **Dusty Miller**
- **Karoly Mirnics**
- **Nicole Muszynski**
- **Diana Neely**
- **Kevin Niswender**
- **Virginia Pensabene**
- **Ronald Reiserer**
- **Philip Samson**
- **David Schaffer**
- **Kevin Seale**
- **Stacy Sherrod**
- **Mingjian Shi**
- **Matthew Shotwell**
- **Veniamin Sidorov**
- **Hak-Joon Sung**
- **David Tabb**
- **Adam Travis**
- **Donna Webb**
- **Hendrik Weitkamp**
- **Erik Werner**
- **John Wikswo**

AstraZeneca

- Matthew Wagoner, Jay Mettetal, Kristin Fabre, Aditya Kolli, Sudhir Deosarkar

CFD Research Corporation

- **Kapil Pant, Prabhakar Pandian**
- Andrzej Przekwas

Charite Hospital, Berlin (2012-2014)

- Katrin Zeilinger, Marc Lubberstadt, Fanny Knöspel

Cleveland Clinic and Flocel Inc.

- Michael Deblock, Kyle Lopin, and Chaitali Ghosh
- Damir Janigro (Flocel)

Harvard/Wyss (2011-2015)

- Don Ingber, Kit Parker, Josh Goss, Geraldine Hamilton, Danny Levner

Johns Hopkins University

- Mark Donowitz

Los Alamos National Laboratory (2012-2014)

- Rashi Iyer

University of Pittsburgh

- Lans Taylor, Albert Gough, Lawrence Vernetti

University of Texas Medical Branch

- Mary Estes

University of Washington

- Jonathan Himmelfarb

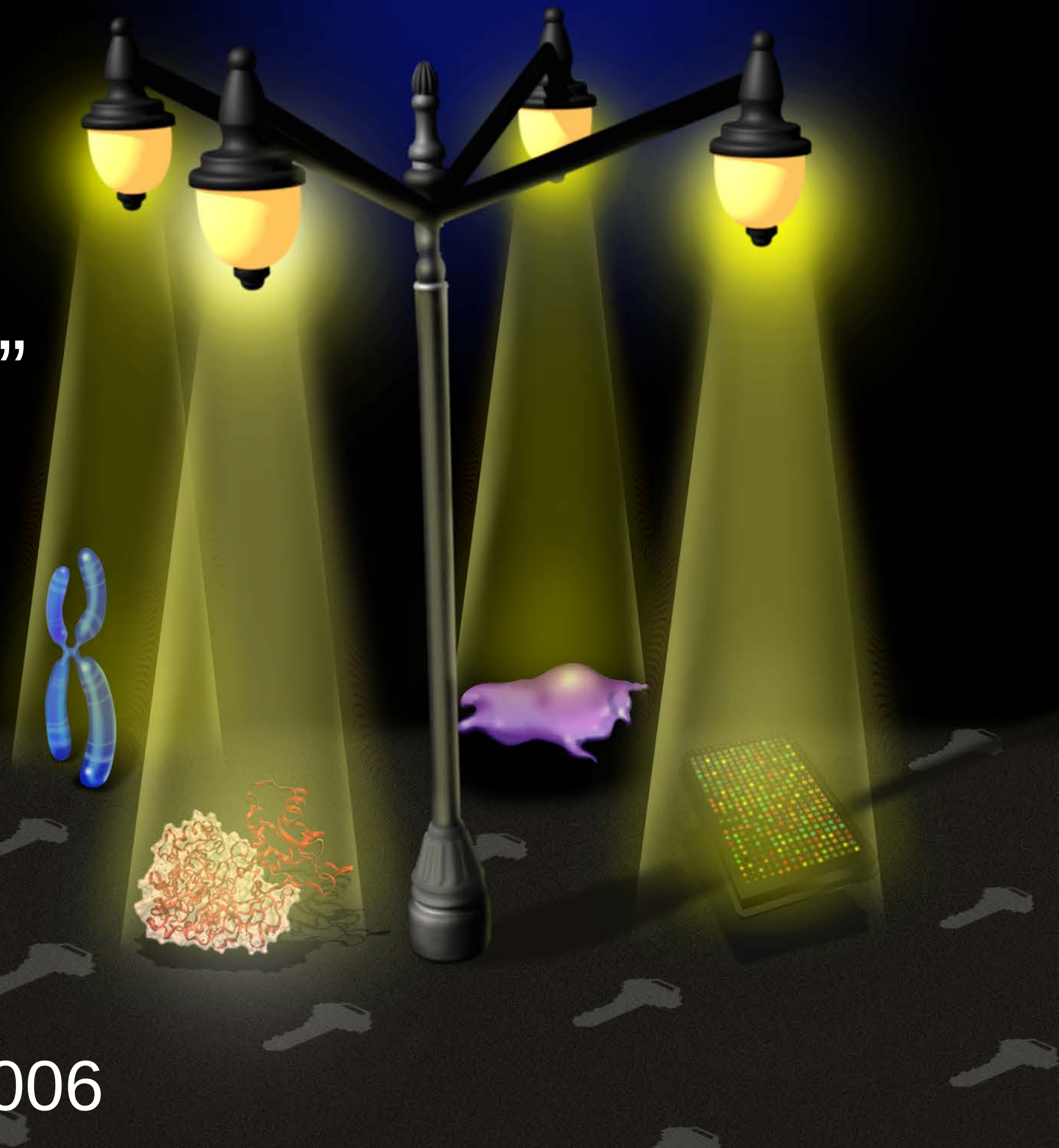
University of Wisconsin

- William Murphy, William Daly

Bold = heavy lifting Blue = PI

Funded by DTRA, DARPA, NIH/NCATS, EPA, AstraZeneca, VIIBRE, SyBBURE-Searle

“Look for the missing keys between the street lamps.”



John Wikswo, 2006