The union of organs-on-chips and mass spectrometric multi-omics: a technological convergence that will advance drug discovery

John Wikswo

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23 May 2018

Julia Wikswo

Abstract

After decades of reliance on animal studies and two-dimensional biology-on-plastic, and measurement techniques such as western blot, ELISA, and targeted mass spectrometry, the drug development pipeline is now primed for two new transformative technologies: organs-on-chips and untargeted, multi-omic reconstruction of drug mechanism of action (MOA). Over the past six years, substantial investments in the US, Europe, and Asia support the development and validation of organ-on-chip, tissue-chip, and organoid technologies. This effort has been motivated by a desire to provide earlier termination of toxic drugs and avoid inappropriate drug terminations. Even more rewarding would be the early identification of problematic human haplotypes and drug–drug interactions for small molecules, and improved prediction of human exposure for compounds and clinical formulations. Possibly the greatest return-on-investment will be the discovery of novel mechanisms of human diseases, identification of novel compounds, and the discovery of on- and off-target of drug candidate MOAs. All will benefit from the convergence of microfluidics, advanced mass spectrometry, and machine learning. Despite technological challenges, the near-term opportunities are exciting. As these technologies are refined and their costs reduced, their combined application to basic science, medicine, and drug development will provide revolutionary advances in an already rapidly moving field.

Bio

John Wikswo is the Gordon A. Cain University Professor at Vanderbilt University and is the founding Director of the Vanderbilt Institute for Integrative Biosystems Research and Education. Trained as a physicist, he received his B.A. degree from the University of Virginia, and his PhD. from Stanford University. He has been on the Vanderbilt faculty since 1977. His research has included superconducting magnetometry, the measurement and modeling of cardiac, neural and gastric electric and magnetic fields, and non-destructive testing of aging aircraft. His group's current work on organ-on-chips focuses on the development of intelligent well plates that serve as perfusion controllers, microclinical analyzers, and microformulators; developing a blood-brain barrier on a chip; and integrating multiple organs to create a milli-homunculus from coupled organs on chips. As a tenured member of the Departments of Biomedical Engineering, Molecular Physiology & Biophysics, and Physics & Astronomy, he is guiding the development of microfabricated devices, optical instruments, and software for studying how living cells interact with each other and their environment and respond to drugs, chemical/biological agents, and other toxins, thereby providing insights into systems biology, physiology, medicine, and toxicology. He has over 200 publications, is a fellow of seven professional societies, and has received 24 patents.

Disclosure

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- The views expressed in this document are solely those of the authors and do not necessarily reflect those of any of the funding agencies or companies. The EPA does not endorse any products or commercial services mentioned.

Why are we here?

Decades of preclinical pharmacology R&D are based on

- Animal studies
- Two-dimensional biology-on-plastic
- Western blot
- ELISA
- NMR
- Targeted mass spectrometry

***In vitro* studies cannot predict with the required accuracy**

- First-in-human dose
- Human drug efficacy
- Human off-target effects

Will new transformative technologies help?

- Organs-on-chips
- Untargeted, multi-omic reconstruction of drug mechanism of action (MOA)
- Machine learning and optimal experimental design

Together they might!

Definition

mass spec·trom·e·ter (noun)

/ˈˌmas ˌspekˈträmədər/

An apparatus for separating isotopes, molecules, and molecular fragments according to mass. The sample is vaporized and ionized, and the ions are accelerated in an electric field and deflected by electric or magnetic fields into a trajectory that produces a distinctive mass spectrum.

Definition

mi·cro·phys·i·o·log·i·cal sys·tem (noun)

/ˈmīkrō ˌfizēəˈläjək(ə)l ˈsɪstəm/

A small-scale *in vitro* model that recapitulates selected functions of living organisms and/or their parts.

Typically implemented as quasi-two-dimensional barriers that support one or more cellular layers, or three-dimensional tissue constructs.

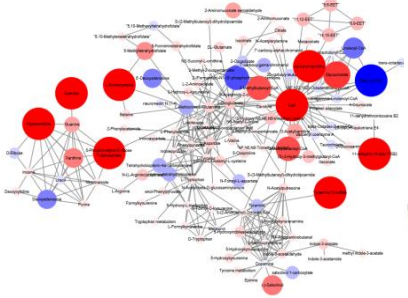
May involve one or more organs-on-chips, tissue chips, organoids, vascularization, electrospun scaffolds, hydrogels, microfluidics, and sensors.

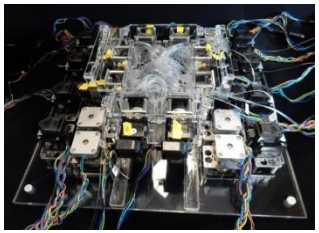
Usually involves fluidic superfusion or perifusion, and possibly perfusion.

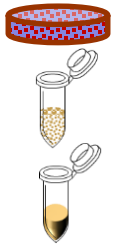
Antonym – two-dimensional biology on plastic.

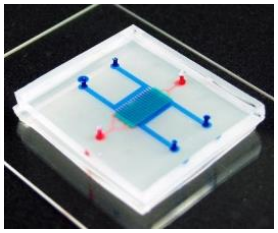
Today's goal: Explain this convergence

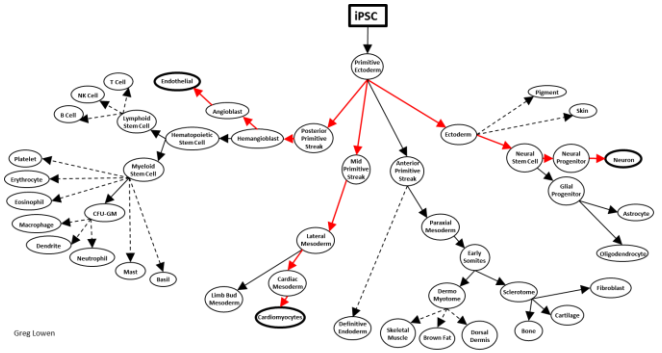
VIVABRE

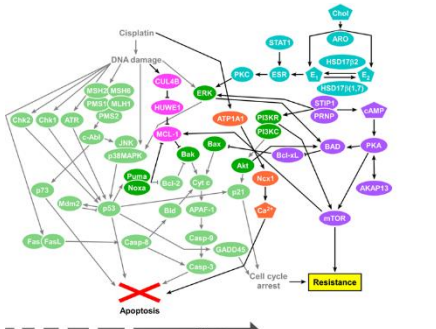


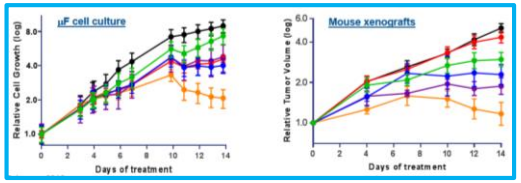


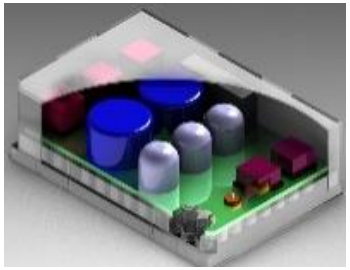


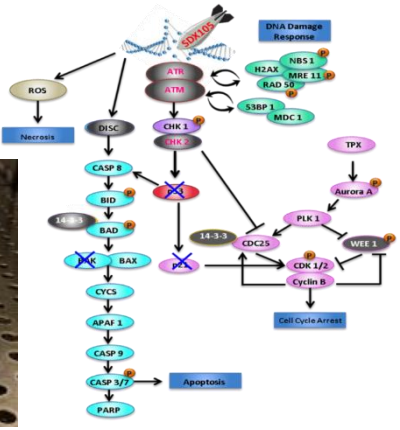


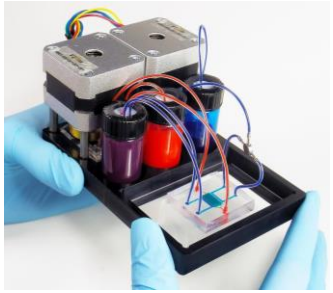


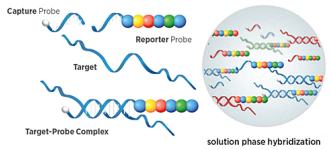













Four themes
1. The complexity of biology
2. MicroPhysiological Systems
3. Multi-Omics
4. Putting it all together
Julia Wikswo


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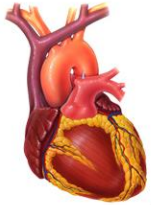
What makes biology so different from physics, chemistry, and engineering?

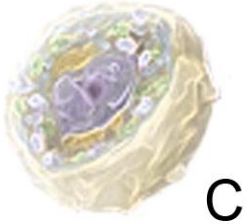
Biology spans lots of space and time

But it more complicated than that…

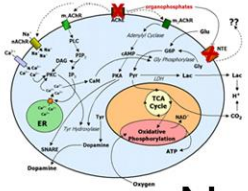
Animals



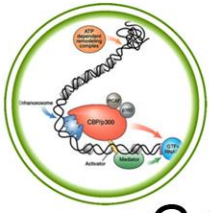
Organs



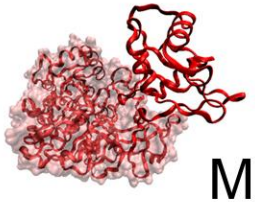
Cells



Networks



Genomes



Molecules

Images by CFDRC and VUMC

10^{17}

Time, s

10^{-9}

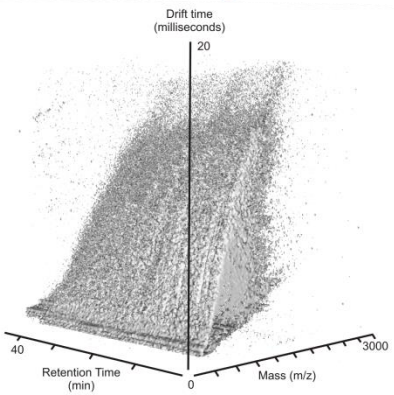
10^{-11}

Length, m

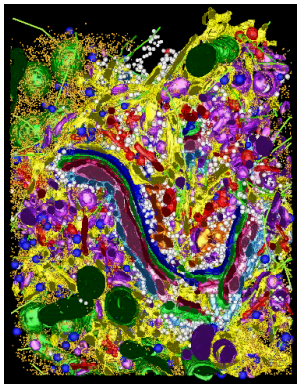
10^{7}

Why is biology so complex, con't?

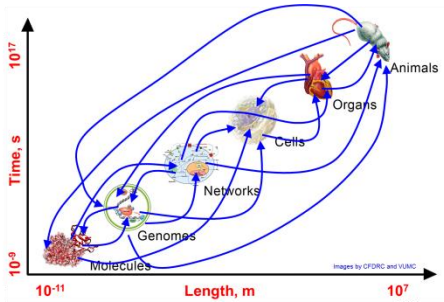
- Today, one can easily detect 100,000 chemical species in 100 µL of rat serum.
- Cells are NOT well-stirred bioreactors but have anomalous diffusion and active transport.
- 10^9 - 10^{11} interacting cells in some organs.
- Cell signaling is dynamic, non-linear, multiscale, redundant, has positive and negative feedback, spans spatial scales …
- Metabolism may have 5,000 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



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



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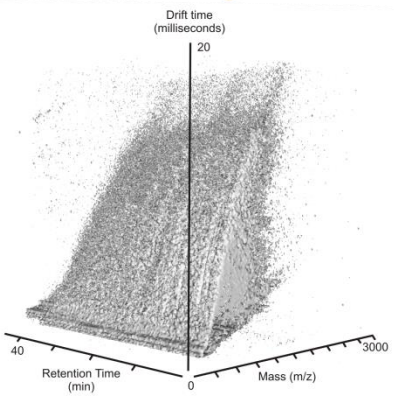

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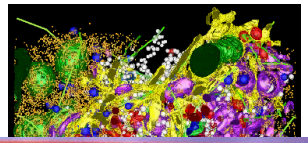
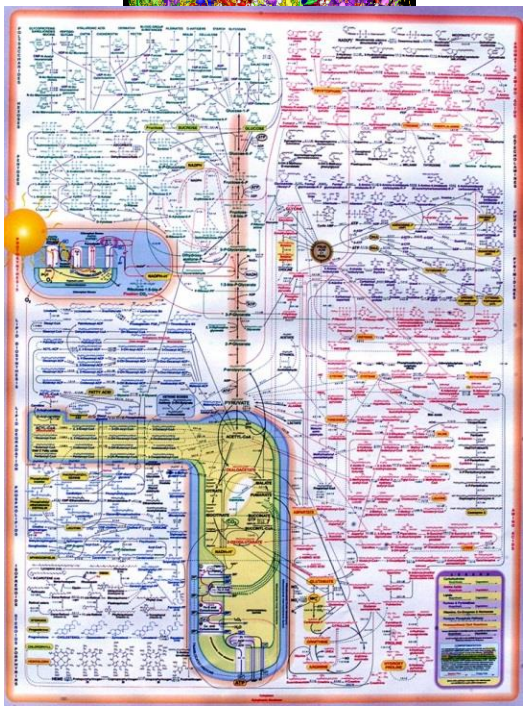




Part of the problem is that human biology is COMPLEX.

Organs, Organs, Organs

Organs, Organs, Organs

Cardiovascular

Heart
Blood
Blood vessels

Digestive

Salivary glands
Esophagus
Stomach
Liver
Gallbladder
Pancreas
Intestines

Excretory

Kidneys
Ureters
Bladder
Urethra

Immune

Leukocytes
Tonsils
Adenoids
Thymus
Spleen
Appendix

Reproductive

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

Respiratory

Organs, Organs, Organs

Colon
Rectum
Anus

Endocrine

Hypothalamus
Pituitary gland
Pineal gland
Thyroid
Parathyroids
Adrenals

Integumentary

Skin
Hair
Nails

Muscular

Muscles
Golgi tendon organ

Nervous

Brain
Spinal cord
Nerves
Eyes

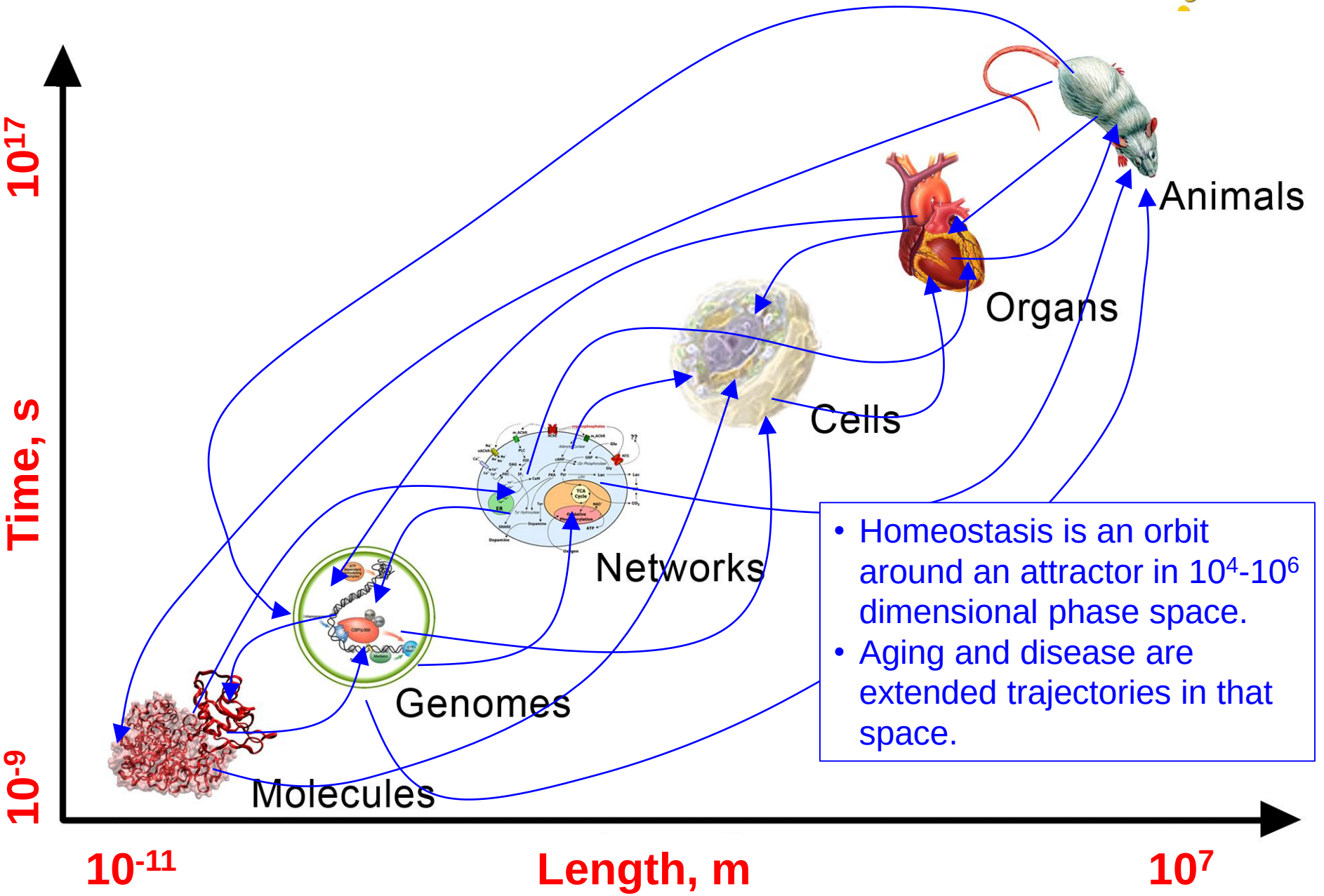
Pharynx
larynx
Trachea
Bronchi
Lungs
Diaphragm

Skeletal

Bones
Cartilage
Ligaments
Tendons

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

Complexity from multiscale interactions



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

Next, “How did Biology get here and where is it going?”

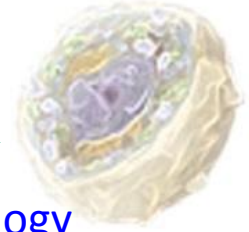
The Hermeneutic Circle of Biology

Anatomy

Animal

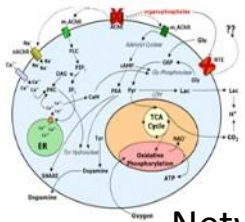
Physiology

Organ



Cell Biology

Cell

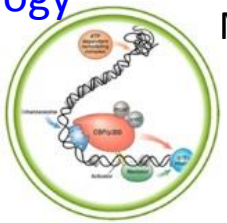


Molecular Biology

Network

Structural Biology

Molecule



Genomics

Genome

Reduction

Construction

Standard biology and medicine

Does this create a problem?

Systems Biology

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

JP Wikswo and AP Porter, *EBM*, 2015

How have we been studying biology?

- People

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

- Animals

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

- Cells *in vitro*

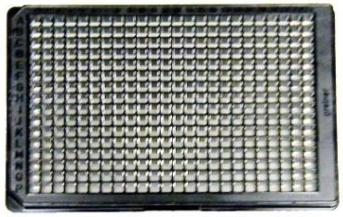
  2D biology on plastic: Many biological experiments are conducted on cells that

  - have cancer,
  - are inbred,
  - are diabetic,
  - are potatoes on a stiff 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.

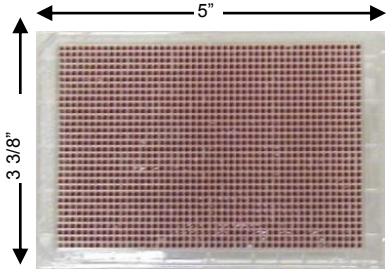
glasslaboratory.com/files/2245127/uploaded/GL-P100%20Petri%20Dish.jpg

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

4ti.co.uk/files/cache/e7199a9f456dacab058c6be0b54e9235.jpg



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


Four themes
1. The complexity of biology
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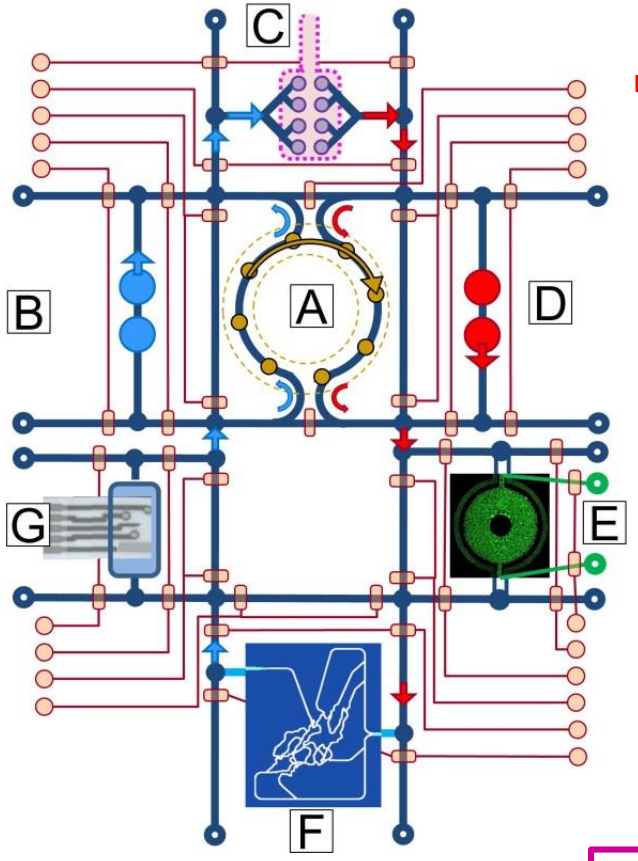
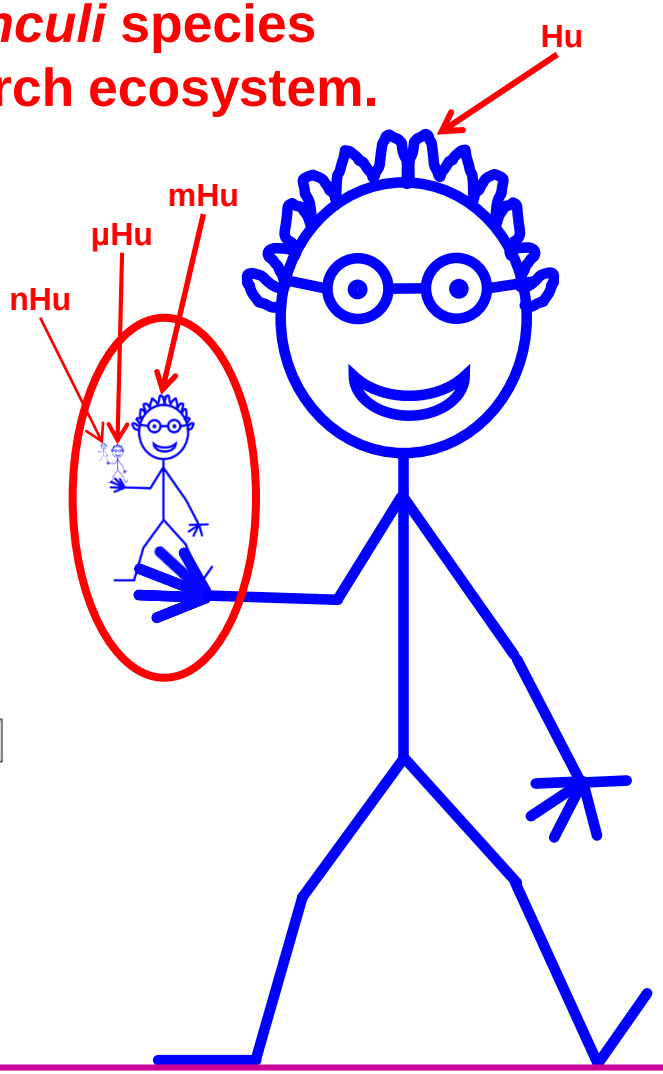
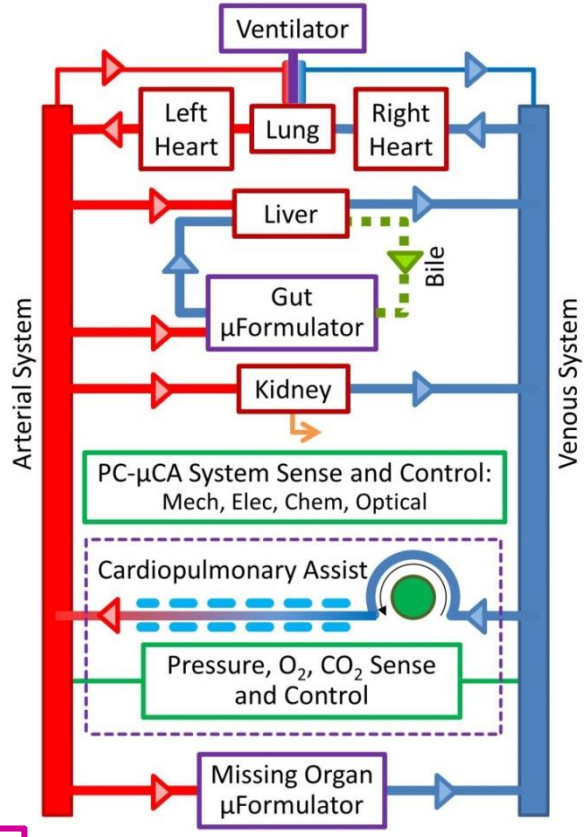
Julia Wikswo

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







Homo chippiens
NanoHuman (nHu)
(Organoids)

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

Homo minutus
MicroHuman (μHu)
(Tissue Chips)

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

Enter organoids

Schmeichel and Bissell "Modeling tissue-specific signaling and organ function in three dimensions."
J Cell Science (2003)

https://www.ted.com/speakers/mina_bissell

Organoids are in the commercial limelight!

2017

CORNING

Rock the Science of 3D

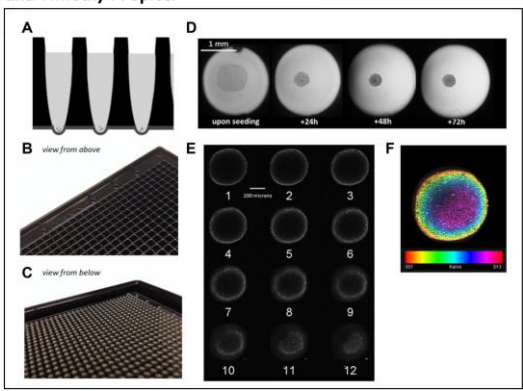
/ Products / Life Sciences Product Portfolio / Life Science Applications: See It All Come Together / Cell Culture / 3D Cell Culture

Create *in vivo*-like functionality with optimized 3D cell culture models

3D Cell Culture, Drug Screening, and Optimization

A 1536-Well 3D Viability Assay to Assess the Cytotoxic Effect of Drugs on Spheroids

Franck Madoux[1,3], Allison Tanner[2], Michelle Vessels[2], Lynsey Willetts[2], Shurong Hou[1], Louis Scampavia[1], and Timothy P. Spicer[1]

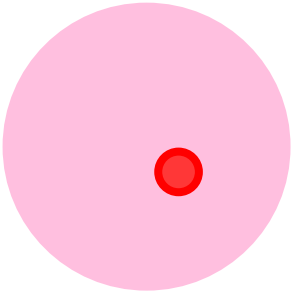


Madoux et al., SLAS Discovery **2017**, Vol. 22(5) 516–524

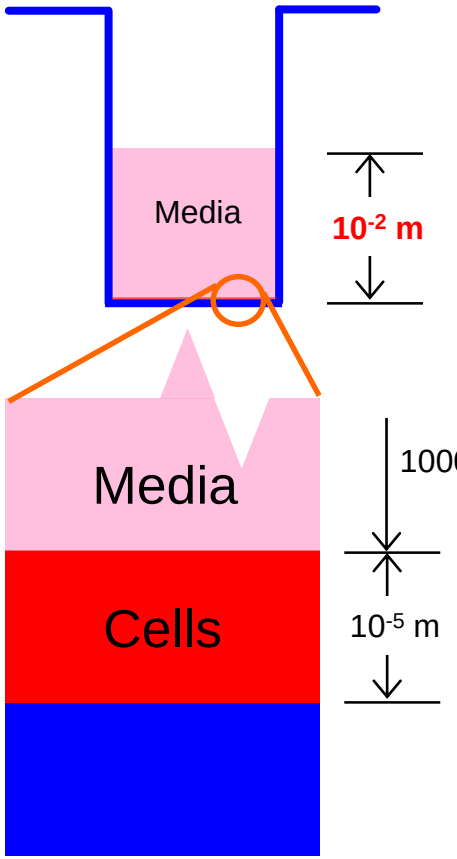
The “Media Volume” problem

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.**

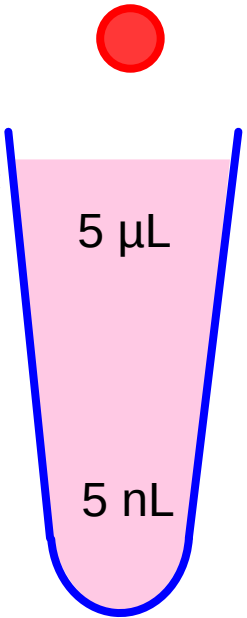


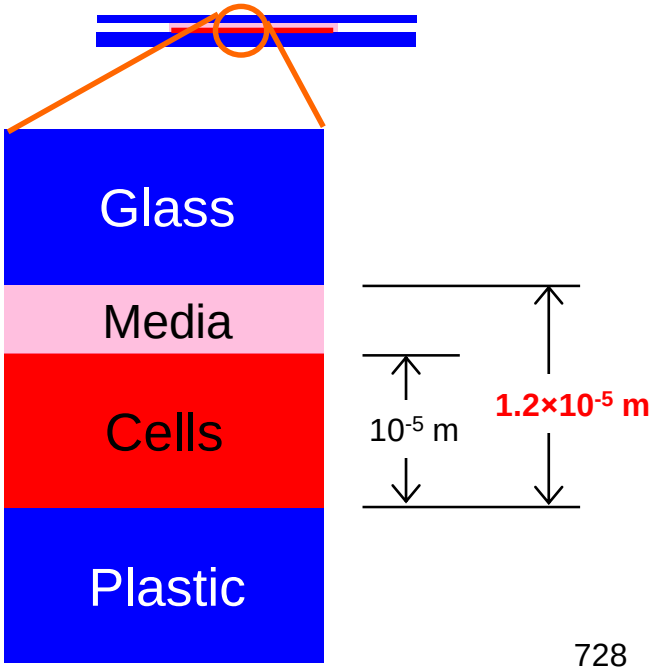
Relative sphere sizes:
nL media vs pL cell



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**



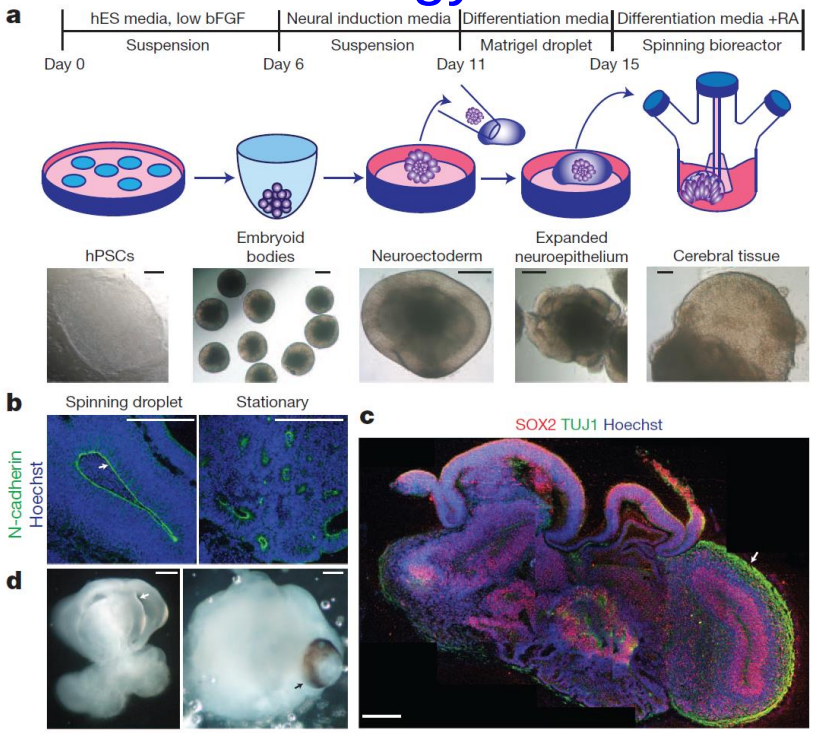


A hot, new *in vitro* model for biology

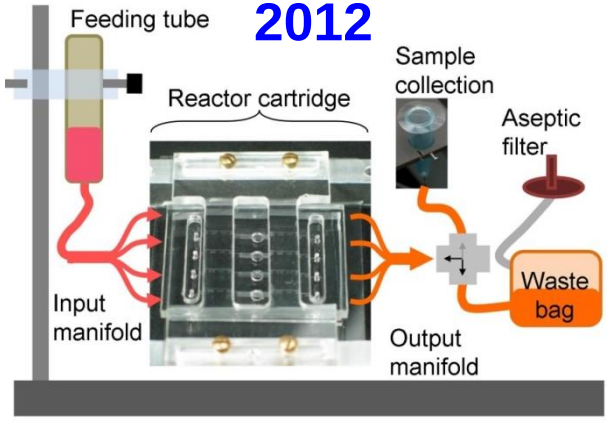
Complex 3D biology is a better model than 2D biology.

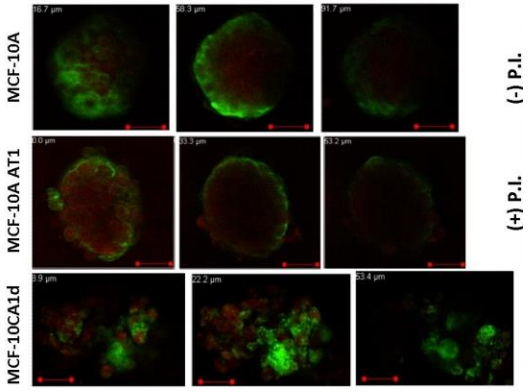
- 3D Organoids
  - Are self-organizing models with tissue-level functions and disease phenotypes.
  - Demonstrate development
  - Can be transplanted
  - Can be a medium-to-high throughput assay
  - Hard to replicate an individual organoid
  - May benefit from engineered hydrogels
  - Hard to perfuse 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

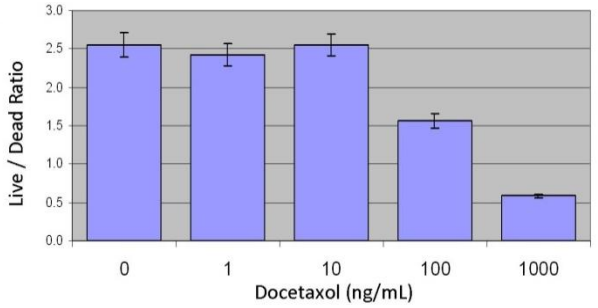
Contributions from Kapil Bharti (NIH/NEI)



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







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

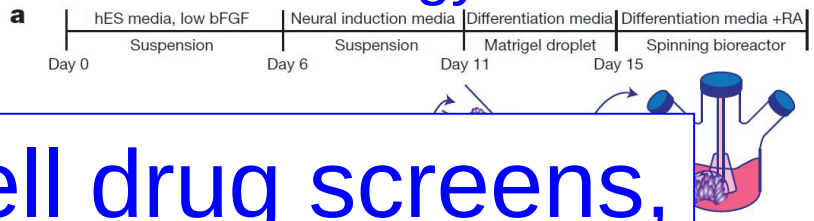
A hot, new *in vitro* model for biology

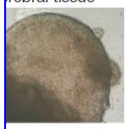
Complex 3D biology is a better model than 2D biology.

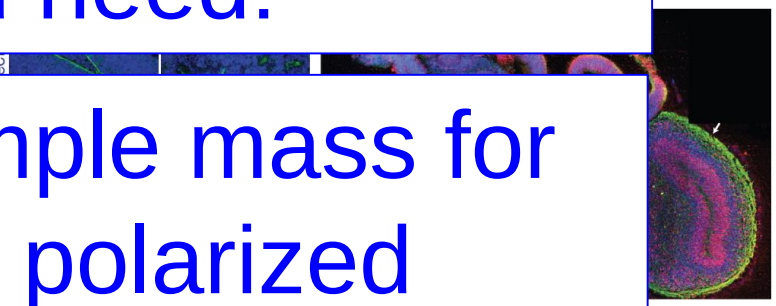
- 3D Organoids
  - Are self-organizing models with tissue-level
    - funct
  - Demon
  - Can be
  - Can be
  - Hard t
  - May be
  - Hard t
  - Hard t
  - Hard t
  - Hard t
    - prop

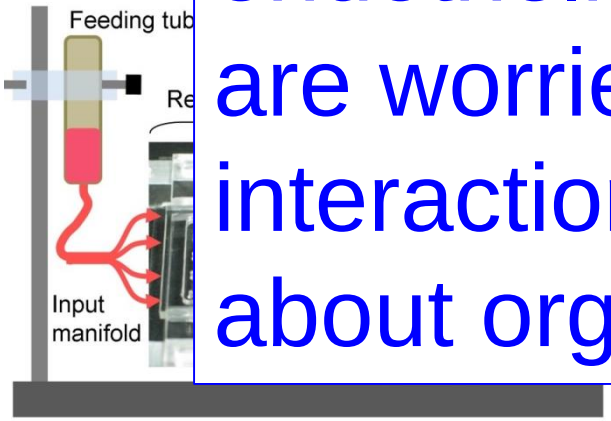
If you need 1536-well drug screens, then 3D spheroids and organoids may be just what you need!

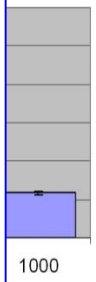
If you need more sample mass for quantitative analysis, polarized endothelial/epithelial barriers, or are worried about organ-organ interactions, then you need to think about organ chips!











delivery. Lab Chip 12:4560-4568. **2012**.

Enter organs on chips

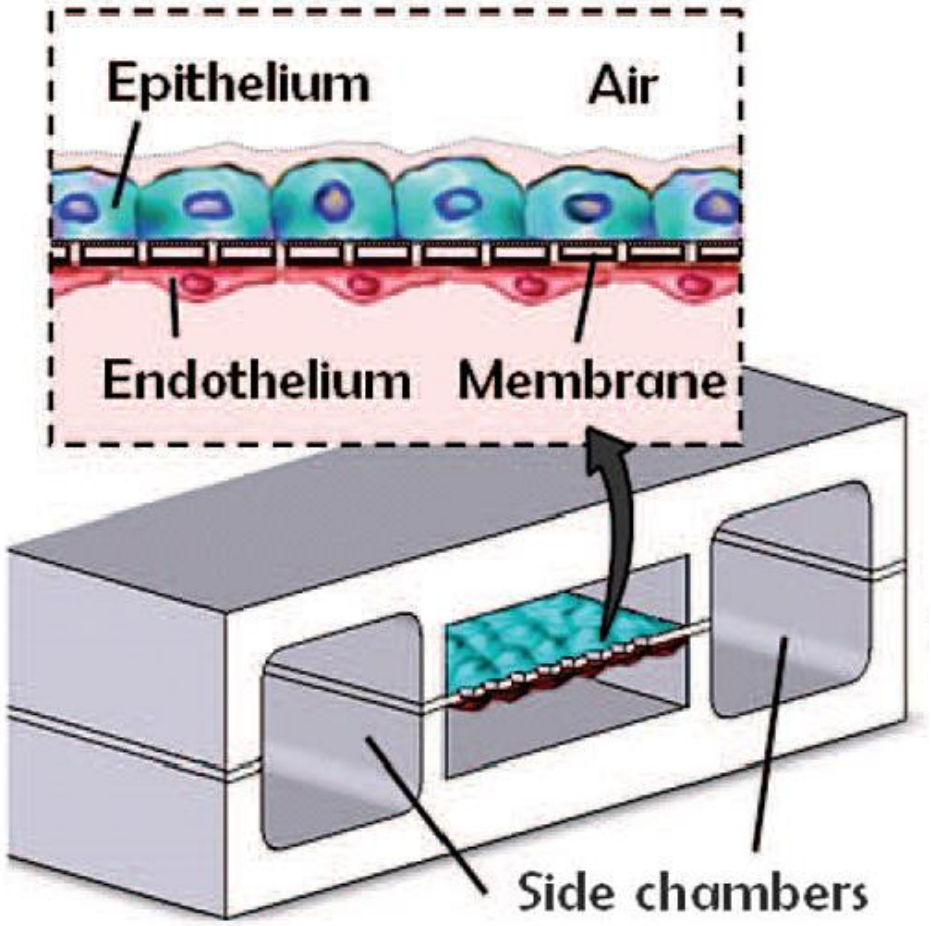
Sin, A., … Shuler, "The Design and Fabrication of Three-Chamber Microscale Cell Culture Analog Devices with Integrated Dissolved Oxygen Sensors." Biotechnology Progress (2004).

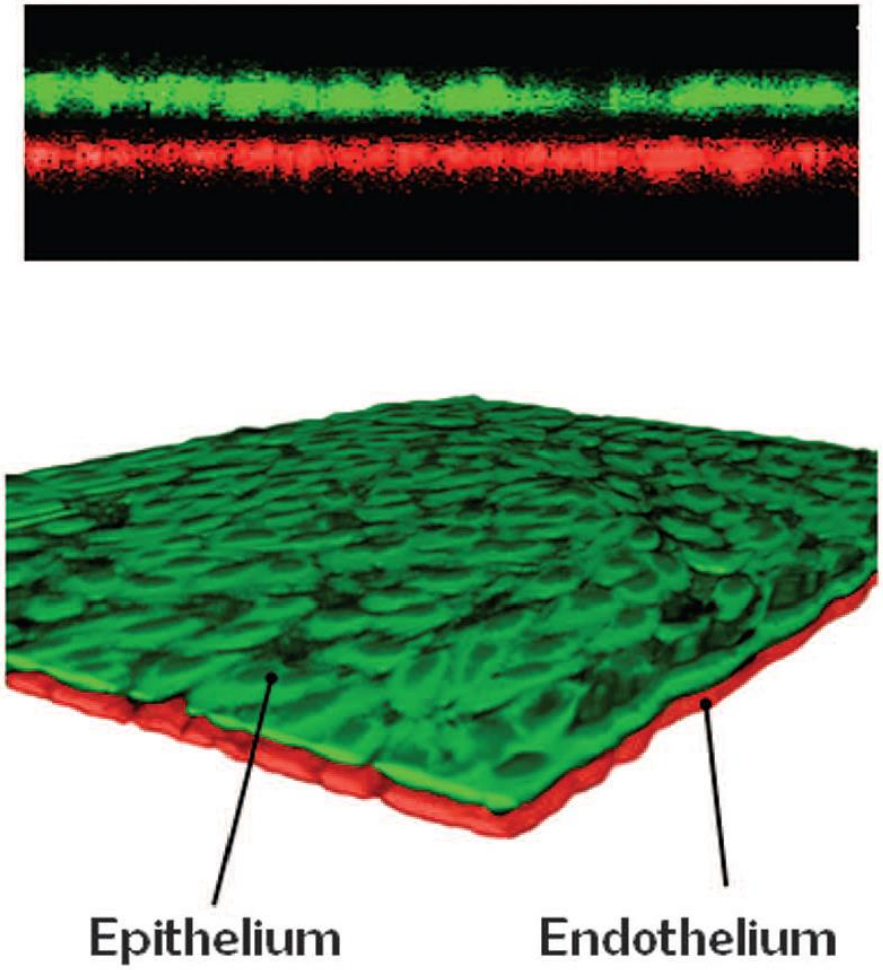
Huh … Ingber, "Reconstituting Organ-Level Lung Functions on a Chip." Science (2010)

What do organs-on-chips look like?

Perforated PDMS membranes support pulmonary endothelial and epithelial cell layers (Ingber group, Wyss, Harvard)

2010





Interfaces are important, and endothelia can protect cells.

Huh et al., Science, **2010**

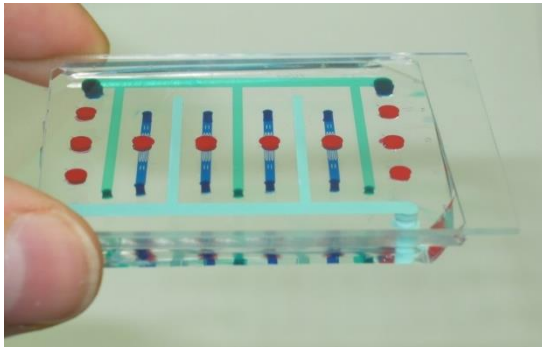
Another hot new *in vitro* model for biology

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

- Organ Chips

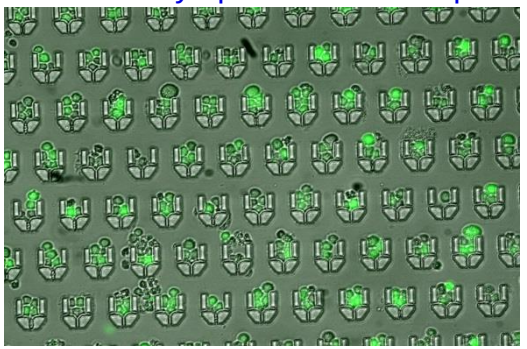
Better than 2D biology
Ideal for barrier functions
Can reproduce physiological flows
Provide a thick ECM for scaffolding and drug/factor binding
Support organ-organ interactions
Sufficient tissue for multi-omics of 10's to 1000's of variables
Can use minimal media volumes
Will be vascularized soon
May 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



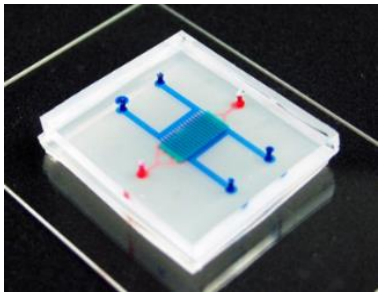
Lisa McCawley and Dmitry Markov, Vanderbilt

T cells in a lymph node on a chip



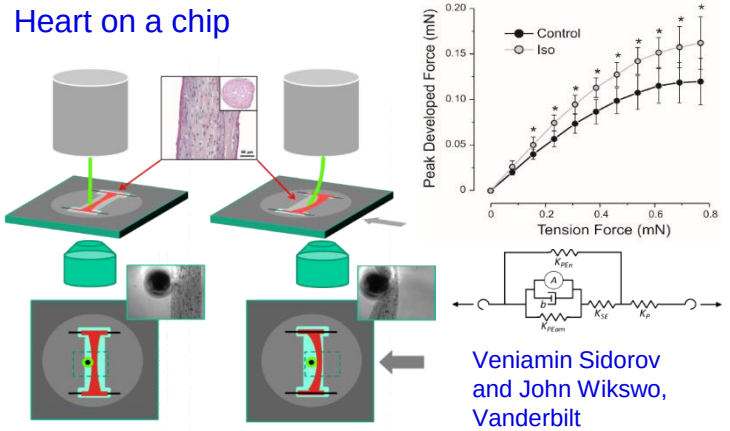
Shannon Faley, Kevin Seale and John Wikswo, Vanderbilt

Brain on a chip



Jacquelyn Brown and John Wikswo, Vanderbilt

Heart on a chip

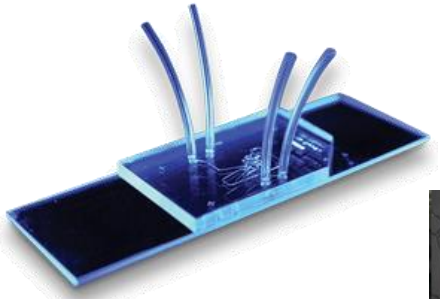


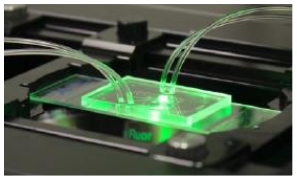
Veniamin Sidorov and John Wikswo, Vanderbilt

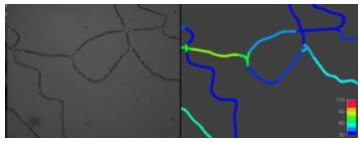
Planar Constructs

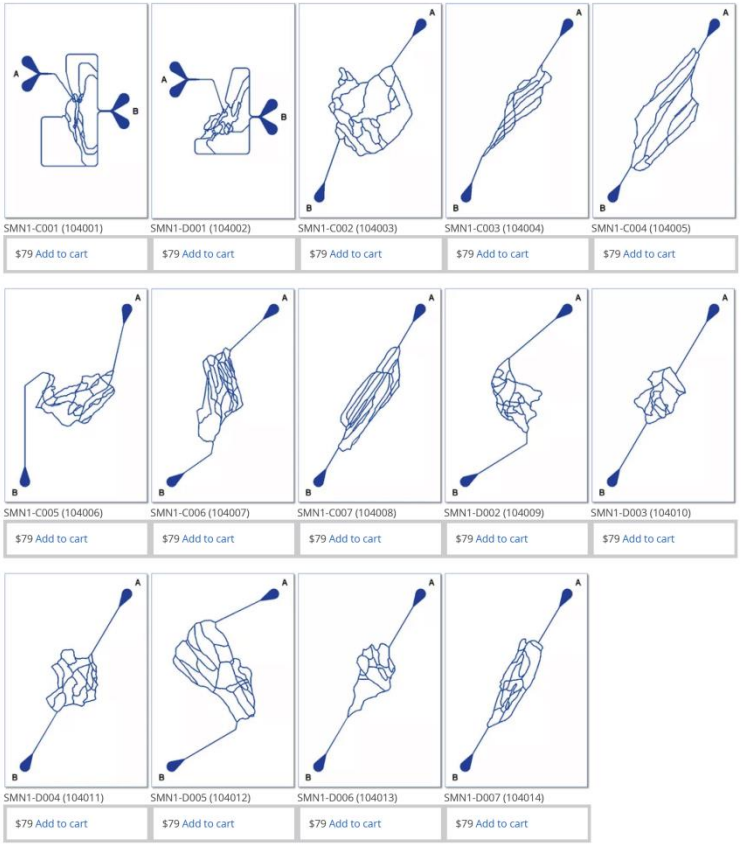
http://www.synvivobio.com

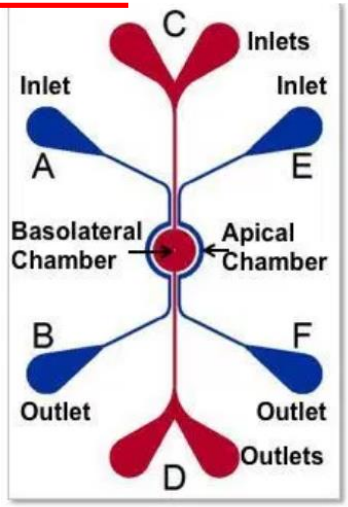
Microvascular nanoparticle delivery assay

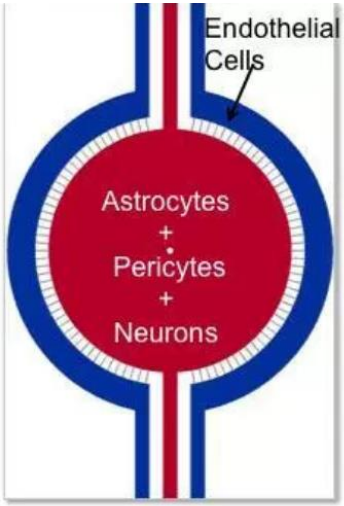


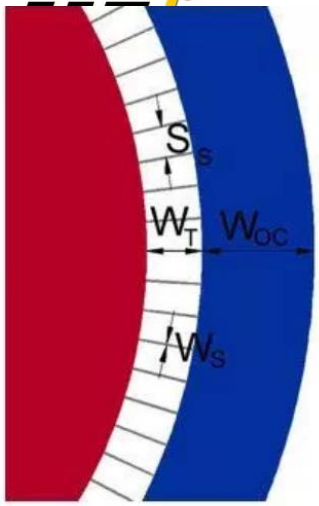




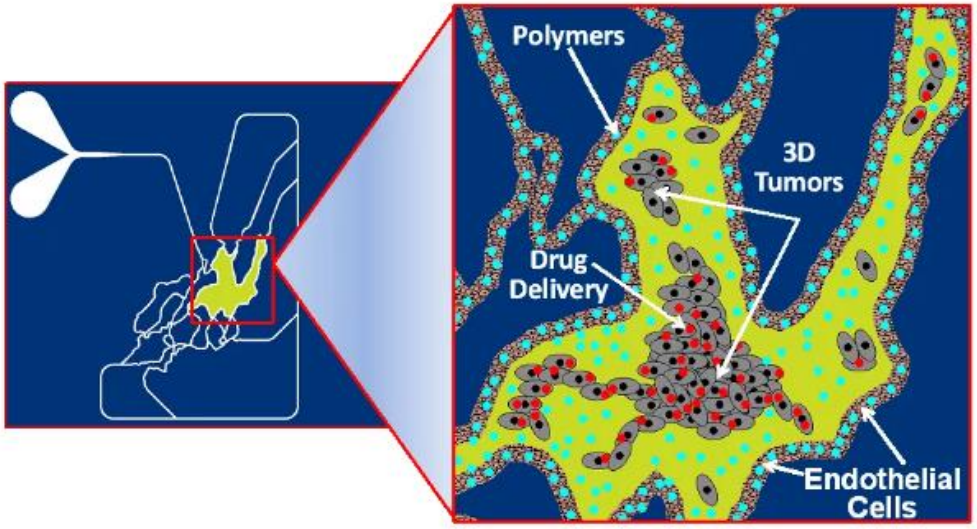






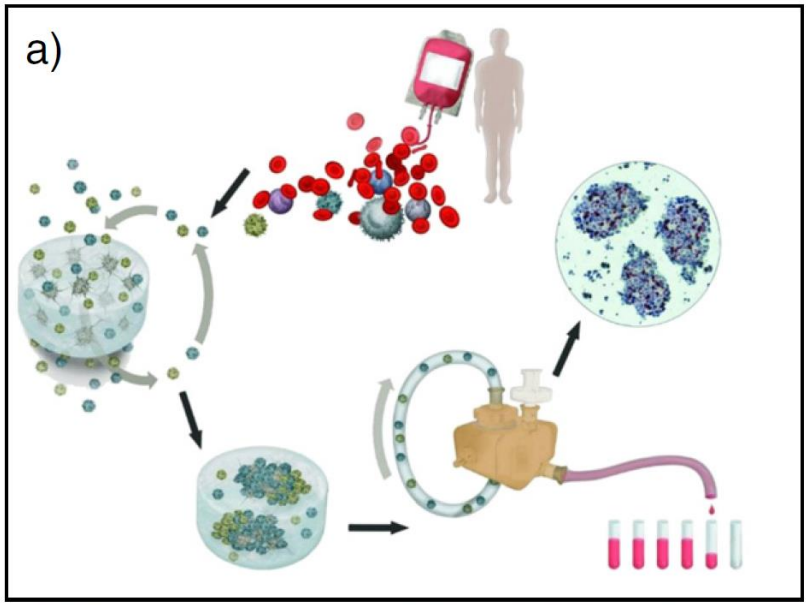


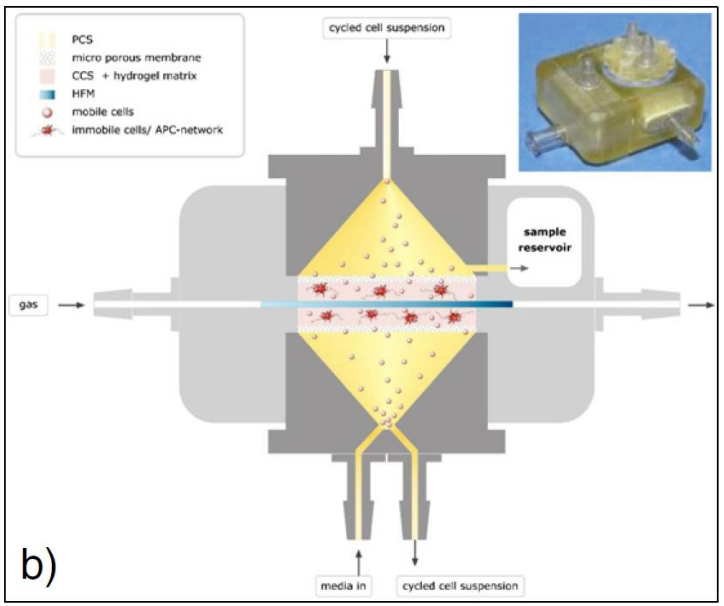
Schematic of the BBB Model. Apical chamber (outer channels) are for culture of vascular (endothelial cells) while basolateral chamber (central chamber) are for culture of brain tissue cells (astrocytes, pericytes, neurons). Porous architecture enables communication between the vascular and tissue cells.

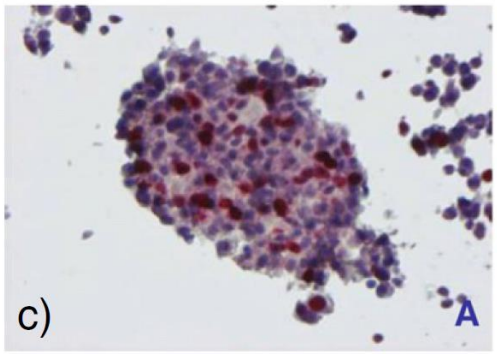


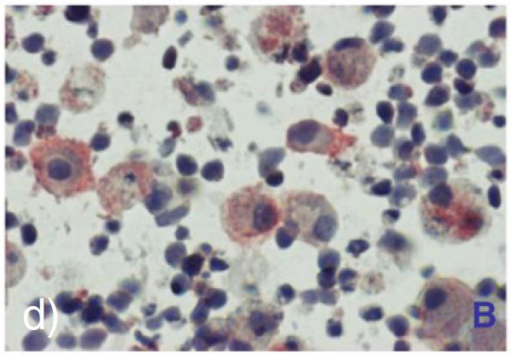
Create a realistic 3D co-culture with real time monitoring of cell-cell interactions between tumor, stromal, vascular and immune cells.

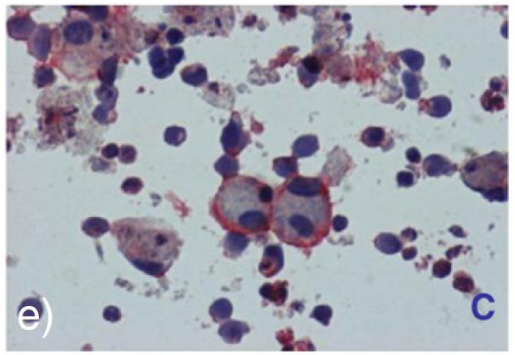
ProBioGen AG, Berlin, Germany, 2010









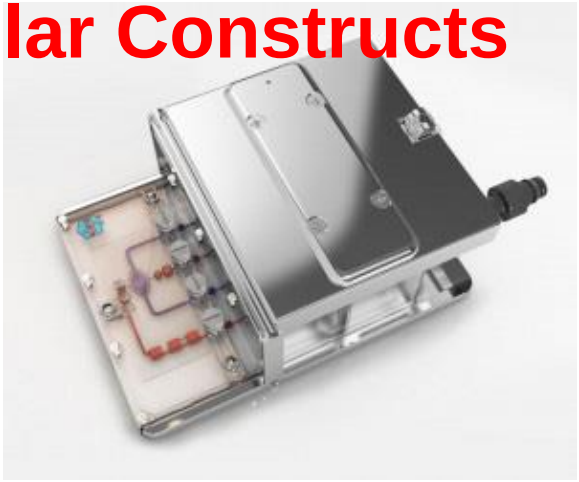


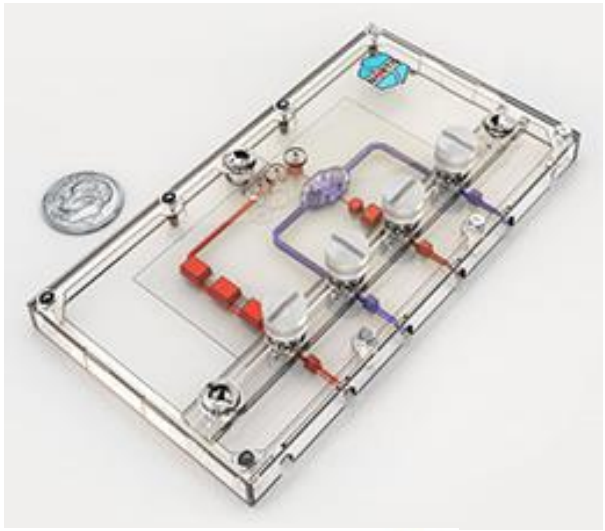
"Immunological substance testing on human lymphatic micro-organoids in vitro," Giese C, Lubitz A, Demmler CD, Reuschel J, Bergner K, Marx U, Journal of Biotechnology 148 (2010) 38–45, as presented in "Human immunity in vitro - Solving immunogenicity and more," Giese C, and Marx U,. Adv. Drug Del. Rev. 69:103-122. 2014.

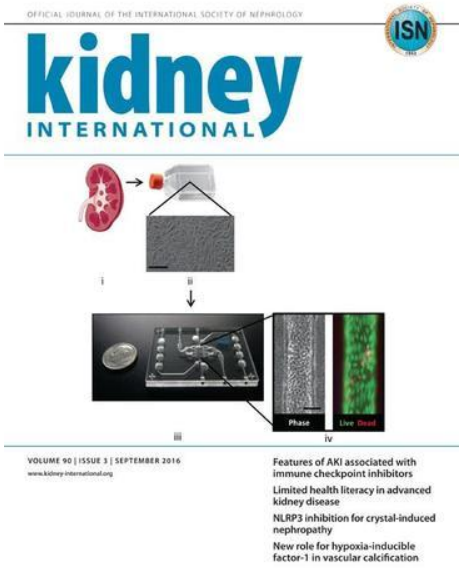
Human Artificial Lymph Node. a) Different cells of the native immunity are separated from donor leukocytes, differentiated into mature cells, seeded into 3D matrices, and mounted into a bioreactor device (b). c) Follicle-like spheroid formation and proliferation (c Ki67; red staining), plasma cell differentiation (c; CD138; red staining) and antigen-specific binding on plasma cells (e; biotynilated CMV-lysate; red staining).

3D Vascular Constructs

https://www.nortisbio.com/

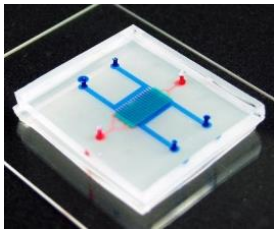


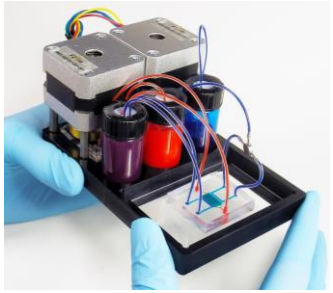




How can we keep a single organ alive for a month?

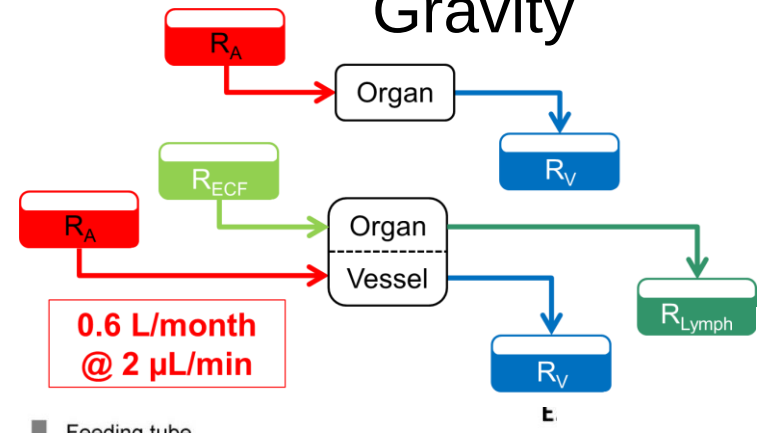
NeuroVascular Unit (NVU) on a Chip

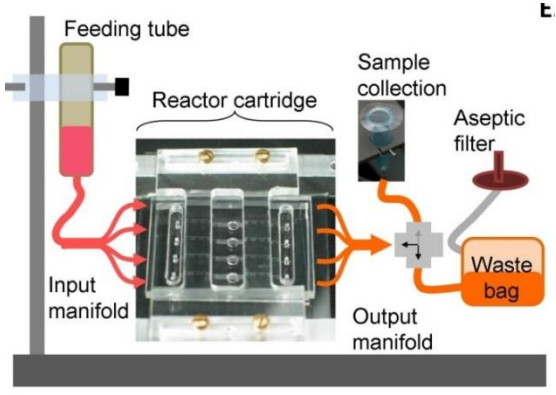




Organ Perfusion Methods

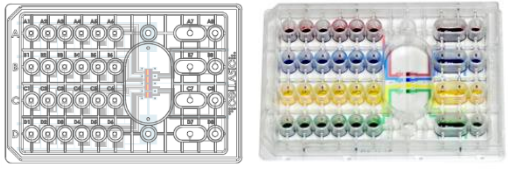
Gravity





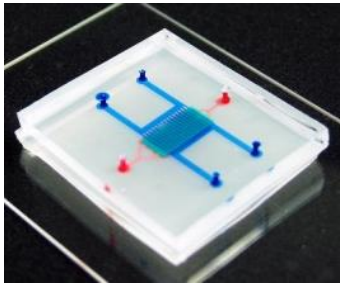
Air Pressure

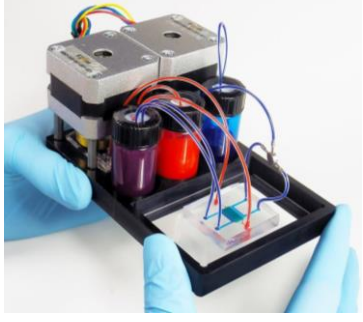
EMD-Millipore CellASIC ONIX™



Pipetting Robot

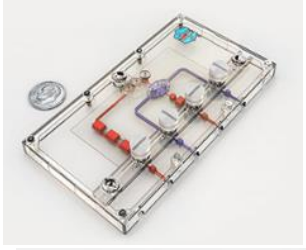
Microfluidic Pumps





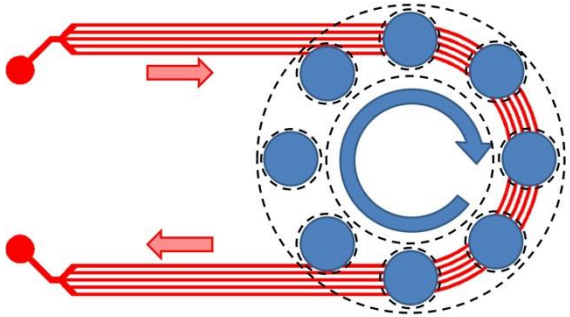
Syringe Pumps

Wyss Institute from long ago…

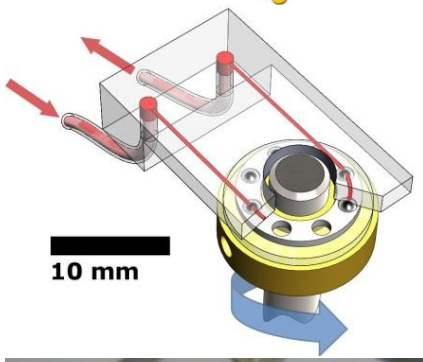


Nortis Bio today…

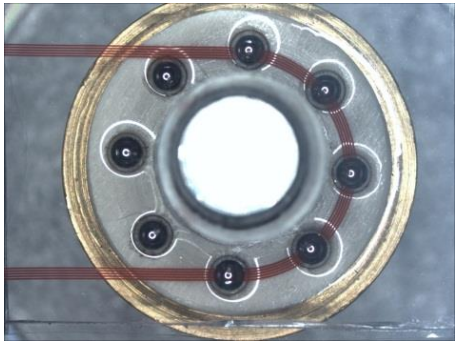
Rotary planar peristaltic micropump (RPPM)



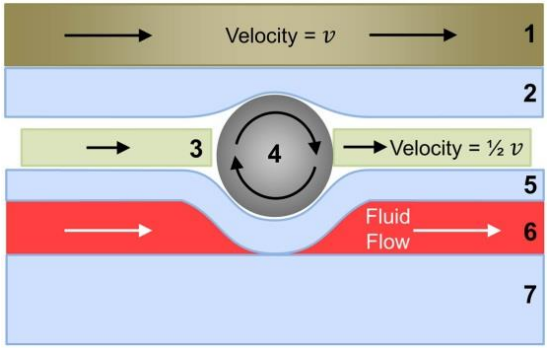
Balls are driven in a circle over microfluidic channels by a rotating disk of PDMS while being held a plastic cage

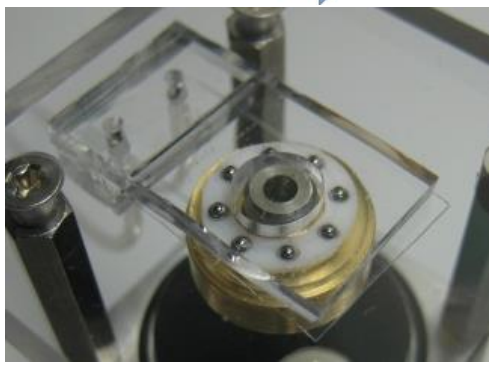


Imagine rolling an orange in a circle between your hands.



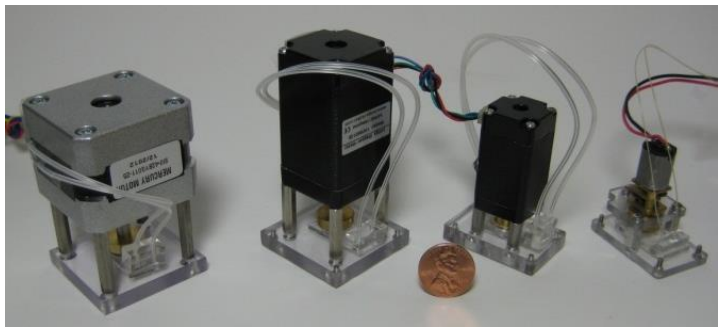
RPPM with dye-filled channels

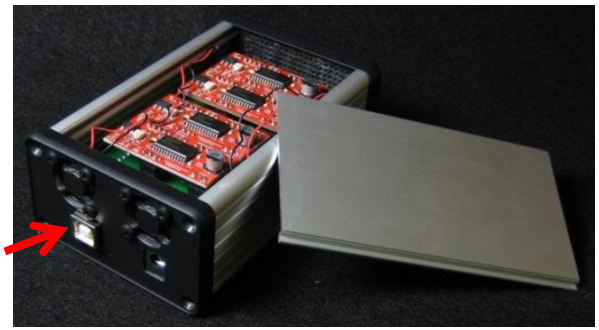




Arduino controller for four RPPMs

Four different sizes so far. Pumps can operate > 2.5 million cycles





Gould, Huang, *et al.*, PCT/US2011/055432

Parker Gould, Loi Hoang, *et al.*, in preparation

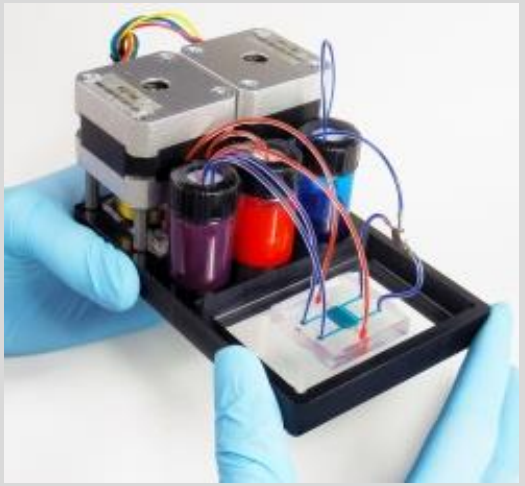
VIIBRE Pumps and Valves 2010-2016…

Challenge: Syringe pumps are expensive and not easy to move during handling.

Solution: Our microfluidic pumps and valves allow for stand-alone IOMs at a low cost.

PUMPS

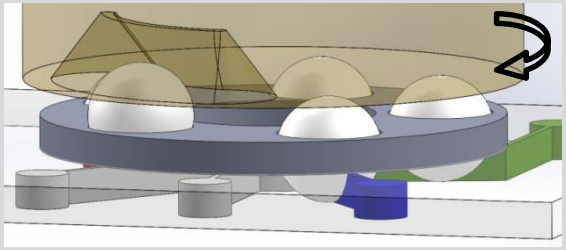
Rotary Planar Peristaltic Pumps enable a Perfusion Controller for a Neurovascular Unit on a Chip.

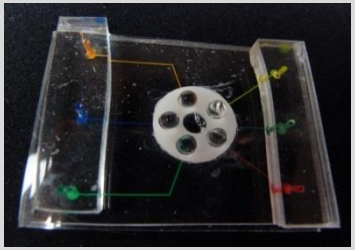


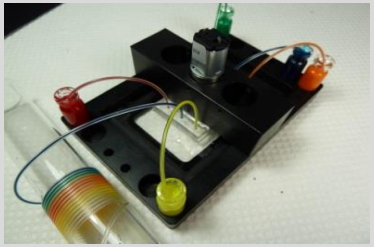
VALVES

Normally-closed rotary planar valves (NC-RPV) allow us to control perfusion, drug delivery, and sampling on-chip.

Patent US 9,618,129







Automated MultiPump Experiment Running Environment (AMPERE)

VIIBRE four-motor microcontroller can readily drive:

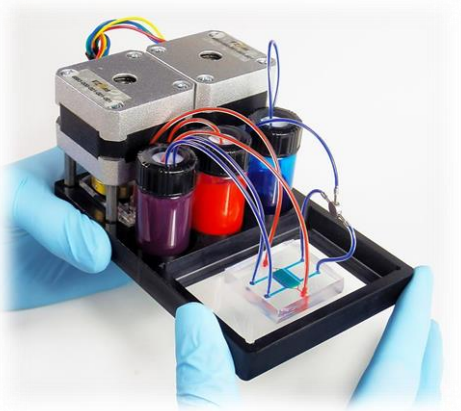
- MicroClinical Analyzer Module
- Microformulator Module
- Perfusion Controller Modules
- Integration Module

AMPERE drives multiple microcontroller modules

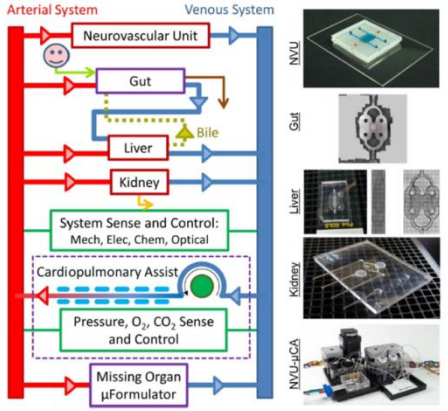
VIIBRE developers since 2013: Erik Werner and Greg Gerken

VIIBRE's organ module concept

- Create general purpose components
  - Pumps, valves, baseplates, bubble traps, microcontroller, software…
  - Assemble components into modules
    - Perfusion Controller, MicroClinical Analyzer, MicroFormulator, InterConnect…
- Each organ operates as an individual module
  - Low-volume on-board pumps and valves
  - Perfusion, oxygenation, waste removal..
  - Recirculation optimizes media conditioning
  - Replace media at a physiological rate
  - Fluidics disposable after use, hardware reusable

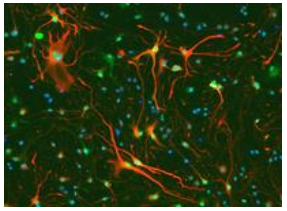


- The organ modules can be coupled together
  - Passive tubing (1 cm of 360 µm PEEK tubing = 20 nL/cm)
  - Can include active valves as required (load, recirculate, sample…)
  - Cardio-pulmonary assist
- System sensing and closed-loop control
  - Mechanical, electrical, chemical, optical
  - Real-time electrochemical metabolic sensing
- Missing Organ MicroFormulator
- Untargeted, in-line, near-real-time analytics



Tissue Chips at Vanderbilt

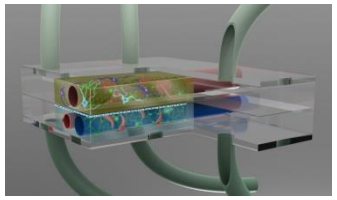
- Human iPSC-derived neuronal cells



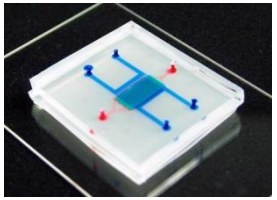
hiPSC glutamatergic neurons

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

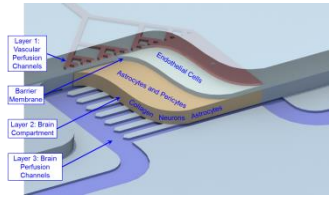
- Bioreactors

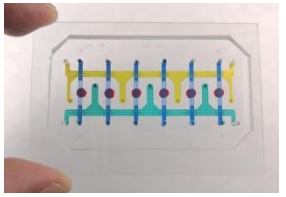


VIIBRE NVU concept 2012



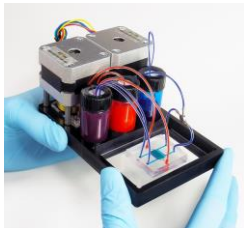
VIIBRE NVU as built 2014





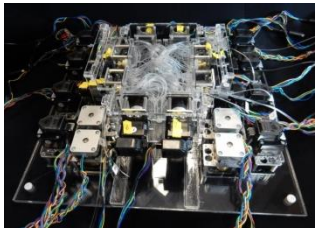
Mammary gland-on-a-chip 2016

- Control hardware

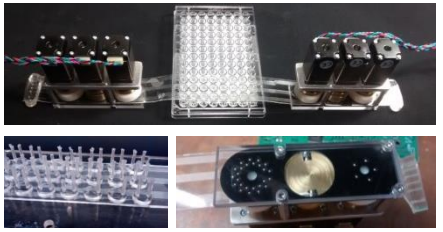


VIIBRE NVU Perfusion Controller 2014

VIIBRE 24-port valve 2015



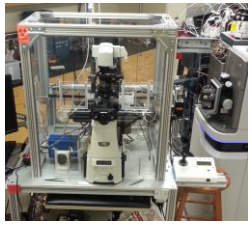
MicroFormulator 1.0, 2015



MicroFormulator 2.0, 2016

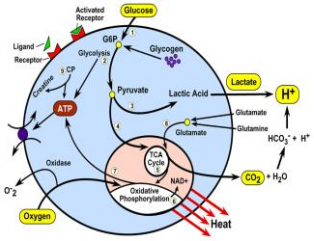
SmartMotor 2.0, 2016

- Analytical chemistry & metabolomics

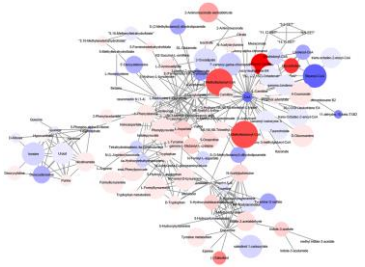


In-line MS of organ chip 2013

VIIBRE MicroClincial Analyzer 2014



Core Carbon Metabolism



MS metabolomics 2016

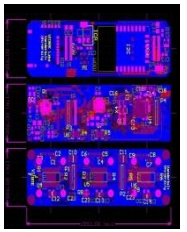
- Translation

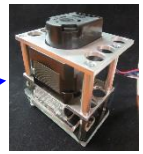
Issued U.S. patents: 7,435,578; 7,534,601; 7,704,745; 7,713,733; 7,790,443; 7,974,003; 7,977,089; 7,981,649; 8,129,179; 8,339,704

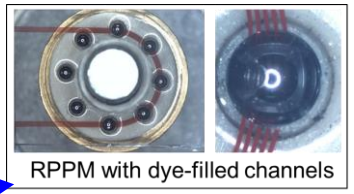
VIIBRE Hardware Overview - 1

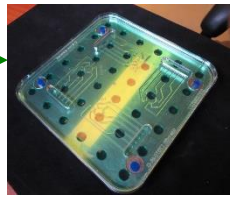
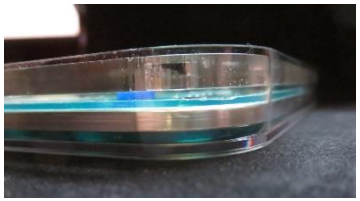
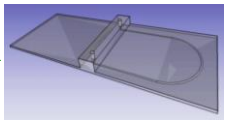
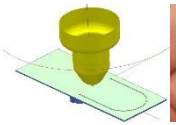
- **Controllers**
  - 4x Arduino for NEMA-17
  - 3x NEMA-8 SmartMotor
  - 1x NEMA-17 SmartMotor
- **Motors**
  - NEMA-17
  - Triple NEMA-8 Smart Motors
  - Single NEMA-17
- **Pumps**
  - NEMA-17 Low Flow Classic (0 to 6 µL/min)
  - NEMA-17 High Flow (0 to 1 mL/min)
  - Advanced molding techniques
  - Non-PDMS
  - Triple NEMA-8
- **Valves**
  - 5 port
  - 25 port
  - New fab techniques
  - 2x8 port
  - Triple NEMA-8 2x NVU recirculating
  - Non-PDMS

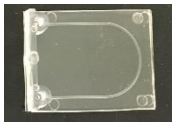
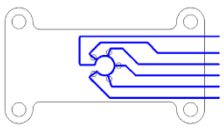
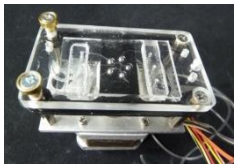
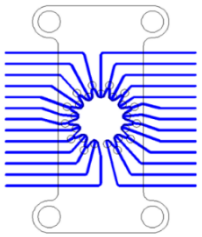
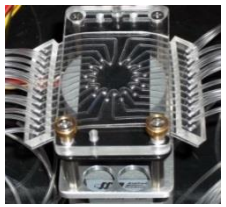


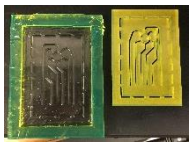
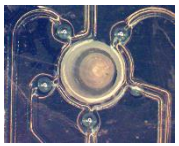
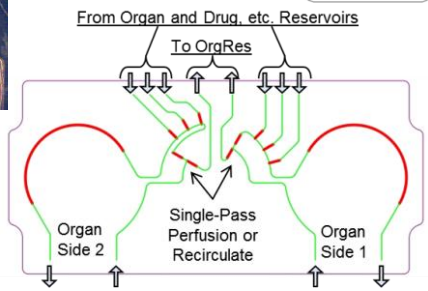


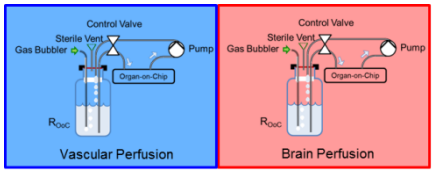





RPPM with dye-filled channels






VIIBRE 24-port valve
2015



- In production
- Under development
- Under consideration

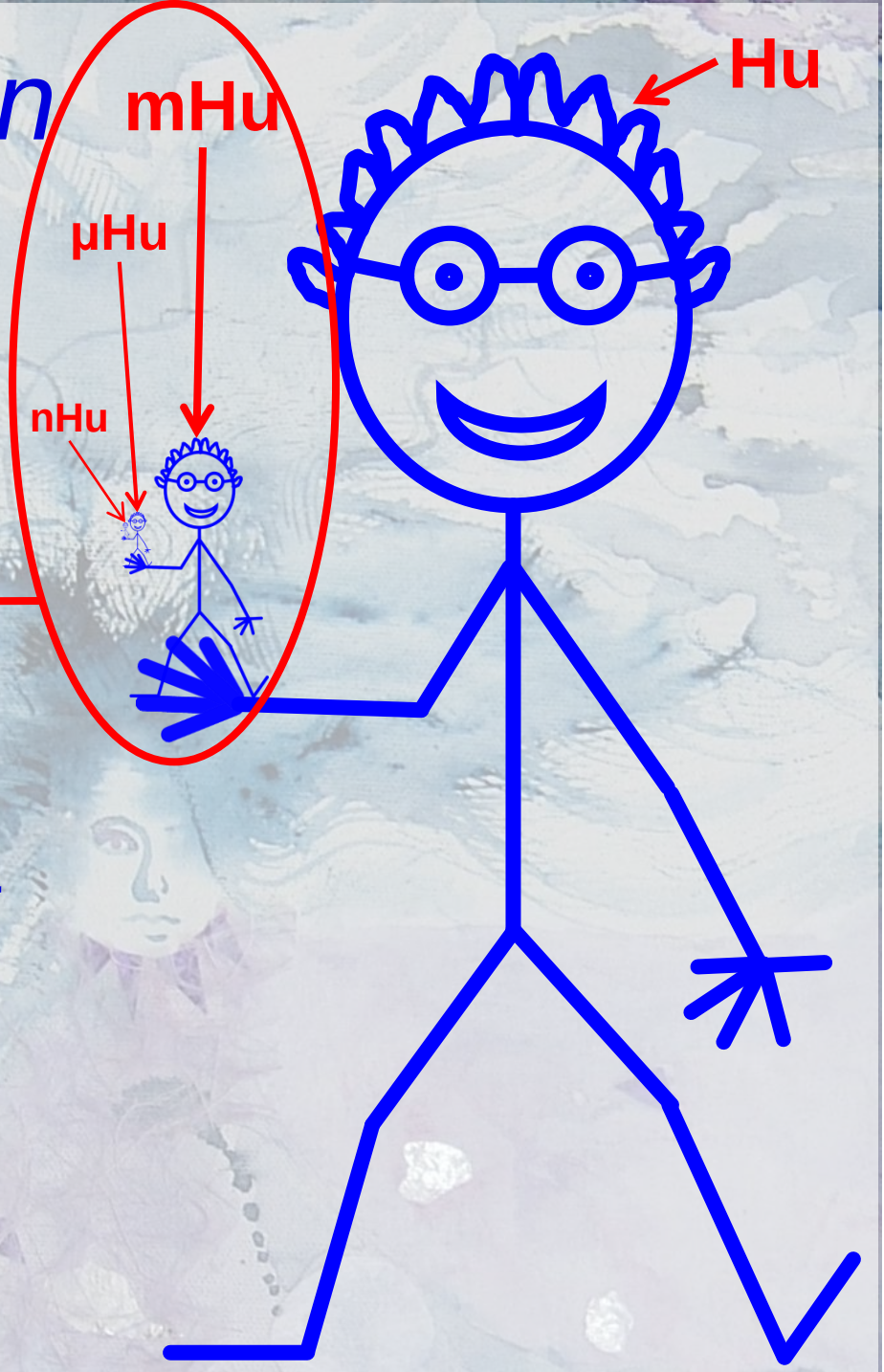
Should we couple organs-on-chips together?

Homunculus, *noun*

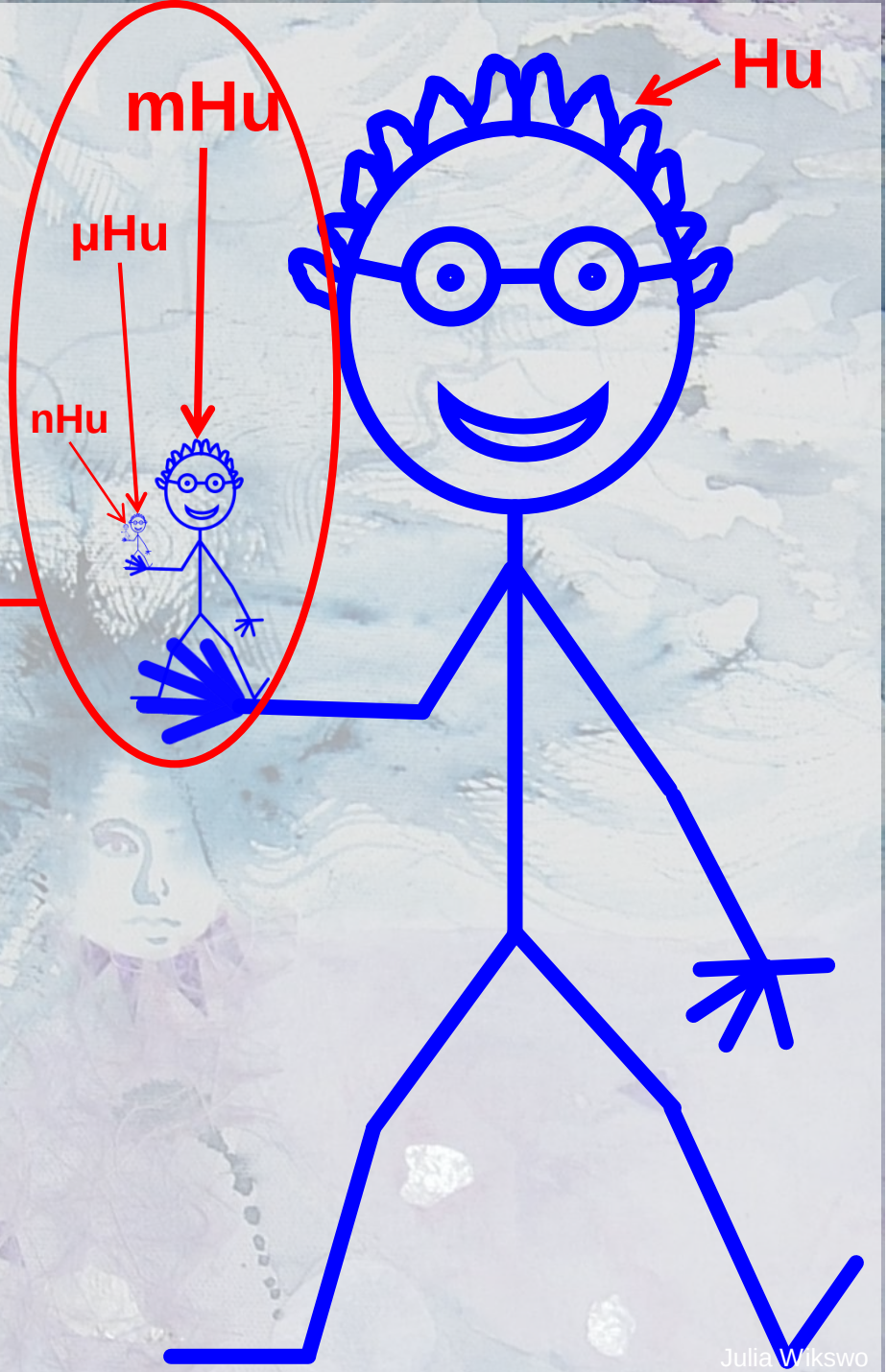
[hō-ˈmən-kyə-ləs]

plural ho·mun·cu·li

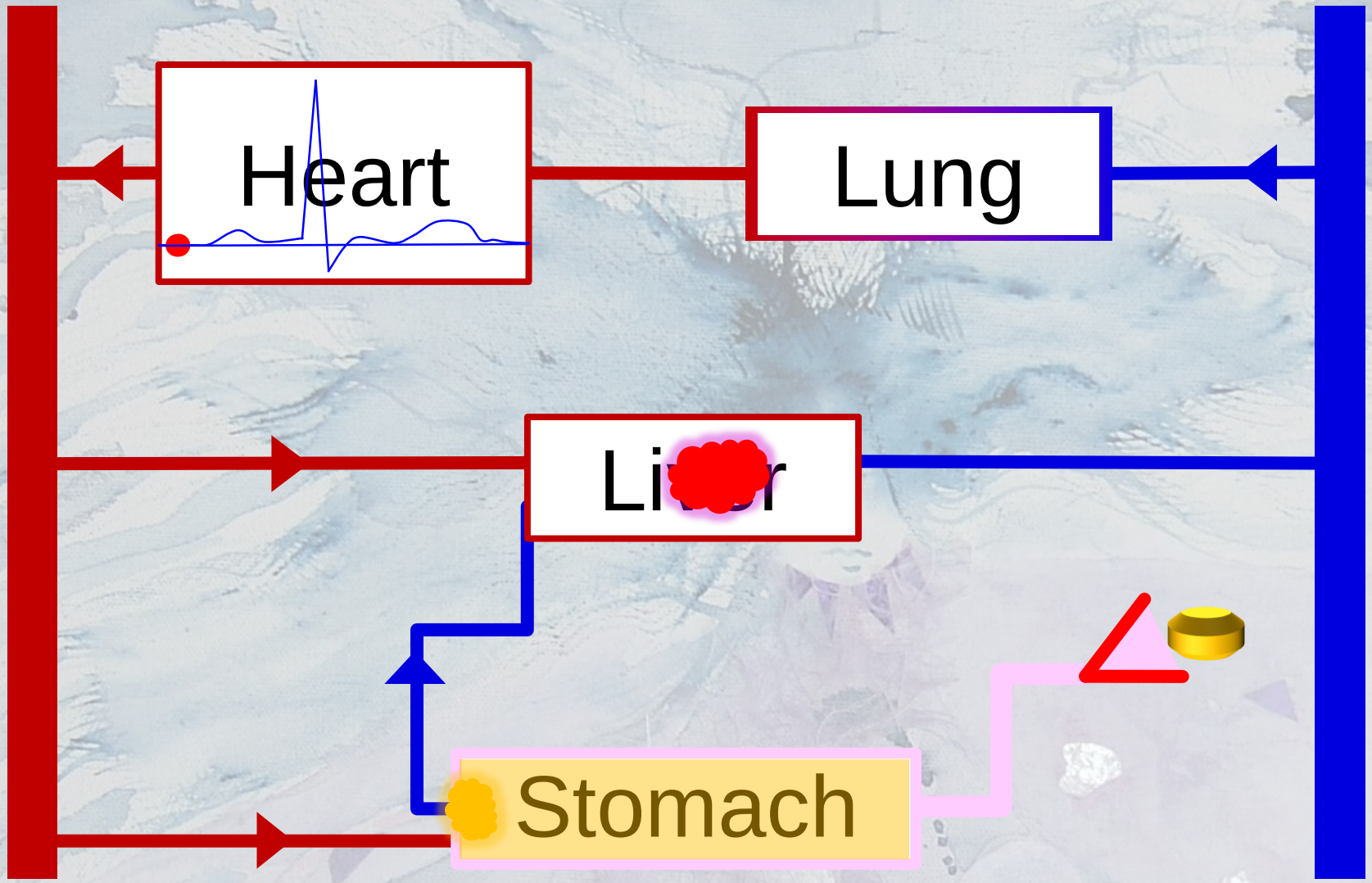
A miniature representation of a human.



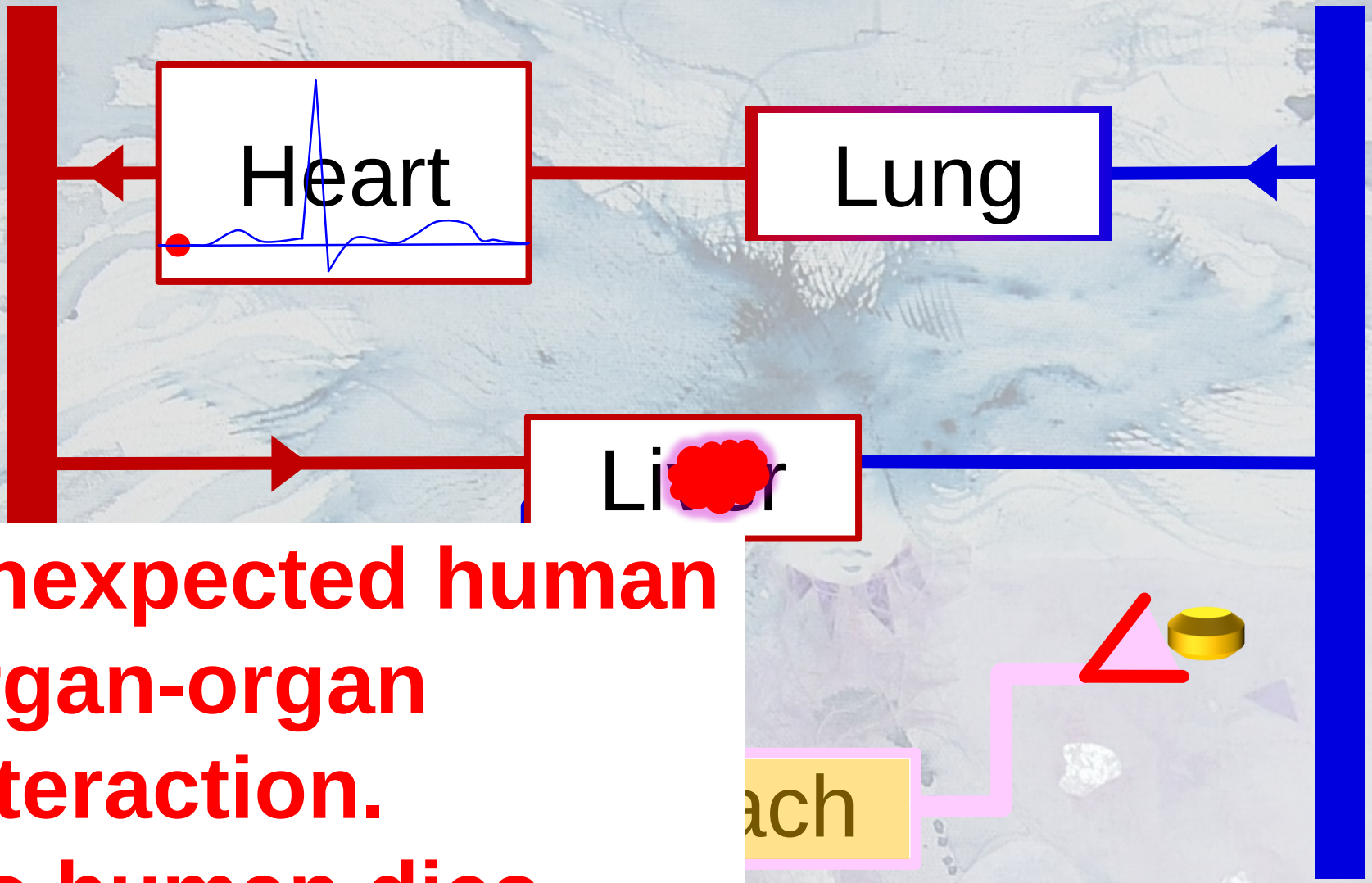
OCM homunculi will be alive, built with human cells!



Test drugs in homunculi!



Test drugs in homunculi!



Unexpected human organ-organ interaction.

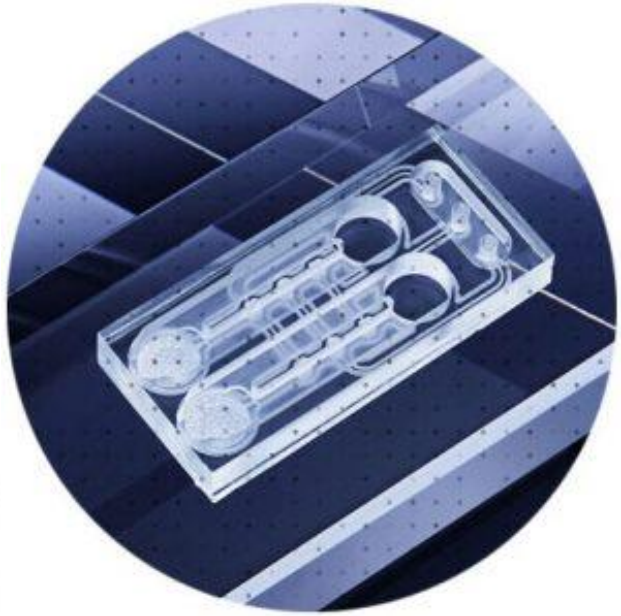
No human dies.

Do we need multiple organs?

- Drugs metabolized by one organ may be toxic to another
- How many?
  - –Two organs are obvious: *e.g.*, liver plus something else (heart, kidney, brain…)
    - Drug metabolism
    - Environmental toxicology
  - –ADMET could benefit from coupled gut, lung, kidney, liver
  - –DoD invested at least ~$120 million to get 4 to 10 or more interconnected organs.
  - –Does Pharma need them today? If not, how soon?
- How are coupled organs useful? How do you do it?

cnBio innovations

VIBRE

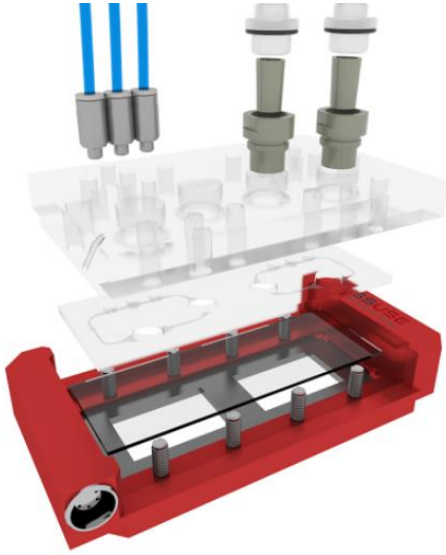


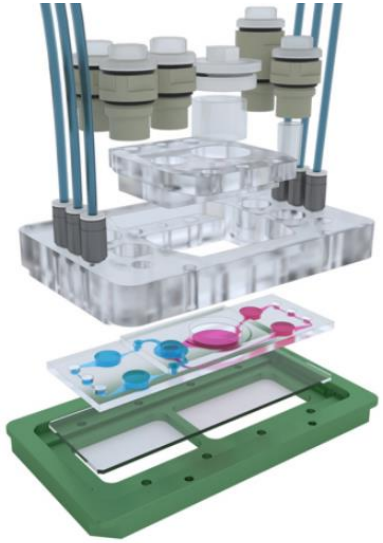
Under license from MIT

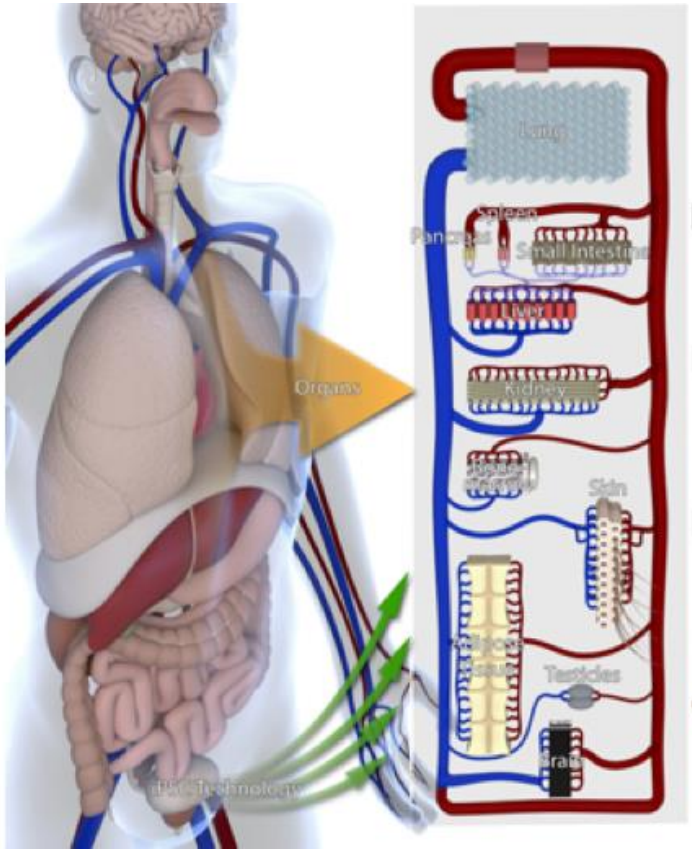
http://cn-bio.com/instruments/

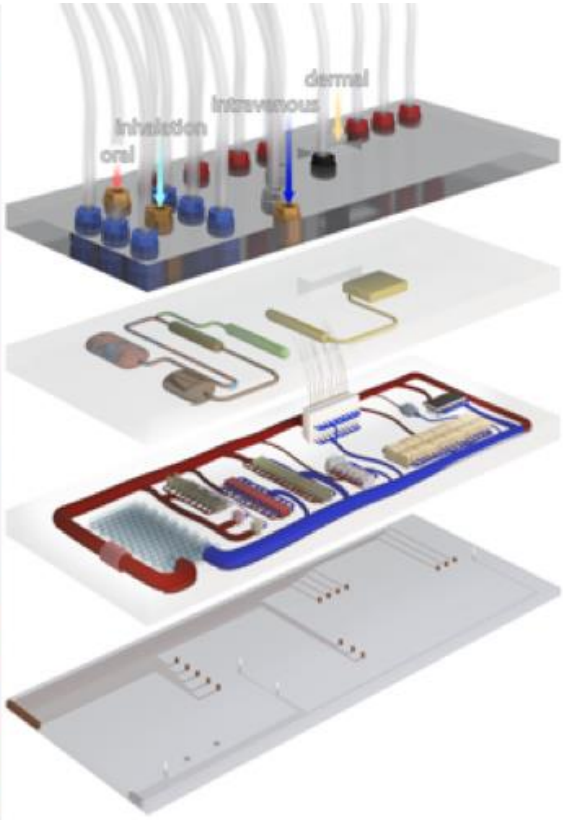
TISSUSE

Emulating Human Biology



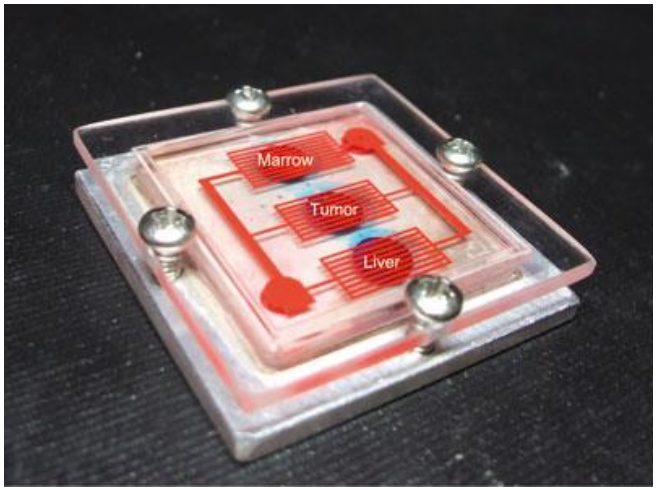




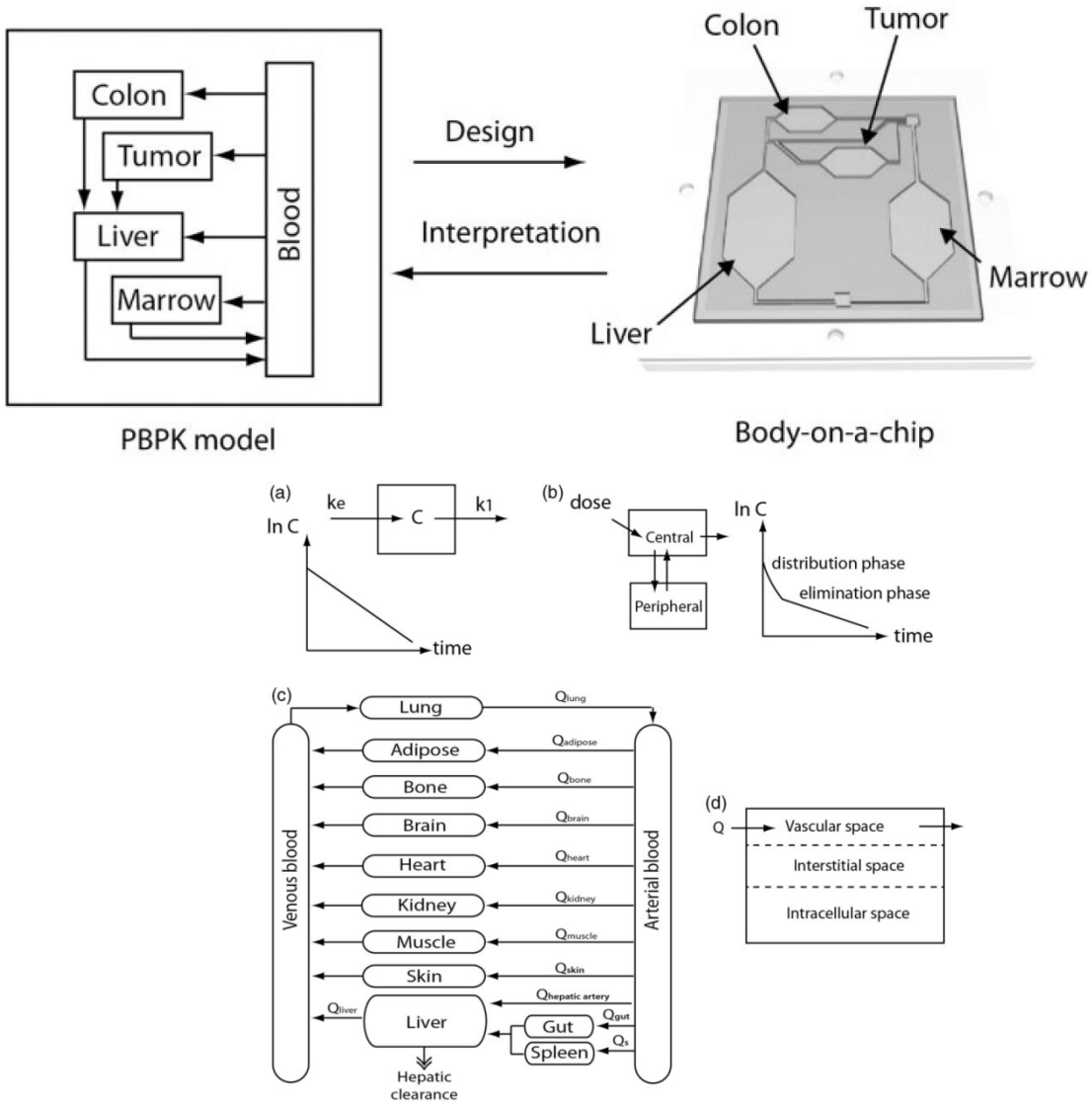


Coupled organs support PKPD

- Coupled organs on a single chip can scale either volumes or media exposure times
- Supports PKPD analyses



Nature 471, 661–665 (31 March 2011)



Sung … Shuler, Hickman. *Exp.Biol.Med. 239 (9):1225-1239, 2014.*

The "Volume problem"

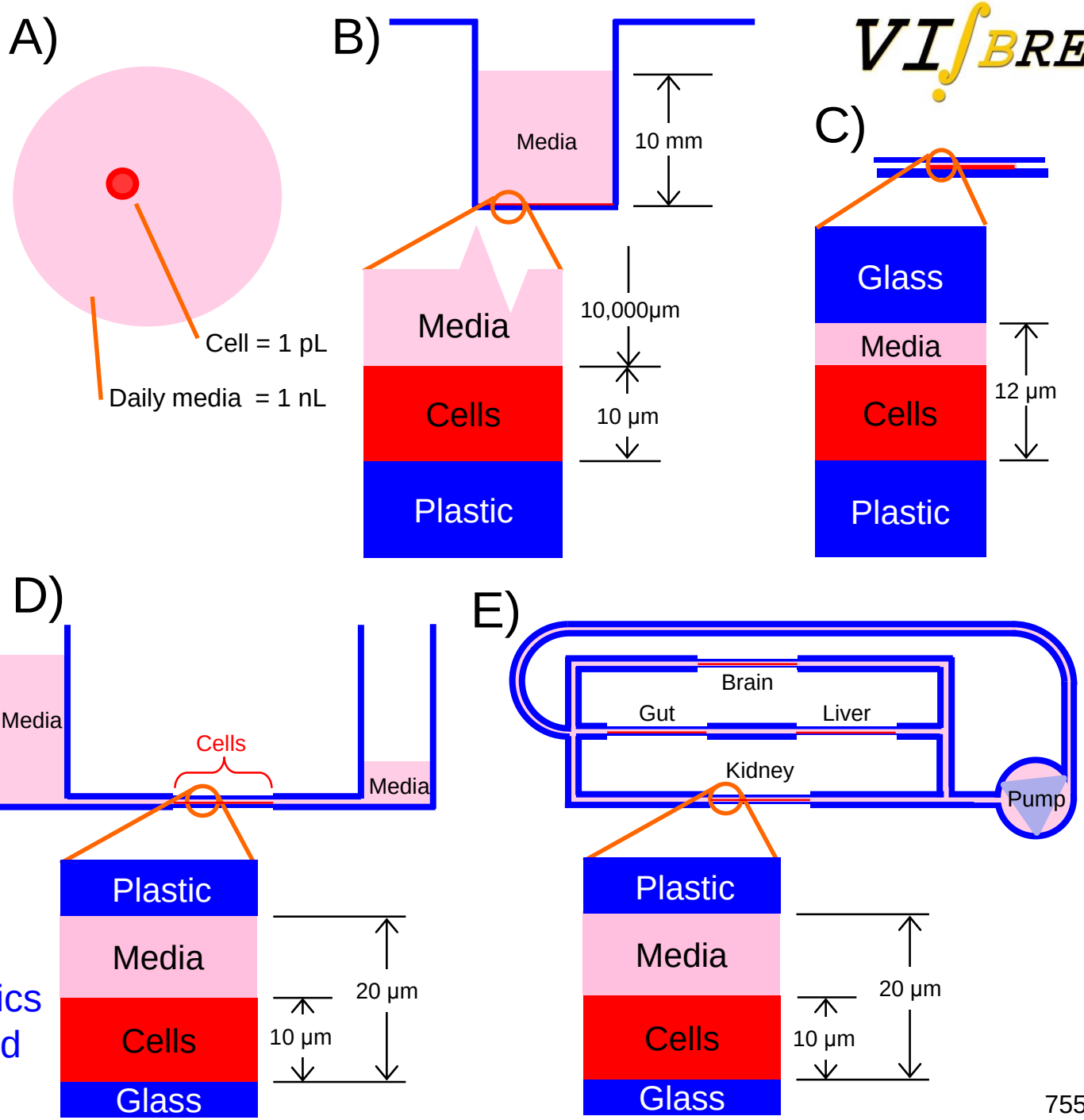
A) A pL cell requires a nL of fresh media each day.

B) The "Volume problem" in dishes and wells: paracrine, autocrine and endocrine factors diluted by a factor of 1000.

C) Microfluidics can reduce the volume of a single organ-on-chip.

D) Pipetting between reservoirs may not solve the volume problem

E) Integrated microfluidics should solve the coupled organ volume problem.

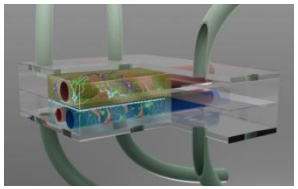


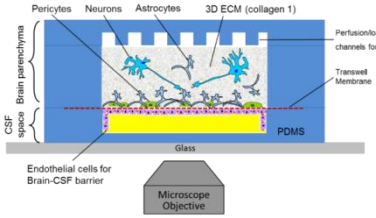
VIIBRE's Neurovascular Unit (NVU)

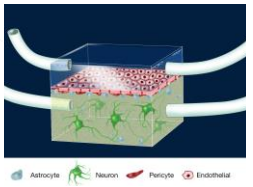
Vanderbilt Institute for Integrative Biosystems Research and Education

VIIBRE

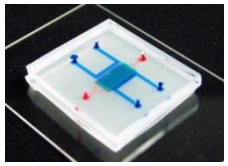
- **Concept**
  - Vascular and brain spaces
  - Planar not hollow fiber
  - Four cell types
  - Could add CSF and choroid plexus

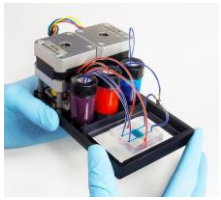


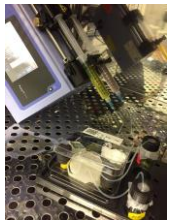


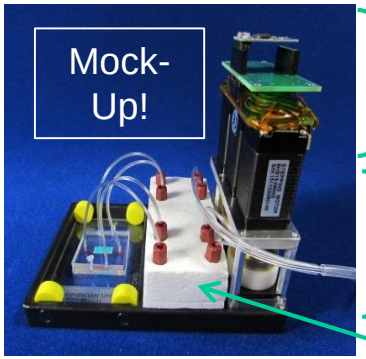


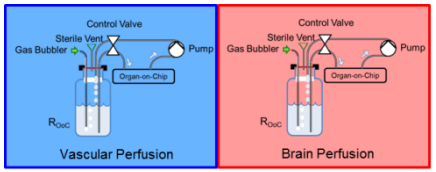
- **Today**
  - Planar µfluidic NVU
  - Perfusion
    - Syringe pumps
    - Two NEMA 17 LoFlow pumps
  - TEER / impedance spectroscopy



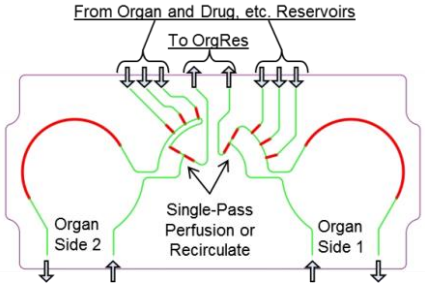


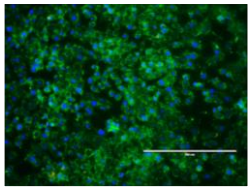


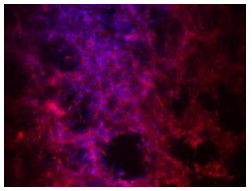




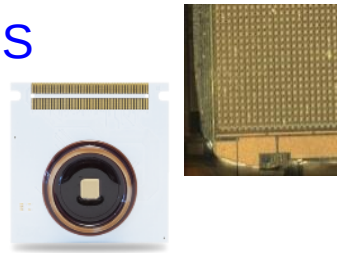
- **Soon**
  - NEMA-8 SmartMotors
  - Drug injection valve
  - Recirculation
  - On-board wireless TEER
  - Transparent membranes

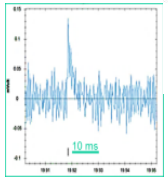


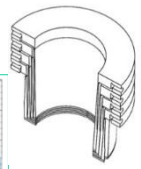


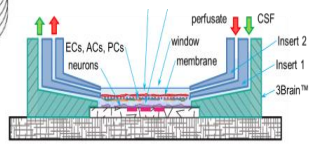


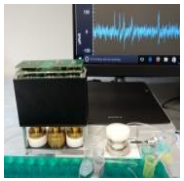
- **Neuro-electric signals**
  - 4096-channel CMOS
  - Stimulation
  - 7 kHz recordings
  - Stackable



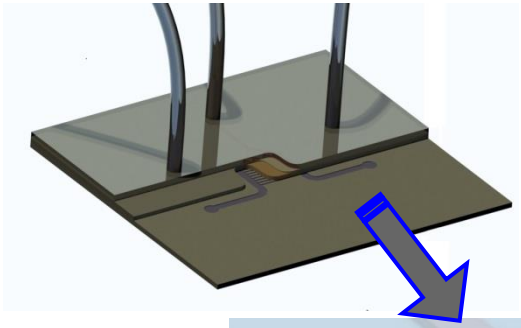


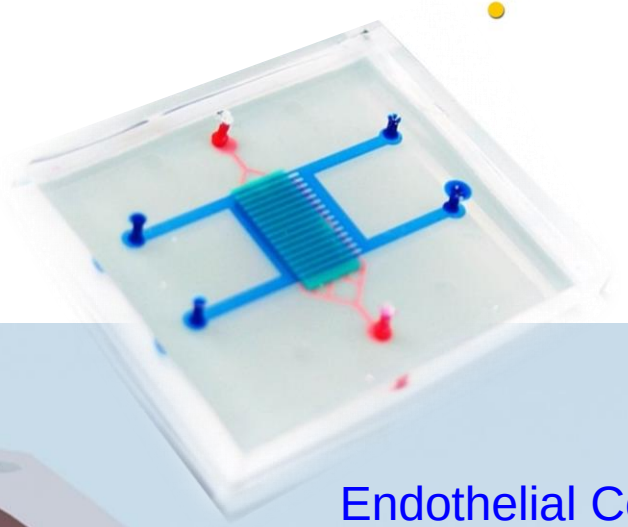


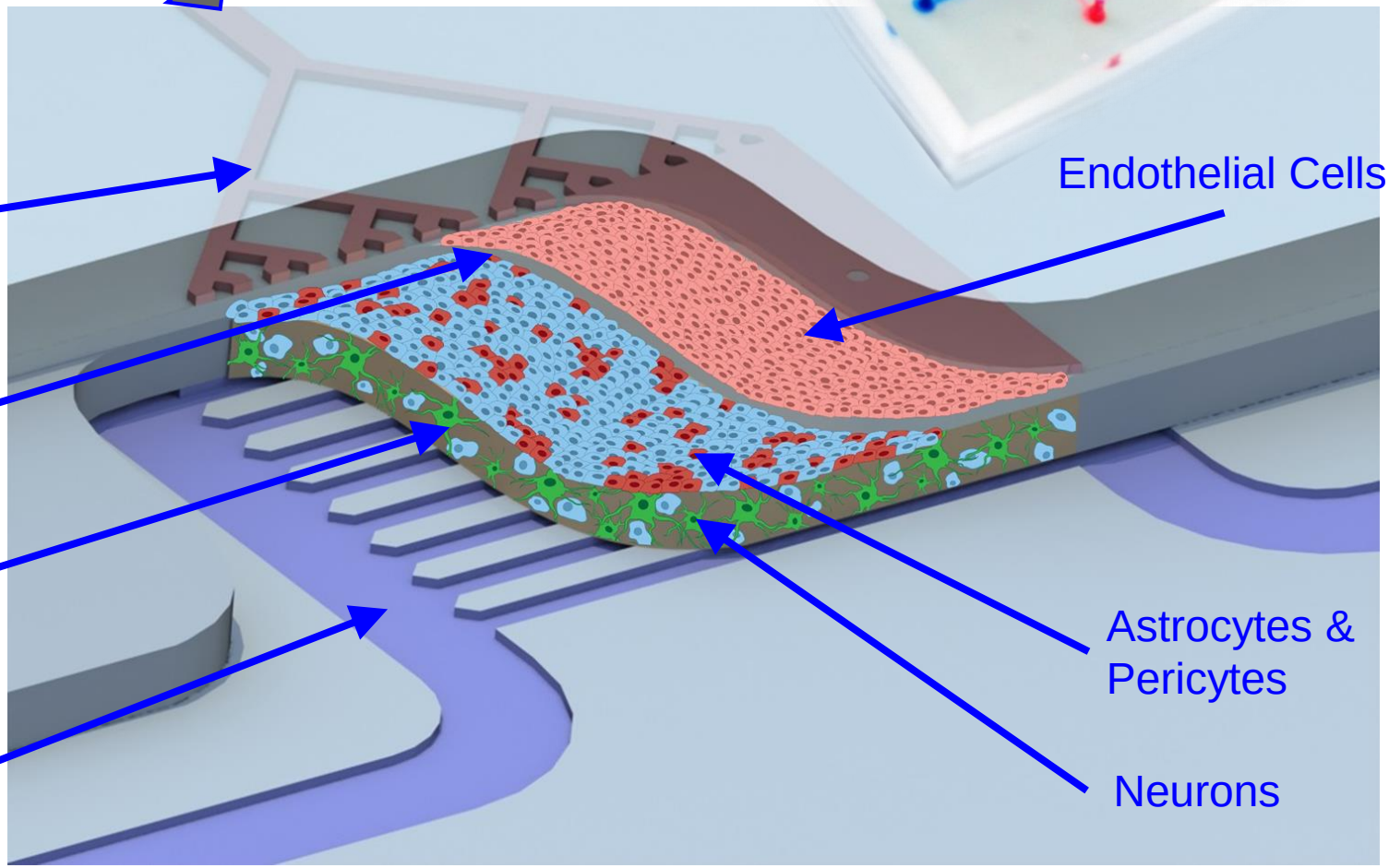




The VIIBRE NVU and BBB

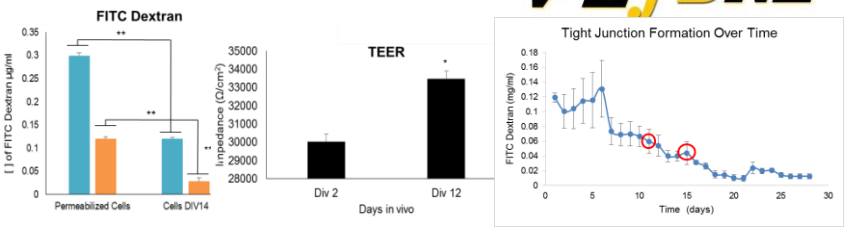


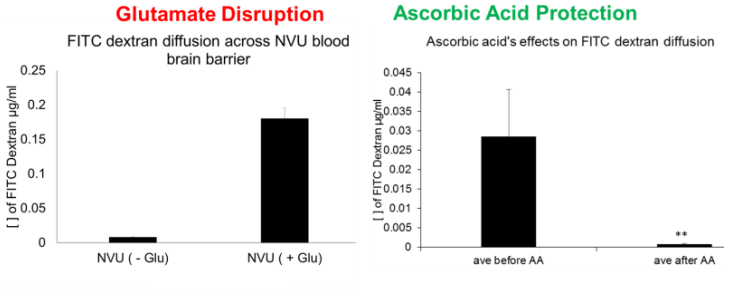


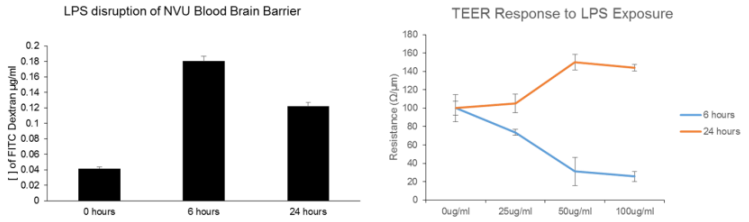


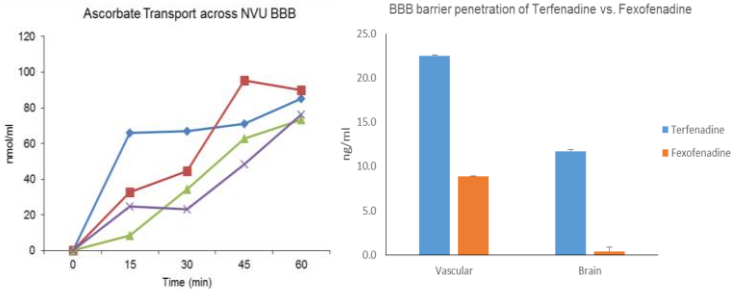
NVU/BBB measurements

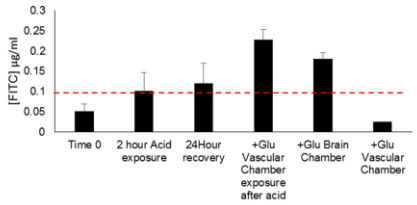
- Tightening of the BBB with time after assembly
- Disruption by glutamate in the brain compartment
- Tightening by ascorbic acid in the vasculature
- Differential responses over time to inflammatory agents (LPS and cytokine cocktails)
- Differential transport across the BBB: ascorbic acid (Y), Terfenadine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



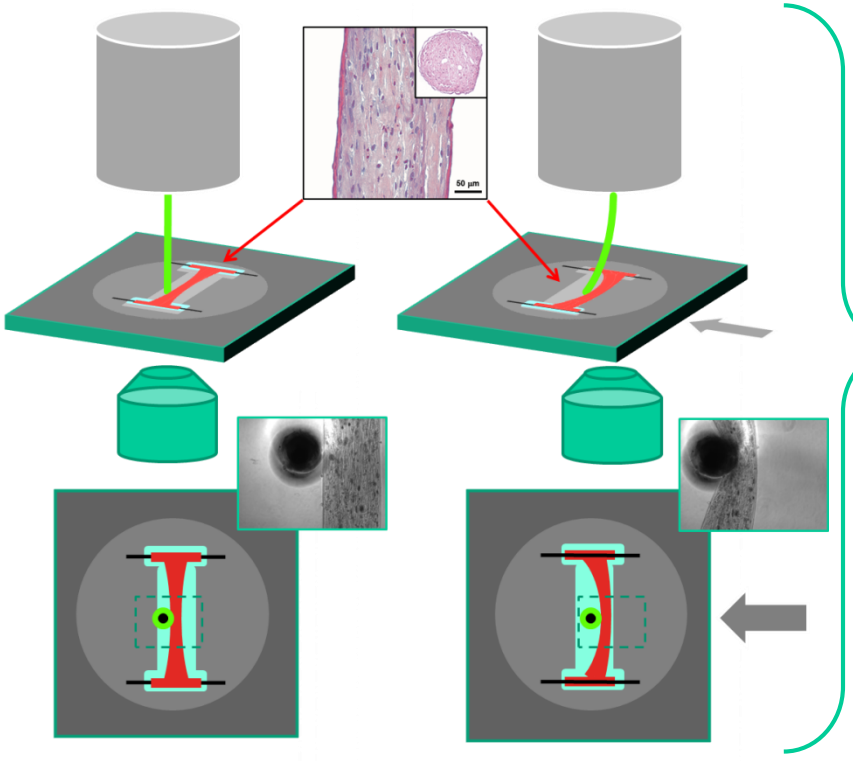






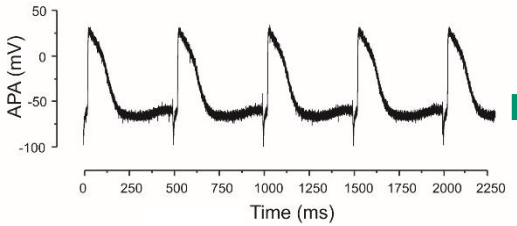


Cardiac I-Wire

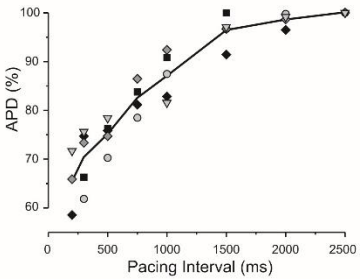


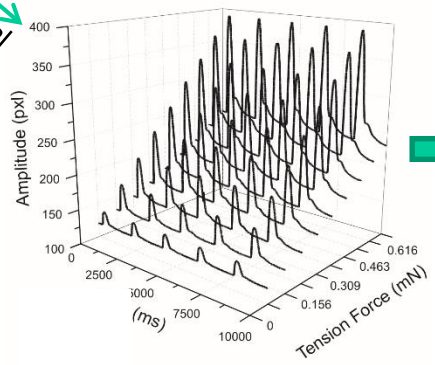
Electrical

Mechanical

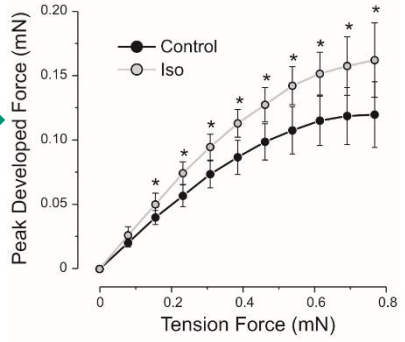


Restitution Curve

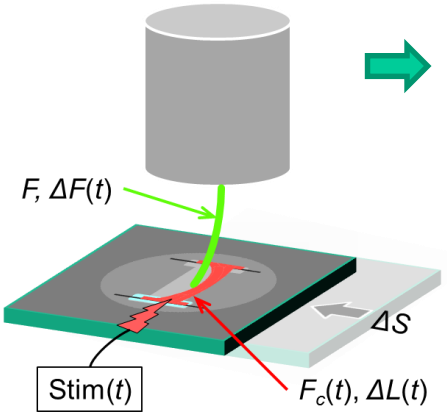




Frank-Starling Curve



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



$$F_P = K_P \Delta s_1$$

$$\Delta s_1 = \Delta s - \Delta s_2 = \Delta s - \sqrt{\Delta L(2L + \Delta L)}$$

$$\Delta s_2 = \sqrt{(L + \Delta L)^2 - L^2} = \sqrt{\Delta L(2L + \Delta L)}$$

$$\Delta L = \sqrt{L^2 + {\Delta s_2}^2} - L = \sqrt{L + \left(\Delta s - \frac{F_P}{K_P}\right)^2} - L$$

$$F_P = K_P \, \Delta s_1 = 2F_C \sin(\theta) = 2F_C \, \frac{\Delta s_2}{L + \Delta L}$$

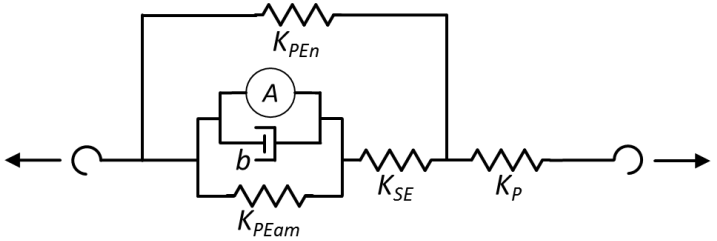
$$F_C = \frac{1}{2} K_P \left(\frac{\Delta s}{\sqrt{\Delta L(2L + \Delta L)}} - 1\right)(L + \Delta L)$$

$$F_C = \frac{\left(K_{PEam} + K_{PEn} + \frac{K_{PEn} K_{PEam}}{K_{SE}}\right)}{1 + \frac{K_{PEam}}{K_{SE}}} \left(\Delta L + \Delta L_{offset}\right)$$

$$\dot{\Delta L} = \frac{\left(1 + \frac{K_{PEam}}{K_{SE}}\right) f_1(\Delta L) - K_M(\Delta L + \Delta L_{offset}) - F_A}{b\left(1 - \frac{f_2(\Delta L) - K_{PEn}}{K_{SE}}\right)}$$

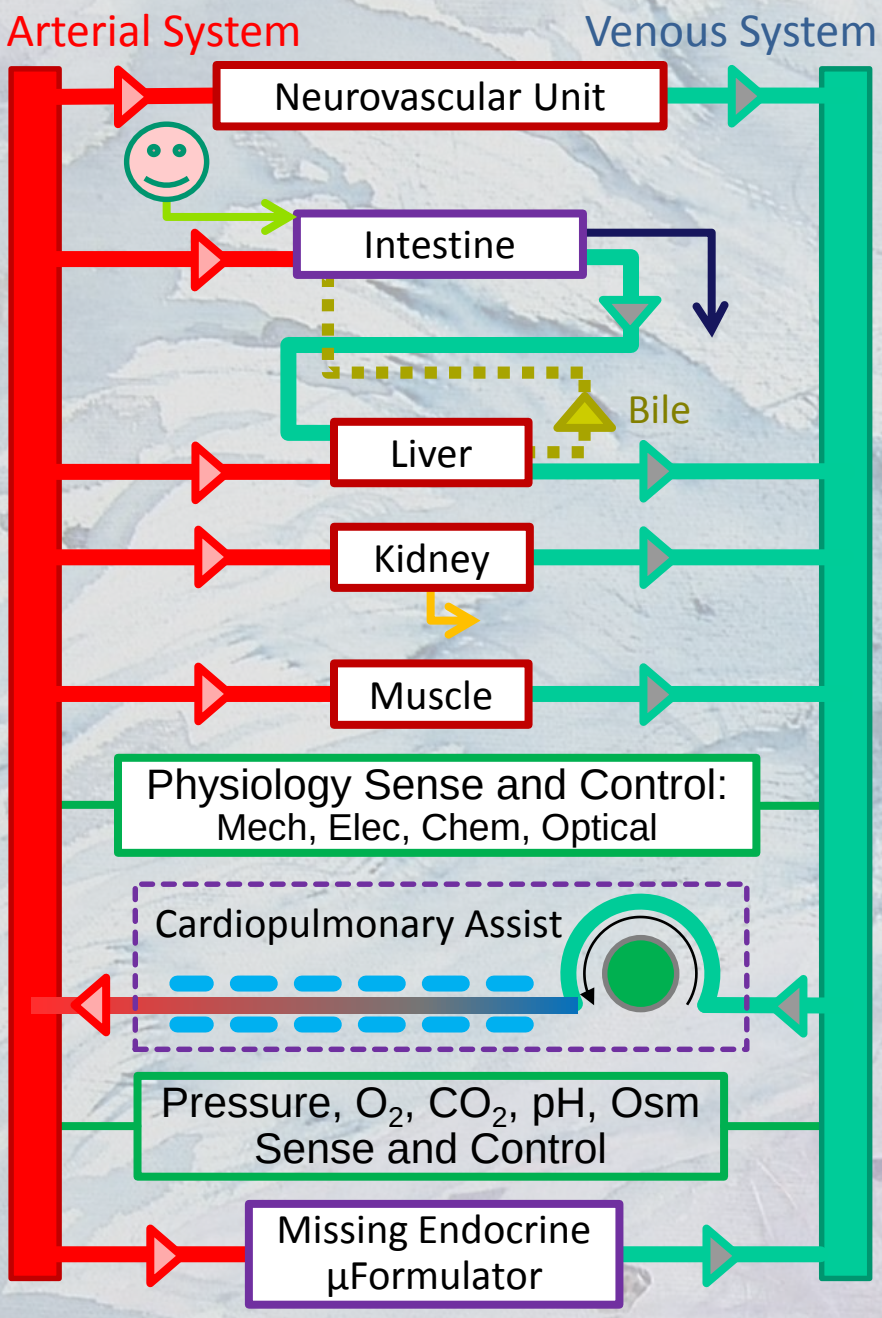
Nashville single-string guitar equation

Cardiac Hill model

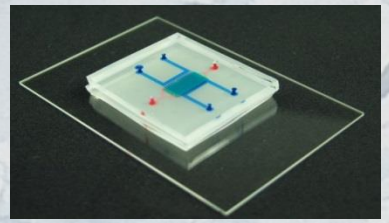


Schroer, *et al.*, Acta Biomaterialia, 2016

NIH-NCATS MPS Integration

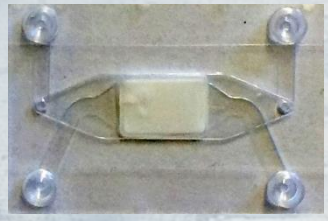


NVU



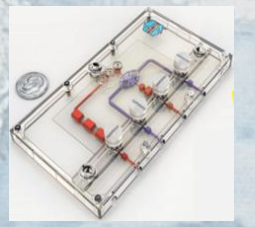
Vanderbilt

Intestine



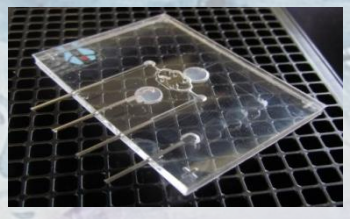
Hopkins, Baylor

Liver



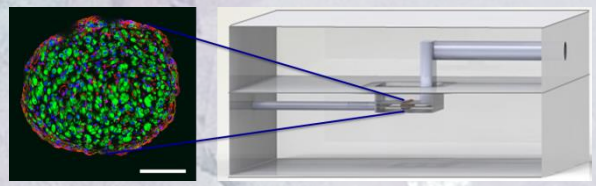
Pittsburgh

Kidney



Washington

Muscle



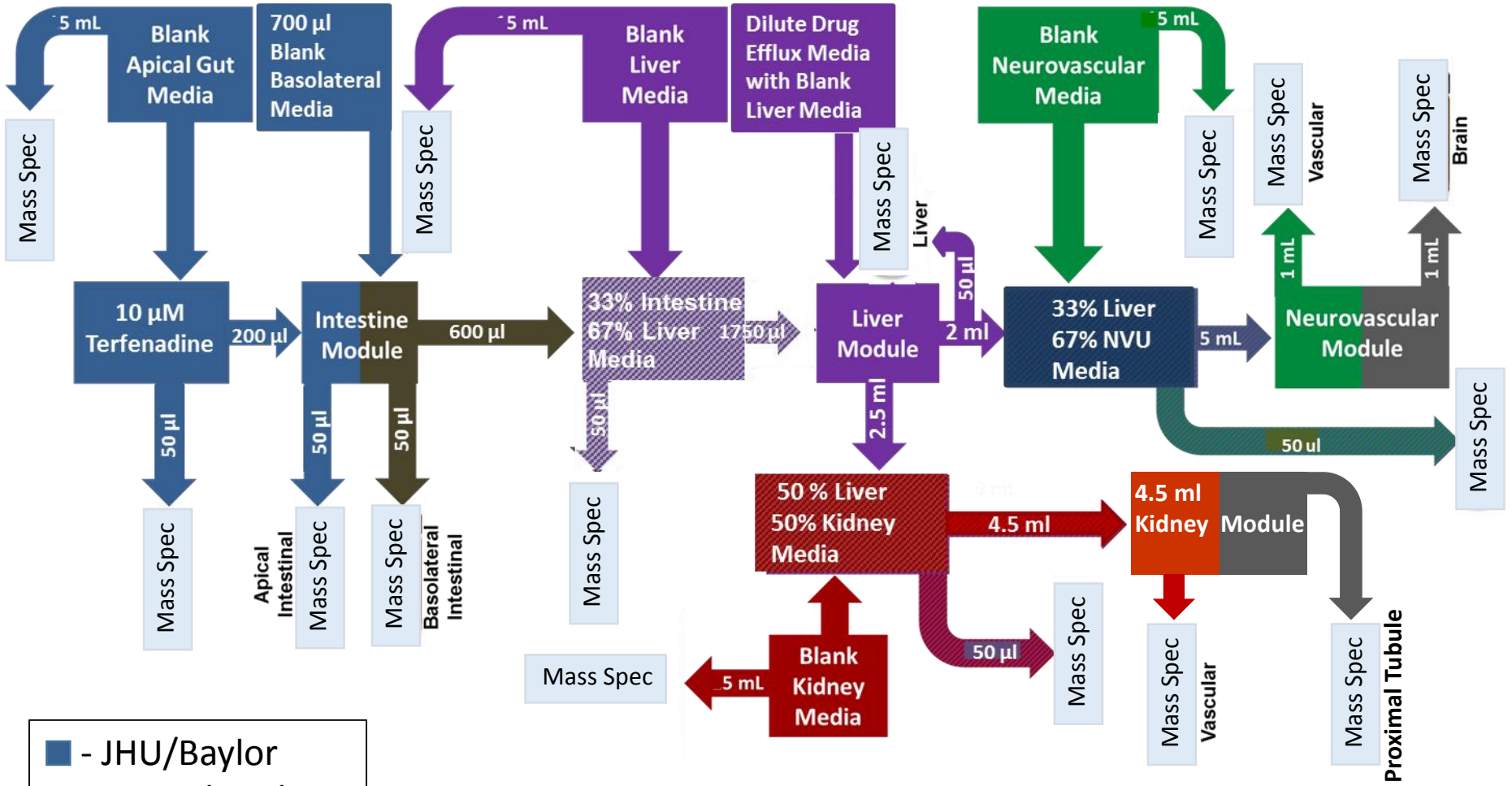
Duke

ME-µF

Vanderbilt

Work Flow for Functional Coupling Experiment

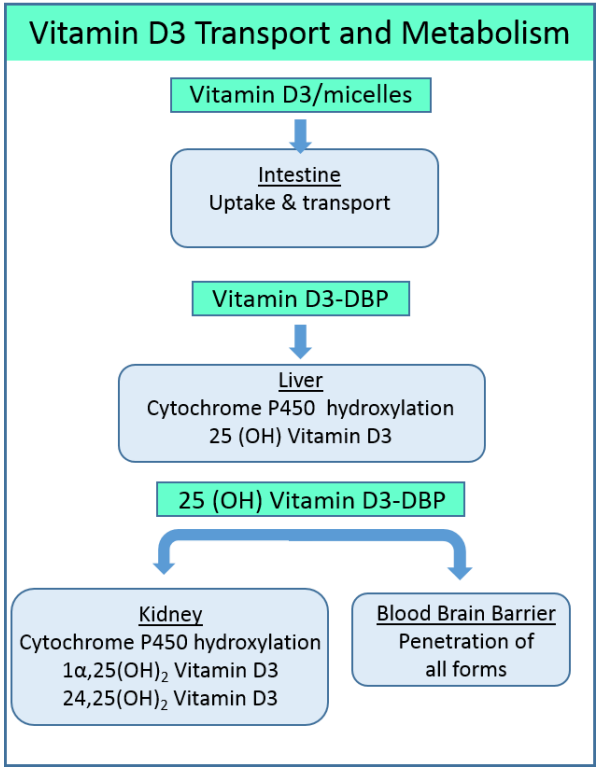
Goal: Couple Gut, Liver, Brain, and Kidney

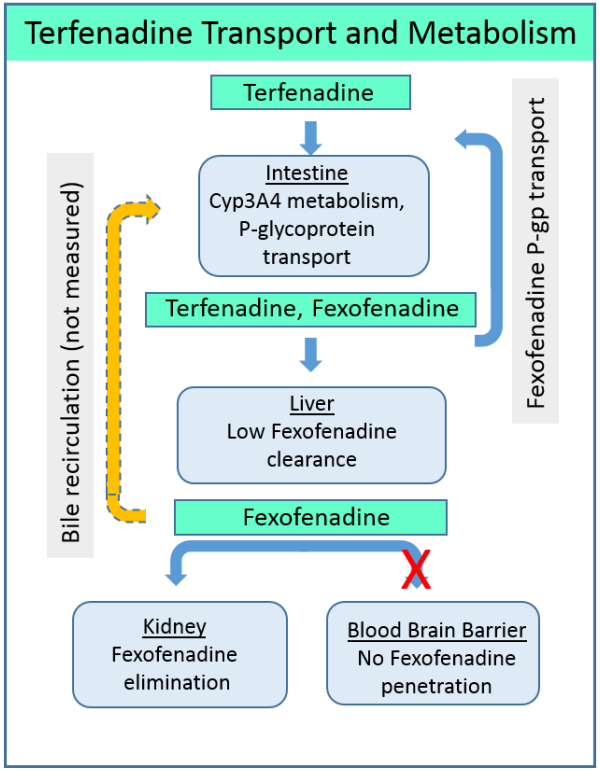


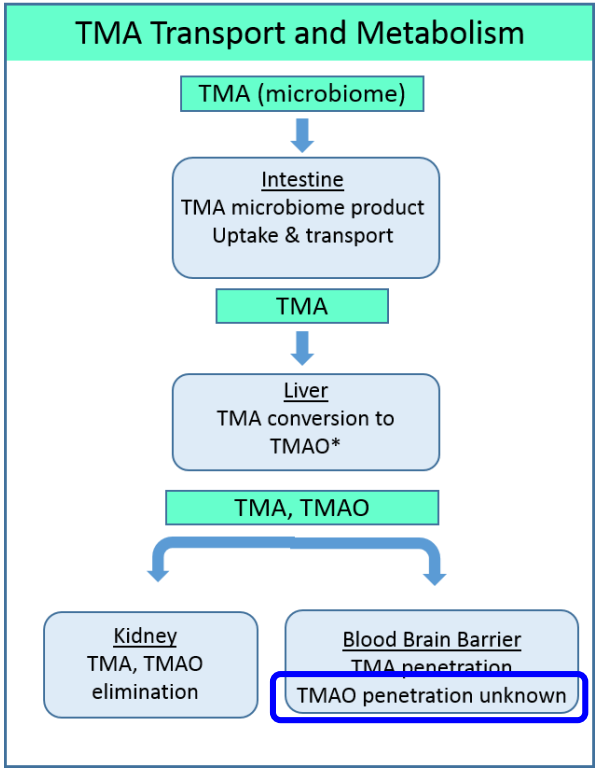
- JHU/Baylor
- U Pittsburgh
- U Washington
- Vanderbilt

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!

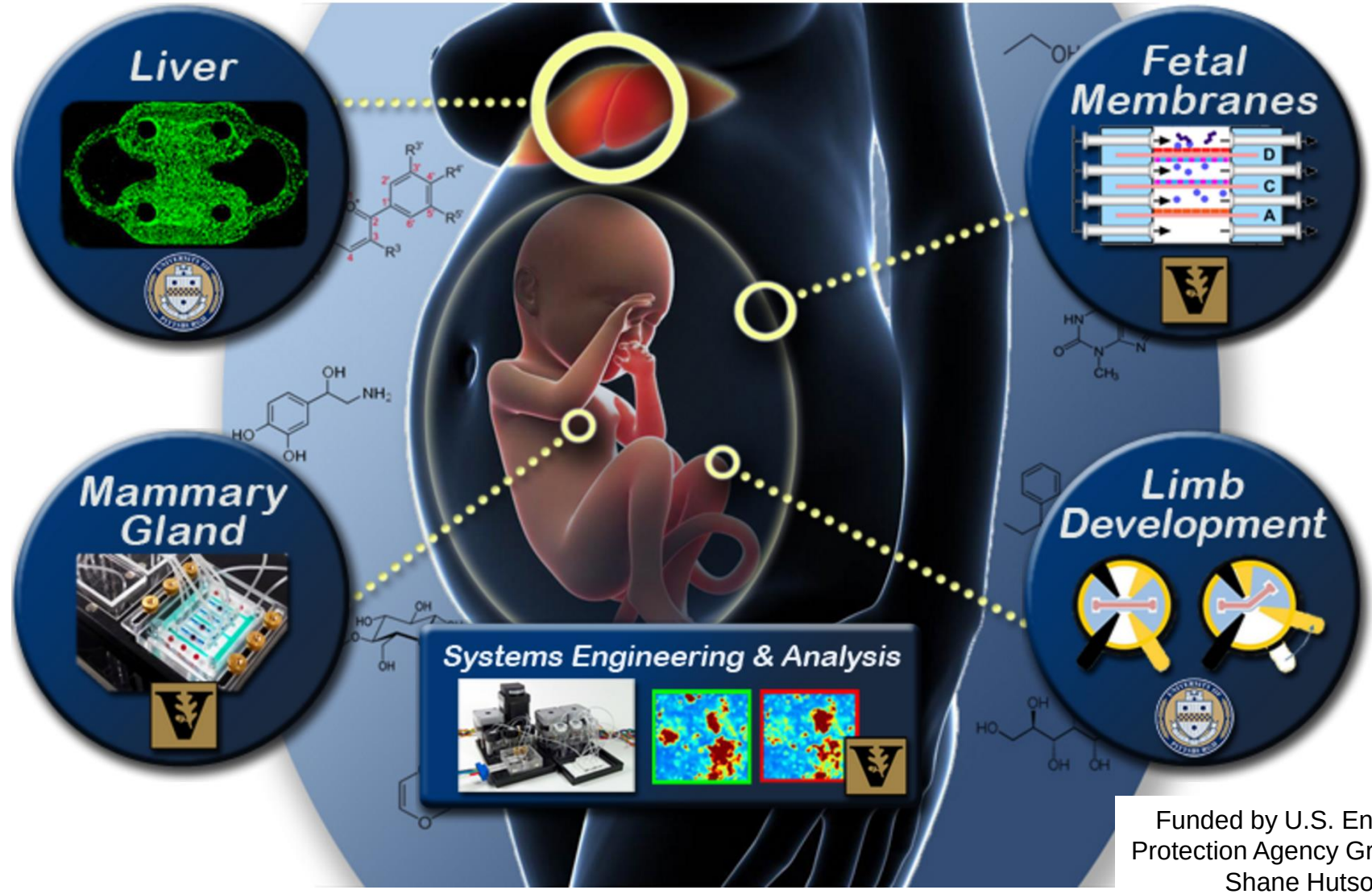
Test Agent/*Metabolites*	Clinical MPS Model	Intestine	Liver	Kidney	BBB
TMA *TMAO*	**Clinical**	Uptake & Transport	**TMA** → *TMAO* < 5% **TMA** Clearance	> 95% *TMAO* Excreted	*TMAO* Penetration: Unknown
	MPS	Uptake & Transport	**TMA** → *TMAO* < 1% TMA Clearance	~46% *TMAO* Excreted	26% *TMAO* Penetration
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

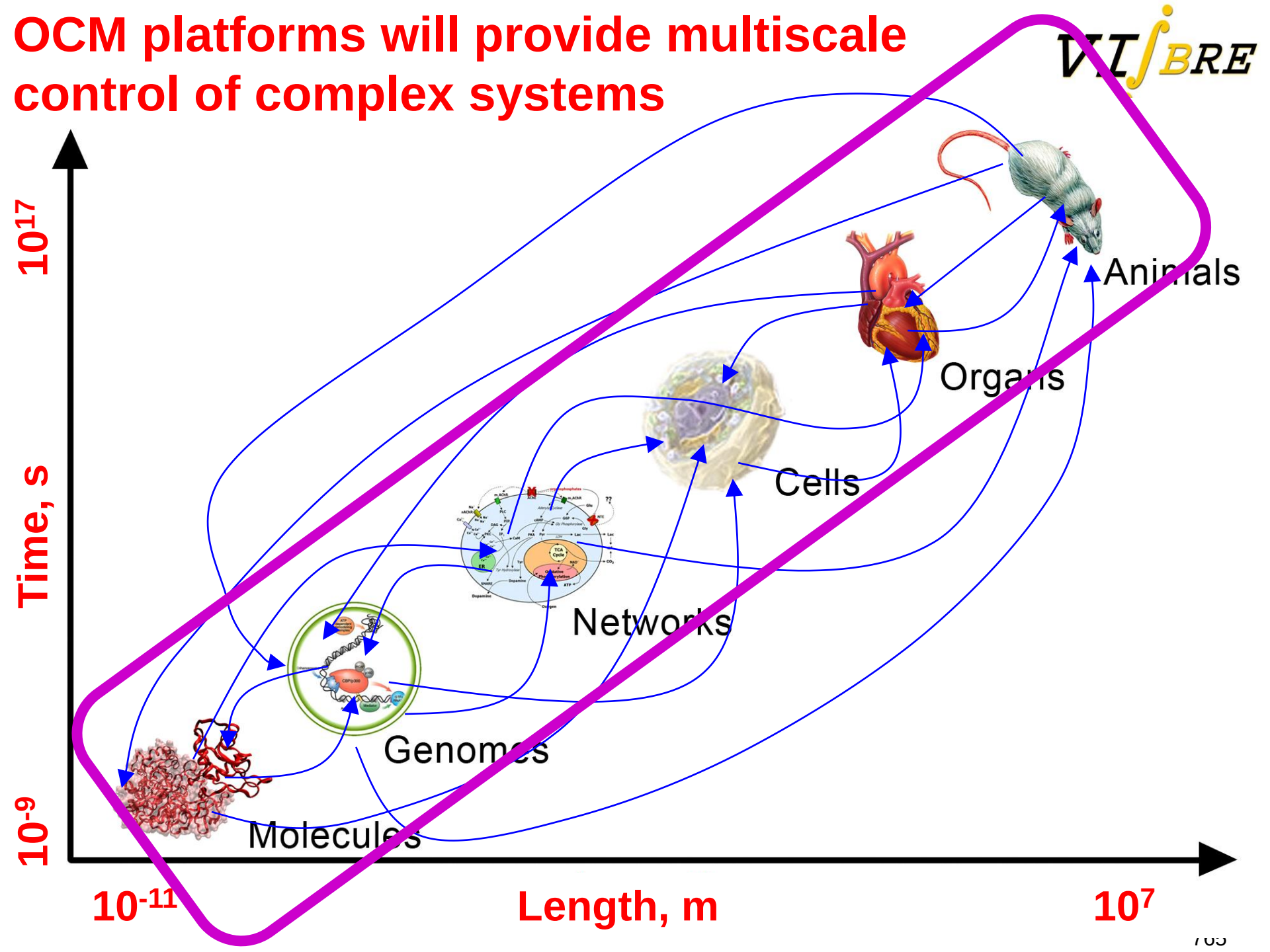
Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology



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

How do you monitor organ health, performance, and response to drugs and toxins?

OCM platforms will provide multiscale control of complex systems

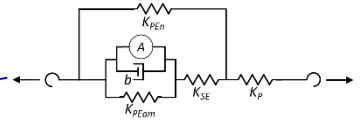


VIIBRE Analytics for Organs on Chips

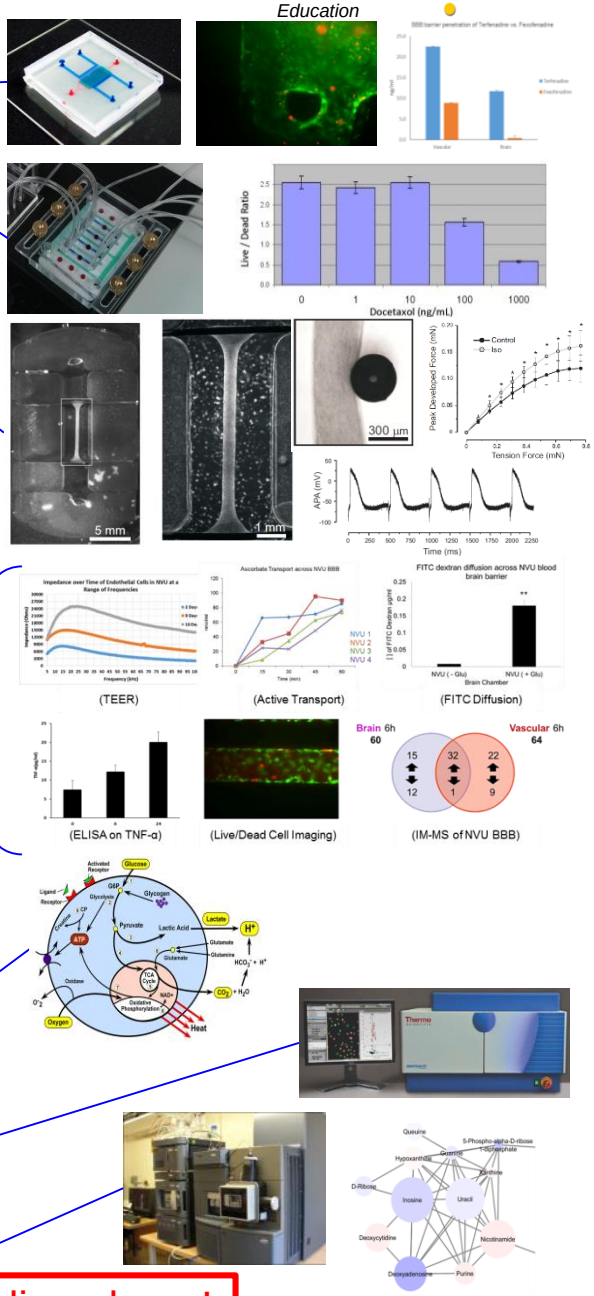
Vanderbilt Institute for Integrative Biosystems Research and Education

VIIBRE

- Organs-on-Chips
  - Brain (in regular production and testing drugs)
  - Mammary Gland (demonstrated, published, moving towards production)
  - Cardiac Muscle 3D construct (demonstrated, published, being parallelized and automated)
  - Fetal Membrane (under development by Osteen @ VU)
  - Gut (In prep with Donowitz @ JHU and Estes @ Baylor; Rericha and Lau @ VU)
  - Developmental bone-joint (In prep with Tuan @ U. Pitt)
- Real-Time Evaluations

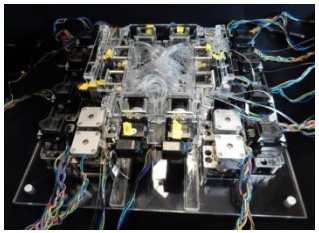


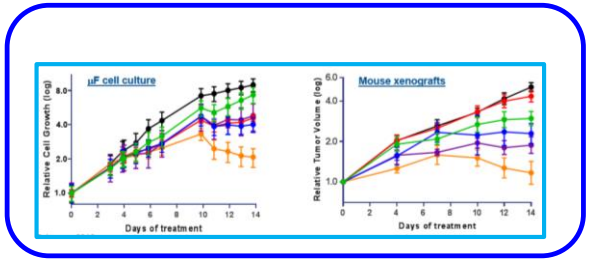
  - Myocardial elastomechanics
  - TEER – transendothelial electrical resistance (real-time)
  - Barrier active transport (off-line microplate or LC-MS)
  - Barrier permeability (FITC dextran diffusion)
  - Cytokines (ELISA)
  - Fluorescence imaging
    - Cell survival – live/dead assay
    - Mitochondrial membrane potential
    - Transmembrane potential
  - Metabolic activity (real-time glucose, lactate, pH, oxygen)
  - Cell morphology
  - Confocal 3D reconstruction
  - Secretome proteomics and metabolomics (UPLC-MS)



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

MultiWell MicroFormulators

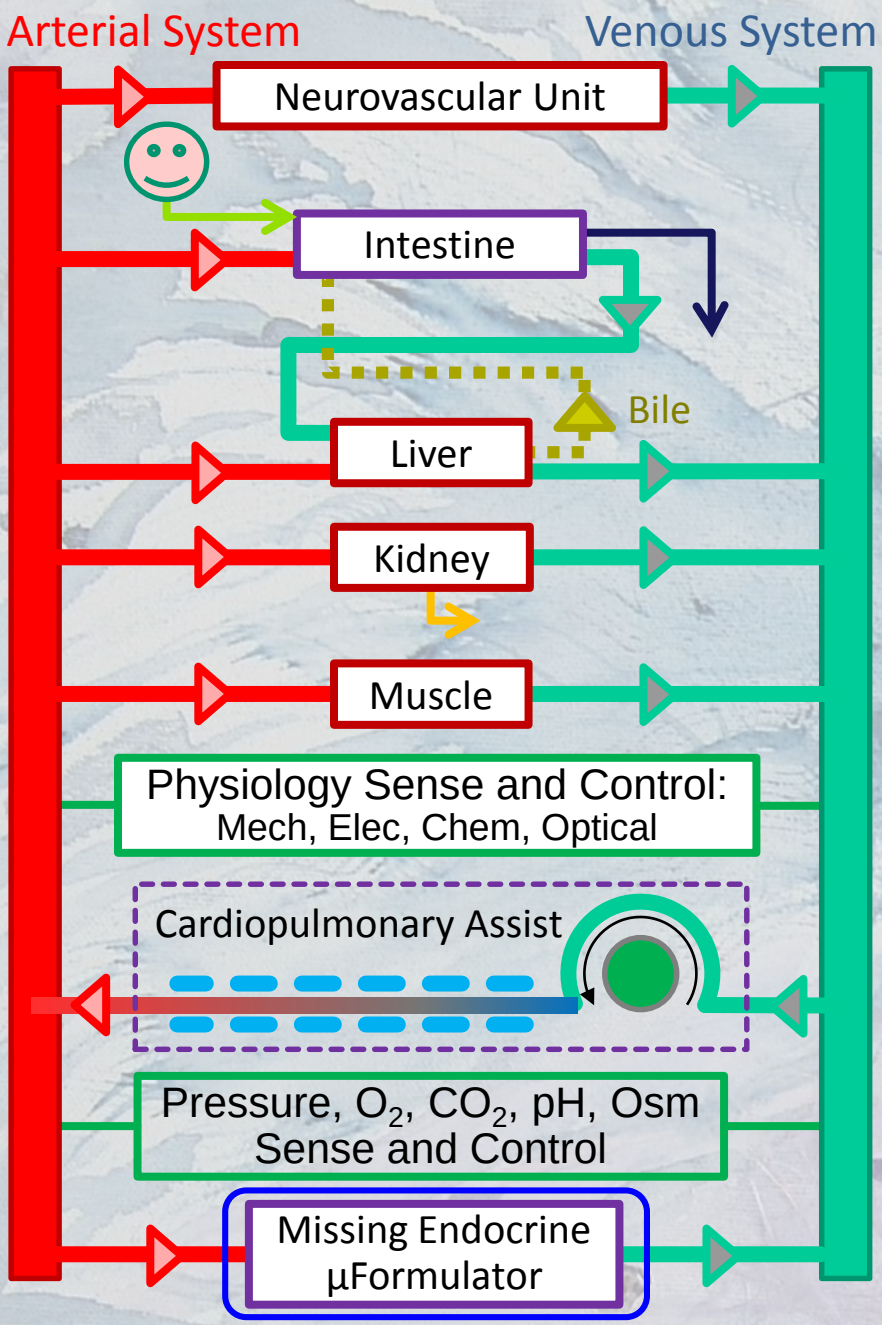




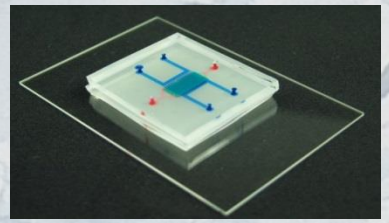
The "Missing Organ" Problem

- 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 secretory organs can be replaced with a ***Missing Organ Microformulator***.
  - Hormones, hormones, hormones

NIH-NCATS MPS Integration

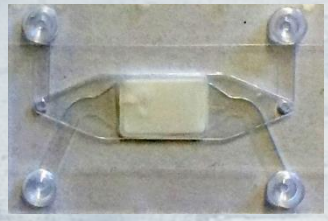


NVU



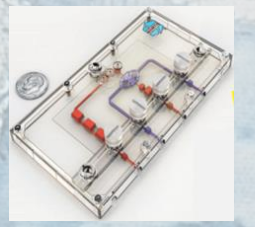
Vanderbilt

Intestine



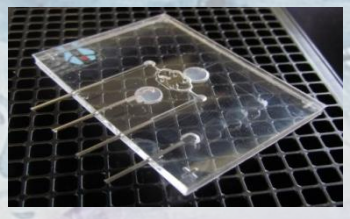
Hopkins, Baylor

Liver



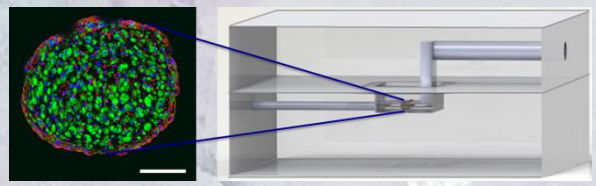
Pittsburgh

Kidney



Washington

Muscle

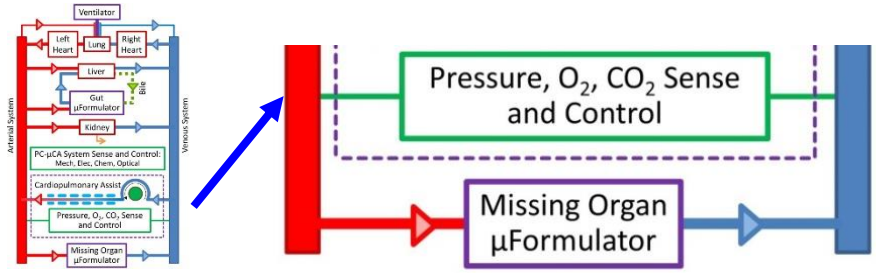


Duke

ME-µF

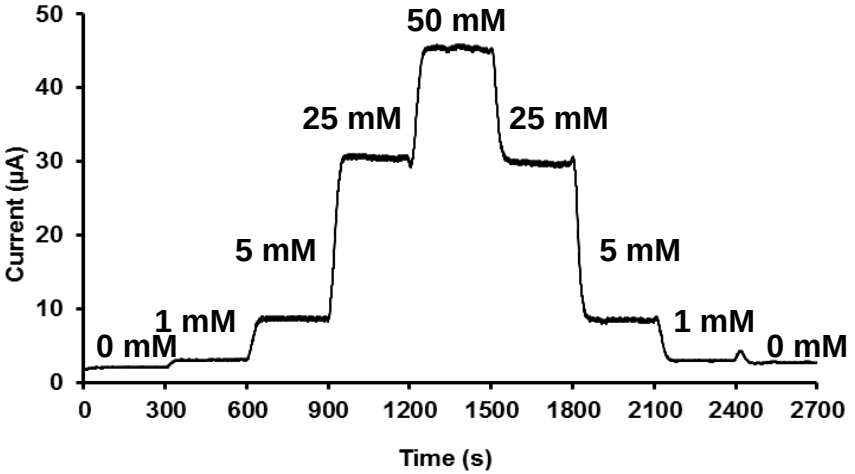
Vanderbilt

Missing Organ MicroFormulator (µF)

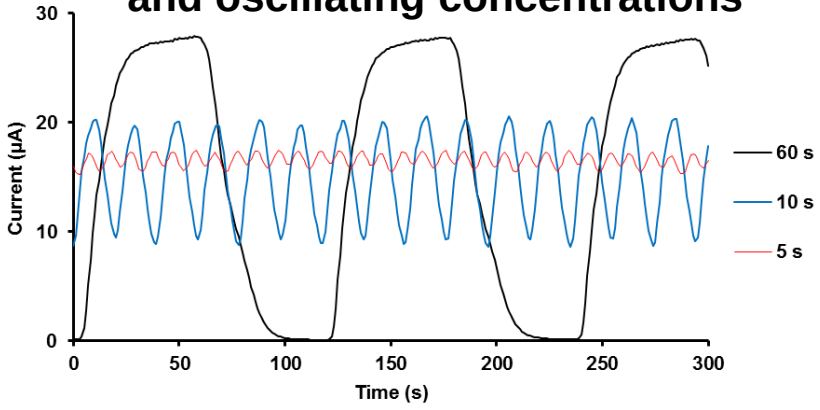


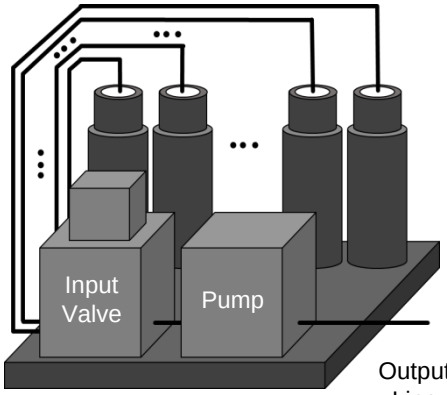
- Cliffel Group:
  - Testing performance with e-chem
  - Reduction of ferricyanide at -0.16V *vs.* Ag quasi-reference.
- Low leakage between ports
- Programming allows rapid switching between ports for dilution, gradients, and calibration of electrochemical sensors

Delivery of desired concentration

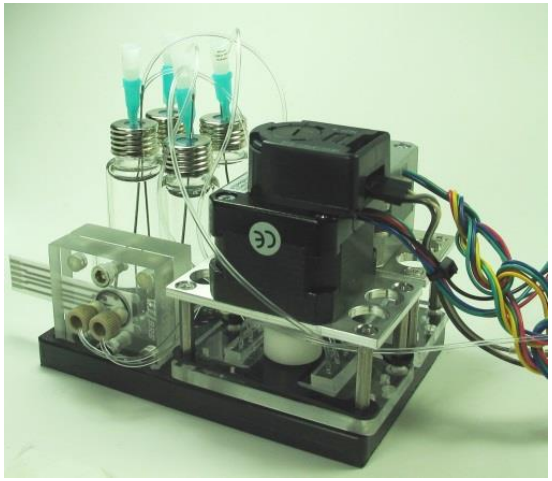


Time-division multiplexing and oscillating concentrations





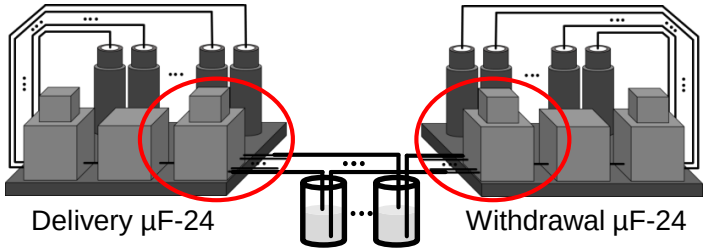
United States Patent, 9,618,129 B2

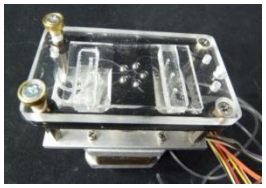


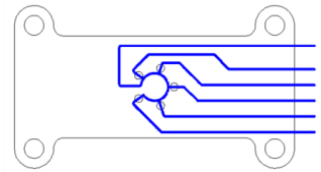
A normally closed rotary planar valve for microfluidic applications, F. E. Block III, J.R. McKenzie, P. C. Samson, D. A. Markov, and J. P. Wikswo, *In Preparation*.

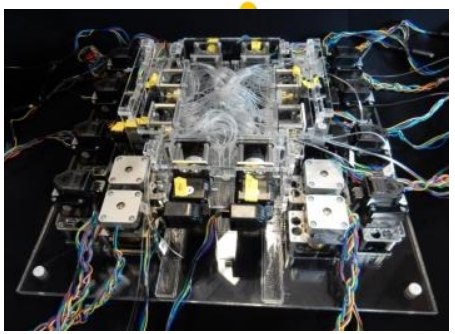
Multi-MicroFormulators for testing the effects of drug timing

- Matt Wagoner – “Your µF is great, but I need 96 channels.”

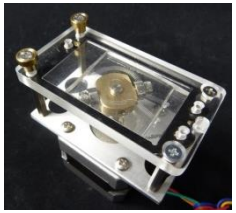


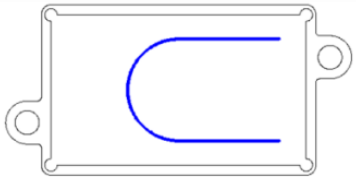




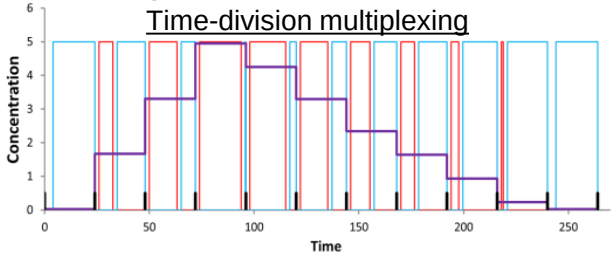


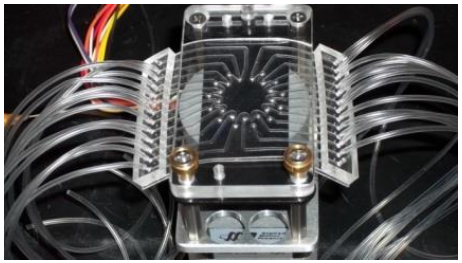
- What can you learn by lengthening or shortening the effective PK profile of a drug *in vitro*?

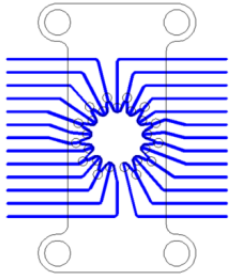


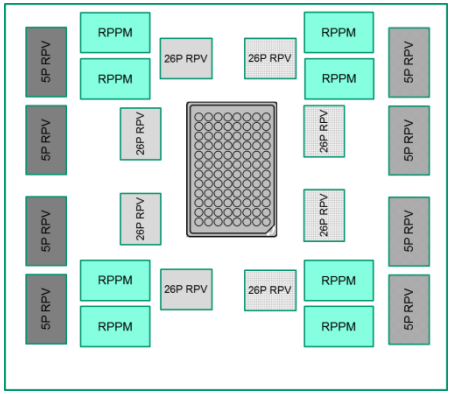


Time-division multiplexing





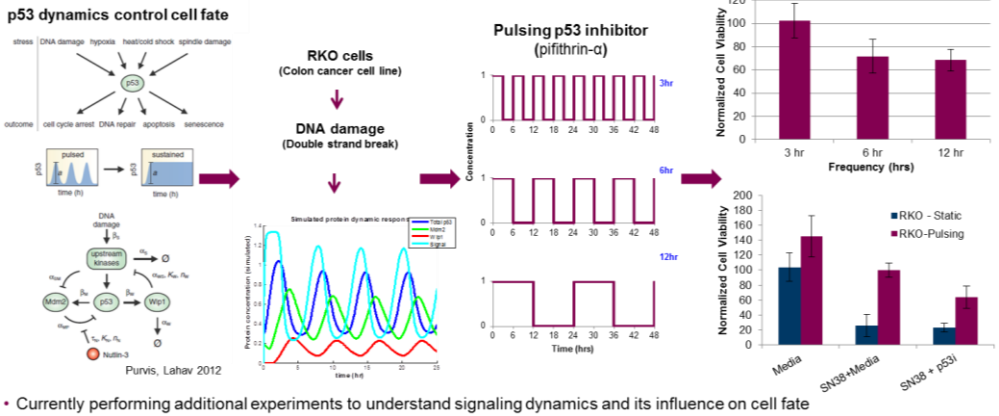




- What is the optimal timing for repeated or multi-drug dosing?

Funded by AstraZeneca.
In operation at AZ - Waltham, MA since January, 2016

Perturbing Cellular Pathways



2016-2018

- Currently performing additional experiments to understand signaling dynamics and its influence on cell fate

Courtesy of Aditya Kolli, Harish Shankaran, Matthew Wagoner, and Jay Mettetal, AstraZeneca

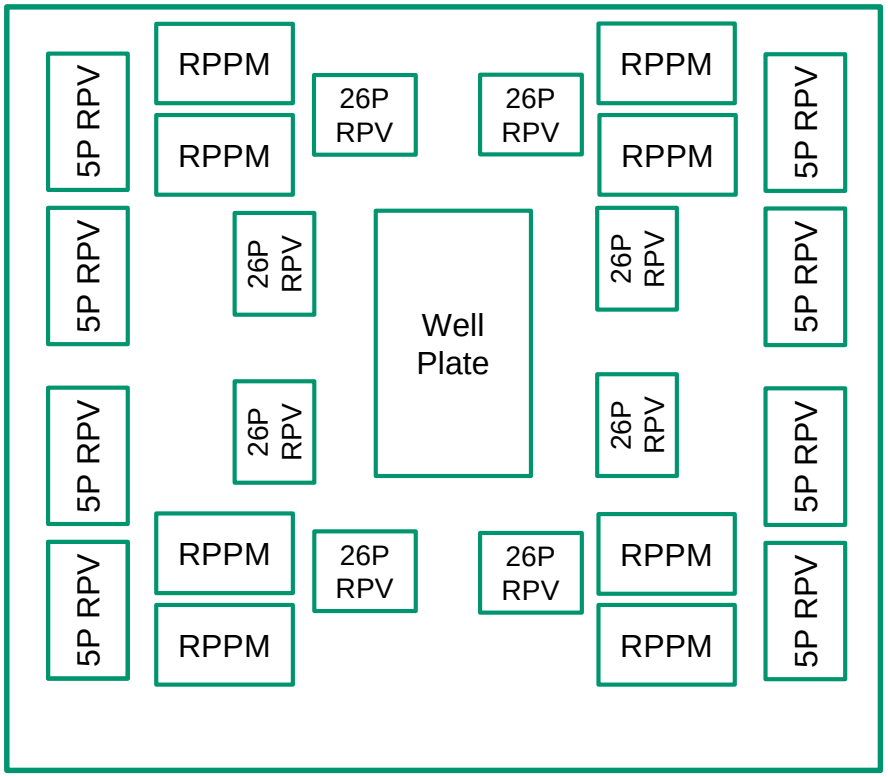
Funded by AstraZeneca and NIH-NCATS/CDFRC

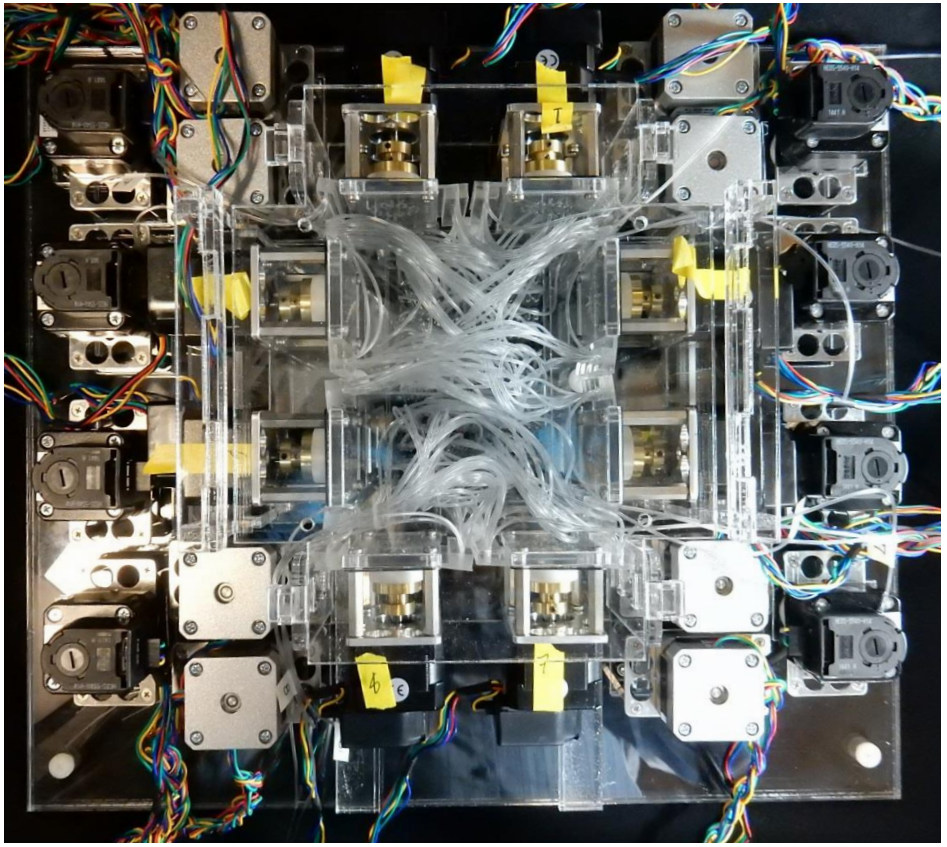
What can you do with a µF-96?

- 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
    - Hanging drop
    - Transwells
- 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
- Create circadian rhythms on a well plate or Petri dish
  - Hormones
  - Nutrients
  - Drugs
  - Substances of abuse

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.





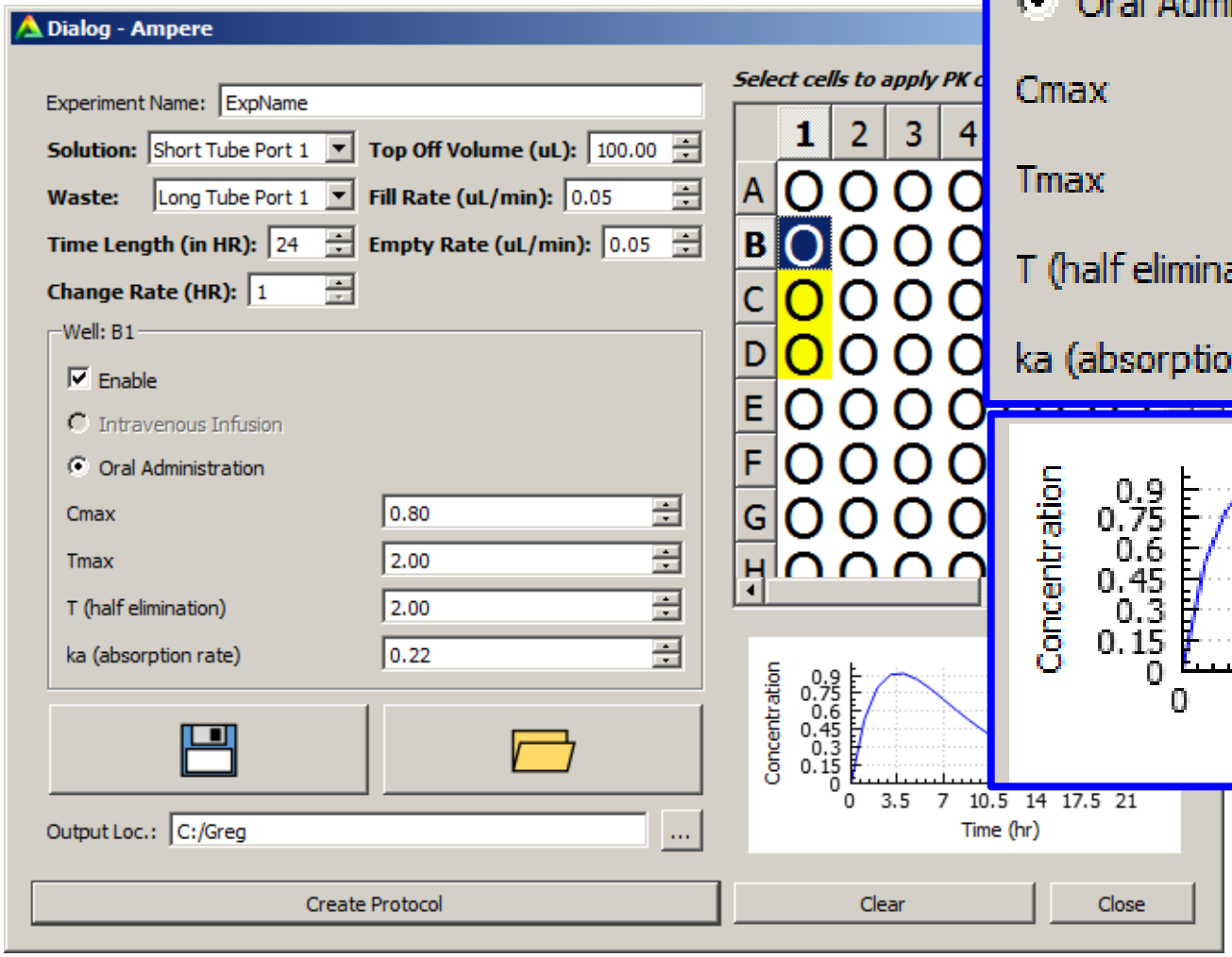


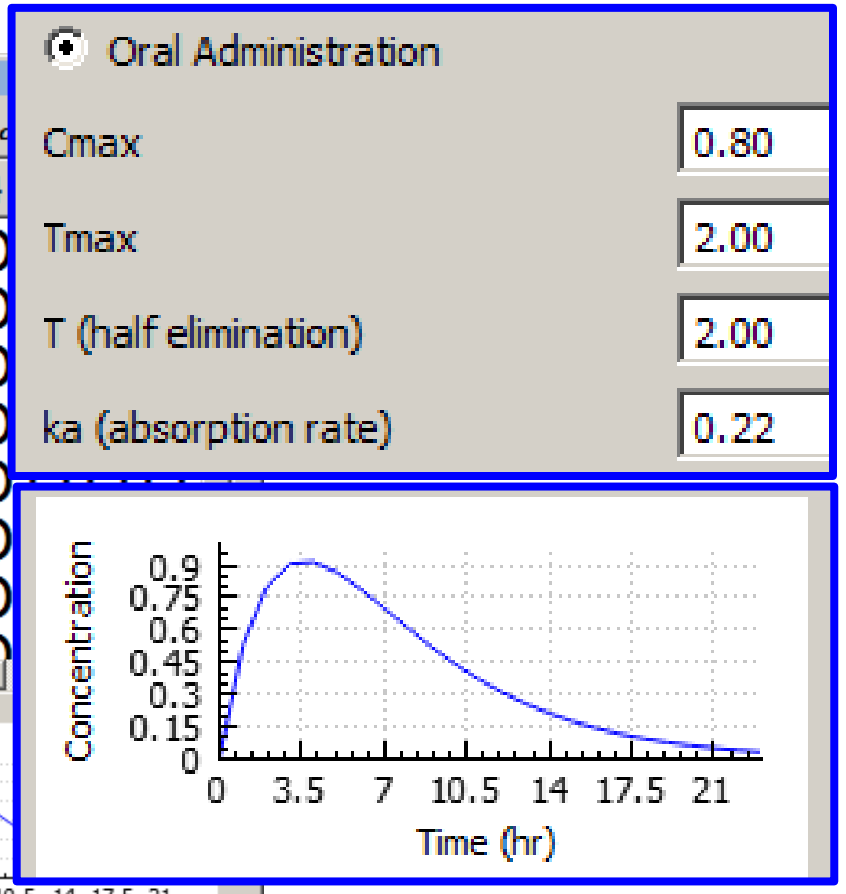



Can individually formulate, deliver, and remove custom media cocktails to each well of a 96-well plate to simulate PK profiles.

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.





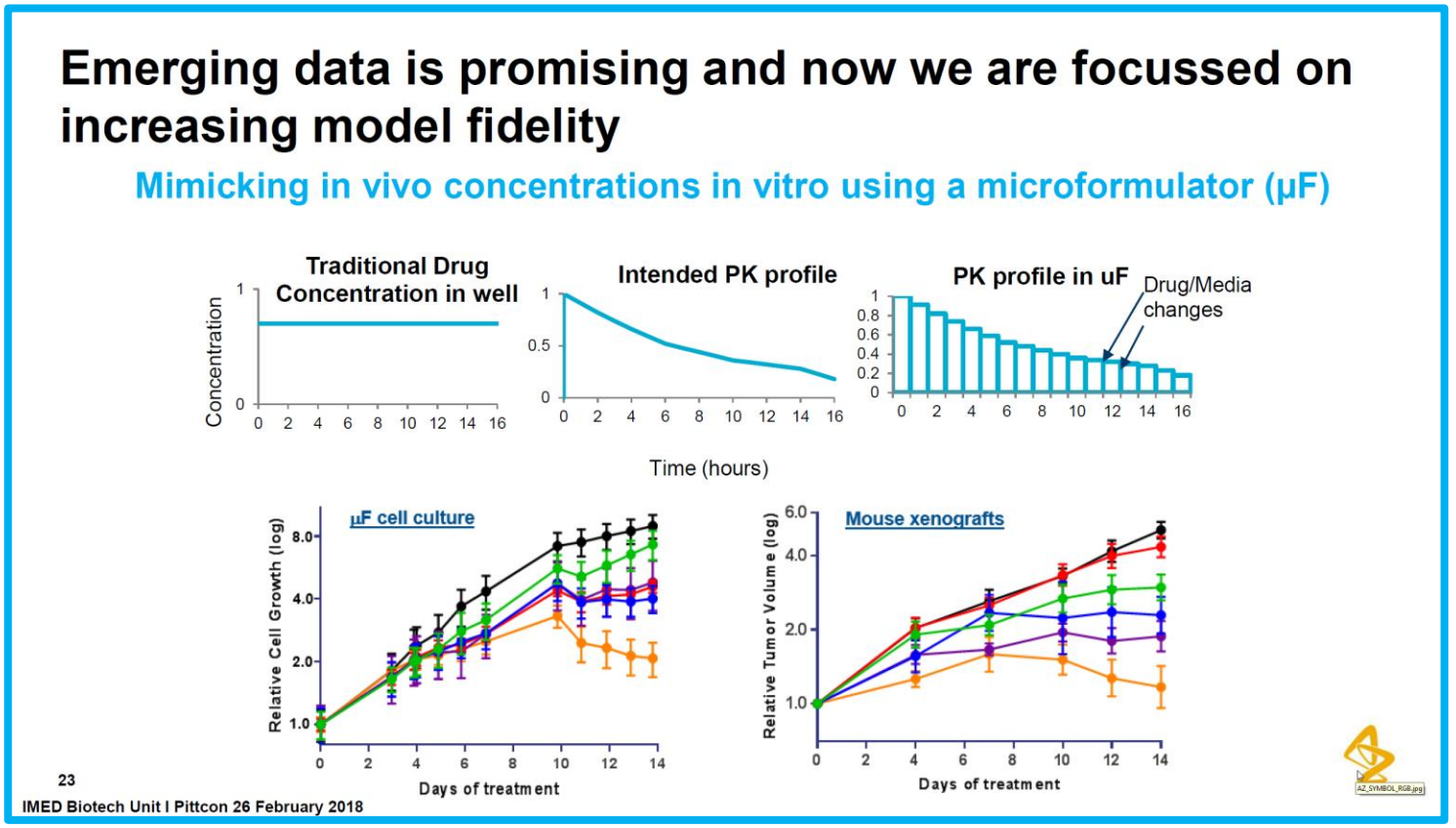
It is straightforward to adjust PK profiles *in vitro*.

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

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

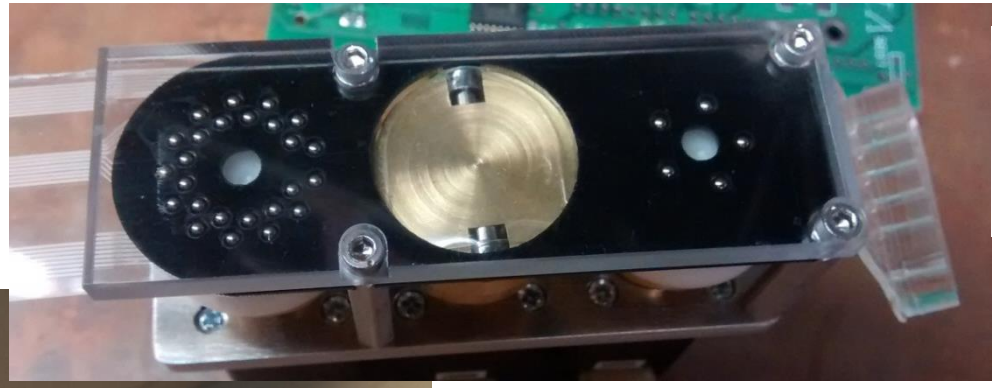
24 wells
18 hours per day for 3 weeks
1.9 million error free instructions

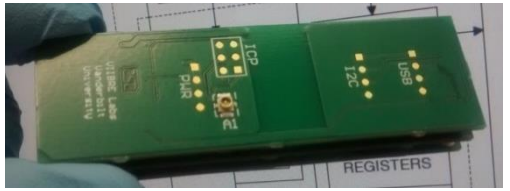


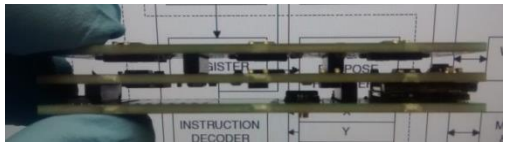
Courtesy of Lorna Ewart, AstraZeneca

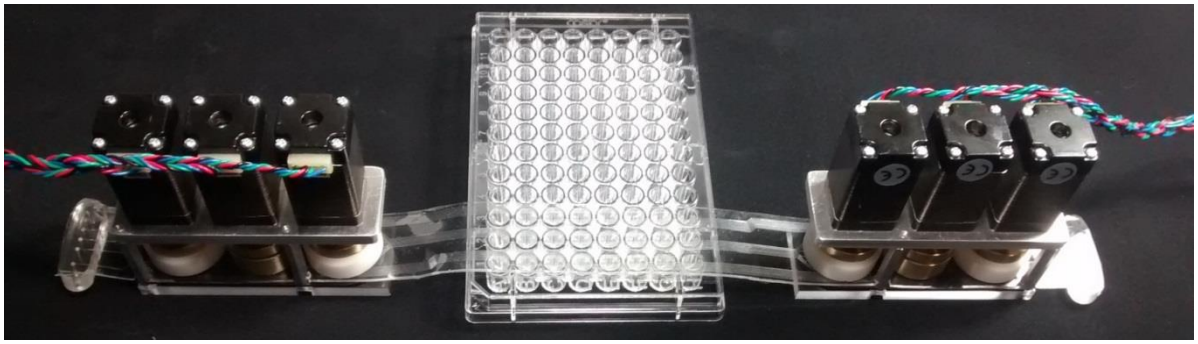
96-Channel MicroFormulator (µF-96), v2.0

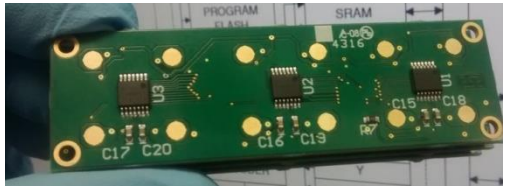
- For each well, formulate a custom media/drug mixture in real time.
- Change 10% of the fluid in each well 40x/day.









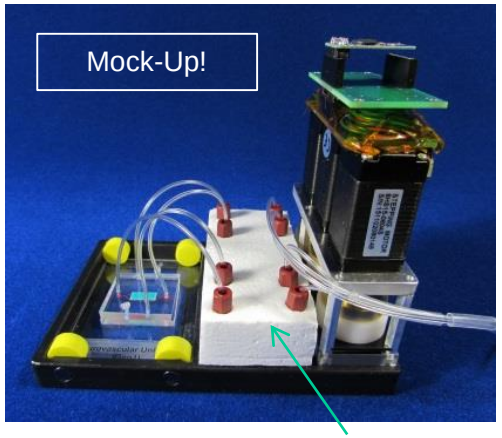


Funded in part by AstraZeneca and an NIH/NCATS SBIR to CFD Research Corporation. Licensed to CN Bio Innovations

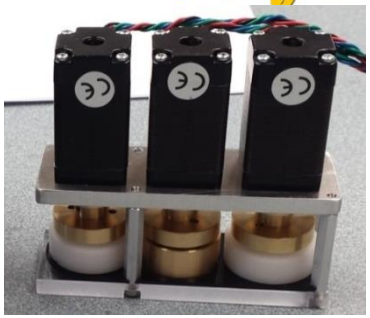
Smart Motors

We are developing the next-generation, universal, NEMA-8, three-motor module:

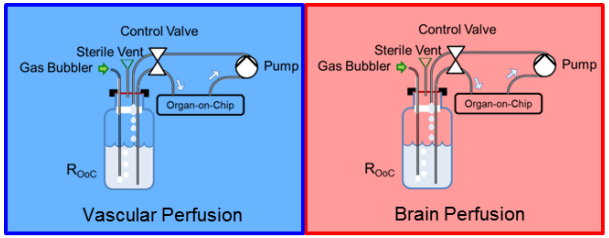
- On-board dedicated TEER
- NVU perfusion controller with a pump for each side of the NVU and a perfusion control valve.
- Independent perfusion, oxygenation, and recirculation for each side.
- Valve select single-pass, recirculation, media injection/removal, osmotic balance, and drug addition
- SmartMotor with five microcontrollers
- Wireless communication will be wireless
- 12 V power
- Battery backup

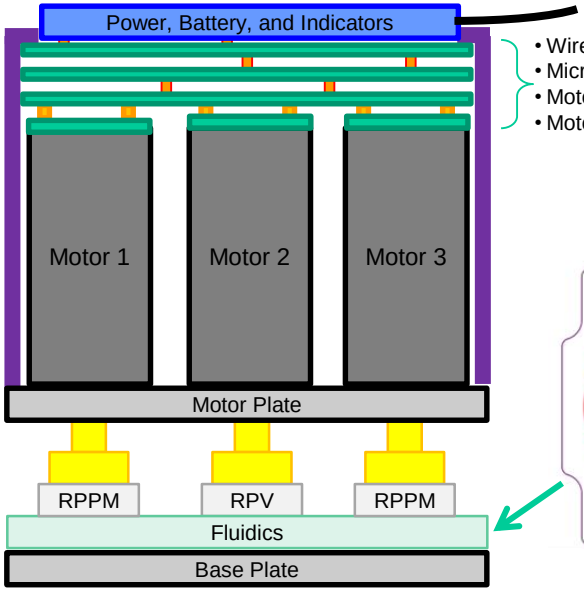


Wireless SmartMotor Controller

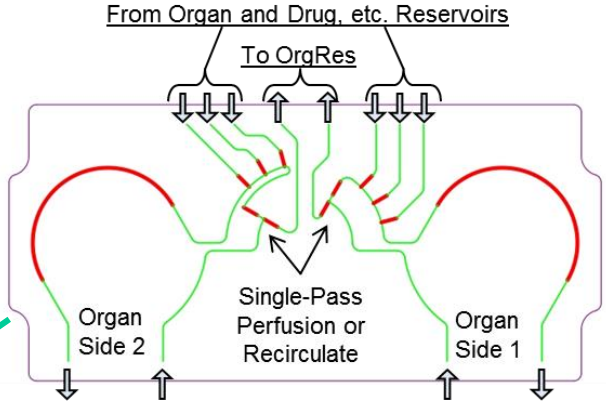


Pump Valve Pump



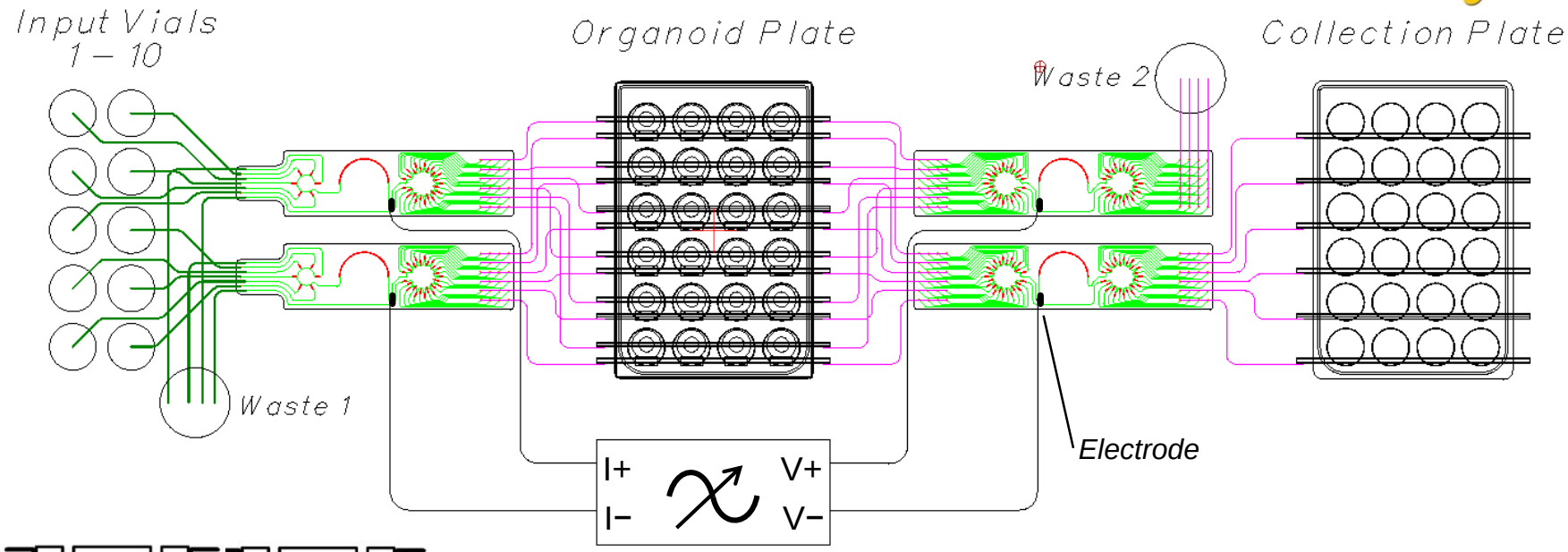


- Wireless communication
- Microcontrollers
- Motor controllers and encoders
- Motor connector



The three NEMA-8 motor unit will simplify production, reduce cost, provide more capabilities, and be better matched to microscopes with large condensers.

Cartridge Combinations for a 24-well TEER µF



- Enables long-term perfusion and sampling of transwell plates
- The fluidic design allows multiplexed TEER measurements
- TEER can provide level verification
- TEER can be measured without removing the plate from the incubator or the lid from the plate
- Fluidics can easily be adapted to any standard plate size up to 96 wells

A team of scientists from Vanderbilt University, led by Professor John Wikswo, have won an R&D 100 Award for their MultiWell MicroFormulator device. The MultiWell MicroFormulator, developed at Vanderbilt and being commercialized by CN Bio Innovations, provides customized real-time formulation, delivery, and removal of cell culture media to each well of a 96-well plate for drug discovery, toxicology research, and personalized medicine. This innovative technology offers a promising alternative to existing fluid-handling systems and greatly reduces the cost and footprint required for long-term cell culture studies. The R&D 100 Awards honor the top 100 most innovative and technologically significant products and advancements each year. Past winners have included the automated teller machine (1973), the liquid crystal display (1980), the Taxol anticancer drug (1993), and HDTV (1998). This is Professor Wikswo's second R&D Award. (VU CTTC announcement)

CN Bio Innovations and the MicroFormulator

What can you do with a µF-96?

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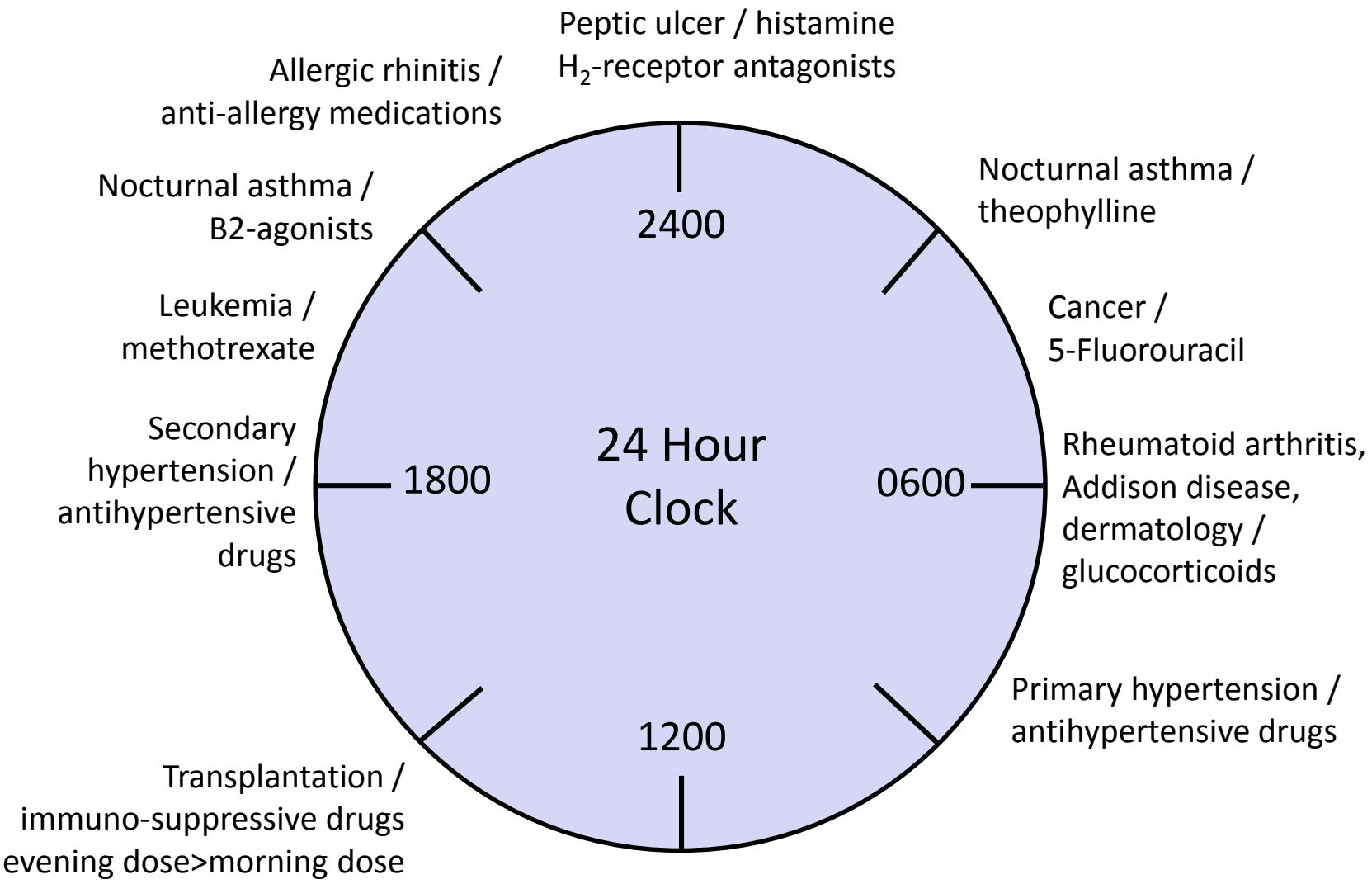
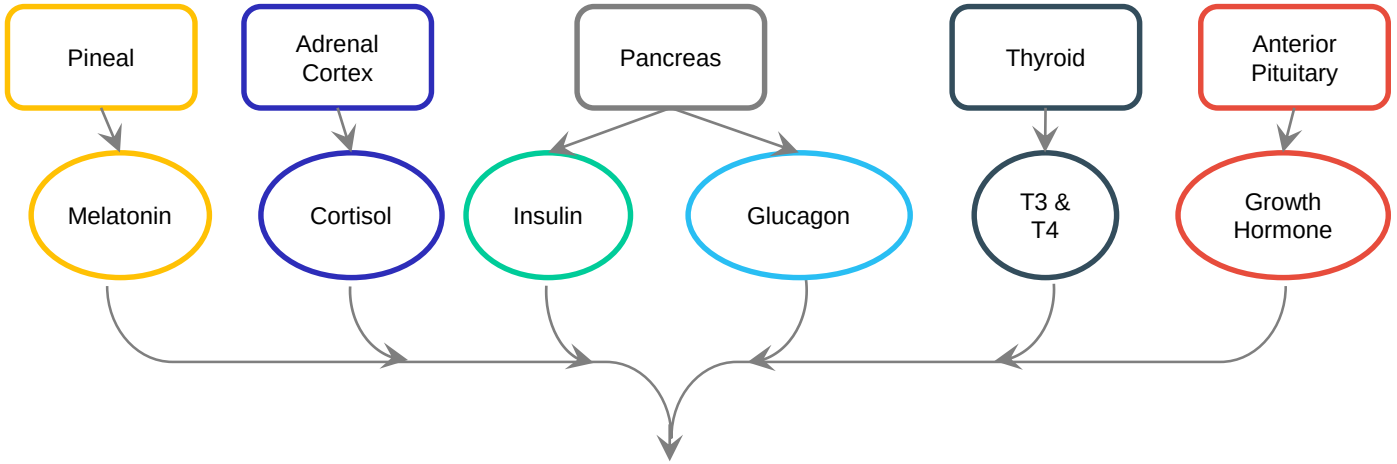
  - Conventional cell culture
  - Massively parallel organs on chips
  - Organoid HTS arrays
    - Hanging drop
    - Transwells
- Create circadian rhythms on a well plate or Petri dish
  - Hormones
  - Nutrients
  - Drugs
  - Substances of abuse
- Explore in a massively parallel manner the multitude of 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

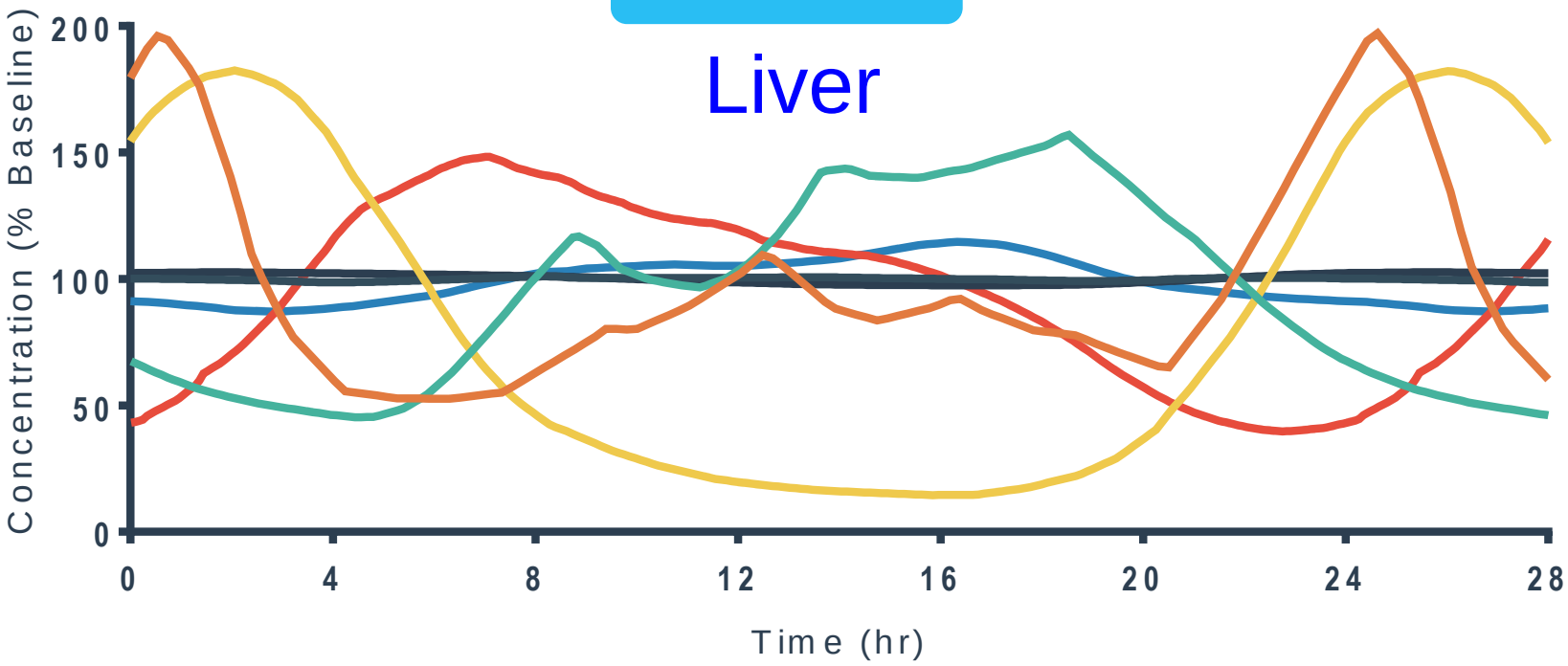
Diseases and Optimal Drug Dosing are Circadian



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,

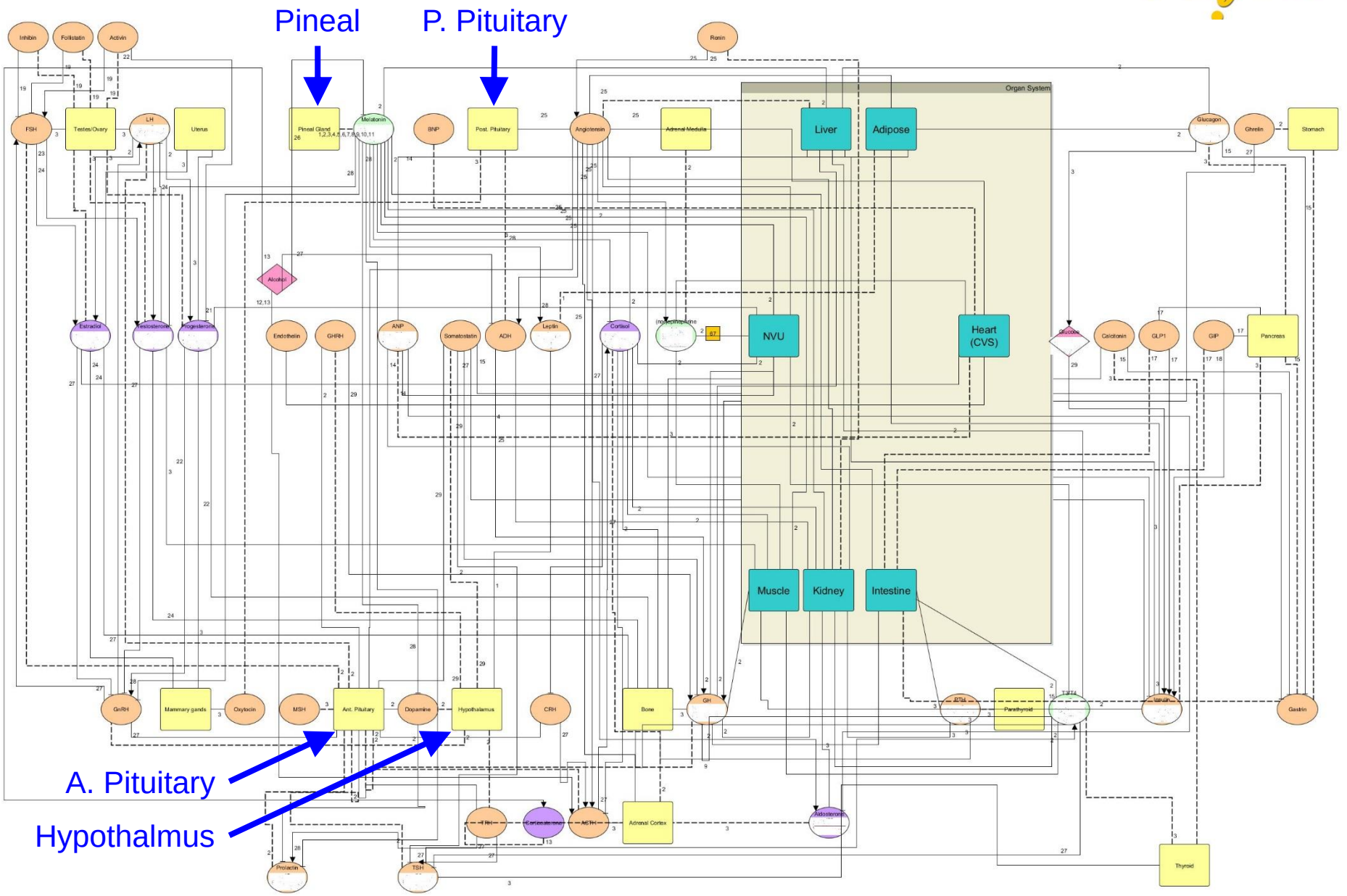
Diurnal Variations of Liver-Regulating Hormones



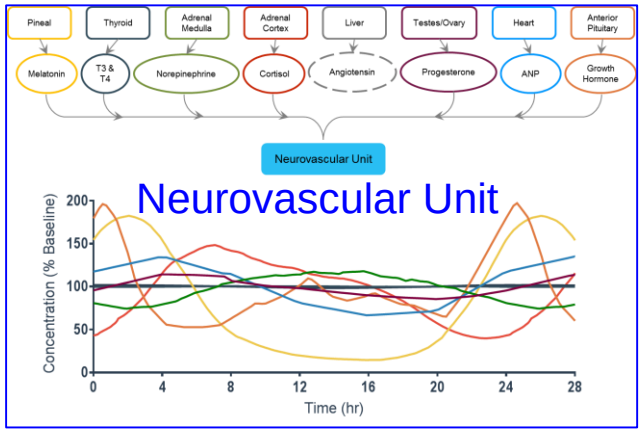


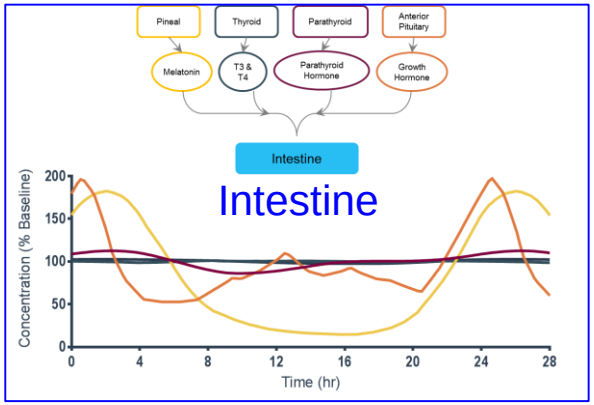
Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

Which endocrine organs / hormones do we need?

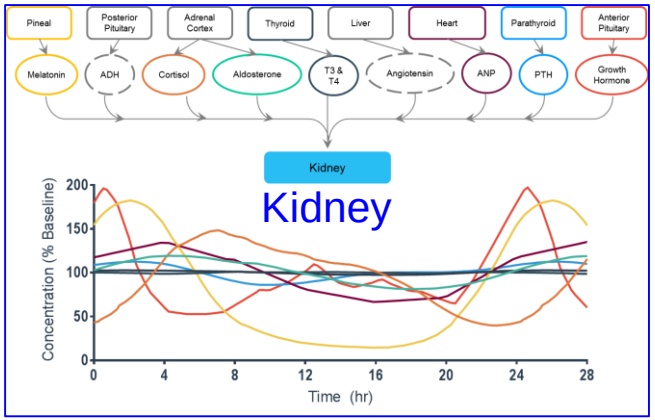


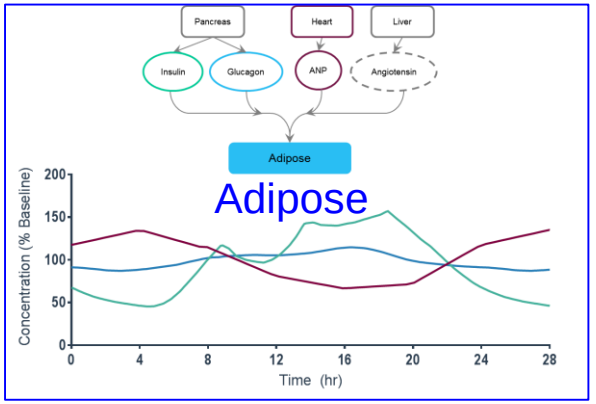
Diurnal Variations of Organ-Regulating Hormones

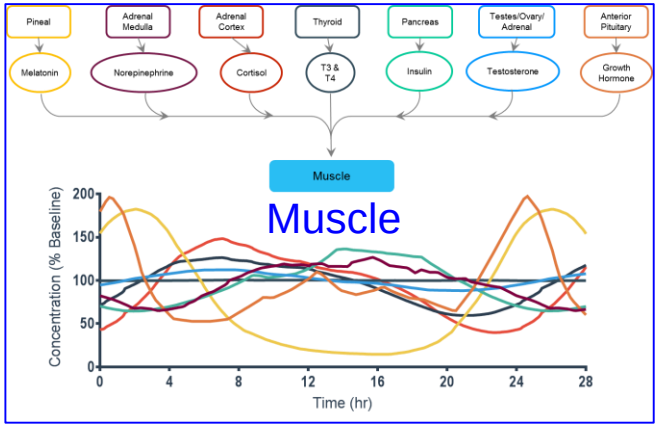


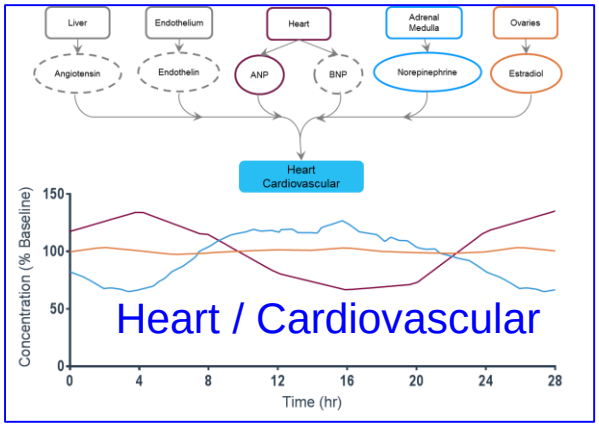


- Neurovascular Unit
- Kidney
- Muscle
- Adipose
- Heart / Cardiovascular

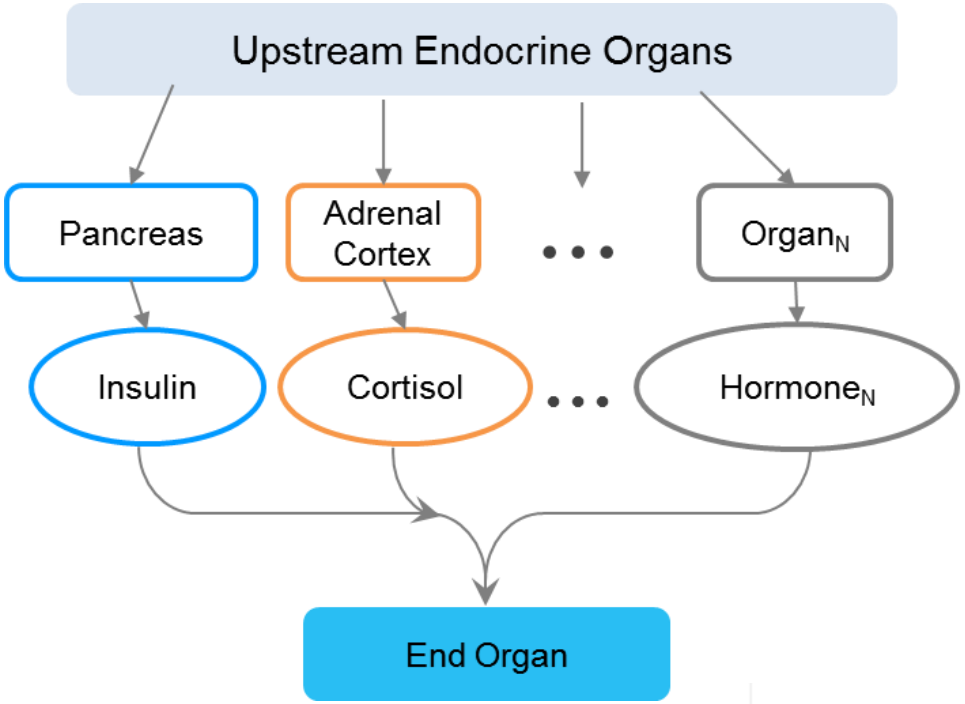


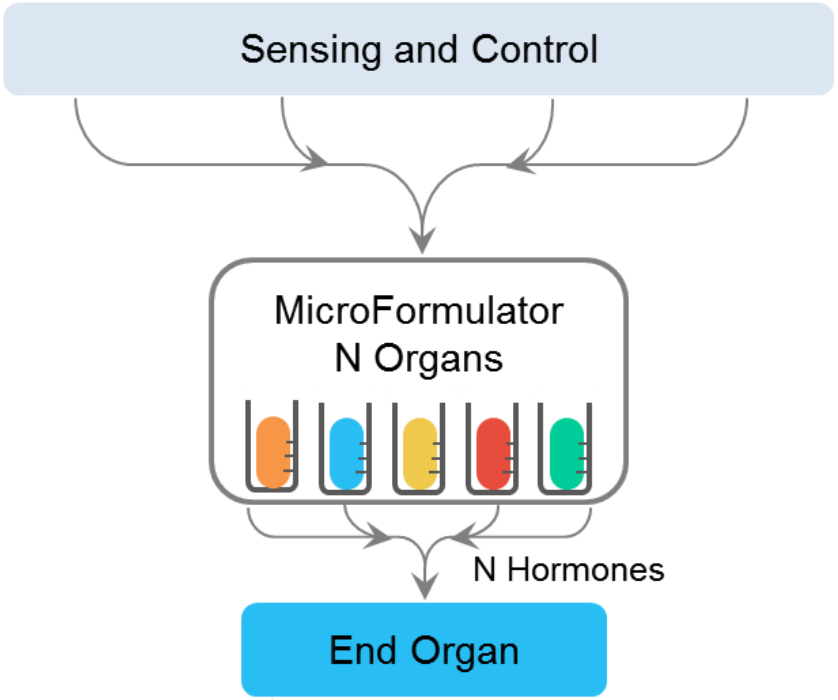


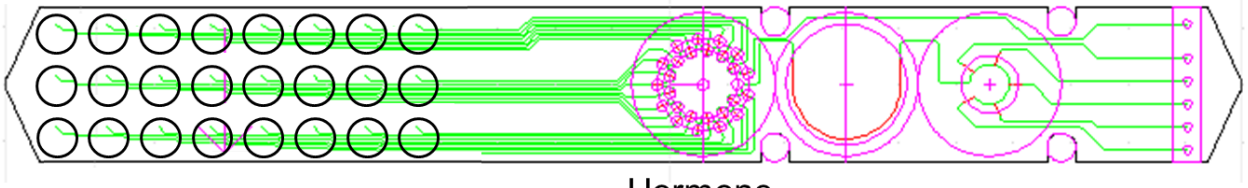


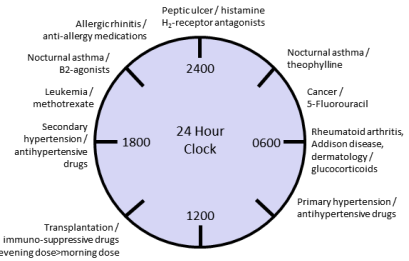


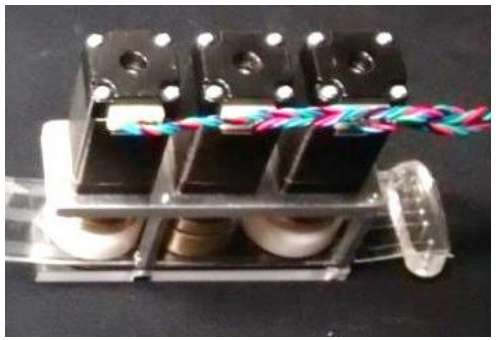
Diurnal Variations of Organ-Regulating Hormones









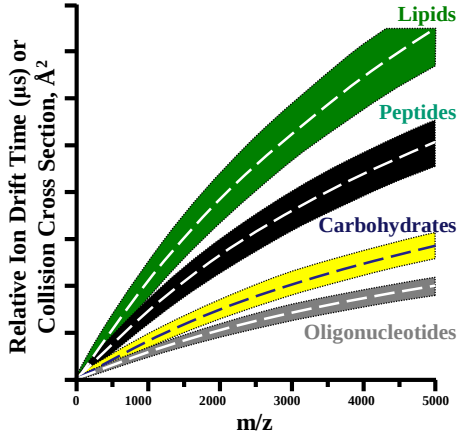


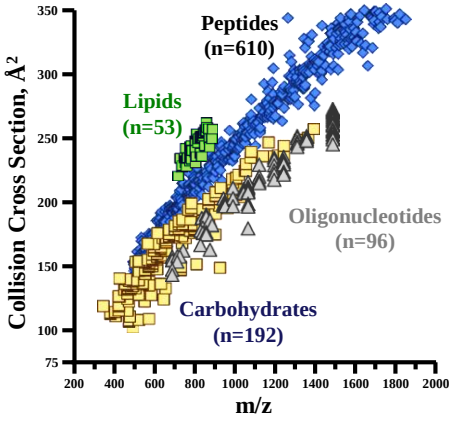
The MicroFormulator can bring diurnal rhythms to biology on plastic.

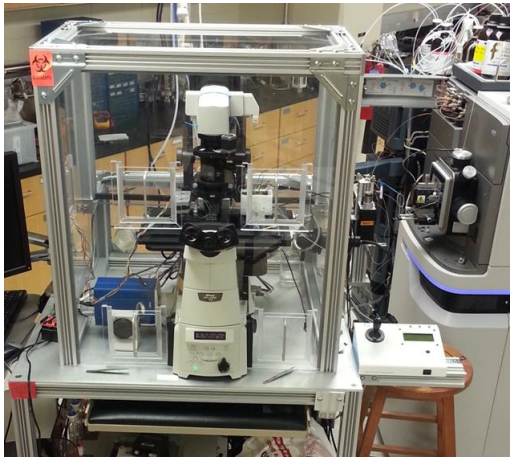
Cyr, Avaldi, and Wikswo, Experimental Biology and Medicine, 2017

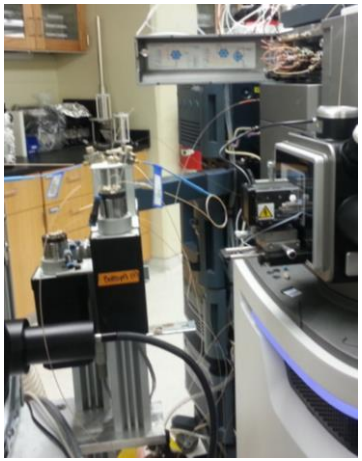

Four themes
1. The complexity of biology
2. MicroPhysiological Systems
3. Multi-Omics
4. Putting it all together
Julia Wikswo

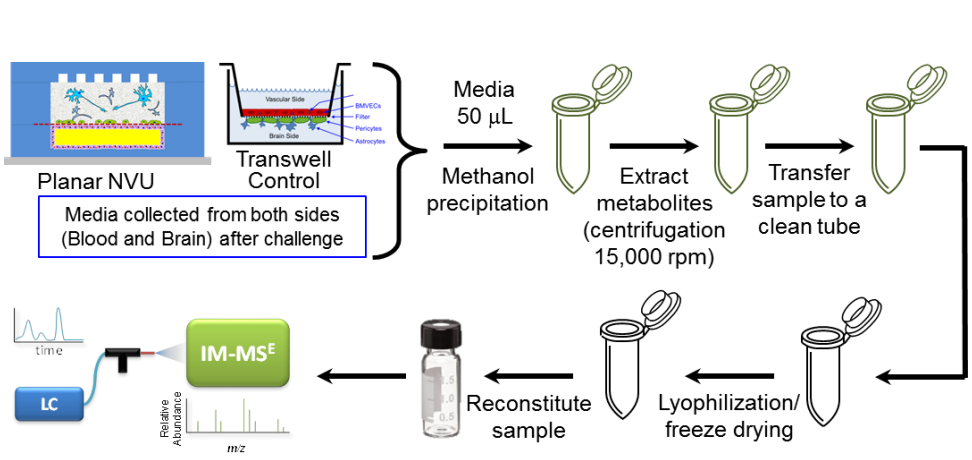
Organs-on-chips are matched to UltraPerformance Liquid Chromatography-Ion Mobility-Mass Spectrometry (UPLC-IM-MS)

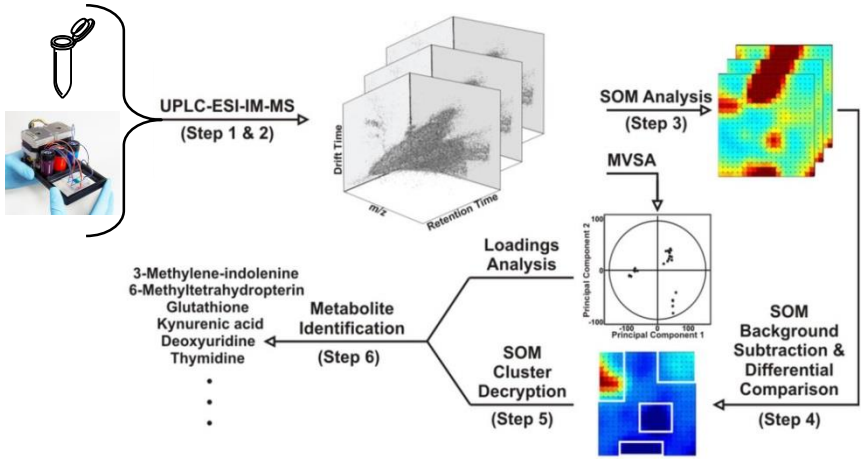












OoC exometabolome can be measured in near-real-time every 10 minutes!

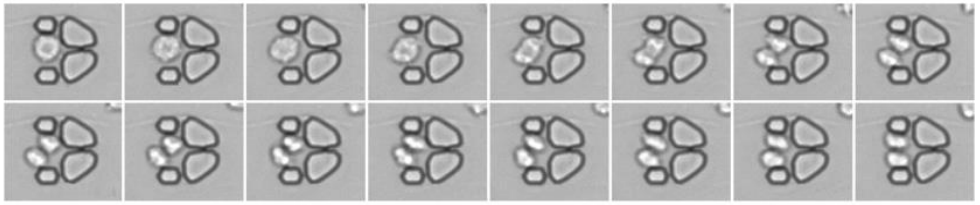
John McLean, Vanderbilt Chemistry

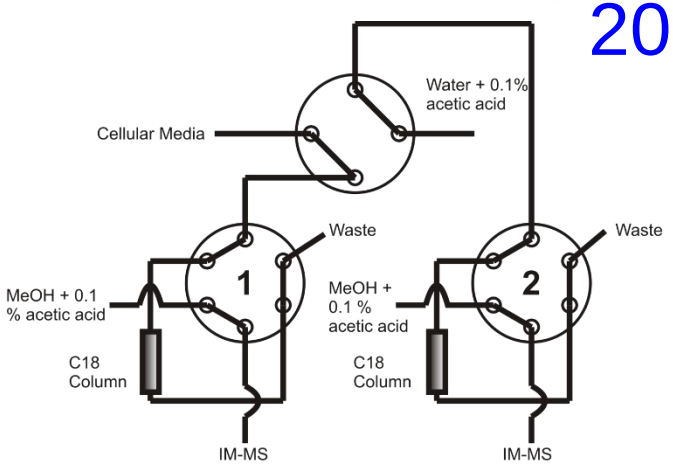
Enders, Marasco, Wikswo, McLean, Anal.Chem, 2012

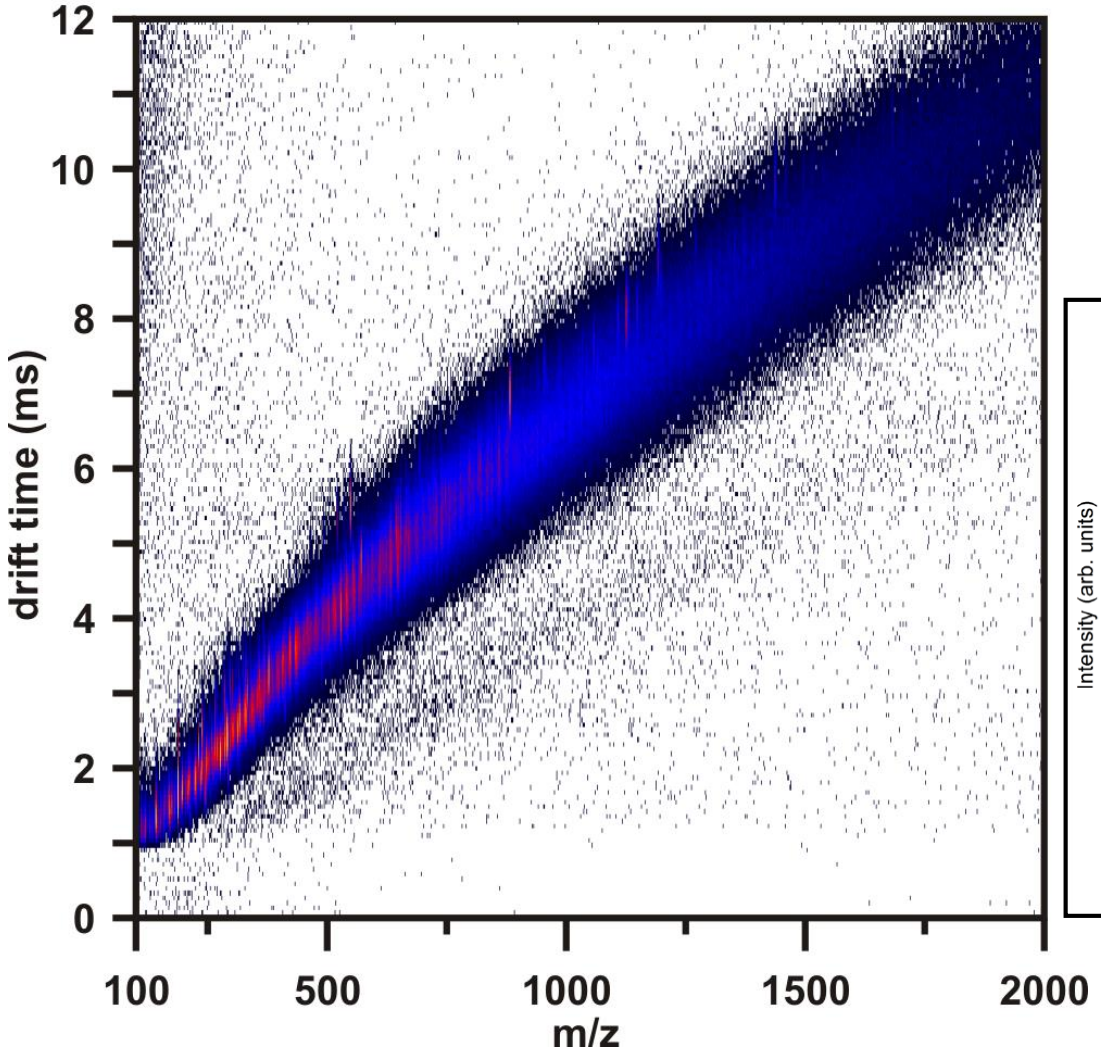
Real-time desalting enables on-line IM-MS spectra from trapped Jurkat cells

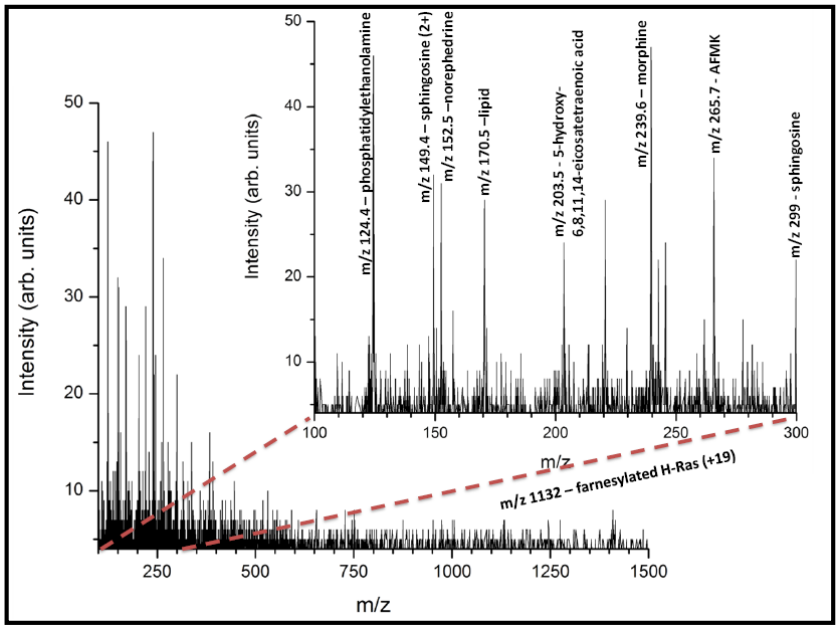
VIBRE

2010



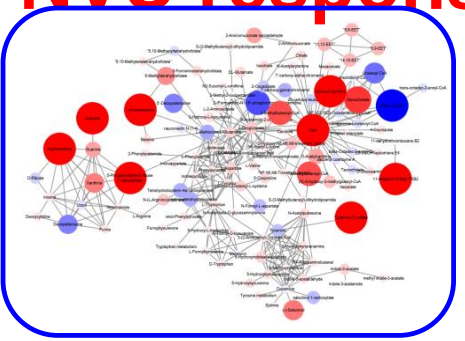


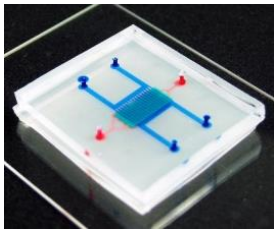


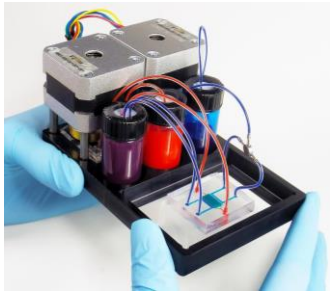


Images from Jeff Enders, Vanderbilt

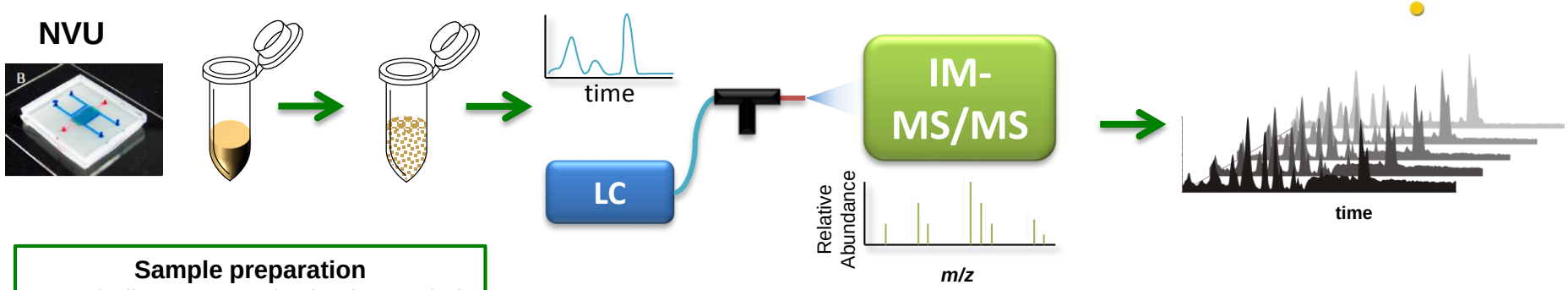
NVU response to inflammatory cytokine







NVU/BBB UPLC-IM-MS workflow



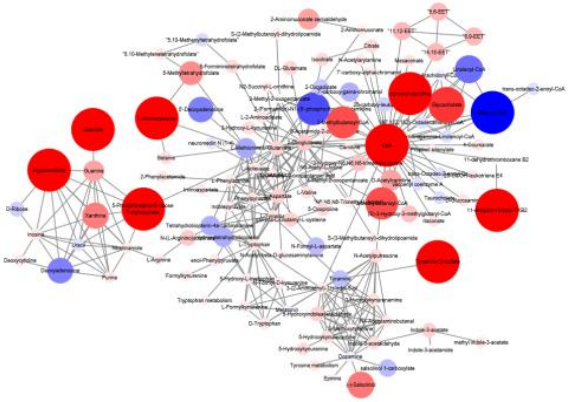
Sample preparation
metabolites extracted using ice cooled methanol:H_2O (80:20),
incubated -80^{0}C overnight,
spun down at 15,000 rpm, 15 min
dried down *in vacuo*

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



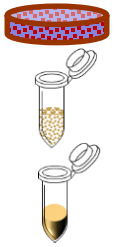
Network & Pathway Module Output

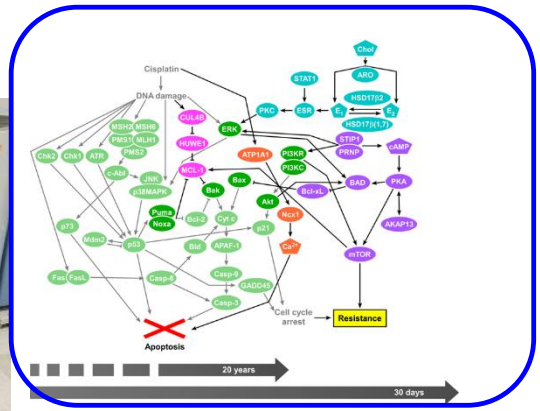
Network and Pathway analysis
Mummichog

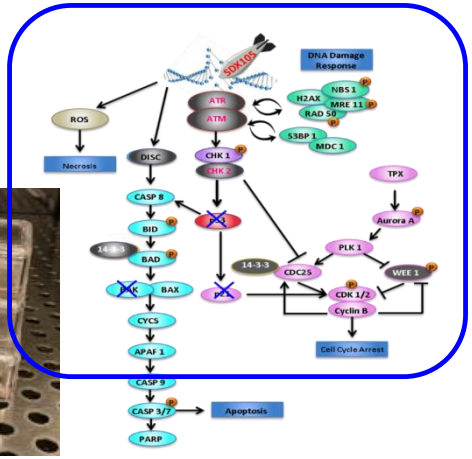
Metabolomic pathway analysis with high mass-accuracy UPLC-IM-MS is accelerating the incorporation of untargeted metabolomics into mechanism of action studies.

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

MultiOmic Mechanism of Action







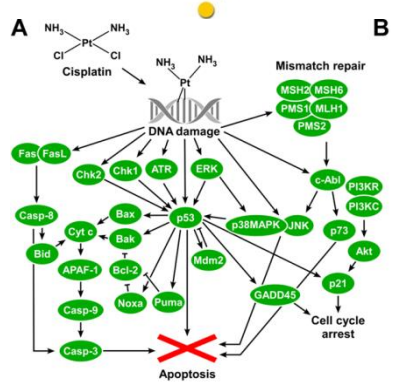
Rapid Threat Assessment (RTA) of MoA

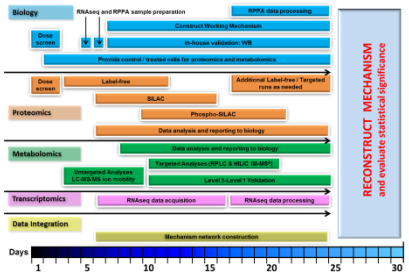
Richard Caprioli, PI, DARPA W911 NF-14-2-0022.

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.

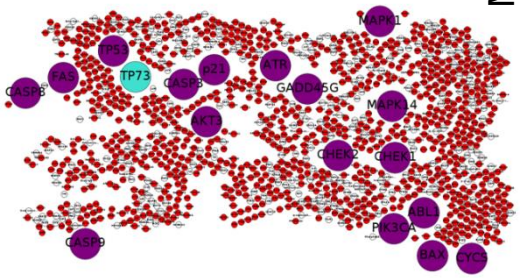
Canonical Cisplatin MoA Siddik 2003 plus literature

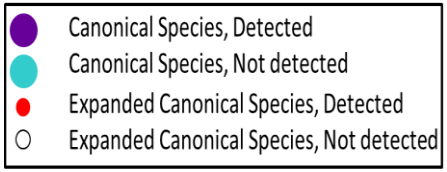




30-day workflow

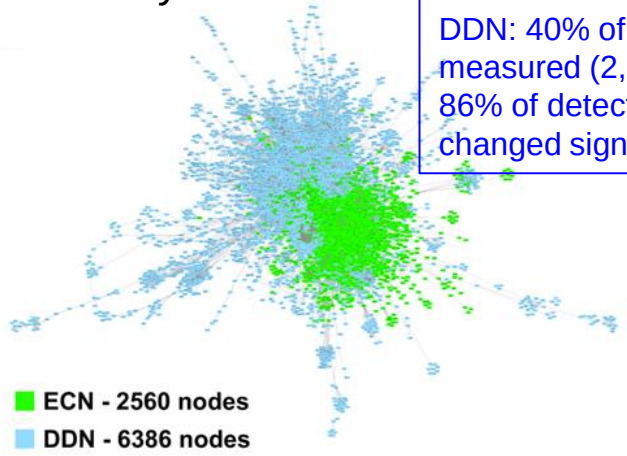
- 254,296 total features
- 55,898 unique species
- 13,483 (24%) species significantly changed



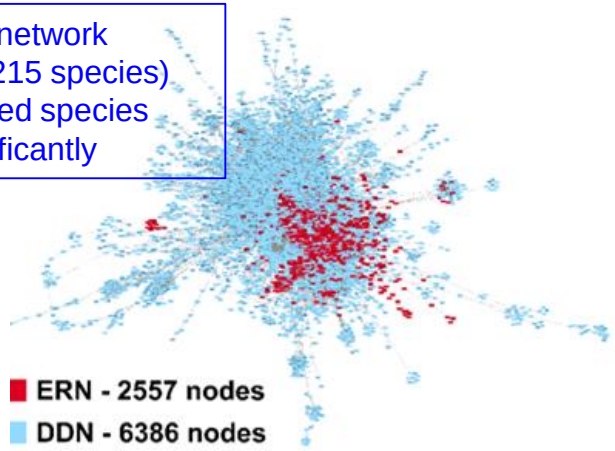


Expanded Canonical, Pino *et al.,* in preparation

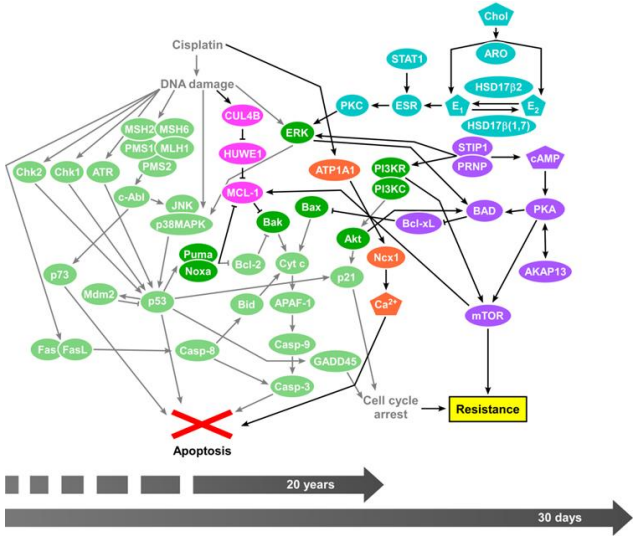
DDN: 40% of network measured (2,215 species) 86% of detected species changed significantly



Expanded Canonical vs Data Driven Network



Expanded Resistance vs Data Driven Networks



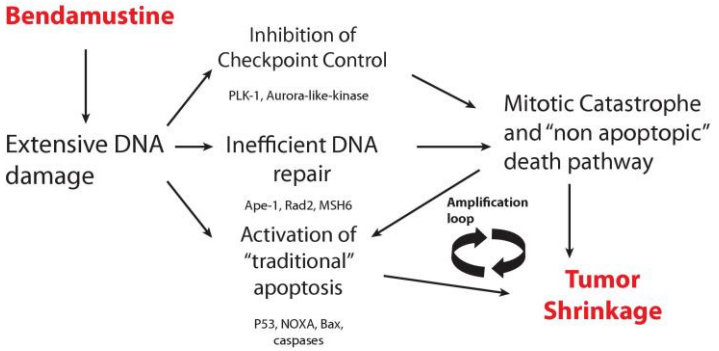
New Canonical Cisplatin MoA

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

Time-resolved omni-omics has great potential!

RTA – Bendamustine MoA

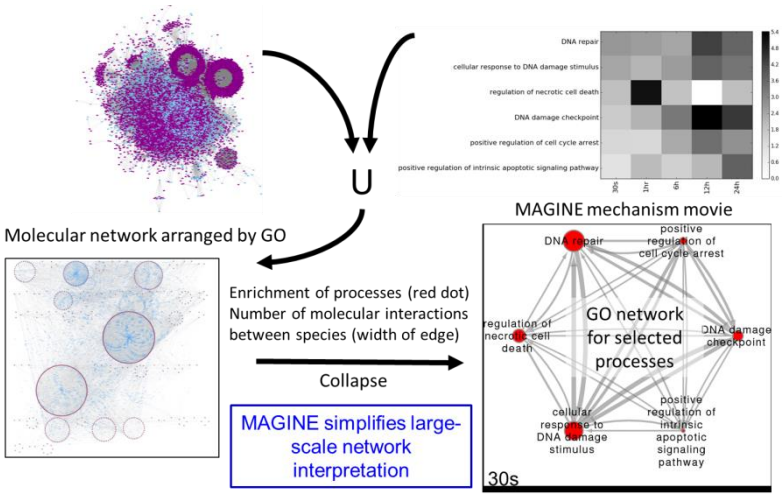
Known Mechanism of Action of Bendamustine



Leoni and Hartley, Seminars in Hematology (2011)

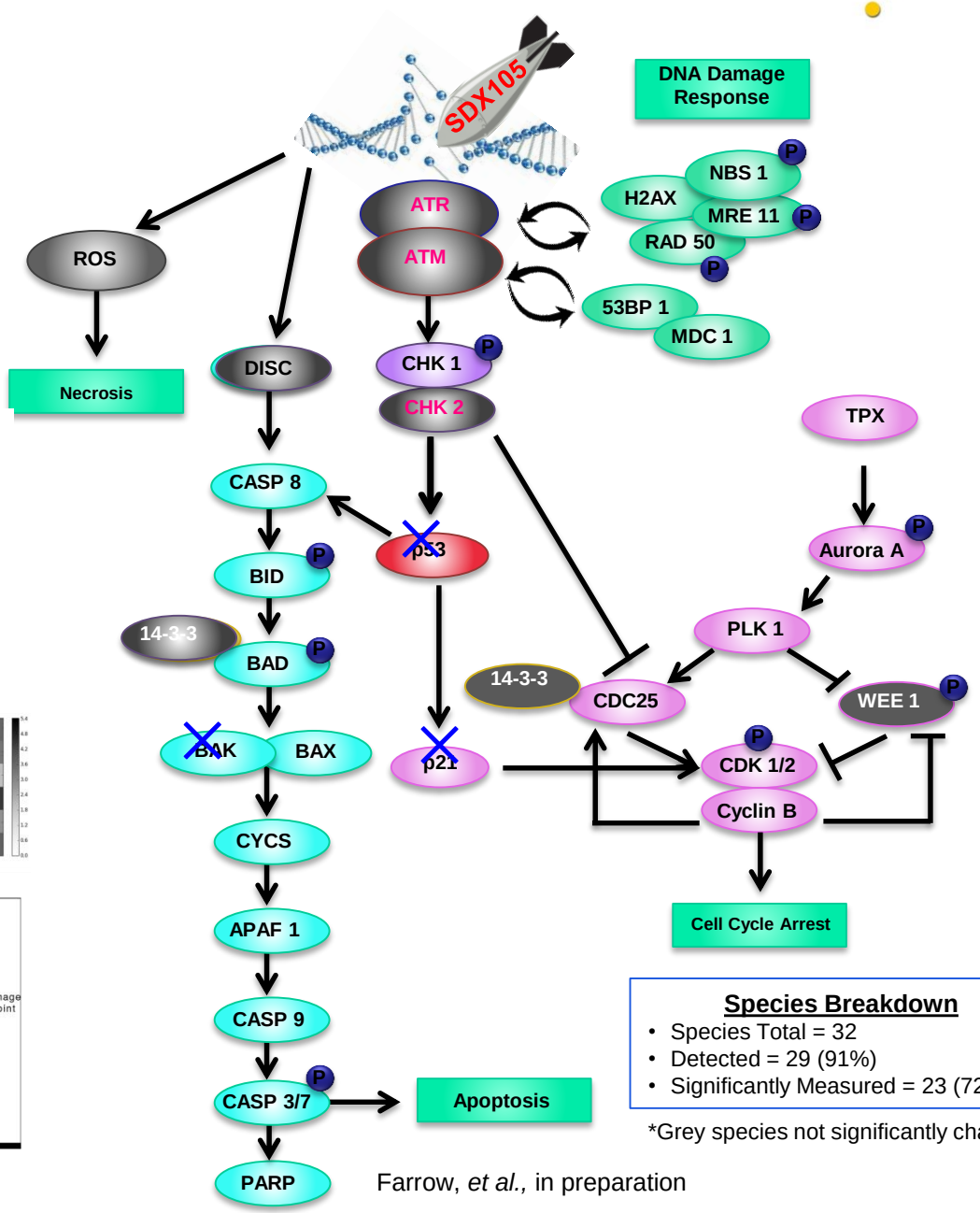
Vanderbilt RTA 2nd 30-day Challenge

- Acquire 781,072 features 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

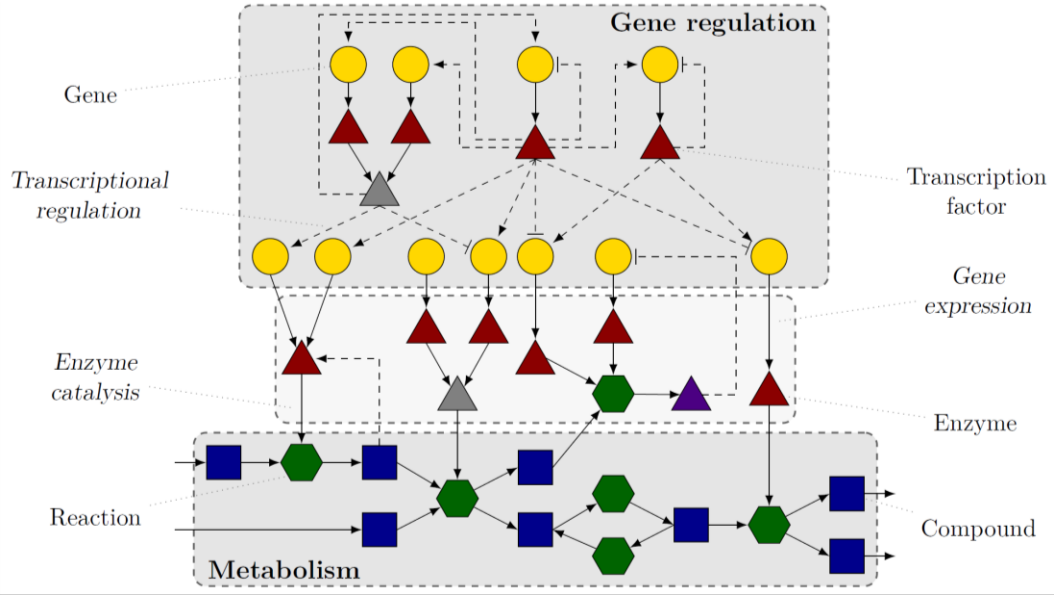
- Species Total = 32
- Detected = 29 (91%)
- Significantly Measured = 23 (72%)

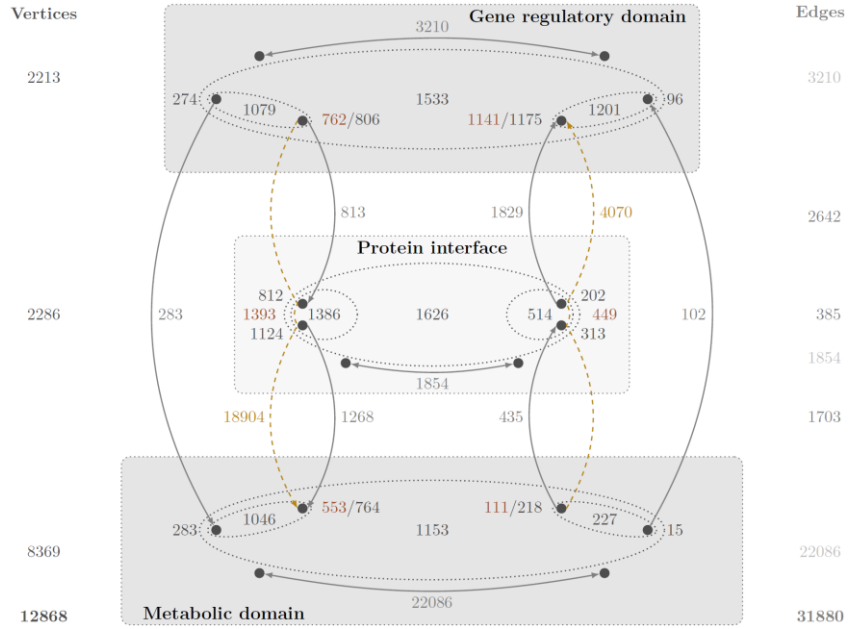
*Grey species not significantly changing

Farrow, *et al.,* in preparation

The challenge is to merge genetic, proteomic and metabolomics networks

E. Coli Gene-Enzyme-Metabolism





Encoding and reaction-associated ----------→

Regulatory ——————→

Genes ●

Protein monomers ▲

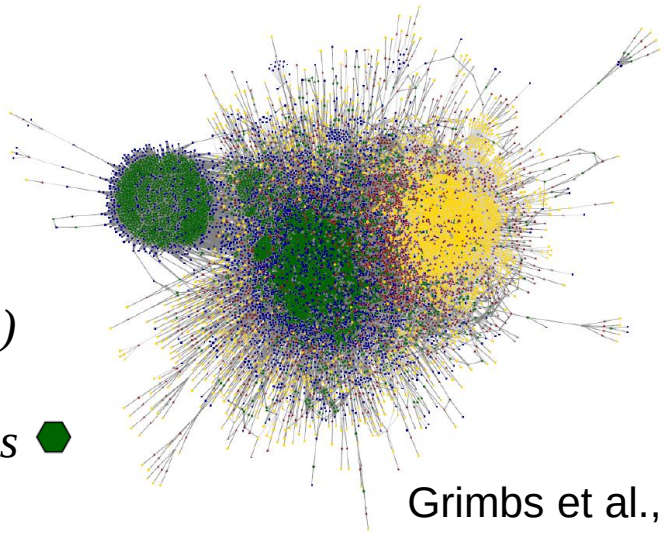
Proteiin complexes ▲

(mainly transcription factors)

small molecules ■

catalyzing biochemical reactions ⬢

modified proteins ▲



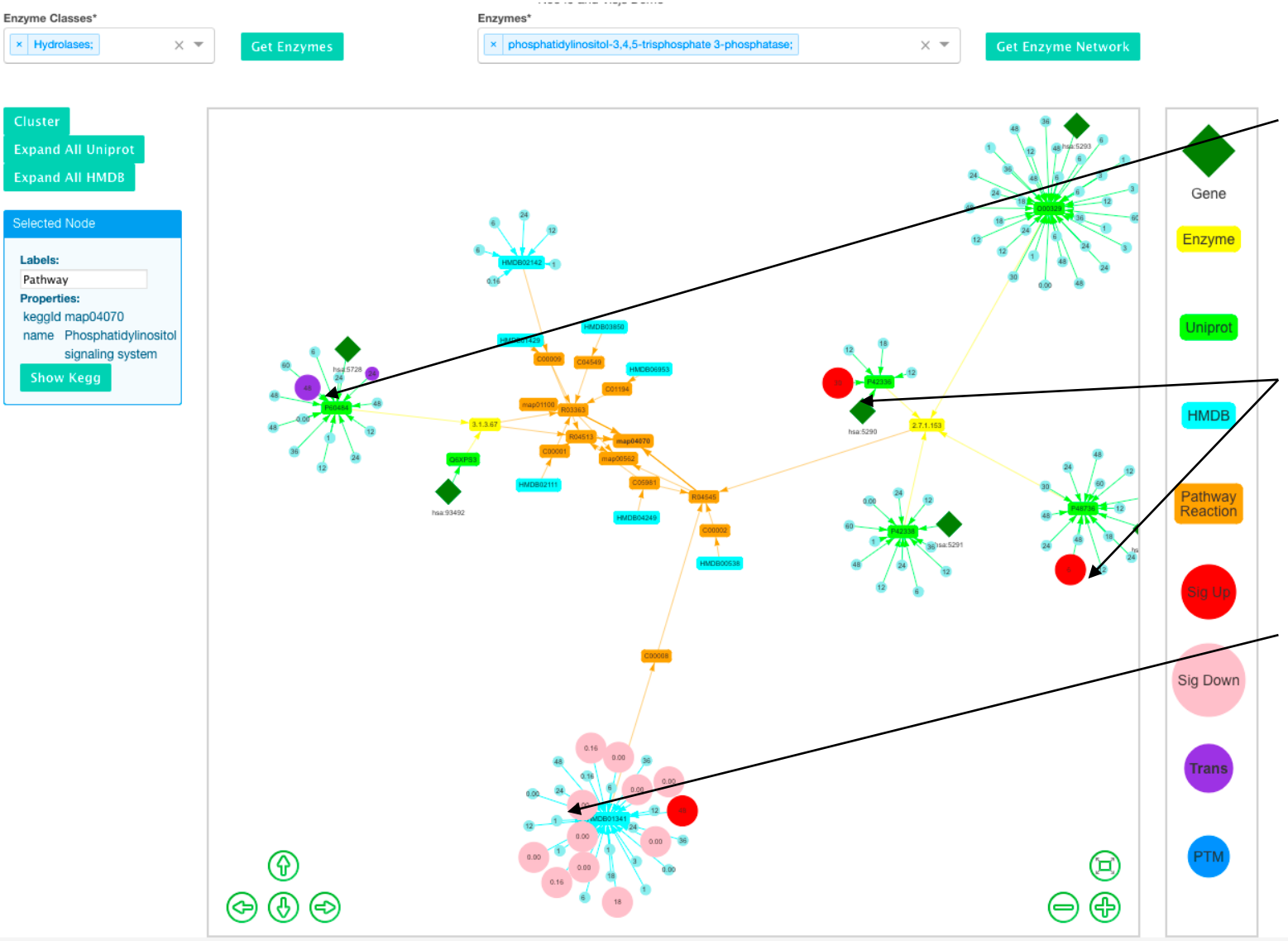
Vertex composition

- Reaction
- Compound
- Gene
- Protein monomer
- Protein-protein complex
- Protein-compound complex
- Protein-RNA complex

Grimbs et al., arXiv 1803.05429v1. 2018.

It is feasible to link the genome, proteome, and metabolome!

HL-60 methotrexate gene-enzyme-metabolism linked to phosphatidylinositol signaling system



Observed translocation event at 24 hr and 48 hr time points

Observed significant up regulations at 6 hr and 30 hr time points

Observed significant up regulation in metabolite at 48 hr time point

Tina Tsui

Mammalian genome-proteome-metabolome linking with Neo4J


Four themes
1. The complexity of biology
2. MicroPhysioligical Systems
3. Multi-Omics
4. Putting it all together
Julia Wikswo

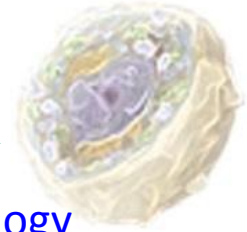
The Hermeneutic Circle of Biology

Anatomy

Animal

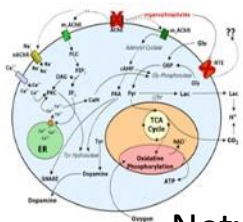
Physiology

Organ



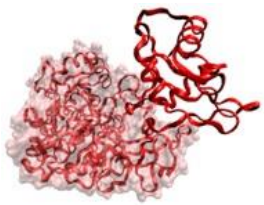
Cell Biology

Cell



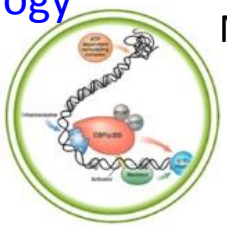
Molecular Biology

Network



Structural Biology

Molecule



Genomics

Genome

Reduction

Construction

Standard biology and medicine

Does this create a problem?

Systems Biology

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

JP Wikswo and AP Porter, *EBM*, 2015

Today

Anatomy

Physiology

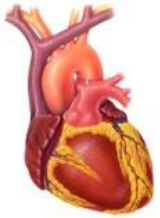
Cell Biology

Molecular Biology

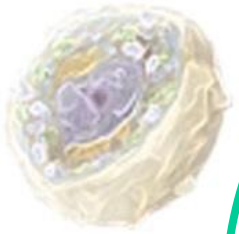
Structural Biology

Genomics

Animal



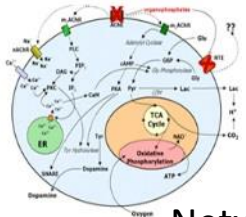
Organ



Cell

1960s

Post-Genomic, Post-Proteomic Biology and Tissue Engineering

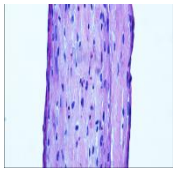


Network

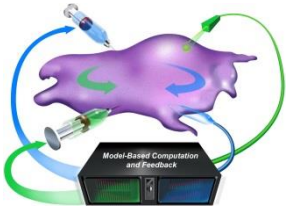
Molecule

Genome

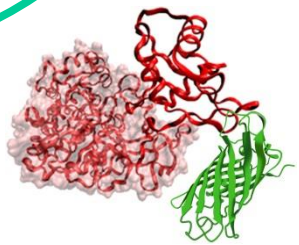
2014



Organotypic Constructs



Control of Biology



Engineered Molecules

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

The next five years

Anatomy

Physiology

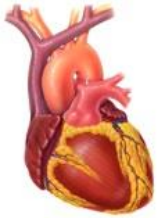
Cell Biology

Molecular Biology

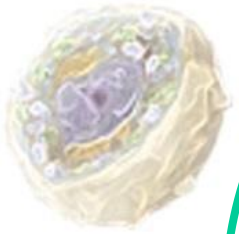
Structural Biology

Genomics

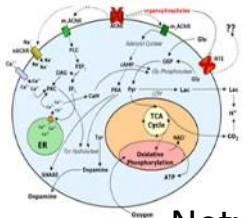
Animal



Organ



Cell

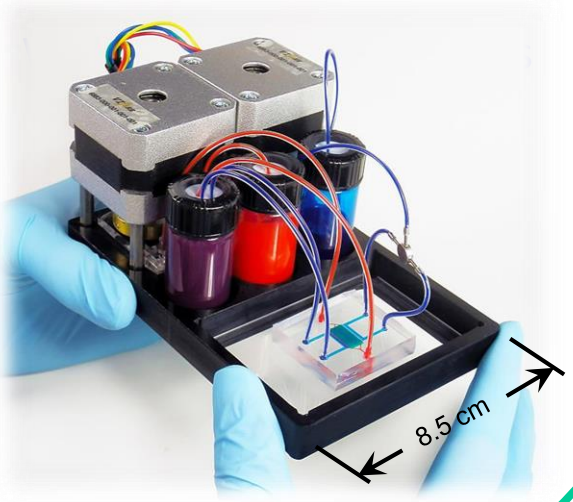


Network

Molecule

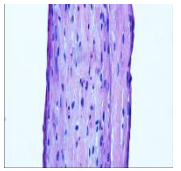
Genome

1960s

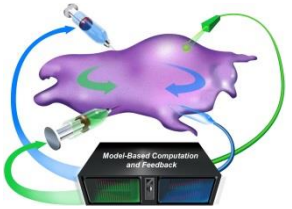


2014

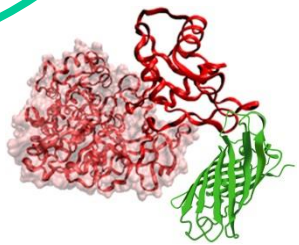
Organ Modules



Organotypic Constructs



Control of Biology



Engineered Molecules

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

Closing the Circle

Anatomy

Physiology

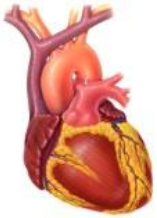
Cell Biology

Molecular Biology

Structural Biology

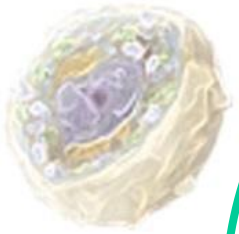
Genomics

Animal

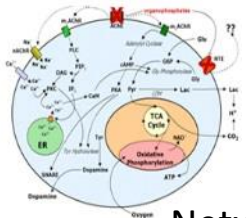


Organ

1960s



Cell



Network

Molecule

Genome

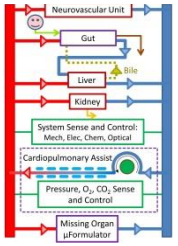
Constructionist Biology

Reductionist Biology

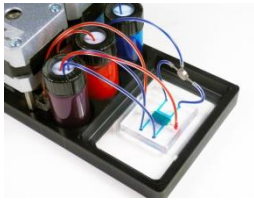
2014

Tomorrow

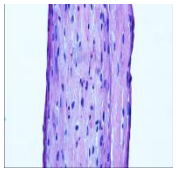
Homunculi!



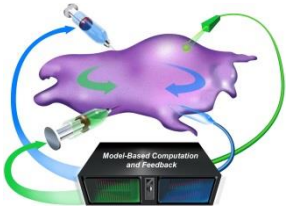
Coupled Human Microphysiological Systems



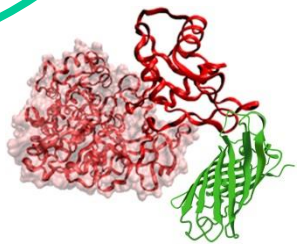
Organ Modules



Organotypic Constructs



Control of Biology



Engineered Molecules

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

The Hermaneutic Circle of Biology

VIBRE

Anatomy

Physiology

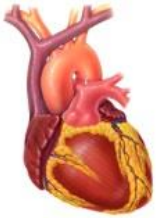
Cell Biology

Molecular Biology

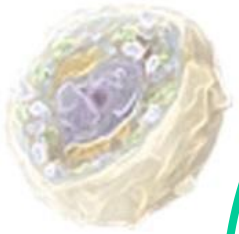
Structural Biology

Genomics

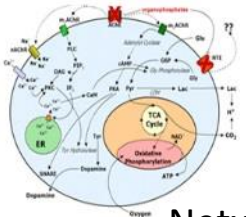
Animal



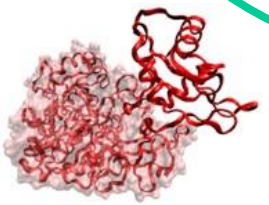
Organ



Cell



Network



Molecule

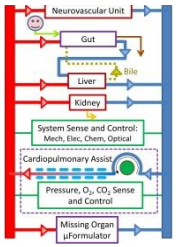
Genome

1960s

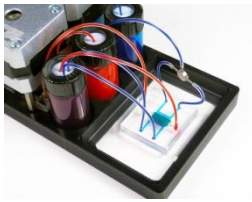
You cannot understand the whole without understanding the parts, and you cannot understand the parts without understanding the whole.

2014

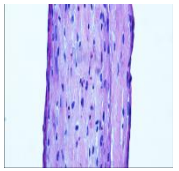
Homunculi!



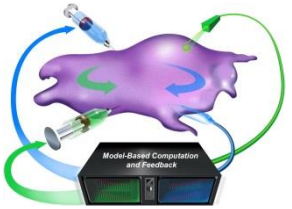
Coupled Human Microphysiological Systems



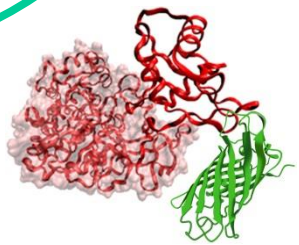
Organ Modules



Organotypic Constructs



Control of Biology



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Experimental Biology and Medicine

Vol. 239 | No. 10 | October 2014
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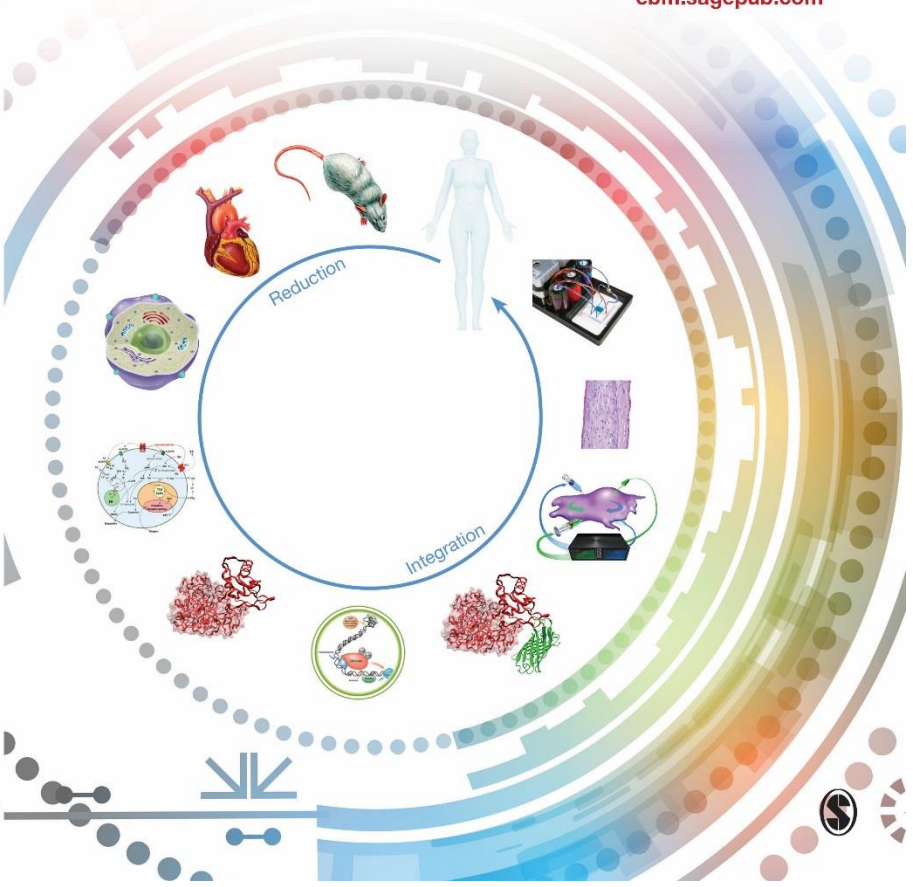

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- Endocrinology and Nutrition
- Pharmacology and Toxicology
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- Bioimaging
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Convergence!

Anatomy

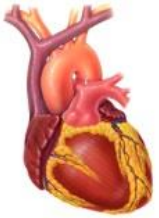
Physiology

Cell Biology

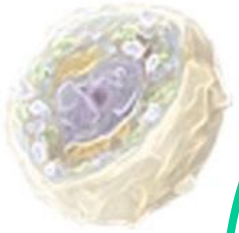
Molecular Biology

Structural Biology

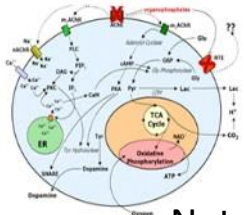
Genomics



Organ

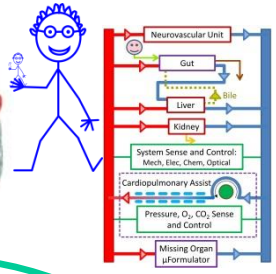


Cell



Network

Animal



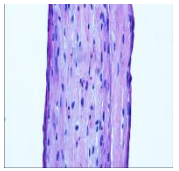
Coupled Human Microphysiological Systems

1960s

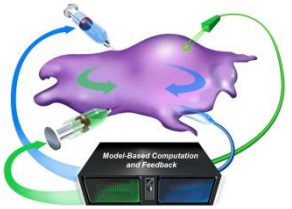
Intracellular and extracellular multi-omics may be the key to closing the hermeneutic circle of biology

2014

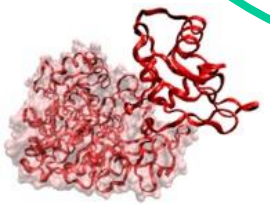
Organ Modules



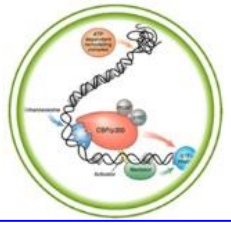
Organotypic Constructs

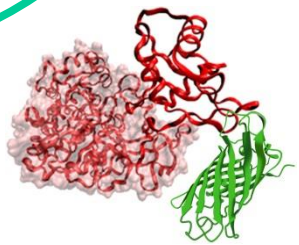


Control of Biology



Molecule

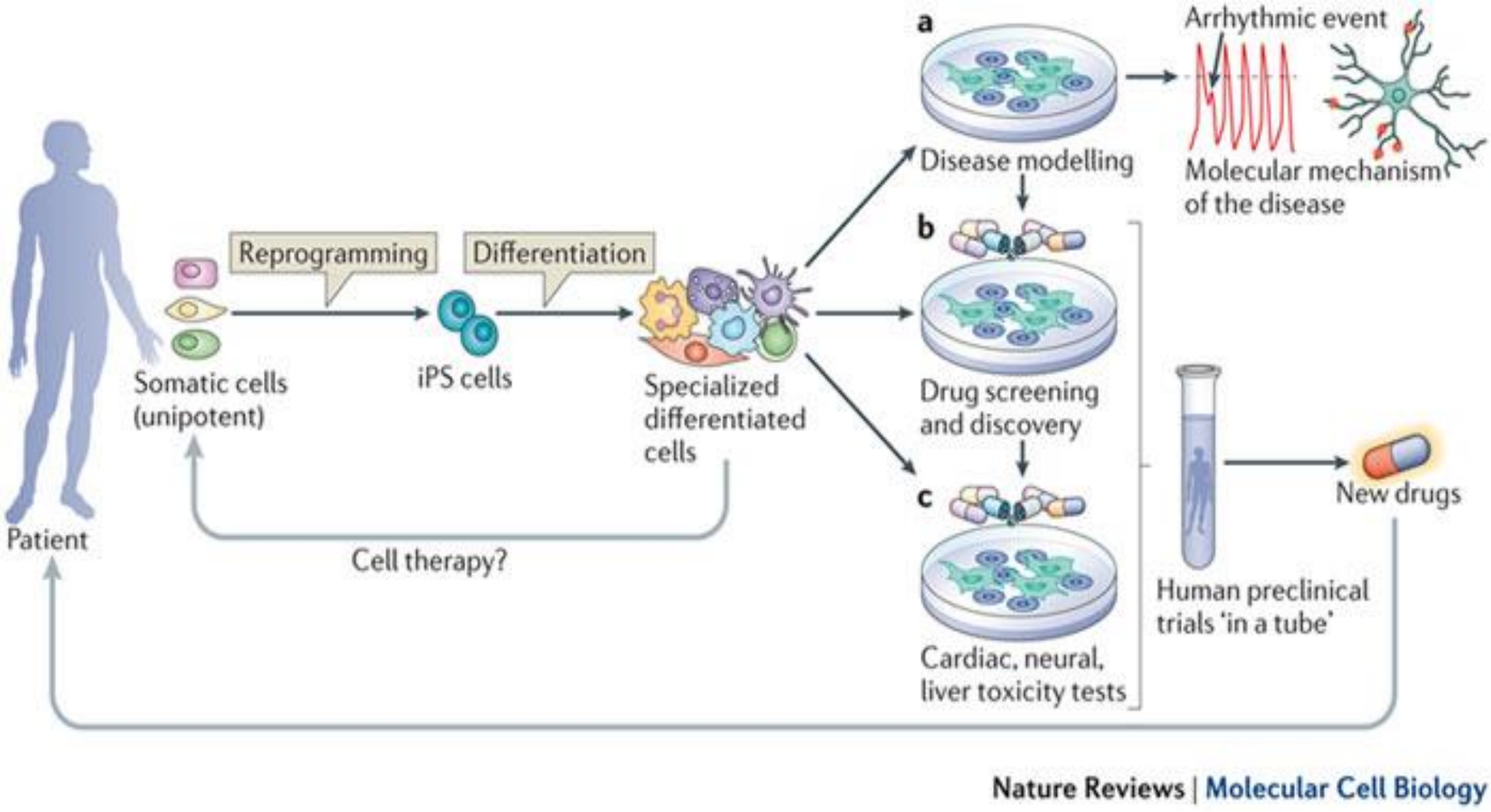




Engineered Molecules

Genome, Epigenome, Transcriptome, Lipidome, Metabolome, Connectome, …

Induced Pluripotent Stem Cells



Bellin et al., Nat Rev Mol Cell Bio, 2012

Neurovascular Unit on a Chip as a Model System for Tuberous Sclerosis Complex

VIBRE

Objective: Create an *in vitro* neurovascular unit (NVU) model of tuberous sclerosis complex (TSC) that replicates the pathology of the disease in the brain and its response to mTOR inhibitors.

Table 1: RDCRN/Dr. Ess available TSC-patient, primary dermal fibroblasts from which iPSC lines have been generated.

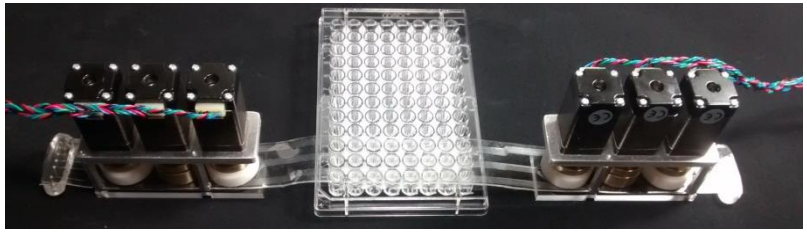
Patient	Gene	GRch38 Genome	mRNA	Protein	rs (if applicable)	Exon
TSP8	*TSC2*	NC_000016.10:g.2081734C>G	NM_000548.3:c.3750C>G	NP_000539.2:p.Tyr1250Ter	rs45517308	31/42
TSP20	*TSC2*	[illegible]	[illegible]	[illegible]		7/42
TSP21	*TSC2*	[illegible]				14/42
TSP23	*TSC2*	[illegible]			65195	27/42
TSP24	*TSC2*	[illegible]				40/42
TSP30	*TSC2*	[illegible]				19/42
TSP31	*TSC2*	[illegible]	[illegible]	[illegible]	rs45437193	34/42
TSP22	*TSC1*	NC_000009.12:g.132921367C>T	NM_000368.4:c.733C>T	NP_000359.1:p.Arg245Ter	rs118203434	8/23

With TC-2, NIH is supporting personalized organs-on-chips!

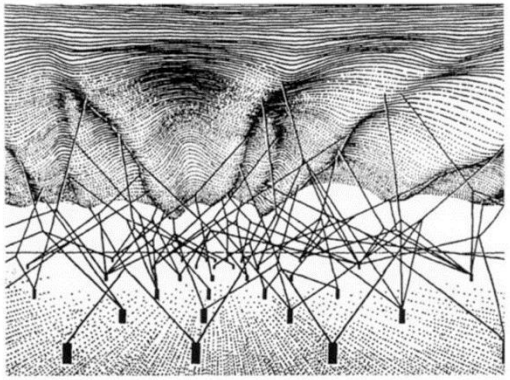
Plan: Generate, for the first time, a basic NVU tissue chip in which all cellular components (ECs, PCs, ACs, Ns) are derived from the same human individual.

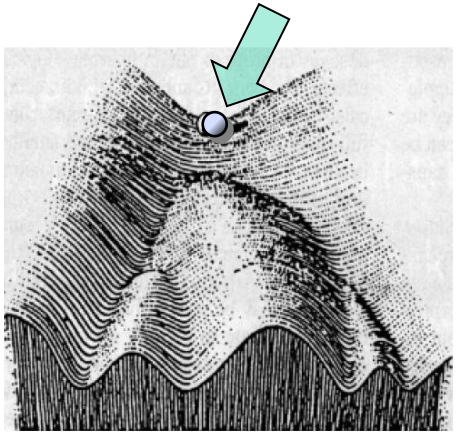
What can you do with a µF-96?

- 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
- Create circadian rhythms on a well plate or Petri dish
  - Hormones
  - Nutrients
  - Drugs
  - Substances of abuse
- 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

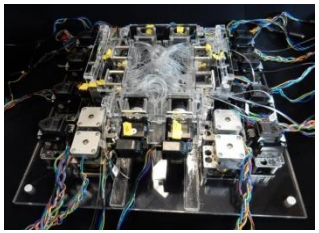


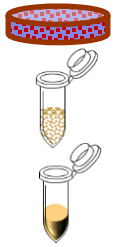


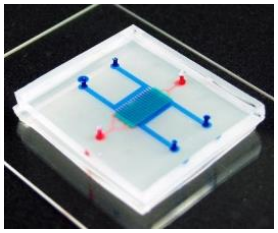
Waddington 1957

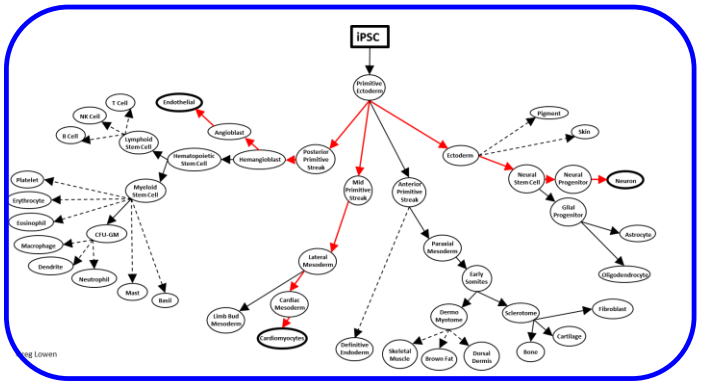
Closed-Loop Control: Neuron Development

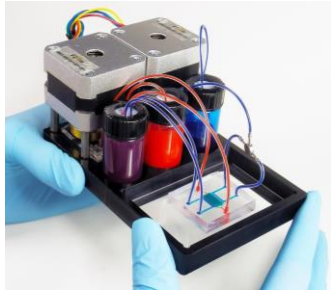
VIBRE

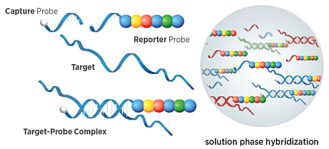




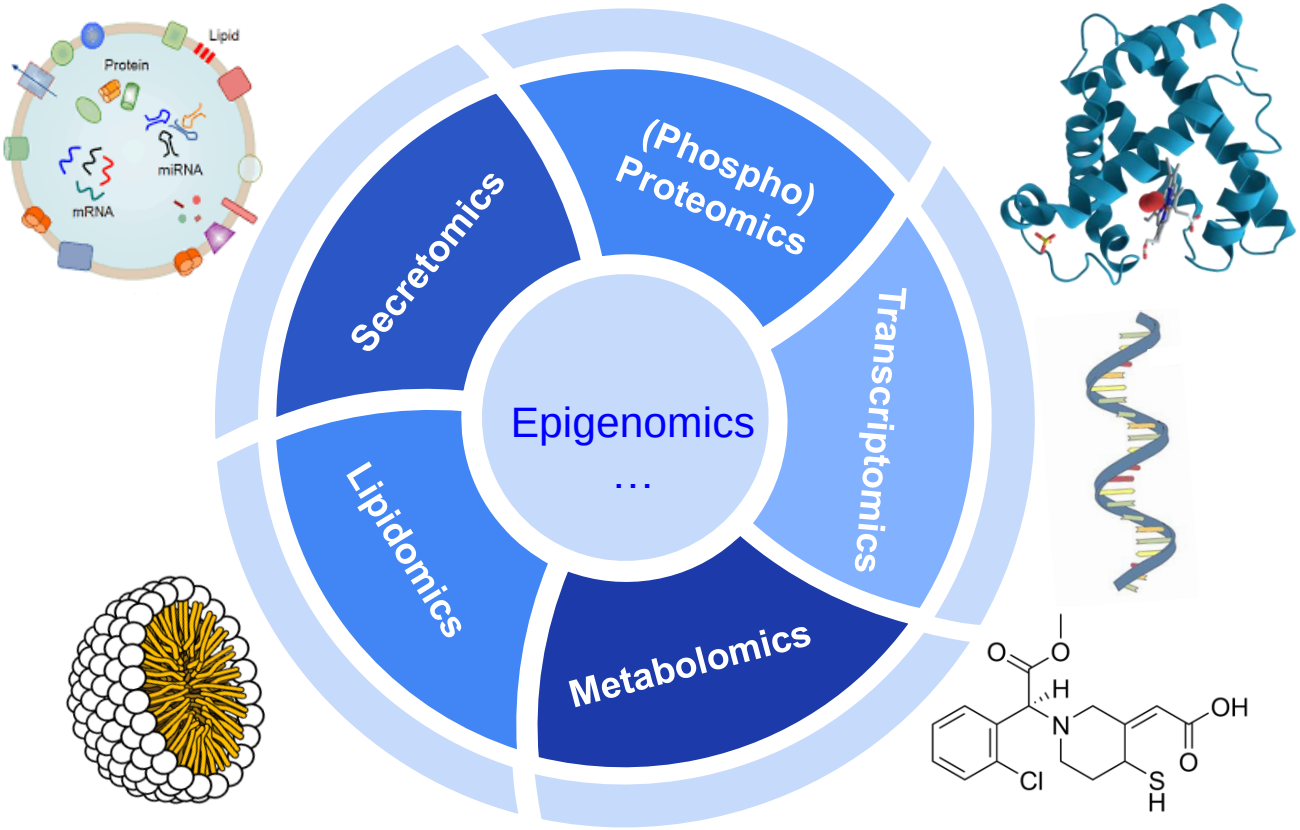








Multi-Omics


Lipid
Protein
miRNA
mRNA
Secretomics
(Phospho) Proteomics
Transcriptomics
Epigenomics
…
Lipidomics
Metabolomics
O
O
H
N
OH
O
Cl
S
H

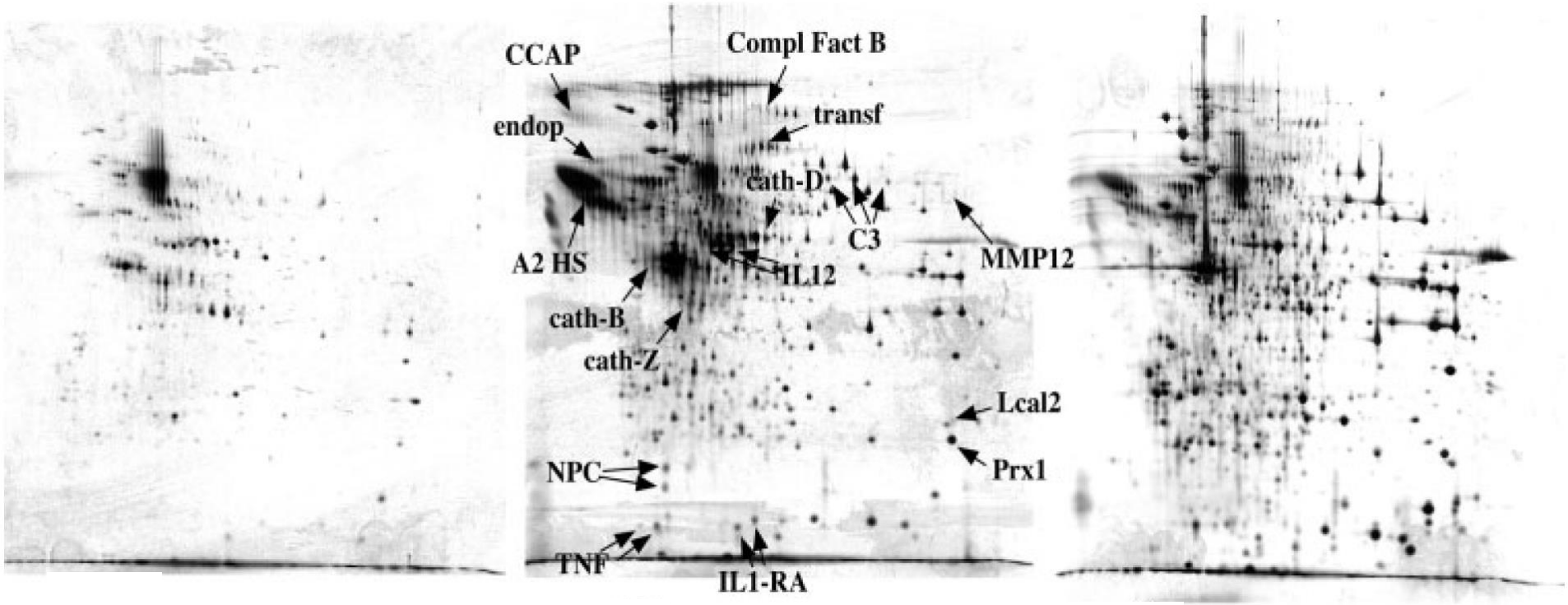
We need to revisit the proteomic and metabolomic secretome to control iPSC differentiation!

Proteins in Secretome vs Cytosol

Protein Secretome of Immature Dendrtitic Cells

Protein Secretome of LPS-Activated Dendrtitic Cells

Proteins From Cell Lysate



Protein Secretome

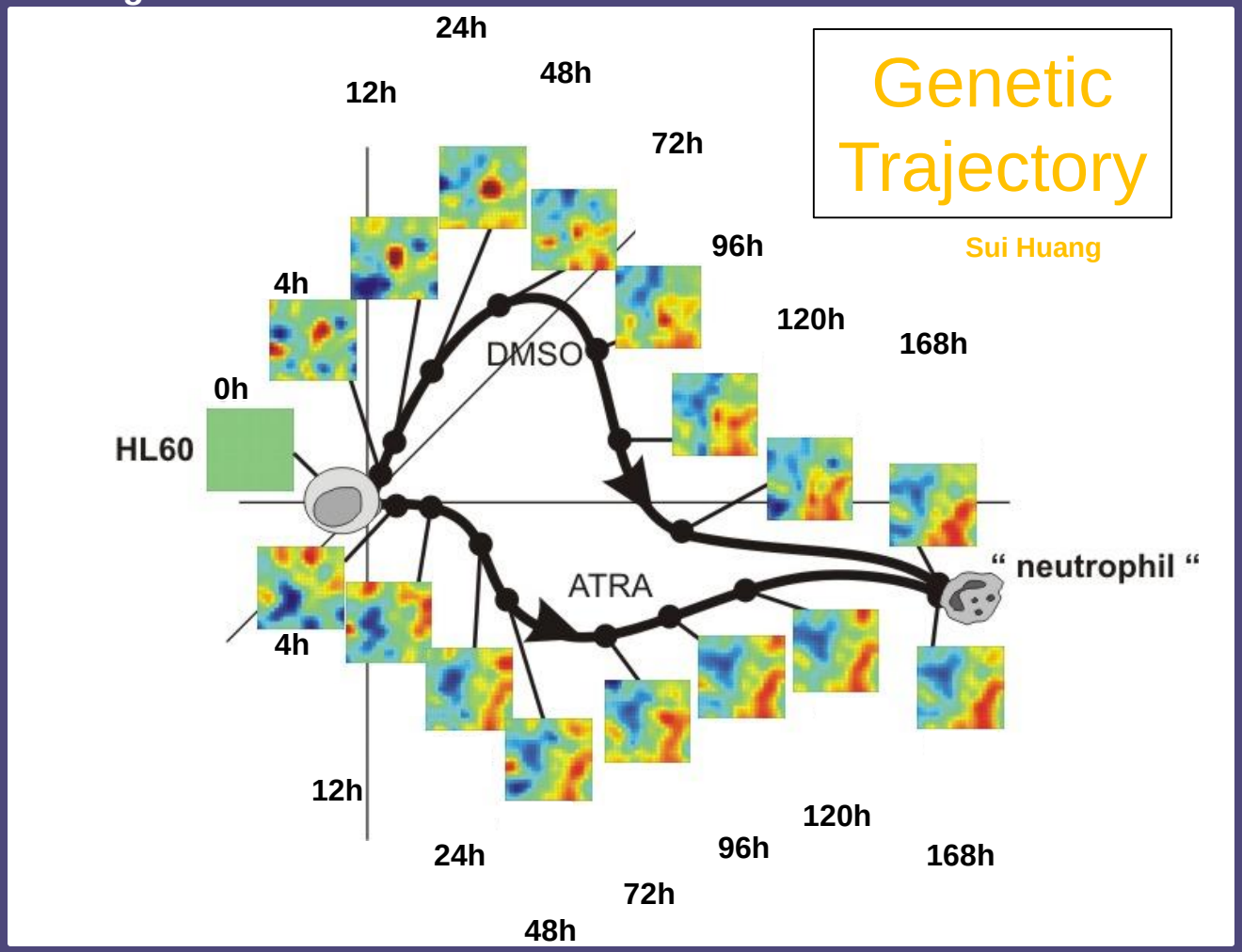
Lysate Proteins

What is the small-molecule, metabolite secretome?

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.



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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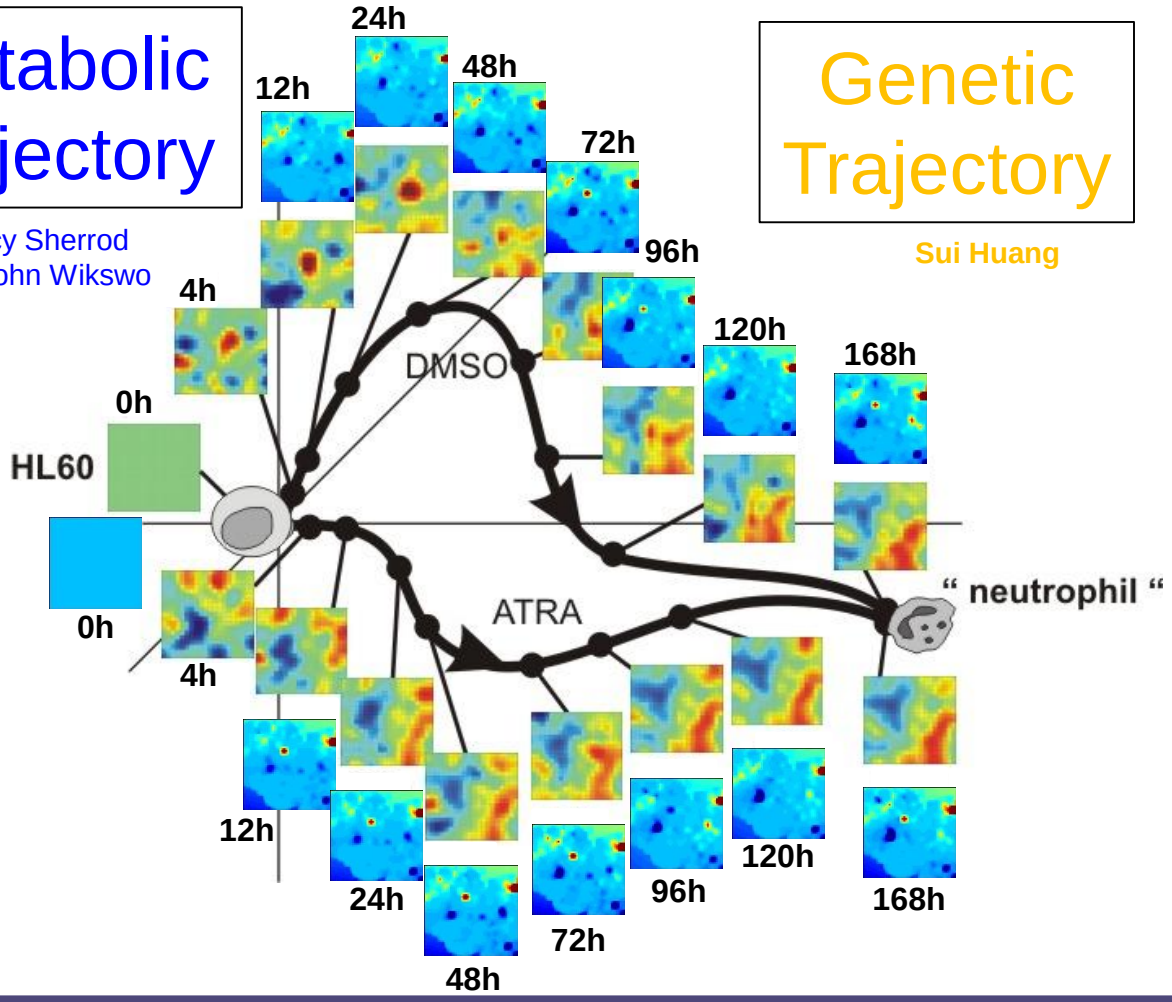
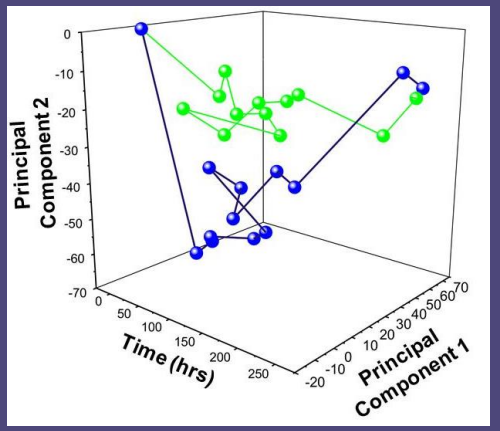
Metabolic Trajectory

Stacy Sherrod and John Wikswo

Genetic Trajectory

Sui Huang





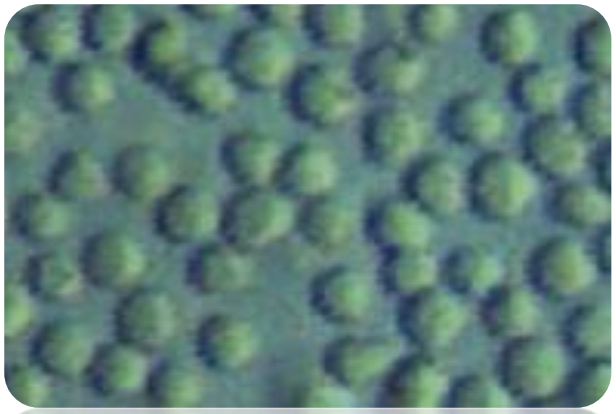
Secretome metabolomics can distinguish transitions in intracellular state

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

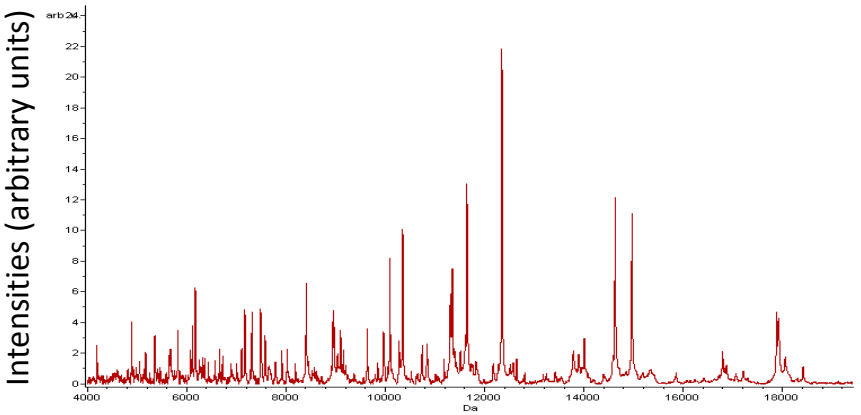
Transcriptomic data from Huang S, Eichler G, Bar-Yam Y, et al. Cell fates as high-dimensional attractor states of a complex gene regulatory network. Phys Rev Lett. 2005 Apr 1;94(12):128701.

Phenotypic MALDI Assay for *In-Vitro* Differentiation

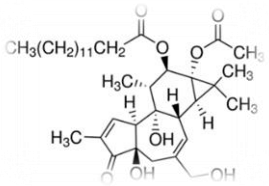
Undifferentiated THP-1 cells



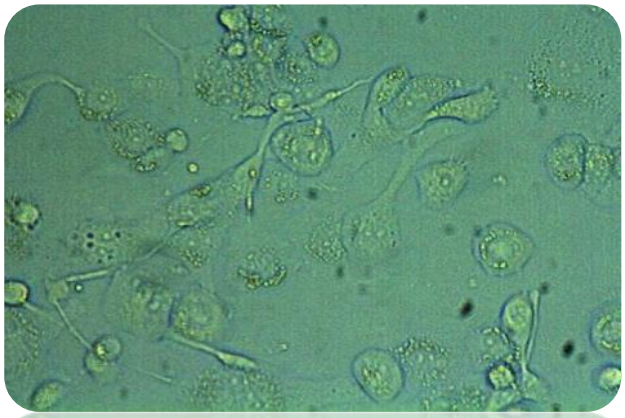
Day 0



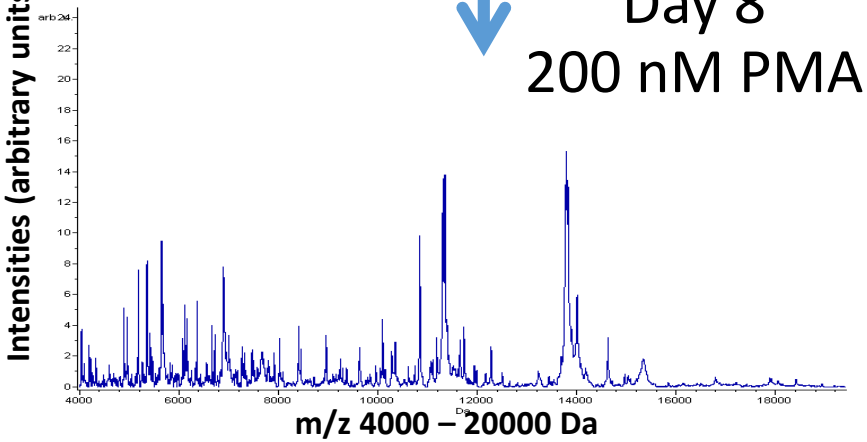
m/z 4000 – 20000 Da



Differentiated cells Macrophage-like



Day 8
200 nM PMA



m/z 4000 – 20000 Da

Prof Carsten Hopf HS Mannheim

Dr. Bogdan Munteanu

Munteanu et al., *Anal. Bioanal. Chem*., 2012

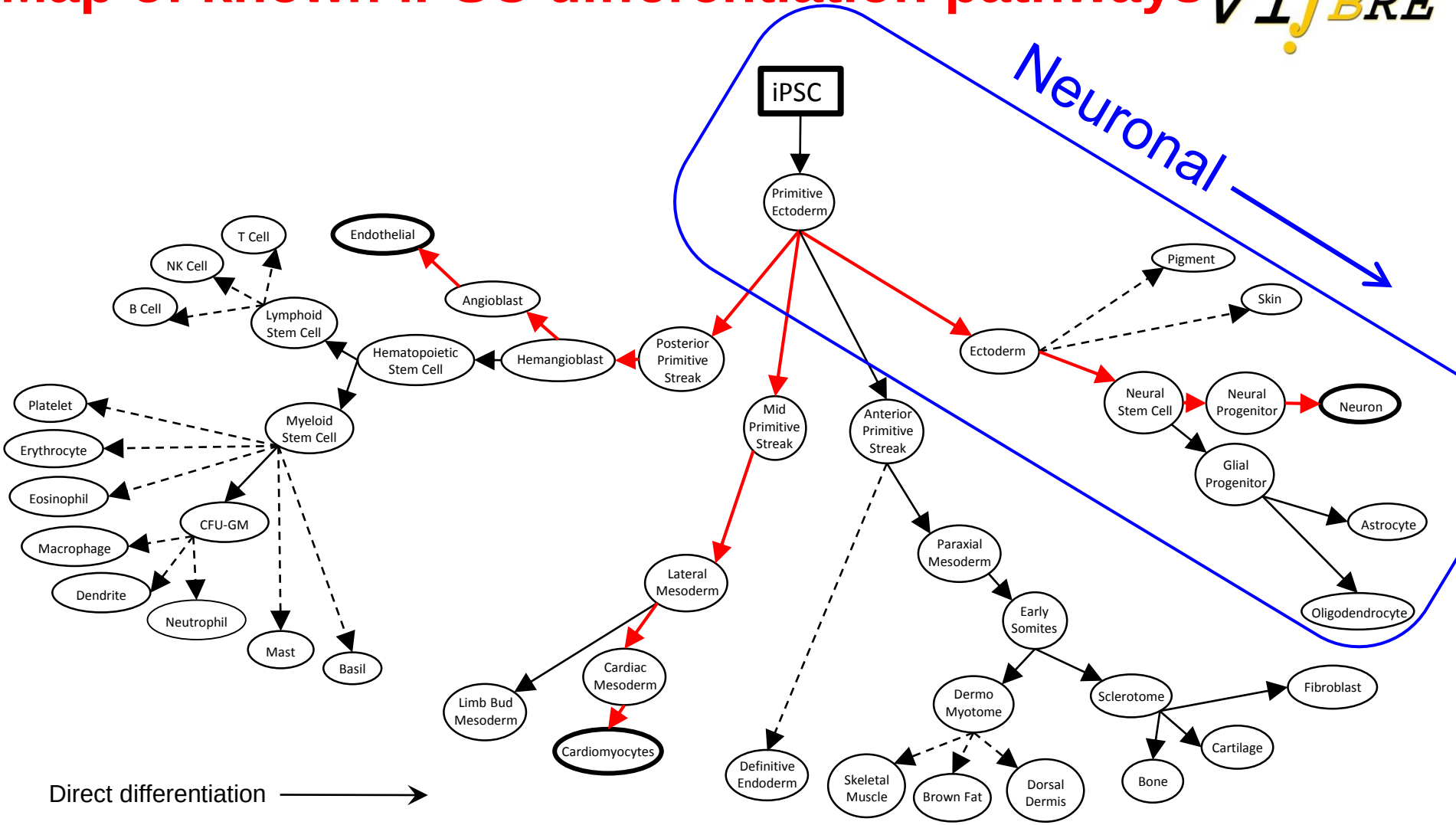
Munteanu and Hopf, *Bioanalysis*, 2013

Munteanu et al., *Anal. Chem*., 2014

Untargeted transcriptomics, IM-MS secretory metabolomics, and MALDI MS proteomics can readily track cellular differentiation!

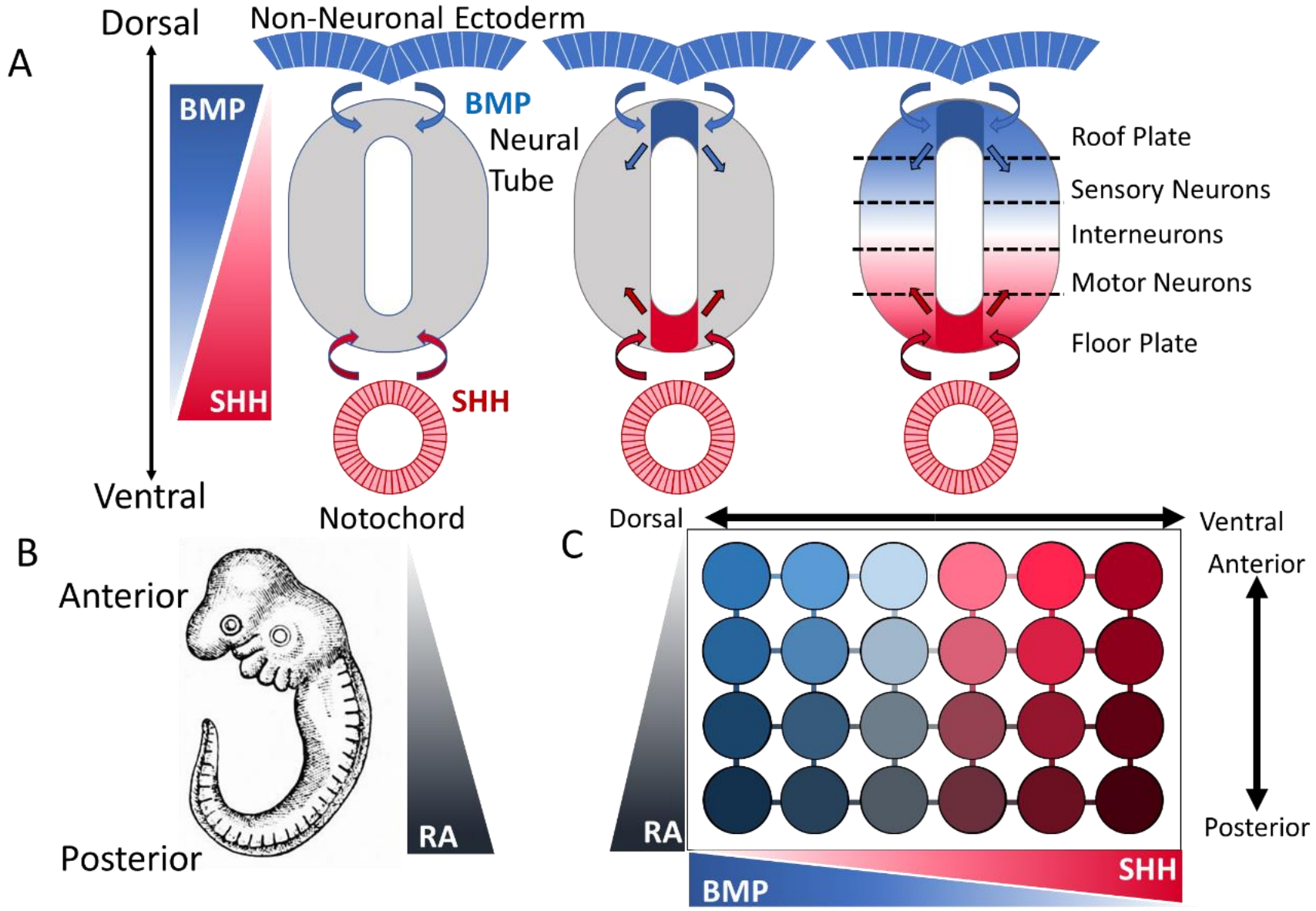
All we need to do is correlate the metabolic and proteomic secretome with the cellular multiome to get a non-destructive control signal!

Map of known iPSC differentiation pathways



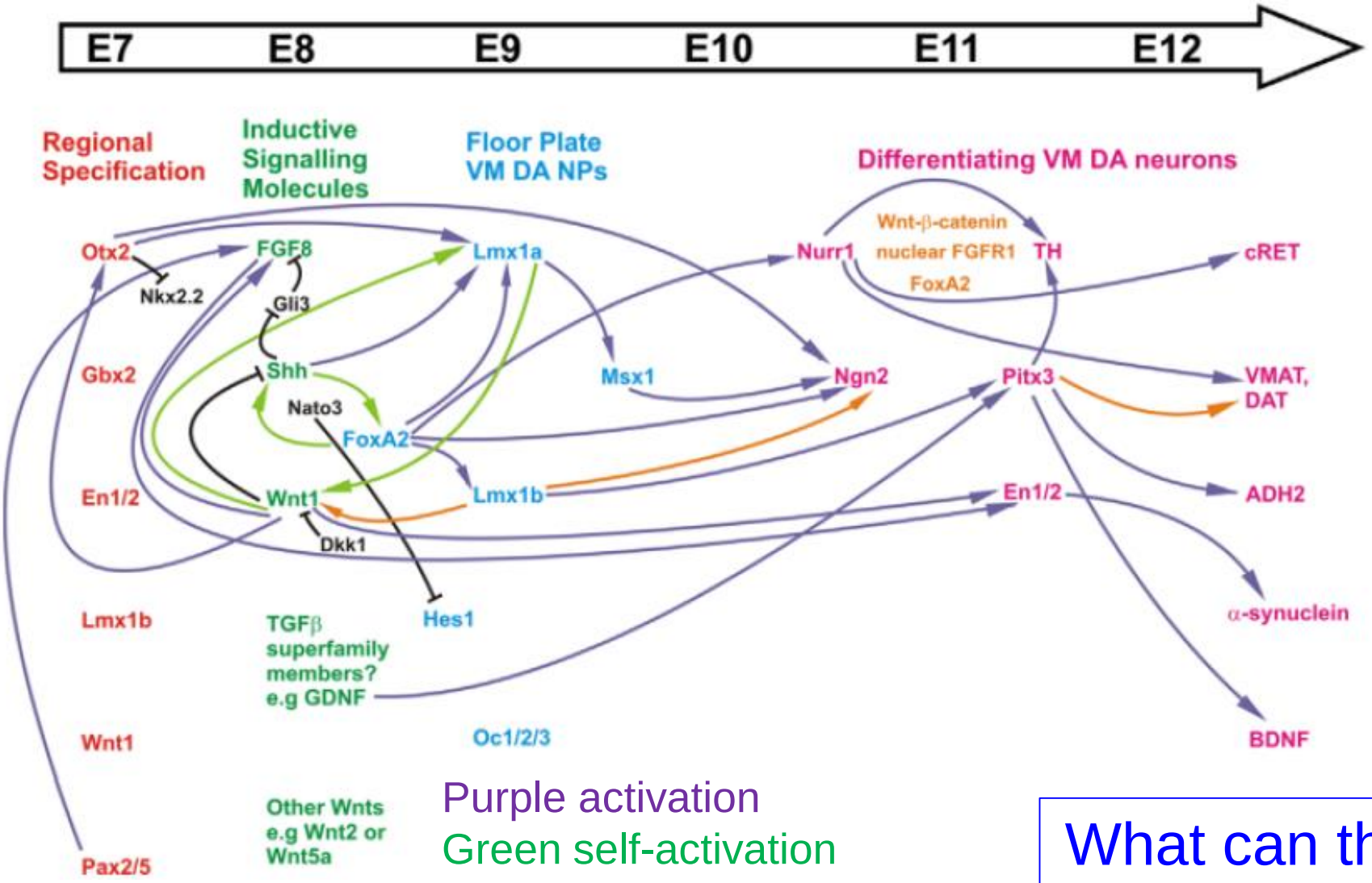
Greg Lowen

Neural Tube Development



Natalie Hawken, plus Polular Science Monthly, 1892

Genetic regulation of ventral midbrain dopaminergic neuron development.

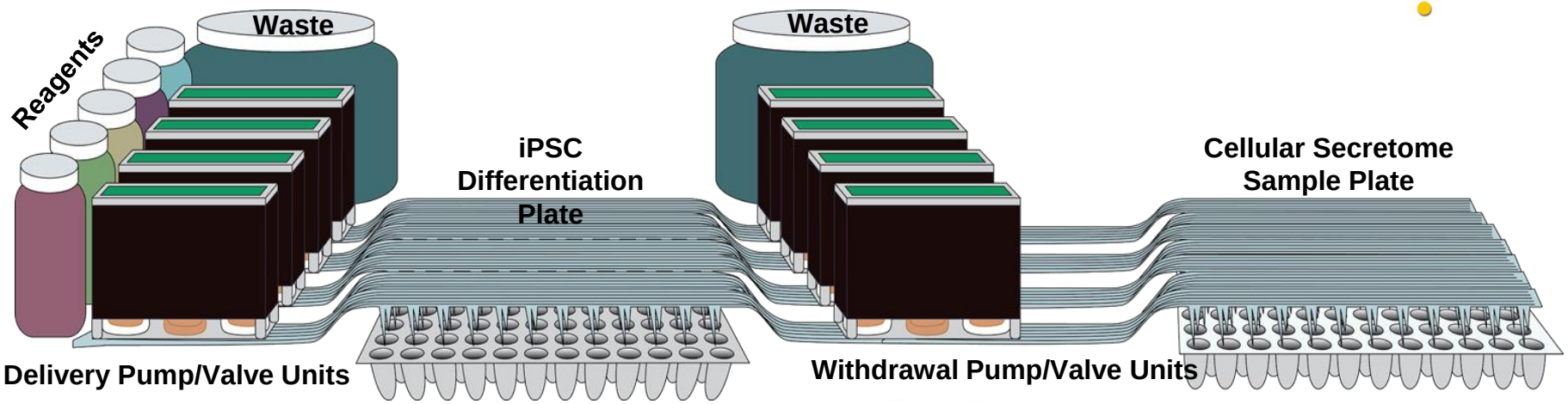


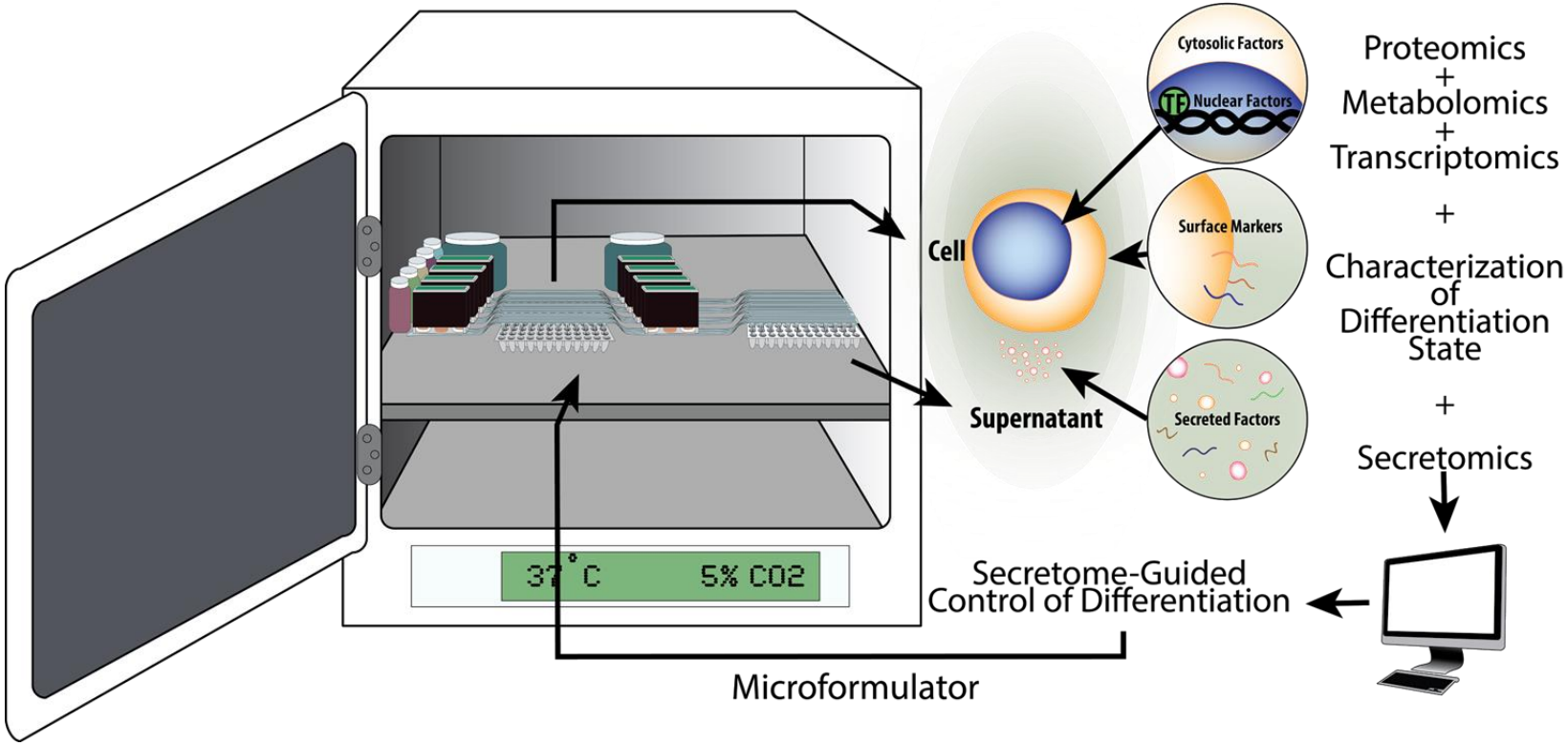
Purple activation
Green self-activation
Orange cooperative regulation
Black arrows inhibition.

What can the µF control?

Hegarty, Sullivan, and O'Keeffe, 2013,

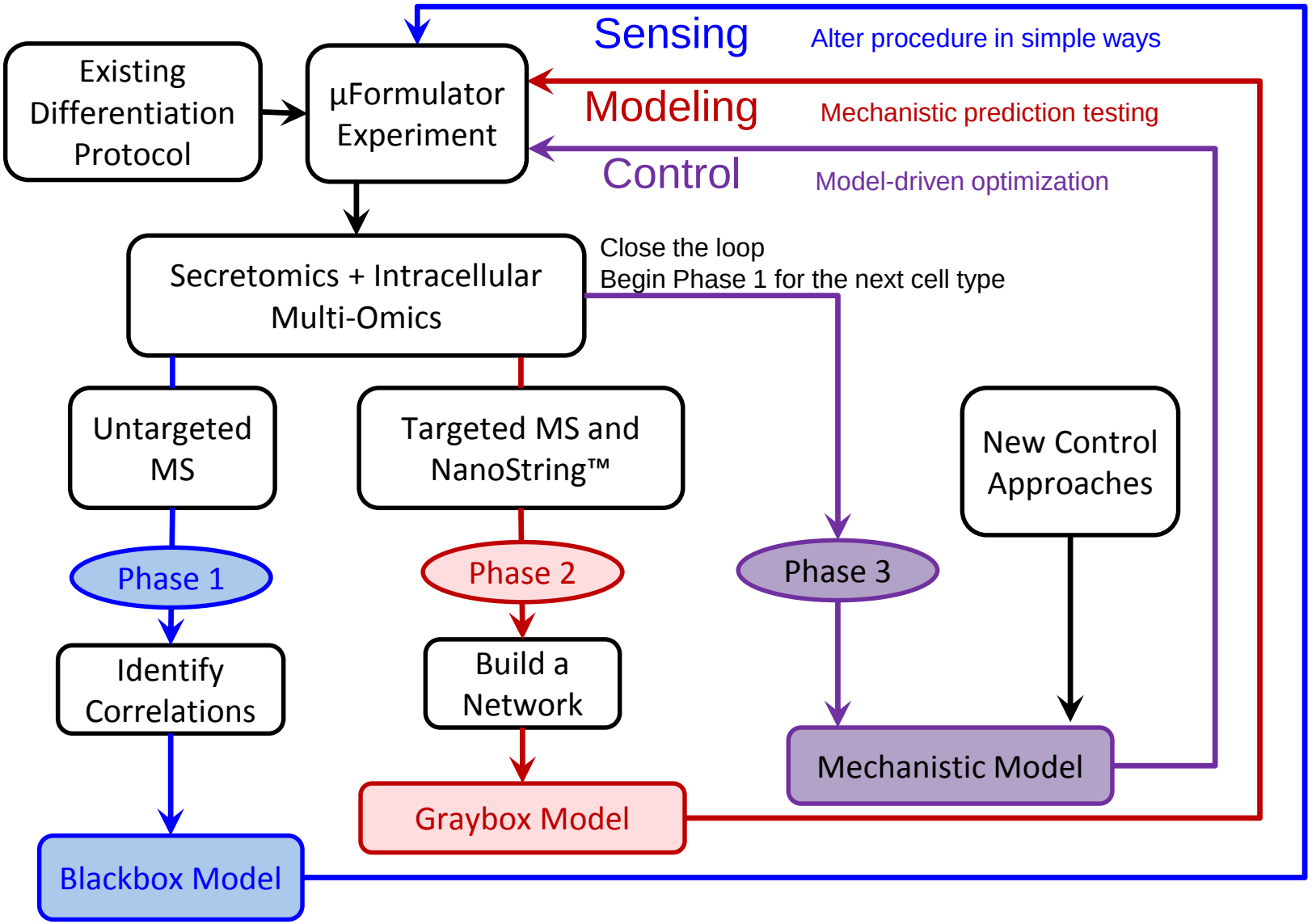
MicroFormulator for iPSC Differentiation





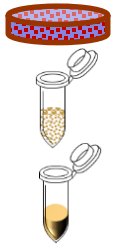
Caleb Casolaro

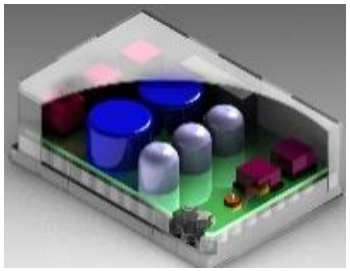
Development of Closed-Loop Control

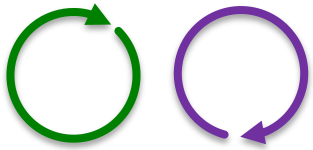


Controlled Bioprinting of 3D Tissues

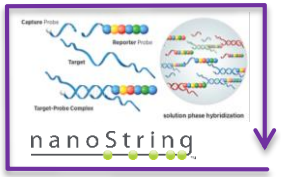
VIBRE

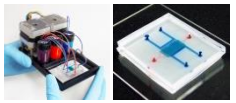


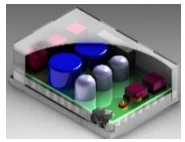




Secretome and Cellular Multi-Ome for Controlling iPSC Differentiation and Bioprinted Tissue Maturation







CFDRC SmartLid Well-Plate Feeder

iPSC Cell Culture → Cell Differentiation → Cell Characterization → Cell Maintenance → Organ Chip & Tissue Bioprinting → Tissue Maturation → Tissue Characterization

MicroFormulator Optimizations of Differentiation Protocols

Data Integration and Modeling

VIIBRE Secretome Metabolomics and Proteomics

MicroFormulator Media Optimization

NCATS Cellular Proteomics and Histology

NCATS MALDI Mass Spectrometry Imaging

Tissue

Cell Pellet

Secretome

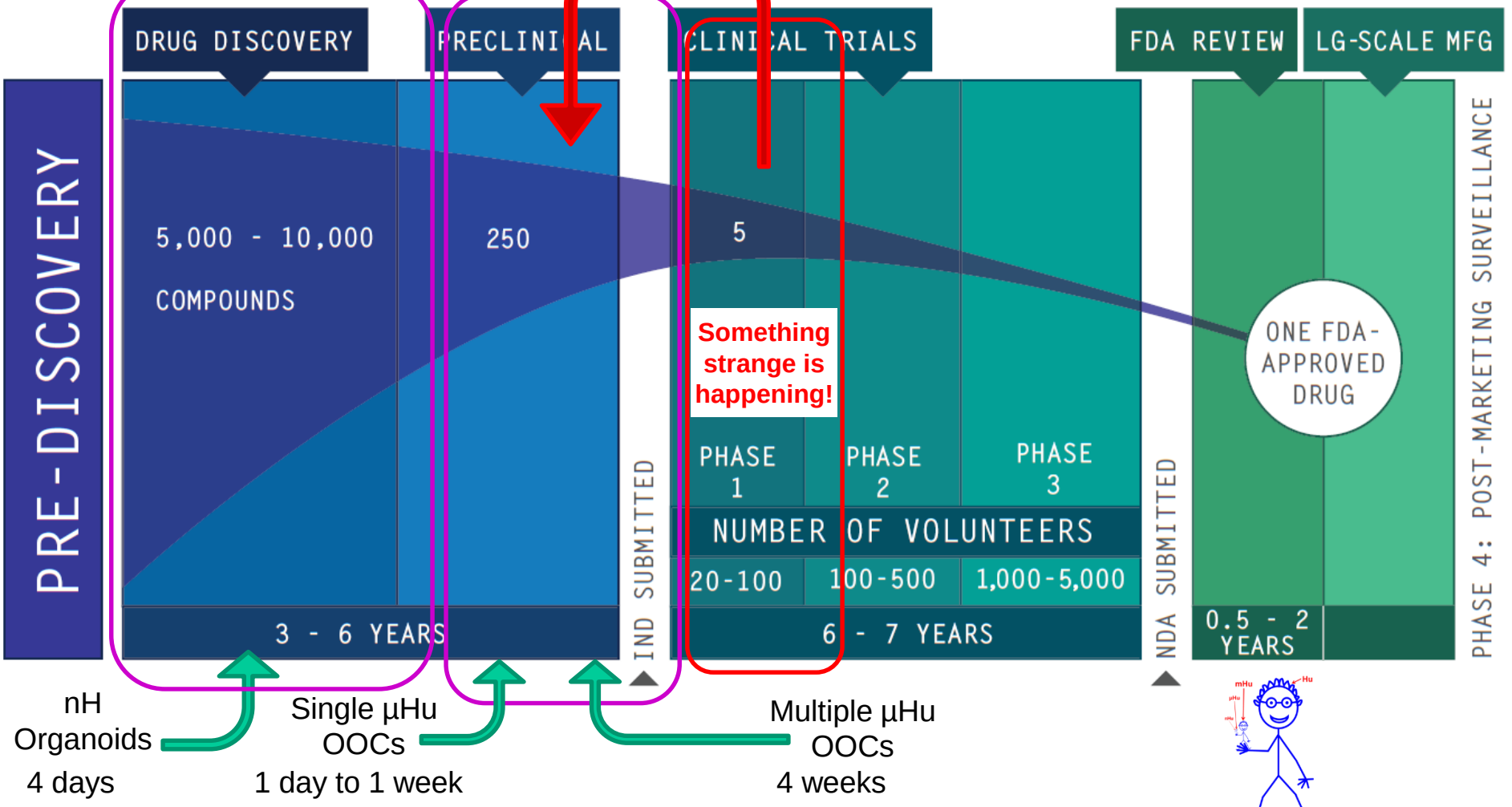
MicroFormulators

Vanderbilt Mass Spectrometers

Where do organs-on-chips fit into the drug discovery and development pipeline?

Where do MPS models belong?

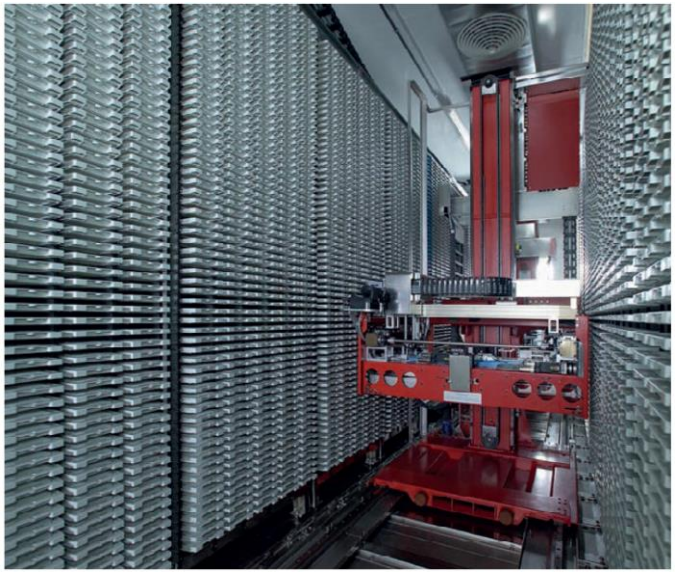
VIBRE



- Improve the transition from cells to animals to humans?
- Introduce human cells and tissue-equivalences earlier?
- Explain mechanism of action when questions arise?

http://www.phrma.org/sites/default/files/pdf/rd_brochure_022307.pdf

Really High Throughput!

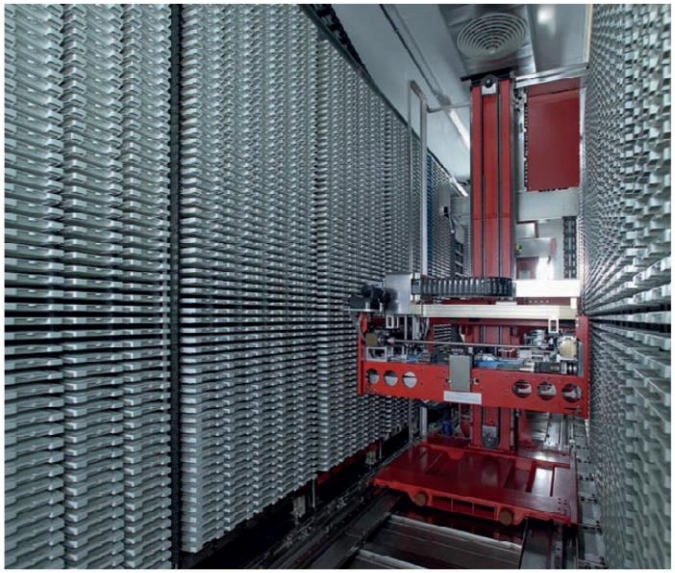


- AstraZeneca has more that two million compounds in its libraries
- Retrieval capacity is approaching 80,000 samples per day
- Trays hold 1,280 acoustic dispensing tubes
- It is possible to plate 100,000 samples in 30 hours
- 2.5 nL increments of compound, up to 1 uL per well

C. Green and P Spencer, Drug Discovery World Winter 2017/2018
G Schneider, Nature Reviews, Drug Discovery, 2018
Dawes, JALA, 2016

Images from Boehringer–Ingelheim Pharma and AstraZeneca.

Really High Throughput!



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- 2.5 nL increments of compound, up to 1 uL per well

If you need these robots and this library in its <u>entirety</u>, you may need organoids, but you <u>don't</u> need organs-on-chips

C. Green and P Spencer, Drug Discovery World Winter 2017/2018
G Schneider, Nature Reviews, Drug Discovery, 2018
Dawes, JALA, 2016

Images from Boehringer–Ingelheim Pharma and AstraZeneca.

The Grand, Organ-on-a-Chip Vision for Drug Development

Imagine instead of animals ….

- A “human-on-a-chip” …
- … using cells from patients who are in the hospital today
- … and your Human-on-a-Chip helps you understand whether that person will have a pharmacologic response to your drug
- … whether that drug is in Phase 3, or Phase 1, or was just this morning synthesized by a medicinal chemist for a novel drug target that may, or may not, bring tremendous value to one or more patients
- … and by the way, you can also predict the absorption, disposition, metabolism, drug-drug interactions, and safety risks for this drug in the intended patient, as well as a panel of 100’s of other patients with that disease.
- And if something goes wrong, you learn this before patients are put at risk.

Dave Watson, 2017

Immediate Applications for MPS models

- Disease Biology / Pharmacology
  - Discovery of novel **mechanisms of human diseases**
  - Identification of novel compounds including probes, leads, clinical candidates
  - Discovery of the **mechanism of action of drug candidates**
    - **On target**
    - **Off target**
- ADME-PK-Clinical Pharmacology
  - Early identification of problematic human haplotypes and drug–drug interactions (DDIs) for small molecules
  - Improved prediction of human exposure for compounds and clinical formulations
- Toxicology
  - Earlier termination of toxic drugs
  - Avoid inappropriate drug terminations

Untargeted MS proteomics and metabolomics may hold the keys!

Dave Watson, Rosemarie Hunziker, and John Wikswo, Experimental Biology and Medicine, 2017

In Vitro Problems that may need today's OoC capabilities

- Access to both sides of barriers polarized by shear flow
  - Blood-brain barrier
  - Blood-testis barrier
  - GI tract
  - Angiogenesis / vasculogenesis
- Mechanically active systems
  - Alveolar interface
  - Gut
  - Skeletal, smooth, and cardiac muscle
  - Developmental bone-joint
- Complex, well-defined heterogeneous 3D cultures
  - Liver
  - Brain → Electrical recordings of neural network activity
  - Skin
  - …
- Coupled organs for drug-drug interactions and ADME-Tox
  - Gut-liver
  - Liver-brain
  - Gut-kidney-liver …

Problems that are at the MPS cutting edge

VIBRE

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
- **Testing immuno-oncology drugs**

How accurately must we recreate adaptive immunity?

  - 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 (Kristina Howard, FDA).

The Payoff

- Organ on chip systems may reduce costs
  - Drug efficacy
  - Drug toxicity
  - Enviromental toxicology
  - Rapid detection of mode of action of hacked CB agents.
- The simultaneous EC and IM-MS measurement of the dynamics of tens to hundreds or even thousands of cellular variables will allow an unprecedented advance in our understanding of living cells
  - Pharmaceuticals, cellular or environmental toxins, CBN agents
  - Toxin-toxin adverse synergism
  - Drugs that are used for toxin prophylaxis and treatment.
- The general application of this technology will support
  - **A deep understanding of biology and complex systems**
  - Development of **new drugs**
  - Screening for unwanted **drug side effects**
  - **More rapid understanding of mechanism of action**
  - **Assessment of yet-unknown effects of environmental toxins.**

Organ-on-Chip Technical Challenges 2013

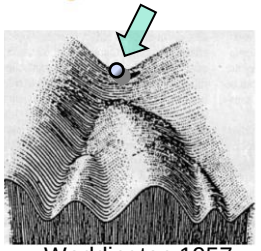
- What is the size of each organ?
  - Scaling criteria
  - Creation and maintenance of cellular heterogeneity
  - Scaling will fail at the single-cell level
- How do you control fluids within the volume and cost budgets?
  - 4.5 mL for milliHuman, 4.5 µL for a microHuman
  - Minimize pump, tubing and interconnect dead volume
  - Fluid makeup after sample withdrawal
  - Eliminate bubbles
  - Need thousands of units operating for a month
- Analytical chemistry in nL bioreactors
  - Electrochemical sensing of pH, glucose, lactate, oxygen
  - Optical monitoring of $[Ca^{2+}]in$
  - UPLC/MALDI/nESI ETD IM-MS/MS Omni-Omics
  - Non-specific analyte binding
  - Integration, mining, and interpretation of Omni-Omic data
- Blood surrogate
  - Universal media without serum
  - Transport protein
  - Osmolarity
  - Perfluorocarbon or hemoglobin O2 carrier
- Putting organs together and controlling each and all of them
  - Scaling laws revisited
  - Delivering oxygen without excess fluid
  - Controlling metabolic activity
  - Maintaining correct salinity
  - Preventing, controlling or utilizing oscillations
  - Utilizing Fisher randomized multiparametric questionnaires
- Accounting for missing organs
  - Adding missing compounds
  - Removing compounds that would be metabolized by missing organs
- Modeling of coupled organ systems
  - Multiphysics to design
  - PK/PD of drugs in multiorgan systems
  - Inverse models for date interpretation
  - Learning from regulatory noise
- How do we diagnose health vs disease?
- What will a milli/microHuman cost?
- Utilizing organs on a chip
- How accurate a mHu or µHu can we produce?

Wikswo *et al.,* IEEE Transactions on Biomedical Engineering, 2013

Tissue Chips Challenges 2018

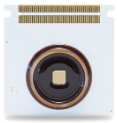
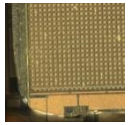
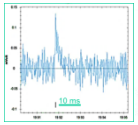
VIBRE

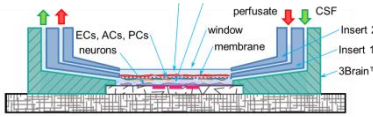
- Human iPSC-derived neuronal cells
  - Reduce costs
  - Shorten time from patient to iPSC to mature phenotypes
  - Develop genotype libraries
  - Learn how to control iPSC differentiation

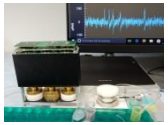


Waddington 1957

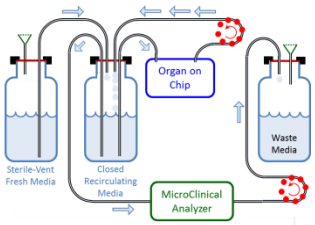
- Bioreactors
  - Reduce volumes
  - Vascularize
  - Eliminate PDMS
  - Add electrodes to the NVU

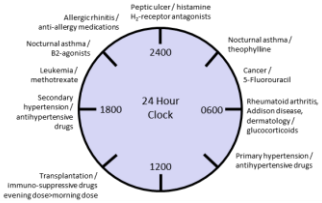




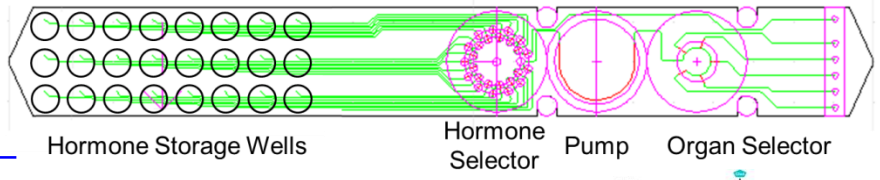




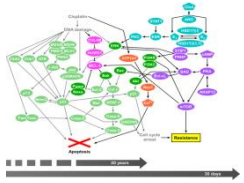
- Control hardware
  - Reduce volumes
  - Reduce size and cost
  - Recirculate
  - Add diurnal hormone and nutrient variations







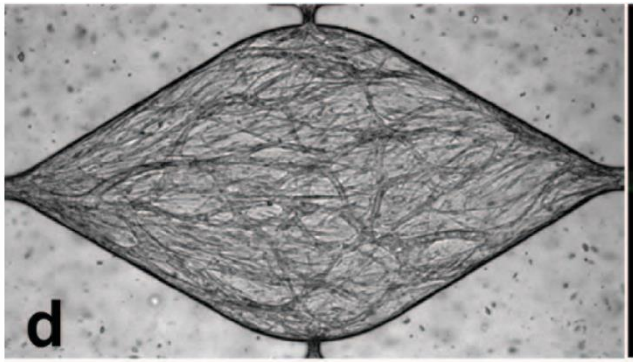
- Analytical chemistry & metabolomics
  - Reduce volumes
  - Detect more analytes on-line at lower cost
  - Infer metabolic and signaling networks

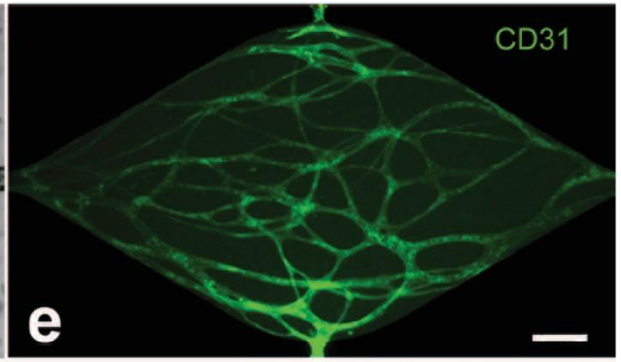


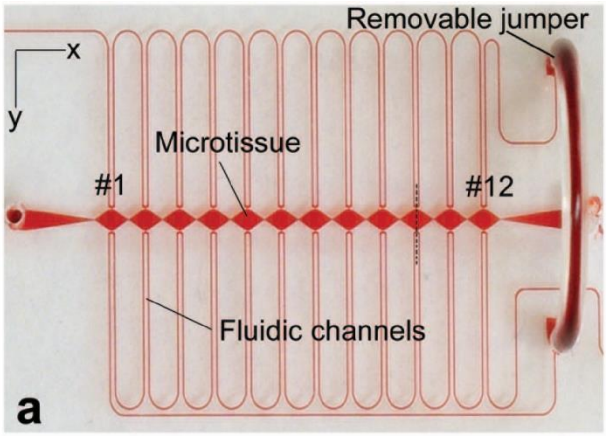
- Translation
  - Make it cost-effective and easy for conventional biologists, toxicologists, and pharmacologists to use organs on chips without a gigantic capital investment or an engineering degree
  - Start answering medical questions and solving medical problems

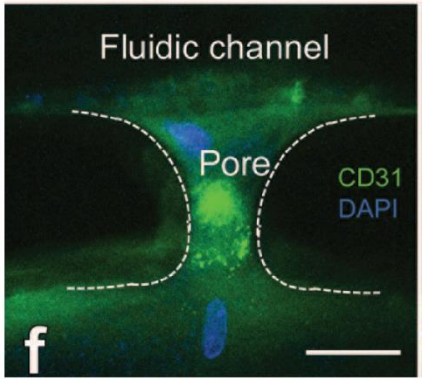
Self-assembling perfused microvasculature in a microfluidic device (George & Hughes, UCI)

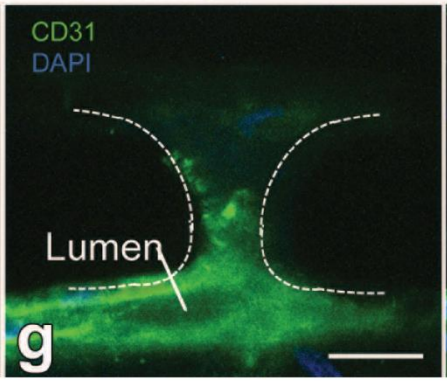
VIBRE

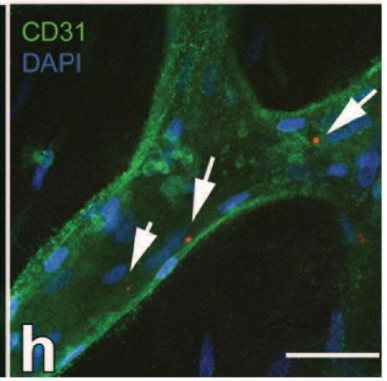


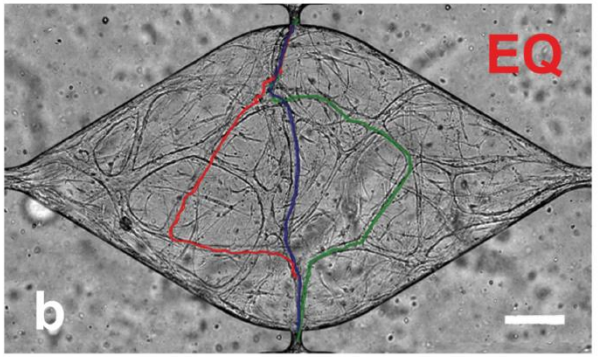


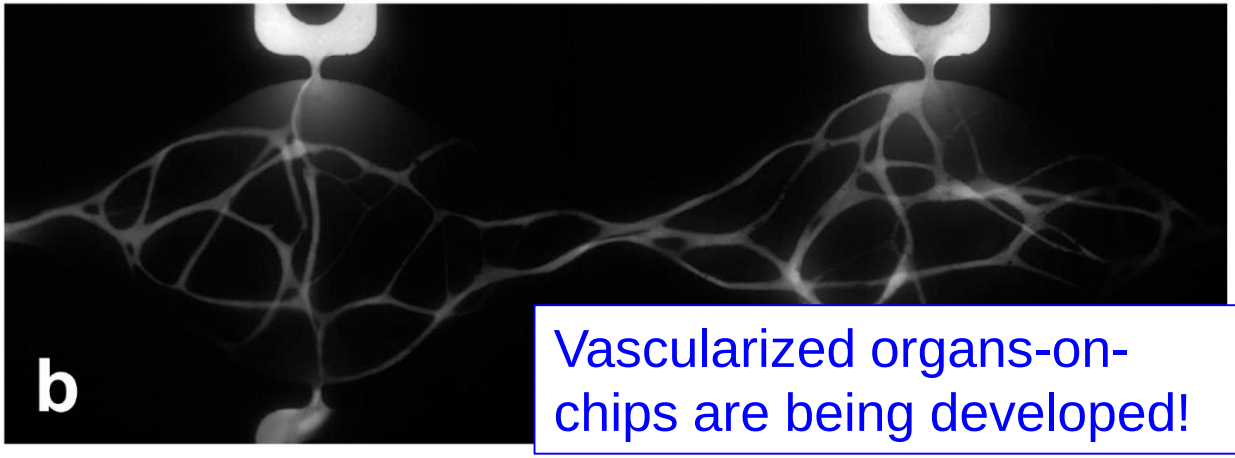


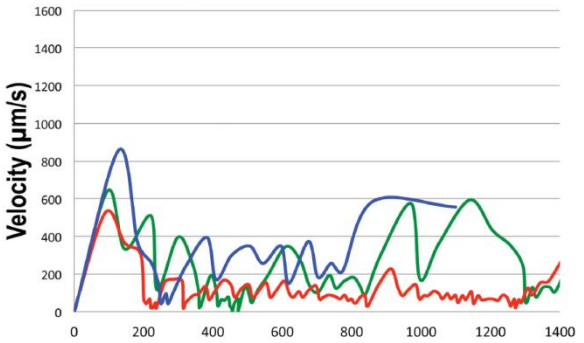










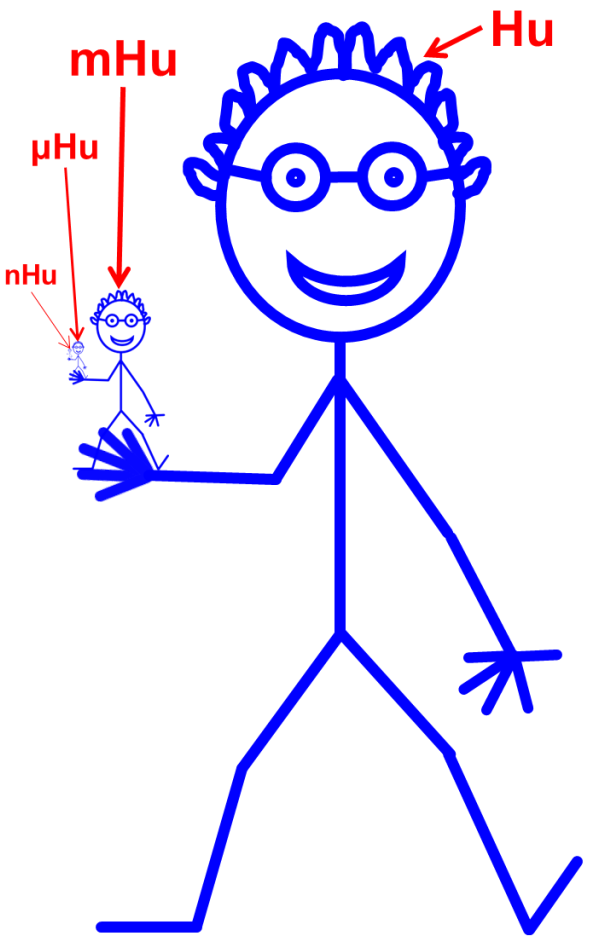


Vascularized organs-on-chips are being developed!

Moya et al., TissEngC, 2013

How kind of model do Pharma need?

- Model type
  - *In vivo*
    - Animal
    - Human
  - *In vitro*
    - Cell
    - Tissue
    - Organ
    - Multi-organ
  - Mathematical
    - Exact, bottoms-up, microscopic functional
    - Top-down, phenomenological
    - Effective
    - Toy
  - Hybrid *in vitro* and mathematical
- How do we use it?
  - Understanding physiology
  - Clarifying a specific mechanism of action
  - Predicting response to drugs and toxins
  - Guide stem cell differentiation
  - Guide cyber drug design
  - Interpret untargeted data
  - Providing a compact representation of a subsystem in a larger synthesis

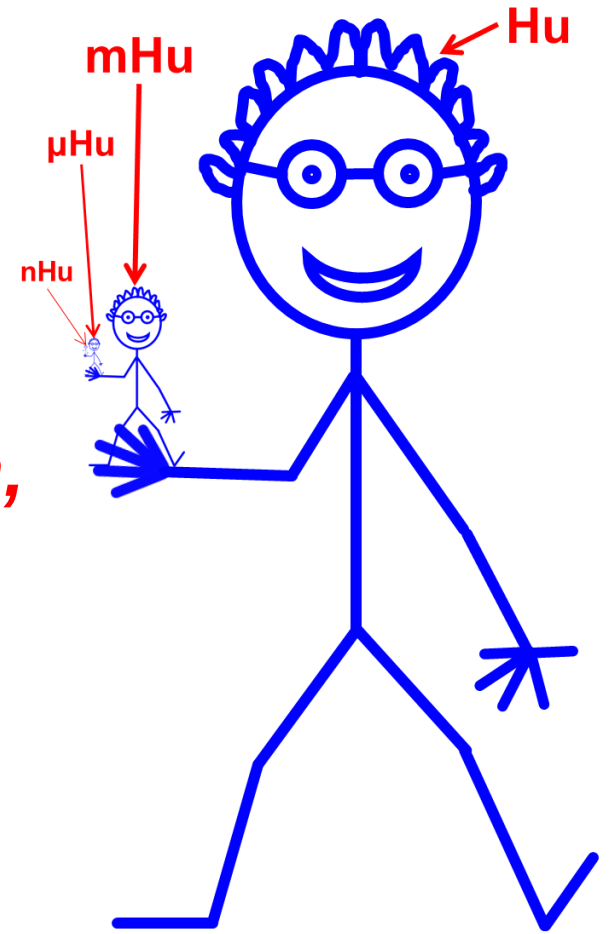


How good a model do we need?

- 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

Which is better?

A. A non-human model that is physiologically incomplete, *e.g.*, rat hepatocytes in a dish.

B. A human model that is physiologically incomplete, and of questionable phenotype, *e.g.*, immortalized, cancer-derived human hepatocytes in a dish.

C. A human model that is physiologically incomplete, and may not be functioning normally, *e.g.*, primary human hepatocytes in a dish.

D. A non-human model that has complete functioning physiology, *e.g.*, a mouse, a rat, or a non-human primate.

E. A model that has fully human physiology but is physiologically incomplete, *e.g.,* a human liver chip.

F. Coupled human organ chips.

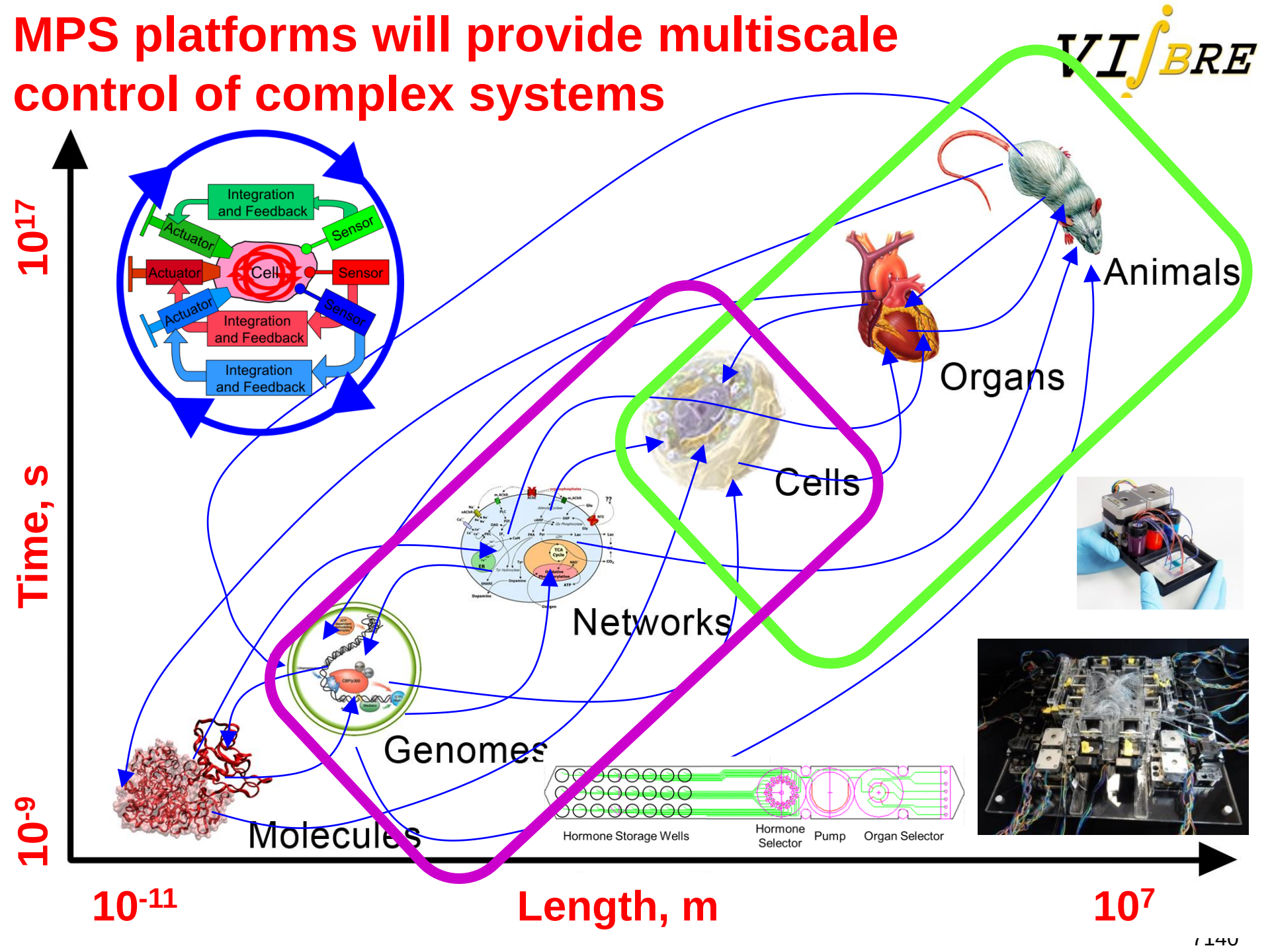
The answer depends upon your budget and your question!

My hypothesis

There will be a major shift in the topology of biological experimental apparatus when the size and portability of modular analytical instruments and system controllers for MPS studies reaches that of a well plate and their cost approaches $100.

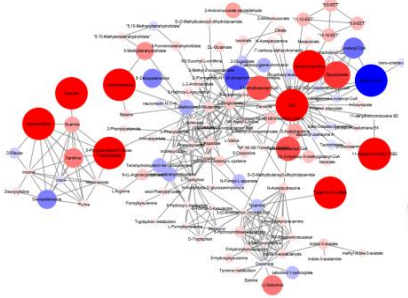
- Instruments will be consumables.
- Each experiment will have dedicated hardware.
- Massively parallel, closed-loop, automated 3D MPS tissue experiments can be made at a realistic cost.
- This will advance drug development and toxicology!

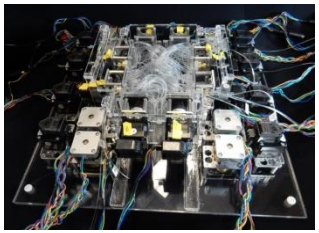
MPS platforms will provide multiscale control of complex systems

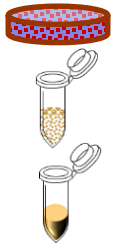


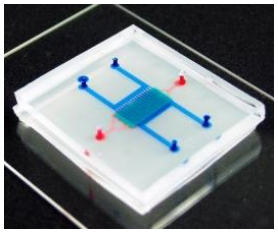
Today's goal: Explain this convergence

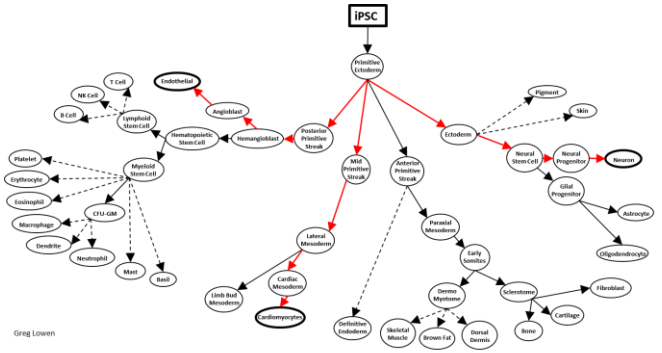
VIVABRE

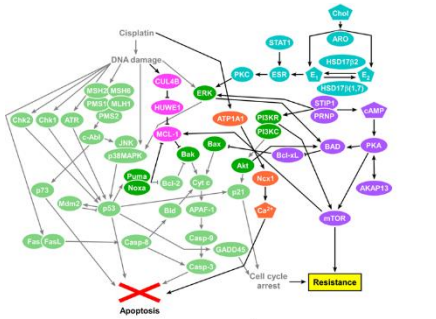


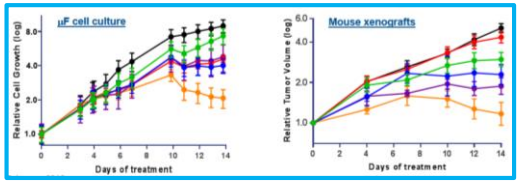


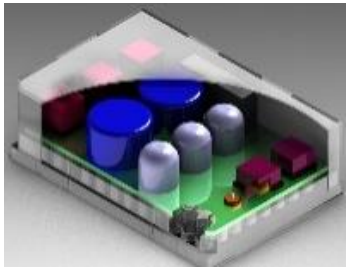


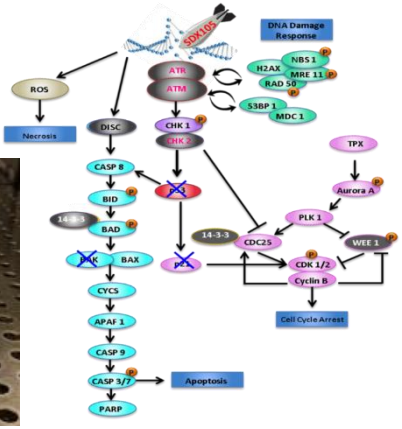


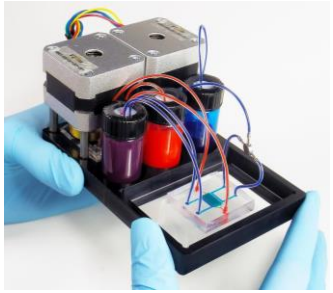


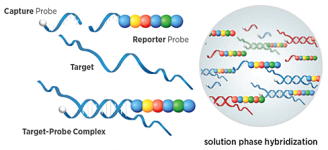












VIIBRE Organ-on-a-Chip Collaborators

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**

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CFD Research Corporation

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- Mark Donowitz

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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**

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