FutureTox-5 V

New Technologies to Evaluate Organ-Specific Effects of Drugs and Chemicals
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In Silico Models of Organ-Specific Effects

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DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

Context: *cell-based in silico models for biosimulation and predictive toxicology*

- Vast collections of bioactivity data from *in vitro* chemical profiling (ToxCast/Tox21) now in hand (https://comptox.epa.gov/dashboard).

- These datasets provide a resource to examine cellular and molecular determinants of organ-specific toxicity (but are convoluted).

- Integrative models are needed to drive biomolecular lesion(s) into higher levels of biological organization for mechanistic prediction.

Virtual reconstitution of a self-organizing system from unidimensional data (embryogeny) remains a challenge for consideration of biological plausibility for causation.

Purpose-built *in silico* microsystems: *autonomous cellular 'agents' in a shared microenvironment that can sustain a biological processes.*

Anatomical homeostasis in a self-regulating 'Virtual Embryo'

Shared by Tim Otter, from Andersen et al. (2006) Am. Assoc. Artif. Intel.

- Morphogenesis is a complex process that causes a tissue to develop its shape from a genetic blueprint and self-regulating (autopoietic) spatial distribution of cells.

- Of paramount importance:
  - genetic, environmental factors influencing cell fate decisions
  - mechanisms by which they are executed
  - biomechanical forces at the cellular level
  - multicellular coordination.

Cellulome: characterization of the network of cells that form an organ system is central for understanding it's development and disease.

Cellular Agent-Based Models (ABMs)

- Nature-inspired *agents* (cells) and *rules* (behaviors) are set into motion as a self-organizing virtual system, using an open-source modeling environment (**CompuCell3d.org**).

- Soft-computing uses 'fuzzy logic' to simulate forces or properties governing cell activity where rules are inexact or knowledge incomplete (**computational intelligence**).

- Can change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions fed into the dynamic model from real world data (**mechanistic causation**).

- Probabilistic rendering of where, when and how a particular condition might lead to an adverse developmental outcome (**cybermorphs**).

Computational Morphodynamics: using computational intelligence and quantitative simulation to establish mechanistic causation in modeling the organ-specific effects of drugs and chemicals (toxicodynamics).

Gastrulating embryo: *remarkable example of a self-organizing system*


Embryoid Body

Epiblast
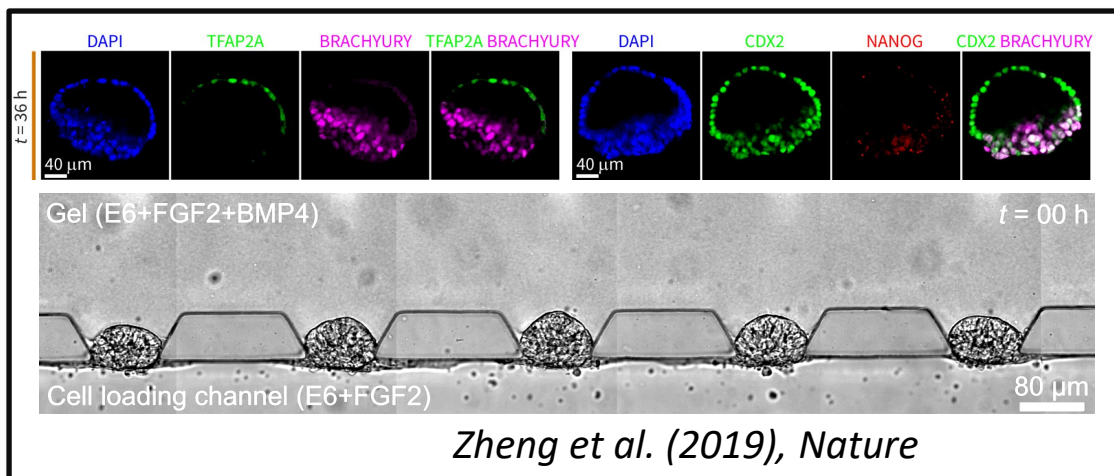
Primitive Streak

Kyoto Collection
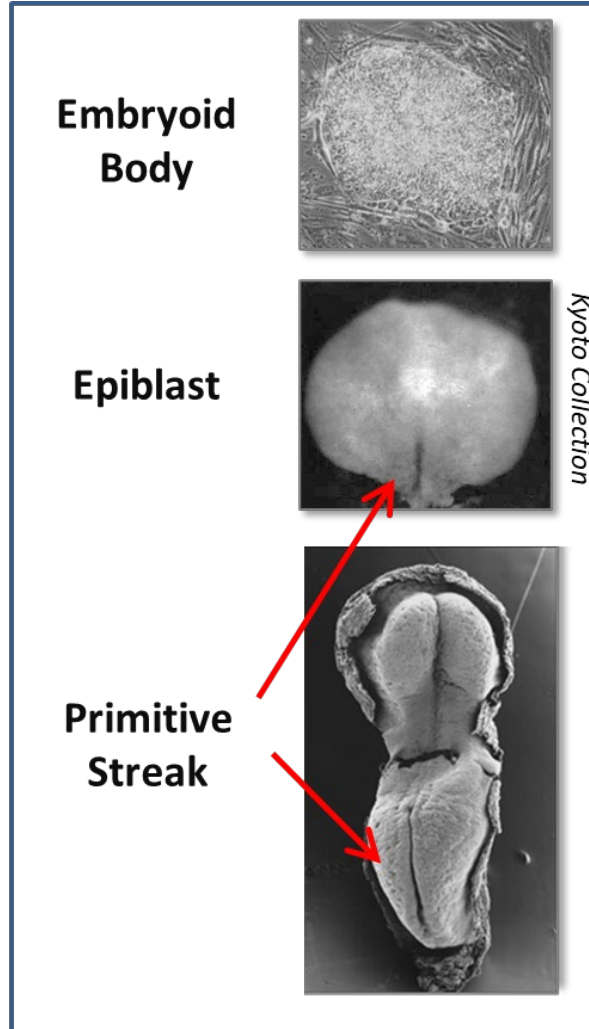
- The molecular biology and behavior of hPSCs in culture most closely resembles the **epiblast** of an early embryo during 'gastrulation'.

- An anatomical hallmark of gastrulation in *Mammals* is the **primitive streak** through which the genomic body plan is established.

- Cell migration through the primitive streak is essential for spatial organization, regional specification, and lineage determination.

- Although cultured hPSCs can form most cell types in the fetus they lack **positional information** of an intact epiblast.

"It is not birth, marriage, or death, but **gastrulation** which is truly the most important time in your life." *- Lewis Wolpert*

Engineered *in vitro* microsystems

Beccari et al. (2018), Nature
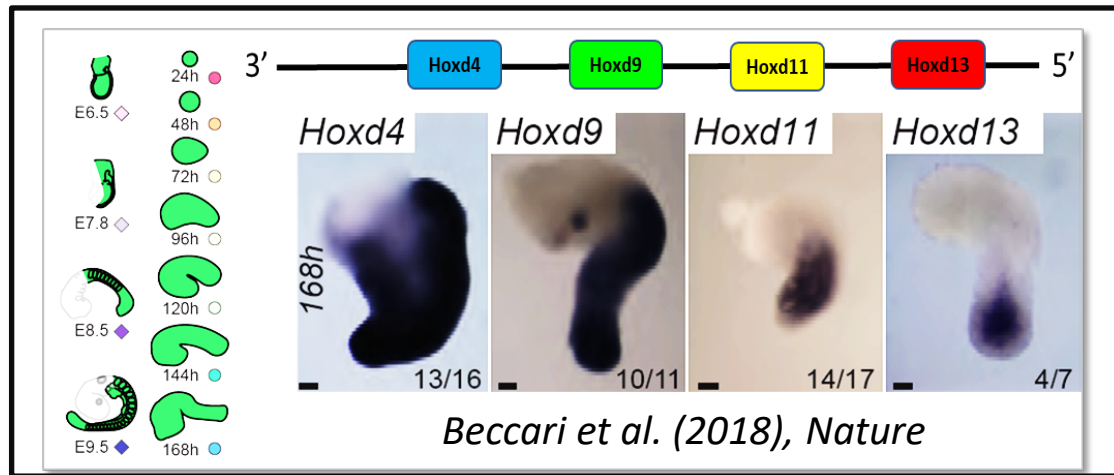
- iPSC-derived microsystems can self-organize at least some positional information.

- **Example**: colinear *Hox* gene expression in 'gastruloids' forming from mESC-aggregate.


Zheng et al. (2019), Nature

- Synthetic epiblast reacts to microfluidic gradients (but no primitive steak forms).

- **Example:** restoring FGF2-BMP4 signaling polarizes a synthetic epiblast from hPSCs.

Computational (*in silico*) epiblast microsystem

Regional fate of endomesoderm

chordo-mesoderm
paraxial mesoderm
lateral plate mesoderm
extraembryonic mesoderm
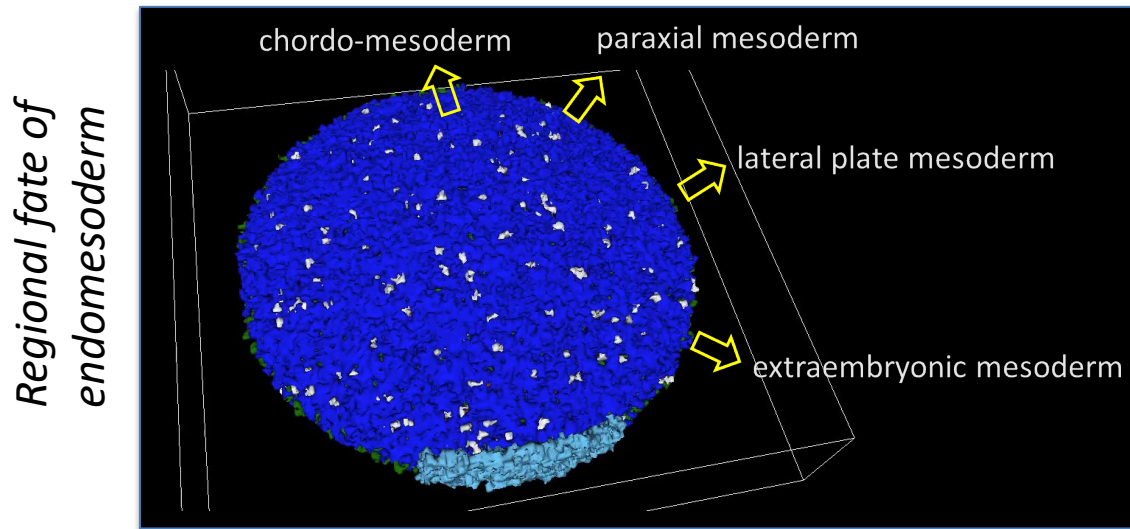
- FGF-BMP signaling network drives cell migration through the primitive streak → mesoderm.

- Timing sets HOX clock for '*decoding the genomic blueprint of the fetal body plan*'.

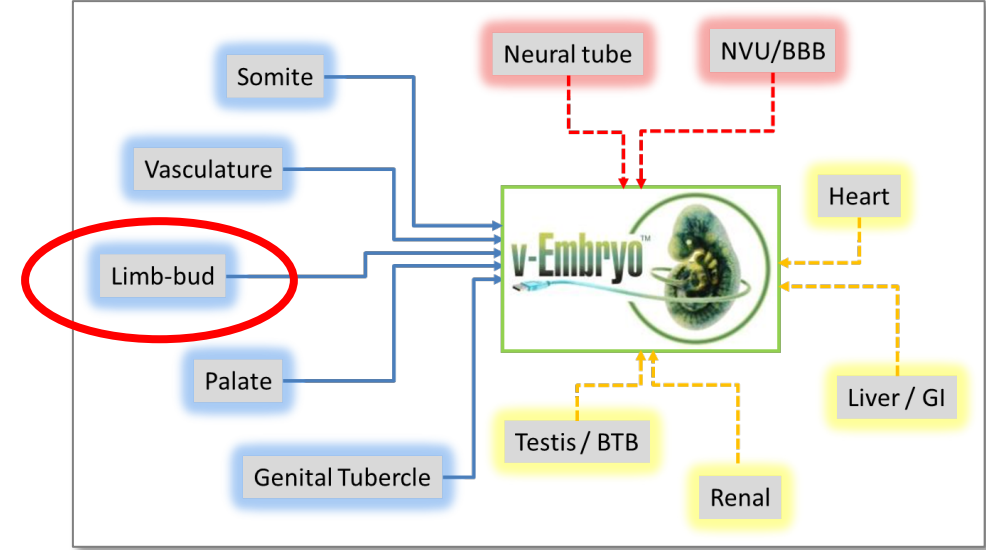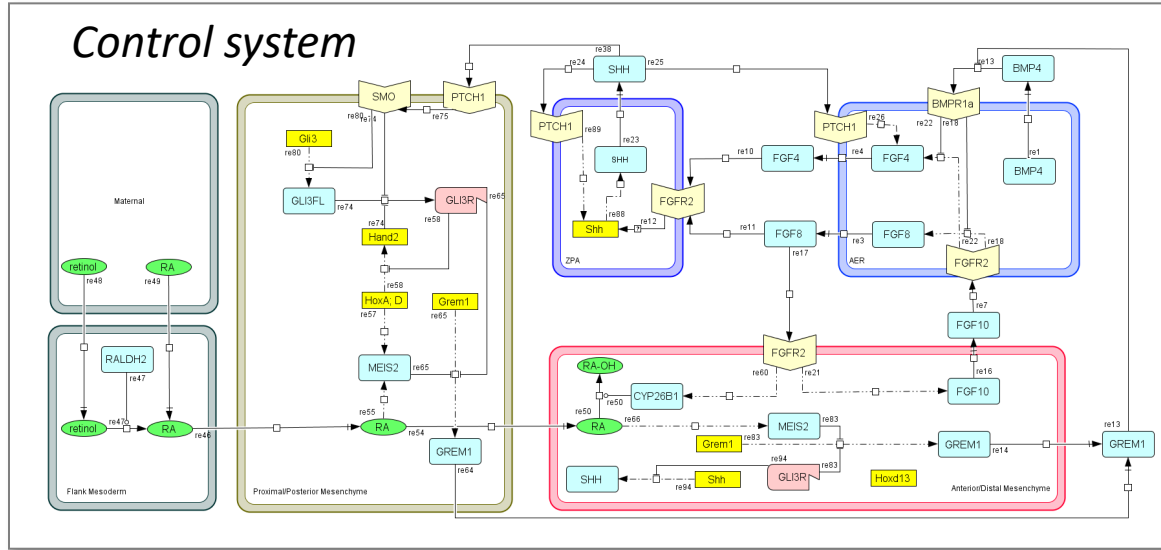- ABM can '*recode the genomic blueprint of the fetal body plan*' for evaluating chemical effects.

Temporal colinearity

3' Hoxd4 Hoxd9 Hoxd11 Hoxd13 5'
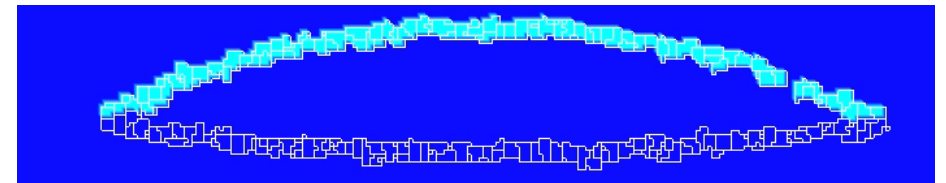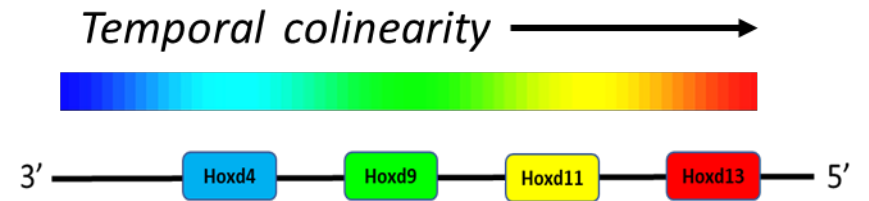
Transverse slice at the organizer node
(4_9_11_13 @5000 MCS)
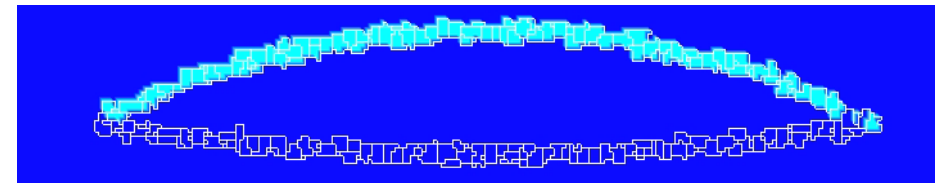
↓FGF signaling slows the Hox clock
(4_9_11_13 @5000 MCS)

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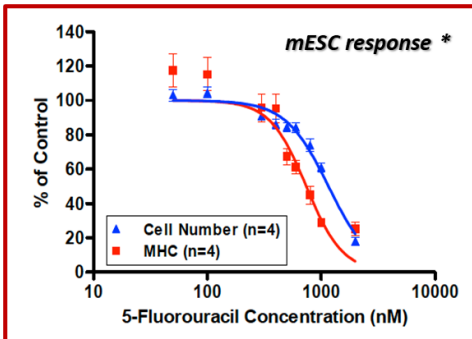# Dynamic knowledge representation: *early limb-bud outgrowth*

EPA/ORD/CCTE control system built with CellDesigner and simulated with CompuCell3D.org
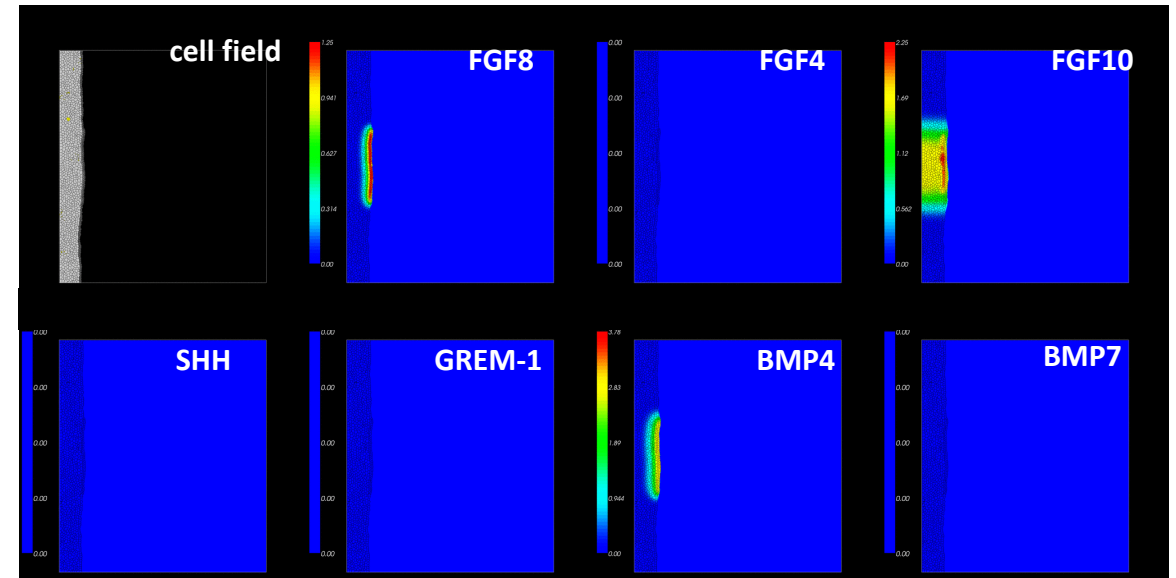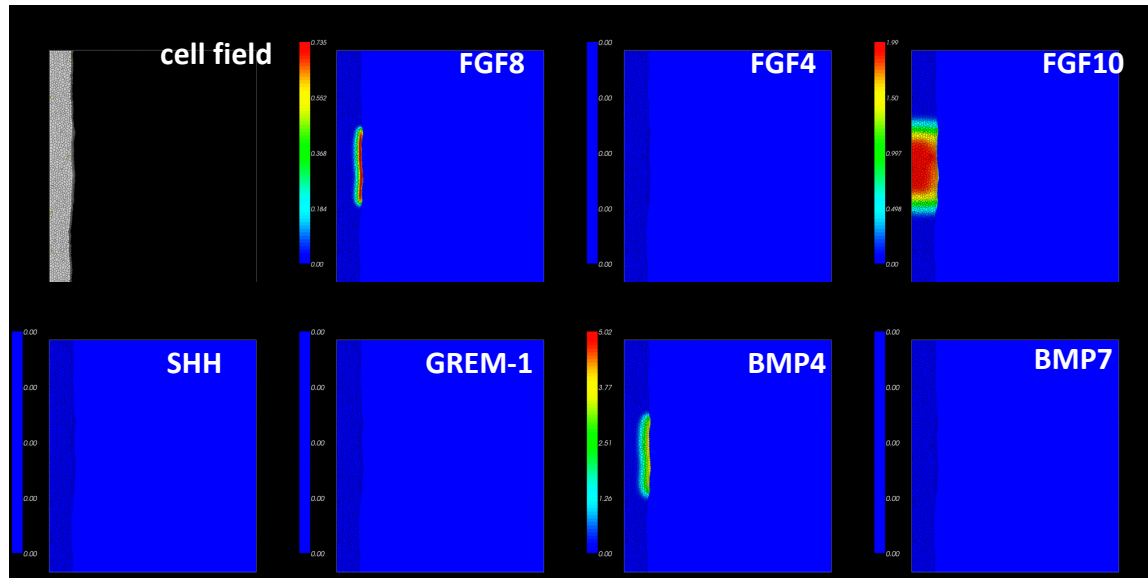
Cybermorphs: *in silico toxicodynamics*

Early limb development (~4-weeks gestation)

mESC response *

* mESC data from S Hunter
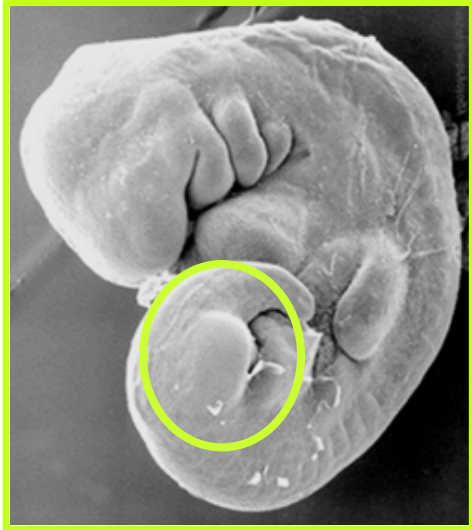
Synthetic dose response (cell level mESC data)

control   weak 5-FU

moderate 5-FU   strong 5-FU
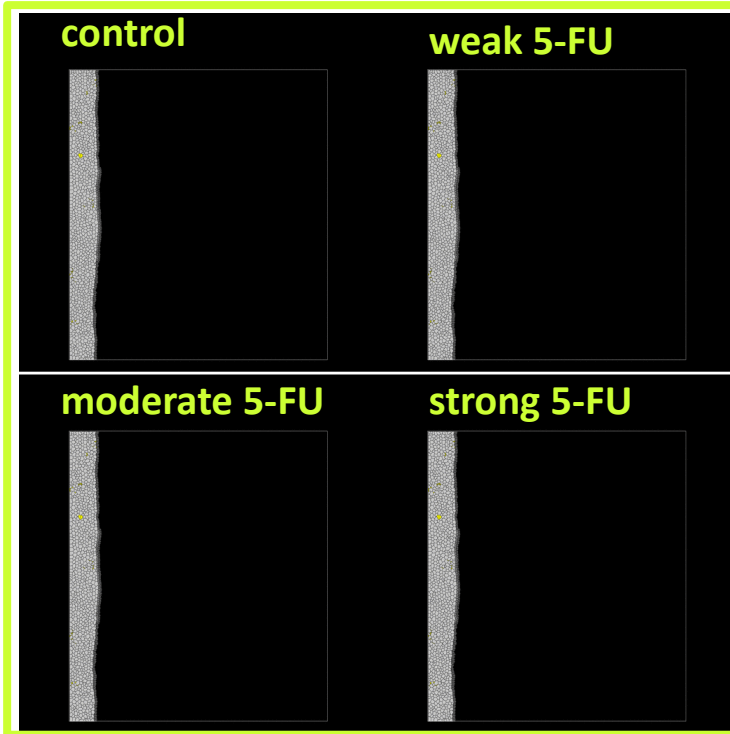
Hacking the network (SHH cybermorphs)

normal   Shh-null

phocomelia

Galli et al. (2010) PLoS Gen
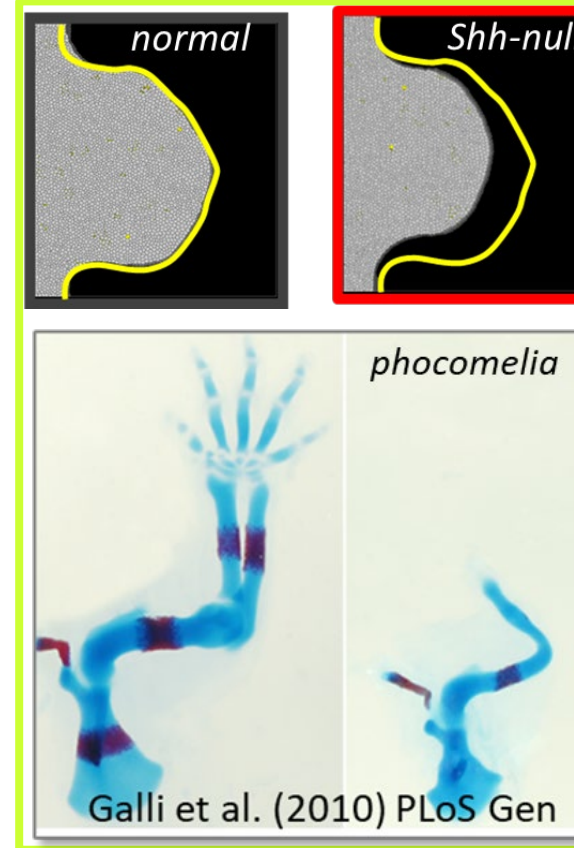
Tweaking the ABM (ATRA overload)

control   ATRA overload

GREM-1   GREM-1

ATRA   ATRA
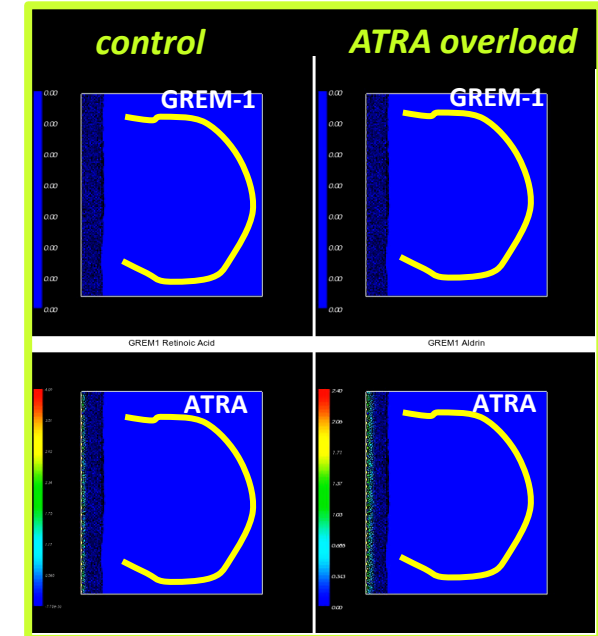
Hacking the model: *medial edge epithelium (MEE) seam breakdown*

K Sulik, University of North Carolina
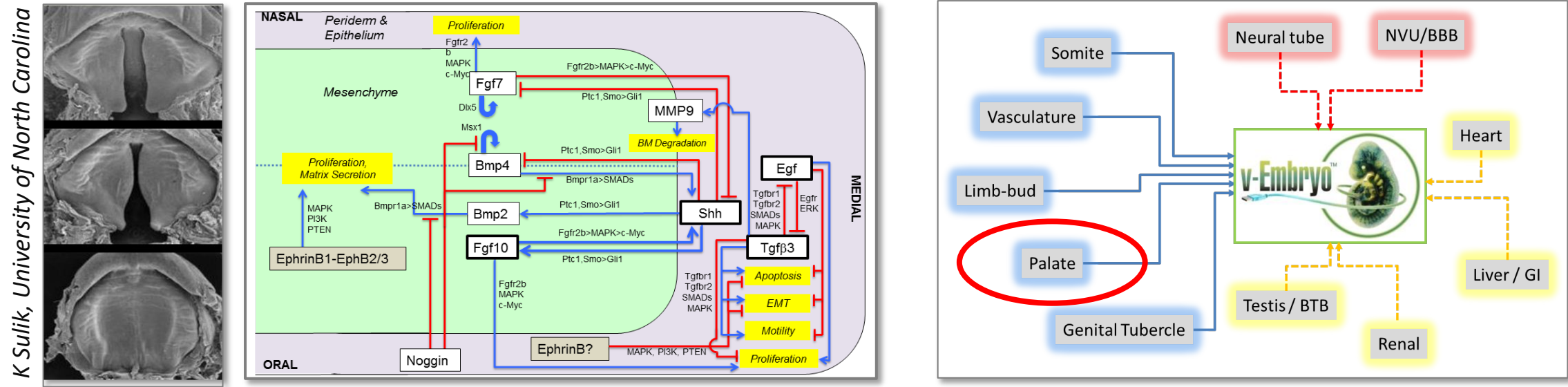
Hacking the control network in silico → cybermorphs

Messin' with the TGFβ/EGF switch (hysteresis)

Hutson et al. (2017) Chem Res Toxicol
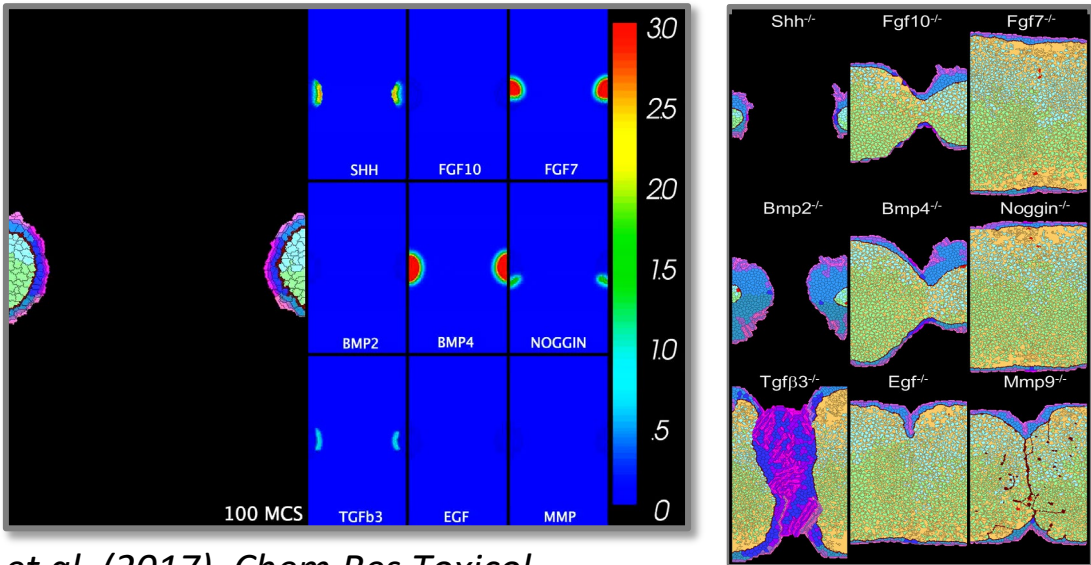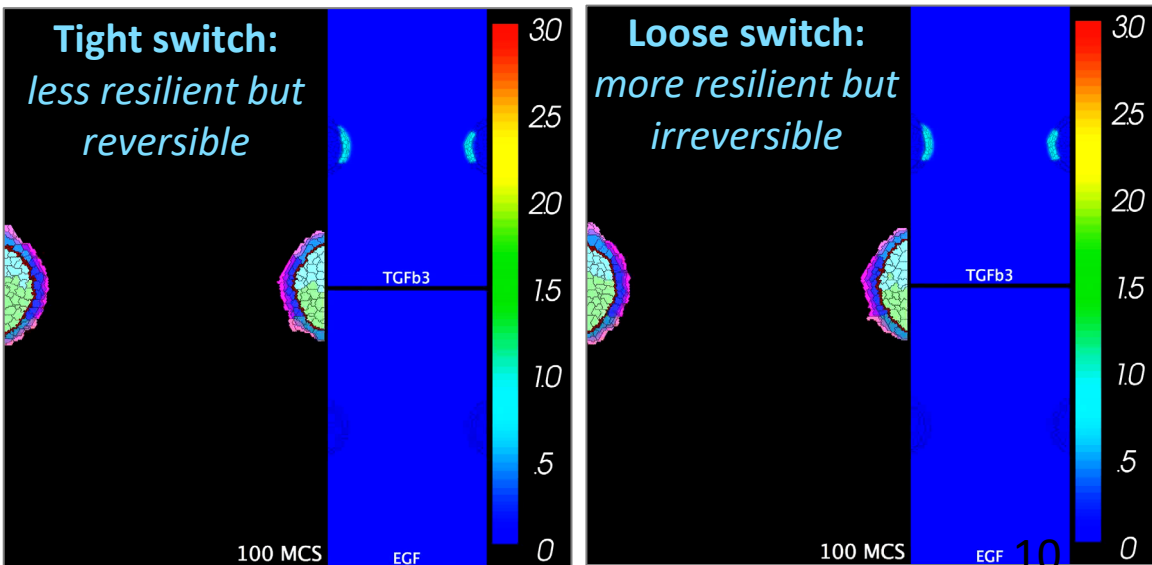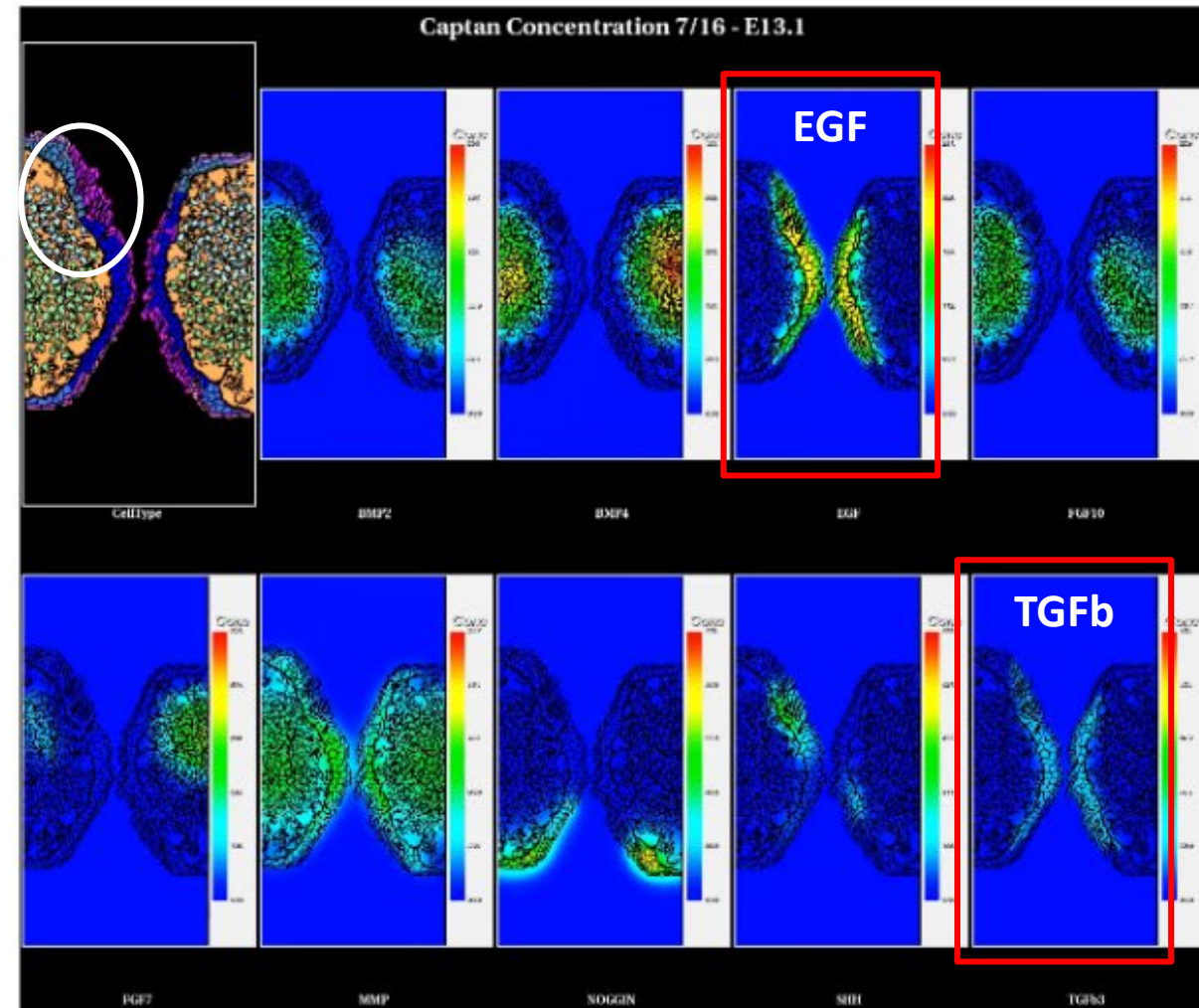
Virtual microscope: *cell signaling (kinematics) and consequences (dosimetry)*

pre-critical dose
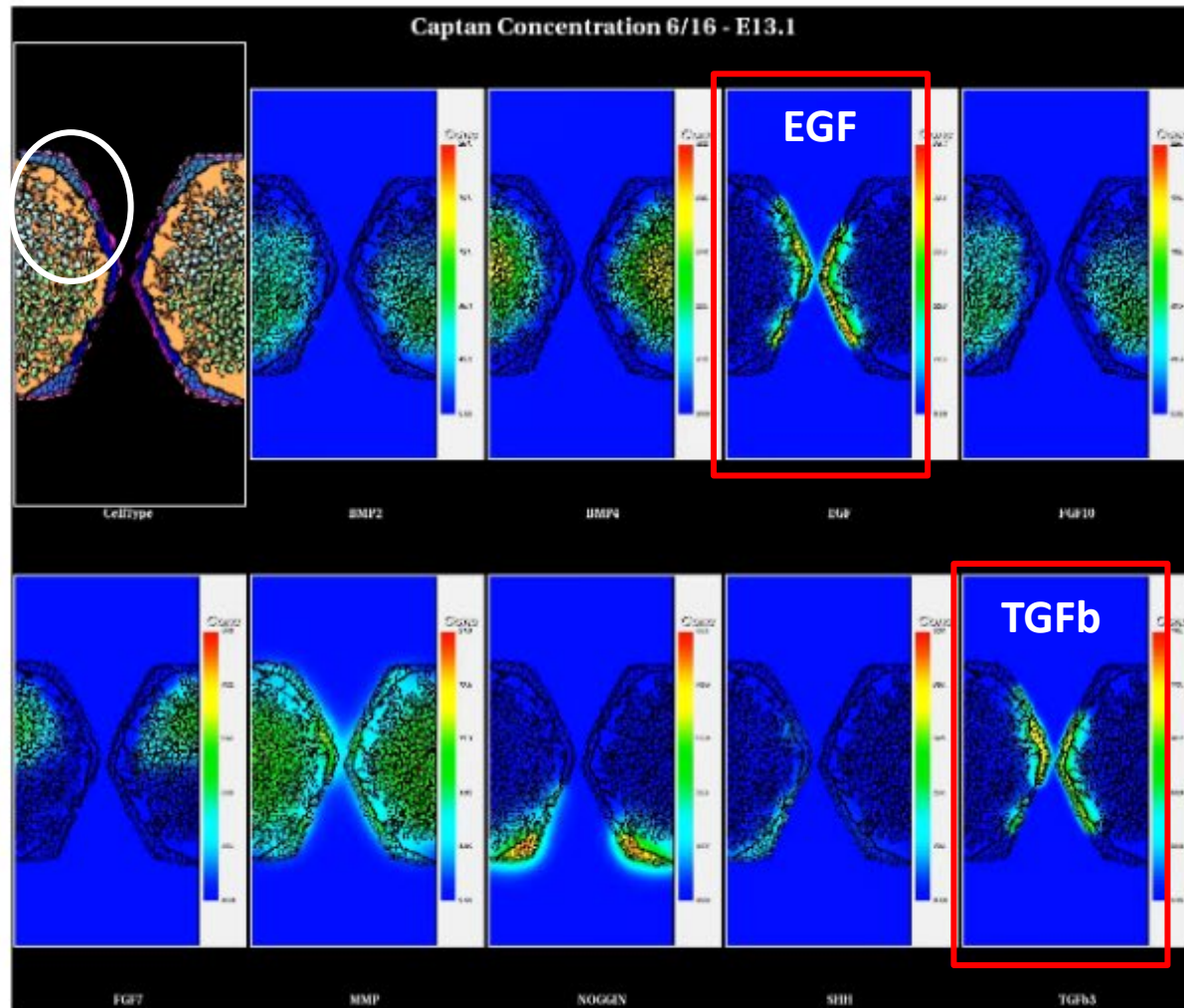
post-critical dose

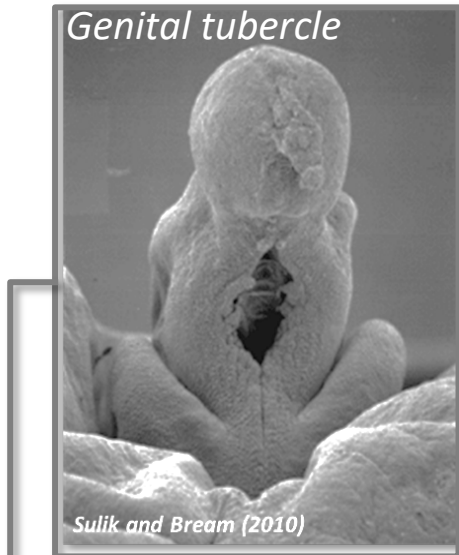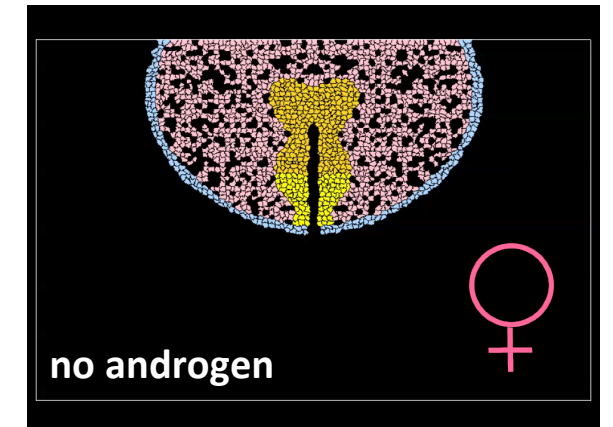# Sexual dimorphism: *genital tubercle morphogenesis*

Genital tubercle

Sulik and Bream (2010)

Apoptosis — Growth

only for eUPE

GLI1/2/3
SMO
PTCH1

UPE/eUPE
FGFR2IIIb
AR

SHH — FGF10 — Androgen

PTCH1
SMO
GLI1/2/3

FGFR1
CM/PM
AR (*only for PM*)

only for CM

Apoptosis — Growth

Somite — Vasculature — Limb-bud — Palate — v-Embryo™

Neural tube — NVU/BBB

Heart — Liver / GI

Testis / BTB — Renal

Genital Tubercle
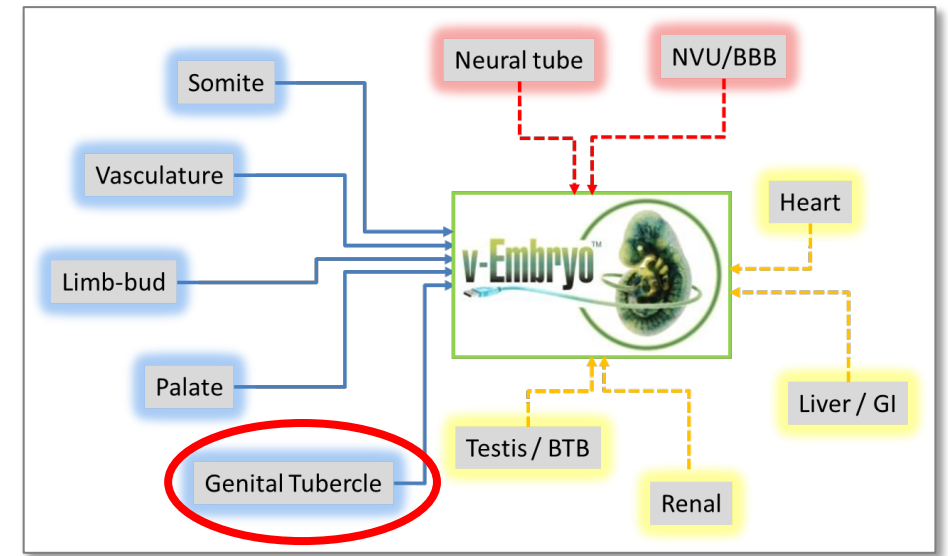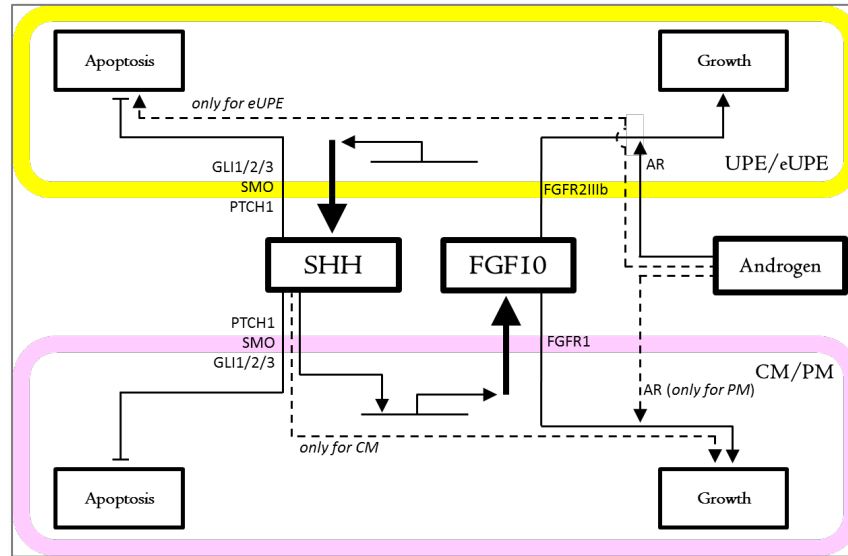
ABM simulation for sexual dimorphism (mouse GD13.5 – 17.5)

androgen ♂

SHH field ♂

FGF10 field ♂

no androgen ♀

Leung et al. (2016), Reproductive Toxicology

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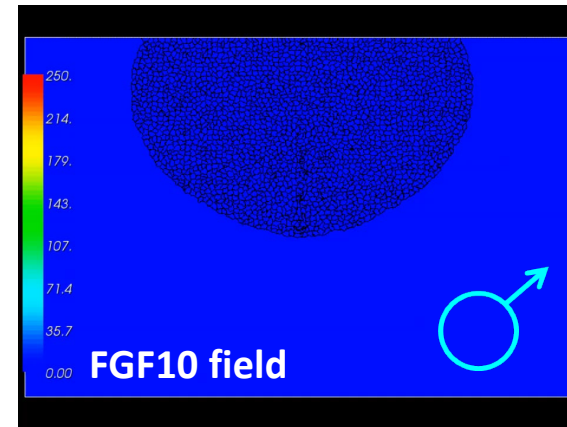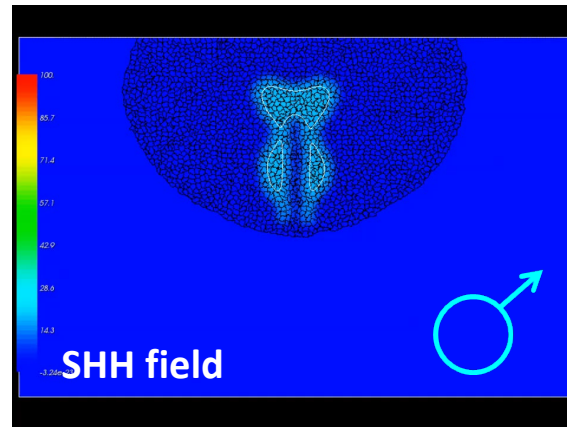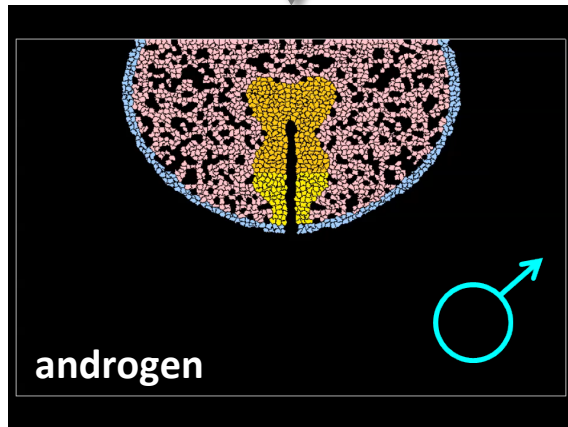
# Androgen disruption: *closure rates @4000 MCS ∫ androgen supply*

100%   67%   33%   0%
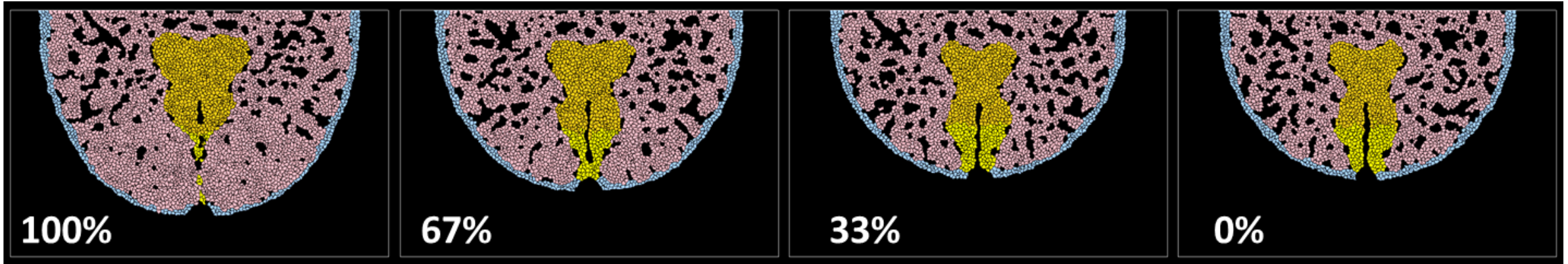
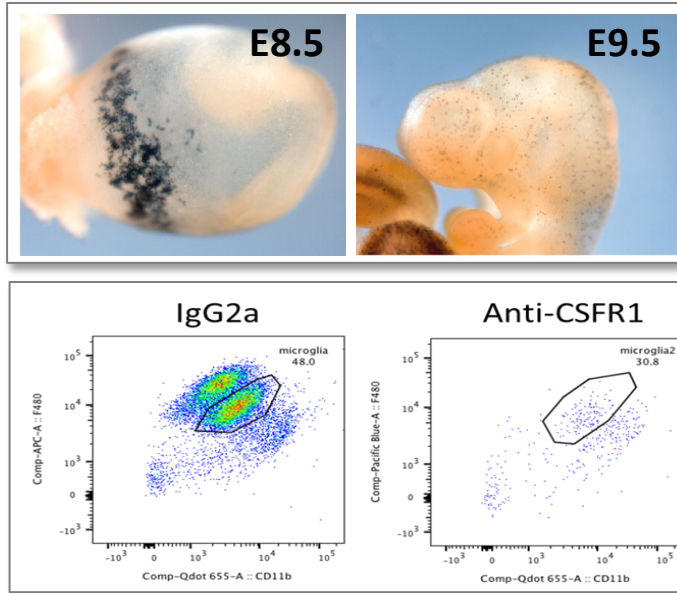*Increased incidence of urethral plate closure defect @ BRR = 33%*

Multi-disturbance plot simulating three individual risk factors for hypospadias:

- genetics (eg, FGFR polymorphism)
- metabolism (eg, ATRA alters SHH, FGF)
- environmental (eg, androgen disrupters)

High risk for hypospadias

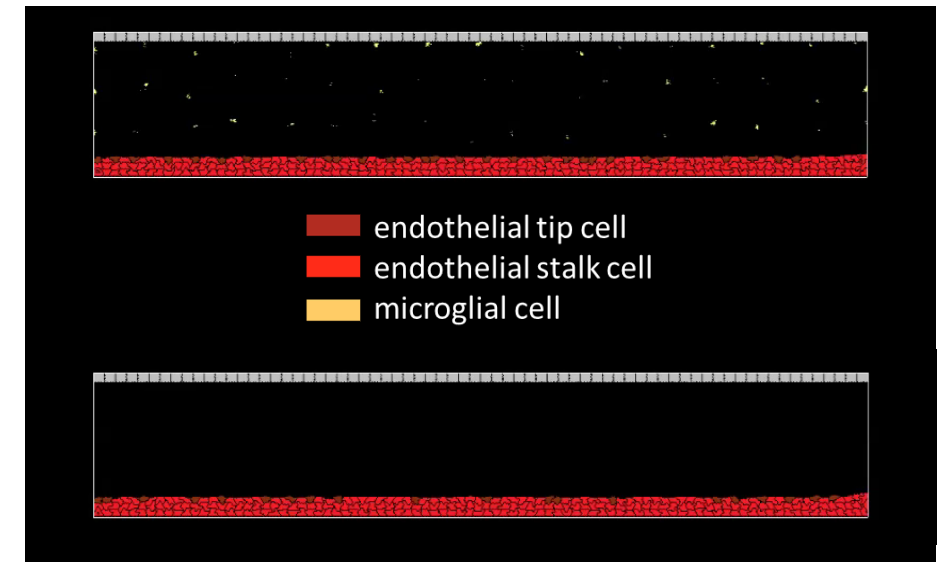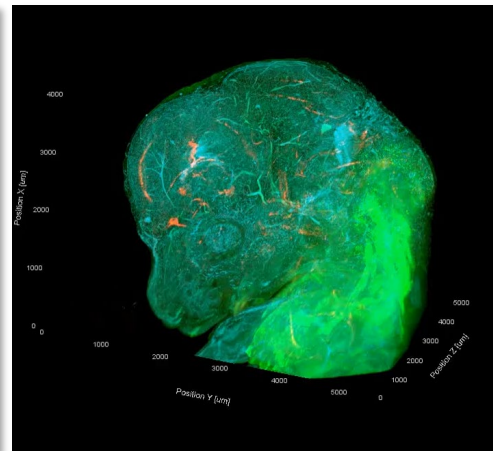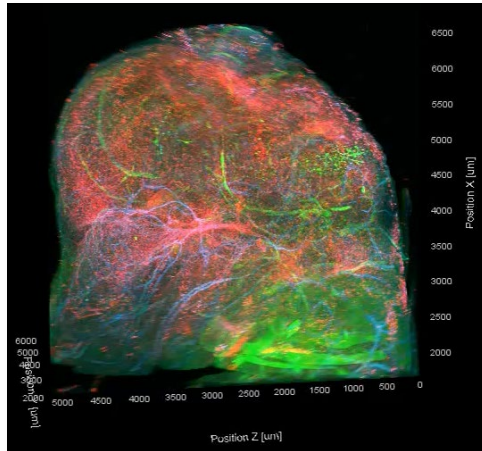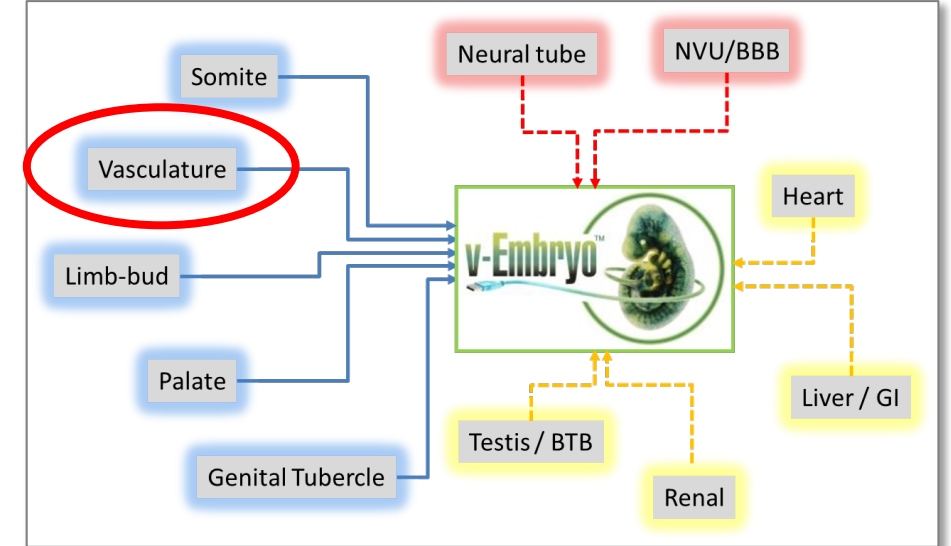*Leung et al. (2016) Reproductive Toxicology*

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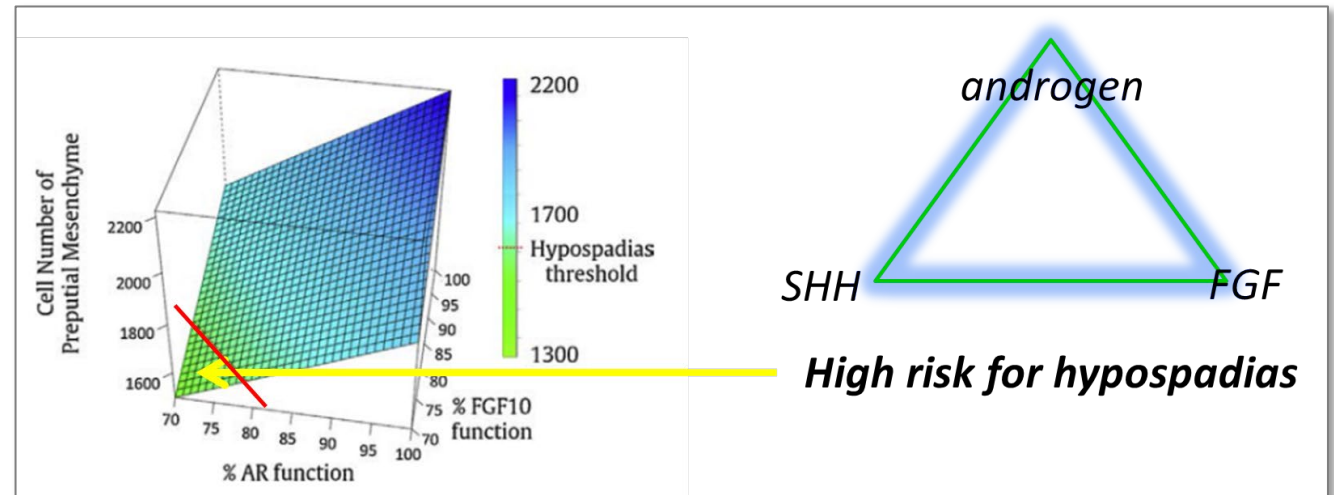
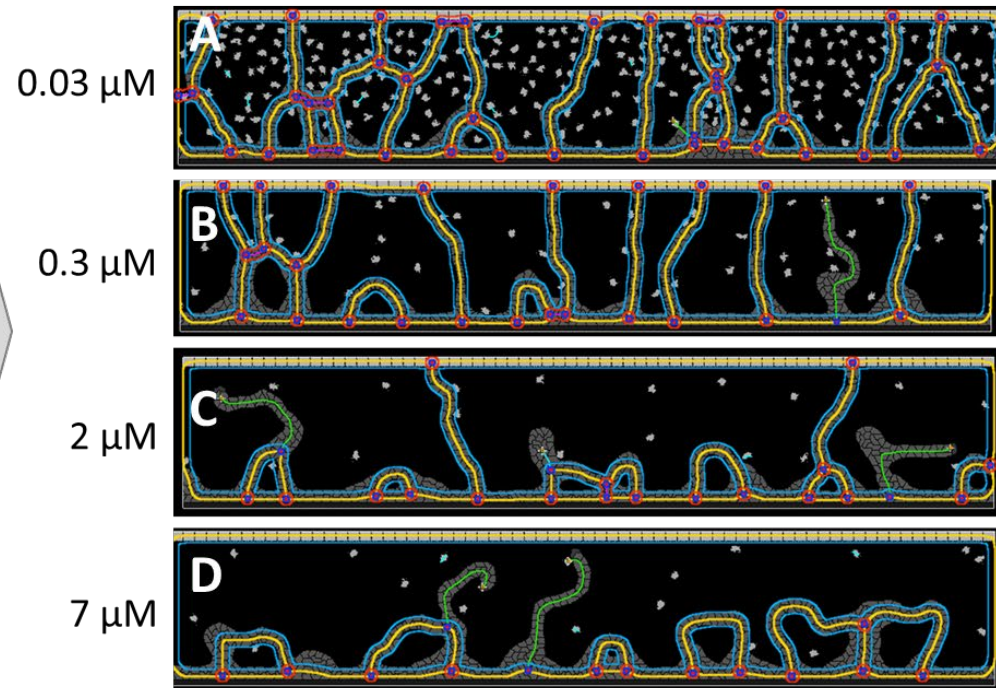# Microglial dynamics: *blood-brain barrier development*

Ginhoux et al 2010, Science
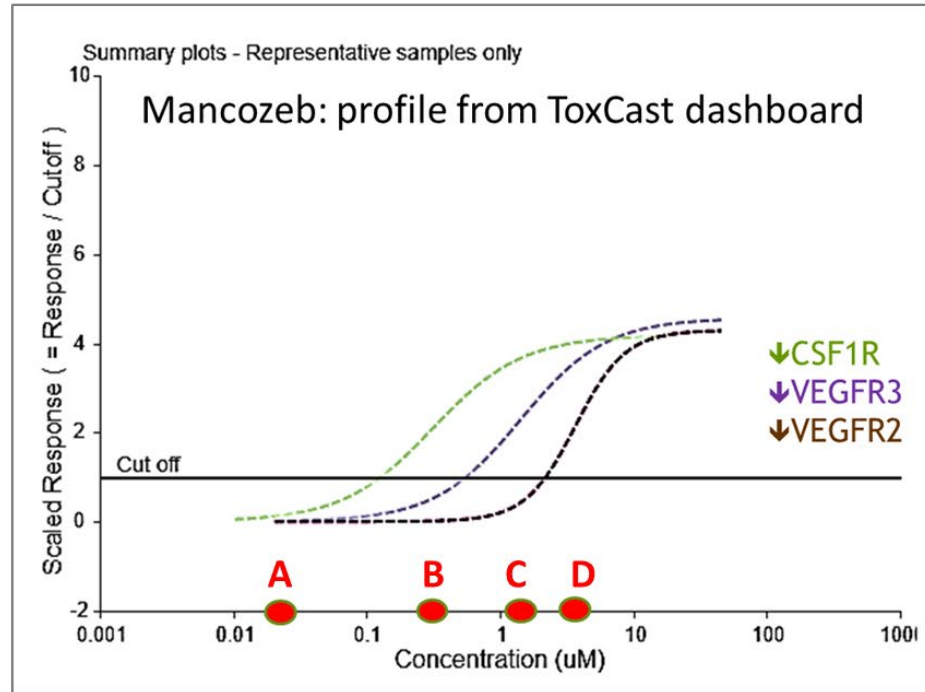
E8.5   E9.5

IgG2a   Anti-CSFR1

Microglia arise in the yolk sac and colonize the neuroepithelium

Experimental microglia deficiency in mouse impairs neurovascular development


Somite — Vasculature — Limb-bud — Palate — Genital Tubercle → v-Embryo™ ← Neural tube, NVU/BBB, Heart, Liver / GI, Renal, Testis / BTB

- endothelial tip cell
- endothelial stalk cell
- microglial cell

Synthetic NVU microsystems: *microglial-vascular-neuronal integration*

Summary plots - Representative samples only

Mancozeb: profile from ToxCast dashboard

- CSF1R
- VEGFR3
- VEGFR2

0.03 μM — A
0.3 μM — B
2 μM — C
7 μM — D

Neuronal layer
Endothelial cells
Microglia

Day 21   Network infiltration

Microglia Migration into Neural Layer

- HTS bioactivity profile for three receptors known to mediate microglial-endothelial interactions (LEL = 0.5 μM).

- Critical effect observed in an engineered PNVP microsystem for microglial migration (LEL = 0.3 μM) [Kaushik et al. (2020)].

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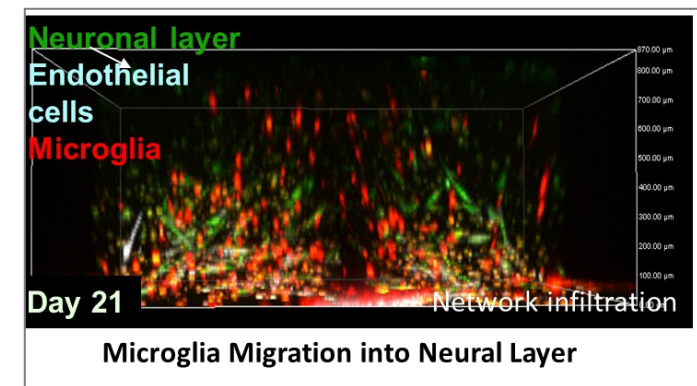
ABMS for *in silico* toxicodynamics

- Computational approach to integrate information generated at one level of biology with concurrent parallel processes to identify critical phenomena in a complex system.

- Different cell types 'inhabit' preorganized structures that resemble tissues and self-organize into emergent phenotypes with minimal explicit programming.

- Dynamic knowledge representation executed bottom-up (agent-by-agent, interaction-by-interaction) tests veracity of presumed mechanisms.

- A fully computable synthetic embryo ('synbryo') may be a distant goal, but modular systems bring spatial biology to life to pinpoint critical phenomena through a virtual lens.

Acknowledgements

Food for thought …

Translational: what do synthetic microsystems of human development - both computational and organoids - bring to future of DART testing?

Investigational: how smart must these models be to support decision-making in the animal-free (3Rs) zone?

Operational: what best practices are needed to implement mechanistic predictions from synthetic microsystems into an integrative decision framework?

Communication: what are the practical considerations for science, engineering, and stakeholder engagement (academics, government, industry, NGOs, policy, …)?