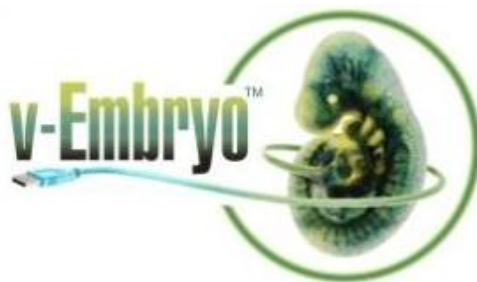


# **Developmental Systems Toxicology: computer simulation in a 'Virtual Embryo' prototype**



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***DISCLAIMER: The views expressed in this presentation are  
those of the presenter and do not necessarily reflect the  
views or policies of the U.S. Environmental Protection Agency***





# Can a computer model of the developing embryo translate molecular and cellular disruptions into a prediction of dysmorphogenesis?

*and if so ...*

How might such models, with high-performance computing, be used analytically (to understand) and theoretically (to predict) adverse developmental outcomes for different exposure scenarios?

*e.g., chemicals, doses, non-chemical stressors, mixtures, stages, sensitive subpopulations, ...*

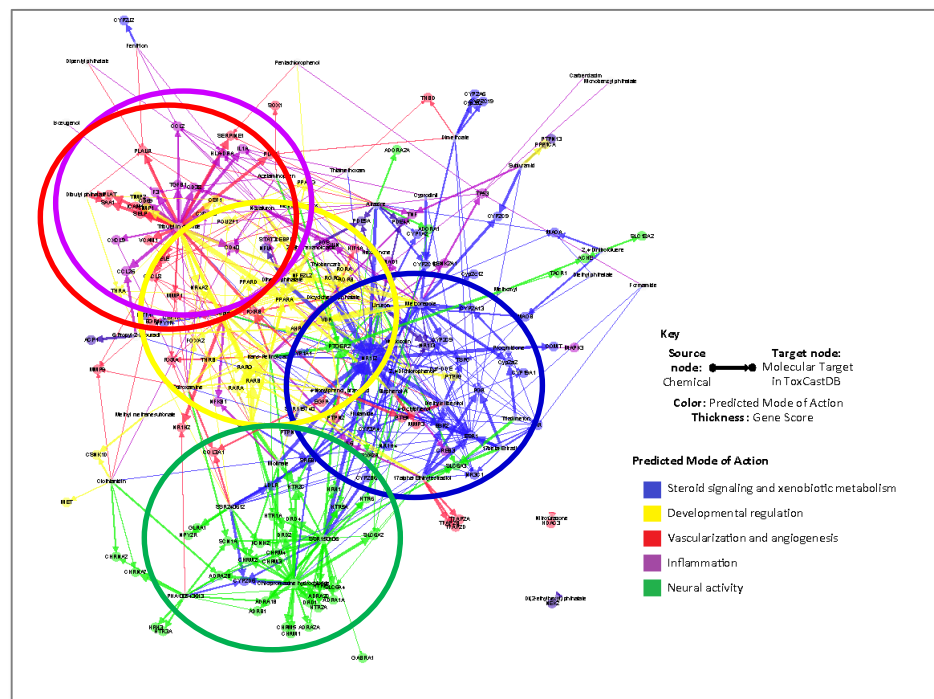


# Strategy

- Build spatially-explicit agent-based models (ABMs) of normal embryology that can simulate developmental systems dynamics.
- Seed computer models with 'big-data' profiles from ToxCast and set up complex and theoretical pathway-level scenarios.
- Run the computer models in parallel to simulate how molecular-cellular dysfunctions could impact tissue structure and function.
- Compare computational outcomes with experimental results to reveal inconsistencies in our understanding of the system.
- Eventually can become part of an integrated testing strategy to address systems-level behaviors not easily studied experimentally.

# Bipartite Network: translates chemical-assay bioactivity profiles into predicted mode-of-action

- Bipartite network for 54 ToxCast chemicals that produce male developmental toxicity.
- Functional annotation revealed 4-5 target biological processes (mode-of-action) from ToxCast assays.

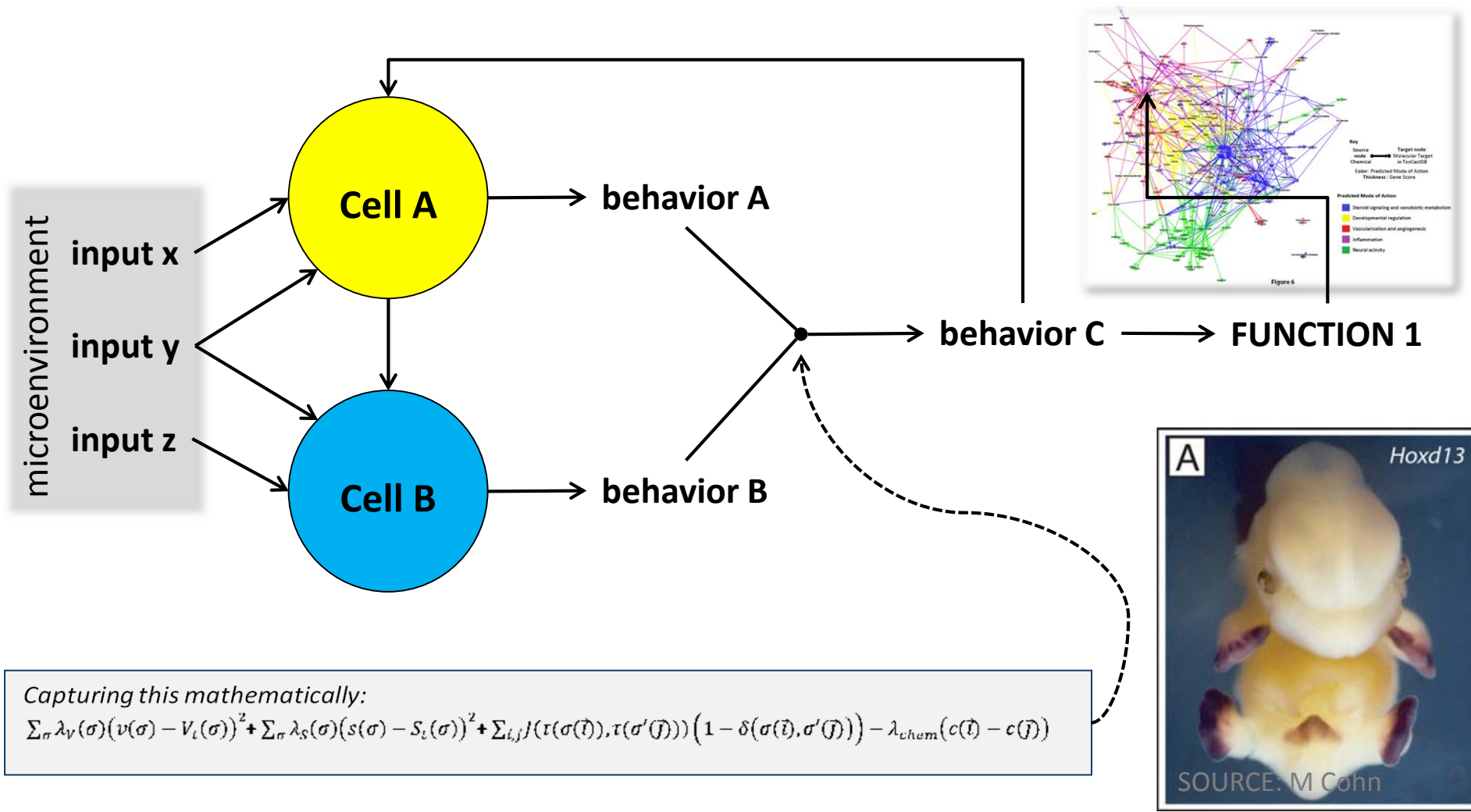


Leung MCK, Phuong J, Baker NC, Sipes NS, Klinefelter GR, Martin MT, McLaurin K, Setzer RW, Darney SP, Judson RS and Knudsen TB (2015) Systems Toxicology of Male Reproductive Development: Profiling 774 Chemicals for Molecular Targets and Adverse Outcomes. Env Hlth Persp (accepted).

## Predicted Mode of Action

- Steroid signaling and xenobiotic metabolism
- Developmental regulation
- Vascularization and angiogenesis
- Inflammation
- Neural activity

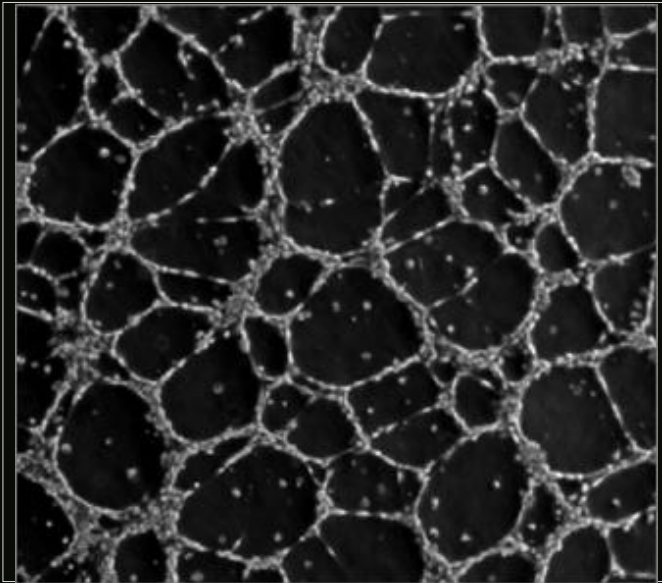
# Cellular Response Network (CRN): how cellular systems translate spatial information into higher-order function



# Cellular Agent-Based Models (ABMs): use in multiscale modeling and dynamical simulation

## EXAMPLE: nascent blood vessel formation

VEGF165  
MMPs  
VEGF121  
sFlit1  
TIE2  
CXCL10  
CCL2



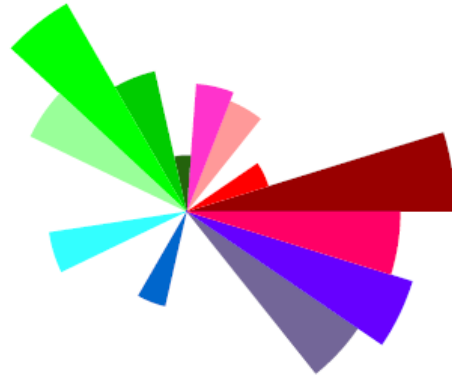
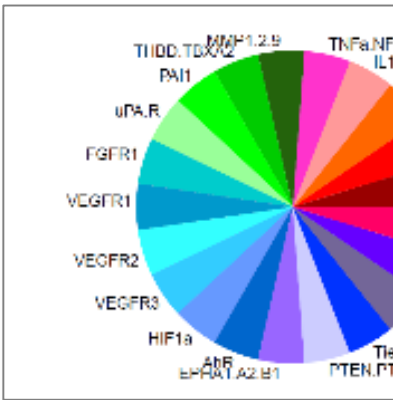
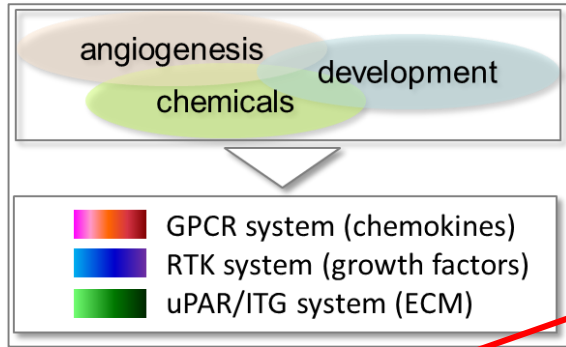
♥ Endothelial Stalk  
♣ Endothelial Tip  
♣ Mural Cell  
♣ Inflammatory Cell

- individual rules are assigned to low-level 'agents' (here = cells)
- agents then interact in a shared environment (CompuCell3D\*)
- running the simulation executes this biology (emergence)
- models run differently each time (stochastic)
- each run reveals one possible solution (outcome)

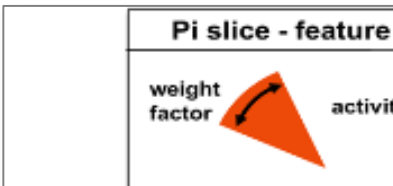
\* *CompuCell3D.org is an open-access environment for cell-oriented modeling developed at Indiana University by J Glazier and colleagues*



**ToxCast:** chemicals sorted by predicted vascular disruption (pVDCs)



**This synthetic thalidomide analogue disrupts microtubule function in endothelial cells of immature blood vessels.**



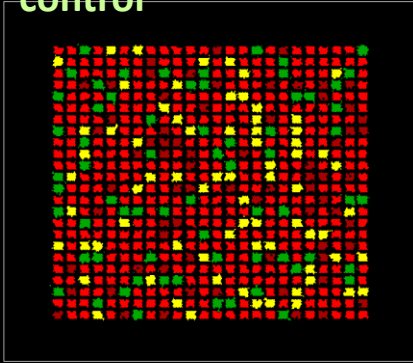
<http://epa.gov/ncn>  
<http://comptox.unc.edu>

5HPP-33 ( 0.327 )

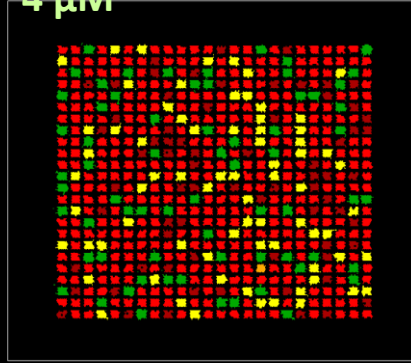


# 5HPP-33 concentration response predicted *in silico* from ToxCast and demonstrated *in vitro* with a human endothelial cell assay

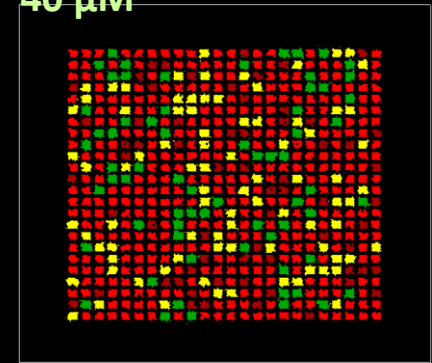
control



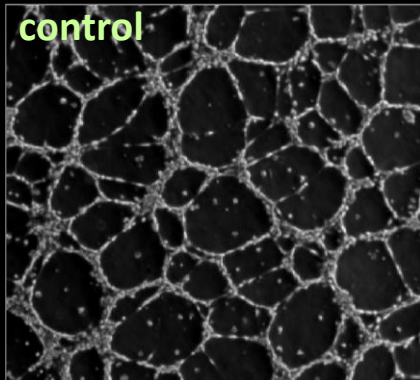
4  $\mu\text{M}$



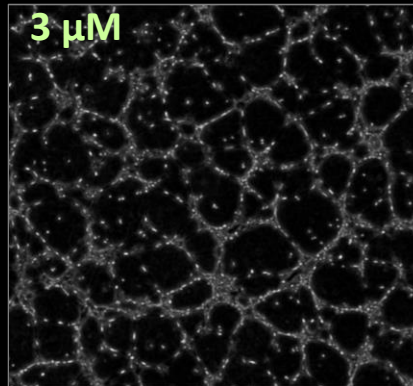
40  $\mu\text{M}$



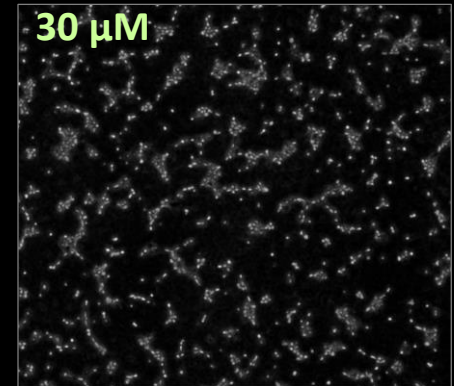
control



3  $\mu\text{M}$



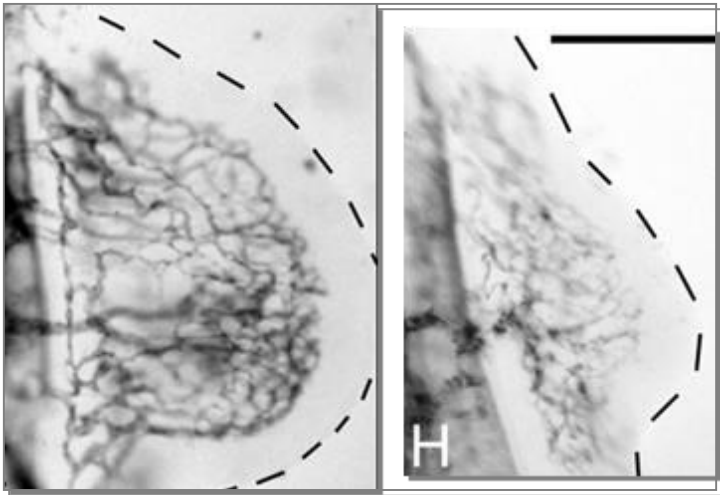
30  $\mu\text{M}$



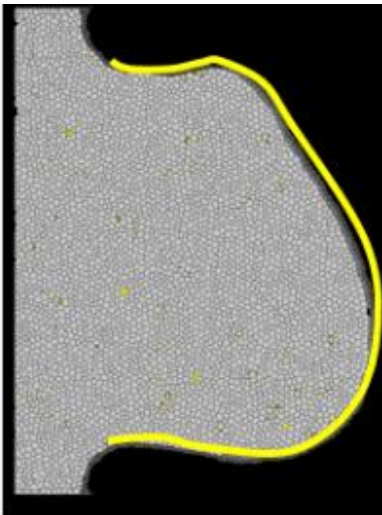


# 'Virtual Embryo' prototype of early limb-bud outgrowth

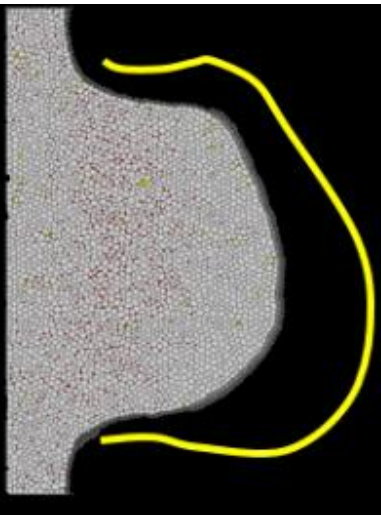
Thalidomide disrupts immature blood vessels *Therapontos et al. (2009)*



*normal*

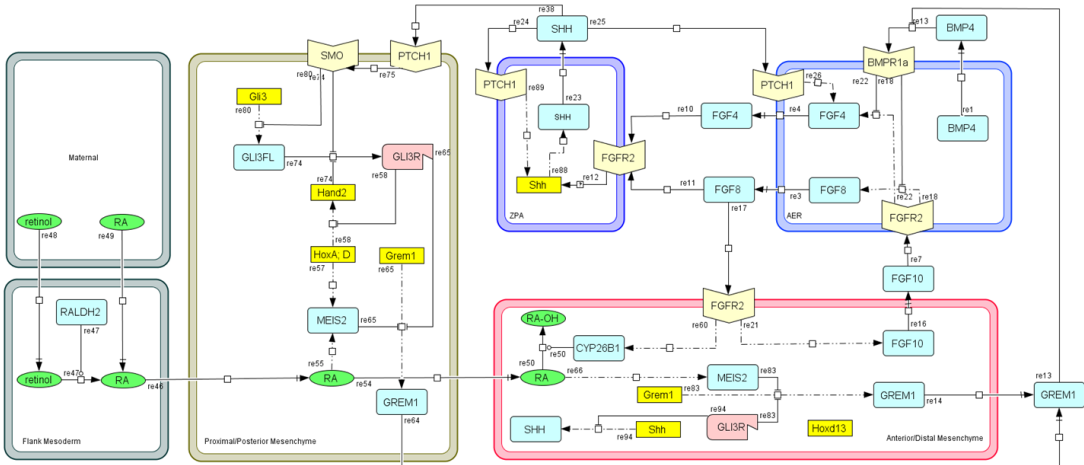
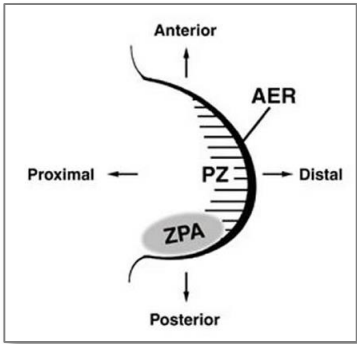


*episodic cell death*

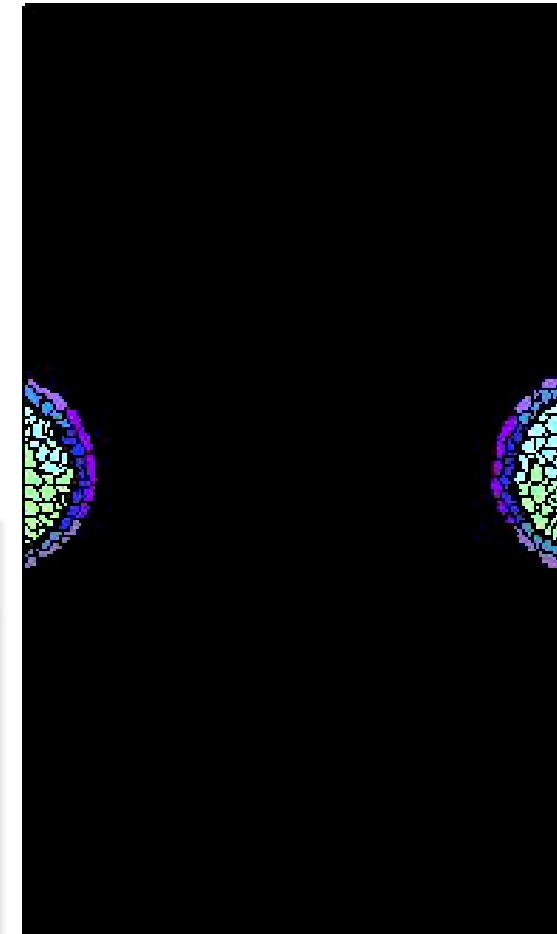
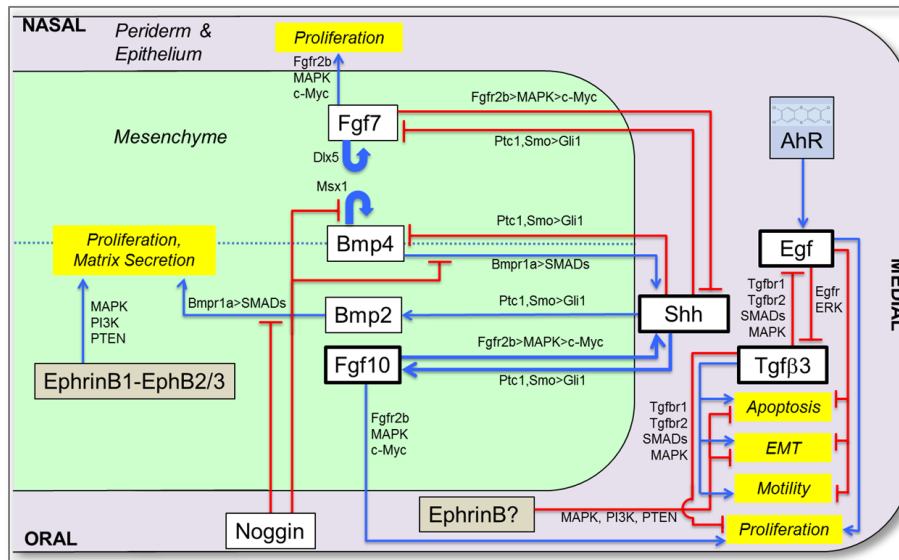
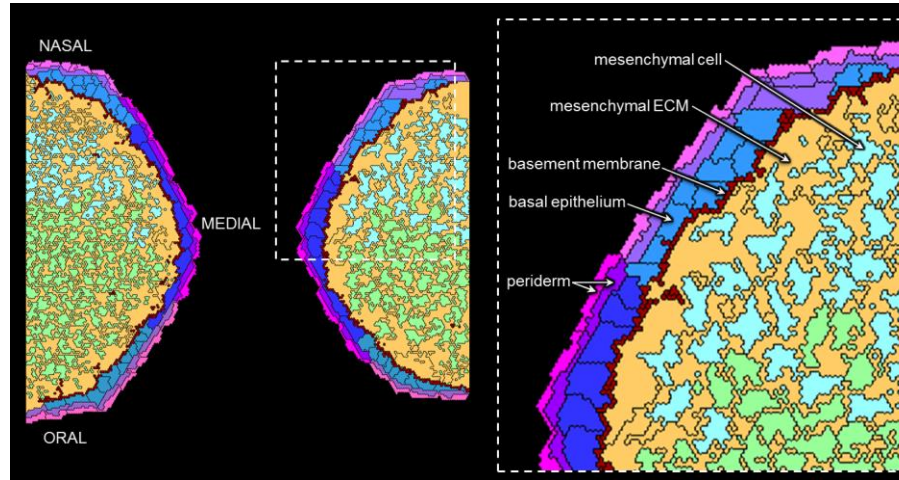
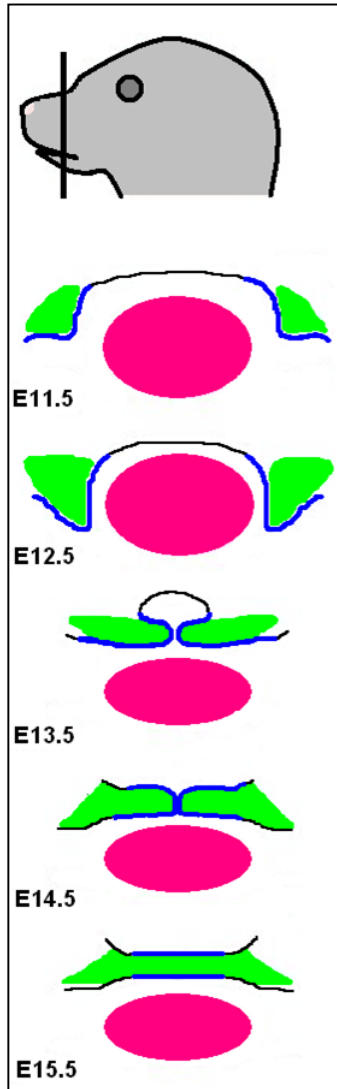


*Dewoskin et al. (in preparation)*

This occurs as the organizing centers are established

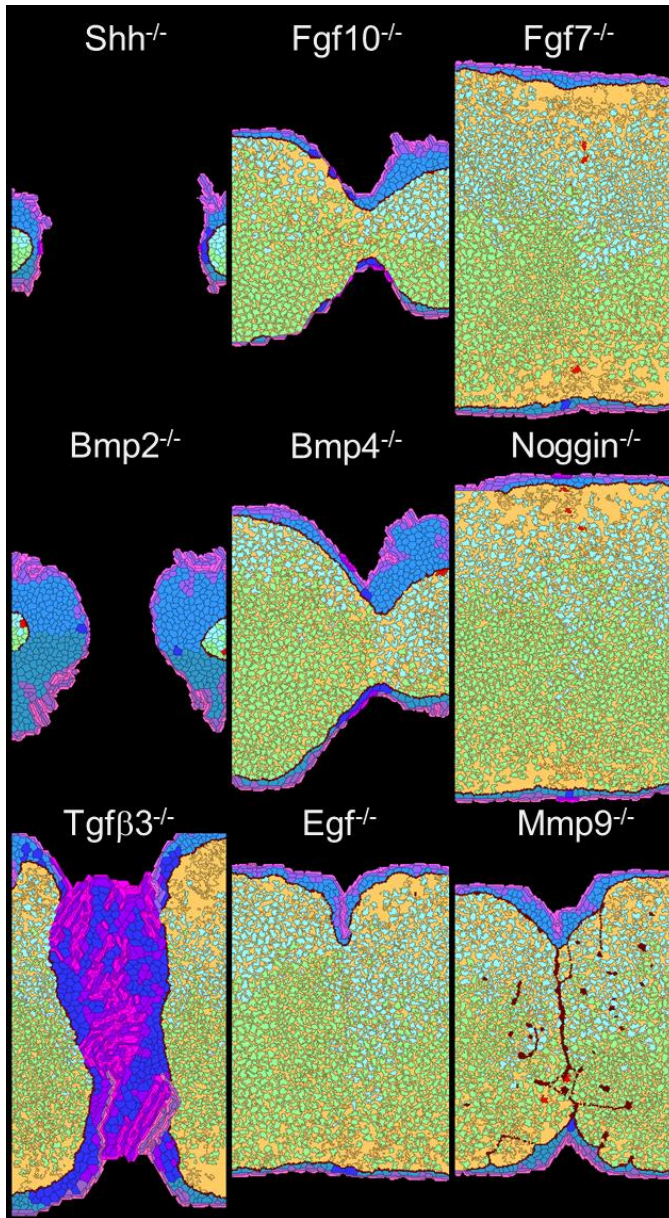


# Palatal Closure: ABM can probe quantitative relationships during Medial Edge Epithelium (MEE) fusion and seam breakdown.

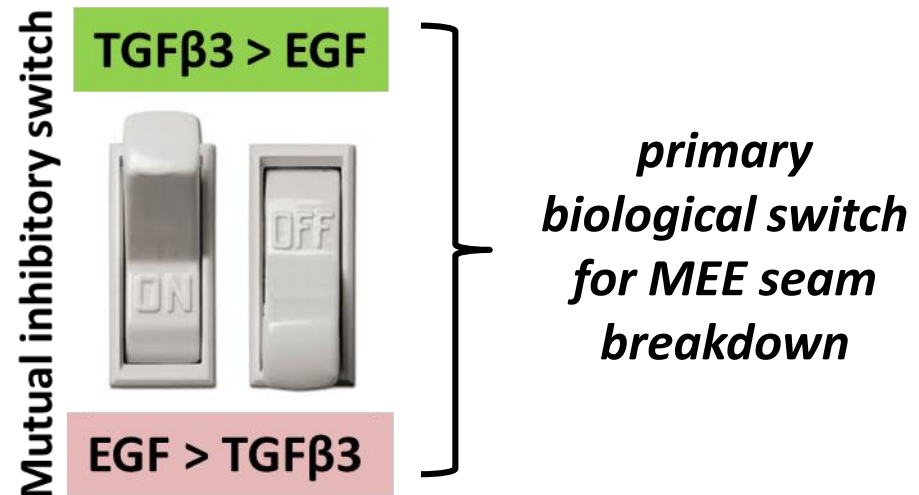


*Hutson et al. (in preparation)*

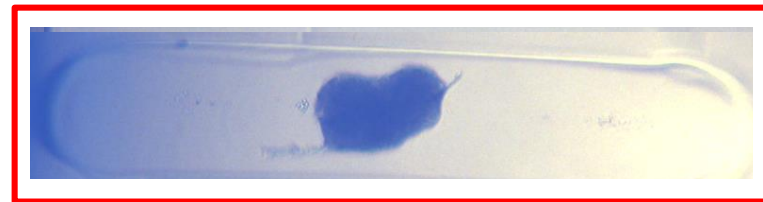
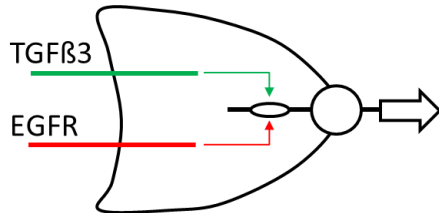
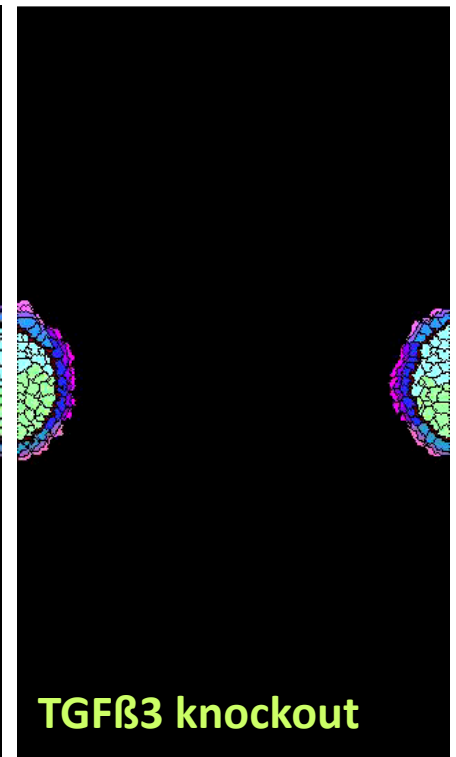
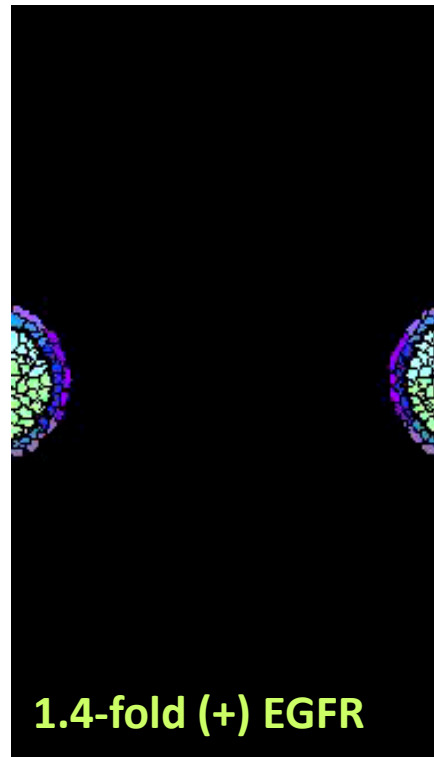
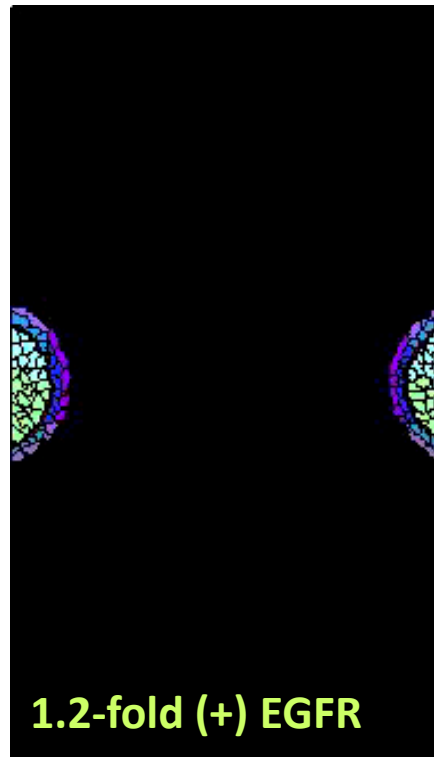
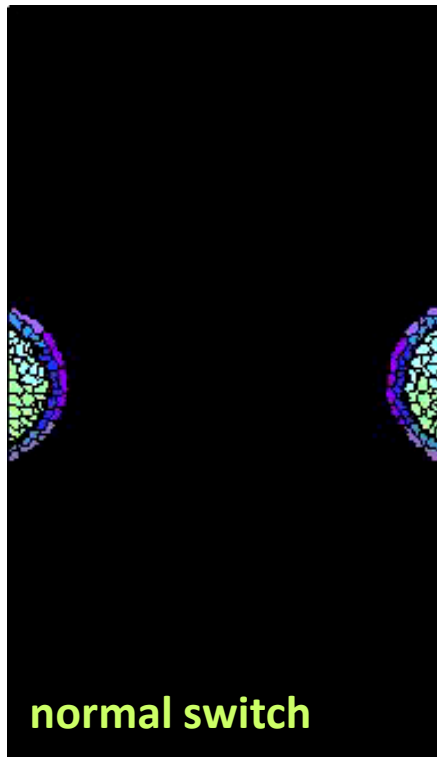
# Hacking the Control Network



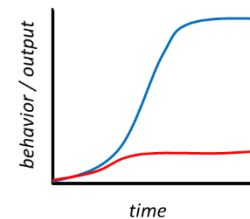
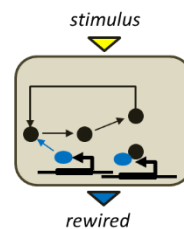
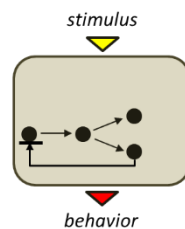
- *in silico* knockouts of elements in the underlying signaling network
- predicted impacts on MEE contact and seam breakdown (critical events)



$$k_{EGF} = 15 + \text{Random}[0,1] - 12 \frac{[Tgf\beta 3]^4}{[Tgf\beta 3]^4 + AC_{50}^4} + k_{EGF-AhR}$$



TGFβ3	EGFR	CP
1.0	1.0	0
0	1.0	1
1.0	1.2	0
1.0	1.4	1
1.0	1.5	1



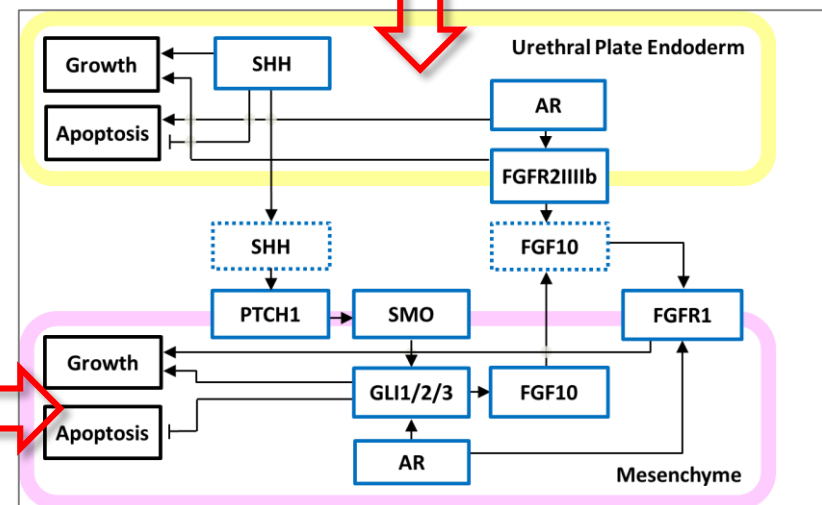
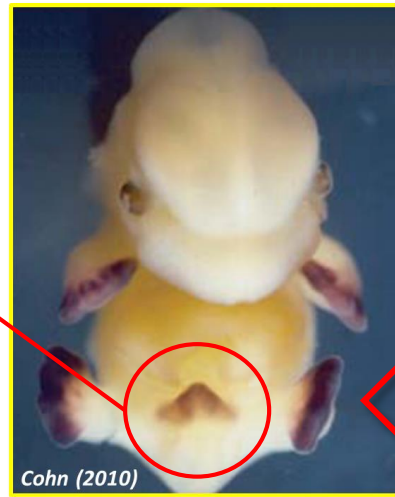
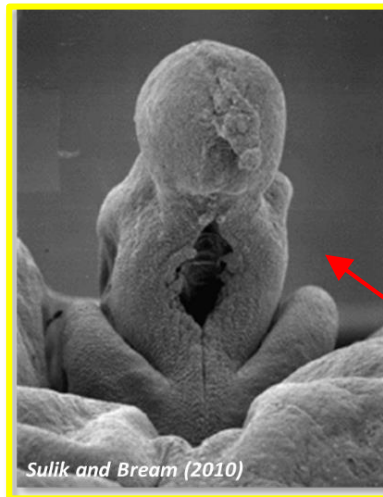
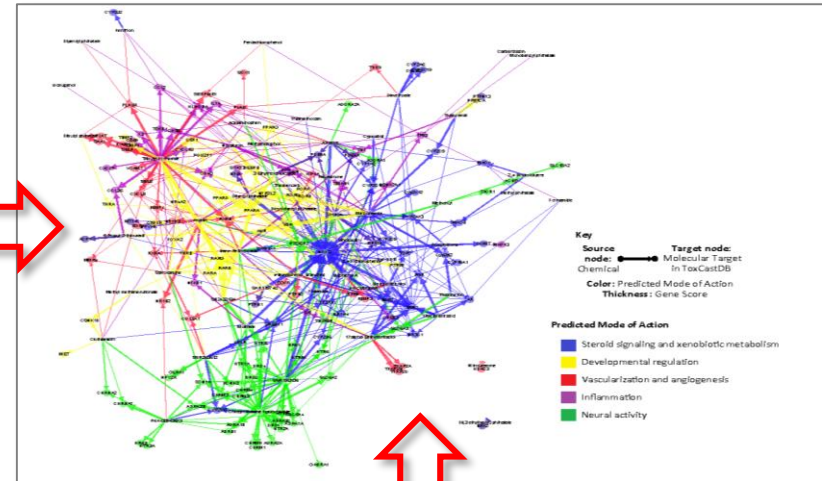
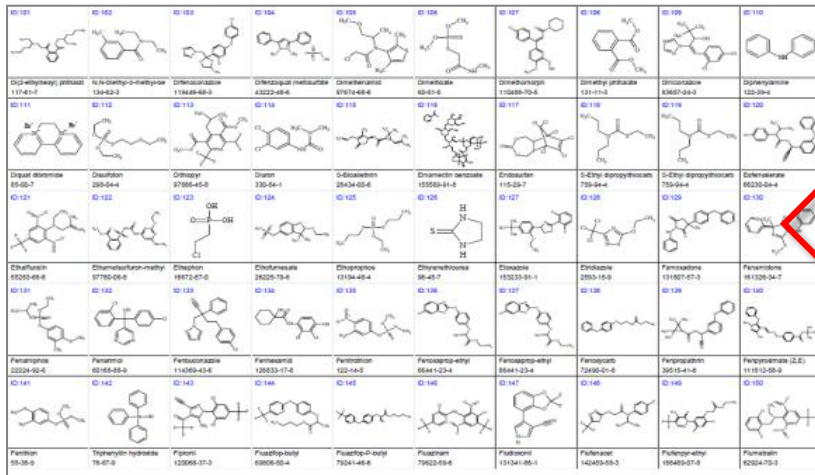
*Validate with  
fusion-competent  
human iPSC-  
microsystems and  
rewire switching*



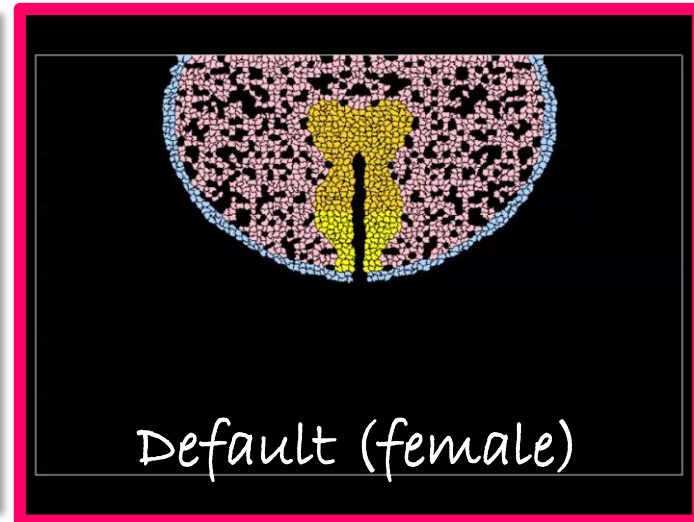
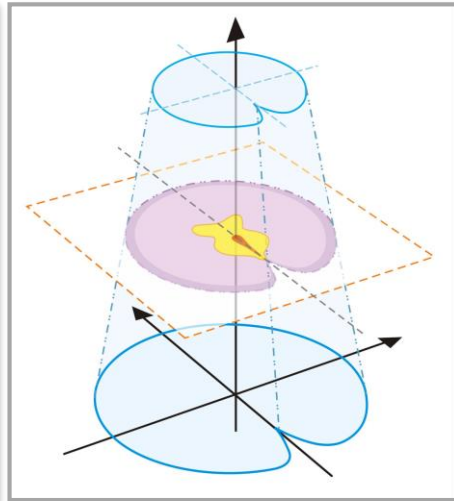
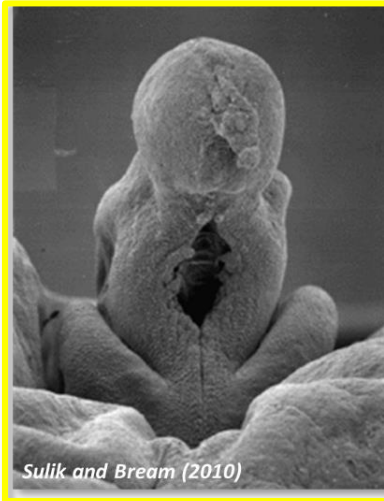
# How do chemical-bioactivity bipartite networks interact with control networks in disrupting development?

**EXAMPLE: hypospadias, a urethral closure defect**

*Leung et al. (in preparation)*

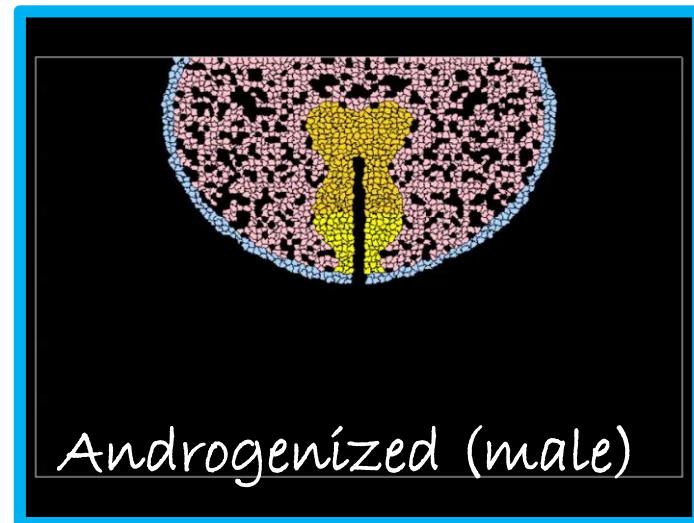


# Cell ABM for Genital Tubercle (GT) development

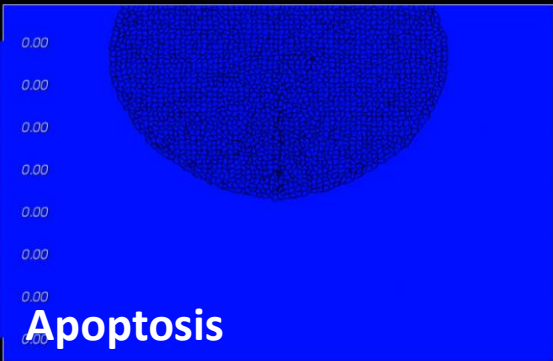
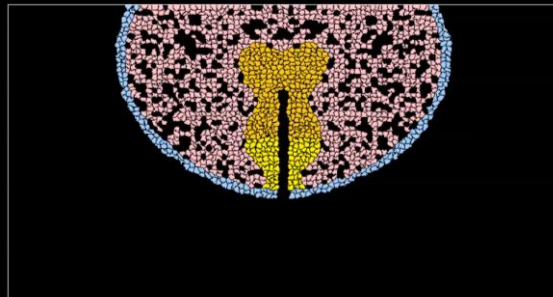


Embryonic GT - Abstraction

Androgen production by fetal testis triggers sexual dimorphism of the GT into male or female phenotypes.

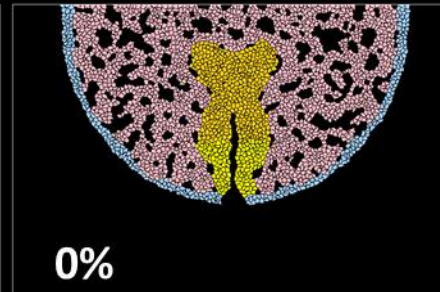
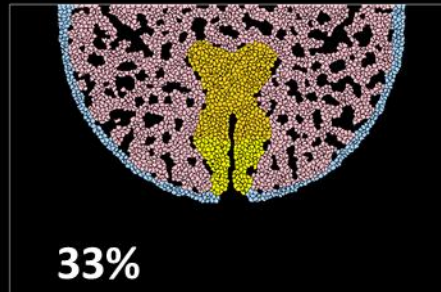
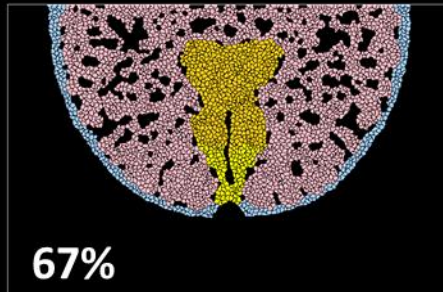
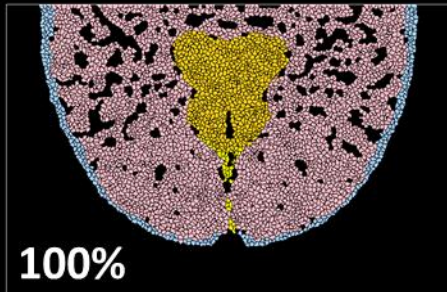


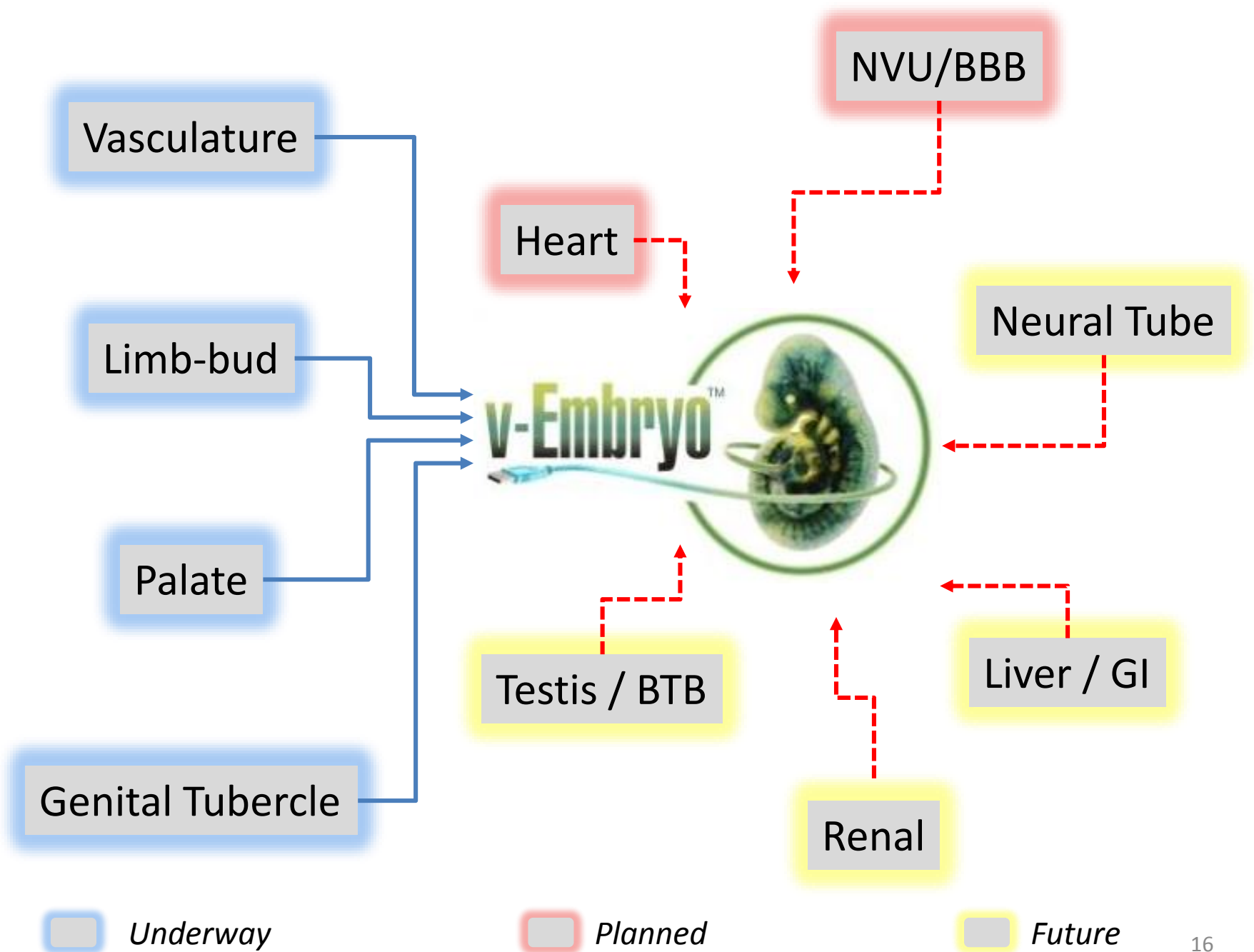




- Endodermal apoptosis & Mesenchymal (preputial) proliferation drives closure and centralization of the urethral rudiment.
- Disruption of SHH, FGF10, or AR signaling leads to closure defect (hypospadias).

### Androgen Sufficiency (theoretical)





# Virtual Tissues Laboratory System

VIRTUAL TISSUES  
LABORATORY SYSTEM

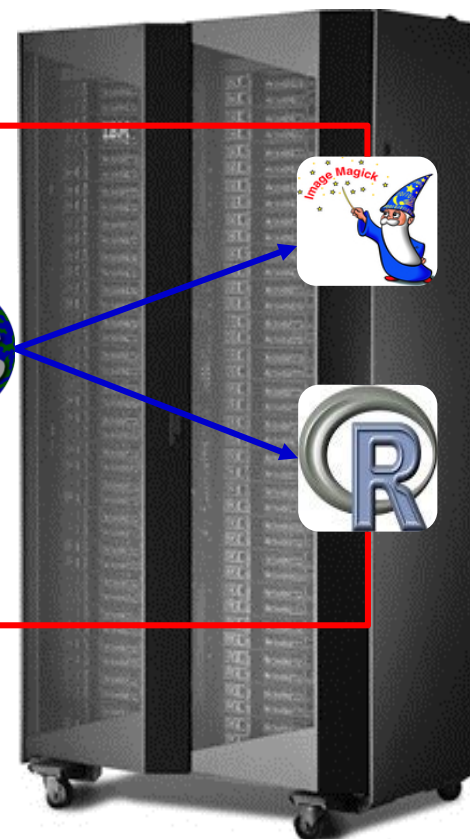
**Virtuoso**

Web Services and Queries



**HPC**

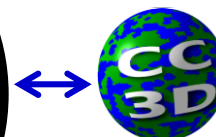
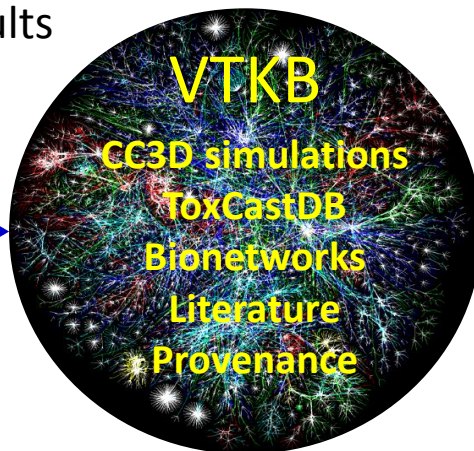
Massively-parallel simulation



Video or 3D Results



Data Analysis



BIO2RDF

A screenshot of a web browser showing the EPA's Virtual Tissues Laboratory System. The page has a blue header with the EPA logo and the text 'The Virtual Tissues Laboratory System'. Below the header, there is a 'Submit Jobs' form with fields for 'Description', 'Tissue Type', 'Chemical', 'Dose', 'Dose Type', 'Dose Unit', 'Dose Rate', 'Dose Duration', 'Dose Frequency', 'Dose Schedule', 'Dose Route', 'Dose Site', 'Dose Type', 'Dose Unit', 'Dose Rate', 'Dose Duration', 'Dose Frequency', 'Dose Schedule', 'Dose Route', 'Dose Site'. There is also a 'Submit' button and a small chemical structure diagram.



# Special Thanks

- Richard Judson – NCCT
- Imran Shah – NCCT
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- Sid Hunter – NHEERL / ISTD
- Dustin Kapraun – NCCT (ORISE)
- Eric Watt – NCCT (ORISE)
- Max Leung – NCCT (ORISE)
- Jill Franzosa – NCCT (ORISE)
- Nicole Kleinstreuer – NCCT (now ILS/NTP)
- Nisha Sipes – NCCT (now NTP)
- Richard Spencer – Lockheed Martin / EMVL
- Nancy Baker – Lockheed Martin / NCCT
- Rob DeWoskin – EPA / NCEA
- Tamara Tal – NHEERL / ISTD
- Monica Linnenbrink – NCCT / CSS
- Christina Baghdikian – NCCT / CSS
- Ed Carney – Dow Chemical Company
- T Heinonen – U Tampere / FICAM
- E Berg – DiscoverX – BioSeek
- A Seifert – U Kentucky
- L Egnash – Stemina Biomarker Discovery
- M Bondesson – U Houston / STAR
- J Glazier – Indiana U / STAR
- Shane Hutson – Vanderbilt U / STAR
- William Murphy – U Wisconsin / STAR
- Randy Ashton – U Wisconsin / STAR
- John Wikswa – Vanderbilt U / STAR



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## science in ACTION

INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE

Virtual Tissue Models: Predicting How Chemicals Impact Human Development

[http://www2.epa.gov/sites/production/files/2015-08/documents/virtual\\_tissue\\_models\\_fact\\_sheet\\_final.pdf](http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf)



**National Center for Computational Toxicology**