Systems Toxicology and Computational Dynamics

What can we learn from a virtual embryo?

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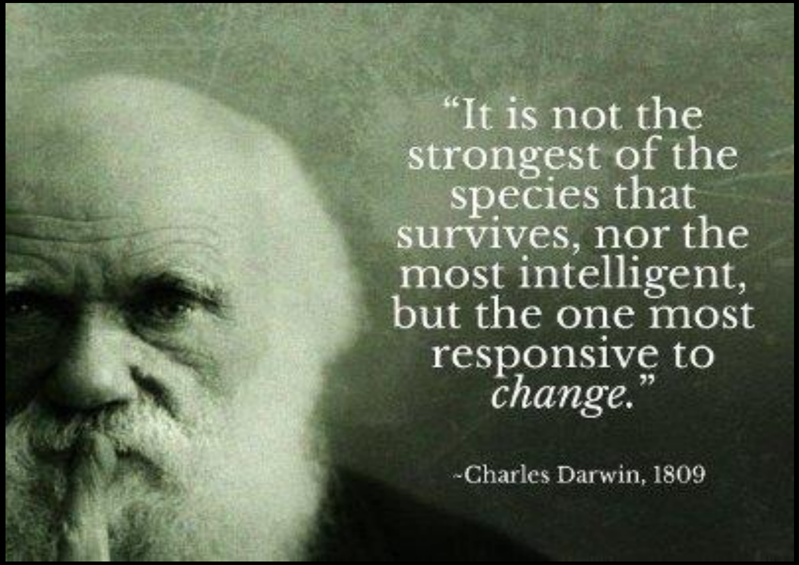
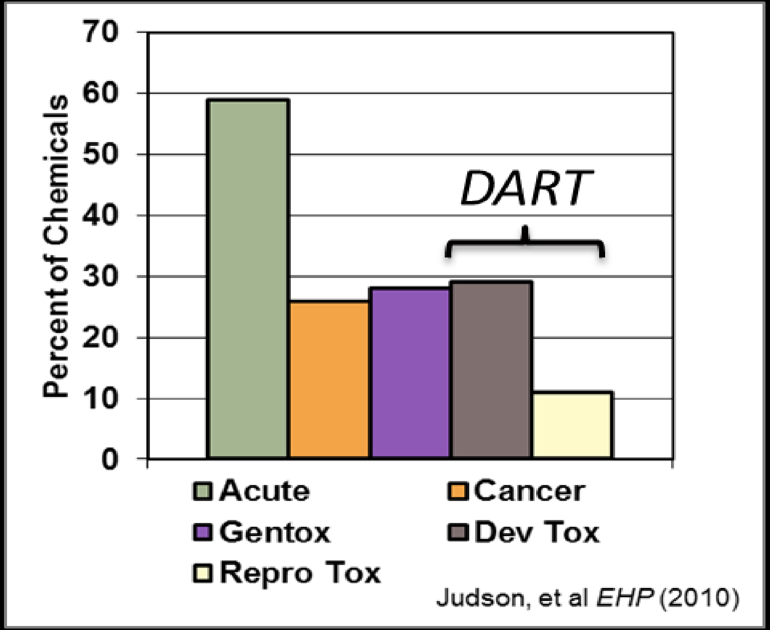
OVSOT 2017, Purdue University – 12/1/2017

Drivers of change

- Chemical regulation is challenged by more than 85,000 chemicals on EPA's inventory of substances that fall under TSCA (Toxic Substances Control Act, amended 2016).

- Animal-based methods for developmental and reproductive toxicity (DART) are resource-intensive and do not scale to the testing problem.

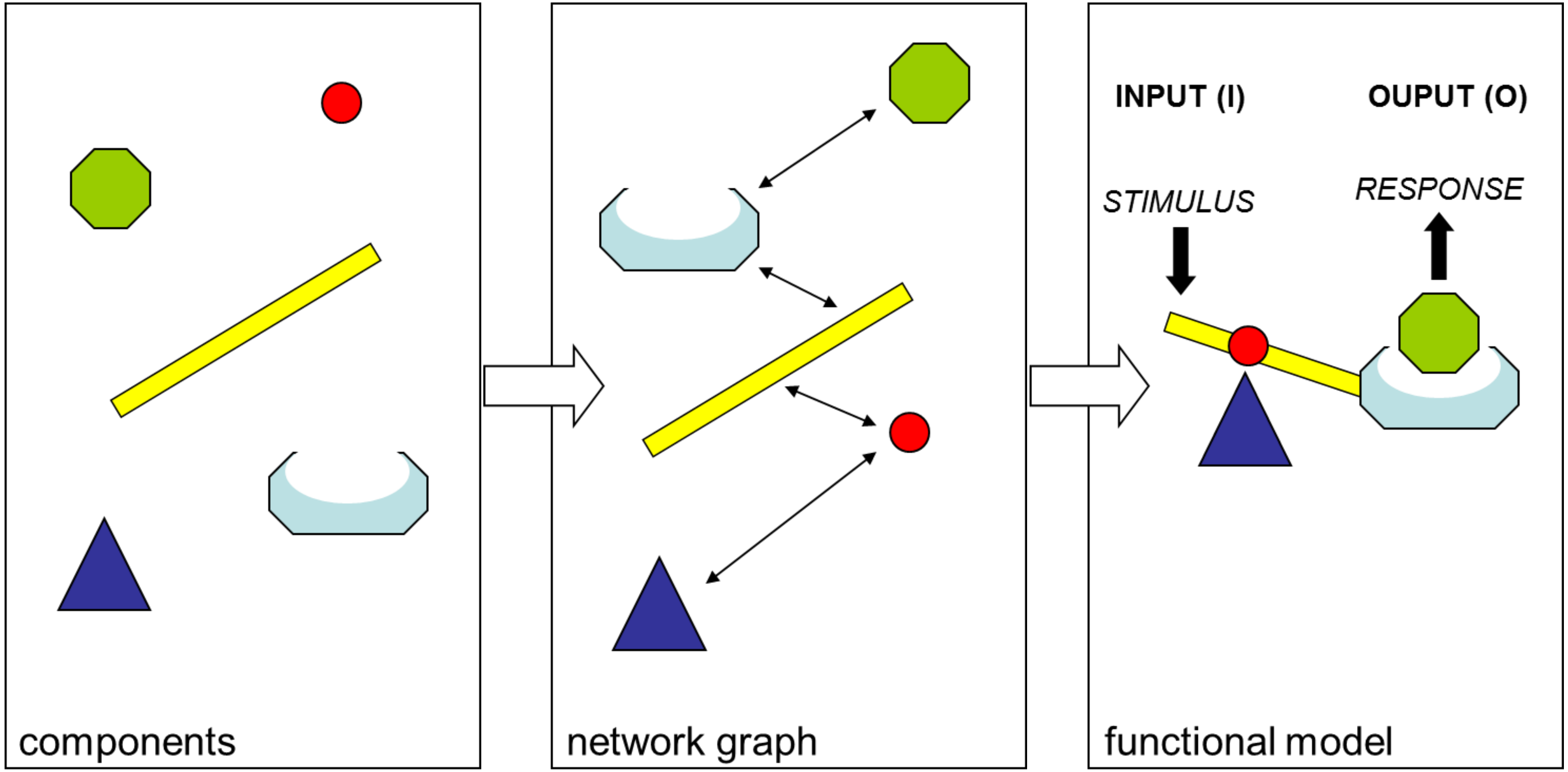




In a nutshell:

- Advances in biomedical, engineering, and computational sciences enable high-throughput screening (HTS) to profile the toxicological landscape (*ToxCast/Tox21*).
- Surfeit of HTS data now in hand, practical need arises to formally translate this information into biological understanding (*predictive toxicology*).
- Information must be collected, organized, and assimilated across multiple levels of biological organization to meet these requirements (*systems toxicology*).
- Computational biology is uniquely position to capture this connectivity and help shift decision-making to mechanistic prediction (*systems modeling*).

Why systems models are needed …



Knudsen and Kavlock 2008, based on MW Covert (2006)

… but the embryo is not so easy!

- **Biological systems are complex:** networks of 'nodes' (molecules) and 'edges' (interactions) operate in nonlinear fashion across space and time to control cellular behavior:
  - cell growth, proliferation, adhesion, differentiation, polarization, motility, apoptosis, …

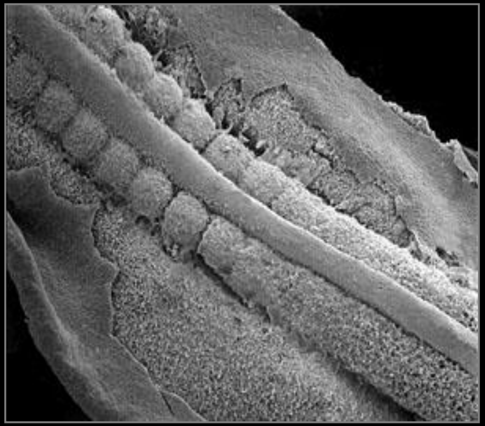
- **Systems are wired for robustness:** cross-talk in cell signaling may accentuate or dampen how a complex adaptive system reacts to chemical perturbation:
  - challenge is quantitative prediction of how cellular injury interacts with developmental dynamics.

- **Agent-Based Models (ABMs):** formal approach to explain/predict how mechanistic changes in a self-organizing system propagate to a critical effect (eg, malformation):
  - the biological unit (cell) is taken as the computational unit (agent) in a dynamical simulation.

Anatomical homeostasis in a self-regulating Virtual Embryo

SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

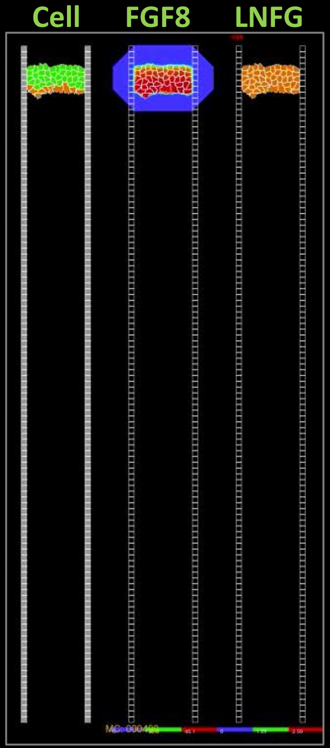
Somite formation



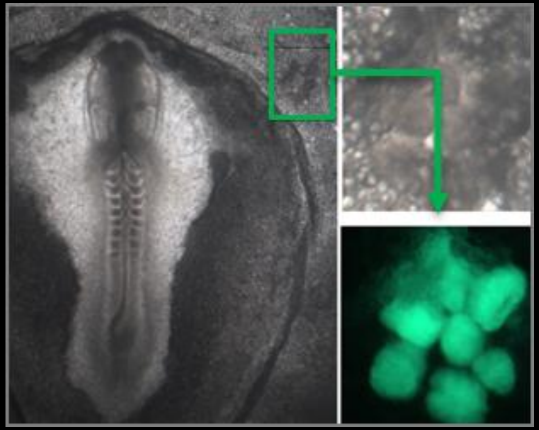

Hes1-EGFP time-lapse (3h)
Masamizu et al. 2006

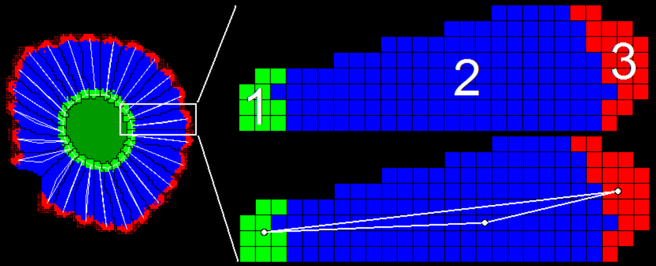
Clock and Wavefront Model

- *oscillating gene expression (eg, Hes1, LNFG)*
- *signal gradients along AP axis (eg, FGF8, RA)*
- *differential cell adhesion (eg, ND, ephrin system)*



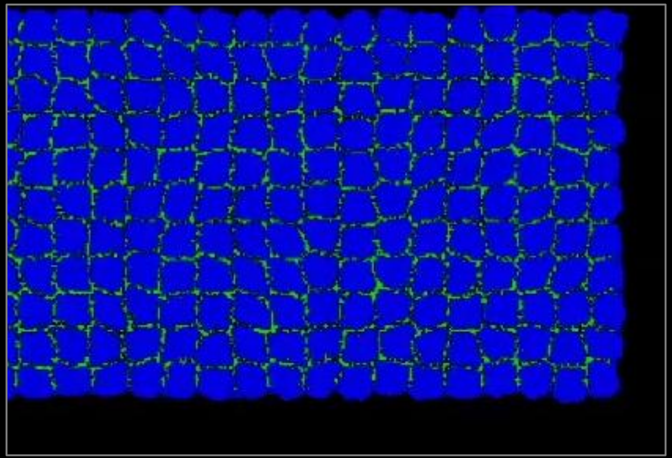
SOURCE: Hester et al. (2011) PLoS Comp Biol



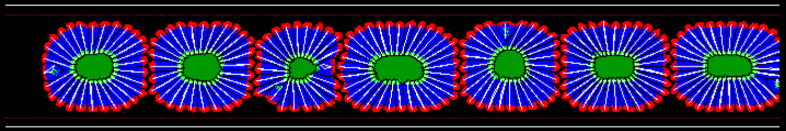


Epithelialization Model

- *clock genes do not oscillate*
- *somites form simultaneously*



- *adding the wavefront restores sequentiality*
- *adding the clock improves regularity*



SOURCE: Dias et al. (2014) Science

Building and testing ABMs for *in silico* DART:

translational applications of a 'virtual embryo'

Can multicellular simulation tame the beast?

- reconstruct tissue development cell-by-cell, interaction-by-interaction
  (emergence)

Multicellular simulation can tame the beast!

- pathogenesis following synthetic knockdown
  (cybermorphs)

- import HTS (ToxCast) data into an embryological simulation
  (toxico-dynamics)

- probabilistic rendering of where, when and how a defect might emerge
  (animal-free mechanistic prediction)

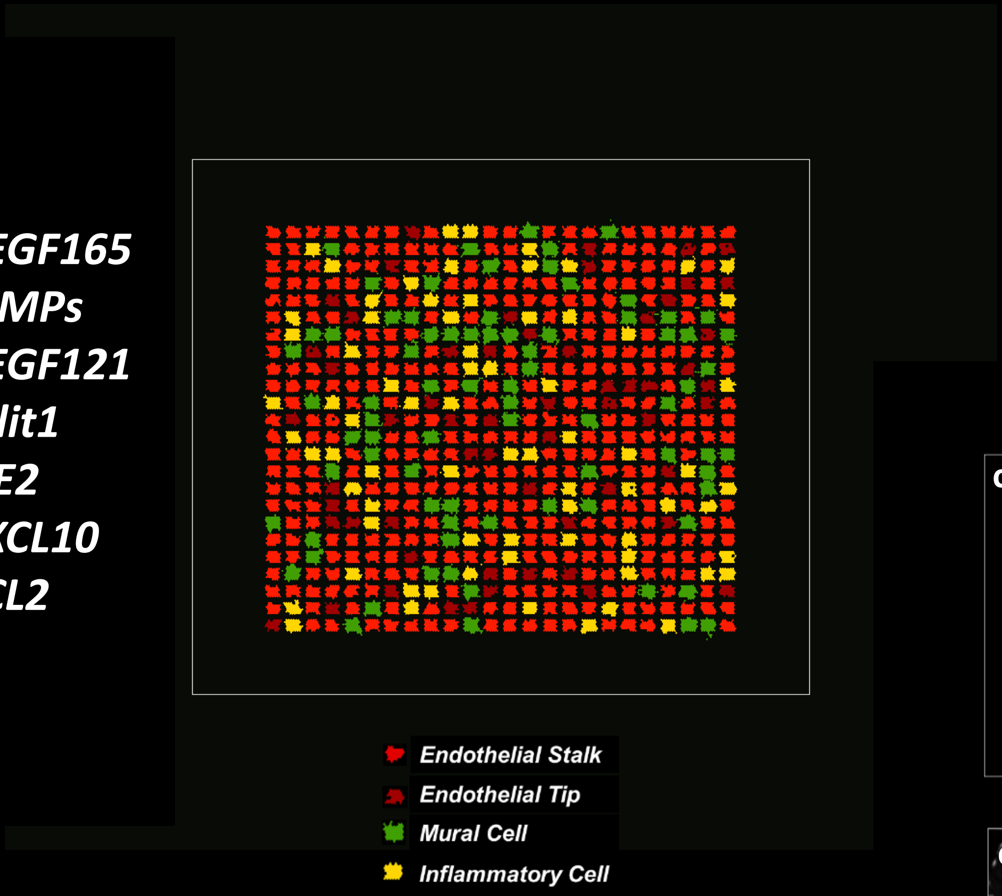
ABM strategies

Forward engineering the system - *suppose we know a molecular effect (eg, ToxCast lesion), how far can an ABM take us to hypothesizing an apical outcome?*

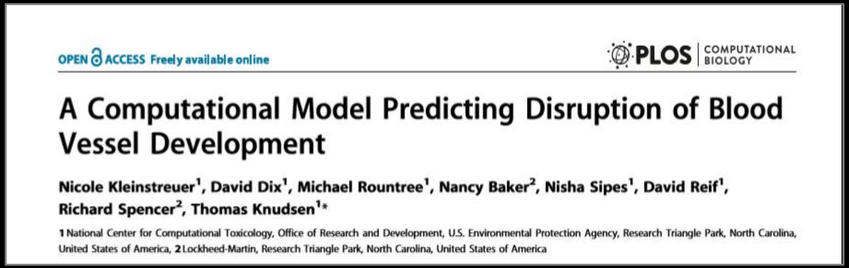
Reverse-engineering the system - *suppose we know an apical outcome (eg, malformation), how far can an ABM take us to inferring a key event quantitatively?*

Developmental angiogenesis

VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2



SOFTWARE: www.CompuCell3D.org
BioComplexity Institute, Indiana U

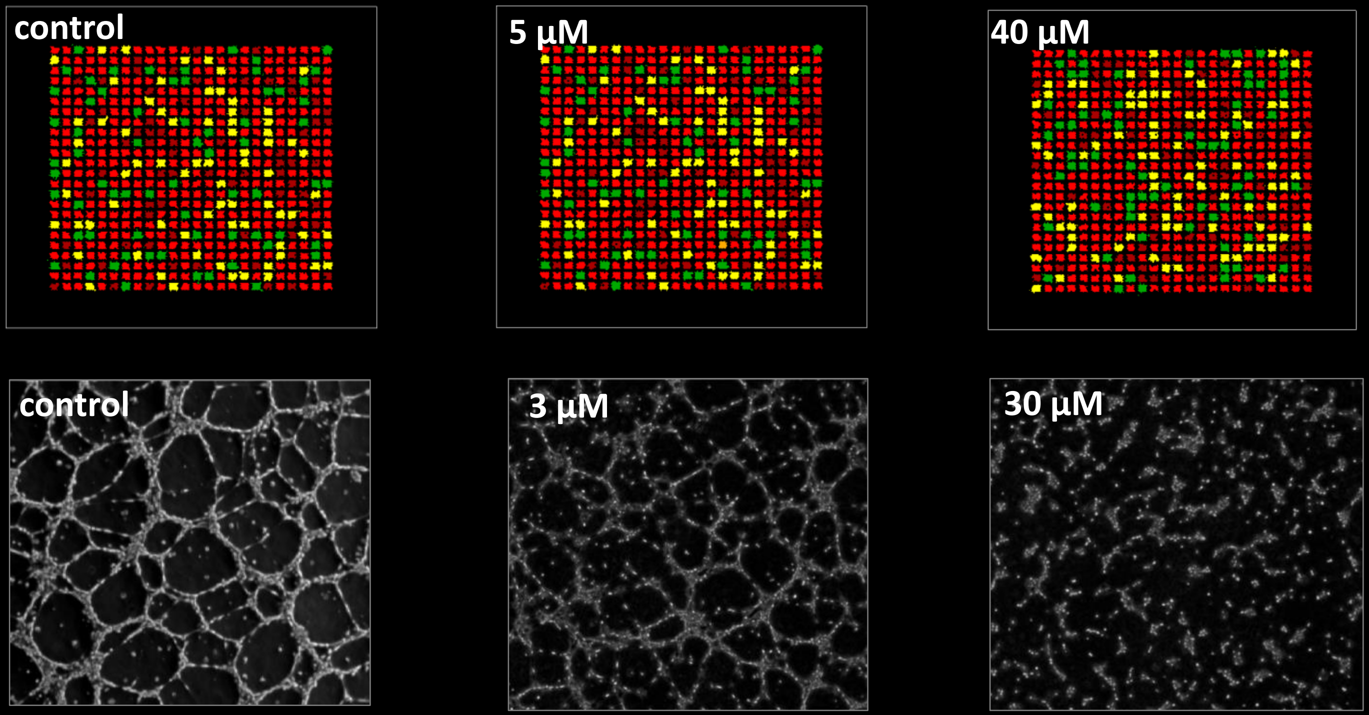

OPEN ACCESS Freely available online PLOS COMPUTATIONAL BIOLOGY

A Computational Model Predicting Disruption of Blood Vessel Development

Nicole Kleinstreuer[1], David Dix[1], Michael Rountree[1], Nancy Baker[2], Nisha Sipes[1], David Reif[1], Richard Spencer[2], Thomas Knudsen[1]*

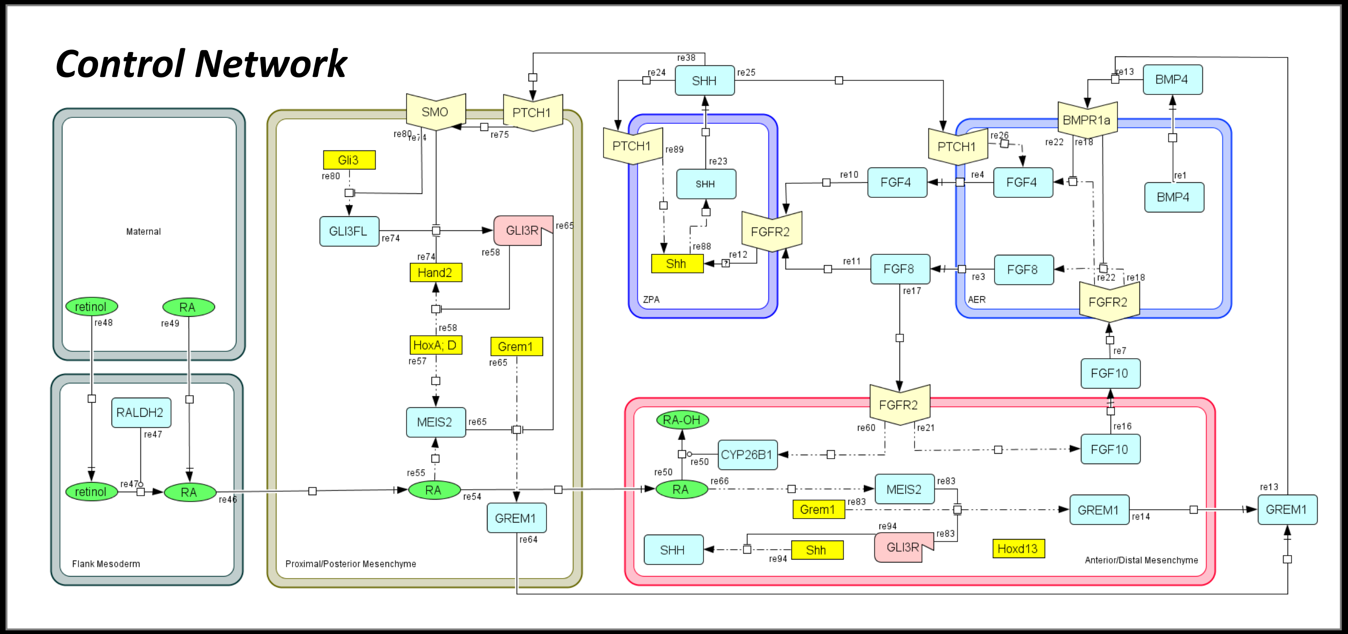
1 National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America, 2 Lockheed-Martin, Research Triangle Park, North Carolina, United States of America

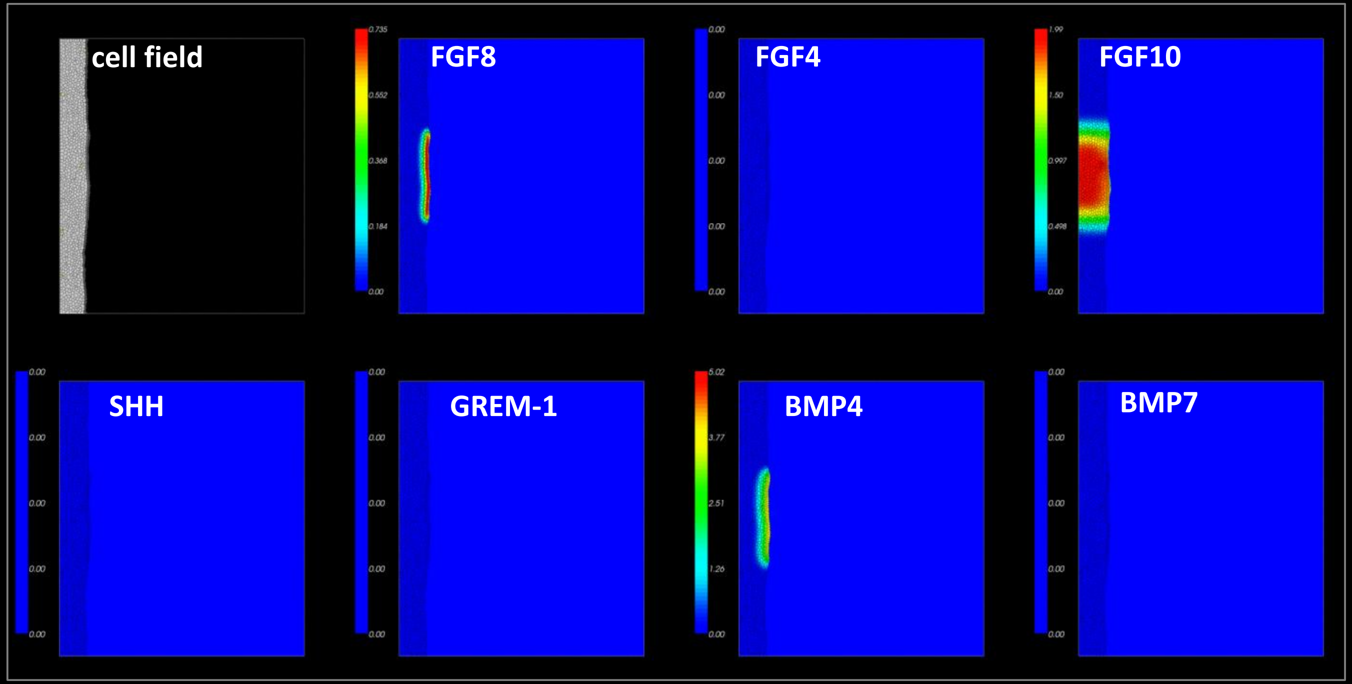
ToxCast bioactivity profile for 5HPP-33
(synthetic thalidomide analog)

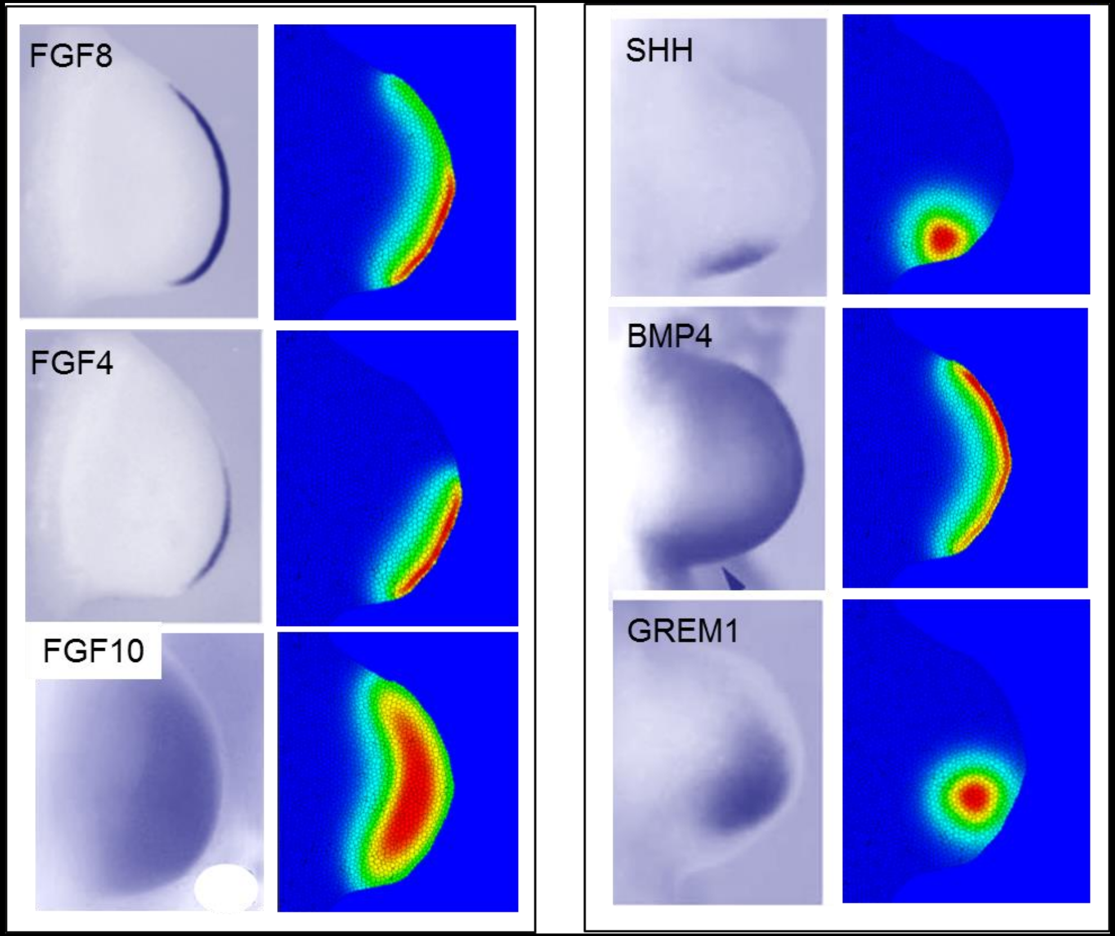


SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996

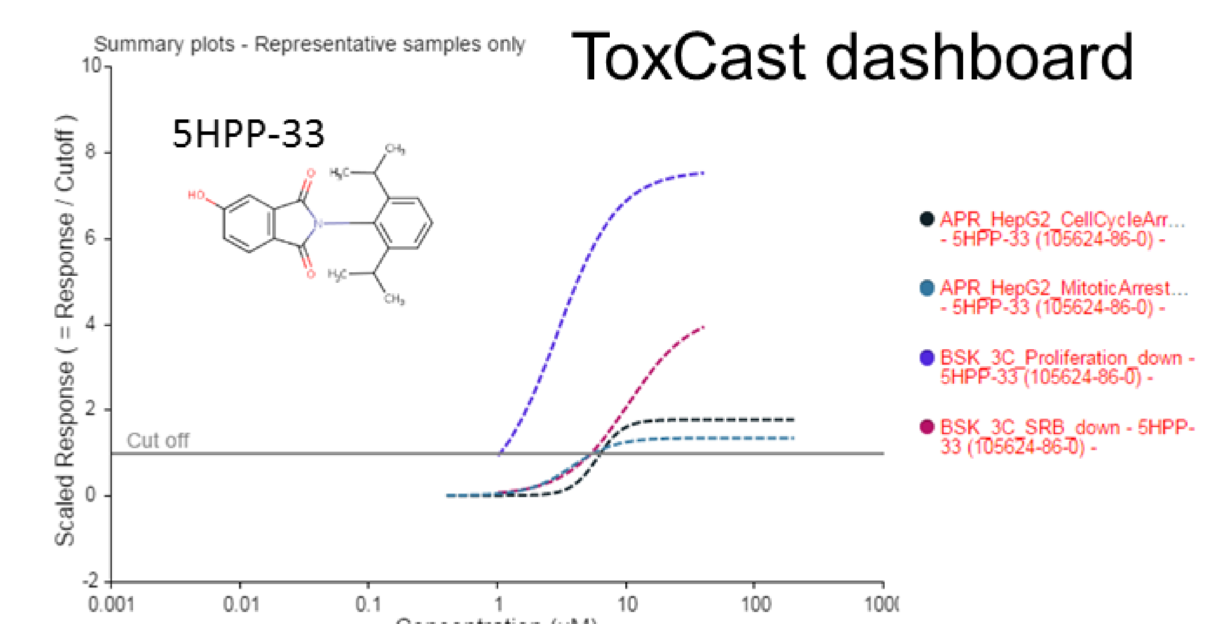
Limb-bud outgrowth



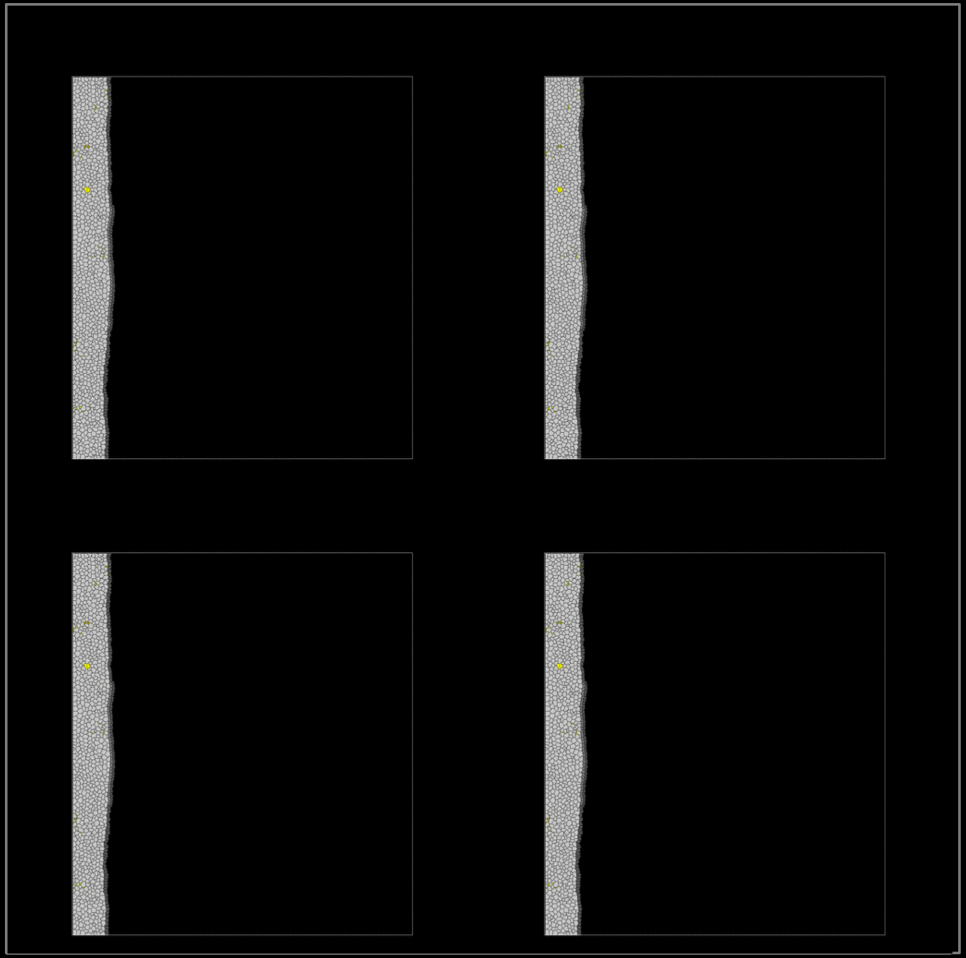




Limb teratogenesis *in silico*



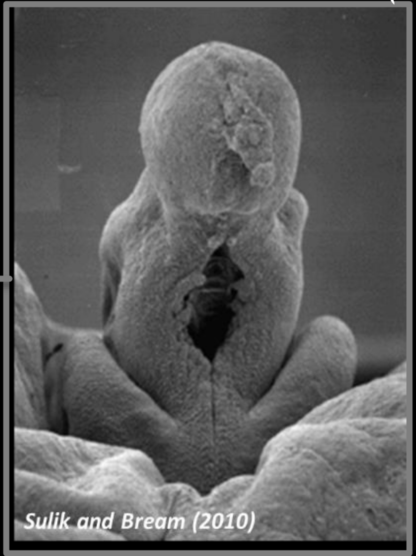
https://actor.epa.gov/dashboard/

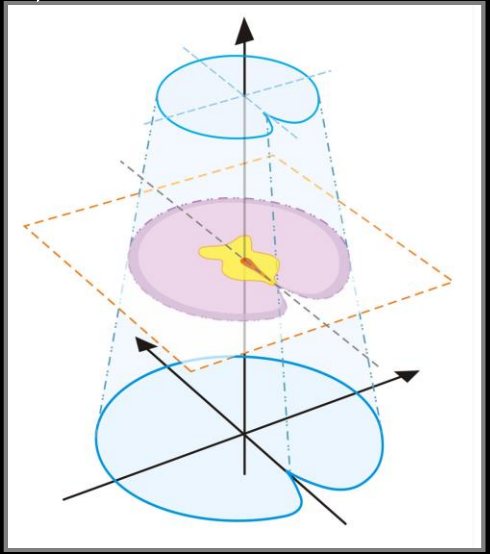


SOURCE: NCCT, unpublished

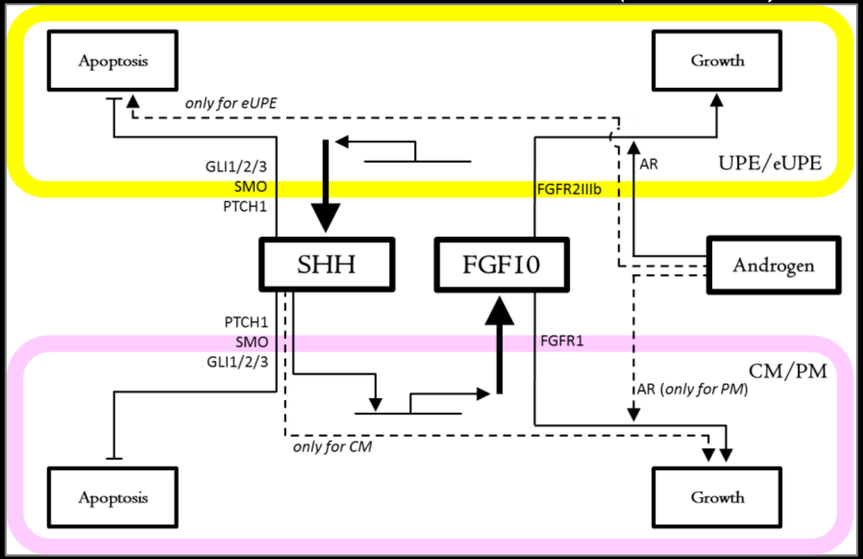
Sexual dimorphism: *genital tubercle morphogenesis*

Genital tubercle (GT)

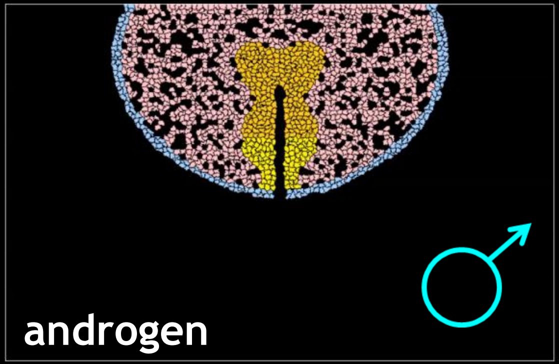


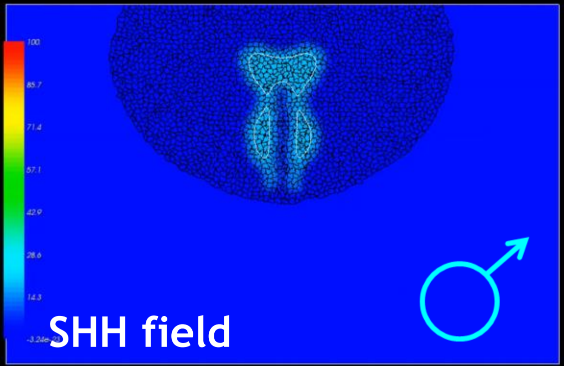


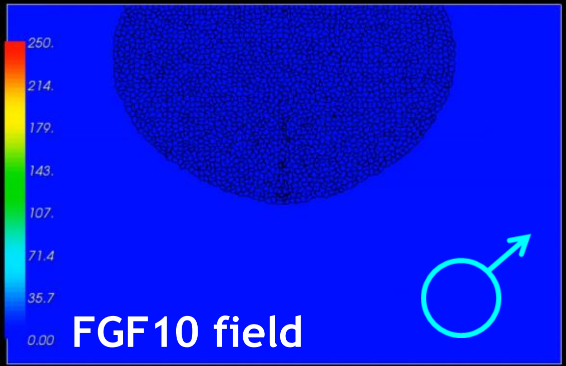
Control Network (mouse)



ABM simulation for sexual dimorphism (mouse GD13.5 - 17.5)



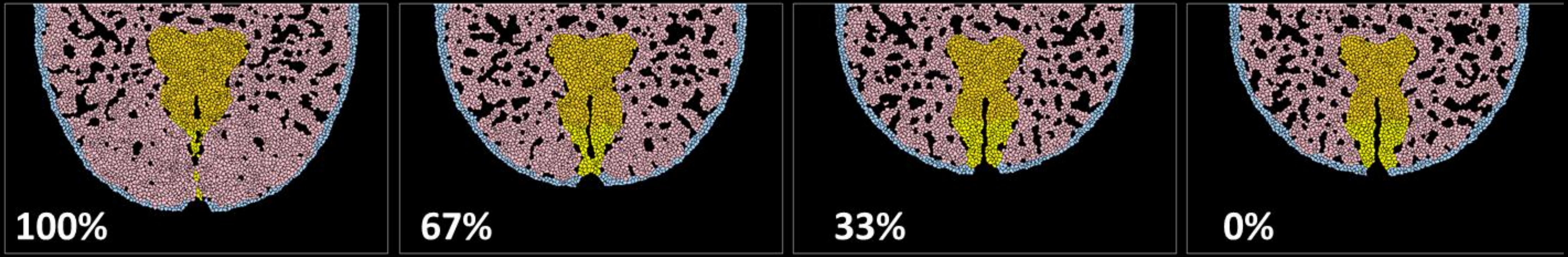




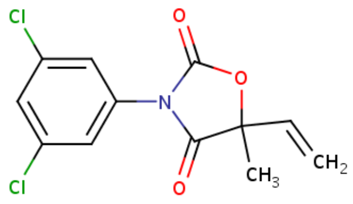
Leung et al. (2016) Reproductive Toxicology

Urethral Closure: *complex process disrupted in 'hypospadias'*

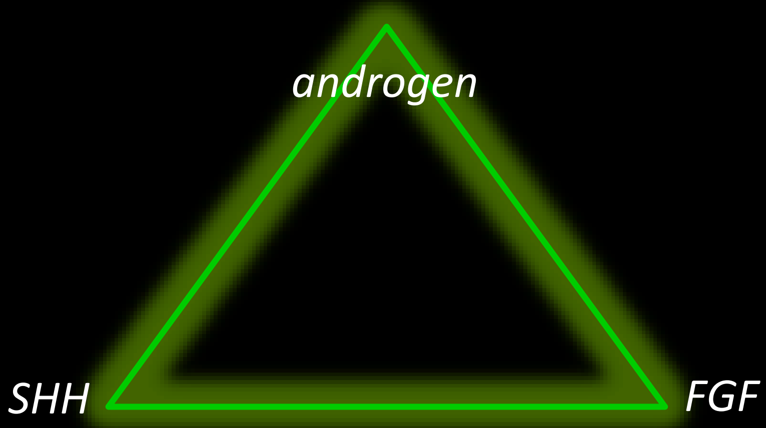
- Driven by urethral endoderm (contact, fusion apoptosis) and androgen-dependent effects on preputial mesenchyme (proliferation, condensation, migration) via FGFR2-IIIb.



vinclozolin



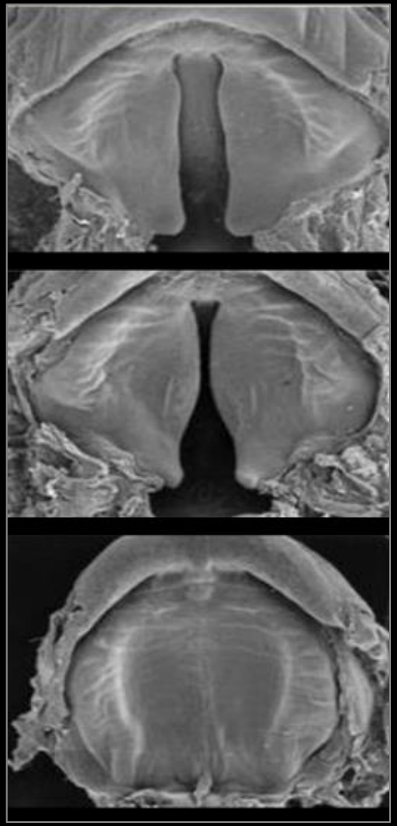
Androgenization *(n = 10 sims)*	Closure Index
100%	0.80
67%	0.57
33%	0.13
0%	0.07



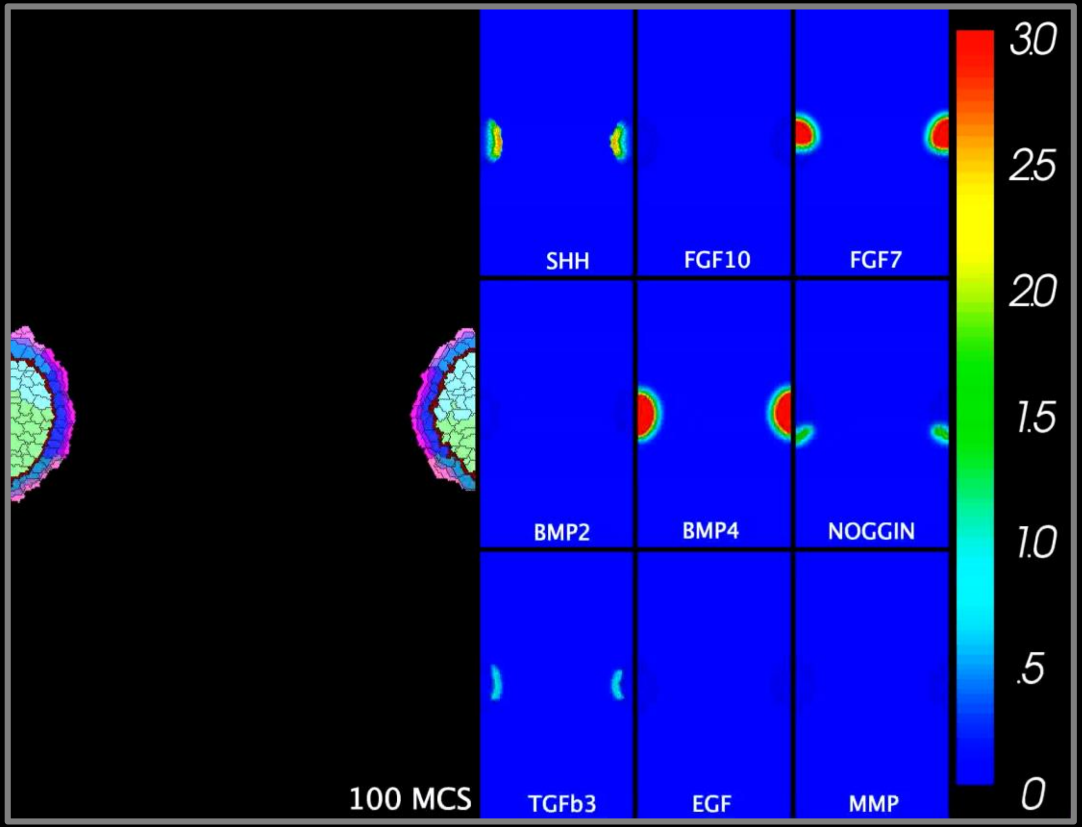
Palatal morphogenesis: *medial edge epithelium (MEE) seam breakdown*

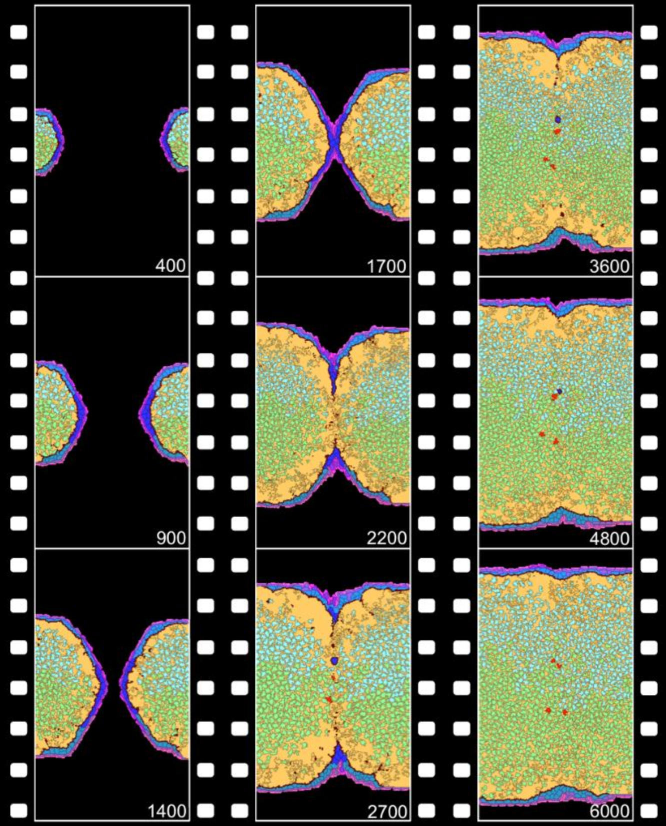
Palatal fusion *in vivo*

SEMs of human palate by K Sulik, UNC



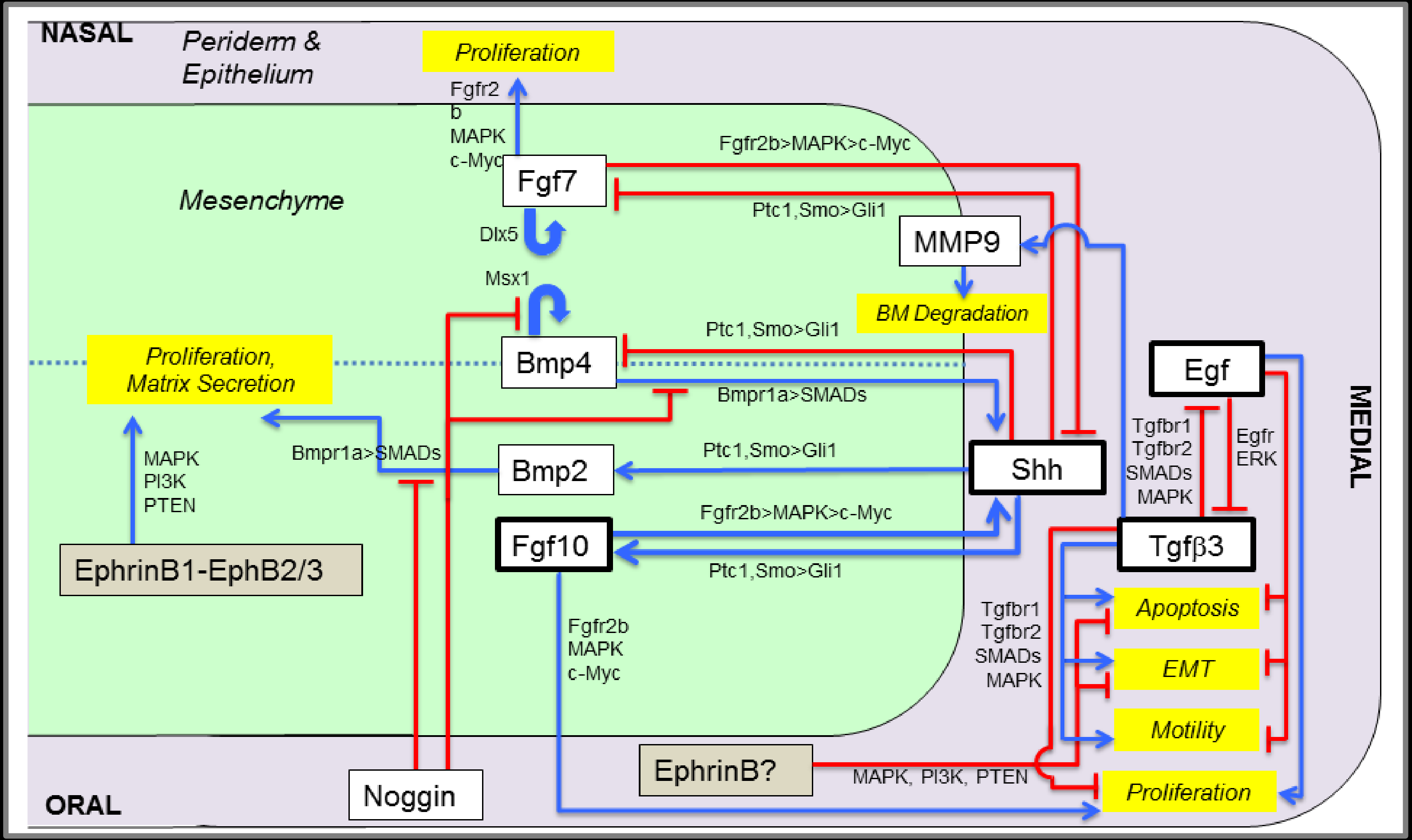
Palatal fusion *in silico*

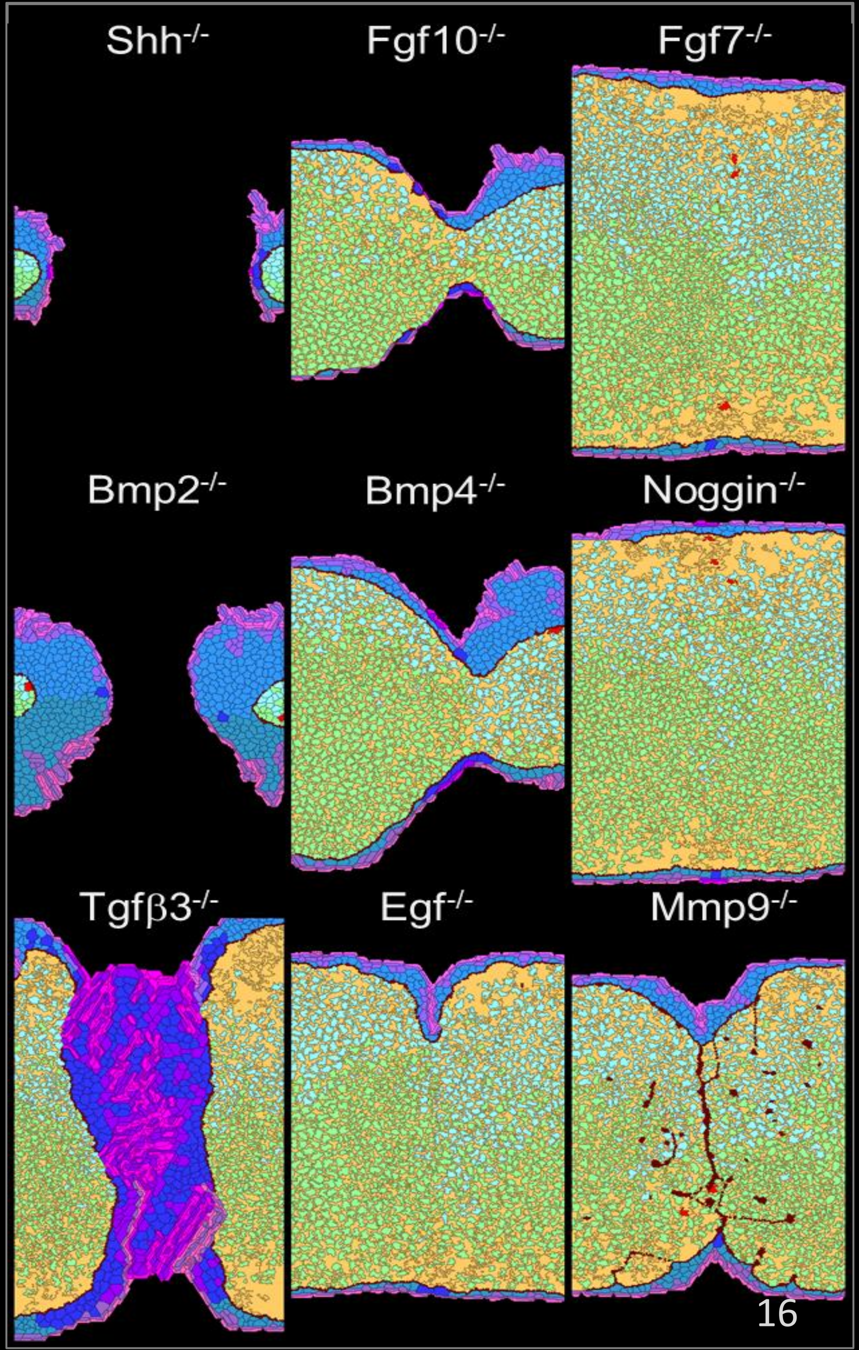




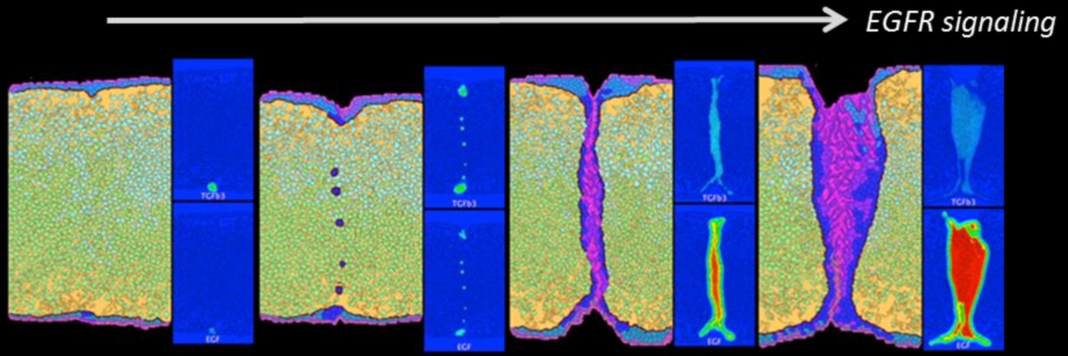
Hutson et al. (2017) Chem Res Toxicol

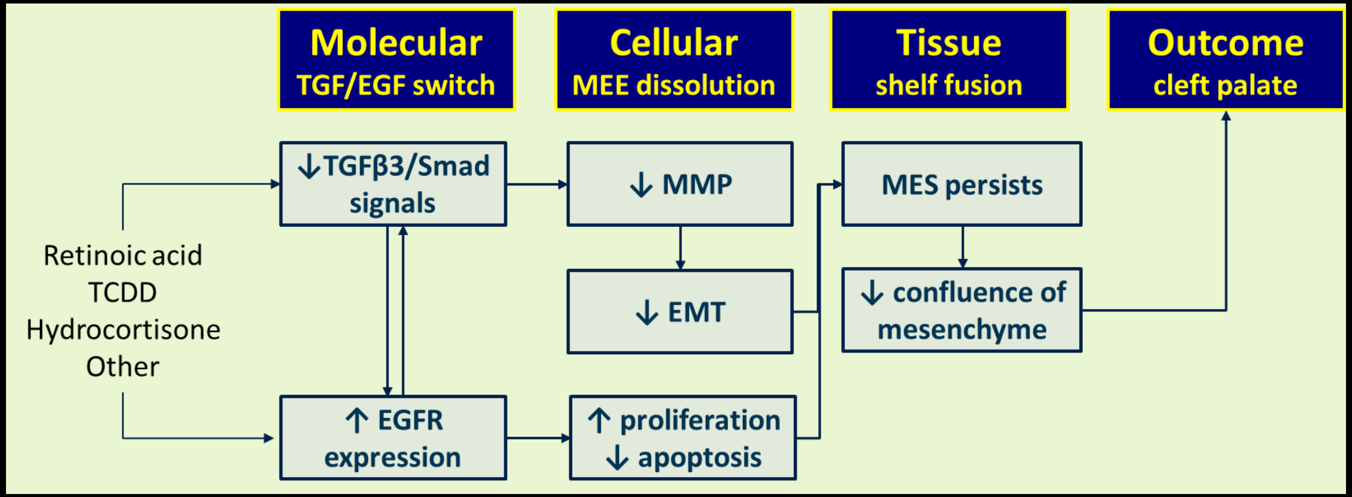
Hacking the control network → 'Cybermorphs'

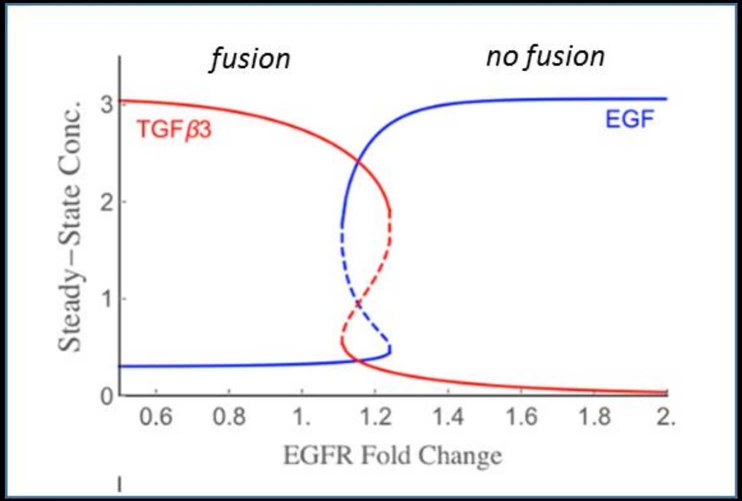




Breathing life into an AOP: *TGFb/EGF flip-flop latch controls MEE breakdown*



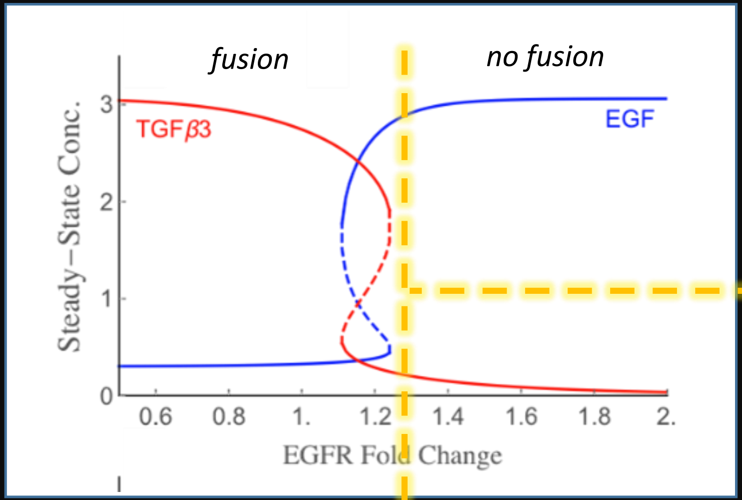




- TGFbeta/EGF signaling is mutually inhibitory
- Sigmoidal threshold leads to bistable response (hysteresis)
- Dynamics converts continuous stimulus into well defined states
- EGF → MEE maintenance; TGFbeta → MEE regression

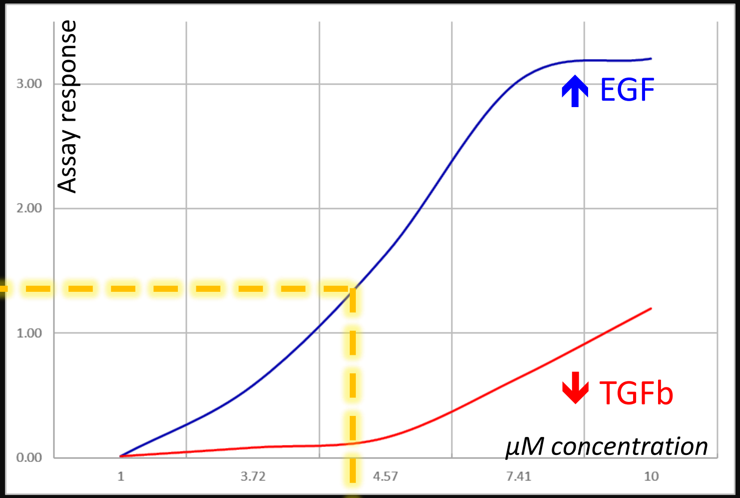
ToxCast lesion: Captan-induced cleft palate in rabbits

INPUT: switch dynamics



tipping point predicted by computational dynamics (hysteresis switch)

INPUT: Captan in ToxCast

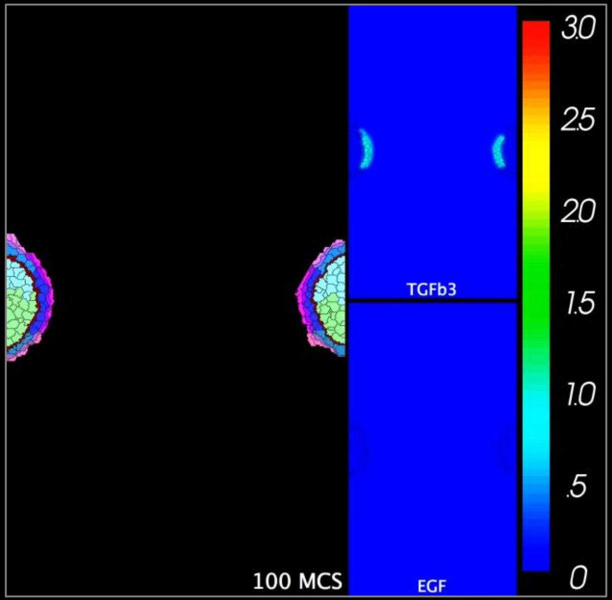


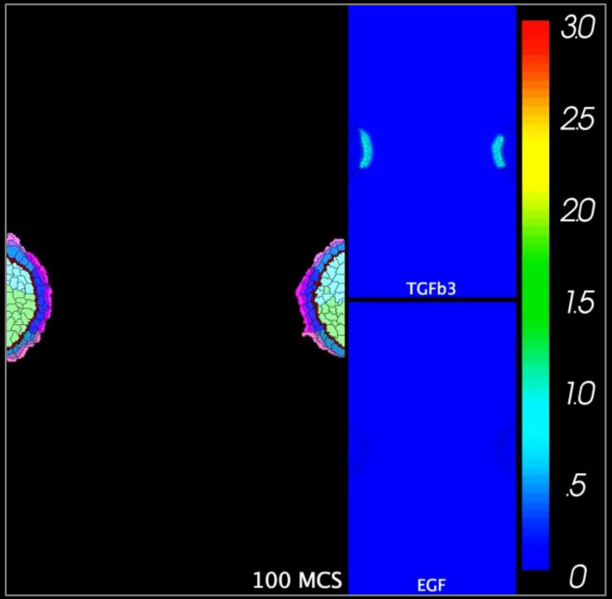
OUTPUT: tipping point mapped to HTS concentration response (4 µM)

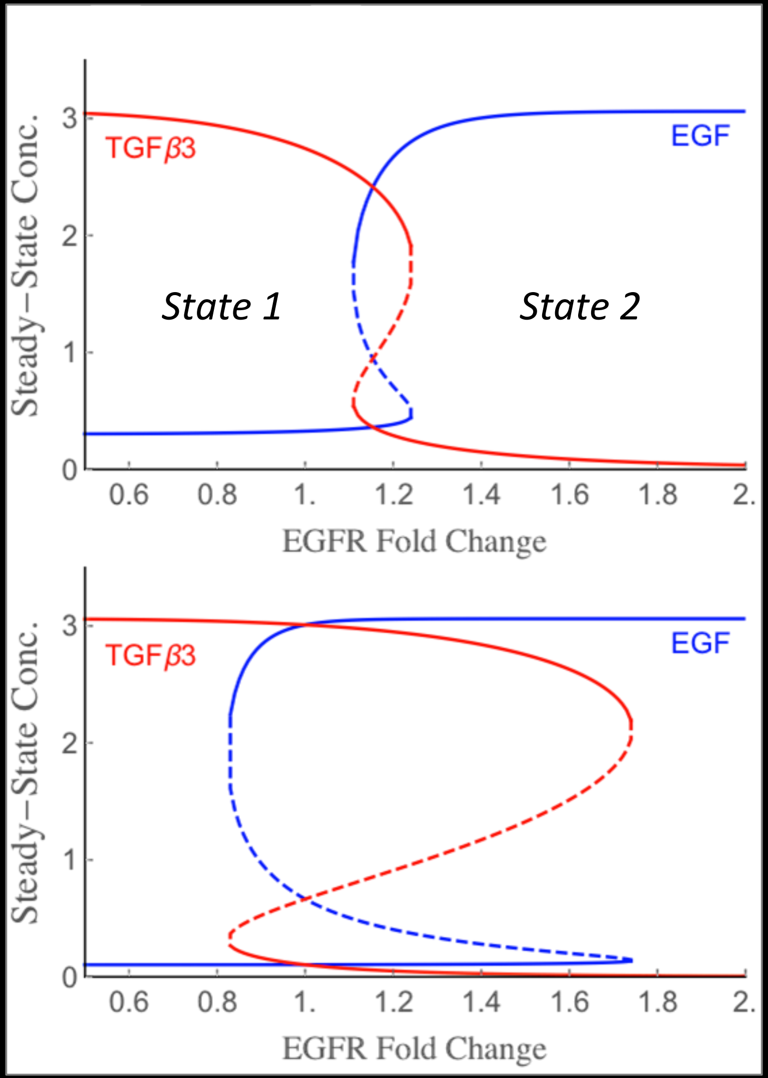
Captan in ToxRefDB
NOAEL = 10 mg/kg/day
LOAEL = 30 mg/kg/day

HTTK pregnancy model predicts **2.39 mg/kg/day** Captan would achieve a steady state concentration of 4 µM in the fetal plasma

Messin' with the switch: two scenarios for teratogenic dynamics



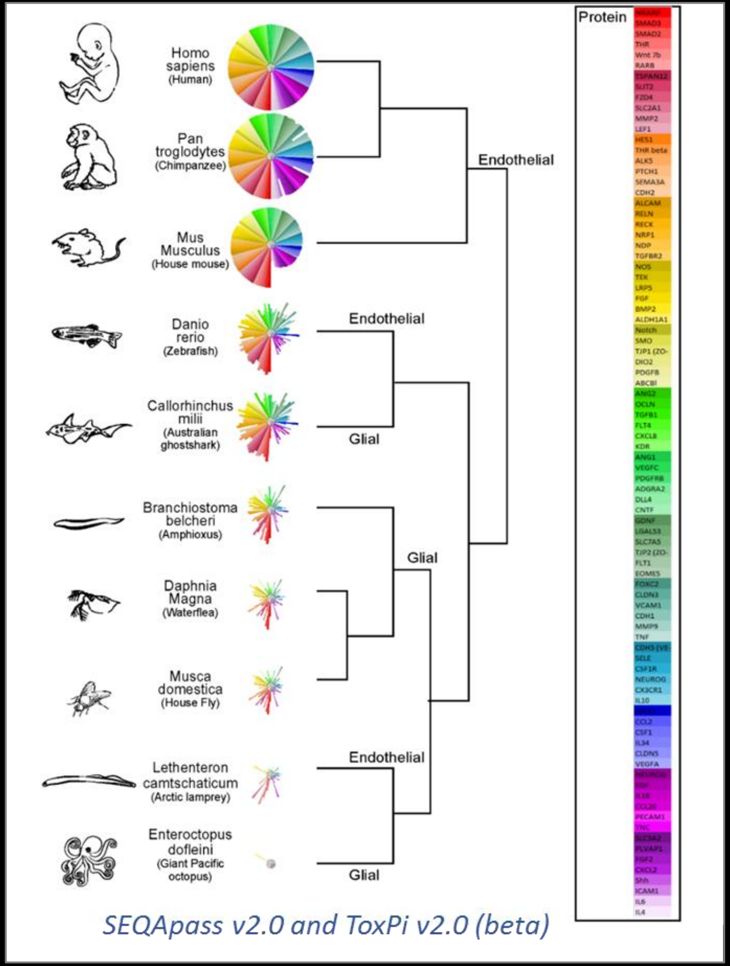




Narrow hysteresis zone:
easier to perturb but reversible

Broad hysteresis zone:
more resilient but irreversible

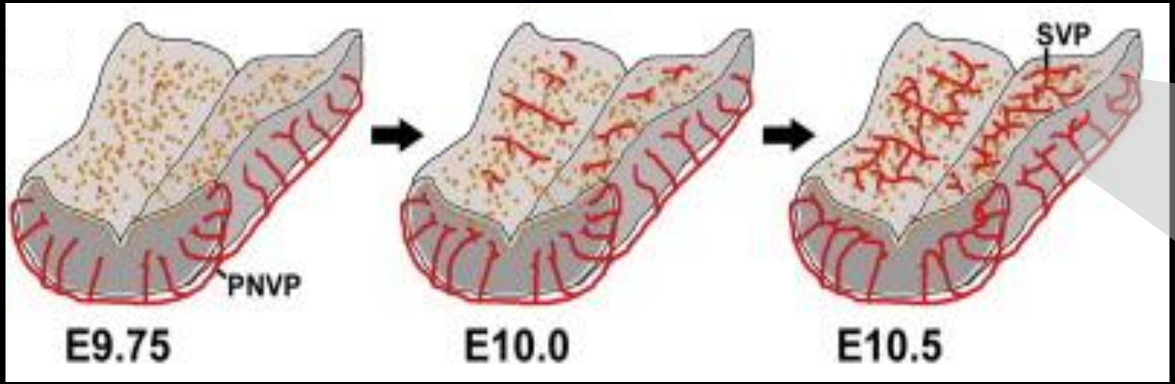
Blood-Brain-Barrier development: *decoding the toxicological blueprint*



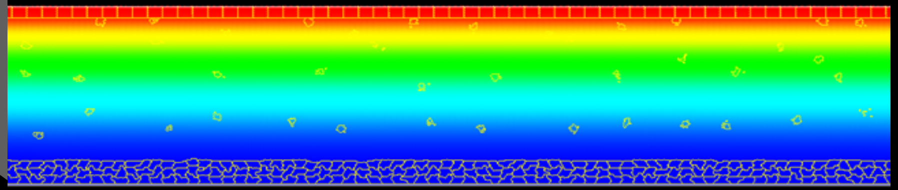
Saili et al. (2017) Birth Defects Res (in press)

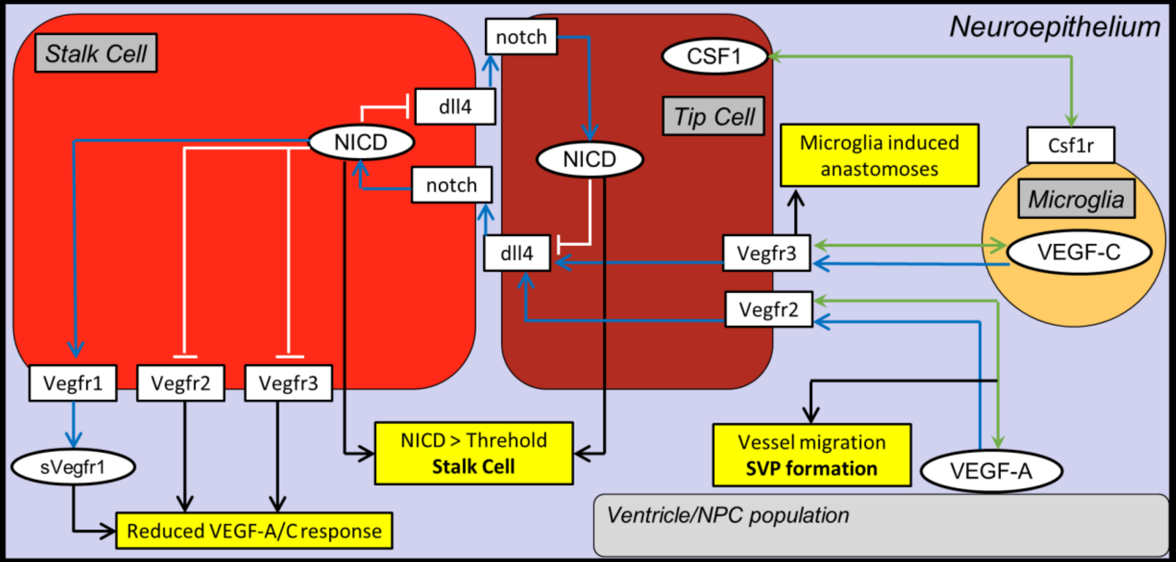
Brain Angiogenesis: *cellular ABM of vascular patterning*

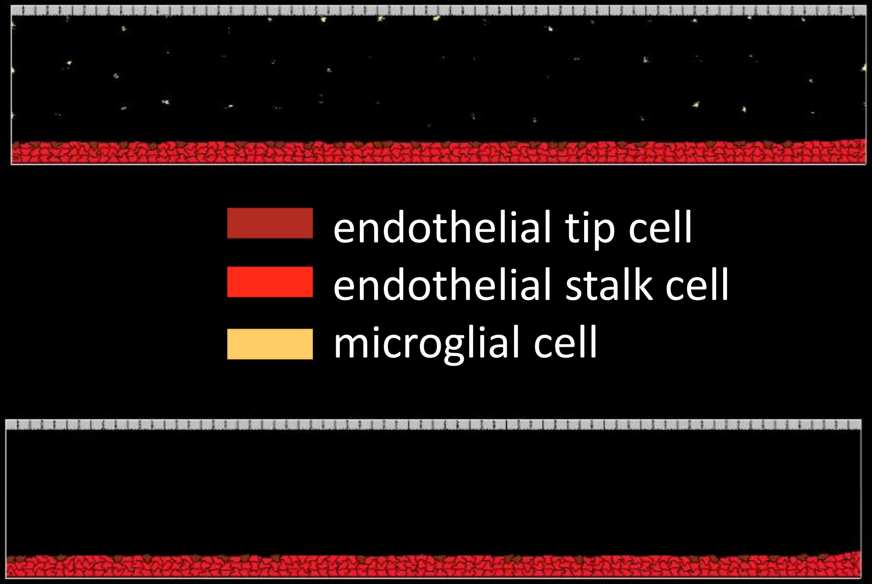
Tata et al. (2015) *Mechanism Devel*

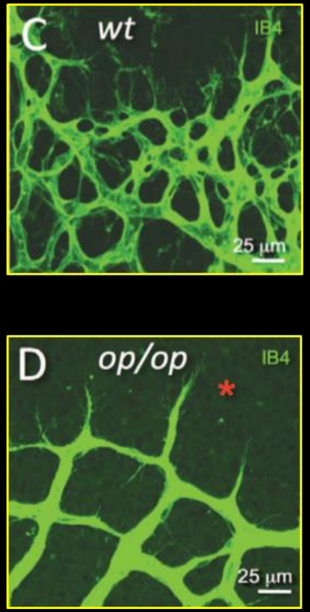


VEGF-A gradient: NPCs in subventricular zone







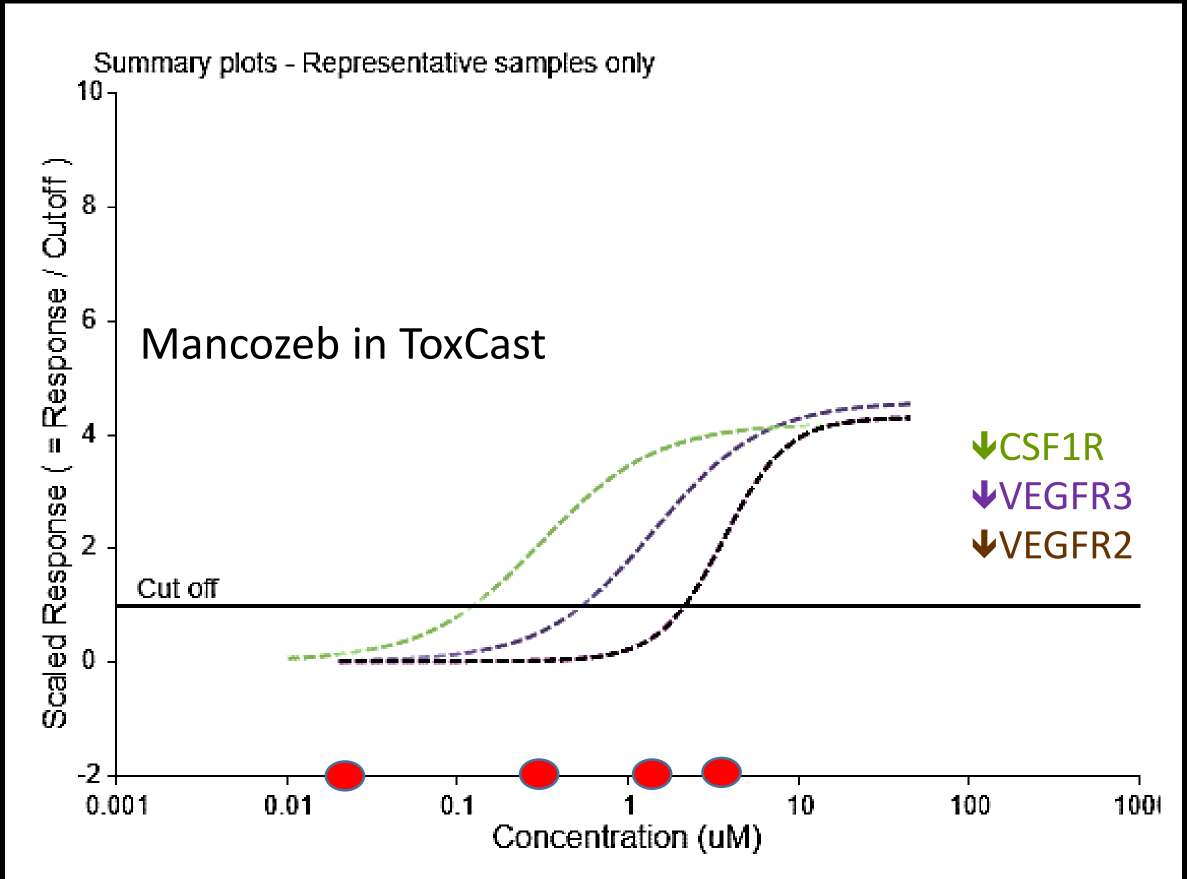


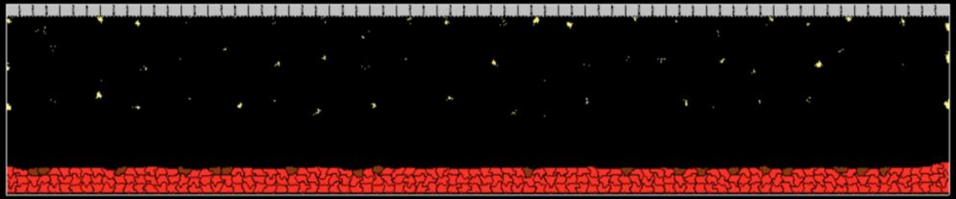
Rymo et al. (2011) PLoS one

SOURCE: T Zurlinden – NCCT (2017)

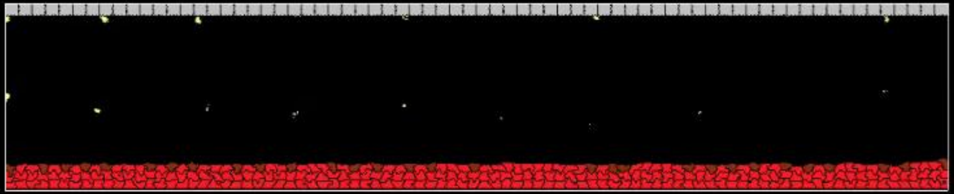
In silico cascading dose scenario

endothelial tip cell
endothelial stalk cell
microglial cell

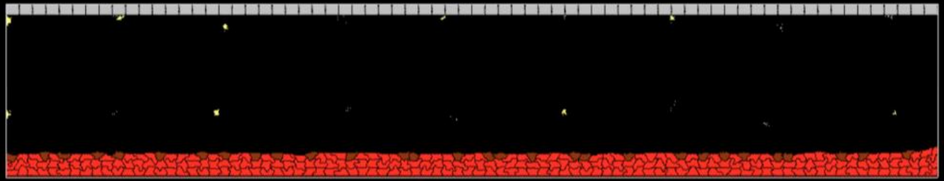




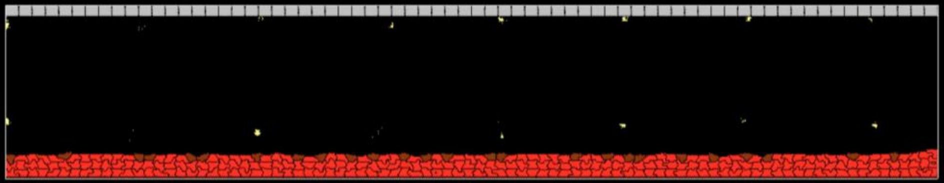
INPUT 0.03 µM
OUTPUT: predicted dNEL



INPUT 0.3 µM: AC50 CSF1R
OUTPUT: fewer microglia drawn to EC-tip cells



INPUT 2.0 µM: AC80 CSF1R + AC50 VEGFR3
OUTPUT: overgrowth of EC-stalk cells

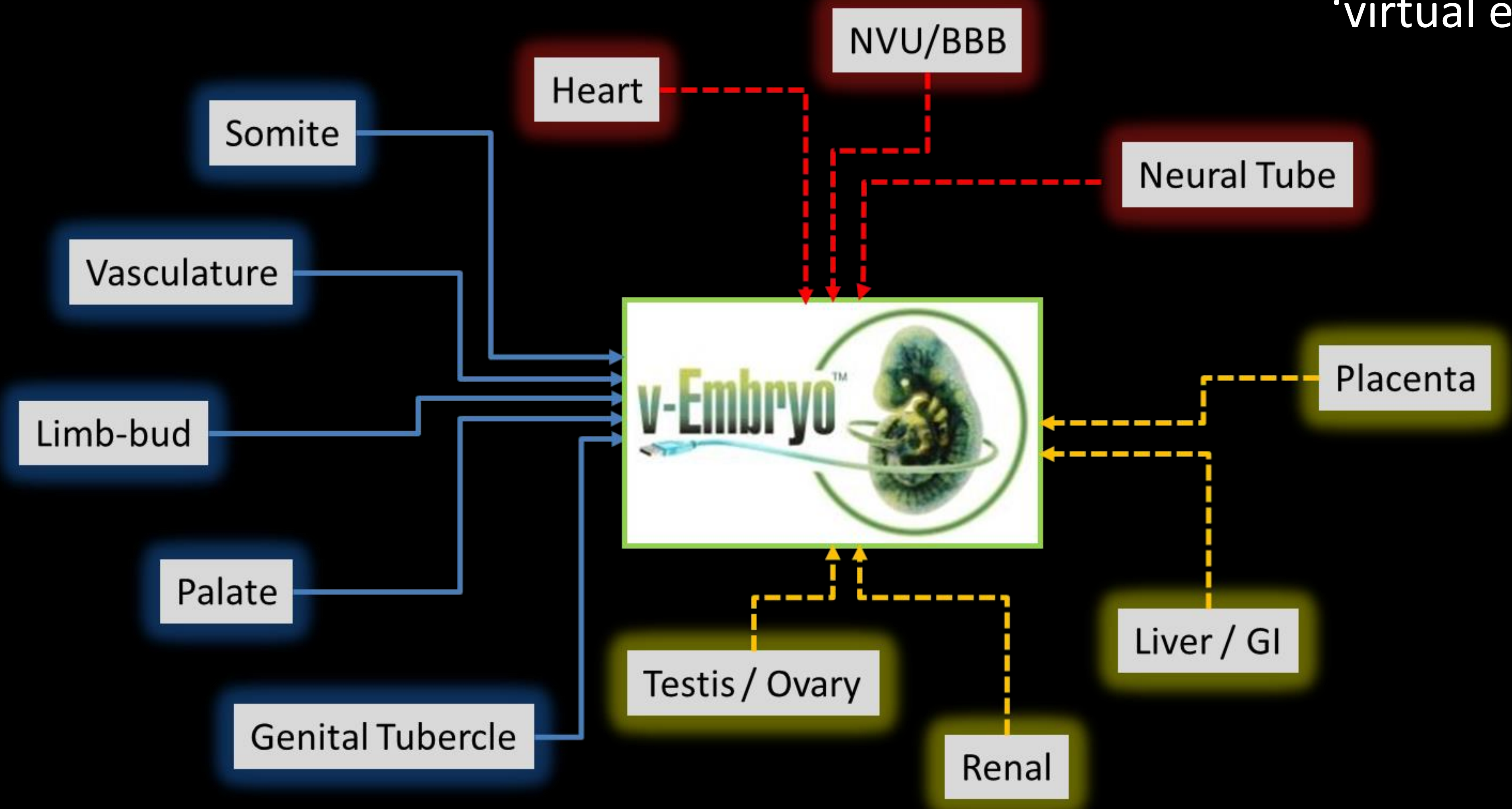


INPUT 6.0 µM: AC95 CSF1R + AC85 VEGFR3 + AC50 VEGFR2
OUTPUT: loss of directional sprouting

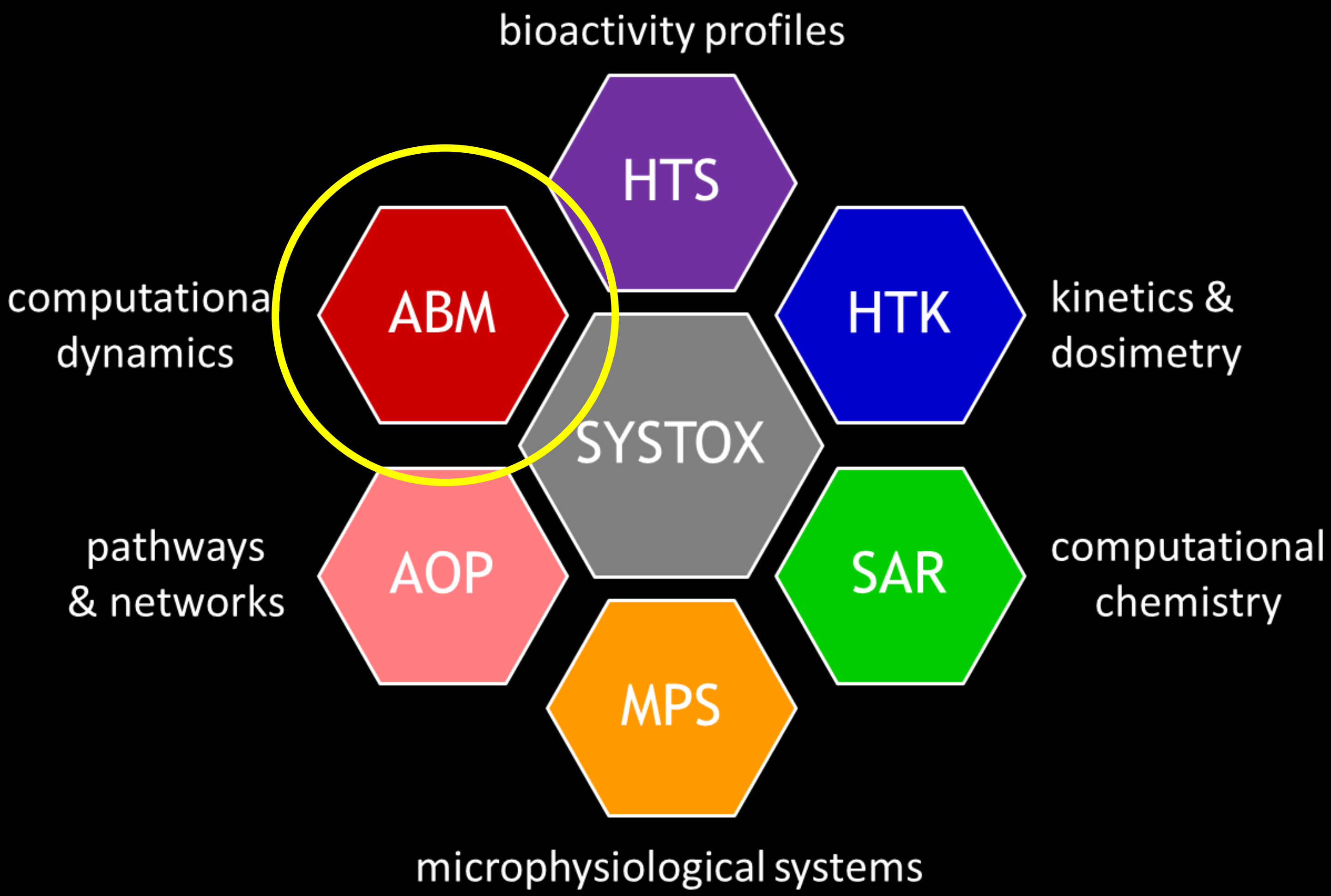
Zurlinden et al. (2017) manuscript in preparation

Grand Challenge:

a predictive
'virtual embryo'



Systems Toxicology



Special Thanks

- Barbara Klieforth – EPA / NCER
- Max Leung – NCCT (now CalEPA)
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- Todd Zurlinden – NCCT
- Nancy Baker – Leidos / NCCT
- Richard Spencer – ARA / EMVL
- James Glazier – Indiana U
- Sid Hunter – NHEERL / ISTD
- Kyle Grode – NHEERL/ISTD
- Andrew Schwab – NHEERL/ISTD
- Barbara Abbott – NHEERL/TAD
- David Belair – NHEERL/TAD
- John Wikswo – Vanderbilt U
- Shane Hutson – Vanderbilt U
- Bill Murphy – U Wisconsin
- Brian Johnson – U Wisconsin
- W Slikker Jr. – FDA / NCTR

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- Shane Hutson – Vanderbilt U (VPROMPT)
- Bill Murphy – U Wisconsin (H-MAPS)
- Elaine Faustman – U Washington (UW-PTC)
- Ivan Rusyn – Texas A&M U (CT-AOP)

EPA www.epa.gov/research 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

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