Computational Modeling and Simulation of Developmental Toxicity

What can we learn from a virtual embryo?

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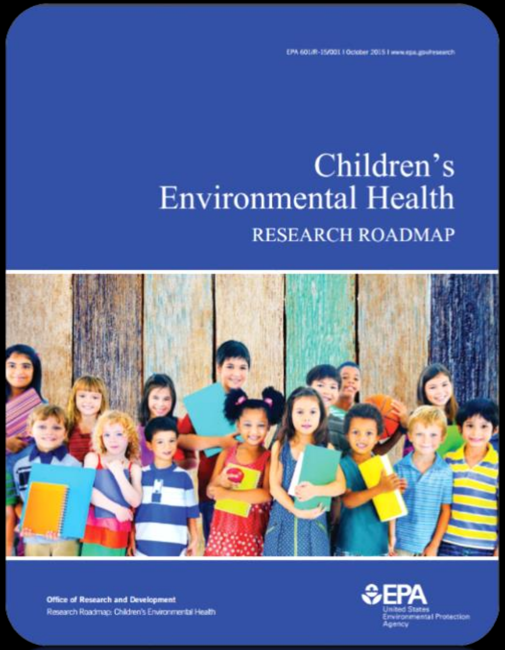
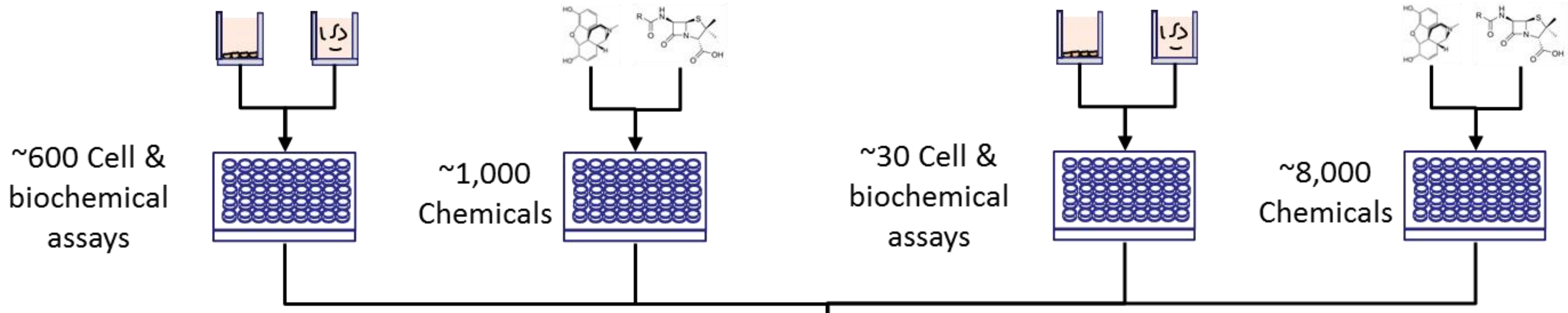
Developmental and Reproductive Toxicity (DART)



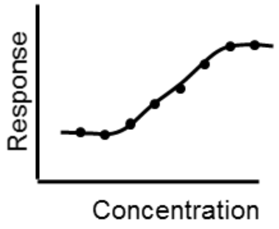
- DART testing is important for assessing the potential consequences of drug and chemical exposure on human health and well-being.
- Complexity of pregnancy and the reproductive cycle makes DART testing challenging and costly for traditional (animal-based) methods.
- A compendium of *in vitro* data from ToxCast/Tox21 high-throughput screening (HTS) programs is available for predictive toxicology.
- 'Predictive DART' will require an integrative strategy that mobilizes HTS data into *in silico* models that capture the relevant embryology.

Shifting to Molecular/Pathway Approaches

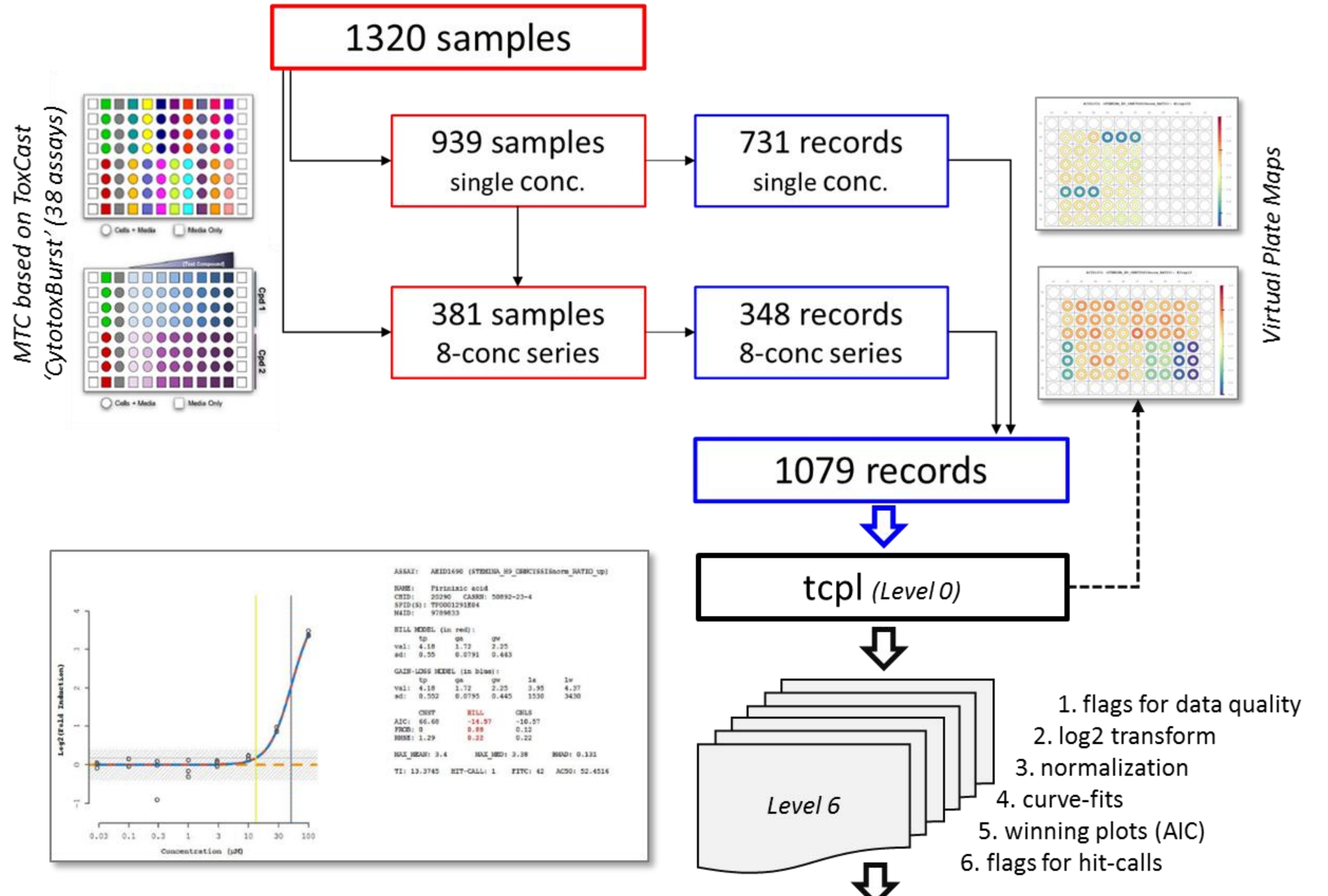
ToxCast | Tox21



Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



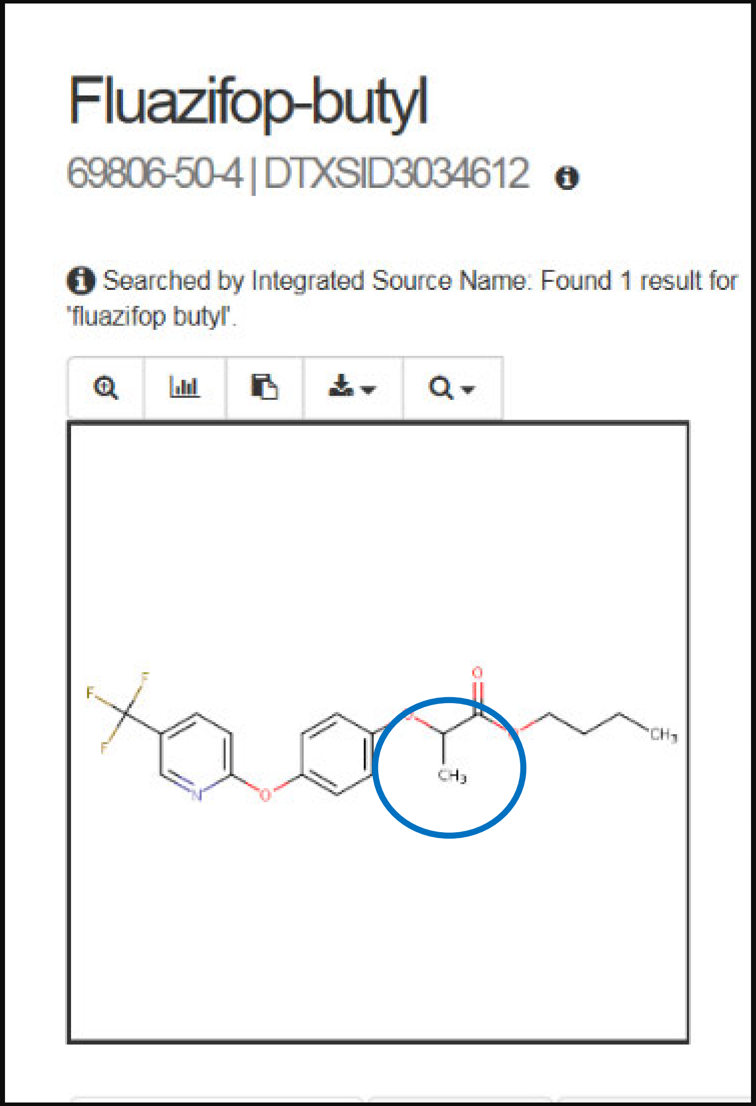
Stemina (STM): hESC (WA09) targeted biomarker (ORN/CYSS secretome)

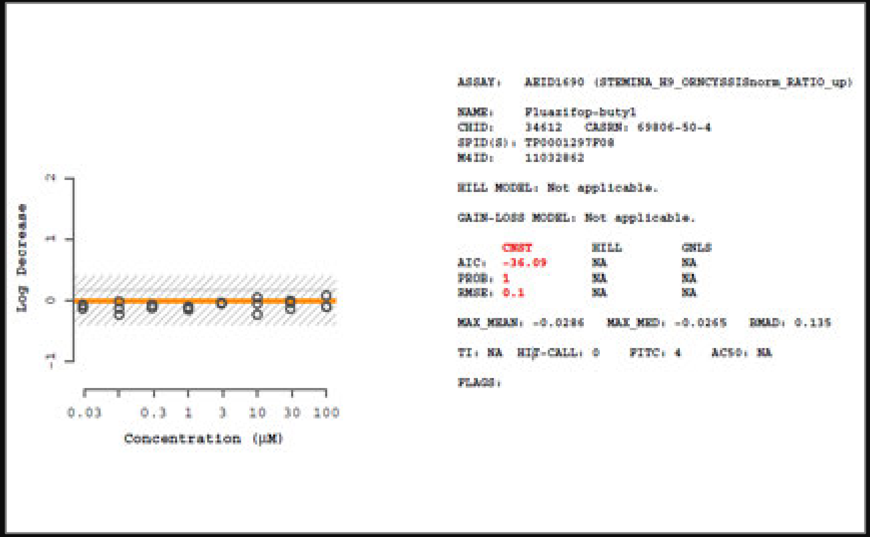


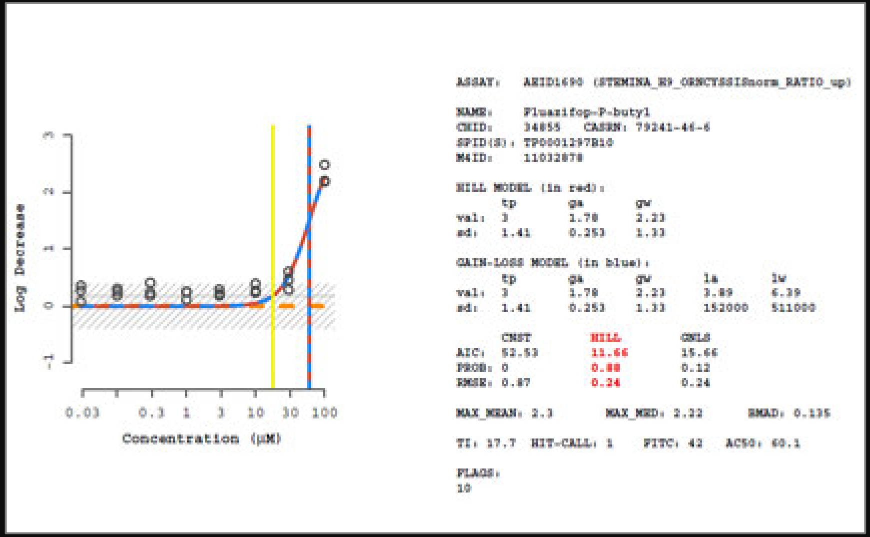
aeid	aenm	resp_unit
1680	STEMINA_H9_CystineISnorm_up	log2_fold_induction
1681	STEMINA_H9_CystineISnorm_dn	log2_fold_induction
1686	STEMINA_H9_OrnithineISnorm_up	log2_fold_induction
1687	STEMINA_H9_OrnithineISnorm_dn	log2_fold_induction
1690	STEMINA_H9_ORNCYSSISnorm_RATIO_up	log2_fold_induction
1691	STEMINA_H9_ORNCYSSISnorm_RATIO_dn	log2_fold_induction
1858	STEMINA_H9_Viability_Norm	percent activity

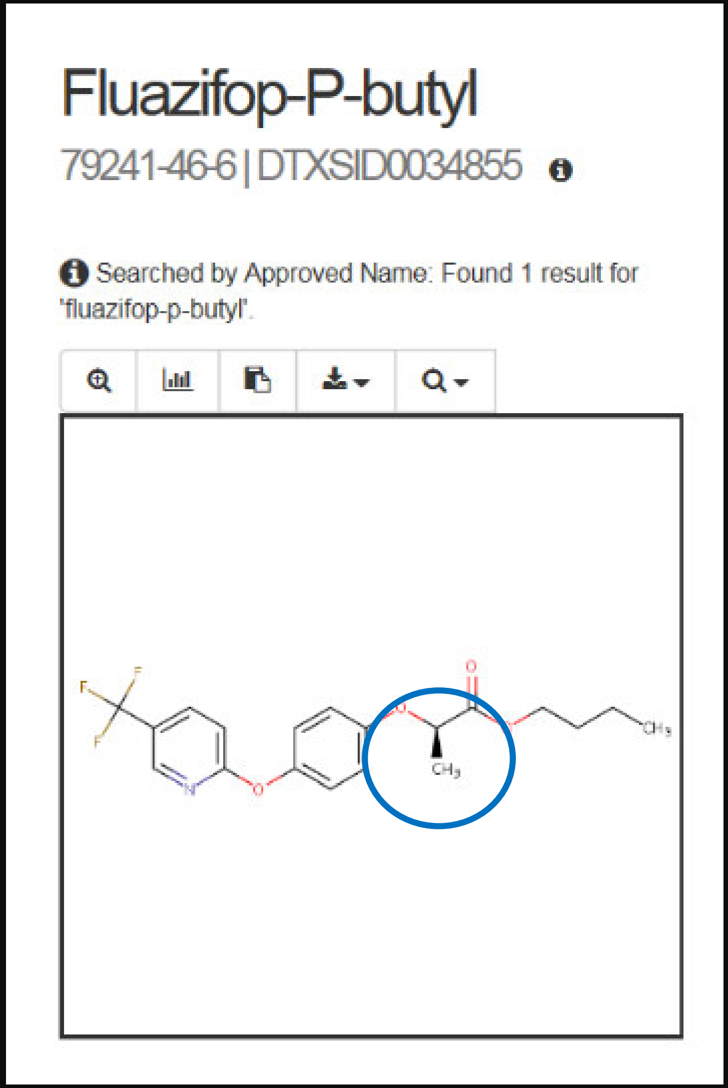
- **Prediction models:** range from 87-91% BA (sens 0.80 to 0.86, spec 0.93 to 1.00 depending on anchor)
- **Initial analysis:** revealed 177 actives (16.4% tested) with several known teratogens and many unknowns
- **ToxRefDB:** preliminary model vs skeletal defects (dLEL ≤ 50 mg/kg) : sensitivity (0.36), specificity (0.86) for BA = 79.3%

Example of “unknown teratogenicity” - stereoisomer pair

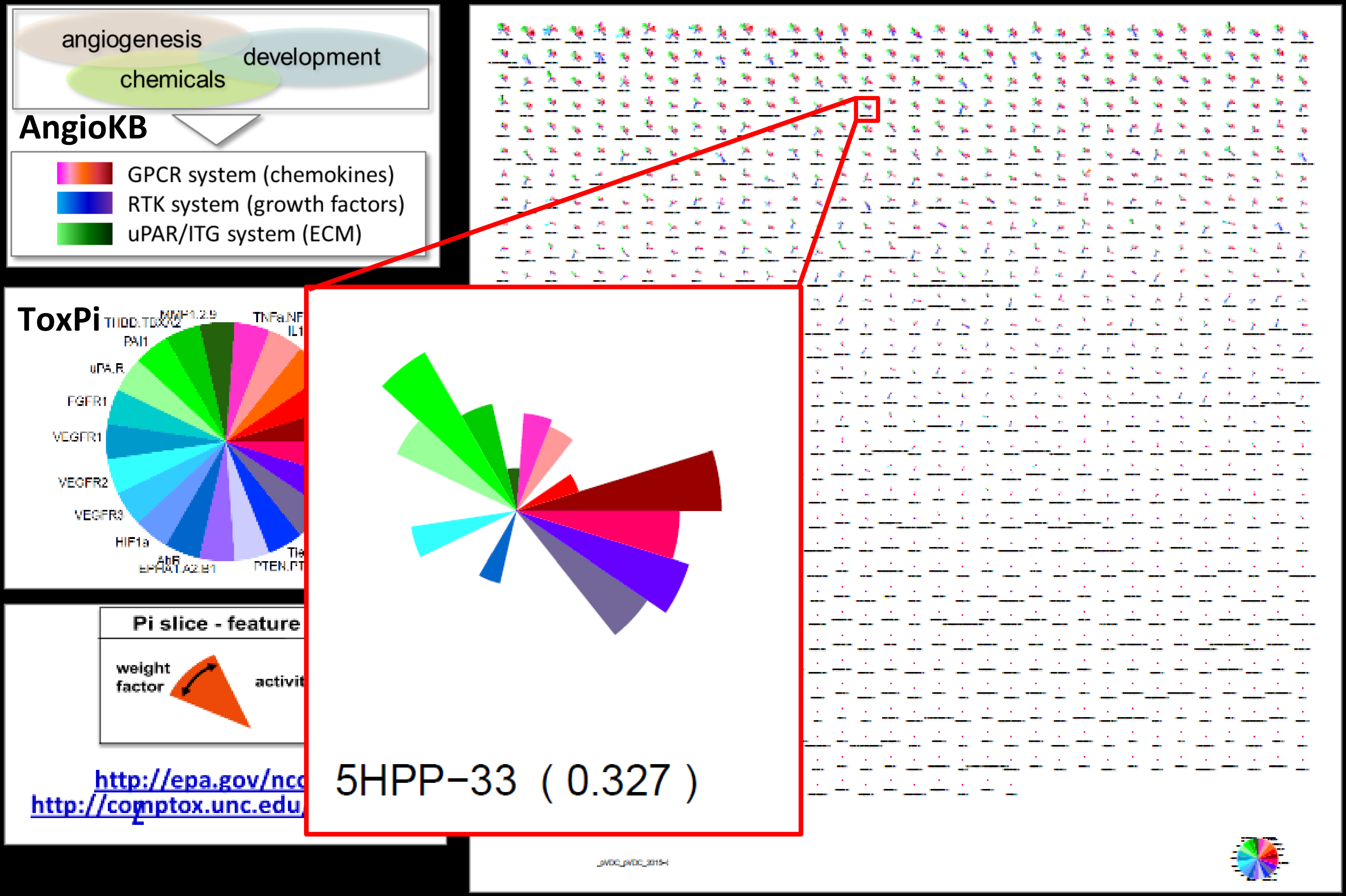




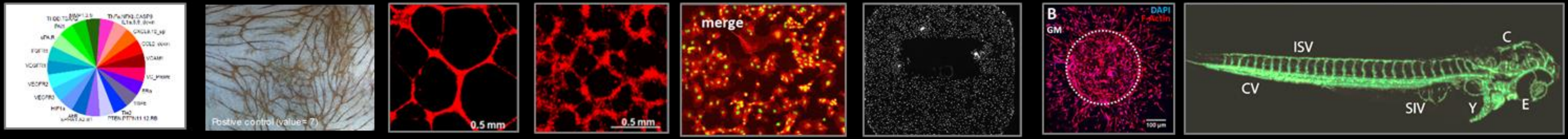


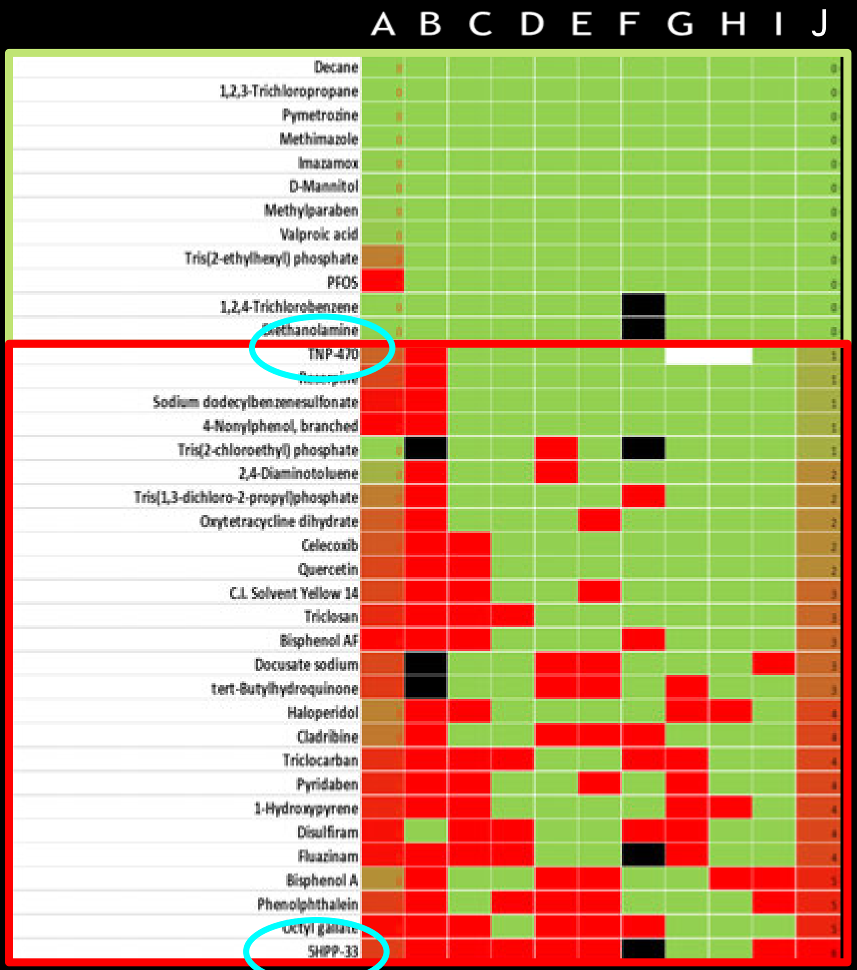


Angiogenesis: chemicals sorted by predicted potential to disrupt angiogenesis (pVDCs)



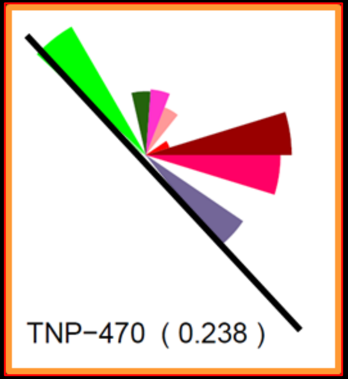
Validation: 38 pVDCs and non-pVDCs evaluated across 8 angiogenic platforms

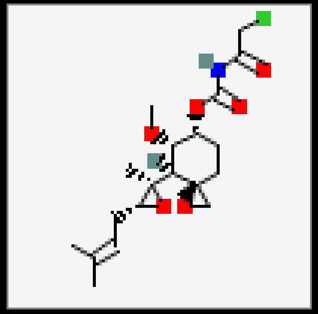


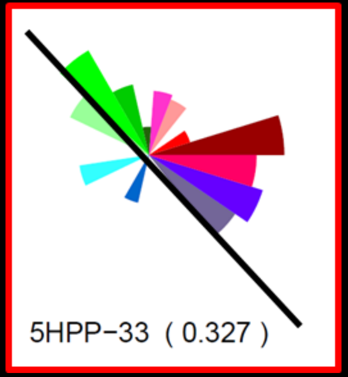


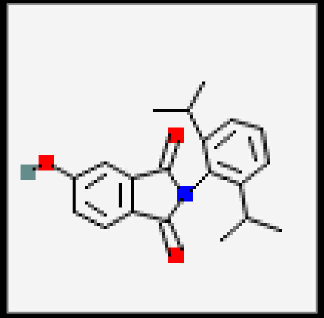
- A ToxPi [1]
- B FICAM tubulogenesis [2]
- C Synthetic tubulogenesis [3]
- D Matrigel tubulogenesis [3]
- E nuCTNB [4]
- F EC migration [4]
- G angiogenic sprouting [5]
- H TG-zebrafish [1]
- I Vala tubulogenesis [2]
- J aggregate (B to I)

[1] Tal et al. Reprod Toxicol (submitted); [2] Knudsen et al., in prep; [3] Nguyen et al. Nature Bioengineering (submitted); [4] Belair et al. (2016) Acta Biomaterialia.

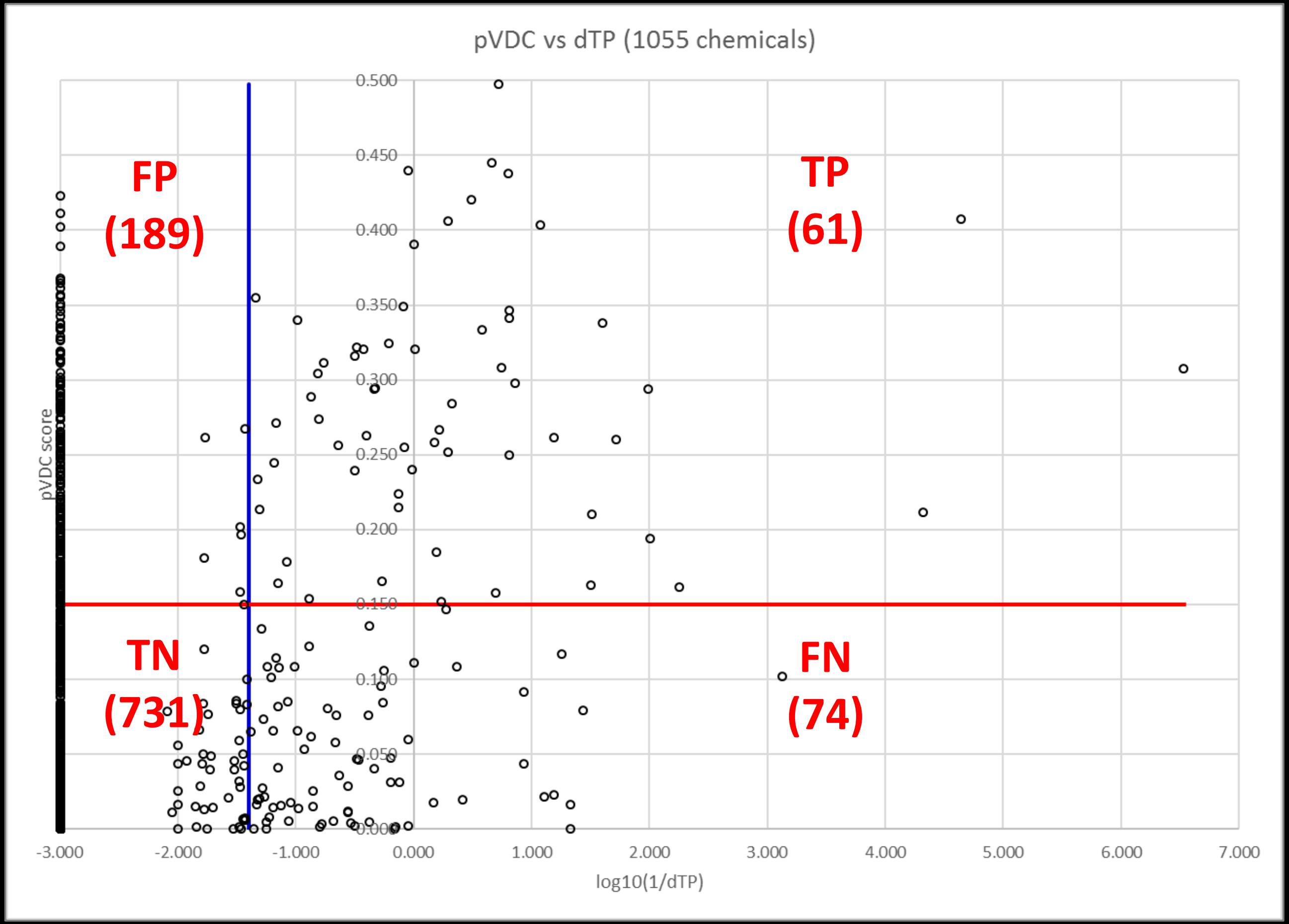








DevTox: how well does pVDC score match teratogenic potential in a human system?



AOP-based **pVDC** score vs **DevTox** potential from the STM hES cell platform

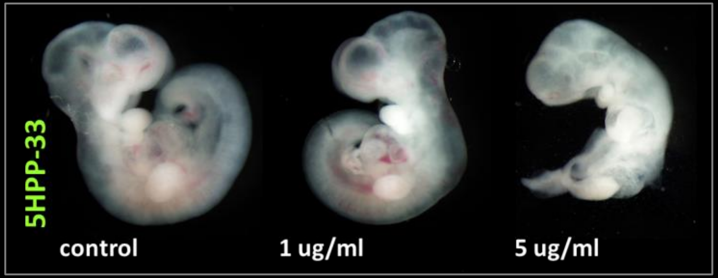
Balanced Accuracy = 75.1%
(modeled on the 38-test set)

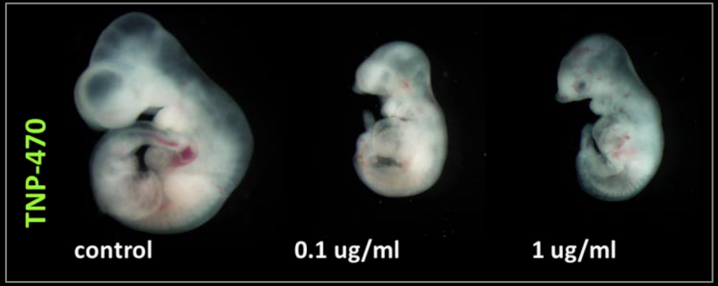
24.4% pVDC(+) also STM(+)
90.8% pVDC(-) also STM(-)

5HPP-33: DevTox (WEC) AC50 = 21.2 uM; STM predicts human teratogenicity at $\geq$ 4.37 uM

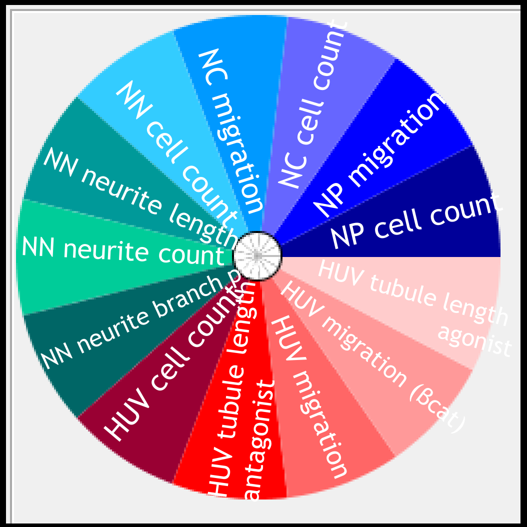
TNP-470: DevTox (WEC) AC50 = 0.038 uM; STM predicts human teratogenicity at $\geq$ 0.01 uM

Preliminary: 46 chemicals tested for activity on angiogenesis (Vala) and neurogenesis (Aruna)

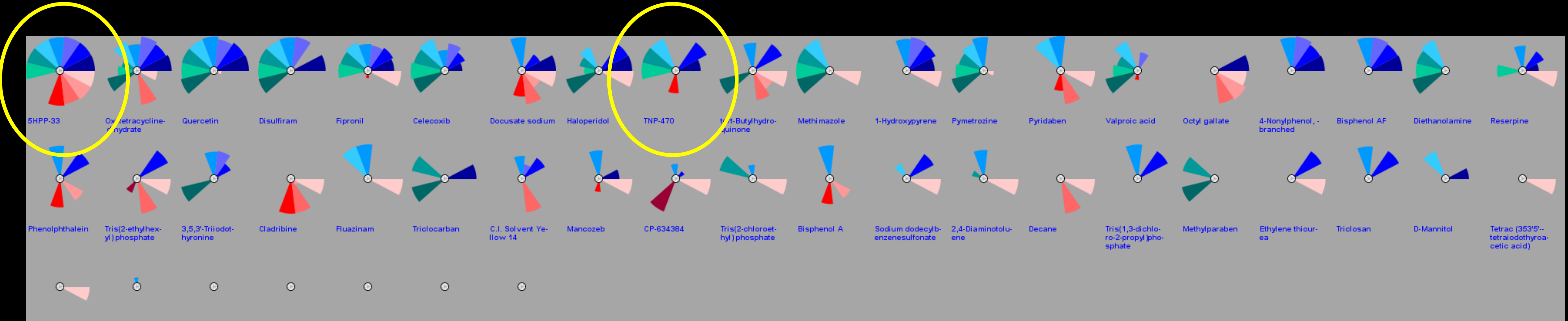




Neurogenesis (blue/green)



Angiogenesis (red)



AC_{20} normalized to control presented as percentage of max potency

Virtual reconstruction of developmental toxicity

- The question of how tissues and organs are shaped during development is crucial for understanding (and predicting) human birth defects.
- While ToxCast HTS data may predict developmental toxicity with reasonable accuracy, mechanistic models are still necessary to capture the relevant biology.
- Subtle microscopic changes induced chemically may amplify to an adverse outcome but coarse changes may override lesion propagation in any complex adaptive system.
- Modeling system dynamics in a developing tissue is a multiscale problem that challenges our ability to predict toxicity from *in vitro* profiling data (ToxCast/Tox21).

Anatomical homeostasis in a self-regulating Virtual Embryo

Mouse Morula
SOURCE: Science Photo Library

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

Breathing life into a ‘Virtual Embryo’

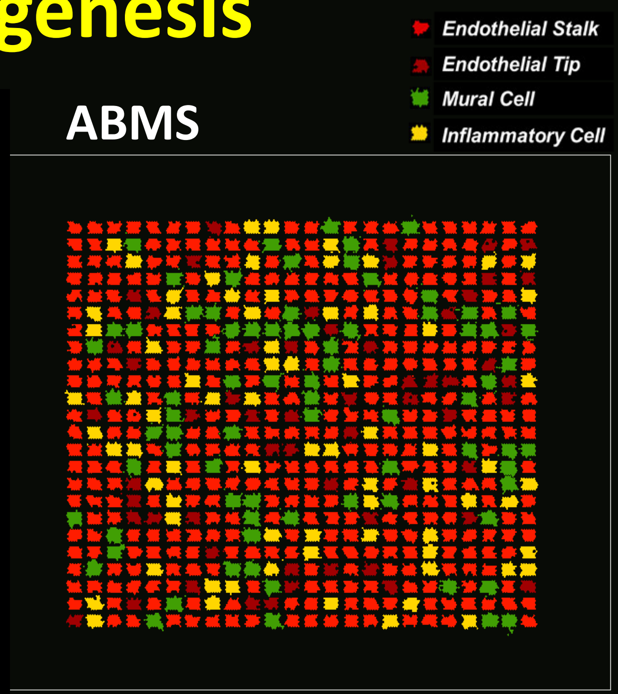
- **Hypothesis:** computer models that recapitulate a morphogenetic series of events can be used analytically (to understand) and theoretically (to predict) developmental toxicity.

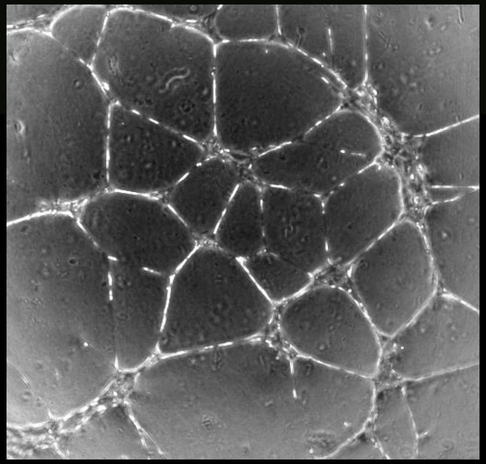
- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics from the bottom-up, cell-by-cell and interaction-by-interaction.

- **CompuCell3D:** open source modeling environment
  - engineered at Indiana University by James Glazier and colleagues;
  - steppables for distinct cell behaviors (growth, proliferation, apoptosis, differentiation, polarization, motility, ECM, signal secretion, …);
  - rules coded in Python for cell-autonomous ‘agents’ that interact in shared microenvironment and self-organize into emergent phenotypes.

Angiogenesis

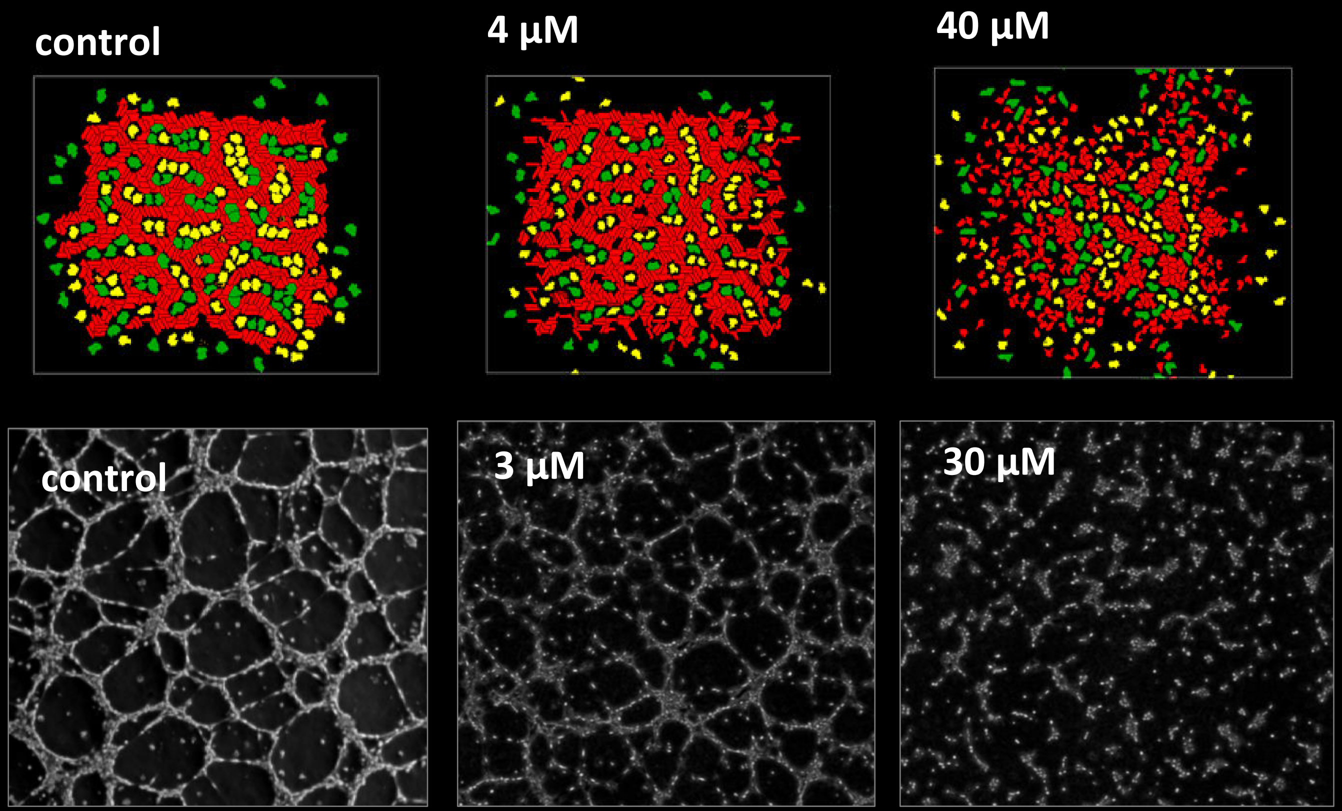
VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2





SOURCE: J Glazier, Indiana University

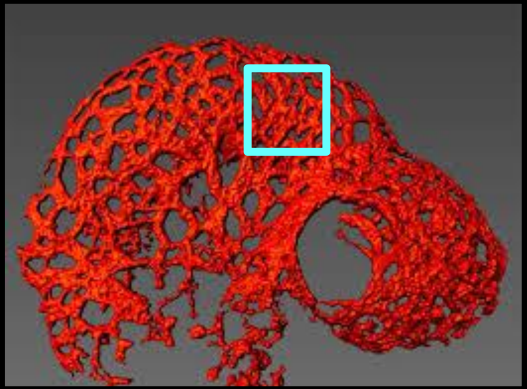
Exposure to 5HPP-33, a synthetic thalidomide analog

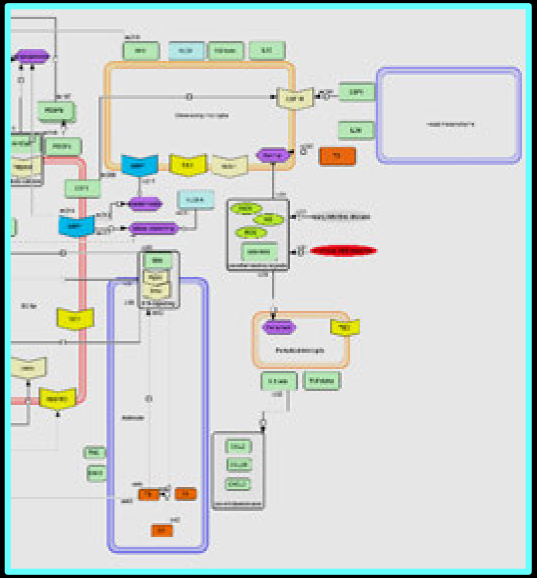


SOURCE: Kleinstreuer et al. 2013, PLoS Comp Biol

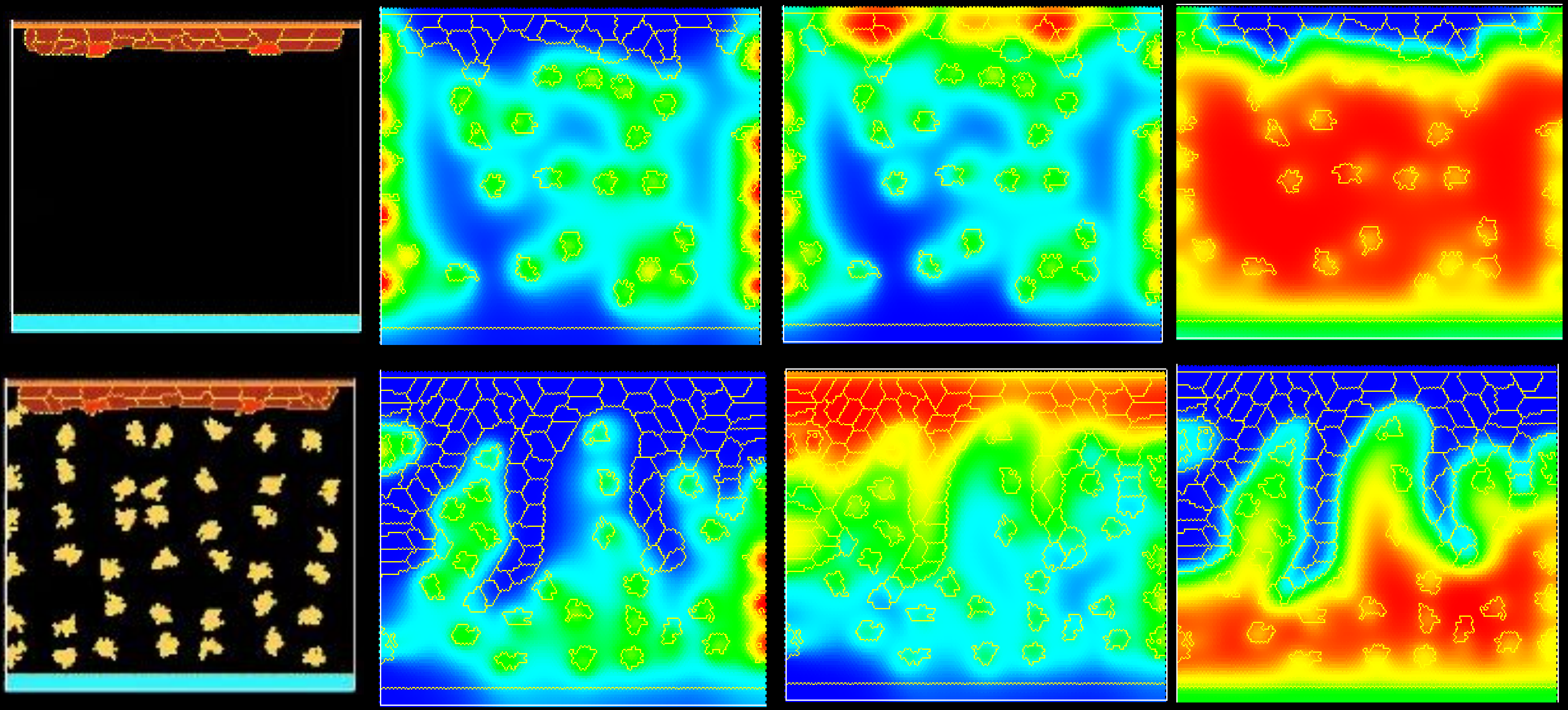
Computational Neurovascular Unit (cNVU): simulating brain angiogenesis with microglia.

BBB development





Cell field | VEGF-C | sVEGFR1 | VEGF-A



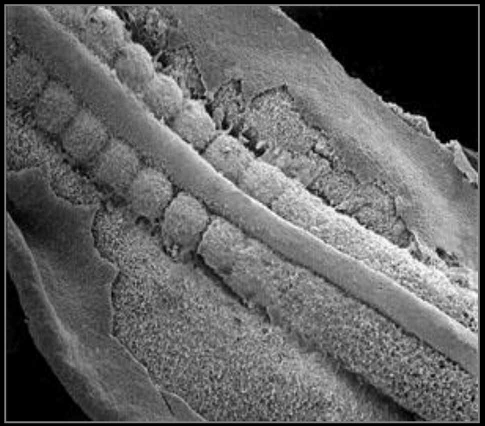
Endothelial (Stalk)

Endothelial (Tip)

Microglia

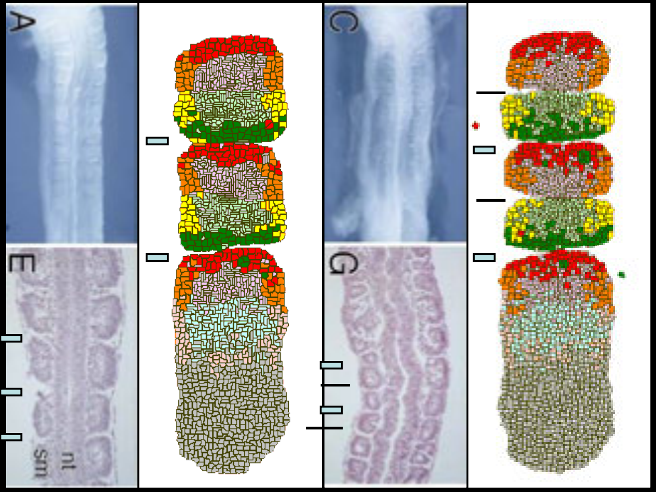
Zurlinden et al. (in preparation)

Somite formation

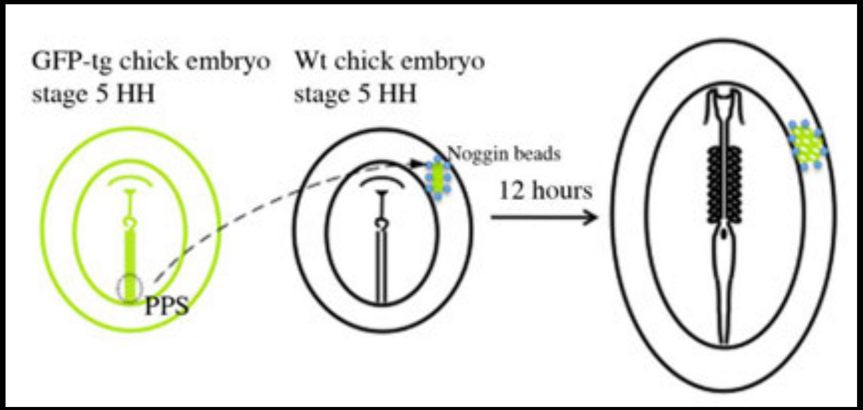


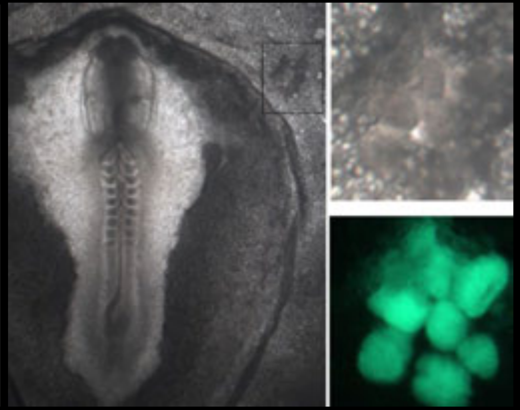
Hes1-EGFP time-lapse (3h)
Masamizu et al. 2006

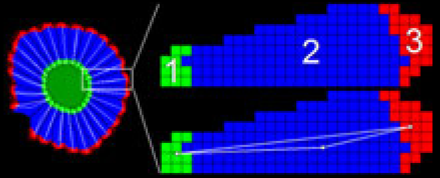
Clock and Wavefront model:
- *signal gradients along AP axis (eg, FGF8, RA)*
- *oscillating gene expression (eg, LFNG, Hes1)*
- *cell adhesion (eg, ND, ephrin system)*



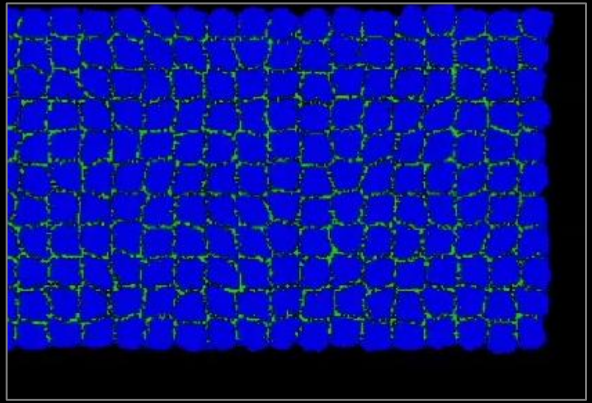
SOURCE: Hester et al. (2011) PLoS Comp Biol



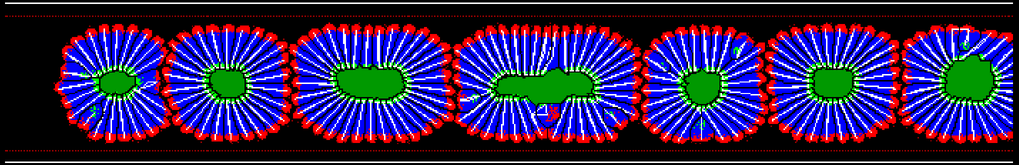




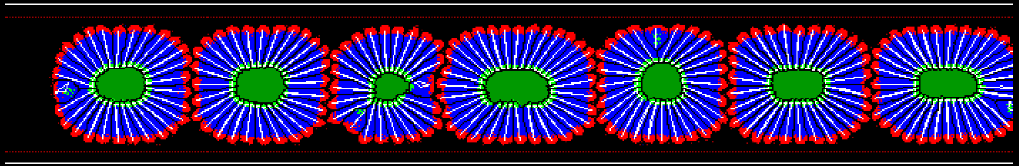
Epithelialization model:
- *clock genes do not oscillate*
- *somites form simultaneously*



Adding the Wavefront:
- *restores sequentiality*

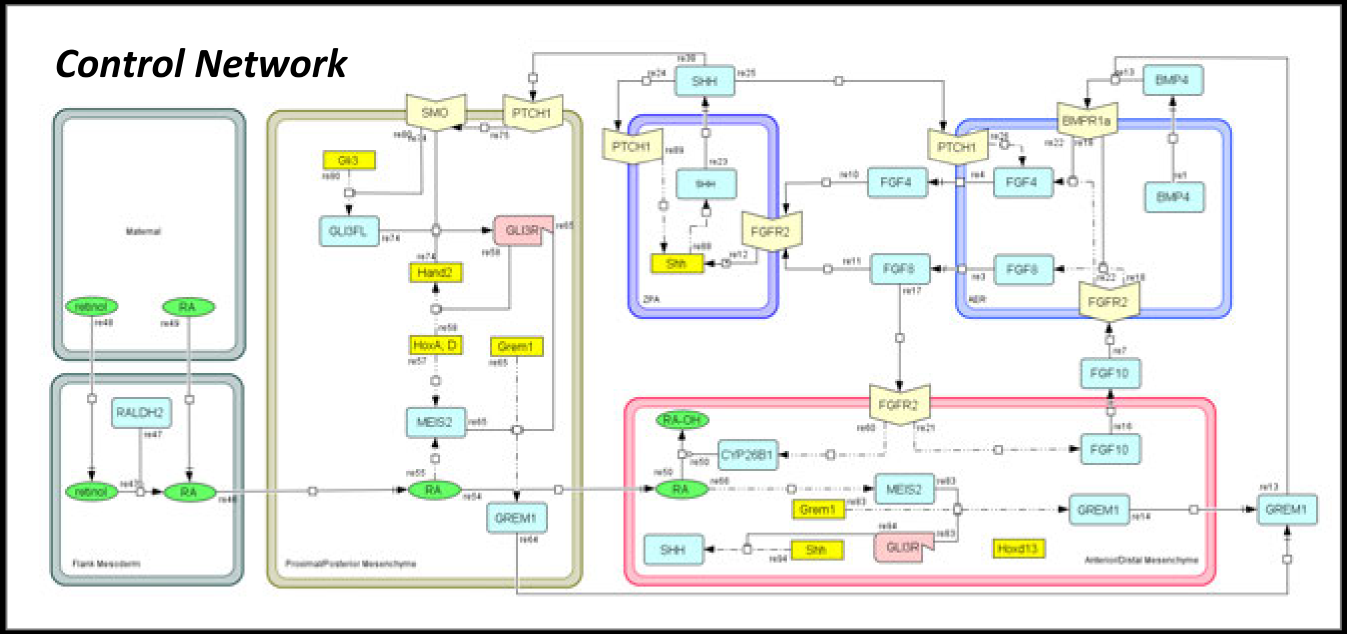


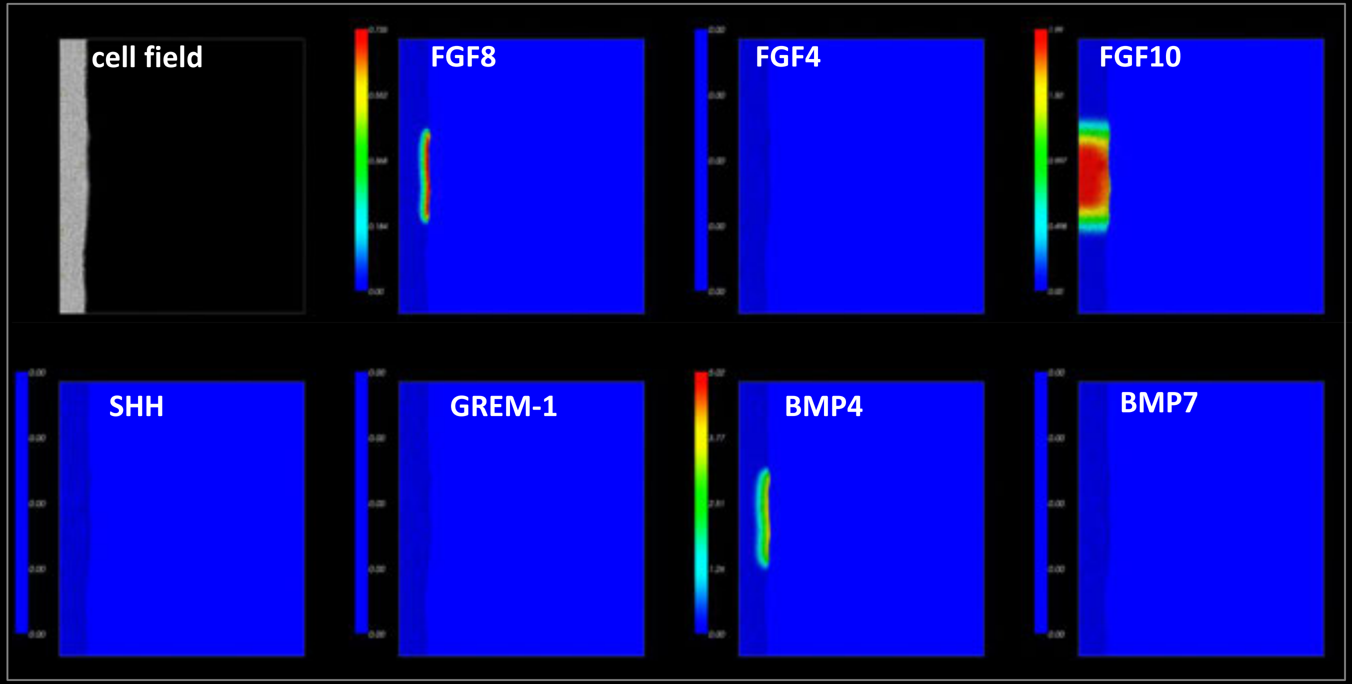
And adding the Clock:
- *improves regularity*

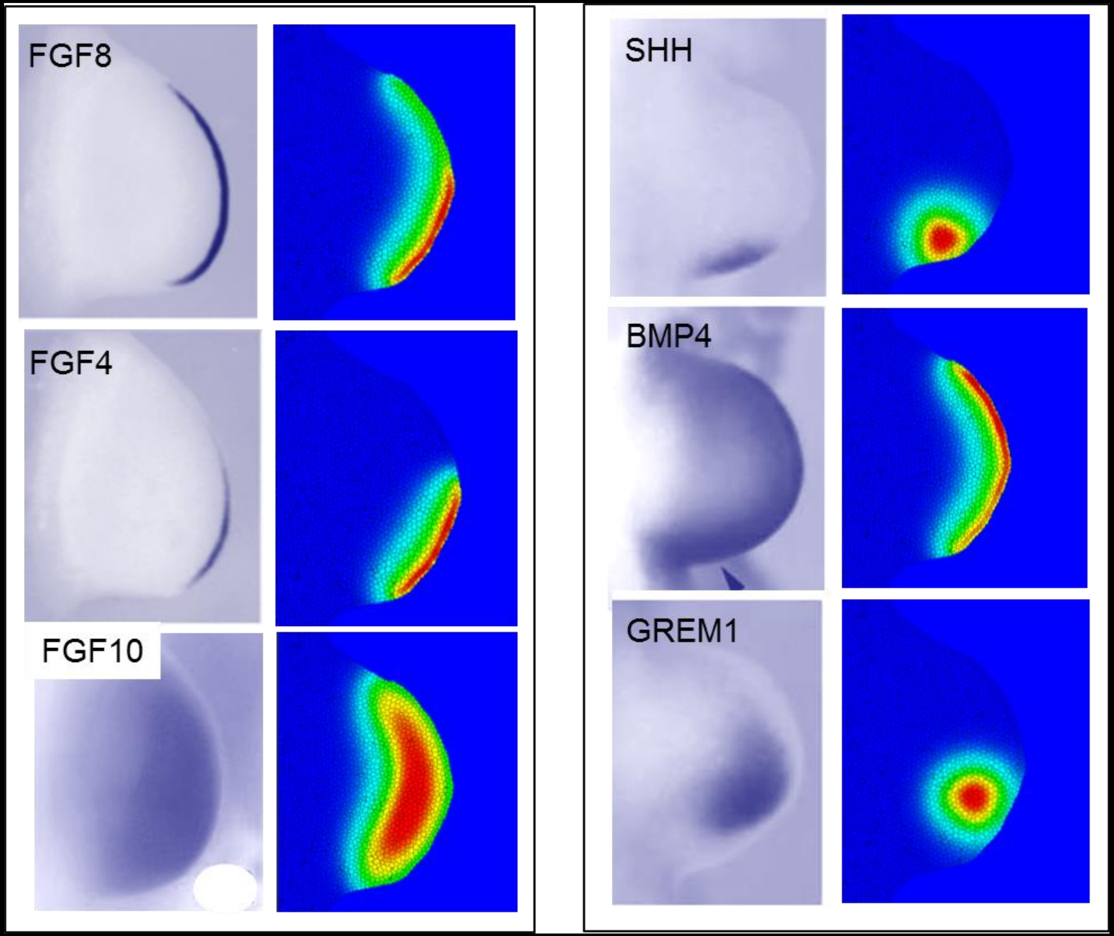


SOURCE: Dias et al. (2014) Science

Limb-bud outgrowth

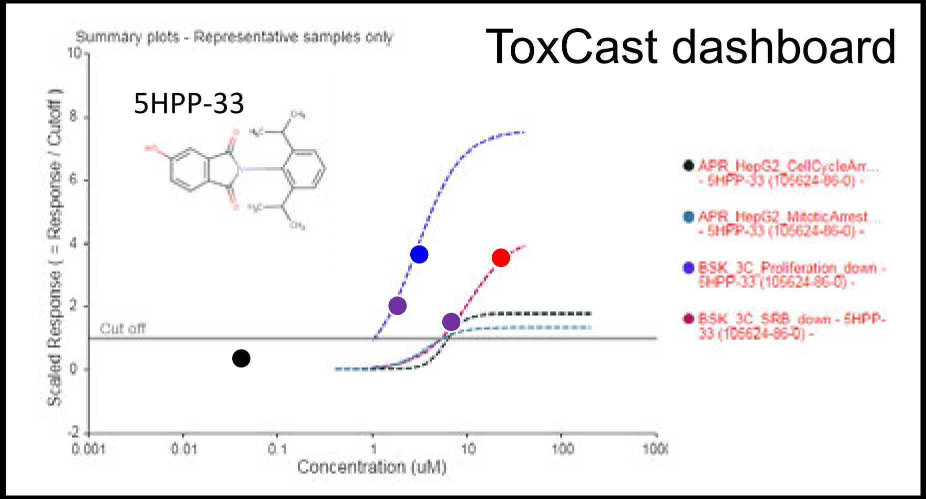


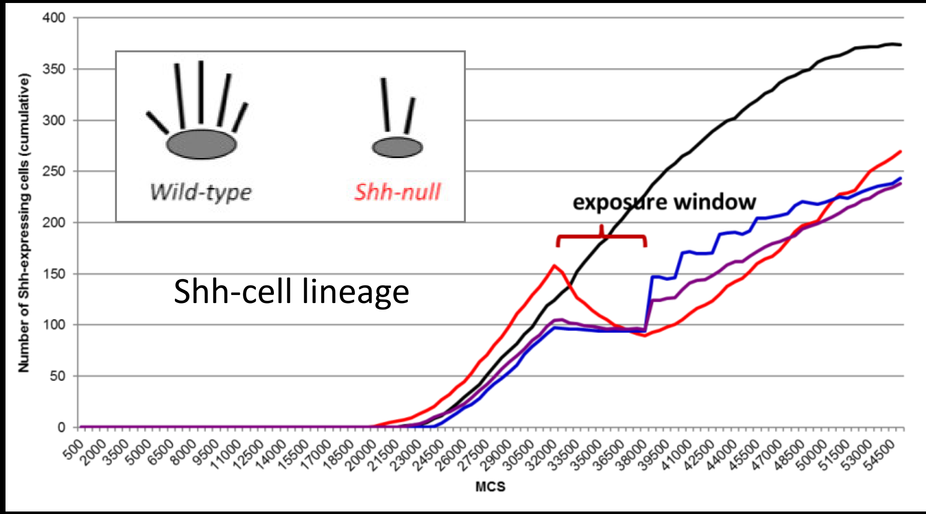


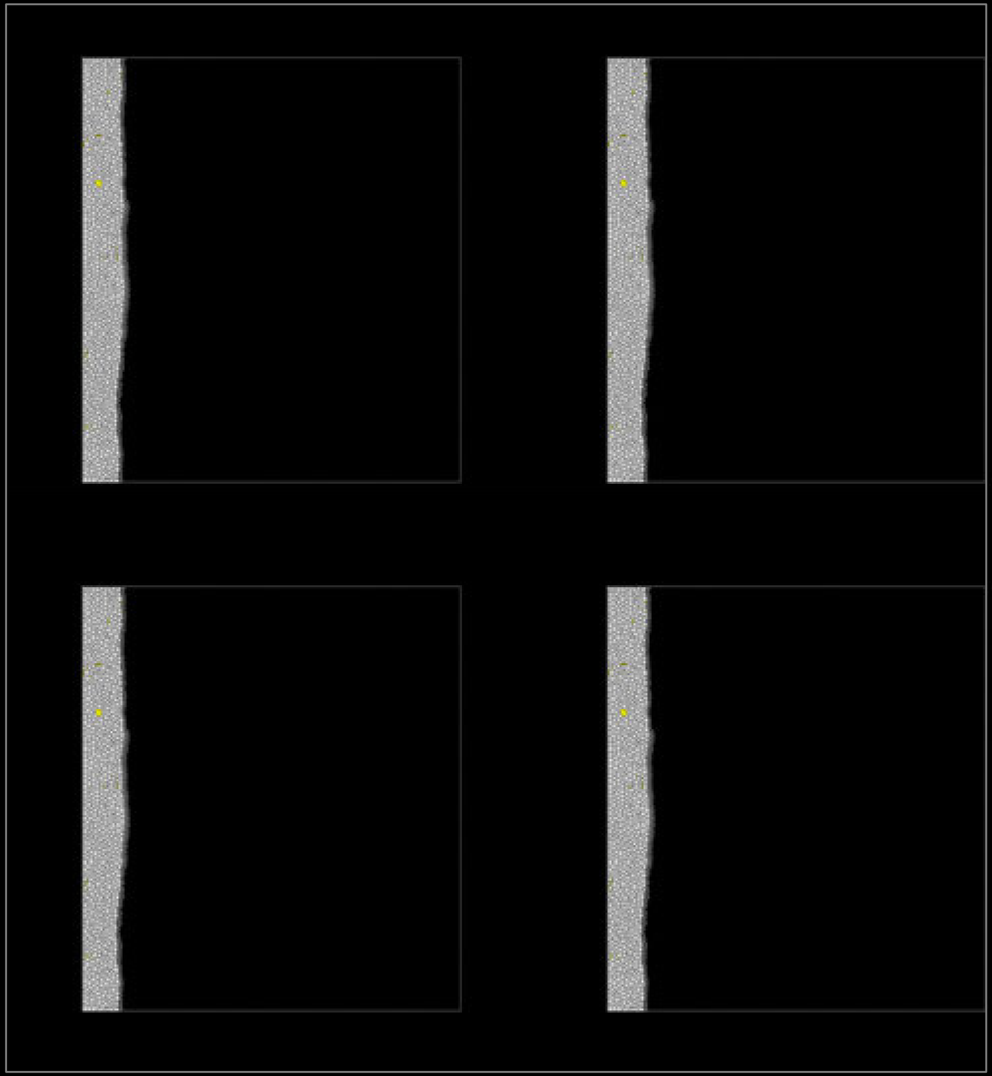


Knudsen et al. (in preparation)

Teratogenesis *in silico*

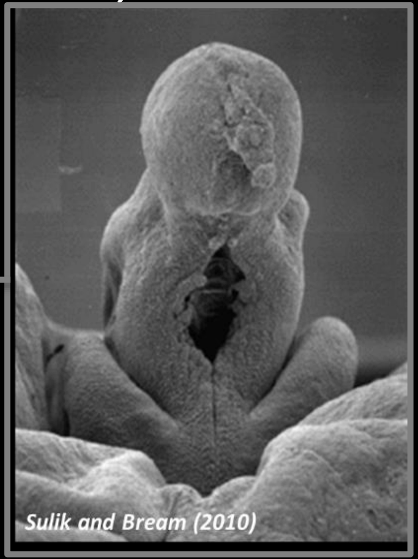




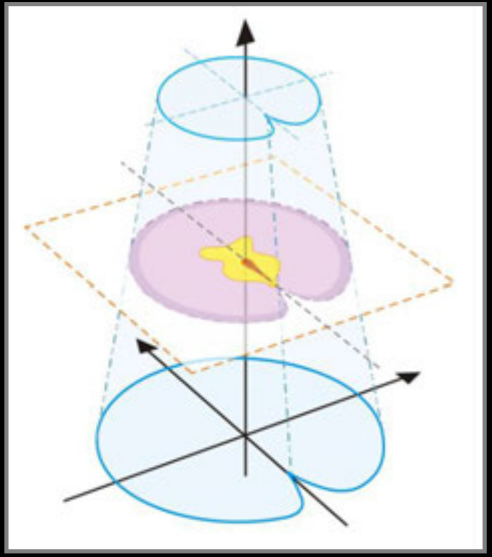


Genital Tubercle (GT) differentiation

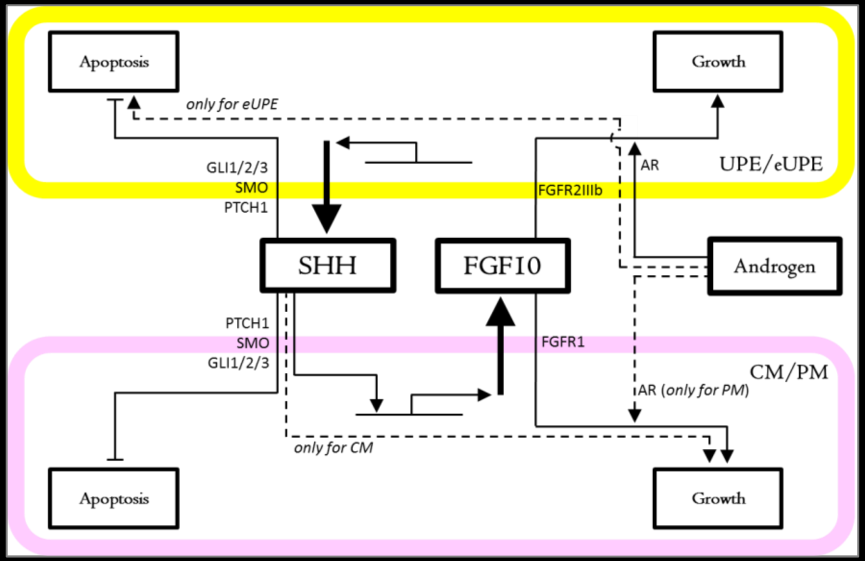
Embryonic GT



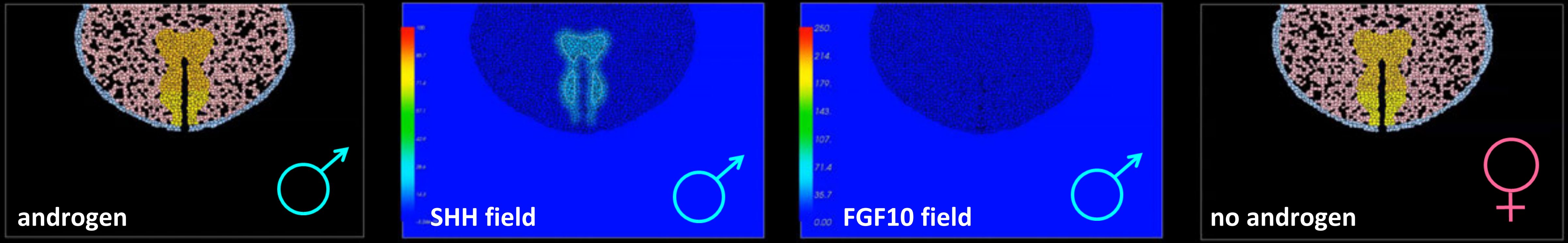
Abstracted GT



Control Network (mouse)

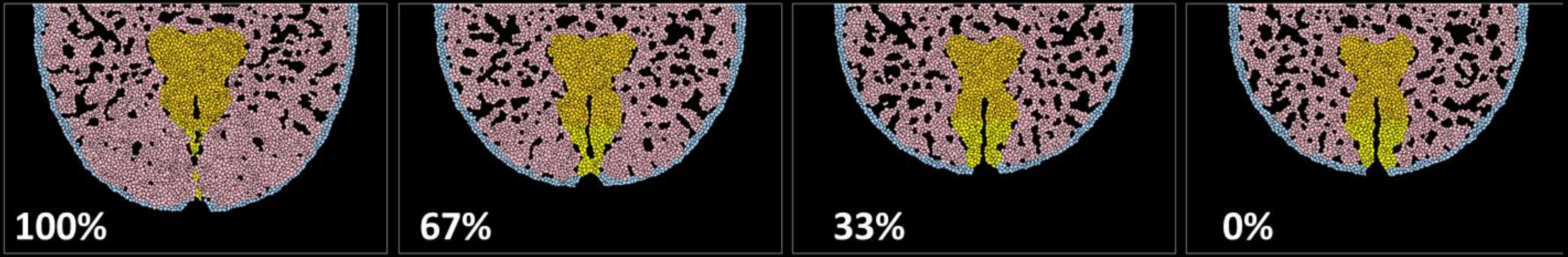


ABM simulation for sexual dimorphism (mouse GD13.5 – 17.5)

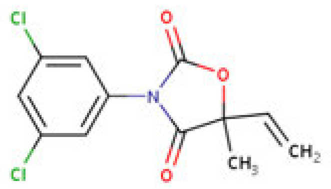


Urethral Closure: complex process disrupted in 'hypospadias'

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

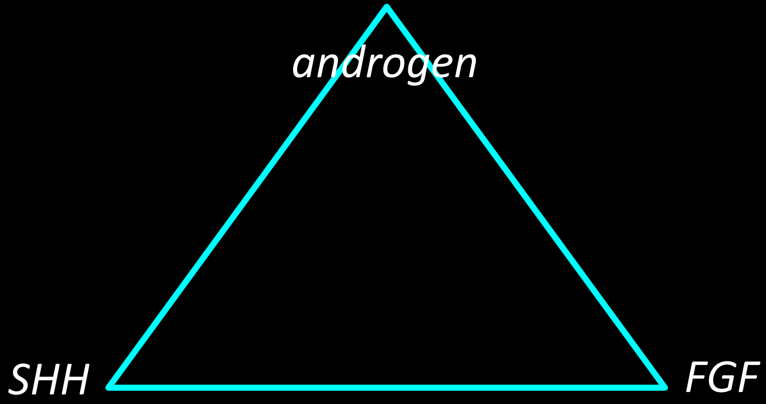


Leung et al. (2016) ***Reproductive Toxicology***

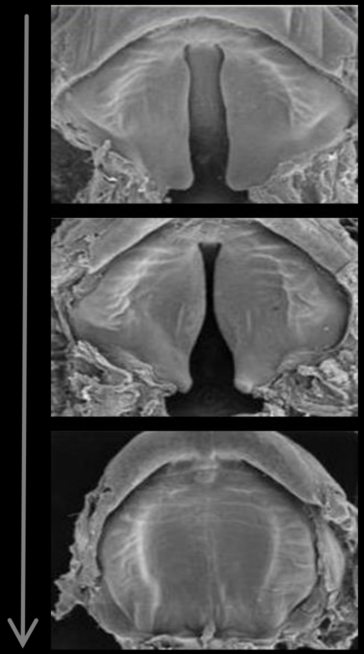


vinclozolin

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

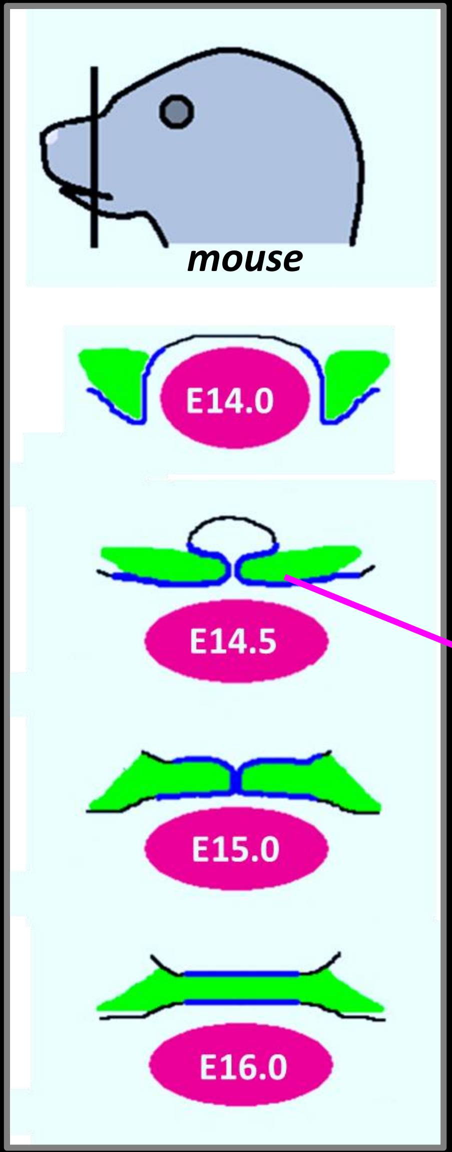


Cleft Palate

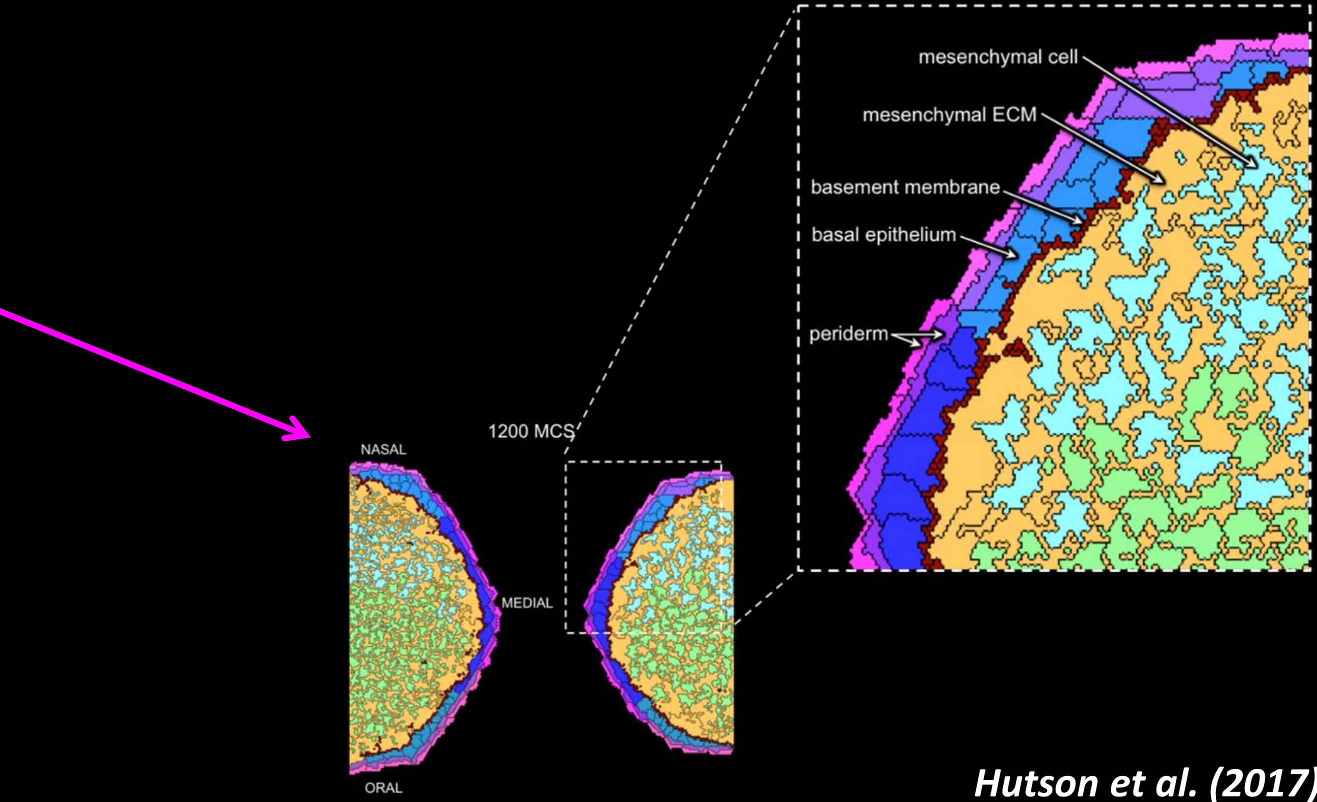


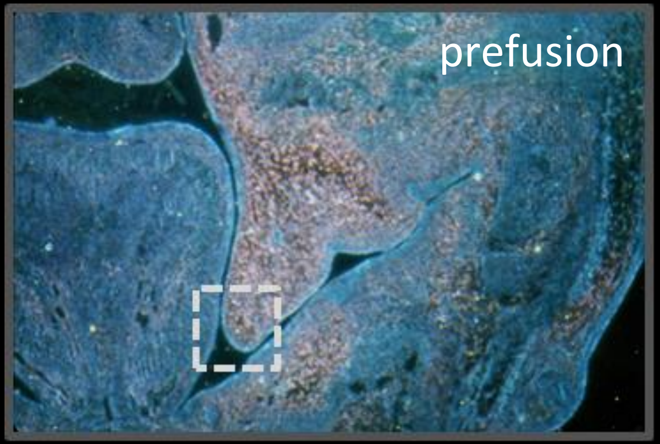
- Most prevalent craniofacial birth defect (annually ~7000 newborns in the USA and ~200,000 worldwide).
- Etiology is multifactorial: interaction of genetic, environmental, and lifestyle factors.
- Vulnerable period encompasses outgrowth of right-left palatal process in the oral cavity of the 1st trimester embryo.
- Local disruption of epithelial-mesenchymal interaction impairs outgrowth and fusion of the palatal processes.

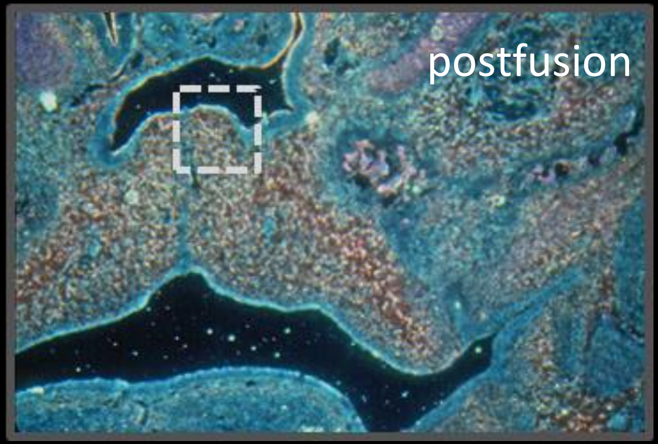
Modeling Palatal Development



- E12.5 initial outgrowth of palatal shelves
- E13.5 expansion alongside the tongue
- E14.5 elevate, meet, and adhere at medial edge
- E15.5 fusion complete, mesenchymal confluence
- E16.5 osteogenic differentiation

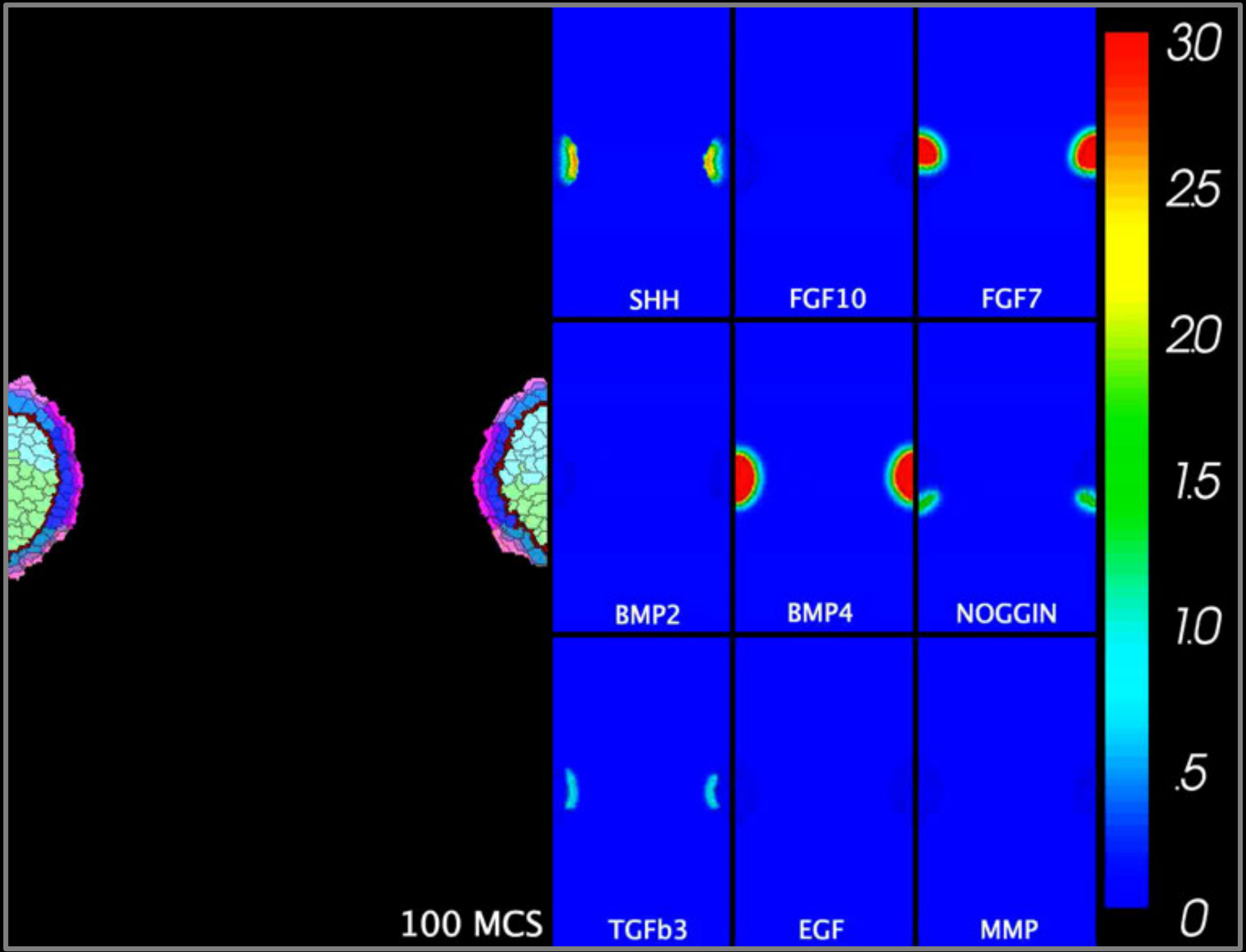






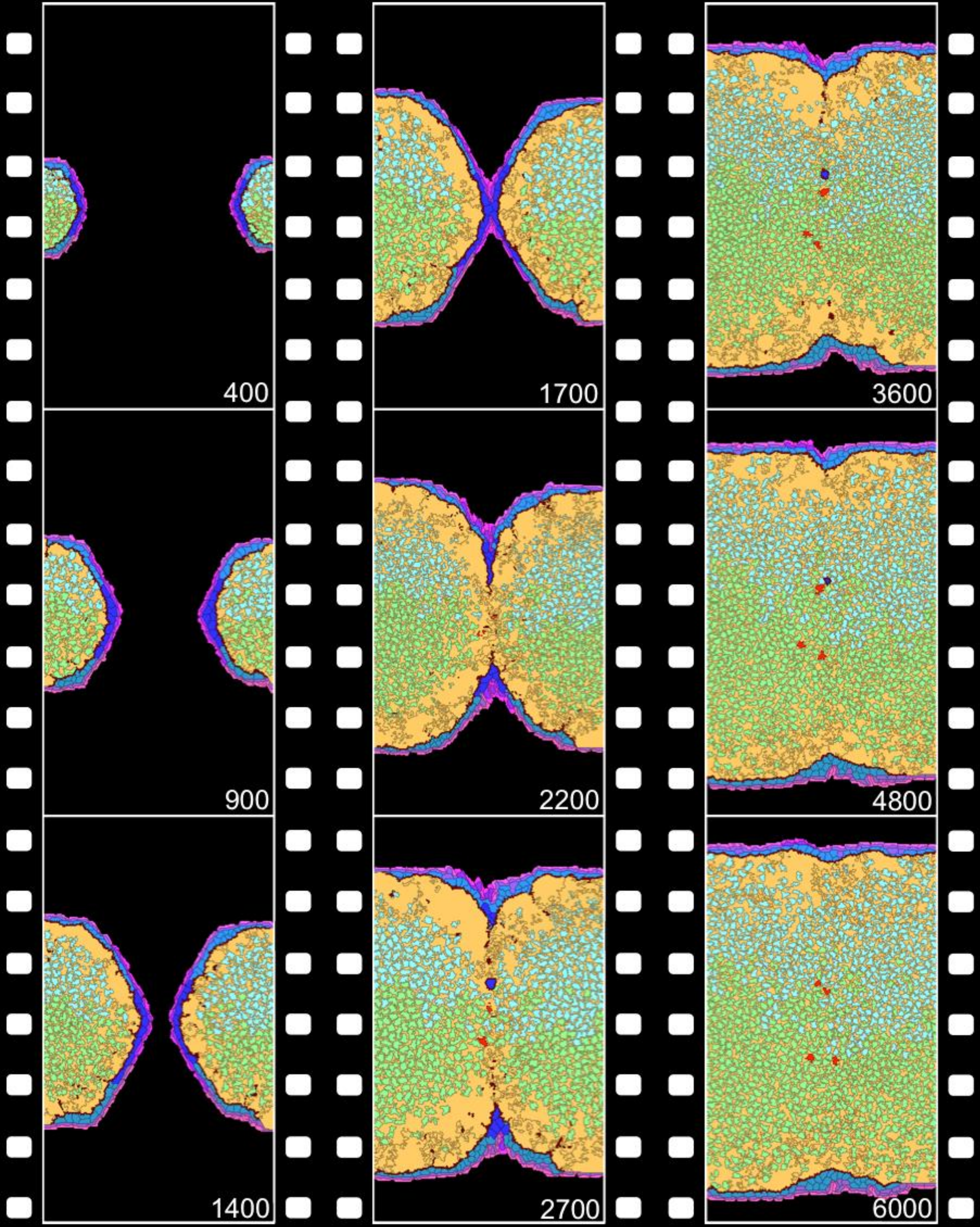
Hutson et al. (2017) (Chem Res Toxicol, in press)

Spatially-dynamic ABMS for palate development

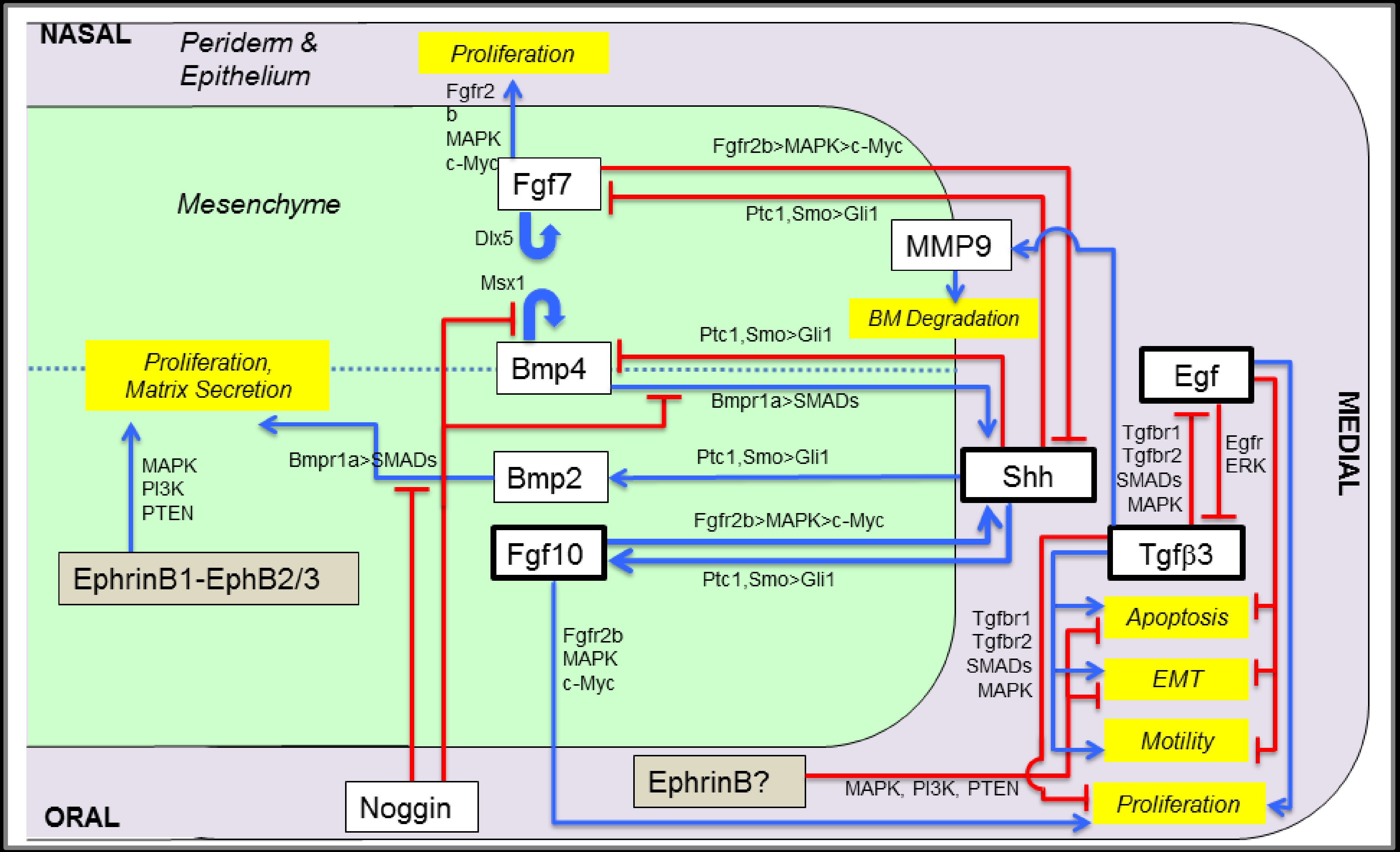


Morphogenetic fusion

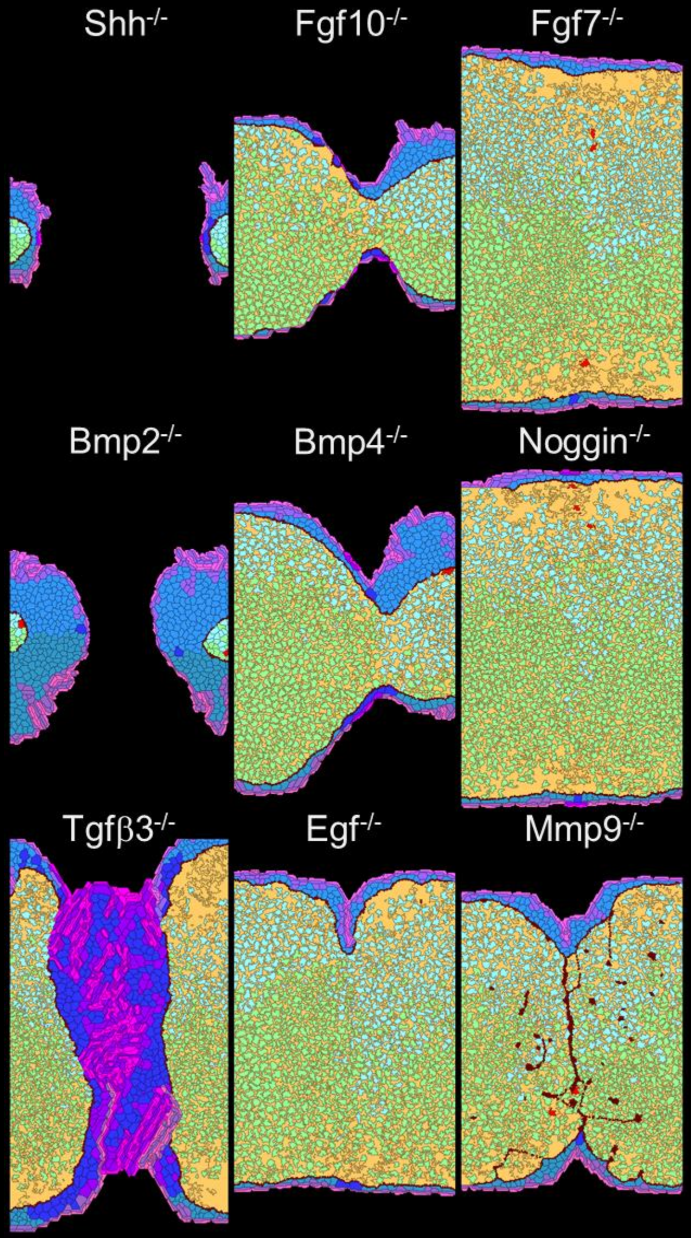
MES breakdown is programmed genetically to coincide with MEE apposition



Control network



Hacking the Control Network: *in silico* knockouts → 'Cybermorphs'



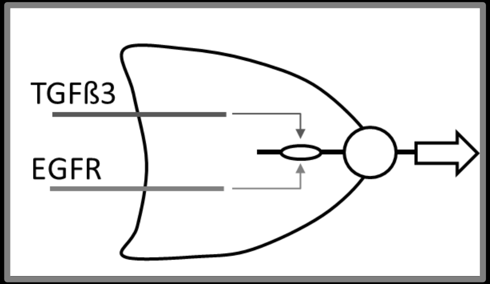
Signals driving outgrowth to apposition and MEE contact (MCS 200-2000)

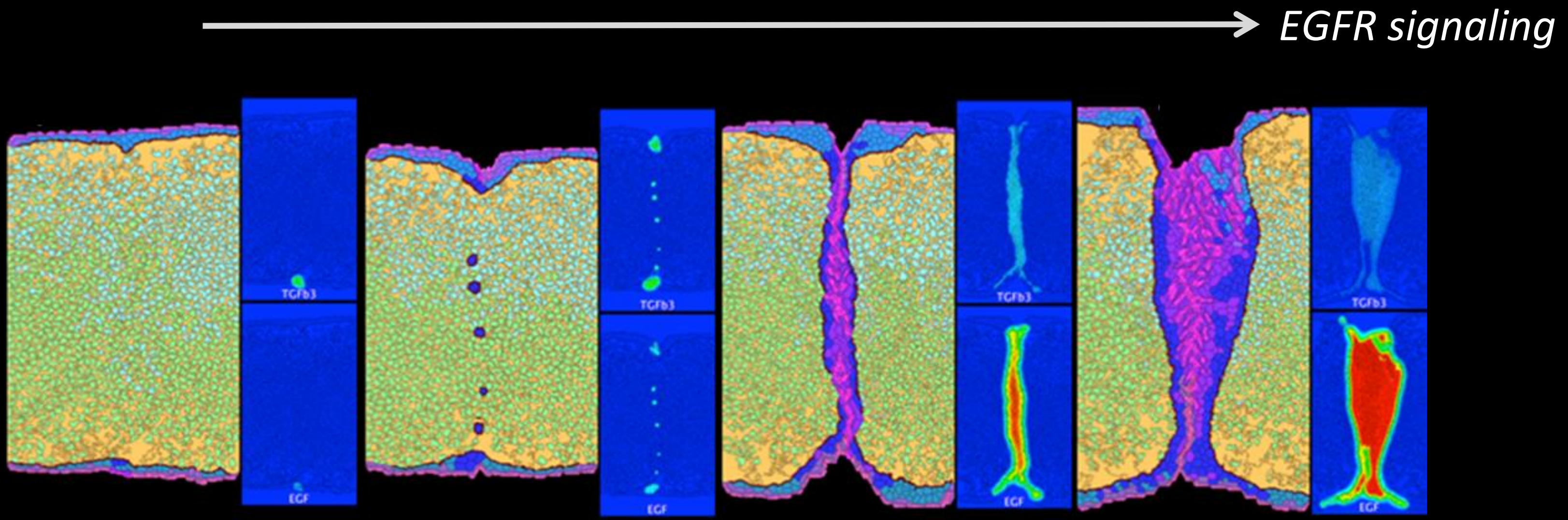
- SHH from the MEE drives mesenchymal proliferation and ECM production via FGFs/BMPs.
- Positive and negative feedback loops modulate epithelial-mesenchymal signaling cell-by-cell and interaction-by-interaction.

Signals driving MES breakdown (MCS 2000-3000)

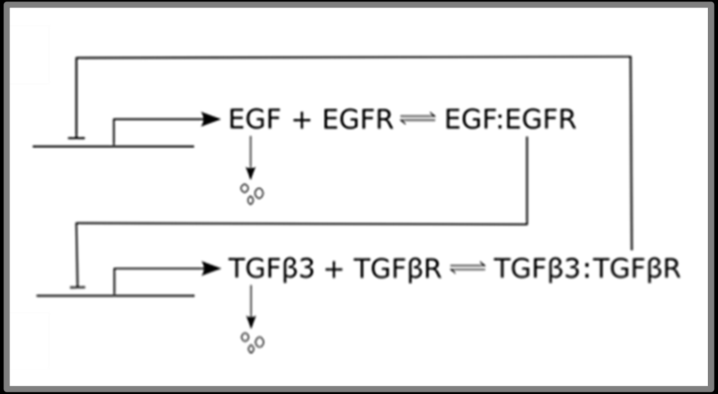
- TGFβ3 triggers MEE cells to undergo apoptosis (PCD), epithelial-mesenchymal transition (EMT), and migration (retraction).
- EGF has the opposite effect, maintaining MEE cell growth, proliferation, and survival.

Messin' with the switch: system fragility and fault tolerance



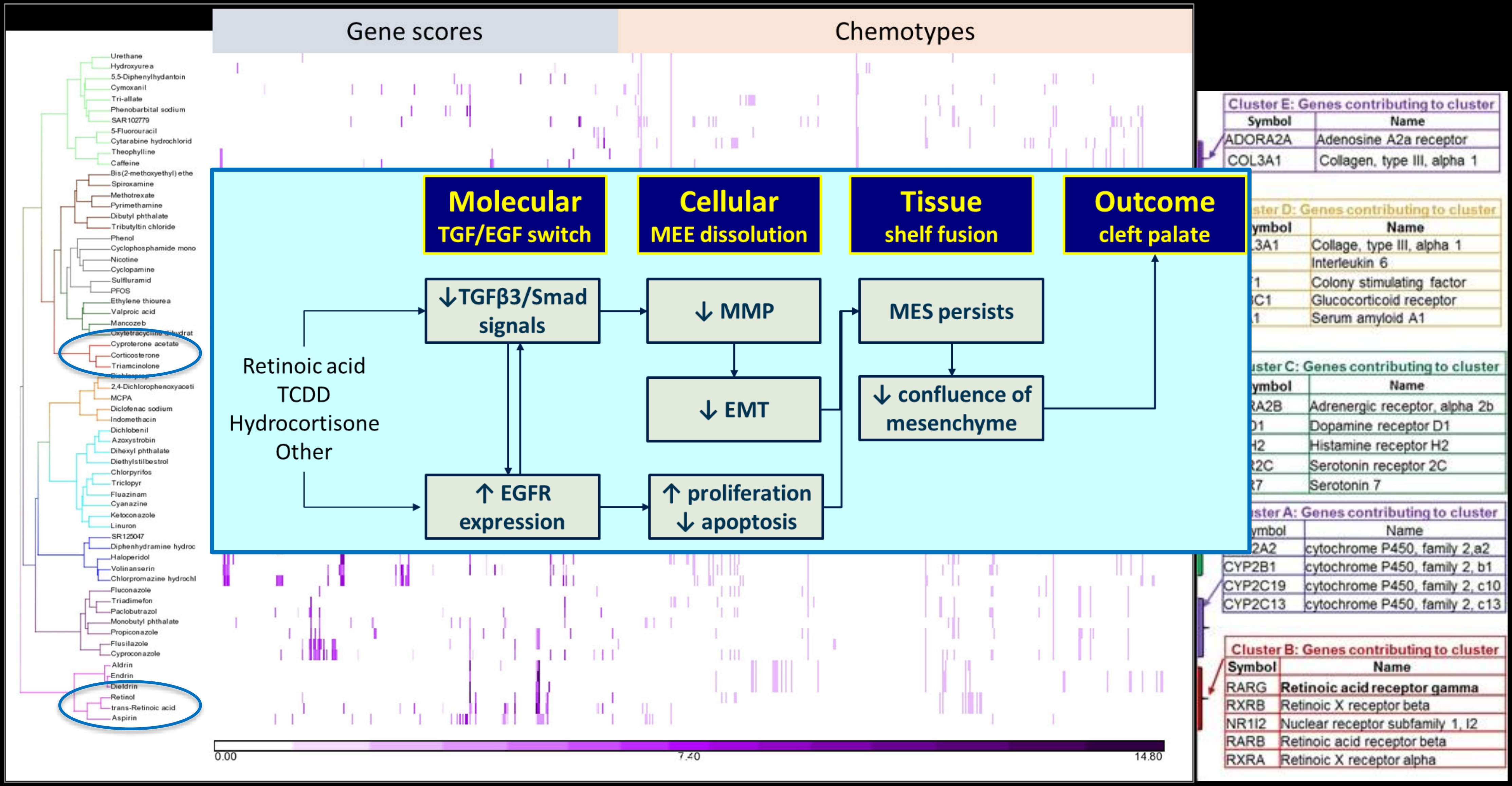


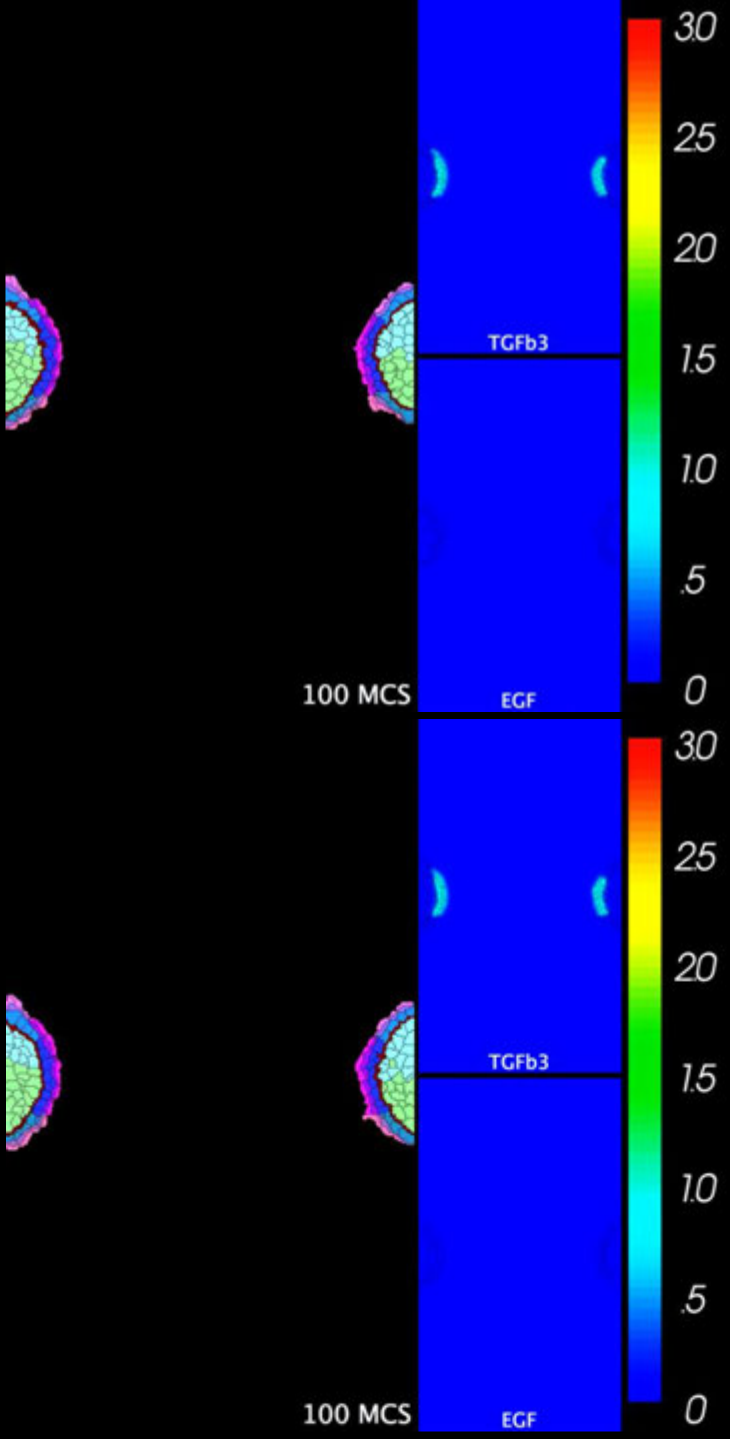
- A mutual inhibitory gene regulatory circuit exhibits switch-like behavior in the MEE.
- EGFR expression normally wanes several hours prior to MEE apposition to flip the switch to the TGFβ3 state.
- Several cleft palate teratogens are known to maintain EGFR expression (Retinoic acid, Hydrocortisone, TCDD) [Abbott 2010]).



63 cleft palate (animal) teratogens in ToxCast

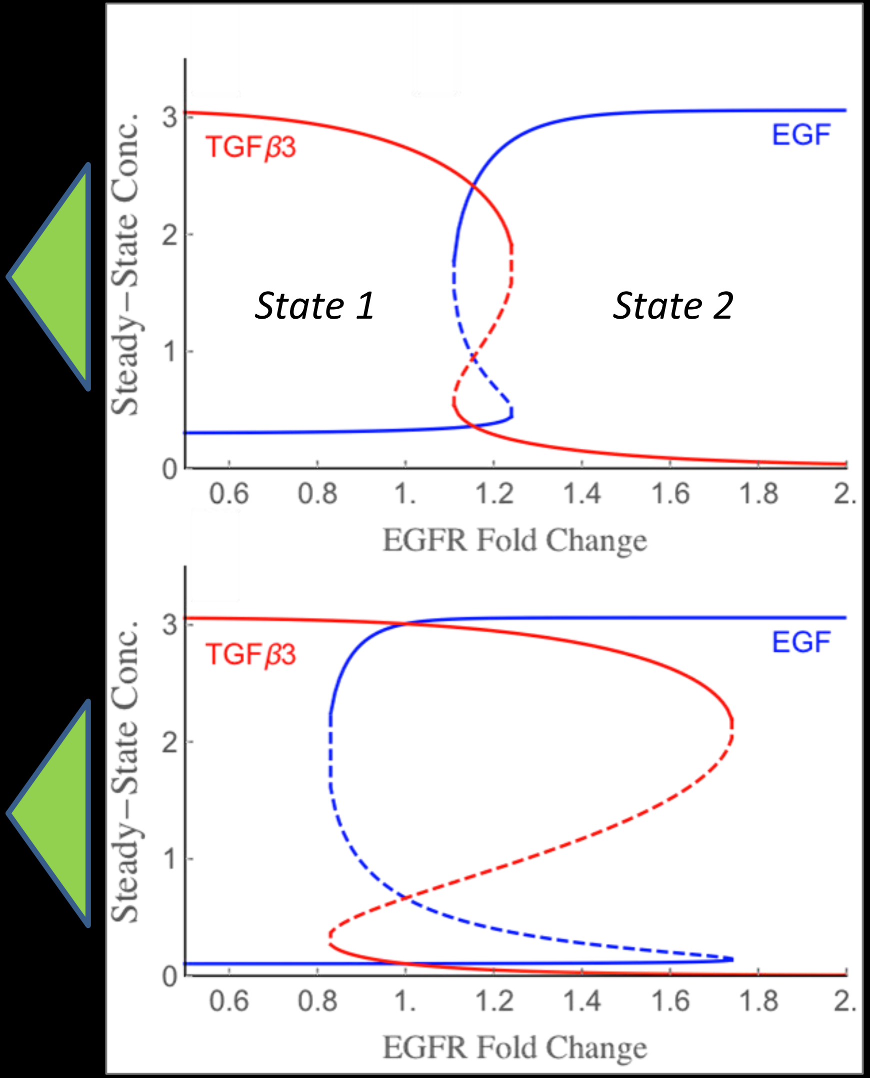
Baker et al. (in preparation)



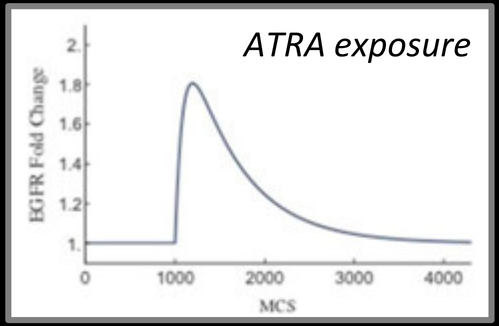


Dissecting circuit dynamics *in silico*:

two scenarios for differential teratogenicity

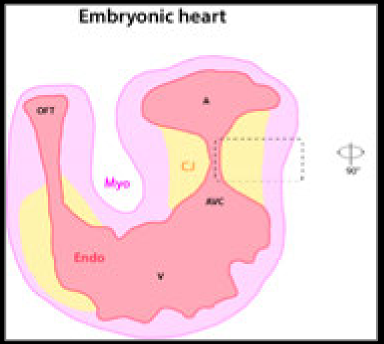


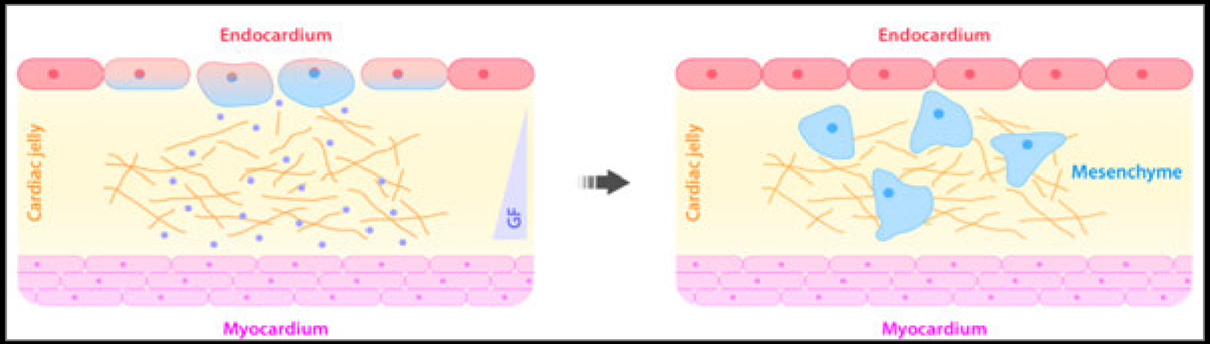
tipping point >1.8x (n=24)
(reversible)



tipping point ~1.5x (n=16)
(not reversible)

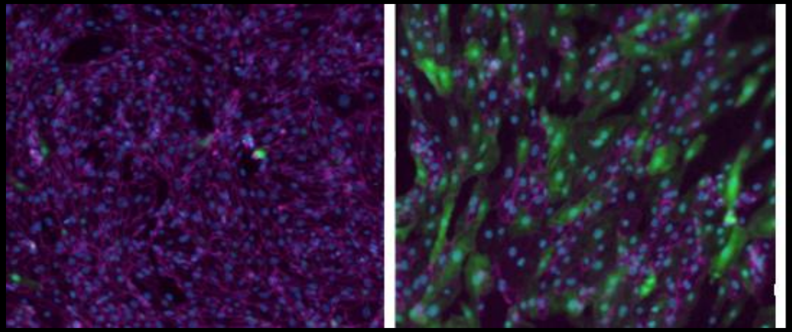
Epithelial-Mesenchymal Transition: delay or disruption underlies some congenital malformations (e.g., valvulo-septal heart defects) …

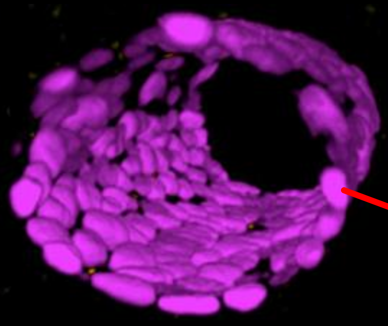


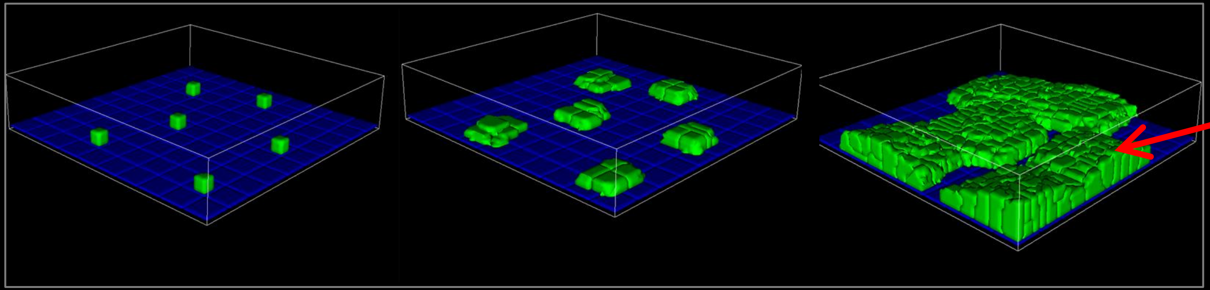


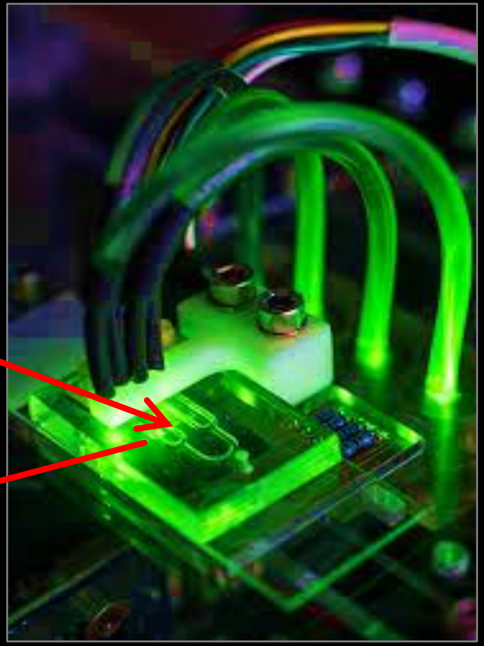
… but endocardial EMT does not occur in a static environment; need to "go with the flow".

TGF-beta
Wnt
FGF
Chemokine/Cytokine
Integrin
Angiogenesis
Cadherin
GPCR
Notch
PDGF



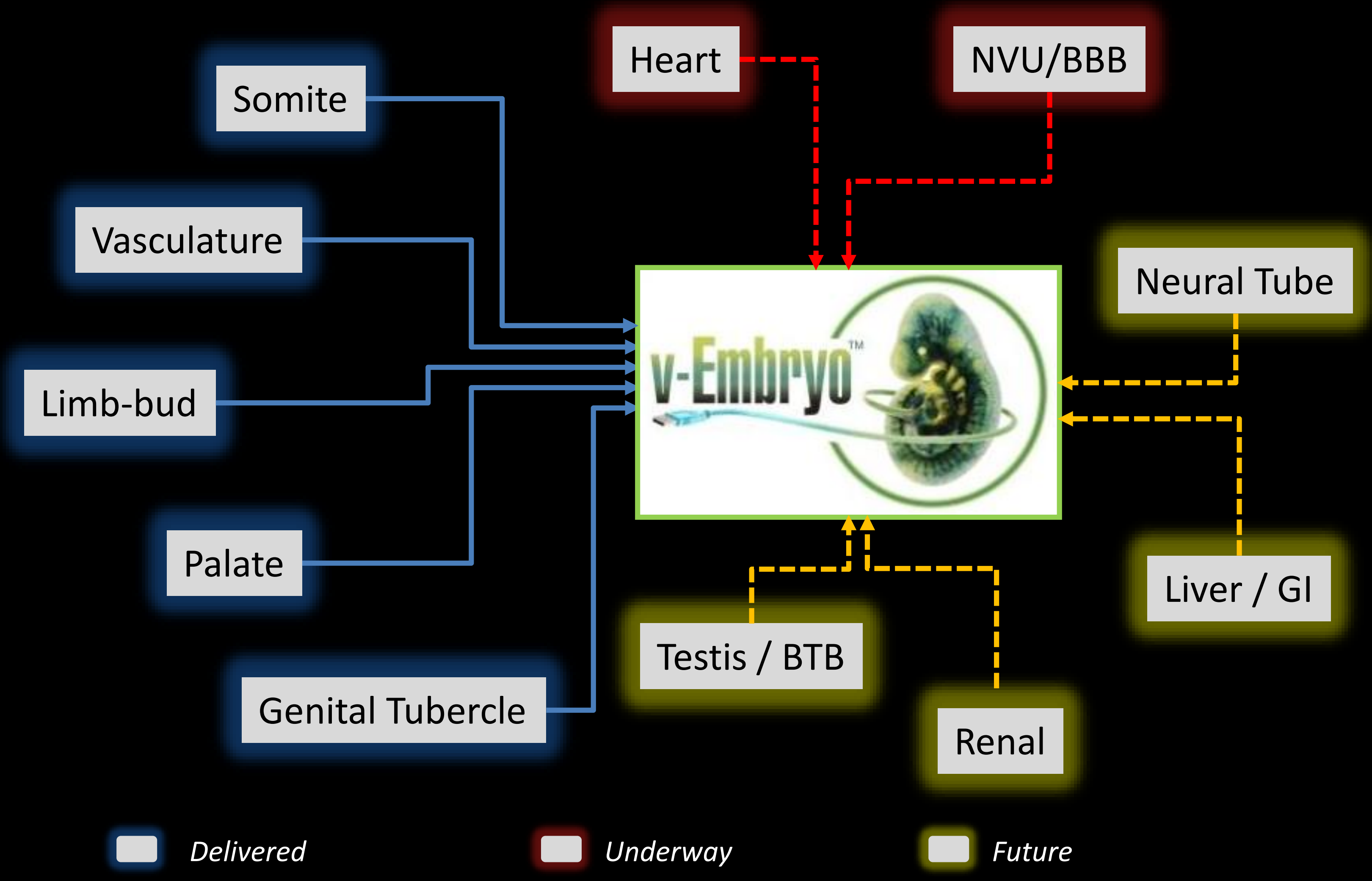






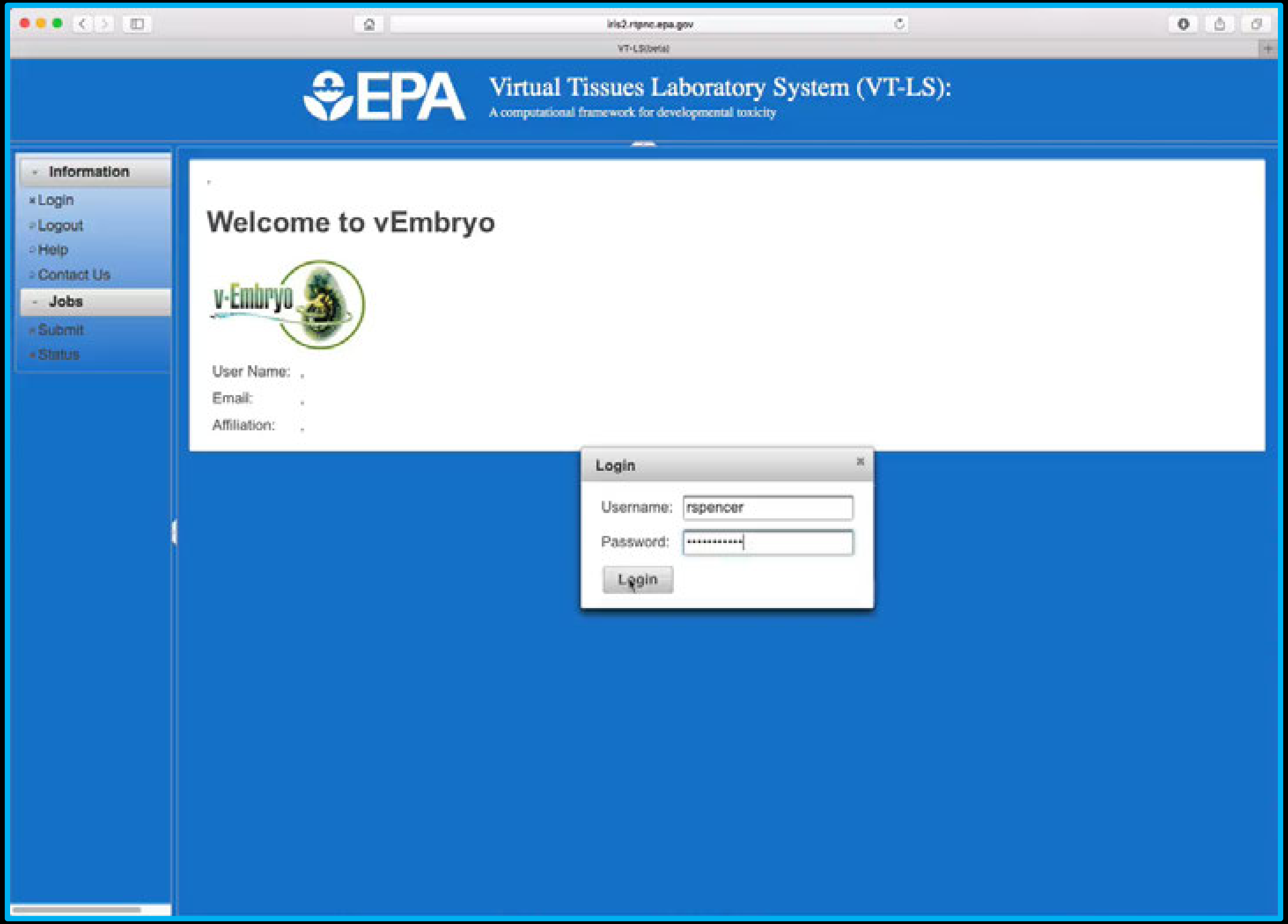
S Hunter – EPA/NHEERL

Toward a 'Virtual Embryo'



- Hester et al. (2011) PLoS Comp Bio; Dias et al (2014) Science
- Kleinstreuer et al. (2013) PLoS Comp Bio.
- Ahir et al. (MS in preparation).
- Hutson et al. (2017) Chem Res Toxicol.
- Leung et al. (2016) Reprod Toxicol.
- Zurlinden/Saili et al. (FY17 product).
- Hunter et al. (FY18 product).
- Your name here.

Virtual Tissue Laboratory System (VTLS)



EMVL: Spencer, Balabin, Cathy, Transue, Howard

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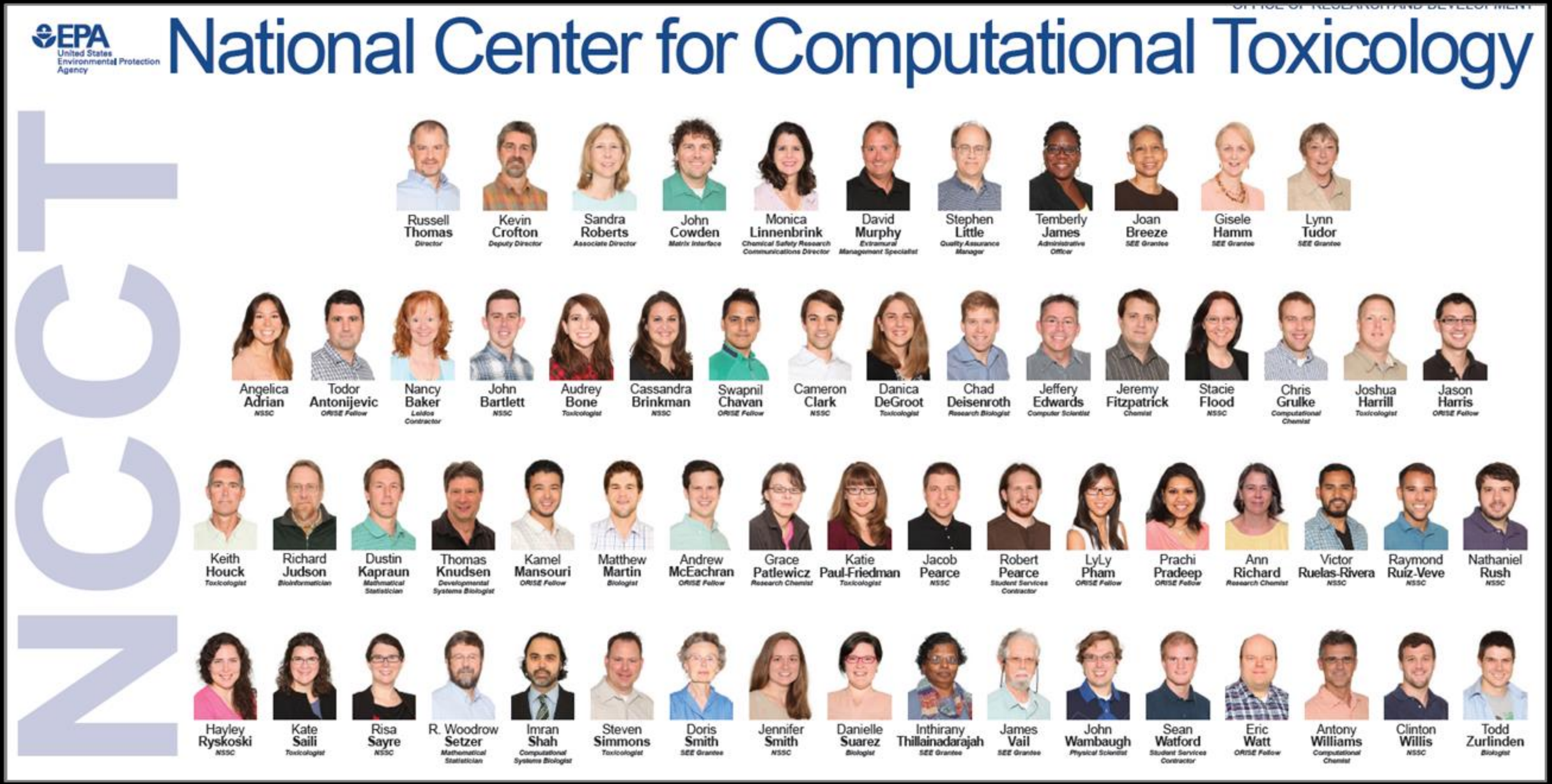

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Virtual Tissue Models: Predicting How Chemicals Impact Human Development

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http://www2.epa.gov/sites/production/files/2015-08/documents/virtual_tissue_models_fact_sheet_final.pdf