United States Environmental Protection Agency

Predictive Toxicology and Computational Biology

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v-Embryo™

Can a multiscale computer model of the embryo (virtual embryo) translate cellular dynamics to simulate a developmental phenotype?

and if so …

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

Predictive Toxicology & Human Development

- Evaluating and assessing impacts to development is a national priority – *EPA's Children's Environmental Health (CEH) Research Roadmap.*

- Too many chemicals (~80K) in production and/or the environment to test each by traditional animal-based methods (cost, time, 3Rs).

- Profile the 'human exposure universe' of chemicals *in vitro* with high-throughput and high-content screening (HTS/HCS) assays.

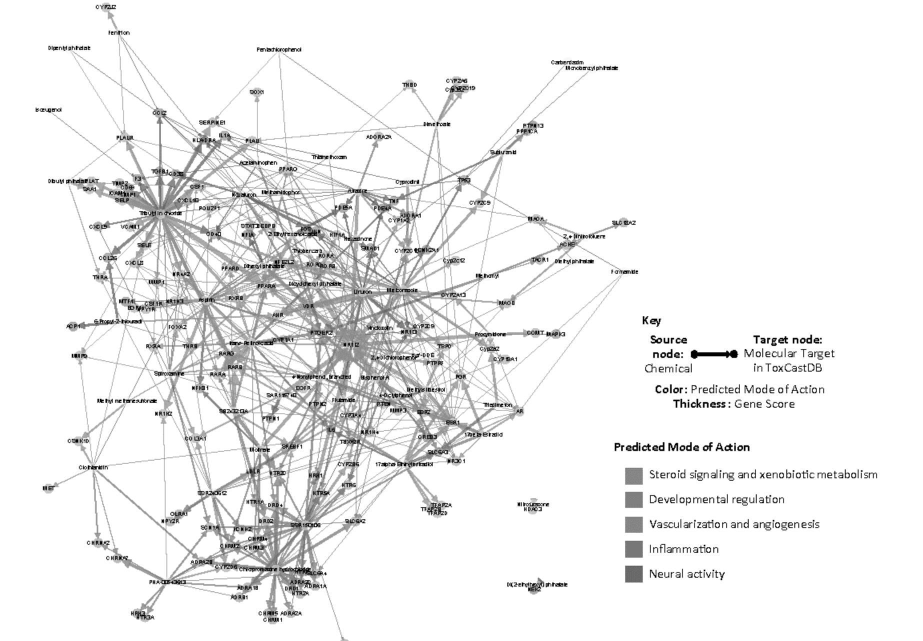
- Build computational (*in silico*) models to integrate *in vitro* data with biological knowledge representing human development.

HTS Chemical Profiling:
under Tox21 federal partnership

- **ToxCast:** 1060 chems in ~600 assays; 2874 chems in ~50-100 assays; **Tox21:** 8599 chems in ~50 assays; 293 assay targets annotated to a distinct gene product.
- **ToxCastDB:** holds >27M data points and ~1.7M concentration response curves in a public database (http://actor.epa.gov/dashboard/).
- **Bipartite network:** translates chemical-assay bioactivity profiles into predicted mode-of-action (e.g., TDS).

Predicted Mode of Action

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

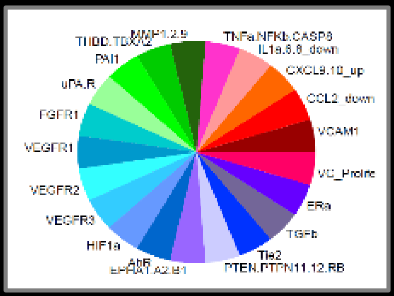


Leung et al. (2015), Env Hlth Persp (accepted)

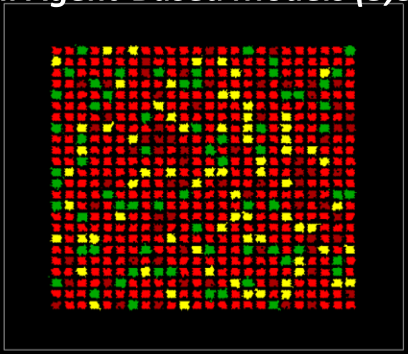
Vascular Development and Disruption:

tiered strategy to predictive toxicology

ToxCast pVDC (1,2)



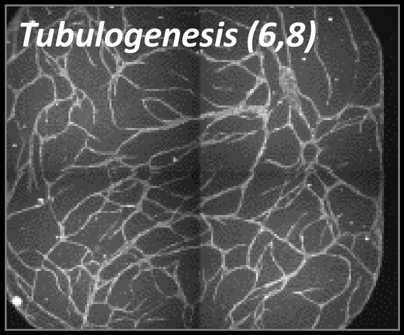
Cell Agent-Based Models (3,8)

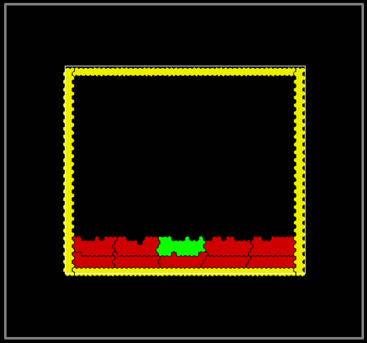


Vascular development (4,5,6)

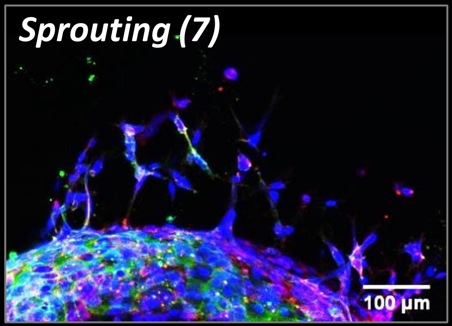
Morphogenesis (9,10)

Tubulogenesis (6,8)





Sprouting (7)



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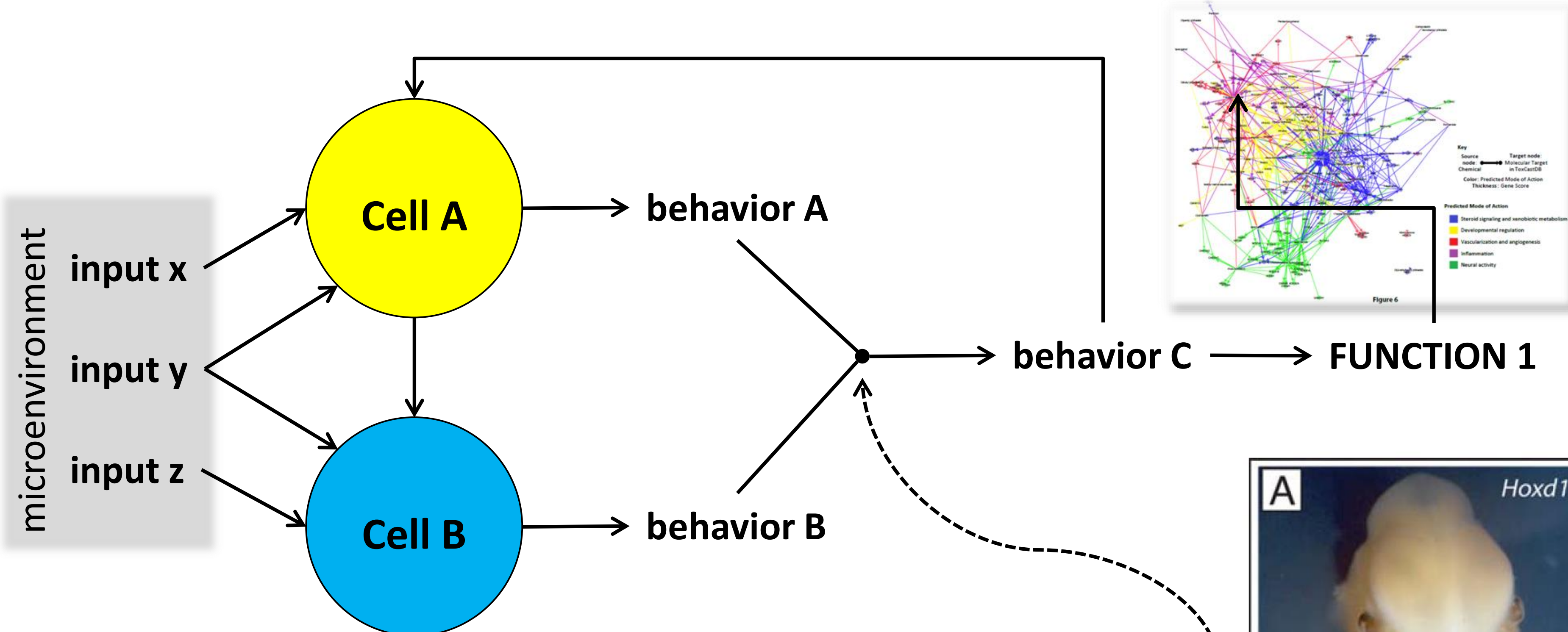
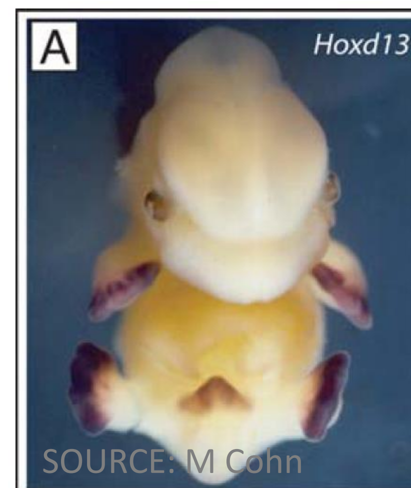
(1) Kleinstreuer et al. (2011) Env Hlth Persp
(2) Knudsen and Kleinstreuer (2011) BDRC
(3) Kleinstreuer et al. (2013) PLoS Comp Biol
(4) Tal et al. (2014) Repro Tox
(5) Tal et al. (in prep)
(6) McCollum et al. (in prep)
(7) Belair et al. (in prep)
(8) Knudsen et al. (in prep)
(9) Ellis-Hutchings et al. (in prep)
(10) Franzosa et al. (in prep)

Cellular Response Networks (CRNs): how cellular systems translate spatial information into higher-order function





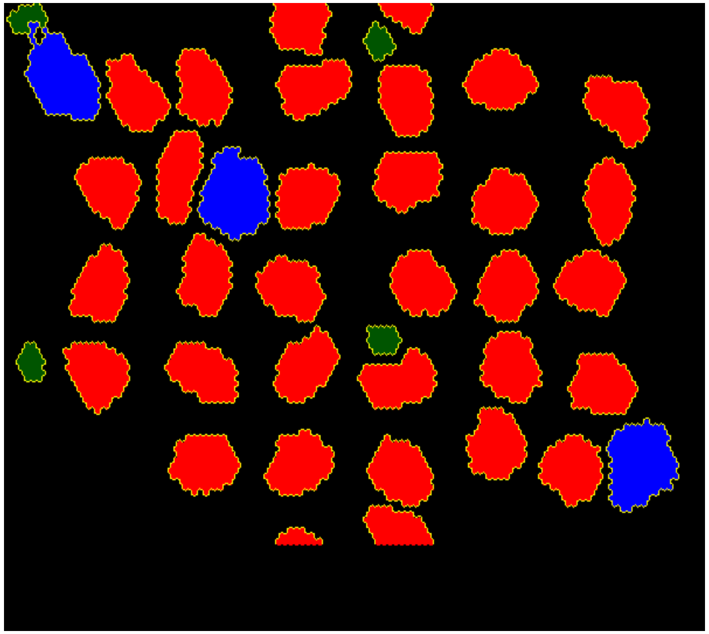
Capturing this mathematically:

$$\sum_\sigma \lambda_V(\sigma)\left(v(\sigma)-V_t(\sigma)\right)^2+\sum_\sigma \lambda_S(\sigma)\left(s(\sigma)-S_t(\sigma)\right)^2+\sum_{i,j} J\left(\tau(\sigma(\vec{i})),\tau(\sigma'(\vec{j}))\right)\left(1-\delta\left(\sigma(\vec{i}),\sigma'(\vec{j})\right)\right)-\lambda_{chem}\left(c(\vec{i})-c(\vec{j})\right)$$

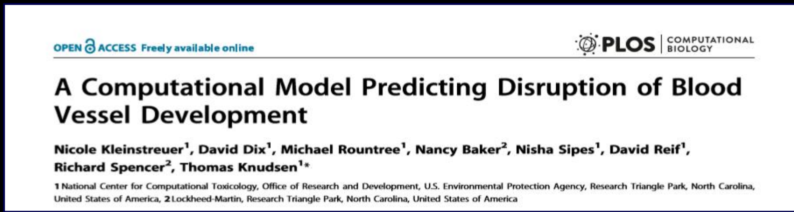
Cellular Agent-Based Models (ABMs)

- rules are assigned to low-level ‘agents’ (cells in this case)
- agents then interact in a shared environment (CompuCell3D)
- simulation recapitulates system-level behaviors (emergence)
- models are stochastic (biological variability)

This example uses CompuCell3D.org simulation environment to model macrophage chemotaxis to a microbe in a field of RBCs.



Many ToxCast assays map to the angiogenic cascade



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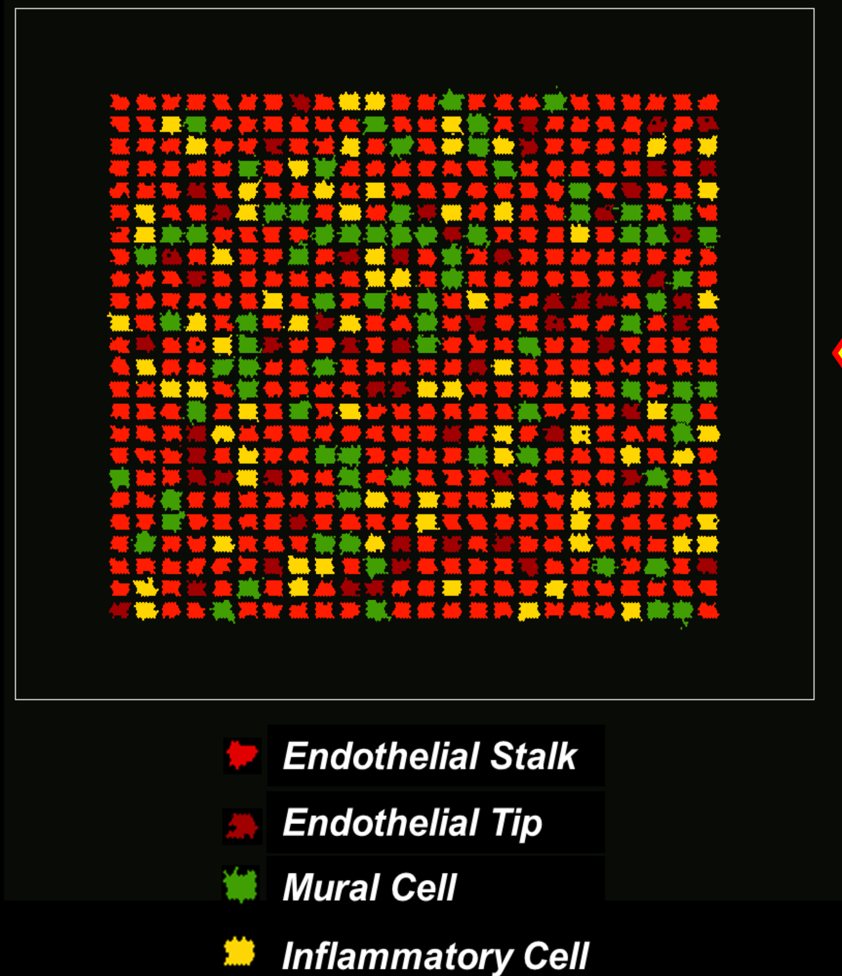
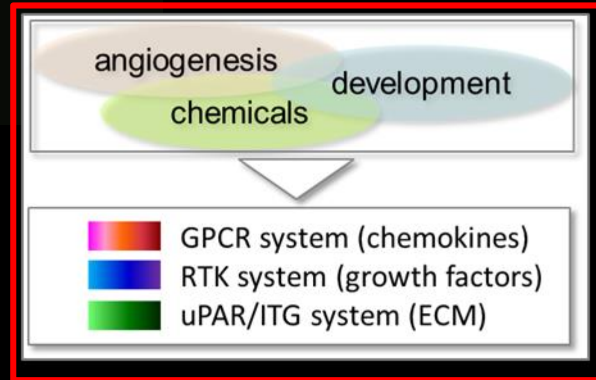
A Computational Model Predicting Disruption of Blood Vessel Development

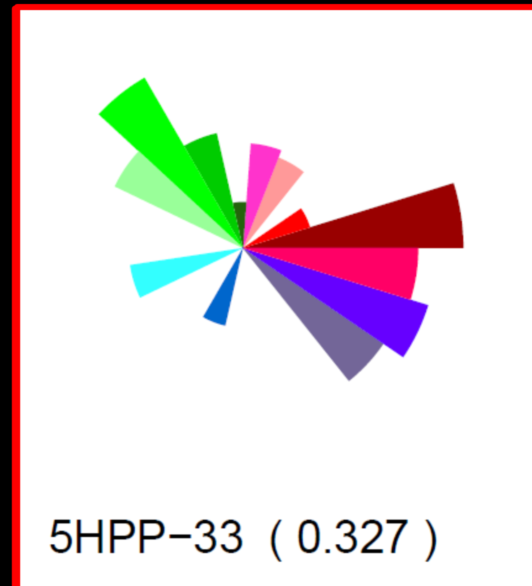
Nicole Kleinstreuer[1], David Dix[1], Michael Rountree[1], Nancy Baker[2], Nisha Sipes[1], David Reif[1], Richard Spencer[2], Thomas Knudsen[1*]

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

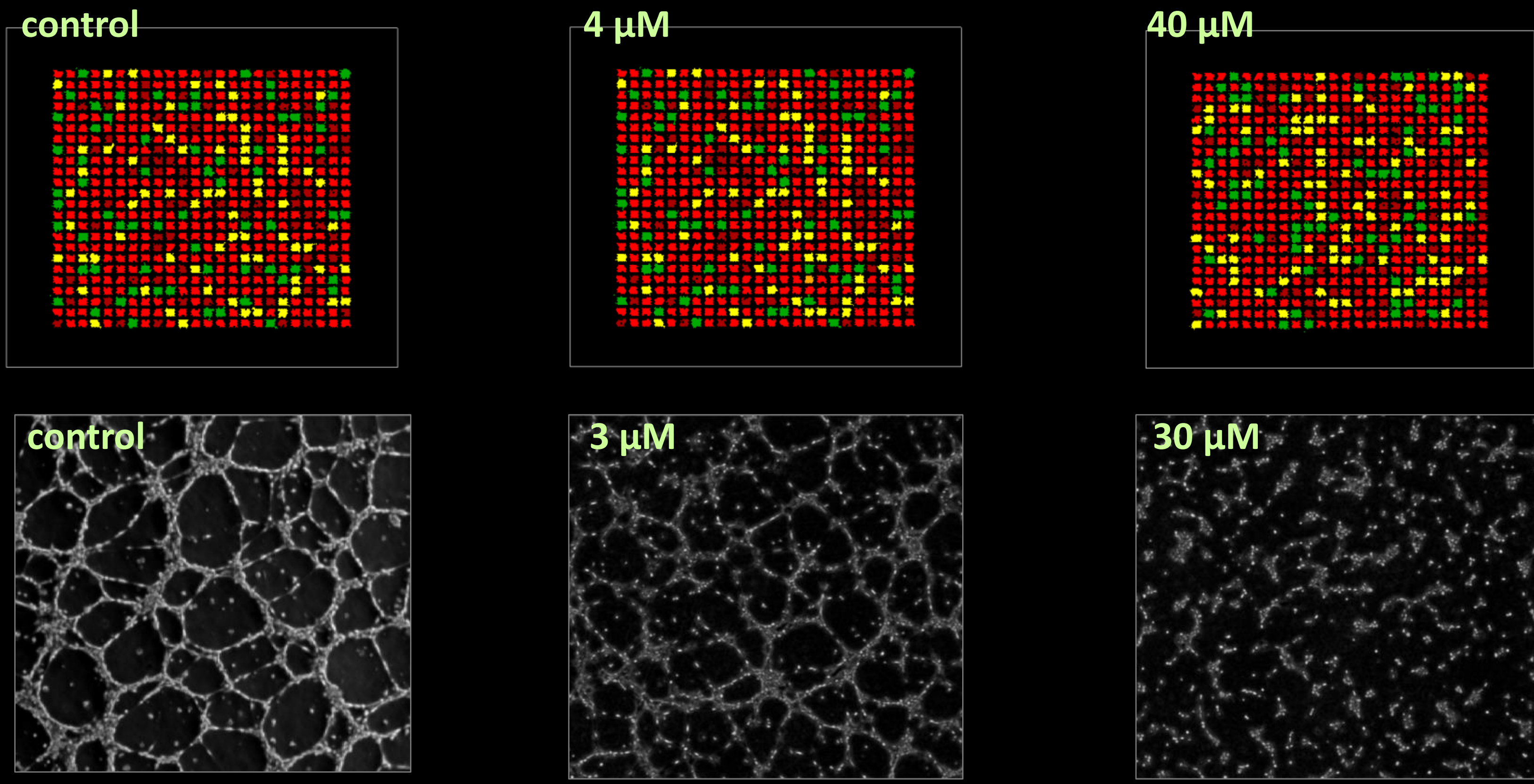
VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2







Simulation of 5HPP-33 Concentration Response



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

Programmed Fusion of Opposing Surfaces

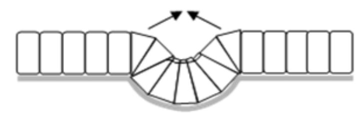
- Organizing principle underlying NTDs, coloboma, cleft palate, valvuloseptal defects, hypospadias, gastroschisis, …
- Emergent property orchestrated by CRNs: EMT, apoptosis, epithelial cell adhesion / migration / intercalation are recurring themes.

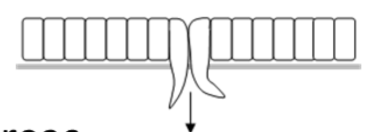
Cellular primitives

- growth (proliferation)
- programmed cell death (apoptosis)
- genetic signals and responses
- differentiation
- cell adhesion
- shape (geometry)
- motility (cell migration)
- ECM (remodeling)

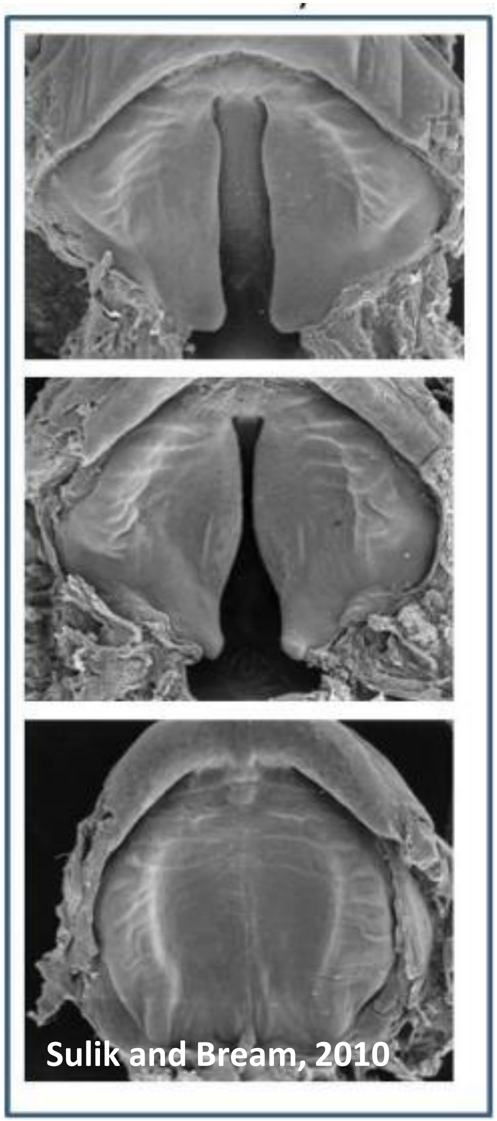
Tissue movements

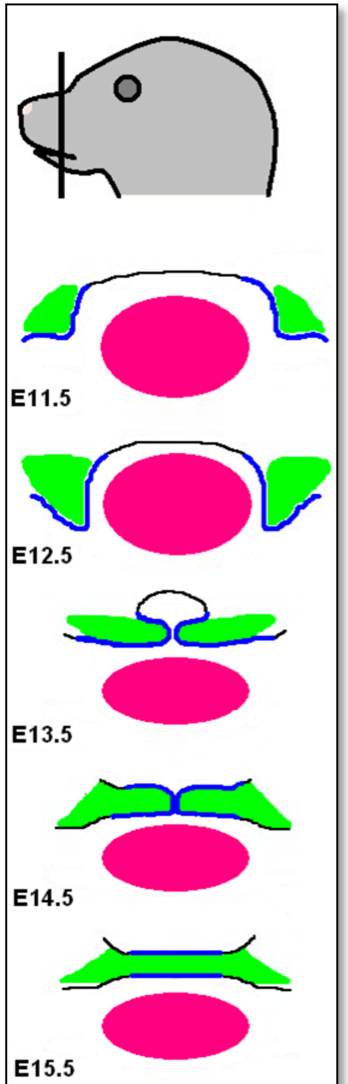
- folding
- epiboly
- convergent extension
- branching morphogenesis
- cell condensation
- cell sorting
- trans-differentiation (EMT)
- cavitation
- involution
- tractional forces
- ...

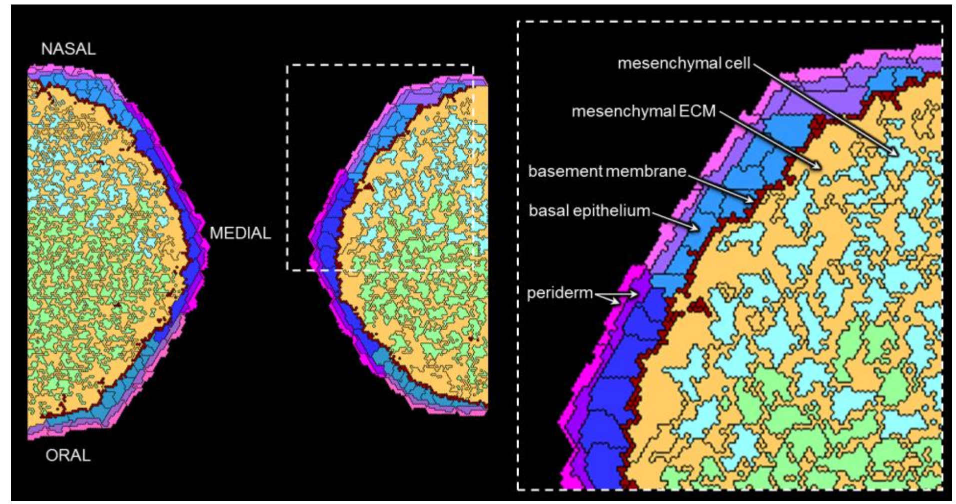


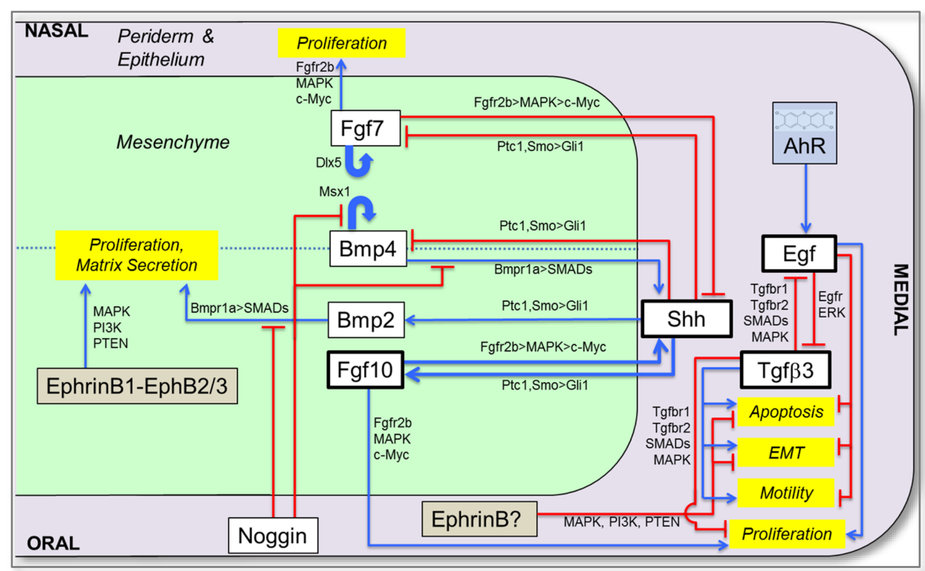


Palatal Closure: ABM can probe quantitative relationships during Medial Edge Epithelium (MEE) fusion and seam breakdown.



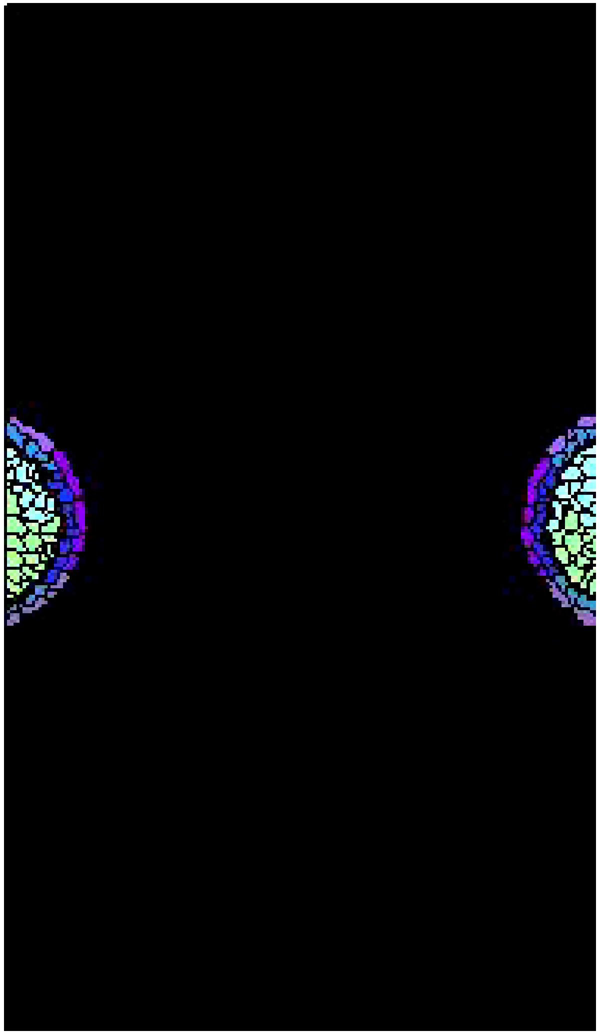


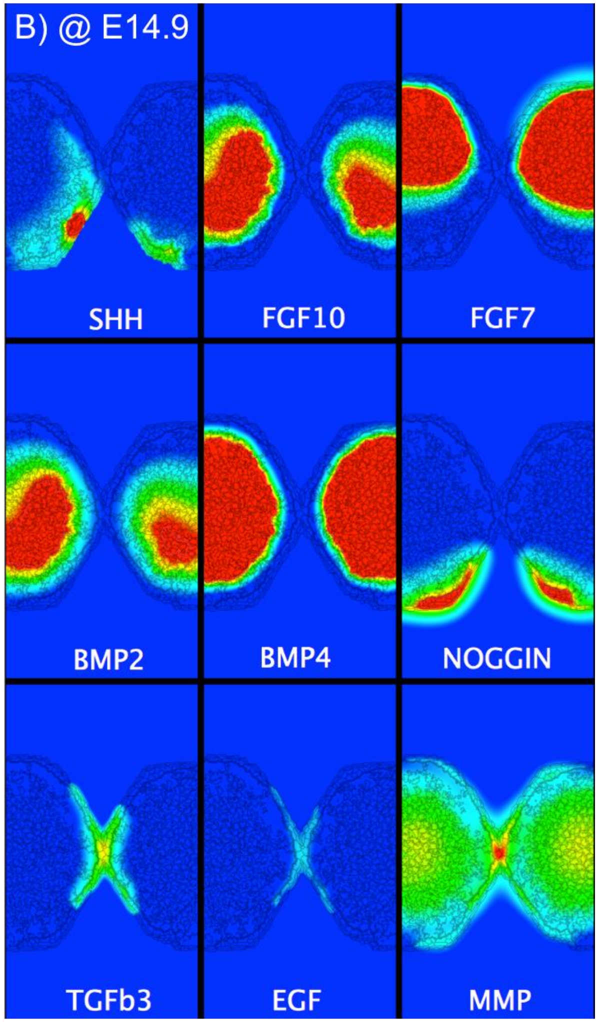


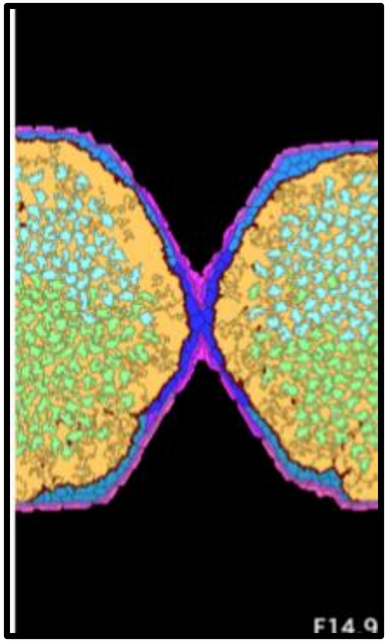


Hutson et al. (in preparation)

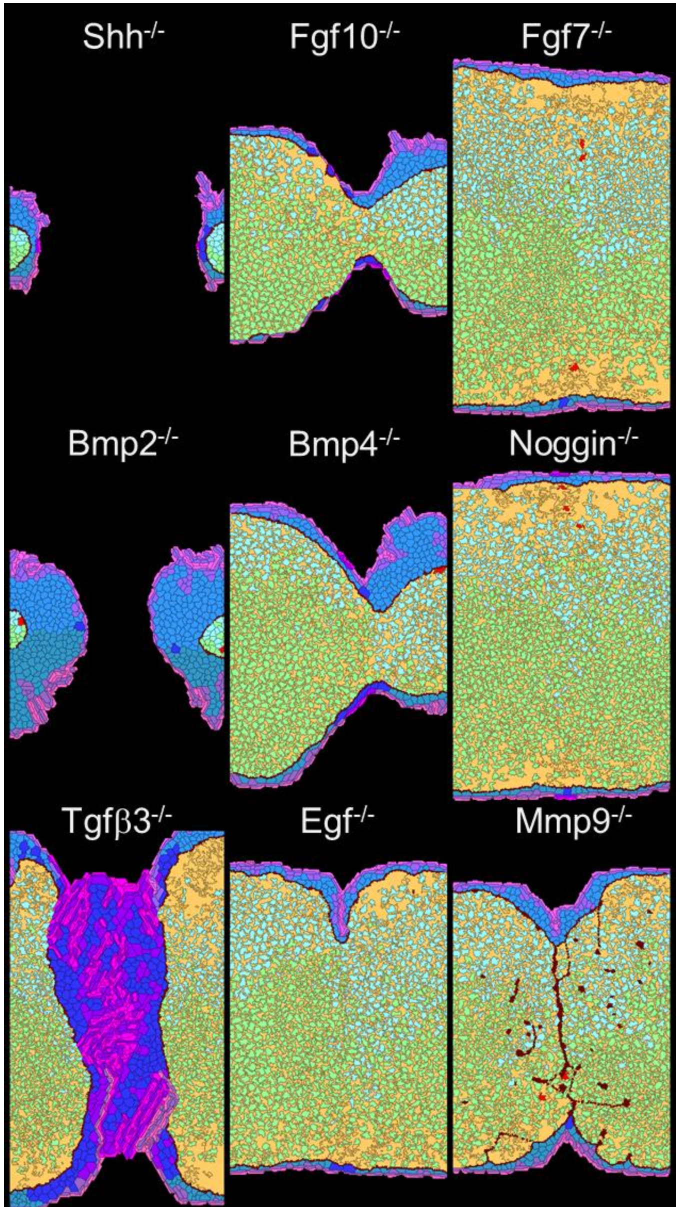
Prefusion Signaling Domains *(at the time of contact)*







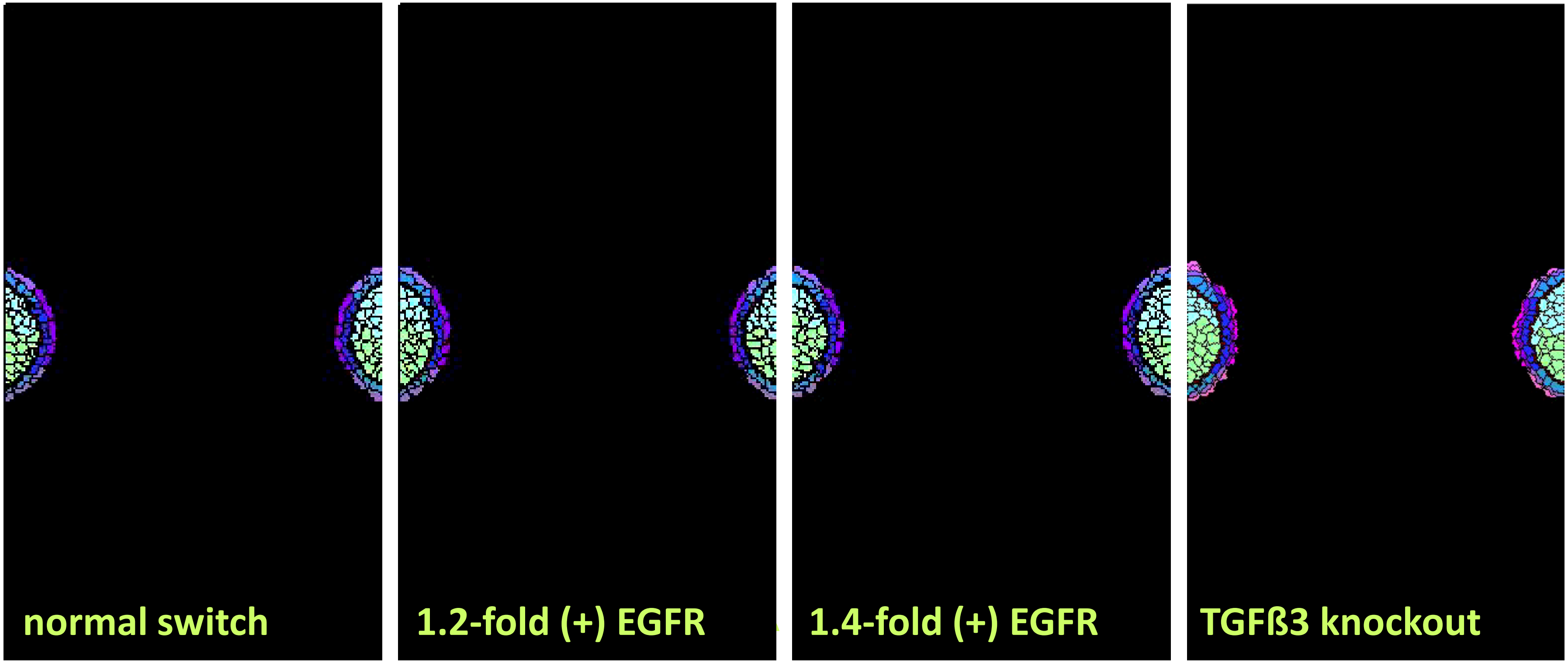
Hacking the Control Network

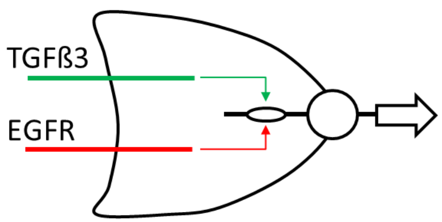


- *in silico* knockouts of elements in the prefusion signaling network
- impacts on prefusion, MEE contact and seam breakdown (critical event)

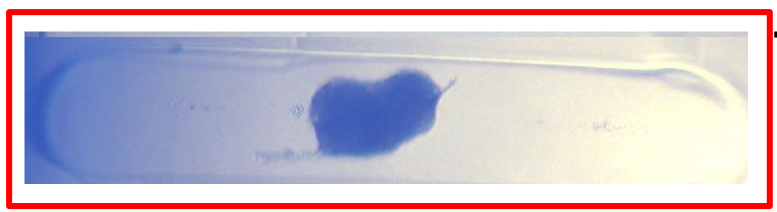
Biological switch driving MEE seam degeneration

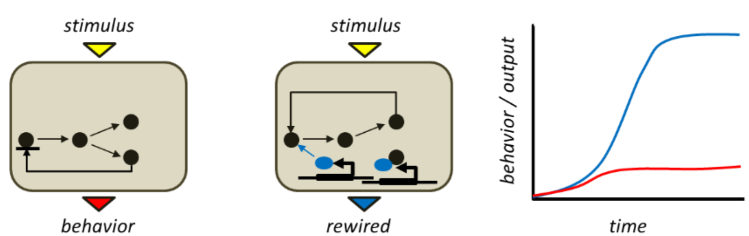
$$k_{EGF} = 15 + \text{Random}[0,1] - 12\frac{[Tgf\,b3]^4}{[Tgf\,b3]^4 + AC_{50}^4} + k_{EGF-AhR}$$





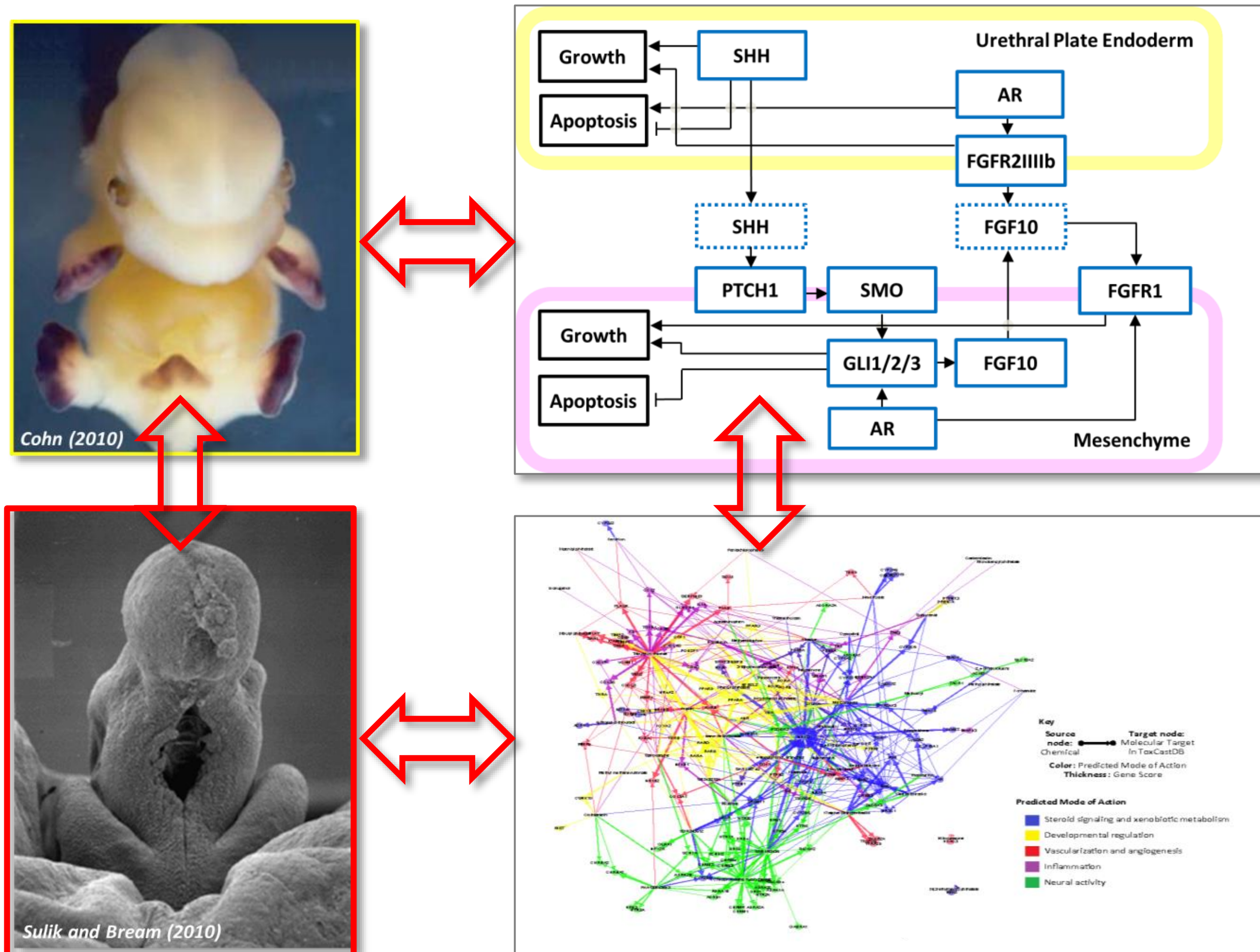
TGFß3	EGFR	CP
1.0	1.0	0
0	1.0	1
1.0	1.2	0
1.0	1.4	1
1.0	1.5	1





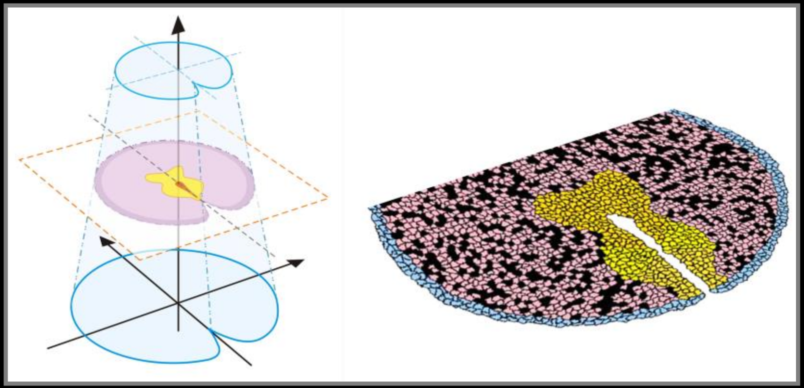
Validate model in fusion-competent human iPSC-miniorganoids (B Abbott)

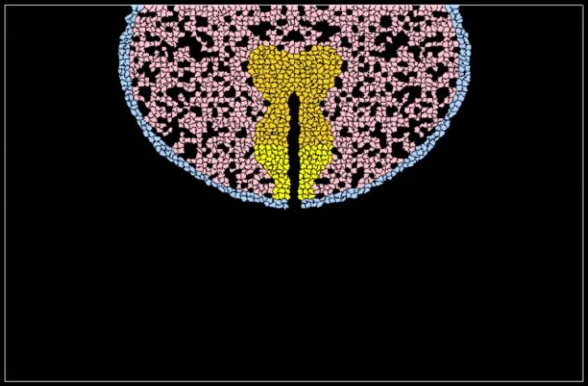
Genital Tubercle (GT) development: how does a chemical-bioactivity *[bipartite network](#)* interact with a *[control network](#)* to induce hypospadias, a urethral closure defect?



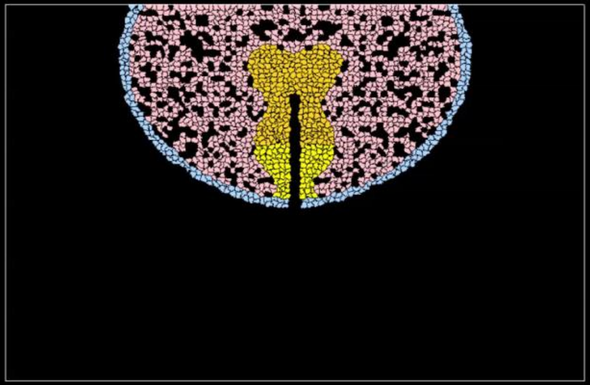
M Leung, manuscript in preparation

Cell ABM for Urethral Closure



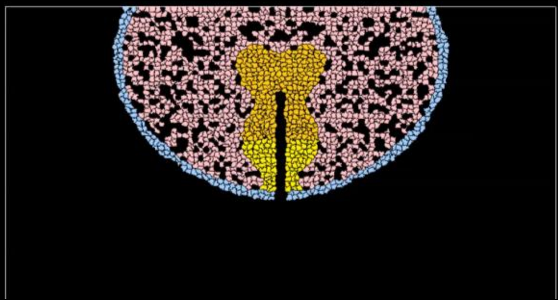


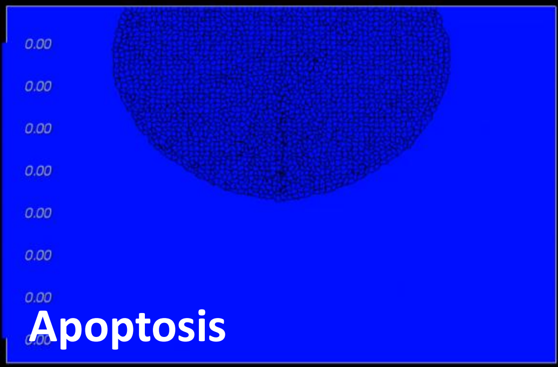
Default
(female)



Androgenized
(male)

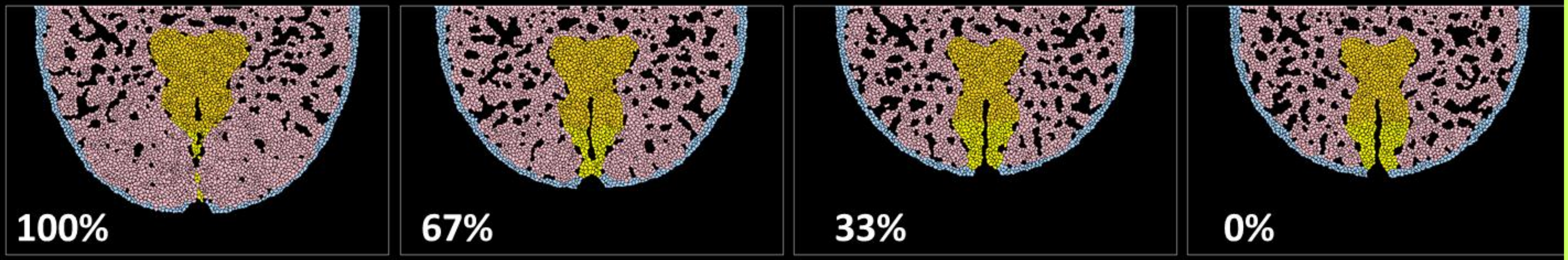
- Androgen production by fetal testis triggers sexual dimorphism of the GT into male or female phenotypes.

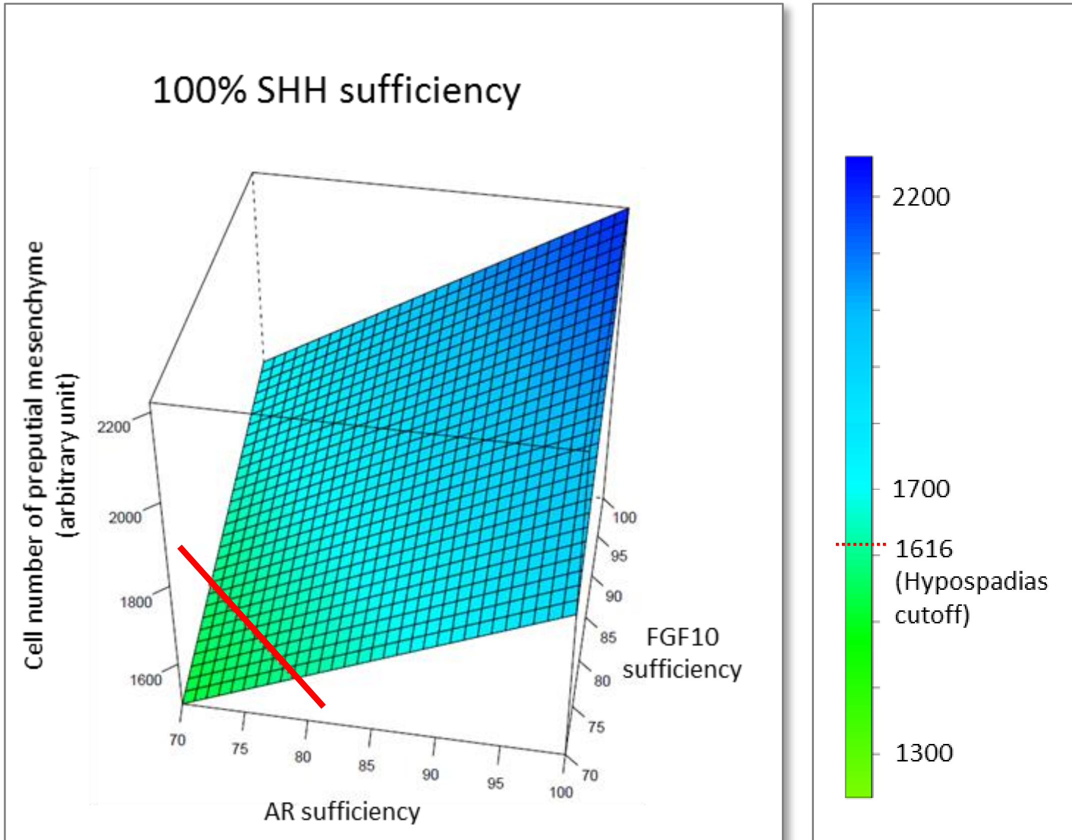




- **Epithelial apoptosis & mesenchymal (preputial) proliferation drives closure and centralization of the urethral plate.**
- **Disruption of SHH, FGF10, or AR signaling leads to closure defect (hypospadias).**

Androgen Sufficiency

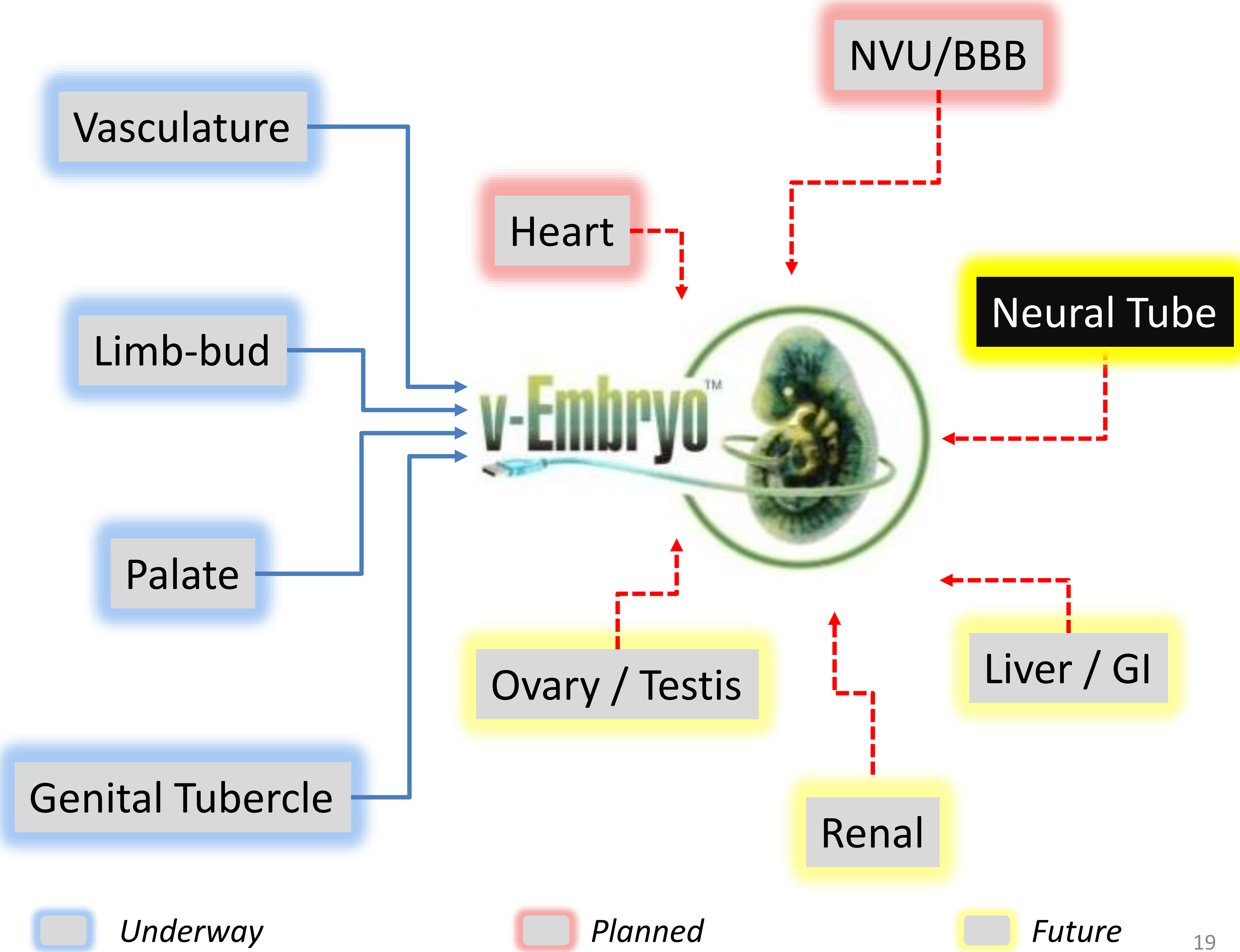




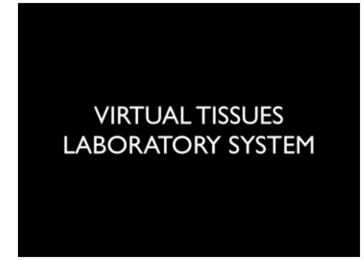
Running the simulation multiple times gives theoretical probability of failed closure in the allotted time

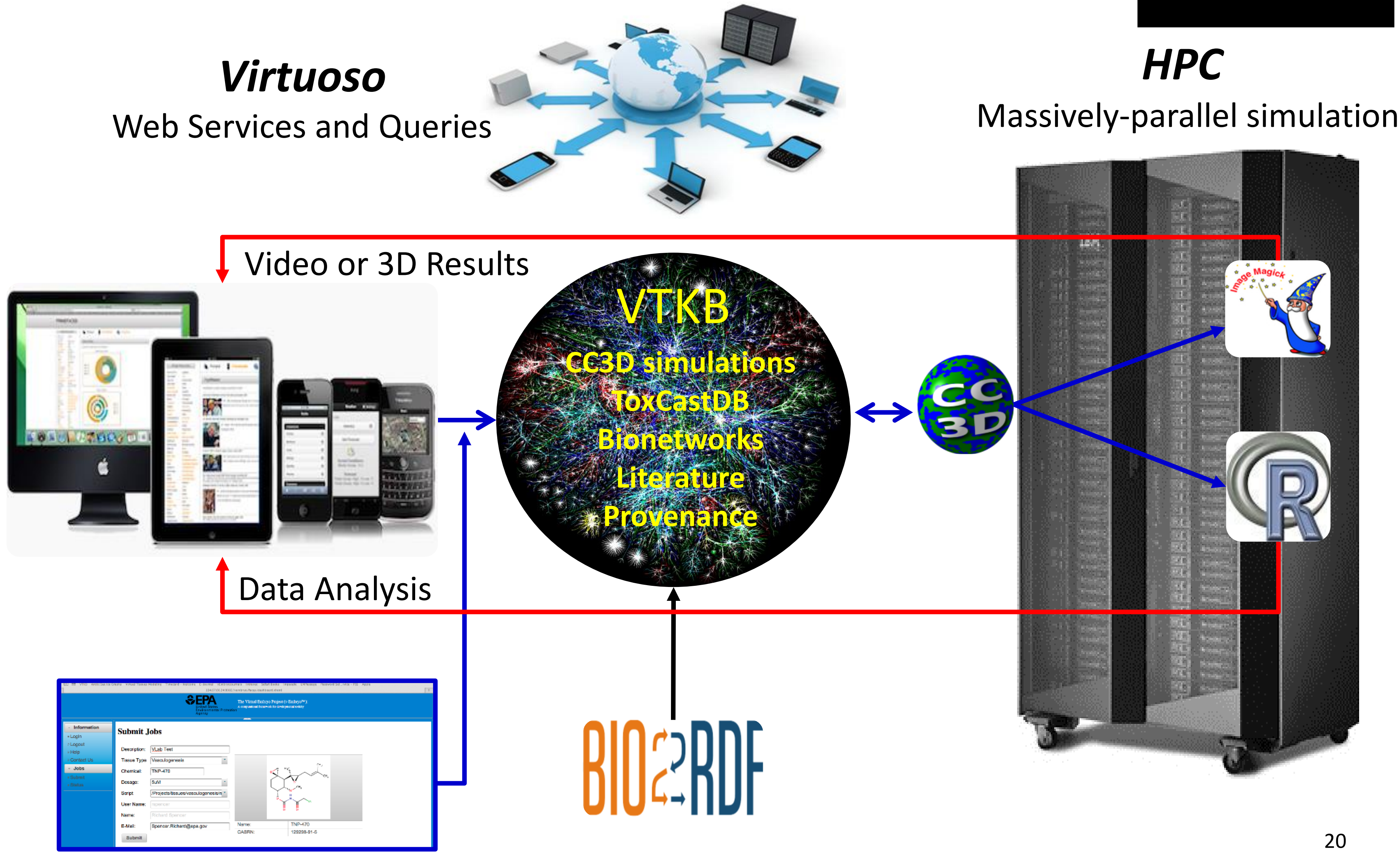
Multi-disturbance surface from an ABM of the developing GT can be used assess individual risks for complex interactions:

- genetics (e.g., FGF10 polymorphisms)
- metabolism (e.g., cholesterol deficiency)
- environmental exposure (e.g., androgen disrupters).


Vasculature
Limb-bud
Palate
Genital Tubercle
Heart
NVU/BBB
v-Embryo™
Neural Tube
Ovary / Testis
Renal
Liver / GI
Underway
Planned
Future

Virtual Tissues Laboratory System





Can a multiscale computer model of the embryo (virtual embryo) translate cellular dynamics to simulate a developmental phenotype?

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

[chemicals, doses, non-chemical stressors, mixtures, stages, sensitive subpopulations, …]

Special Thanks

- Richard Judson – 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)
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- Nancy Baker – Lockheed Martin / NCCT
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- A Seifert – U Kentucky
- L Egnash – Stemina Biomarker Discovery
- M Bondesson – U Houston / STAR
- J Glazier – Indiana U / STAR
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- Randy Ashton – U Wisconsin / STAR
- John Wikswo – Vanderbilt U / STAR

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

National Center for Computational Toxicology