Predictive Toxicology and Computational Biology

Thomas B. Knudsen, PhD
Developmental Systems Biologist
National Center for Computational Toxicology
US EPA, Research Triangle Park NC
knudsen.thomas@epa.gov

'Delivering Superior Consumer Benefits by Exploiting Life Science Innovation'
October 21-22, 2015 West Chester OH

Office of Research and Development
National Center for Computational Toxicology

Predictive Toxicology & Human Development

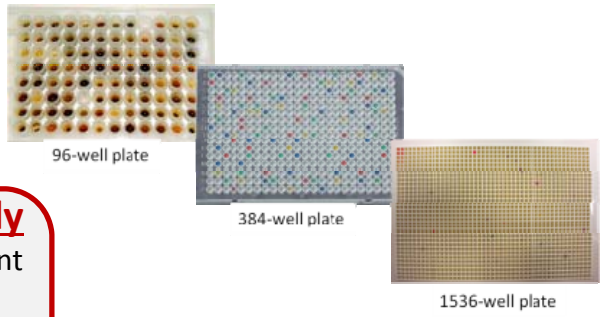
- Evaluating and assessing impacts to development is a national priority – *EPA's Children's Environmental Health (CEH) Research Roadmap.*
- Too many chemicals (~80K) in production and/or the environment to test each by traditional animal-based methods (cost, time, 3Rs).
- Profiling the 'human exposure universe' chemicals with automated high-throughput and high-content screening (HTS/HCS) assays.
- Grows the need for computational models to integrate *in vitro* data with biological knowledge representing human development.

HTS Chemical Profiling:
under the Tox21 federal partnership

- ToxCastDB holds >27M data points and ~1.7M concentration response curves in a public database (http://actor.epa.gov/dashboard/).
- Assays mostly use human cells or cell-extracts, but include complex co-culture systems (and newer organotypic culture microsystems).

	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~1100	2011	Now
ToxCast Phase II	767	~600	~1100	2014	Now
ToxCast Phase III	2074	~100	~250	Ongoing	2016
E1K (endocrine)	800	~50	~120	2013	Now
Tox21	8,599	~50	~75	Ongoing	Ongoing

ToxCast Assay Portfolio



Assay Provider
ACEA
Apredica
Attagene
BioReliance
DiscoverX (BioSeek)
CeeTox
CellzDirect
Tox21/NCATS
NHEERL (mES, ZFE)
Perkin Elmer (NovaScreen)
Odyssey Thera
Vala Sciences

Biological Response
cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
prot
oxidati
report
gene e
re
re
st

Target Family
response Element
transporter
cytokines
kinases

Assay Design
porter
reporter
reporter
porter
ntial reporter
porter
eporter
ecretome

To increase the diversity of *in vitro* assays used to assess developmental toxicity, 4 new platforms added to ToxCast. (preliminary analysis of 1069 compounds showed ~15% active in the hES platform)

Readout Type
single
multiplexed
multiparametric

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
Metabolomics
TR-FRET

DevTox
Stemina (hES)
Aruna (hNPs)
Vala (differentiation)
OT (gene expression)

List of assays and related information at: **http://www.epa.gov/ncct/**

Bipartite network: translating ToxCast chemical-assay bioactivity profiles into predicted mode-of-action for an adverse outcome

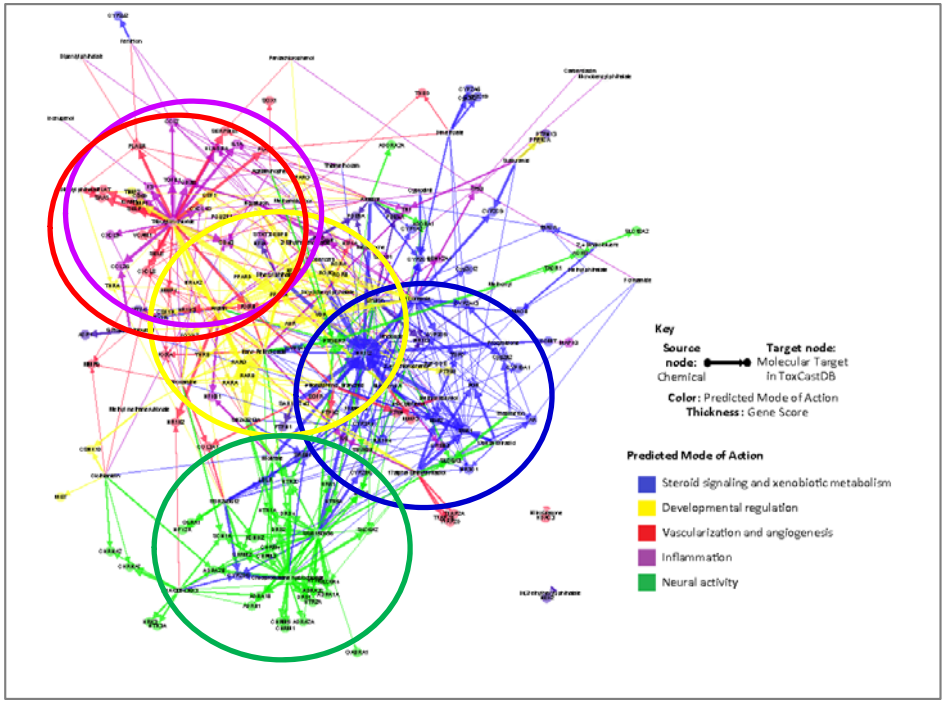
Toxicity Forecaster Database (ToxCastDB)
1,860 chemicals with data for 328 HTS assays
795 cellular features, 293 molecular targets

↓

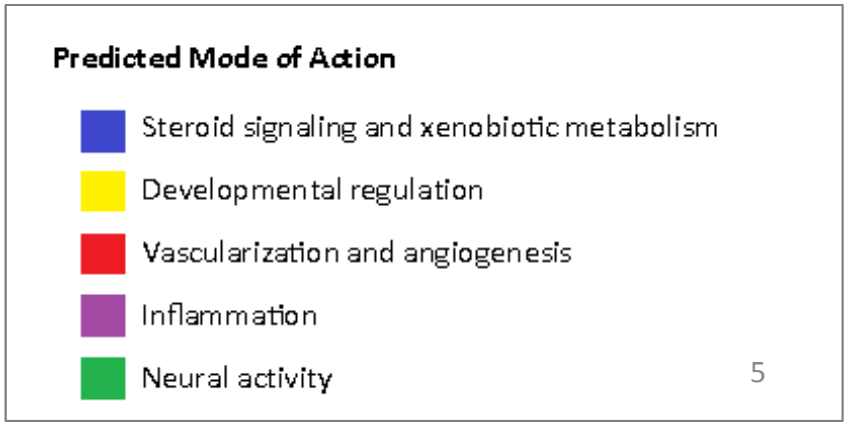
Adverse Developmental Outcomes (ToxRefDB)
Example: 54 chemicals with male reproductive developmental outcomes (TDS)

↓

Chemical-Target Bipartite Network
TDS bipartite network with 156 molecular targets functionally annotated by biological process (GO)



EXAMPLE: modes of action predicted for animal endpoints correlating with human Testicular Dysgenesis Syndrome (TDS)

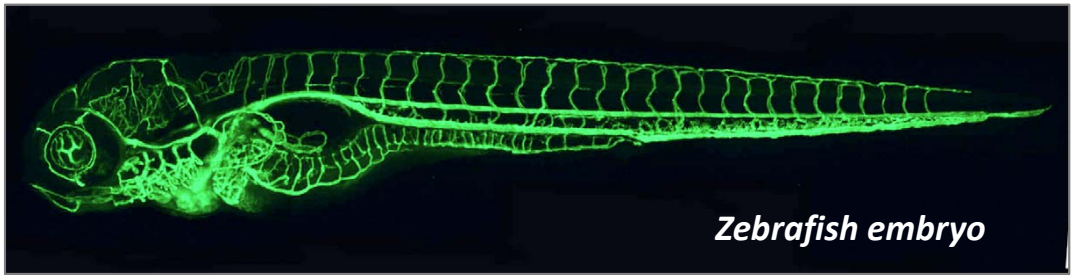


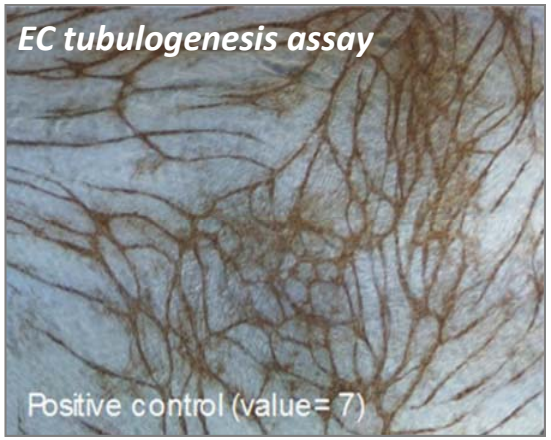
SOURCE: Leung et al. (2015), Env Hlth Persp (accepted)

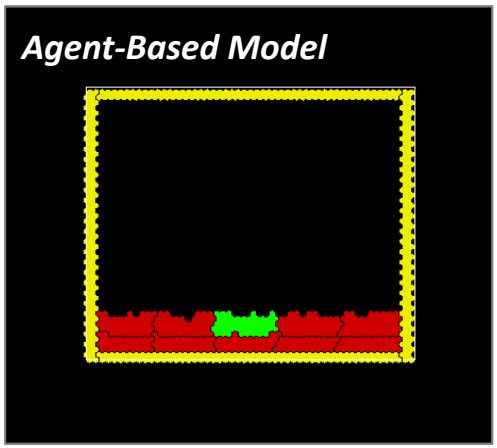
Blood vessel development

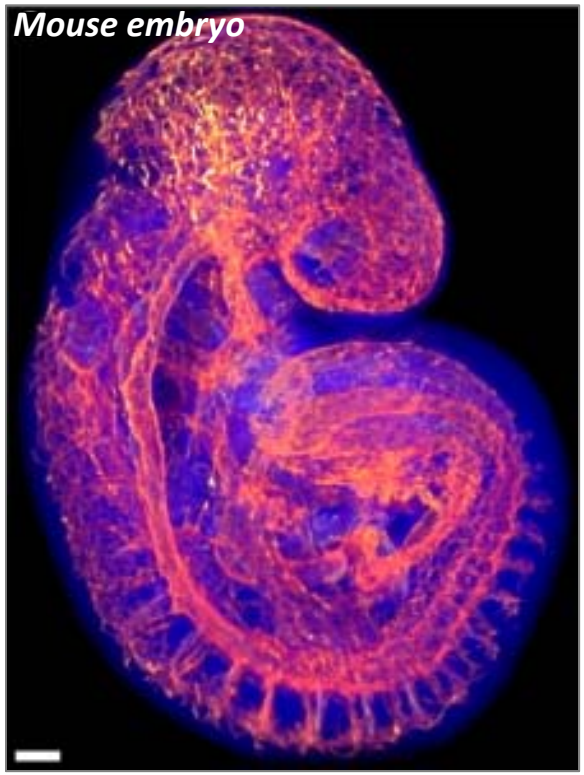
- Many ToxCast assays map to pathways used for neovascularization
  - *Knudsen and Kleinstreuer (2011) Birth Def Res C.; Tal et al. (2014) Repro Tox.*

- Direct correlations can be drawn with developmental toxicity
  - *Kleinstreuer et al. (2011) Env Hlt Persp.; Tal et al. (2015) in submission.*

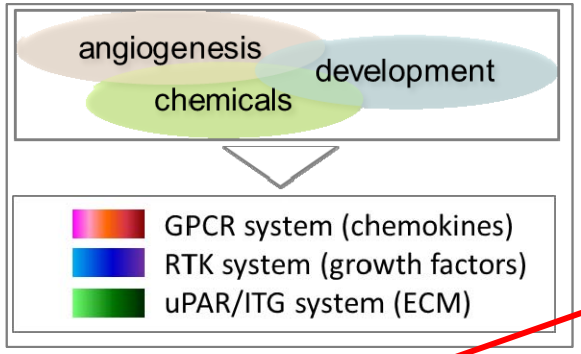




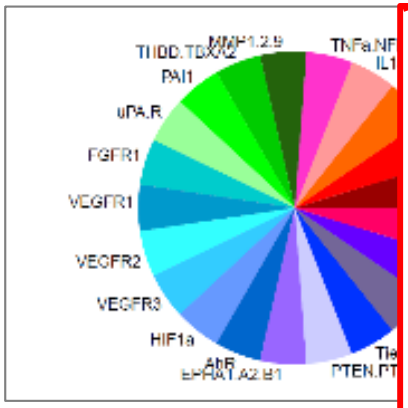


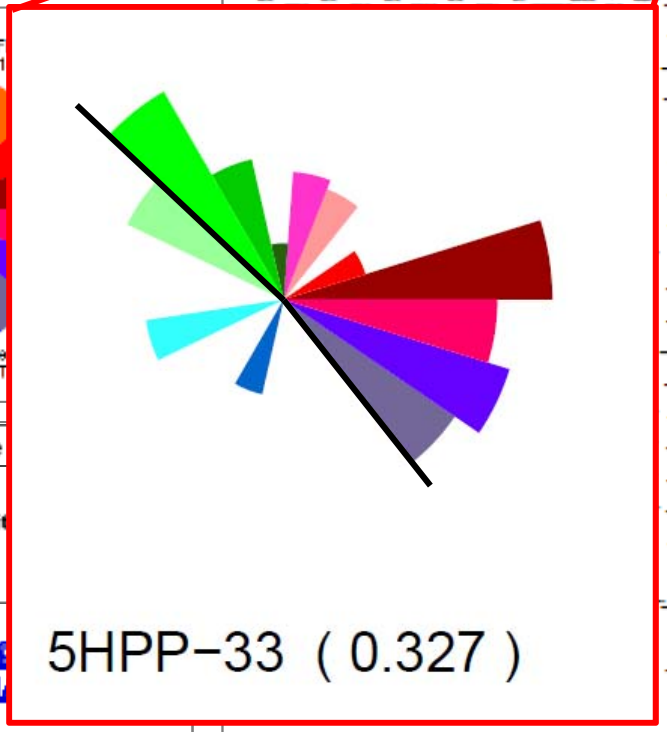


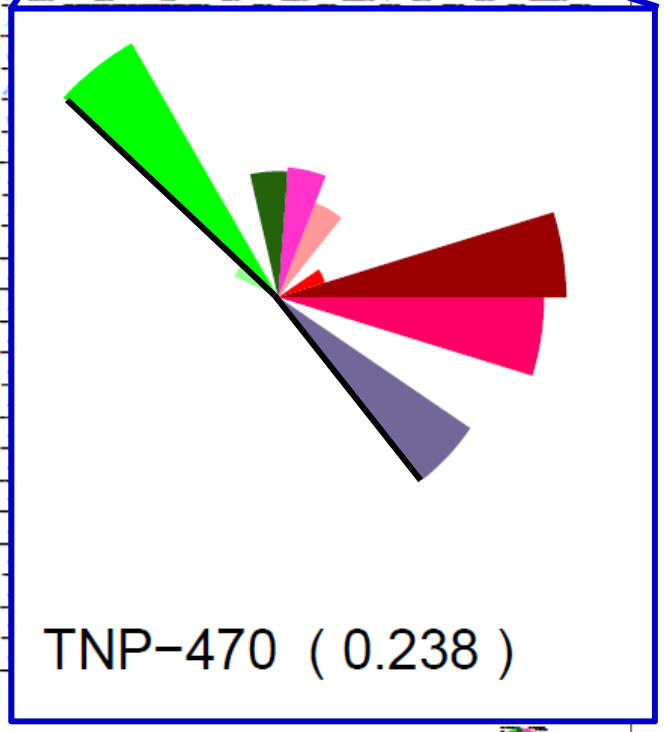
ToxCast Chemicals sorted by pVDC ranking



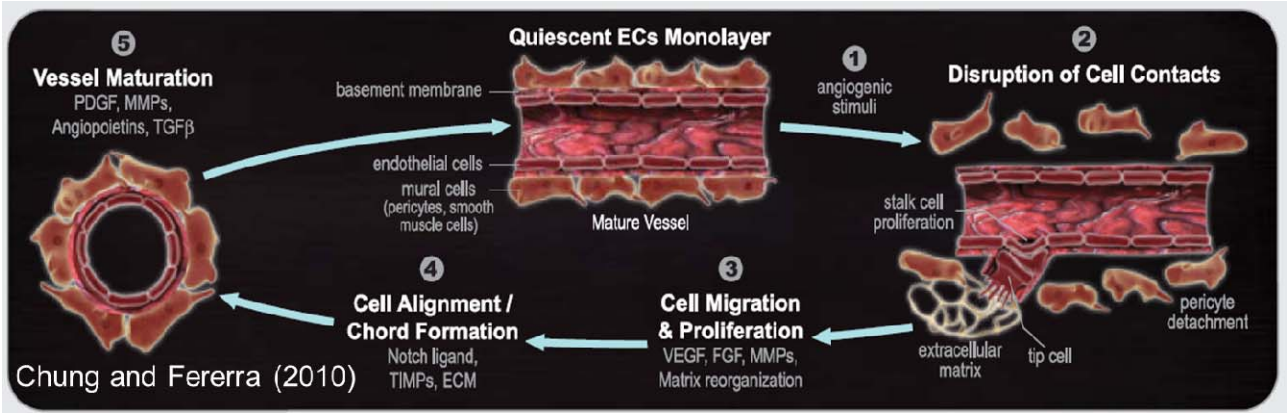
An evaluation of 36 chemicals + 2 reference compounds yielded a balanced accuracy of 85% in a human angiogenesis platform (FICAM - 0.91 sensitivity, 0.79 specificity).

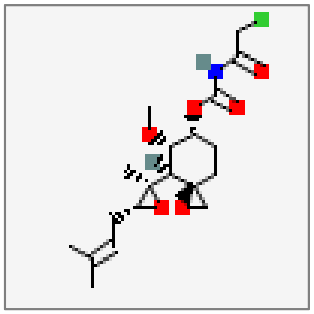






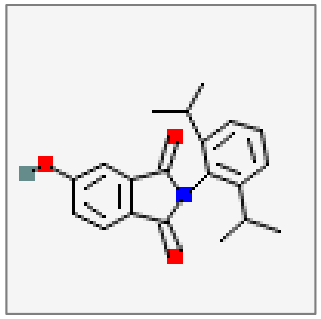
Angiogenesis Cascade





5HPP-33: synthetic thalidomide analog, destabilizes the tubulin network and disrupts endothelial tubulogenesis [Noguchi et al. (2005)].

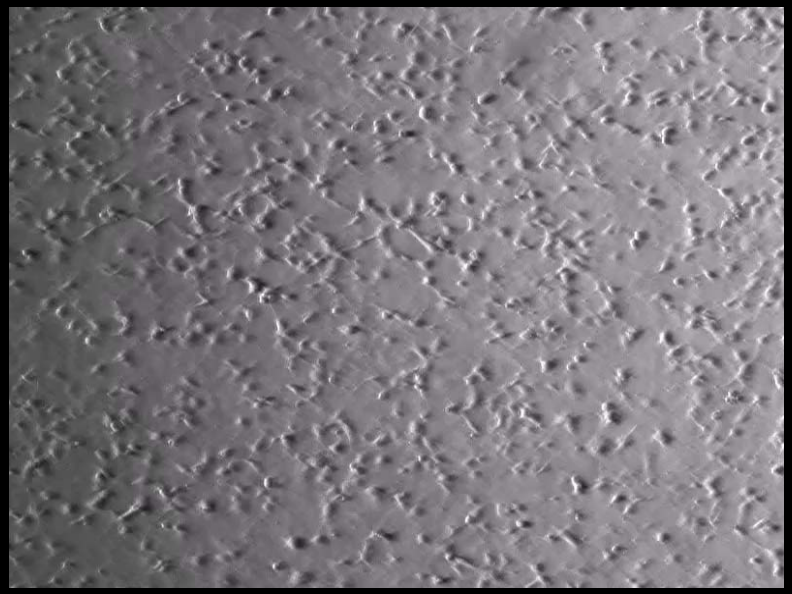
- stronger effect on endothelial network assembly



TNP-470: synthetic fumagillin analog, inhibits MetAP2 and disrupts endothelial proliferation in response to Wnt signals [Griffith et al. (1998)].

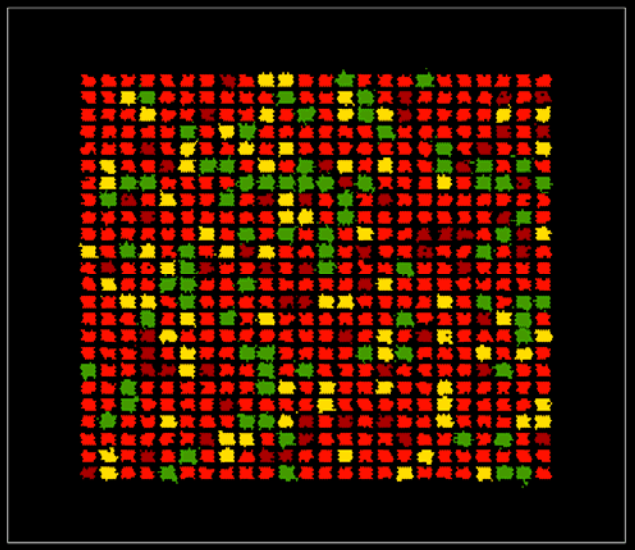
- stronger effect on microvessel outgrowth

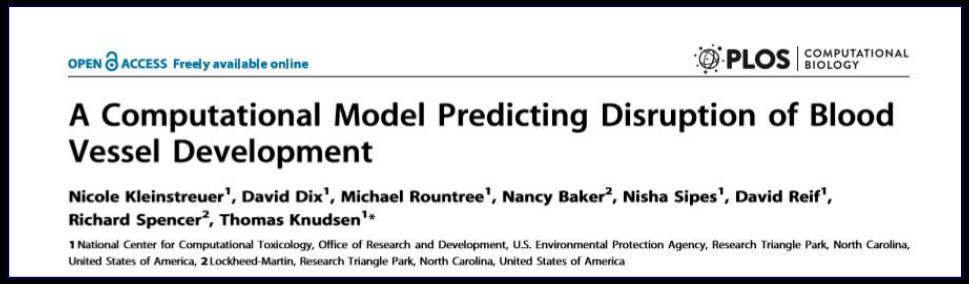
Agent-Based Models (ABMs): rules assigned to low-level 'agents' that then interact in a shared environment to recapitulate higher-order (emergent) behaviors.



in vitro tubulogenesis
J Glazier (Indiana U)

VEGF165
MMPs
VEGF121
sFlit1
TIE2
CXCL10
CCL2





OPEN ACCESS Freely available online

PLOS | COMPUTATIONAL BIOLOGY

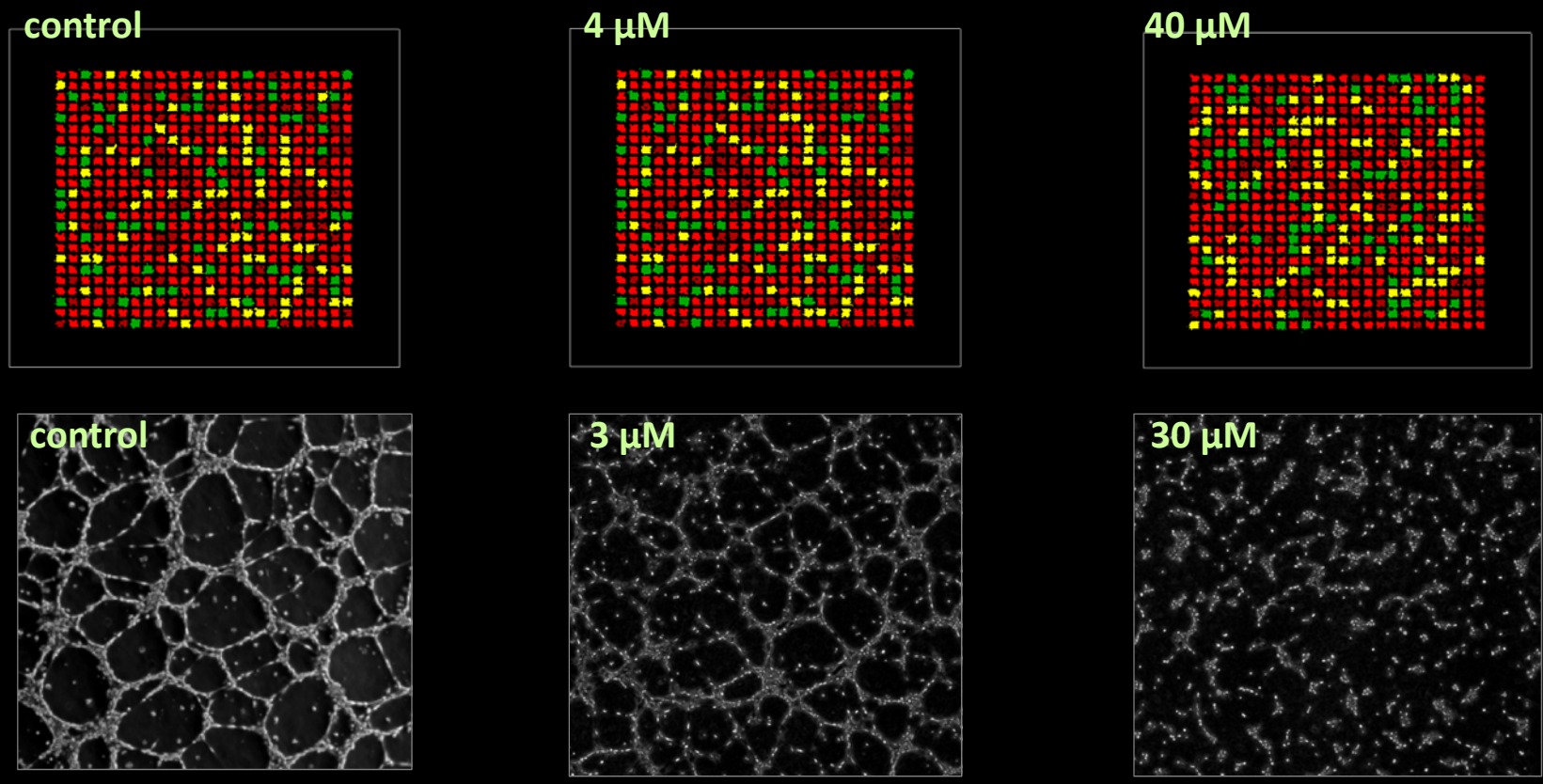
A Computational Model Predicting Disruption of Blood Vessel Development

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

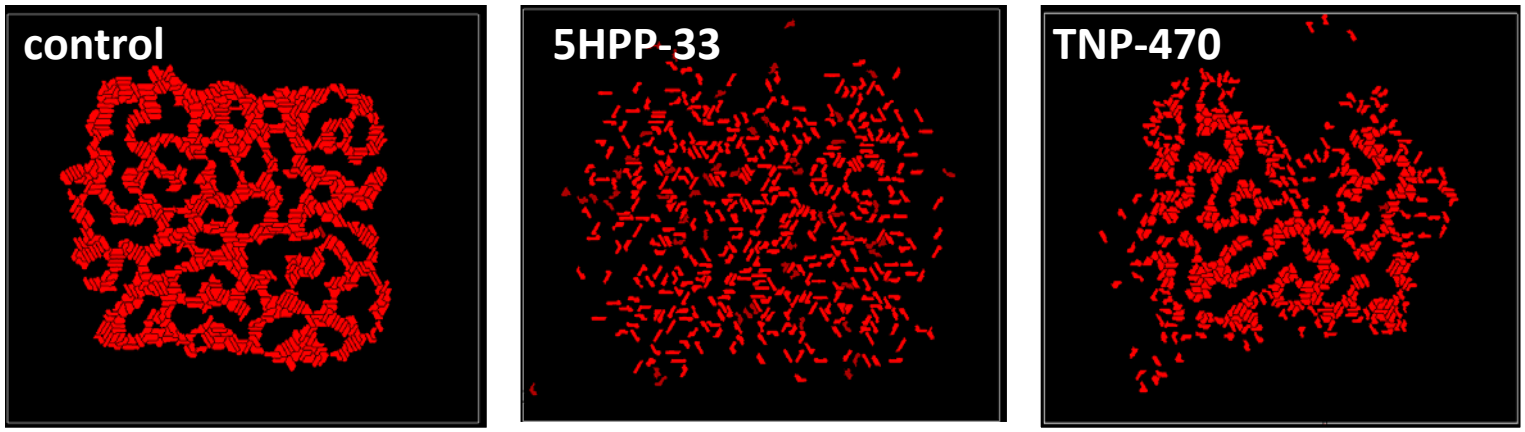
SOFTWARE: www.CompuCell3D.org

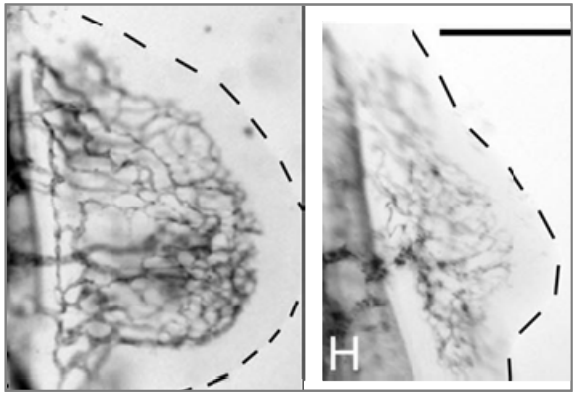
Simulation of 5HPP-33 Concentration Response



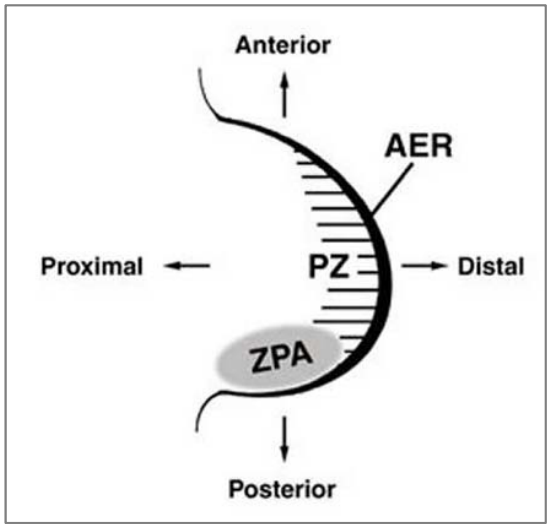
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol

Translating cellular effects into a measureable tissue response for a spatially-dynamic embryological system



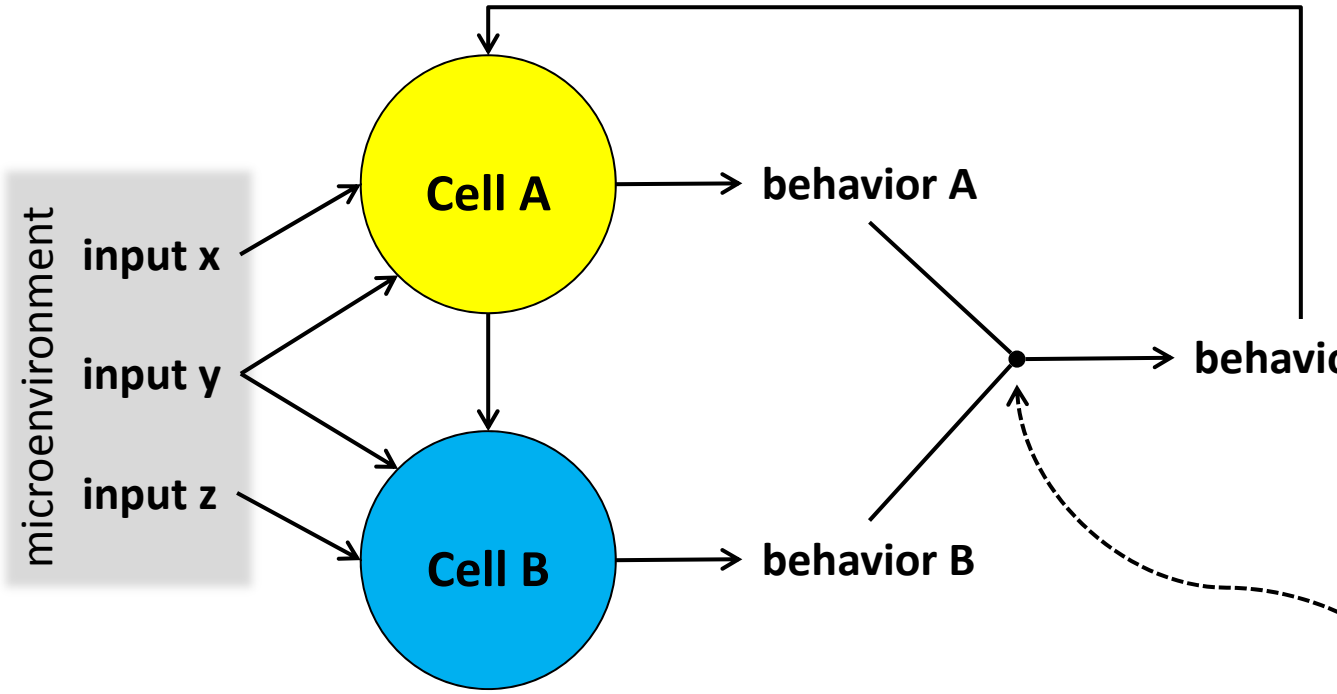


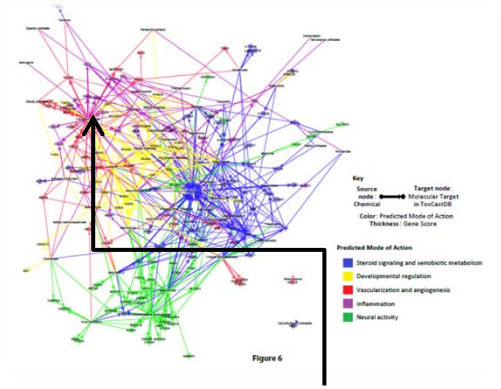
Thalidomide disrupts immature blood vessels – ***Therapontos et al. (2009) Proc Natl Acad Sci USA***



This occurs as the organizing centers of morphogenesis are critical

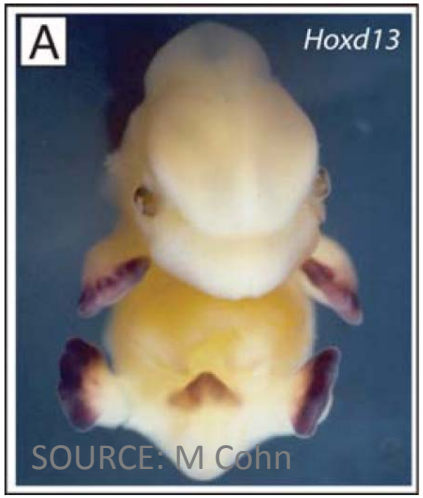
Cellular Response Networks (CRNs): how cellular systems translate spatial information into higher-order function





Capturing this mathematically:

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



12

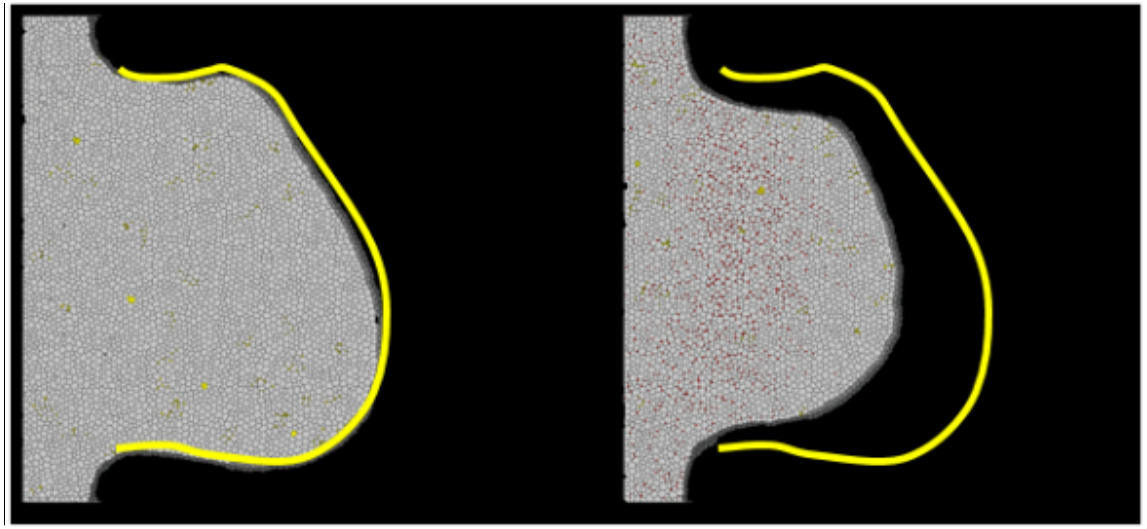
Can a spatially-dynamic computational model of an embryonic system (a 'virtual embryo') effectively simulate developmental toxicity?

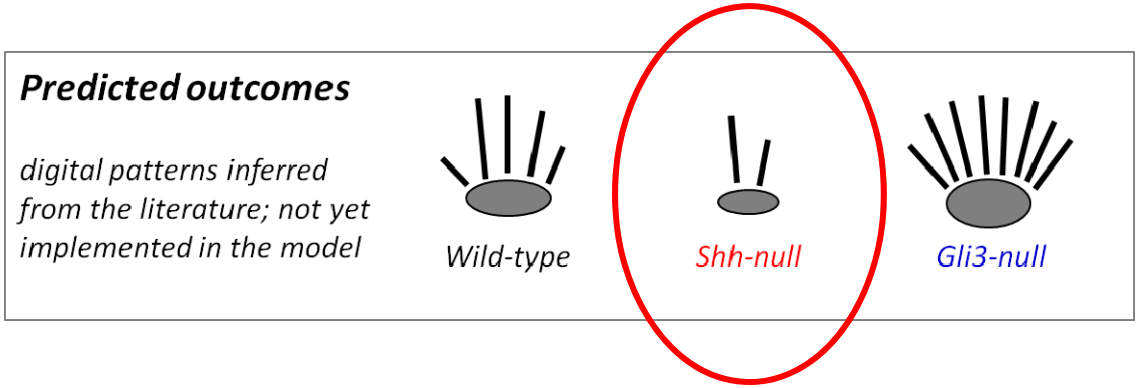
and if so …

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

[chemicals, doses, mixtures, stages, subpopulations, …]

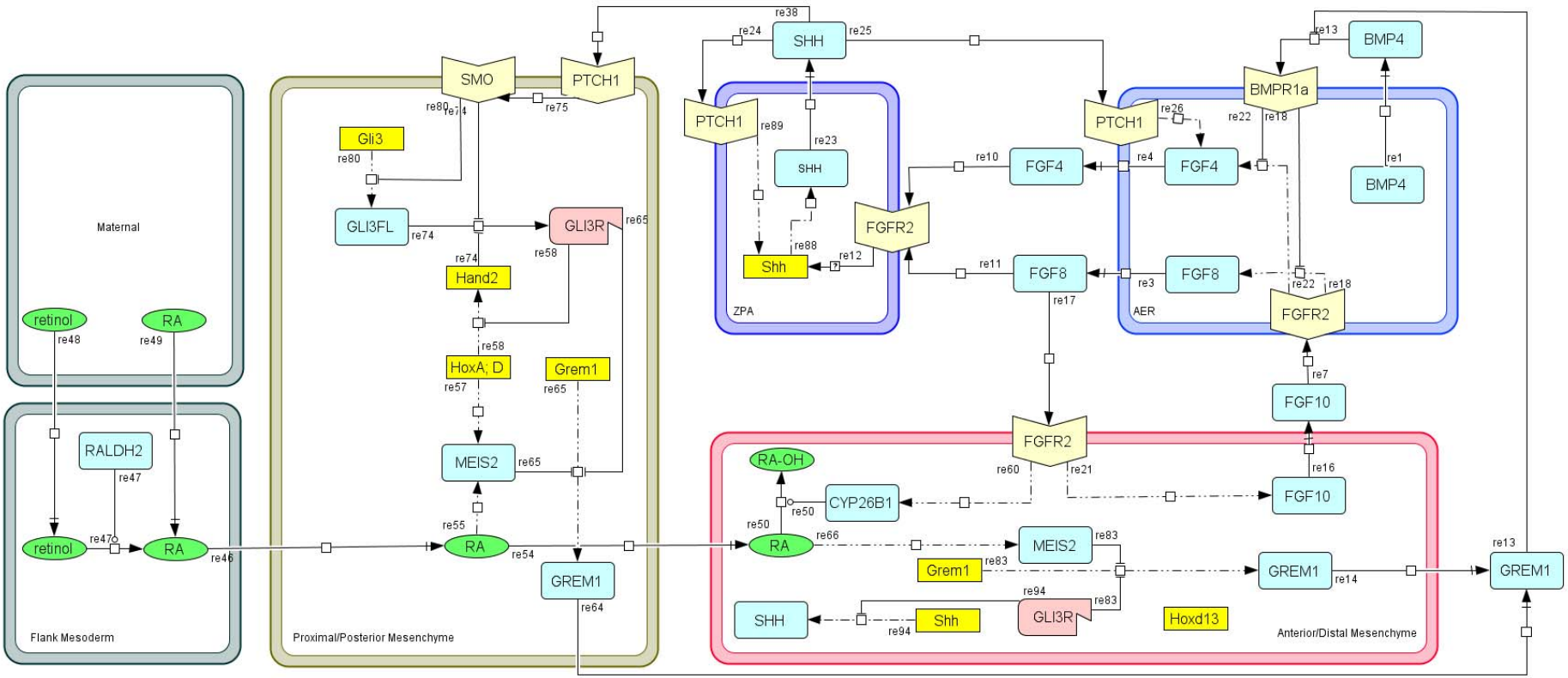
Computer Simulation: translating quantitative cellular injury into a measureable tissue response based on spatial dynamics.



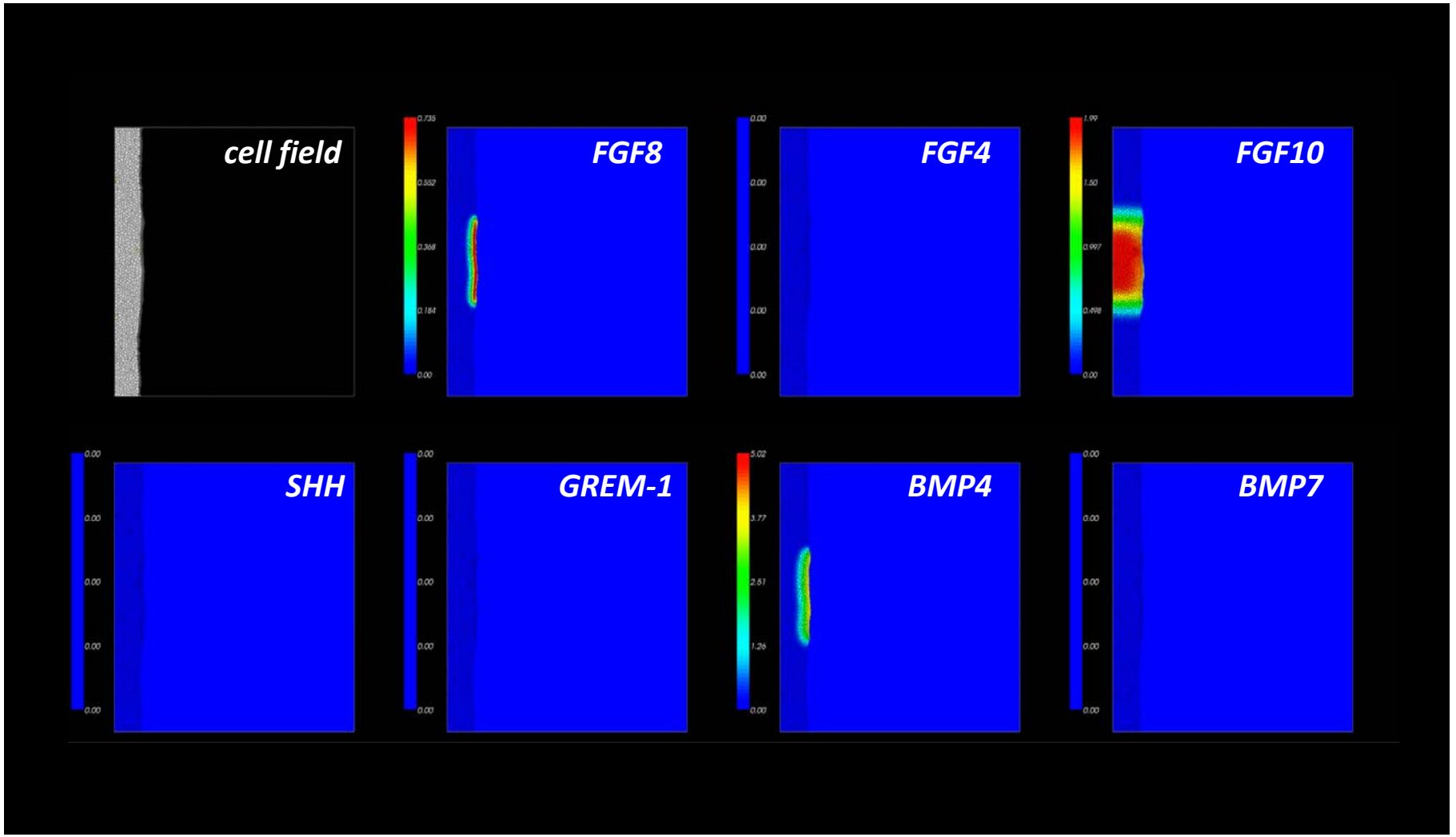


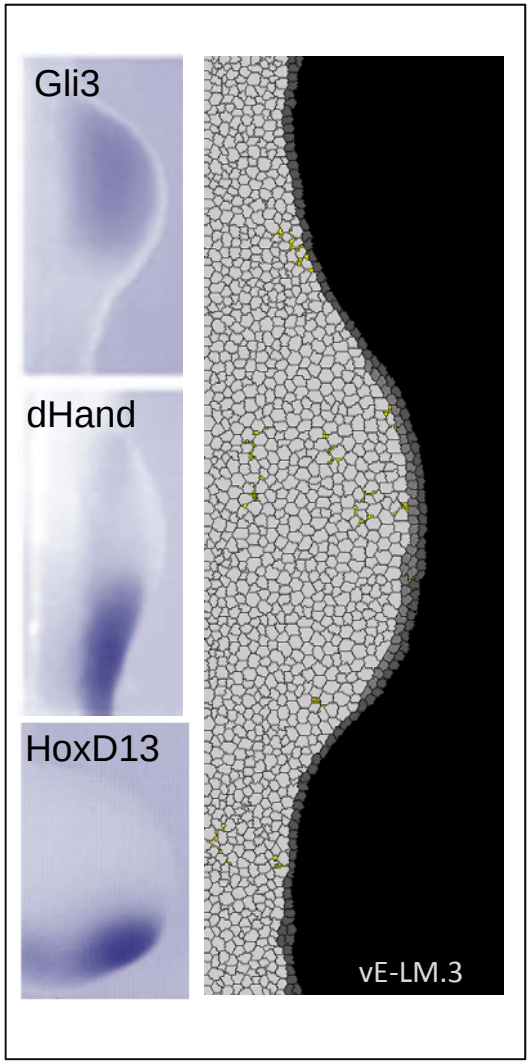
SOURCE: Dewoskin et al. (2015) in preparation

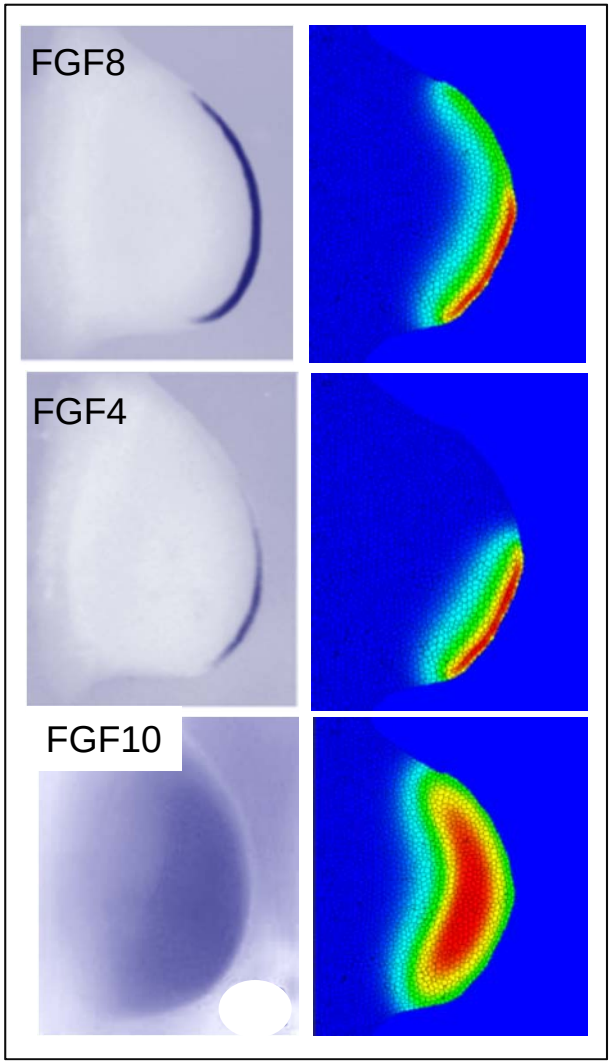
Control Network: limb-bud signaling modeled in Cell Designer

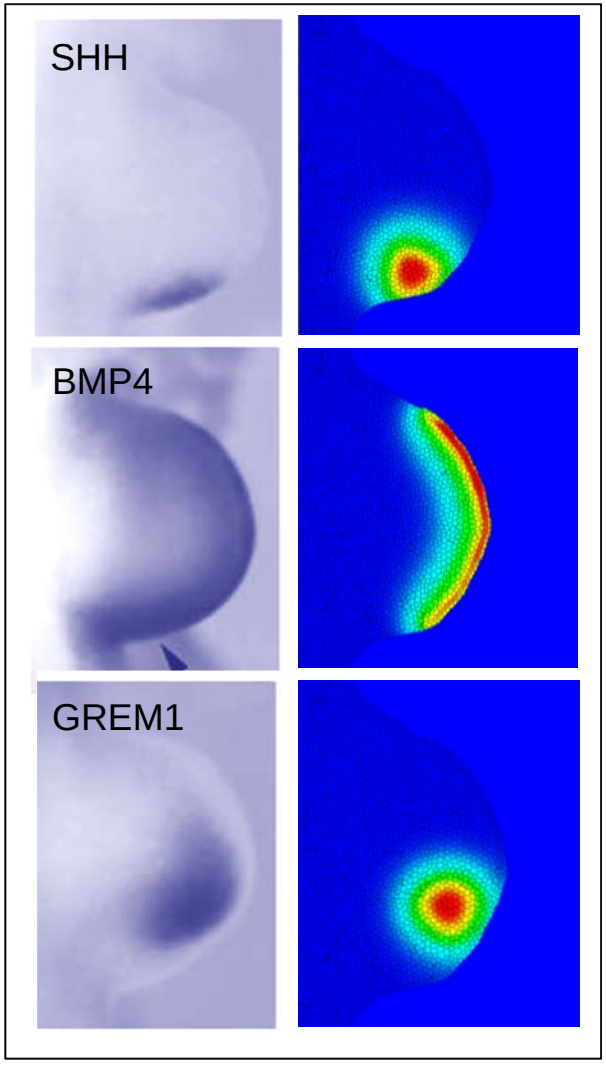


Translating the control network



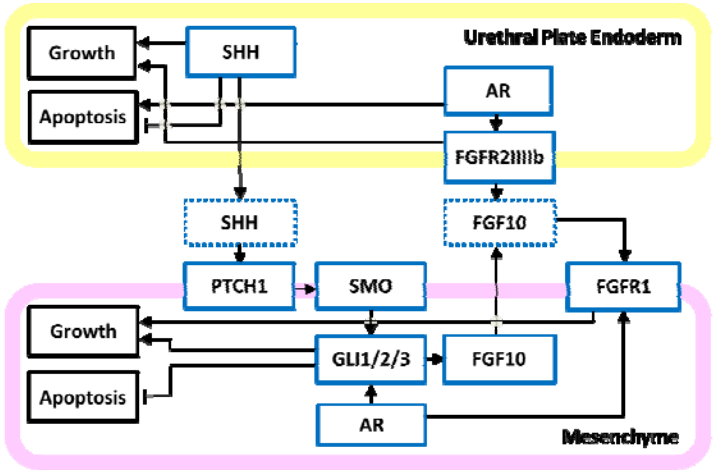


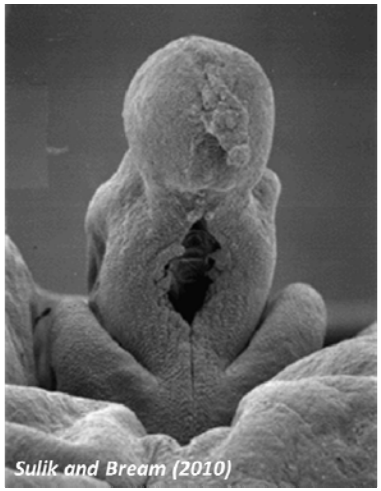


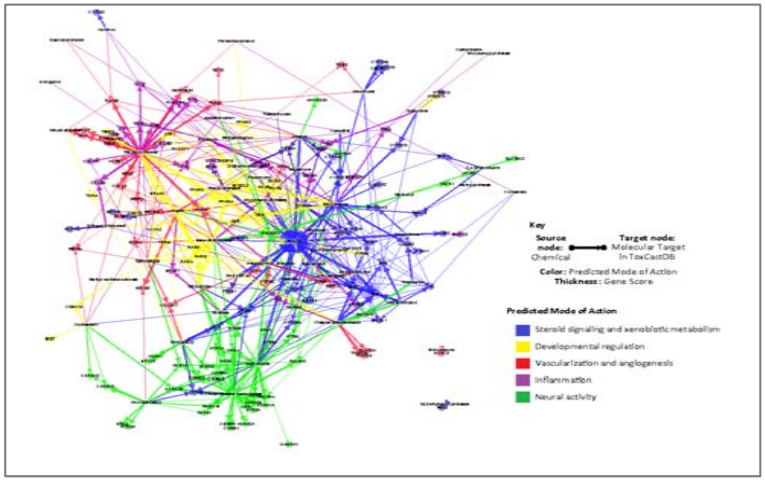


ISH (mouse literature) vs ABM

Genital Tubercle (GT) development: cell ABM can explore how the *bipartite network* might interact with the *control network* to induce hypospadias, a urethral closure defect.

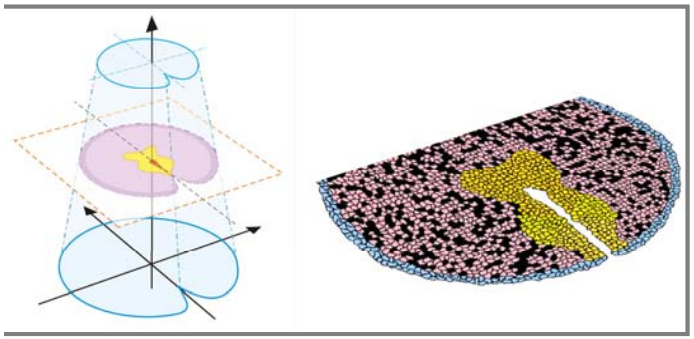


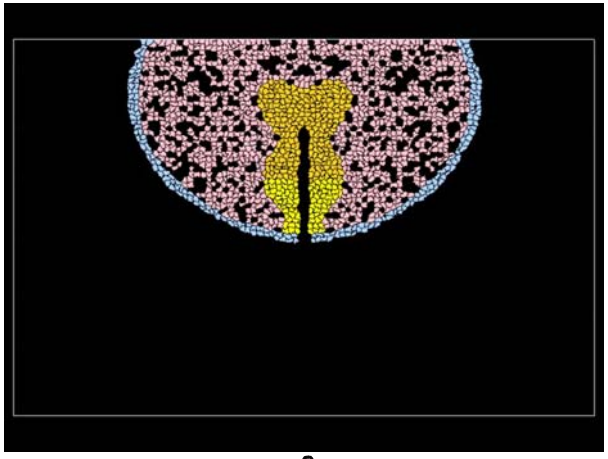




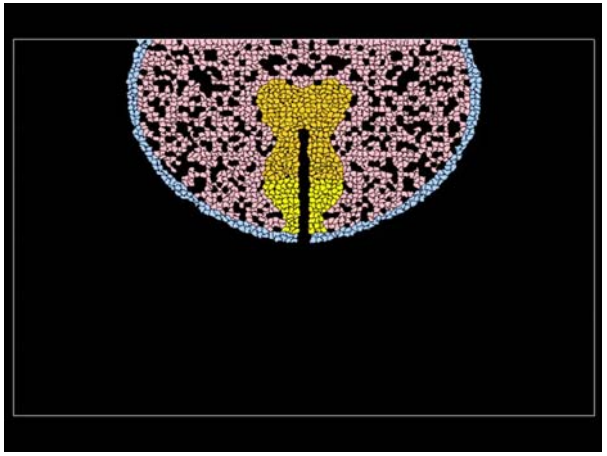
SOURCE: M Leung, manuscript in preparation

Cell ABM for Urethral Closure



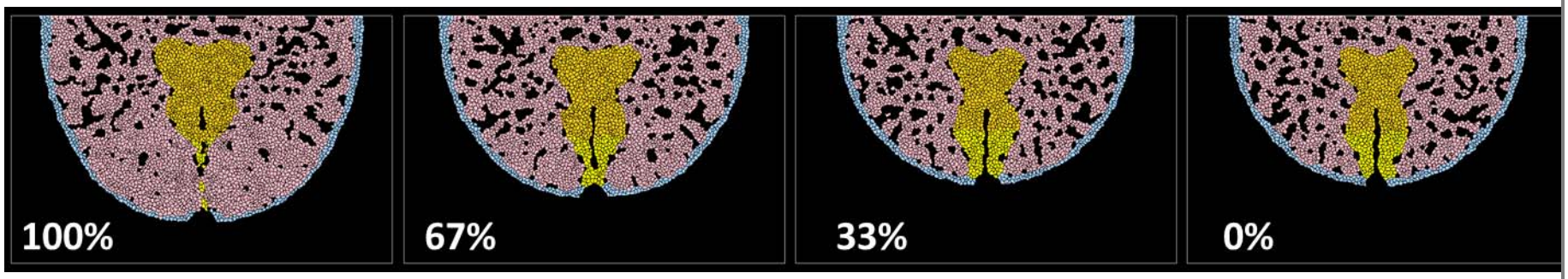


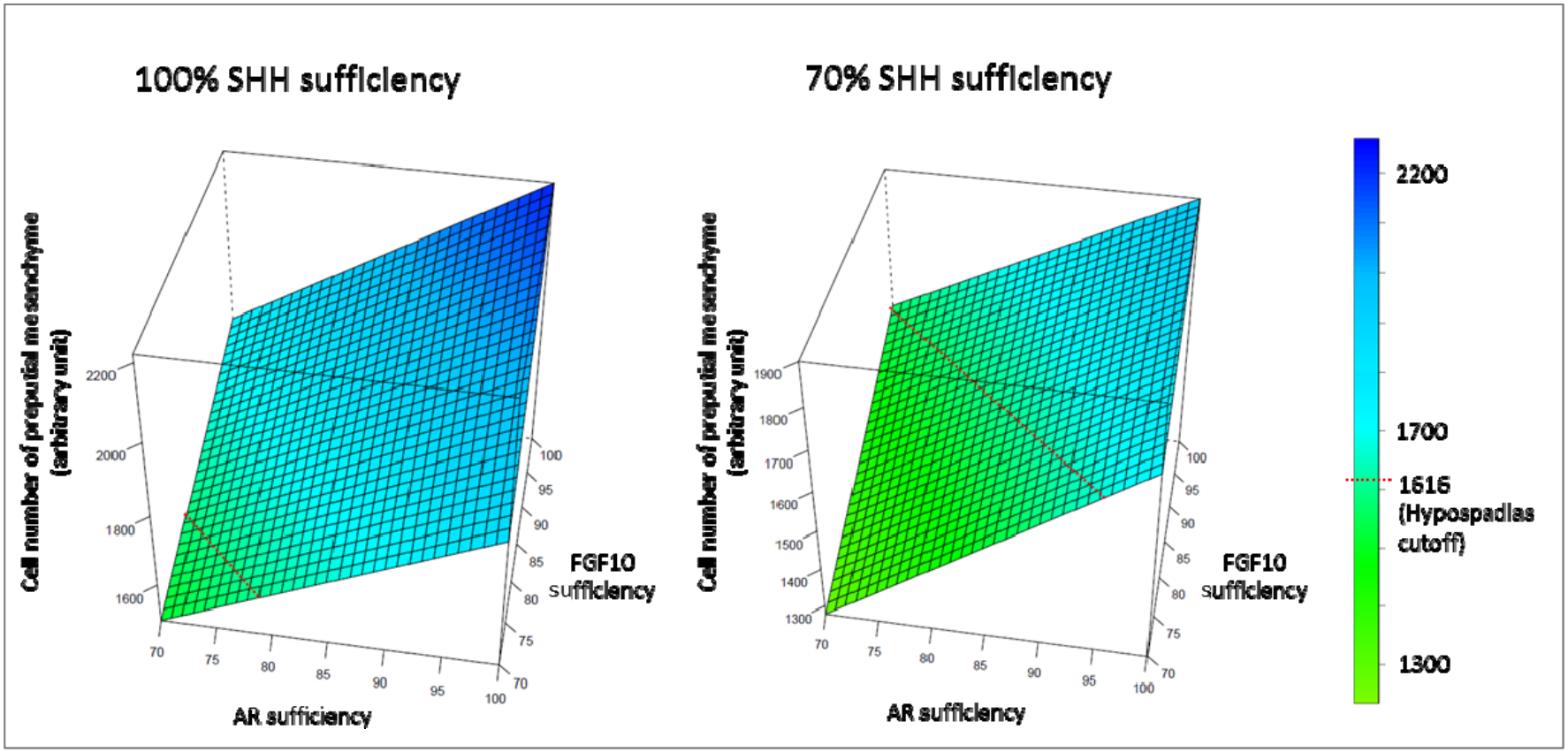
Default
(female)



Androgenized
(male)

- Androgen production by fetal testis triggers sexual dimorphism of the GT into male or female phenotypes (%-normal sufficiency).

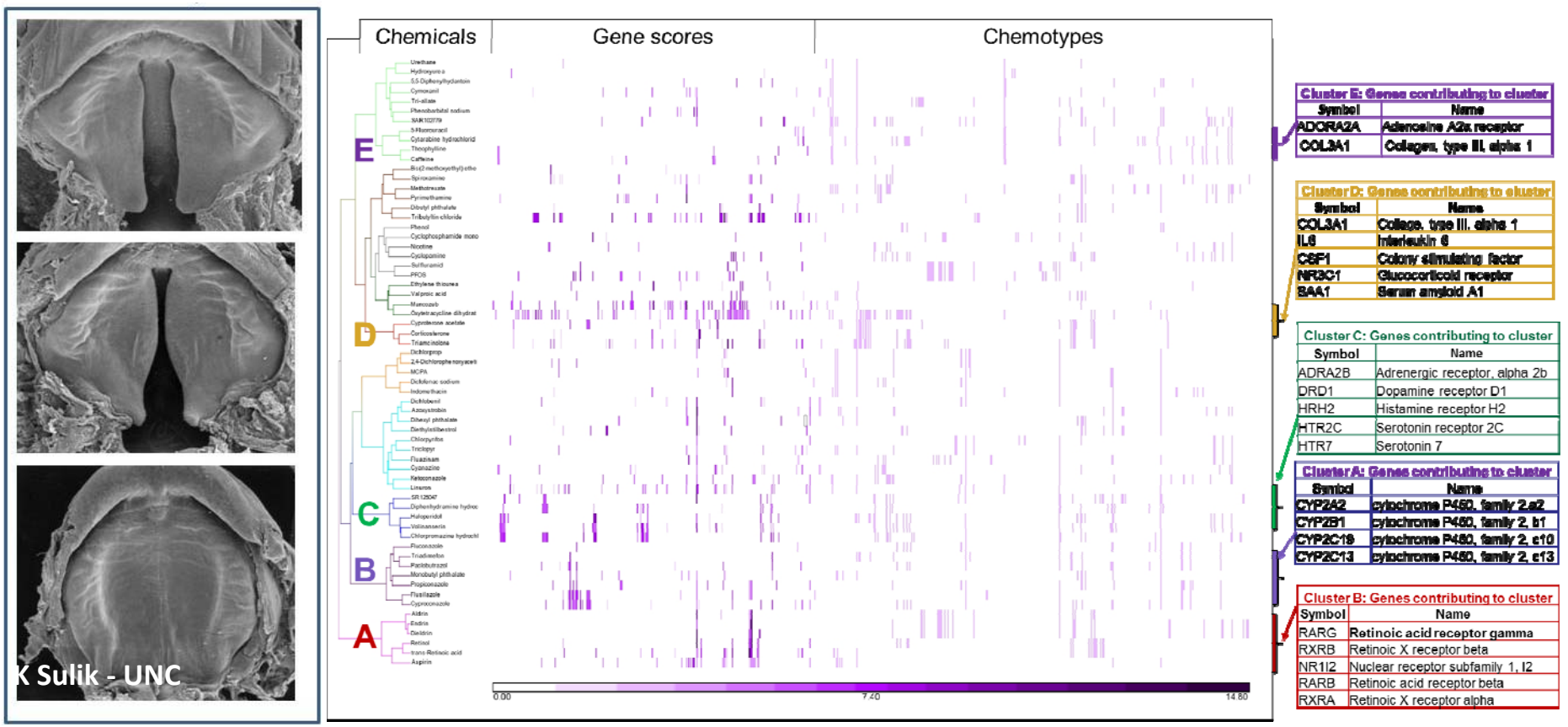




Multi-disturbance surfaces from an ABM of the developing GT can be used assess individual risks for complex interactions:

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

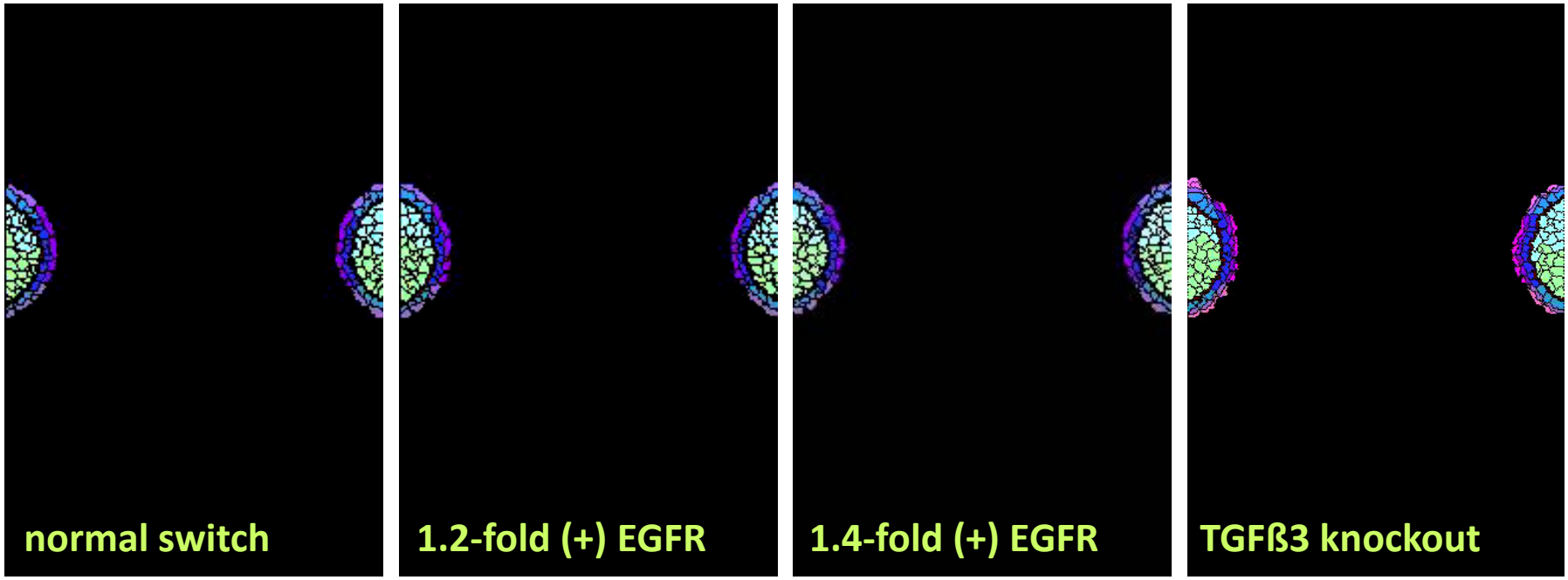
Cleft Palate: 63 actives in ToxCast have been used to produce a chemical-target biopartite network.

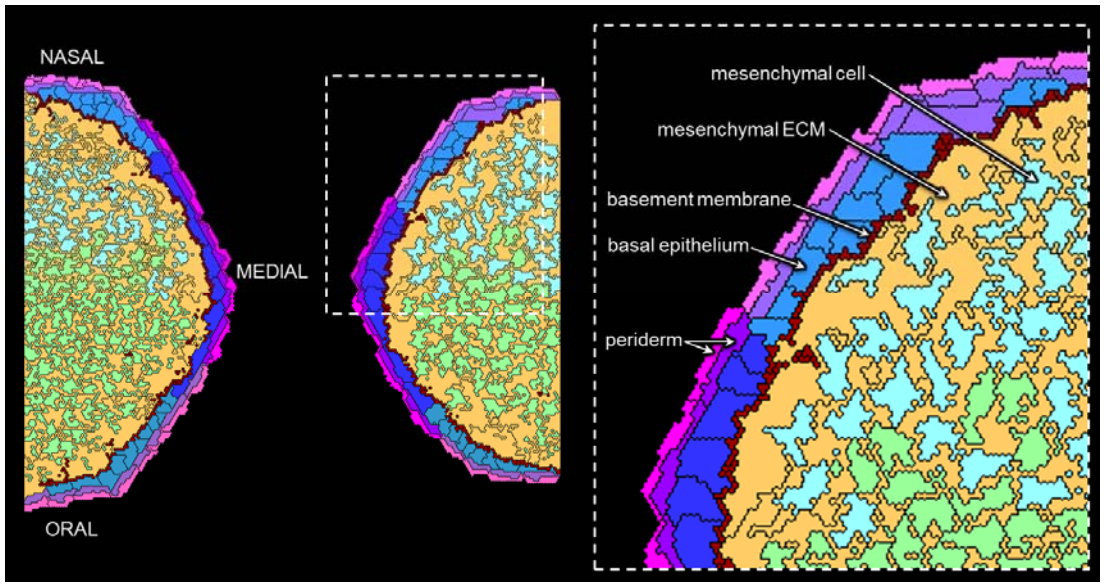


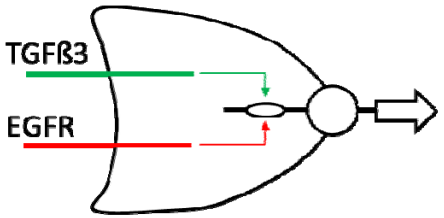
CYPs (conazoles), RARs (retinoids, OC insecticides), anti-angiogenic/inflammatory cytokines (steroids), GPCRs (adrenergic/serotonergic)

SOURCE: N Baker, manuscript in preparation

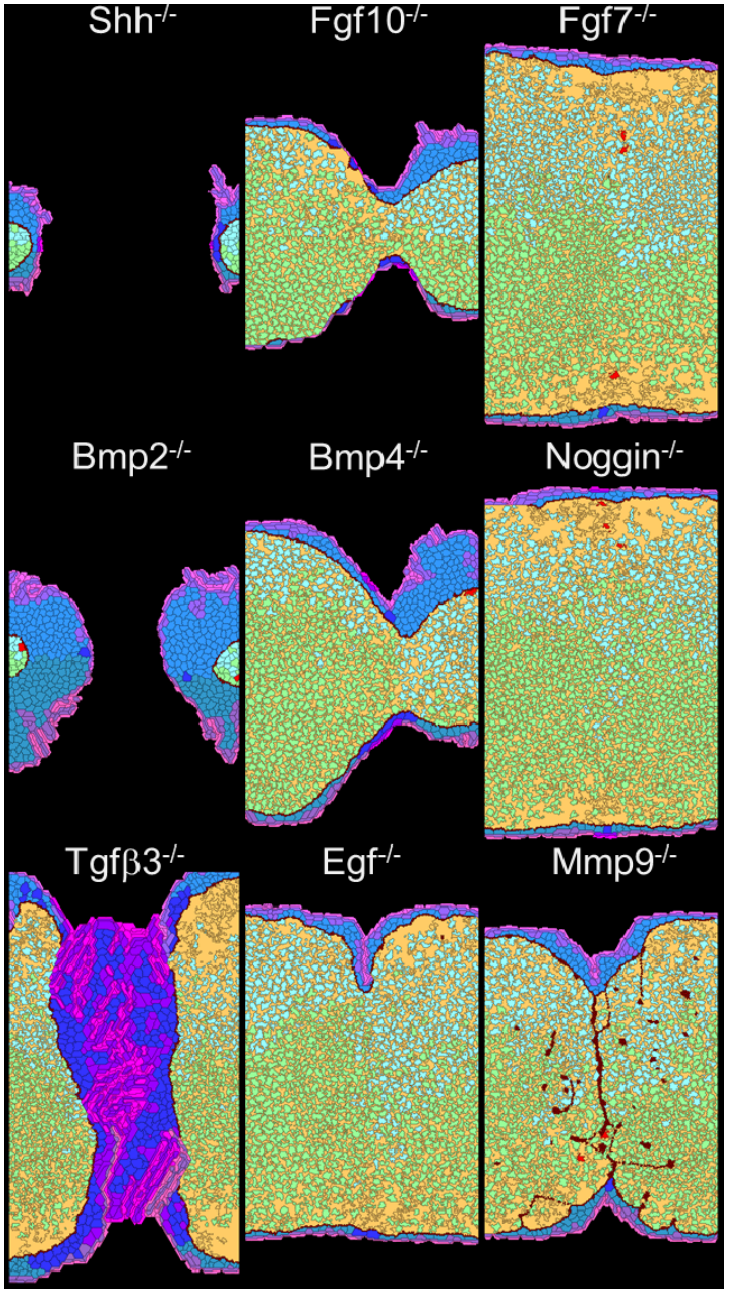
Cell ABM for Palatal Closure



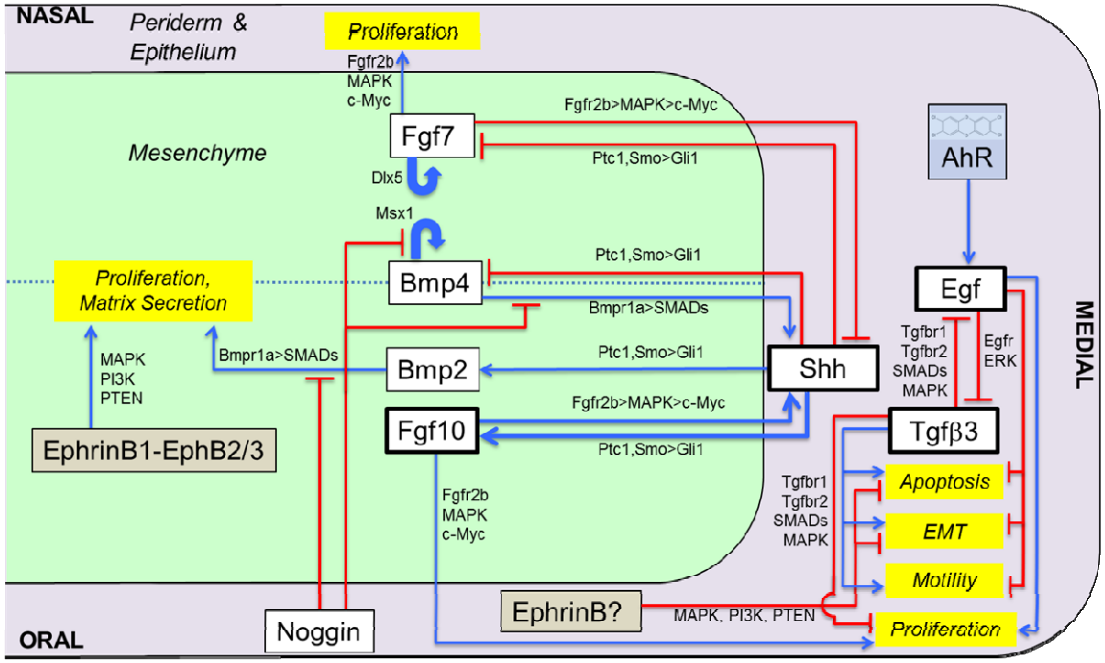




TGFß3	EGFR	CP
1.0	1.0	0
0	1.0	1
1.0	1.2	0
1.0	1.4	1
1.0	1.5	1



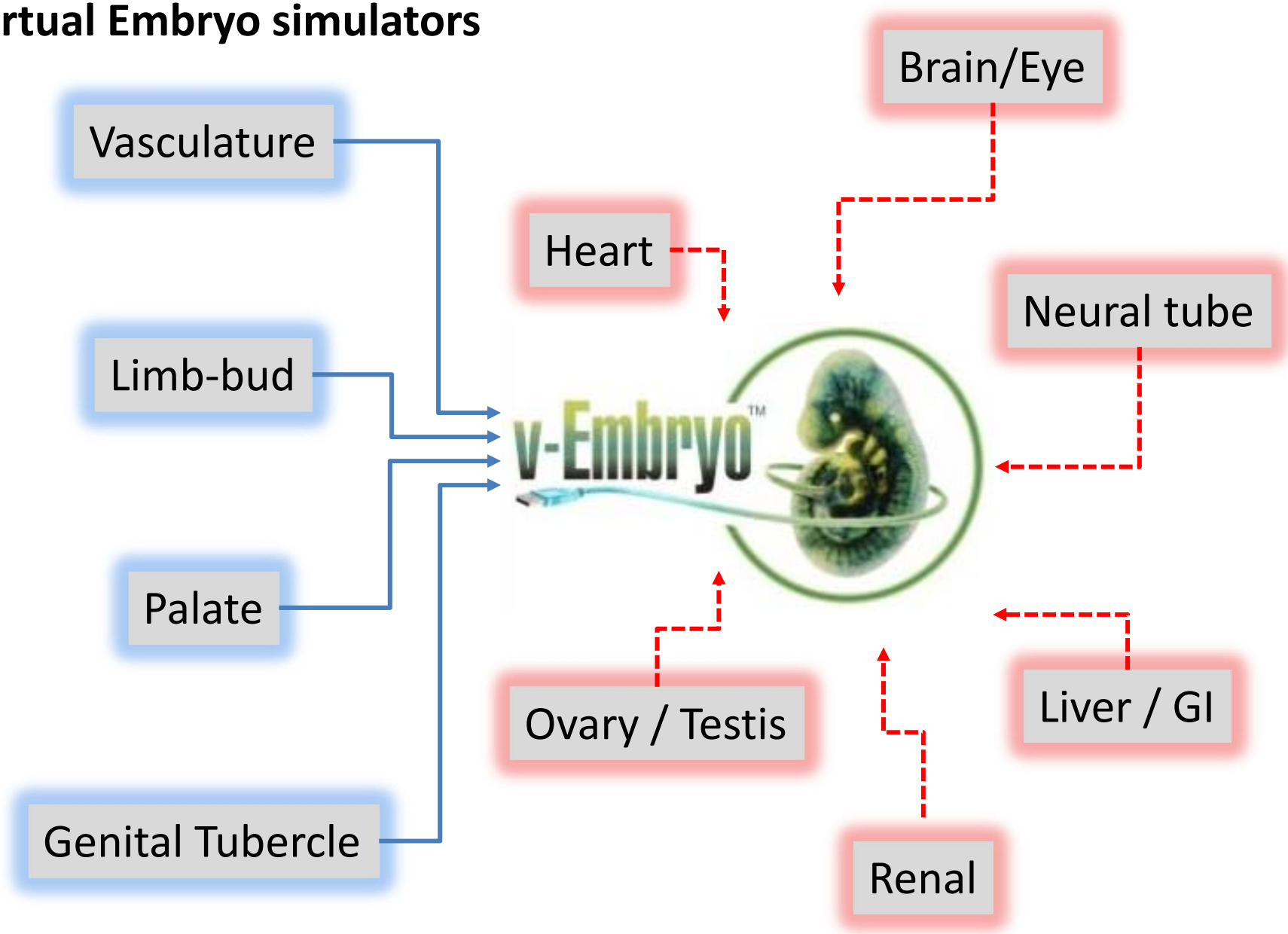
Regulatory network



Hacking the network

SOURCE: S Hutson manuscript in preparation

Virtual Embryo simulators



Virtual Tissues Laboratory System

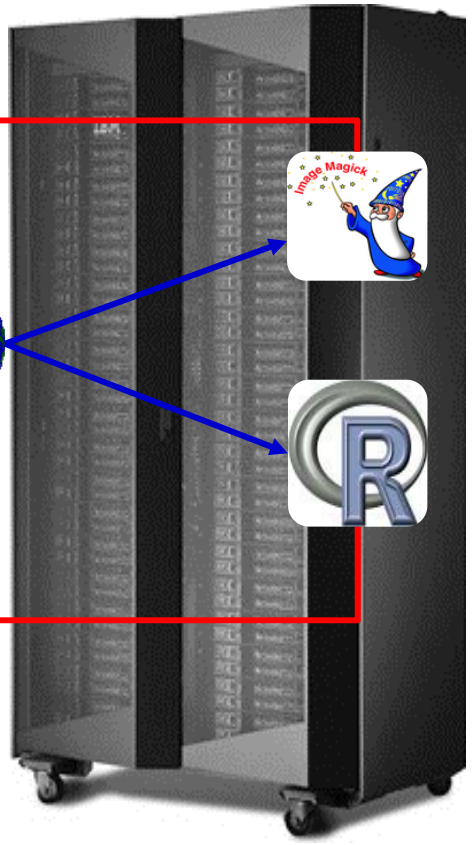
VIRTUAL TISSUES LABORATORY SYSTEM

Virtuoso

Web Services and Queries

HPC

Massively-parallel simulation

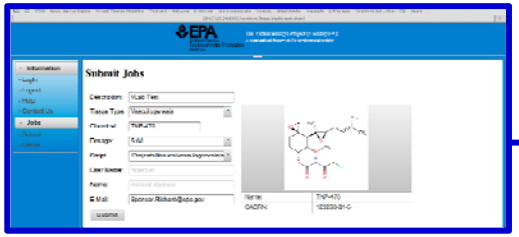


Video or 3D Results

VTKB

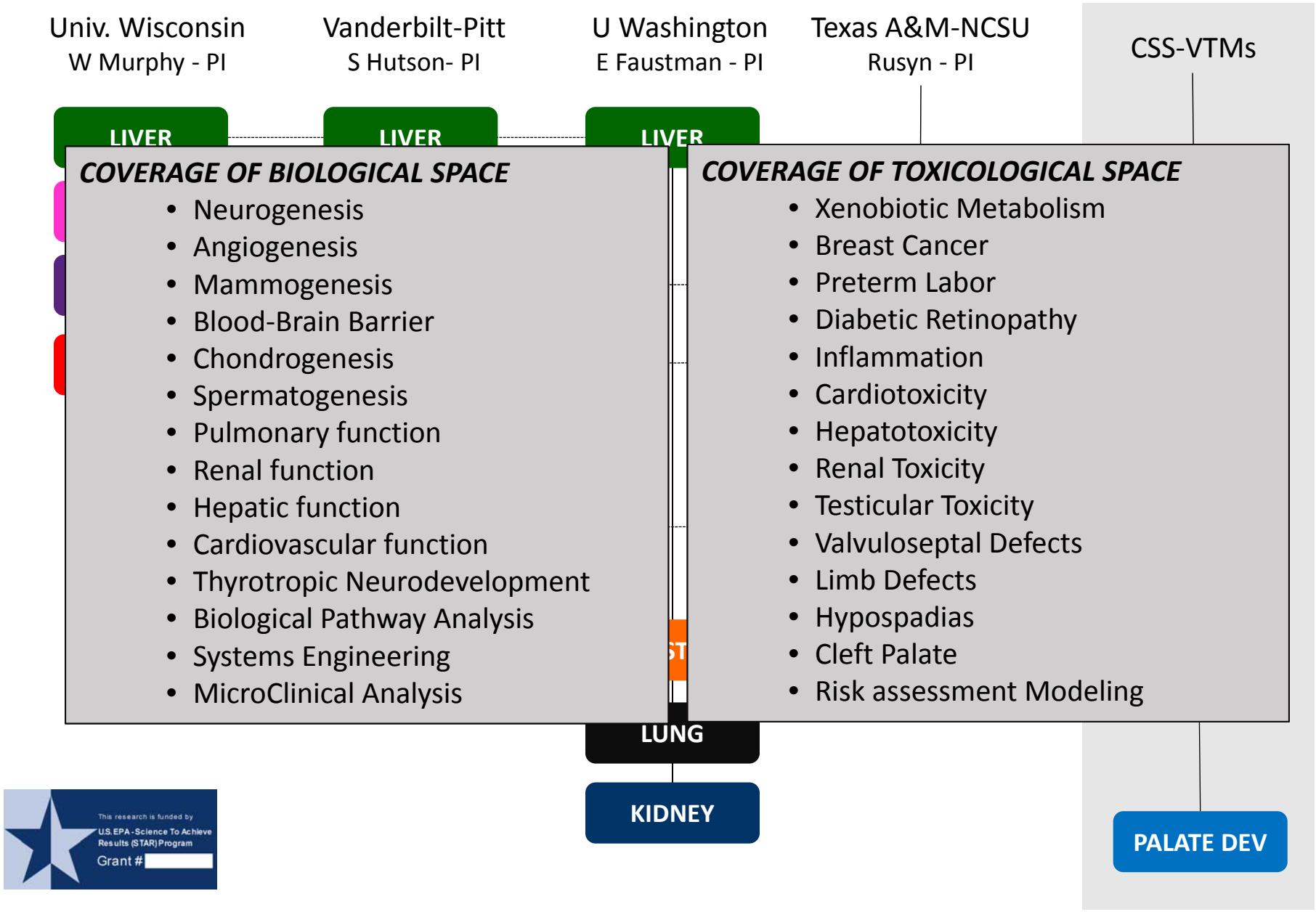
CC3D simulations
ToxCastDB
Bionetworks
Literature
Provenance

Data Analysis

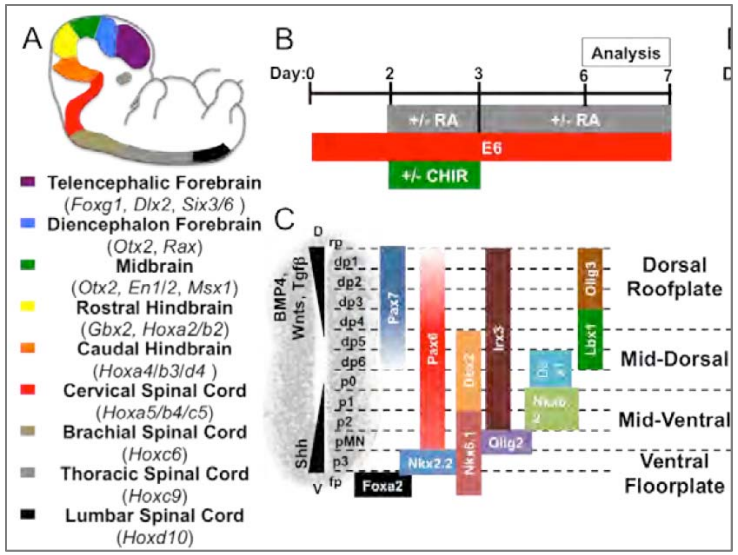


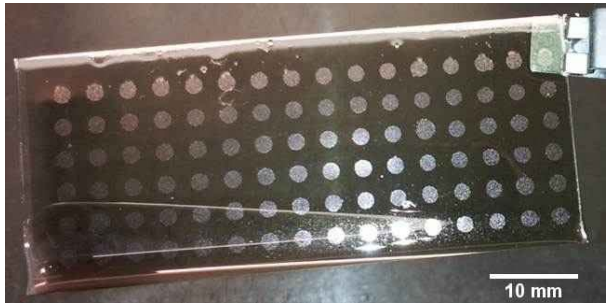
BIO2RDF

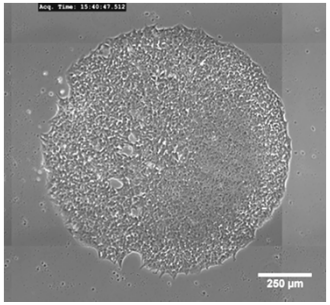
new OCM Centers awarded EPA STAR funding in FY15



Microscale and Microphysiological Systems (MPS)





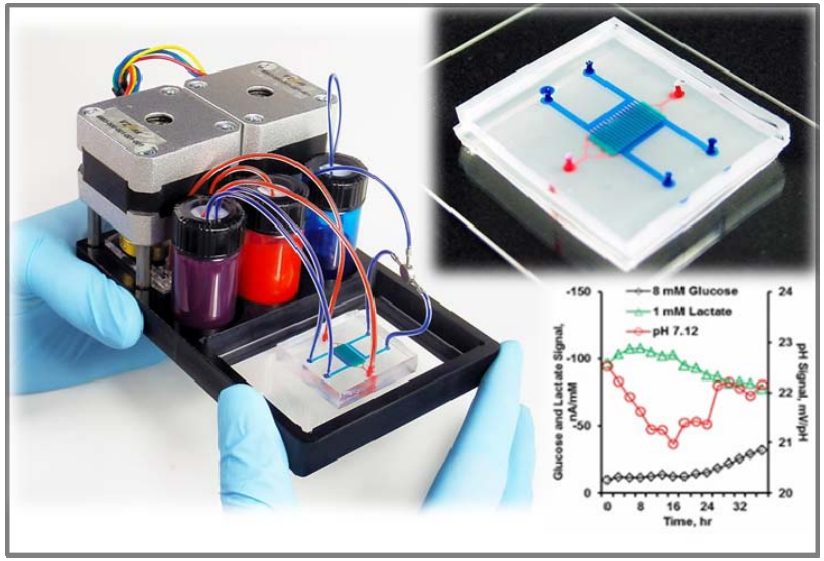


Minibrain arrays

Bill Murphy – U Wisconsin

Phenotypic diversity arrays

Randy Ashton – U Wisconsin



Synthetic NVU on a chip

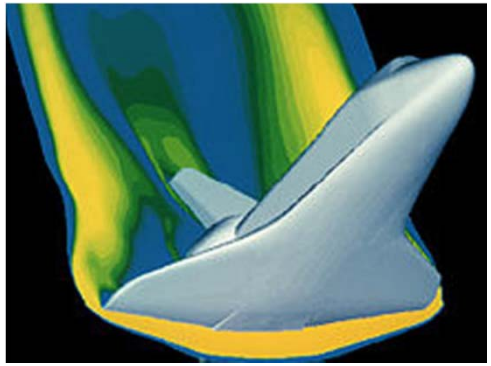
John Wikswo – Vanderbilt U

v-Embryo™

Some Benefits and Challenges For Predictive Toxicology:

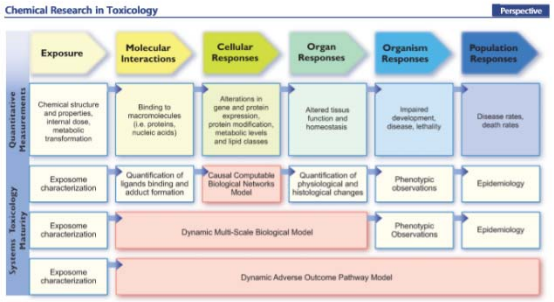
- multicellular ABMs elevate HTS data to spatially dynamic systems
- sensitivity analysis to unravel complex CRNs
- pinpoint nascent events underlying 'emergent' biology
- high-throughput hypothesis generation (susceptibility)
- surrogate for missing data (compute the 'un-measureable')
- simulating different exposure scenarios (kinetics, mixtures)

- not a living entity
- can only code rules as we understand them
- need to enable but not over-specify model performance
- what is the optimal degree of biological complexity
- additional lifestage considerations / life-course impacts



Engineers commonly design, model and test complex systems *in silico* before building them.

- *Woltosz (2012) J Computer Aided Molecular Design*



Toxicologists commonly build conceptual models of complex systems *in silico* after testing them.

- *Sturla (2014) Chemical Research in Toxicology*

We can design, model and test biological complex systems *in silico* for predictive toxicology.

- *Hypothesis and Motivation for a Virtual Embryo toolbox*

Special Thanks

- Richard Judson – NCCT
- Imran Shah – NCCT
- Barbara Abbott – NHEERL / TAD
- 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 Wikswo – Vanderbilt U / STAR

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

EPA's National Center for Computational Toxicology