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Symposium: "Digitalisation meets Pathology and Developmental Toxicology"
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Status and Prospect of the Virtual Embryo Program

Thomas B. Knudsen, PhD
Developmental Systems Biologist
National Center for Computational Toxicology
US Environmental Protection Agency
Research Triangle Park, NC 27711

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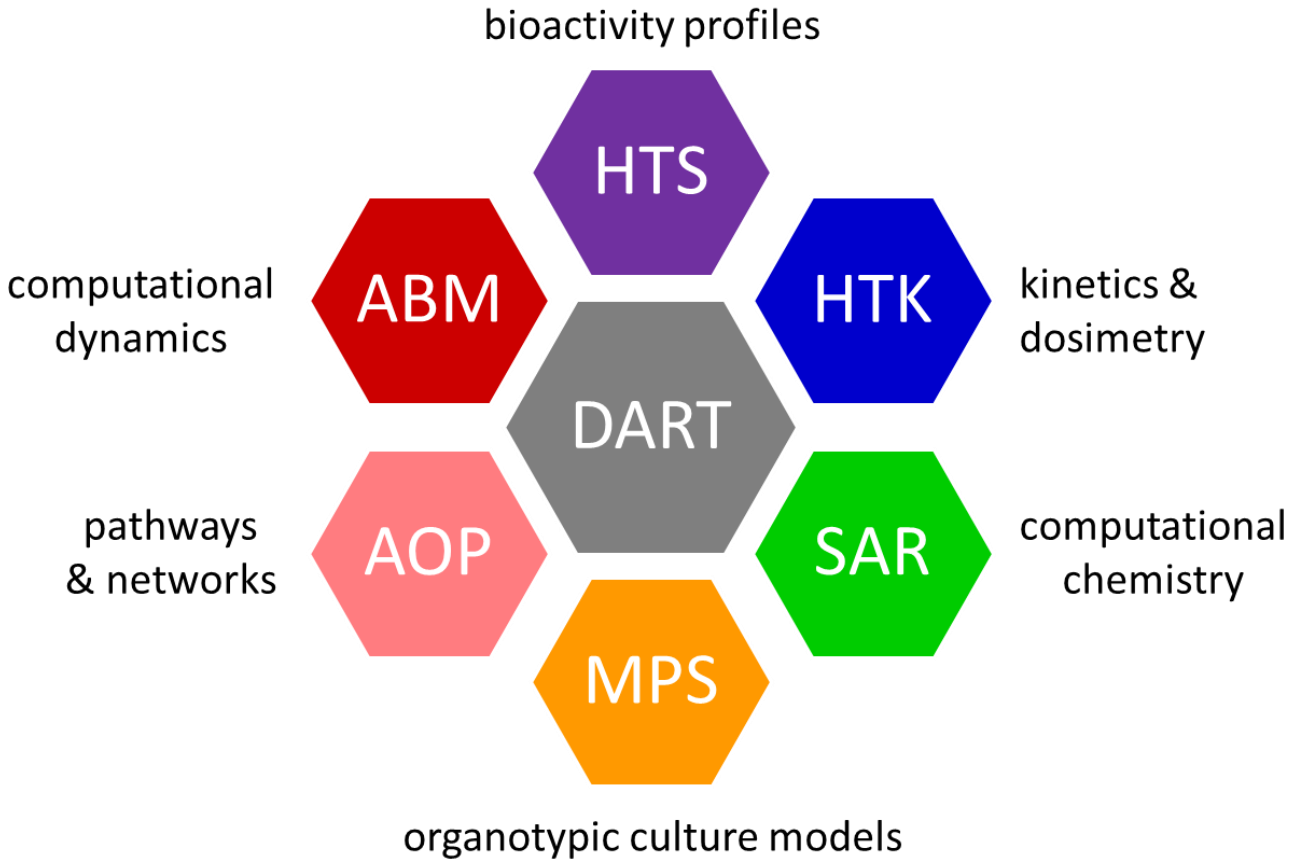
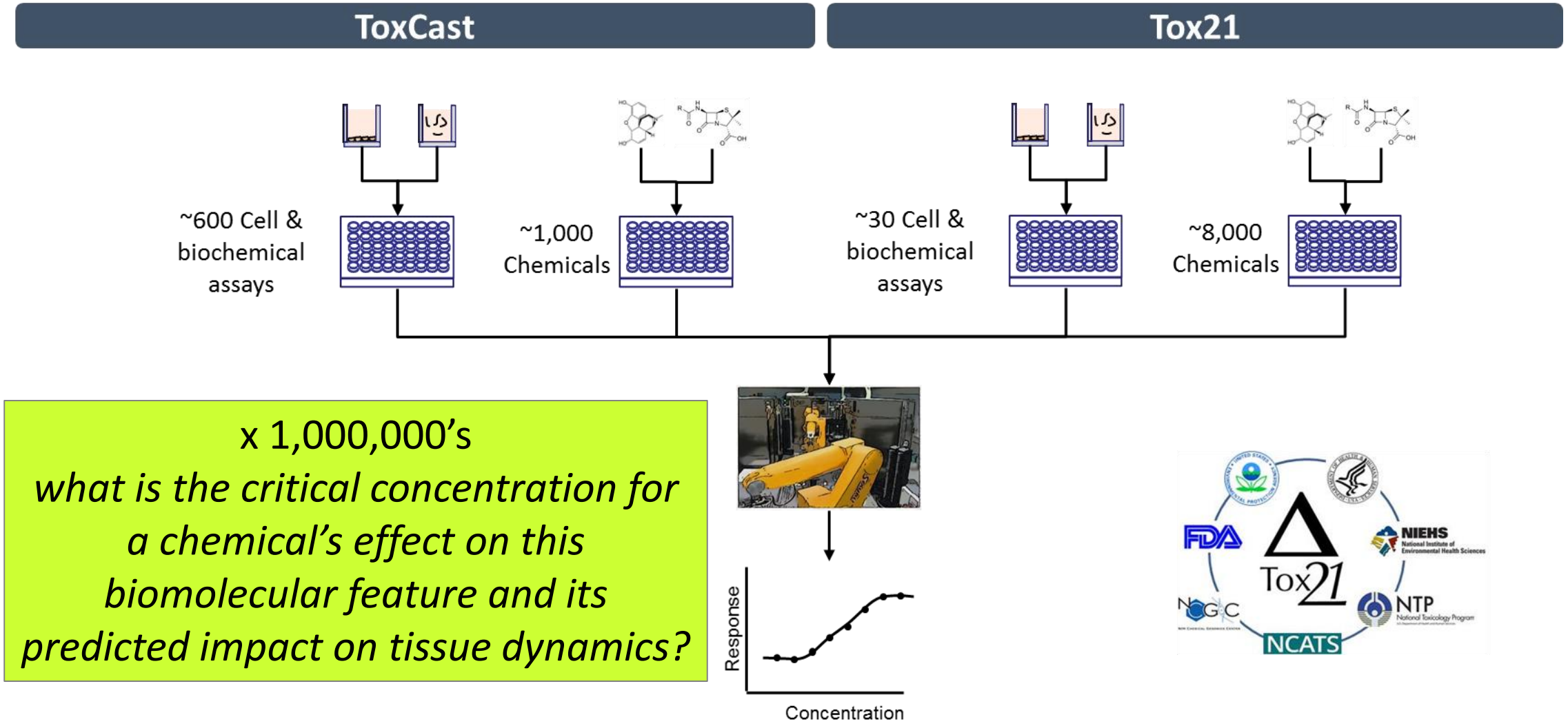
Predictive toxicology



- New Approach Methodologies (NAMs) for toxicity assessment based largely on *in vitro* data and *in silico* models.
- Science challenge - translating complex data and information into predictive models for human toxicity.
- For predictive DART, this means virtually extrapolating biomolecular lesion(s) into adverse developmental outcome(s).

2

Shifting toxicology to pathway-based approaches



x 1,000,000's
what is the critical concentration for a chemical's effect on this biomolecular feature and its predicted impact on tissue dynamics?

https://www.epa.gov/chemical-research/toxcast-dashboard

AI and 'big-data' connectivity in biology

https://www.thescientist.com May, 2019

- AI refers to the ability of a computer to learn from data or empirical observation ... and do so without falling prey to a lot of meaningless connections.

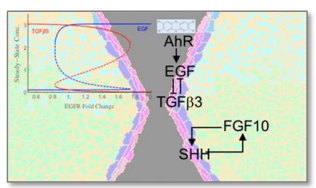
- Requirements to find relevant biological patterns across complex datasets and derive wisdom from information:

  1. availability, type and quality of data for analysis;
  2. ontologies for systematic organization of data;
  3. algorithms that can handle complex agent behaviors;
  4. sophisticated models to reconstruct system dynamics.

1. Profiling the ToxCast library

Objective: increase the diversity and relevance of assays in ToxCast that can be used to profile chemicals for potential adverse effects on human embryonic development.

2. Translating cellular lesions into quantitative phenotypes



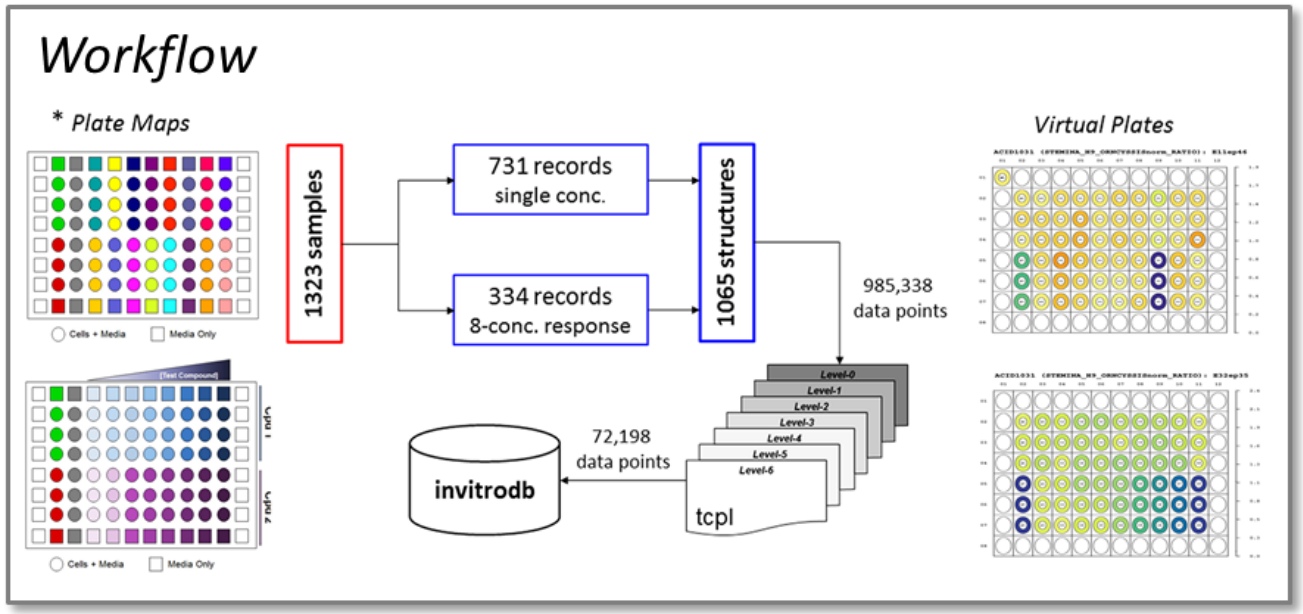
Objective: build and test computer models of complex tissues that advance critical phenomena (specificity, canalization, plasticity) in quantitative prediction for virtual screening and *in silico* testing.

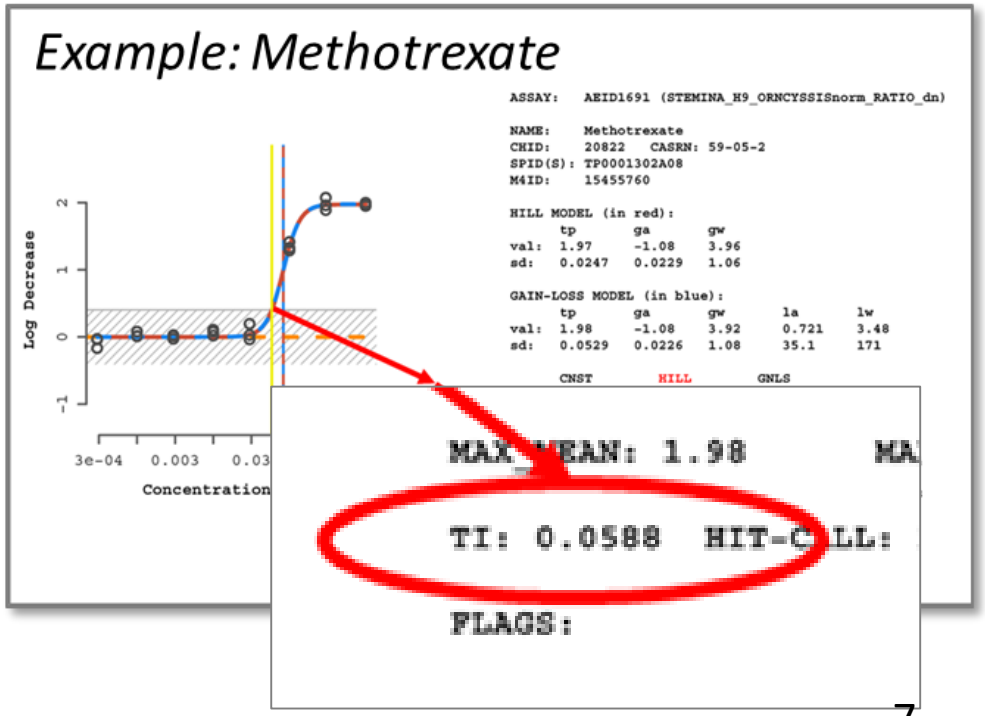
1. Profiling the ToxCast library

Objective: increase the diversity and relevance of assays in ToxCast that can be used to profile chemicals for potential adverse effects on human embryonic development.

ToxCast_STM assay

- devTOX^{qP} assay from Stemina Biomarker Discovery [Palmer et al. 2013]
- pluripotent stem cells exposed to chemicals for 3-days
- critical drop in ornithine : cystine ratio is the targeted readout
- **Key point:** 183 of 1065 (17%) ToxCast chemicals (tested to date) were positive

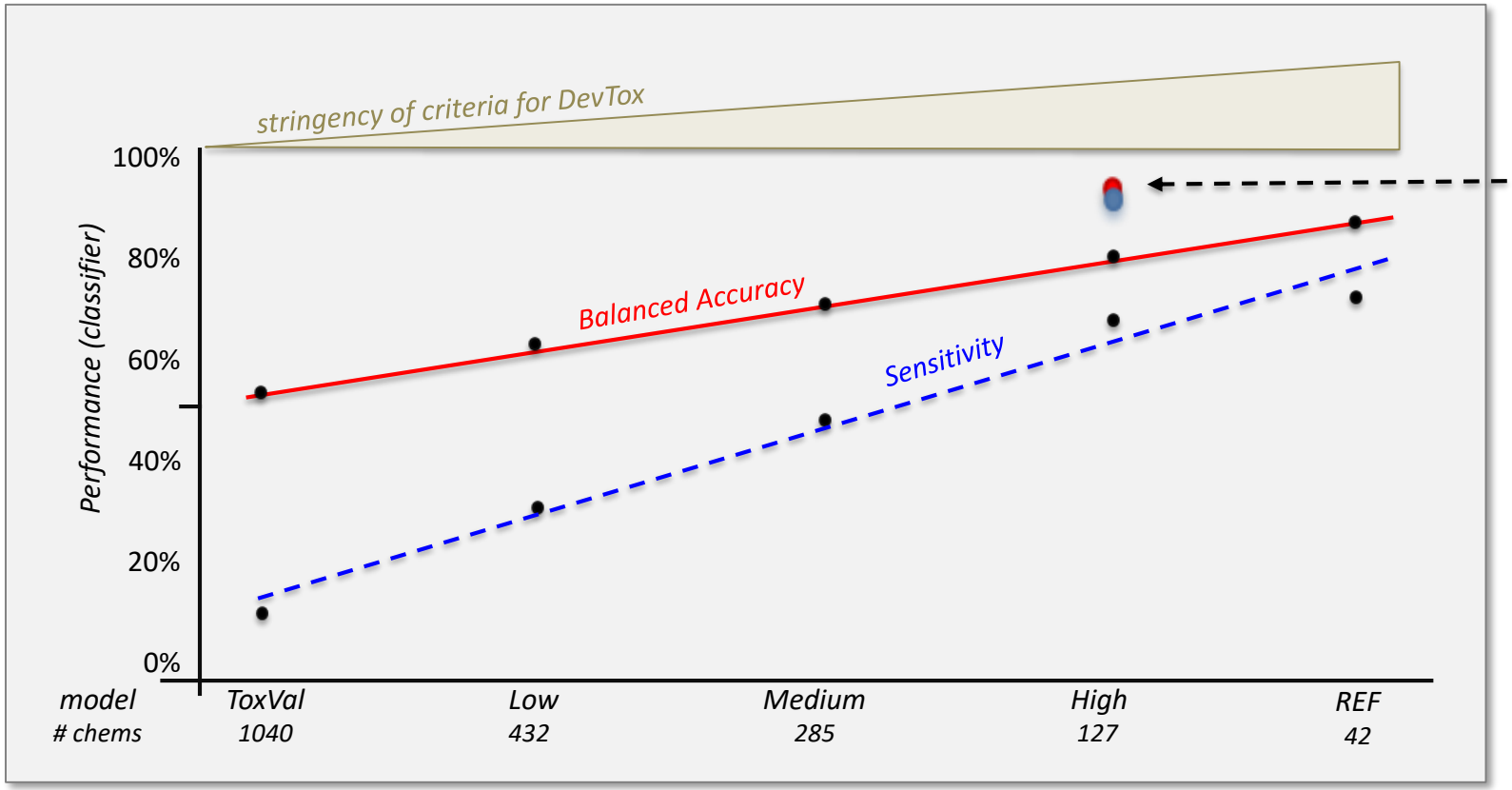




SOURCE: Zurlinden et al. (submitted)

Performance-based classifier

- **Key point:** ToxCast_STM does a pretty good job on its own classifying hazard potential when the animal model for human DevTox is strong, but sensitivity drops as criteria weaken.



BAC & sensitivity augmented with 43 ToxCast features selected by machine learning

Bring in the embryology to better understand and translate NAMs

SOURCE: Zurlinden et al. (submitted)

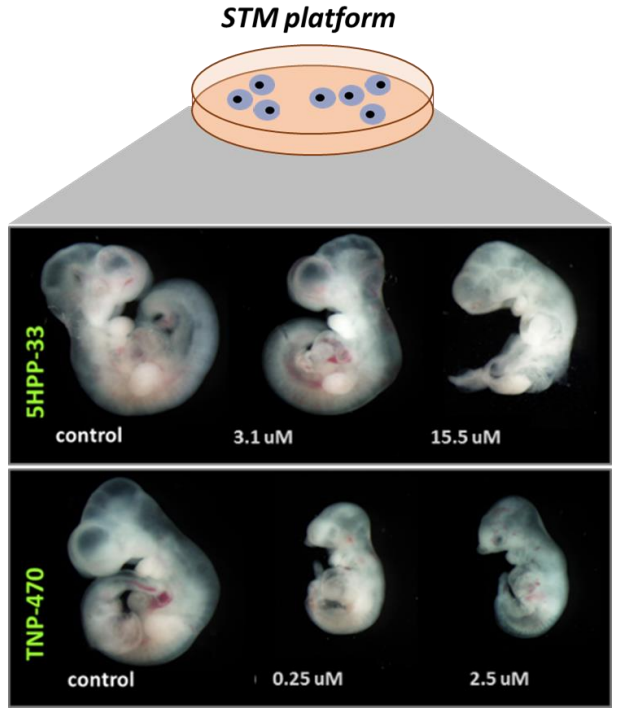
hESC (predicted) vs rat WEC (observed)

5HPP-33: *synthetic thalidomide analog*

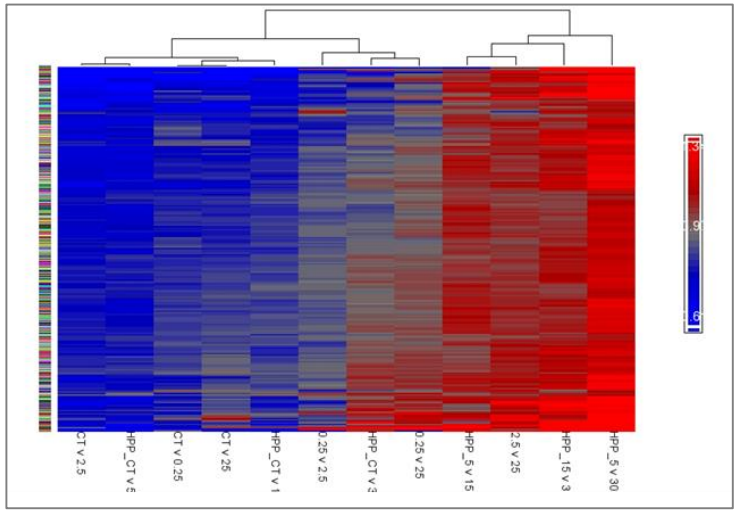
- T.I. predicted 9.5 µM
- AC50 observed 21.2 µM (embryo viability)

TNP-470: *synthetic fumagillin analog*

- T.I. predicted 0.01 µM
- AC50 observed 0.04 µM (dysmorphogenesis)



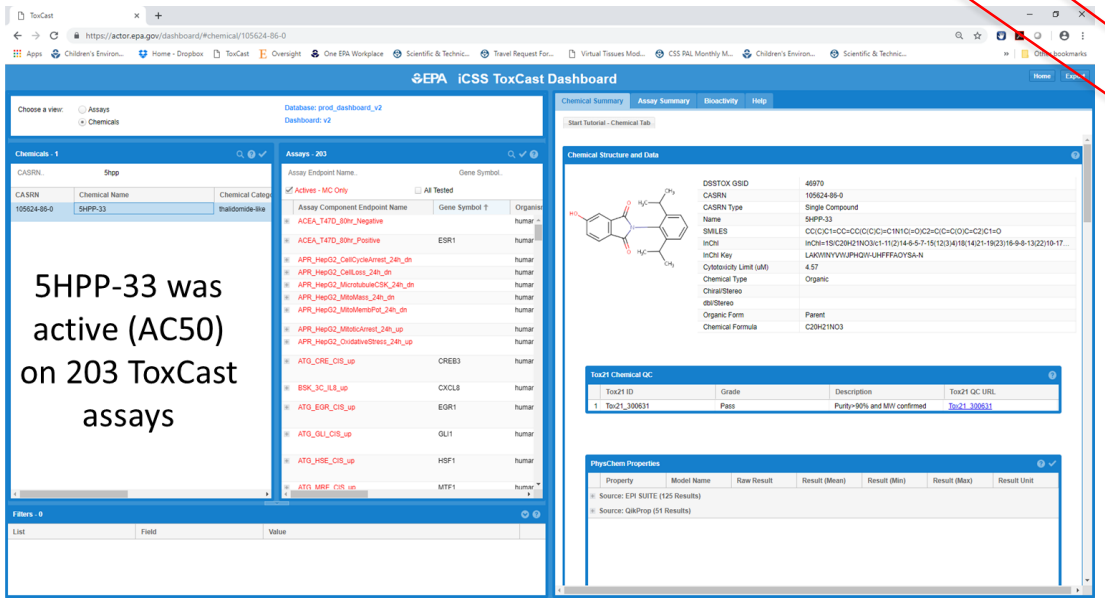
RNAseq: exposure-based potential for DevTox at 4h correlated with changes in common for pathways regulating splicesome-RNA metabolism and proteasome-ubiqutination.

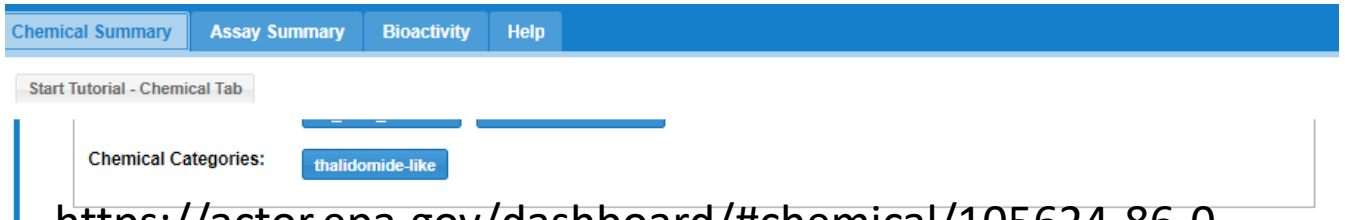


SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol; Siali et al. (2019) Curr Opin Toxicol

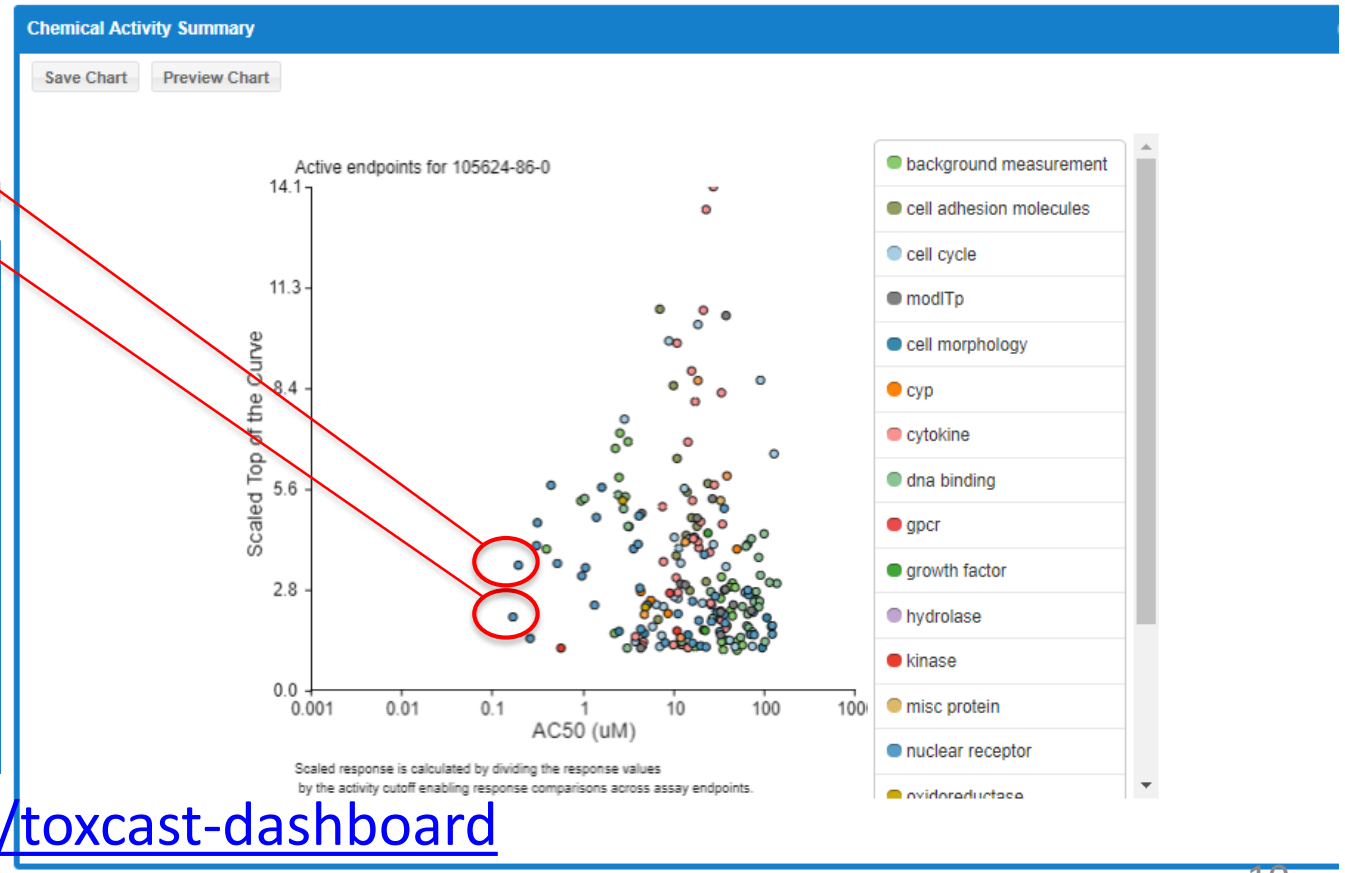
Potential MIE targets for 5HPP-33 in ToxCast

↑TOX21_ERa agonist
↑ATG_ERa transactivation





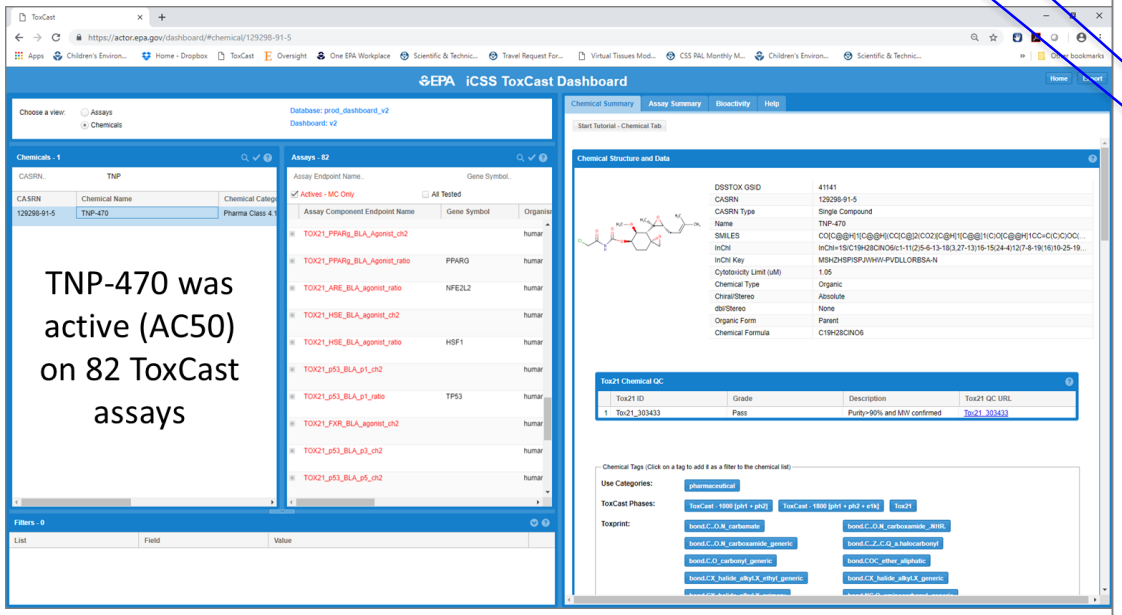
https://actor.epa.gov/dashboard/#chemical/105624-86-0



https://www.epa.gov/chemical-research/toxcast-dashboard

Potential MIE targets for TNP-470 in ToxCast

↓BSK_3C (coronary) proliferation
zebrafish yolk sac edema



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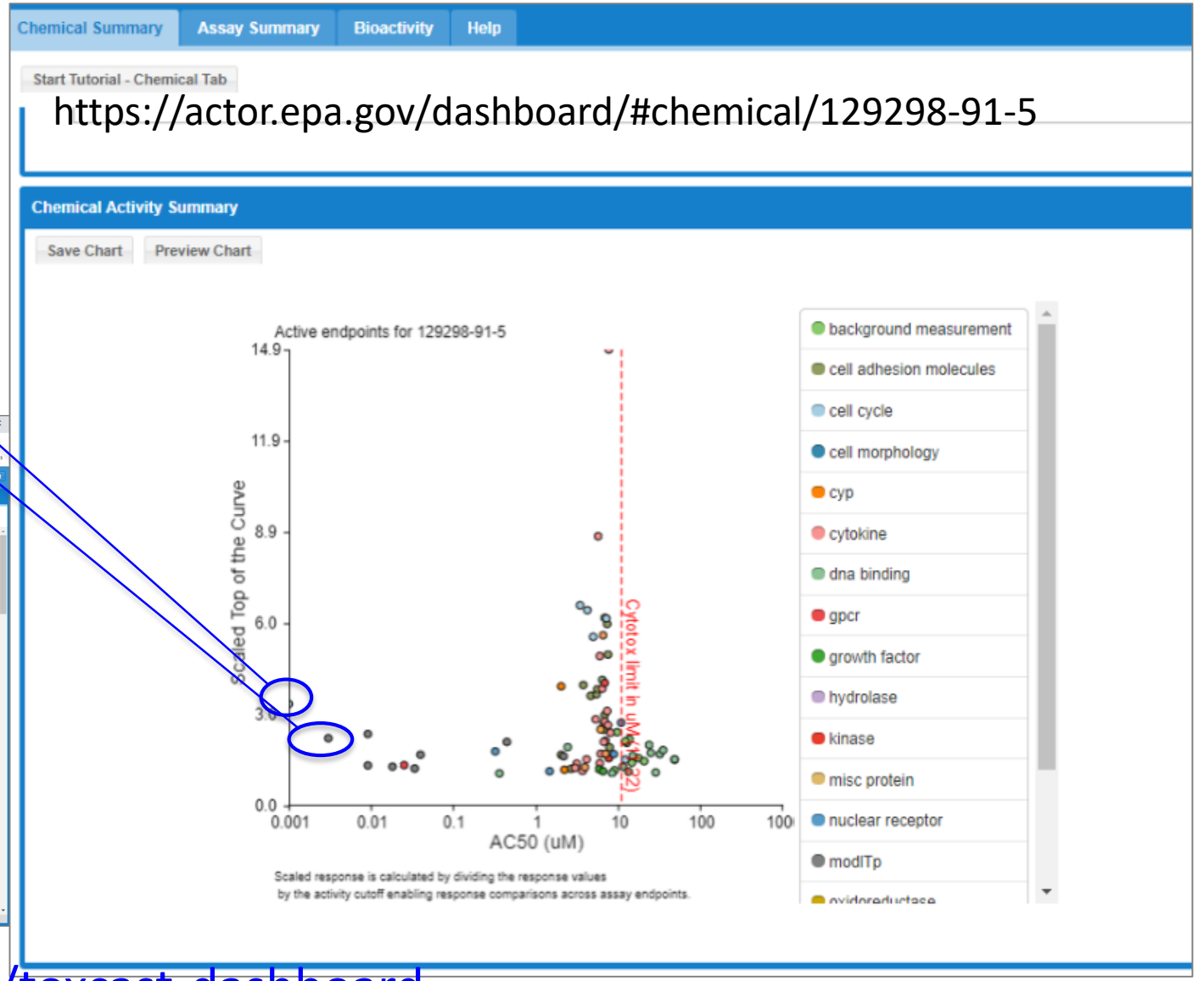


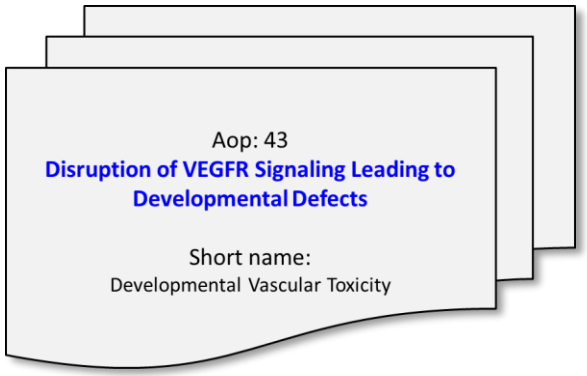
https://www.epa.gov/chemical-research/toxcast-dashboard

Vascular Development

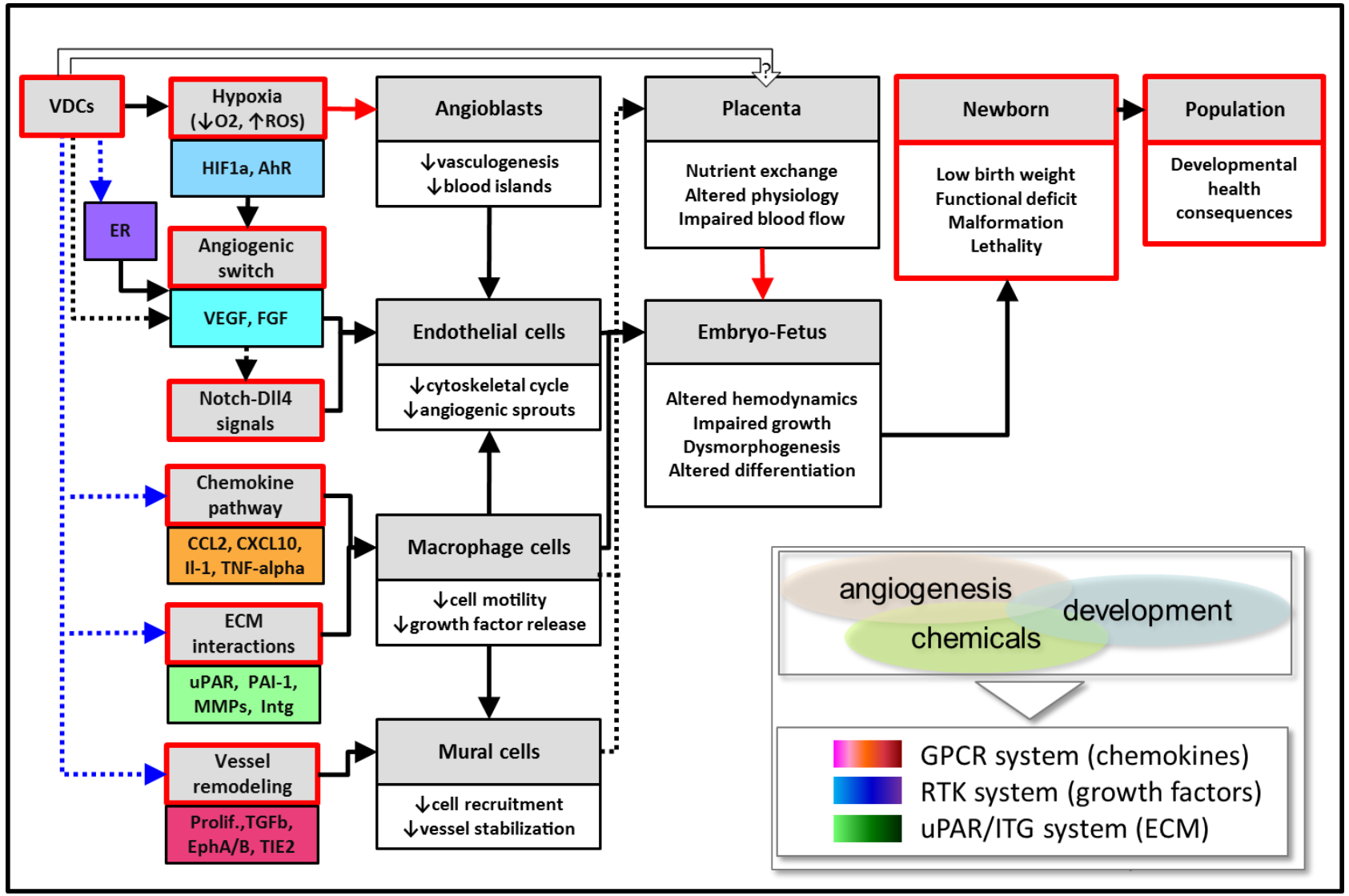
- Blood vessel formation is essential to embryogenesis (cardiovascular is first functioning organ system across *Vertebrate* species).

- Vascular insufficiency is tied to many disease processes (diabetes, preeclampsia, neonatal respiratory distress, osteoporosis, teratogenesis, …).

- Aop43: one of 28 AOPs included in the OECD work plan with status ‘open for citation & comment’ [https://aopwiki.org/aops/43].



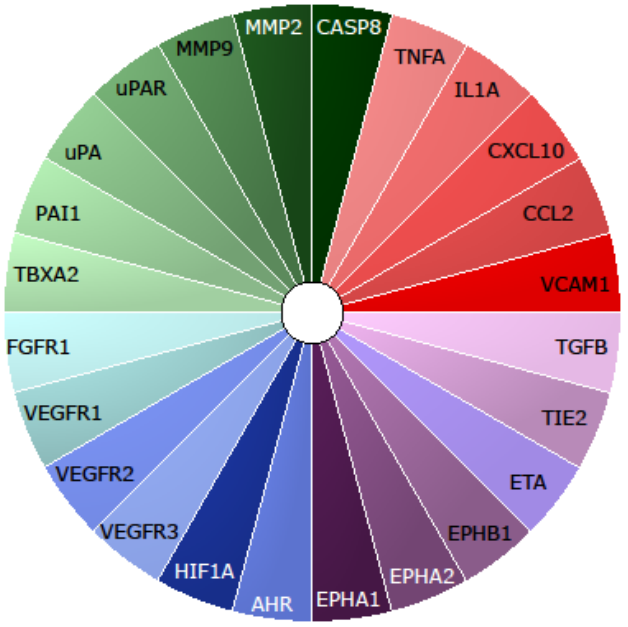
Aop43 framework: *developmental vascular toxicity (DVT)*



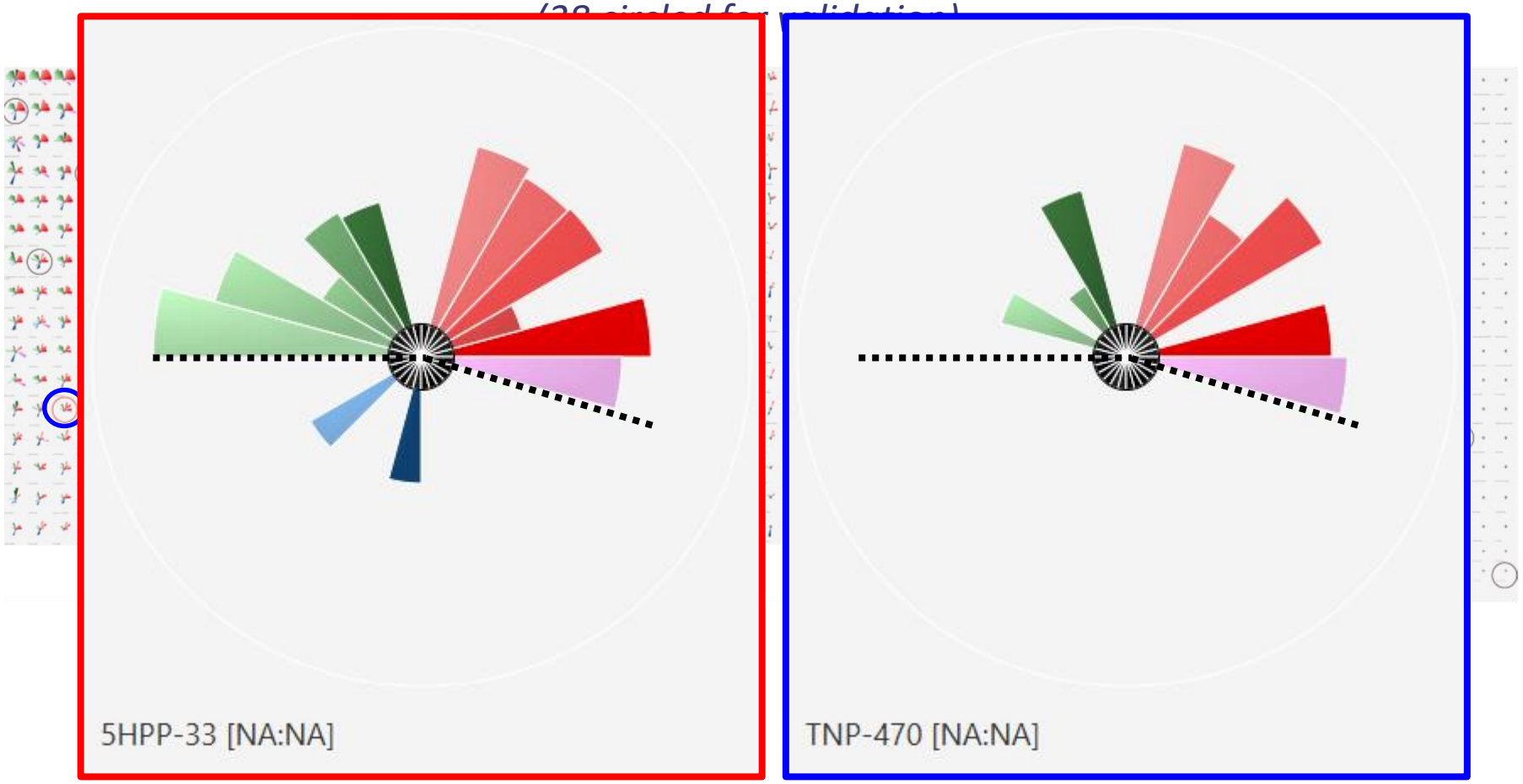
SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res

AOP-based ranking: *predicted vascular disrupting chemicals (pVDCs)*

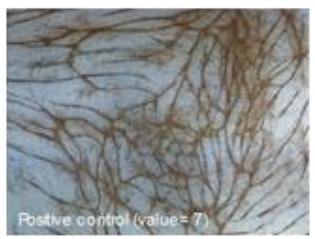
24 ToxCast target assays (pVDC ToxPi)

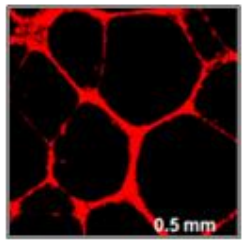


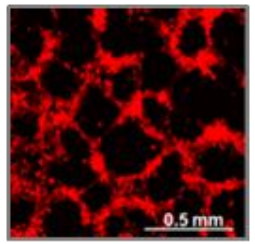
1058 ToxCast chemicals ranked by pVDC ToxPi (28 singled for validation)

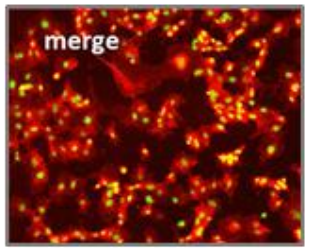


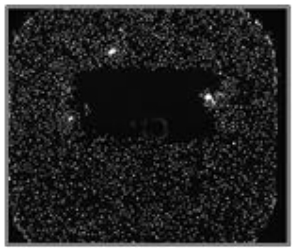
SOURCE: Saili et al. (submitted)

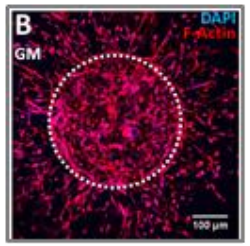


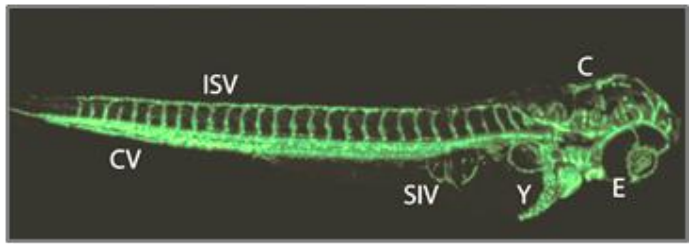




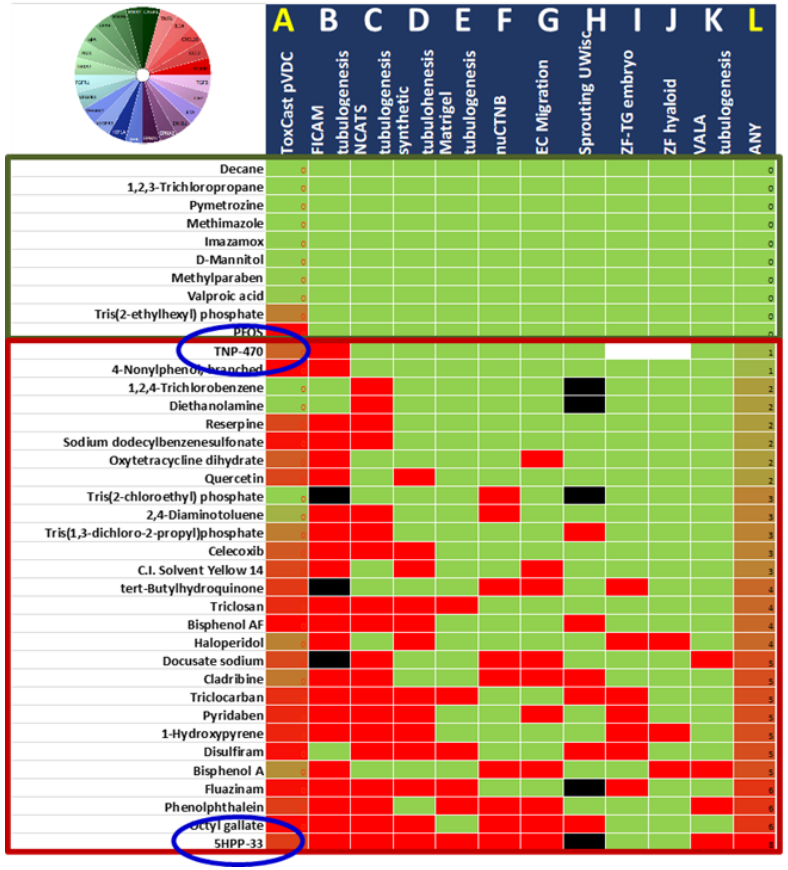








- inactive
- active
- cytotoxic
- no data

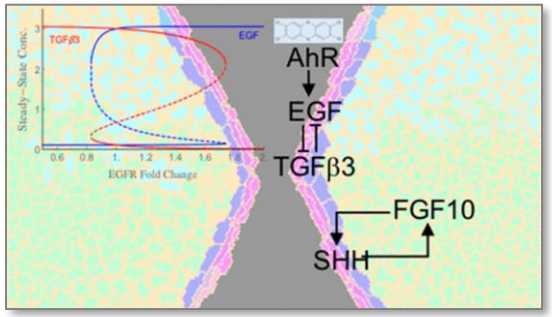


A pVDC ToxPi
B HUVEC tubulogenesis (FICAM)
C HUVEC tubulogenesis (NCATS)
D tubulogenesis in synthetic matrices (HMAPS)
E tubulogenesis in Matrigel (HMAPS)
F nuCTNB biomarker (VALA)
G endothelial cell migration (VALA)
H iPSC endothelial sprouting (HMAPS)
I ISV reporter zebrafish (NHEERL)
J reporter zebrafish (UDUBLIN)
K HUVEC tubulogenesis (VALA)
L ANY (B to K)

sensitivity 0.89, specificity 0.80
overall accuracy 87% (PPV 93%, NPV 73%)

SOURCE: Saili et al. (submitted)

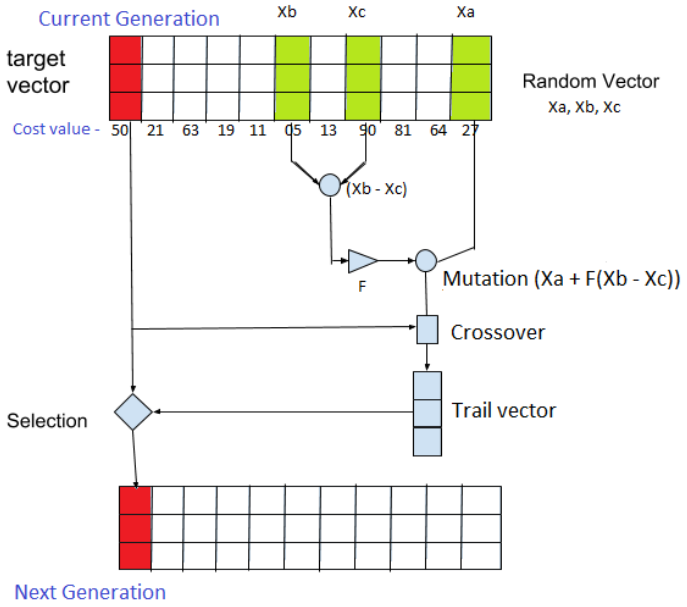
2. Translating cellular lesions into quantitative phenotypes

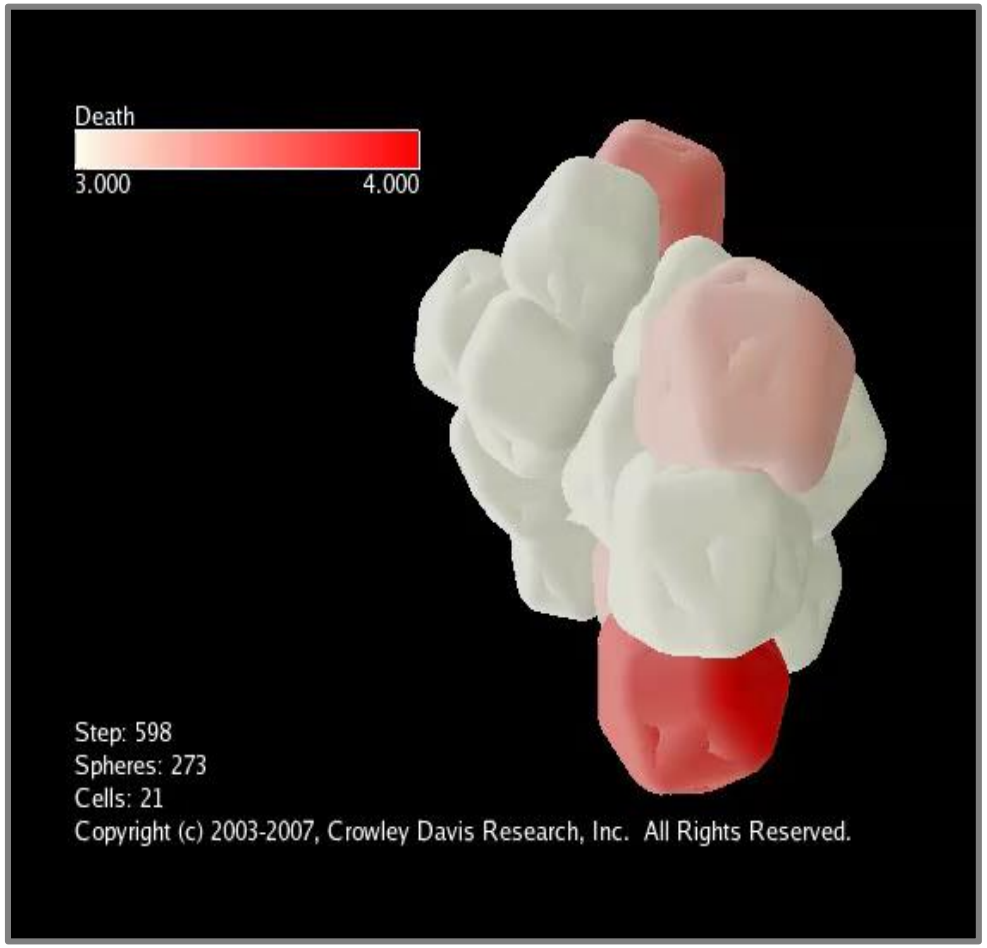


Objective: build and test computer models of complex tissues that advance critical phenomena (specificity, canalization, plasticity) in quantitative prediction for virtual screening and *in silico* testing.

Anatomical homeostasis in a self-regulating ‘Virtual Embryo’

- EA for self-regulation (fitness measure) - simulation executes randomly paired agents (parent cells) that generate daughter cells mutated in their rules.
- You only need to specify the goal of the computation; EA searches rule-space using ‘survival of the fittest’ (good solutions propagate, poor solutions discarded).

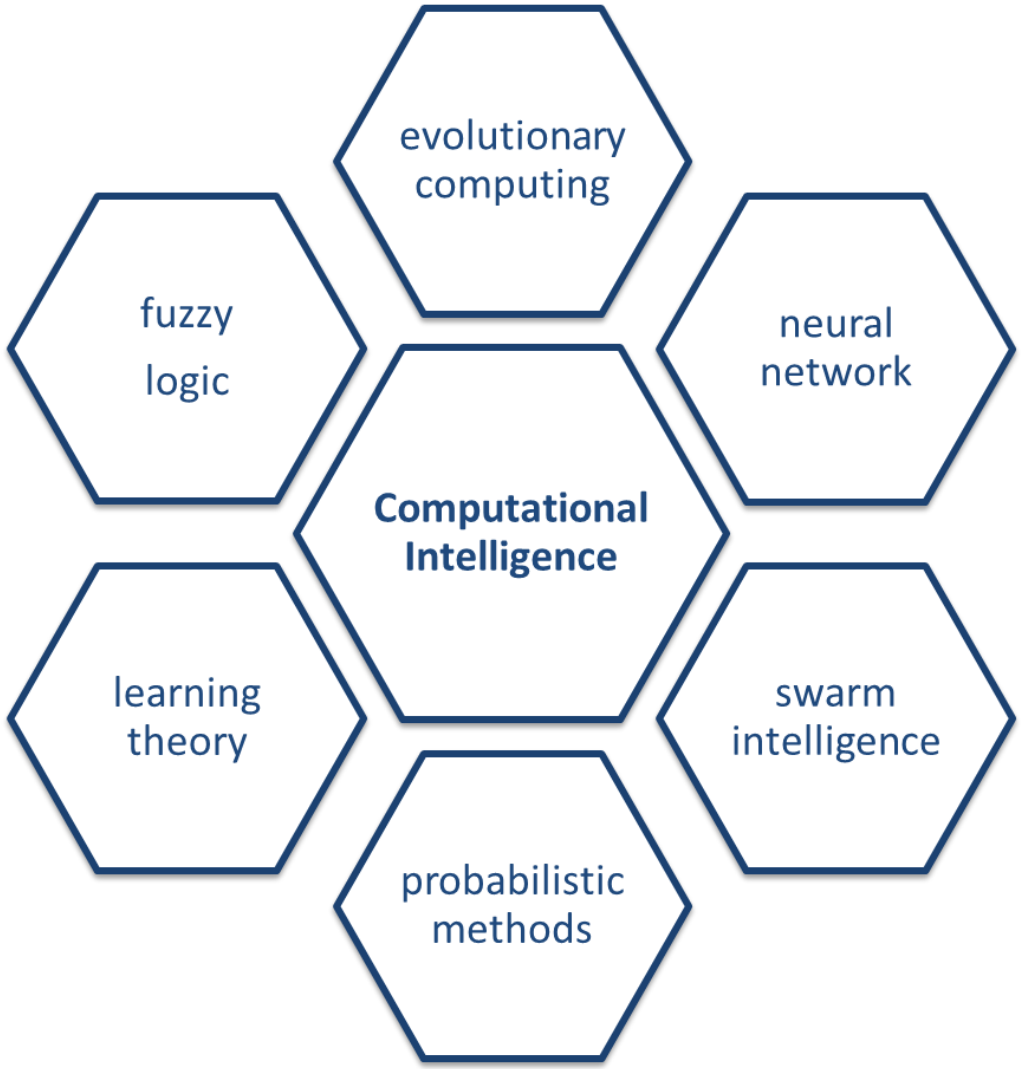




SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Computational intelligence (C.I.)

- Deep learning algorithms can be used for data-driven predictions.
- Different kinds of AI models will be needed for predictive toxicology.
- Soft-computing [‘smart models’] can fill in for missing or inexact data.

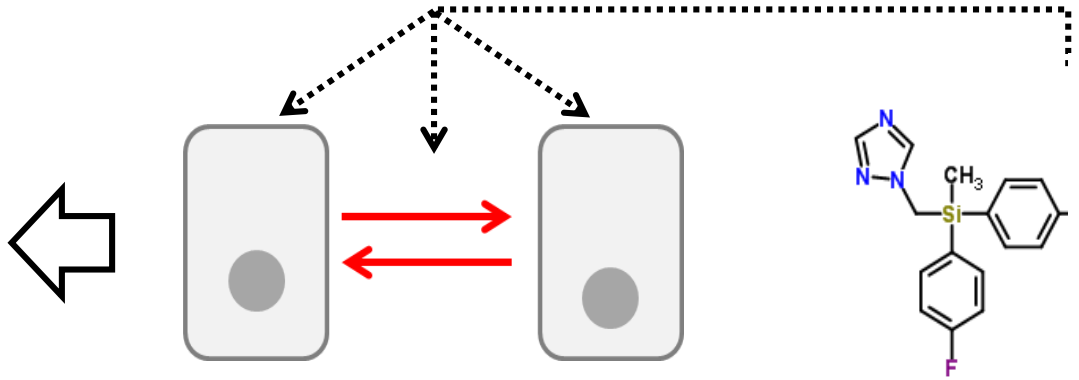


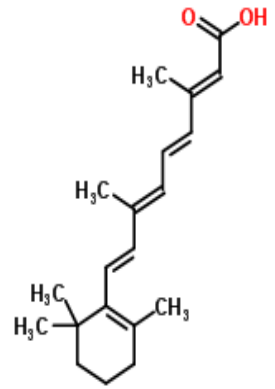
Organizing principles

1. In patterning the embryo, genetic signals setup spatial information that cells then translate into a coordinated biological response.

2. A hallmark of multicellular organization is the ability of cells to interact with one another via well-conserved signaling pathways.

3. Just as ‘the Cell’ is the fundamental unit of biology, so too should it be the computational unit (‘Agent’) for modeling embryogenesis.

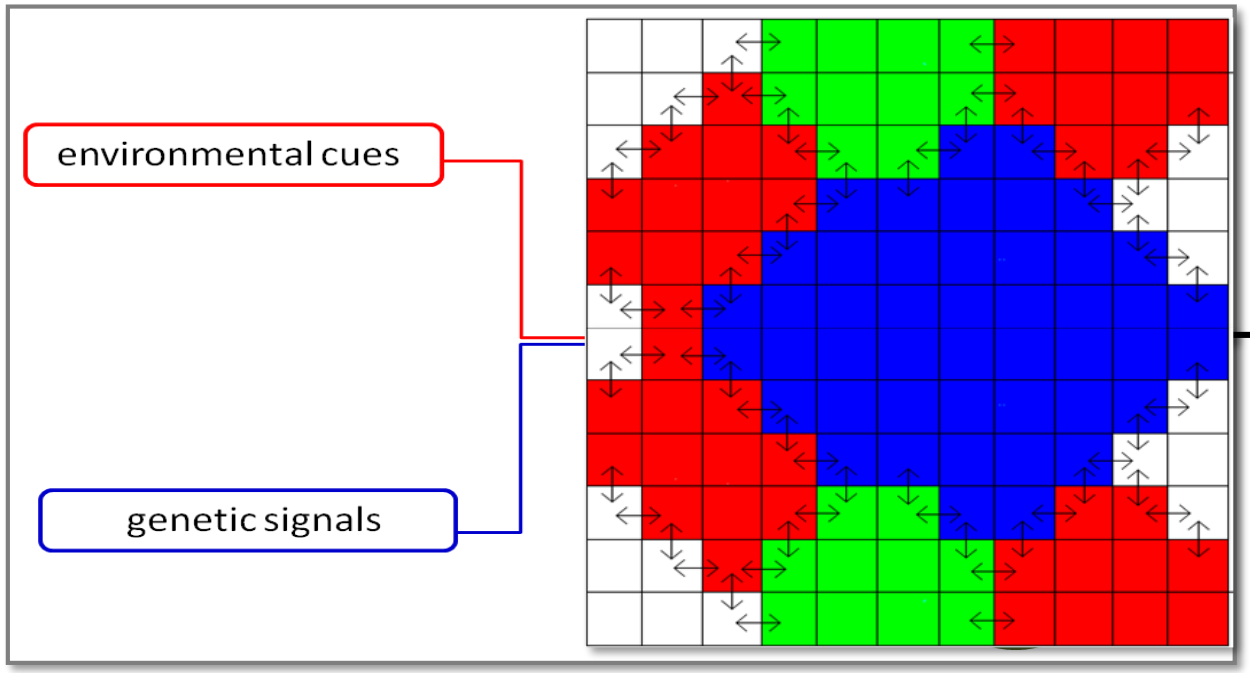




Morphogenesis is fundamentally complex

computational

Cell = biological unit (agent)



Capturing this mathematically:

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

Cellular behaviors

Core Developmental Processes

Patterning (Sets up Future Events)
Timing (Clocks and Oscillators)
Differentiation (Cell Diversification)
Morphogenesis (Tissue Organization)

Cellular Primitives

Growth (Proliferation)
Growth (Volume Increase)
Death (Apoptosis)
Differentiation (Function)
Adhesion (Differential Hypothesis)
Shape (Geometry)
Motility (Cell Migration)
Extra Cellular Matrix (Remodeling)

Morphogenetic Movement

Folding
Epiboly
Convergent Extension
Branching Morphogenesis
Cell Condensation
Cell Sorting
Trans-Differentiation
Cavitation
Involution/Invagination
Tractional Forces

Directed Cell Movement

Contact Guidance (Boundaries)
Haptotaxis (ECM Tracks)
Chemotaxis (Chemical Signals)

Agent-Based Models (ABMs)

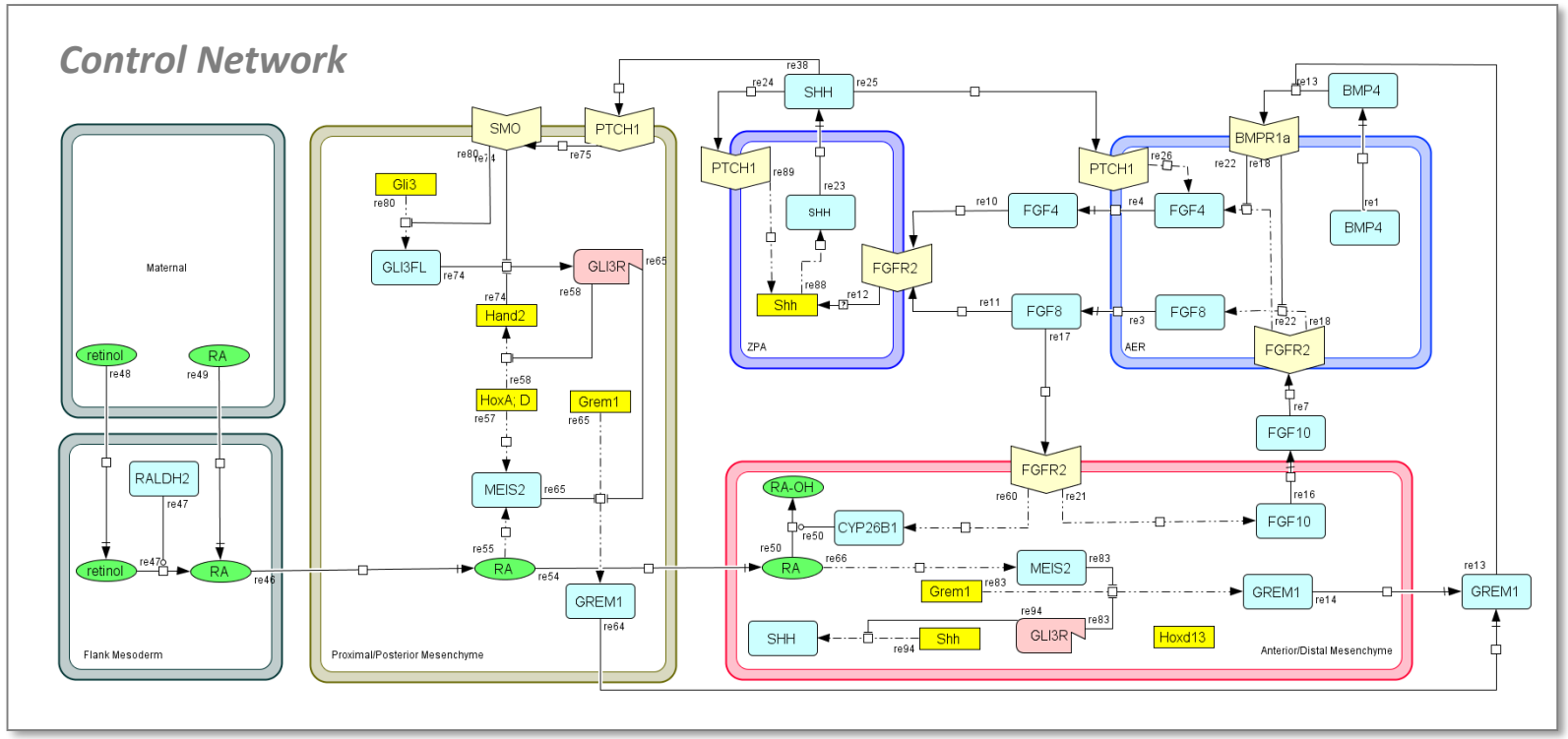
- nature-inspired *agents* (cells) and *rules* (behaviors) are set into motion as a self-organizing virtual system, using an open-source modeling environment (CompuCell3d.org).

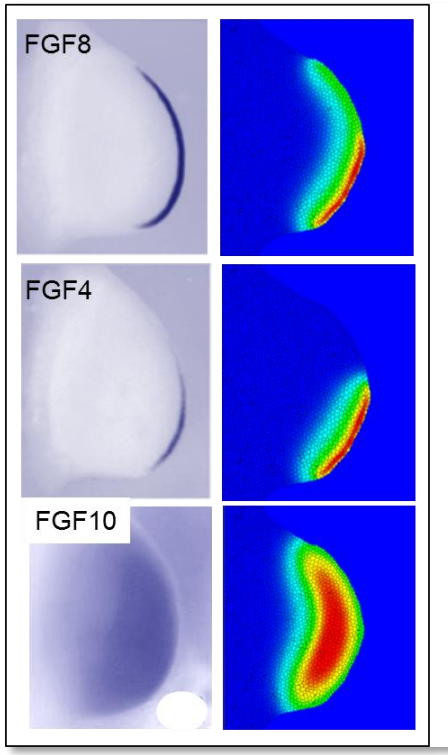
- soft-computing uses ‘fuzzy logic’ to simulate forces or properties governing cell fate and behavior where rules are inexact or knowledge incomplete (computational intelligence).

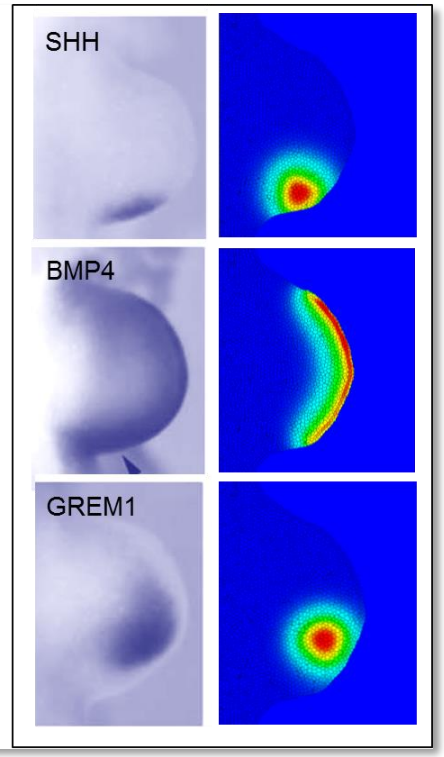
- can change course in response to a particular situation or stimulus, such as genetic errors or biomolecular lesions introduced from real world data (dynamic translation).

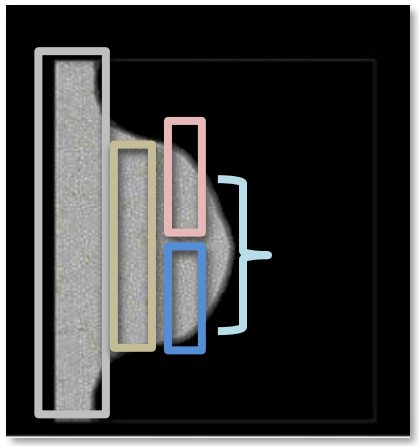
- probabilistic rendering of where, when and how a particular condition might lead to an adverse developmental outcome (cybermorphs).

Translating genetic control circuits into phenotypes with C.I.

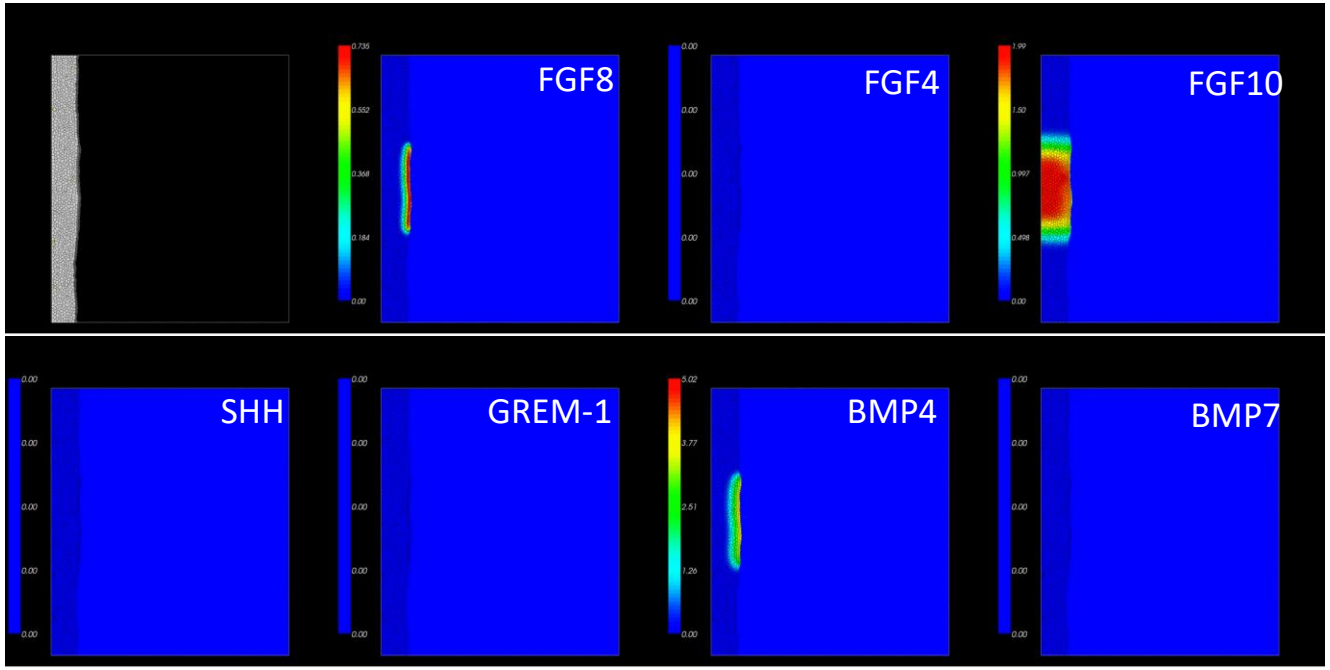








- biological wiring diagram maps cell-cell signaling
- we code the signal-response for individual cell types
- and enable ‘steppables’ of individual cell behavior in CompuCell3d.org
- executing the simulation triggers signal-response behaviors
- can quantitatively monitor emergent properties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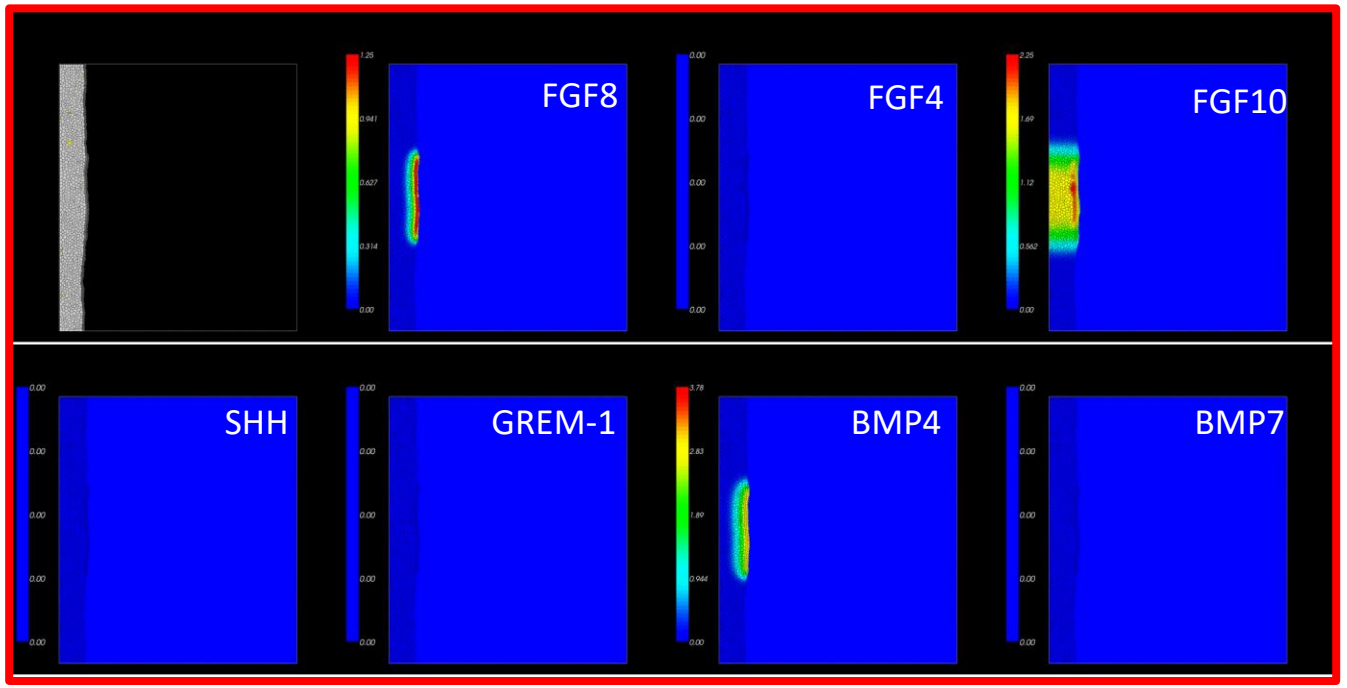
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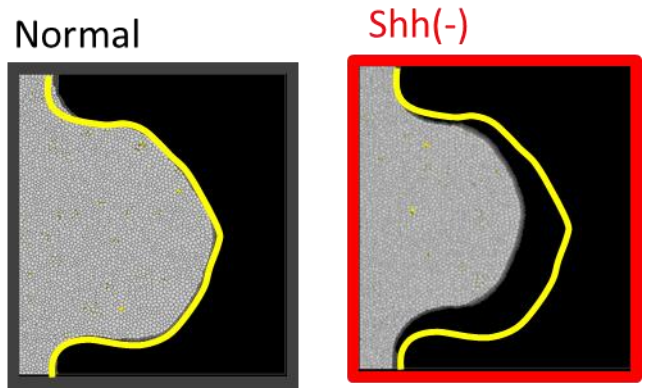
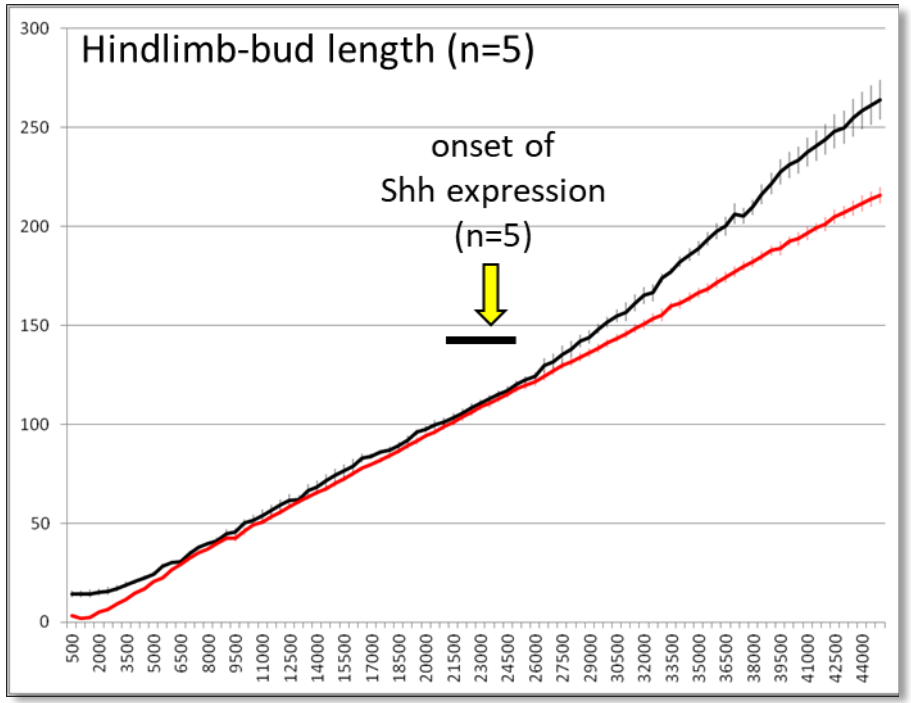
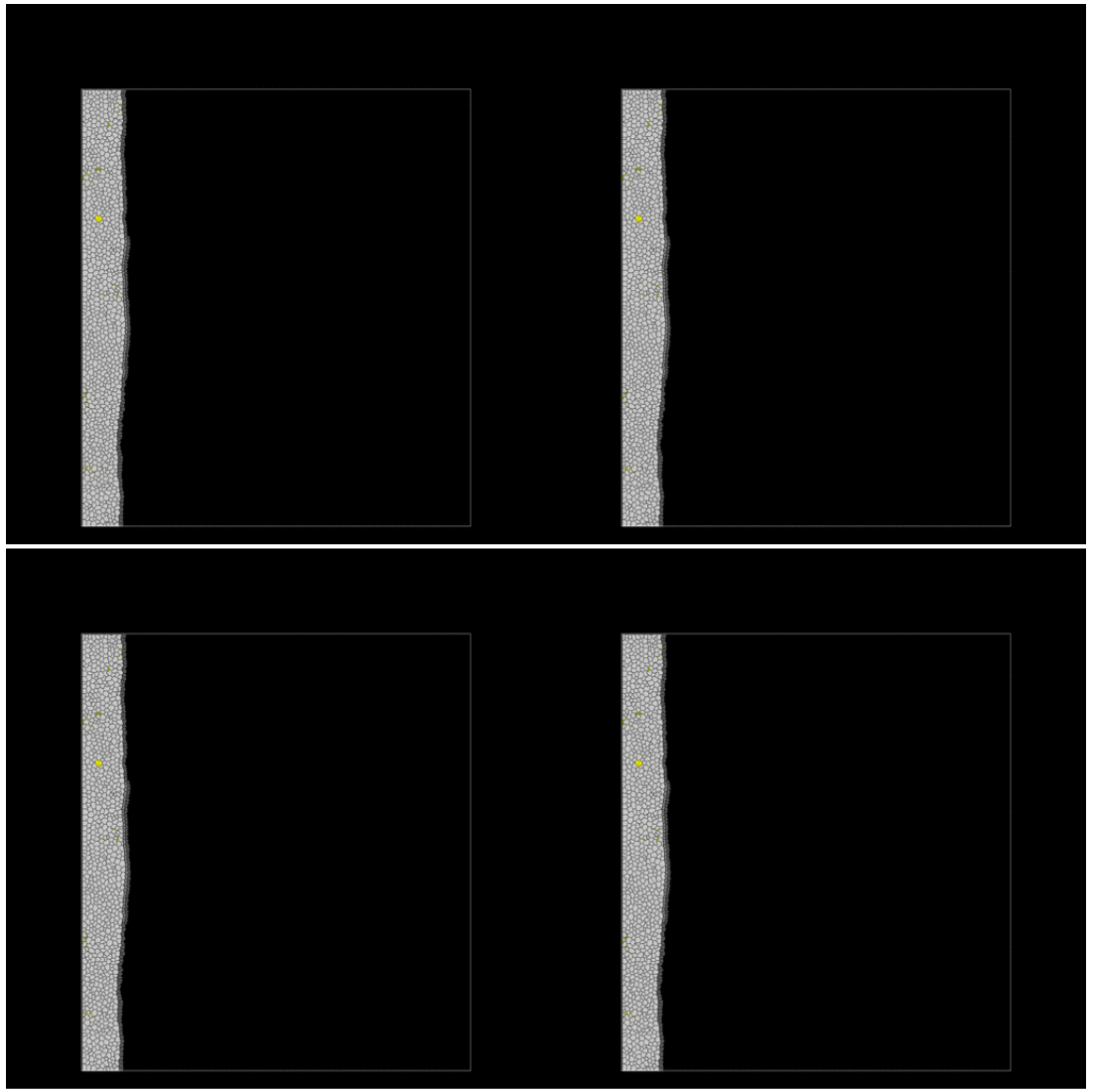

Normal
Shh(-)


Hindlimb-bud length (n=5)
onset of
Shh expression
(n=5)
300
250
200
150
100
50
0
500
2000
3500
5000
6500
8000
9500
11000
12500
14000
15500
17000
18500
20000
21500
23000
24500
26000
27500
29000
30500
32000
33500
35000
36500
38000
39500
41000
42500
44000

Introducing cellular lesions into the swarm …

- SI addresses collective behavior of a complex self-organizing system emerging from local interactions.
- Agents work together in closed-loop systems (e.g., flocks, schools, colonies, swarms) → phenotype.
- Subtle details in the simulation can greatly influence the outcome (checkpoints?).



PLOS | COMPUTATIONAL BIOLOGY

REVIEW

Agent-based modeling of morphogenetic systems: Advantages and challenges

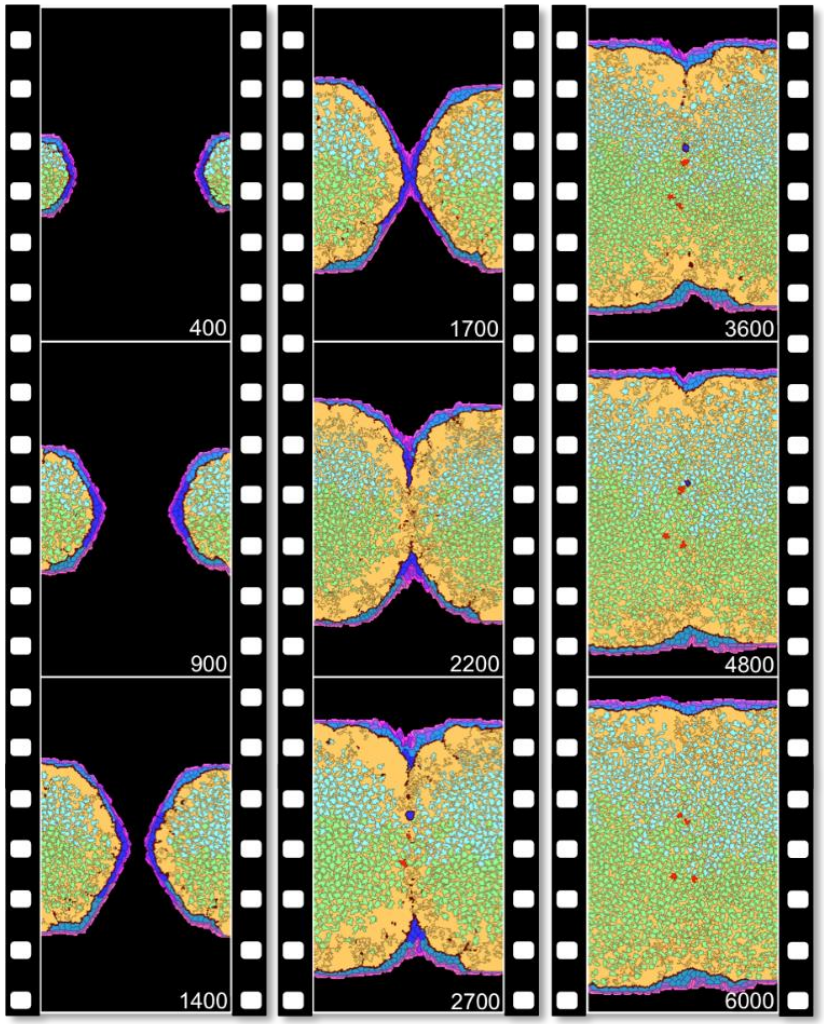
Chad M. Glen, Melissa L. Kemp*, Eberhard O. Voit*

The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, United States of America

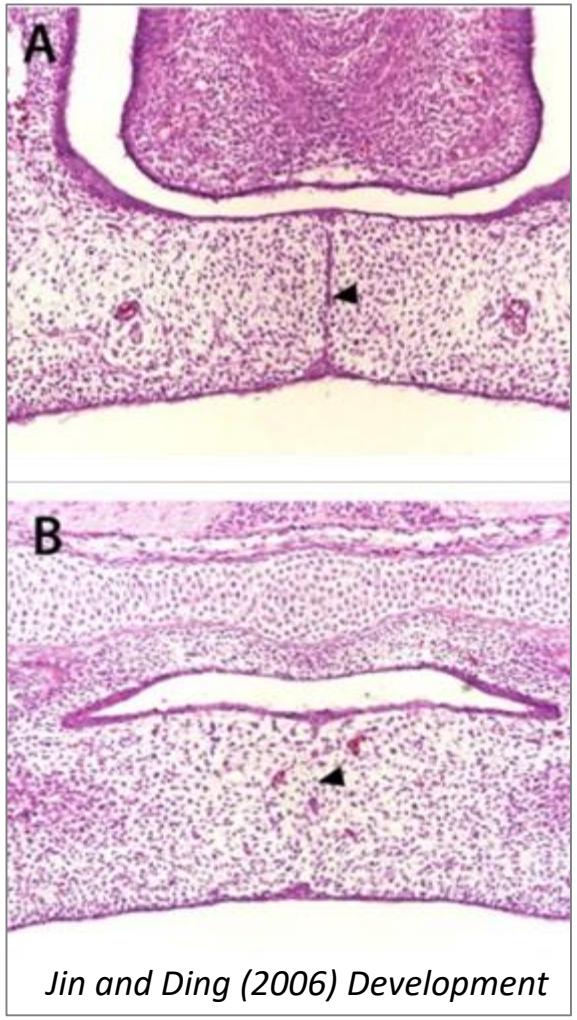
"Agent-based models (ABMs) … display unparalleled flexibility for studying various morphogenetic phenomena at multiple levels …"
PLoS Comp Biol, 2019

Morphogenetic fusion (palate)

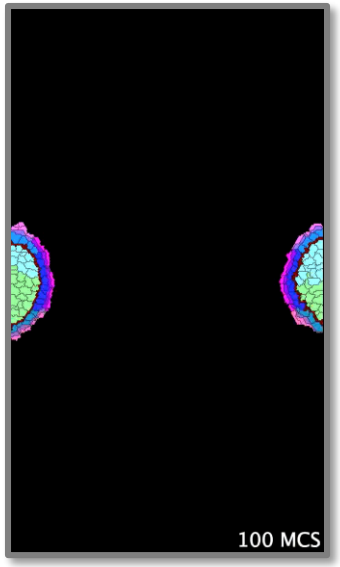
in silico

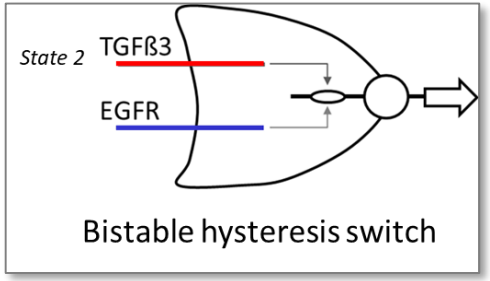


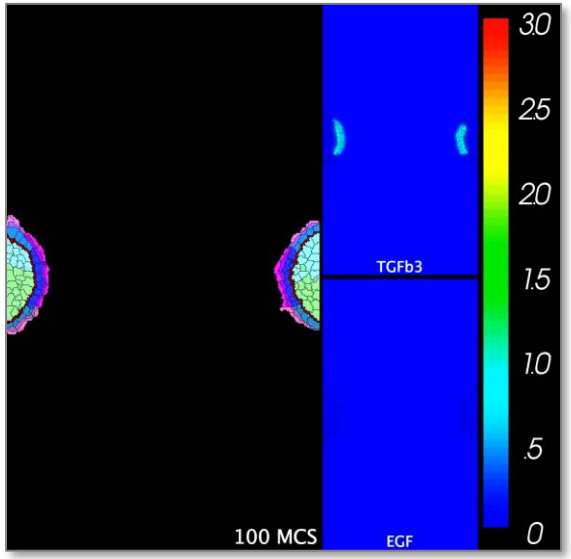
in vivo



Jin and Ding (2006) Development







SOURCE: Hutson et al. (2017) Chem Res Toxicol

Smart model …

Chemical Research in Toxicology

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Article

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Computational Model of Secondary Palate Fusion and Disruption

M. Shane Hutson,*[†,‡] Maxwell C. K. Leung,[‡] Nancy C. Baker,[§] Richard M. Spencer,[§] and Thomas B. Knudsen*[,∥]

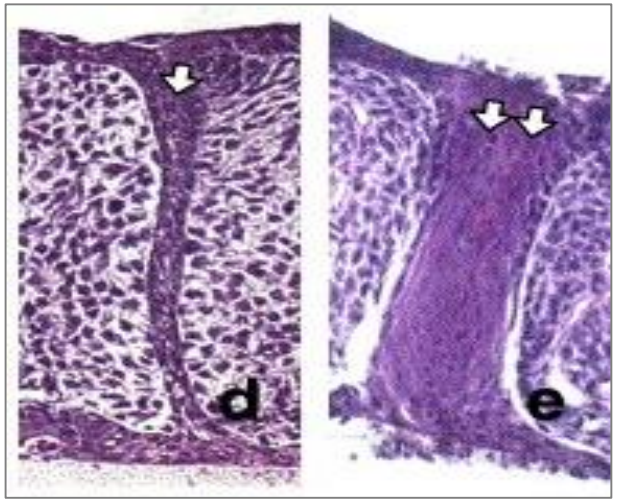
[†]Department of Physics & Astronomy, Department of Biological Sciences and Vanderbilt Institute for Integrative Biosystem Research & Education, Vanderbilt University, Nashville, Tennessee 37235, United States
[‡]Oak Ridge Institute for Science & Education, Oak Ridge, Tennessee 37832, United States
[§]Leidos, Research Triangle Park, Durham, North Carolina 27711 United States
[∥]National Center for Computational Toxicology, Office of Research & Development, U.S. Environmental Protection Agency, Research Triangle Park, Durham, North Carolina 27711, United States

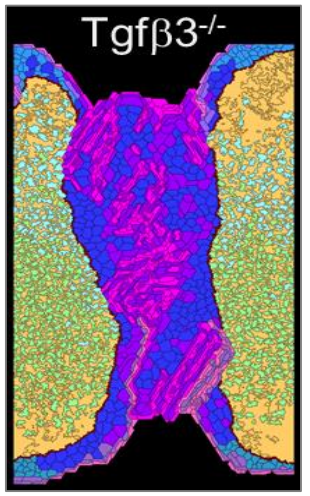
Supporting Information

ABSTRACT: Morphogenetic events are driven by cell-generated physical forces and complex cellular dynamics. To improve our capacity to predict developmental effects from chemical-induced cellular alterations, we built a multicellular agent-based model in CompuCell3D that recapitulates the cellular networks and collective cell behavior underlying growth and fusion of the mammalian secondary palate. The model incorporated multiple signaling pathways (TGFβ, BMP, FGF, EGF, and SHH) in a biological framework to recapitulate morphogenetic events from palatal outgrowth through midline fusion. It effectively simulated higher-level phenotypes (e.g., midline contact, medial edge seam (MES) breakdown, mesenchymal confluence, and fusion defects) in response to genetic or environmental perturbations. Perturbation analysis of various control features revealed model functionality with respect to cell signaling systems and feedback loops for growth and fusion, diverse individual cell behaviors and collective cellular behavior leading to physical contact and midline fusion, and quantitative analysis of the TGF/EGF switch that controls MES breakdown—a key event in morphogenetic fusion. The virtual palate model was then executed with theoretical chemical perturbation scenarios to simulate switch behavior leading to a disruption of fusion following chronic (e.g., dioxin) and acute (e.g., retinoic acid) chemical exposures. This computer model adds to similar systems models toward an integrative "virtual embryo" for simulation and quantitative prediction of adverse

Reviewer Comment: *"Crucial mechanisms occurring during palate fusion, especially opposing palatal shelf adhesion, are not considered in the model. In fact, the main reason why Tgf-b3 KO mice have cleft palate is a failure of opposing MEE adhesion, leading to separation of palatal shelves after their initial contact. Even in those strains in which palatal shelves adhere partially, I have never seen a MES as the one shown in Fig. 5."*

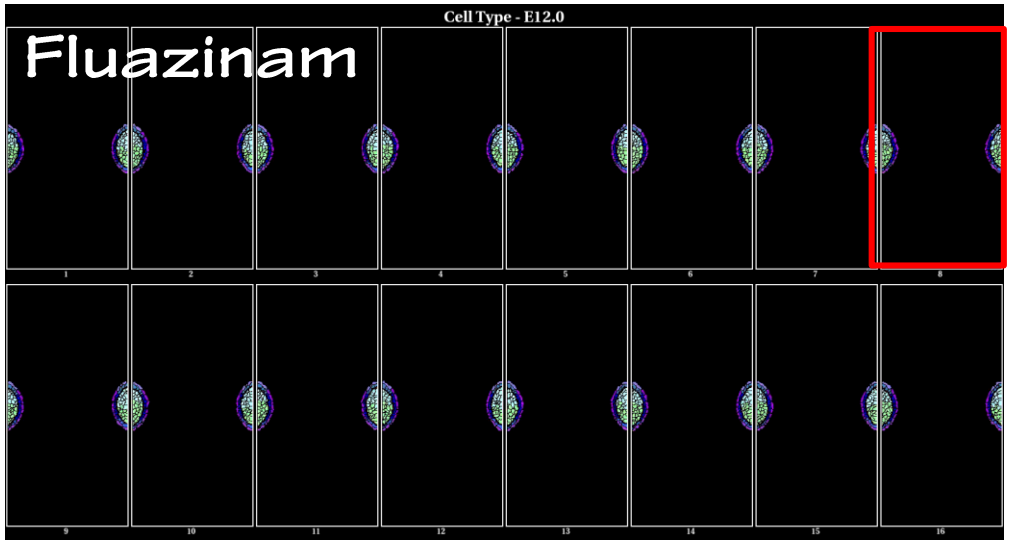
Our Response: TGF-b3 knockout mouse palates transduced with ALK vectors *in vitro. (from Dudas et al. 2004).*

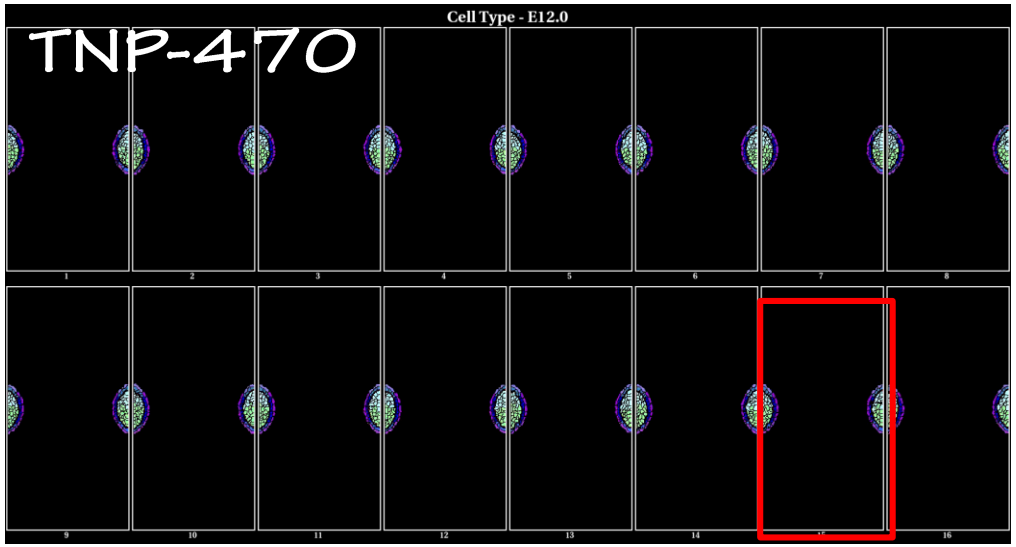


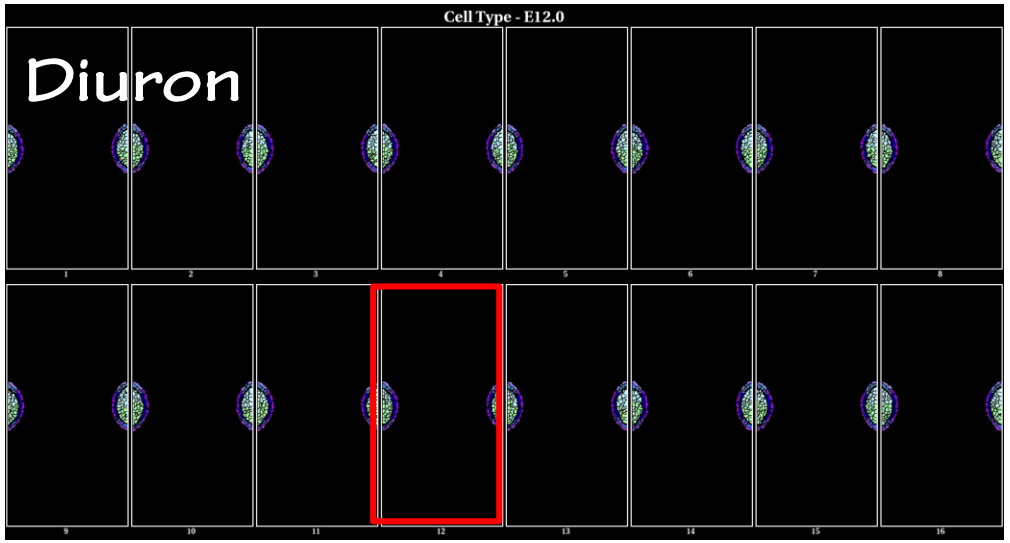


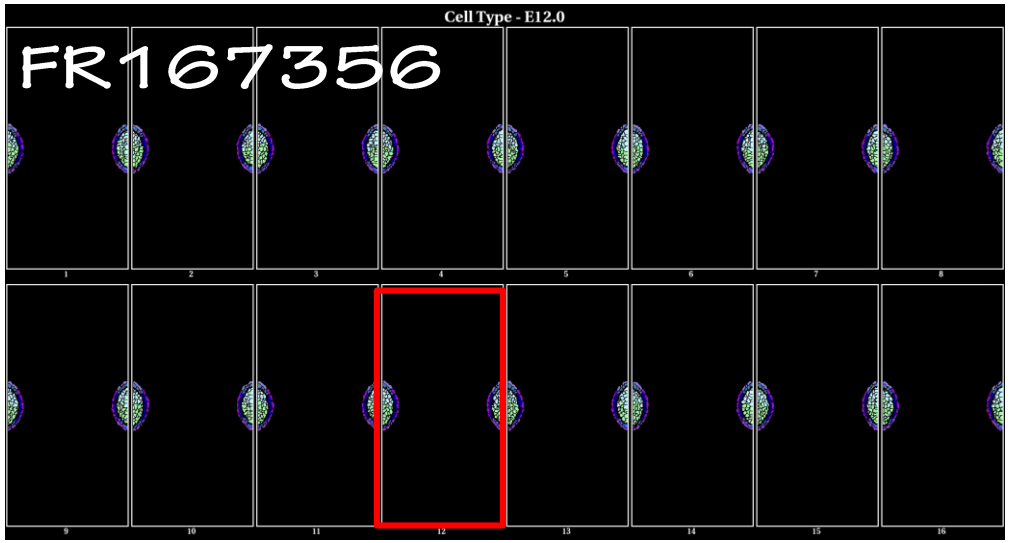
SOURCE: Hutson et al. (2017) Chem Res Toxicol

In silico dose-response: *translating ↑EGF/TGFb in vitro profile into a critical effect*



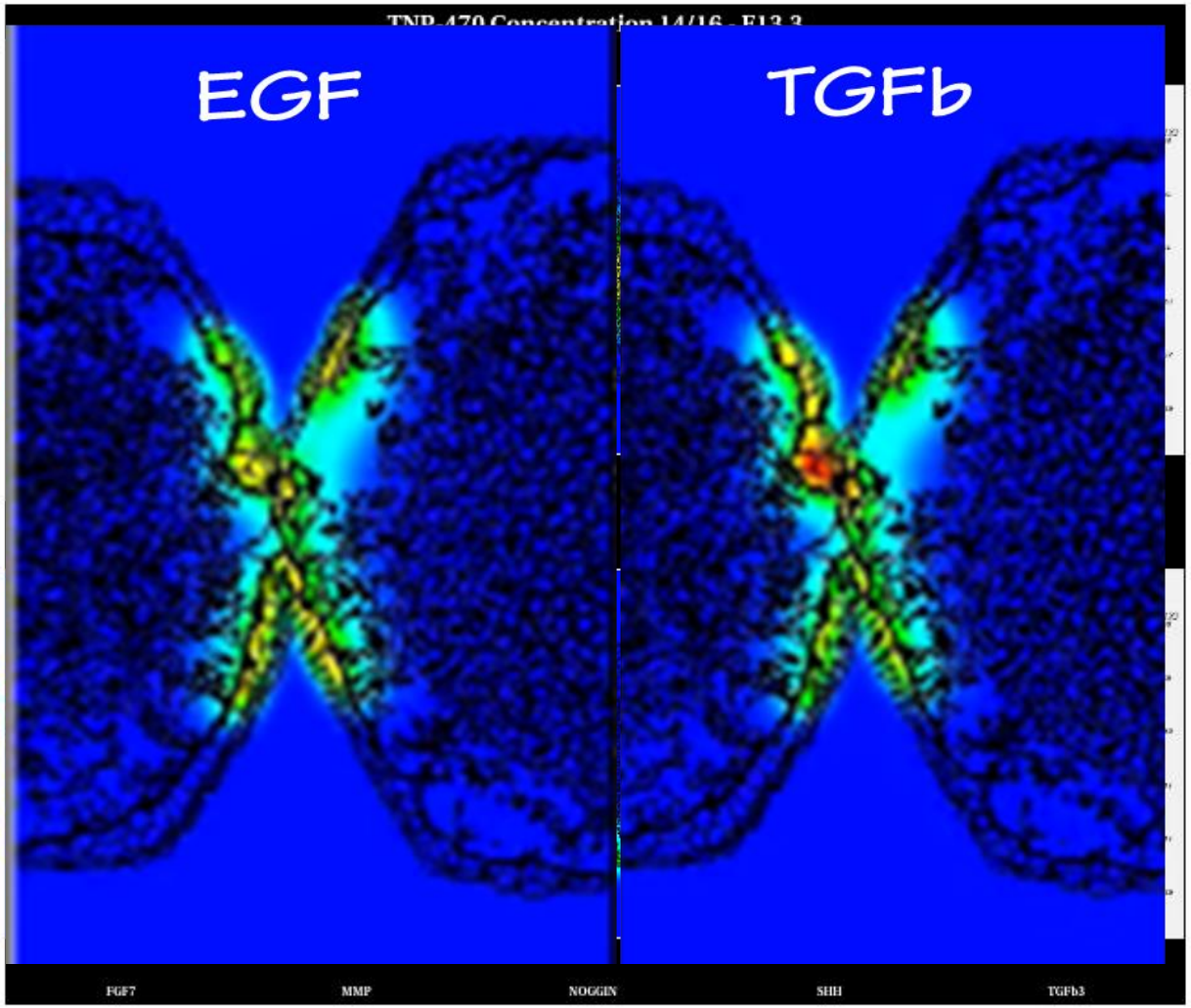




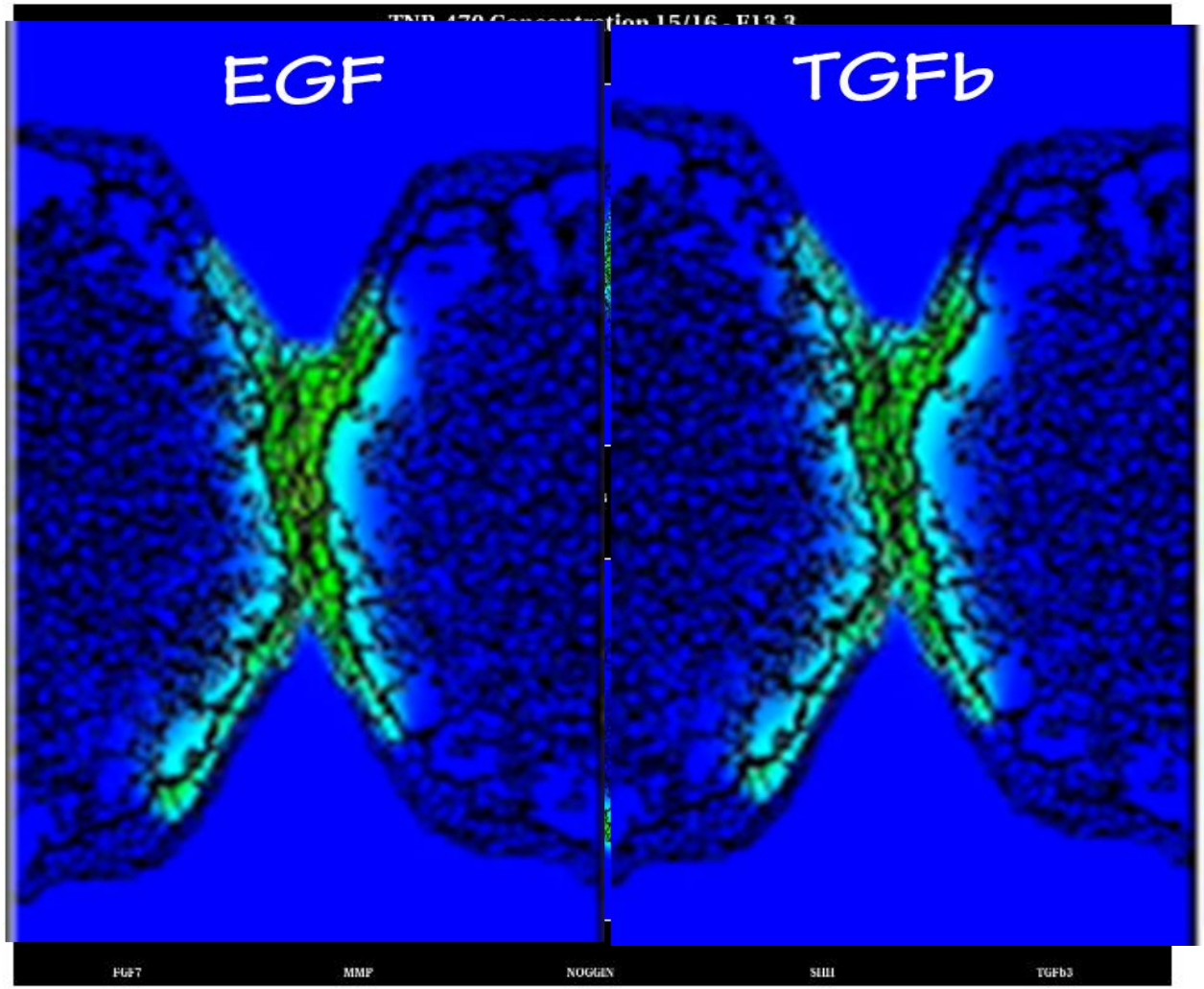


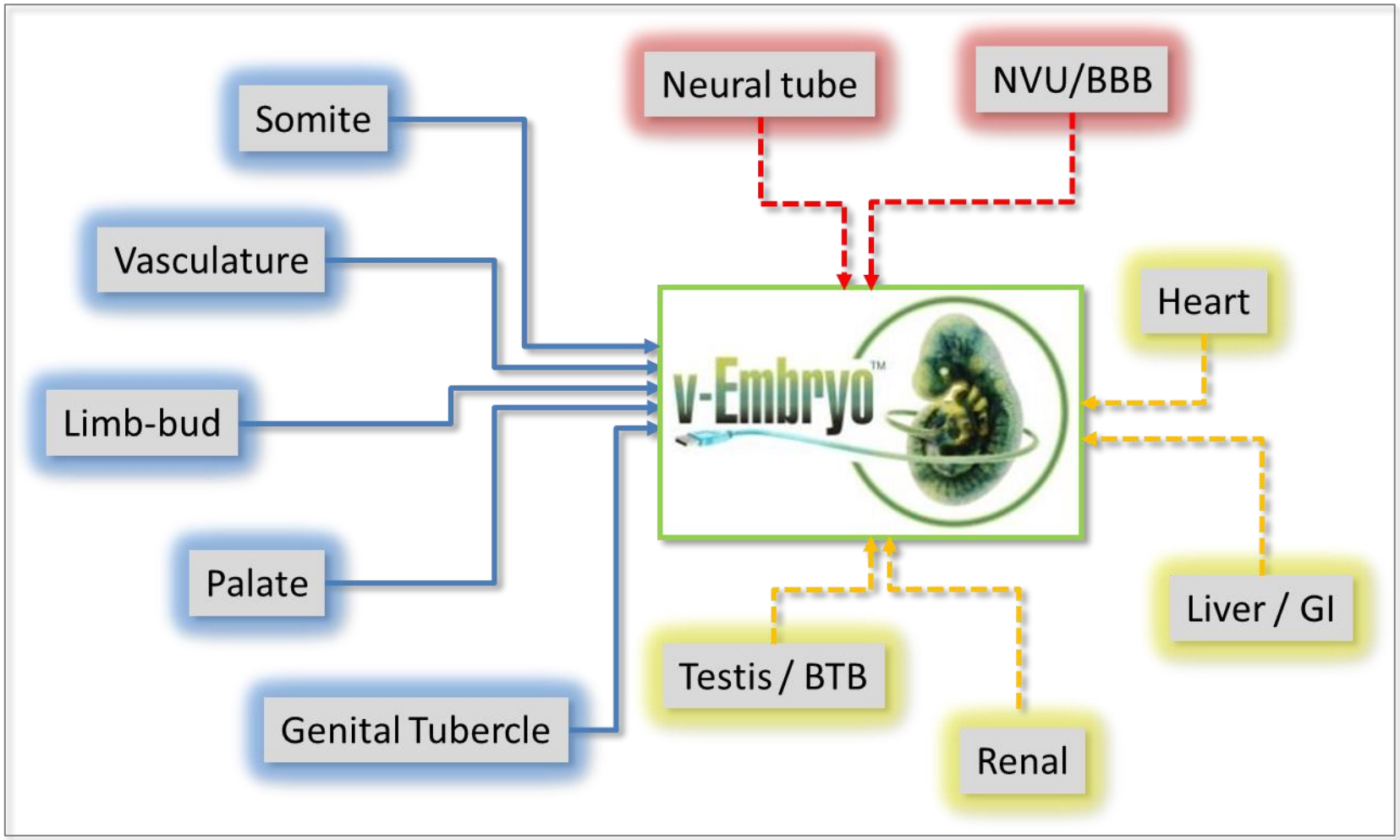
Pathogenesis: *simulating critical alterations in tissue function (eg, TNP-470)*

pre-critical dose



post-critical dose

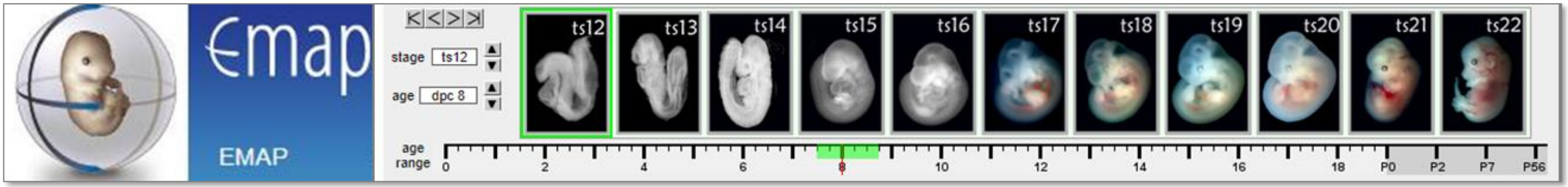




VTLS

- *access to models & simulations*
- *VT-KB (knowledgebase)*
- *Literature mining*
- *tied to ToxCastDB*
- *high-performance computing*

vtls.epa.gov/



https://www.emouseatlas.org/emap/ema/home.php

Predictive DART

- Computer models that integrate high-dimensional data with current knowledge of embryology provide new ways to understand and predict developmental toxicity.
- Agent-based models (ABMs) have surfaced as a core technology conceptualized in a ‘virtual embryo’ with computational artificial intelligence for *in silico* DART.
- These models convey specific lesions from ToxCast/Tox21 *in vitro* data into critical phenomena leading to birth defects such as cleft palate or hypospadias.
- A comprehensive suite of human-relevant synthetic models can be made, but how smart must they be to support decision-making in the animal-free (3Rs) zone?

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Yvonne Staal – RIVM
Harm Heusinkveld - RIVM