2021 *In Vitro* Toxicology Lecture and Luncheon

Monday, March 22

Computational Intelligence:
Building 'Smart Models' for Toxicology in the Era of Big-Data

Thomas B. Knudsen, PhD

Developmental Systems Biologist

US EPA, Center for Computational Toxicology and Exposure

Biomolecular and Computational Toxicology Division

Research Triangle Park, NC 27711

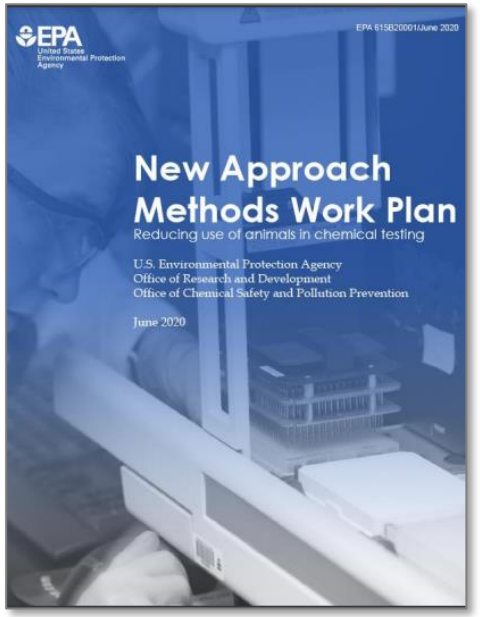
knudsen.thomas@epa.gov

ORCID 0000-0002-5036-596x

DISCLAIMER: The views expressed are those of the presenter and do not reflect Agency policy.

Shifting toxicity testing to *in vitro* data and *in silico* models

- **June 2016:** *Frank R. Lautenberg Chemical Safety for the 21st Century Act* enacted to advance chemical safety evaluations with novel methods that reduce testing on vertebrate animals and are translatable to vulnerable populations / lifestages.
- **September 2019:** directive issued by USEPA Administrator Wheeler set a vision to reduce mammalian study requests 30% by the year 2025 and eliminate them by 2035.



- **June 2020:** USEPA work plan to accelerate scientifically valid *New Approach Methods* (NAMs) for assessing toxicity of large numbers of chemicals with less reliance on animal testing.
- **Today's lecture:** focuses on the predictive power of computational models and computer simulation for human-relevant pathways underlying developmental toxicity.

Can the computer eliminate the lab animal?

technology feature

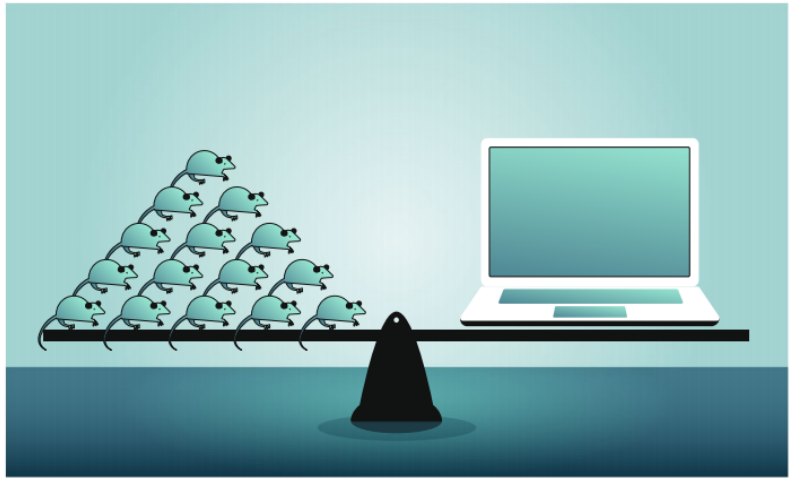
Toxicology testing steps towards computers

Can the computer eliminate the lab animal? As computational methods become more advanced and data more freely available, in silico modeling approaches have growing potential to help reduce the number of animals needed to test chemical toxicity.

Jim Kling

The 2016 overhaul of the United States Toxic Substances Control Act (TSCA), originally passed in 1976, was meant to help curb animal use in determining the potential toxicity of drugs and other chemicals. But in the short term, at least, the opposite seems to have happened. *Science* reported[1] a surge in animal testing, from 7,000 animals used in a few dozen tests in 2016, to more than 300 conducted a year later that involved about 75,000 rats, rabbits and other animals.

The specific cause of the jump in animal testing is unknown, but it is ironic given that the law also required the Environmental Protection Agency (EPA) to "reduce, refine, or replace" animals in toxicological testing. The trend is alarming to animal welfare and industry groups, and frustrating to researchers working on alternatives. One such alternative avenue that has made strides in recent years is to move *in vivo* toxicology studies *in silico*: a number of computational methods have been developed that could be



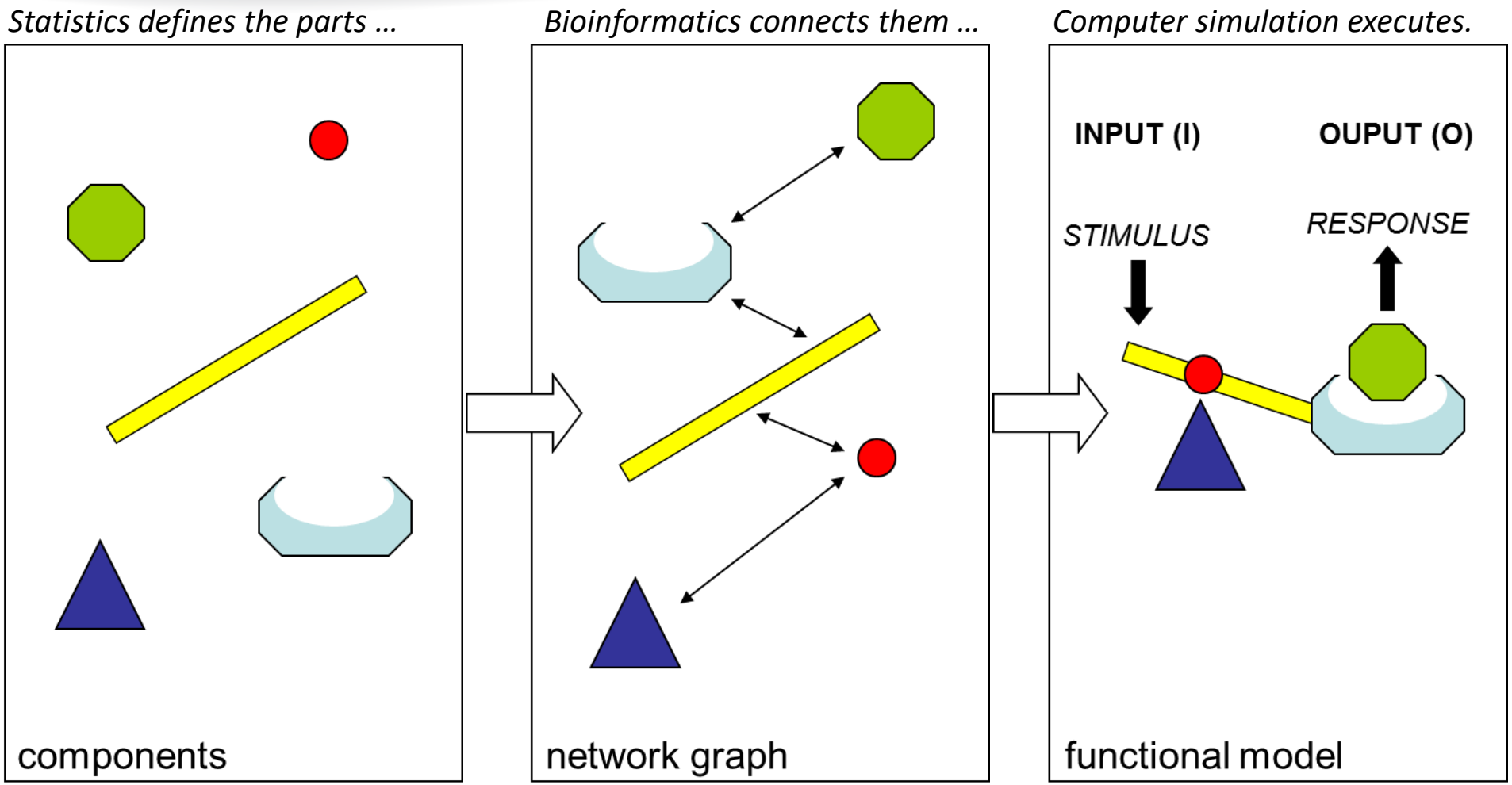
In vivo* vs. *in silico: Computer models are in the works that might help shift the balance away from animal use in toxicity testing. Credit: E. Dewalt/Springer Nature

Nature (Lab Animal) 48: 40-42, February 2019

"… in silico modeling approaches have growing potential to help reduce the number of animals needed to test chemical toxicity."

- machine-learning (A.I.): capitalizes on computing power and vast amounts of data in the public domain.
- research challenge: improve accuracy and trust in toxicological predictions made by computers.

Why computational models are needed ...



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

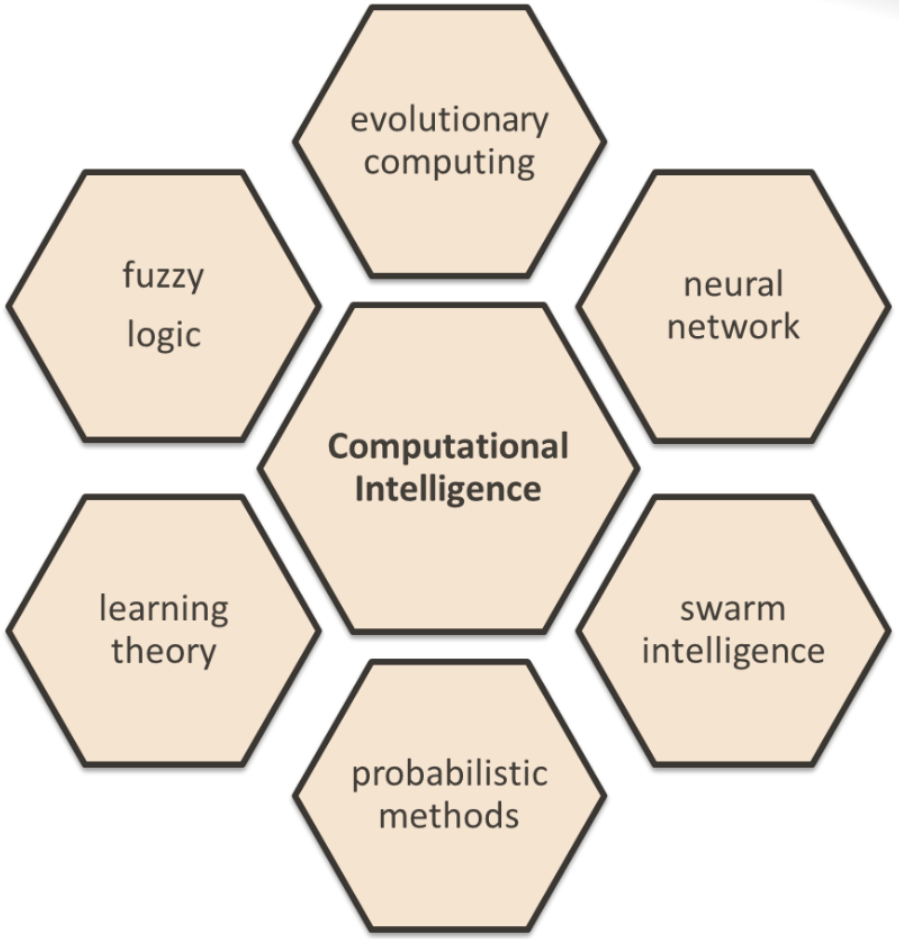
Computational Intelligence (C.I.)

- Deep-learning is the dominant AI technique today for mining data-driven ***correlations*** in predictive toxicology

  See for example:
  Luechtefeld et al. (2018) Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility. Toxicol Sci 165: 198-212.

  Ciallella and Zhu (2019) Advancing computational toxicology in the big data era by artificial intelligence: data-driven and mechanism-driven modeling for chemical toxicity. Chem Res Toxicol 32: 536-547.

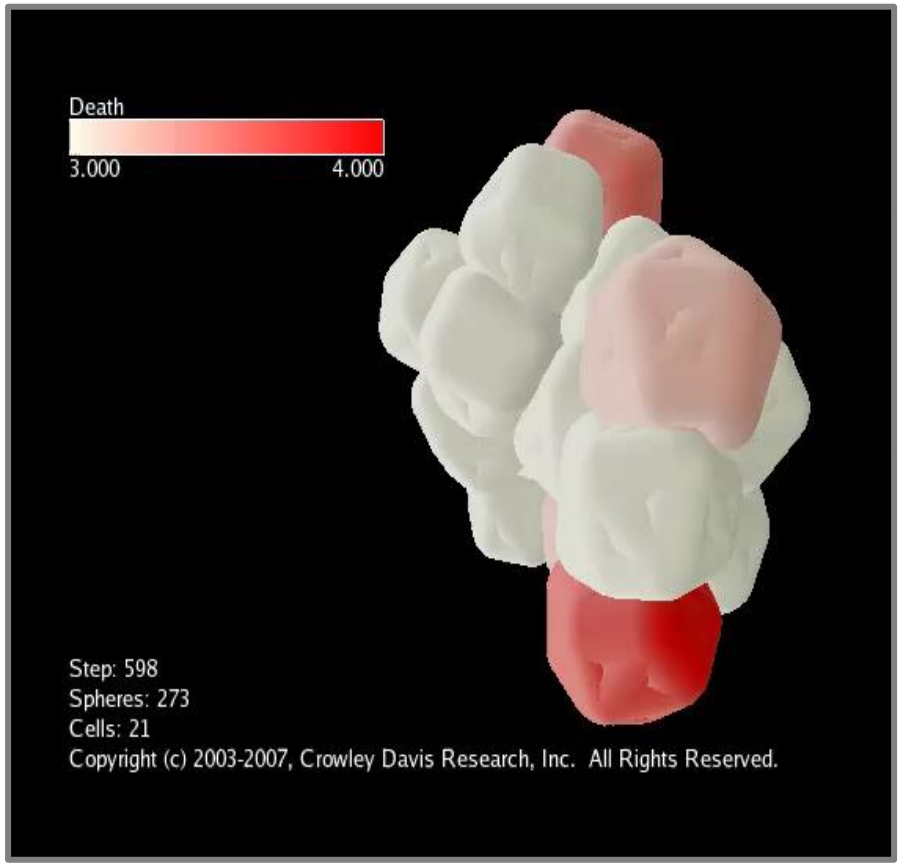
- However, to get at ***causality*** we'll want to go beyond the data, utilizing novel C.I. approaches that can handle randomly determined (stochastic) behaviors of cells in a complex setting.



Cellular Agent-Based Models (ABMs)

- nature-inspired *agents* (cells) and *rules* (behaviors) set into motion as a self-organizing system (virtual tissue)
- enough information is coded into the model's blueprint to execute a complex morphogenetic series of events
- soft-computing uses 'fuzzy logic' to fill-in for missing information (inexact rules, incomplete knowledge)
- readout is a phenotype that can unravel precisely where, when and how a particular change emerged

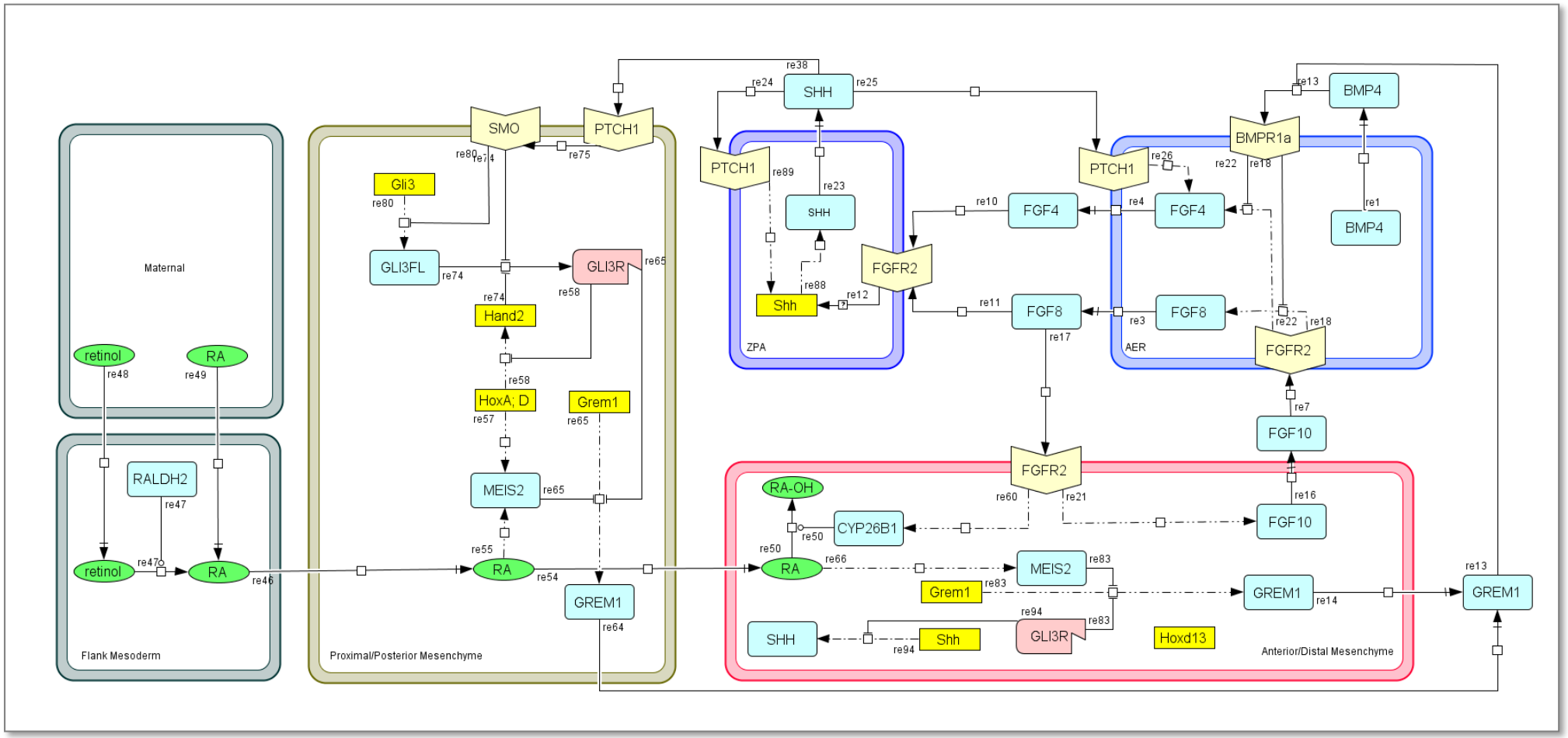
Example: anatomical homeostasis in a self-regulating 'virtual embryo'



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

Biological blueprints are complex: *cells, signals, responses, biomechanics, …*

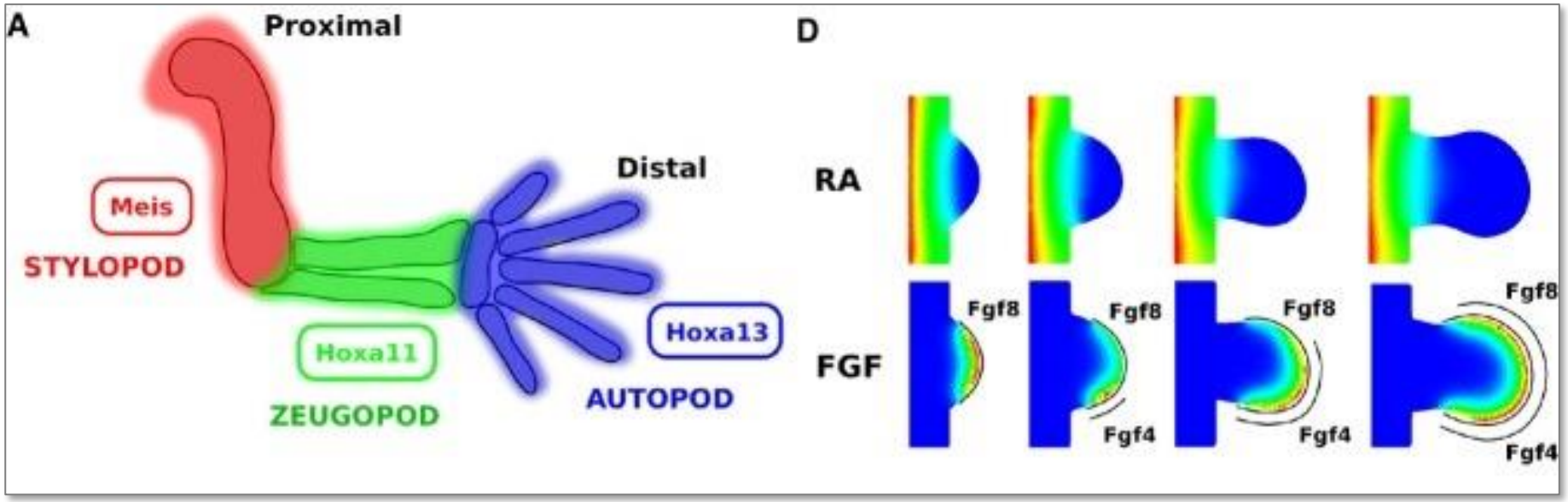
Biological network driving embryonic limb-bud outgrowth



Mapped in CellDesigner [www.celldesigner.org]

Data-driven mathematical model: *embryonic limb-bud outgrowth*

Patterns of retinoic acid (RA) and fibroblast growth factor (FGF4, FGF8) signaling gradients reverse-engineered from gene expression data (in situ hybridization)

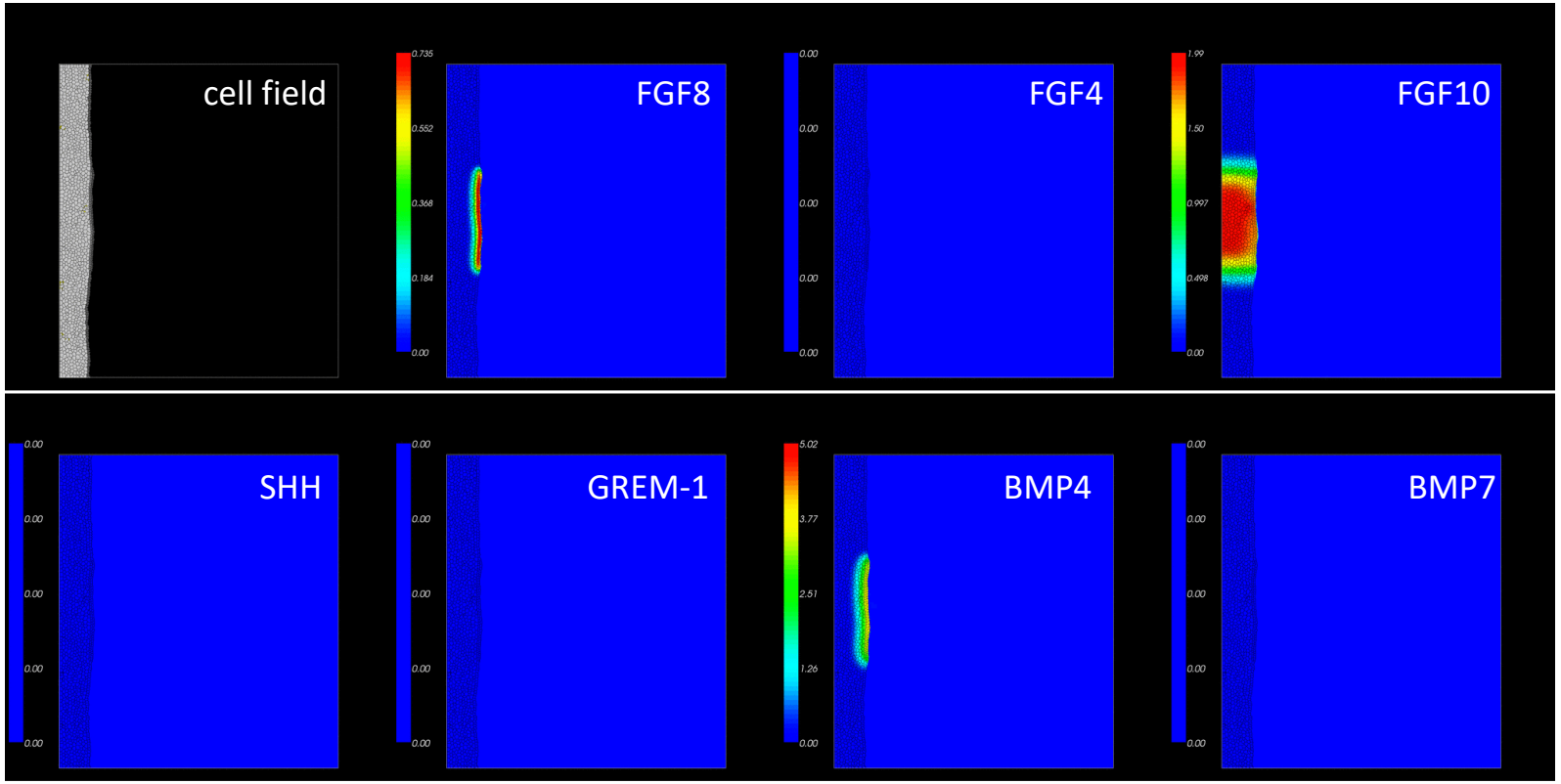


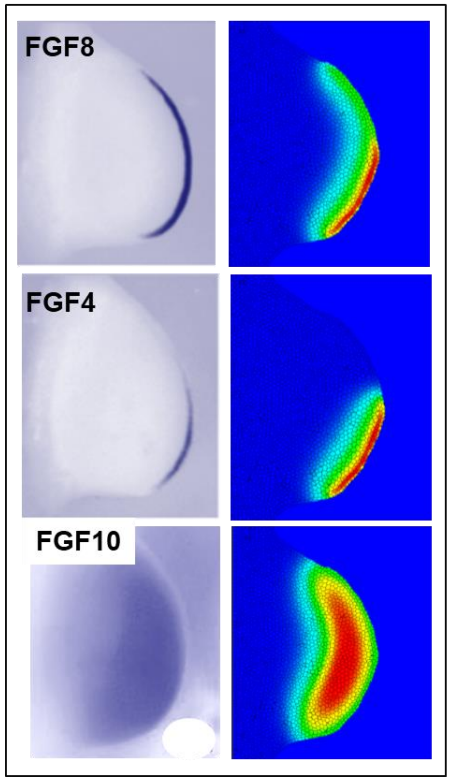
SOURCE: Uzkudun, Marcon and Sharpe (2015) Mol Systems Biol

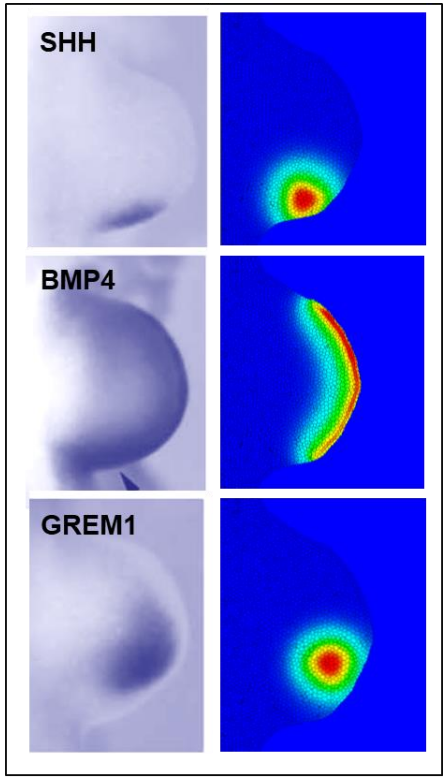
EPA's 'Virtual Embryo' project is taking this philosophy a step further, aiming to simulate how chemicals might affect development, and what exposure thresholds pose a threat.

Virtual Embryo: *limb-bud outgrowth module*

Patterns of gene expression forward-engineered from in situ hybridization images (literature)

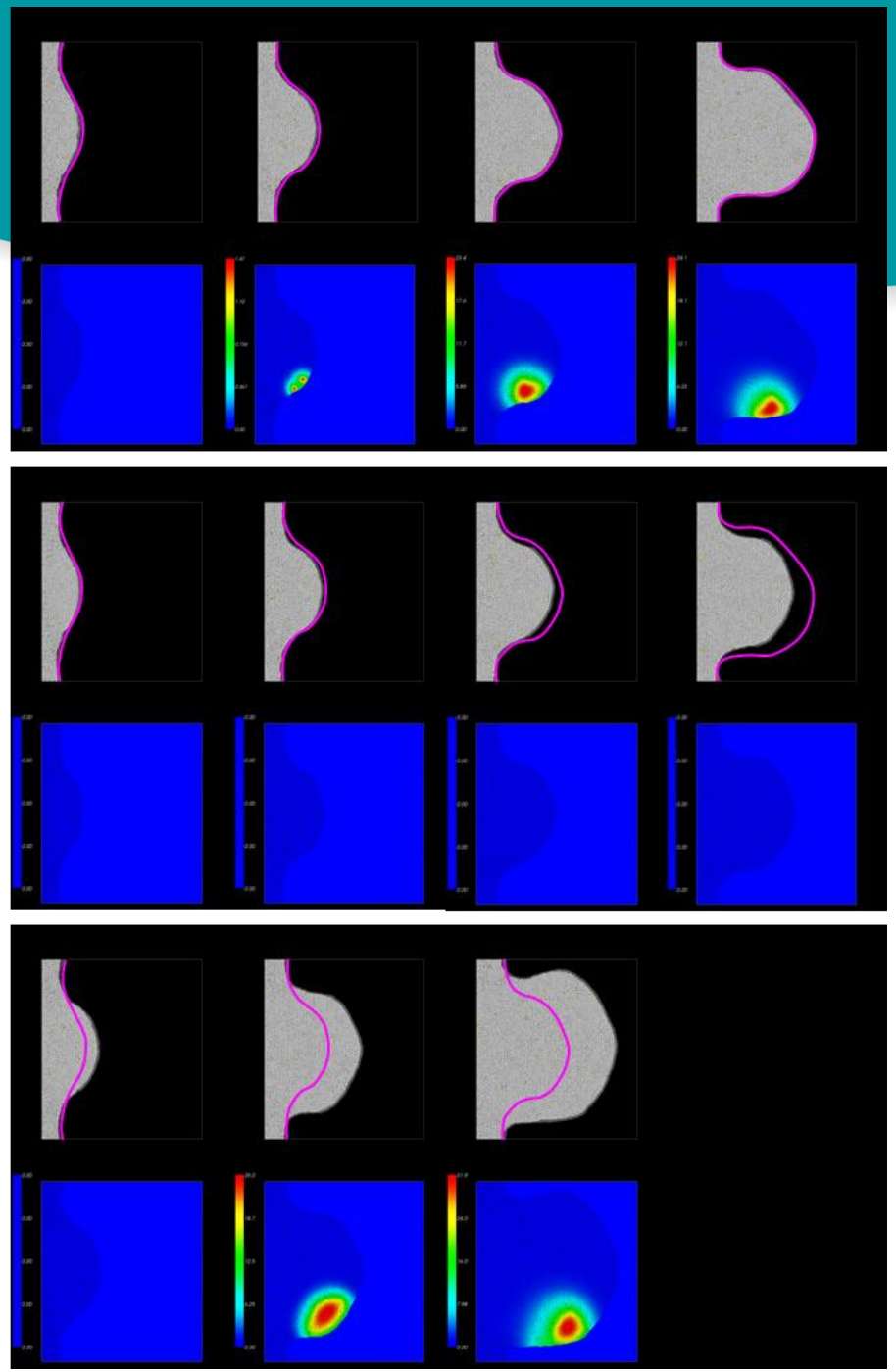




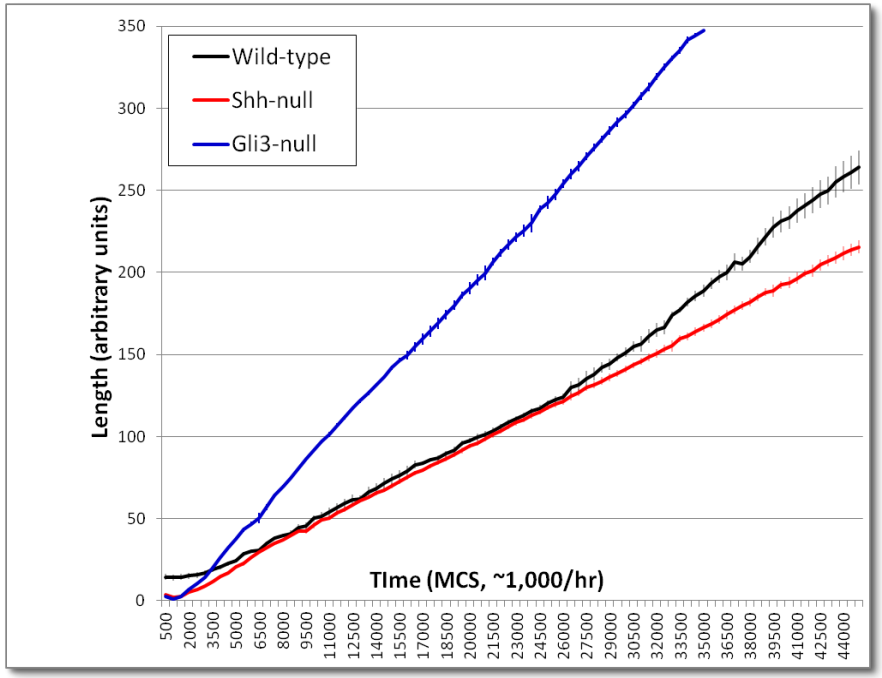


Coded in Python, simulated in the CompuCell3D modeling environment [www.compucell3d.org]

Hacking the control network
(cybermorphs)



Growth trajectories (n=5 simulations)

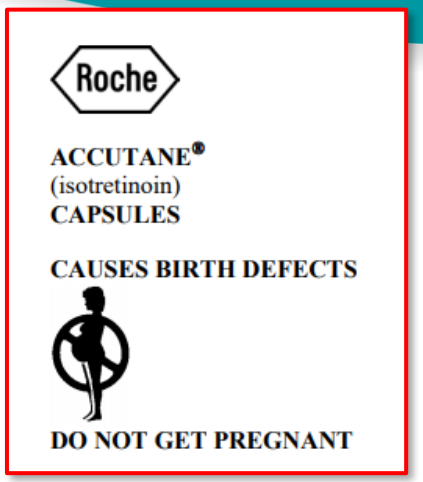


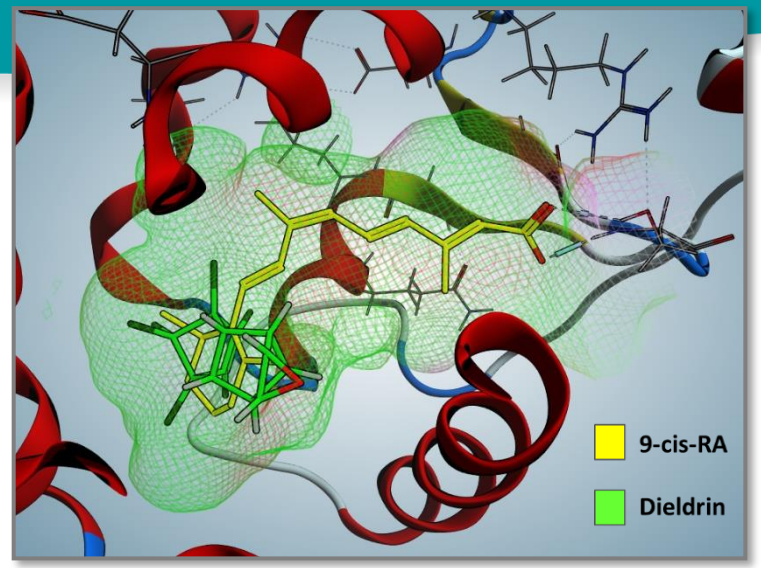
Predicted outcomes

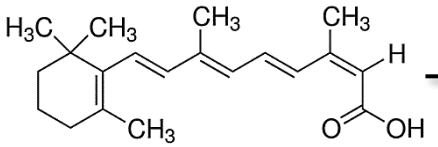
digital patterns inferred from the literature; not yet implemented in the model

Wild-type *Shh-null* *Gli3-null*

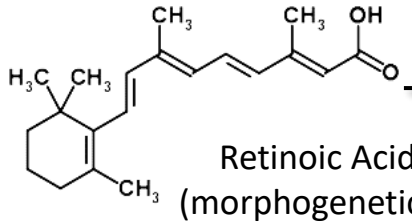
In silico toxicodynamics: *disruption of RA signaling*



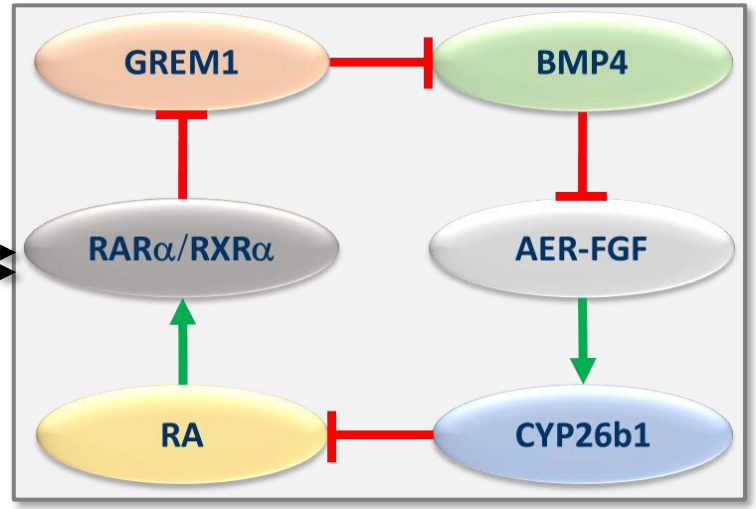


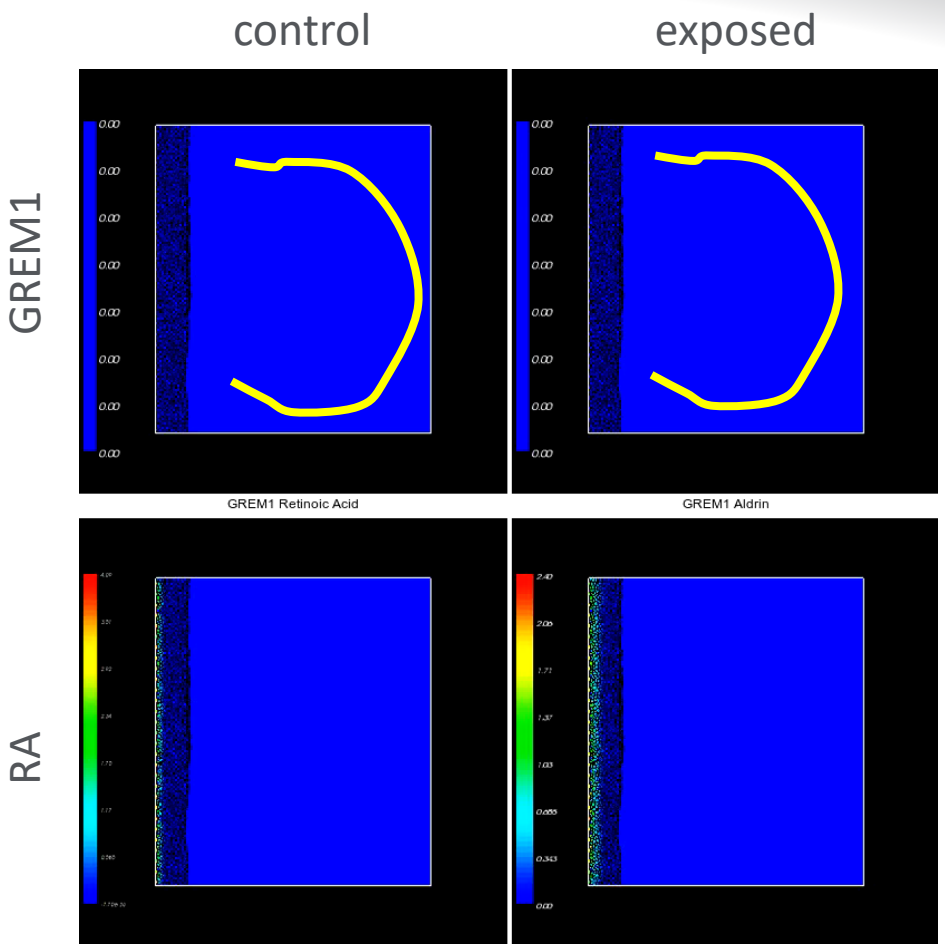


Accutane
(13-cis Retinoic Acid)

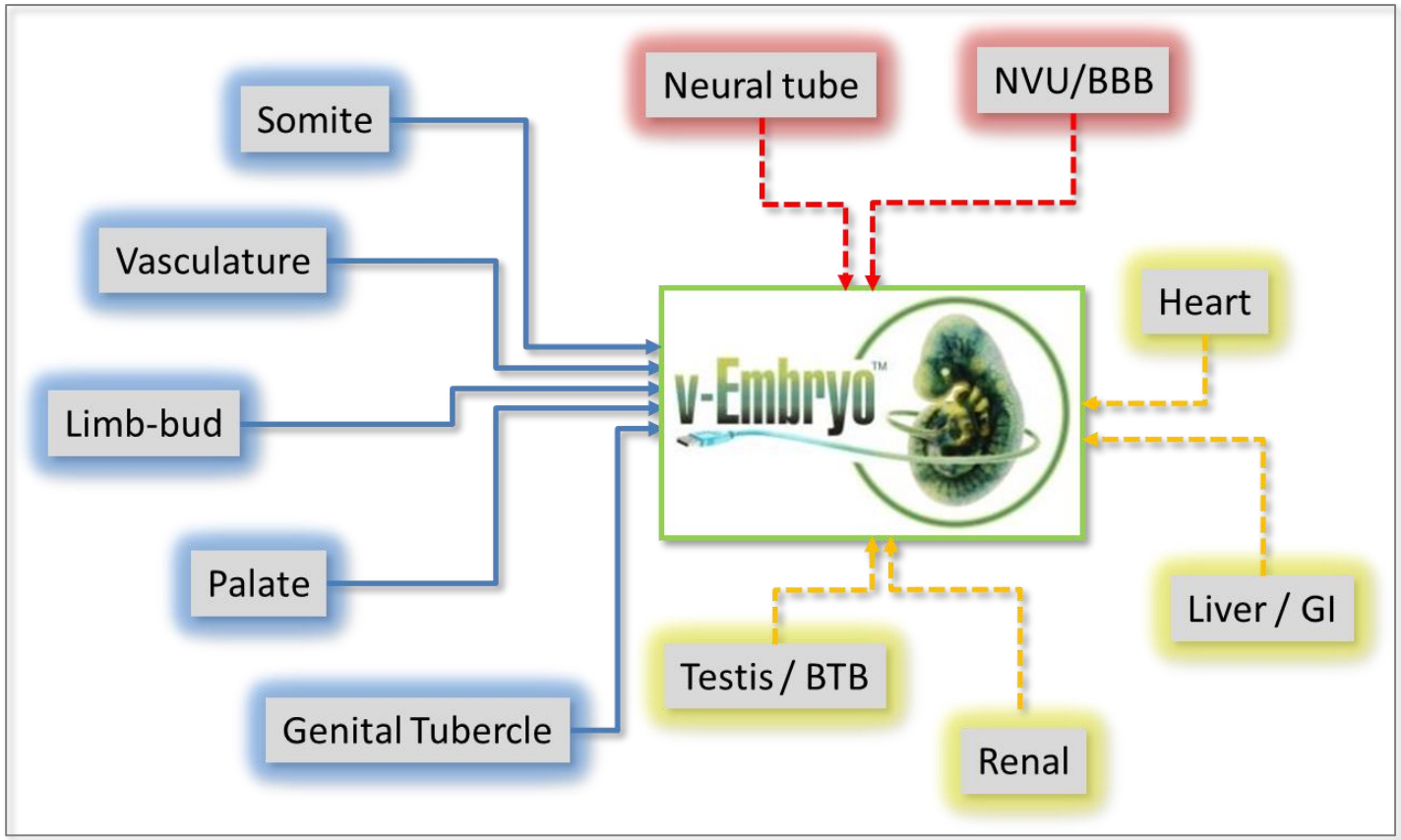


Retinoic Acid (RA)
(morphogenetic signal)





Take home message: <u>in silico toxicodynamics</u>
Computational models with sufficient biological intelligence can quantitatively simulate multiscale dynamics of biomolecular perturbation(s), predicting an in vivo phenotype.



Virtual Embryo toolbox will encompass a range of developing systems

Acknowledgements

Kate Saili – CCTE
Todd Zurlinden – CCTE
Jocylin Pierro – CCTE
Sid Hunter - CCTE
Nancy Baker – Leidos
Richard Spencer – General Dynamics
James Glazier – Indiana U
Max Leung – CCTE (now CalEPA)
Nicole Kleinstreuer (now NTP/NICEATM)
Shane Hutson – Vanderbilt U
Aldert Piersma –RIVM
George Daston – P&G

Opening the 'black-box' with *Computational Intelligence*

- **Translational:** what could a comprehensive suite of human-relevant synthetic (*in silico*) models bring to the future of toxicity testing?
- **Investigational:** how smart must these models be to support decision-making with reduced animal testing (3Rs)?
- **Operational:** what best practices are needed to implement a virtual toxicodynamics platform into integrated decision frameworks?

Communication: given successful proof-of-concept, what factors would make stakeholders more (or less) comfortable using these types of computer models versus a whole organism?