Society of Toxicology 2019, ITS Symposium
'Understanding the utility of in vitro developmental toxicity assays and building integrated testing strategies'

Mining and Modeling ToxCast/Tox21 data for developmental toxicity

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

knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

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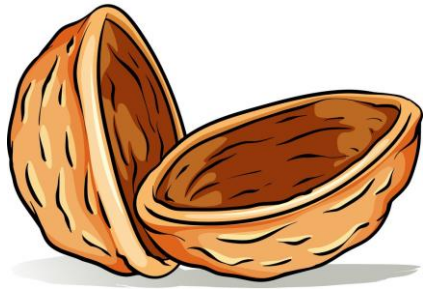


In a nutshell …

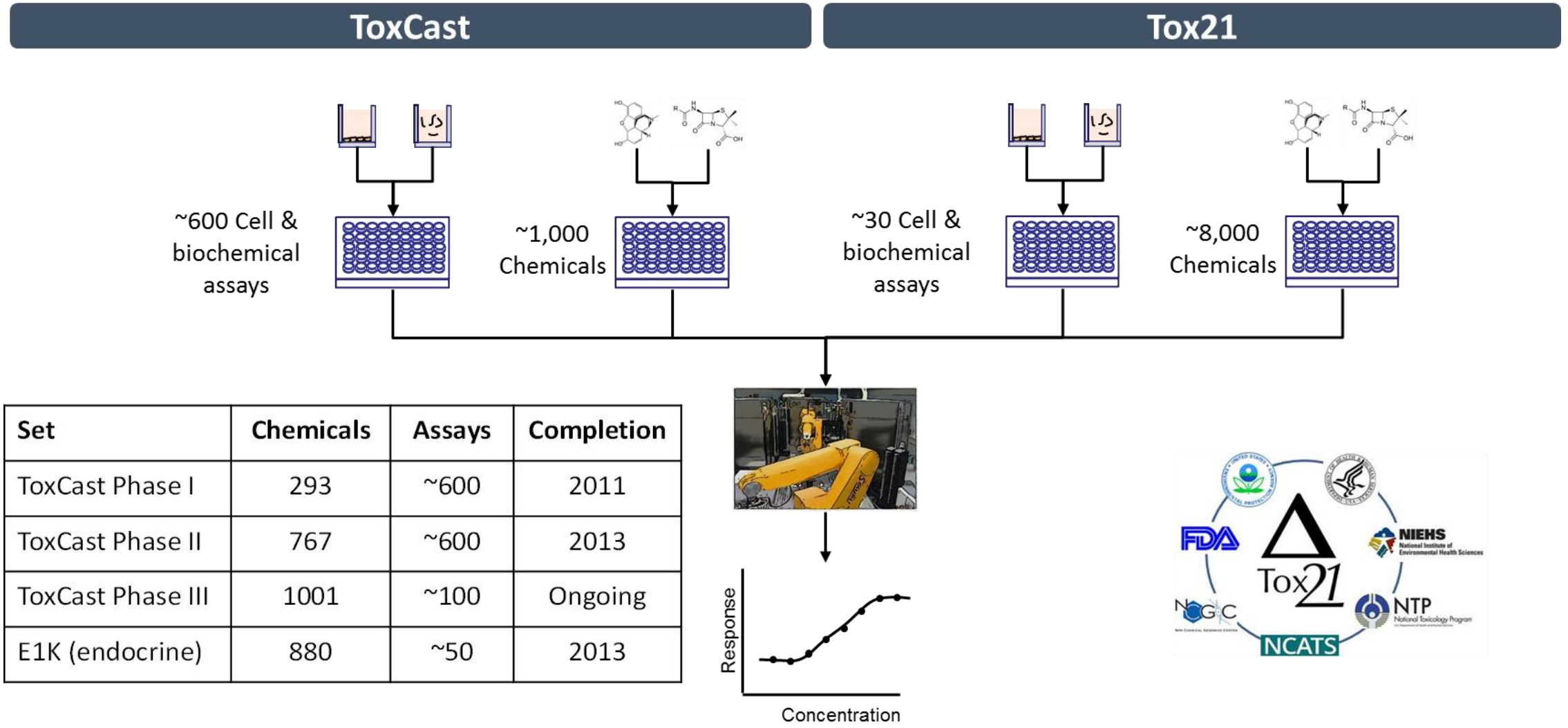
- Chemical exposure to a pregnant woman has the potential to affect her unborn child, leading to adverse birth outcomes and/or risks to early child development.

- Vast amounts of HTS data from ToxCast/Tox21 can be used for quantitative modeling of toxicological pathways and processes [https://comptox.epa.gov/dashboard].

- Translatability into human-predictive models of developmental toxicity must deal with the embryo as a complex self-organizing system that computes with genetic circuits.

- Computational systems models can help define the applicability domain of HTS data in support of understanding the utility of *in vitro* developmental toxicity assays.

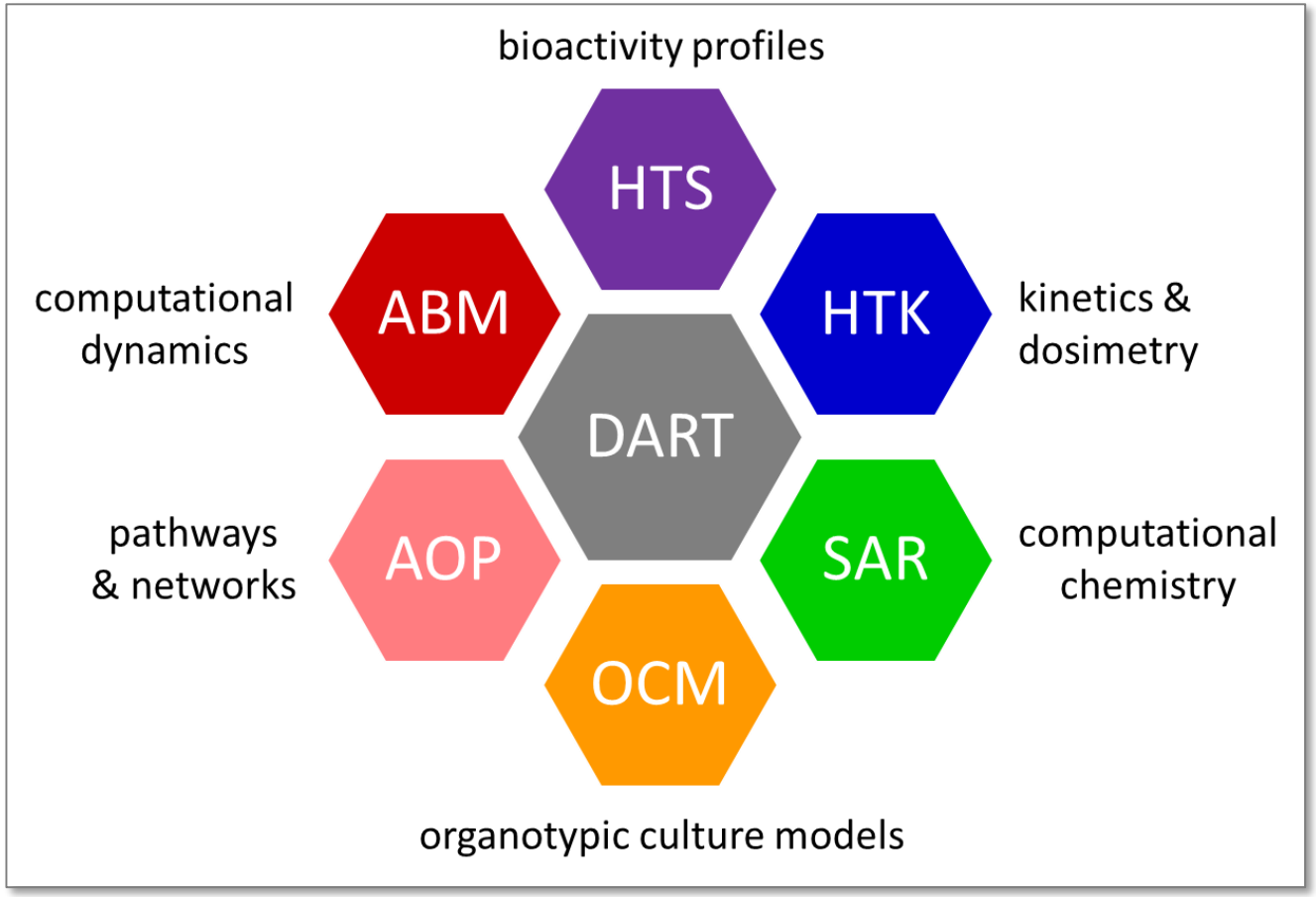
Shifting toxicology to pathway-based approaches



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

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

Computational synthesis and integration



Fundamental principles:

- initiating mechanisms (MIEs)
- genetic susceptibility (species, individual)
- critical periods (patterning, differentiation)
- bioavailability (chemistry, ADME)
- apical outcomes (pregnancy outcomes)

Case examples:

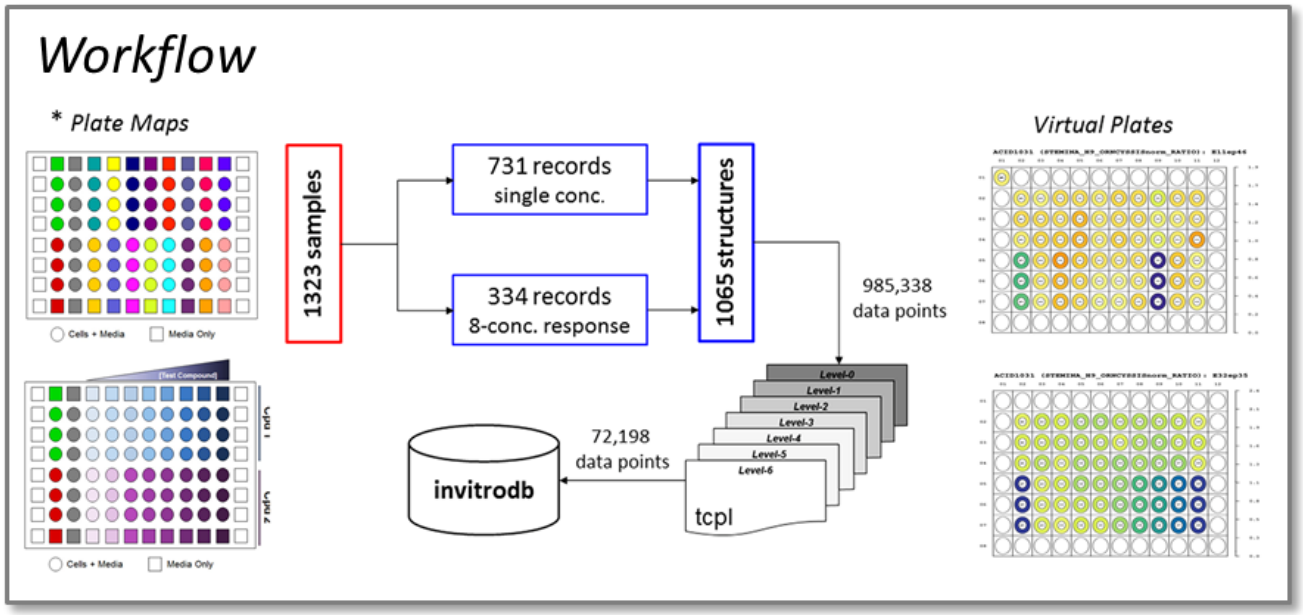
- explore predictive power of ToxCast HTS data when integrated with relevant knowledge;
- inform additional data needs to support regulatory decisions.

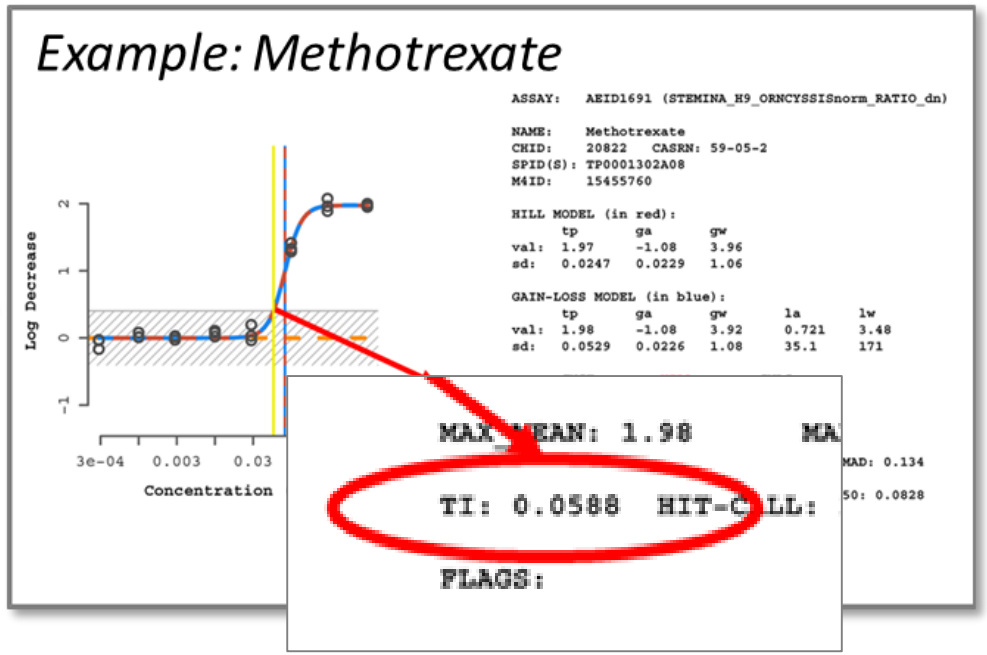
1. Profiling the ToxCast library with a pluripotent human (H9) embryonic stem cell assay

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: devTOX^{qP} assay, Stemina Biomarker Discovery, EPA contract EP-D-13-055

- pluripotent H9 human embryonic stem cells exposed for 3-days
- critical drop in ornithine : cystine ratio is the teratogenic index (TI) [Palmer et al. 2013]
- data processed through the ToxCast pipeline (tcpl, level 6)
- **Key point:** 183 of 1065 (17%) ToxCast chemicals tested positive





SOURCE: Zurlinden et al. (manuscript in clearance)

STM versus rat WEC

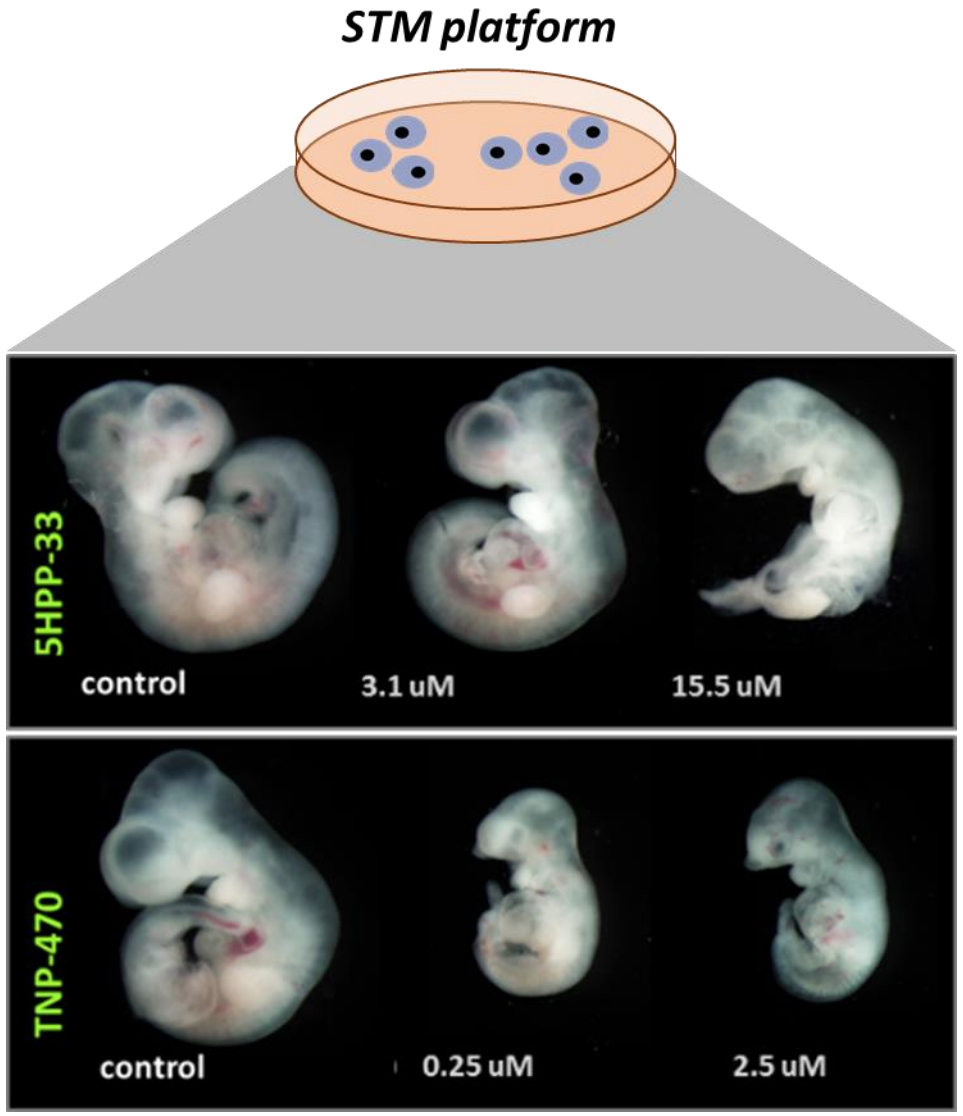
Key point: exposure-based potential for DevTox predicted by hESC assay on-the-mark both qualitatively and quantitatively.

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)



SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol

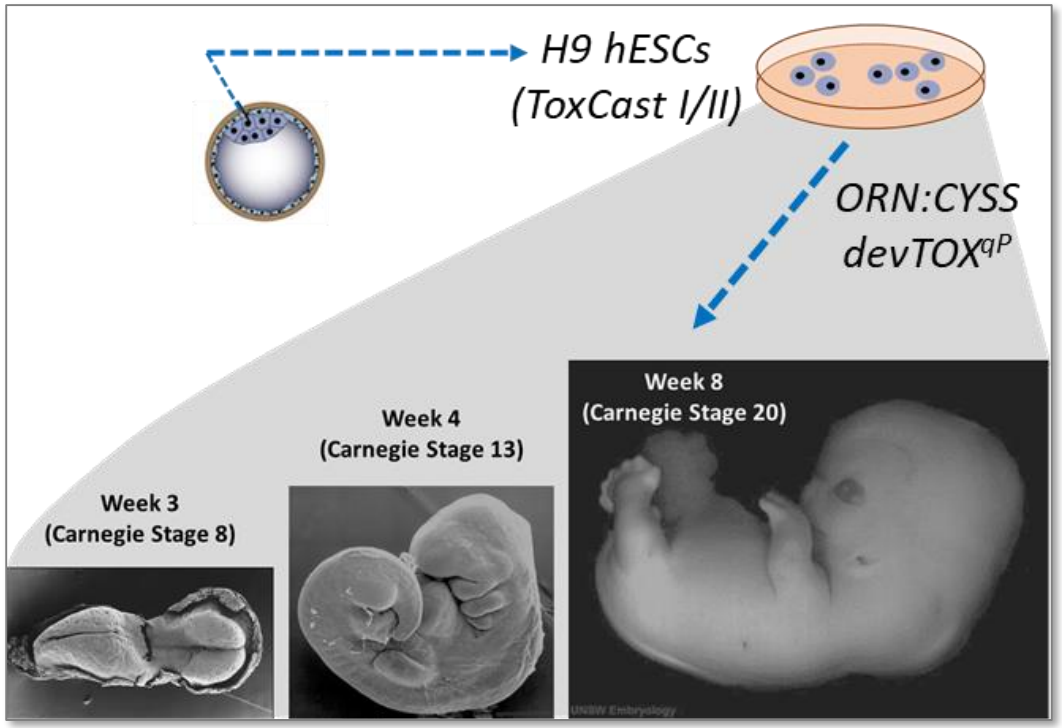
Anchoring STM performance to DevTox *(ToxRefDB v1 endpoint summary)*

- **Key point**: sensitivity (hence balanced accuracy) improves with evidence for DevTox

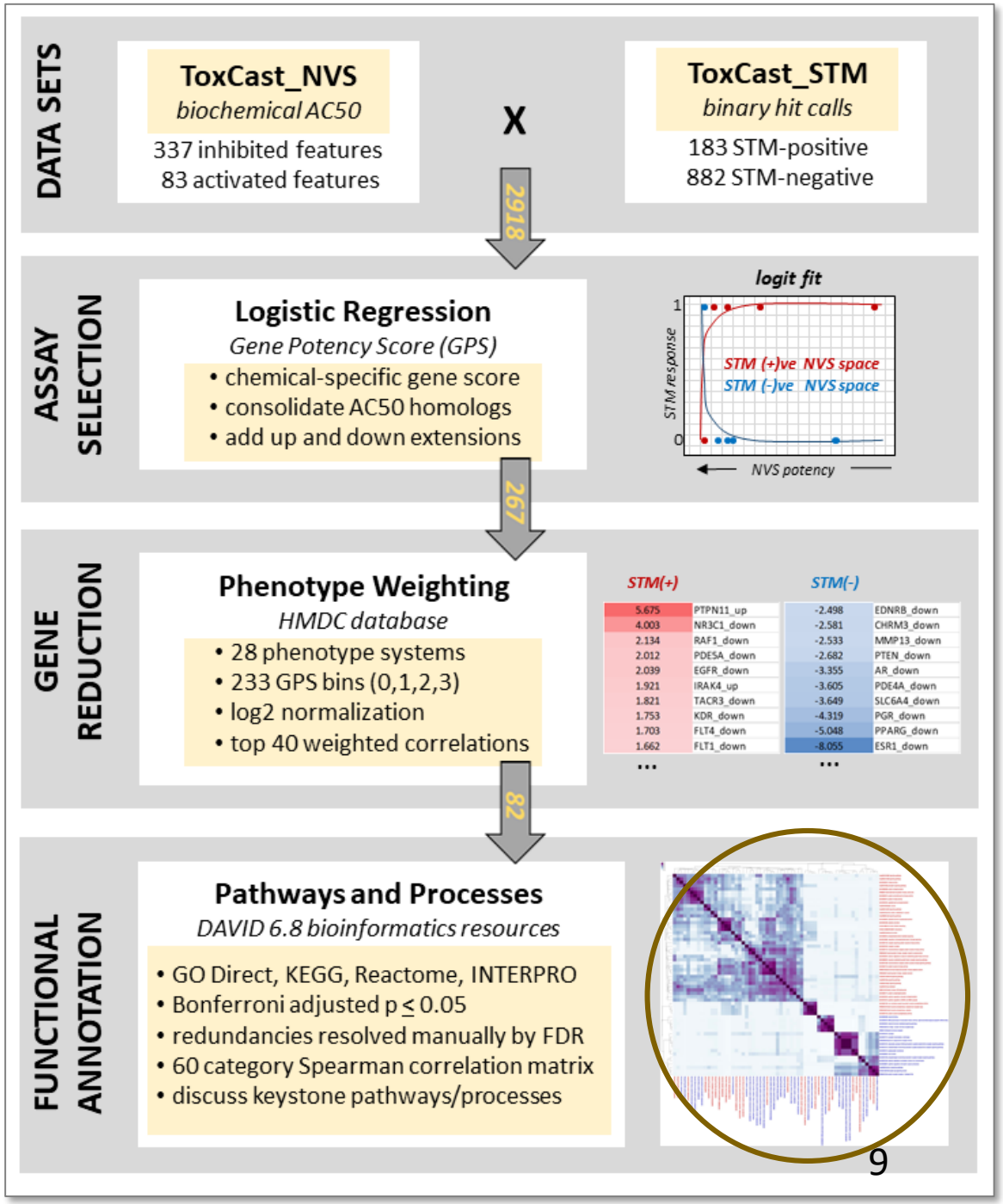
	in vivo	
in vitro	TP	FP
	FN	TN

	Stringency Filter Applied to DevTox Anchor			
***Condition*[2]**	***Base***	***Low***	***Medium***	***High***
TP	85	60	35	19
FP	14	37	23	9
FN	217	127	51	11
TN	116	208	176	88
n	432	432	285	127
sensitivity	0.281	0.321	0.407	0.633
specificity	0.892	0.849	0.884	0.907
PPV	0.859	0.619	0.603	0.679
NPV	0.348	0.621	0.775	0.889
ACC	46.5%	62.0%	74.0%	84.3%
MCC	0.190	0.202	0.332	0.554
	any dLEL rat OR rabbit	SOME evidence rat OR rabbit	CLEAR evidence rat OR rabbit	CLEAR evidence rat AND rabbit

Biochemical determinants *(inferred)*



Key point: sensitive pathways can be inferred from functional annotation of MIEs in the STM-positive and STM-negative domains.



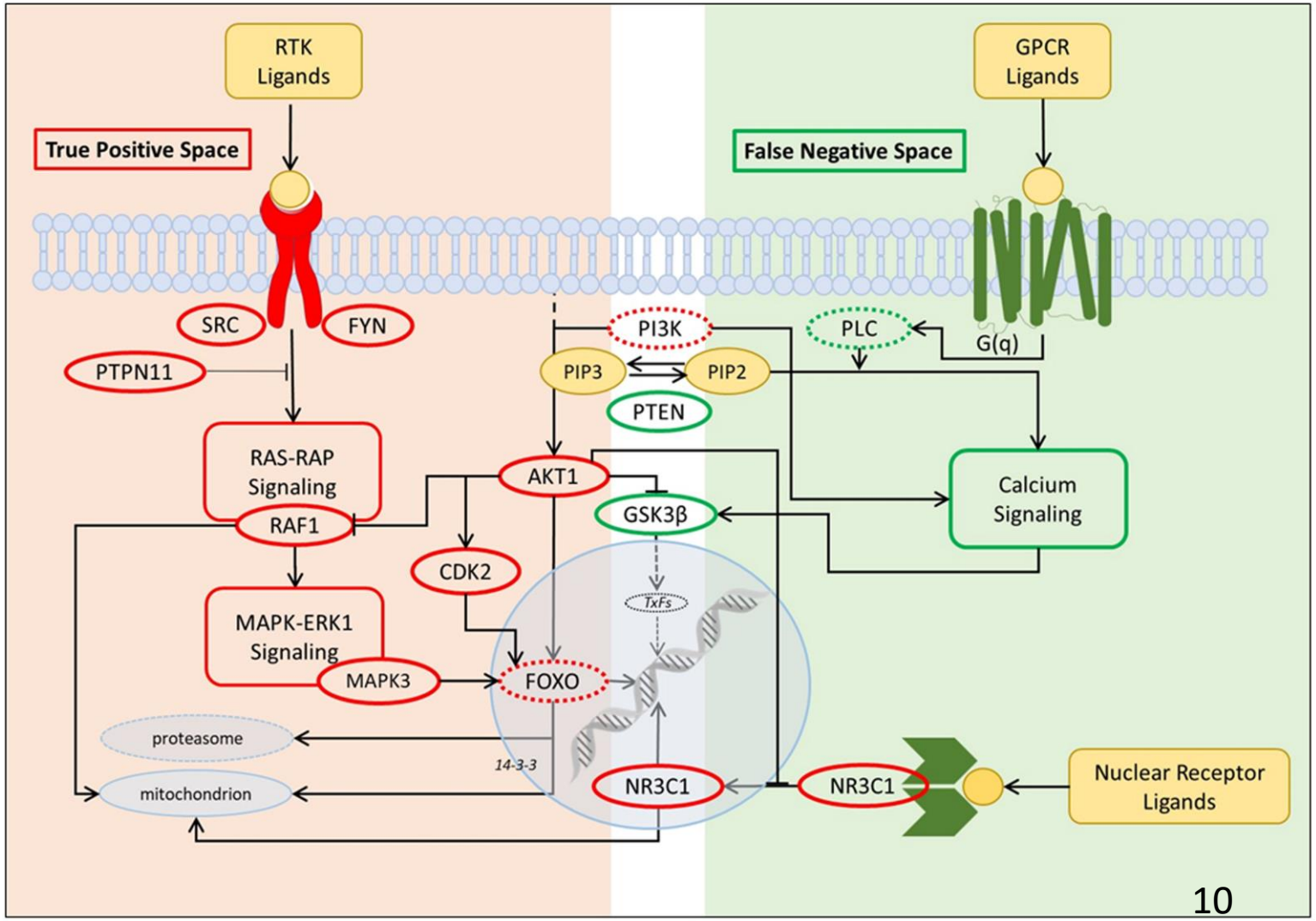
Keystone Pathways

Annotation System	Keystone Pathway / Process	# MIEs	Class
GOTERM_BP_DIRECT	GO:0014066~regulation of phosphatidylinositol 3-kinase signaling	6	TP
KEGG_PATHWAY	hsa04068:FoxO signaling pathway	8	TP
KEGG_PATHWAY	hsa04510:Focal adhesion	13	TP
GOTERM_BP_DIRECT	GO:0007200~phospholipase C-activating G-protein coupled receptor signaling pathway	10	FN
INTERPRO	IPR001723:Steroid hormone receptor	7	FN
GOTERM_MF_DIRECT	GO:0005496~steroid binding	5	FN

Sensitive domain: flow of regulatory information points to AKT/FoxO signaling and focal adhesion in the applicability domain (RTK signaling);

Insensitive domain: GPCR signaling via G(q) pathways and most steroid receptors (aside from NR3C1) fall outside the applicability domain.

Key point: integration of MIEs into biological pathways and processes can help define the applicability domain of the hESC response.



SOURCE: Zurlinden et al. (manuscript in clearance)

On understanding the utility of the STM (hESC) assay

[1] 17% of 1065 ToxCast chemicals tested here yielded an exposure-based prediction of developmental toxicity.

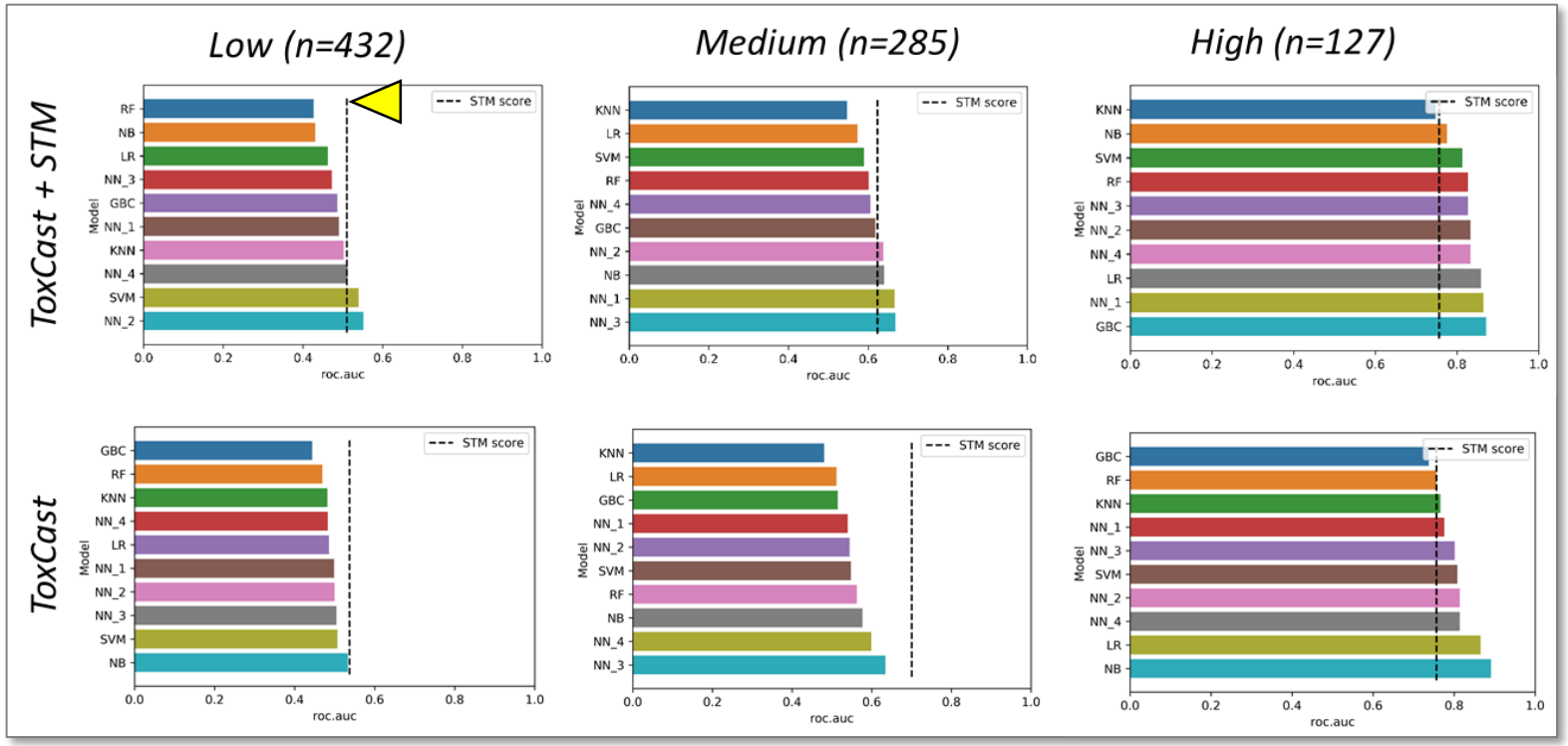
[2] Model performance reached 76% to 84% balanced accuracy with excellent specificity (>88%) but modest sensitivity (<66%) when anchored to apical endpoints in DevTox.

[3] Sensitivity of the STM model improved as more stringent acceptance criteria were applied to the anchoring DevTox animal studies.

[4] Statistical analysis of the most potent NVS MIEs demarcated positivity or negativity of the STM response, but did not clearly resolve true positives from false negatives.

[5] Integration of these MIEs across multiple annotation systems revealed insights into pathways and processes in the applicability domain of the STM assay.

Utilizing the STM assay to build an integrative testing strategy



Algorithms

KNN	K Nearest Neighbors
NB	Naive Bayes
SVM	Support Vector Machine
NN	Neural Network (n hidden layers)
RF	Random Forest
LR	Logistic Regression
GBC	Gradient Boosting Classification

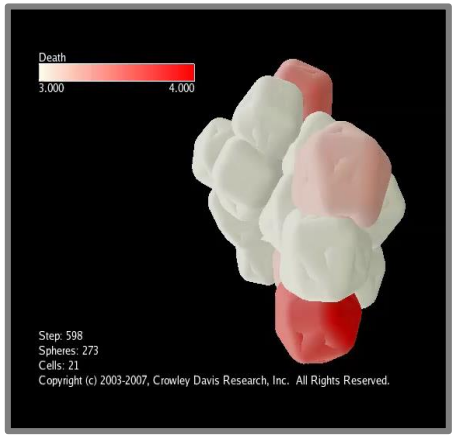
(----) ROC AUC for DevTox prediction using STM hit call alone.

- Machine learning algorithms for ToxCast/Tox21 assay portfolio (>800 features) fit and evaluated using a train/test split of low, medium, and high stringency DevTox models (~200 features selected).

- **Key point:** STM itself out performs ToxCast alone & augments ToxCast for Low / Medium stringency DevTox models; and points to HTS features that augment the High stringency DevTox model.

SOURCE: Todd Zurlinden, NCCT – work in progress

2. Translating *in vitro* MIE(s) into quantitative phenotypes for DevTox



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

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

Cell agent-based models (ABMs)

- **Approach:** build and test self-organizing morphogenetic systems *in silico* using cell-oriented computational dynamic systems [www.compucell3d.org].
- **Input:** A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties to reconstruct developmental progression.
- **Emergence:** simulation resolves into normal or perturbed phenotypes reading *in vitro* data input from specific ToxCast assays (cybermorphs).
- **Output:** probabilistic rendering of where, when and how a developmental defect might occur (critical phenomena).

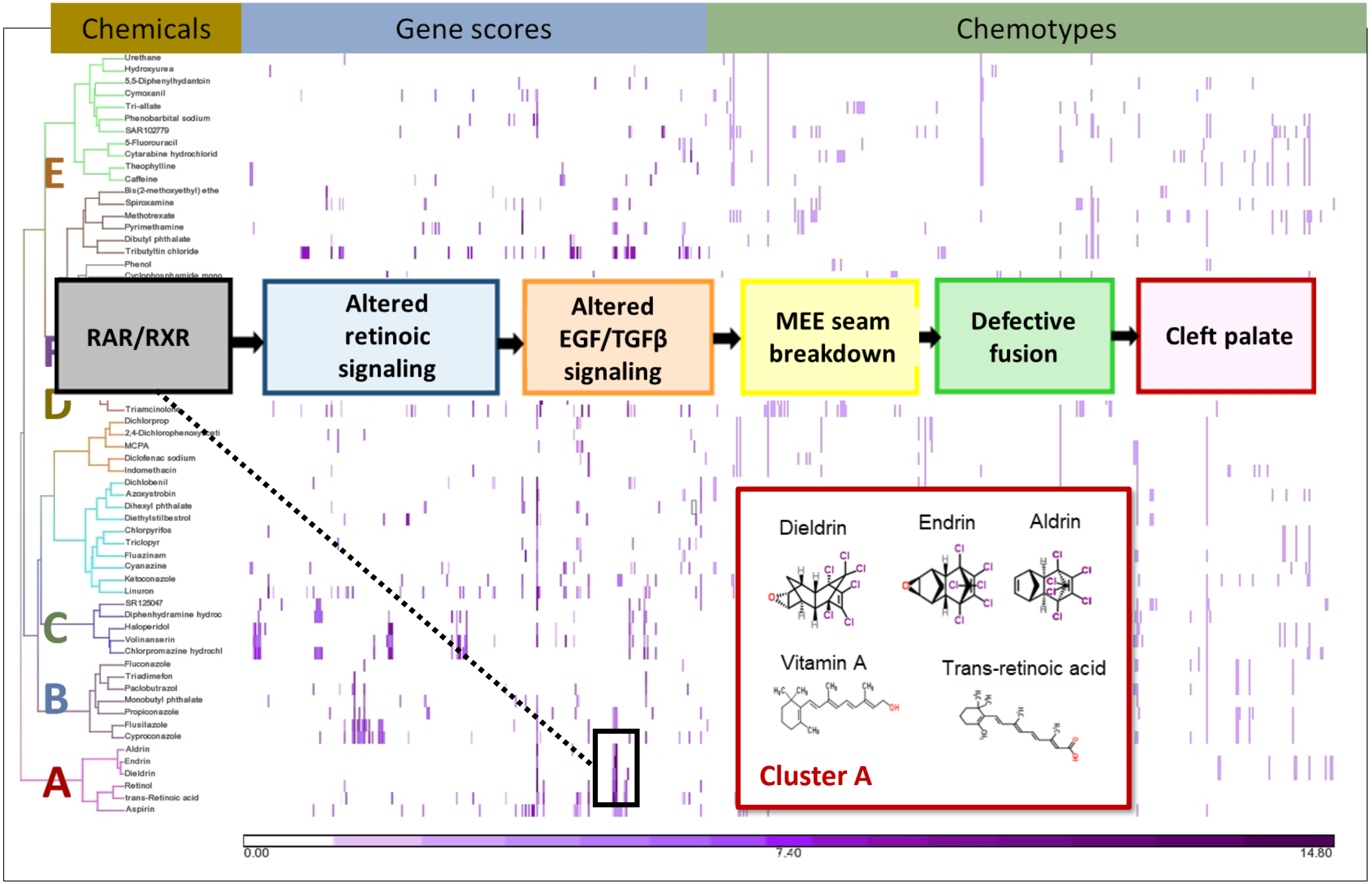
AOP framework: *cleft palate as an example*

ToxCast Chemicals
500 chemicals summarized by ToxCast gene score and chemotype for machine-learning

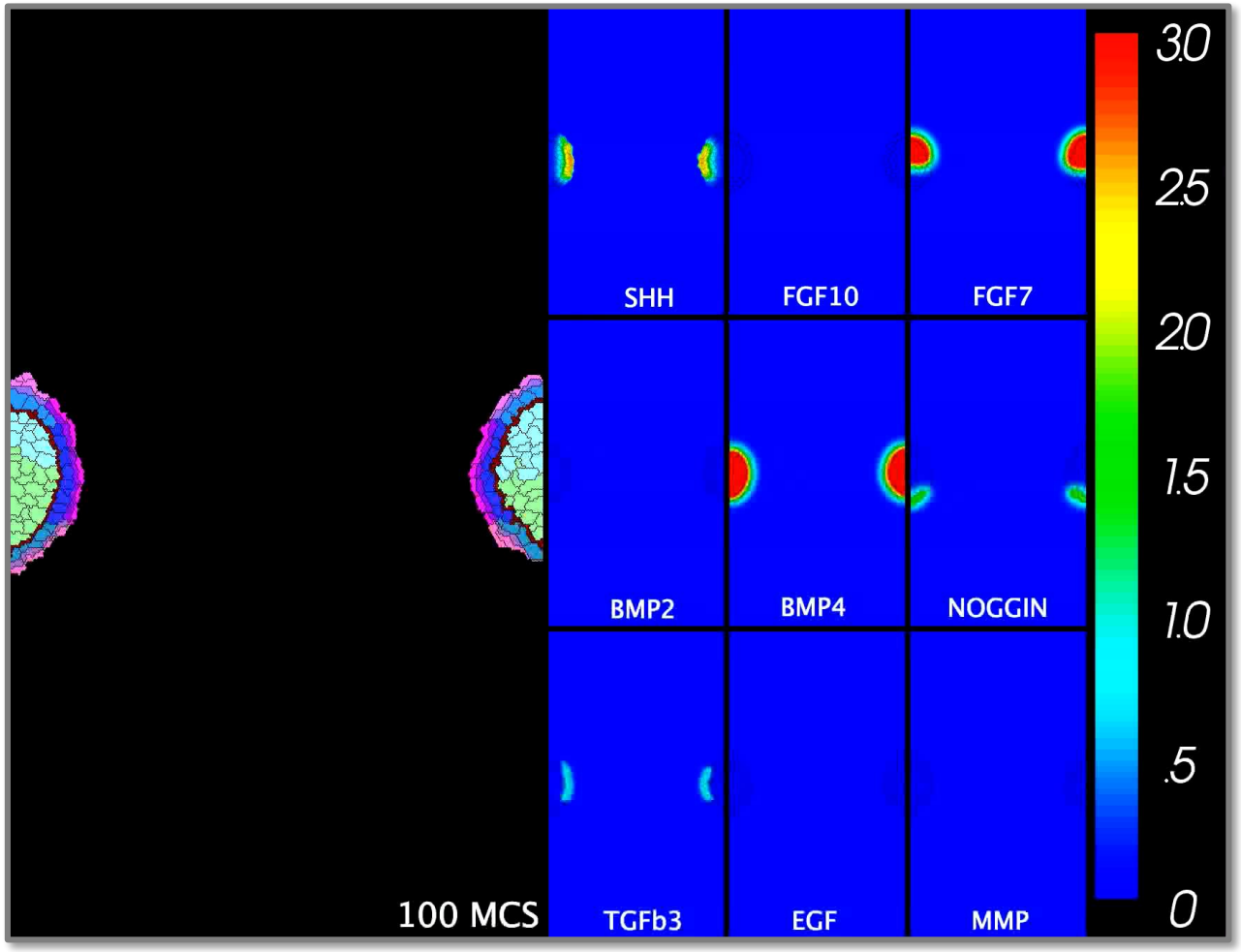
Animal studies
63 chemicals associated with cleft palate in ToxRefDB or open literature

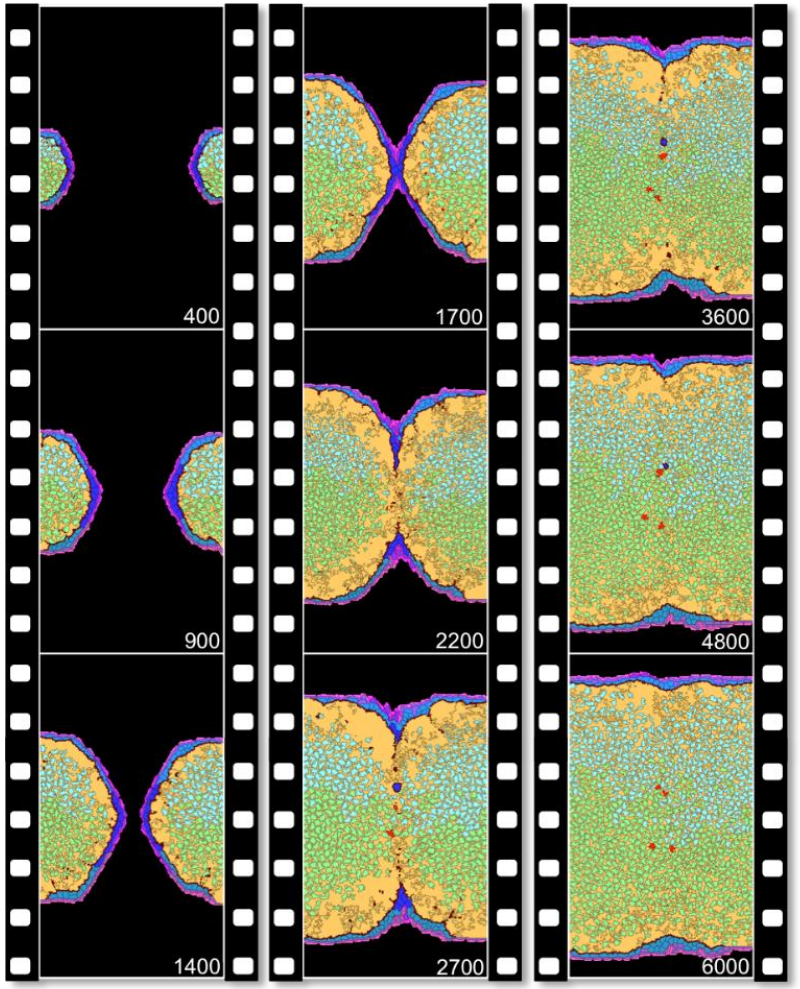
AOP clusters
6 mechanistic pathways inferred from integration of HTS data with chemical structure.

SOURCE: Baker et al. (manuscript)


Chemicals
Gene scores
Chemotypes
Urethane
Hydroxyurea
5,5-Diphenylhydantoin
Cymoxanil
Tri-allate
Phenobarbital sodium
SAR102779
5-Fluorouracil
Cytarabine hydrochlorid
Theophylline
Caffeine
Bis(2-methoxyethyl) ethe
Spiroxamine
Methotrexate
Pyrimethamine
Dibutyl phthalate
Tributyltin chloride
Phenol
E
RAR/RXR
Altered retinoic signaling
Altered EGF/TGFβ signaling
MEE seam breakdown
Defective fusion
Cleft palate
Triamcinolone
Dichlorprop
2,4-Dichlorophenoxyacti
MCPA
Diclofenac sodium
Indomethacin
Dichlobenil
Azoxystrobin
Dihexyl phthalate
Diethylstilbestrol
Chlorpyrifos
Triclopyr
Fluazinam
Cyanazine
Ketoconazole
Linuron
C
SR125047
Diphenhydramine hydroc
Haloperidol
Volinanserin
Chlorpromazine hydrochl
B
Fluconazole
Triadimefon
Paclobutrazol
Monobutyl phthalate
Propiconazole
Flusilazole
Cyproconazole
A
Aldrin
Endrin
Dieldrin
Retinol
trans-Retinoic acid
Aspirin
Dieldrin
Endrin
Aldrin
Vitamin A
Trans-retinoic acid
Cluster A
0.00
7.40
14.80

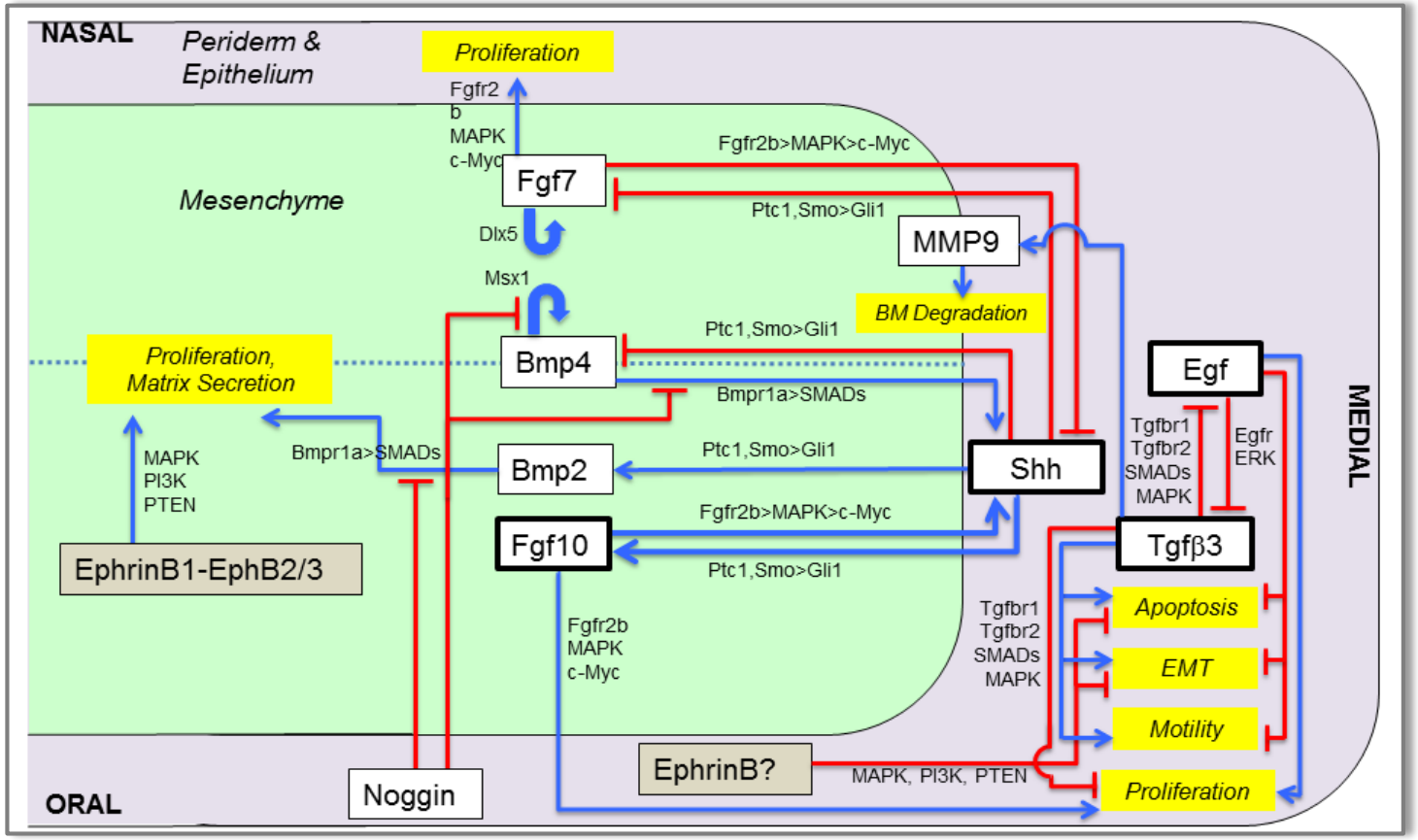
Palate fusion: *epithelial seam breakdown and mesenchymal confluence*

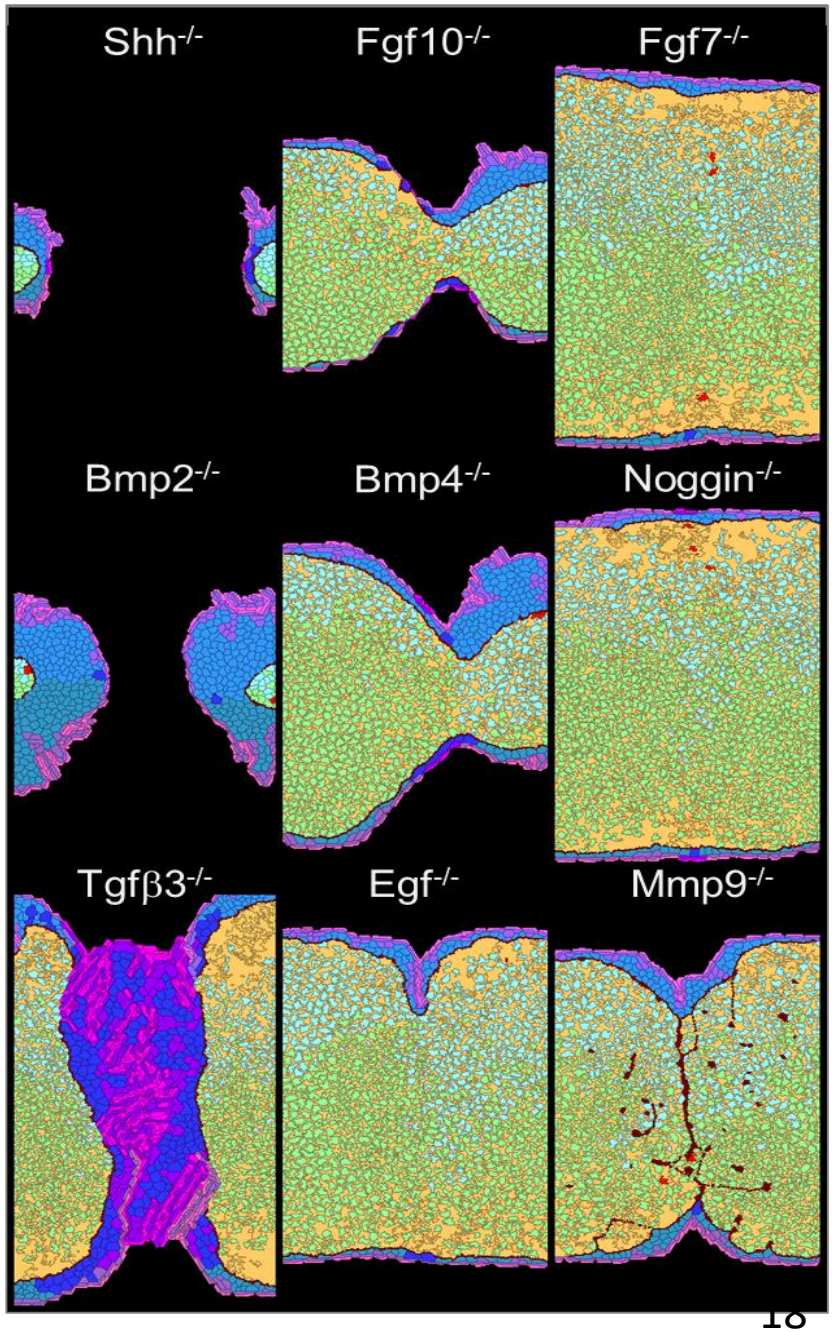




SOURCE: Hutson et al. (2017) Chem Res Toxicol

Hacking the control network

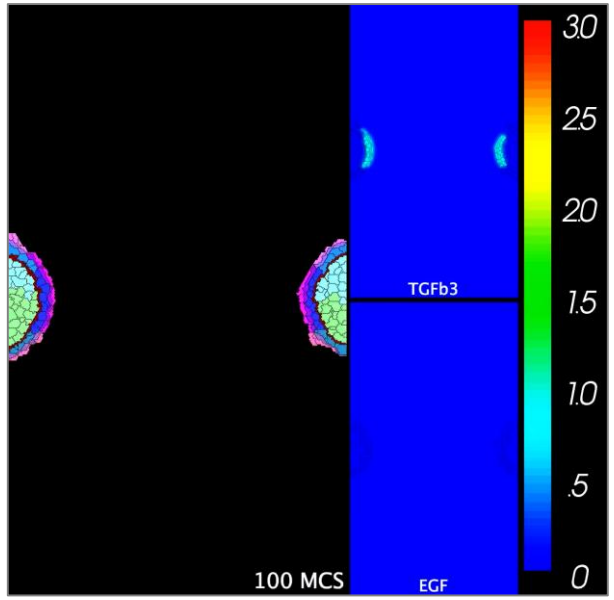


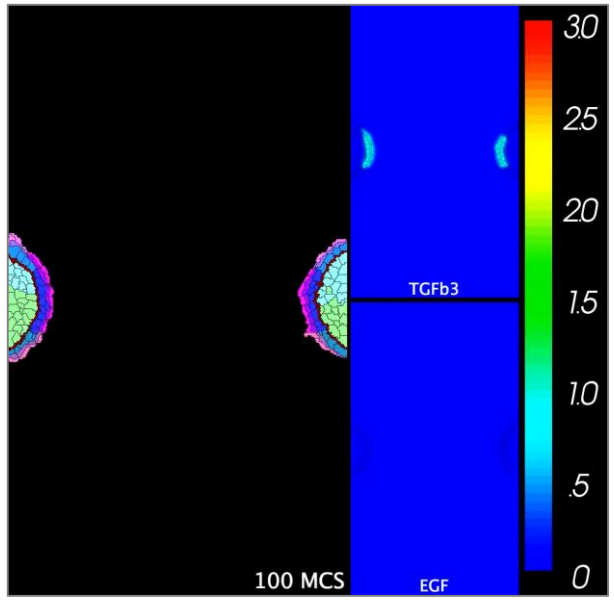


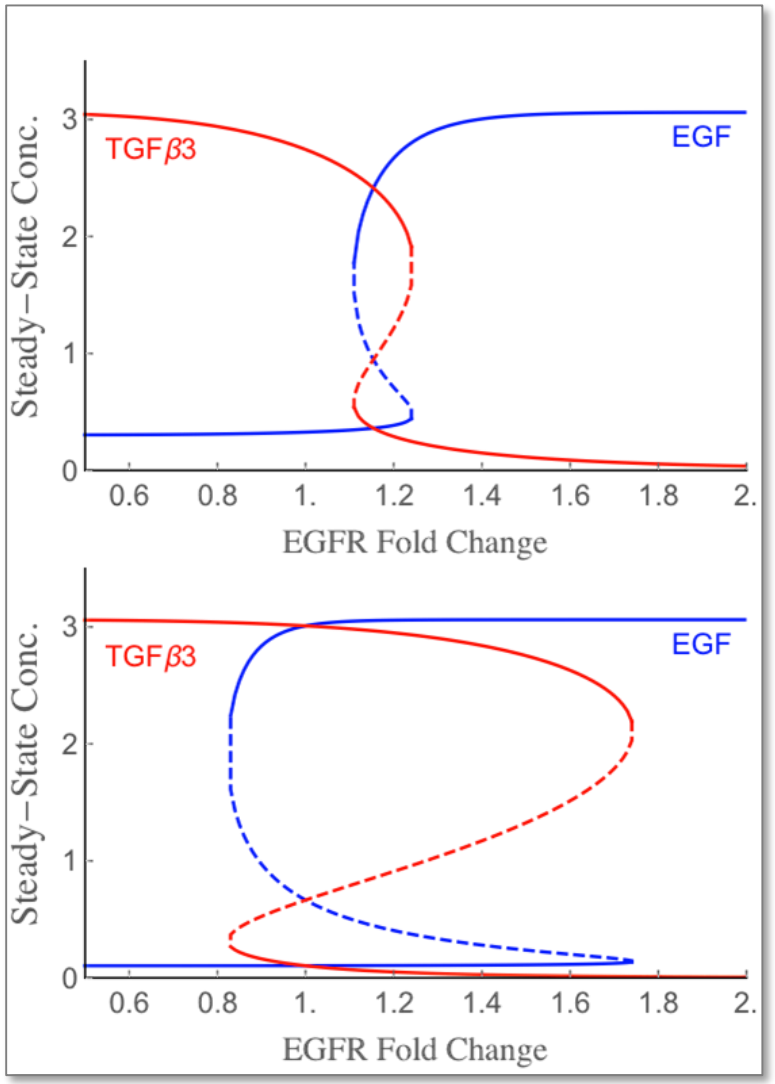
A.I. = synthetic cell signaling networks

Cybermorphs = simulated loss of function

Messin' with the switch: *two scenarios for bistable dynamics*







Narrow hysteresis:
less resilient but reversible

Broad hysteresis:
more resilient but irreversible

ToxCast dataset: *54 ↑EGF-signaling; some also ↓TGF-beta signaling*

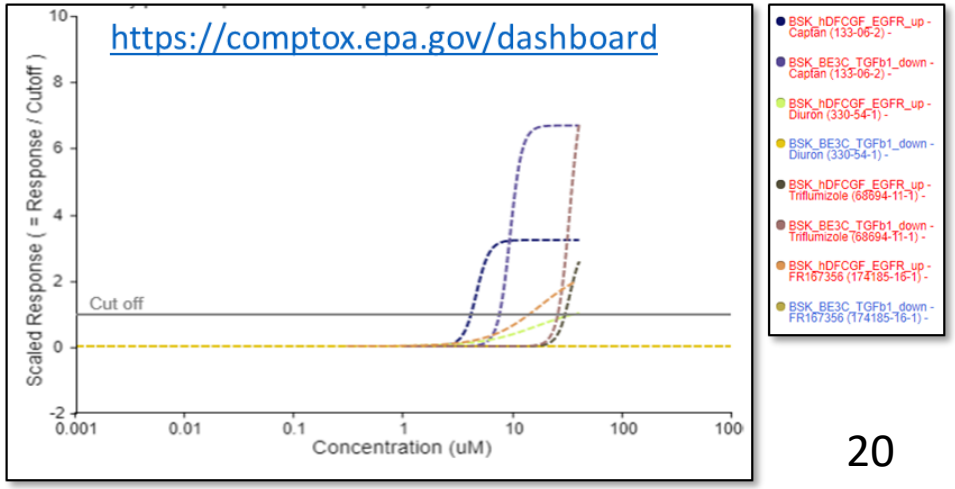
ChemicalName	FR_up(A	GFR_up(	1_down	b1_dow	ToxRef(lo
Methylene bis(thiocyanate)	1.14	2.13	5.93	4.26	NEG
Zoxamide	14.22	1.85	17.37	9.69	NEG
2-(Thiocyanomethylthio)benzothiazole	2.28	1.54	6.48	7.21	NEG
Diphenylamine	32.71	1.49	5.95	1.63	NEG
Azamethiphos	0.89	1.81	1000.00	1000.00	NEG
Bromacil	20.50	1.57	1000.00	1000.00	NEG
Forchlorfenuron	0.02	1.53	1000.00	1000.00	NEG
Methyl isothiocyanate	4.60	1.44	1000.00	1000.00	NEG
Diuron	16.51	1.44	1000.00	1000.00	NEG
Rotenone	0.82	1.42	1000.00	1000.00	NEG
Captan	4.59	2.57	7.15	7.25	POS
Triflumizole	32.71	2.48	19.88	19.88	POS
Butachlor	32.71	2.47	17.85	17.85	POS
Captafol	1.02	2.20	3.76	3.25	POS
Thiram	4.45	1.96	6.95	5.38	POS
Raloxifene hydrochloride	12.40	1.91	15.94	10.94	POS
Fluazinam	2.39	1.61	2.48	4.84	POS
Carbaryl	0.07	1.55	1000.00	1000.00	POS
Linuron	10.91	1.46	1000.00	1000.00	POS
Maneb	0.01	1.46	1000.00	1000.00	POS
Bendiocarb	8.75	1.43	1000.00	1000.00	POS
Fipronil	1.18	1.43	1000.00	1000.00	POS
Propoxur	1.67	1.43	1000.00	1000.00	POS
TNP-470	7.78	1.57	3.97	3.61	x
1-(2,3,8,8-Tetramethyl-1,2,3,4,5,6,7,8-octal	8.33	2.10	9.74	1.88	x
Trimethylolpropane triacrylate	2.02	1.80	5.17	1.41	x
Diiodomethyl 4-methylphenyl sulfone	3.15	1.77	3.74	17.68	x
1,2-Benzisothiazolin-3-one	8.22	1.74	11.91	14.70	x
Tralopyril	18.30	1.68	0.87	1.08	x
Bis(trichloromethyl)sulfone	1.95	1.61	4.49	5.74	x
N,N,N-Trimethyloctadecan-1-aminium chl	2.22	1.56	1.77	1.45	x
beta-Nitrostyrene	7.12	1.52	2.01	2.34	x
4,5-Dichloro-3H-1,2-dithiol-3-one	2.71	1.47	6.42	6.56	x
Tri-o-cresyl phosphate	8.95	1.45	9.54	1.56	x
Isobornyl methacrylate	13.66	1.44	21.86	1.97	x
SAR102779	0.05	1.43	12.95	14.97	x
PharmaGSID_48511	12.19	1.37	11.22	17.33	x
Perfluoroundecanoic acid	6.81	1.35	4.76	5.04	x
FR167356	17.65	2.06	1000.00	1000.00	x
Monobutyl phthalate	0.01	1.35	1000.00	1000.00	x
Niclosamide	0.58	2.14	1000.00	1000.00	x
Tripropylene glycol diacrylate	26.52	2.09	1000.00	1000.00	x
CP-457920	3.50	1.92	1000.00	1000.00	x
Trimethylolpropane trimethacrylate	32.85	1.81	1000.00	1000.00	x
alpha-Terpinyl acetate	39.18	1.64	1000.00	1000.00	x
3-(4-tert-Butylphenyl)-2-methylpropanal	35.26	1.62	1000.00	1000.00	x
1,4-Dinitrobenzene	2.95	1.54	1000.00	1000.00	x
SB281832	34.72	1.54	1000.00	1000.00	x
2-(Morpholin-4-yldithio)-1,3-benzothiazol	5.61	1.52	1000.00	1000.00	x
Tolclofos-methyl	7.71	1.49	1000.00	1000.00	x
1,1':3',1''-Terphenyl	11.98	1.38	1000.00	1000.00	x
Estrone	0.03	1.35	1000.00	1000.00	x

	↑EGFR		↓TGFβ1		ToxRefDB
µM effect in vitro	*AC50*	*top*	*AC50*	*top*	*DevTox*
Diuron	16.51	1.44	1000.00	1000.00	NEG
Rotenone	0.82	1.42	1000.00	1000.00	NEG
Captan	4.59	2.57	7.15	7.25	POS
Triflumizole	32.71	2.48	19.88	19.88	POS
Butachlor	32.71	2.47	17.85	17.85	POS
Captafol	1.02	2.20	3.76	3.25	POS
Thiram	4.45	1.96	6.95	5.38	POS
Raloxifene hydrochloride	12.40	1.91	15.94	10.94	POS
Fluazinam	2.39	1.61	2.48	4.84	POS
FR167356	17.65	2.06	1000.00	1000.00	x
Monobutyl phthalate	0.01	1.35	1000.00	1000.00	x

DevTox-negative in ToxRefDB

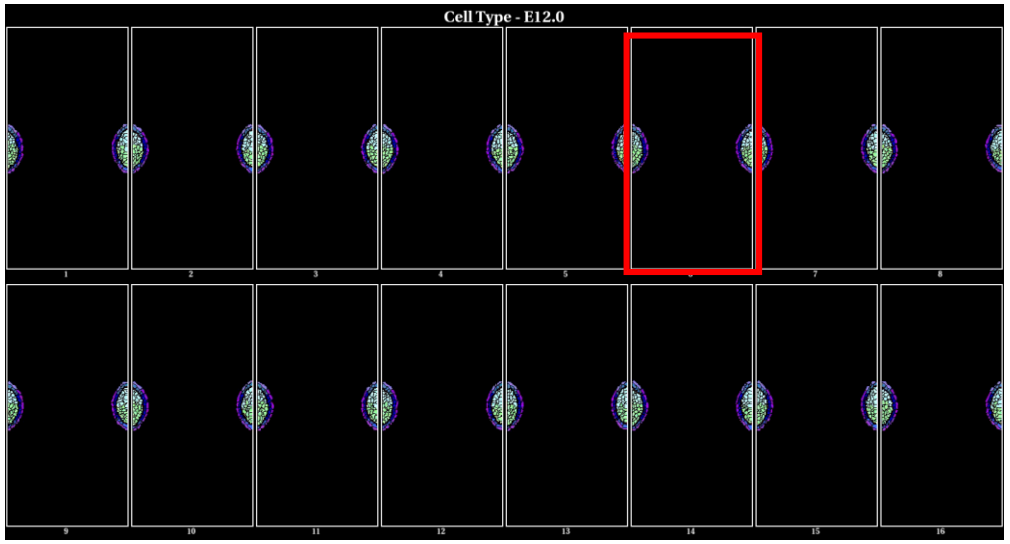
DevTox-positive in ToxRefDB

no DevTox data in ToxRefDB

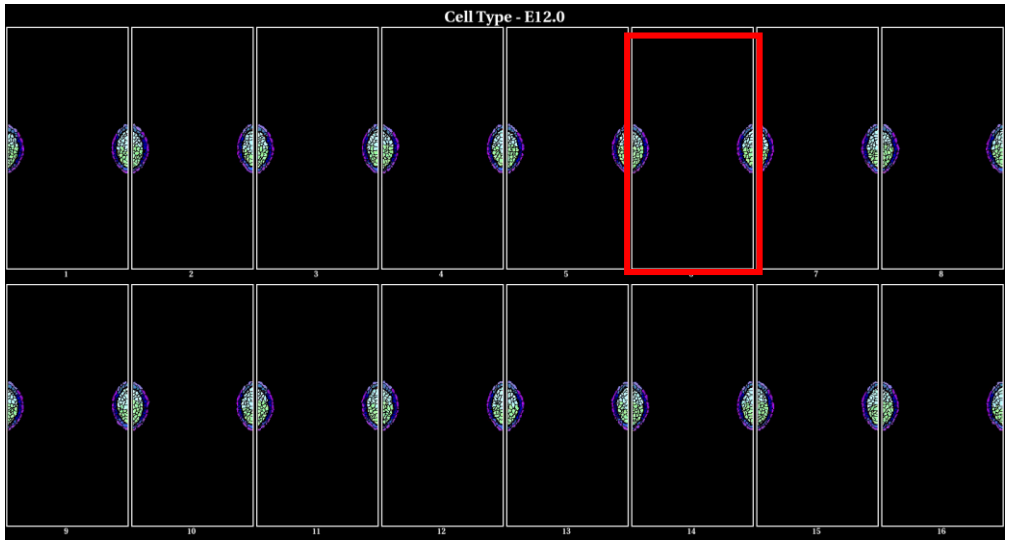


***In silico* dose-response:** translating ↑*EGFR conc. profile into a critical dose*

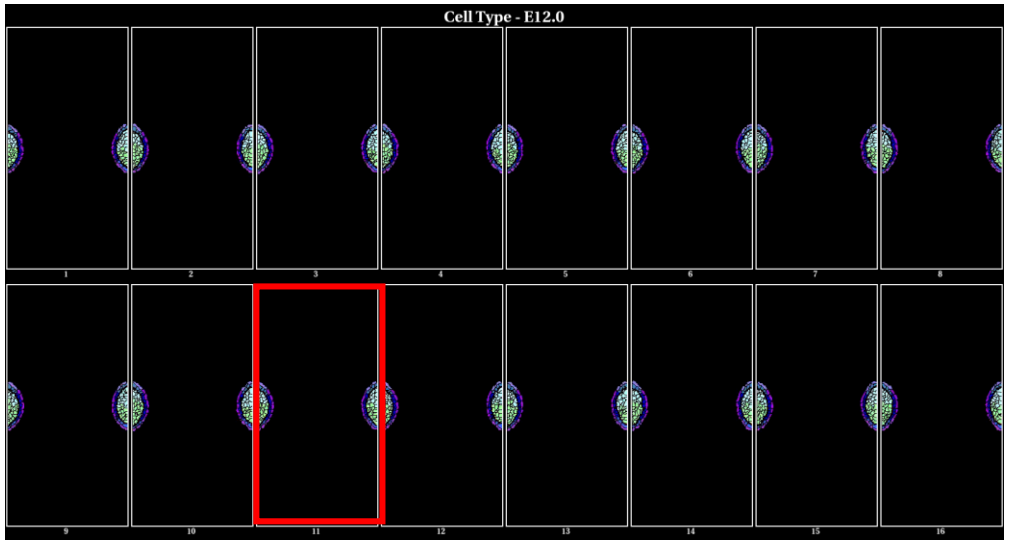
Captan



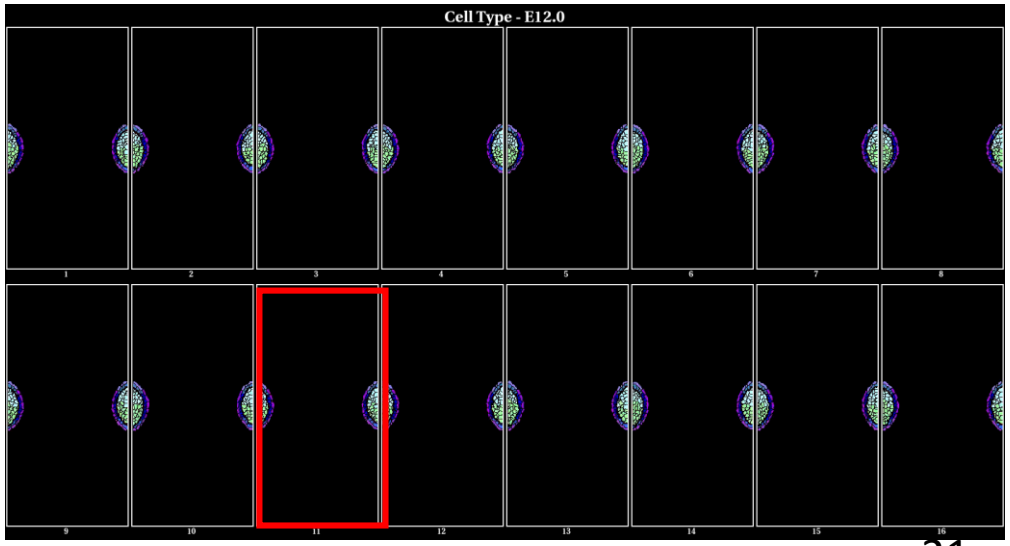
Fluazinam



Diuron

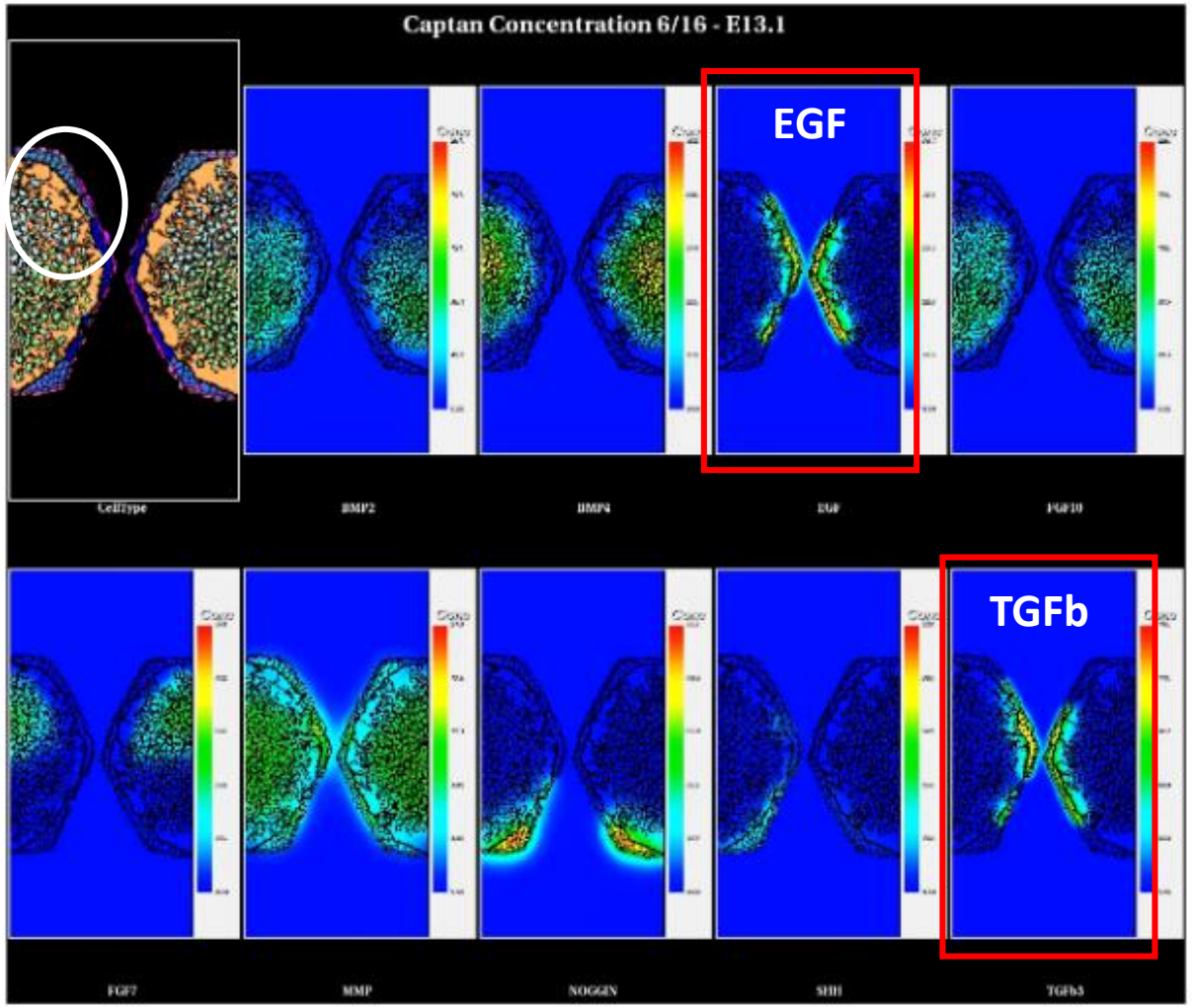


FR167356

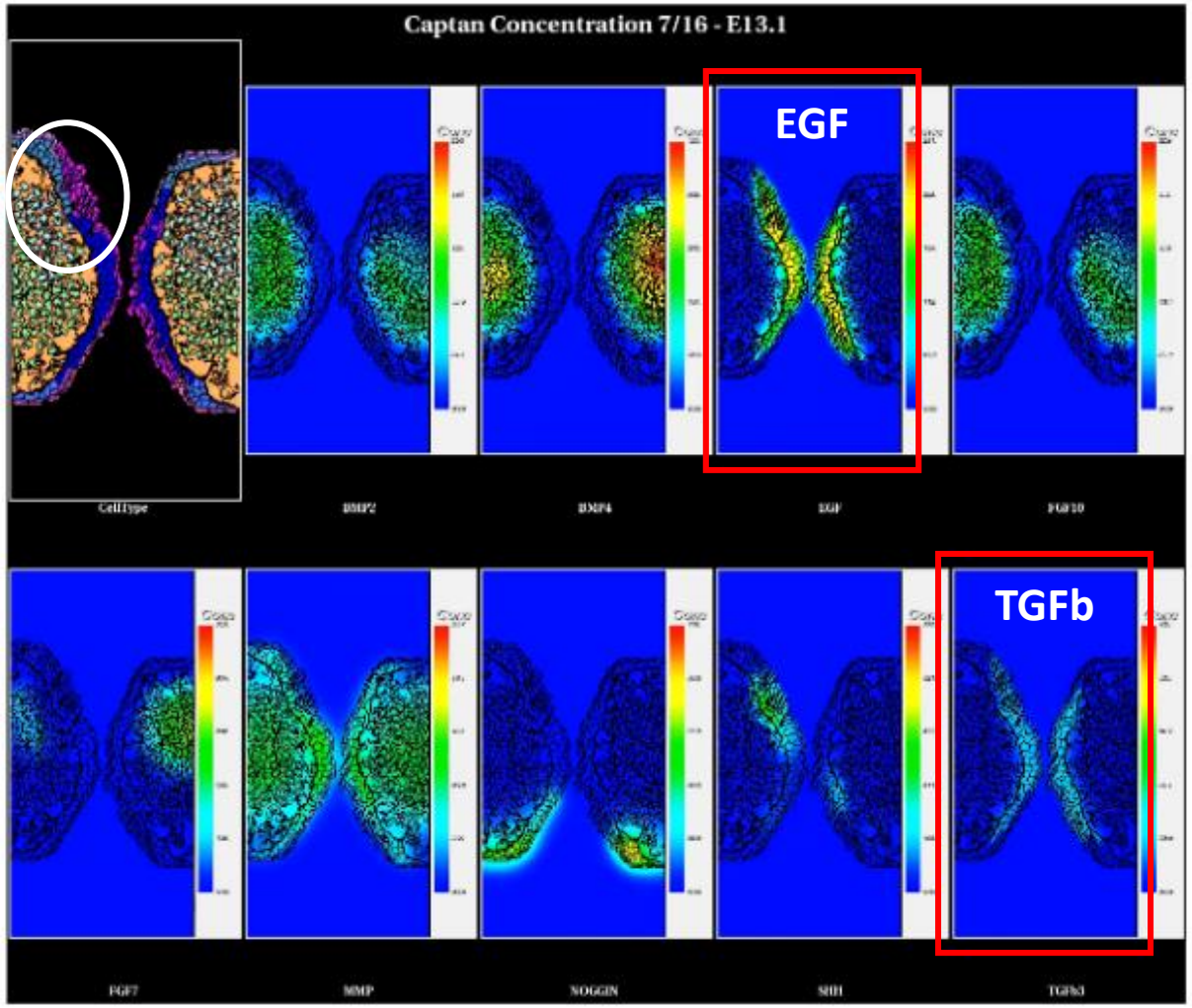


Pathogenesis: *simulating the prefusion alterations*

pre-critical dose

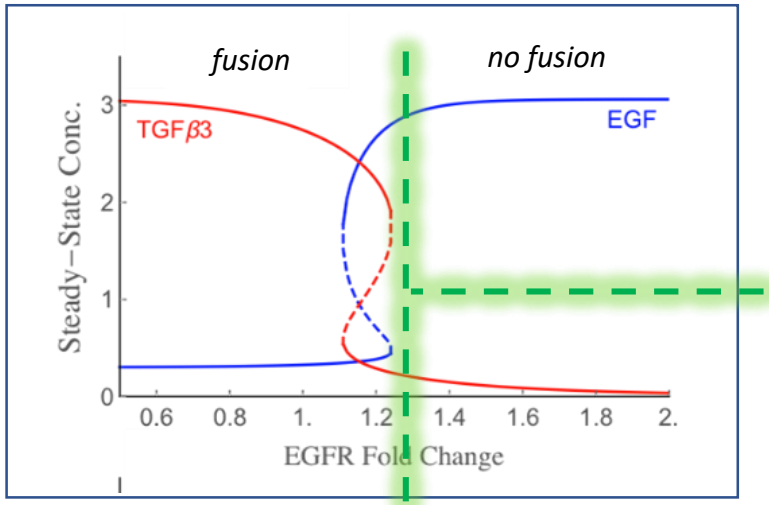


post-critical dose



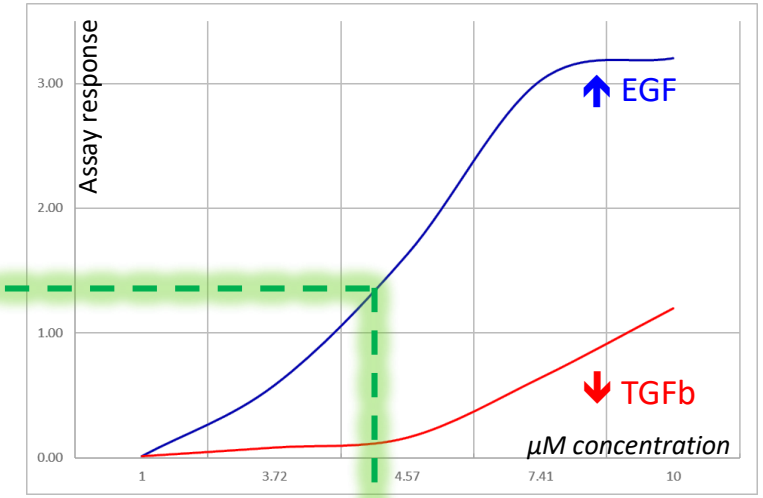
Predictive model: *modeling the critical phenomenon*

INPUT: switch dynamics

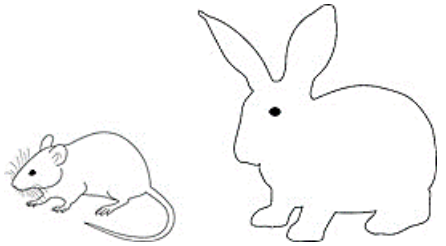


tipping point predicted by computational dynamics (hysteresis switch)

Captan in ToxCast



OUTPUT: tipping point mapped to concentration response (4 μM)

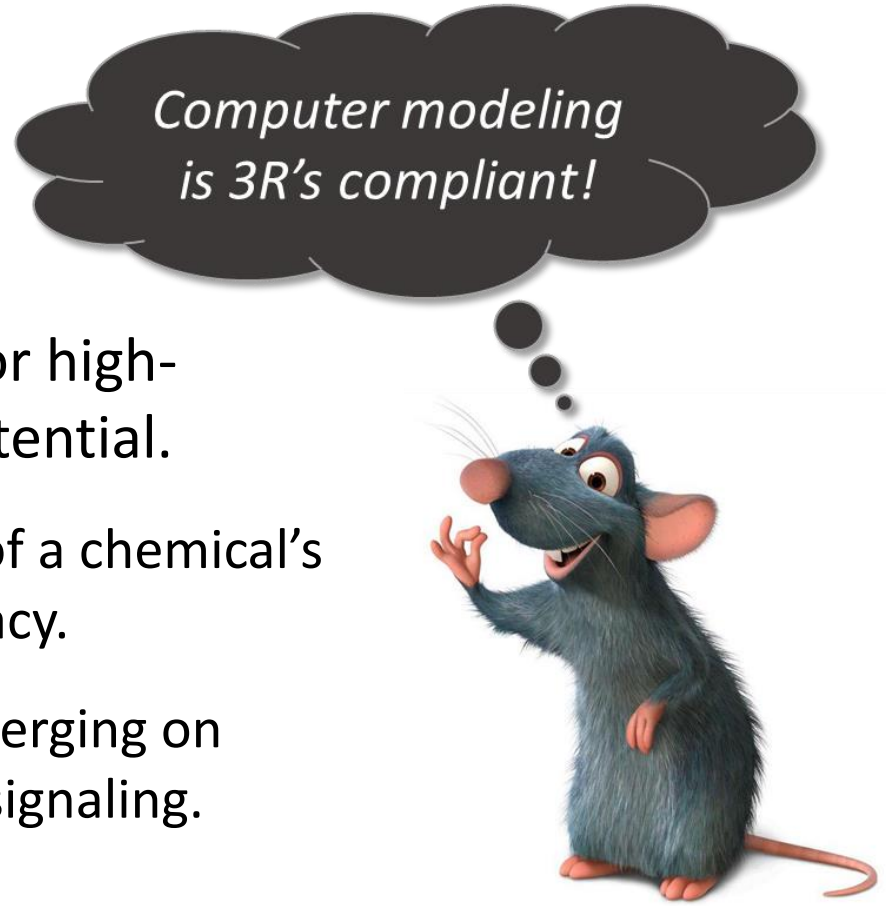


Captan in ToxRefDB
NEL = 10 mg/kg/day
LEL = 30 mg/kg/day

human HTTK model
2.39 mg/kg/day would achieve a steady state of 4 μM in fetal plasma

CompTox Chemicals Dashboard exposure prediction
0.88×10^{-7} mg/kg/day

Summary and Conclusions



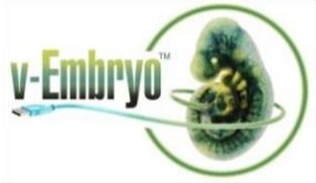
1. Several new approach methods (NAMs) are available for high-throughput screening chemical inventories for DevTox potential.

- STM assay in ToxCast gives an exposure-based readout of a chemical's DevTox hazard potential with up to 84% balanced accuracy.
- Assay sensitivity predicted high for kinase signaling converging on FoxO signaling but weak for estrogenic (ESR1) and G(q) signaling.

2. Cell ABMs recapitulate morphogenesis cell-by-cell and interaction-by-interaction in an embryonic system that self-organizes over time.

- These computer models translate MIEs through key events to simulate DevTox phenotypes and points of departure from HTS data.

Special Thanks



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Michael Colwell – Stemina

Jessica Palmer - Stemina

Nancy Baker – Leidos / NCCT

Richard Spencer - ARA / EMVL

John Cowden – NCCT/CSS

Keith Houck - NCCT

Sid Hunter – NHEERL / ISTD

Richard Judson – NCCT

Parth Kothiya – NCCT (now Indiana U)

Ann Richard – NCCT

Nathan Rush - NCCT

Kate Saili – NCCT

Rusty Thomas – NCCT

Todd Zurlinden – NCCT

Tox21 Cross-Partner Project #6

Nicole Kleinstreuer – NICEATM / NTP / NIEHS

Thomas Knudsen – NCCT / ORD / EPA

Annie Lumen – NCTR / FDA

Menghang Xia – NCATS / NIH