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

Todd J. Zurlinden, PhD
Computational Biologist
Center for Computational Toxicology and Exposure
Research Triangle Park NC 27711

4th Annual STAR Organotypic Culture Models (OCM) for Predictive Toxicology Research Centers

zurlinden.todd@epa.gov
ORCID 0000-0003-1372-3913

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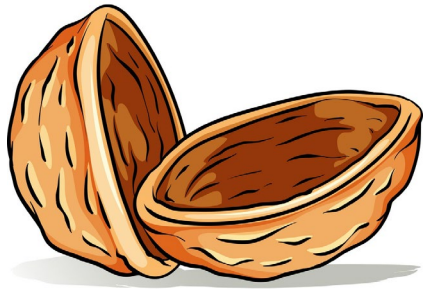


In a nutshell …

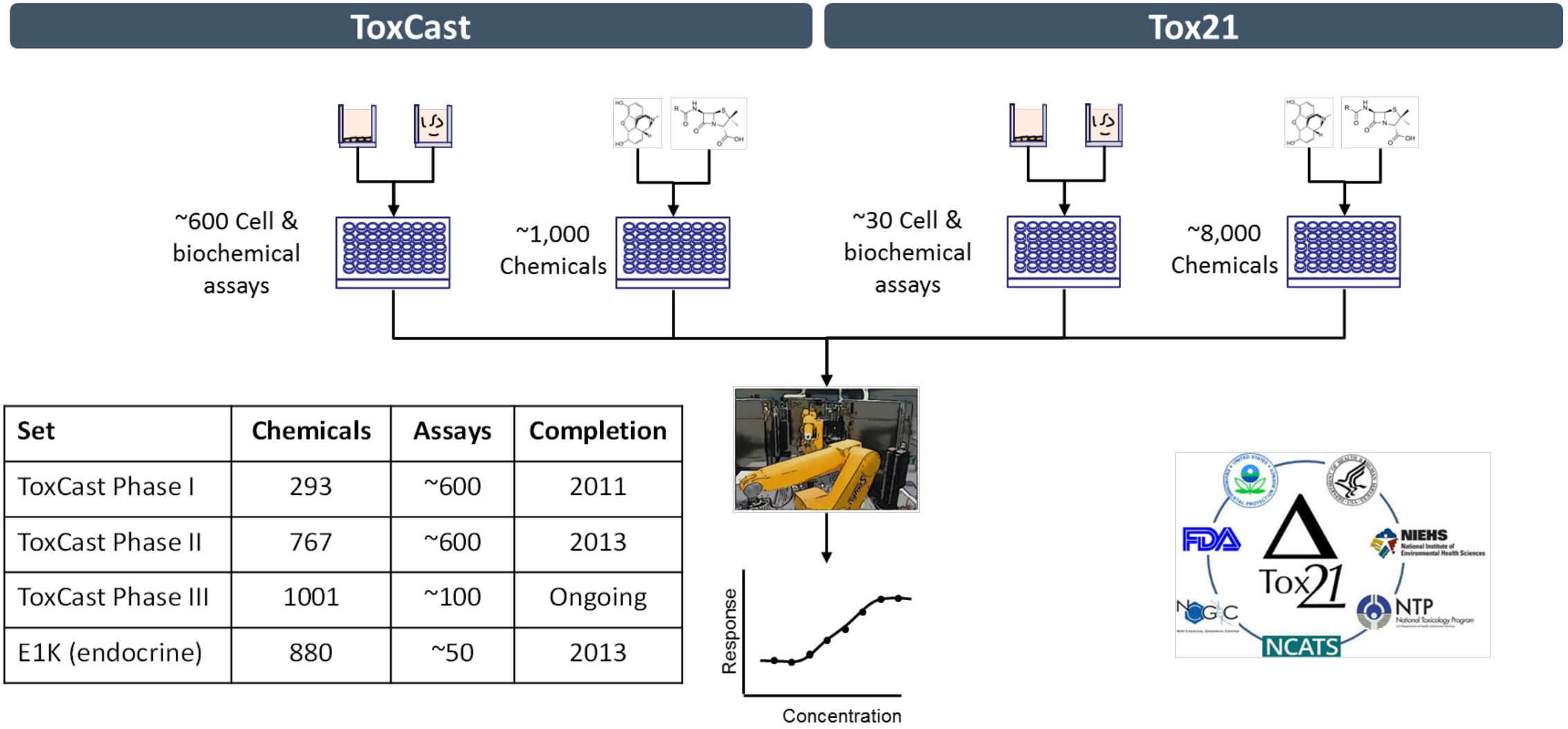
- Chemical exposure to a pregnant woman has the potential to affect her unborn child, leading to adverse birth outcomes and/or risks to early child development.

- 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

October is National Children's Health Month

Problem statement: *predictive DART*

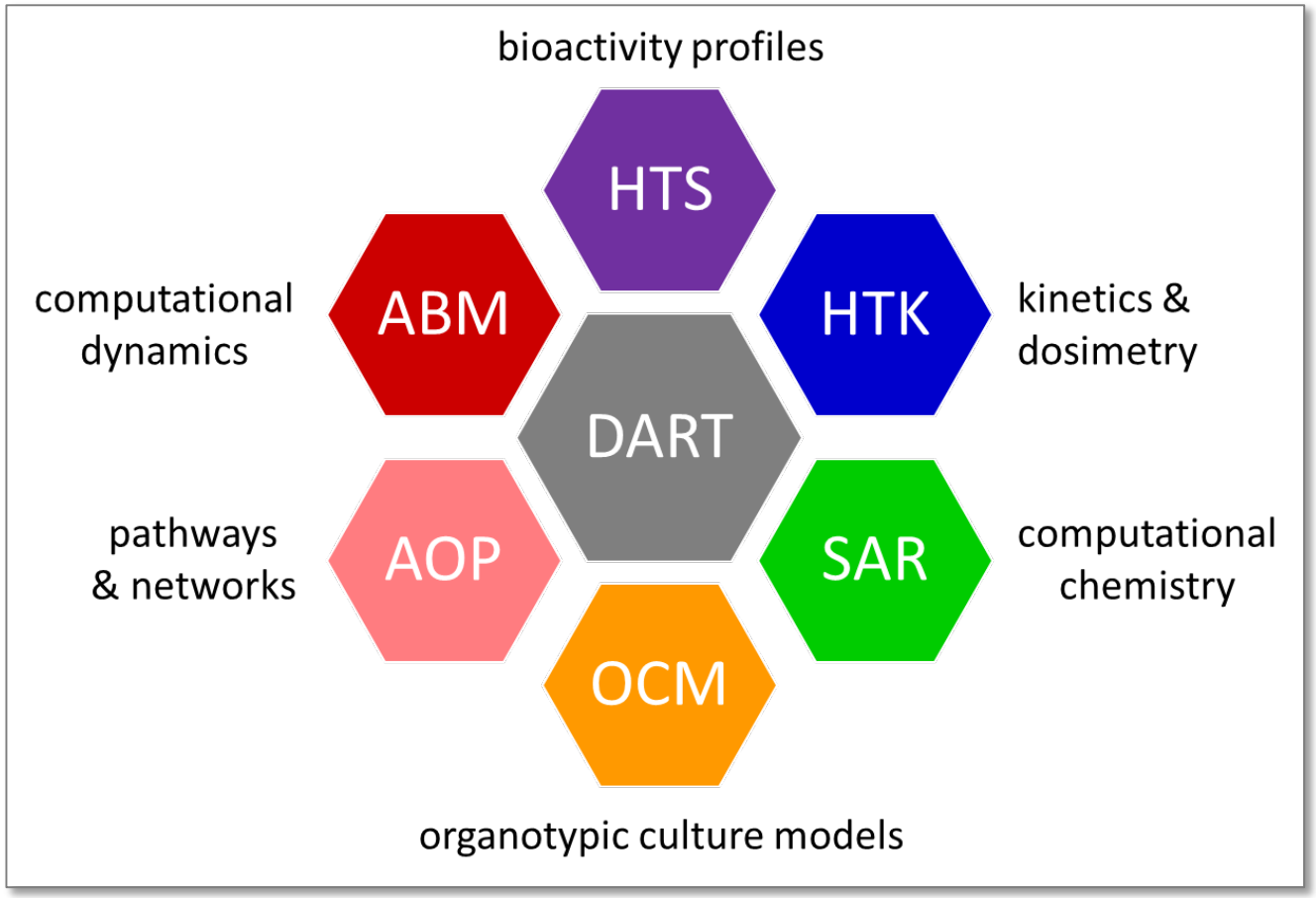
- **Objective:** increase the diversity and relevance of assays in ToxCast that can be used to profile chemicals for potential adverse effects on human embryonic development.

- 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.

- Traditional animal-based methods for assessing prenatal developmental toxicity (OECD TG 414) expose pregnant rats and/or rabbits during organogenesis and necropsy at term.

- Under reauthorized TSCA (2016) EPA must accelerate development of scientifically valid test methods to prioritize large numbers of chemicals with less reliance on animal testing.

Computational synthesis and integration



Fundamental principles:

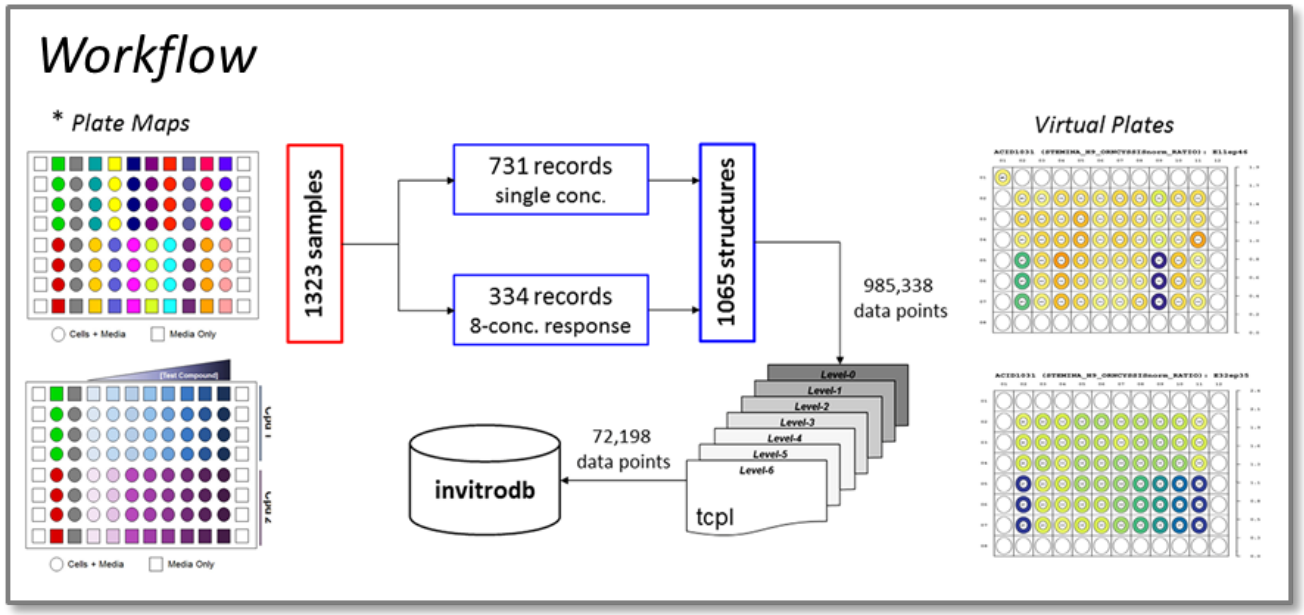
- initiating mechanisms (MIEs)
- genetic susceptibility (species, individual)
- critical periods (patterning, differentiation)
- bioavailability (chemistry, ADME)
- apical outcomes (pregnancy outcomes)

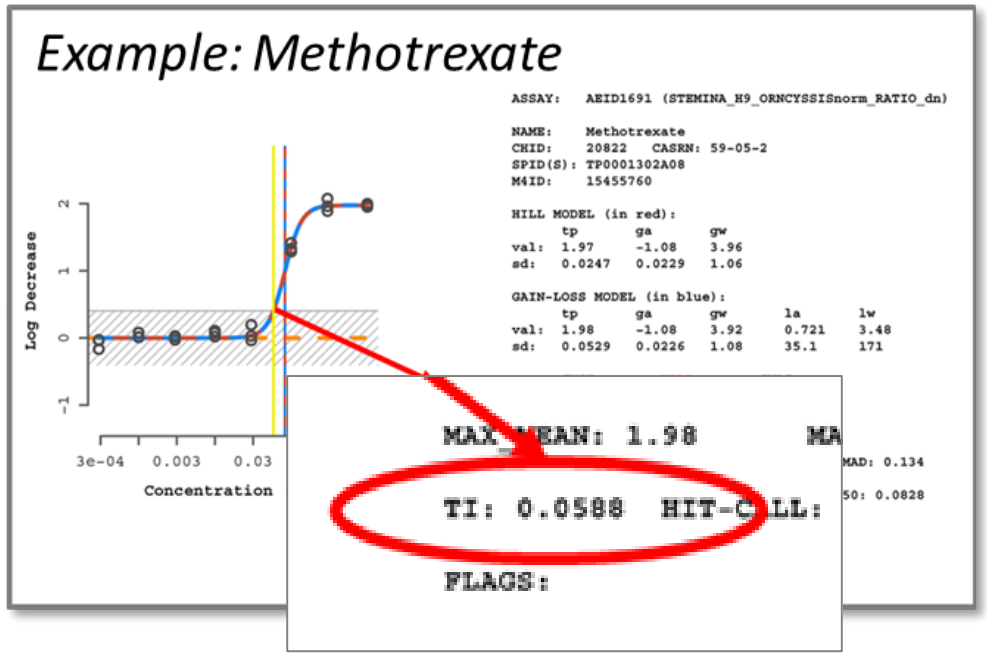
Case examples:

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

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

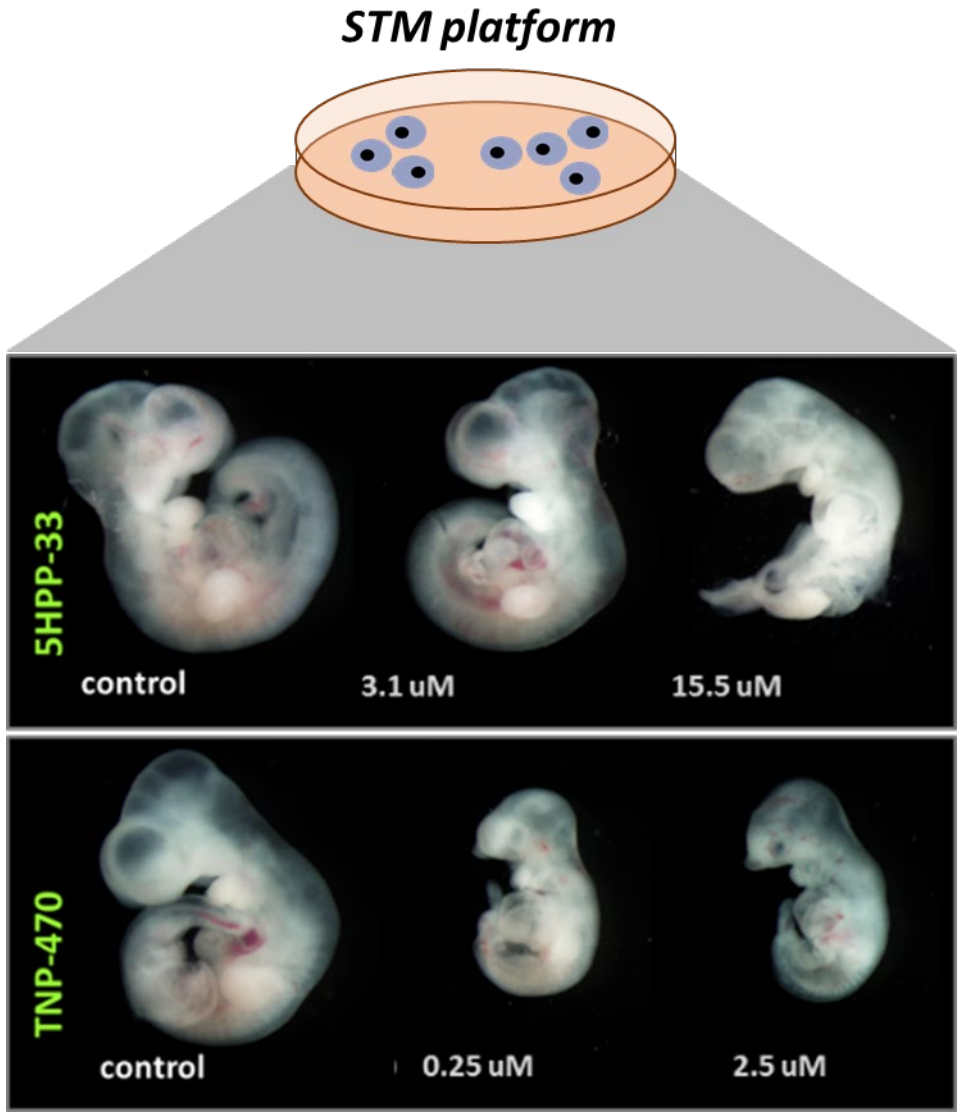
Key point: exposure-based potential for DevTox predicted by hESC assay on-the-mark both qualitatively and quantitatively.

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)*

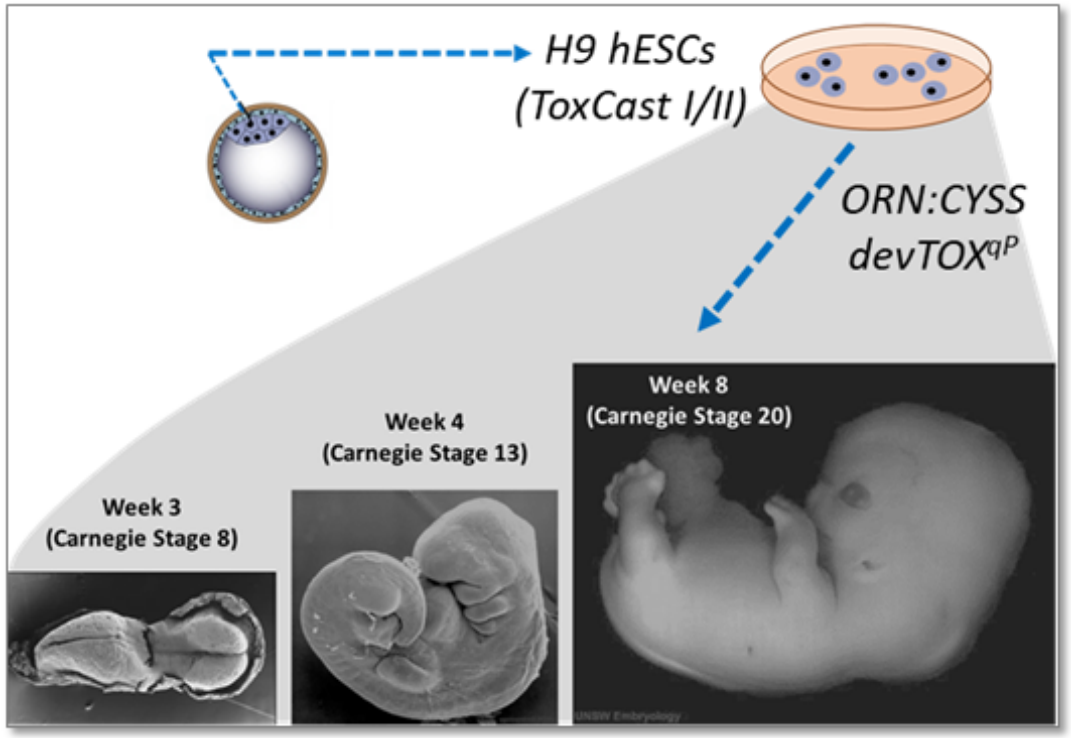
- **Key point**: sensitivity (hence balanced accuracy) improves with evidence for DevTox

in vivo / *in vitro*

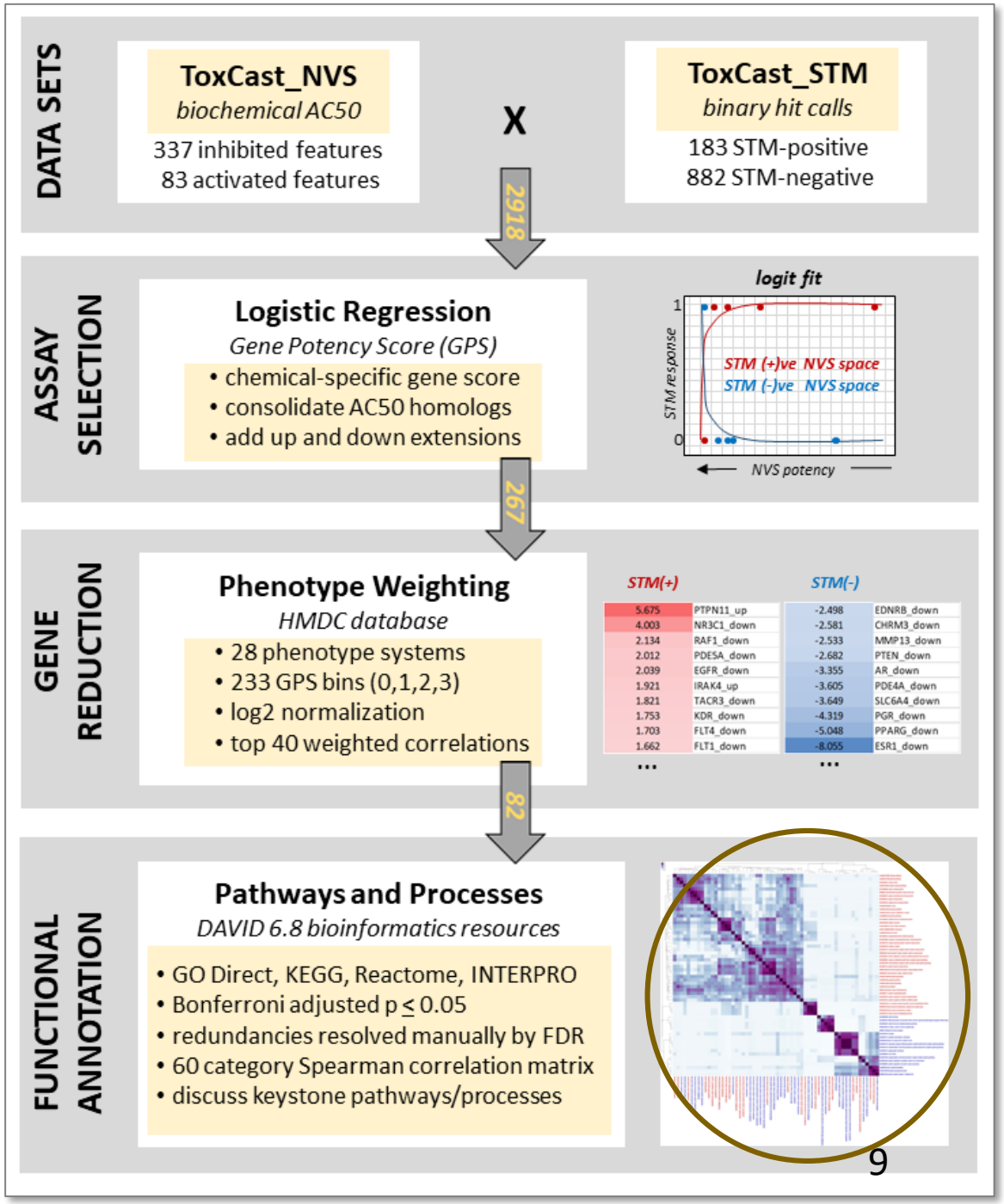
	TP	FP
	FN	TN

	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.



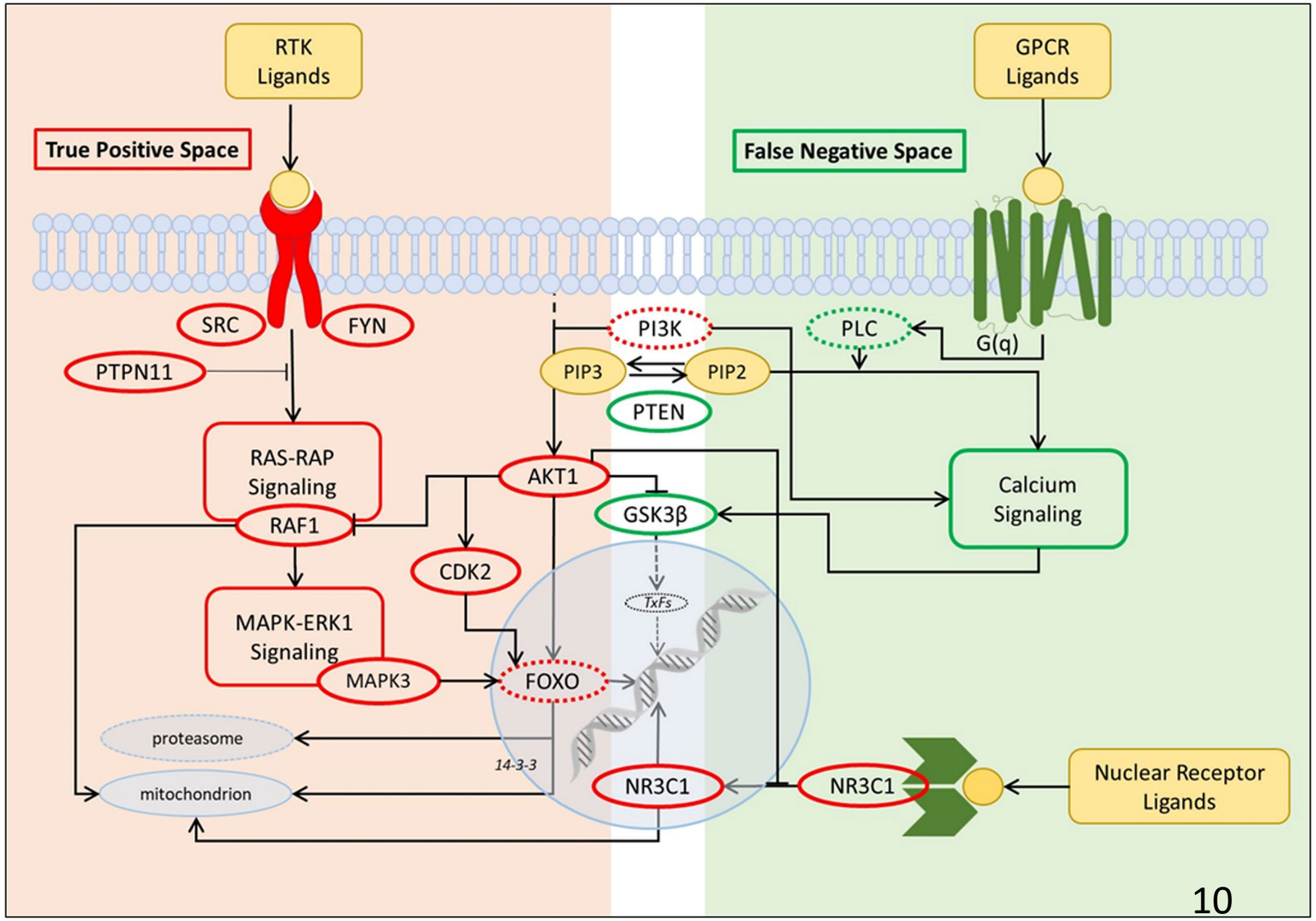
Keystone Pathways

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.

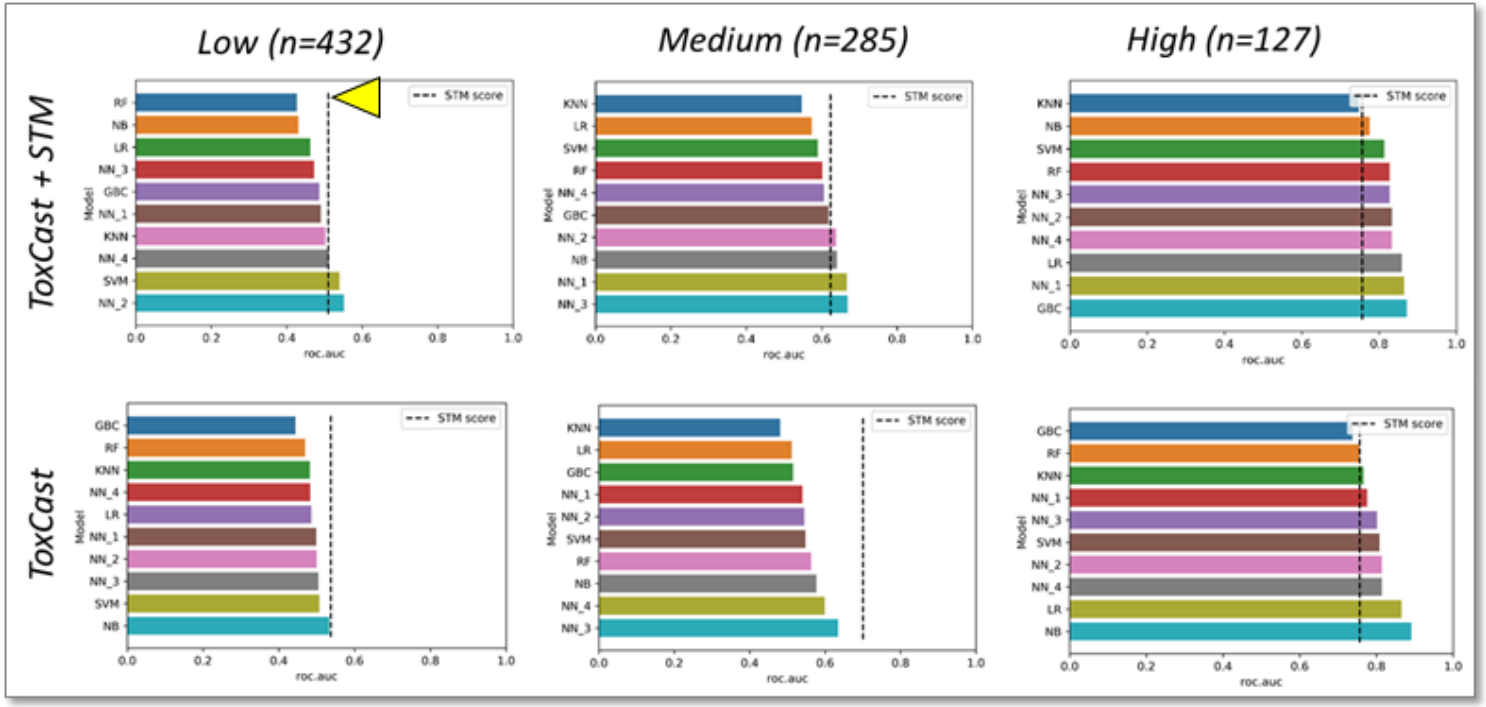
[2] Model performance reached 76% to 84% balanced accuracy with excellent specificity (>88%) but modest sensitivity (<66%) when anchored to apical endpoints in DevTox.

[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

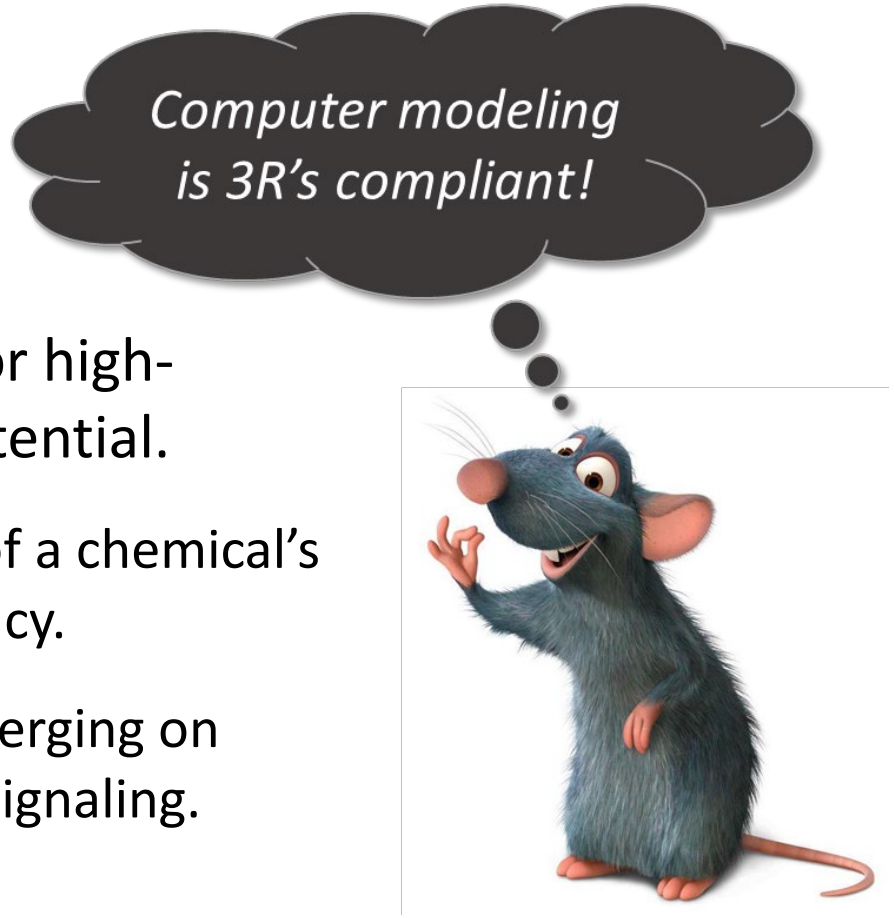
(----) ROC AUC for DevTox prediction using STM hit call alone.

- 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

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.

Special Thanks

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

EPA contract EP-D-13-055
Michael Colwell – Stemina
Jessica Palmer - Stemina

Tox21 Cross-Partner Project #6
Nicole Kleinstreuer – NICEATM / NTP / NIEHS
Thomas Knudsen – NCCT / ORD / EPA
Annie Lumen – NCTR / FDA
Menghang Xia – NCATS / NIH