Integrating *in vitro* and *in silico* data in a model of neurovascular developmental toxicity

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Background

Blood-brain barrier (BBB) development is mediated by complex signals commuted by diverse cell types of the embryonic neuroepithelium (neuroprogenitor cells/radial glia) and invading vasculature (endothelial cells, pericytes). Macrophage-like microglia cells derived from the hematopoietic lineage are incorporated into the rudimentary neurovascular structure. These cells are believed to play a dual role in neurovascular patterning, both as a local source of cytokine/chemokine signals that mediate self-organizing cell behaviors at the developing neurovascular interface and as an adaptive response to alterations in the system. Here, we describe a predictive model for building and testing the **hypothesis that microglia are critical transducers of developmental neurotoxicity in response to prenatal exposure(s).**

Methods

Build
- Literature curation identified 86 proteins important for BBB development (see biowiring diagram produced in CellDesigner v4.4)
- Compared amino acid sequence % similarity across species (SEQApass.epa.gov)
- Identified represented ToxCast assays (yellow in biowiring diagram)

Predict
- Identified putative microglia-mediated BBB toxicants from ToxCast chemical dataset (1857 chemicals) based on 23 ToxCast assay features mapping to the microglial compartment of the biowiring diagram
- Classified ToxCast chemicals according to toxicity signatures (i.e., assay hits and relative potency) for each ToxCast chemical using ToxPi 2.0 (beta)
- Clustered top 73 chemicals (highest ToxPi scores) based on signature similarity using Ward.D algorithm

Translate
- Used *in vitro* angiogenesis and neurogenesis studies (see below) as a surrogate of BBB development
- Filtered active chemical-associated ToxCast assays as a signature of NVU activity to begin to evaluate *in silico* predictions

38 ToxCast Chemicals EXPOSURES: Control: 0.1% DMSO; ArunA and VALA: 0.1 - 100 µM (5 conc.); FICAM: 0.0001 – 500 µM (5+ conc.)

Neurogenesis

ArunA — Assay source code (A)

hESC (H9) → hNP1, hNC → Cell titer (CT); Cell migration/proliferation (MG)

hNN2 → Neurite outgrowth (NC, NL, NN, BN)

Angiogenesis

VALA Sciences — Assay source code (V)

HUVEC (iPSC (in progress)) → Cell titer (CT); Tubule formation agonist (TUB+); tubule length (TL); Tubule formation antagonist (TUB-); tubule length (TL); Cell migration/proliferation (MG) wound area (wa), β-catenin (bc)

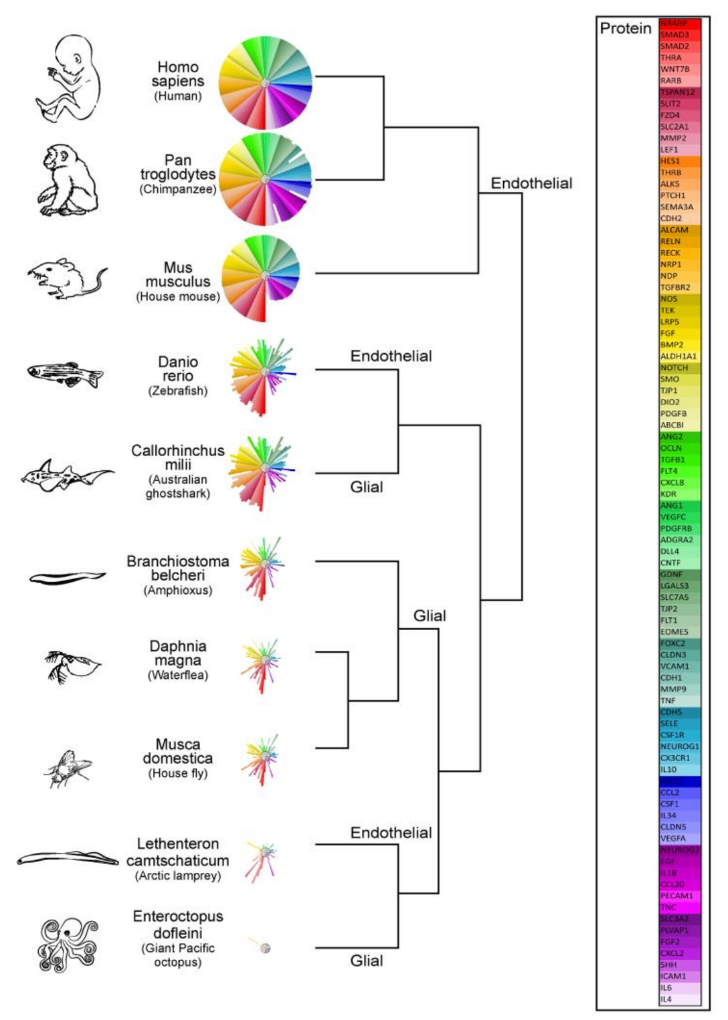
Ficam — Assay source code (F)

HUVEC → Cell titer (CT); Tubule formation antagonist (TUB-); tubule length (TL)

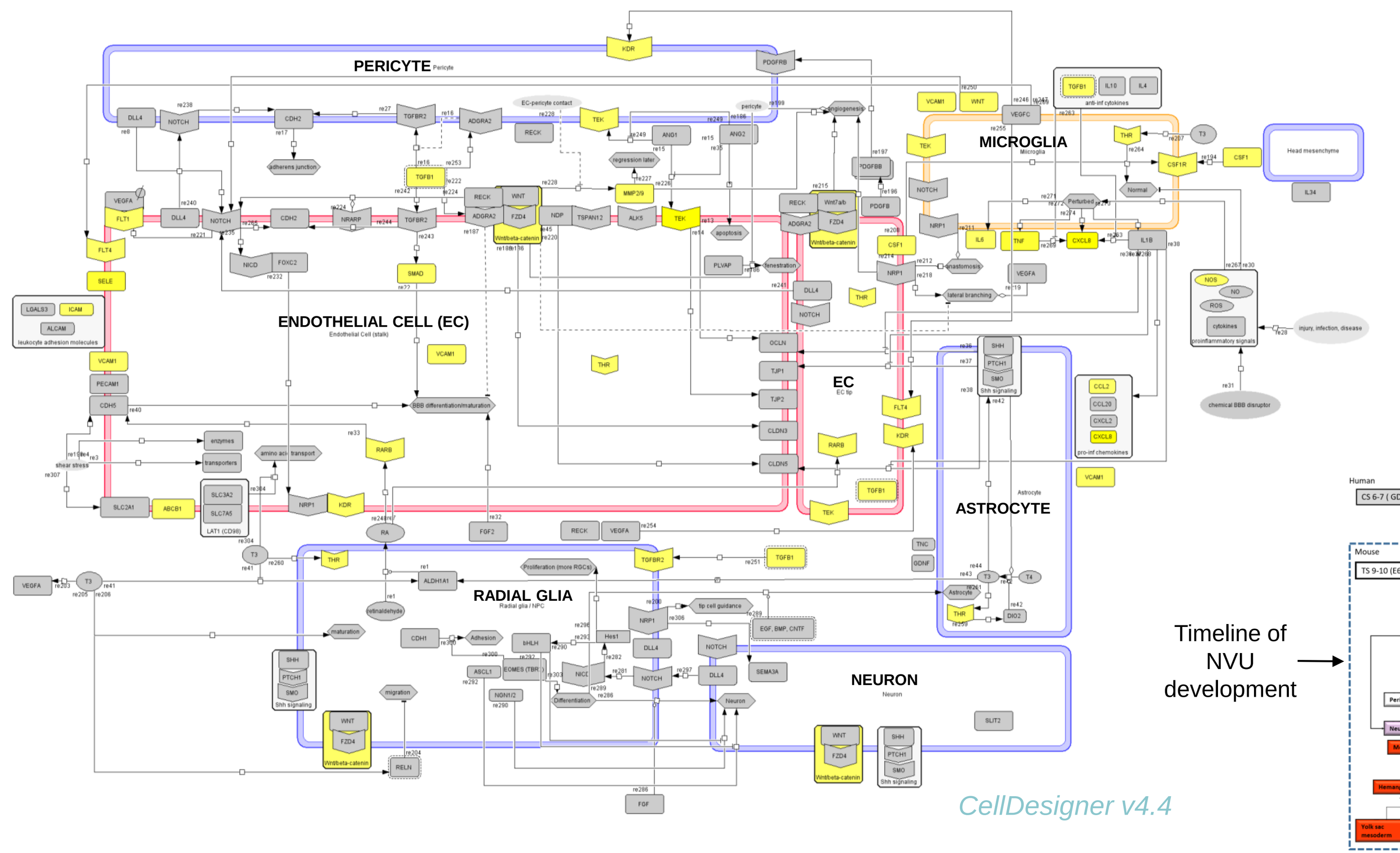
***In vitro* ToxPi analysis:** Chemical clustering was based on transformed (–log10(x)+6) values (x = µM concentration for each chemical-assay feature, where bioactivity is benchmarked relative to vehicle (DMSO) controls as a point of departure from background noise (i.e., ACB values). Assay annotation: [SOURCE_CELL TYPE_FEATURE_BENCHMARK]

Results

Building a model of molecular interactions leading to BBB development [1]



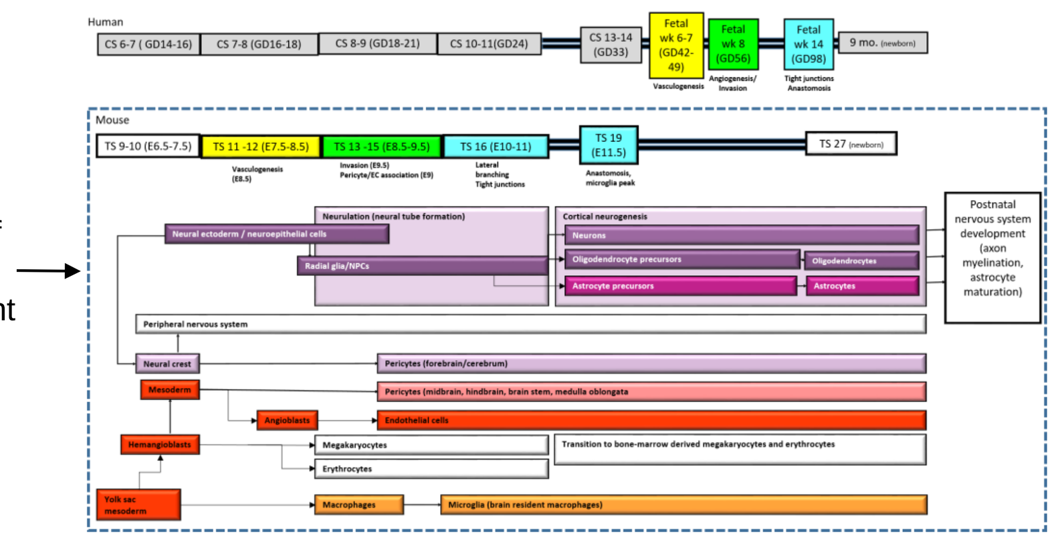
SEQApass and ToxPi v2.0 (beta)



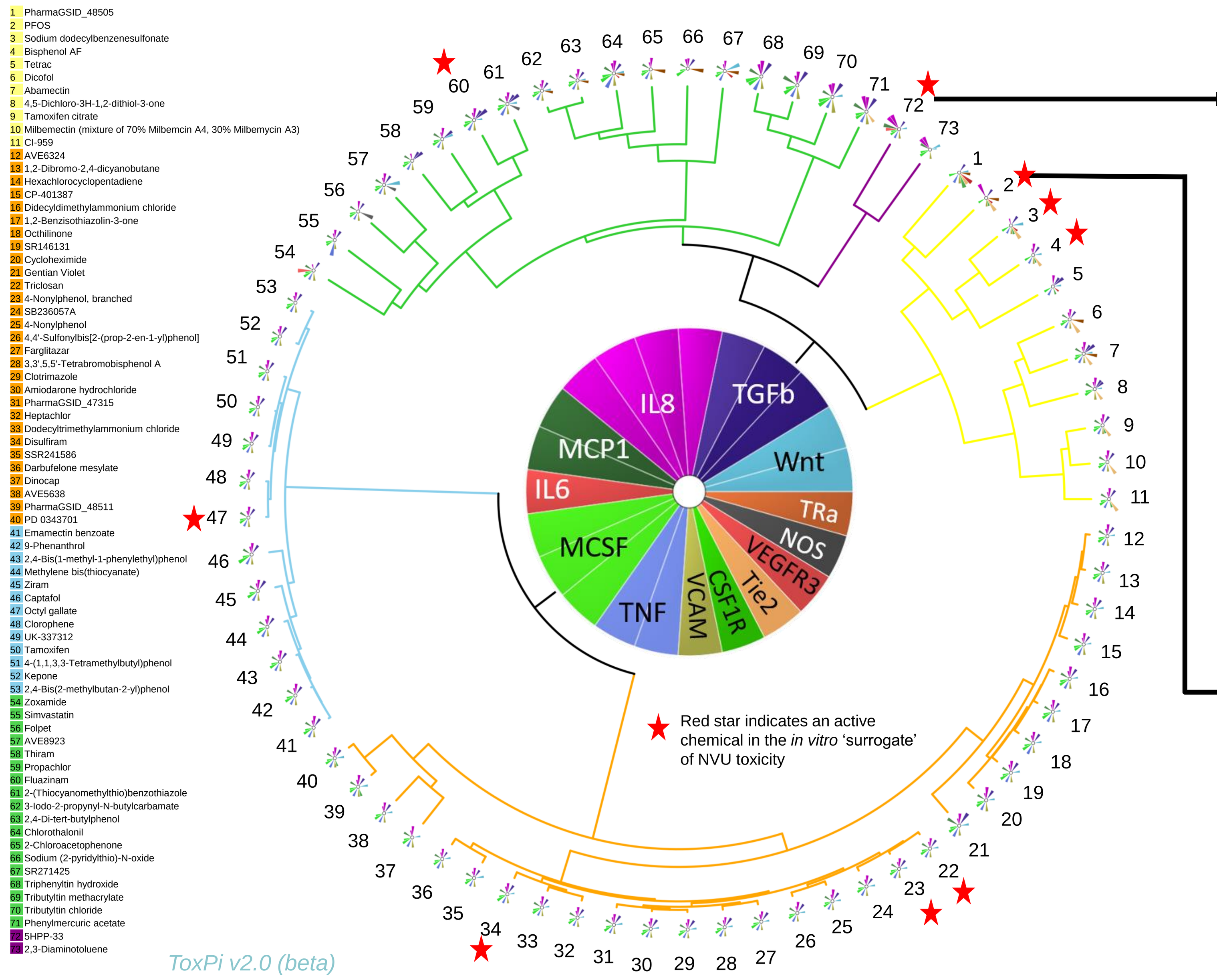
CellDesigner v4.4

CONTROL NETWORK FOR NEUROVASCULAR DEVELOPMENT: Biowiring diagram representing the main molecular events between cells of the neurovascular unit (NVU) during blood-brain barrier (BBB) development. Yellow: 32 ToxCast assays available for model development

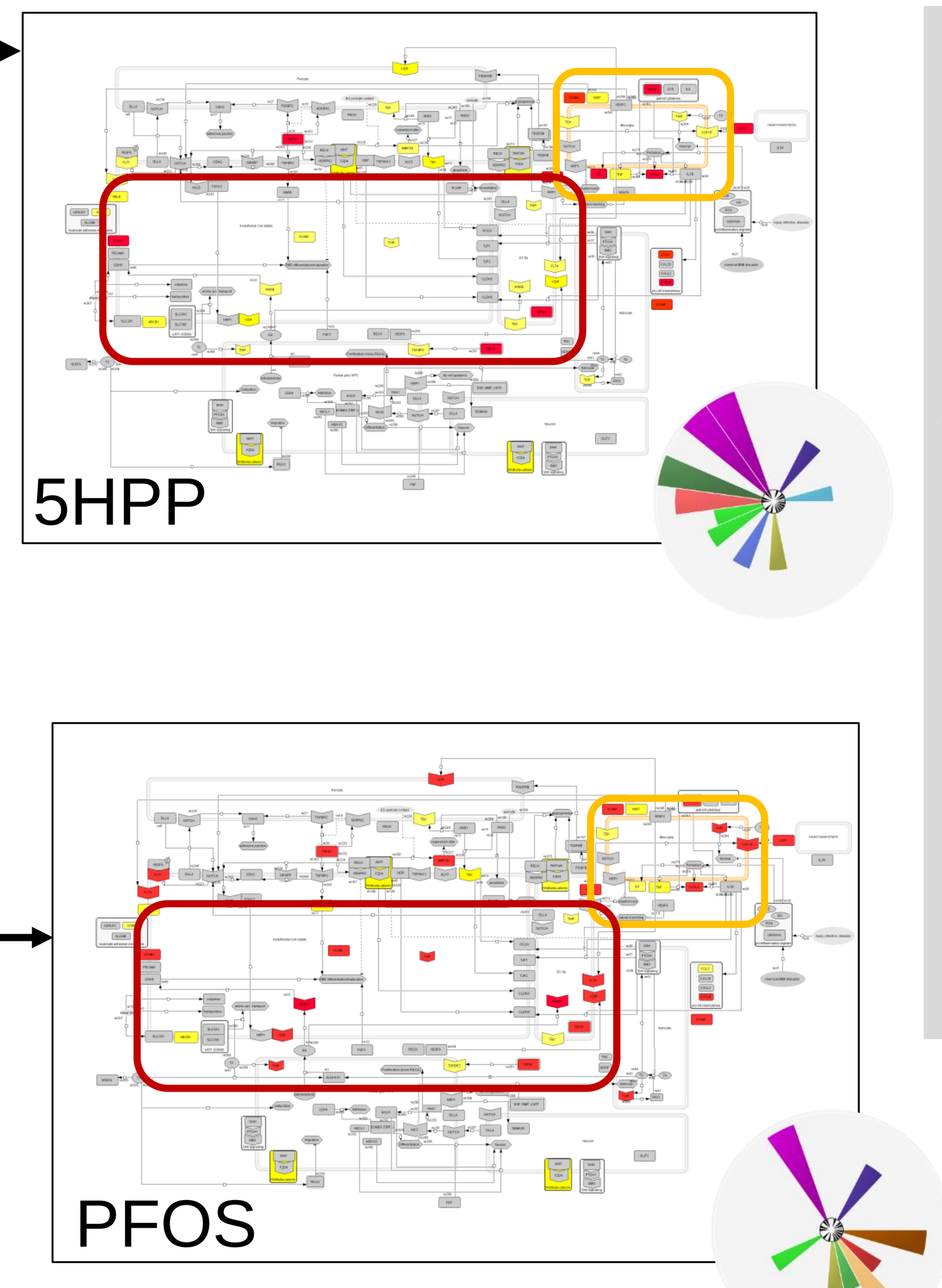
Timeline of NVU development



Predicting putative microglia-mediated BBB disrupting chemicals (*in silico* model)



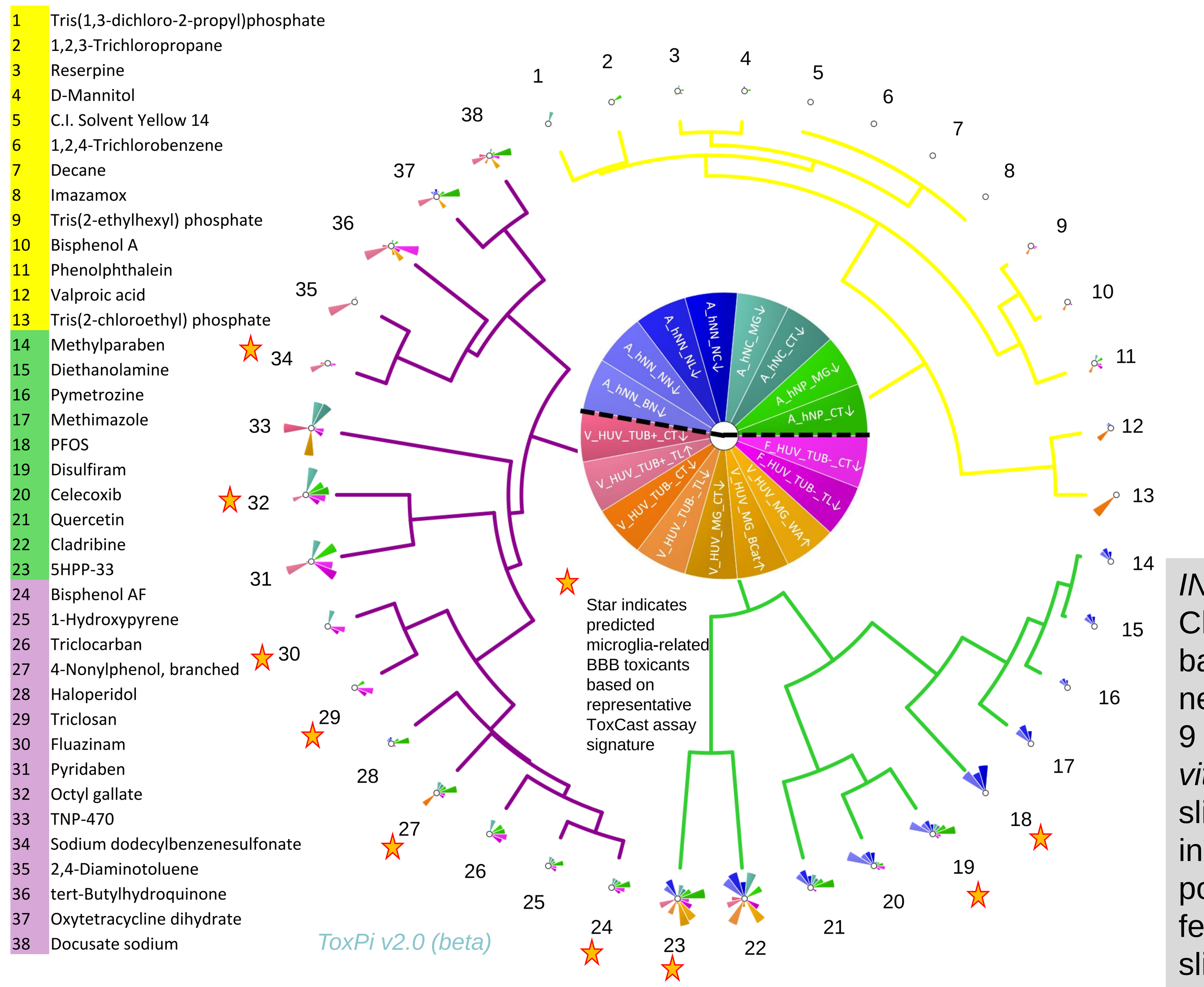
ToxPi v2.0 (beta)



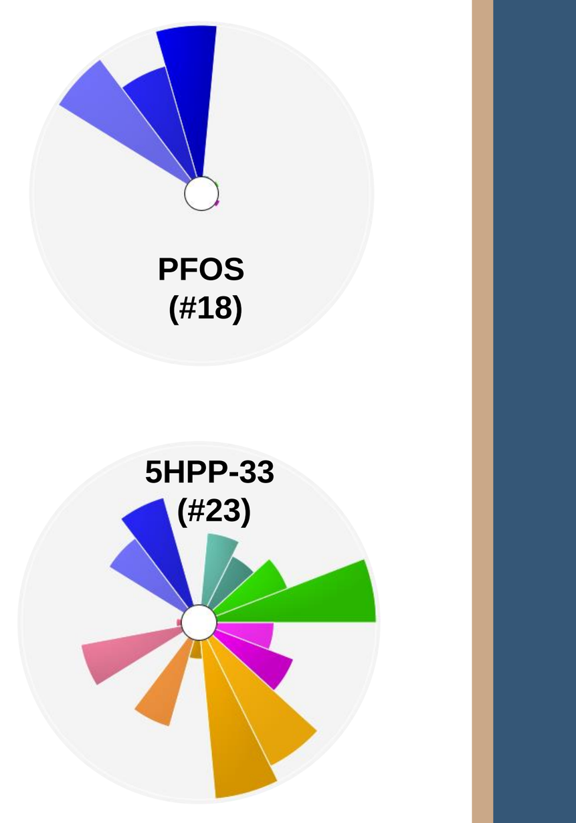
IN SILICO MODEL: Chemical clustering based on 23 microglia-related ToxCast assays. Pie slice length indicates relative potency for a given feature (longest slice = max potency). Examples include 5HPP-33, an analogue of thalidomide, which is known to disrupt angiogenesis and CNS development [2]; and PFOS, an industrial surfactant known to disrupt BBB integrity [3]

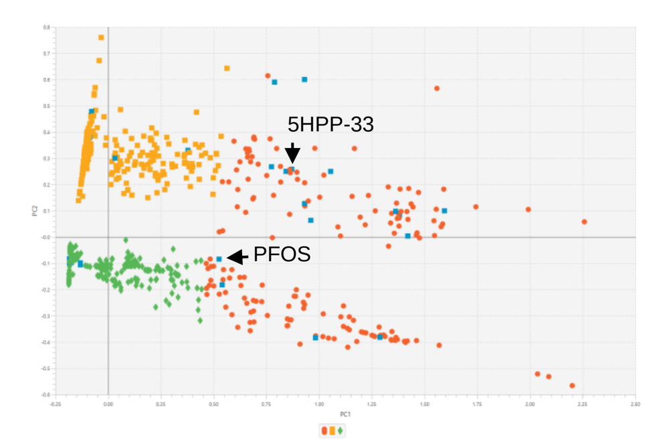
ToxPi v2.0 (beta) software provided by David Reif (dmreif@ncsu.edu) as part of U.S. EPA STAR #R835802.

Translating predictions with an *in vitro* surrogate of BBB toxicity



ToxPi v2.0 (beta)





K-means clustering of 1857 ToxCast chemicals based on 23 microglia-related ToxCast assays, with all 25 *in vitro* 'positives' in blue

IN VITRO DATA: Chemical clustering based on 8 neurogenesis and 9 angiogenesis *in vitro* assays. Pie slice length indicates relative potency for a given feature (longest slice = max potency). *In vitro* signatures for 5HPP-33 and PFOS are included for comparison to *in silico* signatures.

Summary and Conclusions

- A systems state map of BBB development was integrated with available ToxCast assays potentially representing microglia activity to predict putative BBB disrupting compounds that may act through microglia perturbation.
- Nine of the 25 'NVU-active' compounds in the *in vitro* assays were predicted to be BBB disrupting compounds based on activity in high throughput screening ToxCast assays.
- The current limitations of the model include inadequate ToxCast assay coverage, absence of key cells (e.g., microglia) from the *in vitro* testing platforms, and a limited *in vitro* chemical test set; adding these components may qualify the prediction and reduce uncertainty in the modeling.

References

[1] Saili, et al. *In submission*. Blood-brain barrier development: Systems modeling and predictive toxicology

[2] Hallene et al. 2006. Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience*

[3] Wang et al. 2011. Perfluorooctane sulfonate triggers tight junction "opening" in brain endothelial cells via phosphatidylinositol 3-kinase. *BBRC*