# Computational Modeling of the Neurovascular Unit to Predict Microglia Mediated Effects on Blood-Brain Barrier Formation

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# Disclaimers

- The authors have no financial or other interests which pose a conflict of interest.
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- Some of the data presented here were collected by Vala Sciences Inc. or ArunA Biomedical under contract to the US EPA.
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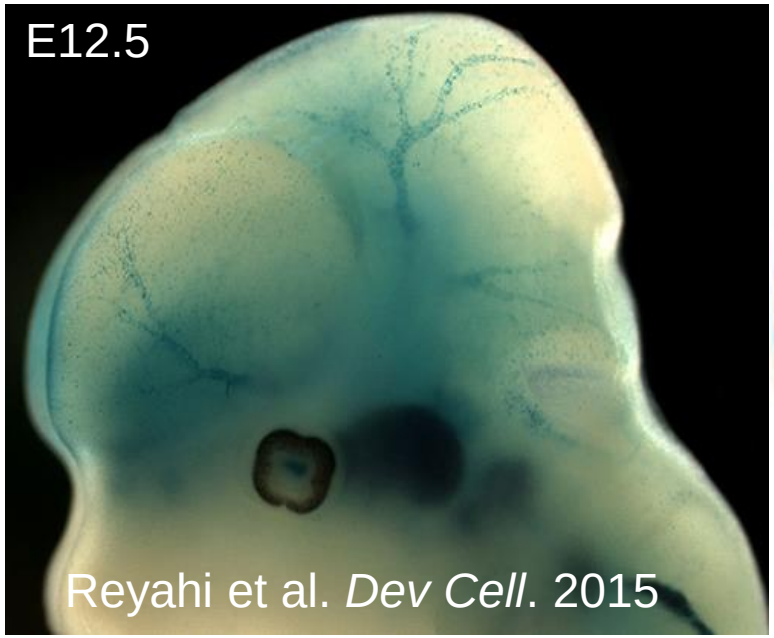
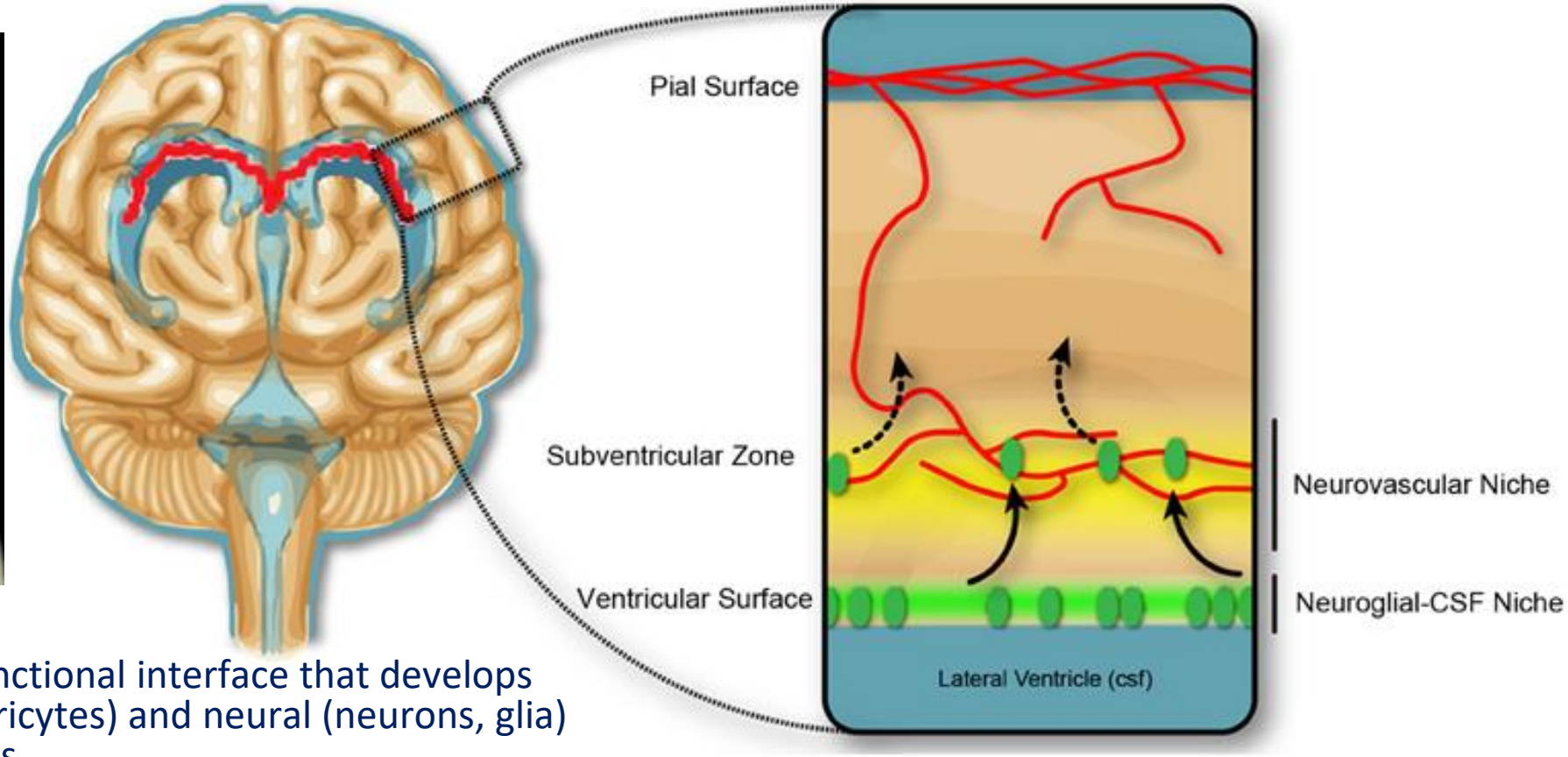
- The views expressed in this presentation do not reflect US EPA policy.

# Computational neurovascular unit (cNVU) focus

Chemical signals from the neuroepithelium (eg, VEGF) initiate brain angiogenesis via sprouting from the PNVP.



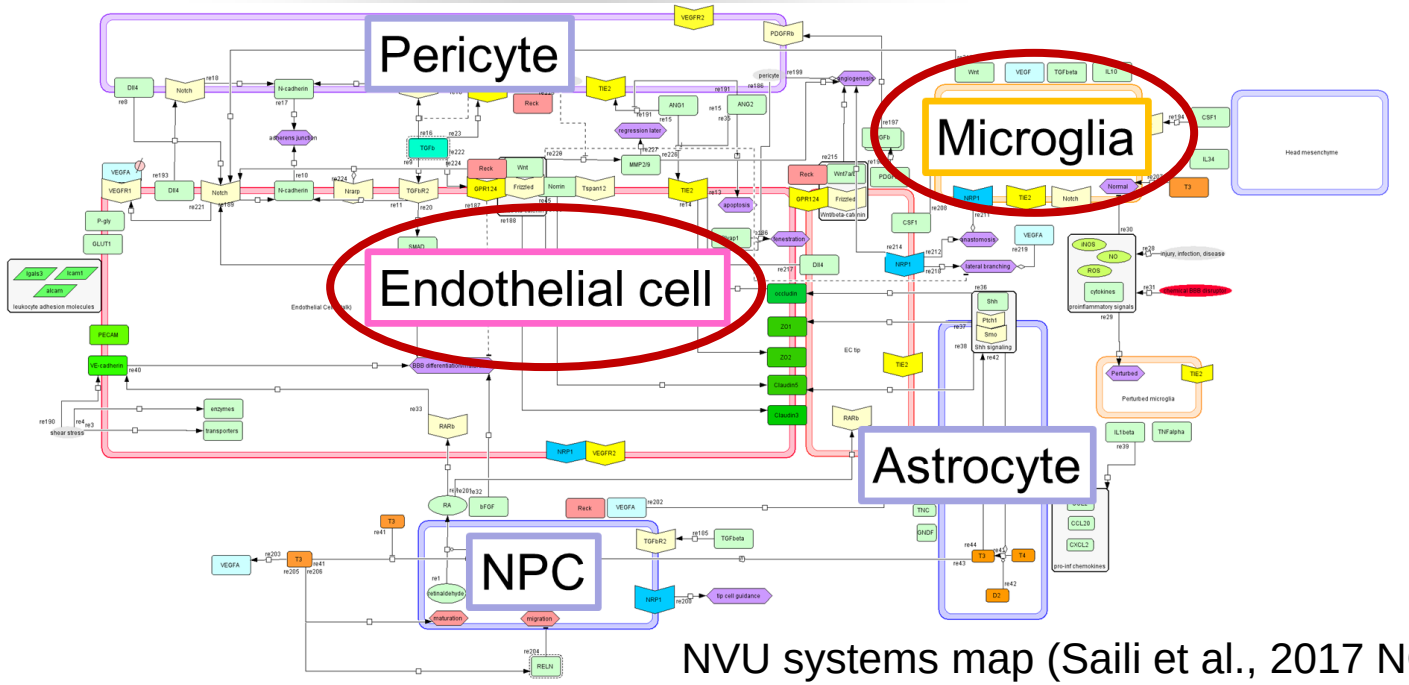




Stolp wt al., *Front. Integr. Neurosci*. 2013

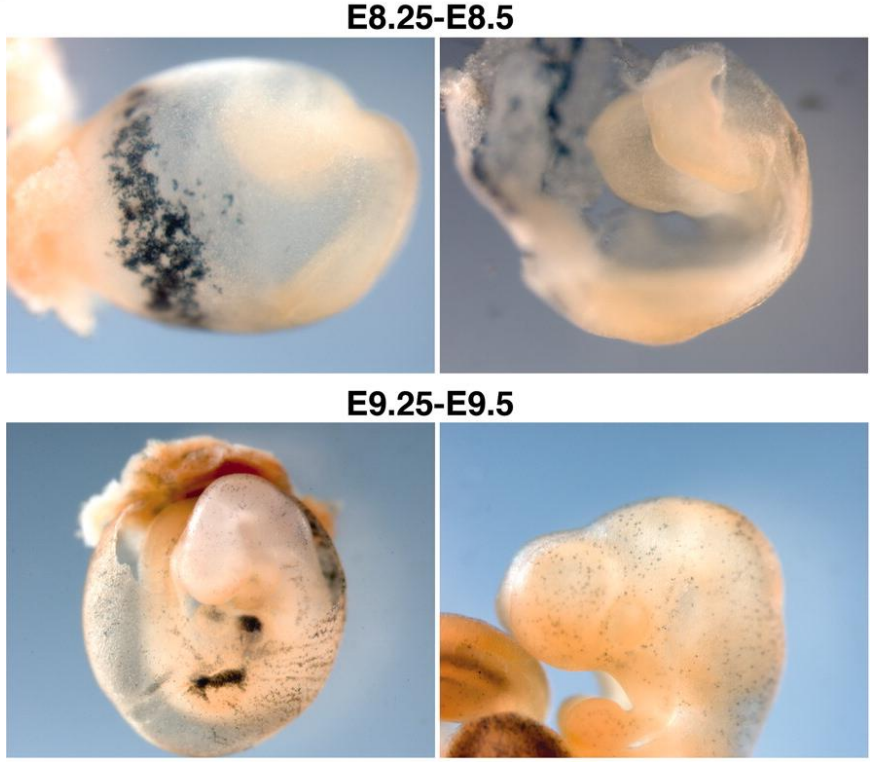
**Neurovascular unit (NVU):** The functional interface that develops between vascular (endothelial, pericytes) and neural (neurons, glia) compartments of the embryo-fetus.

**Hypothesis:** Chemical disruption of NVU development adversely impacts blood-brain-barrier (BBB) formation leading to abnormal brain development and function.

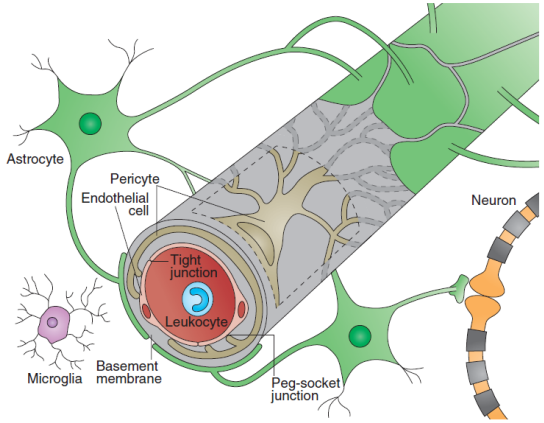
# Cell-Cell interactions of the NVU




NVU systems map (Saili et al., 2017 NCCT)



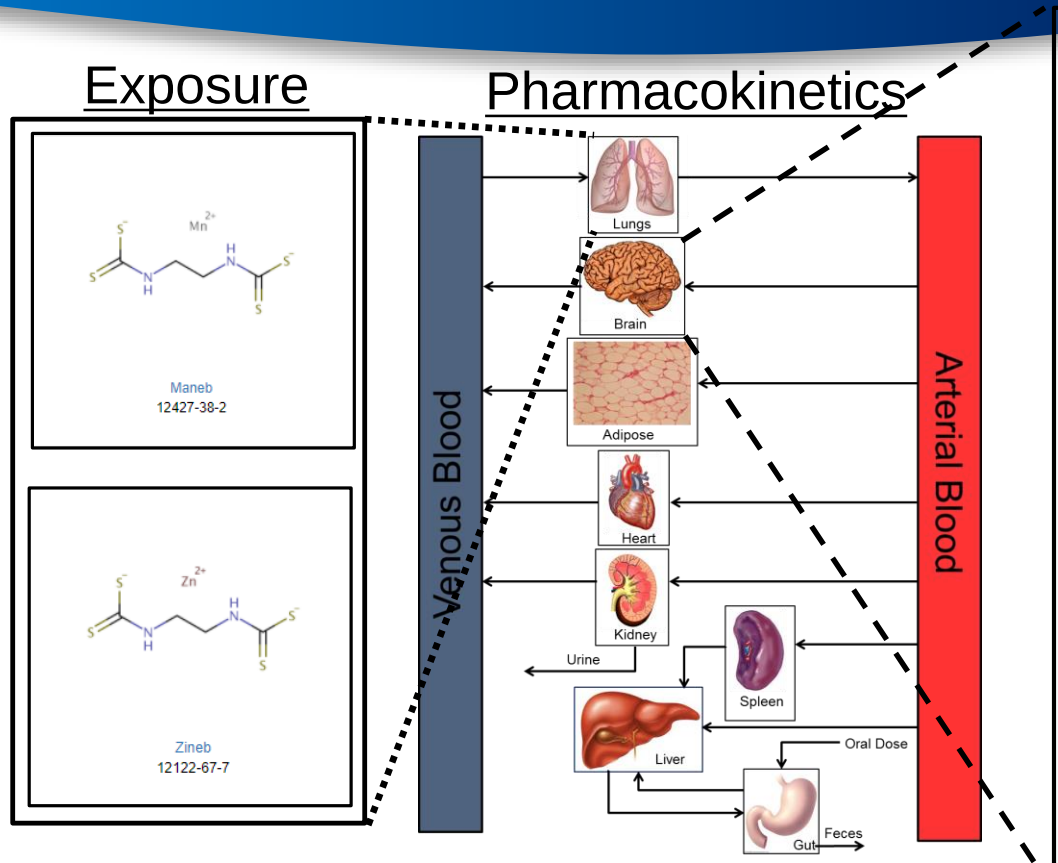

Ginhoux et al., *Science*, 2010




Obermeier et al, 2013

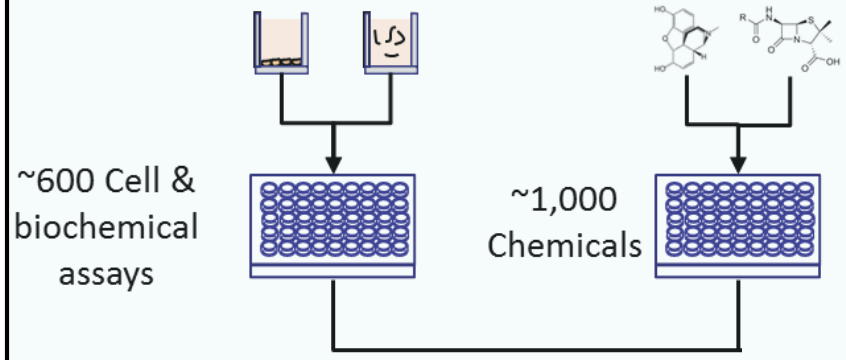
- Microglia, resident macrophages of the brain.
- During development…
  - Orchestrate neurovascular ramifications, surveillance of local injury where hyperactivation can invoke an adverse neuroinflammatory response
  - Are they mediators of developmental toxicity?

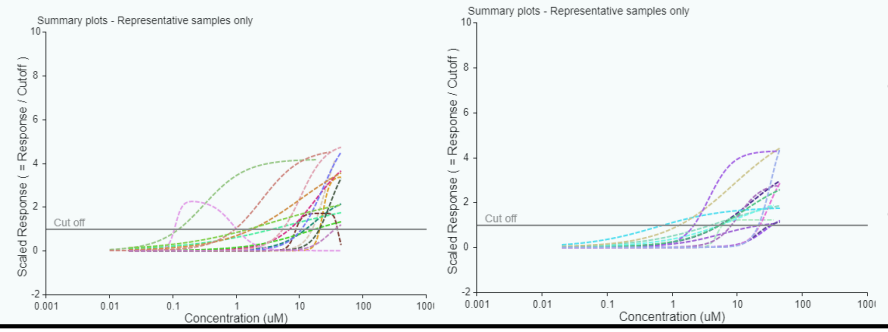
# Computational source-to-outcome framework




## Translation

*In vitro* assays




Pharmacodynamics

- Apoptosis
- Blood-brain barrier integrity
- Cell growth
- Dysmorphogenesis
- Endothelial cell migration

Utilize screening techniques to predict a concentration-dependent disruption of neurovascular development.

*In vitro:* Characterize chemical effects on cell-based phenotypes.

*In silico:* Use mechanistic information to translate HTS data into cell/tissue predictions.

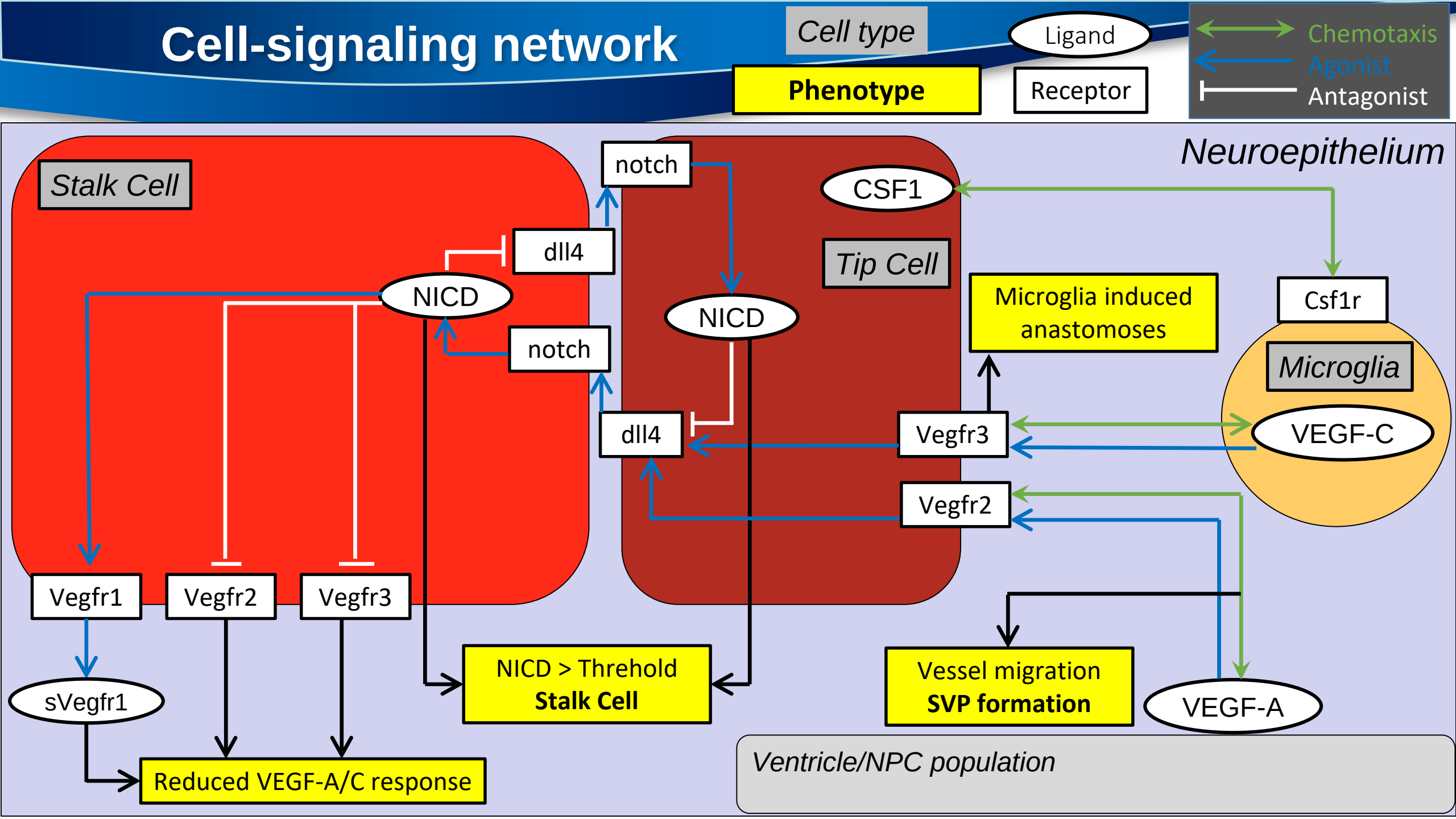
# Cell Agent-Based Modeling

- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics using knowledge of biochemistry and cell-by-cell interactions.
  - Program each *agent* (cell) to follow specific rules
  - Interactions of agents gives rise to *emergent features* (phenotypic outcomes)
  - Qualify emergent feature with experimentally derived phenotypes (tissue level morphology)
  - Make toxicodynamic predictions by integrating biological knowledge & high throughput data

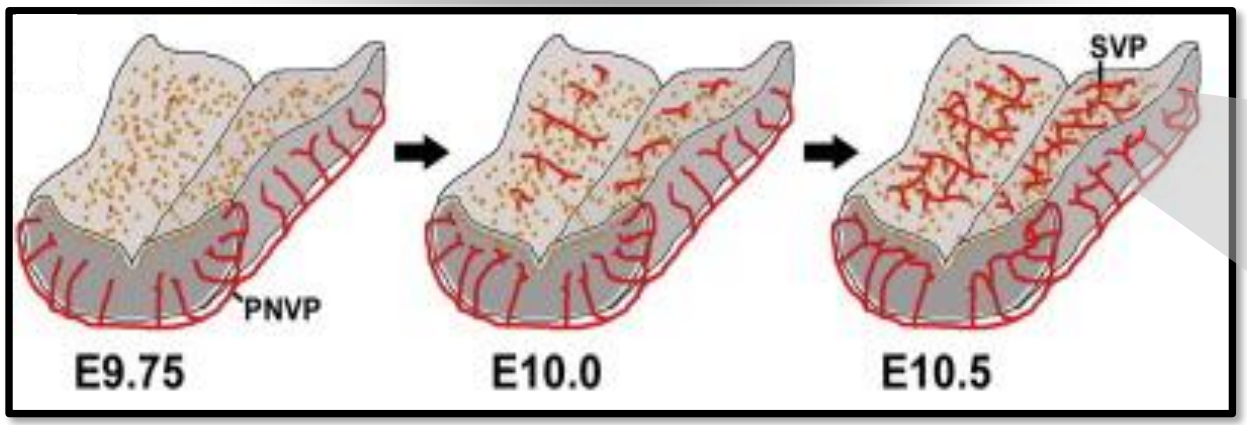
- **CompuCell3D*:** open source modeling environment
  - Rules (steppables) for distinct cell behaviors (growth, proliferation, apoptosis, differentiation, polarization, motility, ECM, signal secretion, …);
  - Rules coded in Python for cell-autonomous 'agents' that interact in shared microenvironment and self-organize into emergent phenotypes.
  - Methodology applied to past systems: vasculogenesis, genital tubercle, palate fusion, etc.

*James Glazier and colleagues, Indiana University

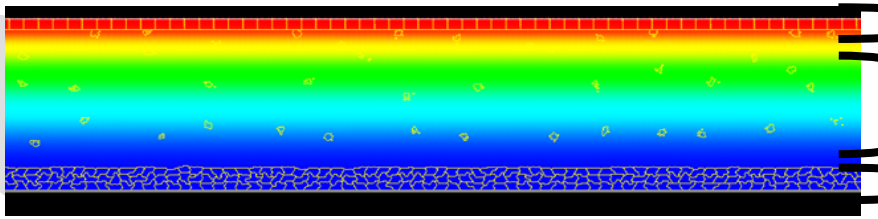
# Cell-signaling network

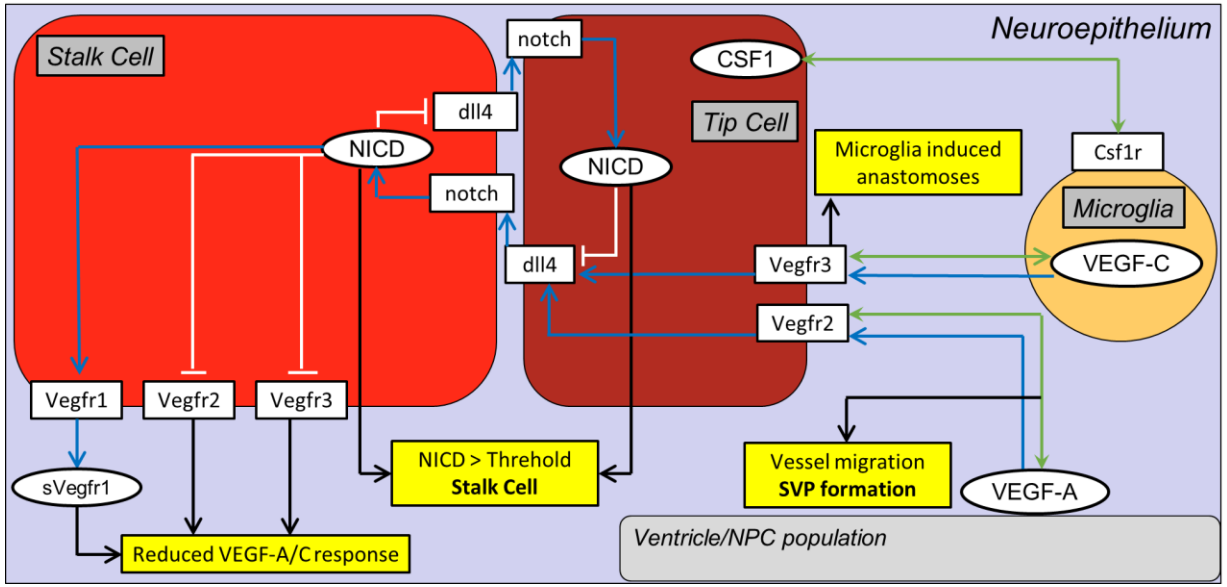


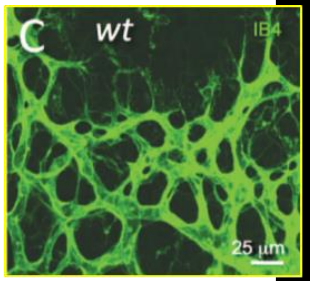
# Modeling Brain Angiogenesis




*VEGF-A gradient*: NPCs in the subventricular zone

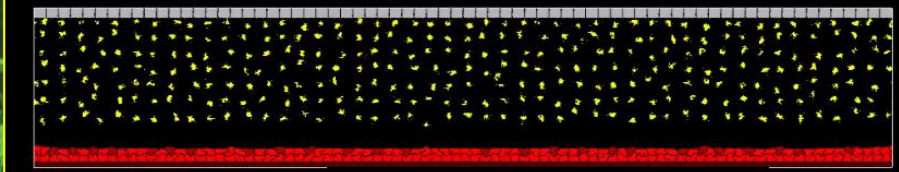


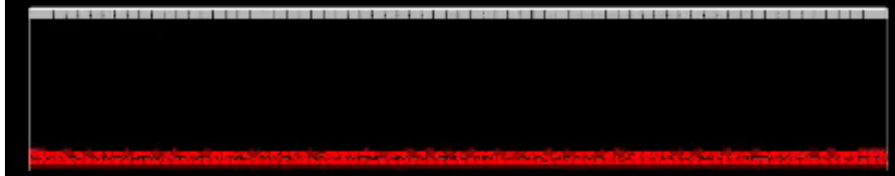




Rymo et al. (2011) PLoS One

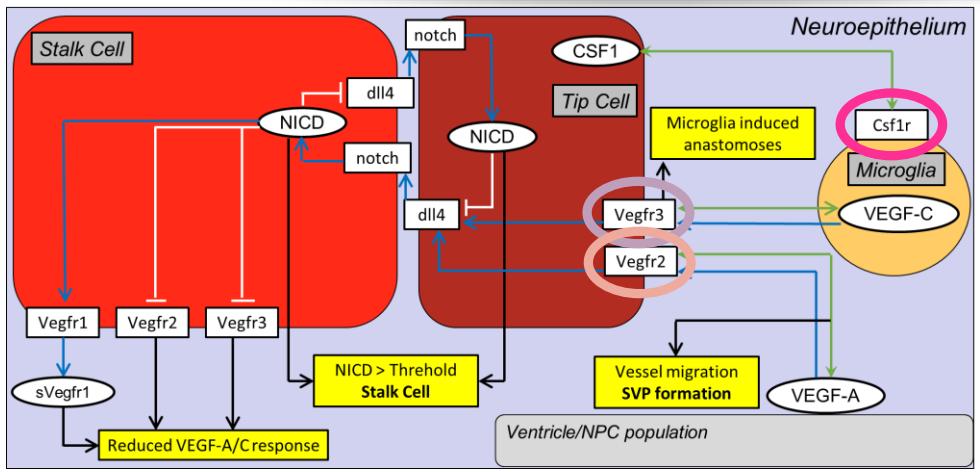
Cell agent Based model of microglia-endothelial interaction

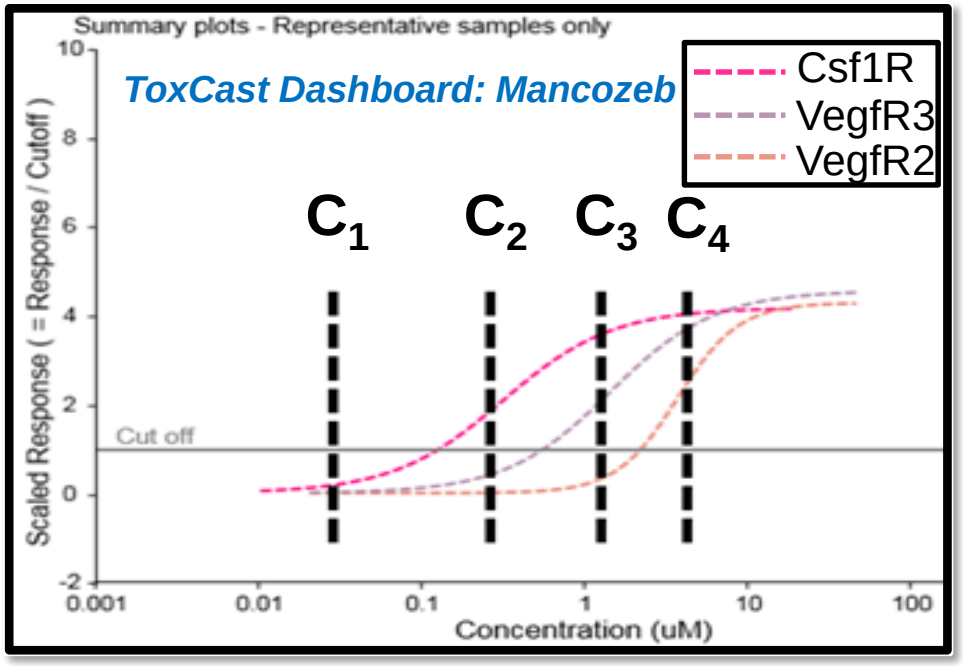


endothelial tip cell
endothelial stalk cell
microglial cell

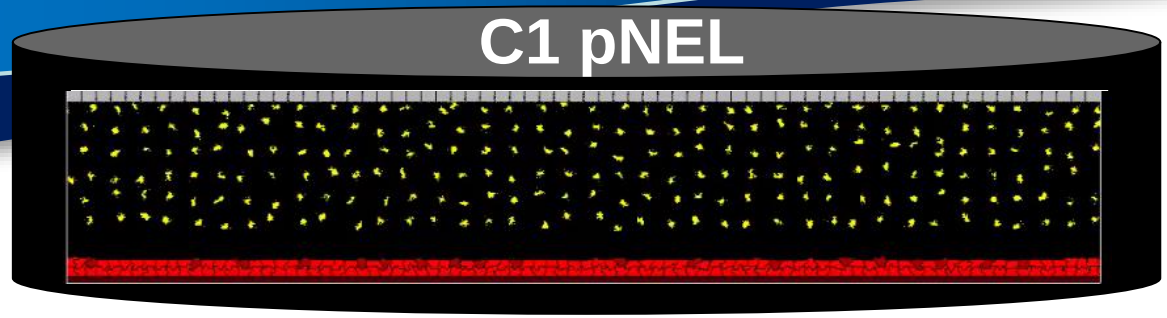


# Translating HTS Data - Mancozeb

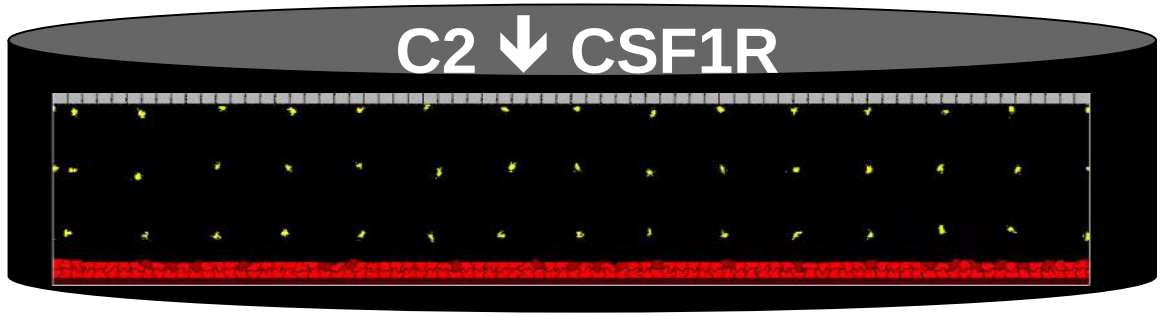


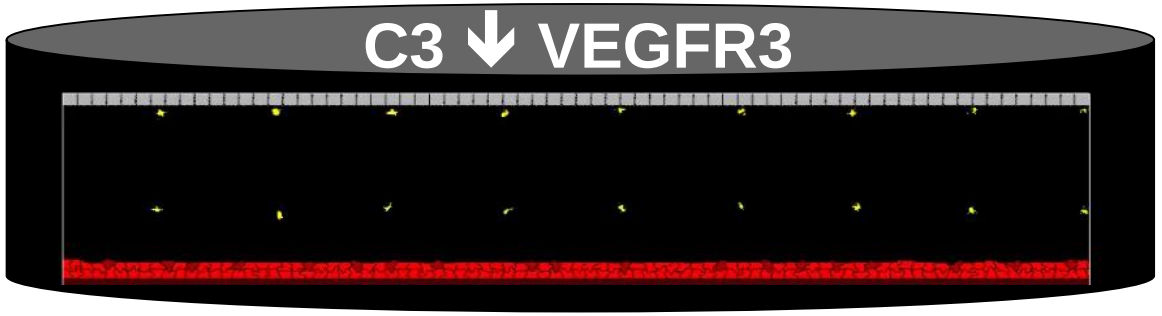

**0.03 µM**
No significant reduction in any receptor



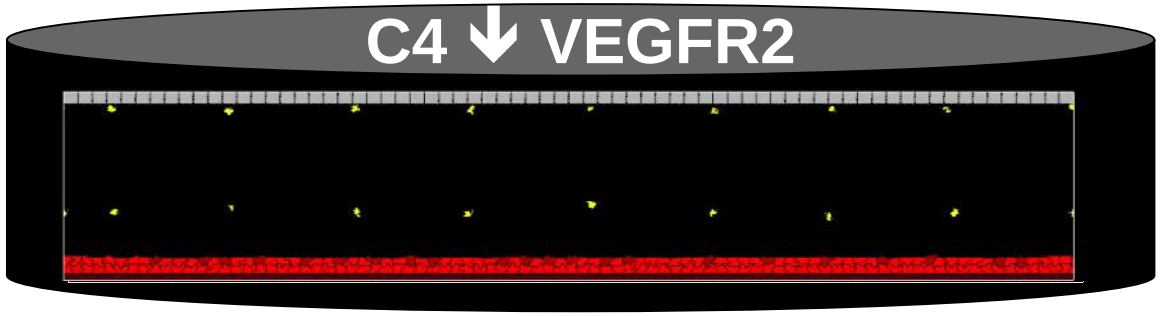

**0.3 µM**
50% ↓ CSF1R



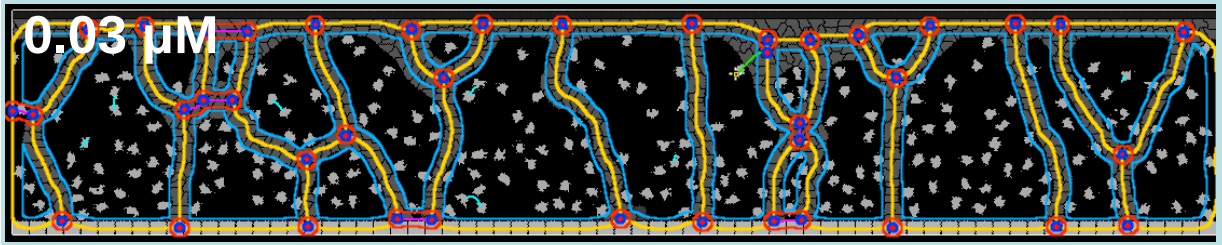

**2 µM**
50% ↓ VEGFR3
80% ↓ CSF1R



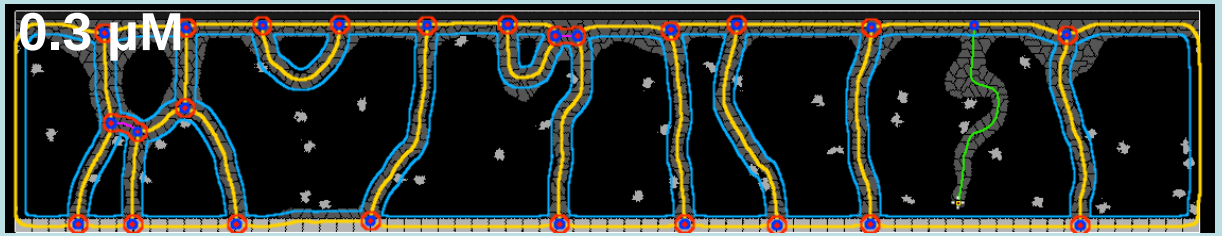

**7 µM**
50% ↓ VEGFR3
85% ↓ VEGFR2
95% ↓ CSF1R



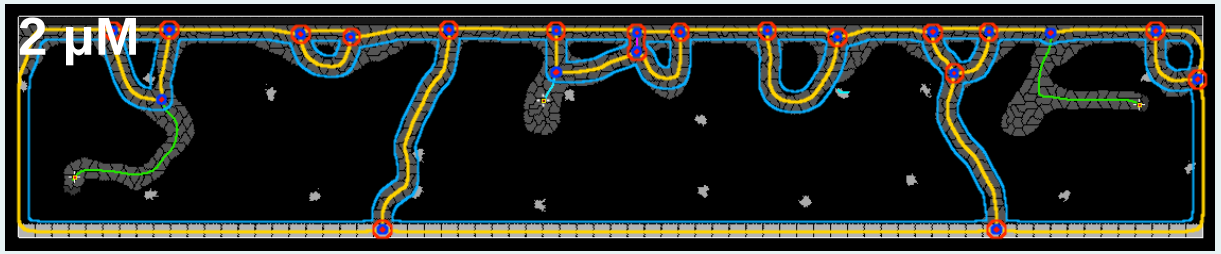
# Vascular Quantitation - Mancozeb



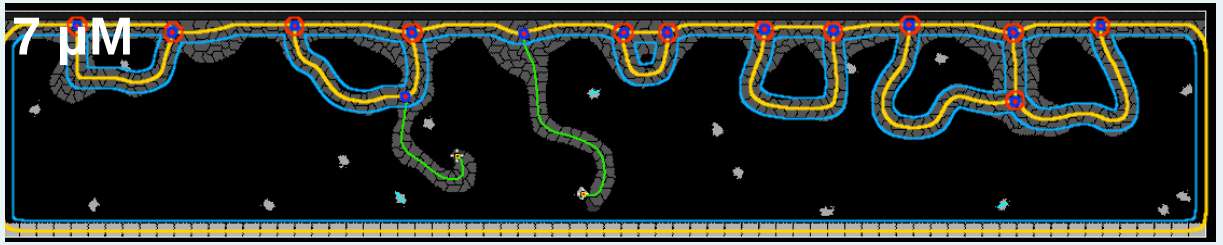

Predicted NEL (pNEL): No changes to vasculature



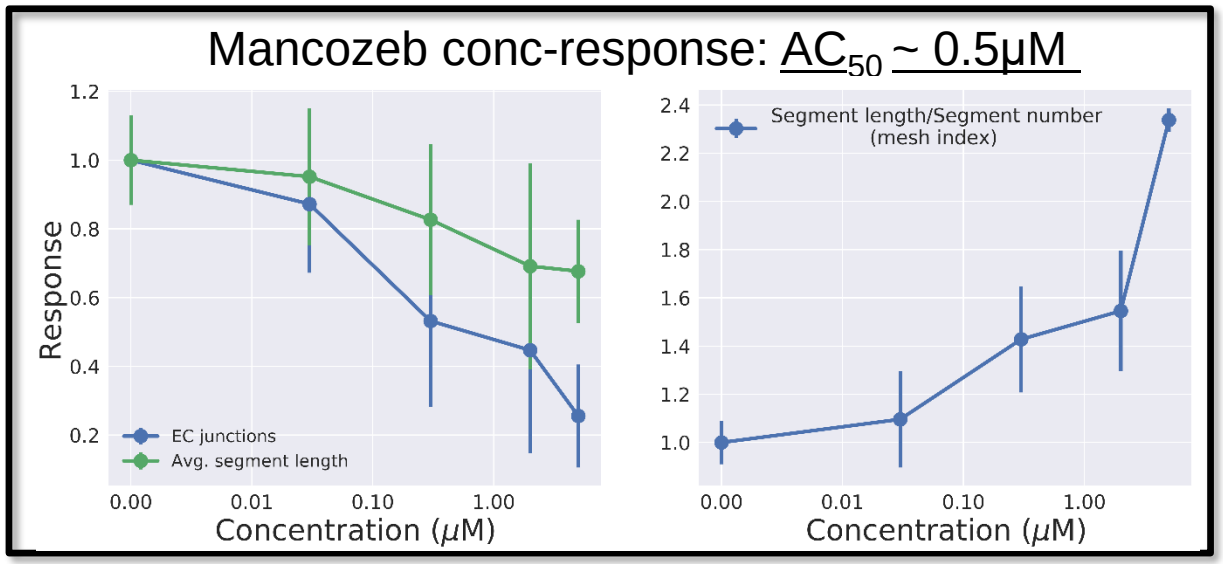

Predicted LEL (pLEL): Reduced tortuosity




Reduction in overall vascular area

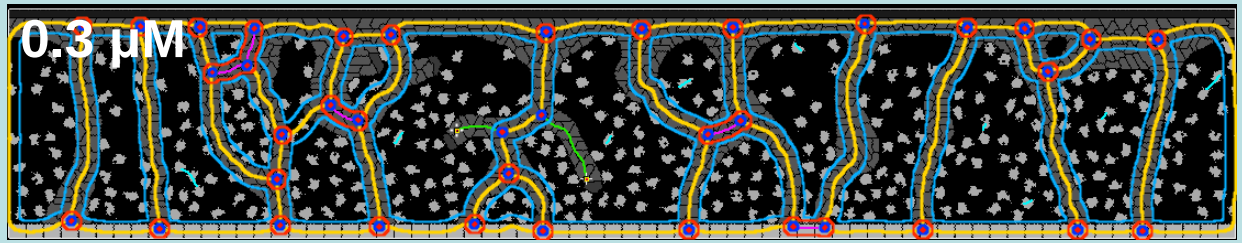



Hypo-vascular angiodysplasia

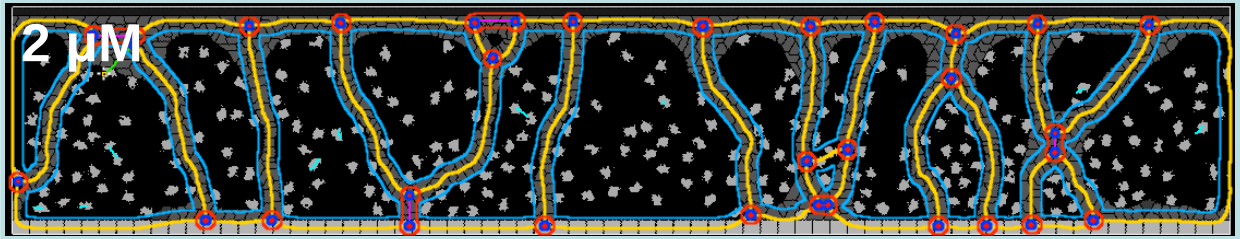



- Quantitate multiple vascular network endpoints in concentration response.
- Running multiple simulations allows us to account for stochastic variability.

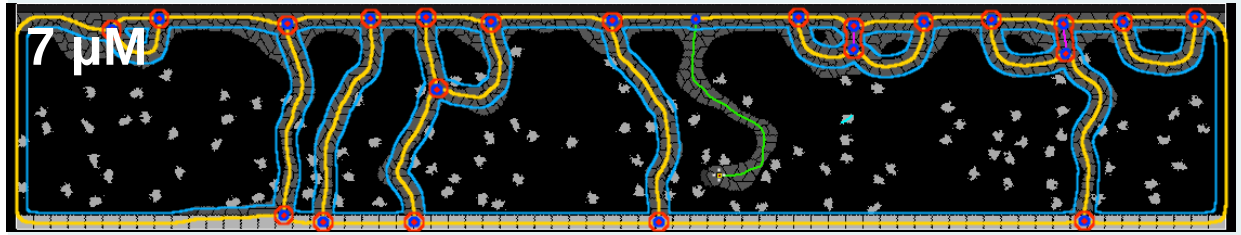
# Vascular Quantitation – Oxytetracycline dihydrate



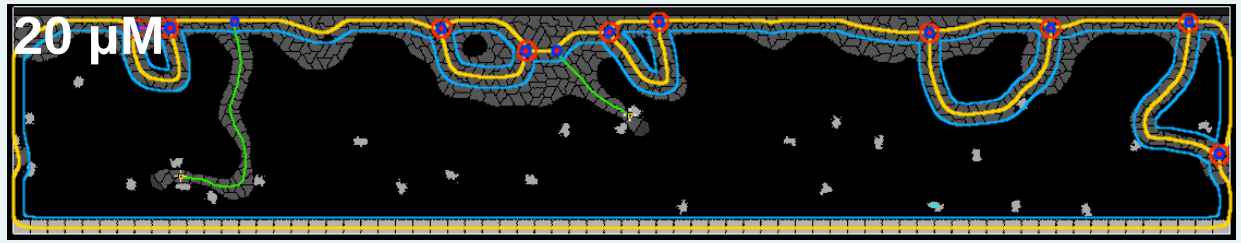

Predicted NEL (pNEL): No changes to vasculature



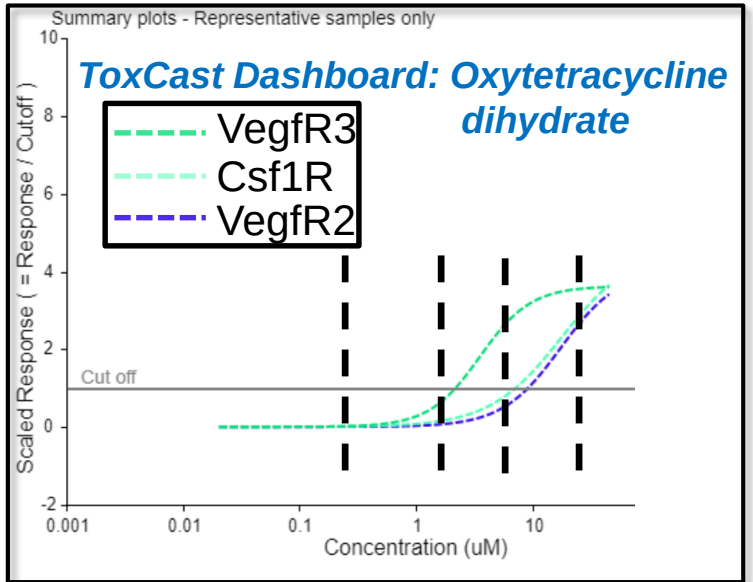

Predicted LEL (pLEL): Reduced vascular area



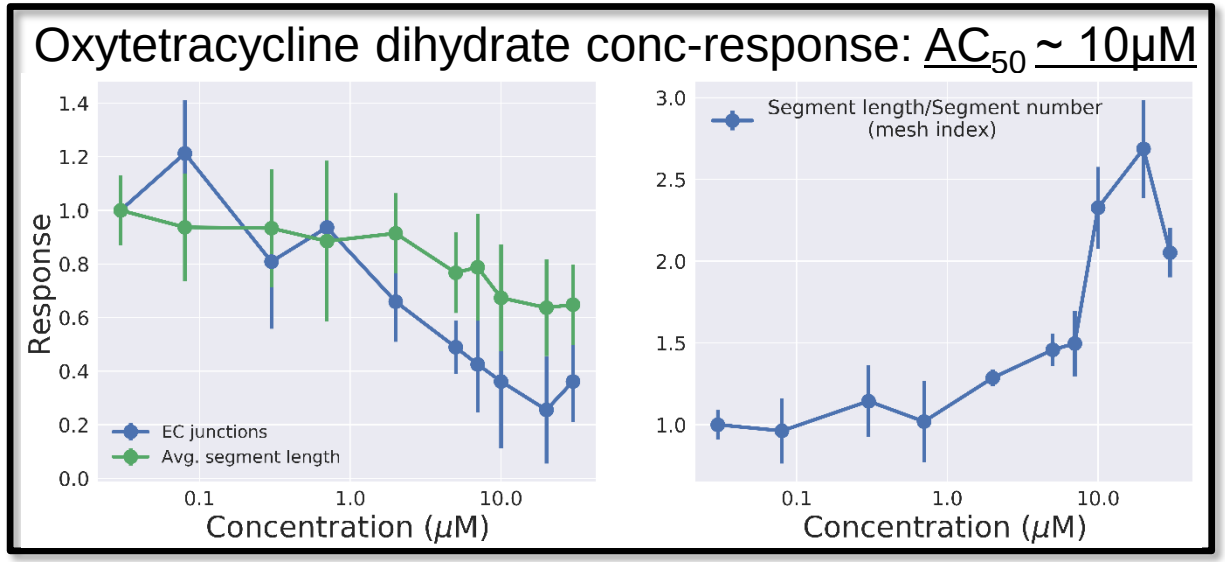

Reduced branching and anastomoses



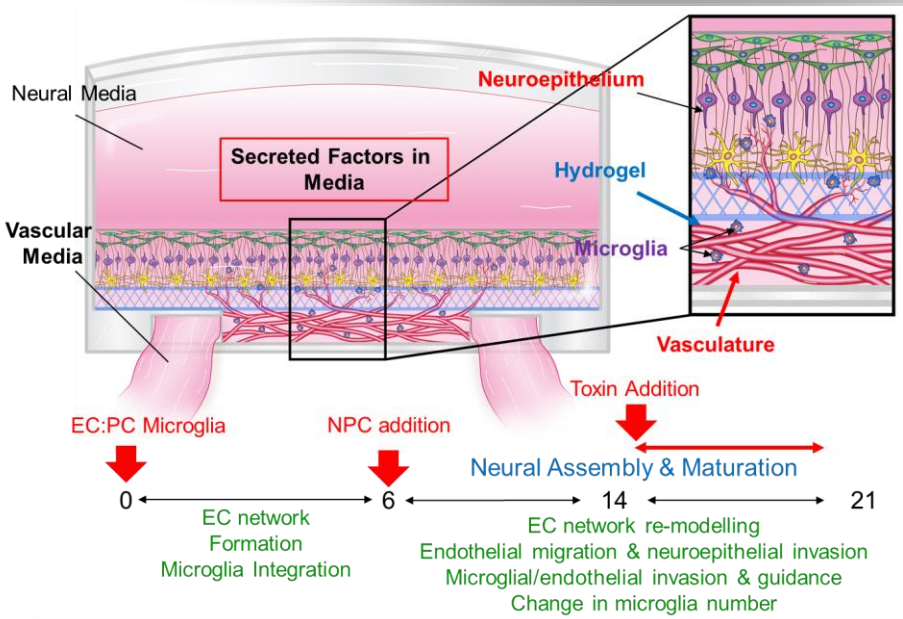

Hypo-vascular angiodysplasia




VEGFR3 serves as the more sensitive angiogenesis endpoint for oxytetracycline dihydrate exposure

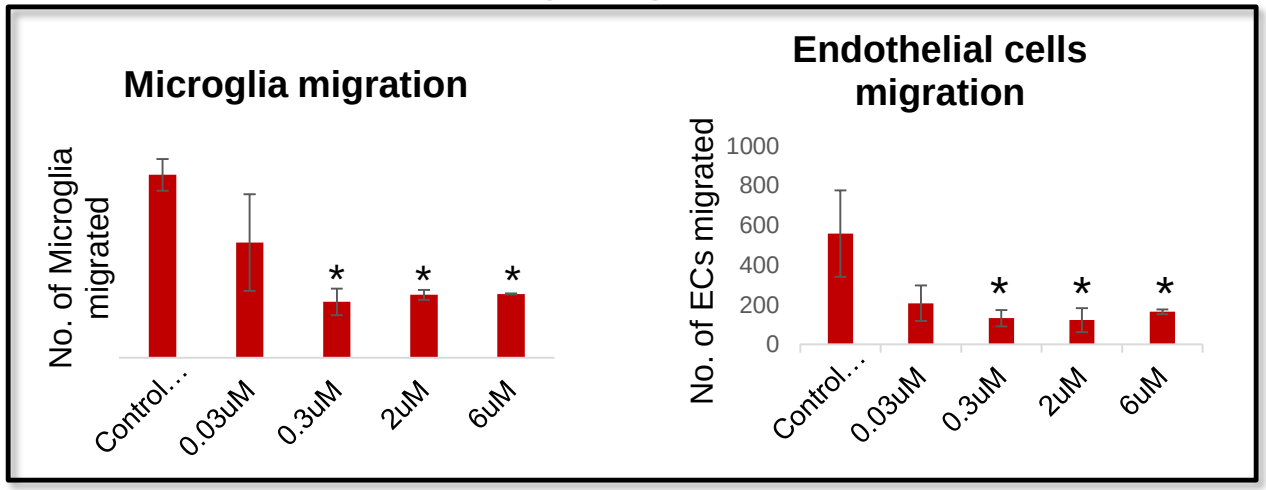


# Experimental comparison

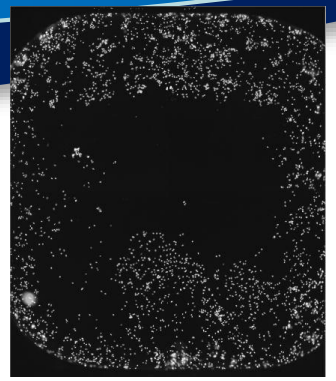


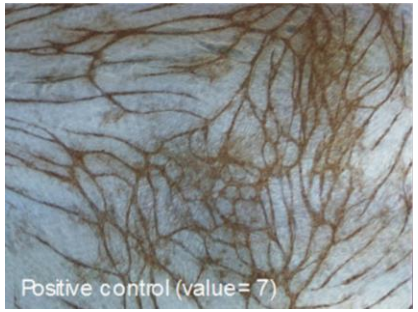

## NVU OCM

- Pilot study using mancozeb concentrations tested *in silico*
- *In vitro*: **~0.3 µM**
- *In silico*: **~0.5 µM**



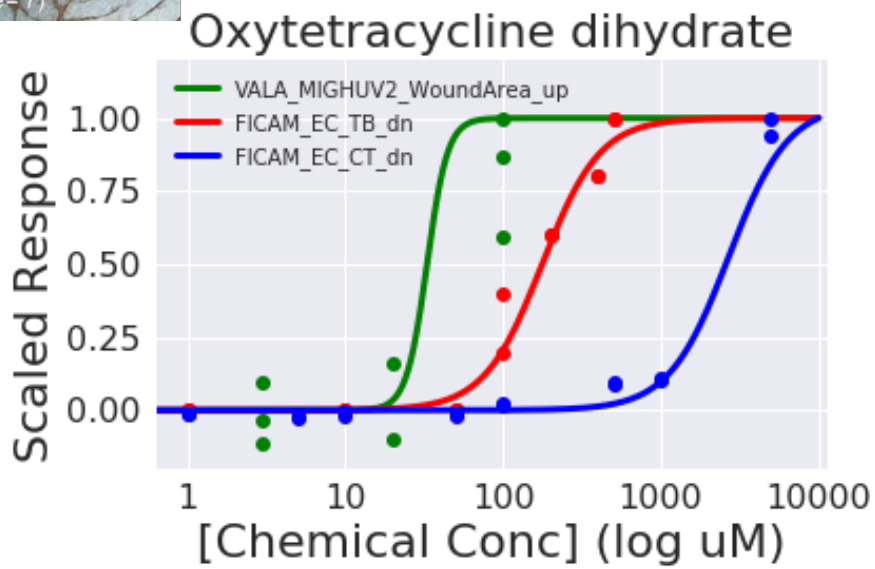

W. Daly, G. Kaushik, UW Madison






## Cell-based assays

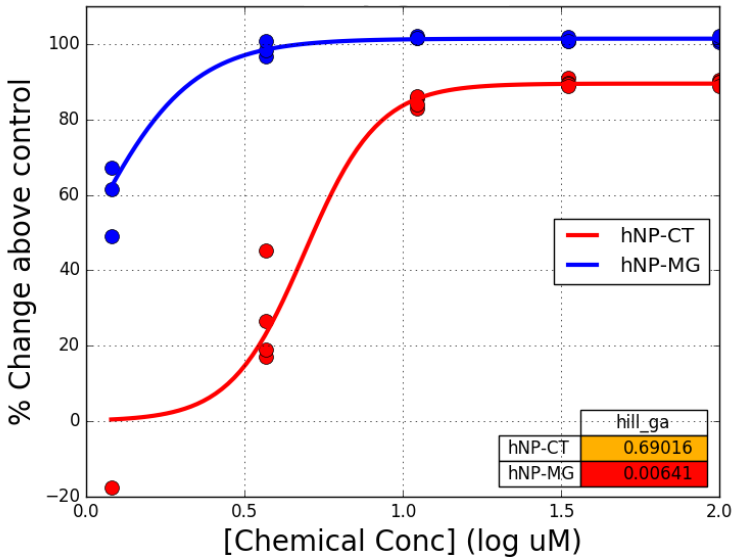
- Endothelial cell tubulogenesis and migration assays
- *In vitro*: **~30 µM**
- *In silico*: **~10 µM**
- No microglia *in vitro*




VALA Sciences, FICAM

# NVU cell-based assays

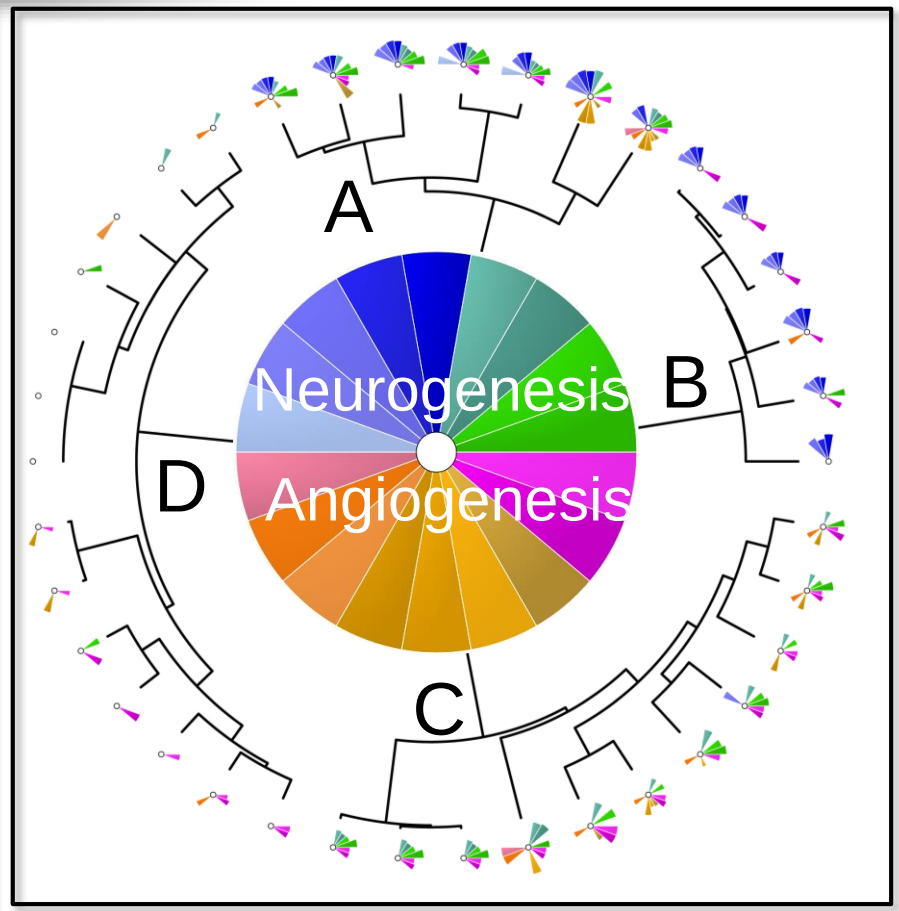
## Cell-based Assays




ArunA: Migration/Proliferation
**hNP/hNC/hNN cells**

VALA: Migration/Proliferation
**HUVEC cells**

FICAM: Tubulogenesis/Proliferation
**HUVEC cells**





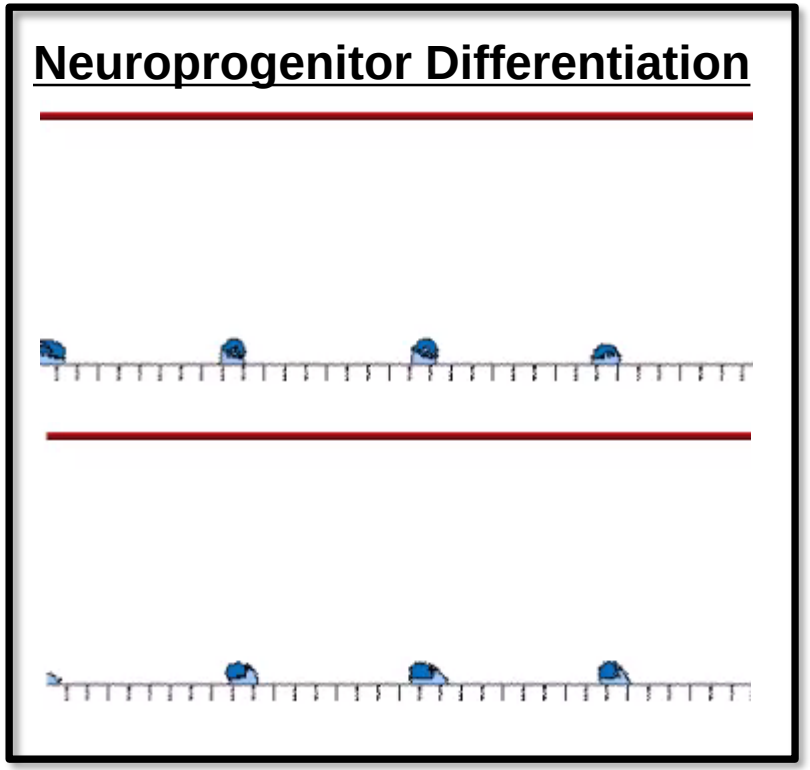
Process data

Cluster through ToxPi

Define in literature

# Towards a functional cNVU model

- Biological pathway perturbations
  - Predict NVU phenotypes from literature fingerprint and cell-agent based model
  - ‘Cybermorphs’ for investigating single pathway knockouts
  - Continuum response following chemical exposure and resulting receptor inhibition
- Neurogenesis submodel
  - Differentiation/migration to neurons and astrocytes
  - Utilize intracellular signaling pathways (cell/centrosome cycle)
  - Endothelial network interacting with neural network (3D)
- Phenotype quantitation
  - Microglia abundance, vessel branch points, network complexity (cortical angiogenesis)
  - Neuron proliferation/differentiation (neurogenesis)
  - Barrier permeation for chemical distribution to neural compartment (barriergenesis)



# Acknowledgements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- Andrew Schwab (NHEERL-ISTD)
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- Richard Spencer (ARA-EMVL)
- Florent Ginhoux (A*STAR)
- Aymeric Silvan (A*STAR)
- Bill Daly (H-MAP, Wisc)
- Eric Nguyen (H-MAP, Wisc)
- Virtual Tissues Modeling Group


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# Thank You

Questions?