United States Environmental Protection Agency

Finding Toxicological Tipping Points from High-Content Imaging Data

WC10
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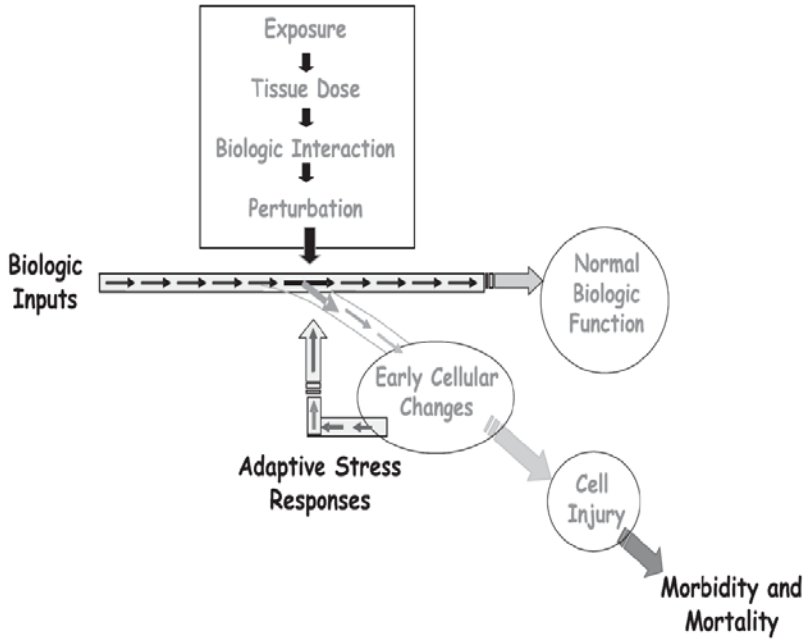
Toxicological Tipping Points

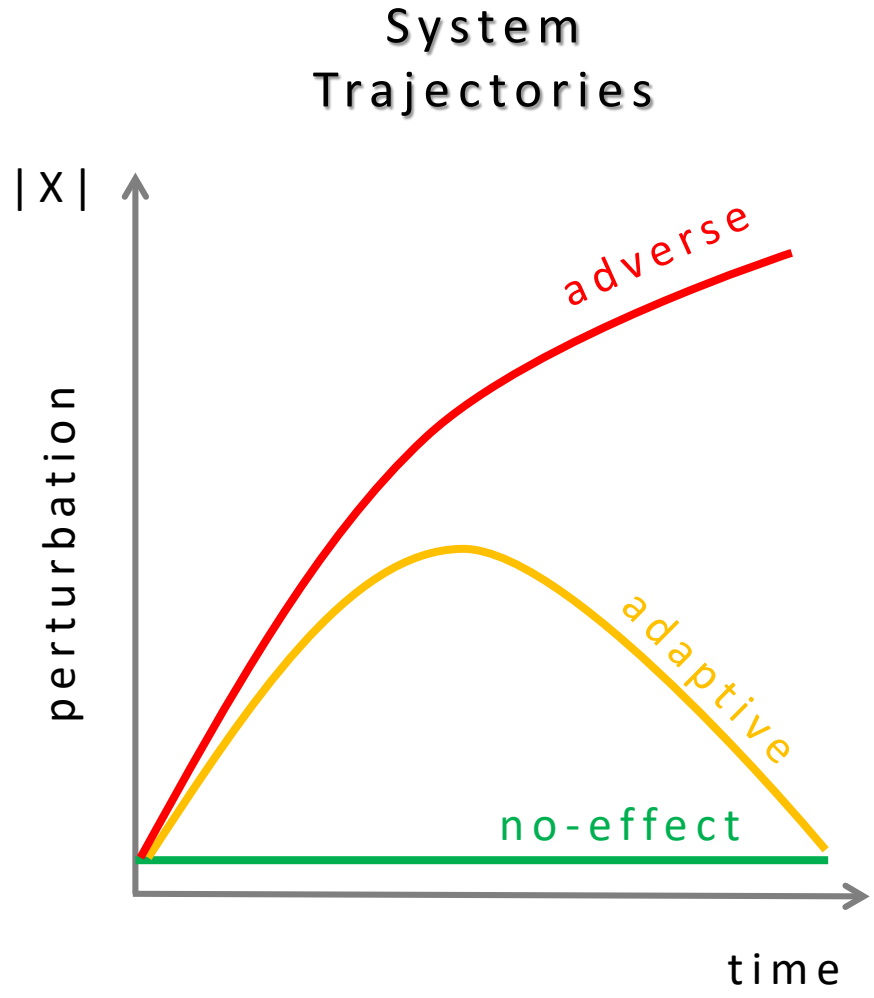
- Biological systems are resilient and adapt to environmental perturbations
- Can we find toxicological tipping points?
- Can a tipping point define a point of departure (PoD) ?



Krewski, Daniel, Daniel Acosta Jr, Melvin Andersen, Henry Anderson, John C Bailar 3rd, Kim Boekelheide, Robert Brent, et al. "Toxicity Testing in the 21st Century: a Vision and a Strategy." Journal of Toxicology and Environmental Health. Part B, Critical Reviews 13, no. 2–4 (February 2010): 51–138.

Tipping Points, *in vitro*

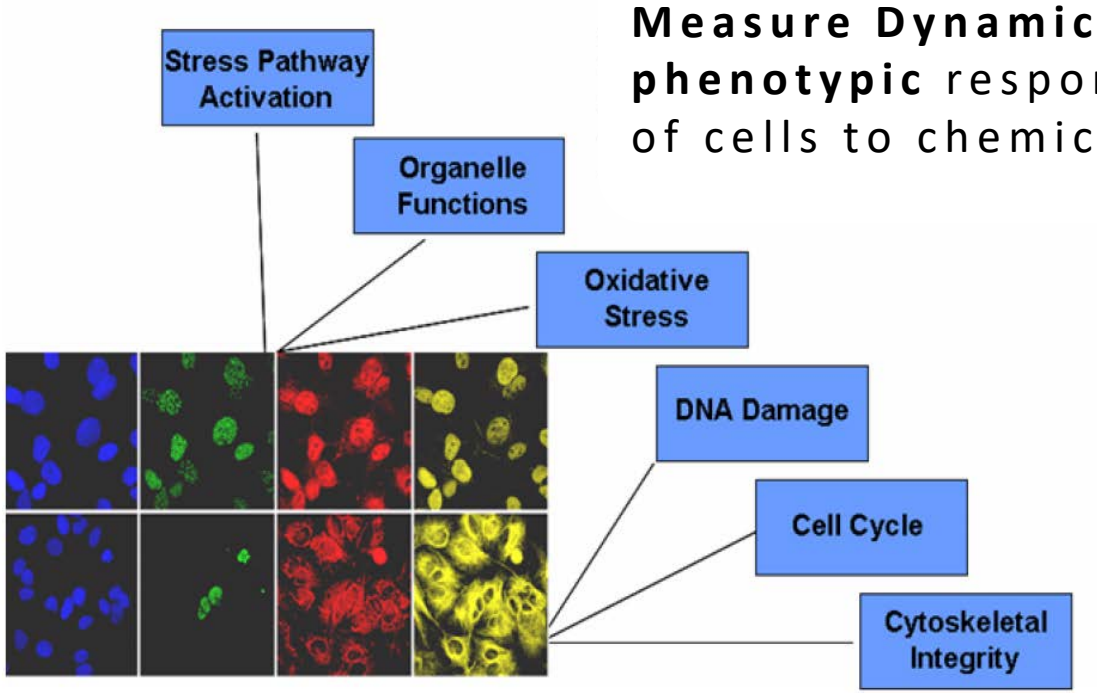
- Used high-content imaging to model system trajectories: no-effect, adaptive and adverse
- Tipping point: critical point between adaptive and adverse trajectories
- How can we use network modeling to analyze tipping points?



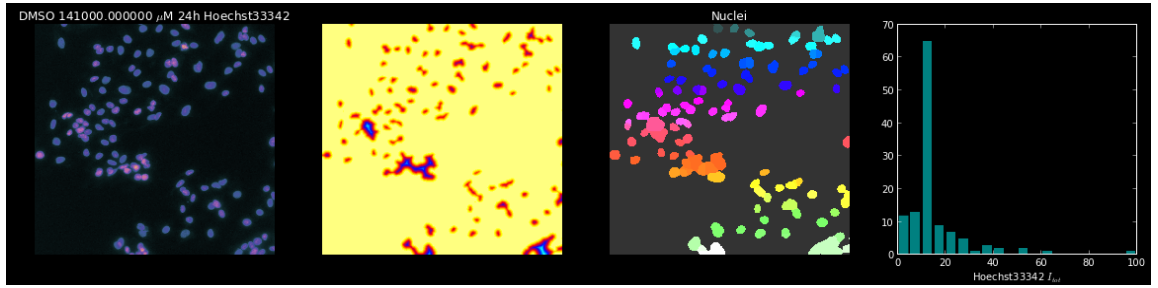
Shah et al, 2016

High Content Imaging (HCI)

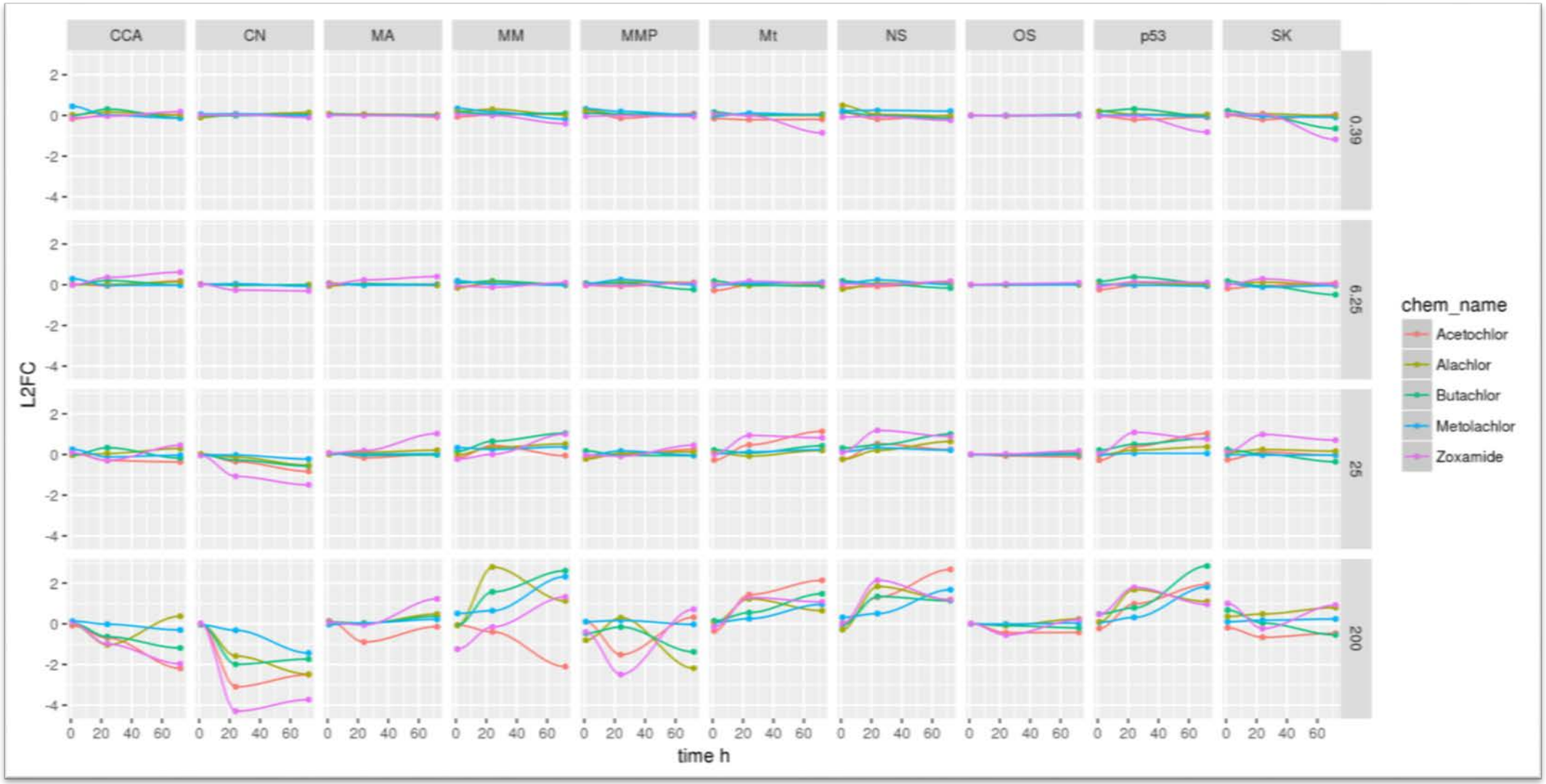
- **Study**
  - HepG2 cell culture
  - 967 chemicals (ToxCast)
  - 10 conc
- **HCI Assays**
  - Health
  - Stress
  - Cell cycle
- **Large-scale data**
  - ~400 plates
  - ~100,000 wells
  - ~2,400,000 images
  - ~30,000 chemical-conc-time-response points



Measure Dynamic phenotypic response of cells to chemicals



Raw Image (Hoechst) → Intensity Analysis → Object Identification → Nuclear intensity distribution



- P53: p53 activity (p53)
- c-Jun: stress kinase (SK)
- Tubulin: microtubule organisation (Mt)
- MitoTracker Red: Mitochondrial memb. pot. (MMP)
- PH3: mitotic arrest (MA)
- Hoechst33342: nuclear size (NS), cell number (CN)
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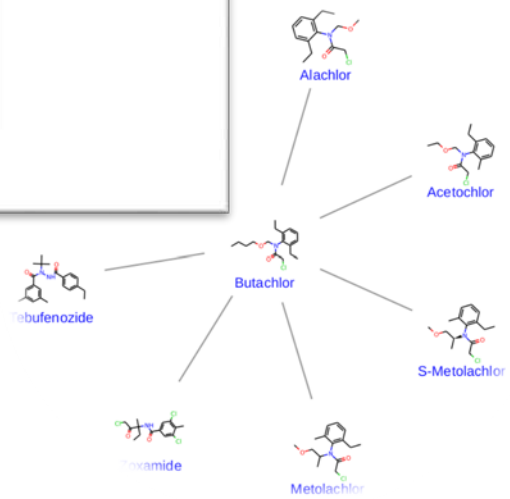
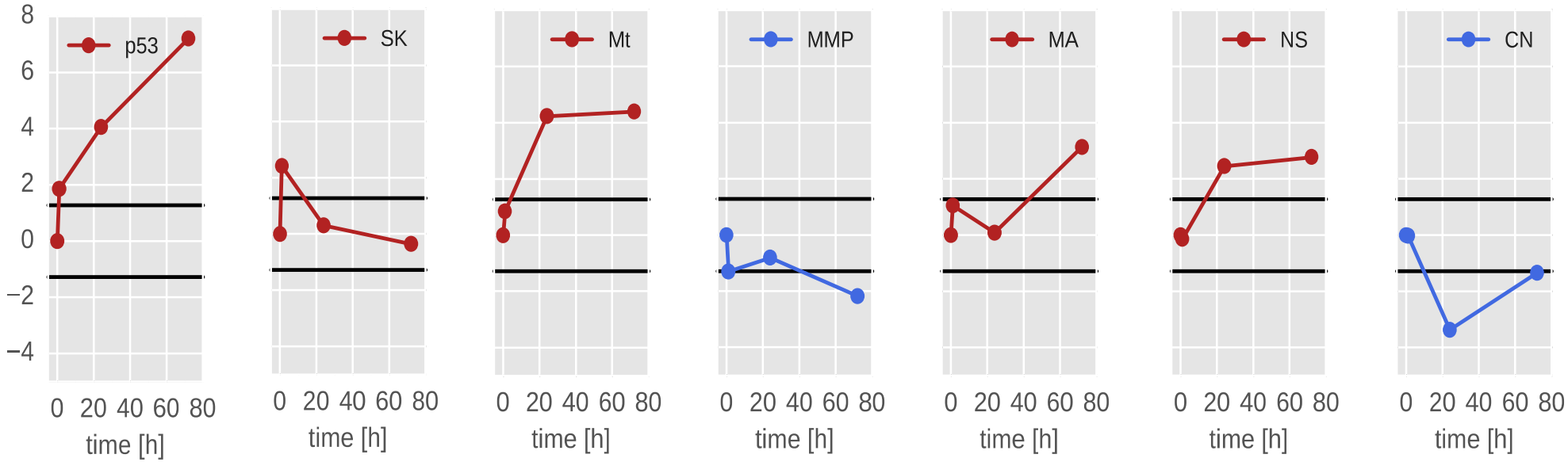


Butachlor 200µM Trajectory



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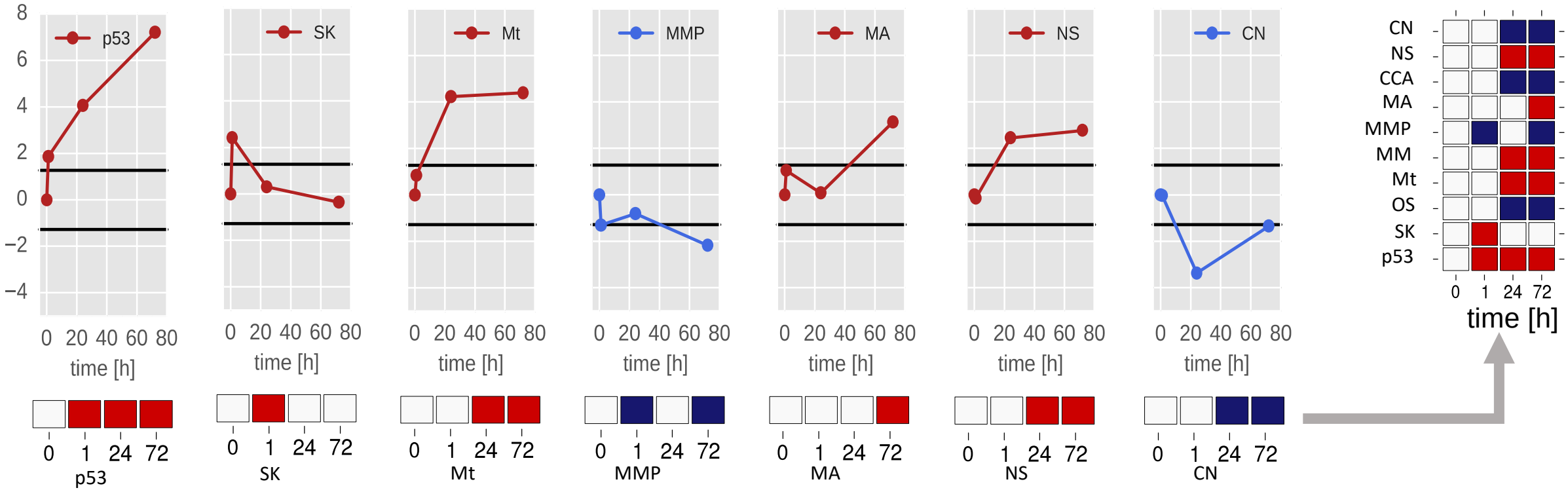
Antonijevic & Shah *(in preparation)*

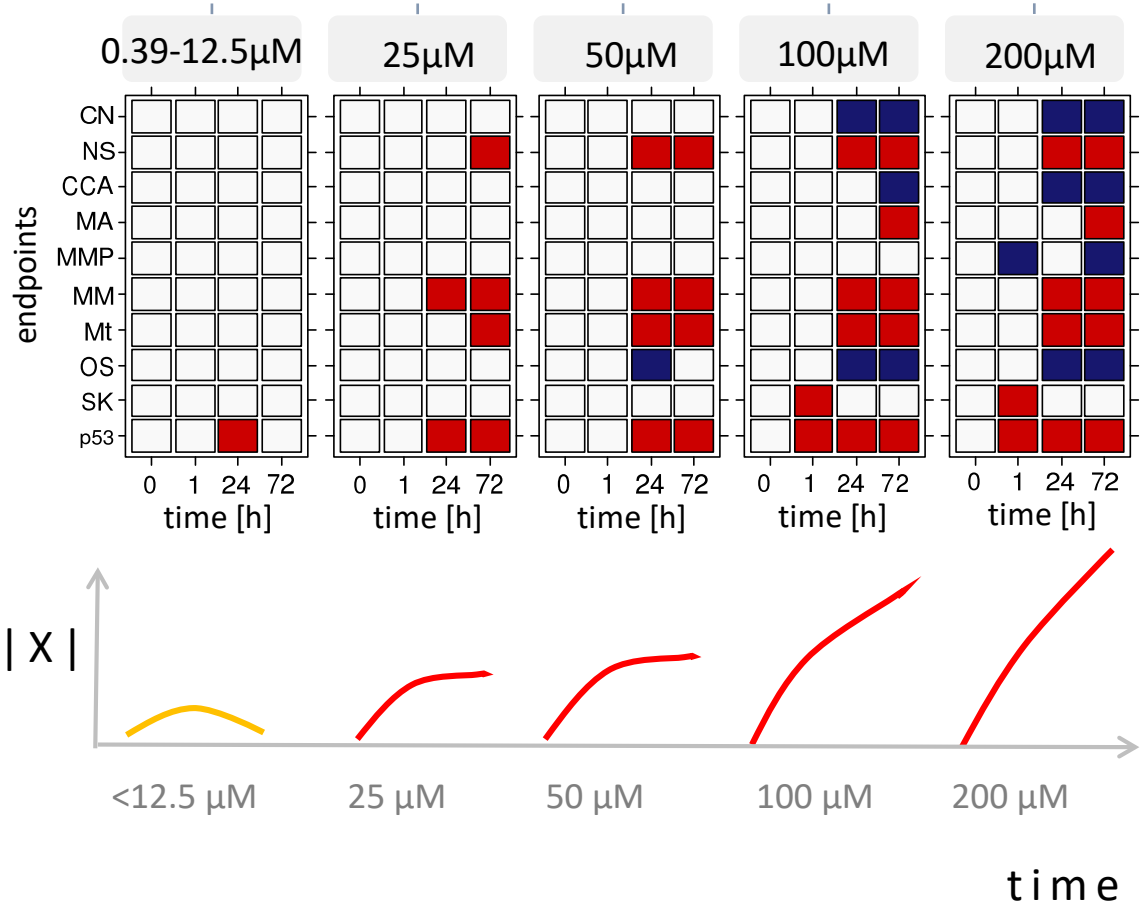
Discretizing Data

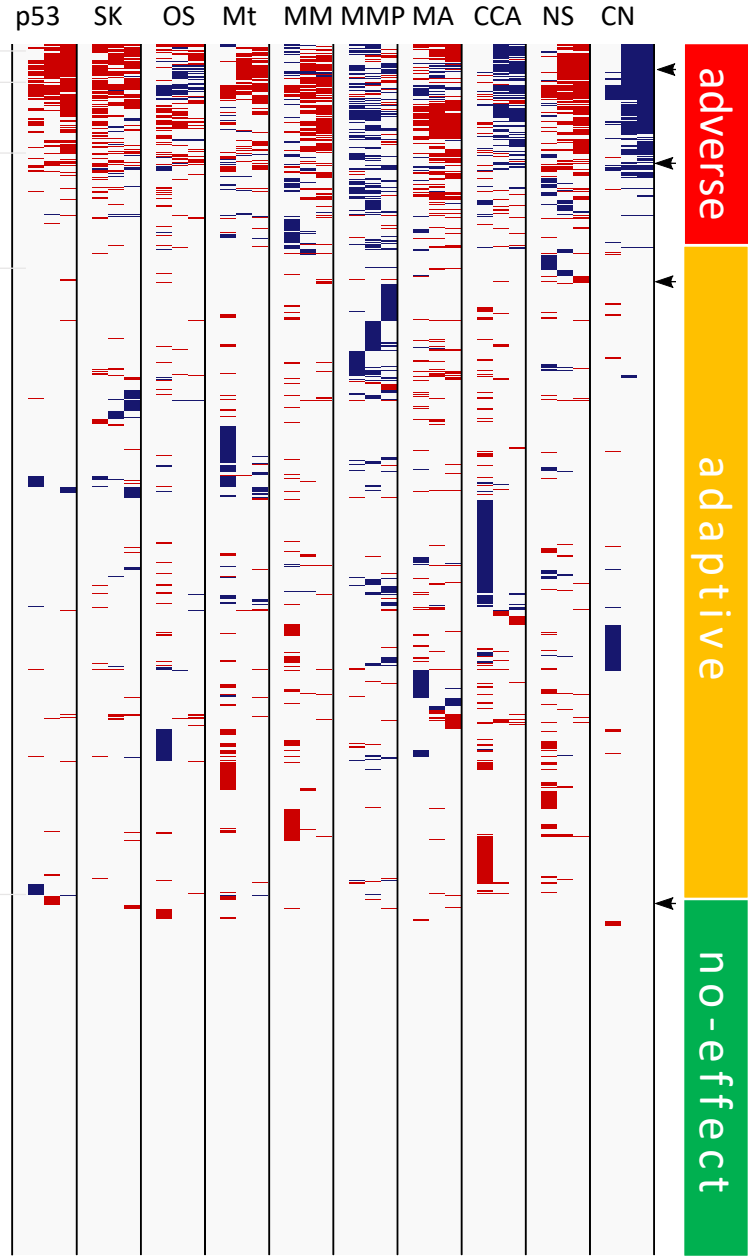


Discretization highlights key responses and reduces noise

Antonijevic & Shah *(in preparation)*

Trajectories for all Chemicals





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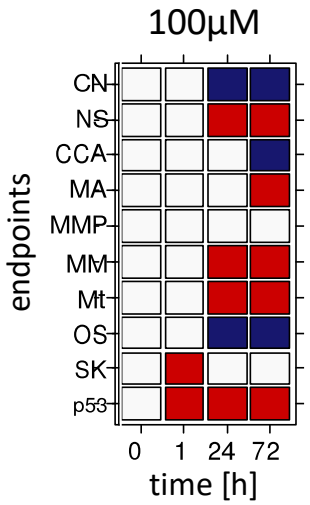
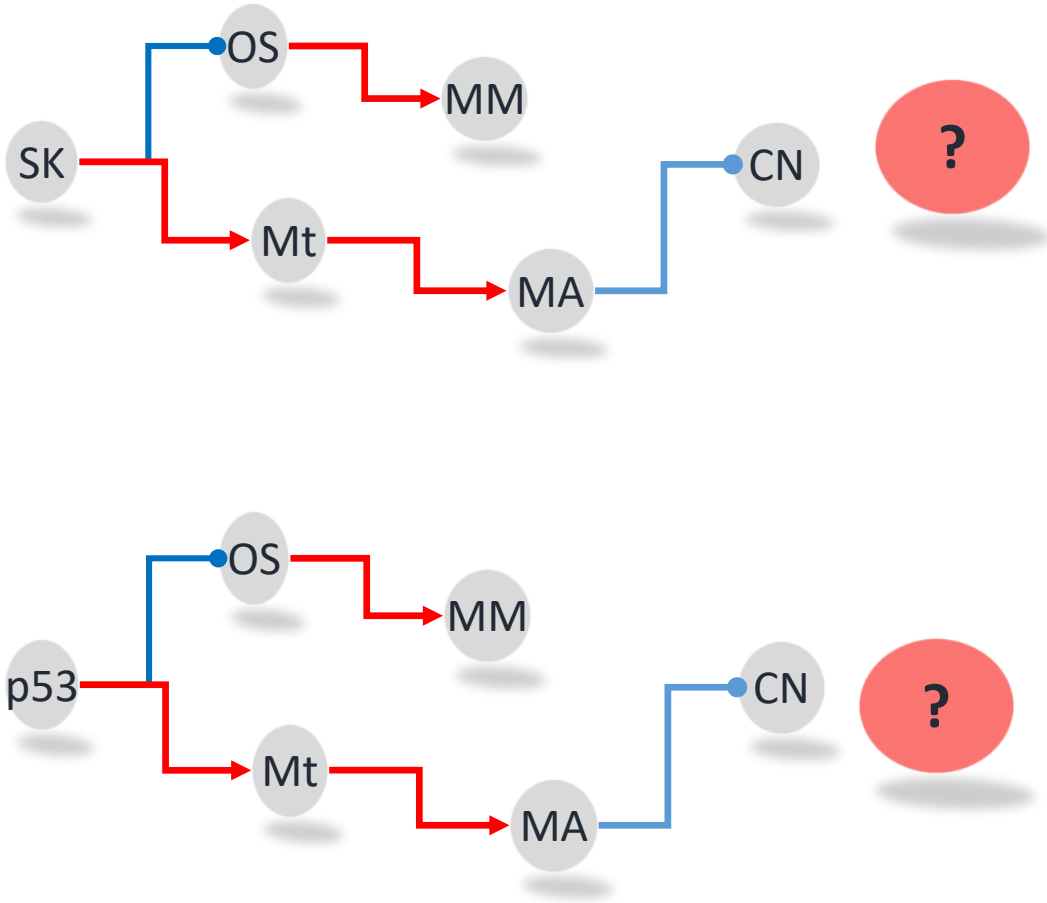
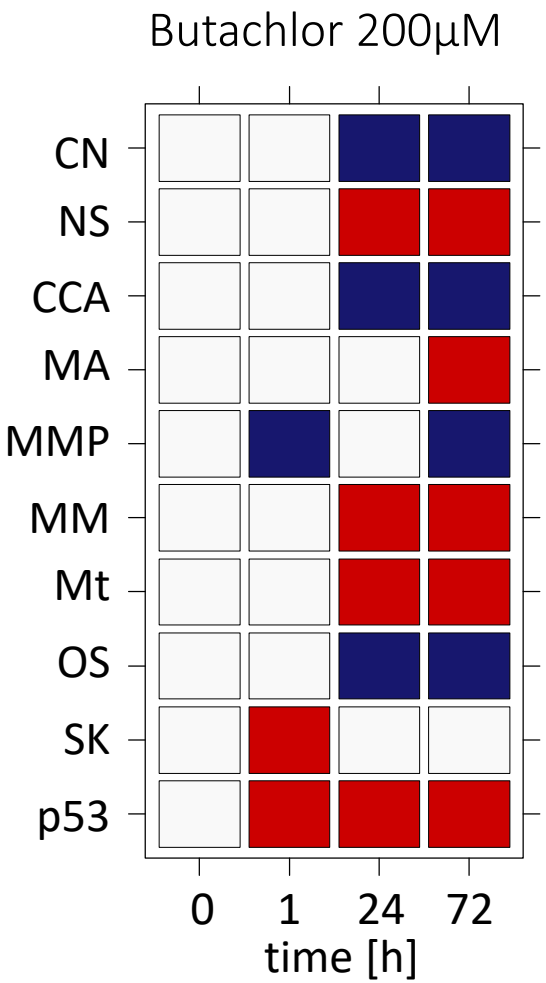
Learning Mechanisms from Data

- Analyse dependencies between endpoints across time
- Tedious to do by hand
- Many network inference approaches
- We used Boolean network (BN) inference using best-fit extension



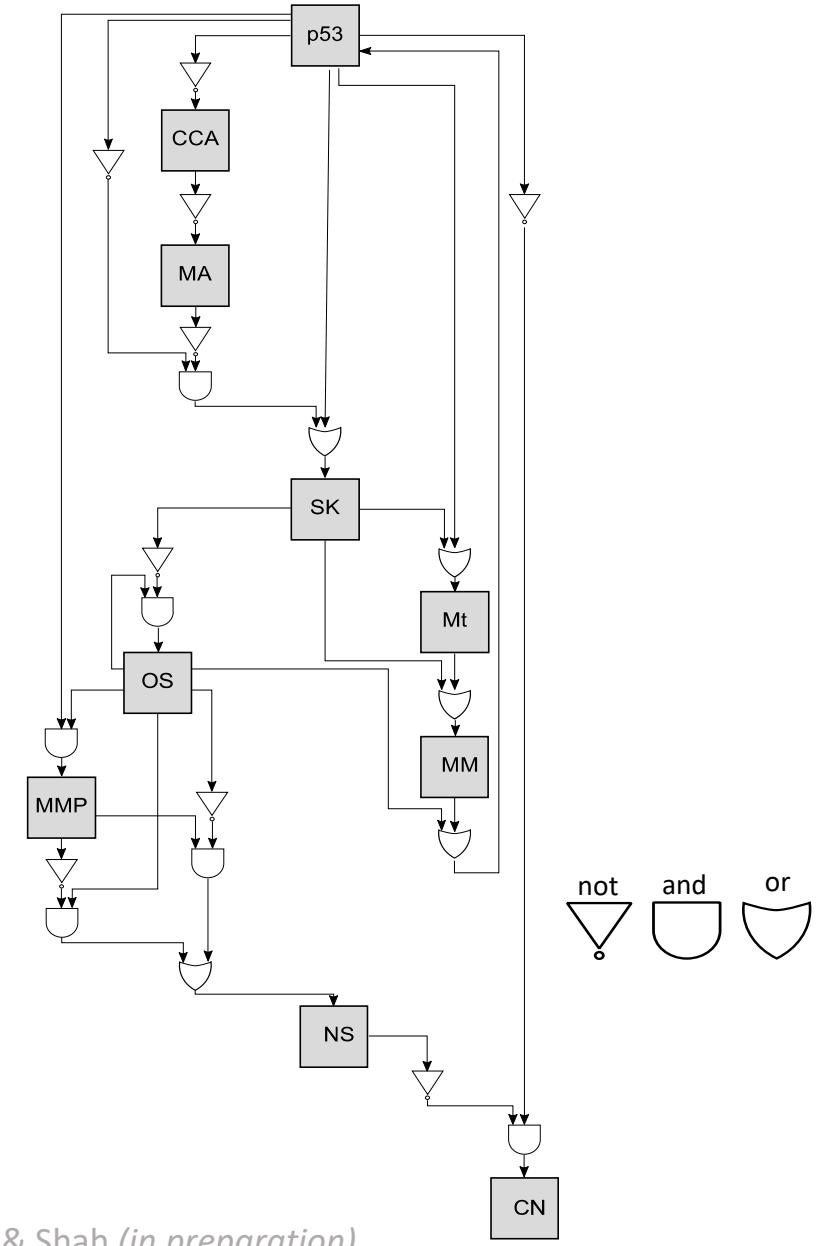


HCI Data



Boolean Network Inference

Predicted Mechanism



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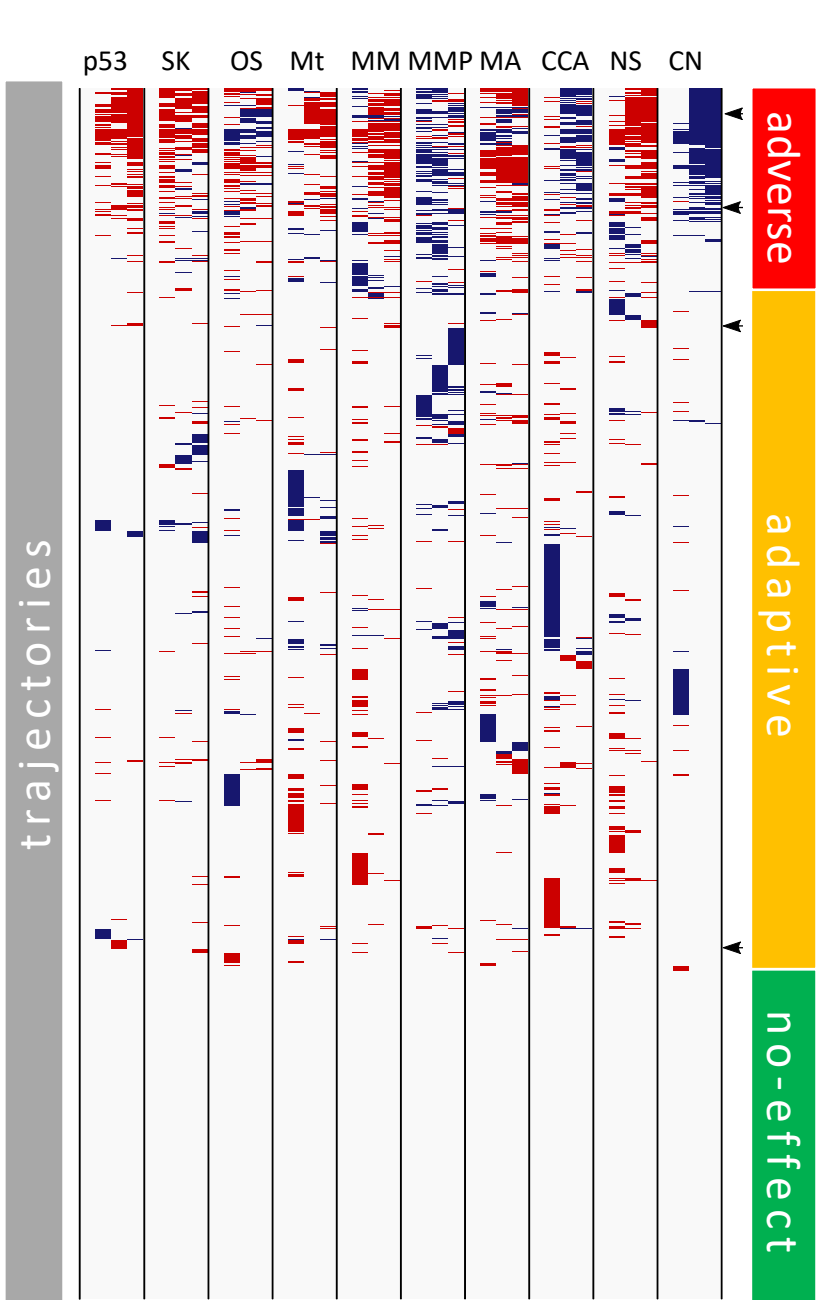
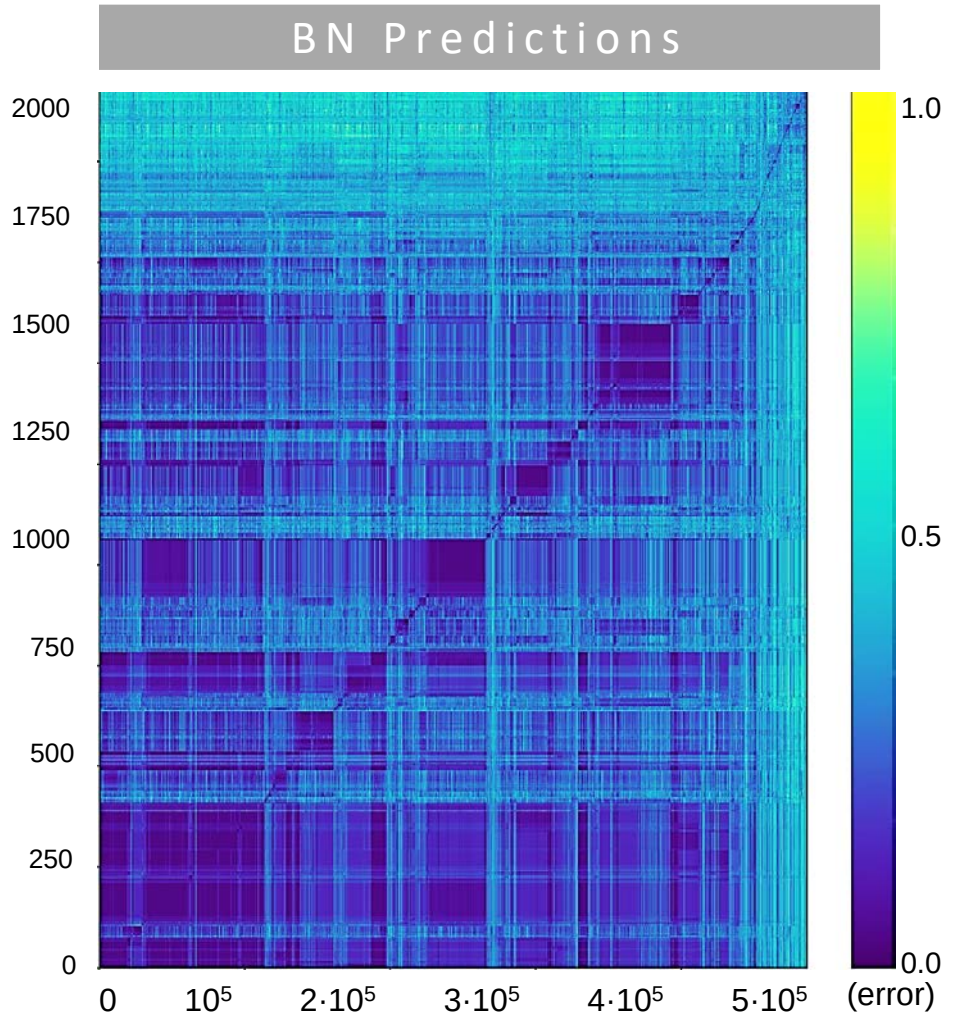
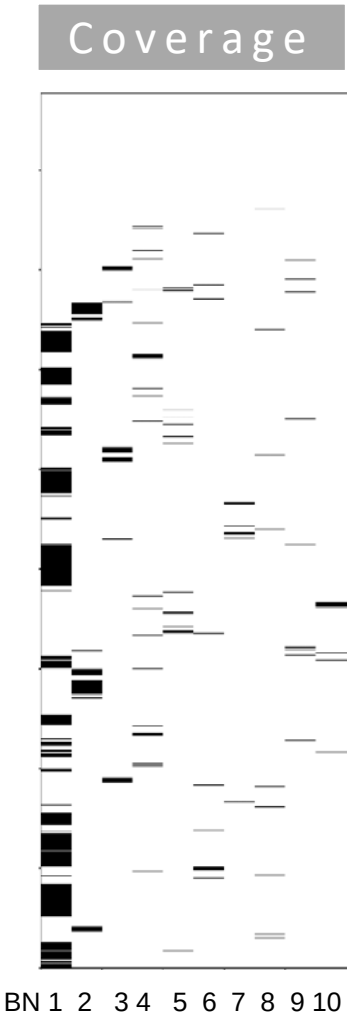






- Predicted 5x10 ^{5} BN for each trajectory (10^9)
- Evaluated each BN against each trajectory
- Just 10 BN predict 80% of all trajectories
- Adverse trajectories

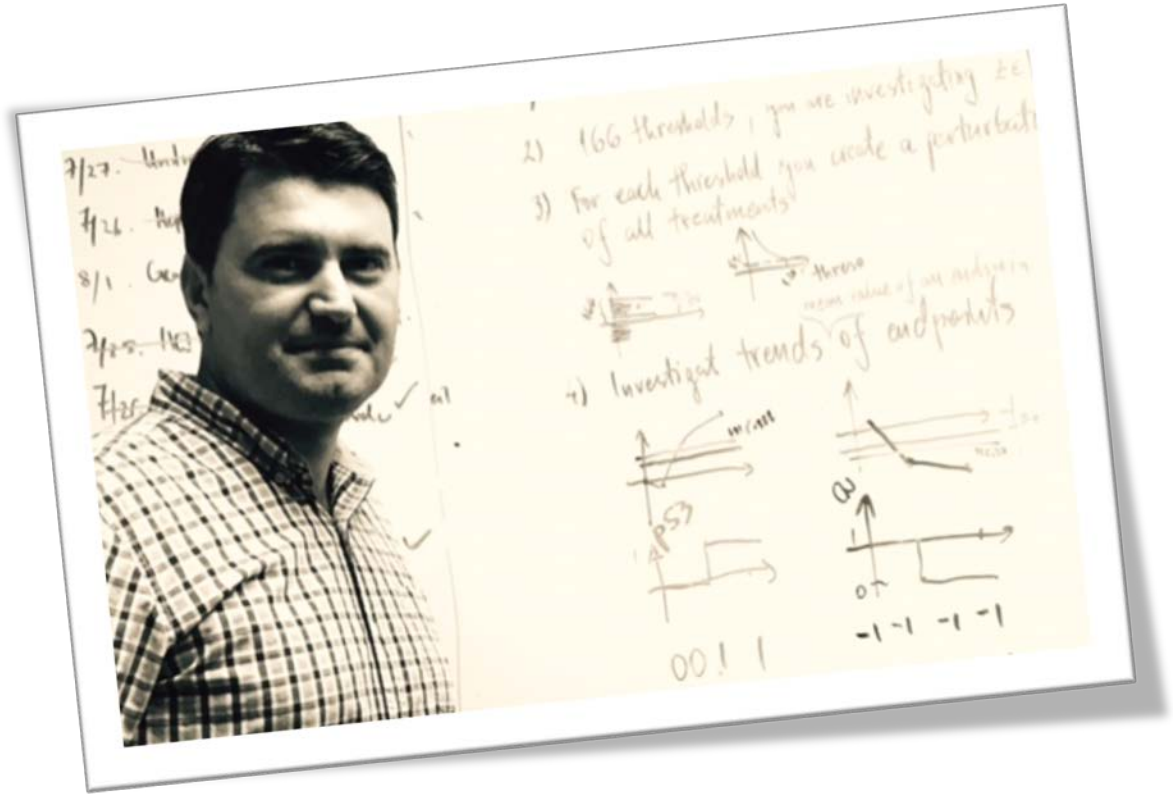
Antonijevic & Shah *(in preparation)*

Summary

- Toxicological tipping points are critical points between adaptive and adverse trajectories
- Adaptive trajectories can be modeled by Boolean Networks
- Feasible to predict tipping points computationally (?)
- Need to understand chemical perturbations alter biological networks

Acknowledgements

Todor Antonijevic



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