Toxicological Tipping Points: Learning Boolean Networks from High-Content Imaging Data.

Shah I and Antonijevic T (NCCT)*

(*) designates the presenters

United States Environmental Protection Agency

Abstracts

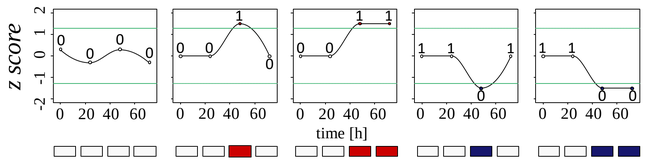
The objective of this work is to elucidate biological networks underlying cellular tipping points using time-course data. We discretized the high-content imaging (HCI) data and inferred Boolean networks (BNs) that could accurately predict dynamic cellular trajectories. We found three main classes of BNs including: cell recovery, adaptation, and injury. We believe biological network analysis can predict critical chemical exposures and mechanisms underlying cellular tipping points.

Disclaimer: this abstract does not reflect EPA policy.

Approach

FY 16/17 Toxicological tipping points using primary rat hepatocytes

1) HCI data[1] were used to study the effect of ToxCast I chemicals on HepG2 cell states by monitoring 10 endpoints across 3 time points (1, 24, and 72h) and 10 concentrations (0.4 to 200µM).
2) Discretization of standardized data.



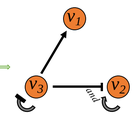
3) Inference of Boolean functions and BNs construction for each trajectory[2,3].

Input			Output		
v_1	v_2	v_3	v_1^*	v_2^*	v_3^*
1	0	0	0	0	1
0	1	0	0	1	1
0	1	1	1	0	0

$v_1^* = v_3$

$v_2^* = v_2$ *and (not* v_3*)*

$v_3^* =$ *not* v_3



5) Estimation of Hamming distance (mismatch) between observed discretized state and BN prediction for:
   1) the trajectory for which BNs were sampled,
   2) all trajectories.
6) Boolean Network Coverage

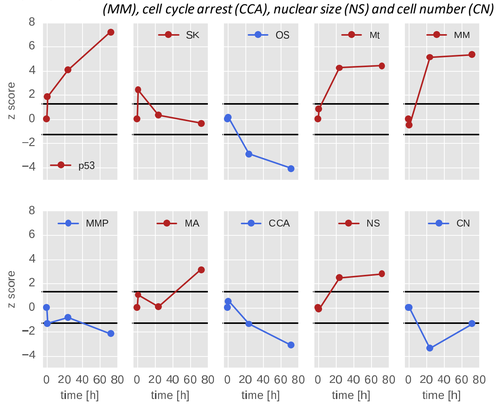
trajectories	1	2	3	4	5	6	7
BN1	1	1	1	0	1	0	0
BN2	0	0	0	1	1	1	0
BN3	0	0	1	0	0	0	1

Preliminary Results

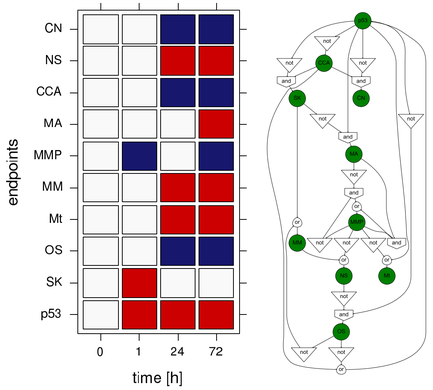
Data Discretization, and BNs in case of Butachlor :

Butachlor 200µM: Measured endpoints

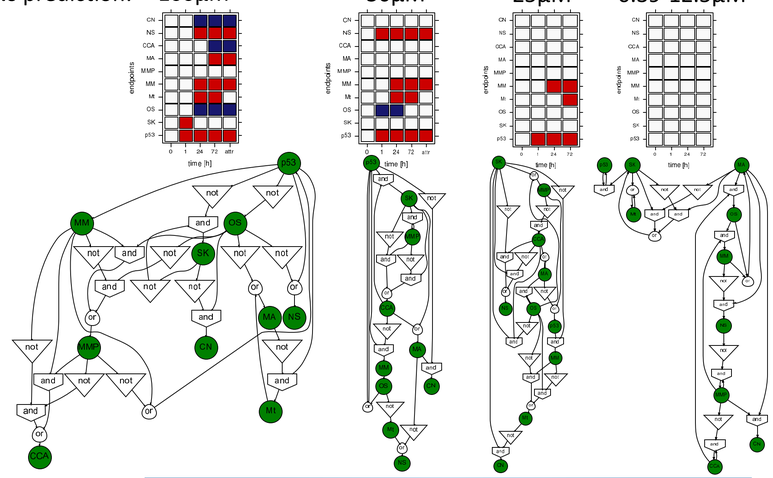
p53 activation (p53), c-Jun activation (SK), phospho-Histone H2A.x (OS), phospho-Histone H3 (MA), phosphorylated α-tubulin (Mt), mitochondrial membrane potential (MMP), mitochondrial mass (MM), cell cycle arrest (CCA), nuclear size (NS) and cell number (CN)



Butachlor 200µM:
Discretized Trajectory and BN

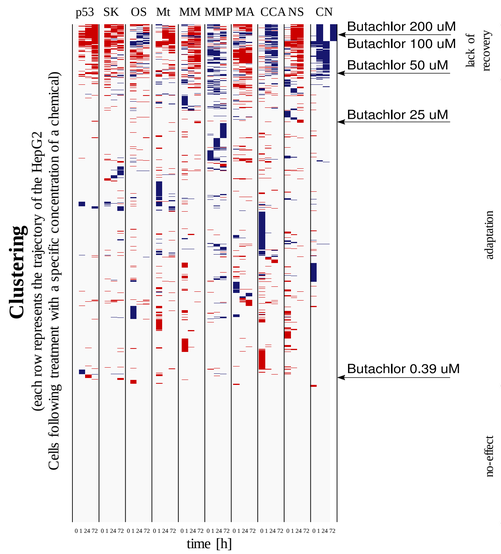


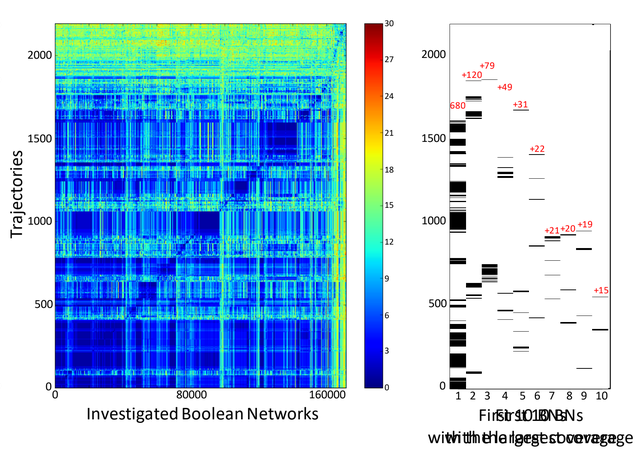
BNs prediction: 100µM 50µM 25µM 0.39-12.5µM



Clustering Trajectories, Hamming Distance Estimation and 10 BNs with the Largest Coverage

Clustering of trajectories suggests that similar perturbation patterns may indicate similar response mechanisms. Furthermore, estimated Hamming distance allows discrimination of BNs that perform well in adaptation region from those that lead toward adverse effect.





Anticipated Products/Impacts

- HepG2 cells showed three phenotypes in response to chemical treatments: no effect, adaptation and injury. We inferred the minimal number of BNs that could explain these phenotypes. We are in the process of identifying the critical network perturbations involved in the transition from adaptation to injury. Understanding these critical network features may enable the prediction of system tipping points with limited time-course data.

References

1. Shah, Imran, et al. "Using ToxCast™ Data to Reconstruct Dynamic Cell State Trajectories and Estimate Toxicological Points of Departure." *Environmental health perspectives* (2015).
2. Akutsu, Tatsuya, Satoru Miyano, and Satoru Kuhara. "Identification of genetic networks from a small number of gene expression patterns under the Boolean network model." *Pacific symposium on biocomputing*. Vol. 4. 1999.
3. Müssel, Christoph, Martin Hopfensitz, and Hans A. Kestler. "BoolNet—an R package for generation, reconstruction and analysis of Boolean networks."*Bioinformatics* 26.10 (2010): 1378-1380.