Inferring Toxicological Responses of HepG2 Cells from ToxCast High Content Imaging Data

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Abstract

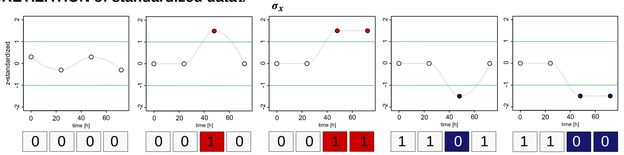
Objective: Understanding the dynamic perturbation of cell states by chemicals can aid in predicting their adverse effects. We Inferred coarse-grained dependencies between cellular functions from high-content imaging data using Boolean Networks (BNs)

Approach: High-content imaging (HCI) was used to measure the state of HepG2 cells at three time points (1, 24, and 72 h) in response to 976 ToxCast chemicals for 10 different concentrations (0.39-200μM). Cell state was characterized by p53 activation (p53), c-Jun activation (SK), phospho-Histone H2A.x (OS), phospho-Histone H3 (MA), alpha tubulin (Mt), mitochondrial membrane potential (MMP), mitochondrial mass (MM), cell cycle arrest (CCA), nuclear size (NS) and cell number (CN). Dynamic cell state perturbations due to each chemical concentration were utilized to infer Boolean networks (BNs) in two steps. First, the data for each state variable were discretized into changed/active (> 1 standard deviation), and unchanged/inactive values. Second, the discretized data were used to learn Boolean relationships between variables. The structure of a BN describes plausible mechanisms by which HepG2 cells respond to chemicals. The output of a BN is the predicted trajectory of HepG2 cells to chemical treatments over time.

This does not necessarily reflect U.S. EPA policy.

Methods

1. **HCI data (Shah I. et al. 2015):**
   The following cellular endpoints were quantified: phosphorylated p53 / p53 activation (p53), phosphorylated c-Jun/c-Jun activation (SK), phospho-Histone H2A.x (OS), phospho-Histone H3 / mitotic arrest (MA), phosphorylated α-tubulin / microtubules (Mt), mitochondrial membrane potential (MMP), mitochondrial mass (MM), cell cycle arrest (CCA), nuclear size (NS) and cell number (CN). MMP and MM were quantified using MitoTracker® Red marker, and NS, CCA and CN were quantified using Hoechst33342 nuclear stain. CCA was defined using the ratio of 2N/4N cells.
2. **DISCRETIZATION of standardized data** $z = \frac{x - x^*}{\sigma_x}$



3. **INFERRING BOOLEAN NETWORKS (Akutsu T. et al. 1999; Shmulevich I. et al. 2002; Müssel C. et al. 2010)**

a. data discretization

INPUT			OUTPUT		
v_1	v_2	v_3	v^*_1	v^*_2	v^*_3
0	0	0	0	0	1
0	0	1	0	0	1
0	1	0	1	0	1
0	1	1	1	0	1
1	0	0	0	0	0
1	0	1	0	1	0
1	1	0	1	0	0
1	1	1	1	1	0

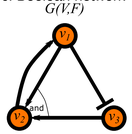
b. inferring Boolean functions

$v^*_1 = v_2$

$v^*_2 = v_1$ ***and*** v_3

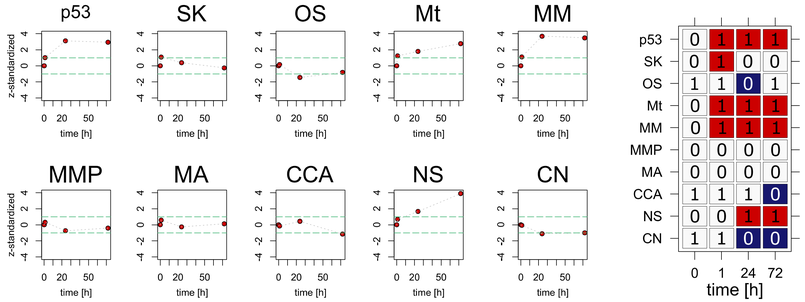
$v^*_3 =$ ***not*** v_1

c. Boolean network $G(V,F)$

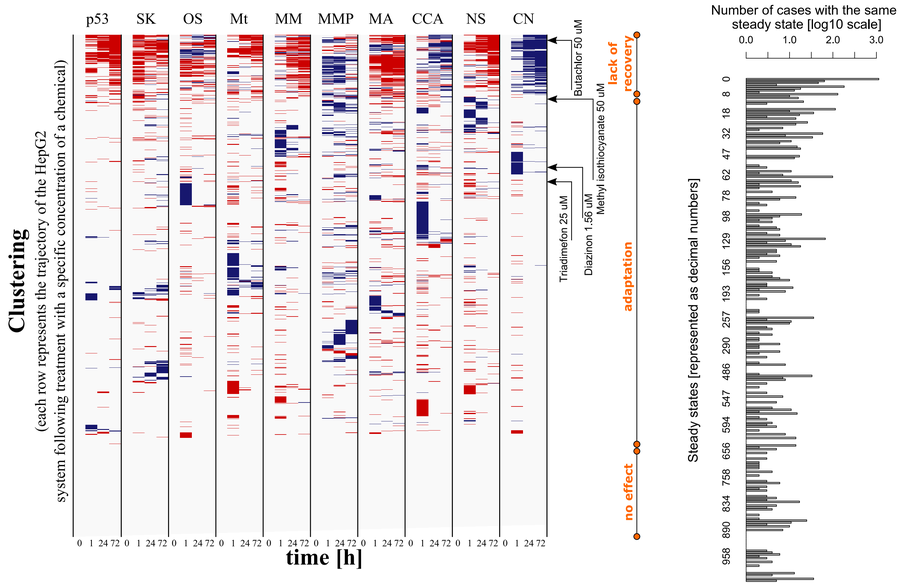


A BN predicts the trajectory of the system. Steady states in trajectories are called “attractors”, which may correspond to phenotypic states of the system.

Data Discretization – case: Butachlor at 50 μM



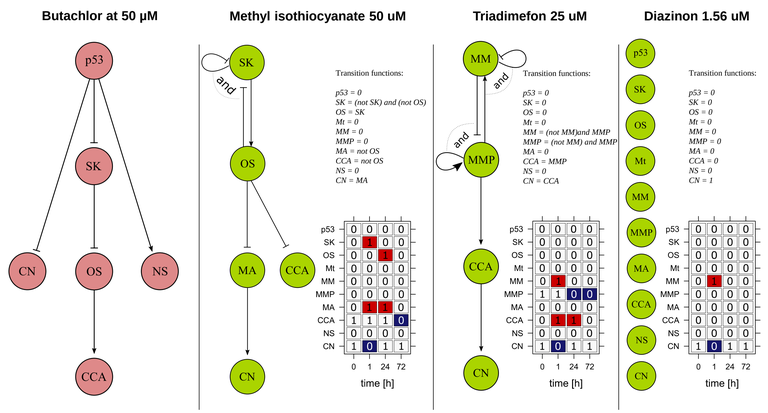
Clustering time-course HCI data



BN Inference and attractor prediction

Lack of recovery

Examples of adaptation (with the same attractor)



Summary & Future Work

- HepG2 cell response shows three temporal trends in HCI data: (i) no effect, (ii) adaptation, and (iii) lack of recovery. We consider lack of recovery toxicity (Shah et al 2015).
- After 72h only 265 unique HepG2 responses (states) were found. These include and cycle BN attractors .
- All attractors may not be biologically-relevant (insufficient time points or cellular end points).
- Boolean networks useful for modeling discrete HCI data sets
- Additional work necessary to predict which BN lead to adaptation vs. toxicity / tipping points.

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. Shmulevich, Ilya, et al. "Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks." *Bioinformatics* 18.2 (2002): 261-274.
4. 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.