Using High-Content Screening Data from ToxCast to Analyze Cell State Trajectories

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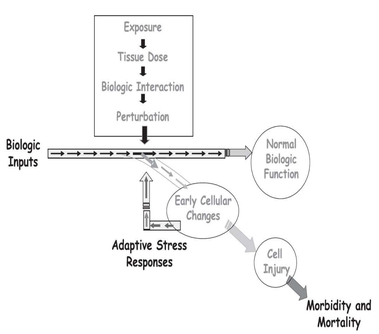
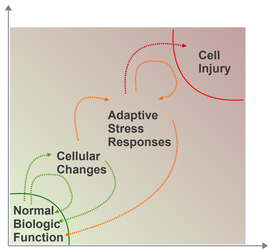
Abstract

We developed a novel approach for analyzing chemical-induced dynamic cell 'state' changes using in vitro data. The ToxCast project conducted a high content screening (HCS) study in which HepG2 cells were treated with 10 concentrations (ranging from 0.4 to 200 μM) of 976 diverse chemicals, and 10 cellular endpoints were measured after 1, 24 and 72 h of exposure. After analyzing the concentration response data we found 669 chemicals significantly changed one or more endpoints including: mitochondrial functions (435), cell cycle (412), microtubule stability (332), c-Jun (253), p53 (253), and cell number (510). In order to capture the overall response to each chemical at a single time point, we constructed a bioactivity profile based on the magnitude and the direction of the change in each endpoint. The bioactivity profiles for all chemicals were clustered by K-means analysis, based on Euclidean distance and different cluster quality metrics were used to identify 15 clusters. We assumed these clusters capture a range phenotypic 'states' of the HepG2 system, from normality to injury (defined by significant cell loss), as it responds to different exposures. By translating bioactivity vectors to cellular states, the temporal effects of each chemical can be described as a sequence of states, called a trajectory. A trajectory summarizes the dynamic sequence of changes in the HepG2 system as it adapts to chemical exposure. Chemicals with similar mechanisms such as, mitochondrial disruptors, microtubule disruptors and stress kinase activators produced similar trajectories. On the other hand, structurally similar chemicals, such as phthalates, produced different trajectories that were consistent with their known toxicity. By analyzing trajectories, it was possible to characterize exposures that caused reversible changes vs those that did not lead to recovery.

Adaptation, adversity and system trajectories

Dynamic multi-dimensional chemical-induced perturbations can be viewed as trajectories. Each point on a trajectory represents the configuration of the system as it adapts to chemical-exposure. Temporal observations (e.g HCS) provide discrete snapshots of the system on a trajectory.





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.

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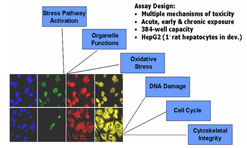
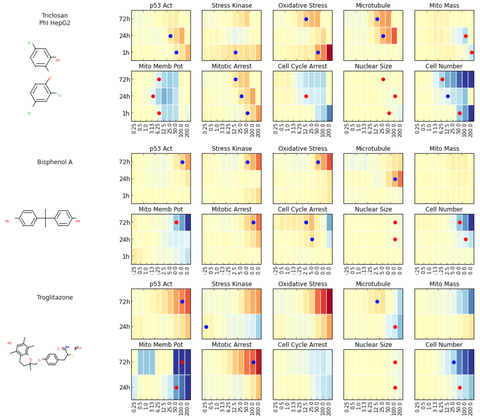
From HCS to dynamic cell system state trajectories

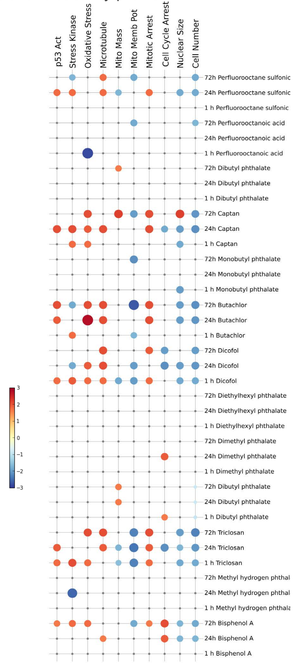
1. High Content Screening: Multiplexed assays capturing 10 cellular endpoints at 1, 24, 72h for 976 chemicals 0.4 to 200μM in HepG2 cells.



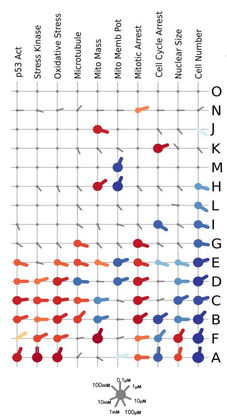
2. Concentration-response profiles: Smoothing, normalization for each chemical, time, and endpoint. Calculate lowest effect concentration (LEC) and efficacy (max(log2(fold change)))



3. Bioactivity profiles: Combine LEC and efficacy for each chemical at each time to generate 10-dimensional bioactivity profile.



4. Bioactivity states: bioactivity profiles clustered into 15 phenotypic states (rows: O-A) defined by 10 endpoints (columns). Circles show the mean efficacy for endpoint across state. Colors signify direction (red=increase, blue=decrease), saturation is median efficacy, and size consistency of effect. The hour hand on circle is 75% potency (12pm=0.1μM, 2pm~1μM, 5pm~100μM).



State O: Normal
State N: Minor perturbation
State H: Mitochondrial disruption
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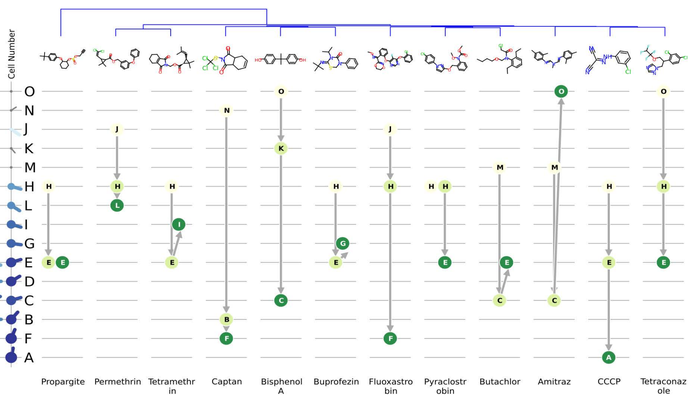
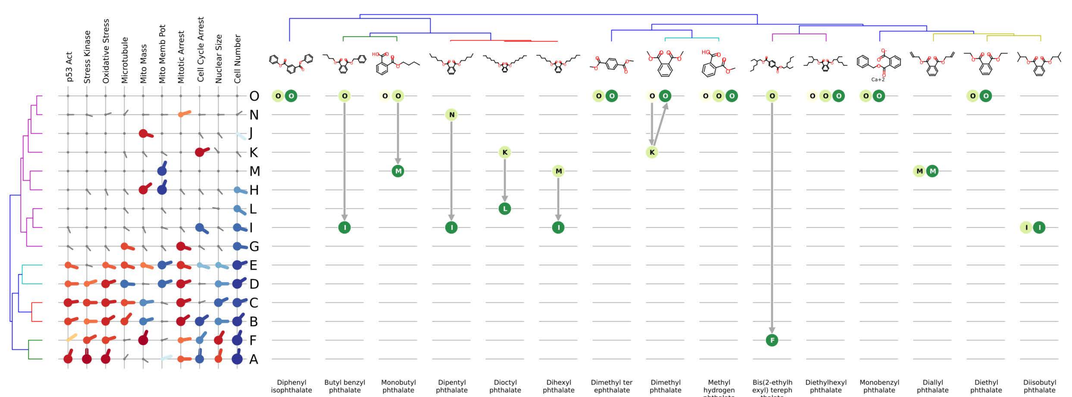
State A: Maximum cell loss

5. Trajectories: The trajectory produced by each chemical is shown as a vertical panel. The color of each circle signifies the time (6, 24, 72h) and the arrows show increasing time. The dendrogram on the top shows similarity between chemicals based on their trajectories.



Propargite produced trajectory H(6h)→E(24h)→E(72h) (or HEE). CCCP (a mitochondrial disruptor) produced trajectory HEA while Amitraz produced trajectory MCO (full recovery). Some chemicals produced increasing state changes from O → A with time (e.g. CCCP). On the other hand, others resulted in partial recovery after perturbation (e.g. famoxadone, tetramethrin and buprofezin).

6. Trajectory analysis of structurally-related chemicals: The trajectories produced by 15 phthalates are shown below. The dendrogram above chemicals was produced by hierarchical clustering using Ward's method, Euclidean distance, and 1,024 bit Morgan fingerprints for representing chemical structures.



Conclusions

Trajectory analysis is a novel tool for interpreting the dynamic response of a living system to chemical-exposure. By assessing multidimensional perturbations in cellular systems, high-content screening (HCS) provides useful information for reconstructing such trajectories. Based on this analysis: 462 chemicals produced no significant changes, 234 chemicals produced partial recovery and 280 chemicals produced trajectories that did not result in recovery (by 72h). We believe trajectory analysis can be used with different high-throughput biological data-streams to characterize "tipping points" and critical concentrations that exceed the adaptive capacity of biological systems.

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