United States Environmental Protection Agency

Using High-Content Imaging to Analyze Toxicological Tipping Points

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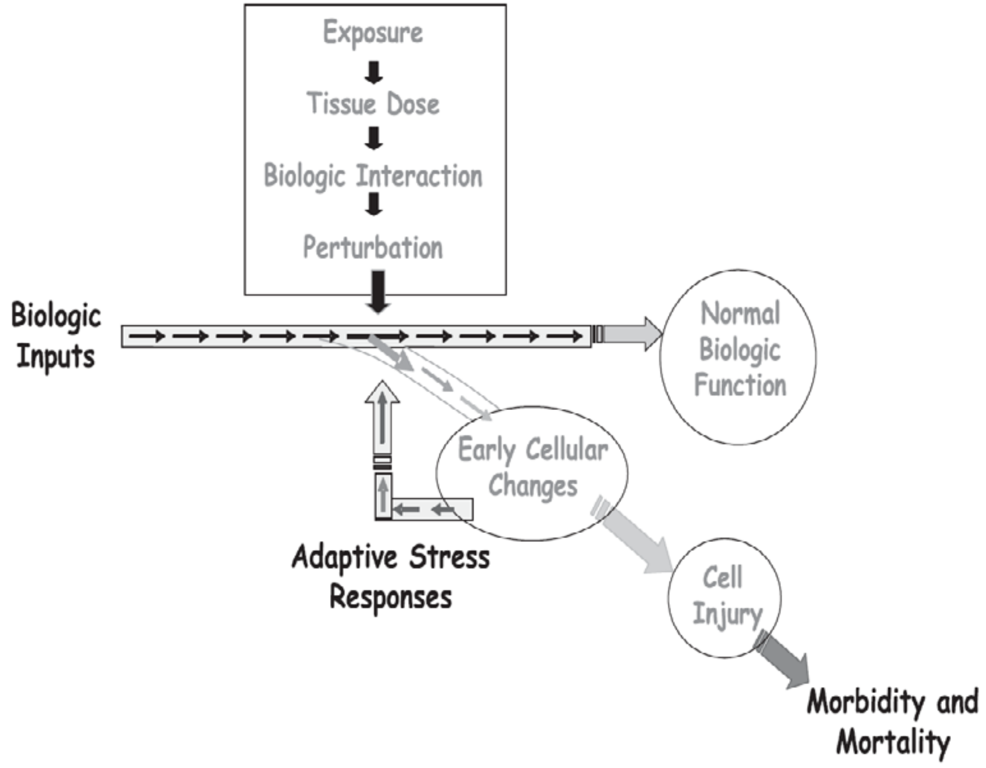
Toxicological Tipping Points

- Current toxicological tests use apical *in vivo* adversity to define a point of departure (PoD) for risk assessment
- Biological systems are resilient and adapt to environmental perturbations
- Threshold between adaptation and adversity: ***"Tipping point"***
- Could toxicological tipping points be used as PoD for risk assessment ?
- *How can we use high-content imaging (HCI) data to find tipping points ?*



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.

High Content Imaging (HCI)

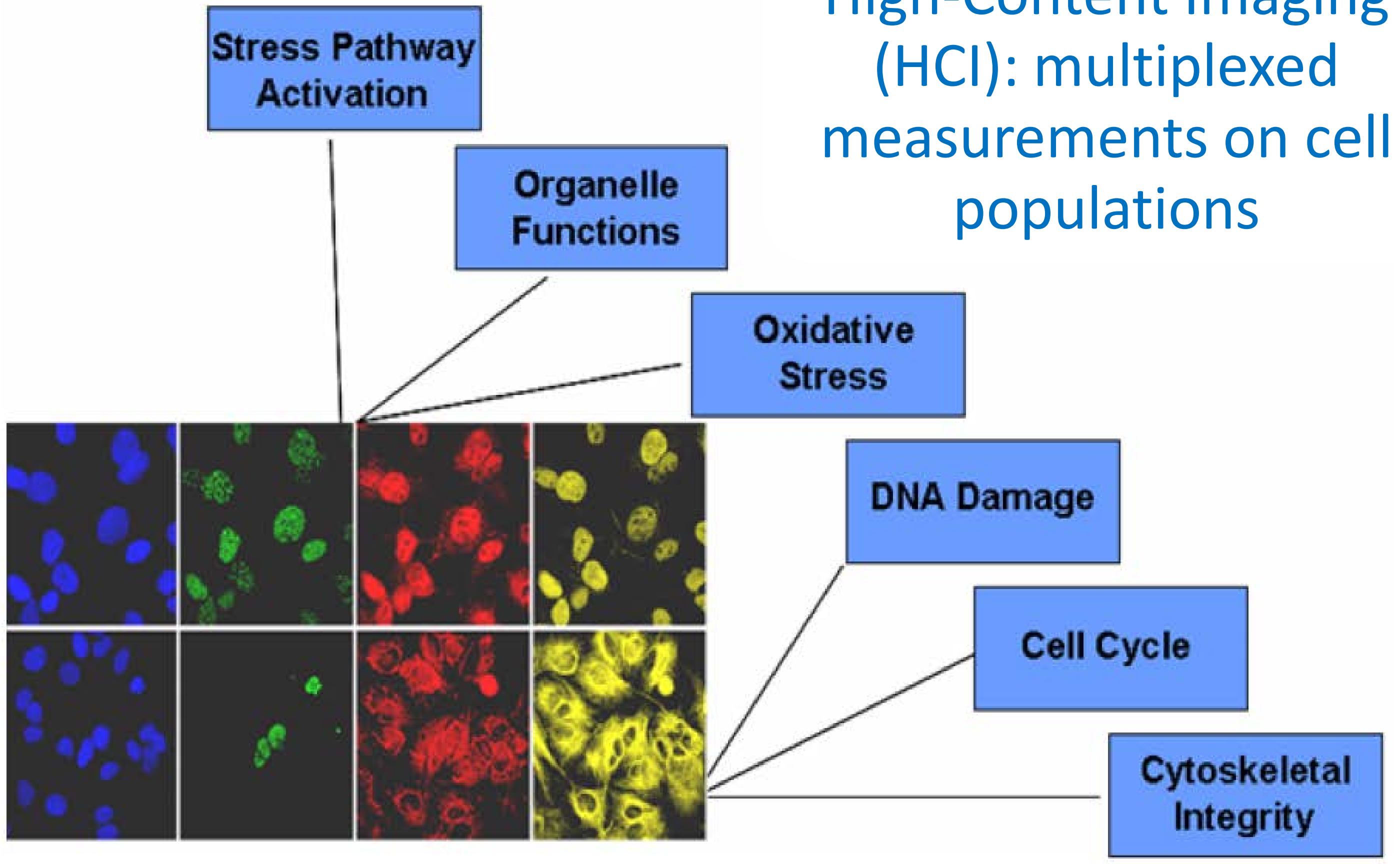
- **Study**
  - HepG2 cell culture
  - 967 chemicals (ToxCast)
  - 10 conc

- **HCI Assays**
  - Health
  - Stress
  - Cellular perturbations

- **Dynamic phenotypic** response of cells to chemicals

- **Large-scale data**
  - ~400 plates
  - ~100,000 wells
  - ~2,400,000 images

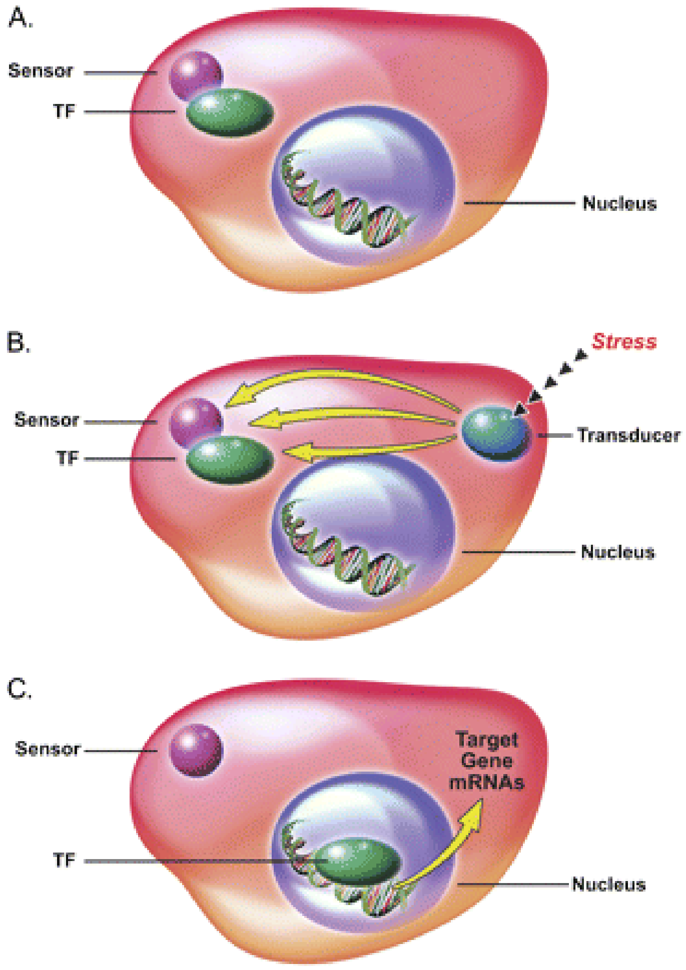
High-Content Imaging (HCI): multiplexed measurements on cell populations



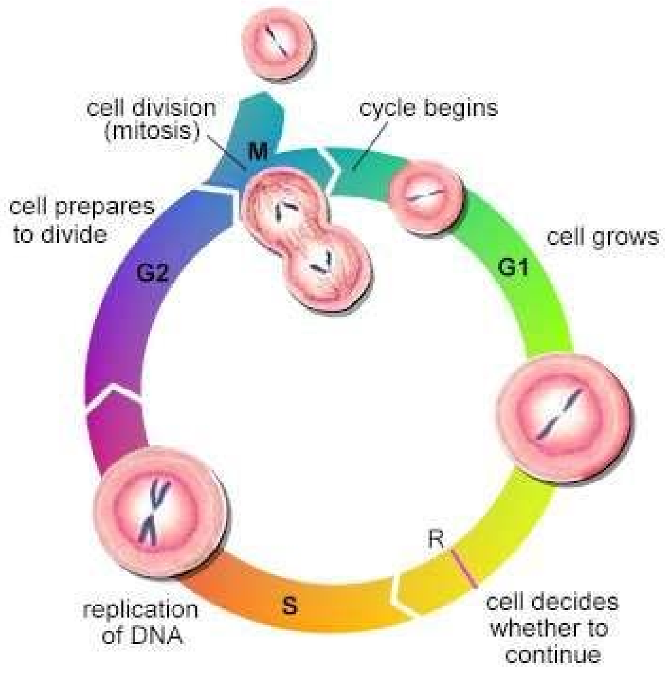
HCI Conducted by Cyprotex, Inc.

HCI Endpoints: Cell State

Stress Response Pathways



Cell Cycle Progression

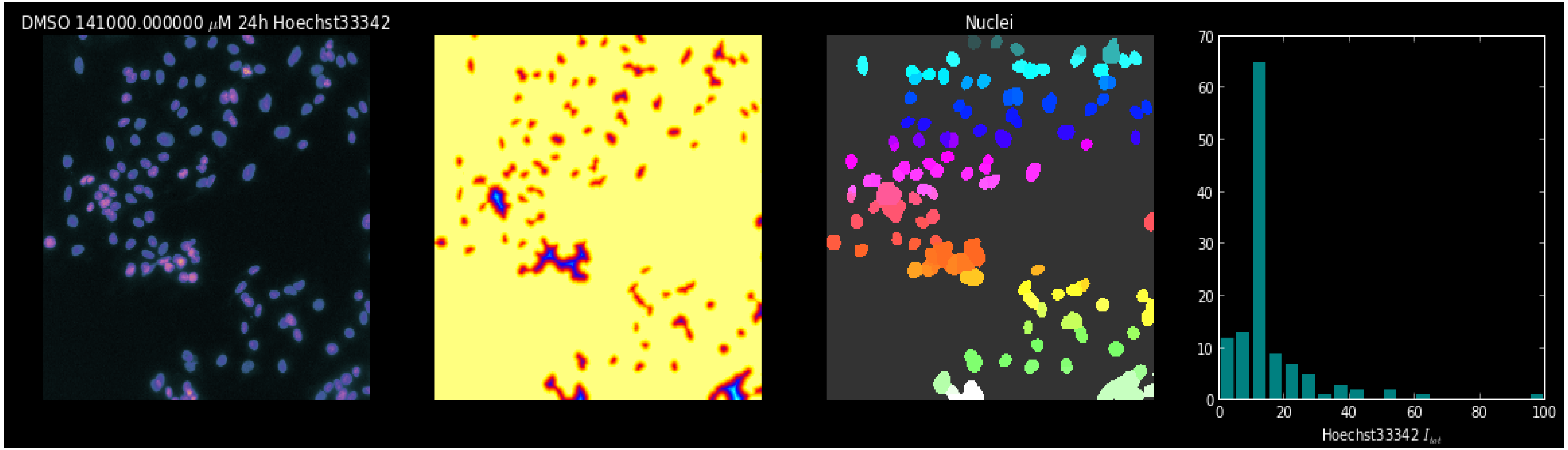


- P53: p53 activity (**p53**)
- c-Jun: stress kinase (**SK**)
- H2AX: oxidative stress (**OS**)
- MitoTracker Red: mitochondrial mass (**MM**), mitochondrial membrane potential (**MMP**)
- Tubulin: microtubule organisation (**Mt**)
- Hoechst33342: nuclear size (**NS**), cell cycle arrest/progression (**CCA**), cell number (**CN**)
- PH3: mitotic arrest (**MA**)

Simmons et al 2009

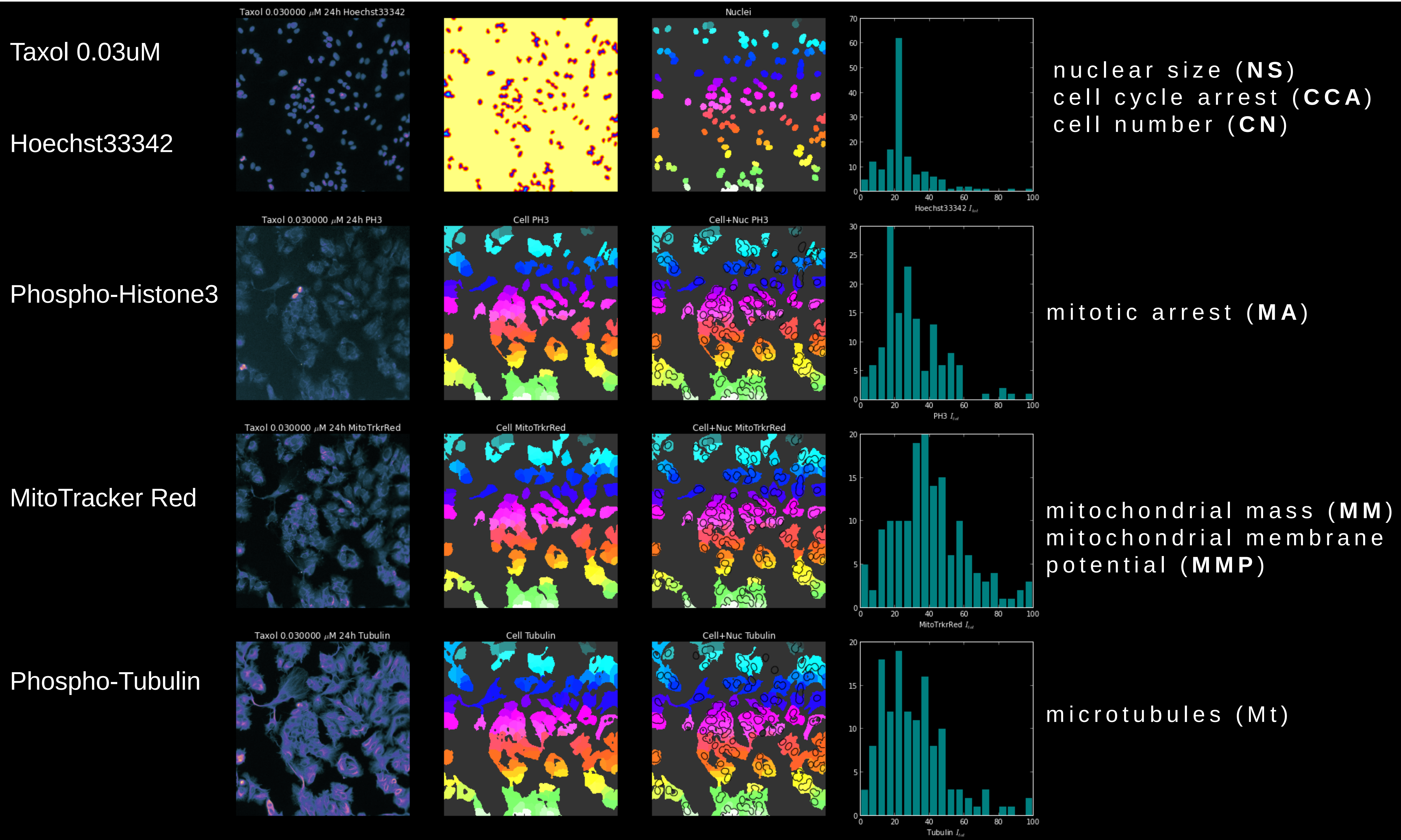
www.flowcytometry.us

Cell-State Data from Images



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

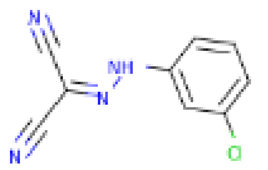
Image analysis and cell level feature feature extraction conducting by Cyprotex Inc. (proprietary software)

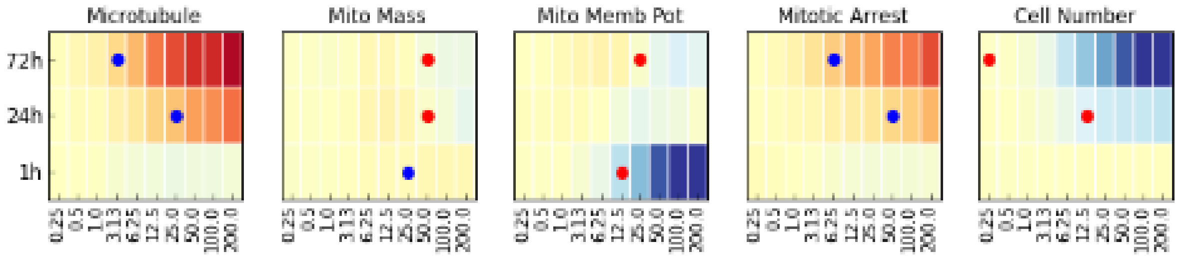


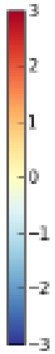
Taxol 0.03uM
Hoechst33342
Phospho-Histone3
MitoTracker Red
Phospho-Tubulin
Taxol 0.030000 μM 24h Hoechst33342
Nuclei
Hoechst33342 I_tot
Taxol 0.030000 μM 24h PH3
Cell PH3
Cell+Nuc PH3
PH3 I_tot
Taxol 0.030000 μM 24h MitoTrkrRed
Cell MitoTrkrRed
Cell+Nuc MitoTrkrRed
MitoTrkrRed I_tot
Taxol 0.030000 μM 24h Tubulin
Cell Tubulin
Cell+Nuc Tubulin
Tubulin I_tot
nuclear size (NS)
cell cycle arrest (CCA)
cell number (CN)
mitotic arrest (MA)
mitochondrial mass (MM)
mitochondrial membrane
potential (MMP)
microtubules (Mt)

Mitochondrial disruptors

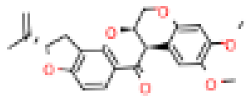
CCCP

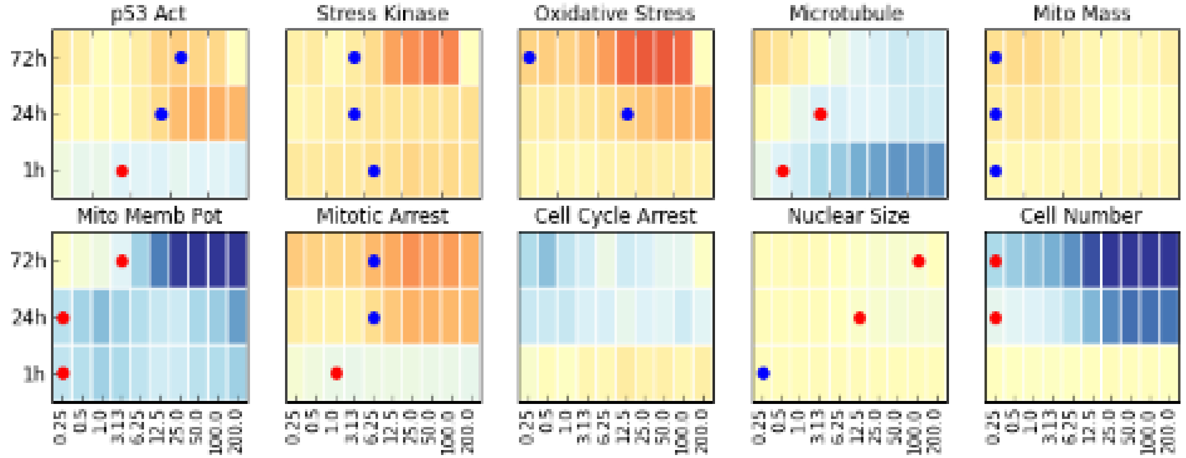






Rotenone





- log2(fold change): decrease (BLUE), increase (RED) or no effect (YELLOW)
- Bioactivity profile: “deviation from normal state” of HepG2 cells

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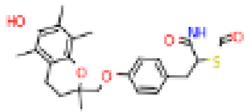
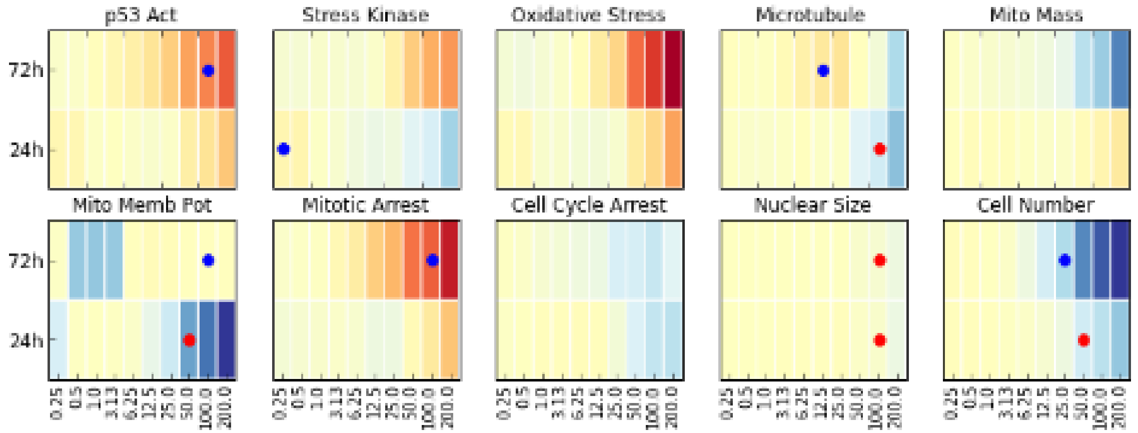
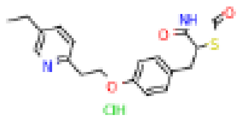
Thiazolidinediones (TZDs)

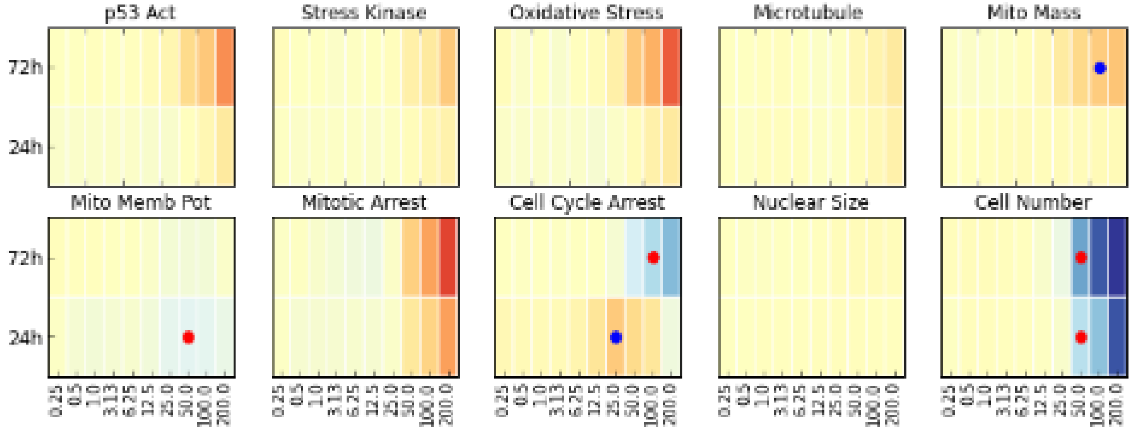
Troglitazone

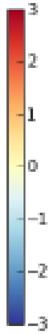




Pioglitazone hydrochloride

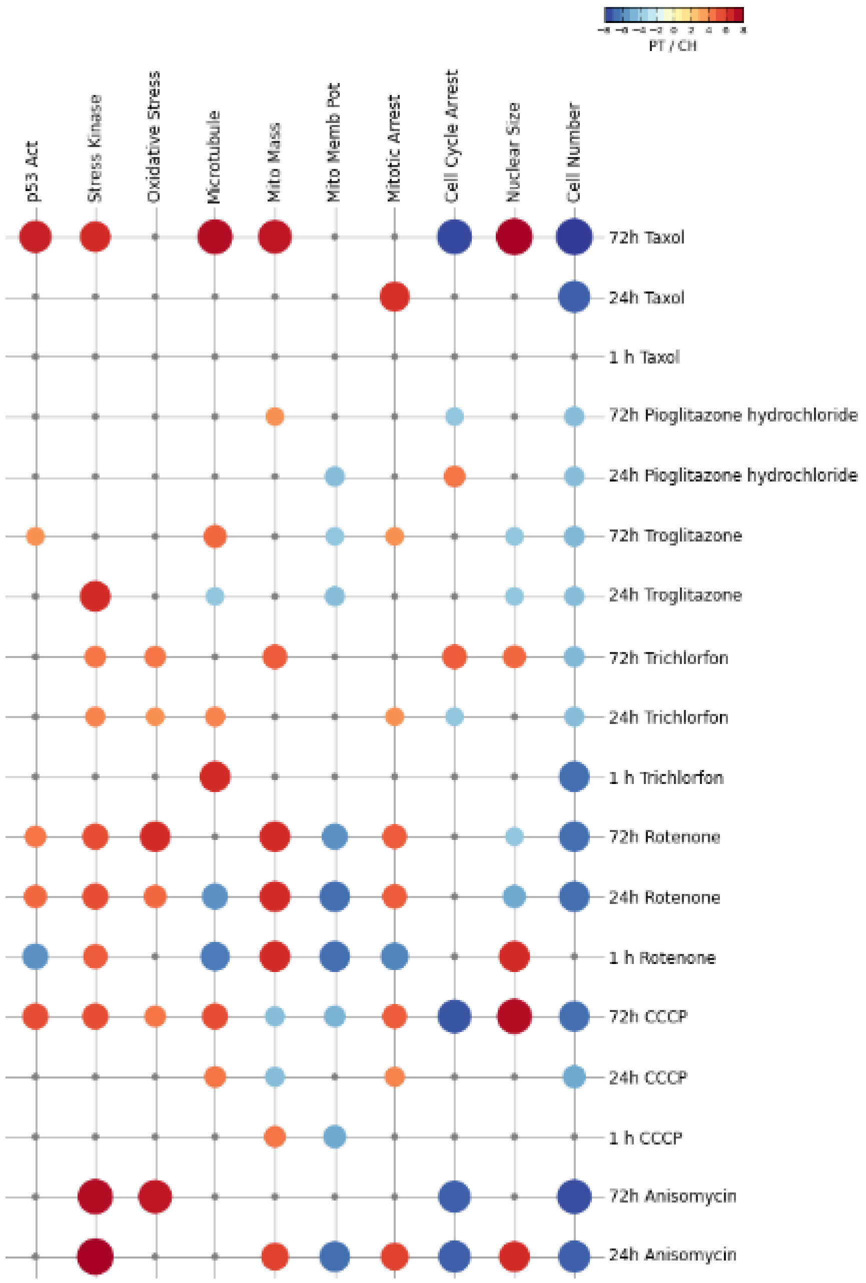






Summarizing Large-Scale HCI Data

- Each chemical produces a 10 dimensional HCI response for each treatment (concentration & time)
- Results in close to 10^5 data points or 10^4 vectors
- How do we summarize and interpret such data?
- How do we predict tipping points using these data ?



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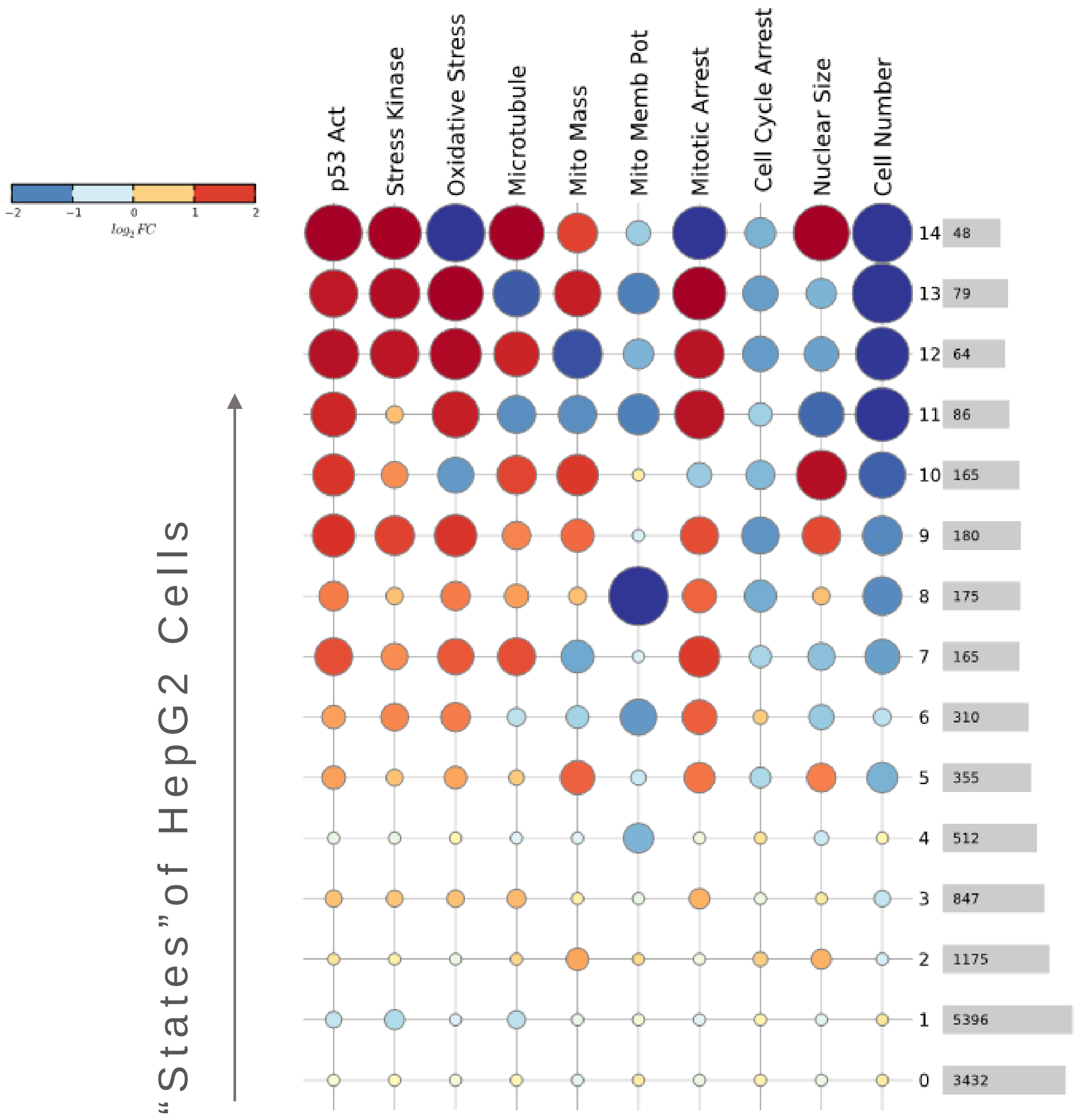
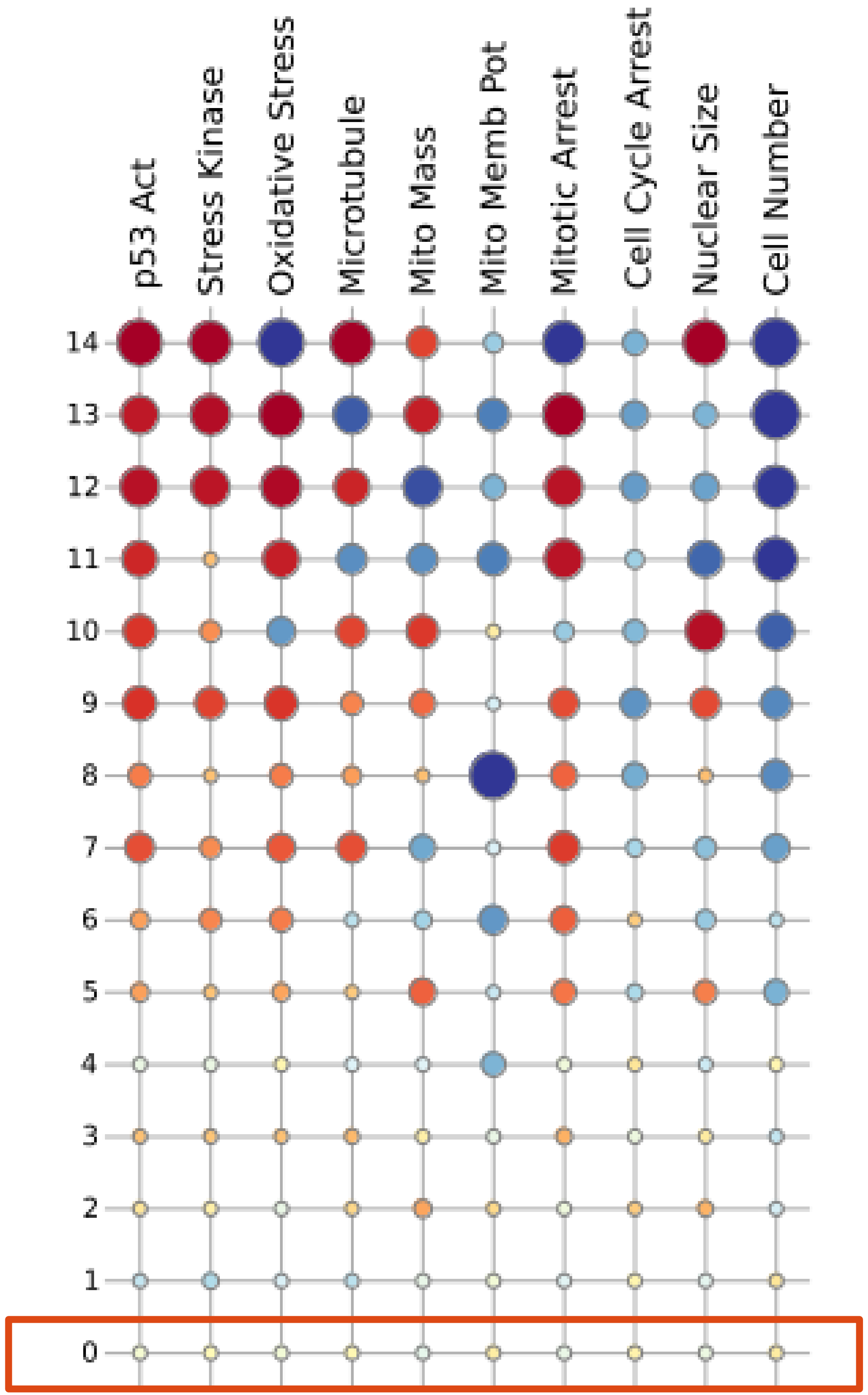
HCI to Phenotypic States

- We used unsupervised learning (clustering)
- Cluster all 10^4 treatment responses
- Derive phenotypic “states” of HepG2 cells
- Phenotypic states *could* represent canonical behaviours of cells
- We could use these phenotypic states to understand the dynamic response to chemicals



“Normal” State

Fluazinam 0.78 uM



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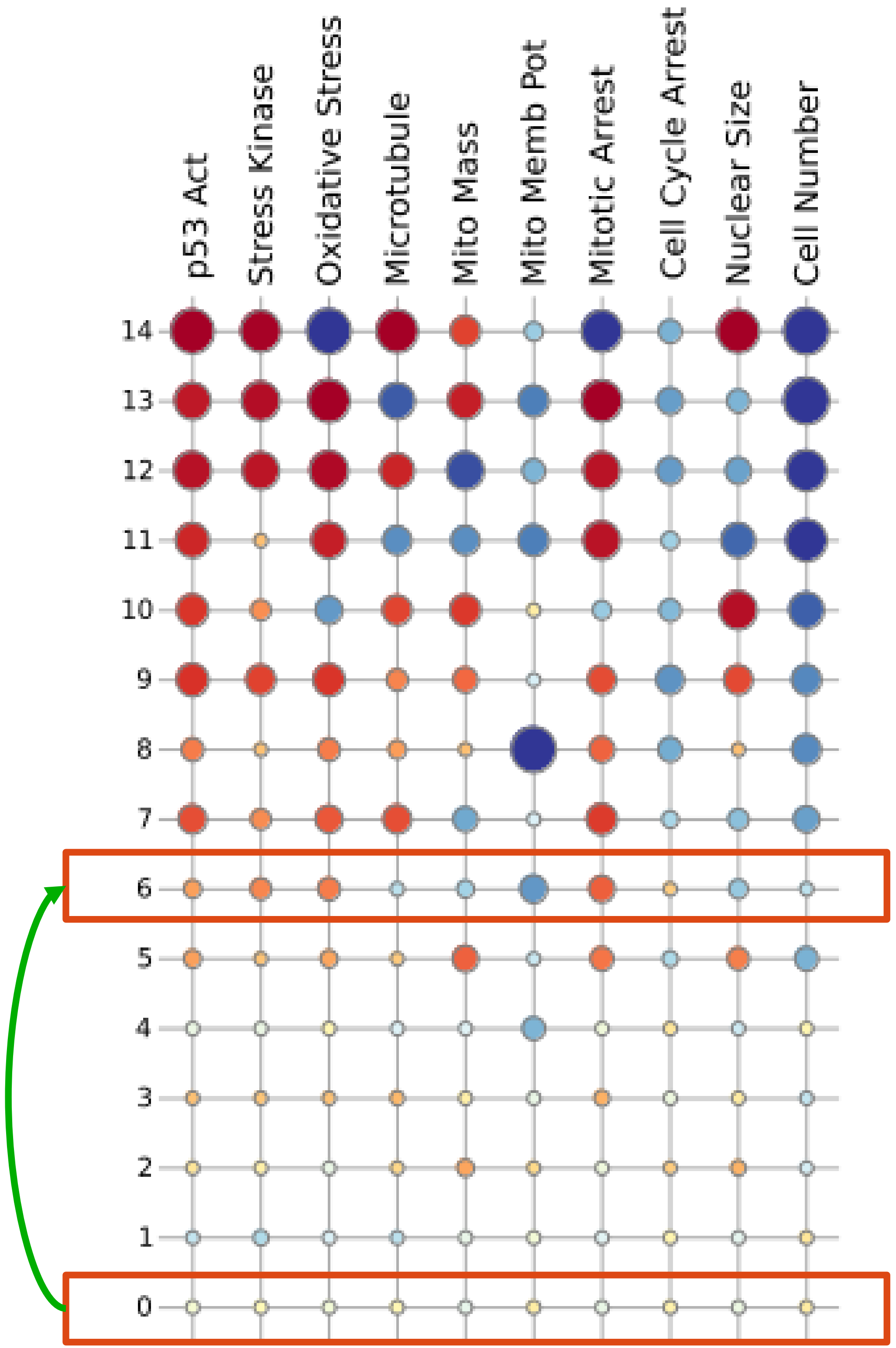
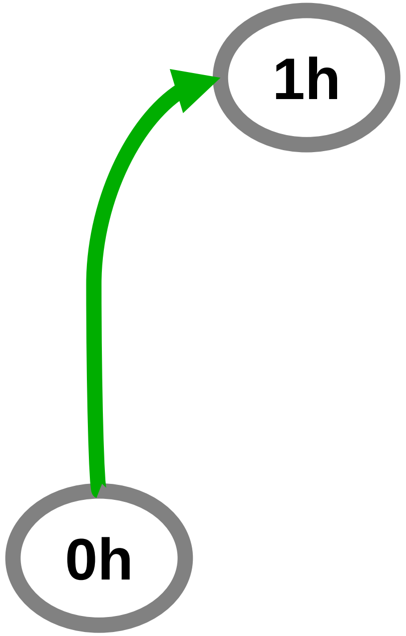
State Transition

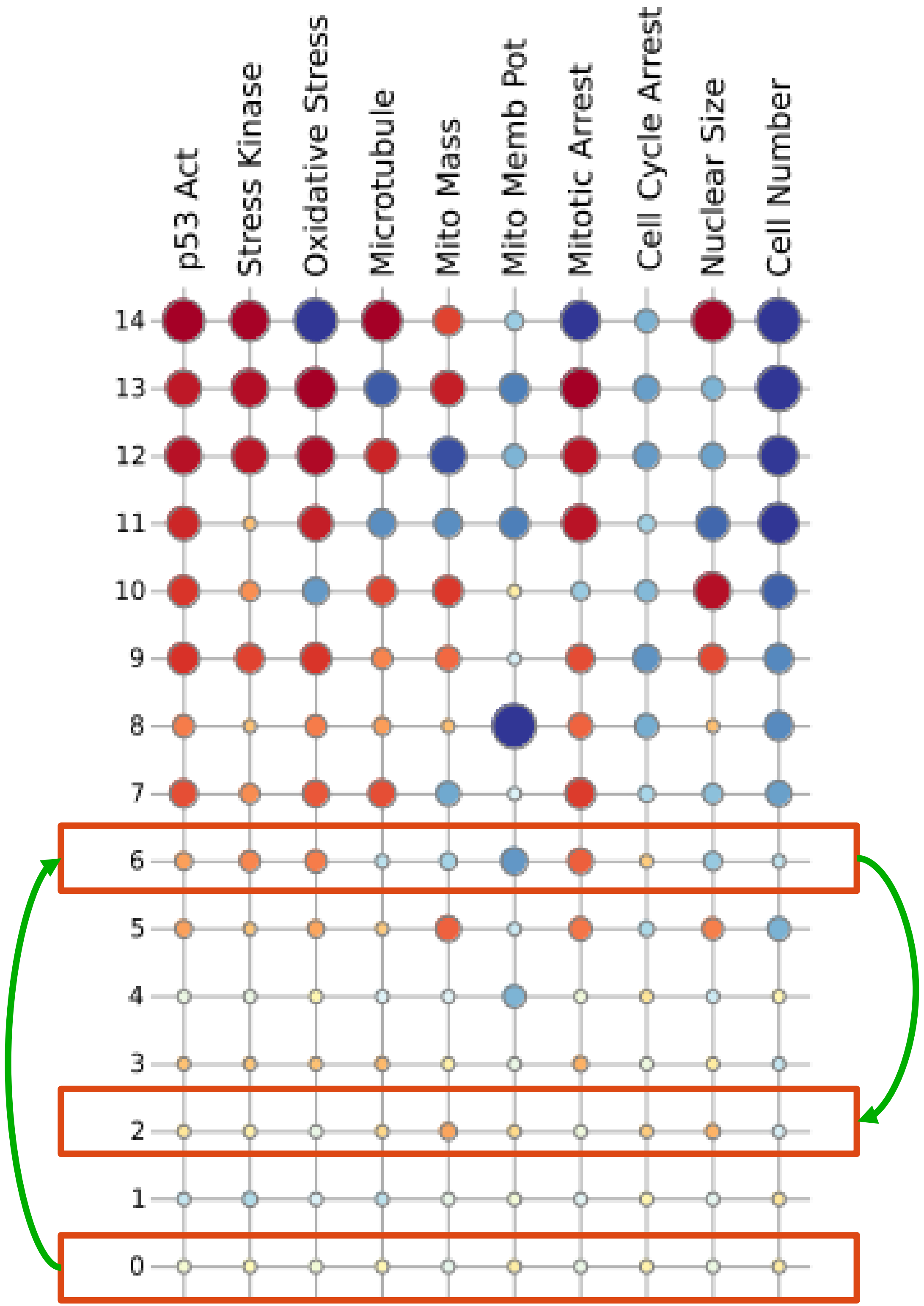
Fluazinam 0.78 uM

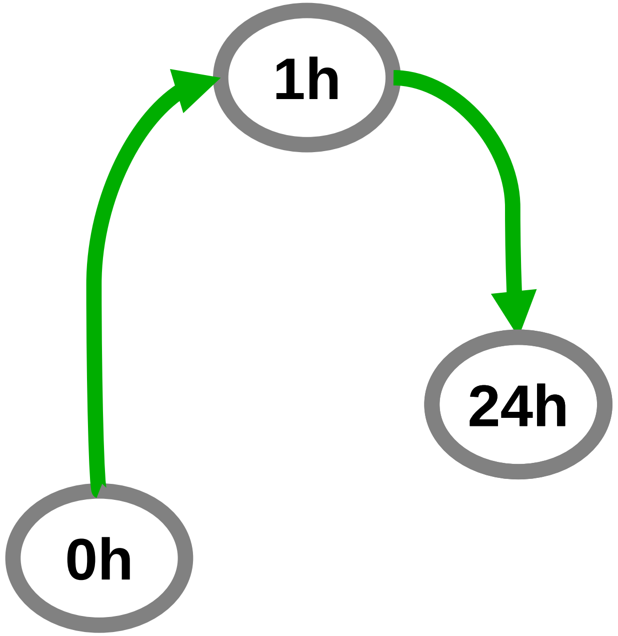




System trajectory

Fluazinam 0.78 uM





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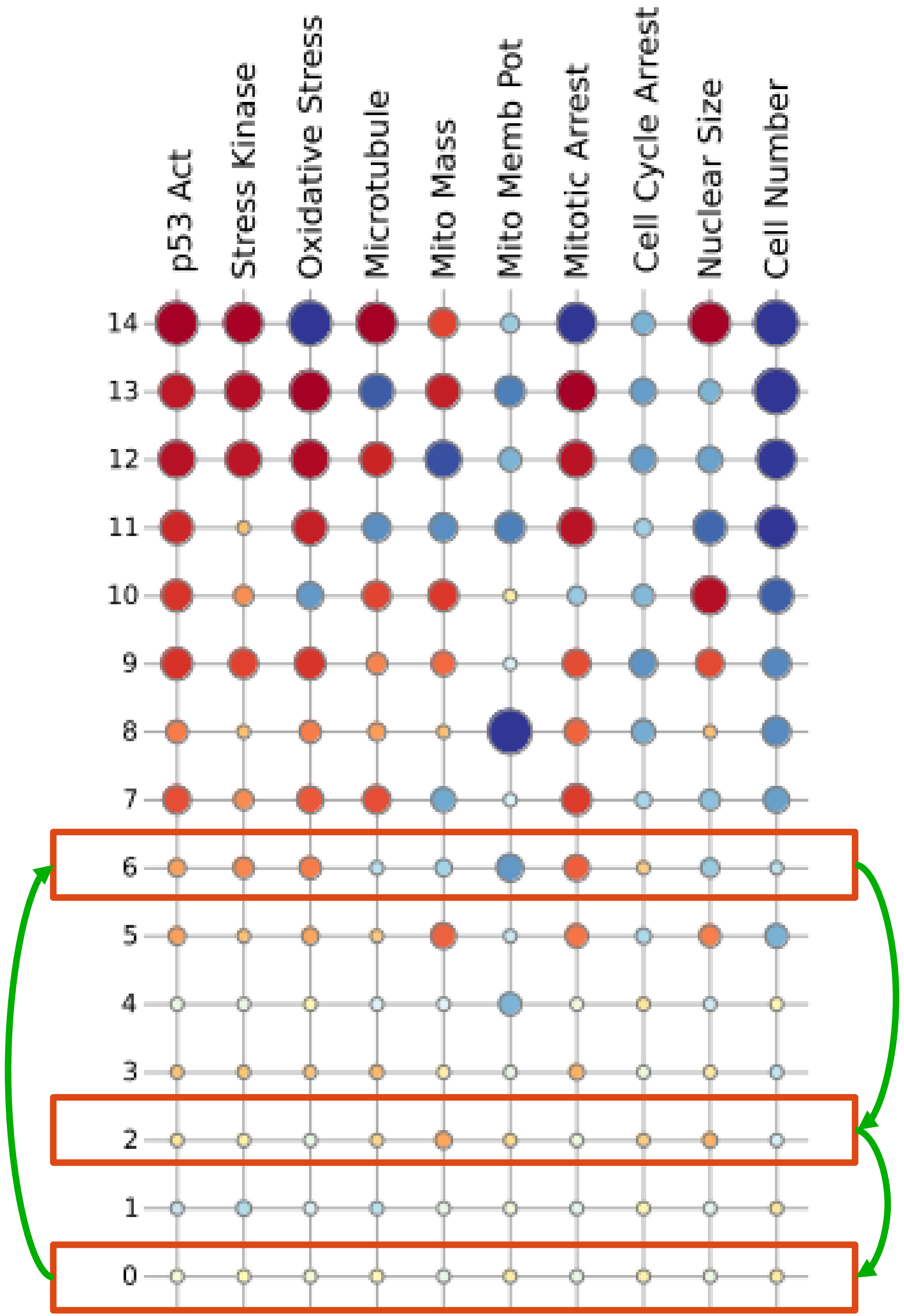
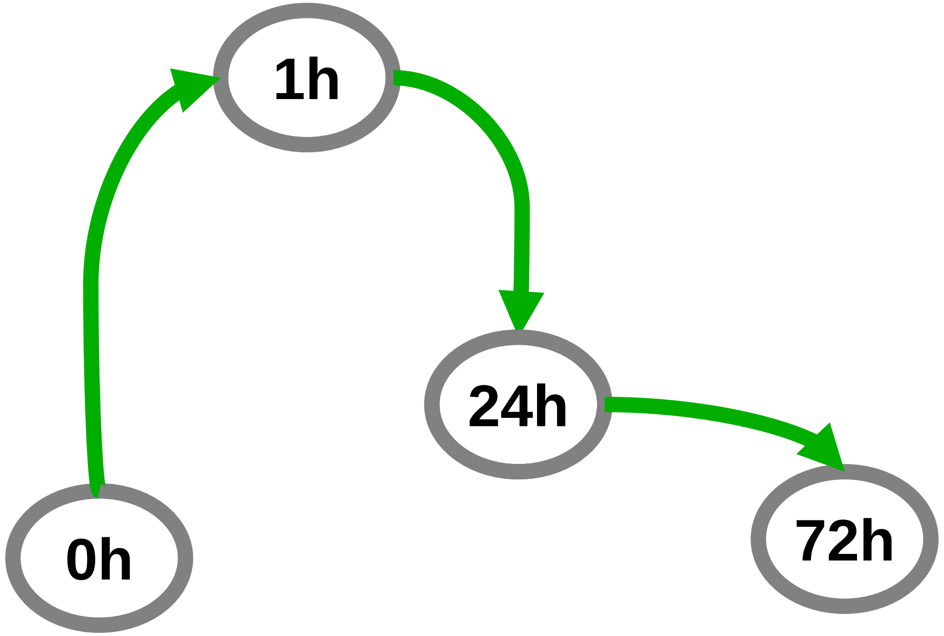
System trajectory

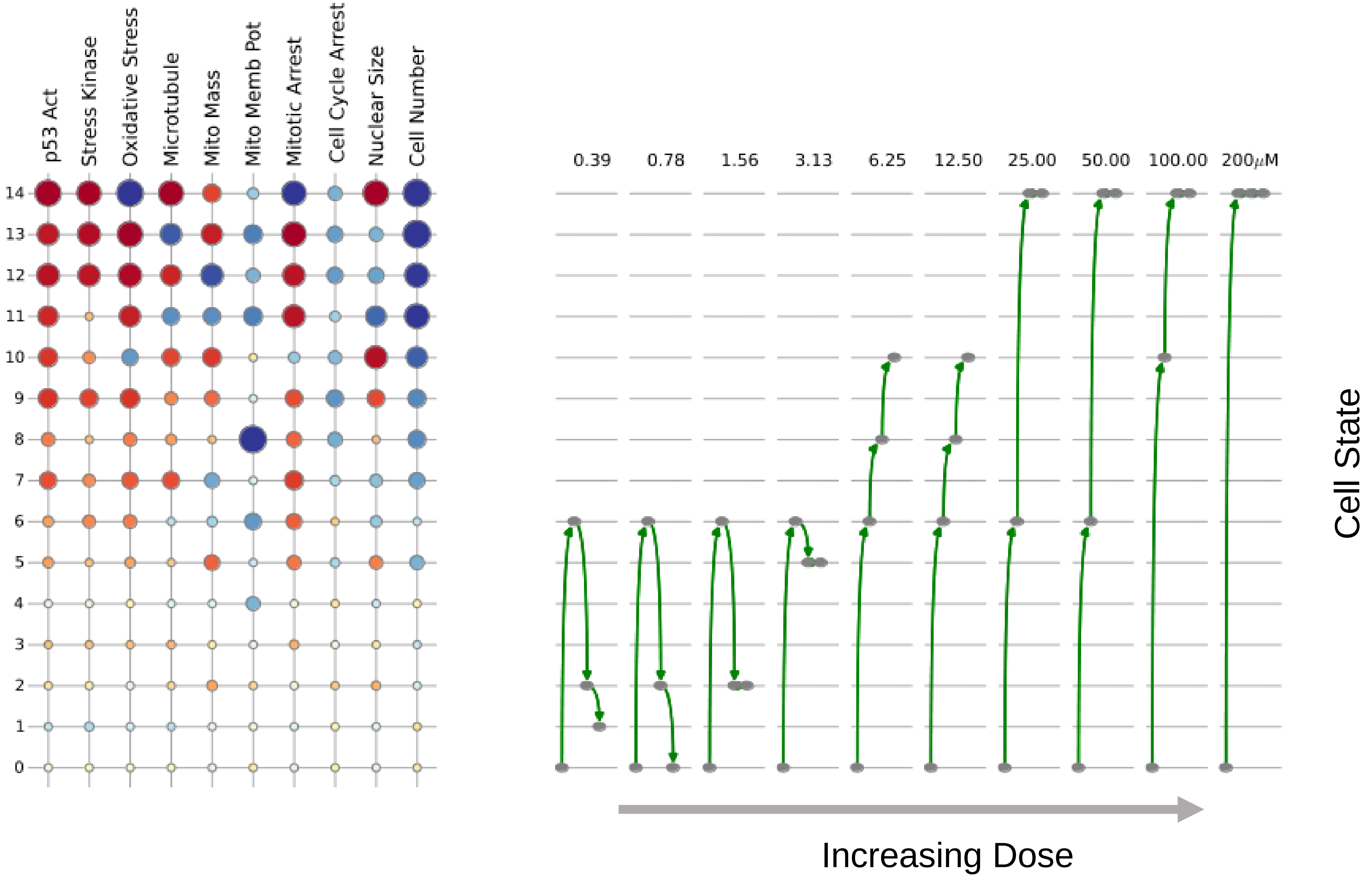
Fluazinam 0.78 uM



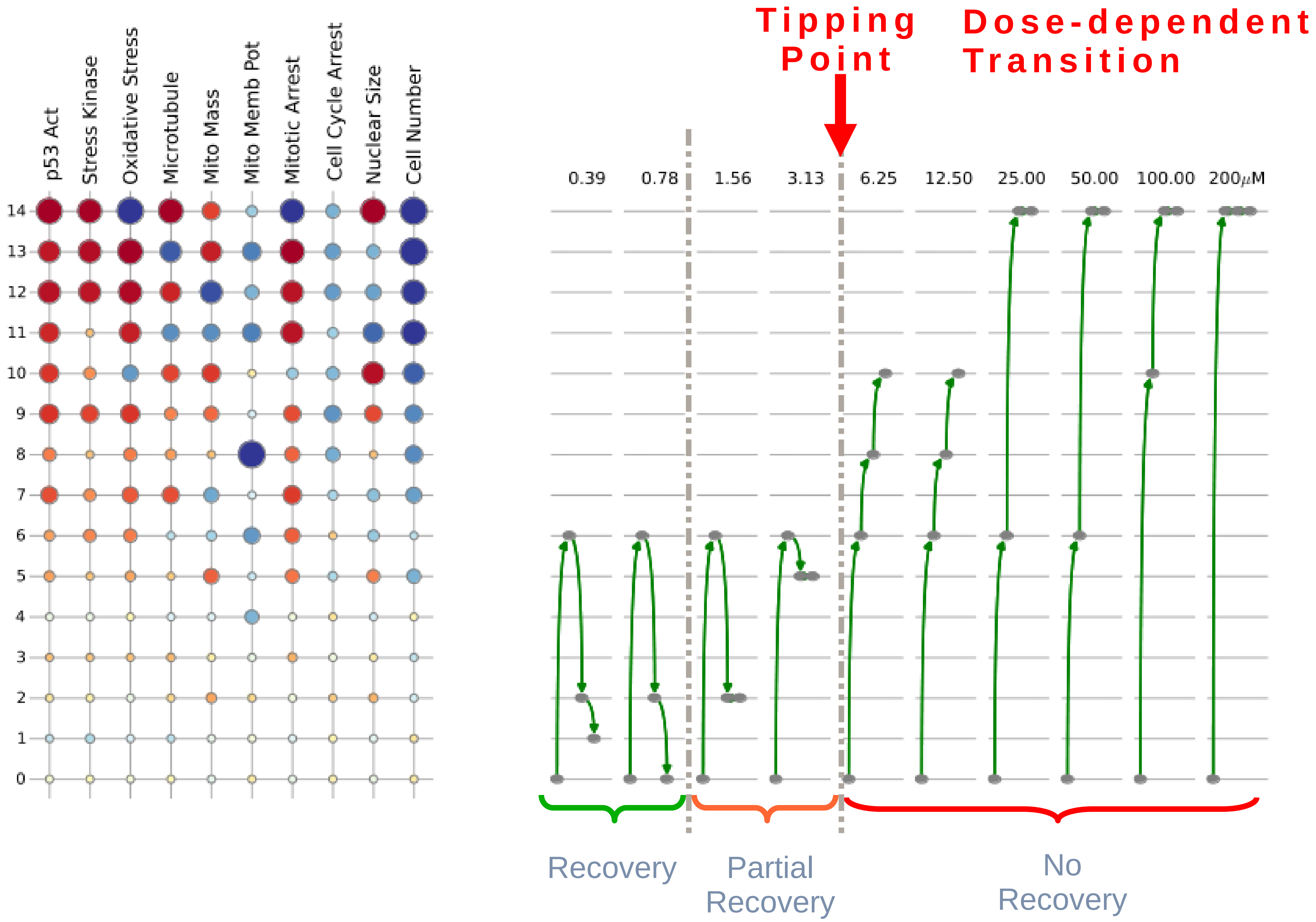
Trajectory=Sequence of states



Fluazinam “Trajectories”



Fluazinam “Trajectories”



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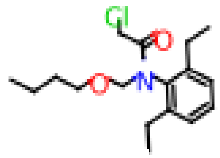
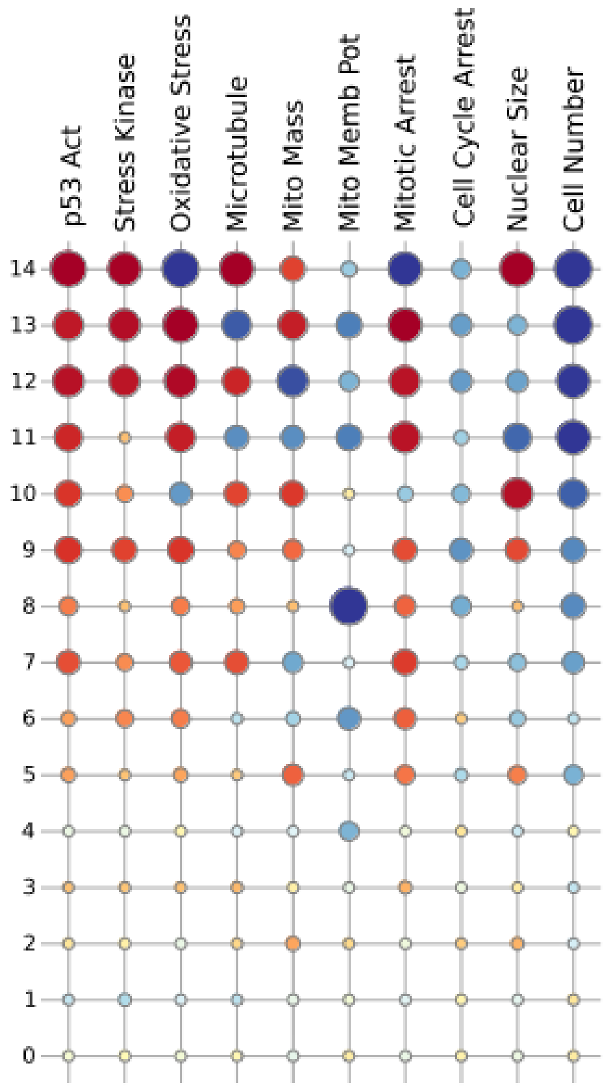
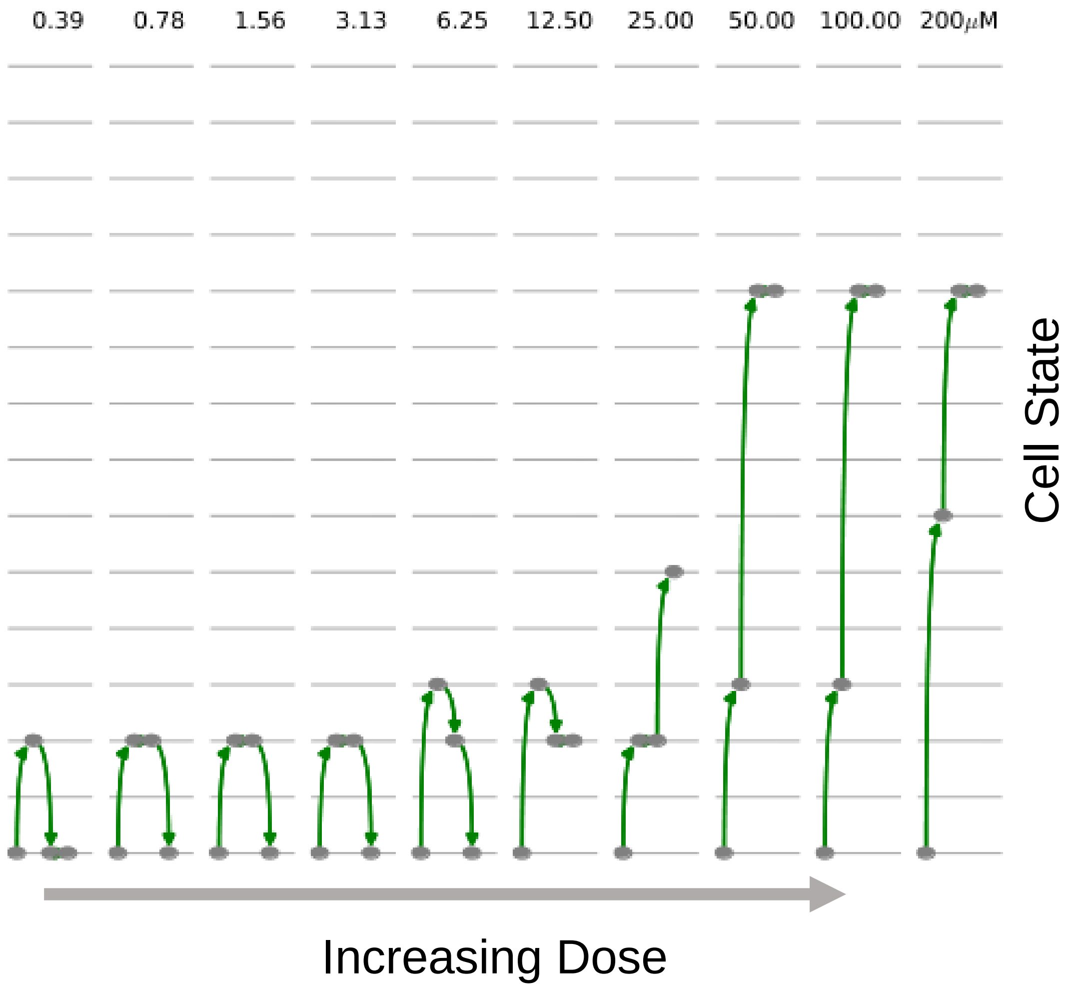
Butachlor Trajectories

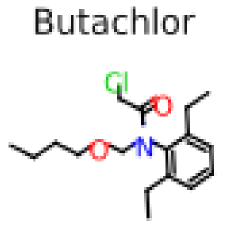
Butachlor

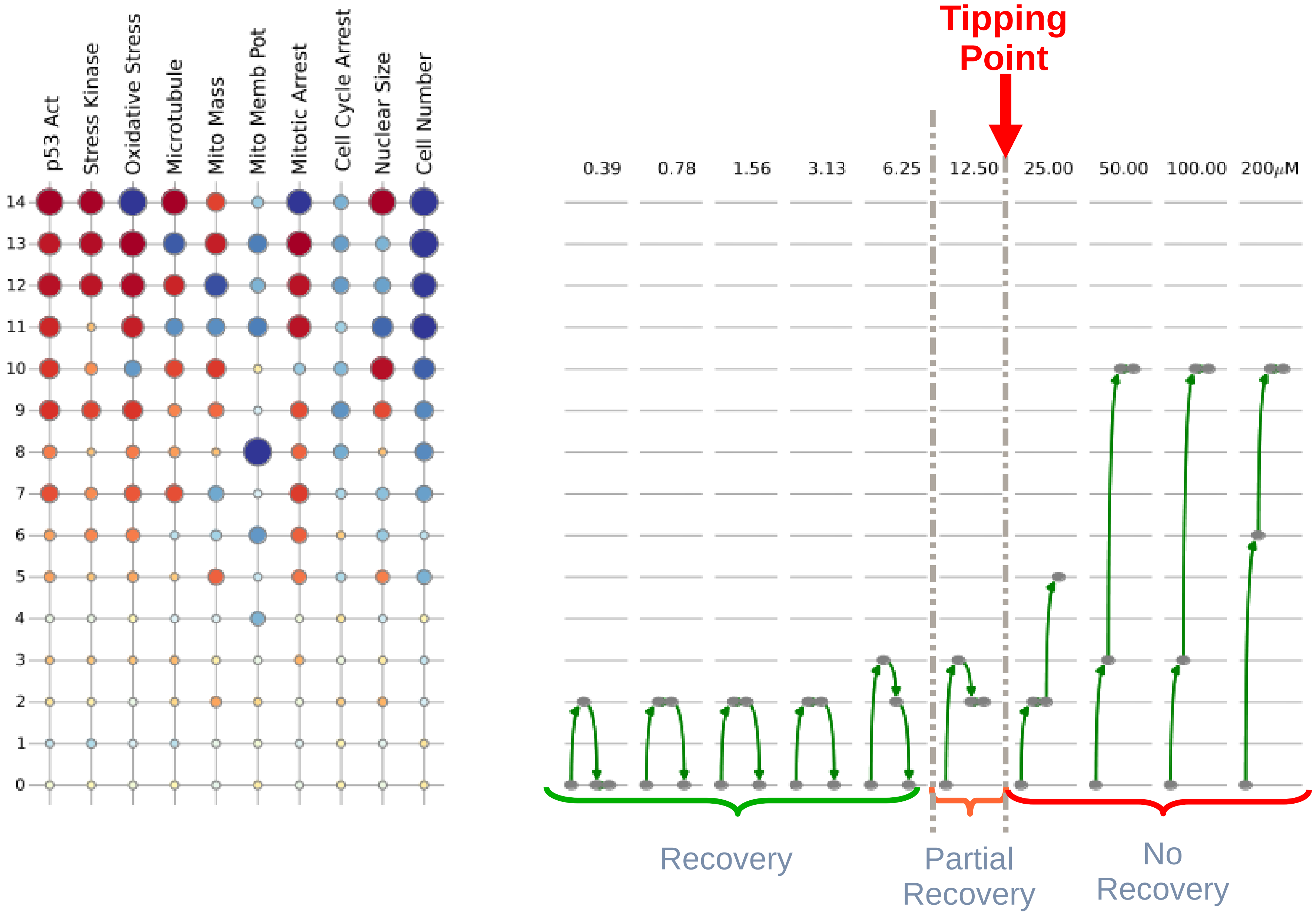






Butachlor Trajectories



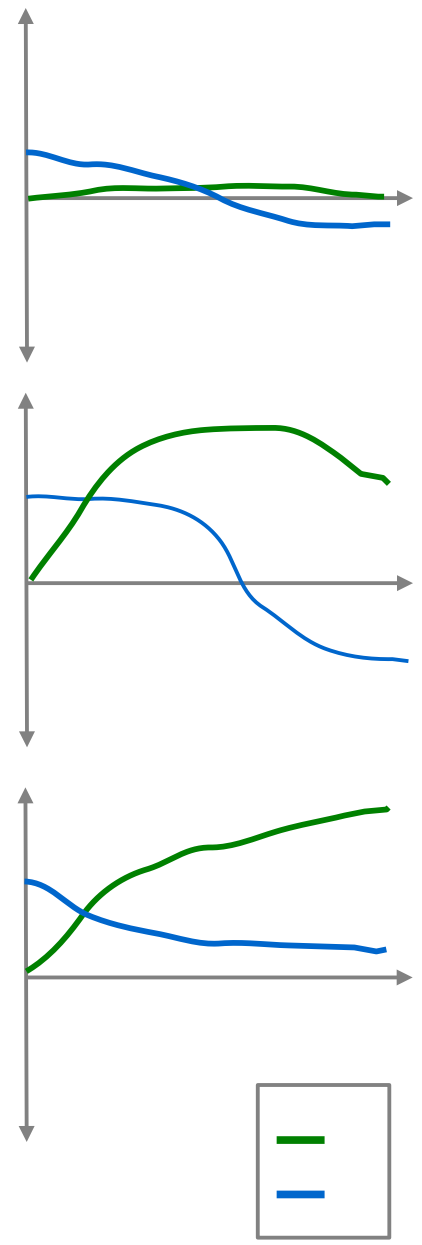


Analyzing Trajectories Quantitatively

Some perturbation
Recovery

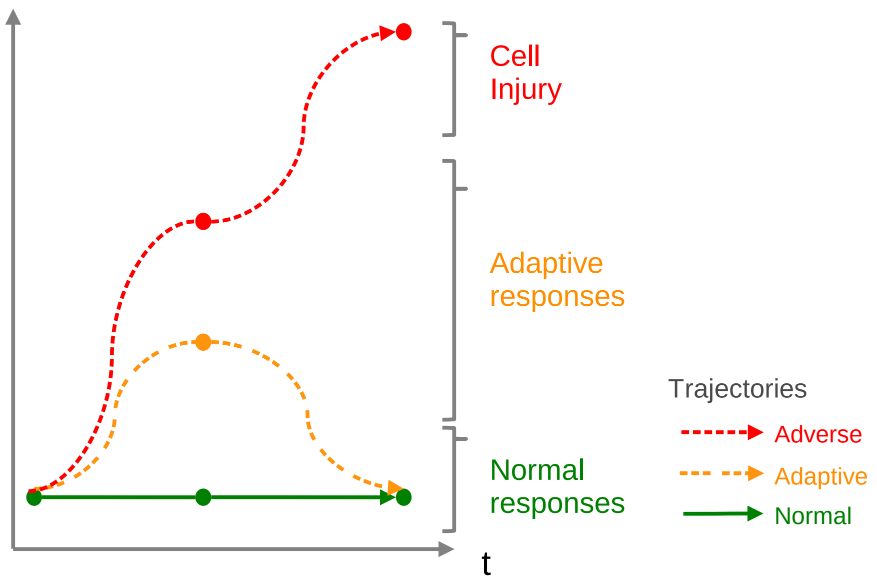
Adaptive stress response?
Partial recovery

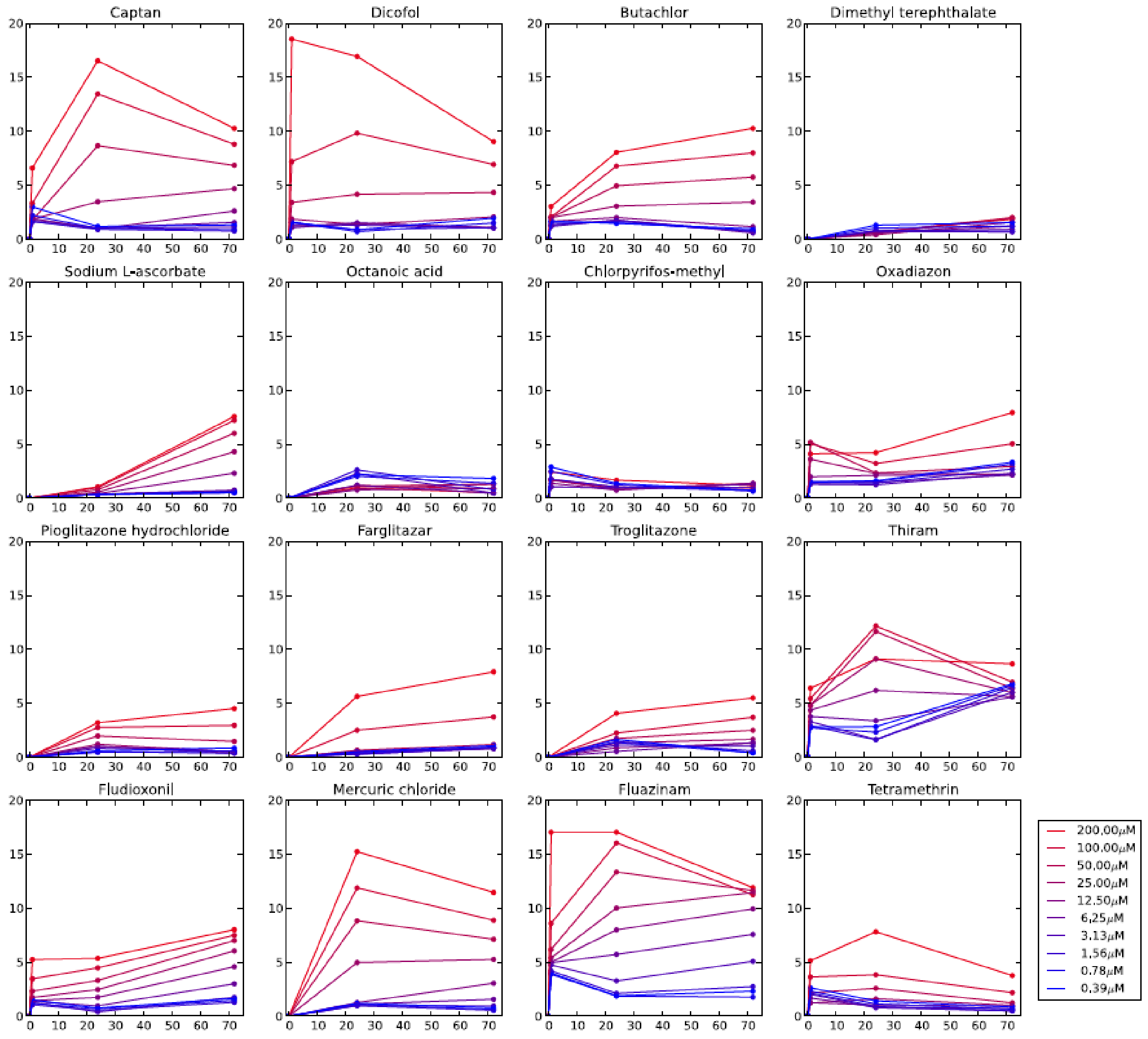
Adaptive capacity exceeded/
Cell injury?
No recovery



Shah et al *2015*

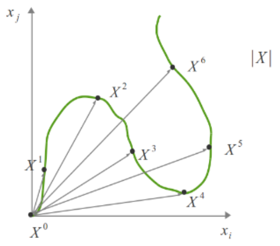
Quantitative Trajectories

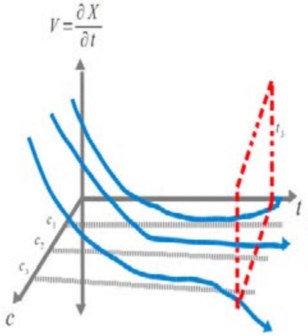


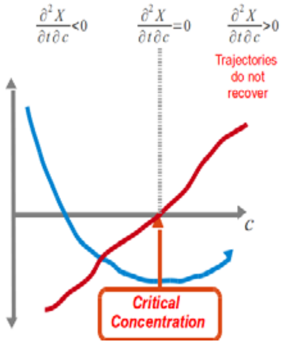


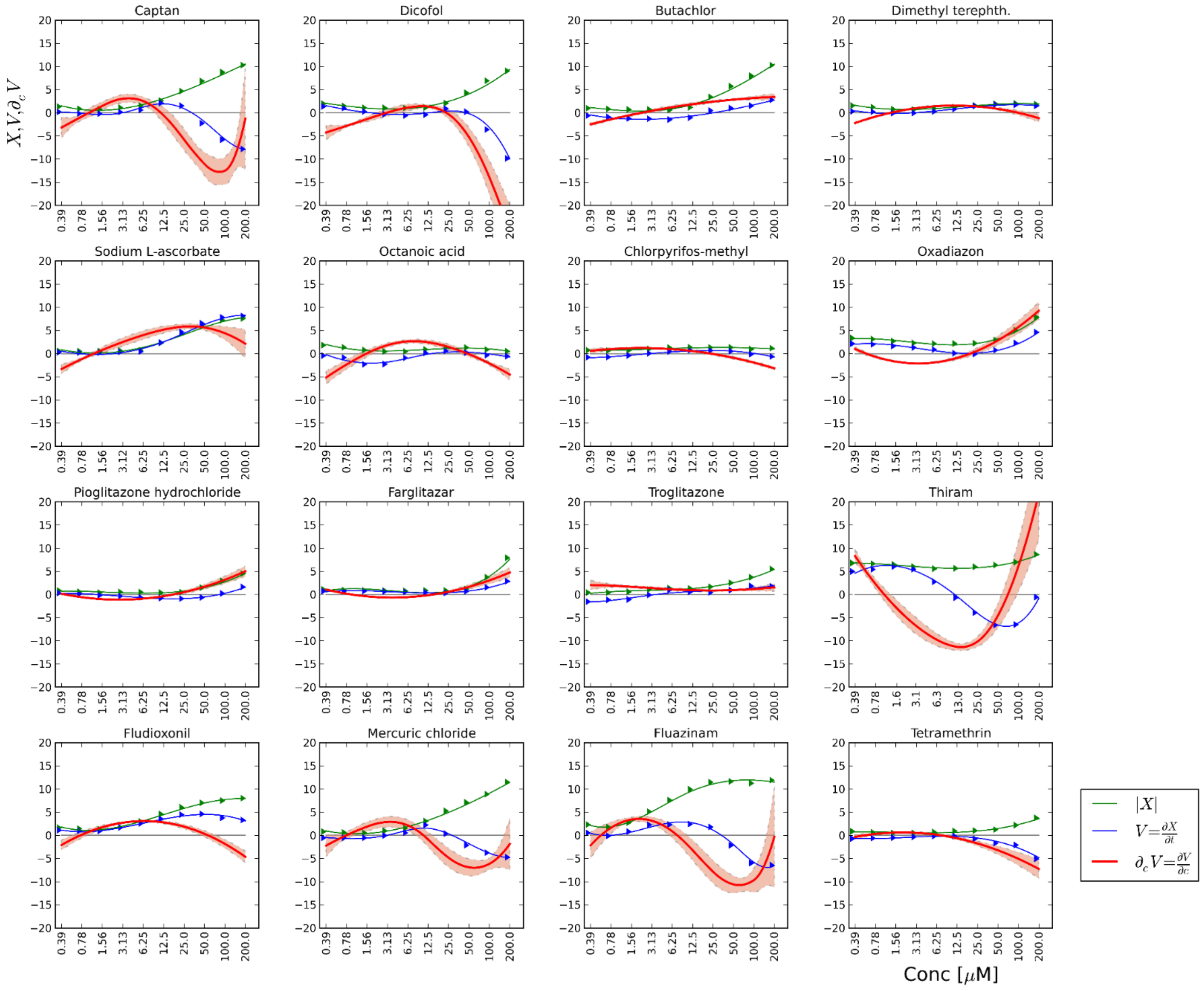
Shah et al *2015*

Dose-Dependent Transition





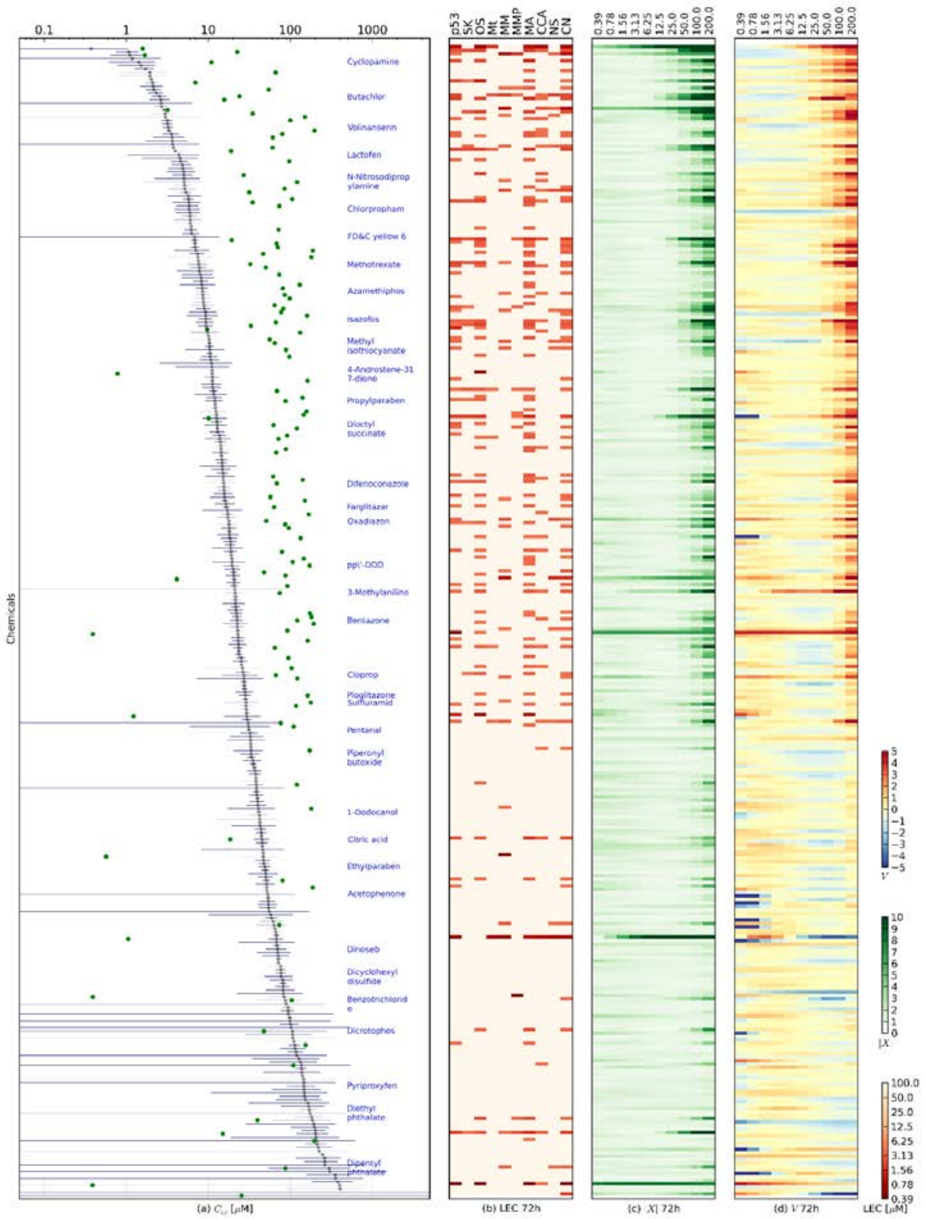




Shah et al *2015*

Critical Concentrations

- Can systematically identify critical concentrations- “tipping points”
- Tipping point precedes concentration that produces significant cell loss



Shah et al *2015*

MANY CHALLENGES…

❑ Study design enhancements:

- More physiologically-relevant cell model
- More holistic cellular stress-response assays (e.g. *–omics)*

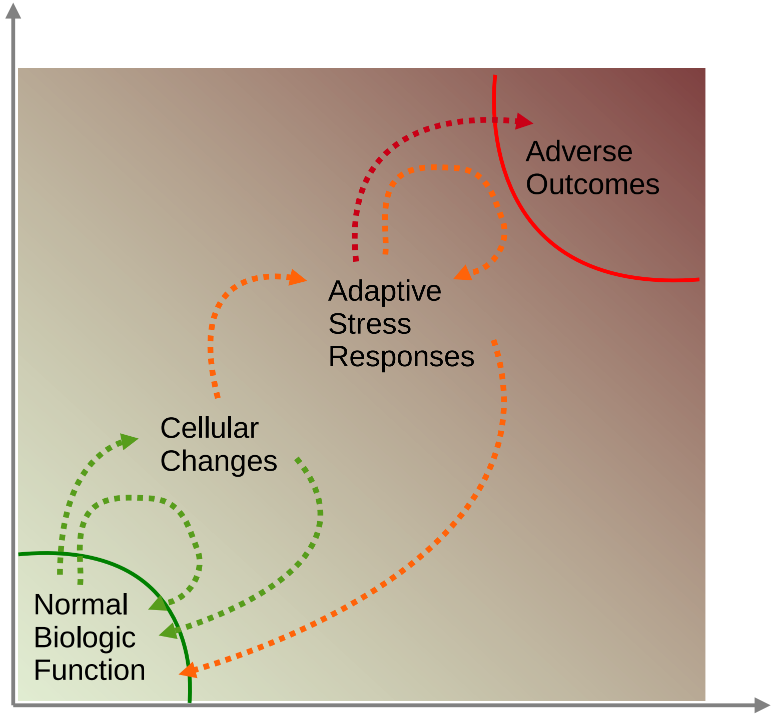
❑ Tipping point evaluation

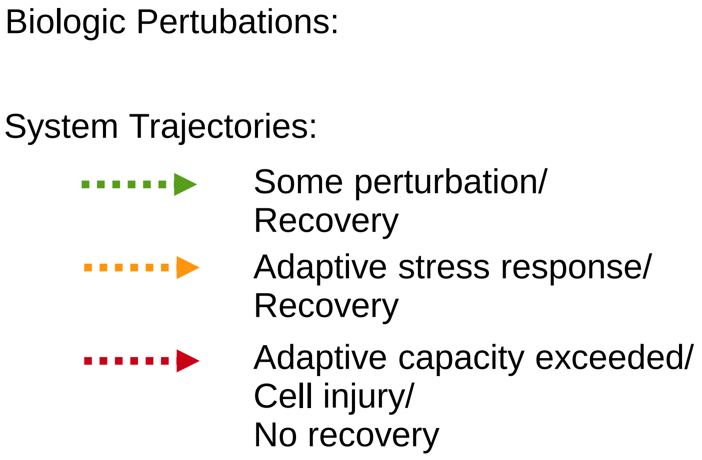
- Differentiating between PK and adaptation
- Reproducibility
- Biological relevance
- *In vivo* Extrapolation

New study underway to address some of these challenges …

Summary

- Complex biological data can offer new insights into cellular state
- Chemical-induced perturbations can be described by dynamic cellular trajectories
- Trajectory analysis can identify "tipping point" of cellular adaptation
- Additional research on linking tipping points with adversity
- Could be used as point of departure for risk assessment





Acknowledgements

- HCI Experiment
  - Keith Houck
  - David Dix
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  - Matt Martin
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Analysis

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Richard Judson
Thomas Knudsen
Robert Kavlock