United States Environmental Protection Agency

# Toxicological Tipping Points

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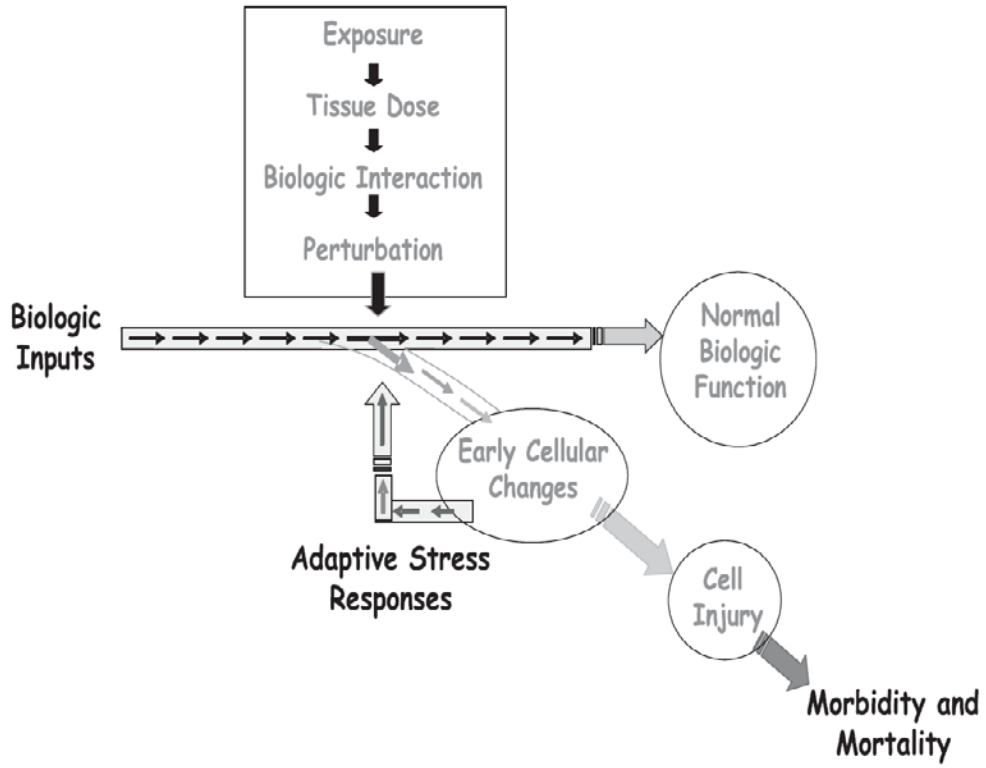
Laboratory Seminar Series

# 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"***
- Toxicological tipping points could 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)

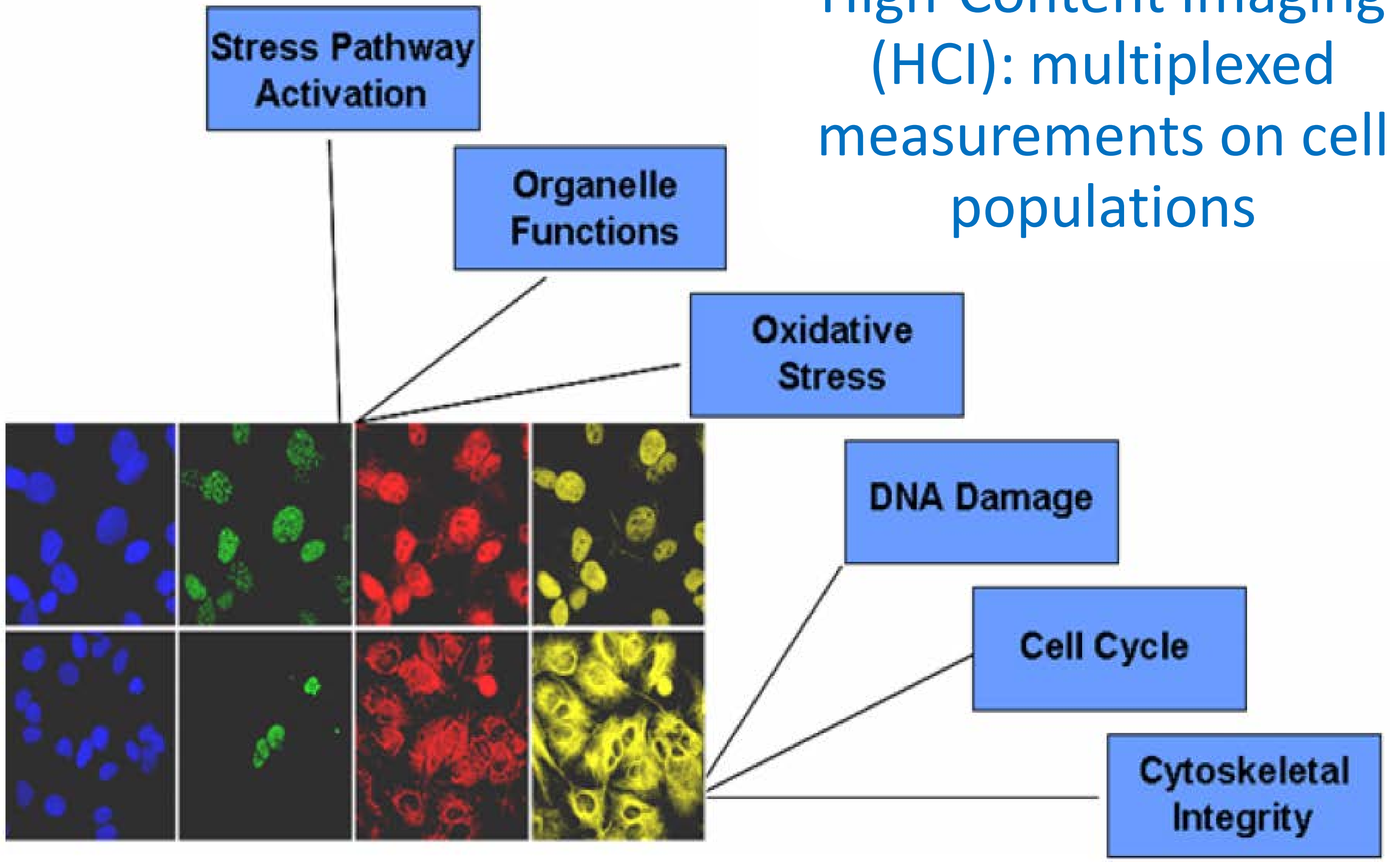
- **Study**
  - HepG2 cell culture
  - 967 chemicals (ToxCast)
  - 10 conc (0.1, 3 times

- **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

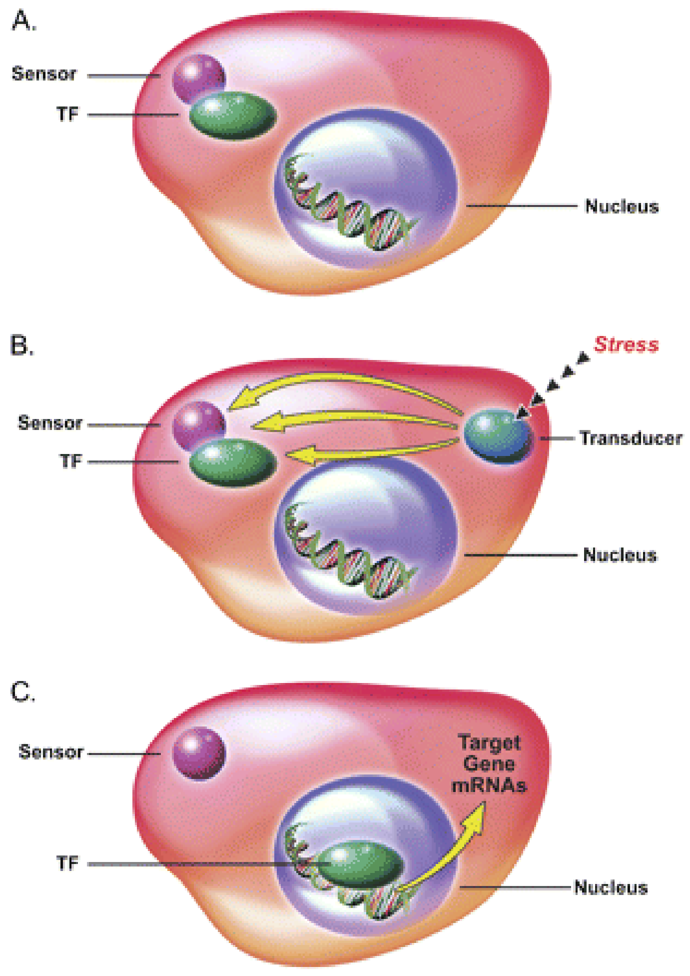



HCI Conducted by Cyprotex, Inc.

# HCI Endpoints: Cell State

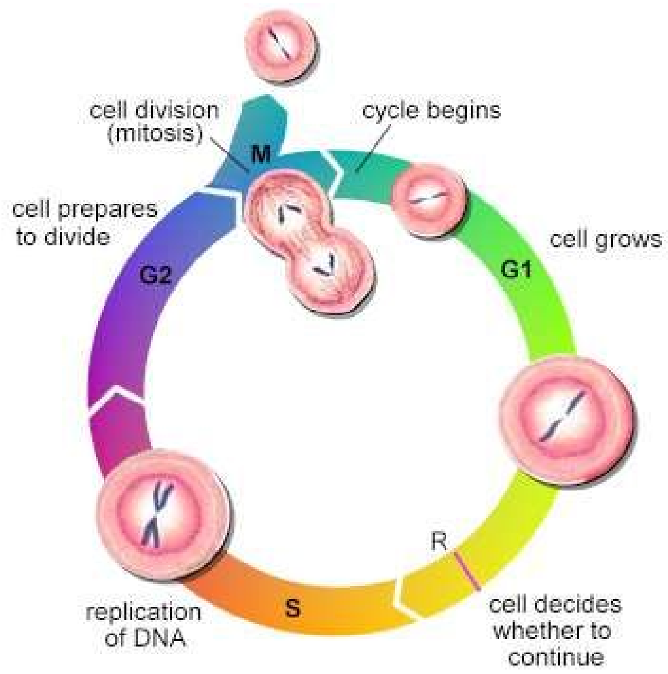
- P53: p53 activity (**p53**)
- c-Jun: stress kinase (**SK**)
- H2AX: oxidative stress (**OS**)
- MitoTracker Red: mitochondrial mass (**MM**), mitochondrial membrane potential (**MMP**)
- Tubulin: microtubule organisation (**Mt**)
- Hoechst33342: nuclear size (**NS**), cell cycle arrest/progression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

### Stress Response Pathways

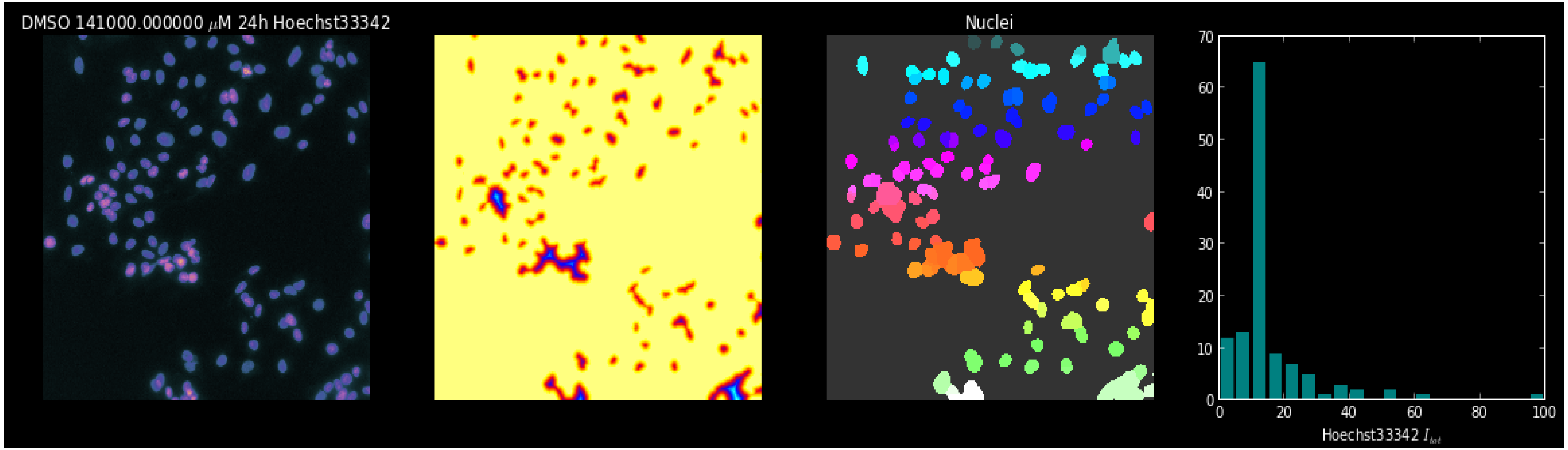



Simmons et al 2009

### Cell Cycle Progression



# 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)

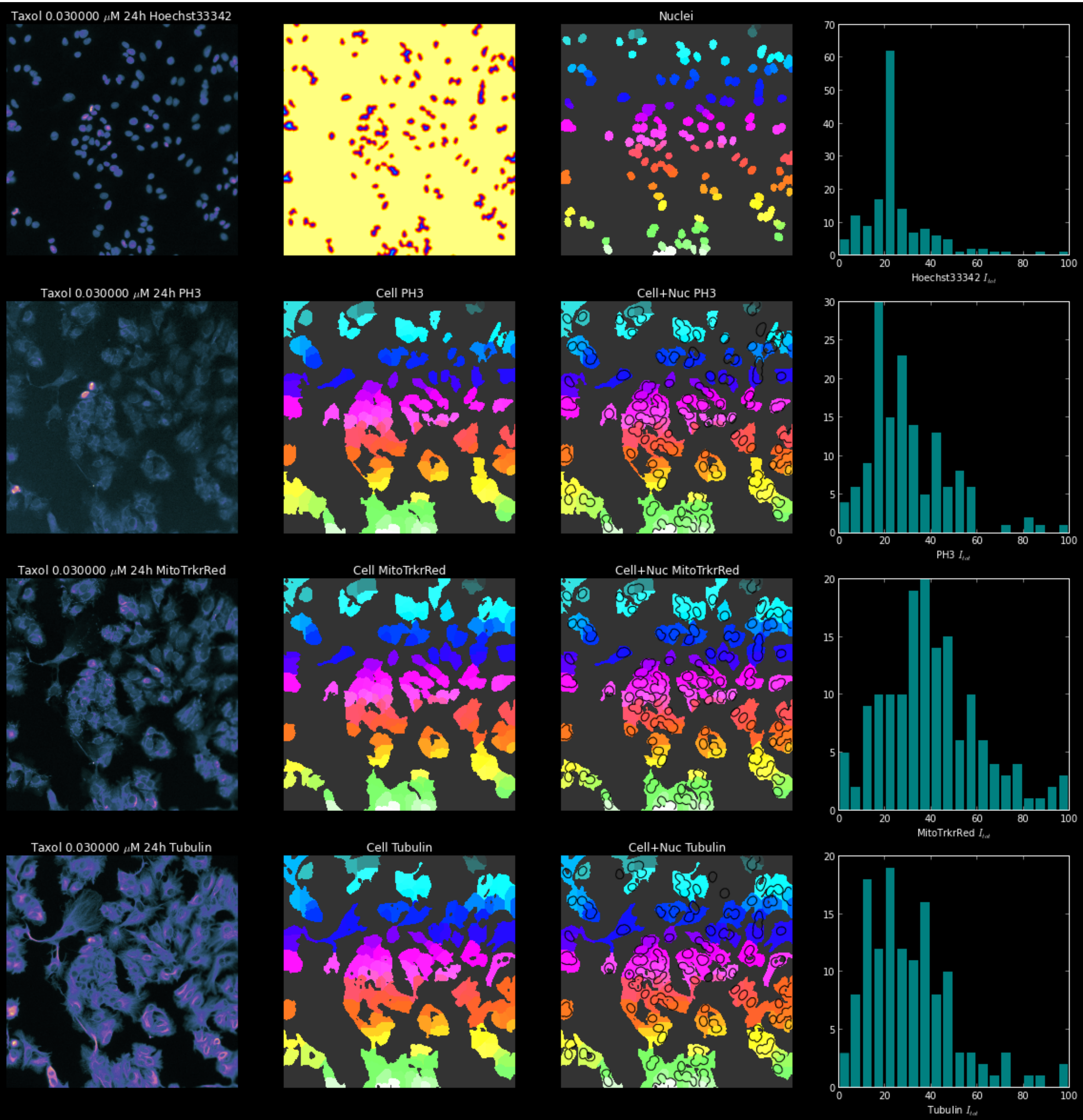
Taxol 0.03uM

Hoechst33342

Phospho-Histone3

MitoTracker Red

Phospho-Tubulin



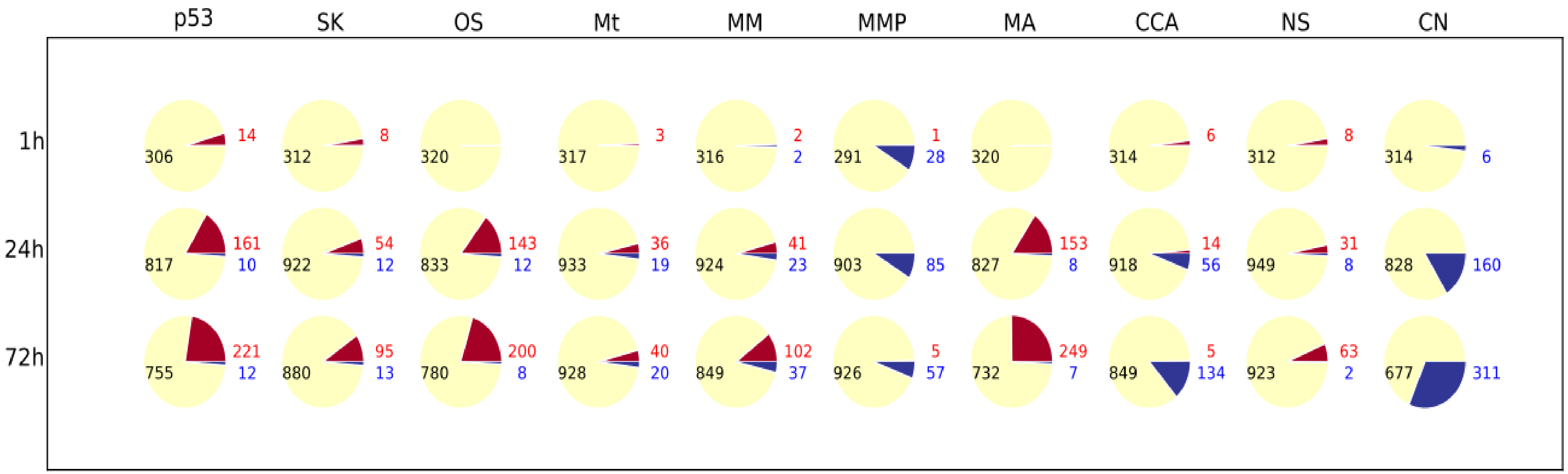

nuclear size (**NS**)
cell cycle arrest (**CCA**)
cell number (**CN**)

mitotic arrest (**MA**)

mitochondrial mass (**MM**)
mitochondrial membrane potential (**MMP**)

microtubules (Mt)

# Summary of HCI Effects




- Early effects: stress pathways & mitochondrial dysfunction
- Intermediate effects: complex
- Late effects: cell number decrease

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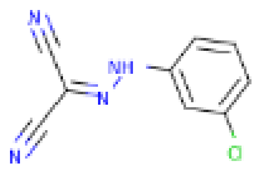
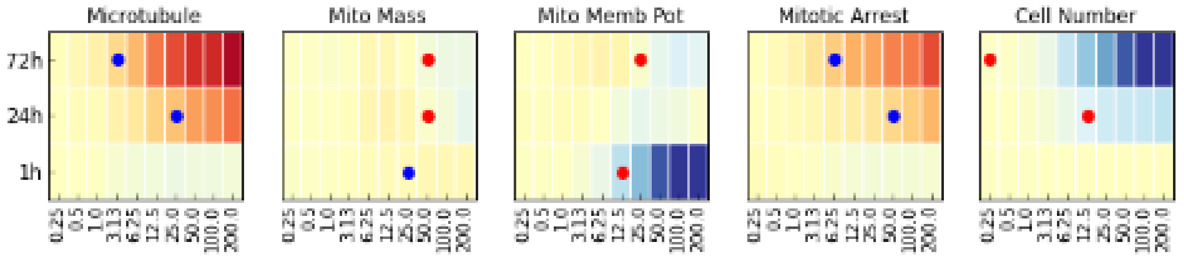
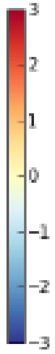
# Mitochondrial disruptors

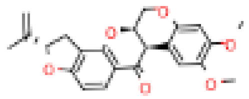
CCCP

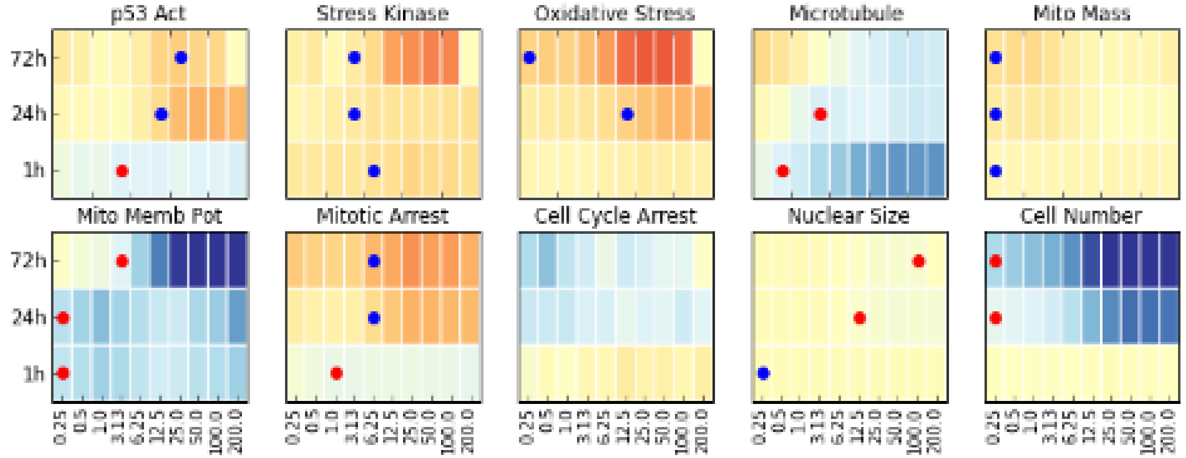



Rotenone

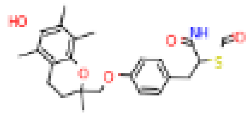


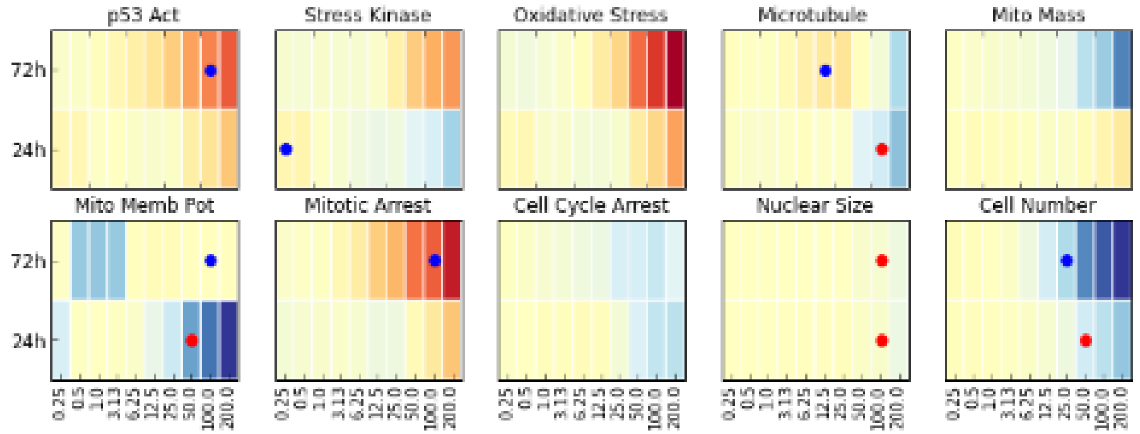



- log2(fold change): decrease (BLUE), increase (RED) or no effect (YELLOW)
- Bioactivity profile: “deviation from normal state” of HepG2 cells

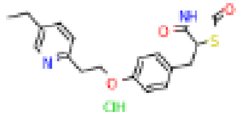
# Thiazolidinediones (TZDs)

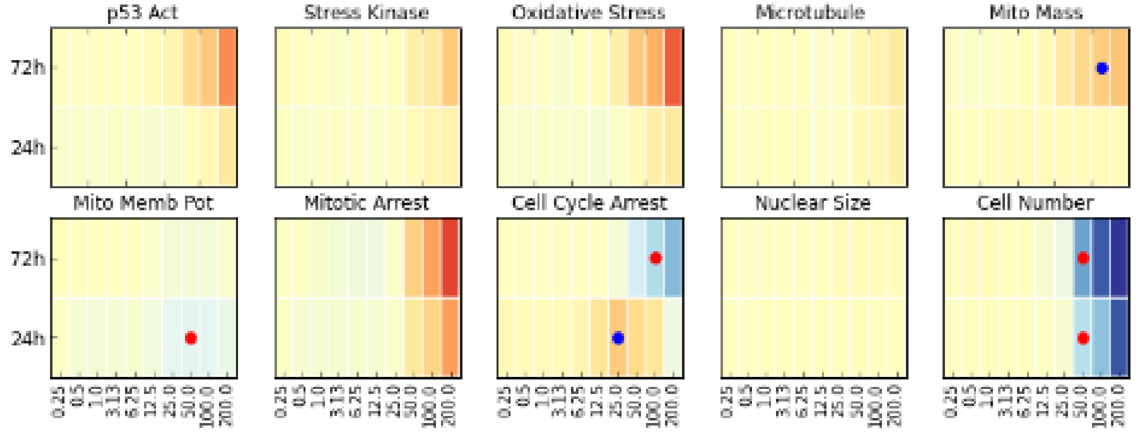
Troglitazone

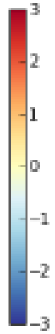



Pioglitazone hydrochloride



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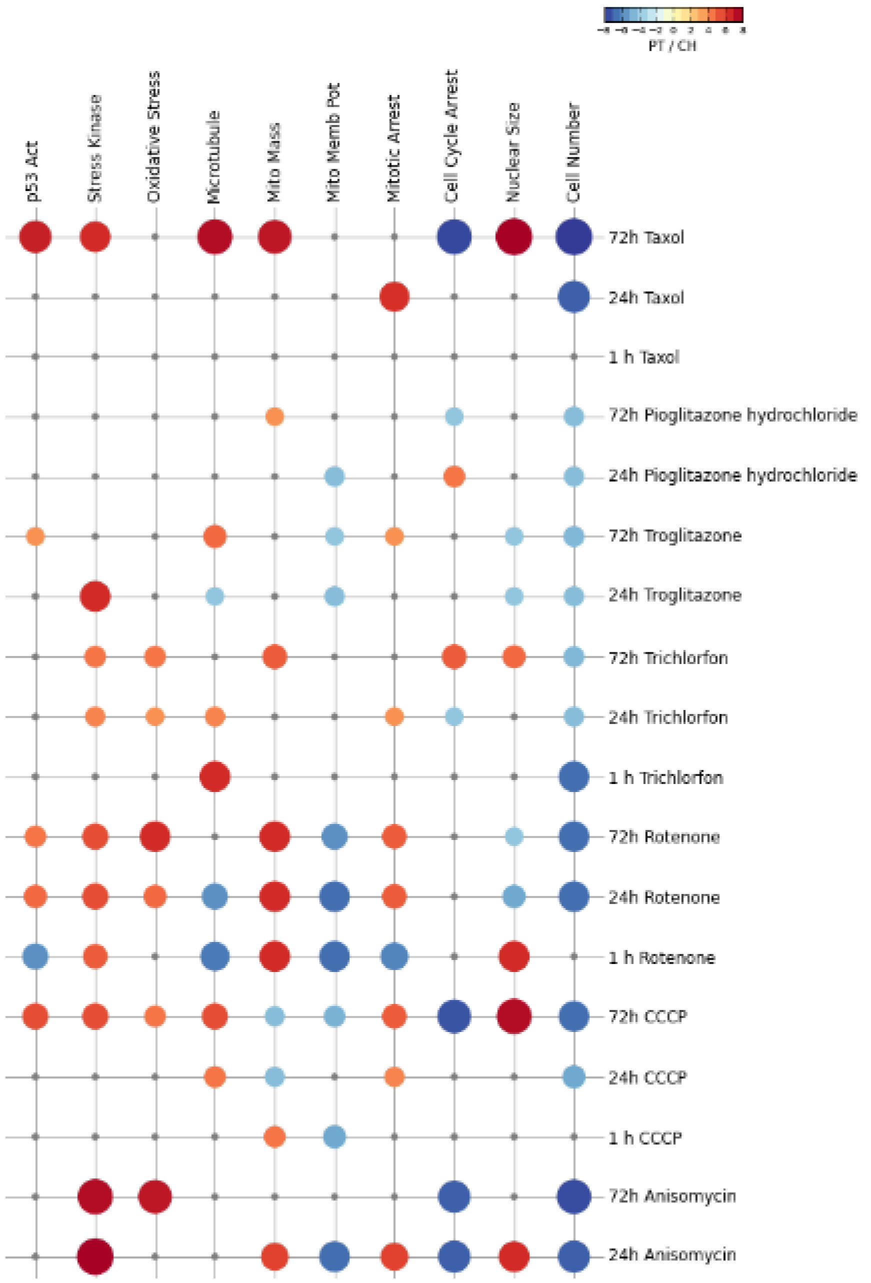
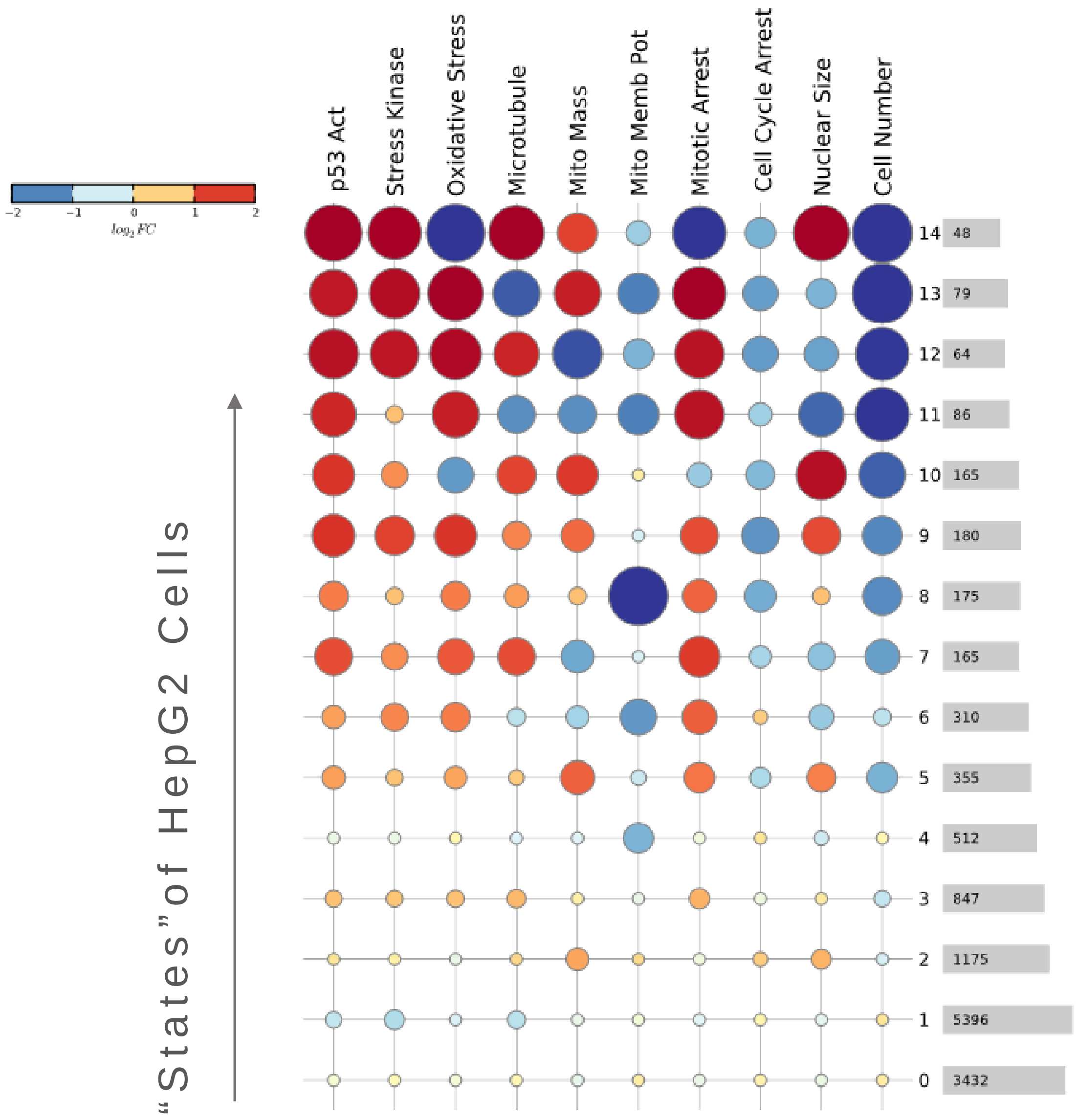
# Summarizing Large-Scale HCI Data

- 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 can we find patterns in the data?
- How do we predict toxicity using these data ?



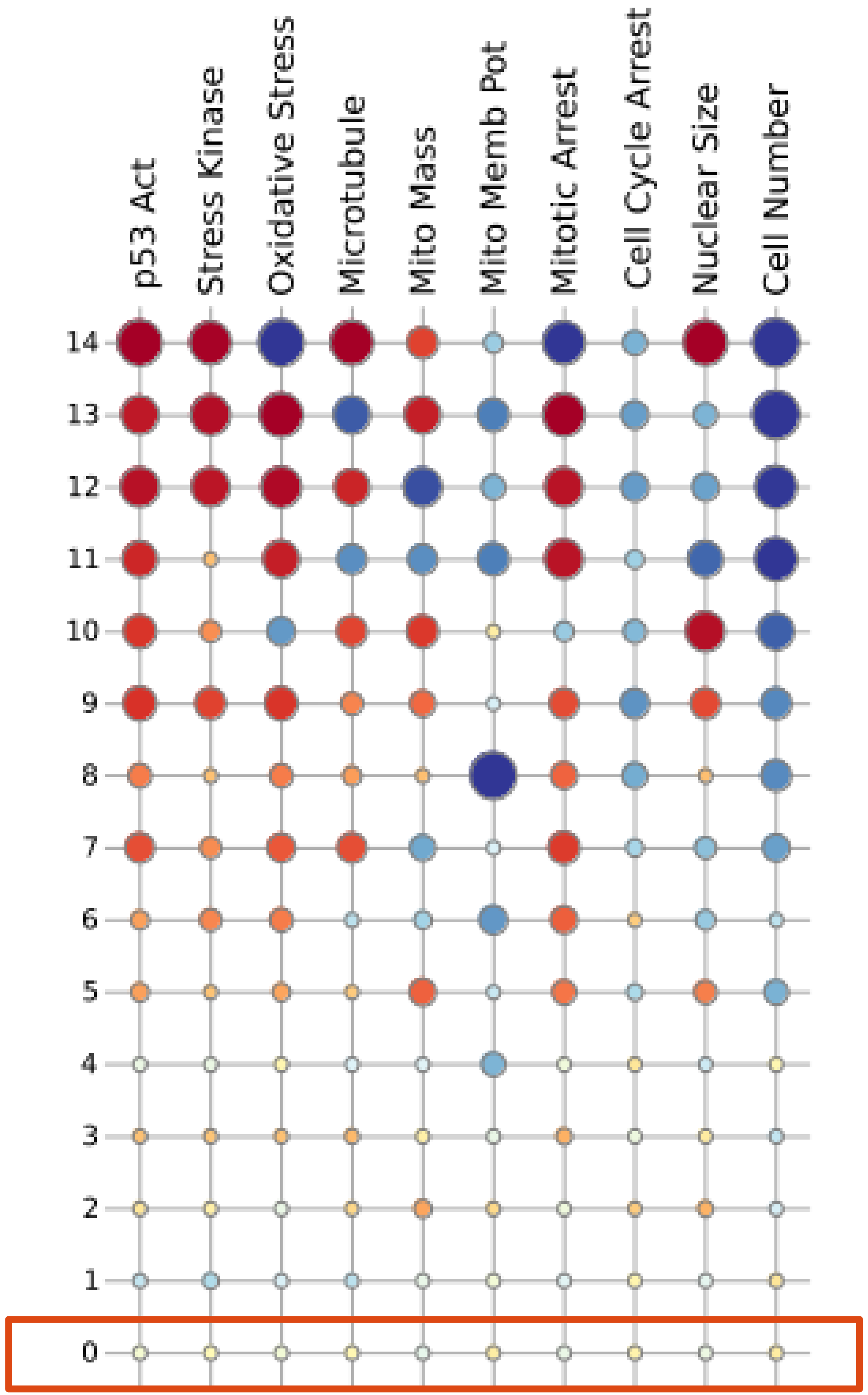
# HCI to Phenotypic States

- 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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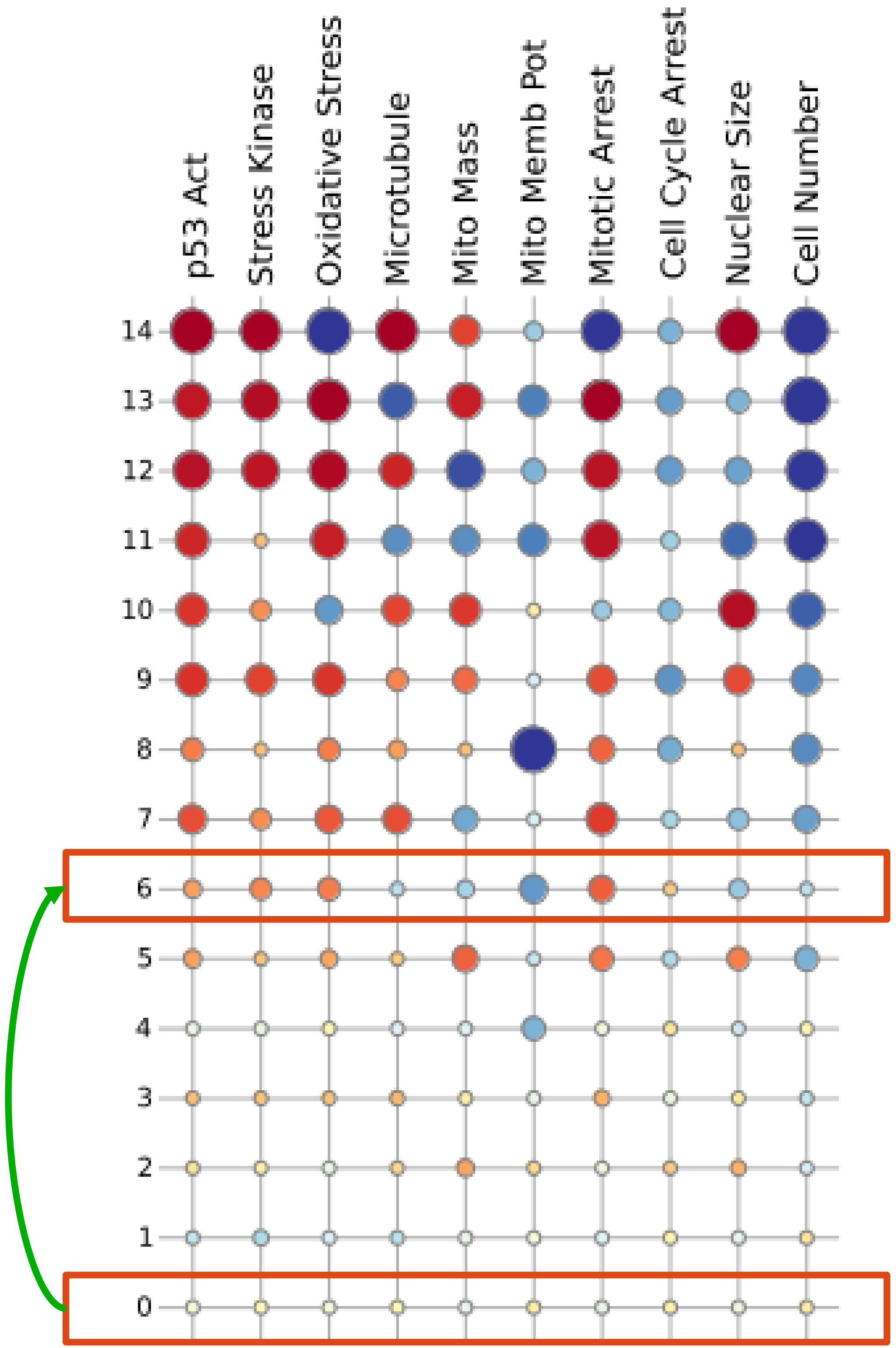
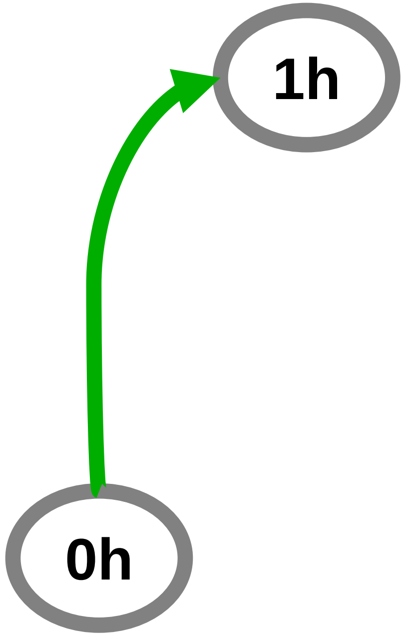
# State Transition

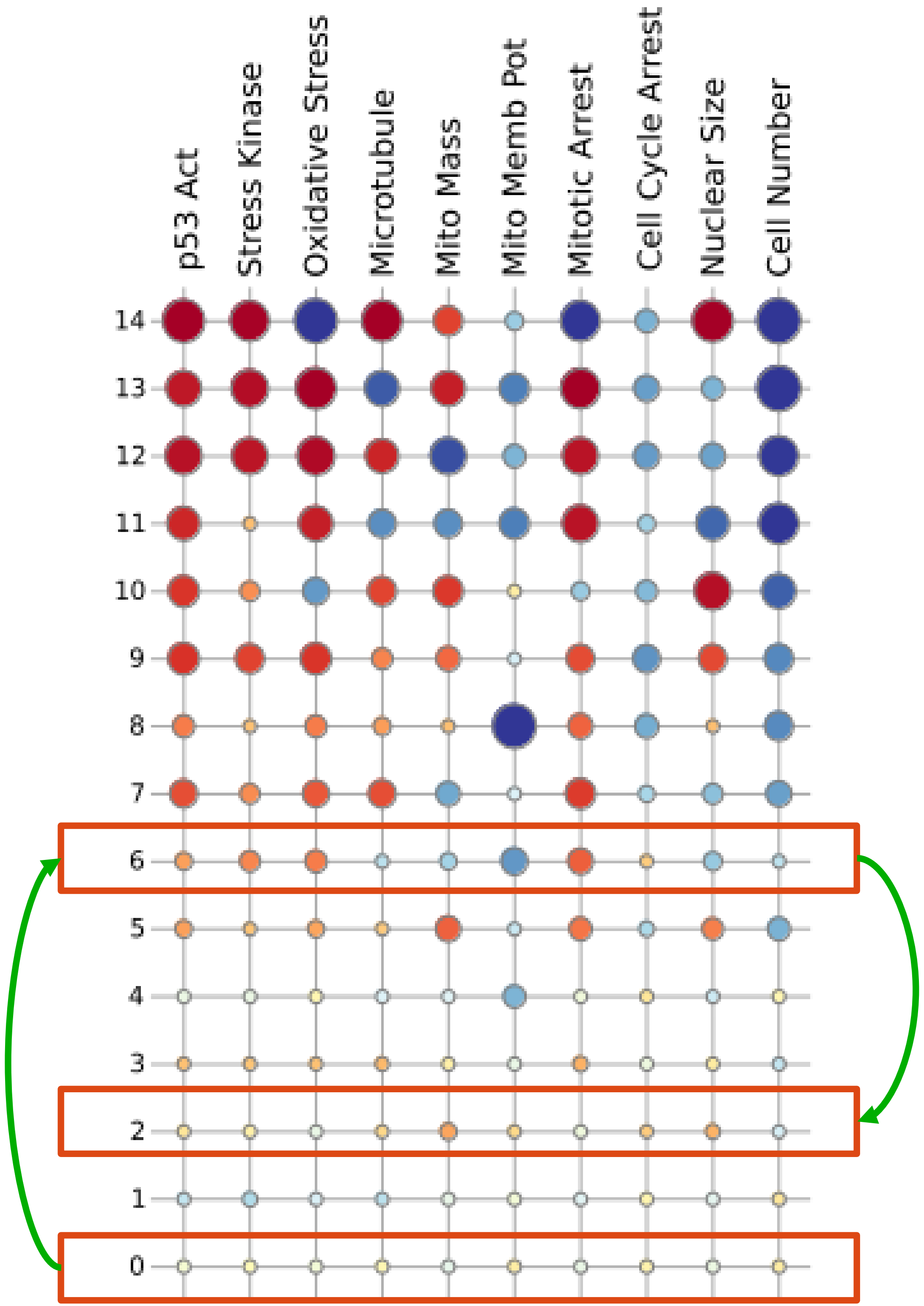
Fluazinam 0.78 uM

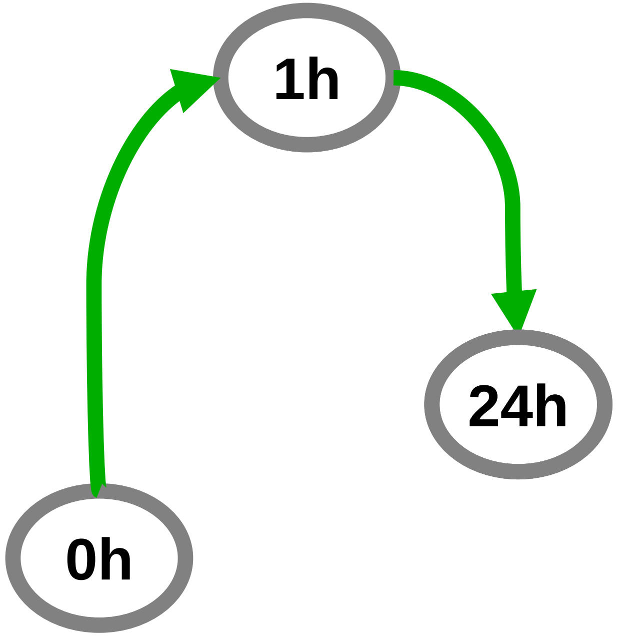


# System trajectory

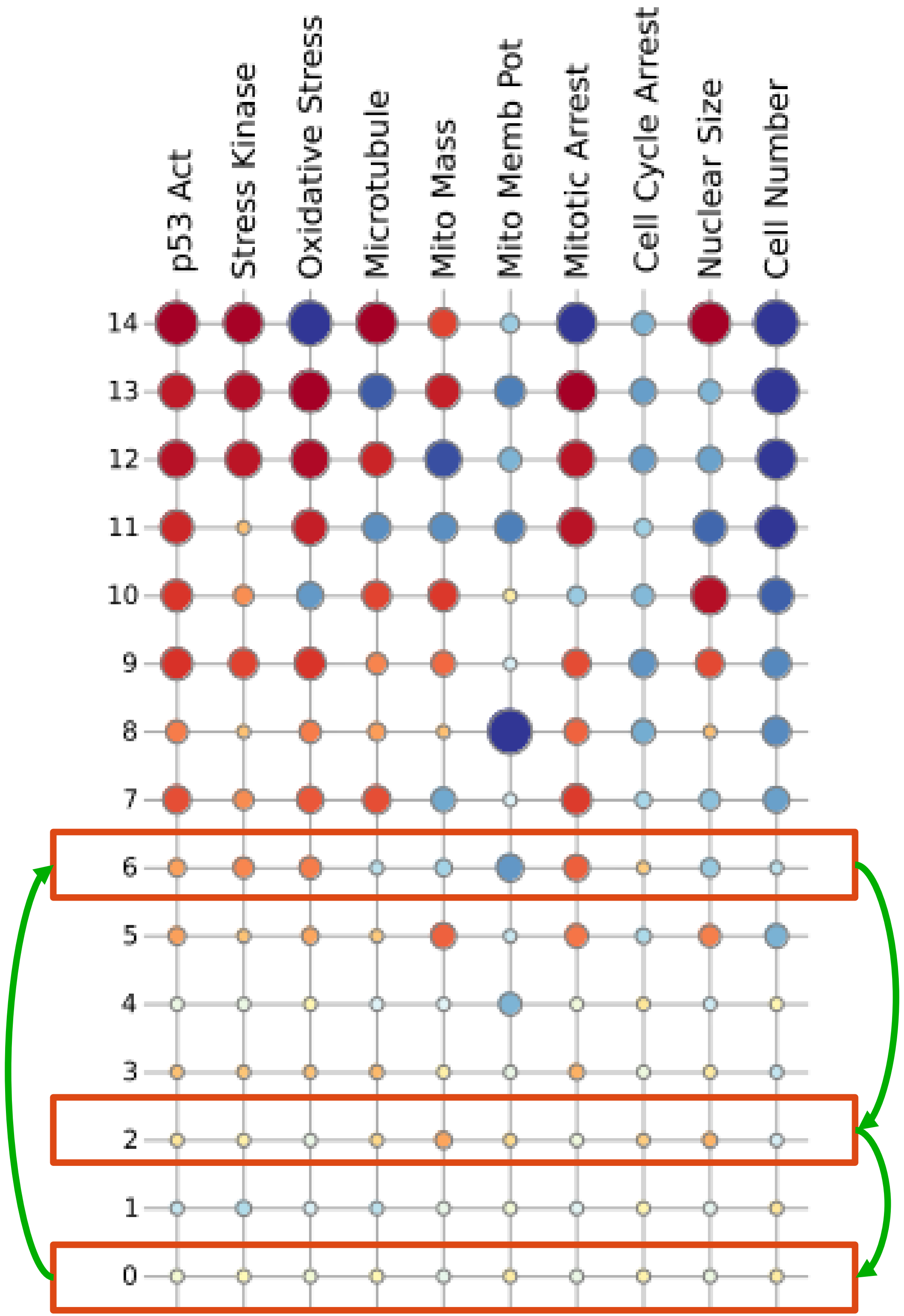
Fluazinam 0.78 uM



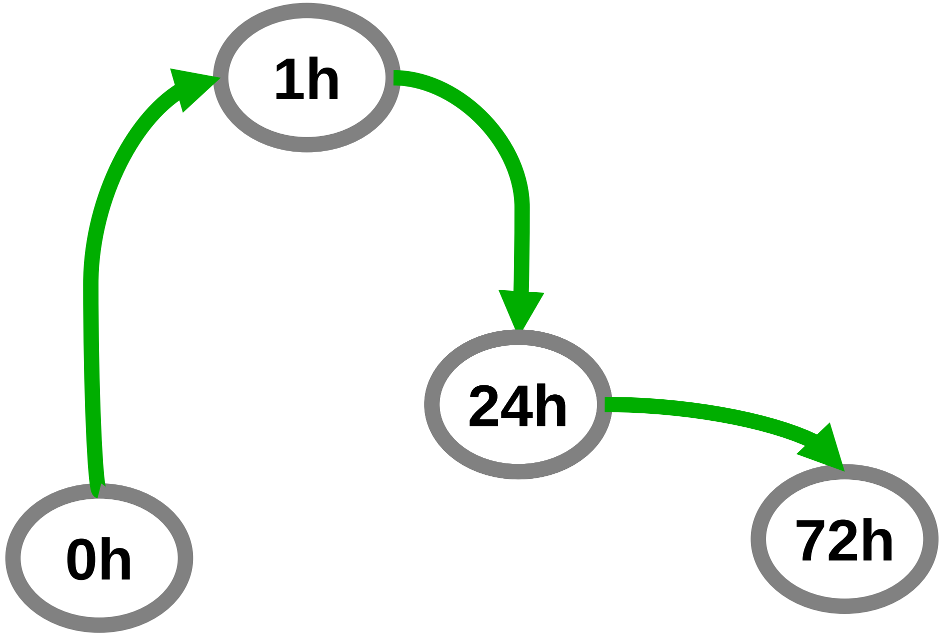



# System trajectory

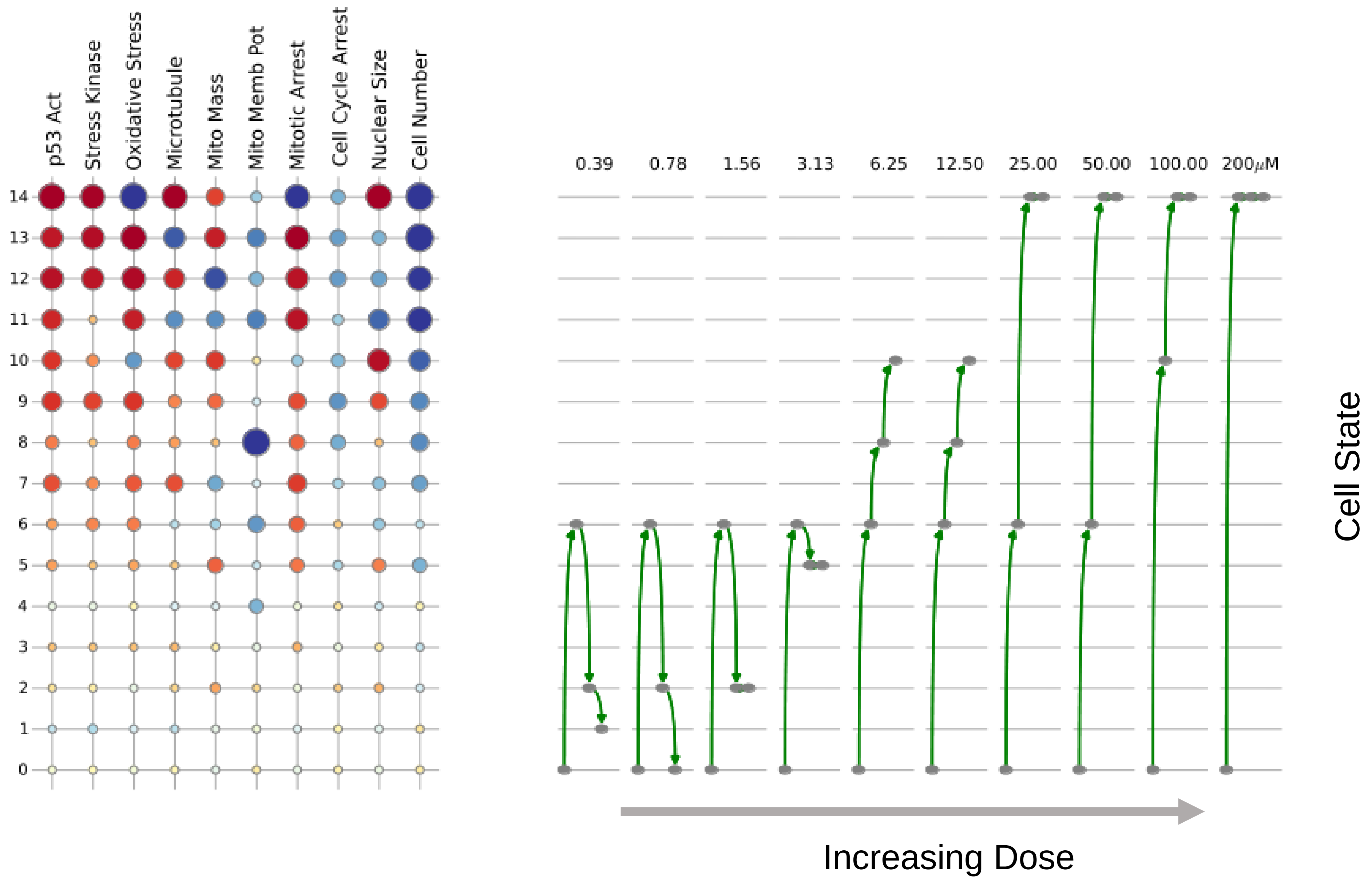
Fluazinam 0.78 uM




*Trajectory=Sequence of states*



# Fluazinam "Trajectories"



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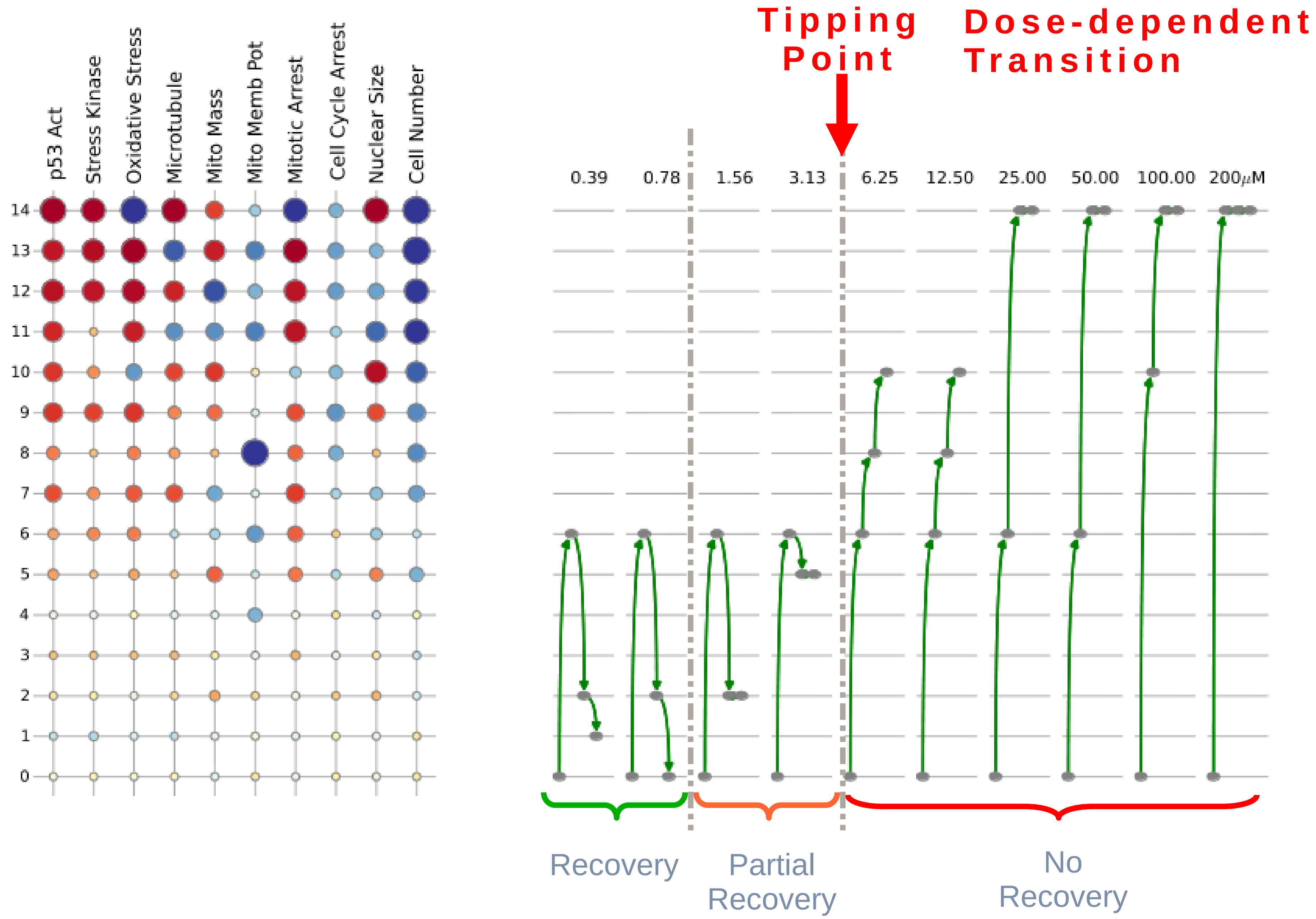

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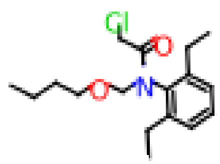
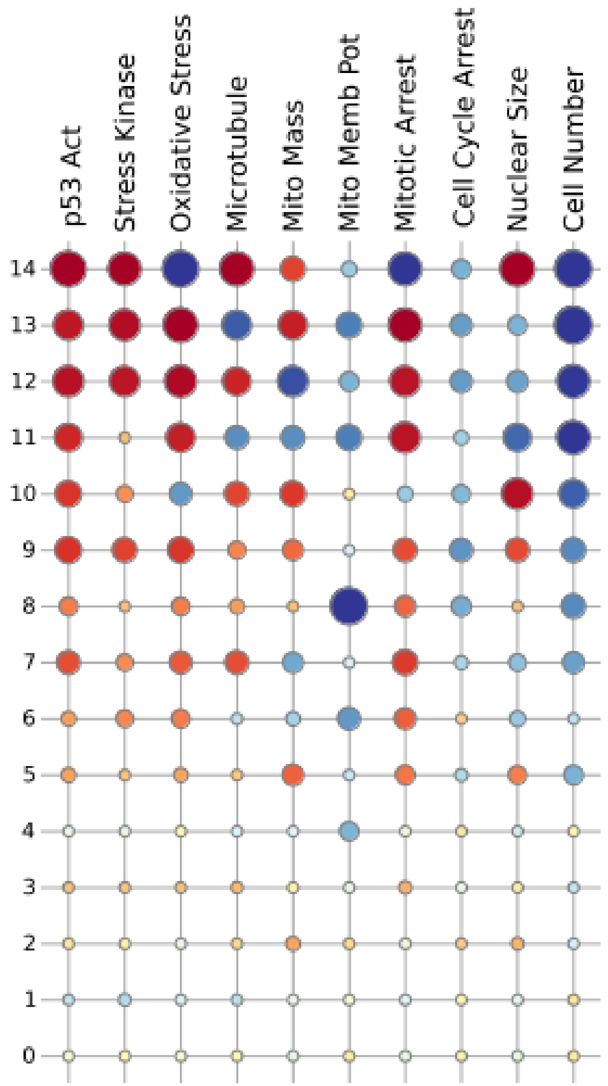
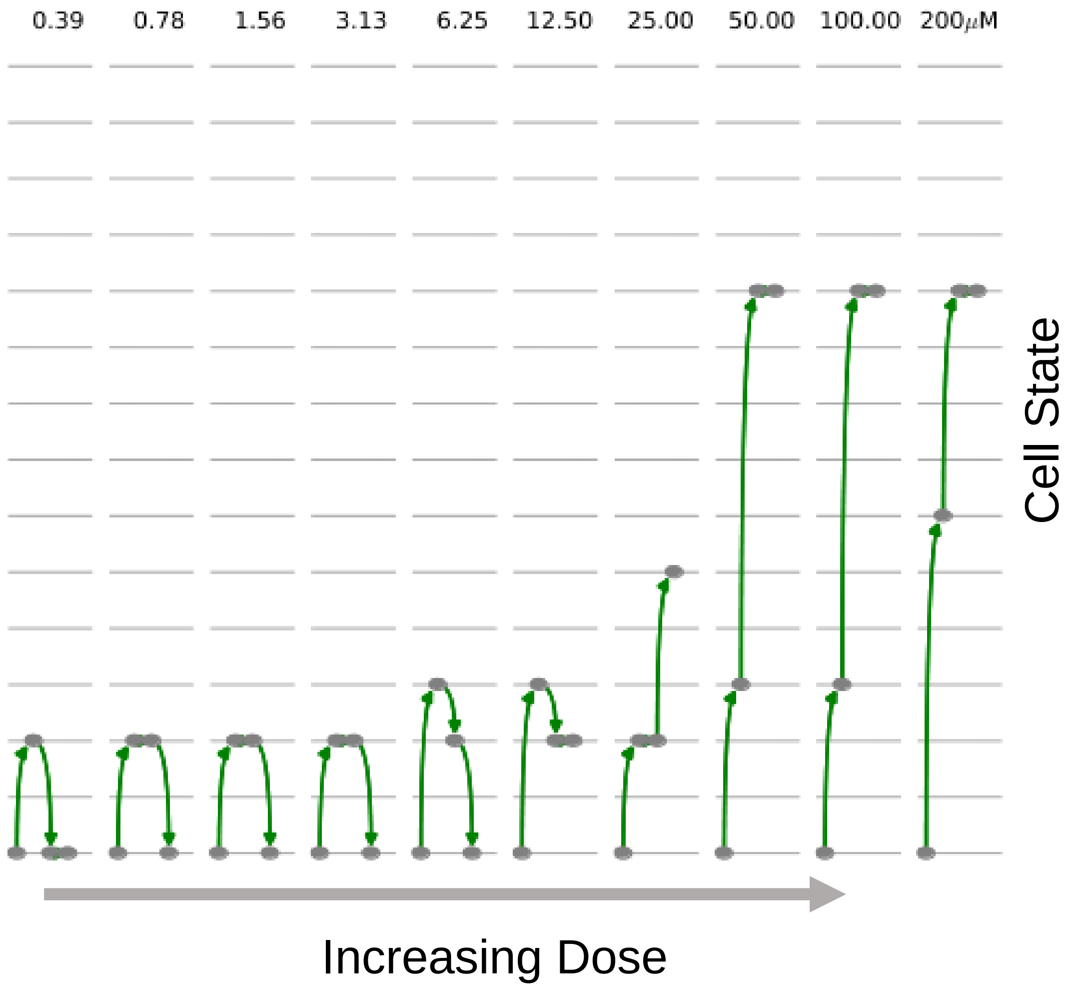
# Butachlor Trajectories

Butachlor





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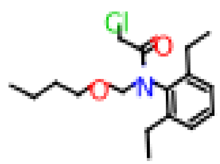
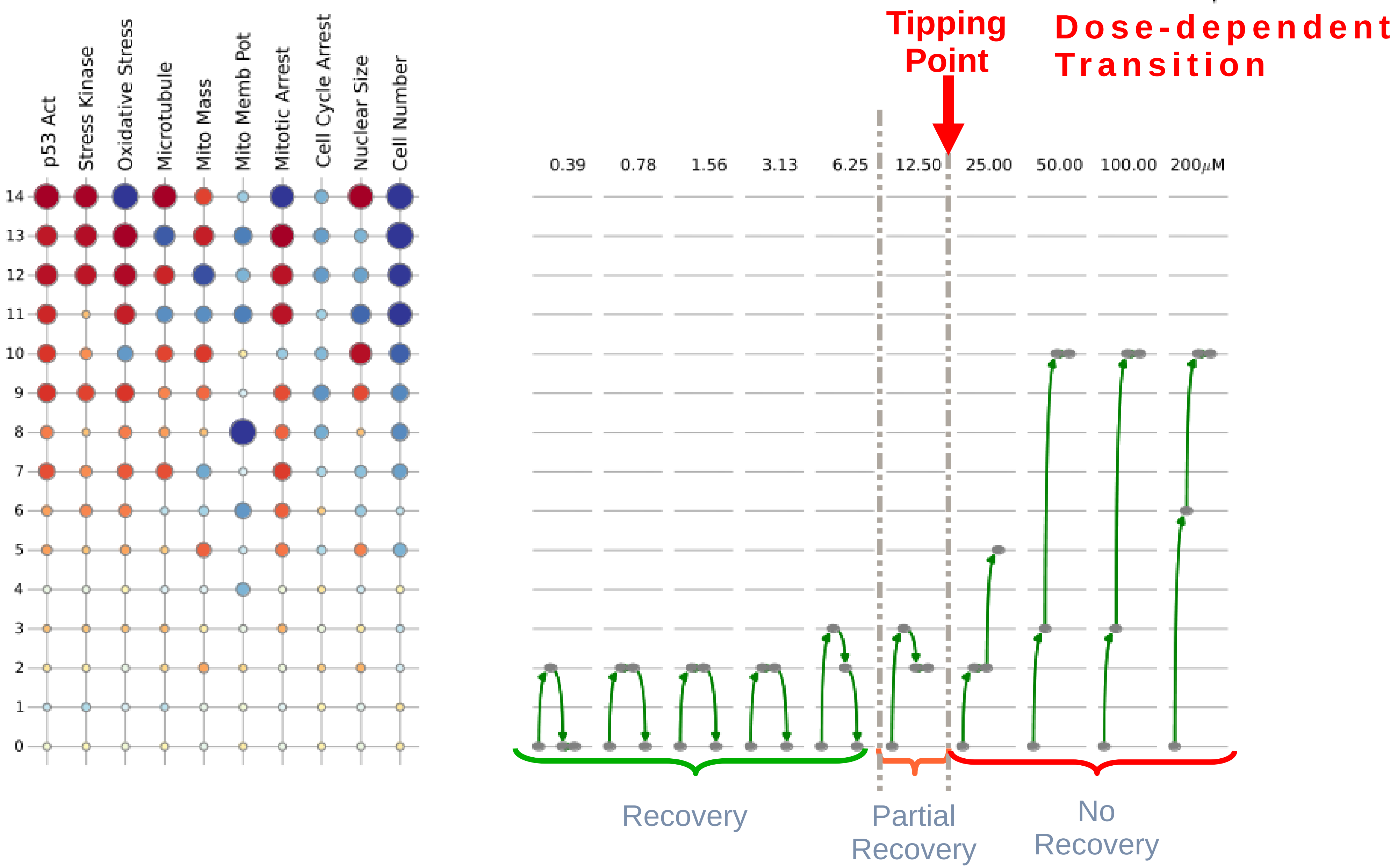
Butachlor



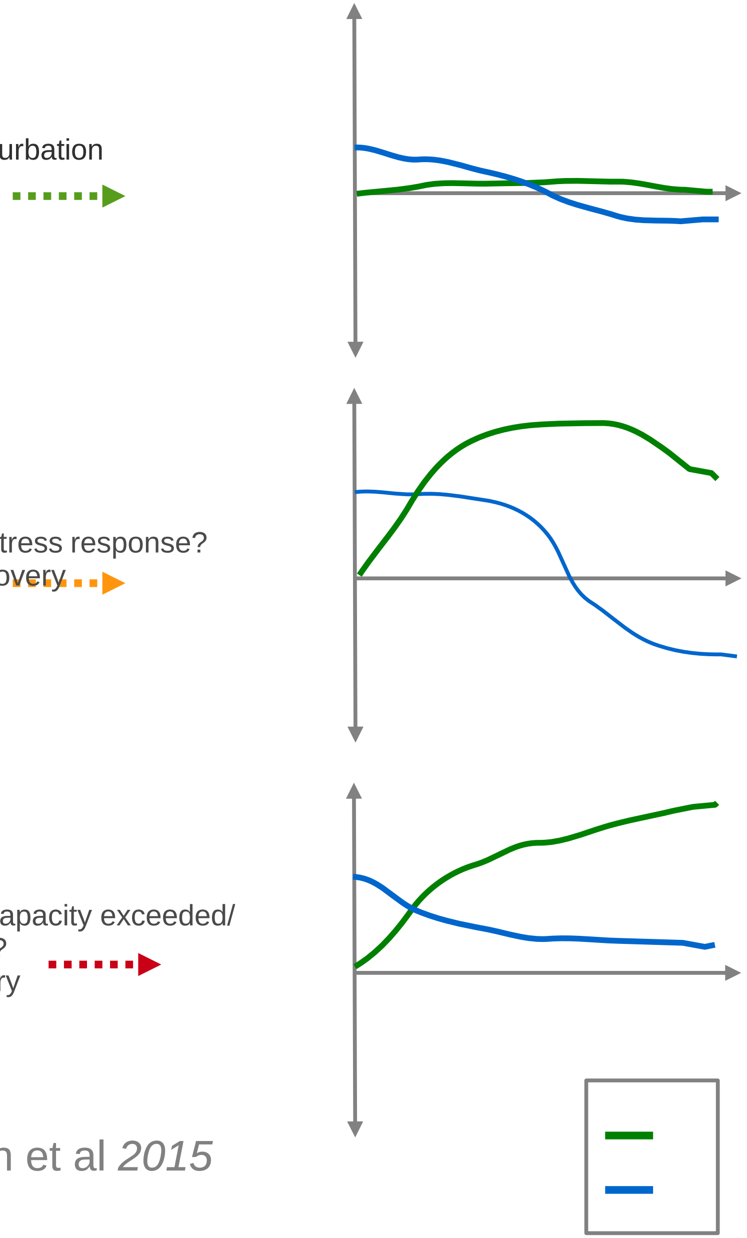



# Analyzing Trajectories Quantitatively

Some perturbation
Recovery

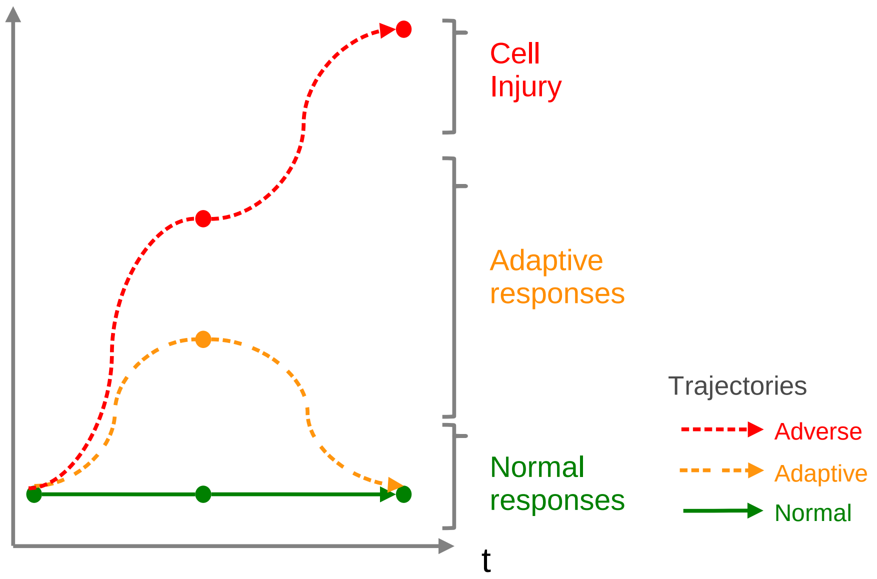
Adaptive stress response?
Partial recovery

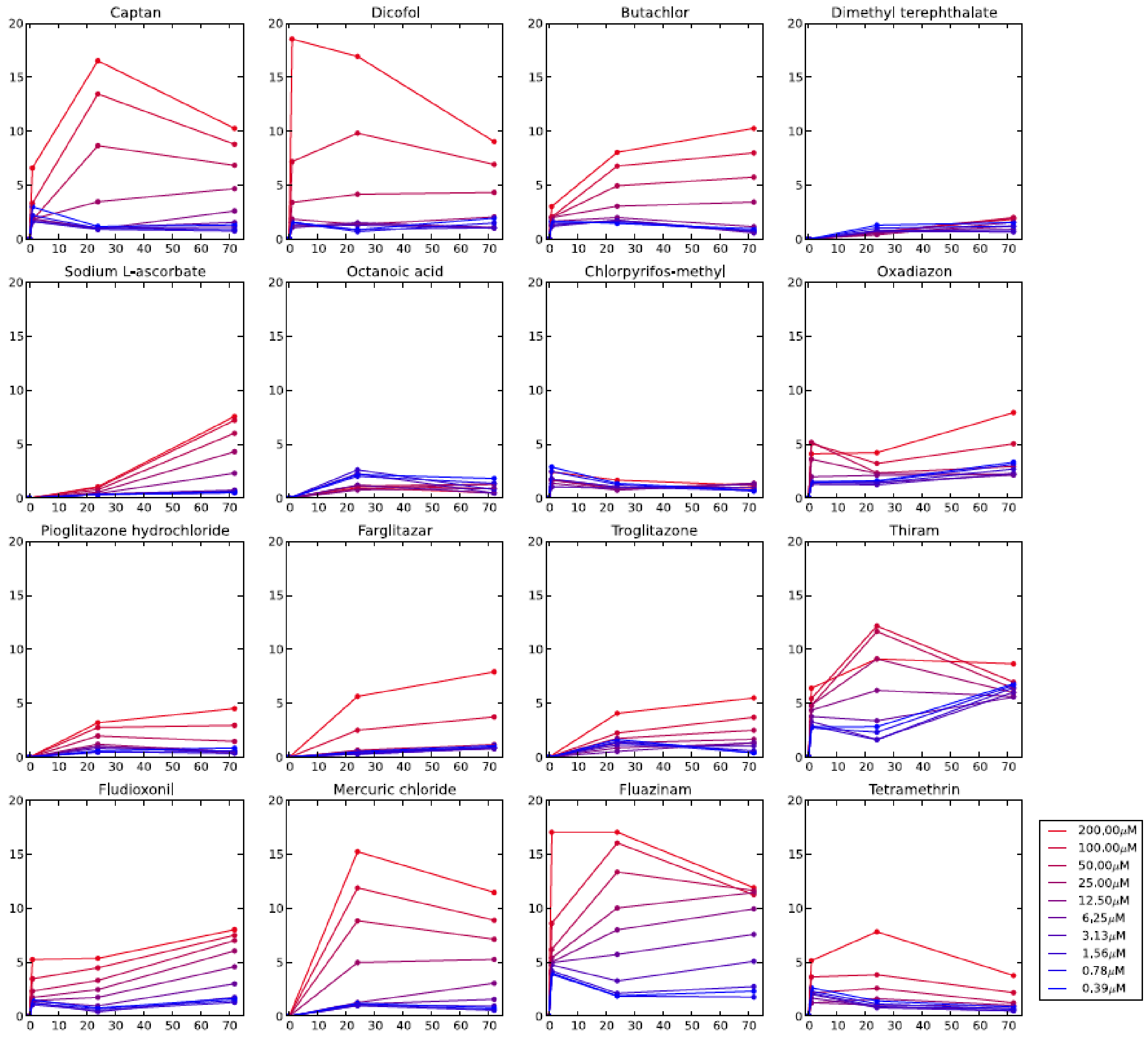
Adaptive capacity exceeded/
Cell injury?
No recovery



Shah et al *2015*

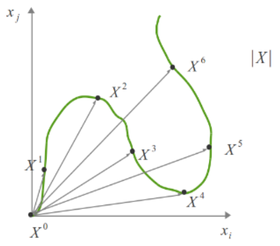
# Quantitative Trajectories

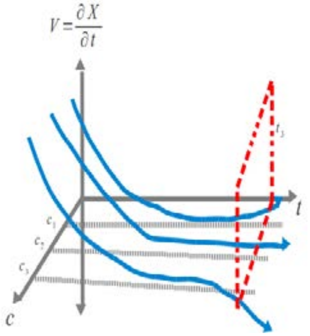


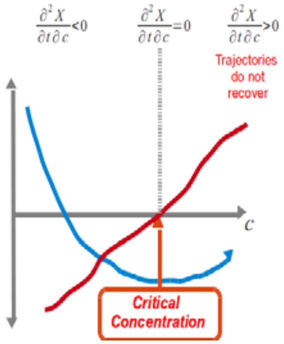



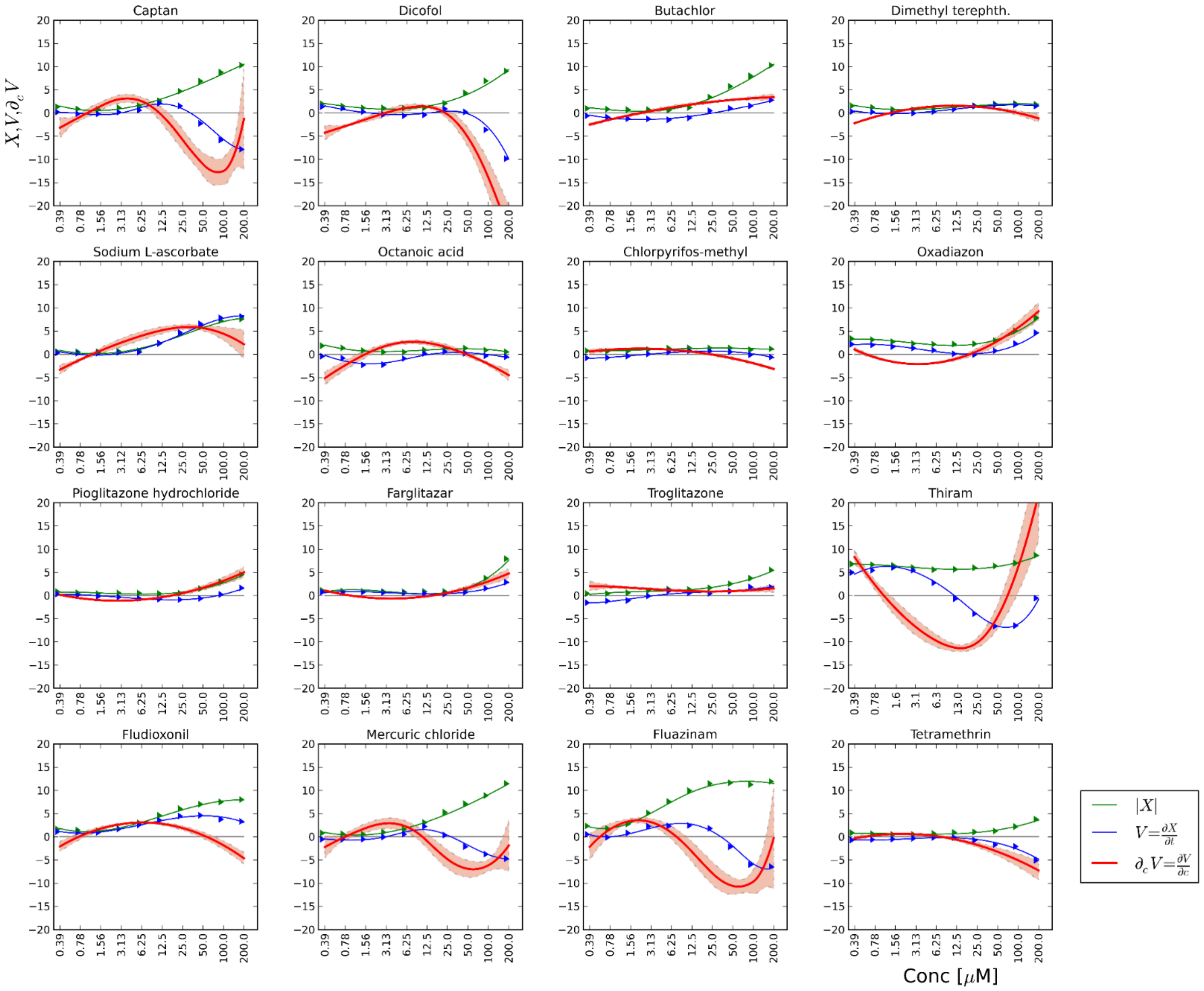

Shah et al *2015*

# Dose-Dependent Transition





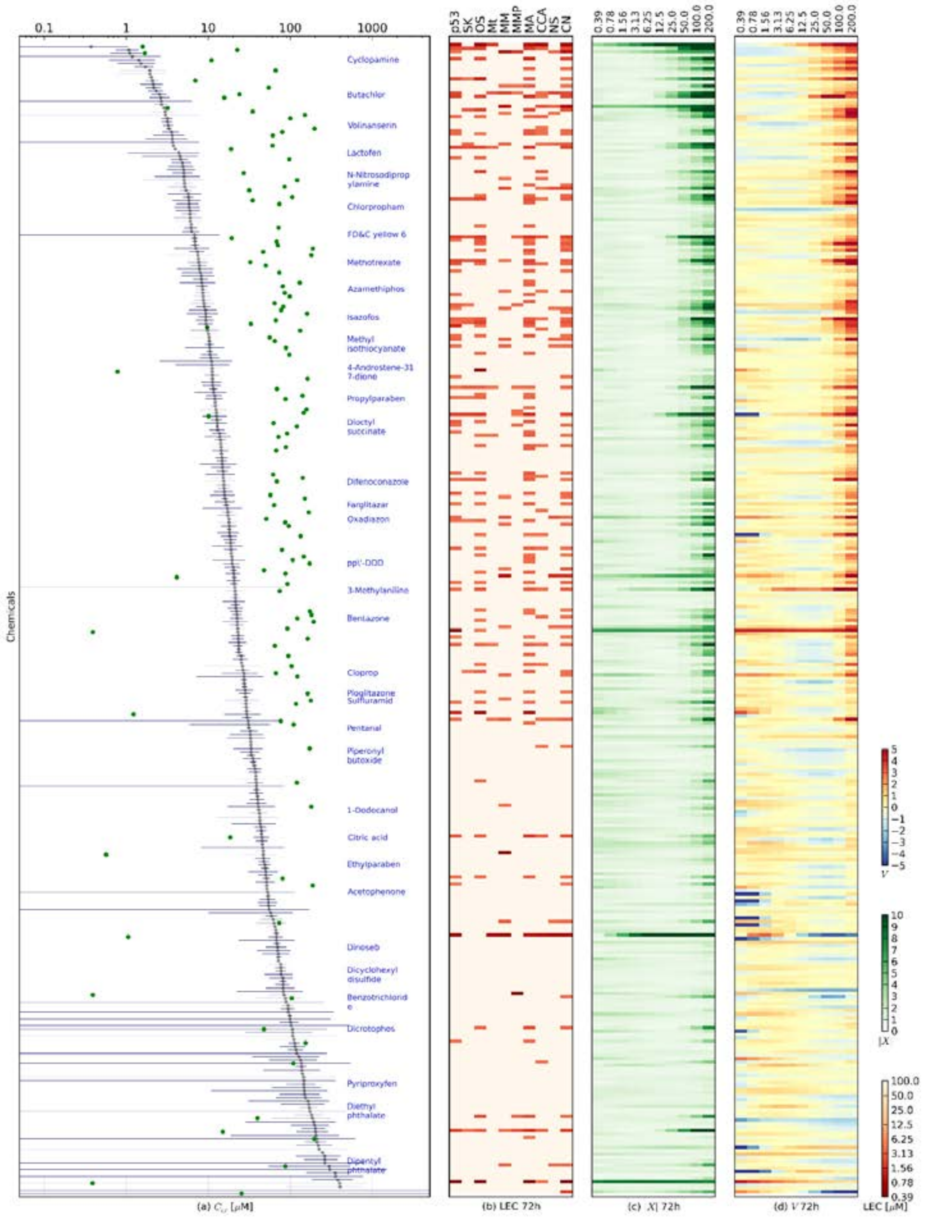



Shah et al *2015*

# 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

- More time points !
- More stress-response markers !
- How to define critical window to identify tipping points ?
- More physiologically-relevant cell model

❑ Tipping point evaluation

- Is it just PK or is there real adaptation ?
- Are they reproducible ?
- Are they biologically relevant (ie. mechanisms)?

*New study underway to address some of these challenges …*

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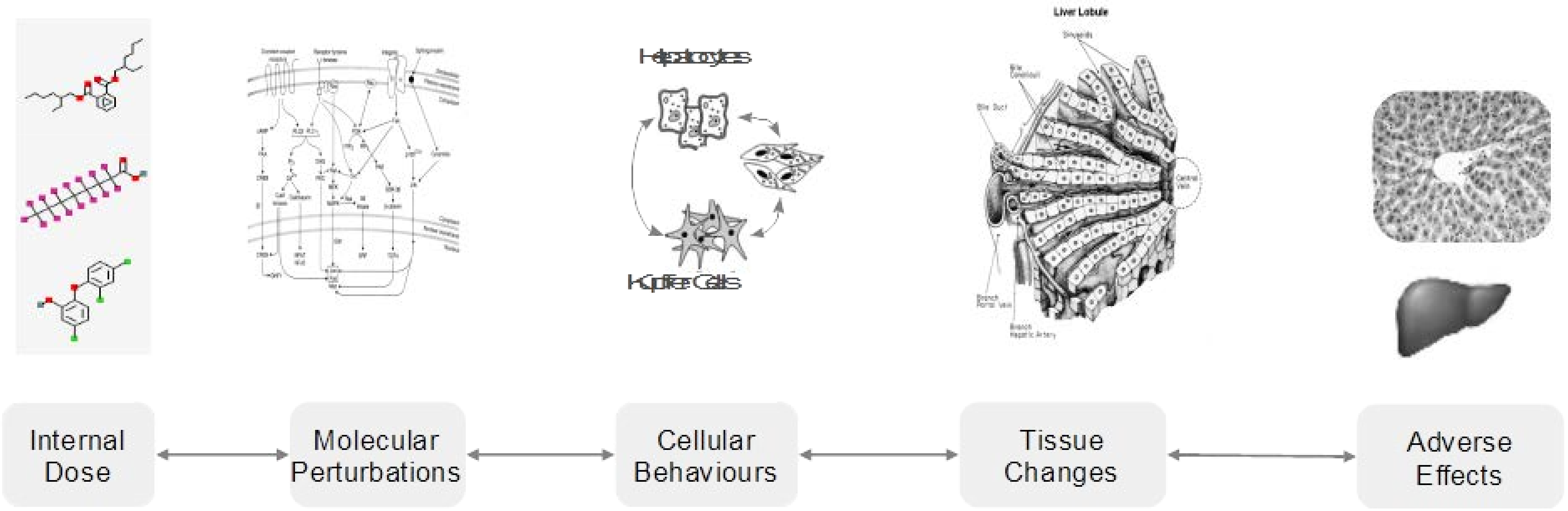
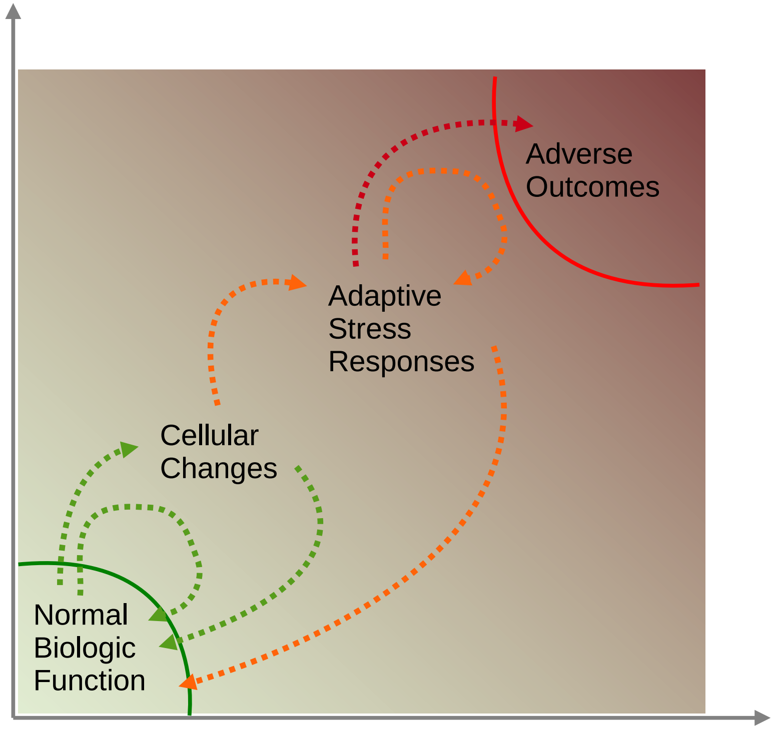
# Translation: *in vivo* Tipping Points ?




- ❑ Adverse effects depends on a complex sequence of events
- ❑ Consider *in vivo* lesions:
  - ❑ Hypertrophy
  - ❑ Necrosis
  - ❑ Inflammation
  - ❑ Steatosis
- ❑ These can be acute effects of chemical exposure or intermediate steps towards chronic injury
- ❑ Differentiating adaptation and adversity is difficult !
- ❑ Transient effects are more likely to be adaptive

# Summary

- Complex biological data: system state
- State pertubations define trajectory
- Trajectory analysis→Tipping Point
- Threshold for homeostatic adaptation
- Linked with adversity
- Could be used as point of departure for risk assessment




Biologic Pertubations:

System Trajectories:

- Some perturbation/ Recovery
- Adaptive stress response/ Recovery
- Adaptive capacity exceeded/ Cell injury/ No recovery

# Acknowledgements


- HCI Experiment
  - Keith Houck
  - David Dix
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Analysis

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Thomas Knudsen
Robert Kavlock