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Toxicological Tipping Points & Cell Stress

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Discriminating between adaptation & adversity
SOT 2016, New Orleans, LA

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Office of Research and Development
National Center for Computational Toxicology
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Toxicological Tipping Points ...

... threshold between adaptation and adversity

... **may** be useful as PoD for risk assessment

... **may** involve stress response pathways

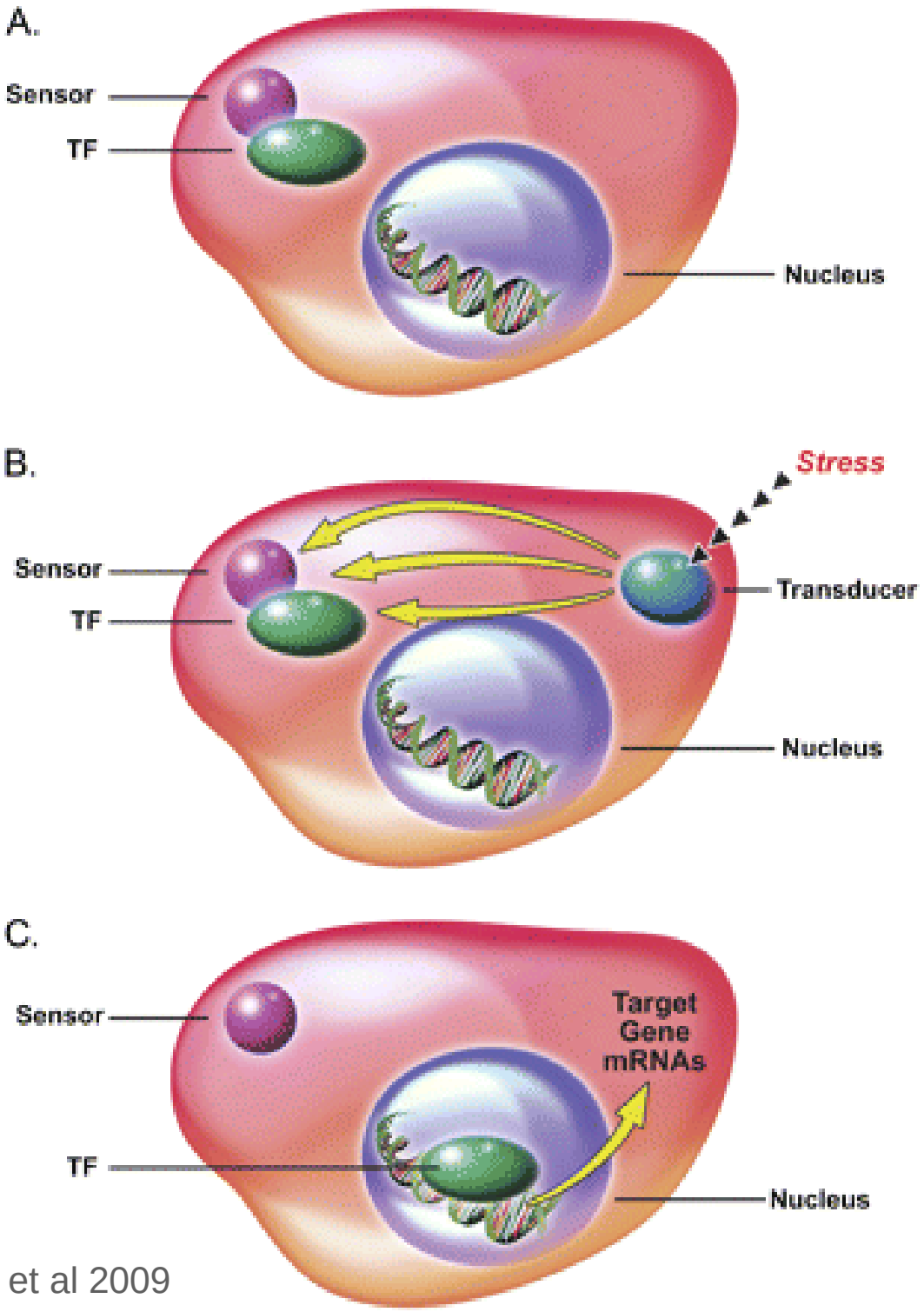
... **could** be found from some *in vitro* data

... needs new computational approaches

- Case-Study: Use high-content data to find tipping points

- Celluar stress response pathways
  - Oxidative stress
  - Hypoxia
  - DNA damage
  - ER stress
  - Mitochondrial stress

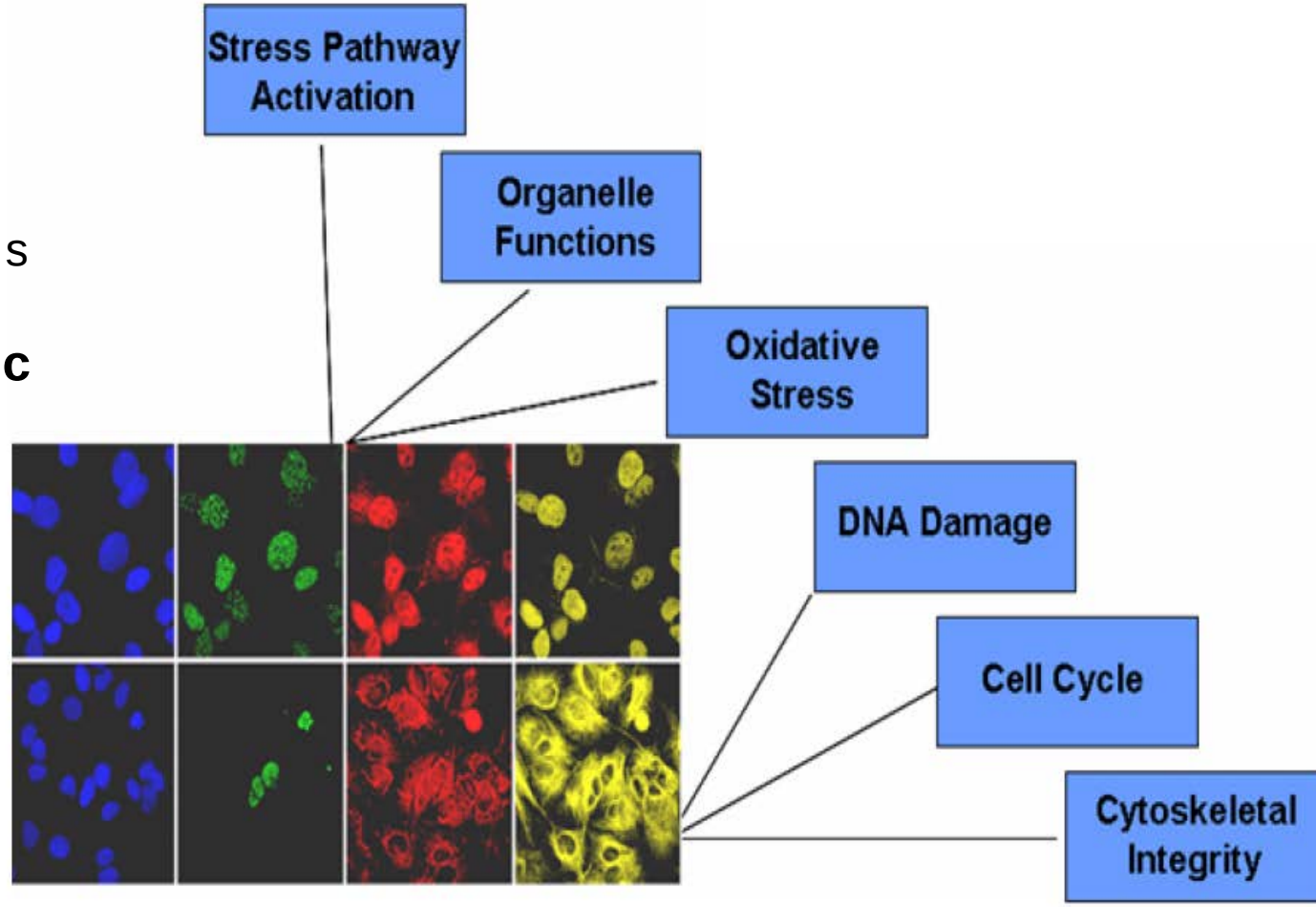
- Induced by chemicals to overcome “perturbation” of normal state



Simmons et al 2009

HCI → Stress Responses?

- **High content imaging**
  - HepG2 cell culture
  - (ToxCast I, II)
  - 10 conc, 3 times
- **HCI Assays**
  - System perturbation
  - Cell health
  - Some stress pathways
- **Dynamic phenotypic** response of cells to chemicals



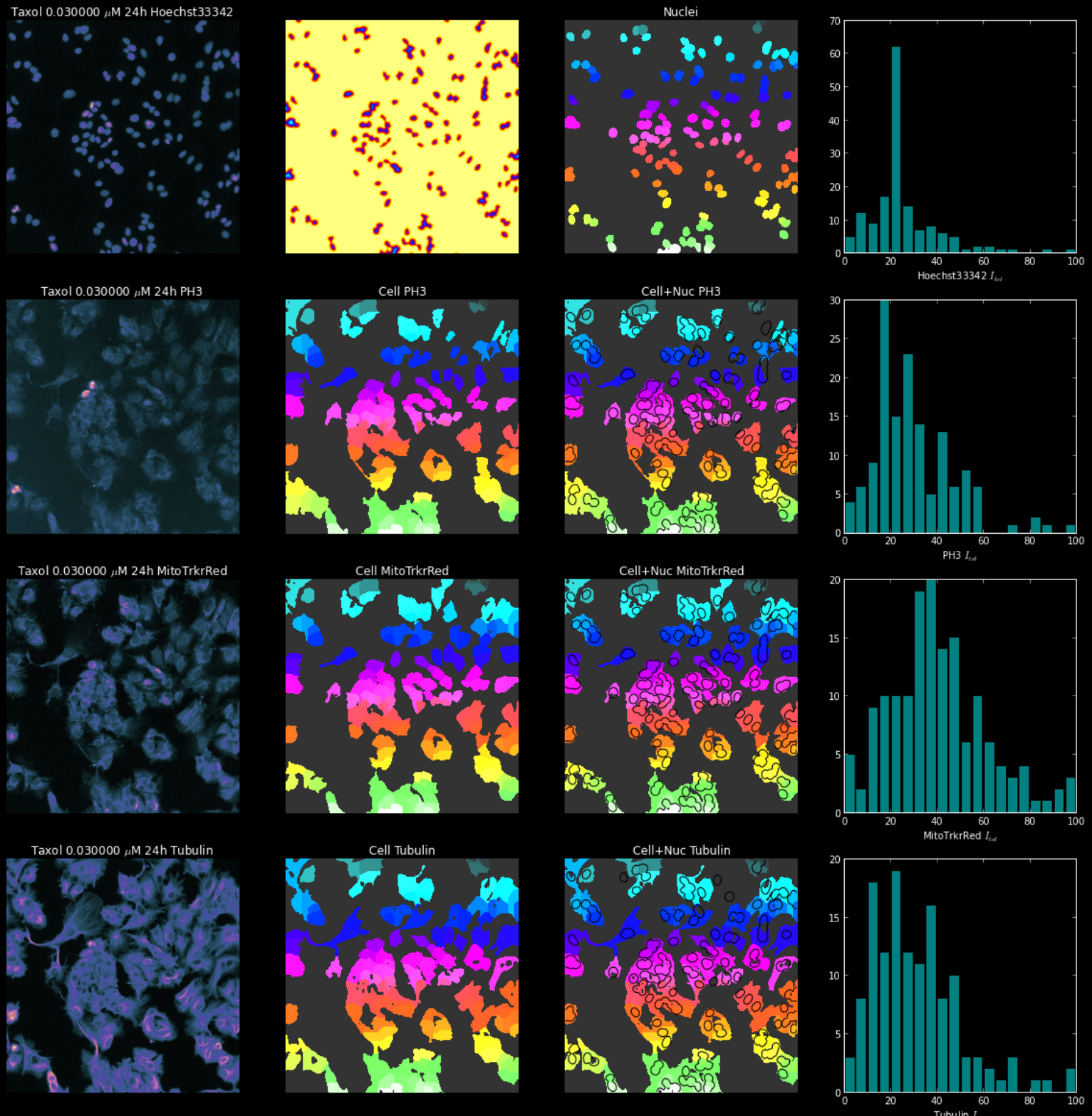
Taxol 0.03uM

Hoechst33342

Phospho-Histone3

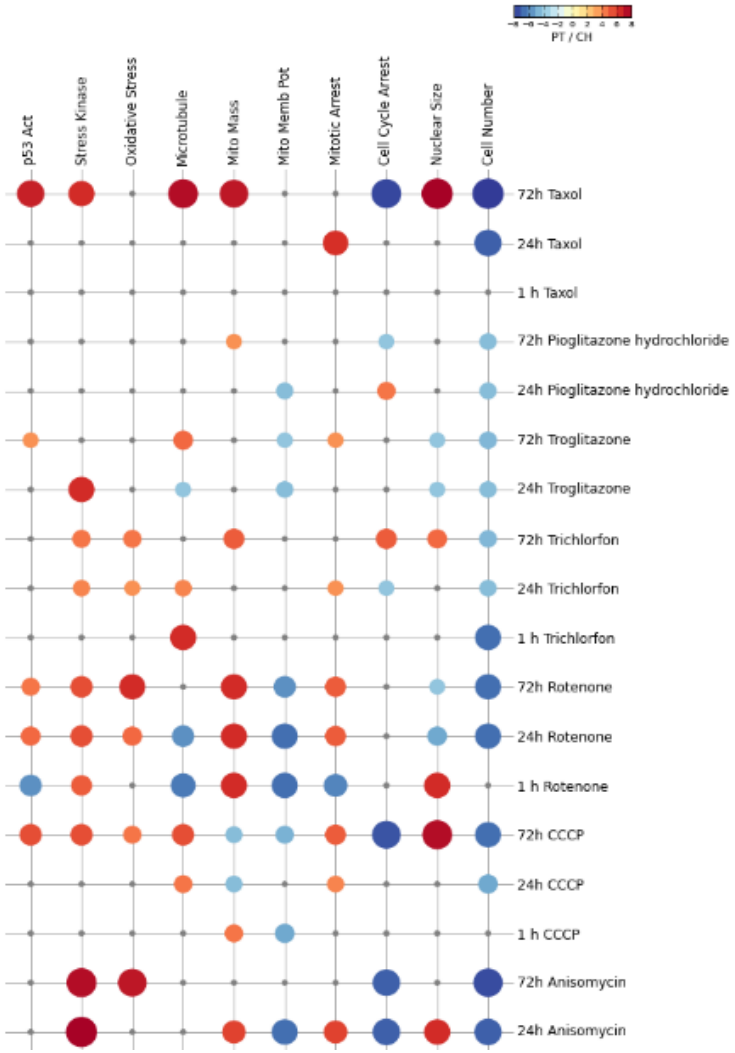
MitoTracker Red

Phospho-Tubulin

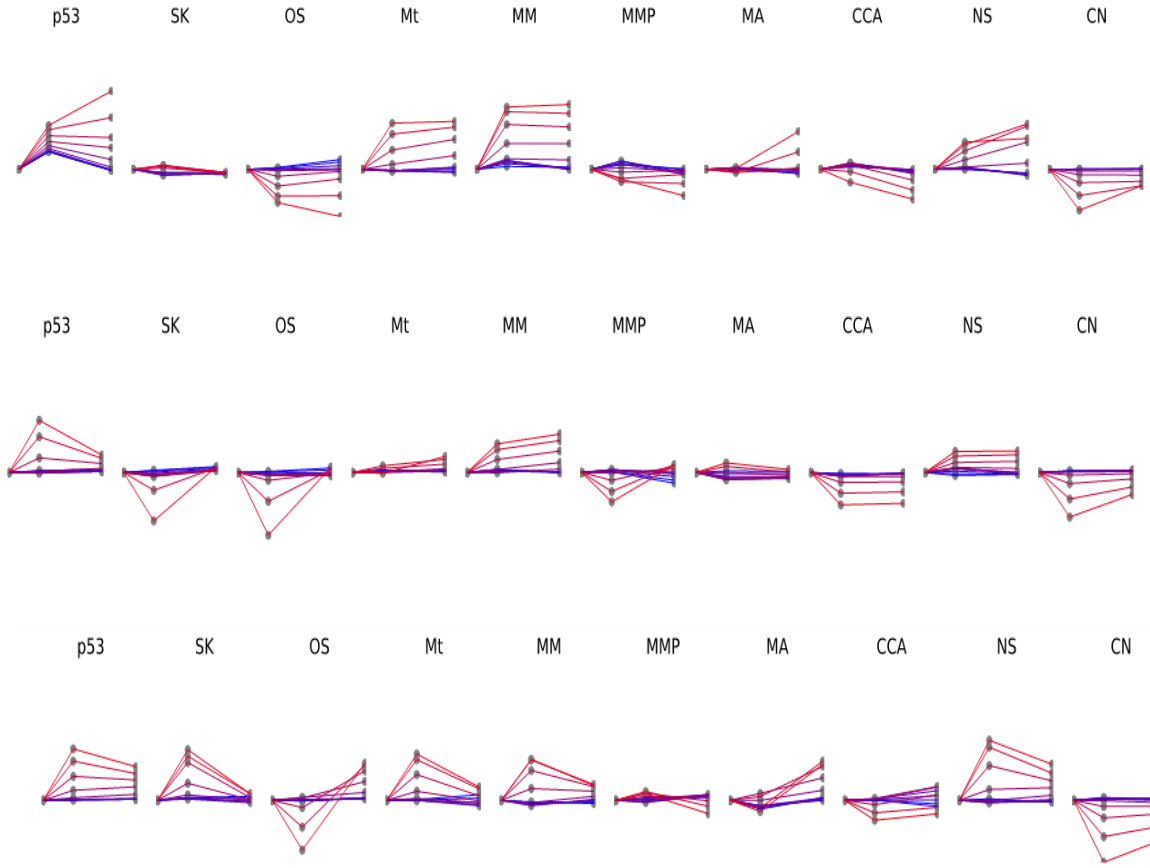


HCI → Multidimensional Data

“Discrete Responses”

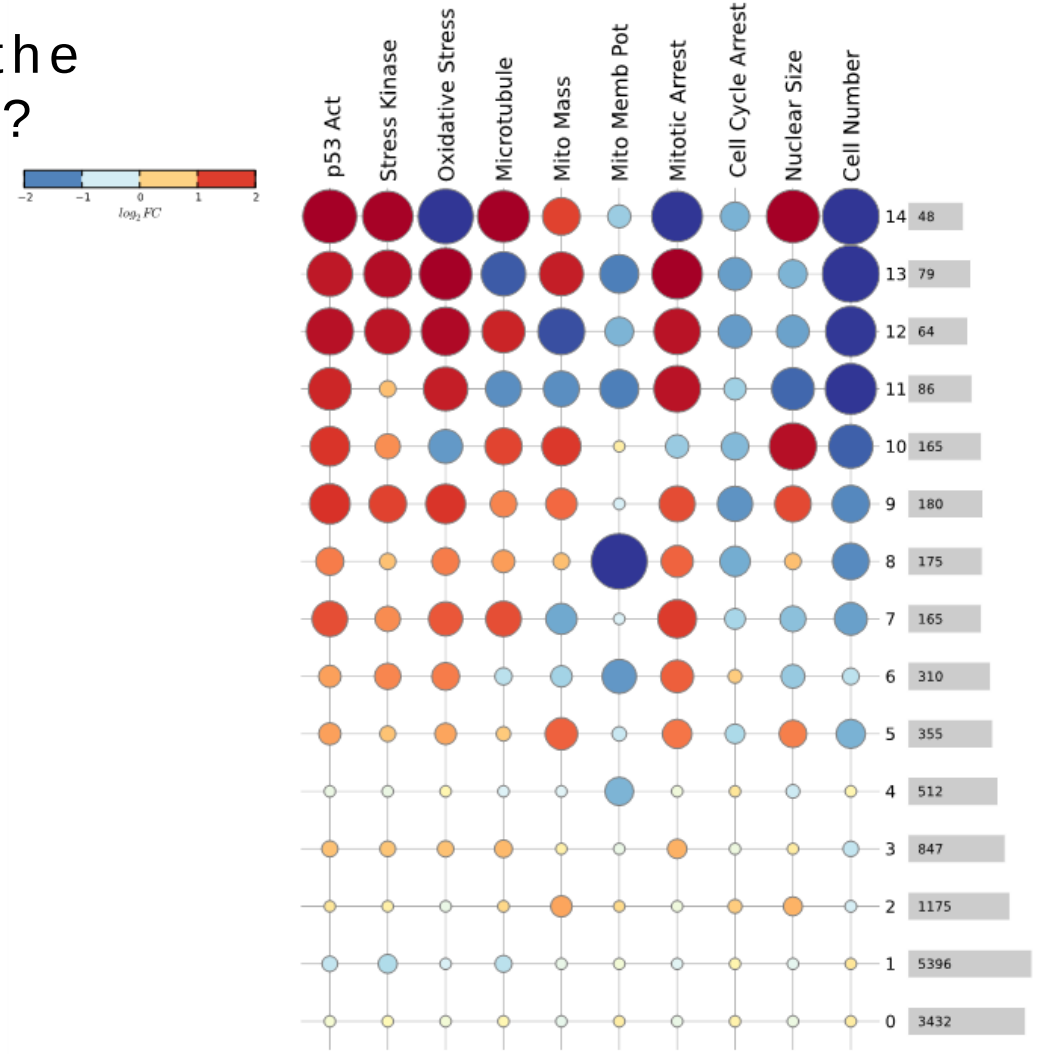


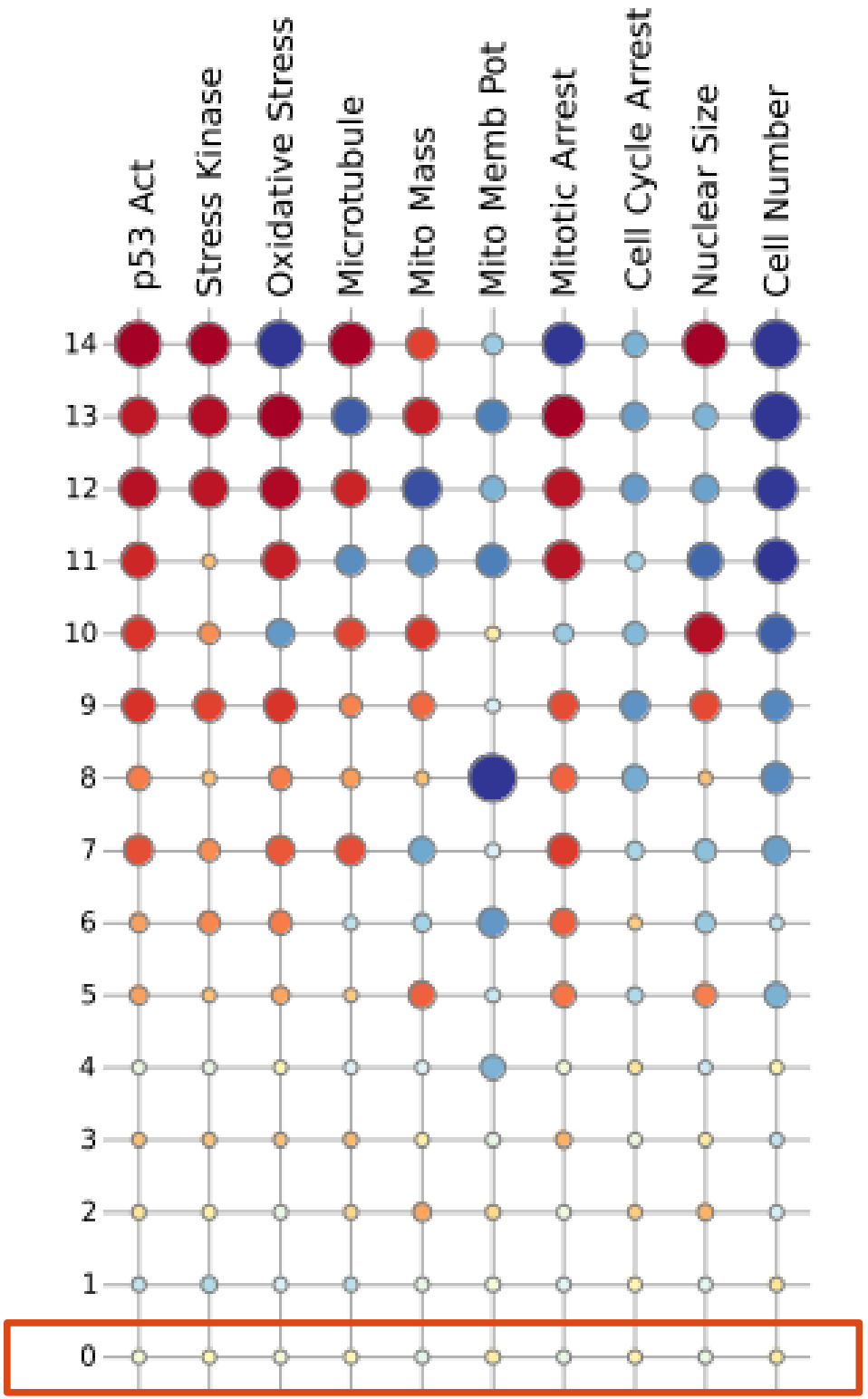
“Continuous Responses”



Discrete States – Phenotypes?

- How can we understand the behavior of HepG2 cells ?
- Cluster all data to derive phenotypic “states” of HepG2 system
- Analyze system trajectories
- Can we define normal adaptive and adverse responses ?





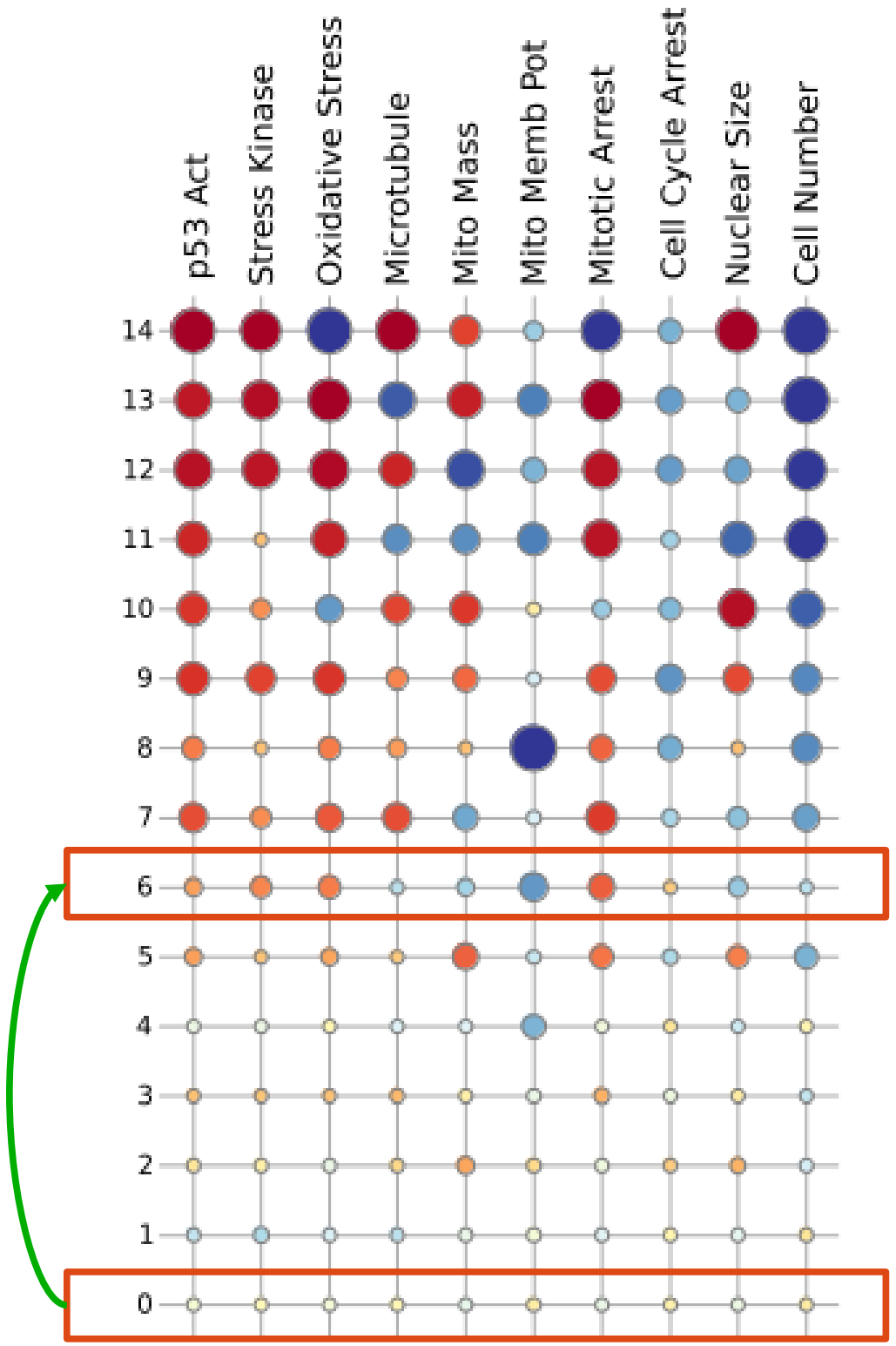
“Normal” State

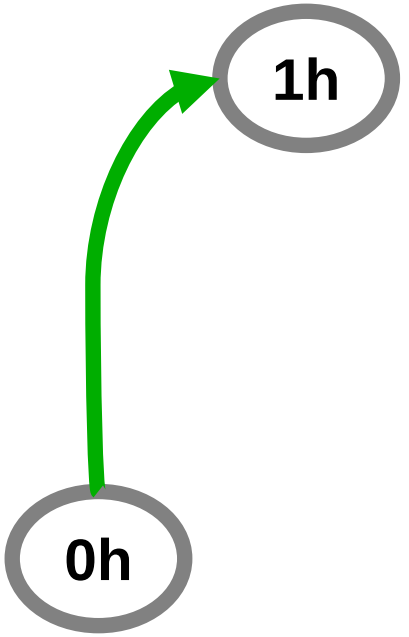
Fluazinam 0.78 uM

0h

State Transition

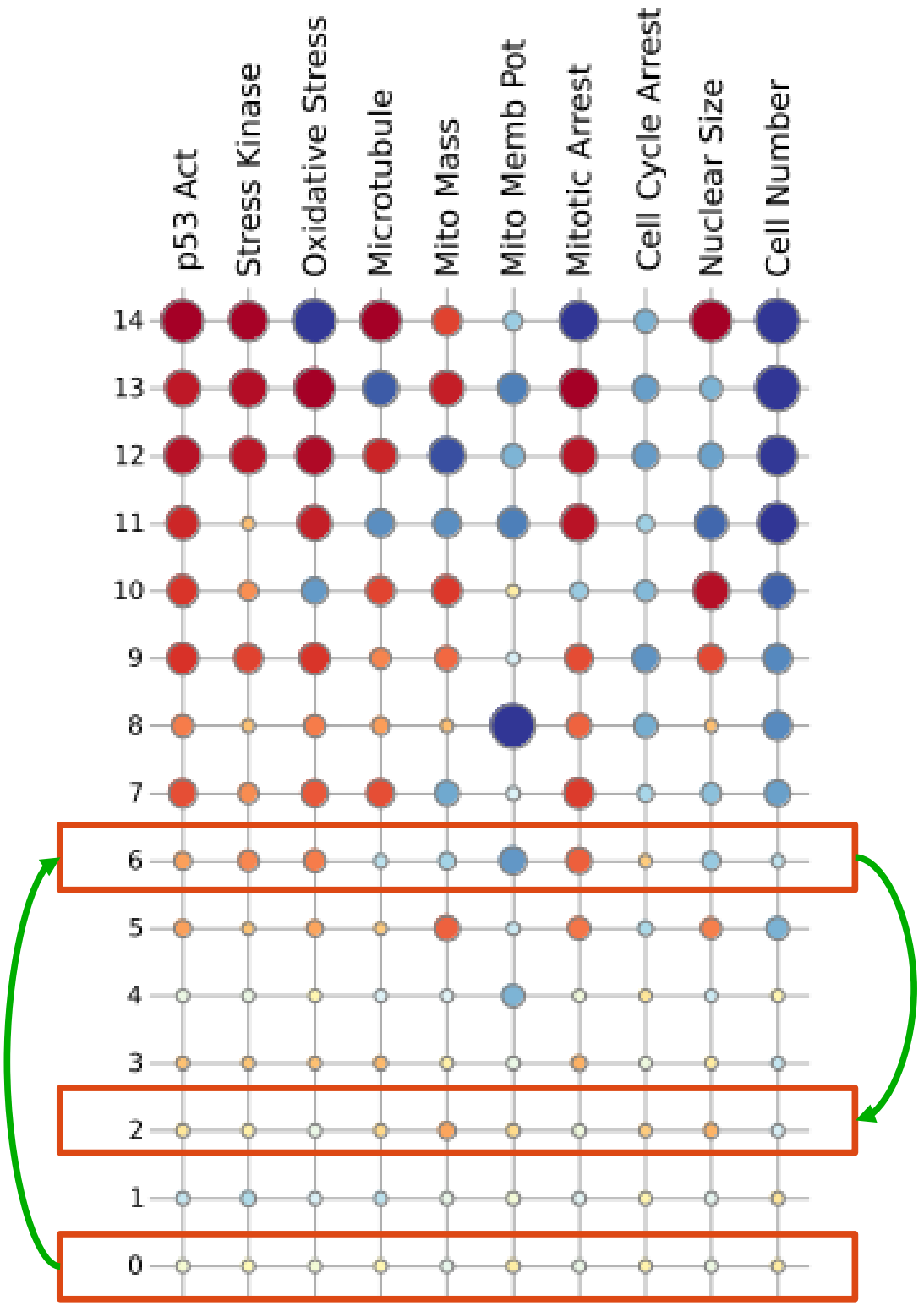
Fluazinam 0.78 uM

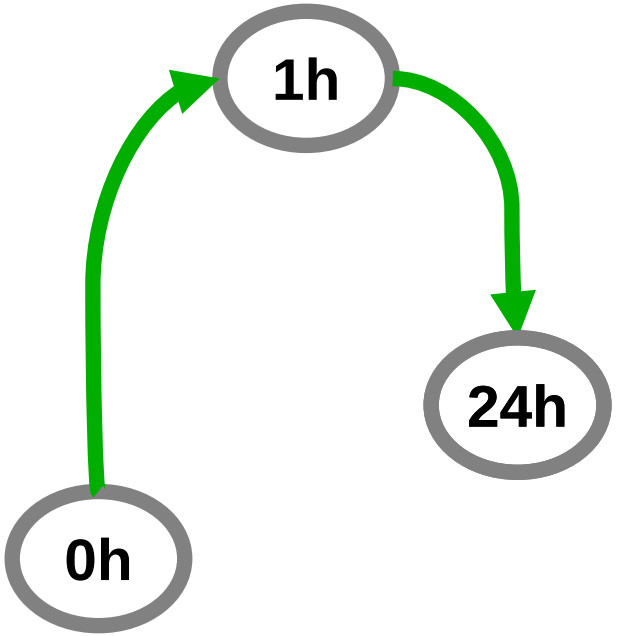


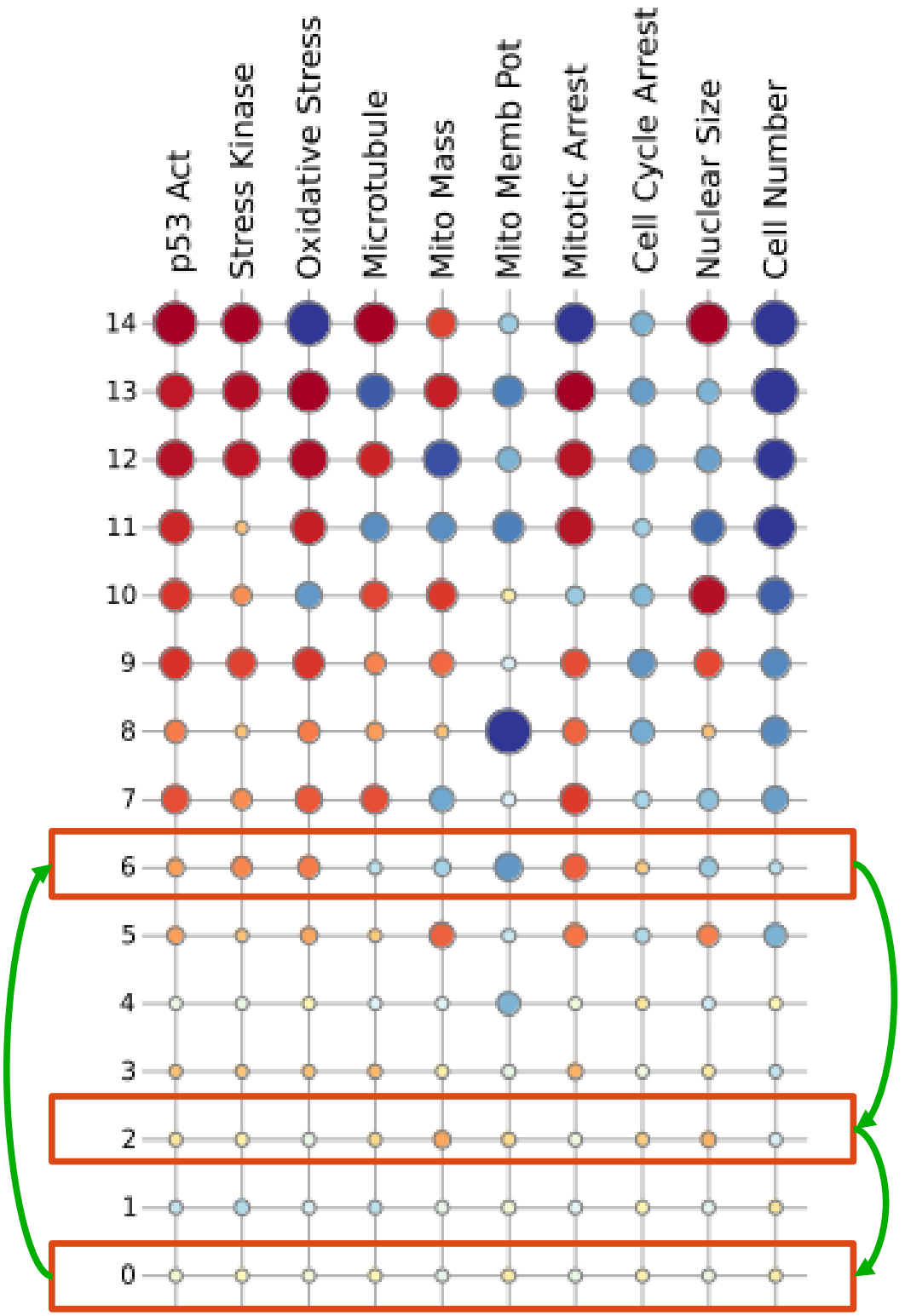


System trajectory

Fluazinam 0.78 uM



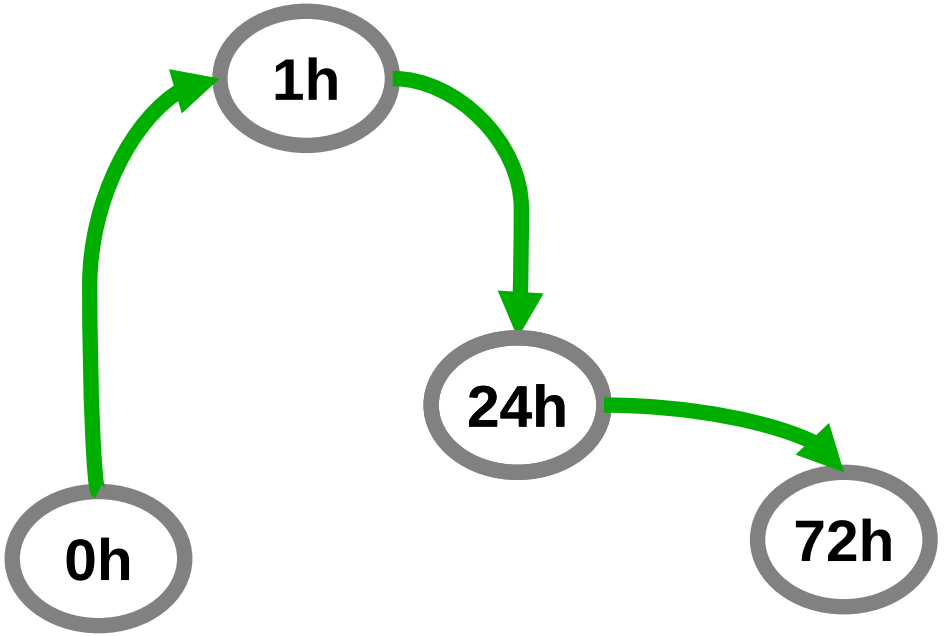




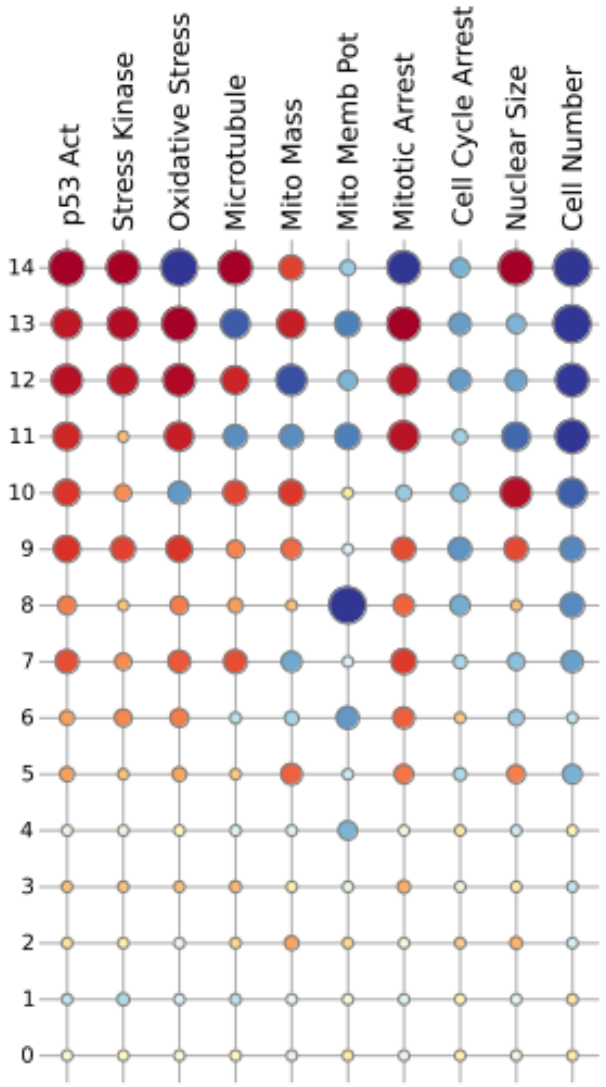
System trajectory

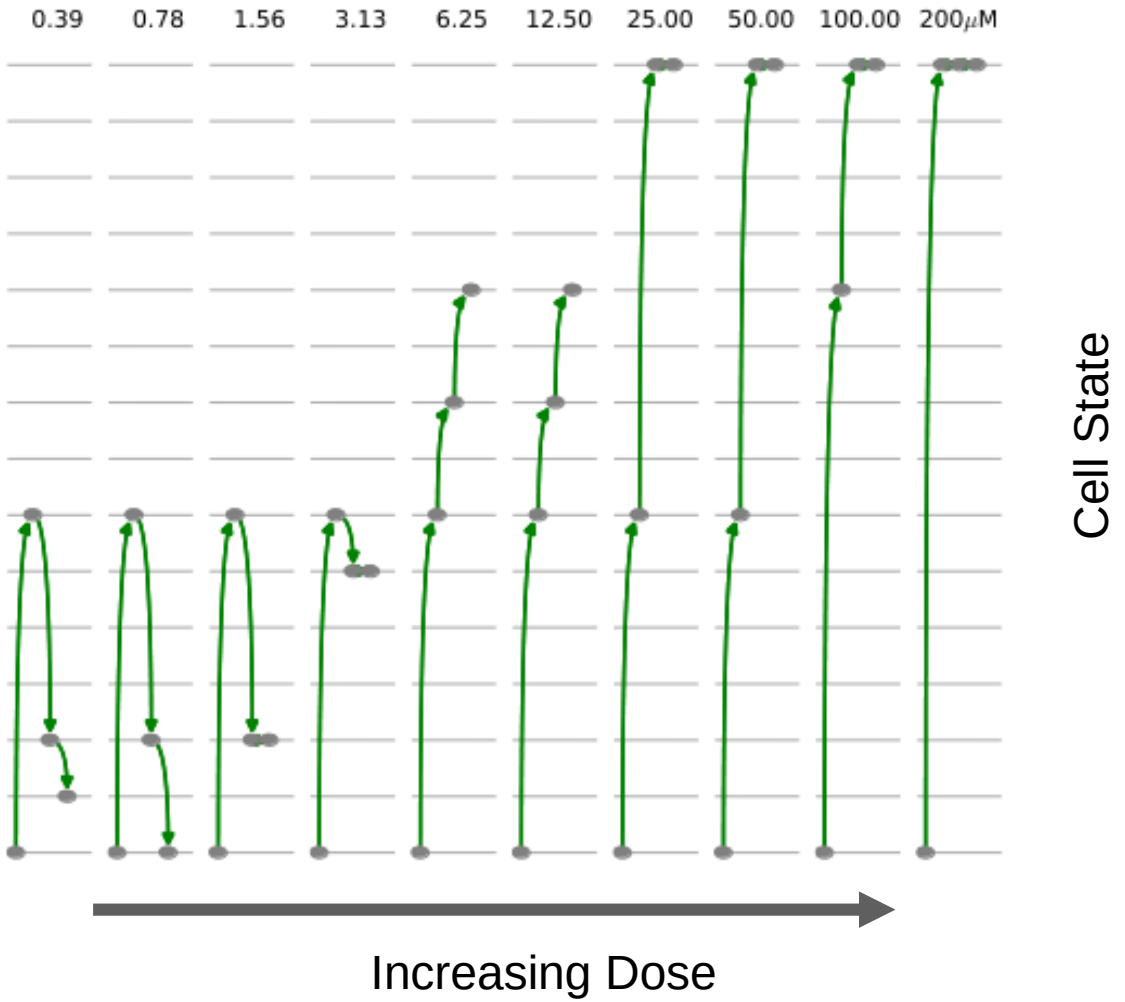
Fluazinam 0.78 uM

Trajectory=Sequence of states

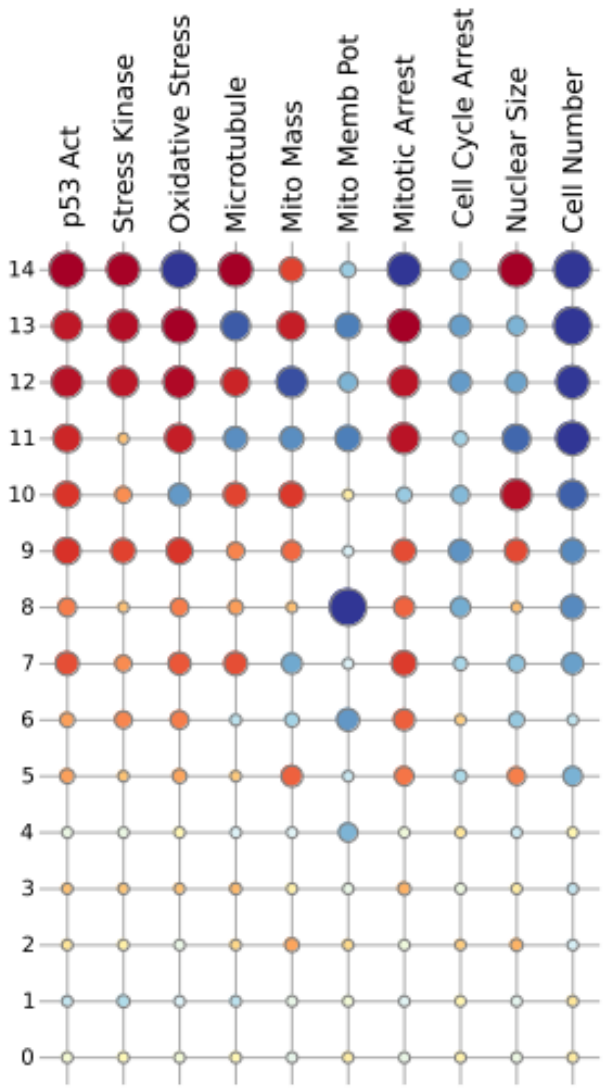


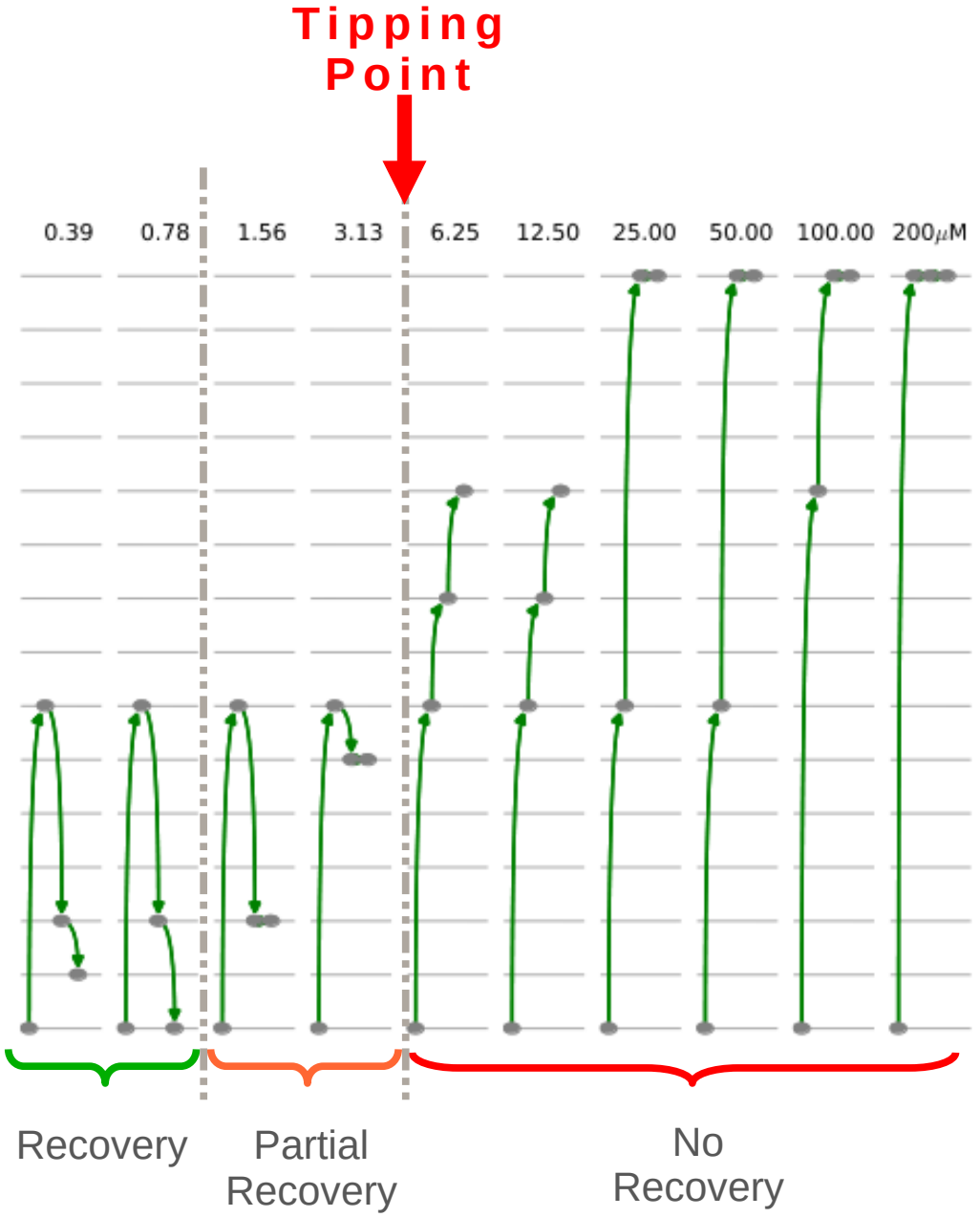
Fluazinam “Trajectories”



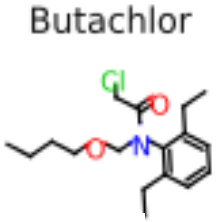


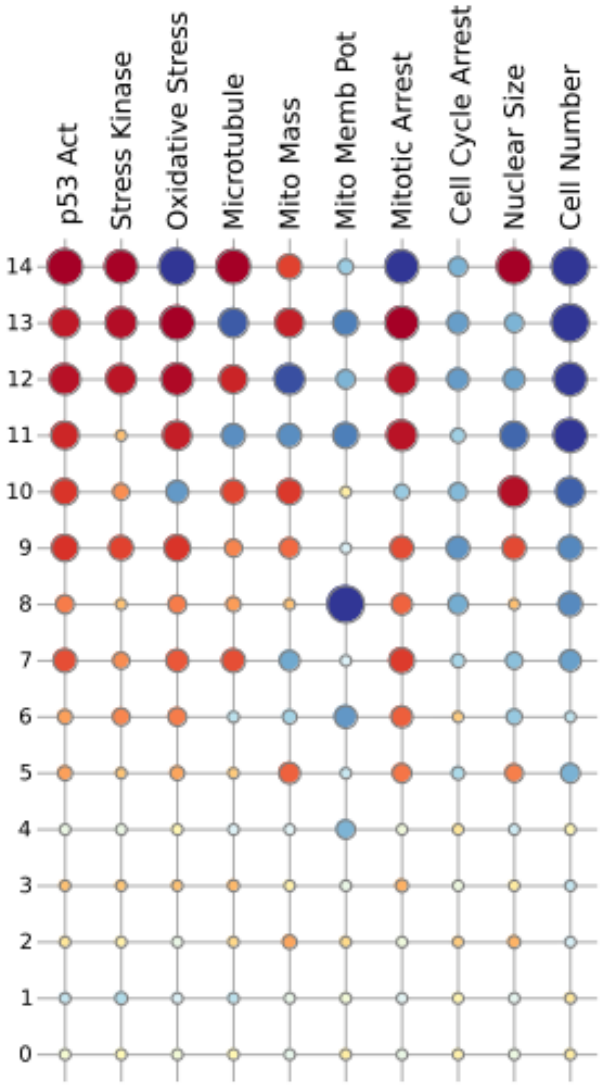
Fluazinam “Trajectories”

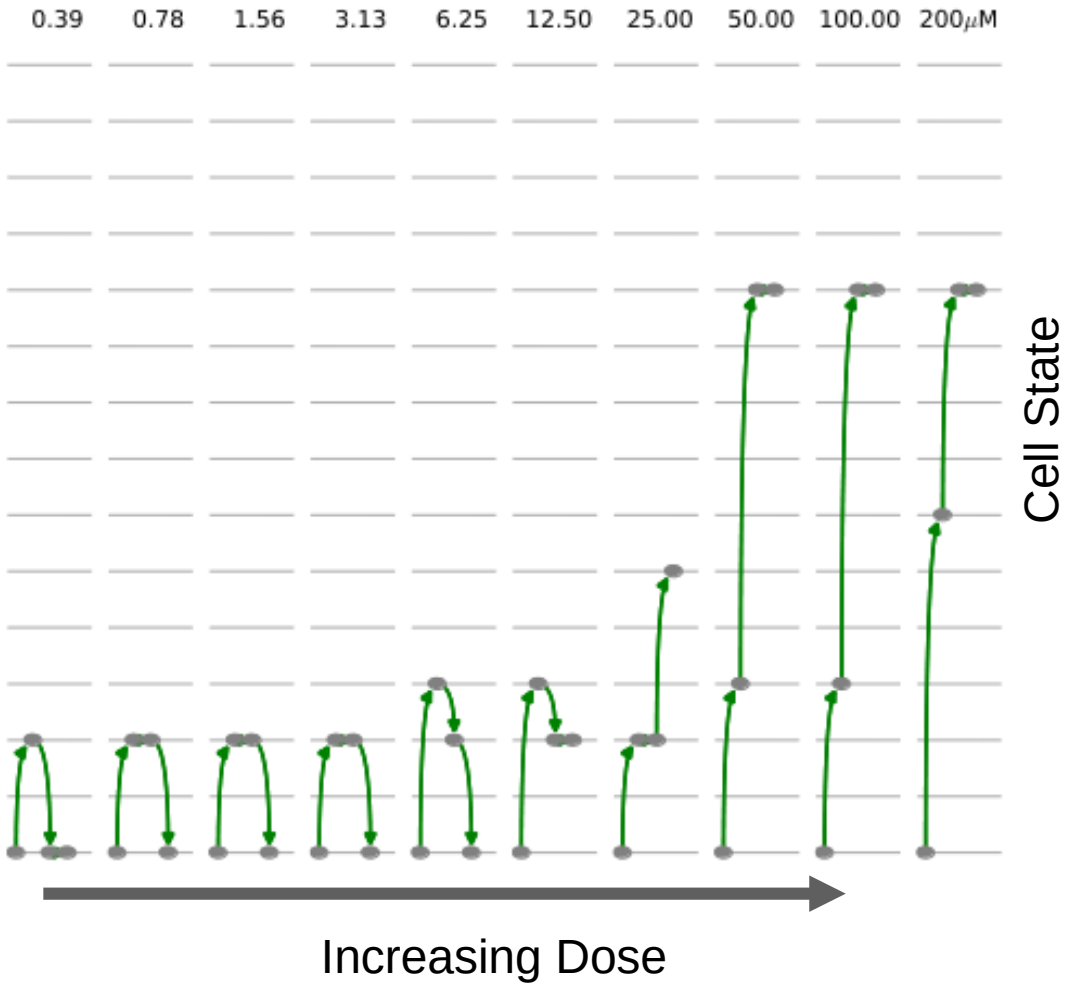




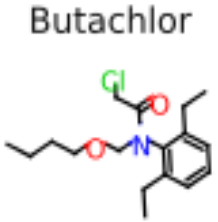
Butachlor Trajectories

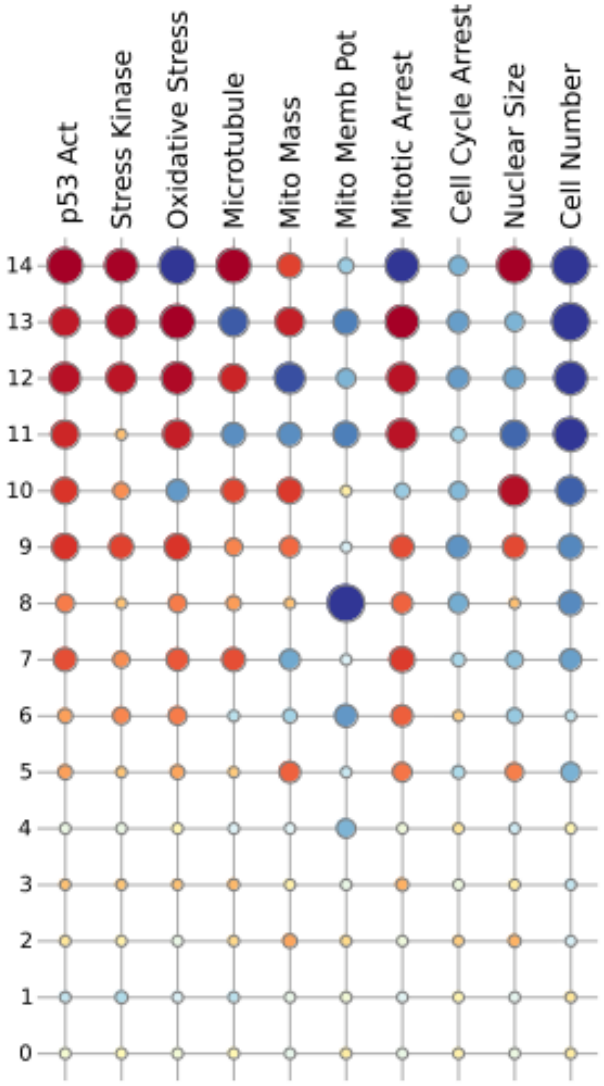


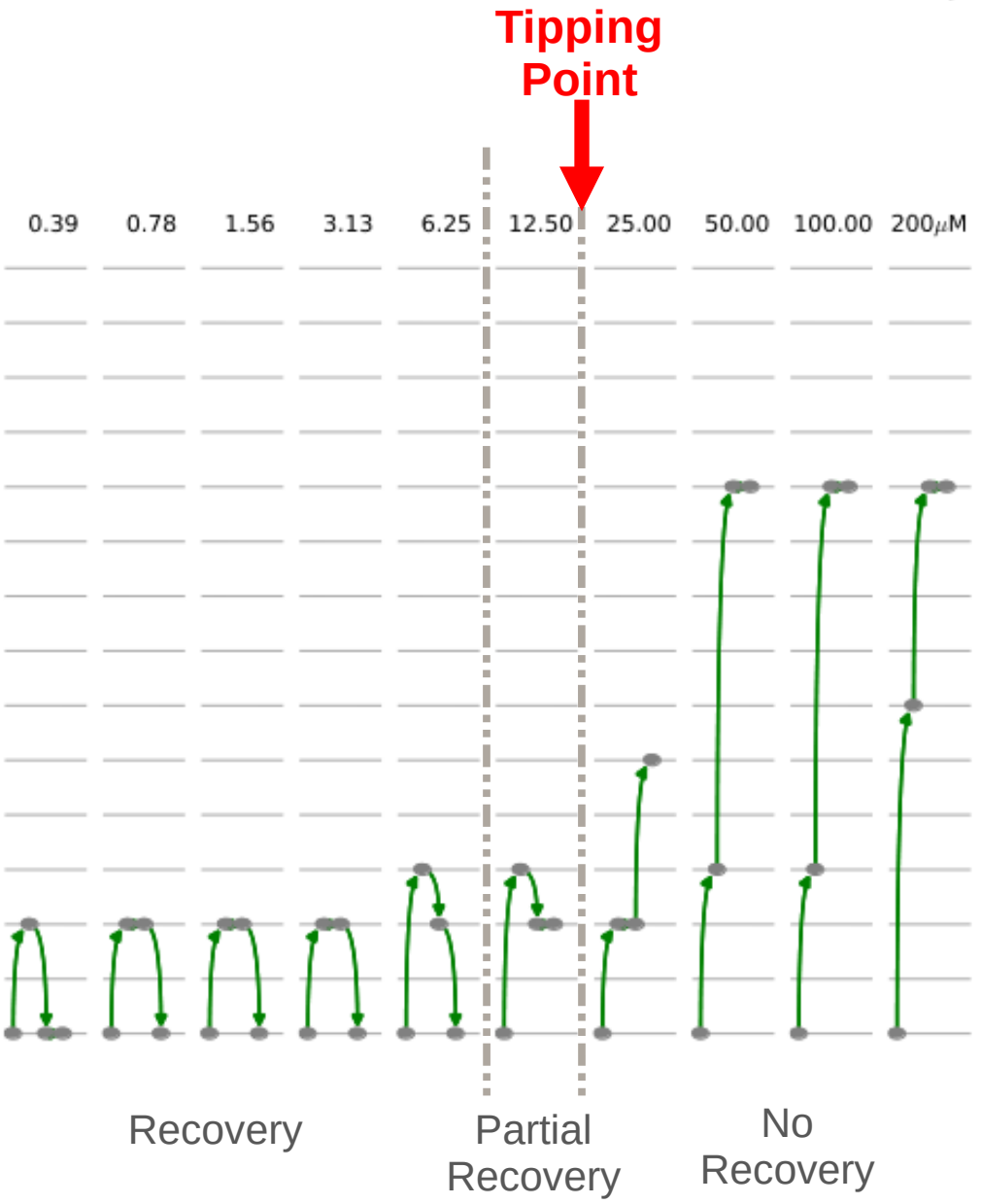




Butachlor Trajectories



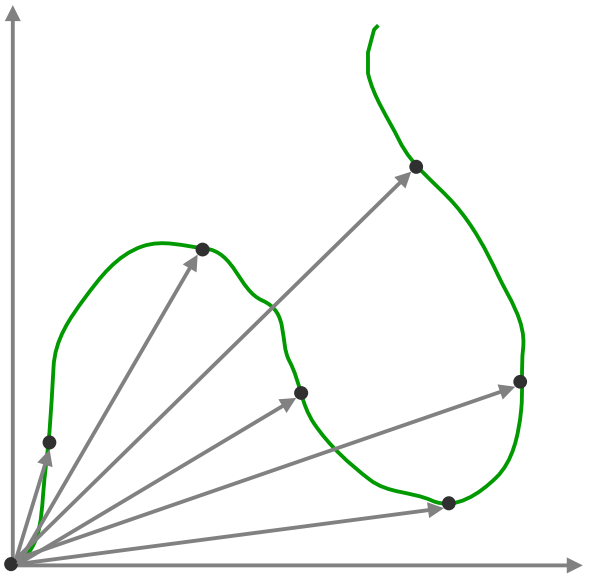




A Systems View…

- HCI data→ System State (***X***)
- Chemical→ System perturbation
- Pertubation → Dynamic response
- Dynamics → Trajectory
- Trajectory → Adaptation/Adversity

System Trajectory



A 2-dimensional projection of trajectory along dimensions (x_i, x_j)

Biologic endpoint,

Biologic alteration,

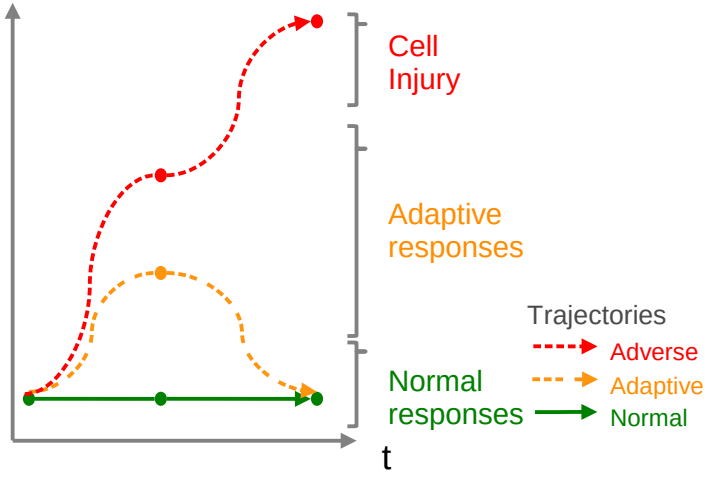
System state, $X^k = [x_1, x_2, x_3, x_4, ..., x_N]$

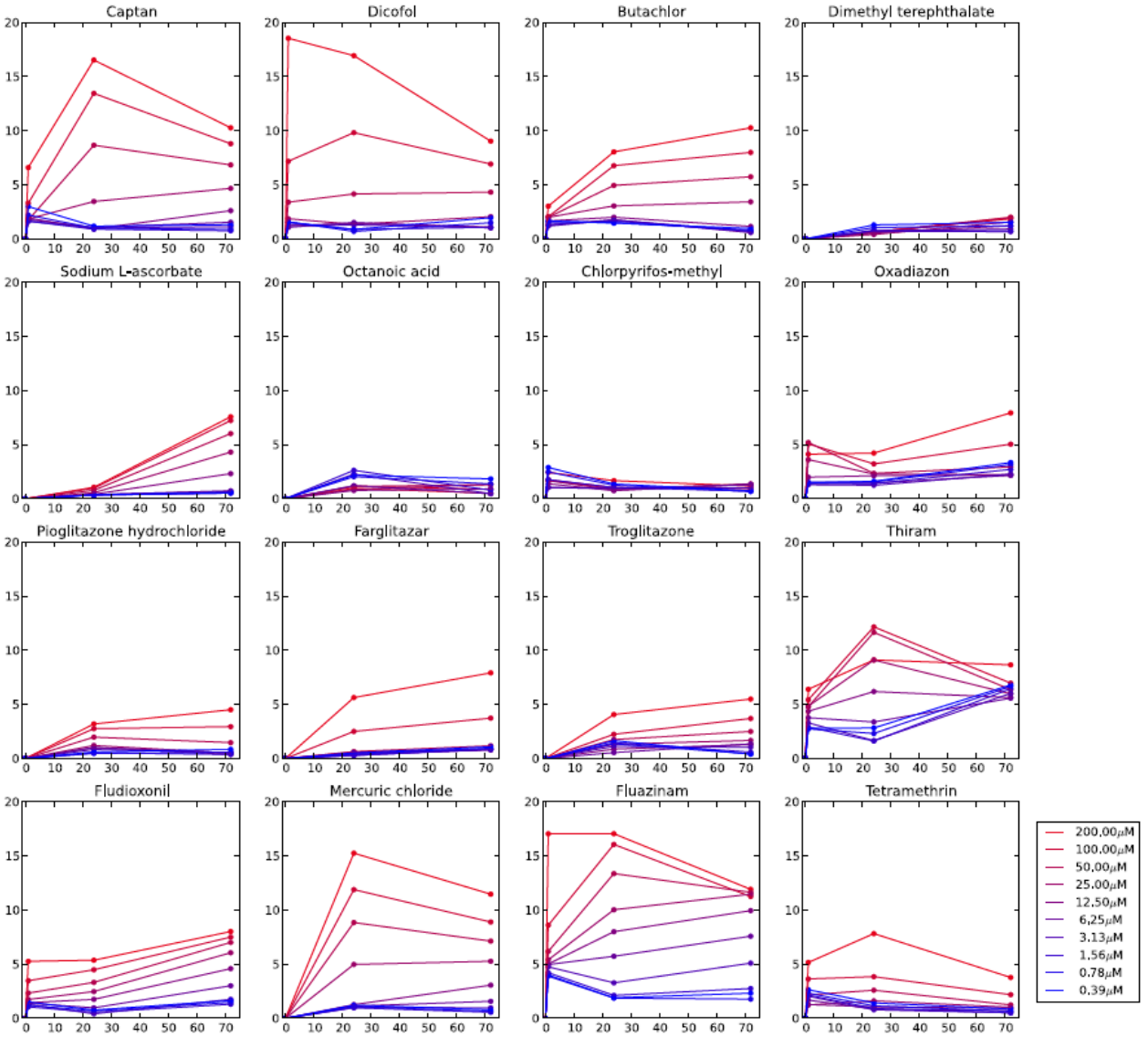
Scalar perturbation, $X^k = |S^k|$

Trajectory, scalar

Shah et al *2015*

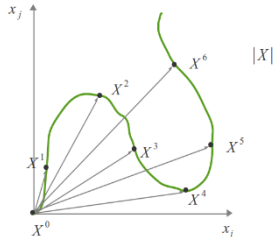
Trajectories & Adaptation

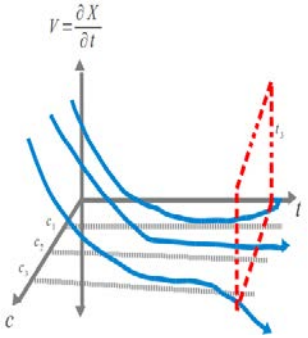


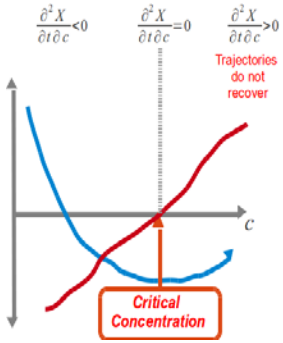


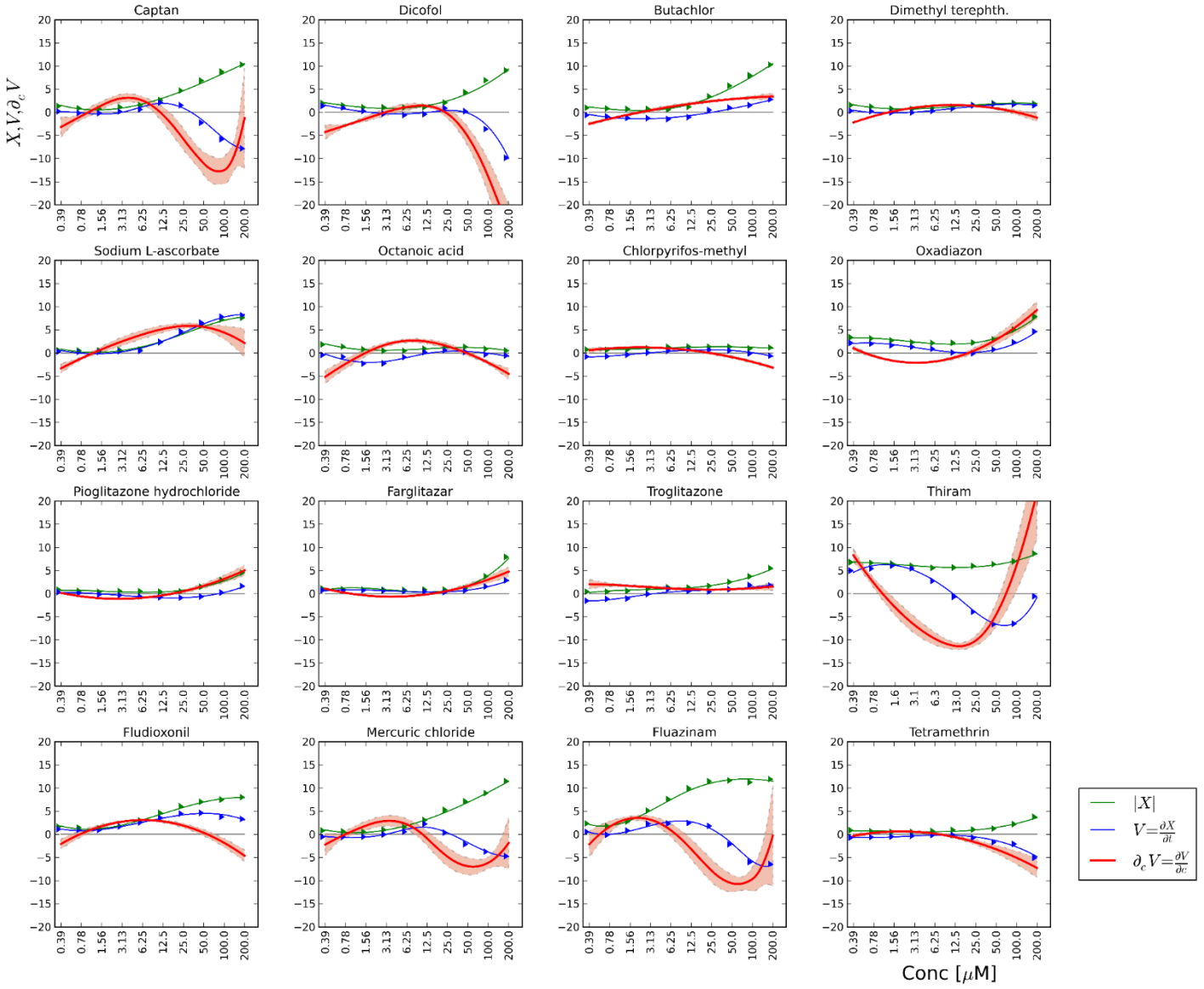
Trajectories & Tipping Points

United States Environmental Protection Agency



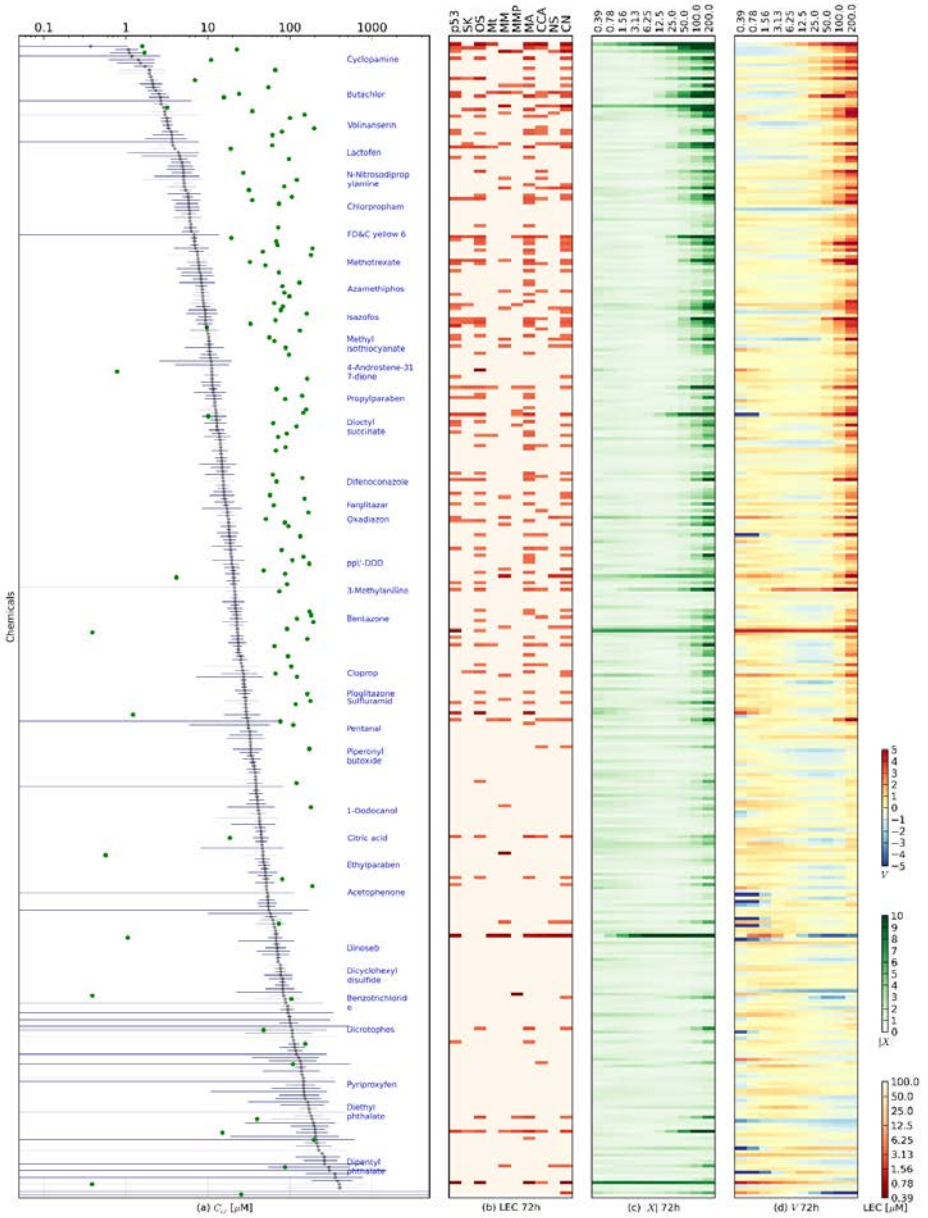






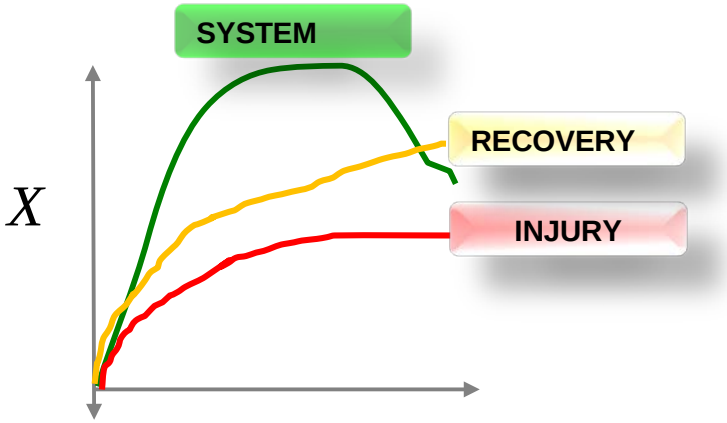
Trajectories & Tipping Points

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

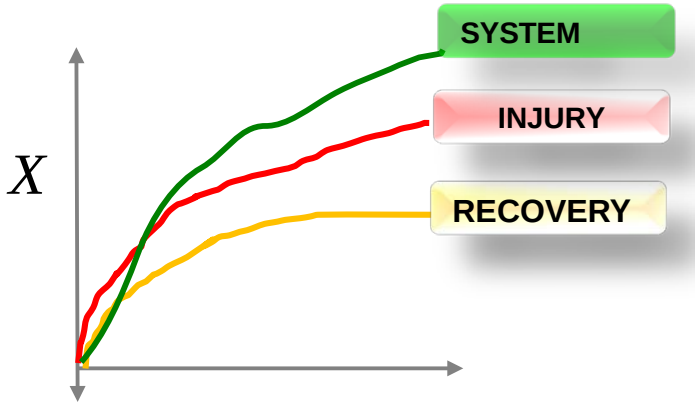


Toxicological Tipping Points

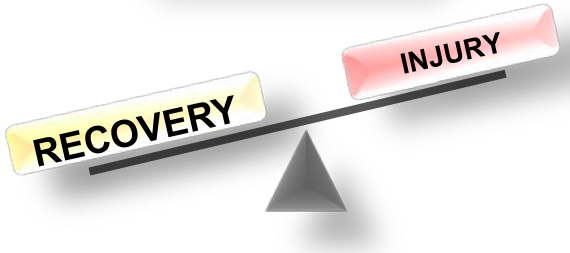
ADAPTATION



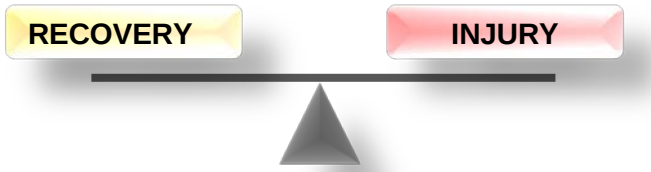
ADVERSITY



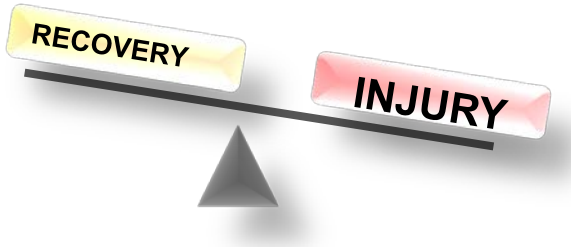
SYSTEM ADAPTATION



SYSTEM TIPPING POINT



SYSTEM ADVERSITY



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)?
- Translation
  - How do *in vitro* tipping points compare *in vivo* PoD ?