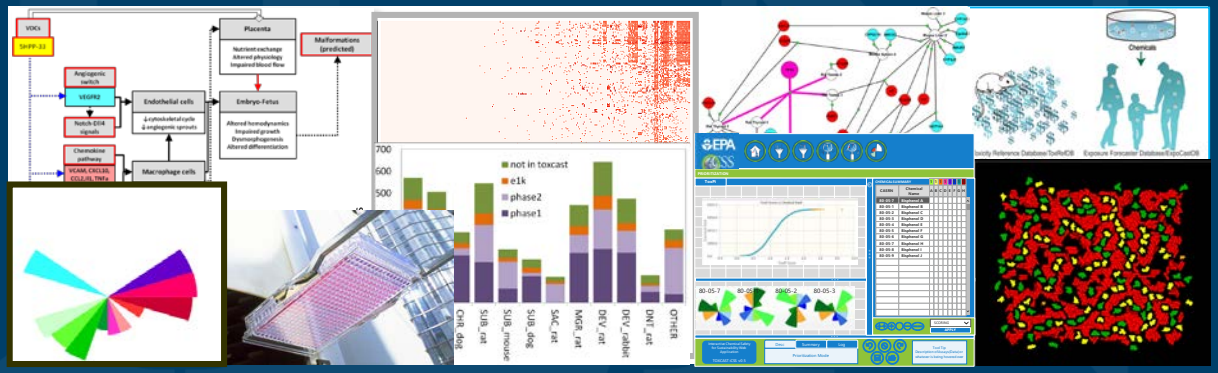
United States Environmental Protection Agency

Filling in the Gaps: The Role Functional Genomics Can Play in 21st Century Toxicology



SOT Symposium: Effectively Leveraging Cellular Functional Genomics Strategies for Elucidating Chemical Mechanisms of Action
March 13, 2018

Keith Houck
National Center for Computational Toxicology

EPA United States Environmental Protection Agency

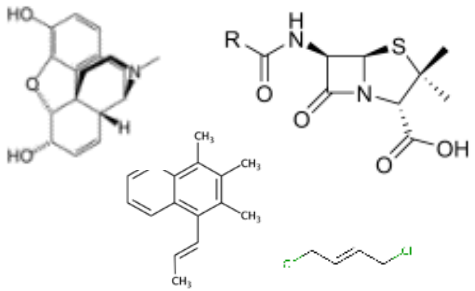
I have no conflicts of interest to disclose!

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

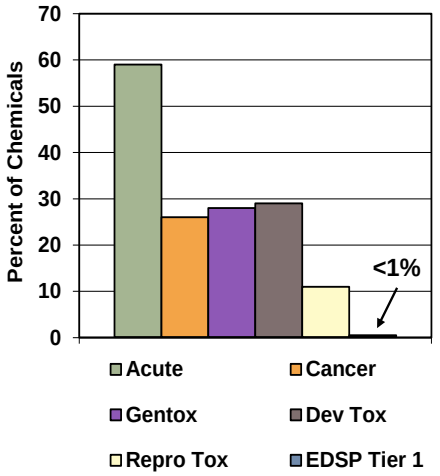
Acknowledged Need to Innovate in Toxicology

United States Environmental Protection Agency

Number of Chemicals/Combinations



Lack of Data

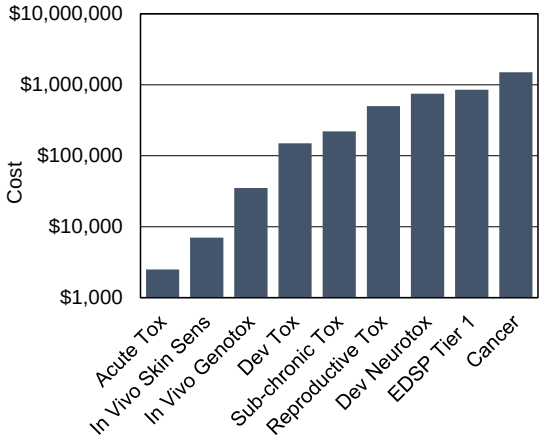


Modified from Judson *et al.*, EHP 2010

Why?

Ethical Concerns

Economics



One Approach: High-Throughput Hazard Screening

United States Environmental Protection Agency

ToxCast | **Tox21**

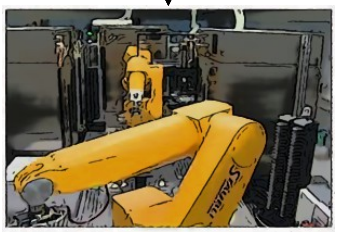
~800 cell & biochemical assays

~3,000 Chemicals

~80 Cell & biochemical assays

~8,000 Chemicals

MIE's/pathways

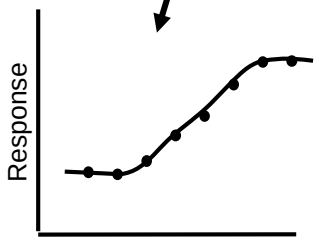


Models

Substances

Assays

Assay Results



FDA | NIEHS National Institute of Environmental Health Sciences | Tox21 | NCGC | NTP National Toxicology Program | NCATS

Challenges Encountered

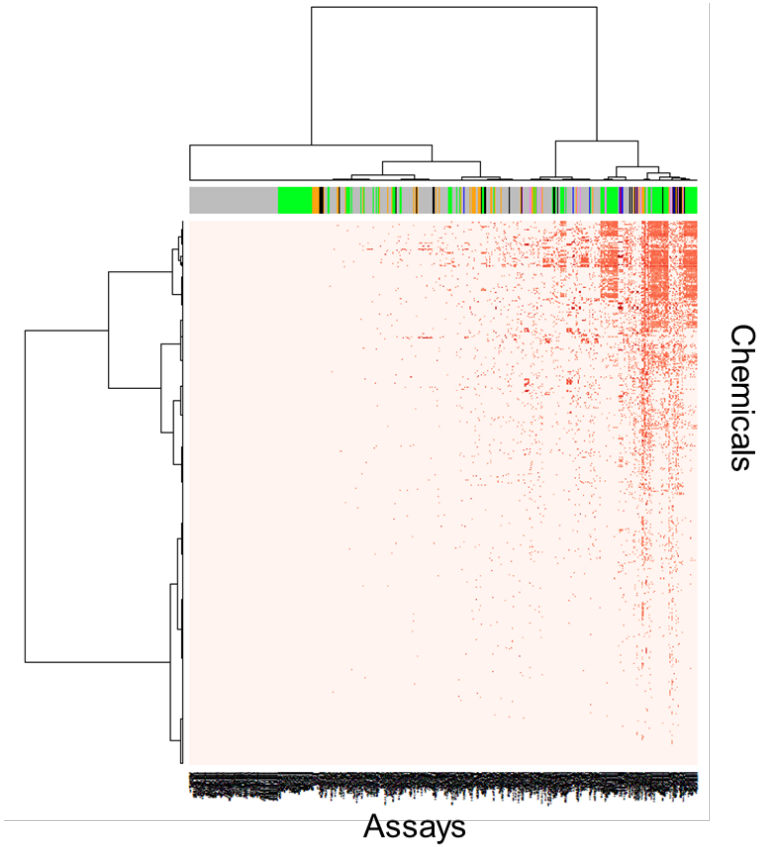
- Promiscuous chemical-target interactions
  - Which are toxicologically relevant?
  - How do we identify assay artifacts?

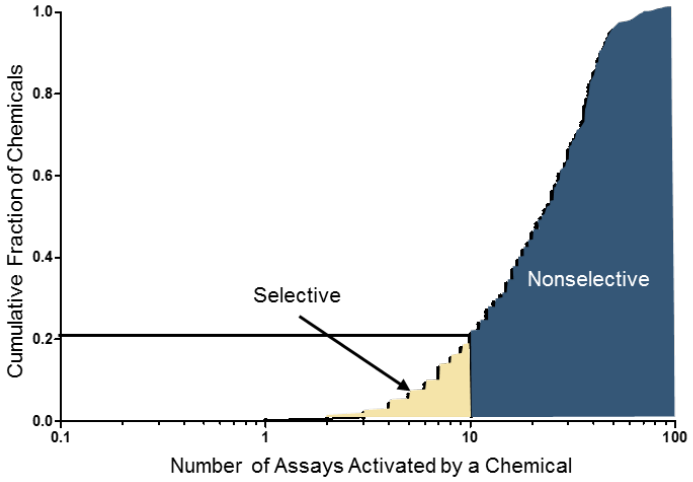
- Limited biological/toxicological diversity screened
  - Thousands of possible MIE's
  - Hundreds of biological pathways

Promiscuous Chemical Response is the Rule

United States Environmental Protection Agency

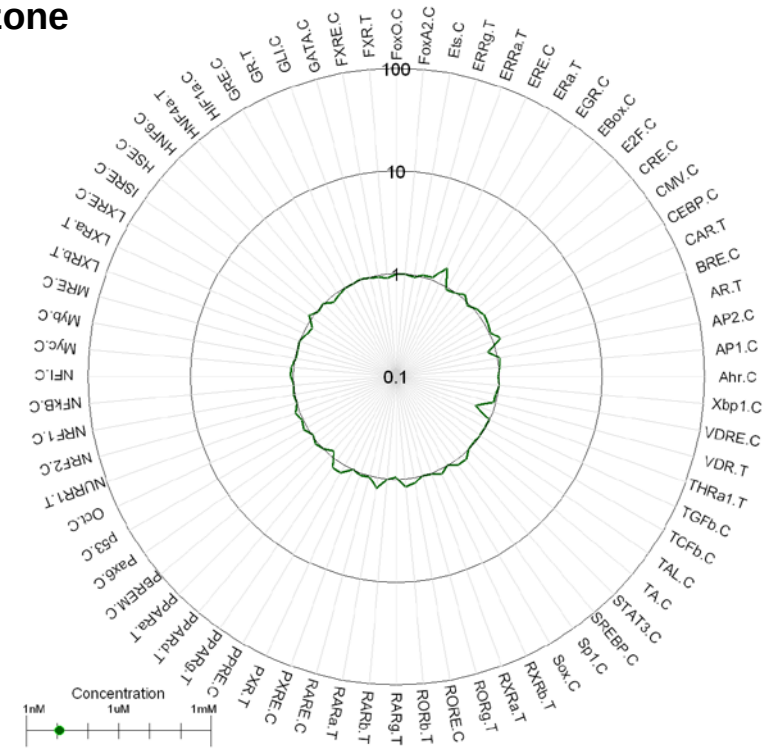
1000 chemicals/
800 assay endpoints





Thomas et al., 2013

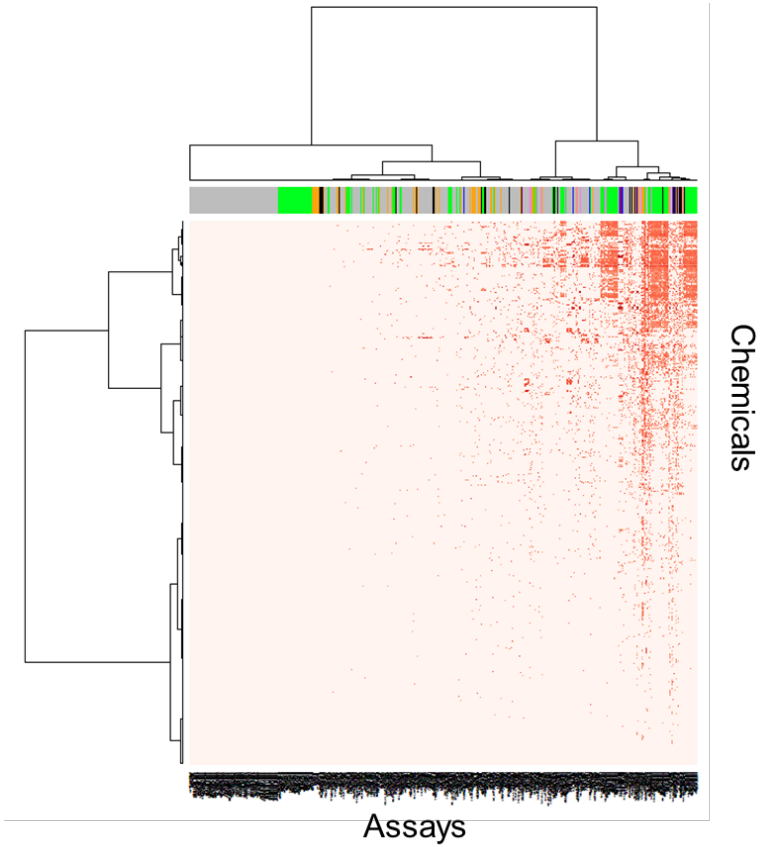
Troglitazone

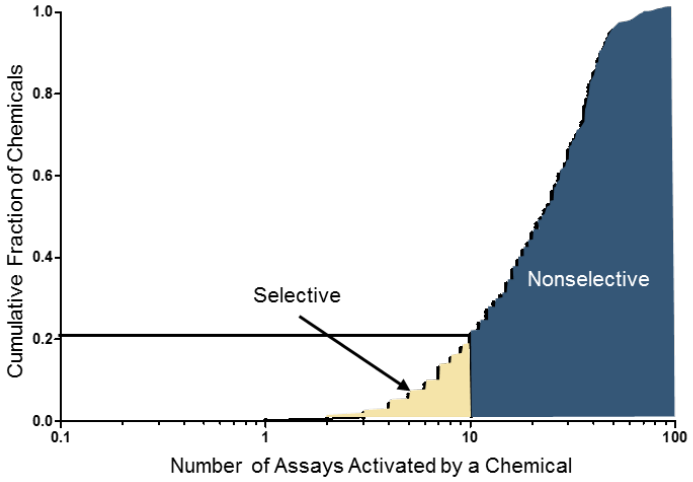


Promiscuous Chemical Response is the Rule

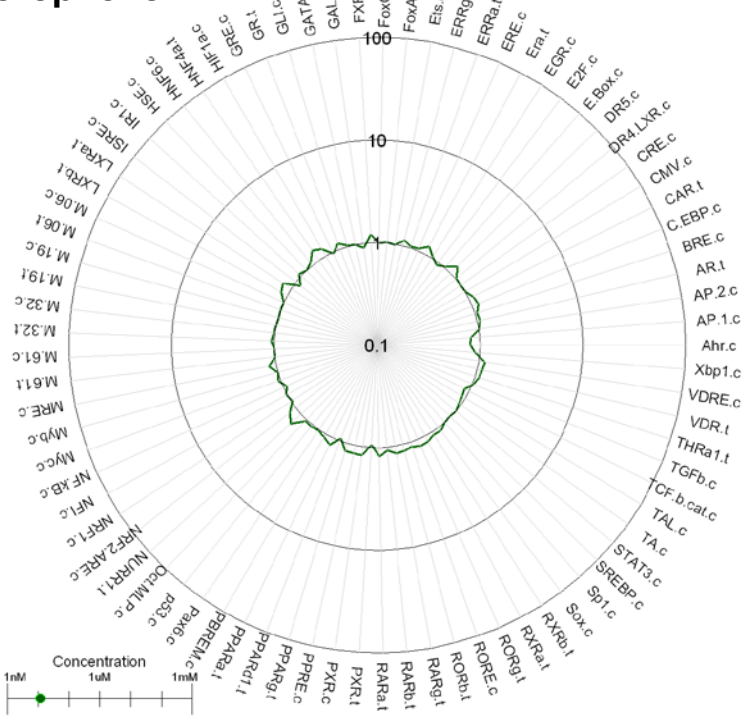
United States Environmental Protection Agency

1000 chemicals/
800 assay endpoints





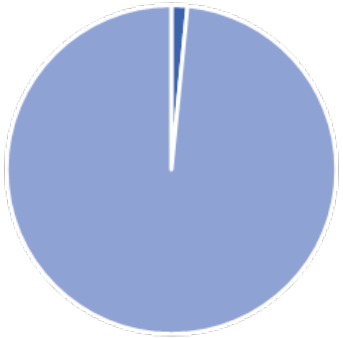
Pentachlorophenol



Beginning to Address Concerns for Increased Biological Coverage

United States Environmental Protection Agency

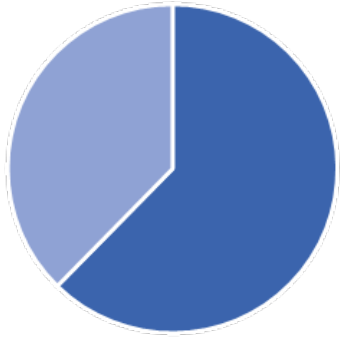
Gene Coverage



ToxCast

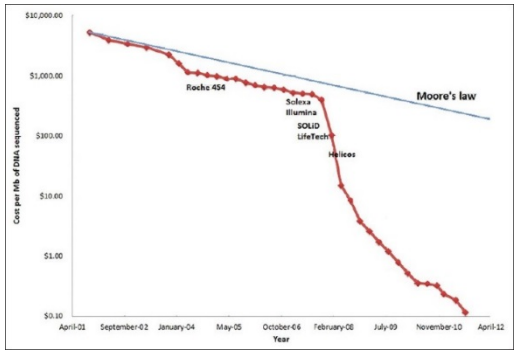
Not in ToxCast

Pathway Coverage*

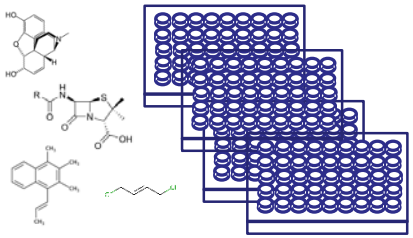


*At least one gene from pathway represented

High-throughput transcriptomics (HTTr)

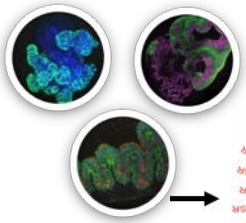


Thousands of chemicals

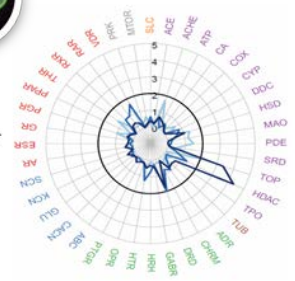


X

Multiple Cell Types



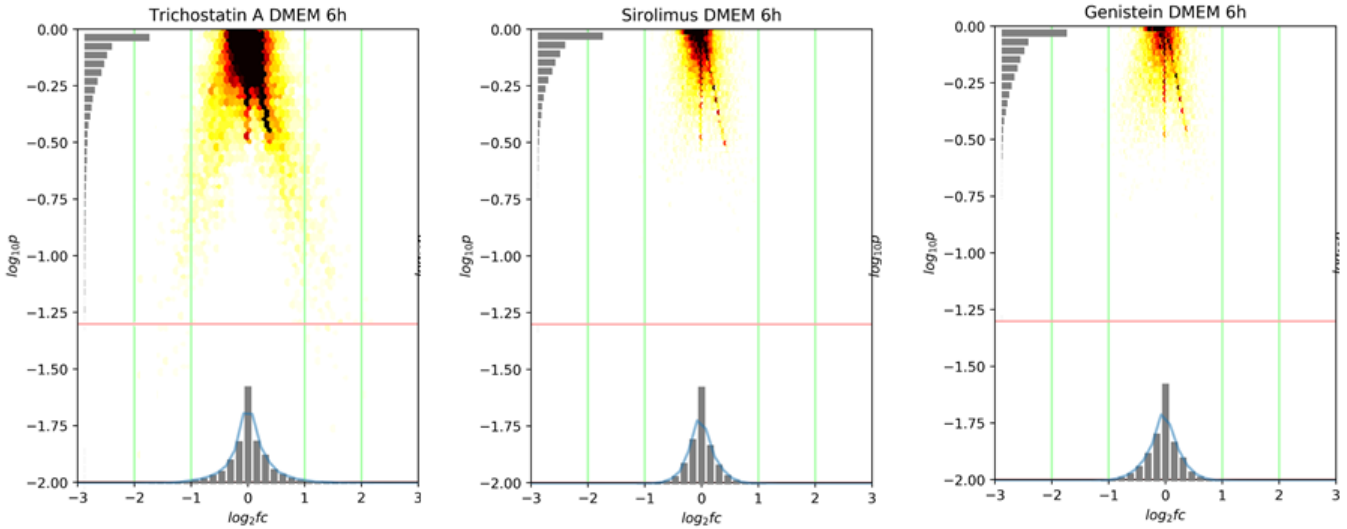
Inferred MOA/MIE

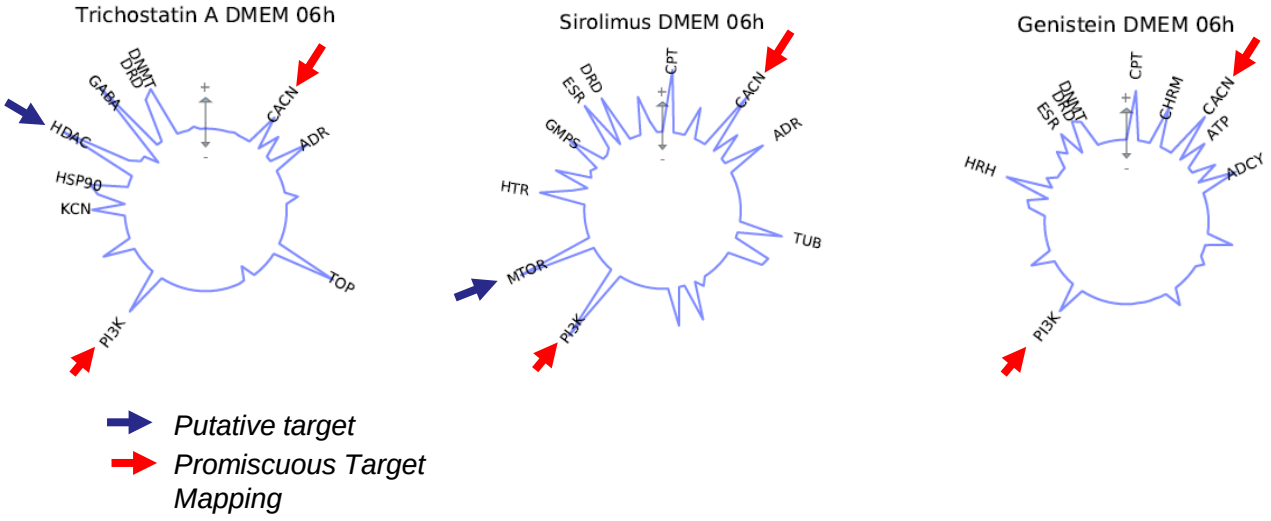


Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Connectivity Mapping (CMAP) Demonstrates Multiple Pathway Matches

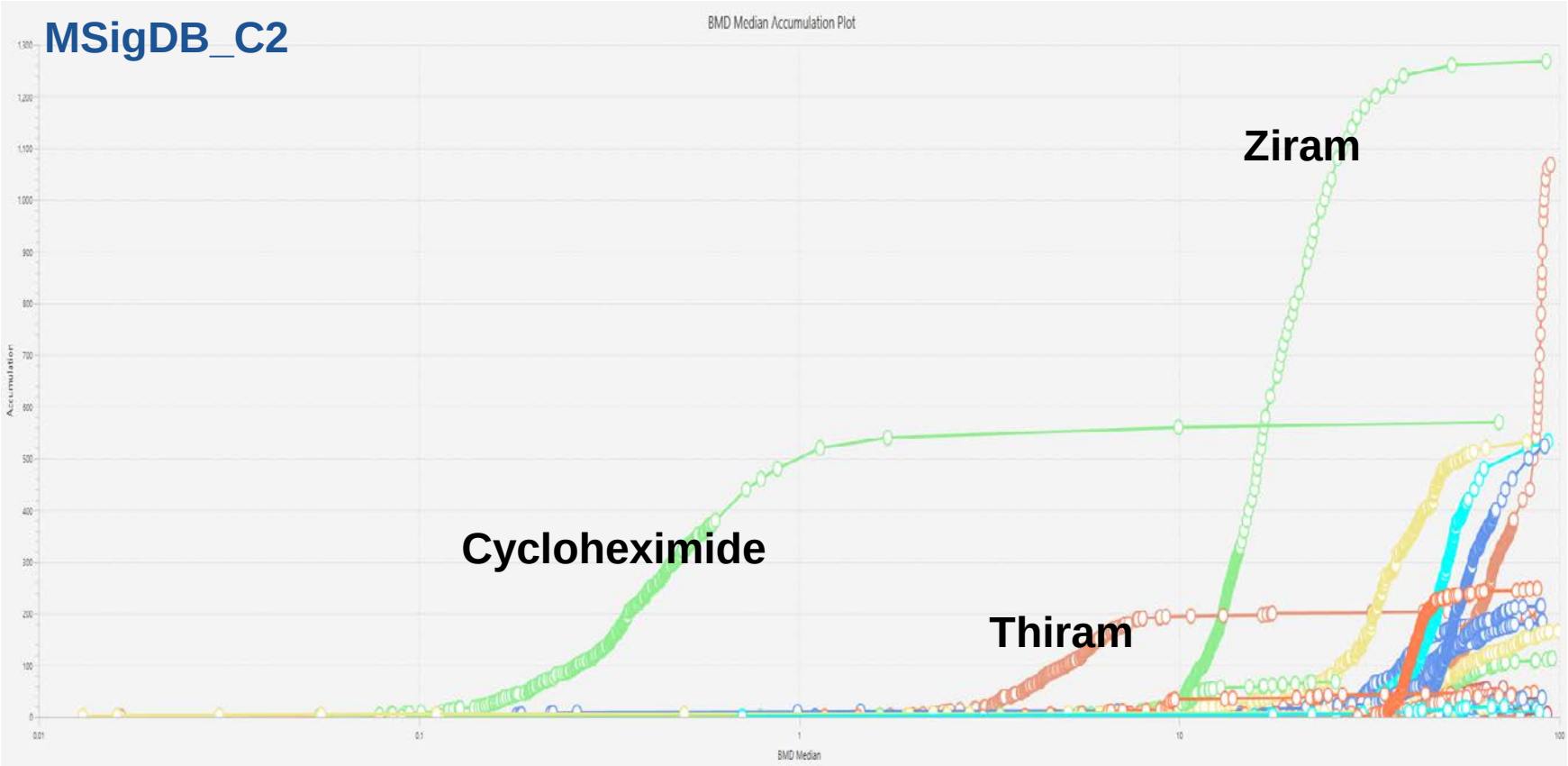




- Differential gene expression observed with reference chemicals
- Putative targets identified using Connectivity Mapping
- Large degree of promiscuity of predicted targets observed
- Currently evaluating additional methods for MIE prediction

Imran Shah, NCCT, preliminary/unpublished

Pathway Potencies by Benchmark Dose (BMD) Analysis: Similar Issues



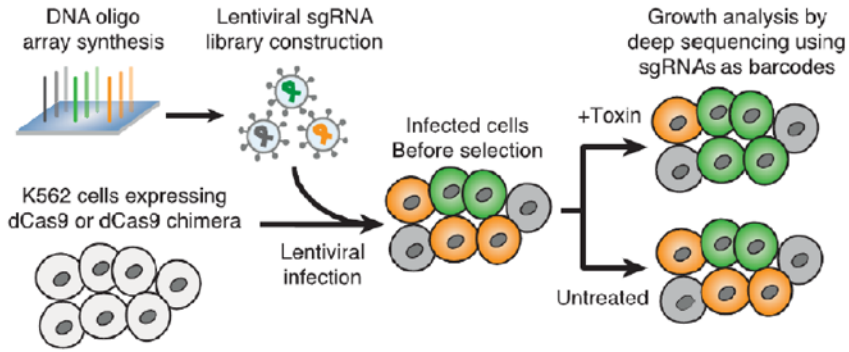
- Broad range of pathway level potency estimates and number of pathways affected across chemicals.

Josh Harrill, NCCT, preliminary/unpublished

Functional genomics: Help with Defining Mechanistic Relevancy?

- Most chemicals have apparent polypharmacology—what is the critical/relevant MOA?
  - Could use potency to define but this may not be linked to adversity
  - Transcriptomics is high content but function is generally inferred

- Functional genomics allows for bridging between genotype and phenotype
- Previously mostly used in prokaryotic systems such as *S. cerevisiae*
- Advent of CRISPR-Cas9 opens door for higher throughput applications in mammalian cells



Gilbert et al., Cell, 2014

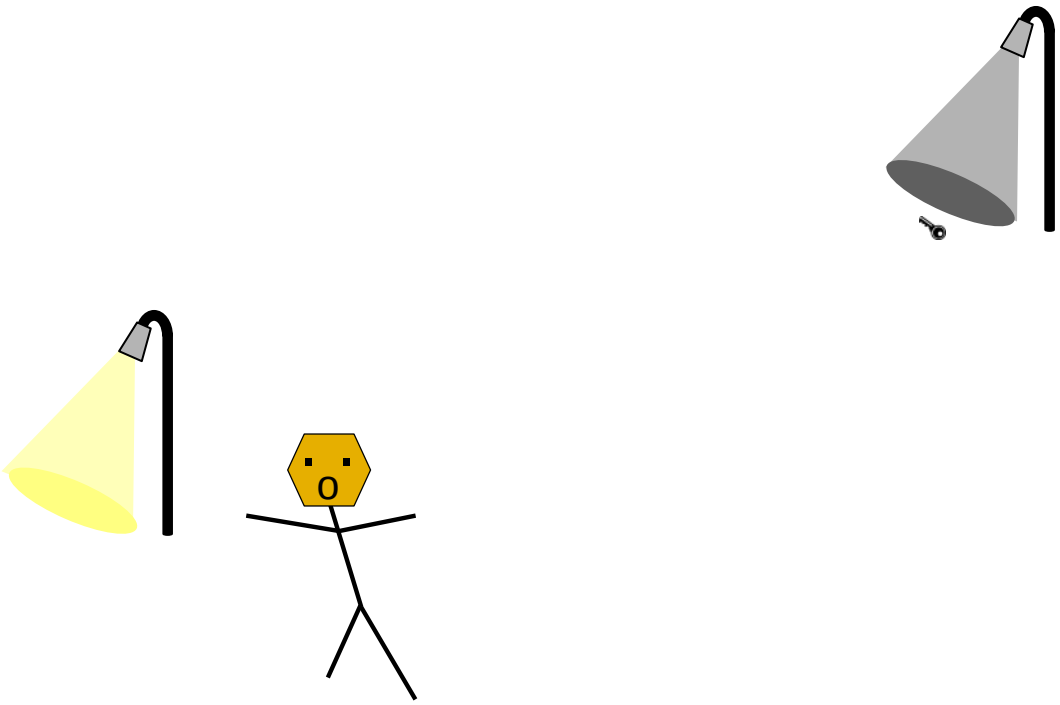
Advantages of Functional Assays

- Non-targeted approach--unbiased (*relatively*)

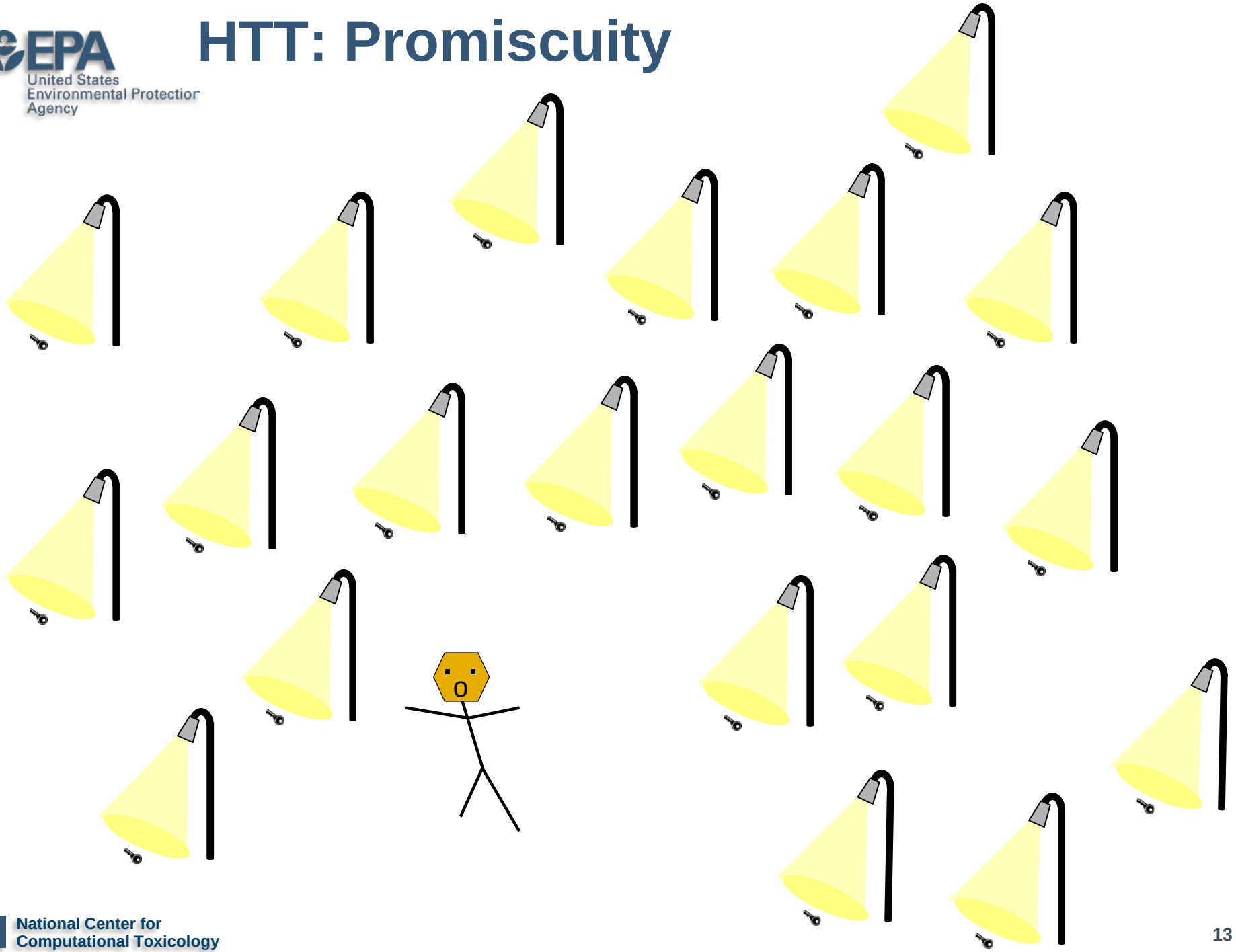
- Genomics
  - Full genome/proteome
  - Critical interactions drive functionality measured
  - Pathway mapping may be necessary (one-to-many problem)

- Chemical Proteomics
  - Full proteome
  - Direct chemical:target interaction
  - Measured in context of cell milieu
  - Identification of MIEs

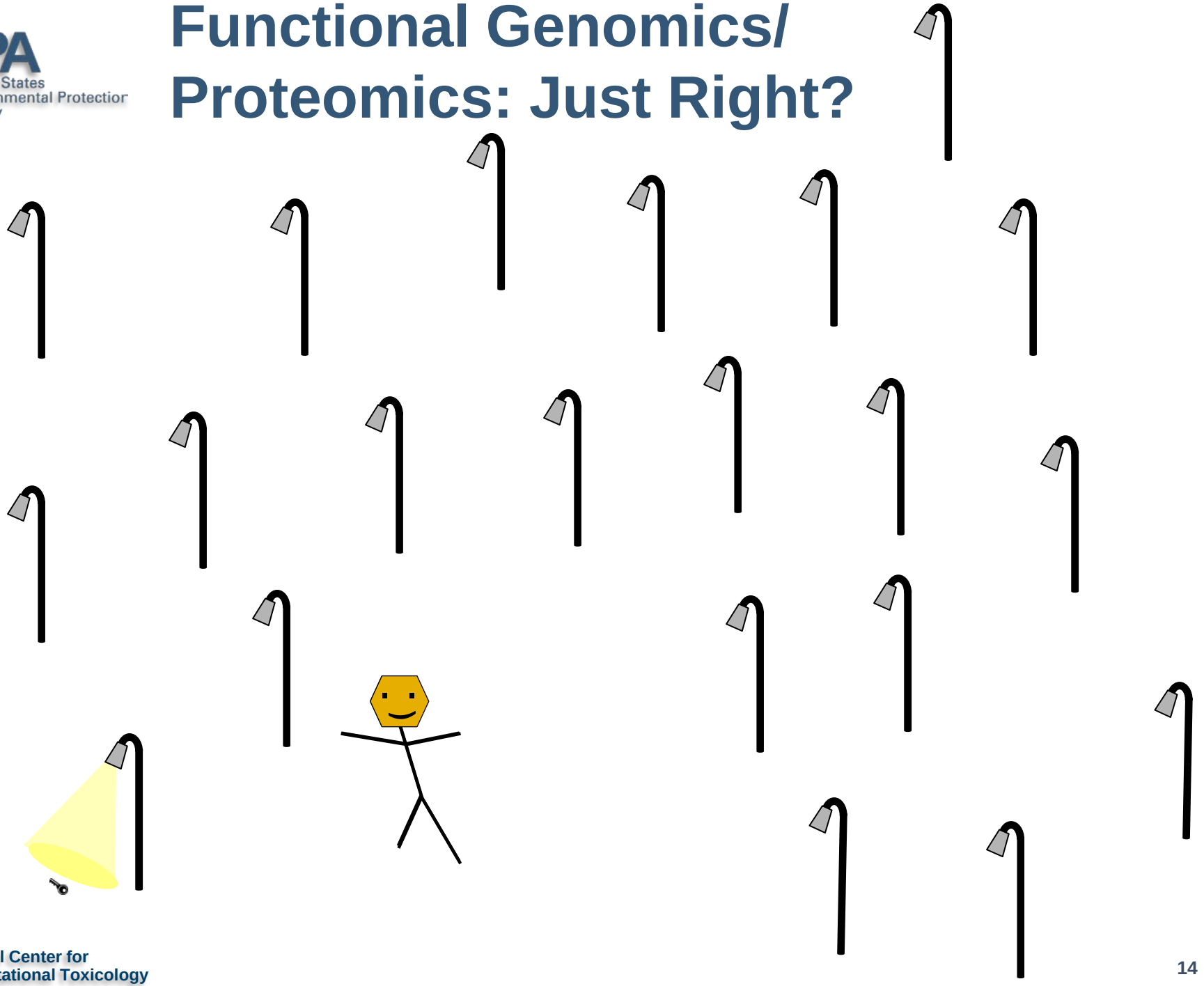
Focused Toxicology: Limited Scope



HTT: Promiscuity



Functional Genomics/ Proteomics: Just Right?



Pathway to Integration of Functional Genomics into Environmental Risk Assessment

- More proof-of-concept and validation studies with well-characterized toxicants
- Development of assay technology that facilitates expansion of functional endpoints beyond cell viability
  - *cell line engineering of pathway reporter lethality assays, sorting on reporter gene expression, sorting/single cell RNA sequencing, etc.*
  - *in vivo* assays in organisms suited to HTT such as *C. elegans* or *Danio rerio*
- Use of physiologically relevant cells and cell systems

Thank You for Your Attention and Now on to the Show!

- **The Use of CRISPR-Cas9 Technology for Validation and De-Validation of Targets from Functional Genomics** Peter Newham, AstraZeneca, Cambridge, United Kingdom.

- **CRISPR Genetic Screens on Cellular Stress Response to Proteo-Toxicants.** Quan Lu, Harvard T.H. Chan School of Public Health, Boston, MA.

- **Genome-Wide CRISPR-Cas9 Screens in Human Cell Lines Provide Novel Mechanistic Insights into Toxic Responses to Arsenic and Acetaldehyde** Amin Sobh, University of California Berkeley, Berkeley, CA.

- **Pooled Genome-Wide Screens as a Powerful Tool for Studying Drug Mechanism of Action in Cancer.** Federica Piccioni, Broad Institute of MIT and Harvard, Cambridge, MA.

- **Development of a Hybrid Chemical Proteomics Platform for Proteome-Wide Identification of Protein Targets of Environmental Chemicals.** David Ross Hall, University of Toronto, Toronto, ON, Canada.

- **Panel Discussion**