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Towards Risk-Based Environmental Monitoring and Technology Assessment – Toxicogenomics and Data Science

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Contaminants of Emerging Concern (CECs)Threat

Problem: Unknown toxicity and risks associated with large and increasing number of contaminants?

- 85,000 chemicals listed in TSCA, most lack of comprehensive toxicological and exposure data
- US EPA ToxCast/ExpoCast program is screening hundreds of chemicals

In Water…

- Current treatments not designed to effectively remove CECs
- CECs are widely-spread, present in mixtures
- Harmful effects exert at very low concentrations
- Various ,many metabolites and transformation intermediates

Challenges in establishing sufficient risk assessment framework and regulations

Need in Risk-based Technology Efficacy Assessment

Problem: Targeted/regulated chemical(s)-based treatment efficacy is not sufficient for risk-reduction/mitigation

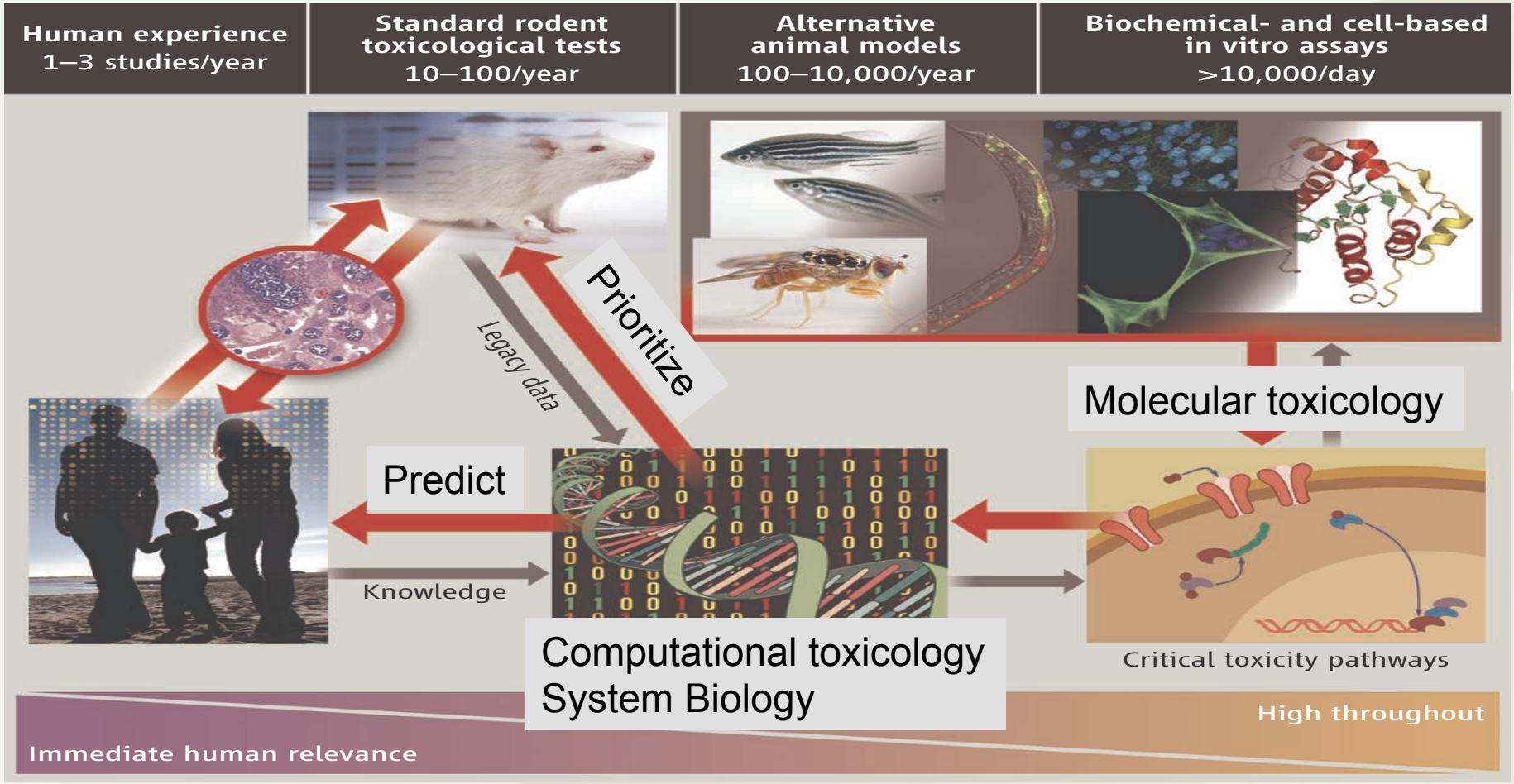
- Treatment designed for targeted pollutants may have unintended impact on water matrix

- The target-chemical-based approach does not considers the complex and broader risks that mixtures of contaminants and transformation products, pose to the environment and human health

Challenge: Lacking feasible tools for evaluating overall toxicity and risk reduction through treatment

Paradigm Shift in Toxicity Testing : Tox21

Balance between certainty and cost



Collins et al., Science (2008)

Objectives of This Study

Develop of a new toxicoomics-based toxicity assessment platform for toxicity evaluation, screening and classification of contaminants, specifically:

1. Develop methods of applying ***real time*** gene/protein expression profiling for toxicity assessment

2. Establish computation methods for quantifying toxicoomic information and determine molecular toxicity endpoints

3. Validate the methods by correlating the endpoints from the proposed methods with conventional methods

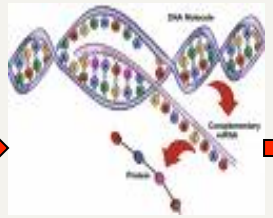
4. Demonstrate the applications of the methods for assessing, emerging contaminants and for exposure assessment (in water)

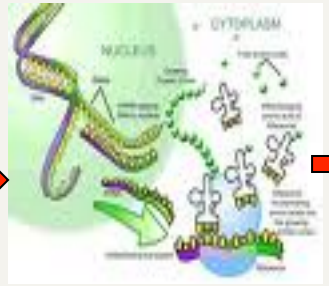
What is, and Why Toxicomics?

Molecular level effects

Cell/organism level effects

Environmental toxicant





Organism (cell)

Transcription Level response

Translation Protein synthesis

Phenotype change

Metabolic change

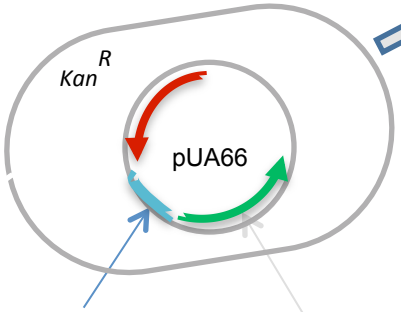
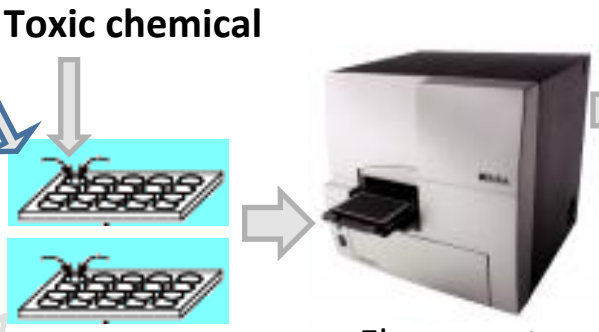
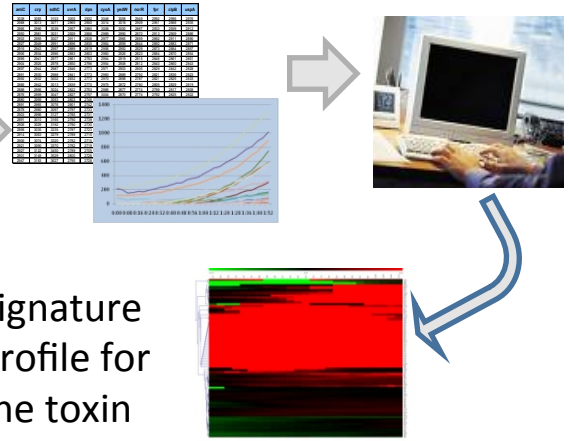
Cellular structure change

Reproductive change

Growth/death

<u>Toxicomics</u>: biological response to toxicants *<u>(sub-cytotoxic levels)</u>* involves changes at molecular level, monitor changes in gene/ protein expression patterns for toxicology assessment

Real Time Gene/protein Expression Profiling via Whole-cell-array

Cell with GFP infusion	Toxicity assessment assays on parallel reporter strains	Data generation, Gene profiling and clustering
		

gfp-transformed *E. coli. Or Yeast* strains for > x1000 genes

Chemical applied on plates, one gene in each well, expression monitored on fluorometer

Chemical-specific gene real-time gene expression profiles generated

Measure: changes in gene expression patterns in exposure to CECs compare to control with no exposure

Part I

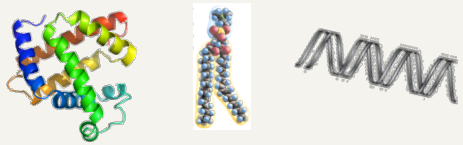
- Stress response pathway ensemble-based assay
- Can molecular disturbance/ stress response pathways be quantified and have dose-response model?

What Pathway(s) to Quantify?
Cellular Response Pathways and Toxicity

AOP- Adverse Outcome Pathway

Chemical

Macromolecular Interactions and damages



Protein Lipid DNA…

Cellular Response

*Receptor activation

*S…n

*D…

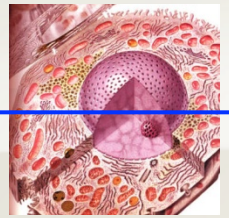
*…

and degradation

* Lipid synthesis

Stress response

Cellular Effects:
Cell stress, dysfunction, apoptosis, …



Toxicity pathways

Mode of action

Restore Homeostasis?

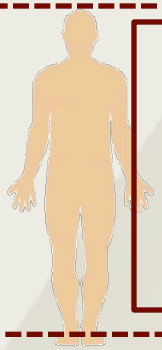
System level response

Yes → Damage repaired

No

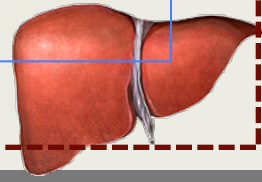
Toxicity Effects

Adverse outcome



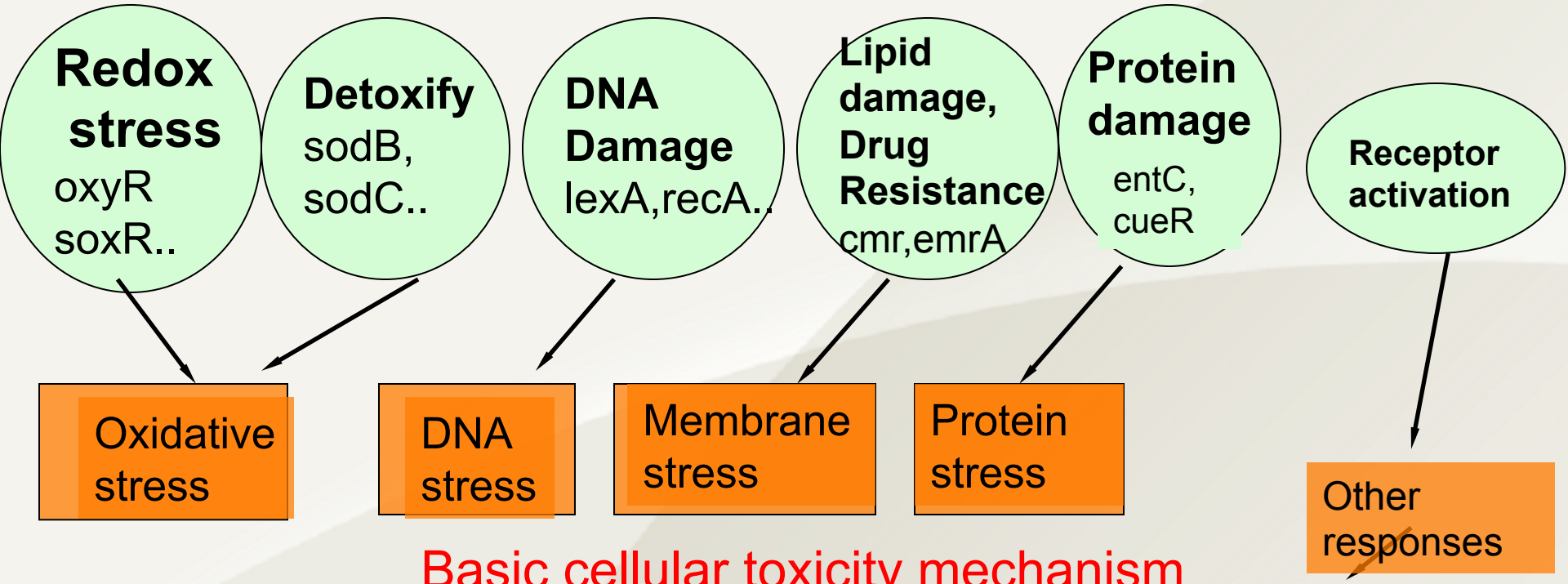
Organism/animal:
Lethality
Impaired Development
Impaired Reproduction
Cancer….

Organ response:
Disrupted homeostasis, physiology, development and function



Stress Response Pathways Ensemble Based Stress Response Library

Genes/pathways that are related to stress responses



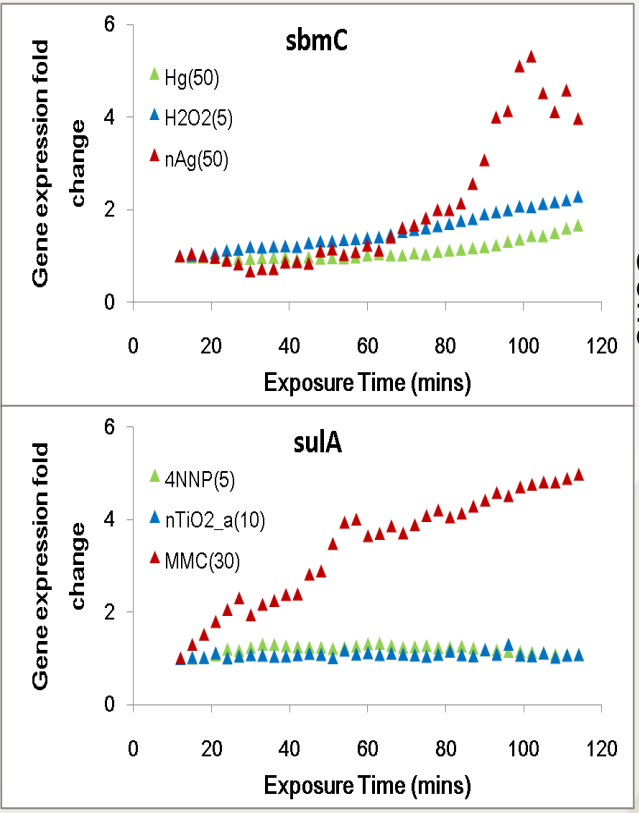
Basic cellular toxicity mechanism

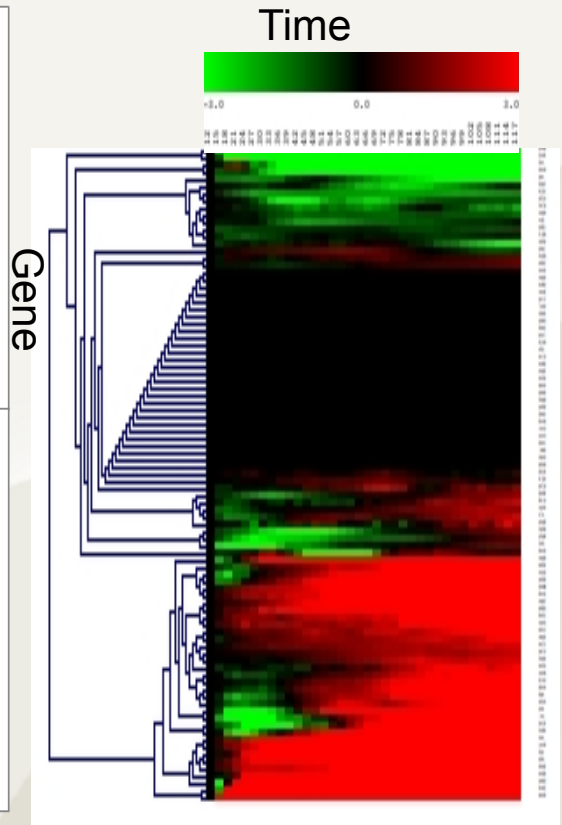
Toxic effect/response characterization

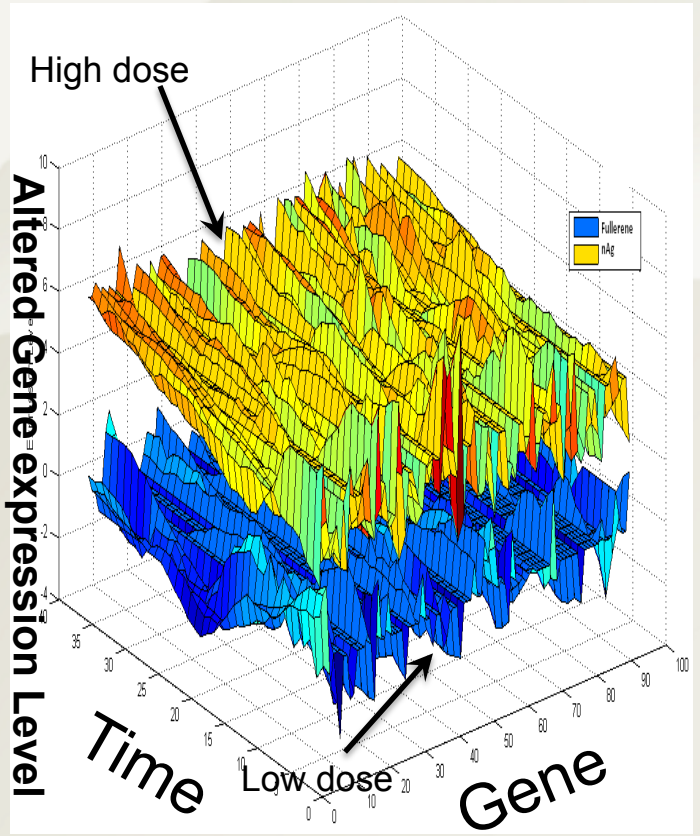
Onnis-Hayden and Gu, 2009,Gou et al.,2011,2014 ES&T., Lan et al., 2014,2015

3-D Toxic Stress Response profiling

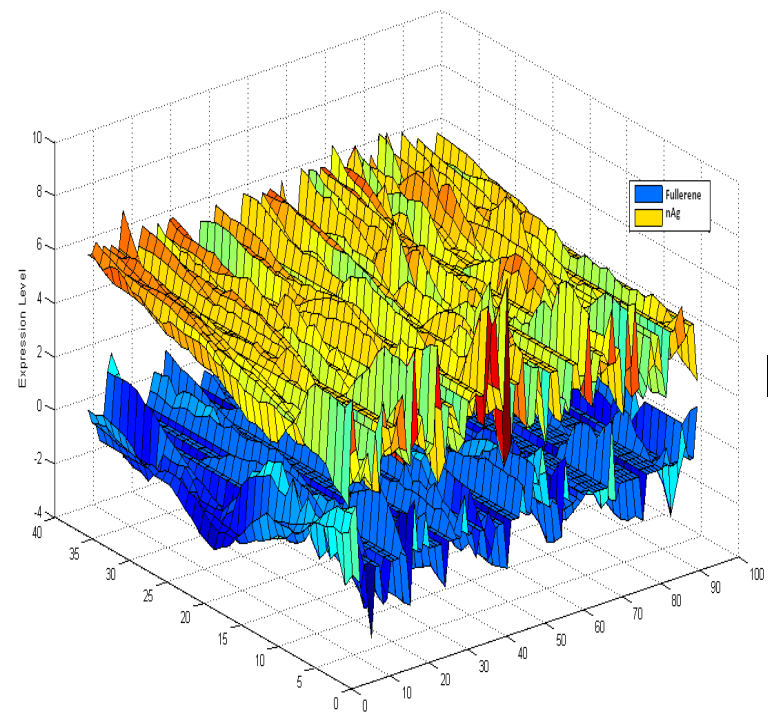
Simultaneous measurements of altered gene/protein expression patterns with temporal resolution yield 3-D toxic response pathway ensemble profiles







A New TELI Index For Quantifying Molecular Response and Pathway Activities



$$TELI_{(genei)} = \frac{\int_{t=0}^{t=2hr} (e^{|\ln(I)|} - e^{|\ln(1)|})}{ExposureTime} \quad (1)$$

$$TELI_{(total)} = \int_{gene(i=1)}^{gene(i=n)} Wi * (TELI_{genei}) \quad (2)$$

TELI –Transcriptional Effect Level Index or PELI considers 3-dimensional data that include:

-Magnitude of gene/protein response.

-Temporal pattern and cumulative effects

-Extent of cellular pathway(s) response

Gou and Gu, 2011,2014 ES&T
Lan et al., 2014,2015,ES&T

Gene Enrichment Analysis To Identify Toxicity Mechanism

Modified gene set enrichment analysis (GSEA) technique for *time series* toxicogenomics data analysis

Toxicant-induced expression profiles are *time, concentration and chemical*-dependent

1)To consider temporal patterns/effects:

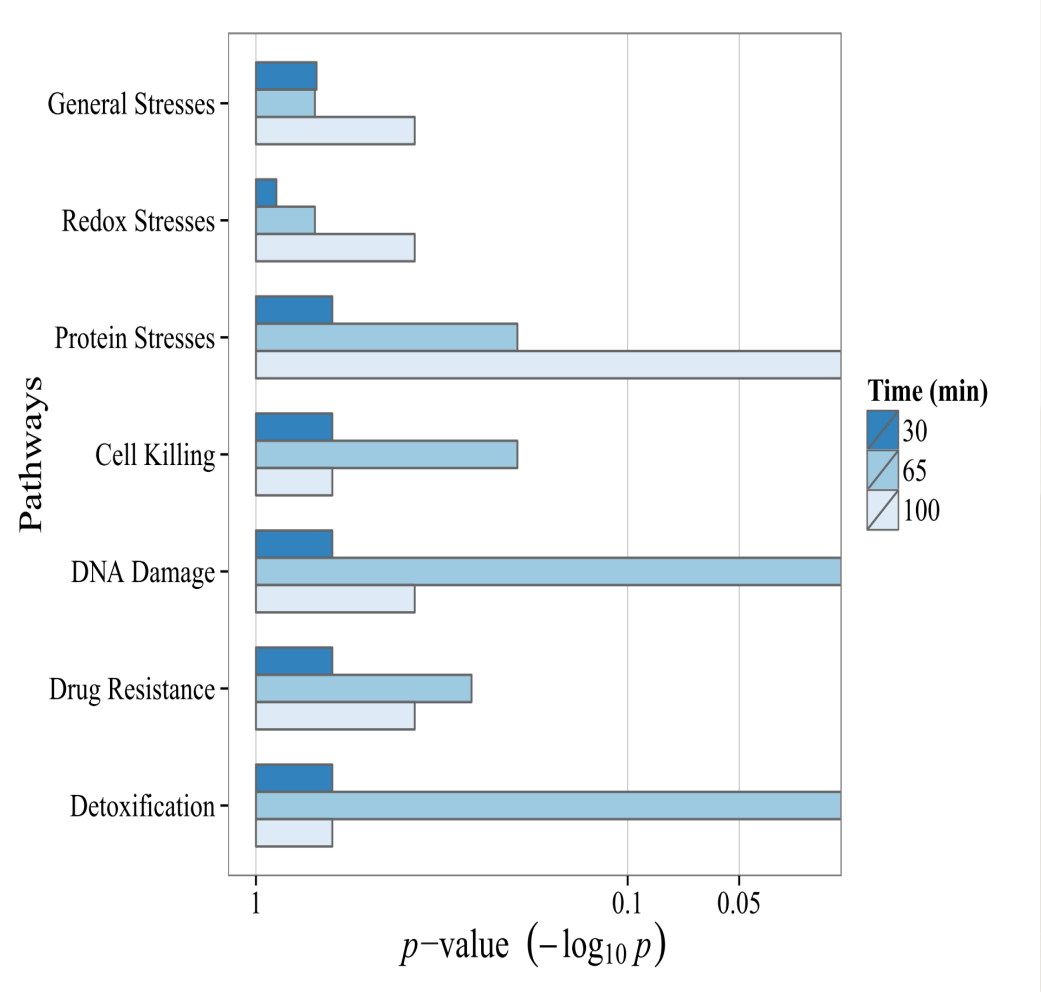
*Propose TELI index, time series modeling

2)To consider different dose concentrations:

**common principal components analysis (CPCA) with different ranking matric (Gao et al., 2015)*

Time-dependent analysis results

MMC (0.5 ng/L)-model genotoxicant



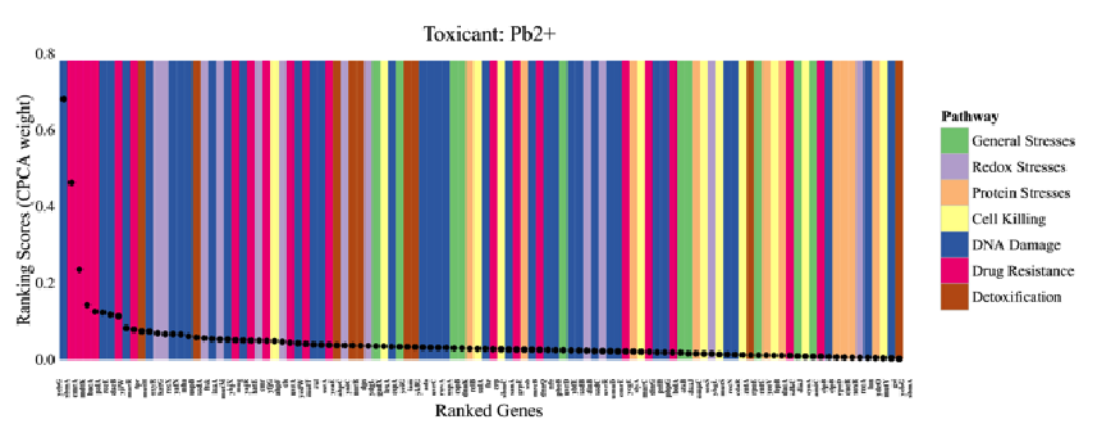
Ranked by TELI values

- Mechanism profile is dynamic, time-dependent
- Single "snap shot" at one time point may be biased
- Temporal variability is just as important as expression level changes

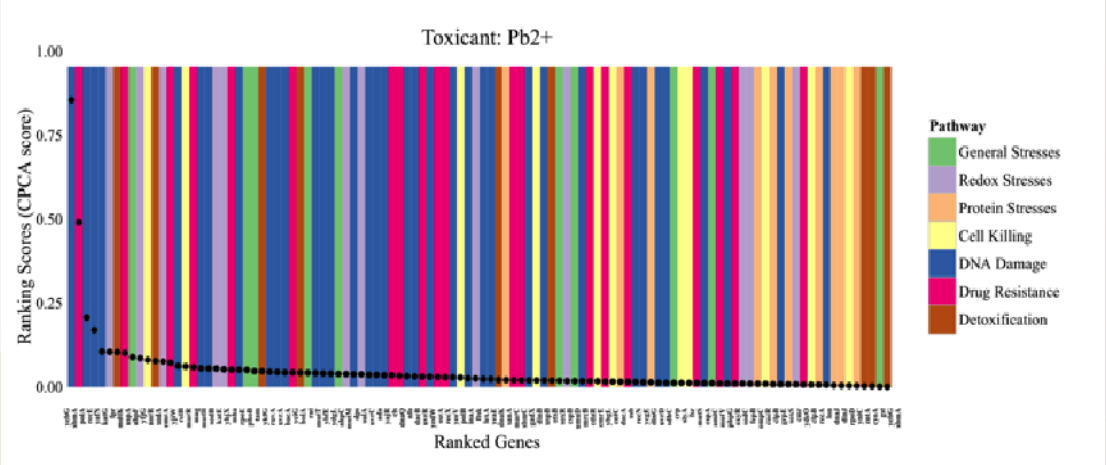
Gao et al., 2015 ES&T

Gene Enrichment Analysis – concentration effects

Pb- 0.125 µg/L



Pb- 6 different concentrations

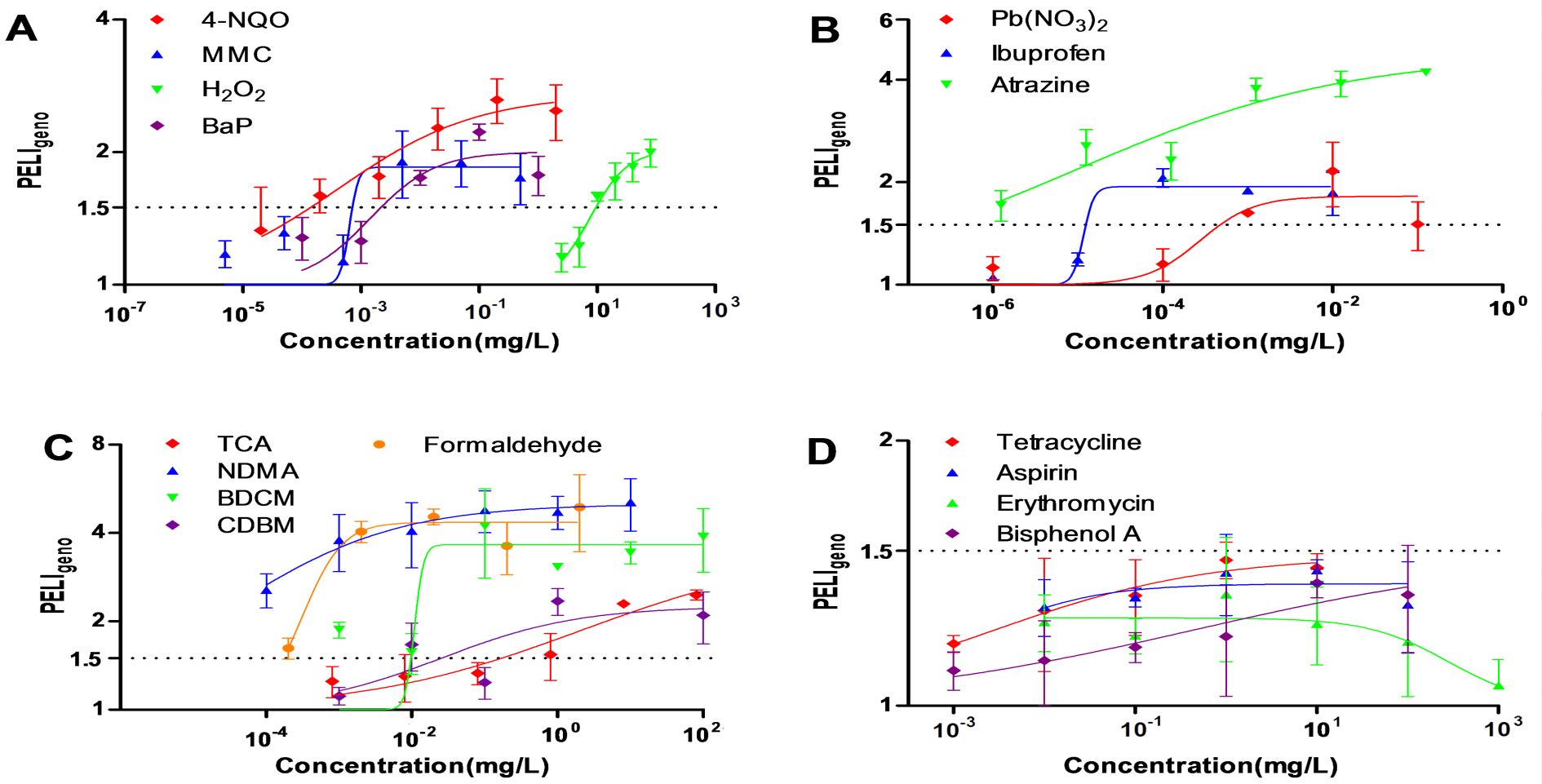


Ranked by CPCA score

- Ranking profiles vary with dose concentrations
- Single concentration result may not be comprehensive
- CPCA may reflect more "conserved" mechanism for a given chemical ?

Gao et al., 2015 ES&T

Dose-response Curves Based On New TELI/PELI



Dose-response relationship of TELI exist and they can quantify toxicity pathway response

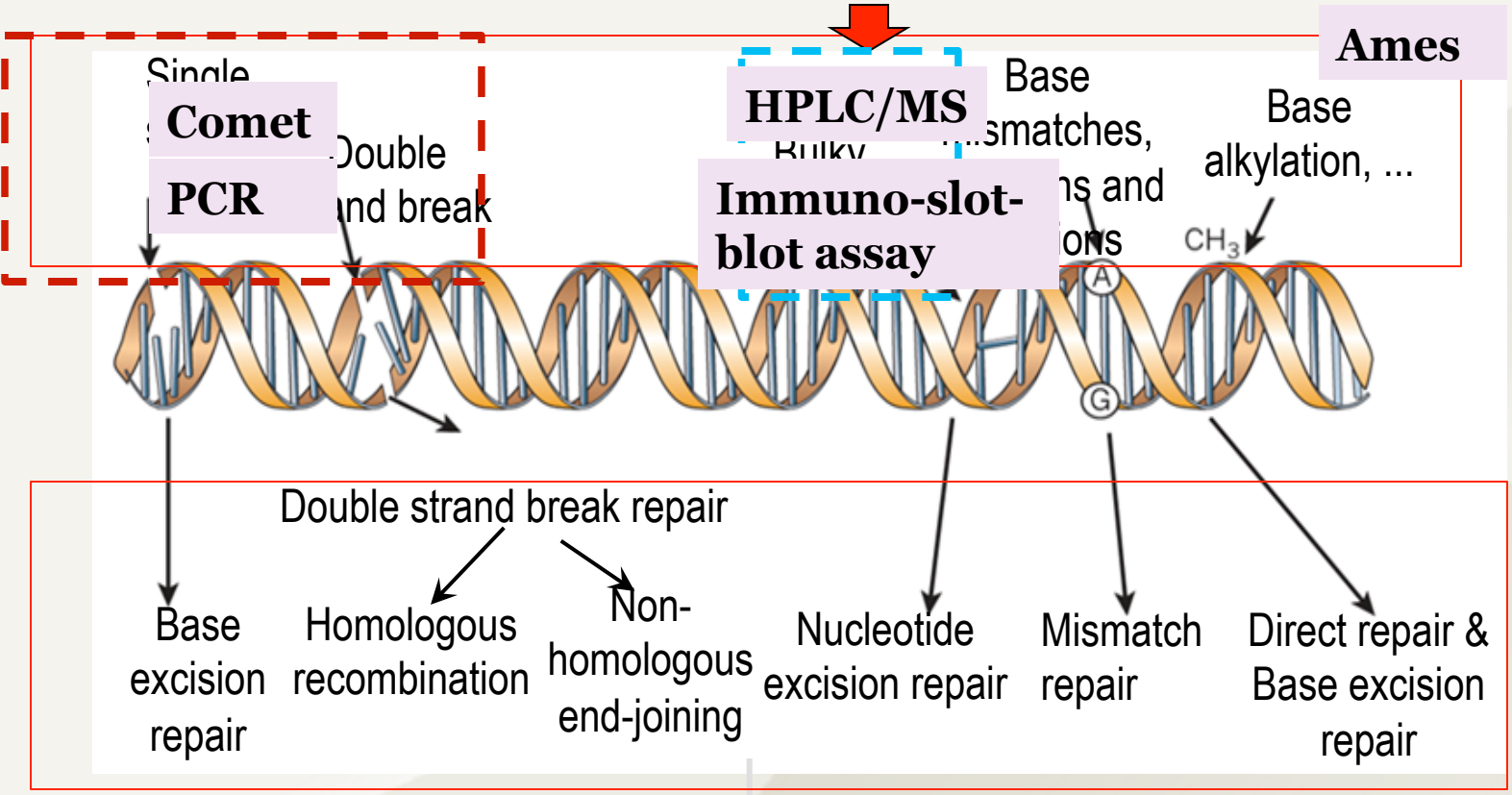
Gou at al., 2011; Lan et al., 2014. ES&T

Part II Phenotype Anchoring

- Do molecular effect-based endpoints correlate with cell/organism level phenotypic endpoints?
- DNA-damage and repair pathways-based PELI correlated with phenotypic endpoints

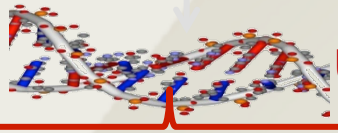
DNA Damage Related AOP

DNA damaging agent(s) Reacts with DNA



DNA damage

DNA repair



Repair failed

Unrepaired DNA

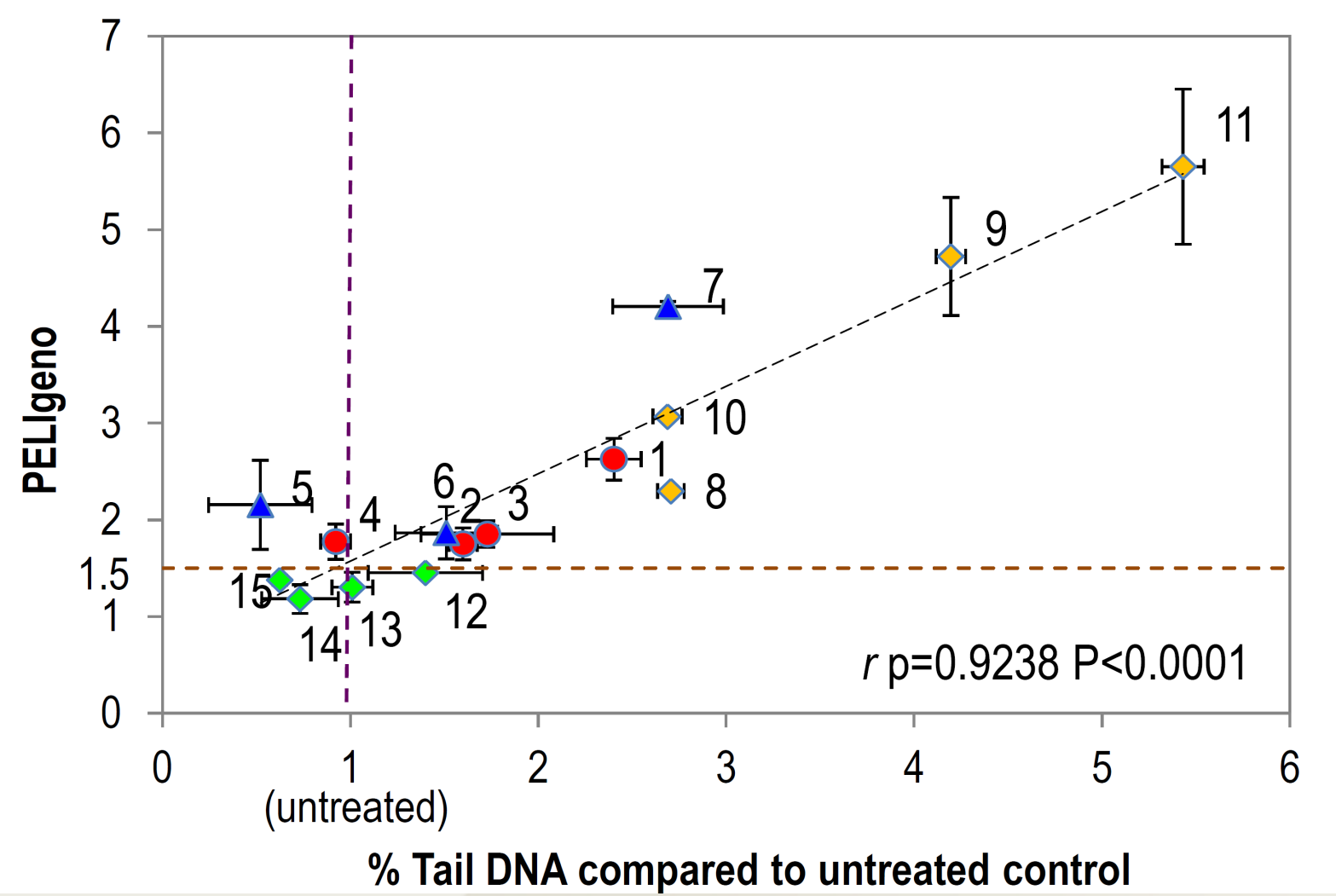
Cell killing

Mutations

Tumor formation

Lethality; Impaired Development; Cancer

Prediction of Genotoxicity



Lan et al., 2014, 2015 ES&T

Prediction of Genotoxicity

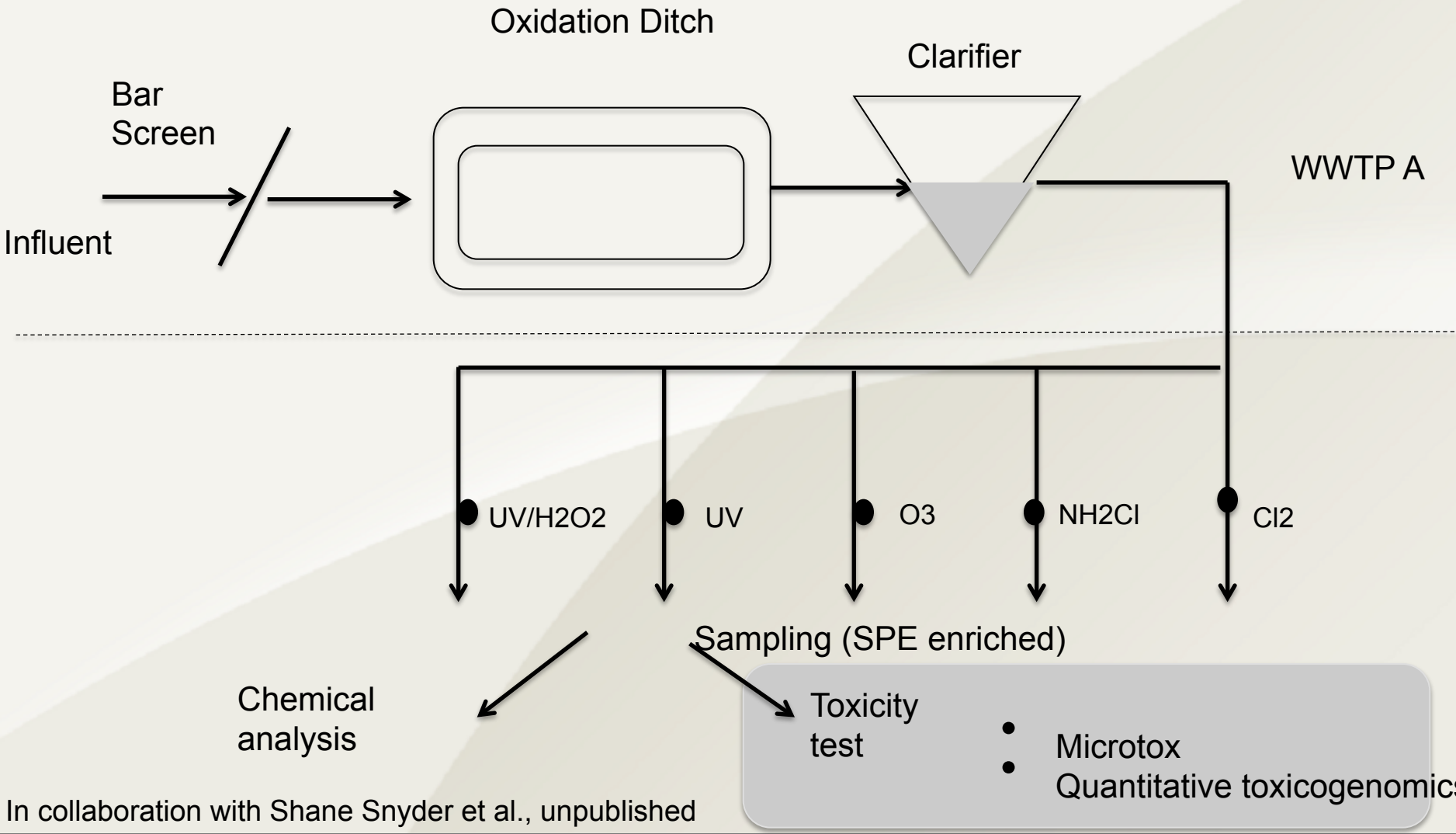
Chemical	Yeast assay in this study [a]	Genotoxicity assay			*In vivo* carcinogenesis assay [21, 57]
		Bacteria	Mammalian cells		
		Ames test [68-74]	Comet test [57, 74-81]	Micronucleus test [2, 57, 74, 80, 82-90]	
4-NQO	+	+	+	+	+
›Mitomycin C	+	+	+	+	+
H_2O_2	+	+	+	+	+
Benzo [a] pyrene (with S9)	+	+ (with S9)	+	+	+
Lead (II) nitrate	+	+	+	+	+
Ibuprofen	+	-	+	+	-
Atrazine	+	-	+	+	+
Trichloroacetic acid	+	-	+	-	+
N-nitrosodimethylamine (NDMA)	+	+	+	+	+
Bromodichloromethane	+	+	-	-	+
Chlorodibromomethane	+	+	-	-	+
Formaldehyde	+	+	+	+	+
Tetracycline hydrochloride	-	-	+	-	-
Aspirin	-	-	+	-	-
Erythromycin	-	-	+	+	-
Bisphenol A	-	-	+	-	-

Lan et al., 2014, 2015 ES&T

Part III Application for Water Quality Monitoring

* Application for Water Toxicity
* Technology Assessment
* Water Quality Monitoring

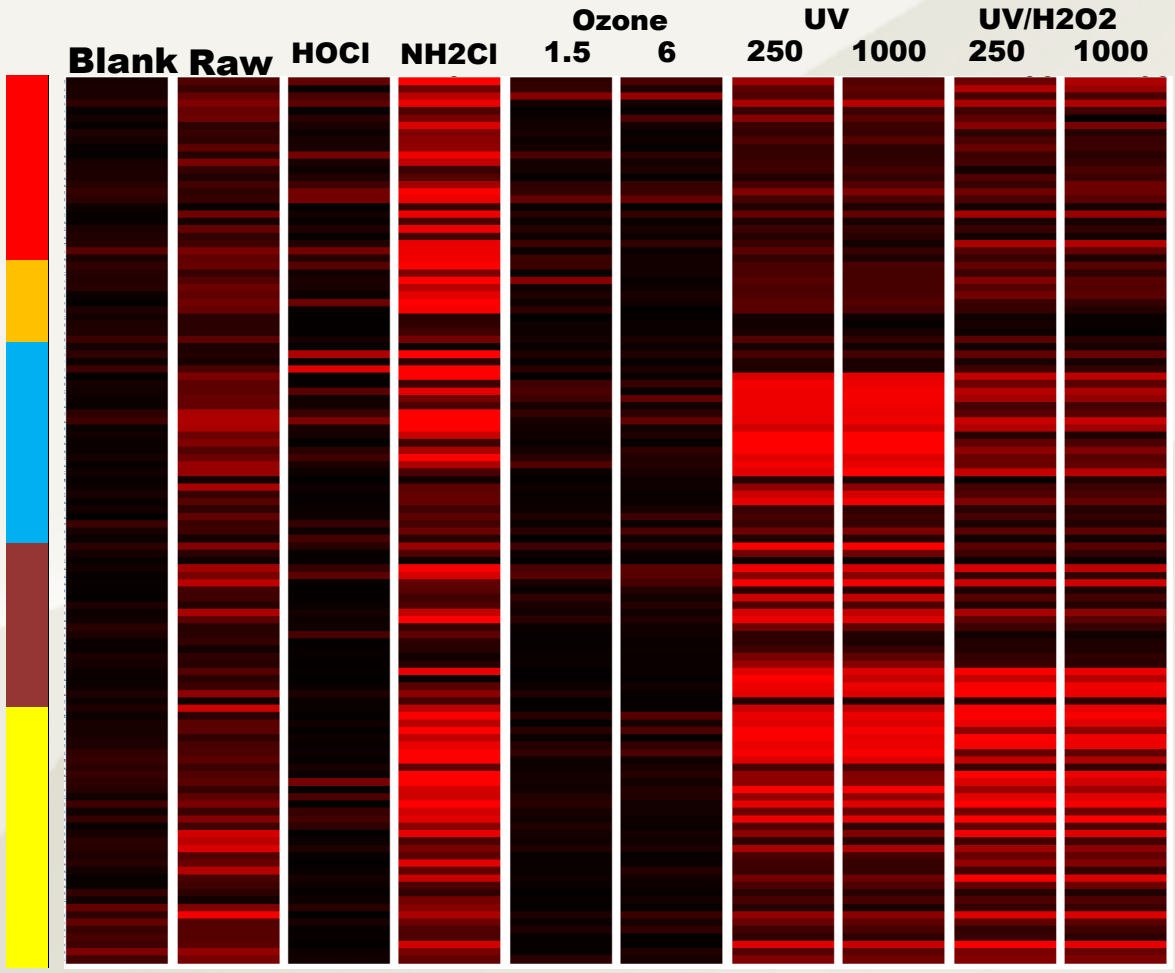
Approach overview – Pilot WWTP with parallel disinfection and oxidation treatments



In collaboration with Shane Snyder et al., unpublished

Technology Efficacy Assessment

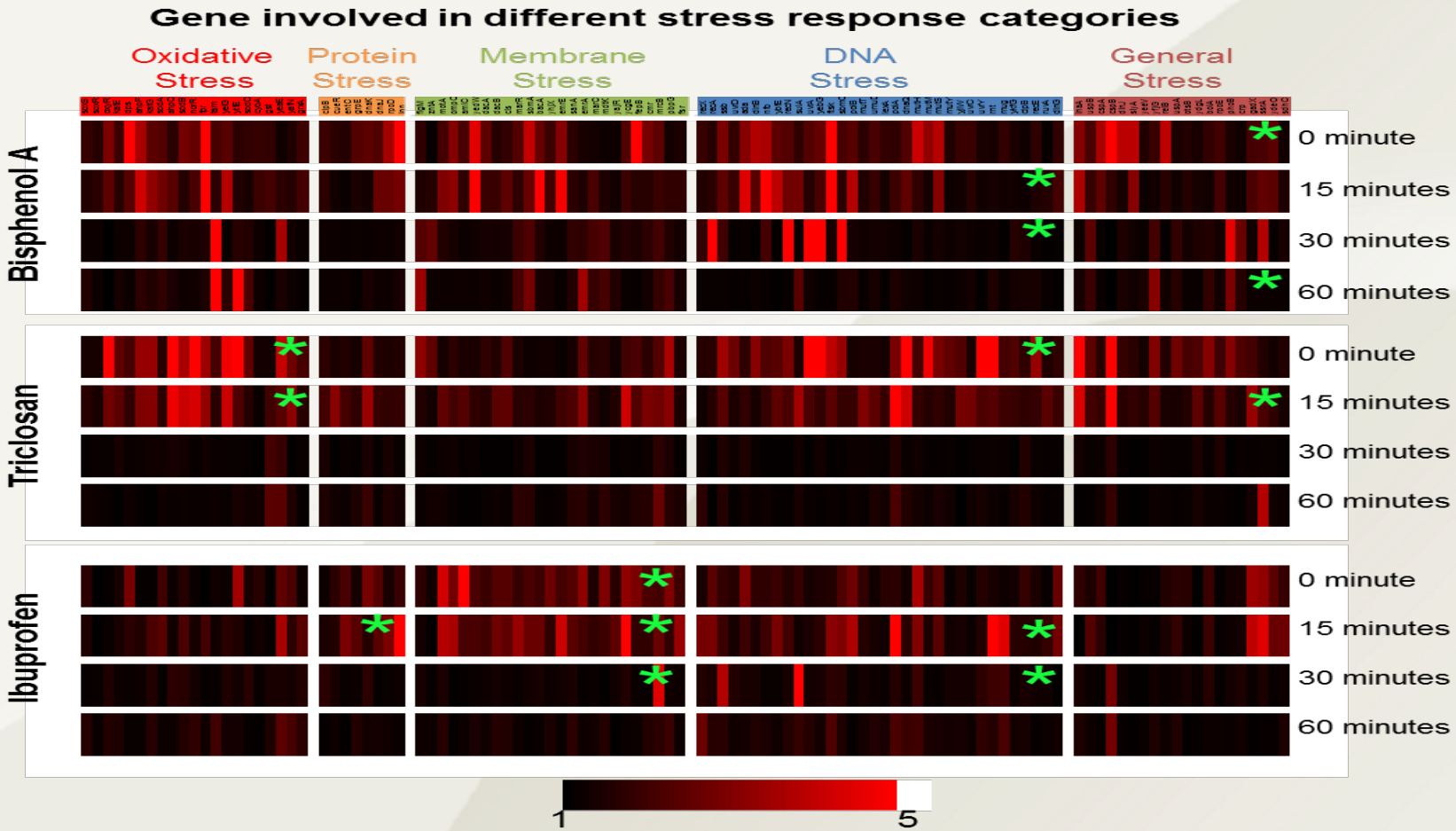
- Technology-dependent effluent toxicity profiles
- Certain process seemed to generate toxic products
- Treatment parameters affect efficacy



In collaboration with Shane Snyder et al., unpublished

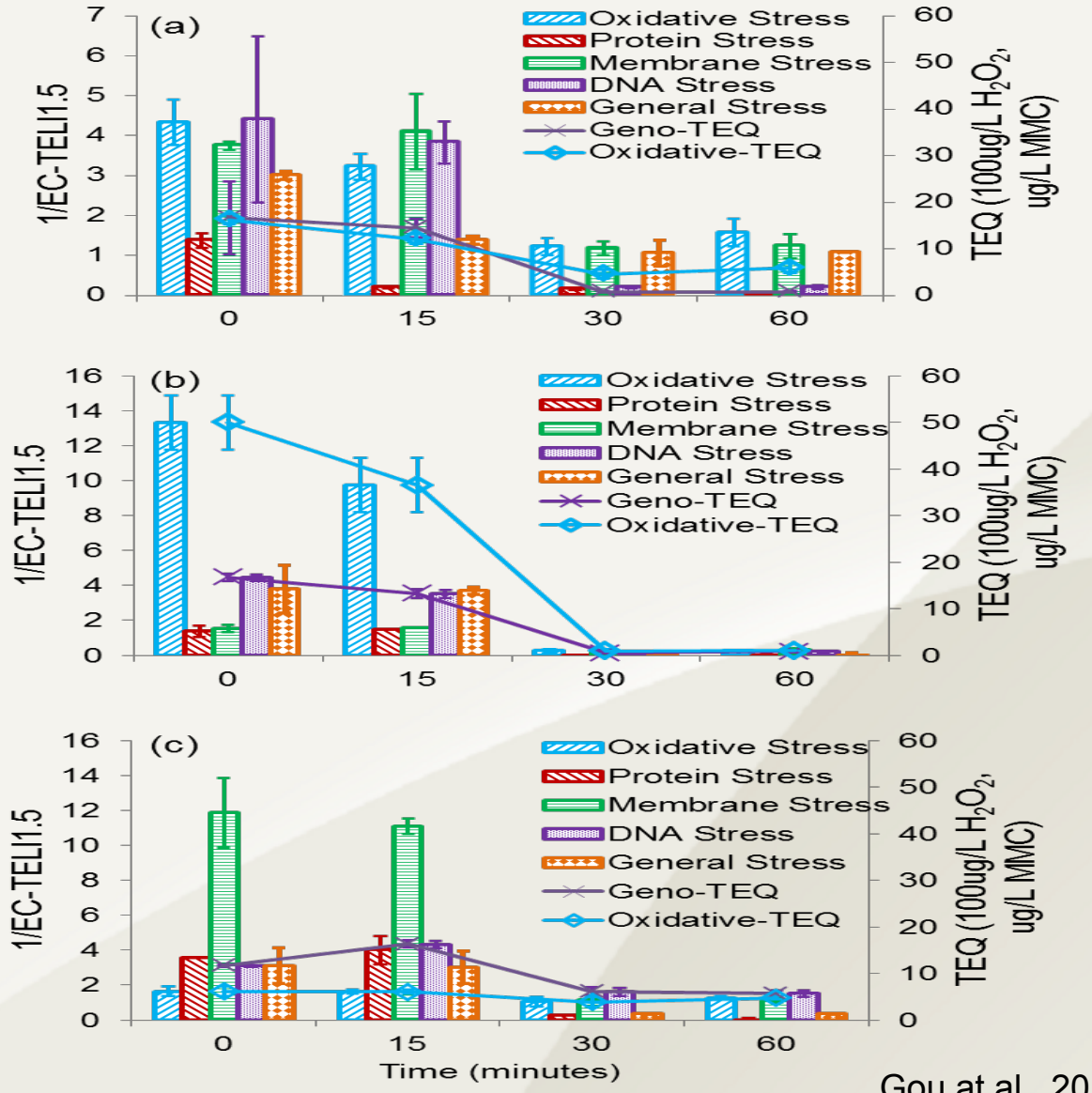
Toxicity Evolution During CEC Degradation

--Advanced Oxidation (Electro-Fenton) Process for CEC degradation
---Treatment efficacy based on temporal toxicity level and profiles



Gou at al., 2014, ES&T, Yuan et al., 2013 Chemosphere

Toxicity Evolution During CEC Degradation



--Advanced Oxidation (Electro-Fenton) Process for CEC degradation

---Treatment efficacy based on temporal toxicity level and profiles

-- Identify causal intermediates

- Optimize treatment strategy and condition

Gou at al., 2014, ES&T, Yuan et al., 2013 Chemosphere

Case Study- Dan River Spill

- The third-largest coal ash spill of U.S. occurred at Eden, N.C on Feb 2nd, 2014

- ~39,000 tons of coal ash and 27 million gallons of wastewater spilled into Dan River

In collaboration with Madeline E. Schreiber (VT), Brian Williams (DRBA) (unpublished)

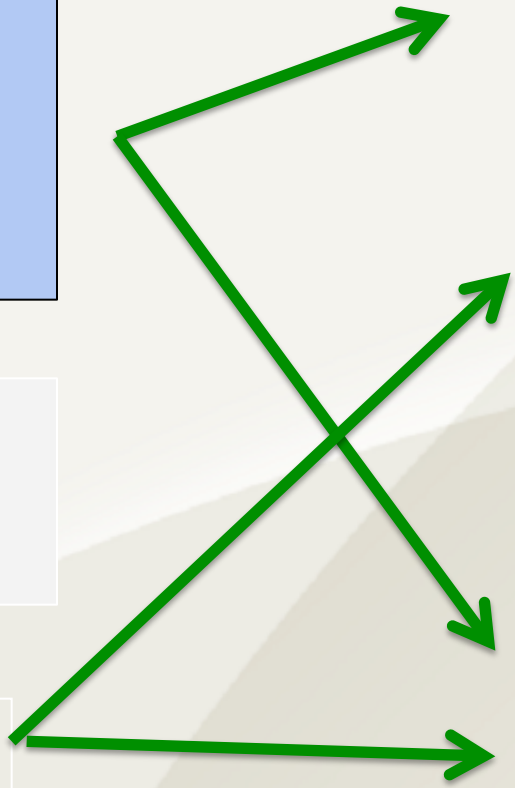
A. Dennis Lemly , Environmental Pollution 197 (2015) 55-61

Methods—Overview

15 surface water samples

14 sediment samples

leachate



Chemical Analysis

Toxicity Assessment

Total organic carbon (TOC)

Dissolved organic matter (DOM-EEM)

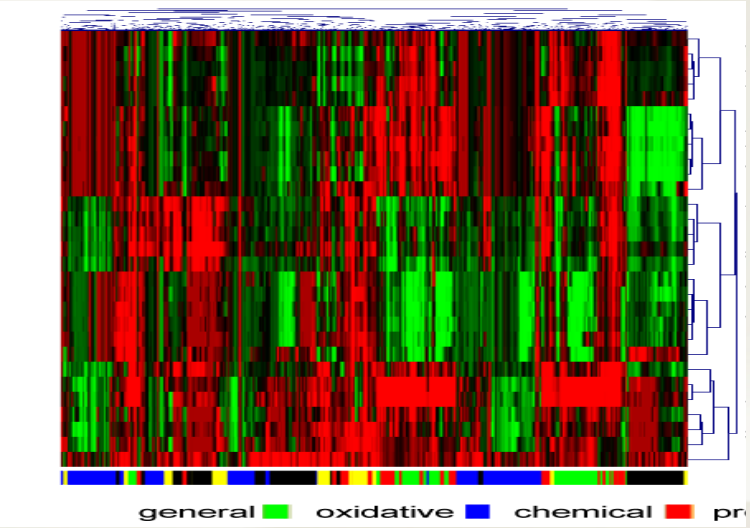
Trace elements (ICP-MS)

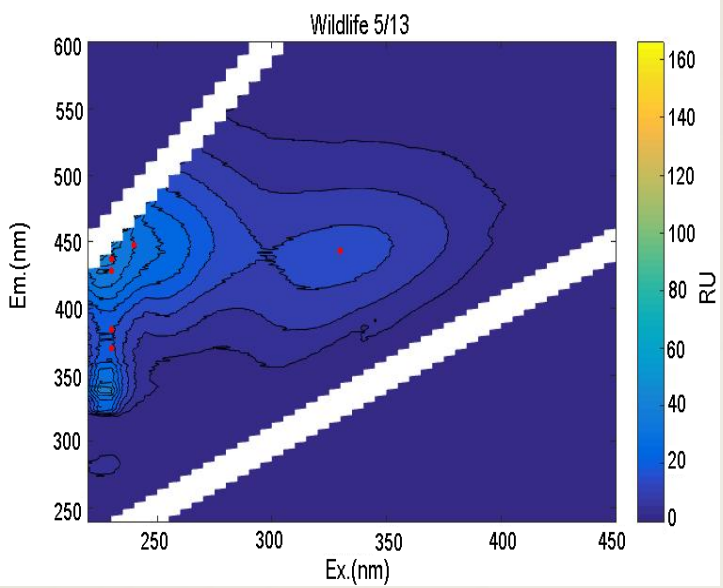
Toxicogenomics Assay

Toxicity test in human cells

Results Highlights

- Temporal and spatial trends of metals, molecular toxicity
- Insights of toxicity profiles in water and sediment
- Statistical and correlation analysis, as well as “iceberg” metal mixtures to examine the potential contribution of metal mixtures
- Explored the correlation between organic matters, metals with toxicity effects detected





In collaboration with Madeline E. Schreiber (VT), Brian Williams (DRBA) (unpublished)

Conclusions

•A quantitative toxicomics-enabled toxicity assessment platform has been explored and developed (preliminarily).

• Fundamental and quantitative understanding of molecular perturbation and correlation with phenotypic toxicity has been explored

• Allow high rate, feasible and economical mechanistic screening of CECs, mixture and exposure assessment

• The technology applicable to exposure assessment, monitoring, technology efficacy evaluation

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