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Application of Toxicogenomics for Toxicity Assessment and Screening of Nanomaterials

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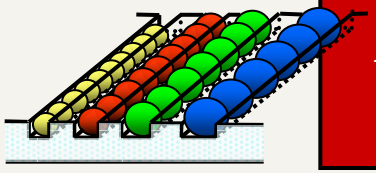
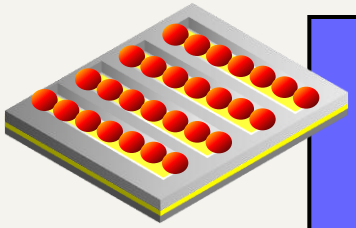
NSF Nanoscale Science and Engineering Center
NU CHN- High Rate Nanomanufacturing



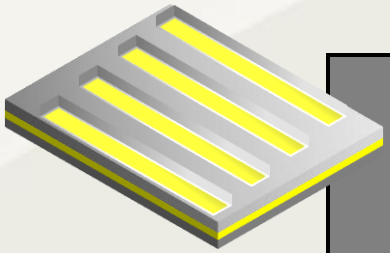
Biosensor

Thrust 3: Testbeds, Applications and Reliability
NEU; UML; UNH



Assembled Particles

Thrust 2: High-rate Assembly and Transfer
NEU; UML; UNH



Trench Template

Thrust 1: Nanoelements and Nanotemplates
NEU; UML; UNH

Thrust 4: Responsible Manufacturing NEU; UML

- Exposure Assessment & Control
- **Toxicity Assessment Screening**
- End-of-Life Impacts
- Environmental and Economic Uncertainties
- Regulatory Issues
- Social & Ethical Issues

Challenges in Toxicity Assessment and Screening of ENMs

How do we measure the increasing, large number of ENMs and their derivatives, evaluate their harmful effects, feasibly?

- Effect-driven aggregated parameter
- Reveal overall toxic effects
- Informative multiple endpoints
- Elucidate toxic mechanisms
- Identify causative agents (NMs)
- Physically, economically feasible

Genomics-based toxicity assessment

Toxicogenomics

Toxicogenomic Technologies

Microarrays- central technology for toxicogenomics

Some Practical Limitations:

(1) Advanced expertise, complex protocol
(2) High cost
(3) Not reusable
(4) Test-condition-sensitive results
(5) Lacks temporal resolution

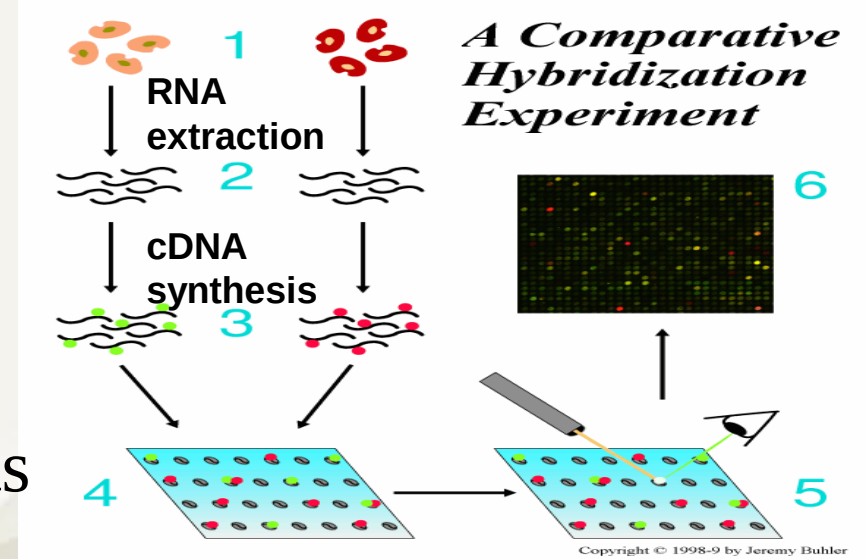
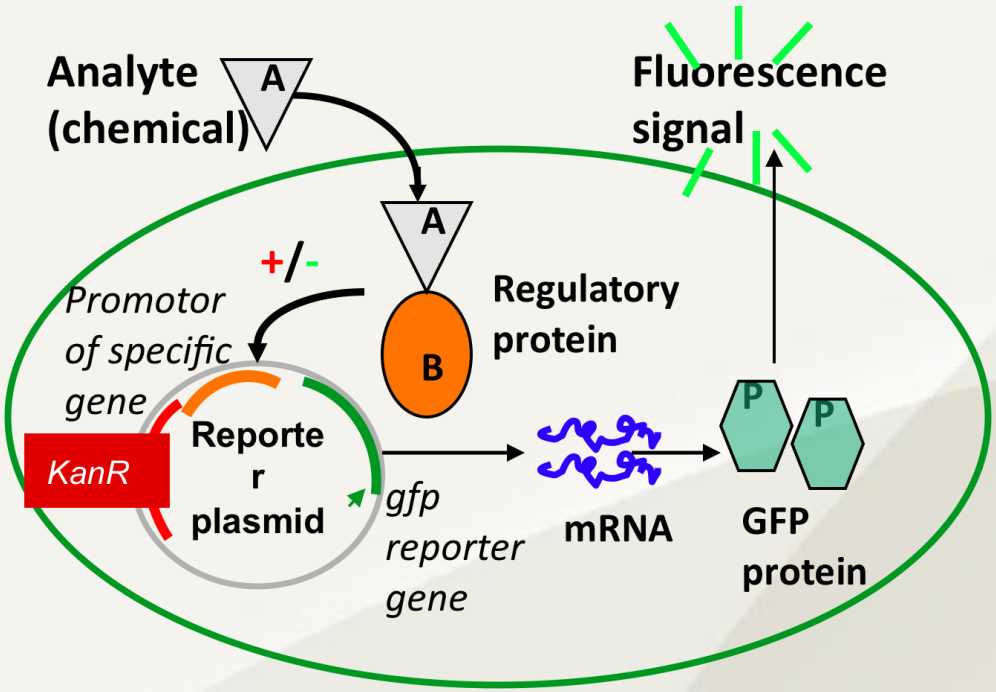


Diagram of microarray

How can we apply toxicogenomics feasibly?
Inexpensive, simple protocols, informative results

Whole Cell Array of GFP-infused Recombinants

GFP-Transformed Bacterial Cells to Monitor Gene Expression



- The cell gives signal if it contains the specific gene that is involved in the response to a ENM
- Reflects cellular level subtle response
- Reflects bioavailability

Prokaryotic Real Time Gene Expression
Time-dependent Gene Expression Pattern

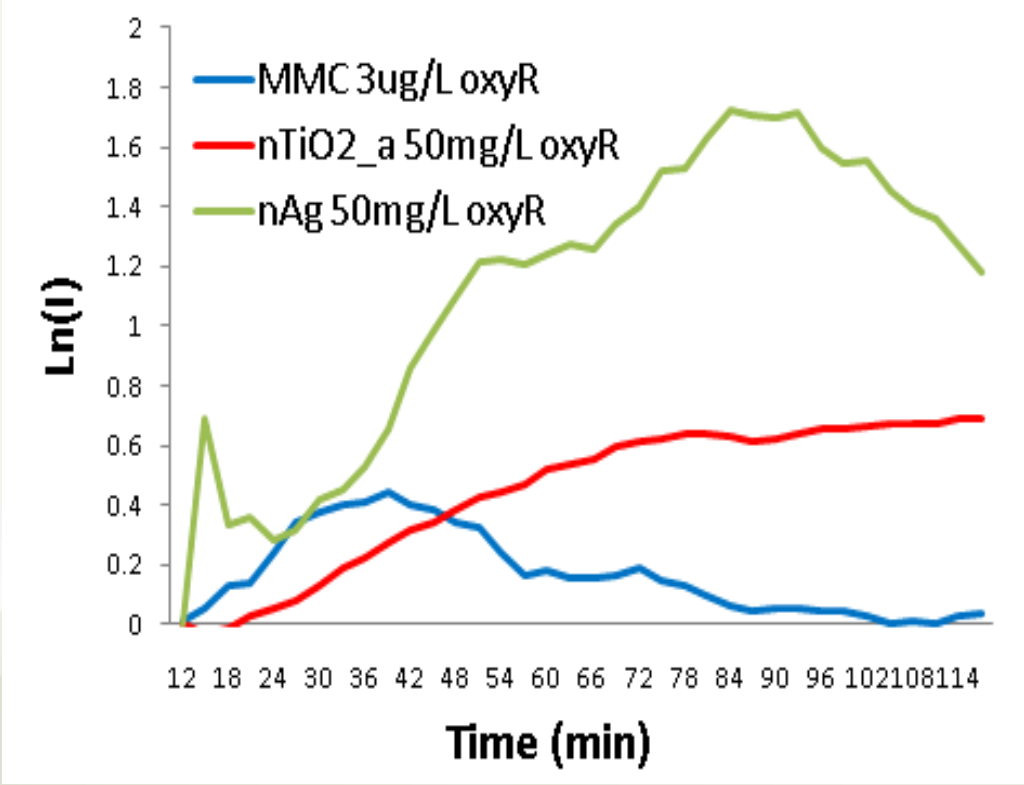
Key findings:

1.Temporal dynamic pattern of gene expression level

2.Varying response to different ENMs

3.Depends on toxic response pathway and sequence of involvement

4.Induction factor (I) seem to be quantitatively correlated to the toxicity level



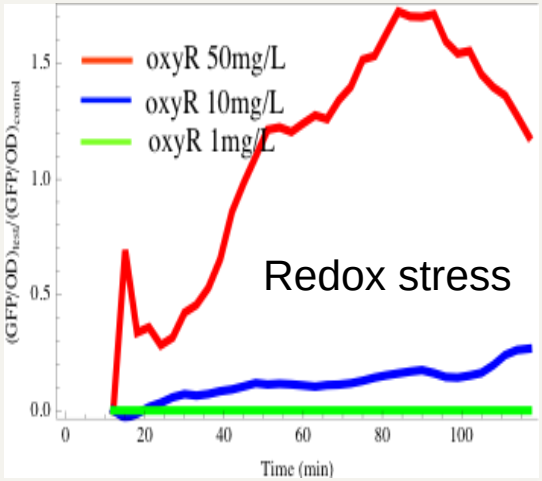
Ln(I), I- induction factor

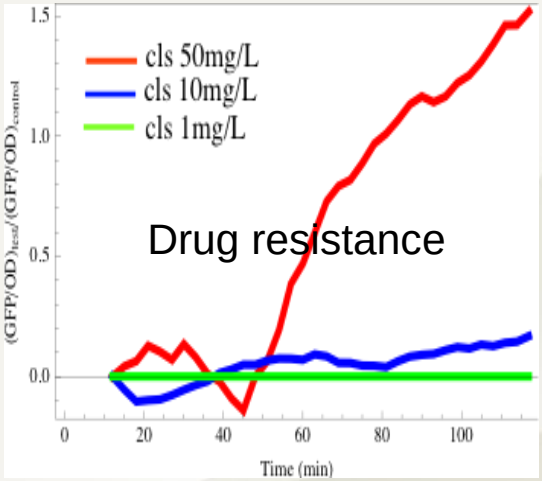
***oxyR*- Belong to redox stress, oxidative stress regulator.**

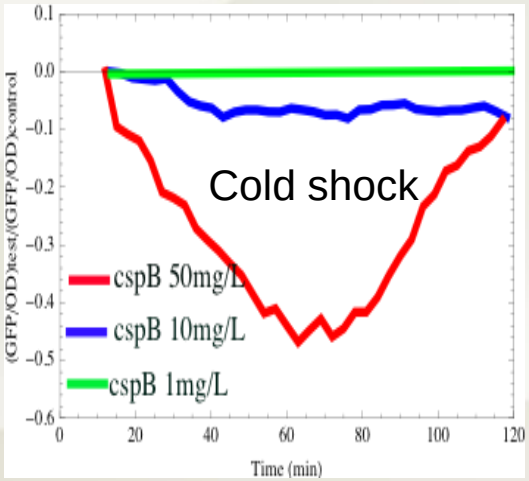
*Onnis-Hayden and Gu, 2009, ES&T; Gou and Gu, 2010, ES&T, in press.

Prokaryotic Real Time Gene Expression
Time and Concentration Dependent Patterns

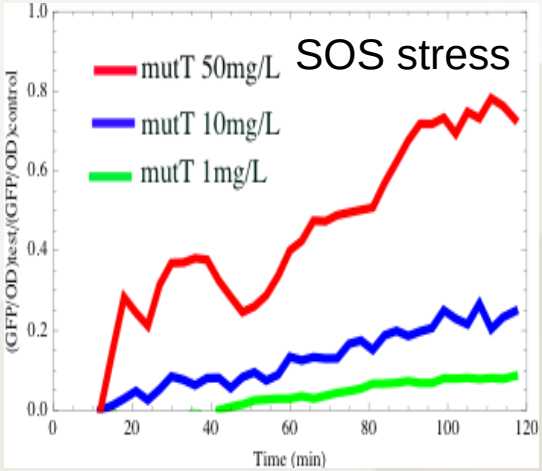
nAg

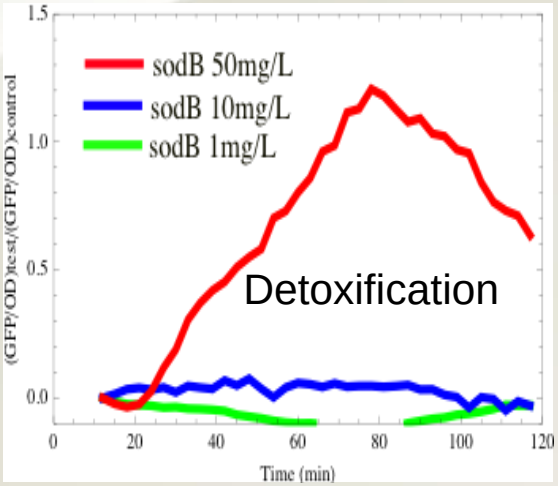


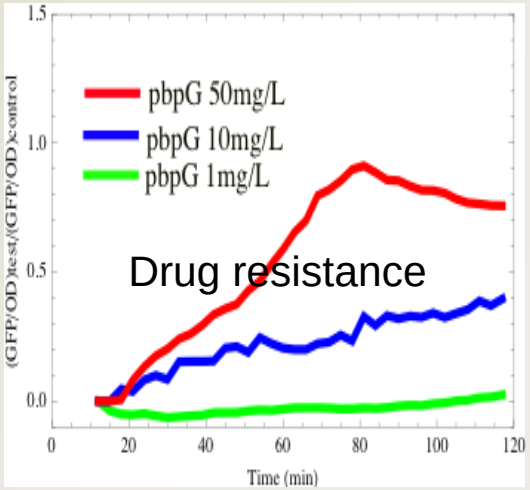




$nTiO_2$_a

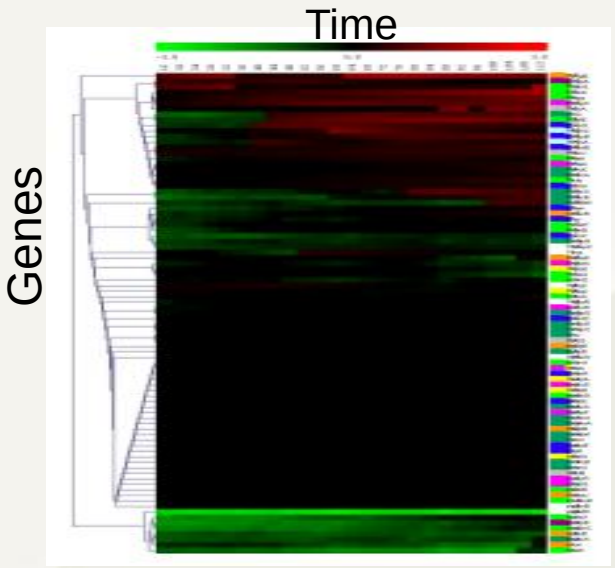




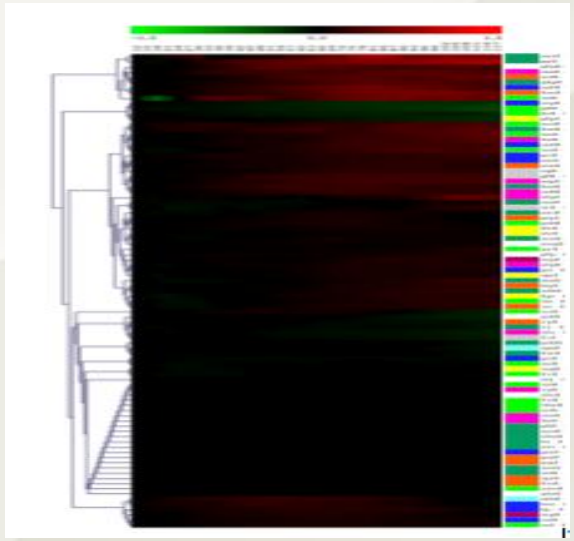


Prokaryotic Real Time Gene Expression Generates Compound-specific signature profile

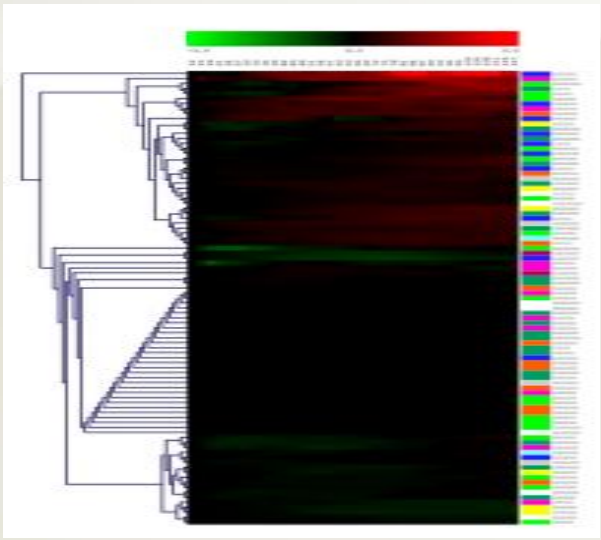
Distinctive compound-specific two-dimension (time and gene) profiles obtained for different toxicants depending on their modes of action (MOA)



mercury 100ug/L



$nTiO_2$, 5 mg/L



nAg, 5 mg/L

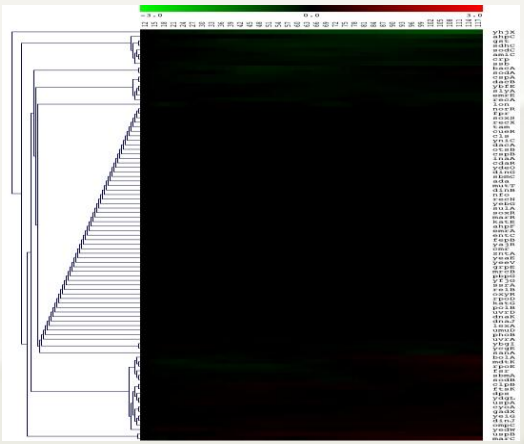
Prokaryotic Real Time Gene Expression Generates Concentration-sensitive profile

Concentration-sensitive response:

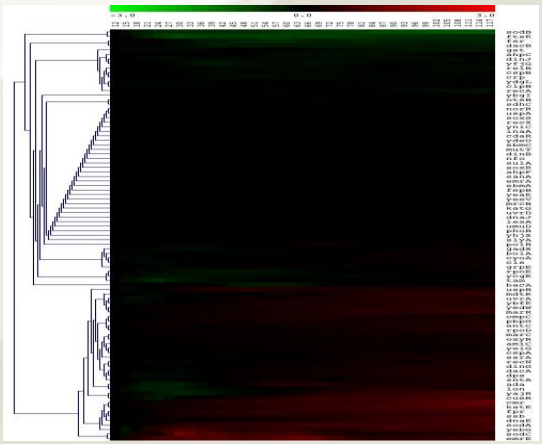
- Distinctive concentration-sensitive profiles obtained for the same toxicant at different concentrations
- Concentration-sensitive response:
  - (1). No-effect level (detection limit-NOTEL),
  - (2). Compound- specific response range (MOA indicative range),
  - (3). Global stress (cellular damage)

Time

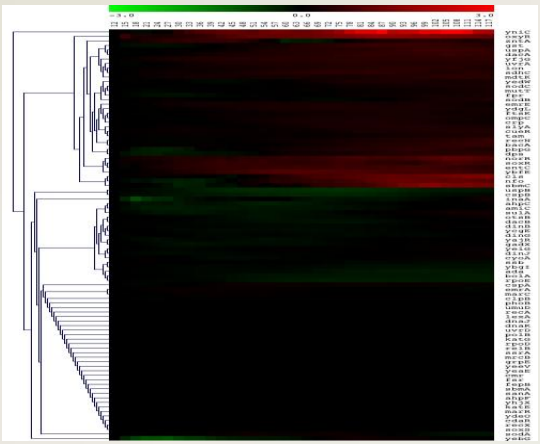
Genes



nAg 1 mg/L



nAg 10 mg/L



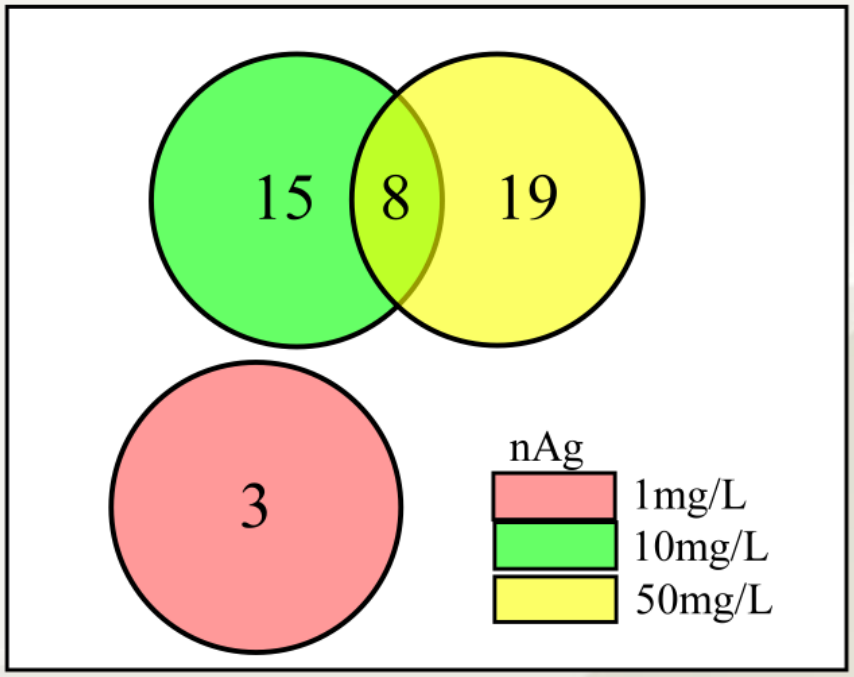
nAg 50 mg/L

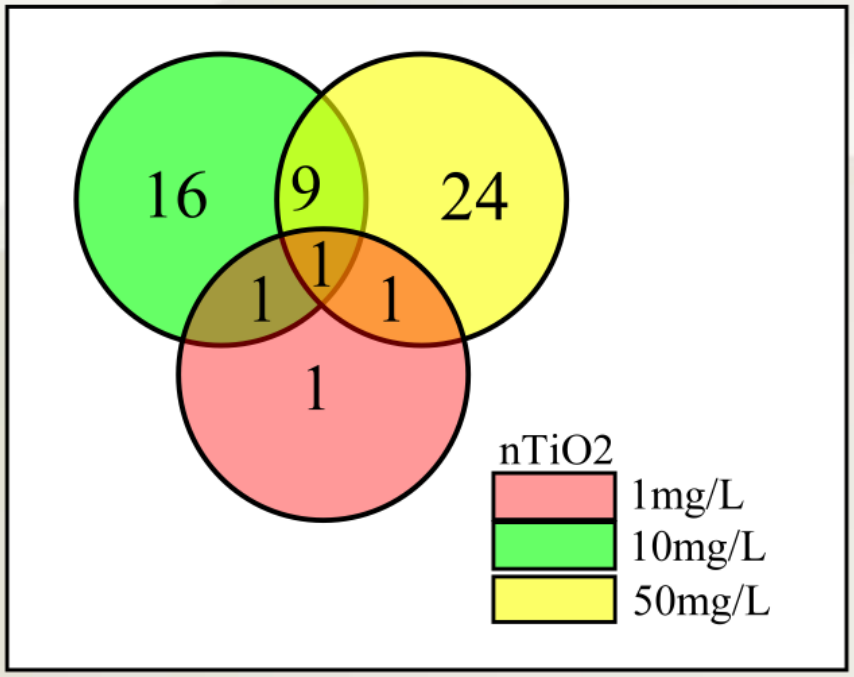
Gou and Gu, unpublished

Mechanistic toxicity assessment of nAg and $nTiO_2$-anatase
Concentration-sensitive response

Different concentrations induce different genes with altered expression

- **The genes response to very low concentrations are different from those at higher concentrations**
- **There are genes that are common to different concentrations- potential biomarkers**





*N. Gou, Onnis-Hayden, A. and A.Z. Gu (2010) Mechanistic Toxicity Evaluation of Nanomaterials via Prokaryotic Gene Expression Profiling. Environ. Sci. Technol. (in press)

Prokaryotic Real Time Gene Expression Generates Concentration-sensitive profile

Concentration-sensitive response:

- The % of genes with altered expression increases with increase in concentration
- Dose-response curve

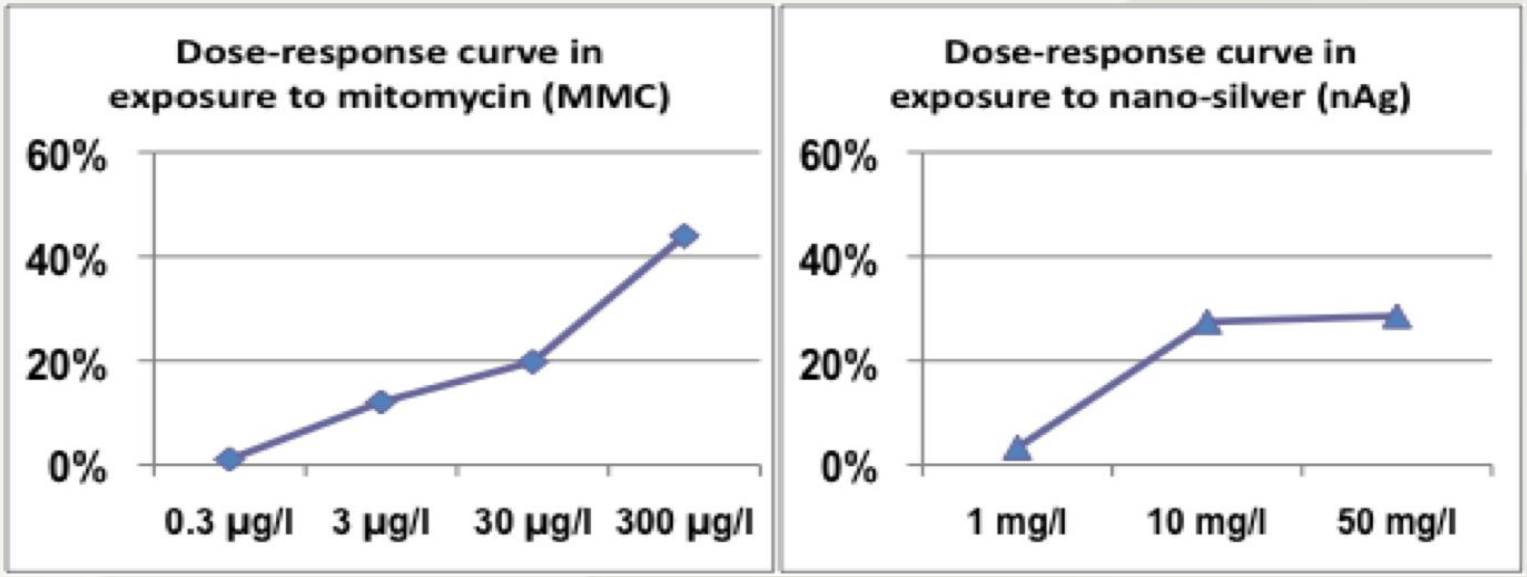


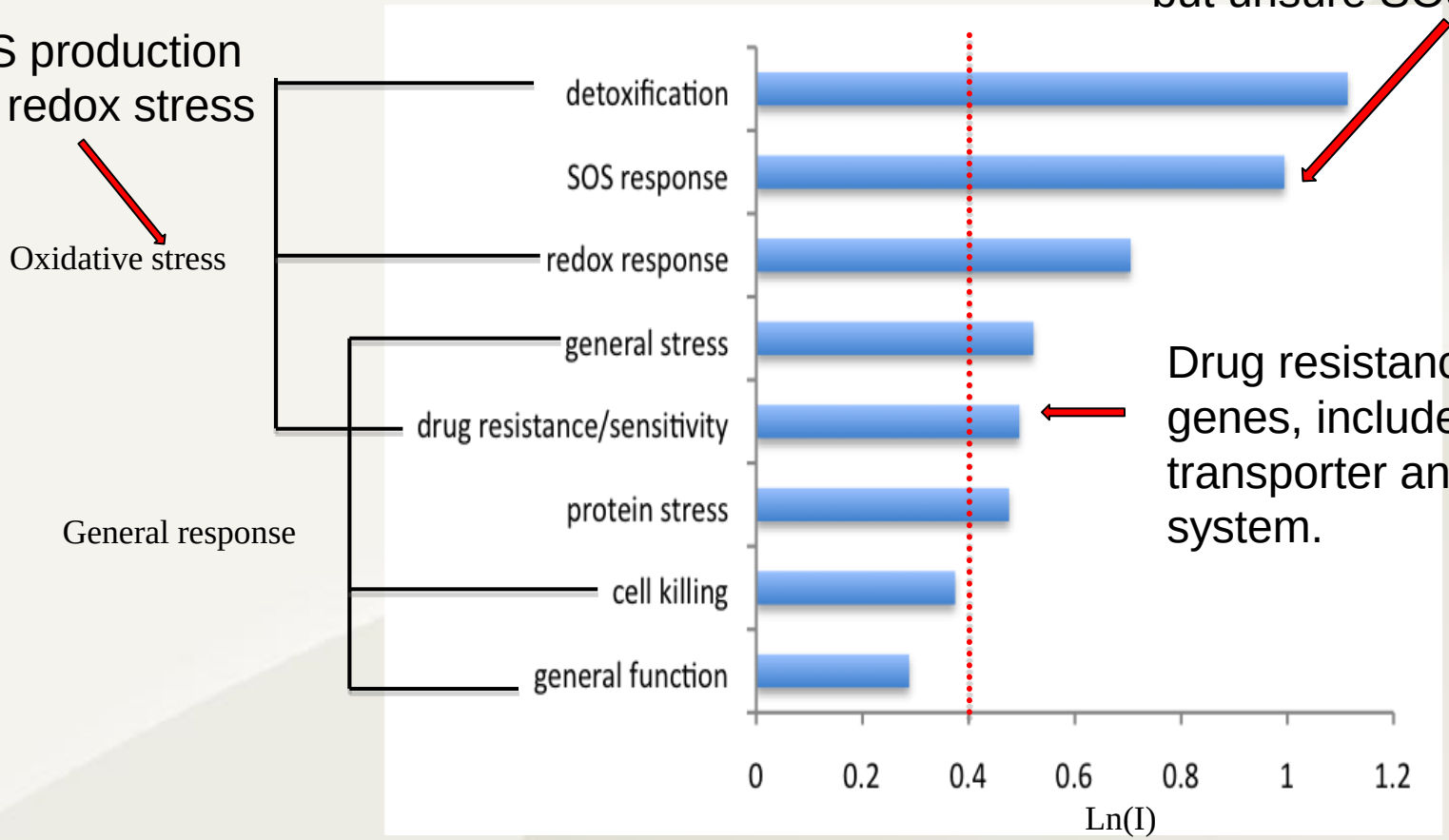
Figure 8 Mitomycin and nano-silver dose-response curves for stress gene expression. Y- % of genes differentially expressed in exposure to toxins compared to control (no toxin)

Gou and Gu, unpublished

Mechanistic Toxicity Assessment of nAg

Genotoxicity- DNA damage, but unsure SOS response

ROS production and redox stress



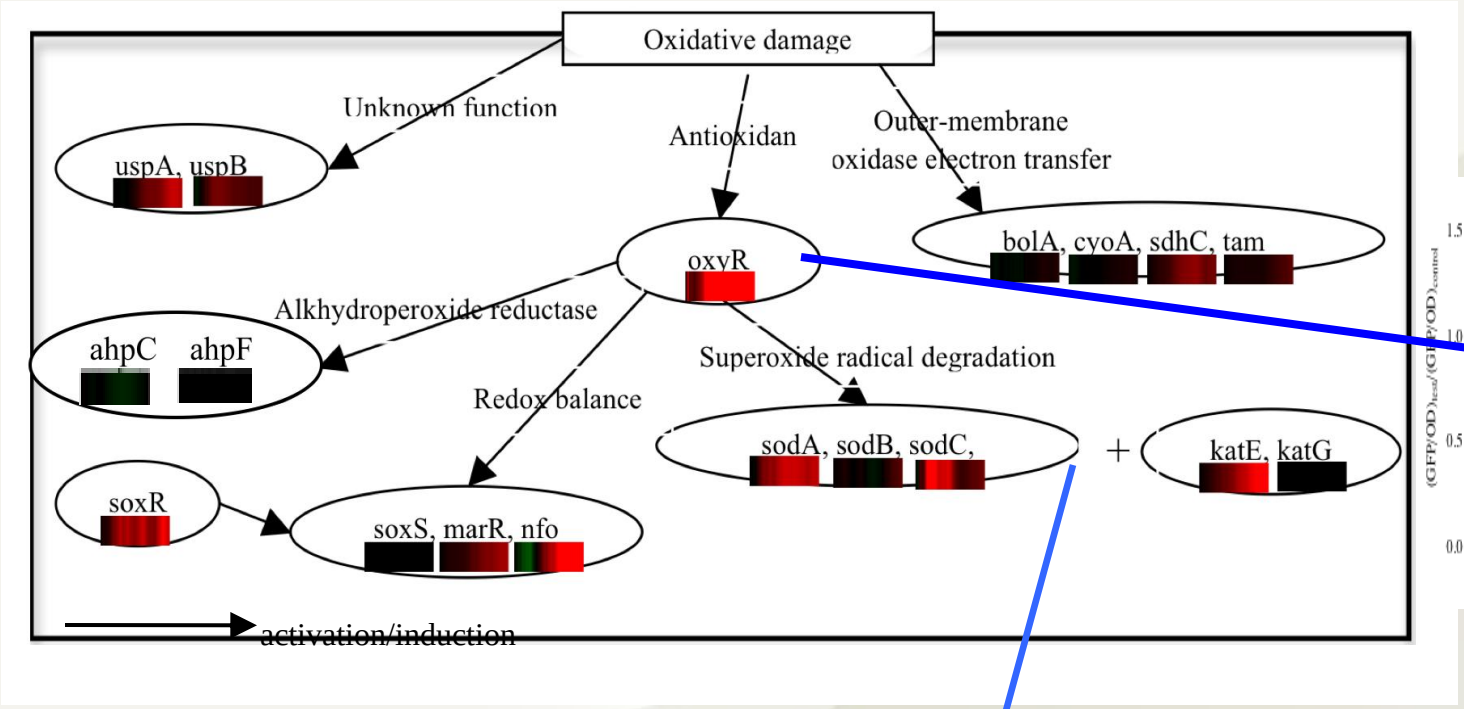
Drug resistance- MFS genes, include multidrug transporter and drug efflux system.

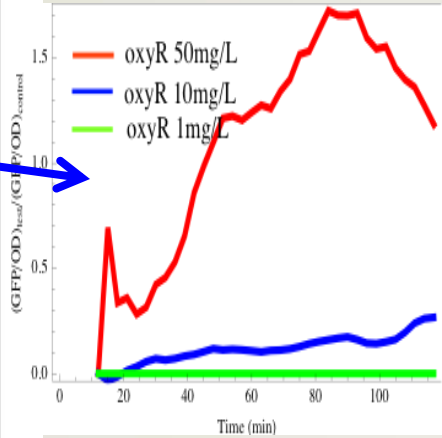
nAg induced stress gene response (10 mg/L)

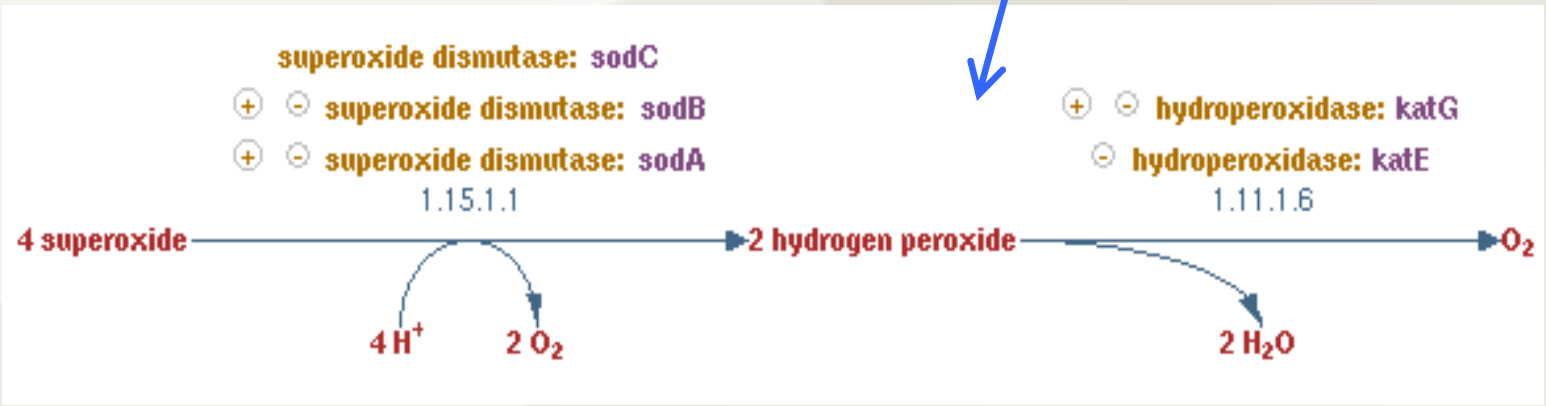
Gou and Gu, ES&T, 2010

Mechanistic toxicity assessment of nAg

Oxidative Stress Response System







Gou and Gu, unpublished

How to Define Toxicity Assessment End-point?
Link to regulatory benchmark

NOTEL -No Observable Transcription Effect Level

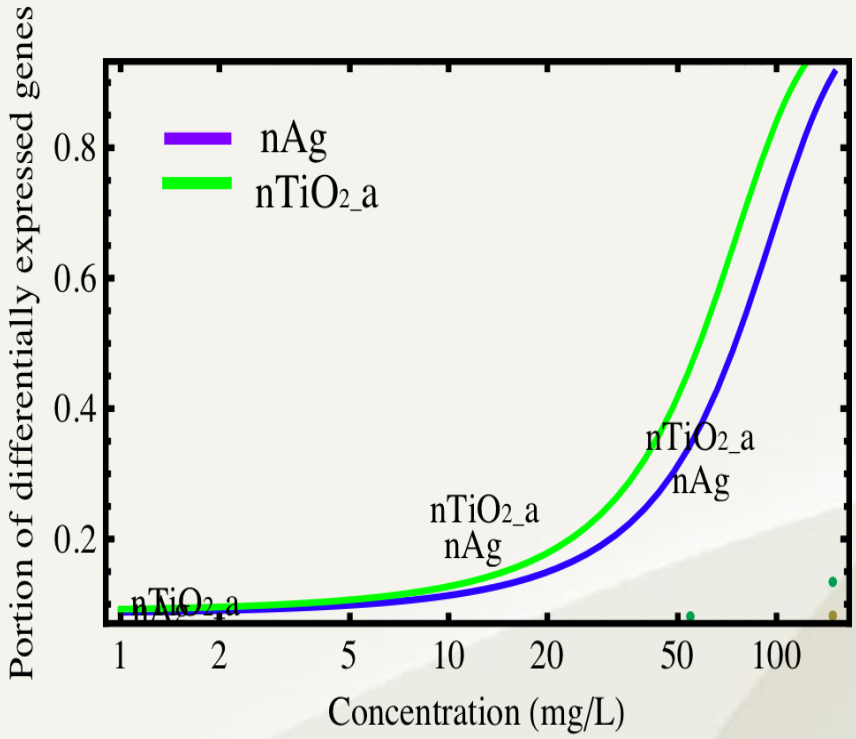
Concept: used by others in toxicogenomics

Definition: the maximum concentration of a chemical at which less than 5% of the genes are differentially expressed

Determination: We fit a dose-response curve % gene differentially expressed vs concentrations

How to Define Toxicity Assessment End-point?
Correlation of NOTEL with Other Endpoints

How to determine NOTEL?



Dose- response curve

Comparison of NOTEL with conventional endpoints

Compound	NOTEL	LC_{50}/ EC_{50} from literature
4-NNP (EDCs)	100 μg/l	EC_{50}: 480 μg/l for bacteria *V.fischeri* EC_{50}: 100-300 μg/l for Crustaceans[97] LC_{50}: 43-170 μg/l for Zooplankton[97]
nAg (Nanomaterials)	0.5 mg/l	LC_{50}: 5mg/L for *E.coli* [96]
MMC (Pharmaceutical)	0.1 μg/l	LC_{50}: 6.7 mg/l for cancer cells[98]
Hg (Heavy metals)	1 μg/l	LC_{50}: 60-700 μg/l for Fish[97] LC_{50}: 4-850 μg/l for Crustaceans[97] LC_{50}: 3.5-600 μg/l for Zooplankton[97]

NOTEL can be an quantitative endpoint linking toxicogenomic results with conventional toxicity assement endpoints

*N. Gou, Onnis-Hayden, A. and A.Z. Gu (2010, ES&T)

How to Define More Informative End-point?
The new INDEX??- TGRI

Although NOTEL seems like a good candidate for endpoint, we feel that the rich information of toxiogenomic are not fully reflected:

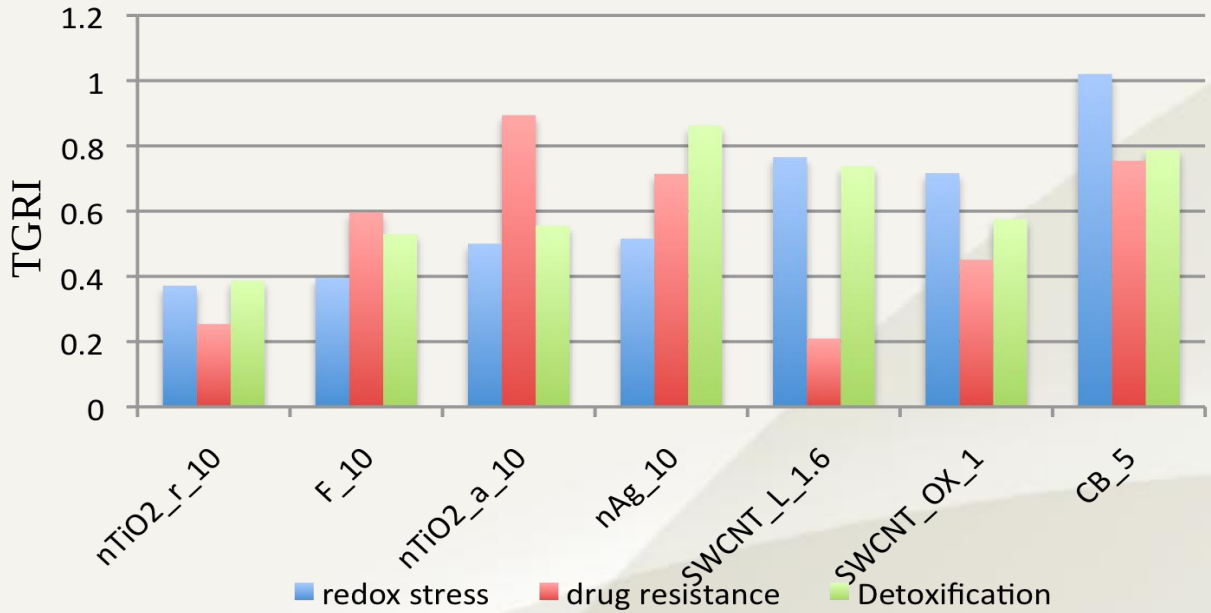
-The number of genes with altered expression level by toxicant?
- The magnitude of changes in the gene expression induced?
- The time factor : temporal change patterns?

ToxicoGenomic Response Indicator (TGRI) – new index proposed

We are using a mathematical manipulation that incorporate both the number and level of genes with altered expression, as well as the time length for the maximum expression level to occur.

The TGRI index converts the multi-dimensional toxicogenomic data to a regulatory toxicity endpoint.

TGRI Allows for Toxicity Level Comparison in Different Toxicity Categories

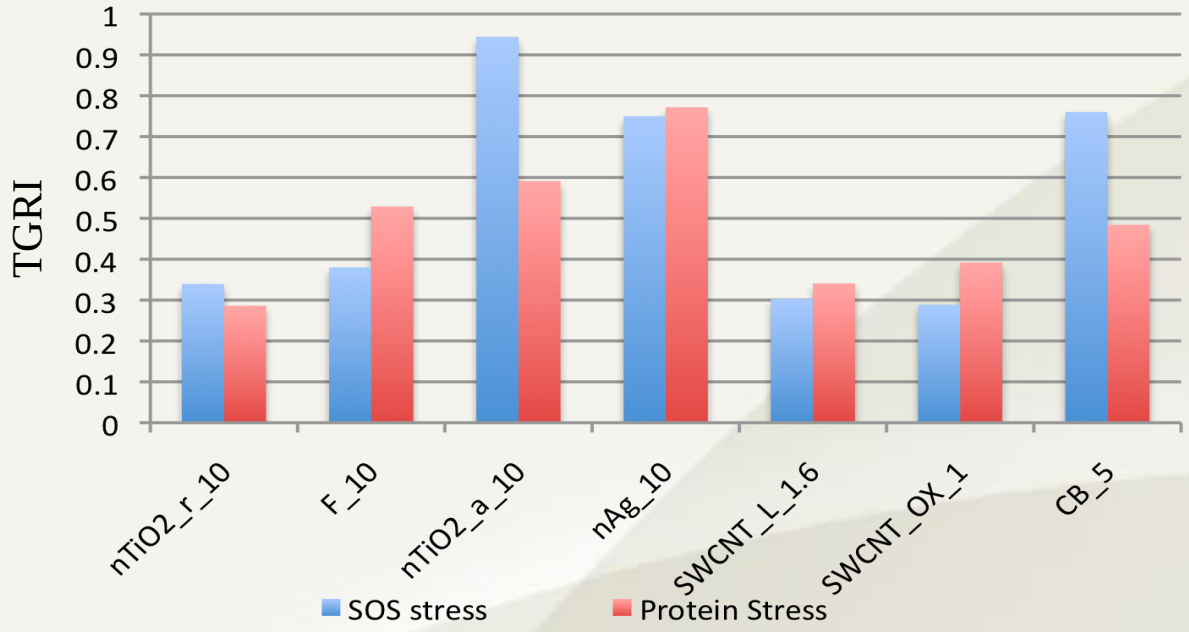


Comparison of toxicity level:

Oxidative stress: CB_5>SWCNT>nAg>$nTiO_2$-a>C_{60}>$nTiO_2$-r

Drug resistance: $nTiO_2$-a>CB_5>nAg >C_{60}>SWCNT-ox >SWCNT>$nTiO_2$-r

TGRI Allows for Toxicity Level Comparison in Different Toxicity Catagories



Comparison of toxicity level:

Genotoxicity: $nTiO_{2\text{-}a}$>CB>nAg> C_{60}>SWCNT

Protein stress: nAg>$nTiO_{2\text{-}a}$>C_{60}>CB

Advantages and Impact of Proposed High Rate Toxicity Screening Method and System

- Fast bioassay (2-3 hrs)
- Sensitive and quantitative assessment of toxicities of ENMs
- Information-rich results reveal mechanism, reflect bioavailability and overall biological response to ENMs
- Easy, simple, inexpensive procedures

Conclusions

- Temporally dynamic gene expression yields NM-specific & concentration-sensitive profile

- The specific yet “conservative” profile allows for potential classification and identification of NMs

- Reveals detailed toxicity mechanisms of various NMs

- Allow high rate, feasible and economical screening of NMs

- Provide information that can be incorporated into risk assessment framework