Fundamental Interactions of Nanomaterials with Organisms: Reducing Uncertainty With High Content Data

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The Opportunities

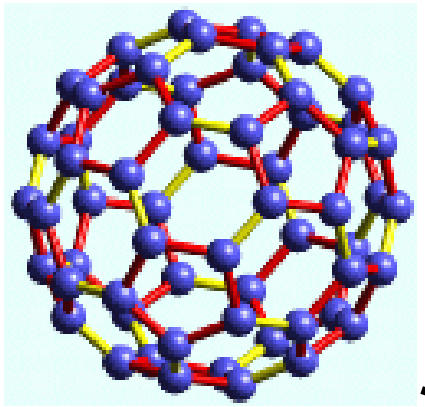
Proactively guide the development of inherently safer nanomaterials

- **Identify the physicochemical properties that drive behaviors – take a global view**
- **Think nanoscience**
- **Develop predictive "behavioral models" from experimental data.**

The Nano Challenge

- **Much in common with the small molecule challenge**
- **One material at a time approach will ultimately fail**
- **Generalizations cannot be made…yet**
- **We need (MUCH) more data**
- **We need paradigm shift in how we assess hazard**
- **Very little of this data will be directly used for risk assessment.**

How a material "behaves" absolutely depends on its physical properties



Agglomeration

Dissolution

Environmental Interactions

Environmental Fate

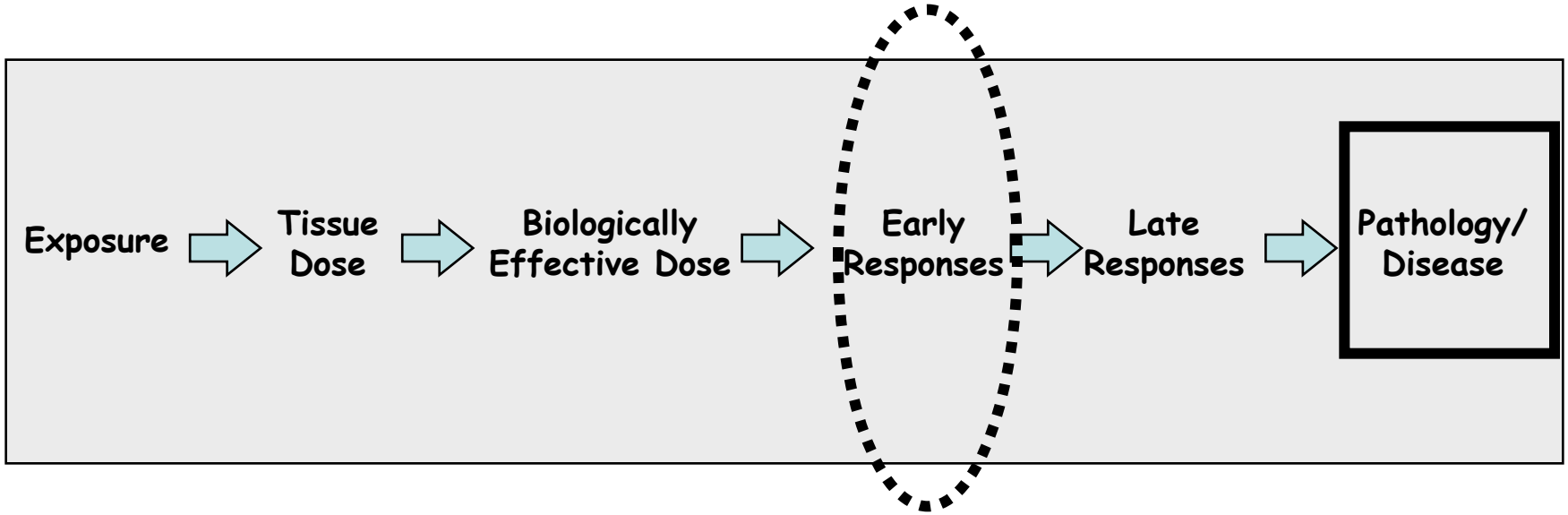
Biological Interactions

Biological Fate

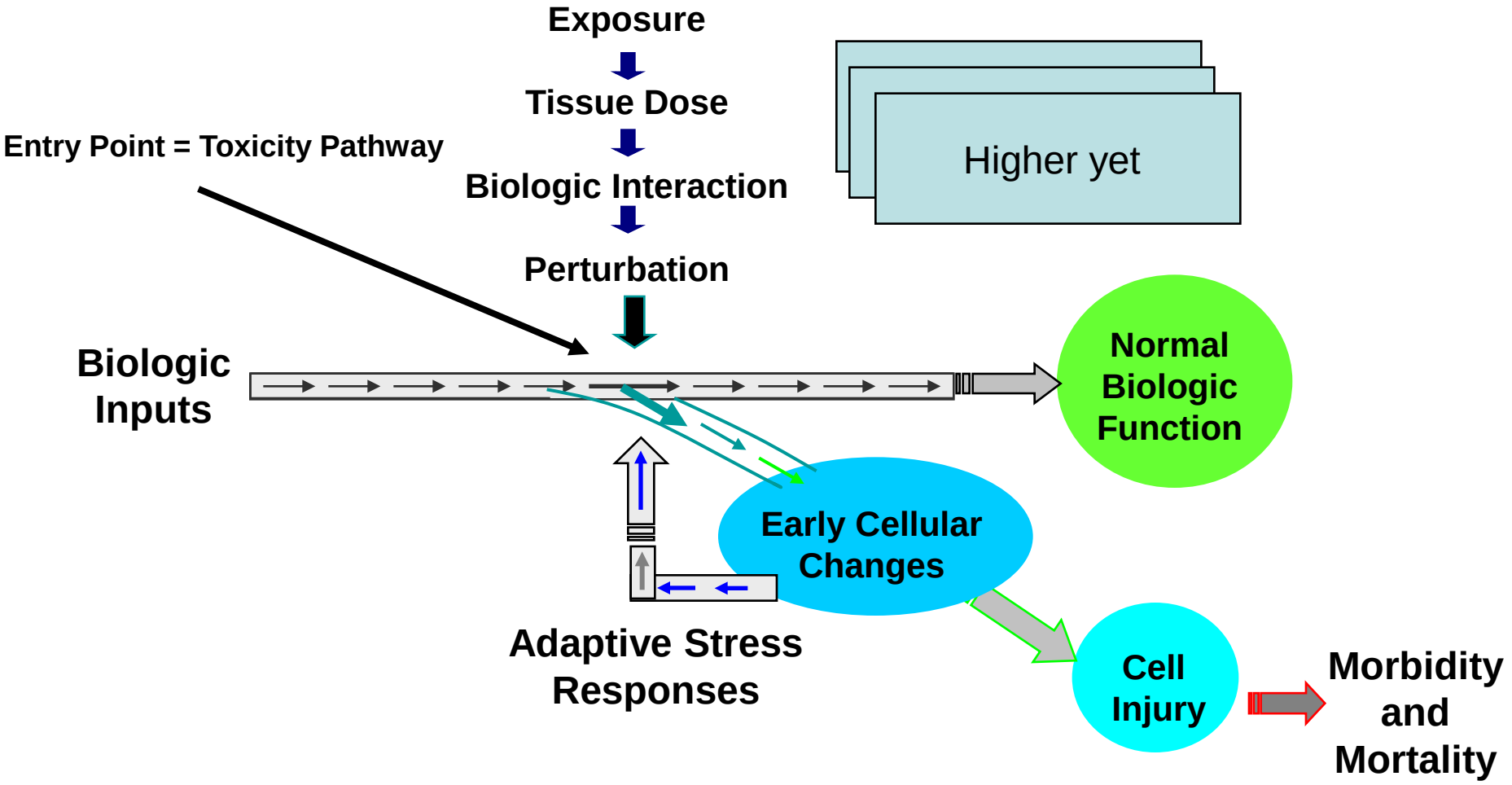
Biological Responses

Goal is to predict these behaviors from inherent properties

Exposures and Biological Responses



Biology is a System that Responds



Adapted from the National Academy
Toxicity Testing for the 21st Century

Toxicity Pathways

Toxicity Pathway: A cellular response pathway that, when sufficiently perturbed, is expected to result in an adverse effect. **National Academy Toxicity Testing for the 21st Century**

- **We need to identify these toxicity pathways**
- **Determine if NP perturb them**

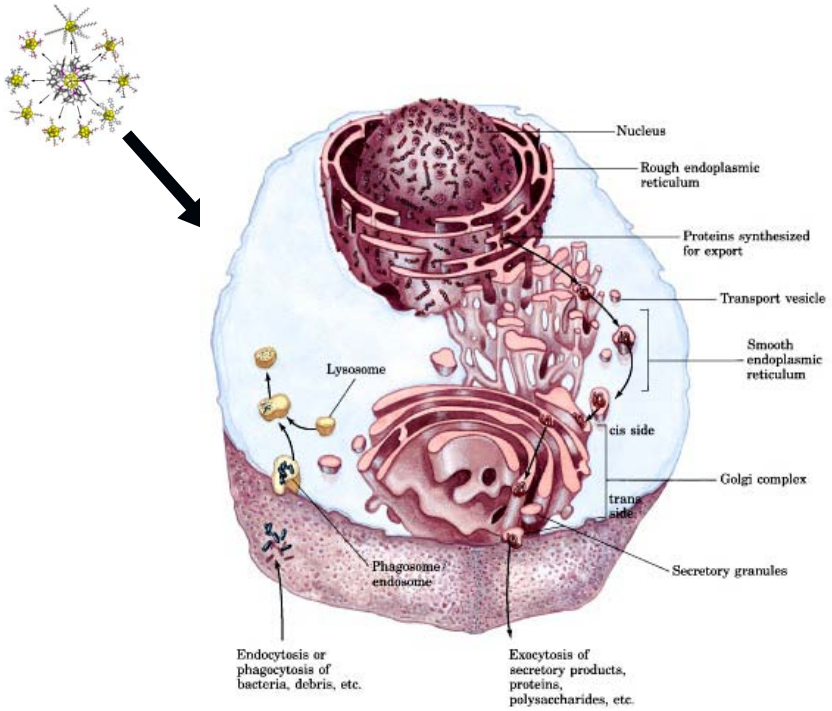
Nanomaterial Biological Assessments Platforms

- *In vitro*
  - Continuous cell culture system
  - Primary cell culture system

- *In vivo*
  - Whole animal studies
  - Rodents- slow and expensive
  - Zebrafish
  - Flies and worms – non vertebrates

Cell-Based Approaches

- Advantages - quick, easy and cheap



Response

Proliferation
Cell death
Metabolism
Gene expression
Phenotypic change

“There are blind spots”

What blind spots?

- **Different cell-cell interactions cannot be evaluated**
- **Indirect effects cannot be evaluated**
- **Cells in culture can only respond using their unique repertoire of expressed gene products – limited potential targets**
- **Practical problem…what cells do you choose?**
- **Tremendous potential for missed data**
- **Need rapid, in vivo model…….**

Collecting Biological Response Data

We need to pick up the pace…

But….

High throughput ≠ high content

For example:

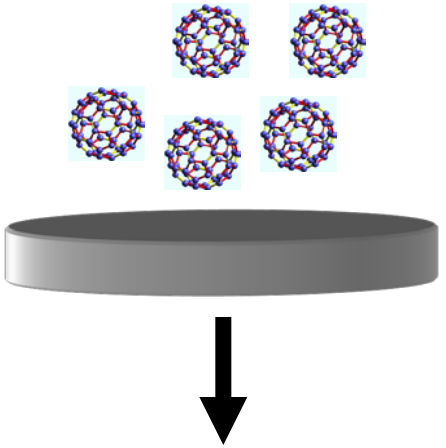
If an assay is developed for a specific responses we are biased……

i.e. Apoptosis, proliferation, ROS, Calcium influx..

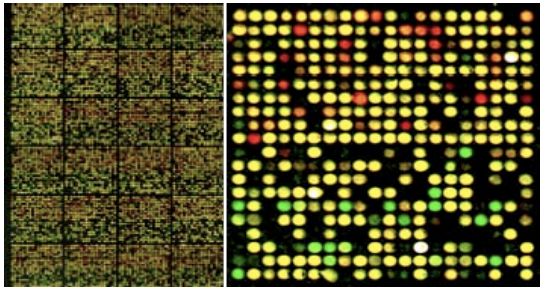
Example

Cultured endothelial cells

Expose and collect “omics data”



Hundreds of gene expression changes



Are these gene expression changes related to an adverse outcome? Do they represent an adaptive response?

What decisions can be made based solely on this information?

Systems Biological Approach
- early embryonic development -

Why?

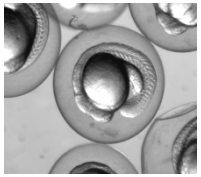
- **Generally more responsive to insult… because**

  Most dynamic life stage…and the full signaling repertoire is expressed and active, therefore fewer blind spots.. Highest potential to detect interactions

- **If a chemical or nanomaterial is developmentally toxic it must influence the activity of a molecular pathway or process.. i.e. hit or influence a "Toxicity Pathway"**

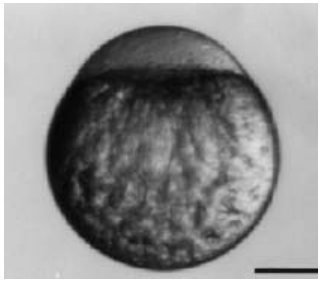
- **Use the biological response to identify the "Toxicity Pathway"**

Why we use Zebrafish

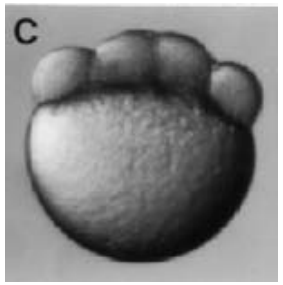


- Share many developmental, anatomical, and physiological characteristics with mammals
- Molecular signaling is conserved across species
- Technical advantages of cell culture - power of in vivo
- Amenable to rapid whole animal mechanistic evaluations
- Focus on responses, then identity the “Toxicity Pathway” underlying it - immediately relevant

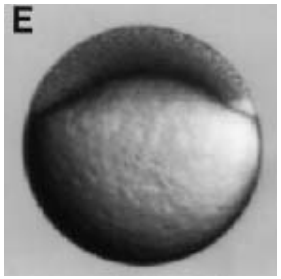
Development Stages of Assessments



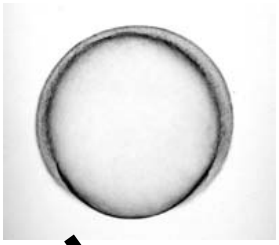
3 min



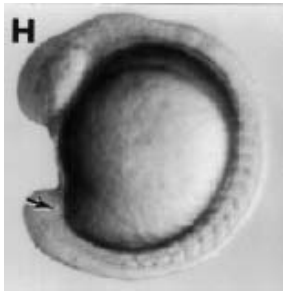
1. 25hr



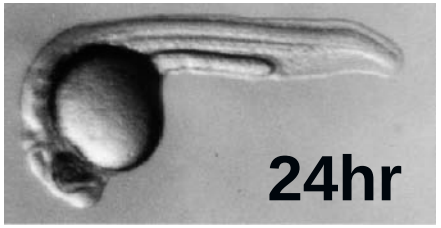
4 hr



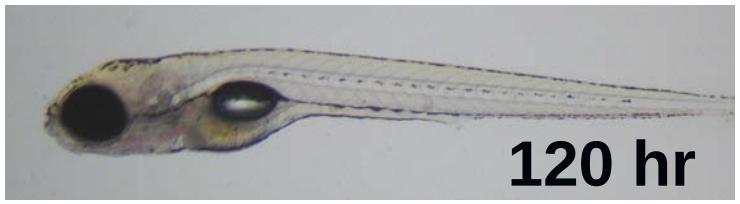
6 hr



19 hr



24hr



120 hr

48hr

Assessing Biological - Nanomaterials Interactions and responses

<u>Tier 1:</u> Toxicity Screening

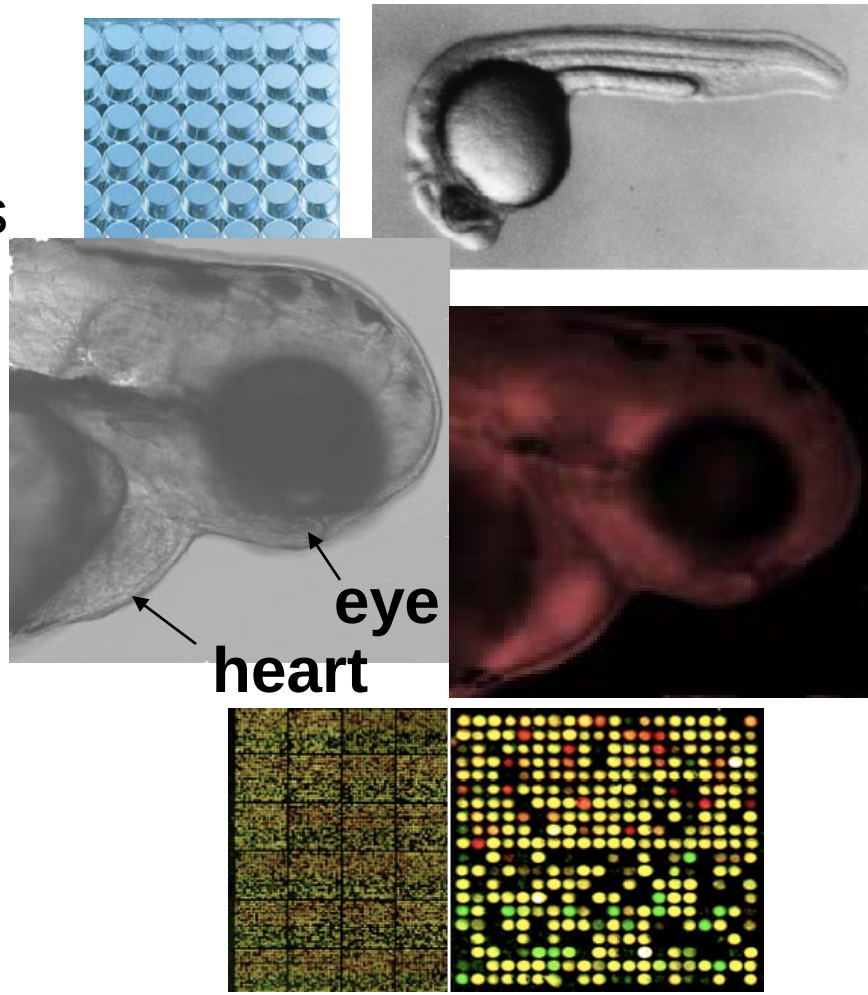
- Toxicity testing whole organisms
  - *In vivo* - zebrafish

<u>Tier 2:</u> Cellular Targets and Distribution

- Defined *in vivo*
  - Fluorescent nanomateials
  - Targeted assays

<u>Tier 3:</u> Molecular Expression

- Genomic Responses
  - Whole animal gene expression profiles



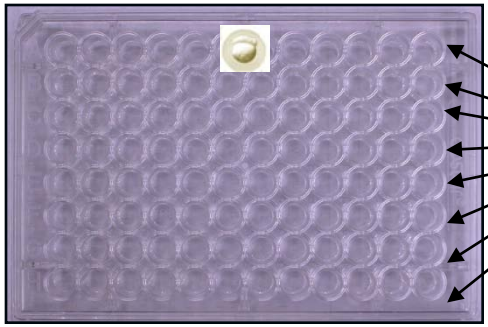
Define Structure Activity Relationships

Toxicity Testing (First Steps)

A large adult colony is required to support testing laboratory

Remove Chorions

1 Embryo/well



Test Materials

Screening for responses 1-5 days

High Content Tier 1 Endpoints (Assessed between 24 and 120 hpf)

Morphological

Malformations

i.e. pericardial edema, yolk sac edema, body axis fin malformations, eye diameter

Circulation

Heart beat (rate)

Developmental progression

Embryo viability

Behavioral

spontaneous movement (18-24 hpf) onset and frequency

touch response (27 hpf)

motility

Automation: To Increase Throughput

Our Recent Technical Advances

- **Embryo Production**
- **Embryo handling**
- **Microinjections**
- **Plate reader based assays**
- **Behavioral assays**

Current Needs

- **Efficient dissemination of shared materials**
- **Reduce the randomness of assessments**
- **Data sharing infrastructure**
- **Comparative analysis with shared data**
- **Define mode of actions of responsive NPs**
- **Develop predictive behavior models**
- **Test predictive models**