nanomaterial–biological interactions

NBI KNOWLEDGEBASE

Integrative Nanotoxicology: Linking Rapid Assays and Informatics to Predict Nanomaterial –Biological Interactions

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ONAMI OREGON NANOSCIENCE AND MICROTECHNOLOGIES INSTITUTE

Nanomaterial-Biological Interactions

"Structure"-"Activity" Relationships

- The holy grail for nanomaterials

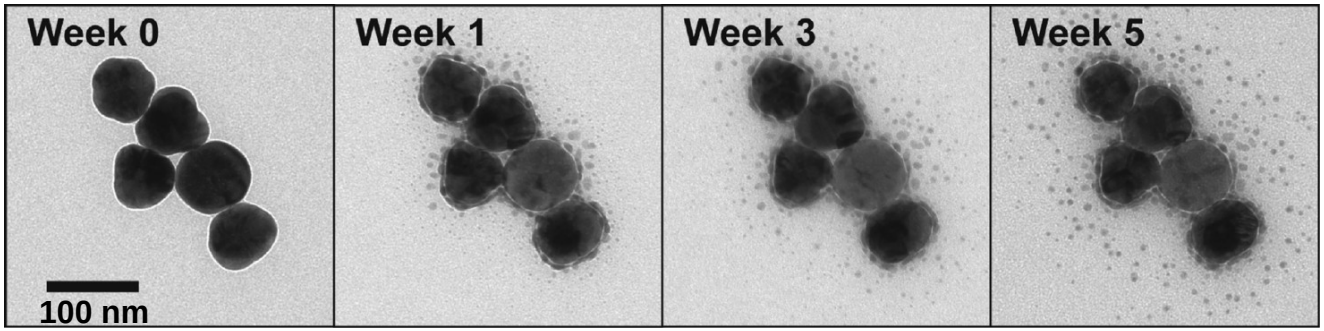
- Structure = inherent properties
- Activity = conditional behavior

nanomaterial-biological interactions

Challenges in Measuring Biological Responses to Nanomaterial Exposure

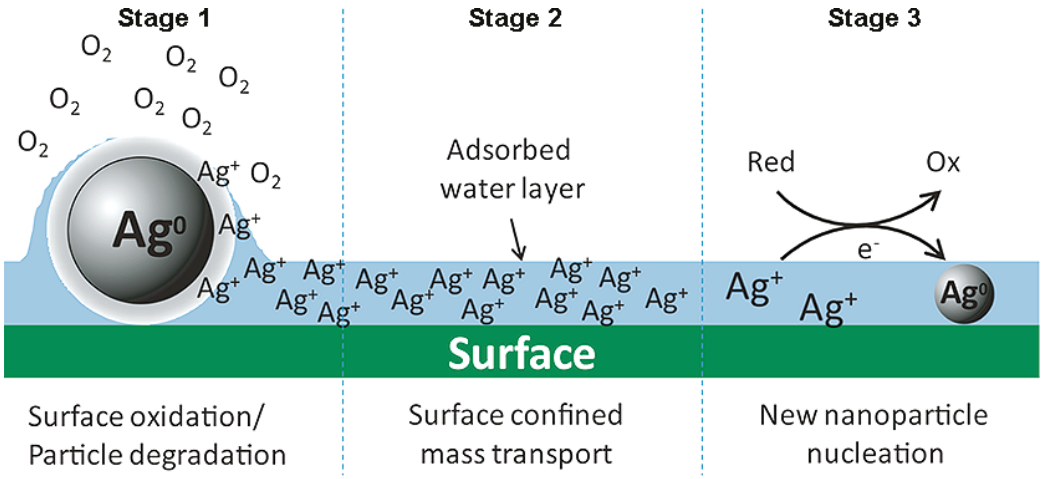
Nanomaterial Stability

Dissolution → particles + ionic species



Glover et al., ACSNano, 2011

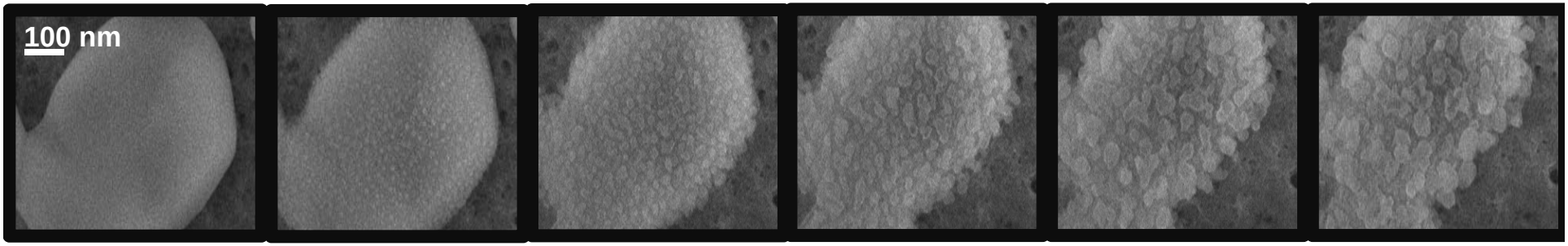
Nucleation → particle formation

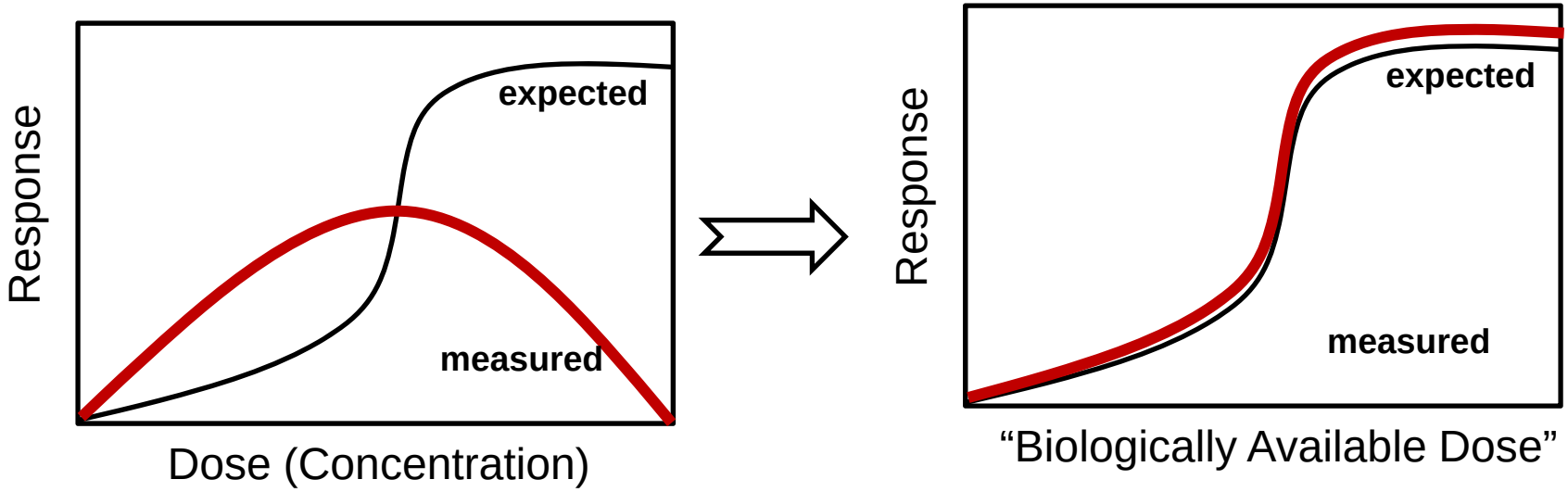


Challenges in Measuring Biological Responses to Nanomaterial Exposure

Nanomaterial Stability

Agglomeration – "Biologically Available Dose?"





Challenges in Measuring Biological Responses to Nanomaterial Exposure

nanomaterial–biological interactions

Influence of Exposure Scenario

- Sample preparation – sonication, vortex, solution
- Experimentation design - timing, duration, organism
- Transformation during exposure
  - Nanomaterials are dynamic – “conditional behavior”
  - Solution vs. dispersion - *not in equilibrium*

Challenges in Measuring Biological Responses to Nanomaterial Exposure

nanomaterial–biological interactions

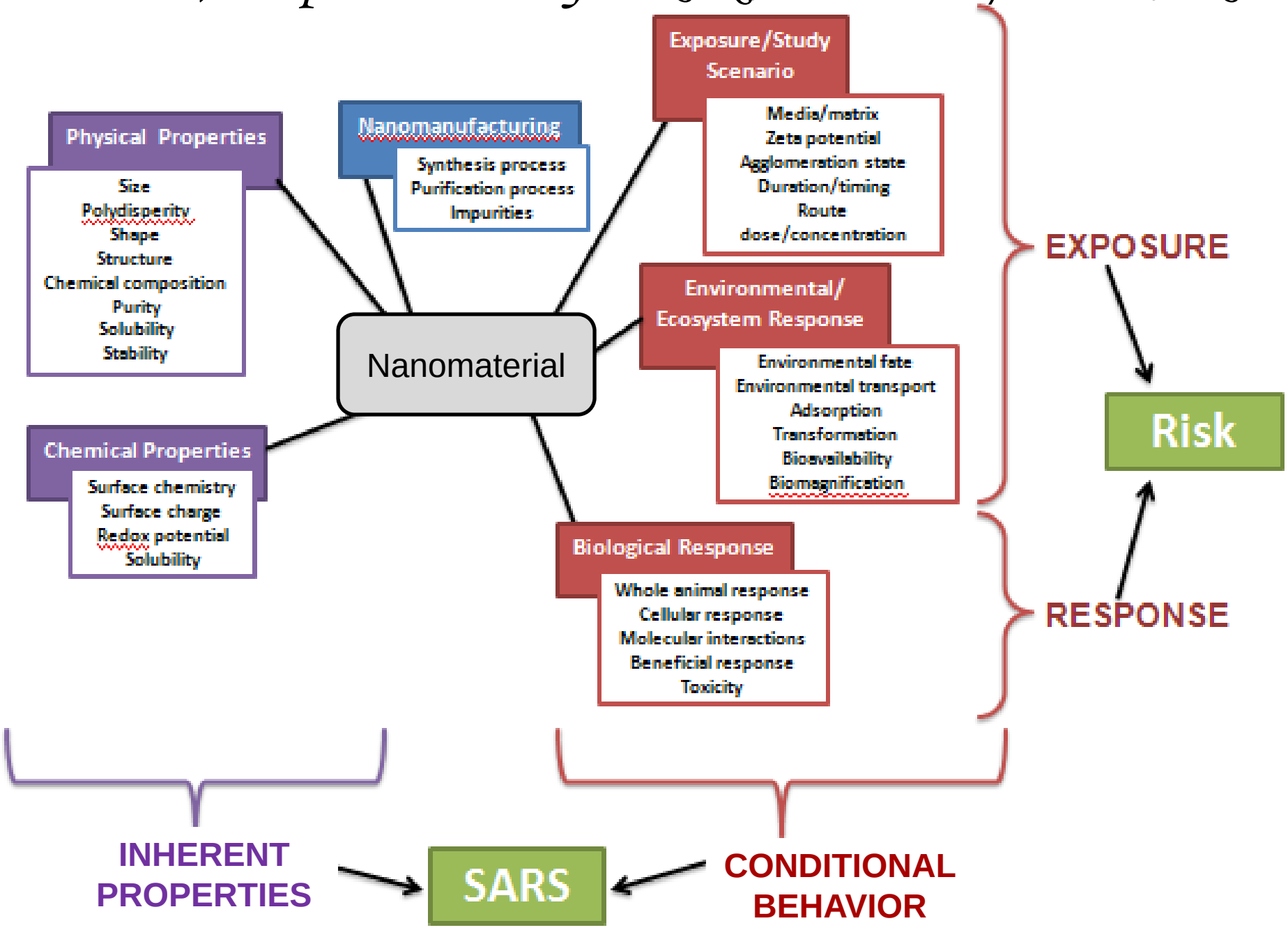
ADME – Tox Limits for Nanomaterials

- Complex matrixes make finding nanomaterials difficult – limits of instrumentation
- Exposure → dose unknown
  - ICP-MS, fluorescent labeling limits
- Biodistribution
  - Fluorescence
  - Microinjection

Nanomaterial Interference

- Optical analysis limited for some nanomaterials

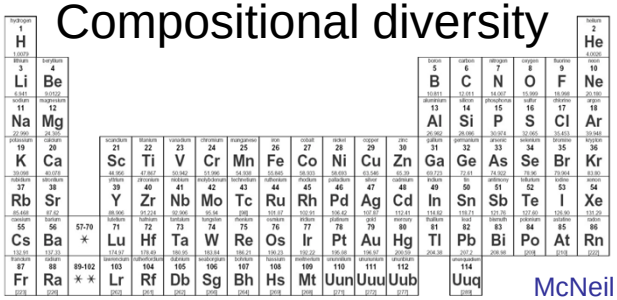
Diverse, Disparate Data for Risk Assessment/ nanoSARs

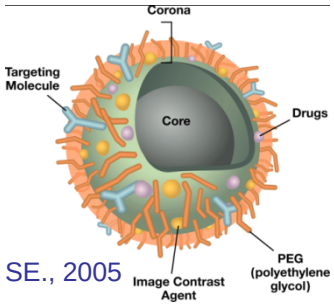


Need for Rapid Assays

Nanomaterial Diversity

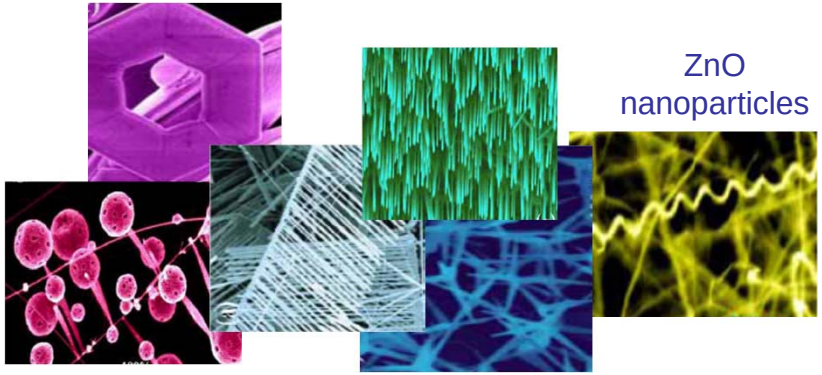
Compositional diversity





McNeil SE., 2005

Synthesis process influences nanoparticle shape



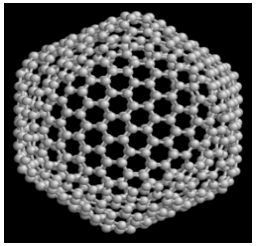
ZnO nanoparticles

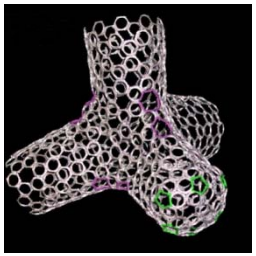
Nanomaterial Complexity

Relative importance of nanomaterial characteristics are unknown

Physicochemical properties

- Chemical Structure
- Core Particle Composition
- Size
- Shape
- Charge
- Surface Chemistry
- Surface Area
- Agglomeration State
- Zeta Potential



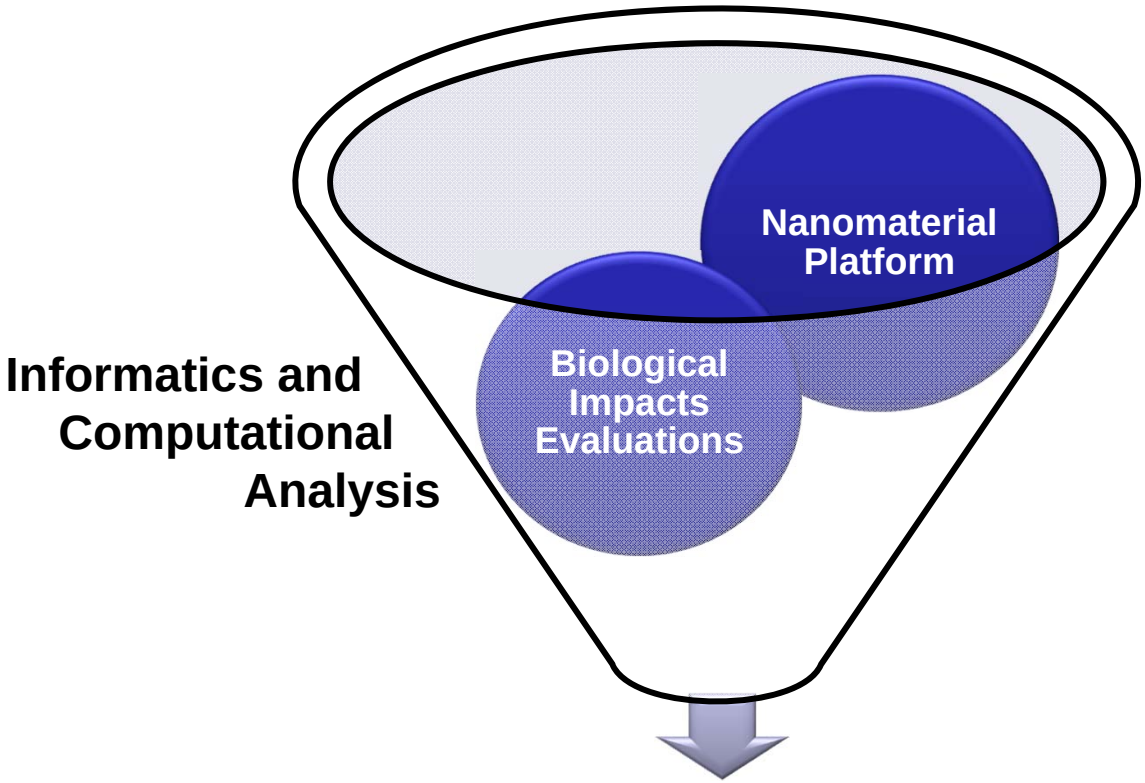


http://www.vincentherr.com/cf/nanomain.html

Small changes in nanomaterial can alter conditional behaviors of nanomaterials (performance, exposure, hazard)

Toward Establishing SARs

H_o: A select set of inherent nanomaterial features can be used to predict their environmental behavior and biological impacts (conditional behavior).



Biological Impacts Evaluations

Embryonic Zebrafish Assay

In vivo system to rapidly screen for biological impacts

General Attributes

Share molecular, cellular and physiological characteristics with other vertebrates

Develop rapidly

Easy to maintain

Toxicity Evaluation

Large sample sizes

Many routes of exposure

Transparent - non-invasive evaluations

Amenable to mechanistic evaluations

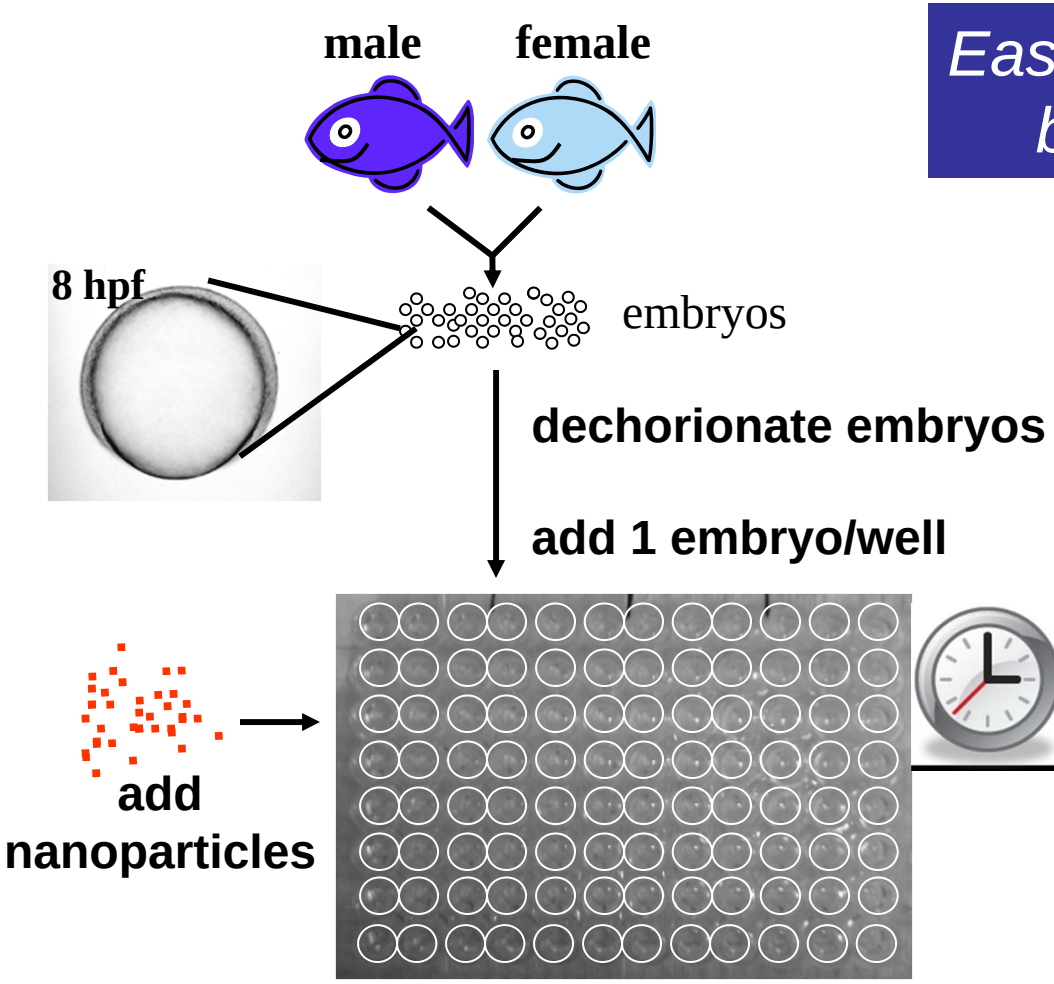
Investigate genomic → whole animal responses in same organism

Full suite of molecular signaling necessary and active early in development

Biological Impacts Evaluations

Embryonic Zebrafish Assay

Experimental Design



Ease of cell culture, benefits of whole animal!

Harper et al., J Exp Nanoscience, 2008

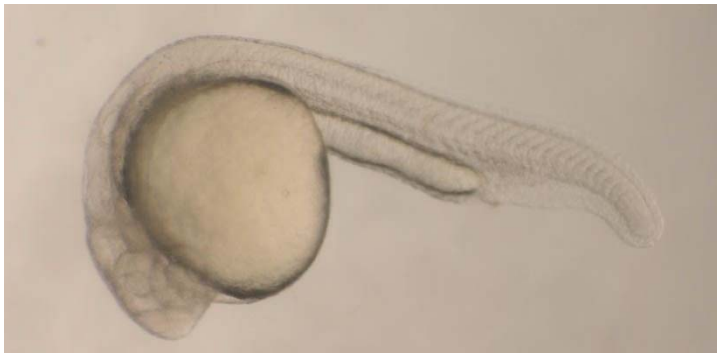
Biological Impacts Evaluations

Embryonic Zebrafish Assay

24 hpf evaluations

Mortality (**mort**)
Developmental progression (**dp**)
Spontaneous movement (**sm**)
Notochord (**nc**)

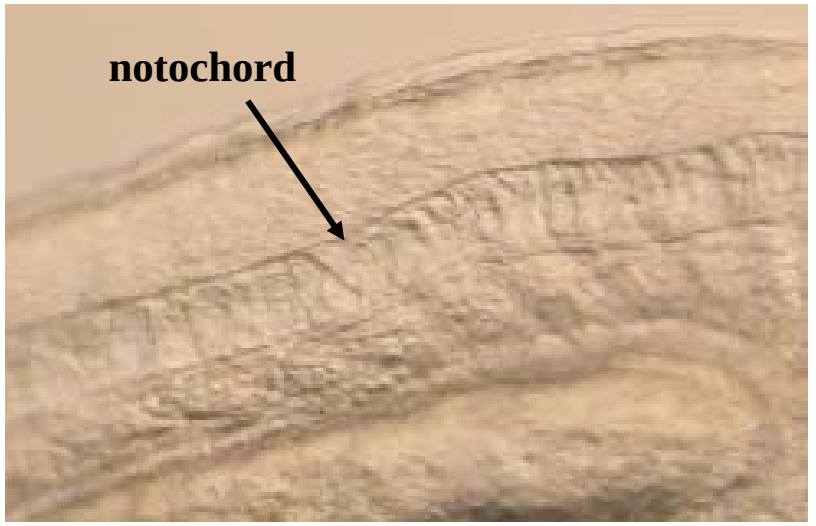
CONTROL



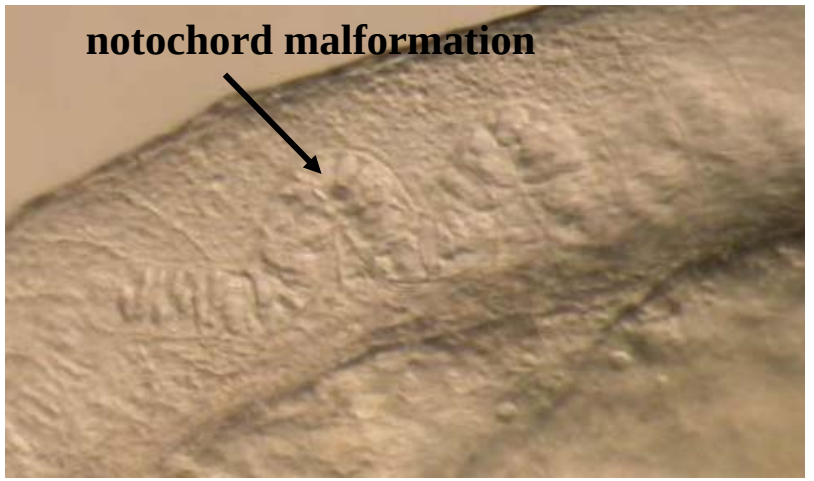
varying degrees of delayed development

EXPOSED

CONTROL



EXPOSED



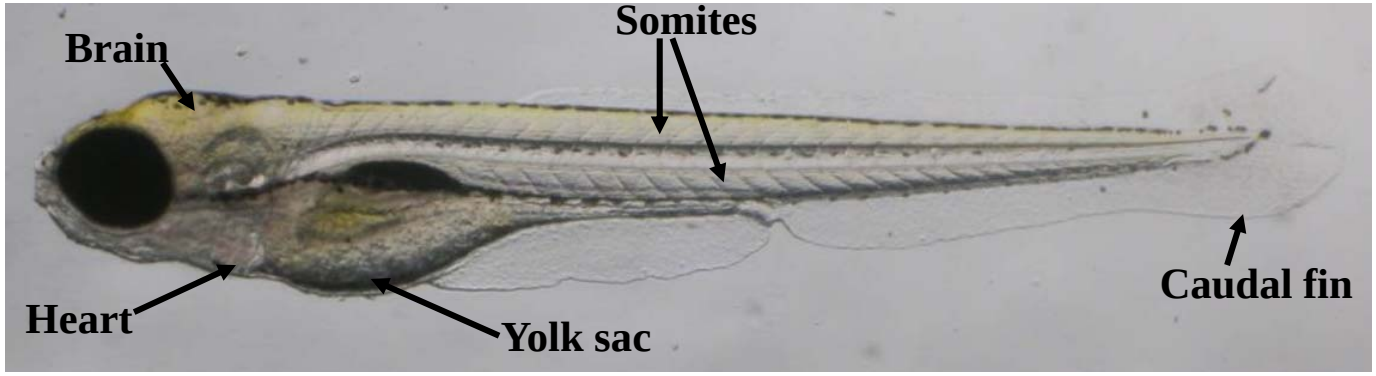
Biological Impacts Evaluations

Embryonic Zebrafish Assay

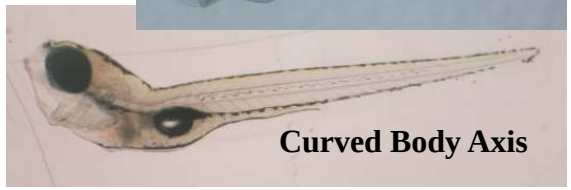
120 hpf evaluations

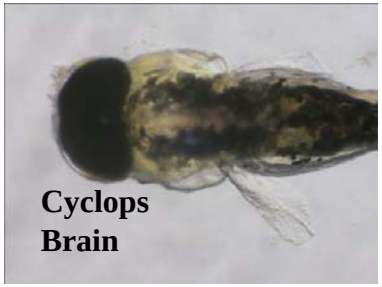
Mortality (**mort**)
Yolk sac edema (**YSE**)
Body axis (**axis**)
Eye
Snout
Jaw
Otic vessicle (**otic**)
Pericardial edema (**PE**)
Brain
Somites
Pectoral fin (**pfin**)
Caudal fin (**cfin**)
Pigmentation (**pig**)
Circulation (**circ**)
Trunk
Swim bladder (**swim**)
Motility (touch response, **tr**)

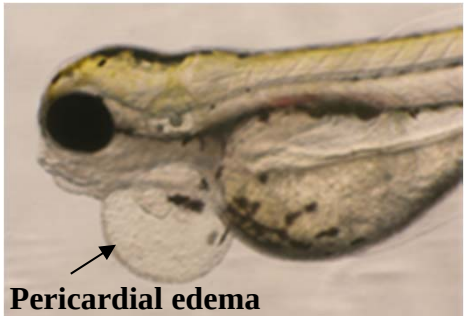
CONTROL

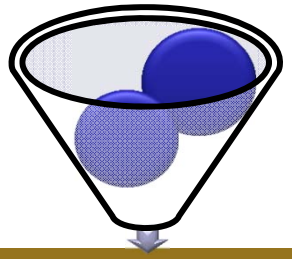


EXPOSED







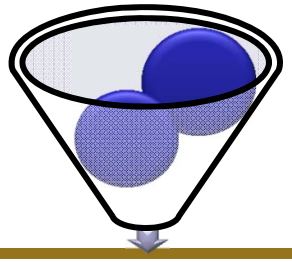


Informatics and Computational Analysis

EZ Metric (embryonic zebrafish metric)
A single metric representative of lethal and sublethal impacts on embryonic zebrafish in a screening-level assay

- Combines 21 endpoints of morbidity and mortality
- Additive EZ Metric
    - Sum of all effects independent of impact
    - *e.g.*, eye malformation = mortality

Concentrations (ppm)	Weighted EZ Metric	Additive EZ Metric
0.00	0.04	0.04
0.02	0.00	0.00
0.08	0.00	0.00
0.40	0.08	0.17
2	0.21	0.38
10	0.34	0.54
50	0.78	1.46
250	1.00	1.00



Informatics and Computational Analysis

EZ Metric

A single metric representative of lethal and sublethal impacts on embryonic zebrafish in a screening-level assay

- Combines 23 endpoints
- Additive EZ Metric
- Weighted EZ Metric
  - Weighted metric based on hierarchical ranking of effects
  - Takes into account embryo survivability

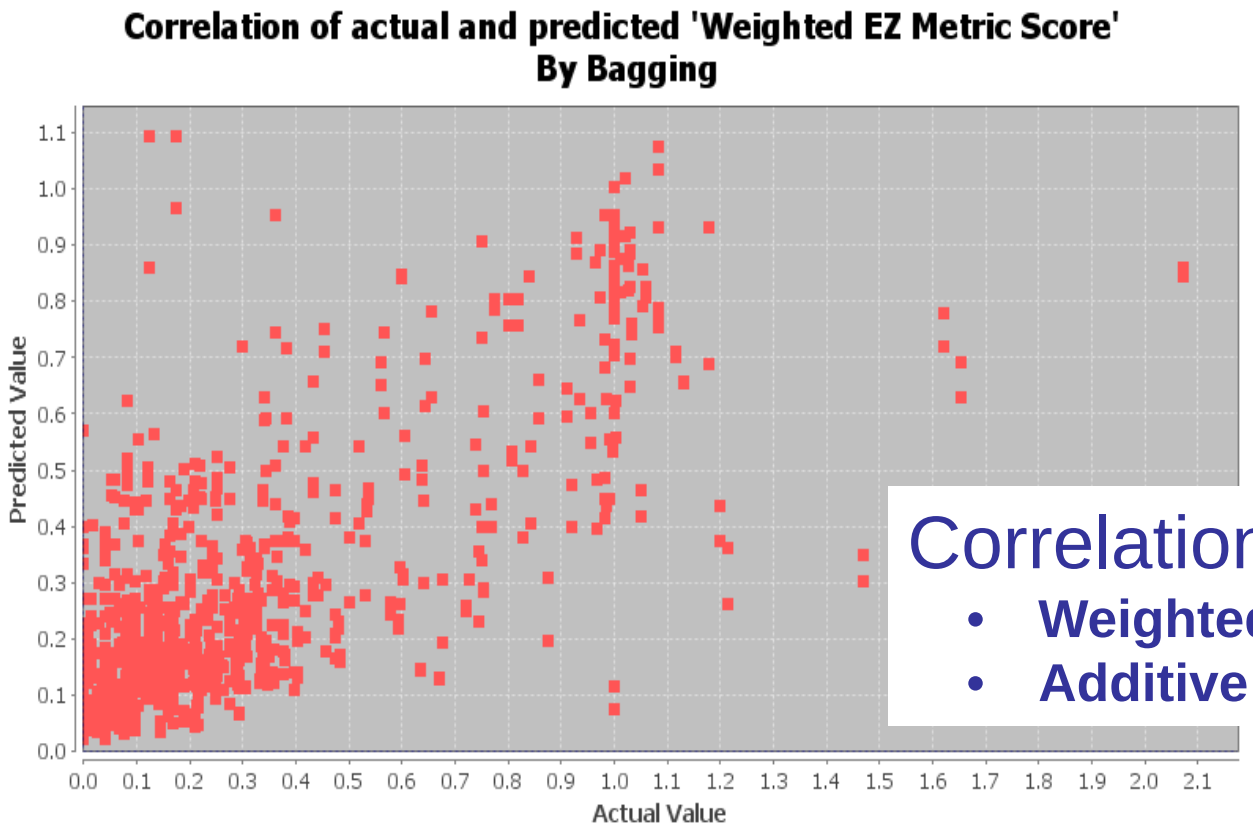
- Calculations:

$$\frac{\text{frequency of effect} \times \text{hierarchical ranking}}{\text{weighted EZ Metric}}$$

Ranking	Effect
1	Mortality – 24 hpf
0.08	Notochord Malformation
0.06	Developmental Progression (delayed development)
0.04	Spontaneous Movement
0.95	Mortality – 120 hpf
0.12	Heart Malformation
0.12	Brain Malformation
0.10	Yolk Sac Edema
0.08	Axis Malformation
0.06	Trunk Malformation
0.04	Eye Malformation
0.04	Jaw Malformation
0.04	Circulation
0.02	Snout Malformation
0.02	Otic Malformation
0.02	Somite Malformation
0.02	Pectoral Fin Malformation
0.02	Caudal Fin Malformation
0.02	Pigmentation
0.02	Swim Bladder
0.02	Touch Response

Embyronic Zebrafish Assay

Weighted EZ Metric more predictive than Additive



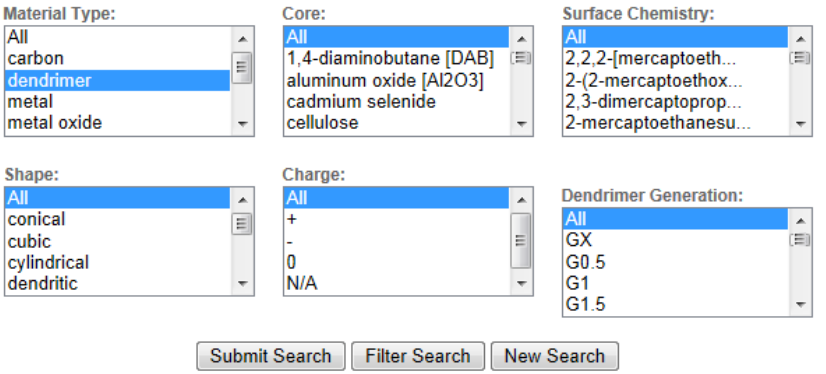
Correlation Map

- **Weighted 0.79**
- **Additive 0.65**

Liu et al., Int J Nanomedicine, 2013
Tang et al., Int J Nanomedicine, 2013

nanomaterial–biological interactions

Nanomaterial-Biological Interactions Knowledgebase



Link to material record

Link to experimental data

Data repository

Nanomaterial							EZ Metric								
ID	Family	Core	Surface Chemistry	Shape	Size	Charge	Concentration								
nbi_0010	metal	gold [Au]	2-mercaptoethanesu...	spherical	1.5	-	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.07	0.08	0.05	0.00	0.08	View
nbi_0004	metal	gold [Au]	2-(2-mercaptoethox...	spherical	0.8	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.04	0.02	0.04	0.04	0.09	0.15	View
nbi_0007	metal	gold [Au]	N,N,N-trimethylamm...	spherical	1.5	+	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.03	0.13	0.36	0.74	0.77	0.98	1.03	1.00	View
nbi_0013	metal	gold [Au]	6-mercaptohexanoic...	spherical	10	-	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.08	0.02	0.10	0.04	0.13	0.57	0.99	View
nbi_0012	metal	gold [Au]	N,N,N-trimethylamm...	spherical	10	+	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.00	0.04	0.08	0.65	0.93	View

http://nbi.oregonstate.edu/

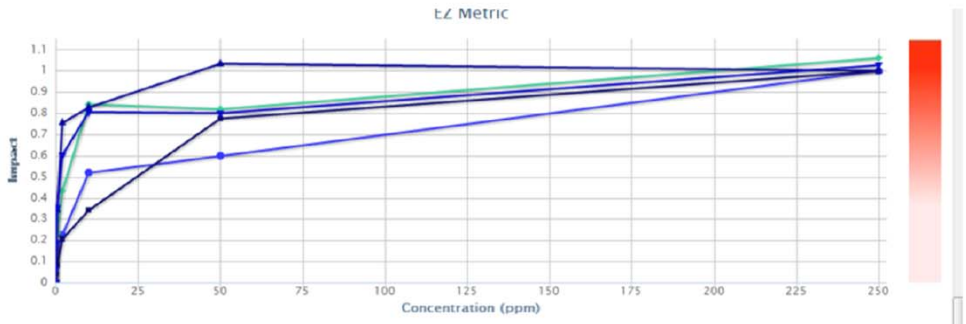
Material Record

Experimental Data

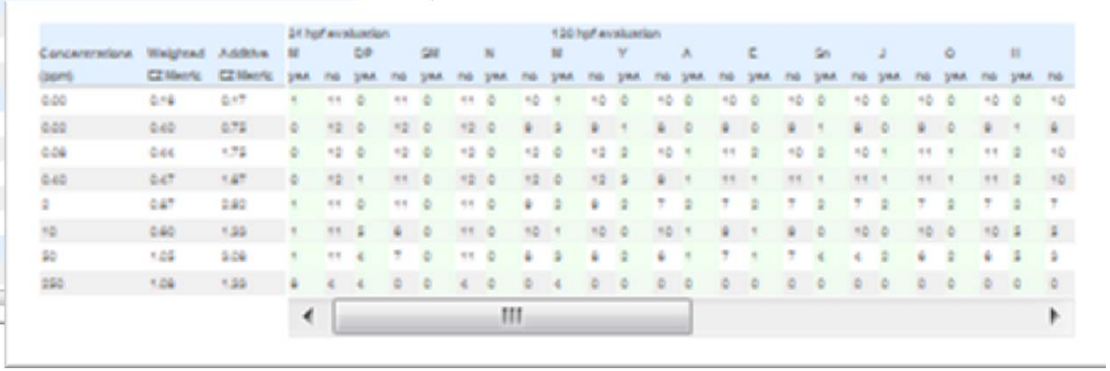
Nanomaterial -Biological Interactions Knowledgebase

NBI Knowledgebase

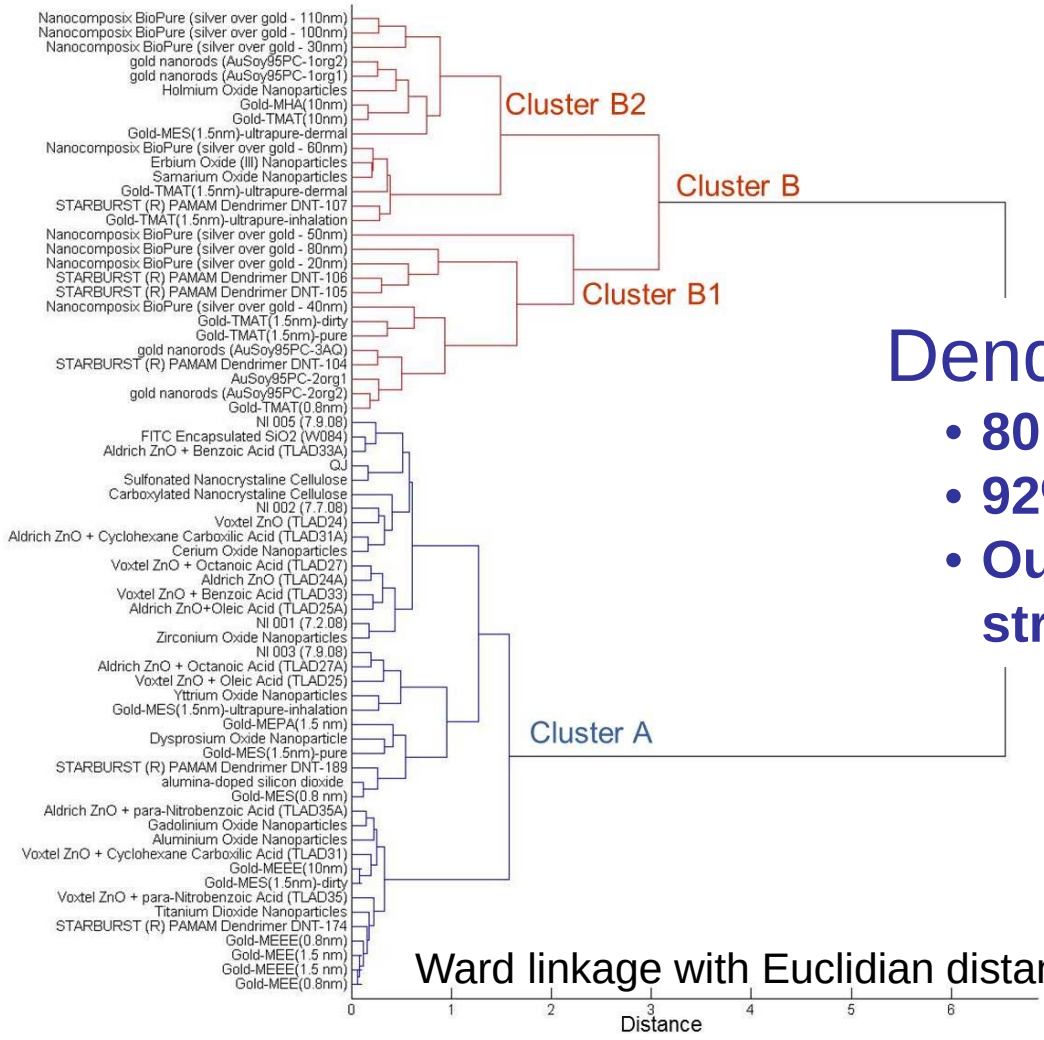
Field	Value
NBI Material Identifier	44
Particle Descriptor	alumina-doped silicon diox
Investigator / Material Data Contributor: Name	Stacey Harper
Investigator / Material Data Contributor: Affiliation	Oregon State University
Investigator / Material Data Contributor: Email	stacey.harper@oregonstat
Material Type	metal oxide
Manufacture Date	
Manufacturer	Sigma-Aldrich
Synthesis Process	
Synthesis Precursors	
Purity	pure
Types of Impurities	
Primary Particle / Material Core Data:	
Primary Particle Size: Avg. (nm)	24.5
Primary Particle Size: Min. (nm)	0
Primary Particle Size: Max (nm)	49
Method of Size Measurement	BET
Instrument Used for Size Measurement	
Core Shape	irregular-angular
Core Structure	
Crystal Structure	
Core Atomic Composition	silicon dioxide [SiO2];aluminum oxide [Al2O3]
Number of Core Atoms	
Mass Core Atoms (ng)	
Core Shell / Coating (if present):	
Shell Composition	
Shell Surface Shape	
Shell Linkage	
Surface Linkages / Ligands (if present):	
Outermost Surface Functional Groups	
Surface Chemistry Linkage Group / Type	
Density of Surface Covered with Ligands (%)	
Minimum Number of Ligands	
Maximum Nunber of Ligands	
Complete Material:	
Mass of Core + Shell + Linkages and Ligands (ng)	



Nanomaterial							EZ Metric								
ID	Family	Core	Surface Chemistry	Shape	Size	Charge	Concentration								
nbi_0073	metal	gold [Au]	phosphatidylcholine	rod	7	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.15	0.23	0.52	0.60	1.00	View
nbi_0075	metal	gold [Au]	phosphatidylcholine	rod	14	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.09	0.24	0.43	0.64	0.82	1.00	View
nbi_0072	metal	gold [Au]	phosphatidylcholine	rod	7	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.04	0.00	0.00	0.08	0.21	0.34	0.78	1.00	View
nbi_0074	metal	gold [Au]	phosphatidylcholine	rod	22	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.34	0.75	0.83	1.03	1.00	View
nbi_0076	metal	gold [Au]	phosphatidylcholine	rod	14	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.06	0.19	0.35	0.60	0.81	0.80	1.03	View



Clustering Analysis of EZ Metrics

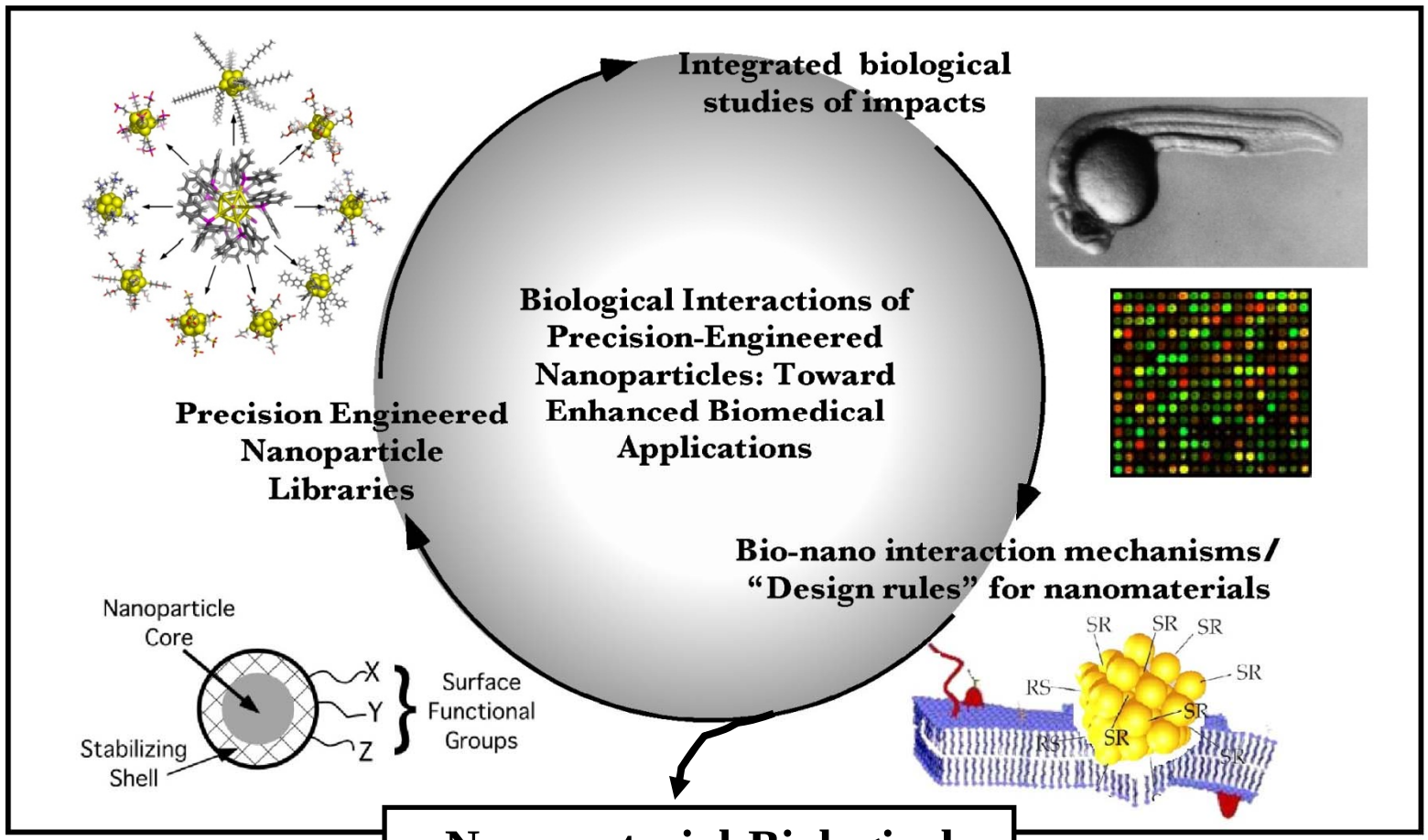


Ward linkage with Euclidian distance measure

Dendrogram plot

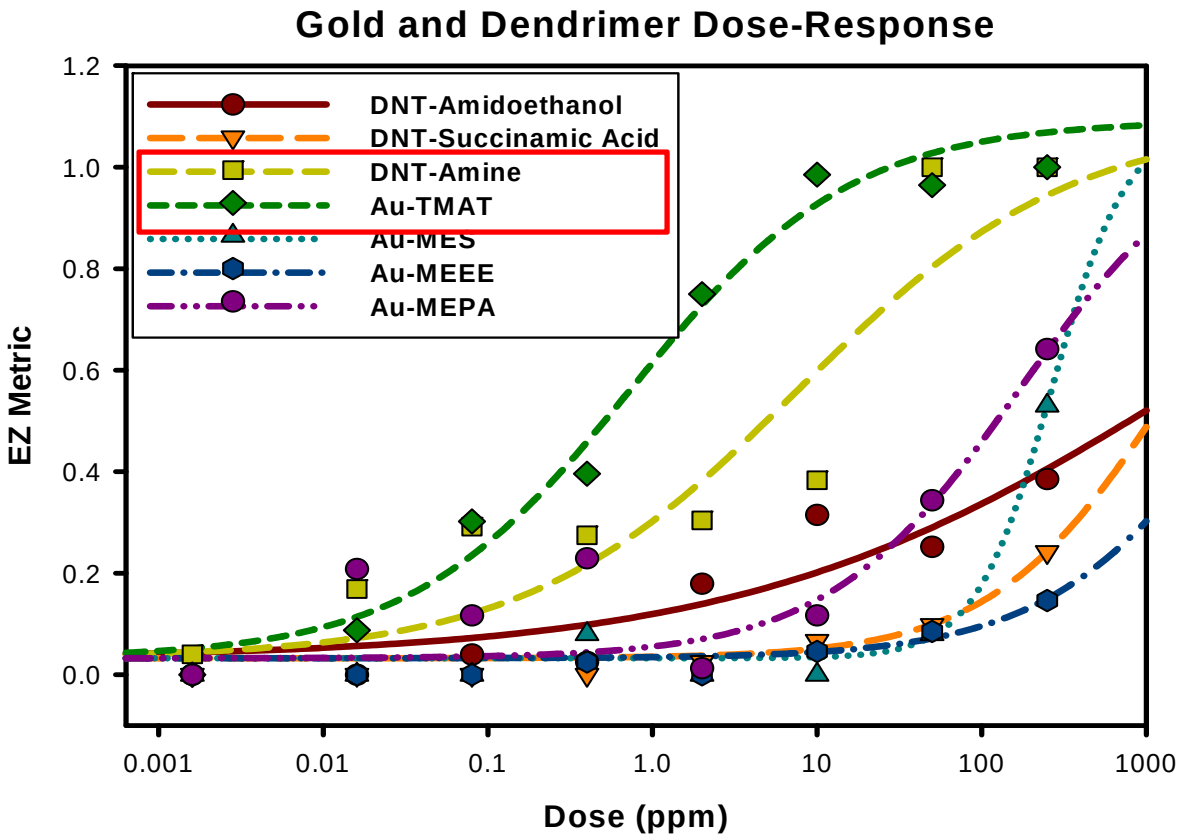
- **80 nanomaterial files**
- **92% accuracy**
- **Outermost surface chemistry strongest predictor**

Embryonic Zebrafish Assay: Iterative Testing to Gain Knowledge

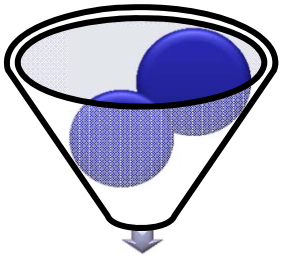


Nanomaterial-Biological Interactions Knowledgebase

Dose-Response of Weighted EZ Metric Scores

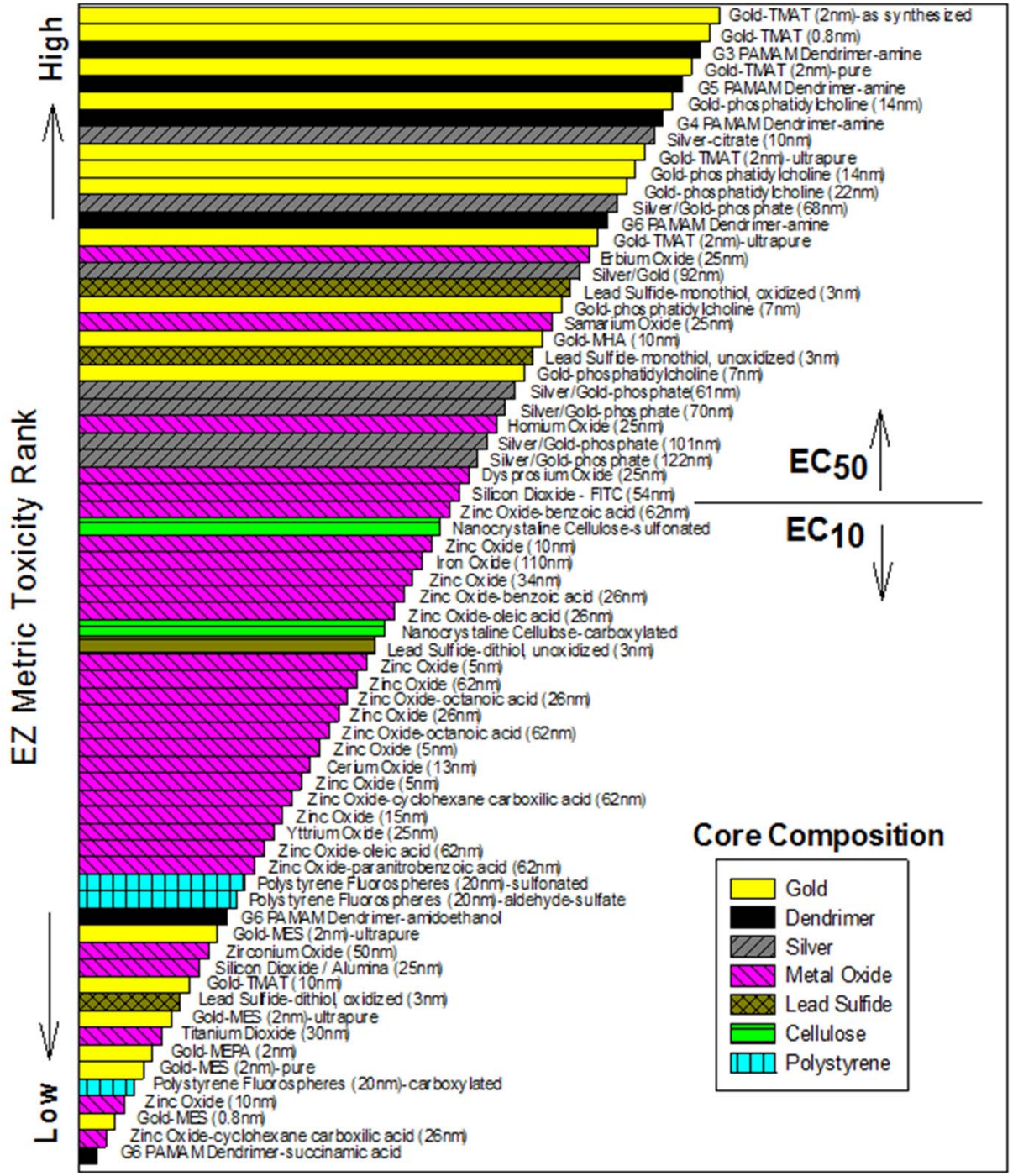


Nanomaterial	Charge
DNT-amidoethanol	neutral
DNT-succinamic acid	negative (-)
DNT-amine	positive (+)
Au-MEEE	neutral
Au-MEPA	negative (-)
Au-MES	negative (-)
Au-TMAT	positive (+)



Nanomaterial Hazard Ranking Based on EZ Metric Scores

Color-coded to core composition



Embyronic Zebrafish Assay

nanomaterial–biological interactions

EZ Metric for Nanomaterial Hazard ID

- A single metric representative of lethal and sublethal impacts on embryonic zebrafish in a screening-level assay
- Utility in hypothesis generation
- Rapid, low-cost whole animal model

*BUT…this is **one** type of organism in **one** exposure scenario*

Movement toward weight-of-the-evidence will require additional datasets to be considered.

Toward a Community-Driven Informatics Framework

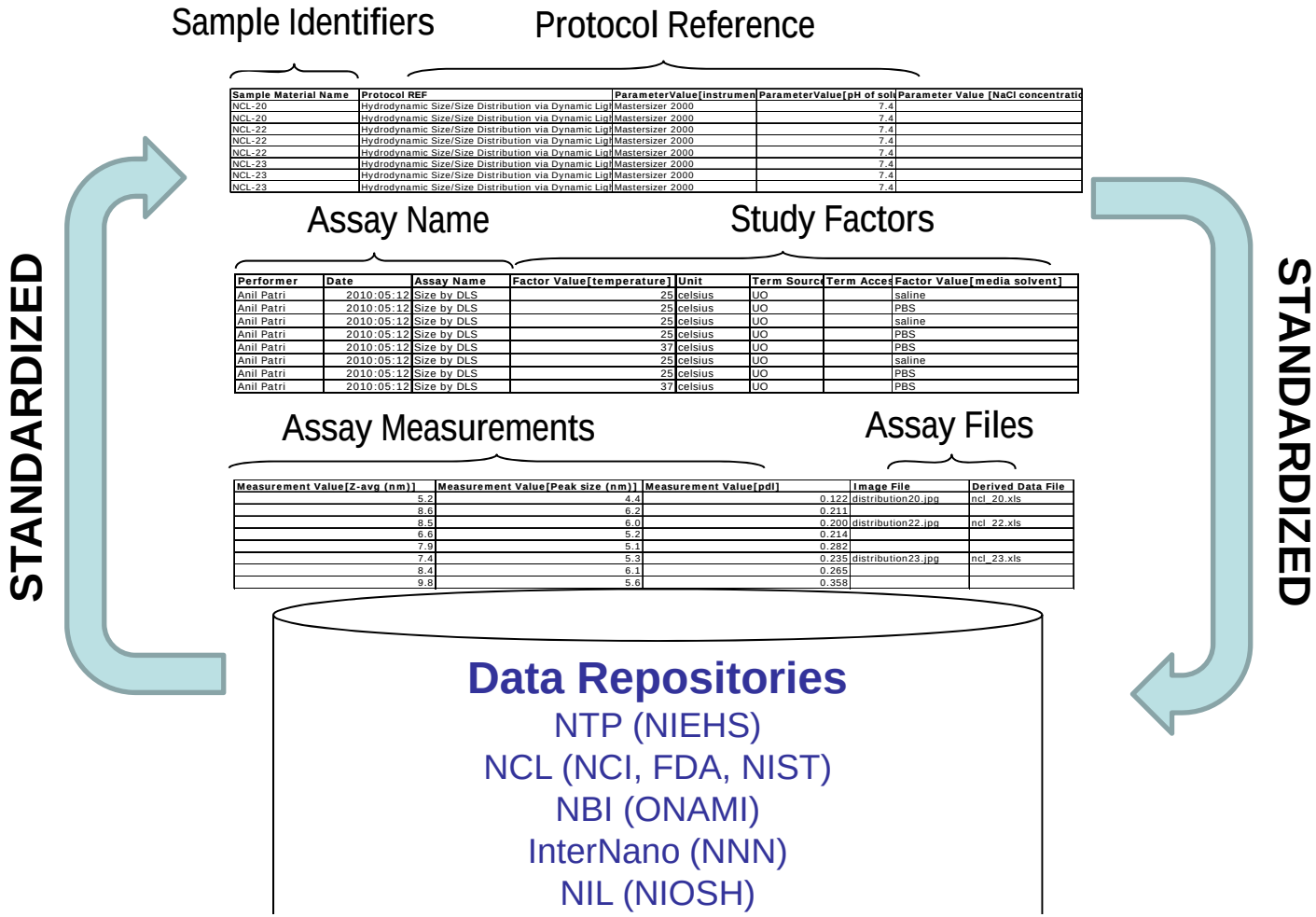
Current Limitations to Data Integration

- Protocols not standardized – lack key information for replication
- Lack of standards for nanomaterial characterization and representing nanomaterial 'structure'
- Lack of standards for data sharing
- Nanomaterials studied often produced in small batches
  - Batch to batch variability
  - Limits sharing of nanomaterials for inter-laboratory testing
  - Material quantity is limited
  - Diversity of data
  - Inconsistency of data

nanomaterial–biological interactions

ISA-TAB-Nano: A standard tab-delimited format for data exchange

Specification to facilitate the import/export of data on nanomaterials and their characterizations to/from nanotechnology resources



Sample Material Name	Protocol REF	ParameterValue[instrumen	ParameterValue[pH of sol	Parameter Value [NaCl concentratio
NCL-20	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-20	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-22	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-22	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-22	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-23	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-23	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	
NCL-23	Hydrodynamic Size/Size Distribution via Dynamic Ligh	Mastersizer 2000	7.4	

Performer	Date	Assay Name	Factor Value[temperature]	Unit	Term Source	Term Acces	Factor Value[media solvent]
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		saline
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		PBS
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		saline
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		PBS
Anil Patri	2010:05:12	Size by DLS	37	celsius	UO		PBS
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		saline
Anil Patri	2010:05:12	Size by DLS	25	celsius	UO		PBS
Anil Patri	2010:05:12	Size by DLS	37	celsius	UO		PBS

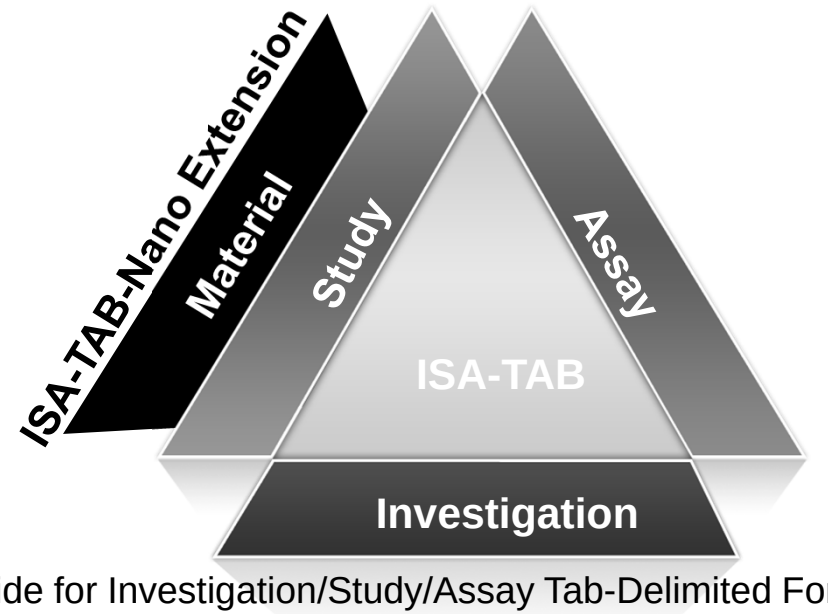
Measurement Value[Z-avg (nm)]	Measurement Value[Peak size (nm)]	Measurement Value[pdl]	Image File	Derived Data File
5.2	4.4	0.122	distribution20.jpg	ncl_20.xls
8.6	6.2	0.211		
8.5	6.0	0.200	distribution22.jpg	ncl_22.xls
6.6	5.2	0.214		
7.9	5.1	0.282		
7.4	5.3	0.235	distribution23.jpg	ncl_23.xls
8.4	6.1	0.265		
9.8	5.6	0.358		

ISA-TAB-Nano Standard
A tab-delimited format for data exchange

Describes data related to investigations, nanomaterials, studies and assays

Leverages and extends the Investigation/Study/Assay (ISA-TAB) developed by the European Bioinformatics

ISA-TAB-Nano supports ontology-based curation; nanomaterials and concepts from the NanoParticle Ontology (NPO)

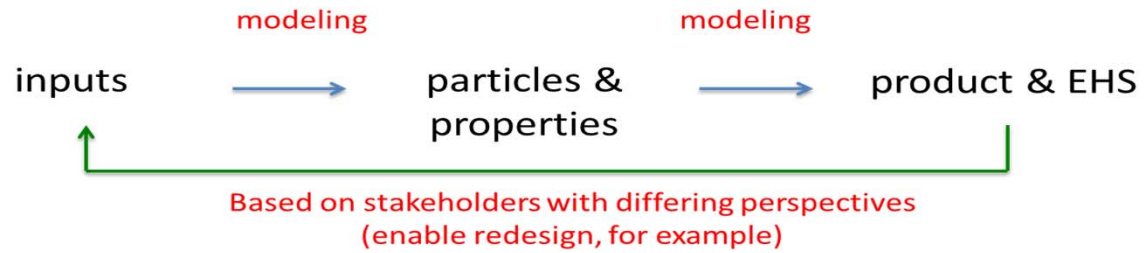


ASTM E2909: Guide for Investigation/Study/Assay Tab-Delimited Format for Nanotechnologies (ISA-TAB-Nano): Standard File Format for the Submission and Exchange of Data on Nanomaterials and Characterizations

Toward a Community-Driven Informatics Framework

Promoting Data Integration

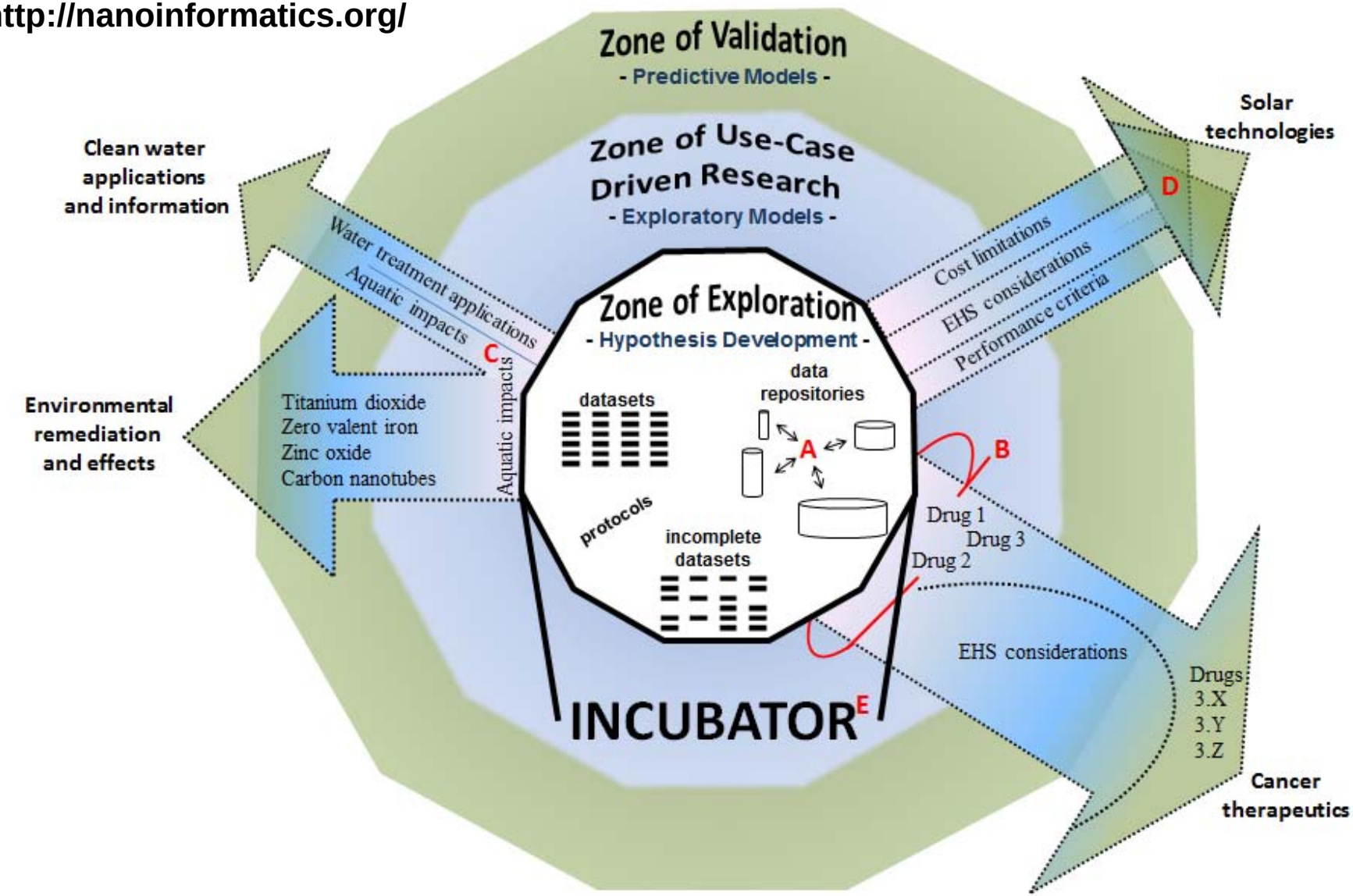
- Informatics framework can provide needed organization for diverse and often disparate data
- Application of weight-of-the-evidence to larger volume of data
- Data sharing mechanisms need to be expanded
  - ISA-TAB-Nano
- Community driven standards
- Use-case driven collaborations to define required information and level of detail
- Value added examples
  - Manufacturing performance:EHS measures



nanomaterial–biological interactions

Thoughts from the 2012 Nanoinformatics Workshop

http://nanoinformatics.org/



Harper, SL, J Hutchison, N Baker, M Ostraat, S Tinkle, J Steevens, MD Hoover, J Adamick , K Rajan, S Gaheen, Y Cohen, A Nel, R Cachau and M Tuominen. 2013. Nanoinformatics infrastructure: Current resources, community needs, and the proposal of a collaborative framework for data sharing and information integration. Computational Science and Discovery, *In press.*

Thank you for your attention

"I'm on board for microbrews, but nanopizza is taking technology a step too far."

Balbus et al. (2005) Issues in Science and Technology