Improving the reliability of in vitro toxicity and ecotoxicity measurements with nanomaterials

**Elijah J. Petersen, Shannon Hanna, Monique Johnson, Gregory Cooksey, Bryan Nelson, John Elliott, Lee Yu**
National Institute of Standards and Technology (NIST)
Presented 3/10/2015

Overview

1. **Introduction/Background**
2. **Use of cause-and-effect analysis to design a robust nanoecotoxicity assay with *C. elegans***
3. **Single-particle ICP-MS method to quantify the size distribution of gold nanoparticle uptake by *C. elegans***

NIST Role in Nano-EHS
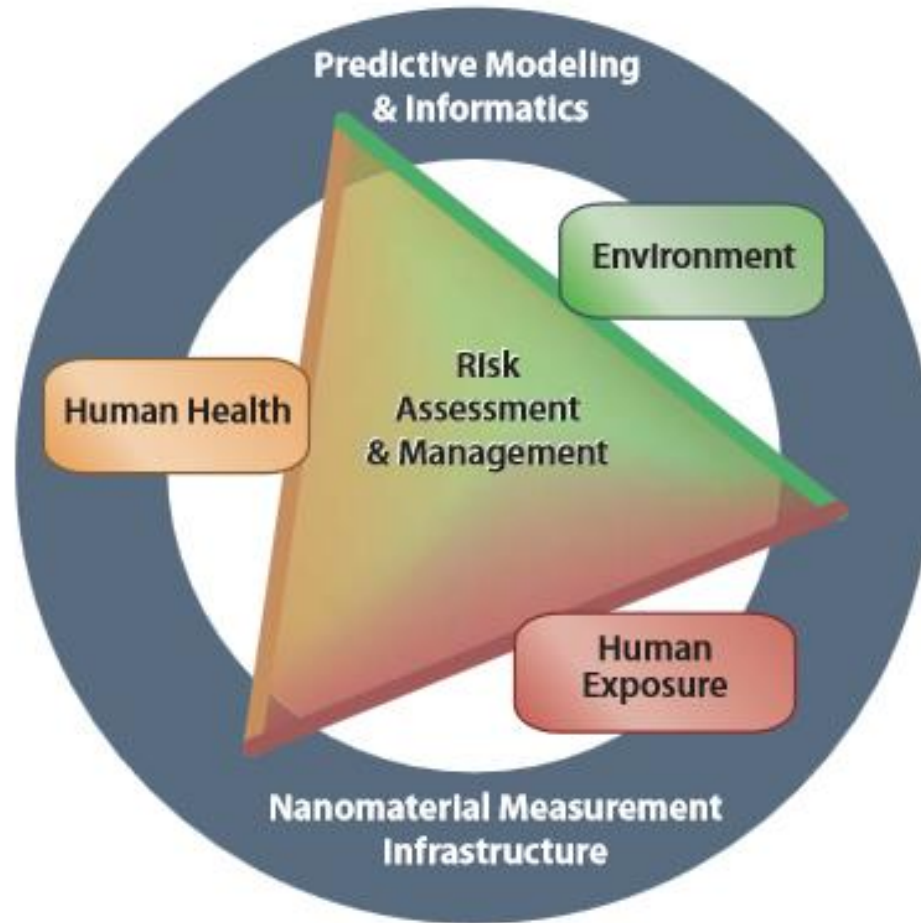
National Nanotechnology Initiative 2011 Environmental Health and Safety Research Strategy

NIST & NanoToxicology

Three Major Areas

- Standards

- Tools for Nanomaterial Characterization

- Methods/Data to inform health and environmental risk models

    - Robust methods to enable reliable toxicity testing

    - Metrology for evaluating transport and transformations of nanomaterials in biological and environmental systems
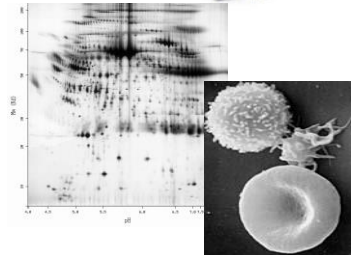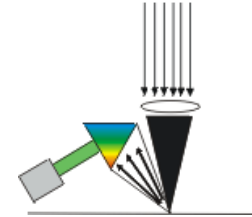
NIST Standard Reference Materials

Gold nanoparticles (10, 30, and 60 nm)

Single-wall carbon nanotube (raw soot) and dispersed into three length populations

Titanium dioxide nanoparticles (made from Degussa P25)

2 nm silicon nanoparticles

Silver nanoparticle (75 nm PVP coated)

Silver nanoparticles (10 nm in preparation)

Multiwall carbon nanotube (in preparation)

Can be useful for interlaboratory comparisons, instrument validation and calibration, and positive and negative controls for nanotoxicity studies

Critical for establishing comparability of nano-related measurements.

Documentary Standards

NIST participates in standards organizations that provide validated documentary standards on a range of topics
- Nanoparticle characterization using a range of instruments for all nanoparticles (DLS, TEM, etc.) through the NIST/NCL protocols
- Sonication protocols that provide reproducible, traceable NP sonication between instruments and laboratories
- MTS assay for cell toxicity from nanomaterials
- Guidance document for aquatic toxicity testing of nanomaterials

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Why *C. elegans*?

- Abundant in soils and sediments
- > 70% of *C. elegans* proteome has human homologues
- 12 out of 17 signal transduction pathways conserved between *C. elegans* and humans
- Complete cell lineage map, large knockout libraries, established genetic methods
- "easy" to culture
- Small size & short lifespan
- Higher-throughput *in vivo* assays
- Transparent

Leung et al, Toxicological Sciences 2009

ISO Method 10872

Preparation

Add *E. coli* to Luria Broth and incubate at 37°C for 17 h

↓

Plate *E. coli* on NGM and incubate overnight

↓

Add Dauer larvae to plate and incubate for 72 h

Assay

Add chemicals, *E. coli*, and J1 nematodes to 12 well plate, incubate at 20°C for 96 h

↓

Add 200 ul Rose Bengal, heat plate at 80°C for 10 min

↓

Allow plate to cool, add 1ml of mineral oil, image plate, and analyze images
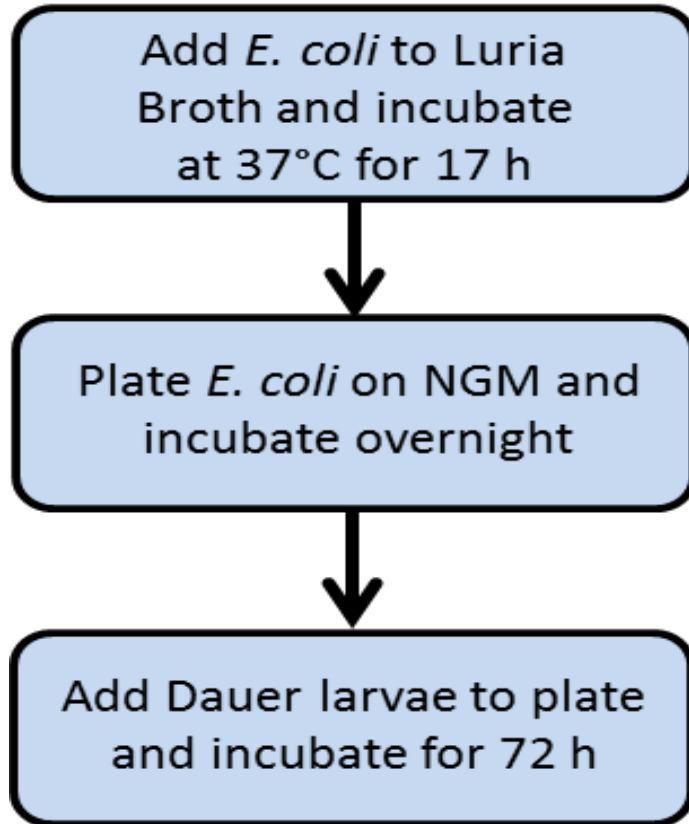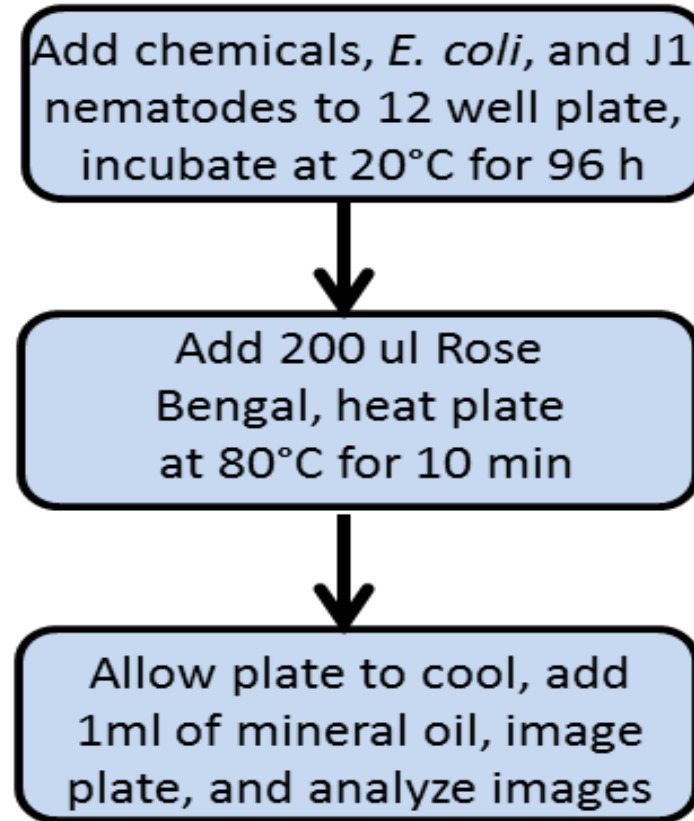
- Uses positive control benzylcetyldimethylammonium chloride (BAC C16 – EC_{50} = 15.1 mg l^{-1})
- Only test specification is growth inhibition of 20-80% at EC_{50} value

Automated *C. elegans* Imaging
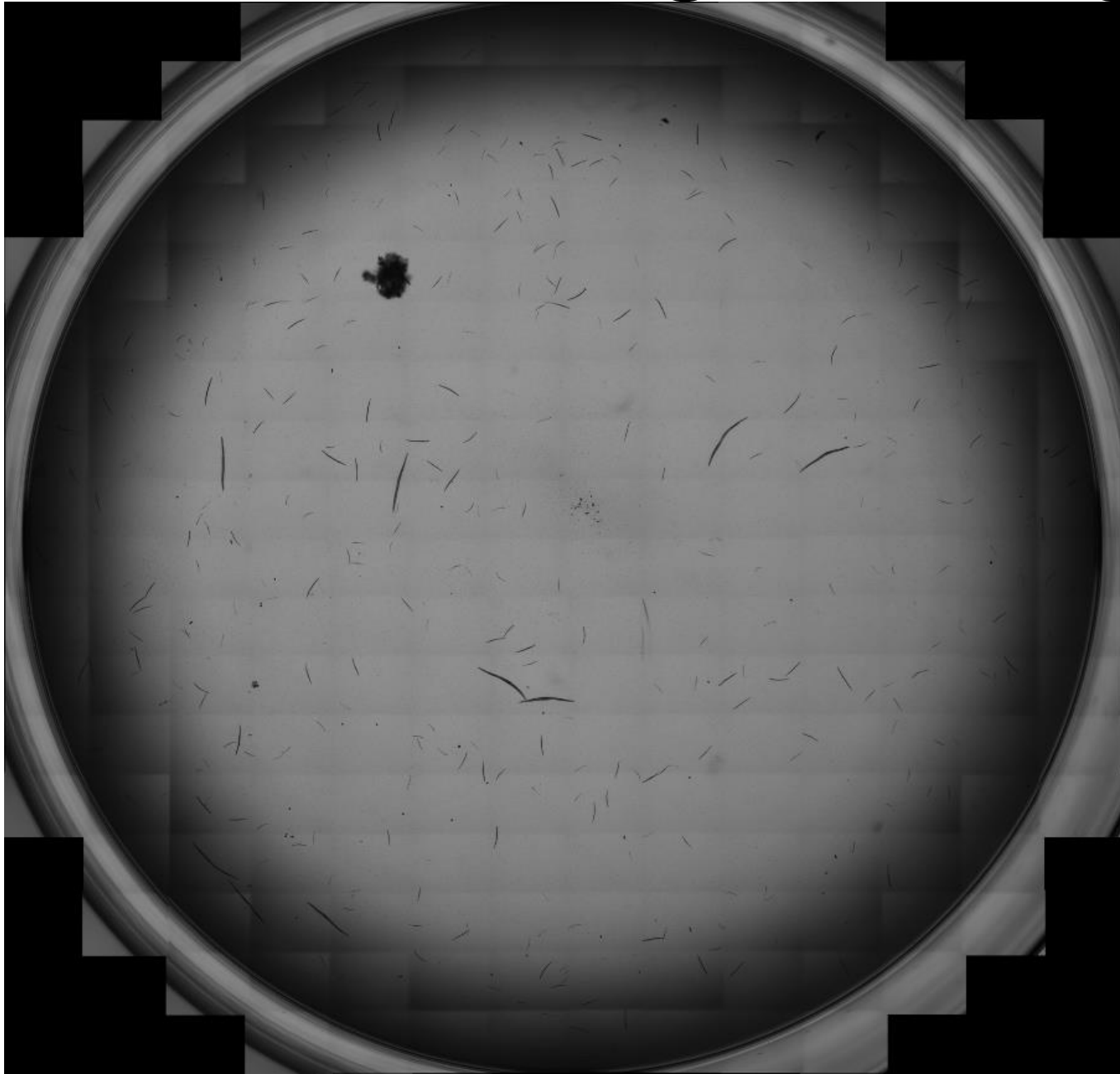
C. elegans Image Stitching
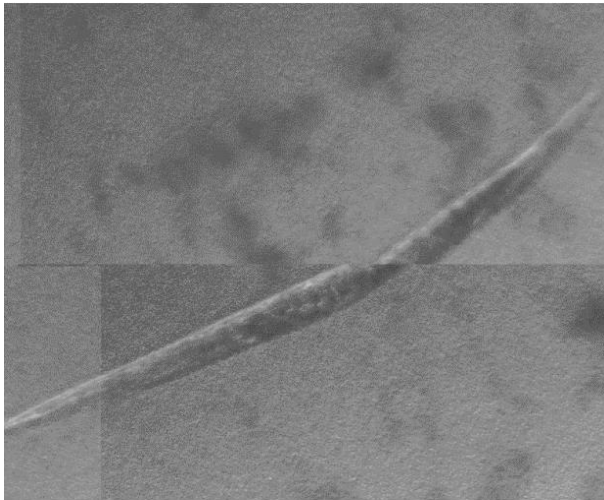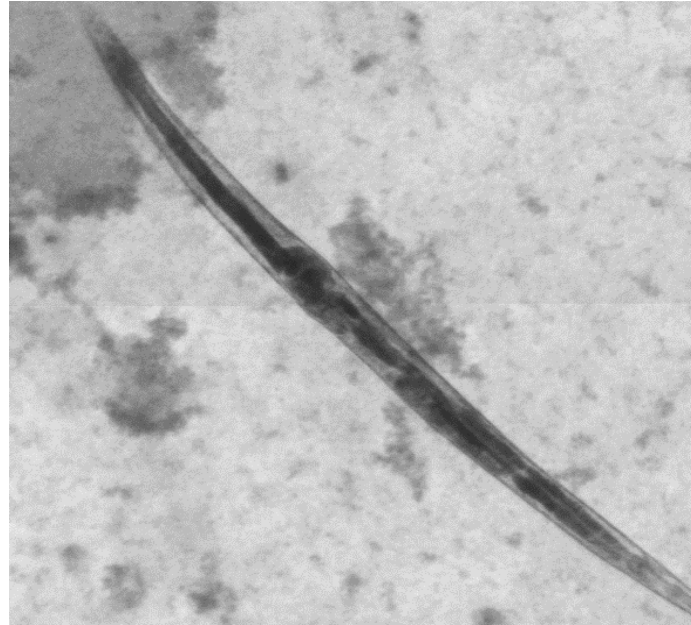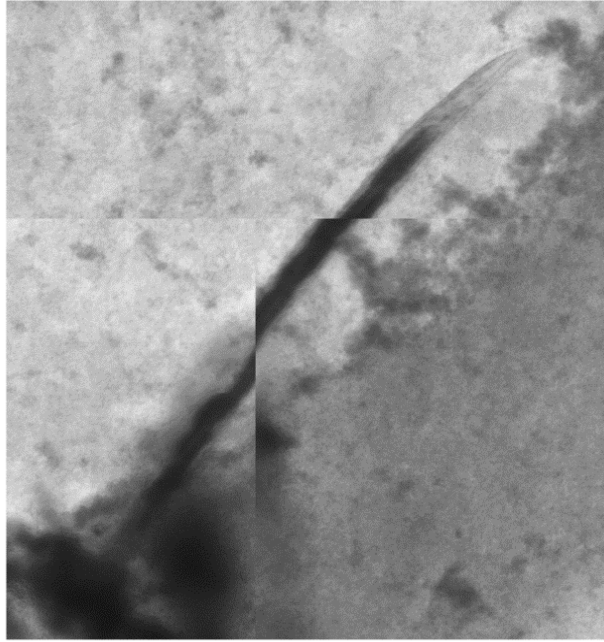
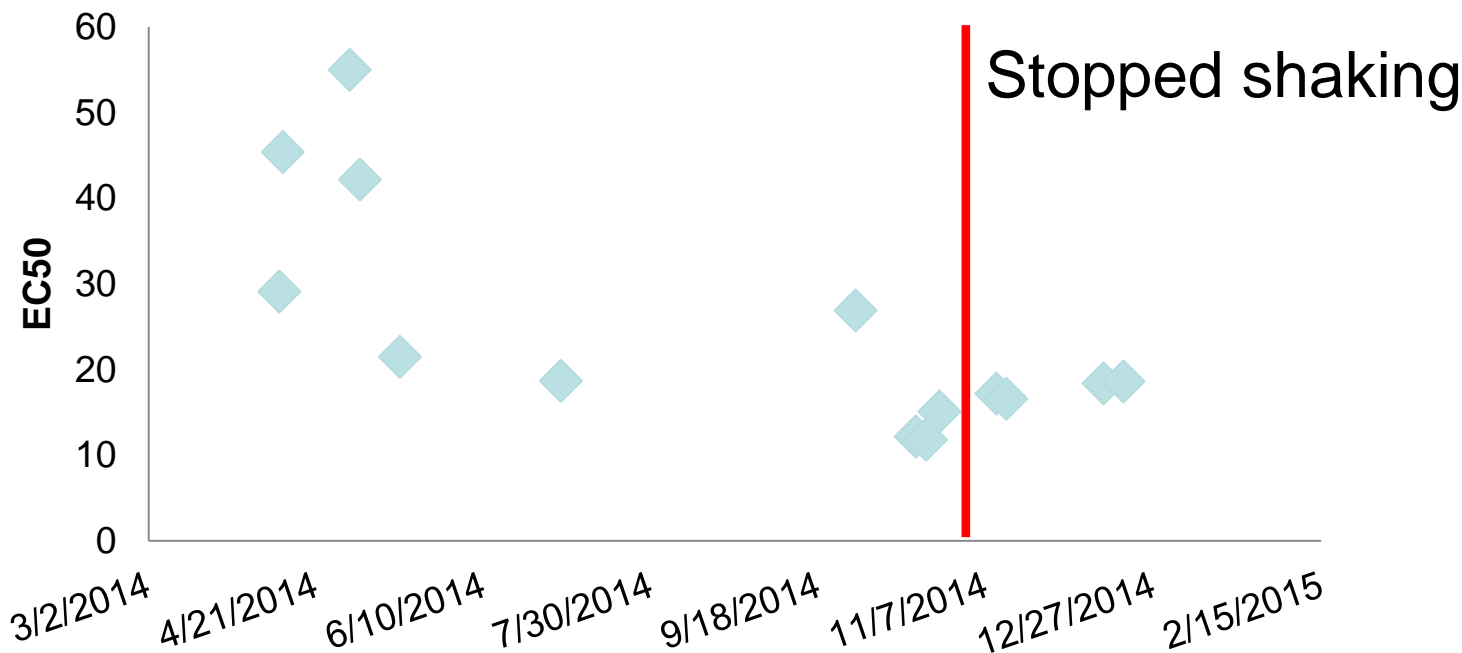# Use of Cause-and-Effect Analysis to Design a High-Quality Nanocytotoxicology Assay

Matthias Rösslein,[†,⊥] John T. Elliott,*[§,⊥] Marc Salit,[∥] Elijah J. Petersen,[§] Cordula Hirsch,[†] Harald F. Krug,[‡] and Peter Wick[†]

Poster: Toward achieving harmonization in a nano-cytoxicity assay measurement through an interlaboratory comparison study

Cause & Effect Analysis of *C. elegans* Assay
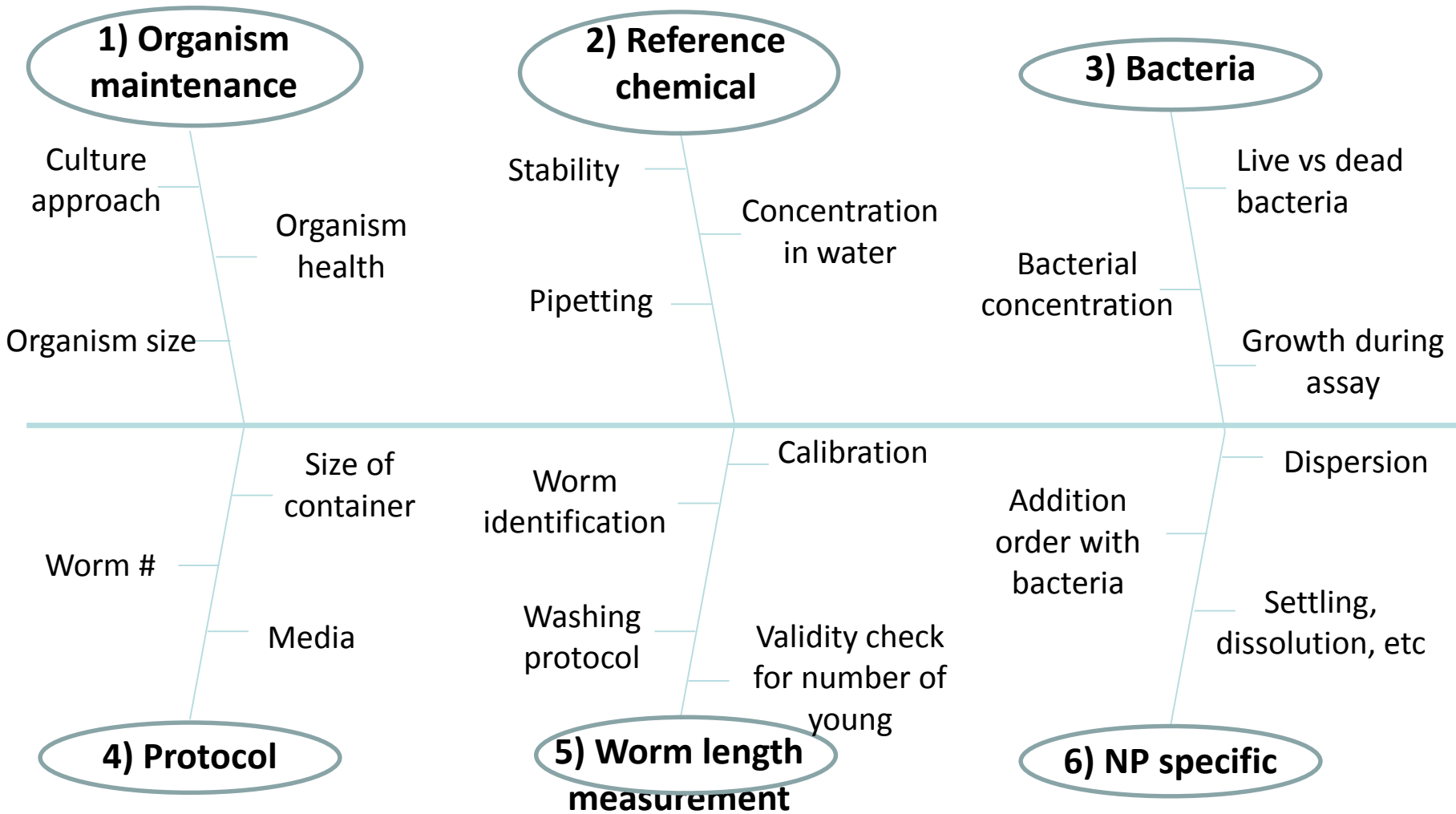
EC$_{50}$ of BAC C16 Dose-Response Curve

Stopped shaking

y-axis: EC50

x-axis: 3/2/2014, 4/21/2014, 6/10/2014, 7/30/2014, 9/18/2014, 11/7/2014, 12/27/2014, 2/15/2015

Growth inhibition of 15mg/L BAC C16

Stopped shaking

y-axis: Growth inhibition (%)

x-axis: 6/10/2014, 7/30/2014, 9/18/2014, 11/7/2014, 12/27/2014
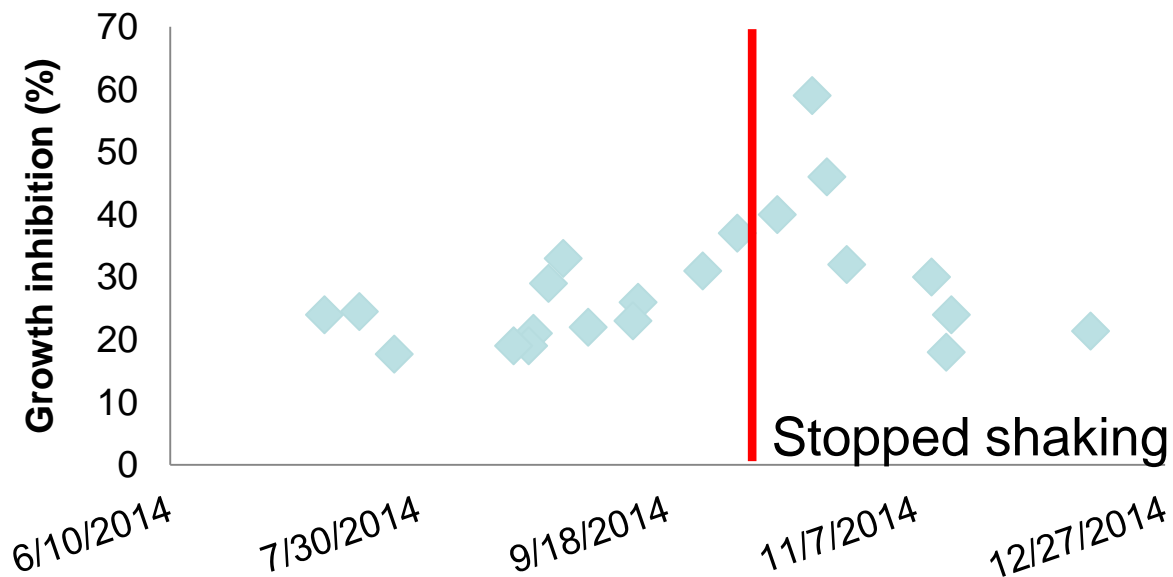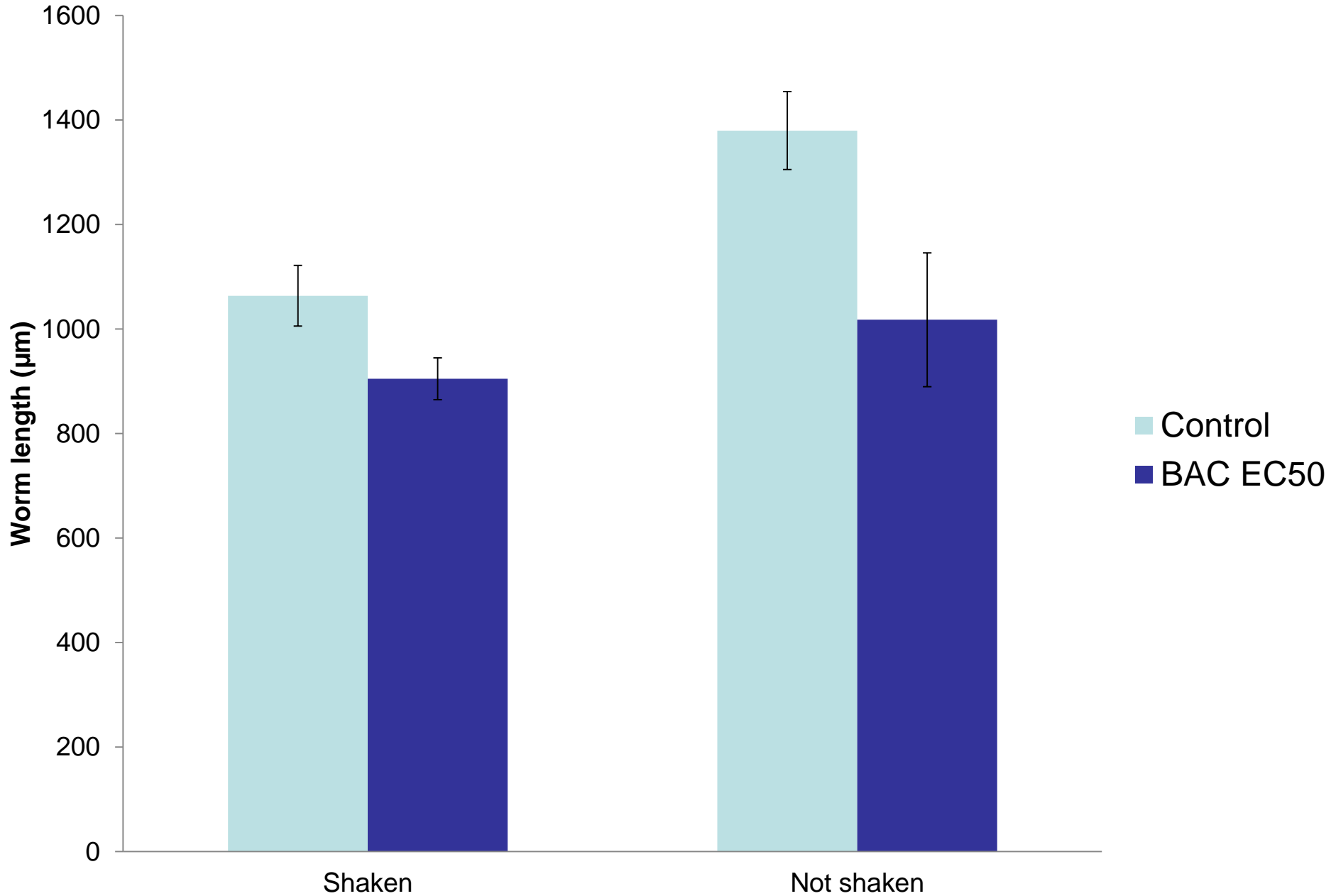
Plate shaking

Influence of media on BAC toxicity
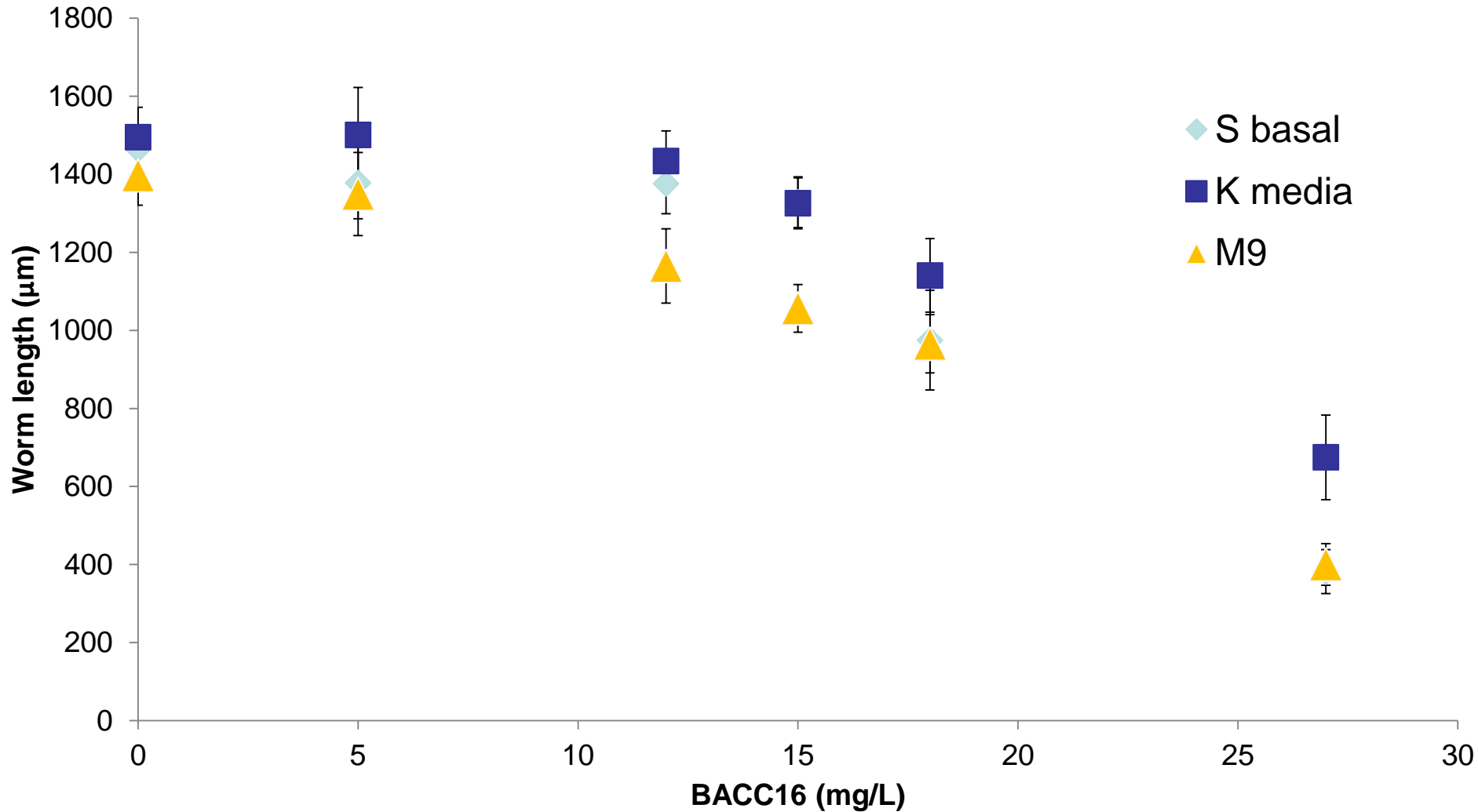
Influence of live/dead feed on bioassay

Worm Length (µm) vs BACC16 (mg/L)

Live
Dead
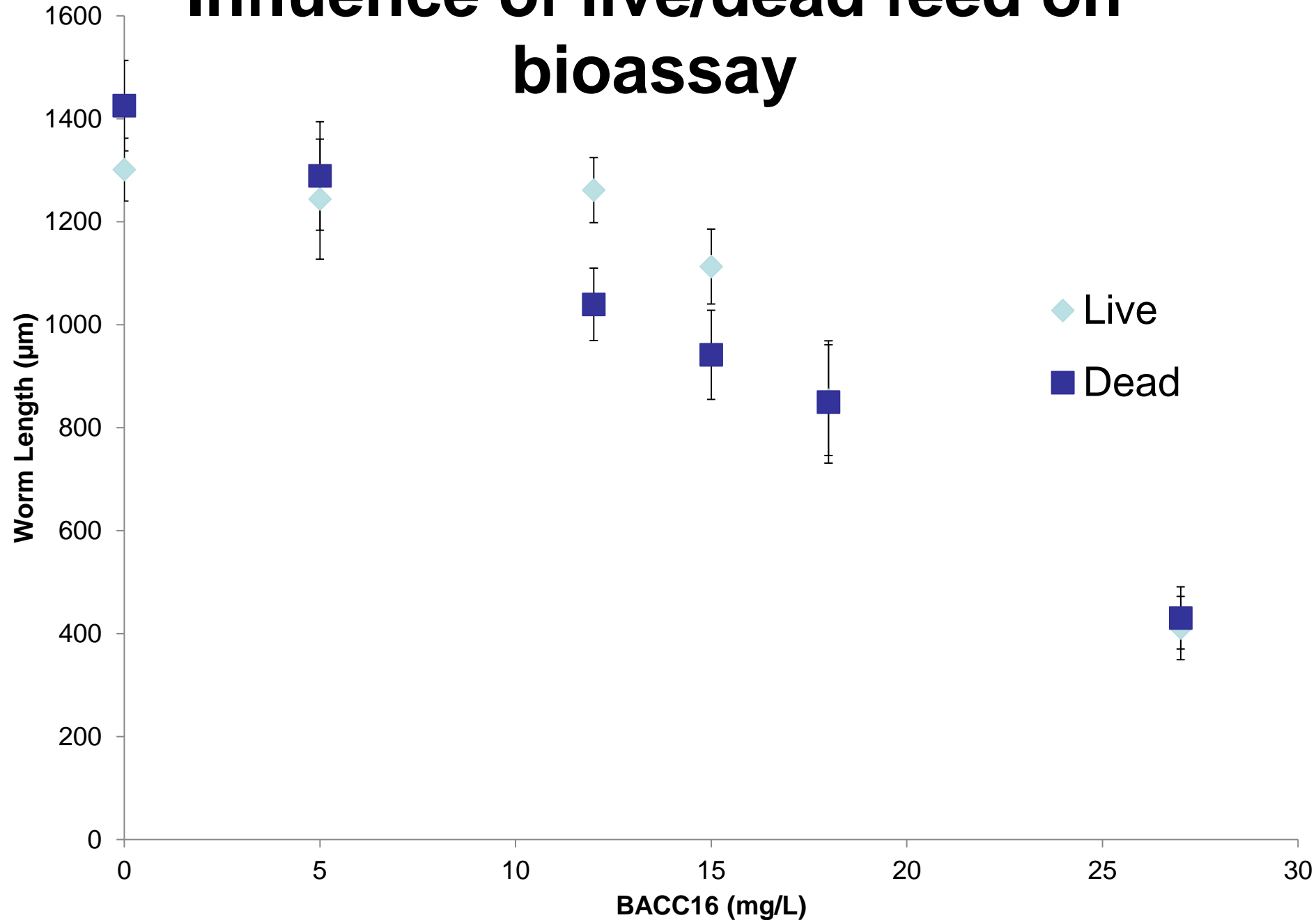
Bioassay feed dependence
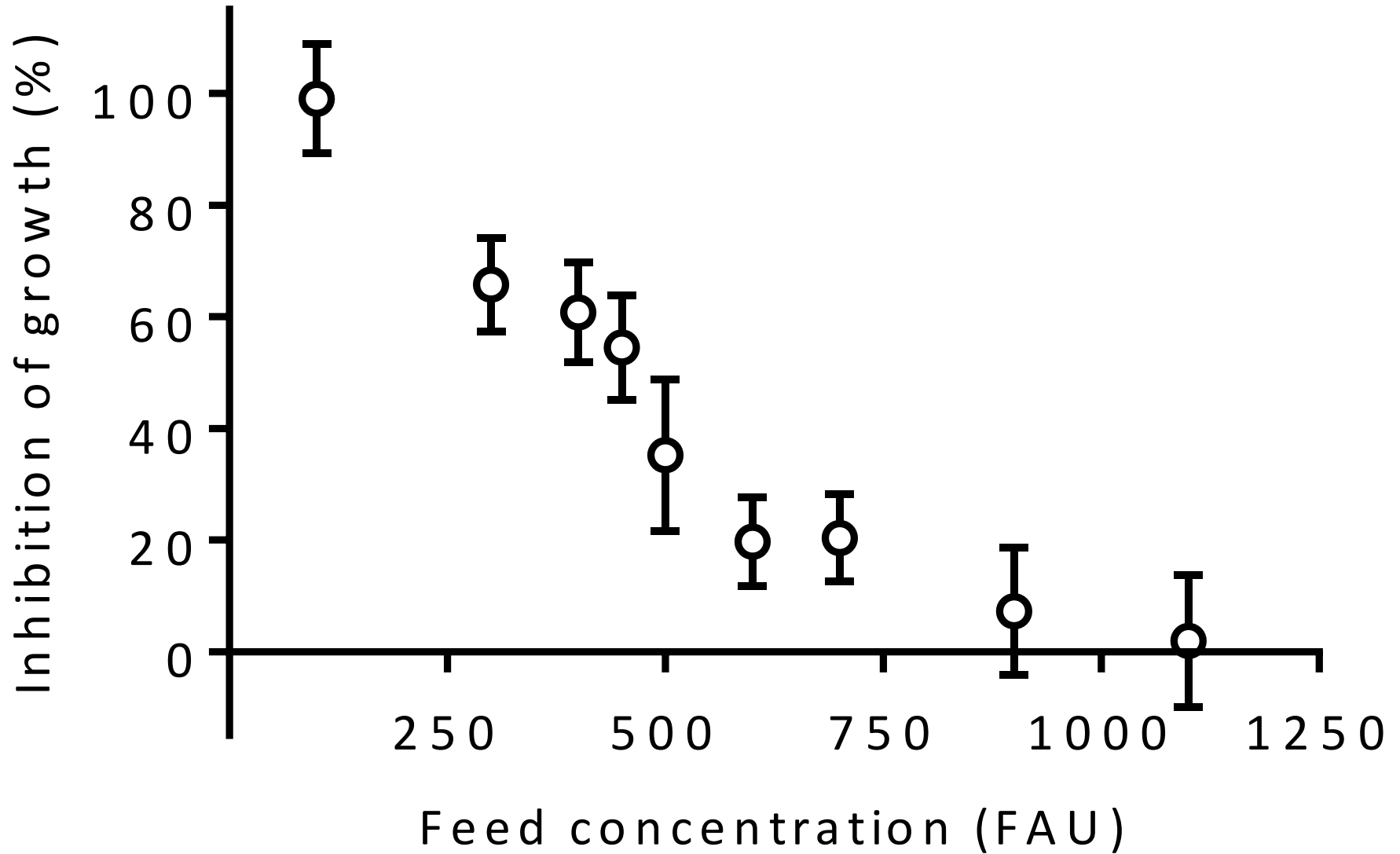
Tests were conducted with 15 mg/L BAC-C16

Impact of worm culture on bioassay

Worm Length (µm) vs BAC C16 (mg/L)

- ◆ fed liquid
- ■ unfed liquid
- ▲ fed plates
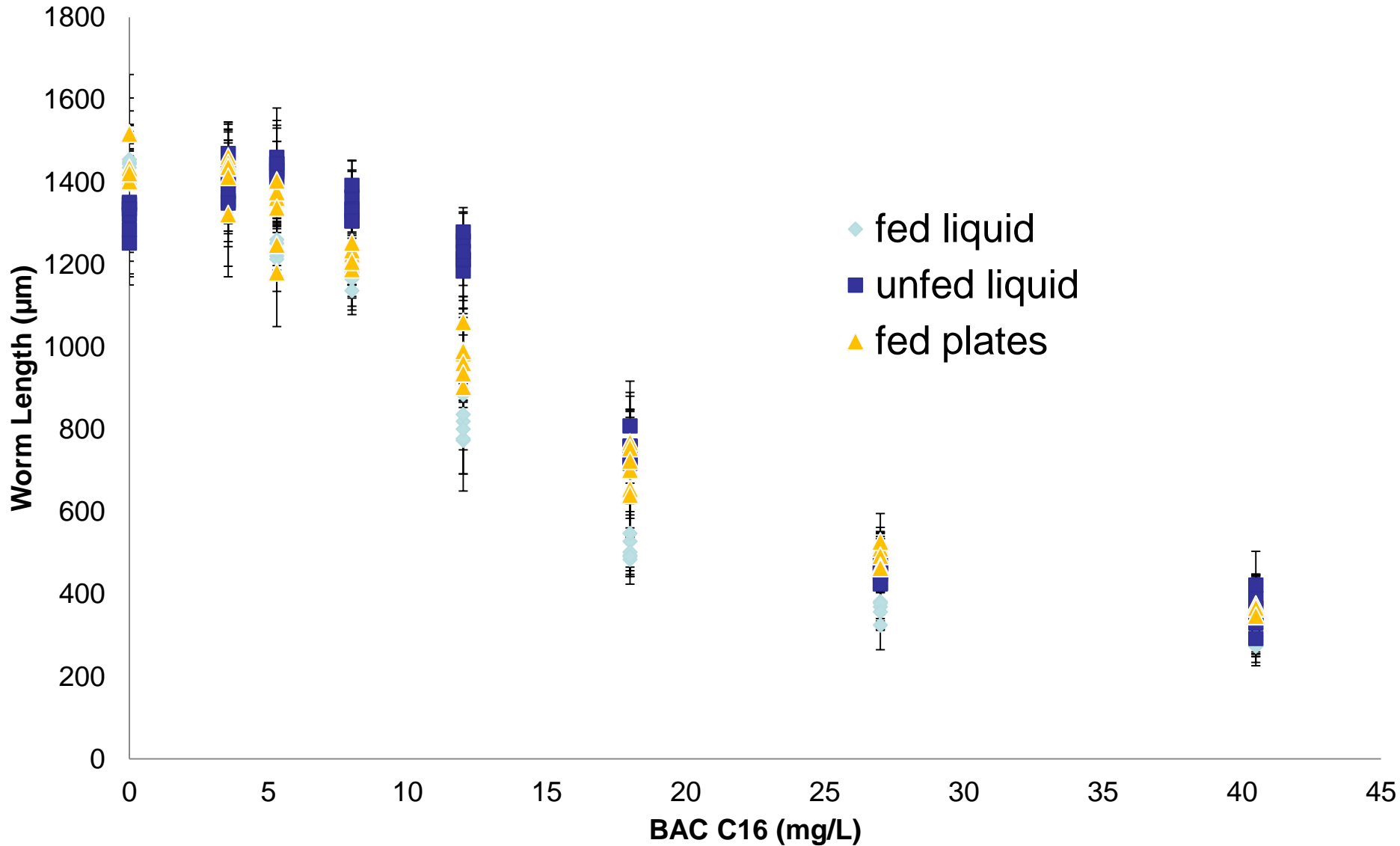
*This experiment was performed with the plates shaking

Plate design to test for NP artifacts
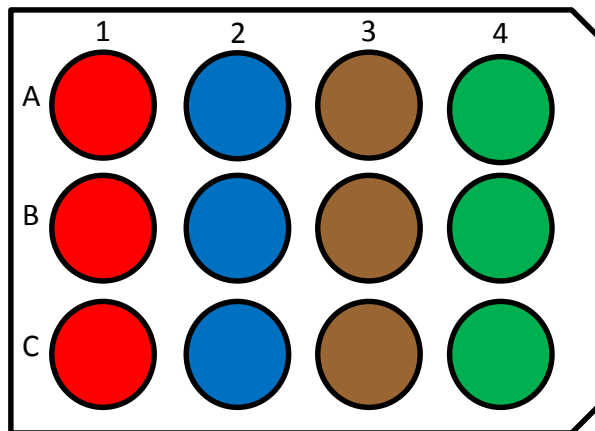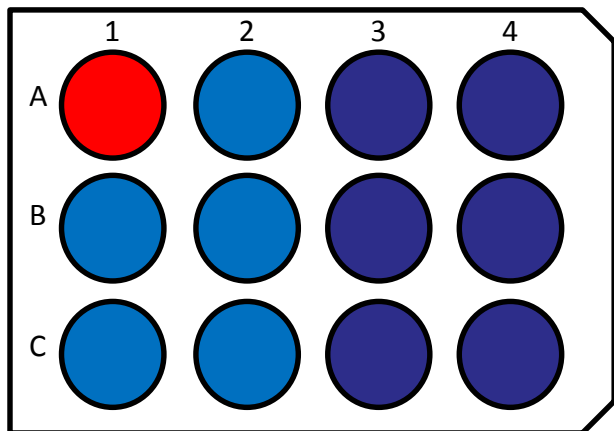
Poster: Identification and avoidance of potential artifacts and misinterpretations in nanomaterial ecotoxicity measurements

Petersen et al., 2014, Environ. Sci. Technol. 48(8), p 4226-4246.
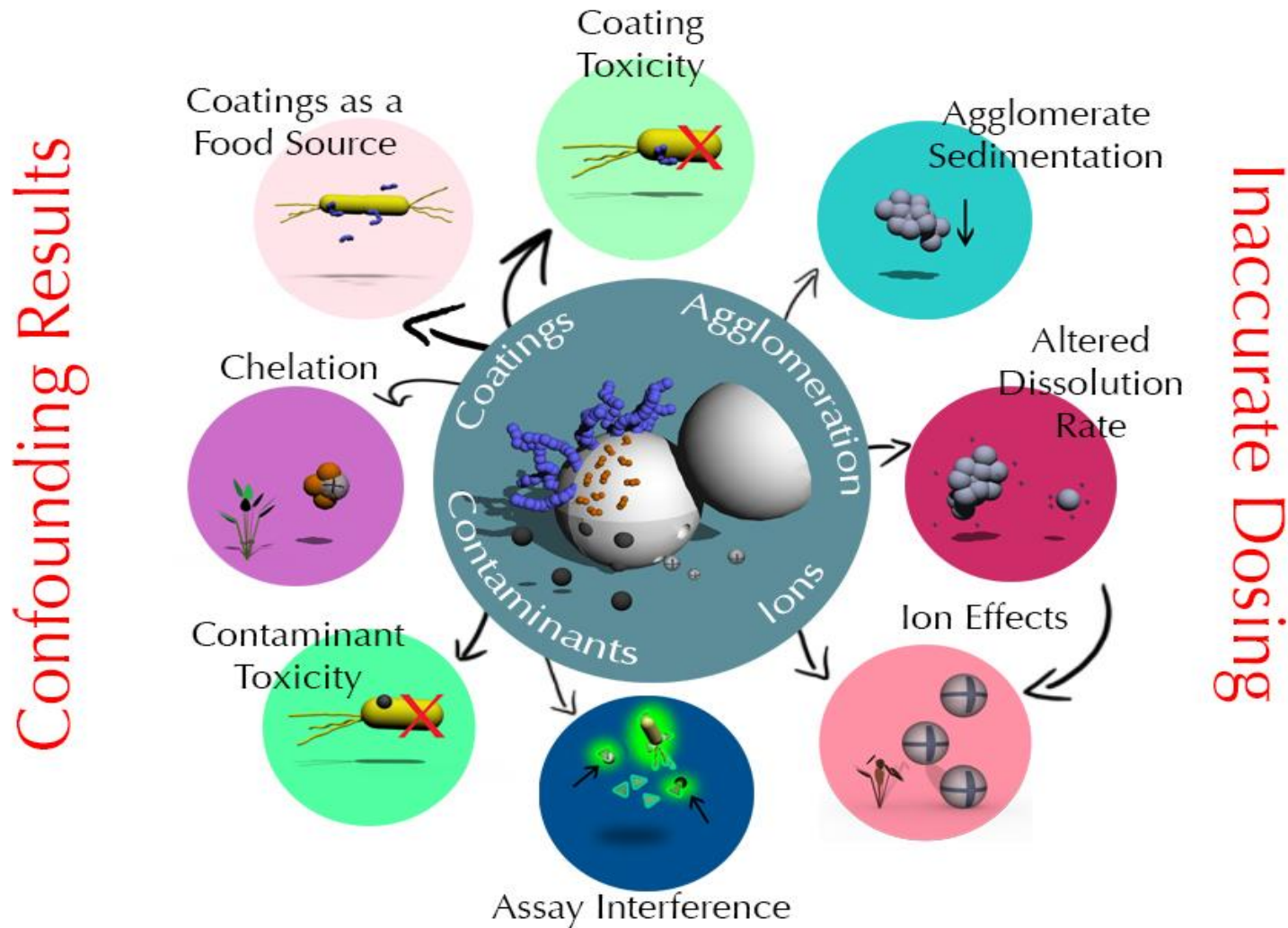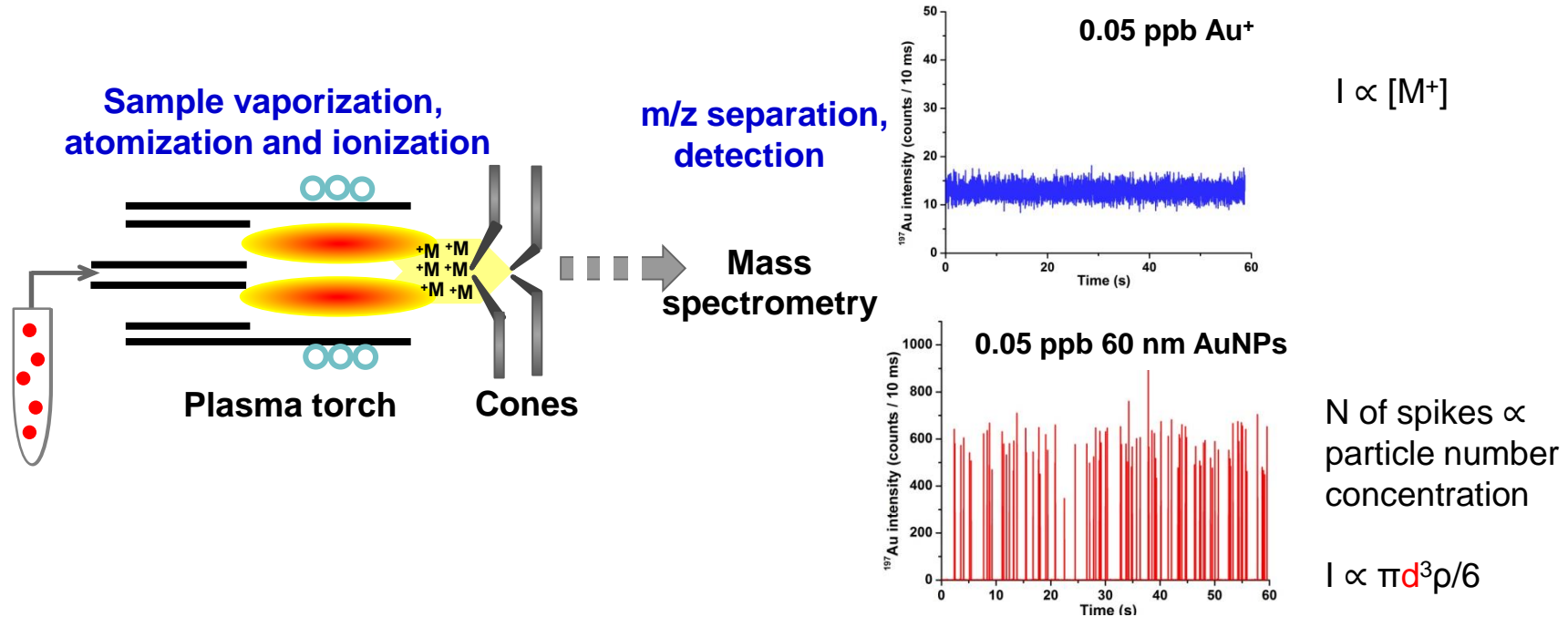
Polystyrene Nanoparticle Toxicity Assay
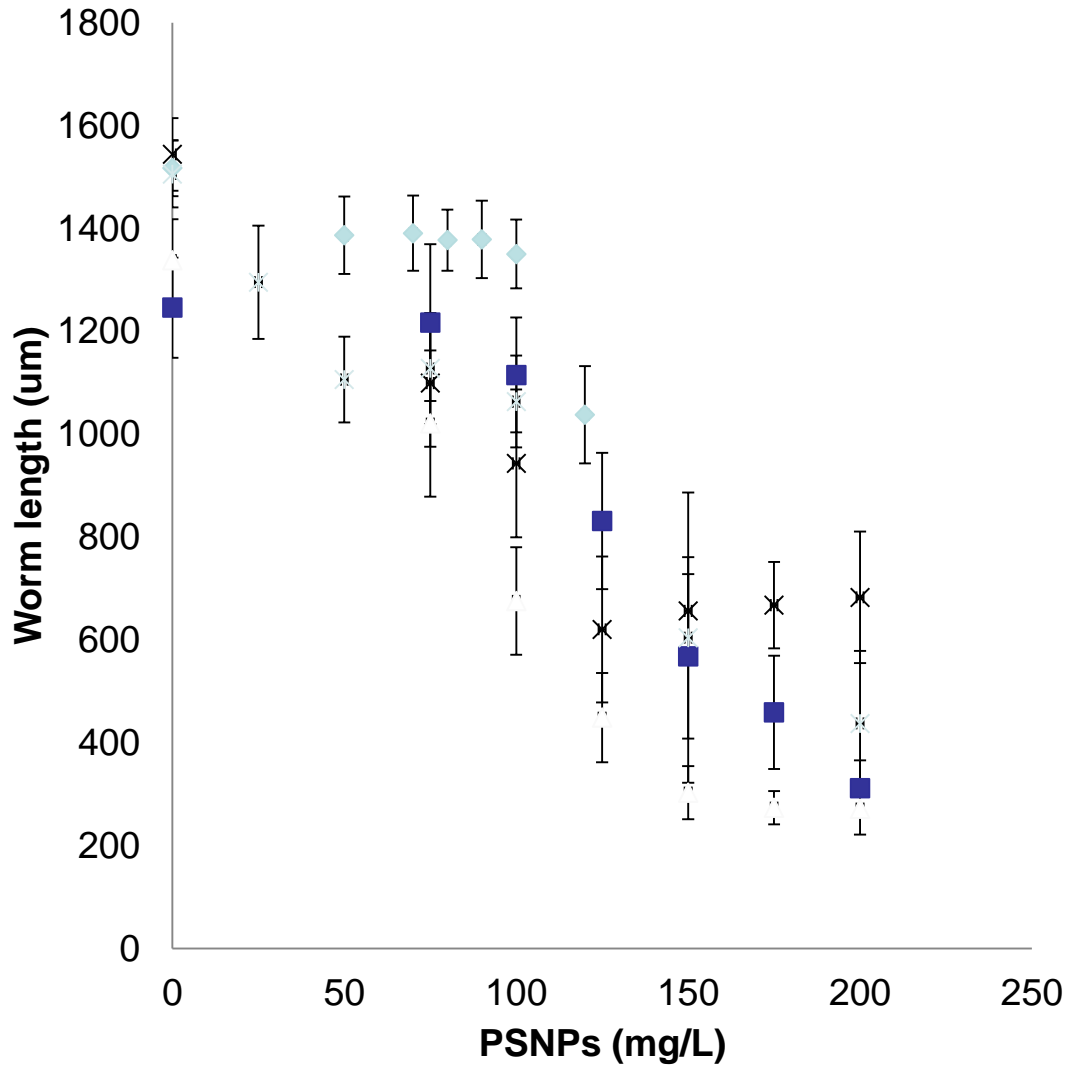
EC50s for growth

12/12/2014   100.7
12/18/2014   125
12/29/2014   81.7
1/2/2015   74.9
1/9/2015   119.6

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Single Particle ICP-MS (spICP-MS)

Sample vaporization, atomization and ionization

m/z separation, detection

Mass spectrometry

Plasma torch

Cones

0.05 ppb Au⁺

$I \propto [M^+]$

0.05 ppb 60 nm AuNPs

N of spikes $\propto$ particle number concentration

$I \propto \pi d^3 \rho / 6$

- Operates ICP-MS in time resolved analysis mode: a particle event lasts 0.4 ms, typical dwell time 3 - 10 ms
- Particle size distribution and number concentration

* *J. Anal. At. Spectrom., 2012, 27, 1143*

# Base Digestion of *C. elegans* for spICP-MS analysis

- 7 % TMAH (w/v) used for dissolution of worm tissue
- Dilution to bring TMAH concentration to <0.1%
- sp-ICP-MS measurement to test NPs accumulation on the organisms

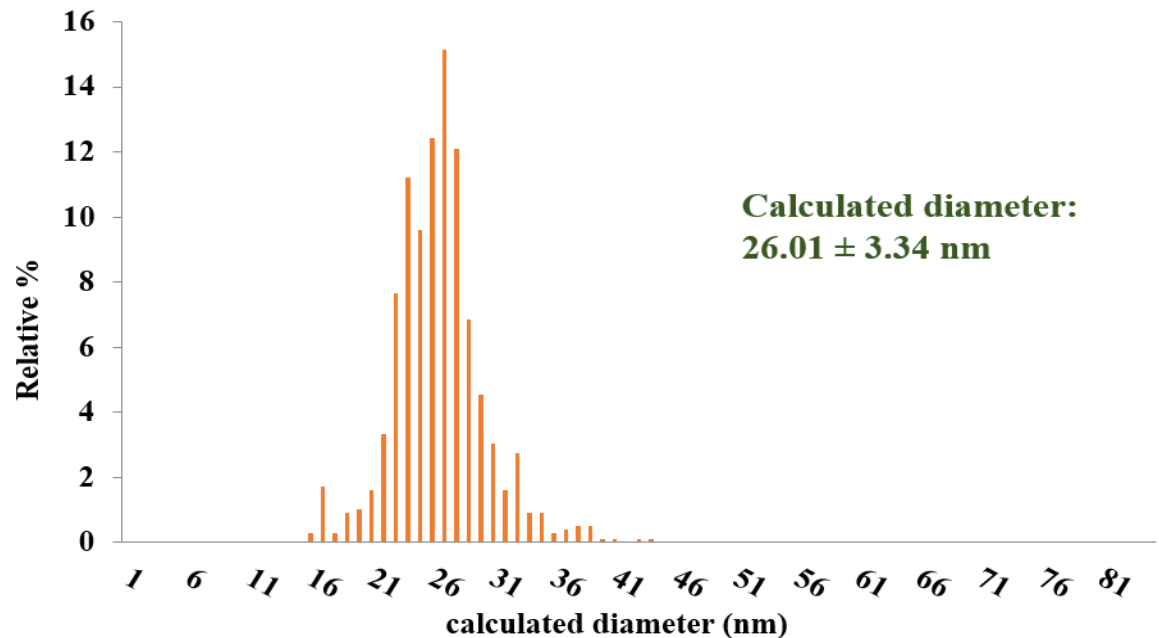# spICP-MS Measurements of control *C. elegans*
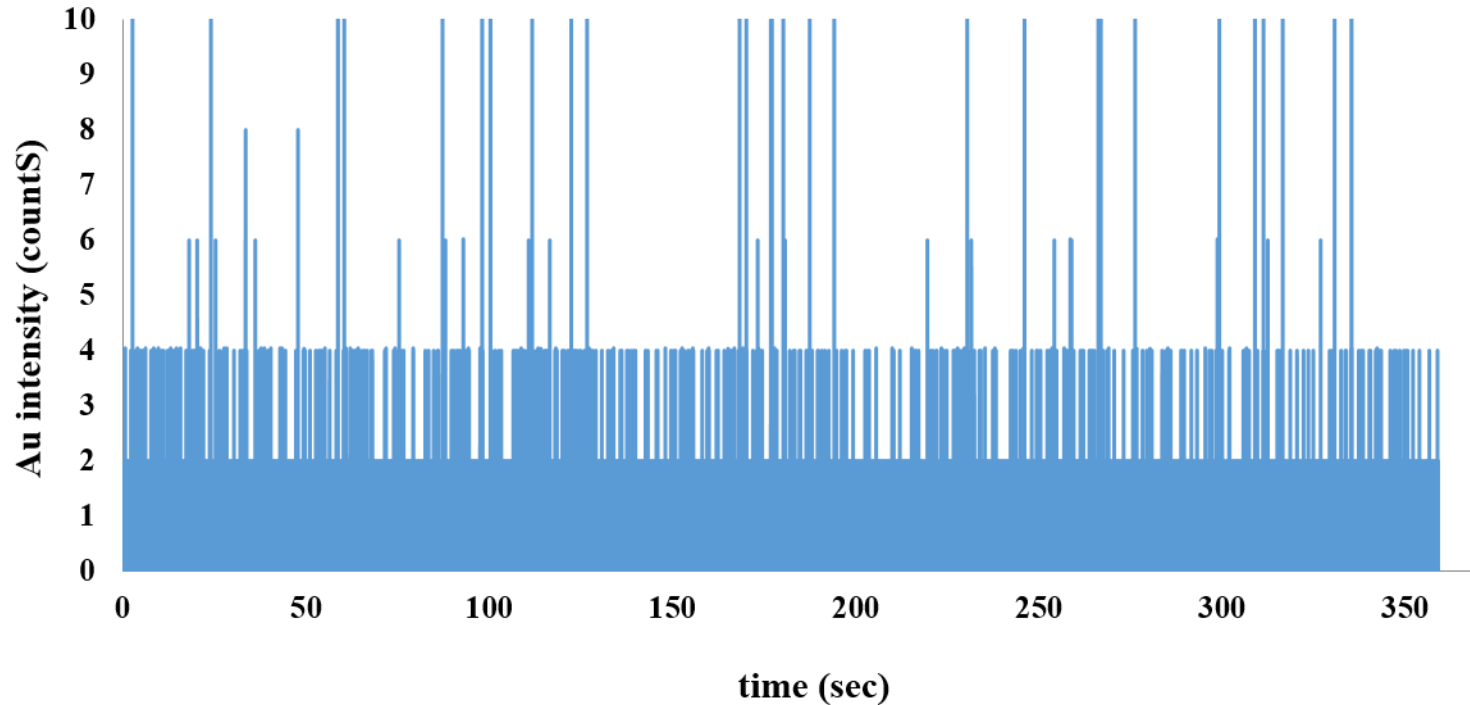
Single particle ICP-MS signals for *C. elegans* in control exposure.
Mean Au intensity: 0.18 counts.   Date of exposure: June 24, 2014

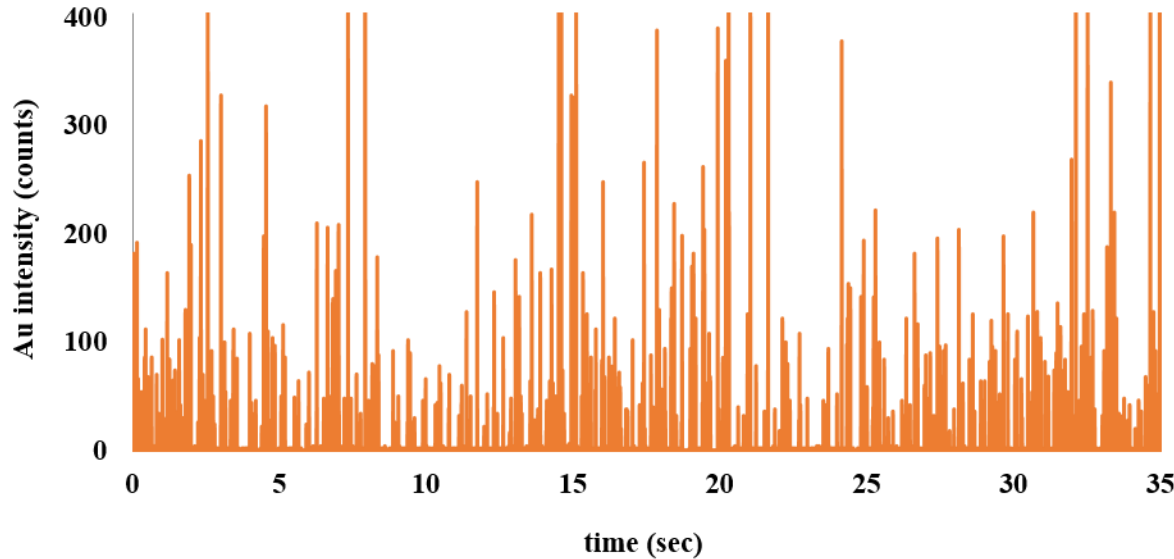# spICP-MS Measurements of 30 nm AuNPs after digestion procedure (no worms)
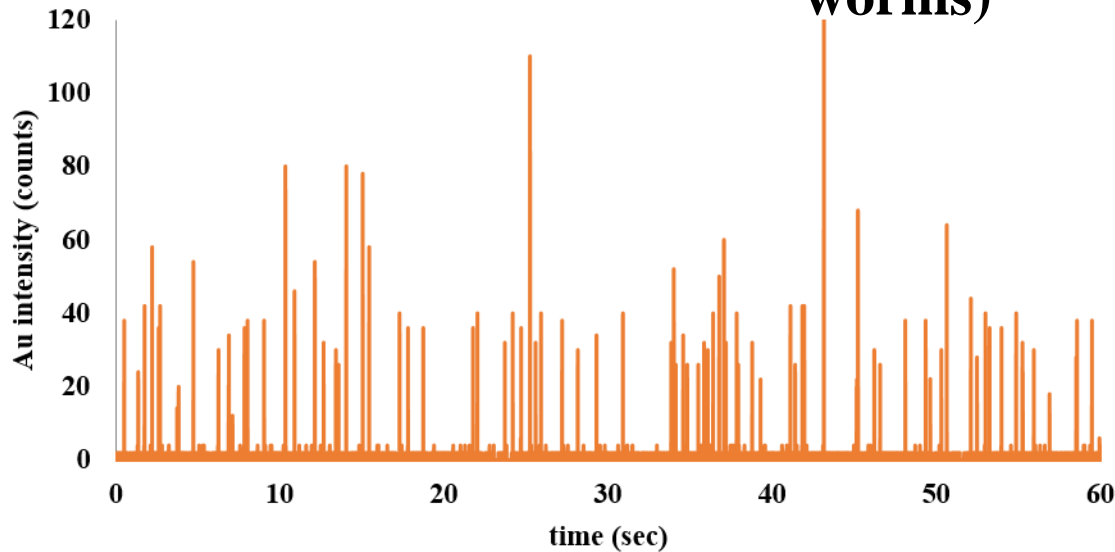
Single particle ICP-MS signals for 30 nm AuNPs (NIST) following treatment with 7% TMAH. **Control digestion, no worms.**

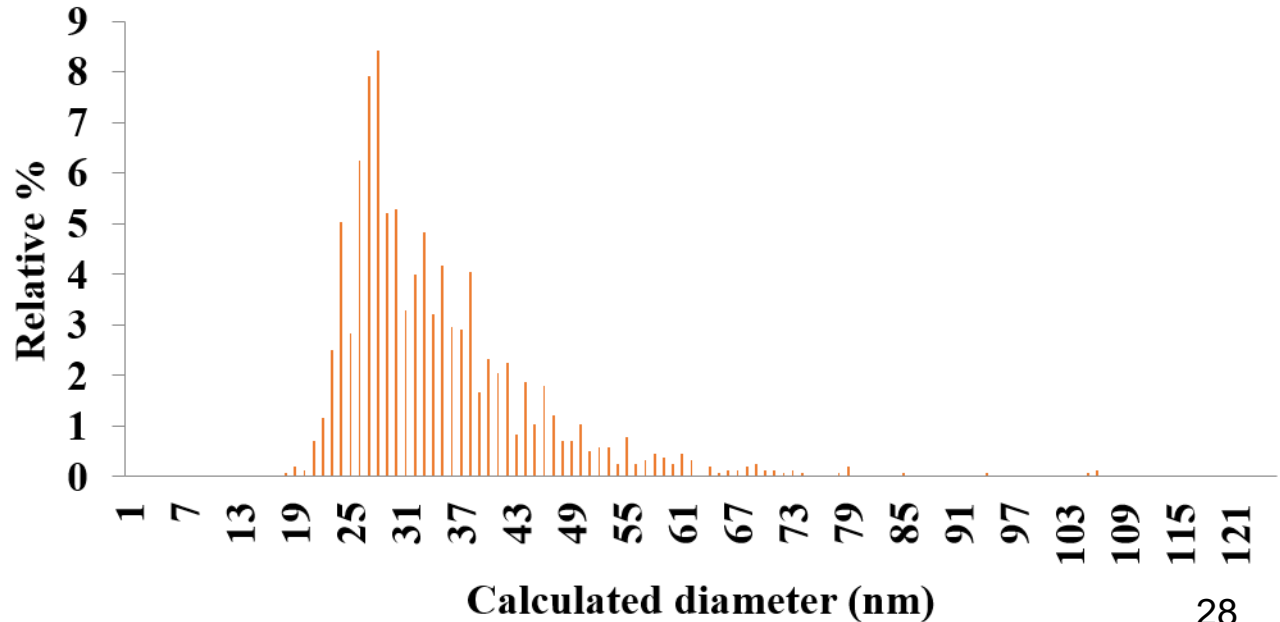Size distribution of 30 nm AuNPs (NIST) extracted following TMAH treatment. **Control digestion, no worms.**

Calculated diameter: 26.01 ± 3.34 nm

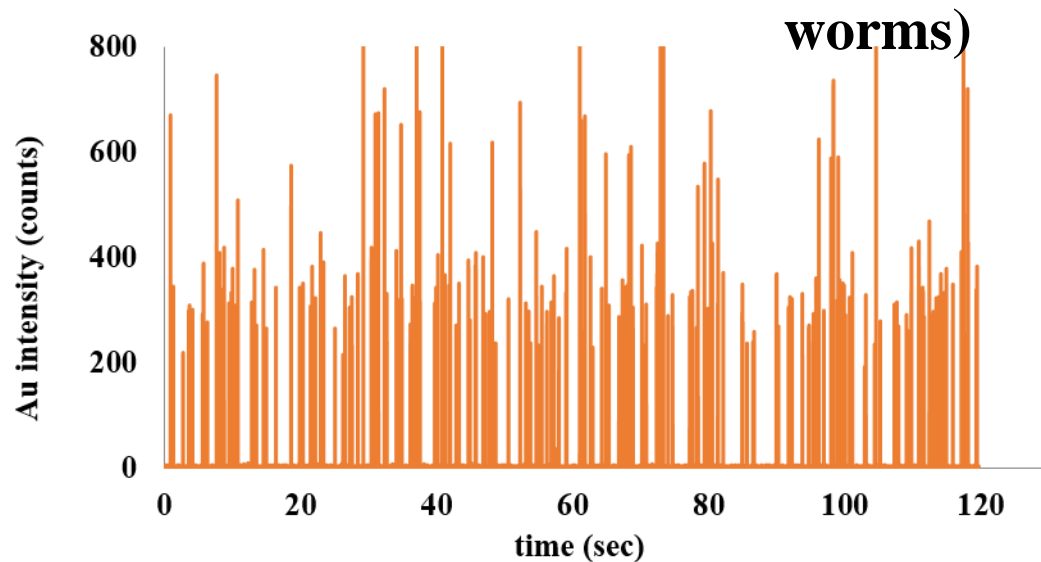# spICP-MS Measurements of *C. elegans* exposed to 30 nm AuNPs

Single particle ICP-MS signals for *C. elegans* spiked with 30 nm AuNPs (NIST). 799 particle events in 60 s. **Diluted by a factor of 1500.**

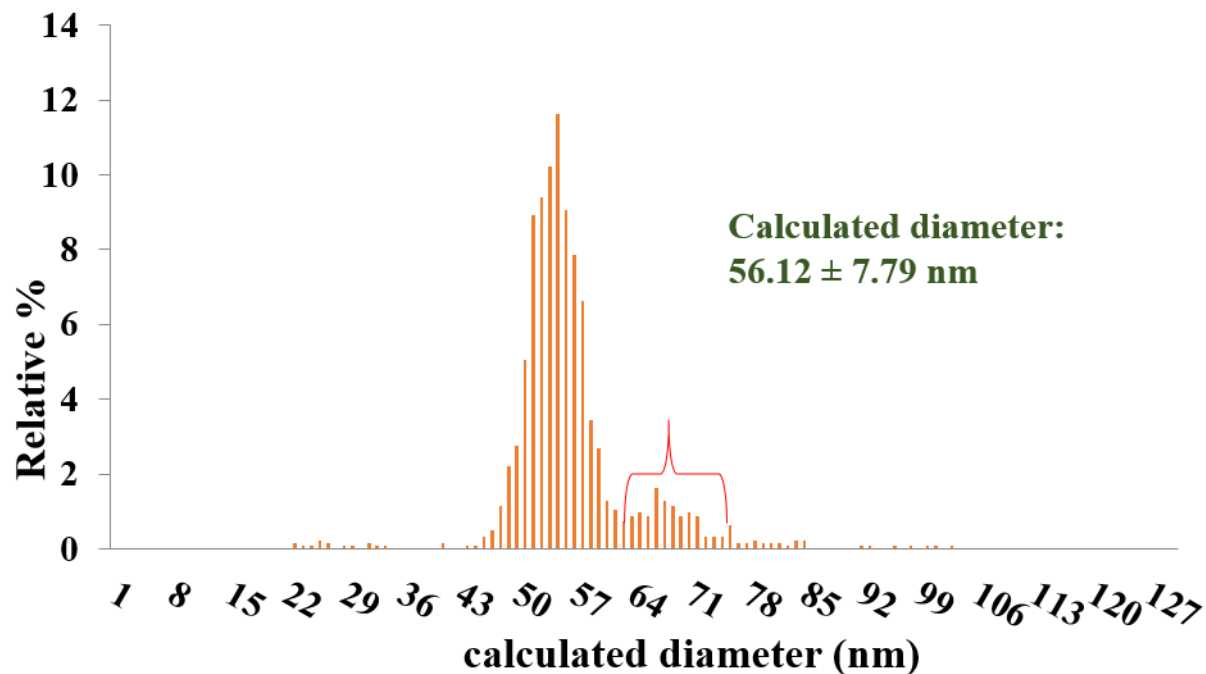Size distribution of NPs extracted from *C. elegans* exposed to 30 nm AuNPs (NIST).

# spICP-MS Measurements of 60 nm AuNPs after digestion procedure (no worms)

Single particle ICP-MS signals for 60 nm AuNPs (NIST) following treatment with 7% TMAH. Control digestion, no worms.

Size distribution of 60 nm AuNPs (NIST) extracted following TMAH treatment. Control digestion, no worms.
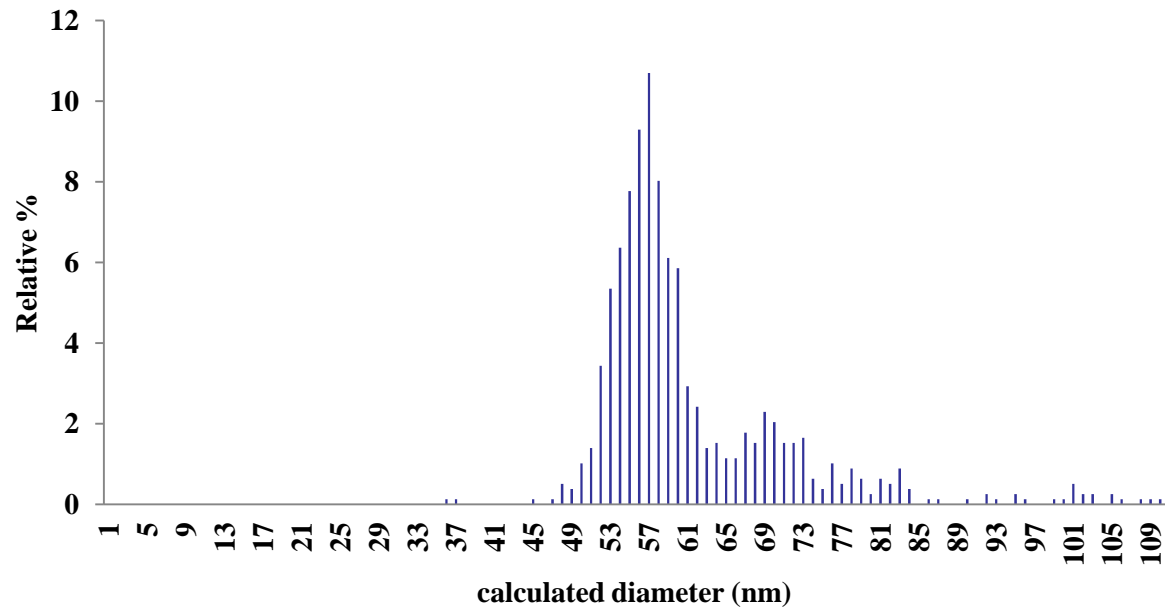

Calculated diameter: 56.12 ± 7.79 nm

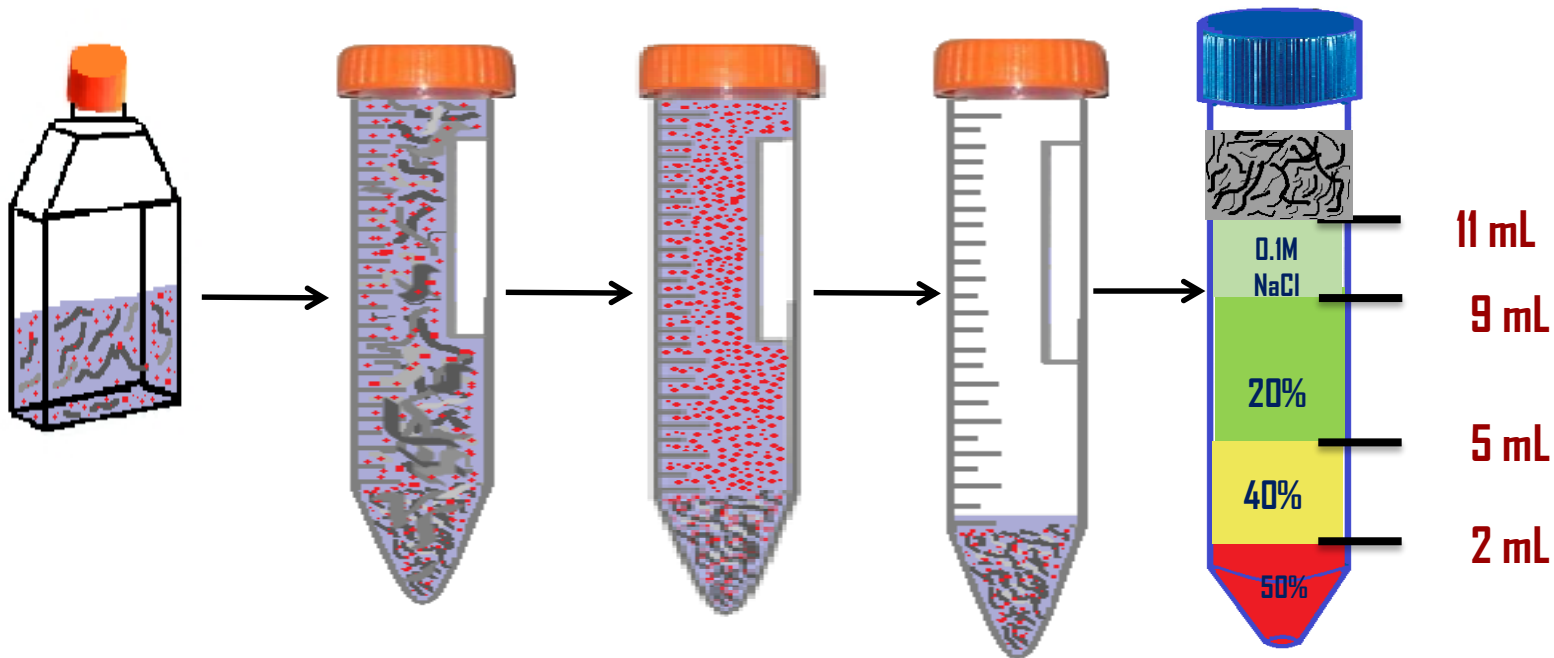# spICP-MS Measurements of *C. elegans* exposed to 60 nm AuNPs
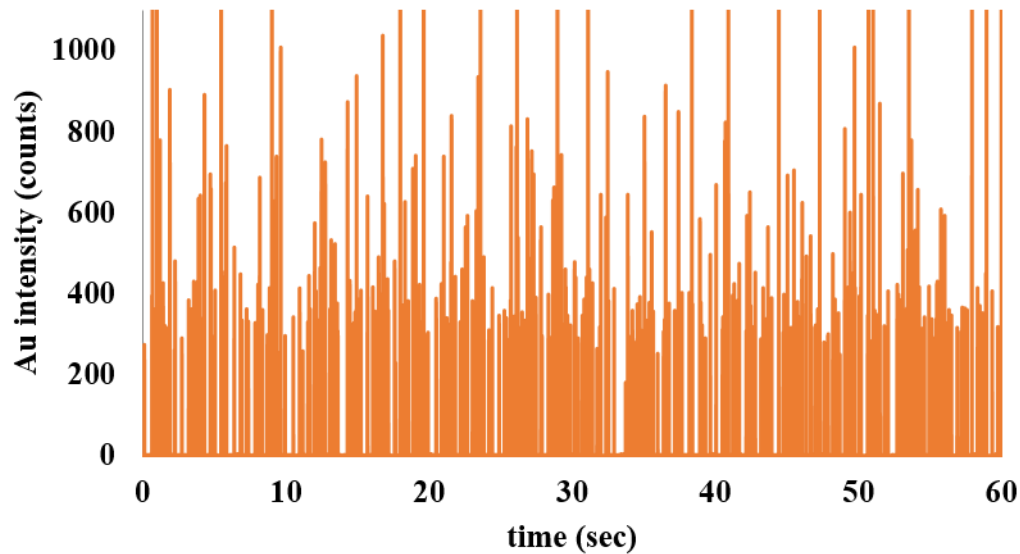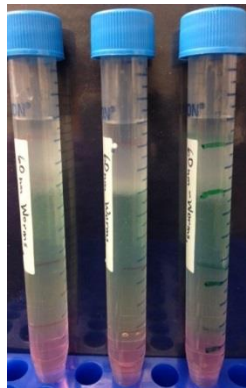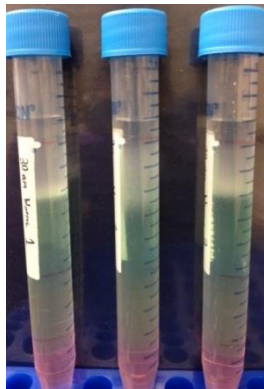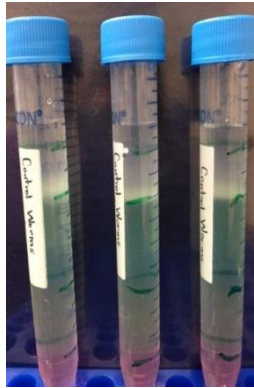

Single particle ICP-MS signals for *C. elegans* spiked with 60 nm AuNPs (NIST). 412 particle events in 60 s. Diluted by a factor of 2500.

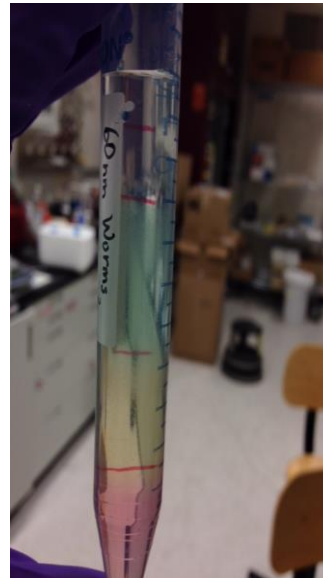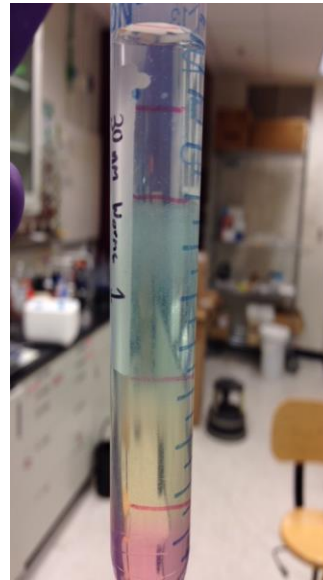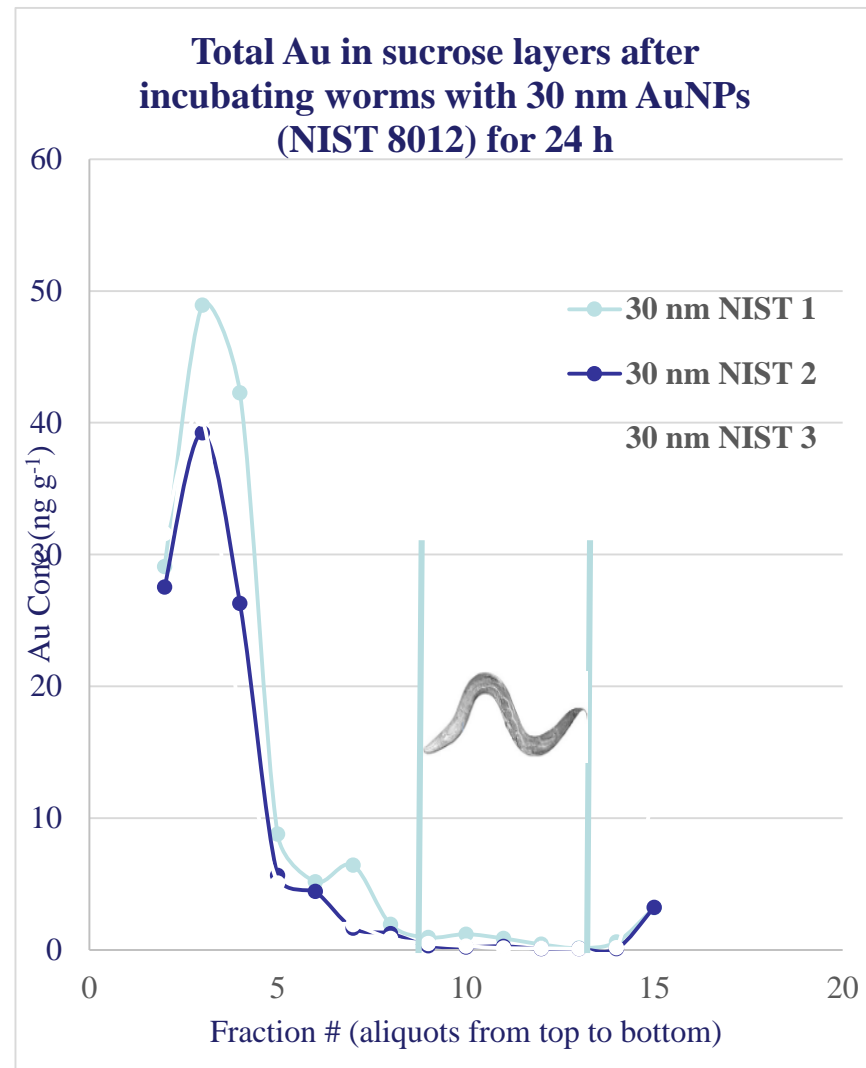Size distribution of NPs extracted from *C. elegans* exposed to 60 nm AuNPs (NIST).
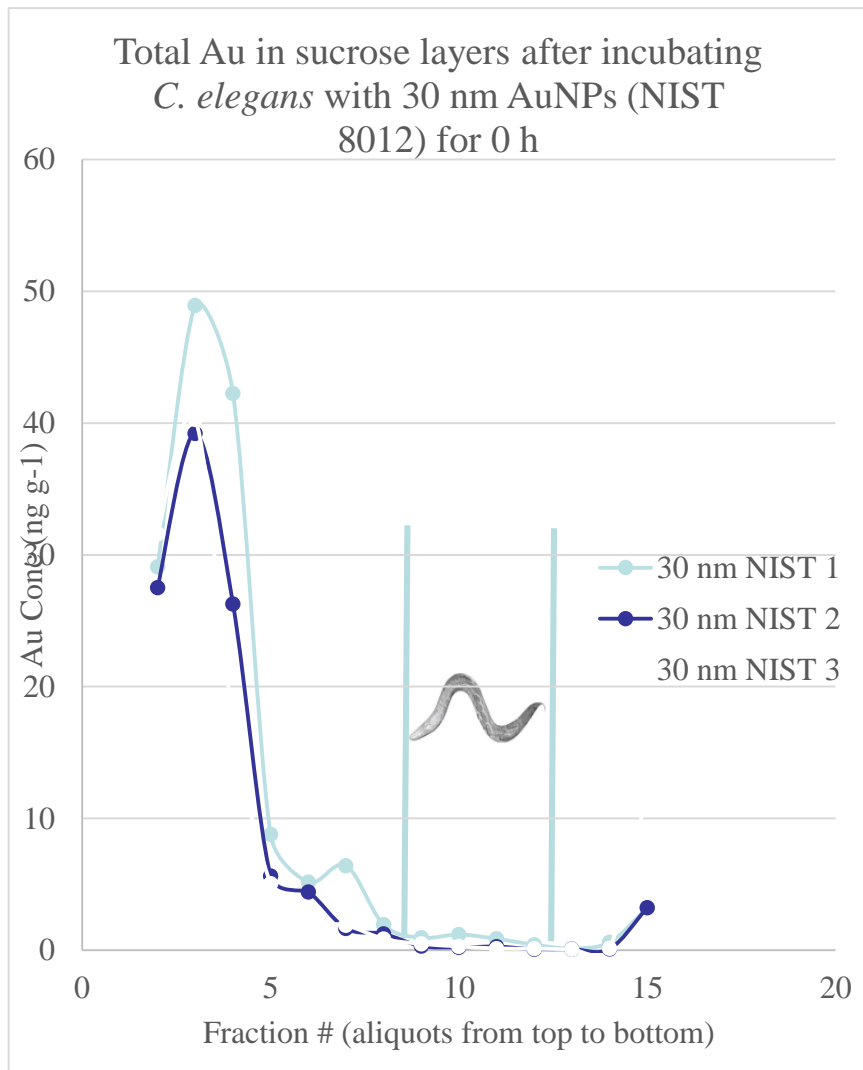
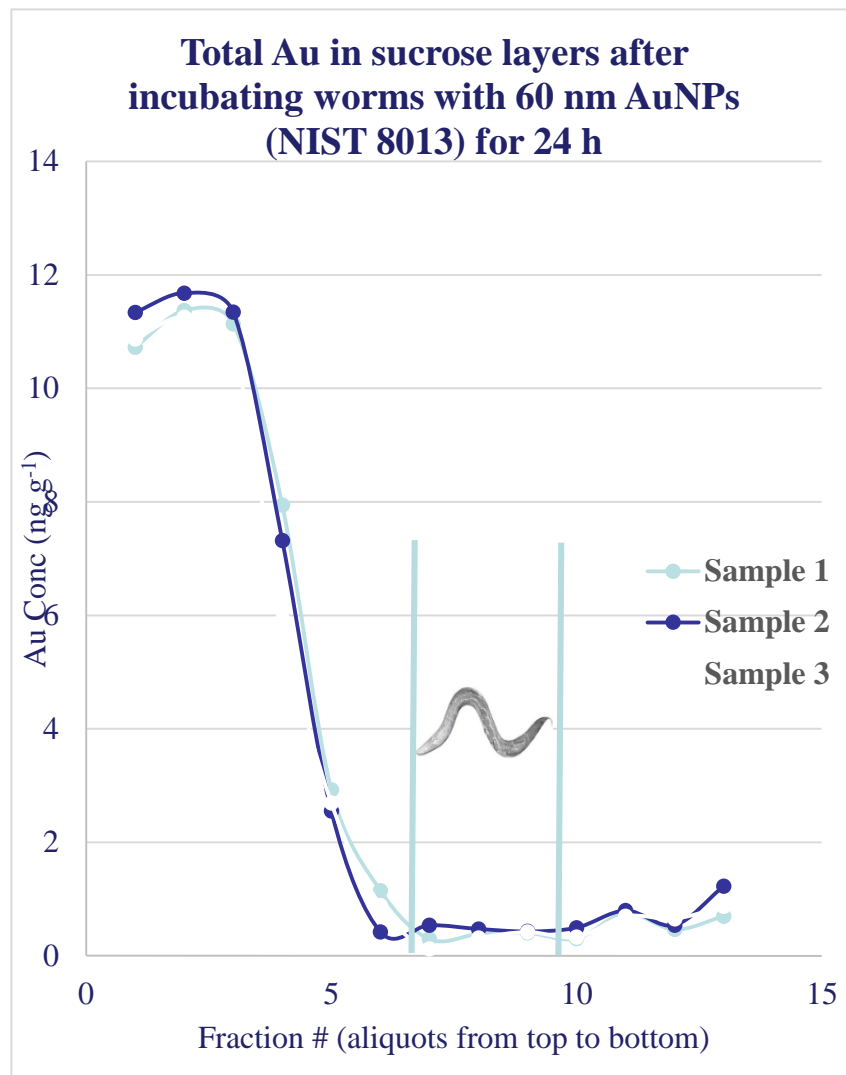# Sucrose density gradient centrifugation to separate *C. elegans* and AuNPs

Spin in centrifuge

Total Au in sucrose layers after incubating *C. elegans* with 30 nm AuNPs (NIST 8012) for 0 h

Total Au in sucrose layers after incubating worms with 30 nm AuNPs (NIST 8012) for 24 h

% Recovery of Au conc: 95.6% ± 3.7%

Total Au in sucrose layers after incubating *C. elegans* with 60 nm AuNPs (NIST 8013) for 0 h

Total Au in sucrose layers after incubating worms with 60 nm AuNPs (NIST 8013) for 24 h
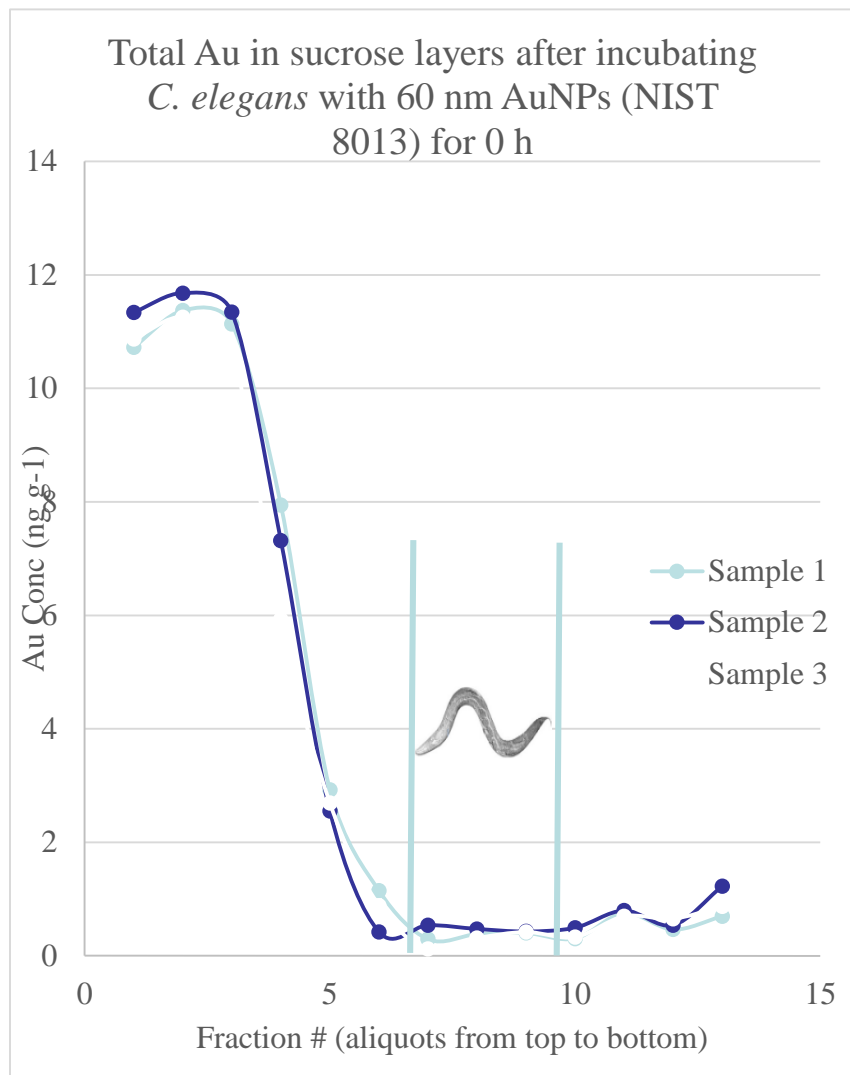
% Recovery: 95.6% ± 3.7%

Conclusions

1. Cause and effect analysis was used to identify major sources of uncertainty in a *C. elegans* standard method

2. Plate shaking and bacteria concentration were shown to have the strongest impact on assay results

3. Growth inhibition variability was similar for BAC-C16 and PSNPs

4. A method was developed to analyze AuNP size distributions in *C. elegans*

Cause & Effect Analysis:
A new approach for developing robust bionano assays

St. Gallen, Switzerland
June 18 & 19, 2015
http://www.empa.ch/plugin/template/empa/22/155222/---/l=2