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

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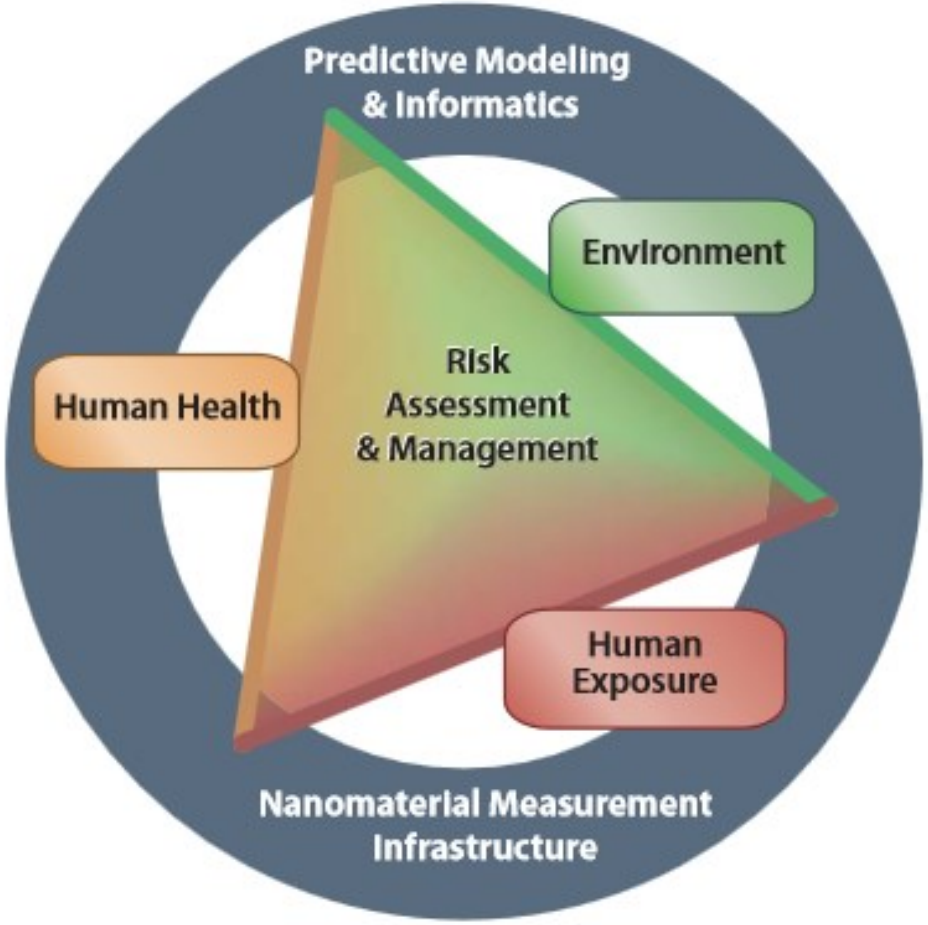
National Institute of Standards and Technology (NIST)
Presented 3/10/2015

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

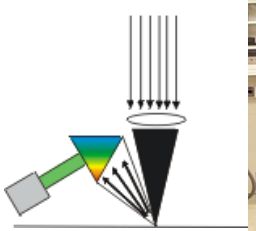


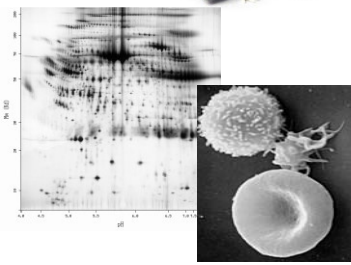
National Nanotechnology Initiative 2011 Environmental Health and Safety Research Strategy

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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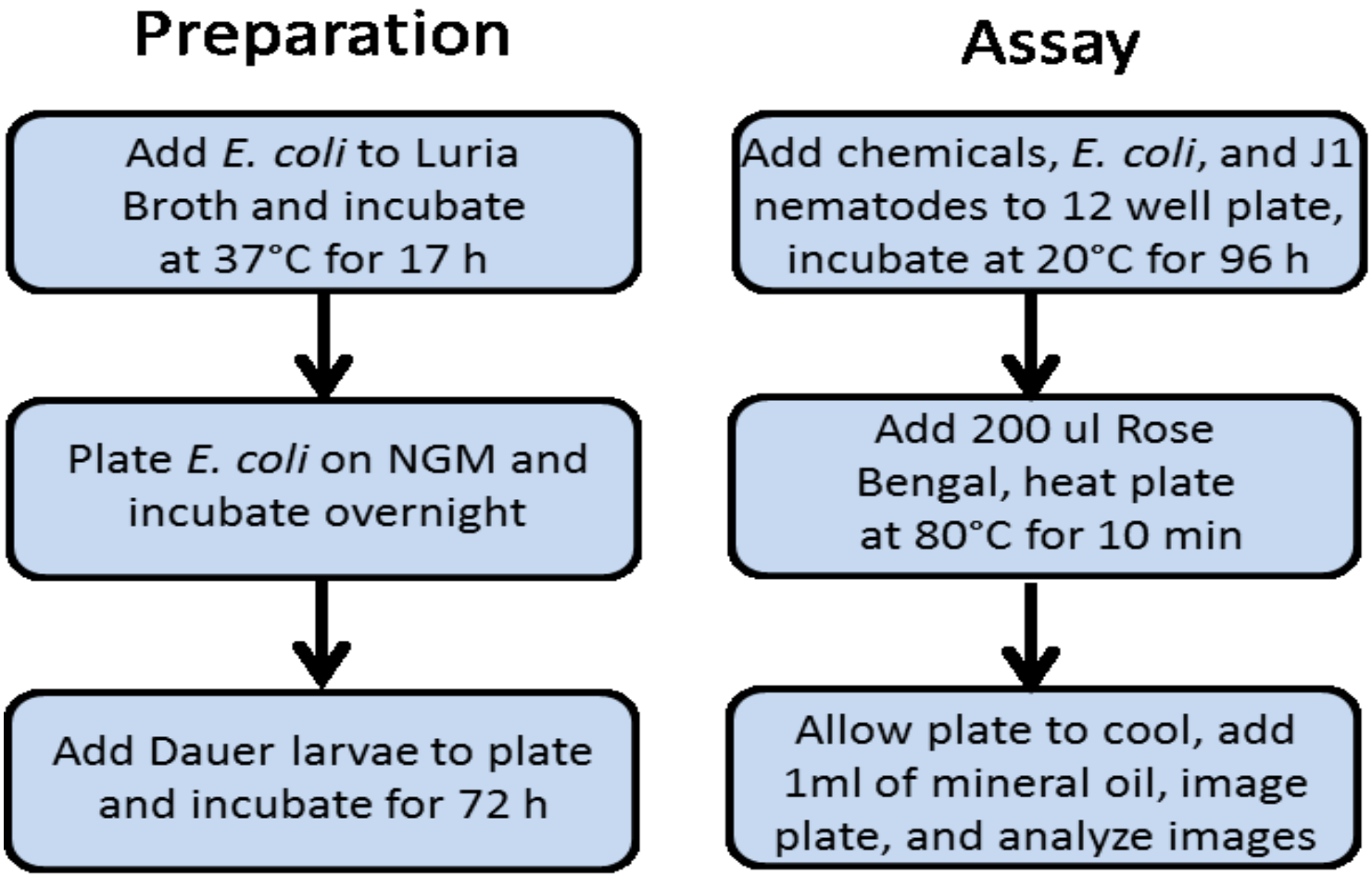
3. **Single-particle ICP-MS method to quantify the size distribution of gold nanoparticle uptake by *C. elegans***

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

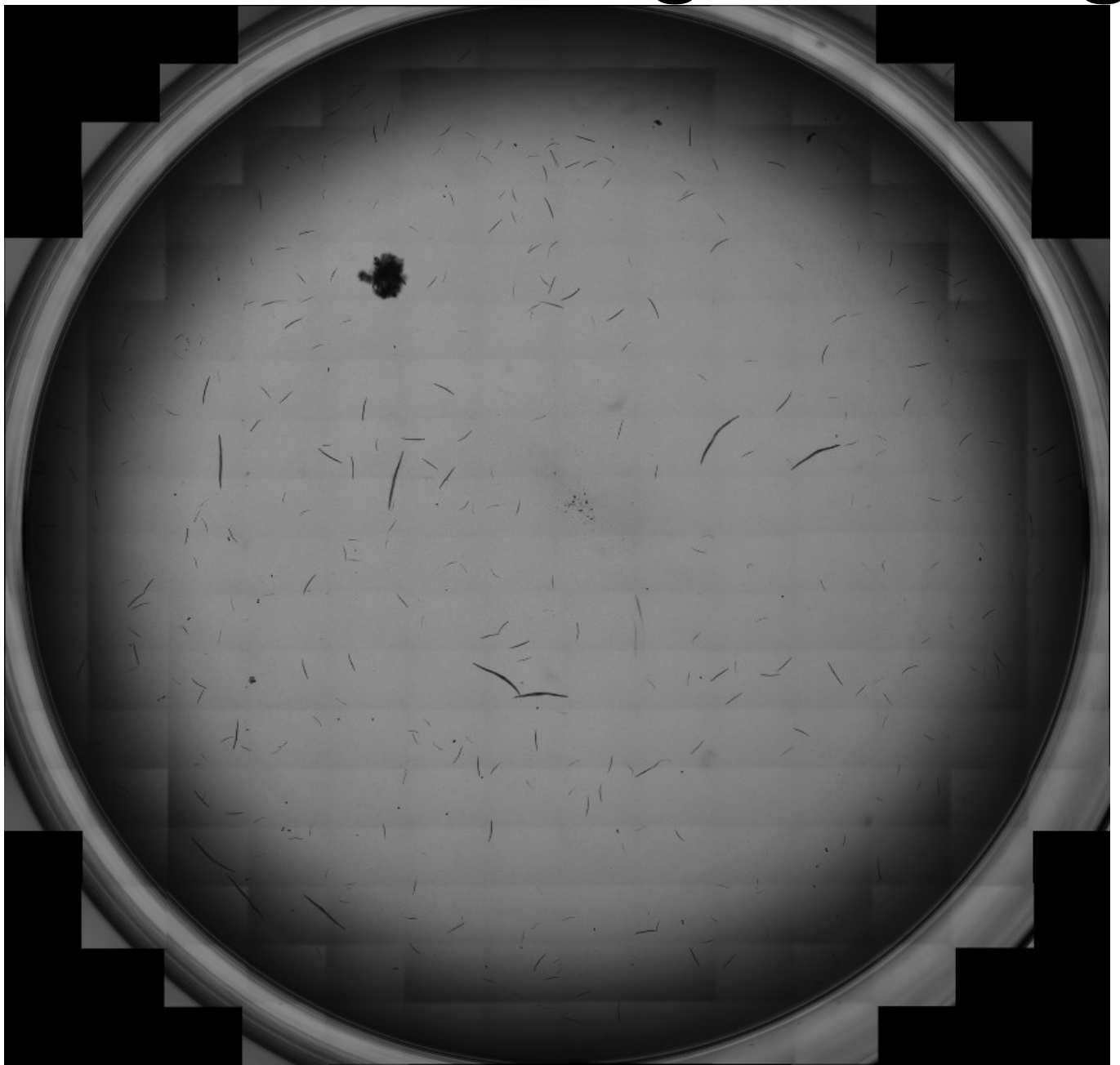
Leung et al, Toxicological Sciences 2009

ISO Method 10872

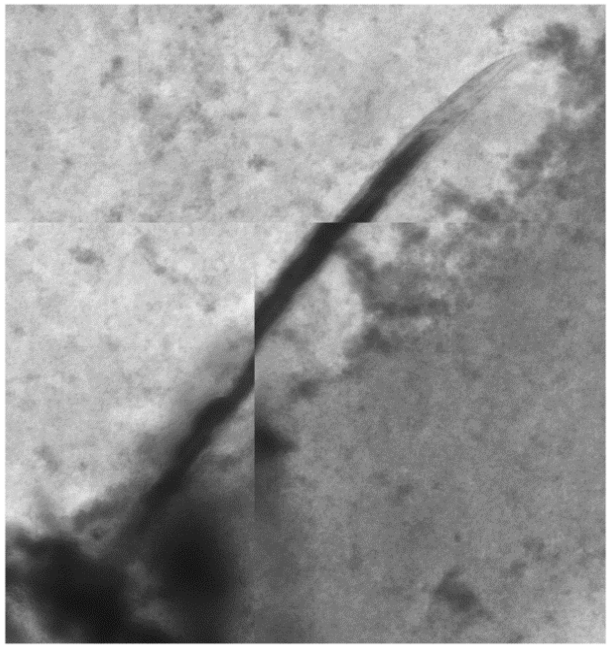


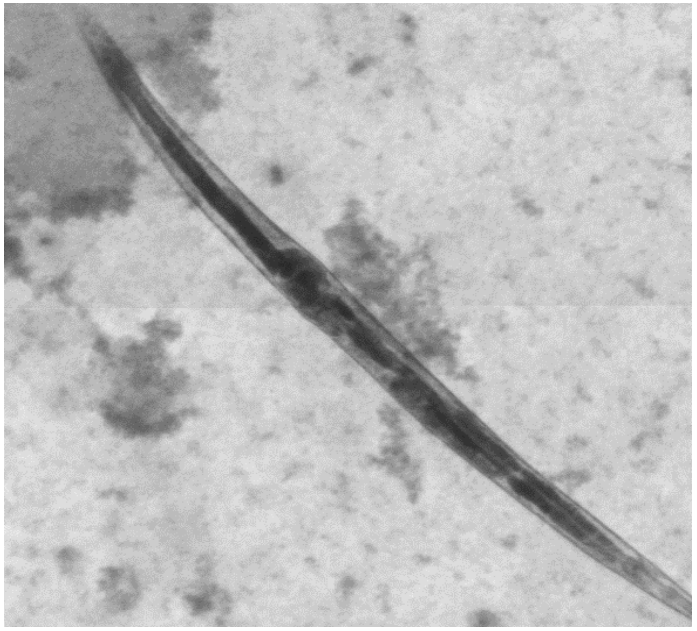
- Uses positive control benzylcetyldimethylammonium chloride (BAC C16 – EC_{50} = 15.1 mg l^{-1})
- Only test specification is growth inhibition of 20-80% at EC_{50} value

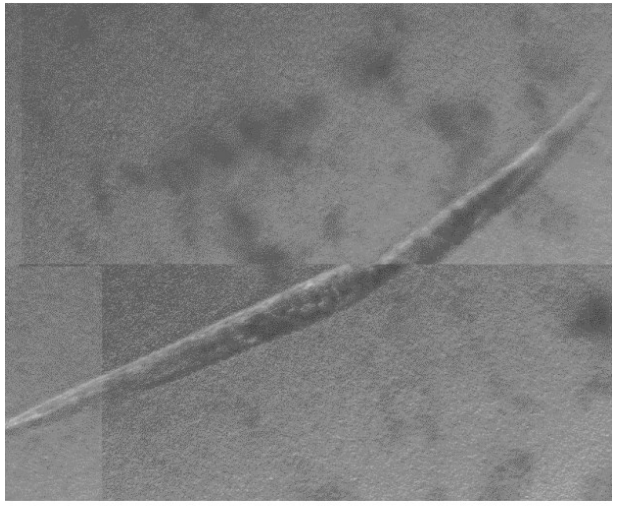
Automated *C. elegans* Imaging



C. elegans Image Stitching







Chemical Research in Toxicology

Perspective

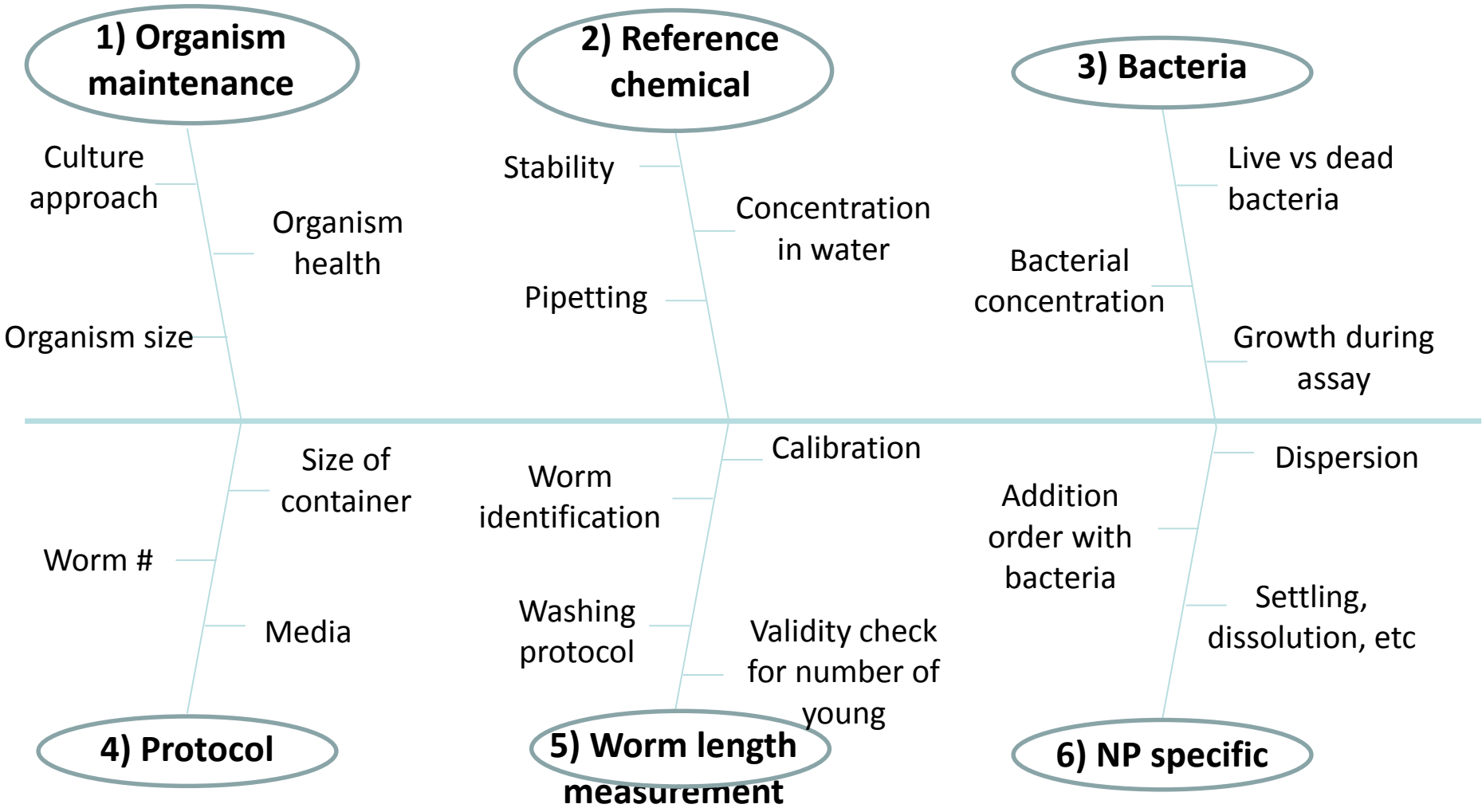
pubs.acs.org/crt

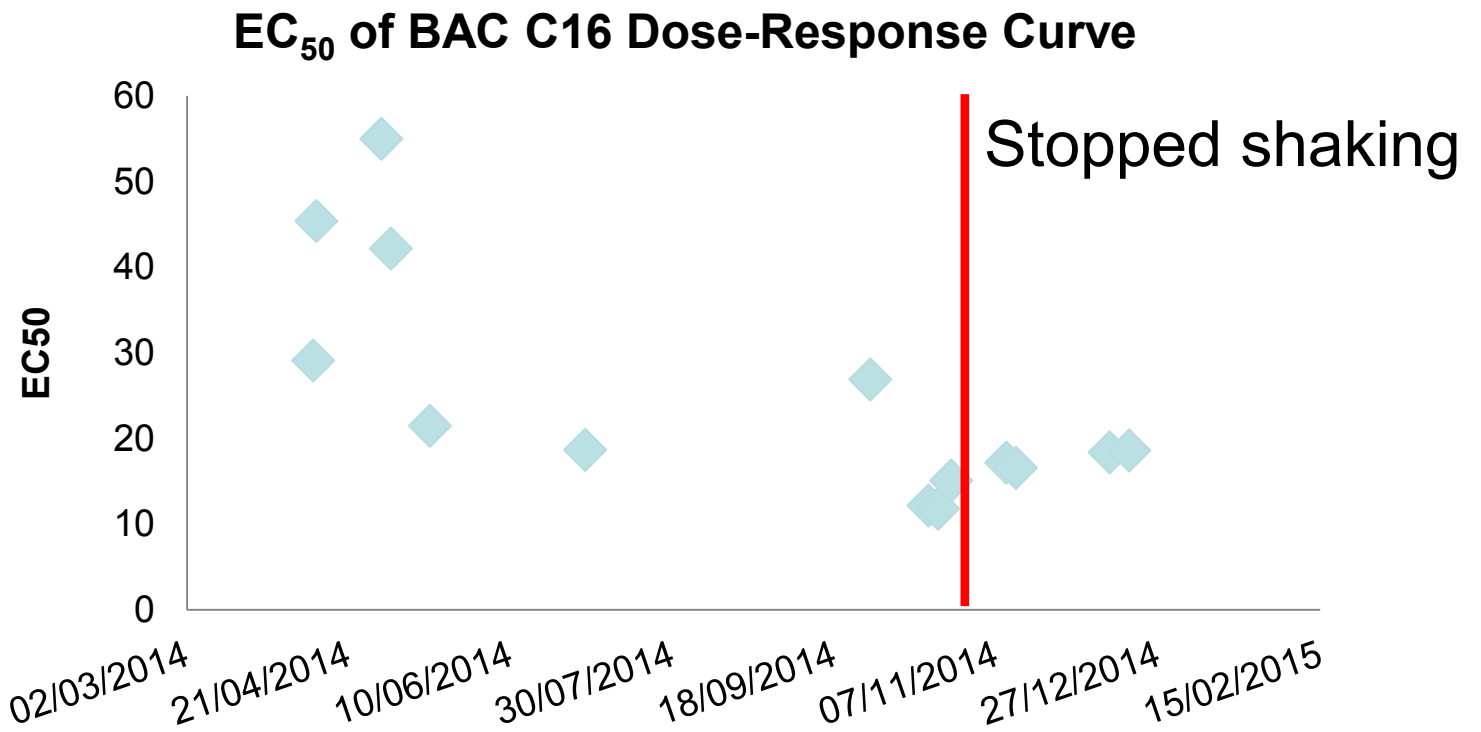
Use of Cause-and-Effect Analysis to Design a High-Quality Nanocytotoxicology Assay

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




EC50 of BAC C16 Dose-Response Curve
EC50
60
50
40
30
20
10
0
Stopped shaking
02/03/2014
21/04/2014
10/06/2014
30/07/2014
18/09/2014
07/11/2014
27/12/2014
15/02/2015

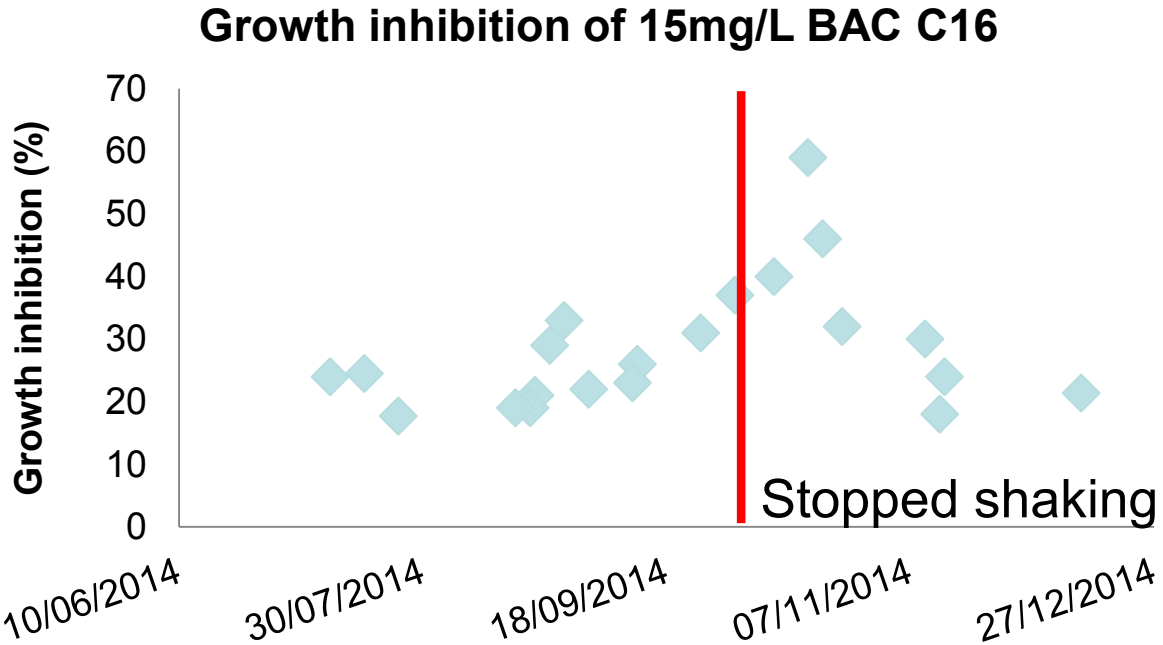
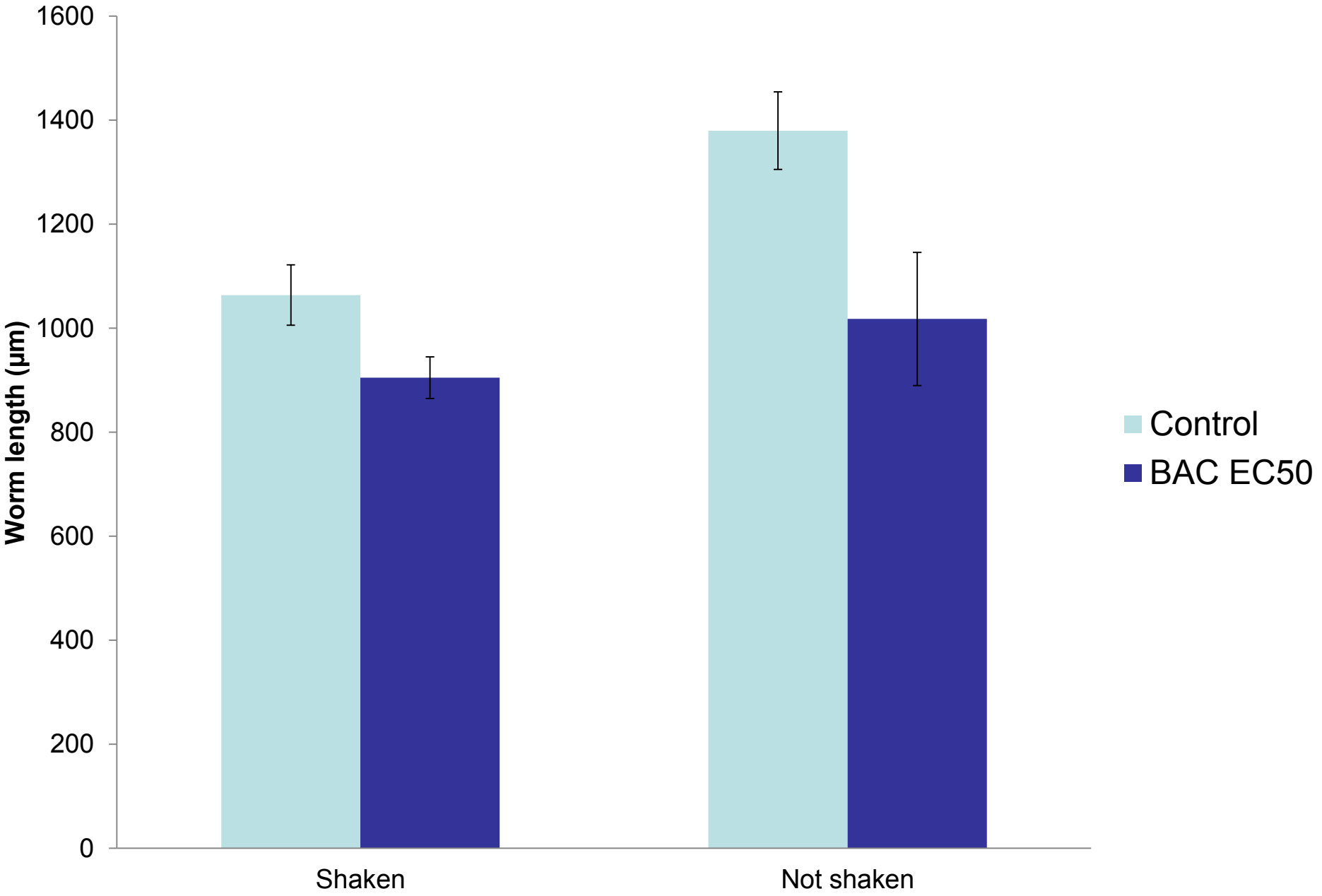
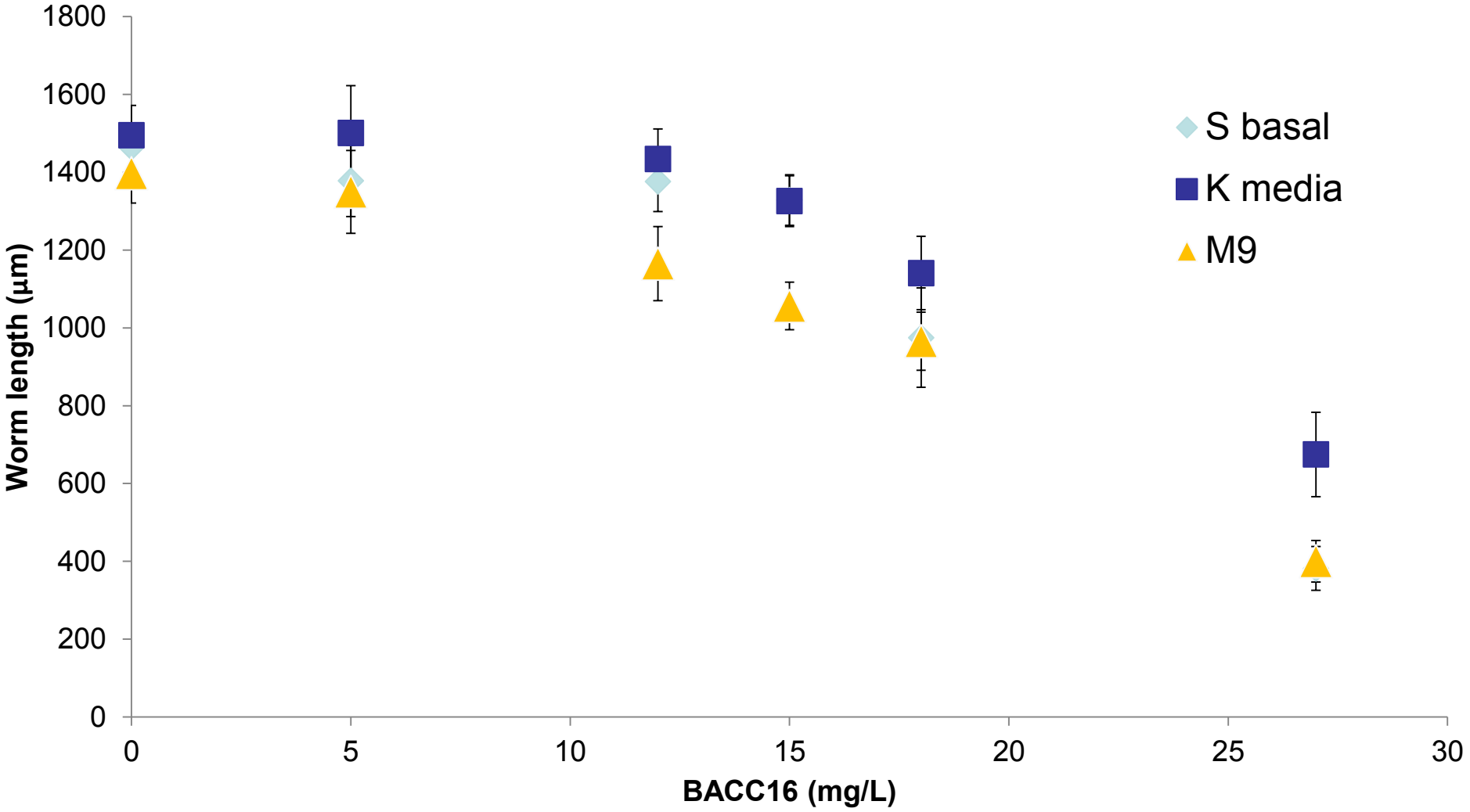

Growth inhibition of 15mg/L BAC C16
Growth inhibition (%)
70
60
50
40
30
20
10
0
Stopped shaking
10/06/2014
30/07/2014
18/09/2014
07/11/2014
27/12/2014

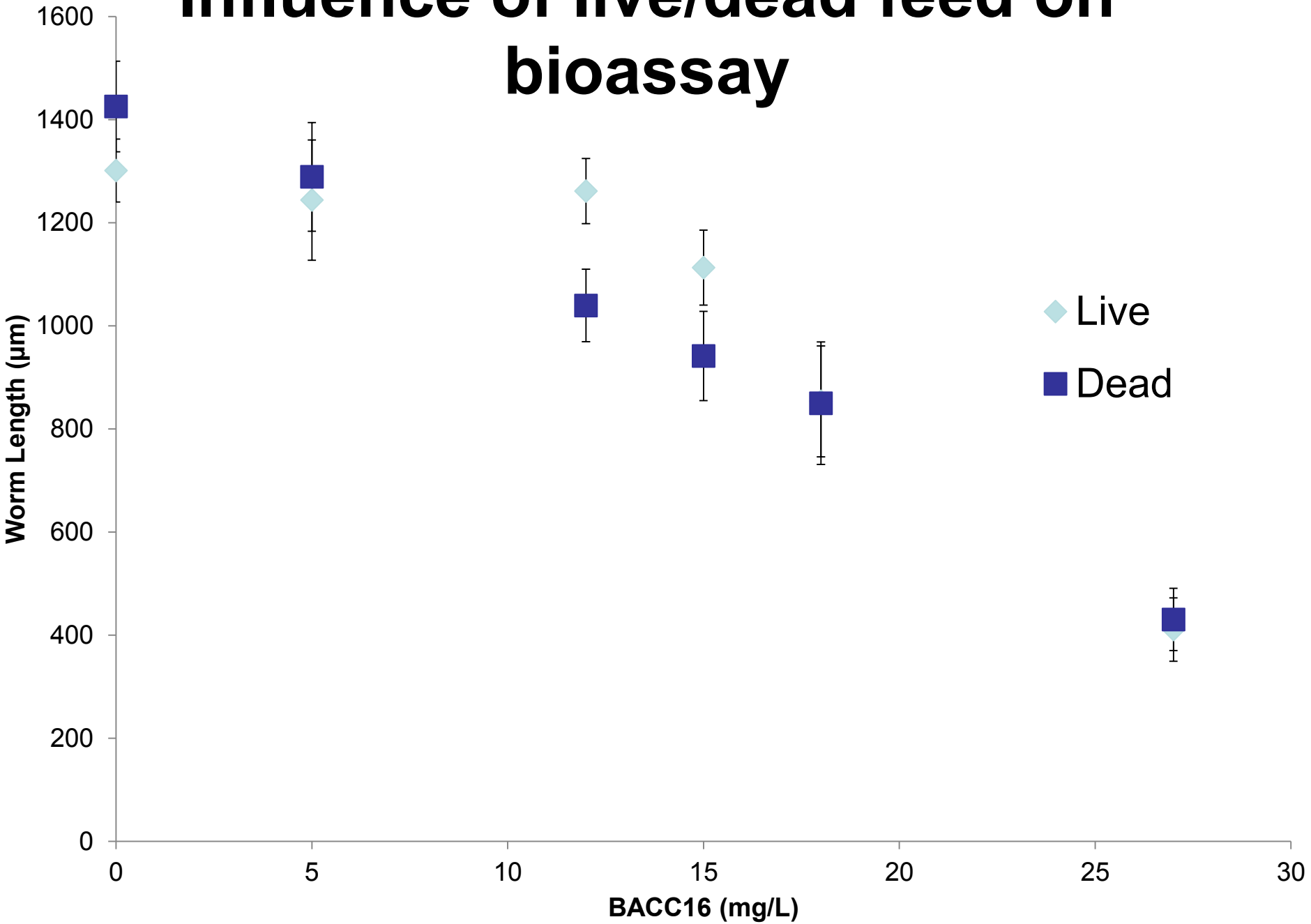
Plate shaking



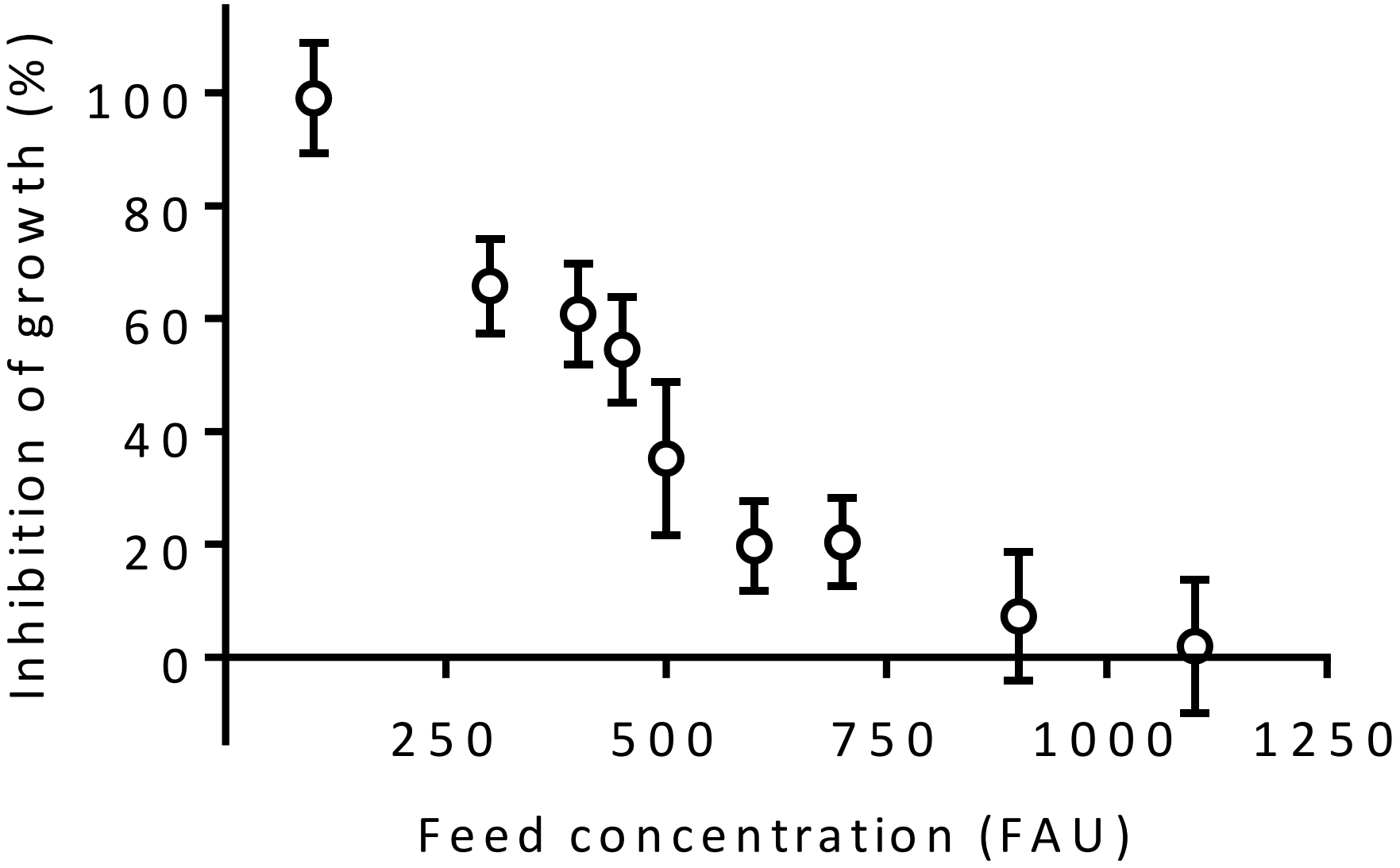
Influence of media on BAC toxicity



Influence of live/dead feed on bioassay

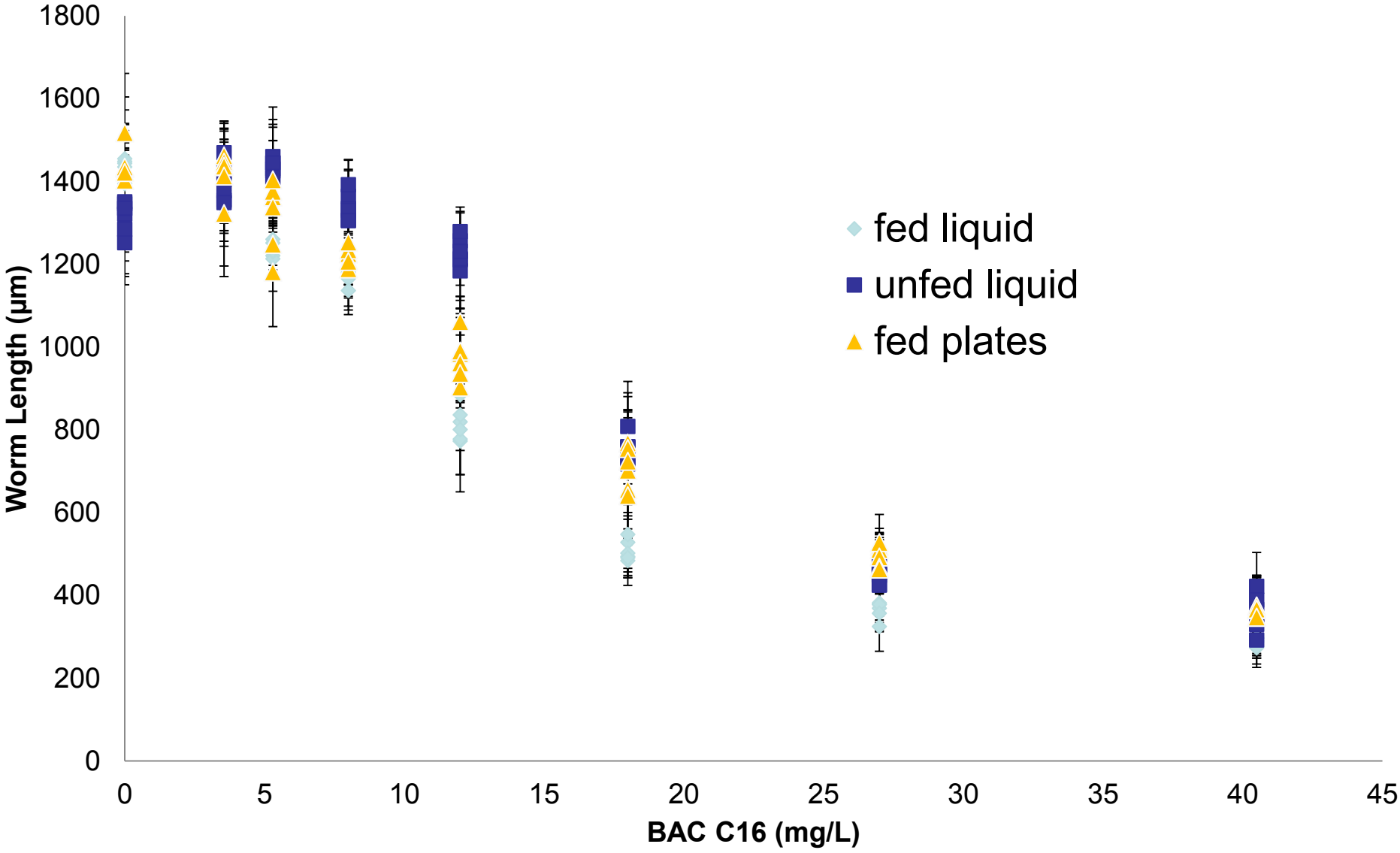


Bioassay feed dependence



Tests were conducted with 15 mg/L BAC-C16

Impact of worm culture on bioassay



*This experiment was performed with the plates shaking

Plate design to test for NP artifacts

Plates 1-3

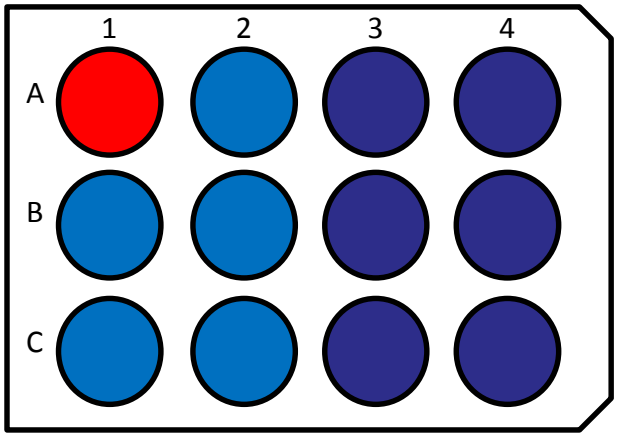
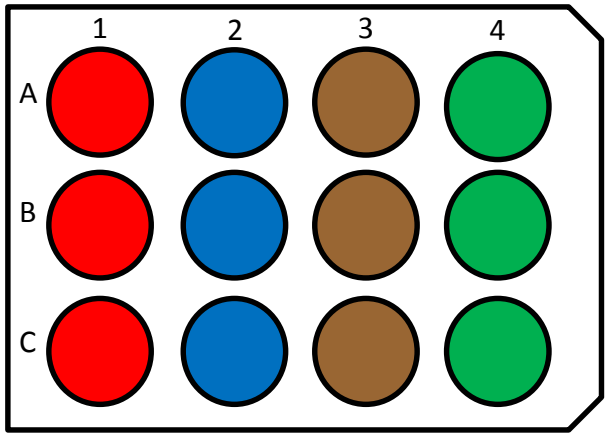
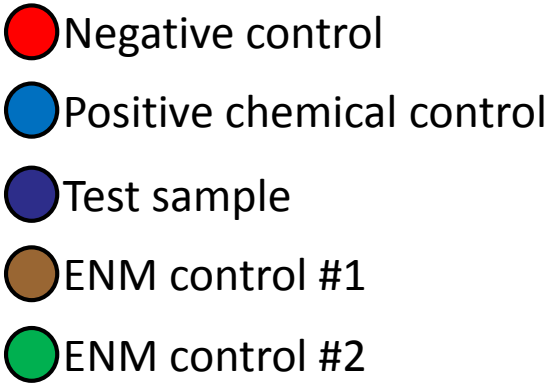
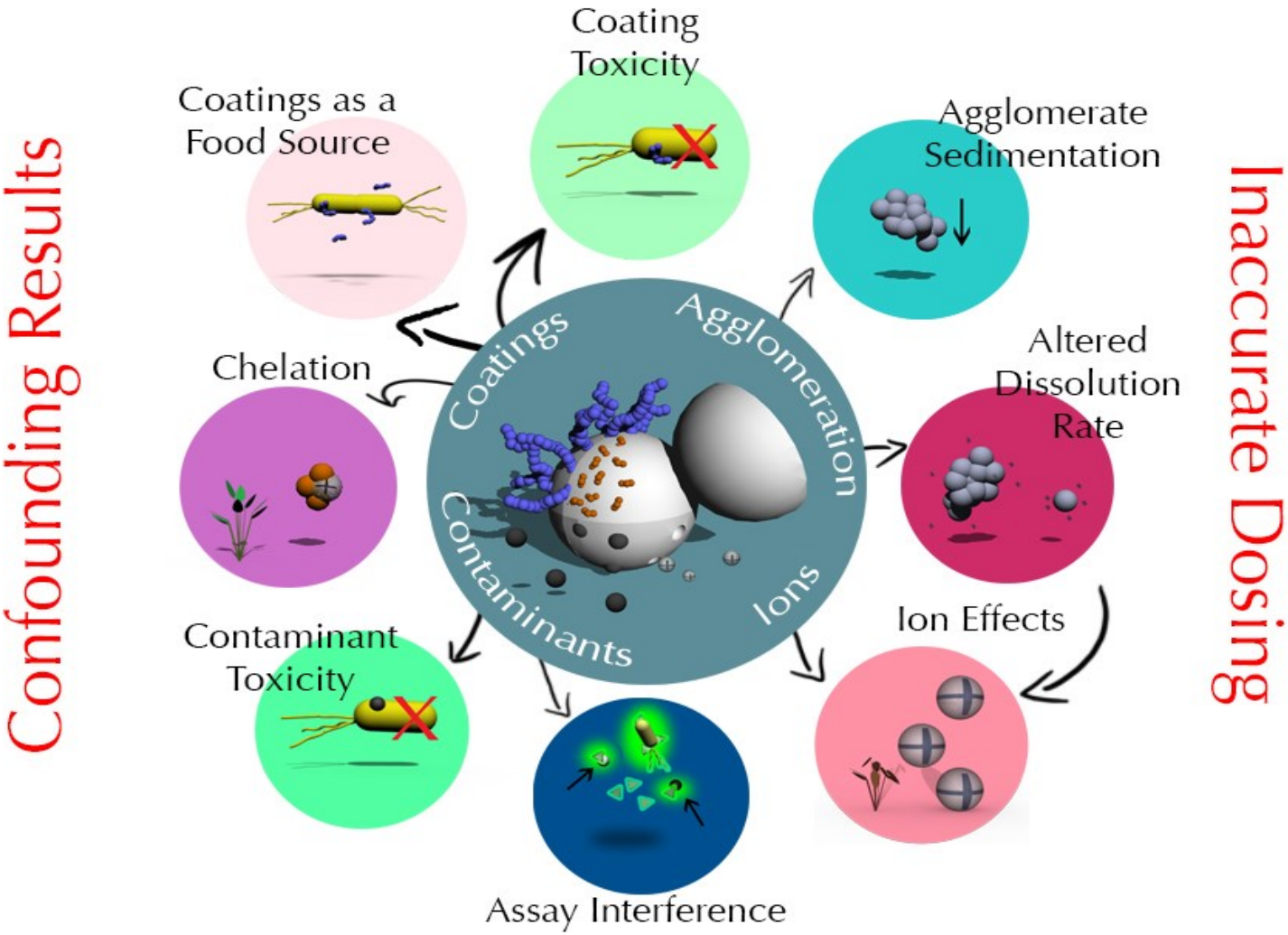


Plate 4

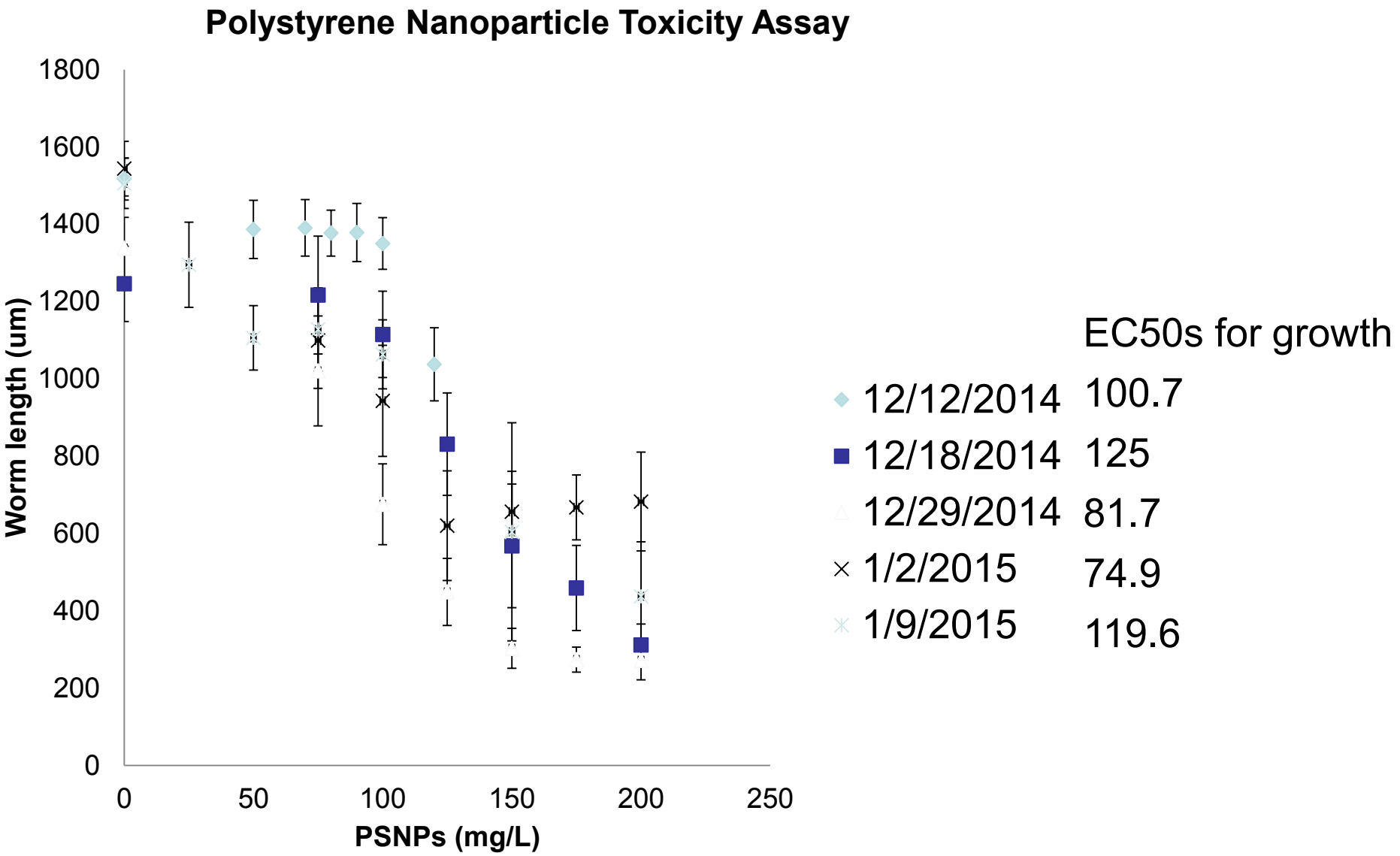




Poster: Identification and avoidance of potential artifacts and misinterpretations in nanomaterial ecotoxicity measurements



Petersen et al., 2014, Environ. Sci. Technol. 48(8), p 4226-4246.


Polystyrene Nanoparticle Toxicity Assay
Worm length (um)
1800
1600
1400
1200
1000
800
600
400
200
0
0
50
100
150
200
250
PSNPs (mg/L)
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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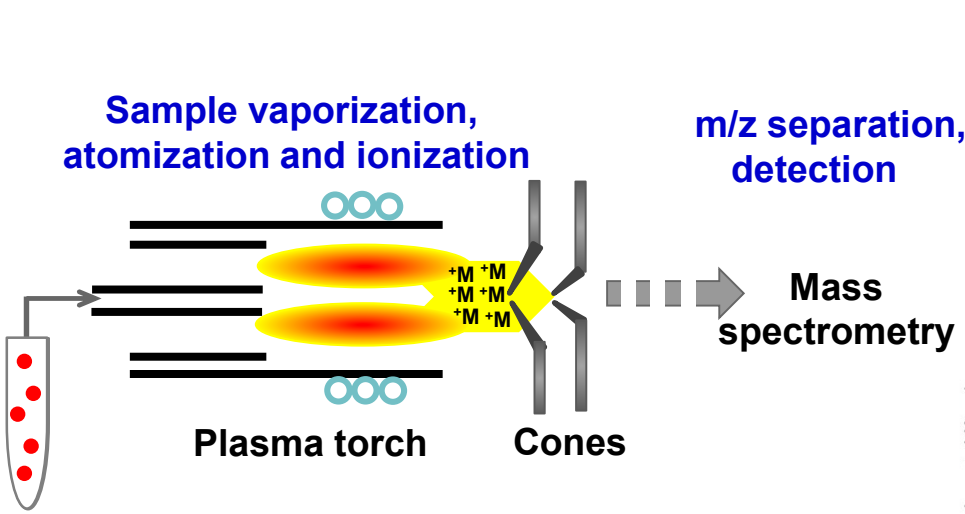
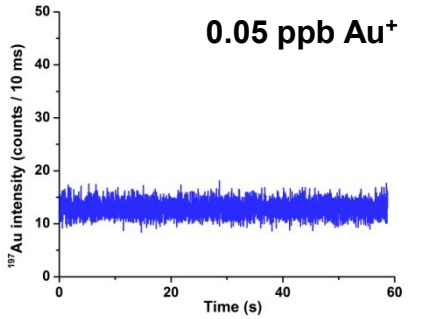
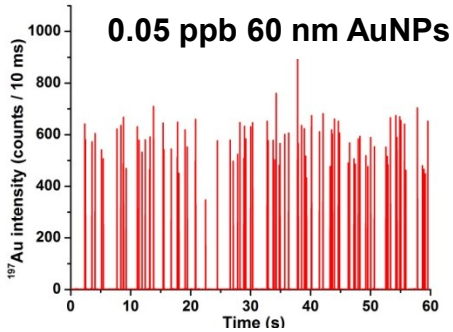
3. **Single-particle ICP-MS method to quantify the size distribution of gold nanoparticle uptake by *C. elegans***

Single Particle ICP-MS (spICP-MS)





$I \propto [M^+]$



N of spikes ∝ particle number concentration

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

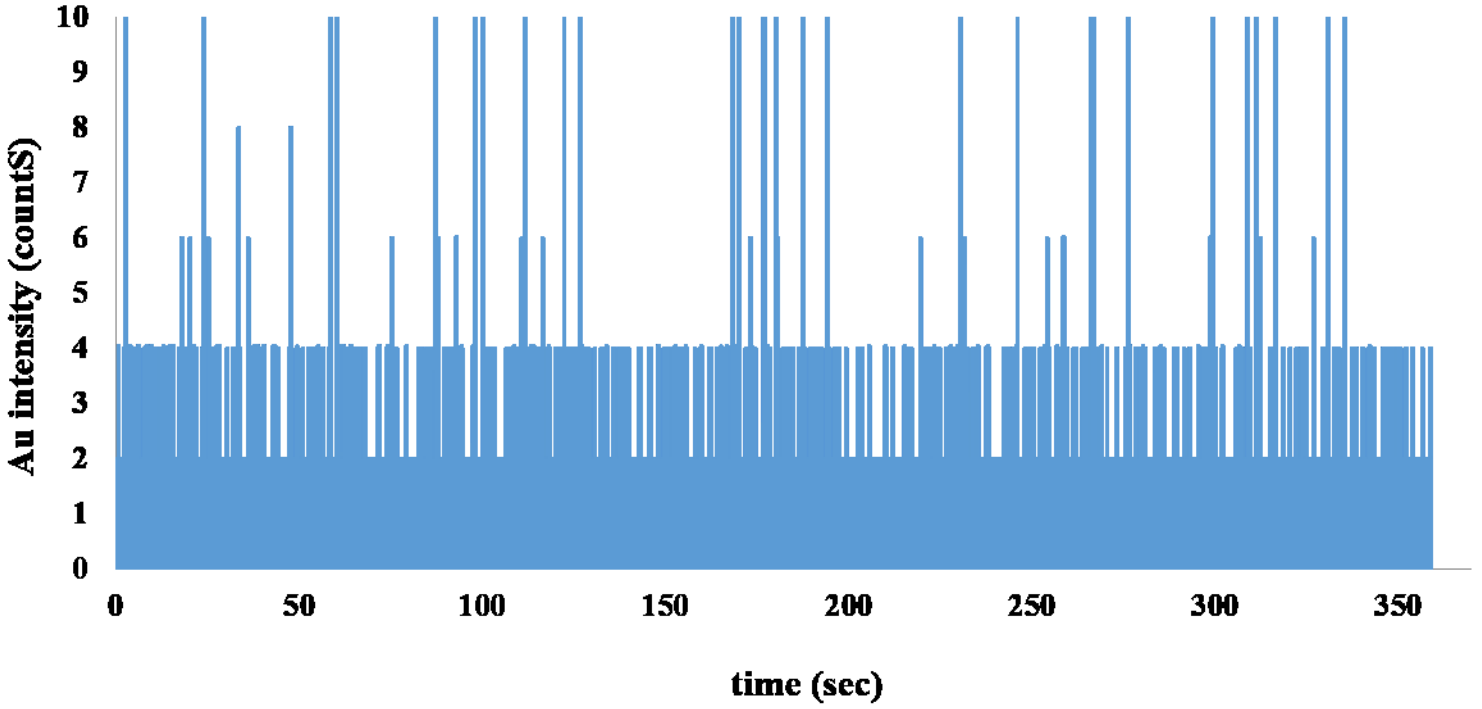
- Operates ICP-MS in time resolved analysis mode: a particle event lasts 0.4 ms, typical dwell time 3 - 10 ms
- Particle size distribution and number concentration

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

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

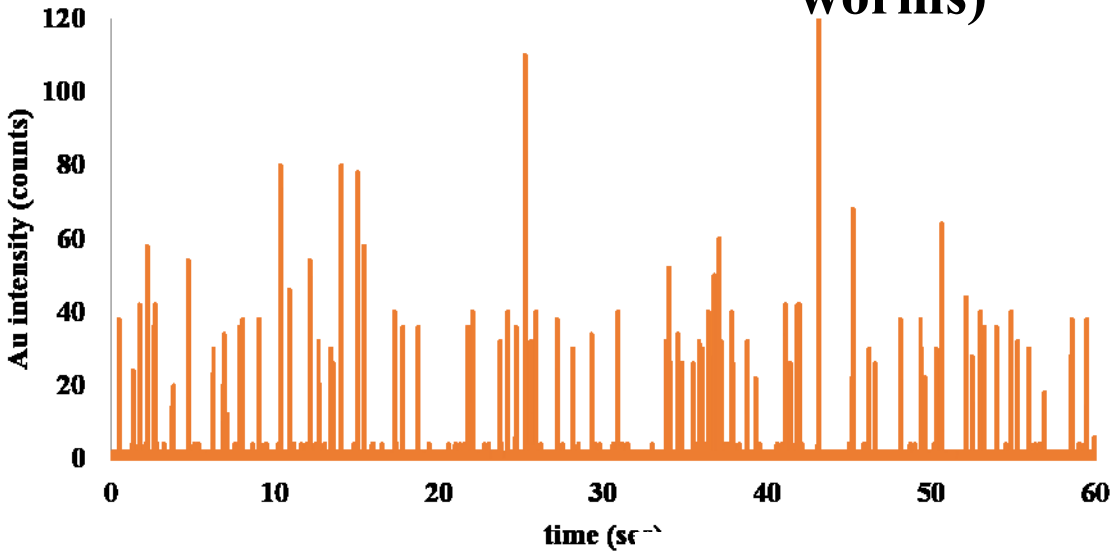
- 7 % TMAH (w/v) used for dissolution of worm tissue
- Dilution to bring TMAH concentration to <0.1%
- sp-ICP-MS measurement to test NPs accumulation on the organisms

spICP-MS Measurements of control *C. elegans*



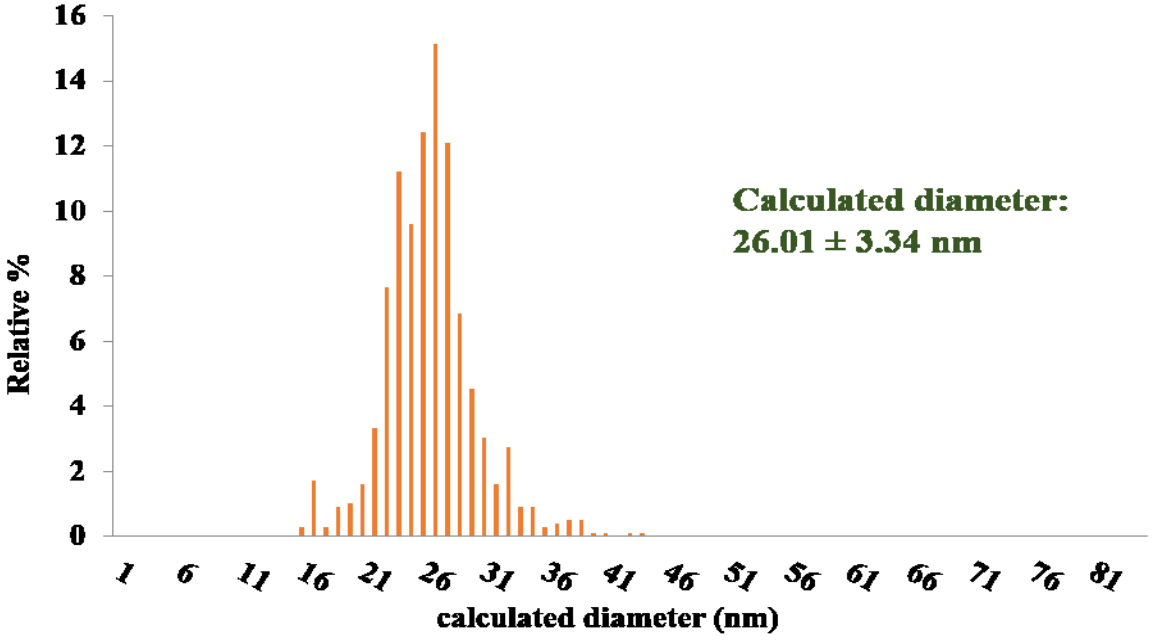
Single particle ICP-MS signals for *C. elegans* in control exposure.
Mean Au intensity: 0.18 counts. Date of exposure: June 24, 2014

spICP-MS Measurements of 30 nm AuNPs after digestion procedure (no worms)



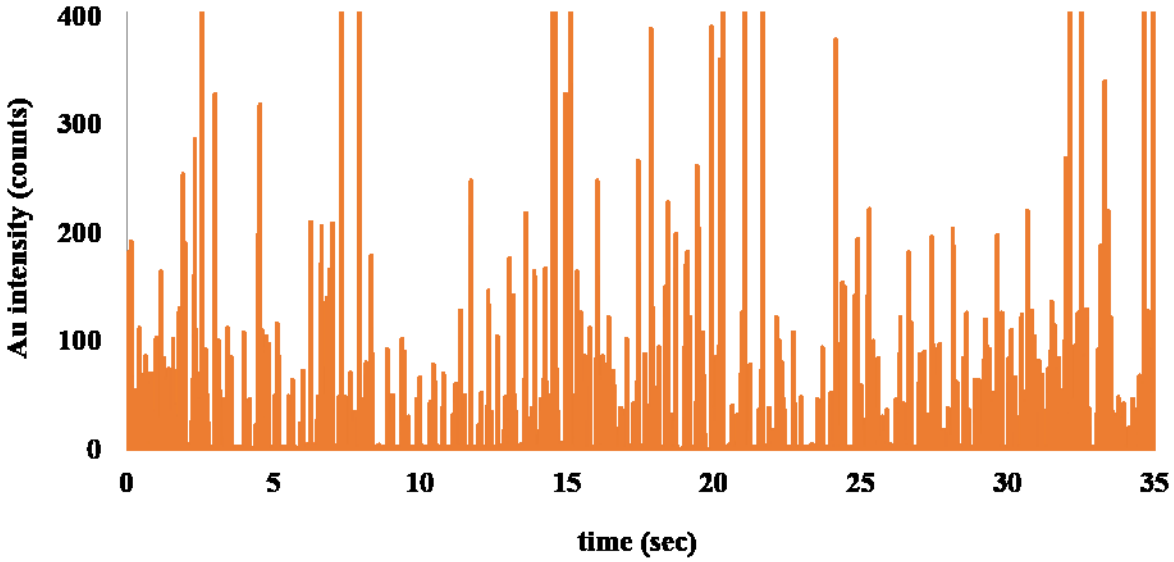
ingle particle ICP-MS signals for
0 nm AuNPs (NIST) following
eatment with 7% TMAH.
Control digestion, no worms.

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



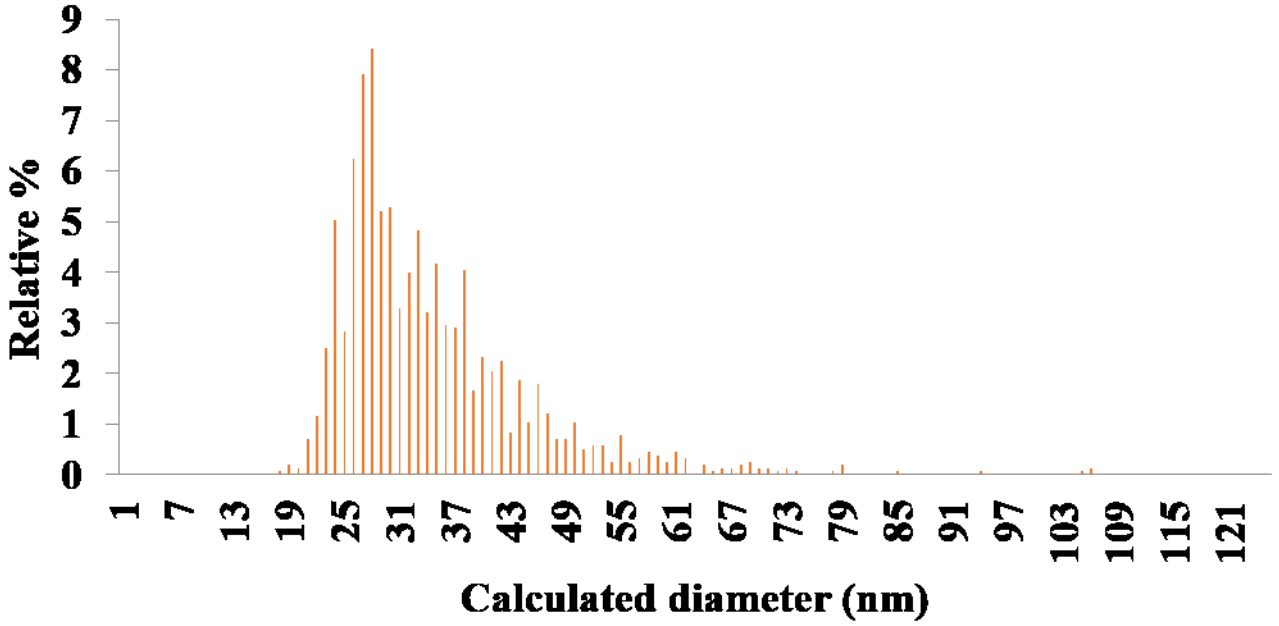
Calculated diameter:
26.01 ± 3.34 nm

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

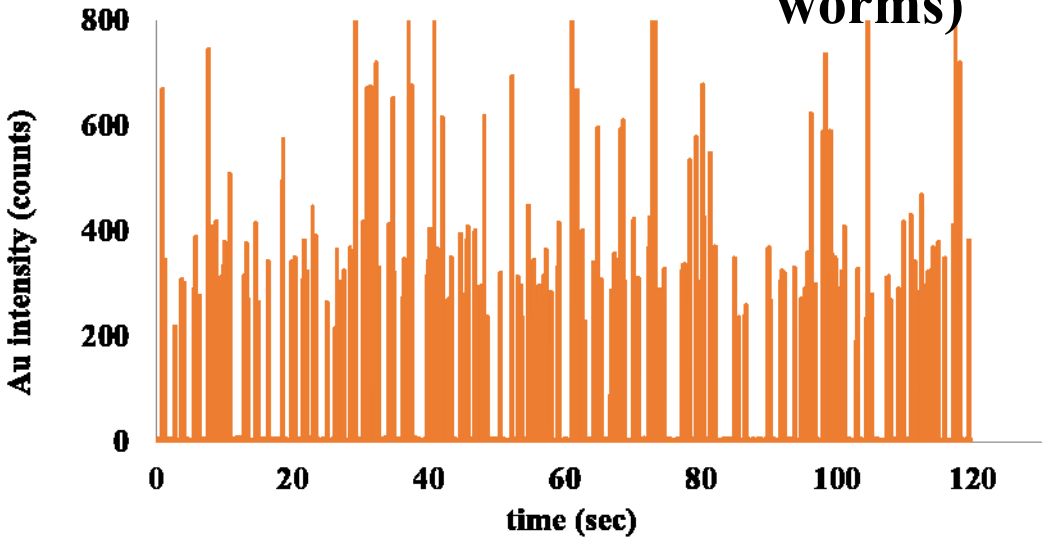


Single particle ICP-MS signals for *C. elegans* spiked with 30 nm AuNPs (NIST). 799 particle events in 60 s. **Diluted by a factor of 1500.**

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

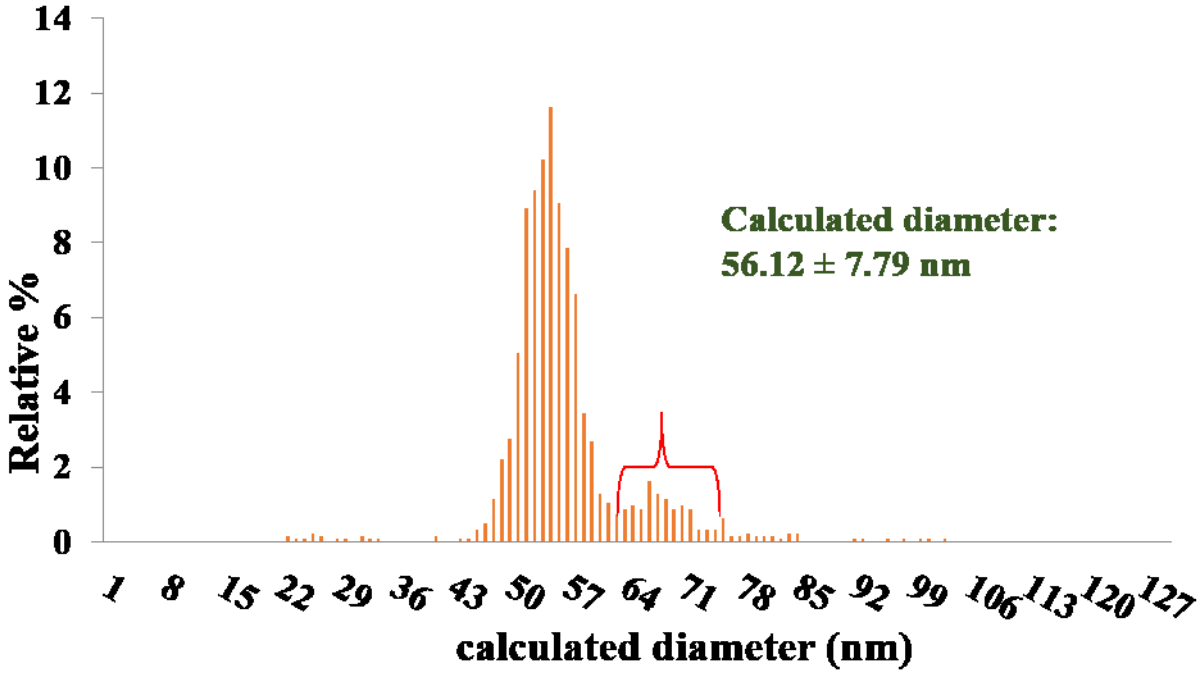


spICP-MS Measurements of 60 nm AuNPs after digestion procedure (no worms)

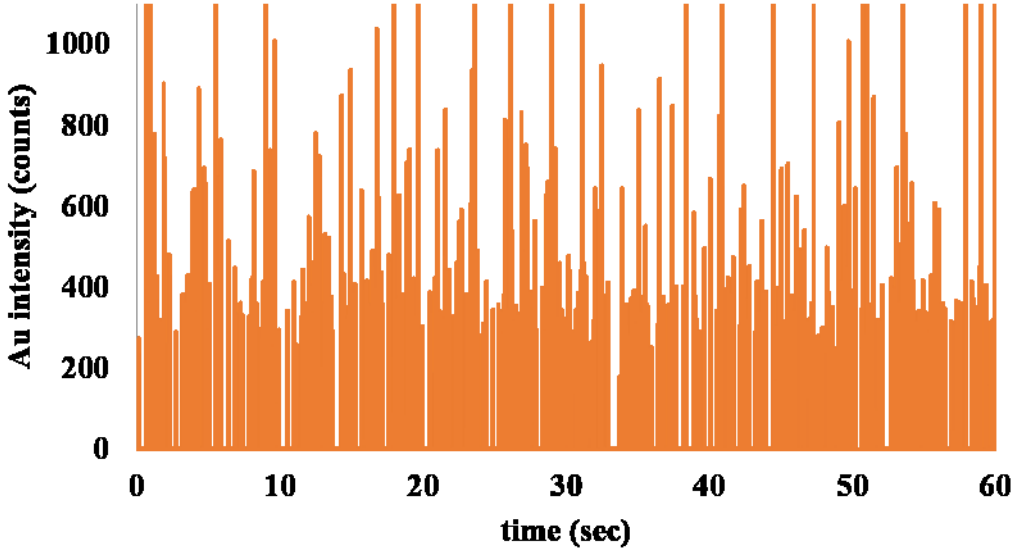


ngle particle ICP-MS signals for
) nm AuNPs (NIST) following
eatment with 7% TMAH.
ontrol digestion, no worms.

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

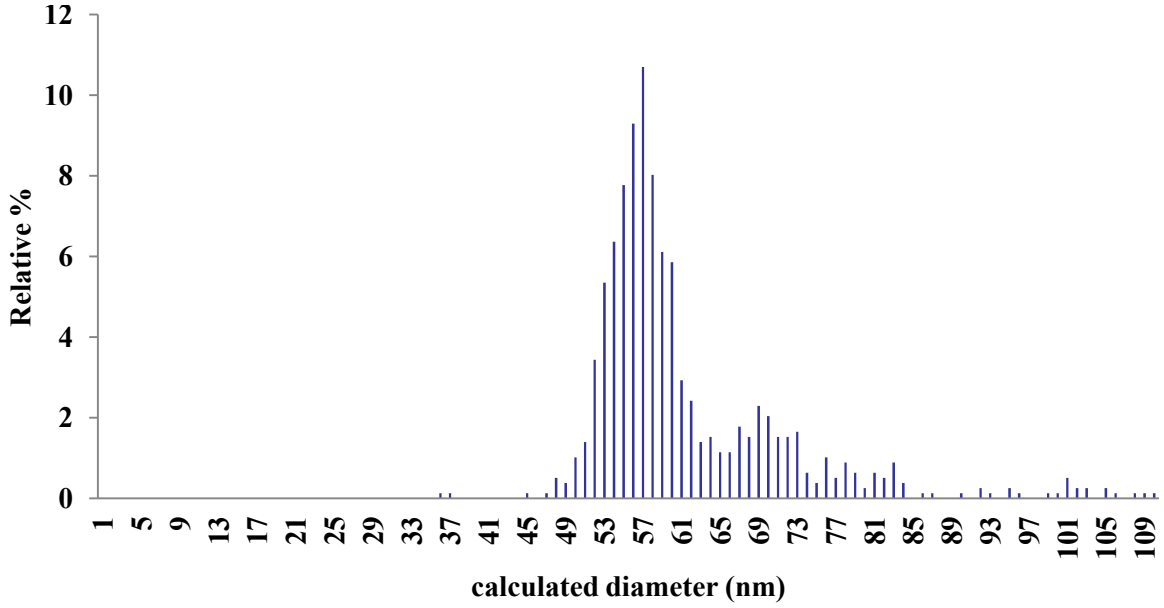


spICP-MS Measurements of *C. elegans* exposed to 60 nm AuNPs

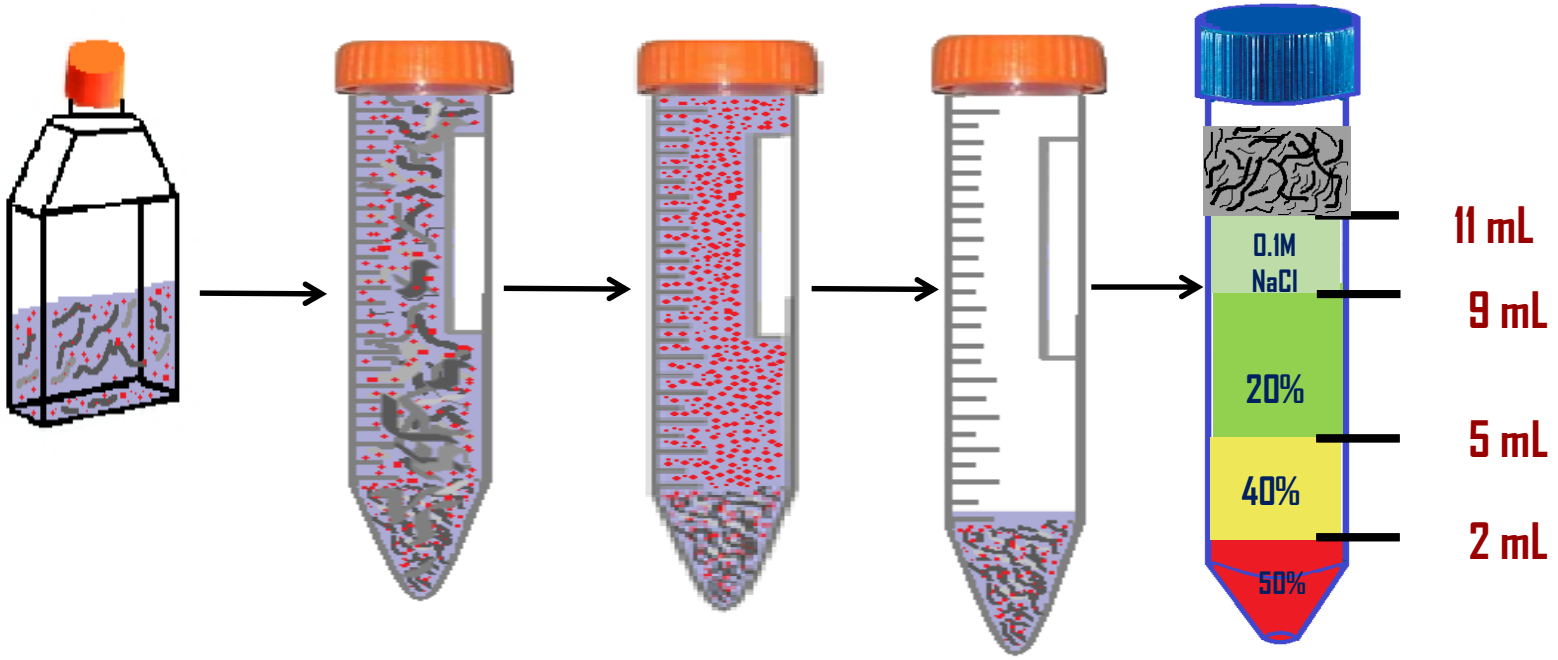


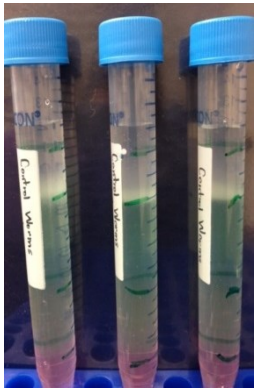
Single particle ICP-MS signals for *C. elegans* spiked with 60 nm AuNPs (NIST). 412 particle events in 60 s. Diluted by a factor of 2500.

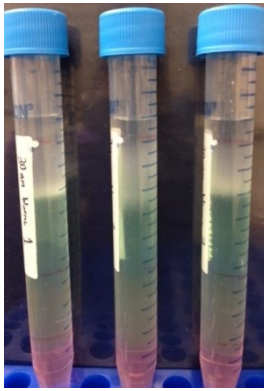
Size distribution of NPs extracted from *C. elegans* exposed to 60 nm AuNPs (NIST).



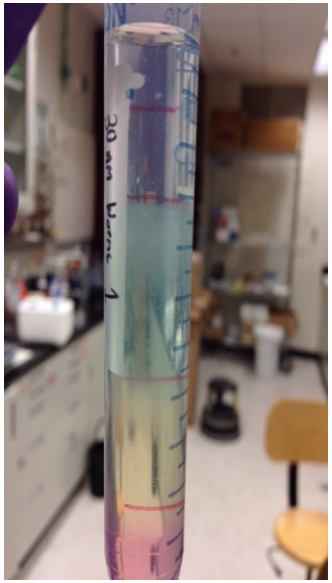
Sucrose density gradient centrifugation to separate *C. elegans* and AuNPs

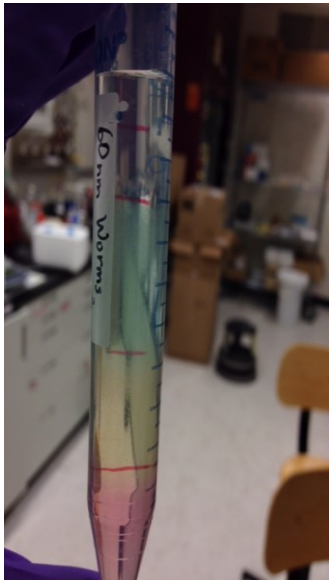


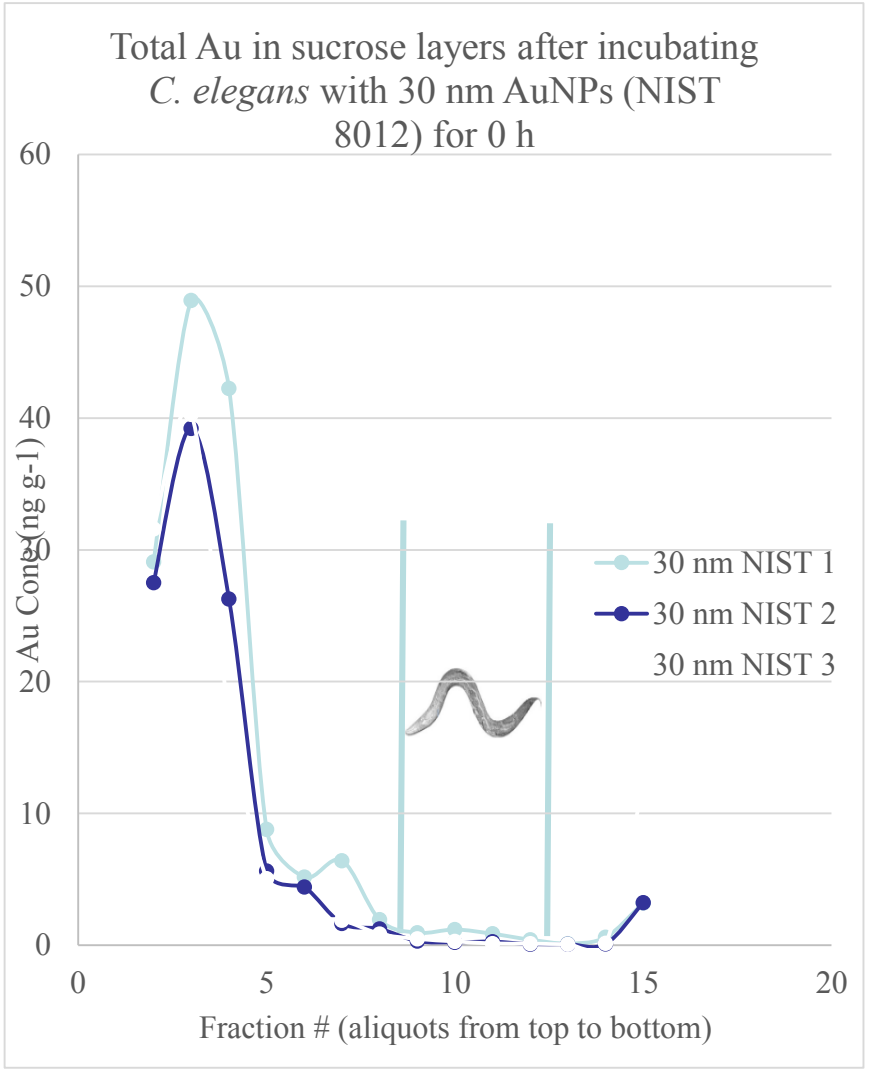


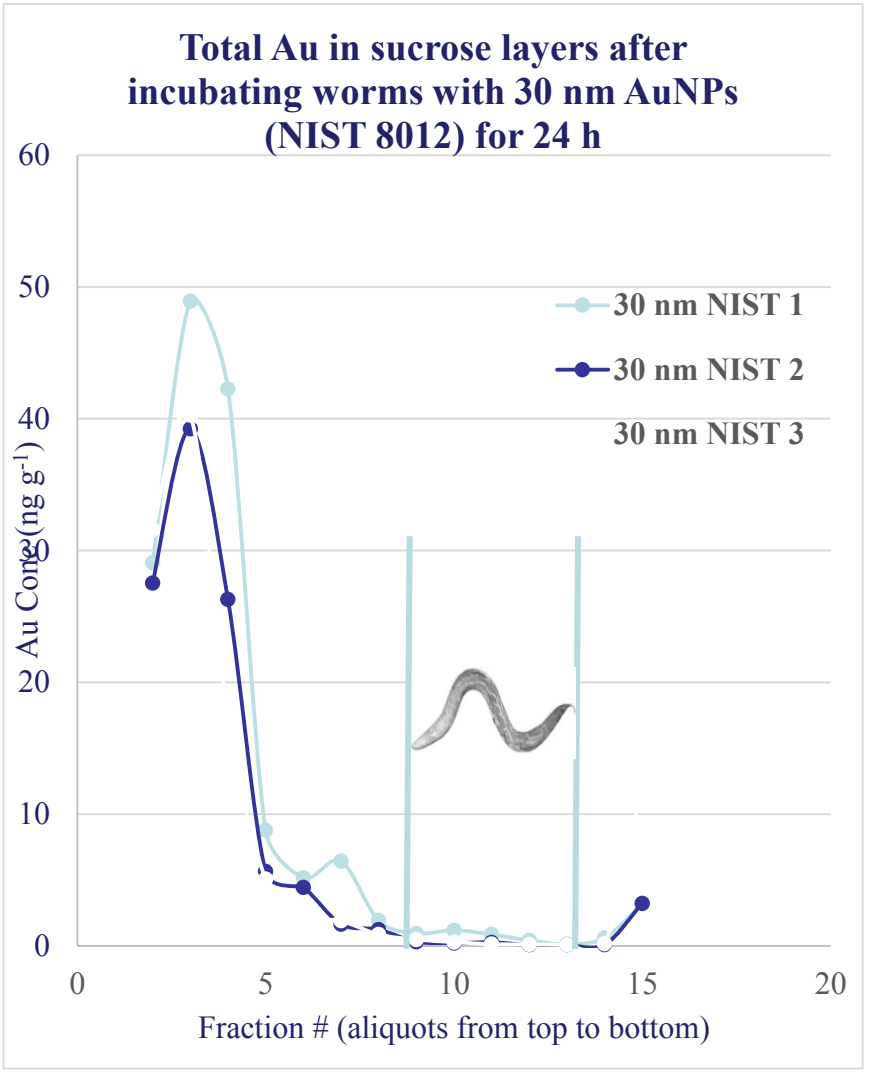


Spin in centrifuge

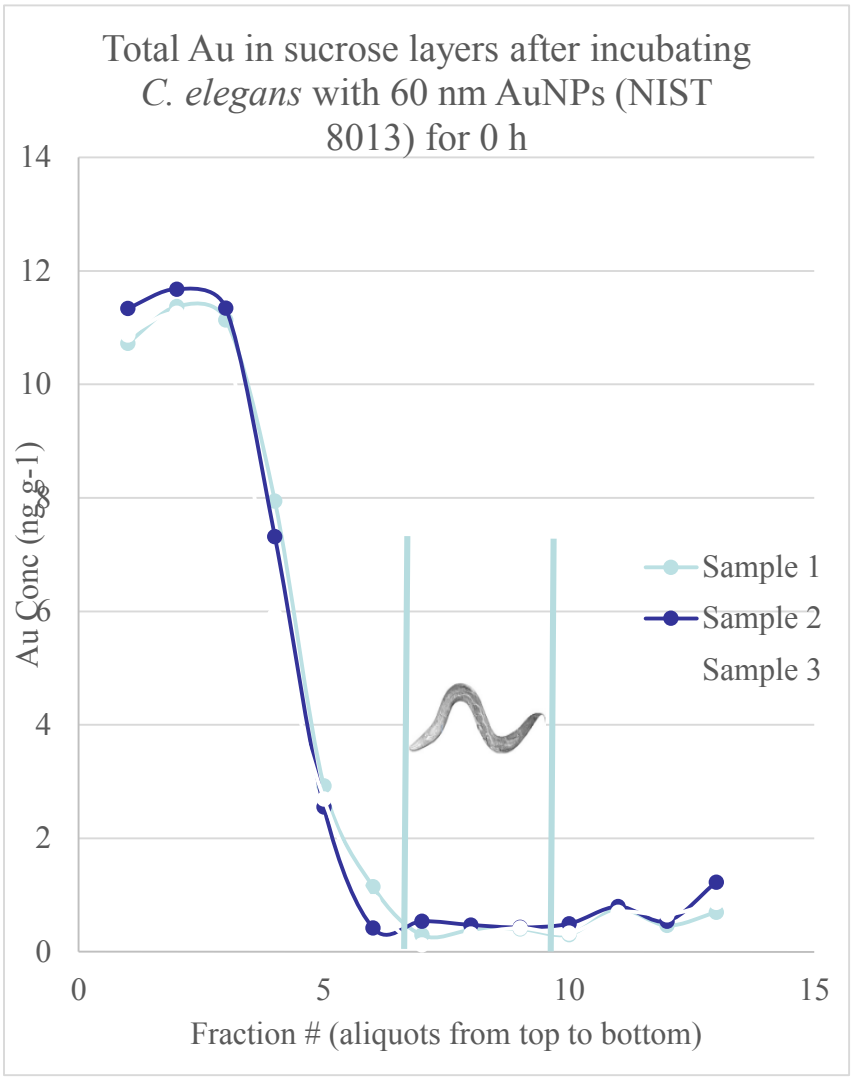


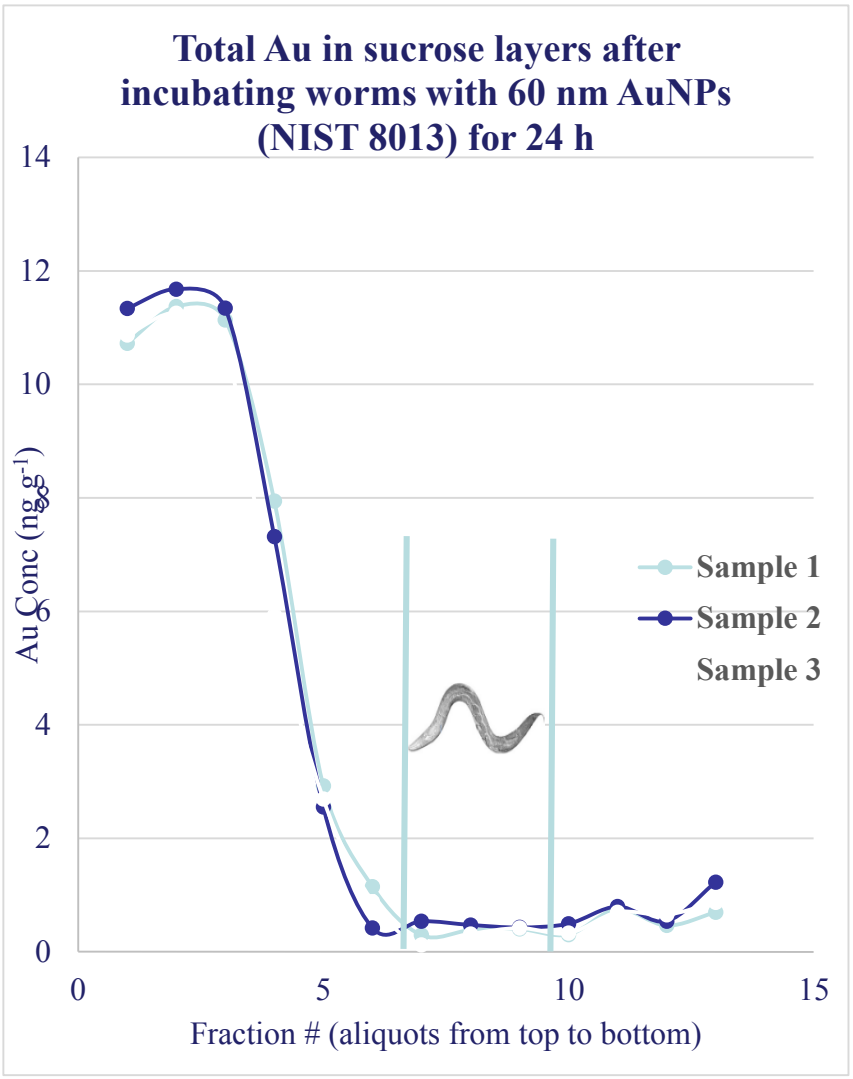






% Recovery of Au conc: 95.6% ± 3.7%





% 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