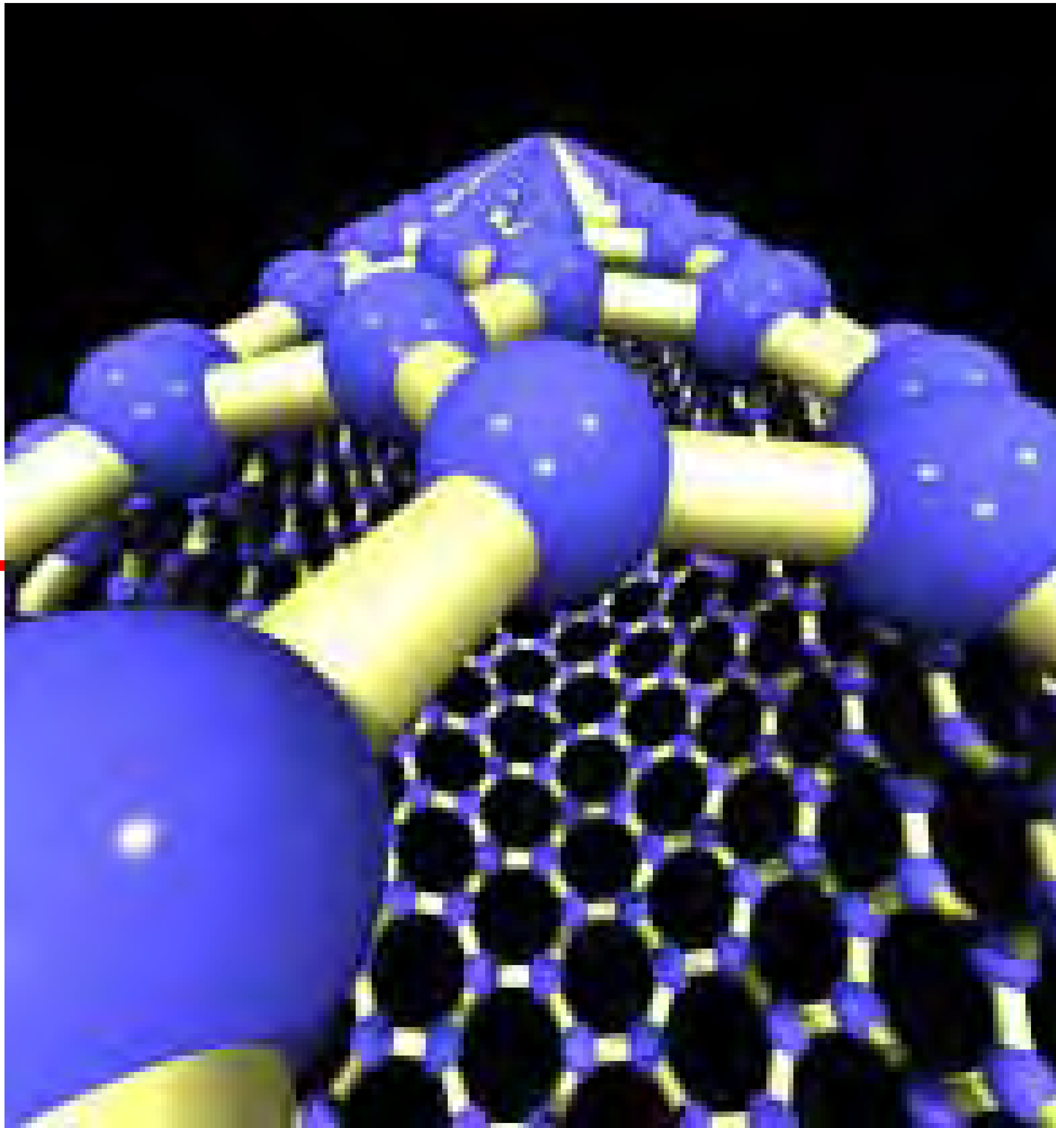
# Nanomaterials with Antimicrobial Properties: Mechanisms, Implications and Applications

USCOE ERDC-EL-MS

18 August 2009



**Pedro J.J. Alvarez**

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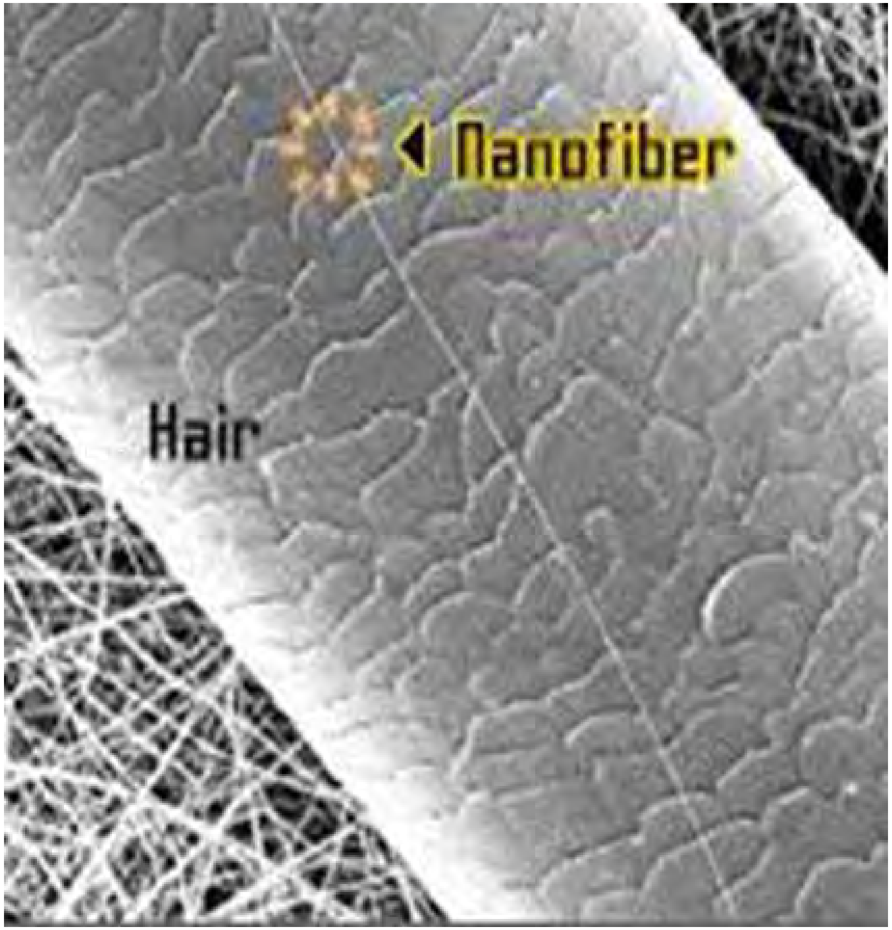
# Acknowledgements: NSF/CBEN, EPA


# Nano = Dwarf (Greek) = $10^{-9}$

"Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications."

-National Nanotechnology Initiative



# Nanomaterials in the Environment

## Engineered

- Carbon-based
  NTs, Fullerenes
- Metal Oxides
- Quantum Dots
- Nanotubes
- Nanowires
- Dendrimers

## Incidental

Particles from:

- Combustion
- Industrial
  Processes
- Vehicles
- Construction

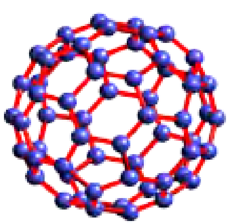
## Natural

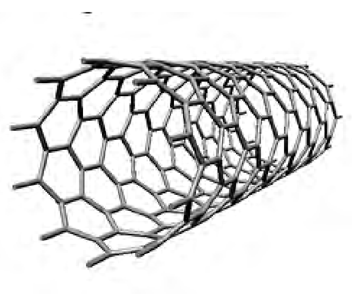
Particles from:

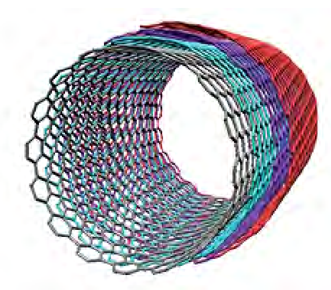
- Plants, Trees
- Oceans, other
  water bodies
- Erosion
- Dust

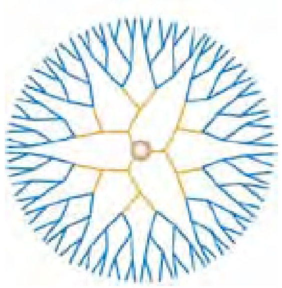
# Unique Properties of Nanoscale Materials

- Chemical reactivity greatly different from macroscopic forms, e.g., gold
- High surface area per unit mass (>100 $m^2/g$)
- Quantum effects (dual behavior, wave- and particle-like) resulting in unique mechanical, electronic, photonic, and magnetic properties



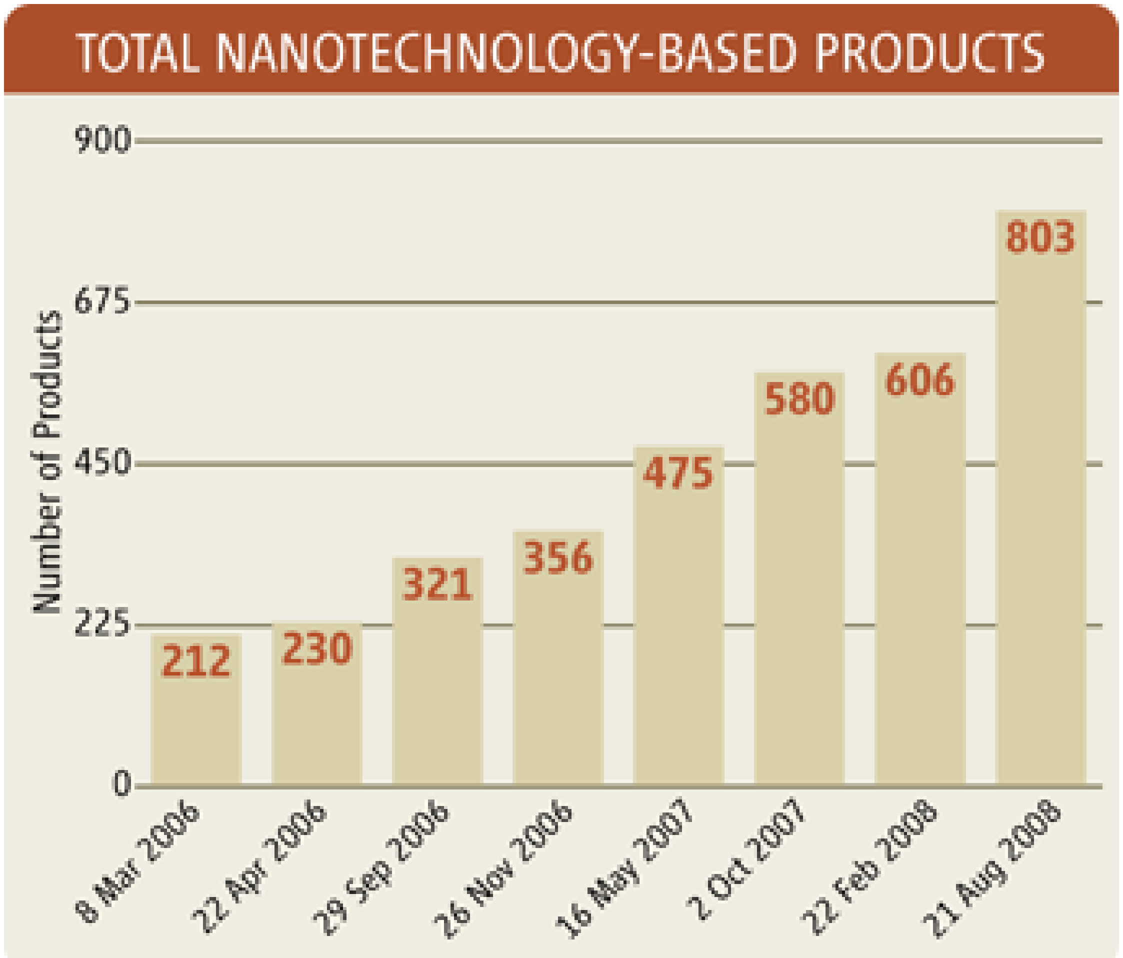






# 800 Commercial products contain nanomaterials

(sensors, electronics, drug delivery, tissue engineering, imaging, catalysis, cosmetics, sunscreens, etc.)




Robert F. Service, 2008, *Science*

# Nano-products on the Market Now

Display Screens
Motorola (NTs)

Automobiles
(BASF's Mincor®
Nanocomposite)

Sunscreen
**($TiO_2$)**

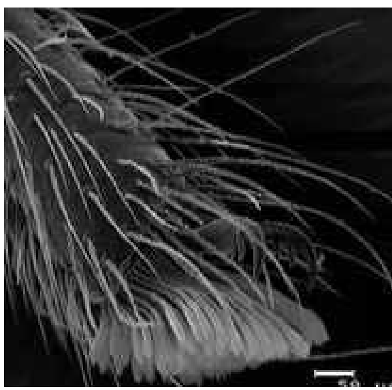
Nano Silver Wash
Samsung Washing Machine

Tennis Rackets
Wilson (C fibers)

# Responsible Nanotechnology

"With Great Power, Comes Great Responsibility"

*Uncle Ben to Peter Parker in Spider Man*



Nanoscale fibers on spider feet


Paul Hermann Muller
Thomas Midgley

(Dr. Nigel Walker, NIEHS NTP)

# Environmental Concerns & Opportunities

1. **Implications:** Create the information needed to use nanomaterials in an *environmentally responsible* and *sustainable manner*

*CBEN Societal Driver: To enable effective risk management for emerging nanotechnologies.*

2. **Applications:** *Enhanced* or *new* capabilities to address existing and future environmental problems.


*CBEN Systems Goal: To develop effective water treatment systems that exploit engineered nanoparticles*

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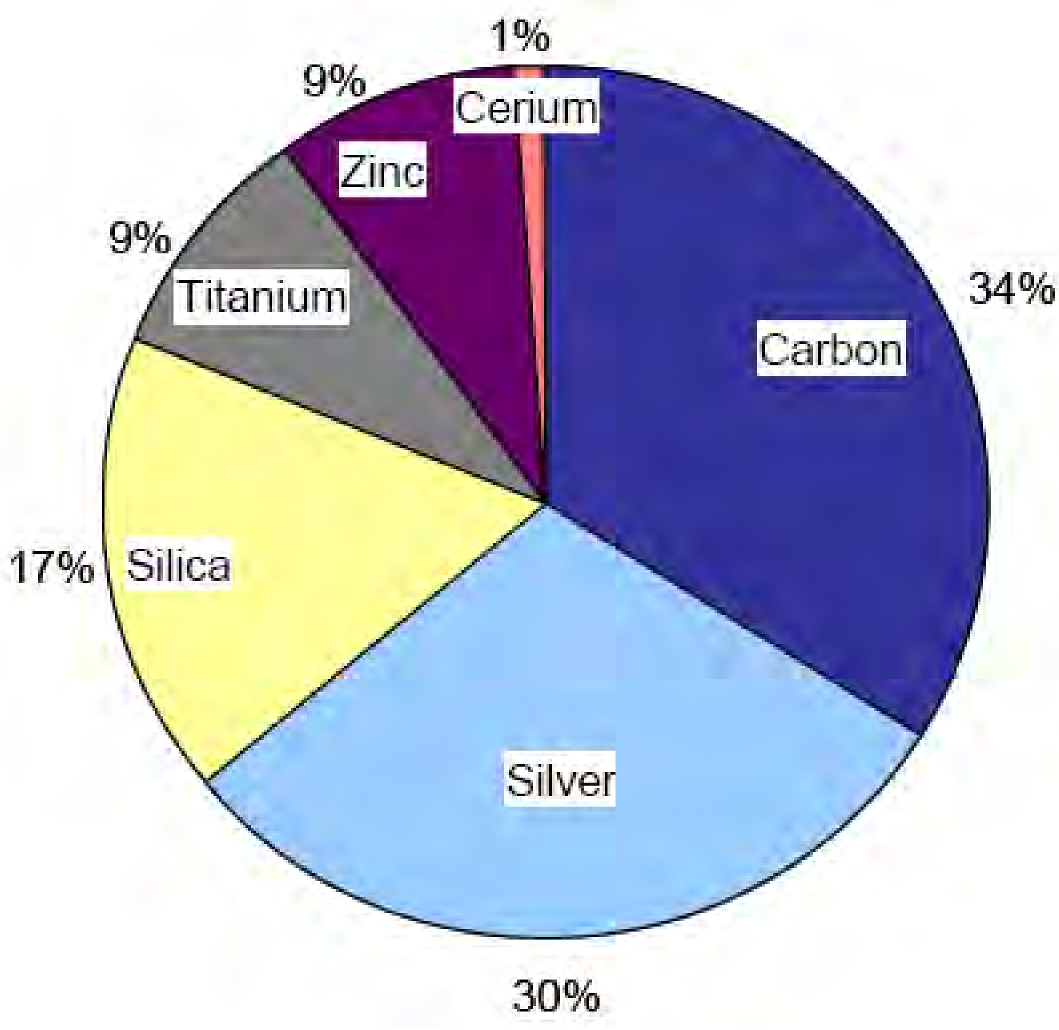
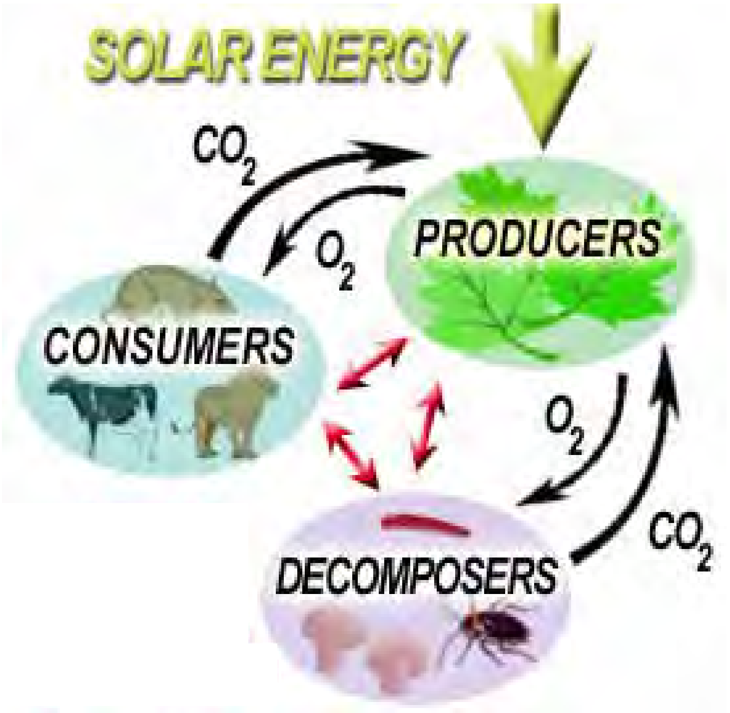
# Which Types of Nanomaterials Should We Focus On?




Source Maynard AD (2006): Nanotechnology: A Research Strategy for Addressing Risk

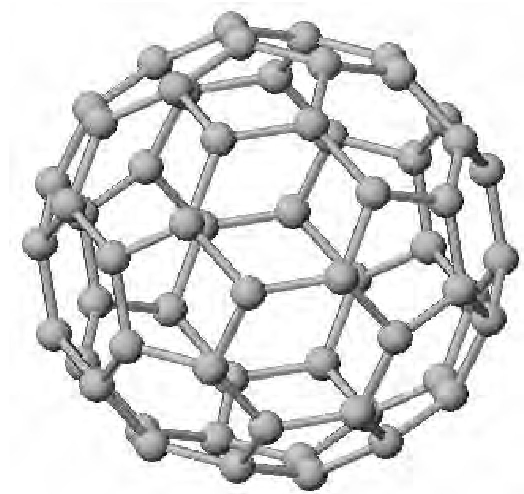
# Microbial-nanoparticle Interactions to Inform Risk Assessment



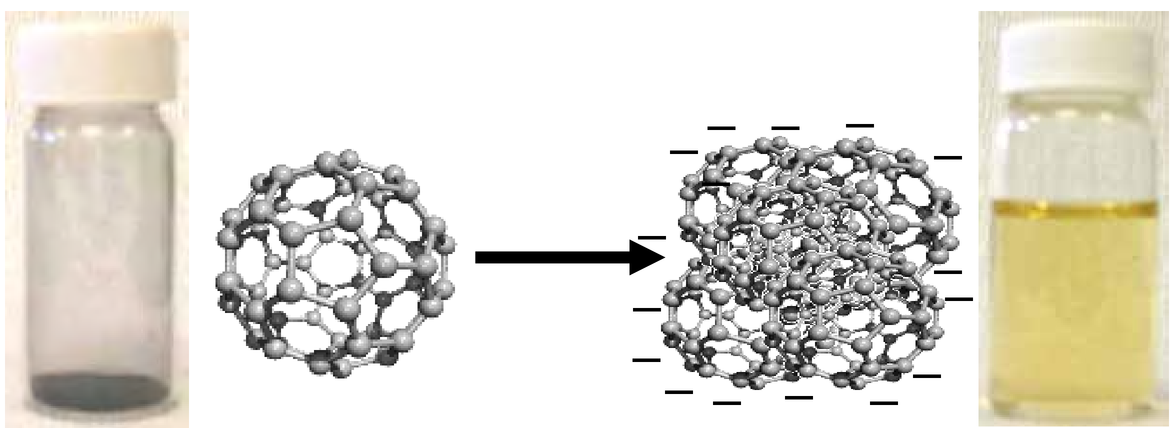

- Bacteria are at the foundation of all ecosystems, and carry out many ecosystem services
- Disposal/discharge can disrupt primary productivity, nutrient cycles, biodegradation, agriculture, etc.
- Antibacterial activity may be indicative of toxicity to higher level organisms

# $C_{60}$ (buckminsterfullerene)

Photocatalyst
and Antioxidant
(sp2 hybridized)



R. Buckminster Fuller (Bucky)



Solid or solution

$nC_{60}$ (20-200 nm)

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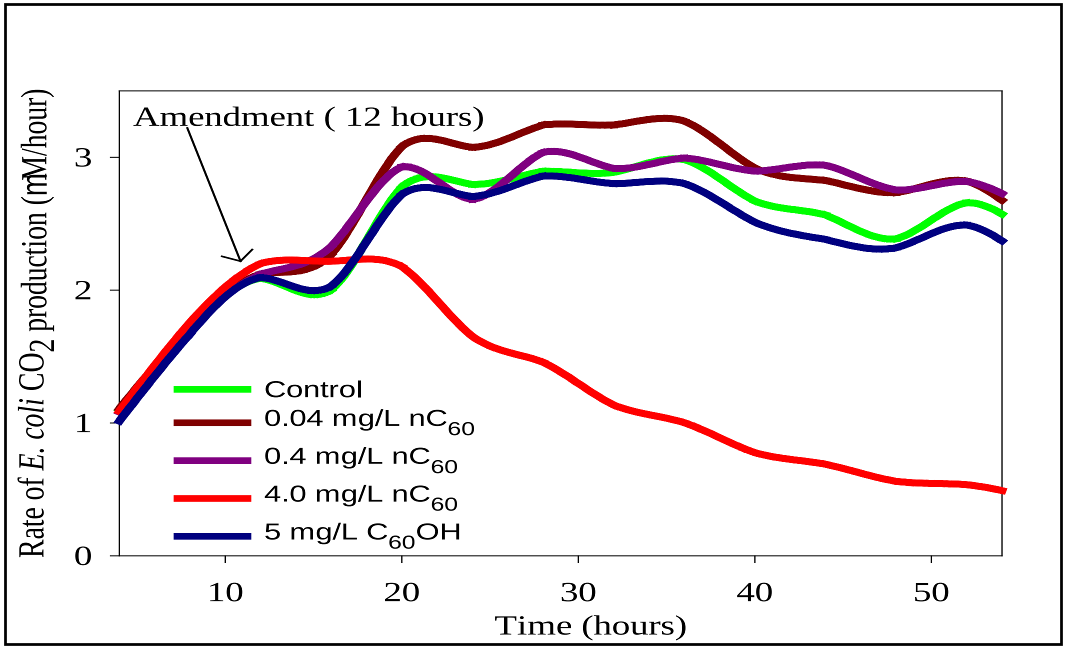
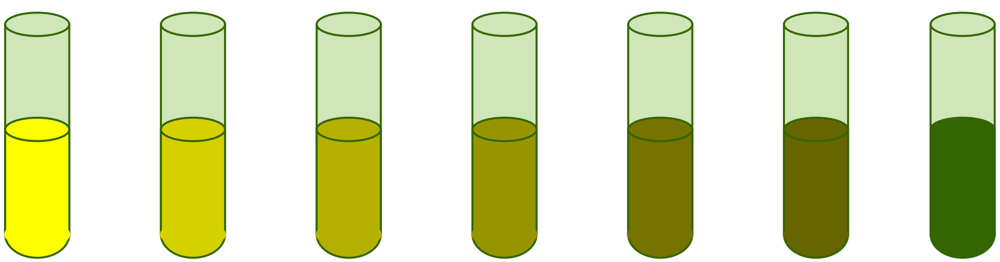
# $nC_{60}$ is antibacterial




*E. coli* respiration ceases after exposure to $nC_{60}$

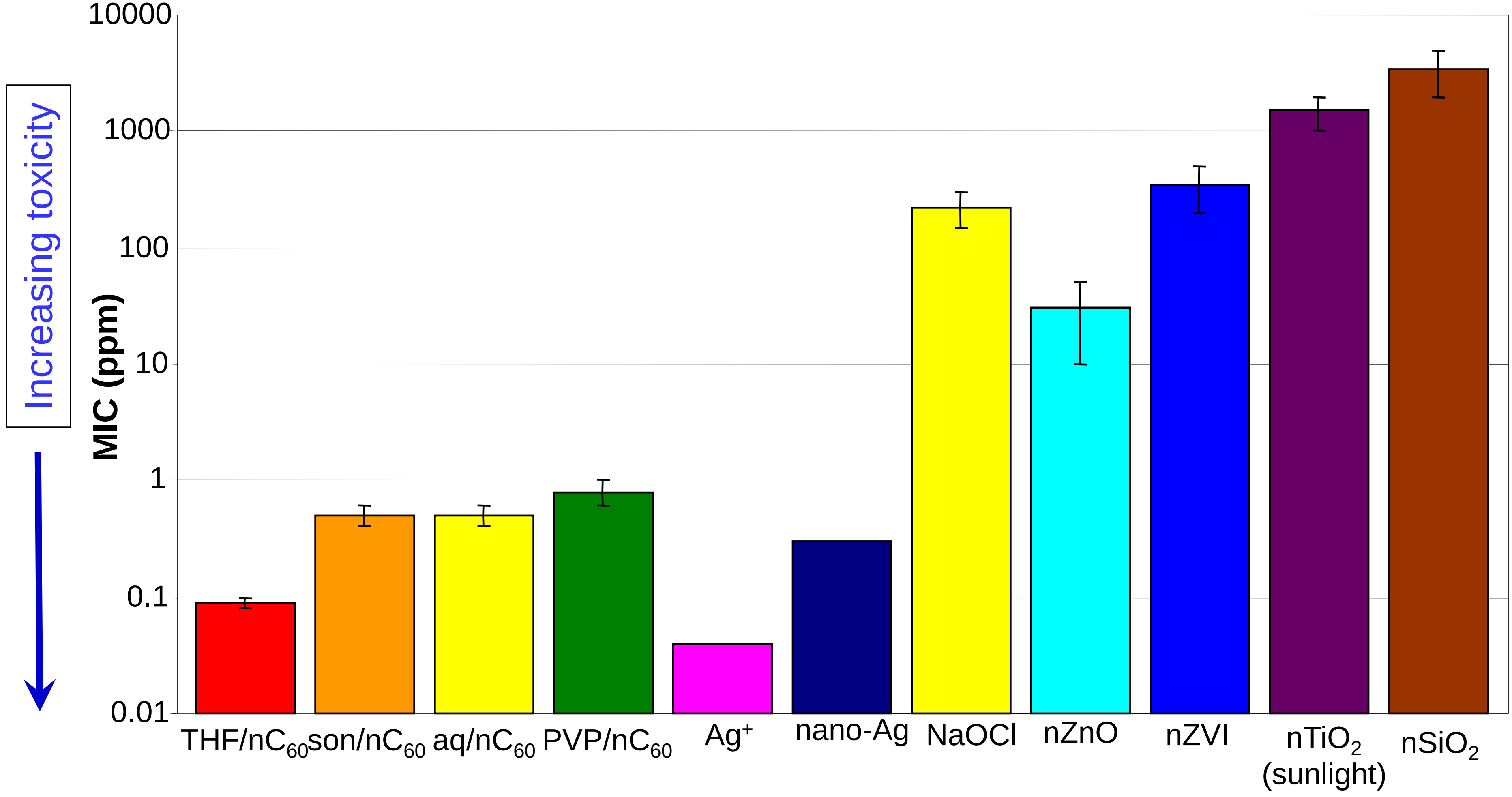
<u>Standardized Microtox Assay</u>



*Vibrio fischeri* (luminescent bacteria) with increasing concentrations of $nC_{60}$

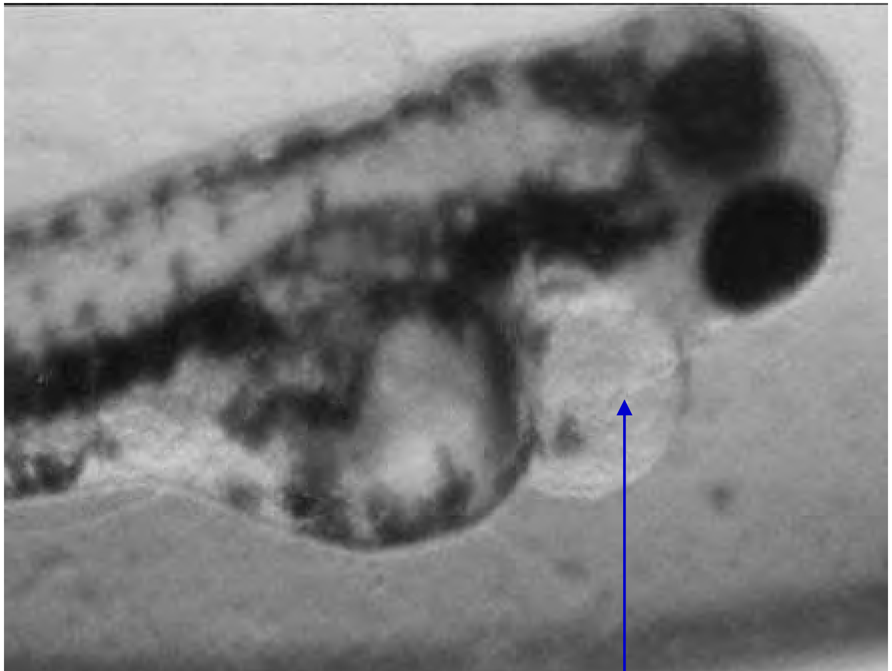
| Compound | $EC_{50}$ (mg/L) |
|---|---|
| $nC_{60}$ | 1.6 |
| Benzene | 2.0 |
| Sodium azide | 43-66 |

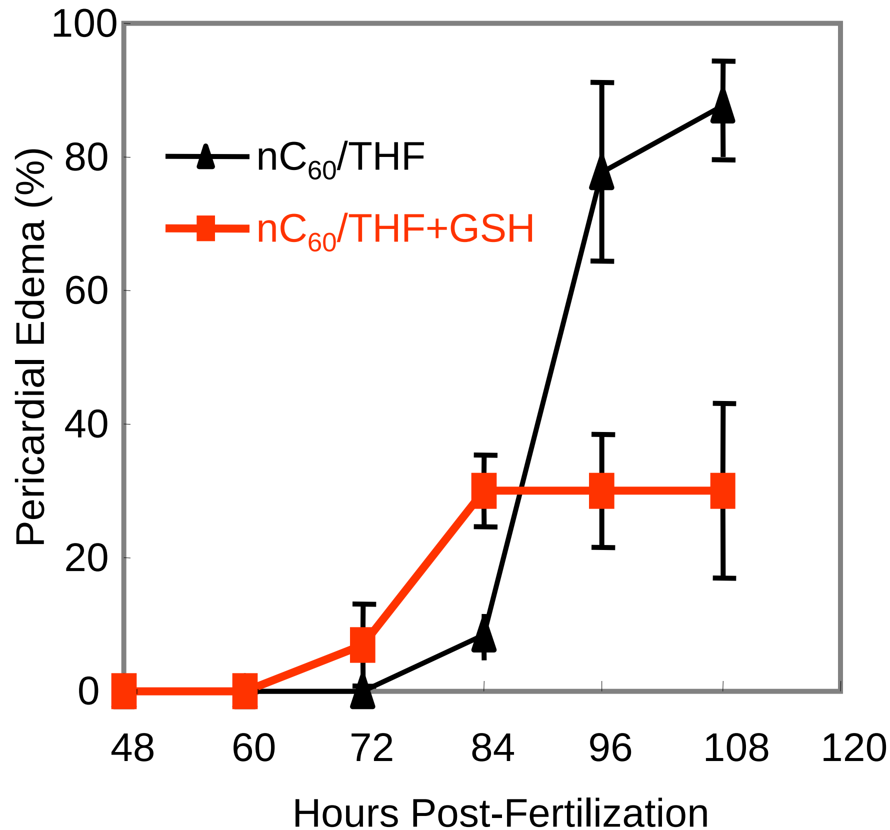
# $nC_{60}$ is more toxic to bacteria than many other common nanomaterials



# Developmental toxicity of $nC_{60}$ (Zebrafish)

**Mitigation by GSH suggest that toxicity is related to oxidative stress**

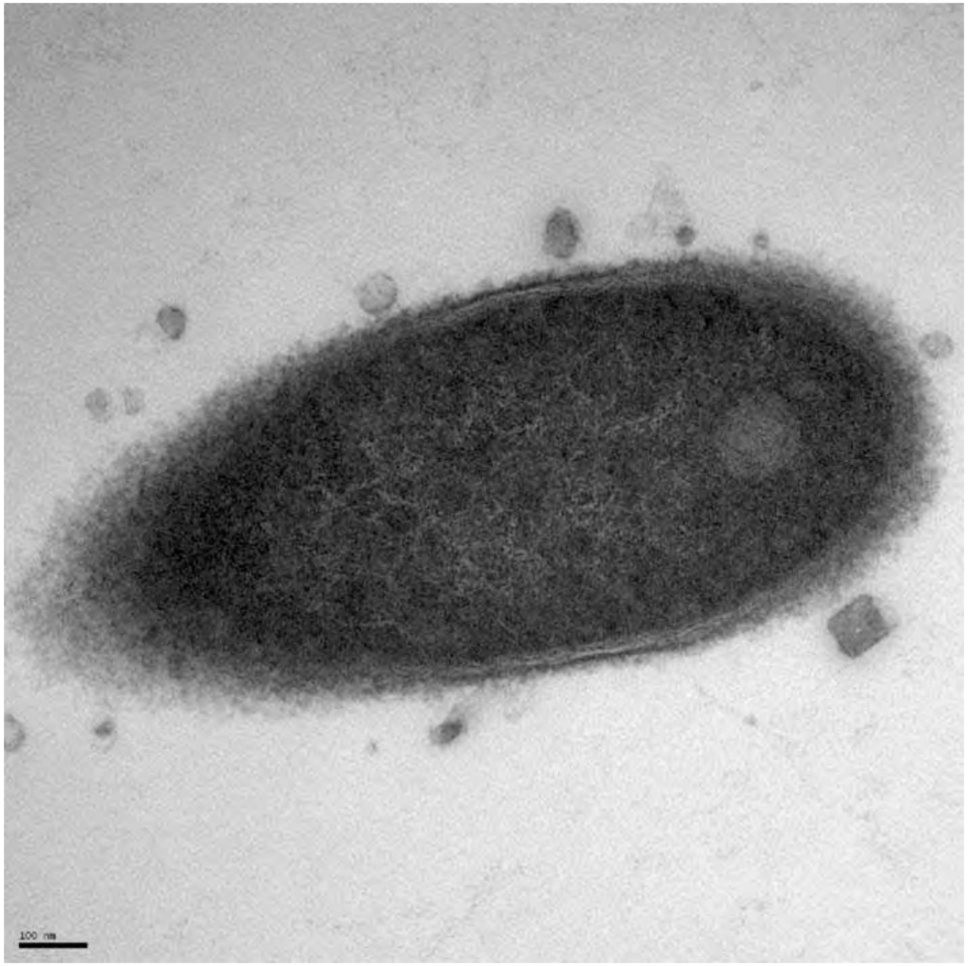


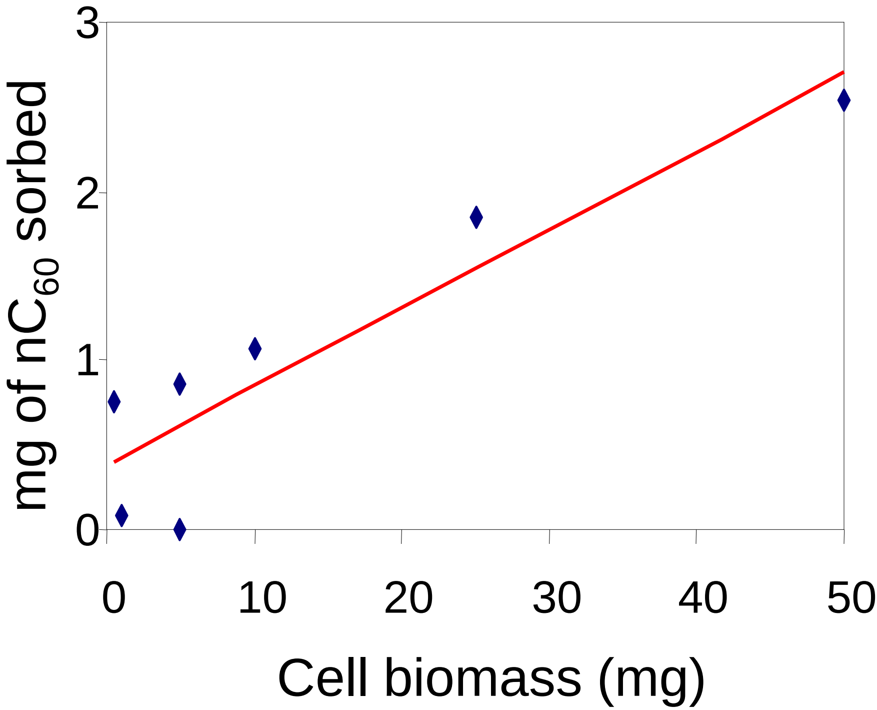



Zebrafish larva with pericardial edema due to $nC_{60}$ exposure (1 mg/L)

X. Zhu, Lin Zhu, Y. Li, Z. Duan, W. Chen and P.J. Alvarez* (2007). *Environ. Toxicol. Chem.* 26(5):976-979

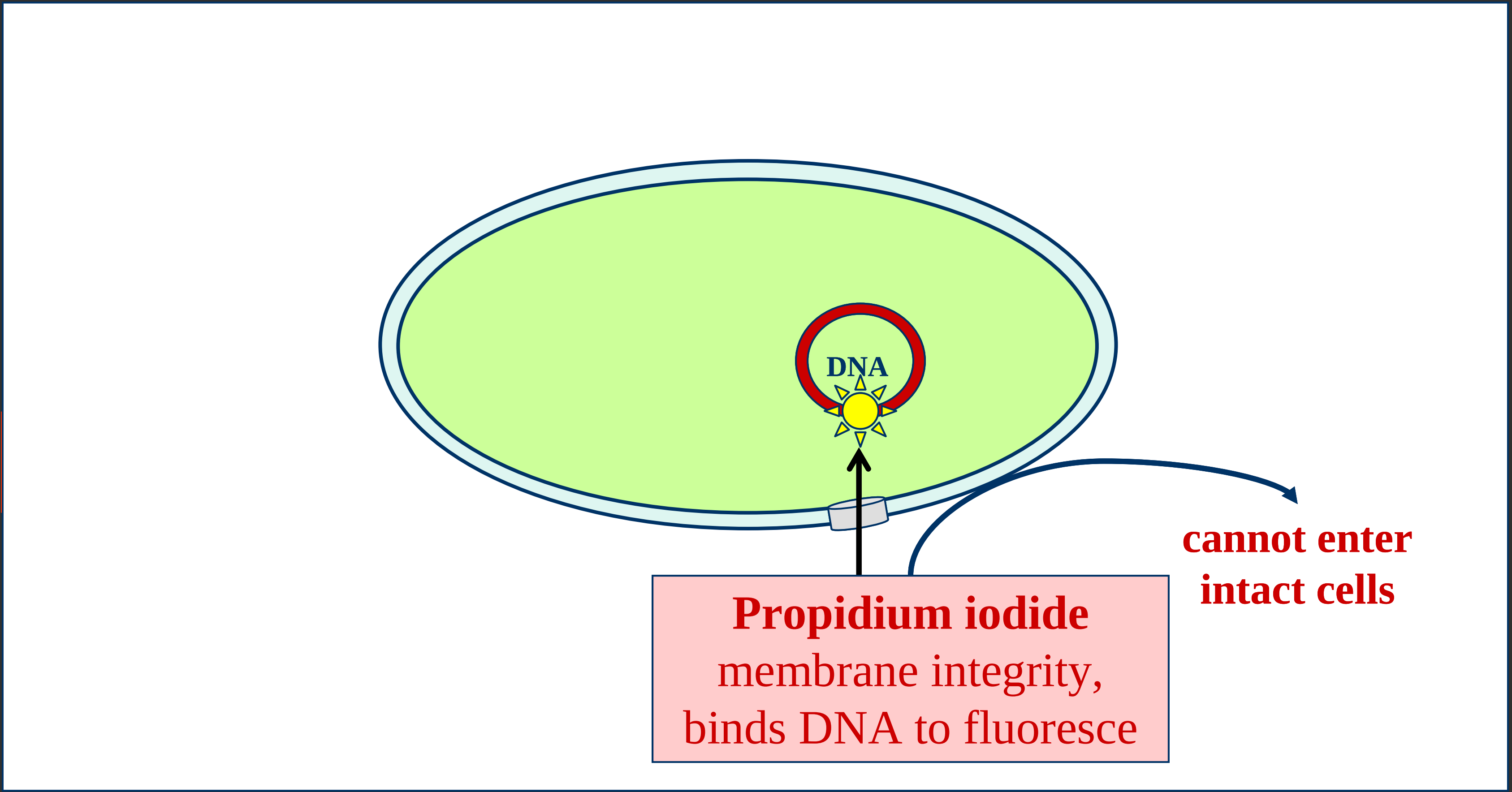
# $nC_{60}$ association with *Bacillus subtilis*



# 1. Does $nC_{60}$ puncture cells?

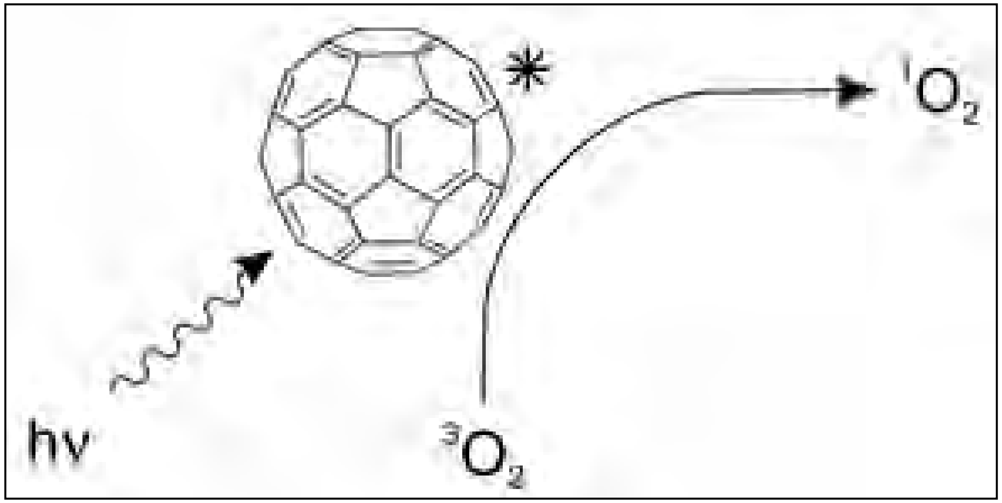
- Propidium iodide enters permeablized cells and stains nucleic acids




Lyon D.Y., L. Brunet, G.W. Hinkal, M.R. Wiesner, and P.J.J. Alvarez (2008). Nanoletters. 8(5): 1539-1543.

# 2. Oxidative Stress Due to ROS?

Photosensitization




- But no ROS was detected from $nC_{60}$

(Used SOSG for $^1O_2$, XTT for $O\cdot_2^-$, and pCBA for $OH\cdot$)

ROS from $C_{60}$ probably gets quenched in aggregate

- Cell death also occurred in the dark without $O_2$.

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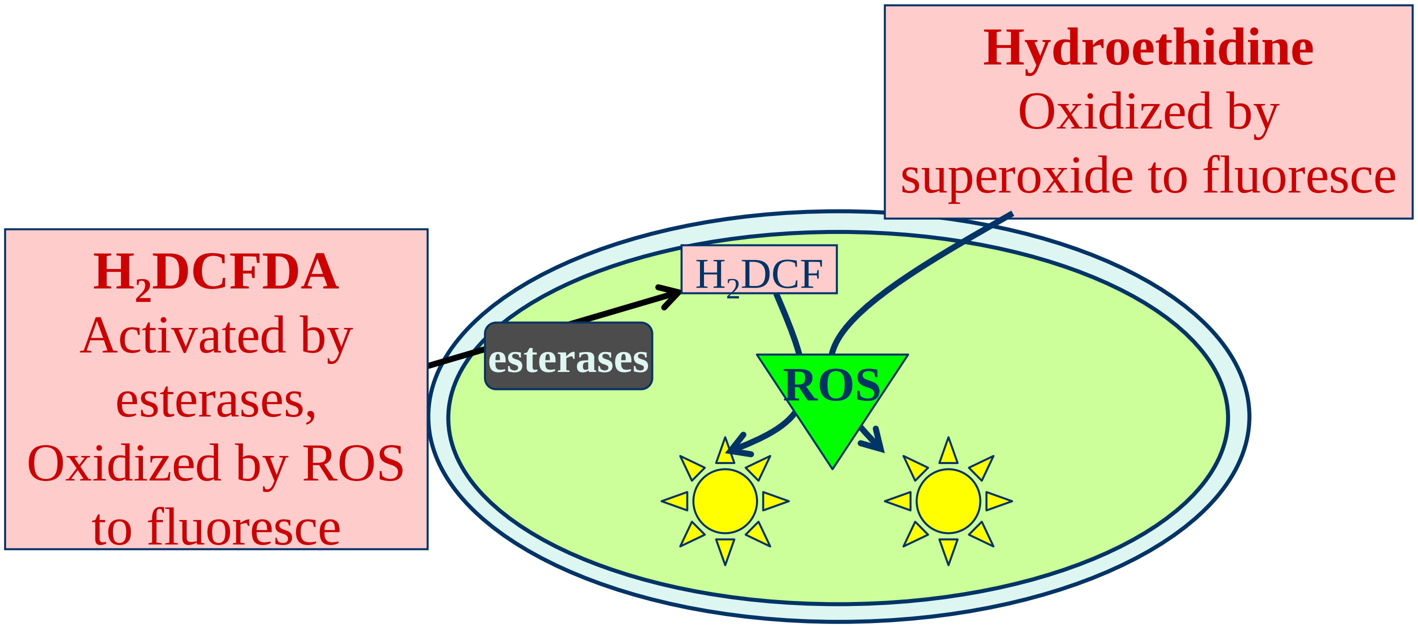
# Endogenous ROS?

$$O_2 \xrightarrow{e^-} O_2^{-} \xrightarrow{e^- + 2H^+} H_2O_2 \xrightarrow{e^-} OH^- + OH\cdot \xrightarrow{e^- + 2H^+} 2H_2O$$

Superoxide      Hydrogen peroxide      Hydroxyl radical

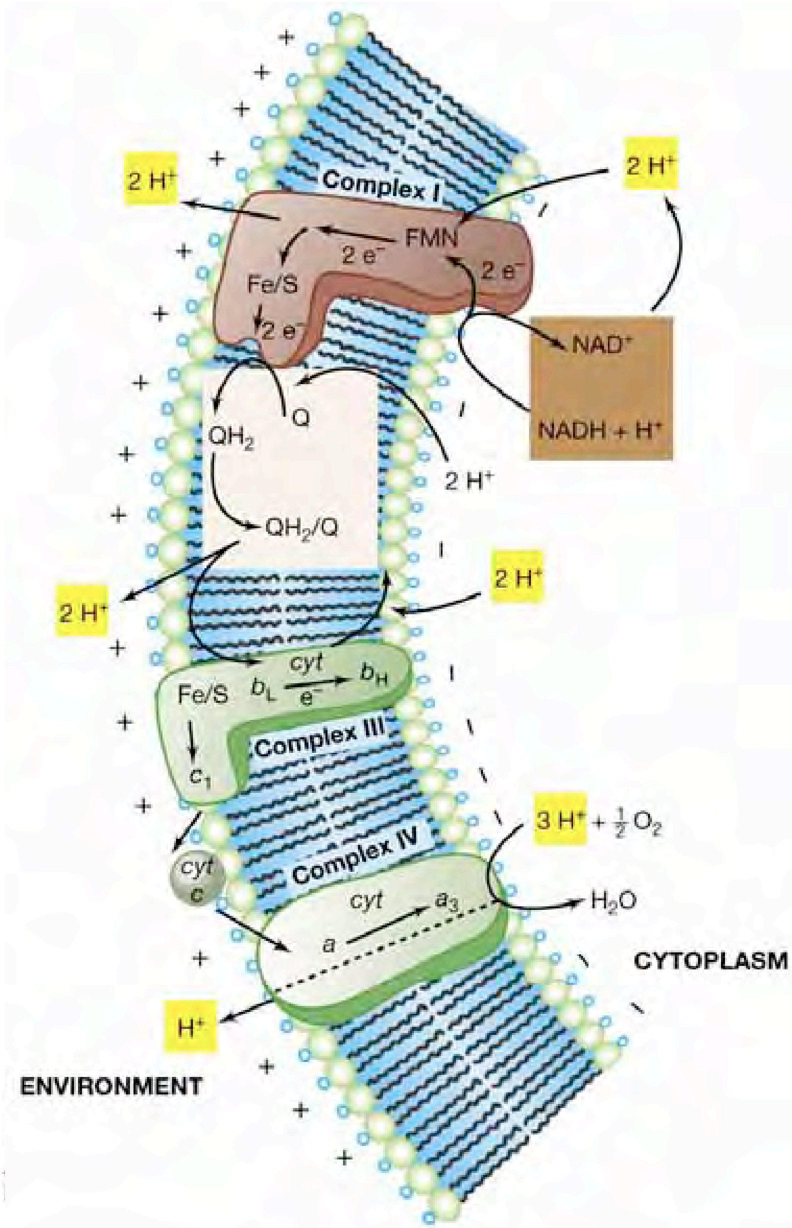
# Does $nC_{60}$ produce ROS inside bacteria?




**Need to need to re-evaluate previous studies that reported ROS-mediated oxidative stress**

Lyon D.Y., L. Brunet, G.W. Hinkal, M.R. Wiesner, and P.J.J. Alvarez (2008). Nanoletters. 8(5**):** 1539-1543.

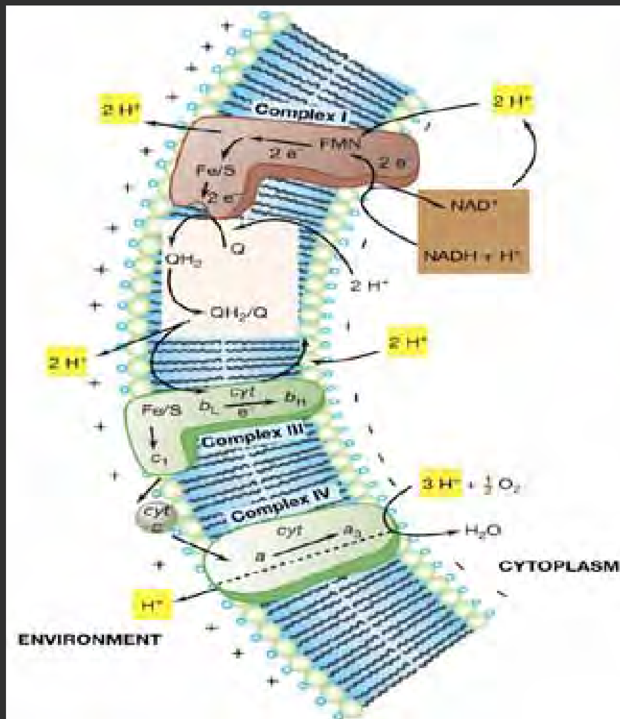
# 3. ROS-Independent Oxidative Stress?



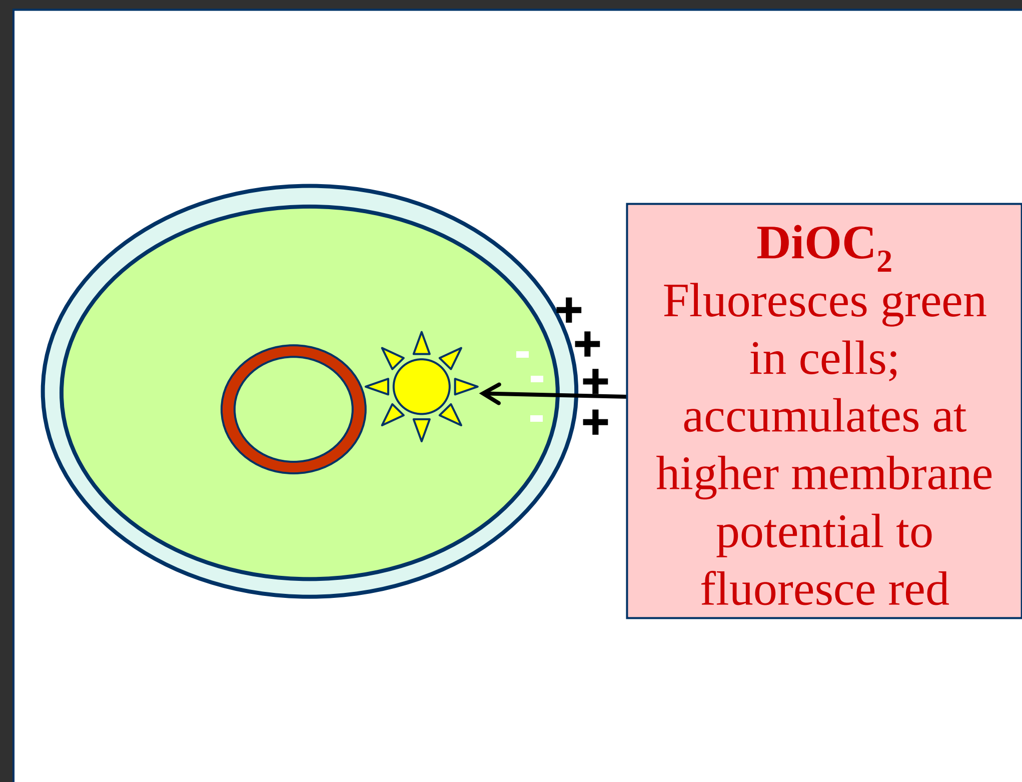

**Review of Bacterial Membrane**

- $e^-$s are transferred from fuel molecules to reduced coenzymes (e.g., NADH) to $e^-$ acceptor (e.g., $O_2$) through a chain of e- carriers (cytochromes, quinones, etc).
- As $e^-$s flow, protons are pumped outside to generate Proton Motive Force (voltage + pH gradient)
- Energized membrane dissipates PMF when $H^+$ flow back in through ATPase, and this potential energy is transferred to the phosphoryl bond of ATP

# Membrane potential Collapse?




- Assay monitors $DiOC_2$
  - Red fluorescence indicates higher membrane potential
- Higher red/green ratio means higher membrane potential
- CCCP is an ionophore (uncoupler)

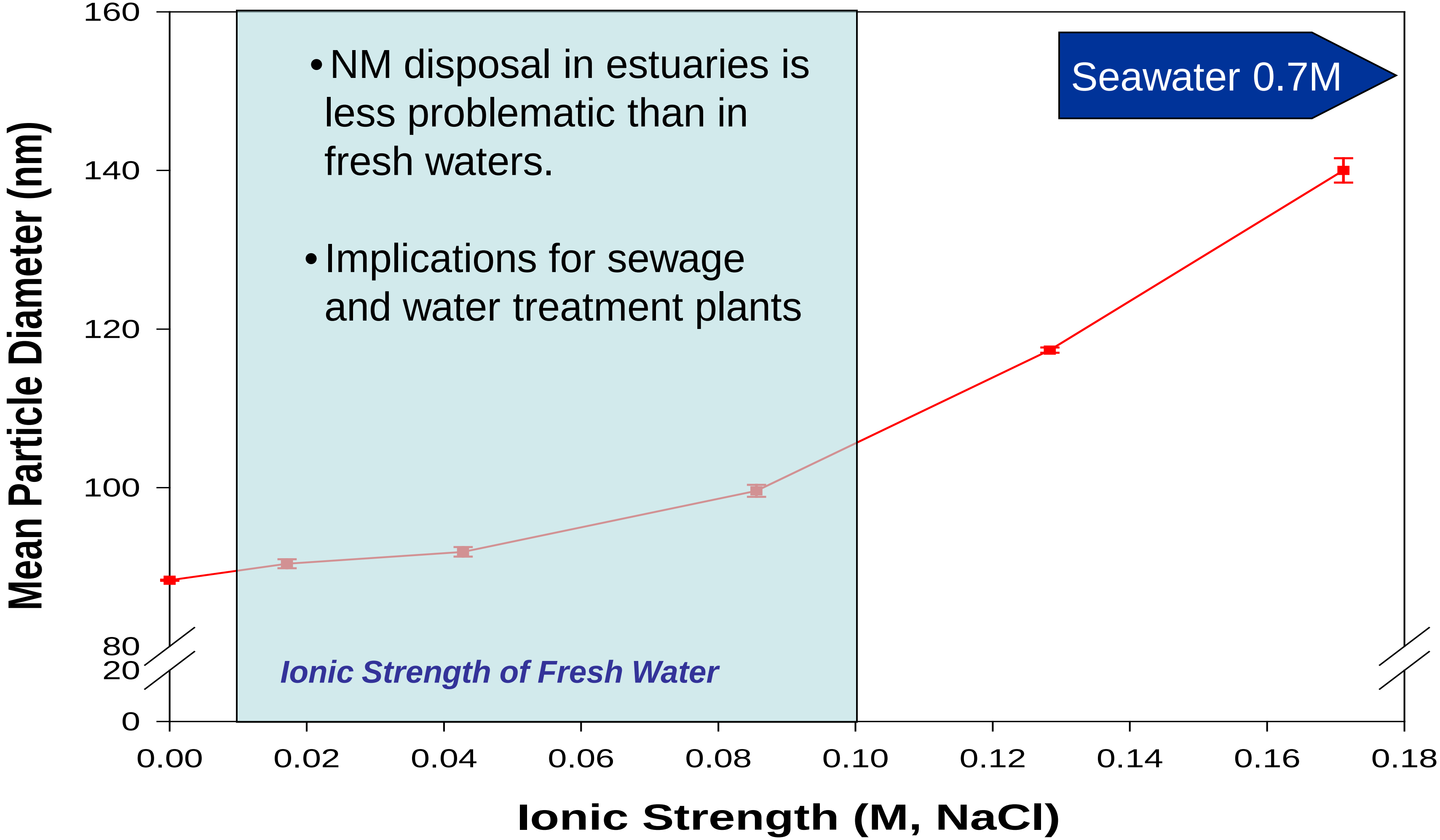



$nC_{60}$ also

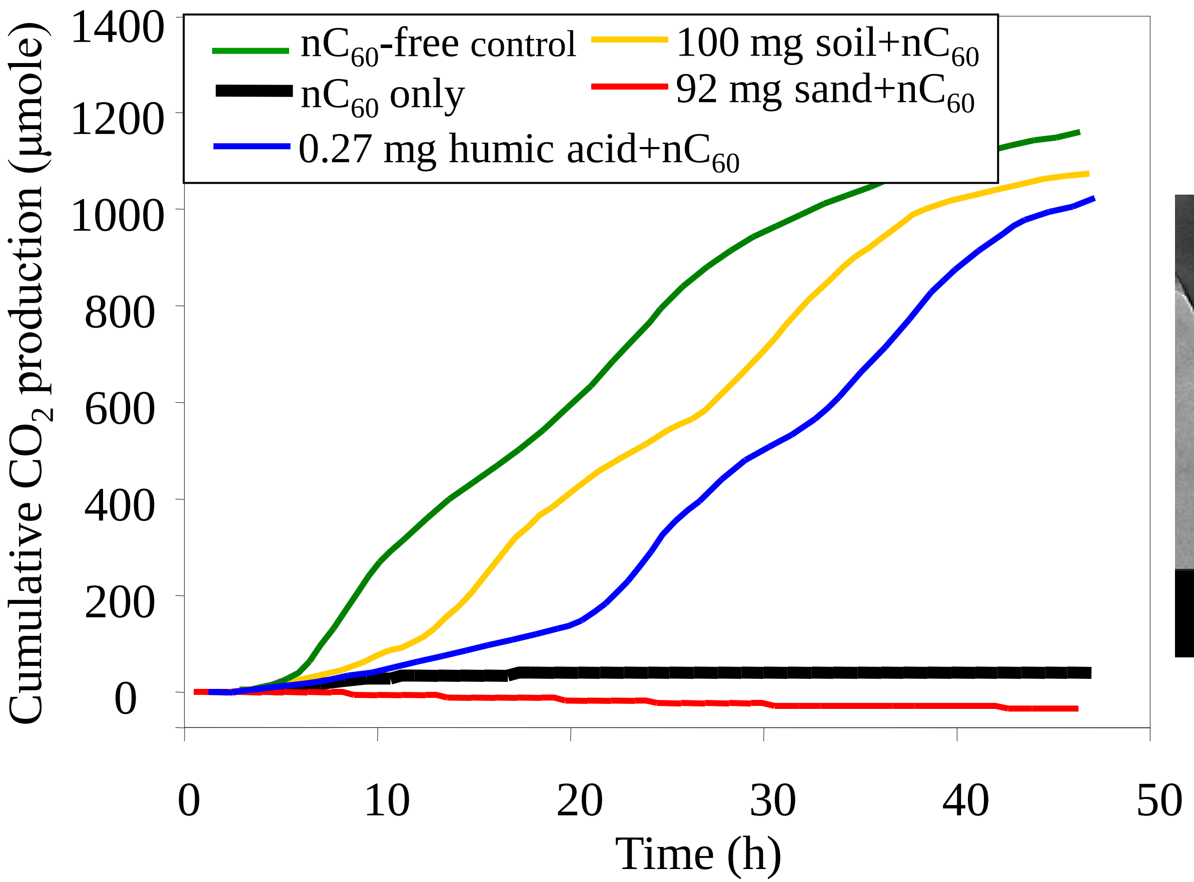
- Decreases electron flow (RET sub-mitochondrial particles)
- Decreases reductase activity (Redox-sensor™ green)
- Oxidizes proteins (thiol loss)

Lyon & Alvarez (2008). ES&T. 42:8127-8132

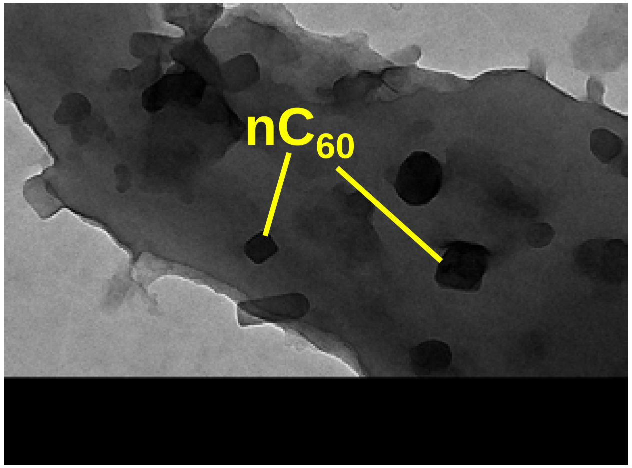
# Salts promote coagulation & precipitation = less toxicity



# NOM reduces bioavailability & toxicity of $nC_{60}$




**$nC_{60}$ trapped by humic colloids**




Humic acid concentrations as low as 0.1 mg/L eliminated toxicity

Li, D., Lyon D.Y., Q. Li, and P.J.J. Alvarez (2008). Environ. Toxicol. Chem. 27(9):1888-1894

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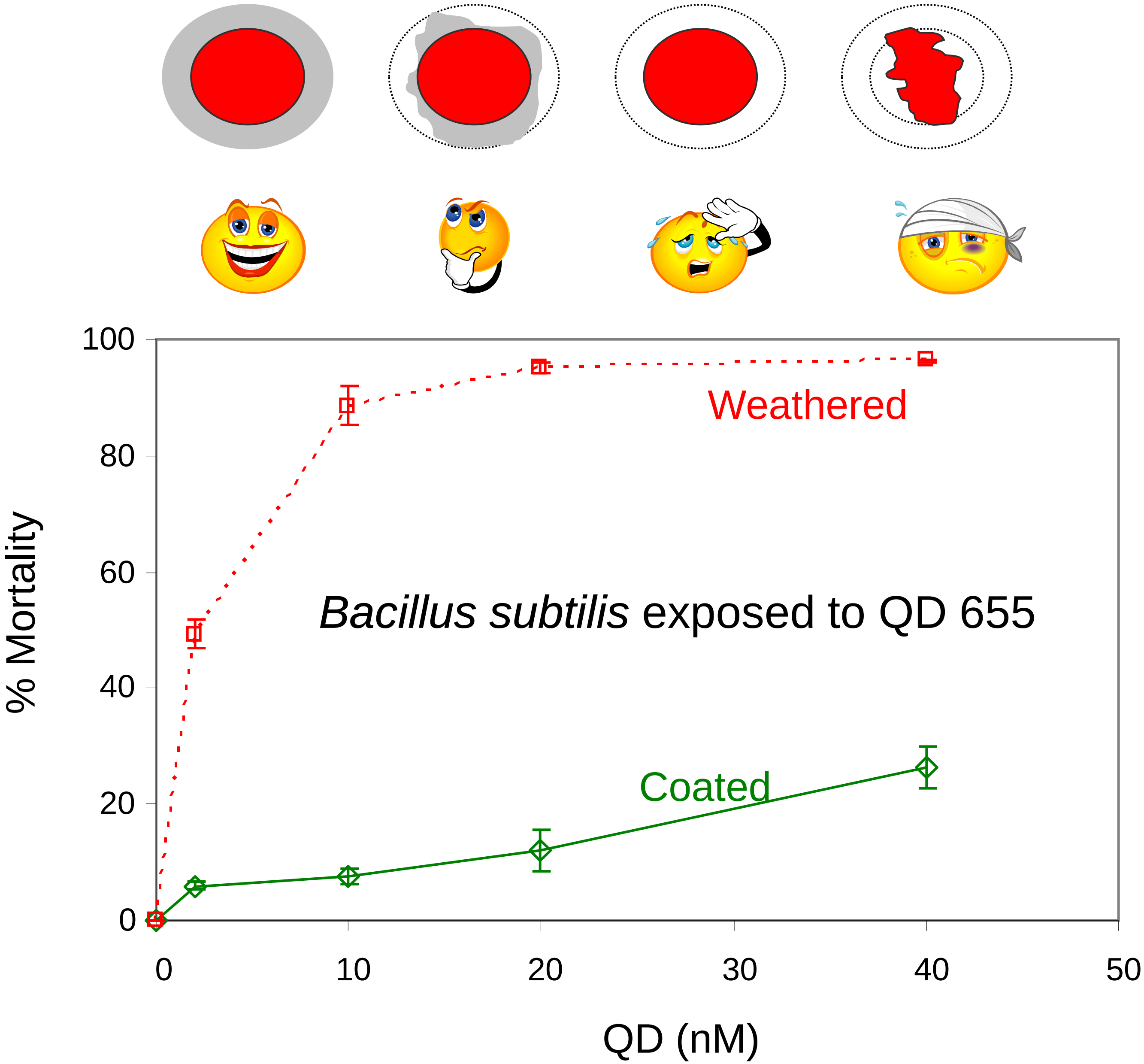
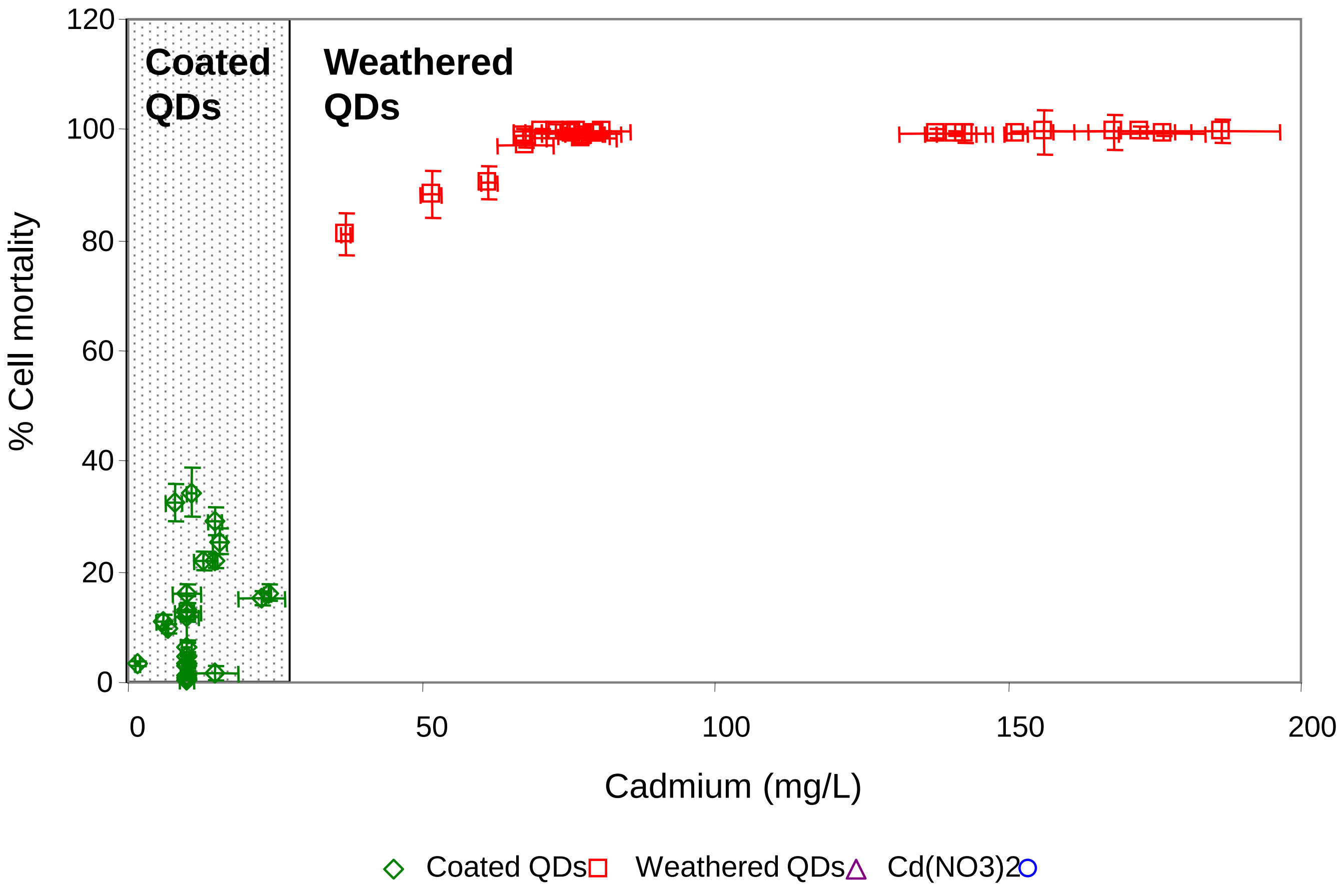
# Dose-Response of QDs

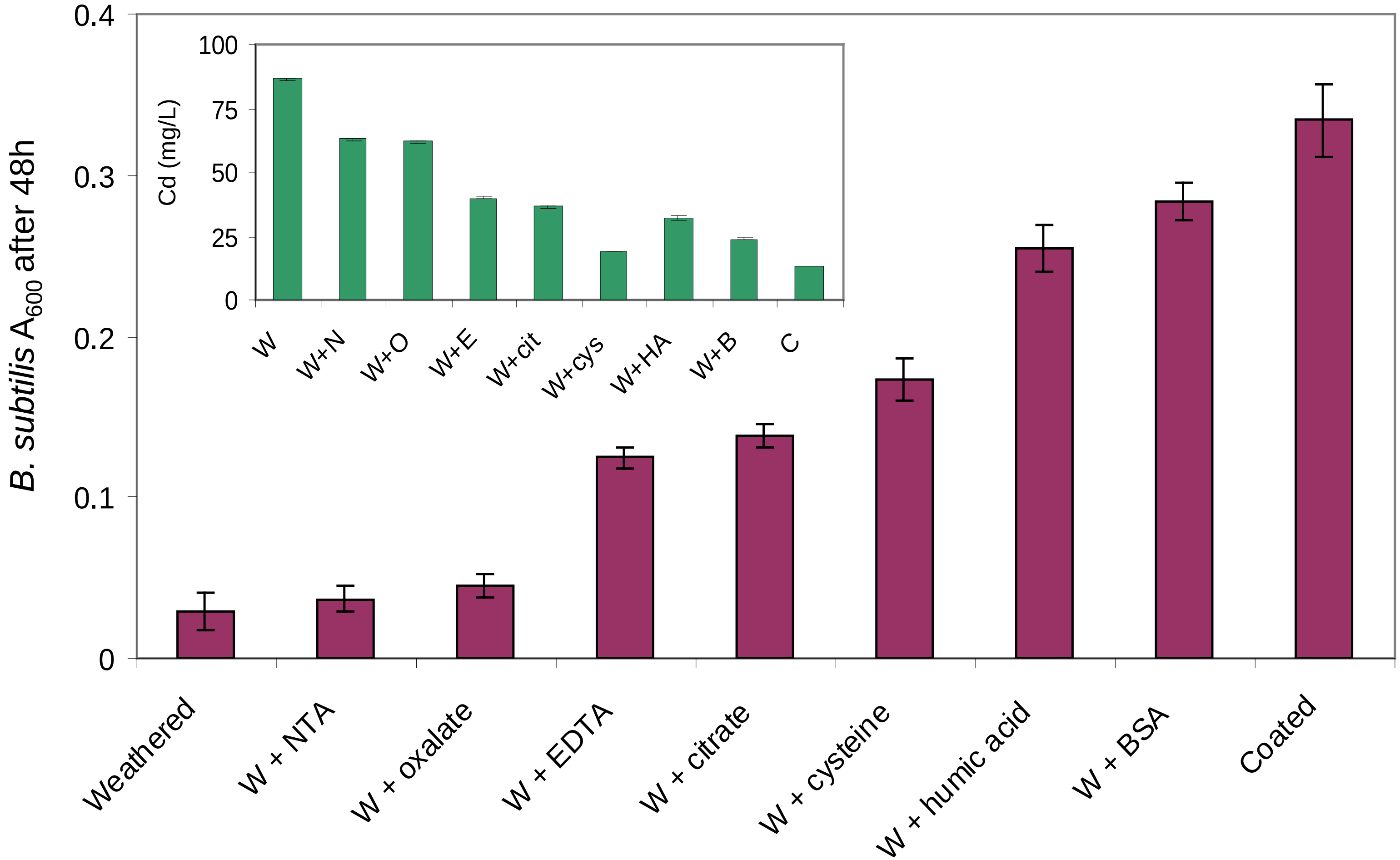


# $Cd^{2+}$ and Selenite Release after pH change (pH <4 & pH >10) Explains Dose-Response




Mahendra S., H. Zhu, V. Colvin and P. J. J. Alvarez (2008). *Environ. Sci. Technol*. 42 (24), 9424-9430

# Organic ligands mitigate toxicity




Mahendra S., H. Zhu, V. Colvin and P. J. J. Alvarez (2008). *Environ. Sci. Technol*. 42 (24), 9424-9430

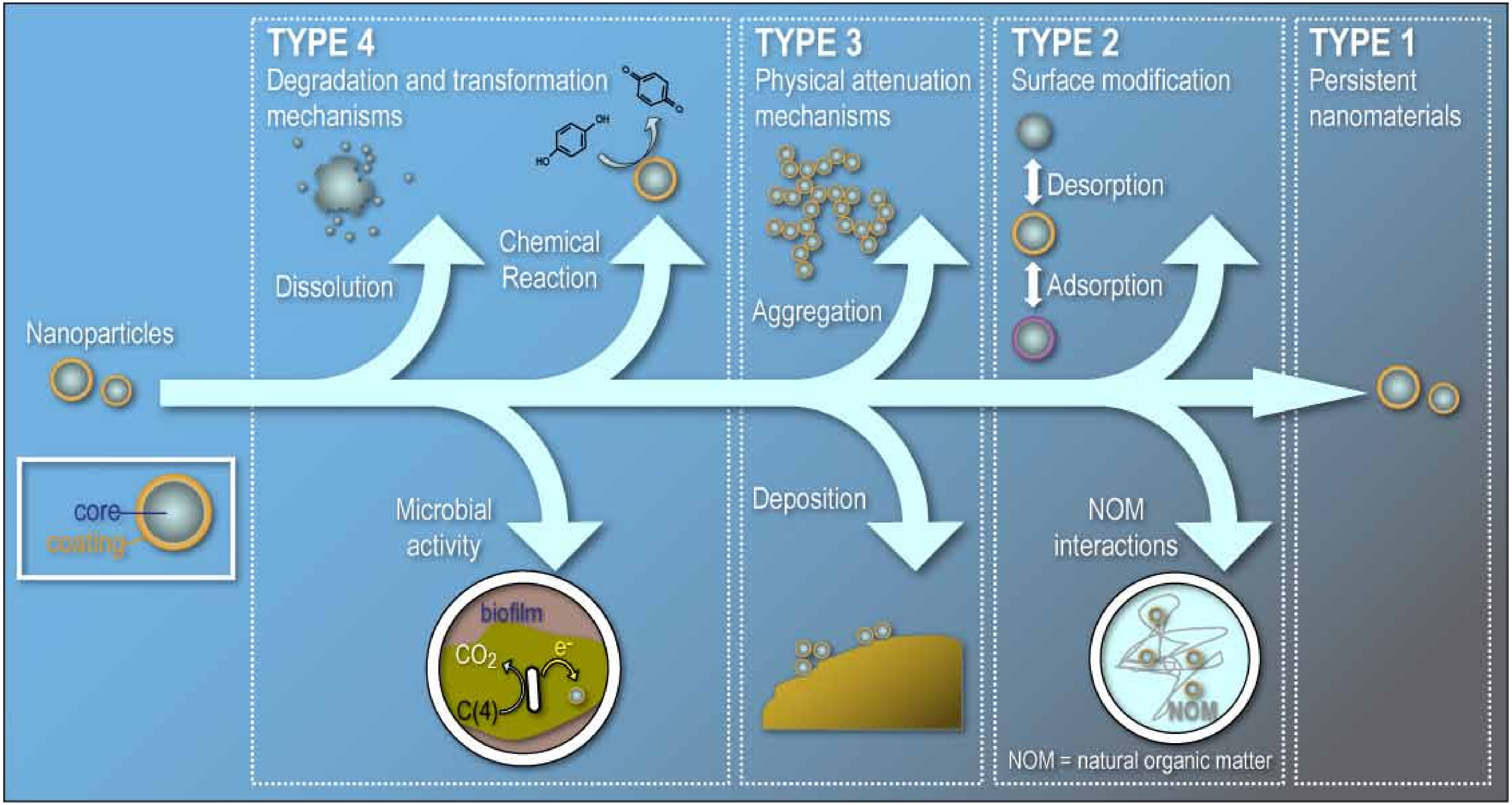
# Risk = Hazard X Exposure

Hazard, but no exposure

Exposure but no hazard

Hazard as well as exposure

# Nanoparticle Modifications in the Environment

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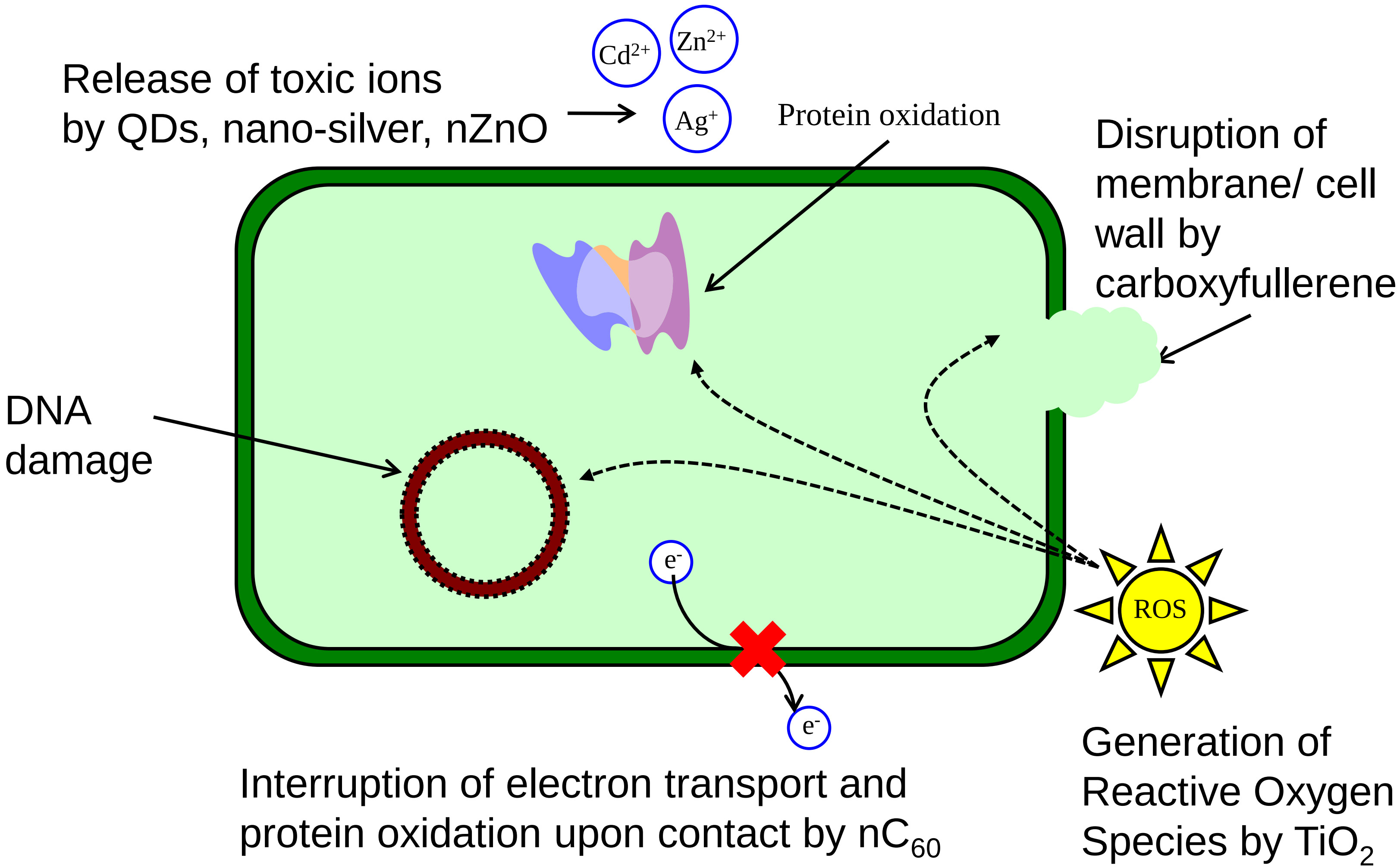
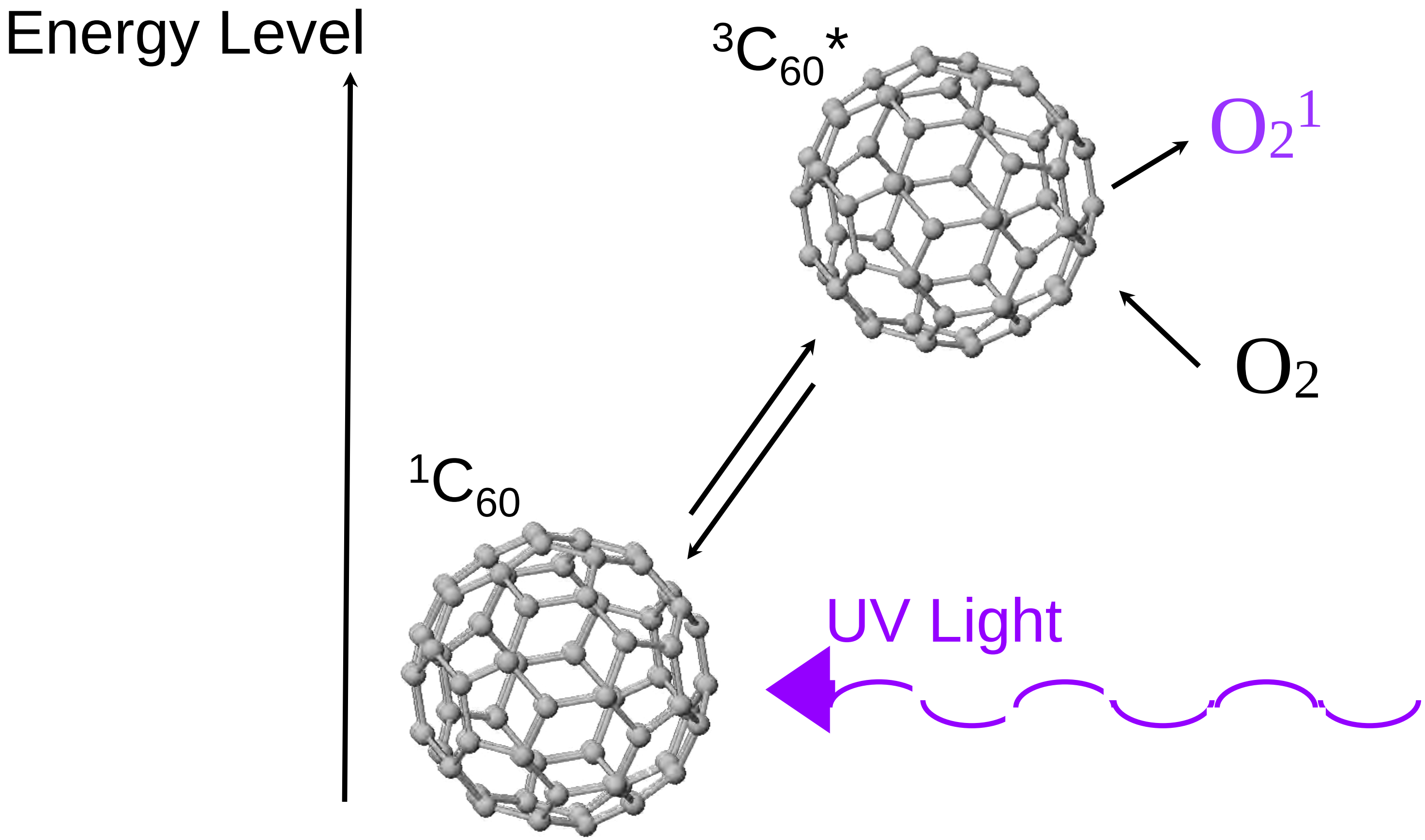
# Bacterial Toxicity Mechanisms



# Potential Leapfrogging Opportunities for Microbial Control and Disinfection

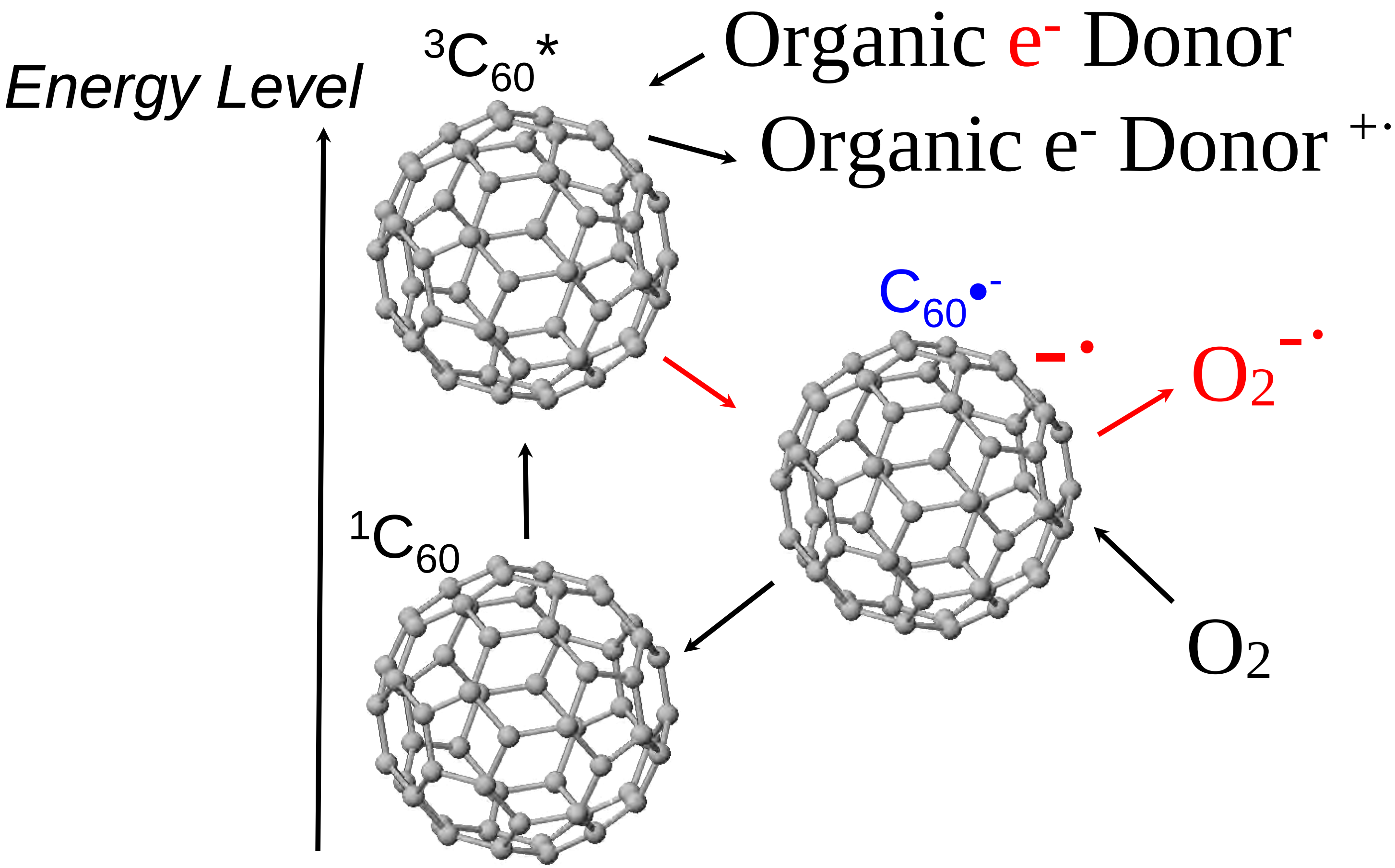
# Potential Application: Enhancing UV Disinfection

- UV disinfection is increasingly used to inactivate cyst-forming protozoa such as *Giardia* and *Cryptosporidium*.
- However, UV is relatively ineffective to treat virus unless the contact time and energy output are significantly increa$ed




Light excites $C_{60}$ to triplet state. Energy transfer between $^3C_{60}^*$ and molecular oxygen gives rise to singlet oxygen ($^1O_2$)

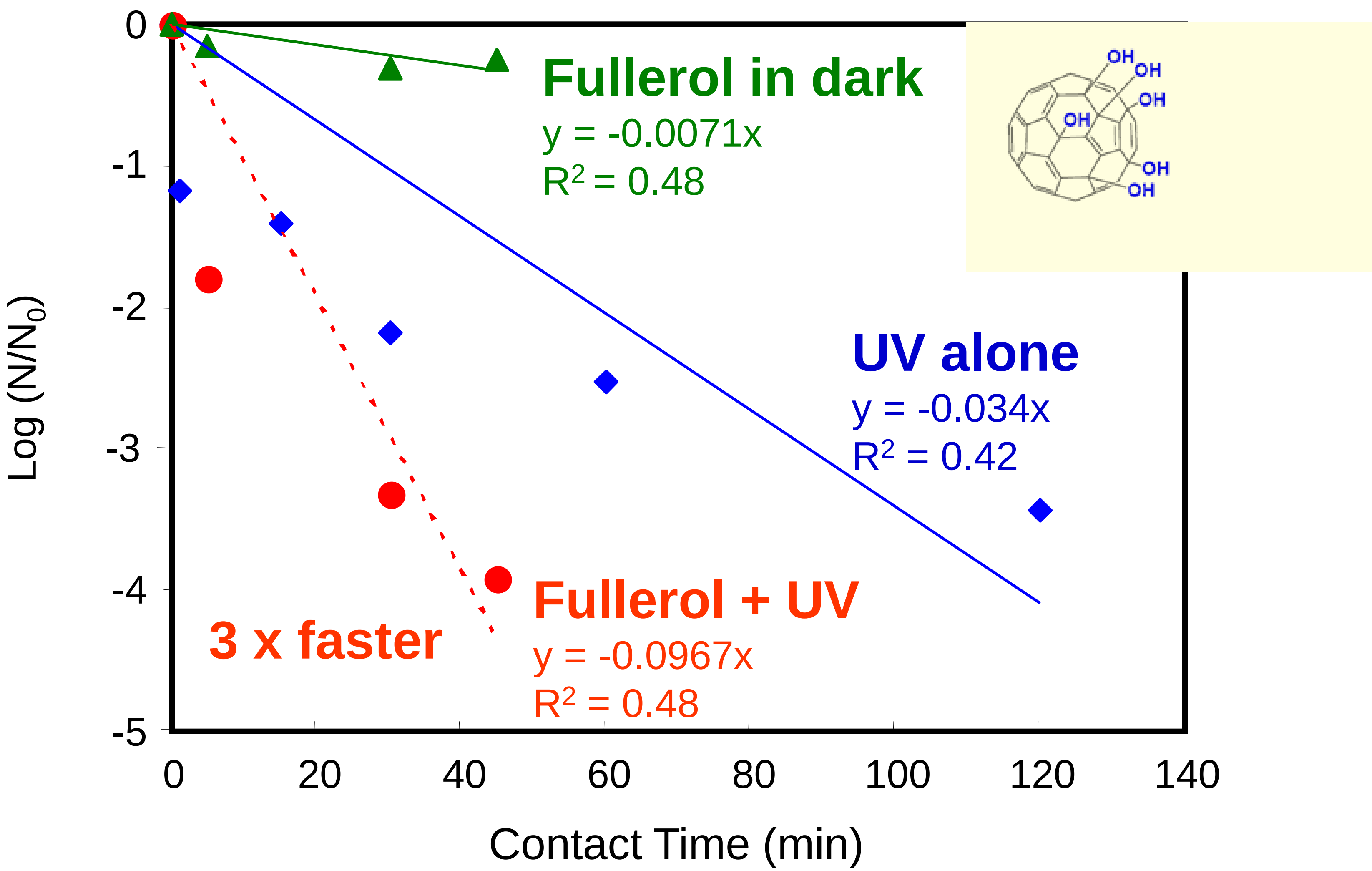
Hotze M., J. Labille, P.J.J. Alvarez and M. Wiesner (2008). Environ. Sci. Technol. 42, 4175–4180




$^3C_{60}^*$ transforms to $C_{60}$ radical anion ($C_{60}\bullet^-$) in the presence of some electron donors, which reduces $O_2$ to superoxide ($O_2^-\bullet$)

Hotze M., J. Labille, P.J.J. Alvarez and M. Wiesner (2008). Environ. Sci. Technol. 42, 4175–4180

# MS2 virus inactivation by UV and fullerol

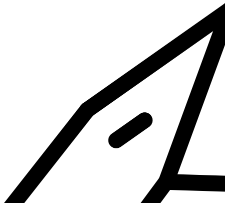



Hotze M., A.R. Badireddy, S. Chelam, P.J.J.Alvarez and M. Wiesner (2007). *Environ. Sci. Technol.* 41(18): 6627-6632

# “Water Soluble” Derivatized Fullerenes



**vs**

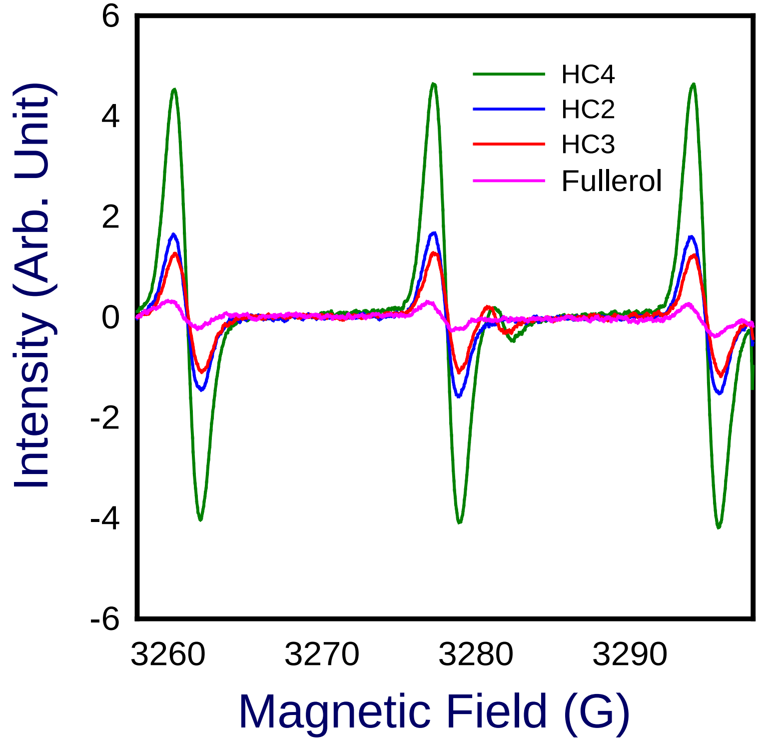


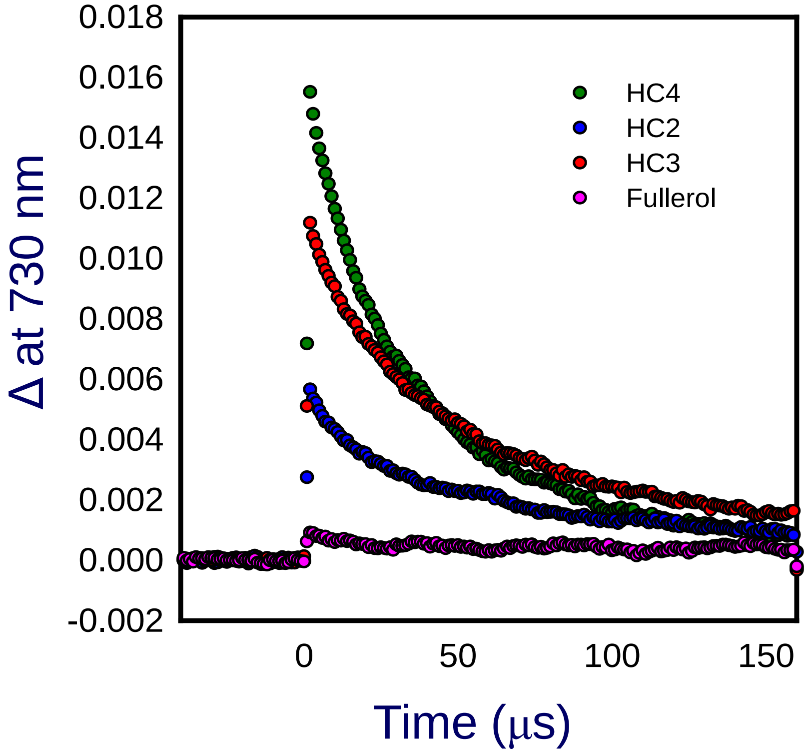
* Commercially Available, MER Corp.

* Synthesized in Lon Wilson’s lab, Dept of Chemistry, Rice University

# Superior Photosensitized $^1O_2$ Production

- **Larger Electron Paramagnetic Resonance Spectra Peaks Correspond to Higher $^1O_2$ Generation (All Outperform Fullerol)**
- **Nanosecond Laser Flash Photolysis Confirms Long-lived Triplet State, Conducive to Efficient ROS Production (HC4 >> Fullerol)**

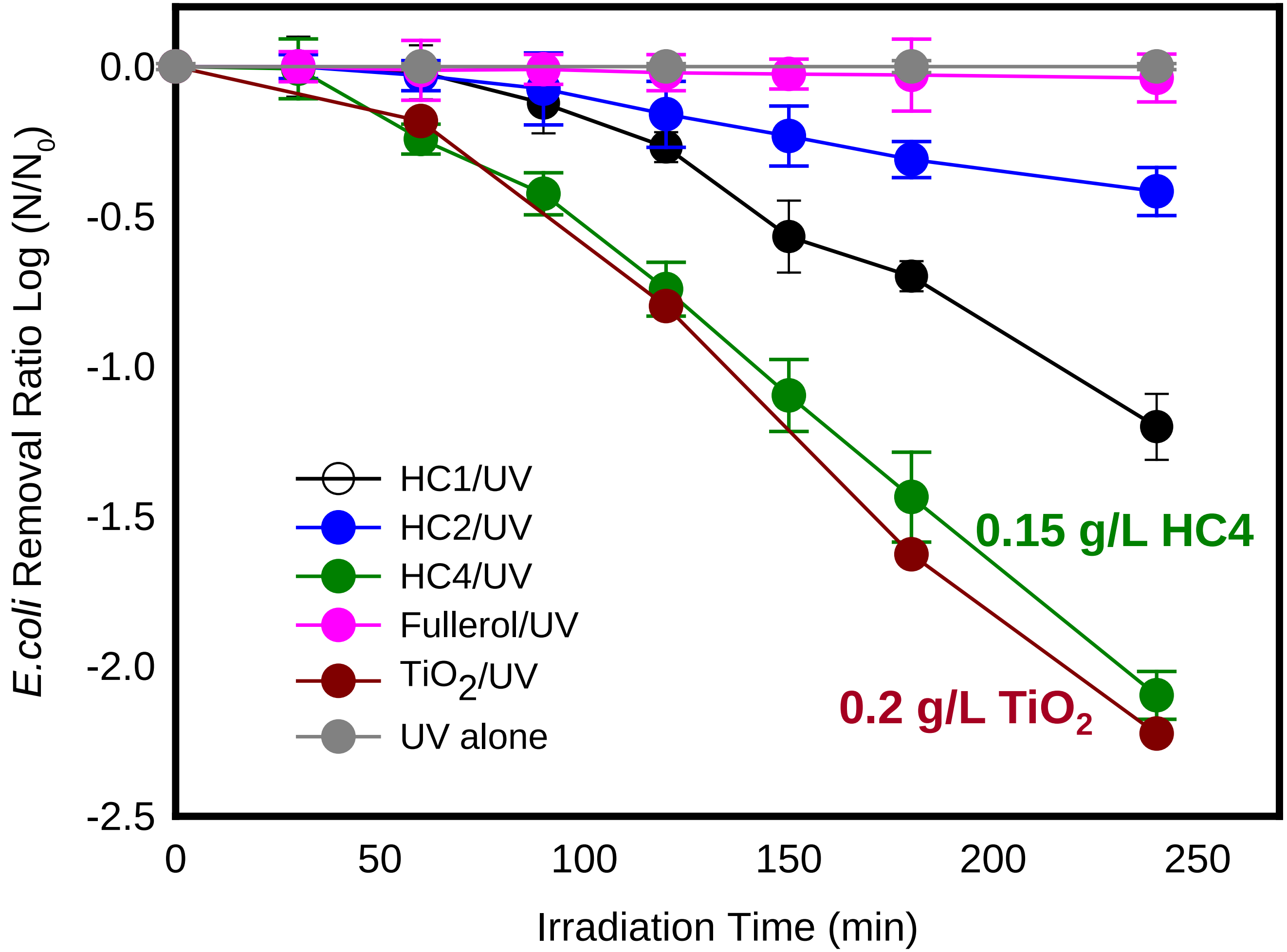



J.Lee, Y.Mackeyev, M.Cho, D.Li, J.-H. Kim, L.J. Wilson, and P.J.J. Alvarez, *Environ. Sci. Technol*. In press.

# Photocatalytic *Escherichia coli* Disinfection

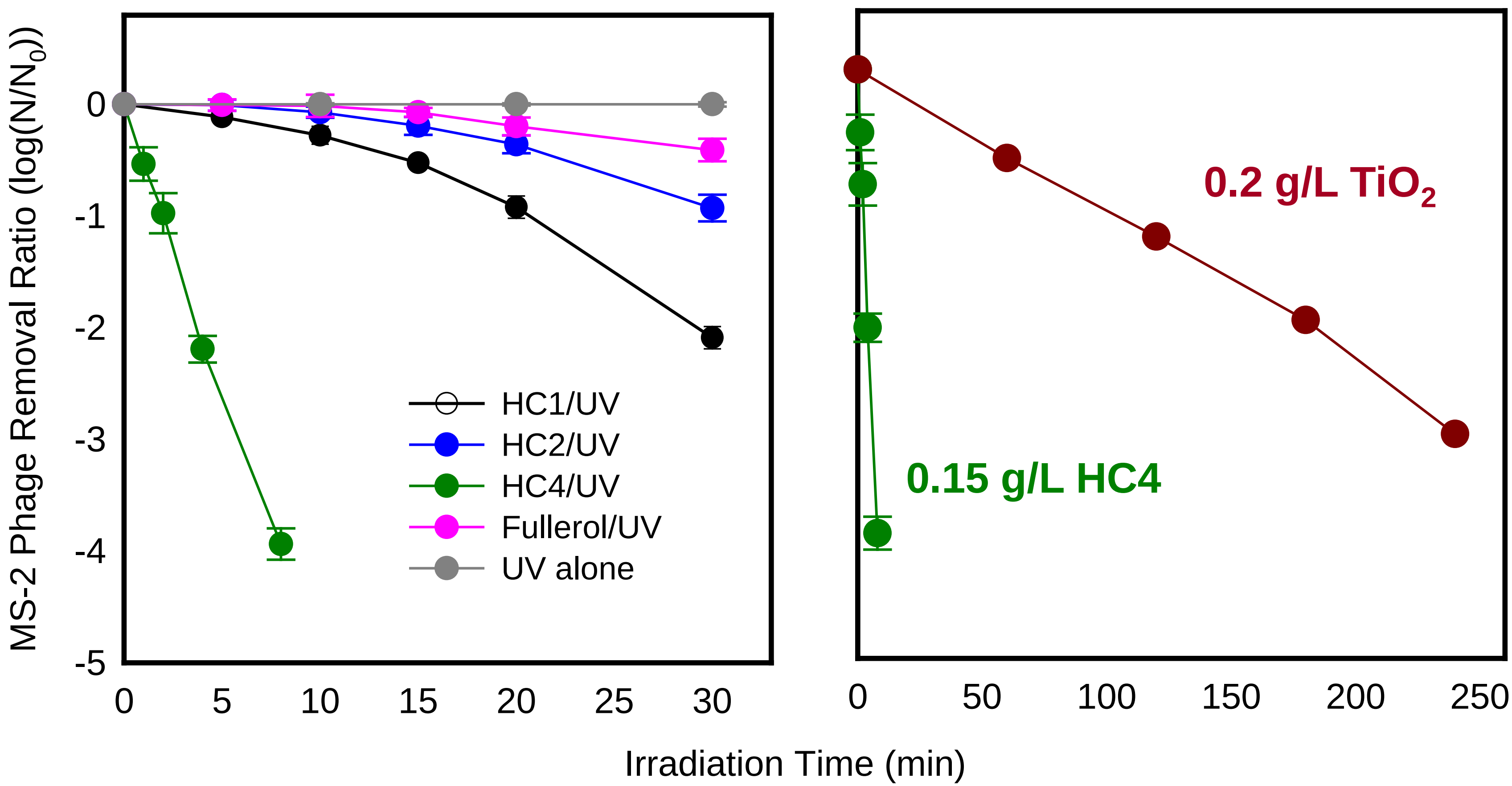
[$C_{60}$ derivative] = 50 mM, BLB lamp 350 - 400 nm, *I* = 6 ´ $10^{-6}$ Einstein×$min^{-1}L^{-1}$




J.Lee, Y.Mackeyev, M.Cho, D.Li, J.-H. Kim, L.J. Wilson, and P.J.J. Alvarez, Submitted.

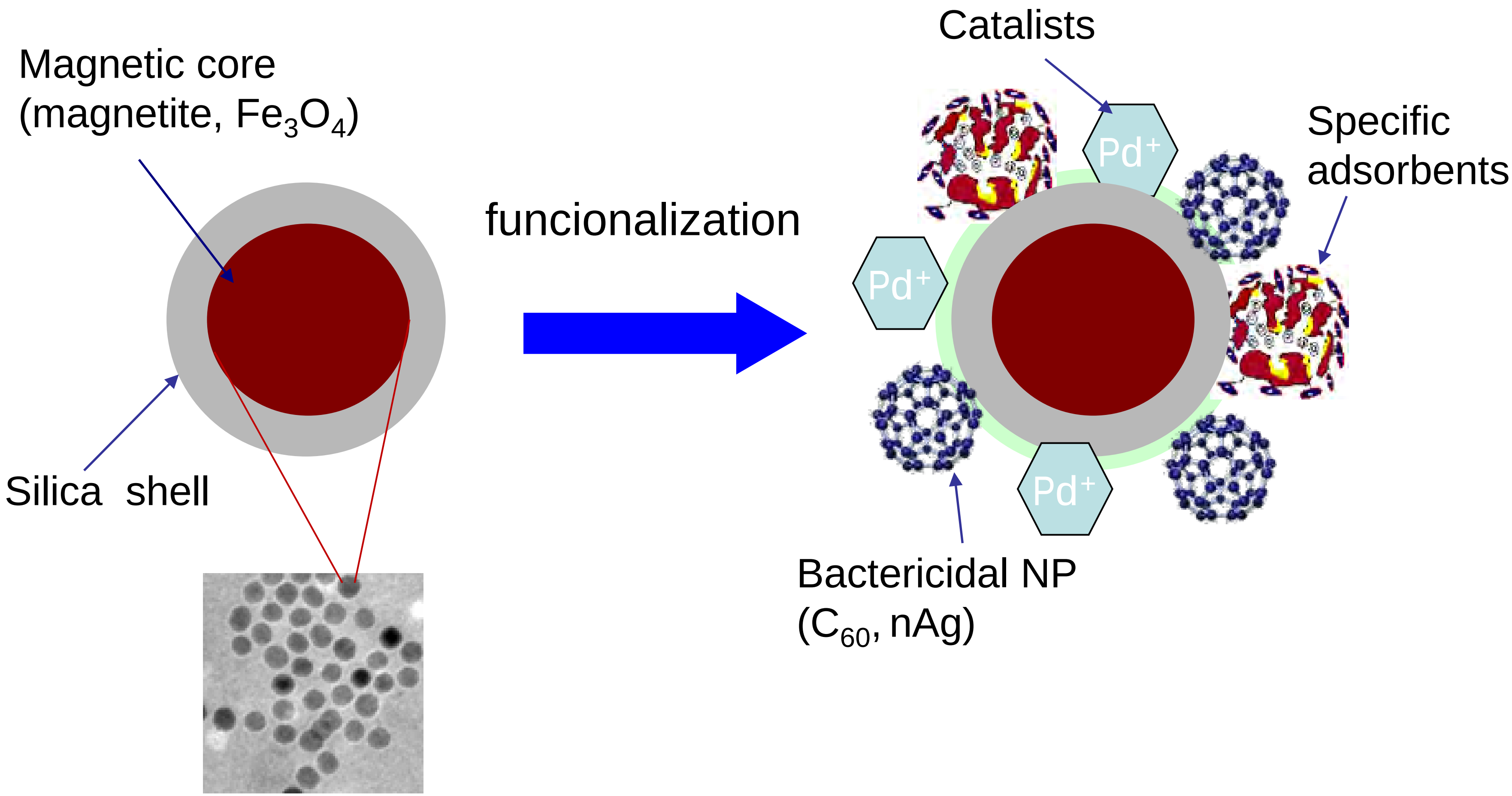
# Photocatalytic MS-2 Bacteriophage Inactivation

Antiviral activity was higher than antibacterial activity (unusual for traditional disinfection with UV, $O_3$, $Cl_2$ etc.)

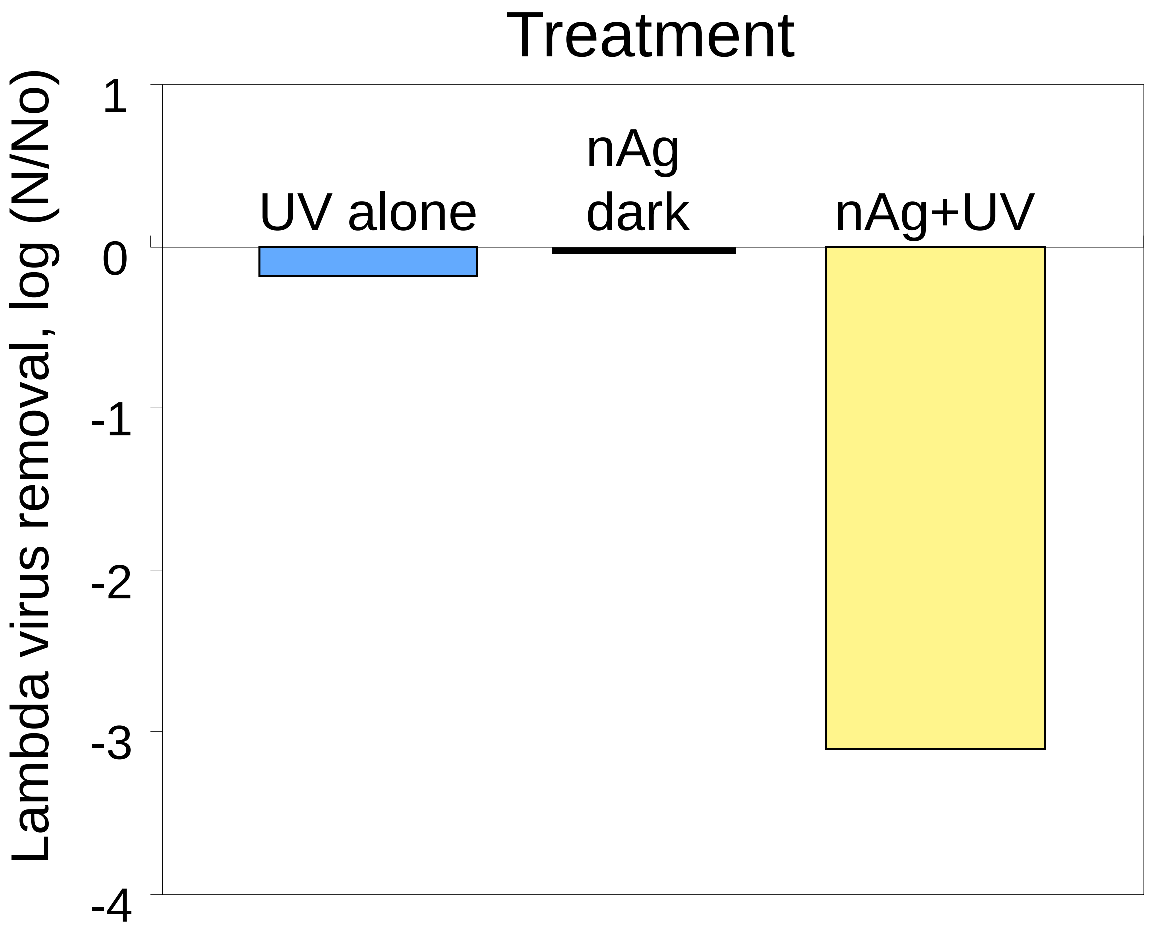



J.Lee, Y.Mackeyev, M.Cho, D.Li, J.-H. Kim, L.J. Wilson, and P.J.J. Alvarez, Submitted.

# Multifunctional Magnetic Nanoparticles

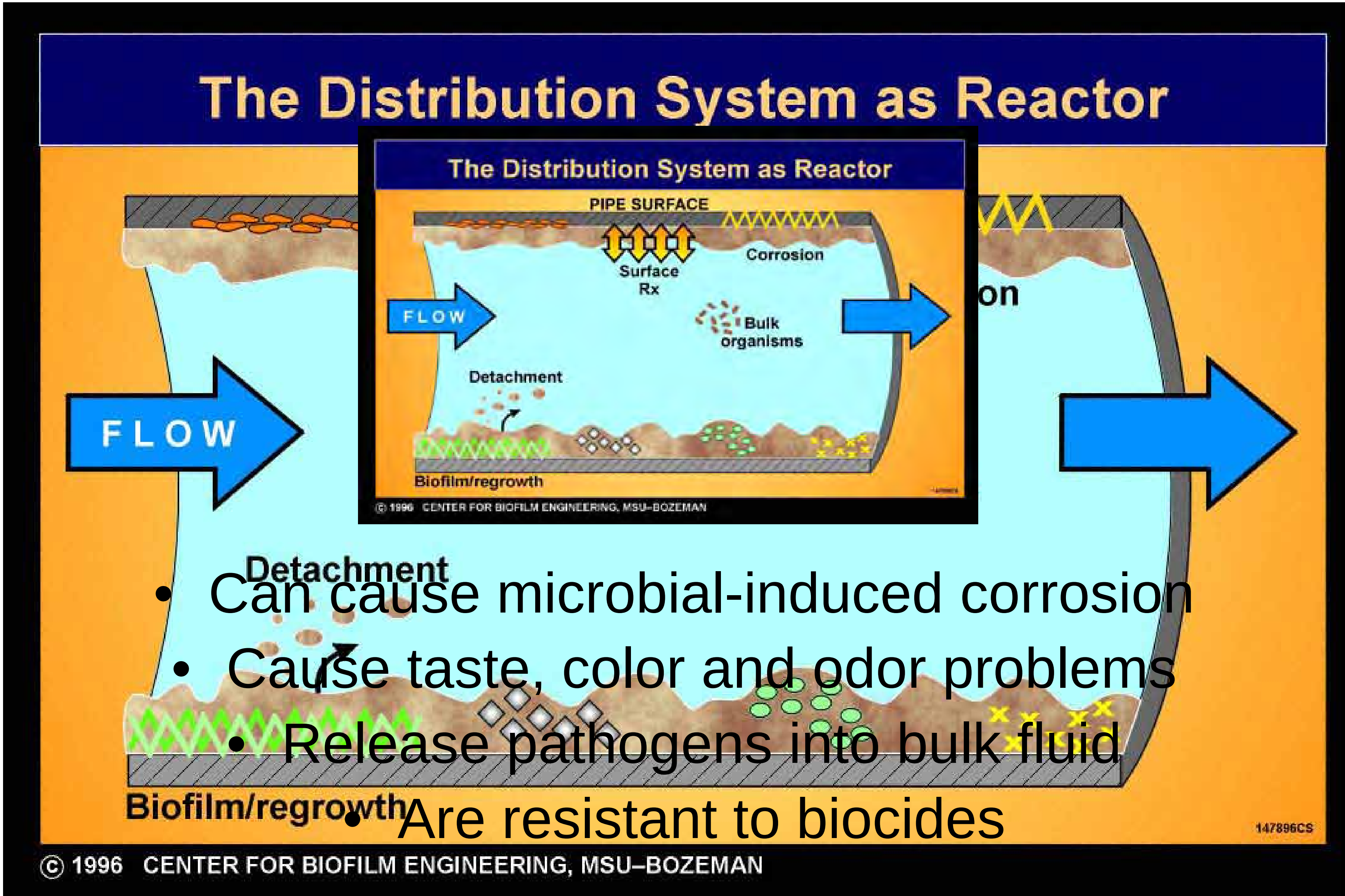


# Synergistic Inactivation of Lambda virus with nano-Ag and UV illumination




nAg mounted on nano-magnetite, 0.5 ppm, 1-h under UV illumination of 24.1 $mW/m^2$

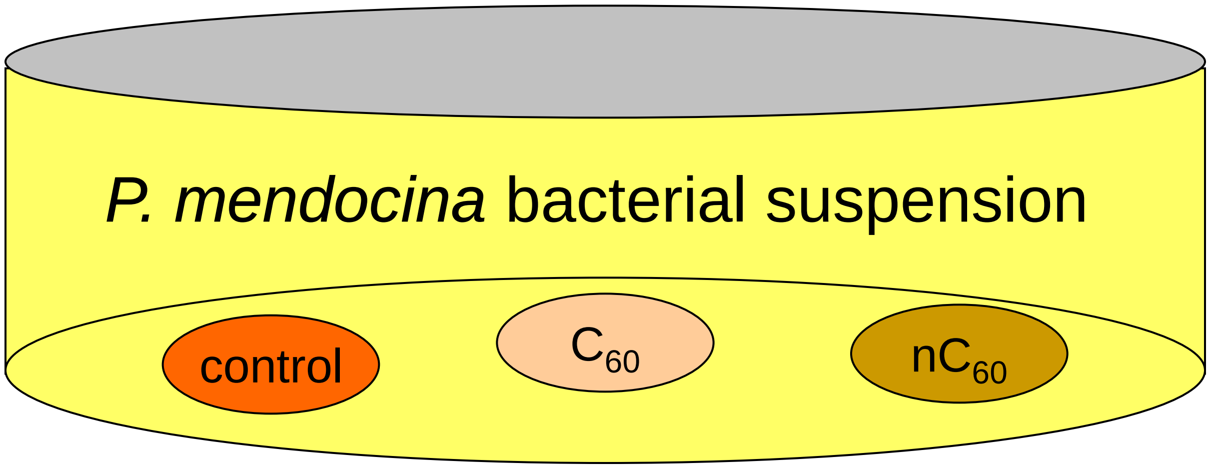
# Biofilms in Water Distribution Systems



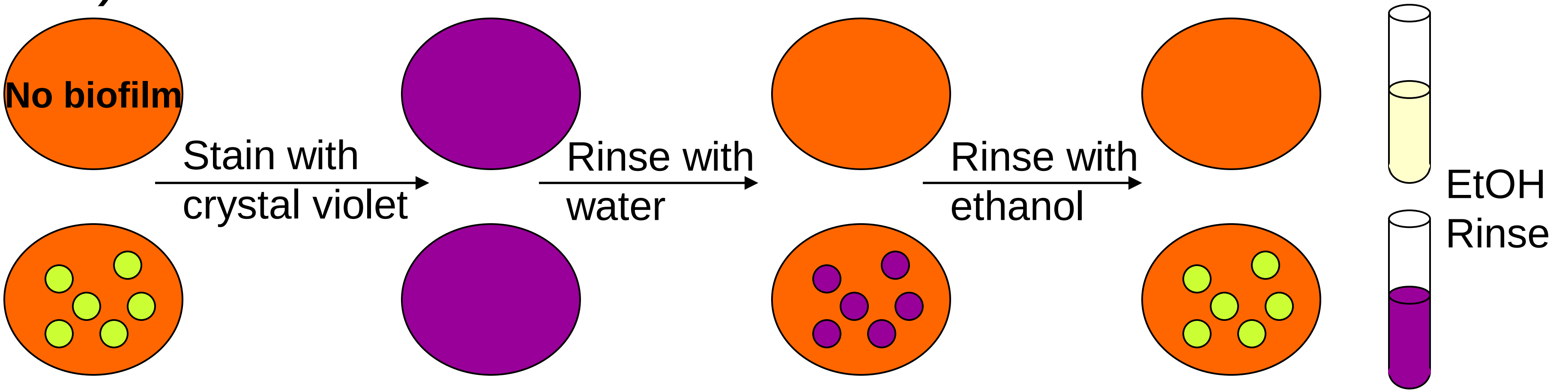

- Can cause microbial-induced corrosion
- Cause taste, color and odor problems
- Release pathogens into bulk fluid
- Are resistant to biocides

# Assessing Biofilm Formation

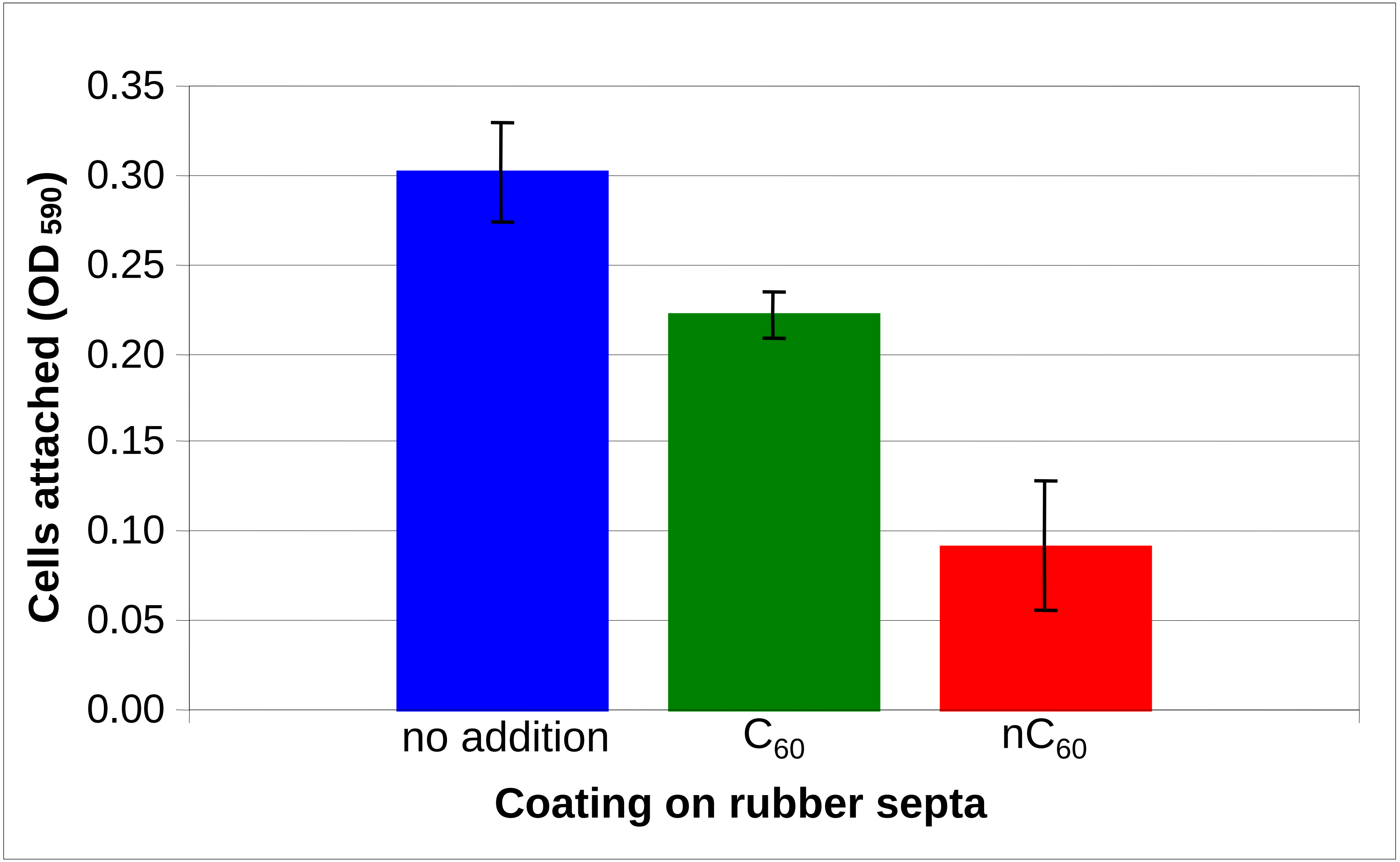
1) Grow biofilm on rubber septa



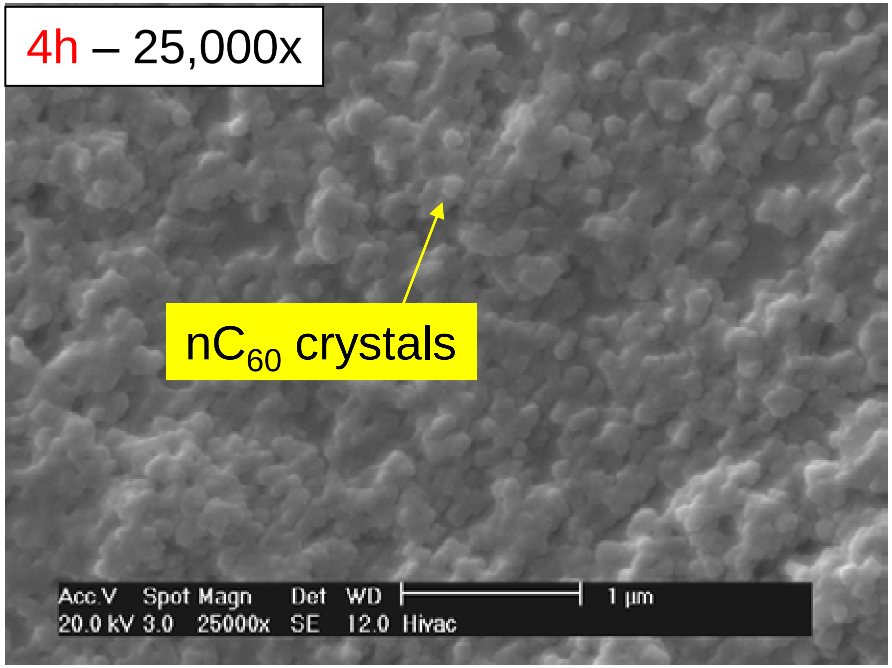

2) Measure amount of biofilm

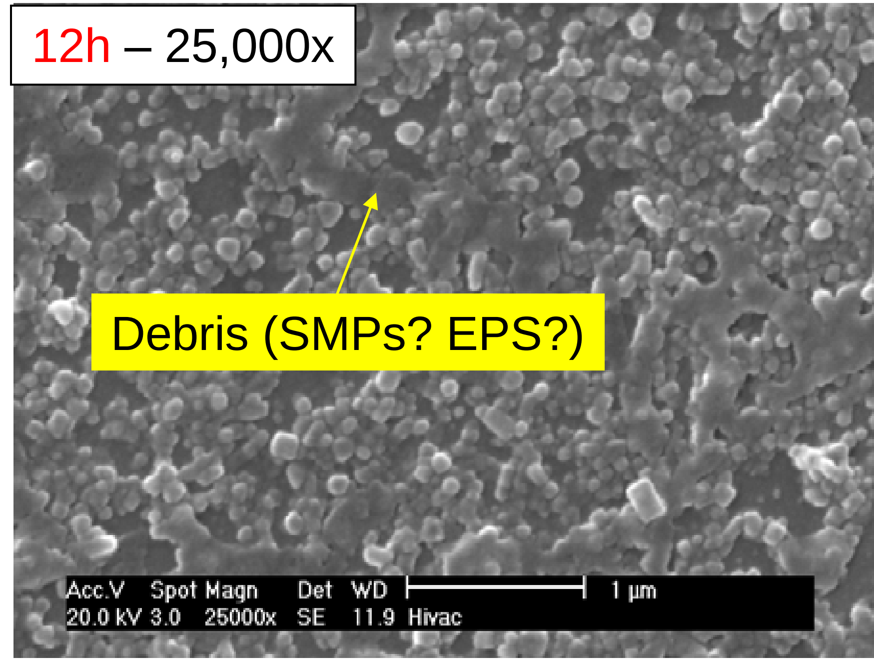


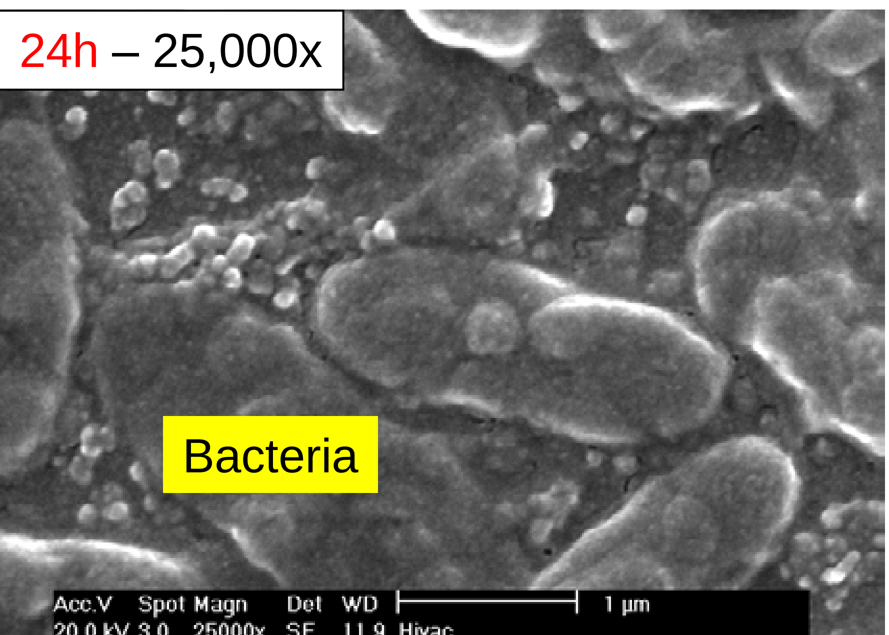
# $nC_{60}$ reduces biofilm formation?

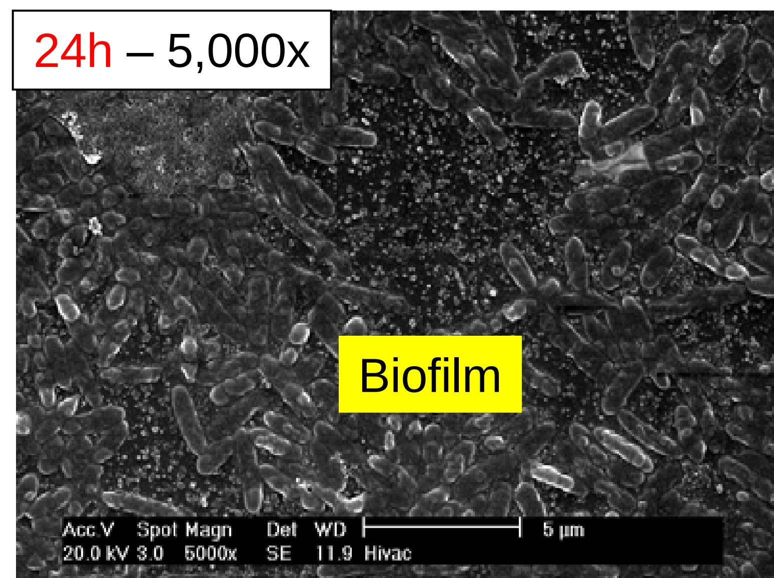


# SEM images of biofilm formation on $nC_{60}$

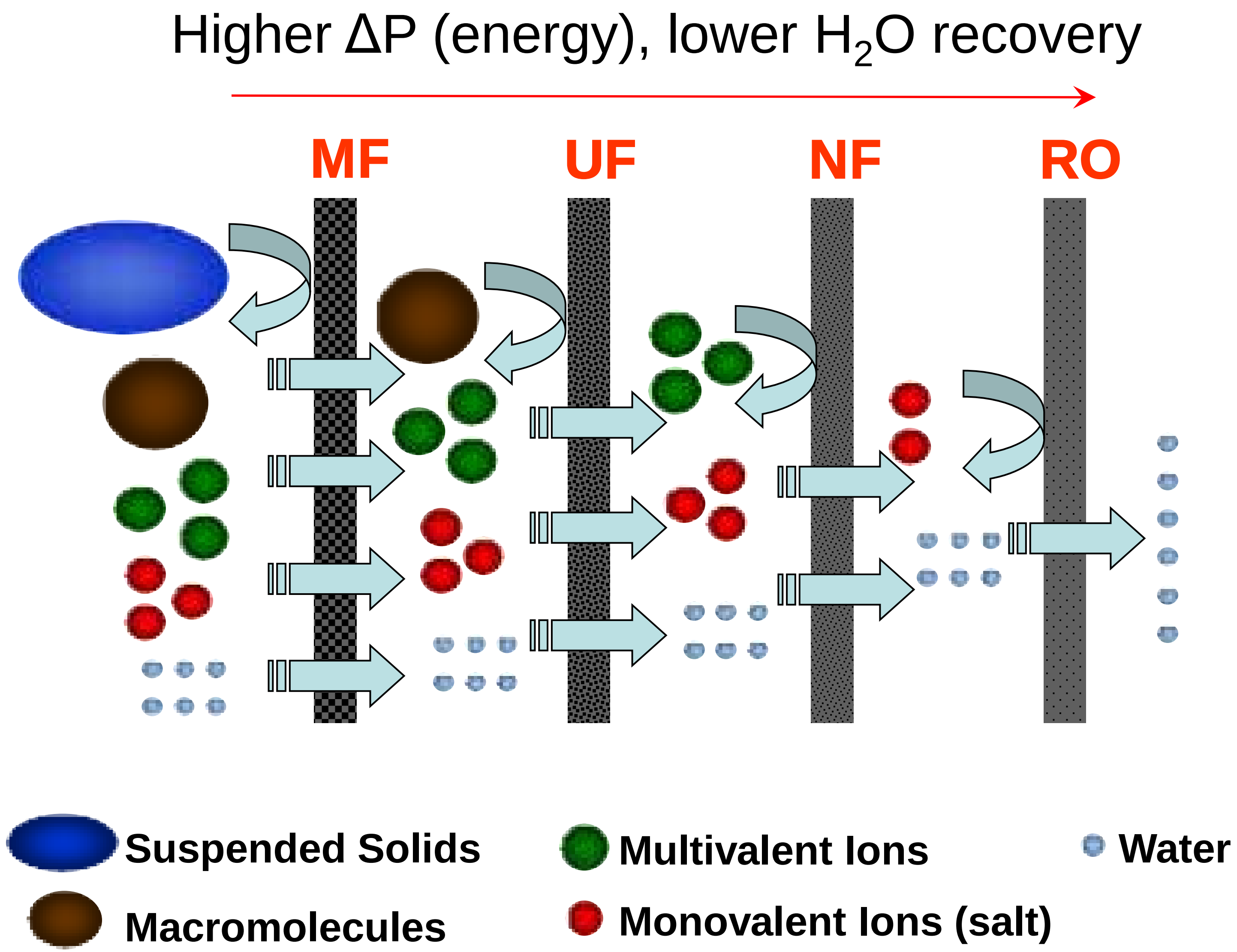


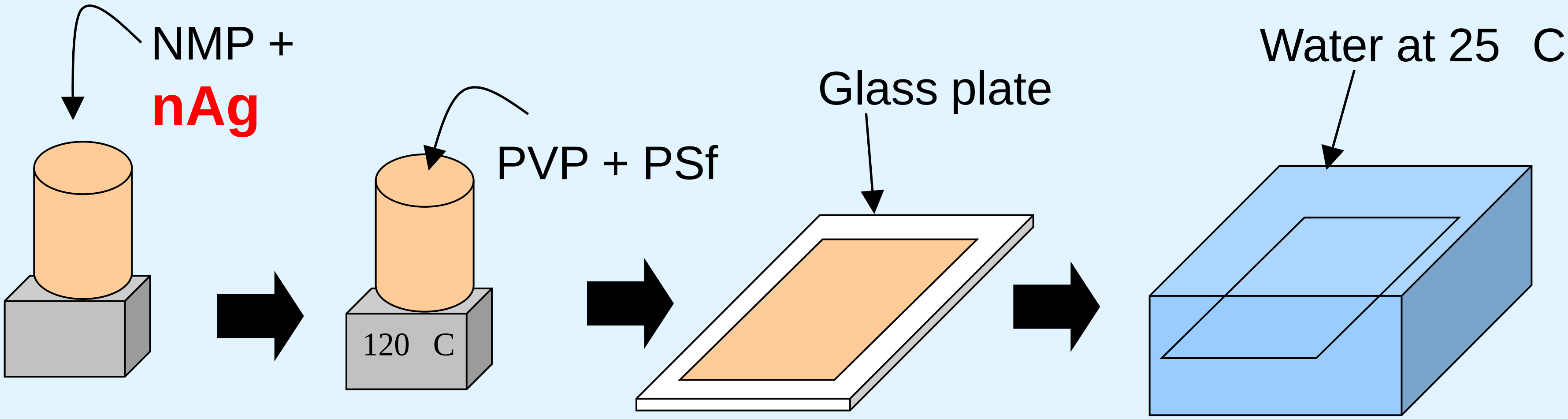



# Water Treatment Membranes

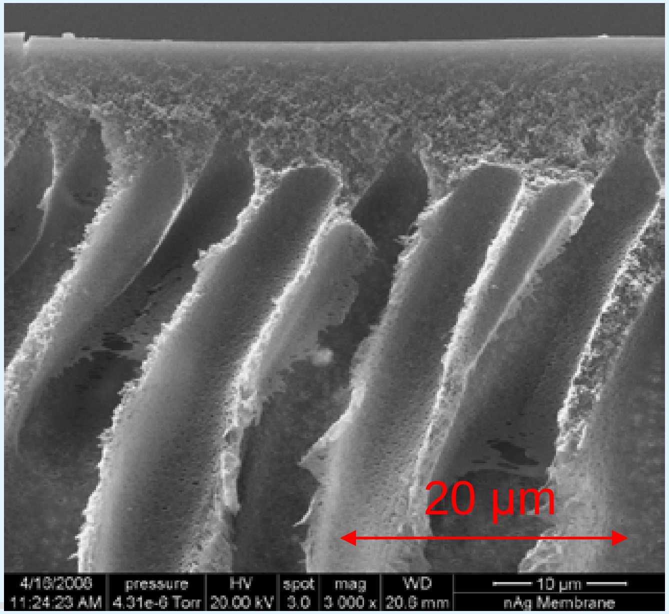


# Fouling-Resistant UF Membrane Fabrication (Wet Phase Inversion)




**1. Collodion preparation :**

**2. Film casting** at room temperature

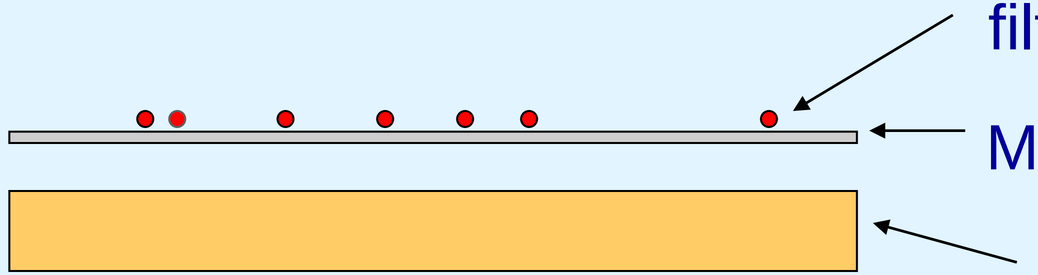


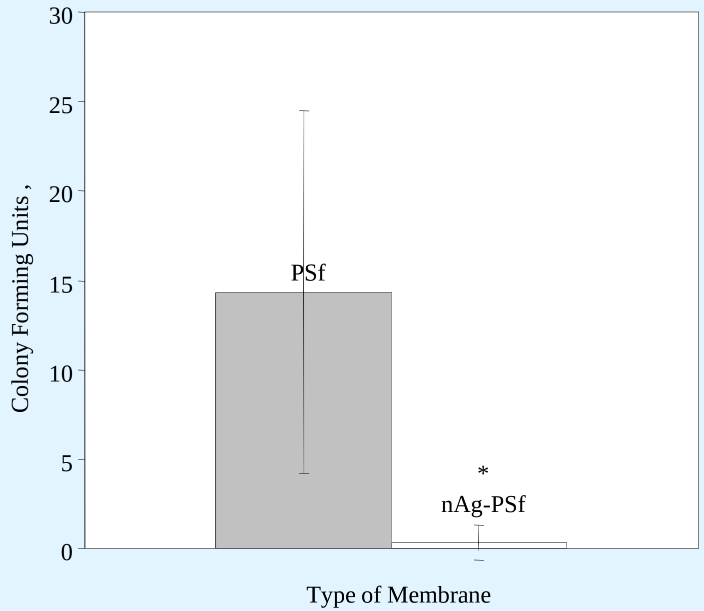

- Nanoparticle solubilisation in NMP. Ultrasonication (100 W, 4 min)
- Addition of PVP at 70 C
- Slow addition of PSF while stirring at 120 C

RICE

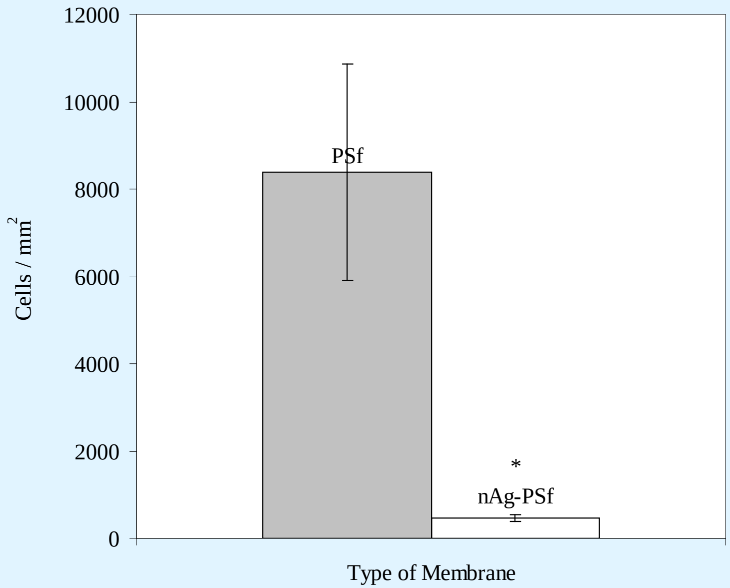
NMP = N-methylpyrrolidone ; PSf = Polysulfone ; PVP=Poly(vinylpyrrolidone)

# Bacterial growth inhibition test




Inhibition of growth of *E. coli*




Inhibition *E. coli* attachment onto membrane surface

Zodrow K., L. Brunet, S. Mahendra, Q. Li, and P.J.J. Álvarez (2009). *Wat. Res* doi:10.1016/j.watres.2008.11.014

# nAg-PSf Membranes Enhance Virus Removal

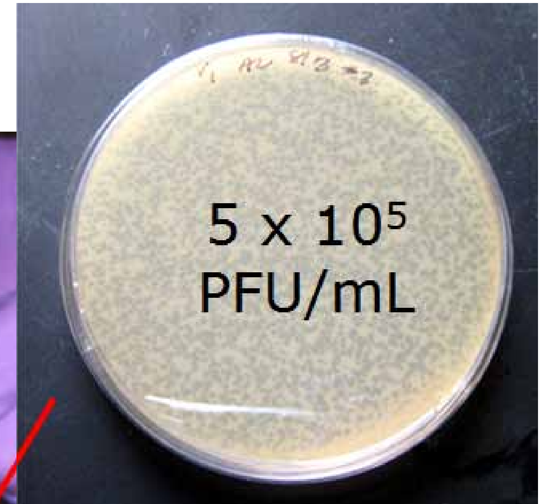
**Removal of MS2:**

**PSf: 99.9%**

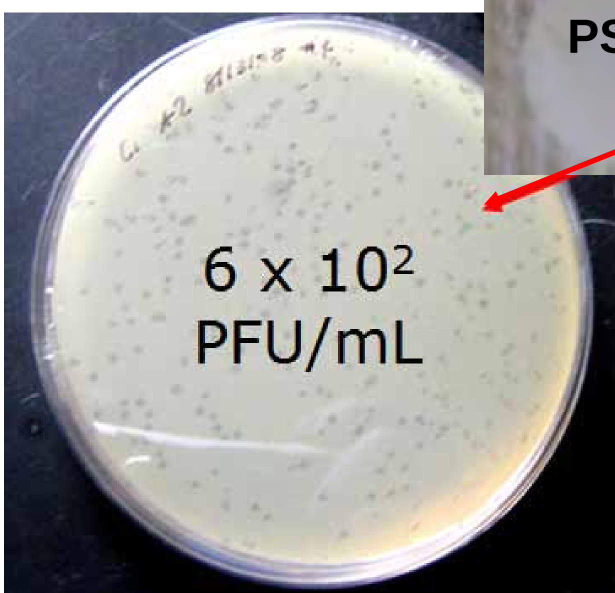
**nAg-PSf: > 99.999%**

**Surface Water Treatment Rule:**

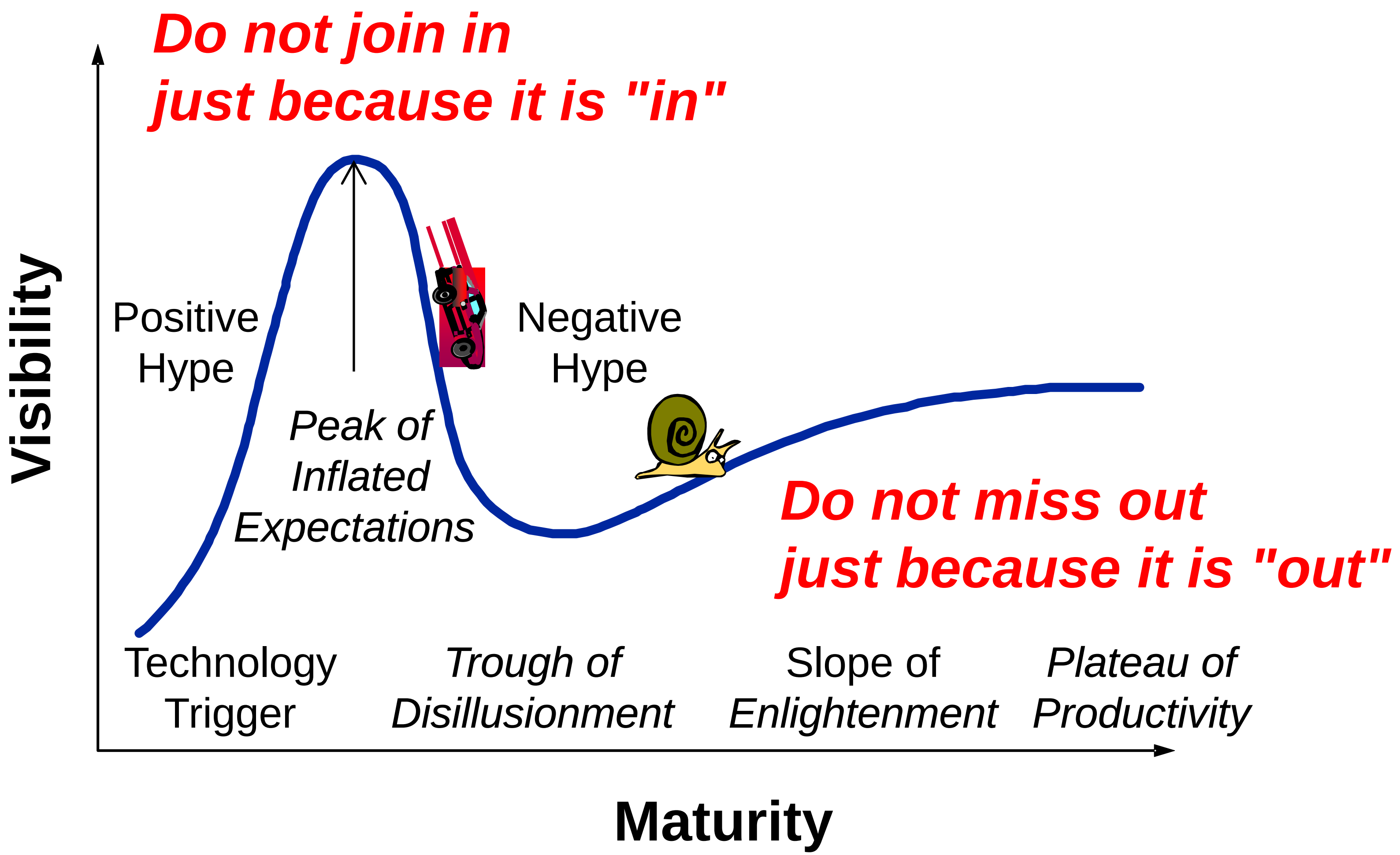
**99.99% Virus Removal**






# Quo Vadis, Nano?

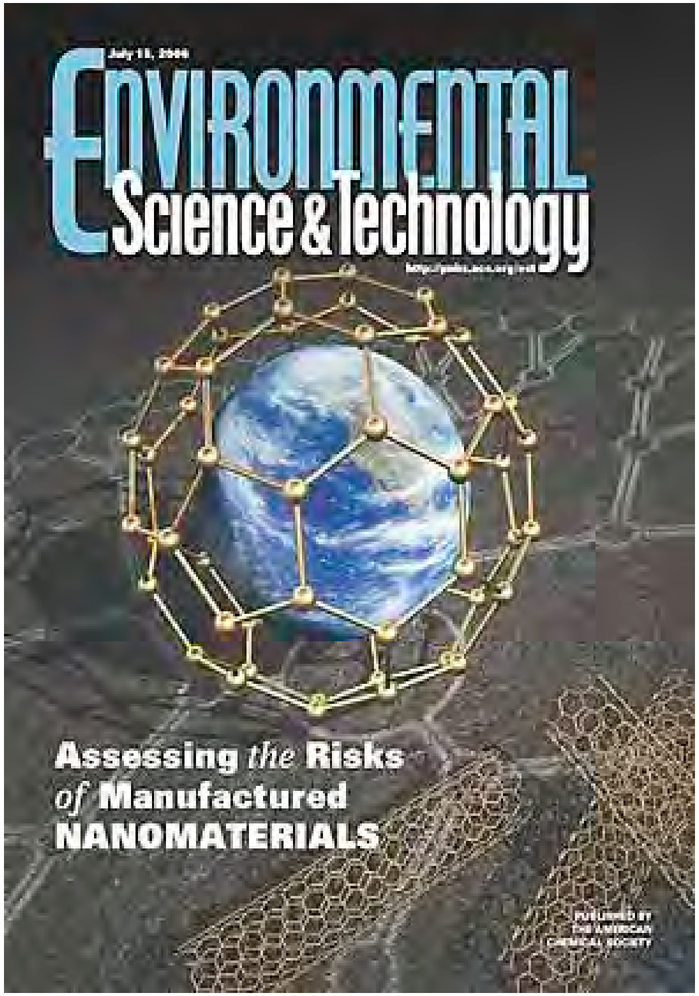



"Nanohype" - Berube

# So What?

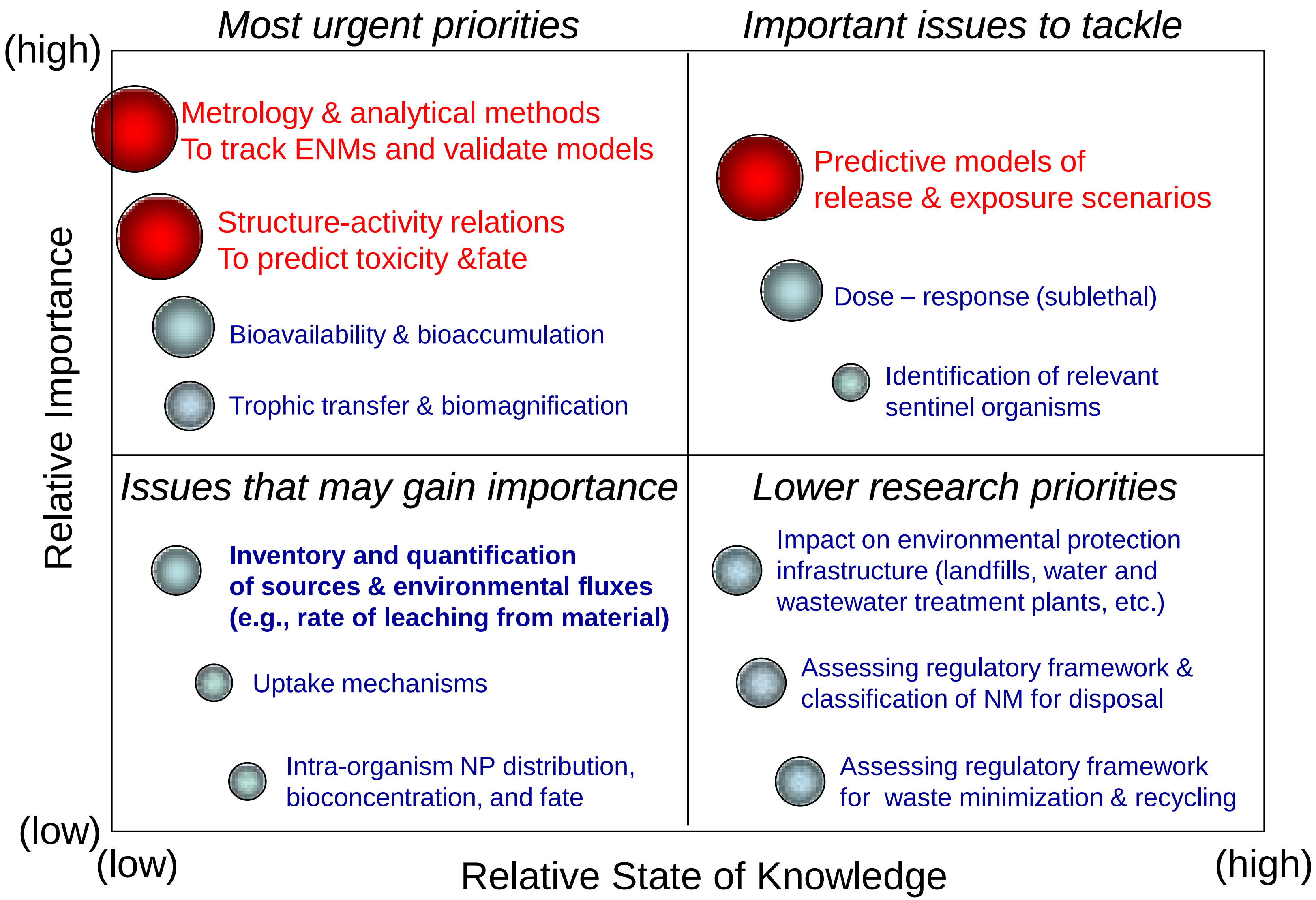
- <u>Implications</u>:
  Ecotoxicology- Biodiversity and food webs?
  Biogeochemical cycling?
  *Mitigated by NOM, salts*

- <u>Applications</u>:
  DBP-free disinfection, antifouling or anticorrosion coatings? Membranes? Bioremediation?



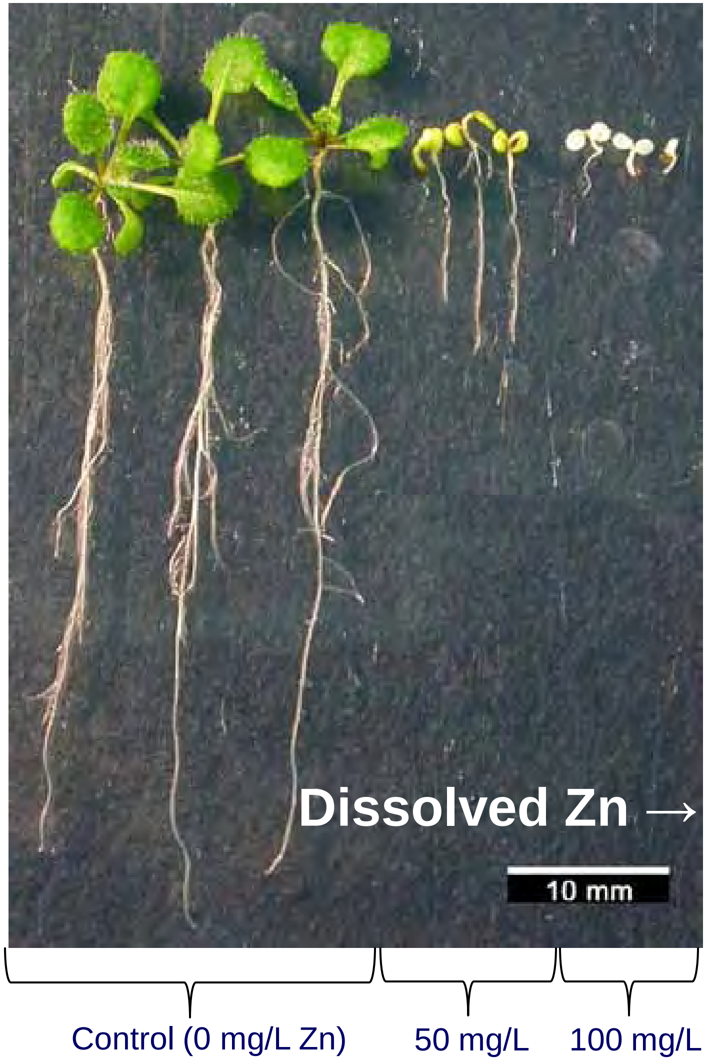
# Any Questions?

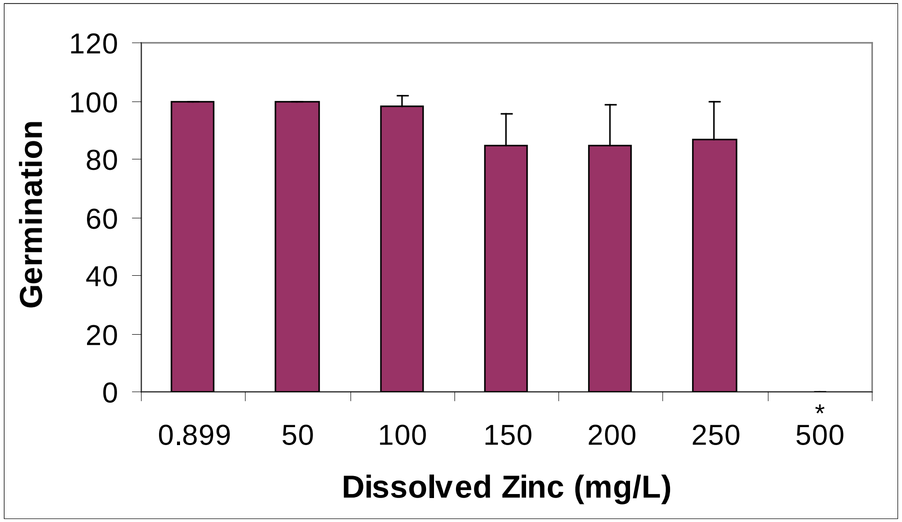
# Towards Ecoresponsible Nanotechnology

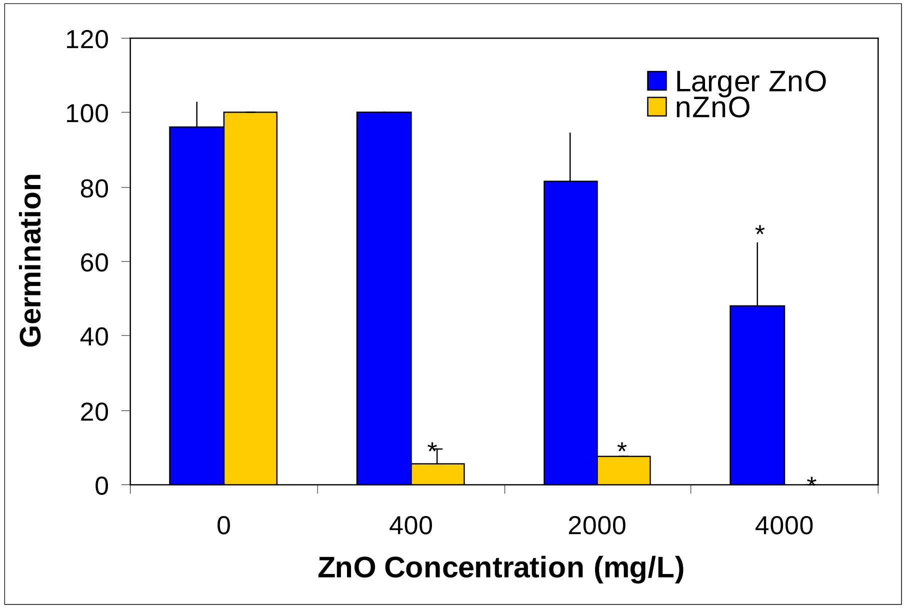


# BACKUP SLIDES

# Nano-scale Metal Oxides are Phytotoxic



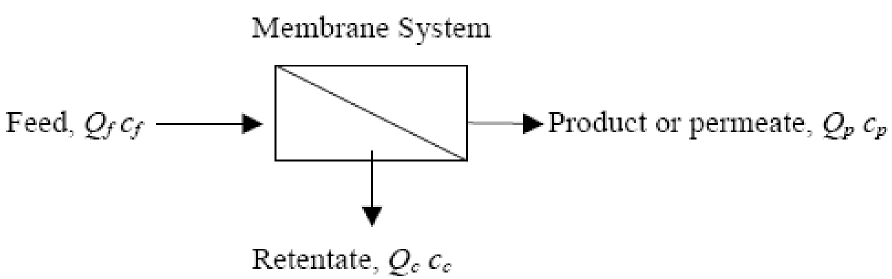



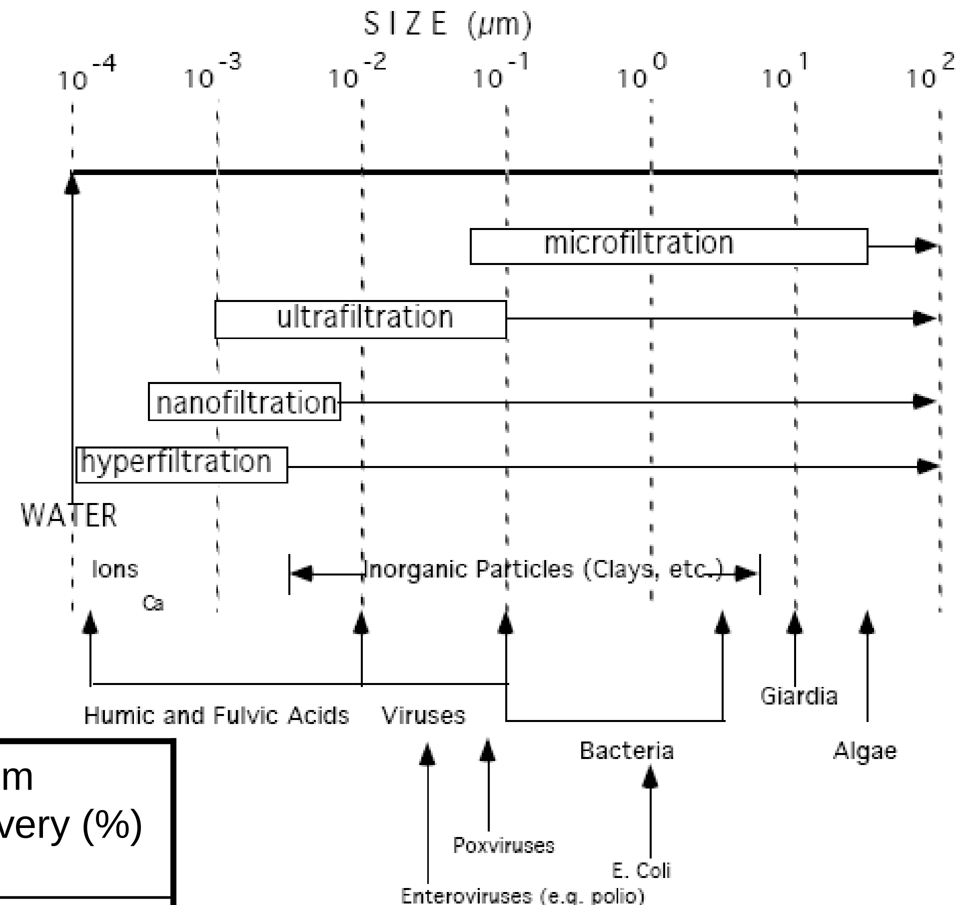
# Water Filtration Membranes

- Pore Size
- Recovery, $r$

$$r = \frac{Q_{pr}}{Q_f}$$



# Water Filtration Membranes

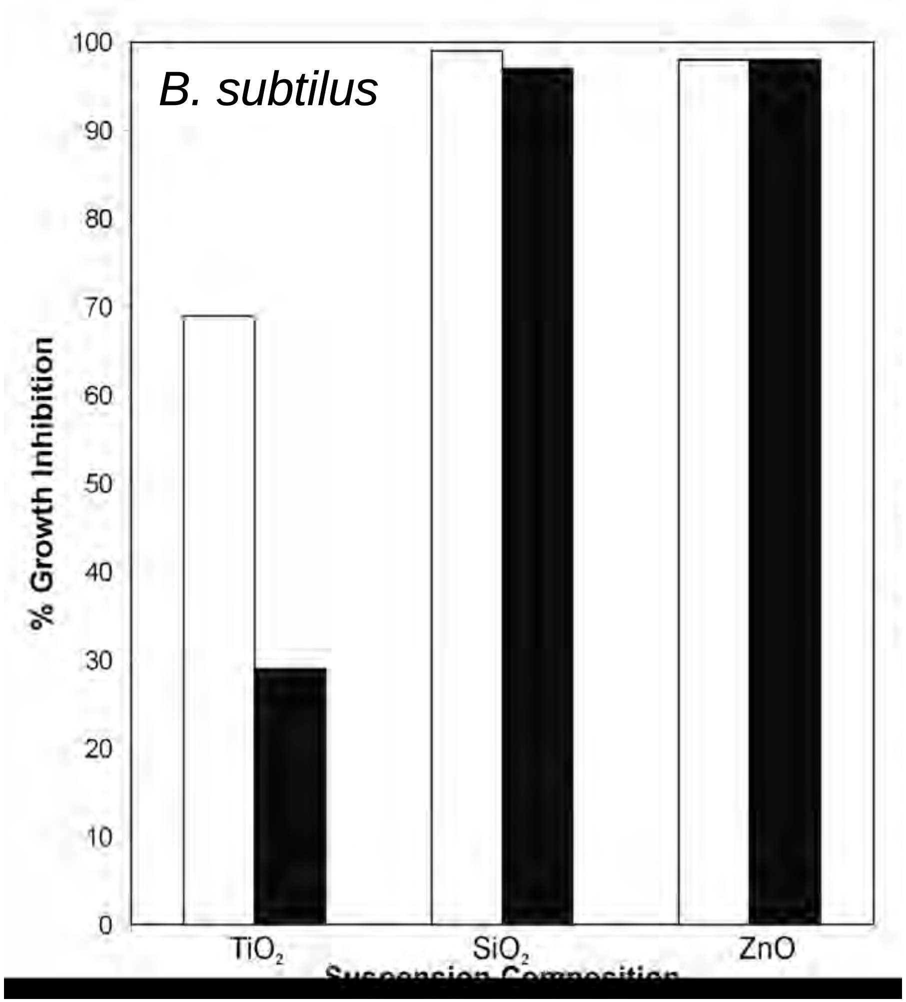



**Membranes with smaller pores have higher ΔP and lower recovery**

| Membrane Process | Transmembrane Pressure, ΔP (psi) | System Recovery (%) |
|---|---|---|
| Reverse Osmosis | 60 to 1,000 | 20 to 80 |
| Nanofiltration | 40 to 150 | 60 to 90 |
| Ultrafiltration | 7 to 40 | 80 to 95+ |
| Microfiltration | 4 to 30 | 80 to 99+ |

# Acknowledgements: NSF/CBEN, EPA

# Effect of illumination on antibacterial activity of photosensitive inorganic nanomaterials




Toxicity: ZnO > $SiO_2$ > $TiO_2$

ROS production: $TiO_2$ > ZnO > $SiO_2$

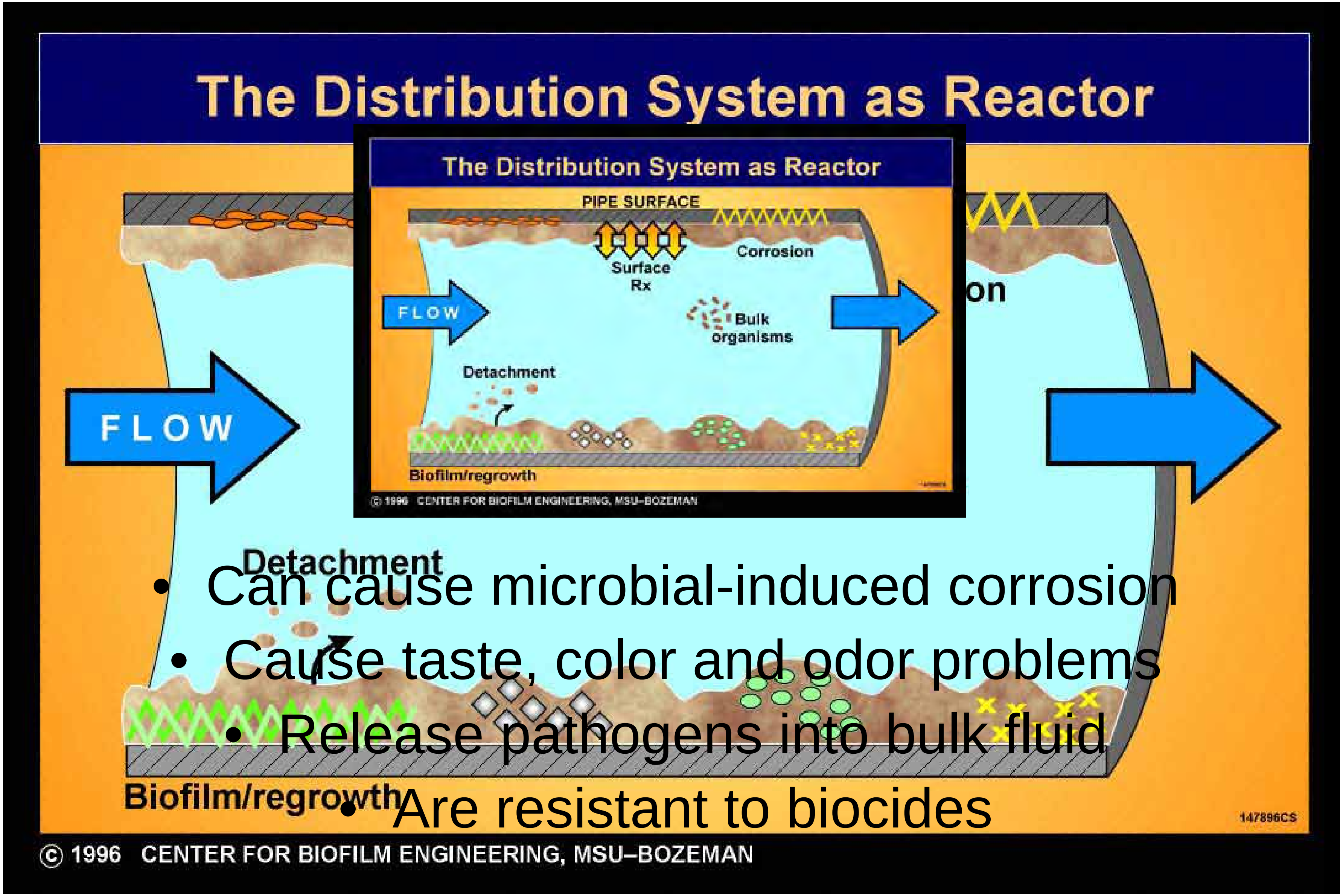
(No correlation)

**Cell death also occurs in the dark and in the absence of $O_2$.**

Thus, an additional mechanism besides photocatalytic ROS production is involved.


Adams L.K. D. Y. Lyon, and P.J.J. Alvarez (2005). *Wat. Res.* 40(19):3527-3532.

# Biofilms in Water Distribution Systems

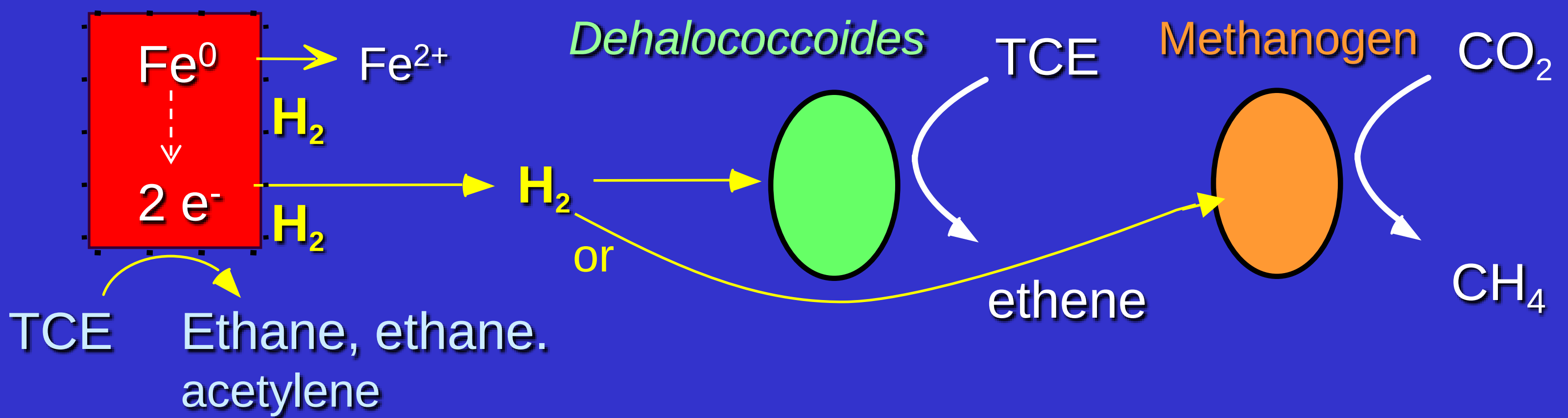



- Can cause microbial-induced corrosion
- Cause taste, color and odor problems
- Release pathogens into bulk fluid
- Are resistant to biocides

# Iron-Stimulated Bioremediation

$H_2$ produced by (anaerobic) NZVI corrosion stimulates anaerobic bioremediation (reductive dechlorination)

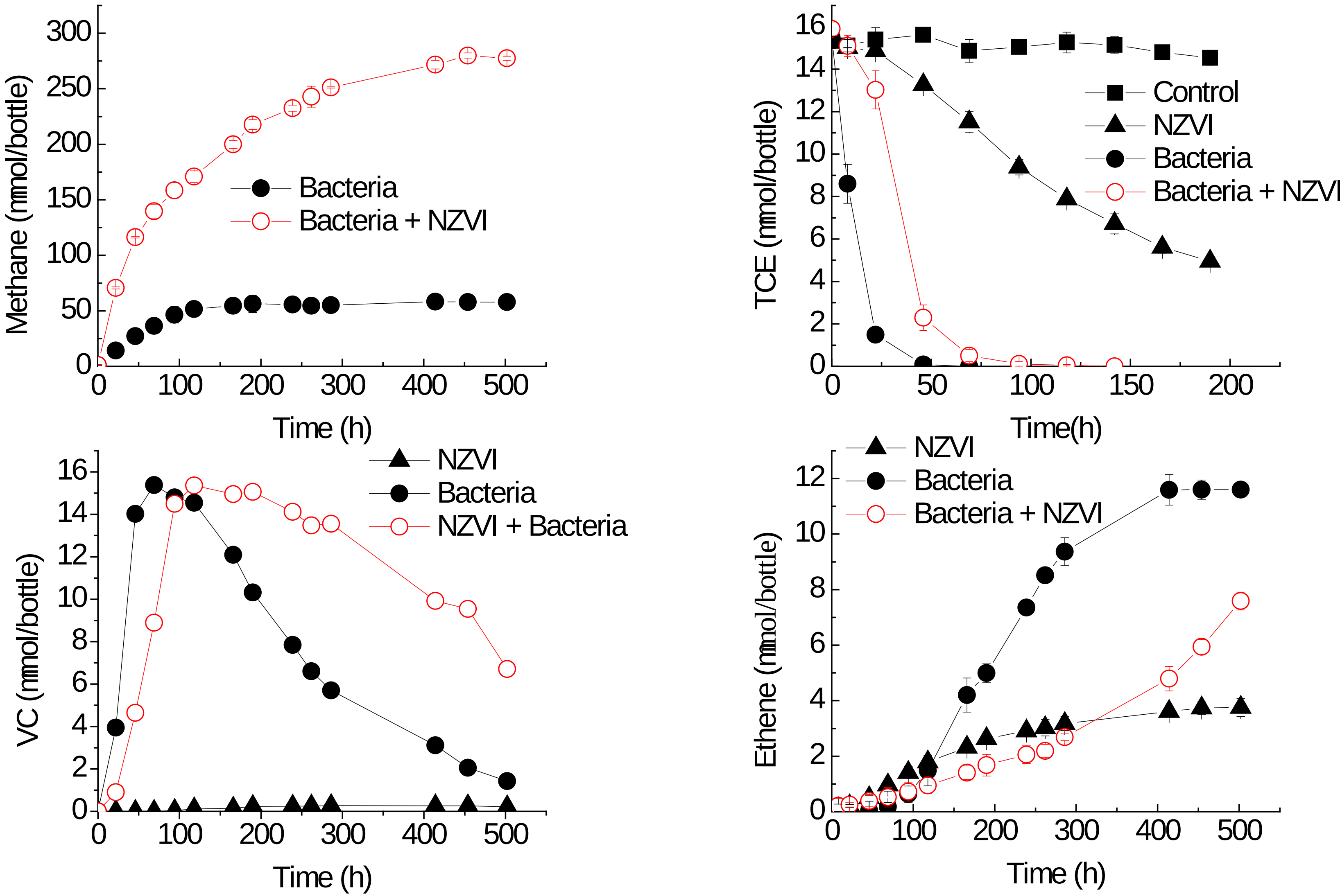
$$Fe^0 + 2H_2O \rightarrow Fe^{+2} + \mathbf{H_2} + 2OH^-$$



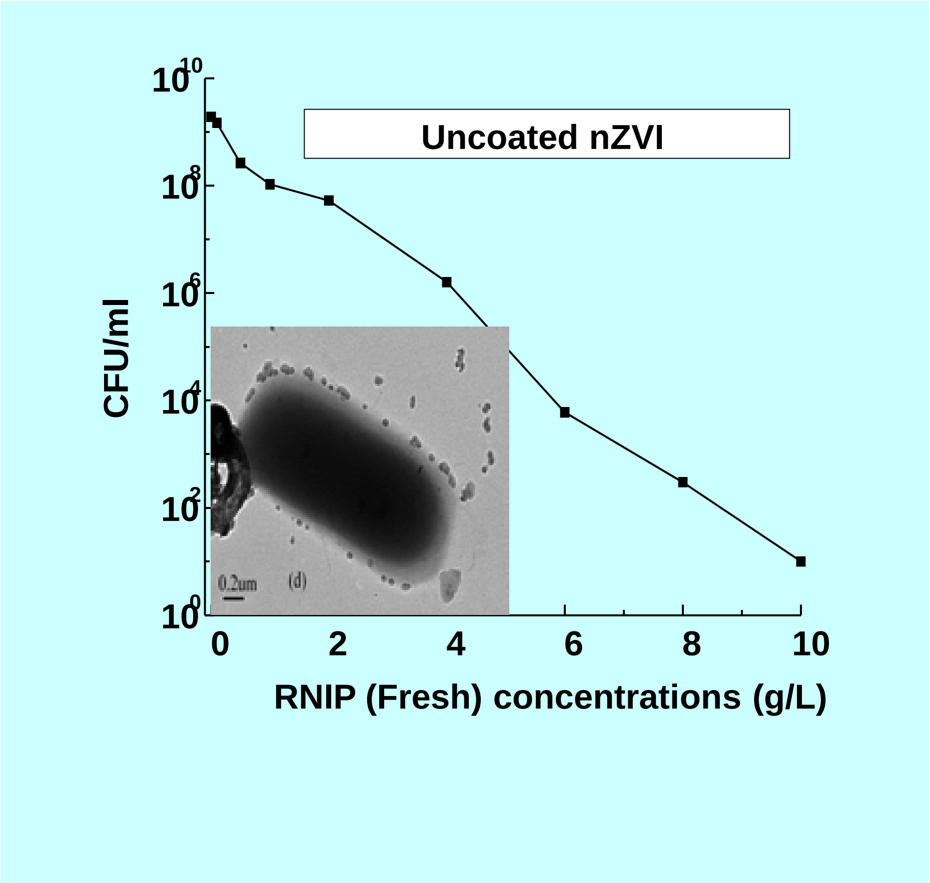

*Abiotic Reduction*

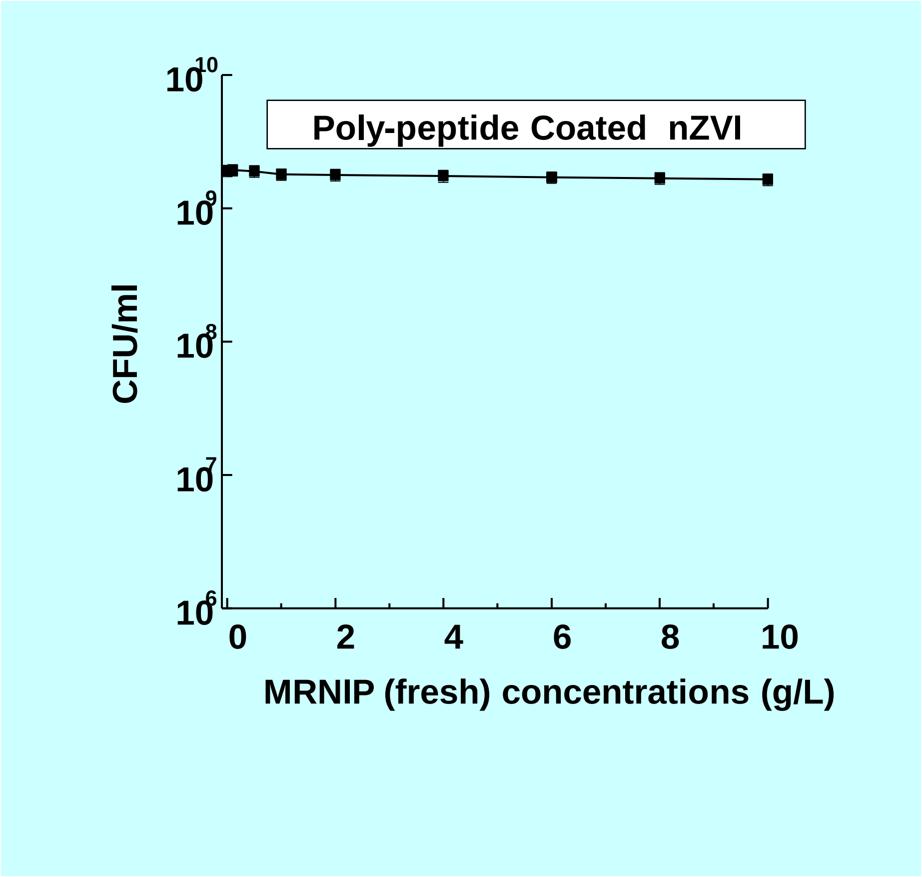
*Microbial Reduction: polishing, less toxic products*

# NZVI Preferentially Biostimulated Methanogens, also Dechlorinators after Inhibitory Period



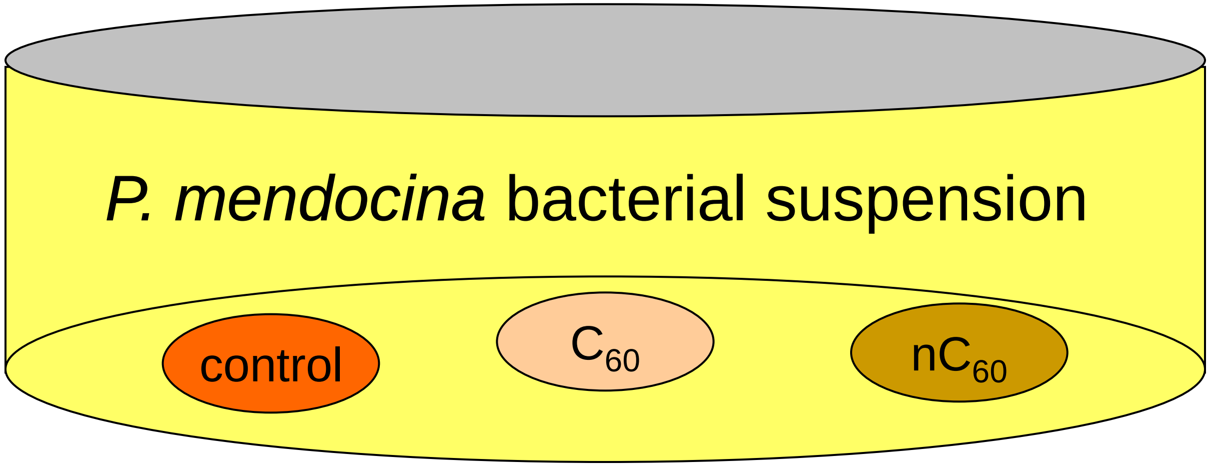
# Dose response of *E. coli* exposed to nZVI



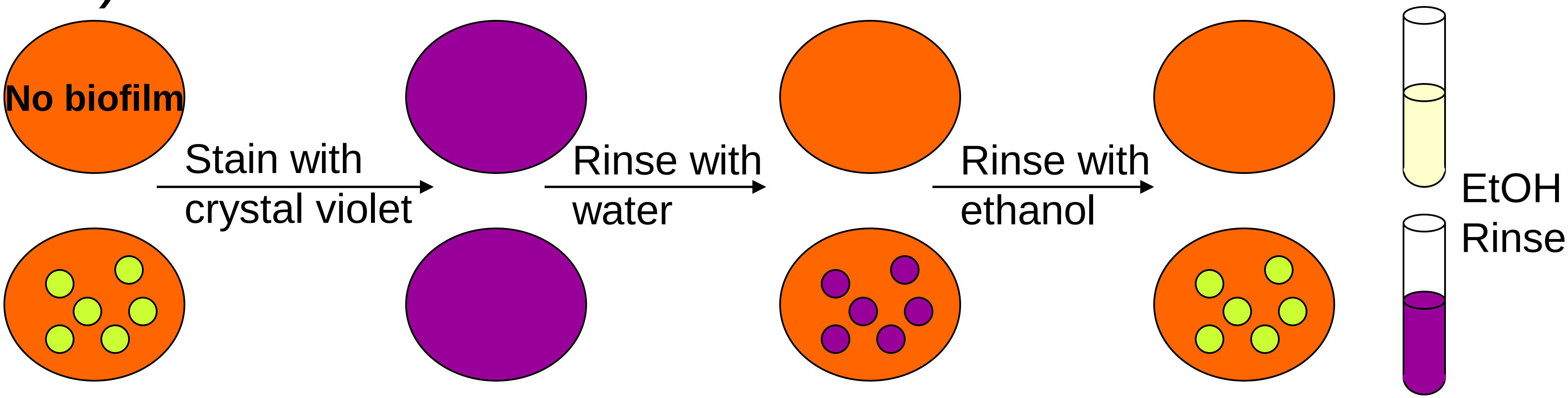



# Assessing Biofilm Formation

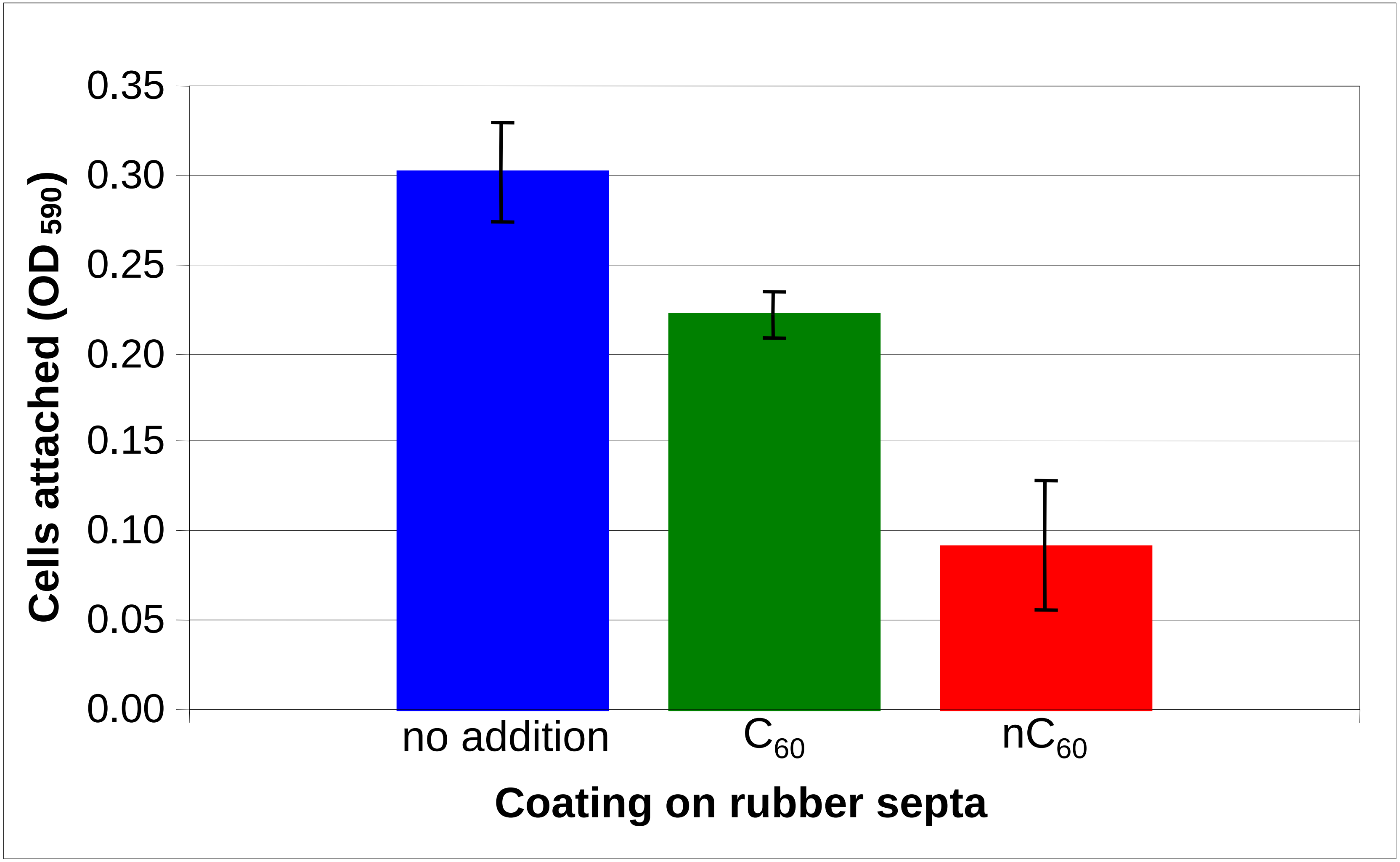
1) Grow biofilm on rubber septa




2) Measure amount of biofilm

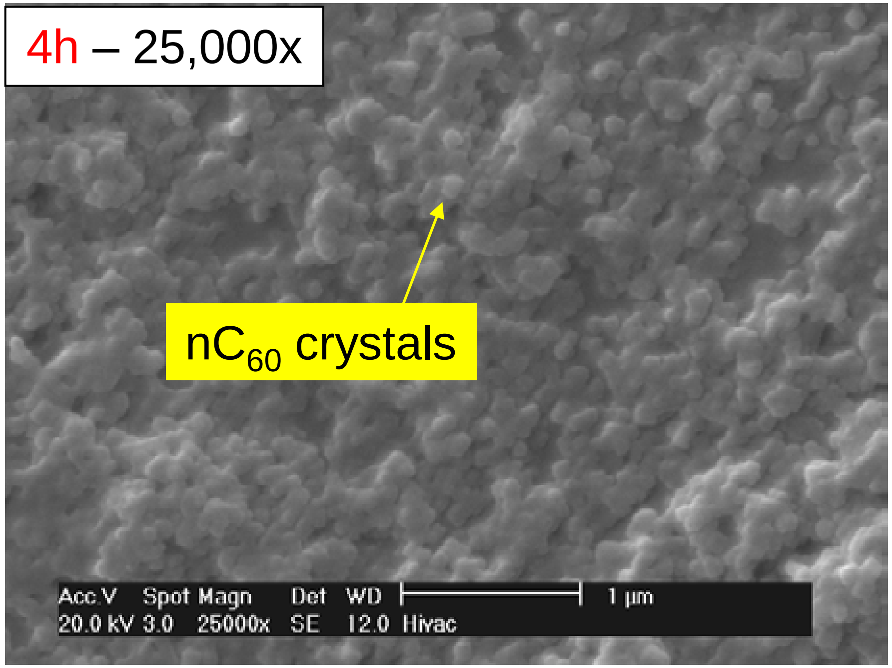


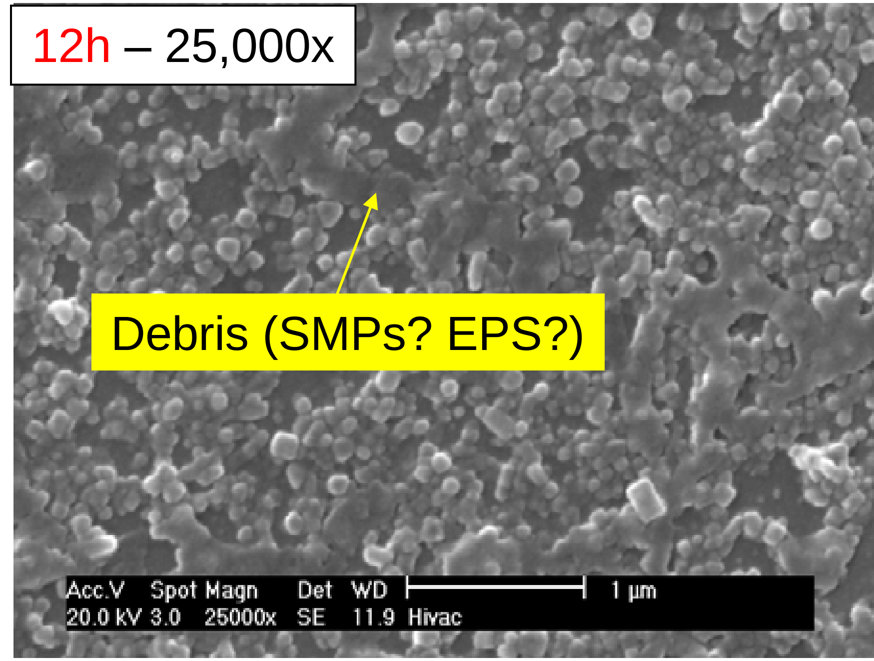
# $nC_{60}$ reduces biofilm formation?

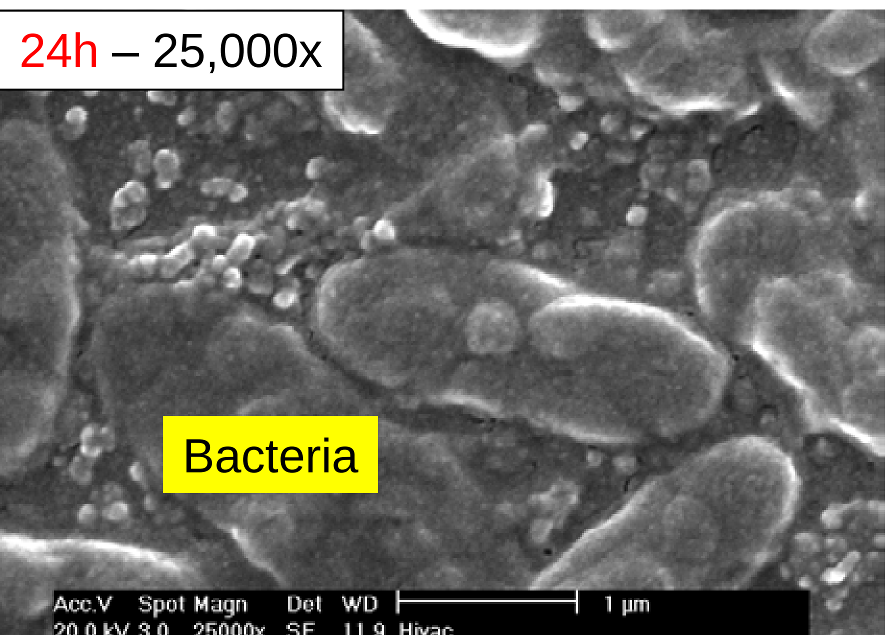


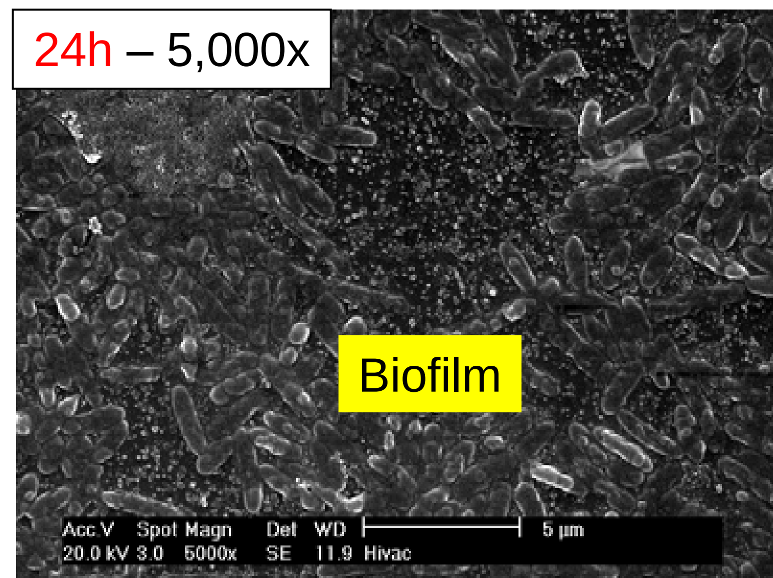

Lyon D.Y., D, Brown, E. Sundstrom, and P. J.J.Alvarez (2008). *Int. Biodeterior. Biodegrad*. 62:475-478

# SEM images of biofilm formation on $nC_{60}$



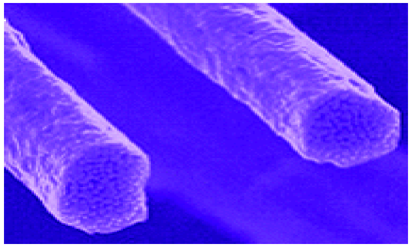



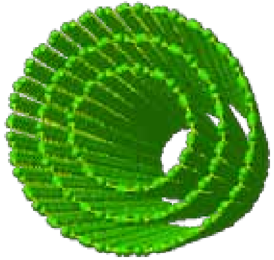



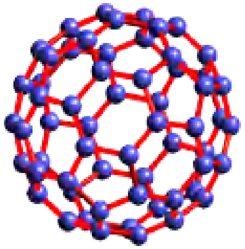
# Which Types of Nanomaterials Should We Focus On?

**Single and multi walled nanotubes**



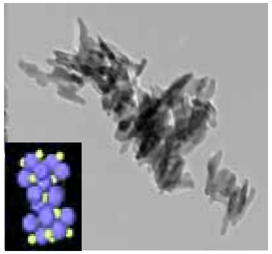


**Fullerenes**

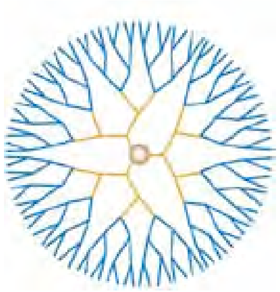


**Nanoshells**

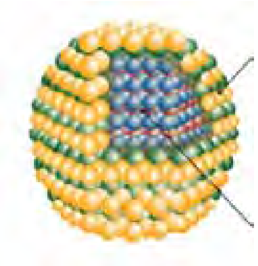
**Metal oxides**



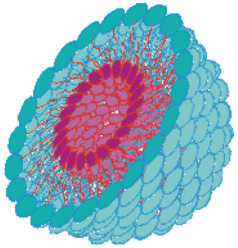
**Dendrimers**



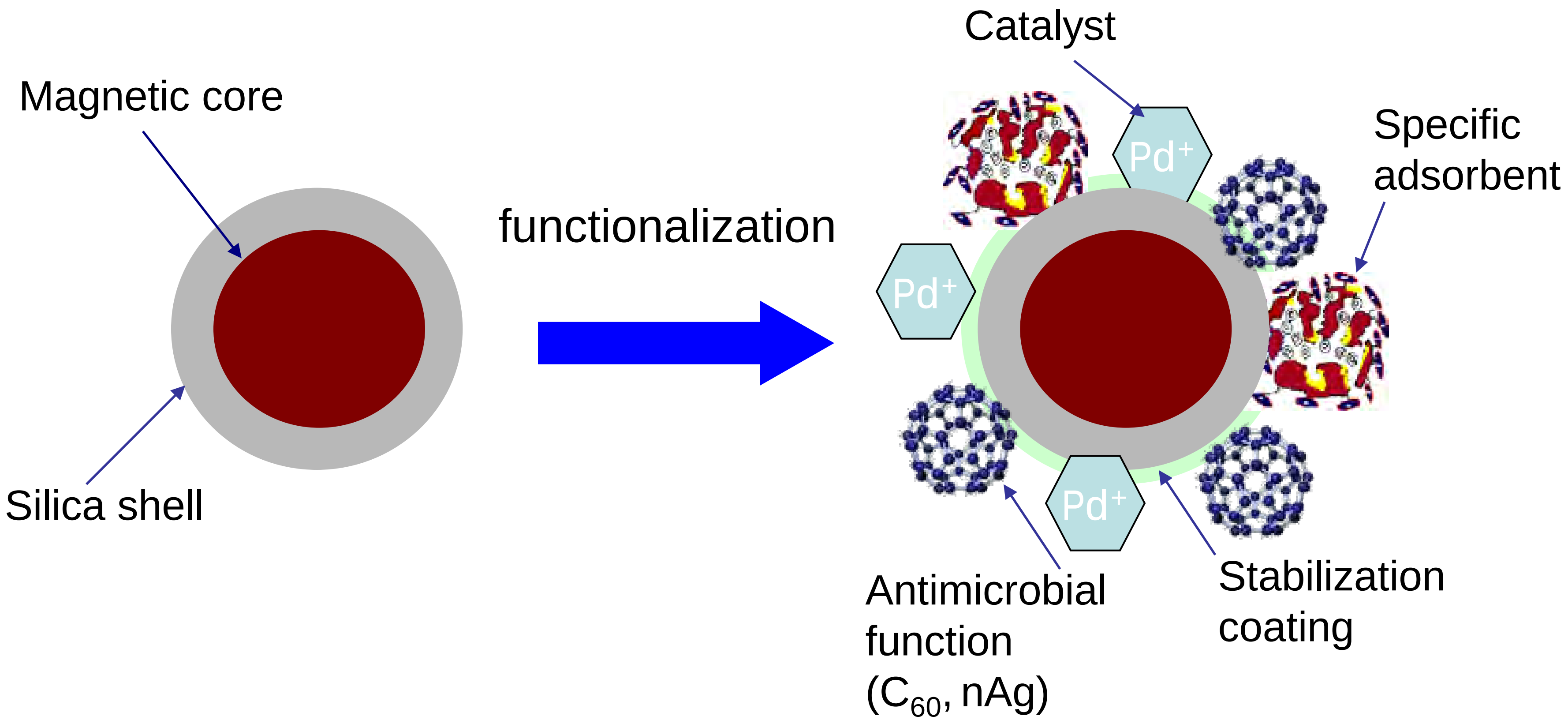
**Quantum dots**



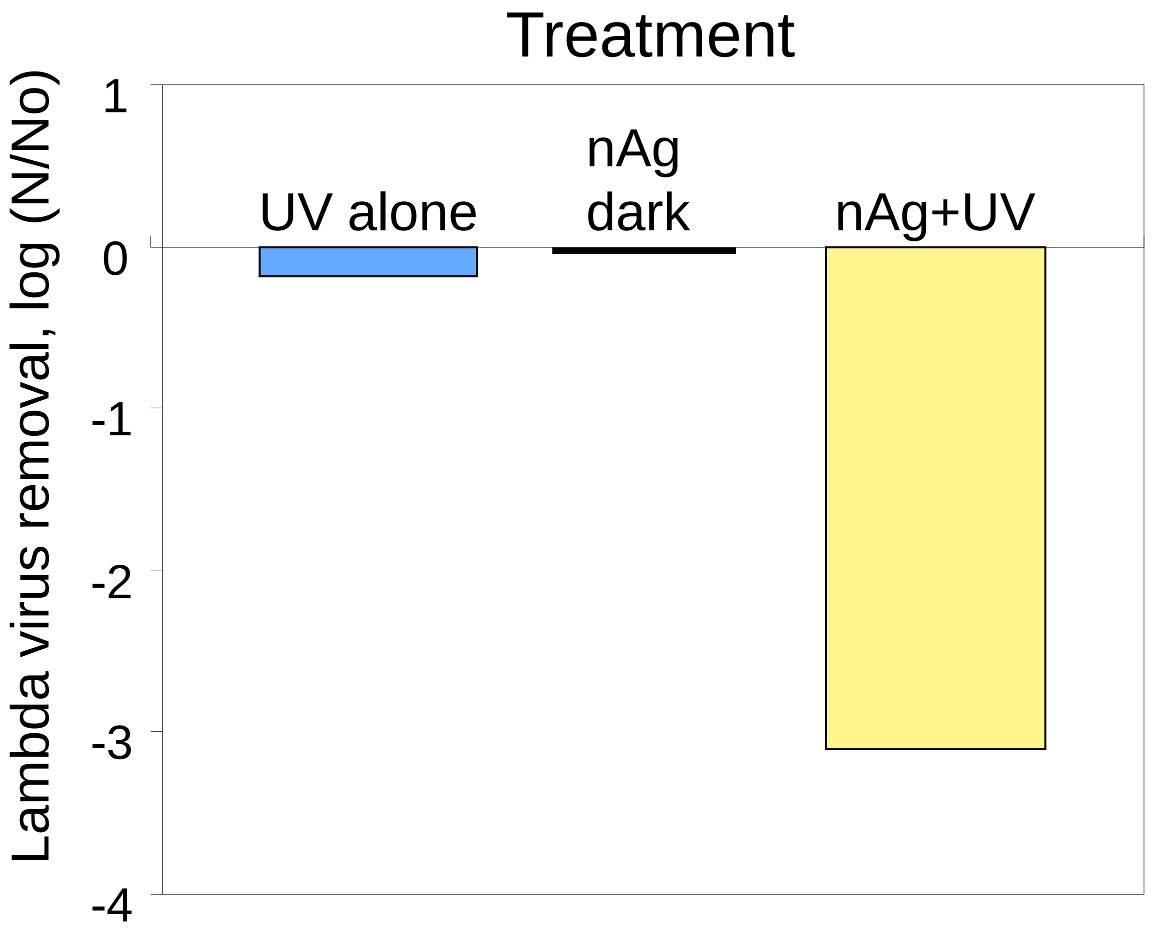
**Nanosomes**



# Multi-functional Magnetic Nanoparticles



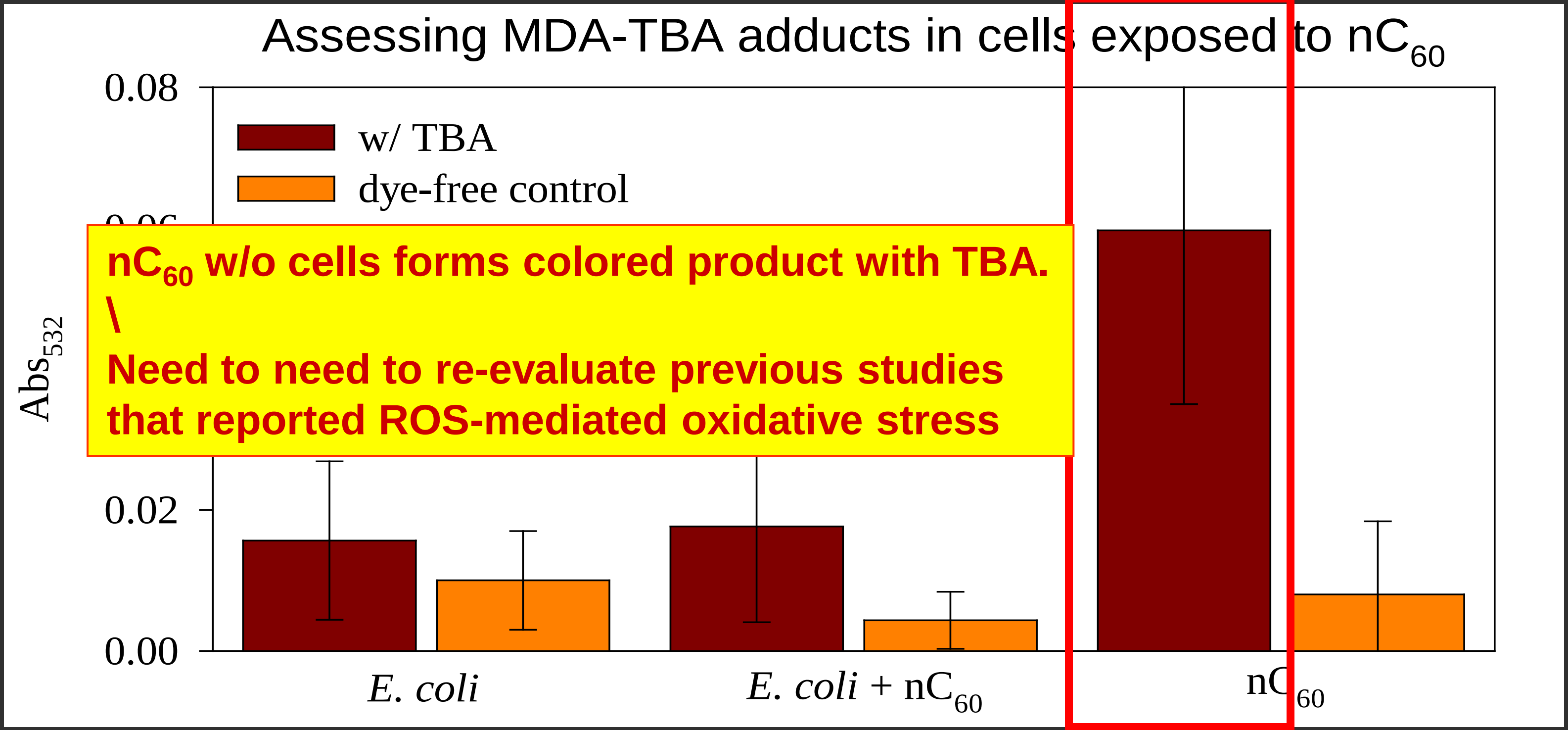
# Synergistic Inactivation of Lambda virus with nano-Ag and UV illumination




nAg mounted on nano-magnetite, 0.5 ppm, 1-h under UV illumination of 24.1 $mW/m^2$

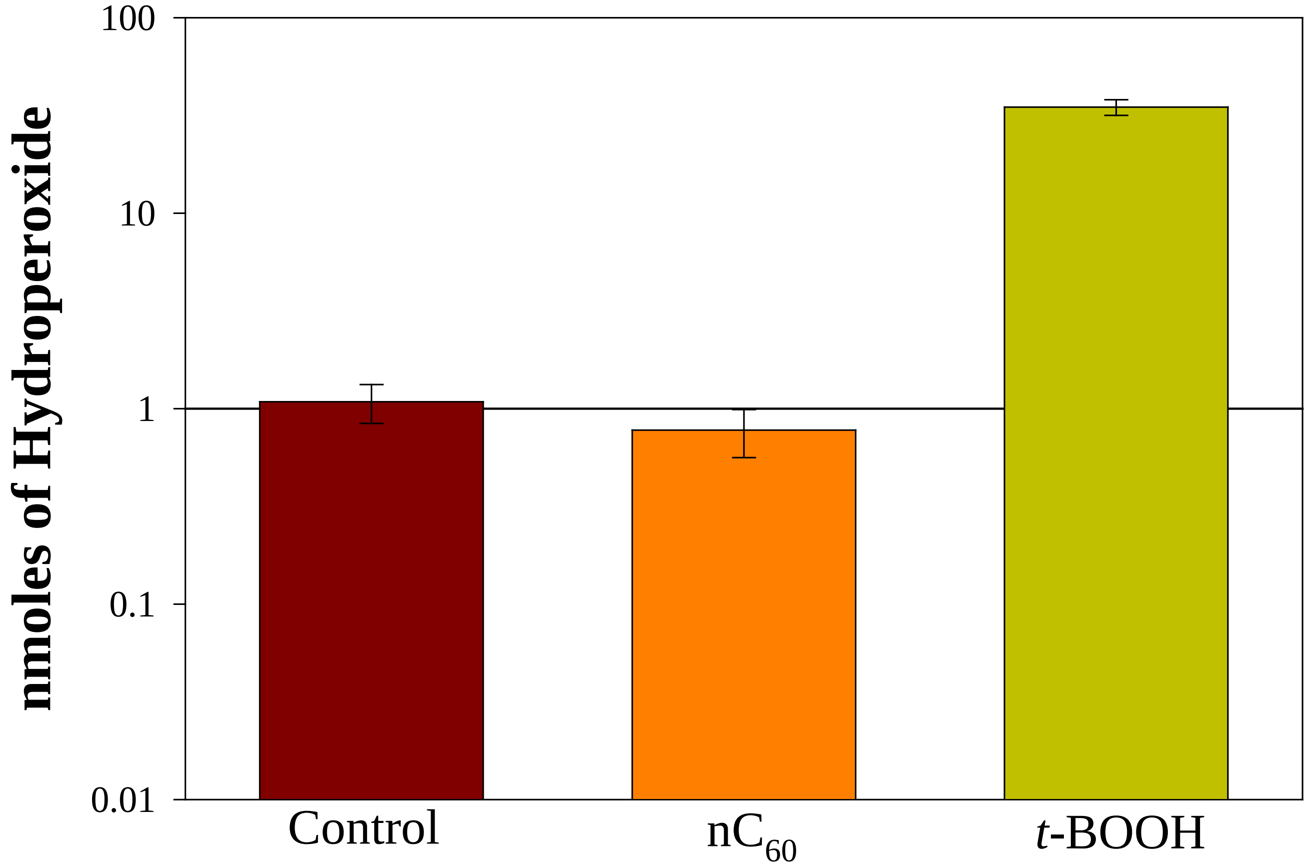
# Looking for lipid peroxidation as evidence of ROS damage

- Hallmark of lipid peroxidation is malonedialdehyde (MDA)
- MDA forms colored adducts with thiobarbituric acid (TBA)




**$nC_{60}$ w/o cells forms colored product with TBA.**
****
**Need to need to re-evaluate previous studies that reported ROS-mediated oxidative stress**

# No Lipid Peroxidation

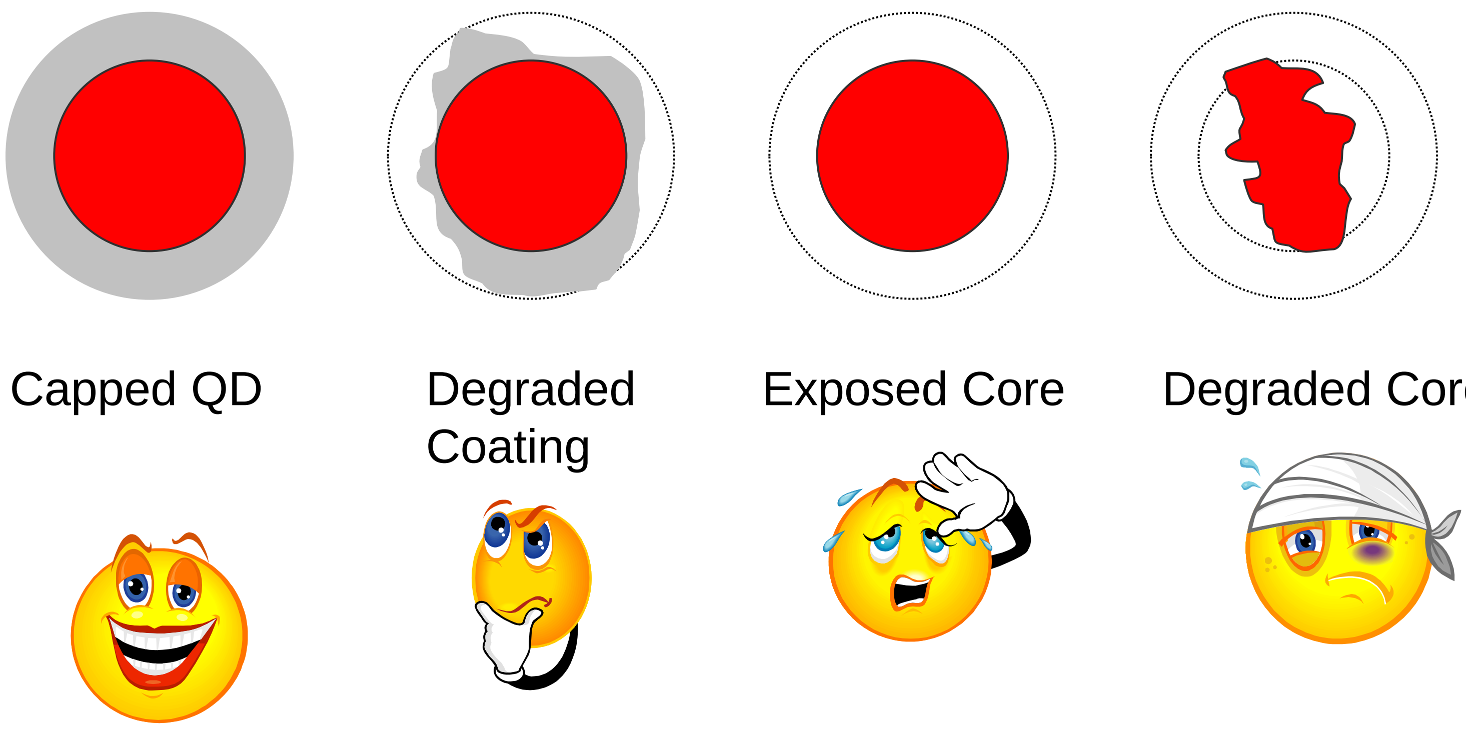



- Chromogenic assay based on hydroperoxide reaction with ferrous ions to make ferric ions
- no lipid perooxidation in $nC_{60}$-exposed samples (P=0.15)
- *Tert*-butylhydroperoxide is positive control

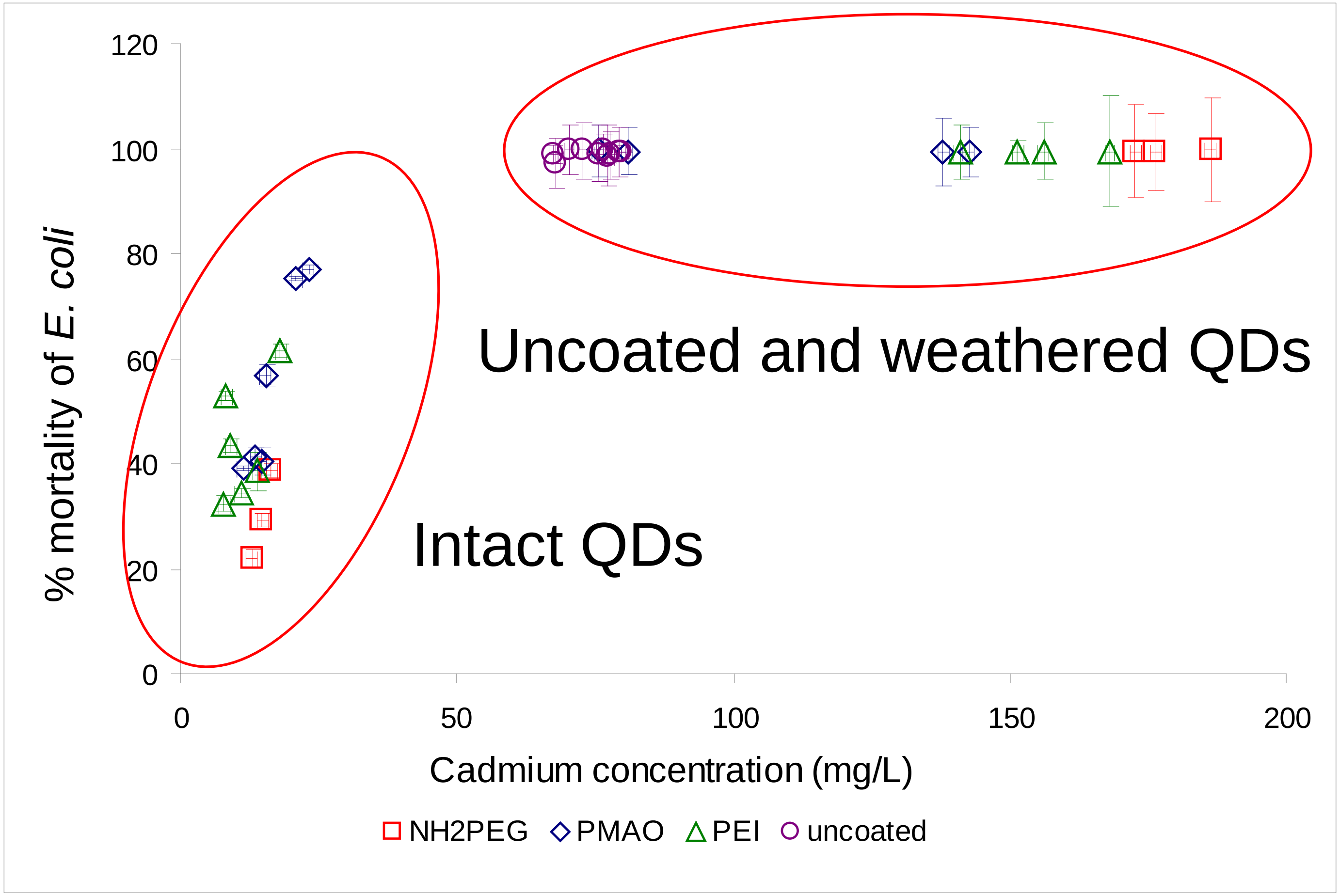
Lyon D.Y. and P.J.J. Alvarez (2008). Environ. Sci. Technol. (In Press)

# Quantum Dot Weathering

***Hypothesis:*** *Toxicity of quantum dots is primarily due to free metal, and environmental weathering of the coating will increase their toxicity to cells*

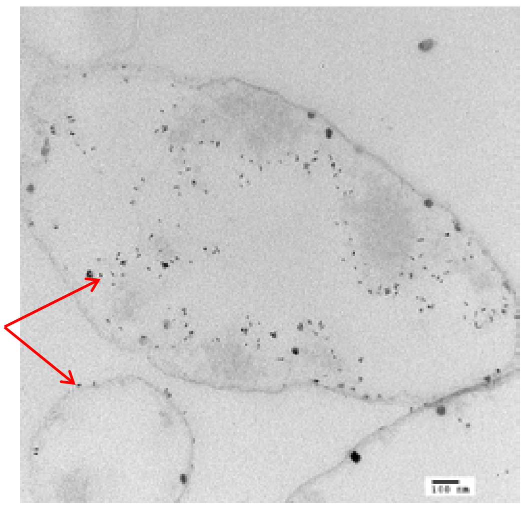


# Cd from QDs is Toxic to Bacteria



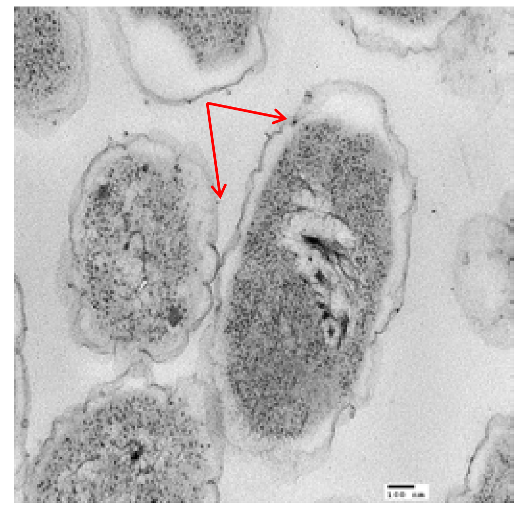
# *E. coli* Takes Up Intact (Coated, Biocompatible) QDs

***E. coli (G-)***

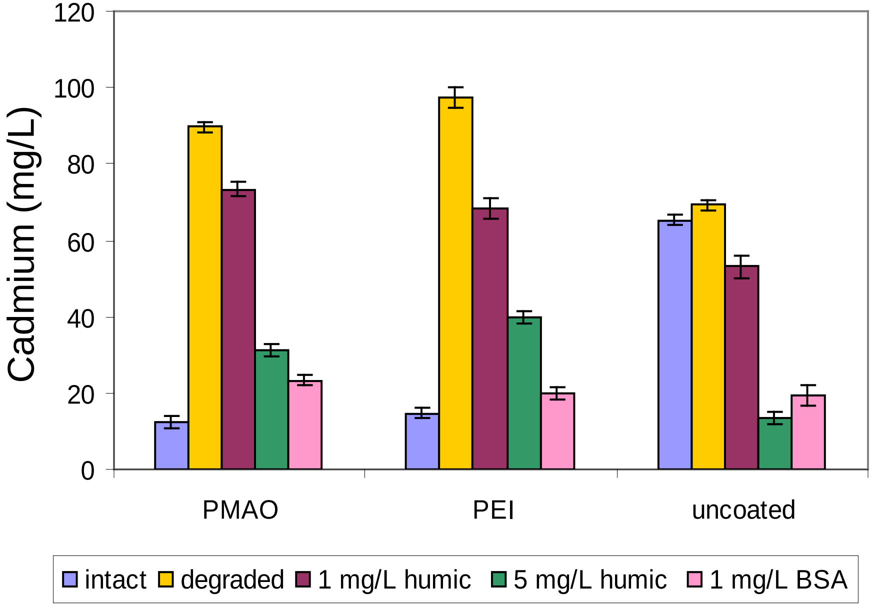


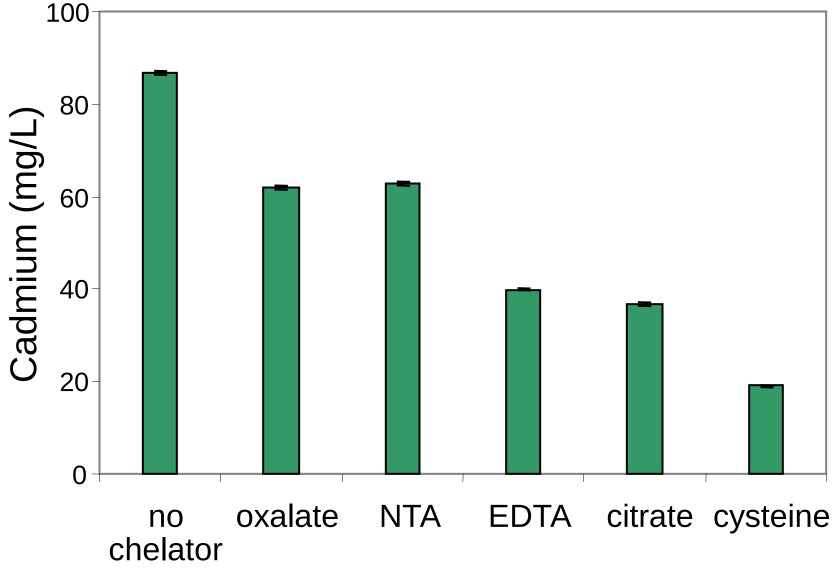
***B. subtilis (G+)***

***P. aeruginosa***

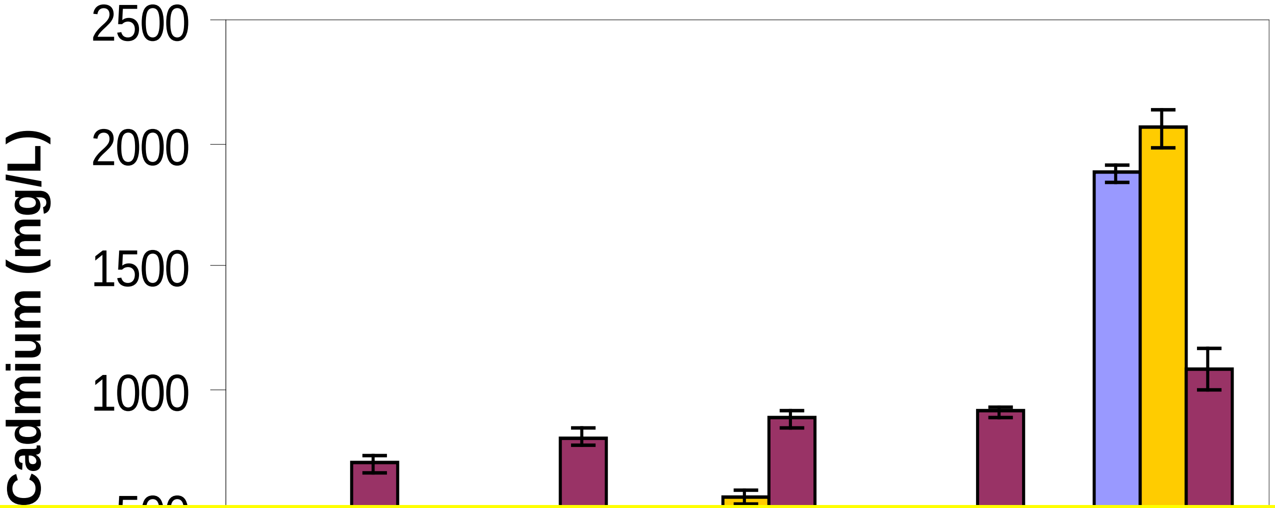


# Humic Acids, Proteins and Chelators Mitigate Cd Release and Toxicity



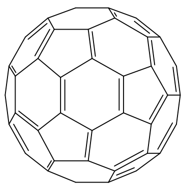



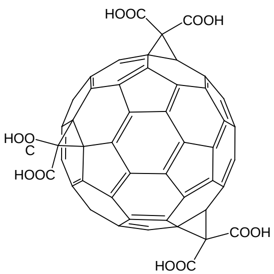
# Salinity Increases QD Aggregation and Cadmium Release

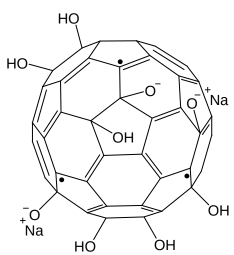


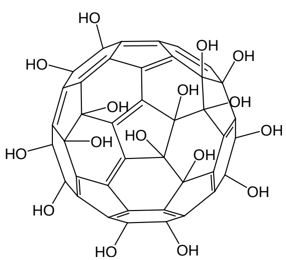

Counter-Intuitive Trend:
Processes that promote QD aggregation (changes in pH, increased salinity) accelerated the degradation of QD coating, resulting in increased metal release and toxicity.

# Dose Response Curve for Fullerenes
## Colvin, West & co-workers, Rice University

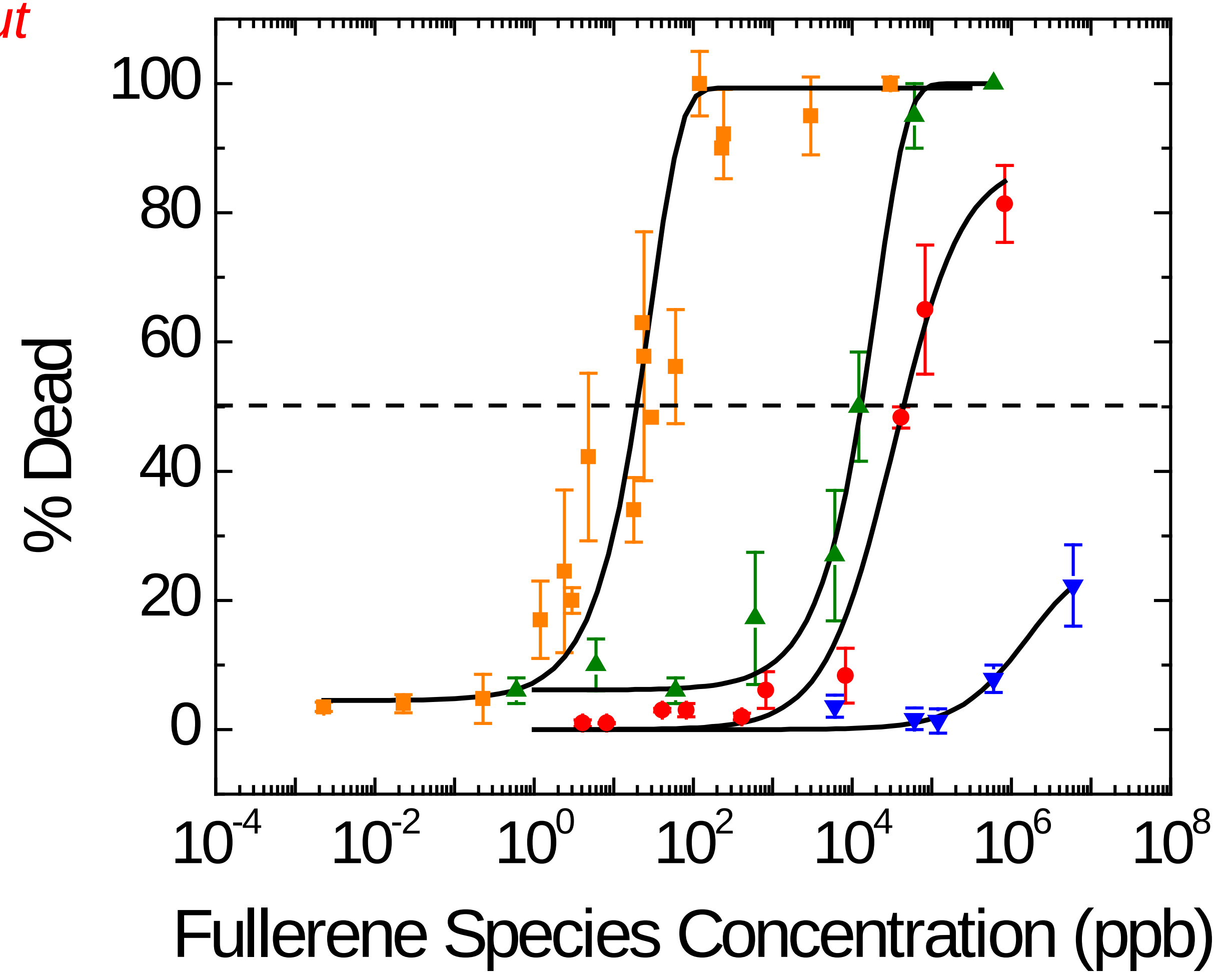




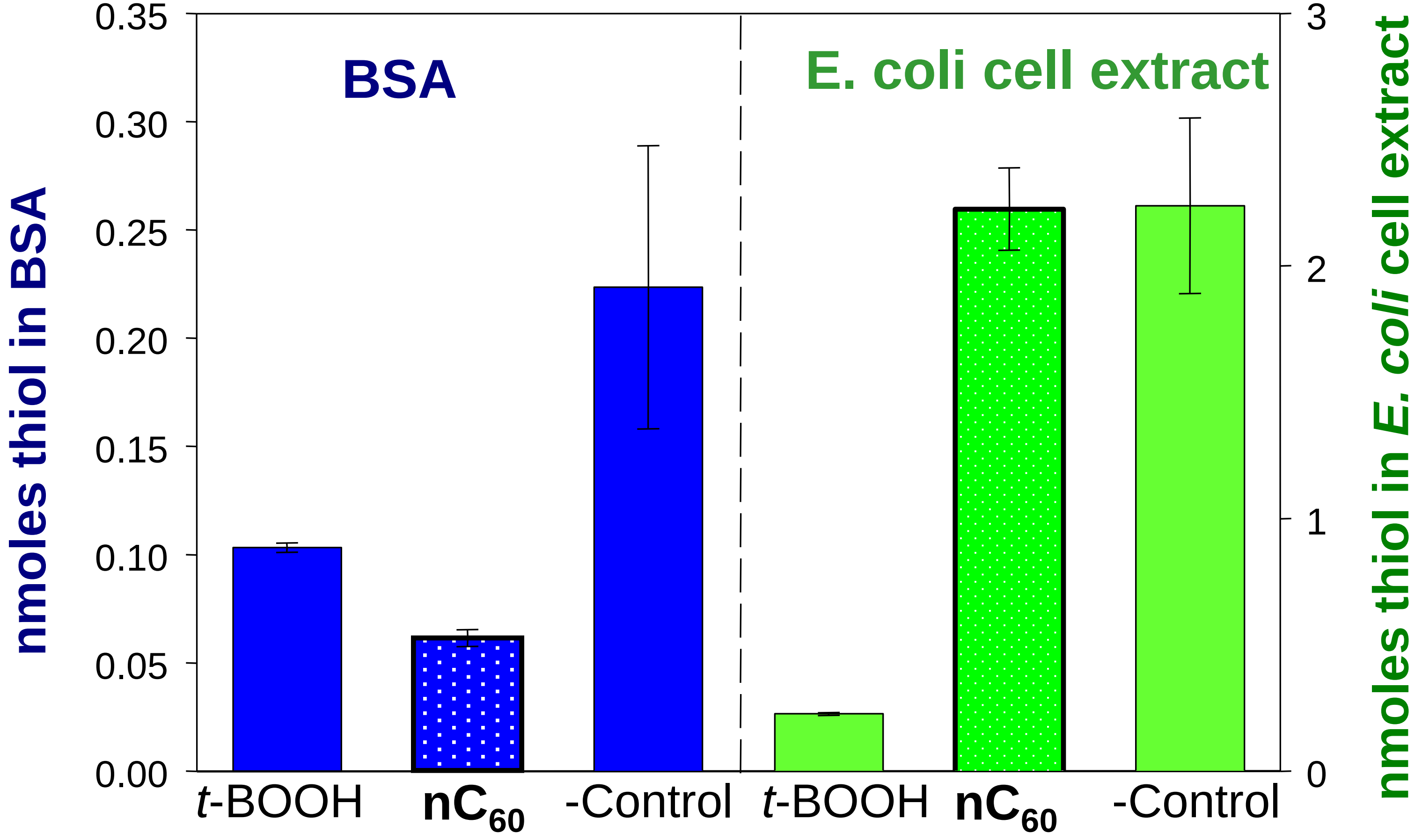




*Less toxic but more mobile*



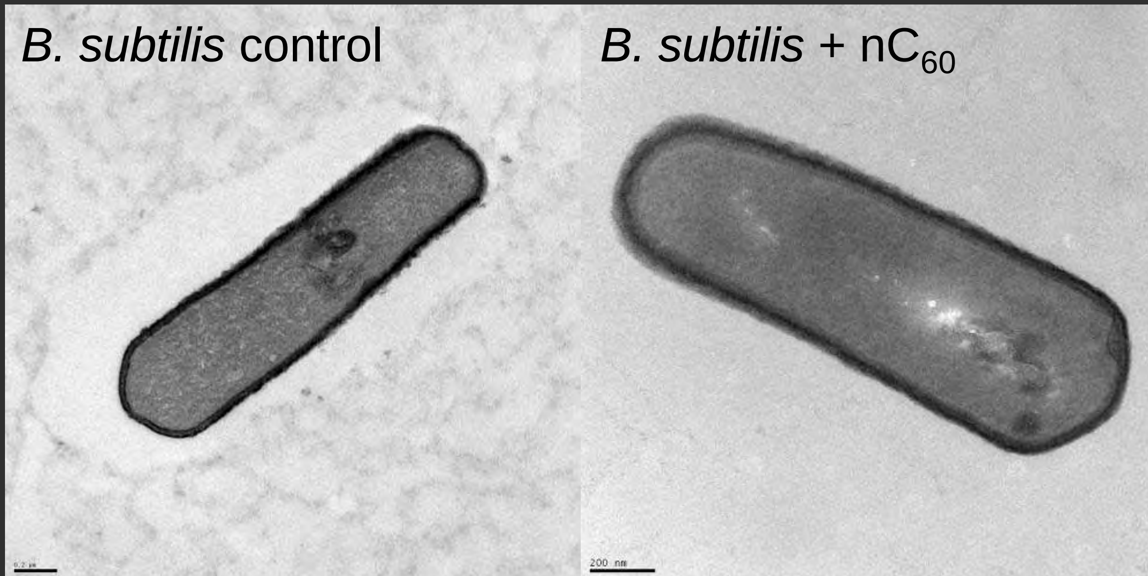

Sayes et al,. *NanoLetters* **2004**, *4*, 1881-1887

# Protein Oxidation

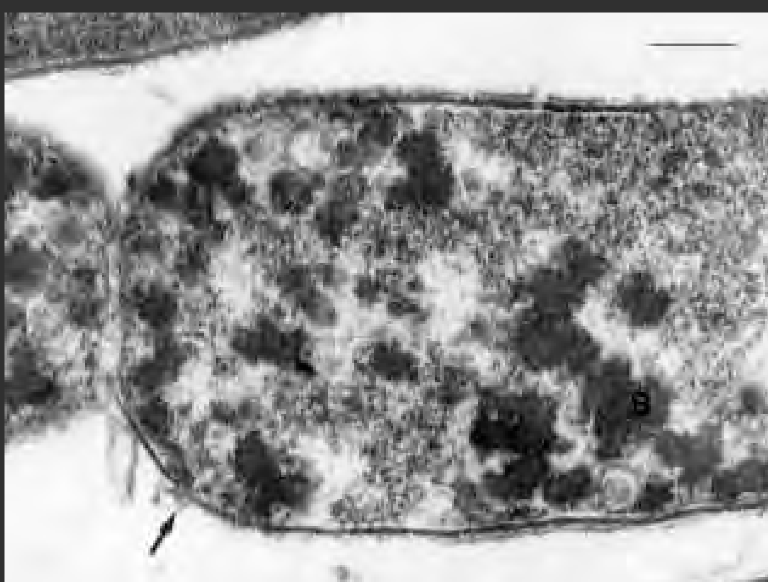



- Loss of thiol groups relative to control reflects oxidation
- *Tert*-butylhydroperoxide is positive (oxidizing) control
- No oxidation in cell extract (cytoplasmic proteins)
- Pure protein (bovine serum albumin) shows oxidation

# TEM shows no membrane holes

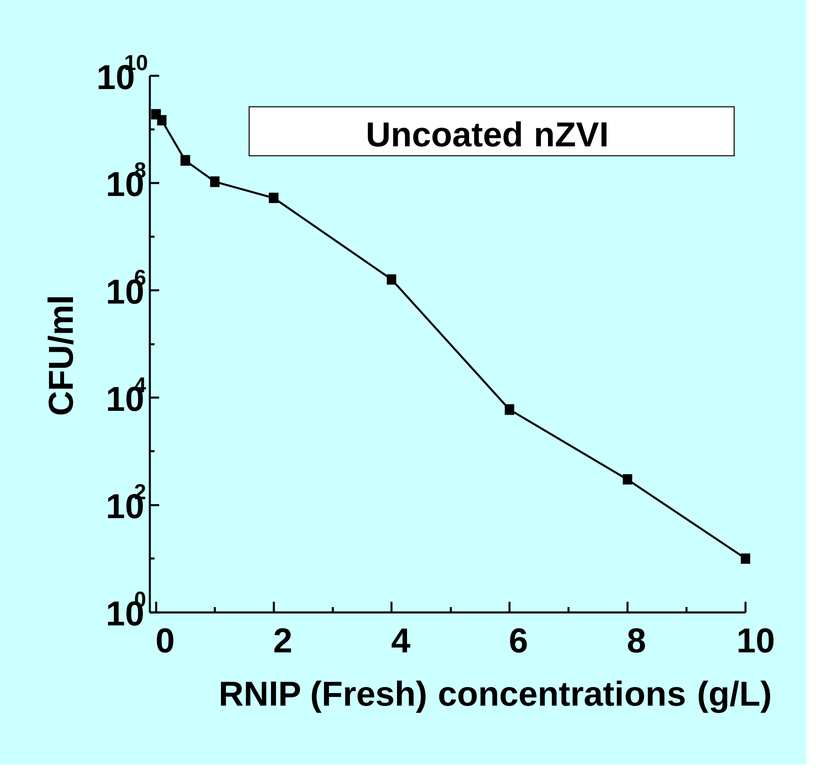


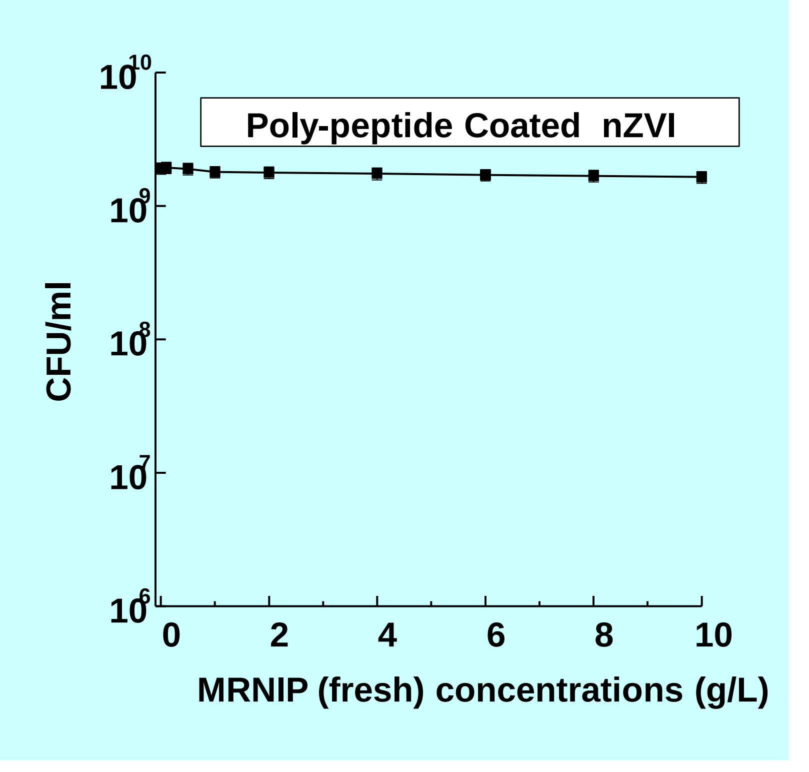

- Samples embedded in agarose and thinly sliced



*Bacillus stearothermophilus* cell treated with tamoxifen (10 µ /140 min). (from Luxo, C., et al. 2003, *Toxicol in Vitro* **17:** 623–628)

# Dose response of *E. coli* exposed to nZVI



# NMs to Improve Disinfection & Biofouling Control

Water-borne infectious diseases are the leading cause of death: 4 billion cases of diarrhea kill 2.2 million children/year

Biofouling = health hazard + corrosion + taste & odor + energy (head) loss

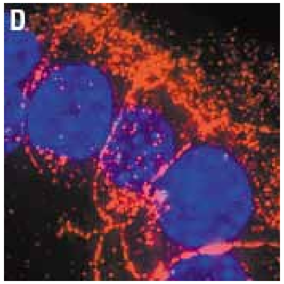
- Existing disinfectants ($Cl_2$, $O_3$) have serious shortcomings (carcinogenic DBPs, fast decay, cannot eradicate biofilms)
- Nanomaterials with large surface to volume ratio, enhanced reactivity, and multifunctionality offer great opportunities for innovation in microbial control

RICE

# Quantum Dots

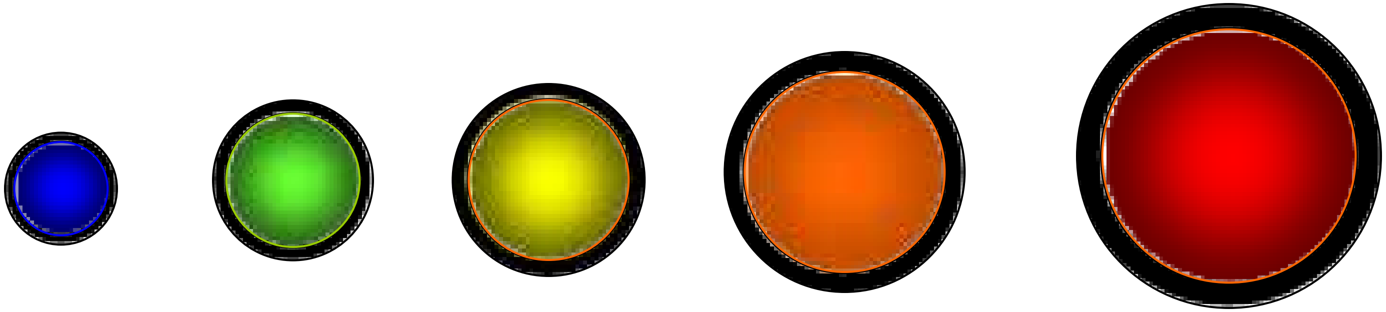
- Fluorescent nanoparticles containing heavy metals
- Surface coatings increase solubility and bioavailability.

Biomedical Applications:
*in-vivo* imaging, immunoassays, targeted gene and drug delivery




Wu et al., 2003

Electronics: solar cells, flat panel LED displays, solid state lighting



# Research Objectives

1. Characterize Weathering

   To describe the kinetics of heavy metal release from quantum dots of variable composition and surface coating under realistic environmental conditions

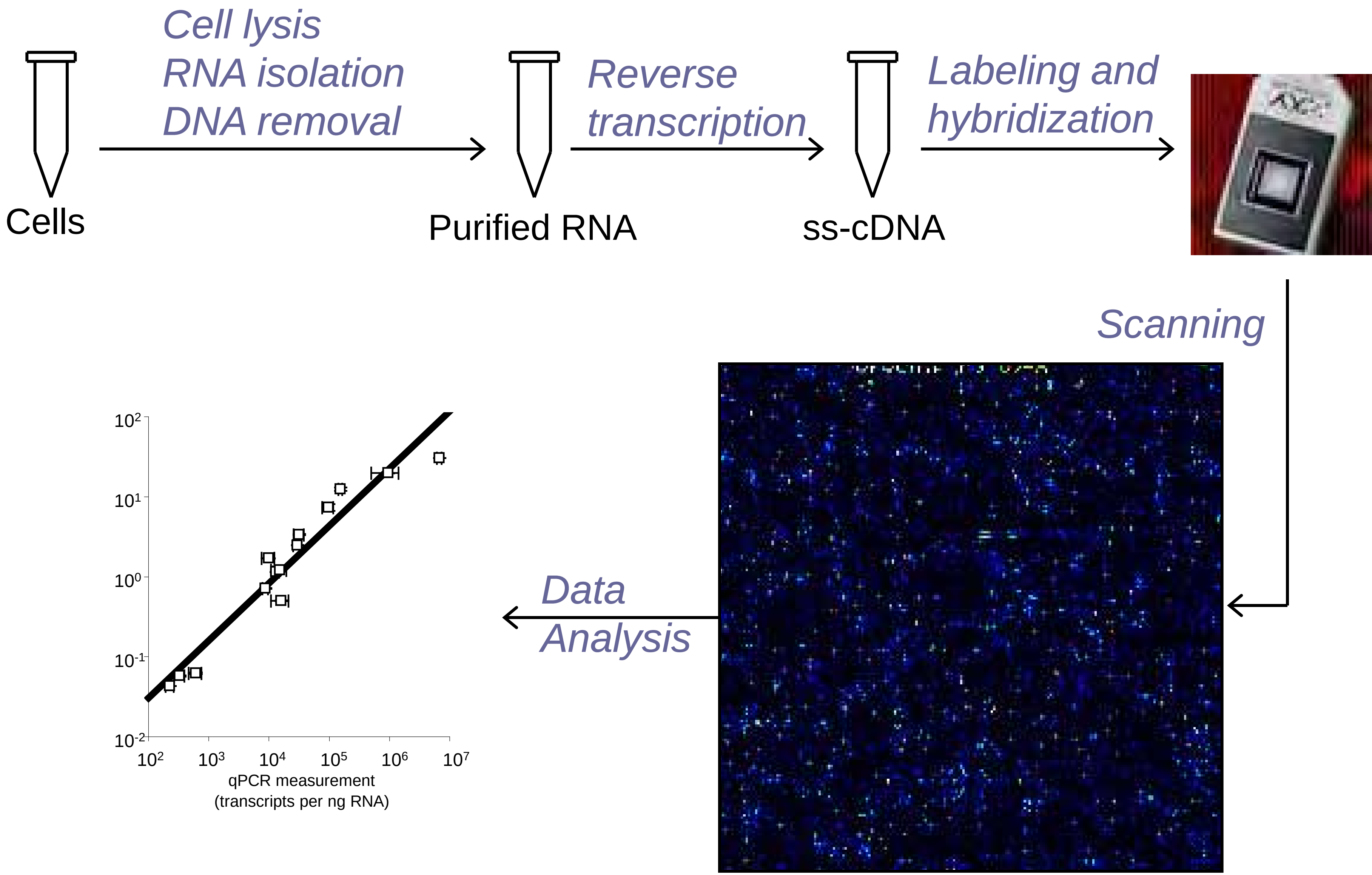
2. Interaction with Cells

   To establish the concept of 'equivalent dose' for quantum dots by exposing laboratory bacteria to quantum dots and evaluating their effects relative to the better characterized heavy metal salts.

3. Impact on Microbial Communities

   To describe the effects of quantum dots and their weathering products microbial community composition and function, specifically, antibiotic resistance, and global biogeochemical cycles.

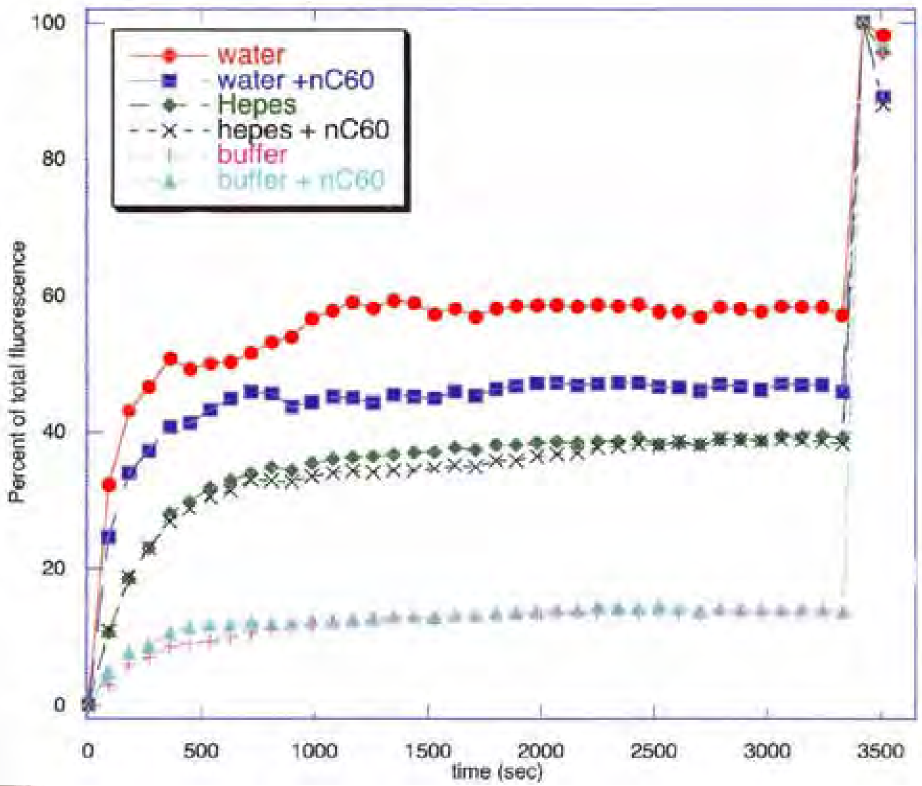
# Future Work

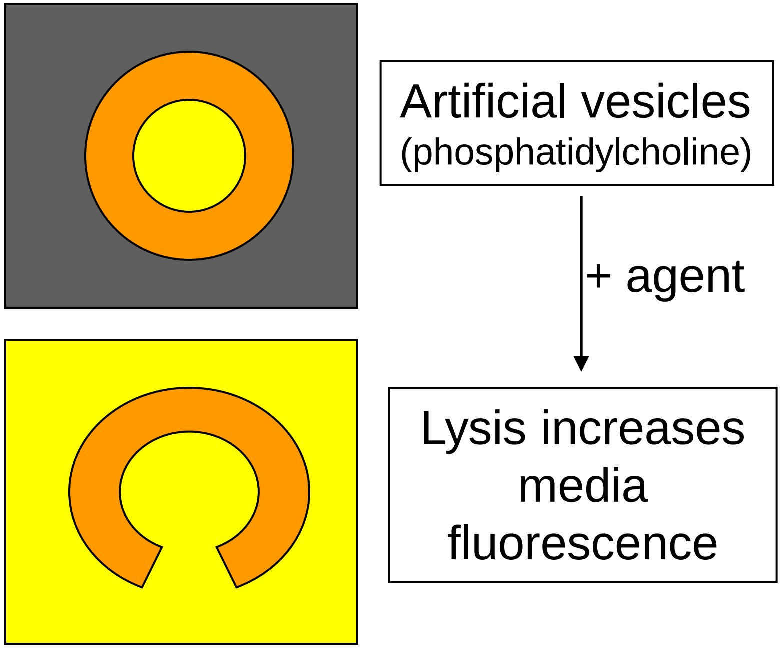


Cell lysis
RNA isolation
DNA removal
Cells
Reverse
transcription
Purified RNA
Labeling and
hybridization
ss-cDNA
Scanning
Data
Analysis
$10^2$
$10^1$
$10^0$
$10^{-1}$
$10^{-2}$
$10^2$ $10^3$ $10^4$ $10^5$ $10^6$ $10^7$
qPCR measurement
(transcripts per ng RNA)

# 1. Does $nC_{60}$ Punctures Membranes?

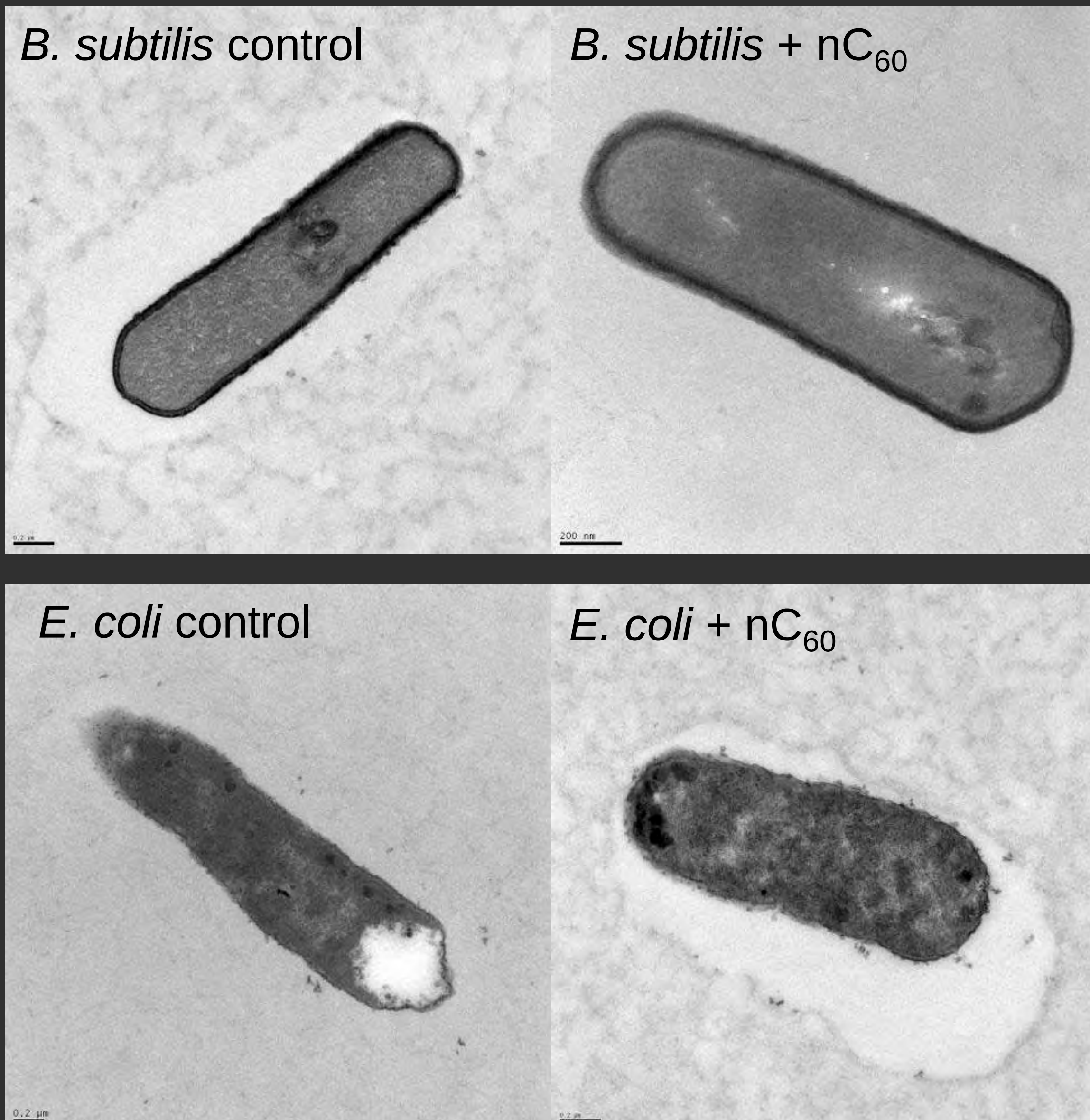
- Fluorescent liposome assay (phosphatidyl choline) for *rapid physical disruption*



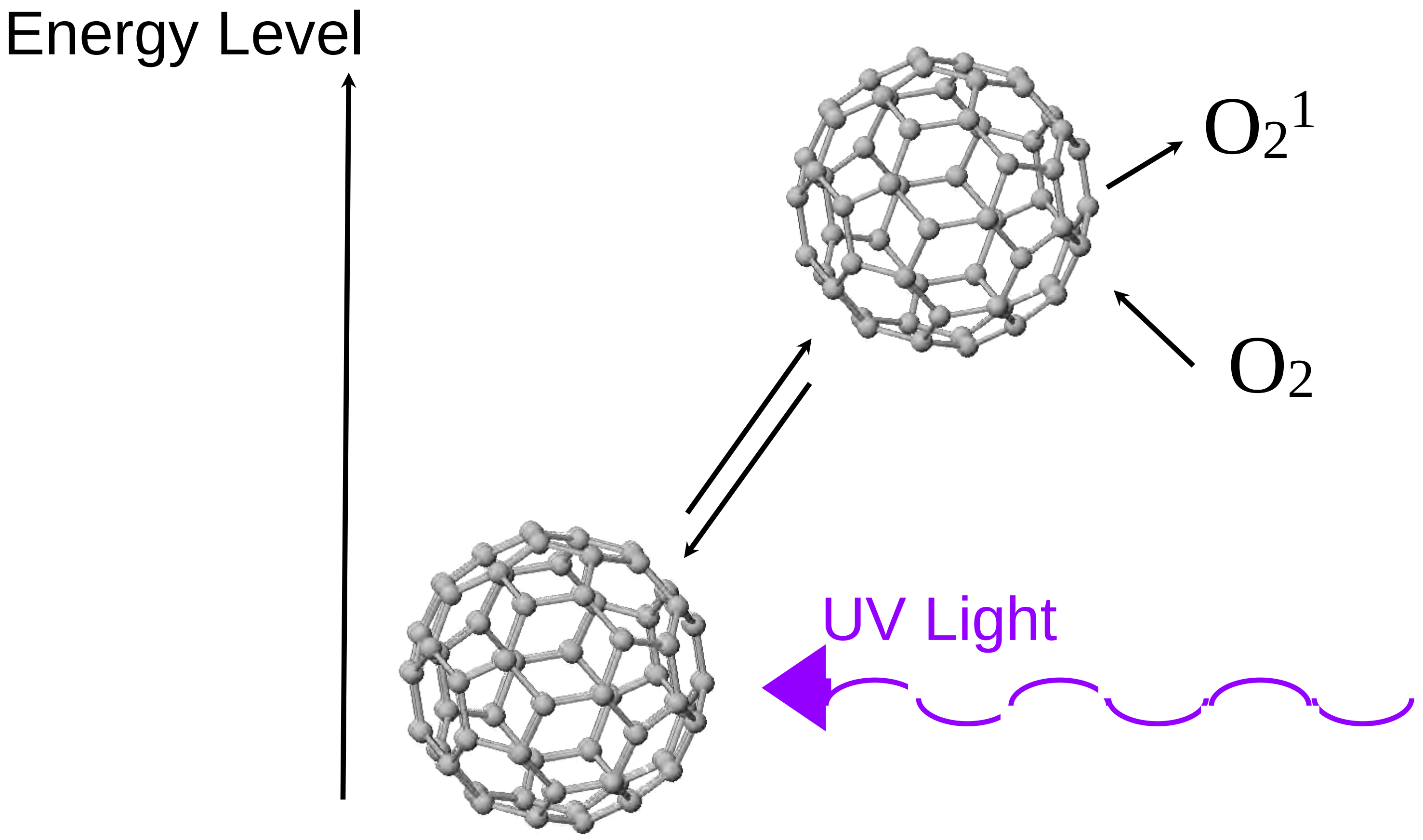




**Vesicles + $nC_{60}$ = no lysis**

# TEM shows no membrane holes



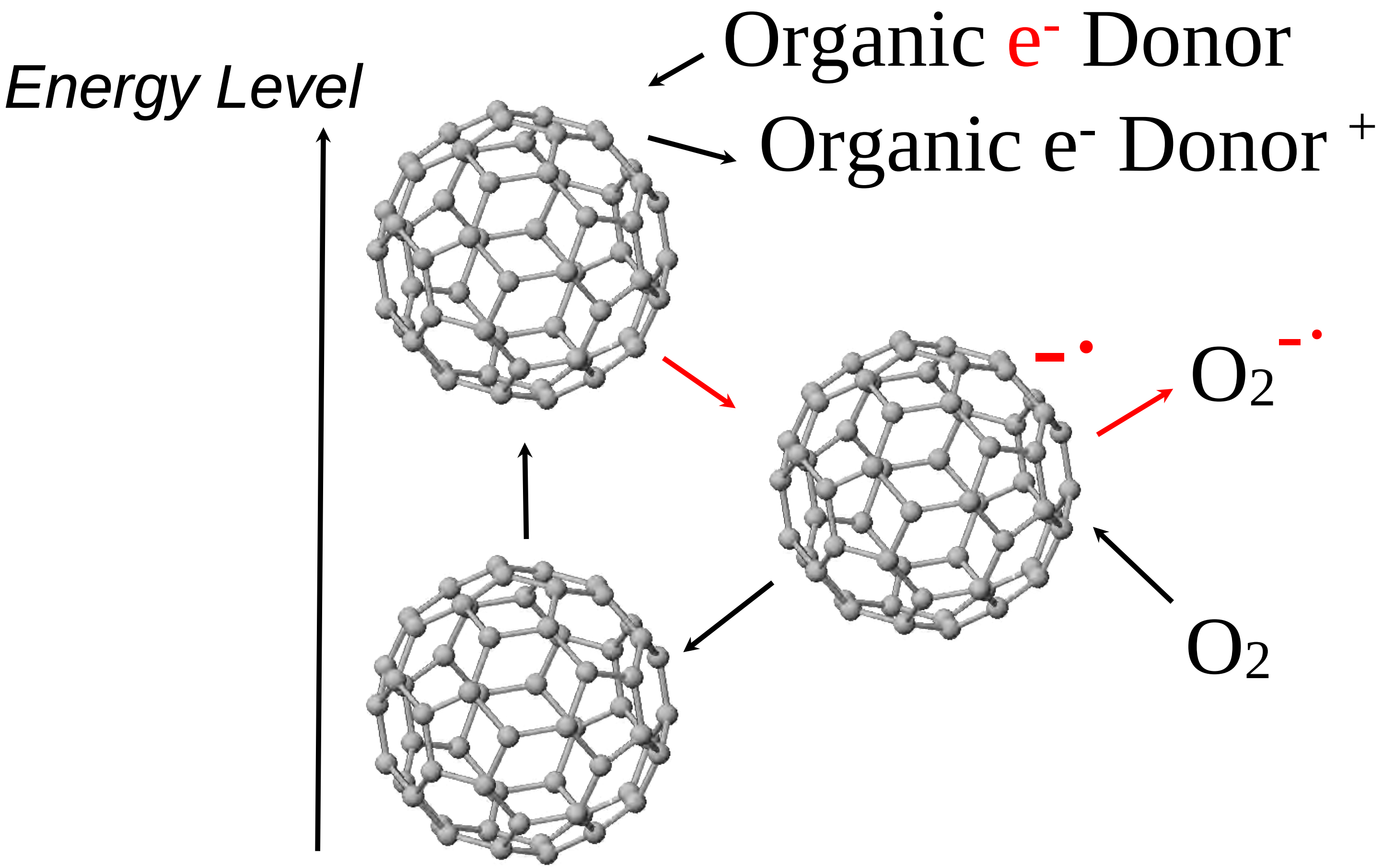

- Samples embedded in agarose and thinly sliced
- Could not differentiate $nC_{60}$ particles from background




# Type II Photosensitization :

deactivation without reaction

Produces singlet oxygen




# Hypothetical Type I mechanism:

electron transfer

Produces superoxide

# Further biofilm-inhibition tests

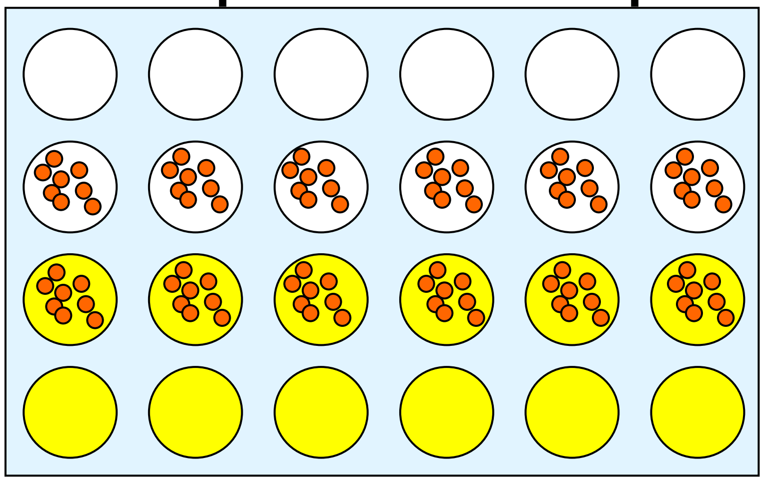
**Experimental set-up**

No $nC_{60}$, no bacteria

No $nC_{60}$, w/bacteria

$nC_{60}$, w/bacteria

$nC_{60}$, no bacteria



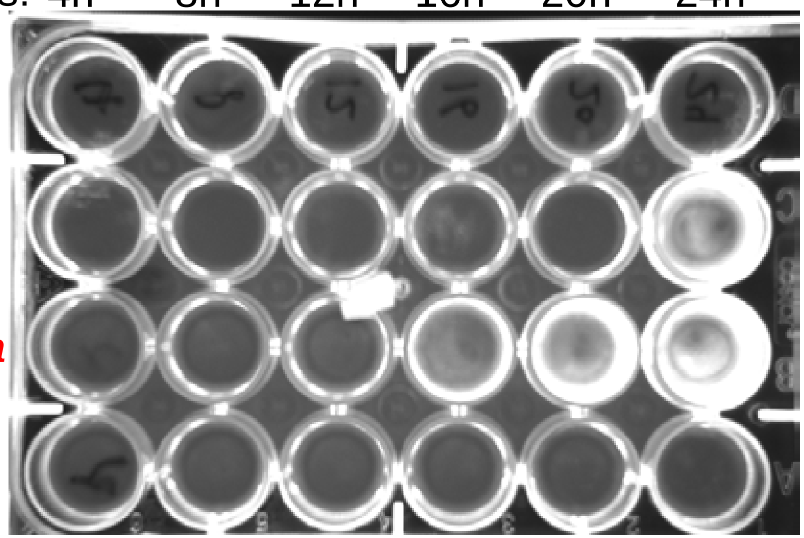
Incubation Times: 4h 8h 12h 16h 20h 24h

No $nC_{60}$, no bacteria

No $nC_{60}$, w/bacteria

***$nC_{60}$, w/bacteria***

$nC_{60}$, no bacteria

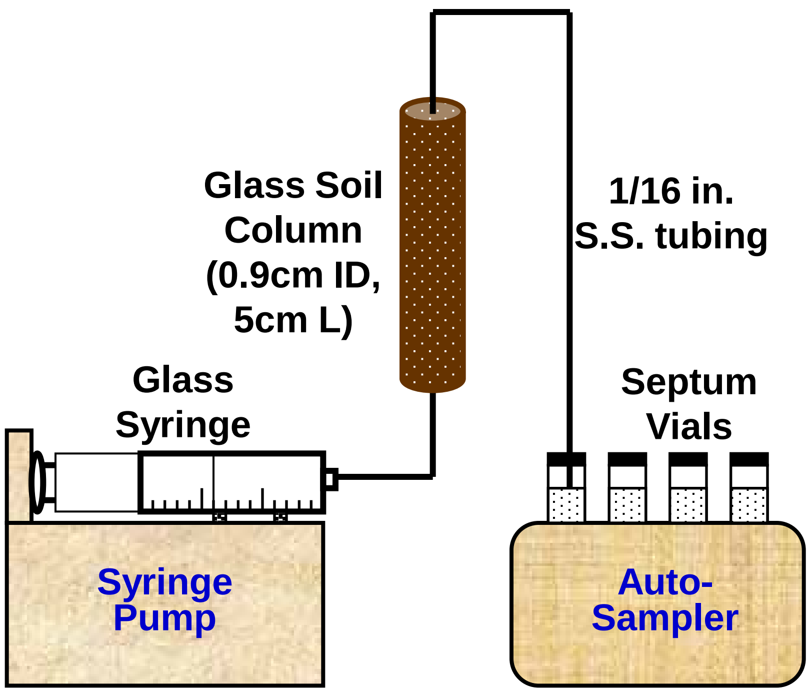


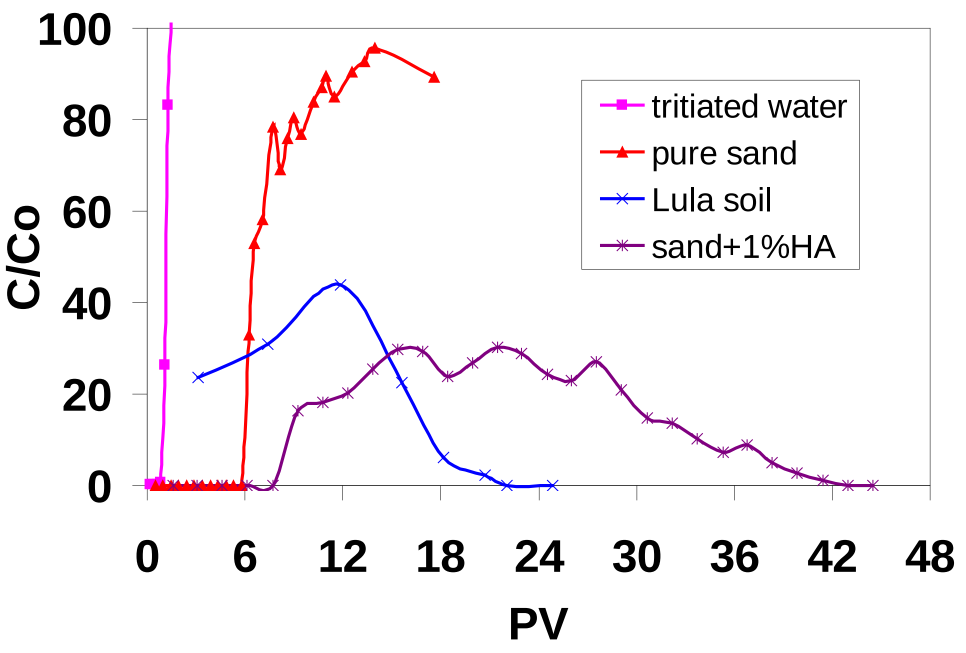
Grow *Pseudomonas mendocina* biofilm on plastic surface. Use ETBR fluorescence to assess biofilm formation

**$nC_{60}$ did not retard biofilm formation; it may have even encouraged it.**

RICE

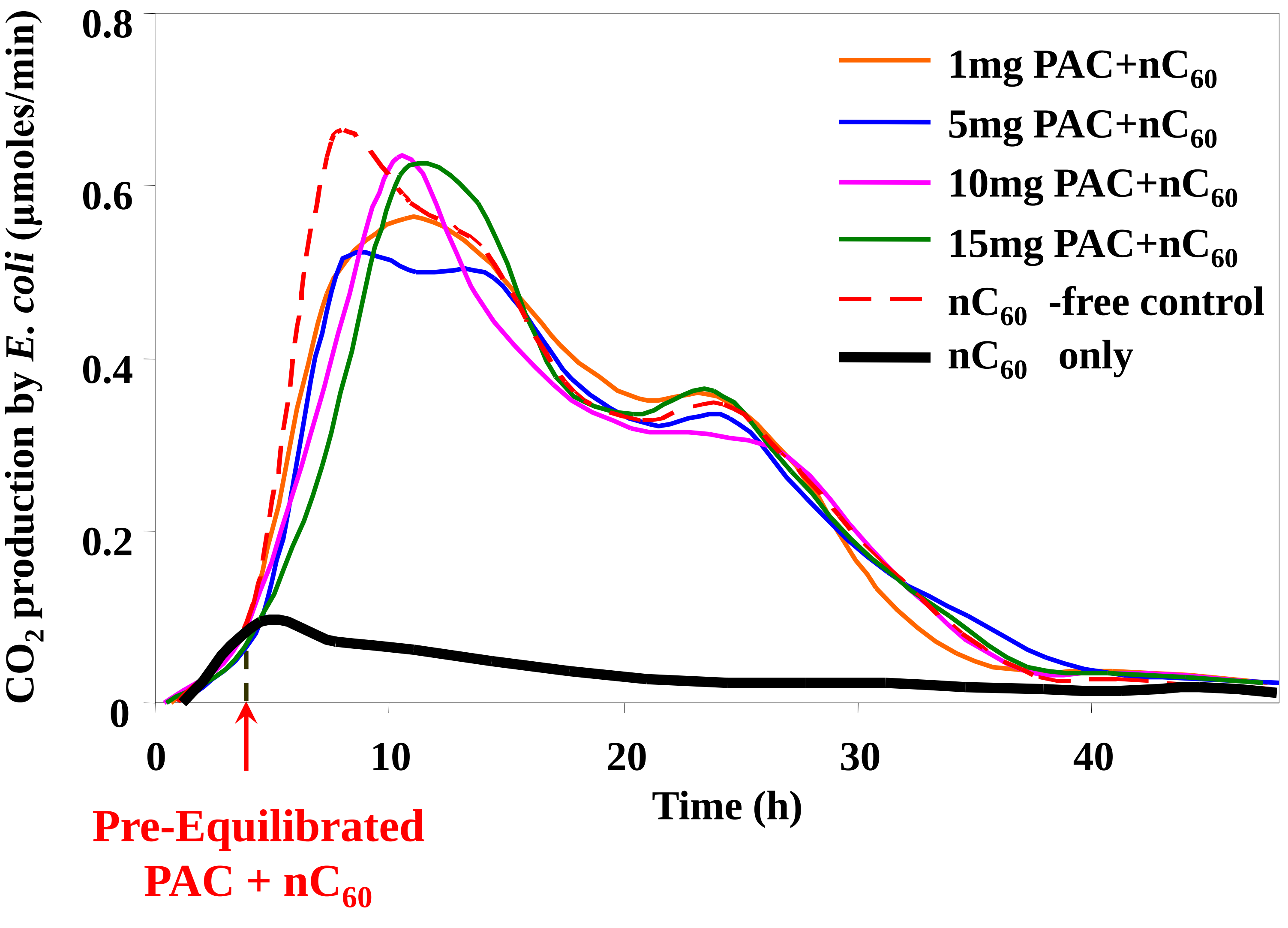
# Filtration of $nC_{60}$

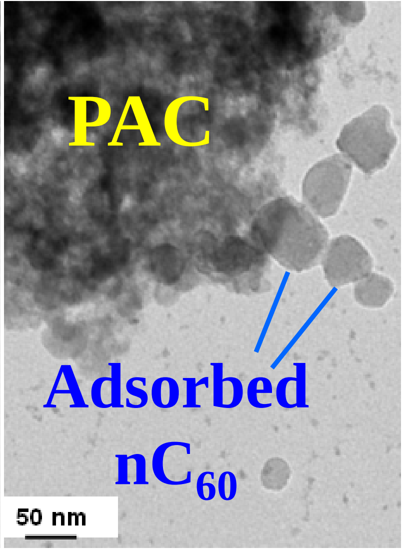


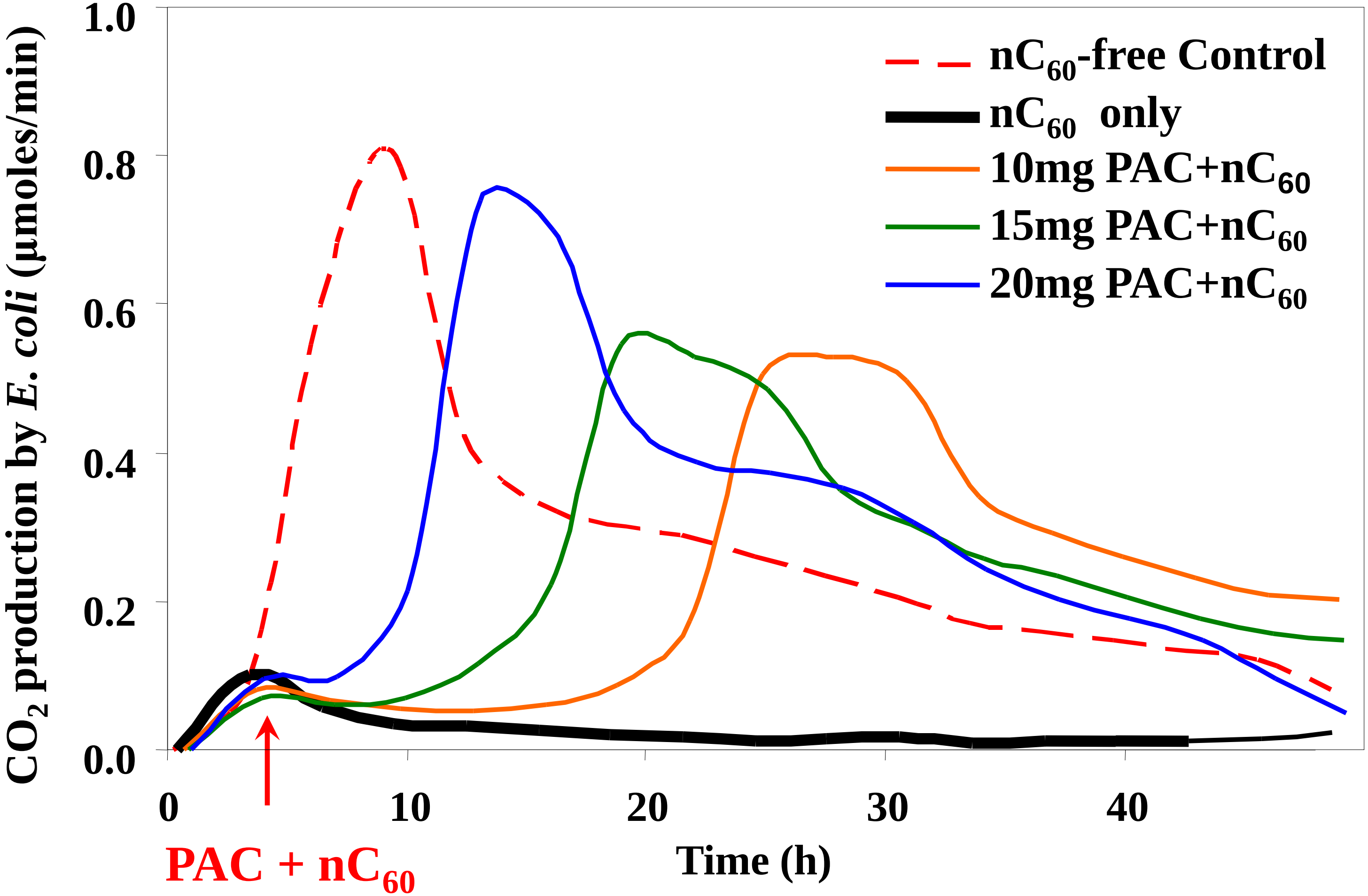

**Transport of $nC_{60}$ in soil shows limited but highly unusual mobility (filter ripening?)**

# Sorption kinetics effect not observed when PAC and $nC_{60}$ were equilibrated for 2 d prior to exposure






# Sorption of $nC_{60}$ onto PAC reduced its bioavailability and toxicity when added concurrently at the time of exposure (more PAC added = more attenuation).



# Environmental Nanotechnology Vision

Use nanotechnology research to:

...Help clean up past environmental damage

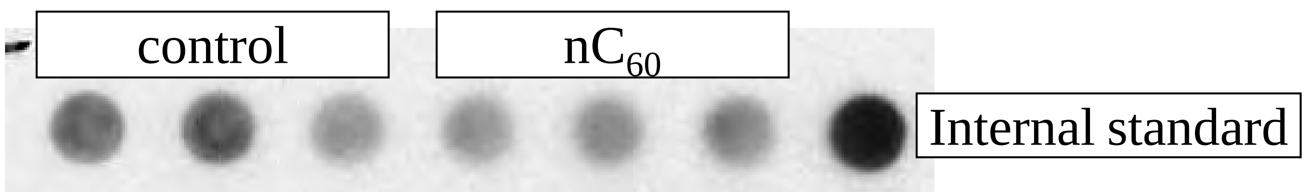
...Correct present environmental problems

...Prevent future environmental impacts (no surprises)

...Help sustain the planet for future generations

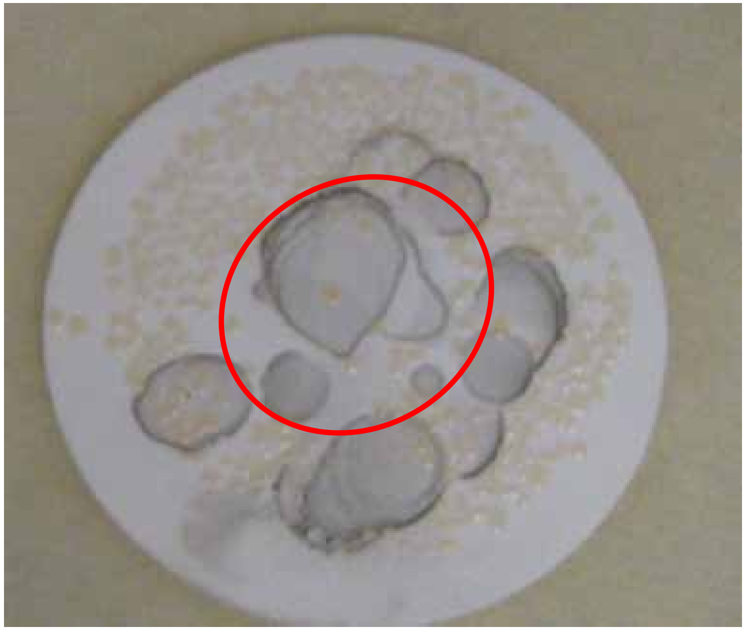
# No Evidence of Oxidative Damage of Cytoplasmic Proteins

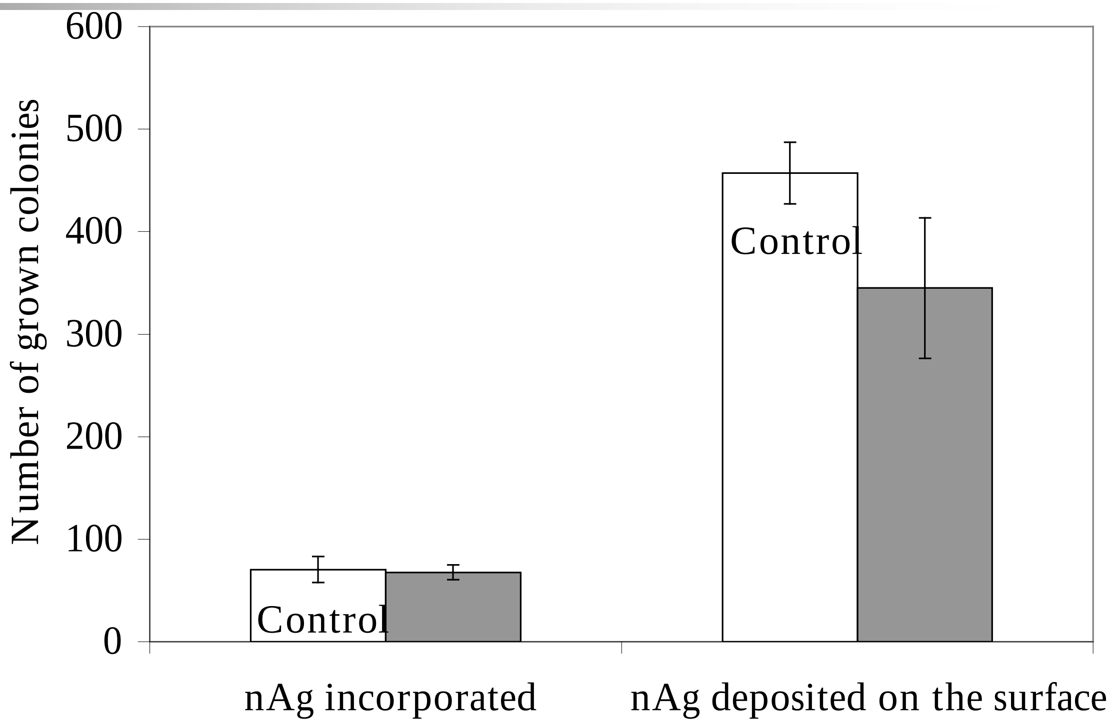
- An immunoassay was used to detect carbonyl groups (evidence of oxidative damage) in cytoplasmic proteins.
- $nC_{60}$ did not cause oxidative damage as compared to the control.




| | control | $nC_{60}$ |
|---|---|---|
| Density of blot | 1925 400 | 1319 92 |

# Nano-silver reduces membrane biofouling

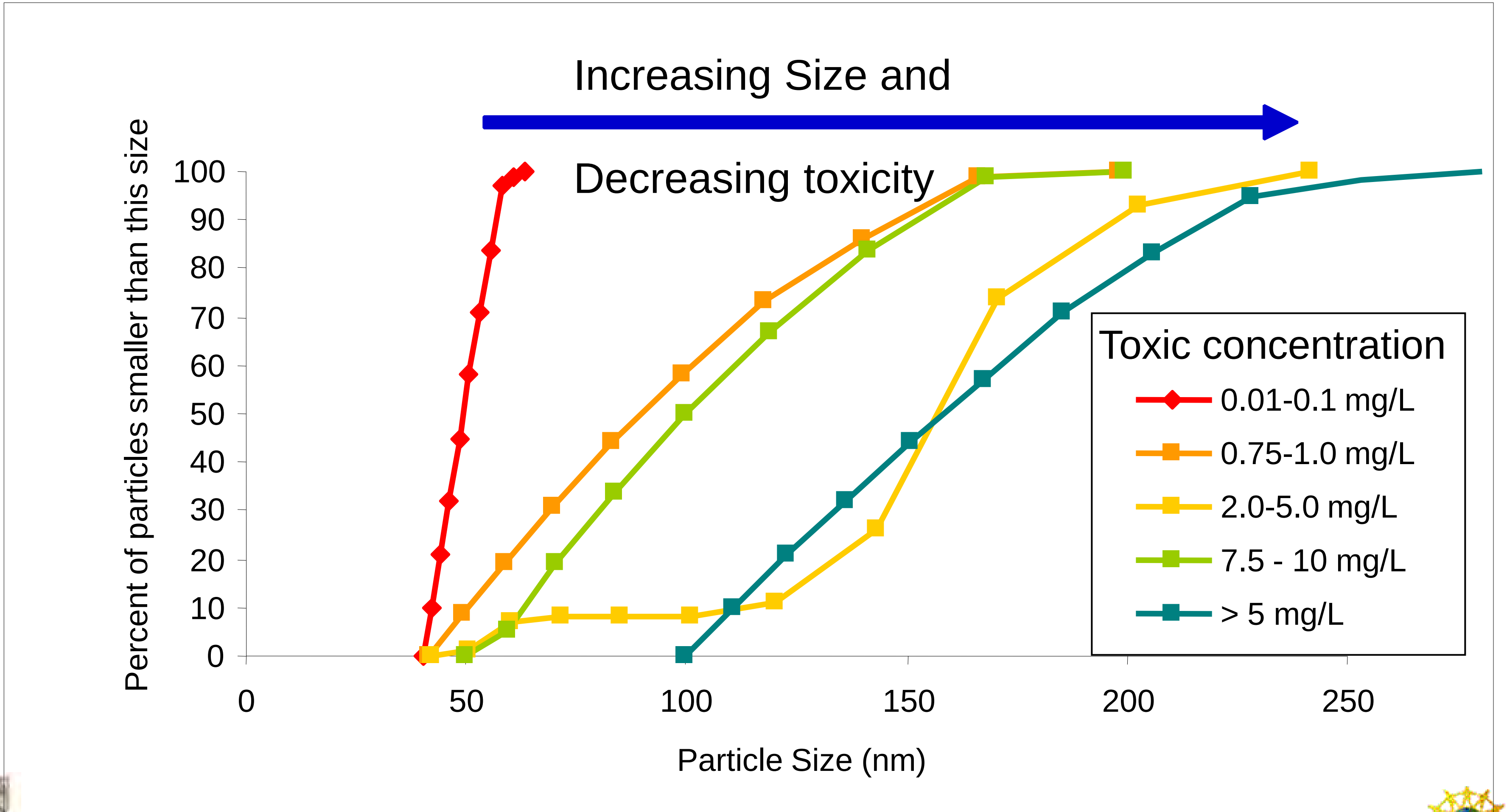





- Membranes with nAg incorporated display almost no antimicrobial properties as nAg is not bioavailable
- When nAg is directly deposited on the surface of the membrane, patches of no growth can be observed
- Polymer coated with nAg (nAg bioavaiable but anchored) could reduce biofouling

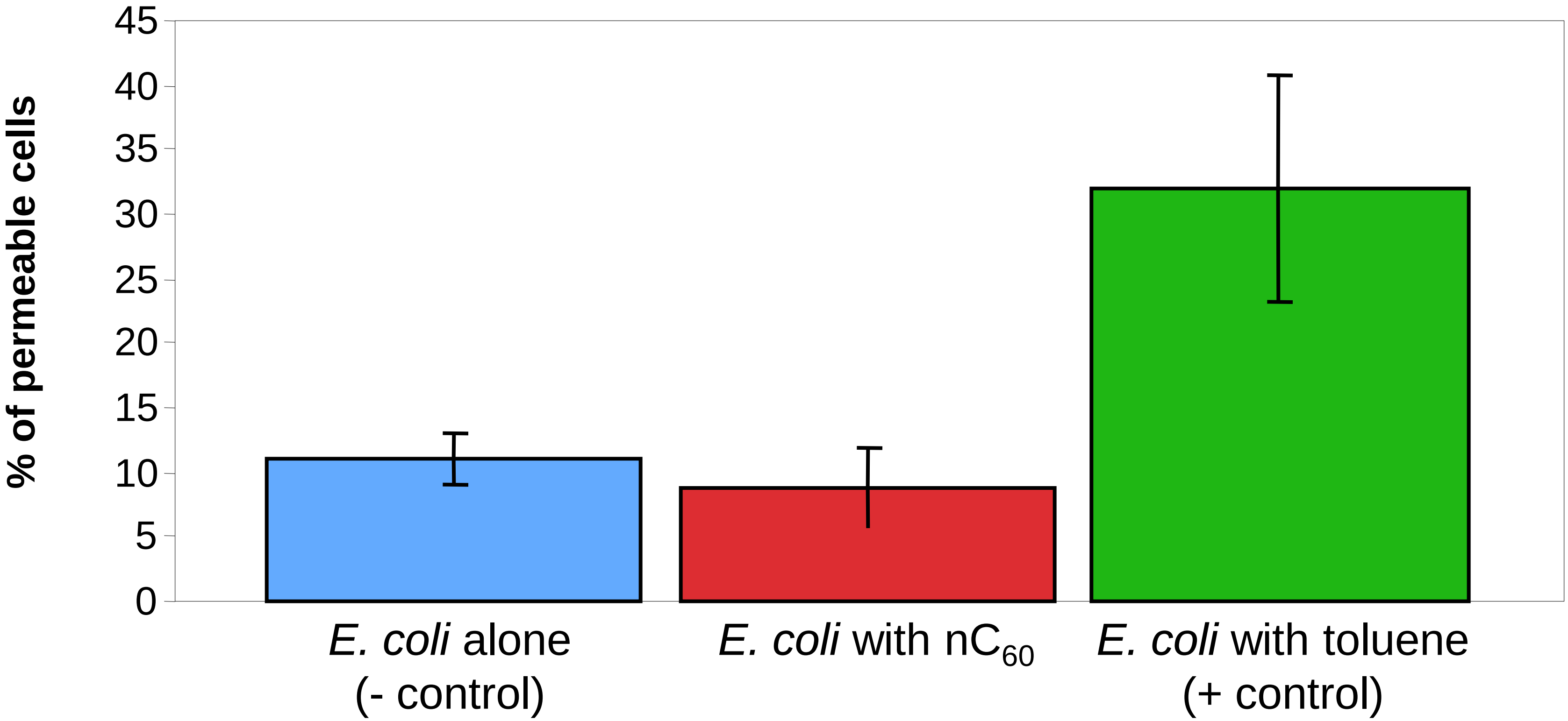
RICE

# THF/$nC_{60}$ Particle Size Affects Toxicity




RICE

# *E. coli* permeability using flow cytometry




- Dye (propidium iodide) uptake indicates permeability (the dye fluoresces when bound to the DNA within the cell)
- Toluene makes cells permeable, but $nC_{60}$ does not

# Anti-fouling membranes: summary & future work

**nAg and nC60 are antibacterial when inserted in polymeric membranes in sufficient quantities**

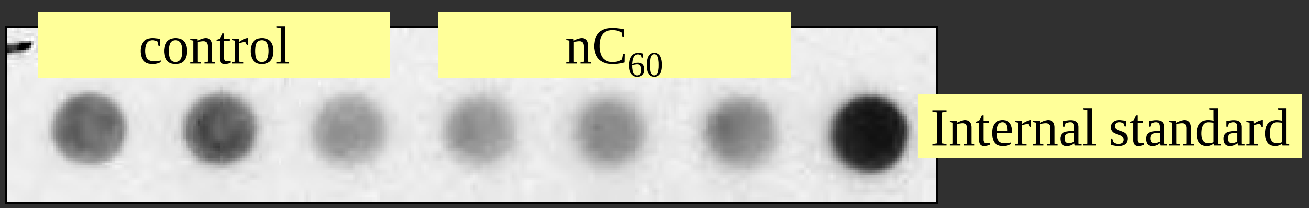
**But,** need to improve incorporation protocol:
- To concentrate NPs in the (top) selective layer
- To anchor NPs and avoid leakage

**Ongoing experiments :**
- Assess proliferation of cells after adhesion
- Assess anti-fouling effect in dead-end filtration cell (decreasing flux assay)

RICE

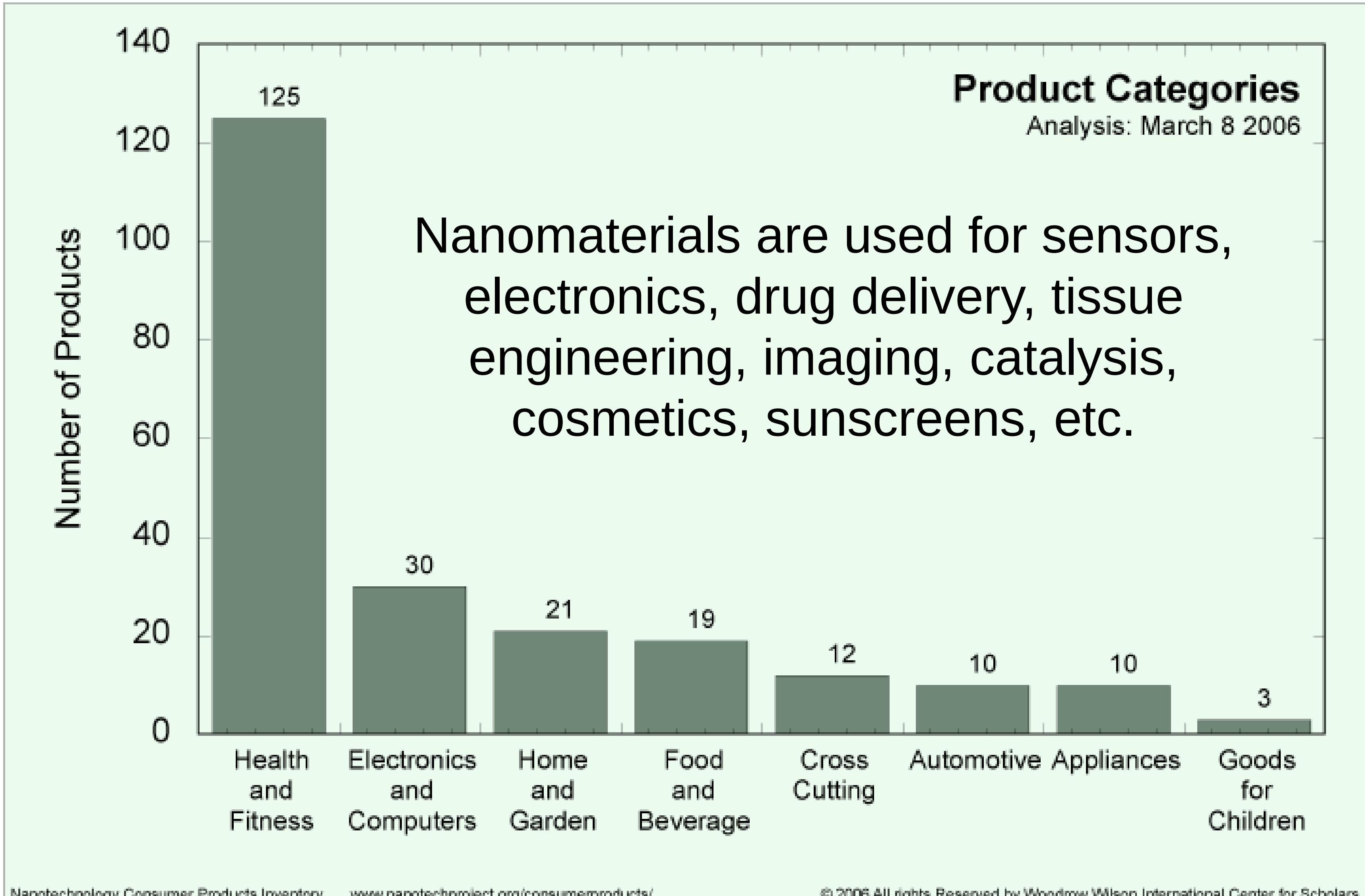
# No Evidence of Oxidative Damage of Cytoplasmic Proteins

- An immunoassay was used to detect carbonyl groups (evidence of ROS damage) in cytoplasmic proteins.
- $nC_{60}$ did not cause oxidative damage as compared to the control.

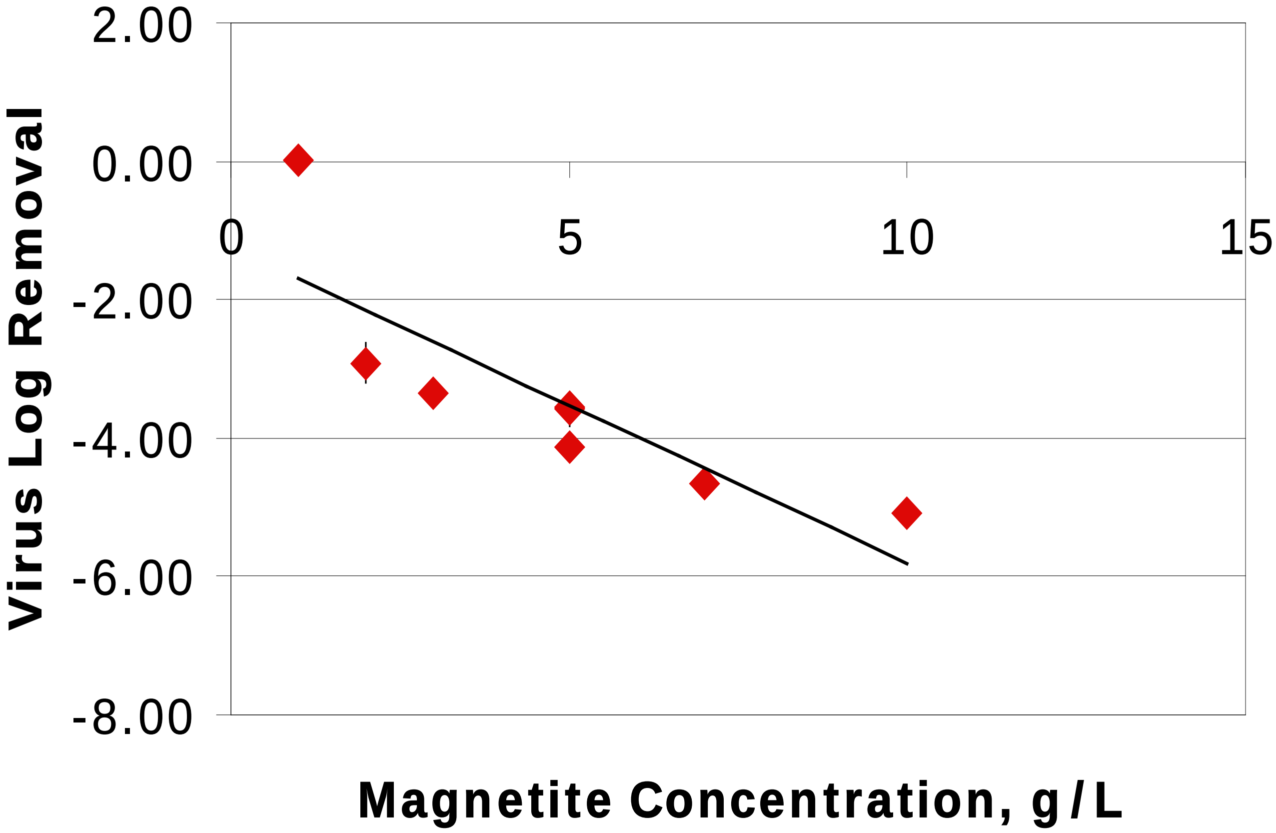


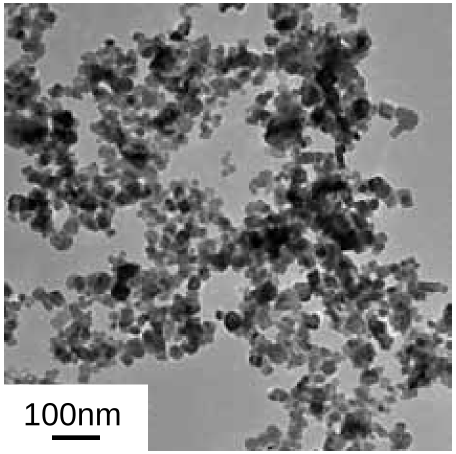

**No conclusive evidence of ROS production or ROS-mediated damage**

# > 475 Products Use of Nanomaterials

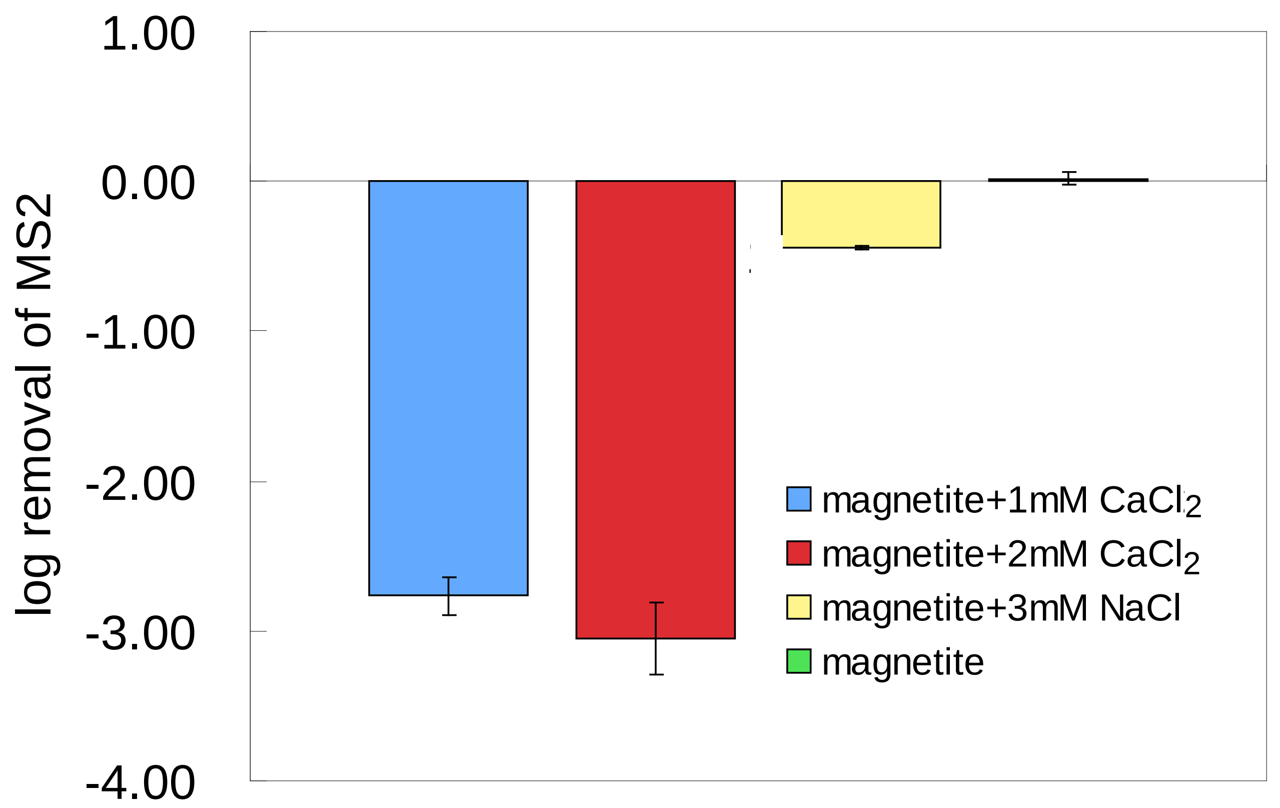


# Viral removal by magnetite nanoparitcles at different concentrations after 1 hour of exposure.



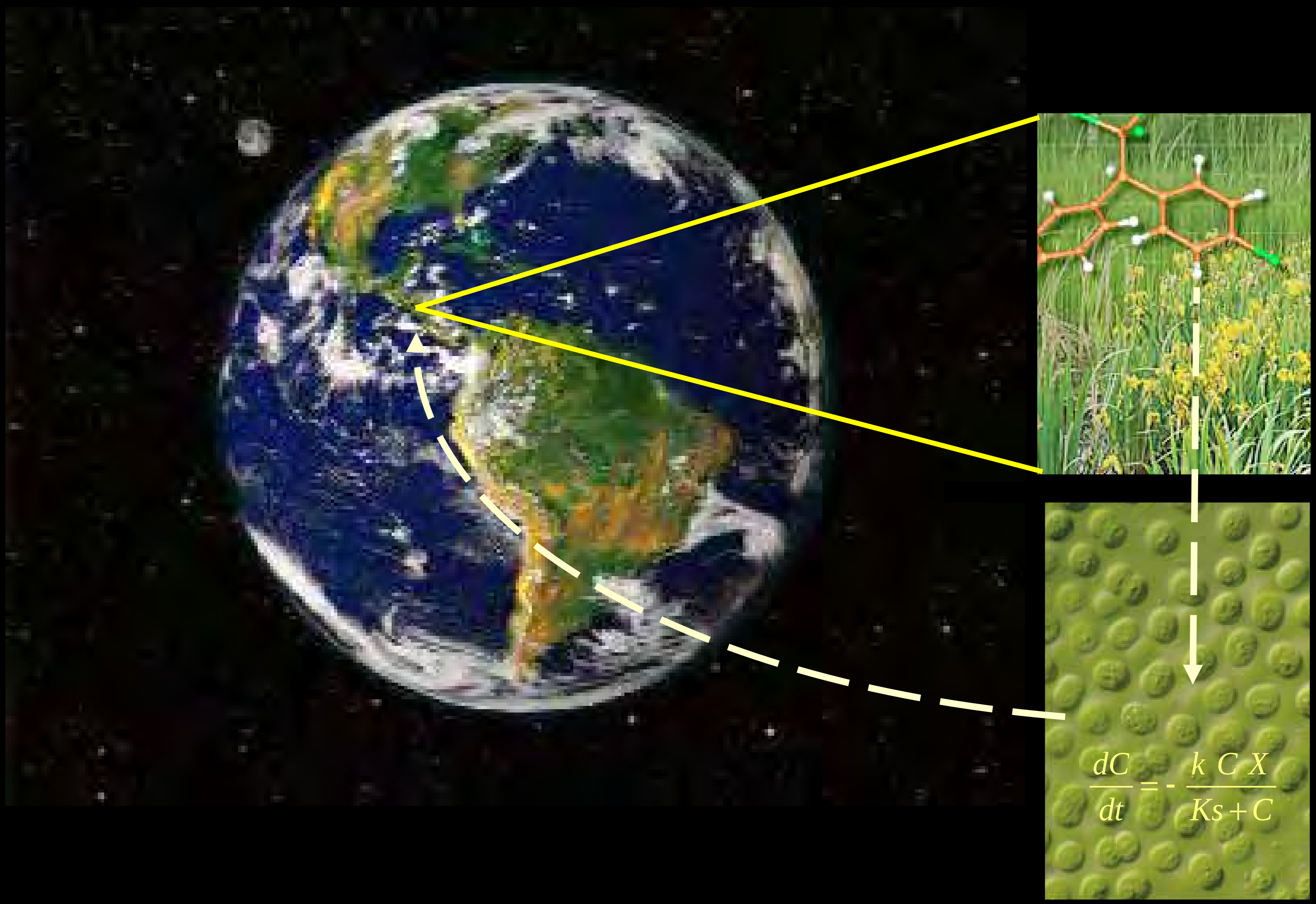

**TEM image of magnetite nanoparticles.**




Effect of $Ca^{2+}$ and $Na^{+}$ on the adsorption of MS2 by magnetite (1 g/L, 1 hour contact time).

# Environmental Biotechnology and Bioremediation

Harnessing natural biogeochemical cycles for sustainability



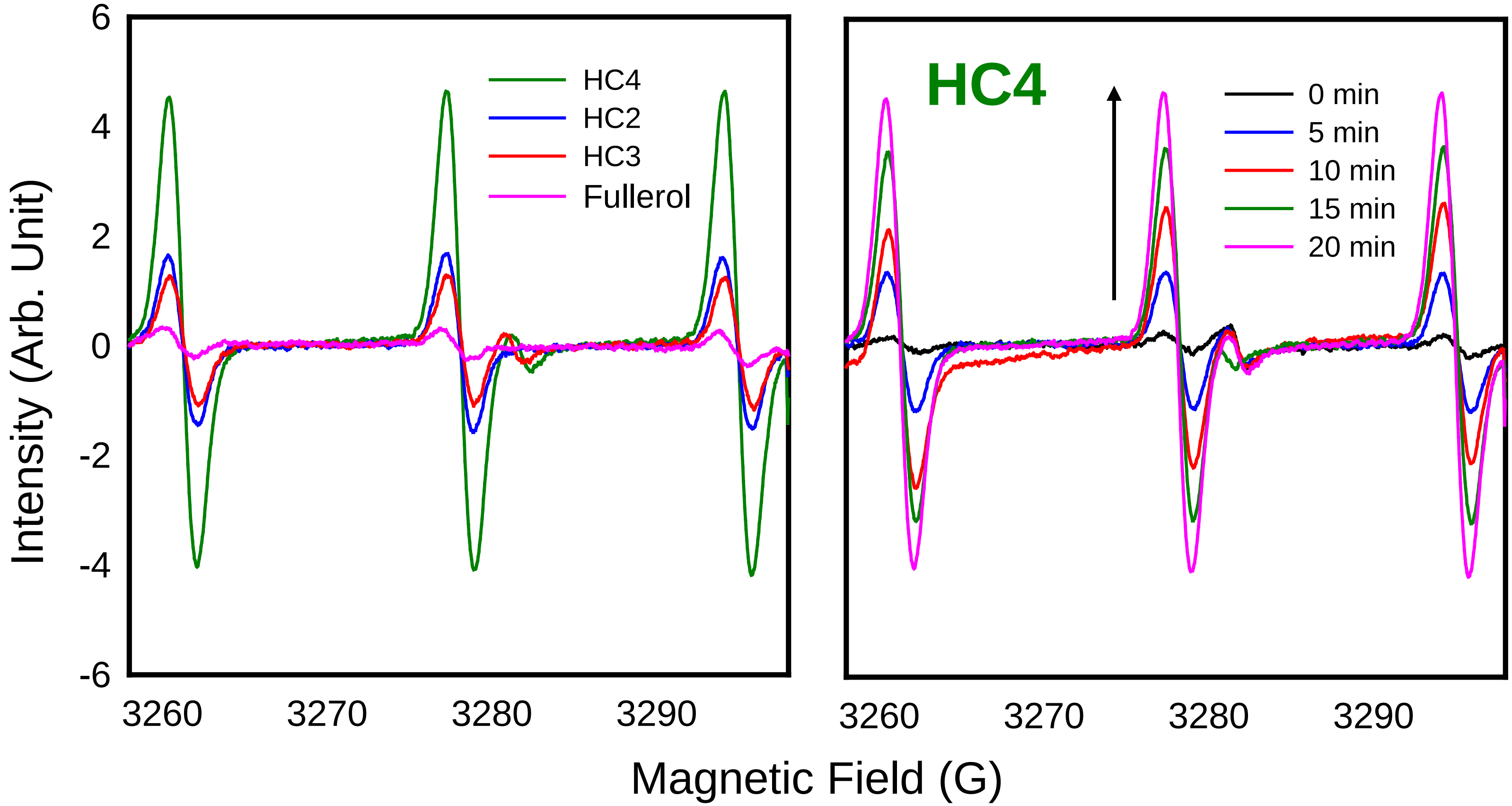
# Selected Research Areas

- Water footprint of biofuels
- Contaminant hydrogeology: modeling substrate interactions BTEX-ethanol (plume dynamics);
- Phytoremediation and rhizoremediation;
- Biogeochemical interactions in permeable iron barriers;
- Fate and transport of antibiotic resistance vectors and amplification and attenuation of resistance reservoirs
- Biomarkers to support of bioremediation forensics and natural attenuation performance assessment.
- Bioaugmentation to degrade recalcitrant pollutants (TCE, DX)
- Medical bioremediation of age-related diseases

Ø Environmental applications & implications of nanotechnology

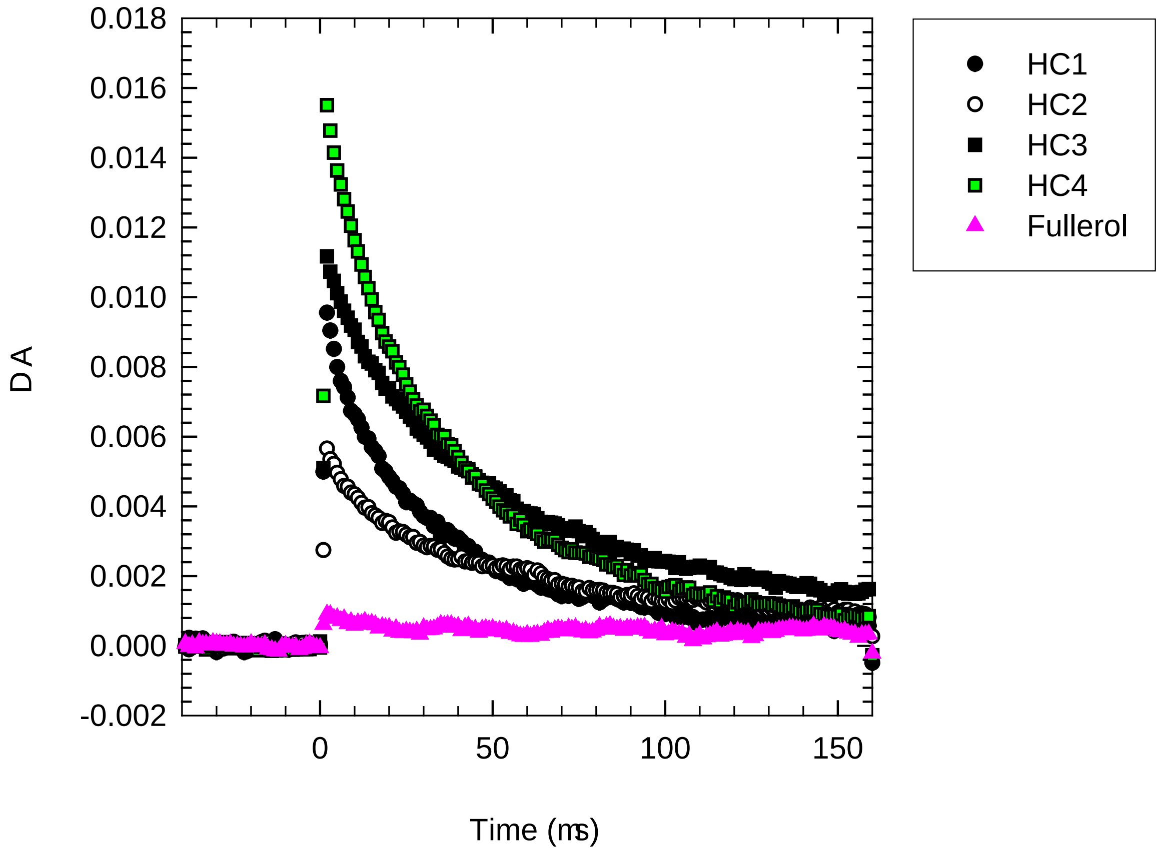
# Photosensitized Singlet Oxygen Production

**Larger Electron Paramagnetic Resonance (EPR) Spectra Peaks Correspond to Higher $^{1}O_2$ Generation (All Outperform Fullerol)**




J.Lee, Y.Mackeyev, M.Cho, D.Li, J.-H. Kim, L.J. Wilson, and P.J.J. Alvarez, Submitted.

# Nanosecond Laser Flash Photolysis (LFP) confirms long-lived triplet state, conducive to efficient ROS production (**HC4** > HC3 > HC1 > HC2 > fullerol)




J.Lee, Y.Mackeyev, M.Cho, D.Li, J.-H. Kim, L.J. Wilson, and P.J.J. Alvarez, Submitted.