Understanding nanoparticle mutagenicity:

Is the Ames test a suitable technique to provide insight into the relative unknown?

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The bacterial-based 'Ames test' is an easily reproducible test for xenobiotic mutagenicity and enables large sample screening quickly [1]. These benefits, compared to the alternative genotoxic testing strategies have led to the Ames test being proposed as a good technique for the screening of nano-object (NO) mutagenicity. Increased debate surrounds the suitability of the Ames test however, as a realistic method for determining NO mutagenicity. A NO is defined as a material with one, two or three external dimensions in the nanoscale (1-100nm) [2]. It is suggested that NOs are unable to penetrate the rigid outer double-membrane of gram negative bacteria and that any mutagenic events observed are not nano-object-specific.

The aim of this study was to (i) assess the potential for the Ames test to predict NO-induced mutagenicity, and (ii) study the interaction of a series of well characterised and dispersed NOs (cerium dioxide (CeO_2)), diesel exhaust particles (DEPs), single and multi-walled carbon nanotubes (SWCNTs/MWCNTs), crocidolite asbestos fibres (CAFs) (all 0.005-0.04mg/mL) and in addition, gold-nanoparticles (Au-NPs) (0.67-26nM)) with *Salmonella (S.) Typhimurium*.

Positive mutagenic effects (two-fold rule for Ames test) were only observed for DEPs. Investigation using transmission electron microscopy found no DEPs inside the *S. Typhimurium*. CAFs were observed to puncture the double lipid-layer membrane of, as well as be present inside *S. Typhimurium*. Interestingly, neither SWCNTs or MWCNTs showed any direct interaction with the *S. Typhimurium* but, similar to the CAFs, the SWCNTs were found to be attached to the double lipid bilayer membrane. The CeO_2 nano-objects however, supported via elemental transmission electron microscopy was identified inside bacteria following 2hrs exposure with no loss to bacterial viability after

48hrs. Au-NPs were also found inside the *S. Typhimurium* however were found to induce significant bacterial death after 48hrs exposure at 26nM (Fig. 1).

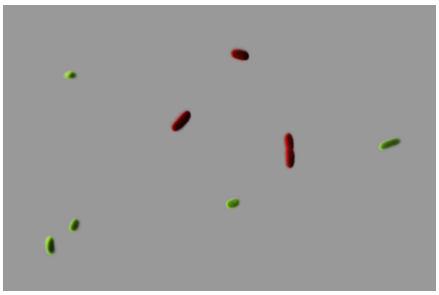

Fig. 1: Bacterial viability. Viability analysis was assessed after nano-object exposure qualitatively by laser scanning microscopy using a Live/Dead *Bac*Light kit (Invitrogen). Viable bacteria are shown in green, dead bacteria in red.

The results of the present study show positive mutagenic effects by DEPs. DEPs however, are unable to gain direct access to the nuclear material of bacteria, suggesting a secondary effect from the DEPs which might be induced by the soluble organic fractions attached to the diesel particulate. In contrast, CAFs, CeO_2 and Au-NPs as well as both SWCNTs and MWCNTs interact and penetrate the double lipid layer membrane of the bacterial wall. Therefore, the findings of the present study show that direct interaction of nano-objects with the nuclear material of bacteria does not correlate to an observed mutagenic effect. We suggest that the Ames test is a good indicator for soluble fractions on DEPs, but not for nano-object specific mutagenic effects.

[1] Ames BN, Lee FD, Durston WE. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc Natl Acad Sci U S A. 1973 Mar;70(3):782-6.

[2] International Organization for Standardization (ISO) Technical Specification (ISO/TS) 27687:2008; Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate; First published 2008-08-15.

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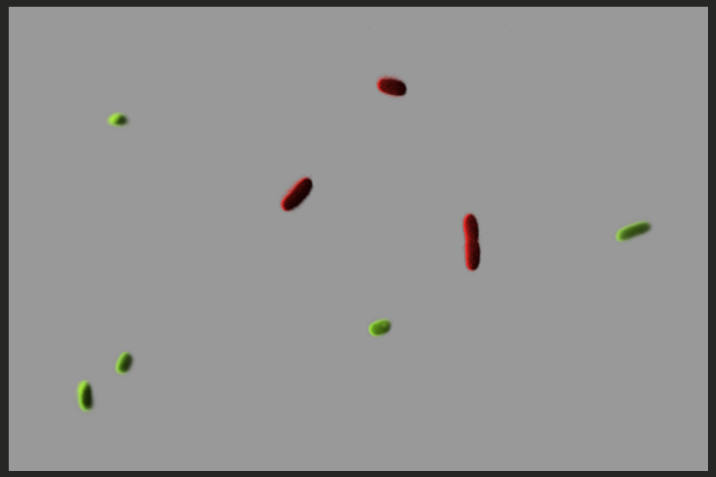
Investigating nanoparticle mutagenicity: Can nanoparticle-bacterial interactions provide an insight into the unknown?



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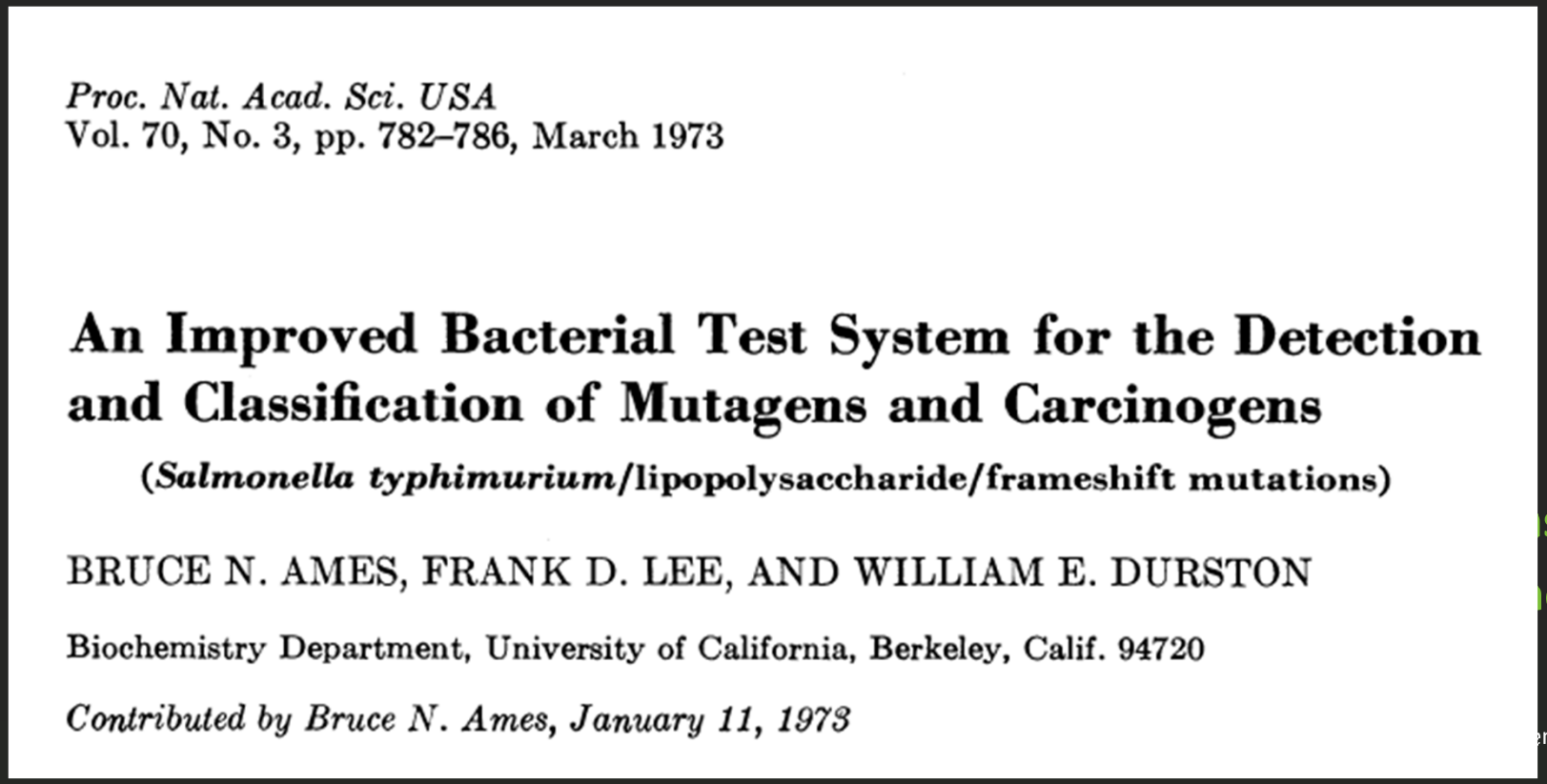
Introduction

- The **Ames test** is a biological assay to assess the **mutagenic potential** of chemical compounds Ames et al. PNAS 1973



Proc. Nat. Acad. Sci. USA
Vol. 70, No. 3, pp. 782–786, March 1973

An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens

(*Salmonella typhimurium*/lipopolysaccharide/frameshift mutations)

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Biochemistry Department, University of California, Berkeley, Calif. 94720

Contributed by Bruce N. Ames, January 11, 1973

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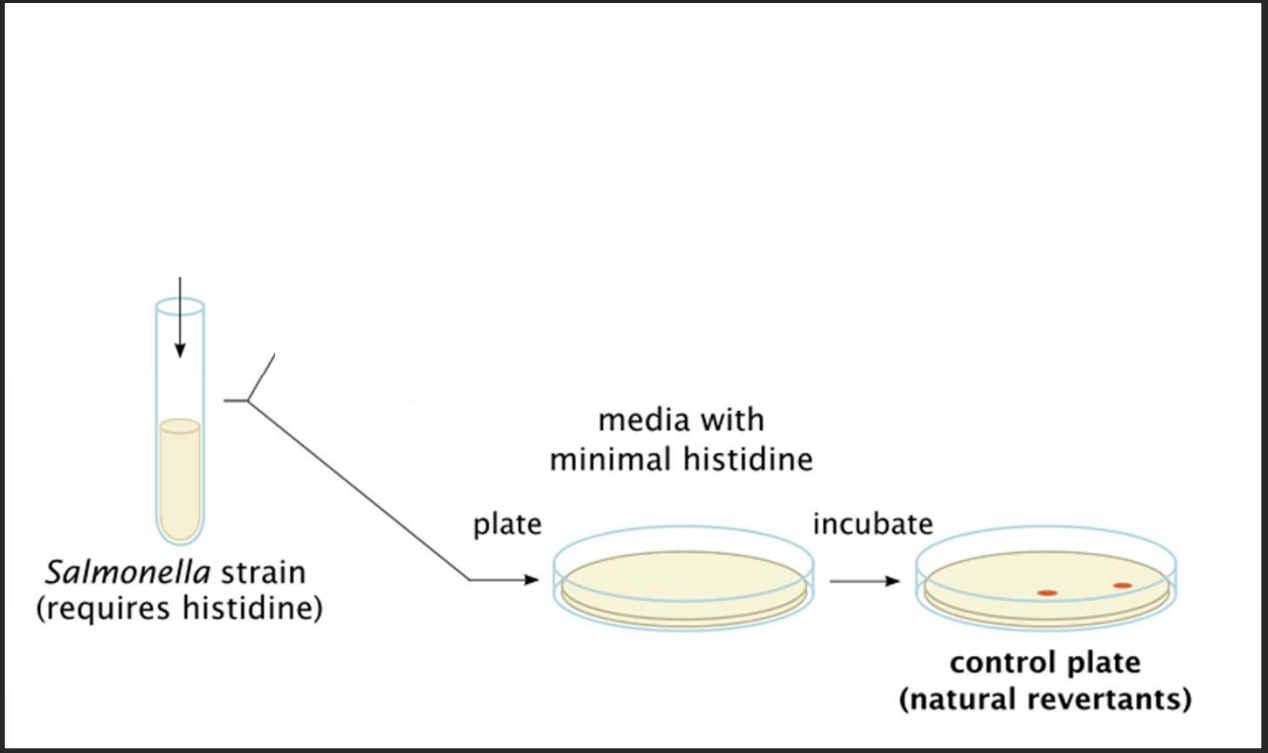
The Ames test

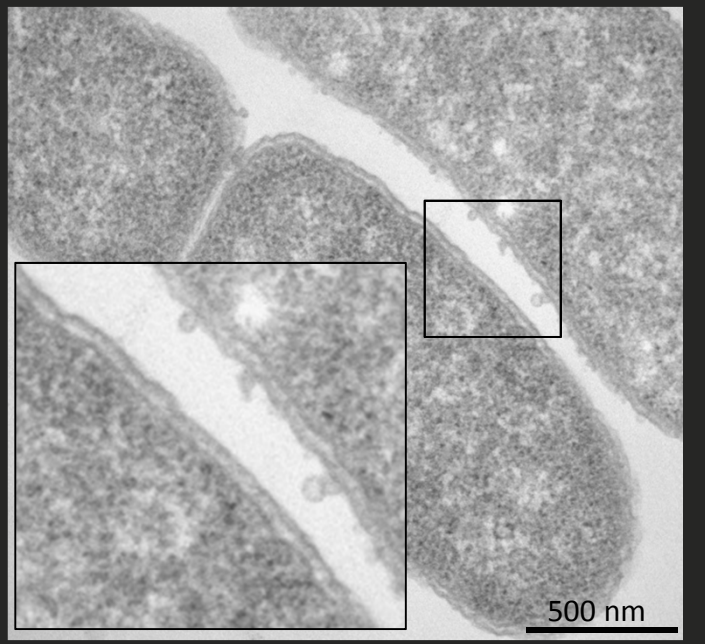
- Several strains of the bacterium ***Salmonella typhimurium*** that carry **mutations** in genes involved in **histidine synthesis**



http://en.wikipedia.org/wiki/File:Ames_test.svg

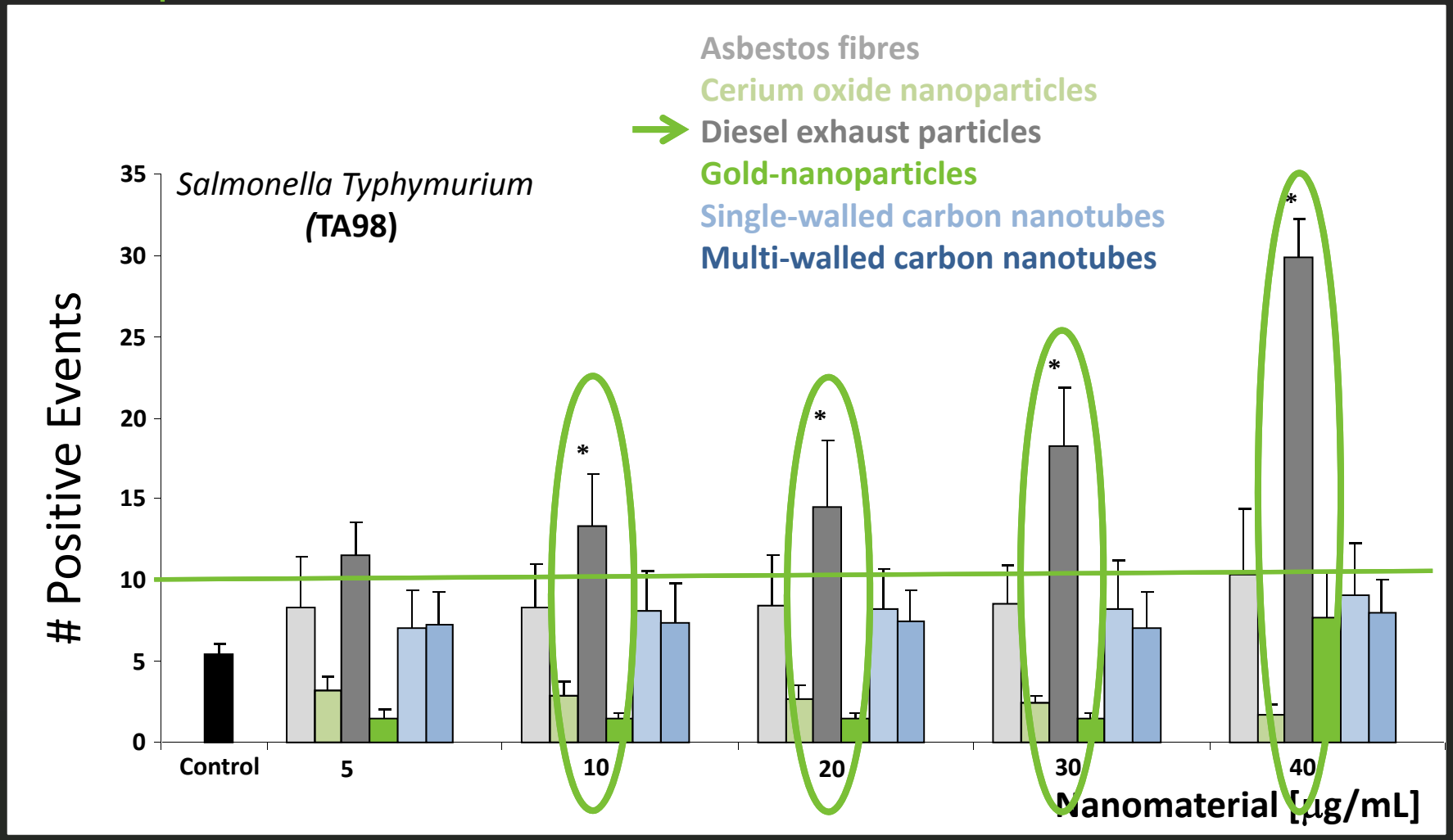
Material and Methods

- ***Salmonella Typhymurium***
  - Gram-negative
  - TA98 (base-pair substitution mutations)
- **Nano-objects**
  - Diesel exhaust particles (NIST #2975)
  - Asbestos (crocilodite) fibres
  - Cerium oxide-nanoparticles
  - Gold-nanoparticles
  - Single-walled carbon nanotubes
  - Multi-walled carbon nanotubes
- **Concentrations:** 5, 10, 20, 30 and 40µg/mL
- **Incubation time:** 2h and 48h post-incubation



- **Inner membrane** (double lipid layer)
- **Thin peptidoglycan layer**
- **Outer membrane** (double lipid layer) with Lipopolysaccharides

The Ames test using different nanomaterials



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Nanomaterial – Bacteria Interactions

Control

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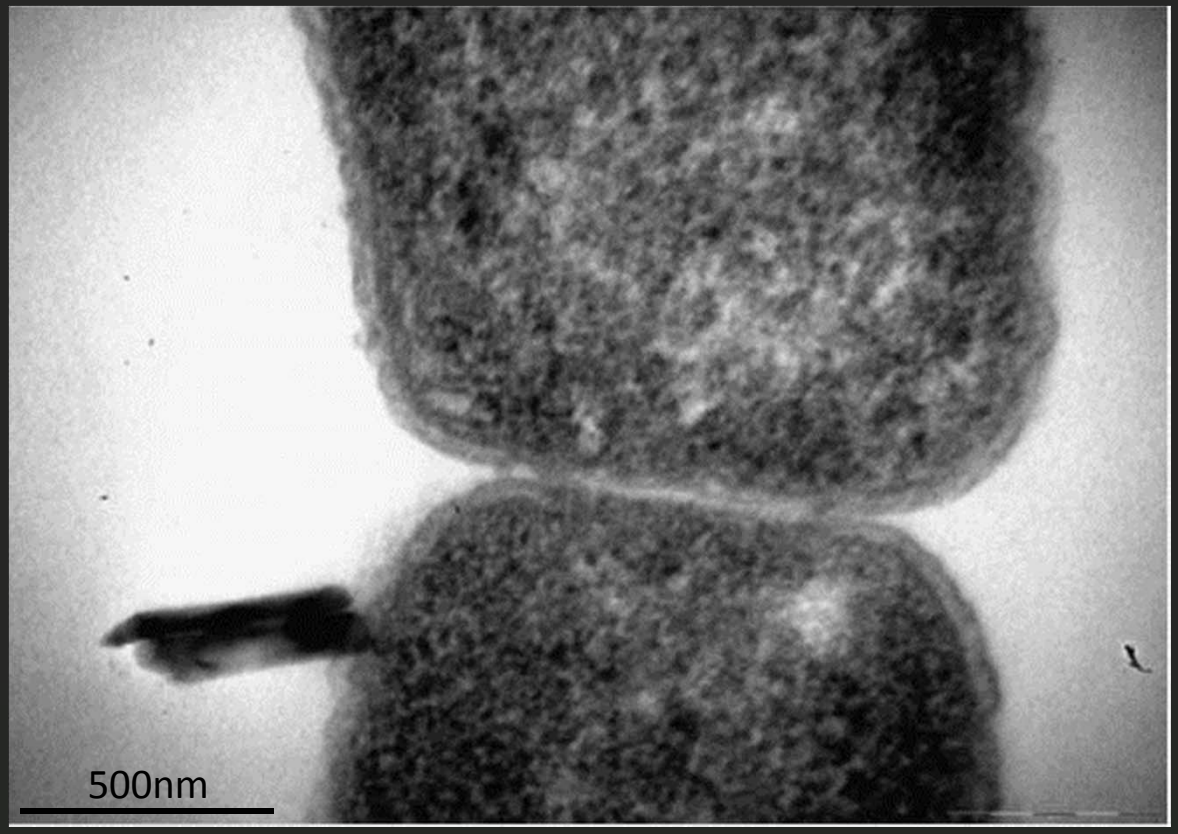
Nanomaterial – Bacteria Interactions

Asbestos fibres



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Nanomaterial – Bacteria Interactions

Diesel exhaust particles

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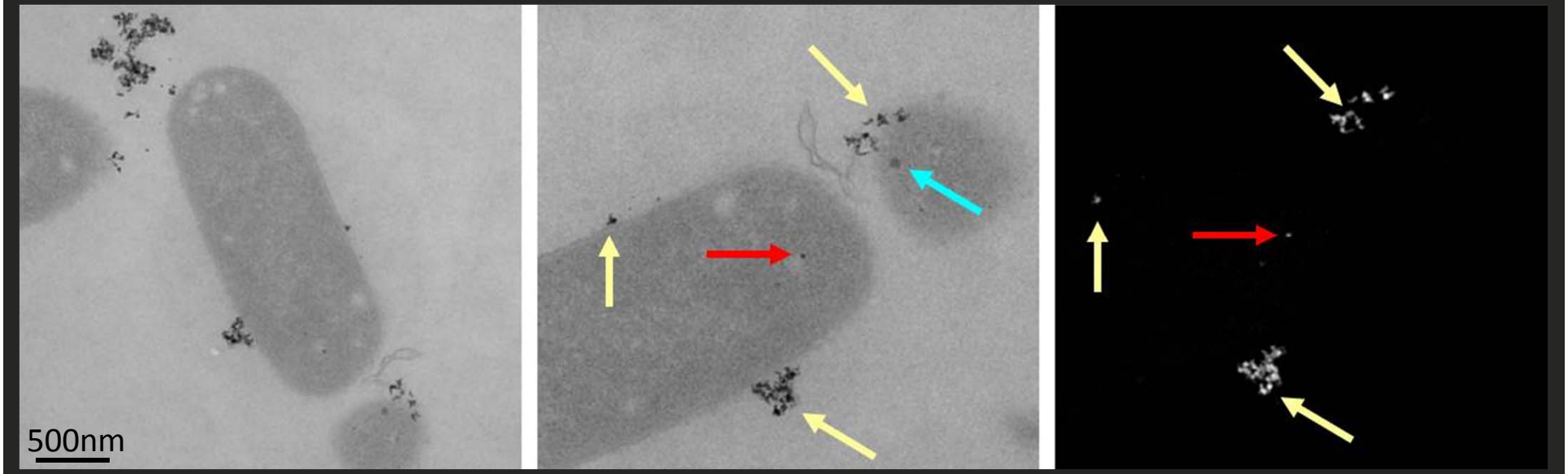
Cerium dioxide-nanoparticles



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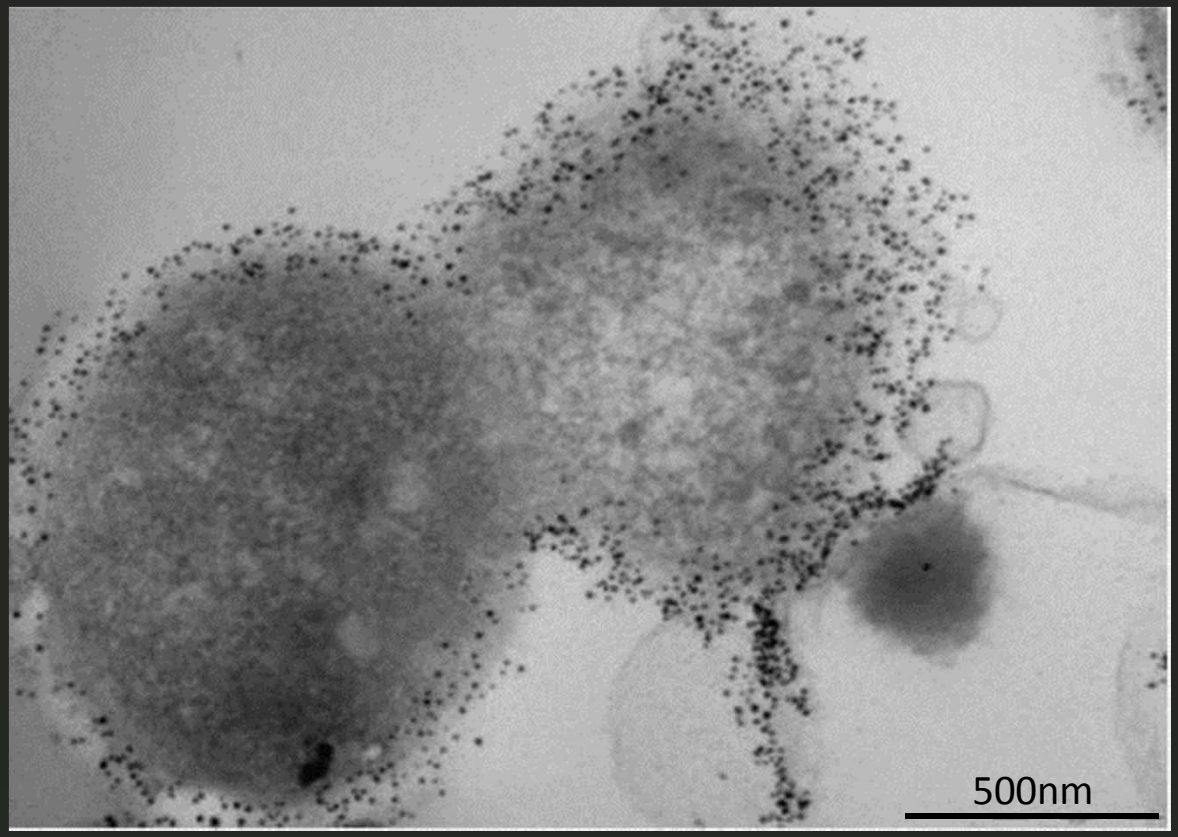
Nanomaterial – Bacteria Interactions

Gold-nanoparticles



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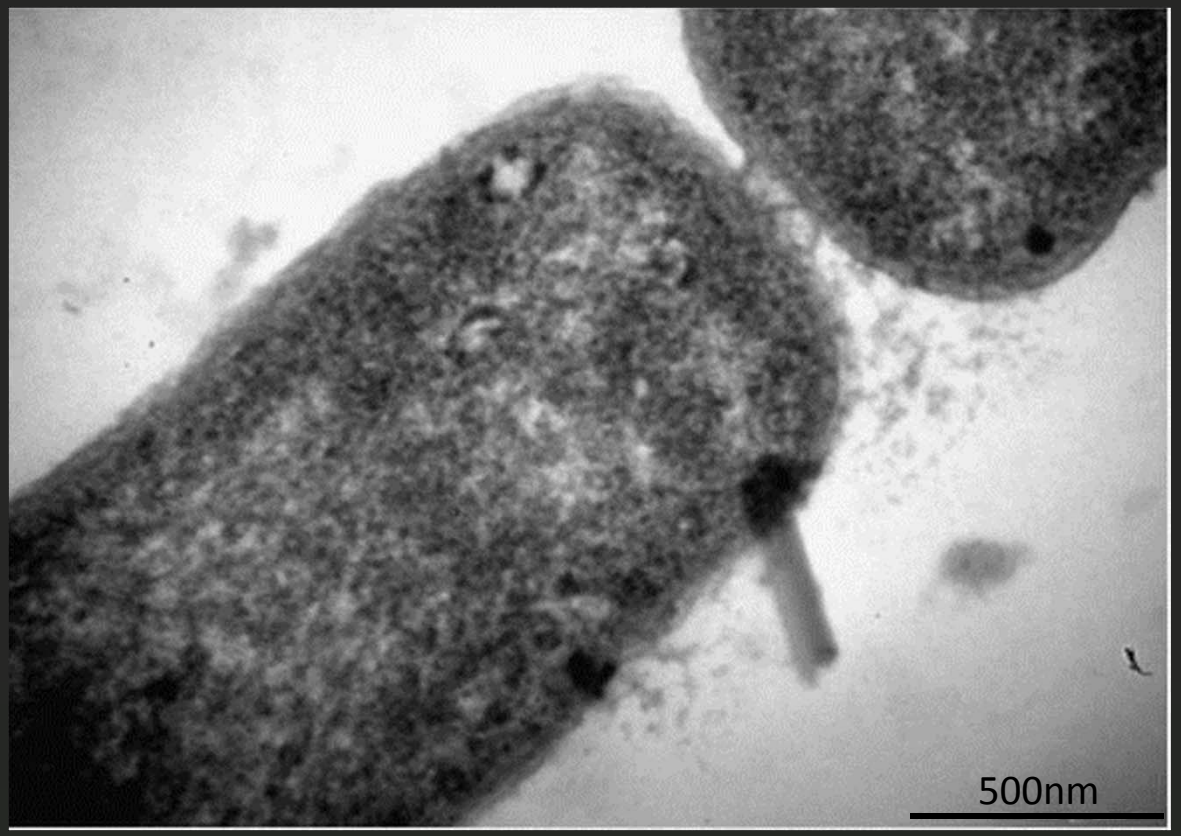
Nanomaterial – Bacteria Interactions

Single-walled carbon nanotubes



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Nanomaterial – Bacteria Interactions

Multi-walled carbon nanotubes

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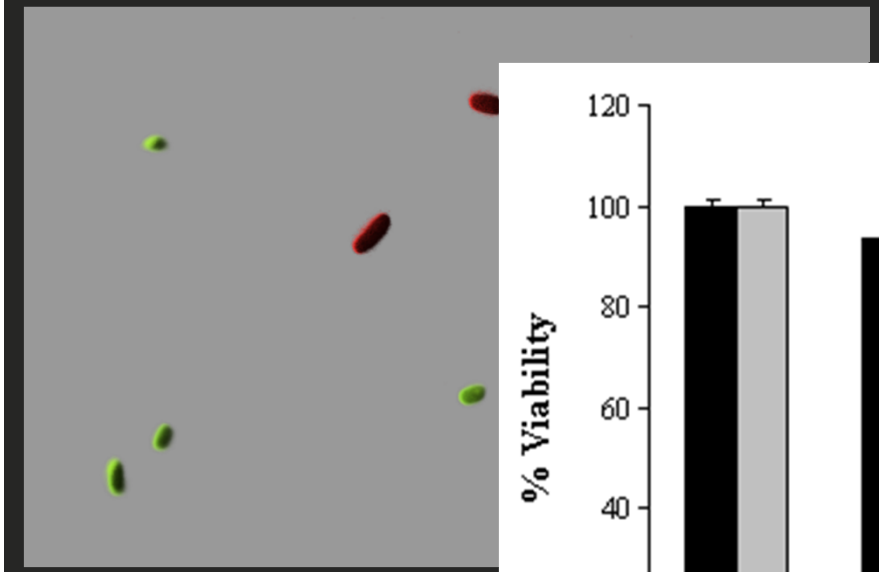
Bacterial viability

Living bacteria Dead bacteria



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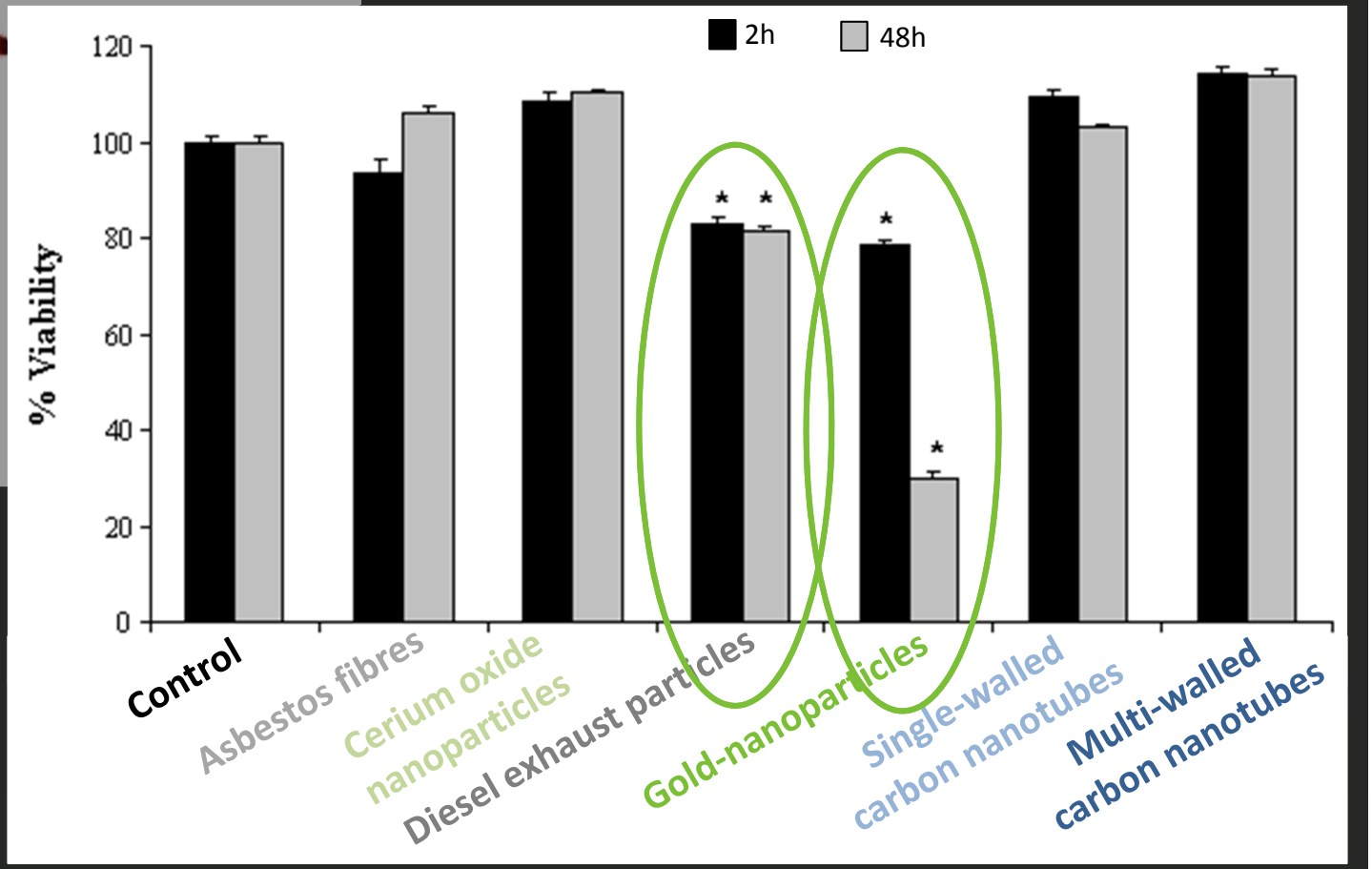
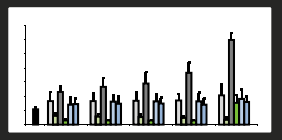


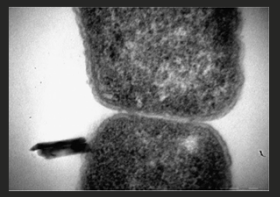
Conclusions

- **Positive mutagenic effects are observed only for diesel exhaust particles, but no diesel exhaust particles inside the bacteria**



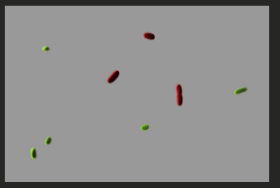
=> Suggesting a secondary effect

- **In contrast, asbestos fibres, cerium dioxide- and gold-nanoparticles as well as single-walled carbon nanotubes interact and penetrate the double lipid-layer membrane of the bacterial-wall**



=> Direct interaction of nano-objects with the nuclear material of bacteria however, does not correlate to an observed mutagenic effect

- **Only diesel exhaust particles and gold-nanoparticles reduced bacteria viability significantly**



The Ames test is a good indicator for soluble fractions on diesel particles but not for nano-object specific mutagenic effects

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