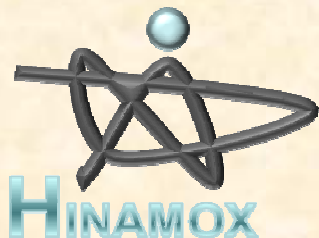
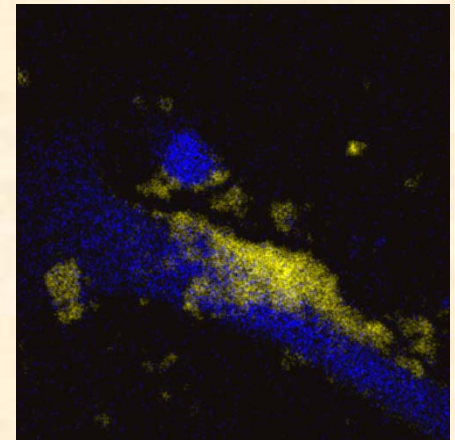


Quantification of Nanoparticle Uptake, Colocalization and Toxicity at the Single Cell Level

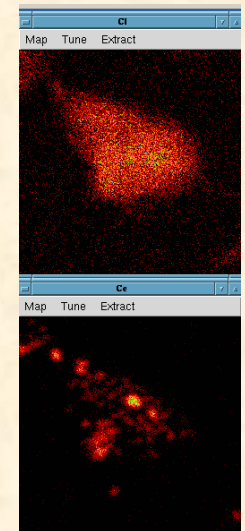
Irina Estrela-Lopis



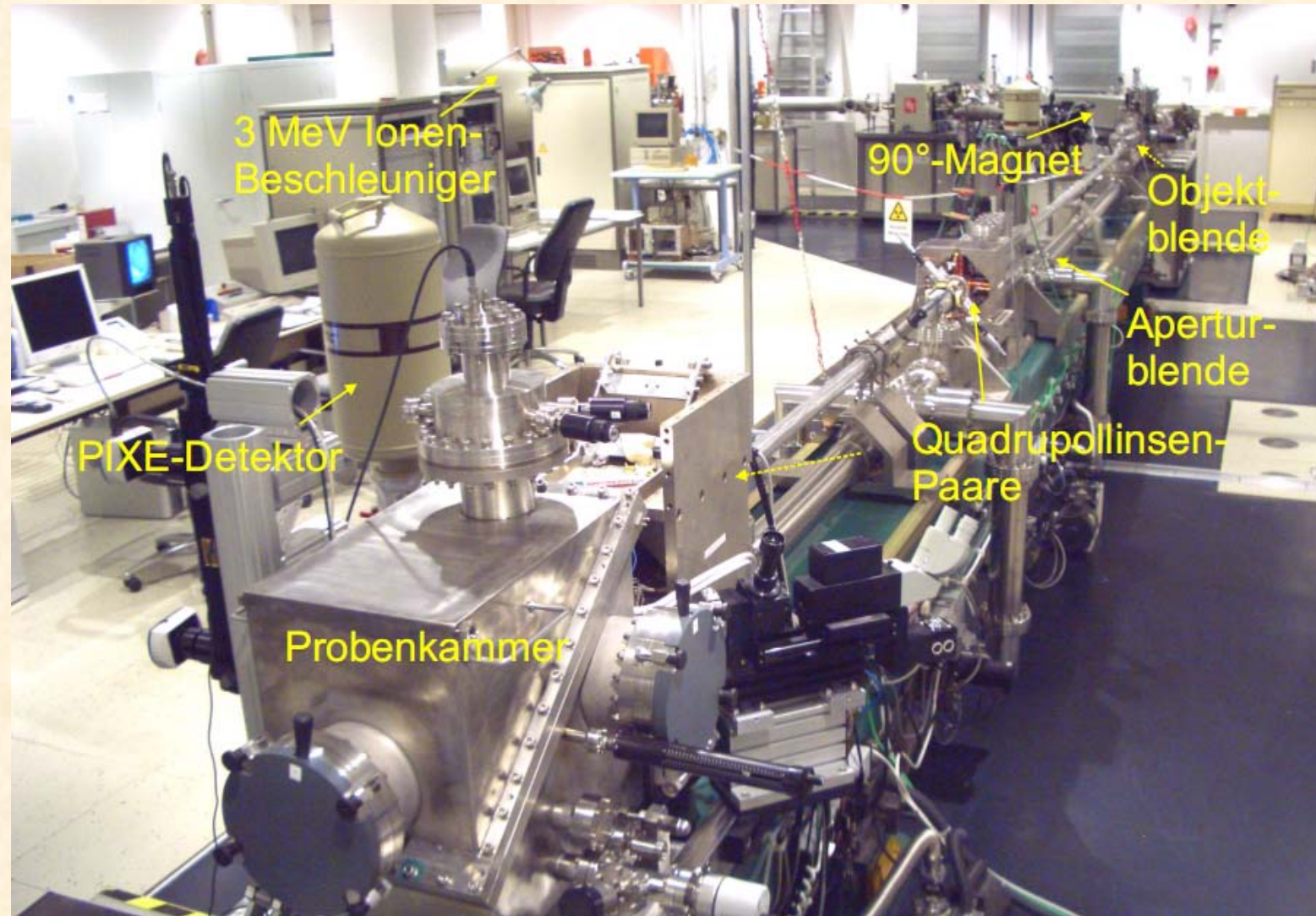
1. Quantification of NP uptake in cell cultures at single cell level (IBM)

- *Calculation of intracellular NPs concentration*
- *Intracellular Dose – effect relationship*
- *NP internalization*

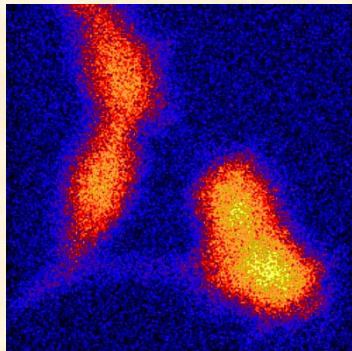
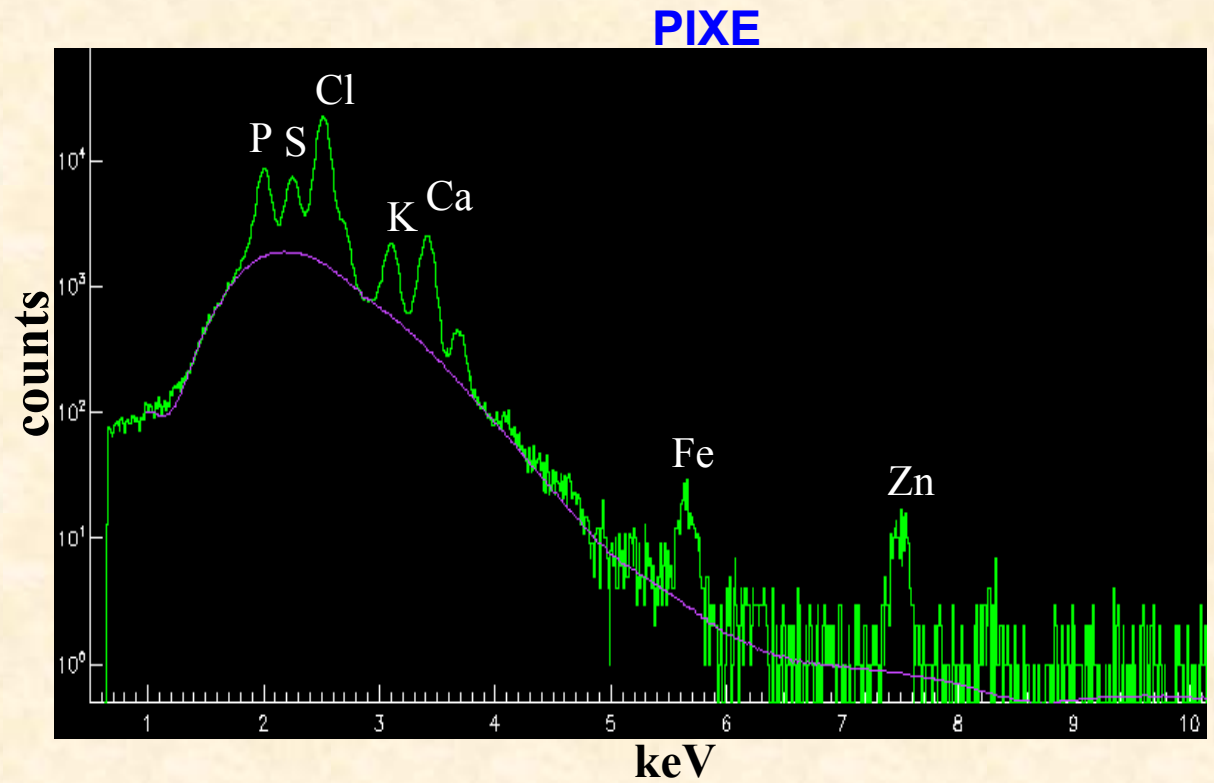
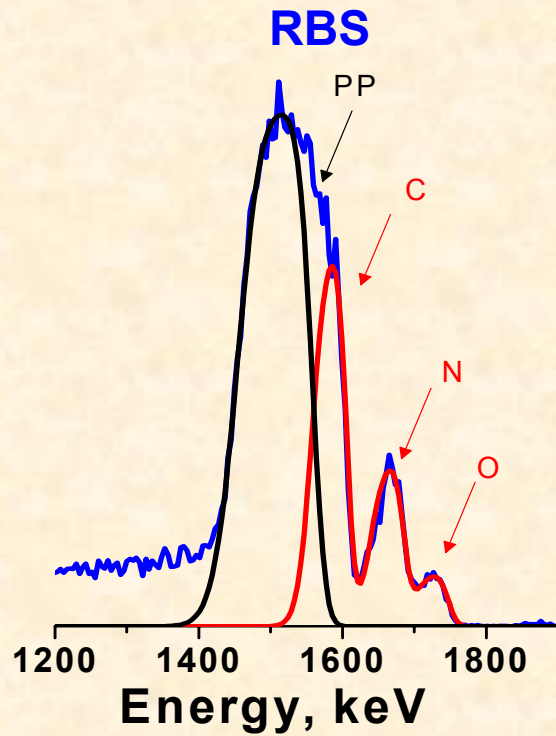
2. NPs uptake, distribution and co-localization with cell components of NPs in cells (Confocal Raman Microspectroscopy)



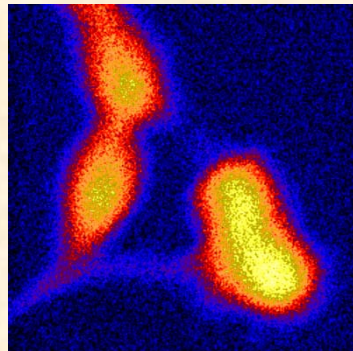
The quantification of the NPs uptake in single cells (IBM)



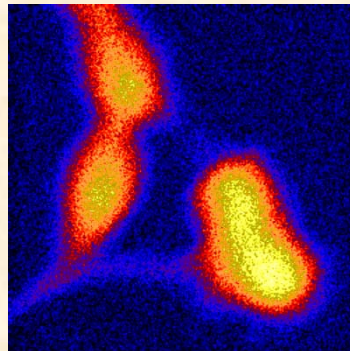
IBM Spectra of control cells (A549)



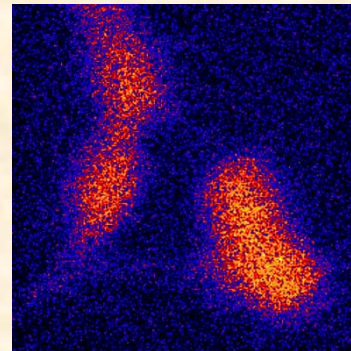
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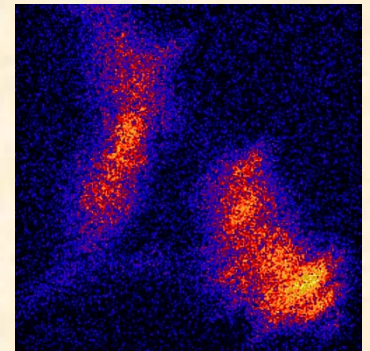
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Cl

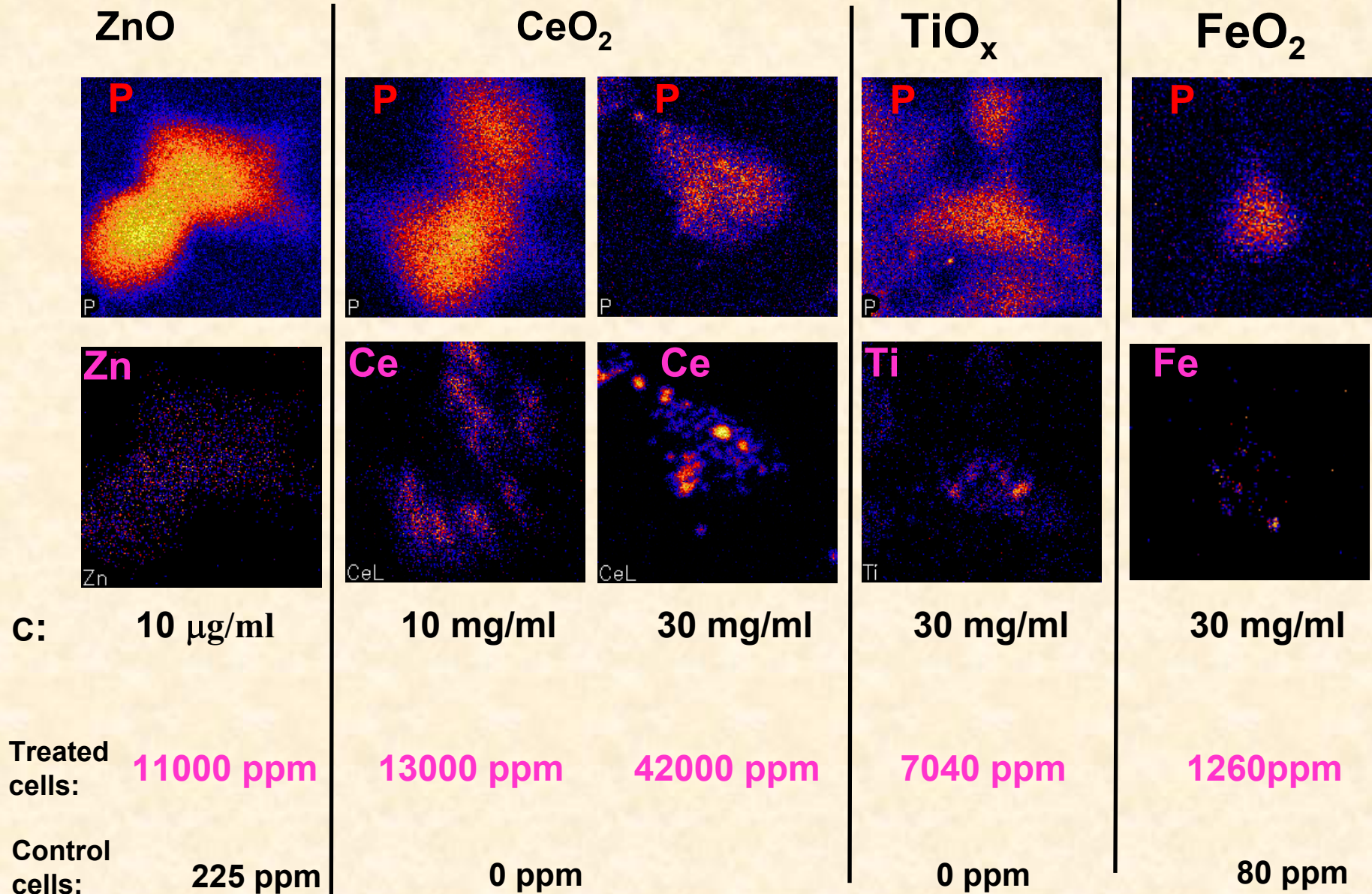


K



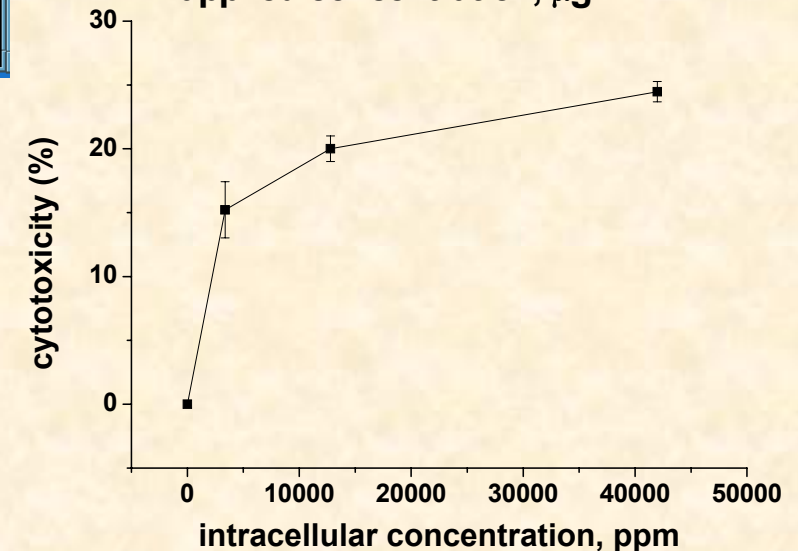
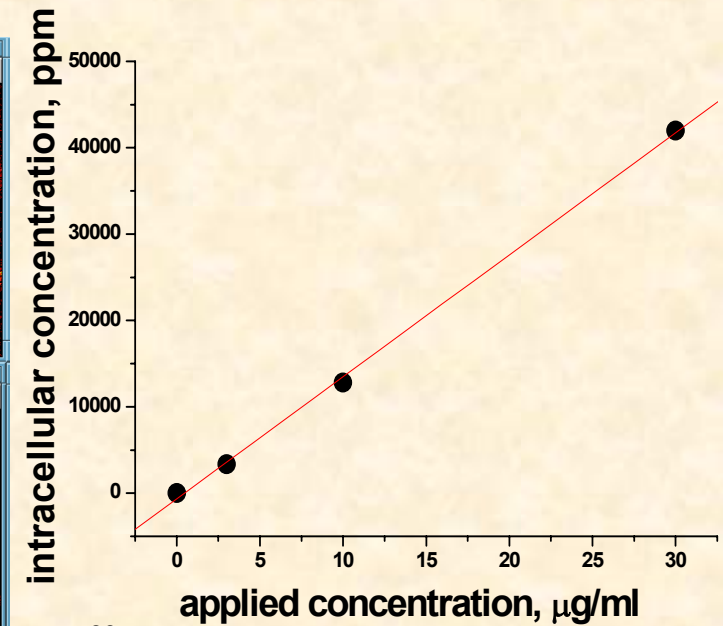
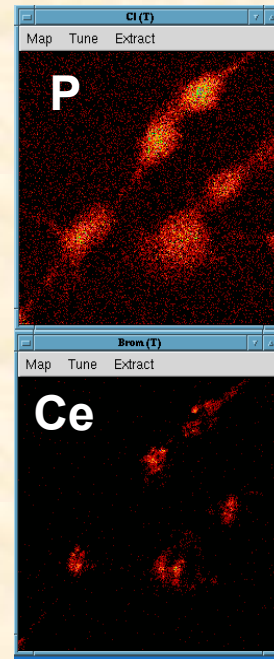
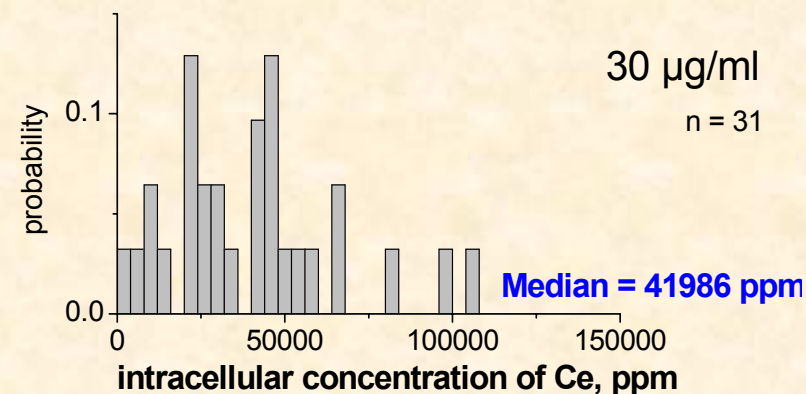
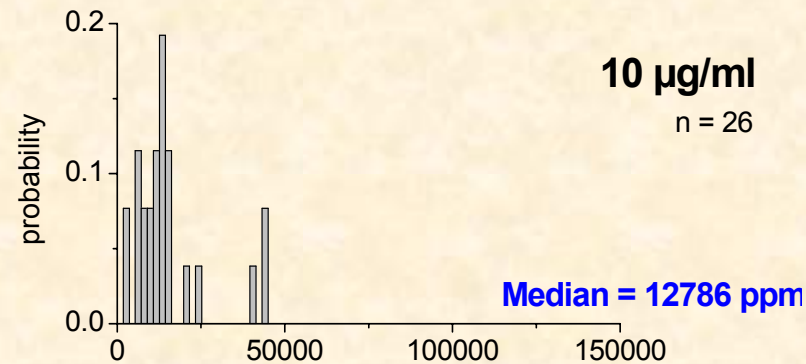
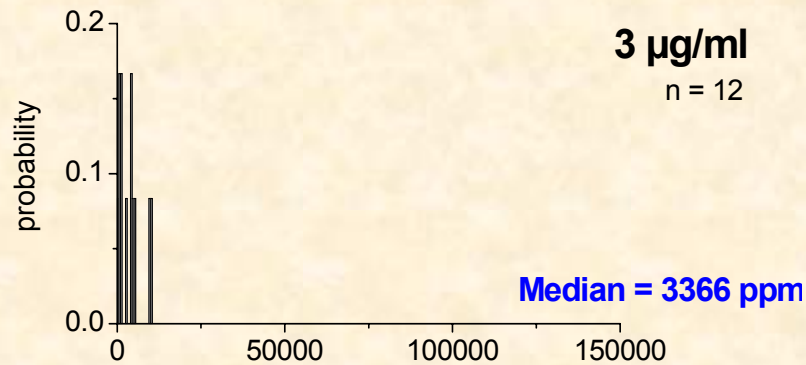
Ca

Detection and Quantification of NPs in cells



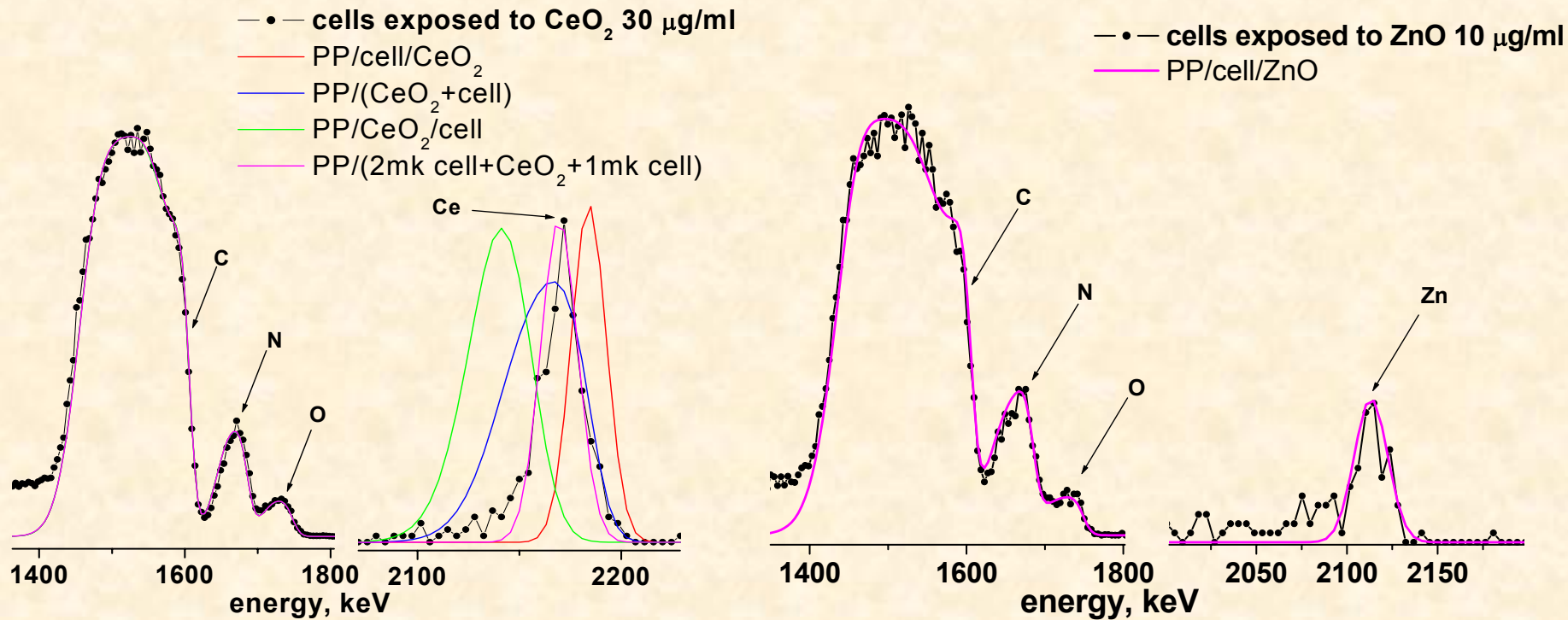
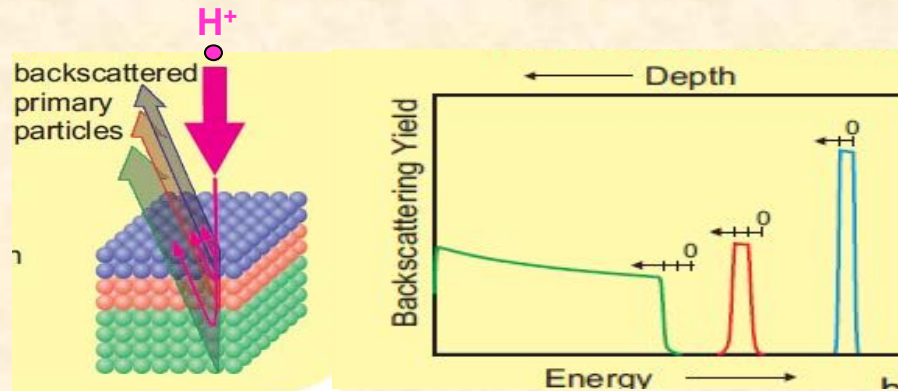
➤Overlapping of major cell elements with NPs can be seen for all particles

Intracellular Dose – Effect relationship in cells exposed to CeO₂ NPs (72 h)



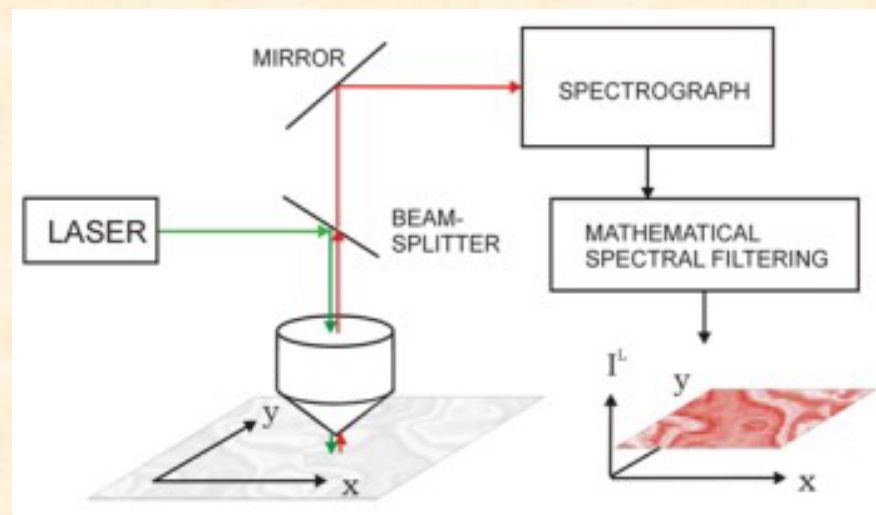
- Median value of intracellular concentration is linearly dependent on exposition dose
- Dose-effect relation demonstrates a saturating growth of toxicity of cells exposed to CeO₂

CeO₂ NP internalization in single cells



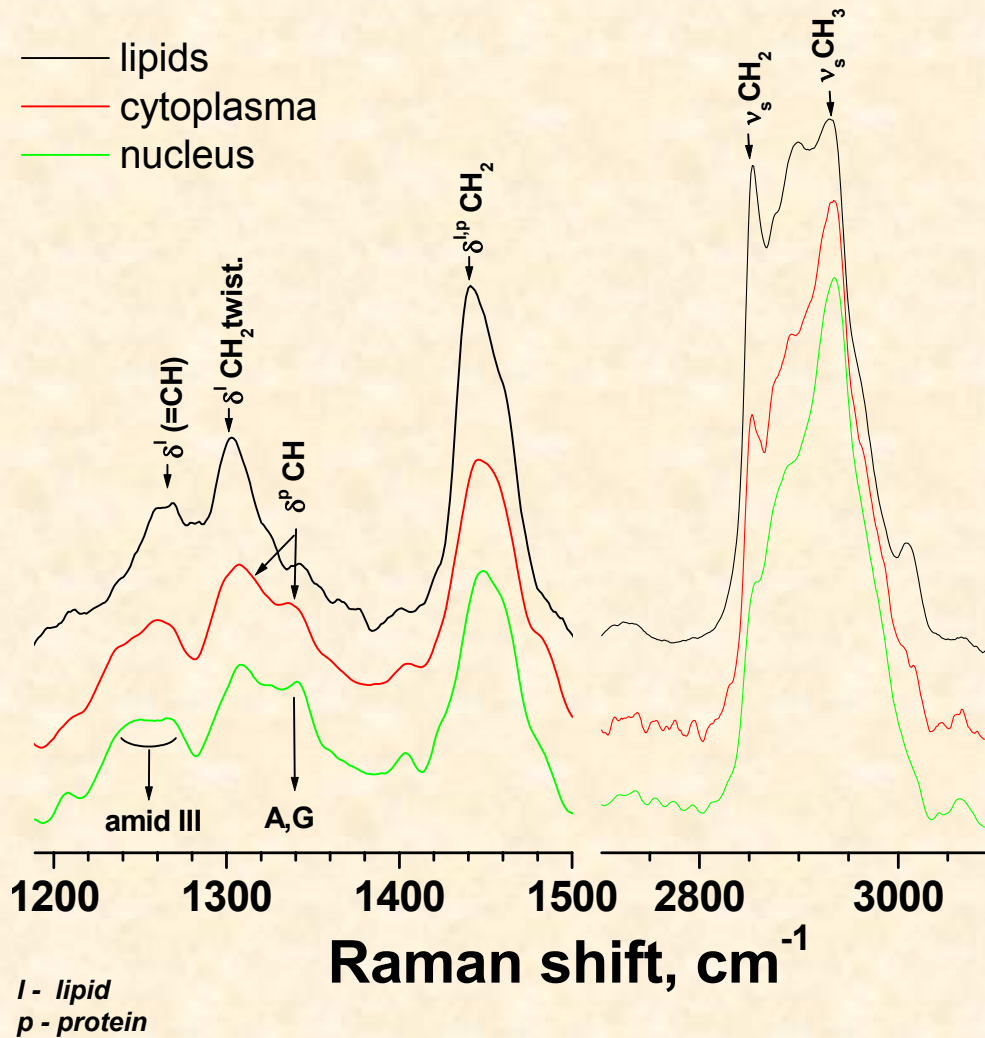
➤ RBS spectra could be a good proof for NP internalization

Renishaw inVia micro-Raman Spectrometer

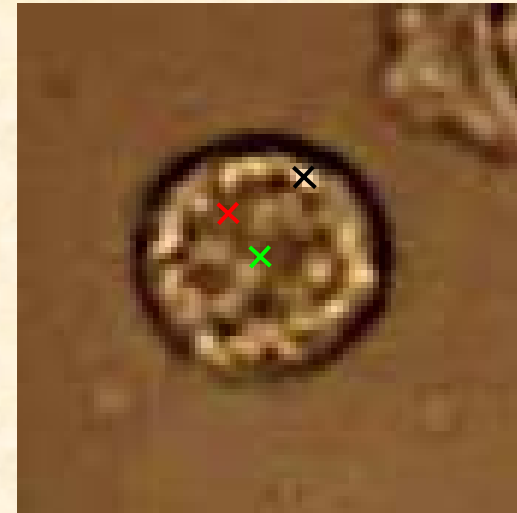


- Label free techniques in single “living cells”
- NP localization
- NP distribution and co-localization in the cells
- NP internalization within cells

Spectroscopic Signature of Cell Compartments

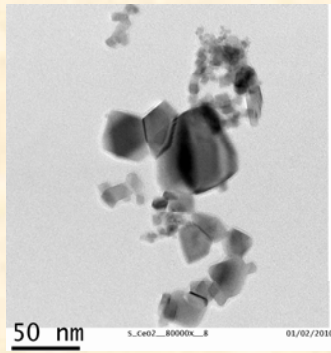


Hepatocytes (HepG2)

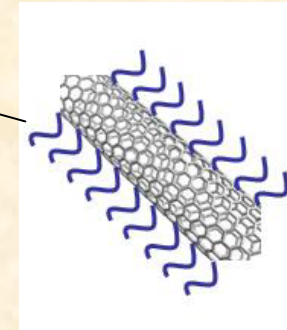


NP Detection in Cells

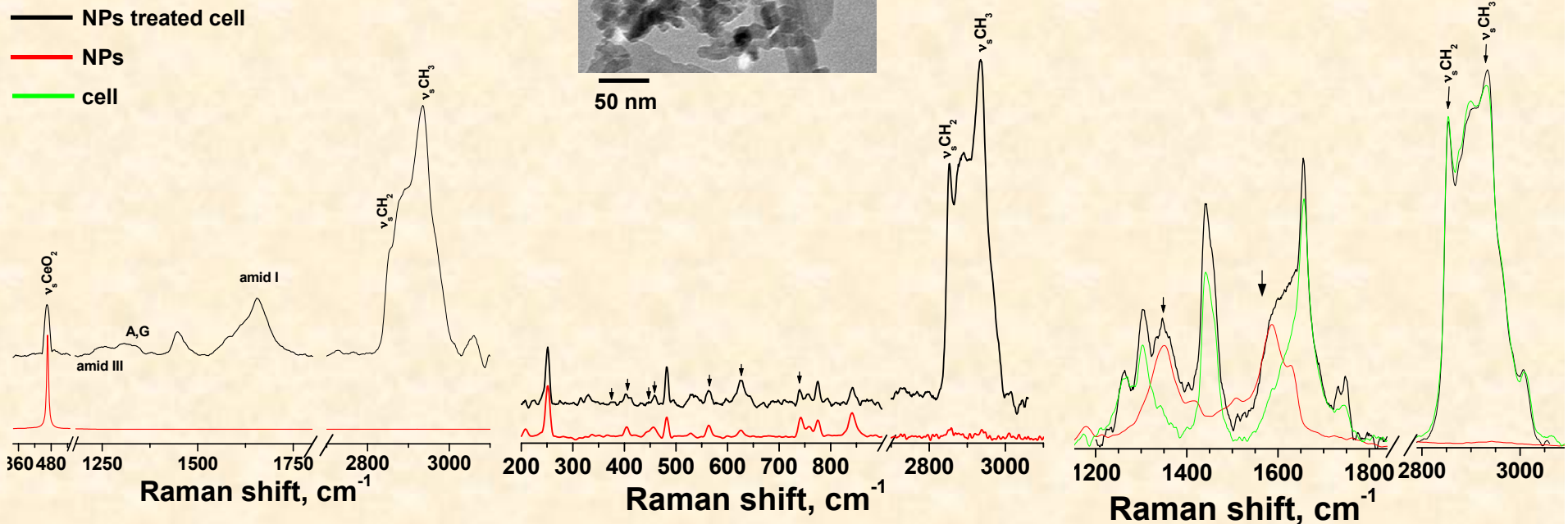
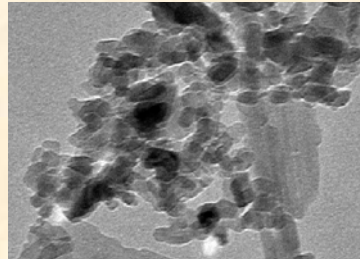
CeO₂ NPs



CNT/PSPM



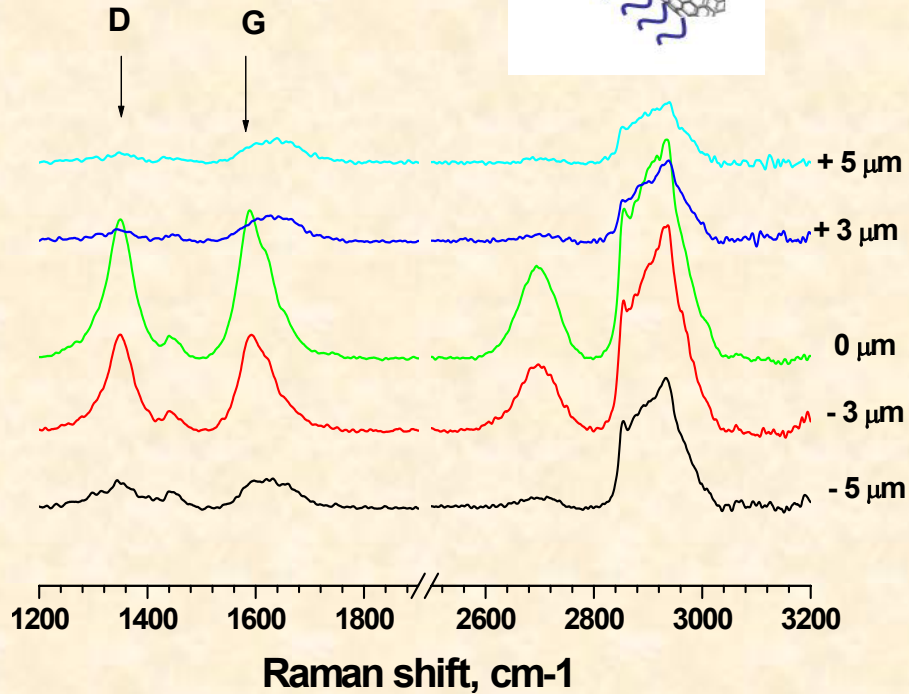
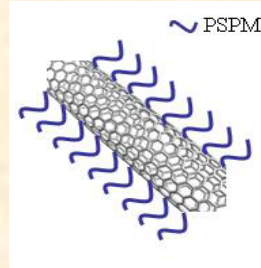
Al₂O₃ NPs



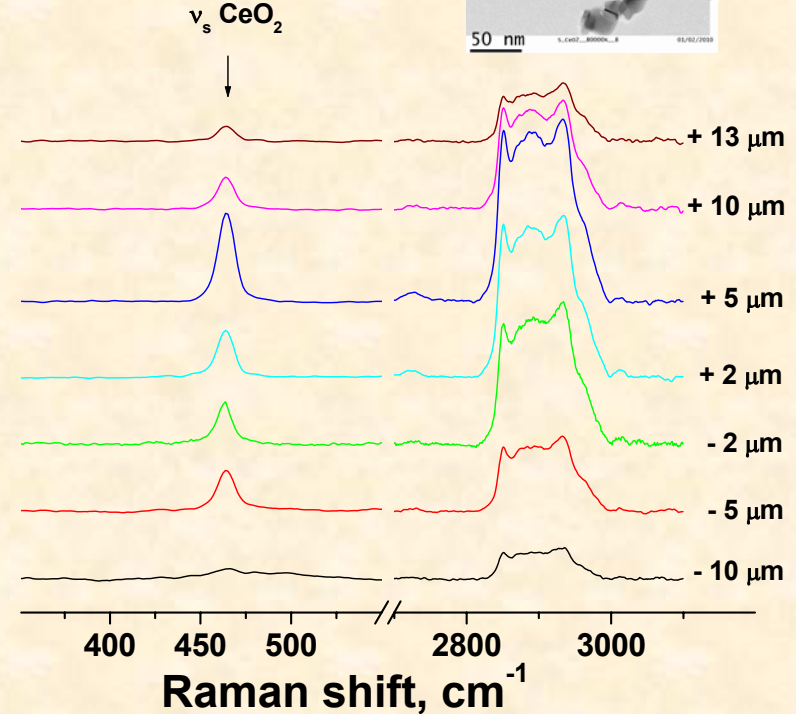
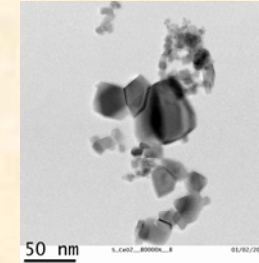
Spectra show the signals of NPs and their environment within cells. Nanoparticle internalization!?

NP Internalization

CNT/PSPM

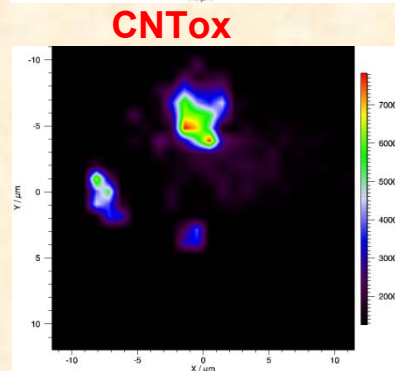
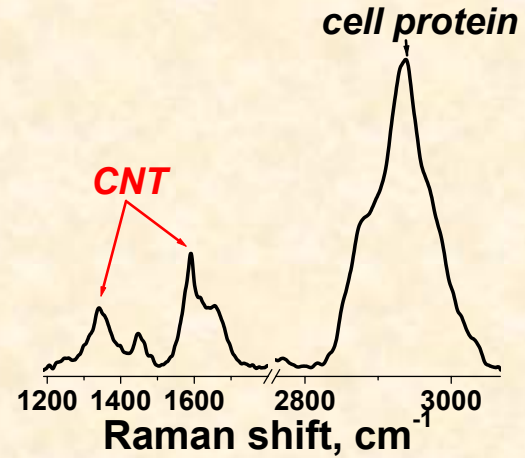
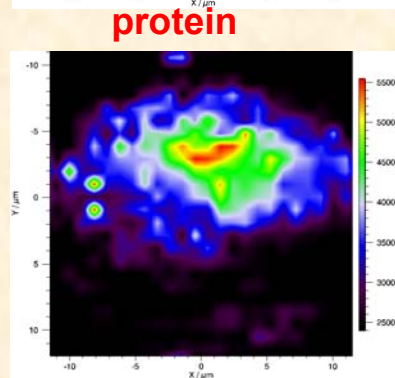
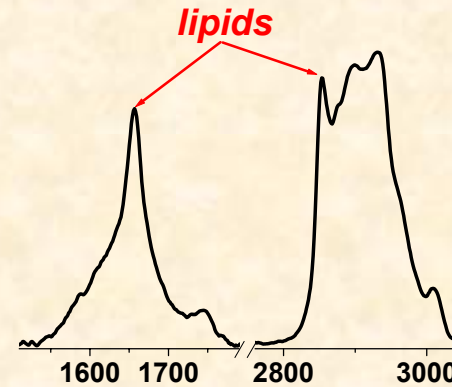
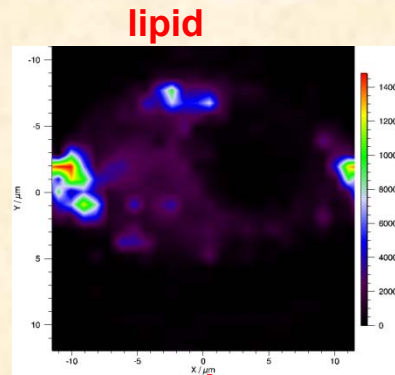
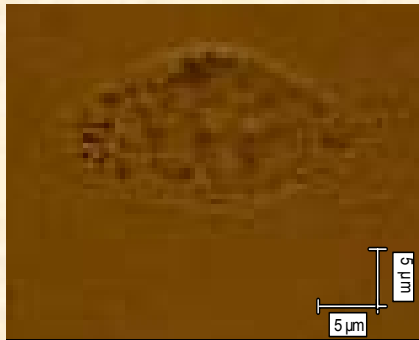


CeO₂ NPs

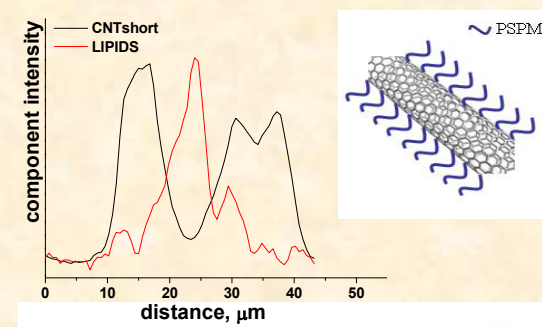
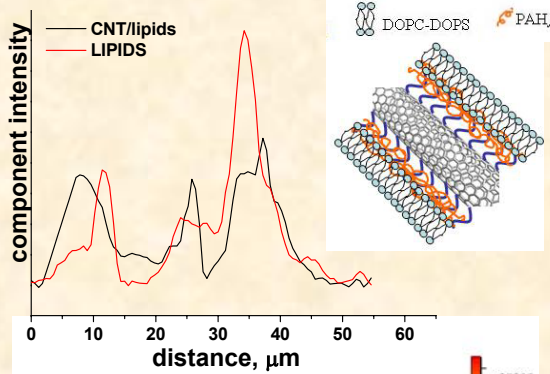


- CNT/PSPM and CeO₂ NPs are inhomogeneously distributed in the cytoplasm
- CeO₂ NPs are internalized in the vicinity of lipid-rich region and CNT/PSPM NPs are not associated with them
- Z-scanning proves NPs internalization

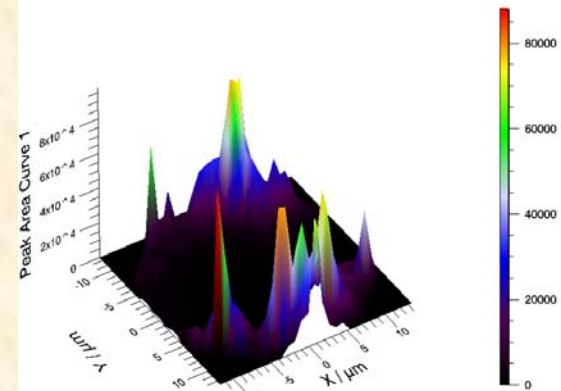
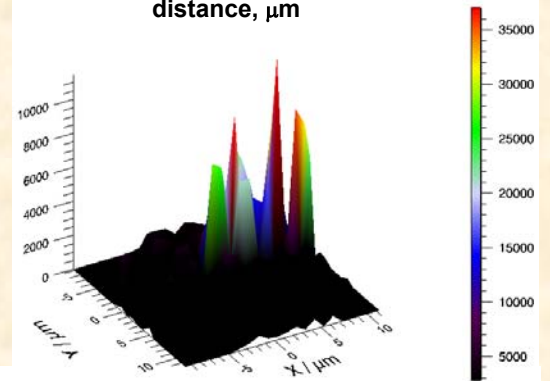
Spectroscopic Mapping of Cell treated with oxidized CNT



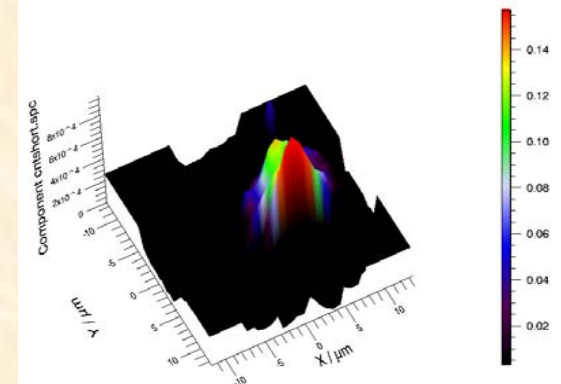
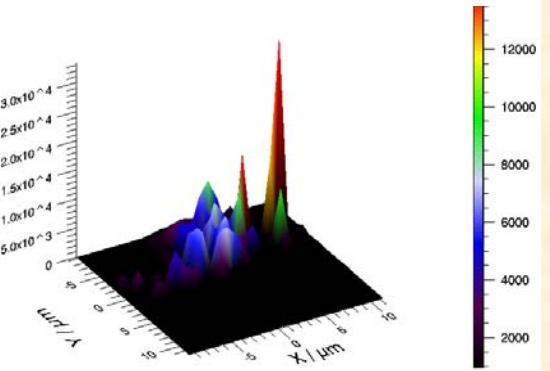
Spectroscopic Mapping of Cell Compartments



lipids

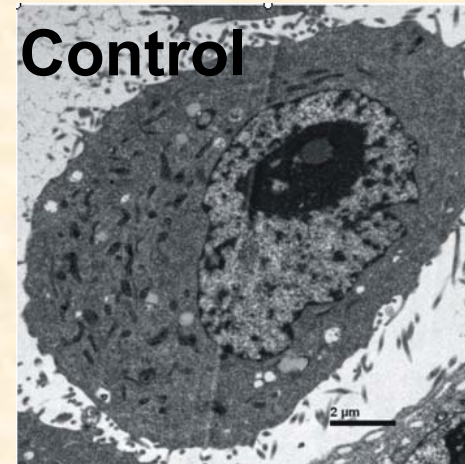
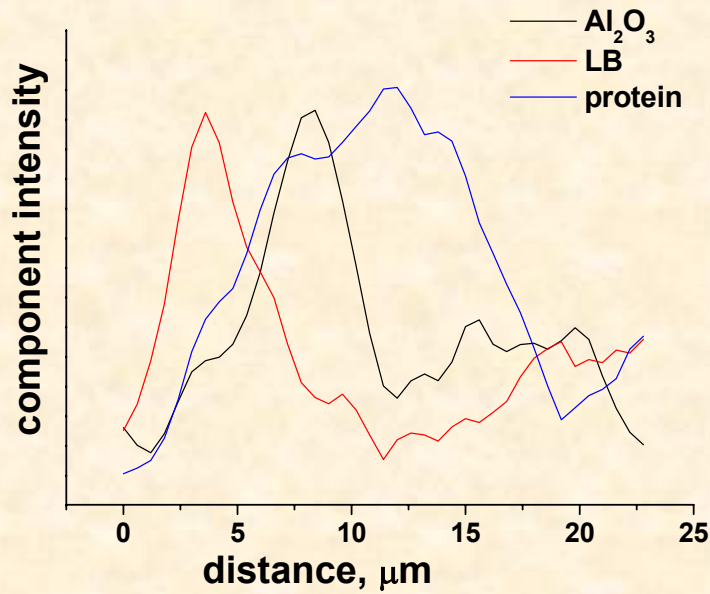


CNT

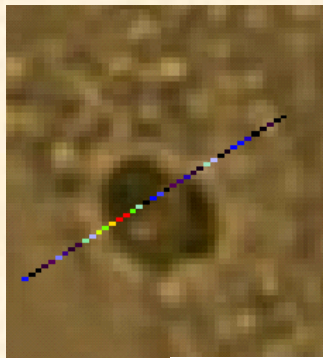


- CNT/PSPM NPs have a tendency to avoid the site where the LBs are preferentially located
- Lipid coated CNTs are found within cells in close proximity of LB accumulations

Colocalization and Al_2O_3 distribution in single cell

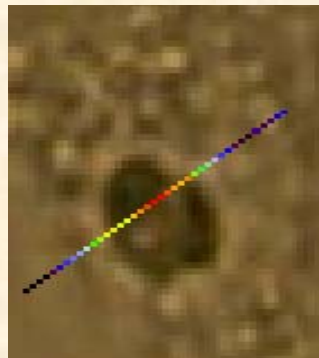


Al_2O_3 NPs line distribution

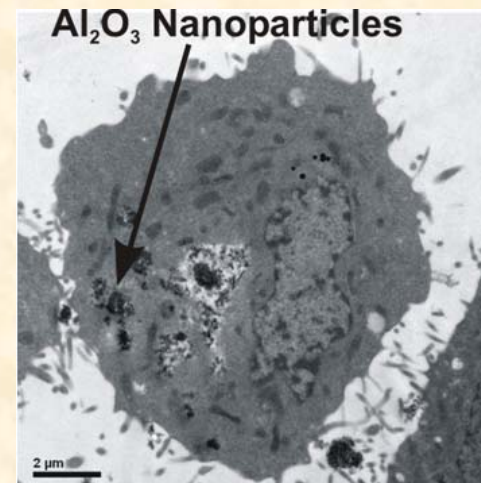


10 μm

Protein line distribution

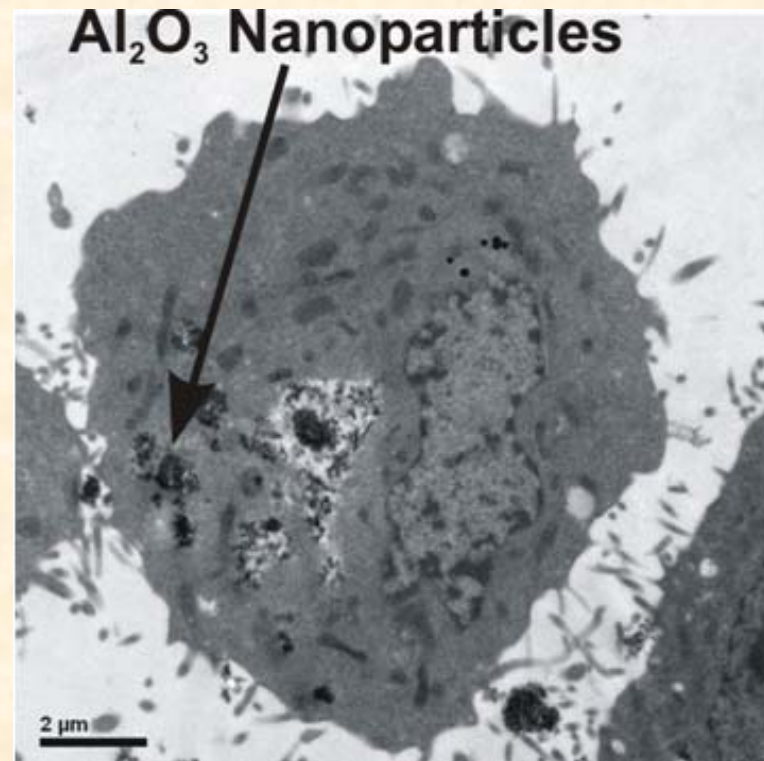
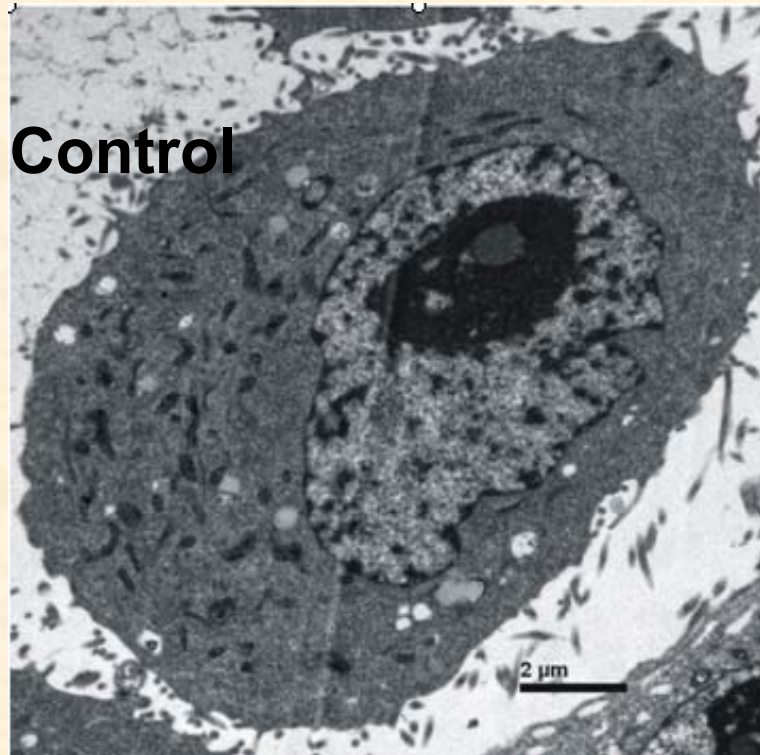


10 μm



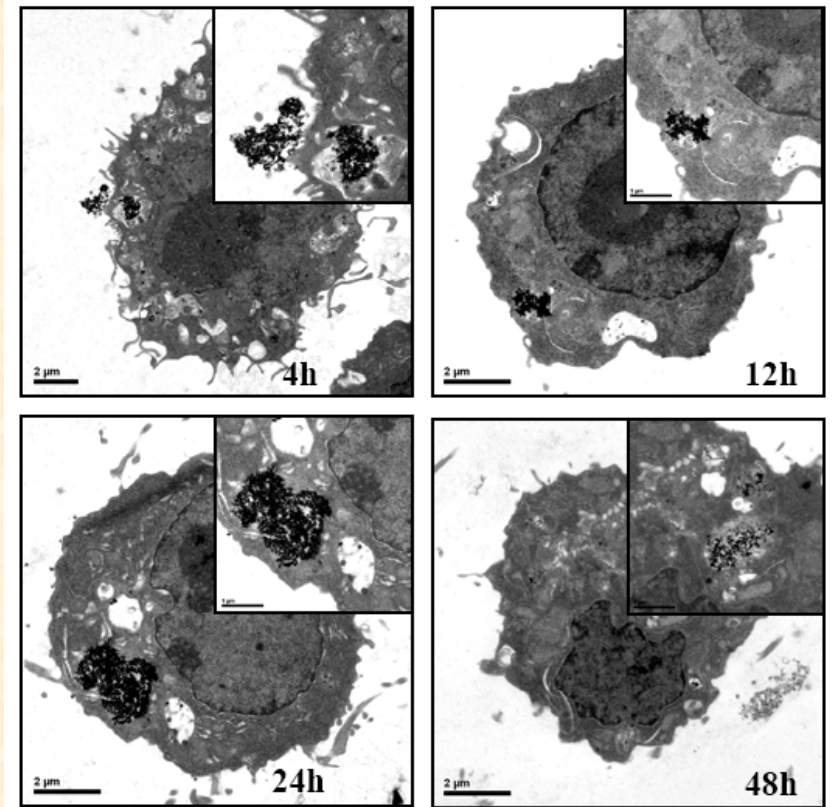
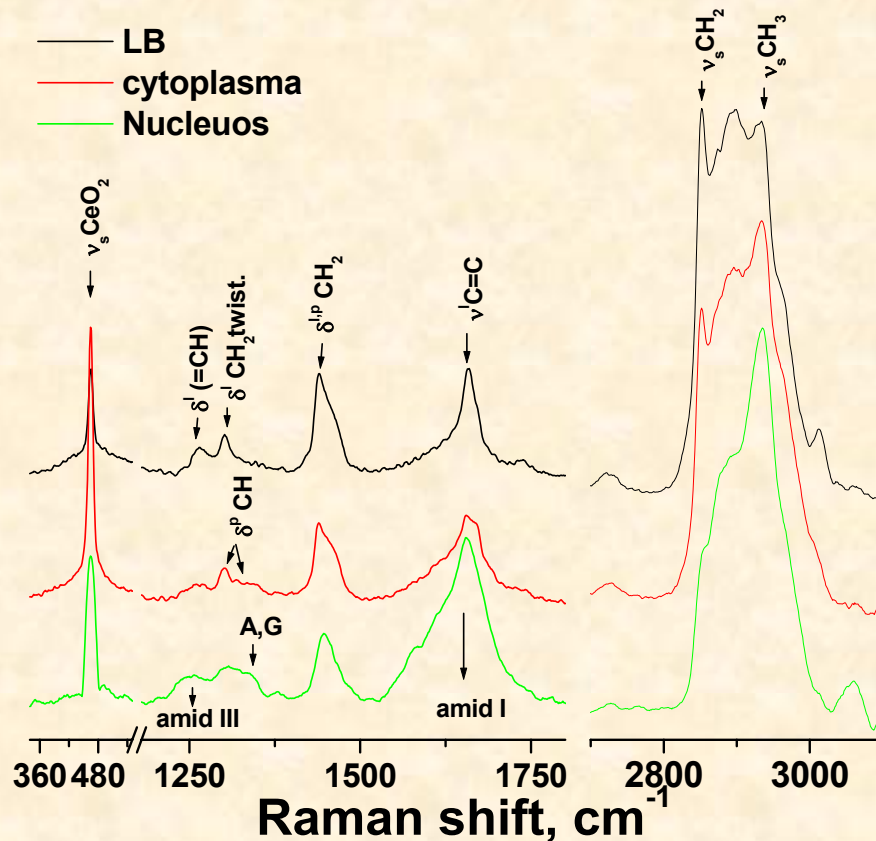
- The image clearly reveals the cytoplasmic distribution of the Al_2O_3 NPs.
- The Al_2O_3 NPs are found as clusters of variable size distributed within the cytoplasm.
- Spatial Pearson coefficient of NPs/protein correlation is about 0.7

Transmission Electron Microscopy (TEM)



- The image clearly reveals the cytoplasmic distribution of the Al₂O₃ NPs.
- The Al₂O₃ NPs are found as clusters of variable size distributed within the cytoplasm.

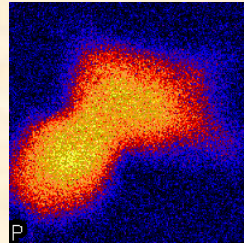
Co-localization and CeO₂ distribution in single cells



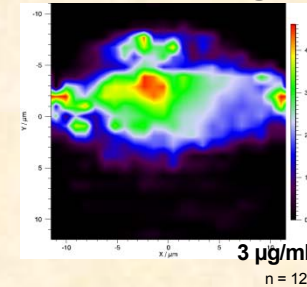
CeO₂ NPs do not show a preferential association with particular cell constituents

Summary

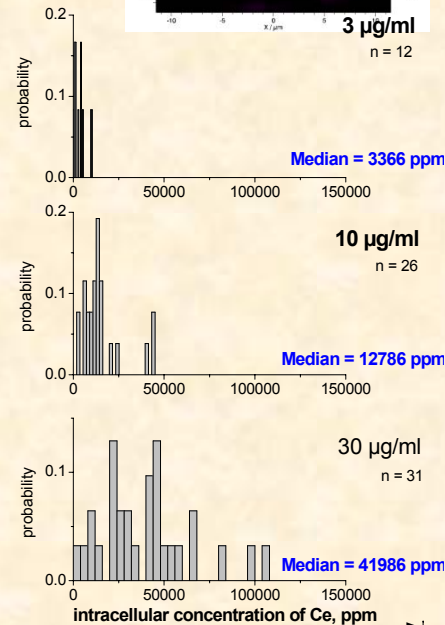
◆ NP Uptake: elemental imaging



chemical imaging

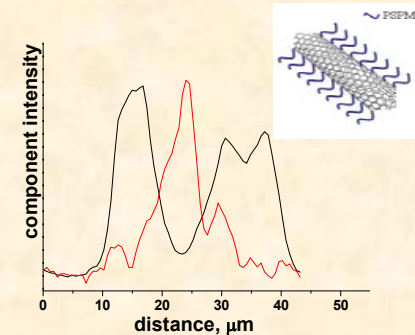


◆ Quantification of NP uptake



◆ Intracellular Dose – effect relationship

◆ NPs distribution and colocalization with cell constituents organelles at single cell level



◆ The potential of RAMAN spectroscopy as a probe of cytotoxicity to NPs exposure

This work was supported by the European Commission in the framework of FP7 Theme 4 – NMP - Nanosciences, Nanotechnologies, Materials and New Production Technologies, Proposal No: CP-FP 228825-2 HINAMOX.



UNIVERSITÄT LEIPZIG

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M. Dorn

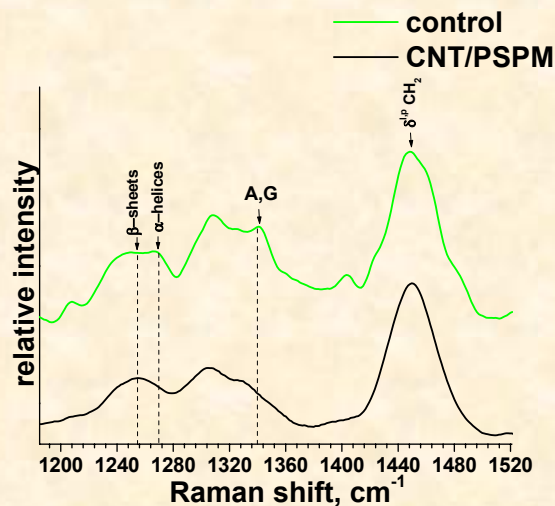
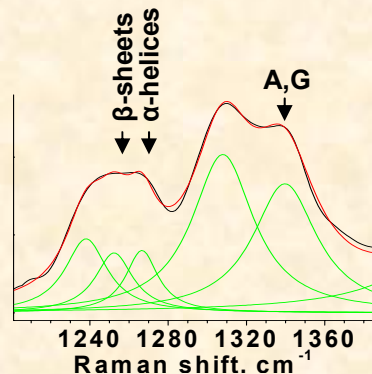


San Sebastian: S. E. Moya
E. Rojas
G. Romero

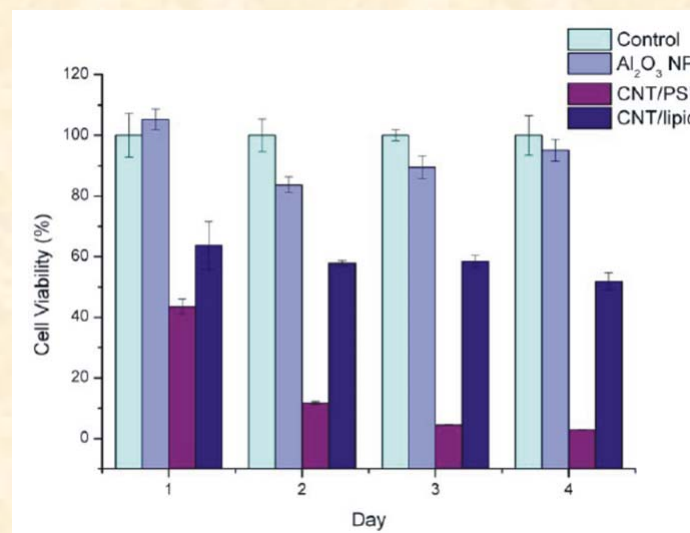
Hangzhou, Zhejiang University:
Ch. Gao, Zh. Mao

Spectroscopic Toxicity Assay

Fitting analysis of a Raman spectrum from the nucleus



Cells	Ratio (A,G/ amide III), %
control	100
PLGA exposed	76
Al ₂ O ₃ exposed	76
CNT/ PSPM exposed	16
CNT/lipid exposed	95

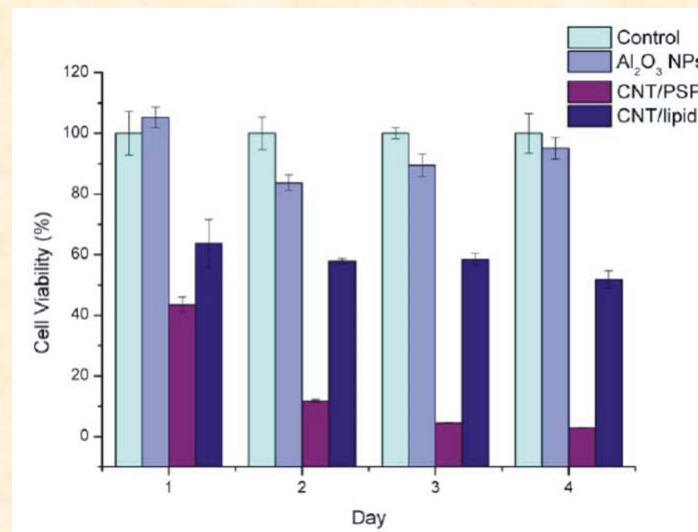


- The peak related to adenine and guanine at 1338 cm⁻¹ decreased markedly in the treated cells
- This decrease can be related to fragmentation of DNA which is known to occur during apoptosis
- The ratio between nucleotide and amide III protein bands can be considered as a marker for the toxic effect and correlates with cytotoxicity tests.

Spectroscopic Toxicity Assay

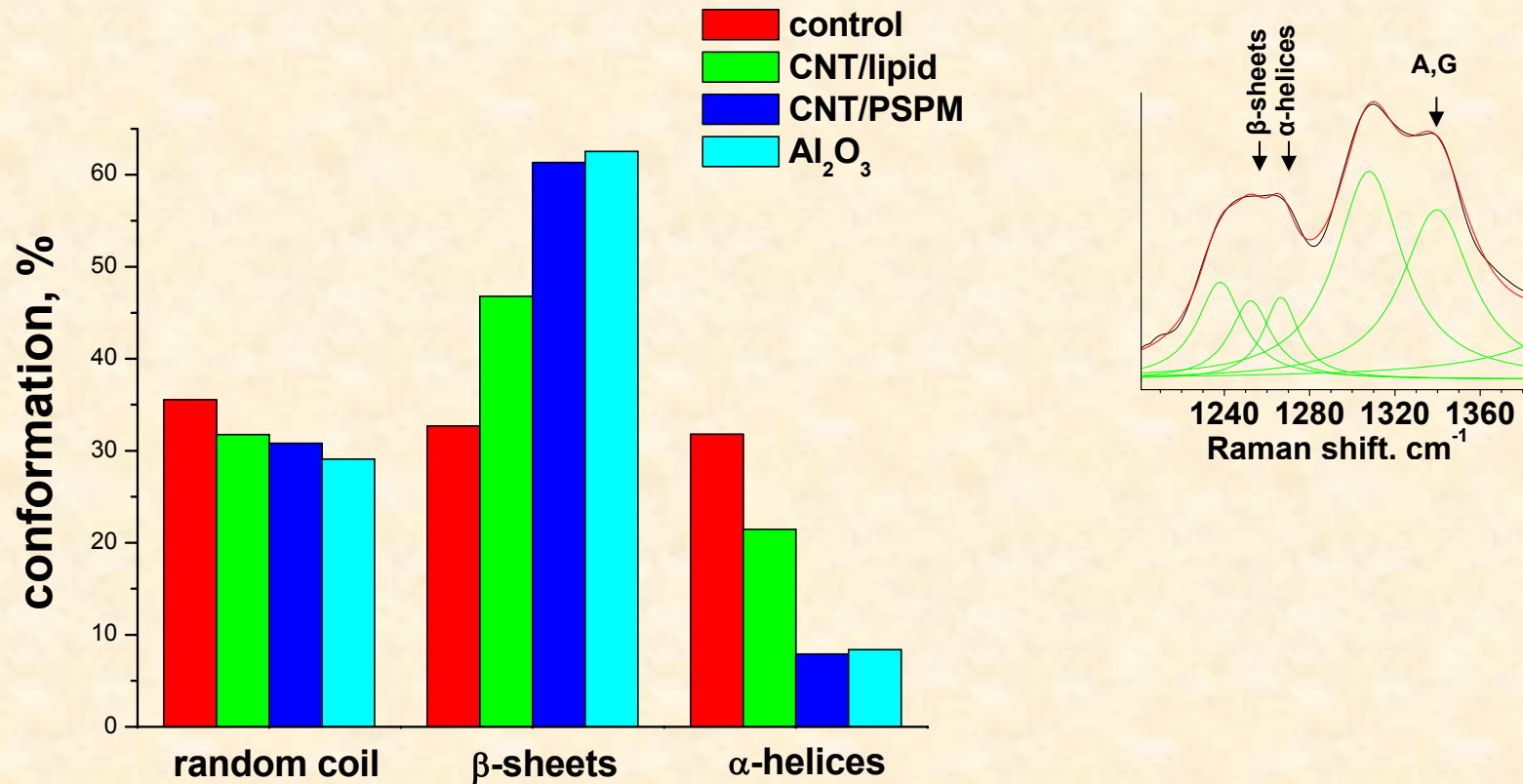
Toxicity evaluation by measuring Raman bands of A, G nucleobases and Protein.

Cells	Ratio (A,G/ amide III), %
control	100
PLGA exposed	76
Al ₂ O ₃ exposed	76
CNT/ PSPM exposed	16
CNT/lipid exposed	95



- Toxicity estimation by Raman Microscopy correlates with cytotoxicity tests
- Biomimetic lipid layers as a protection for the toxic CNT

Protein Conformation State of Cell exposed to NPs



- Relative contribution of α-helices to the amide III band compares to β-sheet decreased
- The shift of the secondary nuclear protein structure may be related
 - to the onset of nuclear fragmentation
 - may reflect a change in the rate of protein synthesis in response to NPs
 - can possibly be considered as an early manifestation of the toxic effect of NPs