Tackling confounding factors in nanomaterial hazard assessment: re-examining dose

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Metrology and dose metrics: Which metrics (metrology) should be used for MNMs in regulatory toxicology?

- **Mass is the most interesting metrics** to express the amount of MNM in contact with cells, tissue and organs.
  - This statement is justified by the fact, that for granular MNM (*aspect ratio* <2) a transfer of mass concentration to surface or number concentration is possible if particles are characterized.
  - Therefore, mass, surface and number are equivalent.

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Metrology and dose metrics: Which metrics (metrology) should be used for MNMs in regulatory toxicology?

- **The dose has to be expressed as the "deposited dose"** (amount/area), whereas the amount could be expressed in mass, surface or number.
- **The deposited dose could be estimated using models or measuring the mass of deposited particles by chemical analysis.**

Deposited dose: includes all particles at the cell surface (after washing) and up-taken

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The deposited dose could be estimated using models or measuring the mass of deposited particles by chemical analysis.

Open questions:

- Are the proposed and accepted characterization methods precise enough and reproducible, especially if the particle properties have to be measured in cell media with serum?
- Is the list of properties which has to be measured and documented (published) well known?
- Can we include the dynamic changes of agglomerate size, protein corona, surface charge?
- How important is the sticking coefficient (if the cells touch the membrane, they stick or partially desorbs)?
- Is a monolayer a relavant dosage?

Computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies

(In vitro Sedimentation, Diffusion and Dosimetry model ISDD model)

Diffusion:
Stokes-Einstein equation

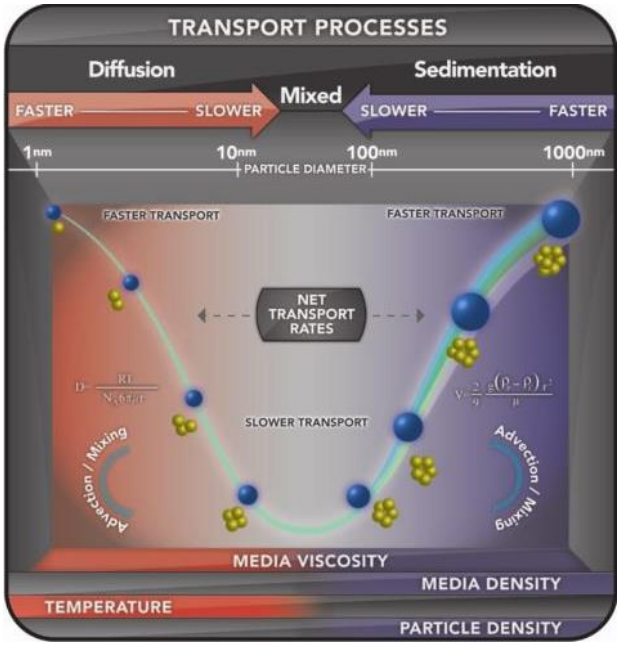
$$D = \frac{RT}{3N_A \pi \mu d_H}$$

Sedimentation:
Stoke´s law

$$u = \frac{g(\rho_p - \rho_f) d_H^2}{18\mu}$$

$$\frac{\partial n}{\partial t} = D\frac{\partial^2 n}{\partial x^2} - u\frac{\partial n}{\partial x}$$

Mason M, Weaver W: The Settling of Small Particles in a Fluid. Phys Rev 1924, 23:412-426



Conditions:

static, Non-interactive particles; No dynamic formation of agglomerates during simulation; **Spherical particles**; Initial uniform particle distribution at t=0

Hinderliter et al. Particle and Fibre Toxicology 2010, http://nanodose.pnnl.gov/default.aspx
DeLoid et al. Particle and Fibre Toxicology (2015) 12:32

Diffusion or sedimentation controlled deposition? Peclet number

The **Péclet number** (**Pe**) is a dimensionless numbers relevant in the study of transport phenomena in a continuum.

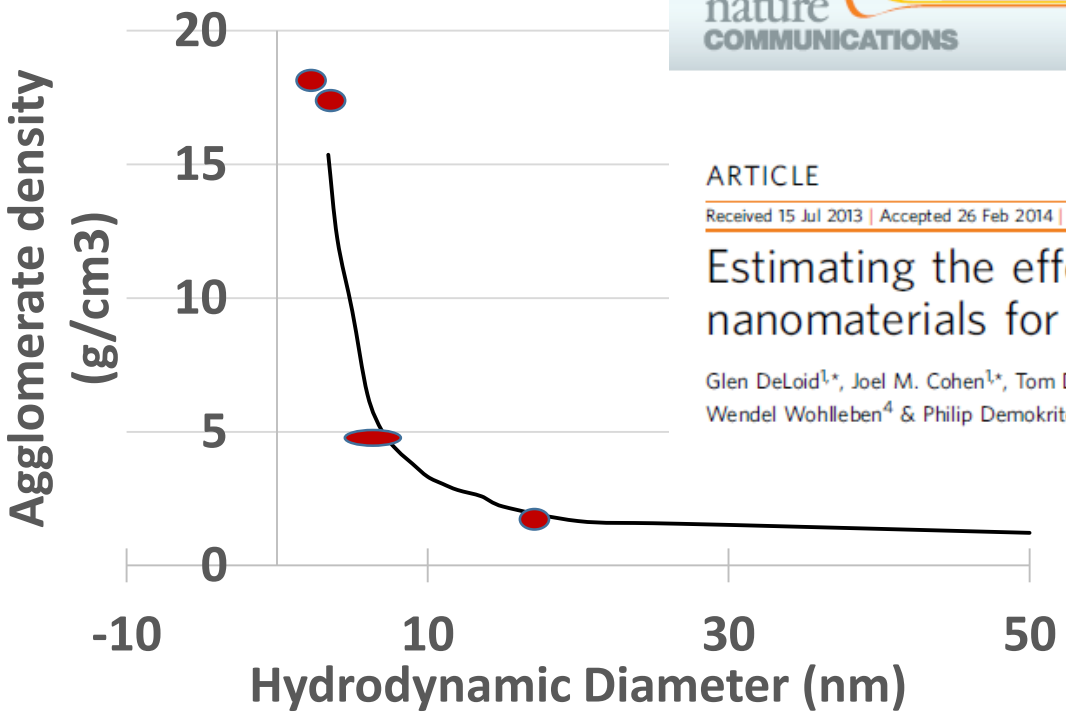
$$Pe = \frac{ul}{D} = \frac{g(\rho_P - \rho_f)d_H^2}{18\mu} l \frac{3\pi\mu d_H}{k_B T} = \frac{3g\pi}{18k_B} \frac{(\rho_P - \rho_f)d_H^3}{T} l$$

$$Pe = 3.72 \square 10^{23} \frac{(\rho_P - \rho_f)d_H^3}{T} l$$

Pe < 1 : Diffusion driven
Pe > 1: Sedimentation driven

ρ density (kg/m^3)
D_h hydrodynamic diameter
T Temperatur (K)
L characteristic length (higth of media, m)

Agglomerate size and effective density Example: Coated Gold particles



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Estimating the effective density of engineered nanomaterials for *in vitro* dosimetry

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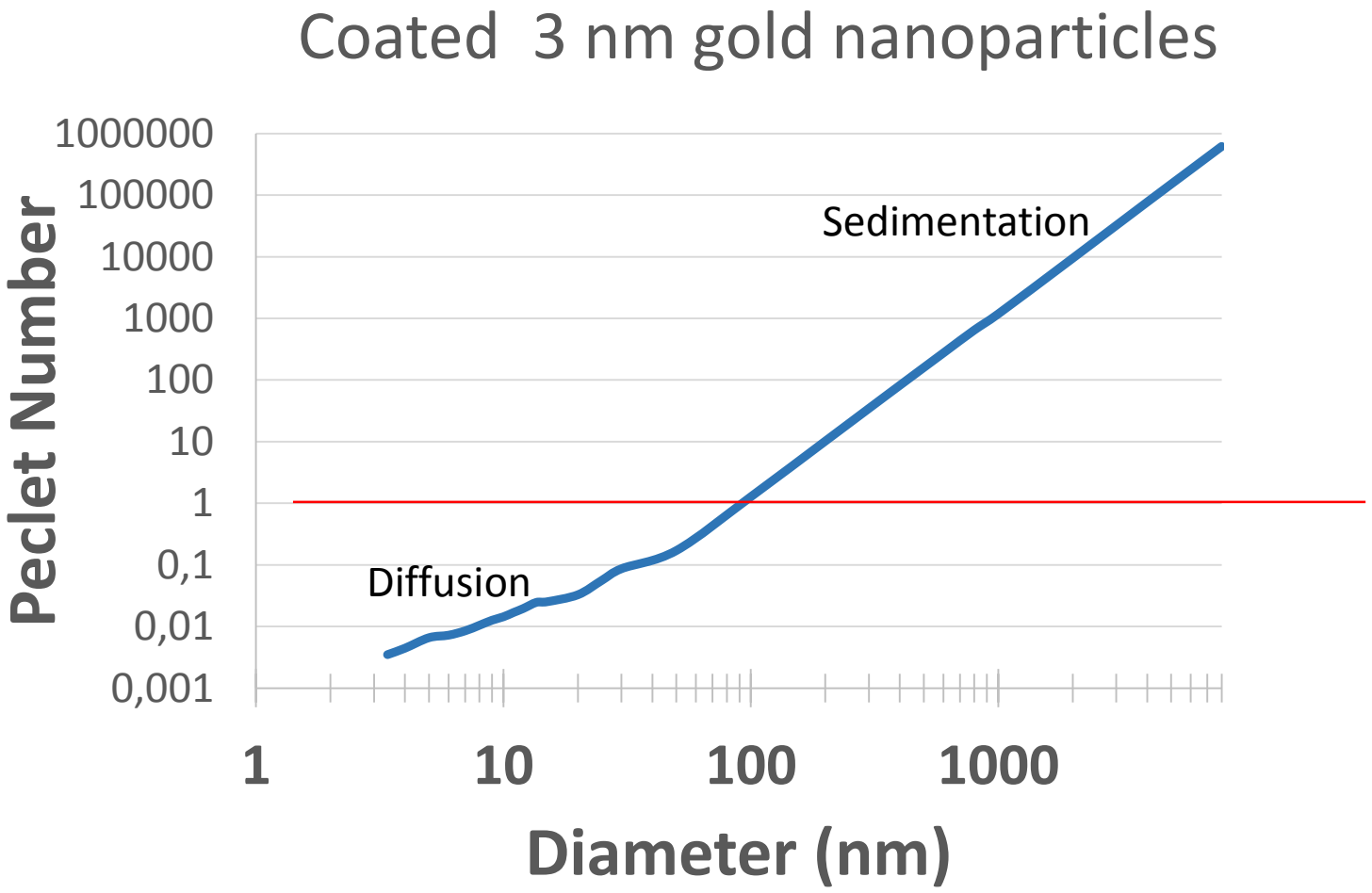
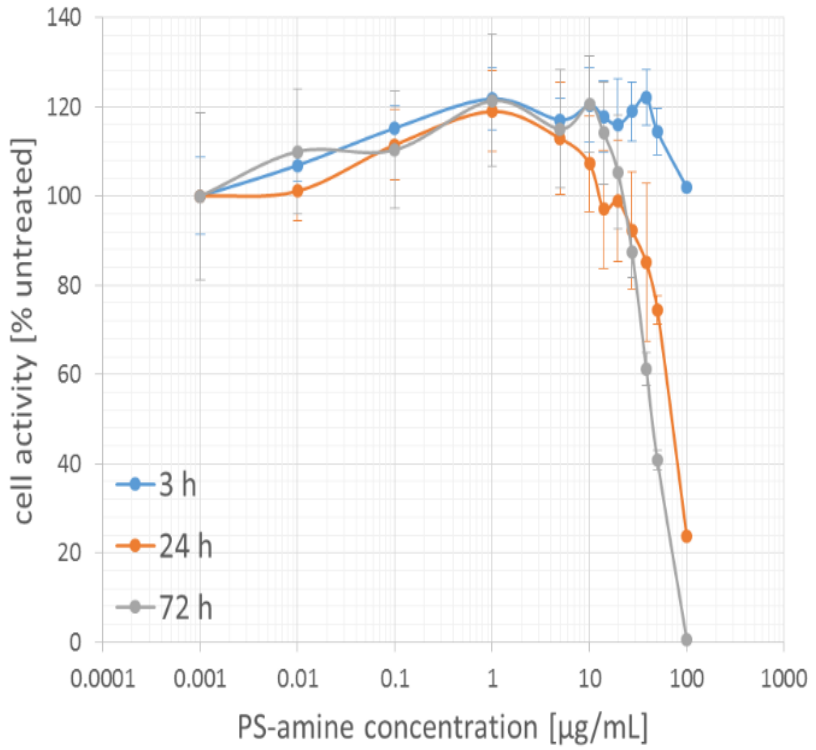
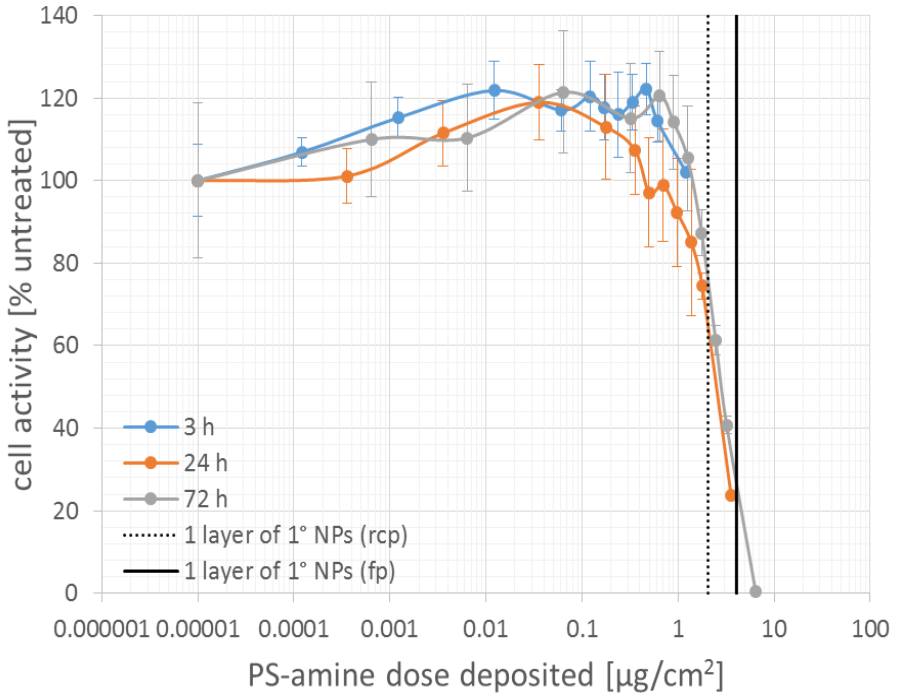
Peclet Number

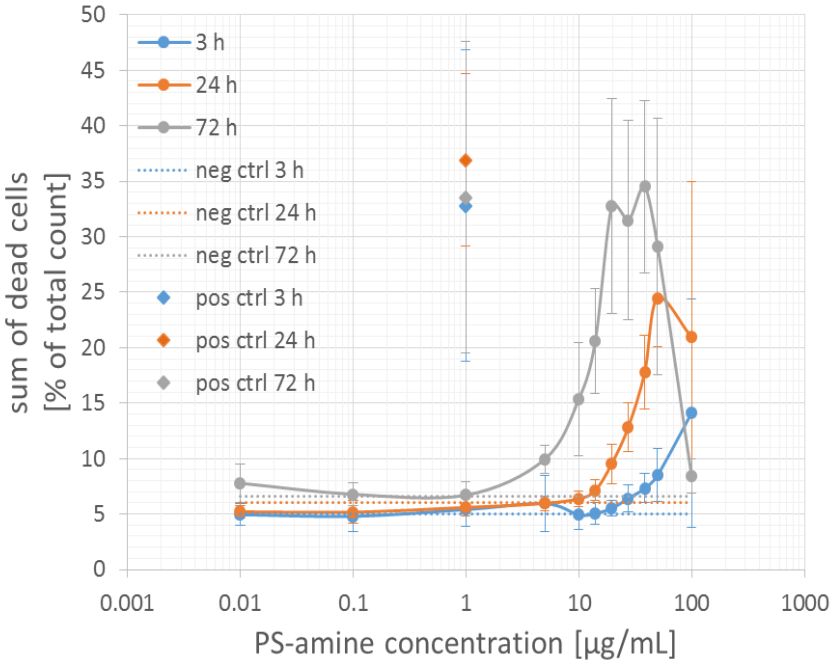


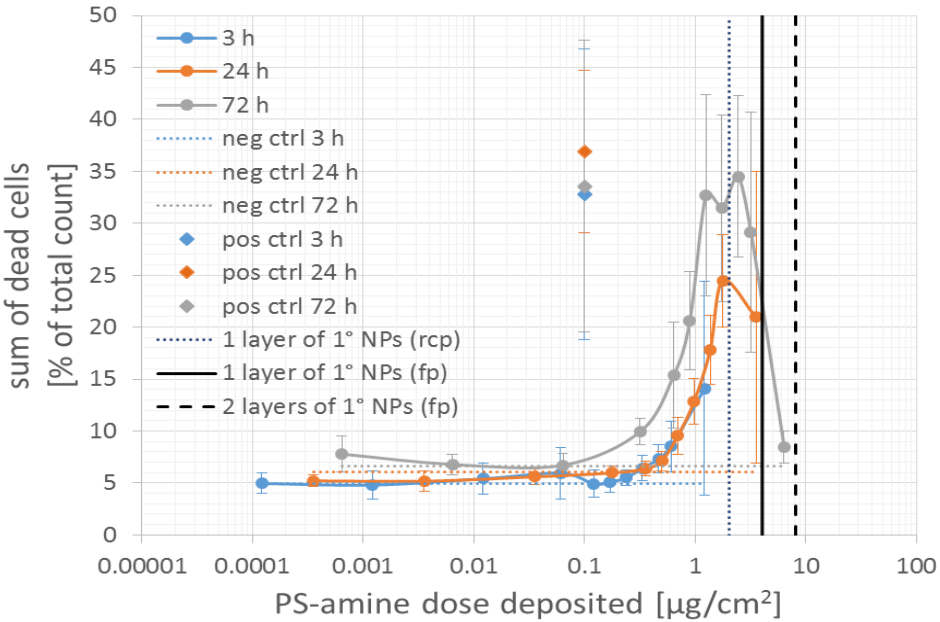
Dose-response curves of the MTS assay with A549 cells (A, B) exposed to increasing concentrations of PS-amine for 3 h, 24 h and 72 h.



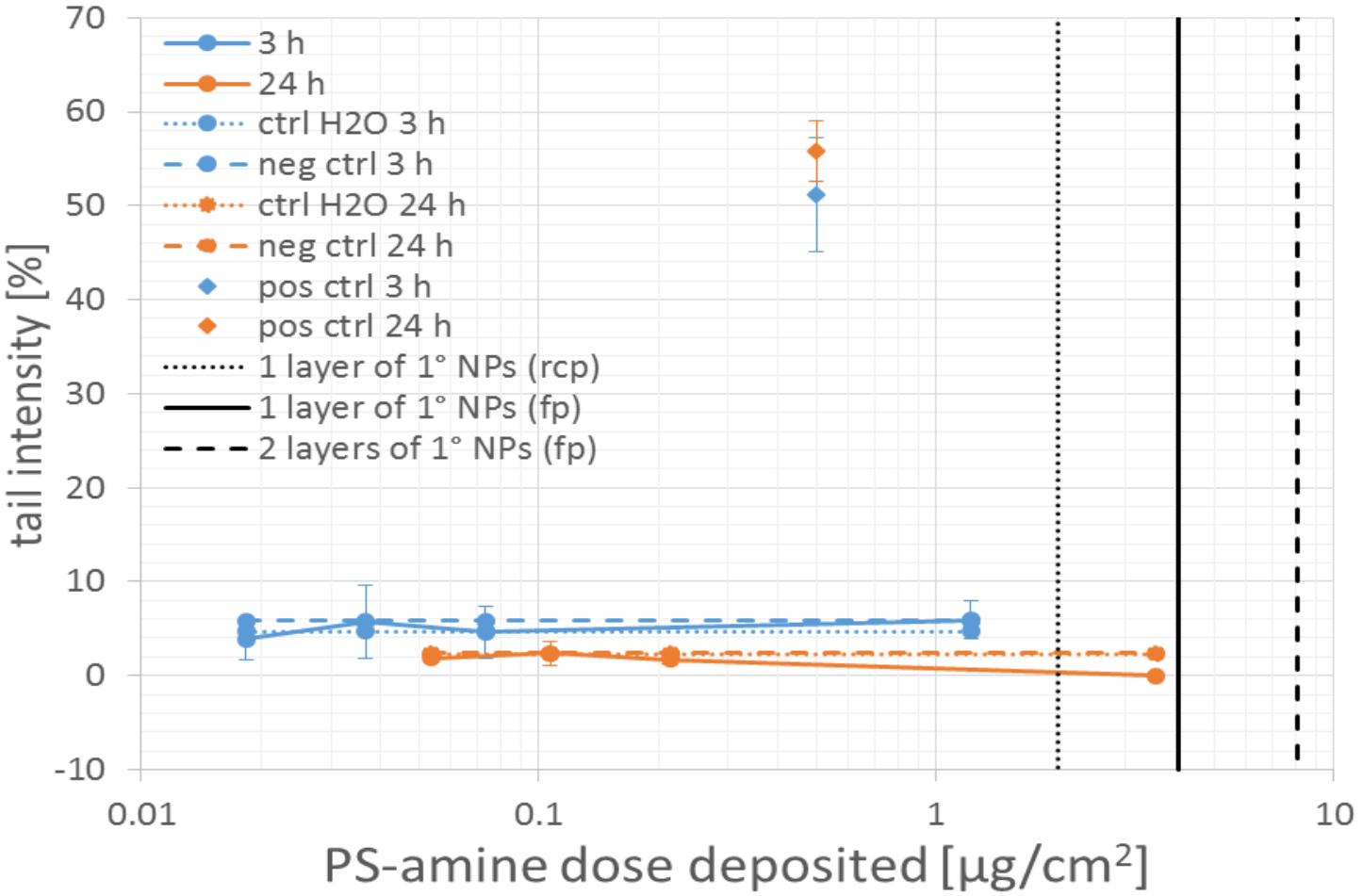


Dose-response curves of the Annexin V/PI assay with A549 cells exposed to increasing concentrations of PS-amine NPs for 3 h, 24 h and 72 h

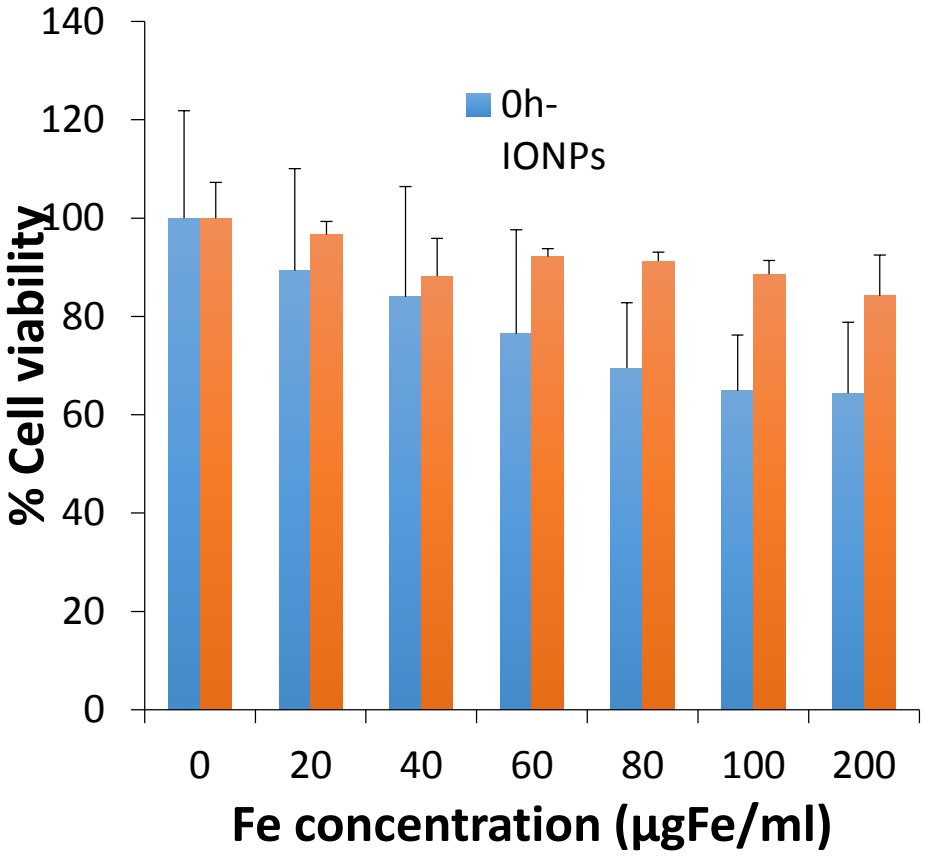


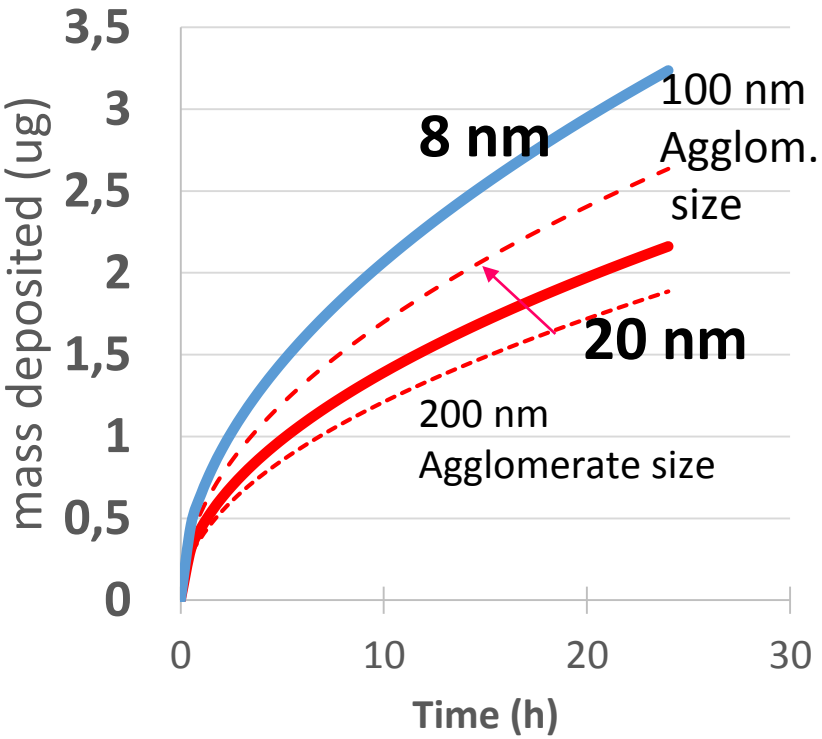


Dose-response curves of the Comet assay with A549 cells exposed to increasing concentrations of PS-amine NPs for 0.5 h, 3 h and 24 h.



Cell viability (Hela Cells) with increasing SPION concentration (8 and 2 nm particles)





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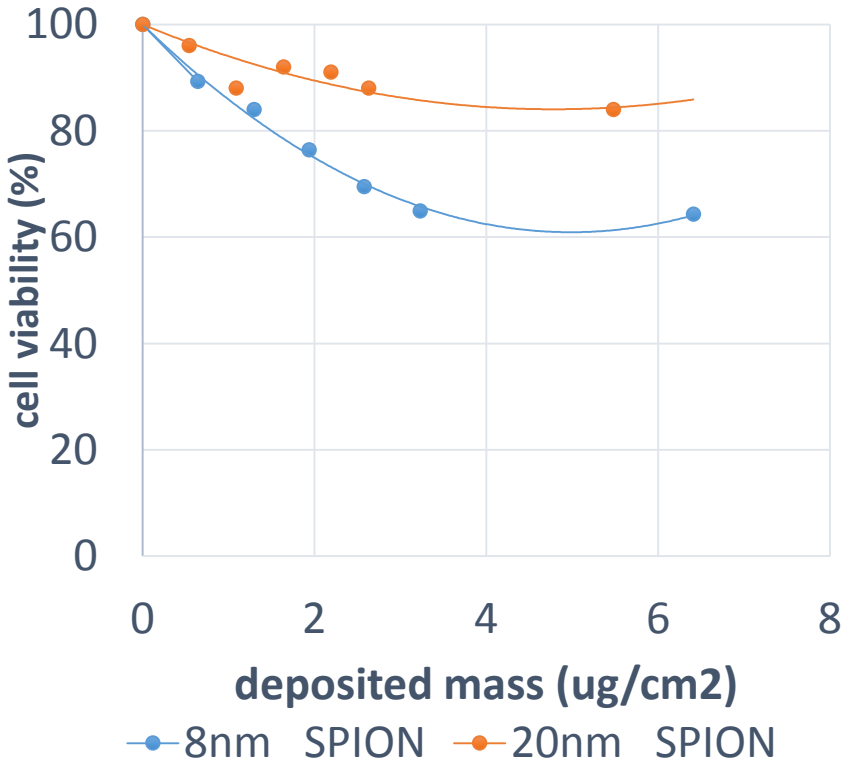
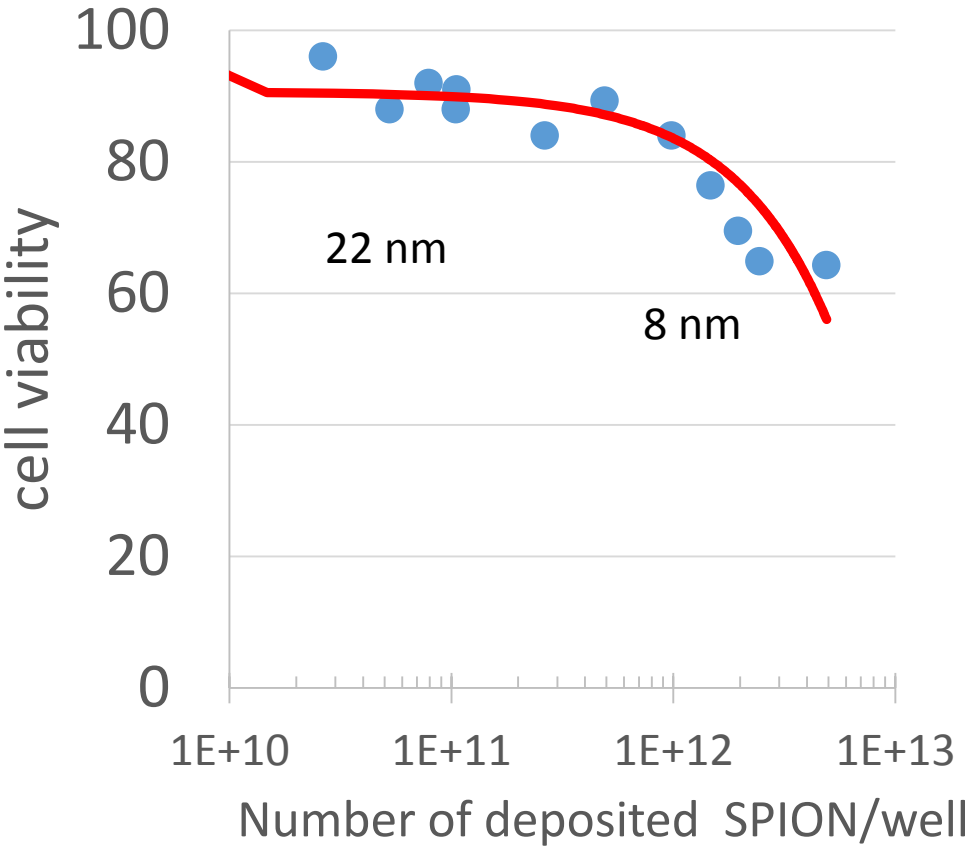
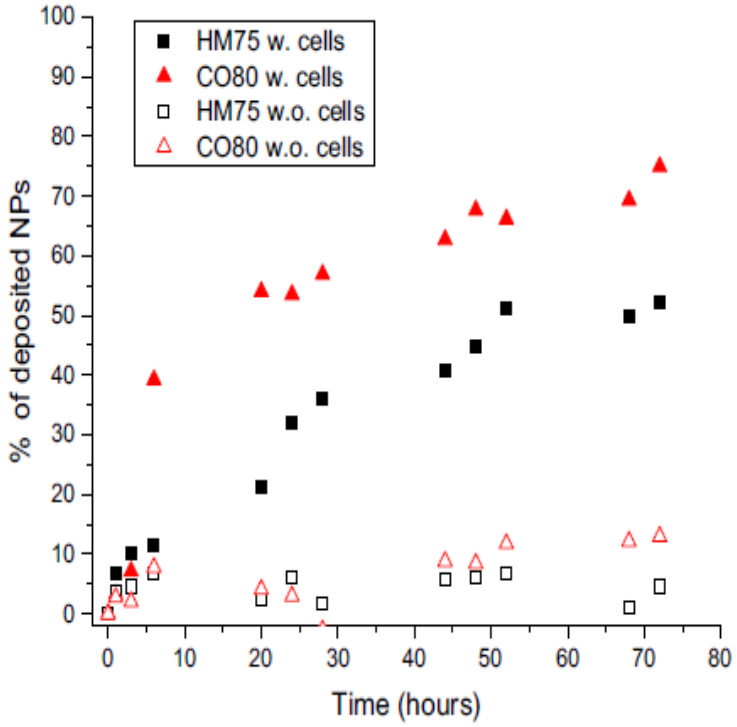




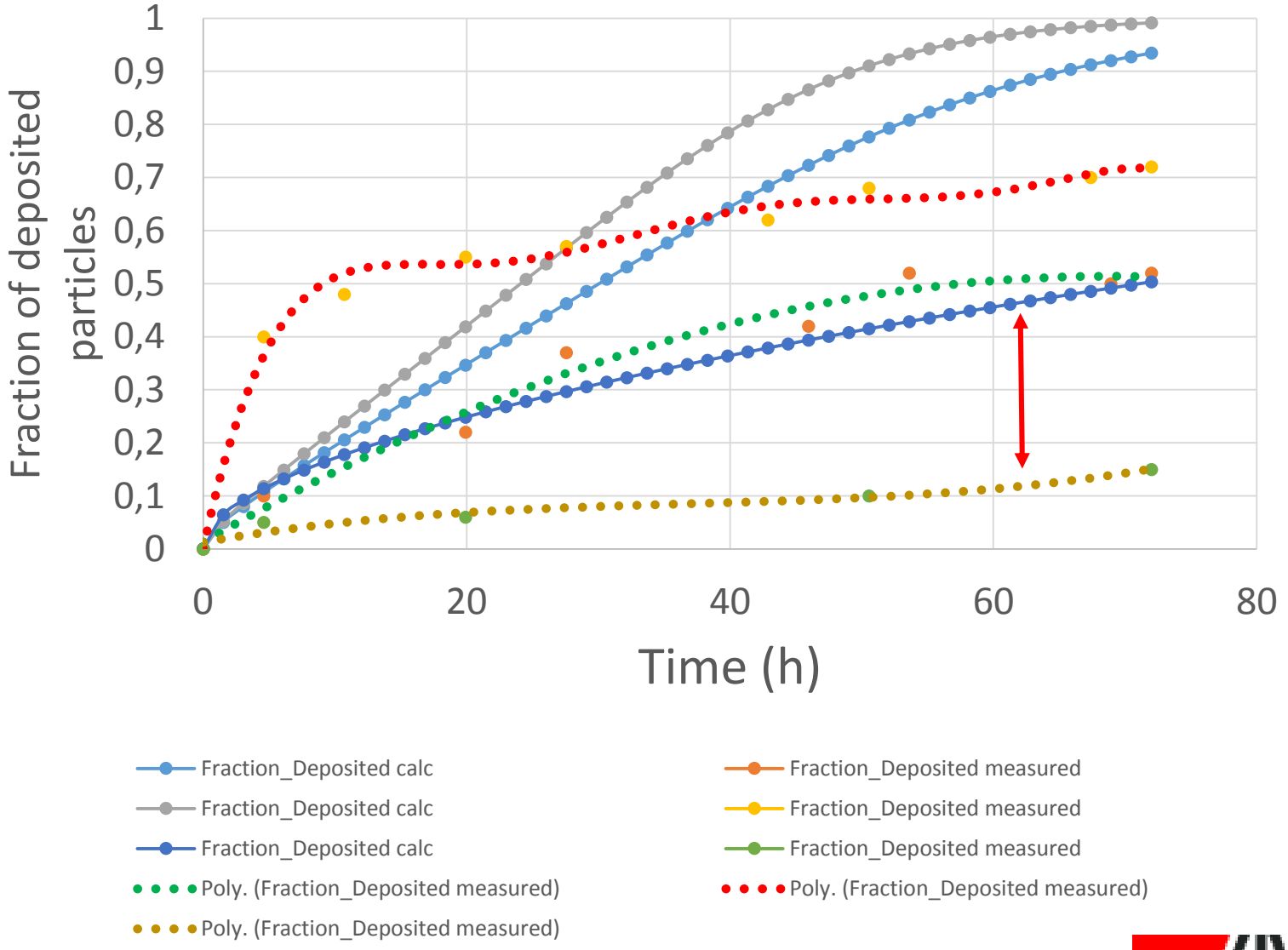
Deposition of Gold nanoparticles Comparison of experiment and calculation



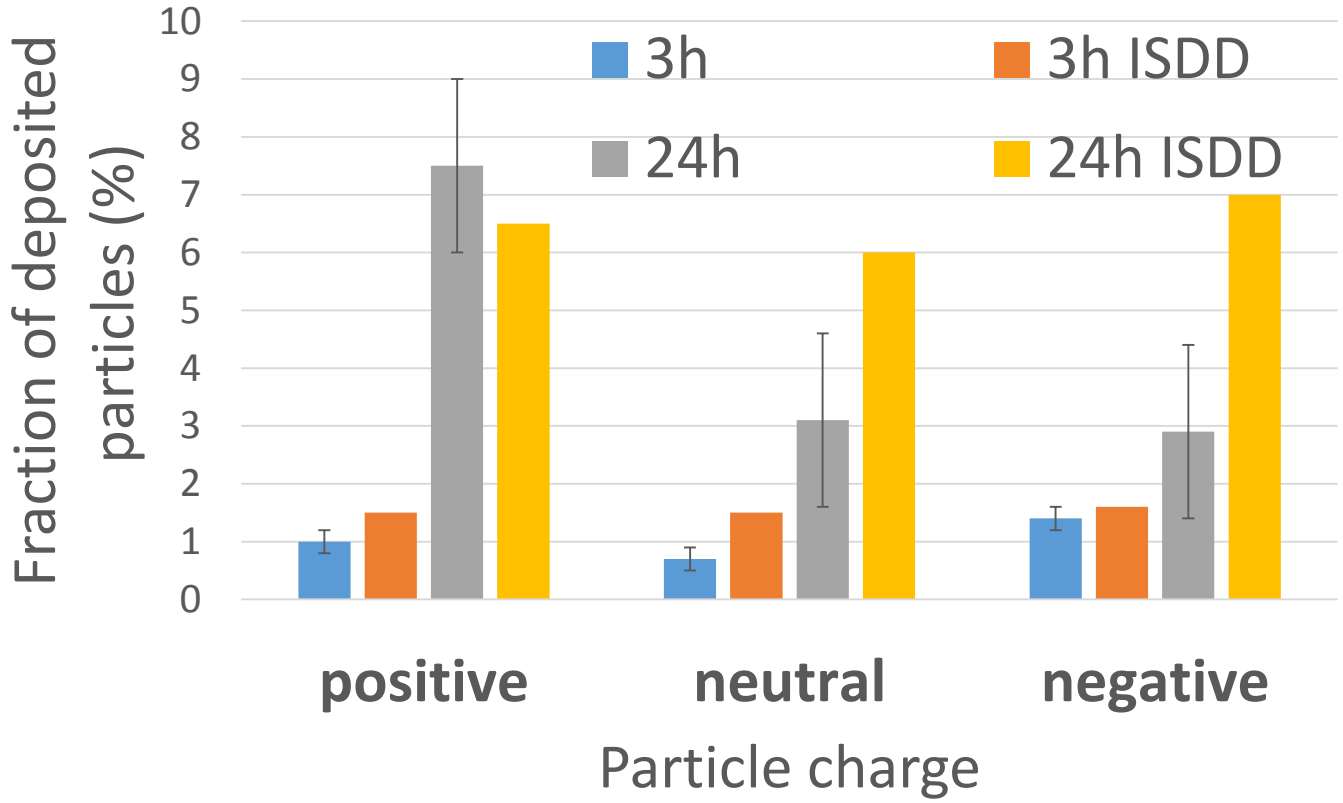
Property	CO80	HM75	CO20
Particle Diameter (nm)	73	69	15.7
Particle Density (g/ml)	19.2	19.2	19.2
Particle concentration (uM)	60	40	60
Dish depth (mm)	5.5	5.5	5.5
Volume (ml)	0.200	0.200	0.200
Temperatur (K)	310	310	310
Viscosity (Pa s)	0.00074	0.00074	0.00074
Density (media) (g/ml)	1	1	1
Agglomerate diameter (nm)	117	102	36
Agglomerate density g/ml	6.7	6.2	4.32

Rischitor et al. Particle and Fibre Toxicology (2016) 13:47

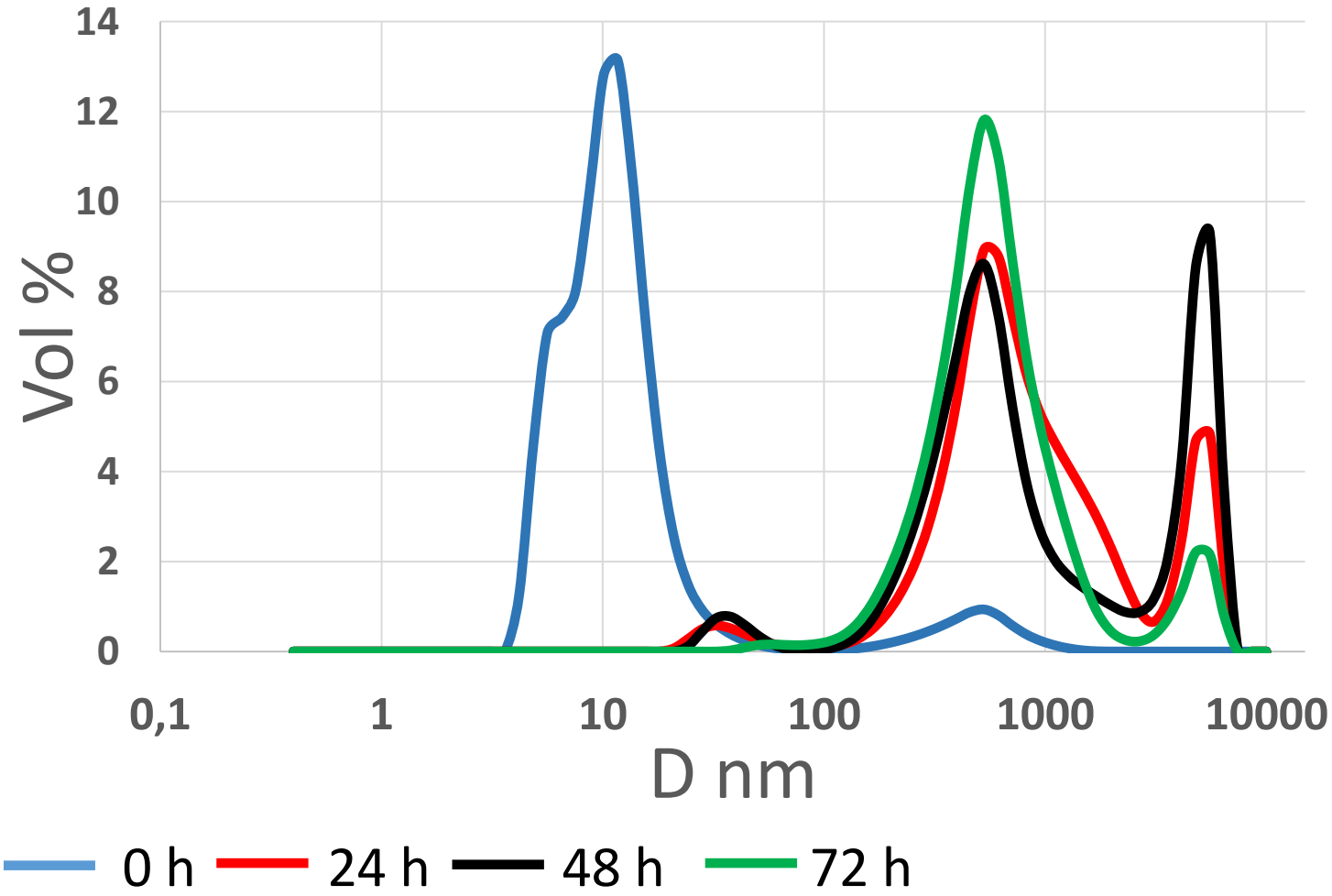
Deposition of Gold nanoparticles Comparison of experiment and calculation



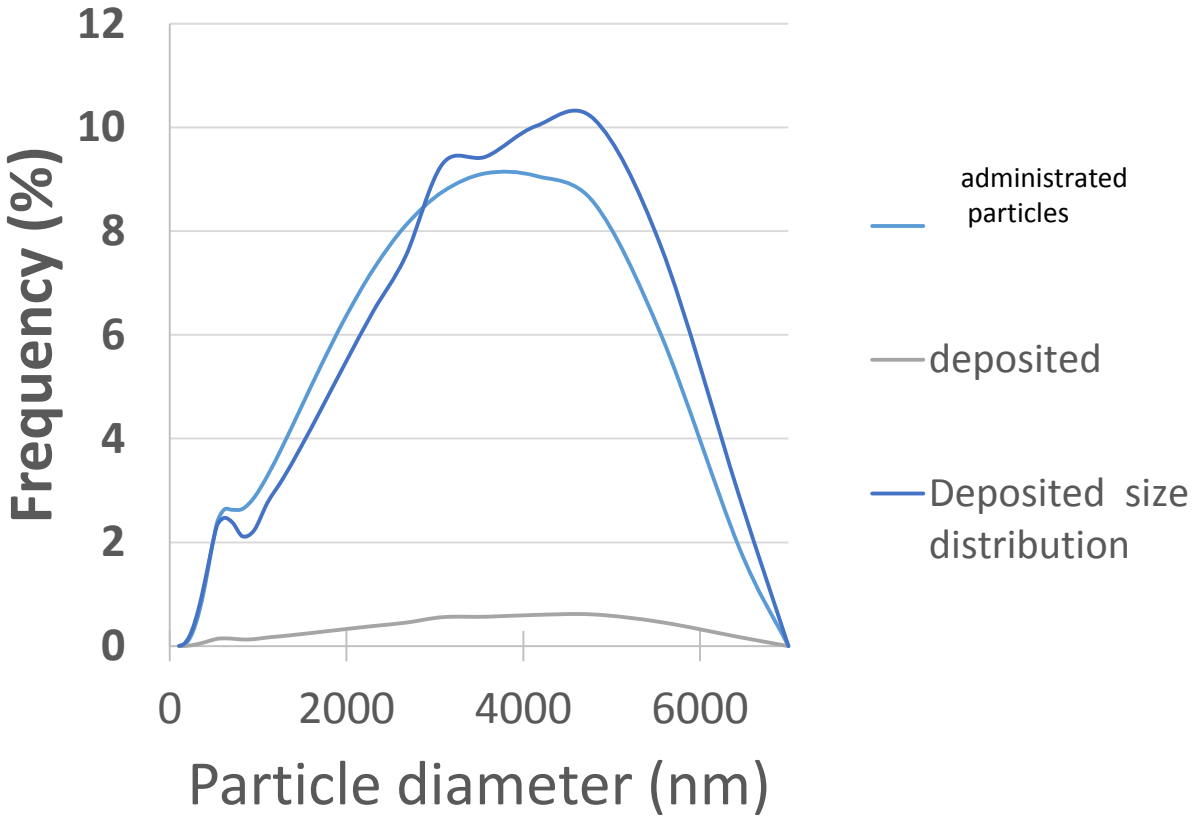
Gold nanoparticles deposited (3 and 24h) measured and calculated (ISDD model)



Agglomeration of coated gold nanoparticles in DMEM with serum



Size distribution of the administrated and deposited particles (agglomerates)



Dosage, different metrics

	3h per well	3h per cm2	24 h per well	24h per cm2
Fraction deposited (wt.-%)	1.5		6	
Number of particles	$1.49\ 10^{13}$	$1.55\ 10^{12}$	$6.96\ 10^{13}$	$7.23\ 10^{12}$
Surface (cm^2)	5.75	0.59	26.77	2.78
projected surface(cm2)	1.439	0.15	6.69	0.69
Mass (mg)	3.22	0.335	14.99	1.55

Calculation based on primary particles (no agglomeration) : deposited fraction = 40%

Conclusions

- Experimental problem with measurement of the amount of deposited nanoparticle still not solved.

- Quality of the prediction depends strongly of the quality of the particle size determination and density.
- In the case of highly agglomerated nanoparticles, the size distribution of agglomerats has no influence on the size distribution of deposited particles (agglomerates!!) → calculation per bin or using the mean value gives similar results,
- **Predicted deposited fraction and experimental measured fraction of administrated particles deposited are in a similar range.**

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- VIGO, Competence Centre of Materials, ETH board
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