Biological fate of Engineered Nanomaterials: tracing aggregation/degradation and nanomaterial dose in vitro and in vivo

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There is an urgent need for a deeper understanding of the impact of engineered nanomaterials (ENMs) on human health resulting from deliberate exposure to ENMs, such as in nanomedicine, or from accidental exposure due to handling or using devices or products containing ENMs. The characteristics of ENMs, such as shape, size, degradability, aggregation, surface and core chemistry determine their interaction with biomolecules and the ENMs fate both intracellularly and at body level. Therefore, for the assessment of ENMs toxicity is necessary to correlate ENMs characteristics with their fate and biological interactions. ENMs fate *in vivo*, distribution per organ, accumulation, biodurability and dose are fundamental to assess how ENMs affect biological functions. The physical state of the ENMs, including aggregation, the interaction with biomolecules in different cellular environments, and guide the intracellular action of nanomaterials. A fundamental aspect for understanding toxicity is to establish the relation between exposure dose of ENMs and the intracellular dose or the dose per organ. The actual dose following an exposure route is the result of the translocation of ENMs across different barriers.

Tracing ENMs in biological matrixes and moreover, determining the intracellular or organ dose of ENMs pose several challenges since ENMs are not easy to visualize and to quantify once in a biological matrix. In this presentation we will focus on the methodologies and experimental techniques, mainly from Molecular Imaging and Biophysics, which are combined to address these issues.

Several aspects of ENMs fate *in vitro* and *in vivo* will be discussed mainly in relation with ENM quantification and ENMs stability.. Cell uptake and intracellular fate of ENMs will be presented. The intracellular dose for metal oxides nanoparticles will be measured with Ion Beam Microscopy. Relations between exposure dose, intracellular dose and cell viability will be established.

Protein corona formation and the aggregation behavior of gold nanoparticles (Au NPs) will be investigated by means of Fluorescence Correlation Spectroscopy (FCS) in cell culture media and in live cells. The behavior in vitro will be compared with the level of aggregation of the NPs intracellularly. Diffusion coefficients of the NPs will be measured following NP

trafficking at different positions in the cell: the endoplasmatic reticulum, the endocytic vesicles, the cytosol and in intracellular vesicles. Fluorescence Cross Correlation Spectroscopy (FCCS) will be applied to study the intracellular stability of protein corona.
The bio distribution, organ accumulation and fate of radiolabelled ENMs will be studied in animal models by means of Positron Emission Tomography (PET). NPs dose per organ will be evaluated. A dual radiolabelling strategy of nanoparticle core and coating will be presented using gamma emitters with non overlapping emission bands. After intravenous administration into rats, energy-discriminant Single-Photon Emission Computerised Tomography (SPECT) resolve each radioisotope independently revealing different fate in vivo for the core and coating, which will be used to evaluate NP integrity.

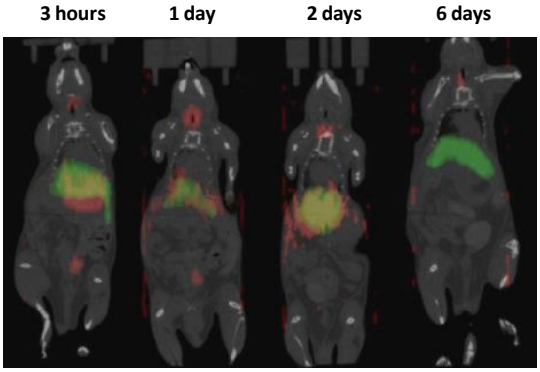

Figure.1 Coronal SPECT images of the biodistribution of dual radiolabelled NPs after intravenous administration in mice; ^{125}I: red colour; ^{111}In: green colour.