European Commission | Horizon 2020 European Union funding for Research & Innovation

SmartNanoTox
Smart Tools for Gauging Nano Hazards

SMART TOOLS FOR GAUGING NANO HAZARDS

NMP-29-2015: Increasing the capacity to perform nano-safety assessment

Figure 1: Partners of the SmartNanoTox project

Scientific and Techological challenges:

Relating the NM descriptors to the toxicity end point is at present practically unfeasible. Most approaches use black-box search for statistical correlations between the descriptors and toxicity indicators. We consider that **progress can be made using adverse outcomes (AO) pathway-based mechanism-aware intelligent QSARs**.

Another challenge is to **determine all the hazardous effects of engineered NMs** using common *in vitro* studies of the toxic effects, which address only acute responses and cytotoxicity. A more complete picture can be obtained using ***in vivo* study of the acute and chronic toxic effects**, with relation to the adverse outcome, in depth analysis of the pathologies.

Understanding of the mechanisms underlying the observed adverse effects from engineered NMs is not possible in most cases as the **state of the NM inside the tissues is not known**. The state of the NM after the initial contact, in particular their biomolecular corona, must be analysed. **NMs must be tracked inside the biological fluids and molecules involved in bionano interactions identified**.

SmartNanoTox approach:

- **Identification of underlying pathways ('toxicity pathways') for NM interactions with living organisms**

  We perform comprehensive analysis (transcriptomics, proteomics, histopathology) of *in vivo* pulmonary toxicity and use systems biology for a number of representative engineered NMs and extensive data mining to identify the TPs and related AOs.

- **Development and demonstration of a mechanism-based understanding of the toxicity**

  We identify and analyse *in vivo* pulmonary toxicity pathways for a number of engineered NMs, track the path of the NMs inside the organism to identify the molecular initiating events/key events (MIE/KE) for each pathway understand how the NM triggers or steers the AOP via inducing the corresponding KEs. The triggering effect will be verified experimentally for the affected pathways.

- **Linkage of the potential for adverse effects to specific physical or chemical nanoscale properties**

  We analyse the whole set of molecular interactions at the bionano interface after the NM uptake in the lungs and into individual cells and their distribution beyond.

- **Creation of a basis for grouping of engineered NMs by the toxic action**

  We systematically study, *in silico*, *in vitro*, and *in vivo*, the main groups of engineered NMs to identify the properties responsible for triggering the toxicity pathways (TP) and determine the groups of NMs by their ability to cause particular MIE/KE.

Project progress:

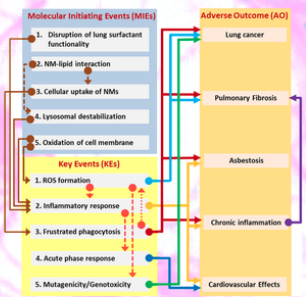
The first 12-month period of the project was focused on an analysis of the existing information and a development of an optimum strategy to identify *in vivo* AOPs for respiratory exposure to NM. We also developed, tested and validated methods for NM tracking inside the biological samples, post-uptake characterisation and bionano interface modelling. Our main achievements for the period are as follows:

- **Work Package 1:**
  - Identified 5 respiratory AOPs that can be addressed within the project
  - Selected a set of relevant NMs suitable for aerosolisation and study of the chosen AOPs
  - Performed transcriptomics analysis of lung samples after in vivo exposure to MWCNT from NANoREG
- **Work Package 2:**
  - Developed a protocol to assess fluorescent probe desorption from NM
  - Applied the protocol for ensuring the quality of labelled NM at three major phases of experiments: functionalisation of NM, labelling and free probe removal
  - Developed a NM labelling technique by embedding europium atoms into the TiO2 crystal lattice during TiO2 NP synthesis, which enables fluorescent imaging of NP after long-exposure in vivo experiments
  - Developed a set of in vitro tests consisting of pristine NPs and model membranes to study the evolution of NP wraps and alleviate the determination of MIEs
  - Refined the procedure for the analysis of NM protein corona
  - Identified 5 respiratory AOPs that can be addressed within the project
  - Selected a set of relevant NMs suitable for aerosolisation and study of the chosen AOPs
  - Performed transcriptomics analysis of lung samples after in vivo exposure to MWCNT from NANoREG
- **Work Package 3:**
  - Selected NMs, doses and protocols to identify the relevant TPs
  - Isolated coated nanoparticles from in vivo samples from mice and identified proteins in corona by mass-spectrometry
  - Performed proteomics analysis of in vivo samples after exposure to asbestos and CNTs
  - Performed pathway reconstruction for several in vivo samples with MWCNTs
- **Work Package 4:**
  - Developed a multiscale method of modelling of NM-biomolecule interaction using two-layer NM model and potentials of mean force NM-aminoacid from atomistic simulations
  - Calculated adsorption free energies of aminoacids to various CNTs
  - Predicted 3D structures for over a 100 plasma proteins identified in NM protein corona

Adverse Outcomes:

We have identified 5 AOs for the study. For each AO pathway, Molecular Initiating Events and Key Events will be determined:

- Chronic inflammation
- Enhanced lung cancer prevalence
- Asbestosis
- Pulmonary fibrosis
- Cardiovascular effects



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grand agreement No. 686098. http://www.smartnanotox.eu/

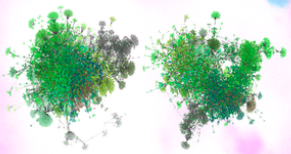
SmartNanoTox materials:

We have selected 61 NM for *in vivo* respiratory toxicity studies:

Asbestos; 28 CNTs: NM400, NM401, NM402, NM403, Mitsui-7, etc.; 12 TiO2: NM101, NM103, NM105, TiO2 tubes, etc.; 5 SiO2s: quartz DQ12, NM200, etc.; Fe2O3; ZnO; Carbon black; etc.

In vitro toxicity studies were performed using cell lines. 17 NMs were first selected (7 metal oxides (NM110 ZnO, NM100 TiO2, NiZnFe4O8, ZnFe2O4, NiFe2O4, Fe2O3-NRCWE-018, Fe2O3-NRCWE-019) and 10 CNT (NM401, NM403, NRCWE-040, NRCWE-042, NRCWE-045, NRCWE-048, NRCWE-049, NRCWE-051, NRCWE-055, Printex 90).

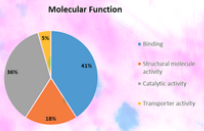
Gene regulation networks:



NM401 NRCWE26

We have generated the gene regulatory networks for two of the NM included in the SmartNanoTox list of NM. NM401 and MCRWE26, are both carbon nanotubes but have different physicochemical properties. Using Cytoscape software, we have visualised the GRN for these NMs and visual comparison already shows that the topology of both networks is clearly specific for both NMs, which can only be explained by the different physicochemical properties of both NMs.

Analysis of NM protein corona:

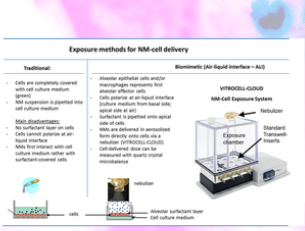


Molecular functions of proteins in the Crocidolite protein corona.

Employing Maxquant the spectra obtained from the peptides bound to the NM were analysed, calculating the relative protein abundance using Maxquant's inbuilt label free quantification. Analysis of the protein corona identified over 360 proteins specifically binding to the crocidolite NM.

Exposure techniques:

Comparison of the traditional (submerged cell culture conditions) - with several physiological disadvantages - and the biomimetic approach (air-liquid interface (ALI) culture conditions) chosen by the SmartNanoTox consortium. ALI cells are supplemented with a realistic amount of alveolar surfactant for modelling of the alveolar tissue barrier. The VITROCELL-CLOUD system allows for dose-controlled, aerosolized NM-cell exposure under physiological conditions (see Figure 2c) and biomimetic modelling of the sequence of molecular events encountered by NMs upon deposition on the lung epithelium.



NM-cell exposure methods and description of SmartNanoTox approach.

Figure 2 (background of the poster): Nanomaterial (blue) tracked within living lung epithelial cells with labeled membrane structures (purple) as seen by superresolution STED microscopy (scale is on the right)

2 µm