Project overview

Horizon 2020 RIA NMBP call “Increasing the capacity to perform nano-safety assessment”
SmartNanoTox: Smart Tools for Gauging Nano Hazards
Overall funding: €8M
Duration: 48 months
Project consortium: 11 partners
Coordinator: University College Dublin
Where we are

• Limited capacity to predict hazard for new materials as the properties of concern are not known

**Standard NM characterisation is not sufficiently informative**

**Toxicity mechanisms are not known**

• Real dosage/NP state after uptake not known
• Many in vitro toxicity endpoints (e.g. EC50) are irrelevant
Mechanistic understanding of nanotoxicity

New level of complexity:

• Knowing the nanomaterial chemistry and structure is not enough: coating, adsorbed materials, surface energy, dielectric properties may be important

• Nanoparticles use specific ways of systemic distribution, which are unavailable for individual molecules or micron-sized particles. Exposure route can be equally important

• Toxicity and adverse outcomes may be related to molecular perturbation of cell structures/pathways and not direct damage
Mechanistic Understanding of Toxicity

New toxicity assessment paradigm

Pathway-based assessment:

Understanding of bionano interactions is needed to address Molecular Initiating Events, systemic transport
Project objectives

• Identify main **pulmonary adverse outcomes** induced by common NMs, and identify associated MIE, KEs and toxicity pathways leading to AO.

• Establish **relationships between physchem properties of NMs and KEs** steering the TP leading to AO, and suggest descriptors for grouping of NMs according to toxicological mode-of-action

• Create a **database of bionano interactions** that will enable development of read-across and QSAR tools for the toxicity assessment of new NMs

• Develop a **smart screening approach**, where predictions of toxicity of a NM can be made on the basis of purely computational or limited *in vitro screening tests focused on crucial bionano interactions*
SmartNanoTox approach
SmartNanoTox methods

Molecular simulation

In vitro exposure

In vivo exposure

Binding free energies of amino acids to NP

Outer corona surface defines further fate of NP
SmartNanoTox methods

Omics, systems biology

NP tracking, post-uptake characterisation

Analysis workflow

Biochemical characterisation of MIEKE

In vitro
Secretome
Targeted proteomics
  - RPPA
  - Multiplexed ELISA

In vivo
Corona Proteomics
Lung lavage

Infer signal transduction networks that connect NP corona, secretome and regulated signalling networks with toxicity pathways

Toxicity readouts
  - Cell proliferation
  - Apoptosis
  - Membrane
  - Cell cycle

Individual NP treatment

Computational network inference by Bayesian variable selection

How nanoparticle-lipid/protein contact will be identified?

- Isolated fluorophores:
- Distant fluorophores:
  - More than 2-4 nm

- Close fluorophores:
  - Less than 2-4 nm

- Nanoparticle-bound fluorophores
- Membrane-bound fluorophores

No identified contact between lipid and NP

Identified contact between lipid and NP

NN401
NCRWE26
Beyond the state-of-the-art

<table>
<thead>
<tr>
<th>S&amp;T Objective</th>
<th>SmartNanoTox offers:</th>
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<tbody>
<tr>
<td>Relating the NM descriptors to the toxicity end point</td>
<td>AO pathway-based mechanism-aware intelligent QSARs</td>
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<tr>
<td>Determining the hazardous effect of engineered NMs</td>
<td>In vivo study of the acute and chronic toxic effects, with relation to the adverse outcome, in depth analysis of the pathologies</td>
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<tr>
<td>Understanding of the mechanisms underlying the observed adverse effects from engineered nanomaterials</td>
<td>The biomolecular corona of the NPs will be analysed, NMs will be tracked inside the biological fluids and molecules involved in bionano interactions identified</td>
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<td>Create basis for grouping of engineered nanomaterials by toxic action</td>
<td>Identify generic properties responsible for pulmonary toxicity for carbonaceous materials</td>
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projected impacts

- Described and validated respiratory AOPs
- Database of bionano interactions for 60+ NMs with proteins and lipids
- Identified NM properties of concern
- Mechanism-aware toxicity assessment tools
- Methods for NM tracking inside the tissues and post-uptake characterization
- Replacement of animal experiments by in vitro/in silico tests
Molecular Initiating Events (MIEs)

1. Disruption of lung surfactant functionality
2. NM-lipid interaction
3. Cellular uptake of NMs
4. Lysosomal destabilization
5. Oxidation of cell membrane

Key Events (KEs)

1. ROS formation
2. Inflammatory response
3. Frustrated phagocytosis
4. Acute phase response
5. Mutagenicity/Genotoxicity

Adverse Outcome (AO)

Lung cancer
Pulmonary Fibrosis
Asbestosis
Chronic inflammation
Cardiovascular Effects
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<th>Detection Assays</th>
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<td>Constrained drop surfactometer, MD simulation</td>
<td>Cell free (Surfactant)</td>
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<td>Alveol. cells</td>
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<td>NM-lipid interaction</td>
<td>Microscopy (FRET, FRET-FMS, pFMS, DLS, EPR), MD simulation</td>
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<td>Cellular uptake of NMs</td>
<td>Microscopic localization (pFMS)</td>
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<td>Lysosomal destabilization</td>
<td>Membrane leakage, cell viability, MD simulation</td>
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<td>Oxidation of cell membrane</td>
<td>Antioxidant depletion, ‘Band Gap’ calculation</td>
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<td>ROS formation</td>
<td>Redox sensitive dyes &amp; marker genes, Electron paramagnetic resonance (EPR)</td>
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<td>Inflammatory response</td>
<td>Inflammatory gene &amp; protein expression</td>
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<td>Frustrated phagocytosis</td>
<td>Lysosomal damage, inflammasome activation</td>
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NP-protein interactions
Bio/Nanoinformatics approach

Prediction of corona content using NP and protein descriptors:

Sequence descriptors (PepStat), 3D structure (I-TASSER)
NP-protein interactions
Bio/Nanoinformatics approach

Prediction of Key Events of the AOP using protein descriptors:

Experimental data from Walkey et al. ACS Nano 2014.
Kamath et al. Current Topics in Medicinal Chemistry, 2015
NP-protein interactions
Bio/Nanoinformatics approach

- Atomistic MD of NM and biomolecule segment interaction
- Ab initio and atomistic MD of NM NM descriptors
- CG model of biomolecules
- Adsorption energies
  - Biomolecule structure biomolecule descriptors
- CG model of biological fluids
- QSARs: Biomolecule adsorption energy, NM fingerprint
  - CG modelling of competitive adsorption, biointerface structure
- NM safety assessment