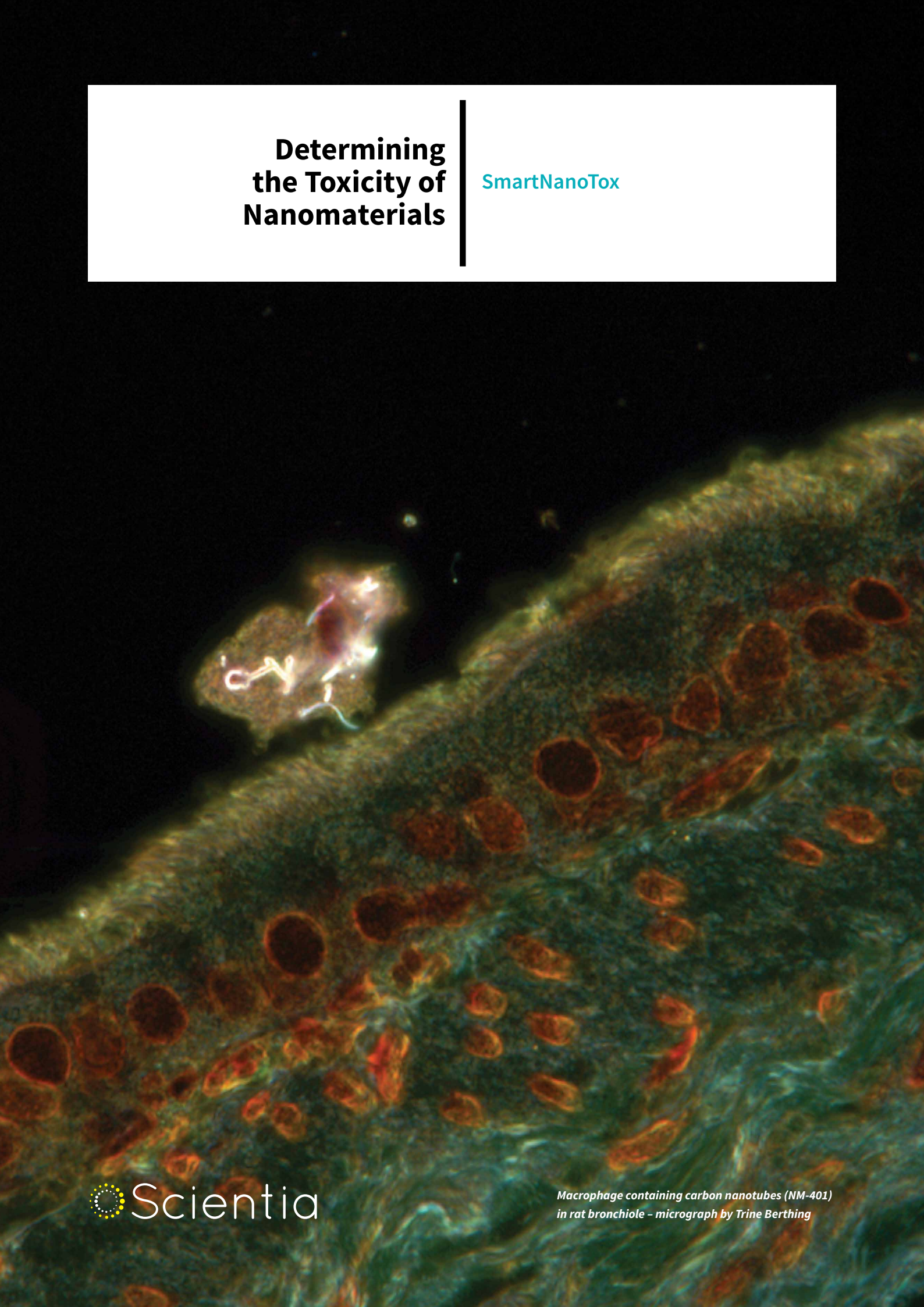
Determining the Toxicity of Nanomaterials

SmartNanoTox



Scientia

Macrophage containing carbon nanotubes (NM-401) in rat bronchiole – micrograph by Trine Berthing

SMARTNANOTOX: DETERMINING THE TOXICITY OF NANOMATERIALS

Nanomaterials, owing to their unique material properties and activities, are popular for applications involving the detection and diagnosis of genetic and life-threatening illnesses, such as cancer. However, there is an academic and public concern over nanomaterial toxicity and their long-term adverse effects on the immune system. **The SmartNanoTox** team, comprising academic and industrial experts in *in-vivo* toxicity, was established to resolve the intricate mechanisms underlying nanotoxicity, and provide an efficient approach to predict the toxicity of nanomaterials.

The term nanomaterial describes a material that is formed of individual units or particles that measure on the scale of 10^{-7}–10^{-9} metres in length. Each particle may be spherical, rod-shaped or fibrous with a relatively large surface area, depending on the fabrication methods and experimental conditions employed. At such dimensions, materials display unique optical, electronic and mechanical properties, often in remarkable contrast to their bulk counterparts. Although these materials are extensively used across the healthcare, environmental and automotive industries, questions about the potential effects that nanomaterials may have on human health, and how severe these effects may be, remain unanswered. This grey area of understanding has led to controversy over the safety of nanomaterials, creating a public perception of fear surrounding these critical functional materials.

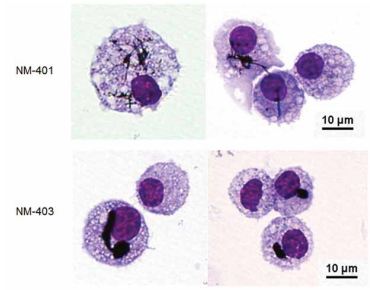
The specific mechanisms within living organisms that lead to adverse health effects after exposure to nanomaterials remain poorly understood. While some common industrial materials such as asbestos or quartz nanodust can cause direct damage to animal or human lungs when inhaled, the effects of the many other nanomaterials can be indirect, delayed and complicated. Nanomaterials can accumulate within tissues and cause a chronic inflammation or distortion of the normal biochemical environment by producing reactive oxygen species or interacting with key biomolecules. There is a current inability to accurately predict, based on their structure and activity, if a particular nanomaterial will display long-term toxicity effects in human cells and tissues.

Nanomaterials can also enter a cell through its membrane receptors and cause adverse effects, such as the deregulation of cellular pathways. Deregulation and deformation of receptors and protein enzymes that participate in regulatory pathways can lead to the formation of neurodegenerative diseases, cancers, fibrosis and diabetes. Hence, a material that interferes with the regulation and function of these pathways in cells is also deemed as toxic. Although these networks have been extensively studied in numerous disease and DNA studies, there remains a distinct lack of understanding of the intrinsic mechanisms that lead to cell deregulation in the presence of nanomaterials.

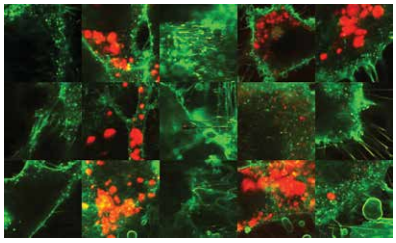
SmartNanoTox: Smart Tools for Gauging Nano Hazards

Dr Vladimir Lobaskin at the School of Physics, University College Dublin, Ireland, coordinates the SmartNanoTox project, which is funded by the European Union's Horizon 2020 research and innovation programme. The SmartNanoTox team is a collaboration between academics and industrial professionals with extensive experience in the field of *in-vivo* nanotoxicity, biophysics, materials science, and industrial marketing. With members in Ireland, France, Germany, Slovenia, Denmark, Finland, Sweden and the UK, the team was recently established to further understand and resolve the underlying mechanisms of nanomaterial toxicity when inhaled. The primary aim of the project is to develop a mechanism-aware toxicity screening approach for nanomaterials.

Existing methods for predicting the toxicity of nanomaterials are based on measurements of toxicity performed on cell cultures *in-vitro*

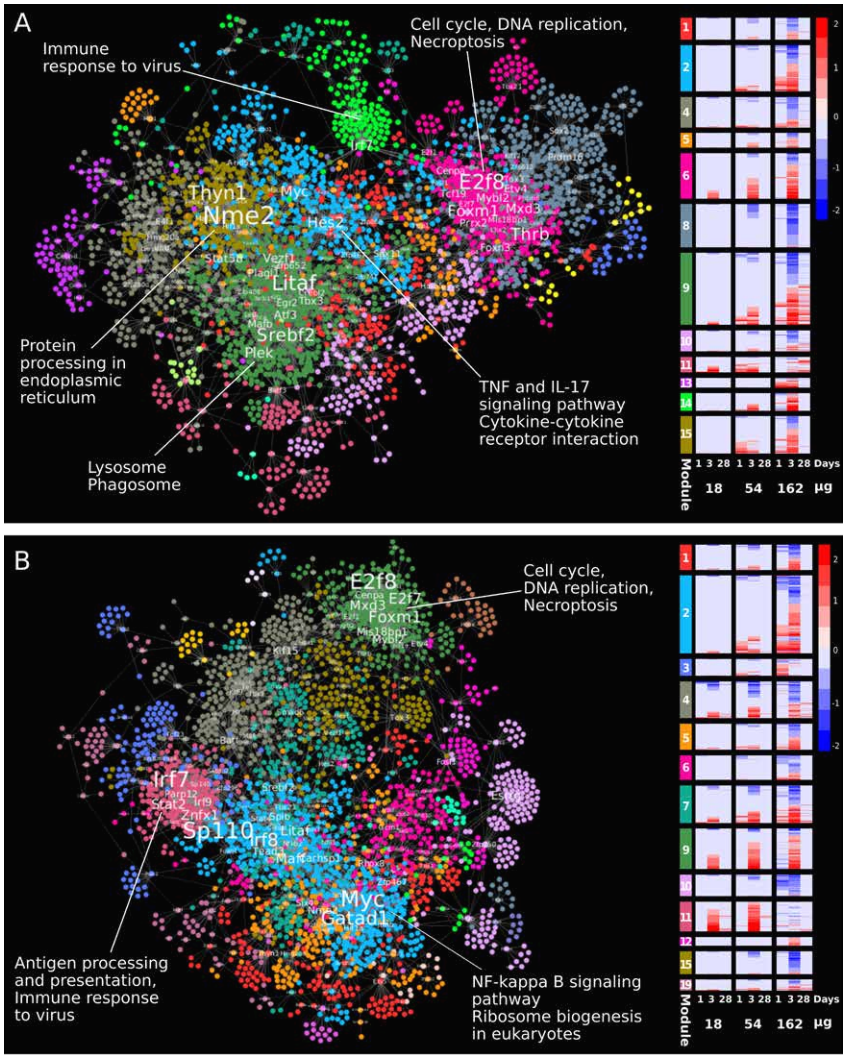


Lung alveolar macrophages containing carbon nanotubes (CNTs). Rats were exposed to nanotube aerosols (NM-401 and NM403).



TiO_2 tubes (red) associate with cell membrane (green).

and on extrapolation of these data to *in-vivo* adverse outcomes. This extrapolation is often hard to justify because the exposure conditions (e.g. the dose and the state of the nanomaterial at the actual contact) and the activated adverse outcome pathways can differ drastically between the *in-vivo* and *in-vitro* setups. Moreover, without the mechanistic understanding of the toxicity, one has to rely on purely statistical correlations between the nanomaterial properties and the toxicity endpoints, where the mathematical model acts like a black box. The key to progress in the development of predictive models lies in the detailed understanding of the response of the organism to nanomaterial exposure from the initial contact to the adverse outcome. The



Inferred gene regulatory networks for NM-401 (A) and NRCWE-26 (B) carbon nanotubes.

main hypothesis of the SmartNanoTox team is that this understanding can be achieved on the basis of system biology and new methods of characterisation and data generation in toxicology that have been developed in recent years. This will finally lead to a new paradigm in the toxicology – a mechanism-aware nanomaterial toxicity screening. Using a combination of *in-vivo, in-vitro* and *in-silico* approaches, the SmartNanoTox team will identify the mechanisms associated with the interactions between the nanomaterials and living organisms. At the systemic level, the team will use transcriptomics and proteomics data from *in-vivo* experiments, complemented by statistical modelling to identify the activated biological pathways and their corresponding molecular initiating and key events. At the molecular level, this will be achieved by analysing the state of the nanomaterial after the uptake, including their biomolecular corona and aggregation state, through both experiments and computational simulations. After this information has been gathered for a subset of nanomaterials, a sufficient set of data will be available, allowing for the identification of interactions leading to the activation of different toxicity pathways.

The SmartNanoTox team also intends to resolve all essential molecular interactions that can occur at the interfaces between biomolecules and nanomaterials, in a complete resolution of the structural deformation that a nanomaterial can cause when inhaled into the pulmonary system. The connection between different adverse outcome pathways and nanomaterial properties will be established through intelligent quantitative structure-activity relationships (QSARs), which will help to identify the properties of concern that should be avoided. The nanomaterials can then be grouped according to their ability to participate in certain interactions and trigger specific adverse outcome pathways or disturb the normal function of the cells by interference in the key events of the pathways. Thus, an accurate scale of the *in-vivo* toxicity of nanomaterials can be defined and predicted without the need for extensive long-term testing.

The overall outcome of the SmartNanoTox project, if successful, will be the definitive ability to predict the *in-vivo* toxicity of a nanomaterial in an accurate and time and cost efficient manner, through the use of QSARs. As a consequence, the need for blanket toxicity testing and animal experiments will be reduced.

The SmartNanoTox team has made substantial progress in achieving the goals in their grant programme, several examples of which will now be detailed.

Computational modeling of gene regulatory networks

Although many studies have investigated mechanisms and signaling pathways of nanomaterials toxicity, the global gene regulation program altered in response to these agents is mainly unknown. This lack of knowledge hinders the development of accurate prediction tests for nanotoxicity evaluation. In order to address these issues, the SmartNanoTox team have developed an integrative computational approach to prioritize key transcription regulators, their associated biological processes and signaling pathways, which were altered in response to toxic nanomaterials. We applied omics-based tools using a systems biology approach that can have a pivotal role in moving toxicity testing away from in vivo to in vitro and in silico models. Our method uses transcriptomics data, generates interaction networks that are specific to each nanomaterial, and is independent from bias in the reference databases for pathway mapping as it infers connections and pathways de novo purely based on the data. Inferred networks are used as a basis for further integration with proteomics data that enhance the power of nanomaterials toxicity prediction models. These results will be used for the development of smart tests addressing the key events.

The Pivotal Role of Mechanism-Aware Dose Metrics and Tissue-Normalised Dose

In a recent editorial in the *Particle and Fibre Toxicology* journal, the SmartNanoTox team has recognised the pivotal role of a biologically relevant dose metric for linking nanomaterial properties with toxicological

Panorama of recently opened super-resolution optical laboratory at the Jozef Stefan Institute, Slovenia.

Device for in vitro exposure of lung cells to aerosolised nanomaterials under physiologically realistic conditions (VITROCELL CLOUD 6).

response. Mass, as the most widely used dose metric, has no biological relevance and is therefore inadequate for structure-based toxicity prediction. The initial interaction between the biosphere and nanomaterials occurs at the bio-nano-interface, which includes binding of biomolecules to the nanomaterial surface suggesting surface area as one of the biologically most relevant dose metrics. This is confirmed by a recent literature review and data of the SmartNanoTox team, which shows that not only acute, but intermediate and long-term (chronic) lung inflammation is scaling with surface area dose. Using surface area as dose metric allows for derivation of nanomaterial-specific toxicity scaling factors – a prerequisite of structure-based toxicity modelling. On the other hand, the relevance of other dose metrics such as number and volume is acknowledged in the context of different modes of action, such as number as a dose metric for frustrated phagocytosis which can be induced by long, rigid and fibre-shaped nanomaterials.

Moreover, scaling of toxicological dose-response curves from *in-vitro* and *in-vivo* models of the lung to human exposure scenarios requires proper scaling of the delivered dose. In a recent publication by the SmartNanoTox team, the pivotal role of normalization of the delivered dose to the surface area of the exposed tissue e.g. lung epithelium was confirmed by observing identical dose-inflammation relationships in mice and rats (based on cm^2-nanomaterial/cm^2-tissue). For regulatory purposes, the surface area-based prediction of nanomaterial toxicity can be converted into mass-based exposure limits.

Affinity of TiO_2 Nanosurfaces Towards Lipids

The controversy of the cell viability tests reported for various TiO2 nanomaterials has driven the SNT teams to focus the research to molecular scale of this problem. By identifying the mechanisms of interaction between nanoparticles' surface and biological molecules we aim to unravel the mechanistic picture of the associated events where the involved supramolecular structures such as lipid membranes, actin and tubulin networks, are changed. For example, lipid-nanoparticle affinity, which was revealed to depend even on the small variations of the surface atoms positions as well as on the type of lipids and their headgroups and saturation of the lipid alkyl chains, has been proven to lead to full or partial wrapping of the nanoparticles by the membranes. While the full wrapping mobilize pieces of membranes and relocate membrane proteins that can later on interfere with different molecular cascades, the partial wrapping can lead to growth of the nanoparticle-biomolecular composites on the epithelial cells followed by macrophage damage, leading to highly probably persistent inflammation. Several advanced techniques has been employed in this research such as super-resolution stimulated emission depletion (STED) microscopy, fluorescence microspectroscopy (FMS) and cross-correlation spectroscopy (F(C)CS), electron and helium ion microscopy (TEM, SEM, HIM) , all-atom and coarse grained molecular dynamics (MD). The team hopes that understanding the mechanisms of the interaction between metal-oxide nanoparticles and biomolecules such as lipids can bring us to prediction of the molecular initiating and key events in nanoparticle exposed lungs triggering adverse outcomes.

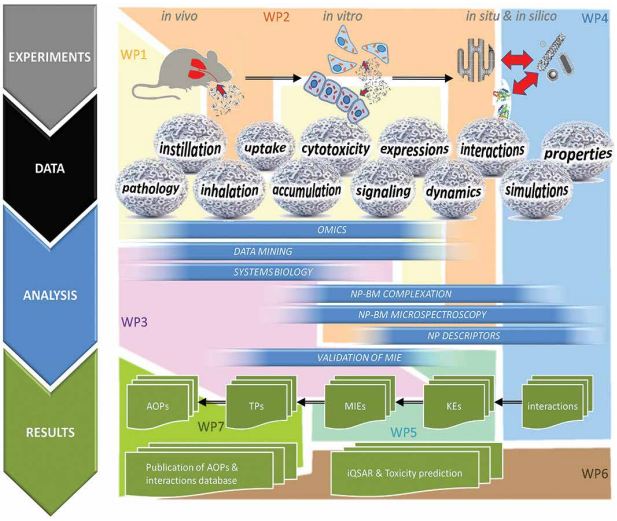
Modelling the Interface Between Cell Membranes and Nanomaterials

Nanomaterials can enter a cell in two different ways, either by active transportation through the receptors in the cell membrane, or passively by adhering to the cell membrane and causing it to bend

Pathology laboratory and chief technician Sauli Savukoski. Finnish Institute of Occupational Health, Finland.

and wrap around the foreign particle. Passive entry into the cell is only possible if the adhesion energy between particle and membrane is sufficient to compensate for the energy required to bend and deform the membrane.

Using molecular dynamic simulations, the SmartNanoTox team has derived a novel method to predict adhesion energies between soft lipid bilayers (which make up the cell membrane) and the solid surfaces of nanomaterials. Their approach involves fixing the two end points of the membrane bilayer being modelled in the simulation, and including a restraining force to allow realistic surface relaxation of the membrane, particularly at a curved interface. To increase the accuracy of the model, the mathematical descriptions of the interactions between the atoms were calibrated with experimental data. The final calculated adhesion energies could then be used to model the process of nanomaterials passively entering into a cell and predict if this process is energetically favourable.



Outline of the project concept. Abbreviations used: WP – work package, BM – biomolecule, NP – nanoparticle, AOP – adverse outcome pathway.

Modelling the Nanomaterial Protein Corona

It is now well accepted that foreign surfaces are modified by the adsorption of biomolecules such as proteins or lipids in a biological environment, and that cellular responses to materials in a biological medium might reflect the adsorbed biomolecule layer, rather than the material itself. The composition of the nanoparticle protein corona is flexible and is determined by many affinity constants and concentrations of the components of the biological fluids such as the blood plasma or the lung lining fluid.

The SmartNanoTox team developed a framework for coarse-grained modelling of interfaces between nanomaterials and biological fluids and membranes. Their model includes united-atom presentations of membrane lipids and proteins, which are based on all-atom structures of the corresponding molecules and are parameterised using experimental data or atomistic simulation results. The nanoparticles are modelled by two-layer objects, where the nanoparticle shell reflects the interaction between the material and the biomolecule in the corresponding fluid, while the core interacts with the biomolecule through van der Waals forces.

The proposed methodology can be used to predict the adsorption energies for dozens of common human blood plasma proteins on nanoparticles of different sizes, as well as the preferred orientation of the molecules upon adsorption. With these energies, scientists will be able to rank the proteins by their binding affinity to nanomaterials, and predict the composition of the nanoparticle protein corona for the corresponding material. Finally, these data will be used to construct a bio-nano interactions database and QSARs for the selected adverse outcome pathways.

Summary

- The measurement of the long-term effects of nanomaterial toxicity on human health is expensive and time consuming, requiring extensive animal testing and verification experiments.
- The SmartNanoTox team, coordinated by Dr Lobaskin at University College Dublin, is funded by a 2020 Horizon European grant to resolve the underlying mechanisms and biochemical pathways that regulate *in-vivo* toxicity of nanomaterials.
- The main focus of the SmartNanoTox team is to provide a mechanism-aware method to predict nanomaterial toxicity based on the generation of quantitative structure-activity relationships between the nanomaterial properties and their ability to trigger adverse outcome pathways.

Meet the researchers

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 686098.
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Dr Vladimir Lobaskin is Head of the SmartNanoTox team and is an Associate Professor in the School of Physics at the University College Dublin, Ireland. Throughout his academic research career, Dr Lobaskin has made significant contributions to the field of theory and modelling of nanostructured biosystems, including the development of computational approaches and software (MOLSIM and ESPResSo) for modelling soft-matter systems.
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Dr Frédéric Cosnier is the Head of the Inhalation and Analytical Toxicology Unit at the National Research and Safety Institute, France. Dr Cosnier has made significant contributions to *in-vivo* studies regarding toxicity associated with either metabolic interactions between chemicals or combined exposures to noise and ototoxic agents. In the nanotoxicology field, Dr Cosnier has contributed to the generation of nanoparticle aerosols and the measurement of their associated toxicity effects.
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Dr Laurent Gaté is the head of the Laboratory of Carcinogenesis, Mutagenesis and Reprotoxicity at French National Research and Safety Institute for the Prevention of Occupational Accidents and Diseases (Institut National de Recherche et de Sécurité), France. Dr Gaté has many research interests including the use of *in-vivo* and *in-vitro* models to assess the pulmonary toxicity and genotoxicity of nanomaterials. Dr Gaté also has significant research experience in cell biology and gene expression profiling.
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Dr Olivier Joubert is an Assistant Professor at Lorraine University, France. Throughout his academic and industrial career, the research conducted by Dr Joubert has specialised on resolving the nanotoxicology, and toxicogenomic of nanoparticles on human cells. In particular, Dr Joubert has focused on the study of the biochemistry and biology of cell membranes when interacting with foreign chemical substances, also known as xenobiotics.
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Dr David Gomez-Matallanas is a Research Fellow and Principal Investigator of Systems Biology Ireland in Dublin, Ireland. Dr Gomez-Matallanas has been actively involved in the study of signal pathways involved in cancer development, and developing models to suppress, regulate and resolve the molecular mechanisms underlying the development of such pathways.

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Dr Marc Meunier

Dr Marc Meunier is a Fellow at Dassault Systemes Biovia Ltd, UK, and has vast career experience in the computational modelling of materials. Dr Meunier's research interests include the simulation of polymeric materials for use in membrane technology, pharmaceutically active molecules, and more recently, within the field of materials informatics.

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Professor Nick Quirke

Professor Nick Quirke is a Professor of Chemical Physics at Imperial College London, UK, a Fellow of the Royal Society of Chemistry, Editor-in-Chief of Molecular Simulation and the Journal of Experimental Nanoscience, and is also Chang Jiang Professor at Xi'an Jiaotong university. Throughout his career in academia, Professor Quirke has used computer modelling and theory to predict electronic and physical properties of materials. In particular, Professor Quirke has studied the interaction of nanomaterials with bio-membranes and polymers.

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Dr Otmar Schmid is an Adjunct Assistant Professor at Missouri University of Science & Technology, USA, and head of a research group on Pulmonary Aerosol Delivery at the Helmholtz Zentrum München, Germany. Dr Schmid has had a prominent career in the field of biotechnology and aerosol physics, and his research has included the development of the ALICE-CLOUD technology to enable rapid delivery of aerosolised drugs to cells at air-liquid interface conditions. Dr Schmid also conducts preclinical *in-vitro* and *in-vivo* studies investigating both therapeutic and toxicological effects of aerosolized substances including experimental drugs, nanomaterials, and cigarette smoke in the lung.

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Dr Henrik Wolff

Dr Henrik Wolff is the Chief Medical Officer for pathology at the Finnish institute of Occupational Health, Finland. His research interests include the study of malignancies in the lungs, pleura and the sinonasal area and their association with exposures to various materials including asbestos and wood dust, and he is also extensively involved in studies concerning the toxicity of nanomaterials. Dr Wolff is also a member of several expert committees, including the Finnish Pathologists Mesothelioma Panel (current chairman), Finnish pneumoconiosis group (current vice-chairman), the working group of pulmonary pathology at the European society of pathology (member) and US-EU Communities of Research (COR) in nanotoxicology (participant).

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Professor Bertrand Rihn is a Professor of Biochemistry and Molecular Biology, at the faculty of Pharmacy in Nancy University, France. He is a leading expert in the field of toxicology, with research topics including safety toxicology, immunotoxicology and investigating the transcriptomic changes that occur due to nanoparticle exposure. In addition to his research, Professor Rihn has also worked as clinical toxicologist, and was awarded the Baratz award (2004) and the E. Taub award (2011) from the National Academy of Medicine. He also served as the President of the French Society of Toxicology from 2007 to 2009, and is a EUROTOX Registered Toxicologist.

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Dr Carole Seidel

Dr Carole Seidel is a Scientific Reseacher at the National Research and Safety Institute, France. Dr Seidel has experience of in vivo and in vitro nanotoxicology, in particular, has focused on the study of the toxicogenomic of in vivo pulmonary models exposed to nanoparticles. Dr Seidel also has extensive experience in study of epigentic mechanisms.

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Professor Håkan Wallin

Professor Håkan Wallin is leader for the group of toxicology at the National Institute of Occupational Health. His research is focussed on the toxicology of inhaled nanomaterials in relation to risk of cancer, cardiovascular disease and reproductive toxicity. The group has a long tradition of working with health effects of inhaled particles. The Institute also investigates exposures at Norwegian work places and is responsible for regulation and advising management of hazardous exposures

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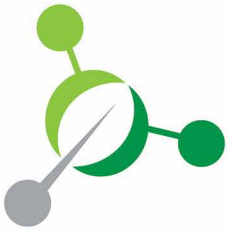

SmartNanoTox

Smart Tools for Gauging Nano Hazards

Stockholm University

Imperial College London

HelmholtzZentrum münchen
German Research Center for Environmental Health