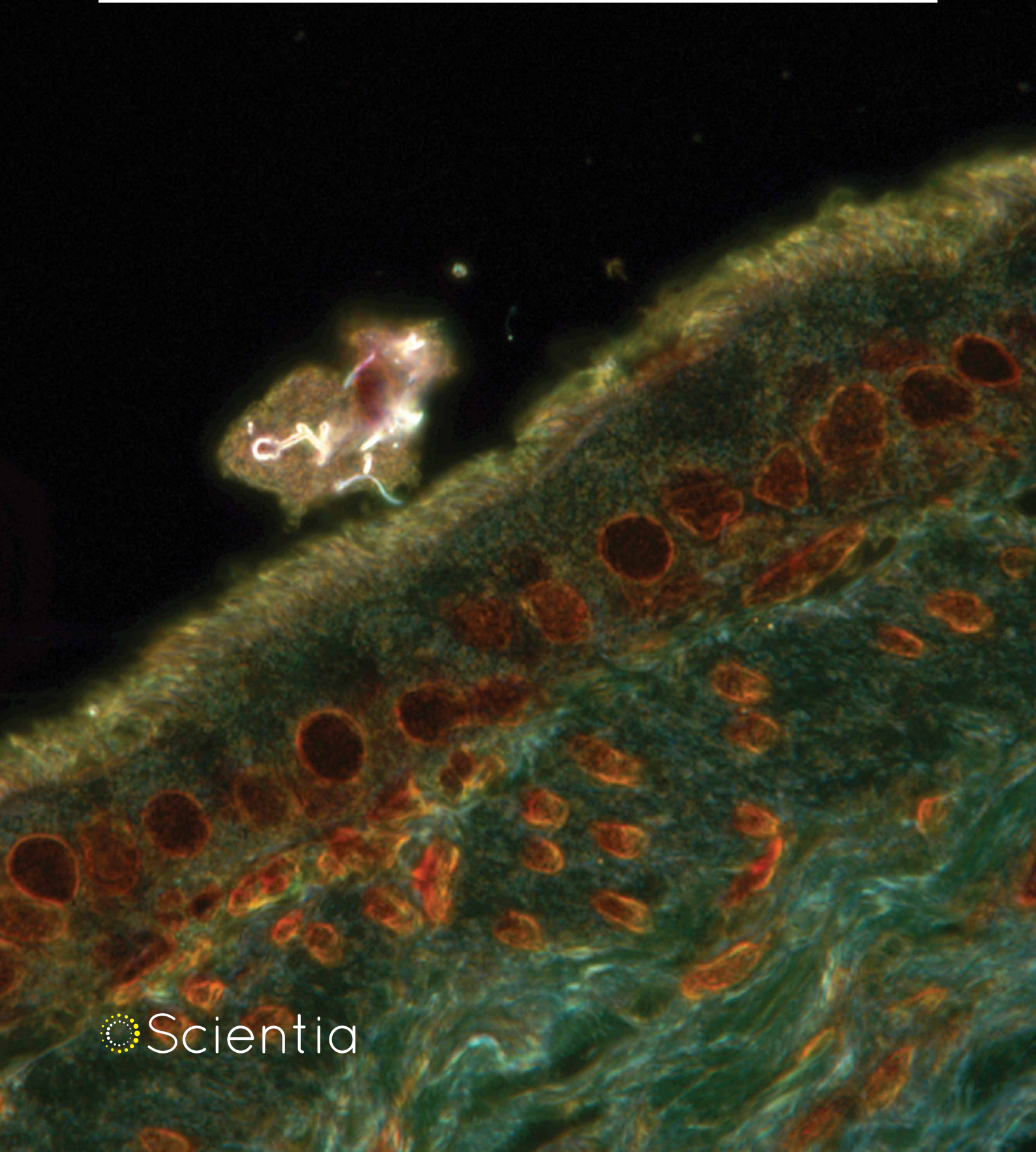


# Determining the Toxicity of Nanomaterials

SmartNanoTox



# 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, which 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.

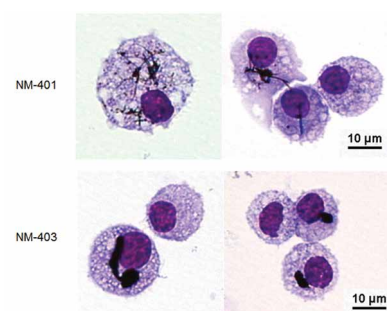
The specific mechanisms within living organisms that lead to adverse health effects after exposure to nanomaterials remain poorly understood. While some common industrial materials such as asbestos or quartz nanodust can cause a direct damage to animal or human lungs when inhaled, the effects of the 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.

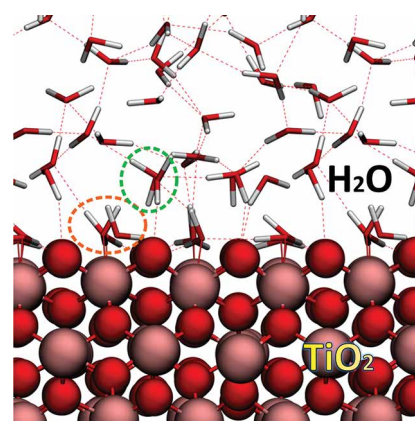
Nanomaterials can also enter a cell through its membrane receptors and cause adverse effects, such as the deregulation of cellular pathways. Deregulation and deformation of receptors and protein enzymes that participate in regulatory pathways can lead to the formation of neurodegenerative diseases, cancers, 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

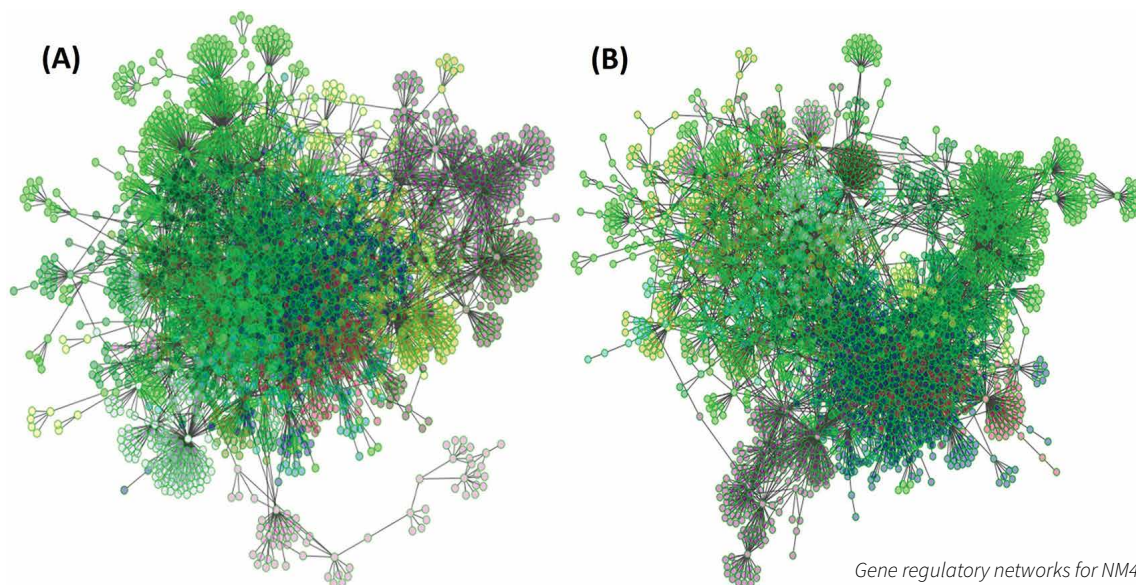
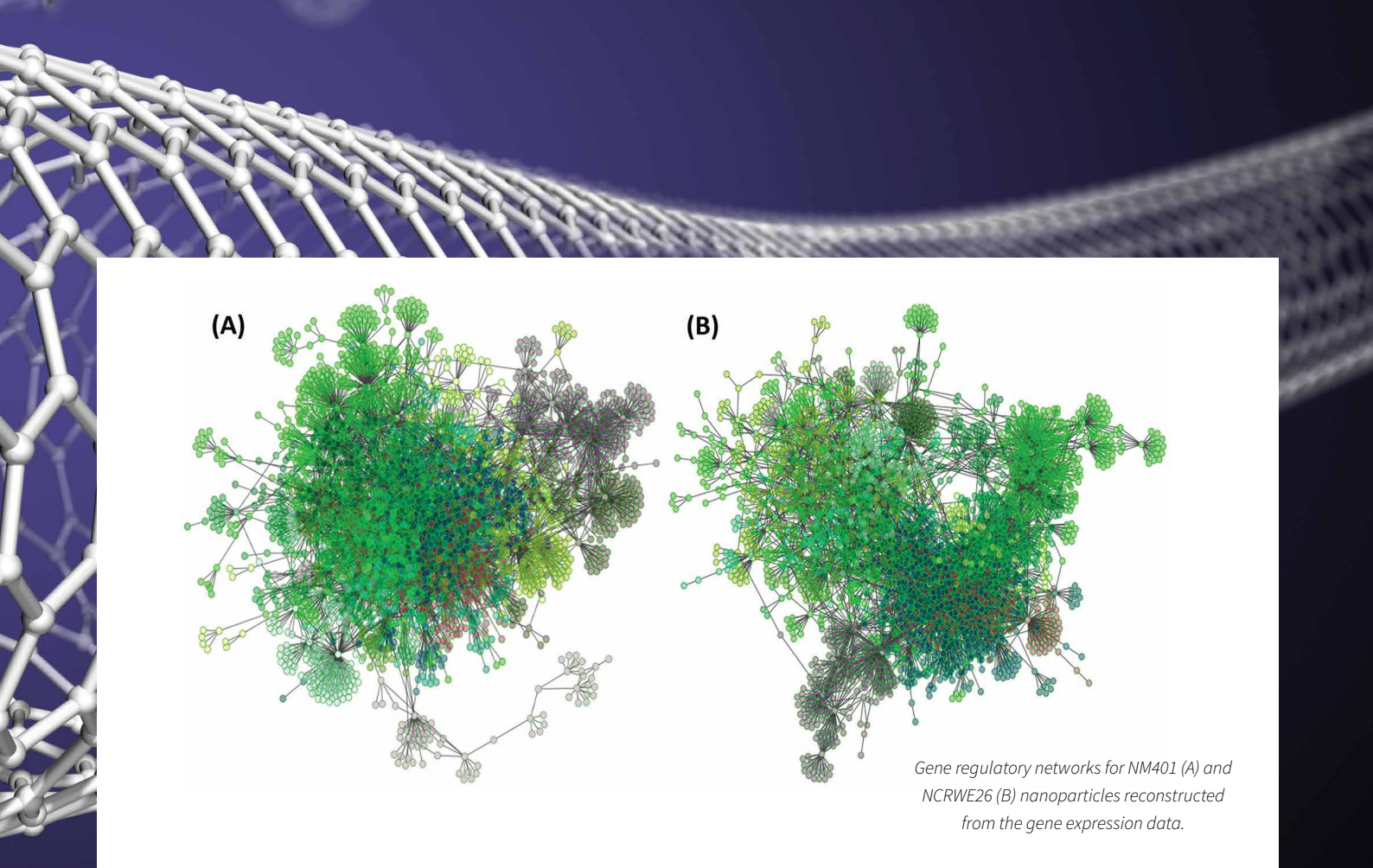


Lung alveolar macrophages containing carbon nanotubes (CNTs). Rats were exposed to nanotube aerosols (NM-401 and NM403).



Wetting of TiO<sub>2</sub> nanoparticles. Ab-initio molecular dynamics reveals details of water structure and reactions at TiO<sub>2</sub> nanosurface with atomistic resolution.





Gene regulatory networks for NM401 (A) and NCRWE26 (B) nanoparticles reconstructed from the gene expression data.

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* 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 the 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 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 the recent years. This will finally lead to a new paradigm in the toxicology – a mechanism-aware nanomaterial toxicity screening.

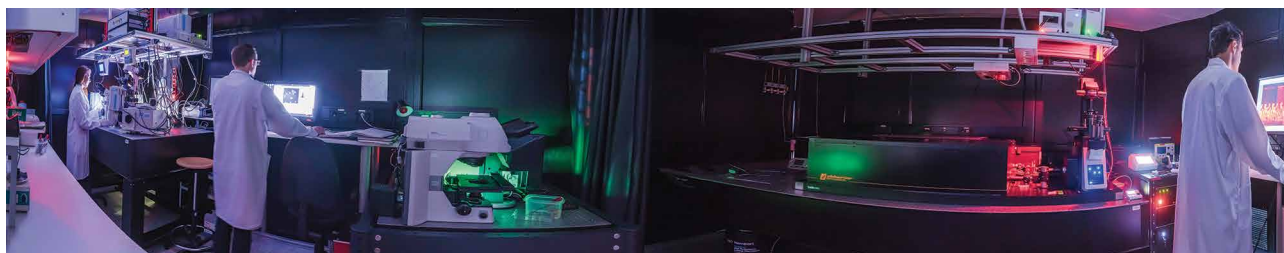
Using a combination of *in-vivo*, *in-vitro* and *in-silico* approaches, the SmartNanoTox team will identify the mechanisms associated with the interactions between the nanomaterials and living organisms. At the systemic level, the team will use transcriptomics and proteomics data from *in-vivo* experiments, 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 have made substantial progress in achieving the goals in their grant programme, several examples of which will now be detailed.



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

### **Wetting TiO<sub>2</sub> nanoparticles**

Titanium dioxide (TiO<sub>2</sub>) is a common nanomaterial used in solar cells, self-cleaning materials and photocatalysis. The surface reactivity of TiO<sub>2</sub> nanoparticles can be tuned by varying the amount of water that is adsorbed to the surface of the material. Understanding the mechanisms that underly the modulation of this surface reactivity is crucial for unveiling the mechanisms that give rise to the toxicity of TiO<sub>2</sub> nanoparticles in the body.

In one particular study, the SmartNanoTox team reported computational simulations predicting the surface reactivity of TiO<sub>2</sub> nanoparticles. Using molecular dynamics modelling, the reaction of TiO<sub>2</sub> nanoparticles with differing concentrations of water can be simulated as a function of time. Simulations were run over a given time frame, and a comparison of initial and final structures were made to analyse the reactivity of the systems. Surface defects, such as missing and displaced atoms, are common in TiO<sub>2</sub>. The team found that missing oxygen atoms promote water dissociation at the nanoparticle surface, and therefore increase reactivity. Furthermore, the concentration of water coverage that gives rise to the highest reactivity of water and the strongest interaction at the nanoparticle surface was identified for each naturally occurring crystal structure of TiO<sub>2</sub>.

This work has demonstrated computational modelling to be a powerful tool for understanding the intricate mechanisms associated with nanoparticle reactivity.

### **Affinity of TiO<sub>2</sub> Nanosurfaces Towards Lipids**

A large number of controversial cell viability tests has been reported for various TiO<sub>2</sub> nanomaterials. Focusing on the specific interaction between the nanomaterial and various plasma proteins and receptors, the Imperial College team has shown that a strong affinity exists between TiO<sub>2</sub> nanosurface and lipids.

With the affinity towards lipids being much stronger than that of silicon dioxide (SiO<sub>2</sub>), the team discovered that at least certain morphological forms of TiO<sub>2</sub> nanoparticles can pull lipids out of membranes despite the free energy penalty arising from hydrophobic interaction and the curvature of the nanoparticles. This result has been proven using several physical methods, including fluorescence microspectroscopy and cross-correlation spectroscopy, super-resolution stimulated-emission depletion (STED) microscopy, and electron paramagnetic resonance spectroscopy (EPR). The team hope that understanding the mechanisms of the interaction between metal-oxide nanoparticles and lipids can predict the molecular initiating events in nanoparticle-exposed lungs, where various lipid structures are abundant. Their work reminds us that even an unspecific interaction between the nanoparticles and biologically relevant molecules can trigger adverse outcomes.



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

### **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 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.

### **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





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

than the material itself. The composition of 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.

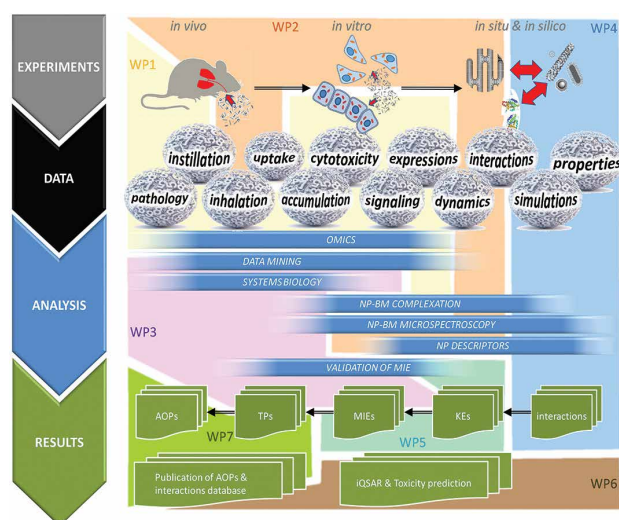
The SmartNanoTox team developed a framework for coarse-grained modelling of interfaces between nanomaterials and biological fluids and membranes. Their model includes united-atom presentations of membrane lipids and proteins, which are based on all-atom structures of the corresponding molecules and are 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.

### Inhibiting the Dynamics of Cell Fate Decisions

Changing the dynamics of protein activation in cellular networked pathways, such as the pathway between RTK/RAS/RAF/MEK/MAPK protein kinases, can lead to protein mutation and disease formation.

On this topic, the SmartNanoTox team reported a review of methods that regulate the dynamics of the MPK kinase, which is associated with cell fate decisions and drug resistance. In particular, the review highlighted that physiological factors can control and modulate cell decisions. Mechanisms within the protein networks can be probed using new techniques such as biosensors and microfluidics, while biochemical responses and their associated timescales under applied external stimulus can also be measured. Mathematical models can also shed light on the expected time scales for each mechanism, thus further resolving the intricate chemical mechanisms associated with cellular protein networks.



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

Hyperactivation of the RAS/MAPK pathway is reported to be the cause for over 30% of human cancers. Inhibitor drugs act to deactivate such hyperactivated pathways, but a serious problem associated with their use is drug resistance. The origin of this resistance is the formation of chemical bonds between two protein kinase complexes, and the SmartNanoTox team has shown that kinase dimerisation is thermodynamically favourable, once an inhibitor molecule is bound to a kinase protein. It is therefore the binding affinity of the inhibitor for the kinase protein that will dictate if drug resistance to pathway inhibitors is thermodynamically favourable.

### 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



## Professor Vladimir Lobaskin

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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## Professor Ulla Birgitte Vogel

Professor Ulla Birgitte Vogel is Professor at the National Research Centre for the Working Environment, Denmark and adjunct professor at the Technical University of Denmark. Her research is focussed on the toxicology of inhaled nanomaterials in relation to risk of cancer, cardiovascular disease and reproductive toxicity. She is a European Registered Toxicologist and advisor of the Danish Working Environment Authorities.

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## Dr Frédéric Cosnier

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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## Professor Luc Ferrari

Professor Luc Ferrari is a Professor of Toxicology at Lorraine University, France, belonging to the Therapeutic targets, Formulation, and Preclinical Expertise of Medicines group (CITHEFOR). Professor Ferrari is also a member of the poison control centre of Nancy, France, and a member of the High Council of Public Health. The research focus of Professor Ferrari is actually on nano toxicology, but also includes studying the effects of inflammation on drug metabolising enzymes, and conducting in-vivo and in-vitro experiments, which evaluate the effects of exposure to genotoxic compounds.

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## Professor Boris Kholodenko

Professor Boris Kholodenko is a Science Foundation Ireland Stokes Professor of Systems Biology and the Deputy Director of Systems Biology Ireland at the University College Dublin, Ireland. His studies are aimed to understand how biological networks compute and control cell-fate decisions.

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## Dr Laurent Gaté

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

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 Kristina Bram Knudsen

Dr Kristina Bram Knudsen is a Postdoctoral Researcher at the National Research Centre for the Working Environment, Denmark. As a trained toxicologist, Dr Knudsen has extensive experience of in-vivo toxicity testing of nanoparticles. In particular, Dr Knudsen's research interests include the study of lung inflammation and soft tissue changes with exposure to popular nanomaterials such as carbon nanotubes, titanium dioxide and nanocellulose.

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## Tobias Krebs

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**Professor Alexander Lyubartsev**

Professor Alexander Lyubartsev is a Professor of Physical Chemistry in the Department of Materials and Environmental Chemistry at Stockholm University, Sweden. Throughout his substantial research career, professor Lyubartsev has developed methodology to allow the simulation of biological systems, and the mechanistic exploration of nanoparticle toxicity.

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**Dr David Gomez-Matallanas**

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**

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 aerosolized 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 Tobias Stöeger**

Dr Tobias Stöeger is a Scientific Researcher and Group Leader at the Institute of Lung Biology and Disease at Helmholtz Zentrum München, Germany, and is the Associate Editor of the Journal of Nanotoxicology and PLoS ONE. Dr Stöeger has focused his research on studying the mechanisms of sterile pulmonary inflammation due to the inhalation of nanoparticles.

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**Professor Janez Štrancar**

Professor Janez Štrancar is a Scientific Researcher and Group Leader at the Jožef Stefan Institute, Slovenia. Professor Štrancar conducts research within the field of molecular biophysics, spectroscopy and advanced microscopy. In particular, his work includes the study of biomembranes, supermolecular structures, physics of biocompatibility and interaction between nanomaterials and biological structures. In the last years professor Štrancar specializes in development of hybrid microspectroscopic techniques for visualising and analysing labelled living cells.

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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**

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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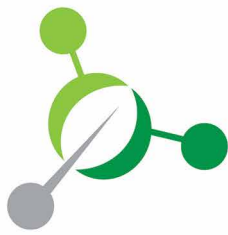
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# SmartNanoTox

Smart Tools for Gauging Nano Hazards



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