Executive Summary for NanoInteract Annual Report

NanoInteract: Development of a platform and toolkit for understanding interactions between nanoparticles and the living world

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The responsible development and implementation of nanotechnology is recognized as a common desire across the European arena of research, and far beyond. The overarching objective of NanoInteract is to create a firm scientific and technical basis for understanding and potentially prediction of likely biological impacts of engineered nanoscale particulates. The NanoInteract program is an EU FP6 STREP funded under the NMP theme, running from January 1st 2007 until 31st December 2009. It involves extensive co-operative laboratory-based research in 9 European academic institutes, 2 National Research Centres, 5 industry partners, and 1 US academic institute, listed in the Table below.

Recognizing the extraordinary degree and quality of inter-disciplinarity required to address the challenge, the program is a network of research-based-interactions in which those researchers best suited to perform a given role, are able to do so, passing on their materials, outputs and knowledge to the others along the value chain. To date the program has lead to no newly identified hazard (solely due to nanoscale elements) for nanoparticles, but it has highlighted several issues requiring further investigation. Numerous examples of apparent hazard, ultimately traced to dispersants or other conventional chemical impurities have been identified and eliminated as factors in the research.

The knowledge and experience gained is now forming the basis for other projects, both in the EU and across the world. The project website is www.nanointeract.net.

Partner	**Country**	**Status**
National University of Ireland / University College Dublin	Ireland	University
Ludwig-Maximilian Universität	Germany	University
Oxford University	UK	University
Trinity College Dublin	Ireland	University
University of Ulster	UK	University
Université Paris-Sud	France	University
Lund University	Sweden	University
National Institute for Public Health and the Environment	Netherlands	Research Centre
Nofer Institute of Occupational Medicine	Poland	Research Centre
Ghent University	Belgium	University
Rice University	United States	University
Glantreo	Ireland	Industry
Medtronic	Netherlands	Industry
L'Oreal	France	Industry
Intel	Ireland	Industry
Umicore	Belgium	Industry
DSM	Netherlands	Industry

The program acknowledges that there are currently significant limitations in the direct application of the traditional toxicological approaches (as applied to pesticides and air pollutants for example) to the assessment of nanoparticle hazard. Besides the potential for new toxicological end-points, there are many problems of a more technical nature that cast uncertainty on current methods are many new issues. Important objectives of the program are therefore to produce controlled and reproducible dispersions of nanoparticles in biological fluids, amongst the most important and challenging aspect of the field, as yet very poorly understood. In particular the program seeks to indentify the role of impurities, state of division, aggregation, and related aspects of nanoparticles in biological media in leading to false positives and false negatives in toxicity testing, eliminating them where necessary.

Work Program

NanoInteract also seeks to identify some of the routes via which nanoparticles enter and accumulate in cells, fauna and the fresh-water system. Then using advanced methods of chemical, physical, biological and toxicological sciences we connect nanoparticle properties to the mechanisms via which they interact with, and disrupt, cellular processes. This knowledge is then connected to the outputs from classical toxicology testing (such as those relevant to REACH and OECD considerations), in order to evaluate whether these existing toxicity tests are sufficient to predict nanoparticle toxicity, or whether they need to re-designed, or supplemented with newer approaches more tailored to the issues implicit in the nanoscale. The interactions and knowledge flows within NanoInteract are summarized in Figure 1. Fundamental to the project is the effort to establish protocols and standards via which every step of the project are being controlled as we seek to eliminate the factors that currently causing irreproducibility. An overview document of these protocols will be published as an output of the project to enhance progress in the field and to share our experiences in this arena with others.

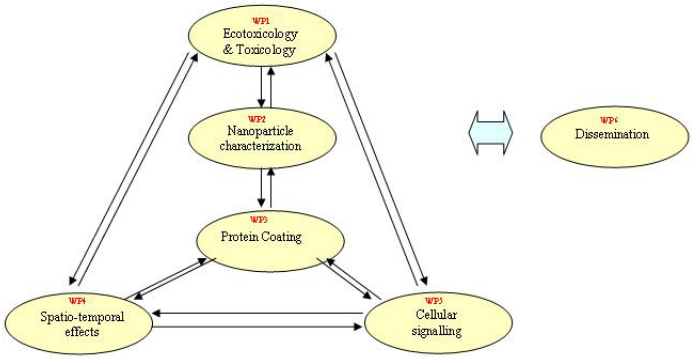

Figure 1 Overview of the interactions and interconnections between the different disciplines within NanoInteract, and the division of the project into workpackages.

The NanoInteract objectives and challenges can be summarized as follows:

- To establish experimental protocols for every aspect of the study of nanoparticle interaction with cells, and several types of aquatic plants and organisms, ensuring complete reproducibility.
- To understand effect of adsorbed protein on nanoparticle stability and nanoparticles on protein conformation and function, ultimately connecting this to biological impacts.
- To connect final cellular location of nanoparticles with the intra- and inter-cellular processes disrupted.
- To combine these results, along with the expertise from diverse disciplines, to point towards a ‘standard approach to nanotoxicology’.

Work performed and some outcomes

The NanoInteract project has begun to develop quite well, with increasing focus and secure processes now in place. In year 1 the work in the project has focussed on several key classes of nanoparticles – silicon dioxide, cerium oxide. Several classes of reference polymeric nanoparticles and quantum dots

have also been studied with success. Some promising (but as yet limited) studies have been carried out with silver and aluminium oxides.

Many different commercial sources of these nanoparticles have been examined (in conjunction with industrial partners), and compared to high quality versions made within the program. More limited studies have been attempted with (single- and multi-walled) carbon nanotubes (CNT's), but so far we have been unable to progress to sufficient level of quality and reproducibility with CNT's for publication. The choices of these particles was based on several factors, including industrial relevance (silica, ceria are already used extensively in industry) ready availability of high quality samples in a range of sizes and in sufficient quantities for distribution throughout the consortium. Very significant efforts have been made to screen, characterise, and validate the reproducibility of every nanoparticle sample entering the program, leading to many new insights, but also a growing awareness of just how challenging work of the highest quality of reproducibility is going to be in this field.

Achievements of significant practical importance from the first year of the project include:

- establishment a solid common ground and understanding for comparing the results from the very different groups and experimental approaches used in the NanoInteract project
- ensuring that all the groups can reproduce the same findings for several sample particle types, and toxicological tests (MTT, LDL, genotoxicity-Comet etc). This has been achieved using the round-robin approach within the toxicology, cell biology and ecotoxicology groupings, using identical nanoparticles, cells (test organisms), common serum, identical nanoparticle dispersion and exposure protocols, and identical test end-points. The challenges are now recognised to be much greater than had been supposed, and major efforts have been expended to make this part of the program successful. A lot has been learned in so doing, and the program is now working smoothly in this regard,
- resulting from this, the first papers from this work and currently being prepared for publication, within the toxicology and ecotoxicology sub-groups. Several issues have been noted in these papers
  - it makes little sense to speak of any 'nanoparticles' of fixed size and polydispersity where biological impacts are concerned. Many other factors such as state of aggregation, surface structure, microstructure (and some factors not yet identified) contribute much more strongly to the biological outcome than had been expected
  - some samples of nominally identical materials are cytotoxic, other are not
  - a major issue, previously suggested, but not emphasized sufficiently, is the presence of trace amounts of impurities, catalysts, dispersants. Failure to characterise their amount precisely leads to irreproducibility and results that are not reproducible if published in the literature.
- One interesting and important hypothesis, which was in the very early stages of at the time of writing the NanoInteract project, is that the biological impacts are related to attachment of proteins (and other biomolecules) to this surface, and the resulting 'corona' of biomolecules which confers a biological identity (see Figure 2a). This concept of the biomolecule corona has now gained considerable interest and support in the international scientific community, and is now incorporated into many new research programmes both in the EU and Internationally. After considerable challenges (again in reproducibility) a standard technique and protocol has been developed to deduce the major components of the nanoparticle-corona.
- The nature of the attached biomolecules (proteins and now lipids) in a range of very different materials, have been identified (for several polymers, silica, alumina etc). A longer term goal of the project is to investigate the potential use of the biomolecule corona as a means to classify nanoparticles, and even to predict their toxicological and biological impacts.
- An additional aspect of the work is that we have observed significant differences in the nature of the adsorbed proteins on nanoparticles compared to flat (bulk) surfaces of identical chemical composition. This work is now in preparation for publication, and will be the first evidence of an impact of curvature or the nanoscale on the biological identity of materials.

- Early results from the high-throughput approaches are intriguing, suggesting that direct application of these methods is promising (high content analysis of nanoparticle interactions with cells).
- Preliminary studies on in-cell transcriptomic and proteomic assessments have lead to complications, suggesting that naive use of these techniques without in depth understanding of the whole chain are irreproducible, and much effort needs to be expended here, particularly in ensuring comparability of results between labs.

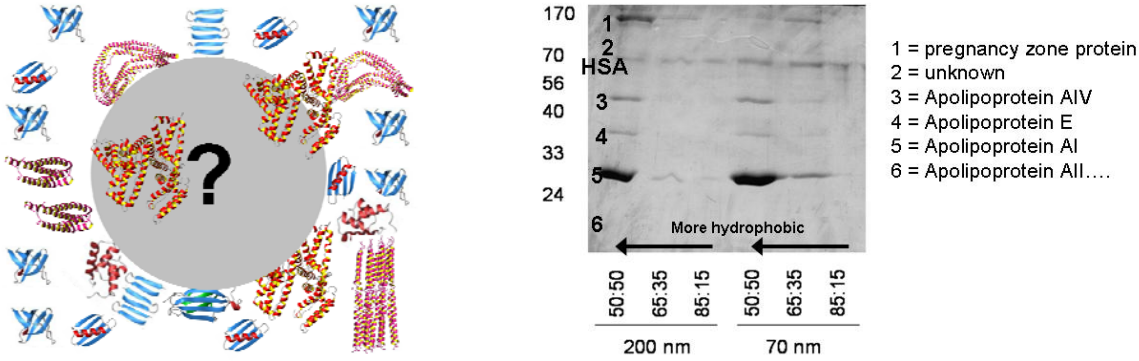

Figure 2 Plasma proteins adsorb to nanoparticles in biological fluids to form a dynamic biomolecule corona. The adsorption appears to be very selective, with several less abundant proteins being preferentially adsorbed over more abundant ones such as human serum albumin.

Overall, the experience of NanoInteract leads us to emphasize the challenging nature of the problems in assessing the potential risks posed by nanoparticles for living systems, which require highly focussed and large multidisciplinary teams that can address all the issues. Significant results are emerging, but it is now clear that to carry out truly useful science in this field, one will need to ensure that a number of different laboratories obtain precisely the same results. This transpires to be a major (but achievable) challenge, but without it, one has to consider carefully the value of publishing the results, and the implications for other scientists seeking to reproduce results. It is hard to predict all the specific research outcomes of NanoInteract, but it is already clear that a major element, not completely foreseen at the time of commencing the work, will be an understanding of *how* to carry out durable, reproducible, collaborative research in the field of nanotoxicology. It also seems likely that the nature, and to some degree the role of the 'protein corona' will clarify and be somewhat resolved within the project. Some early results of nanoparticle impacts on simple aquatic species will also be evident.

Teaching and Training; A significant element of the program, not fully foreseen in the original plan, has been the consequence of quite different background and training of different students and researchers on the ground in the laboratories. Communicating a common understanding of the need for protocols, the variability of samples and the need for controls at all levels has been a significant issue. The current informal documentation will be formalised and transmuted into a training course across the whole Program within the next few months. All young researchers will be invited to attend.

Dissemination; Results are being disseminated as appropriate in the literature. Prior to each result being published (especially if it is in any way surprising) they are being repeated in two or three independent laboratories, as a fundamental check on the quality and validity of the research. To speed up the process of dissemination, more informal routes involve conferences, working groups in EU, OECD, and other instruments. At a recent ESF international meeting on nanotoxicology (organized by the PI of NanoInteract, and another partner) some advances from NanoInteract were communicated, and extensive discussion took place about the need for protocols. In the next year a more focussed conference will take place later in the year in Lodz in which further dissemination will take place.