The need for *in situ* characterisation in nanosafety assessment: Funded Transnational Access via the QNano research infrastructure

Kenneth Dawson, Sergio Anguissola and Iseult Lynch
Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland
Kenneth.A.Dawson@cbni.ucd.ie, ta@qnano-ri.eu

The first concerns regarding potential toxicities of nanomaterials were raised almost 10 years ago, as fears that the small size of nanoparticles might allow them unique access to organisms, cells, cellular barriers and sub-cellular organelles with potentially harmful consequences resulting from quantum properties.(Colvin, 2003) Much has been learned in the intervening period, and indeed nanosafety assessment is almost at the stage of being a quantitative and mechanism-based scientific discipline, where nanoparticle dispersions can be generated reproducibly to ensure known nanoparticle doses are delivered,(Ramirez-Garcia, 2011) nanoparticle uptake can be quantified and modelled,(Elsaesser, 2010) and impacts from the presence of nanoparticles in cells and tissues can be teased out in a time-resolved manner.(Xia, 2008) However, significant work is still needed to ensure that this knowledge becomes formulated into standardised and agreed protocols that are widely disseminated to the growing research community addressing topics related to nanosafety and the interactions of nanomaterials with living systems, be they intentional (e.g. nanomedicine) or unintended (e.g. nanoparticles produced via combustion).

QNano, a Seventh Framework Programme Capacities project, is an analytical research infrastructure for characterisation of nanomaterials for nanosafety assessment (Grant agreement n° INFRA-2010-262163) established in early 2011 to address this issue of formulating and disseminating best practice for nanosafety assessment. QNano consists of 29 funded and 25 additional partners from around Europe, with expertise ranging from nanomaterials synthesis, dispersion, labelling (with optical, isotope and radio-labels), characterisation of nanomaterials in both their pristine state and *in situ* in complex milieu (including in the environment and in consumer products), using a range of advanced techniques[1], as well as a suite of approaches for nanomaterials exposure assessment in both *in vitro* and *in vivo* models. QNano is an analytical infrastructure dedicated to quality in all aspects of nanomaterials safety assessment, with characterisation of the nanoparticles at all stages of exposure being a key focus, in order to connect the actual dose experienced by living systems with the observed impacts. A key pillar of activity within QNano is the provision of funded Transnational Access to approximately 400 researchers from the nanosafety and nanomedicine communities for characterisation of nanomaterials *in situ*. Each transnational access visit will be approximately 5-8 working days.

The importance of characterisation of nanomaterials in the relevant biological dispersant (i.e. under the exposure conditions) is increasingly accepted as being necessary in order to determine the "available" nanoparticle dose. Scientists increasingly recognise that nanoparticles immediately absorb proteins or other biomolecules from their surroundings to lower their surface energy (Rivera Gil, 2010) with important consequences for nanoparticle stability in dispersion.(Kendall, 2011) Note that for ecotoxicological studies, natural organic matter plays much the same role as proteins for *in vitro* and *in vivo* toxicology studies, modulating the nanoparticle surface (free energy) and thus the dispersibility of nanomaterials.(Baalousha, 2008) As a consequence, nanoparticles dispersed in biofluids containing proteins, lipids, polysaccharides

[1] The full list of nanoparticle equipment available through QNano Transnational Access partners can be found at www.qnano-ri.eu/access. Examples of some of the more specialised pieces include a wide range of Electron Microscopies, Spectroscopies, cyclotrons for radiolabelling of nanoparticles, Particle Induced X-ray Emission etc.

etc. can have a very different dispersion profile than the same nanoparticles dispersed in reference buffers, as shown in Figure 1, which can lead to very different effective or available doses of nanoparticles for interaction with living systems.

The composition of the biomolecule corona, and the subsequent stability, available dose and consequent biological interactions of nanoparticles, have been found to depend on the specific details of the biofluid in which the nanoparticles are dispersed, which may account for much of the contradictory reports present in the literature for nominally identical materials. Thus, the same (batch of) nanoparticles dispersed in different cell culture media (e.g. DMEM or RPMI) containing identical concentrations of Foetal Bovine Serum (FBS) have been shown to result in quite different coronas, both in terms of their thickness and dynamics.(Maiorano, 2010) The authors of that study observed that DMEM elicits the formation of a large time-dependent protein corona, while RPMI shows different dynamics with reduced protein coating. These different coronas had implications for uptake and impact, with the protein-NP complexes formed in RPMI being more abundantly internalized in cells as compared to protein-NP complexes formed in DMEM, consequently exerting overall higher cytotoxic effects.(Maiorano, 2010) These results suggest that cell culture medium composition and ionic strength can alter adsorption of proteins onto the nanoparticle surface, which can impact on the particle agglomeration and potentially alter the available dose of nanoparticles under the different exposure conditions. Thus, by not having the characterisation of the nanoparticles in the two different media, it is not possible to make any interpretation of the data on the basis of whether the different protein coronas result in different available doses, which could potentially explain the different observed impacts. Nanoparticle dispersions in biofluids can be made quite reproducibly, and the protocols exist and are being shared through QNano and its sister FP7 project NanoImpactNet, and *in situ* dispersion characterisation gives a more complete description of the available dose than even very detailed characterisation in physiological buffer, which can provide an additional correlation with observed impacts data.

While multiple publications have highlighted the need for detailed characterisation of nanomaterials in order to perform safety and impact assessments,(Warheit, 2008, Stone, 2010) these have typically suggested characterisation in water or physiological buffer. However, as shown in Figure 1, the particle size and size distribution in physiological buffer does not reflect the particle size and size distribution of the particles under the exposure conditions (for example, dispersed in cell culture medium containing serum proteins). Thus, there is a pressing need for researchers working to assess the biological impacts and fate and behaviour of nanomaterials to characterise their nanomaterials *in situ* in the dispersion media in which they will be presented to the test system.

The QNano research infrastructure (www.qnano-ri.eu) identified this limited application of characterisation methods to nanomaterials *in situ* at all stages of their processing, presentation to living systems and impact analysis as one of the major roadblocks to clarification of the potential impacts of nanomaterials. QNano recognised many of advanced characterisation technologies will remain outside of the reach of the research community of biologists and toxicologists. As such, QNano proposed the establishment of a resource that would allow the research community to access the knowledge, expertise and equipment required for detailed characterisation of nanoparticles *in situ* in complex biological fluids and during exposure to, and interaction with, living systems, via funded User Access.

Via QNano, the EU is providing funded Transnational Access to 15 of Europe's leading nanoparticle characterisation facilities (as shown in Figure 2), together with the appropriate technical support and

expertise, and protocols for nanoparticle dispersion and characterisation, for researchers working in nanosafety assessment or nanomedicine at European institutions[2]. Access is being offered to a range of nanomaterials characterisation tools suitable for assessment of nanoparticles *in situ* in complex biofluids and living systems, such as the aforementioned DCS and TEM, all types of advanced microscopy, spectroscopy and imaging approaches, not only for characterisation of nanomaterials, but also for the assessment of exposure *in vitro* and *in vivo*, as well as a host of analytical tools. The full list is available through www.qnano-ri.eu/access. Access is granted on the basis that the funded research will be published and thus publically available.

Application for Transnational Access is via the QNano Access portal at www.qnano-ri.eu/access, and is offered via 6-monthly calls for proposals which are reviewed by a panel of external experts and selected based on scientific excellence and demonstrated need for access to the relevant equipment. Applications are made for visits of average duration of 5-8 days. The first call will close on 31st January 2012, with the 2nd call opening on 1st May 2012 with a closing date of 31st July 2012. There will be 6-monthly calls for access proposals thereafter until July 2014, and successful applicants will have 1 year from being granted access to undertake their research visit.

References

BAALOUSHA, M., MANCIULEA, A., CUMBERLAND, S., KENDALL, K., LEAD, J.R. (2008) Aggregation and surface properties of iron oxide NPs: Influence of pH and natural organic matter. *Environmental Toxicology and Chemistry,* 27**,** 1875-1882.

COLVIN, V. L. (2003) The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.,* 21**,** 1166-1170.

ELSAESSER, A., TAYLOR, A., STAATS DE YANÉS, G., GEORGE MCKERR, G., KIM, E-M., O'HARE, E., HOWARD, C.V. (2010) Quantification of nanoparticle uptake by cells using microscopical and analytical techniques. *Nanomedicine (London),* 5**,** 1447-1457.

KENDALL, M., DING, P., KENDALL, K. (2011) Particle and nanoparticle interactions with fibrinogen: the importance of aggregation in nanotoxicology. *Nanotoxicology,* 5**,** 55-65.

MAIORANO, M., STEFANIA SABELLA, S., SORCE, B., BRUNETTI, V., MALVINDI, M.A., CINGOLANI, R., POMPA, P.P. (2010) Effects of Cell Culture Media on the Dynamic Formation of Protein−Nanoparticle Complexes and Influence on the Cellular Response. *ACS Nano,* 4**,** 7481-7491.

RAMIREZ-GARCIA, S., CHEN, L., MORRIS, M.A., DAWSON, K.A. (2011) A new methodology for studying nanoparticle interactions in biological systems: Dispersing titania in biocompatible media using chemical stabilisers. *Nanoscale***,** 4617-4624.

RIVERA GIL, P., OBERDÖRSTER, G., ELDER, A., PUNTES, V., PARAK, W.J. (2010) Correlating Physico-Chemical with Toxicological Properties of Nanoparticles: The Present and the Future. *ACS Nano,* 4**,** 5527-5531.

STONE, V., NOWACK, B., BAUN, A., VAN DEN BRINK, N., KAMMER, F., DUSINSKA, M., HANDY, R., HANKIN, S., HASSELLÖV, M., JONER, E., FERNANDES, T.F. (2010) Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation. *Sci. Total Environ.,* 408**,** 1745-1754.

WALCZYK, D., BALDELLI-BOMBELLI, F., CAMPBELL, A., LYNCH, I., DAWSON, K.A. (2010) What the Cell "Sees" in Bionanoscience. *JACS,* 132**,** 5761-5768.

WARHEIT, D. B. (2008) How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? *Toxicol Sci,* 101**,** 183-185.

XIA, T., KOVOCHICH, LIONG, M., ZINK, J.I., NEL, A.E. (2008) Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. *ACS Nano,* 2**,** 85-96.

[2] Potential applicants from outside the EU should contact the QNano Project Office for details of whether they are eligible to apply.

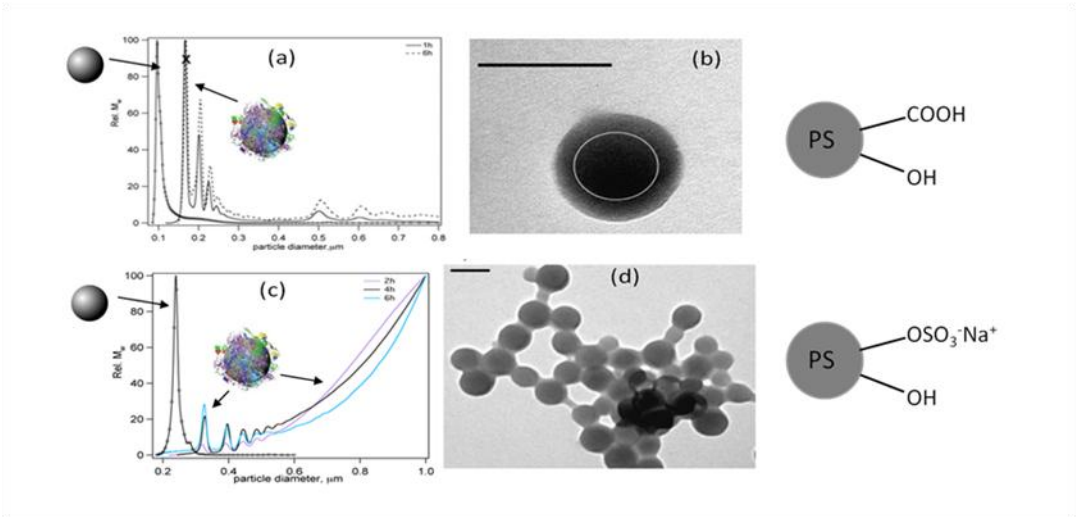

Figure 1: Differential Centrifugal Sedimentation (DCS) data for two 100nm polystyrene nanoparticles with different surface modification dispersed in physiological buffer or human plasma diluted 50 times in physiological buffer. In the first case (top), particles remained well dispersed, with the majority of the particles as monomers, and some dimers and trimers, and a very small amount of larger agglomerates, suggesting that most of the original particle dose is still available. An increase in effective particle size was observed as a result of the formation of a "corona" of proteins and other biomolecules around the nanoparticles. In the second case (bottom) the particles agglomerated very quickly in the presence of proteins, with almost no particle monomers remaining, suggesting that the available dose of nanoparticles is very different in this case, as also confirmed by the Transmission Electron Microscopy (TEM) image (Scale bar 100 nm in b and d). From the peak intensity, some approximation of the fraction of particles in the nanoscale, i.e. the available dose, relative to the initial particle concentration, can be made. Redrawn from (Walczyk, 2010). Note that QNano partners[3] UCD, FUNDP, VITO and UNIVLEEDS offer access to DCS, and partners KIT, TCD, ICN, UU, NHM, UNIVLEEDS and UCD offer access to TEM.

[3] See Figure 2 for all TA partners. UCD – University College Dublin, FUNDP - Facultés Universitaires Notre-Dame de la Paix de Namur, VITO - Vlaamse Instelling voor Technologisch Onderzoek , UNIVLEEDS – University of Leeds, KIT – Karlsruhe Institute of technology, TCD – Trinity College Dublin, ICN - Institut Català de Nanotecnologia (ICN), UU – University of Uppsala, NHM – Natural History Museum.

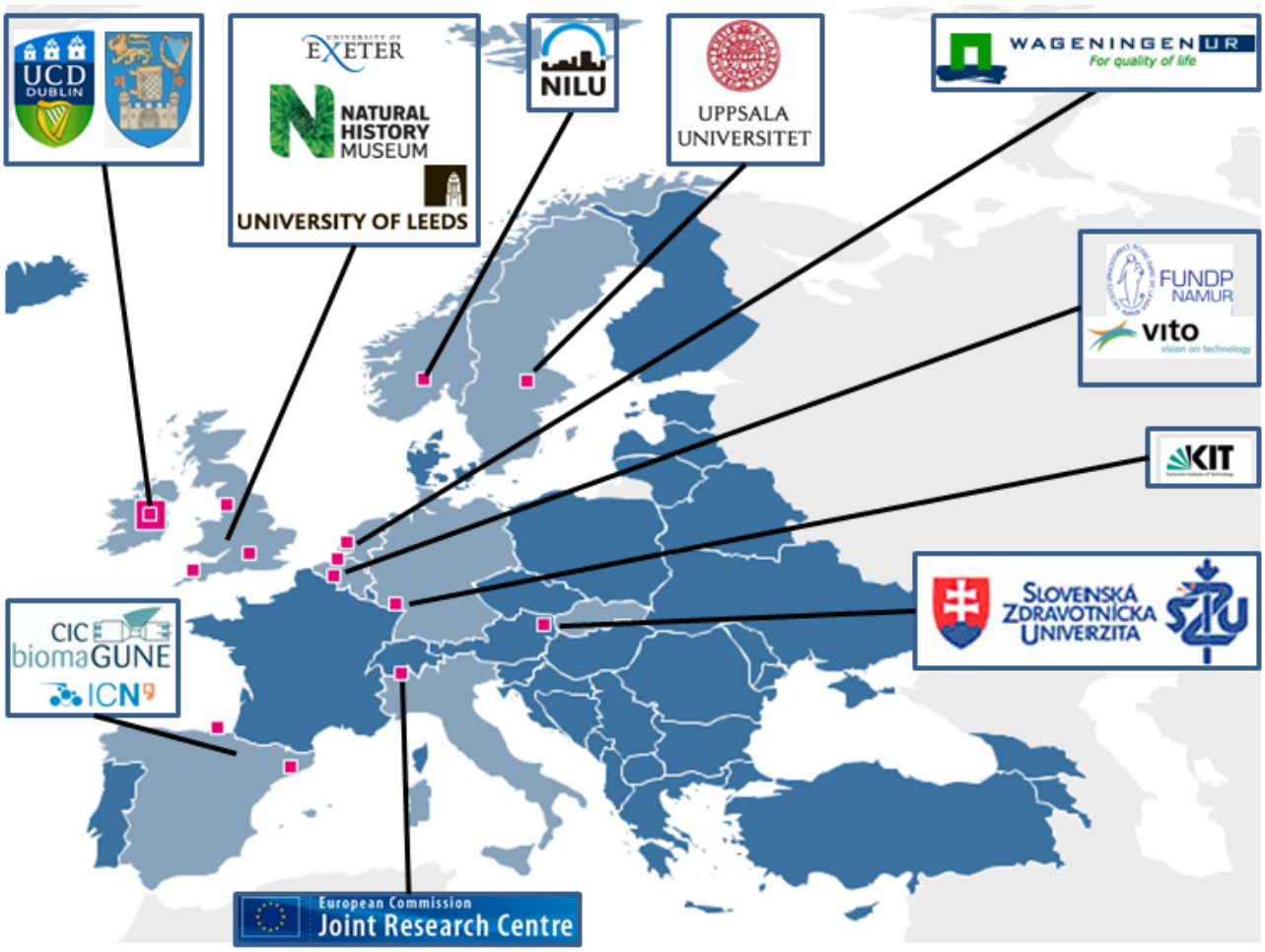

Figure 2: Map showing the locations of the institutes offering QNano-funded Transnational Access to state of the art characterisation facilities for nanomaterials in contact with living systems. Note that a potential User cannot request to visit a facility in the country in which they work.